Molecular pathogenesis, diagnostics and targeted therapies

**Myeloproliferative neoplasms**

- **Clonal hematopoietic stem cell disorders**
- Characterized by proliferation of one or more of the myeloid lineages (i.e. granulocytic, erythroid, megakaryocytic and mast cells)
- Primarily neoplasms of adults (peak frequency in the 5th to 7th decade)
- Initially hypercellular bone marrow with effective hematopoietic maturation, increased granulocytes, red blood cells and/or platelets
- Despite insidious onset, each MPN has the potential to undergo a stepwise progression that terminates in bone marrow failure

**2008 WHO classification of myeloid malignancies**

- Acute myeloid leukemia (AML) and related precursor neoplasms
- Myeloproliferative neoplasms (MPN)
- Myelodysplastic syndromes (MDS)
- MDS/MPN overlap
- Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRα, PDGFRβ or FGFR1

**2008 WHO classification MPN**

- Chronic myelogenous leukemia, BCR-ABL1 positive (CML)
- Polycythemia vera (PV)
- Primary myelofibrosis (PMF)
- Essential thrombocythemia (ET)
- Chronic neutrophilic leukemia (CNL)
- Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
- Mastocytosis
- Myeloproliferative neoplasm, unclassifiable (MPN-U)

**Chronic myeloid leukemia (CML)**

- Myeloproliferative neoplasms characterized by abnormal growth of myeloid cells
- Phases:
  - Chronic phase
  - Accelerated phase
  - Blast phase

**Evolution of disease process in CML**

- Leucocytosis with left shift (neutrophils in different stages of maturation, <2% blast)
- Absolute basophilia, commonly absolute eosinophilia
- <5% blasts in BM
- PLT: normal to > 1000 x10⁹/L
- Increased BM cellularity
- Aberrant megakaryocytes (small, hypolobated)

**Natural history of untreated CML**

- Bi- or triphasic
Myeloproliferative neoplasms characterized by an increased red blood cell production independent of the mechanisms that normally regulate erythropoiesis

or PRIMARY ERYTHROCYTOSIS

or MORBUS VACQUEZ

Polycythemia vera (PV)

Phases
- Early phases:
  - Pre-polycythemic phase
  - Overt polycythemia
- Late phase
  - Post-polycythemic myelofibrosis

Evolution of disease process in CML

Evolution of disease process in CML

Polycythemia

- PRIMARY ERYTHROCYTOSIS
  - increased RBC production, independent of mechanisms that normally regulate erythropoiesis

- RELATIVE ERYTHROCYTOSIS
  - reduced plasma volume

- SECONDARY ERYTHROCYTOSIS
  - normal hematopoietic stem cell, extrinsic factors increase RBC production
    - appropriate EPO production (at high altitude, chronic hypoxic disorders, ...)
    - inappropriate EPO production (non-neoplastic conditions of kidney eg. hydronefrosis, cysts, neoplasms of kidney, liver, ...)

Evolution of disease process in PV

Evolution of disease process in PV
Early phase

- Panmyelosis: proliferation of erythroid, myeloid and megakaryocytic lineages (high BM cellularity)
  - Neutrophilia
  - Prominent thrombocytosis (mimicking ET)
  - High RBC mass
  - Spleenomegaly

Late phase

- Post-polycythemic myelofibrosis (MF) ~20%
  - Proliferation of erythroid cells decreases
  - Normal to decreased RBC mass
  - Leuco-erythroblastosis PB smear
  - Teardrop-shaped RBC
  - Often hypocellular BM
  - ↑↑ fibrosis
  - ↑↑ splenomegaly (extramedullary hematopoiesis)
  - Blastic transformation <10%

Primary myelofibrosis (PMF)

- Clonal myeloproliferative neoplasm characterized by a proliferation of predominantly MgK and granulocytes, and in fully developed disease associated with reactive deposition of fibrous connective tissue and extramedullary hematopoiesis
  - Leuco-erythroblastosis

Prefibrotic phase

- Hypercellular BM, absent/minimal reticulin fibrosis

Fibrotic phase

- Marked reticulin or collagen fibrosis in BM, leuco-erythroblastosis, HSM

Dynamics of disease process in PMF
**Thrombocytosis**

- **Clonal thrombocytosis**
  - *essential thrombocythemia / primary thrombocytosis*
  - other myeloproliferative disorders
    - CML
    - PV
    - Myelofibrosis
- **Reactive /secondary thrombocytosis**
  - inflammatory conditions
  - certain neoplasms
  - auto-immune disorders
  - post-splenectomy / hyposplenism
  - iron deficiency

**Essential thrombocytosis (ET)**

- Sustained platelet count > 450 x 10^9/L
- BM aspirate / biopsy: proliferation of mainly MgK lineage with increased numbers of enlarged, mature MgK
- No evidence of PV, primary MF, CML, MDS
- Demonstrating JAK2 V617F mutation, or in absence of the mutation; no evidence of reactive thrombocytosis

**Mastocytosis**

- Myeloproliferative neoplasms characterized by abnormal growth of mast cells
- Mast cell
  - derives from a (CD117-positive) hematopoietic stem cell
  - leaves BM before complete maturation (› other hematopoietic cells)
  - “home” for well-vascularized tissue (in epithelium -close to vessels, nerves, smooth muscle cells and glandular tissue, no circulation in PB)

**Mastocytosis**

- Myeloproliferative neoplasms characterized by abnormal growth of mast cells
- Mast cell
- morphology

**Mastocytosis**

- Categories recognized by distribution of the disease and clinical manifestations:
  - Cutaneous mastocytosis (CM): only skin involvement
  - Systemic mastocytosis (SM): extracutaneous involvement of organs (bone marrow, spleen, liver, lymph nodes, mucosa GI tract, skin (>50%))
  - Insolent systemic mastocytosis
  - Aggressive systemic mastocytosis
  - Systemic mastocytosis with associated clonal haematological non-mast-cell lineage disease
  - Mast cell leukemia
  - Mast cell sarcoma

**Chronic myeloproliferative neoplasms (MPN)**

Clonal hematopoietic stem cell disorders characterized by proliferation of one or more of the myeloid lineage (i.e. granulocytic, erythroid, megakaryocytic and mast cell)

**Proliferation?**
**Molecular pathogenesis**

- **Mast cells** MASTOCYTOSIS
- **Red blood cells** PV
- **Platelets** ET
- **Eosinophils** CEL
- **Neutrophils** CML
- **PMF**

**Activating mutations in MPN**

- **Mast cells** MASTOCYTOSIS
- **Red blood cells** PV
- **Platelets** ET
- **Eosinophils** CEL
- **Neutrophils** CML

**t(9;22) with BCR-ABL1**

- 1973 discovery that the Phi chromosome results from a reciprocal translocation of chromosomes

**Transcription and translation BCR-ABL1**

- **BCR**
- **ABL**
- **BCR-ABL**

**BCR-ABL1 signaling**

- BCR-ABL has a small pocket where ATP can bind (ATP binds to the kinase domain)
- Binding of the substrate
- Transfer of phosphate to the tyrosine residue of the substrate
- Phosphosubstrate binds to downstream molecules
- Activation of downstream effector molecules

**Activating mutations in MPN**

- **Mast cells** MASTOCYTOSIS
- **Red blood cells** PV
- **Platelets** ET
- **Eosinophils** CEL
- **Neutrophils** CML

In CML: BCR-ABL1 fusion protein

- Constitutively ACTIVE TYROSINE KINASE
- Cell proliferation
Activating mutations in MPN

<table>
<thead>
<tr>
<th>MPN</th>
<th>Activating mutation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cells</td>
<td>Mastocytosis</td>
<td>95%</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>JAK2 V617F</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Platelets</td>
<td>ET</td>
<td>&gt;40-50%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>CEL</td>
<td>50%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>CML</td>
<td>100%</td>
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JAK2 mutations

- JAK2 V617F (exon 14)
  - (2005) acquired somatic mutation
  - guanine-to-thymidine substitution, resulting in a substitution of valine (V) to phenylalanine (F) at codon 617 of JAK2

<table>
<thead>
<tr>
<th>DNA allele specific PCR</th>
</tr>
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<tbody>
<tr>
<td>JAK2 - Wildtype</td>
</tr>
<tr>
<td>Y G V C F C G O</td>
</tr>
<tr>
<td>JAK2 - Mutant</td>
</tr>
<tr>
<td>Y G V C F C G G</td>
</tr>
</tbody>
</table>

- JAK2 exon 12
  - in 3% of PV (JAK2 V617F negative PV)
  - most frequent mutations: N542-E543del (23%), E543-D544del (11%), F537-K539delinsL (10%), K539L (10%) en R541-E543delins (8%)

Normal activation of JAK2

Ligand dependent activation

- Cytokine ligand binding to appropriate cytokine receptor
- Jak kinase phosphorylation and activation
- Recruitment and phosphorylation of Stat proteins
- Phosphorylation and activation of downstream signaling proteins

Activation via JAK2 mutations

Ligand independent activation

- JAK2V617F mutant and JAK2 exon12 mutant kinases bind to cytokine receptors
- Phosphorylation in absence of ligand
- Recruitment and phosphorylation of Stat proteins
- Phosphorylation and activation of downstream signaling proteins

Activating mutations in MPN

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<td>50%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>CML</td>
<td>100%</td>
</tr>
</tbody>
</table>
**MPL gene and mutations**

- **MPL**
  - myeloproliferative leukemia virus oncogene
  - on chromosome 1p34, encoding the thrombopoietine receptor
  - important role in the development of megakaryocytes and platelets

- **MPL mutations**
  - clustered in exon 10: vast majority W515L (68%) and W515K in 4% of JAK2 V617F negative ET or PMF

DNA PCR => Sanger sequencing

**Activation via MPL mutations**

- Ligand independent activation
- MPL W515L/K mutant thrombopoietin receptors phosphorylate wild type JAK in absence of thrombopoietine
- phosphorylation in absence of ligand
- recruitment and phosphorylation of Stat proteins
- phosphorylation and activation of downstream signaling proteins

**Distribution of JAK2, MPL, and CALR mutations in BCR-ABL1 negative MPN**

Proportion of patients with PV, ET, or PMF carrying different genetic abnormalities related to JAK-STAT signaling.

**Somatic mutations in PMF, ET, PV**

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Chromosome location</th>
<th>Frequency in PV</th>
<th>Frequency in ET</th>
<th>Frequency in PMF</th>
<th>Frequency in BP-PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2 V617F</td>
<td>9p24</td>
<td>~96%</td>
<td>~55%</td>
<td>~65%</td>
<td>~50%</td>
</tr>
<tr>
<td>MPL exon 10</td>
<td>1p34</td>
<td>~3%</td>
<td>~3%</td>
<td>~10%</td>
<td>~5%</td>
</tr>
<tr>
<td>CALR</td>
<td>19p13.3</td>
<td>rare</td>
<td>rare</td>
<td>rare</td>
<td>~19%</td>
</tr>
</tbody>
</table>

**Frequency of CALR mutations in myeloid neoplasms**

A: distribution of JAK2, MPL, and CALR mutations in the three classical entities of myeloproliferative neoplasms.

- CALR: another piece of the MPN puzzle

B: frequency of CALR mutations in various myeloid cancers.

- CALR: never in PV

C: distribution of mutations in JAK2, MPL, CALR, and SF3B1 among 24 patients with RARS-T.

- JAK2, MPL, CALR: mutually exclusive, also in ET and PMF

**Mutations in CALR**

A: genomic positions of all 36 mutation types detected in the current study (deletions, insertions, and somatically acquired substitutions). Most frequent mutations: type 1 and type 2.

B: relative frequencies of all 36 mutation types observed in CALR.

- mutations in ET and PMF result in a frameshift and are clustered in exon 9

**Proportion of patients with PV, ET, or PMF carrying different genetic abnormalities related to JAK-STAT signaling.**

Gäbler K. et al. JAKSTAT 2013; 2(3): e25025
**CALR mutations**

- Role of CALR mutations in pathogenesis in MPN?
- How do CALR mutations lead to a clonal advantage in hematopoietic cells?
- Which role in regulation of STAT signaling pathways?

**Mutations in CALR: clinical impact?**

Compared to JAK2 mutated patients (retrospective studies)
- more indolent / benign clinical course
- lower risk of thrombosis
- lower Hb levels, lower WBC count
- longer overall survival

- incorporation into existing prognostic scorings systems for PMF and ET?
- guiding therapeutic decisions?

**Activating mutations in MPN**

<table>
<thead>
<tr>
<th>MPN</th>
<th>Activating mutation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cells</td>
<td>Mastocytosis</td>
<td>KIT D816V</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>PV</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>ET</td>
<td>JAK2 V617F</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>CEL</td>
<td>FIP1L1-PDGRA</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>CML</td>
<td>BCR-ABL1</td>
</tr>
<tr>
<td>PMF</td>
<td></td>
<td>MPL W151L/K</td>
</tr>
</tbody>
</table>

**Activating D816V c-KIT mutation in mastocytosis**

- an amino acid substitution at position 816 in KIT from an aspartic acid (D) to a valine (V)

**Activating D816V c-KIT mutation in mastocytosis**

- KIT D816V mutation
- Ligand-independent tyrosine kinase activity
  - Autophosphorylation of KIT
  - Uncontrolled cell proliferation
  - Stimulation of downstream signaling pathways

**D816V KIT mutation**

- KIT D816V mutation
- Ligand-independent autophosphorylation of KIT
- Uncontrolled cell proliferation
- Stimulation of downstream signaling pathways

- also specific quantitative real-time qPCR
2008 WHO diagnostic criteria for BCR-ABL1 negative MPN

<table>
<thead>
<tr>
<th>Major criteria</th>
<th>Essential thrombocythemia (ET)</th>
<th>Primary myelofibrosis (PMF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hemoglobin ≥4.5 g/dl (men) or ≥4.7 g/dl (women)</td>
<td>Platelet count ≥450 x 10^9/l</td>
<td>Myelofibrosis and atypia, accompanied by either BM fibrosis and/or collagen fibrosis, not meeting WHO criteria for CML, ET, PMF or other myeloid neoplasms, or other clonal marker, or no evidence of reactive bone marrow</td>
</tr>
<tr>
<td>2. Presence of JAK2 or other MPL mutation</td>
<td>High proliferation with large and normal morphology, not meeting WHO criteria for CML, ET, PMF, or other myeloid neoplasms, or other clonal marker, or no evidence of reactive bone marrow</td>
<td></td>
</tr>
<tr>
<td>3. Presence of ≥1 mutation</td>
<td>Not meeting WHO criteria for CML, ET, PMF, or other myeloid neoplasms, or other clonal marker, or no evidence of reactive bone marrow</td>
<td></td>
</tr>
</tbody>
</table>

Minor criteria

<table>
<thead>
<tr>
<th>#</th>
<th>Minor criteria</th>
<th>Major + 1 minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BM or peripheral or peripheral or platelet count 90%</td>
<td>Leukemoblasts</td>
</tr>
<tr>
<td>2</td>
<td>Abnormal serum erythropoietin level</td>
<td>Increased serum erythropoietin level</td>
</tr>
<tr>
<td>3</td>
<td>Evidence of abnormal karyotype or absence of evidence for reactive thrombocytosis</td>
<td>Anemia</td>
</tr>
<tr>
<td>4</td>
<td>Presence of anemia or palpable splenomegaly</td>
<td>Palpable splenomegaly</td>
</tr>
</tbody>
</table>

Diagnostic work-up of BCR-ABL1 negative MPN

- Presence of abnormal erythropoietin levels
- Increased serum erythropoietin levels
- Presence of anemia or palpable splenomegaly

Molecularly targeted therapies

- Blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with all rapidly dividing cells (e.g. with traditional chemotherapy)
- Expected to be more effective than current treatments and less harmful to normal cells

Tyrosine kinase inhibitor (imatinib): mechanism of action

- Imatinib = ATP-mimetic agent
- Binding to BCR-ABL to the ATP binding site
- Prevention of phosphorylation of substrate
- Blocking of downstream signal transduction pathways

Tyrosine kinase inhibitors in CML

<table>
<thead>
<tr>
<th>TKI</th>
<th>1st generation</th>
<th>2nd generation</th>
<th>3rd generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib (Gleevec®)</td>
<td>Nilotinib (Tasigna®)</td>
<td>Dasatinib (Sprycel®)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bosutinib (Bosulif®)</td>
<td>Ponatinib (Iclusig®)</td>
<td></td>
</tr>
</tbody>
</table>
**ELN recommendations for the management of CML (update 2013)**

<table>
<thead>
<tr>
<th>OPTIMAL RESPONSE</th>
<th>WARNING</th>
<th>FAILURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline NA</td>
<td>High risk, or CCA/Ph+, major route</td>
<td>NA</td>
</tr>
<tr>
<td>3 months</td>
<td>BCR-ABL ≤ 5%, and/or Ph ≤ 35%</td>
<td>BCR-ABL &gt; 5%, and/or Ph &gt; 35%</td>
</tr>
<tr>
<td>6 months</td>
<td>BCR-ABL ≤ 5%, and/or Ph ≤ 35%</td>
<td>BCR-ABL &gt; 5%, and/or Ph &gt; 35%</td>
</tr>
<tr>
<td>12 months</td>
<td>BCR-ABL ≤ 0.1%</td>
<td>BCR-ABL &gt; 0%, and/or Ph &gt; 35%</td>
</tr>
<tr>
<td>Then, and at any time</td>
<td>BCR-ABL ≤ 0.1%</td>
<td>CCA/Ph+ (≤ 7, or 7q-)</td>
</tr>
</tbody>
</table>

**Tyrosine Kinase Inhibitors (TKIs) in systemic mastocytosis**

<table>
<thead>
<tr>
<th>TKI</th>
<th>Activity against wild-type KIT</th>
<th>Inhibits wild-type KIT</th>
<th>Less effective against D816V KIT mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib</td>
<td>Activity against wild-type KIT</td>
<td>Inhibits wild-type KIT</td>
<td>Less effective against D816V KIT mutation</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>Activity against wild-type KIT</td>
<td>Not sufficient to inhibit growth in case</td>
<td></td>
</tr>
<tr>
<td>Dasatinib</td>
<td>In vitro activity against both WT and D816V KIT</td>
<td>In vitro activity against both WT and D816V KIT</td>
<td></td>
</tr>
<tr>
<td>Midostaurin</td>
<td>In vitro effective in inhibiting both WT and D816V KIT</td>
<td>In vitro effective in inhibiting both WT and D816V KIT</td>
<td></td>
</tr>
</tbody>
</table>

**JAK inhibitors**

- **INCB018424** (ruxolitinib)
  - JAK1 (1)
  - JAK3 (98)
  - TYK2 (9.3)
  - Tested diseases: PMF, PV, ET, cutaneous inflammation, leukemia, rheumatoid diseases
  - Unique effects: Response rates are similar regardless of MF subtype and regardless of JAK2 V617F mutation presence

- **CEP701** (lestaurtinib)
  - JAK3 (3)
  - I/II
  - Tested diseases: PMF, PV, ET, Hodgkin lymphoma, solid tumors, hematological malignancies, autoimmune diseases
  - Unique effects: Well tolerated; no decrease in JAK2 V617F allele burden

- **SB1518**
  - JAK1 (58)
  - JAK3 (24)
  - I/II
  - Tested diseases: PMF, lymphoma
  - Unique effects: Well tolerated; promising efficacy in symptomatic MF patients with splenomegaly

- **SAR302503**
  - JAK1 (35)
  - JAK3 (332)
  - TYK2 (135)
  - I/II
  - Tested diseases: PMF, mast cell leukemia
  - Unique effects: Improvement of baseline constitutional symptoms

- **XL019**
  - JAK1 (105)
  - JAK3 (996)
  - Discontinued
  - Tested diseases: PMF, PV, post-PV/ET MF
  - Unique effects: Clinical studies discontinued due to high rate of neurotoxicity

- **CYT387**
  - JAK1 (0.6)
  - JAK3 (8.6)
  - I/II
  - Tested diseases: PMF, post-PV/ET MF
  - Unique effects: Significant improvement rates in anemia and splenomegaly, and it has a favorable toxicity profile

- **AZD1480**
  - JAK1 (5)
  - JAK3 (15)
  - I/II
  - Tested diseases: PMF, post-PV/ET MF, glioblastoma, solid tumors
  - Unique effects: The first trial evaluating AZD1480 in humans is still ongoing

- **INCB028050**
  - JAK1 (5.9)
  - Preclinical
  - Tested diseases: Rheumatoid arthritis (in rodent models)

- **INCB16562**
  - JAK1 (2.2)
  - JAK3 (10.1)
  - TYK2 (2.7)
  - Preclinical
  - Tested diseases: PMF, multiple myeloma

- **CP-690550 (Tasocitinib)**
  - Preclinical
  - Tested diseases: Rheumatic and autoimmune diseases, kidney transplantation, pulmonary eosinophilia, organ transplant rejection

- **NVP-BSK805**
  - Preclinical
  - Tested diseases: PV
  - Unique effects: –