To the editor:

Biclonal expansion and heterogeneous lineage involvement in a case of chronic myeloproliferative disease with concurrent MPL$^{W515L}$/JAK2$^{V617F}$ mutation

Chronic myeloproliferative diseases (CMPDs)/myeloproliferative neoplasms (MPNs) are caused by clonal mutations of tyrosine kinase or receptor genes such as BCR-ABL, JAK2$^{V617F}$, and MPL$^{W515L}$. Recently, we and others showed that these mutations may be combined in hematopoietic cells of some MPN cases.1,2 It is not yet clear whether, in cases with a double mutation, subclonal evolution has taken place or whether 2 unrelated clones proliferate independently. To address this issue, bone marrow and peripheral blood cells in a case of unclassifiable MPN (Figure S1, available on the Blood website; see the Supplemental Materials link at the top of the online article) with combined JAK2$^{V617F}$ and MPL$^{W515L}$ mutation were separated by laser microdissection as well as fluorescence-activated cell sorting, followed by quantitative analysis of mutated allele burden by pyrosequencing.3

Hematopoietic lineages demonstrated a heterogeneous involvement of MPL$^{W515L}$ and JAK2$^{V617F}$ (Table 1). Besides double-mutated cell types (CD34+, CD14+, CD14−/CD15+/CD16+), lineage-specific isolates with only one of the 2 mutations were observed affecting either JAK2$^{V617F}$ (megakaryocytes, erythroid cells, and eosinophils; Figure S2) or MPL$^{W515L}$ (T and B lymphocytes).

CD34+ precursor cells exhibited 94% MPL$^{W515L}$ and 44% JAK2$^{V617F}$ alleles. This is consistent with the existence of double-mutated cells. Even if trisomy 9 with 3 mutated JAK2$^{V617F}$ alleles is taken into account, at least 20% of the cells should be affected by both mutations. Single-cell analysis showed MPL$^{W515K/L}$/JAK2$^{V617F}$ in one granulocyte (data not shown). These findings indicate, besides mutated stem cell clones, nonmutated clones were still proliferating, and that the wild-type alleles detectable in BM cells and CD34+ cells did not belong completely to hemizygously mutated cells. Heterozygosity for JAK2$^{V617F}$ was found in megakaryocytes whereby the value below 50% might be due to polyploidy and cutting artefacts.3

Different patterns of lineage involvement may be explained by varying localization of the mutations on divergent levels of the hematopoietic maturation hierarchy. When colony formation by CD34+ precursor cells from MPL$^{W515K/L}$-mutated CMPD was compared with JAK2$^{V617F}$-mutated cases, it was recently found that MPL$^{W515K/L}$ might affect more primitive cells than the JAK2$^{V617F}$ mutation,6 which is in accordance with the results of mutation analysis in lymphocytes in this case which were positive only for MPL$^{W515L}$ but not for JAK2$^{V617F}$. Heterogeneous lineage involvement in this case, amounting to various combinations of mutations of both MPL$^{W515L}$ and JAK2$^{V617F}$ with each other or wild-type, is consistent with the simultaneous proliferation of at least 2 unrelated stem cell clones with either mutation. The findings argue strongly against a concept of clonal evolution with emerging subclones which have subsequently acquired MPL$^{W515L}$ and JAK2$^{V617F}$. Interestingly, the independently coexisting JAK2$^{V617F}$ and MPL$^{W515L}$-mutated precursor cell clones in this case demonstrated divergent preferences to differentiate into the main hematopoietic lineages. Restriction of the JAK2$^{V617F}$ mutation to 1 lineage or sparing of a single lineage has been reported previously.7,8

It has been hypothesized that different gene dosages9 or a varying combination of different molecular defects in one pathologic stem cell might be responsible for the phenotypic heterogeneity of CMPD.10 This study lends support to the concept that, at least in a subfraction of CMPD, heterogeneity might also be caused by parallel proliferation of unrelated abnormal hematopoietic stem cell clones.

Table 1. Hematologic lineages in MPL$^{W515L}$/JAK2$^{V617F}$ double-mutated Ph+ CMPD

<table>
<thead>
<tr>
<th>Type of blood or bone marrow cell</th>
<th>Source</th>
<th>Mode of separation</th>
<th>MPL$^{W515L}$ (% mutant alleles)$^\dagger$</th>
<th>JAK2$^{V617F}$ (% mutant alleles)$^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mononuclear cells</td>
<td>PB</td>
<td>DGC</td>
<td>52</td>
<td>14</td>
</tr>
<tr>
<td>B lymphocytes (CD19+, purity ~95%)</td>
<td>PB</td>
<td>FACS</td>
<td>37</td>
<td>&lt;1</td>
</tr>
<tr>
<td>T lymphocytes (CD3+, purity ~95%)</td>
<td>PB</td>
<td>FACS</td>
<td>52</td>
<td>&lt;1</td>
</tr>
<tr>
<td>CD34+ cells (~300 cells)</td>
<td>PB</td>
<td>FACS</td>
<td>94</td>
<td>44</td>
</tr>
<tr>
<td>Granulocytes (CD14+/CD15+/CD16+, purity ~95%)</td>
<td>PB</td>
<td>FACS</td>
<td>47</td>
<td>62</td>
</tr>
<tr>
<td>Monocytes (CD14+, purity ~95%)</td>
<td>PB</td>
<td>FACS</td>
<td>69</td>
<td>53</td>
</tr>
<tr>
<td>Eosinophils (~100 cells)</td>
<td>PB</td>
<td>LMD</td>
<td>&lt;1</td>
<td>92</td>
</tr>
<tr>
<td>Total bone marrow cells</td>
<td>BM</td>
<td></td>
<td>58</td>
<td>57</td>
</tr>
<tr>
<td>Erythrocytes (hemoglobin-positive, ~100 cells)</td>
<td>BM</td>
<td>LMD</td>
<td>&lt;1</td>
<td>58</td>
</tr>
<tr>
<td>Megakaryocytes (~100 cells)</td>
<td>BM</td>
<td>LMD</td>
<td>&lt;1</td>
<td>30</td>
</tr>
</tbody>
</table>

The 67-year-old female patient presented with splenomegaly and leukocytosis (hemoglobin 151 g/L; leukocytes 13.6 × 10$^9$/L; eosinophils 0.4-0.65 × 10$^9$/L; platelets 136 × 10$^9$/L; no blasts). Cytogenetic analysis revealed trisomy 9 [47, XX, +9, 6/47, idem, del (13)(q13;q21) 546,XX 4. nucle: 1gg4 (ABL × 3), 22q11 (BCR × 2) 78/100].

BM indicates bone marrow; DGC, density gradient centrifugation; FACS, fluorescence-activated cell sorting (FACS; FACSAria, Becton Dickinson, NJ); LMD, laser microdissection (LMD, SmartCutPlus System based on a CKX41 inverse microscope, Olympus, Hamburg, Germany); and PB, peripheral blood.

*Isolated from May-Grünwald-Giemsa–stained cytospins (Figure S2).
†Isolated from bone marrow trephine sections.
‡Allele burden was determined by pyrosequencing (Biotage, Uppsala, Sweden) as recently described.5

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References

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