ANTICANCER RESEARCH *29*: 1643-1650 (2009)

Comparison of Clofarabine Activity in Childhood and Adult Acute Leukemia: Individual Tumor Response Study

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Abstract. *Background: Clofarabine is a second-generation nucleoside analogue. The aim of the study was the analysis of ex vivo activity of clofarabine and 14 other anticancer drugs in pediatric and adult acute lymphoblastic (ALL) and myeloid (AML) leukemia. Patients and Methods: The ex vivo drug resistance profile was analyzed in 282 patients, including 201 children with ALL de novo, 24 children with relapsed ALL, 25 children with AML de novo and 32 adults with AML. Cellular ex vivo drug resistance was tested by means of the MTT assay. Results: Clofarabine had comparable ex vivo activity against lymphoblasts and myeloblasts, both on initial diagnosis and at relapse, both in children and in adults. Its activity in acute myeloid leukemia was independent of patient age. No significant differences in drug resistance to clofarabine between pediatric age-based subgroups of ALL were detected, while it was observed for most of other drugs. An activity of clofarabine in relapsed pediatric ALL patients was as good as in newly-diagnosed ones. Conclusion: In comparison to childhood acute lymphoblastic leukemia, lack of differences in ex vivo activity gives rationale for use of clofarabine in refractory and relapsed pediatric and adult patients with acute myeloid leukemia.*

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Key Words: Acute lymphoblastic leukemia, acute myeloid leukemia, children, adults, drug resistance, individual tumor response testing.

The treatment of pediatric acute leukemias has improved considerably in recent decades, with complete response rates approaching approximately 90% in some subgroups. However, about 20-25% children with ALL and 40-50% with AML still relapse (1-3). The results in adults with acute leukemia are worse, and hematopoietic stem cell transplantation (HSCT) has not improved the outcome significantly (4-6). Treatment of relapse after HSCT is still very poor (7). The discovery and development of clofarabine, a second-generation nucleoside analogue, provides an important chance for relapsed patients (8). This drug received accelerated approval from the US FDA at the end of 2004 for the treatment of pediatric patients 1-21 years old with relapsed or refractory acute lymphoblastic leukemia

after at least two prior regimens. It is the first such drug to be approved for pediatric leukemia in more than a decade, and the first to receive approval for pediatric use before adult use (9, 10). Clofarabine has demonstrated significant activity in children (11-15) and adults (16-18) with refractory lymphoid and myeloid leukemia in early clinical trials. Originally developed to capture the best qualities of cladribine and fludarabine, clofarabine contains halogenated carbons, rendering it resistant to inactivating enzymes and maintaining its stability in acidic environments. Like other adenosine nucleosides, clofarabine acts by inhibiting ribonucleotide reductase and DNA polymerase, thereby depleting the amount of intracellular deoxynucleoside triphosphates available for DNA replication, resulting in premature DNA chain termination and inhibition of DNA repair. Clofarabine has also been shown to induce apoptosis in transformed cell lines, indicating that this drug results in cell death in both cycling and non-cycling cells (19).

The aim of the study was the analysis of *in vitro* activity of clofarabine and 14 other anticancer drugs in pediatric and adult acute leukemia.

Patients and Methods

Patients and leukemic cells. A total of 282 patients were tested for the *ex vivo* drug resistance profile. The group included 201 children with ALL *de novo* (median age 6 years, range 1-18 years), 24 children with relapsed ALL (median age 9 years, range 4-18 years), 25 children with AML *de novo* (median age 13 years, range 1-18 years) and 32 adults with AML (both *de novo* and relapsed) (median age 39 years, range 18-52 years). Adult AML patients were partially included in a previous report (20), while it was not the case for children. Since *ex vivo* drug resistance in adult AML *de novo* and at relapse are comparable (20, 21), all adult AML patients were pooled together in one group for further analysis. Patients aged <1 year or with ALL with L3 (FAB) morphology were not included into the study.

Samples of bone marrow (BM) were collected in a heparinised tube (or 15-20 U heparine was used for 1 mL BM). Before testing, the samples were diluted 1:1 or more with RPMI-1640 (Sigma, St Louis, MO, USA). In case of the presence of small clots, the sample of BM was first filtered through a cell strainer (70 μm nylonfilter; Falcon, Franklin Lakes, New Jersey, USA) using RPMI-1640 to rinse off the strainer. Leukemic cells were separated on Ficoll gradient at 540g for 20 minutes at room temperature. After centrifugation, cells were washed twice with RPMI-1640. The viability and recovery of the cells was tested by trypan blue exclusion assay. Cell morphology and percentage of blasts was analysed on cytospine slides stained with May-Grunwald-Giemsa (MGG) method. Only samples with at least 90% lymphoblasts and 70% myeloblasts, both in the beginning and at the end of the assay, were included into the study. The study was approved by local Ethics Committee and informed consent was obtained from all patients.

Cells. Cells were maintained in RPMI 1640 medium, supplemented with 2 mM glutamine and 20% FBS. Culture medium was supplemented with 100 U/mL penicillin (Polfa Tarchomin, Poland), 100 μg/mL streptomycin (Polfa Tarchomin, Poland), 200 μg/mL gentamycin (Krka, Slovenia) and 0.125 μg/mL amphotericine B. The culture was carried out in conditions of 5% CO_2, 37°C and 95% humidity.

Drugs. The following 15 drugs were used: prednisolone (Jelfa, Jelenia Gora, Poland; concentrations tested: 0.0076-250 μg/mL), vincristine (Gedeon Richter, Budapest, Hungary; 0.019-20 μg/mL), L-asparaginase (Medac, Hamburg, Germany; 0.0032-10 IU/mL), daunorubicin (Rhone-Poulenc Rorer, France; 0.0019-2 μg/mL), doxorubicin (Pharmacia Italia S.p.A., Milan, Italy; 0.031-40 μg/mL), cytarabine (Upjohn, Puurs, Belgium; 0.24-250 μg/mL), cladribine (Bioton, Warsaw, Poland; 0.0004-40 μg/mL), etoposide (Bristol-Myers Squibb, Sermoneta, Italy; 0.048-50 μg/mL), 4-HOO-cyclophosphamide (Asta Medica, Hamburg, Germany; 0.096-100 μg/mL), 4-HOO-ifosfamide (Asta Medica; 0.096-100 μg/mL), fludarabine phosphate (Schering AG, Berlin, Germany; 0.019-20 μg/mL), idarubicin (Pharmacia; 0.0019-2 μg/mL), mitoxantrone (Jelfa; 0.001-1 μg/mL), 6-thioguanine (Sigma; nr A4882, 1.56-50 μg/mL) and clofarabine (Evoltra,

Table I. *Drug resistance in pediatric and adult AML.*

Drug	Pediatric AML (n=25)	Adult AML (n=32)	RR	*p*-value
Prednisolone	226.96 (n=15) 13.92->250	181.45 (n=20) 21.48->250	0.8	0.259
Vincristine	3.71 (n=14) 0.41->20	5.315 (n=21) 0.63-11.89	1.4	0.575
Idarubicin	0.155 (n=21) 0.06->2	0.13 (n=24) 0.02->2	0.8	0.256
Daunorubicin	0.31 (n=14) 0.09->2	0.285 (n=21) 0.01->2	0.9	0.098
Doxorubicin	0.975 (n=8) 0.4->8	1.1 (n=18) 0.39->8	1.1	0.911
Mitoxantrone	0.1285 (n=23) 0.0086->1	0.0709 (n=22) 0.021->1	0.6	0.603
Etoposide	5.15 (n=14) 1.45->50	8.748 (n=21) 0.86->50	1.7	0.145
L-asparaginase	1.19 (n=14) 0.04->10	1.47 (n=20) 0.04->10	1.2	0.324
6-Thioguanine	4.23 (n=8) 1.56->50	8.85 (n=16) 2.52->50	2.1	0.176
4-HOO-cyclopho-sphamide	1.88 (n=8) 0.35-2.27	2.8 (n=19) 0.17-4.63	1.5	0.119
4-HOO-ifosfamide	13.505 (n=4) 10.62-16.39	16.395 (n=16) 2.91-33.82	1.2	0.704
Cytarabine	0.27 (n=23) 0.03->10	1.15 (n=25) 0.05->10	4.3	0.198
Fludarabine	0.3125 (n=23) 0.1477->20	0.9509 (n=24) 0.0984->20	3.0	0.536
Cladribine	0.404 (n=23) 0.0022->40	1.237 (n=24) 0.0006->40	3.1	0.753
Clofarabine	0.055 (n=17) 0.01->12.5	0.06 (n=24) 0.01->12.5	1.1	0.954

Concentration of L-asparaginase is given in IU/mL, clofarabine in μM, and all other drugs in μg/mL. Drug resistance is given as median and range of IC_{50}; n – number of patients, RR – relative resistance.

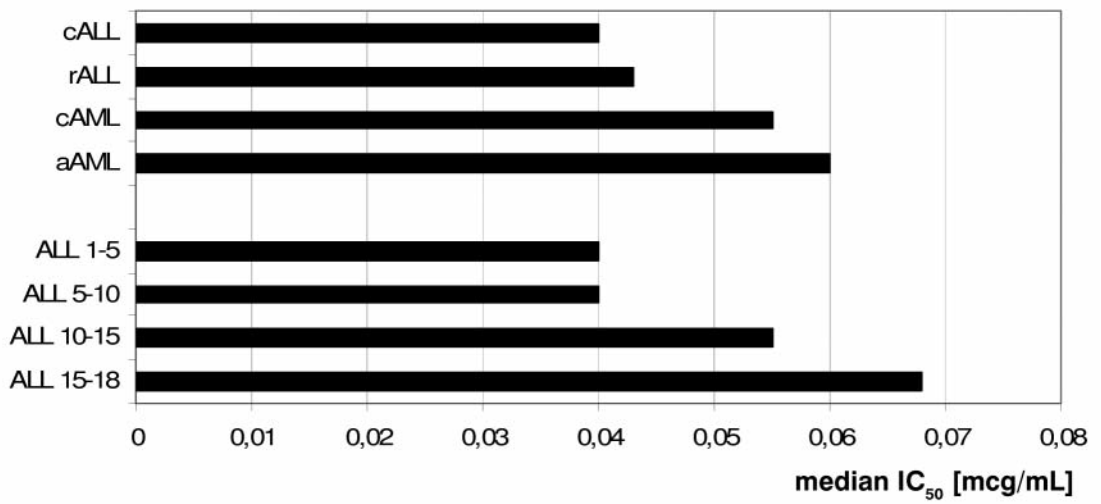

Figure 1. *Comparative values of median cytotoxicity of clofarabine with respect to diagnosis and age. cALL – childhood ALL de novo, rALL – relapsed ALL, cAML – childhood AML de novo, aAML – adult AML; age groups: 1-5, 5-10, 10-15, 15-18 years.*

Bioenvision, Edinburgh, UK; 0.0122-12.5 μM). Before the assay was done, most drug stock solutions were stored frozen in small aliquots at –20˚C, except cladribine, which was stored at +4˚C. Stock solutions were prepared in water for injection, further dilution was made in respective medium.

The MTT assay. Cellular drug resistance was tested by means of the MTT assay. The procedure of the assay has been described previously (22). The concentration of drug that was inhibitory to 50% of the cells (IC_{50}) was calculated from the dose-response curve and was used as a measure for *ex vivo* drug resistance in each sample. Relative resistance (RR) between analyzed groups for each drug was calculated as a ratio of median value of IC_{50} for this drug.

Statistical analysis. The Mann-Whitney *U*-test and Kruskal-Wallis test were performed to compare differences in drug sensitivity between analyzed groups. All tests were 2-sided with *p*-value of 0.05 being considered as significant.

Results

In comparison to childhood AML, no significant differences were reported in the *ex vivo* drug resistance in adult AML (Table I). There were also no differences in cellular drug resistance between *de novo* and relapsed adult AML. Activity of clofarabine was comparable in pediatric and adult AML samples (Figure 1). No difference in drug *ex vivo* activity against myeloblasts were observed between age groups of AML patients, both in children (Tables II and III) and in adults.

Pediatric AML was more *ex vivo* resistant to most drugs, reaching statistical difference for prednisolone, vincristine, L-asparaginase, daunorubicin, idarubicin and etoposide (Table IV). Activity of clofarabine in ALL and AML *de novo* in pediatric samples was comparable.

Table II. *Drug resistance in children with acute myeloid leukemia with respect to age.*

Drug	<10 years (n=10)	>10 years (n=15)	RR	*p*-value
Prednisolone	214.57 (n=6) 31.25->250	238.5 (n=9) 13.92->250	1.1	0.589
Vincristine	4.17 (n=6) 3.1->20	2.845 (n=8) 0.41-6.84	0.7	0.118
Idarubicin	0.095 (n=9) 0.08->2	0.195 (n=12) 0.06->2	2.1	0.271
Daunorubicin	0.23 (n=6) 0.09->2	0.416 (n=8) 0.28->2	1.8	0.105
Doxorubicin	0.73 (n=6) 0.42->8	1.52 (n=2) 1.52-1.52	2.1	0.495
Mitoxantrone	0.0905 (n=8) 0.037->1	0.144 (n=15) 0.009-1	1.6	0.783
Etoposide	4.23 (n=6) 1.61->50	6.125 (n=8) 1.45->50	1.4	0.566
L-asparaginase	0.84 (n=6) 0.04->10	1.325 (n=8) 0.07->10	1.6	0.431
6-Thioguanine	2.65 (n=6) 1.56->50	5.81 (n=2) 5.81-5.81	2.2	0.495
4-HOO-Cyclo-phosphamide	2.385 (n=4) 0.35->100	1.805 (n=4) 1.34-2.27	0.8	0.218
4-HOO-Ifosfamide	10.62 (n=2) 10.62->100	16.39 (n=2) 16.39-16.39	1.5	0.183
Cytarabine	0.135 (n=8) 0.03->10	0.44 (n=15) 0.04->10	3.3	0.269
Fludarabine	0.1914 (n=9) 0.148->20	0.525 (n=14) 0.159->20	2.7	0.131
Cladribine	0.2265 (n=8) 0.003->40	0.54 (n=15) 0.002->40	2.4	0.251
Clofarabine	0.042 (n=8) 0.01->12.5	0.056 (n=9) 0.03->12.5	1.3	0.226

Concentration of L-asparaginase is given in IU/mL, clofarabine in μM, and all other drugs in μg/mL. Drug resistance is given as median and range of IC_{50}; n – number of patients, RR – relative resistance.

Table III. *Drug resistance in children over 10 years and adults with AML.*

Drug	10-18 years (n=15)	Adults (n=32)	RR	*p*-value
Prednisolone	238.5 (n=9) 13.92->250	181.45 (n=20) 21.48->250	0.8	0.765
Vincristine	2.845 (n=8) 0.41-6.84	5.315 (n=21) 0.63-11.89	1.9	0.154
Idarubicin	0.195 (n=12) 0.06->2	0.13 (n=24) 0.02->2	0.7	0.138
Daunorubicin	0.416 (n=8) 0.28->2	0.285 (n=21) 0.01->2	0.7	0.308
Doxorubicin	1.52 (n=2) 1.52-1.52	1.1 (n=18) 0.39->8	0.7	0.206
Mitoxantrone	0.144 (n=15) 0.009-1	0.0709 (n=22) 0.021->1	0.5	0.433
Etoposide	6.125 (n=8) 1.45->50	8.748 (n=21) 0.86->50	1.4	0.149
L-Asparaginase	1.325 (n=8) 0.07->10	1.47 (n=20) 0.04->10	1.1	0.124
6-Thioguanine	5.81 (n=2) 5.81-5.81	8.85 (n=16) 2.52->50	1.5	0.259
4-HOO-Cyclo-phosphamide	1.805 (n=4) 1.34-2.27	2.8 (n=19) 0.17-4.63	1.6	0.495
4-HOO-Ifosfamide	16.39 (n=2) 16.39-16.39	16.395 (n=16) 2.91-33.82	1.0	1.000
Cytarabine	0.44 (n=15) 0.04->10	1.15 (n=25) 0.05->10	2.6	0.463
Fludarabine	0.525 (n=14) 0.159->20	0.9509 (n=24) 0.0984->20	1.8	0.855
Cladribine	0.54 (n=15) 0.002->40	1.237 (n=24) 0.0006->40	2.3	0.320
Clofarabine	0.056 (n=9) 0.03->12.5	0.06 (n=24) 0.01->12.5	1.1	0.125

Concentration of L-asparaginase is given in IU/mL, clofarabine in μM, and all other drugs in μg/mL. Drug resistance is given as median and range of IC_{50}; n – number of patients, RR – relative resistance.

Table IV. *Drug resistance in pediatric ALL and AML de novo samples.*

Drug	ALL *de novo* (n=201)	AML *de novo* (n=25)	RR	*p*-value
Prednisolone	27.59 (n=159) 0.01->250	226.96 (n=15) 13.92->250	8.2	0.021
Vincristine	0.35 (n=158) 0.01953->20	3.71 (n=14) 0.41->20	10.6	0.006
Idarubicin	0.06 (n=114) 0.01->2	0.155 (n=21) 0.06->2	2.6	0.016
Daunorubicin	0.18 (n=157) 0.01->2	0.31 (n=14) 0.09->2	1.7	0.051
Doxorubicin	0.81 (n=90) 0.1->8	0.975 (n=8) 0.4->8	1.2	0.958
Mitoxantrone	0.043 (n=112) 0.0034->1	0.1285 (n=23) 0.0086->1	3.0	0.287
Etoposide	1.2 (n=154) 0.048->50	5.15 (n=14) 1.45->50	4.3	0.006
L-asparaginase	0.19 (n=159) 0.032->10	1.19 (n=14) 0.04->10	6.3	0.018
6-Thioguanine	3.25 (n=84) 1.5625-50	4.23 (n=8) 1.56->50	1.3	0.739
4-HOO-Cyclo-phosphamide	0.8 (n=83) 0.19->100	1.88 (n=8) 0.35-2.27	2.4	0.335
4-HOO-Ifosfamide	8.28 (n=42) 0.33->100	13.505 (n=4) 10.62-16.39	1.6	0.349
Cytarabine	0.85 (n=158) 0.05->10	0.27 (n=23) 0.03->10	0.3	0.152
Fludarabine	0.525 (n=114) 0.01953-20	0.3125 (n=23) 0.1477->20	0.6	0.399
Cladribine	0.075 (n=112) 0.0025-40	0.404 (n=23) 0.0022->40	5.4	0.104
Clofarabine	0.04 (n=128) 0.01->12.5	0.055 (n=17) 0.01->12.5	1.4	0.916

Concentration of L-asparaginase is given in IU/mL, clofarabine in μM, and all other drugs in μg/mL. Drug resistance is given as median and range of IC_{50}; n – number of patients, RR – relative resistance.

With respect to age groups in ALL, it was found that emerging *ex vivo* drug resistance occurs with age for prednisolone, vincristine and L-asparaginase (Table V). For all other drugs, this trend was also observed. Activity of clofarabine against lymphoblasts was comparable in all age groups. Activity of cytarabine was highest in patients with age 1-5, while for all other age groups, it was comparable. Activity of fludarabine and cladribine was decreased in patients aged over 10.

Relapsed ALL samples were more *ex vivo* resistant to all tested drugs in comparison to *de novo* ALL samples for prednisolone, vincristine, L-asparaginase and daunorubicin (Table VI). Activity of clofarabine in newly-diagnosed and relapsed lymphoblasts was similar (Figure 1). There were no significant differences in relapsed blasts to cytarabine, fludarabine and cladribine, when compared to ALL *de novo* samples.

Discussion

The development of drug resistance is the limiting factor in the therapy of ALL and AML, both in children and in adults. Although a proportion of the acute leukemias occurring predominantly in children are presently curable by chemotherapy, relapse still remains the main problem. In adults, more than 50% AML patients are at risk of therapy failure. In spite of continuous improvement in hematopoietic stem cell transplantation procedures and targeted therapy, there is a need to search for the new drugs in conventional chemotherapy. A second-generation nucleoside analogue, clofarabine, might be a valuable chemotherapeutic option due to its activity in various subtypes of acute leukemia.

Cellular drug resistance in AML cells seems to be similar throughout all age groups, however greater age has a worse

Table V. *Drug activity in pediatric ALL with respect to age groups.*

Drug	1-5 years (n=71)	5-10 years (n=63)	10-15 years (n=26)	15-18 years (n=31)	*p*-value
Prednisolone	25.49 (n=58) 0.08->250	28.4 (n=53) 0.28->250	61.24 (n=21) 0.01->250	148.03 (n=27) 0.19->250	0.031
Vincristine	0.345 (n=57) 0.03-9.8	0.36 (n=52) 0.02-9.33	1.88 (n=21) 0.0195->20	2.19 (n=28) 0.08->20	0.004
Idarubicin	0.075 (n=38) 0.02->2	0.05 (n=39) 0.02-0.63	0.09 (n=14) 0.01->2	0.08 (n=23) 0.06->2	0.035
Daunorubicin	0.215 (n=58) 0.02->2	0.135 (n=52) 0.04->2	0.27 (n=19) 0.01->2	0.22 (n=28) 0.05->2	0.252
Doxorubicin	0.825 (n=26) 0.1->8	0.77 (n=31) 0.23->8	1.03 (n=11) 0.4->8	1.13 (n=22) 0.43->8	0.093
Mitoxantrone	0.0504 (n=38) 0.0084->1	0.0345 (n=38) 0.003->1	0.0592 (n=14) 0.0053->1	0.0501 (n=22) 0.0092->1	0.559
Etoposide	1.38 (n=56) 0.032->50	0.81 (n=52) 0.048->50	2.93 (n=18) 0.06->50	2.06 (n=28) 0.11->50	0.265
L-Asparaginase	0.075 (n=59) 0.04->10	0.395 (n=52) 0.04->10	0.71 (n=20) 0.05->10	3.65 (n=28) 0.07->10	0.001
6-Thioguanine	3.79 (n=26) 1.5625->50	2.07 (n=27) 1.563->50	6.82 (n=9) 1.5625->50	5.285 (n=22) 3.13-18.37	0.064
4-HOO-Cyclophosphamide	0.985 (n=25) 0.28->100	0.865 (n=32) 0.26->100	0.8 (n=10) 0.19-23.4	0.64 (n=16) 0.45-6.25	0.873
4-HOO-Ifosfamide	6.91 (n=15) 0.33->100	13.845 (n=16) 12.5-15.19	10.335 (n=6) 1.56-49.12	5.25 (n=5) 4.79-19.43	0.461
Cytarabine	0.595 (n=38) 0.07->10	1.49 (n=39) 0.06->10	2.28 (n=14) 0.05->10	1.3 (n=23) 0.23->10	0.029
Fludarabine	0.31815 (n=37) 0.0881->20	0.625 (n=40) 0.02->20	1.0511 (n=15) 0.0886->20	0.7875 (n=22) 0.1477->20	0.111
Cladribine	0.0988 (n=38) 0.0117-12.65	0.0244 (n=38) 0.003->40	0.4 (n=14) 0.0068->40	5.0357 (n=22) 0.0132->40	0.020
Clofarabine	0.04 (n=44) 0.01-8.61	0.04 (n=43) 0.01->12.5	0.055 (n=15) 0.01->12.5	0.068 (n=26) 0.01->12.5	0.372

Concentration of L-asparaginase is given in IU/mL, clofarabine in μM, and all other drugs in μg/mL. Drug resistance is given as median and range of IC_{50}; n - number of patients. *p*-value was calculated by Kruskal-Wallis test.

clinical outcome. Previous and current studies suggest that adult AML is not more *in vitro* resistant than childhood AML (reviewed in (21)). In childhood AML, no drug is more effective in comparison to ALL, and cellular drug resistance is partially related to chromosomal abnormalities (23, 24). Pediatric AML is equally resistant as adult AML. Pediatric and adult AML are possibly equally drug resistant on initial diagnosis and at relapse (21, 25). Thus, the difference in outcome between childhood and adult AML cannot be explained by clinically significant differences in an *in vitro* drug resistance measured with the MTT assay and therefore might reflect other differences such as pharmacokinetics. Another explanation may be that a small subpopulation of resistant cells cannot be detected using the MTT assay.

Contrary to children, adults with AML in 20-50% cases are primarily resistant to induction chemotherapy, and clinical resistance to the first cycle of induction chemotherapy is an independent prognostic factor. The resistance to chemotherapy is represented by a characteristic gene-expression profile which distinguishes AML patients with good or poor responses. Genes highly expressed in poor responders are also overexpressed in hematopoietic stem/progenitor cells. In multivariate analysis, the treatment-response signature was an independent prognostic factor. Resistance to chemotherapy in AML can be identified by gene-expression profiling before treatment and seems to be mediated by a transcriptional program active in hematopoietic stem/progenitor cells (26). Microarray technology should lead to the identification of additional gene targets linked to the treatment response of specific cytogenetic leukemia subgroups.

Cellular drug resistance is one of the main causes of the frequent ultimate failure of chemotherapy in childhood AML. It appears that cellular drug resistance differs between AML and ALL. Taking into account both children and adults, age is adversely related to therapy outcome in AML, and the percentage of patients with favorable cytogenetics decreases with age; however age is positively correlated with multi-drug

Table VI. *Drug activity in de novo and relapsed childhood ALL.*

Drug	ALL de novo (n=201)	ALL relapsed (n=24)	RR	*p*-value
Prednisolone	27.59 (n=159)	137.39 (n=21)	5.0	0.002
	0.01->250	31.25->250		
Vincristine	0.35 (n=158)	2.26 (n=22)	6.5	0.037
	0.01953->20	0.12->20		
Idarubicin	0.06 (n=114)	0.13 (n=14)	2.2	0.110
	0.01->2	0.03->2		
Daunorubicin	0.18 (n=157)	0.875 (n=21)	4.9	0.024
	0.01->2	0.07->2		
Doxorubicin	0.81 (n=90)	1.92 (n=13)	2.4	0.240
	0.1->8	0.09->8		
Mitoxantrone	0.043 (n=112)	0.0602 (n=14)	1.4	0.471
	0.0034->1	0.0025->1		
Etoposide	1.2 (n=154)	2.65 (n=18)	2.2	0.170
	0.048->50	0.13->50		
L-Asparaginase	0.19 (n=159)	0.72 (n=22)	3.8	0.015
	0.032->10	0.06->10		
6-Thioguanine	3.25 (n=84)	5.86 (n=11)	1.8	0.133
	1.5625-50	2.37->50		
4-HOO-Cyclo-phosphamide	0.8 (n=83)	2.1 (n=10)	2.6	0.175
	0.19->100	0.11->100		
4-HOO-Ifosfamide	8.28 (n=42)	18.985 (n=8)	2.3	0.144
	0.33->100	4.69->100		
Cytarabine	0.85 (n=158)	1.63 (n=14)	1.9	0.175
	0.05->10	0.04->10		
Fludarabine	0.525 (n=114)	0.711 (n=14)	1.4	0.304
	0.01953-20	0.1226->20		
Cladribine	0.075 (n=112)	0.1719 (n=14)	2.3	0.840
	0.0025-40	0.0016->40		
Clofarabine	0.04 (n=128)	0.043 (n=17)	1.1	0.908
	0.01->12.5	0.01->12.5		

Concentration of L-asparaginase is given in IU/mL, clofarabine in μM, and all other drugs in μg/mL. Drug resistance is given as median and range of IC_{50}; n – number of patients, RR – relative resistance.

resistance and the proportion of patients with unfavorable cytogenetics. Increased incidence of unfavorable cytogenetics contributes to the poor outcome in AML. Within each cytogenetic risk group, treatment outcome deteriorates markedly with age. The distinct biology and clinical responses seen argue for age-specific assessments when evaluating therapies for AML.

Pediatric AML is more resistant than pediatric ALL (23, 27, 28). When *ex vivo* drug resistance profile in childhood ALL and AML were compared, almost no drug was found to be more effective in AML in comparison to ALL. Childhood acute lymphoblastic and myeloid leukemia have different cellular drug resistance profiles. Using the MTT assay, it has been shown that AML cells were significantly more resistant than ALL cells to glucocorticoids, vincristine, L-asparaginase, anthracyclines, mitoxantrone, etoposide and ifosfamide; while the sensitivity was equal for cytarabine. These findings were reported previously (23). On the other hand, clofarabine was as active in AML as in ALL.

In the comparative study of adults with ALL, AML and B-lineage CLL (chronic lymphocytic leukemia), B-CLL cells were found to be more sensitive than cells from both AML and ALL to cytarabine, cladribine, fludarabine, doxorubicin, idarubicin, vincristine and cyclophosphamide (29). No difference in cellular drug resistance was found between B-CLL and ALL cells for prednisolone, whereas AML cells were more resistant. However, B-CLL cells acquired cellular drug resistance during therapy, as cells obtained from patients who had received previous chemotherapy were more resistant to almost all tested drugs as compared to cells from treatment-naive patients (29).

In the study of Beesley *et al*. (30) on pediatric ALL cell lines, clofarabine was marginally more active in B-lineage than T-lineage ALL. The differences in clofarabine *ex vivo* activity between precursor-B-lineage and T-lineage ALL was not observed in the presented pediatric cohort, and this might contribute to general profile of good clofarabine activity against lymphoblasts.

Pediatric relapsed ALL is regarded as highly drug resistant diseases, both in *ex vivo* individual tumor response testing and in clinical practice (31, 32). No differences in *ex vivo* activity of clofarabine between *de novo* and relapsed ALL patients were observed, while high resistance to prednisolone, vincristine, daunorubicin and L-asparaginase was found on relapse. Adult ALL samples were not tested in this study. Adult ALL is a more drug resistant disease than childhood ALL at first diagnosis (33, 34). There are some discrepancies in results comparing cellular drug resistance in adult ALL between initial diagnosis and relapse (28, 29), yet there are probably no differences between these two groups of patients (29).

In conclusion, it has been shown that clofarabine has comparable activity against lymphoblasts and myeloblasts, both on initial diagnosis and at relapse, and both in children and in adults, which suggests the potential for use of clofarabine in refractory and relapsed patients with acute leukemia. Although a trend was observed for increasing resistance to clofarabine in adolescents with ALL, the difference was smaller than for most of drugs, which are currently in use of therapy of childhood ALL. On the other hand, a large variability of IC_{50} values was observed in all tested groups, thus considerable overlap occurred between all patients. Differences in drug resistance to clofarabine between pediatric age-based subgroups of ALL were not detected.

Acknowledgements

The authors thank Beata Kolodziej, Beata Rafinska and Malgorzata Kubicka for their technical support. In addition to the list of authors, this study was performed with the significant

contribution of the following researchers, who provided patient samples and their data: Edyta Juraszewska, Benigna Konatkowska, Marta Kuzmicz, Tomasz Szczepanski, Elzbieta Stanczak, Iwona Malinowska, Igor Olejnik, Tomasz Ociepa, Agnieszka Mizia-Malarz and Lucyna Kapuscinska. This study was partially supported by grant MNiSW N407 078 32/2964.

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Received November 4, 2008
Revised January 19, 2009
Accepted February 17, 2009