Dr. Larissa Liontos, MD, PhD
Department of Laboratory Medicine & Pathobiology
The University of Toronto
PGY4 Hematological Pathology Resident

Dr. Hubert Tsui, MD, PhD, FRCPC
Department of Laboratory Medicine and Pathobiology
University Health Network, University of Toronto, Toronto, ON, Canada
65 yo F presented to the hospital with shortness of breath, cough and weakness, placed on antibiotics for presumed pneumonia

Only significant past medical hx: thymic radiation at age 5

CBC at presentation: Hb 4.5 g/dL, WBC: 174x10^9/L with left-shift and circulating blasts, Platelets: 55

Other labs: Retic: 10, LDH 809 U/L

CT chest-bilateral lung infiltrates (bronchoalveolar lavage showed: immature granulocytes)

Abdomen Ultrasound: No hepatosplenomegaly
Peripheral blood findings

- Left-shift with dysplasia
  - WBC = 125 x 10^9/L
  - Anemia Hb 7.8 g/dL
- Thrombocytopenia, PLT = 28 x 10^9/L
- Blasts 6%
- Eosinophils & Basophils within reference range.
  - Monocytes 3.26 x 10^9/L
Bone Marrow Aspirate Findings

- Reduced erythroid lineage
- Dysplastic forms
- Granulocytic hyperplasia with dysplasia
Blasts 4%
Large nucleoli

Megakaryocytes (not shown) had normal morphology
Gating of blasts difficult due to increased immature, dysplastic myeloid precursors.

Blasts were CD34- & CD117+ and ~3% of total CD33 and CD13 bright.

Altered granulocyte maturation pattern, showing “granulocytes” in brown, “myeloid precursors” in red and “blasts” in green.
Trephine Biopsy Findings

Hypercellular biopsy

Megakaryocyte with normal morphology
CD117 immunohistochemistry

Expanded paratrabecular cuffs of immature cells
Preliminary diagnosis

- Favors Atypical Chronic Myeloid Leukemia, chronic phase. Molecular and cytogenetics is pending.
PB leukocytosis due to increased numbers of neutrophils & their precursors comprising >10%

Dysgranulopoiesis, which may include chromatin clumping

No or minimal absolute basophilia; basophils <2%

No or minimal absolute monocytosis; monocytes <10%

Hypercellular BM with granulocytic proliferation & dysplasia with or without dysplasia in erythroid & mega lineages

<20% blasts in PB and BM

No evidence of PDGFRA, PDGFRB, or FGFR1 or PCM1-JAK2

Not meeting criteria for BCR-ABL+ CML, PMF, PV, ET
Cytogenetics: 46XX [20]

PCR: Negative for BCR-ABL1 (p190 and p210), Negative for FLT3-ITD, **Positive for NPM1 mutation**

NGS panel (53 additional genes) pending
<table>
<thead>
<tr>
<th>Reference</th>
<th>Genes mutated in order of frequency</th>
<th>Gene mutations not detected</th>
<th>CMML</th>
<th>CNL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al. 2014</td>
<td>NRAS, JAK2 V617F, FLT3, CEBPA</td>
<td>CSF3R, CALR, MPL, KIT, IDH1/IDH2</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Targeted sequencing N=65 aCML</td>
<td></td>
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<tr>
<td>Piazza et al. 2013</td>
<td>TET2, ASXL1, SETBP1, EZH2, NRAS, CBL, IDH2, CEBPA</td>
<td>NPM1, JAK2, FLT3, DNMT3A, IDH1</td>
<td>SETBP1</td>
<td>SETBP1</td>
</tr>
<tr>
<td>Targeted sequencing N=61 aCML</td>
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<td></td>
<td></td>
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<tr>
<td>Meggendorfer et al. 2013</td>
<td>ASXL1, SETBP1, CBL</td>
<td>JAK2 exon 12</td>
<td>SETBP1</td>
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<tr>
<td>Targeted sanger sequencing N=60 aCML</td>
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<tr>
<td>Gambacorti-Passerini et al. 2015</td>
<td>SETBP1, NRAS, EZH2, ASXL1, ETNK1, U2AF1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Whole-exome sequencing N=15 aCML</td>
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<tr>
<td>Patnaik et al. 2017</td>
<td>ASXL1, TET2, NRAS-16%, SETBP1-12%, RUNX1, ETNK1, TP53, CALR, MPL, IDH1, TP53, CALR, MPL, IDH1, NPM1, CEBPA, CBL</td>
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<tr>
<td>Targeted panel N=25 aCML</td>
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</tbody>
</table>
## Next generation sequencing

<table>
<thead>
<tr>
<th>Gene</th>
<th>NM id</th>
<th>Variant (cDNA)</th>
<th>Variant (AA)</th>
<th>Variant Allele frequency</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS</td>
<td>NM_002524.3</td>
<td>c.35G&gt;A</td>
<td>p.Gly12Asp (G12D)</td>
<td>46.8</td>
<td>1</td>
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<tr>
<td>DNMT3A</td>
<td>NM_022552.4</td>
<td>c.2645G&gt;A</td>
<td>p.Arg882His (R882H)</td>
<td>47</td>
<td>1 or 2</td>
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<tr>
<td>IDH1</td>
<td>NM_005896.3</td>
<td>c.395G&gt;A</td>
<td>p.Arg132His (R132H)</td>
<td>45.7</td>
<td>1 or 2</td>
</tr>
<tr>
<td>NPM1</td>
<td>NM_002520.6</td>
<td>c.860_863dupTCTG</td>
<td>p.Trp288Cysfs*12</td>
<td>40.3</td>
<td>1 or 2</td>
</tr>
<tr>
<td>ASXL1*</td>
<td>NM_015338.5</td>
<td>c.4247A&gt;G</td>
<td>p.Glu1416Gly (E1416G)</td>
<td>49.9*</td>
<td>3B or 4B</td>
</tr>
</tbody>
</table>

Sequencing was performed using the Illumina TruSight Myeloid Sequencing Panel (54 genes) *reported as an extremely rare SNP
PCR: Negative for BCR-ABL1 (p190 and p210), Negative for FLT3-ITD, **Positive for NPM1 mutation**

NGS panel (53 additional genes):
- 5 variants identified (4 with suspected pathogenic effects)
- NPM1 mutation confirmed
- SETBP1 mutation germline
- ETNK1 mutation not on NGS panel and not available at our institution
NPM1 is one of the most frequently mutated genes in AML (1/3 of cases) & is associated with good prognosis in cases with normal karyotype and absence of FLT3-ITD mutation.

Falini et al. 2010: 85% of NPM1-mutated AML cases show normal karyotype

NPM1 mutations are most often seen in de novo AML, however, several studies have now shown NPM1 mutation in MDS or MDS/MPN prior to blast counts reaching the threshold for AML diagnosis (Schnittger et al. 2011; Lin et al. 2015)

Caudill et al. 2006: Shorter time to progression to AML in patients harboring NPM1 mutations in CMML (no studies on prognosis/significance in aCML)
aCML-Diagnostic & Prognostic dilemmas

Diagnostic-
- No mutation completely characteristic of aCML- SETBP1 & ETNK1 are only seen in a small fraction of cases.
- The most common mutations ASXL1, DNMT3A, TET2 are not specific for aCML or even MDS and can be present in clonal hematopoiesis of indeterminate significance (CHIP).

Prognostic-
- aCML is a rare disease with studies to date only including small samples of patients; the largest, a multicenter study, had 65 patients.
- Typically aCML has a poor prognosis with overall survival between 14-30 months and a rate of progression to AML of 40%.
- ASXL1 & SETBP1 mutations may be associated with worse prognosis.
- The prognostic significance of a NPM1 mutation is unclear since it is found so infrequently in MDS/MPNs. In CMML, it may represent accelerated disease with shorter progression to AML.

Discussion point-
- Should NPM1 mutation be AML disease defining regardless of blast count (and therefore part of the exclusionary criteria for aCML)?
How to best classify this case?

- Atypical CML (BCR-ABL1-negative)
  - Morphology is consistent, genetic alterations (SETBP1 not present, ETNK1 not in NGS panel)
  - 40% of cases have N-RAS mutations, although none have been reported to be NPM1-mutated

- Chronic myelomonocytic Leukemia (CMML)
  - Monocytosis present, but less than 10% in PB
  - Neutrophilia is marked in aspirate & biopsy
  - Genetic alterations consistent with this diagnosis

- MDS/MPN-unclassifiable

- Are any of these genetic alterations sufficient for a diagnosis of AML with <20% blasts?
Our Diagnosis: Hypercellular bone marrow with features of MDS/MPN and NPM1 mutation

Panel Diagnosis: Atypical Chronic Myeloid Leukemia
Patient received on hydroxyurea and followed up at their community hospital 2 months later

A bone marrow was sent to our institution for consultation

Consultant diagnosis: AML with myelodysplasia-related changes

- Blasts with monocytic features/promonocytes greater than 30%

- Eight months since initial diagnosis, patient is alive on palliative azacytidine
Acknowledgements

UHN Toronto General Hospital Hematopathology Group
- Dr. Graeme Quest
- Dr. Rashmi Goswami
- Dr. Anne Tierens
- Dr. David Barth

Dr. Michael Rauh (KGH/Queen’s University)

UHN advanced molecular diagnostics group
- Dr. Tracey Stockley
- Nisha Kanwar


5. Meggendorfer, M. et al. SETBP1 mutations occur in 9% of MDS/MPN and in 4% of MPN cases and are strongly associated with atypical CML, monosomy 7, isochromosome i(17)(q10), ASXL1 and CBL mutations. *Leukemia*. **27**, 1852–1860 (2013).


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Dr. Liontos

Atypical chronic myeloid leukemia, BCR-ABL1-negative