RESEARCH ARTICLE

Cytokines, growth, and environment factors in bone marrow plasma of acute lymphoblastic leukemia pediatric patients

Martin Kováč[1,2], Martina Vášková[1,2], Denisa Petráčková[3], Vendula Pelková[1,2], Ester Mejstříková[1,2], Tomáš Kalina[1,2], Markéta Žaliová[1,2], Jaroslav Weiser[3], Jan Starý[2], Ondřej Hrušák[1,2]

[1] CLIP – Childhood Leukemia Investigation Prague
[2] Department of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic
[3] Institute of Microbiology v.v.i., Czech Academy of Sciences, Prague, Czech Republic

Correspondence: O. Hrušák. Department of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic
<ondrej.hrusak@lfmotol.cuni.cz>

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ABSTRACT. Acute lymphoblastic leukemia (ALL) cells depend on the microenvironment of the host *in vivo* and do not survive in *in vitro* culture. Conversely, the suppression of non-malignant tissues is one of the leading characteristics of the course of ALL. Both the non-malignant suppression and malignant cell survival may be partly affected by soluble factors within the bone marrow (BM) environment. Here, we aimed to identify proteins in BM plasma of children with ALL that may contribute to ALL aggressiveness and/or the microenvironment-mediated survival of ALL cells. LBMp (leukemic bone marrow plasma) at the time of ALL diagnosis was compared to control plasma of bone marrow (CBMp) or peripheral blood (CPBp) using a cytokine antibody array. The cytokine antibody array enabled simultaneous detection of 79 proteins per sample. Candidate proteins exhibiting significantly different profiles were further analyzed and confirmed by ELISA. mRNA expression of one of the candidate proteins (TIMP1) was studied using quantitative reverse transcriptase polymerase chain reaction (qRTPCR). The cytokine antibody array experiments identified 23 proteins that differed significantly ($p<0.05$); of these, two proteins (TIMP1 and LIF) withstood the Bonferroni correction. In contrast, little difference was observed between CBMp and CPBp. At the diagnosis of ALL, changes in the soluble microenvironment are detectable in BM plasma. These changes probably participate in the pathogenesis and/or result from the changes in the cell composition.

Key words: pediatric acute lymphoblastic leukemia, bone marrow plasma, cytokine antibody array

Leukemia, a malignant disorder of lymphoid or myeloid progenitor cells, is the most frequent malignant disease of childhood. The most common subtype, acute lymphoblastic leukemia (ALL), represents 75% to 80% of all pediatric cases; the remaining subtypes are derived from a myeloid lineage [1]. ALL is a heterogenic disease characterized by the clonal expansion of transformed hematopoietic precursors of the T- or B-cells [1]. The dominant form of ALL is derived from B-precursors. The advances in chemotherapy, together with the increased understanding of the biological behavior of leukemic cells, have led to a significant decrease in mortality over the last five decades. Although the natural course of ALL is uniformly fatal, contemporary treatment rescues over 75% children with the disease. At present, the main goal of hematology is to define the reasons for treatment failure in 20% of ALL cases and to find new treatment options with potent anti-leukemic effects and minor side effects.

Malignant transformation of leukemic cells is associated with chromosomal abnormalities, aberrant gene expression patterns, and abnormal cell surface marker profiles [2]. The two most common genetic abnormalities (each accounting for a quarter of pediatric cases) are the TEL-AML1 fusion gene, which results from the t(12;21) chromosomal translocation, and high hyperdiploidy, in which the number of chromosomes is pathologically increased to between 51 and 65 [3]. Both of these genotypes confer a favorable prognosis. Less frequent genetic lesions include the prognostically unfavorable BCR-ABL and MLL-AF4 gene fusions [1].

The burden of leukemic cells impacts the patient and leads to several known clinical and laboratory manifestations, such as the suppression of nonmalignant hematopoiesis and metabolic effects. Leukemic cells are dependent on the microenvironment of the host; therefore, most leukemic cells die during the initial days following the transfer to *in vitro* conditions [4-6]. The factors responsible for the survival of leukemic cells have not been fully identified to date. As most of the attention has been paid to the biology of the leukemic cells themselves, the importance of soluble factors produced by leukemic cells or by their surroundings is largely unclear. Data showing a suppressive effect of adult acute myeloid leukemia patient bone marrow (BM) plasma on the growth of hematopoietic progenitor cells have been reported. This effect was abolished by anti-TNF-alpha and anti-adiponectin antibodies [7].

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Cytokines play a major role in leukemogenesis. Normally, hematopoietic cells require certain cytokines for their viability and growth. When the viability factors are withdrawn, apoptotic cell death naturally occurs. Prevention of programmed cell death by the abnormal production of a cytokine may release the cell from normal growth control leading to malignant transformation. As a first step to decipher the importance of soluble factors in ALL, we used a cytokine antibody array as a proteomic approach. The technical difficulties of plasma proteomic analysis should be taken into account. One problem with routine specimens of BM or PB (peripheral blood) is hemolysis, which occurs in a variable proportion of samples taken from patients or from healthy individuals. As a result of hemolysis, the contents of erythrocytes are released into the plasma, and the differences in the concentrations of such proteins should not be over-interpreted. The main aim of this study was to compare differential expression profiles of the soluble proteins in BM among various types of ALL and control samples. The newly identified molecules may then be considered as putative diagnostic markers for leukemia. In addition, these molecules could potentially serve as therapeutic targets if they are demonstrated to support the survival of leukemic cells.

MATERIALS AND METHODS

Patients

We collected diagnostic samples from children with B-precursor ALL (LBMp) and control samples (CBMp, patients with no signs of malignant disease with a BM check-up more than one year after BM transplantation). Standard immunophenotyping and molecular genetic investigation were performed as previously described [8]. All samples were collected with informed consent at the Department of Pediatric Hematology and Oncology in Prague. CPBp samples were obtained from healthy adult volunteers.

Each of the patient and control samples was tested individually by cytokine antibody array. 10 LBMp samples and 40 CBMp samples were also measured by ELISA, as confirmation.

Processing cells

The LBMp, CBMp and CPBp were collected from supernatants after Ficoll-Paque Plus (GE Healthcare Bio-Sciences, Wauwatosa, WI, USA) density centrifugation at 500 *g* for 35 minutes. Supernatants were centrifuged again after the first density centrifugation to dispose of particles. Plasma was stored at -80°C until analysis by cytokine antibody array.

Cytokine antibody array

The RayBiotech kit, RayBio Human cytokine antibody array (RayBiotech Inc, Norcross, GA, USA), which is composed of array membranes spotted with antibodies to 79 cytokines (human cytokine antibody arrays), was used for screening the concentrations of soluble proteins in the plasma samples. The complete list of cytokines with their full names is available at www.raybiotech.com. We tested soluble proteins in patients (LBMp, n = 15) and control samples (CBMp, n = 9 and CPBp, n = 5). Plasma was added to an antibody-coated membrane and incubated at room temperature on a plate shaker. After a 14-minute incubation with a cocktail of biotinylated antibodies and labeled-streptavidin, the signal was detected by chemiluminescence. The analysis of spot signal intensities was performed using a CCD camera by FluorChem FC2 (Alpha Innotech, Kasendorf, Germany) and software by AlphaEase FC.

Quantitative PCR (qPCR)

Real-time quantitative RT-PCR was performed using a LightCycler rapid thermal cycler system (Roche Diagnostic GmbH, Mannheim, Germany) with SYBR Green DNA-binding dye for quantification of TIMP1 mRNA. The data were normalized using oligonucleotide hybridization probes for β2 microglobulin quantification (β2 microglobulin house-keeping gene). The forward primer sequence for TIMP1 was GGACTGGAAGCCCTTTTCA, and the reverse primer sequence was ACACTGTTGGCTGTGAGGAA. The normalized TIMP1 expression was determined as a ratio between TIMP1 and β2microglobulin expression levels.

Protein validation

The concentrations of proteins with higher expression in patient samples detected by cytokine antibody array (TIMP1, LIF) were measured and confirmed by an ELISA kit (Bender MedSystems, Burlingame, CA, USA) according the manufacturer's instructions.

RESULTS

The protein profile of bone marrow plasma at the diagnosis of leukemia and in the absence of malignancy as detected by a cytokine antibody array

The cytokine antibody array results demonstrated a remarkable similarity between the CBMp and the CPBp of healthy individuals. Of the 79 proteins examined, the concentrations in CBMp and CPBp were no different at a level of significance of 0.05 (Mann-Whitney) for 78 of the proteins. The sole exception was angiogenin, which showed a significantly higher concentration in CPBp (0.8 ± 0.2) than in CBMp (0.47 ± 0.23, $p<0.05$). No CBMp:CPBp difference in individual protein concentrations withstood the Bonferroni correction for multiple observations (p level: 0.000633).

In contrast, the concentrations of 23 proteins (Ang, CXCL13, FGF4, CXCL1, CCL1, IGFBP1, IGFBP2, IGFBP4, IL1B, IL5, IL6, IL7, IL12, LIF, TNFSF14, CCL2, CCL8, MIF, MIP-1gamma, TGFB1, TGFB2, TIMP1, THPO) differed significantly ($p<0.05$) between LBMp and CBMp. Of these, TIMP1 (tissue inhibitor of metalloproteinases) and LIF (leukemia inhibitory factor) both differed at a level of significance that considered multiple comparisons ($p<0.000633$). In total, three proteins (IL1 A, MCSF, and FGF9) did not show detectable signals in any of the specimens. These proteins did not affect protein clustering and are omitted from *Figure 1*.

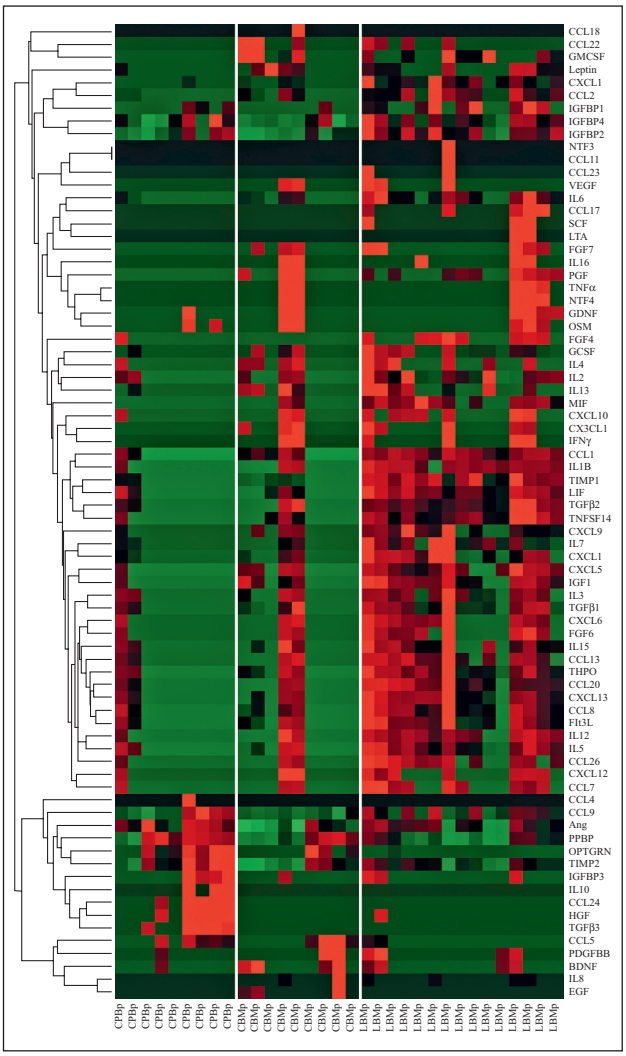

Figure 1

Heat map of cytokine antibody array results. Protein concentrations in control peripheral blood (CPBp) or bone marrow (CBMp) plasma and in bone marrow plasma isolated at the diagnosis of leukemia (LBMp) detected by a cytokine antibody array. Each cell represents a protein concentration in a given specimen; the results are shown as differences between the individual values and the mean concentration of each protein in multiples of standard deviations ([value] - [mean])/[standard deviation]). Individual signals were normalized using the internal negative and positive controls (the difference between each signal value and the mean negative control was divided by the difference between the mean values of the positive and negative controls; values lower than the mean negative control or lower than the median signal obtained by investigating plain buffer were considered to be zero). Proteins are hierarchically clustered (by Euclidean distance).

Protein confirmation by immunological methods

The concentration of TIMP1 was confirmed by ELISA to be significantly higher in LBMp (10 samples) than in CBMp (40 samples) (p = 0.0003, *Figure 2A*). However, differences in the concentration of LIF between LBMp and CBMp were not confirmed by ELISA (p = 0.75) (*figure 2B*).

Expression at the mRNA level, comparison of proteomics data and expression profiling

The concentration of TIMP1 was confirmed to be higher in LBMp than in CBMp, by at least one immunological method. These differences could be attributed to expression by leukemic cells themselves or by non-malignant cells in BM or elsewhere. To determine whether the difference in concentration is more likely to result from expression in leukemia cells or in non-malignant surrounding BM cells, the proteins with a significantly higher concentration in leukemia were further studied using publicly available expression profiling data [9-11]. We chose these studies as they used identical U133A and U133B chips (Affymetrix), and we included control genes that are either generally highly expressed in ALL compared to non-malignant status (positive controls) or genes with no anticipated connection to leukemia (negative controls). As shown in *Table 1*, TIMP1 may be produced by non-malignant BM cells. The higher TIMP1 expression in non-malignant BM cells compared to ALL cells was confirmed by qPCR (n = 8, p = 0.0023, data not shown).

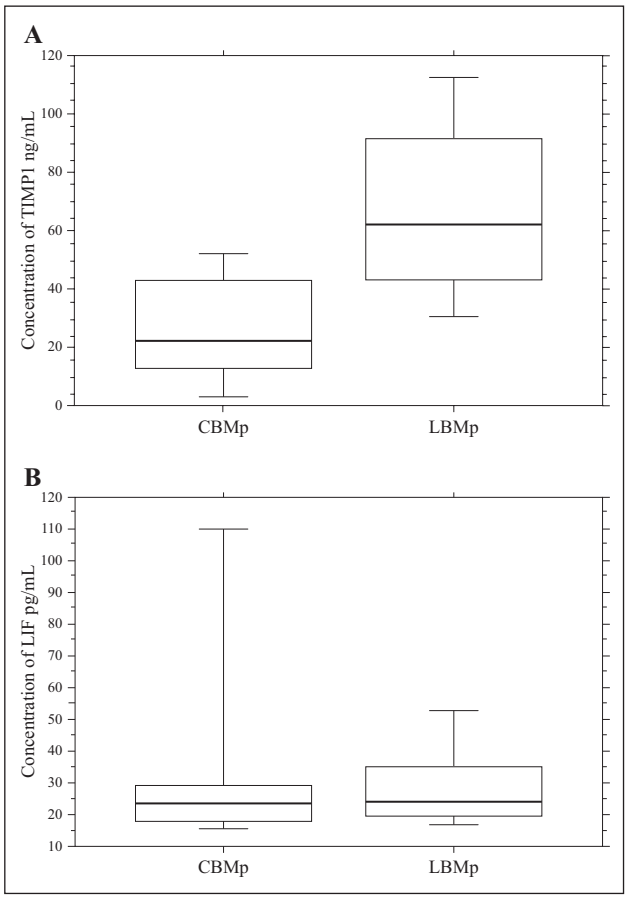

Figure 2

The concentrations of TIMP1 and LIF measured by ELISA in LBMp (10 samples) and CBMp (40 samples). (A) TIMP1 was found to be significantly more abundant in LBMp (p = 0.0003). **(B)** No significant differences were found in the concentration of LIF between LBMp and CBMp.

DISCUSSION

The main aim of the present study was to identify the soluble proteins selectively enriched in LBMp relative to CBMp. We compared the protein profiles of plasma harvested from children at the time of the diagnosis of B-precursor ALL, with those of children one year or more after receiving a BM transplant as a control (CBMp). BMp is a rich source of proteins from the BM

Table 1
Comparison of the expression levels of TIMP1.

Protein, GENE name (probe set)	Leukemic *versus* bone marrow cells	Leukemic *versus* B precursor
Proteins/genes with higher concentration in LMP:		
TIMP1, TIMP1 (201666_at)	Lower	Higher
Proteins/genes with no expected association with leukemia (negative control genes):		
Keratin 15, KRT15 (204734_at)	No difference	No difference
Keratin 19, KRT19 (201650_at)	No difference	No difference
Proteins/genes frequently highly expressed in leukemia (positive control genes):		
CD10, CALLA (203435_s_at)	Higher	No difference or higher
CD19, CD19 molecule (206398_s_at)	Higher	Higher
CD79a, IGA (205049_s_at)	Higher	Higher

Comparison of TIMP1 expression levels using expression profiles of leukemias, non-malignant BM, and B-precursors. Keratin was used as a negative control. CD10, CD19 and CD79a were used as positive controls. This analysis was based on publicly available expression profiling data [10, 11, 12]

micro-environment. Although it has not been used in standard diagnostics to date, the BMp proteome comprises the soluble molecules of this micro-environment.

We used cytokine antibody array analysis and determined the profiles of soluble proteins such as cytokines, chemokines and growth factors, even at very low concentrations.

The cytokine antibody array yielded 23 proteins that showed a significantly higher expression in patients than in the control group. Although some of these differences may simply reflect the large number of comparisons, CBMp was notably similar to CPBp when evaluated by the same technique. The differences in LIF and TIMP1 protein concentrations showed high statistical significance. However, only TIMP1 was confirmed by an independent immunological method that compared CBMp and LBMp. This discrepancy may be caused by inter-individual differences. The BM is a dynamic network of stromal cells, growth factors, cytokines, providing a permissive environment for leukemogenesis. Stromal cells are an essential component of the BM microenvironment, which regulate development of immature hematopoietic progenitor cells [12, 13]. BM stroma cells influence leukemic cell survival through their production of soluble molecules including cytokines and chemokines, and through direct physical interactions [14]. For example, IL7, which is higher in LBMp, may act in concert with stromal cells to support ALL cells viability via Bcl-2 expression [5]. Another support of ALL cell viability may come from IGFBP1, IGFBP2, IGFBP4, which were also present in higher concentrations in LBMp. IGFBP proteins possibly act as carriers though insulin-like growth factor 1 (IGF1) [15]. Binding of IGFBPs prolongs the half-life of the IGFs and alters their interaction with cell surface receptors. Leukemic blasts and BM stroma promote angiogenesis, which is increased in ALL and AML [16]. The serum expression of Ang is closely related to occurrence and development of acute leukemia [17, 18]. Interestingly, Ang levels in LBMp are still lower than in CPBp. Although much is known about the mechanisms by which Ang stimulates angiogenesis, the physiological importance of blood levels of Ang has not been fully determined. Of note, Ang expression in liver is higher than in PB by two orders of magnitude [11].

Chemokines may favor tumor growth and metastasis by promoting tumor cell proliferation, migration or neovascularization in tumor tissue. E.g., increases in specific chemokines and receptors were shown in AML [19]. Analogously, LBMp demonstrated an increased concentration of two CXC subtypes members of the chemokine superfamily: CXCL13 (B-cell attracting chemokine and CXCL1). High expression of CXCL13 was also observed in B-cell lymphomas and diseases with B-cell activation [20], and involvement of CXCL1 in angiogenesis and tumorigenesis has been described [21]. Another chemoattractant protein CCL2, which has been shown to support survival of chronic lymphatic leukemia cells *in vitro* [22] was also higher in LBMp. Inflammatory chemokines MIF, CCL9, CCL1 (which attracts, among others, immature B cells), and CCL8 were all increased in LBMp, whereas no difference was observed in CCL5 and IL8, and in concentrations of other chemokines (ENA78, CCL26, CCL7, CXCL12, CXCL6, CCL13, CCL8; B cell chemoattractants CXCL13 and CCL20) individually did not reach statistical significance, but clustered together with other proteins with higher concentration in LBMp.

Various cytokines have an unspecific cell proliferation effect and/or stimulate specifically progenitor cells. Of these, only thrombopoietin (THPO) and FGF4 were increased in LBMp ($p<0.05$), whereas GMCSF, GCSF or FGF7 did not differ between CBMp and LBMp. Cytokines IL3 and FGF6 were high in LBMp, however, their concentration did not differ from CBMp. Thrombopoietin induced proliferation of AML cells, which may be augmented by adding the other hematopoietic growth factors such as IL3, IL6, stem cell factor, or granulocyte-macrophage colony-stimulating factor [23].

A general T helper cytokine IL2 was not changed in LBMp. Similarly, although the IL15 concentration was high in LBMp, its difference from that in CBMp was not significant. In one study, the intracellular cytokine profile of T cells from children with ALL suggests a dysregulation in the functionality of Th1 and Th2 cells with an expansion in Th2 [24]. Of the Th1 cytokines, IL12 and IL1B were increased in LBMp, but others (IFNG, TNF alpha and TNF beta) did not change significantly. The Th2 cytokines IL5 and IL6 were higher in LBMp ($p<0.05$), but concentrations of IL4, IL10 and IL13 did not differ from those found in CBMp. Both TGFB1 and TGFB2 (but not TGFB3, which was even higher in CPBp than in BMp) were increased in LBMp; of these, TGFB1 was also found to affect myeloblast cell survival [25]. Another molecule involved in T cell physiology - TNFSF14 - was increased in LBMp. Taken together, although individual cytokines appear to be altered in LBMp, our data could not support a general dominance of either Th2 or of any other Th functional subset.

LIF is a multifunctional glycoprotein that is also active in tissues unrelated to hematopoiesis and/or leukemia. The basic functions of this remarkable pleiotropic molecule go beyond the boundaries of both malignant and non-malignant hematopoiesis and include, but are not limited to, the stimulation of proliferation and differentiation of some hematopoietic cells (chiefly thrombocytes), an inhibitory influence on myeloid leukemia cells (not entirely proved so far), enabling the implantation of the blastocyst, maintaining the totipotency of embryonic stem cells, stimulating osteoblasts and osteoclasts, and stimulating the production of adrenocorticotropic hormone (ACTH). LIF may be produced by various cells, such as fibroblasts, T-lymphocytes, osteoblasts, stromal cells and some types of tumor cells. LIF-producing stromal cells are a part of the extracellular matrix (ECM), a dynamic network consisting mainly of collagen, fibronectin, laminin and proteoglycan [26, 27]. The increased expression of LIF in LBMp in a subset of patients may not be due to the leukemic cells, but may come from non-malignant BM cells, namely the stromal cells. This hypothesis is supported by the expression profiling data showing that in leukemias, LIF has a lower expression level compared to that of non-malignant BM cells and B-precursors. Hence, LIF may be one of the factors contributing to the extended survival of leukemic cells in the BM micro-environment, as opposed to the rapid apoptosis that occur under *in vitro* conditions [5].

The TIMP1 protein is an important inhibitor of matrix metalloproteinases (MMPs), but it also potentiates erythroid activity and stimulates the growth of various types of cells, such as fibroblasts and hematolymphoid cells [28-30]. We detected higher concentrations of TIMP1 in the LBMp in ALL. A significantly higher concentration of TIMP1 had been previously found in the blood of both ALL and AML patients, as well as in lymphomas and normal activated B-cells [31]. Reanalyzing the available expression data showed that TIMP1 is less expressed in leukemic cells than in the non-malignant BM cells, but shows greater expression than in B-precursors.

Our study utilized a cytokine antibody array, a profile of soluble proteins in LBMp and the CBMp of children. In the cytokine antibody array, only pre-selected proteins are analyzed. The cytokine antibody array detected remarkable similarities between CPBp and CBMp. However, LBMp differed from CBMp in a number of proteins, of which two differed significantly after a Bonferroni correction. TIMP1 and LIF demonstrated a higher concentration in LBMp. Thus, changes in the BM microenvironment can be detected in the BMp proteome.

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REFERENCES

1. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet* 2008; 371: 1030.
2. Schultz KR, Pullen DJ, Sather HN, Shuster JJ, Devidas M, Borowitz MJ, Carroll AJ, Heerema NA, Rubnitz JE, Loh ML, Raetz EA, Winick NJ, Hunger SP, Carroll WL, Gaynon PS, Camitta BM. Risk- and response-based classification of childhood b-precursor acute lymphoblastic leukemia: A combined analysis of prognostic markers from the pediatric oncology group (pog) and children's cancer group (ccg). *Blood* 2007; 109: 926.
3. Zelent A, Greaves M, Enver T. Role of the tel-aml1 fusion gene in the molecular pathogenesis of childhood acute lymphoblastic leukaemia. *Oncogene* 2004; 23: 4275.
4. Ito C, Kumagai M, Manabe A, Coustan-Smith E, Raimondi SC, Behm FG, Murti KG, Rubnitz JE, Pui CH, Campana D. Hyperdiploid acute lymphoblastic leukemia with 51 to 65 chromosomes: A distinct biological entity with a marked propensity to undergo apoptosis. *Blood* 1999; 93: 315.
5. Nishii K, Katayama N, Miwa H, Shikami M, Masuya M, Shiku H, Kita K. Survival of human leukaemic b-cell precursors is supported by stromal cells and cytokines: Association with the expression of bcl-2 protein. *Brit J Haematol* 1999; 105: 701.
6. Nishigaki H, Ito C, Manabe A, Kumagai M, Coustan-Smith E, Yanishevski Y, Behm FG, Raimondi SC, Pui CH, Campana D. Prevalence and growth characteristics of malignant stem cells in b-lineage acute lymphoblastic leukemia. *Blood* 1997; 89: 3735.
7. Iversen PO, Wiig H. Tumor necrosis factor alpha and adiponectin in bone marrow interstitial fluid from patients with acute myeloid leukemia inhibit normal hematopoiesis. *Clin Cancer Res* 2005; 11: 6793.
8. Fronkova E, Mejstrikova E, Avigad S, Chik KW, Castillo L, Manor S, Reznickova L, Valova T, Zdrahalova K, Hrusak O, Jabali Y, Schrappe M, Conter V, Izraeli S, Li CK, Stark B, Stary J, Trka J. Minimal residual disease (mrd) analysis in the non-mrd-based all ic-bfm 2002 protocol for childhood all: Is it possible to avoid mrd testing? *Leukemia* 2008; 22: 989.
9. Ross ME, Zhou X, Song G, Shurtleff SA, Girtman K, Williams WK, Liu HC, Mahfouz R, Raimondi SC, Lenny N, Patel A, Downing JR. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. *Blood* 2003; 102: 2951.
10. van Zelm MC, van der Burg M, de Ridder D, Barendregt BH, de Haas EF, Reinders MJ, Lankester AC, Revesz T, Staal FJ, van Dongen JJ. Ig gene rearrangement steps are initiated in early human precursor b cell subsets and correlate with specific transcription factor expression. *J Immunol* 2005; 175: 5912.
11. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, Cooke MP, Walker JR, Hogenesch JB. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci U S A* 2004; 101: 6062.

12. Ashley DM, Bol SJ, Kannourakis G. Viable bone marrow stromal cells are required for the in vitro survival of b-cell precursor acute lymphoblastic leukemic cells. *Leukemia Res* 1995; 19: 113.

13. Kumagai M, Manabe A, Pui CH, Behm FG, Raimondi SC, Hancock ML, Mahmoud H, Crist WM, Campana D. Stroma-supported culture in childhood b-lineage acute lymphoblastic leukemia cells predicts treatment outcome. *J Clin Invest* 1996; 97: 755.

14. Gibson LF. Survival of b lineage leukemic cells: Signals from the bone marrow microenvironment. *Leuk Lymphoma* 2002; 43: 19.

15. Kelley KW, Arkins S, Minshall C, Liu Q, Dantzer R. Growth hormone, growth factors and hematopoiesis. *Horm Res* 1996; 45: 38.

16. Ayala F, Dewar R, Kieran M, Kalluri R. Contribution of bone microenvironment to leukemogenesis and leukemia progression. *Leukemia* 2009; 23: 2233.

17. Wang P, Ruan LH and Zhao XQ. [expression of angiogenin-2 and vegf in acute leukemia and its significance]. *Zhongguo shi yan xue ye xue za zhi /Zhongguo bing li sheng li xue hui = J Exp Hematol/Chin Ass Pathophysiol* 2010; 18: 11.

18. Brunner B, Gunsilius E, Schumacher P, Zwierzina H, Gastl G, Stauder R. Blood levels of angiogenin and vascular endothelial growth factor are elevated in myelodysplastic syndromes and in acute myeloid leukemia. *J Hematother Stem Cell Res* 2002; 11: 119.

19. Kittang AO, Hatfield K, Sand K, Reikvam H, Bruserud O. The chemokine network in acute myelogenous leukemia: Molecular mechanisms involved in leukemogenesis and therapeutic implications. *Curr Top Microbiol Immunol* 2010; 341: 149.

20. Smith JR, Braziel RM, Paoletti S, Lipp M, Uguccioni M, Rosenbaum JT. Expression of b-cell-attracting chemokine 1 (cxcl13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma. *Blood* 2003; 101: 815.

21. Wang JM, Deng X, Gong W, Su S. Chemokines and their role in tumor growth and metastasis. *J Immunol Methods* 1998; 220: 1.

22. Burgess M, Cheung C, Chambers L, Ravindranath K, Minhas G, Knop L, Mollee P, McMillan NA, Gill D. Ccl2 and cxcl2 enhance survival of primary chronic lymphocytic leukemia cells in vitro. *Leuk Lymphoma* 2012; 53: 1988.

23. Matsumura I, Ikeda H, Kanakura Y. The effects of thrombopoietin on the growth of acute myeloblastic leukemia cells. *Leuk Lymphoma* 1996; 23: 533.

24. Zhang XL, Komada Y, Chipeta J, Li QS, Inaba H, Azuma E, Yamamoto H, Sakurai M. Intracellular cytokine profile of t cells from children with acute lymphoblastic leukemia. *Cancer Immunol Immunother* 2000; 49: 165.

25. Peled A, Lee BC, Sternberg D, Toledo J, Aracil M, Zipori D. Interactions between leukemia cells and bone marrow stromal cells: Stroma-supported growth vs. Serum dependence and the roles of tgf-beta and m-csf. *Exp Hematol* 1996; 24: 728.

26. Metcalf D. The leukemia inhibitory factor (lif). *Int J Cell Cloning* 1991; 9: 95.

27. Metcalf D. The unsolved enigmas of leukemia inhibitory factor. *Stem Cells* 2003; 21: 5.

28. Murate T, Hayakawa T. Multiple functions of tissue inhibitors of metalloproteinases (timps): New aspects in hematopoiesis. *Platelets* 1999; 10: 5.

29. Hayakawa T, Yamashita K, Ohuchi E, Shinagawa A. Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (timp-2). *J Cell Sci* 1994; 107(Pt 9): 2373.

30. Stetler-Stevenson WG, Bersch N, Golde DW. Tissue inhibitor of metalloproteinase-2 (timp-2) has erythroid-potentiating activity. *FEBS letters* 1992; 296: 231.

31. Guedez L, Courtemanch L, Stetler-Stevenson M. Tissue inhibitor of metalloproteinase (timp)-1 induces differentiation and an anti-apoptotic phenotype in germinal center b cells. *Blood* 1998; 92: 1342.