A CASE OF PERSISTANT NEUTROPHILIA: 
BCR-ABL NEGATIVE

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CLINICAL HISTORY

• An 80 year old male presented with anaemia and persistently raised white cell count (70.2 x 10^9/l)

• Blood film
  • **Differential:**
    • Neutrophils 81% (57 x 10^9/l)
    • Lymphocytes 3.6%
    • Monocytes 2%
    • Eosinophils 2%
    • Promyelocytes 1.6%
    • Myelocytes 4.3%
    • Metamyelocytes 5%
    • Blasts 0.5%
    • No basophils

• **Neutrophil morphology:**
  • Some very well granulated/toxic granulation
  • Some dysplastic
    • Hypogranular cytoplasm
    • Hypolobated nuclei
    • Pseudo-Pelger Huet forms

• **Molecular testing:**
  • No evidence of BCR-ABL fusion
  • CALR normal
  • JAK2 V617F normal
**BONE MARROW ASPIRATE AND TREPHINE**

**Flow Cytometry:**
Neoplastic myeloid progenitors = 1.0% of total cells, composite phenotype:
- CD45+CD34+CD117+ CD15-CD13+HLADR+ CD33+/-CD7+/- CD64-CD56-
- Monocytic cells (CD64+CD14(78%)+CD56-) = 3.44% of total cells.

**Trephine:**
Hypercellular marrow with expanded myeloid series and increased and atypical megakaryocytes
Dysplastic changes in myeloid series (no ring sideroblasts)
ADDITIONAL RESULTS

Cytogenetic/FISH:
• Normal male karyotype

Targeted High throughput sequencing:
• CSF3R mutated (p.Thr618Ile, c1853C>T)
• SETBP1 mutated (p.Asp868Asn, c2602G>A)

Sanger sequencing:
• SRSF2 mutated (p.P95L, c.284C>T)
• MPL exon 10 normal
PROPOSED DIAGNOSIS

ATYPICAL CHRONIC MYELOID LEUKAEMIA

No follow-up available

Diagnosis of atypical CML generally associated with poor prognosis

- Overall median survival approx. 25 months
- 40% transform to acute leukaemia

(Breccia M et al, Haematologica 2006)
Peripheral blood leukocytosis ≥ 13 x 10⁹

- Due to increased neutrophils and precursors ✔
  - Dysgranulopoiesis, may include abnormal chromatin clumping ✔
  - Immature granulocytes (promyelocytes, myelocytes, metamyelocytes) account for ≥ 10% of white cells ✔
  - Myeloblasts <20% white cells ✔

No or minimal absolute basophilia; <2% leukocytes ✔

No or minimal monocytosis; monocytes <10% of leukocytes ✔

Hypercellular bone marrow

- Granulocytic proliferation and dysplasia +/- dysplasia in erythroid and megakaryocyte lineages ✔
- Myeloblasts <20% nucleated cells ✔

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

Not meeting criteria for BCR-ABL1+ CML, PMF, or ET ✔
CSF3R MUTATIONS (Dwivedi P and Greis KD, Exp Hematol 2017;46:9-20)

CSF3R is the receptor for Granulocyte Colony Stimulating Factor

- Transmembrane protein of 813 amino acids
- Binding by ligand induces conformational change and activation of downstream pathways

![Diagram of CSF3R]

- **JAK/STAT**
- **MAPK/ERK**
- **PI3K/AXT**

**Neutropenia**
(severe congenital neutropenia & chronic idiopathic neutropenia)

**Myeloid neoplasms**
(constitutive activation of JAK/STAT)

e.g. T618I

**SCN ➔ AML/MDS**
(SRC Kinase activity↑)

**GRANULOCYTE PROLIFERATION**

**GRANULOCYTE DIFFERENTIATION**
**CSF3R MUTATIONS IN ATYPICAL CML**

**ORIGINAL ARTICLE**

Oncogenic CSF3R Mutations in Chronic Neutrophilic Leukemia and Atypical CML

Julia E. Maxson, Ph.D., Jason Gotlib, M.D., Daniel A. Pollyea, M.D., Angela G. Fleischman, M.D., Ph.D., Anupriya Agarwal, Ph.D., Christopher A. Eide, B.A., Daniel Bottomly, M.S., Beth Wilmot, Ph.D., Shannon K. McWeeny, Ph.D., Cristina E. Tognon, Ph.D., J. Blake Pond, M.S., Robert H. Collins, M.D., Basem Goueli, M.D., Ph.D., Stephen T. Oh, M.D., Ph.D., Michael W. Deininger, M.D., Ph.D., Bill H. Chang, M.D., Ph.D., Marc M. Loriaux, M.D., Ph.D., Brian J. Druker, M.D., and Jeffrey W. Tyner, Ph.D.

2013: 1st descriptions of CSF3R mutations in myeloid neoplasms

- 8/17 (47%) cases of aCML (n=4) or suspected aCML (n=3)
- 8/9 (89%) cases of CNL


Later studies suggest real incidence of CSF3R mutations in aCML likely to be much lower

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of cases harbouring mutated CSF3R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pardanani A et al, Leukemia 2013</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td>Wang SA et al, Blood 2014</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td>Meggendorfer M et al, Haematologica 2014</td>
<td>2/58 (3%)</td>
</tr>
<tr>
<td>Patnaik MM et al, Am J Hematol 2017</td>
<td>2/25 (8%)</td>
</tr>
<tr>
<td>Gambacorti-Passerini CD et al, Blood 2015</td>
<td>0/15 (0%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>4/134 (3.0%) (7.9% incl Maxon cases)</strong></td>
</tr>
</tbody>
</table>
**CSF3R MUTATIONS IN CNL AND OTHER MYELOID NEOPLASMS**

*CSF3R* mutations in myeloid neoplasms (combined results)

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>%</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNL</td>
<td>32/49</td>
<td>65%</td>
<td>1-3,7</td>
</tr>
<tr>
<td>aCML</td>
<td>12/151</td>
<td>7.9%</td>
<td>1-3</td>
</tr>
<tr>
<td>CMML/JMML</td>
<td>8/470</td>
<td>1.7%</td>
<td>1,3,5,6</td>
</tr>
<tr>
<td>MDS</td>
<td>0/88</td>
<td>0%</td>
<td>5,6</td>
</tr>
<tr>
<td>PMF</td>
<td>0/76</td>
<td>0%</td>
<td>1</td>
</tr>
<tr>
<td>ET</td>
<td>0/21</td>
<td>0%</td>
<td>5</td>
</tr>
<tr>
<td>De novo AML</td>
<td>20/2364</td>
<td>0.8%</td>
<td>2,4-6</td>
</tr>
</tbody>
</table>

1. Pardanani A, Leukemia 2013
2. Maxson JE, NEJM 2013
4. Tefferi A, Haematologica 2013
6. Hwang SY, Ann Hematol 2015
7. Cui Y, J Hematol Oncol
8. Kosmider O, Leukemia 2013
10. Patnaik MM, Hematology 2017

**CSF3R mutations are relatively specific for CNL amongst myeloid neoplasms**
Initial studies indicated relatively high frequency in aCML, e.g.

<table>
<thead>
<tr>
<th>Study</th>
<th>Count</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piazza R et al, Nat Genet 2013</td>
<td>17/70</td>
<td>24.3%</td>
</tr>
<tr>
<td>Gambacorti-Passerini CB et al, Blood 2015</td>
<td>4/15</td>
<td>26.7%</td>
</tr>
<tr>
<td>Meggendorfer M et al, Leukemia 2013</td>
<td>19/60</td>
<td>31.7%</td>
</tr>
</tbody>
</table>

May also present in CNL, e.g.

<table>
<thead>
<tr>
<th>Study</th>
<th>Count</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piazza R et al, Nat Genet 2013</td>
<td>1/4</td>
<td>25%</td>
</tr>
<tr>
<td>Pardanani A et al, Leukemia 2013</td>
<td>4/12</td>
<td>33%</td>
</tr>
</tbody>
</table>

Low incidence/not present in other myeloid neoplasms, e.g./
# SETBP1 MUTATIONS IN MYELOID NEOPLASIA

Piazza R et al, Nat Genet 2013.45.18-24

## Table 1  Frequency of SETBP1 mutations in 644 patient samples and 344 cancer cell lines

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Number of samples</th>
<th>Number of mutated samples</th>
<th>Percent mutated samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>106</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ALL</td>
<td>62</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CLL</td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MDS</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MPN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PMF</td>
<td>33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PV</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ET</td>
<td>36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CNL</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>MDS/MPN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCML</td>
<td>70</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>Unclassified MDS/MPN</td>
<td>30</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>CMML</td>
<td>82</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>JMML</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
**CSF3R AND SETBP1 MUTATIONS MAY CO-EXIST IN aCML & CNL**

Cases of CNL with *CSF3R* mutation often also harbour mutation of *SETBP1*
- Pardanani A et al 2013: 40% (4/10 cases)
- Cui Y et al 2014: 60% (6/8 cases)

Cases of aCML with *SETBP1* mutation may also have *CSF3R* mutation, e.g.
- Meggendorfer M et al, 2014

Hypothesized that presence of both mutations infers bad prognosis/resistance to JAK inhibitor (Ruxolitib) in CNL and aCML:

Chronic neutrophilic leukemia with concurrent *CSF3R* and *SETBP1* mutations: single colony clonality studies, *in vitro* sensitivity to JAK inhibitors and lack of treatment response to ruxolitinib

Lasho TL et al, Leukemia 2014

Ammatuna E et al, Ann Hematol 2015
Initial study of this case raised a differential diagnosis (aCML vs CNL)

Targeted molecular testing, performed to help refine the diagnosis, revealed mutations of $SETBP1$ and $CSF3R$

A diagnosis of aCML made in light of:
- Appropriate morphological findings
  - Dysplastic features present
  - Left shift in peripheral blood
- Compatible/suggestive mutational profile, i.e.
  - $SETBP1$ mutations associated with aCML
  - $CSF3R$ mutations more specific for CNL
  - But both mutations may co-exist in aCML

Highlights the importance of interpreting molecular abnormalities in the context of other findings
ACKNOWLEDGEMENTS

HMDS, St James’s University Hospital, Leeds
Jan Taylor
Paul Evans
Sharon Barrans
Matthew Cullen
FINAL PANEL DIAGNOSIS

Atypical chronic myeloid leukemia, *BCR-ABL1*-negative