LETTER TO THE EDITOR

Interference with pre-B-cell receptor signaling offers a therapeutic option for TCF3-rearranged childhood acute lymphoblastic leukemia

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Rearrangements of TCF3 (E2A) occur in <5% of childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cases.1 In 90–95% of these rearranged cases, TCF3 (chromosome 19p13) is fused to PBX1 (chromosome 1q23).2 This TCF3-rearranged subtype is characterized by the expression of cytoplasmic immunoglobulin heavy chain (CyIg) in more than 80% of pediatric patients.3,4 This CyIg positivity is a consequence of an in-frame VDJ rearrangement of the immunoglobulin heavy chain locus (IGH),3 IgI and the surrogate light chain together constitute the pre-B-cell receptor (pre-BCR).5 Activation of this pre-BCR triggers the clonal expansion of pre-B cells in the bone marrow, after which these cells further mature by initiating the rearrangement of the light chain locus genes IGL and IGL.6 The transcription factor TCF3 is essential in the differentiation process of common lymphoid progenitors into B-lineage cells and is a key regulator of further B-cell development. TCF3-deficient cells are impaired in rearranging both the immunoglobulin heavy and light chain genes, and wild-type TCF3 regulates the expression of genes important in B-cell differentiation such as EBF1 and PAX5.7,8 These findings imply that TCF3-rearranged cases might be affected in pre-BCR-mediated signaling, which in turn could point to new therapeutic targets for this subtype of BCP-ALL. To address this hypothesis, we investigated components of the pre-BCR pathway in leukemic cells obtained from newly diagnosed children with TCF3-rearranged BCP-ALL and non-TCF3-rearranged BCP-ALL.

In a representative childhood BCP-ALL cohort, 11/13 (84.6%) of TCF3-rearranged cases were positive for CyIg compared with 68/325 (20.9%) of non-TCF3-rearranged cases (P < 0.001; Figure 1a), thereby confirming previous studies.3,4 We used a previously validated PCR heteroduplex analysis9 of VDJ rearrangement patterns of the IGH, IGL and IGL genes to determine a difference in the heavy and light chain rearrangement patterns of TCF3 and non-TCF3-rearranged cases. Four stages at which the rearrangement had been arrested were defined: samples with only rearrangements of IGH (IGH), samples with VJ-J rearrangements but no IGL-Kde or VJ-J rearrangements (IGK), samples with IGK-Kde and no VJ-J rearrangements (IGK), and samples with VJ-J rearrangements (IGL). Strikingly, the immunoglobulin rearrangement pattern of TCF3-rearranged cases was arrested at the IGH stage in 86.4% (19/22) of the cases. Only one case was assigned to the IGK group and two cases to the IGL group. In contrast, only 17.9% (34/190) of the non-TCF3-rearranged cases were arrested at the IGH stage and the frequency of cases with light chain rearrangements was significantly increased to 18.4% (35/190) for samples arrested at IGK, 38.4% (73/190) for IGK-Kde and 24.7% (47/190) for IGL (P < 0.001). In one sample, that was representing a BCR-ABL1-like patient, rearrangements of the heavy chain (IGH) and light chain genes (IGK, IGL) did not occur (Figure 1b). These data demonstrate that the majority of TCF3-rearranged BCP-ALL cases express the immunoglobulin heavy chain without further initiating the rearrangement and expression of the light chain needed to mature further and form a functional B-cell receptor. This suggests a divergent role for the pre-BCR and its downstream signaling in TCF3-rearranged BCP-ALL.

Next, we analyzed the expression levels of several key components of the pre-BCR signaling complex: ZAP70, SYK, LYN, SLP65 (BLNK), BTK, PLC-γ2, PI3K,p110δ, and IRF4 by reverse phase protein arrays10 of leukemic cell lysates (containing >90% leukemic blasts) obtained from 19 TCF3-rearranged and 113 non-TCF3 BCP-ALL pediatric patients. As visualized in Figure 1c, most of these proteins were expressed significantly more in TCF3-rearranged compared with non-TCF3-rearranged BCP-ALL: ZAP70 1.3-fold (P < 0.001), SLP65 3.4-fold (P = 0.002), BTK 1.5-fold (P < 0.001), PI3K,p110δ 1.2-fold (P = 0.02) and IRF4 5.2-fold (P < 0.001). In addition, the expression of the TCF3-target gene EBF1 was 2.7-fold increased in TCF3-rearranged BCP-ALL cases (P < 0.001; Figure 1c).

The activity of this divergent pre-BCR pathway was evaluated by responsiveness to the BTK inhibitor Ibrutinib (PCI-32765, Selleckchem, Houston, TX, USA). Ibrutinib was demonstrated to be of clinical benefit to patients suffering from chronic lymphoblastic leukemia,11,12 B-cell non-Hodgkin lymphomas13 and multiple myeloma.14 We tested in primary BCP-ALL samples and leukemic cell lines in a 72-h in vitro drug cytotoxicity assay using methyl-thiazol-tetrazolium conversion as read-out.14 In correspondence with preclinical studies showing effective anti-leukemic effect of Ibrutinib in primary chronic lymphoblastic leukemia cells,15 a concentration range between 0 and 50 μM Ibrutinib was used. In our study the growth and viability of the TCF3-rearranged cell line MHH-CALL3 decreased upon exposure to increasing concentrations of Ibrutinib (Figure 2a), resulting in a growth inhibitory concentration (GI50) of 43.5 μM. In contrast, no GI50 value was reached for non-TCF3-rearranged cell lines (Nalm6 and MHH-CALL4) nor the TCF3-rearranged cell line 697 (Figure 2a). This latter cell line is known for its lack of functional BTK16,17 suggesting relative specificity of Ibrutinib for BTK-mediated pre-BCR signaling in BCP-ALL. In correspondence, the level of phosphorylated ERK1/2, which is an important downstream effector of BTK contributing to cell survival, decreased upon a 4-h exposure to Ibrutinib in the TCF3-rearranged MHH-CALL3 but not in both non-TCF3-rearranged cell lines Nalm6 and MHH-CALL4 (as shown on western blot, Figure 2c). Figure 2b shows the differential cytotoxic effect of Ibrutinib in primary patients’ leukemic cells of six TCF3-rearranged and six non-TCF3-rearranged BCP-ALL patients (>90% leukemic blasts) taken at the initial diagnosis (measured by methyl-thiazol-tetrazolium assay). All TCF3-rearranged samples expressed CyIg, in contrast to 50% (3/6) non-TCF3 samples, which represented two hyperdiploid (>50 chromosomes), two ETV6-RUNX1-translocated, one BCR-ABL1-like and one BCR-ABL1-positive case. Ibrutinib significantly reduced the cell viability of all TCF3-rearranged cases at each inhibitor concentration compared with non-TCF3-rearranged cases (P < 0.001). As leukemic cells of patients do not proliferate in ex vivo culture conditions, the concentration of Ibrutinib, which is
Figure 1. Cytoplasmic Igμ expression in TCF3 non-TCF3-rearranged B-cell precursor ALL (a). Patients were considered Cyμg positive if >30% of leukemic cells were stained positively for this marker as detected by flow cytometry. Distribution of immunoglobulin rearrangement pattern of TCF3-rearranged and non-TCF3-rearranged BCP-ALL cases (b). IGH group contains samples with IGH rearrangement only. IGK group consists of cases with Vκ-Jκ rearrangements without IGK-Kde or Vλ-Jλ; IGK-Kde group contains IGK-deleted cases without Vλ-Jλ rearrangements. IGL group contains cases with Vλ-Jλ-rearranged IGL locus. V(D)J rearrangement patterns were detected by genomic PCR heteroduplex analysis as previously described.

Expression level of proteins involved in pre-BCR signaling in TCF3-rearranged and non-TCF3-rearranged BCP-ALL samples (c). Expression levels of indicated proteins were determined by reverse phase protein arrays using leukemic cell lysates of newly diagnosed BCP-ALL patients. The protein level was detected in six spots per patient by specific antibodies and was normalized for total protein loaded on each array. Red line indicates the median expression level per group. ***P < 0.001, **P < 0.01, *P < 0.05 (a and b: χ²-test; c: Mann–Whitney U-test). See Supplementary Materials and methods document for more information.

Figure 2. Sensitivity of leukemic cells to the BTK inhibitor Ibrutinib. (a) Leukemic cell lines. (b) Patients’ leukemic cells. Experiments were performed in duplicate. Dashed line indicates the Ibrutinib concentration affecting growth (GI50, cell lines) or inducing cell death (LC50, patients’ cells) in 50% of the cells. Bars indicate 95% confidence interval. P-value was calculated by one-way analysis of variance test with repeated measurements. Effect of Ibrutinib on ERK protein levels in leukemic cell lines (c). Cell lines were exposed to 0, 50 and 150 μM Ibrutinib for 4 h. The level of phosphorylated ERK1/2 (p-ERK1/2) was reduced in TCF3-rearranged MHH-CALL3 compared with non-TCF3-rearranged Nalm6 and MHH-CALL4 cell lines. β-Actin served as a loading control. P-ERK/β-actin: ratio of the intensities. See Supplementary Materials and methods document for more information.
lethal to 50% (LC50) of the primary leukemic cells, was calculated. The median LC50 value was 16.7 μM for TCF3-rearranged cases, whereas virtually no cell death was induced up to 50.0 μM of Ibrutinib in leukemic samples of non-TCF3-rearranged patients (P < 0.001; Figure 2b). Strikingly, in our study the leukemic cell samples of patients were more sensitive to cell death induced by Ibrutinib than the growth inhibitory concentration needed for cell lines. This suggests that TCF3-rearranged patients’ leukemic cells have an activated pre-BCR signaling pathway. The observed LC50 values for TCF3-rearranged cells are comparable to previously reported drug cytotoxicity data in primary chronic lymphoblastic leukemia cells. In addition, Ibrutinib was shown to impair the migration and adhesion of mature B cells to mesenchymal stromal cells at sub-micromolar levels, these data suggest that Ibrutinib is highly effective to target leukemic cells by interfering with (pre)BCR-driven survival/proliferation signaling and with migration/adhesion pathways.

Remarkably, we observed that TCF3-rearranged BCP-ALL cases intrinsically express higher protein levels of pre-BCR pathway including IRF4 (Figure 1c). In normal B-cell development, IRF4 triggers the rearrangement of the immunoglobulin light chain genes IGK and IGL, thereby contributing to the maturation of the B-cell receptor, whereas the present study shows that these light chain genes are infrequently rearranged in TCF3-rearranged BCP-ALL. Inhibition of TCF3 is essential for the successful production of high-affinity immunoglobulins, a process that is mediated via a negative-feedback loop of the mature B-cell receptor. Given the presently available data, we would like to postulate that the differentiation of TCF3-rearranged BCP-ALL is arrested at an early stage in B-cell development, most likely resembling the compartment of pre-B-II-large cells in normal hematopoiesis before IRF4 triggers the rearrangements of immunoglobulin light chains. The aberrantly high levels of IRF4 together with activated pre-BCR signaling may trigger an uncontrolled expansion of TCF3-rearranged cells in vivo at the expense of differentiation into more mature B cells. In correspondence, the relative sensitivity of TCF3-rearranged leukemic cells for Ibrutinib—without need for external stimuli other than those provided by the culture medium—and the aberrant expression profile of pre-BCR-related proteins suggests that the pre-BCR pathway is more activated in TCF3-rearranged compared with non-TCF3-rearranged leukemic cells.

The prognosis of children with TCF3-rearranged leukemia has improved during consecutive trials by optimizing the regimens with traditional chemotherapeutic drugs. Implementation of more specific, that is, targeted, drugs may further improve prognosis and/or reduce the side effects of current chemotherapy regimens. This suggests that TCF3-rearranged BCP-ALL cases are comparable to previously reported drug cytotoxicity data in primary chronic lymphoblastic leukemia cells.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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REFERENCES

1. Pui CH, Carroll WL, Mesenchin S, Arceri RJ. Biology, stratification, and therapy of pediatric acute leukemias: an update. J Clin Oncol 2011; 29: 551–565.
2. Barber KE, Harrison CJ, Broadfield ZJ, Stewart AR, Wright SL, Martinou M et al. Molecular cytogenetic characterization of TCF3 (E2A/19p13.3) rearrangements in B-cell precursor acute lymphoblastic leukemia. Genes Chromosomes Cancer 2007; 46: 478–486.
3. Williams DL, Look AT, Melvin SL, Roberson PK, Dahl G, Flake T et al. New chromosomal translocations correlate with specific immunophenotypes of childhood acute lymphoblastic leukemia. Cell 1984; 36: 101–109.
4. Crist WM, Carroll AJ, Shuster JJ, Behm FG, Whitehead M, Vierti TJ et al. Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13): A Pediatric Oncology Group study. Blood 1990; 76: 117–122.
5. Sakaguchi N, Melchers F, Lambda 5, a new light-chain-related locus selectively expressed in pre-B lymphocytes. Nature 1986; 324: 579–582.
6. Herzog S, Reth M, Jurma H. Regulation of B-cell proliferation and differentiation by pre-B-cell receptor signalling. Nat Rev Immunol 2006; 9: 195-205.
7. Kwok K, Hutter C, Sun Q, Bilic I, Cobaleda C, Malin S et al. Instructive role of the transcription factor E2A in early B lymphopoiesis and germinal center B cell development. Immunity 2008; 28: 751–762.
8. Aspland SE, Bendall HH, Murre C. The role of E2A-PBX1 in leukemogenesis. Oncogene 2001; 20: 5708–5717.
9. van der Velden VH, van Dongen JJ. MRD detection in acute lymphoblastic leukemia patients using Ig/TCR gene rearrangements as targets for real-time quantitative PCR. Methods Mol Biol 2009; 538: 115–150.
10. Petricoin EF, Espina V, Araujo RP, Mudra B, Yeung C, Wan X et al. Phosphoprotein pathway mapping: Akt/mammalian target of rapamycin activation is negatively associated with childhood rhabdomyosarcoma survival. Cancer Res 2007; 67: 3431–3440.
11. Advani RH, Buggy JJ, Sherrington JP, Smith SM, Boyd TE, Grant B et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. J Clin Oncol 2013; 31: 88–94.
12. Ponader S, Chen SS,显示参考文献的完整列表。
17 Kim E, Koehler S, Rosin NV, Thomas DA, Ravandi F, Kornblau SM et al. Activity of Bruton’s tyrosine kinase (BTK) inhibitor ibrutinib (PCI-32765) in B-cell acute lymphoblastic leukemia (B-ALL). ASH Annual Meeting Abstracts 2012; 120: 2569.

18 Chang BY, Francesco M, De Rooij MF, Magadala P, Steggerda SM, Huang MM et al. Egress of CD19(+)CD5(+) cells into peripheral blood following treatment with the Bruton tyrosine kinase inhibitor ibrutinib in mantle cell lymphoma patients. Blood 2013; 122: 2412–2424.

19 de Rooij MF, Kuil A, Geest CR, Eldering E, Chang BY, Buggy JJ et al. The clinically active BTK inhibitor PCI-32765 targets B-cell receptor- and chemokine-controlled adhesion and migration in chronic lymphocytic leukemia. Blood 2012; 119: 2590–2594.

20 Ma S, Turetsky A, Trinh L, Lu R. IFN regulatory factor 4 and 8 promote Ig light chain kappa locus activation in pre-B cell development. J Immunol 2006; 177: 7898–7904.

21 Verma-Gaur J, Hauser J, Grundstrom T. Negative feedback regulation of antigen receptors through calmodulin inhibition of E2A. J Immunol 2012; 188: 6175–6183.

22 Moorman AV, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol 2010; 11: 429–438.

23 Byrd JC, Furman RR, Coutre SE, Filimn IW, Burger JA, Blum KA et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med 2013; 369: 32–42.

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