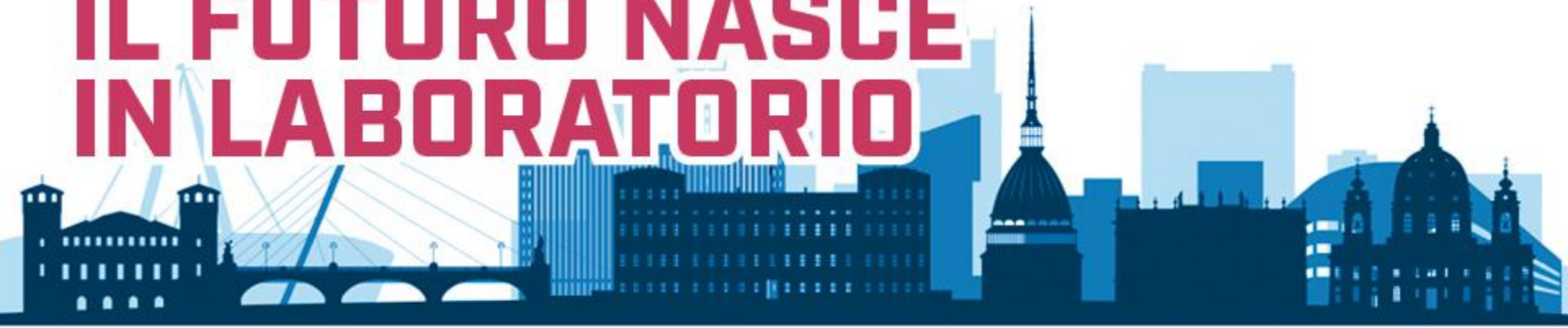
REGIONALE SIES – Delegazione Piemonte

YOUNG SCIENCE FORUM: IL FUTURO NASCE IN LABORATORIO



Diagnostica molecolare della mastocitosi sistemica e dell'iper-alfa-triptasemia ereditaria (HαT)

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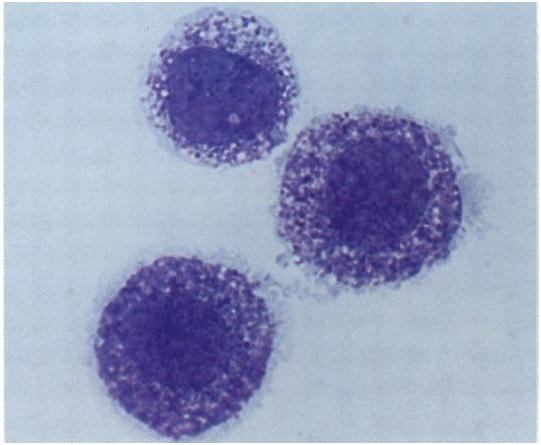
TORINO, ACCADEMIA DI MEDICINA | 4-5 GIUGNO 2026

KIT plays a critical role in mast cell development and activation

- Mast cells are hematopoietic cells of myeloid origin
- Mast cells express KIT, a tyrosine kinase receptor
- Normal KIT signaling drives mast cell proliferation survival, and activation in immune response

Activated mast cells release granules containing proinflammatory mediators

Mast Cells

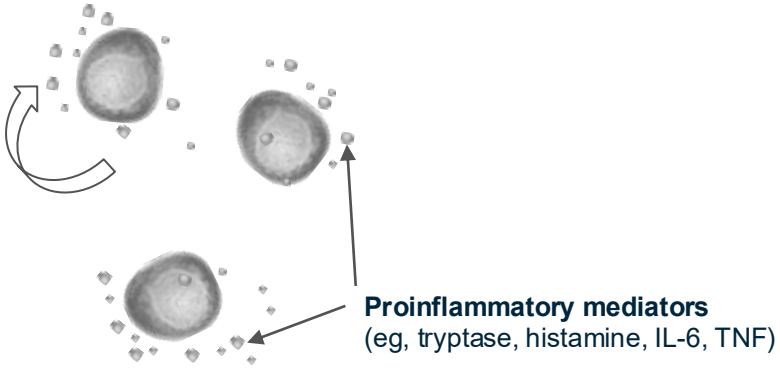


(May-Grunwald/Giemsa staining, ×3750)

Furitsu T et al. *Proc Natl Acad Sci*. 1989;86(24):10039–10043.

Systemic mastocytosis is a rare, clonal mast cell neoplasm driven by KIT D816V

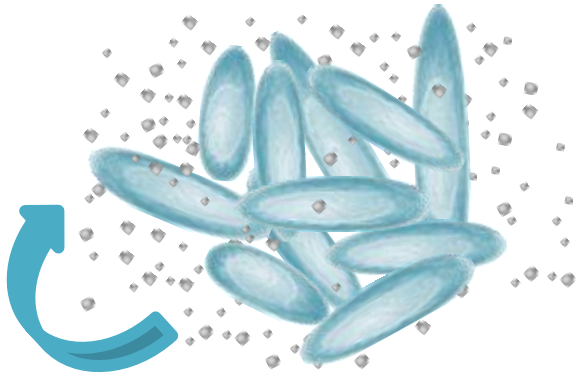
Normal mast cell physiology



Mast cells

Ligand-dependent wildtype KIT

Systemic mastocytosis



Neoplastic mast cell infiltrates/aggregates
CD25 positive
Elevated tryptase

KIT D816V mutation

Theoharides TC et al. *N Engl J Med*. 2015;373(2):163–172;
Jara-Acevedo M et al. *Mod Pathol*. 2015;28(8):1138–1149.

WHO criteria consist of major and minor criteria based on clinical and pathological laboratory findings

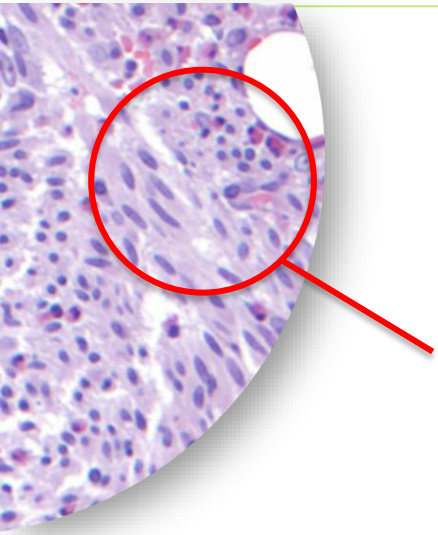


Image courtesy of Tracy George, MD, University of Utah.

WHO Systemic Mastocytosis Diagnostic Criteria
(1 major + ≥1 minor or ≥3 minor)

Major criterion

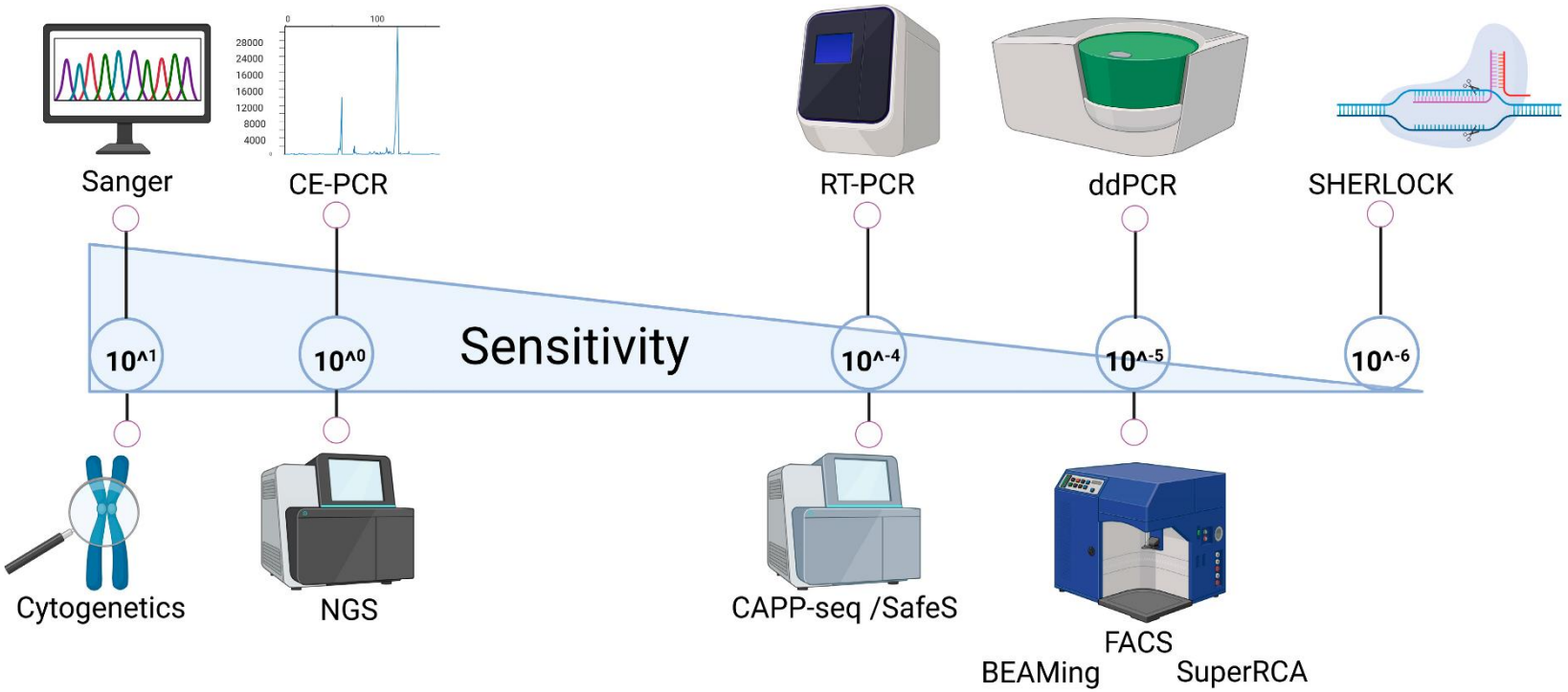
- **Multifocal dense infiltrates of MCs**

Minor criteria

- MCs in the infiltrate are **spindle-shaped; have atypical morphologic features**
- **Serum total tryptase**
- Detection of a point mutation at **codon 816 in KIT** in bone marrow, blood, or another extracutaneous organ
- MCs **express CD25**

What do we need for the diagnosis of mastocytosis?

- Histology → Invasive ✗
- *Tryptase levels* → Misleading ✗
- *KIT mutation* → Good, but low VAF ✓



Ferrando et al., 2026

KIT D816V analysis is ideally performed using a high-sensitivity assay on PB or BM

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)[1]: Myeloid mutation panels are not recommended for the detection of KIT D816V because such NGS assays exhibit low sensitivity (≈5%)

Preferred methods to detect KIT D816V include:

- Allele-specific oligonucleotide quantitative RT-PCR **(ASO-qPCR)**
- Digital droplet PCR (**ddPCR)**
- The limit of detection (LOD) shall be less than or equal to **0.01% VAF**

High-sensitivity KIT D816V assay

LOD< 0.01%

Impact of *KIT D816V* VAF

- VAF correlates with MC infiltration, especially in tissues
- VAF is higher in AdvSM compared to ISM and is an independent predictor of survival
- VAF is a surrogate for the extent of multilineage involvement

multilineage involvement in ISM is one of the most important prognostic factors that impacts on the probability of disease progression.

Initiate a full systemic mastocytosis work-up in all patients with incidental *KIT* mutation findings on myeloid next generation sequencing panel

≈10% of patients with advanced systemic mastocytosis have an associated hematologic neoplasm[1]

CMML | MDS/MPN | CEL | MPN | MDS | AML

KIT mutation on myeloid NGS panel

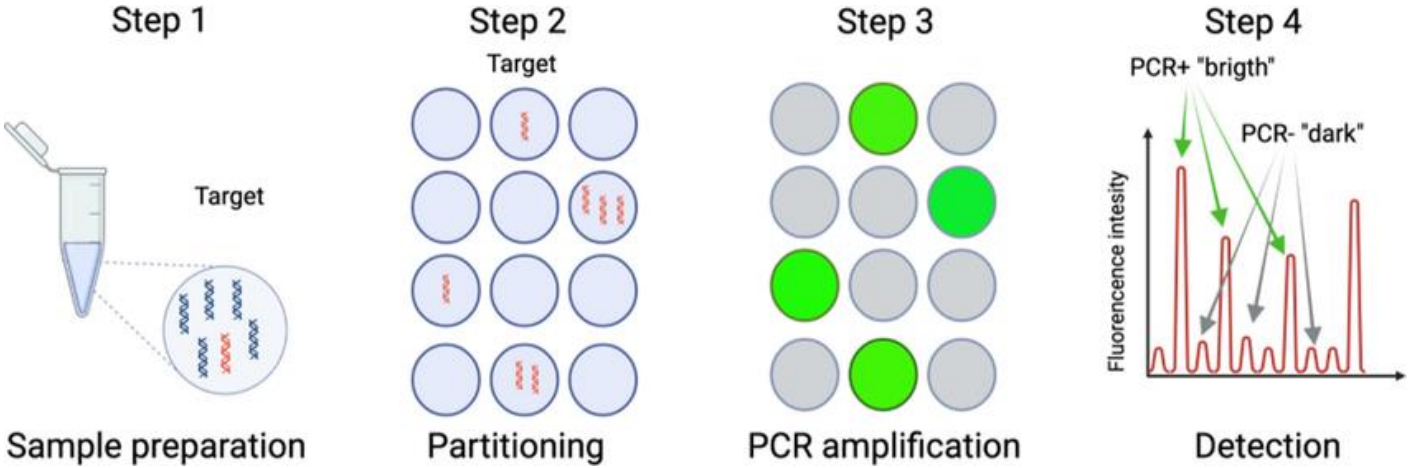
Incidental KIT finding should trigger a full diagnostic work-up for systemic mastocytosis[2]

- Given the low sensitivity (≈5%) of NGS assays for the detection of KIT D816V, some patients presenting with cytopenia(s) with suspected myeloid neoplasm may be missed on myeloid panels[3]
- Continue to monitor for signs and symptoms of systemic mastocytosis and perform mutational testing with high-sensitivity KIT D816V assay if systemic mastocytosis is suspected[3]

AML, acute myeloid leukemia; CEL, chronic eosinophilic leukemia; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasms; NGS, next generation sequencing.

1. Sperr WR, et al. *Lancet Haematol*. 2019;6(12):e638–e649. 2. Craig JW et al. *Modern Pathol*. 2020;10.1038/s41379-019-0447-x. Accessed February 12, 2020. 3. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Systemic Mastocytosis V.1.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed August 13, 2020. To view the most recent and complete version of the guideline, go online to NCCN.org.

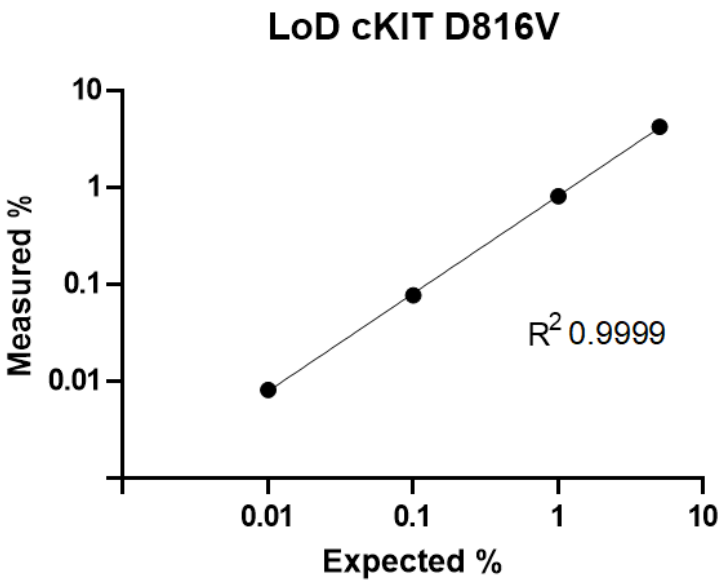
ddPCR for KIT mutations



14.20 **LECTURE II**
Nuove tecnologie in oncoematologia: dalla citofluorimetria convenzionale a super RCA, NGS, ddPCR
Erica Torchiaro, Novara

Experimental cKIT D816V LoD quantification

expected %	measured %
5%	4,264
1%	0,816
0.1%	0,077
0.01%	0,008
0.005%	0,012
0.001%	0,005



Patient ID	Kit VAF %
323	0,6662
329	0,2271
381	0,2732
389	44,016
390	52,656
391	0,0101
392	0,0611
393	0,1212

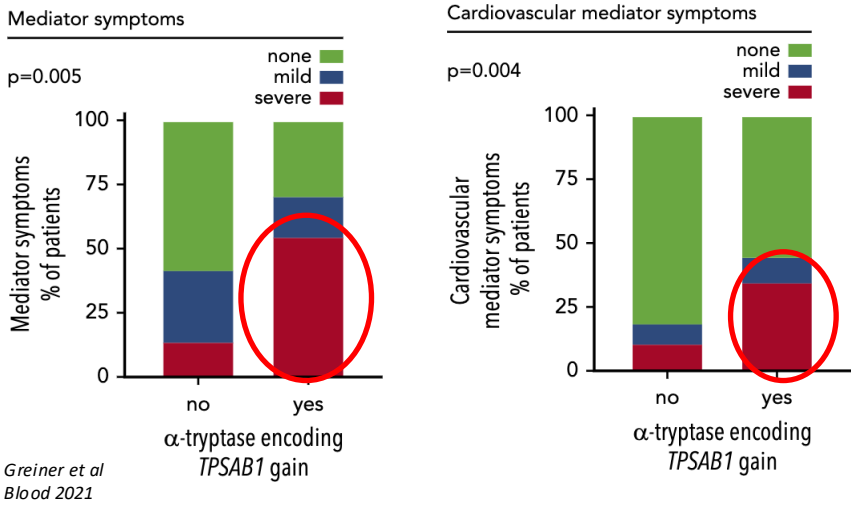
Hereditary alpha tryptasemia

- Is common in the Western population (4-6% of all individuals)
- Increased copy number of ***TPSAB1*** gene coding for a-tryptase
- Mast cell mediator release
- Increased levels of serum tryptase
- High risk for severe hymenoptera venom-induced anaphylaxis
- Increased risk of developing SM
- 12-17% of the patients with SM have concomitant HaT (rate over twice of the general population)
- High number of mast cells in BM
- Production of more mediators and cytokines

Arock M et al. AJ All Ast and Immunol 2022

Hereditary alpha-tryptasemia (HaT) in patients with systemic mastocytosis

✓ Disease features and implications: clinical manifestations



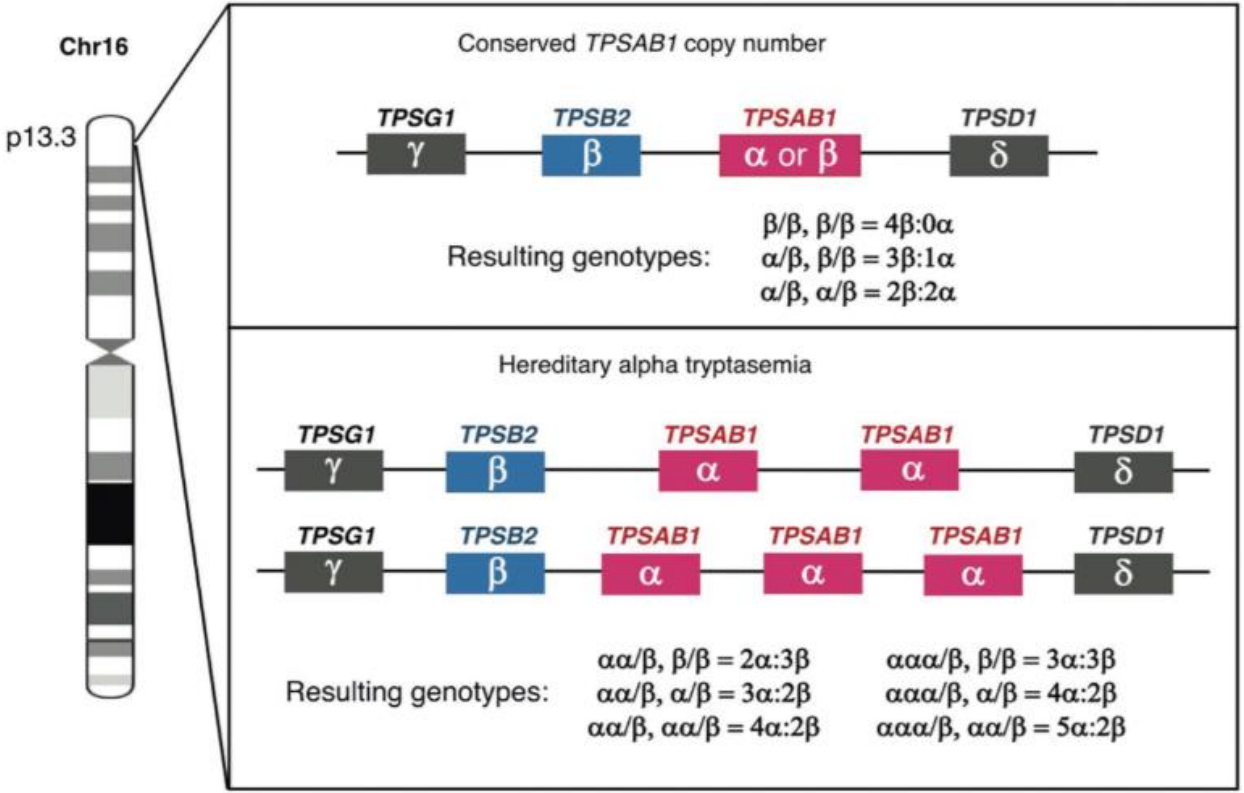
Greiner et al Blood 2021

	HαT+ (# 59) 13.3%	HαT- (# 385) 86.7%	P value
Anaphylaxis (overall incidence)	**61.0%** (36/59)	**40.8%** (36/59)	**0.005**
Mediator symptoms (anaphylaxis excluded)	70.2%	80.6%	0.080
Osteoporosis	28.0%	40.5%	0.119
GI symptoms	29.8%	35.4%	0.458

- HαT+ pts with SM are at **higher risk** or life-threatening complications as **anaphylaxis** (synergistic effect on the triggering of severe reactions)

Sordi et al JACI 2022

HαT genotipes



HαT genotype reconstruction ddPCR assay

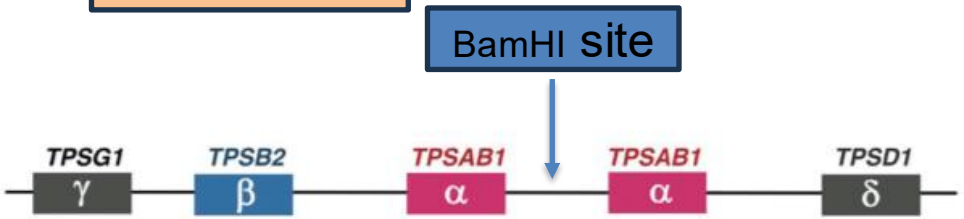
For each sample (patient)

a+hk

a+b

a+hk+BamHI

a+b+ BamHI



hk=AP3B1
(double copy)

a+hk → a/(hk/2) → n° copy of a → 2 = a, a

a+hk+BamHI → Δa=(a+bam)- a → If Δ>X, a duplication → Δ>X, amplified a on same locus

aa, x / x, x

aa, b / b, b

a+b → a/b → Ratio a/b Infer n° copy b → Ratio a/b = 0.66

0.66 “a” for each “b”

2a + 3b

a+b+ BamHI → Δb=(b+bam)-b → If Δ>X, b is double in copy → Δ changes 2b on same locus

Conclusions

- Detection requires **high-sensitivity molecular techniques** (ddPCR)
- Identification of **KIT mutations in myeloproliferative neoplasms** should prompt investigation for underlying mastocytosis
- In patients with persistently elevated tryptase, evaluation for **hereditary alpha-tryptasemia** should be considered
- ddPCR is **not** always available
- **No available** diagnosis grade kit for ddPCR

Thank you for your attention

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