Anemia in Myelofibrosis

The Role of Immunomodulatory Drugs

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No Disclosures
Summary

• Myelofibrosis Anno 2012
  - early stage
  - late stage
• Mechanisms of Anemia
• The Role of Chronic Inflammation
• Treatment of Anemia
• The Role of IMiDs

IMiDs in Myelofibrosis

• Thalidomide
• Lenalidomide
• Pomalidomide
• Prednisolone
• Danazol
• Cyclosporine
• Erythropoietin
  • Interferon-alpha2
  • JAK1-2 Inhibitors
  • HDACi
### Symptoms and Clinical Findings

<table>
<thead>
<tr>
<th>Early Prefibrotic Primary Myelofibrosis</th>
<th>Advanced Primary Myelofibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombocytosis</strong></td>
<td><strong>Thrombocytopenia</strong></td>
</tr>
<tr>
<td>• Hypermetabolic Symptoms</td>
<td>• <strong>Hypermetabolic Symptoms</strong></td>
</tr>
<tr>
<td>• Myeloid Metaplasia</td>
<td>• <strong>Myeloid Metaplasia</strong></td>
</tr>
<tr>
<td>• Bone marrow failure</td>
<td>• <strong>Bone marrow failure</strong></td>
</tr>
<tr>
<td><strong>Anemia</strong></td>
<td><strong>Anemia</strong></td>
</tr>
<tr>
<td>• Moderate Leukocytosis</td>
<td></td>
</tr>
<tr>
<td>• Elevated LDH</td>
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</table>

Anemia
JAK2 V617F positive disorders: One mutation, three phenotypes – a 2012 model?

Unknown genetic event  JAK2 V617F

Lympho-myeloid precursor Stem cell

ET  PV  Post PV MF  AML

100%

% V617F alleles

Chronic Inflammation  Chronic phase  Accelerated phase

Chronic Inflammation
Potential Cell-Cytokine Interactions in the Pathogenesis of Myelofibrosis with Myeloid Metaplasia

A simplistic model of hematopoietic stem cell niches

A simplistic model of hematopoietic stem cell niches

The chicks are flying prematurely (escaping) from the burning nest
Neutrophil Granules

MMM

• Mobilization

• Metastasis

• Myeloid Metaplasia

The Inflamed Circulation

"Young birds "

5/15/2012
Pathophysiological Mechanisms

Symptoms

- Anemia
- Hypermetabolism (Fatigue, weight loss, low-grade fever, night sweats, hyperuricemia)
- Splenomegaly (mechanical discomfort, pain)
- Thrombohemorrhagic complications
- Infectious complications
- Autoimmune phenomena/disease (eg arthritis and vasculitis)

Pathophysiological Mechanisms

Anemia

- Deficiency of iron, folate, vitamin B12
- Bone marrow failure (quantitative/qualitative; inflammation)
- Hemodilution (plasma volume expansion)
- Splenic pooling/sequestration
- Hemolysis (hypersplenism, intrinsic red cell defects, red cell sensitization)
- Blood loss (platelet and/or coagulation deficiency and/or defects)
- Portal hypertension (hypertensive gastropathy, ulcer, oesophageal varices)
Anemia of Inflammation

The Inflamed Bone Marrow
Cytokine Storm
Bone Marrow Failure
Myelofibrosis with huge splenomegaly

Anemia: bone marrow failure, hemodilution, pooling, sequestration, hyperhemolysis, portal hypertension, bleeding

Immune-mediated Anemia in Myelofibrosis

- Decreased RBC-production?
- Increased RBC-destruction?
- A combination of these mechanisms?
- Other mechanisms?

Small non-para-trabecular lymphoid aggregate composed of small lymphocytes (H&E, original magnification ×200) in a person with primary myelofibrosis. Barosi G Leuk Res 2010; 34(9):119-1120
Autoimmunity in Myelofibrosis

Immune-Related Abnormalities

- Antibodies to RBCs (detected in the Coombs test)
- Anti-nuclear and -mitochondrial antibodies (ANA and AMA)
- Rheumatoid factor, lupus-like anti-coagulant
- Low levels of complement
- Increased levels of immune complexes

Cytokine Profiling Study in Myelofibrosis

Elevated Cytokines

- IL-1, IL-1RA, IL-2R, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15
- TNF-alpha
- G-CSF
- IFN-alpha
- IFN-inducible protein 10 (IP-10)
- Macrophage inflammatory protein 1 (MIP-1)
- Monokine induced by IFN-gamma (MIG)
- Monocyte chemotactic protein 1 (MCP-1)
- Hepatocyte growth factor (HGF)
- Vascular endothelial growth factor (VEGF)

Autoimmunity in Myelofibrosis
Previous or concurrent autoimmune or chronic inflammatory diseases

- Systemic lupus erythematosus
- Progressive systemic sclerosis
- Primary biliary cirrhosis
- Ulcerative colitis, mb. Crohn
- Nephritic syndrome
- Polyarteritis nodosa
- Sjogren syndrome
- Rheumatoid arthritis

Primary Autoimmune Myelofibrosis

- Non-clonal, non-neoplastic disease
- Anemia /cytopenias
- Auto-antibodies suggesting systemic auto-immunity
- Absent or mild splenomegaly
- Bone marrow MPN-like histology (fibrosis, hypercellularity, megakaryocyte clusters)
- Bone marrow lymphoid aggregates
## Treatment

### Anaemia

- Substitution with building stones for blood cell production (iron, B12, folic acid)
- Danazol
- Prednisolone
- Erythropoietin
- Thalidomide/Lenalidomide/Pomalidomide +/- Prednisolon
- Splenectomy

## Anemia in Myelofibrosis

### Conventional Drugs

- Danazol /Androgens
- Prednisolone
- Erythropoietin

- Response rate 20-40%
- Response duration 1 year
### Danazol

<table>
<thead>
<tr>
<th>Mechanisms of Action</th>
<th>Efficacy and Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Semisynthetic androgen</td>
<td>• 200 mg x 3/day</td>
</tr>
<tr>
<td>• Erythropoiesis-stimulating</td>
<td>• Median time to response 3 months (1-9 months)</td>
</tr>
<tr>
<td>• Immunomodulating</td>
<td>• Response rate about 40%</td>
</tr>
<tr>
<td>• Anti-inflammatory</td>
<td>• Response duration about 1 year</td>
</tr>
<tr>
<td></td>
<td>• Moderate toxicity (liver)</td>
</tr>
<tr>
<td></td>
<td>• Effective and well tolerated in a substantial proportion of patients with anaemia</td>
</tr>
<tr>
<td></td>
<td>• PSA !</td>
</tr>
</tbody>
</table>

### Erythropoietin

<table>
<thead>
<tr>
<th>Mechanisms of Action</th>
<th>Efficacy and Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Erythropoiesis-stimulating</td>
<td>• Moderate non-transfusion-dependent anemia</td>
</tr>
<tr>
<td>• Immunomodulating</td>
<td>• Low (125 U/L) serum Epo</td>
</tr>
<tr>
<td></td>
<td>• Response rate about 40%</td>
</tr>
<tr>
<td></td>
<td>• Response duration about 1 year</td>
</tr>
<tr>
<td></td>
<td>• Effective and well tolerated</td>
</tr>
<tr>
<td></td>
<td>• Obs – enlargement of the spleen</td>
</tr>
</tbody>
</table>
Treatment
Clonal Myeloproliferation

Conventional Medical
- Hydroxyurea (Elderly)
- Interferon-Alpha2 (Younger)

Surgery
- Splenectomy

Irradiation
- Huge splenomegaly

Stem Cell Transplantation
- Allogeneic
- Minitransplant

New Agents
- JAK1-2-inhibitors
- HDACi

IMiDs in Myelofibrosis

- Thalidomide
- Lenalidomide
- Pomalidomide

+/- Prednisolone
IMiDs in Myelofibrosis
Pleotropic properties in Cancer Models

- Anti-angiogenic
- Anti-proliferative
- Anti-inflammatory
- Anti-T regulatory cell activity
- Pro T-cell and Pro NK-cell activities
- Pro-erythropoietic activity

The precise mechanism of their therapeutic activity poorly understood

- Immunomodulatory drugs (IMiDs) induce growth arrest and/or apoptosis in multiple myeloma (MM) cells and inhibit adhesion of MM cells to bone-marrow stromal cells
- Stromal-cell expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) is reduced by IMiDs, which decreases angiogenesis
- Expression of interleukin-6 (IL-6) and tumour-necrosis factor-α (TNF-α) by the stromal cells is reduced
- The IMiDs enhance T-cell stimulation and proliferation
- The activated T cells release IL-2 and interferon-γ (IFN-γ), which activate natural-killer (NK) cells

IMiDs and Tregs


Thalidomide
Thalidomide

**High Dose (100-400 mg/day)**

- A high drop-out rate
- Sedation
- Obstipation
- Neuropathy

**References**


**Low-dose (50mg/day + Pred)**

- Low-dose (50mg/day) thalidomide in combination with prednisone is better tolerated
- Alleviates anemia in a quarter of treated patients with MF
- Long-term use associated with neuropathy

**References**

Lenalidomide

- Lenalidomide (5–10 mg/day) with or without prednisone improves anemia in about 25% of patients with MF

- No resolution of disease-related fibrosis or angiogenesis

- The drug’s utility is limited by substantial myelosuppression

- Lenalidomide is most useful in del(5q)-associated MF


Lenalidomide in Myelofibrosis

*Table 1. Treatment details of lenalidomide (CC-5013) therapy in myelosclerosis with myeloid metaplasia involving 27 patients from Mayo Clinic and 41 patients from MDACC*

<table>
<thead>
<tr>
<th>No.</th>
<th>Mayo Clinic</th>
<th>MDACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Planned treatment schedule</td>
<td>Three cycles for all patients</td>
</tr>
<tr>
<td>2.</td>
<td>One cycle of treatment</td>
<td>Lenalidomide 10 mg/day × 28 days</td>
</tr>
<tr>
<td>3.</td>
<td>Preferred eligibility criteria</td>
<td>ABR, PFPM, or P75+/M</td>
</tr>
<tr>
<td>4.</td>
<td>No. patients who received</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Less than 1 cycle of treatment</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>1 to less than 3 cycles</td>
<td>11</td>
</tr>
<tr>
<td>7.</td>
<td>3 to 6 cycles</td>
<td>14</td>
</tr>
<tr>
<td>8.</td>
<td>More than 6 cycles</td>
<td>Treatment stopped after 6 cycles</td>
</tr>
<tr>
<td>9.</td>
<td>No. patients who required dose modification</td>
<td>6 (22%)</td>
</tr>
</tbody>
</table>

- Single-agent lenalidomide (CC-5013, Revlimid) in a total of 68 patients with symptomatic myelofibrosis
- Overall response rates were 22% for anemia, 33% for splenomegaly, and 50% for thrombocytopenia
- Response in anemia impressive in 8 patients whose hemoglobin level normalized
- Resolution of leukoerythroblastosis (4 patients),
- A decrease in medullary fibrosis and angiogenesis (2 patients)
- Cytogenetic remission (del(5q)(q13q33)) (1 patient)


Lenalidomide and Prednisolone in Myelofibrosis

*Table 2. Patients With Myelofibrosis Who Responded to Lenalidomide and Prednisone Therapy*

| No. | Age (yr) | Prior Myelofibrosis-Directed Therapy | Splenomegaly (%), WBC (×10^9/L), Hemoglobin (g/L), Platelets (×10^9/L), JAK2V617F Allele %, No. of Cycles to Response, Response Duration (months) |
|-----|---------|--------------------------------------|--------------------------------------------------|-------------------------------------------------------------------|-----------------------------------------------|
| 1.  | 72      | Darbepoetin                          | 10.4, 7.8, 202, 46.7 | 7 | Hemoglobin, spleen | 14+ |
| 2.  | 65      | Peg-IFN-α2b                          | 10.5, 7.1, 180, 82 | 2 | Neutrophil, apheresis | 20+ |
| 3.  | 45      | IFN-α                               | 11.5, 11.6, 150, 81.2 | 6 | Tripelennet | 20+ |
| 4.  | 52      | None                                | 12.5, 11.5, 150, 81.2 | 6 | Tripelennet | 20+ |
| 5.  | 66      | None                                | 10, 9.1, 95, 82 | 3 | Peg-IFN-α2b | 21+ |
| 6.  | 76      | None                                | 15, 31.5, 17.3, 167, 89 | 3 | Peg-IFN-α2b | 20+ |
| 7.  | 71      | Darbepoetin                          | 12.8, 12.2, 359 | 0 | Peg-IFN-α2b | 19+ |
| 8.  | 56      | None                                | 22, 22.3, 10.3, 704, 86.2 | 4 | Peg-IFN-α2b | 20+ |
| 9.  | 69      | Hydroxyurea, dexamethasone           | 10, 30.3, 18.8, 140, 89.15 | 3 | Peg-IFN-α2b | 20+ |
| 10. | 72      | Thalidomide + PRD, darbepoetin      | 7.2, 8.1, 309 | 0 | Peg-IFN-α2b | 6 |
| 11. | 56      | None                                | 10, 9.1, 90, 86.2 | 4 | Peg-IFN-α2b | 12+ |
| 12. | 96      | Hydroxyurea, darbepoetin            | 20, 17.4, 89, 90.6, 86.2 | 4 | Peg-IFN-α2b | 12+ |

- Forty patients with MF
- The median follow-up 22 months (range, 6 to 27)
- Responses in 12 patients (30%); 30% for anemia and 42% for splenomegaly
- The median time to response 12 weeks (range, 2 to 32)
- Reduction in reticulin fibrosis (from grade 4 to grade 2 in 10/32)
- Reduction in JAK2V617F allele burden, greater than 50% in four, and in 1 the mutation became undetectable
- The combination of lenalidomide and prednisone induces durable clinical, molecular, and pathologic responses in MF

Alfonso Quinta’s-Cardama et al. Lenalidomide Plus Prednisone Results in Durable Clinical, Histopathologic, and Molecular Responses in Patients With Myelofibrosis J Clin Oncol 2009;27:4760-4766
Pomalidomide

Pomalidomide +/- Pred/DXM

- Pomalidomide at 2 mg/day in combination with dexamethasone has remarkable activity in relapsed multiple myeloma, including patients refractory to either lenalidomide or bortezomib


- The maximum tolerated dose of pomalidomide 3 mg/day in MF


- Pomalidomide either alone (2 mg/day) or in combination with prednisone safe (no neuropathy and < 10% severe myelosuppression) and effective (25% response rate) in treating anemia associated with MF

Pomalidomide
Monotherapy 0.5 mg/day

Begna KH, Mesa RA, Pardanani A, Hogan WJ, Litzow MR, McClure RF, Tefferi A.
A phase-2 trial of low-dose pomalidomide in myelofibrosis.
Leukemia (2011) 25, 301–304.

Pomalidomide
Monotherapy (0.5 mg/day)

- Transfusion-dependency or hemoglobin < 10gm per 100 ml
- Pomalidomide (0.5 mg/ day) was given to 58 patients (median age 68 years)
- 46 (79%) were transfusion-dependent and 42 were JAK2V617F positive
- 9 of the 10 anemia responders became transfusion independent
- Little effect on leukocyte count, serum LDH or spleen size
Pomalidomide
Monotherapy 0.5 mg/day

- Anemia response only in the presence of JAK2V617F (24 vs 0) but was not further affected by mutant allele burden
- Anemia response in JAK2V617F-positive patients predicted by the presence of pomalidomide-induced basophilia in the first month of therapy (38 vs 6%; \( P = 0.02 \)) or absence of marked splenomegaly (38 vs 11%; \( P = 0.05 \))
- A total of 14 (58%) of 24 patients with a platelet count of < 100 Mia/L experienced a > 50% increment in platelet count
- No spleen responses
- Grade 3 or 4 thrombocytopenia/neutropenia occurred in 2%/ 0%

- Low-dose pomalidomide is effective in the treatment of anemia associated with JAK2V617F-positive MF
- Response is predicted by early drug-induced basophilia

Pomalidomide

Long-Term Outcome of Pomalidomide Therapy in Myelofibrosis

Begna KB, Pardanani A, Mesa R et al.
Am J Hematol 2012; 87:66–68
Pomalidomide

Pomalidomide (0.5 mg 3.5 mg/day)

• Ninety-four Mayo Clinic patients with myelofibrosis (MF) participated in two consecutive clinical trials of pomalidomide (0.5–3.5 mg/day), with or without prednisone

• Overall anemia response was 27%

• Anemia response was 53% in JAK2V617F-positive patients with <10 cm palpable splenomegaly and <5% circulating blasts

• Anemia response rate was 0% in mutation-negative patients with either >10 cm splenomegaly or > 5% circulating blasts

• Median duration of anemia response 16 months

• Treatment effect on splenomegaly negligible

• Pomalidomide therapy has been discontinued in 86 (91%) patients at a rate of 68% at 1 year and 89% at 2 years
Cytokine Profiling Study in Myelofibrosis

Specific Cytokine-Phenotype Associations

- Constitutional symptoms: IL-8
- Marked splenomegaly: HGF, MIG, IL-1RA
- Leukocytosis: IL-2R, IL-8
- > 5% blasts: IL-8
- Thrombocytopenia: IP-10
- JAK2V617F: IL-1RA, IL-2R, IP-10, MIP-1
- Transfusion need: IL-2R, IL-12

- Independently predictive of inferior survival
  IL-2R, IL-8, IL-12, IL-15, IP-10


Pomalidomide

Circulating Levels of MCP-1, sIL-2R, IL-15, and IL-8 Predict Anemia Response to Pomalidomide Therapy in Myelofibrosis

Pomalidomide

<table>
<thead>
<tr>
<th>Good</th>
<th>Anemia Response</th>
<th>Bad</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2V617F-positive</td>
<td></td>
<td>JAK2V617F-negative</td>
</tr>
<tr>
<td>Moderate splenomegaly</td>
<td></td>
<td>Marked splenomegaly</td>
</tr>
<tr>
<td></td>
<td>Lower levels of IL-2R, IL-8</td>
<td>Increased serum LDH</td>
</tr>
<tr>
<td></td>
<td>IL-15, MCP-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High levels of IL-2R, IL-8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-15, MCP-1, VEGF</td>
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</tbody>
</table>

Treatment of Anemia in The Future?

**Early Stage**
- Prevention of Anemia
  - Statins ?
  - Interferon-alpha2 ?

**Advanced Stage**
- Treatment of anemia
  - Danazol
  - Prednisolone
  - Epo
  - Thalidomide + Pred
  - Lenalidomide + Pred
  - Pomalidomide + Pred
  - Combination therapies
    - eg. IMiDs + JAK1-2-inhibitor
Prevention of Anemia?

- Early intervention?
- Dampen chronic inflammation?
- Quell the fire?

- Interferon-alpha2
- Statins?

Symptoms and Clinical Findings

Early Prefibrotic Primary Myelofibrosis  
Advanced Primary Myelofibrosis
JAK2 V617F Positive Neoplasms: Minimal Residual Disease?

100%

% V617F alleles

Unknown genetic event

Lympho-myeloid precursor
Stem cell

Chronic Inflammation

ET

PV

Post PV MF

AML

Chronic phase

Accelerated phase

Stem Cell Wake up Call

IFN-alpha

Myeloproliferative disorders

IFN-alpha

BCR-ABL

JAK2 V617F

Unknown mutation

Chronic myeloid leukemia

JAK2-positive thrombocytopenia

JAK2-positive polycythemia

JAK2-negative myeloproliferative disorder

Chronic phase

Accelerated phase

Blast crisis

Leukemic transformation

IFN-alpha

myelofibrosis

infiltrating tissues

increasing white cells

?
Sustained Molecular Response in Polycythemia Vera treated with Interferon Alfa-2b

Figure 1: Bone marrow histomorphology from patient 1 at a) time of diagnosis 1996 and b) just prior to treatment with IFN alfa-2b. Both panels demonstrate classical PV features with hyperplasia and clustering of morphological abnormal megakaryocytes. Panel c) shows the morphologically normal bone marrow from August 2007 (after eight years of treatment with IFN-alfa 2b) with total regression of PV features (Larsen T et al Ann Hematol 2008; 87: 847–850)

Rationale for Early Intervention
IFN-alpha2

• Major /complete molecular remissions after long-term treatment (> 3 -5 years)

• Sustained molecular remissions after discontinuation of IFN-alpha2

• Minimal residual disease /cure ?

• ET – the early phase of PV ?/ the early phase of myelofibrosis ?
Rationale for Early Intervention
IFN-alpha2

- JAK2V617F-positive ET – the early phase of PV?
- Half ET - the early phase of myelofibrosis?
- Early prefibrotic myelofibrosis inferior survival as compared to ET

Rationale for Early Intervention
IFN-alpha2

- MPNs Associated with an Increased Risk of Second Cancer
- JAK2V617F Tumor Promoter?
- IFN-alpha2 Enhancer of "Tumor Immune Surveillance"
- Early Intervention with IFN-alpha2 Decreases the Risk of Second Cancer?
Conclusions

• Anemia is multifactorial (deficiency of iron, folate, B12, bone marrow failure, inflammation, hemolysis, hemodilution, splenic pooling and sequestration)

• Conventional drugs (danazol, prednisolone, Epo):
  - response rates approximately 20-40%
  - response duration 1 year

Conclusions

• Thalidomide 50 mg/day + Prednisolone:
  - Anemia response in 20%
  - Thrombocytopenia response 20%
  - Spleen response in 10%
  - Sedation and neuropathy
Conclusions

- Lenalidomide 5-10 mg +/- Prednisolone :
  - Anemia response 25 %
  - Thrombocytopenia response 50 %
  - Spleen response 30 %
  - Most useful in del(5q)-associated MF
  - Substantial myelosuppression

Conclusions

- Pomalidomide 0.5 mg/day + prednisone for 3 cycles :
  - Anemia response 40%
    - JAK2V617F-positive
    - Absence of marked splenomegaly
    - Absence of highly elevated cytokines (IL-2R, IL-8, IL-15, MCP-1)
  - Thrombocytopenia response 58 %
  - Spleen response 0 %
  - Well-tolerated
  - Significant advance in the treatment of anemia in JAK2V617F-positive MF without huge splenomegaly
Conclusions

The Future?

• Early intervention (IFN-alpha2 + statins?) to prevent advanced phase disease

• Combination therapies of anemia in advanced phase
  - eg. JAK1-2 inhibitor + pomalidomide
  - eg. JAK1-2 inhibitor + danazol
Thank you for

your attention