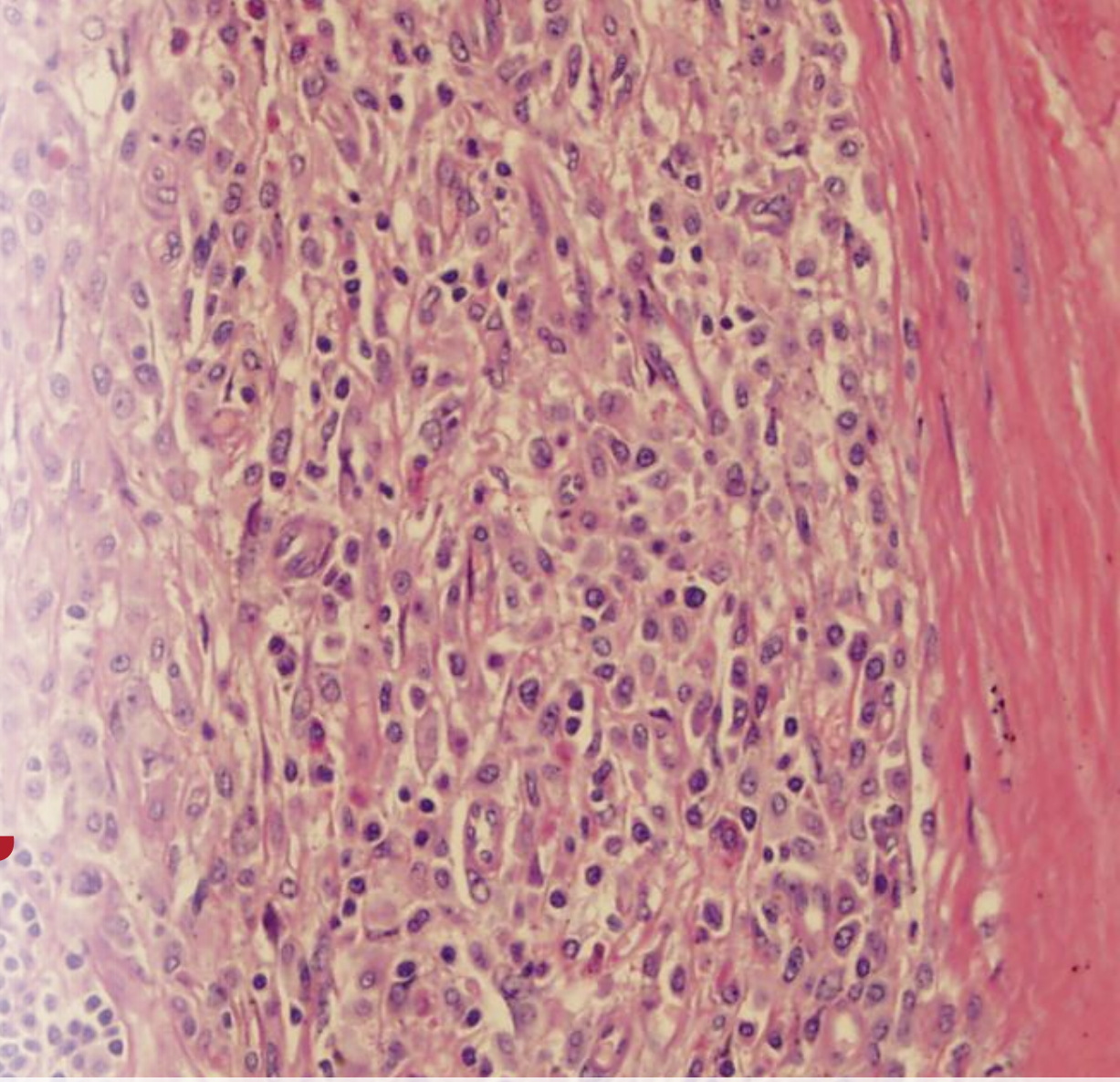
FOR ALLERGISTS/ IMMUNOLOGISTS

Diagnosing Systemic Mastocytosis



Tracy I. George, MD
Chief Scientific Officer and President-Innovation
Professor of Pathology, University of Utah

ARUP LABORATORIES

AUGUST 2025

DISCLOSURES

- Blueprint Medicines, consultant
- Cogent Biosciences, consultant

Agenda

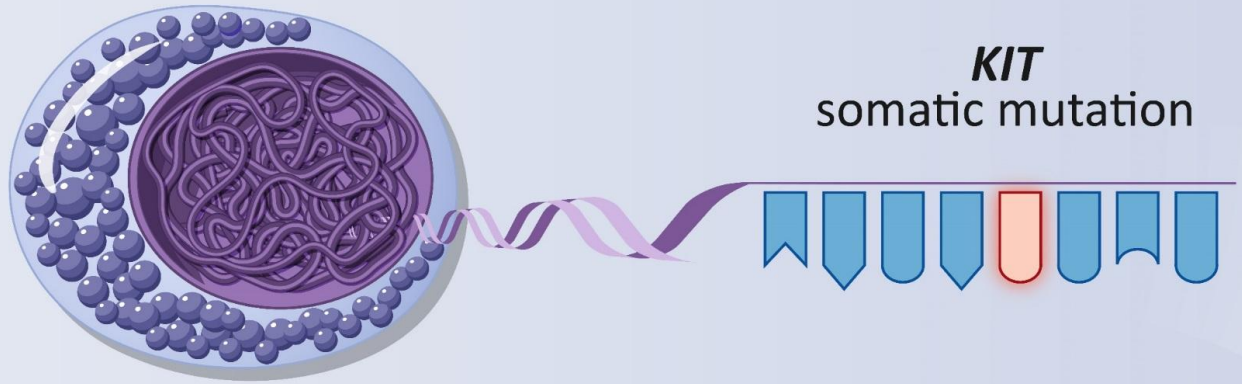
WHO & ICC Classifications

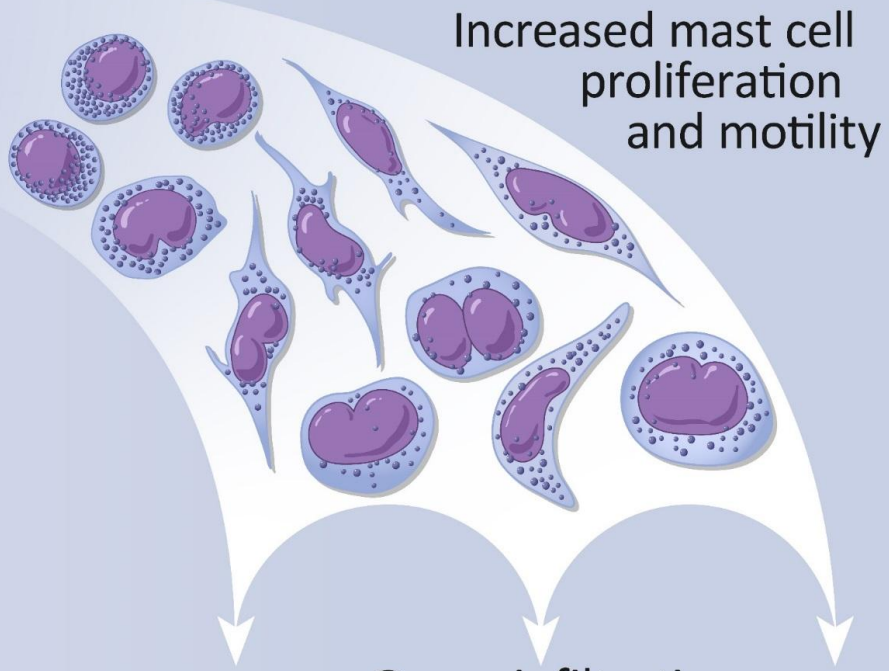
Systemic mastocytosis and subtypes

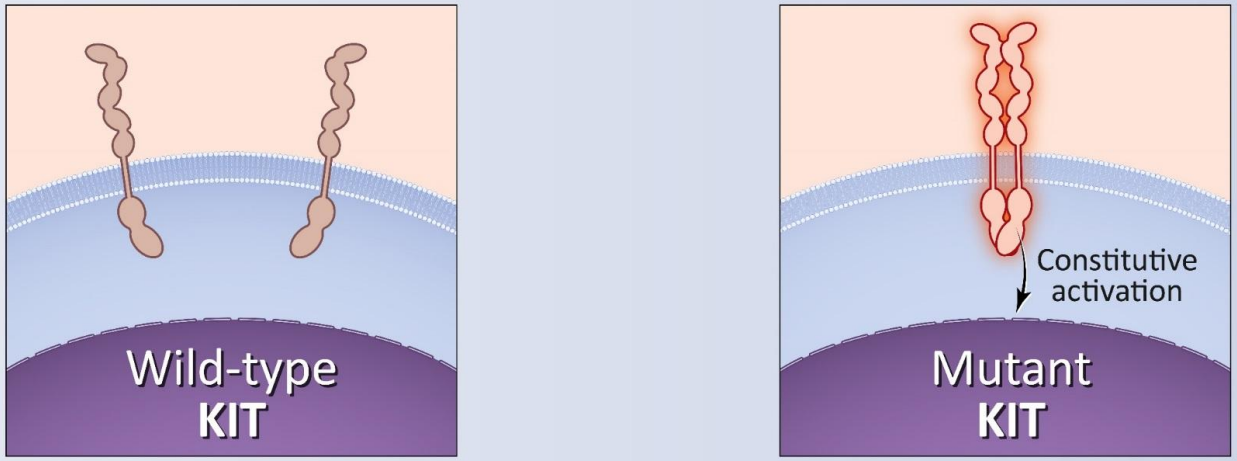
Immunohistochemistry and molecular diagnosis

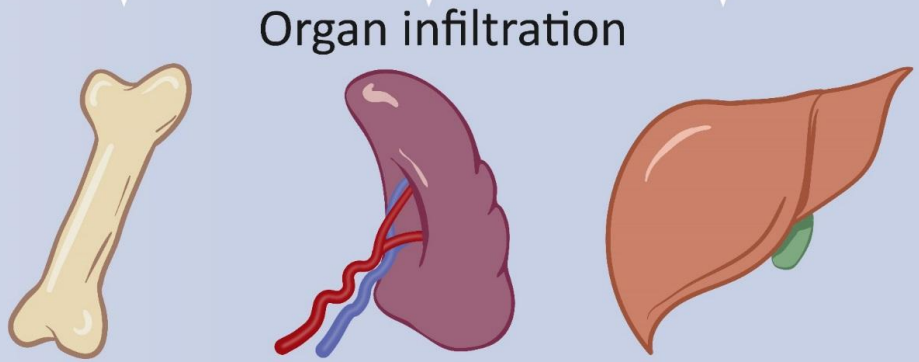
Challenges of diagnosis in non-advanced systemic mastocytosis

KIT D816V is present in >95% of patient with systemic mastocytosis



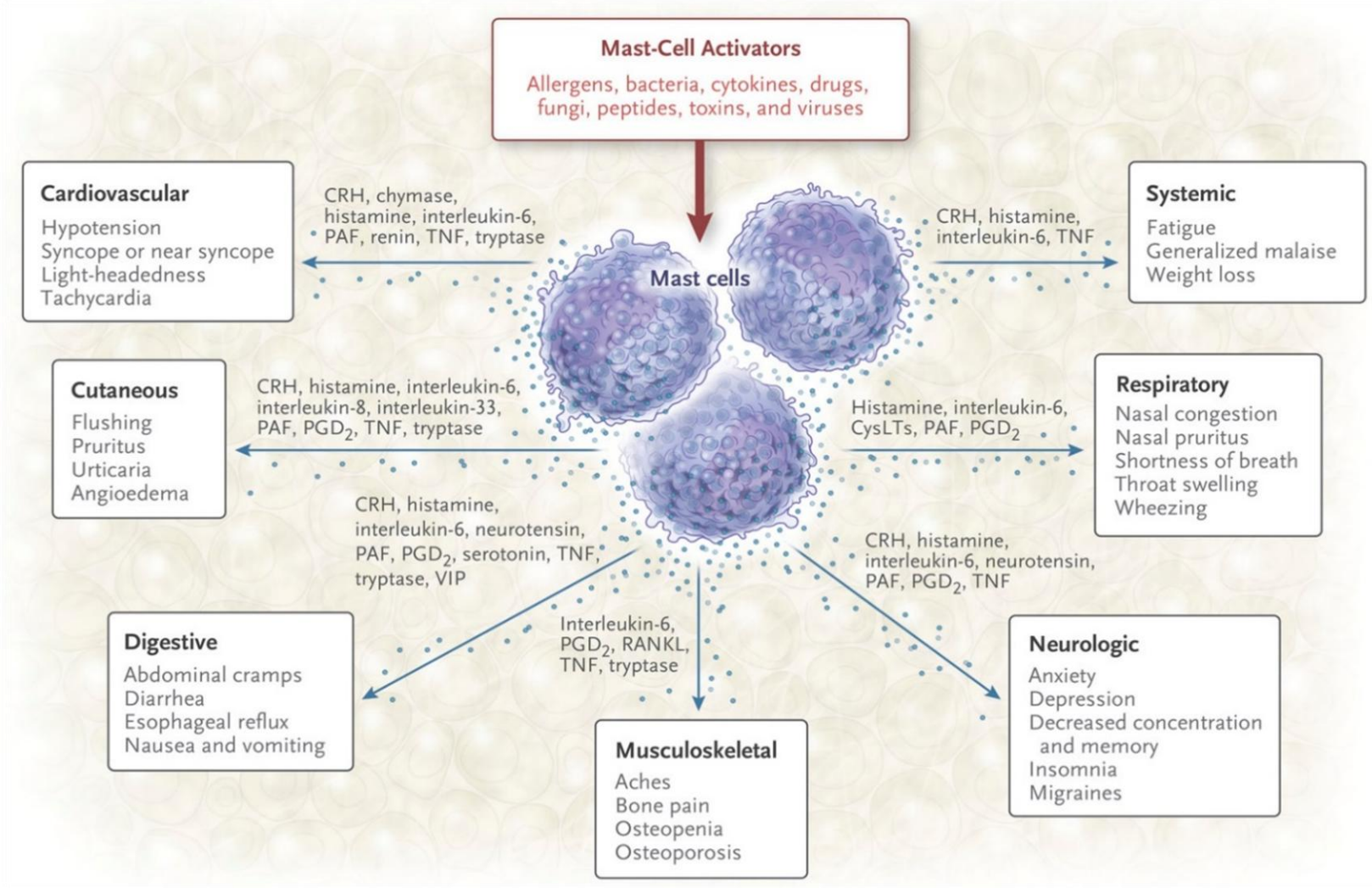






Mastocytosis pathogenesis

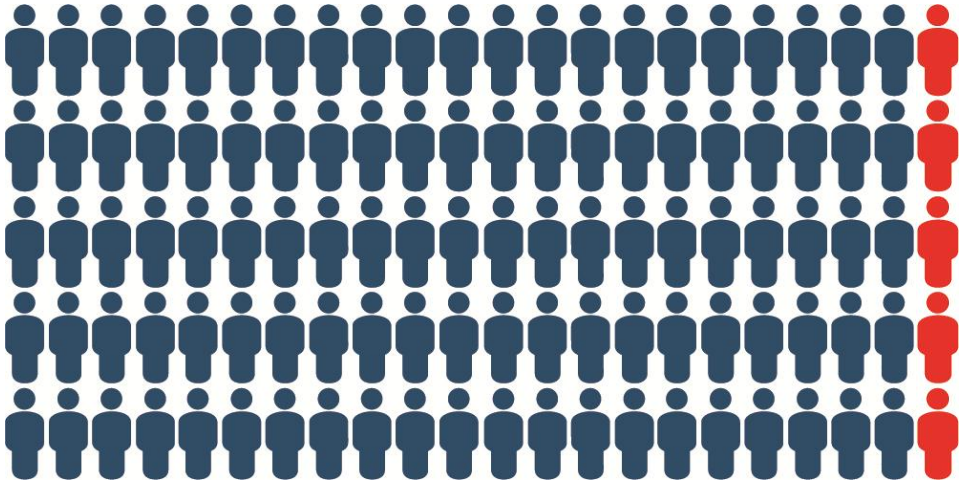
Mast cell mediators and effects



Theoharides TC et al. N Engl J Med 2015.

Systemic Mastocytosis Prevalence

SM Prevalence of ~1:10,000
~30,000 estimated in US

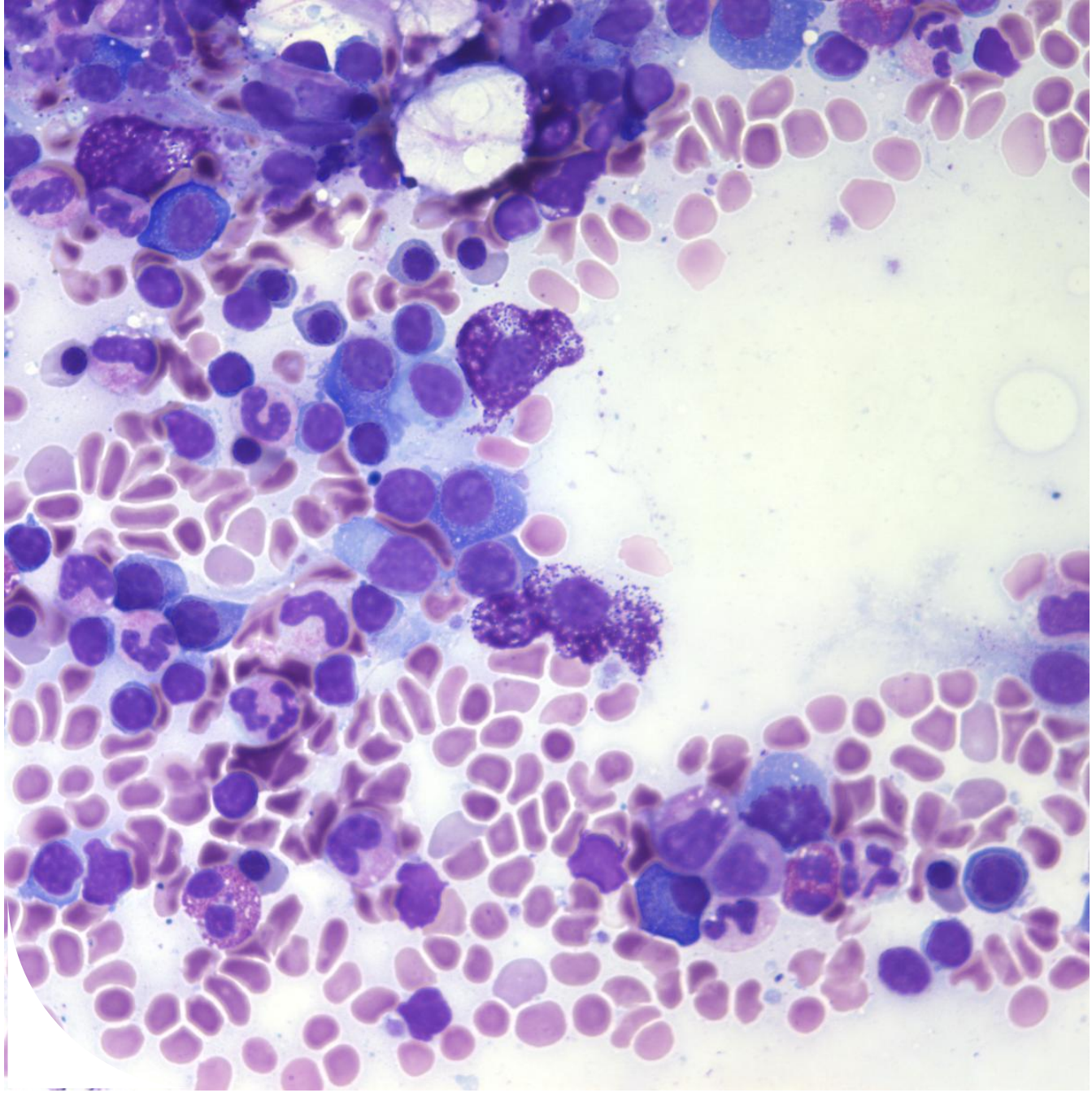


~5% Advanced SM
Organ damage and decreased survival

~95% Non-advanced SM
Indolent and Smoldering SM

K Brockow. *Immunol Allergy Clin North Am* 2014; CF Schuler et al. *J Allergy Clin Immunol* 2021; R Zanotti et al. *Cancers (Basel)* 2021.

WHO & ICC Classifications



Diagnostic criteria for systemic mastocytosis

Major	Multifocal dense aggregates of mast cells
Minor	1) >25% mast cells with atypical morphology
	2) Activating *KIT* mutation
	3) CD2, CD25 and/or CD30 expression on mast cells
	4) Serum total tryptase >20 ng/mL* (unless an associated myeloid neoplasm)

Valent P et al. Leuk Res 2001; Khoury JD et al. Leukemia 2022; Arber DA et al. Blood 2022

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	WHO 5th edition	ICC
Requirements for diagnosis	1 Major + 1 Minor *or* 3 Minor criteria	1 Major *or* 3 Minor criteria
Major criterion		Requires mast cell identification by CD117 and/or tryptase
***KIT* mutation**		If *KIT* negative, the presence of TK gene fusions must be excluded
Serum total tryptase	Adjust if HaT+	

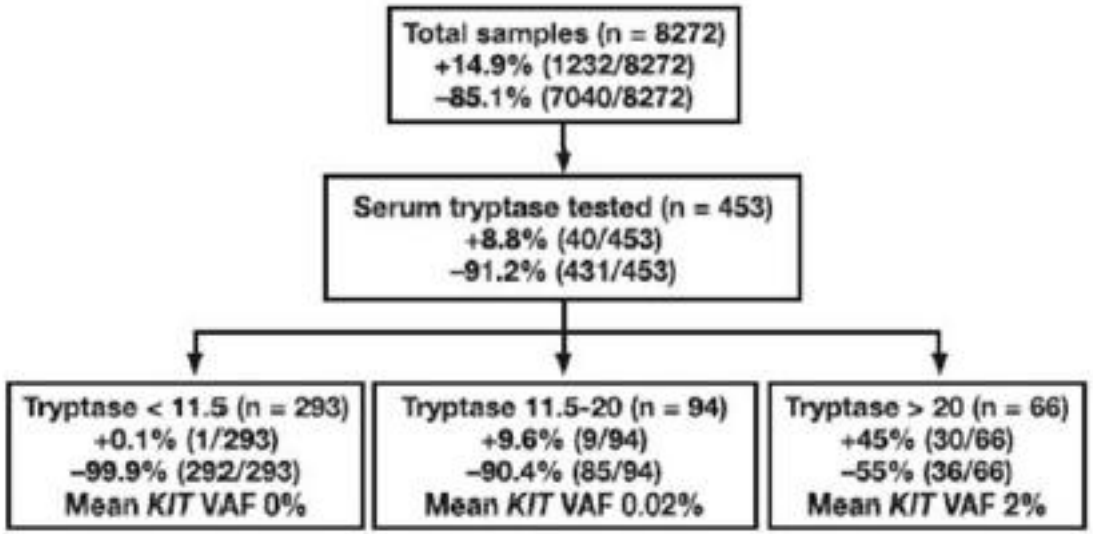
Valent P et al. Leuk Res 2001; Khoury JD et al. Leukemia 2022; Arber DA et al. Blood 2022; Valent P et al JACI Pract 2024; Lyons JJ et al. Nat Genet 2016

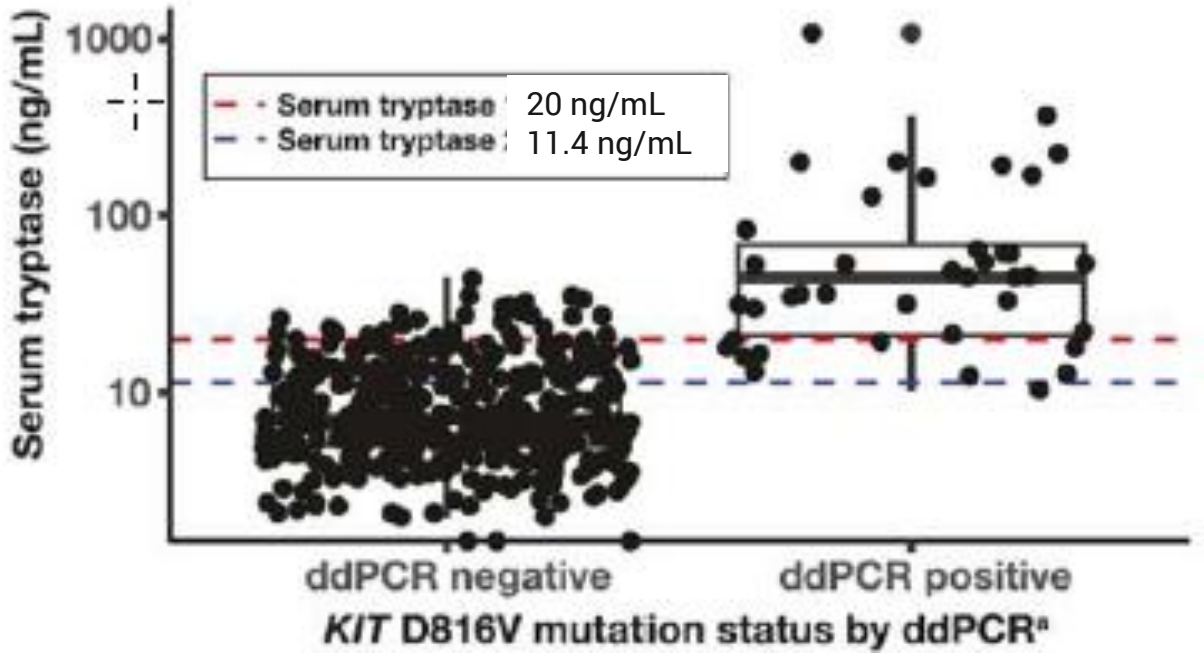
Serum tryptase levels

- Interpretation can be challenging, as hereditary alpha-tryptasemia affects 4-6% of the population and has an elevated serum tryptase levels
- Poor sensitivity: up to 30% of patients with ISM may have tryptase < 20
- Elevations in tryptase may be caused by unrelated factors, including renal dysfunction

Lyons JJ et al. Nat Genet 2016.
Lyons JJ et al. J Allergy Clin Immunol 2021.
Sanchez-Munoz L et al. Mod Pathol 2011.

Serum tryptase cut-off of 20 ng/mL shows limited sensitivity for KIT D816V detection by ddPCR

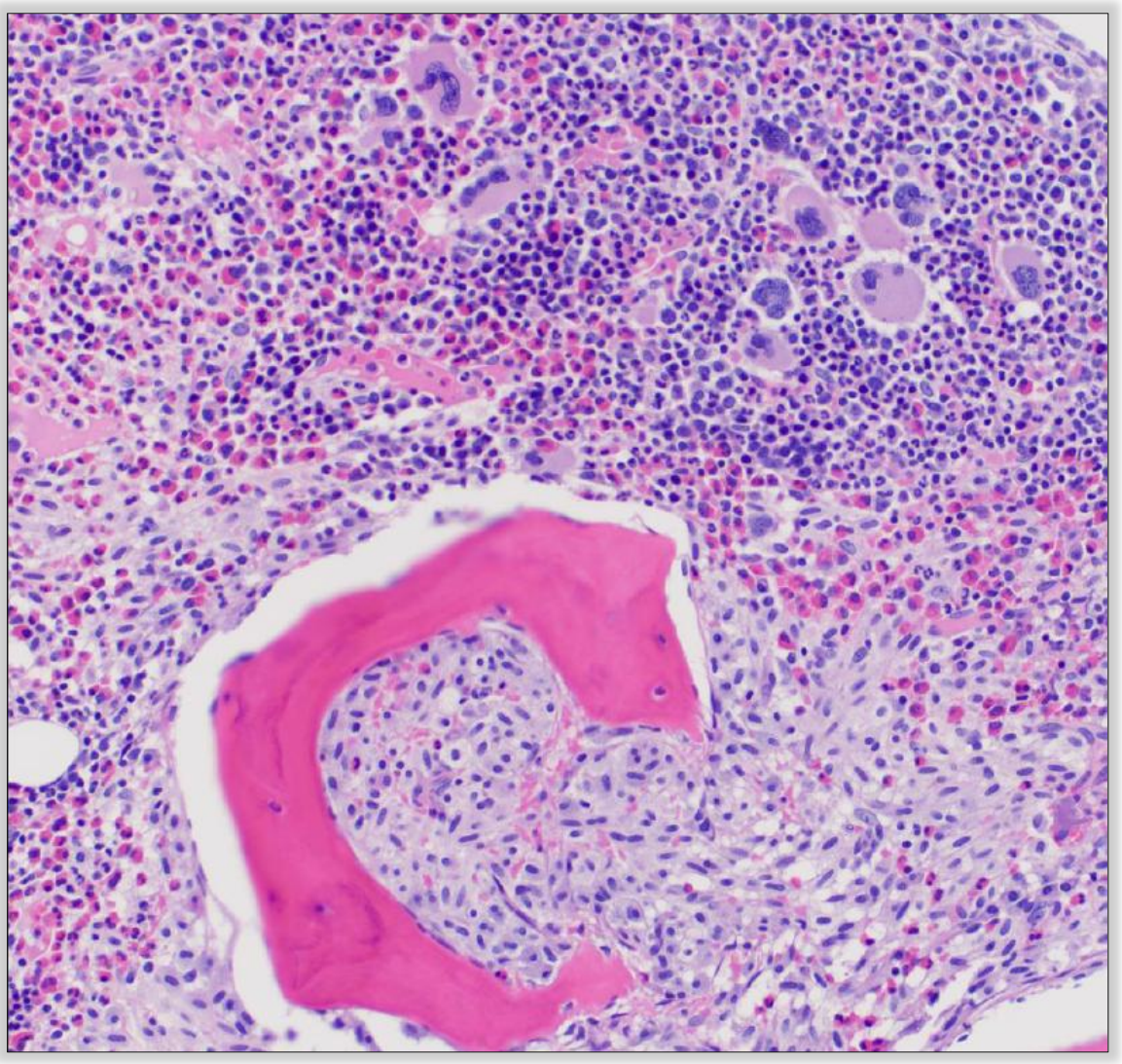


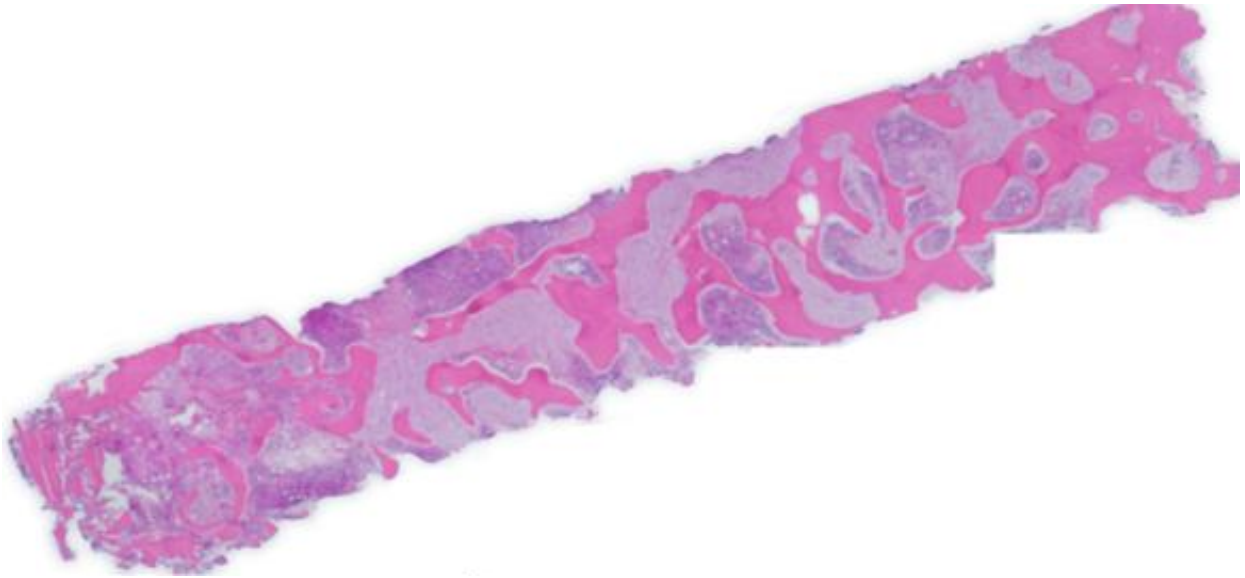


- 20 ng/mL serum tryptase threshold: sensitivity 74%, specificity 91% for detecting *KIT* D816V
- 11.5 ng/mL serum tryptase threshold: sensitivity 98%, specificity 71%

Shean RC et al. Am J Clin Pathol 2025.

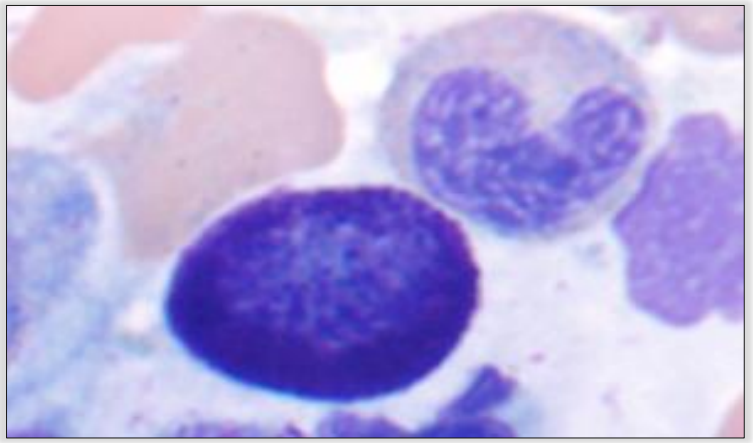
Major criterion: multifocal dense mast cell aggregates



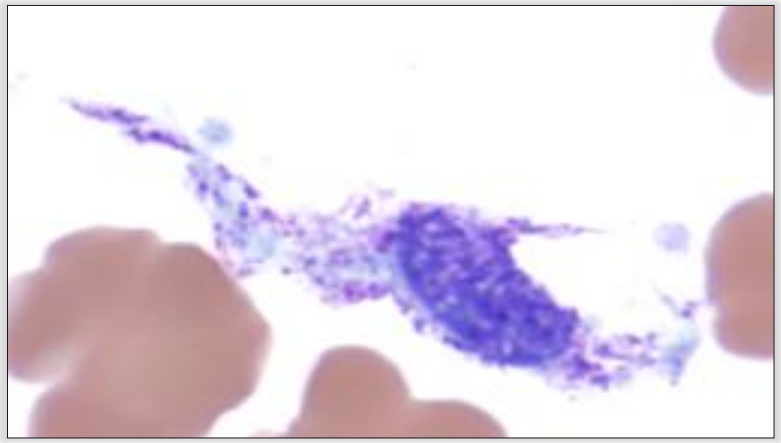


Atypical mast cell morphology

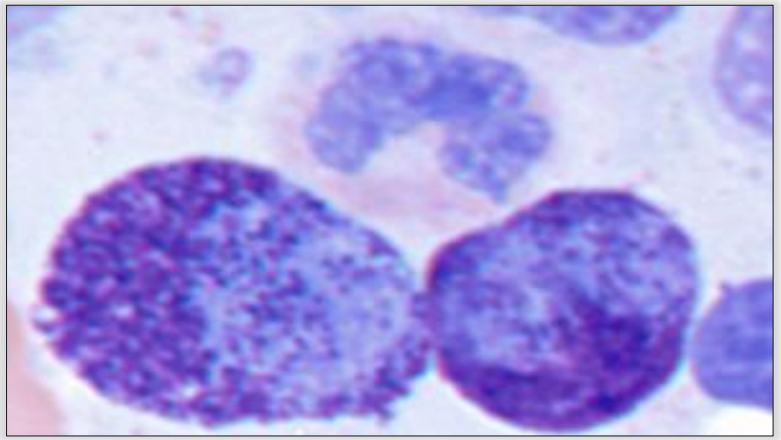
Normal/well-differentiated



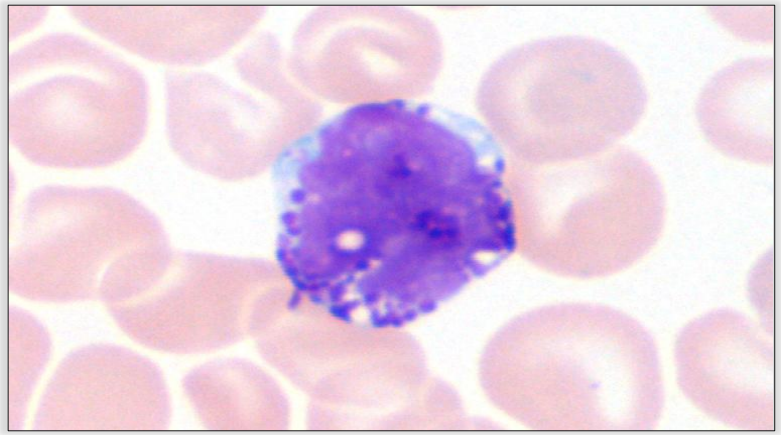
Atypical type I



Atypical type II



Metachromatic blast



George TI and Horny H-P. Hematol Oncol N Am 2011

Classification of Mastocytosis

WHO 5th EDITION	ICC
Mastocytosis	

Cutaneous mastocytosis
• Urticaria pigmentosa/maculopapular cutaneous mastocytosis
  ○ Monomorphic
  ○ Polymorphic
• Diffuse cutaneous mastocytosis
• Cutaneous mastocytoma
  ○ Isolated mastocytoma
  ○ Multilocalized mastocytoma
	Cutaneous mastocytosis
	• Urticaria pigmentosa/maculopapular cutaneous mastocytosis
	• Diffuse cutaneous mastocytosis
	• Mastocytoma of skin

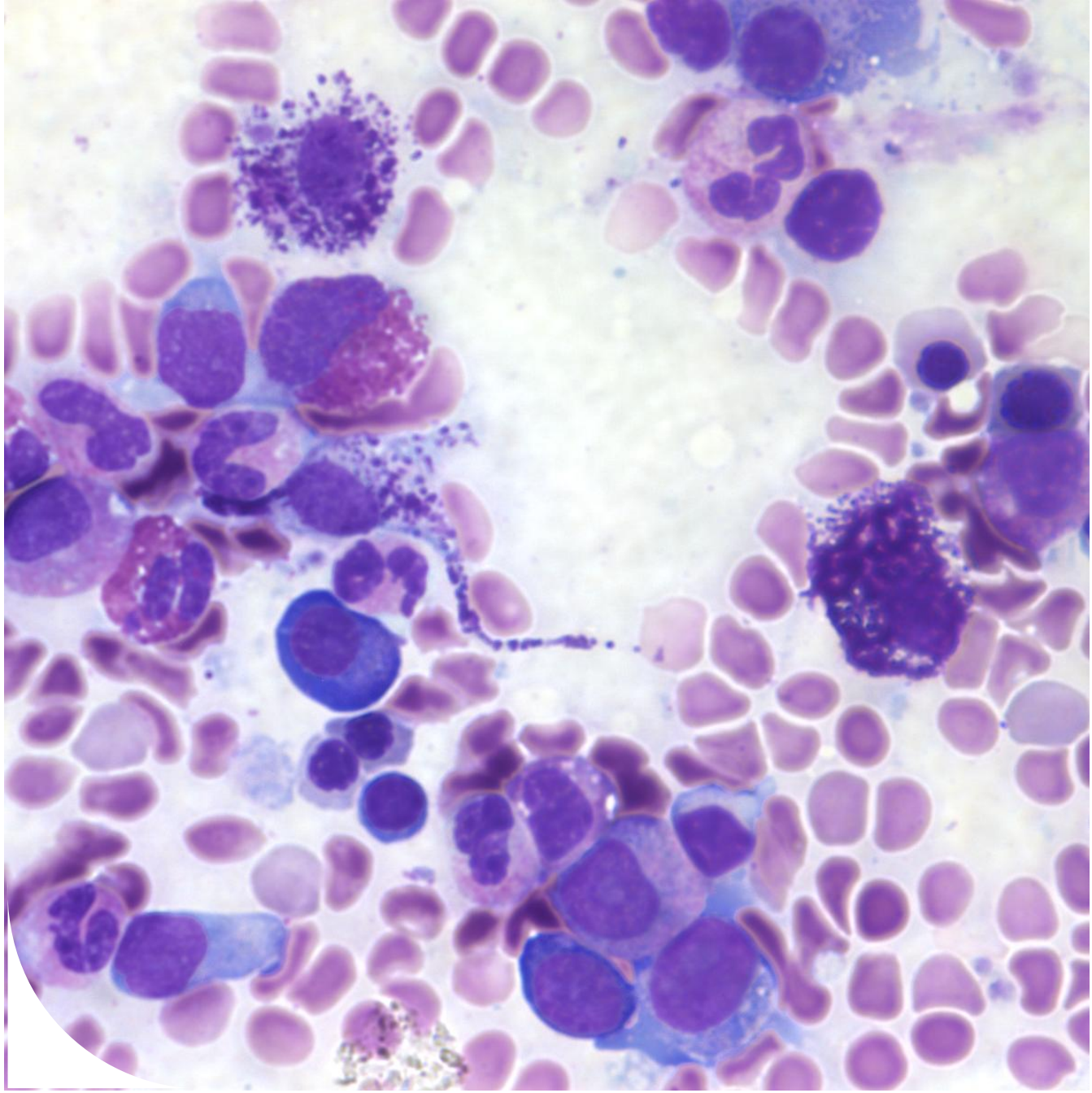
Systemic mastocytosis (SM) • Bone marrow mastocytosis • Indolent SM • Smoldering SM • Aggressive SM • Mast cell leukemia • SM with an associated hematologic neoplasm (SM-AHN)	Systemic mastocytosis (SM) • Indolent SM ○ Bone marrow mastocytosis • Smoldering SM • Aggressive SM • Mast cell leukemia • SM with an associated myeloid neoplasm (SM-AMN)
Mast cell sarcoma	Mast cell sarcoma

Khoury JD et al. Leukemia 2022; Arber DA et al. Blood 2022

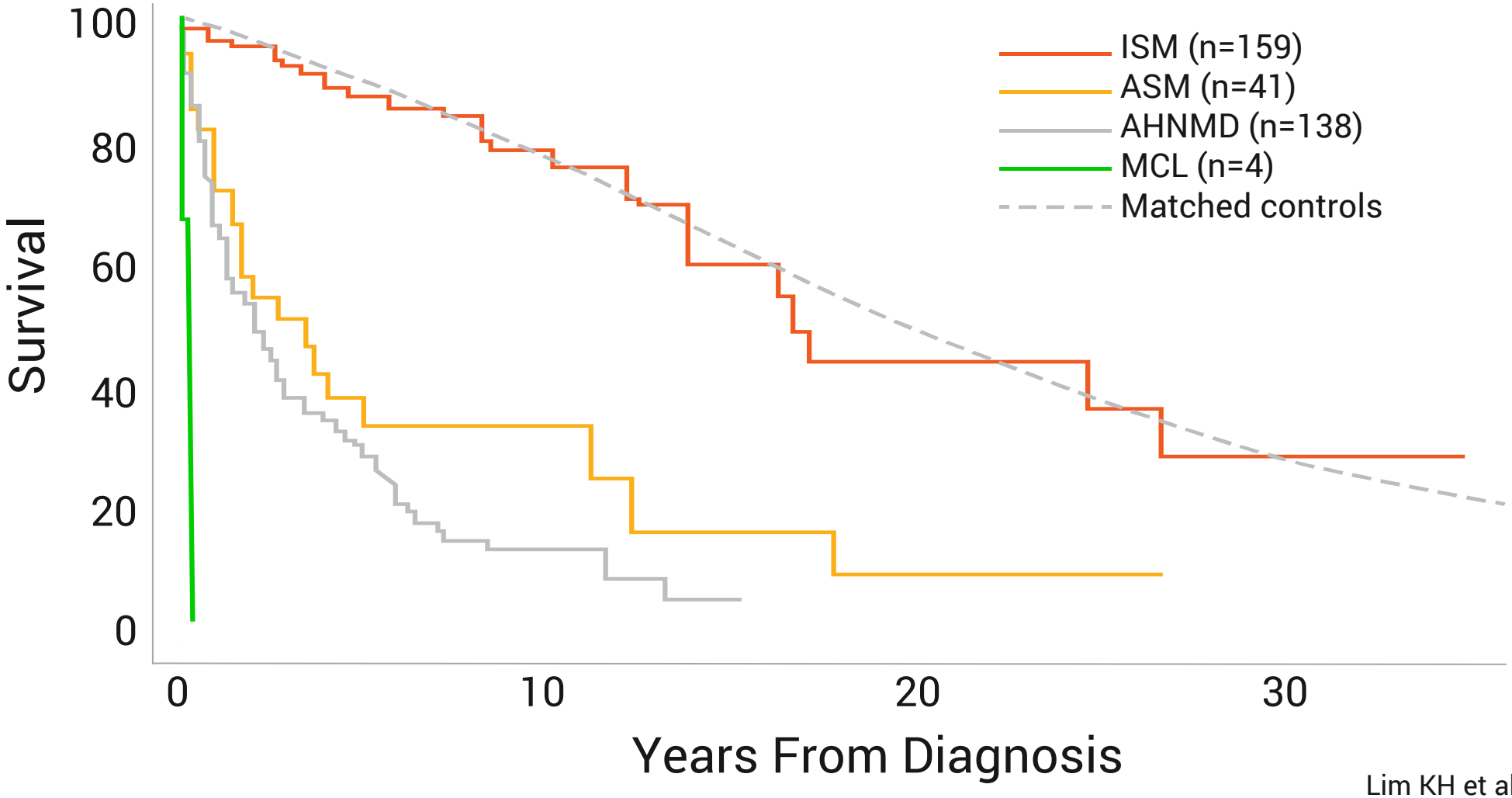
	WHO 5th edition	ICC
B	≥30% MC in BM **and/or** Serum tryptase ≥200 ng/mL **and/or** ***KIT* p.D816V VAF ≥ 10% in BM/PB**	>30% MCs in BM **and** Serum tryptase >200 ng/mL
B	Signs of myeloproliferation and/or myelodysplasia not meeting criteria for an AHN	Cytopenia(s) not meeting criteria for C-findings. Reactive causes excluded. Criteria for other myeloid neoplasms not met
B	Hepatomegaly w/o ascites or organ damage and/or Splenomegaly w/o hypersplenism and w/o weight loss and/or Lymphadenopathy **>2cm**	Hepatomegaly w/o impaired liver function, or Splenomegaly w/o hypersplenism and/or Lymphadenopathy (**>1cm**)
C	≥1 cytopenia (ANC <1.0 x 10^9/L, Hb <10g/dL and/or PLT <100 x 10^9/L)	same
C	Hepatopathy: ascites and elevated LFTs +/- hepatomegaly or cirrhosis +/- portal hypertension	Palpable hepatomegaly w/ impaired liver function, ascites and/or portal hypertension
C	Palpable splenomegaly w/ hypersplenism +/- weight loss +/- hypoalbuminemia	Palpable splenomegaly w/ hypersplenism
C	Malabsorption w/ hypoalbuminemia +/- weight loss	Malabsorption w/ weight loss due to GI MC infiltrates
C	Large osteolysis (**≥ 2cm**) +/- fracture +/- bone pain	Skeletal involvement w/ large osteolysis +/- fractures

Khoury JD et al. Leukemia 2022; Arber DA et al. Blood 2022

Systemic mastocytosis and subtypes



Overall Survival



Lim KH et al. Blood 2009

Mastocytosis classification

Cutaneous mastocytosis (CM)

Systemic mastocytosis (SM)	
Bone marrow mastocytosis (BMM) Indolent SM	*More indolent*
Smoldering SM	
SM with an associated hematologic neoplasm (SM-AHN)	*"Advanced"*
Aggressive SM	
Mast cell leukemia	
Mast cell sarcoma	

Mastocytosis classification

Non AdvSM	Diagnostic features
BMM	0 B-findings, no skin lesions, serum tryptase <125 ng/mL
ISM	<2 B-findings, typical skin lesions
SSM	≥2 B-findings, often high MC burden

Advanced Systemic Mastocytosis

Mast cell leukemia

≥ 20% mast cells on aspirate/PB*

SM + AHN/AMN

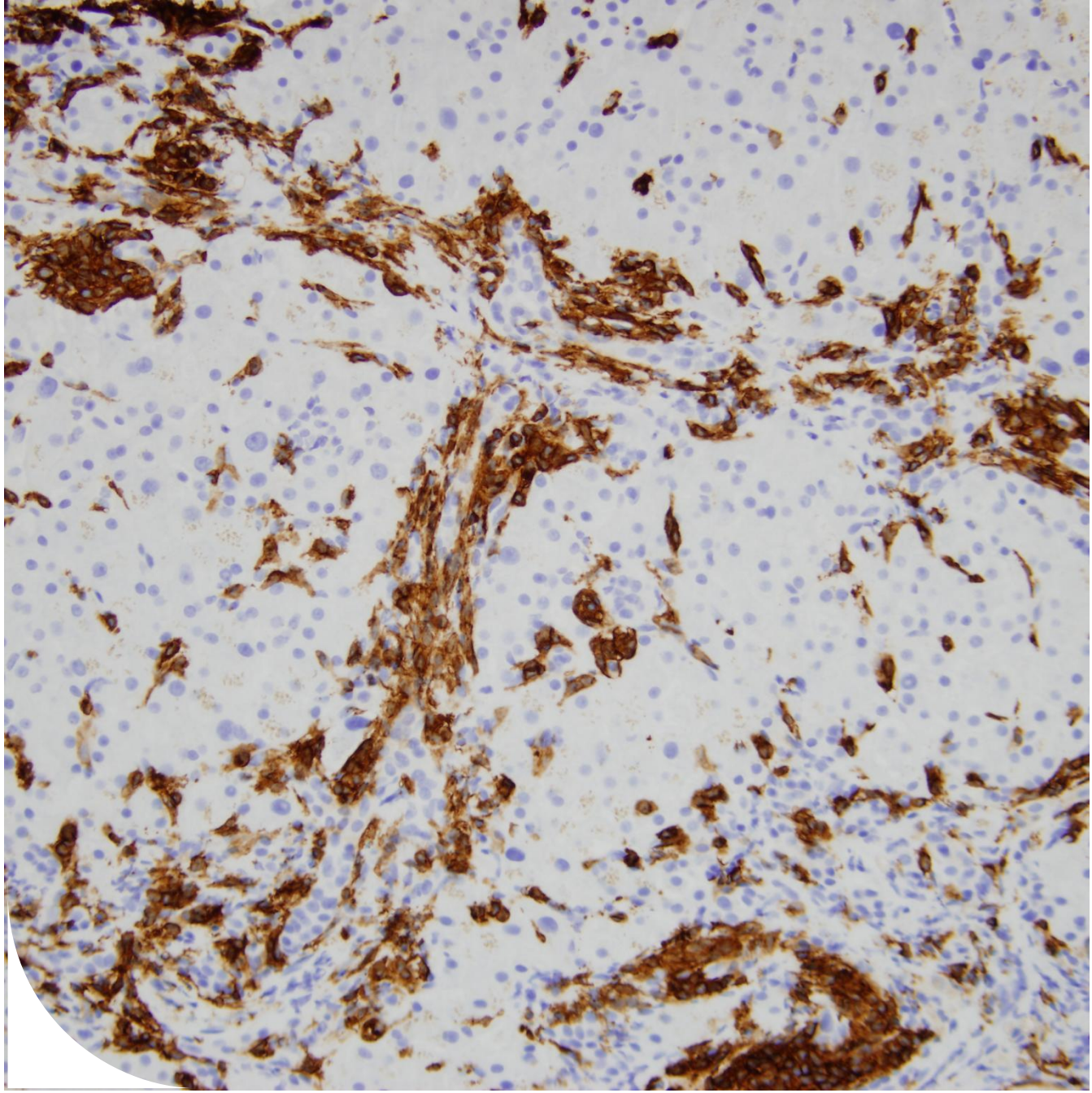
- Meets WHO criteria for an associated hematological neoplasm
- Meets SM criteria

ASM (1+ C-findings)

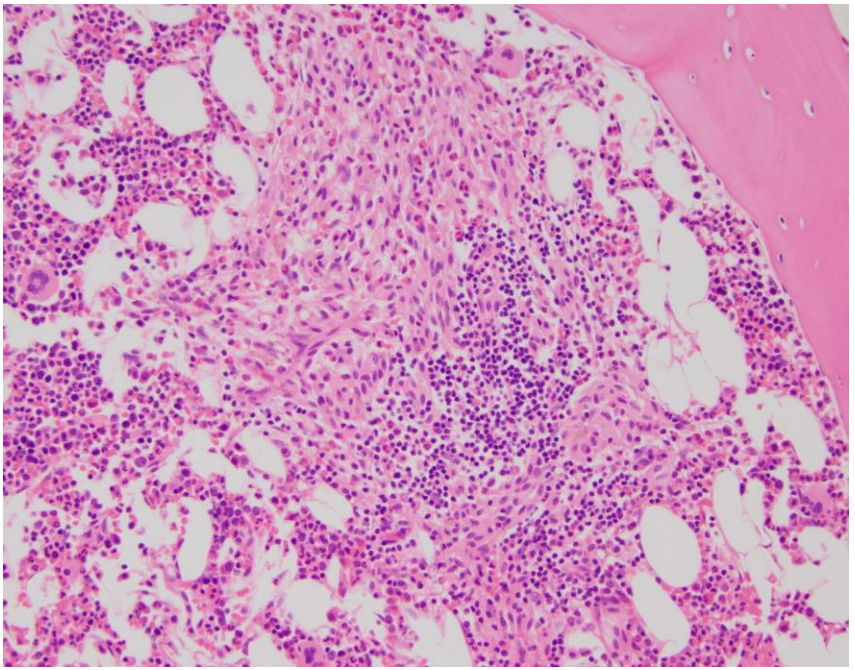
- Cytopenias
- Hepatomegaly with impaired liver function
- Skeletal involvement → osteolytic lesions and/or pathological fractures
- Splenomegaly with hypersplenism
- Malabsorption due to GI mast cell infiltrates

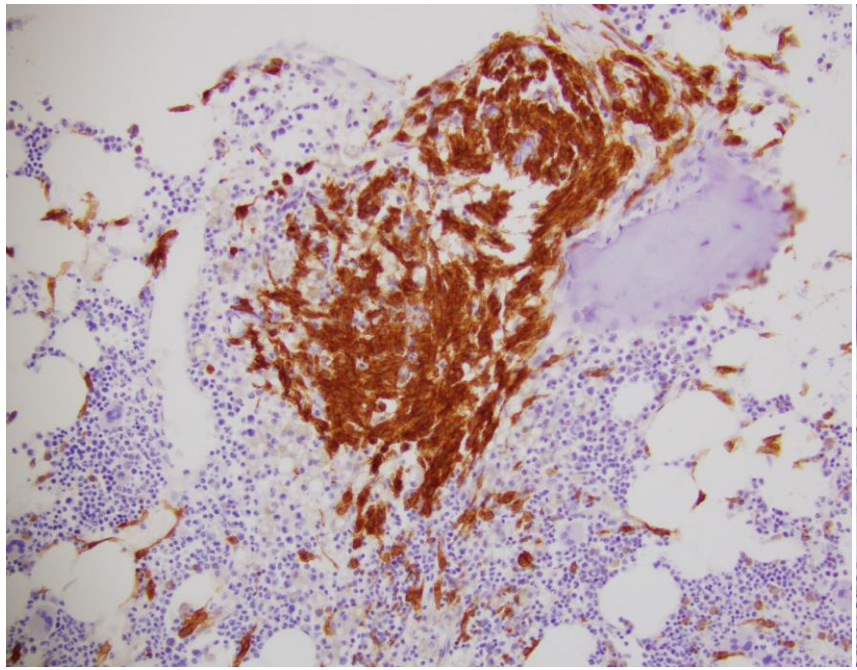
*MCs must be immature per ICC only

Immunohistochemistry and molecular diagnosis

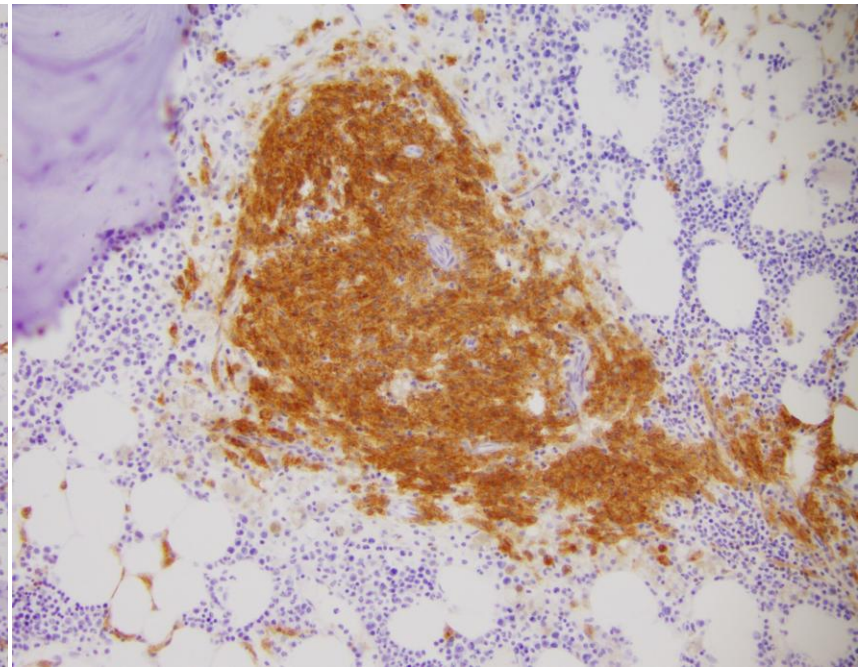


BM core biopsy



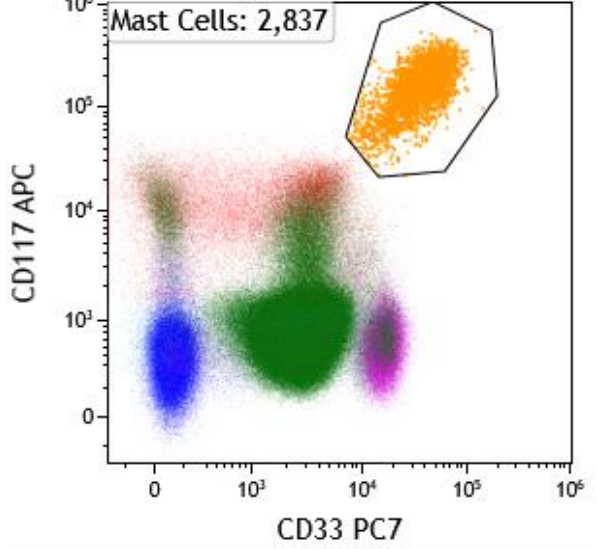


CD117

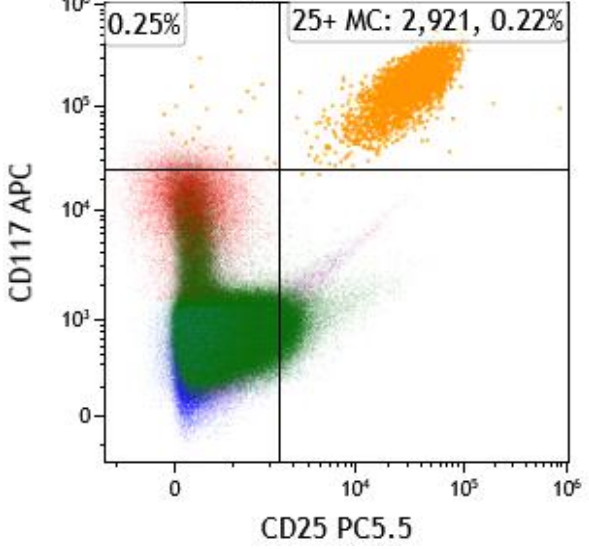


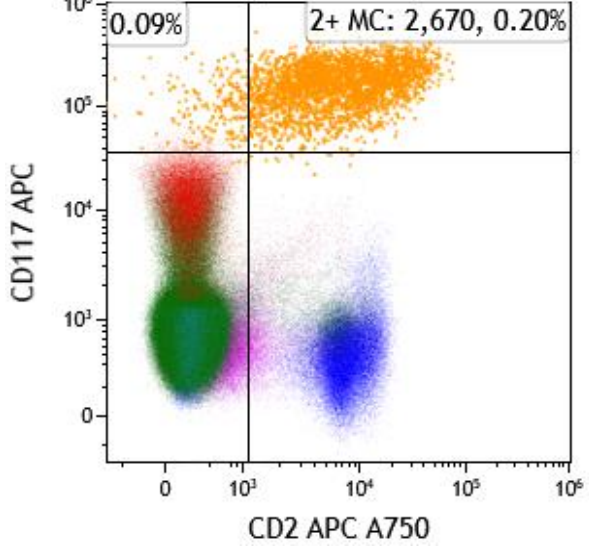
CD25

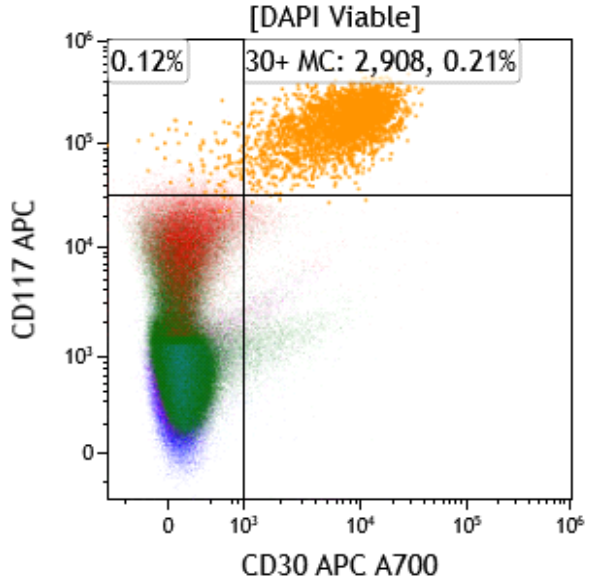
Aberrant expression of CD2 and/or CD25 and/or CD30 in mast cells

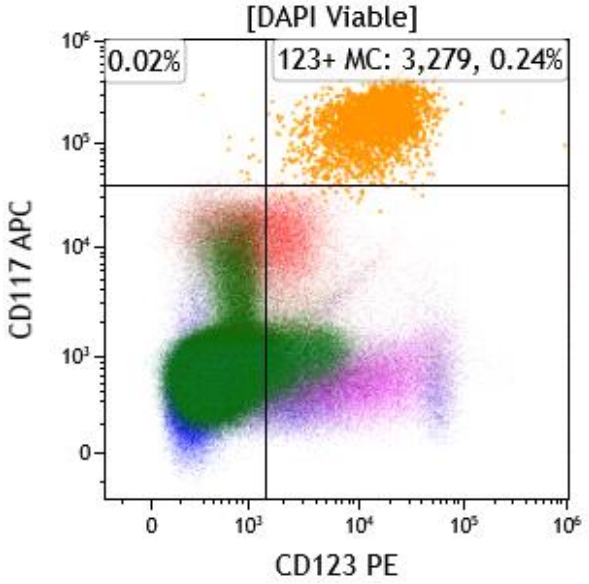


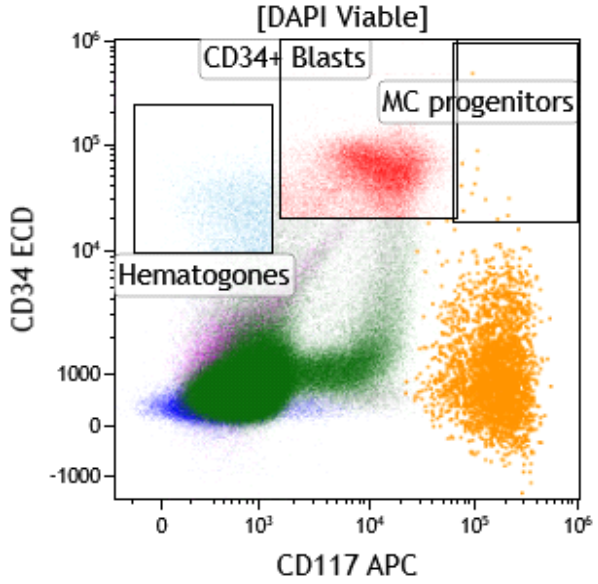
Gate	Number	%Gated
All	1,357,935	100.00
Mast Cells	2,837	0.21









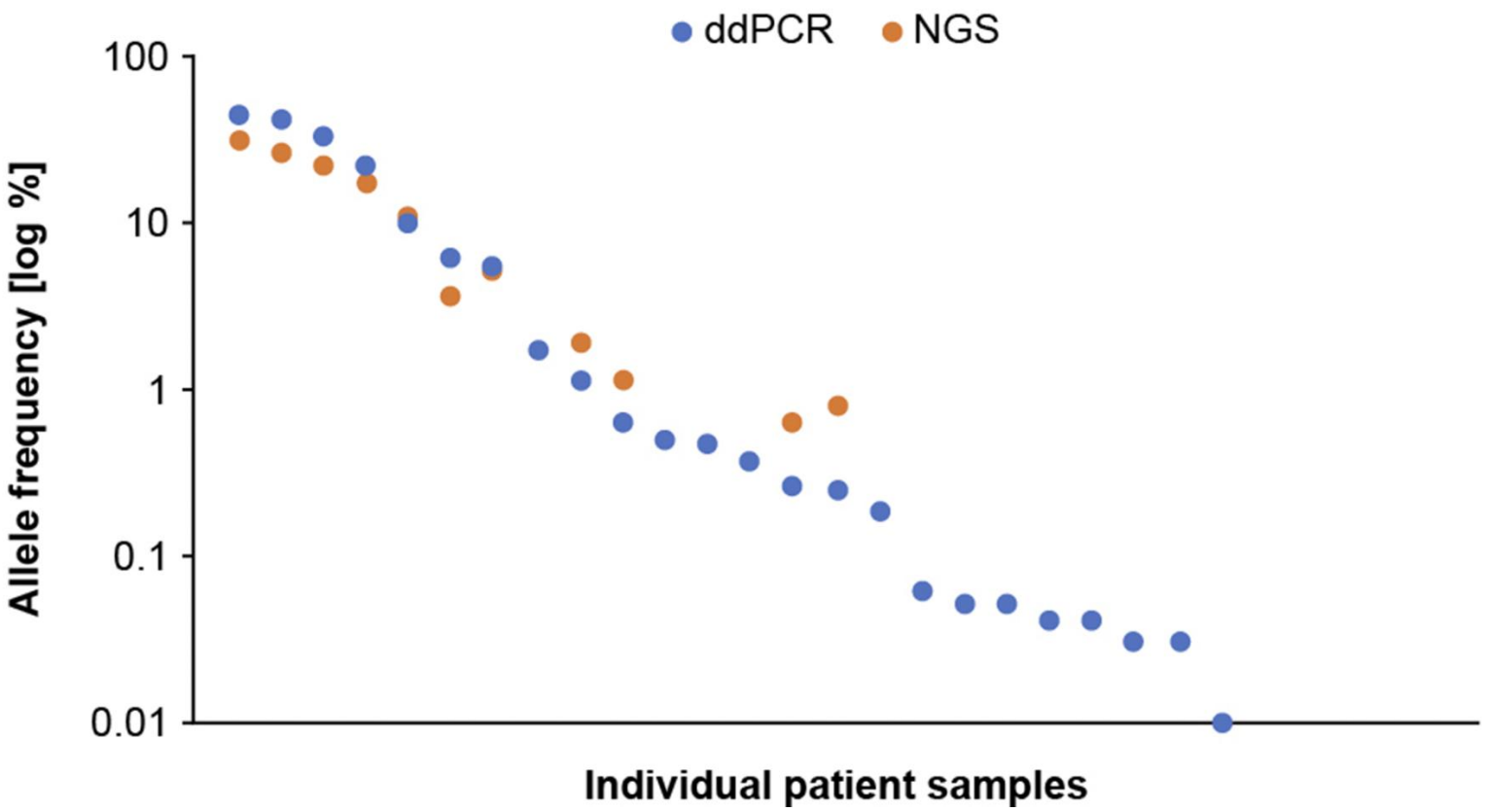


Gate	Number	%Gated
All	1,357,935	100.00
CD34+ Blasts	25,580	1.88
Hematogones	5,415	0.40
MC progenitors	116	0.01

Courtesy of David Ng (Univ Utah)

Increased detection of KIT D816V in ISM using a high sensitivity ddPCR assay (95%) vs NGS (28%)

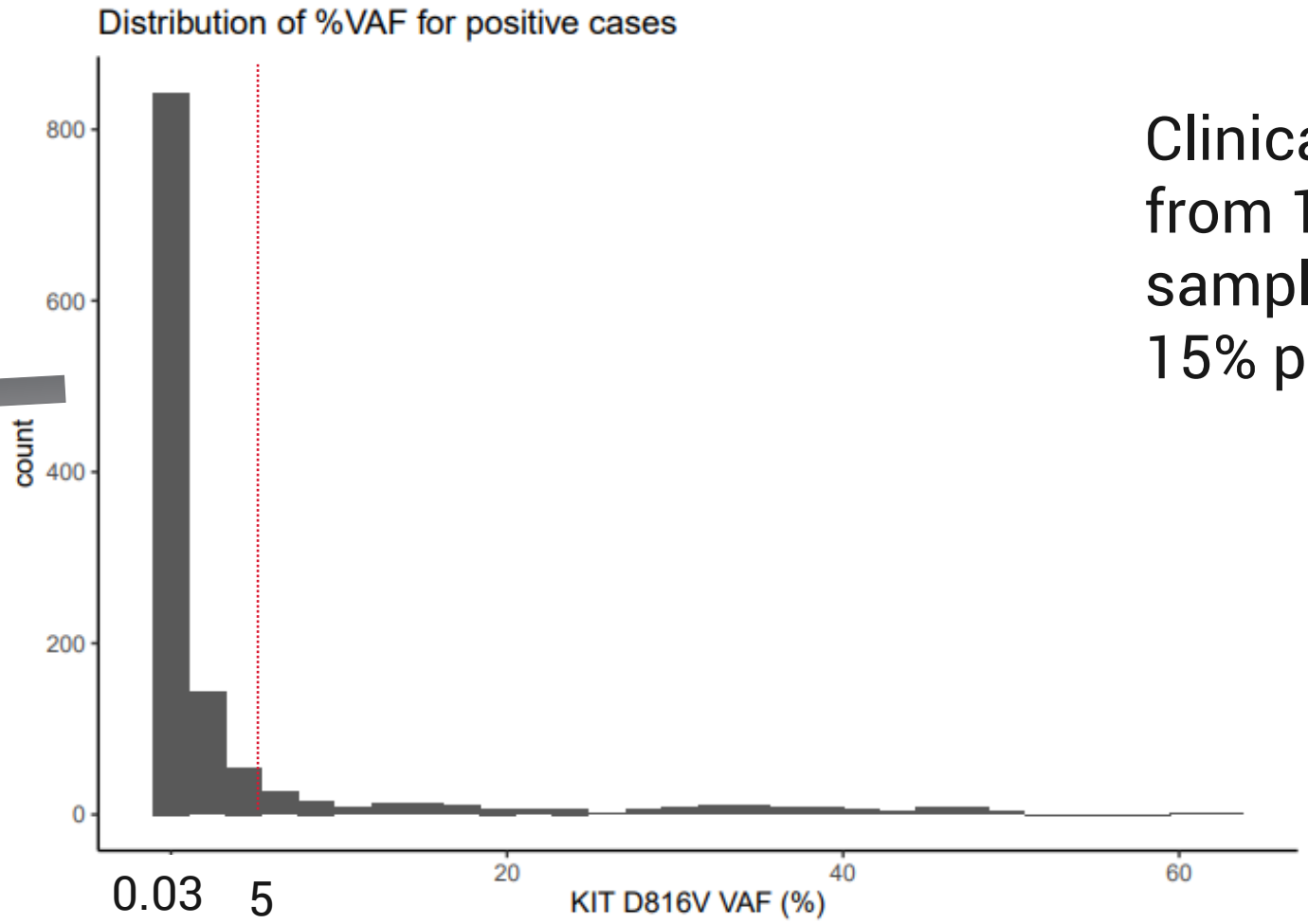
Figure. Performance of central ddPCR and NGS detection of *KIT* D816V VAF in PB samples from patients enrolled in part 1 of PIONEER



George TI et al. Blood 2020, abstract.

High sensitivity *KIT* D816V testing by ddPCR

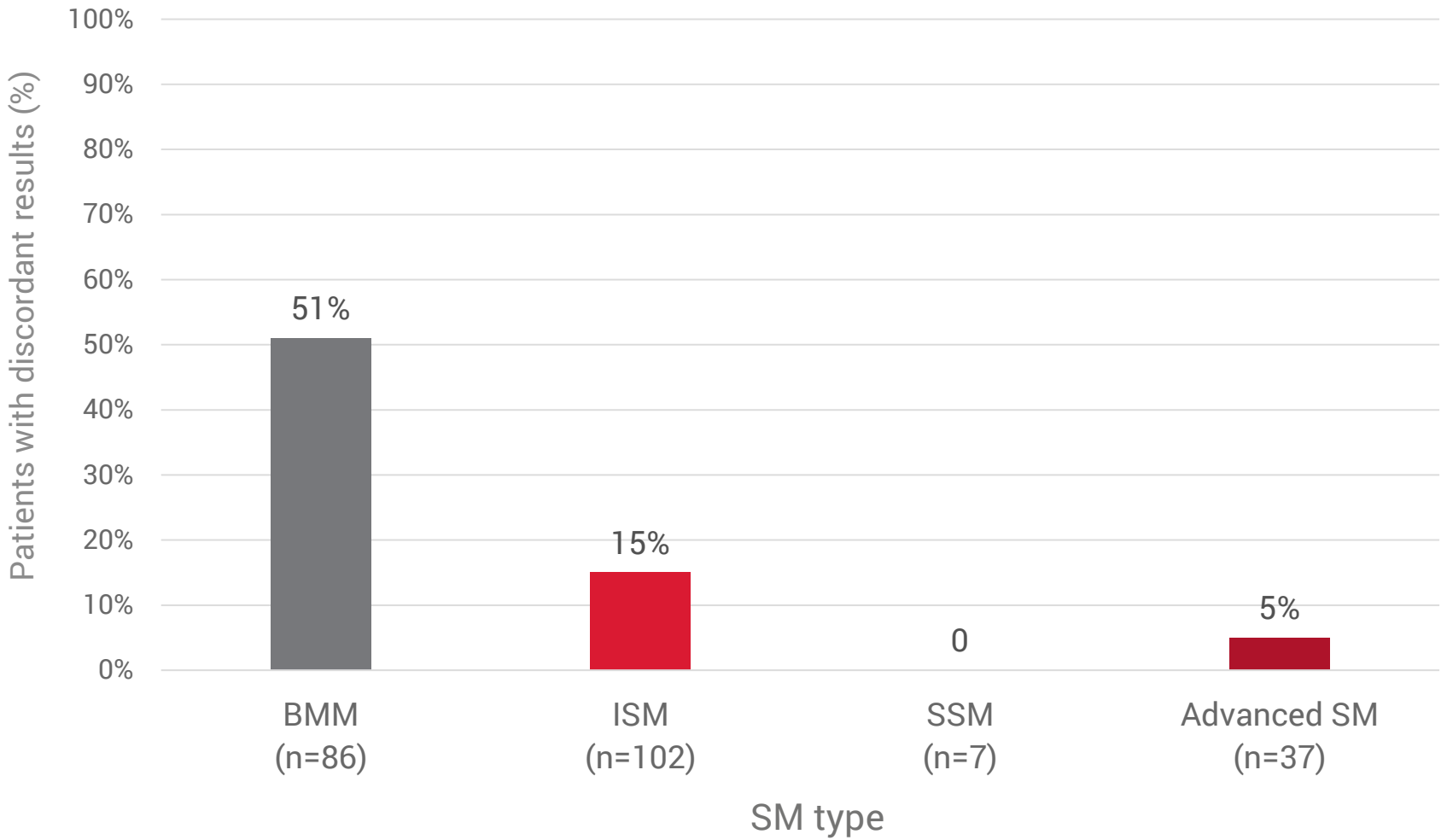
1031/1232 (84%) of positive samples with <5% VAF



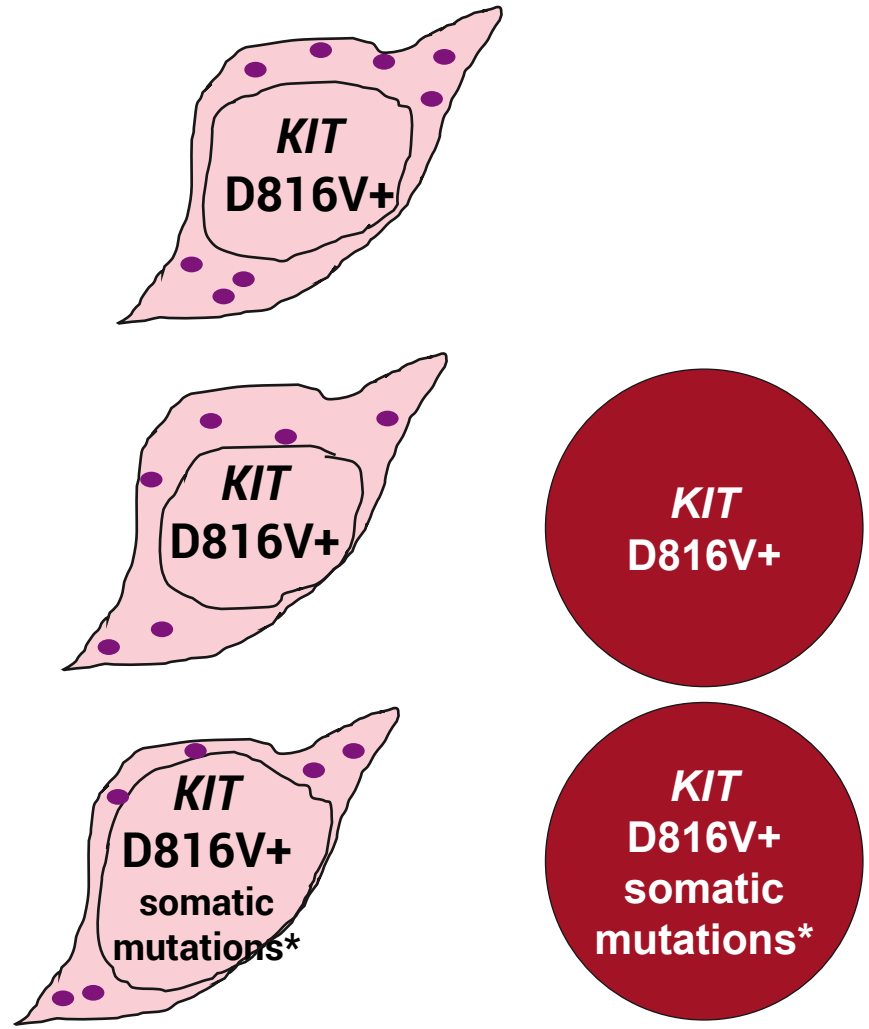
Clinical testing performed from 1/2021-3/2024: 8,272 samples (PB, BM) with a 15% positivity rate overall.

Shean R et al. Am J Clin Pathol 2025.

Higher discordance rates in peripheral blood vs bone marrow *KIT* D186V testing in Non-Advanced SM



Navarro-Navarro P et al. Allergy 2023.



Involvement of mast cell lineage

- **ISM**
- SSM
- MCL

Multilineage involvement

- ISM
- **SSM**
- **SM-AMN**

PB *KIT* D816V qPCR

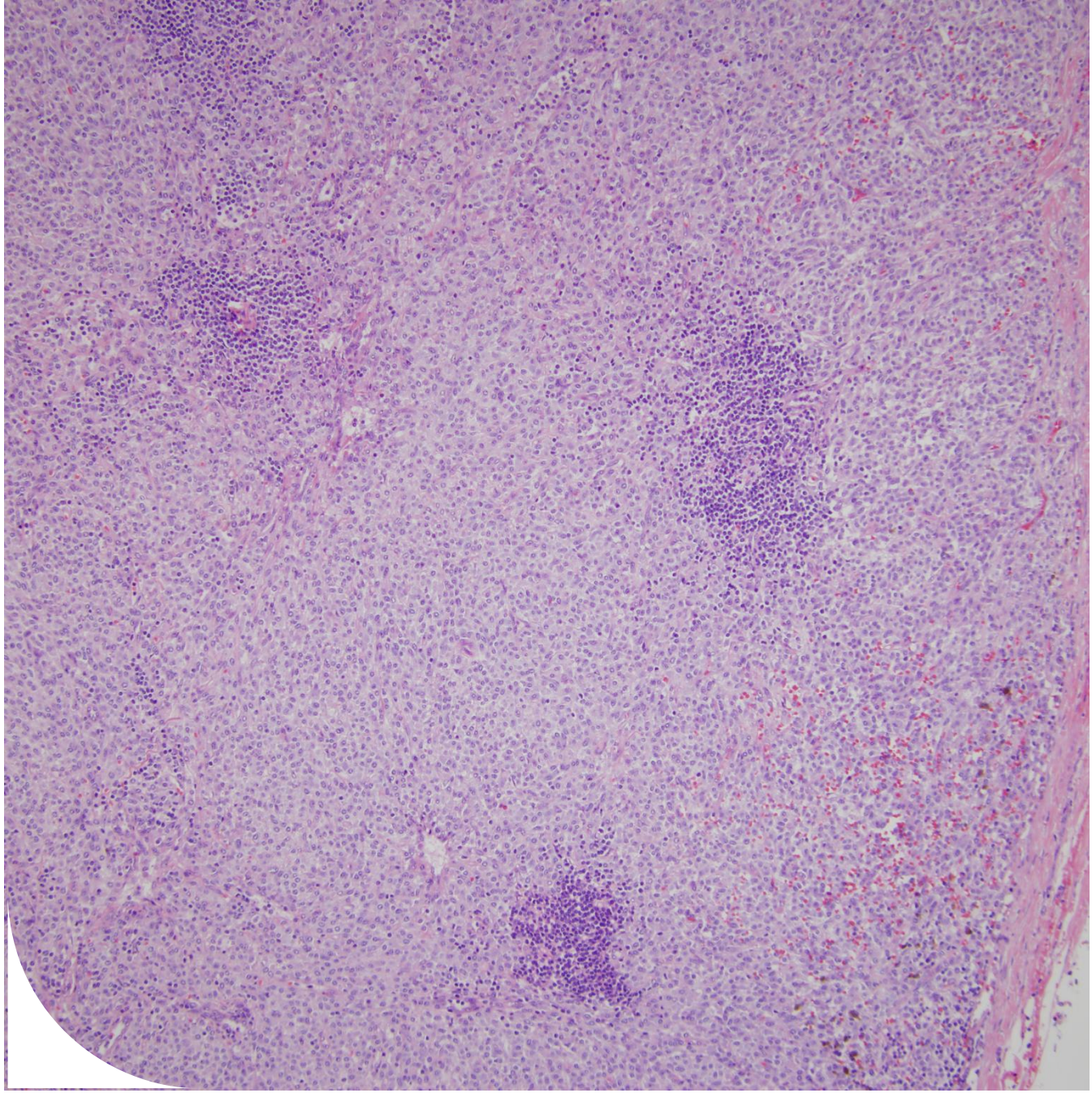
Multilineage involvement + multi-mutated

- **SM-AHN/AMN**
- **MCL ± AHN/AMN**

*additional somatic mutations:
e.g., *SRSF2*, *ASXL1*, *RUNX1*, *CBL*, *JAK2*, *EZH2*

Slide adapted from A. Reiter with permission. Reiter A, George TI, Gotlib. Blood 2020.

Challenges of diagnosis

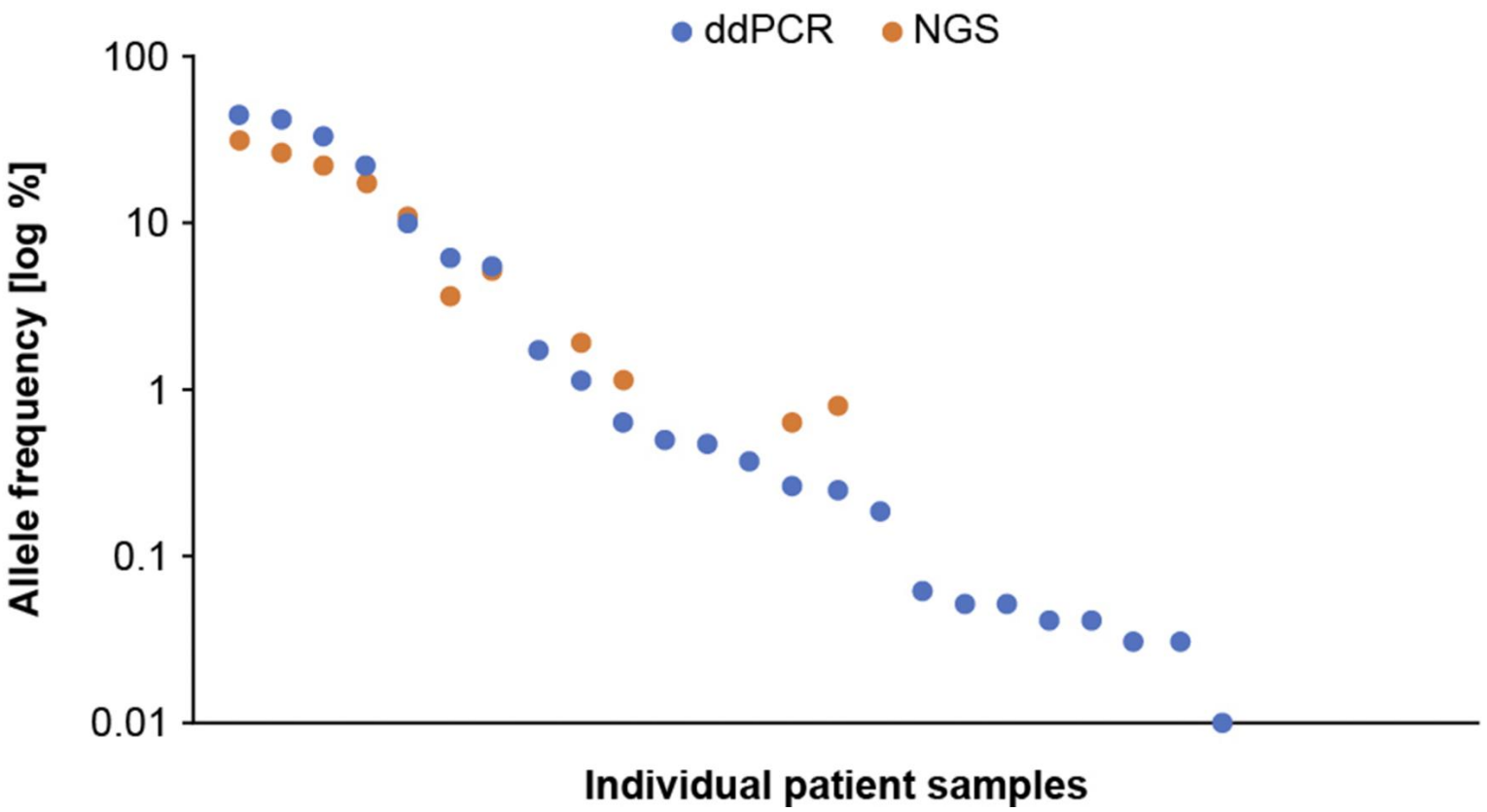


Challenges diagnosing mast cell disease in the NonAdvSM realm

1) Using less sensitive methods for detection of *KIT* p.D816V
2) Requiring serum tryptase levels > 20 ng/mL for a diagnosis of SM
3) Morphologic mimics

Increased detection of KIT D816V in ISM using a high sensitivity ddPCR assay (95%) vs NGS (28%)

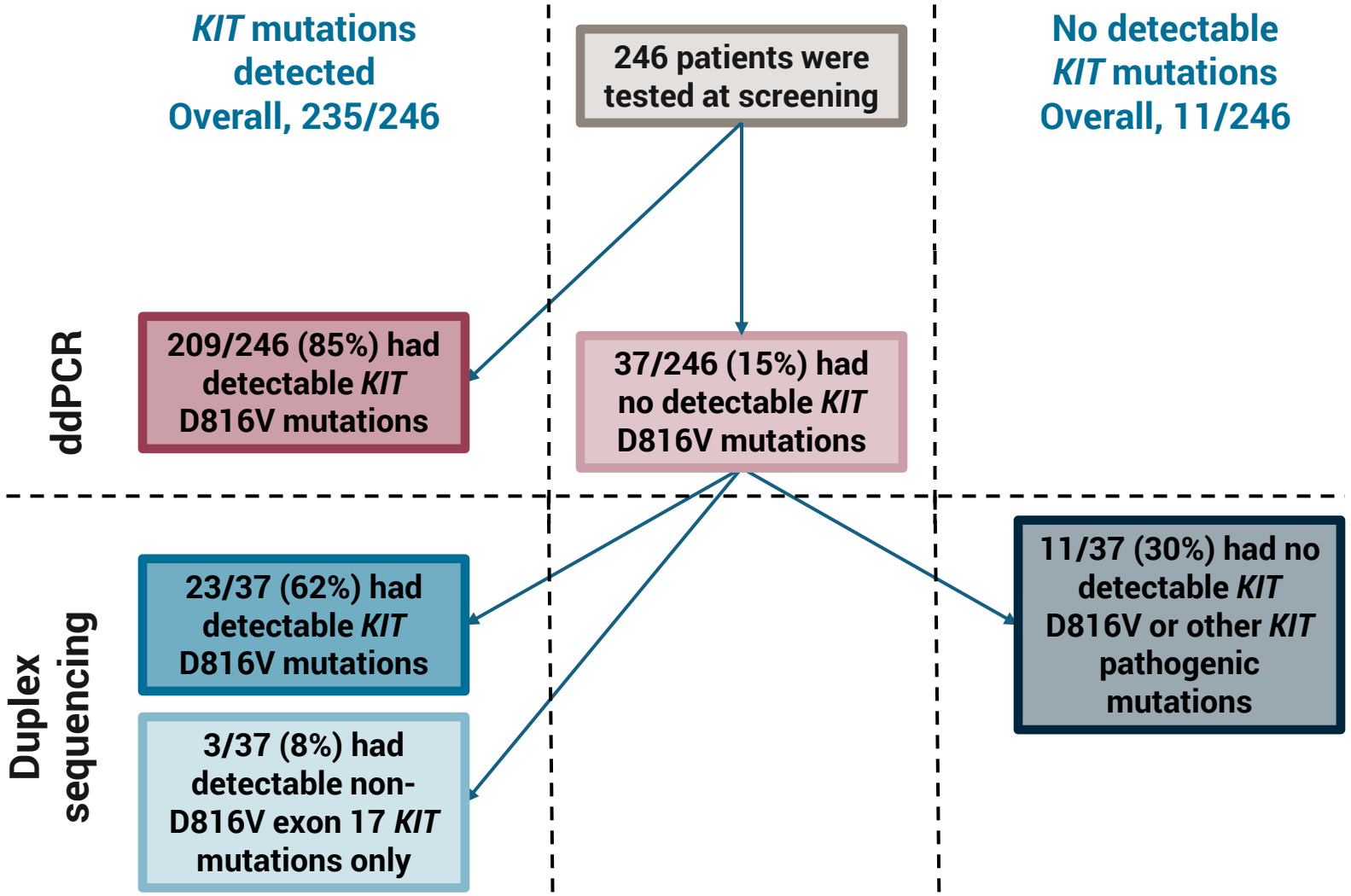
Figure. Performance of central ddPCR and NGS detection of *KIT* D816V VAF in PB samples from patients enrolled in part 1 of PIONEER



George TI et al. Blood 2020, abstract.

Ultra-high sensitivity testing improves upon sensitivity of ddPCR for detection of *KIT* D816V

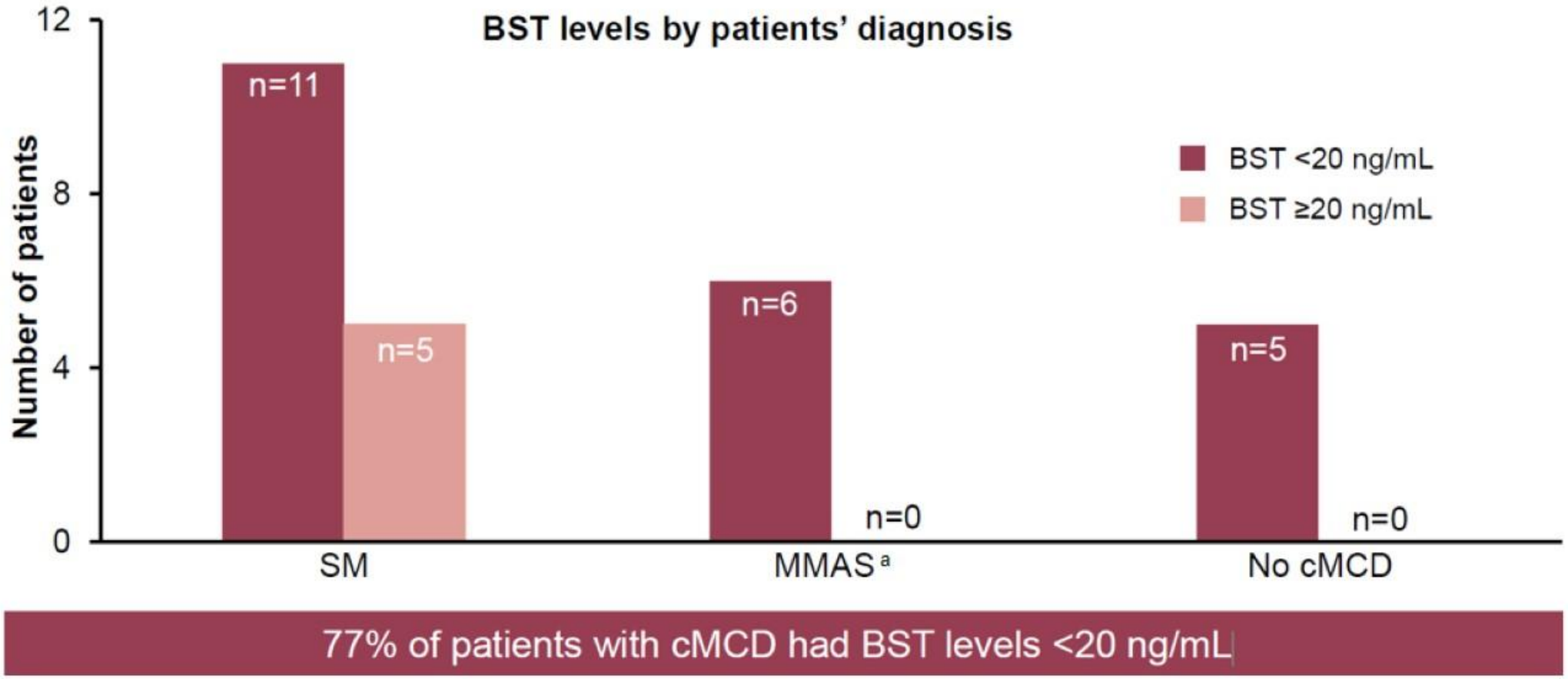
- Patients who had no detectable *KIT* mutations in PB by ddPCR were further tested with duplex sequencing
- Of 37 patients with no detectable *KIT* mutations by ddPCR, 26 had *KIT* mutations detectable by duplex sequencing
- Combining results from clinical ddPCR testing and research duplex sequencing showed that 97% of patients from PIONEER had detectable *KIT* activating mutations



Pongdee T et al. AAAAI 2025

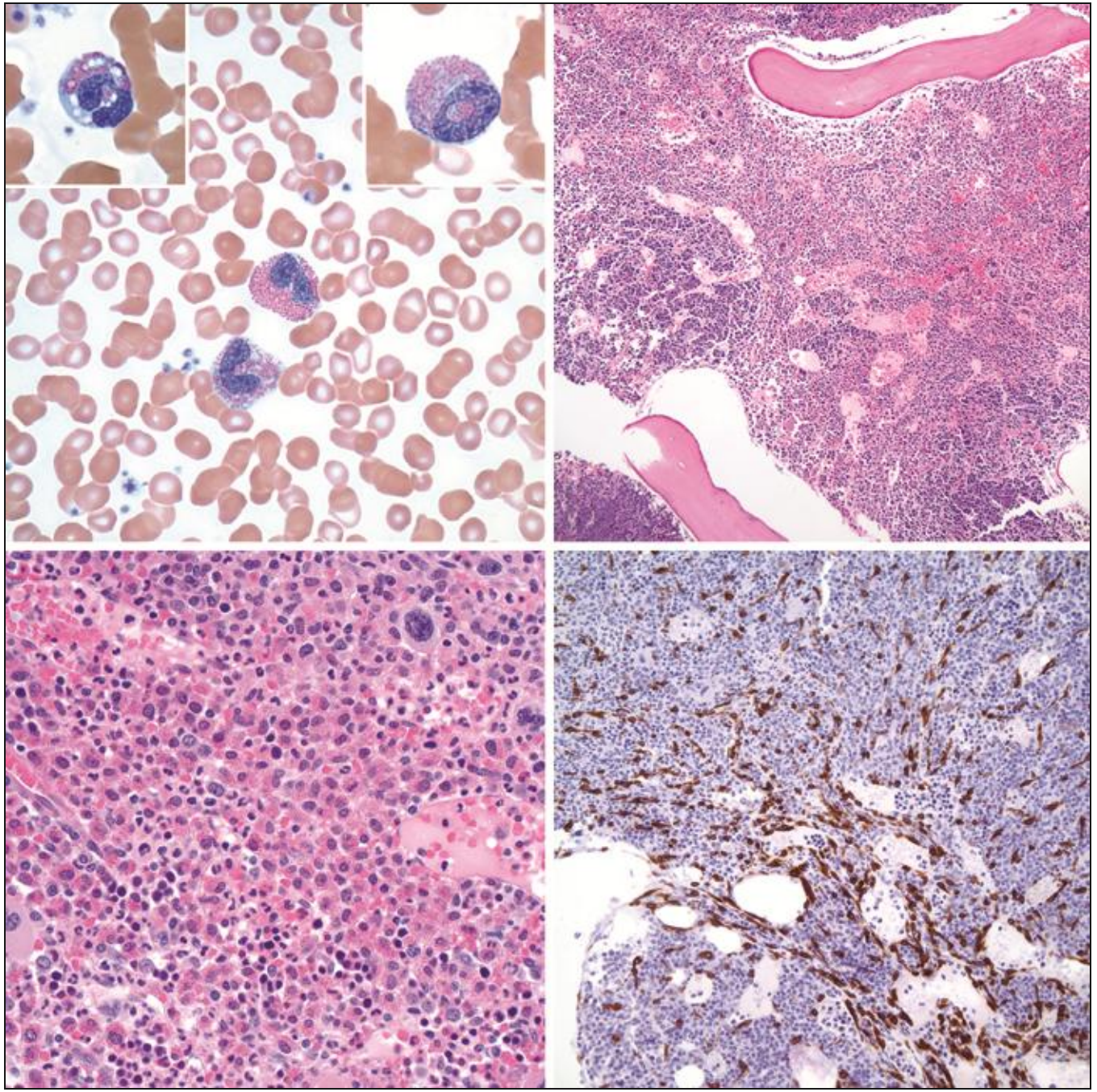
BST levels in patients with BST >11.4 ng/mL and no HαT

- Of the 22 patients diagnosed with cMCD, 17 (77%) had BST <20 ng/mL



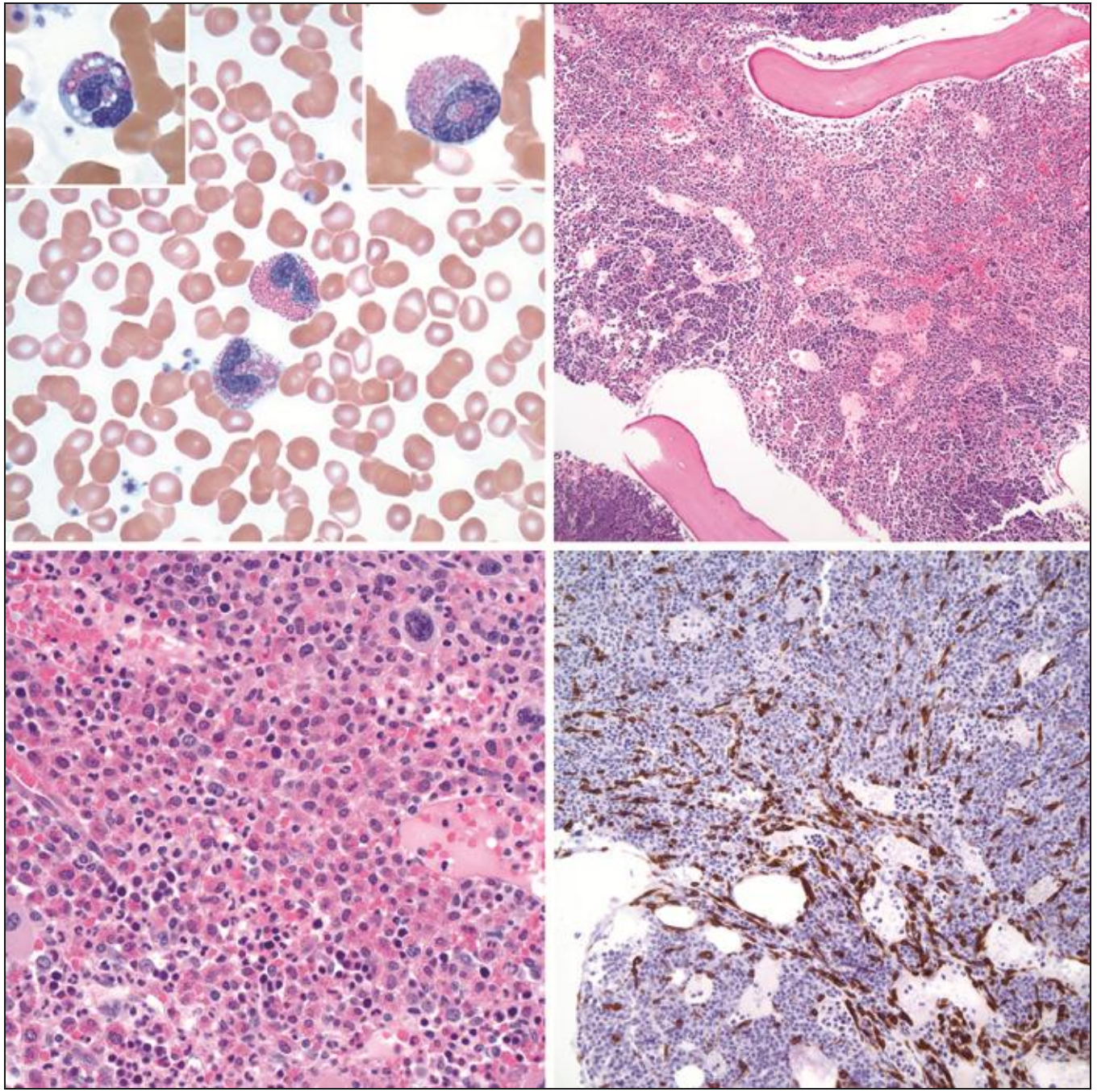
BST, basal serum tryptase; cMCD, clonal mast cell disease; HαT, hereditary α-tryptasemia; MMAS, monoclonal mast cell activation syndrome; SM, systemic mastocytosis.
[a]Two patients classified with MMAS had confirmed clonality (detected *KIT* D816V) but incomplete assessment for SM.

Hartmann K et al. AAAAI/WAO 2025



Diagnosis?

CD117



Chronic eosinophilic leukemia with *FIP1L1-PDGFRA*

CD117

Conclusions

01	WHO & ICC Classifications
02	Systemic mastocytosis and subtypes
03	Immunohistochemistry and molecular diagnosis
04	Challenges of diagnosis

Thank you!

American
Initiative in
Mast Cell Diseases

www.AIMcd.net

ARUP® LABORATORIES

A nonprofit enterprise of the University of Utah and its Department of Pathology