Myeloproliferative Neoplasms (MPN and MDS/MPN)

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DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

- Speaking Bureau: Novartis Oncology
- Past research support from GlaxoSmithKline

None has any relevance in this presentation
Presentation will focus on:

– Myeloproliferative neoplasms

– Myelodysplastic/myeloproliferative neoplasms

Myeloproliferative Neoplasms*

- Chronic myeloid leukemia (CML), \( BCR-ABL1 \) pos.
- Chronic neutrophilic leukemia (CNL)
- Polycythemia vera (PV)
- Primary myelofibrosis (PMF)
- Essential thrombocytopenia (ET)
- Myeloproliferative neoplasm, unclassifiable (MPN-U)
- Chronic eosinophilic leukemia, NOS (CEL-NOS)

*Note: In the 2016 update mastocytosis has been moved out into a separate category
Chronic Myeloid Leukemia, \textit{BCR-ABL1} positive (update 2016)

- Name change to “\textit{myeloid}”
- New definition of lymphoid blast crisis
  - Any lymphoblast(s) in the PB raise concern for blast crisis
  - Cases with \textgreater5\% lymphoblasts in the BM should be diagnosed as blast crisis
- Resistance to TKI treatment is included in the definition of disease progression

Courtesy of Daniel Arber, Stanford University
Criteria for CML, Accelerated Phase

Any one or more of the following hematologic/cytogenetic criteria or response-to-TKI criteria:

- Persistent or increasing WBC (>10 X 10⁹/L), unresponsive to therapy
- Persistent or increasing splenomegaly, unresponsive to therapy
- Persistent thrombocytosis (>1000 X 10⁹/L), unresponsive to therapy
- Persistent thrombocytopenia (<100 X 10⁹/L) unrelated to therapy
- 20% or more basophils in the PB
- 10-19% blasts* in the PB and/or BM
- Additional clonal chromosomal abnormalities in Ph+ cells at diagnosis that include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2
- Any new clonal chromosomal abnormality in Ph+ cells that occurs during therapy

"Provisional" Response-to-TKI Criteria

- Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response** to the first TKI) or
- Any hematological, cytogenetic or molecular indications of resistance to two sequential TKIs or
- Occurrence of two or more mutations in BCR-ABL1 during TKI therapy

Large clusters or sheets of small, abnormal megakaryocytes, associated with marked reticulin or collagen fibrosis in biopsy specimens may be considered as presumptive evidence of AP, although these findings are usually associated with one or more of the criteria listed above.

*The finding of bona fide lymphoblasts in the blood or marrow, even if less than 10%, should prompt concern that lymphoblastic transformation may be imminent and warrants further clinical and genetic investigation; 20% or more blasts in blood or bone marrow, or an infiltrative proliferation of blasts in an extramedullary site is CML, blast phase. **Complete hematologic response: WBC <10x10⁹/L, Platelet count <450 x 10⁹/L, no immature granulocytes in the differential, and spleen non-palpable.
Chronic Neutrophilic Leukemia (CNL)

- True CNL: >90% CSF3R mutations
- Membrane proximal mutation: Empirical trial with JAK2 inhibitors (ruxolitinib)
- Truncation mutation(s): Empirical trial with SRC inhibitors (dasatinib)
- CSF3R is often co-mutated (SETBP1, ASXL1)

Lasho TL, Leukemia, 2014
Tefferi, Curr Opin Hematol. 2015
Elliott MA, Am J Hematol. 2015
CNL: WHO 2008 requirements

- No identifiable cause for physiologic neutrophilia
- No infectious or inflammatory process. No underlying tumor
- No evidence of a plasma cell neoplasm
- Hepatosplenomegaly
Diagnostic criteria for CNL (update 2016)

- Peripheral blood WBC \( \geq 25 \times 10^9 / L \) with segmented neutrophils plus band forms \( \geq 80\% \) of WBC. They lack dysplasia but may have toxic granulations
- Promyelocytes, myelocytes, metamyelocytes) <10\% of WBC
- Myeloblasts rarely observed in PB. In the BM <5\%
- Monocyte count <1 \( \times 10^9 / L \)
- Hypercellular bone marrow with neutrophil granulocytes increased in percentage and number. Neutrophil maturation appears normal
- No BCR-ABL1 or rearrangement of PDGFRA, PDGFRB or FGFR1, or PCM1-JAK2
- Presence of \textbf{CSF3R T618I} or other activating \textbf{CSF3R} mutation*
- Not meeting WHO criteria for PV, ET or PMF. A previous history of MPN, the presence of MPN features in the bone marrow and/or MPN-associated mutations (in JAK2, CALR or MPL) tend to exclude a diagnosis of CNL

*In the absence of a \textit{CSFR3R} mutation, persistent neutrophilia (at least 3 months), splenomegaly and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies

Adapted from Arber DA, Orazi A, Hasserjian RP et al. \textit{Blood}, April 27, 2016
The Classical $BCR/ABL1$ neg. MPNs

PV, PMF and ET
Diagnosed according to WHO, they are distinct diseases with different natural history.

Update 2016

• The integration of mutational data in diagnostic algorithms for the classical MPN
• The identification of early PV
• Refinements in the diagnosis of PMF
Driver Mutations in MPN Subtypes

**JAK2**
- 95% PV have JAK2 V617F (exon 14) mutation; 5% have JAK2 exon 12 mutation
- 60% PMF and ET have JAK2 V617F mutation

**CALR**
- 20-30% PMF and ET have CALR mutations*

**MPL**
- 5-10% PMF and ET have MPL exon 10 mutation

**Triple negative**
- 10-15% are “triple negative” In this cases, mutational analysis for a broad panel of genes associated with myeloid neoplasms is indicated

PMF: Comparison of survival among patients stratified by their mutational status


ET: Survival is the longest for triple-negative and shortest for MPL-mutated patients. Median survival: 19 years for JAK2 and 20 years for CALR-mutated cases (P=0.32)

Figure 2. Kaplan–Meier estimates of overall survival in 299 patients with ET stratified by mutation type.

Prognostically detrimental mutated genes: *ASXL1, EZH2, SRSF2 and IDH1/2*

The presence of two or more mutations predicted the worst survival:

- Two mutations: median 2.6 years (hazard ratio (HR) 3.8, 95% confidence interval (CI) 2.6-5.7)
- One mutation: 7.0 years (HR 1.9, 95% CI 1.4-2.6)
- No mutation: 12.3 years

Updates for PV
Polycythemia Vera: capturing the early disease phase

Modified from: Thiele J, Kvasnicka HM, Orazi A et al. WHO 2008
BMB correlates well with RCM. In a mildly erythrocytotic patient with JAK2 mutation in the proper clinical context, BM biopsy allows diagnostic confirmation of PV.
PV criteria (update 2016)

1. Increased red cell production
   - Hemoglobin >16.5/16.0 g/dL in men/women or hematocrit >49/48% in men/women
   - Hemoglobin >17/15 g/dL in men/women, with sustained increase of 2 g/dL over baseline
   - Increased red cell mass (>25% above normal)
   - Hemoglobin or hematocrit >99%le

2. Bone marrow showing PV histology

3. JAK2 mutation

Minor
- Decreased serum EPO levels
- Endogenous erythroid colony formation

Diagnosis requires the presence of the three major criteria. If JAK2 cannot be obtained, the first two major criteria plus the minor criterion (decreased EPO). If Hb levels > 18.5 g/dL in men (Hct 55.5 %) or >16.5 g/dL in women (Hct 49.5 %), BM biopsy may not be necessary for diagnosis if major criterion 3 and the minor criterion are present.
Updates for PMF
WHO criteria for prefibrotic/early primary myelofibrosis (pre-PMF)

Major criteria:
1. Megakaryocytic proliferation and atypia, without reticulin fibrosis > grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation and often decreased erythropoiesis
2. Not meeting the WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of JAK2, CALR or MPL mutation or in the absence of these mutations, presence of another clonal marker* or absence of minor reactive BM reticulin fibrosis **

Minor criteria:
Presence of at least one of the following, confirmed in two consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis \( >11 \times 10^9/L \)
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range

Diagnosis of pre-PMF requires meeting all three major criteria, and at least one minor criterion. *In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (e.g. ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1) are of help in determining the clonal nature of the disease **minor (grade 1) reticulin fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

For a diagnosis of overt phase PMF: presence of either reticulin and/or collagen fibrosis grades 2 or 3; L/E represents one additional minor criterion for overt PMF.
More comprehensive assessment of fibrosis and osteosclerosis
Summary of the changes for MPN

- Inclusion of novel molecular findings in addition to *JAK2* and *MPL* mutations; in particular in *JAK* negative cases, the *CALR* mutation provides proof of clonality, and has diagnostic and prognosis importance
- *CSF3R* mutation and its strong association with CNL
- PV was under-diagnosed using the Hb or HCT levels published in the 4th edition. New cut-offs plus BM morphology as new criteria for diagnosing early PV
- Only one minor criterion to diagnose pre-PMF
- Various minor refinements

- Myeloproliferative neoplasms
- Myelodysplastic/myeloproliferative neoplasms
MDS/MPN

• Chronic myelomonocytic leukemia (CMML)
• Atypical chronic myeloid leukemia, \textit{BCR-ABL1} negative (aCML)
• Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
• Myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U)
• Juvenile myelomonocytic leukemia (JMML)
CMML 2016: main updates

- Diagnostic/prognostic refinements
- Integration of molecular results
- Better exclusion of “mimics” such as MPN with monocytosis
CMML update 2016: diagnostic/prognostic refinements

Monocytosis (PB)
- Both $\geq 1 \times 10^9/L$ and $\geq 10\%$ of the WBC

MDS- vs. MPN-like (PB)
- CMML dysplastic (WBC, $<13 \times 10^9/L$)
- CMML proliferative ($\geq 13 \times 10^9/L$); this subtype has more frequent RAS or JAK2 mutations and splenomegaly

Refined blast count for prognosis (PB and BM)
- CMML-0: $<2\%$ blasts in PB; $<5\%$ blasts in BM
- CMML-1: 2–4$\%$ blasts in PB; 5–9$\%$ blasts in BM
- CMML-2: 5–19$\%$ blasts in PB; 10–19$\%$ in BM, or when Auer rods are present irrespective of the blast count

Storniolo AM, et al. Leukemia. 1990
Molecular correlates: mutations of *SRSF2, TET2, ASXL1*

- One of the three above mutations can be identified in at least 90% of CMML cases.
- Other mutations seen less frequently are *SETBP1, RAS, RUNX1, CBL, and EZH2*. They can be helpful diagnostic adjuncts in difficult cases, particularly given the frequently normal karyotype of CMML.
- Co-mutation of *TET2* and *SRSF2* is seen in about one-third of CMML cases and is a specific predictor of the diagnosis.
- *ASXL1* is a predictor of aggressive disease behavior and has been incorporated into a prognostic scoring system alongside karyotype and clinicopathologic parameters.
- Of note, *NPM1* mutation is seen in a rare subset of CMML (3-5%) and appears to herald a worst prognosis.
Mimic: Primary Myelofibrosis with Monocytosis (CMML-like)


JAK-V617F pos.; Monocytes >10% and >1.0 x10⁹/L
Karyotype: 46, XY [20]
Diagnostic criteria for CMML (Update 2016)

- Persistent PB monocytosis \(>1 \times 10^9/L\) and \(>10\%\) of the WBC
- Not meeting WHO criteria for \(BCR-ABL1\) pos. CML, PMF, PV or ET\(^a/b\)
- No rearrangement of \(PDGFRA, PDGFRB, FGFR1\), or \(PCM1-JAK2\)
- Fewer than 20% blasts \(^b\) in the PB and BM
- Dysplasia in \(>\) myeloid lineages. If myelodysplasia is absent, the diagnosis of CMML may still be made if the other requirements are met, and an acquired, clonal cytogenetic or molecular genetic abnormality \(^c\) is detected, --or-- the monocytosis has persisted for at least 3 months and all other causes of monocytosis have been excluded.

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\(^a\) Cases of MPN can be associated with monocytosis or they can develop it during the course of the disease. These cases may simulate CMML. In these rare instances, a previous documented history of MPN excludes CMML, while the presence of MPN features in the bone marrow and/or of MPN-associated mutations (\(JAK2, CALR\) or \(MPL\)) tend to support MPN with monocytosis rather than CMML.

\(^b\) Blasts and blast equivalents include myeloblasts, monoblasts and promonocytes. Promonocytes are monocytic precursors with abundant light grey or slightly basophilic cytoplasm with a few scattered, fine lilac-coloured granules, finely-distributed, stippled nuclear chromatin, variably prominent nucleoli, and delicate nuclear folding or creasing. Abnormal monocytes are excluded from the blast count.

\(^c\) The presence of mutations in genes often associated with CMML (e.g. \(TET2, SRSF2, ASXL1, SETBP1\)) in the proper clinical context can be used to support a diagnosis. It should be noted however, that some of the mutations can be age related or be present in subclones. Therefore caution would have to be used in the interpretation of these genetic results.
aCML 2016: main updates

• Integration of molecular data
• Separation from CNL
• Separation from other mimics (e.g. MPN with neutrophilia)
Atypical CML (*BCR/ABL1* neg)

- *SETBP1* mutations in 15-32% and *ETNK1* in 9% of cases
- *ETNK1* coexistent with *SETBP1* in 33% of cases
- *CSF3R* mutations absent or very rare (<10%)

Main distinction is with chronic neutrophilic leukemia (CNL)

Separation from CNL

**CNL**
- Neutrophilia with no significant dysplasia (toxic granulations)

**aCML**
- Neutrophilia with immature myeloid cells and dysplasia

*CSF3R (90%)*
additional *SETBP1 +/- (50%)*

*SETBP1 (15-32%)*
*ETNK1 (9%)*; in one third of these, coexists with *SETBP1* *CSF3R (<10%)*
Mimic: Polycythemia Vera with Neutrophilic Leukocytosis (CNL-like)

WBC > 25 x10⁹/L; FISH for BCR-ABL1 neg.; Karyotype: 46, XY [20]

Boiocchi et al. Mod Pathol. 2015;28:1448-57
Diagnostic criteria for aCML, BCR-ABL1 negative (update 2016)

- Leukocytosis ≥ 13×10⁹/L due to neutrophilia
- Granulocytic precursors (promyelocytes, myelocytes and metamyelocytes) ≥10% of WBC
- Dysgranulopoiesis (may include abnormal chromatin clumping)

Exclusions:
- No basophilia; basophils usually <2% of PB leukocytes; No monocytosis; monocytes <10% of PB leukocyte; Less than 20% blasts in the blood and bone marrow
- No BCR-ABL1, PDGFRA, PDGFRB or FGFR1 rearrangement, or PCM1-JAK2
- No Primary Myelofibrosis, Polycythemia Vera or Essential Thrombocythemia*

*Cases of MPN, particularly those in AP and/or in PV/ET myelofibrosis, if neutrophilic, may simulate aCML. A previous history of MPN, the presence of MPN features in the bone marrow tend to exclude a diagnosis of aCML. MPN-associated mutations in JAK2, CALR or MPL tend also to exclude a diagnosis of aCML. Conversely, a diagnosis of aCML is supported by the presence of SETBP1 and/or ETNK1 mutations. The presence of a CSF3R mutation is uncommon in aCML and if detected should prompt a careful morphologic review to exclude an alternative diagnosis of CNL or of other myeloid neoplasm.

SF3B1 mutation:

- Present in the vast majority (>80%) of MDS/MPN-RS-T
- Often co-mutated with JAK2 V617F (rarely MPL and CALR)
- Median survival is better in SF3B1-mutated patients than in SF3B1-non-mutated patients (6.9 and 3.3 years; P=0.003)

Malcovati L Blood 2011;118:6239
Patniak MM Blood 2012;119:5674
Cazzola M Blood 2013;121:260
Broséus J, et al. Leukemia. 2013
Diagnostic criteria for MDS/MPN-RS-T (update 2016)

- Anaemia associated with erythroid lineage dysplasia with or without multilineage dysplasia; ≥15% ring sideroblasts in BM; <1% blasts in PB and <5% blasts in BM

- Persistent thrombocytosis with platelet count ≥450 x 10⁹/L

- Presence of a *SF3B1* mutation*

- No *BCR-ABL1* fusion gene, no rearrangement of *PDGFRA, PDGFRB* or *FGFR1*; or *PCM1-JAK2*; no (3;3)(q21;q26), inv(3)(q21q26) or del(5q)**

- No history of MPN, MDS (except MDS-RS), or other type MDS/MPN

- In the absence of *SF3B1* mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features

*A diagnosis of MDS/MPN-RS-T is strongly supported by the presence of *SF3B1* mutation together with a mutation in *JAK2 V617F, CALR* or *MPL* genes

**In a case which otherwise fulfills the diagnostic criteria for MDS with isolated del(5q)

Summary: MDS/MPN revision

- Chronic myelomonocytic leukemia
- Atypical CML, $BCR-ABL_1$ negative
- Refractory anemia with ring sideroblasts associated with marked thrombocytosis (new name: MDS/MPN-RS-T)

- Mutation profile (SRSF2/TET2/ASXL1) helpful in supporting diagnosis and providing prognosis
- Cases with NPM1 mutation or 11q23 rearrangement should be followed carefully for AML
- Emphasize careful blast/promonocyte/monocyte count to distinguish from AML
- CMML-0,-1,-2; CMML MDS and MP subtypes
- Integration of NGS: SETBP1, CSF3R, ETNK1
- CNL: common co-mutation CSF3R/SETBP1
- Moved from a provisional to a full entity and new name
- Common co-mutation of JAK2 and SF3B1. Others.

Courtesy of Robert Hasserjian, MGH
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Thank you for your attention!