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Acute Lymphoid Leukemia

**Effect of the histone deacetylase inhibitor depsipeptide on B-cell differentiation in both TEL-AML1-positive and negative childhood acute lymphoblastic leukemia**

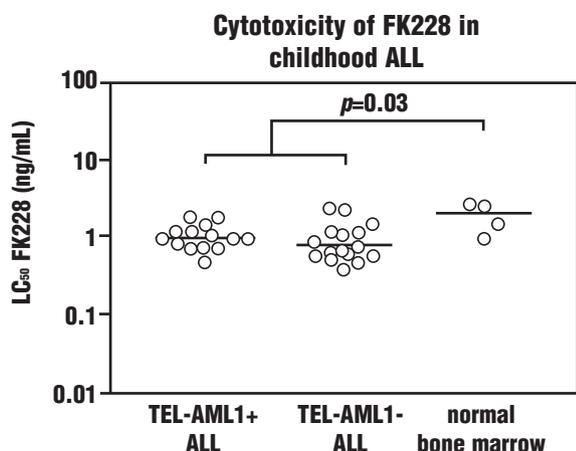
**The fusion protein TEL-AML1 in t(12;21)+ acute lymphoblastic leukemia (ALL) recruits co-repressors and histone deacetylases (HDAC), which transrepress AML1 target genes. Normal bone marrow cells were more resistant to HDAC inhibitor FK228 induced cell killing than were cells from ALL patients with or without t(12;21). FK228 induced differentiation in ALL, irrespective of the presence of t(12;21).**

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The t(12;21)(p13;q22) [*TEL-AML1*] is the most common (~25%) translocation in childhood acute lymphoblastic leukemia (ALL), and is restricted to precursor B-cell lineage leukemia. Like AML1, the abnormal TEL-AML1 fusion protein can bind to core enhancer sequences, but instead of activating transcription through recruitment of co-activators and histone acetylases, it recruits co-repressors and histone deacetylases (HDAC). This induces closure of the chromatin resulting in inhibition of transcription.<sup>1,2</sup> TEL-AML1-positive ALL might benefit from treatment with HDAC inhibitors since the TEL-AML1-induced transcriptional repression was shown to be reversed by HDAC inhibitors.<sup>2</sup> HDAC inhibitors such as



**Figure 1.** Cytotoxicity of FK228 in childhood ALL. The cytotoxicity of FK228 was measured in 14 TEL-AML1-positive (median LC<sub>50</sub> value 1.0 ng/mL), 15 TEL-AML1-negative (median LC<sub>50</sub> value 0.8 ng/mL) and 4 normal bone marrow samples (median LC<sub>50</sub> value 2.0 ng/mL). TEL-AML1-positive versus negative ALL cells:  $p=0.3$ ; TEL-AML1 positive and negative ALL cells versus normal bone marrow samples:  $p=0.03$ .

FK228 (FR901228, depsipeptide) have been shown to induce cell differentiation in myeloid leukemias, to inhibit cell proliferation and to enhance apoptosis in several types of cancer cells.<sup>2</sup>

We studied the cytotoxic effect of HDAC inhibition alone and in combination with L-asparaginase in TEL-AML1-negative and positive leukemic cells of children with ALL at initial diagnosis to gain more insight into the potential use of HDAC inhibitors in the treatment of TEL-AML1-positive ALL. In addition, the effect of HDAC inhibition on differentiation of these B-lineage leukemic cells was studied.

Leukemic cells of 29 children with common/pre B-ALL at initial diagnosis were collected at the Erasmus MC - Sophia Children's Hospital, the Dutch Childhood Oncology Group (DCOG) and the German COALL study group. Bone marrow samples from four healthy children were included as controls. The *in vitro* cytotoxicity of the HDAC inhibitors FK228 and sodium butyrate as well as L-asparaginase was determined using the MTT assay as described before.<sup>3</sup> Despite the presence of more binding

sites for HDAC inhibitors on the *TEL-AML1* fusion gene compared to wild type *TEL* or *AML1* genes, the cytotoxicity of the HDAC inhibitor FK228 did not differ between TEL-AML1 positive (median LC<sub>50</sub> value 1.0 ng/mL, P25-P75: 0.8-1.3) and TEL-AML1-negative ALL cells (median LC<sub>50</sub> value 0.8 ng/mL, P25-P75: 0.6-1.2;  $p=0.3$ ) (Figure 1). Normal bone marrow samples were a median 2-fold more resistant than were ALL cases (median LC<sub>50</sub> value 2.0 ng/mL, P25-P75: 1.1-2.6 and median LC<sub>50</sub> value 1.0 ng/mL, P25-P75: 0.7-1.2 respectively;  $p=0.03$ ). Similar results were obtained with sodium butyrate, another HDAC inhibitor.

TEL-AML1-positive ALL patients are known to be more sensitive to L-asparaginase than are TEL-AML1-negative ALL patients,<sup>4,5</sup> and the mechanism of action of L-asparaginase is different in these genetic subtypes.<sup>6</sup> We observed that FK228 sensitized both TEL-AML1-positive and negative ALL cases to the cytotoxic effect of L-asparaginase. However, this sensitizing effect was not caused by a synergism between FK228 and L-asparaginase, but could be explained by an additive effect caused by the cytotoxicity of FK228 itself.

Since FK228 was shown to induce myeloid differentiation, we studied the B-cell differentiation effect of FK228 in samples from five TEL-AML1-positive and five TEL-AML1-negative ALL patients (matched for age 1-10 years, common/preB immunophenotype, no hyperdiploidy (>50 chromosomes), absence of *MLL* rearrangement and t(9;22) [*BCR-ABL*]). After 4 days of exposure to FK228 or control medium, the expression of B-cell differentiation markers was measured by flow cytometry. Three selected quadruple immunostainings were used to analyze FK228-induced changes in the differentiation stage of precursor B-cells as described previously.<sup>7,8</sup> Incubation with 0.1 or 0.4 ng/mL FK228 had no differentiation effect compared to exposure to culture medium only. However, exposure to 1.1 ng/mL FK228 resulted in a clear and significant effect on the expression of CD10, CD19, and TdT (Table 1). The decreases in CD19, TdT, and CD10 as well as the slight increases in CD20, CD22 and CD45 expression corresponded to the induction of differentiation towards a more mature B-cell stage.<sup>7</sup> This effect of 1.1 ng/mL FK228 was not, however, different between TEL-AML1-positive and negative ALL cells. This suggests that the effect of HDAC inhibitors is independent of the genetic subtype. Studies in acute myeloid leukemia also found that HDAC inhibition restores the differentiation process independently of the genetic subtype.<sup>9</sup>

**Table 1.** FK228-induced B-cell differentiation in childhood ALL.

Marker	Fluorescence Index* unexposed median (P25-P75)	Fluorescence Index* exposed to 1.1 ng/mL FK228 median (P25-P75)	fold** change	paired t-test p value
CD10	751.1 (558.3-964.4)	550.3 (305.2-834.1)	-1.4	<u>0.007</u>
CD19	208.8 (136.7-358.1)	131.8 (76.1-242.2)	-1.6	<u>&lt;0.001</u>
CD20	10.1 (3.8-24.2)	18.6 (5.4-24.2)	1.8	0.15
CD22	112.6 (100.3-316.1)	122.4 (83.3-273.2)	1.1	0.82
CD34	32.2 (8.6-85.1)	31.0 (13.3-70.5)	1.0	0.10
CD45	93.3 (42.7-111.2)	104.6 (64.9-130.9)	1.1	0.09
TdT	50.8 (26.1-67.4)	19.4 (12.2-31.2)	-2.6	<u>&lt;0.001</u>
CyIgG <sub>u</sub>	24.8 (14.7-56.7)	26.3 (15.4-36.7)	1.1	0.15
SmlgM	10.6 (8.0-13.1)	9.0 (7.7-11.1)	1.2	0.16

\* The fluorescence index (FI) is the mean fluorescence intensity of the specific antibody by its isotypic control antibody. \*\*The fold-change value for each differentiation marker represents the ratio between the FI of FK228 exposed cells compared to the FI of culture medium (control)-treated cells. Statistically significant p-values are underlined.

Although our results showed that FK228 induced differentiation of malignant B-cells, the effect of this HDAC inhibitor was not selective for TEL-AML1-positive ALL patients. However, the 2-fold difference in toxicity of FK228 between ALL and normal bone marrow samples may be indicative of a therapeutic advantage from using FK228 in the treatment of ALL. The additive effect of FK228 on L-asparaginase cytotoxicity as well as the induction of B-cell differentiation encourages further studies on the efficacy of HDAC inhibitors in the treatment of precursor B-ALL, irrespective of *TEL-AML1* status.

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*Key words:* histone deacetylase inhibitor, FK228, FR901228, depsipeptide, acute lymphoblastic leukemia, *TEL-AML1*.

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