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Report

In vitro activity of the novel cytotoxic agent CHS 828 in childhood acute leukemia

B-M Frost,[1] G Lönnerholm,[1] P Nygren,[2] R Larsson[3] and E Lindhagen[3]
Departments of [1]Women's and Children's Health, [2]Oncology, Radiology and Clinical Immunology, and [3]Medical Sciences, Section of Pharmacology, University hospital, 751 85 Uppsala, Sweden.

CHS 828, a pyridyl cyanoguanidine, is a new drug candidate now in phase I/II trials, that has shown promising anticancer activity in experimental tumor models and primary cultures of cancer cells from patients. In this study the fluorometric microculture cytotoxicity assay was used for evaluation of CHS 828 in primary cell cultures from children with acute leukemia. The activity of and interaction with the standard drugs, doxorubicin, melphalan, etoposide and cytosine arabinoside (Ara-C), were also assessed. Samples from 65 patients, 42 with acute lymphocytic leukemia (ALL) and 23 with acute myelocytic leukemia (AML) were tested with 72-h continuous drug exposure. There was 50% cell kill at very low CHS 828 concentrations; median IC_{50} was 0.01 μM in ALL and 0.03 in AML samples (NS) with large interindividual variability in both groups. ALL samples were significantly more sensitive than AML samples to melphalan, doxorubicin and etoposide, but not to Ara-C. In AML samples, combinations between CHS 828 and each of the four standard drugs resulted in significantly lower cell survival than either drug alone. This was also observed in ALL samples, except for Ara-C. Using the additive interaction model, CHS 828 showed a synergistic effect with melphalan in 67%, doxorubicin in 47%, etoposide in 38% and Ara-C in 14% of AML samples. In most ALL samples subadditive effects were found. Further exploration of CHS 828 in childhood leukemia is warranted, especially in AML. [© 2002 Lippincott Williams & Wilkins.]

Key words: Anticancer drug, childhood leukemia, CHS 828, cytotoxicity assay, drug development.

Introduction

The success rate in the treatment of childhood leukemia has improved continuously over the past decades, and today the disease-free survival is 70–80% in acute lymphocytic leukemia (ALL) and 40–60% in acute myelocytic leukemia (AML).[1–3] However, during the last 10 years new treatment protocols seem to have implied changes in doses of existing cytotoxic drugs rather than the introduction of new treatment components. Although the success rate has increased, there is still far to go before all children are cured. Thus, there is a need to find new compounds or new drug combinations that might further improve the outcome. Unfortunately, the new anticancer drugs are often analogs of those already available and probably have limited potential for improvement.

CHS 828 (*N*-(6-(4-chlorophenoxy)hexyl)-*N′*-cyano-*N″*-4-pyridylguanidine) is a pyridyl cyanoguanidine that has shown properties as a potential anticancer agent, both *in vitro* and *in vivo*.[4] CHS 828 neither displays structural similarities nor substantial cross-resistance with cytotoxic agents in clinical use.[4,5] Cytotoxic activity of CHS 828 *in vitro* was observed in the nanomolar and micromolar range, and the typical shape of the dose–response curves was a sharp decrease in cell survival followed by a plateau in both human tumor cell lines and primary cultures of tumor cells from patients.[4,5] In a study *in vitro*, tumor cells from hematological malignancies, mainly chronic lymphocytic leukemia (CLL), were found to be more sensitive than solid tumor cells.[5] The first clinical trial of CHS 828 was initiated in 1998[6] and the first phase II study is ongoing in adult patients with CLL.

Tumor cell lines are the most commonly used model system in cancer drug development, but the use of primary cultures of tumor cells from patients has gained increasing interest.[7,8] Previous studies indicate that primary cultures might more adequately

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Correspondence to B-M Frost, Department of Women's and Children's Health, University Hospital, 751 85 Uppsala, Sweden.
Tel: (+46) 18 6115883; Fax: (+46) 18 6115853;
E-mail: Britt-Marie.Frost@kbh.uu.se

reflect the disease-specific activity of new compounds[7] and may therefore be more relevant than cell lines when the effect of new drugs on different tumor types is studied.

The non-clonogenic fluorometric microculture cytotoxicity assay (FMCA) has been applied in drug development to study the effects *in vitro* of both single drugs and combinations of cytotoxic agents, in tumor lines as well as in primary cultures of human tumor cells.[7,9,11] In the present study this assay was used to investigate the activity of CHS 828 in primary tumor cells from patients with childhood acute leukemia. The *in vitro* activity of CHS 828 in ALL and AML samples was compared and interactions between CHS 828 and each of four standard cytotoxic agents, i.e. melphalan, doxorubicin, etoposide and cytosine arabinoside (Ara-C), were studied.

Patients and methods

Patients

Malignant cells were obtained from 65 children with leukemia, 33 girls and 32 boys, and were successfully analyzed between June 1996 and November 2000. The diagnosis was ALL in 42 patients and AML in 23 (Table 1). Of the ALL patients (median age was 4.8), 36 (86%) were newly diagnosed and the other six (14%) were in first relapse. Of the AML patients (median age was 8.4 years), 16 (70%) had newly diagnosed leukemia and seven (30%) were in first relapse. As the amount of cells varied and was a limiting factor, not all drugs and concentrations could be tested in all samples.

Cell preparation

Bone marrow aspirates or peripheral blood was collected in heparinized glass tubes. The samples generally reached the analyzing laboratory so that cell preparation could start within 24 h. Mononuclear cells were obtained by density gradient centrifugation with 1.077 g/ml Ficoll-Isopaque (Pharmacia-Upjohn, Uppsala, Sweden). Viability was determined by the Trypan blue exclusion test and the density gradient centrifugation generally yielded suspensions of greater than 85% leukemic cells. In most cases (65%), fresh samples were used for the *in vitro* assay, but 35% of the samples were cryopreserved in culture medium containing 10% dimethylsulfoxide (DMSO) and 90% fetal calf serum by initial freezing for 24 h at −70°C followed by storage in liquid nitrogen. Cryopreservation does not affect *in vitro* sensitivity to standard drugs.[12]

Table 1. Patient characteristics

	ALL	AML
No. patients	42	23
Sex (female/male)	19/23	14/9
Age (years) [median (range)]	4.8 (0.5–16.5)	8.4 (0.2–15.0)
Newly diagnosed/relapse	36/6	16/7

Reagents and drugs

CHS 828 was obtained from Leo Pharmaceutical (Copenhagen, Denmark). It was dissolved in 100% DMSO and was kept at −20°C as a stock solution of 10 mM, and further dilutions were made in 30% DMSO (1 mM CHS 828) and phosphate-buffered saline (PBS). CHS 828 was tested at six 10-fold dilutions starting from 1 μM. For comparison and in combination experiments, four standard drugs from commercial sources were chosen, representing different mechanistic classes: the alkylating agent melphalan, the antimetabolite Ara-C, the anthracycline doxorubicin and the topoisomerase II inhibitor etoposide. The concentrations of the four drugs tested (doxorubicin 0.9 μM, melphalan 8.2 μM, etoposide 8.5 μM and Ara-C 2.1 μM), were chosen to provide optimal separation of sensitive from resistant samples and are slightly higher than the clinically achievable concentrations.[13]

All drugs and combinations were prepared at 10 times the final concentration in triplicates and deposited into 96-well plates, and the plates were stored at −70°C. Fluorescein diacetate (FDA) (Sigma, St Louis, MO) was dissolved in DMSO 10 μg/ml (Sigma) and the solution was kept frozen (−20°C) as a stock solution protected from light.

FMCA

The semi-automated FMCA is based on measurement of fluorescence generated from hydrolysis of FDA to fluorescein by living cells. The principal steps of the assay procedure have been described previously.[7,10,14] A 180-μl cell suspension, containing 1×10^5 ALL cells or 0.5×10^5 AML cells, was seeded into the wells of microtiter plates prepared with drugs as described above. Six blank wells received only culture medium and six wells with cells but without drugs served as controls.

After 72-h incubation the plates were centrifuged and the medium was removed. After one wash in PBS, FDA was added. Subsequently the plates were incubated for 40 min and the fluorescence was then read in a scanning fluorometer, Fluoroscan II (Labsystems, Helsinki, Finland). Quality criteria for a successful assay included a signal in control cultures of greater than 5 times mean blank values, a mean coefficient of variation in control cultures of less than 30%, and a proportion of leukemic cells of greater than 70% prior to and at the end of incubation. The results are presented as survival index (SI), calculated as the proportion (%) of surviving cells compared to the survival in control cultures with blank values subtracted. Low numerical values indicate a high cytotoxic effect.

Data and statistical methods

Non-parametric methods were used throughout. The Spearman rank-test (two-tailed) was used to examine correlation. The Mann–Whitney *U*-test was applied to compare values from two independent groups and the Wilcoxon signed ranks test to compare groups of paired samples. The SPSS 10.0 software package was used for the calculations. The level of statistical significance was set at $p < 0.05$. IC_{50} was defined as the drug concentration resulting in 50% cell survival compared to the control, interpolated from the concentration–effect curves.

The additive model was used to study the interaction between two drugs.[9,15] This model predicts that the effect of a combination will be equal to the product of the effects of its constituents. For example, if a drug combination is composed of drugs producing SI values of 40 and 60%, respectively, the combination would be expected to result in an SI value of 24% (0.4×0.6). An observed combination effect that is larger than predicted by the additive model indicates synergism, whereas a smaller effect represents a subadditive effect.[15] A ratio between the observed SI value and the SI predicted by the additive model was calculated for all combinations. If the ratio exceeded 1.2, the interaction was classified as subadditive, and if it was below 0.8, as synergistic. Ratios between 0.8 and 1.2 were considered to indicate additive interactions, and this interval was set to take into account the intra-assay variability.[16] An SI value for the combination exceeding the SI of the most effective drug alone was classified as antagonism.

Results

The *in vitro* drug sensitivity for any drug did not differ between samples from male and female patients or between samples obtained at diagnosis and relapse, neither in ALL nor AML. Therefore, data for all samples were pooled for each diagnosis and, for correlation analysis, for all samples.

The cytotoxic activity of CHS 828 was dose dependent with a plateau at the highest concentrations (Figures 1 and 2). There was no significant difference in cell survival between ALL and AML samples at any CHS 828 concentration tested (Figure 1). The median IC_{50} for CHS 828 was 0.01 μM in ALL samples and 0.03 μM in AML samples (derived from Figure 1).

There was a large interindividual variation in CHS 828 sensitivity in both ALL and AML samples (Figure 2a and b). The leukemic cells of some patients were very sensitive, with substantially decreased SI values already at 0.0001 μM. In other samples, more than 50% of the cells survived at 10 μM CHS 828.

The median effects of the four standard drugs and CHS 828 are compared between ALL and AML samples in Figure 3. ALL were more sensitive than AML samples to doxorubicin ($p < 0.05$), melphalan ($p < 0.01$) and etoposide ($p < 0.01$), while there was no significant difference for Ara-C and CHS 828.

The largest difference was seen for etoposide, with median cell survivals of 34 and 63% in ALL and AML samples respectively.

Correlations between the effects of the five drugs are shown in Table 2. The cytotoxic effects of etoposide, melphalan and doxorubicin were strongly

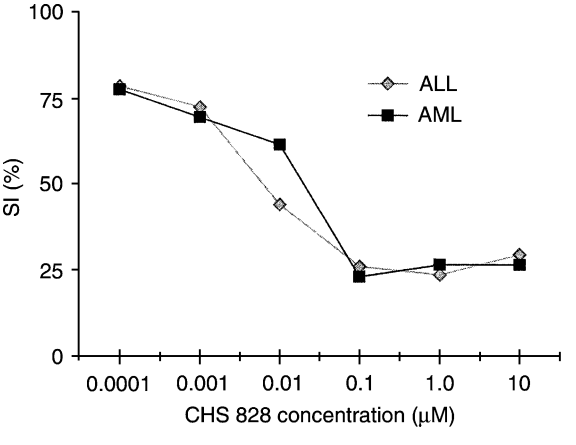

Figure 1. *In vitro* concentration–effect curves for CHS 828. Median values for samples from patients with ALL ($n = 28$) and AML ($n = 22$). The effect is expressed as survival index (SI%), defined as the proportion of surviving cells compared to the control.

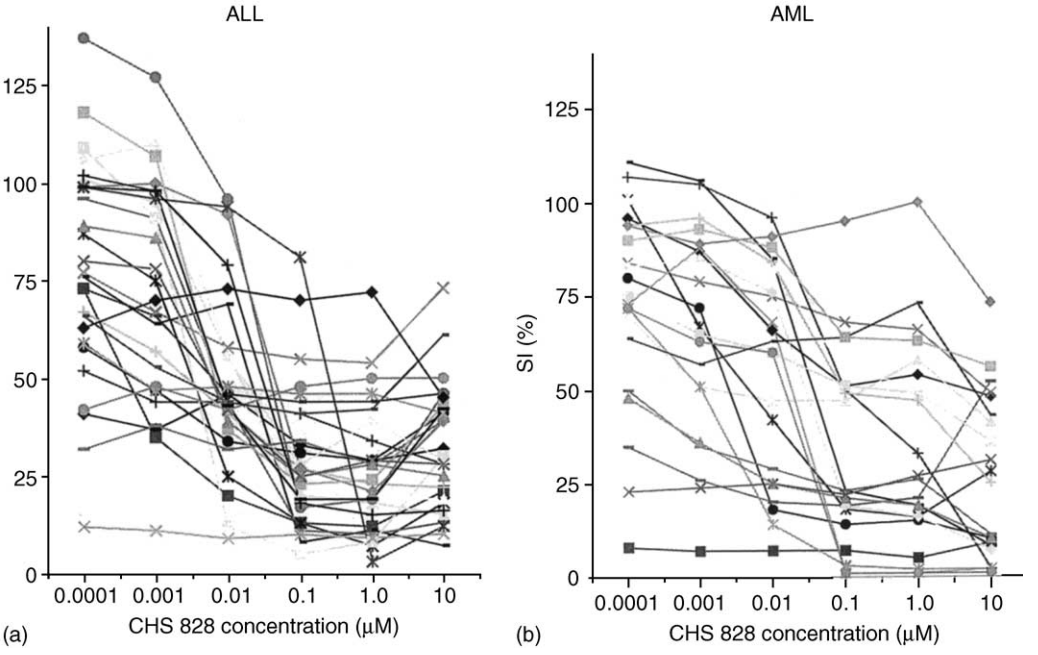

Figure 2. Individual concentration–effect curves for CHS 828 in samples from patients with ALL ($n=28$) (a) and AML ($n=22$) (b). SI = survival index.

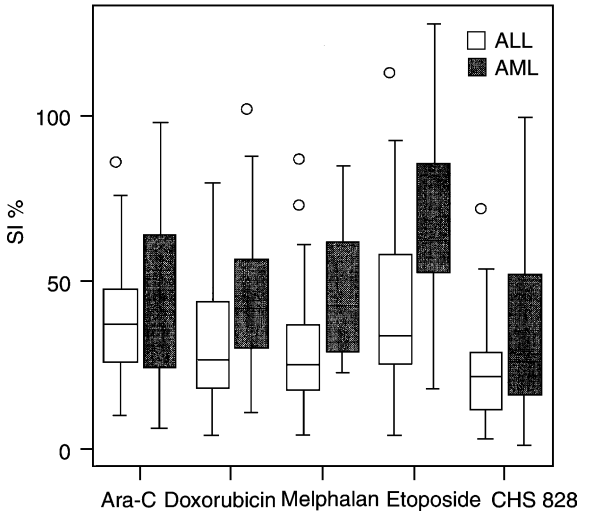

Figure 3. Drug effects of CHS 828 and the four clinically used cytotoxic drugs on cell survival, expressed as survival index (SI%) in ALL ($n=23$–42) and AML ($n=17$–23) samples. Concentrations used: CHS 828 1 μM, etoposide 8.5 μM, Ara-C 2.1 μM, melphalan 8.2 μM and doxorubicin 0.92 μM. The box-and-whisker plot shows median, first and third quartiles; whiskers extend to the highest and lowest value, excluding outliers, which are denoted by circles.

correlated ($\rho=0.7$–0.8), whereas the effects of CHS 828 and Ara-C showed lower correlation to those of the other drugs ($\rho=0.1$–0.5). CHS 828 and Ara-C were significantly correlated ($\rho=0.6$).

Figure 4 illustrates the cytotoxic effects of CHS 828 (1 μM), and the four standard drugs separately and in combination in ALL and AML samples. In the AML samples, all combinations resulted in significantly lower cell survival than either drug alone. In ALL samples, the combination was significantly more effective than either drug alone for all drugs except Ara-C, for which the cell survival after exposure to CHS 828 + Ara-C showed no difference from that after exposure to CHS 828 alone (Figure 4a).

According to the additive interaction model, CHS 828 was found to have a synergistic effect with melphalan in 67%, with doxorubicin in 47%, with etoposide in 38% and with Ara-C in 14% of the AML samples (Table 3). In ALL samples the proportions of synergistic interactions ranged only 0–18%. In some samples additive interactions were found, but most of the interactions were considered subadditive. None of the combinations produced antagonistic effects in any patient sample.

Discussion

Children with AML have a worse prognosis than those with ALL. Event-free survival for AML is below 55%, whereas the cure rate for children with ALL is near 80%. The complete remission rate differs also, with 5–10% induction failures due to refractory disease and toxicity in AML, compared to 1–2% in

Table 2. Correlation between the *in vitro* cytotoxic effect of CHS 828 and that of four standard cytotoxic drugs in pooled ALL and AML samples

	Correlation coefficient (ρ)				
	CHS 828	Doxorubicin	Melphalan	Etoposide	Ara-C
CHS 828	–	0.2	0.1	0.2	0.6**
Doxorubicin	0.2	–	0.8**	0.7**	0.5**
Melphalan	0.1	0.8**	–	0.7**	0.2
Etoposide	0.2	0.7**	0.7**	–	0.3*
Ara-C	0.6**	0.5**	0.2	0.3*	–

Spearman rank-test was used to correlate SI values in all patients ($n=40$–65). **$p<0.01$; *$p<0.05$.

ALL.[17] The differences in treatment outcome may reflect differences in cellular drug resistance, and in a recent publication Zwaan *et al.* reported that childhood AML cells were relatively more resistant to a large number of drugs than ALL cells, notably to 'typical' ALL drugs such as the glucocorticoids, vincristine and L-asparginase, but also anthracycline, etoposide and some alkylating agents.[18] Because AML was found to be resistant to many different classes of drugs, this was thought to reflect a defect in the final common pathway of cytotoxicity, possibly in the drug-induced apoptotic pathways. A notable exception was Ara-C, for which equal sensitivity was found in the AML and ALL cells. This corresponds well with the important role of Ara-C in childhood AML.[18]

The present results, demonstrating that AML samples were significantly less sensitive than ALL samples to melphalan, doxorubicin and etoposide, but with equal sensitivity to Ara-C, agree well with those of Zwaan *et al.*[18] These results add to the cumulated experience that drug response testing *in vitro* by non-clonogenic cytotoxicity assays yields reproducible and clinically relevant drug sensitivity data.

The CHS 828 concentration–response curves in both ALL and AML were typically plateau shaped, a finding conforming with previous reports from studies in human tumor cell lines and in samples from patients both with solid tumors and with CLL.[4,5] This indicates that the cell-killing mechanisms are similar irrespective of the types of tumor cells. CHS 828 appears to induce both active and delayed cell death with features not compatible with classical apoptosis. However, in spite of extensive research the mechanism of action of CHS 828 is still not fully elucidated.[19–24] The effect of CHS 828 did not correlate to the effect of melphalan, doxorubicin or etoposide, which may indicate that CHS 828 has a different mechanism of action. The correlation between CHS 828 and Ara-C was of moderate degree ($\rho=0.6$) and this correlation does not strongly support an antimetabolite-like action of CHS 828, since drugs sharing common mechanism of action generally show correlation coefficients above 0.8.[25]

From a clinical point of view it was interesting to note that the IC_{50}s of CHS 828 in both ALL and AML samples were of the same magnitude as the plasma levels of the drug achievable in the clinic.[6] The inter-individual variation in sensitivity to CHS 828 was large in both ALL and AML, with responses occurring at very low concentrations in some samples. This suggests that selected patients with CHS 828-sensitive cells might be efficiently treatable with potentially non-toxic CHS 828 doses. If these data apply to the clinical situation, the possibility of individualized, tailored therapy should be further explored.

High frequencies of additive and synergistic interactions between CHS 828 and standard cytotoxic agents such as etoposide and mitomycin/amiloride have previously been found in human tumor cell lines (unpublished data). In the present study high frequencies of synergistic interactions were observed between CHS 828 and standard drugs in AML samples, whereas the frequencies were very low in the ALL samples. However, it should be noted that the high degree of cell kill by single drugs alone in ALL solely might make the detection of synergistic interactions difficult, on account of the insufficient sensitivity of the method at very low SI values.[15] A ratio below 0.8 between the observed SI and the SI value predicted by the additive model was considered to indicate a synergistic effect. Recalculation of the frequencies of synergistic interactions using a ratio below 1.0 instead of below 0.8 did not produce a major change (not shown), thus indicating a true difference between ALL and AML.

The alkylating agent melphalan showed the highest fraction of synergistic interactions with CHS 828, followed by doxorubicin. Melphalan is not at present

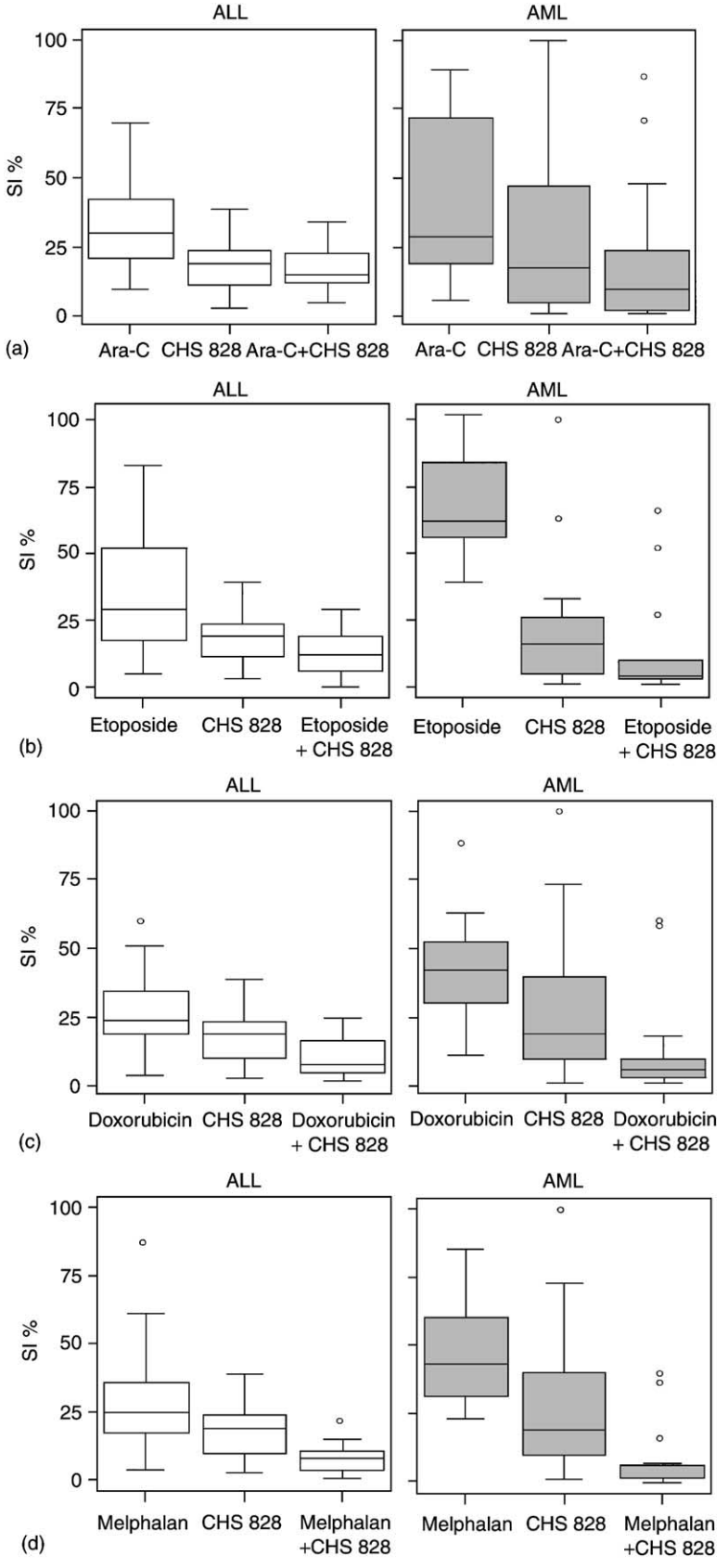
ALL
AML
SI %
100
75
50
25
0
Ara-C
CHS 828
Ara-C+CHS 828
(a)
Etoposide
Etoposide + CHS 828
(b)
Doxorubicin
Doxorubicin + CHS 828
(c)
Melphalan
Melphalan +CHS 828
(d)

Table 3. Interactions between CHS 828 1 μM and the four standard cytotoxic drugs

Drug combination with CHS 828	*n*	Synergistic interactions	Additive interactions	Subadditive interactions
Etoposide				
ALL	15	1 (7%)	2 (13%)	12 (80%)
AML	13	5 (38%)	4 (31%)	4 (31%)
Ara-C				
ALL	17	0 (0%)	2 (12%)	15 (88%)
AML	14	2 (14%)	4 (29%)	8 (57%)
Melphalan				
ALL	17	3 (18%)	4 (23%)	10 (59%)
AML	15	10 (67%)	0 (0%)	5 (33%)
Doxorubicin				
ALL	19	0 (0%)	2 (21%)	17 (89%)
AML	15	7 (47%)	2 (13%)	6 (40%)

Analysis of interaction was done according to the additive model. No samples showed antagonism (see Methods). Concentrations: CHS 828 1 μM, etoposide 8.5 μM, Ara-C 2.1 μM, melphalan 8.2 μM and doxorubicin 0.92 μM.

a first-line treatment in childhood leukemia, but there are studies suggesting that high-dose melphalan is a valuable conditioning agent in AML prior to bone marrow transplantation.[26] It has also been shown to be useful in the treatment of ALL after relapse.[27]

To summarize, the FMCA seems to adequately reflect the clinically well-known difference in treatment outcome between childhood AML and ALL. The new agent CHS 828 was equally active *in vitro* in these two forms of leukemia, with IC_{50} values achievable *in vivo*. CHS 828 also produced high frequencies of synergistic interactions with standard drugs in AML. A further exploration of CHS 828 in childhood leukemia seems warranted especially in AML both as single drug and combined with other drugs.

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References

1. Lie SO, Jonmundsson G, Mellander L, *et al.* A population-based study of 272 children with acute myeloid leukaemia treated on two consecutive protocols with different intensity: best outcome in girls infants and children with Down's syndrome. Nordic Society of Paediatric Haematology and Oncology (NOPHO). *Br J Haematol* 1996; **94**: 82–8.
2. Pui CH, Evans WE. Acute lymphoblastic leukemia. *N Engl J Med* 1998; **339**: 605–15.
3. Gustafsson G, Kreuger A, Clausen N, *et al.* Intensified treatment of acute childhood lymphoblastic leukaemia has improved prognosis especially in non-high-risk patients: the Nordic experience of 2648 patients diagnosed between 1981 and 1996. Nordic Society of Paediatric Haematology and Oncology (NOPHO). *Acta Paediatr* 1998; **87**: 1151–61.
4. Hjarnaa PJ, Jonsson E, Latini S, *et al.* CHS 828 a novel pyridyl cyanoguanidine with potent antitumor activity *in vitro* and *in vivo*. *Cancer Res* 1999; **59**: 5751–7.
5. Aleskog A, Bashir-Hassan S, Hovstadius P, *et al.* Activity of CHS 828 in primary cultures of human hematological and solid tumors *in vitro*. *Anti-Cancer Drugs* 2001; **10**: 821–7.
6. Ahlgren JHP, Hansen K, *et al.* A phase I safety study of CHS 828 in patients with solid tumor malignancy. *Proc Am Ass Cancer Res* 2001; **92**: abstr 2920.
7. Fridborg H, Jonsson E, Nygren P, *et al.* Relationship between diagnosis-specific activity of cytotoxic drugs in fresh human tumour cells *ex vivo* and in the clinic. *Eur J Cancer* 1999; **35**: 424–32.
8. Bosanquet AG, Buriton AR, Bell PB, *et al. Ex vivo* cytotoxic drug evaluation by DiSC assay to expedite identification of clinical targets: results with 8-chloro-cAMP. *Br J Cancer* 1997; **76**: 511–8.
9. Jonsson E, Fridborg H, Nygren P, *et al.* Synergistic interactions of combinations of topotecan with standard drugs in primary cultures of human tumor

Figure 4. Effects of CHS 828, and (a) Ara-C, (b) etoposide, (c) doxorubicin and (d) melphalan as single agents and in combination in ALL (n=13–15) and AML (n=15–19) samples. Drug concentrations as in Figure 3. The box-and-whisker plot shows median, first and third quartiles; whiskers extend to the highest and lowest value, excluding outliers, which are denoted by circles. The SI values of the drug combinations were compared with these of CHS 828 alone. (For ALL; doxorubicin p=0.001, melphalan p=0.001, etoposide p=0.02, Ara-C=0.15. For AML; doxorubicin p=0.005, melphalan p=0.002, etoposide p=0.03 and Ara-C p=0.02.)

cells from patients. *Eur J Clin Pharmacol* 1998; **54**: 509–14.
10. Nygren P, Fridborg H, Csoka K, *et al.* Detection of tumor-specific cytotoxic drug activity *in vitro* using the fluorometric microculture cytotoxicity assay and primary cultures of tumor cells from patients. *Int J Cancer* 1994; **56**: 715–20.
11. Larsson R, Fridborg H, Kristensen J, *et al. In vitro* testing of chemotherapeutic drug combinations in acute myelocytic leukaemia using the fluorometric microculture cytotoxicity assay (FMCA). *Br J Cancer* 1993; **67**: 969–74.
12. Nygren P, Kristensen J, Jonsson B, *et al.* Feasibility of the fluorometric microculture cytotoxicity assay (FMCA) for cytotoxic drug sensitivity testing of tumor cells from patients with acute lymphoblastic leukemia. *Leukemia* 1992; **6**: 1121–8.
13. Larsson R, Kristensen J, Sandberg C, *et al.* Laboratory determination of chemotherapeutic drug resistance in tumor cells from patients with leukemia using a fluorometric microculture cytotoxicity assay (FMCA). *Int J Cancer* 1992; **50**: 177–85.
14. Larsson R, Nygren P. Pharmacological modification of multi-drug resistance (MDR) *in vitro* detected by a novel fluorometric microculture cytotoxicity assay. Reversal of resistance and selective cytotoxic actions of cyclosporin A and verapamil on MDR leukemia T-cells. *Int J Cancer* 1990; **46**: 67–72.
15. Valeriote F, Lin H. Synergistic interaction of anticancer agents: a cellular perspective. *Cancer Chemother Rep* 1975; **59**: 895–900.
16. Lepri E, Barzi A, Menconi E, *et al. In vitro* synergistic activity of PDN-IFN alpha and NM + IFN alpha combinations on fresh bone-marrow samples from multiple myeloma patients. *Hematol Oncol* 1991; **9**: 79–86.
17. Lie S, GJ, LM, *et al.* A population-based study of 272 children with acute myeloid leukaemia. *Br J Haematol* 1996; **94**: 82–8.
18. Zwaan CM, Kaspers GJ, Pieters R, *et al.* Cellular drug resistance profiles in childhood acute myeloid leukemia: differences between FAB types and comparison with acute lymphoblastic leukemia. *Blood* 2000; **96**: 2879–86.
19. Martinsson P, de la Torre M, Binderup L, *et al.* Cell death with atypical features induced by the novel antitumoral drug CHS 828 in human U-937 GTB cells. *Eur J Pharmacol* 2001; **417**: 181–7.
20. Martinsson P, Liminga G, Dhar S, *et al.* Temporal effects of the novel antitumour pyridyl cyanoguanidine (CHS 828) on human lymphoma cells. *Eur J Cancer* 2001; **37**: 260–7.
21. Martinsson P, Liminga G, Nygren P, *et al.* Characteristics of etoposide-induced apoptotic cell death in the U-937 human lymphoma cell line. *Anti-Cancer Drugs* 2001; **12**: 699–705.
22. Ekelund S, Liminga G, Bjorkling F, *et al.* Early stimulation of acidification rate by novel cytotoxic pyridyl cyanoguanidines in human tumor cells: comparison with *m*-iodobenzylguanidine. *Biochem Pharmacol* 2000; **60**: 839–49.
23. Ekelund S, Nygren P, Larsson R, *et al.* Guanidino-containing drugs in cancer chemotherapy: biochemical and clinical pharmacology. *Biochem Pharmacol* 2001; **61**: 1183–93.
24. Hansen CM, Hansen D, Holm PK, *et al.* Cyanoguanidine CHS 828 induces programmed cell death with apoptotic features in human breast cancer cells *in vitro*. *Anticancer Res* 2000; **20**(6B): 4211–20.
25. Jonsson B, GL, KC, *et al.* Cytotoxic activity of calcein acetoxymethyl ester (Calcein/AM) on primary. *Eur J Cancer* 1996; **32A**: 883–7.
26. Tiedemann K, Waters KD, Tauro GP, *et al.* Results of intensive therapy in childhood acute myeloid leukemia incorporating high-dose melphalan and autologous bone marrow transplantation in first complete remission. *Blood* 1993; **82**: 3730–8.
27. Schroeder H, Pinkerton CR, Powles RL, *et al.* High dose melphalan and total body irradiation with autologous marrow rescue in childhood acute lymphoblastic leukaemia after relapse. *Bone Marrow Transplant* 1991; **7**: 11–5.

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