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KIT D816V testing in Systemic Mastocytosis patients

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Prof. Dr. Hans Michael Kvasnika is director of the Institute of Pathology and Molecular Pathology Helios University Hospital Wuppertal and chair holder of pathology at the University of Witten/Herdecke. Previously, Dr Kvasnicka was chief physician and deputy director of the Senckenberg Institute of Pathology at the University of Frankfurt. There he headed the Department of Hematopopathology and Tumor Pathology. He is a consultant to a number of international and clinical-pathological studies on behalf of the World Health Organization (WHO) and is the departing chairman of the European Bone Marrow Committee (EBMWG). He has an impressive list of publications in journals and was co-author of the WHO classification of tumors of hematopoietic and lymphoid tissues.

Personal conflict of interest disclaimer

I have the following relevant financial relationships to disclose:

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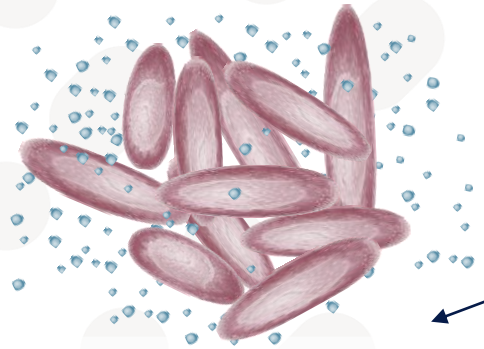
What are the characteristics of systemic mastocytosis?

Rare, clonal mast cell proliferation, with **KIT D816V mutation**[1,2]

Abnormal activation of mast cells can lead to debilitating and potentially life-threatening symptoms[2-4]

Symptom-oriented therapies offer **limited disease control** and associated symptoms[2,4,5]

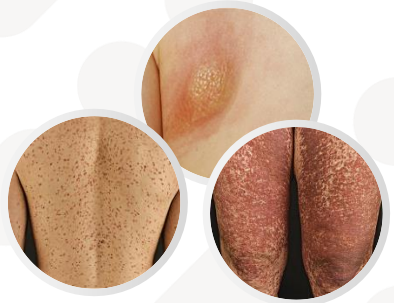
The uncontrolled proliferation and activation of mast cells leads to severe and unpredictable symptoms[2,5,6]



Abnormal mast cells[2]

Release of pro-inflammatory messenger substances (e.g. tryptase, histamine, IL-6, TNF)

Anaphylaxis with hypotension[5]



Maculopapular lesions with Darier's sign[5,6,a]

Nausea, vomiting and/or diarrhea[5]

Muscle and bone pain, osteolytic lesions[2,3]

74% of 158 patients reported living in fear of anaphylaxis and may isolate/stay at home to protect themselves from unpredictable triggers, to manage frequent diarrhea, and/or to manage fear of anaphylaxis[5,7]

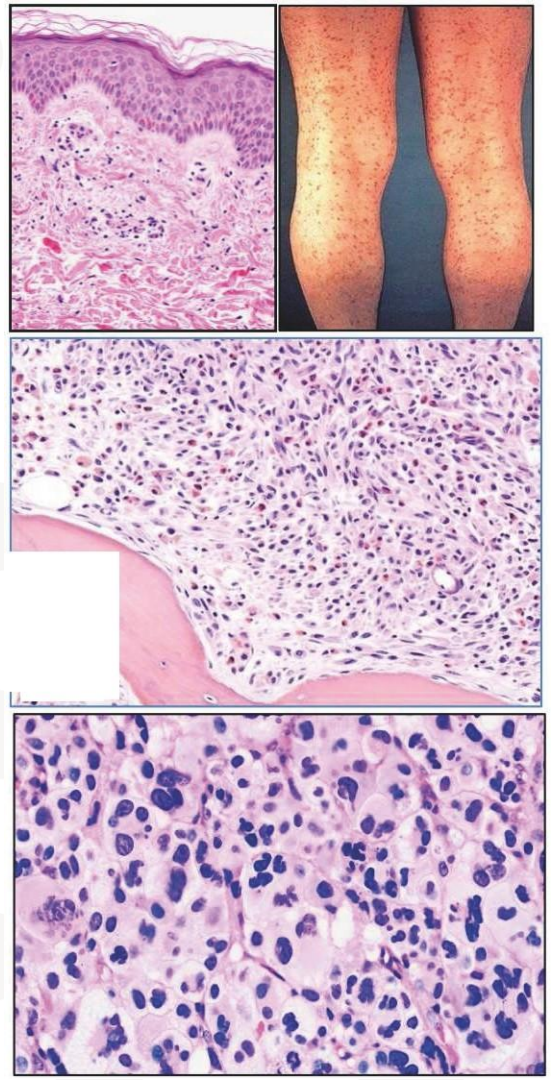
IL-6: Interleukin-6; TNF: Tumor-Necrose-Factor; KIT: KIT proto oncogene, Tyrosin kinase.

[a] Nachgedruckt aus Hartmann K et al. *J Allergy Clin Immunol.* 2016;137(1):35-45.

1. Rossignol J et al. *F1000Research*. 2019:8. 2. Pardanani A. *Am J Hematol* . 2023 Jul;98(7):1097-1116. 3. Theoharides TC et al. *N Engl J Med.* 2015;373(2):163-172. 4. Valent P. *Clin Exp Allergy.* 2014;44:914-920. 5. Reprinted from Jennings SV et al. *Immunol Allergy Clin North Am.* 2018;38(3):505-525. 6. Hartmann K et al. J Allergy Clin Immunol. 2016;137(1):35-45. 7. Siebenhaar F et al. *Allergy*. 2016;71:869-877

Mastocytosis: general types and classifications[1]

- Cutaneous mastocytosis
- Systemic mastocytosis
- Mast cell sarcoma
- WHO-HAEM5 and ICC criteria

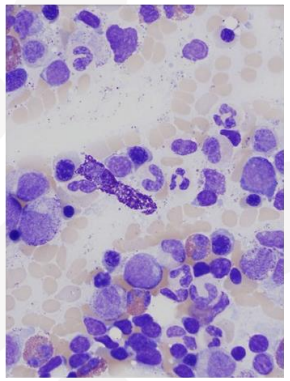


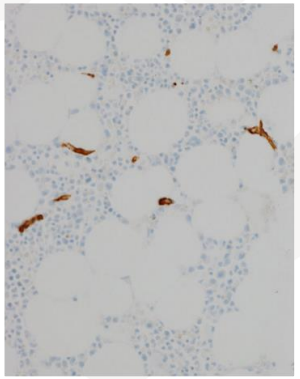
Mast Cell Activation Syndrome

Criterion A: Typical clinical signs of severe, recurrent (episodic) systemic MCA are present (often in form of anaphylaxis) (definition of systemic: involving at least 2 organ systems)

Criterion B: Involvement of MC is documented by biochemical studies: preferred marker: increase in serum tryptase level from the individual's baseline to plus 20% + 2 ng/ml

Criterion C: Response of symptoms to therapy with MC-stabilizing agents, drugs directed against MC mediator production, or drugs blocking mediator release or effects of MC-derived mediators





Monoclonal Mast Cell Activation Syndrome (MMCAS)

WHO criteria states "In some patients with MCAS the diagnostic criteria for SM are not fulfill but clonal MC **with** the ***KIT*** D816V mutation **or** aberrant surface CD25 are found; these patients are diagnosed with MMCAS."

20% of Indolent SM patients **lack** BM **MC clusters** and approximately **30%** exhibit a serum **tryptase** level lower than **20 ng/ml**

Images are property of the speaker

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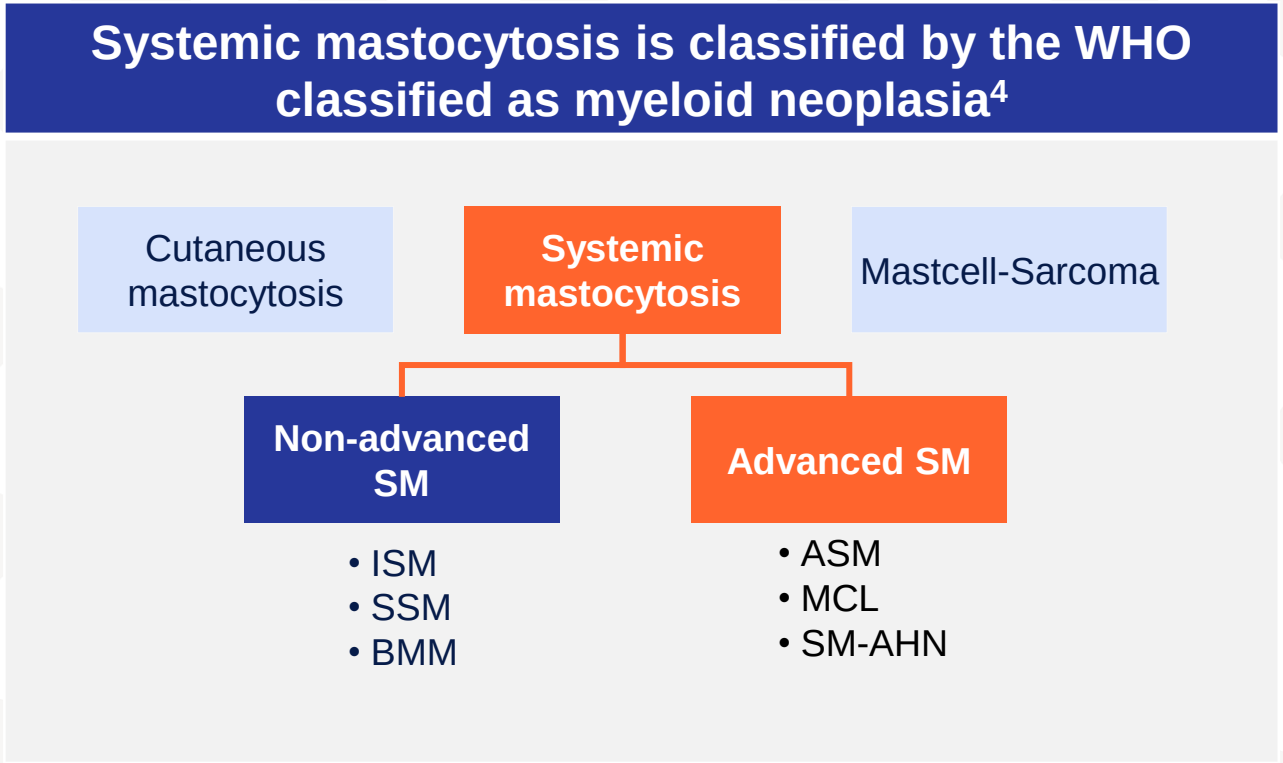
[1]Arber DA et al, Blood (2022) 140 (11):1200-1228; [2]Valent P, Annu Rev Pathol. 2023 Jan 24;18:361-386

Non-advanced systemic mastocytosis is the most common subtype of SM, with 80-90% of patients[1,2]

- The prevalence of systemic mastocytosis is estimated to be ≈ 1 in 10,000 adults[1]
- According to registry data (n=1,454), the majority of patients with non-advanced systemic mastocytosis have ISM (~95%) and ~5% have SSM[2]
- ~70% of advanced SM cases are SM-AHN[3]



ASM: Aggressive systemic mastocytosis; BMM: Bone marrow mastocytosis; ISM: Indolent systemic mastocytosis; MCL: Mast cell leukemia; SM: Systemic mastocytosis; SM-AHN: Systemic mastocytosis with associated hematological neoplasia; SSM: Smouldering systemic mastocytosis; WHO: World Health Organization.

1. Cohen SS et al. *Br J Haematol*. 2014;166:521-528. 2. Sperr WR et al. *Lancet Haematol*. 2019;6(12):e638–e649. 3. Reiter A et al. *Blood*. 2020;135(16):1365-1376. 4. Khoury JD et al. *Leukemia.* 2022;36(7):1703-1719.

WHO diagnostic criteria for systemic mastocytosis

Major criterion[1-3]

- Histological detection of multifocal compact infiltrates from mast cells (≥15) in bone marrow (BM) or another extracutaneous organ

Minor criteria[1,2]

- Detection of atypical spindle-shaped mast cells (≥25% of all mast cells): histologically in BM or other extracutaneous organs or cytologically in the BM smear
- Detection of an activating point mutation in codon 816 or other critical regions* of KIT (usually KIT D816V) in BM, blood, or other extracutaneous organs
- Mast cells in BM, blood or other extracutaneous organs express one or more of these surface markers: CD2, CD25, CD30*
- Serum tryptase levels persistent >20 µg/l**B

Diagnosis[1-3]

Prerequisite:

- The main criterion and at least 1 secondary criterion are fulfilled

OR

- ≥ 3 secondary criteria are fulfilled

*According to (1) in the 2022 WHO classification
**Does not apply in the case of an SM-AHN
BM: bone marrow; CD: cluster of differentiation; WHO: World Health Organization

1. Horny HP, et al. Mastocytosis. In: Swerdlow SH, et al. eds. WHO classification of tumors of hematopoietic and lymphoid tissues. International Agency for Research and Cancer. 2017:62-69. 2. Valent P, et al. Hemasphere. 2021; 5(11):E646. 3. Onkopedia Guideline Systemic Mastocytosis, as of January 2024

Diagnosis: B and C criteria for SM

ISM / SSM

B-criteria[1,2]

- **Mast cell infiltration in BM>30% and tryptase >200µg/l or KIT D816V VAF ≥10% in peripheral blood**
- **Dysmyelopoiesis**: Hypercellular bone marrow with signs of myelodysplasia or myeloproliferation, but no criteria for MDS or MPN. Blood count normal or slightly abnormal
- **Organomegaly** (without impairment of organ function): hepatomegaly (without ascites), splenomegaly (palpable), lymphadenopathy (>2 cm on CT or sonography)

AdvSM

C-criteria[1,2]

- **≥1 Cytopenia**: neutrophils <1,000/µl; Hb<10 g/dl; platelets<100,000/µl
- **Hepatopathy**: Enlarged liver with ascites, elevated liver enzymes +/- portal hypertension
- **Organopathy of the spleen**: splenomegaly with hypersplenism
- **Malabsorption with hypoalbuminemia** and weight loss
- **Severe osteolysis** +/- severe osteoporosis +/- pathological fractures

If there are 2 or 3 B criteria but no C criteria, Smouldering SM (SSM) can be diagnosed

ISM: Indolent systemic mastocytosis; SM: Systemic mastocytosis; SSM: Smouldering systemic mastocytosis; AdvSM: Advanced systemic mastrocytosis; BM: bone marrow; MDS: Myelodysplastic syndrome; MPN: Myeloproliferative disease; Hb: hemoglobin; VAF: Allele frequency variant, KIT: KIT proto-oncogene, tyrosine kinase; CT: Computed tomography.

1. Valent P et al. Hemasphere. 2021; 5(11):E646. 2. Onkopedia Guideline Systemic Mastocytosis, as of Jan 2024

SM subtypes (variants)

- Indolent systemic mastocytosis (ISM)
  - (includes bone marrow mastocytosis; BMM)
- Smoldering systemic mastocytosis (SSM)
- Aggressive systemic mastocytosis (ASM)
- Mast cell leukemia (MCL)
- Systemic mastocytosis with an associated myeloid neoplasm (SM-AMN) *

indolent SM

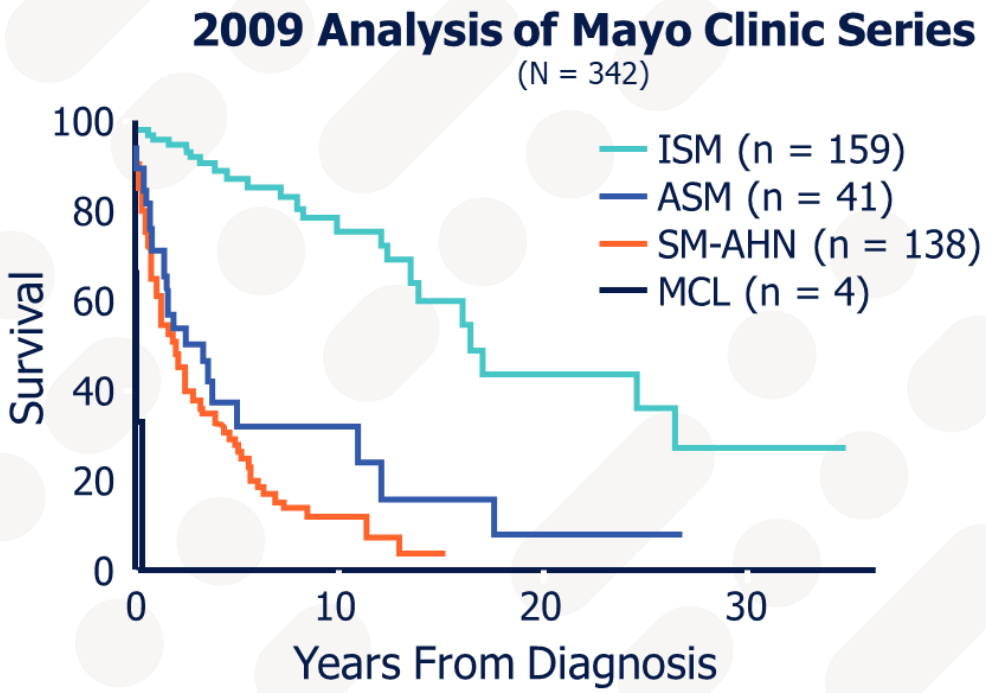
Advanced SM

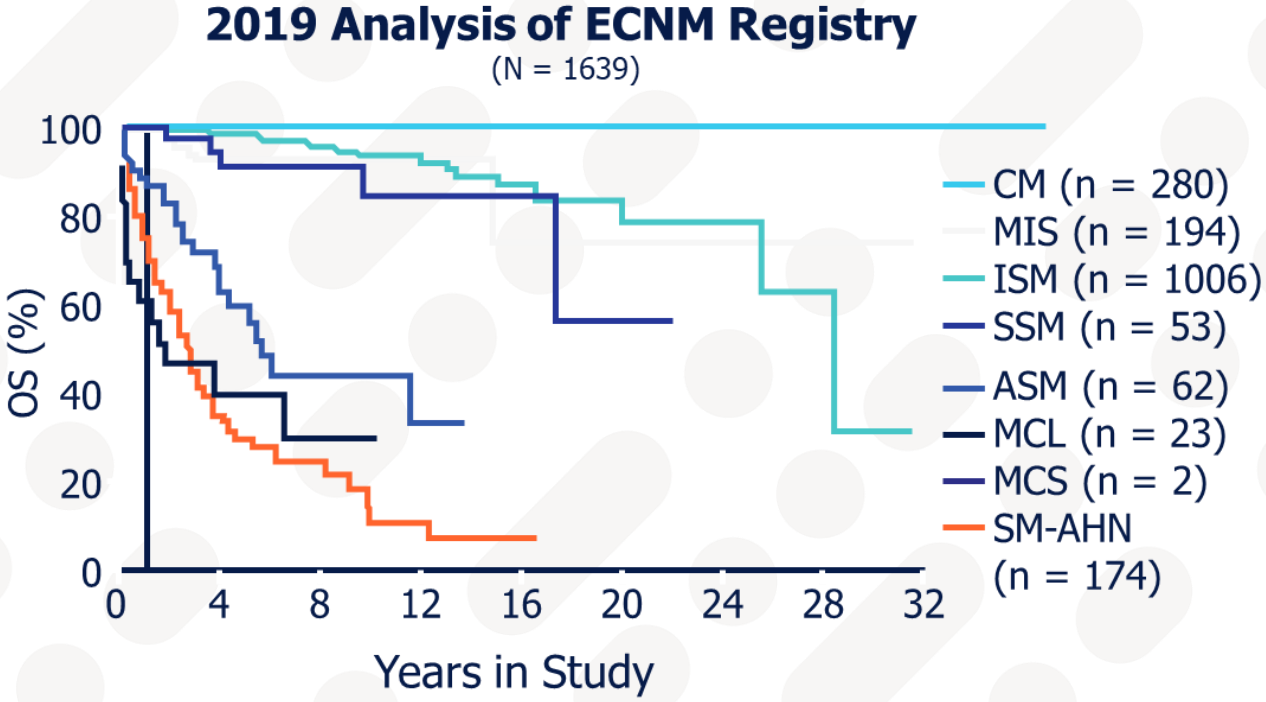
SM subtyping is not stand-alone pathology but depends on the presence/absence of "B" and "C" findings (B=burden of disease; C=cytoreduction required)

* In WH0-HAEM4 and in WHO-HAEM5 called SM with an associated hematologic neoplasm (which includes lymphoid neoplasms). The AMN may be present at disease outset (can be occult) or represent progression.

Arber DA et al, Blood (2022) 140 (11):1200-1228

Prognosis varies by SM subtype





Lim KH et al., *Blood*, 2009, 113(23):5727-5736; Sperr WR et al., *Lancet Haematol,* 2019;6(12):e638-e649.

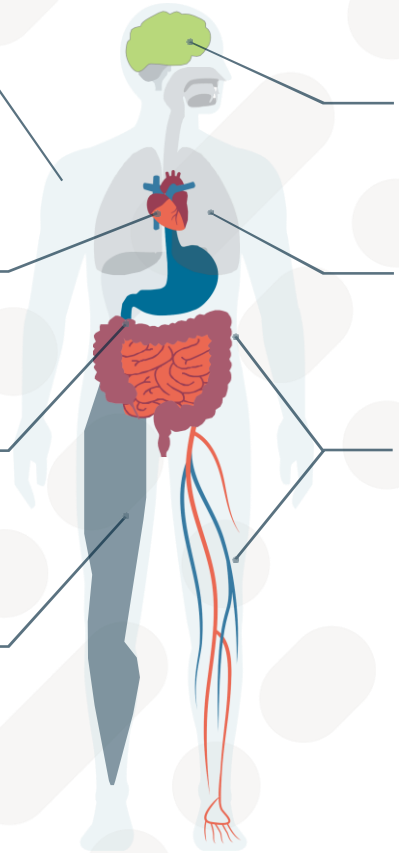
Systemic mastocytosis can affect multiple organ systems[1-3]

Skin:
Itching, redness, maculopapular lesions

Cardiovascular:
Syncope, dizziness, palpitations

Gastrointestinal:
Nausea/vomiting, diarrhea, abdominal cramps, reflux

Musculoskeletal:
Muscle and bone pain, osteolytic lesions



Neuro-psychiatric:
Cognitive impairment, depression, migraine, insomnia

Anaphylaxis:
Hypotension, angioedema, stings caused by hymenoptera, medicines, food

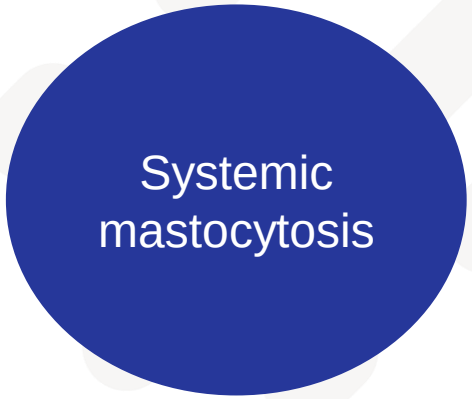
Systemic:
Fatigue, weight loss, general feeling of illness

The presented symptoms may occur with systemic mastocytosis. Depending on the subtype of systemic mastocytosis and the aggressiveness of the disease, interindividual differences may occur

1. Pardanani A. Am J Hematol. 2023 Jul;98(7):1097-1116. 2. Jennings SV, et al. Immunol Allergy Clin North Am. 2018;38(3):505–525. 3. Onkopedia Guideline Systemic Mastocytosis, as of January 2024

Diagnosing systemic mastocytosis can be challenging

- Due to heterogeneous and non-specific symptoms, systemic mastocytosis can be confused with other conditions[1,2]
- In some patients, the disease may go undetected until they are exposed to a trigger (e.g., bee/wasp sting) and suffer anaphylactic reactions or even death[3,4]
- To avoid misdiagnosis and delayed treatment, a detailed medical history and diagnostics are required



Symptoms of SM can mimic other disorders[2,5]:

- Idiopathic MCAS
- Monoclonal MCAS
- Inflammatory bowel disease
- Inflammatory bowel syndrome
- Urticaria
- Cryptogenic cirrhosis
- Malabsorption
- Myeloproliferative disease
- Endocrine disorders
- Hereditary alphatryptasemia
- Idiopathic anaphylaxis

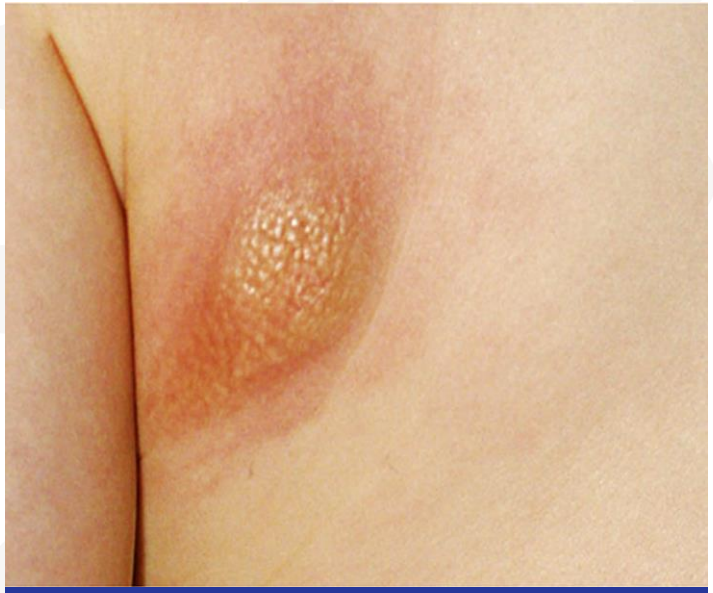
MCAS=mast cell activation syndrome.

1.TheoharidesTC et al. *N EnglJ Med*. 2015;373(2):163-172. 2.NORD. Rare disease database: mastocytosis.. https://rarediseases.org/rare-diseases/mastocytosis/ 3.Reiter N et al. *Lancet*. 2013;382(9901):1380. 4.Kors JW et al. *J Intern Med*. 1993;233(3):255-258. 5. Jiang ZG et al. *Gastroenterology*. 2018;155(1):23-24.e1.

Patients with SM often present with skin involvement

A complete bone marrow workup is required in patients with cutaneous involvement to confirm or deny systemic mast cell infiltration[1]

- Highlight mast cells using tryptase and CD117/KIT[2]
- Expression of CD25 on mast cells is a marker of their neoplastic nature and is predictive of SM in adults but is not a consistent marker for mutated mast cells in the skin[2]
- The distribution, shape, and expression of CD25 are more important than specific mast cell counts[2]



The presence of classic skin lesions with Darier's sign is typically enough to establish cutaneous involvement[1,3]

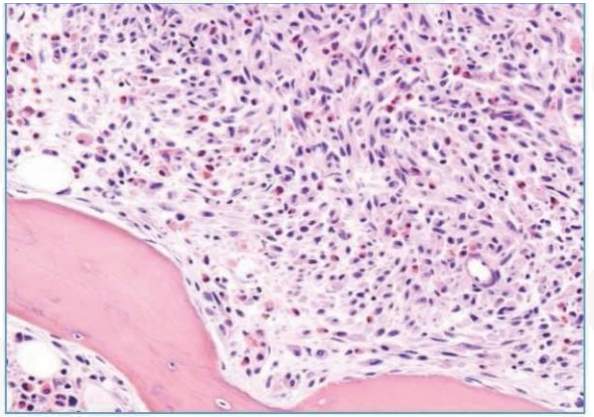
Image adapted from Hartmann K et al. 2016

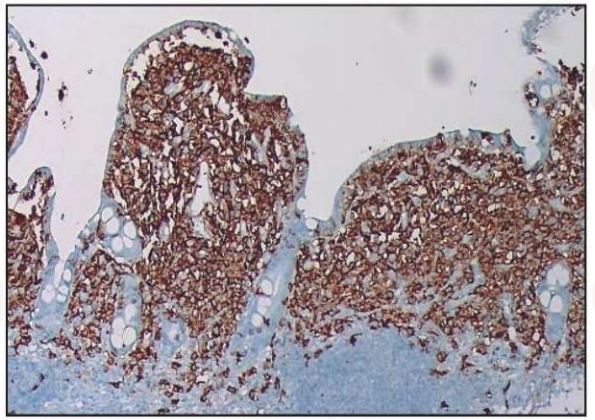
1. Sotlar K et al. *J Allergy Clin Immunol Pract*. 2022;10(8):1986-1998.e2. 2. Zimmermann N et al. *J Allergy Clin Immunol*. 2021;148(4):964-983. 3. Hartmann K et al. *J Allergy Clin Immunol*. 2016;137(1):35-45.

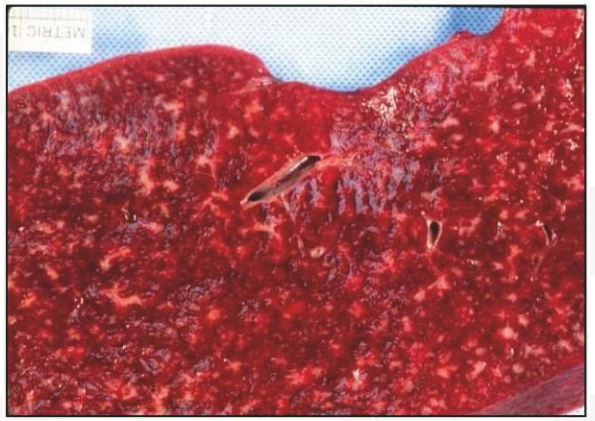
SM: frequency by subtype

- ISM: 46%
- ASM: 15%
- MCL: 1%
- SM-AHN: 34% (32% AMN)

- Different frequencies reported from nation wide studies:
  - ISM up to 89%
  - advanced SM 7% (SM-AMN, up to 6%)



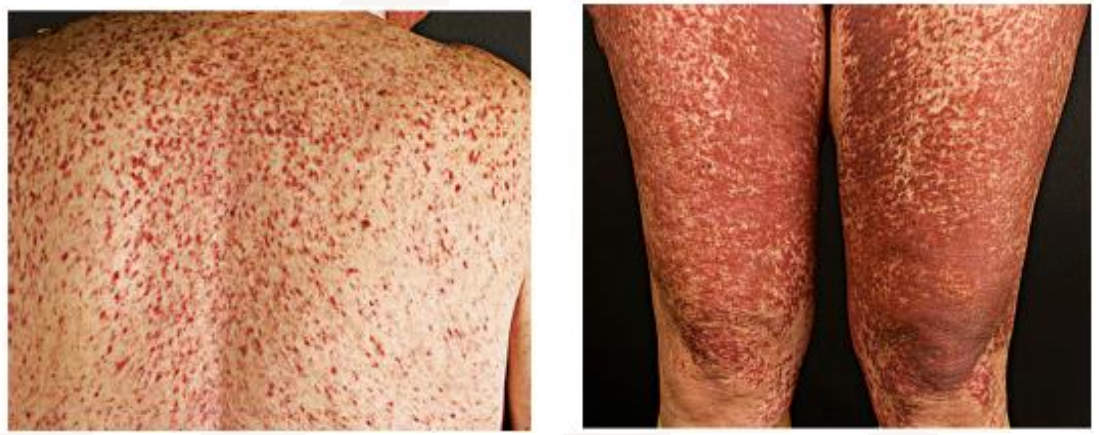




Leguit R et al. Pathobiology 2020;87:2-19

If SM is suspected, a KIT D816V test can be ordered on peripheral blood[1]

- If skin lesions are ambiguous, increased mast cells and/or an activating KIT mutation found in a skin biopsy are required to confirm skin involvement[2]
- Repeat biopsies if clinical suspicion is high, as the infiltrate can be sparse in some cases[3]
- Until a full diagnostic workup has been performed, a provisional diagnosis of mastocytosis in the skin (MIS) can be made[2]

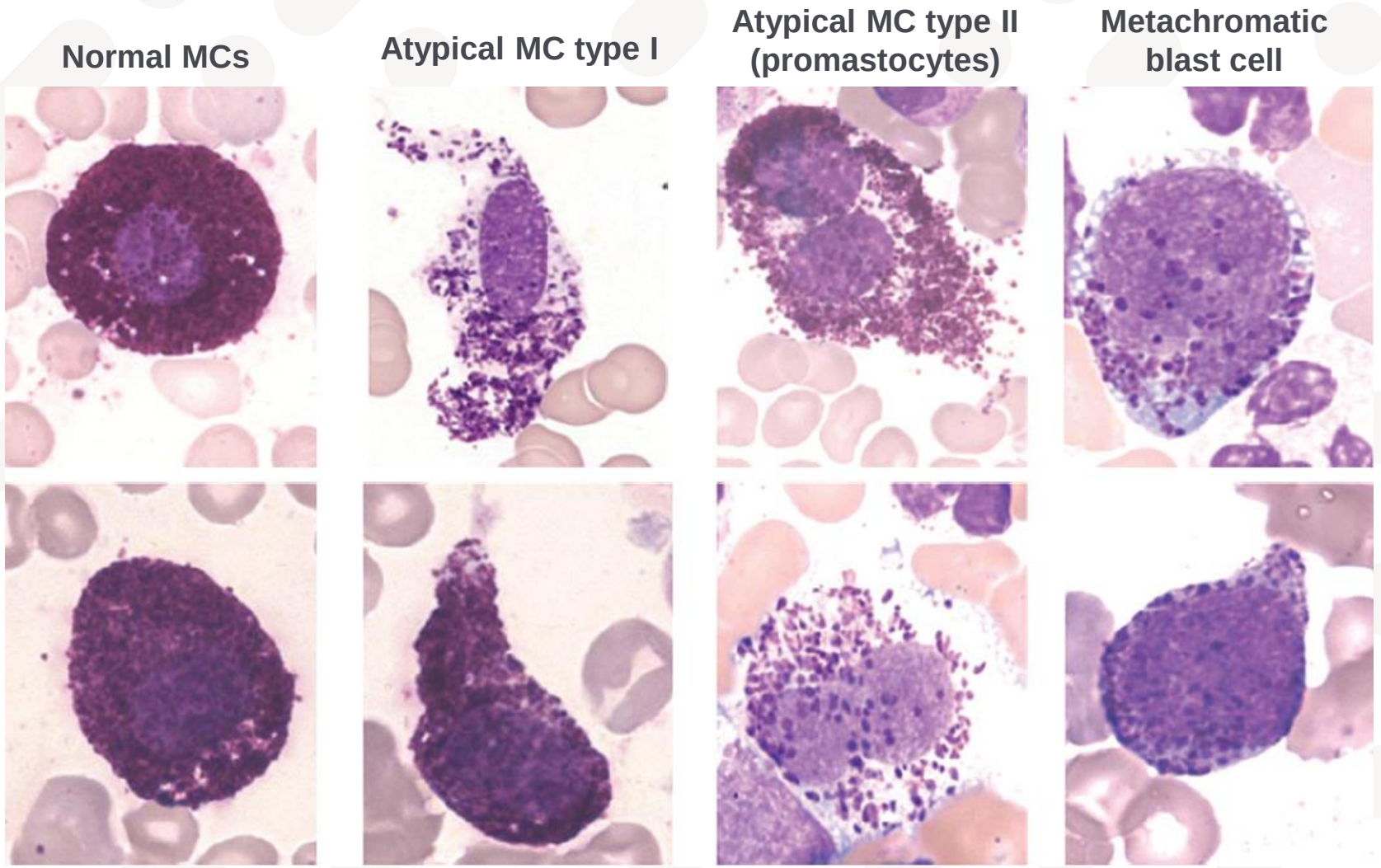


Images adapted from Hartmann K et al. 2016

Small monomorphic lesions appear on the thighs or trunk of the body[4]

1. Sotlar K et al. *J Allergy Clin Immunol Pract*. 2022;10(8):1986-1998.e2. 2. Zimmermann N et al. *J Allergy Clin Immunol*. 2021;148(4):964-983. 3. Hoermann G et al. *J Allergy Clin Immunol Pract*. 2022;10(8):1953-1963. 4. Hartmann K et al. *J Allergy Clin Immunol*. 2016;137(1):35-45.

Mast cell cytology: BM aspirate



Valent P, et al. Annals of Oncology. 2014;25:1691–1700.

Normal MC

- Mature appearing MC
- Round MC
- Round to ovoid nucleus
- No granulation defects

Atypical MC type I

- Spindle-shaped MC
- Ovoid nucleus
- Variably hypogranulated

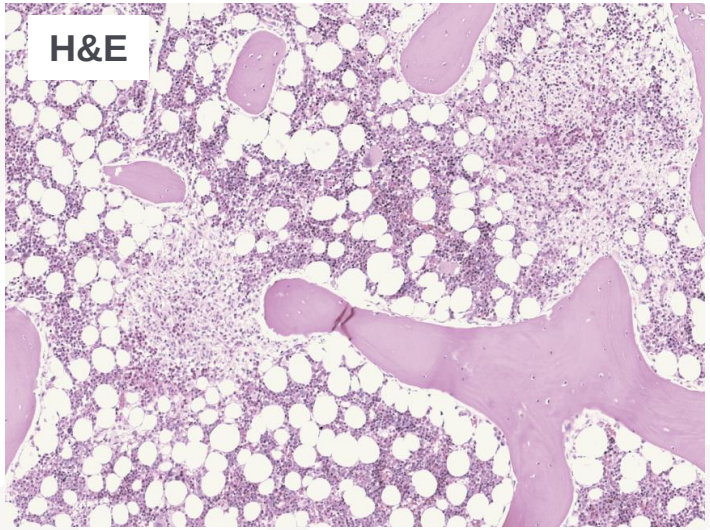
Atypical MC type II

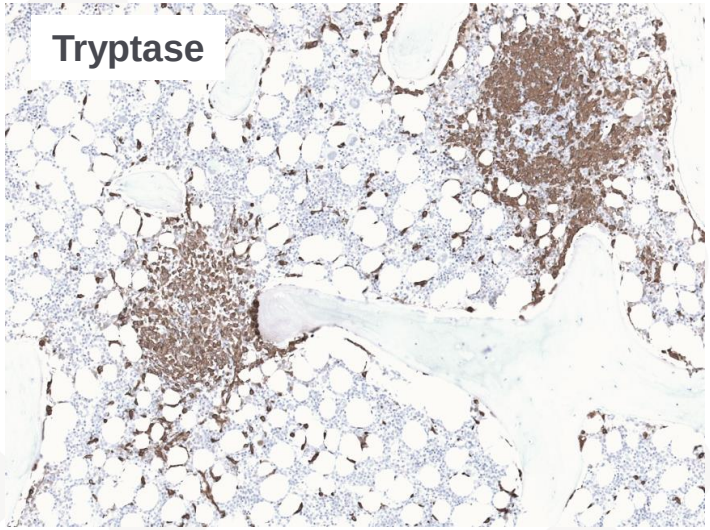
- Bi-/multilobated MC
- Variably hypogranulated

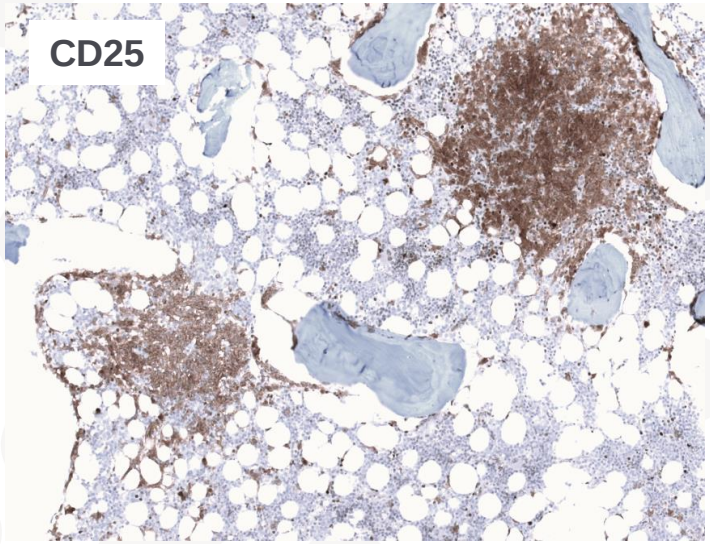
Metachromatic blast cell

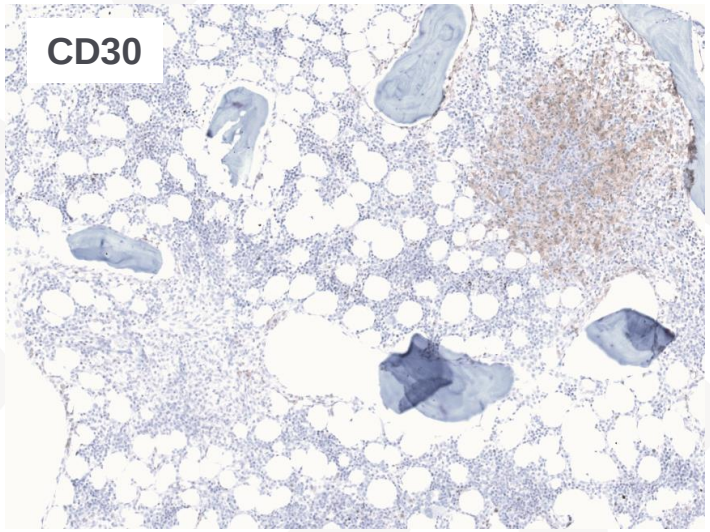
- Blastoid cell
- High nucleus: cytoplasmic ratio
- Metachromatic granules
- Hypogranulation
- Indistinguishable from basophil precursors

Important IHC biomarkers for SM diagnosis









Normal MC

- Tryptase, CD117/KIT

Neoplastic MC

- **Tryptase, CD117/KIT, CD25**, CD2, **CD30** (in a number of cases; frequently expressed in WDSM)

IHC markers in hemato-pathology that may lead to misinterpretations:

- CD9, CD14, CD26, CD45, CD52, CD68, CD99, CD123, CD131 and 2D7

Sotlar K, et al. Mod Pathol. 2011;24(4):585–595. Horny HP, et al. Immunol Allergy Clin North Am. 2014;34(2):315–321.

The pathologist's role in making an accurate SM diagnosis is critical

A thorough workup is required for definitive diagnosis of SM[1,2]

Evaluation for SM may include:

- **Serum tryptase level**[2-4]
- **Bone marrow biopsy** with mast cell immunophenotyping IHC, specifically markers for: **CD117, tryptase, CD2, CD25, and CD30**[3-5]
- **KIT D816V testing** with a high-sensitivity molecular assay can first be undertaken on peripheral blood. If negative, KIT mutational analysis may also be performed on the bone marrow aspirate[6]
- **NGS** for detection of somatic mutations in genes other than KIT[6]

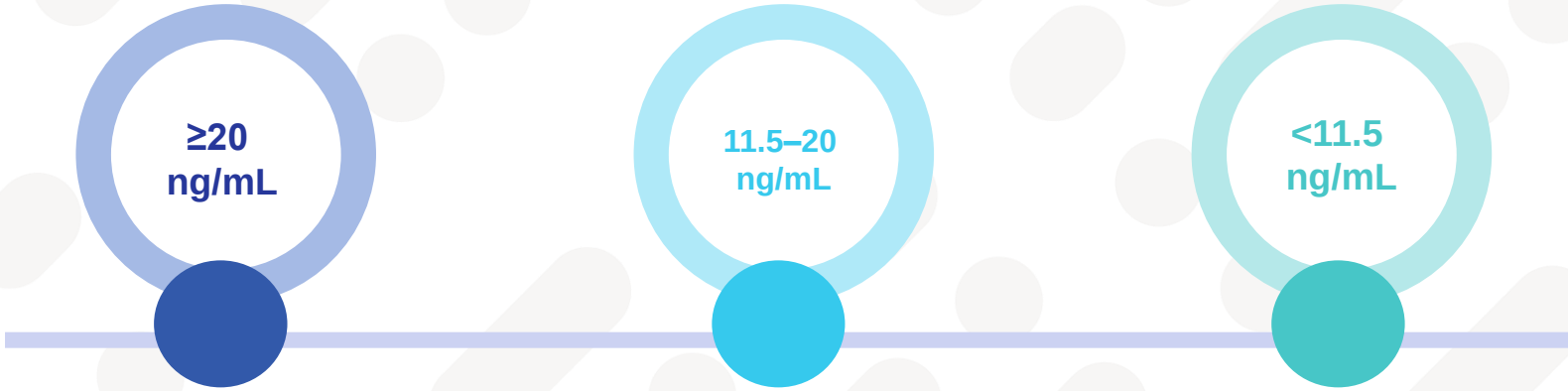
Recommend a high-sensitivity KIT D816V test if not initially requested by the treating clinician[6]
Using high-sensitivity testing (eg, ASO-qPCR or ddPCR), KIT D816V can be detected in the peripheral blood of most patients with SM, serving as a reliable tool for screening

ASO-qPCR: allele-specific oligonucleotide quantitative polymerase chain reaction; ddPCR: droplet digital PCR; NGS: next-generation sequencing.

1. Valent P et al. *Blood*. 2017;129(11):1420-1427.2. Gotlib J et al. *Blood*. 2013;121(13):2393-2401. 3.Pardanani A. *Am J Hematol*. 2023;98(7):1097-1116. 4.Theoharides TC et al. *N Engl J Med*. 2015;373(2):163-172.5. Sotlar K et al. *J Allergy Clin Immunol Pract*. 2022;10(8):1986-1998.e2.6. Hoermann G et al. *J Allergy Clin Immunol Pract*. 2022;10(8):1953-1963.

Serum tryptase should be determined if systemic mastocytosis is suspected[1]

WHO criteria for the diagnosis of systemic mastocytosis
The total tryptase in serum[a] is:



- **Bone marrow biopsy and screen for KIT D816V mutation**[1]
- SM possible - screen for KIT D816V in peripheral blood with high-sensitivity assay[1]
- Mastocytosis unlikely, but cannot be ruled out[1,2]

Serum tryptase alone is not a sufficient screen for SM, and results should be interpreted alongside other clinical and laboratory findings

[a] Unless associated myeloid neoplasia is present; in this case, this parameter is not valid

1. Theoharides TC et al. *N Engl J Med*. 2015;373(2):163-172. 2. Akin C et al. *Immunol Allergy Clin North Am*. 2014;34(2):207-218. 3. Pardanani A. *Am J Hematol*. 2023;98(7):1097-1116.

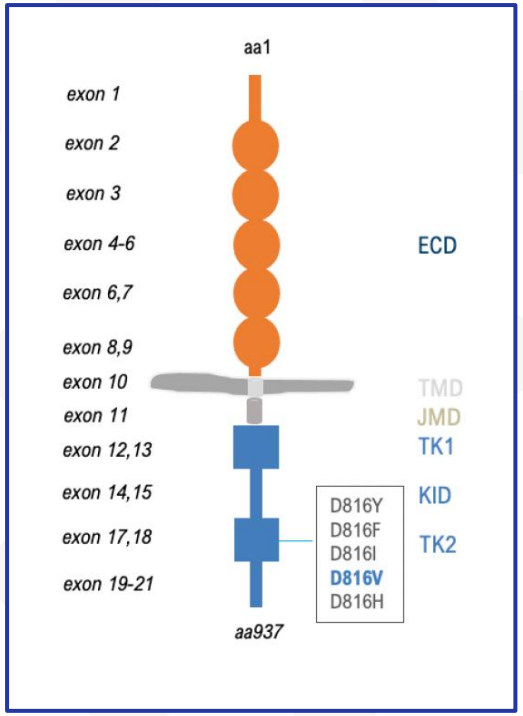
Clinical utility of *KIT* D816V in SM

- The KIT D816V mutation is present in ~95% of patients with SM and is an underlying driver of disease[1]
- The D816V mutation causes structural changes that result in constitutive activation of KIT[2]

Mast cells harboring the KIT D816V mutation have constitutive KIT activation/signaling resulting in uncontrolled and abnormal mast cell proliferation and activation[3,4]

The **detection of KIT D816V** in SM patients is not only important diagnostically but also **for therapeutic decision making** as the D816V mutation also confers resistance to some Tyrosine Kinase Inhibitors (TKI)

Structure of the KIT receptor and position of D816V mutation[5]



ECD=extracellular domain; JMD=juxtamembrane domain; KID=kinase insert domain; KIT=KIT proto-oncogene, receptor tyrosine kinase; TK1=tyrosine kinase catalytic domain 1; TK2=tyrosine kinase catalytic domain 2; TMD=transmembrane domain.

1. Garcia-Montero AC et al. *Blood*. 2006;108(7):2366-2372. 2. Laine E et al. *PLoS Comput Biol*. 2011;7(6):e1002068. 3. Cruse G et al. *Immunol Allergy Clin North Am*. 2014;34(2):219-237. 4. Theoharides TC et al. *N Engl J Med*. 2015;373(2):163-172. 5. Ustun C et al. *Haematologica*. 2016;101(10):1133-1143.

Identification of the KIT D816V mutation is a key step in the diagnosis of SM[1,2]

International Consensus Classification[3]

- To avoid a false negative, the ICC recommends the use of a **high-sensitivity assay to detect KIT D816V** in peripheral blood when SM is suspected

Methods to detect KIT D816V[2]

- ASO-qPCR
- ddPCR

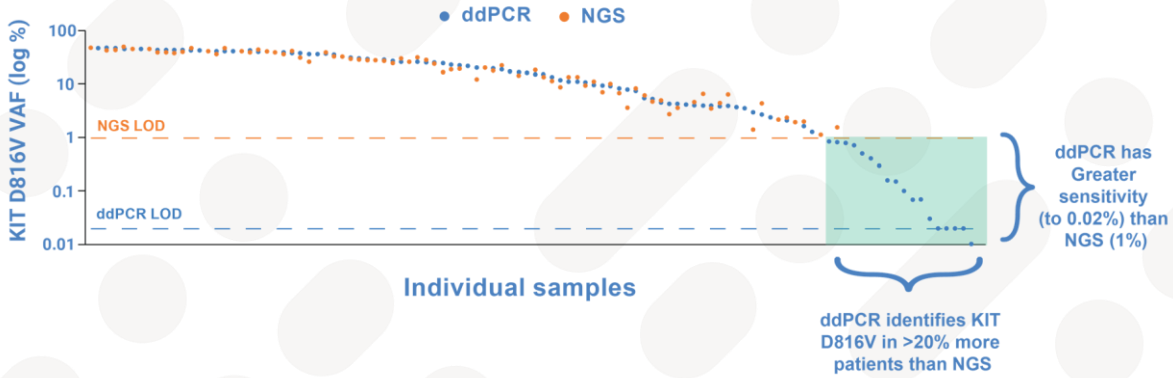
The KIT D816V variant allele frequency (VAF)
is a reliable prognostic marker of SM[2]

1. Kristensen T et al. *J Mol Diagn*. 2011;13(2):180-188. 2. Hoermann G et al. *J Allergy Clin Immunol Pract*. 2022;10(8):1953-1963. 3. Arber DA et al. *Blood*. 2022;140(11):1200-1228.

ddPCR shows greater sensitivity for detecting KIT D816V in peripheral blood[1-3]

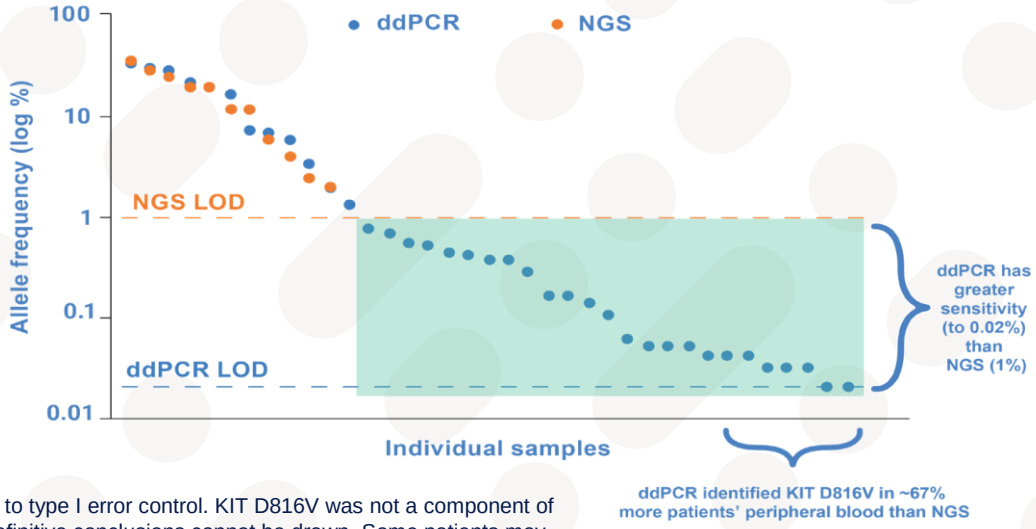
AdvSM

Baseline KIT D816V VAFs*	NGS n (%)	ddPCR n (%)
Patients analysed	107	106
KIT D816V reported	77 (72)	104 (98)



ISM

Detection of KIT D816V MAF†	NGS n (%)	ddPCR n (%)
Patients analysed	39	39
KIT D816V detected	11 (28)	37 (95)
KIT D816V not detected	28 (72)	2 (5)



*Data shown from post hoc analyses of KIT D816V VAF measures, which were not statistically powered and therefore not subject to type I error control. KIT D816V was not a component of mIWG criteria. Bone marrow mast cell aggregates are part of mIWG criteria. These results could represent chance findings and definitive conclusions cannot be drawn. Some patients may have had a reduction in KIT D816V VAF without achieving a response. Data cutoff: April 20, 2021.
Data are from a Blueprint Medicines clinical study in ISM. Results were expressed as the percentage of patient peripheral blood samples testing positive for KIT D816V mutation (all genomic assays) and the log percent of MAF as measured by both central assay methods. NGS data at screening and ddPCR values at C1D1 were plotted for the scatter graph.
ddPCR=droplet digital PCR; LOD=limit of detection; MAF=mutant allele frequency; mIWG=modified International Working Group; NGS=next-generation sequencing.

1. Data on file. Blueprint Medicines Corporation, Cambridge, MA. 2023. 2. Data on file. Blueprint Medicines Corporation, Cambridge, MA. 2020. 3. Hoermann G et al. J Allergy Clin Immunol Pract. 2022;10(8):1953-1963.

What is the role of NGS testing?

While NGS can exhibit a low sensitivity for detecting KIT D816V, it is still recommended as part of the diagnostic workup

- **~70% of patients with Advanced SM** had an AHN (CMML, MDS/MPN unclassifiable, CEL, MPN, MDS, AML) and **may require NGS testing for accurate diagnosis**
- NGS is recommended for **identifying additional genetic mutations other than KIT** that may be involved in disease pathogenesis
    - Co-mutations in KIT D816V and *SRSF2, ASXL1*, and/or *RUNX1* in patients with SM are associated with reduced overall survival (OS)

NGS can help provide prognostic information when diagnosing SM

AHN: associated hematologic neoplasms; AML: acute myeloid leukemia; CEL: chronic eosinophilic leukemia; CMML: chronic myelomonocytic leukemia; MDS: myelodysplastic syndromes; MPN: myeloproliferative neoplasms; NGS: next - generation sequencing; OS: overall survival.

1. Hoermann G et al. *J Allergy Clin Immunol Pract*. 2022;10(8):1953-1963. 2. Reiter A et al. *Blood*. 2020;135(16):1365-1376.

Systemic mastocytosis with an associated myeloid neoplasm (SM-AMN)

- Meets the diagnostic criteria for systemic mastocytosis
- Meets the criteria for an associated myeloid neoplasm e.g., chronic myelomonocytic leukemia or other myelodysplastic/myeloproliferative neoplasm, myelodysplastic syndrome, myeloproliferative neoplasm, acute myeloid leukemia, or other myeloid neoplasm [a]
- **The associated myeloid neoplasm should be fully classified according to established criteria [b]**

[a] High degree of suspicion can be raised by the presence of monocytosis, eosinophilia, splenomegaly, elevated LDH, high *KIT* D816V variant allele frequency, and additional somatic mutations in genes associated with myeloid malignancies (particularly if occurring in combination) as they could be signs of an AMN.

[b] If eosinophilia is present, the presence of tyrosine kinase gene fusions associated with M/LN should be excluded. Although usually mutually exclusive, rare cases with both a *KIT* mutation and a gene fusion associated with M/LN-Eo have been reported. In these rare instances, the M/LN-Eo would represent the SM-associated AMN, but it is recommended assigning such cases only when both a *KIT* mutation and a M/LN-Eo gene fusion are present.

Arber DA et al. Blood 2022

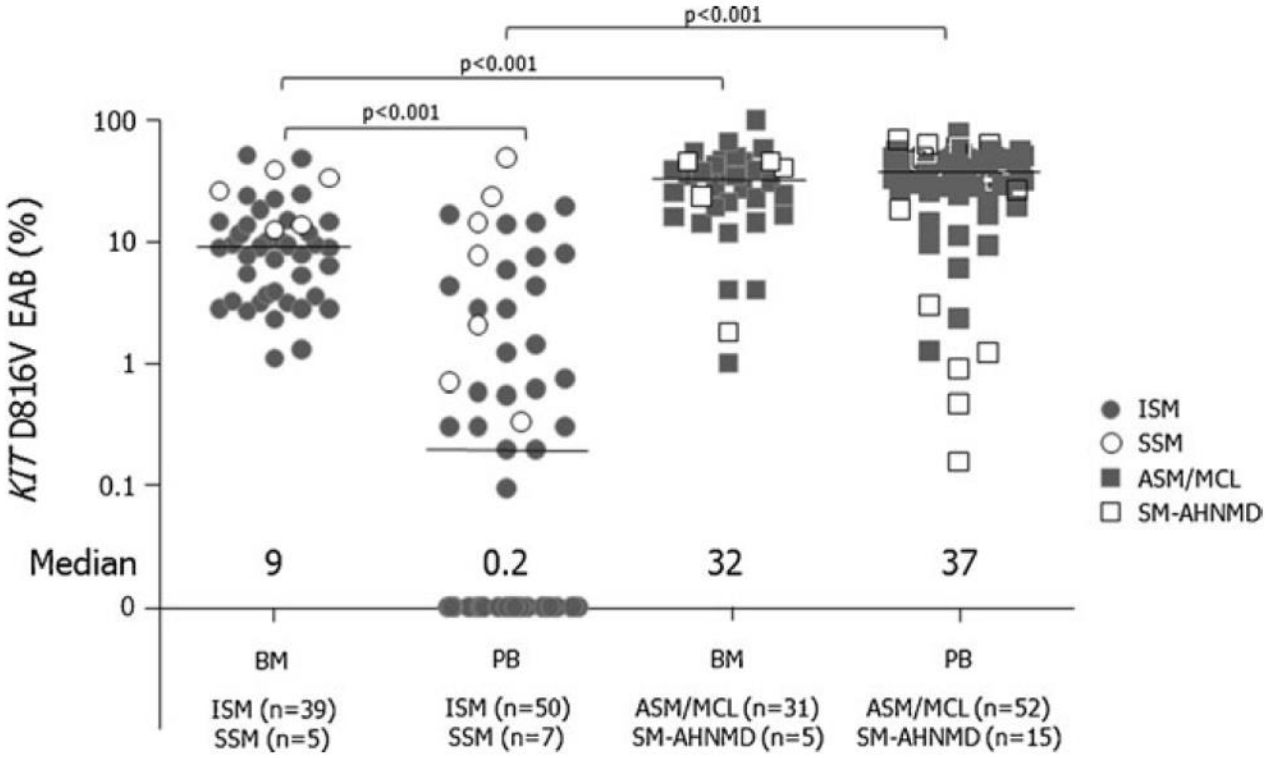
SM-AMN: General features

- More frequent in men (70%) than women (30%)
- **Skin lesion in 65% of patients**
- The two component are diagnosed concomitantly in 67% of pts. In the others there may be a variable interval (3-370 months) between SM and AMN diagnosis, with the latter representing disease progression
- In some cases, the SM can be "occult" e.g. in cases where the AMN is AML, in others is the AMN that is not easily identifiable
- 13% of AML t(8;21) *RUNX1*::*RUNX1T1* have an associated SM
- In most instances, prognosis depends on the AMN. The most aggressive combination is MCL-AML

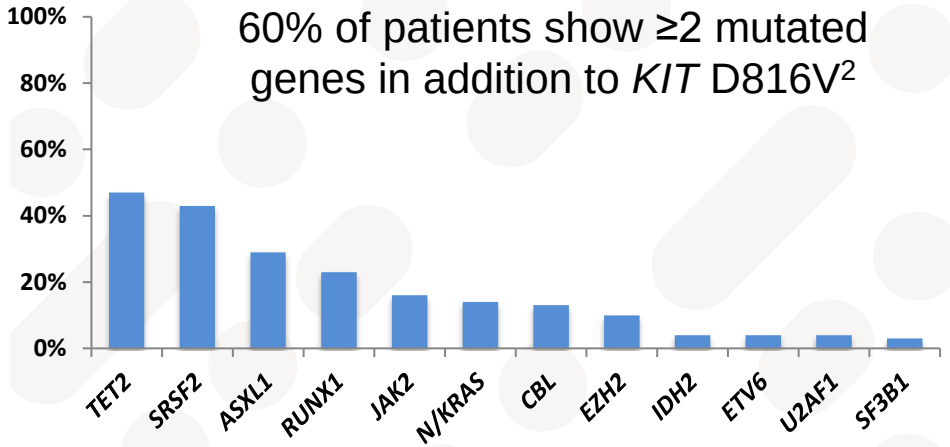
Arber DA et al, Blood (2022) 140 (11):1200-1228

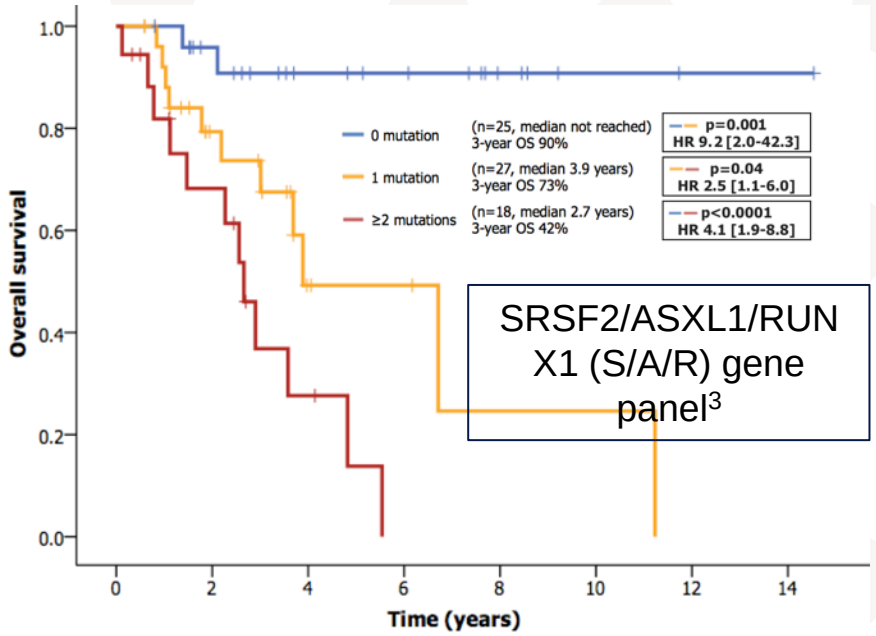
KIT D816V in SM

Expressed allele burden of *KIT* D816V[1]



60% of patients show ≥2 mutated genes in addition to *KIT* D816V[2]

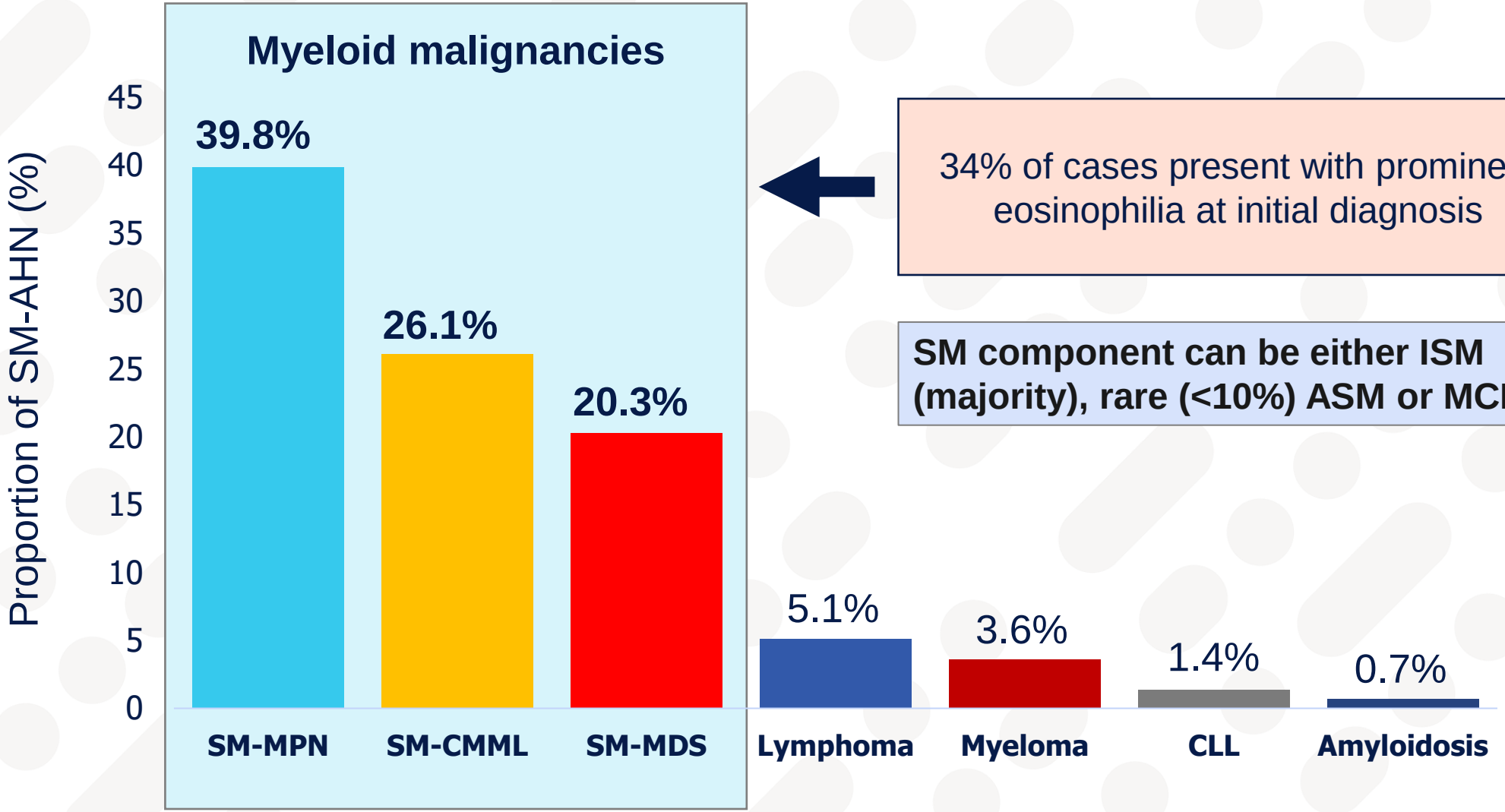




SRSF2/ASXL1/RUNX1 (S/A/R) gene panel[3]

[1]Erben P et al., *Ann Hematol*, 2014, 93(1):81-88;
[2]Schwaab J et al., *Blood*, 2013, 122(14):2460-2466;
[3]Jawhar M et al., *Leukemia*, 2016, 30:136-143.

SM with an associated hematologic neoplasm

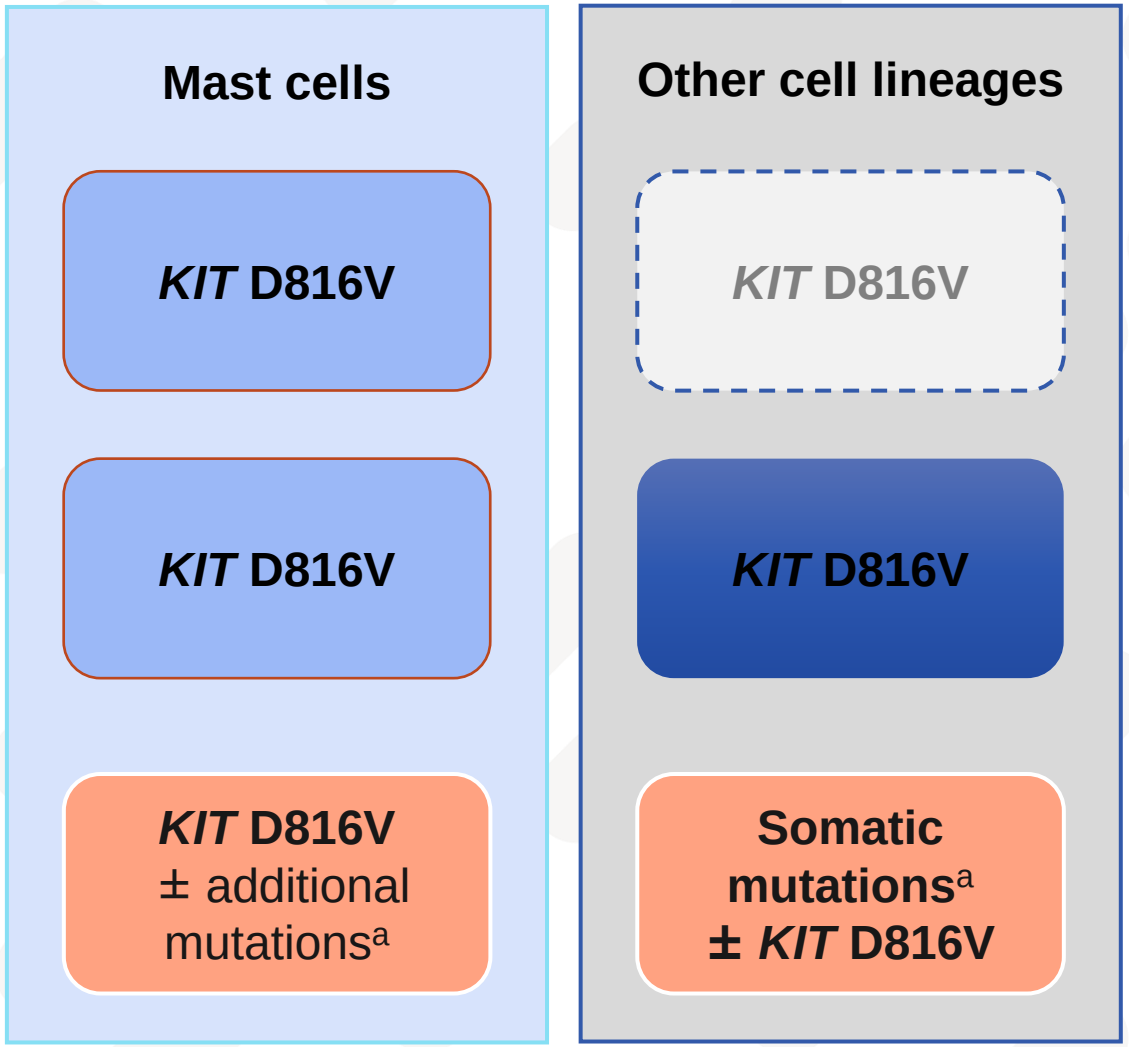


34% of cases present with prominent eosinophilia at initial diagnosis

SM component can be either ISM (majority), rare (<10%) ASM or MCL

Leguit R et al. Pathobiology 2020;87:2-19; Pardanani A, Am J Hematol, 2021, 96(4):508-525; Tzankov A et al. Am J Clin Pathol. 2021;155:239-266

Complex genetics of systemic mastocytosis



Involvement of mast cell lineage
- ISM
- SSM
- MCL

Multilineage involvement
- ISM
- SSM
- SM-AMN (CMML, MDS/MPN, CEL)

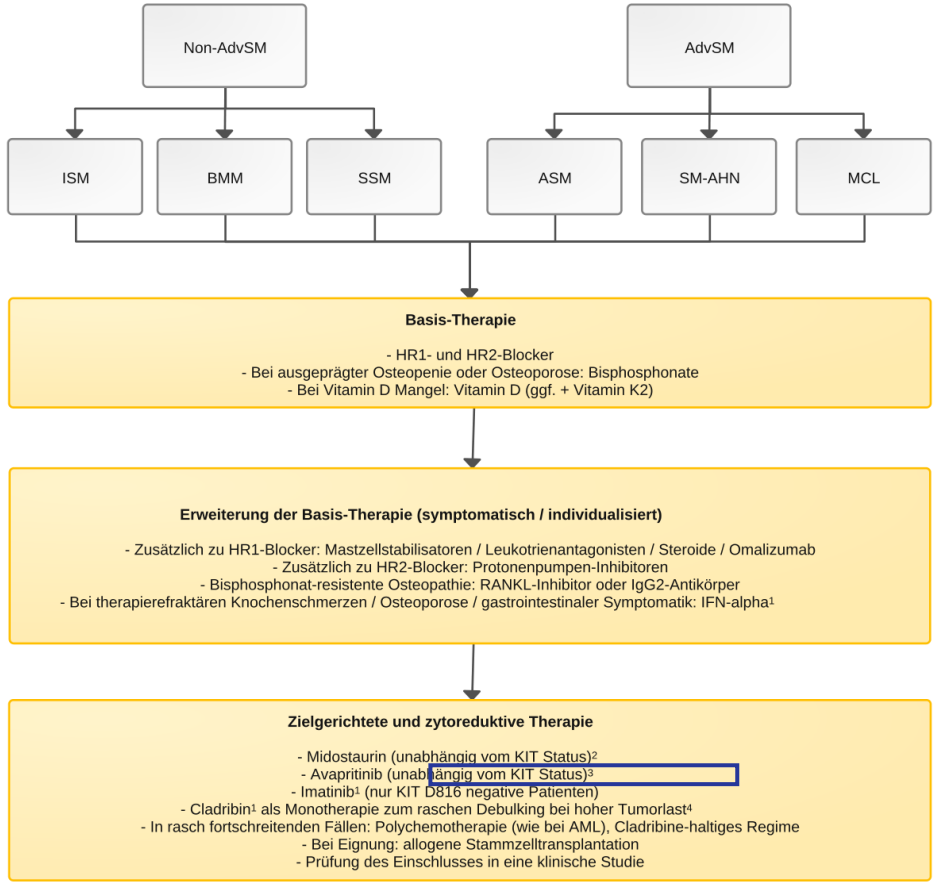
Multilineage involvement + multimutated
- SM-AMN
- ASM ± AMN (…, secondary AML)
- MCL ± AMN

[a]SRSF2, ASXL1, RUNX1, CBL, JAK2, EZH2

Reiter A et al., *Blood*, 2020, 135(16):1365-1376.

German clinical guidelines on SM

Therapy algorithm of non-advanced (non-AdvSM) and advanced SM (AdvSM)[1]



Legende:
ISM, indolente SM; SSM, smoldering SM; ASM, aggressive SM; SM-AHN,
SM mit assoziierter hämatologischer Neoplasie; MCL, Mastzell-Leukämie.

[1] nur off-label;

[2] insbesondere bei hoher KIT D816V Mutationslast, z.B. ≥5-10% im peripheren Blut und Nachweis einer AdvSM sollte Midostaurin im Therapiemanagement (ggf. additiv) berücksichtigt werden.

[3] zugelassen nach einer systemischen Vortherapie bei der AdvSM in einer Startdosis von 200 mg tgl.; bei der ISM mit mittelschweren bis schweren Symptomen, bei denen mit einer symptomatischen Behandlung keine ausreichende Kontrolle erzielt werden kann (Startdosis von 25 mg tgl.).

[4] weitere konventionelle Therapiemöglichkeiten zur Zytoreduktion: Hydroxyurea, Interferon-alpha (off-label) u.a., siehe Kapitel 6.2.2.2.

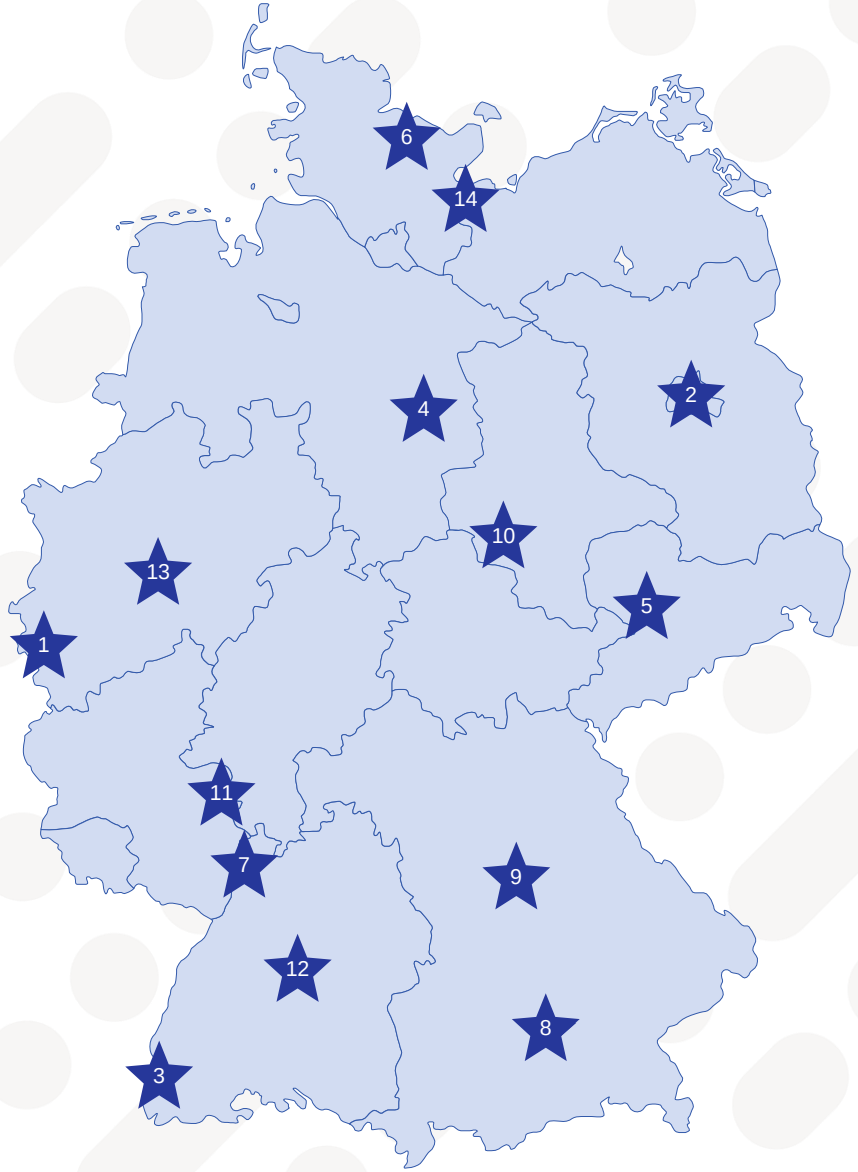
Highlight - new update:
- Avapritinib (regardless of KIT status)
[3]approved after a systemic pre-therapy with AdvSM in a starting dose of 200 mg daily; in ISM with moderate to severe symptoms in which sufficient control cannot be achieved with symptomatic treatment (starting dose of 25 mg daily).

Advanced systemic mastocytosis (AdvSM):
Avapritinib is *indicated as monotherapy for the treatment of adult patients with aggressive systemic mastocytosis (ASM), systemic mastocytosis with an associated haematological neoplasm (SM-AHN) or mast cell leukaemia (MCL), after at least one systemic therapy.*[2]

Indolent systemic mastocytosis (ISM):
Avapritinib is *indicated for the treatment of adult patients with indolent systemic mastocytosis (ISM) with moderate to severe symptoms inadequately controlled on symptomatic treatment.*[2]

1. Onkopedia Guideline Systemic Mastocytosis, as of January 2024.
2. SMPC Ayvakyt 2024.

Centers of excellence for SM in Germany

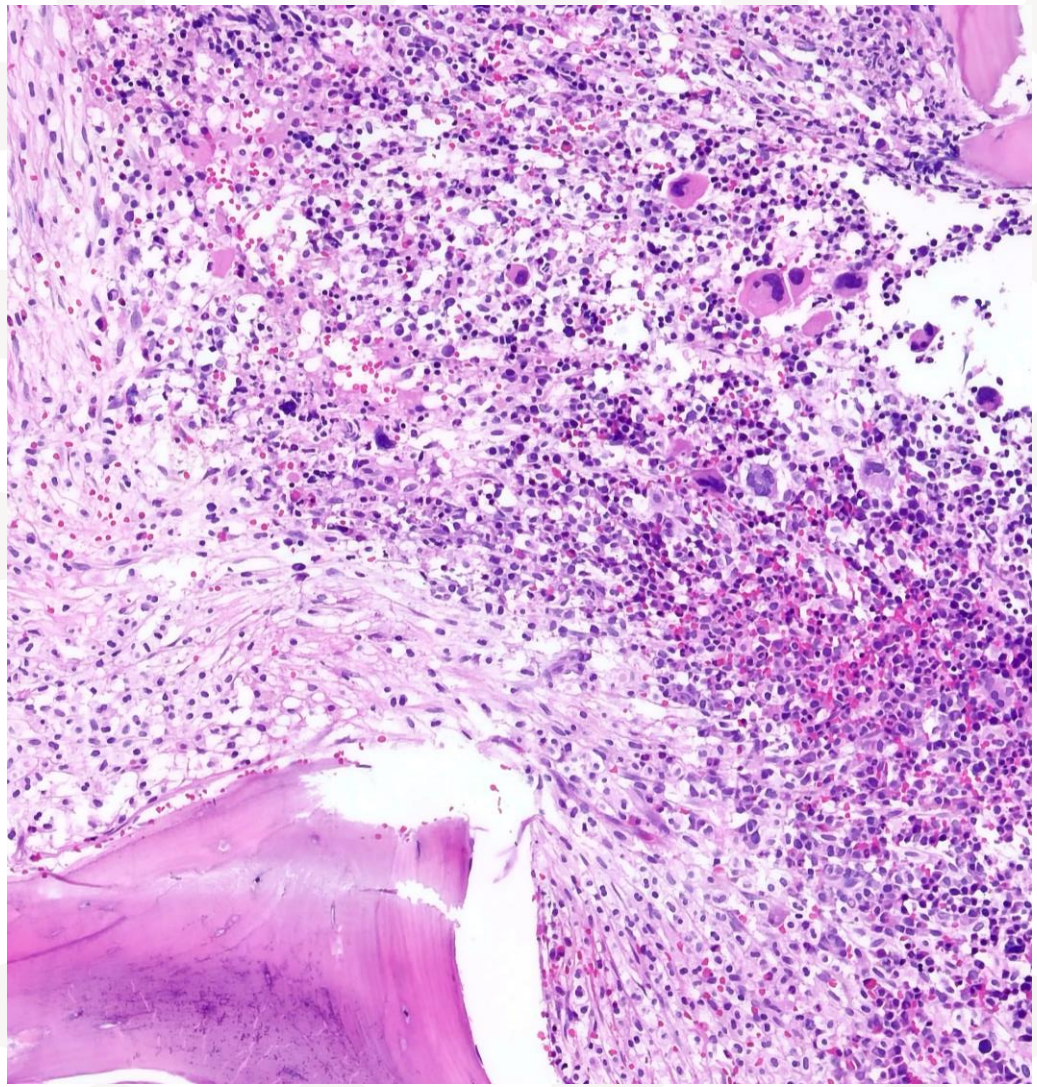


nr	Location	Name
1	Aachen	Uniklinik RWTH Aachen - Klinik für Hämatologie, Onkologie, Hämostaseologie und Stammzelltransplantation
2	Berlin	Charité Universitätsmedizin Berlin - Institut für Allergieforschung
3	Freiburg	Universitätsklinikum Freiburg - Klinik für Innere Medizin I
4	Hannover	Medizinische Hochschule Hannover
5	Leipzig	Universität Leipzig
6	Kiel	University Clinic Schleswig-Holstein, Campus Lübeck and Campus Kiel
7	Mannheim	UMM Universitätsmedizin Mannheim
8	München	Technische Universität München
9	Erlangen	Universitätsklinikum Erlangen
10	Göttingen	Universitätsklinik für Dermatologie, Venerologie und Allergologie Universitätsmedizin Göttingen
11	Mainz	Hautklinik und Poliklinik der Universitätsmedizin Mainz
12	Tübingen	Universitäts-Hautklinik Tübingen
13	Köln	Universität zu Köln
14	Lübeck	Universitätsklinikum Schleswig-Holstein, Campus Kiel

Special Article European Competence Network on Mastocytosis (ECNM): 20-Year Jubilee, Updates, and Future Perspectives

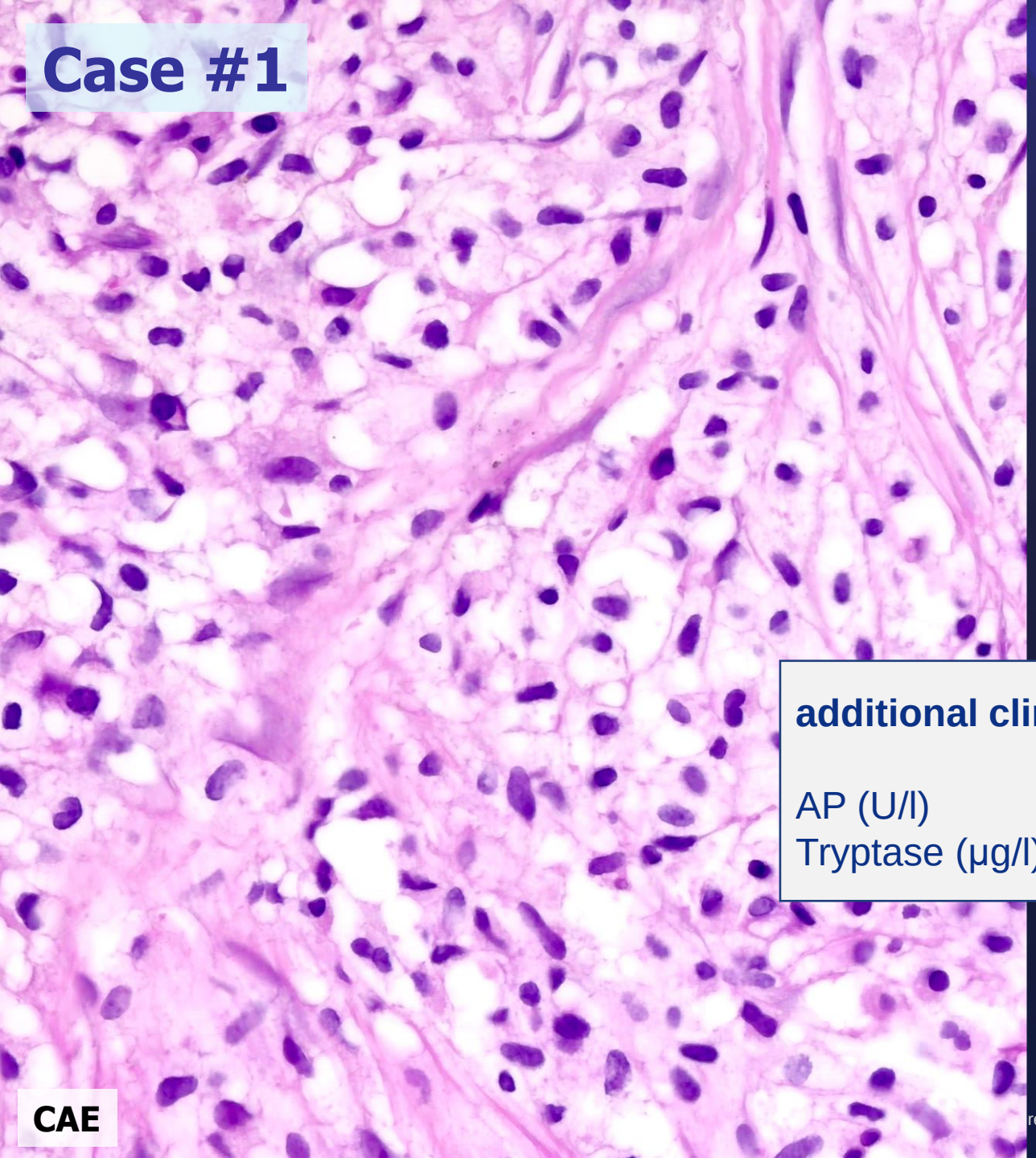
Case #1

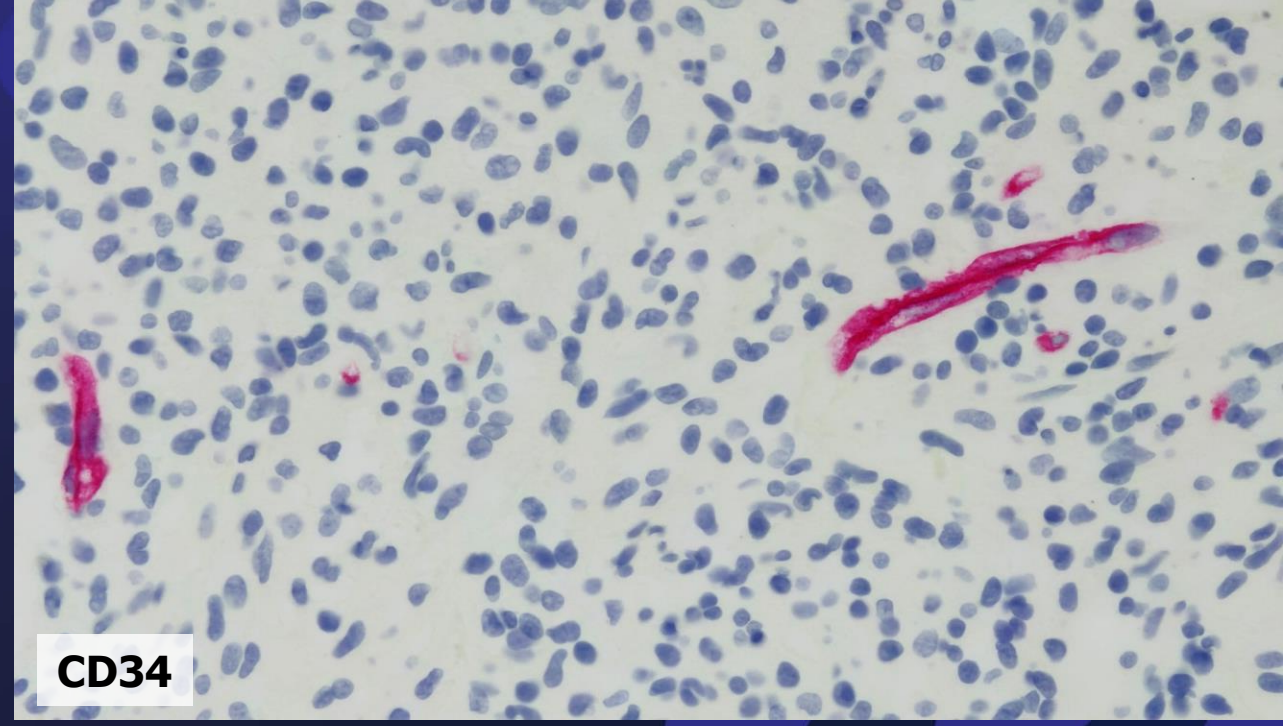
- male, 71 yrs. presenting with skin lesions, constitutional symptoms and splenomegaly (19 x 7 cm)
- WBC 2.9
  Hb 10.9
  PLT 63
  Monocytes 22%
  Eosinophiles 3 %
  leukoerythroblastosis (3% blasts)
- negative molecular testing for JAK2, CALR, MPL
- Clinical differential:
  - triple negative MPN
  - MDS/MPN overlap

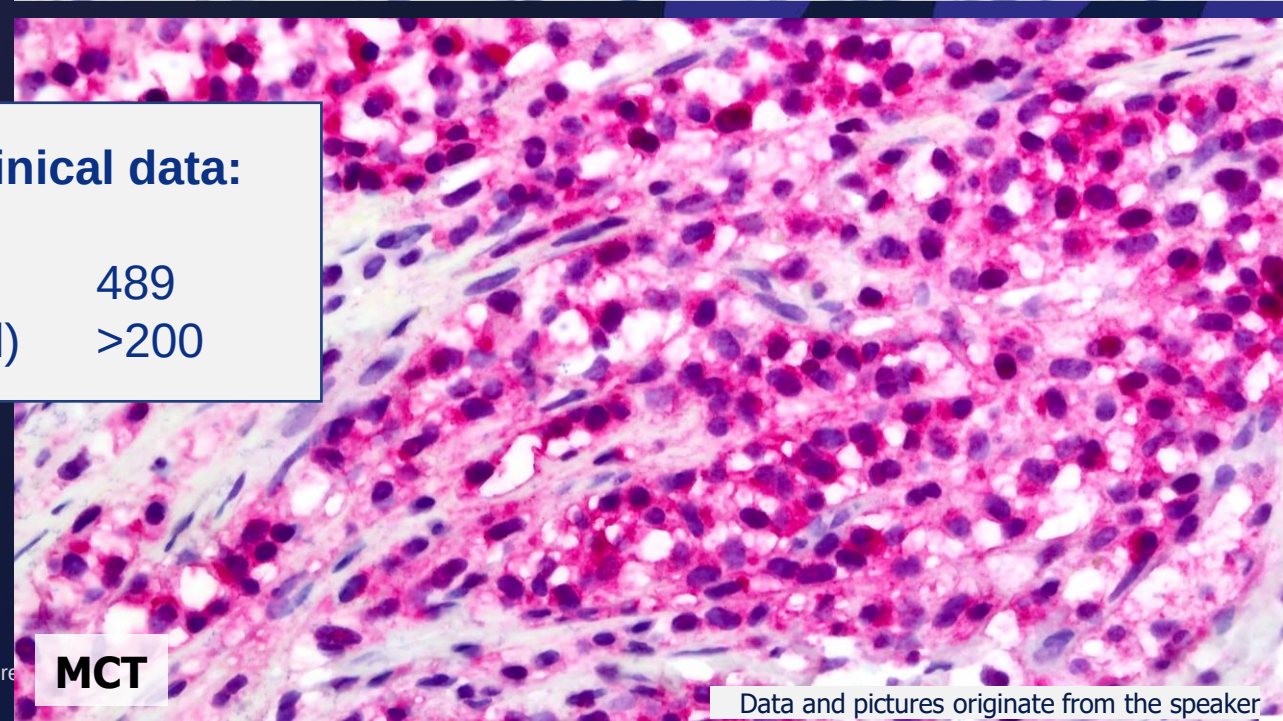


Data and pictures originate from the speaker

Case #1







additional clinical data:

AP (U/l)	489
Tryptase (µg/l)	>200

Data and pictures originate from the speaker

Case #1 – aggressive mastocytosis (ASM)

Molecular profile:

- *BCR::ABL1* negative
- **KIT D816V** **VAF 31%**
- normal karyotype, no fusions detected by FISH
- NGS
  - **SRSF2** **c.284C>A/p.Pro95His** **VAF 32%**
  - CBL c.1096-1G>T VAF 4.9%
  - CBL c.1210T>C/p.Cys404Arg VAF 5.1%
  - CSMD1 c.1040A>G/p.Asp347Gly VAF 22%
  - JAK2 p.Val617Phe VAF 0.11%
- **skin lesions with mast cell infiltrates**

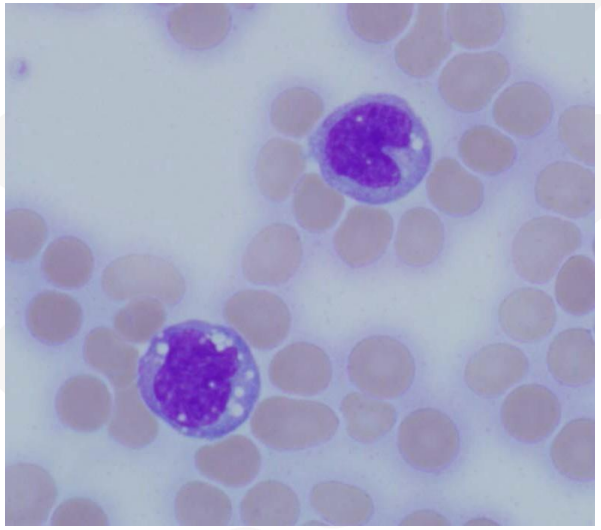
- C-findings:
  - cytopenia
  - splenomegaly
- monocytosis
- AP ↑↑
- Tryptase ↑↑

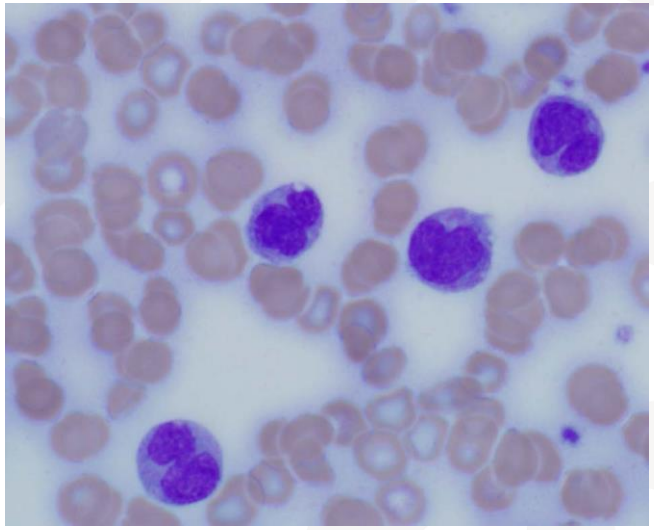
Data and pictures originate from the speaker

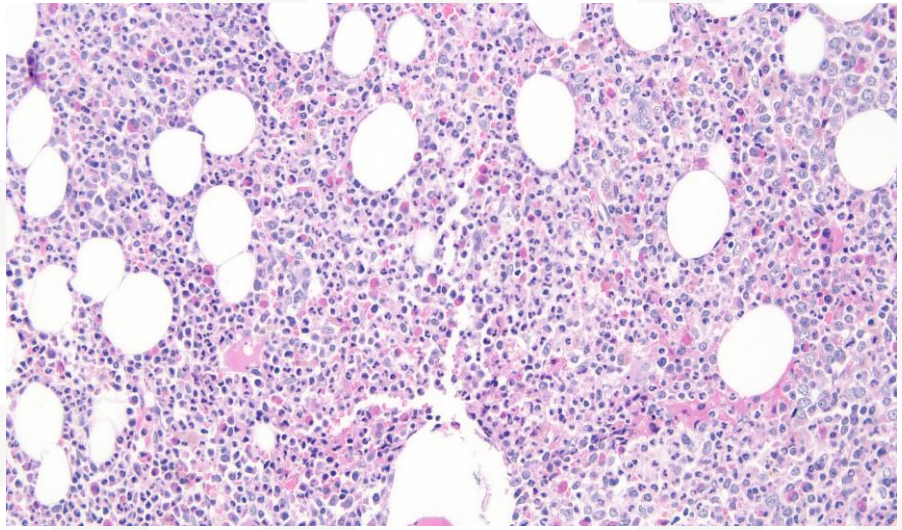
Case #2

- 55 yrs, m, presenting with anemia
- no splenomegaly
- Hb 9.3
  PLT 194
  WBC 16.9

  no blasts
  29% monocytes
  8% eosinophils
- BM biopsy was performed to exclude MDS/MPN, i.e. CMML







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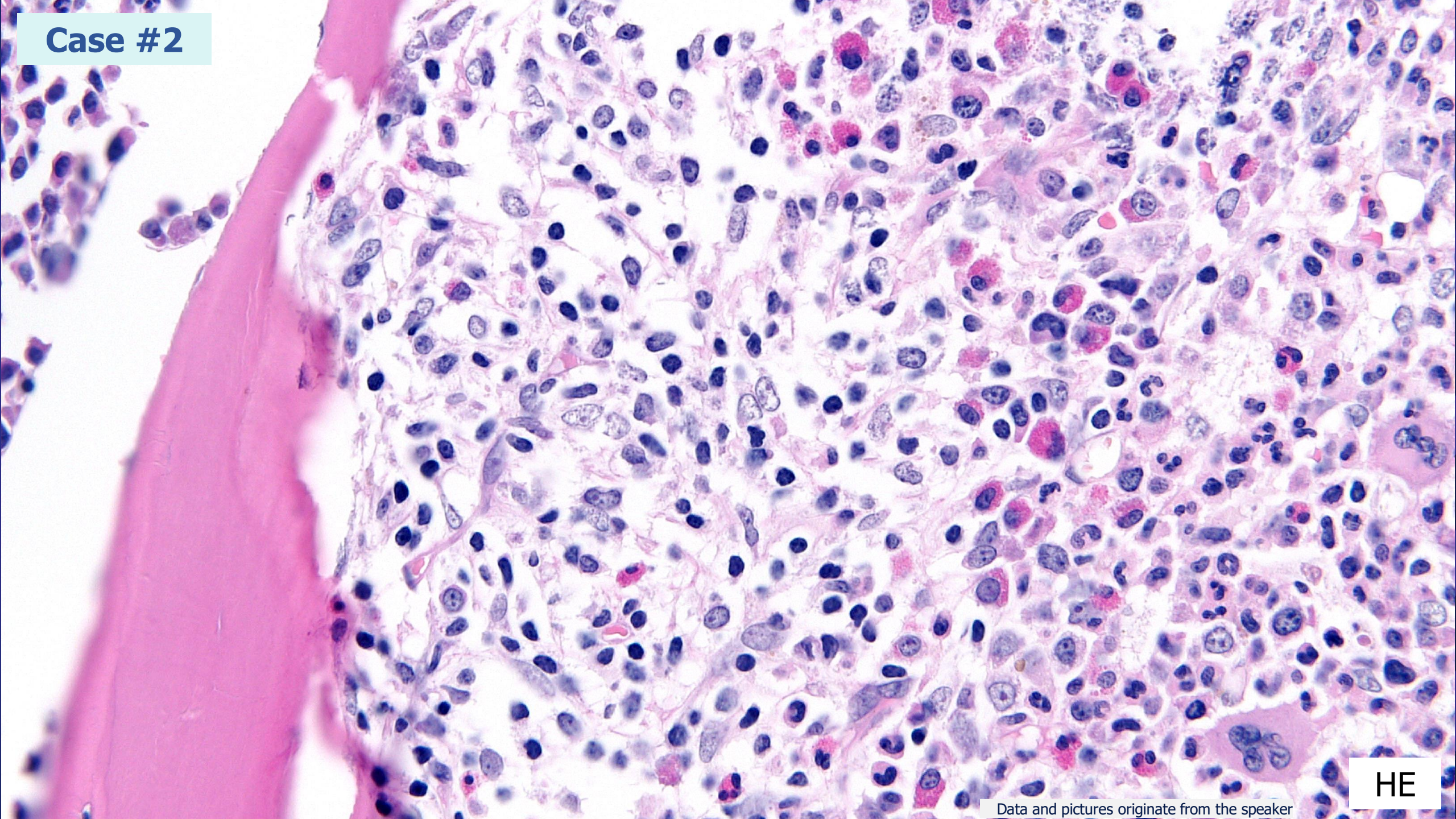
Case #2



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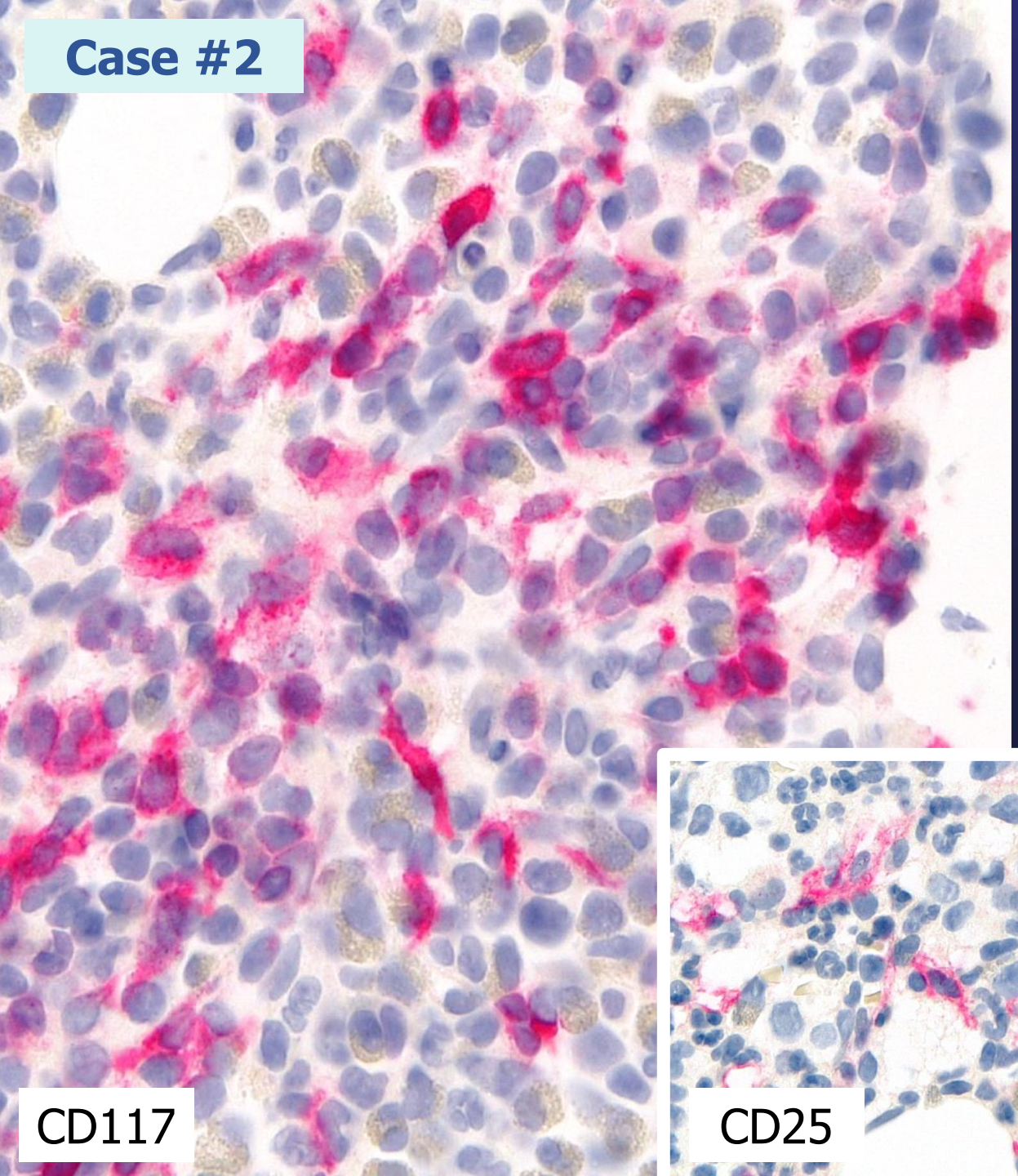
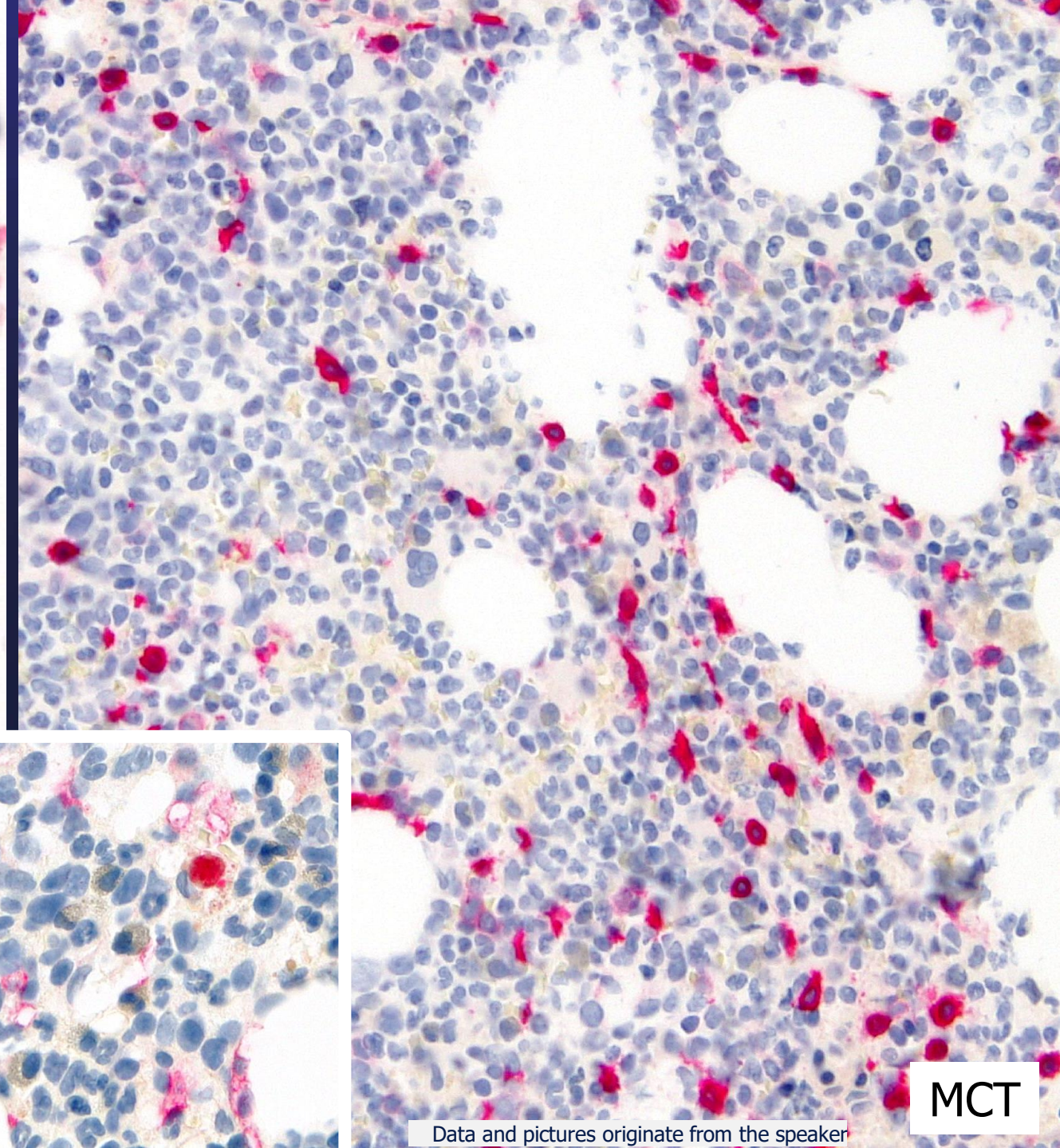
Data and pictures originate from the speaker

Case #2

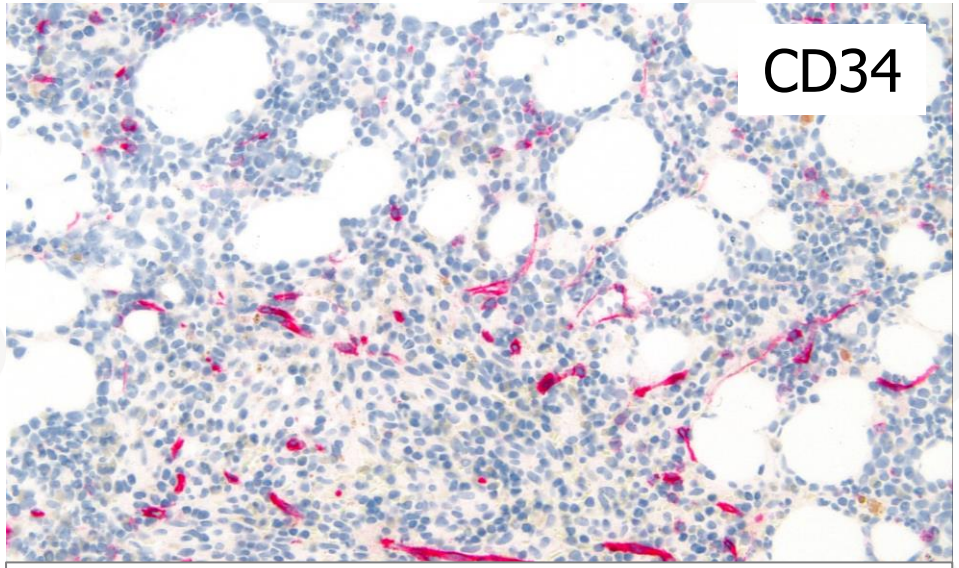




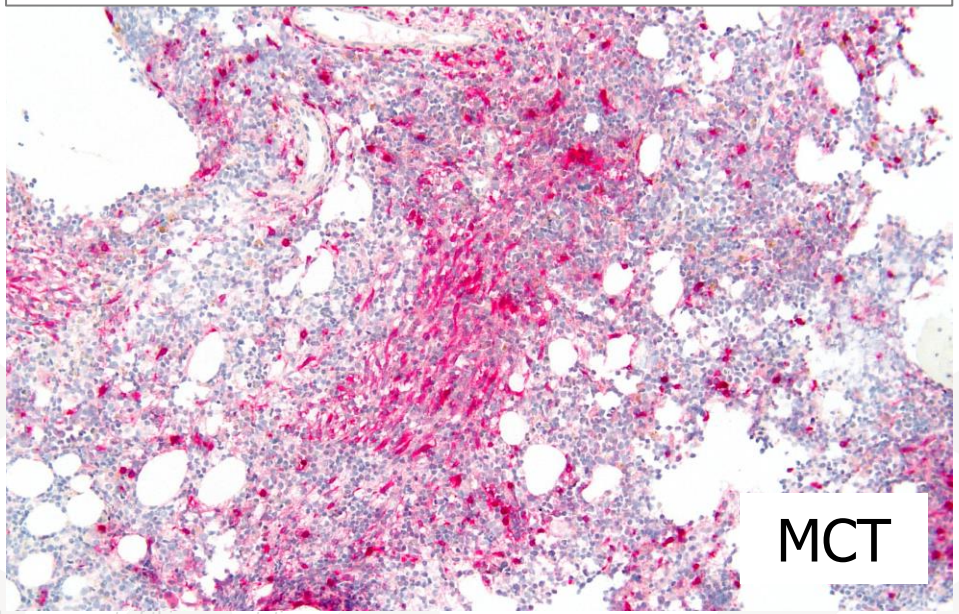
Data and pictures originate from the speaker

Case #2

- SM-AHN -> SM (**ASM**) + AHN (**CMML**)
- Molecular workup by NGS
  - ASXL1 VAF 31%
  - KIT ^{D816V} VAF 25%
- Serum tryptase elevated (> 50)
- Major + Minor criteria + B-findings (anemia) + C-findings



Case #2 – follow-up (6 months)



Data and pictures originate from the speaker

Detection and diagnosis of SM

Skin, bone, GI tract,
anaphylaxis,
AMN in SM,
SM in myeloid neoplasm

Monocytosis,
eosinophilia,
AP, albumin, LDH,
splenomegaly

Tryptase (serum / IHC)
Quantitative *KIT* D816V in PB

Peripheral blood	**KIT D816V low**	**KIT D816V high**
Tryptase low	ISM	SM-AMN
Tryptase high	SSM, ASM, MCL	(A)SM/MCL-AMN

German Registry on Disorders of Eosinophils and Mast Cells

Key messages

Systemic mastocytosis can affect multiple organ systems

The uncontrolled proliferation and activation of mast cells leads to severe and unpredictable symptoms

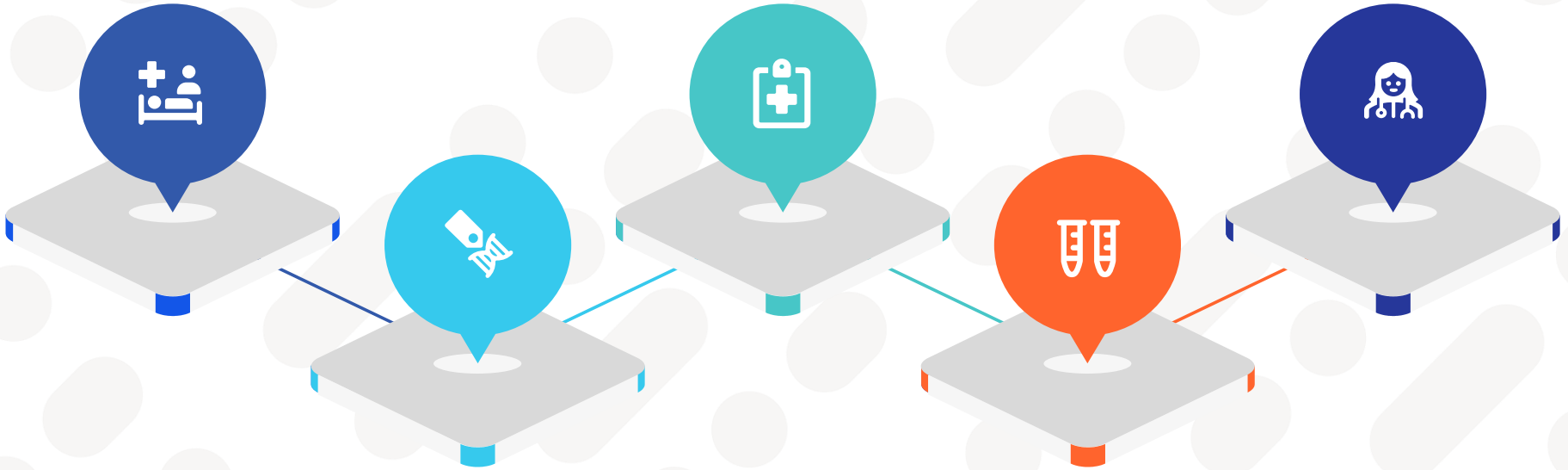
Diagnosing systemic mastocytosis can be challenging

Due to heterogeneous and non-specific symptoms, **SM can be confused with other conditions**

Avoid misdiagnosis and delayed treatment

2024 update on German clinical guidelines for SM includes recommendation for targeted therapy

Avapritinib (regardless of KIT status)



KIT D816V mutation is present in ~95% of patients with SM

Timely and correct diagnosis is important for patients to benefit from targeted therapeutic options

It is recommended to use a high-sensitivity assay to detect KIT D816V

ASO-qPCR

ddPCR