Chronic Myelomonocytic Leukemia with molecular abnormalities

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Patient History

- A 68 year old male with normocytic normochromic anemia for 5 years without a specific etiology (Normal iron and Vit B12/Folate studies)

- NO fatigue, night sweats, splenomegaly etc.

- A bone marrow biopsy 7 months back revealed a hypercellular bone marrow with minimal dysplasia not meeting criteria for MDS/MPN.

- A new bone marrow biopsy was performed and NGS Myeloid panel (targeted sequencing) was ordered on the bone marrow
**Hemoglobin (g/dl)**

Most recent bone marrow biopsy

**Platelet count (K/ul)**

Most recent bone marrow biopsy

### DIFFERENTIAL COUNT
- Neutrophil, %: 57
- Lymphocyte, %: 22
- Monocyte, %: 18
- Eosinophil, %: 2
- Basophi, %: 1
Monocyte count (normal range, 0-0.8 K/ul)

Intermittent monocytosis for 5 years; sustained monocytosis for 3 months above 1k/ul was seen before dropping to the most recent monocyte count of 0.83k/ul.
## Bone marrow aspirate count (1000 cells)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>2%</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>6%</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>7%</td>
</tr>
<tr>
<td>Metamyelocyte</td>
<td>4%</td>
</tr>
<tr>
<td>Bands</td>
<td>15%</td>
</tr>
<tr>
<td>PMN:</td>
<td>13%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1%</td>
</tr>
<tr>
<td>Basophils</td>
<td>%</td>
</tr>
<tr>
<td>Promonocytes</td>
<td>2%</td>
</tr>
<tr>
<td>Monocytes</td>
<td><strong>11%</strong></td>
</tr>
<tr>
<td>Erythroid</td>
<td>37%</td>
</tr>
<tr>
<td>Lymphoblast</td>
<td>%</td>
</tr>
<tr>
<td>Prolymphocyte</td>
<td>%</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>2%</td>
</tr>
<tr>
<td>Atypical Lymph</td>
<td>%</td>
</tr>
<tr>
<td>Plasmablast</td>
<td>%</td>
</tr>
<tr>
<td>Proplasmacyte</td>
<td>%</td>
</tr>
<tr>
<td>Plasma Cell:</td>
<td>1%</td>
</tr>
</tbody>
</table>

**M:E ratio, 1.6:1**

**Rare ringed sideroblasts noted**
Monoblasts and Promonocytes, 1000x
Promonocytes, 1000x
Atypical and immature monocytes, 500x
Dysplastic megakaryocytes, 500x

> 10% Dysplastic megakaryocytes

Rare dyspoietic erythroid cells and rare hypogranular neutrophils (less than 10%)
Bone marrow biopsy, 20x

Average cellularity, 60-70%
Bone marrow biopsy, 200x

Occasional monolobated megakaryocytes
CD34 staining

Reticulin staining - NO fibrosis (MF grade 0/3)
• Chromosomal analysis: 46, XY
• FISH for MDS: NO abnormalities detected
• **NGS**- Trusight myeloid panel from Illumina. PCR products sequenced on MiSeqDx sequencer (Technical sensitivity of 5%).
• Annotation was done using Cartagenia Bench Lab
NGS mutations (Dr. Juan Gomez)

1. **SRSF2** c.284C>A p.P95H
   Variant Allele fraction 53.4%

2. **TET2** c.3195_3202dupGACTAGAC p.Q1068Rfs*17
   Variant Allele fraction 72.1%

3. **TET2** c.3972_3973dupTC p.H1325Lfs*39
   Variant allele fraction 11.5%

4. **ASXL1** c.2290delC p.L764Yfs*8
   Variant Allele fraction 48.15%
Proposed Diagnosis
Chronic Myelomonocytic Leukemia-0

Panel Diagnosis
Chronic Myelomonocytic Leukemia-0. The mutational Spectrum is characteristic of CMML
Patient Followup

- NO active treatment; observation only
- Absolute monocyte count up from 0.83 to 0.86 K/ul
- HgB down from 12 g/dl to 11.4 g/dl
- Platelet count down from 139 K/ul to 130 K/ul
Chronic Myelomonocytic Leukemia

Table 11. Diagnostic criteria for CMML

<table>
<thead>
<tr>
<th>CMML diagnostic criteria</th>
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</thead>
<tbody>
<tr>
<td>• Persistent PB monocyteosis $\geq 1 \times 10^9$/L, with monocytes accounting for $\geq 10%$ of the WBC count</td>
</tr>
<tr>
<td>• Not meeting WHO criteria for $BCR-ABL1^+$ CML, PMF, PV, or ET*</td>
</tr>
<tr>
<td>• No evidence of $PDGFRA$, $PDGFRB$, or $FGFR1$ rearrangement or $PCM1-JAK2$ (should be specifically excluded in cases with eosinophilia)</td>
</tr>
<tr>
<td>• $&lt;20%$ blasts in the blood and BM†</td>
</tr>
<tr>
<td>• Dysplasia in 1 or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are met and</td>
</tr>
<tr>
<td>• An acquired clonal cytogenetic or molecular genetic abnormality is present in hemopoietic cells‡</td>
</tr>
<tr>
<td>or</td>
</tr>
<tr>
<td>• The monocytosis (as previously defined) has persisted for at least 3 mo and</td>
</tr>
<tr>
<td>• All other causes of monocytosis have been excluded</td>
</tr>
</tbody>
</table>

Chronic Myelomonocytic Leukemia

- **CMML**: **proliferative type** (WBC $\geq 13K/ul$) vs. **dysplastic type** (WBC $<13K/ul$) *(Cervera et al. Am J Hematol 2014)*

- Better prognostication: **CMML-0** (<2% blasts in PB and <5% blasts in BM), **CMML-1** (2-4% blasts in PB and/or 5-9% blasts in BM) and **CMML-2** (5-19% blasts in PB and/or 10-19% blasts in BM) *(Schuler et al. Leukemia Res 2014, Cervera et al. Am J Hematol 2014)*

- Clonal cytogenetic abnormality seen in **30%** of cases (trisomy 8, -Y, monosomy 7 and del7q, trisomy 21 and complex karyotypes)

- Gene mutations seen in **$>90\%$** of CMML cases *(Patnaik and Tefferi, Blood Cancer Journal 2016)*
CMML mutation spectrum

Methylation & Hydroxy-methylation:
1. TET2 (~60%)
2. IDH1
3. IDH2
4. DNMT3A

Spliceosome components:
1. SRSF2 (~50%)
2. SF3B1
3. U2AF1
4. ZRSR2

Chromatin & Histone modification:
1. ASXL1 (~40%)
2. EZH2
3. SUZ12
4. EED
5. UTX

Tumor suppressor genes:
1. Tp53
2. PHF6

Cohesin complex:
1. STAG2
2. BCOR
3. SMC1A
4. RAD21

Others:
1. RUNX1 (~15%)
2. SETBP1 (~10-15%)

Cell signalling:
1. RAS (~30%)
2. CBL (~15%)
3. JAK2
4. FLT3
5. SH2B3

Mutational evolution and clonal hierarchy in CMML

Epigenetic mutations
- TET2
- ASXL1
- EZH2
- IDH1/2

Splicing abnormalities
- SRSF2
- SF3B1
- U2AF1
- ZRSR2

Transcription factors
- RUNXI

Tumor suppressor genes
- PHF6
- Tp53

Cell signalling & Tyrosine kinases
- RAS
- CBL
- JAK2

SRSF2 mutations

• Spliceosome component mutations which affects pre-mRNA splicing

• MDS (15-20%), **CMML (50%)**, primary myelofibrosis (15-20%) and AML

• CMML: association with increased age and diploid karyotype

ASXL1 mutations

• Epigenetic regulator gene which regulates chromatin by interacting with polycomb group repressive complex proteins (PRC1 and PRC2)

• MDS (15-20%), CMML (40-50%), primary myelofibrosis (20-35%) and AML (5-10%)

• ASXL mutations associated with a proliferative phenotype (higher WBC, absolute monocyte count and immature myeloid cells)

• Multivariate analyses, ASXL1 mutations (frameshift and nonsense) impact survival (Molecular Mayo model and Groupe Francais des Myelodysplasies (GFM) model)

TET2 mutations

- Epigenetic regulator gene which impacts DNA methylation and hydroxy-methylation

- MDS (15%), CMML (60%), polycythemia vera (15%), primary myelofibrosis (15%) and AML (20%) and systemic mastocytosis (30%)

- No clear impact on either OS or leukemia free survival in CMML (Itzykson T et al. JCO 2013)

Differential diagnoses

- **Reactive monocytosis** with a non-neoplastic cause of the megakaryocytic dyspoiesis (especially considering that most recent monocyte count is less than 1 K/ul and monocytosis is intermittent)
  - *Detection of characteristic mutations help in establishing CMML diagnosis in this case (i.e. clonal nature of disease)*
  - *Caveat: Context, Context and Context considering that these mutations can be seen in healthy older individuals (CHIP)*
    (Jaiswal et al. NEJM 2014 and Genovese et al. NEJM 2014)

- **MDS with monocytosis** (MDS criteria, PB monocytes < 1 K/ul)
  - *Is there a mutational spectrum that differentiates MDS from CMML?*
  - *Higher mutation burden in CMML as compared to MDS (Reinig et al. AJCP, 2016, Malcovati et al. Blood 2014)*
  - *In the context of dysplasia, combination of TET2 and SRSF2 is highly specific for CMML disease phenotype (specificity, 97.6%) (Malcovati et al. Blood 2014)*

- **MDS/MPN unclassifiable**
Oligomonocytic chronic myelomonocytic leukemia (chronic myelomonocytic leukemia without absolute monocytosis) displays a similar clinicopathologic and mutational profile to classical chronic myelomonocytic leukemia

Julia T Geyer¹, Wayne Tam¹, Yen-Chun Liu¹, Zhengming Chen², Sa A Wang³, Carlos Buésò-Ramos³, Jean Oak⁴, Daniel A Arber⁵, Eric Hsi⁶, Heesun J Rogers⁶, Katherine Levinson⁷, Adam Bagg⁷, Duane C Hassane¹, Robert P Hasserjian⁸ and Attilio Orazi¹

¹Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY, USA; ²Division of Biostatistics and Epidemiology, Department of Healthcare Policy & Research, Weill Cornell Medical College, New York, NY, USA; ³Department of Hematopathology, the University of Texas MD Anderson Cancer Center, Houston, TX, USA; ⁴Department of Pathology, Stanford University, Stanford, CA, USA; ⁵Department of Pathology, University of Chicago, Chicago, IL, USA; ⁶Department of Laboratory Medicine, Cleveland Clinic, Cleveland, OH, USA; ⁷Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA and ⁸Department of Pathology, Massachusetts General Hospital, Boston, MA, USA
Oligomonocytic CMML vs. CMML (Geyer et al. Mod Path Path 2017)

- Oligomonocytic CMML, 44 cases (relative monocytosis ≥ 10%, Absolute monocyte 0.5-1K/ul)
- Lower WBC count and ANC but similar monocyte percentage, HgB and Plt count
- M:E ratio normal or decreased vs. CMML
- 38% progressed to CMML (median: 12 months)
- Percentage of mutations lower as compared to CMML
- TET2, ASXL1 and SRSF2 mutations most common
- CBL mutations exclusive to CMML group
- A subset of oligomonocytic CMML represent early phase of “dysplastic” CMML
Conclusion

• NGS can be helpful in cases where all criteria are not fulfilled for CMML diagnosis (to establish clonal nature of the disease)
• NGS can help prognosticate CMML (ASXL1 mutations)
• Not all mutations are created equal (ASXL1 frameshift and nonsense mutations are prognostically important)
Questions?

Panel Diagnosis

Chronic Myelomonocytic Leukemia-0.
The mutational Spectrum is characteristic of CMML
### Table 1: Gene Regions Assessed by the TruSight Myeloid Sequencing Panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Target Region (exon)</th>
<th>Gene</th>
<th>Target Region (exon)</th>
<th>Gene</th>
<th>Target Region (exon)</th>
<th>Gene</th>
<th>Target Region (exon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL1</td>
<td>4-8</td>
<td>DNMT3A</td>
<td>full</td>
<td>KDM5A</td>
<td>full</td>
<td>RAD2I</td>
<td>full</td>
</tr>
<tr>
<td>ASXL1</td>
<td>12</td>
<td>ETV6/TEL</td>
<td>full</td>
<td>KIT</td>
<td>2, 8-11, 13 + 17</td>
<td>RUNX1</td>
<td>full</td>
</tr>
<tr>
<td>ATRX</td>
<td>B-10 and 17-31</td>
<td>EZH2</td>
<td>full</td>
<td>KRAS</td>
<td>2 + 3</td>
<td>SETBP1</td>
<td>4 (partial)</td>
</tr>
<tr>
<td>BCCOR</td>
<td>full</td>
<td>FBMW7</td>
<td>9 + 10 + 11</td>
<td>MLL</td>
<td>5-8</td>
<td>SF3B1</td>
<td>13-18</td>
</tr>
<tr>
<td>BCO8L1</td>
<td>full</td>
<td>FLT3</td>
<td>14 + 15 + 20</td>
<td>MPL</td>
<td>10</td>
<td>SMC1A</td>
<td>2, 11, 16 + 17</td>
</tr>
<tr>
<td>BRAF</td>
<td>15</td>
<td>GATA1</td>
<td>2</td>
<td>MYD88</td>
<td>3-5</td>
<td>SMC3</td>
<td>10, 13, 19, 23, 25 + 28</td>
</tr>
<tr>
<td>CALR</td>
<td>9</td>
<td>GATA2</td>
<td>2-8</td>
<td>NOTCH1</td>
<td>28-28 + 34</td>
<td>SRSF2</td>
<td>1</td>
</tr>
<tr>
<td>CBL</td>
<td>B + 9</td>
<td>GNAS</td>
<td>B + 9</td>
<td>NPM1</td>
<td>12</td>
<td>STAG2</td>
<td>full</td>
</tr>
<tr>
<td>CBLB</td>
<td>9, 10</td>
<td>HRAS</td>
<td>2 + 3</td>
<td>NRAS</td>
<td>2 + 3</td>
<td>TET2</td>
<td>3-11</td>
</tr>
<tr>
<td>CBLC</td>
<td>9, 10</td>
<td>IDH1</td>
<td>4</td>
<td>PDGFRA</td>
<td>12, 14, 18</td>
<td>TP53</td>
<td>2-11</td>
</tr>
<tr>
<td>CCKNI2A</td>
<td>full</td>
<td>IDH2</td>
<td>4</td>
<td>PHF5</td>
<td>full</td>
<td>U2AF1</td>
<td>2 + 6</td>
</tr>
<tr>
<td>CEBPA</td>
<td>full</td>
<td>IKZF1</td>
<td>full</td>
<td>PTEN</td>
<td>5 + 7</td>
<td>WT1</td>
<td>7 + 9</td>
</tr>
<tr>
<td>CSF3R</td>
<td>14-17</td>
<td>JAK2</td>
<td>12 + 14</td>
<td>PTPN11</td>
<td>3 + 13</td>
<td>ZRSR2</td>
<td>full</td>
</tr>
<tr>
<td>CLX1</td>
<td>full</td>
<td>JAK3</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. SRSF2 c.284C>A p.P95H
Additional information:
1-2. 17q25.1
3. HG19
4. NM_003016.4
7. 3502 reads
8. 3051 reads
9. 3502/6553=53.4%

2. TET2 c.3195_3202dupGACTAGAC p.Q1068Rfs*17
Additional information:
1-2. 4q24
3. HG19
4. NM_001127208.2
7. 6333 reads
8. 2450 reads
9. 6333/8783=72.1%

3. TET2 c.3972_3973dupTC p.H1325Lfs*39
Additional information:
1-2. 4q24
3. HG19
4. NM_001127208.2
7. 1634 reads
8. 12554 reads
9. 1634/14188=11.5%

4. ASXL1 c.2290delC p.L764Yfs*8
Additional information:
1-2. 20q11.21
3. HG19
4. NM_015338.5
7. 8749 reads
8. 9433 reads
9. 8749/18182=48.15%
Taken from reference 1. It shows the position of TET2 mutations in myeloid neoplasms.

Red arrow is the mutations in our case.

Taken from cBioPortal (MSKCC database). It shows the position of TET2 mutations in AML and MDS (this database does not have many myeloid neoplasms yet). The green box is the double stranded B-helix dioxygenase domain which is affected by
Taken from reference 2. It shows the position of ASXL1 mutations in myeloid neoplasms as well as the domains L764Yfs*8

Taken from cBioPortal (MSKCC database which is publicly available). It shows the position of ASXL1 mutations in AML and MDS. The blue box is the PHD domain which is the one that needs to be lost in order for the protein to lose its function.