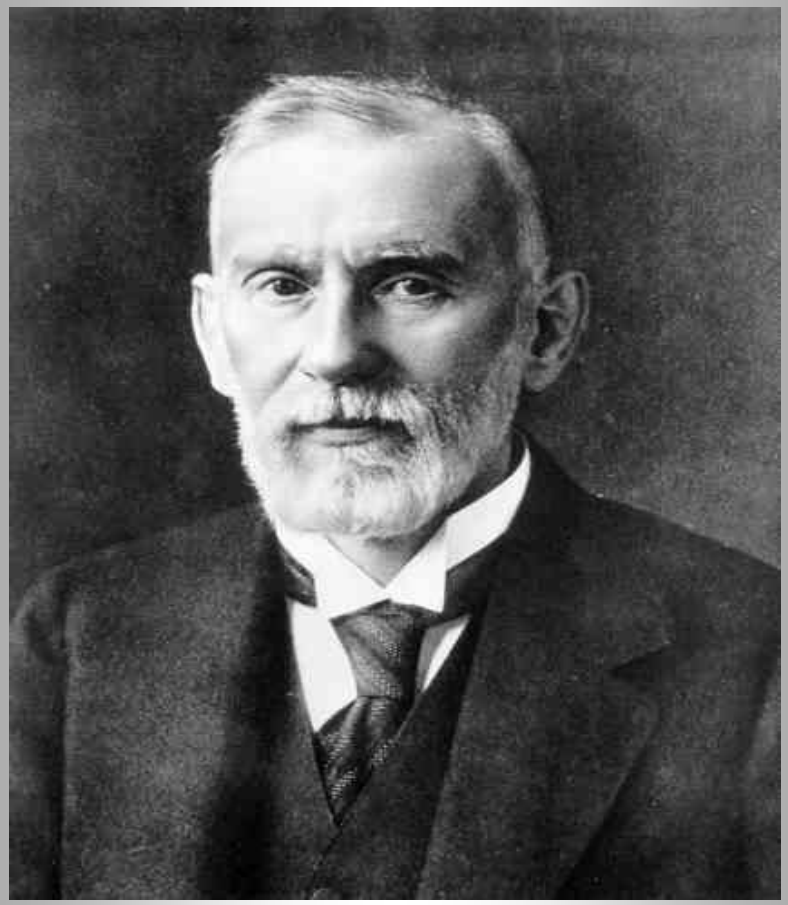
MASTOCYTOSIS

DIAGNOSIS, CLASSIFICATION, AND THERAPY

Peter Valent

HISTORY: MAST CELLS AND MASTERS

PAUL EHRLICH (1854-1915)



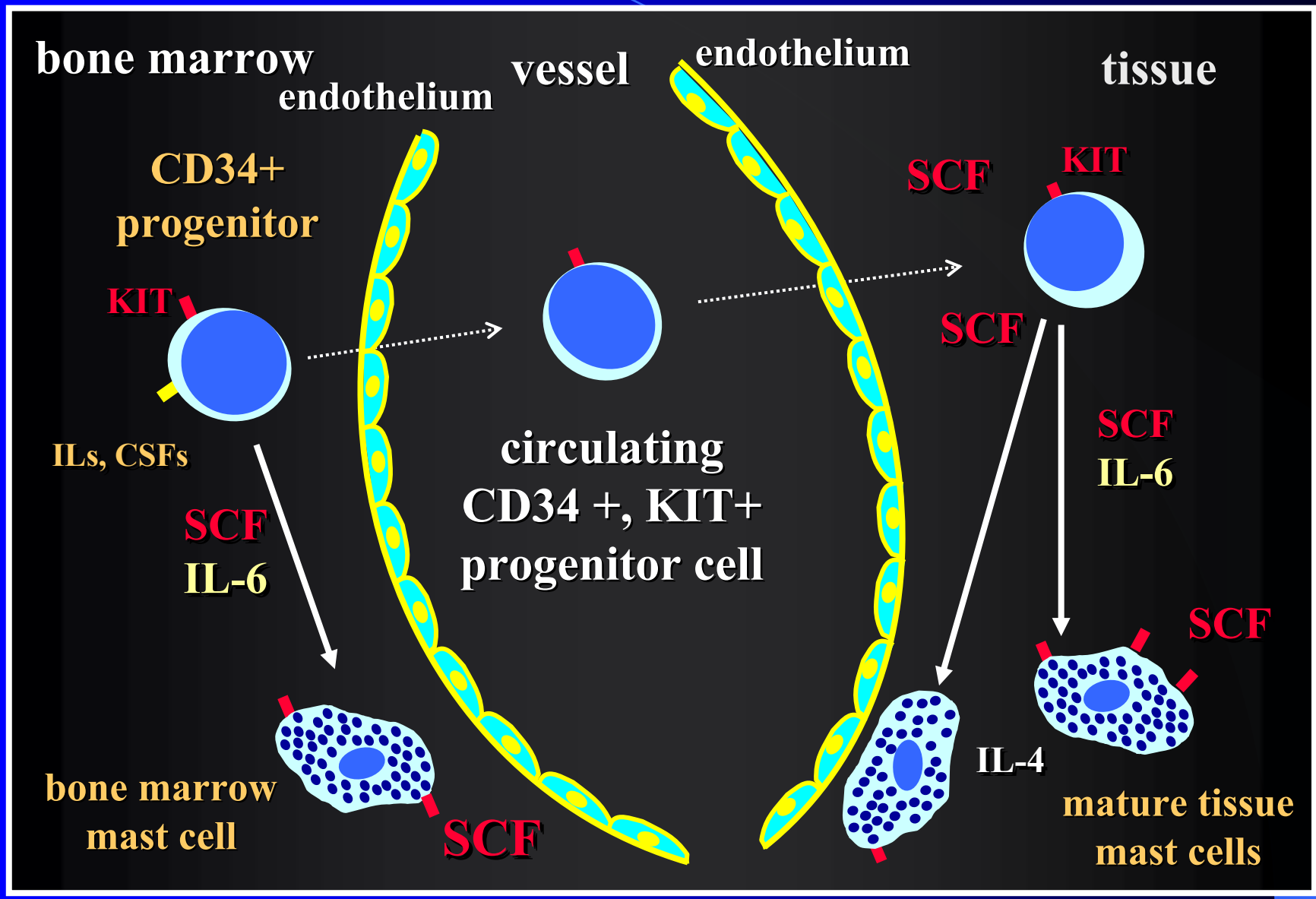
- 1869 - Nettelship — Rare Form of Urticaria
- 1878 - Sangster — Urticaria Pigmentosa UP
- 1879 - Ehrlich — Mast Cells (Mastzellen)
- 1887 - Unna — Mast Cells in UP
- 1949 - Ellis — Systemic Mastocytosis
- 1979 - Lennert — Kiel Classification
- 1991 - Metcalfe — Consensus Classification
- 1996 - Longley — c-kit D816V in SM
- 1998 - Escribano — CD2/CD25 on MC in SM

- 1990-2000 — Criteria Established
- 2000 — Working Conference
- 2001 — WHO Classification

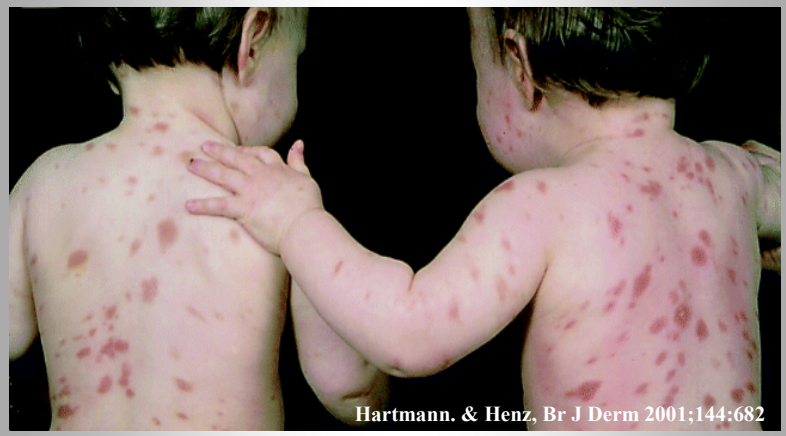
MAST CELLS - BIOLOGY

- Hematopoietic Cells - Leukocytes
- Directly derive from $CD34^+$ Progenitor Cells
- Distributed in Connective Tissues
- Express Stem Cell Factor (SCF) Receptor = Kit
- Differentiate in the presence of SCF
- Are extremely long-lived Cells (contrasting basophils)
- Express Vasoactive Mediators (Histamine, others)
- Release Mediators on Activation (IgER, Kit, ….)

DIFFERENTIATION OF MAST CELLS



Cutaneous Mastocytosis (CM) *vs* Systemic Mastocytosis (SM) !

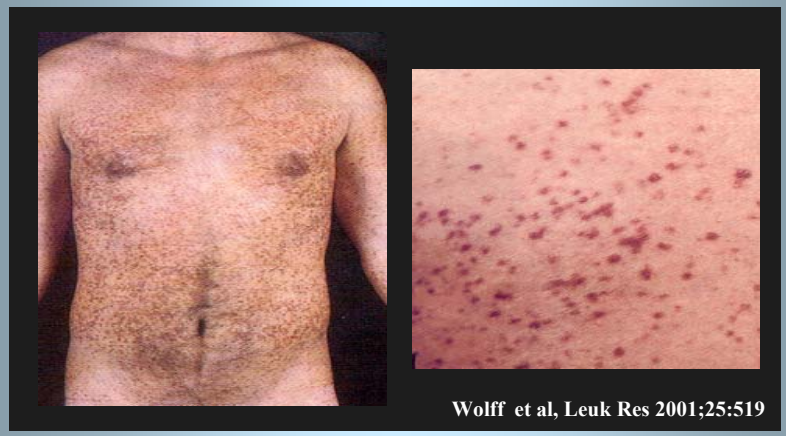


Mostly Children (monoclonal?)

Diagnosis: Skin only

- Biopsy of Skin
- Serum Tryptase
- Usually no BM Biopsy

Cutaneous Mastocytosis



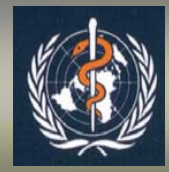
Mostly Adults (c-kit D816V)

Diagnosis: MPD

- Biopsy of BM (and Skin)
- Apply SM Criteria
- Define SM Variant

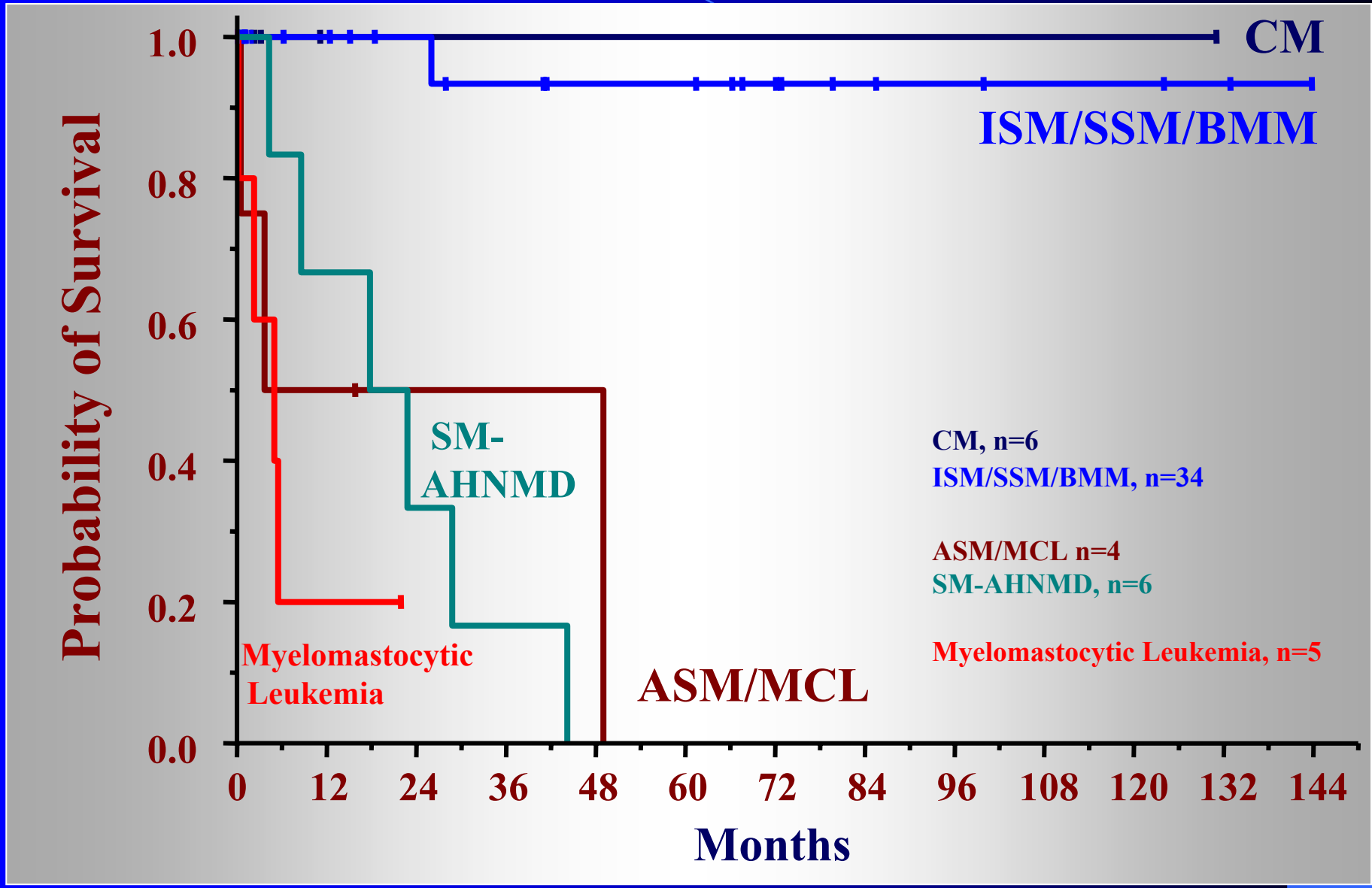
Systemic Mastocytosis

WHO CLASSIFICATION



- Cutaneous Mastocytosis (CM)
- Indolent Systemic Mastocytosis (ISM)
- SM with an Associated Hematologic non Mast Cell Lineage Disease (SM-AHNMD)
- Aggressive Systemic Mastocytosis (ASM)
- Mast Cell Leukemia (MCL)
- Mast Cell Sarcoma (MCS)
- Extracutaneous Mastocytoma

Survival of Patients with Mast Cell Disorders defined by WHO Criteria



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Major Criteria

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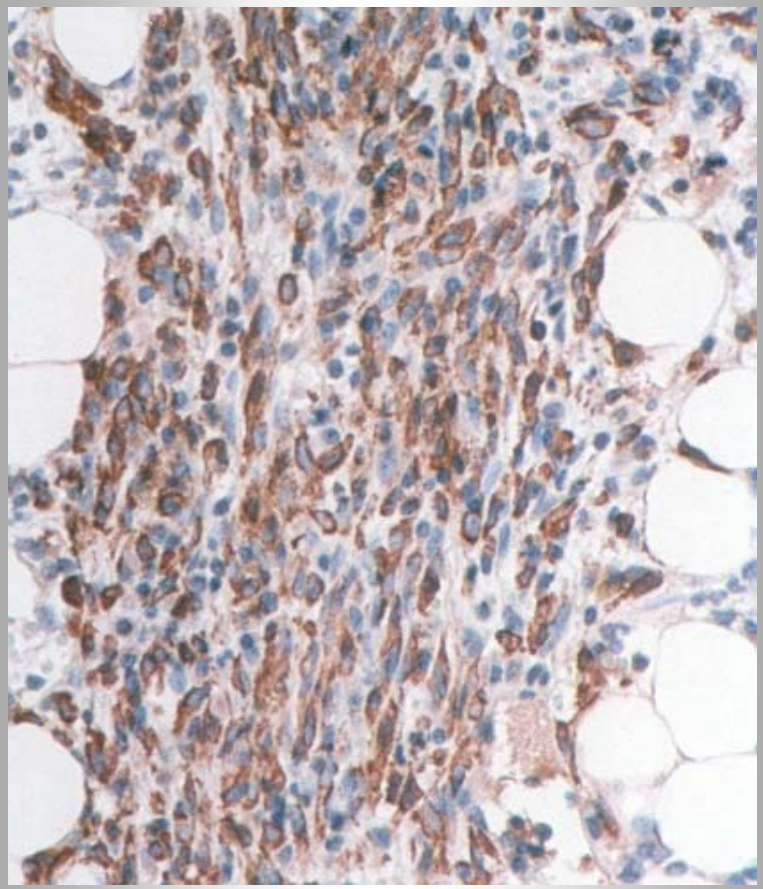
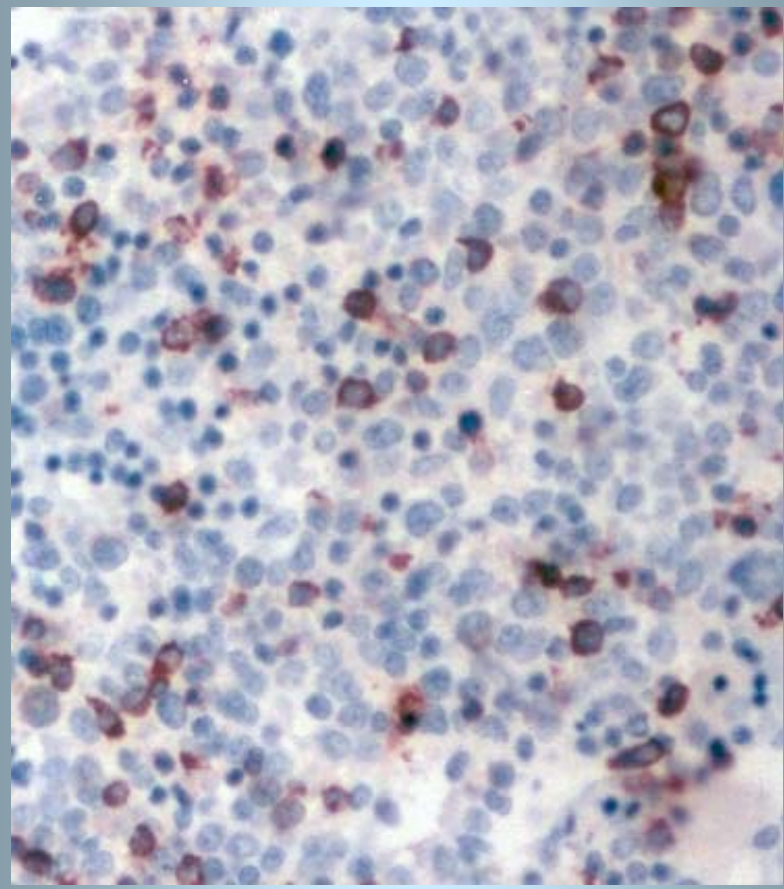
The diagnosis Systemic Mastocytosis is established if at least 1 major and 1 minor or 3 minor criteria are fulfilled

Analysis of Bone Marrow Sections: Tryptase-Immunohistochemistry



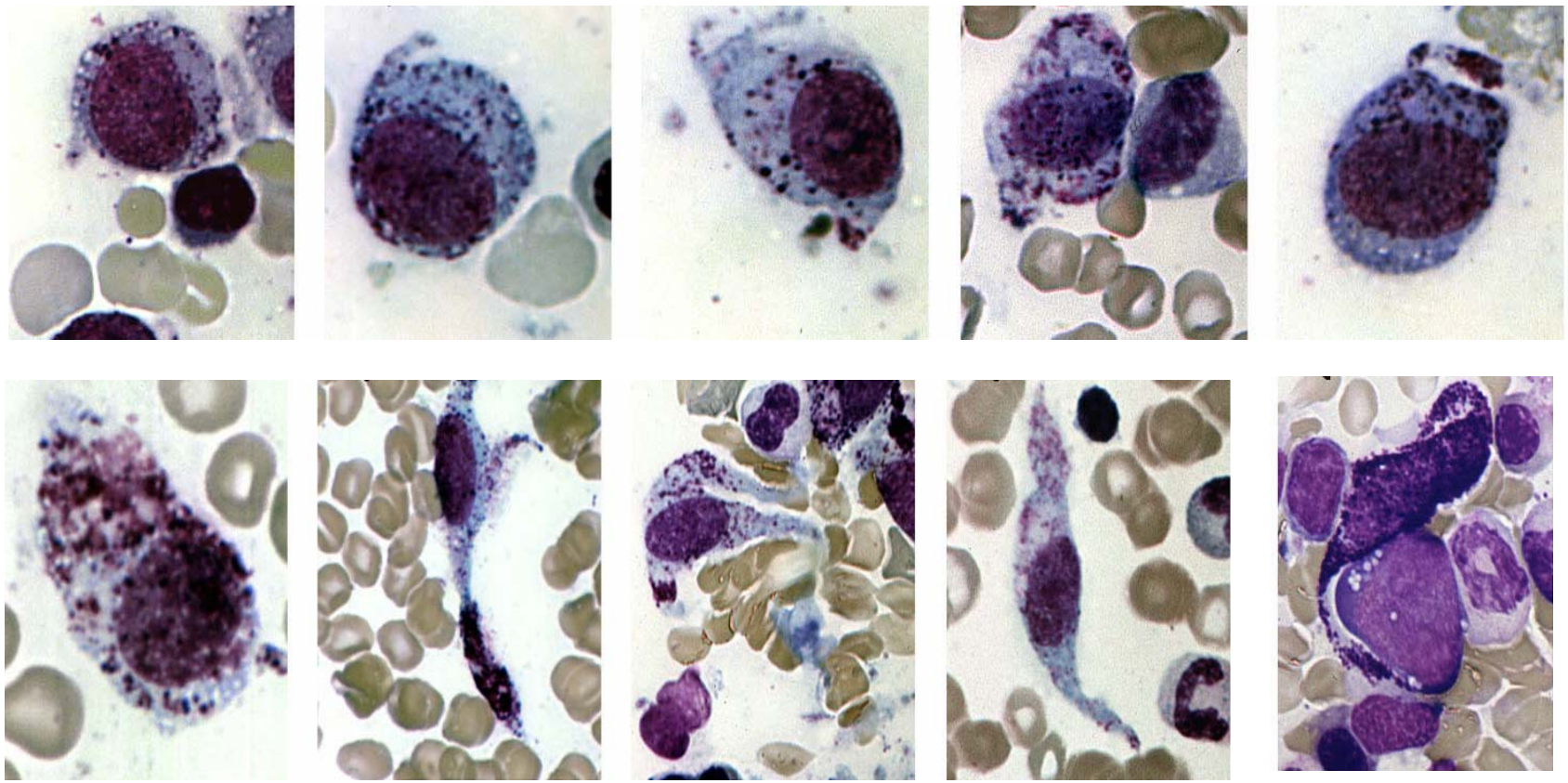
Systemic Mastocytosis



SM-AHNMD ? Myelomastocytic ?

Bone Marrow Smear: Atypical Mast Cells in Systemic Mastocytosis

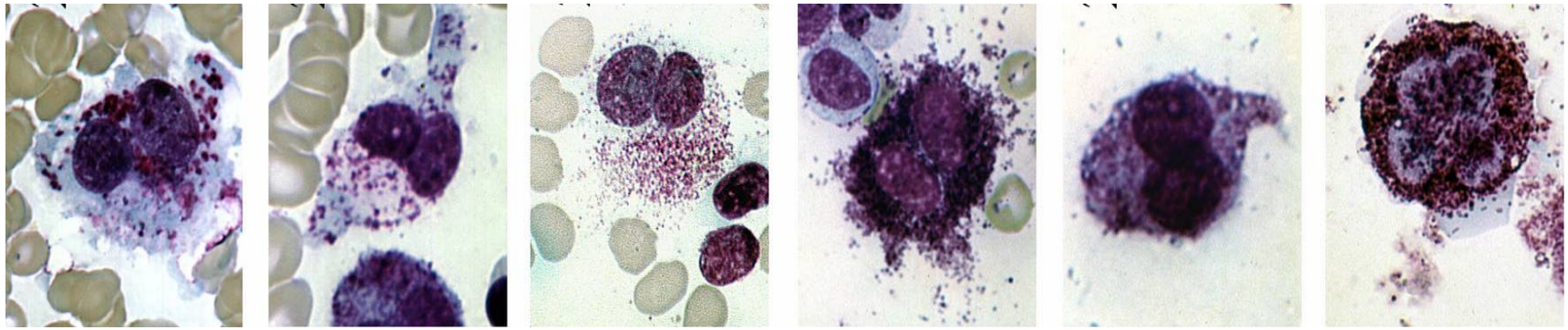
Criteria for Atypical Mast Cells Type I in Bone Marrow Smears:
A: Oval Nucleus, B: Cytoplasmic Extensions, C: Hypogranulated (2/3)



Sperr et al, Leuk Res 2001;25:529

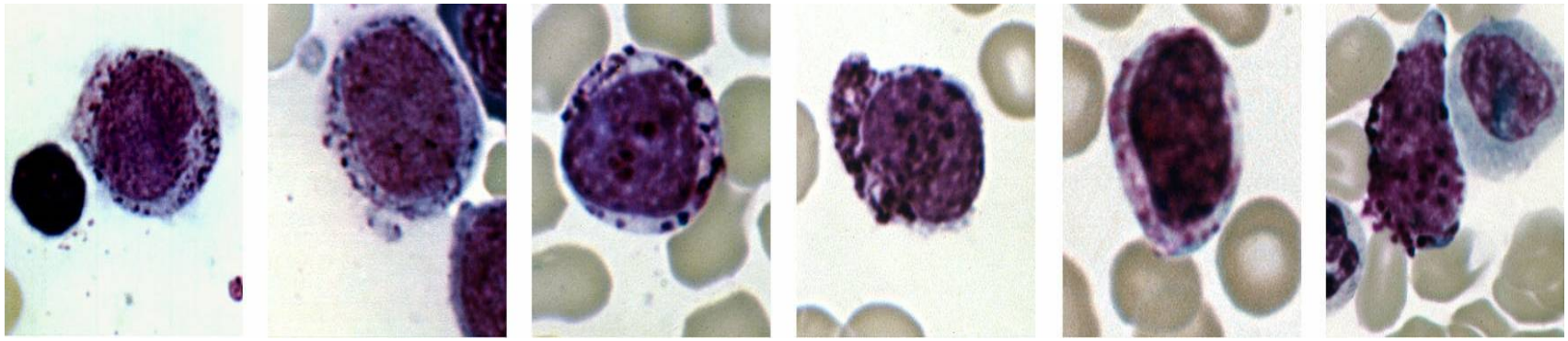
Bone Marrow Smear: Atypical Mast Cells Type II and Metachromatic Blasts

Atypical Mast Cells Type II = Promastocytes in Bone Marrow Smears



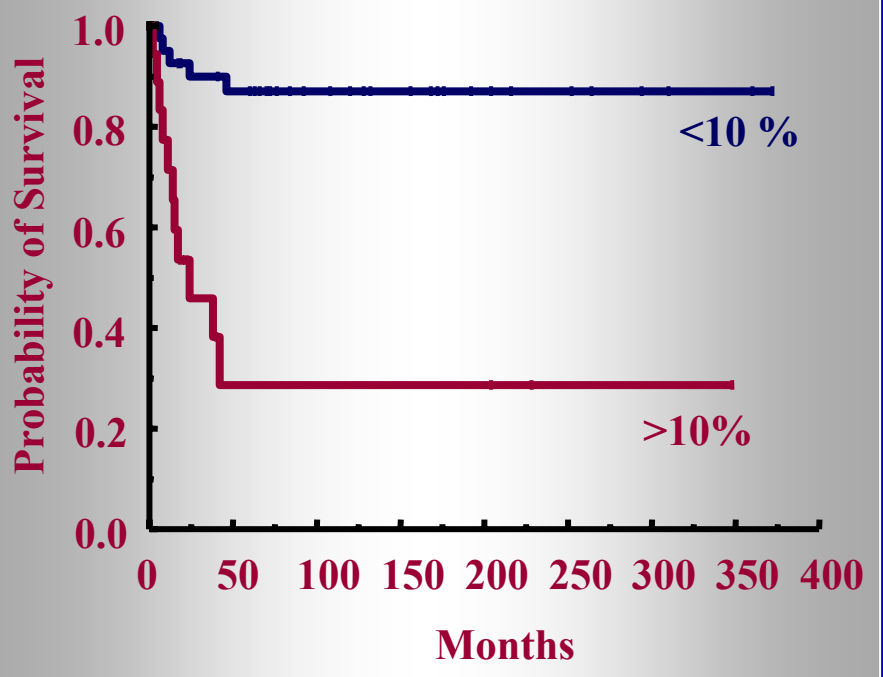
Sperr et al, Leuk Res 2001;25:529

Metachromatic Blasts in Bone Marrow Smears

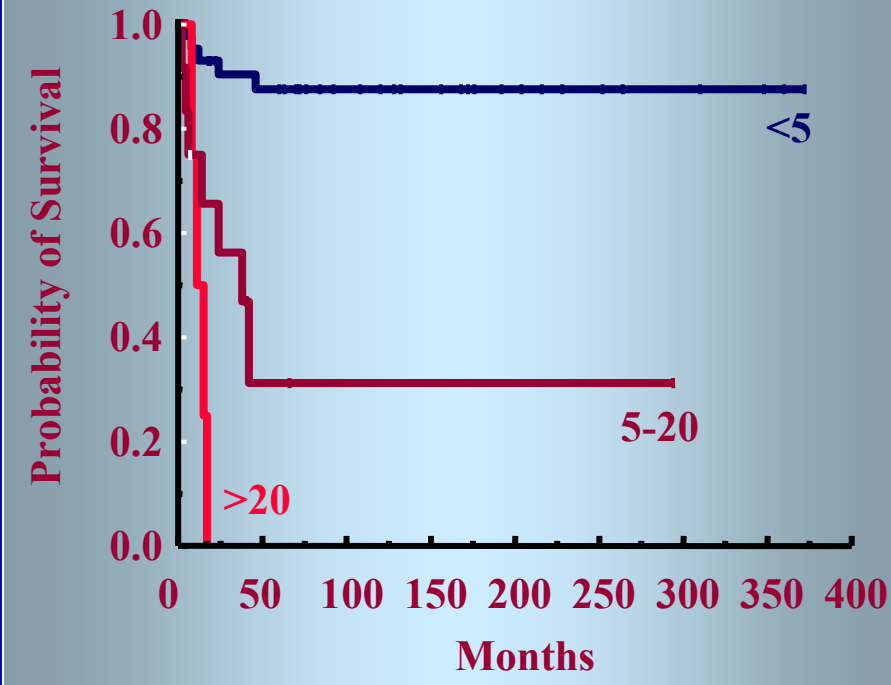


Mast Cell Numbers in Bone Marrow Smears in Patients with SM: Clinical Significance

Survival of patients with varying percentages of pro-mastocytes (of all mast cells) in bone marrow smears



Survival of patients with varying percentages of mast cells (of all nucleated cells) in bone marrow smears



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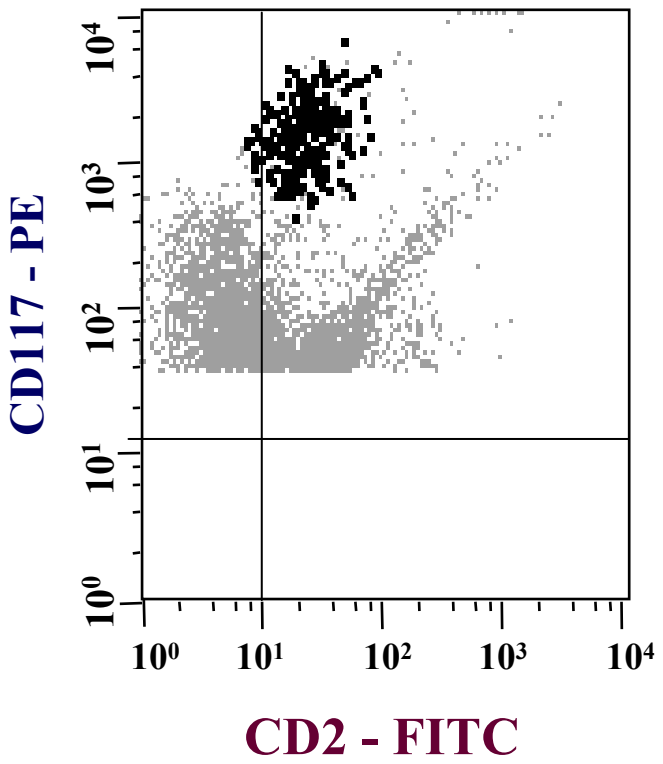
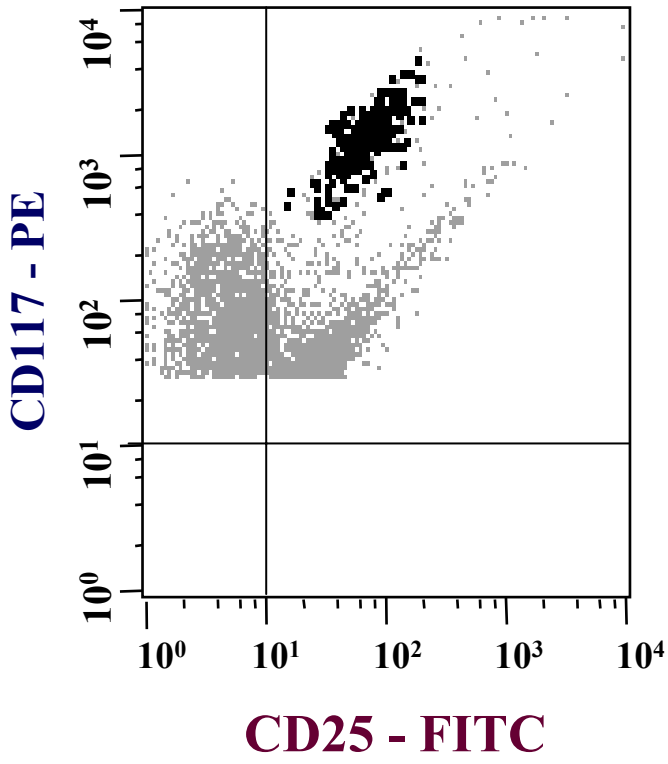
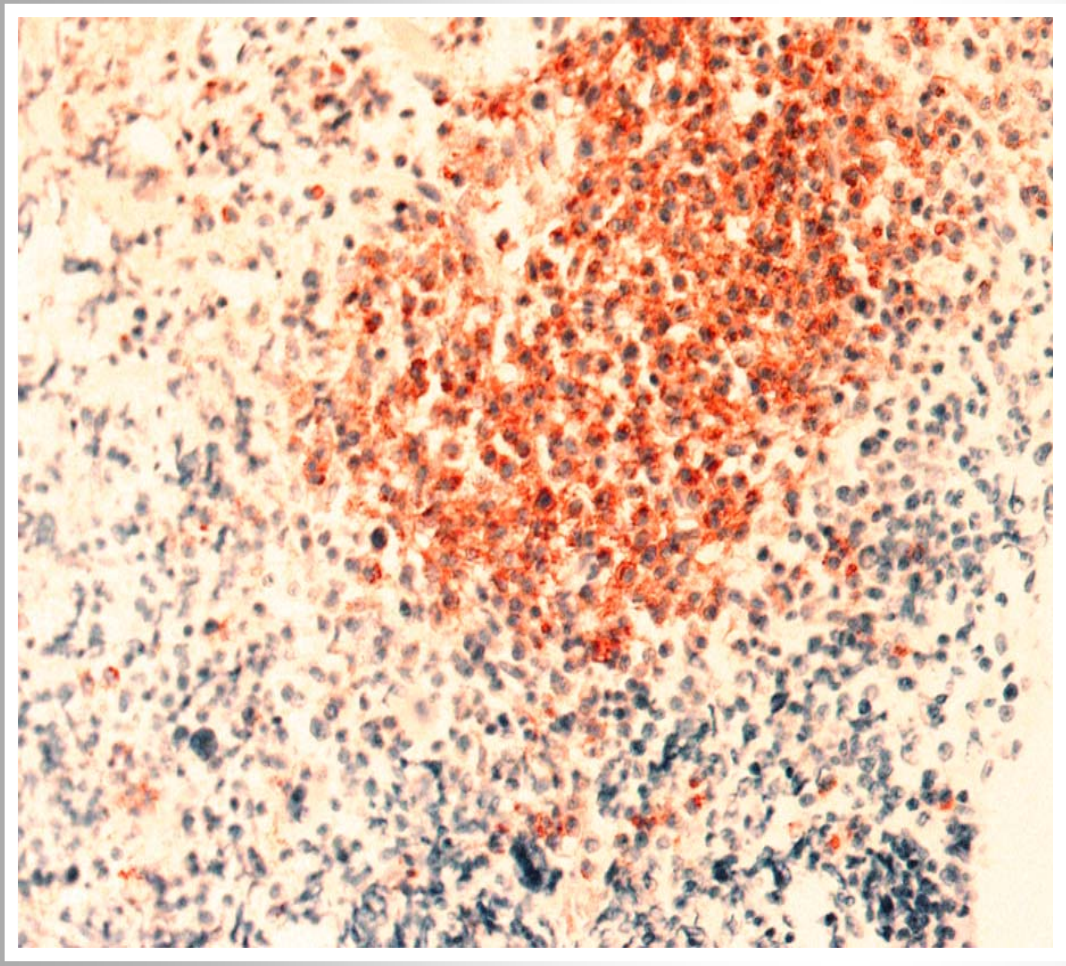
Expression of CD2 and CD25 on Bone Marrow Mast Cells in a Patient with SM - Flow Cytometry





Detection of CD25 in Neoplastic Bone Marrow Mast Cells by Immunohistochemistry (IHC)



CD25-IHC :

- **Easy Test**
- **Highly Specific (>95%) for neoplastic MC in SM**
- **MC in Myelomastocytic Leukemia & reactive MC Hyperplasia are $CD25^-$**
- **Highly Sensitive and superior to CD2**
- **May be equally diagnostic compared to flow-cytometry analysis**
- **not yet accepted as a minor SM criterion**

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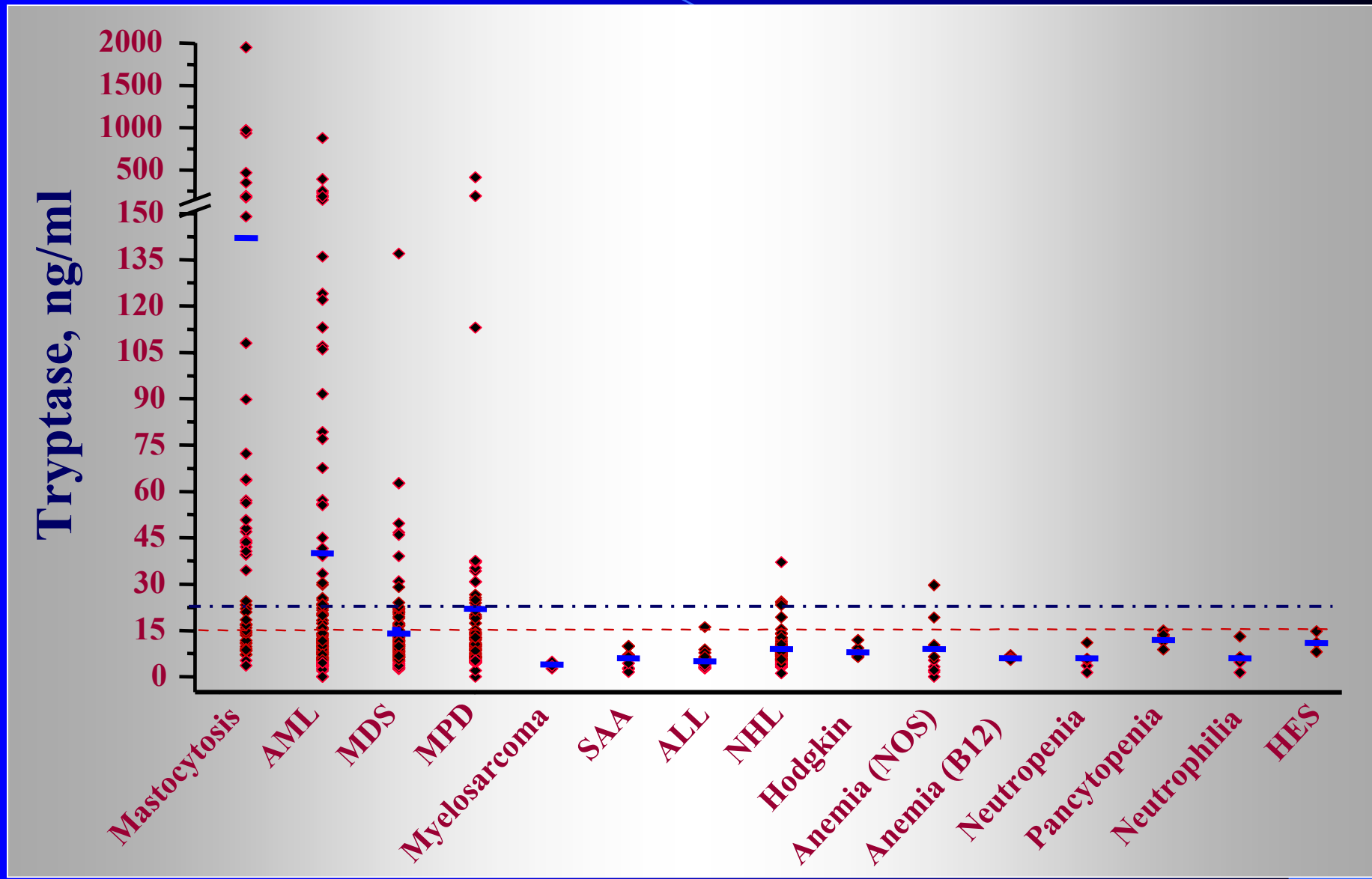
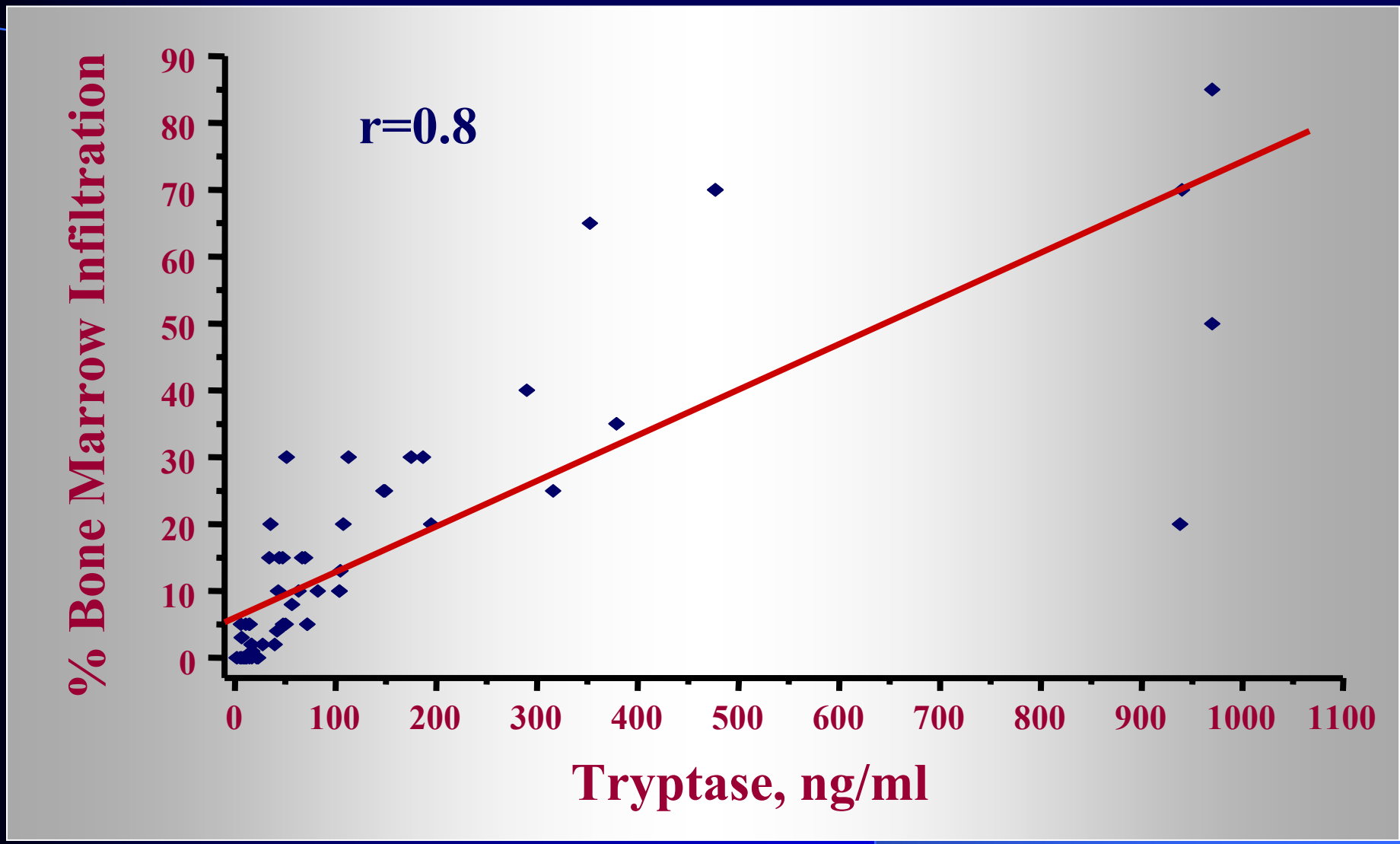
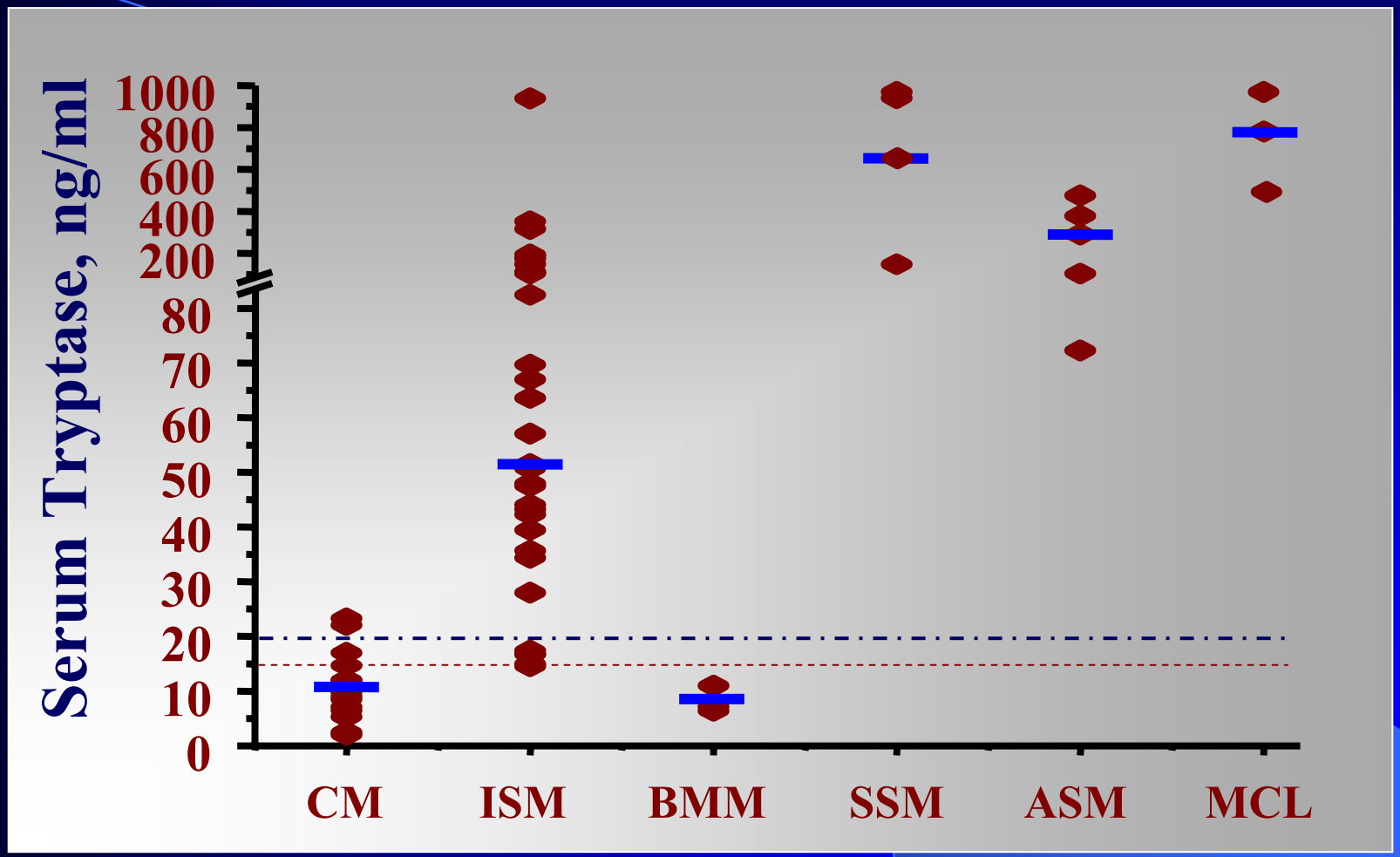
Serum Tryptase Levels in Hematologic Disorders



Correlation between Serum Tryptase Levels and Percentage of Mast Cell Infiltrates



Serum Tryptase Levels in various Groups of Patients with SM



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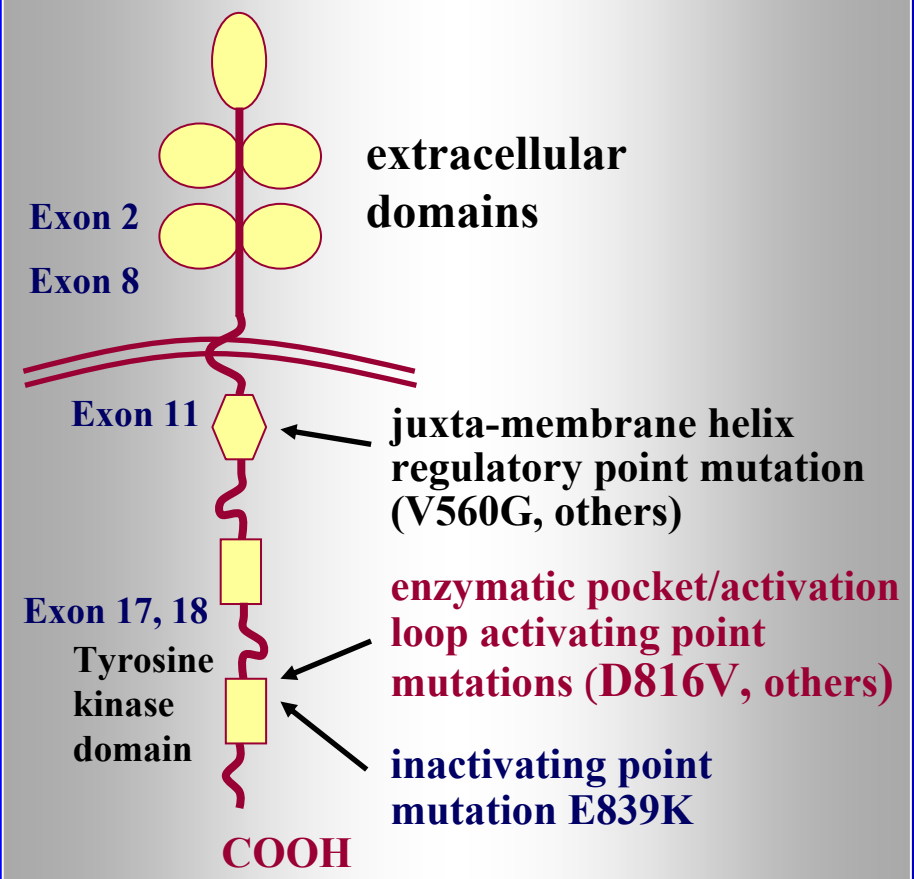
c-kit Point Mutations in Mastocytosis



Proposed Standards (D816V)

- Bone marrow (bm) cells
- MNC or unfractionated bm cells analyzed
- In suspected smouldering SM or mast cell leukemia, peripheral blood (MNC) should also be analyzed
- RT-PCR and RFLP

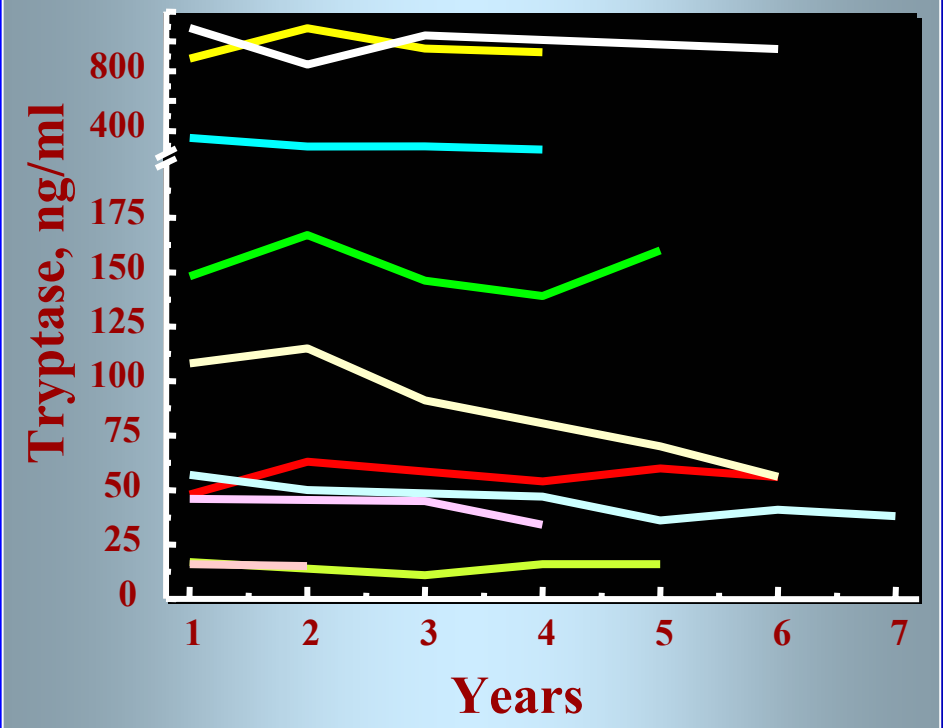
  (in D816V-negative patients → sequencing of *c-kit*)

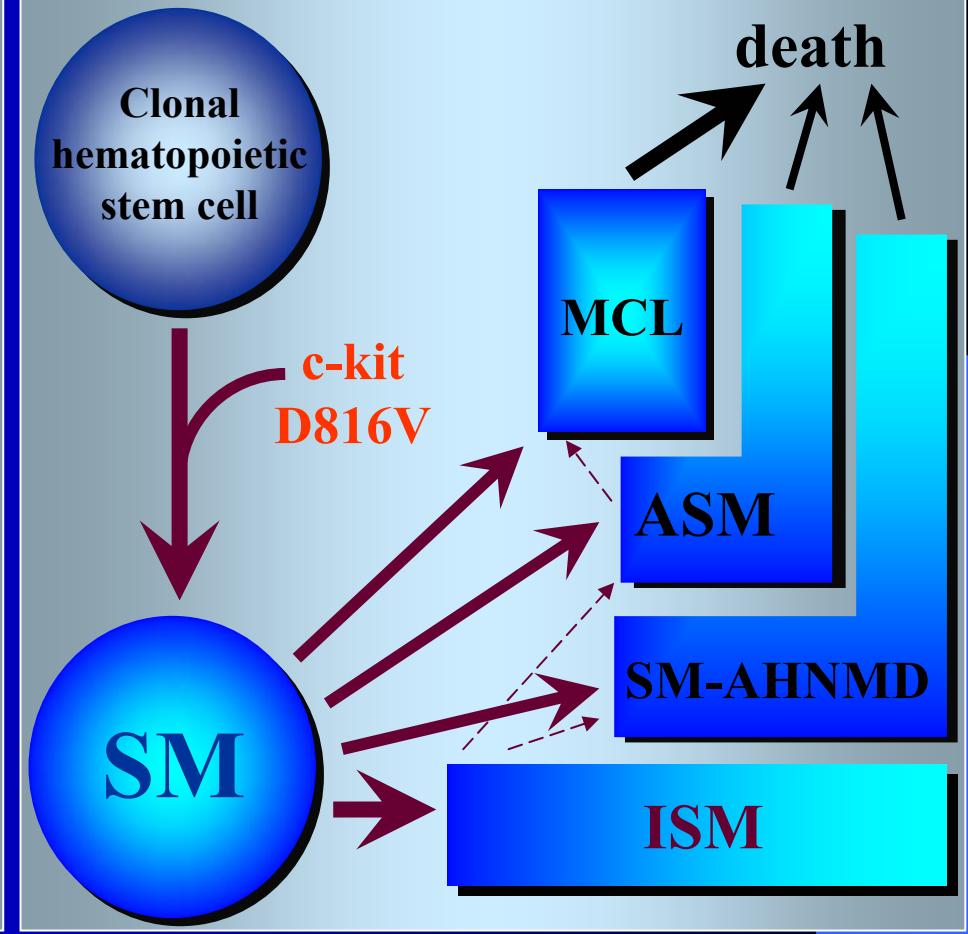
c-kit Point Mutations in Mastocytosis

Mutation	Reported in	Frequency in SM
c-kit D816V	all variants of SM some cases of CM	>80%
c-kit D816Y	ISM, SM-AHNMD, CM ?	<5%
c-kit D816F	ISM, CM ?	<5%
c-kit D816H	SM-AHNMD	<5%
c-kit D812G	SM/ASM	<5%
c-kit D560G	SM/ISM	<5%
c-kit F522C	SM/ISM	<5%
c-kit E839K	CM	<5%
c-kit V531I	SM-AHNMD	<5%
c-kit K509I	ISM/ASM	<5%

THE NATURAL CLINICAL COURSE IN SYSTEMIC MASTOCYTOSIS

Serum Tryptase Levels in Patients with ISM (D816V+)





Doxycycline-Induced Expression of c-kit D816V in Ba/F3 Cells

Effects of c-kit D816V in Ba/F3 cells (Ton.Kit-D816V):

- Cluster Formation

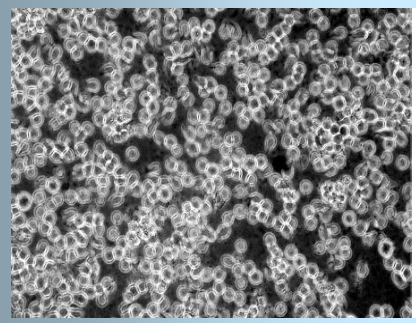
- Differentiation

  (early MC differentiation, histamine, …)

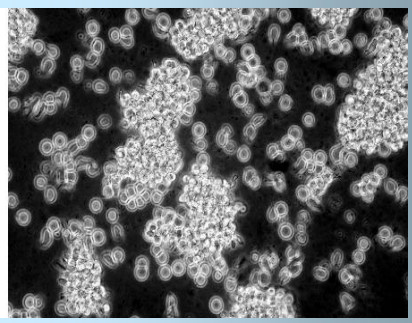
- No Proliferation (!)

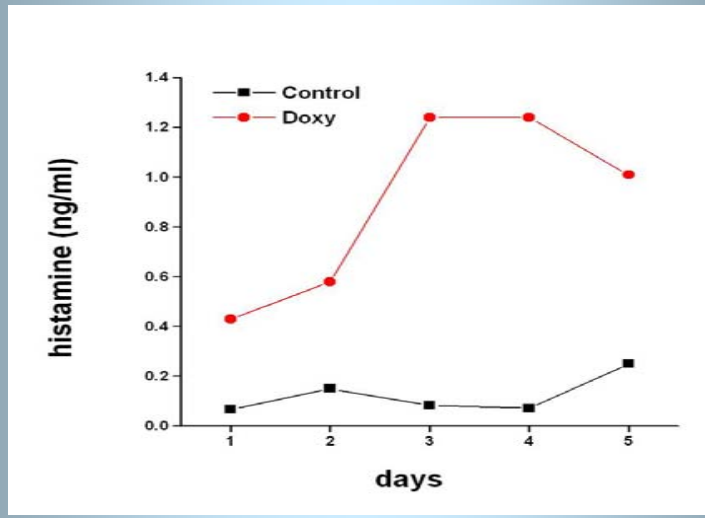
ASH 2004: Mayerhofer et al, Abstract no # 485

control



Doxycycline

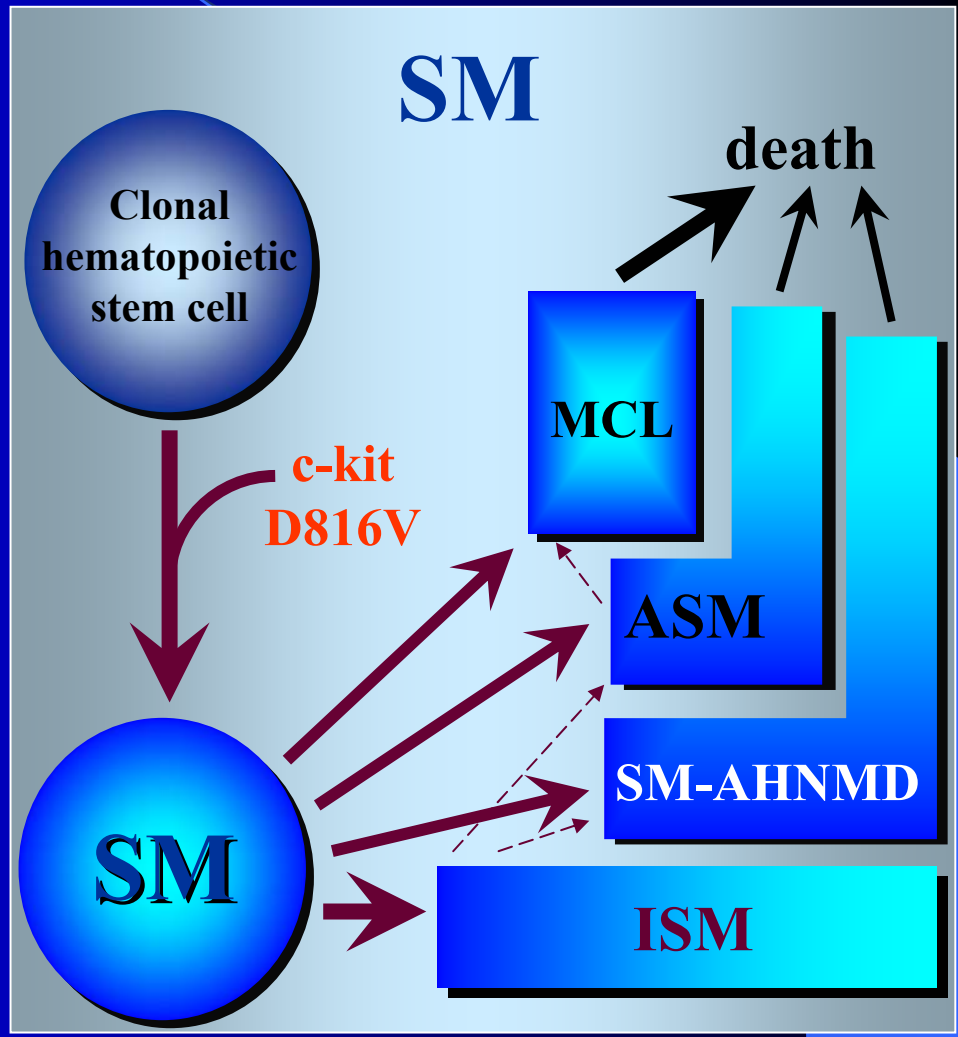




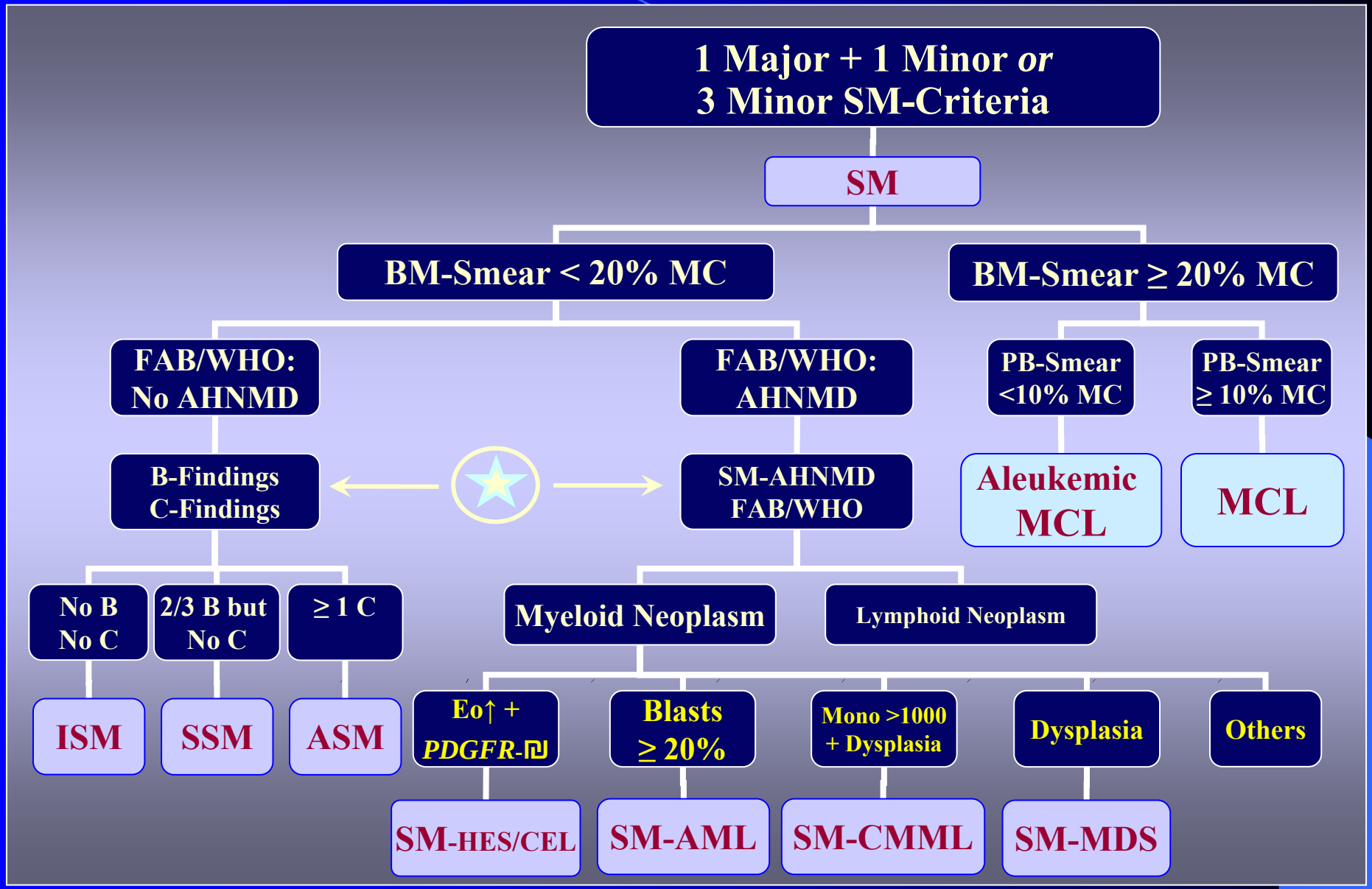
PATHOGENETIC CONCEPTS: ROLE OF OTHER DEFECTS

What factors and defects are responsible for the development of a high grade (mast cell-) disease in patients with c-kit-D816V^{+} SM

?



Stepwise Approach in Defining Subvariant of SM: Proposed Algorithm using WHO - Criteria



B-Findings (Borderline-Benign) and C-Findings (Consider Cytoreduction)

B-Findings:

- **Infiltration grade (MC) in BM>30% and serum tryptase >200 ng/ml**
- **Dysmyelopoiesis:** Hypercellular marrow with signs of myelodysplasia or myelo-proliferation, but no criteria for MDS or MPD. Blood picture normal or slightly abnormal
- **Organomegaly (without impair-ment of organ function):** Hepatomegaly (without ascites), splenomegaly (palpable), lymphadenopathy (>2 cm in CT or US)

When 2 or 3 B-Findings but no C-Findings are recorded, the final diagnosis is Smouldering SM

C-Findings:

- **One or more Cytopenias:** ANC<1000/µl; Hb<10 g/dl; Plt<100000/µl
- **Hepatopathy:** Enlarged liver with ascites, elevated liver enzymes +/- portal hypertension
- **Organopathy of Spleen:** Splenomegaly with hypersplenism
- **Malabsorbtion with** hypalbuminemia and weight loss
- Large **osteolysis** and/or severe **osteoporosis** & pathologic fractures

Two Distinct Entities: Mast Cell Leukemia and Myelomastocytic Leukemia

Mast Cell Leukemia:

- SM Criteria Fulfilled
- c-kit Mutations (D816V)
- Karyotype often normal
- Atypical MC, MC-Blasts
- ≥ 20 % MC in BM smears
- Circulating Mast Cells

- No AHNMD no MDS/AML

- Usually, no CR after Induction Chemotherapy

Myelomastocytic Leukemia:

- Criteria of SM not met
- No c-kit Mutations
- Complex Karyotype
- Metachromatic Blasts
- ≥ 10 % MC in BM Smears or in Peripheral Blood

- AML or MDS-RAEB

- Usually, CR after Induction Chemotherapy

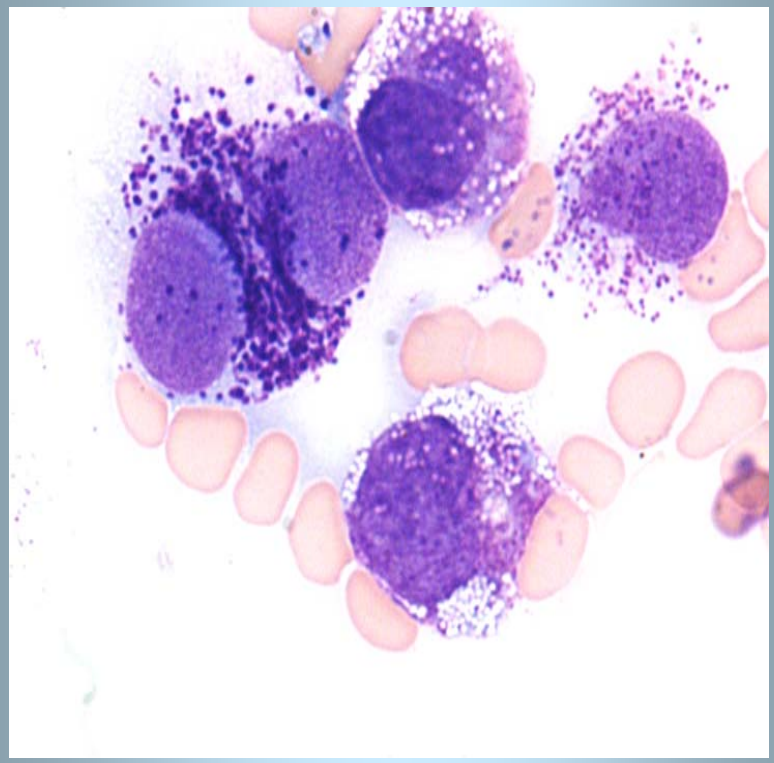
Mast Cell Leukemia (MCL)

Findings in MCL:

- SM Criteria Fulfilled
- Organopathy - C-Finding(s)
- Atypical MC + MC Blasts
- ≥ 20 % MC in BM smears
- No AHNMD
- No Skin Lesions (!)

- Circulating MC:
  - ≥10% = Typical MCL
  - <10% = Aleukemic MCL

Bone marrow smear in MCL: Wright-Giemsa stain



Treatment of Patients with Mastocytosis

A: Treatment of Mediator-Related Symptoms:

Drugs Targeting – Mediator Production
– Mediator Release
– Mediator Effects

B: Cytoreductive Therapy

Drugs Targeting – Neoplastic Stem Cells
– Progenitor Cells (IFNs)
– Mast Cells
– Specific Molecular Targets

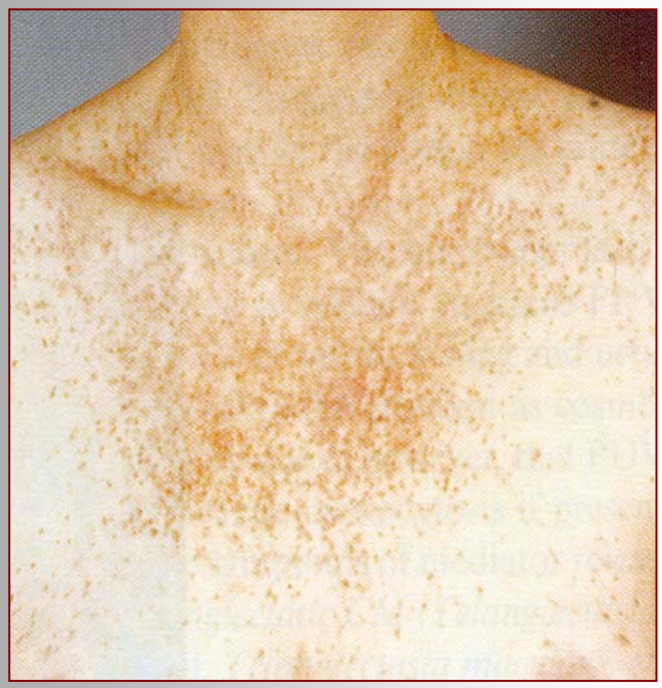
Treatment of Mastocytosis: Mediator-Targeting Drugs

Pruritus, flushing	H_1 + H_2 antihistamines, ketotifen, topical glucocorticoids, avoid triggering factors
Recurrent hypotension and tachycardia	H_1 + H_2 antihistamines, glucocorticoids, avoidance of triggering factors
Recurrent shock	H_1 + H_2 antihistamines, glucocorticoids, epinephrin
Co-existing allergy	H_1 + H_2 antihistamines, glucocorticoids, avoidance of triggering factors, hyposensitization
Peptic ulcer disease	H_1 + H_2 antihistamines, proton pump inhibitors
Diarrhea, abdominal pain, cramping, nausea	H_1 + H_2 antihistamines, oral cromolyn sodium, glucocorticoids, leukotriene antagonists
Bone pain	Analgetics, radiation for severe localized bone pain
Osteopenia, osteoporosis	Vitamin D, calcium, estrogen, testosteron, biphosphonates, low dose interferon-alpha (IFNα)
Neurologic symptoms	H_1 + H_2 antihistamines, oral cromolyn sodium

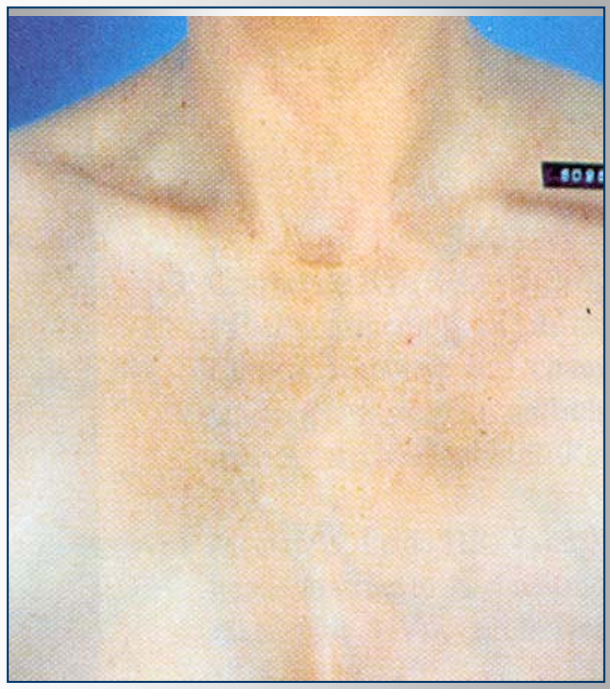
Therapy of Skin Lesions with Psoralen and UV-A Irradiation (PUVA)

Urticaria pigmentosa-like skin lesions in a patient with SM

prior to PUVA therapy



3 months after PUVA



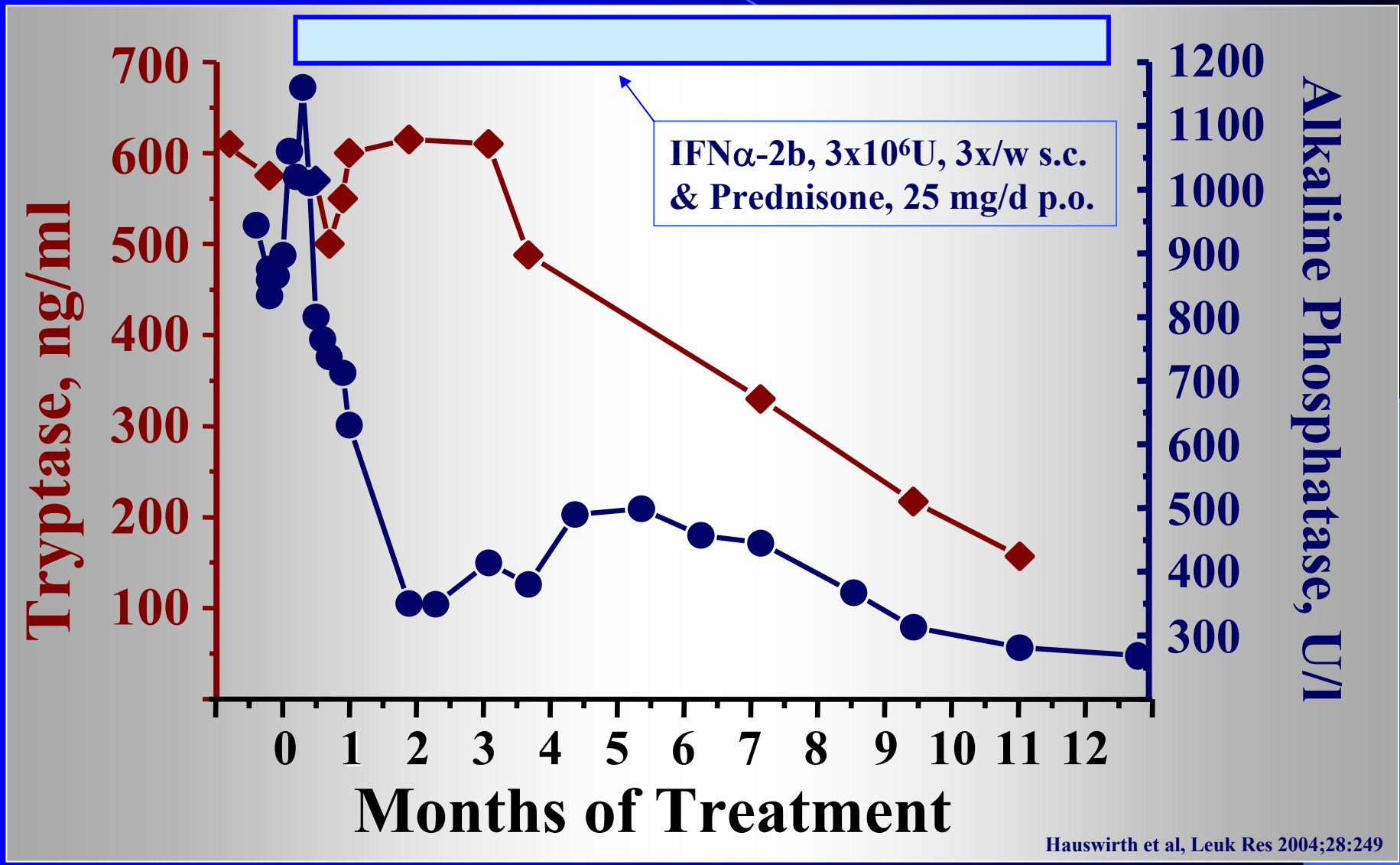
K.Wolff et al., Int Arch Allergy Immunol 2002;127: 115

Treatment of Mastocytosis: Cytoreductive Drugs

ISM	**NO cytoreductive treatment** (exception: severe osteopenia with risk of pathologic fracture, life threatening recurrent shock ?)
SSM	**Wait and watch** in most cases. In select cases (with progression) consider IFNα, 2CdA, or targeted drugs
SM-AHNMD	**Treat AHNMD as if no SM is present, and SM as if no AHNMD had been diagnosed (e.g. ASM-AHNMD !)**
ASM with slow progression	**IFNα**+glucocorticoids, **2CdA**, in case of hypersplenism due to splenomegaly (MC infiltrates) consider splenectomy **(in the absence of D816V - consider Imatinib)**
ASM with rapid progression	**Polychemotherapy** ± 2CdA, IFNα, or glucocorticoids - in responding patients - **consider stem cell transplantation (in the absence of D816V - consider Imatinib)**
MCL	**Polychemotherapy** or 2CdA (±IFNα or corticoids) - in responding patients - **consider stem cell transplantation (in the absence of D816V, consider Imatinib)**

In all categories, mediator-targeting drugs are given as adjunct to cytoreductive therapy

Follow up of a Patient with ASM treated with Interferon-alpha-2b and Prednisone



Hauswirth et al, Leuk Res 2004;28:249

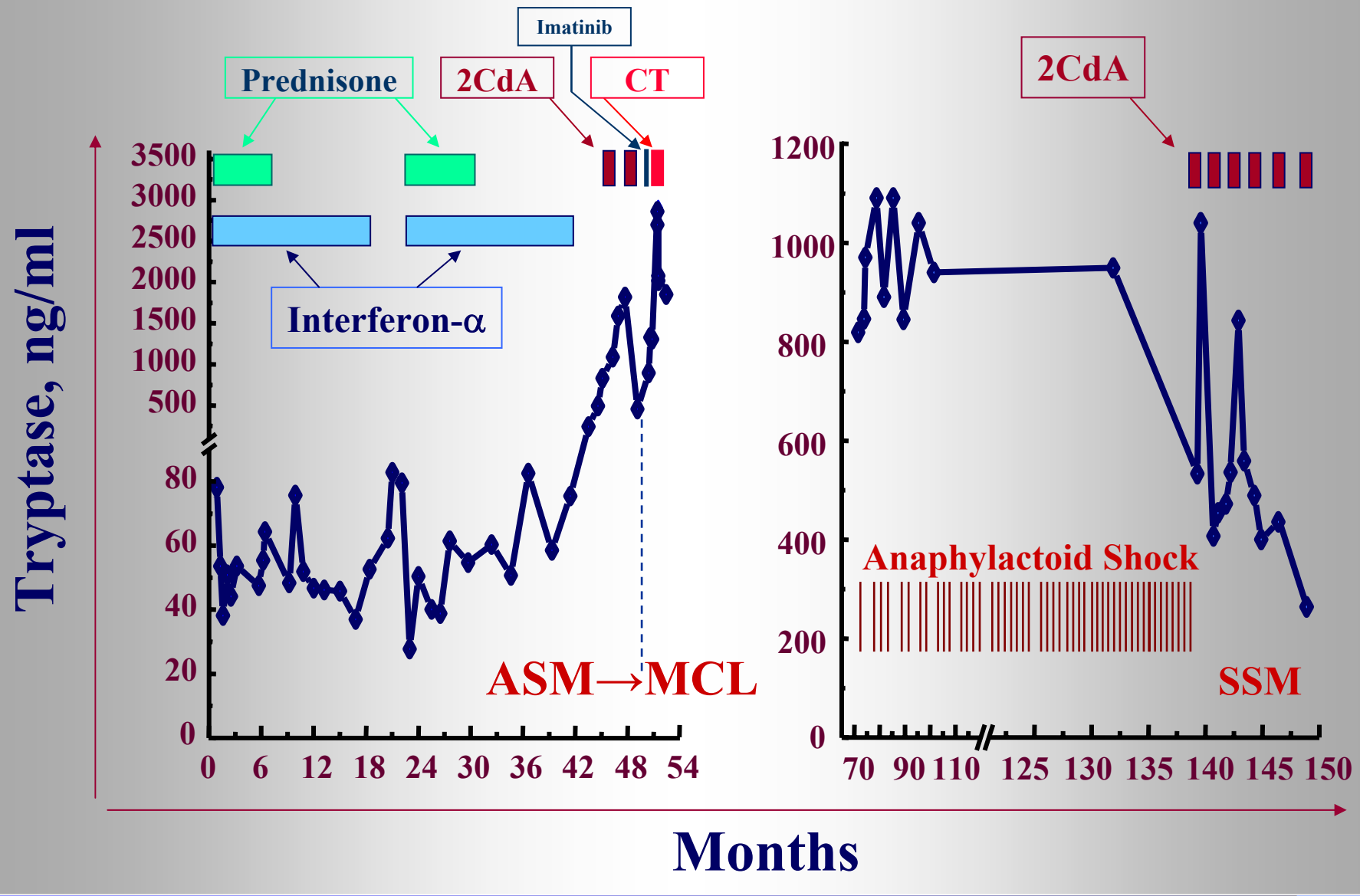
Reported Effects of 2-Chloro-2´-Deoxyadenosine (2CdA) = Cladribine in Patients with Mastocytosis

Report	Patients	2CdA Schedule	Response
Tefferi et al. NEJM 2001;344:307	ASM, n=1	0.13 mg/kg/d 2 h-infusion, d1-5 4 cycles	MR
Escribano et al. Leuk Res 2002;26:1043	SM-NHL, n=1	0.15 mg/kg/d 3 h-infusion, d 1-5 5 cycles	not applicable (MC phenotype switch from CD2+ to CD2-)
Kluin-Nelemans et al. Blood 2003;102:4270	ISM/SSM, n=4, ASM, n=3, SM-AHNMD, n=3	0.1-0.13 mg/kg/d 2 h-infusion, d 1-5 up to 6 cycles	in ASM: MR (2/3), GPR (1/3)
Pardanani et al. Leuk Res 2004;28:127	ASM, n=4	0.14 mg/kg/d 2 h-infusion, d 1-5 3-6 cycles	MR (2/4), GPR (1/4) NR (1/4)
Lortholary et al. ASH 2004 # 661	ISM/SSM, n=6 ASM/MCL, n=23 SM-AHNMD, n=4	0.15 mg/kg/d 2 h-infusion, d 1-5 1-6 cycles	in all patients: MR in >50%

Major Responses (MR) in Patients with Aggressive Systemic Mastocytosis

Therapy in ASM	MR rate
Interferon-alpha 2b s.c. (9-42 million units per week) plus glucocorticoids	15-20 %
2-Chloro-2´-Deoxyadenosine (2CdA) = Cladribine i.v. (2-3 h) 0.1-0.15 mg/kg/d, d 1-5, 1-6 cycles	~ 50 %

Follow up of 2 Patients with SM during Therapy



Targeted Drugs: Imatinib/STI571 *and others*

- Targets of Imatinib detectable in Mastocytosis:
  - wt KIT (tyrosine kinase activation domain)
  - mutated variants of KIT (D560G, F522C, ..)
  - FIPL1/PDGFRA (SM-HES, SM-CEL, SM-eo)
  - BCR/ABL (rare subvariant: SM-CML)

- D816V confers (relative) resistance against STI571

- New Kinase Inhibitors (*PKC412, AMN107, BMS-354825, ….*)

- Other Targets and Targeted Drugs (n > 100)

Targeted Drugs in Mastocytosis: Future Perspectives

TARGETED DRUG	DRUG TARGET(s)	SM VARIANT(s)
PKC412	**wt KIT, KIT[mut]**	**- ASM, MCL, SM-AHNMD**
AMN107	**wt KIT, KIT[mut]**	**- ASM, MCL, SM-AHNMD**
BMS354825	**wt KIT, KIT[mut]**	**- ASM, MCL, SM-AHNMD**
AP23464	**KIT[mut], wt KIT**	**- ASM, MCL, SM-AHNMD**
17AAG	**Chaperone, KIT[mut]**	**- ASM, MCL, SM-AHNMD**
Targeting Antibodies	**CD25, CD33, CD44, CD52, ..**	**- ASM, MCL, SM-AHNMD**
Antisense, siRNA	**KIT, MITF, MCl-1, ….**	**- ASM, MCL, SM-AHNMD**
VEGF-targeting Drugs	**VEGF, Angiogenesis**	**- ASM, MCL, SM-AHNMD**
FTIs (R115777), FTS	**Farnesyltransferase**	**- ASM, MCL, SM-AHNMD**
Rapamycin & Derivatives	**mTOR, VEGF**	**- ASM, MCL, SM-AHNMD**