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Pure erythroid leukemia is characterized by biallelic TP53 inactivation and abnormal p53 expression patterns in de novo and secondary cases

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Data Sharing Statement: All data are available for sharing upon request to the corresponding author.
Pure erythroid leukemia (PEL) is a rare type of acute myeloid leukemia (AML) characterized by a neoplastic proliferation of immature erythroblasts associated with a complex karyotype and a poor prognosis\textsuperscript{1-3}. PEL can arise \textit{de novo}, but more frequently occurs as a therapy-related neoplasm or transformation from myelodysplastic syndromes (MDS)\textsuperscript{4,5}. To date, the potential differences between \textit{de novo} and secondary (therapy-related or MDS-derived) PEL have not been well explored. Recent studies have shown that TP53 mutations are common in PEL\textsuperscript{1,5} and that p53 overexpression is also frequent\textsuperscript{6}. Strong p53 expression shown by immunohistochemistry has become an important clue in the initial workup of PEL. However, we have observed some PEL cases lacking p53 expression despite the presence of TP53 mutations. We conducted the current study to investigate TP53 mutation characteristics and p53 protein expression in PEL and to examine whether secondary PEL cases differ from \textit{de novo} disease. Another aim of this study was to address PEL as an entity that should be histopathologically and genetically defined, versus classifying these cases following the current classification scheme in which a history of prior chemoradiation therapy or MDS is relatively more emphasized.

We collected 22 cases of PEL, defined by a predominant proliferation of neoplastic erythroblasts that formed sheets in bone marrow, of which 30% or more were pronormoblasts. The clinical characteristics of our patients are summarized in Table 1. There were 14 men and 8 women with a median age of 69 years (range, 37-81). Eleven (50%) patients had a history of chemotherapy for other malignancies (therapy-related), 5 (23%) had MDS, 1 (4%) had primary myelofibrosis (PMF), and 5 (23%) occurred \textit{de novo}. Among 21 patients with treatment information available, 5 (24%) did not receive any therapy due to poor performance status or poor response to prior treatments for MDS. The remaining 16 patients received treatments after the diagnosis of PEL (Table 1). Four (25%) patients achieved a complete response with incomplete hematologic recovery; the response in 3 patients (#3, 12, 20) was transient and 1 patient (#22) remained in complete response with incomplete hematologic recovery at last follow-up, 4.7 months after diagnosis. None of the patients was eligible for stem cell transplantation. Twenty-one patients had clinical follow-up: 20 had died at last follow-up and one patient (#22) was alive. The median survival time for this entire cohort was 2.8 months (range, 0.2-7.3); 2.3 months (range, 0.2-7.3) for therapy-related, 2.6 months (range, 0.4-4.9) for patients with a history of MDS, and 3.9 months (range, 2.2-5.5) for \textit{de novo} PEL.

Targeted next generation sequencing (NGS) with panels composed of genes commonly mutated in myeloid neoplasms was performed on bone marrow samples from 20 patients (19 using an 81-gene panel and 1 using a 28-gene panel) at the time of PEL diagnosis as previously described\textsuperscript{7}. One case was tested for TP53 mutation using Sanger sequencing. In total, 21 cases were tested and all patients had TP53 mutation(s) (Table 2). A total of 25 TP53 mutations were detected: 18 patients had one TP53 mutation, 2 patients (#15 and 17) had two mutations, and 1 (#5) patient had three mutations. Twenty-two (88%) mutations occurred in the DNA binding domain (exons 5-8), including 12 in exon 5, 1 in exon 6, 4 in exon 7, and 5 in exon 8. The remaining 3 mutations occurred in exon 4, exon 10, and a splice site, respectively. The types of TP53 mutations included 19 (76%) missense, 1 (4%) nonsense, 1 (4%) splice site, and 4 (16%) small deletion. Among the 4 cases with small deletion mutations, 3 caused frameshift. The median variant allele frequency (VAF) of TP53 mutations was 35% (range, 1% to 92.3%). One patient (#13) was not assessed for TP53 mutation, but immunohistochemistry showed strong and diffuse p53 expression, suggestive of TP53 mutation. The detailed TP53 mutational profiles are summarized in Table 2. Among the 16 patients who received treatment, 11 (#3, 4, 7, 8, 11, 15, \textit{...})
17, 18, 20-22) had repeat TP53 mutation analysis by NGS after treatment and all showed persistent TP53 mutations.

Among the 20 cases tested by NGS, additional gene mutations were detected in 9 (45%) patients (Supplementary Table 1), including DNMT3A (n=3; VAF 10.3-29.3%), NRAS (n=2; VAF 5% and 26.6%), TET2 (n=1; VAF <3%), FLT3 (n=1; VAF 1.7%), PRPF40B (n=1; VAF 40.8%), KMT2A (n=1; VAF 15.2%), and GATA2 (n=1; VAF 1.8%). Patient #22 had a history of PMF that was positive for JAK2 V617F (VAF 32%) and negative for TP53. At the time of progression to PEL, JAK2 V617F was detected with a VAF of 1%, and TP53 mutation was acquired (VAF 80.6%).

Twenty cases underwent conventional karyotyping at the time of PEL diagnosis and all (100%) had complex karyotypes (Supplementary Table 1). Among 19 cases with karyotype data available, 12 (63%) had -5/5q-, 12 (63%) had -7/7q-, and 9 (47%) had concomitant -5/5q- and -7/7q-. The status of 17p/TP53 was assessed by conventional karyotyping and/or fluorescence in situ hybridization (FISH) in 17 cases: deletion of 17p and/or TP53 was detected in 13 (76%) cases (Table 2). The remaining 4 patients were negative but 3 (cases # 5, 15, and 17, Table 2) had more than one TP53 mutation by NGS, raising the possibility that both alleles were affected by TP53 mutations. In one patient (#12), the status of 17p/TP53 was unknown, but the VAF of TP53 mutation was 92.3%, consistent with the loss of wild type TP53.

We performed p53 immunohistochemistry on 21 cases and correlated the results with TP53 mutation types (Table 2). Sixteen (76%) cases of PEL were strongly and uniformly positive for p53; 15 had missense mutations and 1 had a deletion mutation but no frameshift (#3). In the remaining 5 (24%) cases, p53 expression was completely absent in the neoplastic cells (null pattern). In cases negative for p53 expression, 3 (#6, 16, 21) had TP53 frameshift mutations, 1 (#18) had a nonsense mutation, and 1 (#1) had a splice site mutation. Representative cases of PEL with p53 overexpression and completely absence of p53 expression are shown in Fig 1A&B. In this study, two patients (cases #3 and 14) had a single TP53 mutation with a VAF less than 15%. In both cases, erythroblasts formed sheets in the core biopsy and were diffusely and strongly positive for p53 by immunohistochemistry. These findings suggest that most of the erythroblasts had mutated TP53 and the low VAF of TP53 mutation may be due to hemodiluted specimen submitted for molecular analysis. However, we also cannot exclude the possibility that only a subclone of leukemic cells had TP53 mutation.

These data demonstrate that PEL is characterized by biallelic TP53 alterations, frequently present as a mutation in one allele and deletion in another allele. In cases with no TP53 deletion, two or more mutations were often detected. Mutations frequently seen in other myeloid neoplasms are less common in PEL, indicating that biallelic loss of TP53 function is a feature of PEL and may play a critical role in the development of PEL. Of note, biallelic TP53 alteration is not specific to PEL and can be seen in other myeloid neoplasms, such as AML and therapy-related MDS. Thus, TP53 mutations alone may not be sufficient to block the differentiation of erythroid lineage and drive pronormoblast proliferation, a pathognomonic feature of PEL. Alterations of other genes (not covered in our mutation panels) or pathways involved in erythroid differentiation likely also play a role in PEL development.

As mutational analysis often takes time, checking p53 expression status by immunohistochemistry has been used as a surrogate to predict the presence of TP53 mutations. One caveat is that TP53 mutations do not always correlate with p53
overexpression. In the current study, approximately one quarter of PEL cases showed a null pattern by immunohistochemistry. In these cases, \textit{TP53} mutations were either frameshift, nonsense, or involved a splice site. Of note, the null pattern of p53 expression can usually be distinguished from the “negative” wild-type pattern which often shows variable p53 expression in a subset of cells and the staining intensity ranges from weak to moderate (Fig 1C). In some cases, however, assessment of p53 using immunohistochemistry can be challenging, especially in cases where PEL is mixed with residual normal hematopoietic cells in the background which have a wild type p53 staining pattern.

Lastly, we suggest that the category of PEL should be preserved, despite the fact that some cases also can be classified as therapy-related AML/MDS or AML with myelodysplasia-related changes (AML-MRC) using the current World Health Organization (WHO) criteria. We believe classifying these cases as something other than PEL does not fully capture the distinctive features of this disease. The rationale for this proposal includes: 1) PEL cases, irrespective of their origin (\textit{de novo} or secondary), share similar clinicopathological features including poor response to treatment, dismal prognosis, complex karyotype, and biallelic \textit{TP53} alterations. By contrast, the WHO defined categories of therapy-related AML/MDS or AML-MRC are highly heterogeneous at the molecular level, and are associated with highly variable prognoses for different patient subsets\textsuperscript{12,13}. We believe that the distinctive clinicopathologic and molecular features of PEL may be obscured when these neoplasms are placed in the therapy-related AML/MDS or AML-MRC WHO categories; 2) the survival of PEL patients with a history of receiving cytotoxic therapy or MDS is similar to \textit{de novo} PEL patients but is worse than patients with therapy-related AML\textsuperscript{12} and AML-MRC\textsuperscript{13}, respectively; 3) All PEL cases, whether they are \textit{de novo} or secondary, share distinctive morphologic features with prominent pronormoblast proliferation. Pronormoblasts have been shown to play an important role in treatment resistance and increased pronormoblasts have contributed to a poorer prognosis in AML patients\textsuperscript{14,15}. By keeping secondary PEL cases in the category of PEL, these cases can be studied together to explore therapeutic strategies targeting the neoplastic pronormoblasts. Of note, the number of \textit{de novo} PEL cases in our study is relatively small and future studies to include more cases will be valuable.

In summary, we show that PEL is characterized by biallelic \textit{TP53} loss-of-function, a complex karyotype, poor response to AML or MDS directed therapy, and a very dismal prognosis. These unique features are the same for \textit{de novo} or secondary cases of PEL, and therefore we advocate for keeping them under the entity of PEL to facilitate further studies and drug discovery. Immunohistochemistry for p53 can be used as a preliminary screening tool to assess \textit{TP53}; strong p53 expression correlates with missense mutations of \textit{TP53} and a null p53 pattern is often associated with frameshift, nonsense, or \textit{TP53} mutations involving splice sites.
REFERENCES

TABLES

Table 1. Clinical characteristics of pure erythroid leukemia

<table>
<thead>
<tr>
<th>Case #</th>
<th>Sex</th>
<th>Age</th>
<th>F/U (months)</th>
<th>Treatment</th>
<th>Response</th>
<th>Status at F/U</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>77</td>
<td>2.8</td>
<td>None</td>
<td>N/A</td>
<td>Dead</td>
<td>Therapy-related (B-ALL and DLBCL)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>66</td>
<td>0.2</td>
<td>None</td>
<td>N/A</td>
<td>Dead</td>
<td>Therapy-related (PCN)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>68</td>
<td>7.3</td>
<td>Decitabine + Venetoclax, 4 cycles</td>
<td>Transient CRi, 2.1 months</td>
<td>Dead</td>
<td>Therapy-related (ovarian cancer)</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>55</td>
<td>1.4</td>
<td>CLIA + Venetoclax, 1 cycle</td>
<td>No</td>
<td>Dead</td>
<td>Therapy-related (DLBCL)</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>70</td>
<td>1.7</td>
<td>Decitabine + Venetoclax, 1 cycle</td>
<td>No</td>
<td>Dead</td>
<td>Therapy-related (PCN)</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>48</td>
<td>2.3</td>
<td>Fludarabine + AraC + Idarubicin, 1 cycle</td>
<td>No</td>
<td>Dead</td>
<td>Therapy-related (AML)</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>54</td>
<td>4.8</td>
<td>Cytarabine + Daunorubicine, 1 cycle</td>
<td>No</td>
<td>Dead</td>
<td>Therapy-related (breast cancer)</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>66</td>
<td>6.3</td>
<td>Azacitidine, 4 cycles</td>
<td>No</td>
<td>Dead</td>
<td>Therapy-related (PCN)</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>81</td>
<td>0.8</td>
<td>None</td>
<td>N/A</td>
<td>Dead</td>
<td>Therapy-related (DLBCL)</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>69</td>
<td>2.1</td>
<td>Low dose Cytarabine + Venetoclax, 1 cycle</td>
<td>No</td>
<td>Dead</td>
<td>Therapy-related (lung cancer)</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>56</td>
<td>4.4</td>
<td>ASTX660 + ASTX727, 1 cycle</td>
<td>No</td>
<td>Dead</td>
<td>Therapy-related (PCN)</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>76</td>
<td>4.9</td>
<td>Sapacitabine, 3 cycles</td>
<td>Transient CRi, 2 months</td>
<td>Dead</td>
<td>MDS</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>37</td>
<td>0.4</td>
<td>None</td>
<td>N/A</td>
<td>Dead</td>
<td>MDS</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>78</td>
<td>2.6</td>
<td>Low dose Cytarabine + Venetoclax, 2 cycles</td>
<td>No</td>
<td>Dead</td>
<td>MDS</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>79</td>
<td>3.6</td>
<td>FF1101 (BET inhibitor), 2 cycles</td>
<td>No</td>
<td>Dead</td>
<td>MDS</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>60</td>
<td>1.3</td>
<td>None</td>
<td>N/A</td>
<td>Dead</td>
<td>MDS</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>78</td>
<td>2.2</td>
<td>Azacitidine + Nivolumab, 2 cycles</td>
<td>No</td>
<td>Dead</td>
<td>De novo</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>59</td>
<td>5.5</td>
<td>Azacitidine, 3 cycles</td>
<td>No</td>
<td>Dead</td>
<td>De novo</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>78</td>
<td>N/A</td>
<td>None</td>
<td>N/K</td>
<td>N/K</td>
<td>De novo</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>65</td>
<td>5.0</td>
<td>Azacitidine + Venetoclax, 2 cycles</td>
<td>Transient CRi, 2.6 months</td>
<td>Dead</td>
<td>De novo</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>72</td>
<td>2.8</td>
<td>Decitabine + Venetoclax, 1 cycle</td>
<td>No</td>
<td>Dead</td>
<td>De novo</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>73</td>
<td>4.7</td>
<td>Azacitidine + Venclexta + Magrolimab, 3 cycles</td>
<td>CRi at the last F/U, 3 months</td>
<td>Alive</td>
<td>PMF</td>
</tr>
</tbody>
</table>

Note: AML, acute myeloid leukemia; B-ALL, B-acute lymphoblastic leukemia; BET, bromodomain and extra-terminal; CLIA, cladribine, idarubicin, and cytarabine; CRi, complete response with incomplete hematologic recovery; DLBCL, diffuse large B-cell lymphoma; FIA, fludarabine, idarubicin, cytarabine; F/U, follow up; MDS, myelodysplastic syndrome; N/A, not applicable; N/K, not known; PCN, plasma cell neoplasm; PMF, primary myelofibrosis
Table 2. TP53 mutational profiles and p53 protein expression in pure erythroid leukemia

<table>
<thead>
<tr>
<th>Case #</th>
<th>Monosomy 17 or TP53 Deletion (karyotype or FISH)*</th>
<th>Number of TP53 Mutation</th>
<th>Biallelic TP53 Inactivation</th>
<th>TP53 Mutation (Ref: NM_000546.5)</th>
<th>Type of Mutation</th>
<th>VAF</th>
<th>Exon(s)</th>
<th>IHC-p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.673-2A&gt;T</td>
<td>splice site</td>
<td>74.9%</td>
<td>splice site</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.405C&gt;G p.C135W</td>
<td>missense</td>
<td>42.1%</td>
<td>5</td>
<td>positive</td>
</tr>
<tr>
<td>3</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.534_536del p.H179del</td>
<td>deletion, no frameshift</td>
<td>11.9%</td>
<td>5</td>
<td>positive</td>
</tr>
<tr>
<td>4</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.818G&gt;A p.R273H</td>
<td>missense</td>
<td>59.6%</td>
<td>8</td>
<td>positive</td>
</tr>
<tr>
<td>5</td>
<td>no</td>
<td>3</td>
<td>likely yes</td>
<td>c.715A&gt;G p.N239D, c.401T&gt;G p.F134C, c.329G&gt;T p.R110L</td>
<td>deletion, no frameshift, missense, missense</td>
<td>1%</td>
<td>29.3%</td>
<td>32%</td>
</tr>
<tr>
<td>6</td>
<td>N/K</td>
<td>1</td>
<td>N/K</td>
<td>c.501del p.Q167fs</td>
<td>deletion, frameshift</td>
<td>62.6%</td>
<td>5</td>
<td>negative</td>
</tr>
<tr>
<td>7</td>
<td>N/K</td>
<td>1</td>
<td>N/K</td>
<td>c.524G&gt;A p.R175H</td>
<td>missense</td>
<td>37.2%</td>
<td>5</td>
<td>positive</td>
</tr>
<tr>
<td>8</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.377A&gt;C p.Y126S</td>
<td>missense</td>
<td>23.0%</td>
<td>5</td>
<td>positive</td>
</tr>
<tr>
<td>9</td>
<td>N/K</td>
<td>1</td>
<td>N/K</td>
<td>c.715A&gt;G p.N239D</td>
<td>missense</td>
<td>42.6%</td>
<td>7</td>
<td>positive</td>
</tr>
<tr>
<td>10</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.818G&gt;C p.R273P</td>
<td>missense</td>
<td>27.2%</td>
<td>8</td>
<td>positive</td>
</tr>
<tr>
<td>11</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.488A&gt;G p.Y163C</td>
<td>missense</td>
<td>48.0%</td>
<td>5</td>
<td>positive</td>
</tr>
<tr>
<td>12</td>
<td>N/K</td>
<td>1</td>
<td>likely yes</td>
<td>c.797G&gt;A p.G266E</td>
<td>missense</td>
<td>92.3%</td>
<td>8</td>
<td>positive</td>
</tr>
<tr>
<td>13</td>
<td>N/K</td>
<td>N/D</td>
<td>N/K</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
<td>positive</td>
</tr>
<tr>
<td>14</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.745A&gt;G p.R249G</td>
<td>missense</td>
<td>8.4%</td>
<td>7</td>
<td>positive</td>
</tr>
<tr>
<td>15</td>
<td>no</td>
<td>2</td>
<td>likely yes</td>
<td>c.434T&gt;G p.L145R, c.1010G&gt;T p.R337P</td>
<td>missense, missense</td>
<td>20.4%</td>
<td>18.5%</td>
<td>5,10</td>
</tr>
<tr>
<td>16</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.455del p.P152fs</td>
<td>deletion, frameshift</td>
<td>70.1%</td>
<td>5</td>
<td>negative</td>
</tr>
<tr>
<td>17</td>
<td>no</td>
<td>2</td>
<td>likely yes</td>
<td>c.844C&gt;T p.R282W, c.734G&gt;T p.G245V</td>
<td>missense, missense</td>
<td>35.1%</td>
<td>14.4%</td>
<td>7,8</td>
</tr>
<tr>
<td>18</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.493C&gt;T p.Q165*</td>
<td>nonsense</td>
<td>39.1%</td>
<td>5</td>
<td>negative</td>
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<tr>
<td>19</td>
<td>no</td>
<td>1</td>
<td>probably no</td>
<td>c.476C&gt;T p.A159V</td>
<td>missense</td>
<td>16.0%</td>
<td>5</td>
<td>positive</td>
</tr>
<tr>
<td>20</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.590T&gt;G p.V197G</td>
<td>missense</td>
<td>15.1%</td>
<td>6</td>
<td>positive</td>
</tr>
<tr>
<td>21</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.558del p.D186fs</td>
<td>deletion, frameshift</td>
<td>47.1%</td>
<td>5</td>
<td>negative</td>
</tr>
<tr>
<td>22</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
<td>c.824G&gt;T p.C275F</td>
<td>missense</td>
<td>80.6%</td>
<td>8</td>
<td>positive</td>
</tr>
</tbody>
</table>

Note: IHC, immunohistochemistry; N/D, not done; N/K, not known; VAF, variant allele frequency

*Detailed karyotype and FISH findings are listed in the Supplementary Table 1*
FIGURE LEGEND

**Fig 1**: The expression pattern of p53 by immunohistochemistry in PEL. Immunohistochemistry shows two patterns of p53 expression: complete absence of p53 expression (case #1, upper panel) and uniform and strong overexpression (case #17, middle panel). Of note, in the case with absence of p53 expression in tumor cells (case #1, upper panel), there were scattered reactive cells in the background variably positive for p53, serving as positive controls. A normal bone marrow and its p53 expression by immunohistochemistry is illustrated in the lower panel, in which p53 is variably expressed in a subset of cells with weak to moderate intensity.
### Supplementary Table 1. Cytogenetic and mutational profiles of pure erythroid leukemia

<table>
<thead>
<tr>
<th>Case #</th>
<th>Conventional Karyotype</th>
<th>FISH*</th>
<th>Other Mutations (VAF)</th>
<th>Platform of Mutation Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44,XY,der(3)(add(3)(p13)del(3)(q12q25),-5,add(7)(q32),-16,18,+mar[2]/59-66,XY,-X,del(1)(q21),add(3)(p13),-5,+6,-7,+8,+9,-10,-12,-13,-14,-15,-17,18,+19,+21,+22,+1-7mar[cp14]/46,XY[4]</td>
<td>N/D</td>
<td>None</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>4</td>
<td>37-51,XY,-3,add(3)(p12),add(4)(q35),del(5)(q12),del(7)(del)(p13)5q15)7q22q34),add(10)(q26),-13,-17,-19,-21,-22,+1-6mar[cp4]/46,XY[7]</td>
<td>N/D</td>
<td>None</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>N/D</td>
<td>None</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>7</td>
<td>Complex karyotype (detail not available)</td>
<td>N/D</td>
<td>None</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>8</td>
<td>41-46,XY,der(7)(17)(p10)(q10),-16,add(17)(p13),-19,-21,+2,+3mar[cp8]</td>
<td>TP53 deletion and Monosomy 17</td>
<td>None</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>12</td>
<td>49,X,-Y,11;16(1p10q10),del(4)(1;4)(q21;q31.3),del(9;13)(p10q10),del(13)(q12q22),del(14)(q24q32),+5mar[19;50],idem,+6[1]</td>
<td>N/D</td>
<td>None</td>
<td>28-gene panel</td>
</tr>
<tr>
<td>13</td>
<td>ND</td>
<td>N/D</td>
<td>None</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>14</td>
<td>45,X,-Y,inv(9)(p12q13)(6;41),idem,-4,del(5)(q13q33),-10,-13,add(17)(p12),-18,add(19)(p13.1),-21,1mar[cp3]/46,XY,inv(9)(q12p13)[5]</td>
<td>TP53 gene deletion</td>
<td>None</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>15</td>
<td>42-57,XY,+Y,+1,+2,del(5)(q13q33),+der(6)(6;11)(q25;q22),+der(6)(6;11)(q25,q22),+10,+13,+15,+17,+18,+19,del(20)(q11.2),-21,add(12)(p11.2),+1-3mar[cp16]/46,XY[4]</td>
<td>Negative for TP53 gene deletion</td>
<td>None</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>16</td>
<td>45,XY,del(17)(p11.2),-18)[10]/67-71,XY,-Y,-1,1-2,1-2,1-2,1-11,-12,-14,-15,16,del(17)(p11.2),-18,+20,+mar[cp4]/90,X,Y,-Y,-1-2,+4-6,+6,-7,-12,-12,-15,-15,del(17)(p11.2)x2[5]/46,XY[1]</td>
<td>N/D</td>
<td>FLT3 Y572S (1.7%) GATA2 R398Q (1.8%) PRPF40B S166G (40.8%)</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>17</td>
<td>46,XX,der(5)(del(5)(q12p23p55)(p15.1;q35),add(16)(p13.3),del(19)(ins(19.5)(p13.3)q31q31)dup(5)(q31q31),-22,+1-2mar[cp8]/46,XX[12]</td>
<td>Negative for TP53 gene deletion</td>
<td>FLT3 D835 (0.03%)</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>19</td>
<td>42,XX,-del(5)(q13q33),del(7)(q22q32),-11,-12,16,add(16)(q24),+2mar[cp3]</td>
<td>Negative for TP53 deletion</td>
<td>DNMT3A R882H (10.3%)</td>
<td>81-gene panel</td>
</tr>
<tr>
<td>22</td>
<td>44,XX,del(1)(q32)(q7)(del)(9)(q13q22),add(11)(p15),-14,-16,-17,add(17)(p12),der(22)(22)(q22q13q13),+1-2mar[cp19]/46,XX[1]</td>
<td>TP53 gene deletion and monosomy 17</td>
<td>JAK2 V617F (1%)</td>
<td>81-gene panel</td>
</tr>
</tbody>
</table>

Note: N/D, not done; VAF, variant allele frequency

*FISH was performed using an LSI TP53(17p13.1)/CEP17(D17Z1) dual color probe (Abbott Molecular, Inc).