Immunophenotypic, cytogenetic and molecular findings of core binding factor leukemia patients

To the editor:

C-kit mutations in core binding factor leukemias

Positivity for CD117(c-kit) expression is present in 80% of acute myeloblastic leukemia (AML) cases, and has been associated with the expression of CD33, a marker of myeloid precursors, and CD13, a myeloid-associated antigen. Combined positivity for the stem cell antigens CD34, CD117, and HLA-DR characterizes AML-M2 with t(8;21) and AML-M4Eo with inv(16), currently designated core binding factor (CBF) leukemias. According to a recent study, the PEBP2b/MYH11 fusion transcript is involved in CD34+/c-kit+ immature cells and may have leukemicogenic potential only in these cells. The same authors pointed out that the disruption of AML1 and PEBP2b genes encoding the α and β subunits of a single transcriptional complex (CBF) affects the same differentiation stage of the myeloid cells. On the basis of published data concerning c-kit (CD117) expression in CBF leukemias, and our finding of the Asp816Tyr activating c-kit mutation in an AML-M2 patient with t(8;21), we recruited 15 patients with either AML-M2 with t(8;21) or AML-M4Eo with inv(16) to screen for c-kit mutations. Mutation screening was targeted on exon 17 as codon 816 was found to be affected by Asp816Val—first detected in the mast cell leukemia cell line HMC-110 and the alternative codon 816 was found to be affected by Asp816Tyr, as previously reported, was present in 100% of blasts. The application of DGGE/CDGE to screen for the presence of the Asp816Tyr mutation did not reveal any other carrier among the patients recruited for this study. The presence of the Asp816Val mutation, which creates an additional HinfI site, leads to a new DNA fragment that is detected by HinfI digestion of c-kit exon 17 PCR products. As a result of HinfI digestion of the mutated allele, the 147 bp DNA fragment was apparent in AML patients 2, 5, 8, 13 and 15 (Figure 1). The molecular test at remission in patients 5 and 15 is also shown. There was a remarkable variability in the intensity of the 147 bp band due to the different expansion of the mutated leukemic clone among the patients. During follow-up monitoring using the same HinfI assay in patients 5 and 15, the mutated allele in purged bone marrow DNA at remission was undetectable (lanes 5a and 15a), indicating a contraction of the mutant clone together with disease. The targeted selection of AML patients with antigenic and karyotypic features of CBF leukemias thus allowed us to identify 6 out of 15 patients carrying either a D816V or a D816Y c-kit mutation. These results confirm the strictly correlation between the stage of myelomonoblast differentiation and the susceptibility to c-kit activation. Our previous analysis of 12 unselected AML patients did not lead to the detection of any c-kit mutation, which is in line with the results of the study reported by Ferrao, who found that only one AML-M2 patient out of 33 AML cases carried the D816V mutation. Mutation screening of all 21 c-kit exons has recently disclosed new c-kit mutations in AML, which underlines their relevance in CBF leukemias.

Immunophenotypic, cytogenetic and molecular findings of core binding factor leukemia patients

<table>
<thead>
<tr>
<th>BMMNC</th>
<th>Asp816Val and Asp816Tyr Status</th>
<th>Patient Sex/Age</th>
<th>CD13</th>
<th>CD33</th>
<th>CD34</th>
<th>CD117</th>
<th>HLA-DR</th>
<th>FAB</th>
<th>Karyotype</th>
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<tbody>
<tr>
<td>Asp816Val and Asp816Tyr negative</td>
<td>5 F/28</td>
<td>36, 7</td>
<td>49, 9</td>
<td>ND</td>
<td>47, 4</td>
<td>AML-M2</td>
<td>46,XXI(8;14;21)</td>
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<td>4 F/72</td>
<td>48, 6</td>
<td>68</td>
<td>29</td>
<td>21, 9</td>
<td>60</td>
<td>AML-M2</td>
<td>46,XXI(8;21)(q22;q22)</td>
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<tr>
<td>Asp816Tyr negative</td>
<td>6 M/26</td>
<td>88, 9</td>
<td>88, 7</td>
<td>87, 4</td>
<td>87, 2</td>
<td>95</td>
<td>AML-M2</td>
<td>45X,Y(8;21)(q22;q22)(15;46XII(8;21)(q22;42)(17;46XII(5)</td>
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<tr>
<td>7 F/31</td>
<td>78</td>
<td>72</td>
<td>68</td>
<td>50</td>
<td>60</td>
<td>AML-M2</td>
<td>46,XXI(8;21)(q22;q22)(40)</td>
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<td>9 M/32</td>
<td>21</td>
<td>53, 5</td>
<td>56, 8</td>
<td>57</td>
<td>ND</td>
<td>AML-M2</td>
<td>45X,Y(8;21)(q22;q22)</td>
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<td>10 F/42</td>
<td>79, 7</td>
<td>28, 9</td>
<td>58, 1</td>
<td>ND</td>
<td>69, 4</td>
<td>AML-M2</td>
<td>46,XXI(8;21)(q22;q22)(20)</td>
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<td>11 M/45</td>
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<td>71, 4</td>
<td>65, 2</td>
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<td>12 F/59</td>
<td>84</td>
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<td>60</td>
<td>61</td>
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<td>14 F/42</td>
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<td>73</td>
<td>30, 7</td>
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<td>Asp816Val positive</td>
<td>2 M/65</td>
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<td>52, 9</td>
<td>ND</td>
<td>ND</td>
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<td>5 M/16</td>
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<td>62, 1</td>
<td>62, 9</td>
<td>39, 7</td>
<td>73, 2</td>
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<td>8 M/40</td>
<td>93, 7</td>
<td>95</td>
<td>33, 5</td>
<td>48</td>
<td>91, 2</td>
<td>AML-M2</td>
<td>46,XXI(8;21)(q22;q22)</td>
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<td>13 M/55</td>
<td>89, 8</td>
<td>56, 5</td>
<td>61, 7</td>
<td>40</td>
<td>10, 5</td>
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<td>46,XXI(8;21)(q22;q22)</td>
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<td>15 M/53</td>
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<td>78, 6</td>
<td>66, 9</td>
<td>73, 8</td>
<td>44</td>
<td>AML-M2</td>
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<td>Asp816Tyr positive</td>
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<td>87</td>
<td>70</td>
<td>87</td>
<td>23</td>
<td>94</td>
<td>AML-M2</td>
<td>47X,Y(8;21)(q22;q22)</td>
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</tbody>
</table>

ND, not determined; BMMNC, bone marrow mononuclear cell.
by their occurrence in a small proportion of blast cells, or whether in a few cases they have a primary involvement in leukemogenesis.

**References**


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Alessandro Beghini, Paolo Peterlongo, Carla B. Ripamonti, Lidia Larizza, Roberto Cairoli, Enrica Morra and Cristina Mecucci

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