Bench to Bedside: Advances in Mastocytosis

Tracy I. George, M.D.
Associate Professor of Pathology
University of New Mexico Health Sciences Center

Tryptase
PD-L1
Disclosures

- Allakos grant
- Blueprint Medicine consulting fees
- Novartis consulting fees
- Novartis steering committee
Outline

- Definition of mastocytosis
- Advanced mastocytosis
- The case that started it all
- Results of clinical trials
- Other potential therapies
What is mastocytosis?

- Clonal, neoplastic proliferation of mast cells
- Heterogeneous disorder:
  - Skin lesions that spontaneously regress to highly aggressive leukemias with short survival and multiorgan failure
- Subtypes determined by distribution and clinical manifestations
Classification of Mastocytosis

- Cutaneous mastocytosis
  - Skin only

- Systemic mastocytosis
  - Extracutaneous (mostly bone marrow)
  - Can have skin involvement

Cutaneous mastocytosis

CD117

Systemic mastocytosis

• Clinical manifestations reflect mediator release from mast cells or infiltration of mast cells into tissues
• Constitutional signs
• Skin lesions
• Mediator-related findings
  – flushing, syncope, diarrhea, hypotension, headache, abdominal pain
• Musculoskeletal pain
Pathogenesis of Systemic Mastocytosis

- Constitutive activation of the receptor tyrosine kinase KIT
  - Somatic KIT point mutations are detectable in neoplastic mast cells in most patients
- Most common activating mutations of KIT: D816V, D816Y
- Activation of c-KIT induces increased mast cell proliferation and motility, resulting in infiltration of neoplastic mast cells into various organs including the BM

Definition of systemic mastocytosis

**Major:** Multifocal dense infiltrates of mast cells

**Minor:**
- >25% of mast cells in biopsy are spindle-shaped or atypical morphology; >25% immature/atypical mast cells in bone marrow aspirate smears
- D816V KIT mutation
- CD25 expression on mast cells
- Serum total tryptase >20 ng/mL (unless associated myeloid disorder)

Cytology of mast cells

Normal/reactive  Atypical type I

Atypical type II  Metachromatic blast

Cytology of mast cells

Normal/reactive

Atypical type I

Atypical type II

Metachromatic blast

Systemic mastocytosis

- Indolent SM
- Advanced SM
  - Aggressive SM
  - Mast cell leukemia
  - SM (indolent or aggressive) + associated hematological neoplasm (SM-AHN)
Advanced Systemic Mastocytosis

**Aggressive systemic mastocytosis** (1+ “C” findings)

- Bone marrow dysfunction → cytopenias
- Palpable hepatomegaly with impairment of liver function, ascites, and/or portal hypertension
- Skeletal involvement with large osteolytic lesions, and/or pathological fractures
- Palpable splenomegaly with hypersplenism
- Malabsorption with weight loss due to GI mast cell infiltrates, hypoalbuminemia

**Mast cell leukemia**

- BM biopsy with a diffuse infiltration of compact, immature, atypical mast cells
- Aspirate smears with >20% mast cells
Indolent systemic mastocytosis

tryptase
ASM-AHN

ASM-MDS/MPN,U

ASM-CMML

CD117

tryptase
Mast cell leukemia

Bone marrow aspirate

Laboratory values:
Hb: 8.8 g/dL
WBC, PLT: Normal
Serum tryptase: 763
Bone marrow biopsy
Tryptase IHC positive
KIT D816V positive

MAST CELL ANALYSIS
Overall Survival

- Kaplan–Meier survival for SM patients classified by WHO disease type compared with the expected age and sex-matched US population’s survival for the entire cohort.

Lim, KH. Blood. 2009 Jun 4;113(23):5727.
**KIT Mutations:**
**Implications for TK Inhibitors**

- **Juxtamembrane domain**
- **Transmembrane domain**
- **Extracellular ligand-binding domain**
- **Tyrosine kinase domain 1**
- **Tyrosine kinase domain 2**
- **Kinase insert**
- **Dimerization domain**

**Mutations:**
- SM V560G; GIST Exon 11
  - Sinonasal NK/T-CL V559I, E561K
  - GIST Exon 13
    - SM D816V, Y
    - AML: D816V
    - Germ cell
    - Sinonasal
- GIST Exon 9
  - AML (Asp 419) Exon 8
- GIST Exon 17
  - AML: D816V
  - Germ cell
  - Sinonasal

**Imatinib sensitive**
- Rare

**Imatinib resistant**
- D816V~80%

**Gene Mutations:**
- GIST: Gastrointestinal stromal tumors
- SM: Systemic Mastocytosis
- AML: acute myelogenous leukemia
- NK/T-CL: Natural killer/T-cell lymphoma

GIST: Gastrointestinal stromal tumors; SM: Systemic Mastocytosis; AML: acute myelogenous leukemia; NK/T-CL: Natural killer/T-cell lymphoma
Treatment

• Challenging due to the diversity and complexity of disease and the lack of a standard and highly effective therapy

• Current therapies include:
  – Observation
  – Topical therapies for cutaneous disease
  – Symptomatic noncytoreductive therapies
  – Cytoreductive therapy
    • Indicated by the presence of organ dysfunction
    • Used to reduce mast cell burden

Sensitivity of c-KIT D816V-Transformed Ba/F3 Cell Lines to Midostaurin and Imatinib

- Midostaurin inhibited growth of all c-KIT-transformed Ba/F3 cell lines
- Cell lines resistant to imatinib due to expression of c-KIT D816V are inhibited by midostaurin
- Results have been confirmed in additional cell lines

IC$_{50}$ for midostaurin: 44 nM
IC$_{50}$ for imatinib: > 1 uM

Midostaurin

- Midostaurin is a potent inhibitor of all common mutant forms of c-KIT, including D816V and D816Y
- Midostaurin may preferentially inhibit cells expressing mutant c-KIT compared to wild-type c-KIT
- Midostaurin counteracts anti-IgE-induced release of histamine in blood basophils and cultured cord blood cell-derived mast cells
  - Effects were dose-dependent and occurred at pharmacologic concentrations

Activity of the tyrosine kinase inhibitor PKC412 in a patient with mast cell leukemia with the D816V *KIT* mutation


The majority of patients with systemic mast cell disease express the imatinib-resistant Asp816Val (D816V) mutation in the KIT receptor tyrosine kinase. Limited treatment options exist for aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL). We evaluated whether PKC412, a small-molecule inhibitor of KIT with a different chemical structure from imatinib, may have therapeutic use in advanced SM with the D816V KIT mutation. We treated a patient with MCL (with an associated myelodysplastic syndrome [MDS]) by using PKC412. The patient exhibited a partial response with significant resolution of liver function abnormalities. In addition, PKC412 treatment resulted in a significant decline in the percentage of peripheral blood mast cells and serum histamine levels and was associated with a decrease in KIT phosphorylation and development of anemia.
Phase II Trial of Midostaurin

- Aggressive Systemic Mastocytosis
- Mast Cell Leukemia
- Aggressive Systemic Mastocytosis with an Associated Hematological Clonal Non-Mast Cell Lineage Disease

Sites
Stanford
Washington University
Dana Farber
University of Michigan

## Patient characteristics

<table>
<thead>
<tr>
<th>No. Enrolled</th>
<th>26</th>
</tr>
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<tbody>
<tr>
<td>M:F</td>
<td>15:11</td>
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<tr>
<td>Median Age (yrs)</td>
<td>62 (24-79)</td>
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### SM Subtypes

<table>
<thead>
<tr>
<th>SM Subtypes</th>
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<tbody>
<tr>
<td>ASM</td>
<td>2</td>
</tr>
<tr>
<td>SM-CMML</td>
<td>14</td>
</tr>
<tr>
<td>SM-MDS</td>
<td>2</td>
</tr>
<tr>
<td>SM-MDS/MPN-U</td>
<td>1</td>
</tr>
<tr>
<td>MCL</td>
<td>7</td>
</tr>
<tr>
<td>Response Type</td>
<td>Count (Percentage)</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>--------------------</td>
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<tr>
<td><strong>Major response</strong></td>
<td>10 (38%)</td>
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<tr>
<td>- Incomplete</td>
<td>6</td>
</tr>
<tr>
<td>- Pure clinical</td>
<td>4</td>
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<tr>
<td><strong>Partial response</strong></td>
<td>8 (31%)</td>
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<tr>
<td>- Good partial</td>
<td>5</td>
</tr>
<tr>
<td>- Minor</td>
<td>3</td>
</tr>
<tr>
<td><strong>Stable disease</strong></td>
<td>4 (15%)</td>
</tr>
<tr>
<td><strong>Progressive disease</strong></td>
<td>4 (15%)</td>
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S-01

MCL-CMML

D816V neg

Good partial clinical
Off drug 3 weeks due to thrombocytopenia
Midostaurin treatment is associated with a significant decrease in CD25 expression on neoplastic mast cells.

CD25 expression on neoplastic mast cells by flow cytometry in patients #1, #2, and #3 before (A-C) and on day 28 (patient #1, D), day 89 (patient #2, E), and day 336 (patient #3, F) of midostaurin therapy.
Midostaurin therapy is associated with sustained decreases in CD25 expression on neoplastic mast cells by mean fluorescence intensity (MFI; A, C, E) and percent of mast cells positive for CD25 (B, D, F) over time in patient #1 (A-B), patient #2 (C-D), and patient #3 (E-F). The shaded area indicates time on midostaurin therapy.
Proposed mechanism for midostaurin-induced CD25 downregulation

Changes in the intracellular localization of STAT5 with midostaurin
>50% Reduction in Marrow MC Burden by IHC (n=10)
Midostaurin Effects on the AHNMD

Patient S-10

CMML with eosinophilia

End of Midostaurin Cycle

Abs Eos Count/mm³

Eos %

Patient D-03

CMML with eosinophilia

End of Midostaurin Cycle

Abs Mono Count/mm³

Abs Eos/mm³

Eosinophils

Monocytes

Pt S-13, SM-CMML with Eosinophilia, D816V KIT+
## KIT MUTATION ANALYSIS (n=26)

<table>
<thead>
<tr>
<th>Mutation status</th>
<th># pts (%)</th>
<th>Clinical Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>D816V KIT Positive</td>
<td>18/26 (69%)</td>
<td>10 MRs (all)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 Good Partial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Good Minor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Stable Disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Progressive Disease</td>
</tr>
<tr>
<td>D816V KIT Negative</td>
<td>7/26 (27%)</td>
<td>3 Good partial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Stable Disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Progressive Disease**</td>
</tr>
<tr>
<td>Novel MPL Mutation</td>
<td>1/26 (4%)</td>
<td>1 Good partial</td>
</tr>
<tr>
<td>(2 amino acid insertion)</td>
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</table>

Association between positivity for D816V KIT and achieving a MR versus any other type of response (p=0.0095, Fisher’s exact test).

*Allele-specific PCR by Dr. Chris Corless, OHSU

**1 patient with ASM had a constitutional t(5;12)(q31;p12) translocation
Midostaurin (PKC412) in Advanced Systemic Mastocytosis (SM): Updated Results of the Global D2201 Trial

Jason Gotlib, Hanneke C Kluin-Nelemans, Tracy I George, Cem Akin, Karl Sotlar, Olivier Hermine, Farrukh Awan, Elizabeth Hexner, Michael Mauro, Rodica Morariu, Margaret Squier, Matthieu Villeneuve, Fabienne Emery-Salbert, John Coombs, Karin Hartmann, Hans-Peter Horny, Peter Valent, and Andreas Reiter

on behalf of the D2201 investigators


Study Design

**Treatment**
- Fleming 2-stage design: stage 1 (n = 40) and extension phase (n = 49)
- Midostaurin 100 mg po bid on 28-d continuous cycles until disease progression/unacceptable toxicity

**Patient eligibility**
- ASM or MCL, with or without an AHN (myeloid neoplasm)
- 1 or more measurable C-findings (organ damage) considered related to SM

**Primary endpoint: ORR**
- Major or partial response in the first 6 cycles of treatment (modified Valent criteria, Cheson criteria)
- Responses must be maintained ≥ 8 weeks
- Eligibility and responses adjudicated by the Study Steering Committee; central pathology review

**Secondary endpoints: duration of response, OS**

**Exploratory endpoints: symptoms, QOL**
## Stage 1: Baseline Disease Characteristics

(N = 40)

<table>
<thead>
<tr>
<th>Histopathology review</th>
<th>Patients, %</th>
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<tbody>
<tr>
<td>ASM</td>
<td>83</td>
</tr>
<tr>
<td>ASM with AHN</td>
<td>68</td>
</tr>
<tr>
<td>MCL</td>
<td>18</td>
</tr>
<tr>
<td>MCL with AHN</td>
<td>8</td>
</tr>
<tr>
<td>AHNMD</td>
<td>75</td>
</tr>
<tr>
<td>CMML</td>
<td>25</td>
</tr>
<tr>
<td>MDS/MPN-U</td>
<td>25</td>
</tr>
<tr>
<td>HES or CEL</td>
<td>10</td>
</tr>
<tr>
<td>MDS</td>
<td>5</td>
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<tr>
<td>PMF</td>
<td>3</td>
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<tr>
<td>MPN-U</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
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<table>
<thead>
<tr>
<th>Kit D816V/Y mutation status</th>
<th>Patients, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>70</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>23</td>
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<table>
<thead>
<tr>
<th>Median tryptase level (range), µg/L</th>
<th>200 (28-1950)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median bone marrow mast cell burden (range), %</td>
<td>50 (10-90)</td>
</tr>
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</table>
# Best Overall Response

**Best Overall Response, Efficacy Population (N = 40)**

<table>
<thead>
<tr>
<th>Patients, %</th>
<th>ORR (= MR + PR), % (95% CI)</th>
<th>60% (43-75)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease control rate (DCR = MR + PR + SD), % (95% CI)</td>
<td>80% (64-91)</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Major response</th>
<th>53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission</td>
<td>0</td>
</tr>
<tr>
<td>Incomplete remission</td>
<td>23</td>
</tr>
<tr>
<td>Pure clinical response</td>
<td>20</td>
</tr>
<tr>
<td>Unspecified</td>
<td>10</td>
</tr>
<tr>
<td>Good partial response</td>
<td>8</td>
</tr>
</tbody>
</table>

| Stable disease | 20 |
| Progressive disease | 8 |
| Not evaluable* | 13 |

**Best Overall Response by SM Subtype, n (%)**

<table>
<thead>
<tr>
<th>ASM (n = 33)</th>
<th>MCL (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>58</td>
</tr>
<tr>
<td>Major response</td>
<td>48</td>
</tr>
<tr>
<td>Partial response</td>
<td>9</td>
</tr>
</tbody>
</table>

* P < .0001.

* Concurrent use of high-dose steroids (n = 2), insufficient follow-up (n = 2), transfusion unrelated to SM confounding response (n = 1).

MR, major response; PR, partial response; SD, stable disease.
Bone marrow mast cell burden was reduced by ≥ 50% in:
15/32 (47%) evaluable patients
10/20 (50%) patients with baseline mast cell burden ≥ 50%

Median best change in serum tryptase level:
−61% (range, −97% to 16%)

Evaluable patients had an assessment at baseline and at least one post-baseline assessment during the first 6 cycles of treatment.
### Overall Survival

**Median OS, Months (Range; 95% CI)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Median OS, Months (Range; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (N = 40)</td>
<td>41 (0-44; 20.3-NR)</td>
</tr>
<tr>
<td>Patients with ASM (n = 33)</td>
<td>41 (0-44; 20.3-NR)</td>
</tr>
<tr>
<td>Patients with MCL (n = 7)</td>
<td>Not reached (4-43; NR)</td>
</tr>
</tbody>
</table>

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**Median duration of follow-up**\(^a\): 35 months (range, 20-46)

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\(^a\): Measured as the time from treatment start to data cut-off.
Outline

• Updates on the classification of mastocytosis
• Advanced mastocytosis
• The case that started it all
• Results of clinical trials
• Other potential therapies
CD30 expression in systemic mastocytosis

CD30 expression in systemic mastocytosis

Clinical trial with anti-CD30 drug in advanced systemic mastocytosis

Clinical sites:
Stanford, MD Anderson

Pathology:
UNM
PD-1/PD-L1 are novel therapeutic targets for mastocytosis

Smoldering systemic mastocytosis

Mast cell leukemia

M Geeze, E Hatch, C Martin, S Perkins, K Hartmann, P Valent, J Gotlib, D Lidke. USCAP 2016
**PD-1/PD-L1 are novel therapeutic targets for mastocytosis**

<table>
<thead>
<tr>
<th>DIAGNOSIS</th>
<th>PD-1</th>
<th>PD-L1</th>
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<tbody>
<tr>
<td>Systemic mastocytosis</td>
<td>0/26</td>
<td>17/22 (77%)</td>
</tr>
<tr>
<td>Cutaneous mastocytosis</td>
<td>6/31 (19%)</td>
<td>25/26 (84%)</td>
</tr>
<tr>
<td>Myelomastocytic leukemia</td>
<td>0/2</td>
<td>1/1</td>
</tr>
<tr>
<td>MMAS</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Normal/reactive BM</td>
<td>0/21</td>
<td>0/15</td>
</tr>
<tr>
<td>MPN</td>
<td>0/17</td>
<td>0/16</td>
</tr>
<tr>
<td>MDS</td>
<td>0/18</td>
<td>0/18</td>
</tr>
<tr>
<td>MDS/MPN</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

**MCL: 3/3**  
**ASM: 2/2**  
**SM-AHD: 8/11**  
**ISM: 4/6**
Thank you collaborators!

Jason Gotlib
Dan Arber
Susan Atwater
Bruno Medeiros
Athena Cherry

Natasha Savage
Farrukh Awan

Chris Corless
Ilana Kepten
Jeffrey Tyner

Peter Valent

Timothy Graubert

Dan Deangelo

Eric Hsi

Hans-Peter Horny
Karl Sotlar

REMA (Spanish Network on Mastocytosis)
## D2201 Acknowledgements

### Investigators

<table>
<thead>
<tr>
<th>United States</th>
<th>The Netherlands</th>
<th>Germany</th>
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</thead>
<tbody>
<tr>
<td>Jason Gotlib</td>
<td>Hanneke C Kluin-Nelemans</td>
<td>Andreas Reiter</td>
</tr>
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<td>Olivier Hermine</td>
<td>Karin Hartmann</td>
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<td>Gandhi Damaj</td>
<td>Philipp LeCoutre</td>
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<tr>
<td>Michael Mauro</td>
<td></td>
<td>Philippe Schaufhausen</td>
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<tr>
<td>Gary Schiller</td>
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<tr>
<td>Daniel J. De Angelo</td>
<td>Ingunn Dybedal</td>
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<td>Harry Erba</td>
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<tr>
<td>Mark Heaney</td>
<td>Andrzej Hellman</td>
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<tr>
<td>Lawrence Schwartz</td>
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<tr>
<td>United Kingdom</td>
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<tr>
<td>Deepti Radia</td>
<td>Akif Yavuz</td>
<td></td>
</tr>
<tr>
<td>Richard Clark</td>
<td>Andrew Wei</td>
<td></td>
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<tr>
<td>Mark Drummond</td>
<td>Harry Land</td>
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</tbody>
</table>

### Study Steering Committee

- Cem Akin
- Tracy George*
- Jason Gotlib
- Karin Hartmann
- Hans-Peter Horny*
- Hanneke C Kluin-Nelemans
- Andreas Reiter
- Peter Valent

* Central pathologists

### KIT Mutation Analysis

- Karl Sotlar