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
- 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

bone marrow
endothelium

vessel
endothelium
tissue

CD34+
progenitor
circulating
CD34+, KIT+
progenitor cell

KIT
ILs, CSFs
SCF
IL-6

SCF
bone marrow
mast cell

SCF
IL-6

SCF
KIT

SCF
IL-4

mature tissue
mast cells

IL-6
Cutaneous Mastocytosis (CM) vs Systemic Mastocytosis (SM)!

Mostly Children (monoclonal?)
Diagnosis: Skin only
- Biopsy of Skin
- Serum Tryptase
- Usually no BM Biopsy

Systemic Mastocytosis

Mostly Adults (c-kit D816V)
Diagnosis: MPD
- Biopsy of BM (and Skin)
- Apply SM Criteria
- Define SM Variant

Cutaneous 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

- CM, n=6
- ISM/SSM/BMM, n=34
- ASM/MCL n=4
- SM-AHNMD, n=6
- Myelomastocytic Leukemia, n=5

Months: 0 12 24 36 48 60 72 84 96 108 120 132 144
Probability of Survival: 0.0 0.2 0.4 0.6 0.8 1.0
WHO Classification: **Criteria** for Systemic Mastocytosis (SM-Criteria)

**Major Criteria**
- Multifocal dense mast cell (MC) infiltrates (≥15 MC/infiltrate) in the bone marrow or in another extracutaneous organ

**Minor Criteria**
- >25% spindle-shaped cells in MC-infiltrates; or >25% of all MC are atypical MC (type I and/or type II) in bone marrow smears
- Expression of CD2 and/or CD25 on bone marrow MC
- Serum tryptase level >20 ng/ml (does not count in cases with an AHNMD)
- *c-kit* point mutation at codon 816 (mostly D816V) in bone marrow or in another extracutaneous organ

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

Metachromatic Blasts in Bone Marrow Smears

Sperr et al, Leuk Res 2001;25:529
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

Sperr et al, Leuk Res 2001;25:529
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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

![Graph showing tryptase levels in various hematologic disorders](image_url)
Correlation between Serum Tryptase Levels and Percentage of Mast Cell Infiltrates

$r=0.8$
Serum Tryptase Levels in various Groups of Patients with SM
WHO Classification: Criteria for Systemic Mastocytosis (SM-Criteria)

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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)
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Reported in</th>
<th>Frequency in SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-kit D816V</td>
<td>all variants of SM</td>
<td>&gt;80%</td>
</tr>
<tr>
<td></td>
<td>some cases of CM</td>
<td></td>
</tr>
<tr>
<td>c-kit D816Y</td>
<td>ISM, SM-AHNMD, CM ?</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>c-kit D816F</td>
<td>ISM, CM ?</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>c-kit D816H</td>
<td>SM-AHNMD</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>c-kit D812G</td>
<td>SM/ASM</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>c-kit D560G</td>
<td>SM/ISM</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>c-kit F522C</td>
<td>SM/ISM</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>c-kit E839K</td>
<td>CM</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>c-kit V531I</td>
<td>SM-AHNMD</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>c-kit K509I</td>
<td>ISM/ASM</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>
THE NATURAL CLINICAL COURSE IN SYSTEMIC MASTOCYTOSIS

Serum Tryptase Levels in Patients with ISM (D816V+)

Clonal hematopoietic stem cell

death

c-kit D816V

MCL

ASM

SM-AHNMD

ISM

SM
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
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

1 Major + 1 Minor or 3 Minor SM-Criteria

BM-Smear < 20% MC
- FAB/WHO: No AHNMD
  - B-Findings
    - No B
    - 2/3 B but No C
  - C-Findings
    - ≥ 1 C
- Myeloid Neoplasm
  - ISM
  - SSM
  - ASM
  - SM-HES/CEL
  - SM-AML

BM-Smear ≥ 20% MC
- FAB/WHO: AHNMD
  - PB-Smear <10% MC
  - PB-Smear ≥ 10% MC
  - SM-AHNMD FAB/WHO
  - Lymphoid Neoplasm
    - Mono >1000 + Dysplasia
    - Dysplasia
    - Others
  - SM-CMML
  - SM-MDS

Aleukemic MCL

MCL
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 myeloproliferation, but no criteria for MDS or MPD. Blood picture normal or slightly abnormal
- **Organomegaly (without impairment 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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pruritus, flushing</td>
<td>H$_1$ + H$_2$ antihistamines, ketotifen, topical glucocorticoids, avoid triggering factors</td>
</tr>
<tr>
<td>Recurrent hypotension and tachycardia</td>
<td>H$_1$ + H$_2$ antihistamines, glucocorticoids, avoidance of triggering factors</td>
</tr>
<tr>
<td>Recurrent shock</td>
<td>H$_1$ + H$_2$ antihistamines, glucocorticoids, epinephrin</td>
</tr>
<tr>
<td>Co-existing allergy</td>
<td>H$_1$ + H$_2$ antihistamines, glucocorticoids, avoidance of triggering factors, hyposensitization</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>H$_1$ + H$_2$ antihistamines, proton pump inhibitors</td>
</tr>
<tr>
<td>Diarrhea, abdominal pain, cramping, nausea</td>
<td>H$_1$ + H$_2$ antihistamines, oral cromolyn sodium, glucocorticoids, leukotriene antagonists</td>
</tr>
<tr>
<td>Bone pain</td>
<td>Analgetics, radiation for severe localized bone pain</td>
</tr>
<tr>
<td>Osteopenia, osteoporosis</td>
<td>Vitamin D, calcium, estrogen, testosteron, biphosphonates, low dose interferon-alpha (IFN$_{\alpha}$)</td>
</tr>
<tr>
<td>Neurologic symptoms</td>
<td>H$_1$ + H$_2$ antihistamines, oral cromolyn sodium</td>
</tr>
</tbody>
</table>
Urticaria pigmentosa-like skin lesions in a patient with SM prior to PUVA therapy

3 months after PUVA
# Treatment of Mastocytosis: Cytoreductive Drugs

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

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

IFNα-2b, 3x10^6U, 3x/w s.c. & Prednisone, 25 mg/d p.o.

[Graph showing the changes in Tryptase and Alkaline Phosphatase levels over 12 months of treatment]
# Reported Effects of 2-Chloro-2´-Deoxyadenosine (2CdA) = Cladribine in Patients with Mastocytosis

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

<table>
<thead>
<tr>
<th>Therapy in ASM</th>
<th>MR rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon-alpha 2b s.c. (9-42 million units per week) plus glucocorticoids</td>
<td>15-20 %</td>
</tr>
<tr>
<td>2-Chloro-2′-Deoxyadenosine (2CdA) = Cladribine i.v. (2-3 h) 0.1-0.15 mg/kg/d, d 1-5, 1-6 cycles</td>
<td>~ 50 %</td>
</tr>
</tbody>
</table>
Follow up of 2 Patients with SM during Therapy

![Graph showing tryptase levels over time with various treatments indicated.](image-url)
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

<table>
<thead>
<tr>
<th>TARGETED DRUG</th>
<th>DRUG TARGET(s)</th>
<th>SM VARIANT(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC412</td>
<td>wt KIT, KIT[mut]</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>AMN107</td>
<td>wt KIT, KIT[mut]</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>BMS354825</td>
<td>wt KIT, KIT[mut]</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>AP23464</td>
<td>KIT[mut], wt KIT</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>17AAG</td>
<td>Chaperone, KIT[mut]</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>Targeting Antibodies</td>
<td>CD25, CD33, CD44, CD52, ..</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>Antisense, siRNA</td>
<td>KIT, MITF, Mcl-1, ....</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>VEGF-targeting Drugs</td>
<td>VEGF, Angiogenesis</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>FTIs (R115777), FTS</td>
<td>Farnesyltransferase</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
<tr>
<td>Rapamycin &amp; Derivatives</td>
<td>mTOR, VEGF</td>
<td>ASM, MCL, SM-AHNMD</td>
</tr>
</tbody>
</table>