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Translocation t(1;19) is related to low cellular drug resistance in childhood acute lymphoblastic leukaemia

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TO THE EDITOR

Cytogenetic and molecular analyses of leukaemic cells have identified therapeutically important subgroups of childhood acute lymphoblastic leukaemia (ALL). One of the most frequently observed translocations is t(1;19)(q23;p13), which occurs in 2–5% of children with B-lineage ALL.[1] Cytogenetically, two forms are found, one balanced t(1;19)(q23;p13) and one unbalanced der(19) t(1;19)(q23;p13), both giving rise to the E2A/PBX1 fusion gene. The resulting aberrant protein product appears to have oncogenic potential by affecting cell differentiation (for a review see Hunger[2]).

The presence of t(1;19) has been associated with unfavourable presenting features, such as high white blood cell (WBC) count and absence of high hyperdiploidy (>51 chromosomes), and early reports indicated that patients with t(1;19) had a poor outcome with use of standard treatment.[3] Differences in outcome between patients with the balanced and unbalanced form have been reported in some studies,[4] but not in others.[5] However, intensified chemotherapy has been shown to improve outcome and may mitigate or nullify the adverse prognostic impact of t(1;19). Thus, event-free survival (EFS) of 75–85% is reported with modern treatment protocols for ALL.[1,4,6,7] The improved outcome has been accomplished by reinforced early therapy and extended combination therapy. It is not known, however, whether addition or dose escalation of any specific drug was the critical factor, or if improved outcome was the result of a general increase in treatment intensity.[6] Test of cellular drug sensitivity *in vitro* might help to clarify this problem, and we have therefore compared patients with and without t(1;19) to see if this translocation is associated with a specific drug-sensitivity profile. To our knowledge, no such data have been published hitherto.

Leukaemic cells from bone marrow or peripheral blood of children (aged 1–17 years) with newly diagnosed ALL were used in this study. All patients were treated according to the NOPHO ALL 1992 protocol.[8] Nordic centres for paediatric oncology participated and provided samples for test of *in vitro* drug resistance from 689 patients between 1992 and December 2002. The patients were representative of all children in this age group diagnosed with ALL in the Nordic countries during the study period, as described in detail elsewhere.[9]

Chromosome banding analyses of bone marrow and/or peripheral blood samples were performed using standard methods. The definition and description of clonal abnormalities followed the recommendations of ISCN (1995). Since 1996 (Sweden) and 2000 (all five Nordic countries), the karyotypes have been centrally reviewed. Fluorescence *in situ* hybridization (FISH), Southern blot, and reverse-transcriptase polymerase chain reaction (RT-PCR) analyses have been increasingly applied to verify or characterize more precisely the chromosomal abnormalities found.[10] The positive diagnosis of t(1;19) was primarily based on banded metaphase karyotyping, in some cases confirmed by either FISH or RT-PCR. In all t(1;19)-negative cases included in the study, either FISH or RT-PCR were applied to verify the negative result.

FMCA is based on measurement of fluorescence generated from hydrolysis of fluorescein diacetate to fluorescein by cells with intact plasma membranes, measured after 72 h of drug exposure, and has been described in detail previously.[9,11] Drugs

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were tested in triplicate. Six wells without drugs served as controls and six wells containing culture medium only served as blanks. The results are presented as survival index (SI), defined as fluorescence in test wells/fluorescence in control wells (blank values subtracted) × 100. Thus, a low numerical value indicates high sensitivity to the cytotoxic effect of the drug.

Cytotoxic drugs were obtained from commercial sources and tested at empirically derived concentrations, chosen from previous studies of leukaemic cells.[11] The active metabolite of cyclophosphamide, 4-hydroperoxy-cyclophosphamide (4-HC), was used.

Nonparametric statistical methods were used throughout. All analyses were two-tailed and the level of statistical significance was set at $P<0.05$. Local ethics committees approved the study.

Valid data for the presence of t(1;19) translocation were available for 230 of the ALL samples sent to our laboratory for test of *in vitro* drug resistance. In all, 219 were from patients negative for t(1;19) by banded karyotyping and targeted analysis (FISH or RT-PCR). A total of 11 cases had t(1;19), either the balanced or unbalanced variant. Thus, in the study cohort of 230 cases the prevalence of t(1;19) was 4.8%.

Testing of cellular drug resistance by FMCA was accomplished successfully in 164 out of the 230 samples. Reasons for failure of *in vitro* testing in the t(1;19)-positive and -negative groups were: total number of cells too small to test any drug ($n=0$ and $n=13$, respectively), transport problems ($n=0$, $n=6$), too low proportion of lymphoblasts before the *in vitro* test ($n=0$, $n=3$), too low proportion of lymphoblasts in control wells after incubation ($n=0$, $n=10$), too low signal-to-noise ratio ($n=2$, $n=23$), and high coefficient of variation in controls ($n=0$, $n=9$). The technical success rate for *in vitro* testing of samples with sufficient number of cells was 82 and 80% in the t(1;19)-positive and -negative groups, respectively. There was no difference in control cell survival between the t(1;19)-positive and -negative groups ($P=0.42$).

The distribution of important clinical and biological parameters within the t(1;19)-positive and -negative cases with a successful *in vitro* test is summarized in Table 1. There was an expected difference in the modal number of chromosomes between the two groups, since the high hyperdiploid karyotype is uncommon in patients with t(1;19). There were also nonsignificant trends to higher age and higher WBC count at diagnosis in t(1;19)-positive patients. All patients in the t(1;19)-positive group displayed the precursor B phenotype; none had CNS or testicular engagement at diagnosis.

Table 2 and Figure 1 show the *in vitro* drug resistance in samples from patients with or without t(1;19). The t(1;19)-positive samples were significantly more sensitive to all drugs tested, except 4-HC. There was a trend in the same direction for this drug, but for technical reasons only four t(1;19)-positive samples were tested.

The next step in the data analysis was to exclude patients with the nonprecursor B phenotype ($n=22$), since the t(1;19) translocation only occurs in patients with precursor B ALL. The results were essentially the same (data not shown). Finally, we excluded patients with chromosomal aberrations known to be of prognostic significance and present almost exclusively in the t(1;19)-negative group: high hyperploidy (>51 chromosomes) ($n=40$), t(9;22)(q34;q11) ($n=4$), t(12;21)(p13;q22) ($n=40$), and 11q23 translocations ($n=5$). Some patients had more than one exclusion criterion. There were no significant differences in age, sex distribution, or WBC count at diagnosis between the remaining patients in the t(1;19)-positive and -negative groups.

Table 1 Characteristics of children with t(1;19)-positive or -negative ALL tested for *in vitro* cellular drug resistance

	t(1;19) positive	*t(1;19) negative*	*P-value*
No. of patients	9	155	
Median age (years) (range)	6.6 (3.8–12.9)	5.5 (1.1–17.0)	0.30
Sex			0.79
Male, *n* (%)	5 (56)	79 (51)	
Female, *n* (%)	4 (44)	76 (49)	
WBC (10^9/l)			
Median	14.3	11.0	0.60
<10, *n* (%)	3 (33)	74 (48)	
10–<50, *n* (%)	5 (56)	53 (34)	
⩾50, *n* (%)	1 (11)	28 (18)	
Modal number			0.058
45–46, *n* (%)	8 (89)	60 (39)	
47–51, *n* (%)	0 (0)	19 (12)	
52–60, *n* (%)	1 (11)	36 (23)	
>60, *n* (%)	0 (0)	3 (2)	
Unknown, *n* (%)	0 (0)	37 (24)	

P values were determined by χ^2 and Mann–Whitney *U*-test.

Table 2 *In vitro* drug resistance in children with ALL positive ($n=9$) or negative ($n=155$) for translocation t(1;19)

Drug	*Conc.*	*t(1;19) positive*		*t(1;19) negative*		*P-value*
		Median	*(25th–75th)*	*Median*	*(25th–75th)*	
Amsacrine	1	11	(6–18)	30	(17–46)	0.002
Cytarabine	0.5	24	(14–41)	58	(42–75)	<0.001
Dexamethasone	1.4	29	(13–35)	51	(30–73)	0.014
Doxorubicin	0.5	12	(6–17)	33	(16–50)	0.001
Etoposide	5	22	(7–29)	43	(23–67)	0.010
Prednisolone	50	18	(11–24)	50	(33–70)	<0.001
Vincristine	0.5	13	(9–31)	50	(38–73)	<0.001
4-HC	2	14	(8–19)	24	(11–44)	0.15
6-thioguanine	10	14	(11–22)	36	(22–54)	0.001

Concentrations of the drugs are in μg/ml.
Median and 25th–75th percentile figures show percentage surviving cells compared to control wells.
4-HC is 4-hydroperoxy-cyclophosphamide; only four t(1;19)-positive and 36 t(1;19)-negative samples were tested with this drug.
P-values were determined by the Mann–Whitney *U*-test.

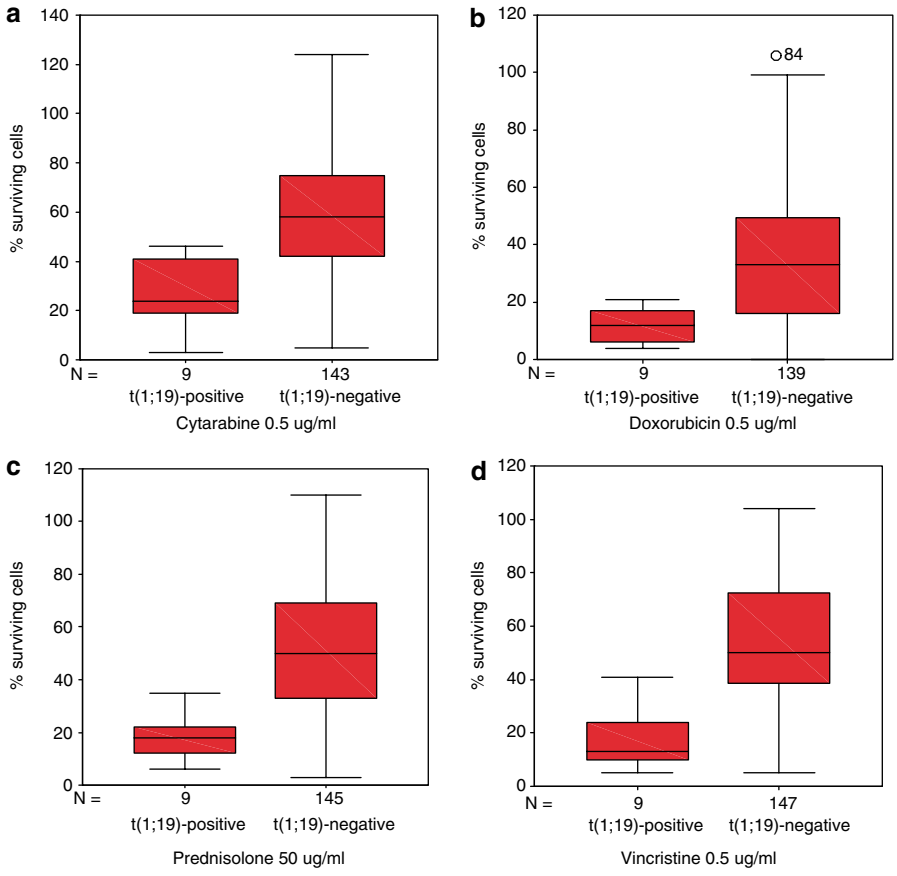

Figure 1 *In vitro* drug resistance in 164 children with ALL positive or negative for translocation t(1;19). Patients with t(1;19) were significantly more sensitive to (a) cytarabine ($P<0.001$), (b) doxorubicin ($P=0.001$), (c) prednisolone ($P<0.001$), and (d) vincristine ($P<0.001$). The box-and-whisker plot shows median, first, and third quartiles; whiskers extend to the highest and lowest value, excluding outliers, which are denoted by circles.

Table 3 *In vitro* drug resistance in children with t(1;19)-positive ($n=8$) or -negative ($n=49$) ALL matched for unevenly distributed risk factors

Drug	*t(1;19) positive*		*t(1;19) negative*		*P-value*
	Median	*(25th–75th)*	*Median*	*(25th–75th)*	
Amsacrine	10	(6–16)	31	(20–43)	<0.001
Cytarabine	26	(12–41)	63	(44–74)	<0.001
Dexamethasone	26	(13–33)	51	(35–79)	0.009
Doxorubicin	14	(6–17)	34	(23–51)	<0.001
Etoposide	21	(6–27)	51	(30–65)	<0.001
Prednisolone	17	(11–25)	51	(34–75)	<0.001
Vincristine	16	(9–35)	45	(39–72)	<0.001
4-HC	18	(8–18)	29	(12–46)	0.28
6-thioguanine	15	(12–22)	42	(25–61)	<0.001

The concentrations of the drugs used are denoted in Table 2.
All the figures are percentage surviving cells compared to control wells, median, and 25th–75th percentile.
4-HC is 4-hydroperoxy-cyclophosphamide; only three t(1;19)-positive and nine t(1;19)-negative patients were tested.
P-values were determined by the Mann–Whitney *U*-test.
For 19 of the t(1;19)-negative patients, data on modal number were missing. Excluding them from the analysis did not change data significantly.

As evident from Table 3, there were very clearcut differences in the cellular drug sensitivity between the two groups. Cell survival was much lower in the t(1;19)-positive samples after exposure to all the drugs tested, with *P*-values <0.01 for eight out of nine drugs.

Six out of nine patients were diagnosed with the unbalanced translocation der(19)t(1;19). Samples from these patients tended to be more sensitive *in vitro* than samples from patients with the balanced t(1;19) translocation, but formal statistical testing was not performed due to the small number of samples (data not shown).

Data regarding bone marrow morphology after 15 days of treatment were available for six t(1;19)-positive patients; five showed M1 (<5% blasts) and one M2 (5–25% blasts) morphology. At treatment day 29, all nine patients showed M1 morphology. One patient died in complete remission on day 48 due to pancreatitis, possibly caused by ongoing treatment with L-asparaginase. Seven patients are alive and well 7–53 months (median 27) after diagnosis. EFS was 0.67 and 0.79 in the t(1;19)-positive and -negative groups, respectively (NS). One patient, the one with M2 bone marrow at day 15, relapsed 32 months after diagnosis, was brought into new remission, and went to bone marrow transplantation. Interestingly, lymphoblasts from this patient showed a relatively less sensitive profile than the other t(1;19)-positive patients, and for vincristine and 6-thioguanine, they were more resistant than blasts from any other patient in the t(1;19)-positive group.

We studied the relation of t(1;19) and *in vitro* drug resistance in ALL patients within the framework of a Nordic multicentre study. The diagnosis of t(1;19) was primarily based on G-banded metaphase karyotyping. The presence of t(1;19) was determined

in 230 out of the 689 samples sent to our laboratory for test of *in vitro* drug resistance (33%). The main reason for the low figure is that only few centres used targeted methods for detection of this translocation before 1997, and several centres started to use them only recently. There is no reason to believe that this introduced any bias in the patient selection. Clinical and biological characteristics of the t(1;19)-positive children included in the present study agree with those of other reports in the literature, in that they are exclusively of precursor B phenotype, lack t(9;22), t(12;21), and 11q23 translocations, and that they mostly have modal number <52 chromosomes.

Our main finding was that tumor cells with the t(1;19) translocation exhibited low drug resistance *in vitro* to a broad panel of antineoplastic drugs. We are aware that the number of t(1;19)-positive patients tested was limited, but the findings were very clearcut. The difference between t(1;19)-positive and -negative patients remained highly significant after elimination of unevenly distributed factors, such as nonprecursor B phenotype, high hyperploidy, Philadelphia chromosome, 11q23 translocations, and t(12;21). One could speculate that t(1;19)-positive cells are generally more susceptible to various unspecific stimuli, and that this might have influenced our results, but this is strongly contradicted by the finding that control cell survival was quite similar in the t(1;19)-positive and -negative groups.

The t(1;19) occurs in both a balanced and an unbalanced form, raising the question of whether these differences might have prognostic import. Pui *et al*[5] did not find differences in presenting features or EFS that would warrant separation of cases with a balanced or unbalanced translocation. However, Uckun *et al*[4] reported that patients with unbalanced der(19)t(1;19) had a significantly better outcome than patients with balanced t(1;19). Owing to the limited number of patients, we analysed all cases with t(1;19) together. Comparison of data for patients with balanced and unbalanced translocations (without formal statistical testing) indicated somewhat higher sensitivity for the latter group, but this preliminary finding must be verified in a larger patient material.

Early reports indicated that patients with t(1;19) had a poor outcome with standard treatment.[3] However, intensified chemotherapy has been shown to overcome this adverse prognostic impact, so that EFS of 75–85% can now be achieved with reinforced early therapy and extended combination therapy.[1,4,6,7] Our data do not explain why patients with t(1;19) need intensified treatment, since the cells were highly sensitive *in vitro*. The inferior cure rate for children with t(1;19)-positive ALL in earlier treatment eras could possibly reflect that t(1;19)-positive ALL benefits less from 6-mercaptopurine/methotrexate maintenance therapy compared to the more common high hyperploidy ALL. During the late 1970s and early 1980s, induction and consolidation therapy was much less intensive and maintenance therapy far more dominating when compared with the treatment regimens used today.[8] After intensification by adding anthracyclines, alkylating agents and/or teniposide/etoposide, patients with t(1;19) have shown a clinical outcome similar to that of other children with precursor B ALL.[4,6,7] Sensitivity to these drugs at the cellular level might be part of the explanation to the remarkable improvement in outcome noted for patients with t(1;19) after intensification of treatment. However, the relative contribution of dose escalation or addition of any specific drug(s) is not possible to pinpoint. Unfortunately, cellular resistance to methotrexate cannot be measured *in vitro* by FMCA and similar methods, since leakage of nucleosides from dying cells provide a salvage pathway for surviving cells.

Factors other than those measured by test of cellular drug resistance *in vitro* probably influence the clinical outcome in t(1;19)-positive patients. Thus, expression of the resulting E2A/PBX1 fusion gene is related to a number of factors of putative pivotal importance for the behaviour of the tumor cells in the patient, for example, differentiation, proliferation, angiogenesis, and stroma interaction pathways. Any of these, or other unknown factors, might explain why patients with t(1;19) need intensified therapy in spite of high drug sensitivity at the cellular level.

A selective sensitivity for the topoisomerase II inhibitors doxorubicin and etoposide has been found in patients with translocation t(12;21), indicating that this cytogenetic aberration induces changes in the cell that affect specific drug action(s).[12] In contrast, the present findings suggest that t(1;19) affects common final pathways for cell death and induces an apoptosis-prone tumor cell phenotype. Modern high-throughput techniques, for example, microarrays for gene expression profiling, should now be used to try to elucidate the mechanisms by which t(1;19) and t(12;21) are linked to these very distinctly different patterns in cellular drug sensitivity.

To summarize, we found that the presence of the t(1;19) translocation in childhood ALL is associated with high sensitivity to a broad spectrum of antineoplastic agents. Factors other than cellular drug resistance should be sought to explain why these patients need intensified therapy.

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