Leukemia in Children with Down Syndrome

Children with Down syndrome (DS) have an increased risk of developing leukemia, which concerns both acute myeloid (AML) as well as acute lymphoblastic leukemia (ALL). DS ALL comprises 1.5-3.2% of children with ALL, and AML in 5-15% (see Table 1 and 2). However, the risk of developing solid tumors appears to be decreased in DS individuals.

Interestingly, children with DS develop distinct biological subtypes of leukemia, when compared with children without DS. As reported by Whitlock et al., children with DS ALL (n=179) less frequently have T-cell disease (7.8 vs. 15.5%, p=0.05) than non-DS ALL (n=8447) patients, and DS ALL does not occur in infants below 1 year of age. Moreover, the cytogenetic abnormalities differ between DS and non-DS ALL: a normal karyotype is found in 45.5% of DS ALL cases vs. 30.3% in non-DS children, high hyperdiploid cases (>50 chromosomes) are rarer in DS (9.1% vs. 25.5%) and no cases of DS ALL with Philadelphia chromosome or t(4;11) have been found. In addition, hypodiploidy and hyperdiploidy (47-50 chromosomes) are more common in DS ALL. The frequency of TEL-AML gene rearrangements is diminished, with 0% in 59 DS ALL patients, versus 19.7% in >2000 non-DS ALL patients.

Similar differences in presentation can be observed for DS AML, where children with DS present at younger age (before the age of 5 years) and with a lower tumor burden than children without DS. There may be a pre-leukemic phase characterized by thrombocytopenia and low numbers of blasts. In addition, DS AML differs in morphology, and is usually classified as M0, M6 and, most frequently, M7. It is however questionable whether the FAB classification is useful in DS AML, and Hasle et al. have proposed to classify the disease as a distinct and unique disease entity named ‘myeloid leukemia of Down syndrome’ (ML DS).

Approximately 10% of children with DS may experience a transient leukemia (TL) in the neonatal period TL usually does not require treatment, unless complications, such as high white counts, massive organomegaly, effusions or liver fibrosis develop. However, these children need to be followed with 3-monthly complete blood counts for at least 3 years, as DS ML may develop in approximately 20% of children who have been diagnosed with TL. It is currently unknown what causes this transition from a pre-leukemic clone, which is thought to arise in fetal liver hematopoietic progenitors, to a malignant bone marrow disease. Moreover, it is unknown whether children with DS only develop DS ML following TL in the neonatal period, or whether this can also arise ‘de novo’ without preceding TL. The extra chromosome 21 in DS children is of maternal origin in >95% of children, and there have been reports suggesting that in DS children with TMD or leukemia, the paternal gene is more often involved. Massey et al. showed that children with additional cytogenetic abnormalities at presentation of TL are at greater risk of developing subsequent leukemia.

Considering prognosis, children with DS ALL seem to do worse than their non-DS counterparts, as summarized in Table 1. This is probably not related to steroid resistance, as the BFM-group showed that the frequency of ‘steroid good responders’ was higher in DS ALL than in non-DS ALL (98.1 vs. 90.7%). It remains questionable whether it is justified to directly compare outcome in DS and non-DS leukemias without taking the genetic differences into account. Especially the lack of hyperdiploidy and TEL-AML1 translocations, which are established good prognostic factors in non-DS ALL, may contribute to differences in prognosis. Several studies point at the poor tolerability for chemotherapy and higher toxic death rates in children with DS ALL (see Table 1). For instance
in the COG-study, death in induction was 3.6 times as frequent in DS vs. non-DS children, due to GI-bleeding, infection and cardiac failure.\textsuperscript{2} Unfortunately, they do not provide data on death in remission, or on complications such as infections or mucositis. Especially methotrexate induced mucositis and aplasia has been described to occur frequently in DS children.\textsuperscript{12-14} Higher susceptibility to infections may be due to altered immune function in children with DS.\textsuperscript{15} In the past, many children with DS ML were not offered adequate anti-leukemic treatment, until it was recognized in the early 1990s that children with DS ML were curable when regular chemotherapy was given.\textsuperscript{16,17} Further studies showed that cure rates in DS ML were higher than in non-DS AML, and that reduced intensity chemotherapy protocols resulted in better outcome than intensive chemotherapy, as summarized in Table 2.\textsuperscript{18} Therefore, children with DS ML should not be eligible for stem-cell transplant in first remission. Some studies showed high toxic death rates, especially in induction (see Table 2).

Recently, somatic mutations in the GATA1 gene, localized to chromosome X, in children with DS TL and ML were documented.\textsuperscript{19,20} These mutations appear to be pathognomonic for DS TL and ML, as they were not found in other leukemias (except for rare cases of FAB M7 AML with acquired trisomy 21, and one single adult case with AML M7 without DS or trisomy 21).\textsuperscript{20,21} The occasional older child with DS and AML may in fact suffer from sporadic AML rather than GATA1 mutated DS ML.\textsuperscript{22} GATA1 is a hematopoietic transcription factor involved in normal erythro- and megakaryopoiesis. The mutations lead to a shorter GATA1 protein, GATA1s, which may contribute to uncontrolled proliferation of DS ML blasts. This also raised

### Table 1: Results of DS ALL studies, compared with non-DS ALL

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Results in DS patients</th>
<th>Results in non-DS patients</th>
<th>Toxicity in DS</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFM</td>
<td>61 vs. 4049 (1.5%)</td>
<td>Good prednisone response 98.1% 6-year pEFS 58% with therapy reduction 46% (± 13%) without therapy reduction 65% (± 9%)</td>
<td>Good prednisone response 90.7% 6-year pEFS 70%</td>
<td>Therapy related death 6.6% in DS vs. 1.8% in non-DS ALL</td>
<td>11</td>
</tr>
<tr>
<td>CCG 1983-1985</td>
<td>179 vs. 8268 (2.1%)</td>
<td>10-year pOS 68% standard risk 70%* high risk 83% Induction failure 2.9%</td>
<td>10-year pOS 77% standard risk 85% high risk 65% Induction failure 0.8%</td>
<td>Death in induction 2.9% in DS ALL vs. 0.8% in non-DS ALL</td>
<td>2</td>
</tr>
<tr>
<td>MRC UK ALL X &amp; XI</td>
<td>55 vs. 3651 (1.5%)</td>
<td>5-years pOS 73%</td>
<td>5-years pOS 82%</td>
<td>Death in remission 11% in DS ALL vs. 2% in non-DS ALL</td>
<td>44</td>
</tr>
<tr>
<td>NOPHO 1984-2001</td>
<td>64 vs. 1915 (3.2%)</td>
<td>10-year pOS 57% Induction failure 14%</td>
<td>10-year pOS 80% Induction failure 2%</td>
<td>No excessive toxicity</td>
<td>45</td>
</tr>
</tbody>
</table>

* DS was a significant adverse prognostic factor at multivariate analysis (RR 2.0, p<0.01 for standard, but not for high-risk ALL, classified according to NCI criteria

### Table 2: Results of DS ML studies, compared with non-DS AML

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Results in DS patients</th>
<th>Results in non-DS patients</th>
<th>Toxicity in DS</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFM 98</td>
<td>67 vs. 907 (6.9%)</td>
<td>3-year pOS 91% Cum. incidence of relapse 7%</td>
<td>3-year pOS 64% Cum. incidence of relapse 28%</td>
<td>No excessive toxicity</td>
<td>5</td>
</tr>
<tr>
<td>CCG 2891</td>
<td>161 vs. 947 (14.5%)</td>
<td>6-year pOS 79% Relapse probability 14%</td>
<td>6-year pOS 43% Relapse probability standard timing 55% intensive timing 37%</td>
<td>No excessive toxicity (with standard timing)</td>
<td>4</td>
</tr>
<tr>
<td>MRC AML 10 and 12</td>
<td>46 vs. 822 (4.8%)*</td>
<td>5-year pOS 74% Relapse rate 5%</td>
<td>5-year pOS 62% Relapse rate 39%</td>
<td>High early death rate (11%, vs. 4% in non-DS), death in CR 15% vs. 9%.</td>
<td>46</td>
</tr>
<tr>
<td>NOPHO 1984-2001</td>
<td>62 vs. 435 (12.9%)</td>
<td>10-year pOS 74% Relapse rate 11%</td>
<td>10-year pOS 53% Relapse rate 41%</td>
<td>No excessive toxicity</td>
<td>45</td>
</tr>
</tbody>
</table>

* Not all children with DS ML were included in this report, hence the true frequency is higher.
the interest in GATA1 target genes, and their role in leukemogenesis and response to therapy.\textsuperscript{23,24} For a more extensive review on GATA1, the reader is referred to a recent review by Crispino.\textsuperscript{25}

### In-vitro drug resistance studies in DS leukemias

Several groups have studied the in-vitro sensitivity profile of DS leukemic blasts, both in DS ALL and AML. For DS ALL, only limited data are available. Zwaan et al. reported data on 9 successfully tested DS ALL samples, and found no significant differences in in-vitro drug resistance profile compared with non-DS B-cell precursor (BCP) ALL samples, for the drugs frequently used in ALL treatment.\textsuperscript{26} However, the control group was unselected, and it is questionable whether that is justified given the skewing of genetic abnormalities in DS ALL when compared with BCP-ALL in general.\textsuperscript{2} This may have rendered the control group too sensitive in comparison with the DS ALL samples. Frost et al. compared 5 DS ALL samples with non-DS ALL and found that DS ALL were more resistant to L-asparaginase and dexamethasone, but not to vincristine and doxorubicin.\textsuperscript{27}

Considering DS ML, Taub et al. reported that DS myeloblasts were 10-fold more sensitive to cytarabine (Ara-C) than non-DS blasts.\textsuperscript{28} They also found approximately 3-fold higher Ara-CTP levels after incubation with 5 micromol/l Ara-C. In a subsequent study, they also reported enhanced sensitivity to daunorubicin (23-fold).\textsuperscript{25} Zwaan et al. reported that DS AML cells were significantly more sensitive to cytarabine (median 12 fold), different 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 (>1.1 fold), than non-DS AML cells.\textsuperscript{26} This general hypersensitivity pattern was confirmed by 2 other groups,\textsuperscript{27,30} and raises several questions:

1) is this sensitivity due to a general mechanisms (such as an enhanced propensity to apoptosis), rather than to different selective and compound related mechanisms?

2) as this general hypersensitivity is not found for DS ALL, this suggests that DS ML has unique biological features, which are related to this specific disease and not to DS in general.

### Pharmacokinetic data

Pharmacokinetics (PK) is defined as the absorption, distribution, metabolism and excretion of drugs. It is well known that there is substantial (2-10-fold range) inter-individual variability in systemic drug exposure of anticancer drugs in children, measured as area under the plasma-concentration curve.\textsuperscript{31} This caused by many different factors such as age-related development of in-body-composition, renal function, and metabolism. Studies from St Jude Children’s Research Hospital have shown that individualized dosing regimens lead to better results when compared with standard dosing based on body surface area (BSA).\textsuperscript{32} Most pediatric studies apply specific dosing guidelines for infants (<1 year of age), as they usually have reduced renal and hepatic excretion, reduced metabolism, decreased protein binding, and a larger volume of distribution.\textsuperscript{33} Some guidelines for infants have been proposed by Reaman and Bleyer:

a) decrease cytarabine by 50%, particularly in high-dose regimens;

b) consider full dose of anthracyclines after 3-6 months of age;

c) decrease etoposide by 50%, or further in case of jaundice.\textsuperscript{34} In the Interfant ALL study, children under 6 months of age get 2/3 of the dose based on BSA, whereas children from 6-12 months of age get 75% of the dose based on BSA.

Children with DS ML have a median age of 1.8 years (range 0.4-16.1 years), hence a reasonable proportion of them will be infants.\textsuperscript{4,5} DS children differ in growth characteristics from non-DS children as growth velocity is mainly reduced between 6 months and 3 years of age and during puberty.\textsuperscript{35,36} At birth, DS children have heights and weights that are approximately -1 to 1.5 SD when compared with non-DS children. A body weight of 10 kg is usually reached between 1.5-2.0 years of age. Non-DS children have a BSA of 1.0 m2 when they are 30 kg and 140 cm, which is usually achieved around the age of 10 years. However, at that age children with DS have a similar weight of 30 kg, but a height of only 130 cm. Additional growth impairment may arise from cardiac disease, growth hormone deficiency, hypothyroidism and celiac disease.

There are very few studies available regarding PK in children DS. One small study compared 5 children with DS and ALL with 3 non-DS matched
controls, and found almost 2-fold higher methotrexate levels 42 hours after the start of a 1 gram/m² infusion (0.47 vs 0.24 mumol/L; P = 0.03). This may have been due to the decreased MTX clearance that was observed in these patients. DS children had significantly more side-effects, despite intensified leucovorin rescue in the DS patients. For etoposide, however, this did not appear to be the case, in the 2 DS patients that were studied.

It is therefore difficult to recommend on the issue of dosing in DS children. In the design of a European DS ML study (see below) we asked the participating groups about their guidelines for DS ML. The UK MRC group gave 25% dose-reduction for DS children under 1 year of age, the AML-BFM SG and NOPHO would dose per kg, rather than per BSA, respectively in children under 1 or under 2 year of age. Based on the heights and weights of children with DS ML in the AML-BFM studies, it was calculated that for 1 gram/m² cytarabine, dosing based on BSA yielded lower dosages for children below 9 kg, whereas above 9 kg higher dosages were given, when compared to dosing in mg/kg. In the new European DS ML proposal we have decided to use dosing in mg/kg in children of 11 kg body weight or less, which in practice means until the age of 2.5-3.0 years. There is however, a clear need of PK-studies in DS individuals.

Pharmacodynamics
Pharmacodynamics is the relation between pharmacokinetics and pharmacological effect (either toxicity or therapeutic effect) of a given drug in the patient. There are clear differences in pharmacodynamics described for children with DS when compared with children without DS. We will here review the available data for methotrexate, cytarabine and anthracyclines.

Methotrexate
Methotrexate (MTX) is transported into the cell by the reduced folate carrier (RFC), which is localized on chromosome 21q22. This has long been associated with potential greater MTX accumulation, both in leukemic cells as well as in other tissues, such as the gastrointestinal mucosa, explaining the enhanced risk of mucositis experienced by DS patients when using MTX. However, Taub et al. studied the mRNA expression levels of certain genes localized to chromosome 21, but found no increase in RFC-transcript levels, although the number of samples that was studied was very limited. Moreover, when in-vitro MTX resistance was measured as the relative inhibition of the enzyme thymidylate synthase (TS), there were no significant differences between DS and non-DS ALL samples. This also does not suggest

Figure 1: Mechanisms of enhanced cytarabine sensitivity in DS ML cells. Cytarabine (Ara-C) enters the cell by a nucleoside transporter (hENT1) and/or passive diffusion, after which it gets phosphorylated to its triphosphate metabolite Ara-CTP, which is incorporated into DNA and leads to chain termination and apoptosis. The rate limiting step in the phosphorylation cascade is deoxycytidine kinase (dCK), which is under negative regulation by deoxycytidine triphosphate (dCTP). DCTP also competes with Ara-CTP for incorporation into DNA. Ara-C can be degraded by deamination through cytidine deaminase (CDA).

DS ML cells are approximately 12-fold more sensitive to cytarabine (Ara-C) than non-DS AML cells, due to several cooperating mechanisms. Cystathionine-â-synthase (CBS) transcript levels are decreased, which indirectly leads to reduced dCTP pools. dCK levels are increased in DS ML. Both CDA and BST2 are potential GATA1 target genes, and are underexpressed in DS AML. BST2 is involved in the interaction between stroma cells and leukemic cells. In stroma cell supported assays downregulation of BST2 resulted in increased Ara-C sensitivity.
enhanced MTX uptake in leukemic cells. This does not exclude tissue-specific effects, and a RFC gene dosage effect could still be responsible for enhanced frequency and severity of mucositis seen in DS children. An alternative hypothesis is that this increased sensitivity is due to pre-existing imbalances in nucleotide pools in DS children, especially due to homocysteine depletion due to a gene-dosage effect of the chromosome 21q22 localized enzyme cystathionine-B synthase (CBS). This enhances folate depletion, and MTX-polyglutamation (polyglutamation of MTX results in increased intra-cellular retention of MTX), and therefore methotrexate sensitivity, although this was debated by other investigators.12,13

Cytarabine
The cytarabine (Ara-C) metabolism in children with DS was extensively studied by Taub and Ravindranath et al., as summarized in Figure 1. They showed that CBS was 12-fold overexpressed in DS-myeloblasts compared to leukemic cells from non-DS AML patients, which questions the 2:3 expected gene dosage effect with regards to chromosome 21 localized genes. CBS deficiency leads to homocystinuria. CBS overexpression indirectly leads to decreased dCTP pools, and therefore to greater Ara-C triphosphate generation and incorporation into DNA, as was also shown with transfection experiments.41 dCTP also has a negative regulatory effect on deoxy cytidine kinase (dCK), the rate limiting enzyme involved in cytarabine phosphorylation. dCK transcript levels were reported to be 2.6-fold higher. Hence, the ratio between dCK and CDA favors activation of cytarabine, leading to higher Ara-CTP levels and greater cytotoxicity.

Apart from CBS, Ara-C sensitivity may be influenced by cytidine deaminase (CDA) levels, which is an enzyme involved in the degradation of Ara-C. Transcript levels appeared to be 5-fold lower in DS ML cells, which could be restored by transfection of the wild-type GATA1 gene in a DS ML cell line. In a recent study, Ge et al. have compared gene expression profiles of 5 DS ML and 5 non-DS AML FAB M7 samples (which is a relatively small sample set for expression profiling studies and may induce error), and found 551 genes that discriminated between the 2 clusters, which were widely localized on various different chromosomes. One gene, the BST2 (bone marrow stromal cell antigen 2) gene, was underexpressed in DS ML. Evidence was provided that BST2 is a GATA1 target gene, and a transfected DS ML cell line (CMK) was rescued from Ara-C induced apoptosis in a stroma cell assay.

These data provide clear evidence for the enhanced sensitivity of DS ML cells. Children with DS ML have a reasonable tolerance for high-dose Ara-C therapy, and, in line with this, Zwaan et al. did not find differences in drug sensitivity between mononuclear bone marrow cells from healthy children with DS compared with non-DS derived marrow mononuclear cells.26

Anthracyclines
The gene encoding for superoxide dismutase (SOD) is localized to chromosome 21q22, and transcript levels in DS myeloblasts were approximately 4-fold higher than in non-DS AML cells.38 SOD is involved in the generation of oxygen radicals, which may be a mechanism involved in the hypersensitivity of DS ML cells in general, and in hypersensitivity to anthracyclines in particular, as they induce cytotoxicity by free radical formation. Moreover, the conversion of daunorubicin to daunorubicinol is mediated through carbonyl reductase (CBR). The CBR gene is localized on chromosome 21 as well.29

Pharmacogenomics
Pharmacogenetics is the study of the inherited basis for interindividual differences in response to chemotherapeutics. A polymorphism has been described in the gene encoding for CBS, i.e. a 68 base pair insertion in exon 8 (844ins68).43 This polymorphism was found in approximately 10% in healthy adults, and in 11% of non-DS AML samples. However the frequency was higher (24%) in mononuclear cells of healthy DS children, and even more frequent in DS ML cells (53%). Within the DS ML group, samples with the polymorphisms were approximately 7-fold more sensitive to Ara-C than the group with a WT-allele.

The European DS ML 2006 Study
Recently, a European DS ML protocol was initiated by investigators from Germany, the UK, France and the Netherlands, with Dr. D. Reinhardt from the AML-BFM SG as principal investigator. This study aims at achieving an
overall survival rate of at least 85% for children with DS ML. The protocol was based on the excellent results (3-year pOS of 91%) obtained with the BFM-98 study for children with DS ML.5 Children with DS ML treated according to this protocol received reduced dosages of anthracyclines, and cranial irradiation, HAM-intensification and stem-cell transplant in first CR were omitted. In the new European protocol some further changes have been introduced, for instance no maintenance therapy will be given. The protocol is outlined in Figure 2. The cumulative dosage of cytarabine is limited to 27.4 gram/m², and anthracyclines to idarubicin 38 mg/m² plus mitoxantrone 14 mg/m². In the non-DS AML BFM-98 study these dosages were for Ara-C 41-47 g/m² (dependent on stratum) and for anthracyclines 64 mg/m² idarubicin and 20 mg/m² mitoxantrone in arm 1, or 50 mg/m² idarubicin and 40 mg/m² mitoxantrone in arm 2.

Figure 2: An overview of the European DS ML 2006 study (courtesy of D. Reinhardt, AML-BFM SG, Hannover, Germany). Outline of the DS ML2006 protocol, which consists of 4 blocks of chemotherapy with reduced dosages of anthracyclines and cytarabine. The protocol is based on the AML-BFM 98 study, with provided separate guidelines for children with DS ML. Please note that the use of this protocol is restricted to study centers, and that not all protocol elements are shown in this graph.

Conclusions

Children with DS have an increased risk of developing leukemias. DS ML is a biologically distinct and unique disease, and is characterized by good clinical outcome with dose-reduced therapy. This is explained by relative sensitivity to cytarabine and anthracyclines, which has been extensively studies. Data for DS ALL mainly show a different cytogenetic distribution when compared to non-DS ALL. DS ALL patients usually do slightly worse than children with non-DS ALL, which may be the results of enhanced toxicity and therapy resistance. There is a considerable paucity of data regarding drug dosing in DS leukemias, especially with regards to DS ALL.

References


