Mdr1/P-glycoprotein, topoisomerase, and glutathione-S-transferase \( \pi \) gene expression in primary and relapsed state adult and childhood leukaemias

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Summary In a variety of adult and childhood leukaemia cell samples collected at different states of the disease, we analysed in a series of sequentially performed slot-blot or Northern-blot hybridisation experiments the expression of genes possibly involved in multiple drug resistance (MDR) (mdr1/P-glycoprotein, DNA topoisomerase II, glutathione-S-transferase \( \pi \)), and the expression of the DNA topoisomerase I and histone 3.1 genes. Occasionally, P-glycoprotein gene expression was additionally examined by indirect immunocytofluorescence using the monoclonal antibody C219. No significant difference in mdr1/P-glycoprotein mRNA levels between primary and relapsed state acute lymphocytic leukaemias (ALL) was seen on average. Second or third relapses, however, showed a distinct tendency to an elevated expression of this multidrug transporter gene (up to 10-fold) in part well beyond the value seen in the moderately cross-resistant T-lymphoblastoid CCRF-CEM subline CCRF VCR 100. Increased mdr1/P-glycoprotein mRNA levels were also found in relapsed state acute myelogenous leukaemias (AML), and in chronic lymphocytic leukaemias (CLL) treated with chlorambucil or prednisone for several years. Topoisomerase I and topoisomerase II mRNA levels were found to be very variable. Whereas in all but one case of CLL topoisomerase II mRNA was not detected by slot-blot hybridisations, strong topoisomerase I and topoisomerase II gene expression levels, frequently exceeding the levels monitored in the CCRF-CEM cell line, were seen in many cell samples of acute leukaemia. If topoisomerase II mRNA was undetectable, expression of topoisomerase I was clearly visible throughout. These observations might be valuable considering the possible treatment with specific topoisomerase I or topoisomerase II inhibitors. Significant positive correlations were found (i) for topoisomerase I and histone 3.1 gene expression levels in general (\( P < 0.001 \)), and (ii) in the CLL samples additionally for the expression levels of the mdr1 gene, and the histone 3.1, topoisomerase I, and glutathione-S-transferase \( \pi \) genes, respectively.

Failure of chemotherapy during treatment of leukaemia is supposedly caused by the resistance of the tumour cells to antineoplastic drugs (Goldie & Coldman, 1984). Multidrug resistant phenotypes of cultured cells selected in \textit{vitro} have been intensely studied in recent years. Two different mechanisms conferring resistance on these cells to a wide variety of structurally unrelated cytotoxic agents have been identified so far at the molecular level, (i) the enhanced expression of the \textit{mdr1} gene coding for an ATP-dependent, transmembrane drug efflux pump called P-glycoprotein, and (ii) an altered activity of the DNA topoisomerase II, a nuclear enzyme possessing DNA double-strand passing activity by an ATP-dependent cleaving and rejoining process. 'Classical' multiple drug resistance (MDR) of cell lines selected in \textit{vitro} is basically mediated by the P-glycoprotein (for review see Endicott & Ling, 1989). The so-called 'atypical' MDR (at-MDR) where the numerous topoisomerase II inhibitors are affected could be associated with a quantitatively or qualitatively altered activity of topoisomerase II (Pommier et al., 1986; Fernandes et al., 1990; DeJong et al., 1990). Other investigations, however, point to further as yet unrecognised mechanisms or a multifactorial emergence of MDR of \textit{in vitro} selected cell lines (McGrath & Center, 1988; Defieu et al., 1988; Harker et al., 1989).

Clinical success of cancer therapy is strongly linked to the type and status of the tumour. This is exemplified by haematological malignancies. In general, childhood acute lymphoblastic leukaemias (ALL) usually respond well to complex protocols of intermittent chemotherapy applying combinations of various antineoplastic drugs, whereas the prognosis for adult ALL is worse. Showing lower curability than ALL the childhood acute myelogenous leukaemias (AML) are still far better responding than adult AML. On the other hand, chronic lymphocytic leukaemias (CLL) which are only seen in adults usually receive either no chemotherapy at all, or if the disease is in progress, a combination of an alkylating agent and prednisone. A cure of CLL is usually not possible. In the case of the most prevalent childhood leukaemia, the acute lymphoblastic leukaemia, currently only about 20% relapse. Without bone marrow transplantation, the prognosis of relapsed leukaemia at present is bad, which actually might be due to the emergence of tumour cells less sensitive to antineoplastic agents. The molecular mechanisms of this clinically observed refractoriness of tumours to chemotherapy are still little understood. Several observations, however, point to the involvement of the P-glycoprotein, as (i) the unresponsiveness of tumours derived from tissues with an intrinsically high P-glycoprotein expression (Fojo et al., 1987), (ii) the emergence of P-glycoprotein expression in specimens of relapsed state malignancies after chemotherapy (Ma et al., 1987; Goldstein et al., 1989; Volm et al., 1989; Musto et al., 1990; Pirker et al., 1991), and (iii) reports on the successful treatment of drug-resistant tumours by including the calcium channel blocker P-glycoprotein binding drug verapamil in chemotherapy protocols (Dalton et al., 1989). However, other studies do not support the view of a frequently occurring link between elevated P-glycoprotein levels and therapy failures of leukaemias (Ito et al., 1989; Ubezio et al., 1989). Moreover, many clinically used antineoplastic drugs like ara C, cisplatin, or most alkylating agents do not even belong to the group of agents involved in the MDR-phenotype of cells selected \textit{in vitro}. Hence, different mechanisms conferring drug resistance on tumour cells \textit{in vivo} have to be considered.

Many antineoplastic drugs used clinically are inhibitors of the topoisomerase II and substrates for the P-glycoprotein mediated efflux as well, for instance, anthracyclines like adriamycin, or epipodophyllotoxins like teniposide. It has been suggested that the clinical response to epipodophyllotoxins could be dependent on a cell's topoisomerase II level. These drugs apparently stabilise an intermediate in the topoisomerase II catalysed reaction with the consequence of DNA
damage which might be lethal to the cell. Thus, reduced levels of the topoisomerase II could confer resistance to drugs targeted at the topoisomerase II by giving better chances to the cell's DNA repair systems (Holden et al., 1990). On the other hand, high topoisomerase II levels vice versa might be indicative for a good response of tumours to these kinds of drugs (Sullivan et al., 1987; Davies et al., 1988). Topoisomerase I is another nuclear enzyme involved in the regulation of DNA topology by relaxation of super-coils in an ATP-independent process (Liu, 1989). Camptothecins are specific inhibitors of topoisomerase I which are currently under investigation for use as antineoplastic drugs (Giovanella et al., 1989), and appear not to be affected in multidrug resistance (Chen et al., 1991).

The activity of the glutathione-S-transferase η was discussed as a further mechanism contributing to the MDR phenotype (Batist et al., 1986). It might, however, rather be involved in resistance of tumour cells to alkylating agents or oxidative stress (Fairchild et al., 1990; Waxman, 1990). The expression of the cell cycle regulated histone 3.1 gene represents an indicator of DNA synthesis activity (Stein et al., 1984; Venturelli et al., 1988) which is an important parameter, because DNA replication is one of the main targets of antineoplastic drugs.

Considering the possible complex genesis of a clinically observed refractoriness of tumours to treatment we analysed the expression of the mdr1/P-glycoprotein gene together with the variety of other genes in different types of leukaemias, at several times during progression of the disease. This might be helpful for the evaluation of drug resistance or an enhanced vulnerability of leukaemic cells to certain antineoplastic agents.

Materials and methods

Materials

Blotting membranes (Hybond N+, Hybond N), labelling kits (Multiprime DNA Labelling System), α-32P]dCTP (specific activity >3000 Ci mmol⁻¹; 1 Ci = 37 GBq) were obtained from Amersham (Braunschweig/Germany). All other chemicals, supplies and tissue culture media were of the purest grade and were purchased from commercial sources.

Cell lines and leukaemia cells samples

The human T-lymphoblastoid cell line CCRF-CEM was obtained from the American Type Culture Collection, Rockville, MD/USA (ATCC CCL 119). The selection of multidrug-resistant CCRF-CEM sublines was published elsewhere (Gekeler et al., 1988; Niethammer et al., 1989). The cell lines used in this work were designated CCRF VCR 100, CCRF VCR 1000, and CCRF ACTD 400 according to the final vincristine or actinomycin D concentration (in ng ml⁻¹), respectively, used for selection and maintenance. A ‘revertant’ subline designated CCRF ACTD (REV) was maintained for more than a year without the drug. It showed a substantial decrease, though not a complete loss of resistance (Kimmig et al., 1990). Peripheral blood specimens or bone marrow aspirates were collected from normal donors and patients suffering from leukaemia in heparin without stabiliser. The mononuclear cells were concentrated by a standard Ficoll-Hypaque technique (Lymphoprep, Nycomed, Oslo/Norway), washed twice, frozen in the presence of 7% DMSO under controlled conditions, and stored in liquid nitrogen until used for analysis.

RNA isolation and analysis

Total cellular RNA was extracted from the cell samples by lysis in guanidine thiocyanate, followed by centrifugation through cesium chloride (Chirgwin et al., 1979). The concentration of RNA in each sample was determined spectrophotometrically. Routinely, the quality of each RNA sample was monitored by ethidium bromide staining after electrophoresis in a 1% agarose/6% formaldehyde gel. For slot-blot hybridisations, 2.5 μg of each RNA sample were fixed onto Hybond N⁺ membranes using the Minifold II slot blotting apparatus (Schleicher & Schüll, Dassel/Germany). For Northern-blot hybridisations 5 μg of total cellular RNA were electrophoresed in a 1% agarose/6% formaldehyde gel, and transferred by electroblotting onto Hybond N⁺ membranes as recombinant (Amersham, Braunschweig/Germany). The RNA was fixed by UV-irradiation of the wet membranes using the Stratalinker 1800 (Stratagene, La Jolla/USA) as recommended by the supplier. Additionally, the membranes were baked thereafter at 80°C for 2 h. Thus, no significant loss of signal intensities were found after reprobing the filters up to six times.

As hybridisation probes we used the gel purified inserts of (i) the plasmid pCD containing a 699 bp EcoRI cDNA fragment starting from position 177 of the human P-glycoprotein mdr1 gene (Chen et al., 1986), (ii) the plasmid p3.2.4(M) containing a 2.2 kb cDNA EcoRI fragment of the human topoisomerase I gene (Romig & Richter, 1990), (iii) the plasmid pHOP2-1 containing a 2.4 kb cDNA EcoRI fragment of the human topoisomerase II gene (Tsai-Pflugfelder et al., 1988), (iv) the plasmid pLK288 containing a 1.7 kb EcoRI fragment of the human histone 3.1 gene, (v) the plasmid pGFP2 containing a 708 bp EcoRI cDNA fragment of the human glutathione-S-transferase class η gene (Kano et al., 1987), and (vi) the plasmid pHBF-A1 containing a 2 kb BamHI fragment of the human β-actin cDNA (Gunning et al., 1983). The probes were labelled with [α-32P]dCTP with a specific activity of 1·2·10⁶ d.p.m. μg⁻¹ by ‘oligolabelling’ (Feinberg & Vogelstein, 1983), and used at a concentration of 1·2·10⁶ d.p.m. ml⁻¹. The hybridisation procedure was performed as communicated earlier (Gekeler et al., 1988), besides 5% instead of 7% SDS (SDS = sodium dodecyl sulphate) were used. The filters were washed to a final stringency of 0.1 × SSC/0.1% SDS at 65°C (SSC = 0.15 M sodium chloride, 0.015 M sodium citrate), and autoradiographed with Hyperfilm MP (Amersham, Braunschweig/Germany) at −80°C. For the quantitative evaluation of the autoradiographs by a Ultrascan XL 2222-20 laserdensitometer (Pharmacia-LKB, Freiburg/Germany) the films were exposed without intensifying screens. As size markers we used RNA-ladders purchased from Boehringer Mannheim/Germany or Gibco-BRL, Freiburg/Germany.

In pilot slot-blot hybridisation experiments we tested the performance of the mdr1 signal intensities using Hybond N⁺ on the Hybond N⁺ membrane of the leukaemia (Amersham) and in relation to the amount of mdr1 mRNA in the sample. Therefore, 100 ng to 15 μg of total RNA obtained from the multidrug-resistant CCRF-CEM subline CCRF ACTD 400 were fixed onto the membranes as described. In the lower range (100 ng to 2.5 μg) Escherichia coli tRNA (Boehringer Mannheim) was added up to a final amount of 2.5 μg. Using the Hybond N⁺ membrane stronger signal intensities together with a better approximation to a linear relationship between the amount of mRNA loaded and the signal intensity were obtained (data not shown).

Indirect immunocytocytosinfluorescence

Cell suspensions of the CCRF-CEM cell lines or mononuclear cell fractions of the leukaemias were washed twice in ice-cold 0.9% sodium chloride, spotted onto gelatine coated slides and fixed in −20°C cold acetone (fluorescence free, Merck, Darmstadt/Germany) and stored at −80°C. According to Volm et al. (1989) we used the streptavidin-biotin-phycoerythrin method (Amersham). The fixed cells were incubated for 2 h with the monoclonal P-glycoprotein-specific antibody C219 at a concentration of 10 μg ml⁻¹ (Isotopen Diagnostik CIS, Dreieich/Germany). After washing, the cells were incubated with biotinylated sheep-anti-mouse secondary antibody, and then with the streptavidin-biotinylated-R-phycoerythrin-complex (Amersham). After addition of a stabiliser (Amersham) to prevent rapid fading of the
phycoerythrin-fluorescence, the slides were dried and cover-slip ped. For control, aliquots of the same cell samples were stained using mouse isotype IgG2a (Coulter Electronics, Krefeld/Germany) instead of the P-glycoprotein-specific antibody C219. Total cells were visualised by phase contrast microscopy.

Statistical analysis

The statistical evaluations were made by student’s t-test. Evaluation of the relationship between the expression values of two genes was done applying Spearman's rank order correlation test.

Results

In series of sequentially performed slot-blot hybridisation experiments the RNA samples prepared from cell lines, healthy donors or leukaemia cell samples were evaluated for gene expression using the mdr1/P-glycoprotein, topoisomerase I, topoisomerase II, histon 3.1, glutathione-S-transferase class, or β-actin specific gene probes. To control the amount of the RNA samples fixed on the membranes we routinely used the hybridisation signal obtained with the β-actin gene probe. This appeared justified according to Venturelli et al. (1988). The hybridisation signal intensities of the samples were compared to the signal intensities obtained with RNA of the multidrug-resistant subline CCRF VCR 100 which were arbitrarily set 100.

A fraction of typical slot-blot hybridisation experiments is shown in Figure 1a and b. The signals seen after sequentially hybridising one and the same membrane with the various gene probes are presented. Part of the RNA samples were additionally analysed by Northern-blot hybridisations as exemplified in Figure 2. Gene expression levels monitored by either method corresponded quite well. So, slot-blot hybridisation was the method of choice for the repeated evaluation of the numerous samples. The results are summarised in the Tables 1–IV. The values listed are usually means obtained in several independently performed experiments. Excepting signal intensities scoring below about 50 arbitrary units, standard deviations were usually less than 20%. The significance of differences will be notified, if it appears important.

The gene expression levels found in the T-lymphoblastoid cell lines and PBMC collected from healthy donors are listed in Table 1. The moderately multidrug-resistant sublines
Figure 1  a. Sequentially performed slot-blot hybridisations of one and the same membrane loaded with RNA prepared from the T-lymphoblastoid cell line CCRF-CEM, multidrug-resistant CCRF sublines, healthy donors, and various cell samples from leukaemias (see Tables) using mdr1/P-glycoprotein, topoisomerase I, topoisomerase II, histone 3.1, and β-actin specific probes of the human genes as described above. b. Sequentially performed slot-blot hybridisations using mdr1/P-glycoprotein, glutathione-S-transferase π and β-actin specific probes.

Figure 2  Sequentially performed Northern-blot hybridisations of RNA prepared from various cell samples from leukaemias (see Tables) using topoisomerase II, topoisomerase I, β-actin, and histone 3.1 specific gene probes.
Table I  Gene expression in multidrug-resistant CCRF-CEM sublines, and peripheral blood mononuclear cells (PBMC) from healthy donors

| Cell sample         | mdr1/P-gp    | Topo II | Topo I | His 3.1 | Gst-π |
|---------------------|--------------|---------|--------|---------|-------|
| CCRF-CEM            | 21/ICF: (−)  | 299     | 127    | 111     | 98    |
| CCRF VCR 100        | 100/ICF: (+) | 100     | 100    | 100     | 100   |
| CCRF ACTD (REV)     | 159/ICF: (+) | 202     | 98     | 109     | 150   |
| Donor A             | 21/ICF: (+)  | (−)     | 117    | 30      | na    |
| Donor B             | 25/ICF: (+)  | (−)     | 85     | 25      | 35    |
| Donor C             | 28/ICF: (+)  | (−)     | 98     | 65      | 38    |

Mdr1/P-glycoprotein (P-gp), topoisomerase (Topo), histone 3.1 (His 3.1), and glutathione-S-transferase π (Gst-π) mRNA levels were estimated as described above.

The signal intensities obtained with material of the multidrug-resistant cell line CCRF VCR 100 were arbitrarily set 100. The values listed are usually means originating from up to seven independent experiments.

P-glycoprotein expression was additionally examined by indirect immunocytofluorescence (ICF) using the monoclonal antibody C219. If no other data were presented, RNA preparation was not possible. The signal intensities were scaled as follows: (−), no stained cell or hybridisation signal visible; (+), weak or moderately, but heterogeneously stained (<25% of the cells scored positive); (+), weakly, or homogeneously stained, ++, moderately or strongly, rather homogeneously stained. na, not assayed.

Table II  Gene expression in acute lymphoblastic leukaemias (ALL)

| Patient     | Age (years) | Blasts (%) | Diagnosis/Status | mdr1/P-gp | Topo II | Topo I | His 3.1 | Gst-π |
|-------------|-------------|------------|------------------|-----------|---------|--------|---------|-------|
| (adult ALL) |             |            |                  |           |         |        |         |       |
| ALL 1 (SK)  | 22          | 90         | cALL, 2nd relapse after BMT | 21        | 362     | 144    | 124     | 90    |
| ALL 2 (BU)  | 25          | 80         | B-ALL, 1st relapse    | 26        | 94      | 206    | 154     | 90    |
| ALL 3 (TO)  | 21          | 80         | ALL, primary leukaemia | 44/ICF: (−) | 98      | 244    | 293     | na    |
| ALL 4-1 (DB) | 34         | 80         | ALL, 1st relapse     | 31        | 109     | 121    | 24      | 61    |
| ALL 4-2 (am) | 34.2     | 2         | remission            | 102       | 258     | 363    | 197     | 43    |
| ALL 4-2b    | 34.2        | none       | remission            | 58        | (−)     | 190    | 18      | na    |
| ALL 4-3     | 34.4        | 35         | 2nd relapse          | 42        | 137     | 108    | na      | 41    |
| (childhood ALL) |         |            |                  |           |         |        |         |       |
| ALL 5 (EW)  | 6.9         | 90         | ALL, primary leukaemia | 144      | na      | na     | na      | 59    |
| ALL 6 (AS)  | 10.7        | 70         | ALL, primary leukaemia | 88       | na      | 734    | na      | 32    |
| ALL 7 (MKA) | 6.1         | 100        | ALL, primary leukaemia | 119      | 201     | 361    | 172     | 46    |
| ALL 8-1 (JB) | 12.3      | 100        | ALL, 1st relapse after 3 years (prior to treatment) | 89/ICF: (+) | 260     | 182    | 90      | 79    |
| ALL 8-2     | 12.4        | 40         | 2nd relapse          | ICF: (+)  |         |        |         |       |
| ALL 9-1 (RK) | 12.9      | 84         | ALL, 1st relapse     | 63        | 72      | 244    | 203     | na    |
| ALL 9-2     | 13.2        | 98         | 2nd relapse          | ICF: (+)  |         |        |         |       |
| ALL 10 (CST) | 8.6       | 90         | ALL, 1st relapse     | 67/ICF: (+) | 252     | 103    | 73      | 46    |
| ALL 11 (DP)  | 0.7        | 94         | ALL, 1st relapse     | 102/ICF: (+) | 62      | 118    | 101     | 53    |
| ALL 12 (UK)  | 17.7       | 93         | ALL, 1st relapse after 5 years | 342/ICF: (+) | 28      | na      | na      | na    |
| ALL 13 (MF)  | 12.1       | 92         | ALL, 1st relapse     | 90/ICF: (+) | 150     | 226    | 157     | 82    |
| ALL 14 (SO)  | 5.3        | 20         | ALL, 1st relapse     | 61/ICF: (+) | 200     | 262    | 156     | 31    |
| ALL 15-1 (SK) | 8.8       | 88         | ALL, 1st relapse     | 64        | na      | na     | na      | 32    |
| ALL 15-2     | 10.1        | 96         | 2nd relapse          | 101       | 98      | 149    | 142     | 56    |
| ALL 16-1 (MS) | 11.3      | 98         | ALL, 1st relapse     | 19        | na      | 127    | na      | 194   |
| ALL 16-2     | 12.3        | 98         | 2nd relapse after ABMT | 79       | 293     | 227    | na      | 266   |
| ALL 17-1 (DL) | 14.1      | 99         | Ph+, 1st relapse     | 20        | na      | 511    | na      | 32    |
| ALL 17-2     | 15.3        | 94         | 2nd relapse          | 262/ICF: (+) | 158     | 742    | 73      | na    |
| ALL 17-3     | 15.8        | 55         | 3rd relapse          | 81/ICF: (+) | 71      | 190    | na      | 45    |
| ALL 17-4     | 16.1        | 70         | 4th relapse          | ICF: (+)  |         |        |         |       |
| ALL 18-1 (CS) | 11.4      | 96         | ALL, 1st relapse     | 27        | 125     | 449    | 209     | na    |
| ALL 18-2     | 11.7        | 90         | 2nd relapse          | 87/ICF: (+) | 75      | 223    | 120     | na    |
| ALL 19-1a (AD) | 5.7       | 73         | T-ALL, primary leukaemia | 45/ICF: (−) | 74      | 162    | 39      | na    |
| ALL 19-1b (bm) | 5.7      | 69         | 2nd relapse          | 269       | 200     | 305    | 168     |     |
| ALL 19-2 (bm) | 6.3       | 95         | 1st relapse          | 184       | 323     | 190    | 141     | 95    |
| ALL 20-1 (AZ) | 7.3       | 93         | ALL, primary leukaemia | 102      | 144     | 282    | 51      | 34    |
| ALL 20-2     | 8.5        | 93         | 1st relapse          | ICF: (+)  |         |        |         |       |
| ALL 20-3     | 9.3        | 85         | 2nd relapse after ABMT | ICF: (+)  |         |        |         |       |
| ALL 21-1 (SH) | 6.1       | 80         | ALL, primary leukaemia | 45        | 100     | 64     | 35      | 48    |
| ALL 21-2 (bm) | 7.1       | 90         | 1st relapse          | 43/ICF: (−) | 349     | 126    | na      | 61    |
| ALL 21-3     | 8.2        | 100        | isolated pleura relapse | na/ICF: (+) | 65      | na     | na      | na    |
| ALL 22 (AG)  | 9.3        | 95         | ALL, 3rd relapse     | ICF: (+)  |         |        |         |       |

See Table I footnotes.

In the relapsed state the leukaemias usually were treated according to an ALL protocol which includes prednisone, vincristine, daunomycin, etoposide, methotrexate, ara C, cyclophosphamide, and asparaginase; bm = bone marrow mononuclear cells; BMT, ABMT = (autologous) bone marrow transplantation; Ph+ = Philadelphia chromosome positive.
Thus, cisplatin, rather than nisone, was used more frequently in the study.

### Table III  Gene expression in peripheral blood mononuclear cells (PBMC) from chronic lymphocytic leukaemias (CLL)

| Patient | Age (years) | Leukocytes (10^6/l) | Lymphocytes (%) | Chemotherapy | topo1/P-gp | Topo II | Topo I | His 3.1 | Gst-α |
|---------|-------------|---------------------|----------------|--------------|-----------|--------|--------|---------|-------|
| CLL 1 (AK) | 58 | 29.6 | 88 | none | 70 | na | 38 | na | 69 |
| CLL 2 (LM) | 75 | 166.0 | 97 | none | 102 | (-) | 190 | 124 | 42 |
| CLL 3 (EJ) | 68 | 140.0 | 88 | none | 77 | (-) | 58 | 121 | 59 |
| CLL 4 (MW) | 57 | 233.2 | 99 | none | 131 | (-) | 157 | 246 | 733 |
| CLL 5 (KB) | 62 | 21.0 | 81 | none | 86 | (-) | 99 | 91 | 182 |
| CLL 6 (AF) | 82 | 45.5 | 81 | none | 68 | (-) | 54 | 33 | 209 |
| CLL 7 (MM) | 68 | 136.3 | 96 | none | 97 | (-) | 150 | 59 | 185 |
| CLL 8 (KU) | 74 | 20.7 | 89 | none | 68 | (-) | 127 | 74 | 141 |
| CLL 9 (HG) | 53 | 28.6 | 85 | Chlorambucil (9/10) | 100 | (-) | 77 | na | 72 |
| CLL 10 (FW) | 63 | 48.4 | 97 | Chlorambucil, PRED | 80 | (-) | na | na | 81 |
| CLL 11 (NS) | 76 | 21.4 | 82 | Chlorambucil, PRED | 49 | (-) | na | 113 | 32 |
| CLL 12 (KHR) | 59 | 17.2 | 92 | Chlorambucil, PRED | 189 | (-) | 114 | 405 | 321 |
| CLL 13 (FG) | 67 | 120.0 | 100 | Chlorambucil, PRED | 119/ICF: (+) | (-) | 186 | 144 | na |
| CLL 14 (WL) | 63 | 12.9 | 99 | Chlorambucil, PRED | 149 | (-) | 155 | 167 | 192 |
| CLL 15 (AM) | 67 | 35.7 | 91 | PRED | 74 | (-) | 180 | 57 | 149 |
| CLL 16 (FS) | 69 | 29.8 | 38 | Chlorambucil, PRED | 180 | (-) | 236 | 238 | 122 |
| CLL 17-1 (HS) | 57 | 85.0 | 97 | Chlorambucil, PRED | 68 | (-) | 55 | na | na |
| CLL 17-2 | 57.2 | 27.3 | 90 | PRED | 176 | (-) | 187 | 292 | 235 |
| CLL 17-3 | 57.4 | 31.0 | 91 | PRED | 83 | (-) | 80 | na | 204 |
| CLL 17-4 | 58 | 85.7 | 97 | PRED | 425 | 67 | 157 | 448 | 128 |
| CLL 18-1 (IG) | 51 | 187.0 | 91 | Chlorambucil, PRED | 66 | (-) | 98 | 100 | 123 |
| CLL 18-2 | 51.2 | 138.0 | 97 | Chlorambucil, PRED | 46 | (-) | 96 | 60 | 101 |
| CLL 18-3 | 52.2 | 35.0 | 75 | Chlorambucil, PRED | 126/ICF: (+) | (-) | 62 | 320 | na |
| CLL 18-4 | 52.7 | 30.2 | 84 | Chlorambucil, PRED | 442 | (-) | 239 | 492 | 560 |

See Table I footnotes.

### Table IV  Gene expression in acute myelogenous leukaemias (AML)

| Patient | Age (years) | Blasts (%) | Diagnosis/Status | topo1/P-gp | Topo II | Topo I | His 3.1 | Gst-α |
|---------|-------------|------------|-----------------|-----------|--------|--------|---------|-------|
| AML 1 (FM) (bm) | 57 | 80 | Primary leukaemia | 43 | 98 | 461 | 39 | na |
| AML 2 (ES) | 51 | 90 | Relapse | 151 | (-) | 102 | 28 | 74 |
| AML 3 (JS) | 19.5 | 66 | Relapse | 476 | 355 | na | na | 59 |
| AML 4 (YD) | 19 | 80 | Relapse after BMT | 54 | 71 | 113 | na | 31 |
| AML 5 (PR) | 15.3 | 96 | Primary leukaemia | 9 | 20 | 129 | 43 | 54 |
| AML 6 (AB) | 11.7 | Primary leukaemia | 59 | 90 | 162 | na | na | 59 |
| AML 7-1 (CD) | 11.3 | 93 | Primary leukaemia | 22/ICF: (+) | 58 | 71 | 36 | 67 |
| AML 7-2 | 11.8 | 100 | Relapse after ABMT | 78/ICF: + + | 62 | 124 | na | 62 |

See Table I footnotes. In the relapsed state AML usually were treated according to the AML protocol which includes ara C, daunomycin, and etoposide.

CCRF VCR 100 and CCRF ACTD (REV) show 'relative resistances' to actinomycin D of 10-fold and 12-fold, to vincristine of 257-fold and 107-fold, and to Adriamycin of 24-fold and 42-fold, respectively, measured by a 72 h growth assay (Kimmig et al., 1990).

**Acute lymphoblastic leukaemias**

We examined four adult and 18 childhood ALL in primary and relapsed states (Table II). With a few exceptions the specimens consisted of >80% leukaemic blast cells. The leukaemias usually were treated by various ALL protocols for primary and relapsed states, respectively, including prednisone, vincristine, Adriamycin, daunomycin, methylprednisolone, cisplatin, asparaginase, ara C, and cyclophosphamide. A rather low mdr1 gene expression was seen in adult ALL. Thus, in specimens originating from three relapsed state leukaemias poorly responding to chemotherapeutic treatment the expression levels were hardly significant at all (ALL 1, ALL 2, and the bone marrow sample ALL 4-1). However, a sample of the ALL 4 at a later presentation in remission after chemotherapy showed distinct mdr1 expression in the bone marrow aspirate, although only 2% blast cells were counted. The patients terminally relapsed, but mdr1 expression remained low. At the same time, a drastic increase in the topoisomerase II mRNA level could be monitored in the PBMC fraction.

Examination of childhood ALL revealed no significant differences in gene expression levels on the average, if the relapsed state leukaemias (18 specimens) were compared to the untreated primary leukaemias (six specimens). All primary leukaemias showed distinct mdr1/P-glycoprotein gene expression, in part even above the value seen in the cell line CCRF VCR 100. Nonetheless, very high values were monitored in two relapsed state leukaemias (ALL 12 and ALL 17-2). It might be worth noting that bone marrow cell samples showed 2- or 3-fold higher mdr1 mRNA levels (ALL 4-2 and ALL 19-1) than the corresponding PBMC fractions collected at the same time. Three relapsed state leukaemias (ALL 16-1, ALL 17-2, ALL 18-1) showed no significant expression of the mdr1 gene below 30 arbitrary units; CCRF VCR 100 = 100 in PBMC samples (96–99% blast cells). However, mdr1 mRNA levels were distinctly, in some cases drastically, elevated throughout, if second or third relapses were examined (ALL 15-2, ALL 16-2, ALL 17-2, ALL 18-2). The increases were all statistically significant. As an example, the values are detailed for the sample ALL 16-1 (19±15 arbitrary units; n = 2), and the sample ALL 16-2 (79±11 arbitrary units; n = 7). This difference is statistically significant at the P<0.001 level.

Topoisomerase II gene expression was quite variable, but usually rather strong especially in some relapsed state ALL, i.e. comparable to the level found in the T-lymphoblastoid cell line CCRF-CEM. A similar observation was made con-
cerning topoisomerase I gene expression in ALL. Thus, a correlation of a low topoisomerase II gene expression with the unresponsiveness of the blast cells to chemotherapy, as suggested to be a mechanism of a topoisomerase II associated multiple drug resistance of cell lines selected in vitro, was not seen in general. An exception is represented by the ALL 12 where, compared to CCRF-CEM cells, about 10-fold lower topoisomerase II mRNA levels were monitored together with a very high mdr1/P-glycoprotein gene expression.

A significant correlation was solely found for topoisomerase I and histone 3.1 mRNA levels ($r = 0.5156, n = 21, P < 0.01$). Histone 3.1 expression most likely corresponds to DNA synthesis and the proliferation status of the tumour cells (Venturrelli et al., 1988). Though constitutively expressed in nucleated cells, the topoisomerase I gene is highly regulated responding to a variety of stimulations (Romig & Richter, 1990). Glutathione-S-transferase $\pi$ gene expression, with two exceptions (relapsed state ALL 16, and the bone marrow sample of the primary ALL 19-1b), were moderate, if compared, however, to the fairly distinct expression found in CCRF-CEM cell lines.

**Chronic lymphocytic leukaemias**

Eighteen CLL samples, all representing a B-cell CLL, were examined, eight of which had not received any chemotherapy (Table III). The drugs applied for the treatment of CLL, the alkylating agent chlorambucil and the corticosteroid prednisone, are not usually affected in multidrug-resistant phenotypes of cell lines selected in vitro. However, two leukaemias (CLL 17 and CLL 18) examined several times during chemotherapy showed quite strong decreases in $r$ determinations of mdr1 expression levels in the most recent cell samples. On average, however, no statistically significant difference was revealed, if the data from untreated and treated leukaemias were compared altogether.

Except the sample CLL 17-4, in all CLL specimens examined topoisomerase II mRNA could not be detected by slot-blot hybridisation experiments, whereas distinct topoisomerase I expression was identified throughout. Surprisingly, several significant positive correlations of gene expression levels were found, i.e. highly significant for the mdr1 and histone 3.1 mRNA levels ($r = 0.8526, n = 19, P < 0.001$), the mdr1 and topoisomerase I mRNA levels ($r = 0.6076, n = 22, P < 0.005$), and mdr1 and glutathione-S-transferase $\pi$ mRNA levels ($r = 0.5415, n = 23, P < 0.005$), and, as in the ALL samples, for topoisomerase I and histone 3.1 mRNA levels ($r = 0.4201, n = 11, P < 0.05$). It appears interesting to note, however, that the significance of correlations turned out to be somewhat different, if untreated or treated CLL were examined separately. Thus, in untreated CLL ($n = 8$) no correlation at all was seen for mdr1 and glutathione-S-transferase $\pi$ mRNA levels, in the chemotherapeutically treated CLL this correlation was significant at the $P < 0.025$ level ($r = 0.6264, n = 13$). For the mdr1 and histone 3.1 mRNA levels the correlation was hardly significant ($r = 0.6786, n = 7, P = 0.05$) in case of the untreated leukaemias, whereas in the treated CLL the positive correlation was highly significant ($r = 0.9021, n = 12, P < 0.001$).

**Acute myelogenous leukaemias**

Four adult and three childhood AML were examined (Table IV). In all relapsed state AML mdr1 mRNA levels were significant (AML 4, AML 7-2) or high (AML 2, AML 3). The patient JS (AML 3) early relapsed after intense chemotherapy. Then, virtually no response of his blast cell population to various combinations of drugs was seen. A strong topoisomerase II gene expression was observed in all cases. The bone marrow mononuclear cells of AML 1, a primary leukaemia, showed very high topoisomerase I expression (see also the Northern-blot hybridisation, Figure 2). Remarkably, in the sample of the relapsed state AML 2 (90% blast cells) no topoisomerase II mRNA was detected (Table IV, Figure 2), whereas the topoisomerase I mRNA level was comparable to the values seen in the CCRF-CEM cell lines. Thus, the AML-2 represents another relapsed state acute leukaemia (see also ALL 12) where the drug resistance might possibly be caused by two rather independent mechanisms, i.e. an increased mdr1/P-glycoprotein, and a distinctly lowered topoisomerase II gene expression. Glutathione-S-transferase $\pi$ mRNA levels did not show peculiarities.

**Indirect immunocytofluorescence**

Part of the cell samples was analysed by indirect immunofluorescence using the P-glycoprotein specific monoclonal antibody C219. Multidrug-resistant sublines of the T-lymphoblastoid cell line CCRF-CEM were taken as a reference for P-glycoprotein expression.

The results are listed in the corresponding tables; some examples are shown in Figure 3. All cell samples were stained and photographed in the same manner. Figure 3a represents the moderate multidrug-resistant subline CCRF VCR 100 which was used as a standard for the evaluation of mRNA levels as detailed above. The limit of sensitivity of this immunofluorescence approach for detecting P-glycoprotein expressing cells seems hereby about to be represented. This is important to note, because the P-glycoprotein expression level in CCRF VCR 100 cells might already be well beyond the value where the resistance of tumour cells to chemotherapy might become a clinical problem. For comparison, stained samples of the highly cross-resistant subline CCRF ACTD 400 (Kimmig et al., 1990) with relative resistances of 571-fold to actinomycin D, 71-fold to adriamycin, or 2831-fold to vincristine (Figure 3b), and the subline CCRF VCR 124 (Kimmig et al., 1990) with relative resistances of 102-fold to actinomycin D, 90-fold to adriamycin, or 1760-fold to vincristine (Figure 3d), are shown as well. In Figure 3c the staining of a cell sample originating from a Russian child suffering from acute lymphoblastic leukaemia is presented. The patient received a continuous chemotherapy at home including methotrexate, prednisone, ara C, cyclophosphamide and various anthracyclines, and arrived in Tübingen in bad condition. The leukaemic blast cell population did not respond any more to chemotherapy, and the child died soon thereafter. Because of the small sample size the preparation of RNA was not possible at this time. However, the immunofluorescent staining of this cell sample revealed a rather homogenous and intense P-glycoprotein expression in nearly every cell examined. Figure 3e shows a sample of the relapsed state leukaemia displayed in Table II (AML 20-2), which again staining was intense (Table II) from which only a minute sample was available as well. Clearly, P-glycoprotein positive cells were seen.

Patient SH came up with an isolated pleura relapse. ICF analysis revealed a distinct staining of the cells (ALL 21-3). Cells collected from the first relapse (ALL 21-2) were 'P-glycoprotein negative' by ICF.

The data obtained by slot-blot hybridisations using RNA of the AML 7 samples (Table IV) were checked by ICF. No significant immunostaining was detected in case of the primary leukaemia (AML 7-1). In the relapsed state after autologous bone marrow transplantation (AML 7-2, 100% blasts), however, the immunostaining of the sample appeared to be inhomogeneous, but about 50% of the cells showed very strong signal intensities (Figure 3f). While a clear elevation of the mdr1 mRNA level was also found here after slot-blot hybridisations, the increase monitored hereby appeared to be less dramatic. This might at least partly be due to the fact that the latter method detects the average expression level in the whole blast cell population.

Cell samples of the parental, sensitive cell line CCRF-CEM did not show a significant P-glycoprotein specific immunofluorescence using our batches of the C219 antibody. In contrast, PBMC fractions of healthy donors showed some immunostaining which appeared to be quite heterogeneous, however, presumably representing the P-glycoprotein expression in specialized subpopulations of haematopoietic cells like macrophages, for example (Schaifer et al., 1990).
Discussion

Gene expression was determined by us mostly at the mRNA level in slot-blot and Northern-blot hybridisation experiments. In a fraction of the samples, P-glycoprotein gene expression was analysed by indirect immunocytofluorescence (ICF) using the monoclonal antibody C219. As the leukaemic cell samples frequently represent more or less heterogeneous cell populations, the analysis of P-glycoprotein expression by an immunocytofluorescence technique appeared to be useful. Even a single drug-resistant tumour cell might pose serious problems in curing the disease. Moreover, an occasional comparison of the data obtained with the two different methodical approaches at the mRNA or protein level, respectively, seemed to be important. In some cases, however, RNA preparation was not possible at all, because of the small sample size.

The monoclonal antibody C219 was used for examination of leukaemia cell samples by others as well (Ma et al., 1987; Volm et al., 1989; Musto et al., 1990; Schlaifer et al., 1990). Some limitations using this reagent have to be considered, though. This antibody apparently cross-reacts with the mdr3 gene product the involvement of which in drug resistance is still unclear (Schinkel et al., 1991). Leukaemias of the B-cell lineage were reported to express the mdr3 gene at significant levels (Herweijer et al., 1990). Nevertheless, we mostly examined mdr1 gene expression at the mRNA level in parallel using a gene probe which was not suspected to cross-react with the mdr3 gene under the highly stringent hybridisation conditions applied in this work according to Southern-blot hybridisation experiments performed with human genomic DNA (data not shown). This takes also account for another problem which might arise by using the C219 antibody, i.e. its cross-reaction to blood group A carbohydrate determinants due to contaminating antibodies in some commercial C219 lots recommending a reevaluation of P-glycoprotein expression data concerning samples of endothelial cells or epithelial tissues which are known to carry blood group antigens (Finstad et al., 1991). However, this appeared not to be a source of error in our work, because we found no link between the immunostaining-intensities of the samples, comprising mononuclear cell fractions consistently, and the blood group of the individuals. Therefore, it appears justified to state that in consideration of the peculiarities of the principally different approaches applied for detecting mdr1/P-glycoprotein gene expression the results were nonetheless similar.

In agreement with the reports from others, a substantial part of the relapsed state acute lymphatic leukaemias examined in this work (the adult relapsed state ALL 1, ALL 2, and ALL 4-1, the childhood relapsed state ALL 16-1, ALL 17-1, ALL 18-1, ALL 21-2) did not show a significant mdr1 gene expression at the mRNA level. A follow-up, however, revealed a clear increase of mdr1/P-glycoprotein mRNA levels in most of the second or third relapses of childhood ALL (ALL 15, ALL 16, ALL 17, ALL 18) indicating that P-glycoprotein expressing and supposedly resistant blasts cells were selected in vivo by prolonged treatment of the disease. A distinct elevation of mdr1/P-glycoprotein mRNA levels was also seen in the few relapsed states of adult or childhood AML analysed by us. The highly significant elevation of mdr1 gene expression after a prolonged treatment with chlorambucil and prednisone in two chronic lymphocytic leukaemias (CLL 17, CLL 18) is in accordance with the work of Holmes et al. (1990a) where a transient increase of mdr1 expression under chemotherapy with chlorambucil or cyclophosphamide was demonstrated. However, the authors suggest that mdr1 expression might be rather linked to as yet unknown factors in the development of CLL because increases of mdr1 mRNA levels were found in untreated CLL as well, which is consistent with the data presented in this work (Table III).

While the implications of P-glycoprotein expression are...
unclear in leukaemias like CLL treated with drugs (chlorambucil, prednisone) usually not associated with the MDR-phenotype of in vitro selected multidrug-resistant cell lines, the expression level of the multidrug transporter might be crucial for the success of the complex, empirically developed protocols for the chemotherapeutic treatment of other types of leukaemias, even if not the whole variety of the drugs applied is affected in the same manner. Furthermore, it is not known at the present time which brink might be for therapy success or failure in terms of the mdrl/P-glycoprotein expression level. No cell sample virtually "mdrl/P-glycoprotein negative" was seen by us applying slot-blot hybridisations. Even the parental T-lymphoblastoid cell line CCRF-CEM showed low mdrl mRNA levels (21 ± 11 arbitrary units, n = 7; Table I) which was proved not to represent unspecified background by a polymerase-chain-reaction (PCR) approach (Gekeler et al., 1990; Noonan et al., 1990). The child C.E.M. from which this cell line was derived, was intensely drug treated without response according to Foley et al. (1965). Thus, the specimens examined by us mostly originate from rather problematic leukaemias. Only three of the acute leukaemias examined in this work (ALL 5, 7, 7, ALL 13) are in remission at present, all of which showed distinct expression of the drug transporter gene, however (Table III). The coincidence of the absence of the mdrl gene expression determined by PCR in untreated nonlymphocytic leukaemias and the remission frequency observed for this type of leukaemia was found (Noonan et al., 1990). These studies suggest that the success of chemotherap in certain leukaemias is associated with the complete silence of the mdrl/P-glycoprotein gene in the tumour cells, and if mdrl expression, whether low or high, is seen at all by any of the methods described so far, the prognosis might be bad in general. Sooner or later chemotherapy would then select for mdrl/P-glycoprotein expressing tumour cells. Therefore, it seems to be necessary to emphasise the analysis of mdrl/P-glycoprotein expression in primary leukaemias which were cured by chemotherapy.

While the conditions of a complete transcriptional 'switch off' of the mdrl gene as yet are unknown, the possible intrinsic variability of mdrl gene expression levels in vivo in response to chemotherapy, or as yet unknown dietary or endogeneous factors has to be considered. Several recent reports describe such rather short term quantitative alterations. Thus, the mdrl gene expression was shown to be inducible in rat liver by carcinogens and cocarcinogens (Fairchild et al., 1987; Burt & Thorpeirson, 1988), after gestation in rat placenta (Van Obberghen, 1988), and by differentiating agents in a colon carcinoma cell line (Mickleby et al., 1989). Moreover, we showed an increase of resistance and mdrl mRNA levels within 72 h in a multidrug-resistant CCRF-CEM subline (Gekeler et al., 1988), and more recently in the parental cell line CCRF-CEM (Noller et al., 1991) after treatment of the cells with actinomycin D. In all these cases the mdrl gene was expressed prior to the treatment albeit at distinctly lower levels. The inducibility of the mdrl gene, however, might contribute to rather short term variations of the mdrl mRNA levels seen in tumour samples due, for instance, to the time of sample collection and the application of drugs. A more frequent gene expression analysis might then answer the question, whether mdrl gene expression increases as a direct consequence of chemotherapy in leukaemias that are not responding to chemotherapy but showing low mdrl gene expression beforehand.

The analysis of topoisoamerase I and II, histone 3.1, and glutathione-S-transferase \( \pi \) gene expression revealed no clear relationship to the status of the leukaemias. However, it might be useful for estimation of (i) target levels for many antineoplastic drugs (topoisoamerasers), (ii) the proliferation status of the other genes (topoisoamerase, histone 3.1), or (iii) the sensitivity to alkylating agents or oxidative stress (glutathione-S-transferase \( \pi \)).

In agreement with our data, a positive correlation was found between mdrl and glutathione-S-transferase \( \pi \) mRNA levels in CLL but not the acute leukaemias by Holmes et al. (1990b). A recent report on a variety of leukaemias, excepting CLL though, suggests that cytotoxic drugs might interfere with the transcription of the glutathione-S-transferase \( \pi \) gene, because a sharp drop of expression was seen following initiation of the chemotherapeutic treatment (McQuaid et al., 1989). A more detailed gene expression analysis in respect to treatment schedules of CLL seems to be useful to unravel these interdependences. The meaning of the positive correlations between the expression levels of the other genes in CLL remains unclear, as well. Our data indicate a highly significant link between mdrl gene expression and the histone 3.1 expression levels in this type of leukaemia, especially if the treated CLL are examined separately. This points to an association of mdrl gene expression with the proliferation status of these tumour cells. Nevertheless, other factors possibly due to the peculiar drug treatment of CLL might be responsible for this phenomenon.

Obviously, the activity of the DNA-synthesis machinery as indicated by the histone 3.1 gene expression levels is correlated to topoisoamerase I gene expression in general. This correlation is significant at the \( P<0.001 \) level for the collection of the whole variety of samples examined by us. The general link, however, between topoisoamerase gene expression and the proliferation status of cells is not to be understood. Our data, however, do indicate a relationship between topoisoamerase II gene expression and the status of DNA-replication which does not exclude an interrelationship to the cycling status of the cells, however. Hwang et al. (1990), reported on the induction of topoisoamerase II gene expression after phytohemagglutinin stimulation of human lymphocytes. In tumour cells, eventually, no difference was seen between a resting and a proliferating state (Liu, 1989), and similar topoisoamerase II activities were found by Holden et al. (1990) in normal or neoplastic tissues. The separate analysis of several topoisoamerase II subtypes which are differently expressed and variously sensitive to drugs (Drake et al., 1989; Holden et al., 1990) might help to unravel the as yet unclear association of topoisoamerase II expression to cell proliferation, and the response of tumours to chemotherapy, also.

Reports on atypical MDR-phenotypes of cell lines selected in vitro showing either a reduced topoisoamerase II expression (Fernandes et al., 1990; DeJong et al., 1990), or the emergence of an altered topoisoamerase II enzyme (Pommier et al., 1986) in these cells, suggest the occurrence of similar phenemens in vivo. So far, only little information is available on topoisoamerase expression in cell samples from leukaemias. Schrappe et al. (1988) described differences in topoisoamerase I levels by Western-immunoblotting in various haematological malignancies including CLL which virtually cannot be cured by chemotherapy at present. Their data nicely correspond to ours, i.e. no significant topoisoamerase II gene expression was found in PBMC samples of CLL patients or normal donors, while distinct topoisoamerase I expression was seen throughout. The authors suggest that a resistance of leukaemias to topoisoamerase II inhibitors like the widely applied drugs adriamycin and etoposide/VP-16 might be attributed to such low topoisoamerase II levels.

Besides in the CLL samples, low or undetectable topoisoamerase II in combination with distinct or high topoisoamerase I mRNA levels were observed by us in other leukaemias as well (AML 2, AML 5). A treatment of such leukaemias showing no significant topoisoamerase II gene expression with specific topoisoamerase II inhibitors might not be useful. It was shown recently that hypersensitivity of tissue culture cells to the topoisoamerase I inhibitor camptothecin is linked to the overexpression of the topoisoamerase I gene (Madden & Champoux, 1992). Thus, if clinically applicable topoisoamerase I specific drugs will be available, their application may in those cases not provoke chemotherapy. This might in general also be true for the numerous acute leukaemias showing a very high topoisoamerase I gene expression.

On the other hand, increased topoisoamerase II levels were parallel by increased sensitivities to intercalating drugs and
epipodophyllotoxins in various hamster cell lines, and the human T-lymphoblastoid cell line CCRF-CEM (Sullivan et al., 1987; Davies et al., 1988). Moreover, a correlation between the clinical response of solid tumours towards adriamycin and the topoisomerase II expression levels were reported by Kim et al. (1991). Hence, an intense application of topoisomerase II inhibitors, at best drugs not transported by the P-glycoprotein, might prove to be advantageous in leukaemias exhibiting strong topoisomerase II gene expression, as monitored by us in numerous acute leukaemias.

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