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Immunophenotypic cell lineage and *in vitro* cellular drug resistance in childhood relapsed acute lymphoblastic leukaemia

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Abstract

At relapse, T-cell acute lymphoblastic leukaemia (ALL) has a worse patient outcome than B-cell precursor (BCP-) ALL. To investigate this further, we compared *in vitro* cellular drug resistance profiles of T-cell and BCP-ALL samples obtained at relapse. We investigated 237 paediatric relapsed ALL cases, including 151 samples taken at first relapse, of which 30 were T-cell ALL. *In vitro* drug resistance was measured using the 4-day methyl-thiazol-tetrazolium (MTT) assay and cellular immunophenotype was determined at central reference laboratories. Similar results were found for first relapsed ALL samples and for the total group: T-cell ALL samples were more resistant to 4-HOO-ifosfamide (1.4-fold, $P = 0.019$) and cisplatin (3.7-fold, $P = 0.005$). The samples were more sensitive to thiopurines such as mercaptopurine (2.1-fold, $P = 0.007$) and thioguanine (1.7-fold, $P = 0.003$). Resistance/sensitivity to 16 other drugs did not differ significantly. These results do not explain the relatively poor prognosis of T-cell ALL at relapse, but do suggest that the more intensive use of thiopurines in relapsed T-cell ALL may be beneficial.

Keywords: Acute lymphoblastic leukaemia; Childhood; Immunophenotype; T-cell ALL; B-cell precursor ALL; Relapse; Drug resistance; MTT assay; Thiopurines; Tailored therapy

1. Introduction

In paediatric relapsed acute lymphoblastic leukaemia (ALL), immunophenotype has prognostic significance. In particular, patients with T-cell ALL have relatively poor treatment success [1]. This is also the case in initial ALL, although more intensive and risk-adapted treatment has diminished the differences [2]. An improvement in prognosis might be achieved by the use of novel agents, but also by a more tailored use of already available drugs, as indicated for instance by cellular drug resistance profiling [3]. We and others have previously reported that relapsed ALL samples are significantly more resistant to several drugs *in vitro* as compared to initial ALL samples [4,5]. In our previous study, this included the thiopurines mercaptopurine and thioguanine, with an increased resistance of 1.3–1.5 fold at relapse, while the differences for other agents like glucocorticoids were much larger (>24-fold) [5]. In addition, within initial cases, T-cell ALL samples were significantly and markedly more resistant to most of the 13 drugs tested *in vitro*, except for thiopurines and for VM26 (teniposide, etoposide not tested), than common/pre-B ALL

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samples [6]. In this study, we have addressed at the time of ALL relapse whether the immunophenotypic cell lineage is related to cellular drug resistance as assayed *in vitro*.

2. Patients and methods

In this study, we have retrospectively reported on a cohort of 237 relapsed ALL cases (including 36 T-cell ALL cases) and successfully tested for *in vitro* drug resistance with informed consent, in a time period of 10 years. We specifically studied the relationship between immunophenotype and resistance to 20 different drugs in the total population and within the group of 151 first relapsed ALL cases (including 30 T-cell, 13 pro-B, 82 common and 25 pre-B ALL samples and one acute undifferentiated leukaemia). The patients had been treated according to four different (largely BFM-based) protocols in either Germany (77% of all cases) or the Netherlands (23% of cases). Since treatment had been heterogeneous and follow-up was sometimes incomplete, we do not report on correlations between *in vitro* drug resistance and clinical outcome. The central reference laboratories of the participating groups determined the sample immunophenotype where B-cell lineage (TdT^+, $CD19^+$, $HLA\text{-}DR^+$) was distinguished from T-cell lineage (TdT^+, cytoplasmic $CD3^+$, $CD7^+$). Data was insufficient to be able to further subdivide T-cell ALL cases based on their maturation status. Blood and/or bone marow samples were sent by courier service at room temperature to the VU university medical center (Amsterdam) and tested for *in vitro* cellular drug resistance within 24–36 hours after sampling. *In vitro* drug resistance was assessed by the total-cell kill 4-day methyl-thiazol-tetrazolium (MTT) assay, which is known to provide clinically relevant information in paediatric ALL [3,7]. A successful assay required at least 70% malignant cells in the control wells (achieved by removal of contaminating normal cells if necessary) [8]. The protocol for MTT assay is described elsewhere [3,5–8]. *In vitro* drug resistance is expressed as the drug concentration lethal to 50% of the cells (LC50). Group differences were considered to be statistically significant in cases where *P*-values were 0.01 or less and of borderline significance where *P*-values were between 0.01 and 0.05, as tested by non-parametric Mann–Whitney *U* test.

Table 1
Differences in cellular drug resistance *in vitro*, as determined by the 4-day methyl-thiazol-tetrazolium (MTT) assay, between T-cell lineage ($n = 30$) and B-cell precursor (BCP-) ALL samples ($n = 121$) obtained at first relapse

Drug	Median LC50[a] values and ranges (in ug/ml)			
	T-cell ALL	BCP-ALL	RR[b]	*P*-value[c]
Prednisolone	155.7	140.6	1.1	0.90
	0.05–250	0.05–250		
Dexamethasone	3.9	>6.0[d]	<0.7	0.95
	0.009–6	0.005–6		
Vincristine	1.39	0.68	2.0	0.23
	0.05–50	0.04–50		
Vindesine	5.68	2.55	2.2	0.68
	0.05–36.8	0.07–50		
Daunorubicin	0.21	0.12	1.8	0.17
	0.01–2	0.02–1.26		
Doxorubicin	0.35	0.30	1.2	0.14
	0.08–8	0.03–1.37		
Idarubicin	0.08	0.08	1.0	0.78
	0.006–2	0.002–2		
Aclarubicin	0.19	0.18	1.1	0.85
	0.07–0.45	0.02–8		
Mitoxantrone	0.07	0.05	1.4	0.48
	0.004–1	0.001–1		
L–asparaginase	0.85	1.06	0.8	0.80
	0.003–10	0.003–10		
Mercaptopurine	79.5	166.7	0.5	0.007
	19.5–278	15.6–500		
Thioguanine	5.0	8.7	0.6	0.003
	1.56–21.5	1.56–50		
Cytarabine	0.78	1.06	0.7	0.62
	0.01–2.5	0.02–2.5		
Cladribine	0.10	0.03	3.3	0.48
	0.004–40	0.003–26.9		
Etoposide	1.65	1.55	1.1	0.72
	0.14–8.64	0.04–50		
Teniposide	0.26	0.28	0.9	0.90
	0.18–8	0.06–8		
4-HOO-ifosfamide	4.94	3.46	1.4	0.02
	1.69–14.9	0.2–18.0		
Cisplatin	5.36	1.46	3.7	0.005
	0.39–12.5	0.39–5.4		
Carboplatin	19.53	18.88	1.0	1.0
	5.7–26.1	12.8–25.0		
Thiotepa	1.70	2.48	0.7	0.19
	0.39–100	0.1–17.1		

[a] Concentration lethal to 50% of the cells, as compared to the control cell survival.
[b] RR, resistance ratio (median LC50 value T-cell ALL/median LC50 value BCP-ALL).
[c] As determined by the non-parametric Mann–Whitney *U* test for unpaired samples.
[d] Indicates a median above the highest concentration of the drug that was tested (i.e., in general it was impossible to kill more than 50% of cells, even at the highest drug concentration).

3. Results

In general, there were marked differences in resistance to drugs between individuals, with a range of LC50 values (Table 1). These differences exceeded group differences and also in instances of statistically significant group differences (see below). The distribution of LC50 values of pro-B, common and pre-B ALL samples were not statistically significantly different and so these were grouped together as B-cell precursor (BCP-) ALL. T-cell and BCP-ALL samples did not show

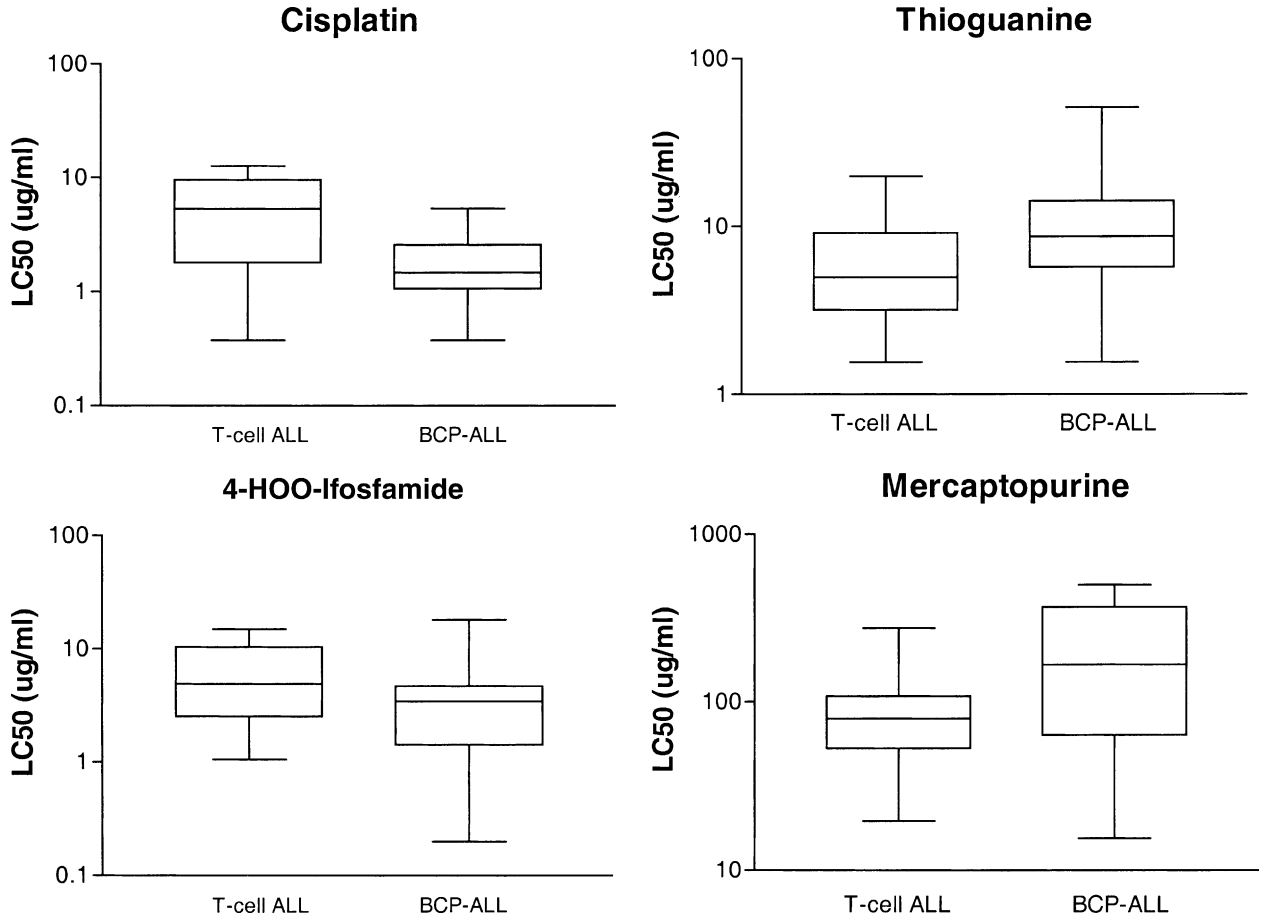

Fig. 1. Differences between T-cell and B-cell precursor (BCP-) ALL samples obtained at first relapse with respect to *in vitro* cellular resistance to the four drugs with statistically (borderline) significant differences: 4-HOO-ifosfamide ($P = 0.02$), cisplatin ($P = 0.005$), mercaptopurine ($P = 0.007$) and thioguanine ($P = 0.003$). The figures show medians, 25th and 75th percentiles, and the minimum and maximum LC50 values.

marked differences in cellular drug resistance *in vitro* apart from the following exceptions: T-cell ALL samples were more resistant to 4-HOO-ifosfamide (1.7-fold, $P = 0.006$) and cisplatin (3.1-fold, $P = 0.002$) and more sensitive to thiotepa (borderline, 1.6-fold, $P = 0.022$), mercaptopurine (borderline, 2.0-fold, $P = 0.03$) and thioguanine (1.7-fold, $P = 0.003$). In the more homogenous group of first relapsed ALL cases only, similar results were found: T-cell ALL samples were more resistant to 4-HOO-ifosfamide (borderline, 1.4-fold, $P = 0.019$) and cisplatin (3.7-fold, $P = 0.005$), but more sensitive to mercaptopurine (2.1-fold, $P = 0.007$) and thioguanine (1.7-fold, $P = 0.003$). These significant differences are illustrated in Fig. 1. *In vitro* resistance to other drugs, including glucocorticoids (prednisolone and dexamethasone), vinca-alkaloids (vincristine and vindesine), anthracyclines (daunorubicin, doxorubicin, idarubicin, aclarubicin), mitoxantrone, amsacrine, l-asparaginase, cytarabine, cladribine and epipodophyllotoxins (VP 16 or etoposide, VM26 or teniposide), was not significantly different between T-cell and BCP-ALL samples (Table 1).

4. Discussion

We previously reported [6] a generally increased *in vitro* drug resistance in T-cell ALL as compared to BCP-ALL samples obtained at initial diagnosis. In that study, T-cell ALL cases were more resistant than BCP-ALL cases to 10 out of 13 drugs tested except for the two thiopurines and teniposide (VM26) tested where both subgroups were equally sensitive. Based on the cohort of paediatric relapsed ALL samples analysis presented here, the differences between the cell lineages has largely disappeared at relapse. In fact, within first relapsed ALL cases, T-cell ALL samples were more resistant to cisplatin ($P = 0.005$) and borderline resistant to 4-HOO-ifosfamide ($P = 0.019$), but significantly more sensitive to thiopurines than BCP-ALL samples. A more detailed comparison with the data that we previously reported on drug resistance at initial diagnosis in relation to cell lineage [6], showed that both T-cell and BCP-ALL subgroups became more resistant to glucocorticoids at relapse. Median LC50 values for T-cell ALL at relapse were 6-fold (prednisolone) to 30-fold

(dexamethasone) higher than those for T-cell ALL samples obtained at initial diagnosis. The increased glucocorticoid resistance at relapse for BCP-ALL samples is even higher at >85-fold. Regarding the other drugs, differences in cellular resistance between T-cell ALL samples obtained at relapse and initial diagnosis are much less pronounced, with median LC50 values differing 1.8-fold (daunorubicin) or less (all other drugs). However, BCP-ALL samples also became more resistant to several other drugs at relapse. Based on a comparison of the median LC50 values for BCP-ALL samples obtained at initial diagnosis [6] and at relapse (this study), an 8-fold increased resistance to l-asparaginase and 2.4-fold increased resistance to cytarabine occurred. While median LC50 values for both thiopurines of T-cell ALL samples obtained at relapse were actually lower than those at initial diagnosis, these values were 1.5 (thioguanine) to 1.8-fold (mercaptopurine) higher for BCP-ALL samples. These data suggest differences between immunophenotypic ALL cell lineages in the development of drug resistance but does not explain the worse prognosis of T-cell ALL at relapse compared with BCP-ALL [1]. We have previously reported on the increased drug resistance *in vitro* at relapsed ALL in general as compared to untreated ALL [5]. In that study, data on paired samples obtained at initial diagnosis and at relapse, from the same patients, showed that both *de novo* and acquired drug resistance occurred [5].

In this study, we have not correlated *in vitro* drug resistance to clinical outcome, since this cohort of relapsed ALL patients were treated by different groups according to different protocols over a long time period. However, large individual patient differences in cellular drug resistance were found, and we and others, have previously reported that increased resistance translated most often to worse prognosis both at initial diagnosis and at relapse [4,5,7,9–11].

Results on the total group of relapsed ALL samples and on those from patients with first relapsed ALL were essentially similar. Within the latter more homogeneous group, T-cell ALL cases were significantly more resistant to cisplatin (although that drug had not been used in the treatment of these patients) and borderline resistant to 4-HOO-ifosfamide. The samples were significantly more sensitive (approximately 2-fold) to thiopurines, than BCP-ALL samples. The latter was found for the two tested thiopurines: mercaptopurine and thioguanine. For samples obtained at initial diagnosis, we have previously reported that T-cell ALL is a relatively drug resistant subgroup as compared to BCP-ALL, but that both subgroups were equally sensitive to thiopurines [6]. Therefore, more intensive use of thiopurines should be considered in T-cell ALL in general, and at relapse in particular, in the hope to improve outcome in this poor prognostic subgroup.

Conflict of interest statement

None declared.

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