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Cytostatic Drug Testing in Human Leukemias by Means of Multiparametric Flow Cytometry*

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Summary. Human bone marrow cells from 20 patients as well as the permanent human B-cell lines RPMI 1788, Raji, Daudi, T-cell lines Molt, CEM, Jurkat and the promyelocytic line HL 60 were assayed by means of a newly developed in vitro flow cytometric cytostatic drug assay. The cells were exposed to cytosine-arabinoside, L-asparaginase, daunorubicin, prednisone or vincristine. Surviving cells were stained after an incubation period of 2 to 7 days with esterase and pH-indicator dye ADB (1,4-diacetoxy-2,3-dicyanobenzene), dead cells with DNA-dye PI (propidium iodide). Dose-response curves were established using percent surviving cells. It was possible to evaluate bone marrow samples from 16 out of 20 patients. Seven samples were leukemic (acute myeloid leukemia (AML) $n = 6$, Non-Hodgkin's Lymphoma (NHL) $n = 1$). Nine samples were from patients either in complete remission or with benign diseases. Daunorubicin and cytosine-arabinoside were cytotoxic in both groups, whereas vincristine was effective mainly in the leukemic group ($p < 0.05$). There was significant heterogeneity in the reactivity of AML-marrow cells from different patients to different drugs. The cell lines exhibited different patterns of sensitivity. Vincristine arrested cells in G2/M-phase, cytosine-arabinoside caused an increase of cells in the S-phase.

Key words: Cytostatic drug testing – Human leukemia – Flow cytometry – Esterase activity

Introduction

The aim of in-vitro drug testing in malignant diseases is to facilitate selection of the best treatment for the individual patient and also to evaluate new cytostatic drugs during preclinical studies. Three main assay systems can be distinguished: a) the short-

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term incubation of patient cancer cells with radioactively labeled DNA-precursors [15]; b) the colony forming assay, in which cells preincubated with cytostatic drugs are plated on agar and the development of tumor cell colonies is monitored [6]; and c) the xenotransplant system, where pieces of human malignant tumors are transplanted into nude mice and tumor growth is observed [17]. The short term assay has the advantage that results are quickly available. The assay is, however, restricted to drugs that influence the cellular DNA-metabolism within a few hours. Cell cycle specific drugs e.g. vincristine, which are important in the treatment of leukemias, cannot be evaluated [13]. Furthermore, the radiation from the labeled thymidine may influence DNA-kinetics [3]. The colony assay is time-consuming and in a significant percentage of cases no tumor cell growth is obtained, e.g. in about 50–60% of acute myeloid leukemias (AML) [5]. In addition, the clonal development in the cell culture may be different from that in the patient and different clones may have different sensitivities to cytostatic drugs [7]. Xenotransplant systems are expensive and time consuming, and not all tumors grow in the nude mouse [17].

Recently, a flow-cytometric in vitro assay has been developed [22], which screens the reduction of vital tumor cells and inflammatory cells under in-vitro cytostatic drug therapy. After an incubation period of 2–7 days, cells are harvested and stained simultaneously with two fluorescent dyes ADB and PI: a) ADB can penetrate the cell membrane of living cells and is cleaved by unspecific esterases into the fluorescent DCH, which is enriched in the cytoplasm [21]. The blue fluorescence of DCH is a measure of esterase activity and the blue to green fluorescence ratio indicates the intracellular pH of vital cells. b) PI stains the DNA of dead cells. The assay has several advantages. It can be performed with most human tumors, the results are available within 1 to 7 days, automated cell staining and measurement seems possible in the future, several parameters can be measured simultaneously and comparatively few cells are needed.

The aim of the present study was to investigate the in vitro response of human leukemia cells from bone marrow samples to five different drugs using the new assay. In addition, several human leukemia cell lines of myeloblastic, as well as T- and B-lymphocytic origin were assayed to compare the responses of cell lines and leukemic bone marrow to the cytostatic drugs.

Materials and Methods

Patient Cells

Twenty-two human bone marrow samples from 20 different patients were assayed. Five specimens could not be evaluated because too few nucleated cells ($< 10000/\mu l$) were present in the 200 μl aspirate. Seventeen samples from 16 different patients could be evaluated. Amongst these were 6 floride AML, 5 AML in complete remission, 1 acute lymphocytic leukemia (ALL) in CR, 1 NHL with bone marrow infiltration and one NHL without bone marrow infiltration, 1 smouldering leukemia (SML) and 1 pernicious anemia; 1 sample could not be classified because no diagnosis was available. Eight samples (from 7 different patients) were from male, and 9 samples were from female patients. Patient age was between 27 and 81 with a mean of 52.6 years. Cytologic diagnosis was obtained from May-Grünwald-Giemsa stained smears. Bone marrow aspirate (150–200 μl) with 10 U/ml heparin was pipetted immediately after removal from the patient into sterile 1.5 ml Eppendorf tubes. Heparinized (10 U/ml) venous blood (10 ml) was additionally taken from each patient. The supernatant plasma was removed after centrifugation of the

blood at 400×*g* for 10 min and diluted with an equal volume of RPMI 1640 medium (Gibco, Frankfurt, FRG). Bone marrow aspirate (10 µl) and 80 µl of the diluted autologous plasma were pipetted into the wells of a 96-well microtiter plate (Costar, Cambridge, MA, USA). Cytostatic drug stock solution (10 µl) (100×, 10× and 1× the concentration of the therapeutic serum concentration) were pipetted into consecutive wells to give final concentrations of 10×, 1× and 0.1× that of therapeutic serum in patients. The following drugs were used (1× therapeutic serum level): cytosine-arabinoside (Ara-C, 0.5 µg/ml) (Mack, Illertissen, FRG), L-asparaginase (0.2 U/ml) (Bayer, Leverkusen, FRG), daunorubicin (0.1 µg/ml) (Farmitalia, Freiburg, FRG), prednisone (10 µg/ml) (Merck, Darmstadt, FRG), vincristine (0.01 µg/ml) (Lilly, Gießen, FRG). RPMI 1640 (10 µl) was pipetted into the control assay instead of the cytostatic drug stock solution. The cells were incubated between 2 and 7 days, with a mean of 4 days, in an incubator at 37°C in air with 5 Vol% CO_2 and saturated humidity.

Cell Lines

The human T-cell-lines Molt, CEM and Jurkat were kindly provided by Dr. Kummer, the human promyelocytic cell line HL 60/16 by Dr. Mailhammer, both from the Institut für Hämatologie der GSF, München, FRG. The human B-cell-lines Daudi, Raji and RPMI 1788 were kind donations from Dr. Johnson and Dr. Ziegler from the Institut for Immunologie, Universität München, FRG. Exponentially growing cells were incubated in 0.9 ml RPMI 1640 with 10% FCS (Gibco), at a cell concentration of $1-4\times10^5$/ml, in a 24-well-titerplate (costar). Cytostatic drug solution (100 µl) of the above concentrations was pipetted into each test assay and 100 µl RPMI 1640 were pipetted into the control assay. The incubation period was 48 h under the same conditions as for the patient cells.

Cell-Staining

The cultured cells were washed twice in the titer-plates with 10 mM HEPES buffered saline (HBS, pH 7.35) by 5 min centrifugation at 200×*g*, followed by resuspension in 250 µl HBS. 5 µl of a dye cocktail (Cyto-P-check, Paesel, Frankfurt, FRG) containing 1 mg/ml of the esterase and intracellular pH-indicator dye 1,4-diacetoxy-2,3-dicyanobenzene (ADB) [21, 23] for vital cells and 2 mg/ml of the DNA dye propidium iodide (PI) for dead cells were added to each well. The cell suspension was stained for 5 min at room temperature. In addition, 10 µl of 5 µm diameter, NH_2-bearing, porous and monosized particles [19] at a final concentration of 2.5×10^5/ml were added as an internal counting, volume and fluorescence standard. The particles were prestained with 20 µg/ml 1,4-dicyano-2,3-hydrochinone (DCH, Paesel, Frankfurt, FRG) solution in HBS-buffer pH 7.4 for 2 h at 0°C.

Flow Cytometry

The assay was measured for 2 min in a Fluvo-Metricell flow cytometer (HEKA-Elektronik, Lambrecht/Pfalz, FRG) [8]. The cell volume was determined electrically in a hydrodynamically-focused cylindrical orifice of 85 µm diameter and 110 µm length at a current of 0.23 mA. The fluorescence of the dyes was excited between 300–400 nm with a HBO – 100 high pressure mercury arc lamp. The blue fluorescence of the DCH was collected between 418 nm to 440 nm and the green fluorescence of the DCH as well as the red fluorescence of PI between 500 nm to 680 nm. The maximum amplitude of each signal was amplified by 2.5 decade logarithmic amplifiers. The digitized amplitudes of the three simultaneously measured signals of each cell were collected on-line in list-mode on magnetic tape. The tapes were evaluated in a second step with a VAX 11/782 (Digital Equipment Corporation, Maynard, MA, USA) by means of FORTRAN programs [20]. The evaluation, described briefly, proceeds as follows. In a first step, a cloud display (Fig. 1) is calculated from the list-mode data on magnetic tape, followed by the projection of the cloud display onto the blue to green fluorescence plain of the cube (not shown). A separation line is drawn which separates the vital and blue stained cells from the dead cells which are mainly red stained by the DNA dye PI. The cube data are then reprocessed such that a cell volume versus blue fluorescence diagram of the vital cells is obtained (Fig. 2), where the blue fluorescence is a measure of the esterase activity of the vital cells. Furthermore, the cell volume versus red fluorescence of the dead cells is plotted (Fig. 3). The red fluorescence is a measure of the cellular DNA content. Surviving cells were calculated from the ratio: number of vital cells in the myeloblastic/granulopoietic area divided by the number of calibration par-

ticles. The surviving cells in the drug assay were expressed as a % of the control assays. Variance analysis was used for the statistical comparison of results obtained from normal and abnormal cell samples.

Results

The flow cytometric measurements were first displayed as a three dimensional cube where the vital and dead cells, the calibration particles and the cell debris could be distinguished (Fig. 1). In a second step the cube was divided by the computer program such that the volume versus esterase activity of the vital cells (Fig. 2) and the volume versus DNA pattern of the dead cells (Fig. 3) were separately displayed. The DNA-distribution (Fig. 4) was obtained from the volume-versus-DNA curve by projection onto the DNA-axis.

The influence of cytostatic drugs was evaluated by calculation of the surviving cells in human bone marrow cells and cell line assays. The influence of cytostatic drugs on the DNA distribution of dead cells was evaluated in the cell line assays only.

1. Human Bone Marrow Samples

Twenty-two bone marrow samples were obtained from 20 patients and incubated with the 5 various drugs previously indicated. Five out of the 22 samples i.e. 4/20 patients could not be evaluated because of nucleated cell counts of less than 10000/μl aspirate. The measurable samples were divided into two groups according to the cytologic diagnosis:

– 7 samples contained more than 20% blast cells (6: AML; 1: NHL with bone marrow infiltration ("leukemic group").

– 9 samples served as "control" samples (5: AML in complete remission; 1: ALL in CR; 1: non infiltrating NHL; 1: pernicious anemia, 1: suspected smouldering leukemia (SML) with 15% blast cells).

– 1 sample could not classified because no diagnosis was available.

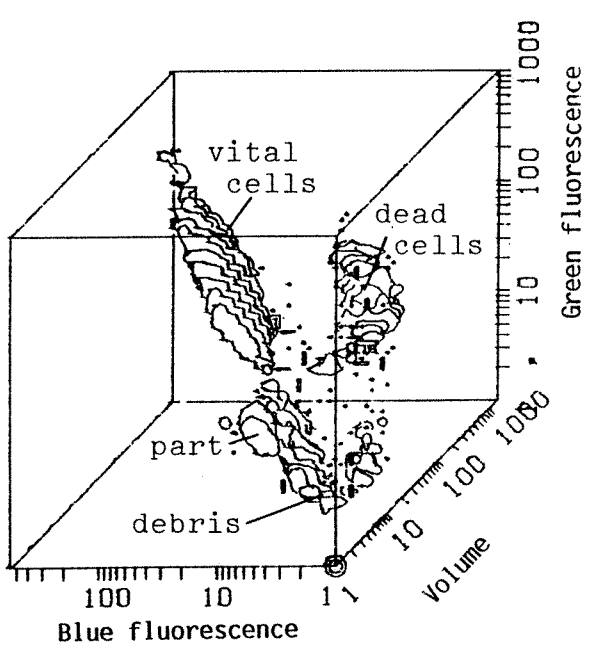

Fig. 1. RPMI 1788 B-cell line after 2 days incubation at 37°C without cytostatic drug. Vital cells and calibration particles can be distinguished from dead cells. A total of 9223 cells and particles was measured. The graph is standardized to the maximum logarithmic channel content (n = 434 particles) and contour lines are plotted at 10% of this value, which indicates a frequency of 2 cells per channel. Thus the position of 98% of all measured cells and particles (n = 9223) is indicated by the clouds

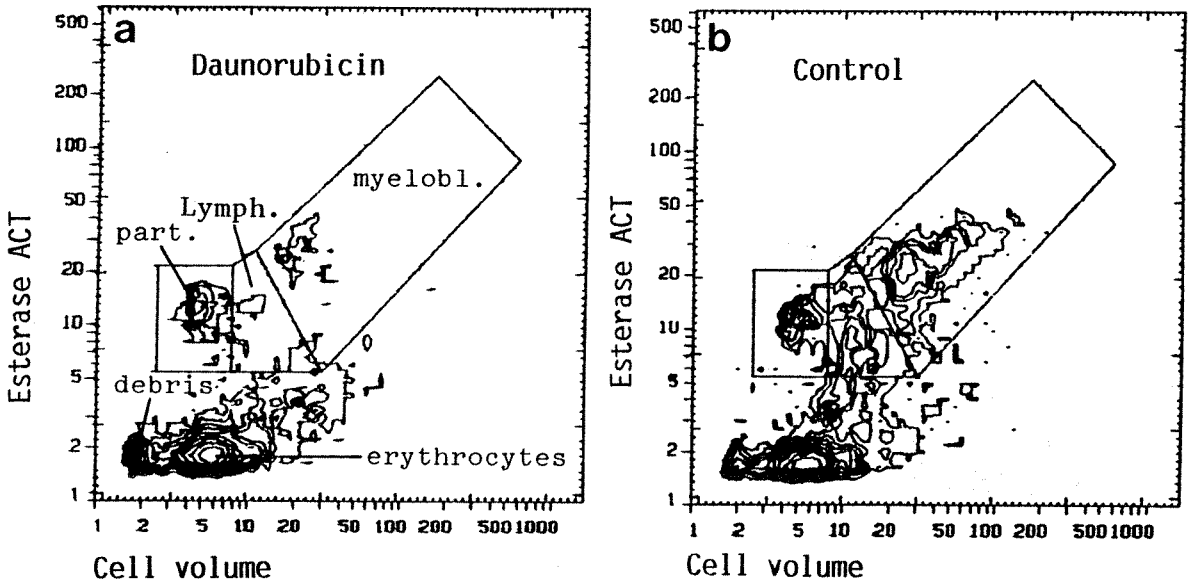

Fig. 2. Esterase activity versus cell volume of vital cells in a daunorubicin (1 μg/ml) **(a)** treated human bone marrow sample and in a control assay **(b)** from a patient with AML in complete remission. Incubation: 4 d at 37°C. The computer program evaluates the number of cells in the different areas separately. One unit on the logarithmic volume scale corresponds to a cell volume of 13 μm^3. The esterase activity is given in arbitrary units. A total of 3766 **(a)** and 4251 **(b)** cells and particles were measured. The graphs are standardized to the maximum logarithmic channel contents (100%) and contour lines are plotted at 10% intervals downwards

There was significant heterogeneity in the sensitivity pattern among patients with AML leukemic bone marrow (Fig. 5 a, 5 b, Table 1). Comparable reductions of surviving cells were seen for daunorubicin and cytosin-arabinoside in both groups. The vincristine assay provided a marked mean reduction in the number of vital cells in patients of the leukemic group compared to patients of the control group, where no significant reduction in the number of vital cells was found ($p < .05$).

Bone marrow specimens from one patient (AML) could be investigated twice at an interval of four weeks. On both occasions a significant reduction of surviving cells was found, by means of daunorubicin to 9% and 21%, and by cytosine-arabinoside to 5% and 18% of the control assay, respectively.

The median of the coefficient of variation for the intraindividually control incubations was 16%.

The DNA distributions of dead cells were not evaluated because very few dead cells (< 5%) were present in treated and untreated bone marrow specimens.

2. Cell Lines

Each cell line had its own sensitivity-pattern (Fig. 5 c, d, Table 2). The promyelocytic leukemia line HL 60/16 was incubated twice on different days. Dose-response curves were similar within 21% with regard to the surviving cells. Cells were estimated sensitive when a decrease of vital cells below 10% of the control assays was observed. According to this criterium the myeloblastic HL 60/16 line, the T-cell lines Jurkat and CEM as well as the B-cell line Raji were sensitive to drug treatment while the T-cell line Molt and the B-cell lines Daudi and RPMI 1788 were more resistant. Besides cell

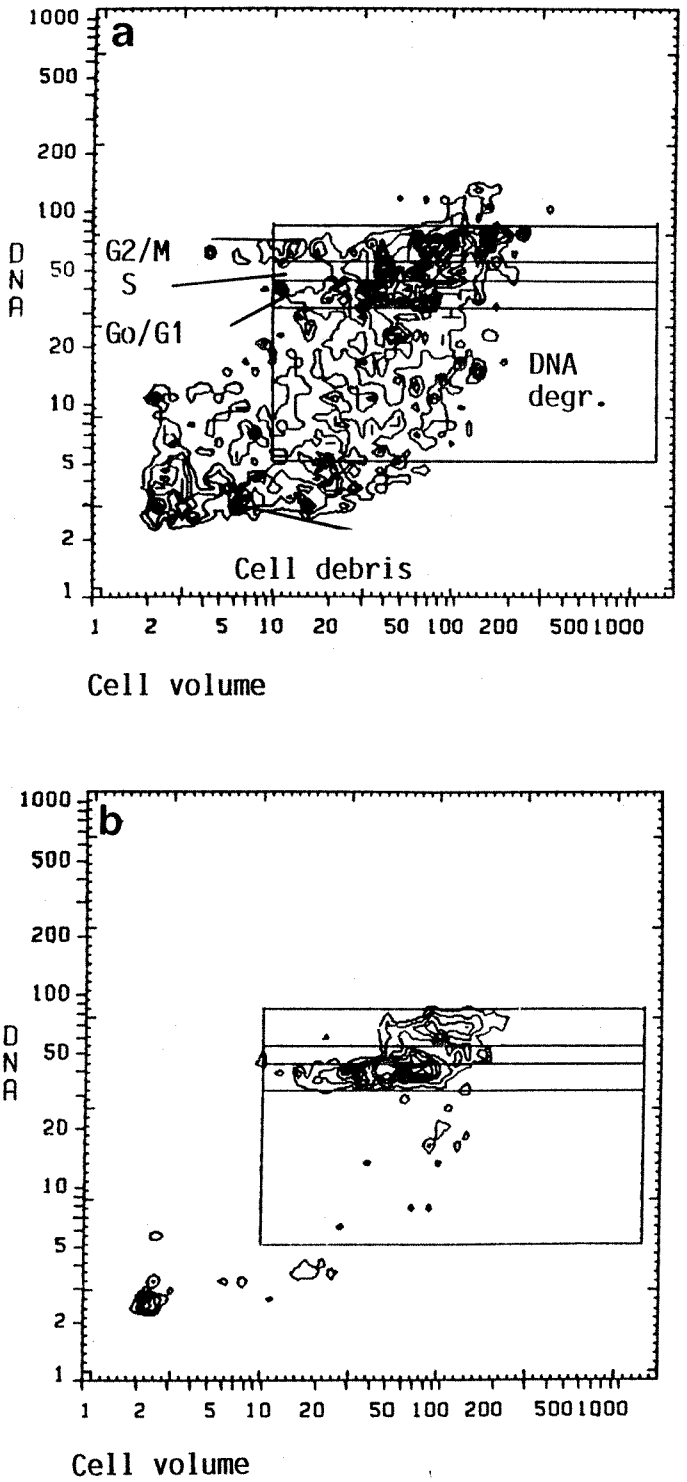

Fig. 3. Volume versus DNA-content of dead cells for the RPMI 1788 B-cell line treated with 0.1 μg/ml vincristine after 2 d incubation **(a)** and of the control incubation without cytostatic drugs **(b)**. The peak of G0/G1 phase is automatically found by the DIAGNOS 1 program system [24]. The limits of the G0/G1 are set 2 DNA histogram channels below and above the maximum fluorescence channel of the G0/G1 cell cycle phase. The upward adjacent area represents the S and G2/M phase (6 DNA channels) of the cell cycle. The lower compartment contains the autolysing dead cells where the DNA is already partially digested. The DNA-distributions are compressed by the logarithmic amplification, necessary for the simultaneous measurement of vital and dead cells

survival, changes in DNA-distribution could be detected, namely accumulation of G2/M-phase cells by means of vincristine and of S-phase cells by means of cytosine-arabinoside for the RPMI 1788 cell line (Fig. 4). Vital cells of the HL 60/16 cell line, furthermore, showed a significant increase in cell volume in the daunorubicin assay. Vital cells of the daunorubicin assay (0.1 μg/ml) provided a mean volume of 2470 μm^3 while vital cells of the 4 control assays had only a mean volume of 1300 μm^3. The volume increase was reproducible.

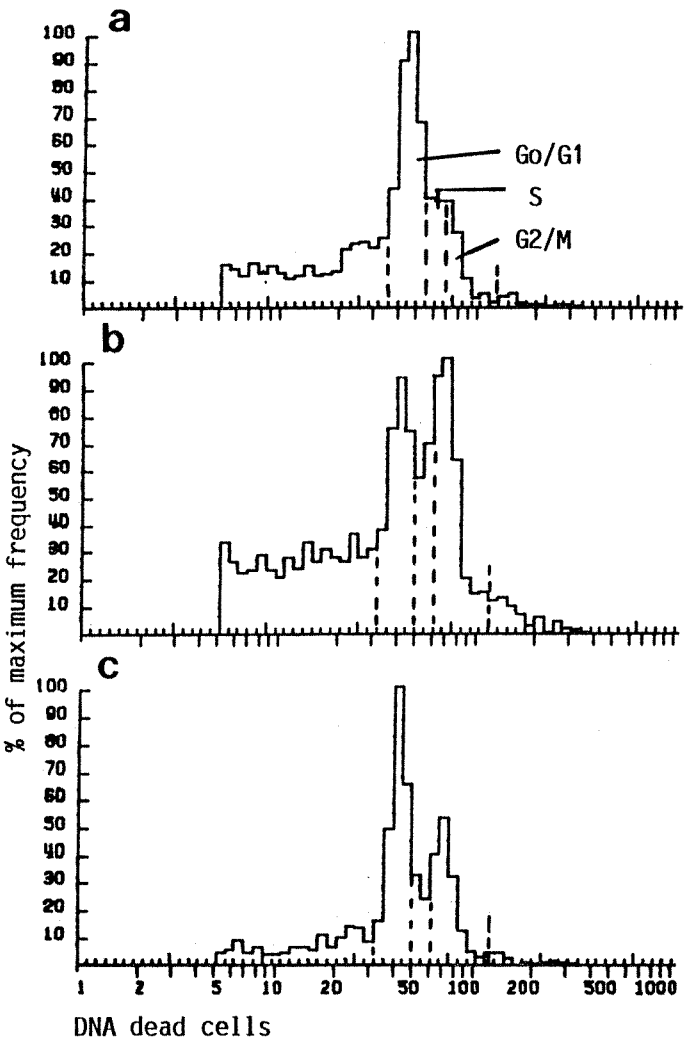

Fig. 4. DNA-distribution curves of the dead cells of an ara-c (5 μg/ml) **(a)**, vincristine (0.1 μg per ml) **(b)** and untreated control assay **(c)** of RPMI 1788 B-cells. The graphs **b** and **c** represent the projection of the histograms of Fig. 3 a, 3 b onto the DNA axis. The DNA distribution is plotted on a logarithmic scale. The *dashed lines* indicate the left and right thresholds of the G0/G1- phase, of the S-phase and of the G2/M-phase of the cell cycle. The thresholds were set as indicated in Fig. 3. The graphs are standardized to the maximum of cell frequency (**a** = 169, **b** = 128 und **c** = 186)

Discussion

The results of the study show that 200 μl bone marrow aspirate with 2×10^6 nucleated cells are sufficient to assay the effects of 5 cytostatic drugs on 78% of the bone marrow aspirates of leukemia patients. In cases with < 10.000, nucleated cells/μl aspirate (5 out of 22 patient samples), it is likely that an increase of aspirate volume to reach ≥ 100.000 nucleated cells per assay will overcome the problem of insufficient cell numbers. The proposed assay can, therefore, be performed in the majority of patients.

AML cells from different patients have different in vitro susceptibility towards cytostatic drugs. This is evident from the broad distribution of percent surviving cells in the assay (Table 1). The quantitative evaluation showed good reproducibility for the number of surviving cells for the cell lines as well as for the patient samples. The median coefficient of variation for all intraindividual control assays was 16% and for the cell lines 20%. Furthermore, bone marrow from one patient could be investigated on two different occasions; the cells from both patient samples were found to exhibit sensitivity towards daunorubicin and cytosine-arabinoside. The reproducibility was also found good in previous studies on malignant solid tumors, ascites and pleural effusions [22, 25]. It is, therefore, believed that a different degree of the reduction of

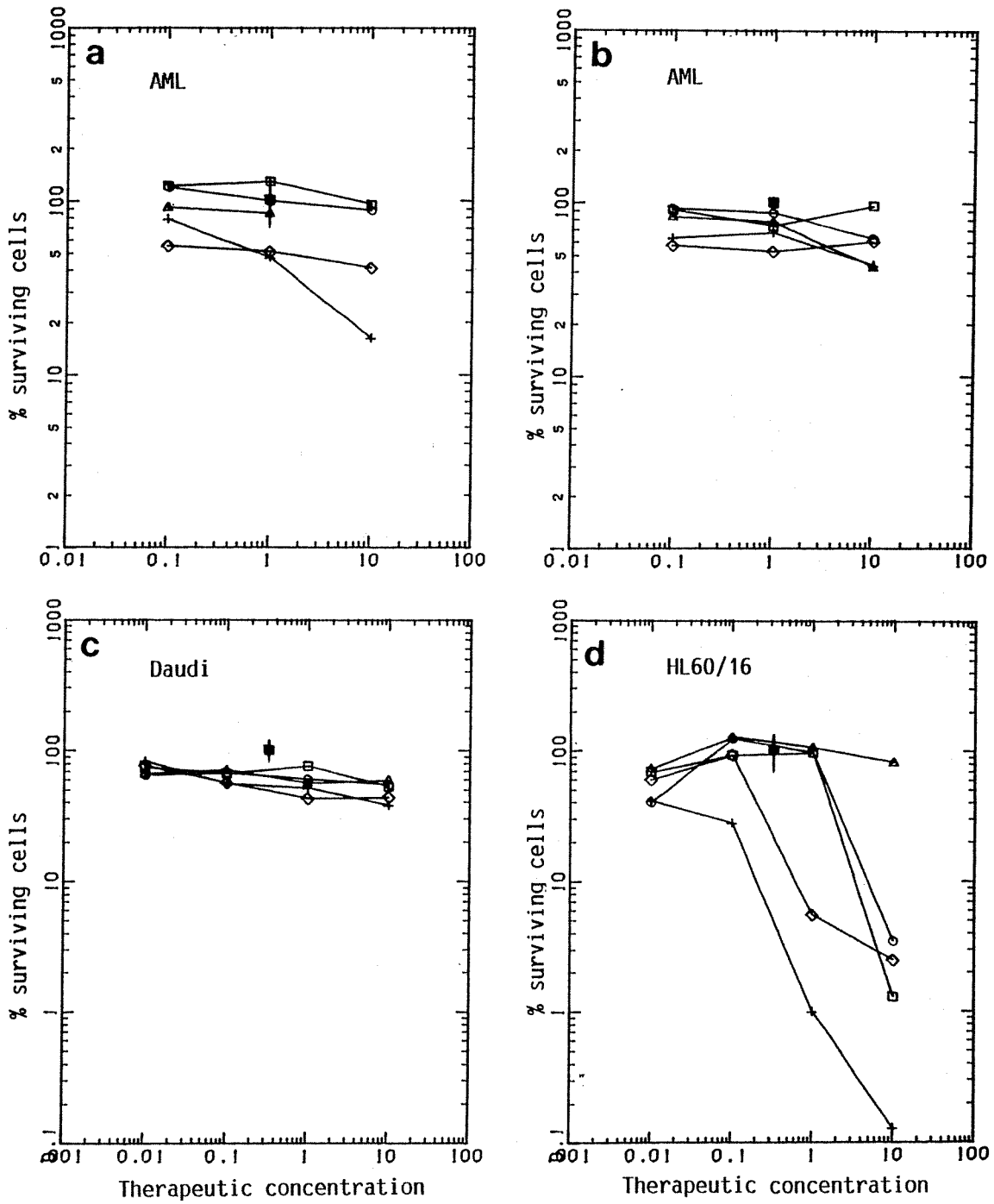

Fig. 5. Dose response curves for two different samples of AML **(a, b)** and two different human cell lines Daudi **(c)** and HL 60/16 **(d)**. The figure shows the percent surviving cells versus concentration of cytostatic drugs. (○) cytosine-arabinoside (0.5 μg/ml 1× therapeutic concentration); (□) l-asparaginase (0.2 U/ml); (+) daunorubicin (0.1 μg/ml); (△) prednisone (10 μg/ml); (◇) vincristine (0.01 μg/ml); (■) 4 control samples +/− SD

Table 1. Sensitivity pattern of patient bone marrow cells towards cytostatic drugs. Given are the percent surviving cells of bone marrow cells from leukemic patients ($n = 7$; 6: AML; 1: NHL with bone marrow infiltration) (*upper half*) as well as from patients in complete remission and with benign diseases ($n = 9$, 5: AML in CR; 1: ALL in CR; 1: non infiltrating NHL; 1: pernicious anemia; 1: suspected smouldering leukemia with 15% blasts) (*lower half*). Data are expressed in percent vital cells as compared to the control assays incubated without cytostatic drugs. Ara-c and daunorubicin were cytotoxic in both groups. Vincristine was significantly more effective in the leukemic cell samples ($p < 0.05$).
PA: pernicious anemia; ne: not evaluable

Diagnosis	%blasts	Surviving cells (%)					
		Ara-c 5 μg/ml	Aspa 2 U/ml	Dauno 1 μg/ml	Predni 100 μg/ml	Vincri 0.1 μg/ml	Mean of controls ($n = 3$) (SD)
Leukemic Patients							
AML	60	5	109	21	130	120	100 (27)
AML	23	51	61	26	94	54	100 (1)
AML	50	63	97	44	43	61	100 (7)
AML	60	98	90	34	127	41	100 (21)
AML	60	63	94	42	59	ne	100 (61)
AML	70	88	95	16	ne	41	100 (27)
AML mean (SD)	($n = 6$)	61 (32)	91 (15)	30 (12)	90 (42)	63 (34)	100 (24)
NHL	70	33	29	15	37	14	100 (1)
CR or normal patients							
AML	< 5	49	59	60	90	77	100 (7)
AML	< 5	18	106	9	90	140	100 (40)
AML	< 5	29	54	4	75	86	100 (40)
AML	< 5	55	57	20	76	67	100 (25)
AML	< 5	56	38	78	48	61	100 (4)
SML	15	66	79	21	85	ne	100 (20)
ALL	< 5	83	138	43	122	104	100 (13)
NHL	< 5	100	ne	54	98	103	100 (8)
PA	< 5	52	57	14	58	99	100 (17)
Mean (SD)	($n = 9$):	56 (24)	73 (33)	33 (24)	82 (21)	92 (23)	100 (19)

the number of surviving cells in vitro reflects the individual sensitivity of the patient cells towards cytostatic drugs. The in vitro sensitivity varied from patient to patient in a significant manner. Other investigators have found similar heterogeneity among individual patient's cells in AML for the colony forming assay [14] as well as for the short term assay [16]. Regarding the cell lines, it is of interest that drug resistance and sensitivity was largely independent of the T-, B-cell or myeloid origin of the cells (Table 2). This is probably not due to different proliferation rates, because all cell lines investigated were exponentially growing cells. RPMI 1788, Daudi and Molt cell lines were resistant, whereas HL 60/16, CEM, Jurkat and Raji cells were sensitive to in-vitro cytostatic treatment. The results with the cell lines show that cytostatic drug test-

Table 2. Sensitivity pattern of different cell lines towards 5 cytostatic drugs. The data are expressed as surviving cells at 10× the therapeutic plasma levels in % of the control assays (= 100%) incubated without drugs. Cell line HL 60/16 was investigated on two different days

	Surviving cells (%)					
Cell line	Ara-c 5 μg/ml	Aspa 2 U/ml	Dauno 1 μg/ml	Predni 100 μg/ml	Vincri 0.1 μg/ml	Mean of controls ($n = 4$) (SD)
Myeloblastic cells						
HL 60/16 I	3	1	1	85	2	100 (13)
II	4	1	1	73	3	100 (33)
B-cells						
RPMI 1788	42	67	23	86	42	100 (6)
DAUDI	55	53	38	60	44	100 (14)
RAJI	18	43	17	92	7	100 (12)
T-cells						
MOLT	21	60	20	190	93	100 (26)
JURKAT	22	28	5	31	22	100 (32)
CEM	4	9	6	52	34	100 (32)

ing in cell lines cannot replace individual testing in patient cells, e.g. in preclinical drug testing.

The increase of G2/M-phase cells in the DNA-distribution was found in all cell lines treated with vincristine (Figs. 3 and 4). Cytosine-arabinoside caused an increase in cells of the S-phase (Fig. 4). This confirms that cytosine-arabinoside acts predominantly on cells in DNA-synthesis [9]. The swelling of surviving HL 60/16 cells to approximately twice the cell volume of control cells in the presence of daunorubicin is of interest. It indicates an effect of the drug on vital cells which is, however, not strong enough to kill them. Whether the cell swelling is caused by damage in ion fluxes or whether metabolic changes are of importance remains to be investigated.

A broad distribution of sensitivity pattern was observed in the 17 patient samples assayed (Fig. 5 a, b, Table 1). It is important to note that leukemic cells were mainly located in the myeloblastic area (Fig. 2 a, b). With the exception of aneuploidy, which occurs in about 15–25% of acute nonlymphocytic leukemias [1, 2, 10, 18], it is not possible without further labels (e.g. fluorescence labeled antibodies) to distinguish between normal myeloblasts and leukemic cells in the assay. It is, therefore, important that the bone marrow aspirates are screened morphologically before sensitivity or resistance of bone marrow cells to cytostatic treatment is interpreted as being specific to leukemic cells. From our experience more than 50% of the nucleated cells should be blast cells to be sure that drug effects on leukemic cells are screened. The sensitivity of normal bone marrow cells varies from patient to patient. It seems of interest to screen bone marrow cells of patients in their leukemic phase and during complete remission.

Unlike the situation in the cell lines, where between 5% and 90% dead cells were present in treated and untreated cultures, very few dead cells were present in the bone marrow aspirates (< 5%) initially and also after cell culture. It was, therefore, diffi-

cult to obtain enough dead cells to establish DNA-distribution curves in fresh cell preparations. The cells had to be fixed in 70% methanol to obtain sufficient cells for DNA measurements. The low degree of dead cells indicates the capacity of either the remaining bone marrow cells to completely digest dead cells in-vitro or of the dead cells to autolyse by intra- and extracellular liberation of enzymes.

When the results from patients with leukemic bone marrow cells were compared to those in complete remission, a significant increase in the vincristine sensitivity was observed for leukemic cells (Table 1). Thus the well known low myelotoxicity of vincristine for normal bone marrow cells and also the well known efficiency of vincristine in reducing leukemic cells is demonstrable by the in vitro assay. The important issue of whether the in vitro sensitivity results of this assay correlates with the clinical sensitivity towards cytostatic treatment cannot be answered at the present time. More samples have to be measured and the clinical course of the disease in each patient has to be correlated with the results. Nevertheless it is believed that this assay has a potential for clinical application; comparatively few cells are needed; practically all cytostatic drugs or their active metabolites as well as combinations of cytostatic drugs may be assayed; the assay conditions are as close as possible to the situation in the patient with regard to the culture medium, temperature and gas phase. The flow-cytometric assay, like the other assays, is mainly intended to detect patients who do not respond to cytostatic drug treatment in vitro, since it is commonly accepted that in vitro cytostatic drug resistance of malignant cells is equivalent to the in-vivo resistance [4, 5, 12, 14, 16]. Unnecessary drugs may be removed from the combination therapy in such cases.

It can be argued that the stem cell assay is superior to other in vitro systems because the proliferative potential of the tumor cells is screened. This is especially important in fast proliferating acute leukemias. However, in practice not only the sensitivity of stem cells, which are a minority of the malignant cells, but also the immediate reduction of tumor mass is a goal of therapy, especially in slowly growing tumors such as chronic leukemias. A practical disadvantage of the stem cell assay is that it takes several weeks to establish cell lines from AML bone marrow samples [5]. This is too long for therapy, which often has to be started as quickly as possible. Moreover not all samples grow to permanent cultures [5]. The flow cytometric assay screens the number of surviving cells as a measure of tumor load. It can be rapidly performed in practically all acute and chronic leukemias. Lower cell numbers are required in this assay compared to the stem cell as well as the short term assay. Biochemical information on drug resistant surviving and cell cycle properties of the killed cells is obtained without additional efforts.

Since the evaluation of the assay is already automated, it seems important to automate sample staining and measurement, which is essential for routine clinical application [11].

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