Different drug sensitivity profiles of acute myeloid and lymphoblastic leukemia and normal peripheral blood mononuclear cells in children with and without Down syndrome


Introduction

Children with Down syndrome (DS) have an increased risk for leukemia. The prognosis for DS acute myeloid leukemia (AML) is better than for non-DS AML, but the clinical outcome of DS acute lymphoblastic leukemia (ALL) is equal to that of non-DS ALL. Differences in prognosis may reflect differences in cellular drug resistance. In vitro drug resistance profiles were successfully investigated on leukemic cells from 13 patients with DS AML and 9 patients with DS ALL and were compared with reference data from 151 non-DS AML and 430 non-DS B-cell precursor (BCP) ALL. DS AML cells were significantly more sensitive to cytarabine (median, 12-fold), the anthracyclines (2-7-fold), mitoxantrone (9-fold), amsacrine (16-fold), etoposide (20-fold), 6-thioguanine (3-fold), busulfan (5-fold), vincristine (23-fold), and prednisolone (more than 1.1-fold), than non-DS AML cells. Compared with DS ALL, DS AML cells were significantly more sensitive to cytarabine only (21-fold). After short-term exposure to methotrexate, DS AML cells were 21-fold more resistant than non-DS AML cells, but no difference was observed after continuous exposure. DS ALL cells and non-DS BCP-ALL cells were equally sensitive to all drugs, including methotrexate. Normal peripheral blood mononuclear cells from DS and non-DS children without leukemia showed highly resistant drug profiles. It was concluded that the better prognosis of DS AML might, at least partially, be explained by a specific, relatively sensitive drug-resistance profile, reflecting the unique biology of this disease. (Blood. 2002;99:245-251)

Patients, materials, and methods

Patient samples

We tested bone marrow or peripheral blood samples from children (newborn to 18 years of age) given diagnoses of de novo AML or ALL, with and without Down syndrome (DS). Three different collaborative groups provided us with patient samples: the AML-BFM Study Group (Münster, Germany), the CoALL Study Group (Hamburg, Germany), and the Dutch Childhood Leukemia Study Group (DCLSG, Den Haag, The Netherlands). Central review of diagnoses, clinical data, and characteristics of disease by immunophenotype (ALL) or FAB classification (AML) was performed by reference laboratories of these groups.

We also tested normal, nonleukemic PBMCs from 9 children with DS and 12 children without DS. Children with inflammation or infection were
excluded from this study. PBMCs were collected in hospitals in 's-Hertogenbosch (EV) and Amsterdam after informed consent was obtained, according to the Institutional Review Board guidelines.

In this study 2 reference groups were used: non-DS children with AML and non-DS children with BCP-ALL. The non-DS AML group consisted of 151 patients (78 boys, 73 girls) with a median age of 8.4 years. Median WBC was 35.5 × 10^9/L, and FAB classification showed the following: 5 patients with M0, 20 patients with M1, 36 patients with M2, 8 patients with M3, 44 patients with M4, 30 patients with M5, 1 patient with M6, 1 patient with M7, and 6 patients unclassified. The non-DS B-cell precursor ALL group consisted of 430 patients (238 boys, 192 girls). Median age was 4.4 years, and median WBC was 13.5 × 10^9/L.

### Cells

Mononuclear cells were isolated by density-gradient centrifugation with ficoll isopaque. In ALL samples with a low (less than 90%) blast percentage (determined morphologically on May-Grünwald-Giemsa (MGG) cytospin slides), we used monoclonal antibodies linked to magnetic beads to remove nonleukemic cells, as described before. A low blast percentage (less than 80%) might have been caused by the presence of mature granulocytes (more than 10%) or lymphocytes (more than 10%). To eliminate granulocytes we performed freezing, in liquid nitrogen, and thawing. Lymphocytes were removed using immunomagnetic beads at room temperature to prevent phagocytosis of the beads by leukemic cells. After these enrichment procedures, the percentage of blasts was determined morphologically (MGG cytospin slides) and was confirmed with immunochemistry if morphologic results were inconclusive. The cutoff level to actually perform the (3-(4,5-dimethylthiazol-2,5-diphenyl) tetrazolium bromide (MTT) assay was set at 80% leukemic cells.

FAB classification was performed according to the criteria of Bennett et al., including the modifications to diagnose FAB M7. Immunophenotype of all cells was determined by flow cytometry or by immunocytochemistry. Patients with DS ALL appeared to have BCP-ALL, defined as sIg-/7TdT+/HLA-Dr+/CD19+ and either CD10+ or CD20+ or both.

### MTT assay

In vitro drug resistance of leukemia and PBMC samples was measured using a 4-day cell culture assay, based on the principle that only viable cells are able to reduce MTT to a colored formazan product that can be determined spectrophotometrically. The MTT assay was successful in 13 DS AML and 9 DS ALL samples. Their patient characteristics are given in Table 1. We also tested normal PBMCs from 9 children with DS and 12 without DS, all without leukemia.

### Results

**Patient samples**

Samples from 22 patients with DS AML and 20 with DS ALL were tested. Two children with transient myeloproliferative disease were included in the DS AML group. MTT assay was successful in 13 DS AML and 9 DS ALL samples. Their patient characteristics are given in Table 1. We also tested normal PBMCs from 9 children with DS and 12 without DS, all without leukemia.

Patients with DS AML were significantly younger (P < .001) than patients with non-DS AML. WBC (P = .17) and sex distribution (P = .9) were not different. Only 1 of the non-DS patients was classified as FAB M7, whereas 64% of the patients with DS AML were classified as such (P < .001).

Patients with DS ALL did not differ significantly from patients with non-DS BCP-ALL for age (P = .72), sex (P = .93), or WBC (P = .47). All 20 patients with DS-ALL had BCP-ALL.

### Cellular drug resistance

**MTT assay.** In DS AML, 59% of samples were tested successfully for at least 1 drug. Reasons for assay failure were too few cells or too low blast count at day 0 (n = 6) or too low blast count after 4 days of culture (n = 3). The 2 patients with transient myeloproliferative disease showed a drug resistance pattern similar to that of...
patients with DS AML. Patients with successful assays had higher WBCs than patients with nonsuccessful assays \((P = .02;\) median, 2.7-fold more sensitive than DS ALL samples \((P = .013).\) DS AML cells were significantly more resistant than DS ALL cells after short-term exposure to MTX (113-fold; \(P = .004);\) however, this was not the case after continuous MTX exposure \((P = .43).\) For the other drugs no significant differences were found, though there was a trend suggesting that DS AML was more resistant to prednisolone than DS ALL \((P = .016).\) When using the criteria for prednisolone sensitivity mentioned in “Patients, materials, and methods,” none of the DS AML samples was highly prednisolone sensitive, and 4 of 10 samples showed intermediate sensitivity. In DS ALL, 44% of the samples were in the highly prednisolone-sensitive group \((\chi^2\) analysis, \(P = .018).\) Median LC50 values are given in Table 2 for DS AML and in Table 3 for DS ALL. Results are depicted in Figure 1.

**DS AML versus non-DS AML.** DS AML was significantly more sensitive than non-DS AML for the following drugs: cytarabine \((\text{median}, 11.5\text{-fold}; \ P < .001),\) daunorubicin \((2.2\text{-fold}; \ P = .005),\) idarubicin \((7.2\text{-fold}; \ P = .002),\) mitoxantrone \((9.2\text{-fold}; \ P = .002),\) etoposide \((20.1\text{-fold}; \ P < .001),\) 6-thioguanine \((2.7\text{-fold}; \ P < .001),\) amsacrine \((16.0\text{-fold}; \ P < .001),\) prednisolone

<table>
<thead>
<tr>
<th>Drug</th>
<th>DS AML</th>
<th>Median (P25-P75)</th>
<th>Non-DS AML</th>
<th>Median (P25-P75)</th>
<th>RR</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>13</td>
<td>0.04 (0.02-0.11)</td>
<td>143</td>
<td>0.46 (0.24-1.21)</td>
<td>11.5</td>
<td>.001</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>12</td>
<td>0.09 (0.05-0.10)</td>
<td>130</td>
<td>0.20 (0.09-0.38)</td>
<td>2.2</td>
<td>.005</td>
</tr>
<tr>
<td>Idarubicin</td>
<td>8</td>
<td>0.017 (0.004-0.025)</td>
<td>94</td>
<td>0.122 (0.039-0.265)</td>
<td>7.2</td>
<td>.002</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>10</td>
<td>0.012 (0.005-0.04)</td>
<td>113</td>
<td>0.11 (0.03-0.35)</td>
<td>9.2</td>
<td>.002</td>
</tr>
<tr>
<td>Etoposide</td>
<td>12</td>
<td>0.37 (0.16-0.60)</td>
<td>132</td>
<td>7.42 (1.92-19.14)</td>
<td>20.1</td>
<td>.001</td>
</tr>
<tr>
<td>6-Thioguanine</td>
<td>12</td>
<td>2.20 (&lt;1.56-2.81)</td>
<td>140</td>
<td>6.03 (4.05-10.54)</td>
<td>2.7</td>
<td>.001</td>
</tr>
<tr>
<td>Amsacrine</td>
<td>8</td>
<td>0.03 (0.02-0.08)</td>
<td>115</td>
<td>0.48 (0.14-1.32)</td>
<td>16.0</td>
<td>.001</td>
</tr>
<tr>
<td>2-Chlorodeoxyadenosine</td>
<td>6</td>
<td>0.016 (0.004-0.023)</td>
<td>103</td>
<td>0.020 (0.004-0.031)</td>
<td>1.3</td>
<td>.42</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>10</td>
<td>231.6 (95.9-400)</td>
<td>146</td>
<td>&gt;250 (&gt;250-250)</td>
<td>&gt;1.1</td>
<td>.006</td>
</tr>
<tr>
<td>Vincristine</td>
<td>11</td>
<td>0.13 (0.05-0.17)</td>
<td>135</td>
<td>2.99 (0.75-23.92)</td>
<td>23.0</td>
<td>.001</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>10</td>
<td>0.76 (0.33-1.19)</td>
<td>123</td>
<td>0.88 (0.23-1.48)</td>
<td>1.2</td>
<td>.83</td>
</tr>
<tr>
<td>Busulfan</td>
<td>6</td>
<td>8.7 (6.1-13.7)</td>
<td>93</td>
<td>37.78 (25.59-59.82)</td>
<td>4.6</td>
<td>.001</td>
</tr>
<tr>
<td>4-hydroperoxy-2-fluorofluridine</td>
<td>8</td>
<td>10.12 (4.69-12.31)</td>
<td>116</td>
<td>11.93 (5.79-14.78)</td>
<td>1.2</td>
<td>.47</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>(TSI50 short) 11</td>
<td>38.32 (32.62-40)</td>
<td>28</td>
<td>1.82 (0.63-18.38)</td>
<td>0.05</td>
<td>.003</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>(TSI50 cont) 9</td>
<td>0.08 (0.04-0.13)</td>
<td>27</td>
<td>0.05 (0.03-0.06)</td>
<td>0.6</td>
<td>.22</td>
</tr>
</tbody>
</table>

LC50 values are given in \(\mu\text{g/mL}.\) For MTX, the TSI50 values \(\mu\text{g/mL}\) are given after short-term \((\text{TSI50 short})\) or long-term \((\text{TSI50 cont})\) exposure. RR, the median LC50 value \((\text{for MTX, TSI50})\) for non-DS AML divided by the median LC50 value \((\text{for MTX, TSI50})\) for DS AML. \(P\) value derived from 2-tailed Mann-Whitney \(U\) test.

L-asparaginase is given in IU/mL, not in \(\mu\text{g/mL}\). N indicates number of samples tested; P25-P75, 25th and 75th percentiles.
## Table 3. Cellular drug resistance in DS ALL versus non-DS BCP-ALL

<table>
<thead>
<tr>
<th>Drug</th>
<th>DS ALL</th>
<th>Non-DS BCP-ALL</th>
<th>RR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Median (P25-P75)</td>
<td>N</td>
<td>Median (P25-P75)</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>7</td>
<td>0.84 (0.45-1.34)</td>
<td>288</td>
<td>0.48 (0.25-1.19)</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>9</td>
<td>0.10 (0.07-0.11)</td>
<td>387</td>
<td>0.09 (0.06-0.15)</td>
</tr>
<tr>
<td>Idarubicin</td>
<td>4</td>
<td>0.021 (0.014-0.046)</td>
<td>145</td>
<td>0.035 (0.022-0.066)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>7</td>
<td>0.26 (0.22-0.28)</td>
<td>193</td>
<td>0.27 (0.12-0.37)</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>6</td>
<td>0.037 (0.019-0.166)</td>
<td>197</td>
<td>0.045 (0.015-0.102)</td>
</tr>
<tr>
<td>6-Thioguanine</td>
<td>7</td>
<td>5.88 (4.12-8.03)</td>
<td>297</td>
<td>5.92 (3.83-8.60)</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>4</td>
<td>158.8 (&lt;15.0-302.6)</td>
<td>278</td>
<td>97.9 (50.6-240.0)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>9</td>
<td>0.12 (0.04-2.37)</td>
<td>352</td>
<td>0.46 (0.19-17.7)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>4</td>
<td>0.07 (0.05-1.59)</td>
<td>239</td>
<td>0.06 (0.01-14.7)</td>
</tr>
<tr>
<td>Vincristine</td>
<td>8</td>
<td>0.53 (0.15-1.79)</td>
<td>371</td>
<td>0.68 (0.24-2.50)</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>9</td>
<td>0.35 (0.03-1.37)</td>
<td>352</td>
<td>0.08 (0.01-1.04)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>10</td>
<td>0.34 (0.17-1.85)</td>
<td>88</td>
<td>0.42 (0.16-1.43)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>11</td>
<td>0.05 (0.02-0.13)</td>
<td>94</td>
<td>0.09 (0.04-0.19)</td>
</tr>
</tbody>
</table>

LC50 values are given in μg/mL. For MTX, the TSI50 values (μM) are given after short-term (TSIshort) or long-term (TSLong) exposure. RR, the median LC50 value (for MTX, TSI50) for non-DS ALL divided by the median LC50 value (for MTX, TSI50) for DS ALL. P-value derived from 2-tailed Mann-Whitney U test. N indicates number of samples tested; P25-P75, 25th and 75th percentiles.

(more than 1.1-fold; P = .006), vincristine (23.0-fold; P < .001), and busulfan (4.6-fold; P < .001). Cells from patients with DS AML were 21.1-fold more resistant to MTX after short-term exposure than cells from patients with non-DS AML (P = .003). However, they were equally sensitive after continuous MTX exposure (P = .22). No significant differences were found for L-asparaginase (P = .83), 2-chlorodeoxyadenosine (P = .42), and 4-hydroperoxy-2-isofosfamide (P = .47). Data are shown in Table 2 and Figure 2.

**DS ALL versus non-DS BCP-ALL.** There were no statistically significant differences in drug resistance between cells from patients with DS ALL and those with non-DS BCP-ALL, including methotrexate, after short- and long-term MTX exposure. Data are shown in Table 3.

**Peripheral blood mononuclear cells.** We found normal nonleukemic PBMCs to be resistant to most tested drugs, with the exception of 2-chlorodeoxyadenosine in cells from patients without DS. For cytarabine, daunorubicin, etoposide, vincristine, 6-thioguanine, and 2-chlorodeoxyadenosine (the latter only in DS PBMCs), the highest concentration of drug was unable to kill more than 50% of the cells in a large proportion of samples. For prednisolone and L-asparaginase, this was so in all samples. Dose-response curves were obtained in all patients for 4-hydroperoxy-2-isofosfamide and amsacrine only. No significant differences were found between DS and non-DS PBMCs. Data are shown in Table 4.

## Discussion

Children with DS AML have a better prognosis with current chemotherapy than patients with non-DS AML. Differences in prognosis may reflect differences in cellular drug resistance. It can be postulated, therefore, that the presence of an extra copy of chromosome 21 results in enhanced sensitivity to chemotherapeutic drugs. However, DS ALL does not carry a better prognosis than non-DS ALL. In the current study, in vitro cellular drug-resistance profiles of DS AML and DS ALL samples were studied to determine whether DS AML and ALL have different resistance patterns than their non-DS counterparts. In addition, PBMCs of nonleukemic children with and without DS were tested; it was speculated that DS PBMCs would be more chemosensitive than non-DSPBMCs, possibly reflecting the higher incidence of chemotherapy-related side effects in children with DS.

DS AML cells were significantly more sensitive to many drugs regularly used in AML treatment than non-DS AML cells. In addition, significant sensitivity to 2 so-called typical ALL drugs was observed—vincristine and prednisolone. The latter may not be clinically relevant, however, because the median LC50 value in DS AML is still approximately 500 times higher than in non-DS ALL. Moreover, none of the DS AML samples was highly sensitive to prednisolone, as defined by our cutoff levels in ALL. DS ALL cells showed drug-resistance profiles that were equally as sensitive as non-DS BCP-ALL cells. BCP-ALL cells, however, are relatively sensitive compared with other immunophenotypes in ALL, such as CD10/cu-negative BCP- and T-cell ALL. These results are in agreement with clinical data showing that patients with DS ALL have prognoses similar to those of patients with non-DS non-high-risk ALL. Both DS and non-DS PBMCs showed resistant drug profiles (except for 2-CdA in non-DS PBMCs). Therefore, testing PBMC sensitivity does not seem an adequate model to predict side effects of anticancer agents.

Two other groups have published data on in vitro drug sensitivity of DS leukemic cells. Taub et al showed that DS AML cells were significantly more sensitive to cytarabine and daunorubicin, the 2 drugs tested in that study. Frost et al reported a study on 5 patients with DS AML and 5 with DS ALL. DS AML cells were significantly more sensitive than non-DS AML cells to cytarabine, daunorubicin, amsacrine, and dexamethasone. For etoposide and 6-thioguanine, a trend for sensitivity of DS AML cells was found. DS ALL was significantly more resistant to dexamethasone and L-asparaginase than non-DS ALL, but numbers were small. Additional studies by Taub et al regarding cytarabine sensitivity showed higher Ara-CTP levels and 12-fold higher cythostatin-synthase (CBS, localized to chromosome 21q22.3) transcript levels in DS versus non-DS AML cells. Moreover, transfection of CBS, in CBS-deficient CCRF-CEM leukemic cells, resulted in enhanced cytarabine sensitivity and increased dCK enzyme activity. In contrast, carbonyl reductase (localized to 21q22.1), which catalyzes the reduction of daunorubicin to daunorubicinol, did not show higher transcript levels in DS myeloblasts when compared with non-DS AML cells. Consequently, the differences between DS and non-DS AML cells did not reflect the predicted 1.5-fold gene-dosage effect. Therefore, other selective regulatory factors of chromosome 21 gene expression appear to be involved in Down syndrome leukemic cells. This might also be disease- or even cell line-related, which is supported by our results on DS PBMCs. These were as resistant as normal non-DS PBMCs. Furthermore, DS AML cells were 21-fold more sensitive to cytarabine than DS AML cells.

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ALL cells, whereas non-DS AML and ALL do not differ in cytarabine resistance.16 For the other drugs, no significant differences were found between DS AML and DS ALL, whereas in an earlier study we found non-DS AML to be relatively resistant when compared with non-DS ALL for a panel of drugs.16 Because of this general pattern of drug sensitivity in DS AML, we suggest that a general mechanism, rather than a specific mechanism for each separate drug, is responsible for drug sensitivity in this particular disease.26 Several of these potential general mechanisms are suggested in the literature: (1) superoxide dismutase overexpression (SOD), localized to 21q22.1, shows 3.8-fold higher transcript levels in DS than in non-DS myeloblasts.26 SOD primarily protects against oxygen radicals, but it also induces the production of hydrogen peroxide, which may lead to enhanced cell death from oxidative damage.31,32 (2) enhanced apoptosis with p53 and CD95 has been detected in the brains of patients with DS, but no studies have been reported on DS leukemic cells.33 In addition, enhanced apoptosis of DS granulocytes has been described.34 (3) defective DNA repair after in vitro mutagen exposure has been reported, and it may be related to the increased cancer susceptibility of DS patients.35 Taken together, these findings may, at least partially, explain the good clinical outcome of children with DS AML, making us
question the need to develop separate treatment protocols for DS AML. The first example of a specific DS AML clinical trial was recently reported by Kojima et al., and it shows that treatment modification toward less intensive therapy is possible without affecting clinical outcome. Patients with DS AML included in the AML93 protocol of the BFM-AML Study Group (n = 41) were treated with a less drug-intensive schedule (dose reduction for anthracyclines and no stem cell transplantation), resulting in a 5-year event-free survival rate of 67% ± 5% (U. Creutzig, personal communication, 2001). It has been noted that the better survival of DS patients coincided with the introduction of the high-dose cytarabine-containing regimens, but this might also reflect such factors as improvements in supportive care and willingness to treat patients with DS AML. Based on our in vitro data and the promising results with dose reductions in clinical trials mentioned above, one might question the need for high-dose regimens with the associated side effects in the DS population.

MTX cannot be evaluated by MTT assay, but we recently described the TSIA to measure MTX sensitivity. The reduced folate carrier (RFC, localized to chromosome 21q22.2-q22.3) transports MTX into the cell, but at higher concentrations passive diffusion also occurs. RFC, localized to chromosome 21q22.2-q22.3 transports MTX into the cell, but at higher concentrations passive diffusion also occurs. The reduced folate carrier (RFC, localized to chromosome 21q22.2-q22.3) transports MTX into the cell, but at higher concentrations passive diffusion also occurs. The reduced folate carrier (RFC, localized to chromosome 21q22.2-q22.3) transports MTX into the cell, but at higher concentrations passive diffusion also occurs.

Studies in hyperdiploid ALL have shown that the high accumulation of MTX polyglutamylation in 2 patients with DS AML (comparable to ALL).39 In non-DS AML samples, we reported earlier that polyglutamylation was low, despite similar sensitivity to non-DS ALL after continuous exposure to MTX.40 It has also been reported that CBS influences MTX metabolism, but transfection of CBS does not lead to enhanced MTX sensitivity.40 We showed here that long-term MTX exposure results in similar TSI50 values for DS-AML and DS ALL and their non-DS counterparts. This suggests that long-term infusion might overcome MTX resistance in DS AML, as we previously reported for non-DS AML.41 One study addressed MTX pharmacokinetics and reported that median MTX plasma concentrations were significantly higher in DS patients than in matched controls with non-DS ALL.

Our results in DS and non-DS ALL might suggest that the mere presence of trisomy 21 (gene dosage effect) does not account for chemosensitivity and that, subsequently, the better prognosis for DS AML is attributed to the distinct and unique biology of this particular disease. However, the clinical outcome of non-DS ALL is better than for non-DS AML, and chromosome 21 is involved in approximately 50% of the non-DS ALL cases, such as the t(12;21)/TEL-AML gene rearrangement, and in nearly all hyperdiploid ALL cases, where it may contribute to improved clinical outcome.42,43 These abnormalities are also associated with specific, relatively sensitive drug-resistance profiles.44 Consequently, it is still possible that abnormalities of chromosome 21 contribute to relatively sensitive drug-resistance profiles in DS AML, DS ALL, and non-DS ALL.

The failure rate of the MTT assay in DS leukemia is higher than we previously reported for non-DS AML or ALL.16,17 In DS AML, failures are mainly caused by a low blast percentage in the bone marrow or by a lack of cells, which can be explained by the myelofibrosis or myelodysplasia, often accompanying DS ALL.2,21 In DS ALL, however, the main cause of assay failure was not surviving the 4-day cell culture. In previous studies, we also described this for hyperdiploid ALL and hypothesized that this might have resulted from a higher propensity for leukemic cells to undergo spontaneous apoptosis.14 Therefore, the results we present here on DS ALL may overestimate drug resistance, based on biased selection of samples surviving the 4-day cell culture.

One drawback of this study is that we were unable to compare children with and without DS based on FAB M7 classification because of the lack of FAB M7 samples in the children without DS. Non-DS FAB M7 is heterogeneous and can be further subdivided into at least 2 groups—children (often infants) with t(1;22) and older children without t(1;22).45 Both groups have poorer prognosis than do children with DS AML.45,46

In conclusion, DS AML has a distinct drug sensitivity profile, with relative sensitivity to drugs frequently used in AML but also to the classic ALL-drug vincristine. This may, at least partially, explain its favorable prognosis over non-DS AML. On the contrary, DS ALL does not show enhanced in vitro drug sensitivity when compared with non-DS BCP-ALL, which is in agreement with clinical studies showing a similar prognosis for these 2 groups of patients.

Acknowledgments

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References