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**PCR’s analysis**

Some or all of the following primers and conditions were used: exon 1 forward 5'-CAGGAAGACGCACATACACAGGA-3' and reverse 5'-GATGGAGCTAGGGTTTGGCAGAT-3' (amplicon 965 base pairs (bp)), annealing temperature 61°C; exon 2 forward 5'-AAAGGAAGGAGGAGAGCAG-3' and reverse 5'-AAGCTTCCAGCCATTCTGTA-3' (amplicon 432 bp), annealing temperature 60°C; Exon 2 forward 5'-GGATTTCTGTGTCTGAGG-3' and reverse 5'-CCAACAGCACCTCAGCCA-3' (amplicon 327 bp), annealing temperature 55°C; Exon 2 forward 5'-GATGGGAGAGGAGATAAGGT-3' and reverse 5'-GGCAACCACCACACTCTCCAGT-3' (amplicon 1103 bp), annealing temperature 61°C; exon 3.1 forward 5'GGAACTTTGGCCACCACATGTTGG-3' and reverse 5'-AGCCGTCTGTCTTCAAAAGTCTC-3' (amplicon 310bp), annealing temperature 58°C; exon 3.2 forward 5'-CTGGATGGAAAAGGCAGCACCA-3' and reverse 5'-GAGCTTAGCCTCAGCTCAGTTTAC-3' (amplicon 304 bp), annealing temperature 58°C.

PCR conditions were; 5–10 minutes at 95°C, 35 cycles of 1 minute at 95°C, 1 minute at the annealing temperature given and 1 minute at 72°C. After the last cycle, an additional step of 5–10 minutes at 72°C was performed. PCR products were verified by gel electrophoresis.

**Sorting of blasts**

Myeloid blasts in TMD and ML-DS show a unique immunophenotype {Langebrake, 2005 #3024} with asynchronous antigen expression of stem/progenitor cells (CD34/CD117), megakaryocytes (CD36, CD41, CD42b, CD61), mature myeloid cells (CD13/CD33) and lineage aberrant antigens (CD7/CD56). For samples with low blast percentage, cells were first gated on the blast gate. Then within the blast gate, a combination of at least three positive antigens (CD34 or CD117/CD13 or CD33/CD7 or CD56) and two negative antigens (CD3 and CD2) was used according to diagnostic immunophenotype.

The lower rate of detection is based on percentage of blasts in the diagnostic sample defined by morphology (down to 5%) and/or immunophenotype (down to 0.1% of blasts in the blast gate on FACS analysis). The lower limit for the detection of mutations was set at 0.5% as this was the lowest blast count for which we could detect a *GATA1* mutation.

**Allelic discrimination assay**

Two TaqMan MGB probes were designed using Primer Express Software (Firma Applied Biosystems), one specific for the *GATA1* wildtype (allele 1) and one specific for *GATA1s* mutation (allele 2). Each of the two probes was labeled with a different fluorescent dye (FAM and VIC dye). For quantitation a standard curve was set up by 10-fold Serial Dilution Series from the patients initial DNA (dilution steps: min 0.5). Assay sensitivity was $10^{-5}$; quantitative range $10^{-4}$; slope of standard curves was between $-3.2$ and $-3.6$. Human embryonic kidney cell line 293T was used as a control.
<table>
<thead>
<tr>
<th>GATA1 mutation characterised</th>
<th>TMD n=134</th>
<th>ML-DS n=103</th>
<th>Total n=237</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Loss of 1st Met</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>0.7011</td>
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<tr>
<td>Splicing errors</td>
<td>14</td>
<td>13</td>
<td>27</td>
<td>0.6741</td>
</tr>
<tr>
<td>PTC 1–3'</td>
<td>37</td>
<td>21</td>
<td>58</td>
<td>0.3388</td>
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<tr>
<td>Total 'High expression'*</td>
<td>56</td>
<td>36</td>
<td>92</td>
<td>0.5534</td>
</tr>
<tr>
<td>PTC1–5'</td>
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<td>92</td>
<td>0.3021</td>
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<tr>
<td>PTC type 2</td>
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<td>0</td>
<td>3</td>
<td>0.2667</td>
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<tr>
<td>Total 'Low expression'*</td>
<td>53</td>
<td>42</td>
<td>95</td>
<td>0.5534</td>
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<tr>
<td>Unknown</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>0.3363</td>
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</tbody>
</table>

Table S1. Analysis of GATA1 mutation types in TMD and ML-DS patients and expected effect on GATA1s expression levels
Patient samples were divided into subgroups based on the type of GATA1 mutation present, according to the classification detailed in Kanezaki et al. Statistical analysis was performed using the Fisher’s exact test.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>T21</th>
<th>Complex cyto.</th>
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<tbody>
<tr>
<td>ML-DS</td>
<td>4</td>
<td>2</td>
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<tr>
<td>CCR</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>7</td>
</tr>
</tbody>
</table>

Table S2. Analysis of correlation between cytogenetics and clinical outcome in TMD patients
Patients were divided into those that presented with T21 as their only cytogenic abnormality and those that had more complex cytogenetics, then separated according to clinical outcome. CCR denotes complete clinical remission. Statistical analysis was performed using Fisher’s exact test.
Figure S1. GATA1s expression in TMD and ML-DS patients is not affected by the GATA1 mutation type

(A) GATA1s expression was analysed in 36 TMD patients with different classifications of GATA1 mutation. PTC 1–3’ n = 10, Splice n = 7, PTC 1–5’ n = 15 and Unknown n = 4. (B) GATA1s expression was analysed in 20 ML-DS patients with different classifications of GATA1 mutation. PTC 1–3’ n = 3, Splice n = 1, PTC 1–5’ n = 14 and Unknown n = 2. No significant differences were found in the expression of GATA1s between the different types of mutation. Filled circles represent each patient and the black bar is mean GATA1s expression.
REFERENCES
