To the editor:

New GATA1 mutation in codon 2 leads to the earliest known premature stop codon in transient myeloproliferative disorder

Recently, Blood published on the decisive pathogenic role of the zinc-finger transcription factor GATA1 in the development of transient myeloproliferative disorders (TMDs) and acute megakaryoblastic leukemias (AMKLs) in the setting of Down syndrome.1,2 Importantly, all mutations so far described cluster in exon 2 and encode a functionally impaired, shorter GATA1 protein, which finally enhances proliferation and stops maturation of hematopoiesis.1,3,4

In our daily routine we diagnosed an intrauterine TMD on a placental specimen submitted for diagnosis after emergency Cesarean section of a preterm boy (32nd gestational week) due to pathologic cardiotocography in the setting of a clinically known Down syndrome. The patient showed characteristics of peripheral blood at the time of birth consistent with TMD, including hyperleukocytosis of 343.80 × 10^9/L with 78.5% atypical cells, erythrocytes of 2.76 × 10^12/L, hemoglobin of 92 g/L, and platelets of 738 × 10^9/L. Atypical cells were characterized as megakaryoblasts by immunohistochemistry on the placental paraffin specimens. We analyzed formalin-fixed, paraffin-embedded blood clots from the umbilical vein, containing high numbers of blasts, for GATA1 exon 2 mutations. The entire region of exon 2 was sequenced using consecutive first and nested PCR. A new point mutation leading to the formation of a premature stop at codon 2 (E2Term) was detected (Figure 1). This mutation results in the earliest known premature stop codon in the sequence of GATA1 compared with the previously reported mutations in the literature.3,4 Because our mutated sequence of GATA1 encodes only for a single methionine amino acid, and because of its position on chromosome X, with no alternatively available allele for GATA1 transcription, this mutation must lead to complete loss of GATA1 protein in TMD blasts. On the protein level, using immunohistochemistry (ab28839, Abcam, rabbit polyclonal) the blasts did not express GATA1, whereas nuclear staining was present in a few maternal blood cells in the intervillous space.

GATA1 is essential in hematopoiesis and performs several different tasks in erythro- and megakaryopoiesis as well as eosinophil and mast cell differentiation.5-8 Complete loss of GATA1 expression in knockout mice leads to early death in embryogenesis due to severe anemia.9 Based on the published data, a remaining GATA1 protein fragment (GATA1s) is required for the development of TMD.10 Since in our case GATA1 expression is completely lost, this might point toward a different pathway in leukemogenesis. The entire GATA1 loss might have been accompanied by loss of important transdifferentiation signals in erythro- and megakaryopoiesis.

Our reported newborn boy suffered from disseminated intravascular coagulation, hepatopathy, hypoalbuminemia, hypercholesterinemia, persisting pulmonary hypertension, extremely high LDH (14 833 U/L), hyperuricaemia as well as renal (hyperkalemia up to 10 mM) and liver failure. He died 2 days after delivery because of multiorgan failure, after it had been decided to withdraw intensive care because of a large intracranial hemorrhage. This poor outcome might point to a more aggressive behavior of TMD with more severe neurologic and hemostaseologic complications, which may be linked to complete GATA1 protein loss.

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Figure 1. Alignment of the selected region of the GATA1 sequence for control cells and blasts cells harboring the E2Term mutation leading to a stop codon in codon 2. (Left) The star indicates the position of a homzygous replacement of the first nucleotide of codon 2 (labeled G in violet) by a T. Below, a part of the GATA1 sequence mRNA is imprinted as light green and the coding region as dark green. (Middle and right) Placental specimen with high numbers of blasts in the fetal vessels in the placental villi with massively elevated proliferation rate in the MIB1 staining, diagnostic for TMD.
We read with interest that Olivieri et al1 observed complete or partial responses to imatinib 100 or 200 mg daily with improvement of lung function in 7 of 11 patients (64%) with pulmonary chronic graft-versus-host disease (cGVHD) of mild severity (lung function score [LFS] of responders, 2-5; percent predicted forced expiratory volume [FEV1], 44-78%).

We have conducted a single-center, prospective, open-label, nonrandomized pilot study of imatinib at 100 to 400 mg daily as antifibrotic treatment targeting the platelet-derived growth factor receptor (PDGFR) and transforming growth factor β (TGFβ) pathways3-5 in patients with refractory cGVHD of the lung. Since November 1995, 9 patients with moderate to severe pulmonary cGVHD have been included (see Table 1). Median age was 45 years (range, 24-50 years); 3 patients were female and 6 male. Peripheral blood stem cell transplantation from sibling (n = 7) or unrelated (n = 2) donors had been performed after myeloablative (n = 7) or reduced-intensity (n = 2) conditioning for acute or chronic myeloid leukemias or lymphomas. All patients had skin, mucosal, visceral, and/or fasciitic manifestations in addition to pulmonary cGVHD; the median duration of pulmonary cGVHD was 6 months (range, 1-28 months). All patients had already received extensive combination therapies with steroids, calcineurin inhibitors, mycophenolate, and/or extracorporeal photopheresis. Additional imatinib was started generally at 100 mg per day and increased monthly up to 400 mg per day, as tolerated. All patients were evaluated monthly for toxicity and response (pulmonary function tests).

Imatinib toxicity (hematologic, nausea, or fluid retention) was mostly mild, except in 2 patients who discontinued the drug due to reversible dyspnea. Dose increase was not possible in a substantial fraction of patients (as has been noted by others6-7); only 3 of 9 reached the target dose of 400 mg. After a median duration of 4 months (range, 1-17+ months) of imatinib treatment, pulmonary function recovered only in 1 patient from severe to moderate. Applying the same response criteria as Olivieri et al, partial responses (ie, possibility of tapering steroids) were found only in

<table>
<thead>
<tr>
<th>UPN</th>
<th>FEV1 before imatinib</th>
<th>Maximum daily dose of imatinib, mg</th>
<th>Duration of imatinib treatment, mo</th>
<th>Side effects CTC &gt; ‘2’</th>
<th>FEV1 after imatinib</th>
<th>Response*</th>
<th>Outcome</th>
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</thead>
<tbody>
<tr>
<td>763</td>
<td>24%</td>
<td>200</td>
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<tr>
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<td>400</td>
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<tr>
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<td>–</td>
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<tr>
<td>736</td>
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<td>Dyspnea ↔ withdrawal</td>
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<tr>
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<td>33%</td>
<td>400</td>
<td>1+</td>
<td>–</td>
<td>21%</td>
<td>none</td>
<td>alive</td>
</tr>
</tbody>
</table>

Pulmonary scoring according to FEV1 (% predicted) or lung function score (LFS): mild, FEV1 60%-79% or LFS 3-5; moderate, FEV1 40%-59% or LFS 6-9; and severe, FEV1 < 40% or LFS 10-12.

CTC > ‘2’ indicates toxicities graded as greater than 2 according to the international Common Toxicity Criteria.

*Response to imatinib treatment, as defined by Olivieri et al.1

Table 1. Details of imatinib therapy and responses
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