MYELOID NEOPLASIA

LIN28B overexpression defines a novel fetal-like subgroup of juvenile myelomonocytic leukemia

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LIN28B is overexpressed in about half of juvenile myelomonocytic leukemia patients and defines a novel fetal-like disease subgroup.

LIN28B expression is correlated with high fetal hemoglobin levels and the absence of monosomy 7.

Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive stem cell disease of early childhood. RAS activation constitutes the core component of oncogenic signaling. In addition, leukemic blasts in one-fourth of JMML patients present with monosomy 7, and more than half of patients show elevated age-adjusted fetal hemoglobin (HbF) levels. Hematopoietic stem cell transplantation is the current standard of care and results in an event-free survival rate of 50% to 60%, indicating that novel molecular-driven therapeutic options are urgently needed. Using gene expression profiling in a series of 82 patient samples, we aimed at understanding the molecular biology behind JMML and identified a previously unrecognized molecular subgroup characterized by high LIN28B expression. LIN28B overexpression was significantly correlated with higher HbF levels, whereas patients with monosomy 7 seldom showed enhanced LIN28B expression. This finding gives a biological explanation of why patients with monosomy 7 are rarely diagnosed with high age-adjusted HbF levels. In addition, this new fetal-like JMML subgroup presented with reduced levels of most members of the let-7 microRNA family and showed characteristic overexpression of genes involved in fetal hematopoiesis and stem cell self-renewal. Lastly, high LIN28B expression was associated with poor clinical outcome in our JMML patient series but was not independent from other prognostic factors such as age and age-adjusted HbF levels. In conclusion, we identified elevated LIN28B expression as a hallmark of a novel fetal-like subgroup in JMML. (Blood. 2016;127(9):1163-1172)

Key Points

- LIN28B is overexpressed in about half of juvenile myelomonocytic leukemia patients and defines a novel fetal-like disease subgroup.
- LIN28B expression is correlated with high fetal hemoglobin levels and the absence of monosomy 7.

Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive blood cancer predominantly affecting infants and young children and is characterized by uncontrolled proliferation within the myeloid and monocytic lineages.1 Hyperactivation of the RAS pathway, a well-known regulator of cell proliferation, is observed in almost 90% of patients as a result of mutations in KRAS, NRAS, PTPN11, NF1, or CBL. Moreover, monosomy of chromosome 7 (monosomy 7) can be detected in one-fourth of JMML patients, whereas elevated fetal hemoglobin (HbF) levels corrected for age occur in more than half of JMML patients.2 In healthy individuals, the β chains of adult hemoglobin α2β2 start substituting for the γ chains of HbF α2γ2 at birth.3 Elevation of HbF levels in JMML is likely due to sustained incorporation of γ chains in the hemoglobin tetramer after birth.2,4

Previous studies have convincingly shown that JMML is a stem cell disease. Indeed, chromosomal abnormalities or somatic mutations identical to the leukemic clone were detected in myelomonocytic cells,


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†P.V.V. and B.D.M. contributed equally to this study.

The data reported in this article have been deposited in the Gene Expression Omnibus database (accession numbers GSE71449, GSE71935, and GSE71452).

The online version of this article contains a data supplement.

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erythroid cells, and, in some cases, B and T lymphocytes. Furthermore, the clonal origin of this hematologic disorder was confirmed by the identification of similar patterns of chromosome X inactivation in monocytes, granulocytes, reticulocytes, platelets, and primitive CD34+CD38- cells. Lastly, a xenograft study showed that human JMML cells can give rise to all blood lineages in immunodeficient mice, thereby suggesting that JMML most probably arises from pluripotent hematopoietic stem cells (HSCs). Consequently, HSC transplantation (HSCT) is currently the only curative therapy available for JMML patients. However, clinical outcome after HSCT is hampered by a high relapse rate occurring in about one-third of the patients. Therefore, new therapies using molecular-targeting antileukemic drugs and demethylating agents are currently being investigated. In the long run, these approaches might either complement or (partially) replace current HSCT approaches. However, further advances in the treatment of JMML will require a better understanding of JMML disease biology.

*LIN28B* is known as an oncofetal gene regulating self-renewal of embryonic, fetal, and cancer stem cells, suggesting a role in stem cell malignancies. The gene encodes a RNA-binding protein that uses different mechanisms of action to execute similar functions. One of the main molecular functions of the *LIN28B* protein consists of inhibition of the *let-7* family of microRNAs (miRNAs) resulting in upregulation of *let-7* targets. Increased protein levels of *let-7* targets such as RAS, MYC, and HMG2A enhance cell metabolism, cell growth, cell cycle, and self-renewal. Self-renewal in fetal HSCs is regulated by the *LIN28B*/*let-7*HMG2A axis, and both *LIN28B* and HMG2A are strongly downregulated after birth. *LIN28B* is mainly expressed in embryonic and fetal stem cell compartments controlling growth and self-renewal. However, *LIN28B* is markedly downregulated after birth and silenced in most adult tissues, including hematopoietic stem and progenitor cells. Ectopic expression of *LIN28B* in adult HSCs induces a fetal phenotype. *LIN28B* overexpression has been linked to neuroblastoma, ovarian cancer, acute myeloid leukemia (AML), peripheral T-cell lymphoma, primitive neuroectodermal tumors, esophagus cancer, non–small cell lung cancer, colon cancer, hepatocellular carcinoma, Wilms tumor, and chronic myeloid leukemia. In this study, we identified a novel fetal-like subgroup of JMML that is characterized by high levels of *LIN28B*, suggesting that this oncofetal protein acts as an important player in the molecular pathogenesis of this juvenile stem cell disease.

### Patients, materials, and methods

#### Patient samples in the discovery cohort

Bone marrow (BM) or peripheral blood (PB) samples from 44 children with previously untreated JMML, and BM samples from 7 healthy controls (siblings screened for transplant; median age of control subjects, 6.8 years) were collected in the discovery cohort of this retrospective study. None of the patients had a germ line *PTPN11* or *CBL* mutation. Diagnostic samples included BM in 36 cases and PB in 8 cases. Twenty-five of the 44 patients are registered in the European Working Group of Myelodysplastic Syndromes in Childhood (EWOG-MDS) studies EWOG-MDS98 and EWOG-MDS2006 (National Institutes of Health trials registered as #NCT00047268 and #NCT00662090 at www.clinicaltrials.gov), and 19 patients are registered in the French national JMML biobank. Informed consent from parents or legal guardians was obtained in accordance with the Declaration of Helsinki, and approval was granted from institutional review committees at each participating center. Detailed information about the patients, including *LIN28B* status, sex, age at diagnosis, HbF values, karyotype, mutation status, percentage blasts in BM, platelet count, cause of death, overall survival (OS) from diagnosis, and event-free survival (EFS) from HSCT can be found in supplemental Table 1, available on the *Blood* Web site.

#### Patient samples in the validation cohort

Results were confirmed in a validation cohort of 38 JMML patients and 9 healthy donors (children without hematologic/oncologic diagnosis; median age of control subjects, 11.3 years). Diagnostic patient samples included BM in 30

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**Figure 1.** *LIN28B* overexpression in JMML. (A) Diagonal plot showing significantly downregulated (blue) and upregulated (red) genes (adjusted *P* < .05) in patients compared with healthy donors. (B) Log2 expression of *LIN28B* in the discovery cohort, measured on Agilent microarray. *LIN28B* expression is shown in 7 healthy donors, 19 *LIN28B*-low, and 25 *LIN28B*-high patients. (C) Log2 expression of *LIN28B* in the validation cohort, measured on Affymetrix microarray. *LIN28B* expression is shown in 9 healthy donors, 21 *LIN28B*-low patients, and 17 *LIN28B*-high patients.
cases and PB in 7 cases (1 patient unknown). All healthy donor samples were derived from BM. All patients were registered in the EWOG-MDS studies mentioned previously. Detailed information about the patients can be found in supplemental Table 1.

RNA isolation, microarray profiling, differential gene expression analysis, complementary DNA synthesis, and reverse transcription quantitative real-time polymerase chain reaction

Detailed information can be found in supplemental Methods. Briefly, mononuclear cells were isolated and total RNA was extracted from different laboratories stored, frozen cells using TRIzol. Samples in the discovery cohort were profiled on a custom-designed Agilent microarray described by Volders et al,21 whereas samples in the validation cohort were hybridized on Affymetrix Human Genome U133 Plus 2.0 arrays. The data have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database22 and are accessible through GEO Series accession number GSE71452.

Table 1. Characteristics of JMML patients with low and high LIN28B expression

<table>
<thead>
<tr>
<th></th>
<th>Total patients, n = 82</th>
<th>LIN28B-low patients, n = 40</th>
<th>LIN28B-high patients, n = 42</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis, y (range)</td>
<td>2.0 (0.1-17.9)</td>
<td>1.3 (0.1-17.9)</td>
<td>2.6 (0.2-6.4)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Median platelets, 10^3/L (range)</td>
<td>57 (5-530)</td>
<td>71 (5-530)</td>
<td>40 (8-197)</td>
<td>.01</td>
</tr>
<tr>
<td>Median blasts in BM, % (range)</td>
<td>5 (0-37)</td>
<td>6 (0-20)</td>
<td>5 (0-37)</td>
<td>NS</td>
</tr>
<tr>
<td>Median HbF, % (range)*</td>
<td>14 (1-70)</td>
<td>4 (1-67)</td>
<td>34 (1-70)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>HbF &gt;97th percentile for age, n (%)</td>
<td>55 (78)</td>
<td>21 (60)</td>
<td>34 (94)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Male/female, n (%)</td>
<td>58/24 (71/29)</td>
<td>30/10 (75/25)</td>
<td>28/14 (67/33)</td>
<td>NS</td>
</tr>
<tr>
<td>Mutational analysis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>.05</td>
</tr>
<tr>
<td>Karyotype, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Monosomy 7 +add†</td>
<td>17 (21)</td>
<td>14 (35)</td>
<td>3 (7)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>54 (66)</td>
<td>25 (63)</td>
<td>29 (69)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>8 (10)</td>
<td>1 (3)</td>
<td>7 (17)</td>
<td></td>
</tr>
<tr>
<td>No result</td>
<td>3 (4)</td>
<td>0 (0)</td>
<td>3 (7)</td>
<td></td>
</tr>
<tr>
<td>Alive at last follow-up, n (%)</td>
<td>40 (49)</td>
<td>23 (58)</td>
<td>17 (41)</td>
<td>NS</td>
</tr>
<tr>
<td>Received HSCT, n (%)</td>
<td>77 (94)</td>
<td>37 (93)</td>
<td>40 (95)</td>
<td>NS</td>
</tr>
<tr>
<td>Alive after HSCT, n (%)</td>
<td>38 (49)</td>
<td>21 (57)</td>
<td>17 (43)</td>
<td>NS</td>
</tr>
<tr>
<td>Events after HSCT, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRM</td>
<td>19 (25)</td>
<td>7 (19)</td>
<td>12 (30)</td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td>23 (30)</td>
<td>10 (27)</td>
<td>13 (33)</td>
<td></td>
</tr>
<tr>
<td>Primary graft failure alive</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td>2 (5)</td>
<td></td>
</tr>
<tr>
<td>No event</td>
<td>33 (43)</td>
<td>20 (54)</td>
<td>13 (33)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.

*Twelve data points missing.
†Eleven data points missing; no HbF percentage was available for 1 data point but was indicated as “normal for age.”25
‡”add” indicates 1 or 2 additional abnormalities in some patients

Correlations between LIN28B expression and all miRNAs were calculated using the Spearman rank correlation. Therefore, miRNAs with undetected values in >60% of the total cohort were excluded. Afterward, these undetected values were replaced by the experiment’s lowest value minus 1 in order to have numerical values for correlation analysis.

Gene set enrichment analysis

We used gene set enrichment analysis software and the Molecular Signature Database (http://www.broad.mit.edu/gsea/).24 In both the discovery and validation cohorts, normalized microarray expression data were used as input and collapsed to gene symbols. Genes were ranked by signal-to-noise ratio, and statistical significance was determined by 1000 phenotype mutations. Gene sets with a false discovery rate <.025 were considered significant. The gene sets used in the different analyses included the human counterparts of differentially expressed genes in Table S1 from Copley et al23 for fetal enrichment (adjusted P < .1 and negative fold change), genes returned after searching for “embryonic hemoglobin” and “fetal hemoglobin” in Homo sapiens in the NCBI Gene portal for HbF enrichment, and c1.all.v5.0 for positional effects on chromosome 7.

Statistics

The discovery and validation cohorts were merged for all analyses related to patient data and survival to achieve more statistical power. For this specific study, the database on patient outcome was locked as of February 1, 2015.

The cutoff for elevated HbF levels for age is based on the method of Huehns and Beaver.25 OS was defined as the time from diagnosis to death or last follow-up. EFS was defined as the time from HSCT to treatment failure or last follow-up. The Kaplan-Meier method was used to estimate survival rates and the 2-sided log-rank test was employed to evaluate the equality of the survivorship functions in different subgroups. Time-to-event outcomes for relapse (cumulative incidence of relapse [CIR]) and treatment-related mortality (TRM) were estimated using cumulative incidence curves with relapse and TRM as competing risks. Differences in the cumulative incidence functions among groups were compared using Gray’s test.
χ² Tests were used to examine the statistical significance of an association between categorized factors. In the case of a 2-by-2 contingency table, Fisher’s exact test was calculated. Median values and ranges were reported, and nonparametric statistics were used to test for differences in continuous variables in terms of LIN28B status (Mann-Whitney U test). Spearman rank correlation was performed to measure the degree of association between 2 continuously...
measured variables. For multivariate analyses, the Cox proportional hazards regression model was used, including all variables with $P < .1$ in the univariate analysis for OS or EFS. All $P$ values were 2-sided, and values $< .05$ were considered statistically significant. $P$ values $>.1$ were reported as nonsignificant, whereas those between .05 and .1 were reported in detail. SPSS for Windows 22.0.0 (IBM) and Number Cruncher Statistical System 2004 (NCSS) were used for the statistical analysis of data.

Results

**LIN28B overexpression in JMML**

Gene expression levels were analyzed in mononuclear cells of 44 JMML patients and 7 healthy children in the initial discovery cohort.
Differential gene expression analysis between patients and healthy donors uncovered that *LIN28B* is one of the genes with higher expression in the JMML patient population (Figure 1A). However, the difference was not statistically significant, possibly indicating that only a subgroup of patients shows enhanced *LIN28B* expression. Detailed analysis revealed that *LIN28B* was indeed not expressed in healthy individuals, in accordance with the reported absence of *LIN28B* expression after birth, and that the range of *LIN28B* expression levels shows gross variation between primary JMML patient samples. When determining the cutoff for high *LIN28B* expression by adding 3 standard deviations to the average *LIN28B* expression level in healthy donors, 25 of 44 JMML patients (57%) showed increased levels of *LIN28B* (Figure 1B). These results were confirmed by reverse transcription quantitative real-time PCR (RT-qPCR) in 14 patients and 1 healthy donor for whom sufficient RNA was available. A positive correlation of *LIN28B* expression between microarray and RT-qPCR in these individual samples was observed (*r* = 0.907, *P* < .01) (supplemental Figure 1, top).

Subsequently, these initial results were validated in an additional cohort of 38 JMML patients and 9 healthy individuals. Gene expression profiles were generated on an alternative microarray platform but yielded similar results, with 17 of 38 JMML patients (45%) showing high expression of *LIN28B* (Figure 1C). The differential *LIN28B* expression was also validated by RT-qPCR, and correlation with the microarray results was very high (*r* = 0.710, *P* < .01) (supplemental Figure 1, bottom).

**LIN28B overexpression is correlated with high HbF levels and the absence of monosomy 7**

Table 1 summarizes the clinical, hematologic, and molecular characteristics of patients with high and low *LIN28B* expression. Of interest, both HbF percentage and age-adjusted HbF levels were significantly higher in patients with high *LIN28B* levels. The median HbF value was 33.7% in *LIN28B*-high patients vs 4.4% in *LIN28B*-low patients (*P* < .01; Figure 2A). In addition, 34 of 42 patients (94%) with high *LIN28B* expression showed elevated age-adjusted HbF levels as compared with 21 of 40 (60%) patients with low *LIN28B* expression (*P* < .01). The 4 outliers for HbF percentage in the *LIN28B*-low group were 4, 7, 8, and 13 weeks old at diagnosis, explaining their high HbF values.

The patient characteristics in this study show, in accordance with previous observations, that HbF levels are rarely elevated in patients with monosomy 7 (Figure 2B). Remarkably, monosomy 7 is also rare in combination with elevated *LIN28B* levels. Indeed, patients overexpressing *LIN28B* are less frequently diagnosed with monosomy 7 (3/42 or 8%) compared with patients with low *LIN28B* levels (14/40 or 35%; *P* < .01; Table 1). When running an unbiased gene set enrichment analysis for positional effects, 6 of 7 significantly enriched gene sets in *LIN28B*-high patients are located on chromosome 7 in both the discovery and validation cohorts (7q11, 7p21, 7q34, 7q22, and 7q32, among others; false discovery rate <.025; supplemental Table 2). The expression of genes located on these specific loci is, in general, higher in patients with *LIN28B* overexpression.

Further comparison of patients with high and low *LIN28B* expression revealed that children with high *LIN28B* levels were diagnosed at a higher median age (2.6 years vs 1.3 years, *P* < .01) and showed a lower platelet count (40 × 10^9/L vs 71 × 10^9/L, *P* < .01) than children with low *LIN28B* levels. It is noteworthy that *PTPN11* mutations tend to be more frequently associated with *LIN28B*-high patients (60% of *LIN28B*-high vs 28% of *LIN28B*-low). An overview of all characteristics and their relationships is shown in a Circos plot (Figure 2C-D).

**LIN28B defines a fetal-like stem cell signature in JMML patients**

Using gene set enrichment analysis, we further investigated the difference in expression of embryonic and HbF genes between *LIN28B*-high
The OS rate in patients with high LIN28B expression was 27% (95% CI, 9%-45%), whereas it was 60% (44%-76%) in the patients with low LIN28B expression (P = .03; Figure 4A). Transplant procedure details were available for 59 EWOG-MDS patients and showed no statistically significant differences between JMML patients with high or low LIN28B expression (supplemental Table 7). The EFS rate was 21% (95% CI, 3%-39%) in LIN28B-high patients vs 56% (95% CI, 43%-69%) in LIN28B-low patients (Figure 4B). Among the transplanted patients, the CIR was 33% (95% CI, 21%-52%) in the patients with high LIN28B levels and 28% (95% CI, 16%-47%) in the LIN28B-low group (P = not significant). However, TRM rates from HSCT tended to be higher in patients with high LIN28B expression (40% [95% CI, 24%-65%] in the LIN28B-high group vs 21% [95% CI, 11%-41%] in the LIN28B-low group, P = .10).

Table 2 summarizes the probability of 5-year OS from diagnosis and EFS from HSCT in JMML patients in relation to LIN28B expression (high vs low), age (<2 years vs ≥2 years), age-adjusted HbF (normal vs elevated), karyotype (normal, monosomy 7, or other), and molecular genetics (PTPN11, KRAS, NRAS, NFI, or quadruple negative). As expected, significantly inferior OS and EFS rates were observed for patients ≥2 years old at diagnosis. In addition, univariate analysis showed that mutational status is significantly associated with inferior outcome. Lastly, in our cohort, there is a trend toward improved survival in patients with normal karyotype and nonelevated age-adjusted HbF levels. Hazard ratios according to Cox regression are listed in supplemental Table 8.

The results from multivariable analyses, including karyotype, age at diagnosis, LIN28B expression, and mutation status are presented in Table 3. In an effort to build a multivariate model for all patients involved in our study (12/82 patients [15%] had missing values for HbF), we excluded HbF percentage and age-adjusted HbF level from the model. In addition, the univariate analysis did not show a statistically significant impact of age-adjusted HbF impact on OS and EFS in our cohort. In our model, we could not show that LIN28B expression is an independent predictor of outcome because of its correlation with other factors such as karyotype and age. In fact,
Table 3. Multivariable analysis of OS from diagnosis and EFS from HSCT

<table>
<thead>
<tr>
<th>OS from diagnosis</th>
<th>P</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIN28B</td>
<td>High</td>
<td>.180 1.8 (0.8-4.2)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>≥2</td>
<td>.040 2.1 (1.0-4.2)</td>
</tr>
<tr>
<td></td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td>Karyotype</td>
<td>Monosomy 7</td>
<td>.027 2.6 (1.1-6.2)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Mutation</td>
<td>Mutation (1)</td>
<td>.557 1.3 (0.6-2.9)</td>
</tr>
<tr>
<td></td>
<td>Other karyotypes</td>
<td>.631 1.3 (0.5-3.5)</td>
</tr>
<tr>
<td>EFS from HSCT</td>
<td>LIN28B</td>
<td>High 1.9 (0.8-4.5)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>≥2</td>
<td>.035 2.1 (1.1-4.2)</td>
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<tr>
<td></td>
<td>&lt;2</td>
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</tr>
<tr>
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<td>Monosomy 7</td>
<td>.033 2.7 (1.1-6.6)</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>Other mutations</td>
<td>.594 1.3 (0.5-3.3)</td>
</tr>
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</table>

monosomy 7 and age ≥2 years at diagnosis were significant predictors for an inferior 5-year OS and EFS from HSCT (Figure 4C). Adding HbF to the multivariate model confirmed the prognostic value of monosomy 7 (supplemental Table 9). However, age ≥2 years at diagnosis lost its significance in predicting survival because of the strong correlation with HbF.

Discussion

JMML is a rare and aggressive childhood cancer associated with elevated HbF levels in more than half of the patients and with monosomy 7 in one-fourth of the patients. Currently, the disease is curable only by HSCT, and survival is hampered by high relapse rates. To develop new therapies, a thorough understanding of the molecular biology behind this stem cell disease is necessary. Therefore, we generated microarray expression profiles of 82 JMML patients and identified a novel fetal-like subgroup characterized by overexpression of the oncofetal gene LIN28B. Fifty-seven percent of patients in the discovery cohort and 45% in the validation cohort presented with high LIN28B levels.

We showed that LIN28B overexpression is significantly correlated with both HbF percentage and age-adjusted HbF levels. Although there was a positive correlation between high LIN28B and high HbF, elevated LIN28B levels are less frequently found in cases with monosomy 7. In addition, genes on chromosome 7 are upregulated in LIN28B-high patients. Reduced LIN28B levels in JMML patients with monosomy 7 are likely the reason why patients with monosomy 7 rarely present with elevated HbF levels, an observation made almost 20 years ago by Niemeyer et al.1 LIN28B, the missing link between monosomy 7 and HbF, has previously been shown to be the master regulator of the HbF transcriptional network and to mediate the expression of HbF through regulation of BCL11A.27 BCL11A expression levels were not different between LIN28B-high and -low JMML patients in our study, but LIN28B seems to drive the aberrant expression of HbF in JMML patients through upregulating embryonic hemoglobin and HbF genes such as HBEB1, HBG1, HBZ, HBQ1, and others.

LIN28B overexpression was accompanied by a downregulation of let-7. RNA-binding proteins and miRNAs are important posttranscriptional regulators able to override preexisting transcriptional networks.28 Aberrant expression of LIN28B and its co-regulated let-7 miRNAs adds an important additional layer to the understanding of JMML biology. LIN28B and let-7 are the core components of several developmental switches throughout hematopoiesis. During lymphopoiesis, the switch from innate-like B-1 B cells and γδ-T cells to adult B-2 B cells and αβ-T cells is characterized by downregulation of LIN28B and upregulation of let-7.16 The same is true for fetal and adult reticulocytes, echoing the fetal-to-adult hemoglobin switch.29 Last, but not least, together with the change of location from fetal liver to adult BM, HSCs lose their high LIN28B and low let-7 levels.15 Evidence suggests a linear relationship between fetal and adult HSCs, indicating a cell intrinsic change rather than the rise of a separate adult HSC population.29 This relationship is especially relevant in the light of the possible prenatal origin of JMML. It has been suggested that a delayed developmental switch can be responsible for the onset of childhood diseases such as neuroblastoma and JMML during early life.29

Our study indicated that stem cell genes important during embryogenesis, such as HMG2 and IGF2BP3, are also highly expressed in LIN28B-high patients. LIN28B is part of a growing number of fetal oncogenes attributing self-renewal capacity to cancer stem cells.18 Similar to maintaining the undifferentiated neuroblast phenotype in neuroblastoma, aberrant LIN28B expression points toward a role in the maintenance of stemness in JMML.30 It remains to be elucidated whether LIN28B overexpression in JMML occurs in the HSCs or in more differentiated common progenitors. On the basis of other findings, it has even been suggested that prenatal non-HSC endothelial-derived erythromyeloid progenitor cells could be the cell of origin of JMML disease.31 Although JMML patients have a high burden of monocyes in their blood, LIN28B overexpression in mice favors common myeloid progenitor differentiation into megakaryocyte-erythroid progenitors rather than granulocyte-macrophage progenitors.32 It is possible that additional regulators define cell fate choices in JMML.

Of note, in our study, patients with higher LIN28B levels had significantly inferior 5-year OS from diagnosis and 5-year EFS from HSCT, which was also the case for older age at diagnosis and molecular genetic status. Recently, the number of somatic mutations at the time of diagnosis was shown to be the strongest predictor of outcome.33,34 In multivariable analysis, only the presence of monosomy 7 and age at diagnosis were significant predictors, not LIN28B expression. The predictive value of age at diagnosis is in line with previous reports, whereas monosomy 7 in the literature is not regarded as a significant predictor for adverse outcome in JMML.1 It should be noted that a higher percentage of monosomy 7 patients presented with an unfavorable mutation (PTPN11, KRAS, or NFI) compared with patients with a normal karyotype, which could be one of the reasons for the inferior outcome of monosomy 7 in our patient population. LIN28B is not an independent prognostic marker because of its...
high correlation with other variables, including age-adjusted Hbf, age at diagnosis, and karyotype. The correlation between Lin28b and hallmarks of JMML such as Hbf and monosomy 7 is important in understanding the biology behind JMML.

An interesting case report in the literature triggers speculation on the possible benefits of eliminating Lin28b in JMML. During the transformation from JMML to AML in a particular patient, a del(6q) karyotype was acquired, occurring with the normalization of high Hbf levels. Subsequently, the patient was treated successfully with intensive chemotherapy and cord blood transplant. The investigators claim that acquisition of new hits by the transformation to AML provides a survival advantage to adult-type hematopoiesis and caused extinction of fetal erythroid progenitors responsible for high Hbf levels. Strikingly, Lin28b is located on 6q21. Consequently, this finding suggests a possible link between the fortuitous loss of Lin28b and the adopted adult-type hematopoiesis in this particular leukemic case.

In conclusion, we identified Lin28b as a crucial molecular player at the heart of a novel fetal-like subgroup of JMML associated with elevated Hbf levels, absence of monosomy 7, and high expression of a fetal transcriptional network. Targeting Lin28b is currently under extensive investigation in a variety of cancers, with promising results in the heart of a novel fetal-like subgroup of JMML associated with Lin28b and karyotype was acquired, co-occurring with the normalization of high Hbf levels.

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H.H.H. is a PhD candidate at Ghent University and this work is submitted in partial fulfillment of the requirement for a PhD.

**Authorship**

**Contribution:** H.H.H., S.B., T.L., G.t.K., P.V.V., and B.D.M. designed the research; H.H.H. and S.B. performed the research and analyzed the data; H.H.H., S.B., T.L., H.C., A.C., F.G., A.d.V., H.H., V.L., R.M., J.S., M.M.v.d.H.-E., J.P., N.V.R., Y.B., F.S., C.N., C.F., G.B., G.t.K., P.V.V., and B.D.M. collected the data; P.N. performed the statistical analysis; and H.H.H., T.L., P.V.V., and B.D.M. wrote the manuscript. All authors critically reviewed and approved the manuscript.

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**References**


LIN28B overexpression defines a novel fetal-like subgroup of juvenile myelomonocytic leukemia


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