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Cellular drug sensitivity in *MLL*-rearranged childhood acute leukaemia is correlated to partner genes and cell lineage

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Summary

Rearrangements in the 11q23 region, the site of the mixed lineage leukaemia (*MLL*) gene, are found in both childhood acute myeloid (AML) and lymphoblastic (ALL) leukaemia. We studied the *in vitro* drug resistance by the fluorometric microculture cytotoxicity assay (FMCA) in 132 children with AML and 178 children with ALL (aged 0–17 years). In AML, children with t(9;11) ($n = 10$) were significantly more sensitive to cytarabine ($P < 0{\cdot}001$) and doxorubicin ($P = 0{\cdot}005$) than non-11q23 rearranged patients ($n = 108$). Children with other 11q23 rearrangements ($n = 14$) differed less from non-rearranged children. The 'AML-profile' common to all three groups included relative resistance to glucocorticoids and vincristine. In ALL, children with 11q23 rearrangement ($n = 22$) were significantly more sensitive to cytarabine ($P = 0{\cdot}026$) than children without 11q23 rearrangement ($n = 156$), also after stratification for white blood cell count. In conclusion, the findings indicate that the cellular drug resistance is correlated to both the cell lineage and the type of 11q23 rearrangement. High cellular sensitivity to cytarabine and doxorubicin might explain the excellent treatment results in children with AML and t(9;11). The present study supports the strategy of contemporary protocols to include high-dose cytarabine in the treatment of 11q23-positive patients both in AML and ALL.

Keywords: acute leukaemia, childhood, drug sensitivity, chromosomal rearrangements, mixed lineage leukaemia.

Genetic and molecular analyses of leukaemic blasts have enhanced our understanding of the pathogenesis of acute leukaemia and have identified therapeutically important subgroups of acute myeloid (AML) and lymphoblastic (ALL) leukaemia. To date, specific genetic abnormalities with clinical relevance have been identified in approximately 75% of childhood ALL cases (Forestier *et al*, 2000; Rubnitz & Pui, 2003). In AML, clonal chromosomal abnormalities are detected in about the same proportion of patients, but they differ markedly from those found in ALL (Grimwade *et al*, 1998; Raimondi *et al*, 1999; Forestier *et al*, 2003). However, abnormalities in the 11q23 region, the site of the mixed lineage leukaemia (*MLL*) gene, occur both in AML and ALL. In childhood AML, 11q23 rearrangements have been reported to occur in 10–24% of the cases. The translocation t(9;11) is the single most frequent xaberration and appears to carry an excellent prognosis, while other 11q23 rearrangements have been associated with an average (Pui *et al*, 2000), or worse than average clinical outcome (Rubnitz *et al*, 2002; Lie *et al*, 2003). In ALL, 11q23 rearrangements have been reported in 4–8% of the whole population of ALL cases (Behm *et al*, 1996; Forestier *et al*, 2000). Translocation t(4;11) is the most

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frequent aberration, followed by t(11;19) and t(9;11). Among infants with ALL, any 11q23 abnormality confers a dismal outcome, but in children >1 year of age the picture is more complex (Pui *et al*, 2002, 2003).

It is not known why infants fare so much worse than older children with the same genetic subtypes of ALL, and why t(9;11) has a favourable prognostic impact in AML, while other 11q23 rearrangements have not. However, *in vitro* studies of cellular drug resistance have indicated that drug resistance at the cellular level might be one factor of importance. Zwaan *et al* (2002) found that t(9;11) samples from children with AML were more sensitive to a number of drugs than other AML samples, and Ramakers-van Woerden *et al* (2004) reported significant differences in the drug sensitivity profile of *MLL*-rearranged samples from children with ALL compared with non-*MLL*-rearranged cases. Both these studies comprised patients treated with a number of different protocols, and no direct comparisons between ALL and AML were made.

We have studied cellular drug resistance in children uniformly treated according to common Nordic protocols for AML and ALL. Leukaemic cells were tested by the fluorometric microculture cytotoxicity assay (FMCA), a rapid and reproducible method for determination of *in vitro* drug sensitivity (Nygren *et al*, 1992; Larsson & Nygren, 1993). Patients with 11q23 rearrangements have been compared to patients without such aberrations, and we have also compared 11q23 subgroups in ALL and AML to investigate the relative importance of cytogenetics and cell lineage. The latter comparison was prompted by a recent study of the gene expression profile in *MLL*-rearranged paediatric AML and ALL, which demonstrated that the lineage of origin was of importance, but that a shared gene expression signature could be identified for cases with *MLL* chimaeric fusion genes (Ross *et al*, 2004).

Materials and methods

Patients and samples

Leukaemic cells from bone marrow or peripheral blood of children (aged 0–17 years) with newly diagnosed leukaemia were collected at Nordic centres for paediatric oncology between 1993–2003 (AML) and 1992–2002 (ALL). Because AML in children with Down's syndrome (DS) differs markedly from other forms of AML, cases with DS were excluded from the study. Samples from 158 non-DS children with *de novo* AML and 690 non-DS children with ALL were received for test of *in vitro* drug resistance. The diagnosis was established at a paediatric oncology centre by analysis of bone marrow aspirates including morphology, immunophenotype and cytogenetics of the leukaemic cells. Immunophenotypes were defined according to the European Group for the Immunological Characterization of Leukemias (Bene *et al*, 1995). Children with AML were treated according to the Nordic Society for Paediatric Haematology and Oncology (NOPHO)-AML-93 protocol (Lie *et al*, 2003), and children with ALL according to the NOPHO-ALL-92 protocol (Gustafsson *et al*, 2000). At participating centres, patients were entered consecutively in a prospective manner. Some Nordic centres did not participate in the study, or did so during the last years of the study period only. Patient characteristics and clinical follow-up data were obtained from annual reports submitted from the treating clinicians to the Nordic registry at the Childhood Cancer Research Unit in Stockholm, and the last day of follow up was 31 December 2003.

The samples were collected in heparinized glass tubes, kept at room temperature, and sent by mail or through international express delivery companies. As a rule they reached the *in vitro* sensitivity laboratory in Uppsala, Sweden, for processing within 24–36 h. Most of the samples (about 90%) were analysed freshly, but for practical reasons some were cryopreserved in culture medium containing 10% dimethyl sulphoxide (DMSO) and 50% fetal calf serum (FCS) by initial freezing for 24 h at −70°C followed by storage in liquid nitrogen. The cells were later thawed and analysed. Previous studies showed that cryopreservation does not affect the *in vitro* sensitivity, and it has also been shown that the source of the leukaemic cells (bone marrow or peripheral blood) does not affect the *in vitro* drug resistance measured (Pieters *et al*, 1988; Kaspers *et al*, 1991; Nygren *et al*, 1992). Leukaemic cells were prepared by 1·077 g/ml Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) density-gradient centrifugation. Viability was determined by Trypan-blue exclusion test. The median viability was 95% and FMCA was performed only when the viability was ≥70%. The median proportion of lymphoblasts after separation was 90% and FMCA was performed only when this proportion was ≥70%.

Cytogenetic investigations

Chromosome banding analyses of bone marrow and/or peripheral blood samples were performed using standard methods in 15 cytogenetic laboratories in the Nordic countries. The definition and description of clonal abnormalities followed the recommendations of the International System for Human Cytogenetic Nomenclature (Mitelman, 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 transcription polymerase chain reaction (RT–PCR) analyses have been increasingly applied to verify or characterize more precisely the chromosomal abnormalities found (Forestier *et al*, 2000, 2003; Mitelman, 2005). Only patients with known 11q23 status were included.

FMCA procedure

The FMCA is based on measurement of fluorescence generated from hydrolysis of fluorescein diacetate (FDA) to fluorescein by cells with intact plasma membranes and has been described

in detail previously (Larsson *et al*, 1992; Larsson & Nygren, 1993; Nygren *et al*, 1994; Fridborg *et al*, 1999). Drugs were tested in triplicate. Six wells without drugs served as controls and six wells containing culture medium only served as blanks. Quality criteria for a technically successful assay included a proportion of leukaemic cells of ≥70% in control wells after 72 h of incubation, a fluorescence signal in control wells of greater than or equal to five times the mean blank value, and a mean coefficient of variation (CV) in control wells of <30%. 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.

Drugs

Cytotoxic drugs were obtained from commercial sources and tested at empirically derived cut-off concentrations, adopted from previous studies of leukaemic cells and chosen to produce a large scatter of SI values among the samples (Nygren *et al*, 1992): amsacrine 1 μg/ml, cytarabine 0·5 μg/ml, dexamethasone 1·4 μg/ml, doxorubicin 0·5 μg/ml, etoposide 5 μg/ml, prednisolone 50 μg/ml, vincristine 0·5 μg/ml, 2-chlorodeoxyadenosine (2-CdA) 0·2 μg/ml and 6-thioguanine 10 μg/ml. The active metabolite of cyclophosphamide, 4-hydroperoxy-cyclophosphamide (4-HC), was used at a concentration of 2 μg/ml.

Cellular sensitivity to methotrexate cannot be tested *in vitro* by FMCA and similar methods, because leakage of nucleosides from dying cells provide a salvage pathway for surviving cells (Hughes *et al*, 1988; Nygren *et al*, 1992; Kaspers & Veerman, 2003).

Statistical analysis

Non-parametric methods were used throughout. Differences in distribution of variables were tested with the Mann–Whitney *U*-test, Kruskal–Wallis H-test or the chi-square test. The Spearman correlation coefficient was used to examine relationships between continuous variables, and the log-rank test to compare survival data generated by the Kaplan–Meier method. The Statistical Package for the Social Sciences (SPSS) version 11.5 software was used for the calculations. All analyses were two-tailed and the level of statistical significance was set at $P < 0{\cdot}05$.

Local ethics committees approved the study.

Results

AML

Data on the presence of 11q23 translocations were available in 155 of the 158 samples sent to our laboratory for test of *in vitro* drug resistance. Eleven patients with t(9;11) and 16 patients with other 11q23 rearrangements were identified, resulting in an overall prevalence of these rearrangements of 7·1 and 10·3% respectively.

Testing of cellular drug resistance by FMCA was successfully accomplished in 132 of the 155 samples. Reasons for failure of *in vitro* testing are given in the legend of Table I. This table shows the characteristics of the study patients, together with those of all children diagnosed with AML in the Nordic countries during the study period ($n = 311$). Included children were somewhat older than those not included, median age 7·2 vs. 6·0 years ($P = 0{\cdot}031$). No statistically significant difference was found for any of the other parameters displayed (*P*-values in Table I), and the same was true for the presence of the non-random chromosomal aberrations inv16, t(8;21), t(15;17) and 11q23 rearrangements (data not shown).

Clinical and laboratory characteristics of the 132 study patients are shown in Table II. As t(9;11) and other 11q23 translocations are known to have different prognostic impacts, they were treated separately. Median age was lower in patients with t(9;11) and other 11q23 translocations than in patients without any 11q23 translocation. The distribution of French-American-British (FAB) groups also differed markedly.

The *in vitro* drug resistance of study patients is shown in Table III. Patients with t(9;11) were significantly more sensitive to cytarabine ($P < 0{\cdot}001$) and doxorubicin ($P = 0{\cdot}005$) than patients without any 11q23 rearrangement. Patients with t(9;11) were, however, more resistant to dexamethasone ($P = 0{\cdot}015$) and prednisolone ($P = 0{\cdot}021$). Patients with other 11q23 translocations were also more sensitive to doxorubicin ($P = 0{\cdot}033$) and more resistant to the glucocorticoids than patients without any 11q23 rearrangement, but did not differ in sensitivity to cytarabine.

ALL

Data on the presence of 11q23 rearrangement were available in 226 of the 690 samples from patients with ALL sent to our laboratory for test of *in vitro* drug resistance. Twenty-four patients with 11q23 rearrangement were identified, resulting in an overall prevalence of 10%.

Testing of cellular drug resistance by FMCA was successful in 178 of the 226 samples. Reasons for failure of *in vitro* testing are given in the legend of Table IV. This table shows clinical and laboratory characteristics of the study patients, i.e. those with available 11q23 data and successful FMCA. As expected, a large proportion of the 11q23-positive patients were infants (59%), and the white blood cell (WBC) count at diagnosis was high, median 171×10^9/l. The translocation t(4;11) was the single most common 11q23 aberration (41%).

We also compared the 178 study patients to the 332 patients with successful FMCA but without available 11q23 data. There were no significant differences in median age, sex, WBC count at diagnosis, immunophenotype, or probability of disease-free survival (p-DFS) at 3 and 5 years (data not shown). We finally compared the 178 study patients to the 48 patients with 11q23 data but unsuccessful FMCA. Again, there were no differences in the characteristics mentioned above (data not shown).

Table I. Characteristics of children with AML included in the study ($n = 132$) compared with those of children not included ($n = 179$).

	Included (drug resistance and 11q23 data available)	Not included	All children with AML	*P*-value
N	132	179	311	
Age (years), median (p 25–75)	7·2 (2·6–12·9)	6·0 (1·5–11·0)	6·1 (2·0–12·1)	0·031
Sex (*n*)				0·78
Male	67	88	155	
Female	65	91	156	
WBC (10^9/l), median (p 25–75)	19·6 (5·7–58)	12·0 (3·9–49)	14·2 (4·9–55)	0·076
FAB (*n*)				0·066
M0	10	10	20	
M1	17	23	40	
M2	31	33	64	
M3	7	9	16	
M4	30	32	62	
M5	29	31	60	
M6	2	5	7	
M7	3	16	19	
RAEB-t	1	6	7	
Unclassifiable	1	6	7	
Missing data	1	8	9	
p-DFS	0·47	0·58	0·53	0·09
p-DFS at 3 years	0·49	0·62	0·57	
p-DFS at 5 years	0·49	0·59	0·55	

p 25–75, 25–75th percentiles; p-DFS, probability of disease-free survival; FMCA, fluorometric microculture cytotoxicity assay; AML, acute myeloid leukaemia; WBC, white blood cell; FAB, French-American-British; RAEB-t, refractory anaemia with excess blasts in transformation.

P-values relate to comparisons between included and not included patients. Reasons for not being included were that no samples were received ($n = 153$), no data on 11q23 status were available for received samples ($n = 3$), or unsuccessful FMCA ($n = 23$). Values for the whole group of children diagnosed with AML in the Nordic countries during the study period are shown for comparison. Reasons for failure of *in vitro* testing in patients with and without 11q23 rearrangement were: total number of cells too small to test any drug ($n = 0$ and $n = 1$, respectively), transport problems ($n = 0$, $n = 6$), too low proportion of blasts before the *in vitro* test ($n = 0$, $n = 2$), too low proportion of blasts in control wells after incubation ($n = 0$, $n = 9$), too low signal-to-noise ratio ($n = 1$, $n = 2$) and high coefficient of variation in controls ($n = 2$, $n = 0$). The technical success rate for *in vitro* testing of samples with sufficient number of cells was 88% and 84% in patients with and without 11q23 rearrangement respectively.

The lymphoblasts of children with 11q23 rearrangement were significantly more sensitive to cytarabine ($P = 0·008$) and 2-CdA ($P = 0·01$) than those of children without any 11q23 rearrangement, with a difference in the same direction for amsacrine, dexamethasone, doxorubicin, etoposide and prednisolone ($P = 0·02$–$0·05$; Table V). When groups of patients with t(4;11), t(11;19) and other 11q23 rearrangements were compared, there was no significant difference or any trend to difference between these groups (data not shown). The influence of age was first examined by comparing children with 11q23 rearrangement who were below or above 1 year of age, respectively; neither a significant difference nor a clear trend was noted for any of the drugs tested. We then compared the *in vitro* sensitivity in infants 0–6 and 6–12 months old respectively. Blast cells of children in the youngest group were significantly more resistant to amsacrine ($P = 0·008$) and doxorubicin ($P = 0·003$), with a trend in the same direction for dexamethasone, etoposide, prednisolone and vincristine. Cytarabine was the only drug for which the two groups showed a similar *in vitro* sensitivity. Median WBC count in 0–6- and 6–12-months-old infants was 508 and 199 $\times 10^9$/l, respectively ($P = 0·4$).

There was an inverse correlation between WBC count at diagnosis and cell survival after drug exposure, i.e. blast cells tended to be more sensitive in patients with high WBC count (tested in all the 178 study patients). The correlation was statistically significant for amsacrine ($P = 0·006$), dexamethasone ($P = 0·004$), etoposide ($P = 0·011$) and prednisolone ($P = 0·011$), with ρ-values ranging between $-0·2$ and $-0·3$, and there was a trend in the same direction for all drugs tested. To further explore the impact of WBC count, we compared patients with 11q23 rearrangement, who all had WBC $\geq 16 \times 10^9$/l, with patients without 11q23 rearrangement and WBC $\geq 16 \times 10^9$/l ($n = 61$). The 11q23-positive

Table II. Characteristics of children with AML for whom data on 11q23 rearrangement and cellular drug resistance were available.

	All patients	t(9;11)	Other 11q23 rearrangements	No 11q23 rearrangement
N	132	10	14	108
Age (years), median (p 25–75)	7·2 (2·6–12·9)	1·8 (0·48–7·2)	2·7 (1·1–8·7)	8·6 (3·2–13·4)
Sex (*n*)				
Male	67	6	3	58
Female	65	4	11	50
WBC (10^9/l), median (p 25–75)	19·6 (5·7–58)	14·4 (3·5–33)	8·1 (6·3–78)	21 (5·7–59)
FAB (*n*)				
M0	10		2	8
M1	17			17
M2	31			31
M3	7			7
M4	30		2	28
M5	29	9	9	11
M6	2			2
M7	3	1		2
RAEB-t	1			1
Unclassifiable	1		1	
Missing data	1			1
p-DFS	0·47	0·68	0·45	0·44
p-DFS at 3 years	0·49	0·68	0·45	0·48
p-DFS at 5 years	0·49	0·68	0·45	0·48

p 25–75, 25–75th percentiles; FAB, French-American-British; RAEB-t, refractory anaemia with excess blasts in transformation; WBC, white blood cell; AML, acute myeloid leukaemia; p-DFS, probability of disease-free survival.

Median age was lower in patients with t(9;11) ($P = 0{\cdot}016$) and other 11q23 rearrangements ($P = 0{\cdot}010$), than in patients without any 11q23 rearrangement. The distribution of FAB groups also differed markedly ($P < 0{\cdot}001$). Other differences between patient groups were non-significant.

Table III. Cellular drug resistance in children with acute myeloid leukaemia (AML).

Drug	t(9;11) ($n = 10$), median (25–75)	*P*-value	No 11q23 rearrangement ($n = 108$), median (25–75)	*P*-value	Other 11q23 rearrangement ($n = 14$), median (25–75)
Amsacrine	36 (24–45)	0·058	48 (33–67)	0·18	39 (26–55)
Cytarabine	27 (23–34)	<0·001	53 (40–67)	0·21	47 (25–65)
Dexamethasone	83 (76–95)	0·015	69 (57–86)	0·014	82 (75–92)
Doxorubicin	28 (16–41)	0·005	50 (34–67)	0·033	33 (28–53)
Etoposide	54 (47–70)	0·46	60 (41–83)	0·18	48 (26–74)
Prednisolone	82 (69–89)	0·021	62 (50–81)	0·026	77 (66–87)
Vincristine	60 (42–66)	0·28	66 (50–82)	0·24	58 (45–71)
2-CdA	28 (21–36)	0·85	30 (17–47)	0·53	22 (15–41)
4-HC	20*	*	58 (43–69)	0·89	56 (29–75)
6-TG	38 (32–43)	0·70	41 (21–57)	0·67	42 (30–57)

Median values and 25–75th percentiles of the survival index, where a low numerical value indicates high cellular sensitivity to the cytotoxic effect of the drug.

2-CdA, 2-chlorodeoxyadenosine; 4-HC, 4-hydroperoxy-cyclophosphamide; 6-TG, 6-thioguanine.

*Only two cases tested.

patients were still more sensitive to cytarabine ($P = 0{\cdot}026$), while there was no statistically significant difference for the other drugs tested.

Samples from the 332 patients without available 11q23 data had an *in vitro* sensitivity profile very similar to that of the 11q23-negative children shown in Table V.

Table IV. Characteristics of children with ALL for whom data on 11q23 rearrangement and cellular drug resistance were available.

	11q23 rearrangement	No 11q23 rearrangement	*P*-value
N	22	156	
Age (years), median (p 25–75)	0·88 (0·46–1·7)	5·4 (3·2–9·8)	<0·001
0–6 months (*n*)	6	0	
6–12 months (*n*)	7	3	
>12 months (*n*)	9	153	
Sex (*n*)			0·052
Male	9	96	
Female	13	60	
WBC (10^9/l), median (p 25–75)	171 (60–489)	7·0 (3·0–33)	<0·001
0–50 (*n*)	3	126	
50–200 (*n*)	10	18	
200+ (*n*)	9	12	
Cytogenetic aberration (*n*)			
t(4;11)	9		
t(11;19)	6		
Other 11q23 rearrangement	7		
Immunophenotype (*n*)			0·66
Precursor B ALL	18	134	
T-ALL	4	22	
p-DFS	0·40	0·84	<0·001
p-DFS at 3 years	0·48	0·87	
p-DFS at 5 years	0·40	0·84	

p 25–75, 25–75th percentiles; p-DFS, probability of disease-free survival; ALL, acute lymphoblastic leukaemia; WBC, white blood cell.

Reasons for failure of *in vitro* testing in patients with and without 11q23 rearrangement were: total number of cells too small to test any drug ($n = 0$ and $n = 8$, respectively), transport problems ($n = 1$, $n = 2$), too low proportion of lymphoblasts before the *in vitro* test ($n = 0$, $n = 2$), too low proportion of lymphoblasts in control wells after incubation ($n = 0$, $n = 8$), too low signal-to-noise ratio ($n = 1$, $n = 21$) and high coefficient of variation in controls ($n = 0$, $n = 5$). The technical success rate for *in vitro* testing of samples with sufficient number of cells was 92% and 77% in patients with and without 11q23 rearrangement, respectively ($P = 0·10$).

Table V. Cellular drug resistance in children with ALL.

Drug	11q23 rearrangement ($n = 22$) median (25–75)	*P*-value	No 11q23 rearrangement ($n = 156$) median (25–75)
Amsacrine	22 (11–34)	0·043	34 (18–47)
Cytarabine	41 (27–54)	0·008	57 (38–72)
Dexamethasone	35 (22–52)	0·048	53 (36–67)
Doxorubicin	20 (10–43)	0·035	34 (19–50)
Etoposide	34 (21–41)	0·12	44 (24–69)
Prednisolone	34 (21–48)	0·022	48 (33–62)
Vincristine	36 (30–58)	0·076	51 (38–69)
2-CdA	19 (13–22)	0·010	40 (32–51)
4-HC	30 (15–60)	0·36	26 (12–33)
6-TG	39 (20–60)	0·88	38 (22–53)

Median values and 25–75th percentiles of the survival index.

2-CdA, 2-chlorodeoxyadenosine; 4-HC, 4-hydroperoxy-cyclophosphamide; 6-TG, 6-thioguanine; ALL, acute lymphoblastic leukaemia.

Comparison between AML and ALL

When 11q23-negative patients were compared, ALL patients were younger than AML patients ($P = 0·019$) and had lower WBC count at diagnosis ($P = 0·001$; see Tables II and IV). There were marked differences in the *in vitro* sensitivity profiles. ALL patients were considerably more sensitive to amsacrine, dexamethasone, doxorubicin, etoposide,

prednisolone, vincristine and 4-HC ($P \leq 0{\cdot}001$ for all drugs; see Tables III and V). Cytarabine and 2-CdA were the only drugs for which AML cells tended to be more sensitive, but the difference was not statistically significant.

When patients with 11q23 rearrangement were compared, ALL patients were younger ($P = 0{\cdot}031$) and had much higher WBC at diagnosis ($P < 0{\cdot}001$). ALL patients were more sensitive to dexamethasone and prednisolone ($P < 0{\cdot}001$), and to amsacrine ($P = 0{\cdot}003$), etoposide (0·020) and vincristine ($P = 0{\cdot}008$). For cytarabine there was a non-significant trend in the other direction (see Tables III and V).

In the AML group there were three cases with t(10;11) and two cases each with t(11;19) and t(11;17), while other aberrations occurred in single patients only. The t(11;19) cases are of interest, because this translocation also occurred in a number of ALL patients. There were no striking differences between the two AML patients with t(11;19) and AML patients without 11q23 rearrangement, i.e. they showed an 'AML-profile' with relatively high resistance to glucocorticoids and vincristine, and sensitivity to cytarabine. Thus, they differed clearly from patients with ALL and t(11;19).

One patient with ALL displayed a t(9;11) translocation. Although the observation was anecdotal, it is interesting to note that this patient was relatively sensitive to cytarabine, etoposide and vincristine, but resistant to glucocorticoids, i.e. showed a profile with a mixture of AML and ALL features.

Discussion

The prevalence of 11q23 abnormalities in the received AML samples was 17·4%, a figure similar to published data (Raimondi *et al*, 1999; Forestier *et al*, 2003). The prevalence of 11q23 abnormalities in the received ALL samples was 10%, which is slightly higher than previously reported (Behm *et al*, 1996; Forestier *et al*, 2000).

A crucial point is whether or not the cases included in this study are representative of the whole population of children with AML and ALL. We therefore compared patient characteristics of the study patients, i.e. those with available 11q23 data and successful FMCA, with data of all other patients diagnosed with AML or ALL in the Nordic countries during the study period. For ALL, a previous report has presented detailed evidence that the samples sent to us for FMCA were representative of the whole population (Frost *et al*, 2003). For AML, study patients showed a slightly higher age than non-study patients, median 7·2 and 6·0 years, respectively, but other baseline data did not differ significantly.

For the AML samples received, data on 11q23 translocations were available in 98% of the cases. For the ALL samples received, data on 11q23 rearrangements were only available in 33% of the cases, and the main reason was that several centres did not use specific methods to detect or exclude 11q23 rearrangements in patients aged more than 1 year, because of the low expectancy of positive findings. We therefore compared the characteristics of study patients to those of patients with successful FMCA but lacking 11q23 data. There were no significant differences, and the *in vitro* sensitivity profiles of these two groups were also very similar. Furthermore, a comparison of patient characteristics for cases with and without successful *in vitro* test did not reveal any significant differences. We therefore conclude that our study groups were representative for childhood AML and ALL patients, with the exception of a slightly higher than expected prevalence of 11q23 aberrations in the ALL group. It is of special interest to note that this included similarities in WBC count at diagnosis (see further below).

AML

Zwaan *et al* (2002) studied cellular drug resistance in childhood AML and found that t(9;11) samples were more sensitive to cytarabine, etoposide, anthracyclines and 2-CdA than other AML samples, while samples with non-t(9;11) 11q23 rearrangements did not differ from AML samples without any 11q23 aberration. Our findings confirm the high sensitivity in t(9;11) samples to cytarabine and doxorubicin, but we also found an increased resistance to glucocorticoids, which has not been reported previously. Because cytarabine and doxorubicin are important drugs in the treatment of childhood AML, the *in vitro* data offer an explanation to the favourable clinical outcome in patients with t(9;11). In Nordic children treated with the NOPHO-AML-93 protocol, the probability of event-free survival (p-EFS) at 7 years was 86% for children with t(9;11) and 36% for children with other 11q23 aberrations, when compared with 49% in the whole AML cohort, DS children excluded (Lie *et al*, 2003). In the present study, patients with other 11q23 aberrations differed from patients with t(9;11) by having a cellular sensitivity to cytarabine comparable to that of patients without 11q23 rearrangement.

ALL

Ramakers-van Woerden *et al* (2004) studied the *in vitro* drug resistance profile in infant ALL in relation to age, *MLL* rearrangements and immunophenotype. They found that *MLL*-rearranged samples were more sensitive to cytarabine, etoposide and 2-CdA, and more resistant to glucocorticoids and L-asparaginase, compared with non-*MLL*-rearranged cases. Our data differed in that children with *MLL* rearrangements were more sensitive to almost all drugs tested, including glucocorticoids. We have demonstrated that our patient material is representative of the whole population of children diagnosed with ALL in the Nordic countries during the study period, including similarities in WBC count at diagnosis. This is of great importance, as there is always a risk of selection bias, so that a higher proportion of samples are received from patients with high WBC count at diagnosis, simply because a large number of tumour cells are more easily available (Kaspers *et al*, 1997). Data for the reference group were not given by Ramakers-van Woerden *et al* (2004), so differences in selection of patients might explain the somewhat diverging results.

How can our data be understood with respect to the well-known fact that ALL patients with 11q23 rearrangements have an unfavourable prognosis? Part of the explanation might be the correlation between cell proliferation and *in vitro* drug resistance described by Kaaijk *et al* (2003). Cell survival after drug exposure correlated negatively to WBC count, i.e. high WBC was correlated to high drug sensitivity *in vitro* in our patient material. As previously discussed (Frost *et al*, 2003), WBC count is thought to reflect cell proliferation, and proliferating cells are generally more sensitive to cytotoxic drugs than non-proliferating cells (Tannock, 1987). Although correlation coefficients were low, indicating that WBC counts accounted for only a small extent of the differences between patients, WBC count might act as a 'confounding factor' when *in vitro* data are correlated to clinical outcome, especially if groups with large differences in WBC count are compared, as in ALL with or without 11q23 rearrangement. In the clinical setting, factors other than cellular drug sensitivity also come into play, such as the rate of tumour cell re-growth and stroma interactions, which might explain why high WBC at diagnosis in childhood ALL strongly correlates with a worse clinical outcome (Preisler *et al*, 1984; Larsson, 1997).

As expected, the WBC count at diagnosis was high in our ALL patients with 11q23 rearrangements, ranging between 16 and 950×10^9/l. When they were compared with a selected group of 11q23-negative patients with WBC $\geq 16 \times 10^9$/l, there was no statistically significant difference for any other drug than cytarabine, for which the 11q23 rearranged patients were still more sensitive.

Our data indicate that factors other than drug resistance at the cellular level are the main reasons why many children with ALL and 11q23 rearrangements relapse. Still, the *in vitro* data might be clinically useful. In full agreement with the findings of Ramakers-van Woerden *et al* (2004), we demonstrated that 11q23-positive patients were particularly sensitive to cytarabine, and thus support the idea of the ongoing clinical trials, where two large prospective international studies in infants (COG-P9407, led by the Children's Oncology Group, Dr ZoAnn Dreyer, Texas Children's Cancer Center, USA and Interfant 99, led by the Dutch Childhood Leukemia Study Group, Dr Rob Pieters, Sophia Children's Hospital in Rotterdam, the Netherlands) are testing the efficacy of intensified therapy that includes high-dose cytarabine.

Within the group of children with ALL and 11q23 rearrangements, infants 0–6 months old were more resistant to anthracyclines than older children, in spite of comparable WBC count at diagnosis. Although groups were small, this suggests that the particularly dismal prognosis in the youngest children partly depends on high cellular drug resistance to this group of agents.

Comparison between ALL and AML

That samples from children with AML and ALL have markedly different drug sensitivity profiles was an early finding (Nygren *et al*, 1992; Kaspers *et al*, 1994) and has recently been described in detail by Zwaan *et al* (2000). Our comparison of patients without 11q23 abnormalities fully support these previous data, showing that AML samples are more resistant to a wide range of drugs including glucocorticoids, vincristine, anthracyclines and etoposide, but equally sensitive to cytarabine and 6-thioguanine as ALL samples. These data are in good agreement with clinical practice and may contribute to the difference in prognosis between childhood ALL and AML.

When patients with 11q23 rearrangements were compared, much of the ALL and AML patterns described above were found. Thus, AML samples were much more resistant to typical 'ALL-drugs' like glucocorticoids and vincristine, while there was a trend in the other direction for cytarabine. Translocation t(11;19) occurs both in AML and ALL, thus allowing a direct comparison. The findings in our few patients indicated that lineage influenced the drug sensitivity profile more than cytogenetics. A single case of ALL with t(9;11), on the other hand, showed a profile with a mixture of AML and ALL features.

Thus, our findings indicate that both cell lineage and the type of *MLL* rearrangement are of importance for the cellular drug resistance. This fits well with recently published studies of the gene expression profile in *MLL*-rearranged paediatric ALL (Armstrong *et al*, 2002; Ferrando *et al*, 2003) and AML (Ross *et al*, 2004), where it was reported that the lineage of origin is of importance, but that a shared gene expression signature can be identified for cases with *MLL* chimaeric fusion genes. This expression signature probably reflects a cellular pathobiology, which is of importance for the *in vitro* sensitivity to cytarabine, shared by *MLL*-rearranged childhood leukaemias. However, direct experimentation will be required to determine which of the identified genes play a mechanistic role for drug sensitivity.

In conclusion, the findings indicate that the cellular drug resistance is correlated to both the cell lineage and the type of 11q23 rearrangement. High cellular sensitivity to cytarabine and doxorubicin might explain the excellent treatment results in children with AML and t(9;11), but our data do not explain the high rate of treatment failures in children with ALL and 11q23 rearrangement, which might be due to factors other than drug resistance at the cellular level. The present study supports the strategy of contemporary protocols to include high-dose cytarabine in the treatment of 11q23-positive patients both in AML and ALL.

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