ANTICANCER RESEARCH *25*: 2253-2258 (2005)

Cytotoxicity of Cortivazol in Childhood Acute Lymphoblastic Leukemia

JAN STYCZYNSKI, ANDRZEJ KURYLAK and MARIUSZ WYSOCKI

Department of Pediatric Hematology and Oncology, Ludwik Rydygier's Collegium Medicum of Bydgoszcz, Nicolaus Copernicus University of Torun, Poland

Abstract. *Background: Glucocorticoids are the most important group of drugs used in the treatment of childhood acute lymphoblastic leukemia (ALL), however, resistance to this group remains the main obstacle in curing the disease. One of the possibilities to circumvent glucocorticoid resistance is the use of new compounds, such as cortivazol (CVZ), which has two binding sites for the glucocorticoid receptor. Aim: Analysis of ex vivo sensitivity to cortivazol and other glucocorticoids in childhood acute lymphoblastic leukemia, as well as the relationship to anticancer therapy outcome. Patients and Methods: Leukemic samples from 60 children with ALL were tested by the MTT assay for glucocorticoid resistance. Cell cycle before and after ex vivo glucocorticoid treatment was analyzed by flow cytometry. Results: Although all tested glucocorticoids presented significant cross-resistance, CVZ showed high antileukemic activity. The equivalent activity of CVZ was 165-fold higher than prednisolone, 7.5-fold higher than dexamethasone and 2.8-fold higher than betamethasone. CVZ showed relatively better cytotoxicity than other glucocorticoids in prednisolone-poor-responders. CVZ, like other glucocorticoids, caused cell cycle arrest in the G1-phase, and increased the percentage of apoptotic cells to a greater extent than other glucocorticoids. The results of antileukemic therapy were strongly related to the ex vivo resistance to all tested glucocorticoids. Conclusion: Cortivazol has potent antileukemic activity in childhood ALL. Its activity is related to cell cycle arrest and induction of apoptosis.*

Glucocorticoids are the most important agents used in the therapy of acute lymphoblastic leukemia (ALL) in children (1, 2). Resistance to this group of drugs is regarded as a one of the strongest prognostic factors, both in *in vivo* (3, 4) and *in vitro* conditions (5, 6).

The mechanisms of antileukemic glucorticoid activity are related, in general, to activation of the glucocorticoid receptor (2, 7), heat shock proteins (2, 8), transcription factors (NF-Î B, AP1) (9), transactivation or transrepression of genes (10) and induction of apoptosis (10). Most investigations on the antileukemic activity of glucocorticoids have been carried out on prednisolone and dexamethasone. A number of clinical studies in childhood ALL were also based on methylprednisolone (11) and high dose of dexamethasone (12).

Cortivazol (CVZ, RU 3625, Figure 1) is a pyrazolosteroid with two binding sites for the glucocorticoid receptor (13, 14), which induces the nuclear translocation and transactivation function of the glucocorticoid receptor, but not of the mineralocorticoid receptor. CVZ interacts with the distinct portion of the ligand binding domain (LBD) and differentially modulates the ligand-dependent interaction between transcription intermediary factor 2 and the LBD when compared with cortisol, dexamethasone and aldosterone (15). CVZ showed significant activity in a glucocorticoid-receptor-deficient cell line (16) and resistance to extrusion by P-glycoprotein (17). It has been shown that CVZ was more active *in vitro* than dexamethasone (13) and betamethasone (18) in cell lines, thus suggesting its promise against childhood ALL (14). Juneja *et al.* (19) have shown that CVZ was successfully used in *ex vivo* purging before autologous stem cell transplantation.

Since most previous studies were performed on cell lines, the *in vitro* activity of CVZ in childhood *de novo* and relapsed ALL samples was analyzed here. The impact of the tested glucocorticoids on the cell cycle was also analyzed. We found that CVZ has potent antileukemic activity and might have good cytotoxic profile in prognostically unfavorable patients.

Patients and Methods

Patient samples. Fresh bone marrow samples from the day of first diagnosis of ALL or relapse were taken from 60 children aged 0.1-17.5 years. The patient characteristics are shown in Table I.

Correspondence to: Dr Jan Styczynski, Department of Pediatric Hematology and Oncology, Ludwik Rydygier's Collegium Medicum of Bydgoszcz, Nicolaus Copernicus University of Torun, ul. Curie-Sklodowskiej 9, 85-094 Bydgoszcz, Poland. Tel: +48 601 222 131, Fax: +48 52 585 4087, e-mail: jan.styczynski@wp.pl

Key Words: Glucocorticoids, cortivazol, prednisolone, dexamethasone, betamethasone, resistance.

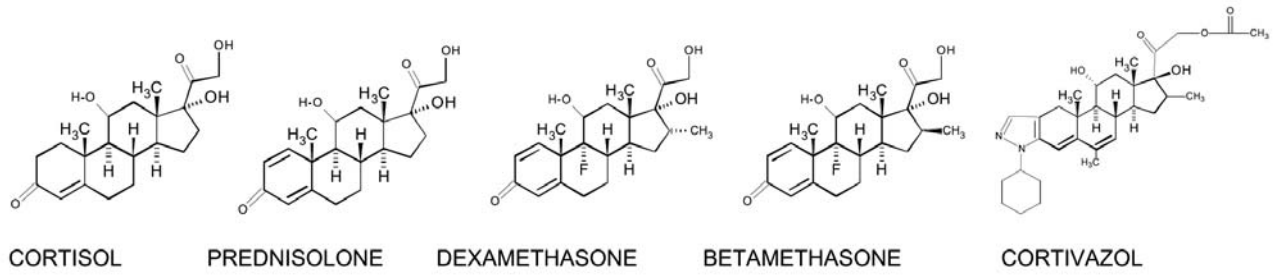

Figure 1. *Chemical structures of cortisol, prednisolone, dexamethasone, betamethasone and cortivazol.*

Table I. *Baseline characteristics.*

Characteristics	Patients (n=60)
Gender (male : female)	30 : 30
Age (median, range) in years	7.8 (0.01-17.2)
Initial : relapsed	46 : 14
FAB morphology	L1 – 36, L2 – 24
Phenotype	B-lineage – 51, T-lineage – 9
Cytogenetics (*)	favorable - 5, unfavorable - 8, other - 47
In vivo response to prednisolone one-week monotherapy (n=33)	good – 27, poor – 6, not done – 27

(*) Cytogenetics: good risk was defined as hyperdiploidy over 50 chromosomes, DNA index ≥1.16 and translocation t(12;21). Poor risk included: translocation t(9:22), bcr-abl rearrangement, translocation t(4:11), hipodiploidy below 45 chromosomes, DNA index ≤0.95. Standard risk was all others.

Leukemic cells were isolated on Ficoll gradient. Only samples which contained at least 90% lymphoblasts on initial diagnosis and at least 80% at relapse were included in the study. Morphology was based on the French-American-British criteria. According to immunophenotype, 51 children were classified as pre-B-lineage, and 9 as T-lineage. Cytogenetics was done by G-banding analysis. The DNA index was calculated by Multicycle software. Children at first diagnosis were treated by the New York II protocol (n=13), or by the ALL-BFM-90 protocol (n=33) (20, 21). For patients treated by BFM-based protocols, prednisolone *in vivo* response after 7 days of monotherapy (with one dose of intrathecal methotrexate) was determined: those who had less than 1000 blasts per μl in peripheral blood were diagnosed as prednisolone good responders (PGR); otherwise, as prednisolone poor responders (PPR). Relapsed patients were treated according to the ALL-BFM-REZ-96 protocol (n=14).

Chemicals. The following glucocorticoids and concentrations were tested: 0.0212–694 μM prednisolone (Fenicort, Jelfa, Jelenia Gora, Poland), 0.5 nM – 15.3 μM dexamethasone (Dexaven, Jelfa, Jelenia Gora, Poland), 0.5 nM – 15.3 μM betamethasone (Bedifos, Jelfa, Jelenia Gora, Poland), 0.094 nM – 9.43 μM cortivazol (Altim, Hoechst Marion Roussel/Aventis, Swindon, UK).

The MTT viability assay. Cytotoxicity was measured by a viability and cell proliferation assay by measuring the ability of the cells to cleave the soluble compound 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT; Serva, Heidelberg, Germany) into an insoluble salt. The ability of cells to cleave MTT is indicative of the degree of mitochondrial/cellular respiration within those cells. The conditions of the assay were similar to those described previously (22). Briefly, cells were incubated for 3 days with various concentrations of glucocorticoids in 96-well plates. Ten ml of MTT (5 μg/ml) were added to each well. The reaction was stopped after 4-h incubation by adding 100 μl of 0.04 N HCl in isopropanol, and the optical density (OD) was measured at 550 nm (reference wavelength 720 nm) with a Multiscan Bichromatic plate reader (Asys Hitech GmbH, Eugendorf, Austria) and DigiWin software (Asys Hitech). All experiments were performed in triplicate, and the data were confirmed to be reproducible. The cytotoxicity was expressed as LC_{50}, *i.e.*, lethal concentration for 50% of cells.

Cell cycle analysis. For DNA content analysis, cells were stained with hypotonic propidium iodine solution (20 μg/ml, DNA-Prep Kit, Lot number PN 6607055, Coulter, Miami, FL, USA) and 20000 events were analyzed with an Epics XL flow cytometer (Coulter) after 24-h incubation. This flow cytometer is equipped with an argon laser with an excitation wavelength of 488 nm. The cell cycle was calculated by Multicycle software (Phoenix Flow Systems, San Diego, CA, USA). The percentage of cells in the G1-, G2- and S-phases were expressed as mean ± s.d.

Statistics. Differences in drug sensitivity were compared by Mann-Whitney and Kruskal-Wallis tests. Correlations in resistance between groups were determined by Spearman's rho coefficient. The Wilcoxon matched-pairs signed ranks test was used to compare changes in cell cycle phases in treated and untreated cells. The probability of disease-free survival (pDFS) was calculated by the Kaplan-Meier method, and differences between curves were compared by log-rank test. Multivariate analysis was performed by the Cox regression model in a backward stepwise manner using the likelihood ratio test at a 0.05 level until all factors in the model were significant. All tests were two-sided with $p<0.05$ regarded as significant.

Results

Ex vivo sensitivity of lymphoblasts to glucocorticoids in childhood ALL samples. Lymphoblasts at relapse showed higher resistance to all glucocorticoids, with relative resistance

Table II. *Sensitivity to glucocorticoids in ALL initial and relapsed samples.*

	ALL *de novo*	Relapsed ALL	RR	*p*
PRN	29.7 (0.5->694)	390.5 (25-610.5)	13.1	0.015
DX	1.35 (0.0005->15.3)	>15.3 (0.07->15.3)	>11.3	0.003
BET	0.51 (0.0007->15.3)	11.7 (0.087->15.3)	22.9	0.001
CVZ	0.18 (0.0002->9.4)	3.11 (0.004->9.4)	17.3	0.003

The value of sensitivity is expressed as median and range of LC_{50} values, given in μM; RR - relative resistance, calculated as median LC_{50} value for relapsed samples divided by median LC_{50} value for *de novo* samples; *p*-value - calculated by Mann Whitney *U*-test.

Table III. *Correlation matrix of LC_{50} values for tested glucocorticoids.*

	PRN	DX	BET	CVZ
PRN		0.893	0.882	0.867
		<0.001	<0.001	<0.001
DX	0.893		0.915	0.893
	<0.001		<0.001	<0.001
BET	0.882	0.915		0.932
	<0.001	<0.001		<0.001
CVZ	0.867	0.893	0.932	
	<0.001	<0.001	<0.001	

Upper value - Spearman's rho; lower value - level of significance (*p*).

ranging at least 11 to 23-fold for each compound (Table II). Equivalent doses of glucocorticoids in *de novo* ALL were as follows: CVZ:BET:DX:PRN = 1 : 2.8 : 7.5 : 165.

Cells of prednisolone-poor-responders (PPR) were more resistant than cells of prednisolone-good-responders (PGR): median 5-fold to prednisolone, 3-fold to dexamethasone and betamethasone, and 1.6-fold to cortivazol. PPR had a higher WBC count (155 G/L *vs* 34.4 G/L, $p=0.035$) than PGR. Common-ALL samples have shown a trend towards better sensitivity to: prednisolone in comparison to pro-B samples (median 4.1-fold) and T-ALL (median 1.3-fold); to dexamethasone (1.5-fold and 1.3-fold, respectively, ns) and to betamethasone (3-fold and 1.8-fold, respectively, ns). No differences in *ex vivo* sensitivity to cortivazol between immunophenotype subgroups were observed.

A highly significant cross-resistance was observed between the tested glucocorticoids in patients with ALL. As the results obtained on cell lines, for each pair of drugs Spearman's rho value was over 0.8 and *p* value was less than 0.001 (Table III).

Analysis of glucocorticoid cell cycle arrest in patient samples. Both on day "0", and after 24 hours, cell cycle phases did not differ between untreated ALL cells. After 24 hours, the

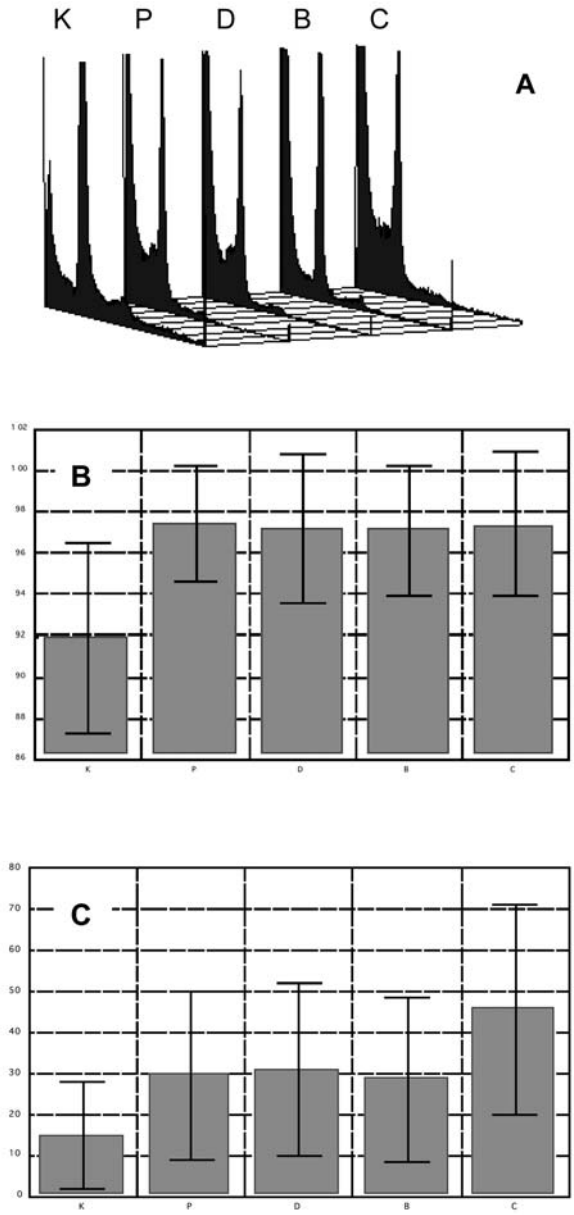

Figure 2. *Cell cycle analysis in childhood ALL samples. (A) Histograms of DNA content and cell cycle arrest after 72 hours of therapy ex vivo. (B) Percentage of cells in G1-phase. (C) Percentage of cells in sub-G1-phase. Each graph shows (from the left), effect in untreated cells (K) and treated with P-prednisolone, D-dexamethasone, B-betamethasone and C-cortivazol.*

percentage of cells undergoing spontaneous apoptosis, presented as sub-G1-phase, increased in all patients ($p<0.001$, Wilcoxon matched-pair test) (Figure 2). The apoptotic phase, expressed as sub-G1-phase, was significantly higher for CVZ than for all other tested glucocorticoids ($p<0.001$ in each case). Each of the tested glucocorticoids caused cell cycle arrest in G0/G1-phase in

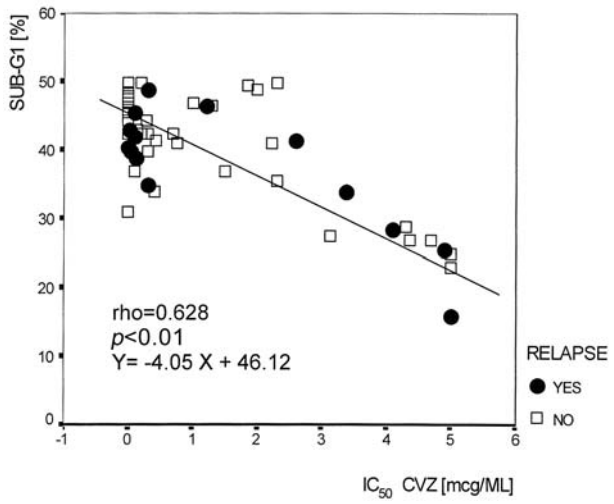

Figure 3. *Correlation between apoptotic phase sub-G1 obtained by flow cytometry and IC_{50} value of cytotoxicity measured by the MTT assay for cortivazol.*

ALL ($p \leq 0.001$, for each compound). Cytotoxicity correlated with the percentage of cells in sub-G1-phase (Figure 3).

Relationship between cellular drug resistance and antileukemic therapy. With cut-off values of 275 μM for prednisolone, 2 μM for cortivazol, 12 μM for dexamethasone and 5 μM for betamethasone, we divided all the tested samples as sensitive or resistant to each respective agent. The impact of *ex vivo* glucocorticoid cellular resistance on pDFS was significant for each of the four agents tested (Table IV). The prognostic significance of cellular resistance to each of the four glucocorticoids was further analyzed in the Cox model. By univariate analysis, sensitivity to each of the glucocorticoids had a significant value (except borderline value for dexamethasone), with *ex vivo* sensitivity to cortivazol being the strongest factor (Table IV). No factor reached significance by multivariate analysis.

Discussion

Glucocorticoids are the most important group of drugs used in the therapy of ALL in children, however, cellular resistance of lymphoblasts to these drugs remains one of the main obstacles in successful therapy. Cortivazol is a glucocorticoid with two binding sites for the glucocorticoid receptor, and is regarded as a potent drug with anti-inflammatory properties. In this study, we compared the antileukemic activity of cortivazol, prednisolone, dexamethasone and betamethasone, as well as their impact on apoptosis induction and cell cycle arrest. We have shown that cortivazol has potent antileukemic properties, and is approximately 165-fold more active than prednisolone and 7.5-fold than dexamethasone in childhood ALL. We have also shown that betamethasone was 2.6-fold more potent in antileukemic activity than dexamethasone. In a few cases, betamethasone was also active against cells resistant either to prednisolone or dexamethasone. All these comparisons were made for childhood initial ALL samples, which are relatively more sensitive to glucocorticoids, even if marked interindividual differences were observed.

Cross-resistance between the tested glucocorticoids was also observed in cell cycle analysis. The apoptotic phase, expressed by sub-G1-phase, was highest after CVZ *ex vivo* therapy in comparison to other glucocorticoids, while cell cycle arrest caused by CVZ was comparable to other compounds.

It is believed that resistance to glucocorticoids plays a role in the etiology and course of various diseases such as autoimmunologic disorders, AIDS, Nelson syndrome, sclerosis multiplex and leukemias (23). It has been shown that primary (hereditary) abnormalities in the glucocorticoid receptor gene make 6.6% of the normal population relatively 'hypersensitive' to glucocorticoids, while 2.3% are relatively 'resistant' (24). These abnormalities might explain the well-known phenomenon that some individuals develop severe adverse effects during therapy with a low dose of glucocorticosteroids, while others do not develop side-effects even during long-term

Table IV. *Estimated 2-year pDFS and univariate analysis in ALL patients with respect to sensitivity vs resistance to each of four glucocorticoids.*

Parameter	Estimated 2-year pDFS(*)	HR (95%CI)(#)
Ex vivo prednisolone sensitivity	0.77±0.09 *vs* 0.50±0.10, $p=0.0075$	1.86 (1.14-3.04), $p=0.0132$
Ex vivo cortivazol sensitivity	0.75±0.08 *vs* 0.45±0.12, $p=0.0034$	1.90 (1.19-3.03), $p=0.0069$
Ex vivo dexamethasone sensitivity	0.71±0.08 *vs* 0.53±0.12, $p=0.0486$	1.57 (0.98-2.50), $p=0.0594$
Ex vivo betamethasone sensitivity	0.76±0.10 *vs* 0.52±0.10, $p=0.0226$	1.71 (1.04-2.80), $p=0.0317$

(*) Analyzed by Kaplan-Meier method and log-rank test.
(#) HR-hazard risk, given with 95% confidence interval in univariate analysis.

therapy with a much higher dose (24). In childhood initial ALL, about 10% of children are regarded as prednisolone-poor-responders to initial therapy, and this rate is much higher at relapse (3, 25). Kaspers *et al.* have shown that glucocorticoid *in vitro* resistance in childhood leukemia, as measured by the MTT assay, is related to both clinical and cell biological features, and to the clinical outcome after multi-drug chemotherapy (26).

In vivo sensitivity to glucocorticoids is thought to be one of the most important prognostic factors in various therapeutic protocols for children with ALL. Several reports have shown that prednisolone resistance, expressed as absolute number of blasts in peripheral blood over 1 g/L in eight days of prednisolone monotherapy, is the strongest single adverse prognostic factor (3, 4, 27). It has been shown previously that therapy with high doses of prednisolone (1 g/day) did not improve the remission rate, but was related to serious adverse effects (28). In a study by Schwartz *et al.*, it was shown that response of ALL to glucocorticoid therapy increased with dose (12). Higher-dose corticosteroid treatment abrogated the effect of relative drug insensitivity and of low glucocorticoid receptor number on peripheral blasts. This was presented with doses of dexamethasone of 18 or 150 mg/m^2/day. In two recent series of *ex vivo* studies, it was shown that a combined resistance profile to prednisolone, vincristine and L-asparaginase was the strongest independent prognostic factor (29, 30).

Our study, based on clinical material, determined an *in vitro* cross-resistance pattern between prednisolone, dexamethasone, betamethasone, and cortivazol in samples of childhood acute leukemia, using the MTT assay. In summary, we have shown that cortivazol cannot circumvent *in vitro* resistance to prednisolone and dexamethasone in childhood ALL, however it shows high antileukemic activity and a favorable cytotoxicity profile in prednisolone-poor-responders.

Acknowledgements

The study was supported by grant BW-01-03 from the Medical University of Bydgoszcz, Poland. The authors thank Malgorzata Kubicka, Beata Rafinska and Beata Kolodziej for their technical support.

References

1 Pieters R, den Boer ML, Durian M, Janka G, Schmiegelow K, Kaspers GJ, van Wering ER and Veerman AJ: Relation between age, immunophenotype and *in vitro* drug resistance in 395 children with acute lymphoblastic leukemia – implications for treatment of infants. Leukemia *12*: 1344-1348, 1998.

2 Tissing WJ, Meijerink JP, den Boer ML and Pieters R: Molecular determinants of glucocorticoid sensitivity and resistance in acute lymphoblastic leukemia. Leukemia *17*: 17-25, 2003.

3 Schrappe M, Reiter A, Zimmermann M, Harbott J, Ludwig WD, Henze G, Gadner H, Odenwald E and Riehm H: Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Berlin-Frankfurt-Munster. Leukemia *14*: 2205-2222, 2000.

4 Riehm H, Reiter A, Schrappe M, Berthold F, Dopfer R, Gerein V, Ludwig R, Ritter J, Stollmann B and Henze G: Corticosteroid-dependent reduction of leukocyte count in blood as a prognostic factor in acute lymphoblastic leukemia in childhood (therapy study ALL-BFM 83). Klin Padiatr *199*: 151-160, 1987.

5 Kaspers GJ, Veerman AJ, Pieters R, Van Zantwijk CH, Smets LA, Van Wering ER and Van Der Does-Van Den Berg A: *In vitro* cellular drug resistance and prognosis in newly diagnosed childhood acute lymphoblastic leukemia. Blood *90*: 2723-2729, 1997.

6 Kaspers GJ, Pieters R, Van Zantwijk CH, Van Wering ER, Van Der Does-Van Den Berg A and Veerman AJ: Prednisolone resistance in childhood acute lymphoblastic leukemia: *vitro-vivo* correlations and cross-resistance to other drugs. Blood *92*: 259-266, 1998.

7 Lauten M, Cario G, Asgedom G, Welte K and Schrappe M: Protein expression of the glucocorticoid receptor in childhood acute lymphoblastic leukemia. Haematologica *88*: 1253-1258, 2003.

8 Lauten M, Beger C, Gerdes K, Asgedom G, Kardinal C, Welte K and Schrappe M: Expression of heat-shock protein 90 in glucocorticoid-sensitive and -resistant childhood acute lymphoblastic leukaemia. Leukemia *17*: 1551-1556, 2003.

9 Bailey S, Hall AG, Pearson AD, Reid MM and Redfern CP: Glucocorticoid resistance and the AP-1 transcription factor in leukaemia. Adv Exp Med Biol *457*: 615-619, 1999.

10 Kofler R: The molecular basis of glucocorticoid-induced apoptosis of lymphoblastic leukemia cells. Histochem Cell Biol *114*: 1-7, 2000.

11 Yetgin S, Tuncer MA, Cetin M, Gumruk F, Yenicesu I, Tunc B, Oner AF, Toksoy H, Koc A, Aslan D, Ozyurek E, Olcay L, Atahan L, Tuncbilek E and Gurgey A: Benefit of high-dose methylprednisolone in comparison with conventional-dose prednisolone during remission induction therapy in childhood acute lymphoblastic leukemia for long-term follow-up. Leukemia *17*: 328-333, 2003.

12 Schwartz CL, Thompson EB, Gelber RD, Young ML, Chilton D, Cohen HJ and Sallan SE: Improved response with higher corticosteroid dose in children with acute lymphoblastic leukemia. J Clin Oncol *19*: 1040-1046, 2001.

13 Schlechte JA, Simons SS, Lewis DA and Thompson EB: [3H]cortivazol: a unique high affinity ligand for the glucocorticoid receptor. Endocrinology *117*: 1355-1362, 1985.

14 Gaynon PS and Carrel AL: Glucocorticosteroid therapy in childhood acute lymphoblastic leukemia. Adv Exp Med Biol *457*: 593-605, 1999.

15 Yoshikawa N, Makino Y, Okamoto K, Morimoto C, Makino I and Tanaka H: Distinct interaction of cortivazol with the ligand binding domain confers glucocorticoid receptor specificity: cortivazol is a specific ligand for the glucocorticoid receptor. J Biol Chem *277*: 5529-5540, 2002.

16 Schlechte JA and Schmidt TJ: Use of [3H]cortivazol to characterize glucocorticoid receptors in a dexamethasone-resistant human leukemic cell line. J Clin Endocrinol Metab *64*: 441-446, 1987.

17 Kralli A, Bohen SP and Yamamoto KR: LEM1, an ATP-binding-cassette transporter, selectively modulates the biological potency of steroid hormones. Proc Natl Acad Sci USA *92*: 4701-4705, 1995.

18 Larsson S, Brattsand R and Linden M: Interleukin-2 and -4 induce resistance of granulocyte-macrophage colony-stimulating factor to corticosteroids. Eur J Pharmacol *334*: 265-271, 1997.

19 Juneja HS, Harvey WH, Brasher WK and Thompson EB: Successful *in vitro* purging of leukemic blasts from marrow by cortivazol, a pyrazolosteroid: a preclinical study for autologous transplantation in acute lymphoblastic leukemia and non-Hodgkin's lymphoma. Leukemia *9*: 1771-1778, 1995.

20 Steinherz PG, Gaynon PS, Breneman JC, Cherlow JM, Grossman NJ, Kersey JH, Johnstone HS, Sather HN, Trigg ME, Uckun FM and Bleyer WA: Treatment of patients with acute lymphoblastic leukemia with bulky extramedullary disease and T-cell phenotype or other poor prognostic features: randomized controlled trial from the Children's Cancer Group. Cancer *82*: 600-612, 1998.

21 Schrappe M, Reiter A, Ludwig WD, Harbott J, Zimmermann M, Hiddemann W, Niemeyer C, Henze G, Feldges A, Zintl F, Kornhuber B, Ritter J, Welte K, Gadner H and Riehm H: Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM Study Group. Blood *95*: 3310-3322, 2000.

22 Styczynski J, Pieters R, Huismans DR, Schuurhuis GJ, Wysocki M and Veerman AJ: *In vitro* drug resistance profiles of adult *versus* childhood acute lymphoblastic leukaemia. Br J Haematol *110*: 813-818, 2000.

23 DeRijk R and Sternberg EM: Corticosteroid resistance and disease. Ann Med *29*: 79-82, 1997.

24 Lamberts SW, Huizenga AT, de Lange P, de Jong FH and Koper J: Clinical aspects of glucocorticoid sensitivity. Steroids *61*: 157-160, 1996.

25 Klumper E, Pieters R, Veerman AJ, Huismans DR, Loonen AH, Hahlen K, Kaspers GJ, van Wering ER, Hartmann R and Henze G: *In vitro* cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukemia. Blood *86*: 3861-3868, 1995.

26 Kaspers GJ, Pieters R, Klumper E, De Waal FC and Veerman AJ: Glucocorticoid resistance in childhood leukemia. Leuk Lymphoma *13*: 187-201, 1994.

27 Dordelmann M, Reiter A, Borkhardt A, Ludwig WD, Gotz N, Viehmann S, Gadner H, Riehm H and Schrappe M: Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. Blood *94*: 1209-1217, 1999.

28 Ranney HM and Gellhorn A: The effect of massive prednisone and prednisolone therapy on acute leukemia and malignant lymphomas. Am J Med *22*: 405-413, 1957.

29 Styczynski J and Wysocki M: Is the *in vitro* drug resistance profile the strongest prognostic factor in childhood acute lymphoblastic leukemia? J Clin Oncol *22*: 963-964, 2004.

30 Den Boer ML, Harms DO, Pieters R, Kazemier KM, Gobel U, Korholz D, Graubner U, Haas RJ, Jorch N, Spaar HJ, Kaspers GJ, Kamps WA, Van der Does-Van den Berg A, Van Wering ER, Veerman AJ and Janka-Schaub GE: Patient stratification based on prednisolone-vincristine-asparaginase resistance profiles in children with acute lymphoblastic leukemia. J Clin Oncol *21*: 3262-3268, 2003.

Received December 20, 2004
Accepted April 5, 2005