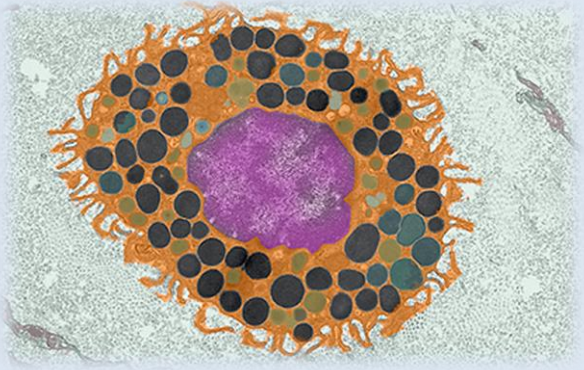
Convegno Regionale SIBioC
Emilia Romagna 2019

Presente e futuro della medicina di laboratorio

Bologna
6 dicembre 2019 - 10:00 - 17:00

Oratorio di San Filippo Neri
via Manzoni 5

Focus sulle Mastocitosi: Inquadramento della patologia ed analisi di laboratorio



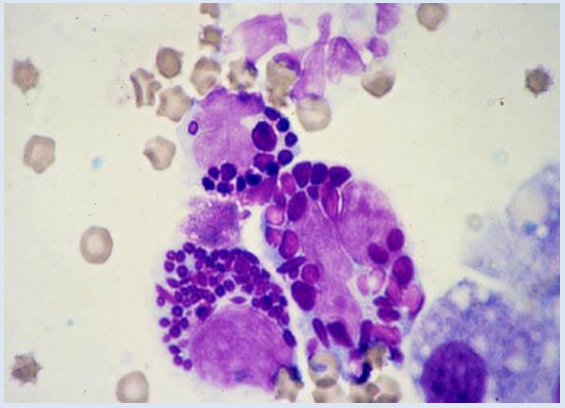
Evita Massari
AUSL Romagna – Laboratorio Unico
U.O. Patologia Clinica
Pievesestina (FC)

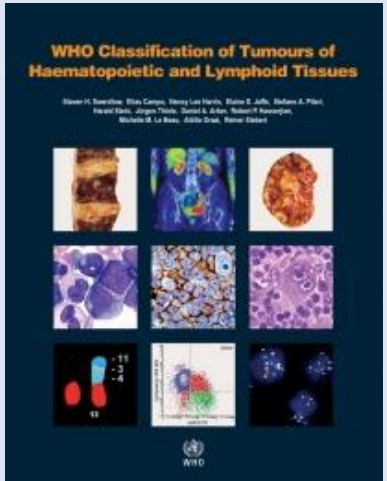
MASTOCITOSI: AGENDA

- LA PATOLOGIA
- LA DIAGNOSI
- LA CLASSIFICAZIONE
- IL RUOLO DEL LABORATORIO

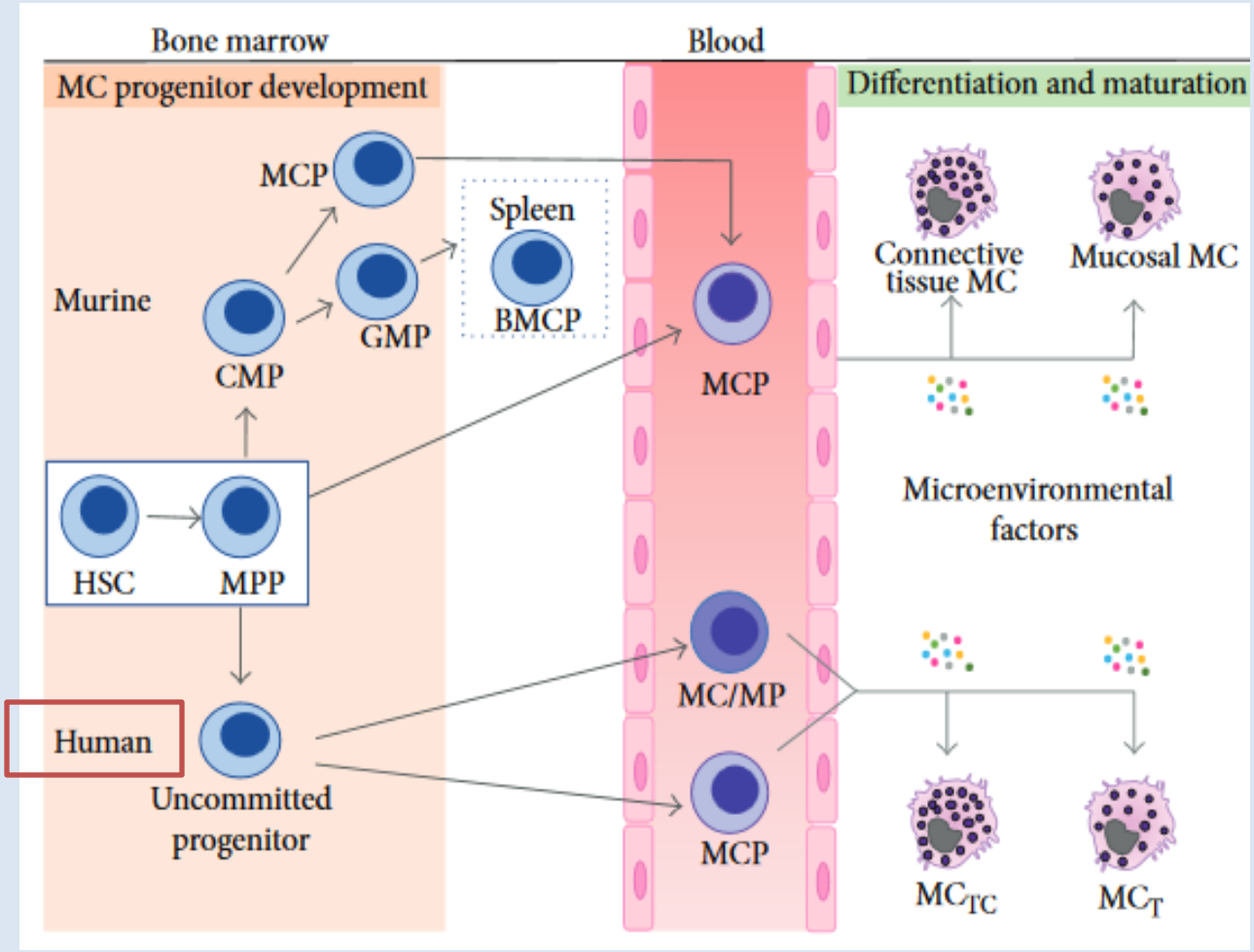
UN PO' DI STORIA…

1869 – Nettleship	**Rare form of Urticaria**
1878 – Sangster	Urticaria Pigmentosa UP
1879 – Ehrlich	**Mast Cells (Mastzellen)**
1887 – Unna	Mast Cells in UP
1949 – Ellis	Systemic Mastocytosis
1979 – Lennert	Kiel Classification
1991 – Metclafe	Consensus Classification
1996 – Longley	**c-kit D816V in SM**
1998 – Escribano	**CD2/CD25 on MC in SM**
1990-2000	Criteria Established
2000	Working Conference
2001	WHO Classification
2008	WHO Classification
2016	**WHO Classification**



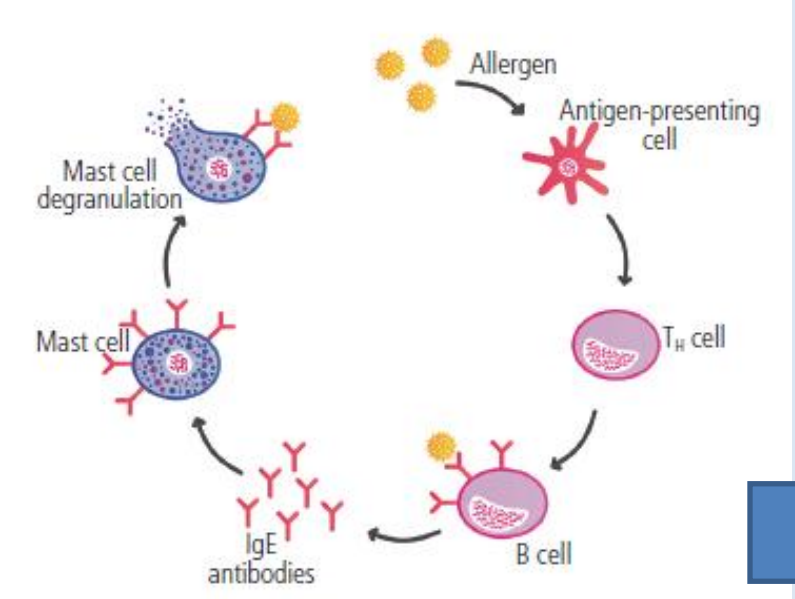


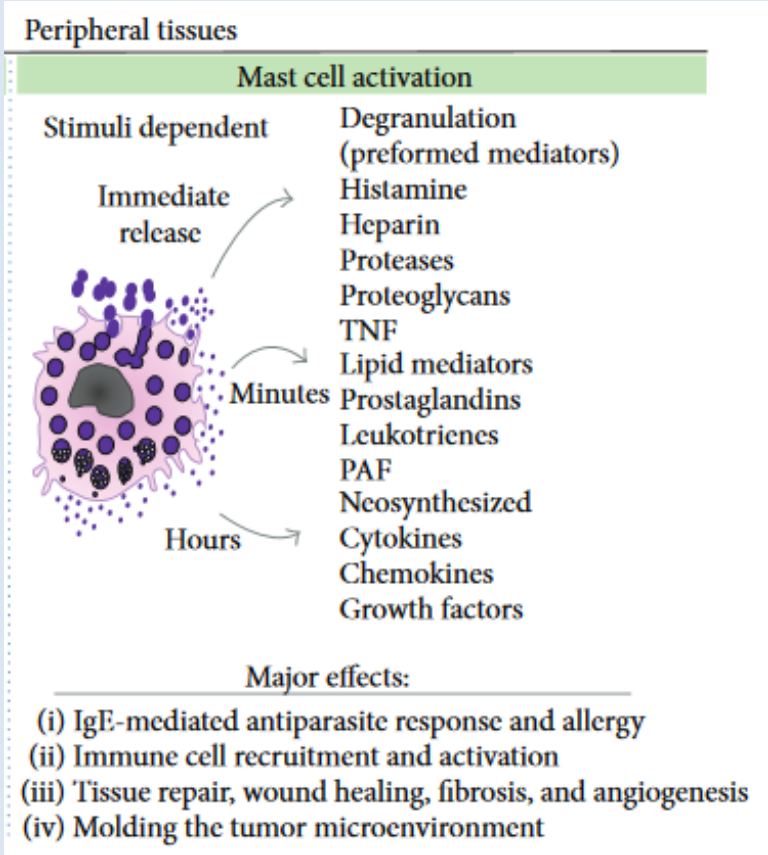
ORIGINE dei MASTOCITI



Aponte-Lopez et al. (2018)

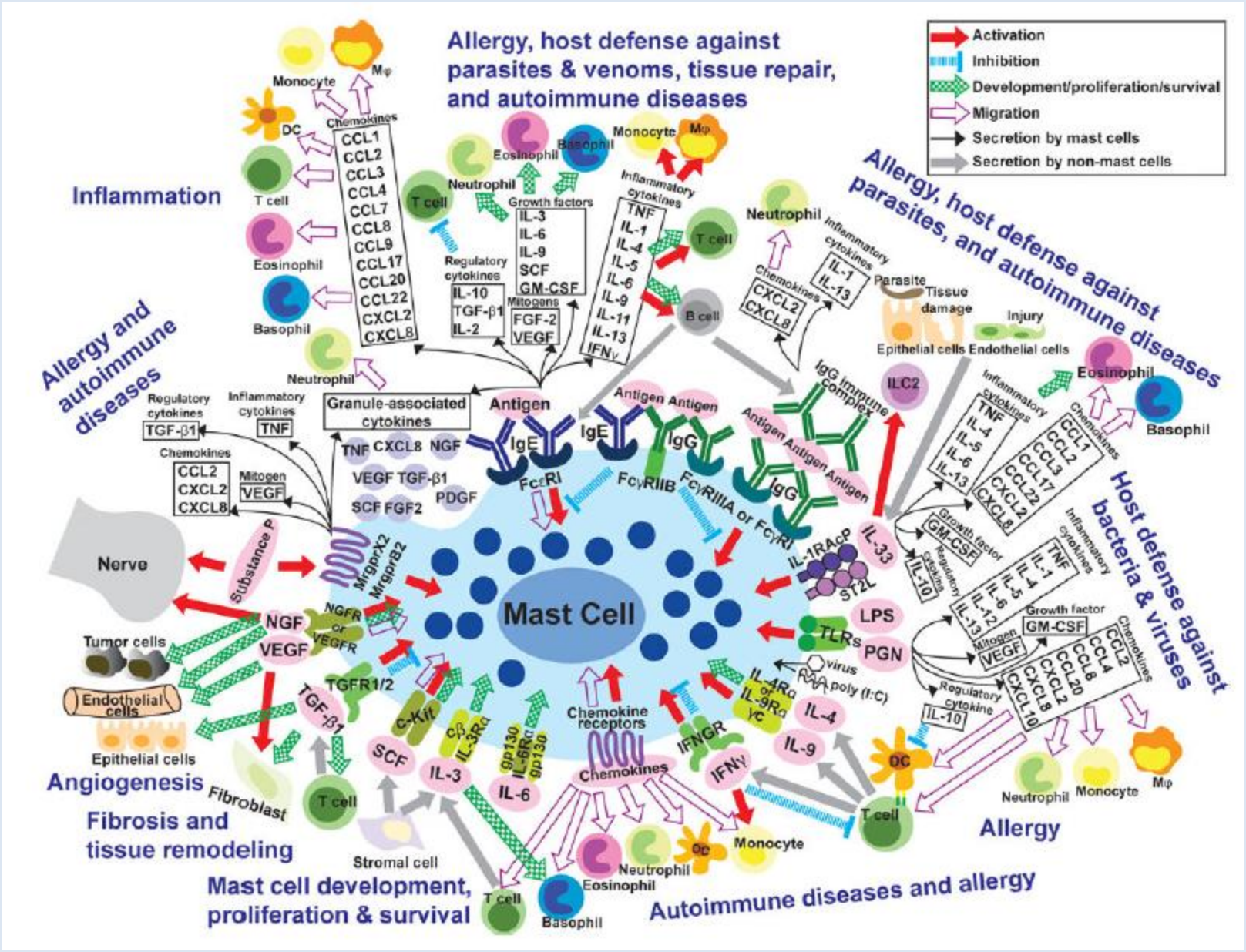
MASTOCITI: ATTIVAZIONE e DEGRANULAZIONE





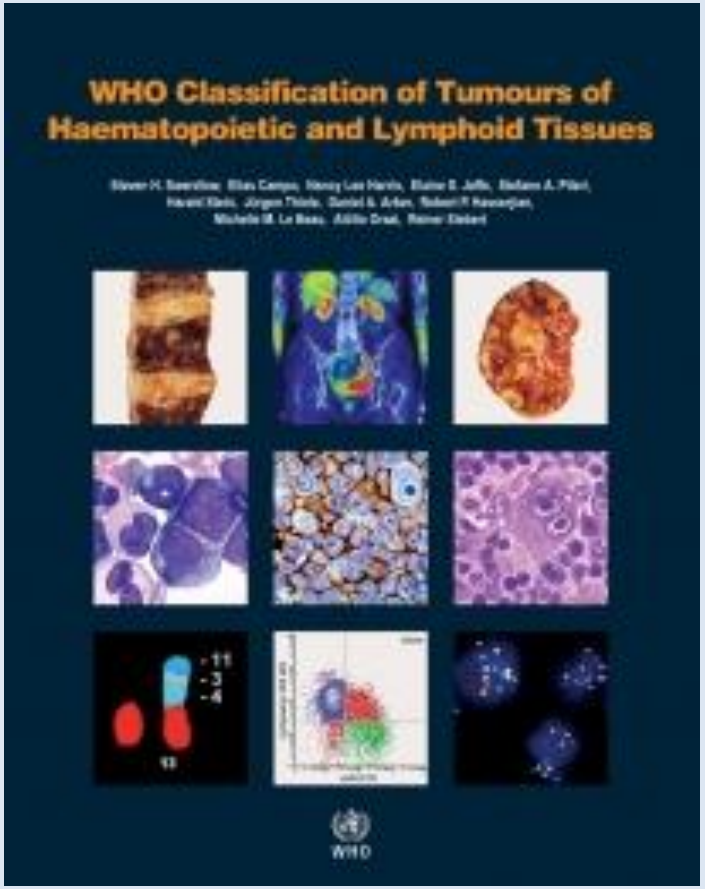
Gastrointestinal tract	Airways	Blood vessels
Increased fluid secretion, increased peristalsis	Decreased diameter, Increased mucus secretion	Increased blood flow, increased permeability
Diarrhea, vomiting	**Wheezing, coughing**	Increased effector response in tissue

Aponte-Lopez et al. (2018)
González-de-Olano et al. (2018)



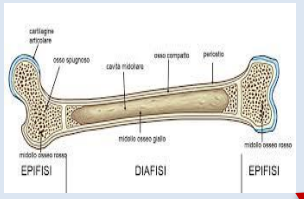
Mukai et al. Immunol Rev (2018)

WHO 2016: CLASSIFICAZIONE MASTOCITOSI



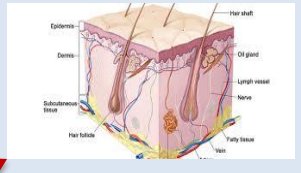
1. Cutaneous mastocytosis (CM):
   a. Urticaria pigmentosa (UP)/Maculopapular cutaneous mastocytosis (MPCM)
   b. Diffuse cutaneous mastocytosis
   c. Solitary mastocytoma of skin
2. Indolent systemic mastocytosis (ISM)
   - Meets criteria for systemic mastocytosis (SM).[a] No "C" findings.[a] No evidence of associated hematological neoplasm. Isolated bone marrow mastocytosis[b]
   - As above (ISM), but with bone marrow involvement and no skin involvement, generally low-burden of MC.
3. Smoldering systemic mastocytosis (SSM)
   - As above (ISM), but with 2 or more "B" findings, and no "C" findings,[1] generally high-burden of MC.
4. Systemic mastocytosis with an associated hematological neoplasm (SM-AHN)
   - Meets criteria for SM and criteria for AHN as a distinct entity per the WHO classification
5. Aggressive systemic mastocytosis (ASM)
   - Meets criteria for SM. One or more "C" findings.[a] No evidence of mast cell leukemia.
6. Mast cell leukemia (MCL)
   - Meets criteria for SM. Bone marrow biopsy shows diffuse infiltration, usually dense, by atypical, immature mast cells. BM aspirate smears show ≥20% mast cells. In classic cases, mast cells account for ≥10% of peripheral blood white cells. Aleukemic MCL variant (<10% circulating mast cells).
7. Mast cell sarcoma (MCS)
   - No evidence of SM. Generally localized destructive growth pattern. High-grade cytology.

Pardanani A, Am J Hematol (2019)



Reumatologo

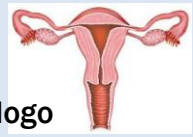
Neurologo



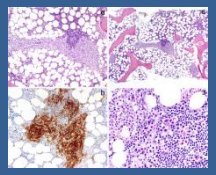
Dermatologo

Anestesista

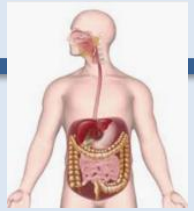
MULTIDISCIPLINAR MANAGEMENT OF MASTOCYTOSIS



Ginecologo

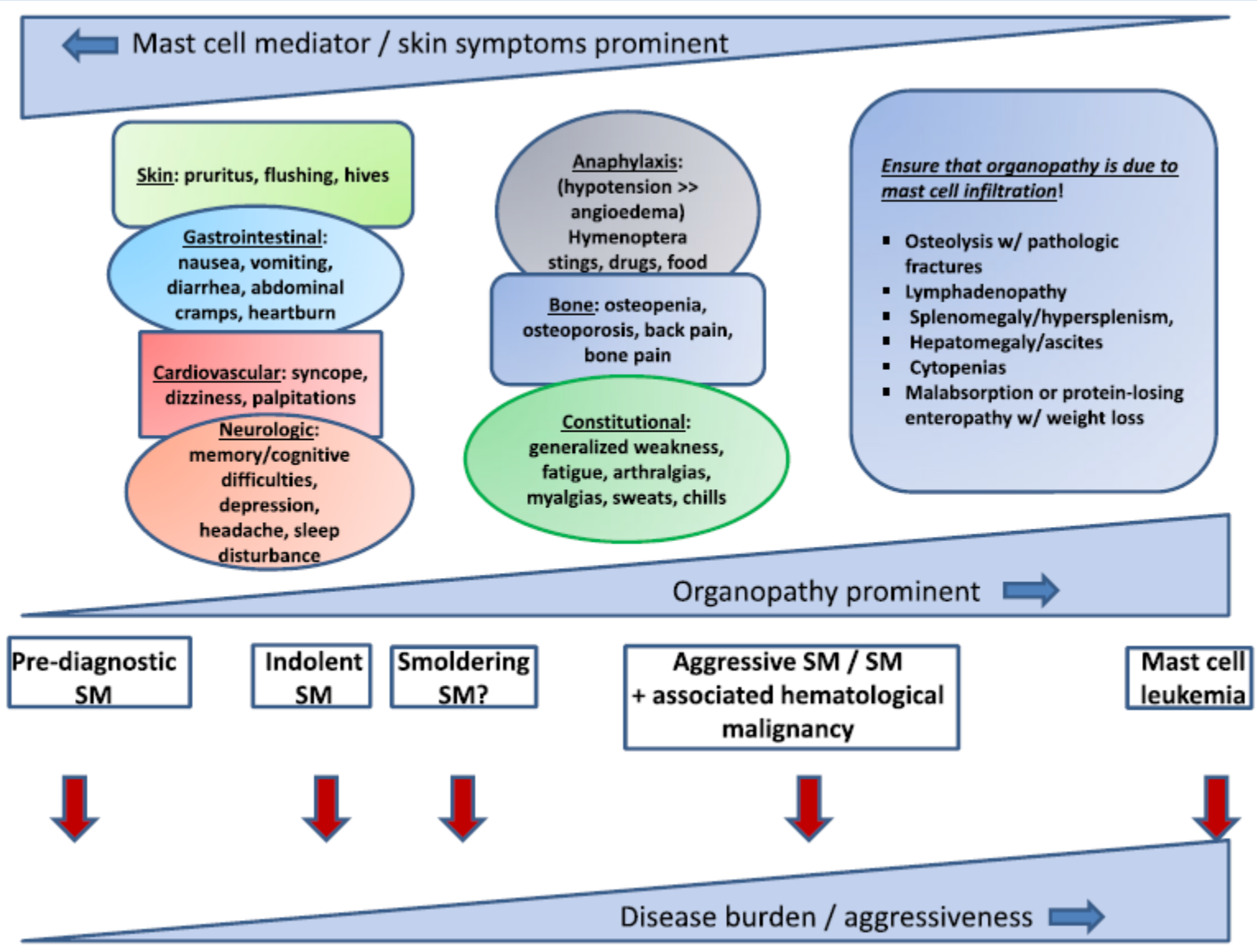


Ematologo



Allergologo

PRESENTAZIONE CLINICA



Pardanani A, Am J Hematol (2019)

SOPRAVVIVENZA

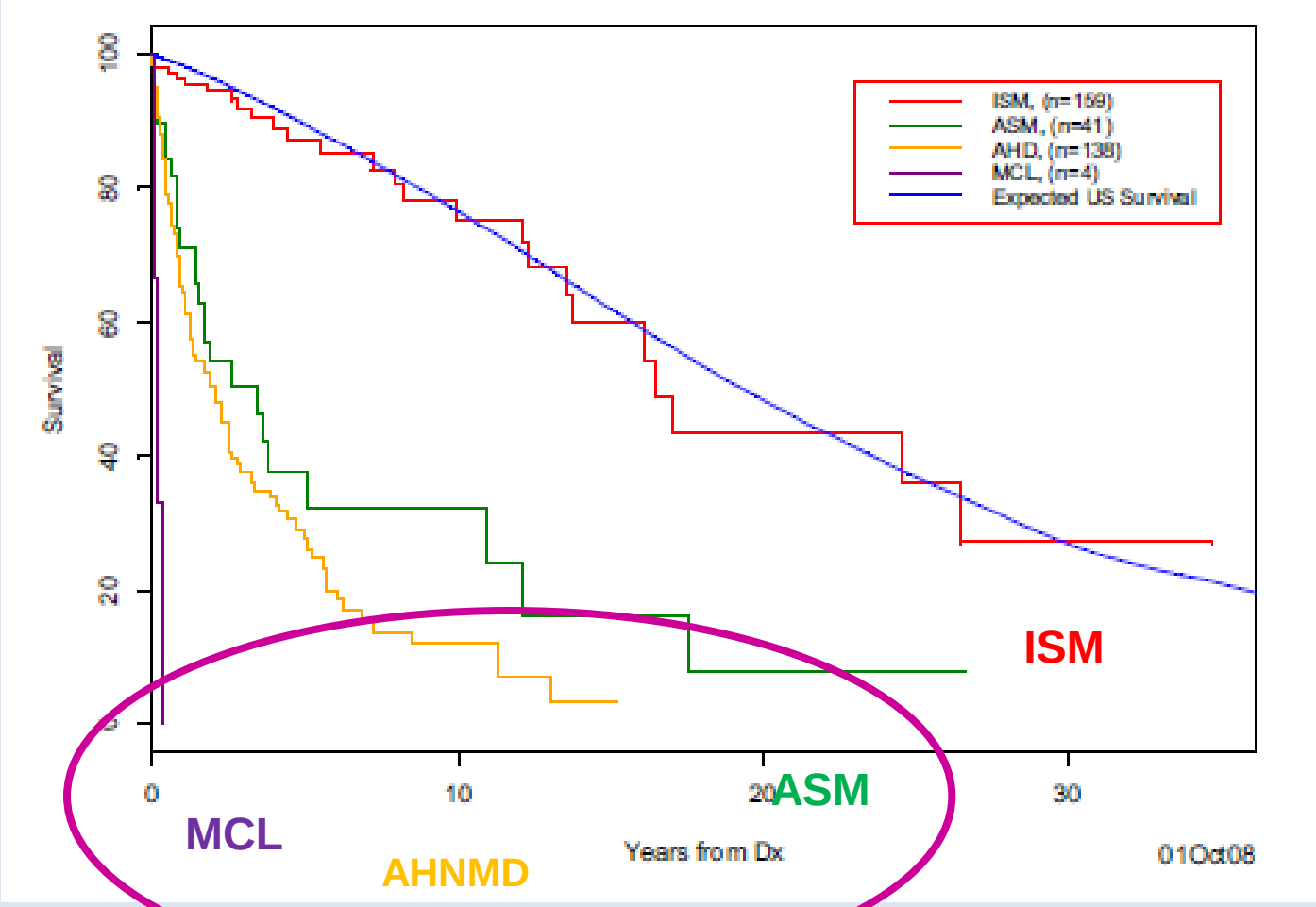
Median survival FU 20.7 months

ISM 198 months

ASM 41 months

AHNMD 24 months

MCL 2 months



Lim K-H, Tefferi A, Lasho T, et al. Systemic mastocytosis in 342 consecutive adults. Blood 2009;113:5727–5736.

MASTOCITOSI SISTEMICA: CRITERI DIAGNOSTICI WHO 2016

CRITERIO MAGGIORE:
Infiltrato mastocitario multifocale (>15 MC aggregati) in BO e/o organi extracutanei

CRITERI MINORI:
>25% dei MC immaturi o atipici
Mutazione KIT816V
MC esprimono CD25 e/o CD2
Triptasi sierica >20ng/mL

"B FINDINGS" (BURDEN)
>30% MC, Triptasi > 200ng/mL
displasia/mieloproliferazione
Epatomegalia, splenomegalia e/o linfadenopatia

"C FINDINGS" (cytoreduction)
BM disfuction
Epatomegalia
Lesioni osteolitiche
Splenomegalia e ipersplenismo
malassorbimento

The diagnosis of SM can be made when the major criterion and one minor criterion or at least three minor criteria are present

Major criterion

Multifocal, dense infiltrates of mast cells (≥15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organs

Minor criteria

a. In biopsy sections of bone marrow or other extracutaneous organs, >25% of the mast cells in the infiltrate are spindle-shaped or have atypical morphology or, of all mast cells in bone marrow aspirate smears, >25% are immature or atypical
b. Detection of an activating point mutation at codon 816 of *KIT* in bone marrow, blood or other extracutaneous organ
c. Mast cells in bone marrow, blood or other extracutaneous organ express CD25 with/without CD2 in addition to normal mast cell markers[a]
d. Serum total tryptase persistently exceeds 20 ng/mL (unless there is an associated myeloid neoplasm, in which case this parameter is not valid)

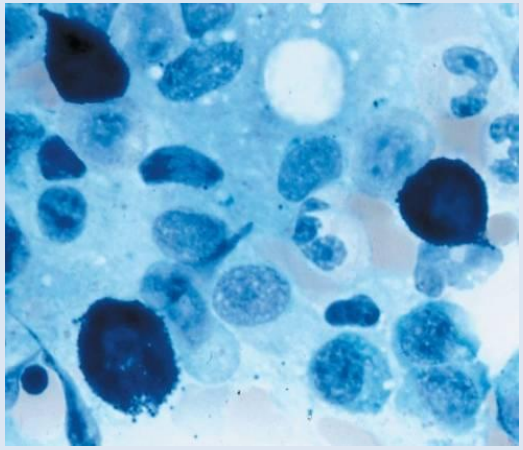
"B" findings

1. High mast cell burden shown on BM biopsy: >30% infiltration of cellularity by mast cells (focal, dense aggregates) and serum total tryptase level > 200 ng/mL
2. Signs of dysplasia or myeloproliferation, in non-mast cell lineage(s), but insufficient criteria for definitive diagnosis of an associated hematological neoplasm (AHN), with normal or only slightly abnormal blood counts.
3. Hepatomegaly without impairment of liver function, palpable splenomegaly without hypersplenism, and/or lymphadenopathy on palpation or imaging.

"C" findings

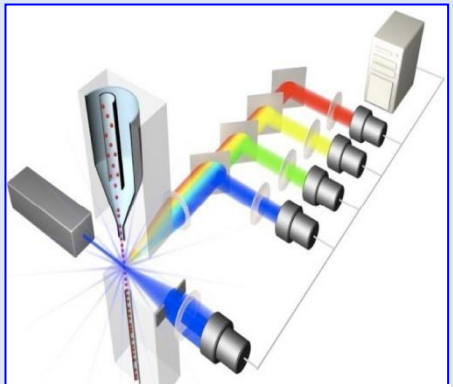
1. Bone marrow dysfunction caused by neoplastic mast cell infiltration, manifested by ≥1 cytopenia(s) (ANC <1.0 × 10^9/L, Hgb <10 g/dL, and/or platelet count <100 × 10^9/L).
2. Palpable hepatomegaly with impairment of liver function, ascites and/or portal hypertension.
3. Skeletal involvement with large osteolytic lesions with/without pathological fractures (pathological fractures caused by osteoporosis do not qualify as a "C" finding.
4. Palpable splenomegaly with hypersplenism.
5. Malabsorption with weight loss due to gastrointestinal mast cell infiltrates.

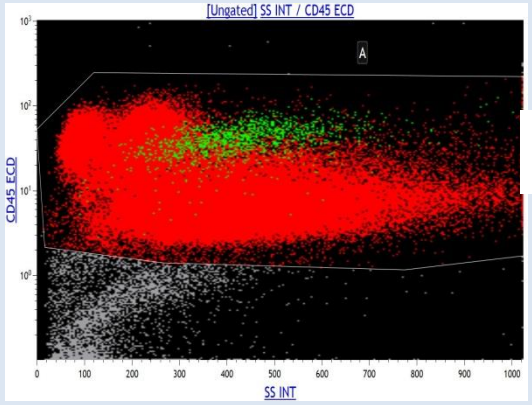
Pardanani A, Am J Hematol (2019)



Morphology

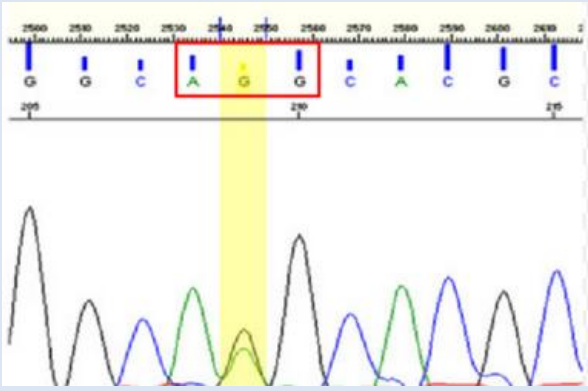
Full Blood Counts and Differential Counts
Serum Biomarkers
Cell morphology
Flow Cytometry
Histo/Immunohistopathology
Cytogenetics/FISH
Molecular Pathology





Flow Cytometry

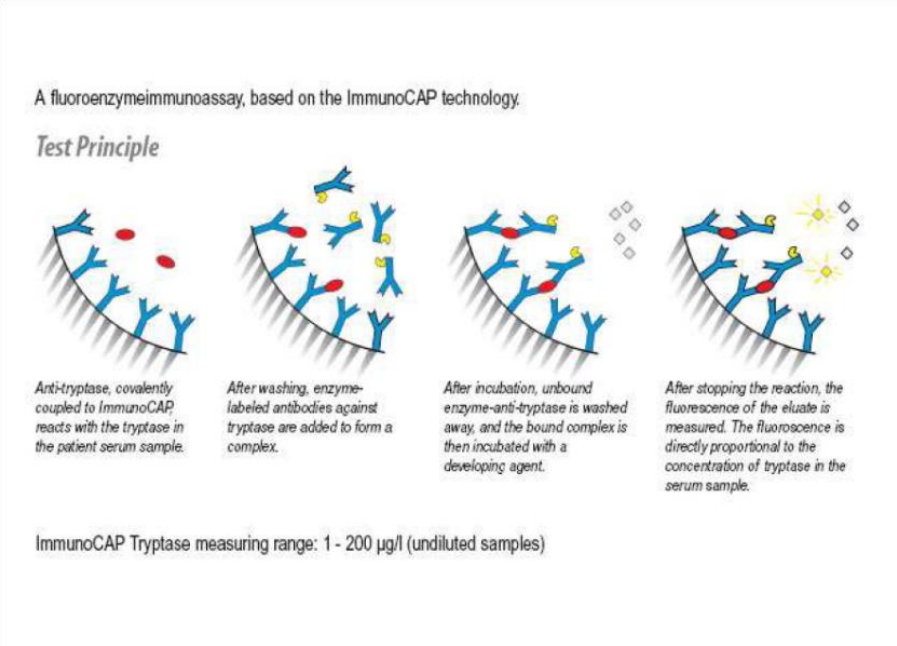
Tryptase



Molecular tests

TRIPTASI

- Serina proteasi
- Nell'uomo: α, β, δ, ε, γ- tryptase
- α and β triptasi espresse e secrete dai MC
- Aumenta nel siero in seguito a degranulazione MC



Triptasi totale nel siero (α + β) indicatore dell'attivazione mastocitaria

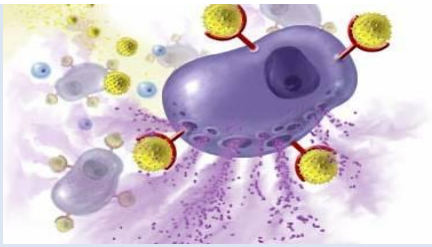
- ❖ Test fluoroimmunoenzimatico
- ❖ Campione stabile dopo centrifugazione
- ❖ Test sensibile e specifico

blood

2009 113: 5727-5736
Prepublished online April 10, 2009;
doi:10.1182/blood-2009-02-205237

Systemic mastocytosis in 342 consecutive adults: survival studies and prognostic factors

Ken-Hong Lim, Ayalew Tefferi, Terra L. Lasho, Christy Finke, Mrinal Patnaik, Joseph H. Butterfield, Rebecca F. McClure, Chin-Yang Li and Animesh Pardanani

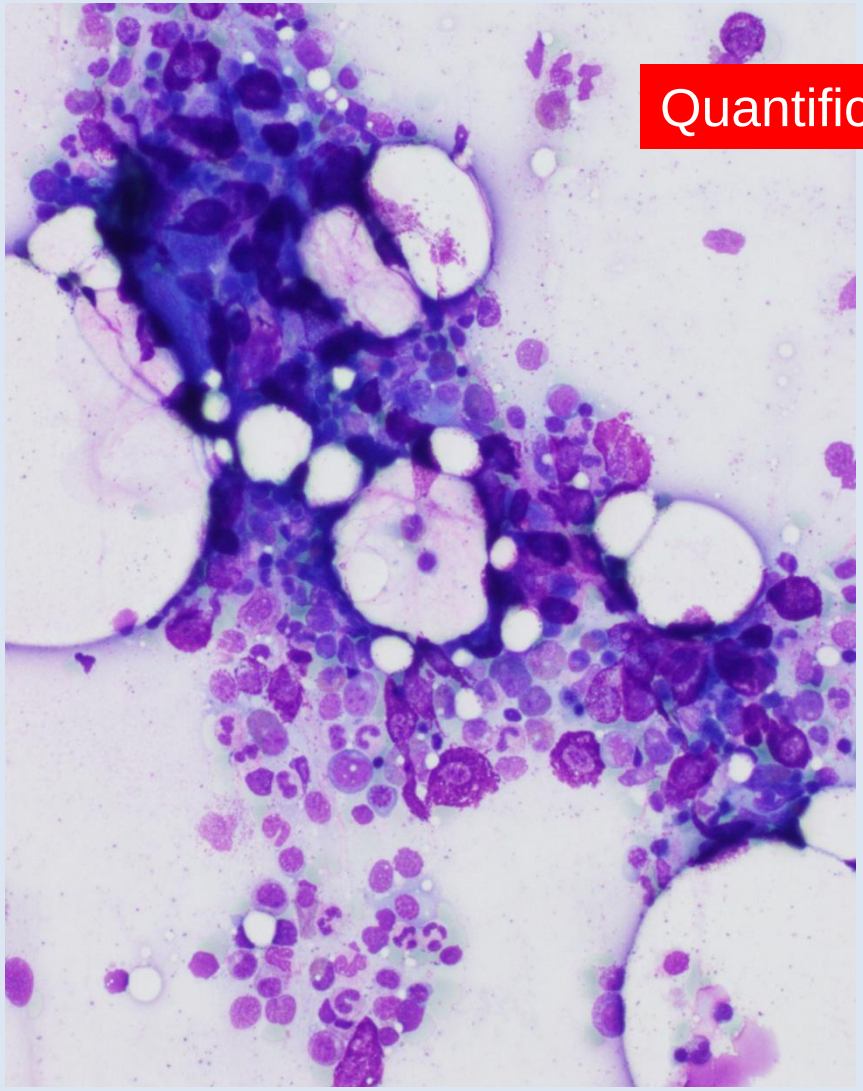


Characteristic	No. (%) of patients	Median (range)	ISM	ASM	SM-AHNMD	*P* for ISM vs ASM	*P* for ASM vs AHNMD	*P* for ISM vs AHNMD	Overall *P*
Serum tryptase, < 11.5 ng/mL	160 (47)	63.6 (3.7-2000)	53.6 (11.4-1410)	145 (10-2000)	75.4 (3.7-1360)	.02	NS	NS	.03
Tryptase 11.5 or more	154 (96)	66.6 (11.9-2000)	89 (99)	14 (93)	49 (92)	NS	NS	NS	.08
Tryptase 200 or more	33 (21)	303 (200-2000)	11 (12)	6 (40)	15 (28)	.007	NS	.02	.009

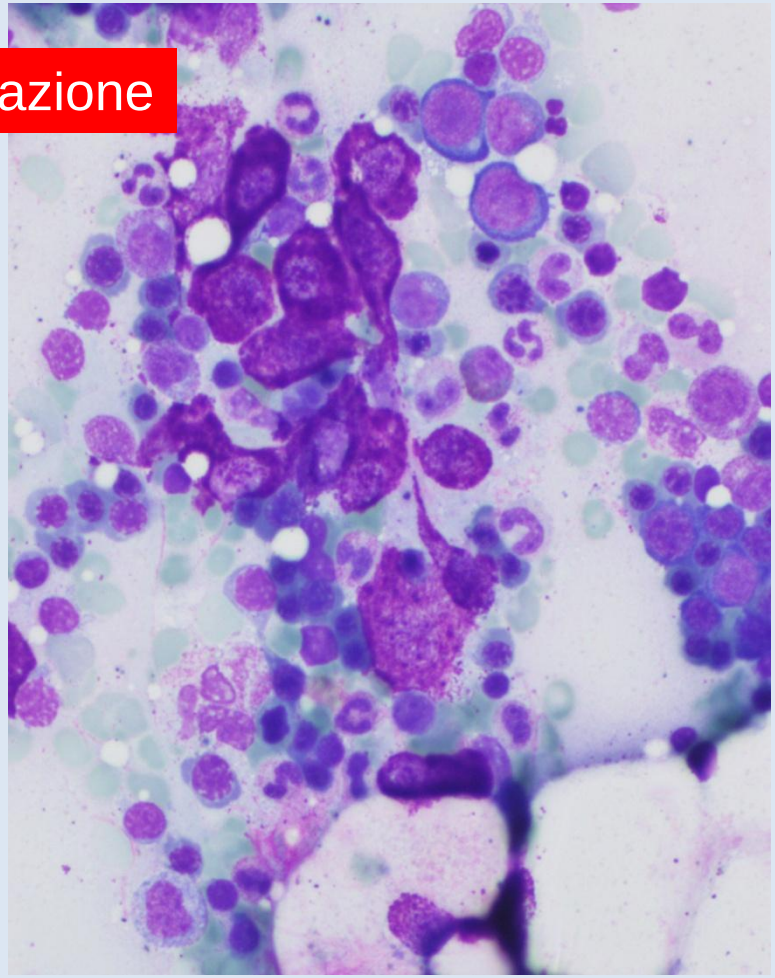
- serum triptase (normal <11.5 ng/mL) was measured in 47% of patients
- 96% with elevated level (median 66 ng/mL)
- greater proportion of ASM and SM-AHN had triptase level ≥200 ng/mL compared with ISM

Caratterizzazione morfologica della malattia

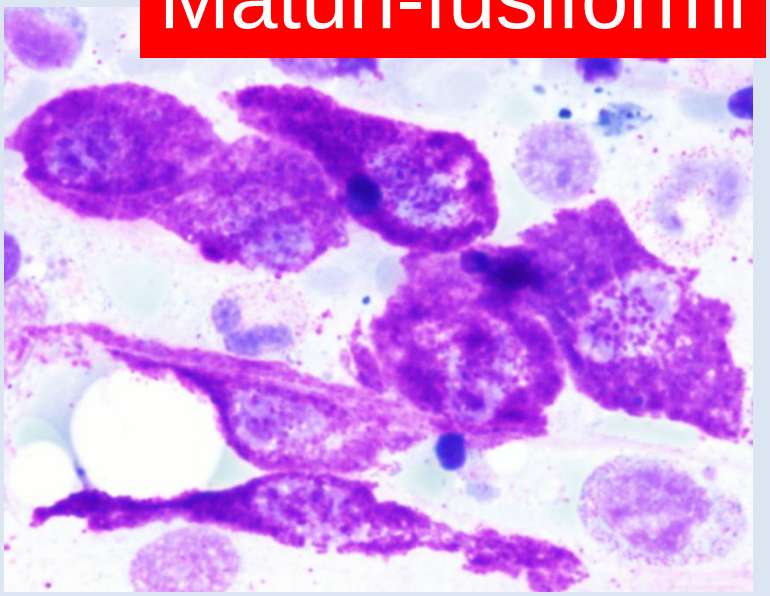
1. Quantificazione infiltrato
2. Quantificazione e descrizione delle atipie morfologiche
3. Esclusione di altre neoplasie ematologiche



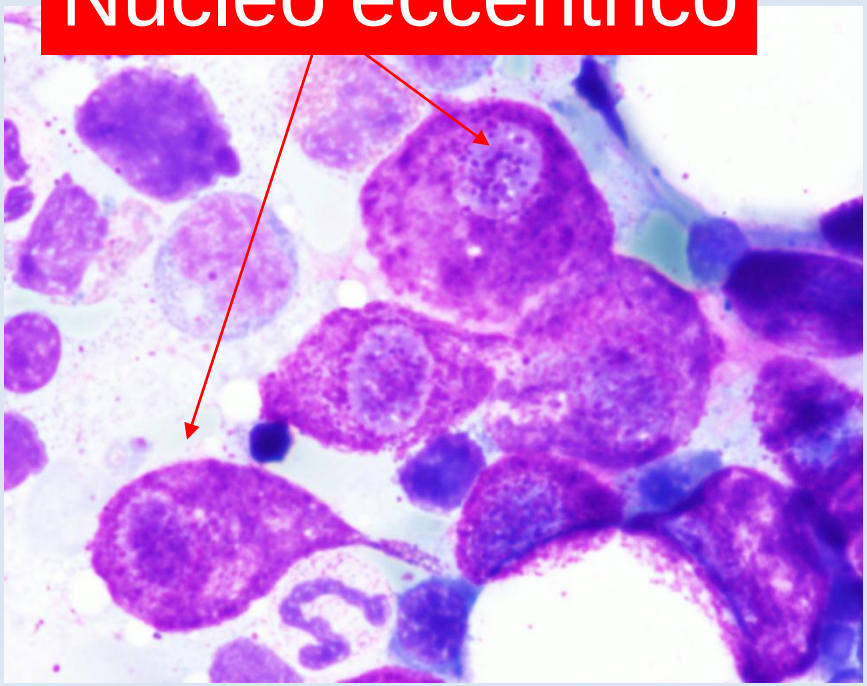
Quantificazione



Maturi-fusiformi



Nucleo eccentrico

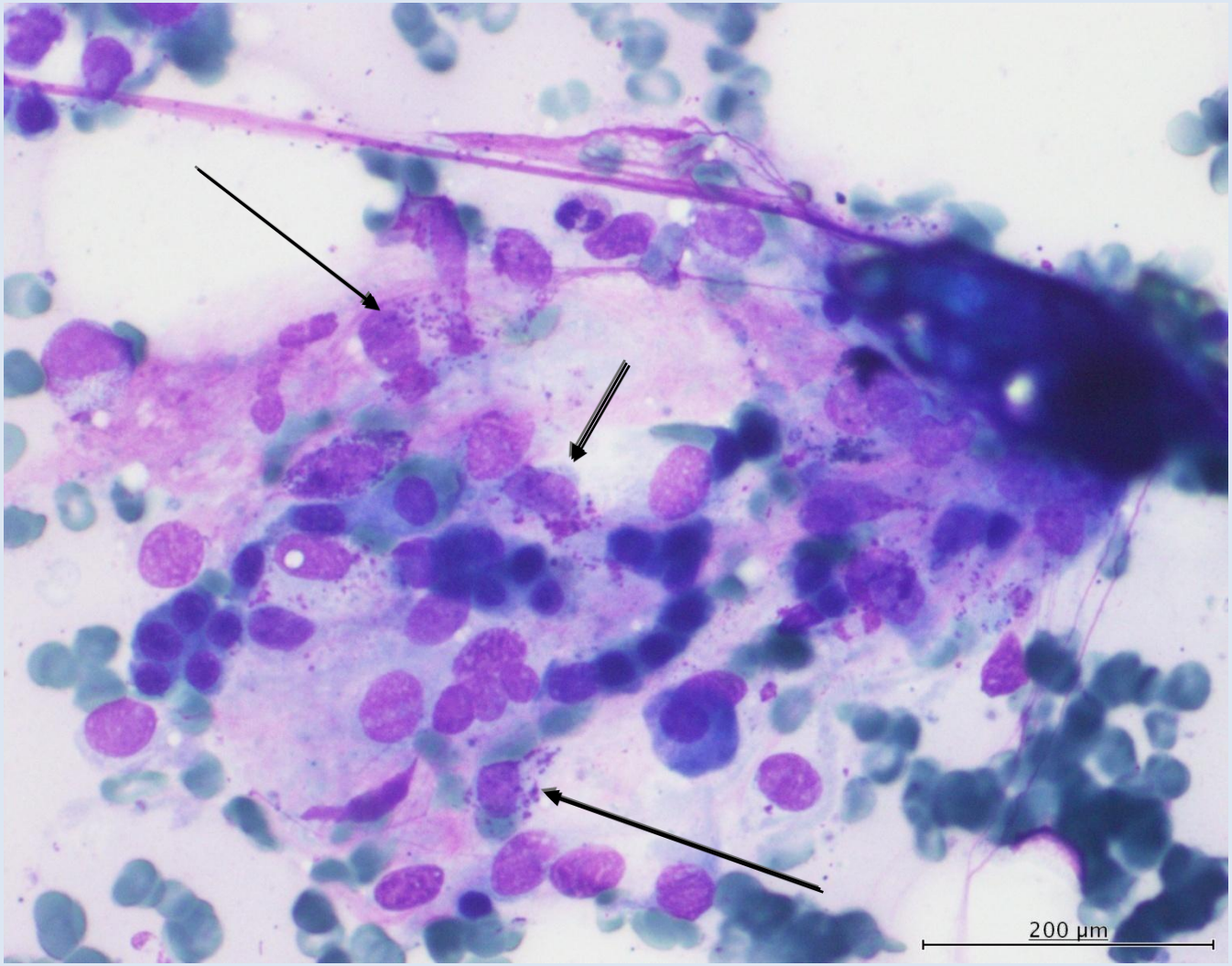


Minor criteria (SM):

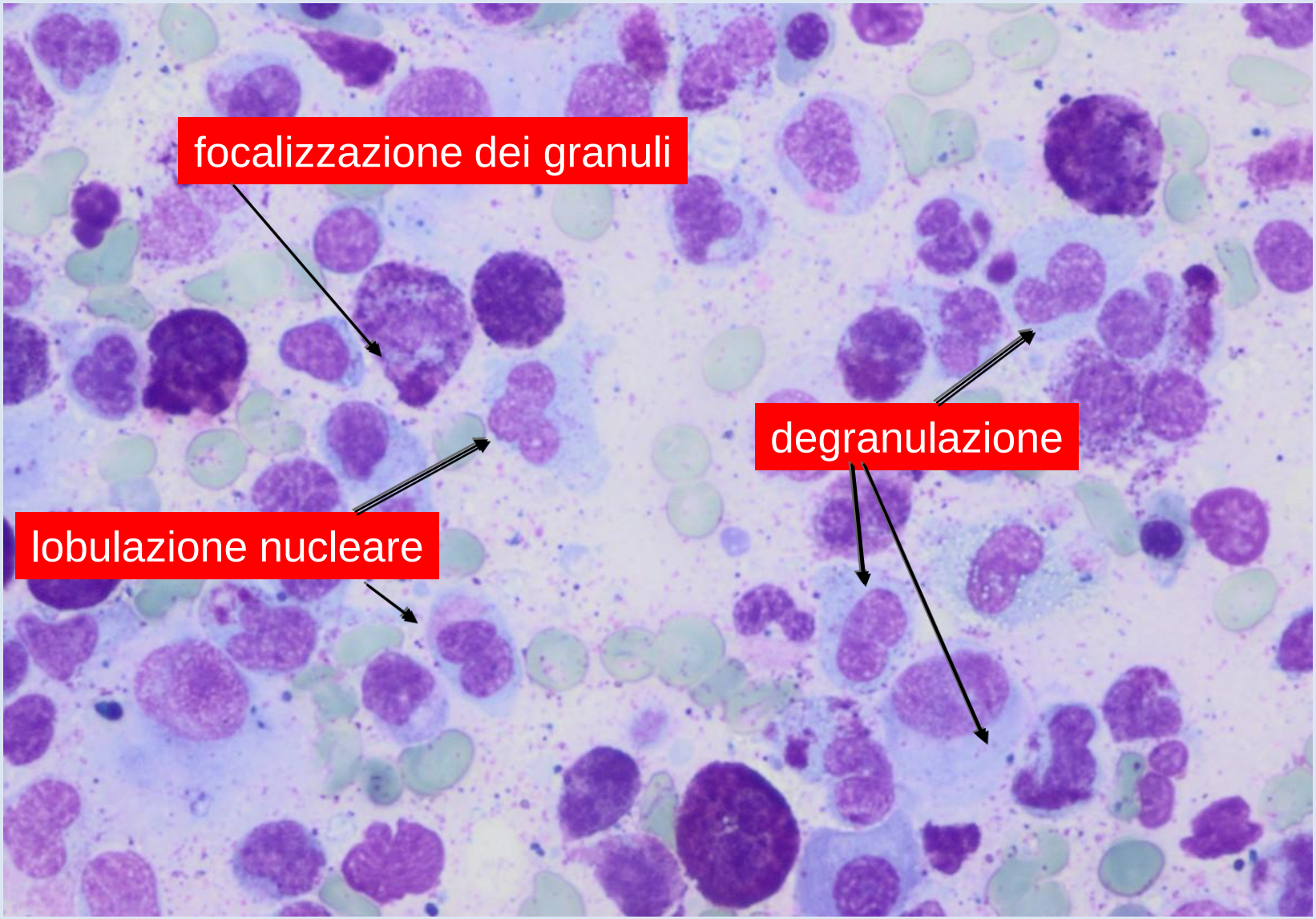
•> 25% of the MCs in the infiltrate are spindle-shaped or atypical

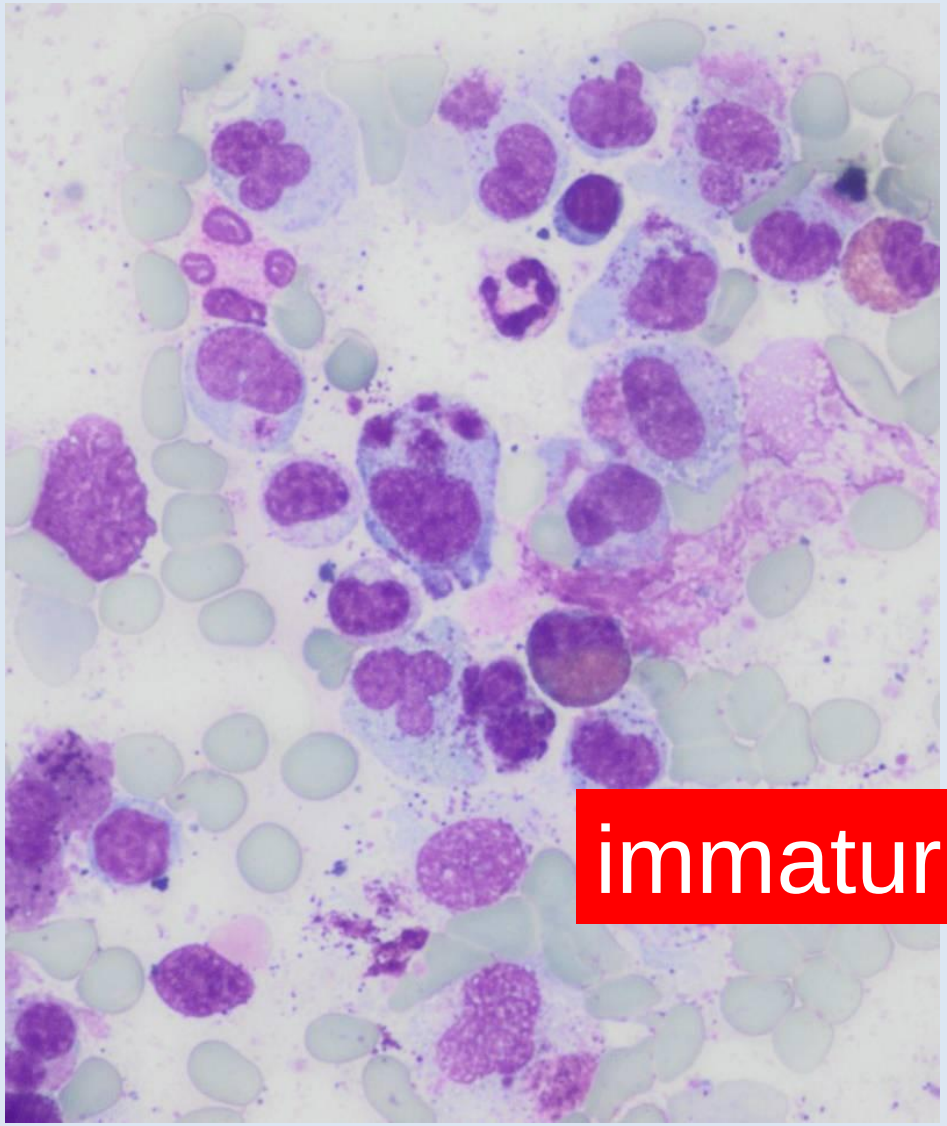
OR

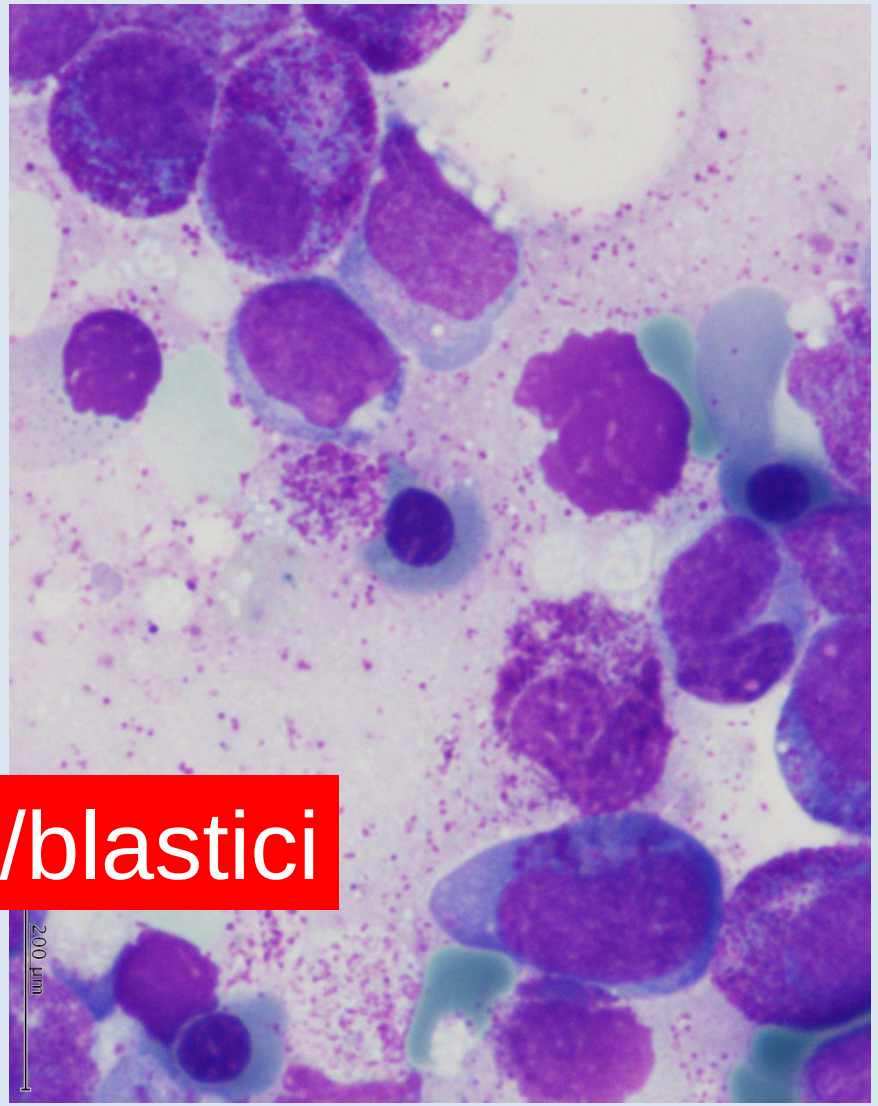
•> 25% of all MCs are immature or atypical



Maturi-ipogranulati


focalizzazione dei granuli
degranulazione
lobulazione nucleare





immaturi/blastici

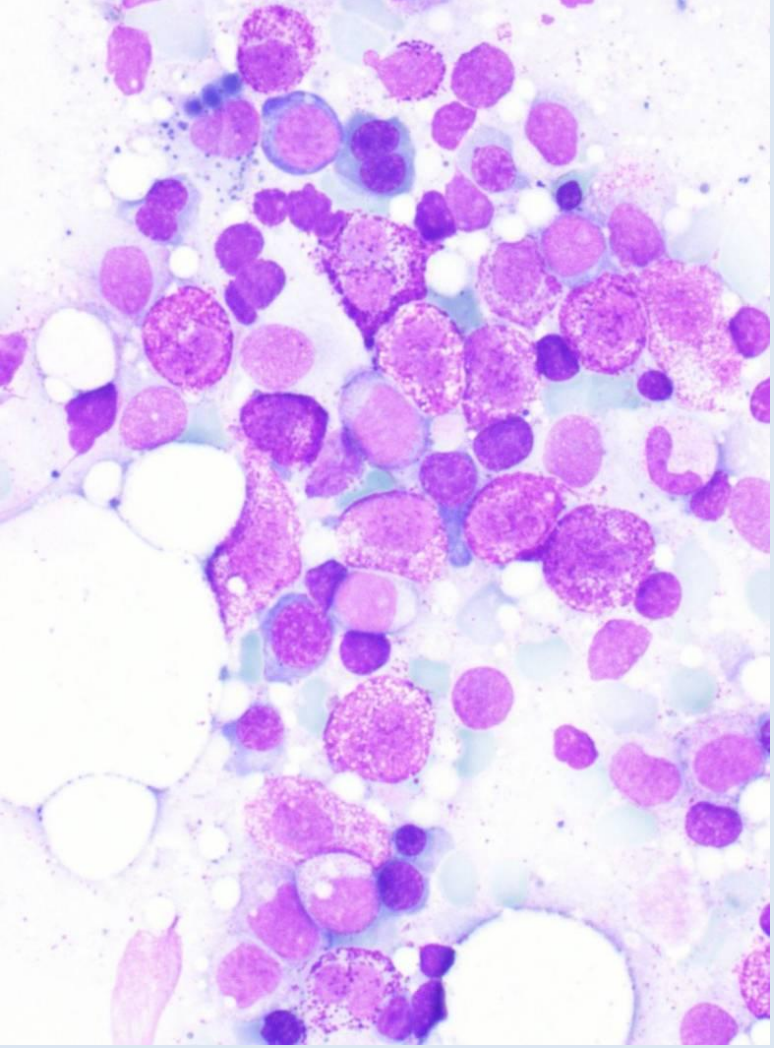
midollo

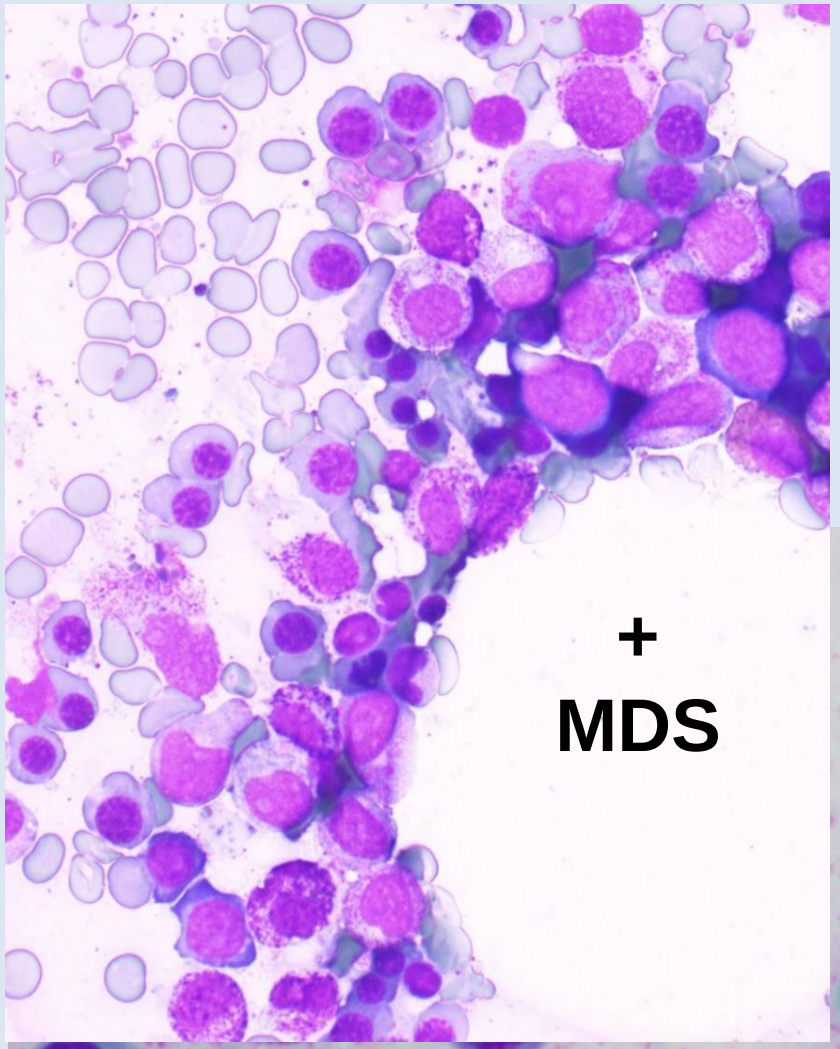
CB >/= 20%

Mast Cell Leukemia

sangue periferico

CB >/= 10%





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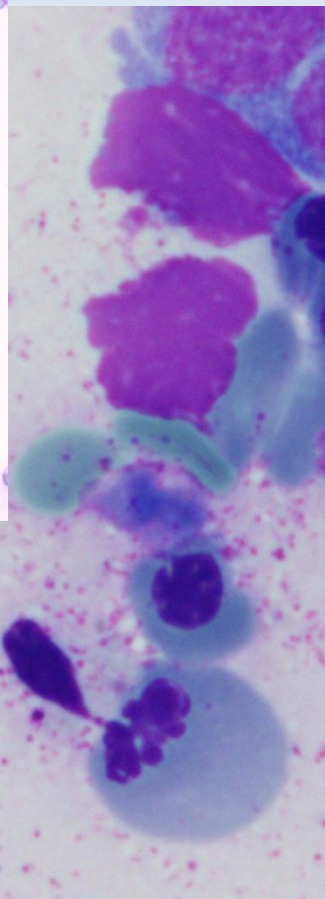
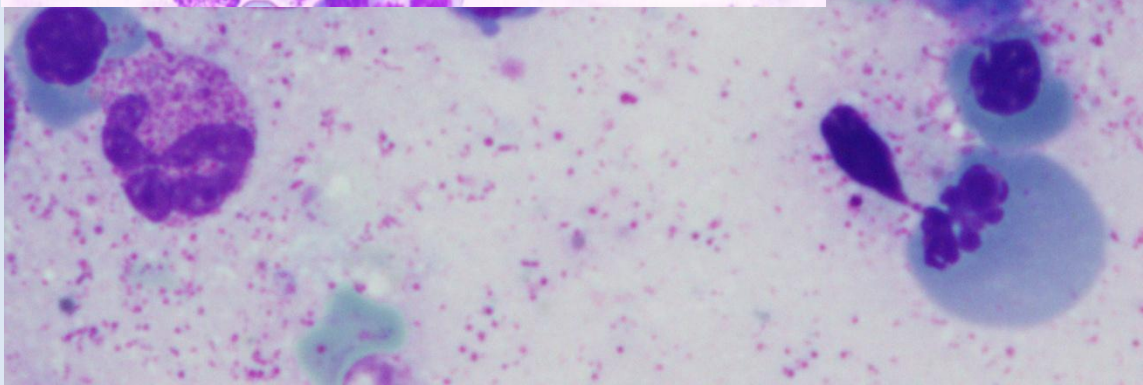
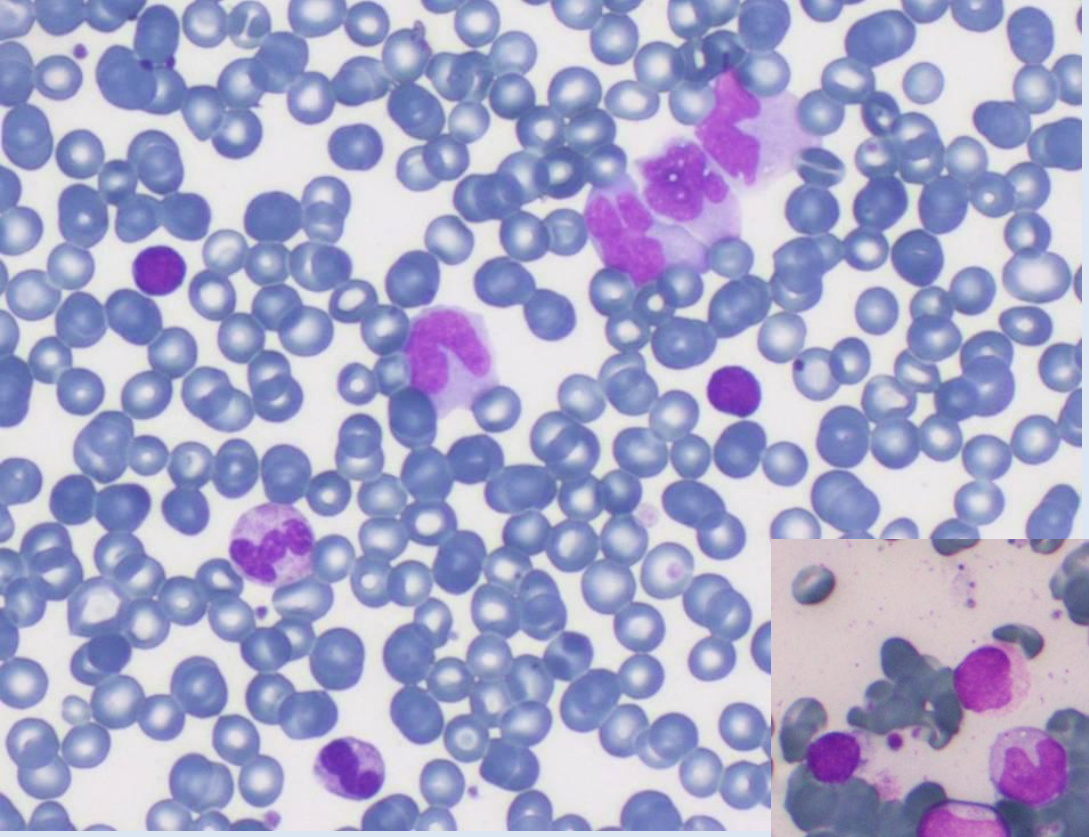
MDS







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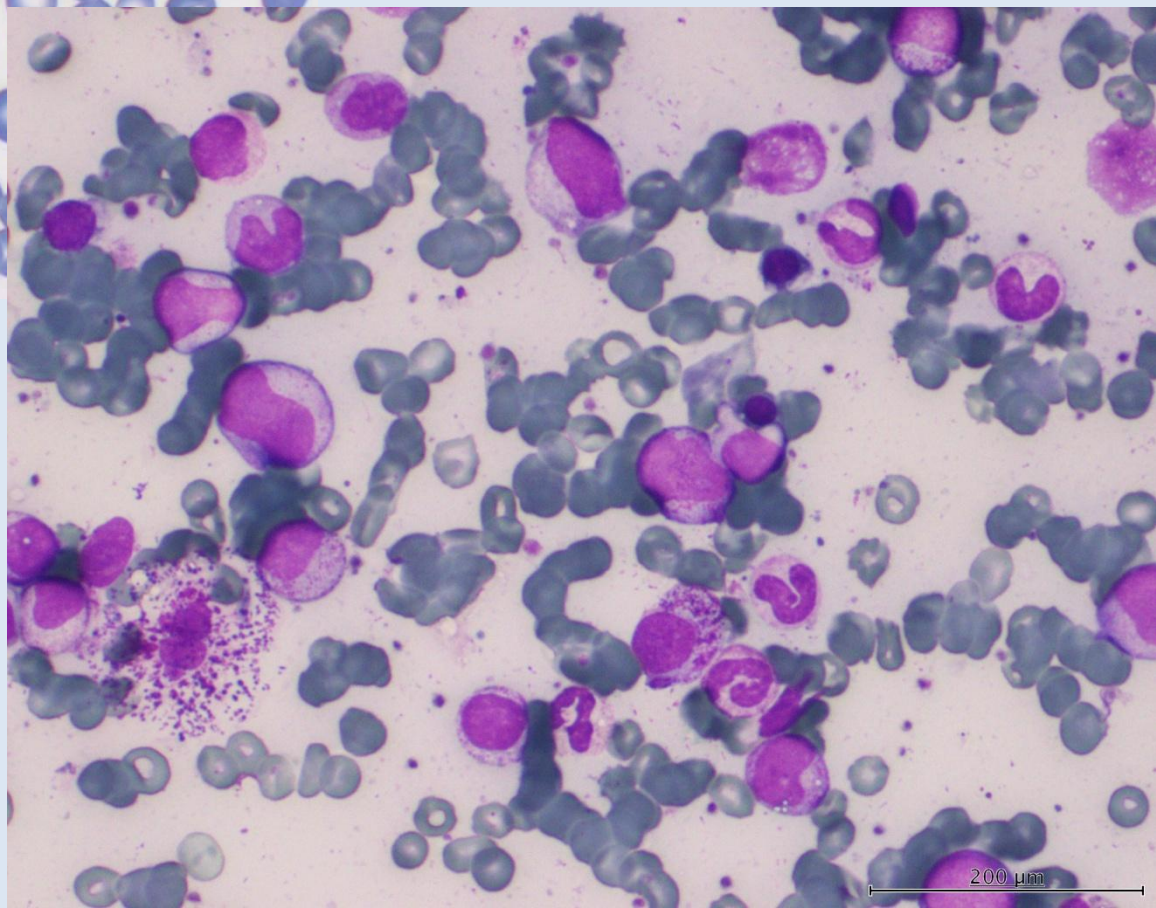
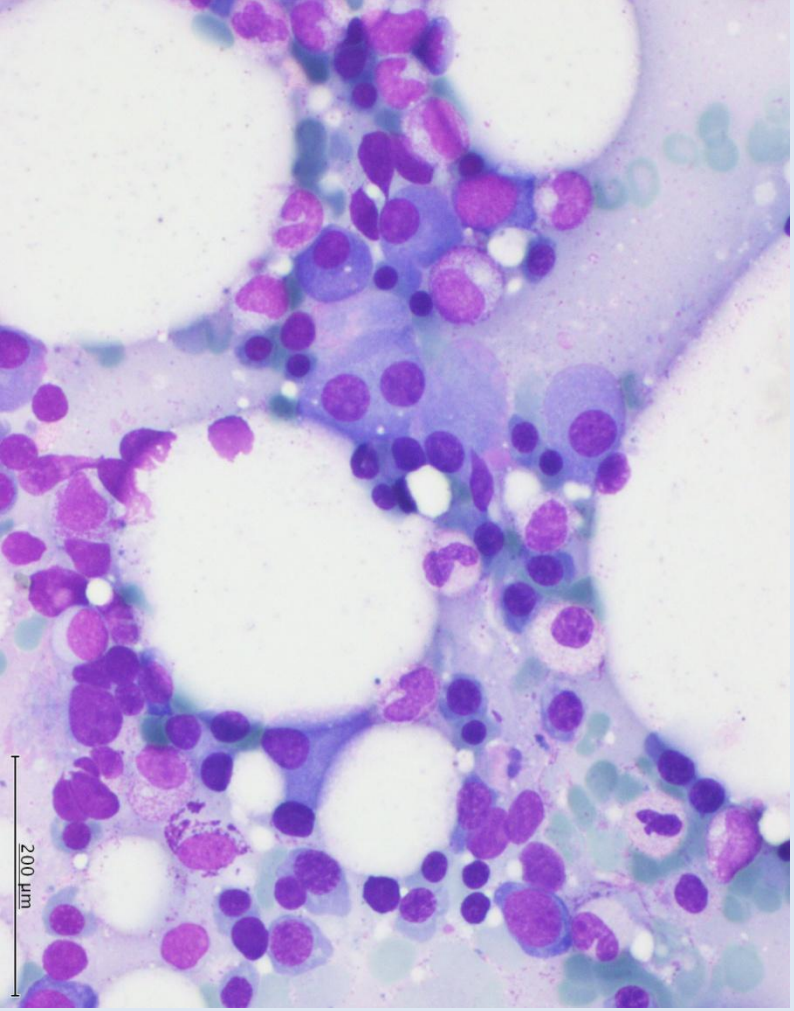
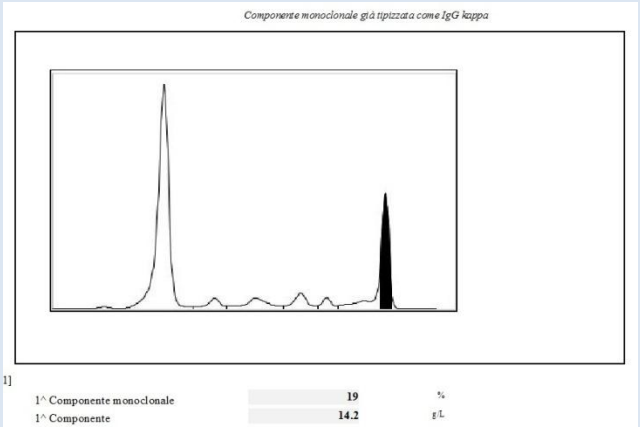
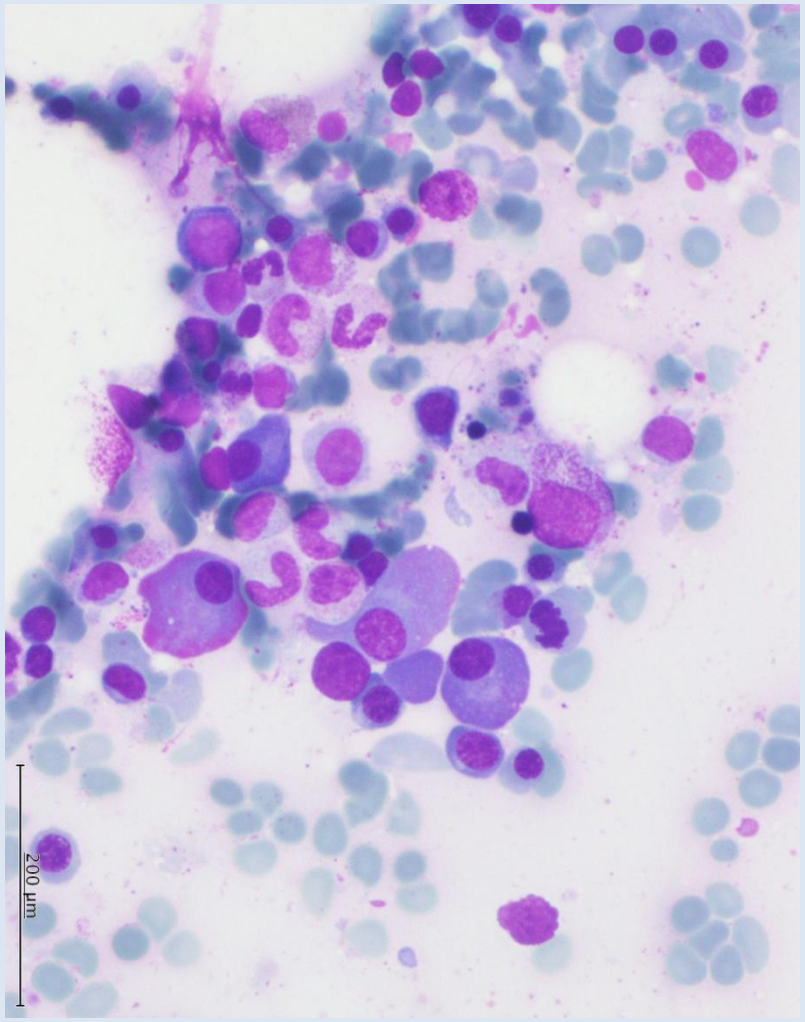
CMML



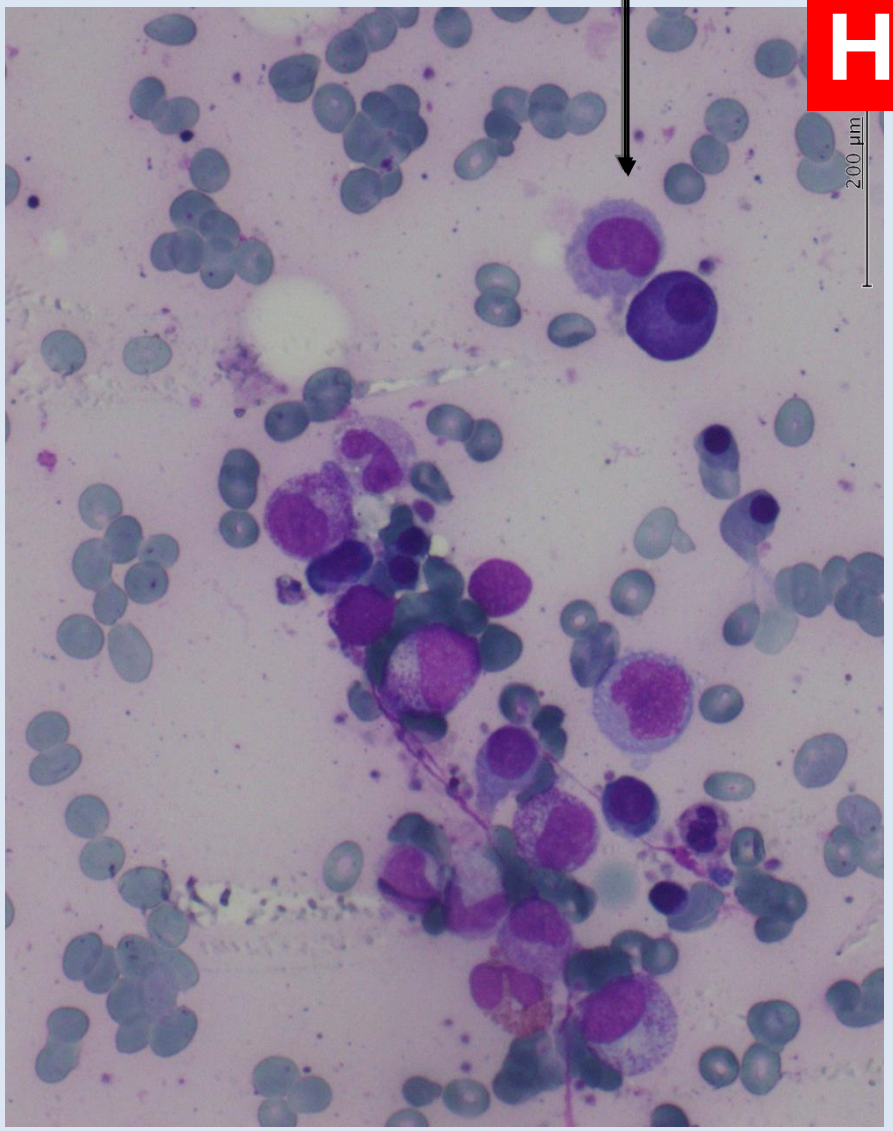
Plasmacellule

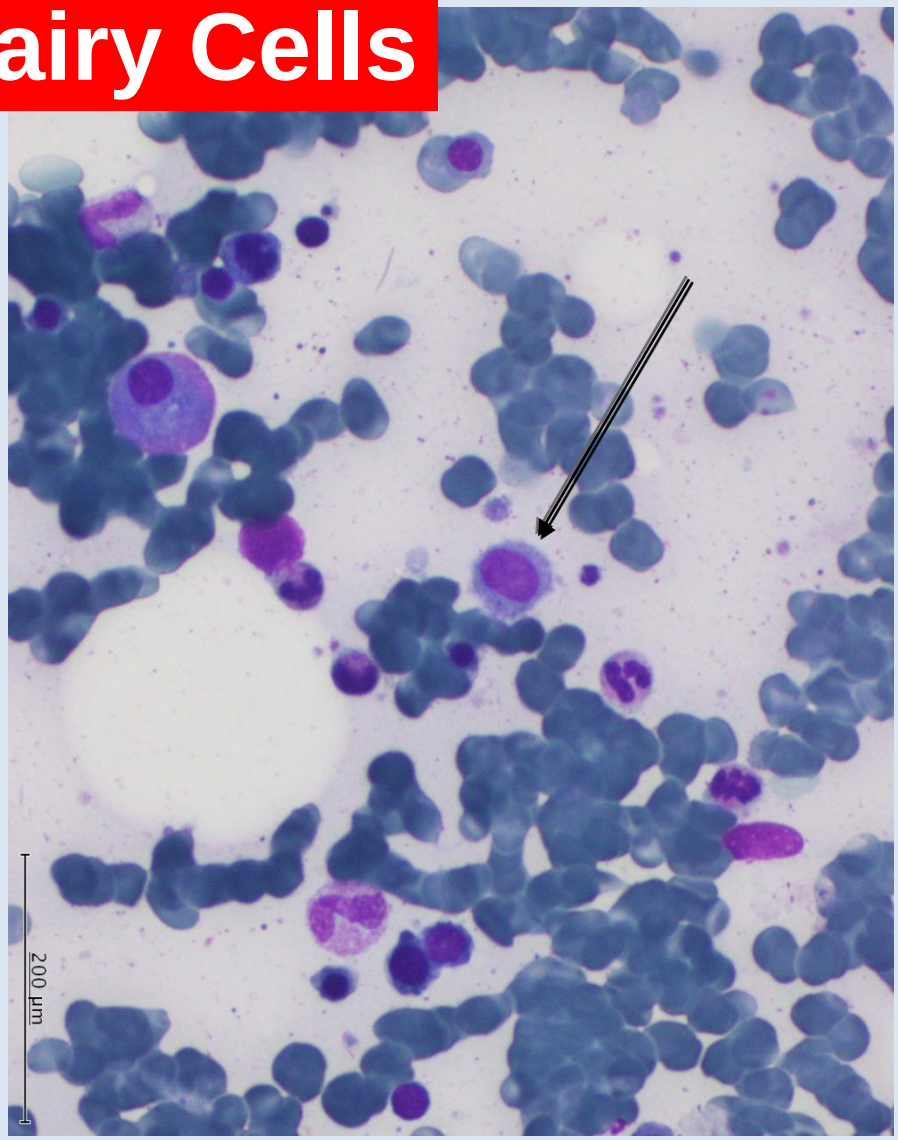




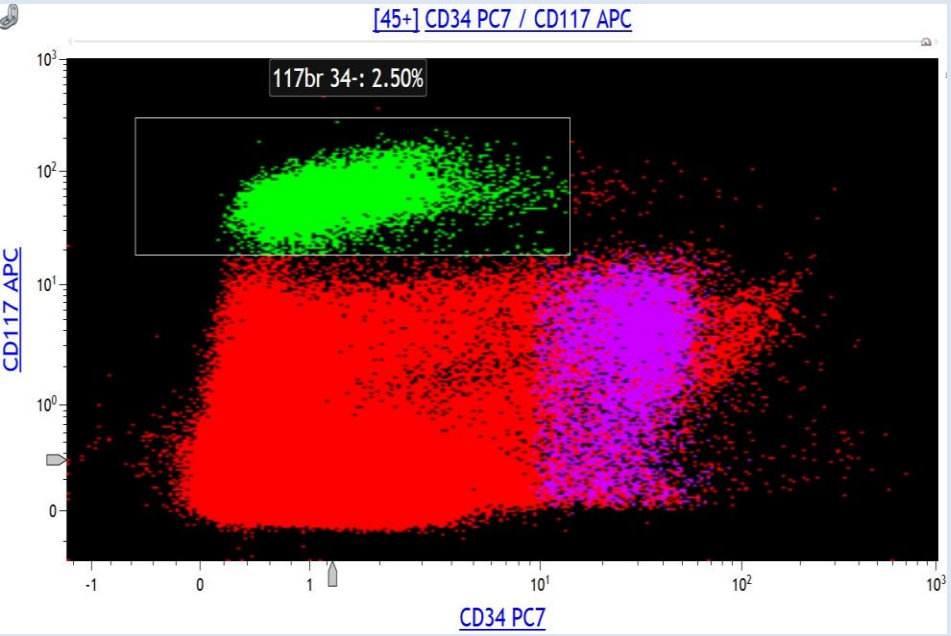


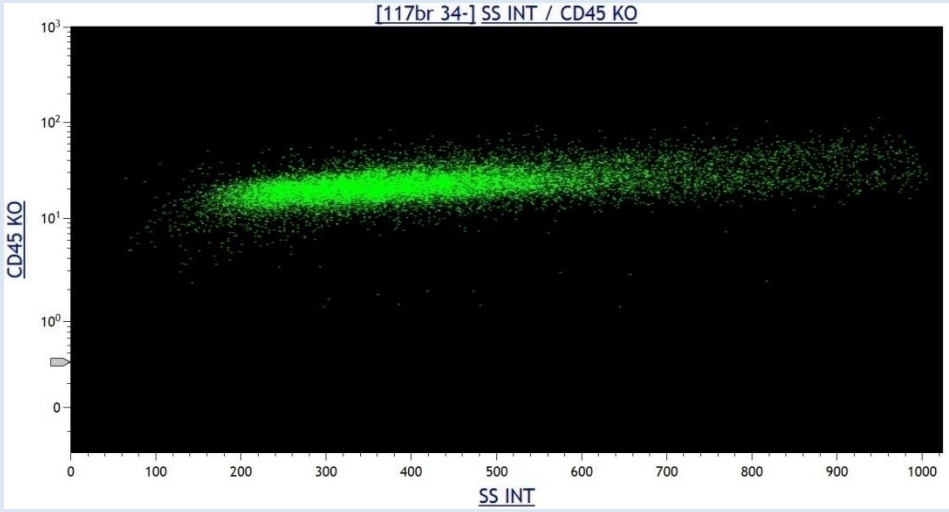
Hairy Cells



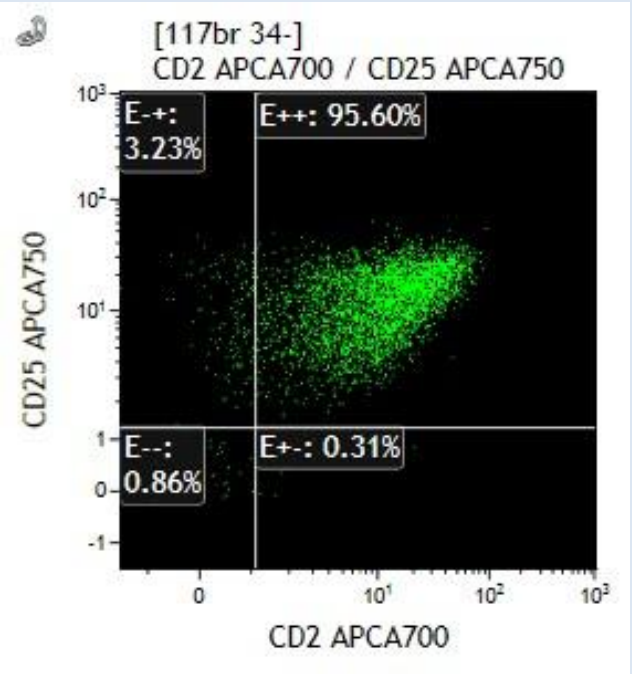


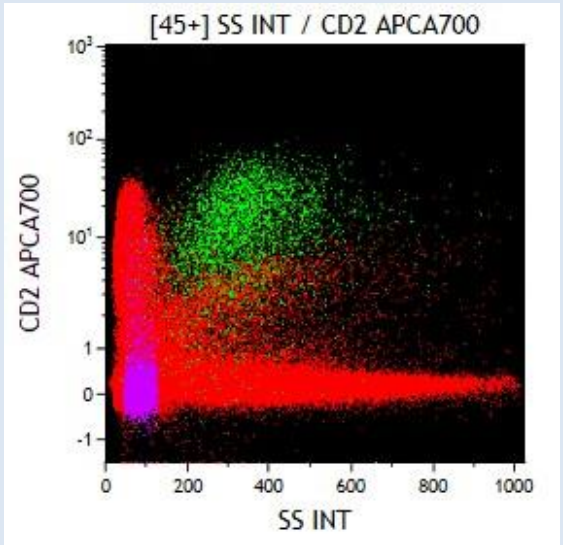
ANALISI CITOFLUORIMETRICA

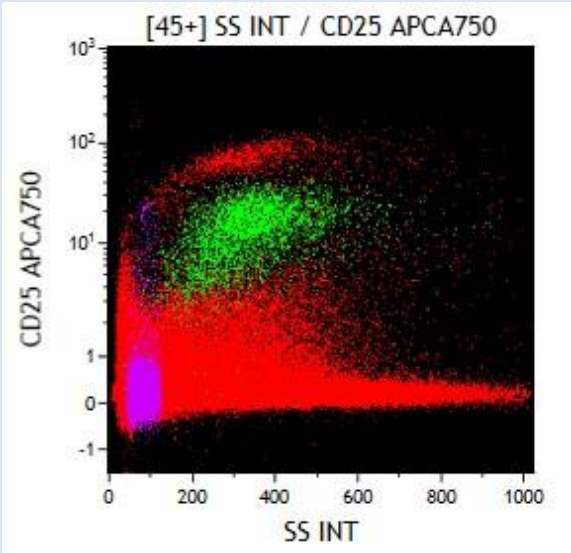


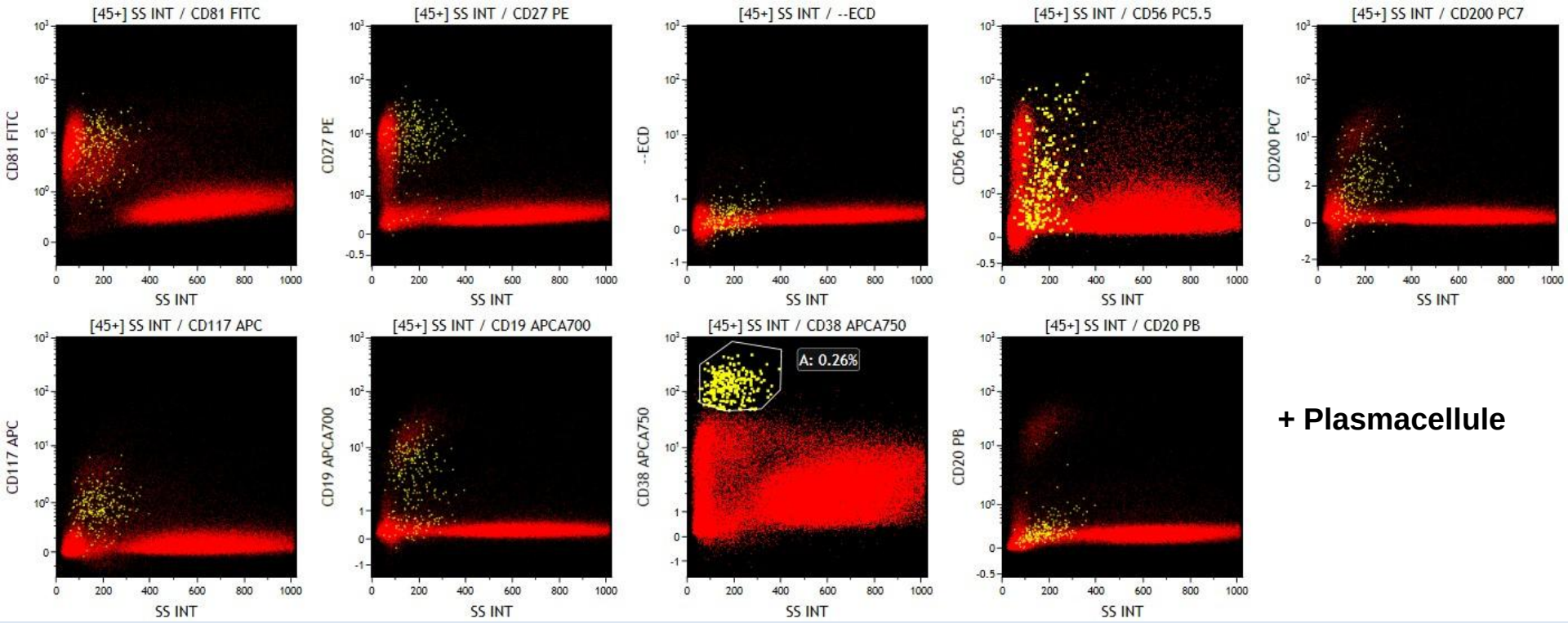


CD45+bright
CD117+bright
CD34-
CD25+ CD2+

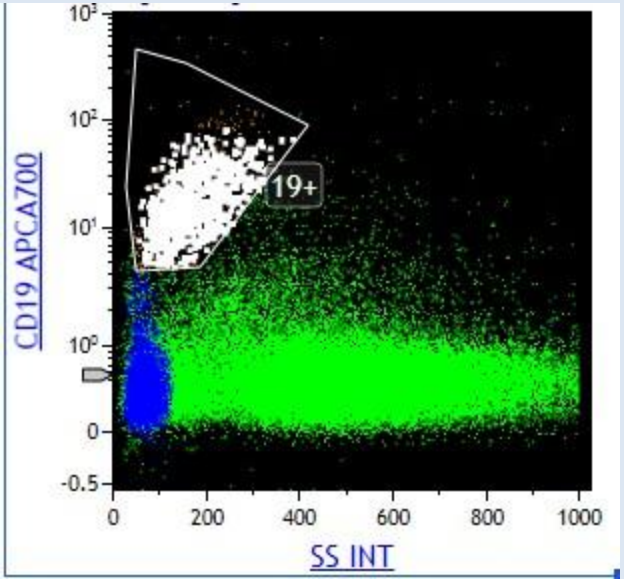


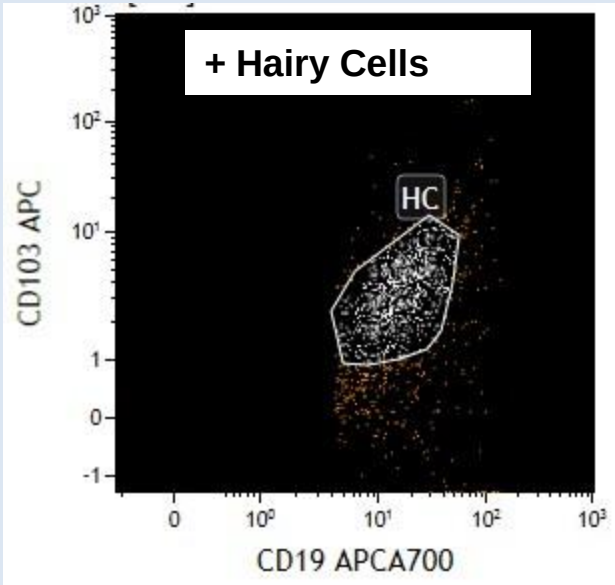


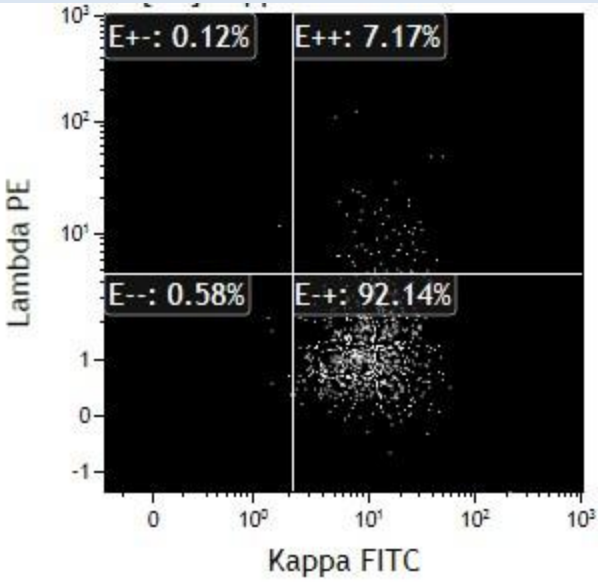




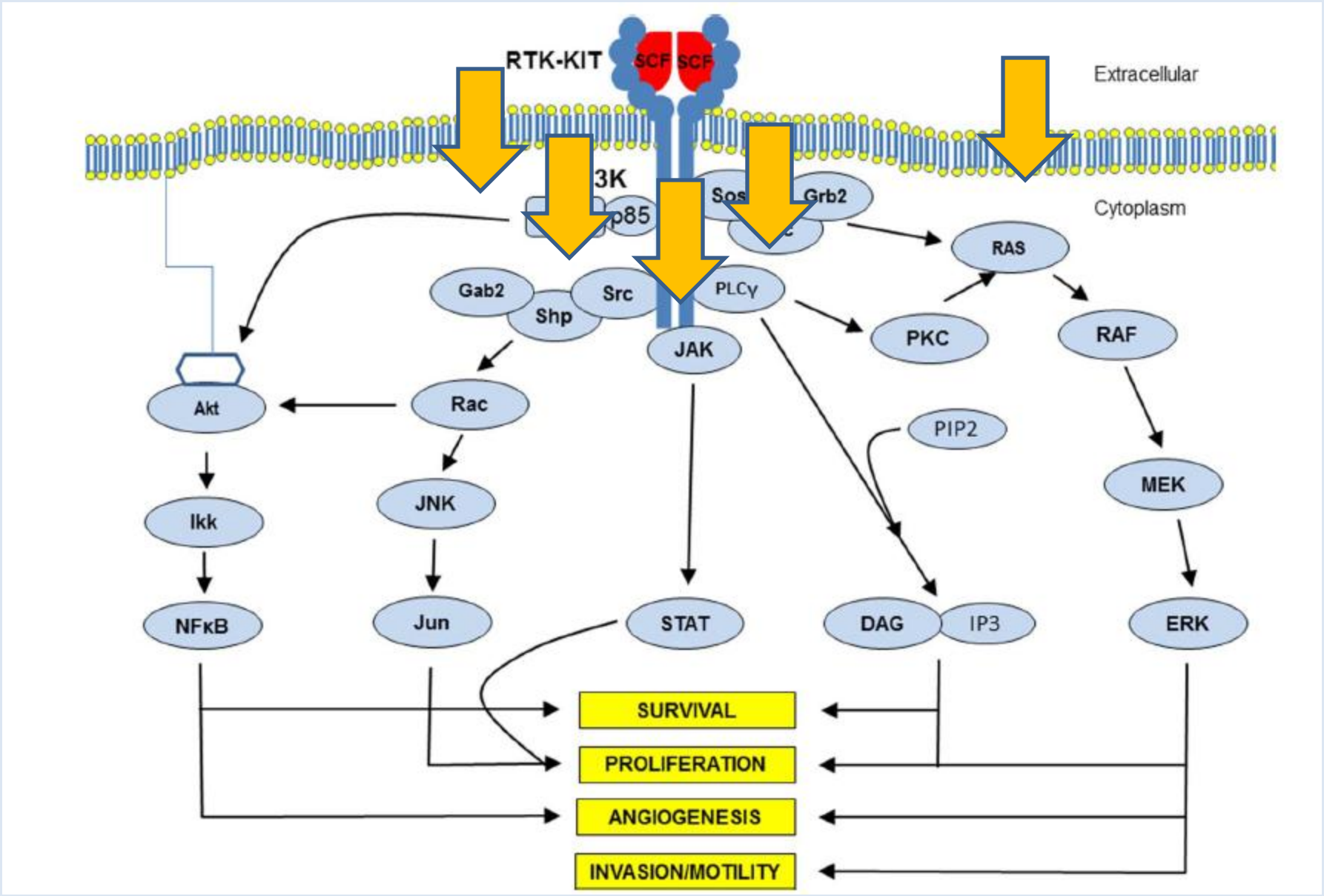
+ Plasmacellule



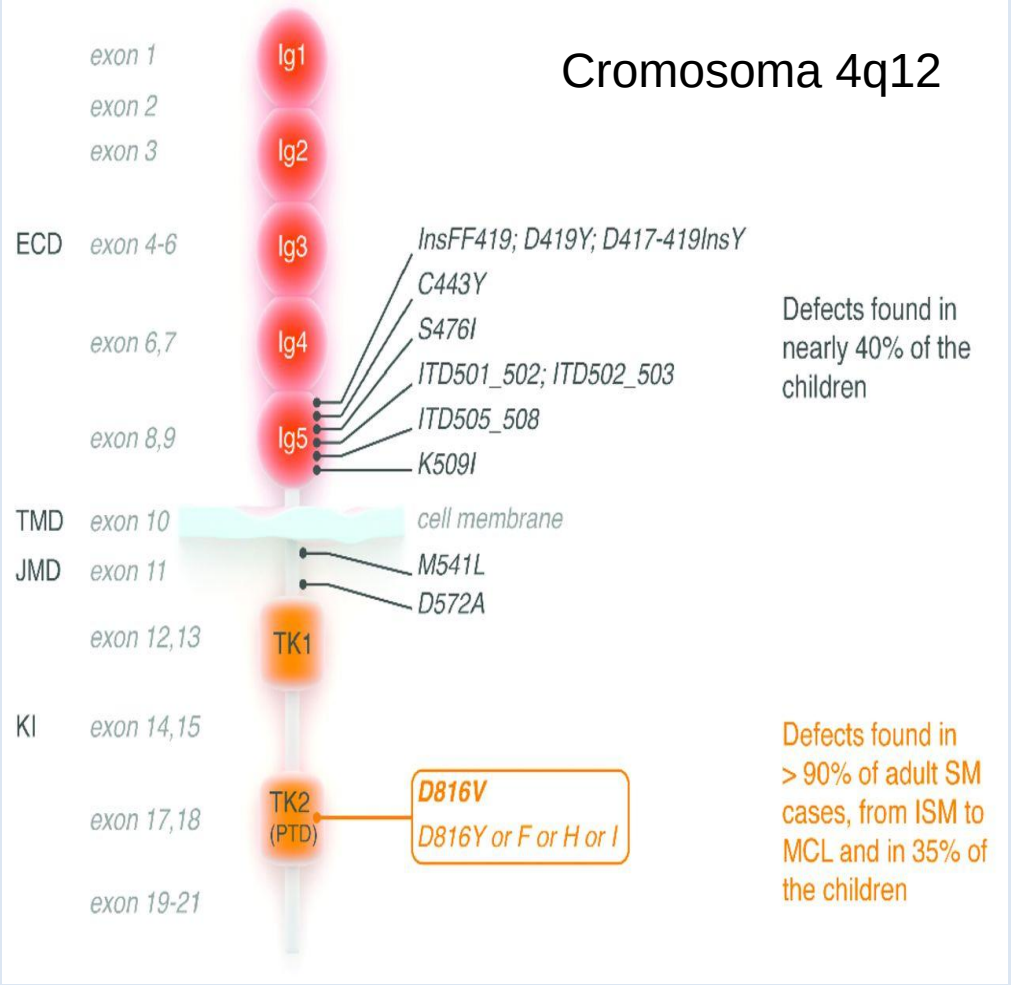




KIT (CD117)



Roberts et al. J Clin Pathol (2015)



- Gain of function mutations in the KIT receptor tyrosine kinase (most frequently, D816V) are detectable in the great majority of patients with SM and are thought to play a key pathogenetic role
- KIT816V confers resistance against Imatinib, Masitinib, other Kit-targeting drugs

- several lines of evidence suggest that KIT D816V may not be a fully transforming oncoprotein

  > clinical courses of ISM vs ASM and MCL vary greatly

  > KIT D816V transgenic mouse models develop ISM rather than ASM or MCL

  (Zappulla, J Exp Med 2005)

Bibi et al. Haematologica (2014)

Molecular somatic lesions in SM

Molecular Abnormality	Reported in patients with	Estimated frequency in patients with SM	reference #
KIT D816V	all SM variants, rarely in CM	>80%	37, 38, 42
KIT D816Y	CM, ISM, SM-AHNMD	<5%	10, 47, 48
KIT D816F	CM	<5%	10, 47, 48
KIT D816H	MCL, ASM, SM-AHNMD	<5%	10, 47
KIT D820G	ASM	<5%	10, 46
KIT V560G	ISM	<5%	10, 46
KIT F522C	ISM	<5%	10, 47
KIT E839K	CM	<5%	10, 47, 48
KIT V530I	SM-AHNMD	<5%	10, 47
KIT K509I	SM (familial type)	<5%	10, 47, 48
Other *KIT* mutations	CM and/or SM variants	<5%	10, 47, 48
FIP1L1/PDGFRA	SM-CEL	<5%	58, 59, 60
AML1/ETO	SM-AML with t(8;21)	<5%	53
JAK2 V617F	SM-PMF	<5%	95
RAS mutations	ASM, SM-AHNMD	<5%	51
TET2 mutations	SM-AHNMD, ISM, ASM	<5%	49, 50
DNMT3A mutations	ISM, SM-AHNMD	<5%	50
ASXL1 mutations	SM-AHNMD	<5%	50
CBL mutations	SM-AHNMD	<5%	50

CM, cutaneous mastocytosis; SM, systemic mastocytosis; SM-AHNMD, SM with an associated haematologic clonal non mast cell lineage disease; CEL, chronic eosinophilic leukemia; AML, acute myeloid leukemia; PMF, primary myelofibrosis.

Am J Cancer Res 2013;3(2):159-172

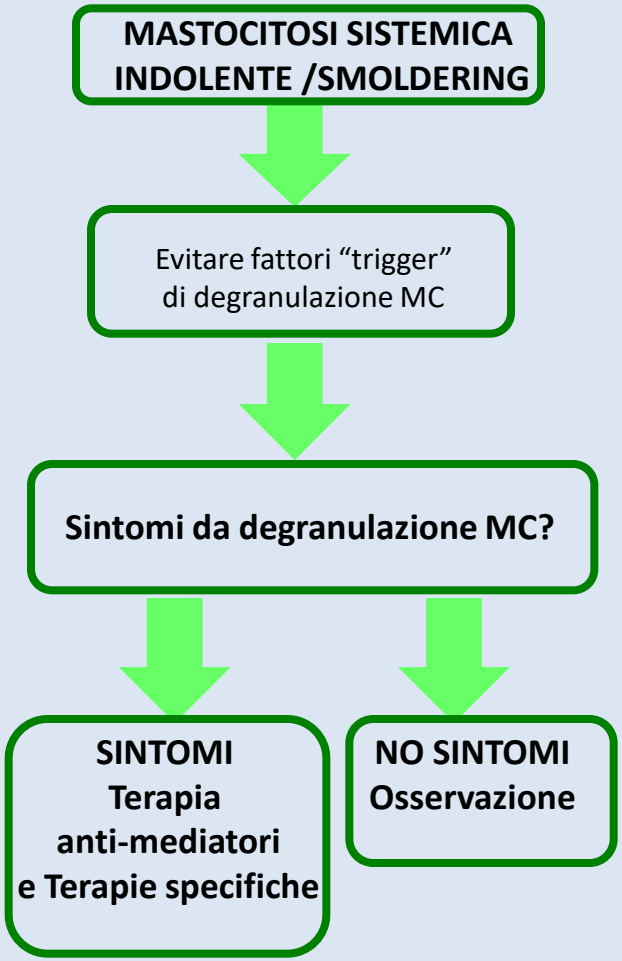
Indagine molecolare KITD816V: tecniche a confronto

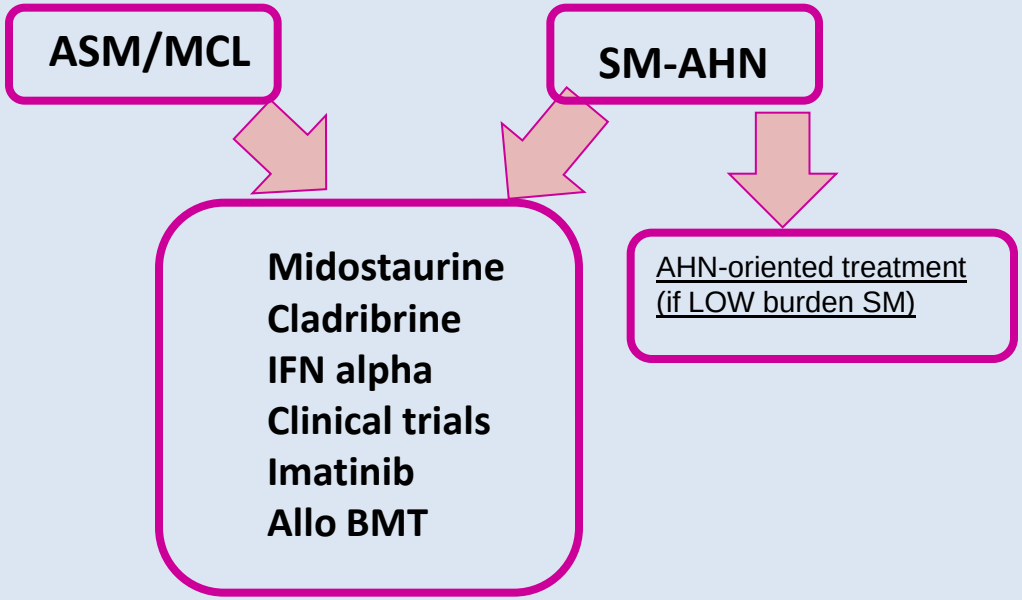
Technique	RT-PCR plus restriction fragment length polymorphism (RFLP)	Nested RT-PCR followed by D-HPLC of PCR amplicons	PNA-mediated PCR	ASO-qPCR on DNA or RNA/cDNA
Advantages	-simple -fast -reliable -cost-saving	-detects different *KIT* mutations at position 816	-allows detection of *KIT* mutations at position 816 or at adjacent positions -recommended for formalin-fixed paraffin-embedded tissues	-simple -fast -cost-saving -highly sensitive test -quantitative: allows the quantification of the *KIT* D816V allele burden in blood or BM at diagnosis or in the follow up
Disadvantages	-detects only *KIT* D816V mutant -not quantitative	-relatively low sensitivity -not quantitative -time-consuming -needs special facilities (HPLC)	-not quantitative -intermediate sensitivity	-detects only *KIT* D816V mutant -needs standardization,validation and harmonization
Sensitivity	± 0.05 %	0.5-1.0 %	± 0.1%	± 0.01%

ASO-qPCR: allele specific-quantitative PCR; BM: bone marrow; D-HPLC: denaturing high performance liquid chromatography; PNA-mediated PCR: peptide nucleic acid-mediated; PCR RT-PCR: reverse transcriptase-polymerase chain reaction.

Arock et al. Leukemia (2015)

Treatment algorithm for SM

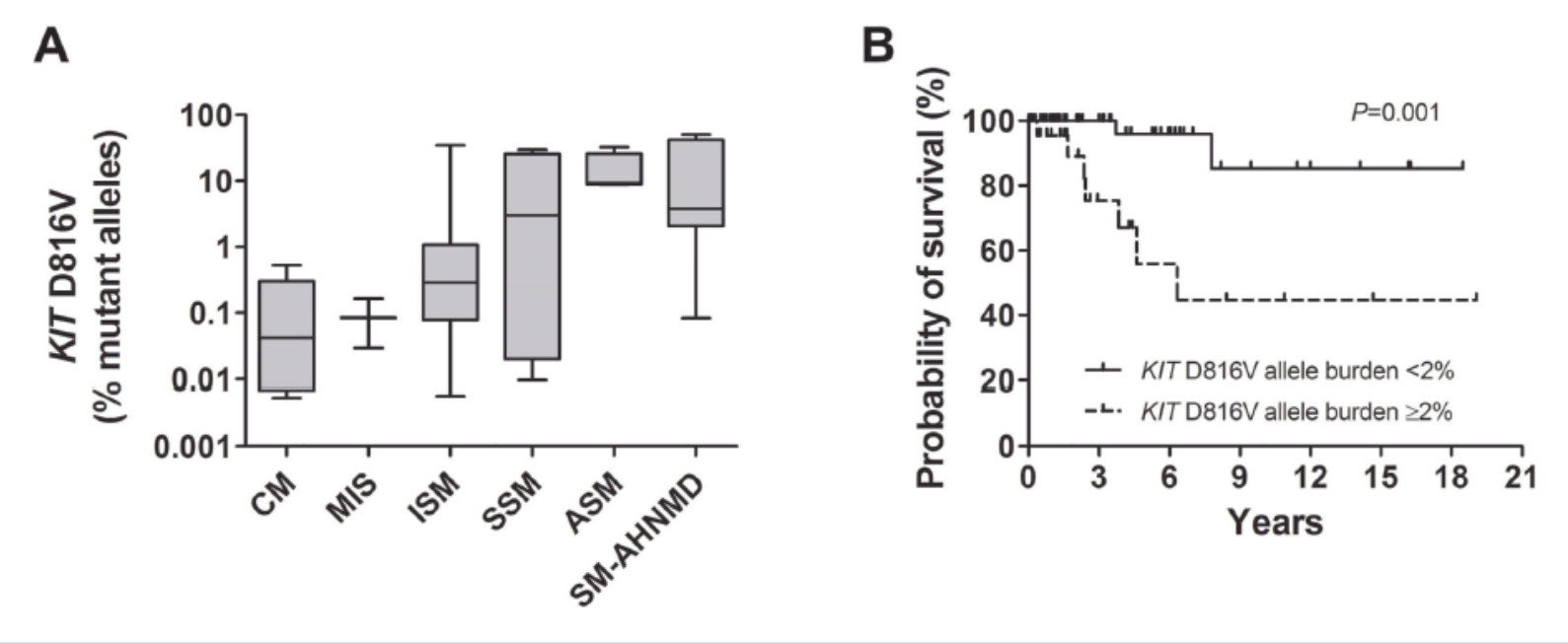




Pardanani A, Am J Hematology, 2019 (modificato)

The value of *KIT* allele burden

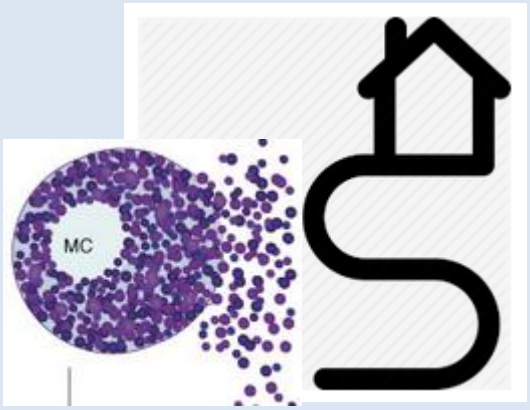
• The *KIT* D816V allele burden in the BM correlates with the WHO type of the disease and predicts survival in patients with mastocytosis



• Good correlation with serum tryptase levels
• No correlation with MC in BM
• Prognostic significance
• Monitoring of Minimal Residual Disease

Hoermann et al, Allergy 2014

Mastocitosi: Take-home messages



- Patologia molto eterogenea
- Diagnosi complessa specialmente in assenza di sintomi cutanei, con triptasi normale, con basso burden MC
- Rilevanza dei test di laboratorio, per la **diagnosi**, la **classificazione**, il **significato prognostico**, il **monitoraggio** della terapia

Grazie a…

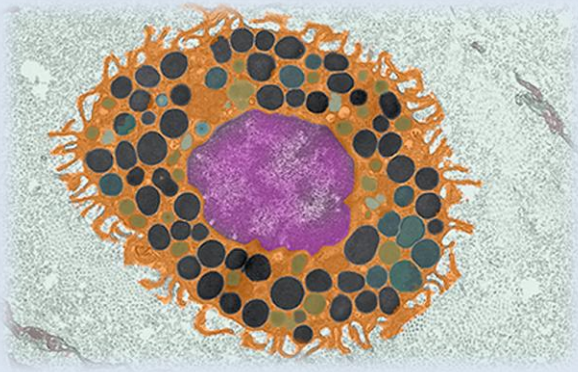
U.O. Patologia Clinica
AUSL Romagna
(direttore Dr. R.M. Dorizzi)

Dr. Giovanni Poletti
Dr. Marco Rosetti
Dr. Franco Monti
Dott.ssa Alice Clementoni
Dr. Luca Baldrati

I nostri tecnici

U.O.C. Ematologia Ravenna
AUSL Romagna
(direttore Dr. F. Lanza)

Dott.ssa Michela Rondoni



…ed a tutti voi per l'attenzione!