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Mapping epigenetic regulator gene mutations in cytogenetically normal pediatric acute myeloid leukemia

Acute myeloid leukemia (AML) accounts for approximately 15-20% of all childhood leukemias, and despite dramatic improvements in treatment outcome, only approximately 70% of children with AML are cured.1-2 AML results from collaborating genetic aberrations in at least 2 different classes: type-I aberrations, inducing uncontrolled cell proliferation and/or survival, and type-II aberrations inhibiting cell differentiation. However, certain aberrations found in AML do not completely fit into our current definition of type-I and type-II aberrations.3 These aberrations concern epigenetic modifier genes involved in DNA methylation and histone modification, such as Ten-Eleven Translocation 2 (TET2), Isocitrate Dehydrogenase 1 (IDH1), IDH2, Enhancer of Zeste Homolog 2 (EZH2), DNA (cytosine-5)-Methyltransferase 3 Alpha (DNMT3A) and Additional Sex Combs Like-1 (ASXL1). Most of these genes are frequently mutated in adult AML patients with a prevalence varying between 12% to 35% per gene.4-8 Reports on prognostic implications of these mutations are ambiguous. It has, however, been shown that patients with IDH mutations have a hypermethylation phenotype. This implies that demethylating agents such as decitabine and azacitidine may be of use in the treatment of IDH1-mutated AML. In addition, a favorable outcome in elderly AML patients with DNMT3A mutations after treatment with decitabine has been reported.7,8

These epigenetic regulator genes were also studied in pediatric AML. Most of these studies focused on the relevance of mutations in a single gene and mutations were detected in 1-4% of the patients.9-13 Although no significant statements can be made since study cohorts were quite heterogeneous, most mutations seemed to occur in cytogenetically normal (CN) AML, a subgroup that comprises 20-25% of all pediatric AML. This distribution corresponds to adult AML data in which most epigenetic regulator gene mutations were also identified in CN patients. So far, no larger study focusing on pediatric CN-AML is available and identifying these genetic aberrations may lead to improved risk stratification and a rationale for the use of demethylating agents in these patients. Therefore, we decided to study the mutation prevalence of epigenetic regulator genes EZH2, IDH1/2, DNMT3A, ASXL1 and TET2 in a well-documented, international cohort of 65 de novo pediatric CN-AML patients.

Patient material was obtained from the Dutch Childhood Oncology Group (DCOG), the AML-Berlin-Frankfurt-Münster Study group (AML-BFM), the Czech Pediatric Hematology Group, and the Saint-Louis Hospital in Paris, France. A minor selection of the discovered mutations had been included in previous reports, but not with a focus on CN-AML and never within one cohort.11-13 Exon 4 of IDH1/2, exon 13 of ASXL1, the SET-domain of EZH2 and the entire coding region of TET2 were screened using Sanger sequencing of genomic DNA. The mutational status of DNMT3A was assessed by screening cDNA and aberrations were verified in genomic DNA. The prevalence of cytogenetic and molecular aberrations was assessed as previously described.14 In our study cohort, aberrations in the genes IDH1 and IDH2 occurred with a frequency of 10.8% (95%CI ± 7.5%) and mutations in TET2 and DNMT3A were found with a frequency of 4.6% (95%CI ± 5.1%) each. While all aberrations in IDH1 and IDH2 were confined to codon 132 and 140, respectively, various mutation loci were observed in TET2 and DNMT3A. All epigenetic regulator gene muta-

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patient</th>
<th>Mutation</th>
<th>Protein change</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>WBC count (x10^9/L)</th>
<th>Other mutations</th>
<th>Survival status</th>
<th>Months of follow up</th>
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<tbody>
<tr>
<td>IDH1</td>
<td>2</td>
<td>c.395G&gt;A</td>
<td>p.R132H</td>
<td>14.3</td>
<td>F</td>
<td>44.8</td>
<td>FLT3-ITD; MLL-PTD</td>
<td>Alive</td>
<td>50</td>
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<tr>
<td>IDH1</td>
<td>4</td>
<td>c.395G&gt;A</td>
<td>p.R132C</td>
<td>12.6</td>
<td>F</td>
<td>3.0</td>
<td>FLT3-ITD</td>
<td>Death after relapse</td>
<td>46</td>
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<tr>
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<td>M</td>
<td>226.0</td>
<td>NUP98-NSD1; FLT3-ITD</td>
<td>Alive</td>
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<tr>
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<td>c.419G&gt;A</td>
<td>p.R140Q</td>
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<td>M</td>
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<td>6.0</td>
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<td>N/A</td>
<td>NPM1; FLT3-ITD</td>
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<tr>
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<td>M</td>
<td>30.8</td>
<td>NPM1; N-RAS</td>
<td>Alive</td>
<td>65</td>
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<tr>
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<td>c.2644C&gt;T</td>
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<td>25.0</td>
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<td>52</td>
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<tr>
<td>DNMT3A</td>
<td>10</td>
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<td>p.R484W</td>
<td>15.7</td>
<td>F</td>
<td>9.5</td>
<td>CEBPA dm</td>
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<tr>
<td>TET2</td>
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<td>163.0</td>
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<td>F</td>
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<tr>
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<td>p.M533I</td>
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<td>M</td>
<td>2.2</td>
<td>FLT3-ITD</td>
<td>Alive</td>
<td>34</td>
</tr>
</tbody>
</table>

yrs: years; F: female; M: male; N/A: not available; WBC: white blood cell count; dm: double mutant. The nucleotide sequence variations are designated according to the recommendations of the Human Genome Variation Society (HGVS) (http://www.hgvs.org/mutnomen/). Numbering of nucleotides is done by using the first base of the translated part of the gene as nucleotide 1. Transcripts used as a reference are: NM_005806.2 for IDH1, NM_002168.2 for IDH2, NM_175629.2 for DNMT3A and NM_001127208.2 for TET2 (National Center for Biotechnology Information). The protein changes are theoretically deduced and designated according to the recommendations of the HGVS.

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tions appeared to be mutually exclusive (Figure 1) and no mutations were identified in EZH2 or ASXL1. The number of patients with a mutation was too small to allow for survival analysis. The specifics of the identified aberrations and the main clinical and genetic features of the patients carrying an epigenetic regulator gene mutation are summarized in Table 1. The epigenetic regulator gene mutations co-occurred with well-known type-I/II aberrations (Figure 1). Out of 13 patients with 10 epigenetic regulator gene mutation also carried a FLT3-ITD (n=6) or RAS mutation (n=4), and 6 of 13 patients were found to have a CEBPA double mutation (n=1), NPM1 mutation (n=3), or NUP98-NSD1 translocation (n=2). Overall, epigenetic regulator gene mutations were shown to co-exist with both a type-I and type-II driver aberration in 7 of 13 patients.

In this study of a substantial pediatric CN-AML cohort, we have identified recurrent somatic mutations in the epigenetic regulator genes IDH1, IDH2, DNMT3A and TET2 in 13 of 65 cases (20%). IDH1/2 mutations occurred significantly more often in pediatric CN-AML than in previously reported pediatric AML cohorts with patients carrying various cytogenetic aberrations (P=0.025). Although the aberration frequency of epigenetic regulator gene mutations is relatively high for a pediatric AML cohort, the prevalence is still evidently lower in pediatric CN-AML than in adult CN-AML. In addition, within adult AML, IDH2, ASXL1 and TET2 mutations are significantly more prevalent in elderly patients, suggesting an increasing prevalence of these mutations as a function of age.

A recent study from Welch et al. identified DNMT3A, IDH1/2, TET2, ASXL1 and NPM1 mutations as possible major initiating or driver mutations in CN-AML, rather than co-operating events acquired at a later stage of clonal evolution. However, we found epigenetic regulator gene mutations in only 20% of our pediatric CN-AML cohort, and in addition, these mutations often co-occurred with other type-I/II aberrations thought to have leukemia driving capabilities, such as NUP98-NSD1 and NPM1. This suggests that there might be other, as yet unidentified driver mutations in pediatric AML, reflecting potential differences in pathophysiology between childhood and adult AML. Future clonal evolution studies of pediatric CN-AML might lead to a better understanding of the initiating and co-operating events in this disease.

Mutations in ASXL1 and EZH2 were absent in our pediatric CN-AML cohort. This corresponds to literature reports that ASXL1 and EZH2 mutations in childhood AML are very rare. EZH2 mutations seem to be highly uncommon in pediatric as well as in adult AML, in contrast to the high prevalence observed in other myeloid malignancies such as adult myelodysplastic syndrome.

In summary, we identified epigenetic regulator gene mutations to be mutually exclusive and recurrent, but infrequent in pediatric CN-AML. IDH1/2 aberrations were more frequent in pediatric CN-AML than in published series of other cytogenetic AML subgroups. The frequency of epigenetic regulator gene mutations is lower in pediatric than in adult CN-AML which underlines the difference in pathophysiology between the two. While there is currently insufficient solid rationale for routine screening of epigenetic regulator gene mutations in newly diagnosed pediatric CN-AML patients, new agents are emerging that may make screening more therapeutically appealing.

Daria G. Valerio, Jenny E. Katsman-Kuipers, Joop H. Jansen, Lonneke J. Verboon, Valerie de Haas, Jan Stary, André Baruchel, Martin Zimmermann, Rob Pieters, Dirk Reinhardt, Marry M. van den Heuvel-Eibrink, and C. Michel Zwaan

Department of Pediatric Oncology, Erasmus MC-Sophia Children’s Hospital, Rotterdam, the Netherlands; Department of Hematology, Radboud UMC, Nijmegen, the Netherlands; Dutch Childhood Oncology Group (DCOG), The Hague, The Netherlands; Pediatric Hematology/Oncology, 2nd Medical School, Charles University, Prague, Czech Republic; AP-HP, Hôpital Robert Debré and Université Paris Diderot, Paris, France; AML-BFM Study Group, Department of Pediatric Oncology/Hematology, Medical High School, Hannover, Germany

Correspondence: c.m.zwaan@erasmusmc.nl
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