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Although the efficacy of nelarabine and clofarabine has been extensively studied in acute lymphoblastic leukaemia (ALL) *in vitro* and *in vivo*, less is known about their possible role in other haematological malignancies. The aim of this study was to perform an analysis of *in vitro* drug sensitivity of nelarabine and clofarabine in lymphoblastic lymphoma and myeloid leukaemia cell lines.
Six cell lines were used for the study of *in vitro* drug sensitivity: two ALL (CCRF-CEM and Jurkat), two lymphoblastic lymphoma cell lines (Raji and Daudi), acute promyelocytic leukaemia (HL60) and chronic myeloid leukaemia (K562) cell lines. *In vitro* drug resistance was tested by the MTT assay.
When compared to ALL cell lines, relatively good *in vitro* activity of clofarabine and nelarabine in both B-cell lymphomas was found. *In vitro* activity of both new purine analogues was favourable in comparison to cytarabine, fludarabine and cladribine activity. Drug sensitivity profiles of Raji and Daudi cell lines were comparable to each other, although overall, Raji cell line was less drug sensitive. There was good activity of clofarabine and nelarabine against HL60 cells, while K562 cell line was resistant to most of the tested drugs. Drugs used in high-dose therapy before haematopoietic stem cell transplantation, such as busulfan, etoposide, treosulfan and cyclophosphamide, presented good activity, when application of high doses during this procedure was taken into account.
In conclusion, these results support the hypothesis of relatively good *in vitro* activity of nelarabine and clofarabine in tested B-cell lymphoma and HL60 cell lines.

Key words: cell lines, leukaemia, lymphoma, clofarabine, nelarabine, drug resistance, MTT assay.

Differential activity of nelarabine and clofarabine in leukaemia and lymphoma cell lines

Zróżnicowana aktywność nelarabiny i klofarabiny w liniach komórkowych białaczek i chłoniaków

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Introduction

The nucleoside analogues are a group of antimetabolite cytotoxics which generally have to be metabolised to the equivalent nucleotide before incorporation into DNA. Nelarabine and clofarabine are purine analogues closely related to fludarabine and cytarabine [1]. Recently, these drugs have demonstrated good activity in pre-clinical studies and have been tested in clinical trials in patients with acute lymphoblastic leukaemia (ALL) with varying success. Clofarabine and nelarabine have been shown to have significant efficacy in both children and adults with refractory leukaemia [2-6]. Clofarabine is the first deoxyadenosine analogue that shows promise in adult and paediatric acute leukaemias without untoward toxicity. Nelarabine, as expected from its design, is a drug that may be directed to T-cell diseases. Clofarabine was granted accelerated approval by the US Food and Drug Administration (FDA) for the treatment of paediatric patients with relapsed or refractory ALL after at least two prior regimens in December 2004 [7, 8]. Nelarabine followed in 2005 with FDA approval for the treatment of refractory T-cell ALL, based on data indicating particular efficacy in this lineage [4, 8-11].

Clofarabine is thought to work via three mechanisms: inhibition of ribonucleotide reductase; incorporation to DNA; and induction of apoptosis. Given these mechanisms of action, clofarabine would be predicted to act synergistically with other chemotherapeutic agents such as other purine nucleoside analogues and DNA damaging or cross-linking agents such as anthracyclines and platinum-based compounds. Intravenous clofarabine also showed significant efficacy in paediatric ALL [12].

Purine nucleoside phosphorylase (PNP) deficiency is a rare, inherited immunodeficiency disorder in which the specific molecular defect was identified. Clinically, a lack of PNP manifests as profound T-cell deficiency with minor or variable changes in the humoral system. Biochemically, the absence of PNP results in an increase in plasma deoxyguanosine (dGuo) and a T-cell-specific increase in intracellular deoxyguanosine triphosphate (dGTP). This observation has been the impetus for the search for potential anti-T-cell-lineage agents. Nelarabine (a PNP-resistant dGuo analogue) proved to be T-cell selective when tested in clinical trials [13-15]. Nelarabine was rapidly converted by cells of lymphoid lineage to its corresponding arabinosylguanine nucleotide triphosphate (araGTP). The triphosphate form of araG acts as a substrate for DNA polymerases and araG is incorporated into the DNA, resulting in inhibition of DNA synthesis and subsequent cytotoxicity [16]. Nelarabine is water soluble and is rapidly converted to araG, which is specifically cytotoxic to T-lymphocytes and T-lymphoblastoid cells. Clinical and pharmacokinetic investigations have established that nelarabine is active

Efektywność nelarabiny i klofarabiny została dobrze określona w ostrej białaczce limfoblastycznej (ALL) *in vitro* oraz *in vivo*. Znacznie mniej jednak wiadomo na temat możliwej roli tych leków w innych nowotworach hematologicznych. Celem pracy była analiza aktywności *in vitro* nelarabiny i klofarabiny w liniach komórkowych chłoniaków B-komórkowych oraz liniach mieloidalnych.
Do badań wrażliwości *in vitro* na cytostatyki użyto 6 linii komórkowych: 2 linii ALL (CCRF-CEM i Jurkat), 2 linii chłoniaków B-komórkowych (Raji i Daudi), linii ostrej białaczki promielocytowej (HL60) oraz przewlekłej białaczki szpikowej (K562). Profil oporności *in vitro* na nelarabinę, klofarabinę oraz 18 innych leków przeciwnowotworowych został określony za pomocą testu cytotoksyczności MTT.
W porównaniu z linią ostrej białaczki limfoblastycznej stwierdzono stosunkowo dobrą aktywność *in vitro* nelarabiny i klofarabiny w obydwu liniach chłoniaków B-komórkowych. W porównaniu z aktywnością cytarabiny, fludarabiny i kladribiny aktywność obydwu nowych analogów purynowych była korzystna. Profil oporności na cytostatyki w liniach Raji i Daudi był podobny, chociaż linia Raji okazała się bardziej wrażliwa na testowane leki. Stwierdzono również dobrą aktywność nelarabiny i klofarabiny wobec komórek linii HL60, natomiast linia K562 wykazywała oporność na większość testowanych leków. Leki używane w kondycjonowaniu przed transplantacją komórek hematopoetycznych, takie jak busulfan, etopozyd, treosulfan i cyklofosfamid, wykazywały dobrą aktywność wobec badanych linii komórkowych, przy uwzględnieniu stosowania wysokich dawek.
Podsumowując, otrzymane wyniki potwierdzają hipotezę o stosunkowo dobrej aktywności *in vitro* nelarabiny i klofarabiny w testowanych liniach komórkowych chłoniaków B-komórkowych i ostrej białaczki promielocytowej.

Słowa kluczowe: linie komórkowe, białaczka, chłoniak, klofarabina, nelarabina, oporność na cytostatyki, test MTT.

as a single agent, which has led to exploration of an expanded role in the treatment of T-cell haematological malignancies [17, 18].

The *in vitro* efficacy of these drugs has been tested in a variety of ALL cell lines and their *in vivo* specific effect in patients with ALL has been well studied. However, less is known about their effects with regard to other haematological malignancies. The aim of this study was to perform an analysis of *in vitro* drug sensitivity of nelarabine and clofarabine in lymphoblastic lymphoma and myeloid leukaemia cell lines, in comparison to sensitivity of paediatric acute lymphoblastic leukaemia cell line, by means of the MTT assay.

Material and methods

Cell lines

Six cell lines were used for the study of *in vitro* drug sensitivity: two acute lymphoblastic leukaemia (CCRF-CEM and Jurkat), two lymphoblastic lymphoma cell lines (Raji and Daudi), acute promyelocytic leukaemia (HL60) and chronic myeloid leukaemia (K562) cell lines.

CCRF-CEM (ECACC No. 85112105) is human acute lymphoblastic leukaemia T-cell line, obtained from the peripheral blood of a 4-year-old Caucasian female with acute lymphoblastoid leukaemia. Cell karyotype 2n = 46. Culture medium for this cell line contained RPMI 1640 medium, supplemented with 2mM glutamine and 20% FBS.

Jurkat (ECACC No. 88042803) cells are an immortalized line of T lymphocyte cells. The Jurkat cell line was established in the late 1970s from the peripheral blood of a 14-year-old boy with T cell leukaemia. Karyotype: pseudodiploid, 2n = 46. Jurkat cells are also useful in science because of their ability to produce interleukin 2. Their primary use is to determine the mechanism of differential susceptibility of cancers to drugs and radiation.

Raji (ECACC No. 85011429) cell is human B lymphocyte Burkitt's lymphoma, established in 1963 from the left maxilla of a 12-year-old African boy with Burkitt's lymphoma. It is the first continuous human haematopoietic cell line. Karyotype: diploid, 2n = 46. This cell line carries the latent Epstein-Barr virus (EBV) genome and is positive for EBNA. Human Raji cells are cultured in RPMI 1640 medium with 2 mM L-glutamine and harvested at the log phase of growth.

Daudi (ECACC No. 85011437) is human Burkitt's lymphoma cell line; cells are lymphoblast-like in morphology. Karyotype: diploid, 2n = 46. Derived from 16-year-old male Negro. Positive for EBNA, carries the Epstein-Barr virus markers, complement receptors, surface bound immunoglobulin and surface markers for the Fc fragment of IgG. Growth medium: RPMI 1640 medium with 2 mM L-glutamine, and 10% fetal bovine serum (FBS).

HL60 (ECACC No. 98070106) cell line was established in 1977 from a 36-year-old Caucasian female with acute promyelocytic leukaemia. The cells largely resemble promyelocytes but can be induced to differentiate terminally *in vitro*. Karyotype: pseudodiploid 2n = 46. Some reagents cause HL60 cells to differentiate to granulocyte-like cells, others to monocyte/macrophage-like cells. The HL60 cell genome contains an amplified c-myc proto-oncogene; c-myc mRNA levels are correspondingly high in undifferentiated cells but decline rapidly following induction of differentiation.

K562 (ECACC No. 89121407) cells were the first human immortalised myelogenous leukaemia line to be established and are a bcr-abl positive erythroleukaemia line, derived from a 53-year-old female chronic myeloid leukaemia (CML) patient in blast crisis [19-21]. Karyotype: diploid, 2n = 46. K562 blasts are multipotential, haematopoietic malignant cells that spontaneously differentiate into recognisable progenitors of the erythrocyte, granulocyte and monocytic series. Growth medium: RPMI with 2 mM L-glutamine and 10% FBS.

Drugs

The following 20 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), fludarabine phosphate (Schering AG, Berlin, Germany, 0.019-20 µg/ml), idarubicin (Pharmacia, Milan, Italy, 0.0019-2 µg/ml), mitoxantrone (Jelfa, Jelenia Gora, Poland, 0.001-1 µg/ml), 6-thioguanine (Sigma, nr A4882, 1.56-50 µg/ml), thiotepa (Lederle Riemser, Griefswald, Germany, 0.032-100 µg/ml), treosulfan (Medac, Hamburg, Germany, 10 pg/µl – 1 µg/ml), bortezomib (Janssen Pharmaceutica N.V., Belgium, 1.9 nM – 2 µM), busulfan (busilvex, Pierre Fabre Medicament, Boulogne, France, 1.17-1200 µg/ml), topotecan (GlaxoSmithKline, UK, 0.097-100 µM), clofarabine (Evoltra, Bioenvision, Edinburgh, UK, 0.0122-12.5 µM), and nelarabine (GlaxoSmithKline, Greenford, UK, 6.1 ng/ml – 200 µg/ml). 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 the respective medium.

The MTT assay

Cellular drug resistance was tested by means of the MTT assay. The procedure of the assay has been described elsewhere [22]. Briefly, in 96-well round-bottomed microculture plates 80 µl of cell suspension at concentration 0.1-0.2 × 10^6/ml were incubated in the absence (control wells) or presence of drugs, being tested at 6 different concentrations in duplicate. To improve culture conditions, insulin (at final concentration 5 µg/ml), transferrin (at final concentration 5 µg/ml) and sodium selenite (at final concentration 5 ng/ml) were added (ITS, Sigma, St. Louis, MO, USA). After 3 days of culture at 37°C in humidified air containing 5% CO_2, 50 µg 3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) was added to each well (final concentration 0.45 mg/ml). Cells were incubated with MTT for 6 hours at 37°C. In these conditions viable, but not dead, cells can reduce yellow MTT into purple formazan. The formazan crystals were dissolved in acidified isopropanol and its quantity 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). The optical density (OD) at 550 nm is linearly related to the number of viable cells.

The average OD of the blank control wells (no drugs, no cells) was subtracted from the average OD of the control wells (cells but no drugs) and the wells containing drugs. The leukaemic cell survival (LCS) in each well was calculated by the equation: LCS = [OD tested well (– blank)] / [mean OD control wells (– blank)] × 100%. The LC50 value, defined as the concentration of drug that was lethal to 50% of the cells, was calculated from the dose-response curve and was used as a measure for *in vitro* drug resistance in each sample. LC50 value was calculated from the formula: [(%LCS above 50%) – 50] / [(%LCS above 50%) – (%LCS below 50%)] × [drug concentration above 50% LCS – drug concentration below 50% LCS] + [drug concentration below 50% LCS].

In cases where 50% cytotoxicity was not achieved by even the lowest or highest dose in a particular experiment, the LC50 was recorded as the lowest or highest concentration tested, respectively. At least 4 independent experiments were performed for each cell line. Results were compared between respective cell lines. Relative resistance (RR) between cell lines for each drug was calculated as a ratio of mean value of LC50 for this drug in analyzed cell lines. A value of RR < 1 for an analyzed drug denotes better sensitivity of the tested cell line to this specific drug in comparison to the other cell line, while a value of RR > 1 denotes higher drug resistance.

Statistical analysis

Student's *t*-test was used to compare differences in drug resistance between groups.

Results

CCRF-CEM was the most drug sensitive cell line to most of the tested drugs, except for Jurkat cell line, which was the most sensitive to nelarabine, cytarabine, asparaginase and 4-HOO-cyclophosphamide, and HL60, which was the most sensitive to treosulfan (Table 1). Raji and Daudi cell lines were in most cases more drug resistant than acute lymphoblastic leukaemia cell lines, with the possible exception of better sensitivity of Daudi cell line to L-asparaginase, doxorubicin and bortezomib. HL60 cell line presented a differential drug sensitivity profile. It was more resistant than lymphoblastic cell lines to most of the tested drugs, but it showed relatively good sensitivity to fludarabine, L-asparaginase, doxorubicin, thioguanine, treosulfan, thiotepa, bortezomib and topotecan. K562 cell line was the most resistant cell line to most of the tested drugs, and only differences in activity of busulfan, treosulfan, bortezomib and topotecan were lower than for the other drugs.

Nelarabine was 2.5-3-fold less active in lymphoma cell lines than in CCRF-CEM, and it had very weak potency in HL60 and K562. Clofarabine was 2-fold less active in Raji and HL60 than in CCRF-CEM cell line, and there were no differences between activity in Daudi and CCRF-CEM. Activity of clofarabine was comparable in Jurkat, Raji, Daudi and HL60 cell lines. No clofarabine activity was observed in K562 cell line. Both nelarabine and clofarabine were more active than cytarabine and fludarabine in Raji and Daudi cell lines, while this was not the case in HL60.

First-line drugs used in therapy of acute lymphoblastic leukaemia, such as prednisolone, L-asparaginase, vincristine, daunorubicin, doxorubicin, thioguanine, 4-HOO-cyclophosphamide and cytarabine proved to show comparable activity in all lymphoblastic cell lines, with the exception of worse activity of cytarabine and thioguanine in Raji cell line. The same activity profile was observed for etoposide, mitoxantrone, and topotecan.

Table 1. Activity of tested drugs in cell lines
Tabela 1. Aktywność badanych leków w liniach komórkowych

Drugs	CCRF	Jurkat	Raji	Daudi	HL60	K562
Nelarabine	0.02 ±0.005	0.01 ±0.005* RR = 0.5	0.06 ±0.02* RR = 3	0.05 ±0.01* RR = 2.5	0.14 ±0.03* RR = 7	0.19 ±0.05* RR = 9.5
Clofarabine##	0.03 ±0.01	0.05 ±0.02* RR = 1.7	0.07 ±0.02* RR = 2.3	0.04 ±0.01 RR = 1.3	0.06 ±0.01* RR = 2	1.28 ±0.68* RR = 42.7
Cytarabine	0.013 ±0.004	< 0.0097 RR < 0.7	0.14 ±0.03* RR = 10.8	0.12 ±0.04* RR = 9.2	0.03 ±0.01* RR = 2.3	> 10* RR > 769
Fludarabine	0.38 ±0.1	2.37 ±0.43* RR = 6.2	> 20* RR > 52	> 20* RR > 52	0.24 ±0.05* RR = 0.6	> 20* RR > 52
Cladribine	0.02 ±0.01	0.13 ±0.03* RR = 6.5	0.8 ±0.2* RR = 40	0.14 ±0.04* RR = 7	0.03 ±0.01 RR = 1.5	3.1 ±1.6* RR = 155
Prednisolone	3.5 ±0.9	19.3 ±6.5* RR = 5.5	42.2 ±10.3* RR = 12.1	43.1 ±1.3* RR = 12.3	108 ±22* RR = 30.9	151.2 ±19.9* RR = 43.2
Vincristine	0.027 ±0.01	0.03 ±0.01 RR = 1.1	0.05 ±0.23 RR = 1.9	0.04 ±0.01 RR = 1.5	0.07 ±0.01* RR = 2.6	0.28 ±0.05* RR = 10.4
Asparaginase#	0.5 ±0.2	0.19 ±0.03* RR = 0.4	0.61 ±0.1 RR = 1.2	0.2 ±0.05* RR = 0.4	0.69 ±0.21 RR = 1.4	5.4 ±2.2* RR = 10.8
Idarubicin	0.04 ±0.01	0.05 ±0.03 RR = 1.3	0.12 ±0.04* RR = 3	0.03 ±0.01 RR = 0.8	0.06 ±0.01* RR = 1.5	0.22 ±0.05* RR = 5.5
Daunorubicin	0.05 ±0.02	0.1 ±0.05* RR = 2	0.13 ±0.06* RR = 2.6	0.07 ±0.01* RR = 1.4	0.15 ±0.02* RR = 30.9	0.41 ±0.13* RR = 8.2
Doxorubicin	0.24 ±0.05	0.48 ±0.09* RR = 2	0.26 ±0.09 RR = 1.1	0.14 ±0.02* RR = 0.6	0.27 ±0.04 RR = 1.1	1.08 ±0.34* RR = 4.5
Mitoxantrone	0.02 ±0.01	0.04 ±0.01* RR = 2	0.06 ±0.02* RR = 3	0.03 ±0.01 RR = 1.5	0.05 ±0.01* RR = 2.5	0.68 ±0.25* RR = 34
Etoposide	0.25 ±0.01	0.12 ±0.04* RR = 0.5	0.22 ±0.04 RR = 0.9	0.34 ±0.08* RR = 1.4	0.35 ±0.04* RR = 1.4	5.41 ±2.15* RR = 21.6
Thioguanine	< 1.5625	< 1.5625 RR = NA	8.9 ±3.2* RR = 5.7	< 1.5625 RR = NA	< 1.5625 RR = NA	7.6 ±1.5* RR = 4.9
4-HOO-cyclophosphamide	0.41 ±0.1	0.34 ±0.12 RR = 0.8	2.02 ±0.2* RR = 4.9	0.41 ±0.12 RR = 1	0.69 ±0.08* RR = 1.7	4.1 ±0.4* RR = 10
Busulfan	10.5 ±4.8	10.3 ±3.2 RR = 1	95.7 ±9.8* RR = 9.1	8.3 ±1.9 RR = 0.8	15.3 ±1.1* RR = 1.5	34.4 ±4.5* RR = 3.3
Treosulfan	0.42 ±0.05	0.29 ±0.09* RR = 0.7	> 1* RR > 2.4	> 1* RR > 2.4	0.05 ±0.02* RR = 0.1	0.87 ± 0.45* RR = 2.1
Thiotepa	0.41 ±0.06	0.88 ±0.25* RR = 2.1	8.3 ±1.8* RR = 20.2	> 100* RR > 243	0.32 ±0.07* RR = 0.8	7.93 ±0.8* RR=19.3
Bortezomib##	514 ±128	711 ±46* RR = 1.4	682 ±114 RR = 1.3	230 ±50* RR = 0.4	613 ±155 RR = 1.2	985 ±89* RR = 1.9
Topotecan##	< 0.097	< 0.097 RR = NA	0.25 ±0.05* RR = 2.6	0.21 ±0.07* RR = 2.2	< 0.097 RR = NA	0.2 ±0.1* RR = 3.1

Mean ± standard deviation of LC50 values is given. RR – relative resistance (in comparison to drug activity in CCRF-CEM cell line). (*) statistical significance (p < 0.05) in comparison to respective LC50 value for CCRF-CEM cell line. (#) concentration of L-asparaginase is given in IU/ml; (##) concentration of bortezomib, clofarabine and topotecan is given in μM. NA – not applicable.

The *in vitro* activity profile of busulfan, treosulfan and bortezomib was similar in all analyzed cell lines, and the differences in cell line resistance did not exceed 3.3-fold activity in CCRF-CEM cell line, with the only exception of busulfan activity in Raji cell line. Both lymphoma cell lines and K652 were highly resistant to thiotepa.

Discussion

The search for more effective and safer anti-leukaemia therapies has led to the identification of several new agents that show activity against specific types of acute lymphoblastic leukaemia (ALL). Recently, two novel purine nucleoside analogues (nelarabine and clofarabine) have

shown promising activity in patients with relapsed or refractory ALL. Of these, clofarabine has shown promising clinical activity in paediatric patients, with an overall response rate of 30%, and some patients are able to proceed to allogeneic haematopoietic cell transplantation. Nelarabine has also shown clinically meaningful benefit in patients with T-cell ALL, with overall response rates ranging from 33% to 60%, the induction of durable complete remissions, and an overall 1-year survival rate of 28% in adults [23, 24].

In this study we undertook the analysis of activity of clofarabine and nelarabine in 6 cell lines, including 2 T-ALL, 2 B-cell lymphoma, AML and CML ones. We also compared activity of other purine antimetabolites, cytarabine, fludarabine and cladribine, as well as other anticancer drugs in these six cell lines. When compared to lymphoblastic cell lines, relatively good *in vitro* activity of clofarabine and nelarabine in both B-cell lymphomas was found. *In vitro* activity of these new purine analogues was promising in comparison to cytarabine, fludarabine and cladribine activity.

A number of clinical attempts have shown good *in vivo* activity of these two compounds. Low-dose clofarabine induced a remission in a patient with T-cell leukaemia who relapsed in the skin and marrow after allogeneic transplant and was refractory to nelarabine, which suggests significant activity for low intermittent dose clofarabine in relapsed patients [25]. Mechanisms of clofarabine antitumour activity, both *in vitro* and *in vivo*, are correlated with its ability to induce apoptosis, particularly *in vivo* [26, 27]. On the other hand, clofarabine is a possible substrate of ABCG2 (breast cancer resistance protein), raising the possibility that this transporter could affect the disposition of nucleoside analogues in patients or cause resistance in tumours [28]. *In vitro* drug sensitivity profiles of Raji and Daudi cell lines were comparable, although overall, Raji cell line seemed to be less sensitive.

To validate the results of drug resistance analyses for clofarabine and nelarabine, we also tested a number of known antileukaemic compounds, routinely used in therapy of ALL and AML. Results of MTT assay for these drugs were comparable with previous studies of our group [22, 29-32] as well as other reports [33-36]. Nucleosides remain the most important class of drugs in acute myeloid leukaemia and the interest in new compounds is strong.

The results of our study might be translatable from the laboratory to a clinical setting, with respect to activity of drugs used in conditioning chemotherapy regimens before haematopoietic stem cell transplantation. Usually 3-10-fold higher doses of cytostatic drugs than in conventional chemotherapy are used in these procedures. Thus, several-fold higher resistance of cancer cells to a specific compound might be overcome successfully by use of high-dose therapy followed by stem cell transplantation.

Nelarabine is also an effective agent in indolent leukaemias [37], acute biphenotypic leukaemia [38], and in adults with refractory T-lineage acute lymphoblastic leukaemia [24, 39]. T-cell malignancies have distinct biochemical, immunological, and clinical features which set them apart from non-T-cell malignancies. In the past, T-cell leukaemia portended a worse prognosis than leukaemia of B-cell origin. Cure rates have improved with intensification of therapy and advanced understanding of the molecular genetics of T-cell malignancies. Further advances in the treatment of T-cell leukaemia will require the development of novel agents that can target specific malignancies without a significant increase in toxicity [17].

The MTT assay used in this study is an end-point type of *in vitro* cytotoxicity assay. It assesses total cell-kill caused by the tested cytotoxic drug. However, it obviously cannot take into account *in vivo* conditions, such as drug binding with proteins, drug distribution, metabolism and elimination. Therefore, the results of the study should be interpreted with caution.

It is likely that nelarabine and clofarabine will be useful drugs in the treatment of resistant/recurrent leukaemia and lymphoma, both as single agents and in combination. Thus both nelarabine and clofarabine are interesting drugs for further studies.

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