### Disclosures for Ayalew Tefferi

<table>
<thead>
<tr>
<th>PI/co-PI</th>
<th>Celgene, Novartis, BMS, YM Biosciences, Sunovion, Aventis, Incyte, PharmaMar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research support</td>
<td>None</td>
</tr>
<tr>
<td>Employee/Consultant</td>
<td>None</td>
</tr>
<tr>
<td>Major Stockholder</td>
<td>None</td>
</tr>
<tr>
<td>Speakers' Bureau</td>
<td>None</td>
</tr>
<tr>
<td>Scientific Advisory Board</td>
<td>None</td>
</tr>
</tbody>
</table>

Presentation includes discussion of the following off-label use of a drug or medical device:
- Hydroxyurea
- Interferon-alpha
- Busulfan
- Ruxolitinib
- Cladribine
- ESAs
- Prednisone
- Danazol
- Androgens
- Thalidomide
- Lenalidomide
- Imatinib
- Alemtuzumab

---

### Myeloproliferative Neoplasms (MPN)

1. Chronic myelogenous leukemia
2. Polycythemia vera
3. Essential thrombocythemia
4. Primary myelofibrosis
5. Mastocytosis
6. Primary eosinophilia
7. Chronic neutrophilic leukemia
8. MPN-U

---

### Myeloid Malignancies

- Acute myeloid leukemia
- Myeloproliferative Neoplasms

### Objectives

1. Pathogenetic mechanisms
2. Contemporary diagnosis
3. Prognostication
4. Management
Diagnosis

The two pathogenetic faces of myelofibrosis

- Secondary inflammatory state
  - Abnormal cytokine milieu
  - Activation of genes important in proliferation and survival

- Mutation-driven clonal myeloproliferation
  - Bone marrow fibrosis
  - Leukocytosis
  - Thrombocytosis
  - Ineffective hematopoiesis (anemia)
  - Extramedullary hematopoiesis (splenomegaly)

Disease-initiating mutations
- BCR-ABL1
- JAK2(+)

Disease-transforming mutations
- JAK2, MPL, TET2, ASXL1, CBL, IDH1, IDH2, DNMT3A, SF3B1, TP53

Phenotype-modifying mutations
- PV, ET, PMF

Disease and Genetic Abnormalities

- Not found in secondary polycythemia
- Not found in reactive thrombocytosis
- Not found in lymphoma or metastatic cancer
- JAK2, MPL, TET2, ASXL1, CBL, IDH1/2, IKZF4, LNK, EZH2, DNMT3A, SF3B1, TP53

Diagnostic algorithm

Polycythemia vera
- Blood JAK2(V617F) and Epo screen
- JAK2(V617F) screen

Essential thrombocythemia
- Blood JAK2(V617F) screen

Myelofibrosis
- BM biopsy

Levine et al. Nat Rev Cancer. 2007;7:673

JAK2, MPL, TET2, ASXL1, CBL, IDH1, IDH2, DNMT3A, SF3B1, TP53

Diagnosis

PV 97% V617F and 3% JAK2 exon 12 mutations
ET 60% V617F and 4% MPL515 mutations
PMF 60% V617F and 8% MPL 515 mutations
NOT FOUND IN SECONDARY POLICYTHEMIA
NOT FOUND IN REACTIVE THROMBOCYTOSIS
NOT FOUND IN LYMPHOMA OR METASTATIC CANCER

JAK2 mutations
- Activation of genes important in proliferation and survival
- Stimulation of signal transduction pathways

Blood JAK2V617F and Epo screen

PV (+) V617F
- Epo subnormal
- Epo normal or elevated

PV (-) V617F
- MPN present
- MPN possible

Polycythemia vera
- V617F, +9 or 13q- = PMF

CML
- Blast phase
- CML

MDS
- e.g. RARS-T

ET VS.

Prefibrotic PMF
- VS.

Myelofibrosis
Survival and risk of leukemic transformation in ET are significantly influenced by accurate morphologic diagnosis: An international study of 1,104 patients. Barbui et al. JCO 2011;29:3179

10-year AML risk <1%
10-year MF risk <1%
10-year AML risk 6%
10-year MF risk 12%

Prognosis

IPSET model for ET

Survival and prognosis in primary myelofibrosis: A Mayo Clinic study of 884 patients

Based on 8 risk factors: Karyotype, Transfusion-dependency, Hgb <10, Plt <100, WBC >25, Circulating blasts >1%, constitutional symptoms, Age >65

DIPSS-plus intermediate-1 risk; n=128
median survival ~ 94 months; 2-year mortality 11%
DIPSS-plus low risk; n=84
median survival ~ 200 months; 2-year mortality 3%
DIPSS-plus high risk; n=298
median survival ~ 23 months; 2-year mortality 53%
DIPSS-plus intermediate-2 risk; n=322
median survival ~ 44 months; 2-year mortality 26%

Very high risk category; n=52
median survival ~ 9 months; 2-year mortality 82%

Survival and prognosis in primary myelofibrosis:

Based on 8 risk factors: Karyotype, Transfusion-dependency, Hgb <10, Plt <100, WBC >25, Circulating blasts >1%, constitutional symptoms, Age >65

DIPSS-plus intermediate-1 risk; n=128
median survival ~ 94 months; 2-year mortality 11%
DIPSS-plus low risk; n=84
median survival ~ 200 months; 2-year mortality 3%
DIPSS-plus high risk; n=298
median survival ~ 23 months; 2-year mortality 53%
DIPSS-plus intermediate-2 risk; n=322
median survival ~ 44 months; 2-year mortality 26%

Very high risk category; n=52
median survival ~ 9 months; 2-year mortality 82%

ASXL1
SRSF2
EZH2
IDH
Management

Issues of relevance in myeloproliferative neoplasms

1. Shortened survival
   - Leukemic transformation
   - Thrombosis

2. Poor quality of life
   - Anemia
   - Splenomegaly
   - Cachexia

Constitutional symptoms
   - Pruritus
   - Anemia
   - Splenomegaly

Thrombosis

CIRN_23506_5_Myelodysplastic Syndrome_DT10 7/18/2005 2:18 PM

*New data suggests a lower risk of thrombosis associated with extreme thrombocytosis, which however is associated with a bleeding diathesis in the presence of AVWS and aspirin therapy. Barbui et al. JCO 2011;29:3179

Risk stratification for thrombosis in ET and PV

<table>
<thead>
<tr>
<th>Category</th>
<th>Criteria</th>
<th>Thrombosis risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk</td>
<td>Age &lt; 60 years and no history of thrombosis and platelet count &lt; 1 million</td>
<td>Not significantly increased compared to controls</td>
</tr>
<tr>
<td>High-risk</td>
<td>Age ≥ 60 years or previous thrombosis</td>
<td>Significantly increased</td>
</tr>
<tr>
<td>Low-risk with extreme thrombocytosis</td>
<td>Platelet count ≥ 1 million/μL</td>
<td>Might be lower?</td>
</tr>
</tbody>
</table>

Observation
Hydroxyurea (Plt < 400k) + ASA
Phlebotomy if PV

My current treatment algorithm in ET and PV

Low-risk without extreme thrombocytosis
ASA

Low-risk with extreme thrombocytosis
Hydroxyurea sensitive

High-risk hydroxyurea sensitive

ASA

Randomized study in high-risk ET

P=0.0001

Hydroxyurea n=58
Controls n=58
Additional management issues in PV and ET

1. What about interferon alpha or JAK inhibitors as first-line therapy?
   - Both have been shown to induce remissions
   - Interferon reduces JAK2 V617F allele burden in a subset of patients
   - The question is, will they be as safe and as effective as hydroxyurea/ASA

2. What about treatment during pregnancy?
   - Low-risk…ASA only
   - High-risk…IFN alpha

3. What about treatment of pruritus?...paroxetine, IFN-alpha, UVB, JAK inhibitor

4. What if you can’t use hydroxyurea (i.e. second-line therapy)
   - Interferon alpha
   - Busulfan
   - JAK inhibitors?

My current treatment algorithm in ET and PV

Myelofibrosis treatment algorithm

Management of Myelofibrosis

Conventional therapy
- Treatment for anemia
  - Erythropoietin
  - Corticosteroids
  - Androgens + Prednisone
  - Darapladib
  - Thalidomide + Prednisone
  - Lenalidomide
- Treatment for splenomegaly
  - Hydroxyurea
  - Splenectomy
- Treatment for extramedullary hematopoiesis
  - Low-dose irradiation
- Supportive care

Experimental drug therapy
1. Pomalidomide
2. JAK inhibitors
3. Others
   - mTOR inhibitors
   - Hypomethylating agents
   - HDAc inhibitors
   - Telomerase inhibitors
   - Etc.

Thalidomide
- Anemia-20%
  - Splanenmangly-5%
- Neoptrophy

Lenalidomide
- Anemia-22%
  - Splanenmangly-33%
- Myeloosuppression
- Less neuropathy

Pomalidomide
- Anemia-25%
  - Splenomegaly 0%
- 3 agents
  - 0.5 mg/kg dose

Thalidomide
- Neoptrophy
- Myeloosuppression
- Less neuropathy

Lenalidomide
- Neoptrophy
- Myeloosuppression
- Less neuropathy

Pomalidomide
- Neoptrophy
- Myeloosuppression
- Less neuropathy

Works best in the presence of 5q-
Pomalidomide 0.5 mg/day
Mayo Clinic study of 58 patients with myelofibrosis and anemia

- Well tolerated with little or no myelosuppression, neuropathy or thrombosis
- Early basophilia predicted anemia response
- 58% platelet response in patients with < 100k platelets
- No spleen responses
- Increasing dose to 2 mg/day did not increase anemia response

<table>
<thead>
<tr>
<th>JAK2V617F</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 patients</td>
<td>Anemia response = 24%</td>
<td></td>
</tr>
<tr>
<td>16 patients</td>
<td>Anemia response = 6%</td>
<td></td>
</tr>
</tbody>
</table>

JAK inhibitors

- Ruxolitinib
  - IC50 values: JAK2 2.8, JAK1 3.3, JAK3 428, TYK2 1948, Other targets
  - Current stage: Symp 42%, Resp 29%
  - Spleen Resp 14%, Anem Resp 18%
  - Side effects: Thrombocytopenia, anemia, rash, headache, transaminitis

- SAR302503
  - IC50 values: JAK2 3, JAK1 105, JAK3 1040, Other targets
  - Current stage: Symp >50%, Resp 39%
  - Spleen Resp 0%, Anem Resp 0%
  - Side effects: Nausea/Diarrhea, anemia, thrombocytopenia, transaminosis, hyper-lipase/amylasemia

- Lestaurtinib
  - Current stage: Symp NR, Resp >18%
  - Side effects: Nausea/Diarrhea, anemia, thrombocytopenia

- CYT387
  - Current stage: Symp >50%, Resp 45%
  - Side effects: Thrombocytopenia, headaches

- SB1518
  - Current stage: Symp >50%, Resp 32%
  - Side effects: Thrombocytopenia

- XL019
  - Current stage: Symp >50%, Resp 33%
  - Side effects: Peripheral neuropathy, transaminosis

- AZD1480
  - Current stage: Symp NR, Resp >22%
  - Side effects: Nausea/Diarrhea, anemia

- BMS911543
  - Current stage: Symp NR, Resp NR
  - Side effects: Nausea/Diarrhea

- NS-018
  - Current stage: Symp NR, Resp NR
  - Side effects: Nausea/Diarrhea, electrolyte abnormalities/TLS?

- XL019
  - Current stage: Symp >50%, Resp 33%
  - Side effects: Peripheral neuropathy

Diagnostic Algorithm for Primary Eosinophilia

1st step
- Peripheral blood screening for FIP1L1-PDGFRA using FISH or RT-PCR

2nd step
- Bone marrow biopsy with cytogenetics

3rd step
- Peripheral blood lymphocyte phenotyping and TCR gene rearrangement studies

Long-term outcome of treatment with ruxolitinib

Comparison of survival between 51 patients with myelofibrosis treated with ruxolitinib versus 410 patients with primary myelofibrosis seen at the Mayo Clinic in the last 10 years (adjusted for DIPSS-plus)

Treatment algorithm in “HES”
When should you suspect mastocytosis?

- Urticaria pigmentosa-like lesions
- Mast cell mediator symptoms  
  - Anaphylactoid symptoms/dizziness/headache  
  - Diarrhea  
  - Flushing/urticaria
- Osteopenia/unexplained fractures

Practical classification of mast cell disease

1. Cutaneous mastocytosis  
   (skin-only disease)
   - Both can manifest mast cell mediator release symptoms

2. Systemic mastocytosis (SM)
   - Indolent SM
   - Aggressive SM (cytopenia, bone disease, organomegaly, etc.)
   - 1. SM without associated 2nd myeloid neoplasm
   - 2. SM with associated 2nd myeloid neoplasm
   - 3. Mast cell leukemia

Survival for 342 systemic mastocytosis patients classified by disease type compared with the expected age and gender matched US Population's survival

Infection with H1 and H2 blockers  
Topical steroids

If this fails, OK to try IFN-α or cladribine

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Mutations</th>
</tr>
</thead>
</table>
| 1. Absolute monocyte count > 10 x 10^9/L  
2. No BCR-ABL1  
3. No PDGFR mutation  
4. < 20% blasts or promonocytes  
5. Dysplasia or abnormal karyotype present |
| 1. ASXL1 (30-50%)  
2. SRSF2 (30-50%)  
3. TET2 (20-40%)  
4. RAS (20-40%)  
5. RUNX1 (20-30%)  
6. CBL (10-20%)  
7. JAK2, EZH2, IDH, etc. (~10%) |

Prognosis (Mayo model ASH 2012)

<table>
<thead>
<tr>
<th>Treatment</th>
</tr>
</thead>
</table>
| 1. Observation (preferred)  
2. Transplant; 5-year OS 20-40%  
3. Investigational drug therapy  
4. Decitabine; OR ~30%  
5. Hydroxyurea |

Prognosis (Mayo model ASH 2012)

<table>
<thead>
<tr>
<th>Treatment</th>
</tr>
</thead>
</table>
| 1. Observation (preferred)  
2. Transplant; 5-year OS 20-40%  
3. Investigational drug therapy  
4. Decitabine; OR ~30%  
5. Hydroxyurea |

Survival for 342 systemic mastocytosis patients classified by disease type compared with the expected age and gender matched US Population's survival.