Molecular Advances in the Diagnosis and Classification of Myeloproliferative Neoplasms

International Society for Laboratory Hematology
Chicago, IL – May 2015

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Major flavors of myeloid neoplasms

AML

MPN

MDS

MPN/MDS

MALNWEA AOPPOF
Myeloproliferative -vs- Myelodysplastic

quantitatively increased hematopoiesis

- effective
  - high peripheral counts
  - MPN

- ineffective
  - low peripheral counts
  - bone marrow hypercellularity
  - MDS

neoplastic hematopoietic stem cell disorders
Flavors of myeloproliferative neoplasms

MPN

"Classical"
- CML
- Ph-neg MPNs
  - PV
  - ET
  - PMF

"Non-classical"
- CEL
- CNL
- Mastocytosis
- Unclassifiable

MPN/MDS

CMML
- aCML
- Unclassifiable

J MML
CML – a peripheral blood diagnosis
CML – bone marrow helpful (but PB better!)
The Philadelphia story
Molecular testing in CML

Diagnosis

Monitoring

Resistance
CML diagnosis: t(9;22) and BCR-ABL1

? CML → cytogenetics → ? t(9;22) → molecular genetics

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? CMML

Avoid term “Ph-neg” CML

? aCML

? CML

✓ CML

~95% +

~5% -

~2.5% +

~2.5% -

✓ CML

✓ CML

× CML

? BCR-ABL1

~95% +

~5% -

~2.5% +

~2.5% -

× CML

✓ CML

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× CML
CML diagnosis: t(9;22) and BCR-ABL1

? CML → cytogenetics → ? t(9;22) → molecular genetics

~95% + → YES!

molecular target for:
1] Rx
2] MRD

~5% - → molecular genetics

→ ? cytogenetics

→ YES!

- clonal “evolution” (ACA) [Ph+; Ph- with imatinib]
Two major forms of therapy …

- **TKI**
  - Initial therapy of choice
  - Does not eradicate/cure CML
  - ? Long-term outcome
  - Minimal toxicity

- **SCT**
  - No longer 1st line Rx
  - Only Rx that cures CML
  - Major toxicity and mortality

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1. cytopenias (~40%), cardiotoxicity, ? mutagenicity [inhibit eph tumor suppressor, Ph (-) clones]
2. indicated when [i] very young, [ii] TKI failure, [iii] AP and BC
3. 10-yr survival ~65%
4. 10-20% mortality even when low risk
CML monitoring: definitions of response

**Complete hematologic**
- platelet: < 450
- WBC: < 10
- diff: no immature granulocytes
- basos: < 5%
- clinical: non-palpable spleen

**Cytogenetic # Ph+**
- none: >95%
- minimal: 66-95%
- minor: 36-65%
- partial: 1-35%
- complete: 0%

**Molecular**
- next slide please ...
<table>
<thead>
<tr>
<th># cells</th>
<th>diagnosis</th>
<th>complete hematologic remission</th>
<th>complete cytogenetic remission</th>
<th>major molecular response</th>
<th>“complete molecular remission” undetectable transcript</th>
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<td>$10^{12}$</td>
<td></td>
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<tr>
<td>$&lt;10^{11}$</td>
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<td>$&lt;10^{7-8}$</td>
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### Log Reduction

- $>3$: 1-log reduction by 3 months is the new Rx goal!
- $<0.1$: yet overall survival ~95%

### BCR-ABL1 Ratio

- $>4.5$: ~25% with 800mg
- $40\%$ @ 12mo
- $55\%$ @ 24mo
- $75\%$ @ 44mo

### Imatinib Responses

- $~98\%$
- $~85\%$
- $~75\%$
- $~40\%$
Mechanisms of resistance to imatinib

1. Kinase domain mutations:
   - most common cause of resistance [~40-90%]
   - spans ~240 aa’s

2. BCR-ABL1 amplification:
   - genomic > transcriptional [~10%]

3. Clonal evolution:
   - other genetic/cellular pathways [LYN]

4. ↓Bioavailability:
   - absorption
   - metabolism [hepatic]
   - plasma binding [$\alpha_1$ acid glycoprotein sequestration]
   - ↓influx ↑efflux [MDR1, PGP, BCRP2/ABCG2, hOCT1, MRP1]
Kinase domain mutations

P = P loop
- ATP-binding site
- ? worst mutations

B = Binding domain
- where imatinib binds

C = Catalytic domain

A = Activation loop
- conformation altered
- affects imatinib binding
- closed: inactive
- open: active

> 100 different mutations

these 6 account for > ~65% of all mutations

> 2-10% of patients

> >10% of patients
Genetic testing in CML: summary

**Diagnosis**
- CC: BM
- RT-PCR: PB qualitative vs quantitative with characterization

**Monitoring**
- CC: BM 3-6 monthly until CCR 6-12 monthly thereafter
- FISH: PB ?? before achieve CCR (no)
- RQ-PCR: PB 3 monthly

**Resistance**
- direct sequencing: PB ∨ BM Rx failure, loss of response, accelerated & blast phase
Molecular and other testing in non-CML MPNs

pre-2005 ...

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Genes</th>
<th>PRV1</th>
<th>EEC</th>
<th>↓ mpl megas</th>
<th>↓ GATA1 megas</th>
<th>↑ circ CD34+</th>
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<td>PV</td>
<td>9p+, +8+9</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
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<tr>
<td>ET</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>[50%]</td>
<td>+/-</td>
<td>?</td>
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<tr>
<td>PMF</td>
<td>del(13q14)</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
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<tr>
<td>PPMF</td>
<td>1q+</td>
<td>?</td>
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Molecular and other testing in non-CML MPNs

post-2005 ...

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JAK2 V617F mutation (etc)
JAK2 is out of the box …

• Just Another Kinase
  - one of many cloned at the time (1989)

• Janus Kinase
  - two-headed Roman god of gates and passages

• non-receptor tyrosine kinase (TK)
• has 2 TK domains (hence the name)
  - most TKs have only 1
• 4 members of JAK family
  - JAK1, JAK2, JAK3 and TYK2
JAK-STAT pathway

**MPL W515**
- ~5-10% PMF/ET

**JAK2 V617F**
- ligand-independent activation
  - ~95% PV
  - ~50% PMF
  - ~50% ET
**But wait, there’s more …**

- **JAK2 exon 12 mutations**
  - only in PV (thus ~100% PV have a JAK2 mutation)

- **CALR mutations**
  - calreticulin protein normally located in ER, cytosol, cell surface
  - functions:
    * Ca++ homeostasis
    * protein folding
    * chaperone
    * cell adhesion
  - mutations cluster in exon 9
  - frameshift -- >80% are either:
    * type 1 52bp del
    * type 2 5bp ins

  - loss of ER retention signal
  - no longer bind Ca

  - JAK-STAT activation
  - evasion from phagocytosis
Mutations in Ph-negative MPNs

- **3 drivers**
  - **JAK2**
    - PV: ~100%
    - PMF: ~60%
    - ET: ~50%
  - **CALR**
    - PV: ~0%
    - PMF: ~20-25%
    - ET: ~20-25%
  - **MPL**
    - PV: ~0%
    - PMF: ~5-10%
    - ET: ~5-10%

- **Epigenetic modifiers**
  - **TET2**
  - **IDH1/2**
  - **ASXL1**
  - **DNMT3A**
  - **EZH2**

- **Others**
  - **LNK**
  - **CBL**
  - **TP53**
  - **SRSF2**

Logical testing sequence:

- **test for prognosis**
PMF: driver mutations are prognostic

Rumi E et al. Blood 2014:124; 1062
Flavor of MPN affects survival: need to distinguish

Tefferi A et al. Blood 2014; 124:2507
Non-CML myeloproliferative neoplasms: histology matters!

- Can mimic ET, but:
  - clusters denser
  - cloud-like nuclei

<table>
<thead>
<tr>
<th>Polycythemia Vera</th>
<th>Peripheral Blood</th>
<th>Bone Marrow (hematoxylin and eosin)</th>
<th>Bone Marrow (reticulin stain)</th>
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<tbody>
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<td>Essential Thrombocythemia</td>
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| Idiopathic Myelofibrosis |                 |                                     |                              | cellular/ prefibrotic phase of PMF
Mixed MDS/MPNs: molecular genetics of JMML

- Ras-GDP
- Ras-GTP
- neurofibromin
- GM-CSF
- GM-CSFR
- SHP-2
- PTPN11
- NRAS/KRAS
- NF1

Juvenile myelomonocytic leukemia mutations

PTPN11 ~35%
NRAS/KRAS ~20%
NF1 ~20%

Mutually exclusive