**High expression of transcription factor 4 (TCF4) is an independent adverse prognostic factor in acute myeloid leukemia that could guide treatment decisions**

Mutations in transcription factor 4 (TCF4) have recently been described in myeloid dysplastic syndromes (MDS) and acute myeloid leukemia (AML). We analyzed the impact of TCF4 mRNA expression on clinical outcome in AML patients (n=525). Patients with high TCF4 expression (TCF4**, defined as the 25% highest TCF4 expressors) had a significantly worse overall survival (OS) and event-free survival (EFS) than patients with lower TCF4 expression (TCF4**, 5-year OS 18% vs. 44%, P<0.0001; 5-year EFS 15% vs. 34%, P<0.0001, respectively). This was confirmed in an independent cohort (n=436). Multivariate analysis showed that TCF4** is an independent prognostic factor for OS and EFS in the whole cohort and in patients carrying a normal karyotype.

Importantly, TCF4** patients benefited most from an allogeneic hematopoietic cell transplantation (HCT), compared to an autologous HCT or additional chemotherapy (CT) (5-year OS 39%, 8%, 10%, P=0.0001; 5-year EFS 31%, 0%, 10%, P=0.001, respectively), while TCF4** patients seemed to benefit most from an autologous HCT, compared to allogeneic HCT or additional CT (5-year OS: 61%, 45%, 39% P=0.002; 5-year EFS: 42%, 32%, 34%, P=0.102, respectively).

We demonstrate that high expression of TCF4 is an independent adverse prognostic factor in AML that could guide treatment decisions.

TCF4 plays a role in a variety of developmental processes, including hematopoiesis. TCF4 is part of the basic helix-loop-helix (bHLH) class 1 family, also called E-proteins. These E-proteins recognize an E-box DNA binding site and function as transcriptional regulators. The bHLH class 1 family includes many genes, such as HES1, EGR1, and TCF4. TCF4 is a key component of the Notch signaling pathway, which regulates cell fate decisions during development and in adult tissues. TCF4 plays a role in various biological processes, including hematopoiesis, neurogenesis, muscle development, and immune function.

Table 1. Multivariate Cox’s regression survival analysis. Factors predicting overall survival and event-free survival in acute myeloid leukemia patients of the first cohort with available complete data of all cytogenetic and molecular parameters (n=506).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall survival (n=506)</th>
<th>Event-free survival (n=506)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>χ² (Wald)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Favorable ELN risk group</td>
<td>40.11</td>
<td>3</td>
</tr>
<tr>
<td>Intermediate-I ELN risk group</td>
<td>16.55</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate-II ELN risk group</td>
<td>9.36</td>
<td>1</td>
</tr>
<tr>
<td>Adverse ELN risk group</td>
<td>39.36</td>
<td>1</td>
</tr>
<tr>
<td>Age (&gt;60 years)</td>
<td>18.06</td>
<td>1</td>
</tr>
<tr>
<td>WBC (&gt;100 *10⁹)</td>
<td>11.02</td>
<td>1</td>
</tr>
<tr>
<td>TCF4** expression</td>
<td>16.07</td>
<td>1</td>
</tr>
</tbody>
</table>

OS: overall survival; EFS: event-free survival; ELN: European LeukemiaNet; DF: degrees of freedom; HR: Hazard Ratio; CI: Confidence Interval; WBC: white blood cell count.
expression levels on survival in the second cohort of 436 AML patients (OS: \(P=0.001\); EFS: \(P<0.0001\)) (Online Supplementary Figure S3). In the multivariate Cox regression analysis, patients classified as TCF4 high had a significantly higher risk of death (HR 1.7, CI: 1.3–2.1; \(P<0.0001\)) or not obtaining a CR than TCF4 low patients (HR 1.6, CI: 1.3–2.0; \(P<0.0001\)) (Table 1A). In addition, multivariate Cox regression analysis revealed TCF4 expression, as a continuous variable per 100 arbitrary units (AU), was a significant predictor of OS and EFS (HR 1.04, CI: 1.01–1.07, \(P=0.024\); HR 1.05, CI: 1.02–1.08, \(P=0.002\), respectively) (Online Supplementary Table S2A). When selecting for AML patients with a normal karyotype, TCF4 high patients again showed a worse OS and EFS than TCF4 low patients (5-year OS 21% vs. 41%, \(P=0.0001\); 5-year EFS 18% vs. 33%, \(P<0.0001\), respectively) (Online Supplementary Figure S4). In the multivariate Cox regression analysis of normal karyotype AML patients, TCF4 expression is also an independent predictor of survival (OS: HR 1.7, CI: 1.2–2.5, \(P=0.003\); EFS: HR 1.7, CI: 1.2–2.4, \(P=0.005\)) (Online Supplementary Table S2B). Also as a continuous variable, TCF4 expression remained an independent prognostic factor in this cohort (OS: HR 1.07 (per 100 AU), CI: 1.02–1.13, \(P=0.003\); (Online Supplementary Table S2C).

Interestingly, survival analysis according to the Kaplan-Meier method showed that TCF4 high and TCF4 low patients of the first cohort demonstrated a different survival benefit depending on the consolidation treatment they received, i.e., an additional cycle of chemotherapy (CT), autologous or allogeneic hematopoietic cell transplantation (autoHCT, alloHCT, respectively) (OS: Figure 1E and F; EFS: Online Supplementary Figure S5). TCF4 high patients who received alloHCT showed a superior survival compared to TCF4 low patients who received autoHCT or who received additional CT (5-year OS 39%, 8%, 10%, \(P<0.0001\); 5-year EFS 31%,...
In addition, patients classified as TCF4<sup>low</sup> showed a trend towards significant survival after autoHCT, compared to TCF4<sup>high</sup> patients who received alloHCT or additional CT (5-year OS: 61%, 45%, 39%; P=0.002; 5-year EFS: 42%, 32%, 34%, P=0.102, respectively). Moreover, this difference in outcome, depending on type of consolidation treatment between the TCF4<sup>low</sup> and the TCF4<sup>high</sup> patients, was confirmed in a multivariate Cox regression analysis (Online Supplementary Table S3). In the second cohort, only 7 patients in the TCF4<sup>high</sup> group received autoHCT, hampering validation of our observations in this subgroup. Nevertheless, also in this cohort, consolidation treatment with alloHCT (n=44) resulted in significantly better OS for TCF4<sup>high</sup> patients compared to TCF4<sup>low</sup> patients who received additional chemotherapy (n=58) (5-year OS 41% vs. 8%, respectively; P<0.0001). Furthermore, in this cohort TCF4<sup>low</sup> patients who received autoHCT (n=52) showed a superior OS compared with those patients who received alloHCT (n=86) or additional CT (n=186) (5-year OS 61%, 48% vs. 26%, respectively; P=0.0001), confirming the observations from the first cohort.

The biological role of TCF4 is poorly understood, and contrasting observations are described in the literature. For example, enforced expression of members of the bHLH class A family, including TCF4, suppresses colony-forming efficiency of various cell lines due to upregulation of p21, p15 and p16, suggesting that these bHLH proteins act as negative regulators of cell growth. In contrast, Tcf4 expression increased in rat-E1A-immortalized RK3E cells following β-catenin induced neoplastic transformation and aberrant expression of Tcf4 promoted neoplastic transformation of RK3E cells. These different observations might be explained by differences in cellular context, or by the different transcript variants of TCF4, which could affect the function of the protein. Possibly, TCF4 can either stimulate or inhibit cell growth, depending on its environment, which might indicate that an aberrant expression is not only a prognostic marker, but also a pathological feature. This would be in line with the report of mutations in TCF4 in MDS and AML.

TCF4 has also been reported to be highly expressed in hematopoietic stem cells (HSC) and to show a decreased expression in committed progenitors. Since the frequency of TCF4 mutations is relatively low (0.5% in AML), obviously not all patients with high expression of TCF4 can have mutated TCF4. Interestingly, in MLL-AF9-mediated transformation of progenitor cells, TCF4 has been shown to be up-regulated. In the first cohort, patients with high TCF4 expression are significantly more classified in the M0 or M1 FAB-subgroups than TCF4<sup>high</sup> patients, suggesting that the leukemic cells of the TCF4<sup>high</sup> patients derive from more immature cells. In addition, TCF4 expression of patients in the TCF4<sup>high</sup> group is comparable to the level of TCF4 expression of hCD34<sup>+</sup> cells. Furthermore, when looking at the CD34 mRNA expression in the first cohort, 73.3% of the TCF4<sup>high</sup> patients show a high CD34 expression (above the median), compared to 42.1% of the TCF4<sup>low</sup> patients. When including CD34 expression in the multivariate Cox regression analysis, CD34 expression is an independent prognostic factor in OS and EFS; nevertheless TCF4 expression also remains an independent prognostic factor (data not shown).

Our observations report on the prognostic relevance of the level of TCF4 expression in AML and demonstrate that high TCF4 expression is associated with a worse survival. In addition, the TCF4 expression levels seem to provide additional information in the response to treatment. Before considering TCF4 expression levels in clinical decision-making, additional validation studies, also to define optimal cut-off levels, are needed. Further mechanistic studies are warranted on the role of TCF4 in myeloid diseases.

References


