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Letter to the Editor

Failure to Detect the P-Glycoprotein Multidrug Resistant Phenotype in Cases of Resistant Childhood Acute Lymphocytic Leukaemia*

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OVEREXPRESSION of the multidrug resistance gene *mdr1*, which encodes the P170 membrane glycoprotein (P-glycoprotein), is associated with decreased sensitivity of tumour cells to natural product drugs such as anthracyclines, Vinca alkaloids, and the epipodophyllotoxins [1, 2]. Reduced intracellular accumulation and retention of drugs, apparently mediated by P170, has been implicated as the basis for this resistance [1, 2]. Although P170 expression has been demonstrated in some human tumours [3–5], the relationship of P170 overexpression to decreased drug accumulation in clinical multidrug resistance remains to be established. Ma *et al.* [4] reported that two acute non-lymphoblastic leukaemias had an MDR phenotype, but no studies are available on childhood acute lymphocytic leukaemia (ALL). We have therefore determined the extent of expression of the *mdr1* gene in lymphoblasts from five cases of ALL that were resistant to chemotherapy, including doxorubicin, and from five other cases that were responsive to initial drug treatments. Intracellular concentrations of doxorubicin were also determined by quantitation of ^{14}C-labelled doxorubicin accumulation and by flow cytometric analysis, allowing evaluation of intracellular drug fluorescence in single cells in a higher number of cases.

Blast samples were obtained from children (age 4–16 years) with ALL. Drug-sensitive patients responded to chemotherapy by achieving complete remissions (minimum follow-up 16 months), whereas patients at second or later relapse were considered to be resistant to chemotherapy. Chemotherapy was administered according to standard protocols, and, in addition to anthracyclines, included Vinca alkaloids, methotrexate, 6-mercaptopurine, and cytosine arabinoside.

As can be seen in Fig. 1, which shows hybridization of an *mdr1* probe with cytoplasmic RNA extracts from leukaemia cells, the *mdr1* gene was expressed at essentially the same low levels, whether lymphoblasts were collected from resistant or sensitive cases. The RNA on which these measurements were based appeared to be of good quality, as judged from expression of the actin gene in the same samples. Similarly, determination of intracellular concentrations of doxorubicin by either method failed to disclose any substantial differences between lymphoblasts from resistant and sensitive cases (Table 1). Flow cytometric determinations following treatments with different concentrations of doxorubicin and daunomicin produced similar results.

Nonetheless, other investigators employing techniques similar to ours [3] have demonstrated that

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Table 1. Intracellular concentration of doxorubicin in leukaemic lymphoblasts

	Relative fluorescence units*		ng/10^6 cells†	
	Mean ± S.D.	Range	Mean ± S.D.	Range
Sensitive cases	2.0 ± 1.0 $n = 20$	0.8–4.4	9.1 ± 3.6 $n = 5$	4.9–14.1
Resistant cases	2.2. ± 0.8 $n = 15$	0.5–3.5	9.2 ± 3.5 $n = 5$	6.4–15.0

*Cells were exposed to 1.25 μg doxorubicin for 4 h and directly analysed by flow cytometry [11, 12] at the end of the treatment, excluding dead cells by propidium iodide staining. The fluorescence frequency distributions obtained were all unimodal with relative S.D.s ranging from 19% to 39% in both groups. The fluorescence values reported are expressed in arbitrary relative units.

†Cells were exposed to 1.25 μg/ml ^{14}C-doxorubicin (specific activity: 2.22 GBq/mmole) for 4 h and directly processed at the end of the treatment as described previously [13].

the frequency of *mdr1* expression in ALL is appreciably lower than in malignancies such as colon carcinoma [3] and sarcoma [5], and a recent work failed to demonstrate gene amplification, *mdr1* expression and P170 overexpression in 19 cases of adult leukaemia at the onset and/or first relapse [14]. In our data, significant increases in the levels of different mdr indicators do not emerge in children at the second relapse of ALL, when the resistant phenotype presumably should already have been induced or selected. We conclude that clinical multidrug resistance in childhood ALL does not appear to be frequently associated with either overexpression of the *mdr1* gene or decreased intracellular concentrations of natural product drugs. An alternative explanation is suggested by studies of human leukaemic cells selected for resistance to teniposide. Such cells are cross-resistant to doxorubicin, displaying an 'atypical' form of multidrug resistance (at-MDR) associated with alterations in the activity of topoisomerase II [15], but do not overexpress of P170 [16] or display decreases in drug accumulation [17]. We suggest that some forms of clinical multidrug resistance in ALL may be characterized by the at-MDR phenotype.

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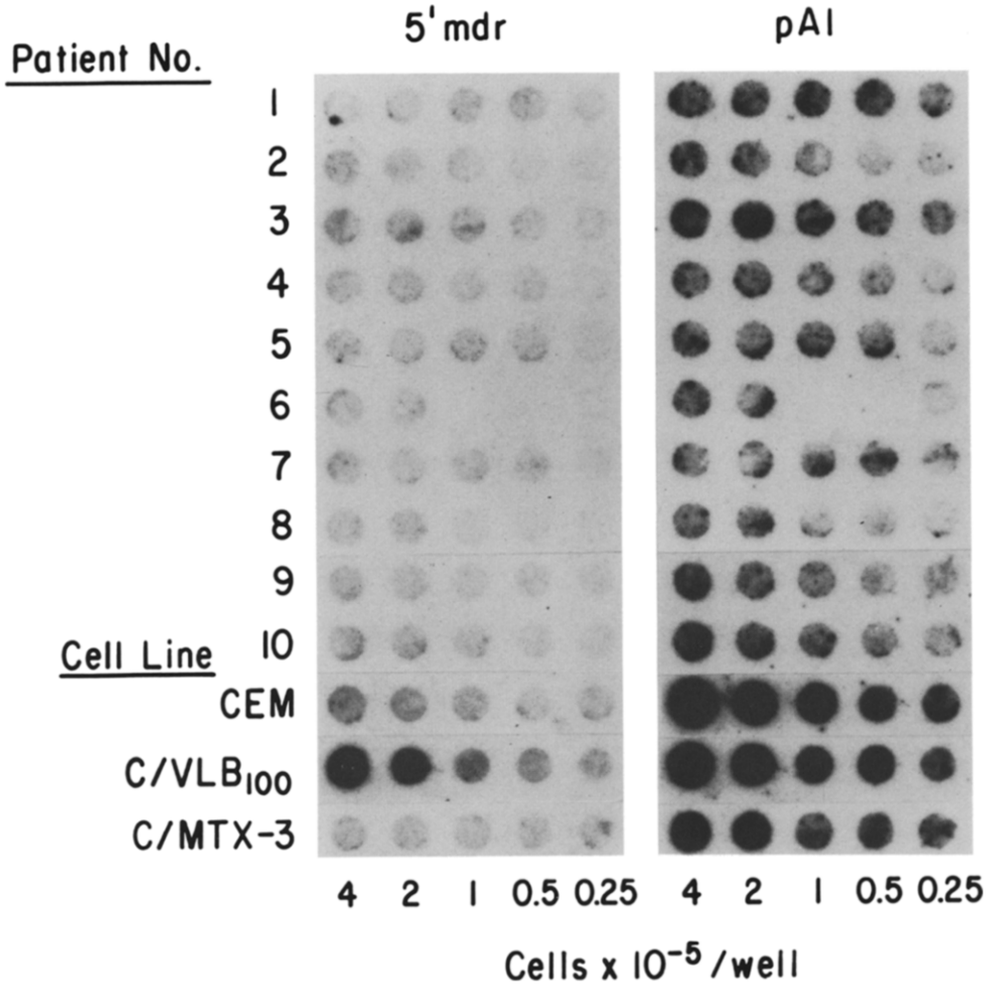

Fig. 1. Mdr1 *gene expression in leukaemic lymphoblasts from drug-sensitive and -resistant patients with ALL. Dot-blots of cytoplasmic RNA extracts of leukaemic blasts were analysed for* mdr1 *expression* [6] *by hybridization with 5'mdr, which is a* 1.2kb *EcoR*1 *fragment containing the 5'portion of the* mdr1 *cDNA sequence. To control for RNA loading, blots were also hybridized with an actin-specific probe, pA*1 [7]. *The drug-sensitive human T-cell line, CCRF-CEM, derived from a patient with ALL* [8] *was used as a negative control for these experiments, as was a methotrexate-resistant derivative, CEM/MTX-*1, *that does not express P*170 [9]. *The CEM/VLB*$_{100}$ *cell line, also derived from CCRF-CEM, was used as a positive control for* mdr1 *gene expression* [10].