EVII expression in childhood acute lymphoblastic leukaemia is not restricted to MLL and BCR/ABL rearrangements and is influenced by age

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EVII is a transcriptional regulator with an important function in haematopoiesis and self-renewal.1 Aberrant overexpression of EVII has been firmly established as one of the most aggressive oncogenes in AML. Importantly, a recent report in Leukemia from Konantz et al.3 suggests that EVII might also have a role in paediatric acute lymphoblastic leukaemia (ALL), where high expression confers apoptosis resistance, and possibly also an adverse prognosis. Rearrangements of the 3q26 region, which encompasses the MECOM (MDS–EVI1 complex) gene that encodes EVII transcripts and that are commonly associated with EVII overexpression in adult AML, rarely occur in childhood ALL or AML.2,4 However, when EVII is expressed in childhood AML it seems to be predominate in MLL-rearranged cases,5,6 which may then confer an adverse prognosis, as illustrated by the correlation with the t(6;11) subtype,7 and with complex karyotype cases. However, in general high EVII expression is not seen in AML with good prognosis cytogenetics such as core-binding factor-rearranged AML with t(8;21) or inv(16)8,9 (Figure 1a). In addition, in chronic myeloid leukaemia, the BCR–ABL fusion tyrosine kinase sustains EVII expression.6

To complement the work reported by Konantz et al.,3 we analysed gene expression data generated from nucleated cells obtained from diagnostic bone marrow aspirates of 70 de novo ALL (31 female subjects, median age at diagnosis: 4.4 years, range: 1.1–14.6 years), using the Affymetrix U133 obtained from diagnostic bone marrow aspirates of 70 different cut-off to analyse a meaningful sample size for comparison. In general, the overlap was greater between T-cell ALL and B-cell ALL than either of these groups with AML (Supplementary Information, Figure 1). Only 12 genes were significantly co-regulated in all the subgroups of childhood leukaemia (P < 0.01, analysis of variance) (Supplementary Information, Figure 1), with seven regulated in different directions between the subgroups. This included SMARCA5, of which the encoded protein recently has been shown to directly interact with the EVII protein in SKOV ovarian cancer cells and K562 leukaemia cells.13

Importantly, we noted that several of the high EVII-expressing ALLs were from patients in late childhood or adolescence. To further explore a possible association of EVII expression with age in de novo childhood ALL, we carried out rank regression analysis by age on our B-cell ALL cases, which is the largest group of our data sets targeted (n = 51), excluding those with MLL or BCR–ABL rearrangements. We found a highly significant increase in EVII expression with age at diagnosis of ALL (Figure 1bi, upper panel) during childhood years (age 1–10, r = 0.29, P = 0.05) but in particular obvious with the onset of adolescence (age 1–14, r = 0.53, P < 0.003). To further explore the relationship of patient age on gene expression patterns in childhood ALL we applied this approach to the entire data set. We identified 415 probe sets corresponding to 341 genes with significant age-associated changes in expression levels (P < 0.01) (Figure 1bii, lower panel). Of these, 130 genes have the highest expression in adolescence (cluster 3). When we associated the upstream regulators of these 130 genes (Ingenuity pathway analysis), we found that 21 are regulated directly by transforming growth factor-β (P = 1.8 × 10−5). This resembles our findings with respect to the impact of age on gene expression patterns in general12 and implies that transforming growth factor-β might in particular
contribute to age-dependent changes in ALL gene expression observed in the adolescent age group. As EVI1 also has been shown to have an impact on transforming growth factor-β signalling, we have started to investigate this in more detail. When we analysed the age association of gene expression patterns in our AML data set (also excluding MLL-rearranged cases), we detected some striking differences to ALL. Although age has an impact on gene expression patterns also distinctly in AML, we see a negative correlation of EVI1 with increasing age (Supplementary Figure 2).
The data set by Hogan et al. shows that in de novo ALL that subsequently relapses EVI1 expression has a significantly wider range compared with the de novo ALL cases that are not selected for subsequent relapse in the other studies (P<0.001). Importantly, paired analysis suggests that at ALL relapse, expression of EVI1 is on average higher than in the corresponding de novo sample (1.3-fold increased, P=0.006, Figure 1c).

In summary, our analysis confirms high EVI1 expression in a group of paediatric ALL, which does not appear to be confined to distinct cytogenetic subtypes. The limited overlap of gene expression profiles associated with high EVI1 expression in different forms of childhood leukaemia and the different levels of expression of some co-regulated genes implies the possibility of tissue specificity, and the impact of cell of origin for EVI1-mediated transcriptional regulation, which has also been suggested for MLL-rearranged AML subtypes. High EVI1 expression itself is likely to be a secondary event in paediatric lymphoblastic and myeloid leukaemia. The higher expression in older children might be linked to the worse prognosis of ALL in this age group. Whereas higher EVI1 expression in relapsed disease might be a function of increasing age, it also implies potentially a more general role in ALL stem cell survival. It will be important to prospectively investigate EVI1 in paediatric ALL for prognostic or therapeutic treatment regimens, and potential therapeutic benefit. In addition, our analysis strongly suggests an impact of age at diagnosis on gene expression patterns in childhood leukaemia. As we are uncertain to what extent this reflects the chronological age of the patient, or that of the cell of origin in childhood leukaemia, we have started to investigate this more comprehensively.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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