Pediatr Blood Cancer 2005;44:1–7

COMMENTARY
Down Syndrome and Leukemia: New Insights Into the Epidemiology, Pathogenesis, and Treatment

INTRODUCTION

Down syndrome is the most common autosomal chromosomal disorder with incidence ranging from 1 in 700 to 1:1,000 live births [1]. The protean manifestation are mental retardation, congenital heart disease, and risk for early death. The predominant cause of death is related to the congenital heart defects followed by hypothyroidism, respiratory infections, and malignancy (particularly, leukemia) [2,3]. Death from leukemia in Down syndrome children is predominately in the younger ages [2]. Down syndrome children account for approximately 3% of children with acute lymphoblastic leukemia (ALL) and 5–8% of children with acute myeloid leukemia (AML) diagnosed in the United States. Despite the fact that the association of increased risk for leukemia with Down syndrome have now been recognized for nearly 50 years, it is this relatively low frequency of the total number of cases and the reluctance to give aggressive chemotherapy in a developmentally challenged youngster hindered the systematic evaluation of the pathogenesis and treatment of leukemia in children with Down syndrome. Within the last two decades, several important developments in the understanding of the biology and treatment of leukemias in Down syndrome children have occurred. These developments in general define the pivotal role played by chromosome 21, both in childhood ALL and AML. The serendipitous discovery of the unique drug sensitivity of AML in Down syndrome [4] provided additional impetus for these studies. What emerges is a fascinating story for increased risk for leukemia on the one hand and the increased sensitivity to chemotherapy on the other [5]. In this issue of the journal, six articles describe some of these developments and provide new insights on the biology and treatment of leukemia in Down syndrome [3,6–10]. It is a privilege to write this overview. The articles will be reviewed in the context of epidemiology, pathogenesis, and treatment/drug sensitivity. Some of the remaining challenges will be identified in the summation.

Epidemiology of Leukemia in Children With Down Syndrome

Ross et al. [3] describe the current status of the epidemiology of leukemia in Down syndrome. First and foremost, there is a 20-fold increased risk of leukemia in individuals with Down syndrome [11,12]. Brewster and Cannon [13] get credited with the first descriptions of the association between Down syndrome and leukemia in 1930. A striking feature of the increased risk for malignancies is that there is an increased risk for leukemia but not for other solid tumors with the exception of testicular cancer, germ cell tumors, and retinoblastoma [14,15]. The increased risk for leukemia appears to be for the most part to a particularly high risk for one type of AML, megakaryocytic leukemia (AMKL, M7 AML) [16]. The Children's Oncology Group additionally show an almost fourfold higher incidence of AML to ALL in Down syndrome children [3]. It is fascinating that while the age adjusted incidence of ALL similar to that seen in non-Down syndrome children, the incidence of AML is highly skewed towards the younger age with very few cases if any beyond the age of 5 years confirming the observations from population based registry of Down syndrome children from Nordic countries [14,17].

Comments. Ross et al. [3] offer several suggestions for further investigation of the increased risk for leukemia in Down syndrome. They correctly point out that as of now, the only potentially shared risk factor for both Down syndrome and childhood leukemia is advanced maternal age. Other avenues of study would include quantitation of the exposure to radiation from the diagnostic X-ray studies in Down syndrome for study of heart disease and studies for investigation of intercurrent infections. Ross et al. [3] focus on the relationship of infections in early infancy and the risk for ALL. The infection hypothesis of Greaves et al. [18,19] suggests that exposure to common infections in early childhood may protect a child against ALL by contributing to normal maturation of the immune system, whereas children whose exposure is delayed will be at comparatively higher risk. Greaves et al. suggest that these may be the basis for the lower incidence of childhood ALL in non-industrialized countries versus industrialized

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Received 13 September 2004; Accepted 13 September 2004

DOI 10.1002/pbc.20242

countries. Children with Down syndrome present a paradox in this regard. On the one hand, the immune deficiency that occurs in Down syndrome may predispose to an increased risk of developing leukemia analogous to the known increased risk of lymphoid malignancies in children with other immune deficiencies. On the other hand, while the age peak for children with Down syndrome and ALL appears to follow the age peak for childhood ALL, the incidence of hyperdiploid ALL and for that matter, ALL with t(12;21) is extremely low or non-existent in Down syndrome children [8,20,21]. It has been suggested that the increased incidence of childhood ALL in the industrialized countries is largely due to the hyperdiploid ALL. Recent studies from India suggest that the incidence of TEL-AML t(12;21) is lower in the Indian children with ALL [22]. It should also be noted that hyperdiploid ALL is also less common in US black children Smith et al. [23]. Thus, it appears that Down syndrome is protective against the occurrence of hyperdiploid ALL and ALL with t(12;21). This parallels the pattern of ALL in less industrialized (and over crowded, under-nutrition) societies. If so, what may be the basis for this? Could there be a linkage with the relative folate deficiency in Down syndrome and the low incidence of hyperdiploid ALL? An intriguing parallel is the pattern of incidence of leukemia and solid tumors in individuals with homozygosity for C677T variant of methylene tetrahydrofolate reductase, a folate pathway enzyme. Recent studies have shown that homozygosity for C677T MTHFR is protective against hyperdiploid ALL [24] and these individuals are also at a low risk for colon cancer, a cancer not found in Down syndrome children in the population based studies from Denmark [14]. Thus it would, indeed, be great interest to study the folate status of children with Down syndrome, the US blacks (sub-Saharan Africans), and the South Asians (Indian sub continent) patient populations and to correlate with the incidence of hyperdiploid ALL, and ALL with t(12;21) in these populations.

Pathogenesis of Transient Leukemia/AML and GATA1 Mutations

Massey [6] reviews the current knowledge of transient leukemia in newborns with Down syndrome and Crispino [7] provides an overview of the relationship of the recently discovered GATA1 mutations and TL/AML in Down syndrome children.

One of the more fascinating manifestations of Down syndrome is the disorder variously known as transient myeloproliferative disorder (TMD or transient leukemia), first described by Schunk and Lehman in 1954 [25]. This disorder is typically seen in newborns associated with a high incidence of spontaneous remissions [26]. The disease is largely clinically silent and frequently discovered by routine blood counts done for other reasons but in some cases the disease is life threatening. In severe cases the infant may be born with hydrops fetalis and show evidence of liver or multi-organ system failure. Retrospective reviews of suggest that neonatal mortality may range from 11 to 55% (the higher figure includes stillborn patients) [27,28]. In the POG 9481 prospective study 8 of 47 patients (17%) experienced early death [29]. And of those who achieve spontaneous remission up to 30% will subsequently develop AMKL [27,28,30]. Megakaryoblastic nature of the neonatal TMD/TL has been clearly established by the electron microscopic studies of Zipursky et al. [31] and as well by the more recent studies using the platelet glycoprotein IIb/IIIA markers CD41/61. Mutations of exon 2 of the *GATA1* gene are universal [32].

Comments. Three important questions remain to be answered. (1) What is the mechanism of the spontaneous resolution of TMD/TL in the majority of the cases? (2) Why do some infants develop hydrops fetalis and liver dysfunction and die? (3) What is the mechanism for the subsequent development of AMKL in up to 30% of the infants with TMD/TL and can this be prevented? All three appear to be linked. The most important new development in the understanding of the biology of AML of Down syndrome is the identification of the truncating mutations involving the hematopoietic transcription factor gene *GATA1* (reviewed by Crispino [7]). GATA1 is located on chromosome X and encodes for a zinc finger transcription factor that is essential for normal erythroid and megakaryocytic differentiation. Mutations in exon 2 of GATA1 have been detected almost exclusively in trisomy 21 associated TMD/TL and in Down syndrome patients with AMKL but not in non-Down syndrome AMKL cases [33]. The mutations result in the introduction of a premature stop codon leading to the exclusive production of a smaller GATA1 isoform named GATA1s measuring 40 kDa compared to the normal full length 50 kDa isoform of GATA1. Remarkably these 40 kDa isoform retains both the zinc fingers that are involved in the DNA binding and the interaction site with an essential cofactor named, friend of GATA1 (FOG 1) [7]. It is interesting that all of the GATA1 alterations reported to date abolished the expression of the full length form but retain the expression of GATA1s. This finding is of critical importance with regard to the pathogenesis of AML. For example, mice that totally lack GATA1 (knockout) die in embryogenesis due to deficiencies in both primitive and definitive erythropoiesis, while mice that express low levels of GATA1 (knockdown) with varying degrees of anemia and thrombocytopenia [34]. In these knockdown mice, the GATA1 deficient megakaryocytes are defective in terminal maturation and exhibit abnormal proliferation when expanded in vitro [35]. In parallel to this, it is of interest that blast cells from Down syndrome children with AMKL and TMD/TL express CD36 [36], in contrast to the

low or lack of expression of CD36 in non-Down syndrome AMKL. Further, the expression of wild type GATA1 in the MGS cell line (derived from a Down syndrome child with AMKL) have been shown to partially rescue differentiation [37]. It is to be noted that an earlier study suggested that low GATA1 expression may be a marker for good response in AML [38].

Based on the above and additional observations in aborted Down syndrome fetuses, twins with Down syndrome and prospective studies of screening for GATA1 mutations at birth, Crispino suggests an origin of TMD/TL fetal liver, which would explain both the spontaneous resolution of TMD/TL and the occurrence and the fatal form of TMD and TL with liver failure [7,39]. Crispino speculates that GATA1 mutations occur in fetal liver hematopoietic progenitors in both Down syndrome and unaffected individuals but that the mutations have a selective advantage only against the backdrop of trisomy 21, because of the increased gene dosage effort of key transcription cofactors AML1 and ETS2 which are localized to chromosome 21 [39]. Spontaneous resolution then reflects the transition from fetal liver erythropoiesis to marrow derived postnatal erythropoiesis. The hepatic dysfunction and hydrops fetalis in some of the cases are due to a high TMD burden in the liver resulting in hepatic fibrosis from excessive PDGF and TGF production [6,40]. The later emergence of true AMKL then is due to persistence of these clones or occurrence of the same GATA1 mutation simultaneously in the fetal and marrow derived hematopoietic precursors. This hypothesis supported by the finding of GATA1 mutations in asymptomatic infants with Down syndrome, demonstration of the same GATA1 mutation both at the initial TMD as well as the latter AMKL [41].

The question of whether the children presenting with severe manifestations of TMD/TL should be treated pharmacologically with chemotherapeutic agents remains unanswered at present. Anecdotal data from Toronto shows that low dose cytosine arabinoside (Ara-C) regimen might induce lasting ''remissions'' in these children and low dose Ara-C regimen has also been shown to be effective in the treatment of myelodysplastic syndrome seen in later infancy in the Down syndrome children [42]. Unpublished data from POG 9481 study also suggests that Down syndrome infants with TMD/TL treated with the low dose Ara-C and who survived have not developed AMKL (G Massey, personal communication). This raises the intriguing possibility that low dose Ara-C,(1–2 courses) may even prevent later occurrence of AMKL. In any case, the treatment of children with hydrops or severe liver dysfunction remains problematic. First, many of these children have far advanced hepatic failure with some autopsy have virtually no viable hepatocytes (unpublished personal observation). In others, there may severe lung disease from either hyperviscosity or pulmonary fibrosis. It is of note that several CCG studies have identified pulmonary toxicity as a feature of the toxicity profile in Down syndrome and leukemia. The unique risk for pulmonary toxicity has not been fully explained although it is of interest that an existing hypothesis for the site of production of platelets suggests that megakaryocytes home in to the lung and then release platelets by explosion [43].

Treatment of ALL and Down Syndrome

Basal et al. [8] review the experience of Down syndrome children treated between 1952 study for NIH consensus standard risk ALL (age ≥ 1 and ≤ 10; initial WBC $\leq 50 \times 10^3/\mu l$). Fifty-nine of 2,174 registered patients or 3% had Down syndrome. The study confirmed the prior observations that hyperdiploid ALL and ALL with TEL/AML1 do not occur in Down syndrome (reviewed by Lange [20]). At the same time, adverse translocations or hypodiploidy was also not observed in the ALL Down syndrome cohort. Prior reviews had suggested that the outcome in Down syndrome children with ALL is either the same or somewhat inferior to ALL in non-Down syndrome, thus contrasting with the markedly superior outcome of AML in Down syndrome versus non-Down syndrome children. In narrowing down into one single study and as well, restricting the analysis to NIH consensus standard risk group, Basal et al. [8] were able to isolate some of the issues. First, among the B lineage ALL patients, as a whole, the ALL Down syndrome cohort had a lower 4 year EFS of compared to the non-Down syndrome ALL cohort but this difference was no longer significant when the groups were adjusted for the presence of either TEL-AML1 or triple trisomies (hyperdiploidy) (78.6% vs. 82.3%, $P = 0.14$). With regard to overall survival, however, non-Down syndrome cohort did better compared to the Down syndrome group. The authors suggested that the contributing factors for the difference in the low overall survival for Down syndrome ALL cohort might be the increased infection rate or less intensive salvage therapy offered for Down syndrome children with relapse. For example, in their study, only 8% of Down syndrome patients relapsed or received a bone marrow transplant compared to 29% of the non-Down syndrome cohort with relapse. Some other differences emerged in this study. No case of T lineage leukemia was observed in this study in Down syndrome children compared to an incidence of 5.9% in the non-Down syndrome cohort on CCG 1952. The well-known methotrexate related toxicity was confirmed. Down syndrome children spent longer in the hospital than non-Down syndrome cohort, and there was a higher incidence of hyperglycemia. There was also a higher incidence of bacteremia in the Down syndrome cohort. Interestingly, the infection risk was far greater during remission in the

post remission phases of the treatment in Down syndrome patients possibly reflecting increased risk of bacteremia resulting from the increased incidence of mucositis. This increased risk of infections, however, did not seem to translate in toxic deaths although Basal et al. [8] do not provide actual numbers toxic deaths for either group.

Comments: The EFS data in Down syndrome ALL are somewhat consistent with the known in vitro drug sensitivity studies, which also did not show a specific difference between the Down syndrome and non-Down syndrome groups in terms of drug sensitivity [44]. These in vitro drug sensitivity data should be treated with some degree of caution. MTT assay was successful in only 45% of the Down syndrome ALL cohort whereas typically investigators found up to 80% of BP ALL samples the test is successful for at least for one drug. In 6 of the 20 tested cases of Down syndrome-ALL, the cell count was too low on Day 4, indicating a high spontaneous apoptosis rate in control wells. Zwaan et al. [44] also did not correct for cytogenetic subgroups (hyperdiploidy or TEL-AML1) between the Down syndrome ALL and the non-Down syndrome ALL cohorts. Further, Basal et al. [8] while presenting an exhaustive summary of the comparison of results between Down syndrome and non-Down syndrome cohorts treated on CCG 1952 did not provide data on the actual relapse rate and the number of withdrawals for toxicity between the two groups nor the actual numbers of deaths during remission.

In any case, current data at best show equivalent results between Down syndrome and non-Down syndrome cohorts in contrast to the markedly superior results in the Down syndrome AML patients compared to non-Down syndrome AML. Potential reasons are: (1) the results are already at a high level for both groups and thus differences may not be easy to appreciate, (2) oxygen radical mediated drug induced apoptosis may be much more evident with high dose chemotherapy as is frequently done in AML compared to the relatively low dose therapy in ALL, (3) Ara-C is not a frequently used drug in the therapy of standard risk ALL, (4) dose adjustments of methotrexate on account of concern for mucositis may actually result in under dosing of Down syndrome ALL patients, and (5) finally as pointed out by Basal et al. [8] relapsed Down syndrome ALL patients may be under treated because of parental and physician concerns.

Drug Sensitivity of AML in Down Syndrome

Taub and Ge [10] provide concise review of the series of drug sensitivity studies done by the Wayne State University group, particularly in relation to the unique and endogenous modulation of Ara-C metabolism in Down syndrome. Taub and Ge also provide the initial evidence for the potential linkage of GATA1 mutation and the increased sensitivity to Ara-C. To summarize briefly, the altered folate metabolism in Down syndrome children on account of the increased activity of cystathionineβ synthase (CBS), results in low levels of endogenous dCTP low *s*-adenosyl methionine. Low endogenous dCTP results in release of the feedback inhibition deoxycytidine cytidine kinase, the enzyme that phosphorylates both deoxycytidine and Ara-C resulting in a ''favorable'' Ara-CTP to dCTP ratio. In addition, Taub et al. have shown that expression levels of the Ara-C degrading enzyme, cytidine deaminase (CDA; gene localized to chromosome 1p), are lower in Down syndrome leukemic cells compared to non-Down syndrome cases, an additional factor contributing to the increased Ara-CTP generation in Down syndrome megakaryoblasts [45]. More recent experiments of Taub et al. provide evidence that increased CDA expression could be linked to decreased co-cooperativity between the mutated GATA1 and a short form promoter of CDA. Stable transfection of the wild type GATA1 coding cDNA into the Down syndrome AMKL cell line, CMK (which contains mutated *GATA1* gene), resulted in increased Ara-C resistance and a threefold lower level of Ara-CTP generation. Consistent with this hypothesis are data from pharmacologic interventions aimed at reducing endogenous dCTP by either prior treatment with methotrexate [46] and hydroxyurea [47] or fludarabine [48] (inhibitors of ribonucleotide reductase), which enhance Ara-C cytotoxicity.

Comments: While clear evidence exists with regard to this unique modulation of Ara-C sensitivity in Down syndrome, less obvious is the generalized increased sensitivity of Down syndrome AML to anthracyclines and as well, other drugs [44,49,50]. A possible reason appears to be the well-known increased generation of oxygen radicals in Down syndrome observed and the well-documented increased spontaneous apoptosis in multiple cell systems including neuronal cells (reviewed by Ravindranath [5] and Taub and Ge [10] in this issue). A modest increase in superoxide dismutase as occurs in Down syndrome in the absence of a concomitant increase in glutathione peroxidase and catalase might result in increased hydroxyl radical formation which itself might be quite toxic. Studies from the Wayne State group provide additional insight to the increased production of reactive oxygen species (ROS) in Down syndrome cells. Chien et al. [51] demonstrate that an increased mitochondrial production and leakage of superoxide in the CMK cell line compared to non-Down syndrome AMKL cell lines MEG-O1 (derived from a Philadelphia chromosome positive CML case in blast crisis) and HL60 erythroleukemia cell line (also derived from a Philadelphia chromosome positive CML patient). The increased to superoxide production correlates with the increased drug sensitivity of CMK versus MEG-O1 versus HL60. In these studies there was no significant increase in hydroxyl radical production in the CMK cell line, presumably related to an

increase in catalase activity. These preliminary data in cell lines suggest that the primary reason for the increased spontaneous apoptosis in Down syndrome is the increased superoxide generation from mitochondrial respiratory activity and that the modest increases in superoxide dismutase and catalase are not sufficient to detoxify all of the ROS generated. Other evidence suggests that ETS2 over-expression in transgenic mice and in Down syndrome predisposes apoptosis via the p53 pathway [52]. ETS2 is also induced by oxidative stress and sensitizes cells to hydrogen peroxide induced apoptosis [53]. Together, these studies suggest that increased ROS production on the background of increased ETS2 expression and imbalance of the antioxidant enzymes SOD1/GPX+ catalase such as occurs in Down syndrome may prime the cells for spontaneous apoptosis and thereby drug induced apoptosis. The studies by Chien et al. suggest that the increased ROS itself is likely from increased mitochondrial respiratory activity possibly linked to the increased gene dosage effect of NADH dehydrogenase ubiquinone flavoprotein 3 (NDUFV3) gene, a 10KDA component of complex 1 localized to chromosome21q22.3 [51].

Treatment of AML in Down Syndrome

This topic is covered within two articles in this issue. The article by Gamis [9] is factual, historical, and comprehensive. It is clear that the unique responsiveness of AML in Down syndrome became obvious only after the general usage of high dose Ara-C in the treatment regimens for AML. In the POG 8498 study all children with Down syndrome and AML entered on study survived event free [4]. Subsequent reports (reviewed by Gamis [9] in this issue) confirmed the superior outcome in AML children with Down syndrome with EFS of 75–80%. With the exception of one study, all used high dose Ara-C post remission. However, given the some what increased toxicity in children with Down syndrome with current AML regimens, some reduction in intensity of treatment seems appropriate. In light of the markedly increased sensitivity in vitro to Ara-C an obvious question is can we reduce toxicity by lowering the cytarabine dose from 3 to 1 g/m^2 in each of the high dose cytarabine courses. The answer is likely yes but needs to be explored in prospective studies to assure that the current high cure rates are not jeopardized. Further future studies would have to recognize that some of the AML cases in children (particularly older children) with Down syndrome may represent the true de novo AML cases and not linked to GATA1 mutations. The response in such cases may be same as can expected from the cytogenetic abnormalities other than constitutional trisomy 21. Absence of the classic AMKL features and low or lack of expression of the megakaryocyte maturation marker CD36 (thrombospondin receptor) may provide a clue [36,54] and in such cases studies of GATA1 mutations may be necessary.

SUMMARY

The discovery of the unique sensitivity of Down syndrome-AML to chemotherapy, the observation of the linkage of reduced function associated GATA1 mutations with Down syndrome-AMKL/TMD have provided a great impetus to understand the mechanistic basis of the pathogenesis of Down syndrome-AML and as well the sensitivity to chemotherapy. The recent studies have proved critical in determining the relationship of folate pathway to cytarabine sensitivity as well the pivotal role of increased ROS in determining cellular sensitivity to chemotherapeutic agents. Several questions that are specific to Down syndrome and leukemia remain to be elucidated. Nevertheless, it is clear that Down syndrome is a unique paradigm for increased risk for leukemogenesis on the one hand, drug sensitivity on the other. Down syndrome may yet become the prototype disorder defining the integral relationship of life (cell proliferation) and death (spontaneous apoptosis).

ACKNOWLEDGMENT

Dr. Ravindranath is supported by Georgie Ginopolis Endowment, and holds the Georgie Ginopolis Chair for Cancer and Hematology at Wayne State University School of Medicine.

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REFERENCES

1. Down syndrome prevalence at birth—United States, 1983–1990. MMWR 1994;43:617–622.
2. Yang Q, Rasmussen SA, Friedman JM. Mortality associated with Down's syndrome in the USA from 1983 to 1997: A population-based study. Lancet 2002;359:1019–1025.
3. Ross A, Spector LG, Robison LL, Olshan AF. Epidemiology of leukemia in children with Down syndrome. Pediatr Blood Cancer 2005;44:8–12.
4. Ravindranath Y, Abella E, Krischer JP, et al. Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: Experience on Pediatric Oncology Group AML Study 8498. Blood 1992;80:2210–2214.
5. Ravindranath Y. Down syndrome and acute myeloid leukemia: The paradox of increased risk for leukemia and heightened sensitivity to chemotherapy. J Clin Oncol 2003;21:3385–3387.
6. Massey GV. Transient leukemia in newborns with Down syndrome. Pediatr Blood Cancer 2005;44:29–32.
7. Crispino JD. GATA1 mutations in Down syndrome. Pediatr Blood Cancer 2005;44:40–44.

8. Basal M. Lymphoblast biology and outcome among children with Down syndrome and ALL treated on CCG-1952. Pediatr Blood Cancer 2005;44:21–28.
9. Gamis A. Acute myeloid leukemia and Down syndrome: Evolution of modern therapy—State of the art review. Pediatr Blood Cancer 2005;44:13–20.
10. Taub JW, Ge Y. Down syndrome, drug metabolism and chromosome 21. Pediatr Blood Cancer 2005;44:33–39.
11. Krivit W, Good RA. Simultaneous occurrence of mongolism and leukemia: Report of a nation wide survey. Am J Dis Child 1957;94: 289–293.
12. Robison LL. Down syndrome and leukemia. Leukemia 1992;6: 5–7.
13. Brewster HFCH. Acute lymphatic leukemia: Report of a case in eleven month Mongolian Idiot. New Orleans Med Surg J 1930;82: 872–873.
14. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet 2000; 355:165–169.
15. Satge D, Sommelet D, Geneix A, et al. A tumor profile in Down syndrome. Am J Med Genet 1998;78:207–216.
16. Zipursky A, Peeters M, Poon A. Megakaryoblastic leukemia and Down's syndrome: A review. Pediatr Hematol Oncol 1987;4:211–230.
17. Rajantie J, Siimes MA. Long-term prognosis of children with Down's syndrome and leukaemia: A 34-year nation-wide experience. J Intellect Dis Res 2003;47:617–621.
18. Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. Nat Rev Cancer 2003;3:639–649.
19. Greaves MF. Biological models for leukaemia and lymphoma. IARC Sci Publ 2004;157:351–372.
20. Lange B. The management of neoplastic disorders of haematopoiesis in children with Down's syndrome. Br J Haematol 2000;110: 512–524.
21. Ragab AH, Abdel-Mageed A, Shuster JJ, et al. Clinical characteristics and treatment outcome of children with acute lymphocytic leukemia and Down's syndrome. A Pediatric Oncology Group study. Cancer 1991;67:1057–1063.
22. Sazawal S, Bhatia K, Gutierrez MI, et al. Paucity of TEL-AML 1 translocation, by multiplex RT-PCR, in B-lineage acute lymphoblastic leukemia (ALL) in Indian patients. Am J Hematol 2004;76: 80–82.
23. Smith MA, Chen T, Simon R. Age-specific incidence of acute lymphoblastic leukemia in US children: In utero initiation model. J Natl Cancer Inst 1997;89:1542–1544.
24. Wiemels JL, Smith RN, Taylor GM, et al. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. Proc Natl Acad Sci USA 2001;98:4004–4009.
25. Schunk GJLW. Mongolism and congenital leukemia. JAMA 1954; 155:250–251.
26. Weinstein HJ. Congenital leukaemia and the neonatal myeloproliferative disorders associated with Down's syndrome. Clin Haematol 1978;7:147–154.
27. Homans AC, Verissimo AM, Vlacha V. Transient abnormal myelopoiesis of infancy associated with trisomy 21. Am J Pediatr Hematol Oncol 1993;15:392–399.
28. Isaacs H, Jr. Fetal and neonatal leukemia. J Pediatr Hematol Oncol 2003;25:348–361.
29. Al-Kasim F, Doyle JJ, Massey GV, et al. Incidence and treatment of potentially lethal diseases in transient leukemia of Down syndrome: Pediatric Oncology Group Study. J Pediatr Hematol Oncol 2002;24:9–13.
30. Zipursky A, Poon A, Doyle J. Leukemia in Down syndrome: A review. Pediatr Hematol Oncol 1992;9:139–149.
31. Zipursky A, Christensen H, De Harven E. Ultrastructural studies of the megakaryoblastic leukemias of Down syndrome. Leuk Lymphoma 1995;18:341–347.
32. Gurbuxani S, Vyas P, Crispino JD. Recent insights into the mechanisms of myeloid leukemogenesis in Down syndrome. Blood 2004;103:399–406.
33. Mundschau G, Gurbuxani S, Gamis AS, et al. Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis. Blood 2003;101:4298–4300.
34. McDevitt MA, Shivdasani RA, Fujiwara Y, et al. A "knockdown" mutation created by cis-element gene targeting reveals the dependence of erythroid cell maturation on the level of transcription factor GATA-1. Proc Natl Acad Sci USA 1997;94:6781–6785.
35. Vyas P, Ault K, Jackson CW, et al. Consequences of GATA-1 deficiency in megakaryocytes and platelets. Blood 1999;93:2867–2875.
36. Savasan SBS, Ravindranath Y. CD36 expression is associated with superior in vitro Ara-C sensitivity in acute megakaryocytic leukemia with and without Down syndrome. Med Pediatr Oncol 2003;41:274–275, abs#O070.
37. Xu G, Nagano M, Kanezaki R, et al. Frequent mutations in the *GATA-1* gene in the transient myeloproliferative disorder of Down syndrome. Blood 2003;102:2960–2968.
38. Shimamoto T, Ohyashiki K, Ohyashiki JH, et al. The expression pattern of erythrocyte/megakaryocyte-related transcription factors GATA-1 and the stem cell leukemia gene correlates with hematopoietic differentiation and is associated with outcome of acute myeloid leukemia. Blood 1995;86:3173–3180.
39. Taub JW, Mundschau G, Ge Y, et al. Prenatal origin of GATA1 mutations may be an initiating step in the development of megakaryocytic leukemia in Down syndrome. Blood 2004;104:1588–1589.
40. Terui T, Niitsu Y, Mahara K, et al. The production of transforming growth factor-beta in acute megakaryoblastic leukemia and its possible implications in myelofibrosis. Blood 1990;75:1540–1548.
41. Hitzler JK, Cheung J, Li Y, et al. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood 2003;101:4301–4304.
42. Tchernia G, Lejeune F, Boccara JF, et al. Erythroblastic and/or megakaryoblastic leukemia in Down syndrome: Treatment with low-dose arabinosyl cytosine. J Pediatr Hematol Oncol 1996;18: 59–62.
43. Zucker-Franklin D, Philipp CS. Platelet production in the pulmonary capillary bed: New ultrastructural evidence for an old concept. Am J Pathol 2000;157:69–74.
44. Zwaan CM, Kaspers GJ, Pieters R, et al. Different drug sensitivity profiles of acute myeloid and lymphoblastic leukemia and normal peripheral blood mononuclear cells in children with and without Down syndrome. Blood 2002;99:245–251.
45. Ge Y, Jensen TL, Stout ML, et al. The role of cytidine deaminase and GATA1 mutations in the increased cytosine arabinoside sensitivity of Down syndrome myeloblasts and leukemia cell lines. Cancer Res 2004;64:728–735.
46. Newman EM, Villacorte DG, Testi AM, et al. Biochemical interactions between methotrexate and 1-beta-D-arabinofuranosylcytosine in hematopoietic cells of children: A Pediatric Oncology Group study. Cancer Chemother Pharmacol 1990;27:60–66.
47. Bhalla K, Swerdlow P, Grant S. Effects of thymidine and hydroxyurea on the metabolism and cytotoxicity of 1-beta-D arabinofuranosylcytosine in highly resistant human leukemia cells. Blood 1991;78:2937–2944.
48. Gandhi V, Estey E, Keating MJ, et al. Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 1993;11:116–124.
49. Taub JW, Stout ML, Buck SA, et al. Myeloblasts from Down syndrome children with acute myeloid leukemia have increased

in vitro sensitivity to cytosine arabinoside and daunorubicin. Leukemia 1997;11:1594–1595.
50. Frost BM, Gustafsson G, Larsson R, et al. Cellular cytotoxic drug sensitivity in children with acute leukemia and Down's syndrome: An explanation to differences in clinical outcome? Leukemia 2000; 14:943–944.
51. Chien MB, S, Johnson RM, Stout M. Increased generation of reactive oxygen species correlates with cytotoxicty in acute myeloid leukemia (AML) of Down syndrome and is augmented by cytotoxic agents affecting the mitochondrial electron transport chain. AACR. Orlando; 2004:ABS#3098.
52. Wolvetang EJ, Wilson TJ, Sanij E, et al. ETS2 overexpression in transgenic models and in Down syndrome predisposes to apoptosis via the p53 pathway. Hum Mol Genet 2003;12:247–255.
53. Sanij E, Hatzistavrou T, Hertzog P, et al. Ets-2 is induced by oxidative stress and sensitizes cells to H(2)O(2)-induced apoptosis: Implications for Down's syndrome. Biochem Biophys Res Commun 2001;287:1003–1008.
54. Karandikar NJ, Aquino DB, McKenna RW, et al. Transient myeloproliferative disorder and acute myeloid leukemia in Down syndrome. An immunophenotypic analysis. Am J Clin Pathol 2001; 116:204–210.