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## Acute myeloid leukaemia in children

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Acute myeloid leukaemia (AML) is characterized by a block in differentiation and an unregulated proliferation of myeloid progenitor cells. While the cause of AML in children is unknown, risk factors that have been identified include exposure to toxins such as ethanol, pesticides and dietary topoisomerase II inhibitors, prior chemotherapy with alkylating agents or topoisomerase II inhibitors, constitutional disorders such as Down's syndrome and type I neurofibromatosis, and haematopoietic failure syndromes such as Fanconi anaemia and severe congenital neutropenia. With intensified chemotherapy including high-dose Ara-C, followed in many cases by bone marrow transplantation, and with improvements in supportive care, current survival rates approach 50%. Future advances in paediatric AML will include better risk stratification to determine optimal treatment and targeted cytotoxic therapy.

**Key words:** acute myeloid leukaemia; leukaemia in children; chemotherapy; stem cell transplantation.

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## INTRODUCTION

AML is the seventh most common paediatric malignancy. While considerable progress has been made in understanding the molecular pathogenesis of the disease, improvements in treatment have come more slowly. Paediatric patients have benefited from enrollment on clinical trials that have demonstrated gains in survival resulting from the intensification of therapy. However, treatment-related mortality and relapse rates remain high. This chapter will review the epidemiology and pathogenesis of AML in children, evaluate treatment options and discuss ongoing areas of study.

EPIDEMIOLOGY

Approximately 500 children/year in the United States develop AML.<sup>1</sup> According to data from the Surveillance, Epidemiology and End Results Program of the National Cancer Institute (SEER), the incidence peaks at 12 cases/million at 2 years of age and decreases to a nadir of approximately 3.8 cases/million at 9 years of age.<sup>2</sup> The incidence begins to rise again after 9 years of age and peaks at approximately 9 cases/million at age 16. Unlike paediatric acute lymphoblastic leukaemia (ALL), significant differences in sex adjusted incidence rates in the United States do not occur. According to the International Association of Cancer Registries<sup>3</sup>, the highest incidence of paediatric AML occurs in the Maori population in New Zealand with an age-standardized incidence rate of 14.4/million. High age-standardized rates are also found in Hawaiian Americans (11.2 cases/million) and among Africans in Zimbabwe (11.0 cases/million). The lowest incidence rates for countries reporting more than 10 cases occurred in Colombia (1.6 cases/million), Bulgaria (2.9 cases/million), and Brazil (3.5 cases/million). Geographical differences in AML subtype also occur. Acute promyelocytic leukaemia (APL) comprises about 30% of all paediatric AML in Latin countries, but less than 10% in non-Latin nations.<sup>4</sup>

RISK FACTORS

Several acquired and congenital risk factors increase the propensity of children to develop AML. Acquired risk factors include parental exposure to pesticides, and in utero exposure to ethanol and dietary topoisomerase II inhibitors.<sup>5-10</sup> Radiation-induced AML, seen in survivors of the atomic bomb and in patients who received high-dose in utero exposure, is less relevant today.<sup>11,12</sup> Therapy-induced secondary AML (t-AML) cases induced by chemotherapy for a prior malignancy are increasingly common. These cases fall into two groups. The first are leukaemias induced by therapy with alkylating agents. These typically present several years after therapy, with a peak incidence at about 5 years, and are characterized by deletions of 5q or 7q.<sup>13</sup> The second group comprises leukaemias induced by topoisomerase II inhibitors, which tend to occur within 2 years after therapy and are characterized by rearrangements involving the MLL gene at 11q23.<sup>14</sup> Both types of secondary AML are difficult to cure.

Table I. Constitutional disorders with an increased risk of AML.

Syndrome	Frequency of AML
Down's syndrome (trisomy 21)	1 : 100-200
Increased chromosomal fragility	
Fanconi anaemia	1 : 10
Bloom syndrome	Unknown
Neurofibromatosis Type I	1 : 200
Severe congenital neutropenia (Kostmann's disease)	1 : 10
Blackfan-Diamond anaemia	Unknown
Paroxysmal nocturnal haemoglobinuria	Unknown
Shwachman-Diamond syndrome	Unknown
Thrombocytopenia-absent radii syndrome	Unknown

Several congenital syndromes carry an increased risk of AML. These are summarized in Table I. Bloom's Syndrome and Fanconi anaemia are characterized by increased chromosomal fragility. Many patients with Fanconi anaemia have aplastic anaemia, and one in ten will develop AML or a myelodysplastic syndrome.<sup>15</sup>

Neurofibromatosis type I results from mutations in the *NF1* gene on chromosome 17q11. Individuals have an increased propensity for central nervous system and peripheral nerve tumours and for AML, particularly juvenile myelomonocytic leukaemia. Patients with neurofibromatosis have a 20-fold increased risk of developing a myeloid disorder compared to normal children.<sup>16</sup>

Down's syndrome results from trisomy of chromosome 21 and carries a 10–18-fold increased risk of leukaemia, with AML predominating in children less than 3 years old.<sup>17</sup> Acute megakaryoblastic leukaemia, an otherwise rare paediatric ALM subtype, is the most common subtype in patients with Down's syndrome.<sup>18,19</sup> Approximately 10% of all cases of paediatric AML occur in patients with Down's syndrome.<sup>20</sup> Individuals with mosaicism of trisomy 21 also have an increased risk of leukaemia in the affected bone marrow cells. The genes that predispose to leukaemia in Down's syndrome patients have not been elucidated. Neonates with Down's syndrome may develop a transient clonal megakaryoblastic myeloproliferative syndrome that closely resembles AML. The syndrome usually resolves within a few months, although patients have an even higher risk, compared to other children with Down's syndrome, of developing acute megakaryoblastic leukaemia between the ages of 1 and 5 years.<sup>21,22</sup>

Identical twin siblings of AML patients have a 5% increased risk of developing AML.<sup>23</sup> While this may reflect a shared genetic predisposition towards AML, the increased risk may also be due to an in utero twin–twin transfer of leukaemic cells. Several cases of identical genetic alterations observed several years prior to the onset of AML have been described.<sup>24</sup>

## **PATHOGENESIS**

The principal defect in AML is a block in the differentiation of primitive myeloid precursor cells. AML blasts also have a proliferative advantage compared to normal myeloid precursors. Studies of leukaemia-specific translocations and of congenital syndromes that carry a high risk of leukaemia have provided insight into the mechanism of leukaemogenesis. In general, two predominant mechanisms have been identified. In many cases, the defect is at the level of transcriptional activation, in which the leukaemia cell changes from a normal pattern of transcriptional activation to one in which transcriptional repressors are recruited to the site of genes involved in differentiation.<sup>25</sup> In other cases, defects are seen in the signalling pathway of haematopoietic growth factors, resulting in growth factor independence or an abnormally high response to physiological levels of growth factor. In particular, the proto-oncogene *Ras*, a critical mediator of haematopoietic growth factor signalling, is mutated in up to one-third of patients with AML. These mutations cause *Ras* to remain in its activated, GTP-bound form rather than reverting to an inactive, GDP-bound form.<sup>26</sup> The elevated levels of *Ras*-GTP result in an abnormally high proliferative response to granulocyte-macrophage colony stimulating factor (GM-CSF).<sup>27,28</sup> Some other examples specific to paediatrics are discussed below.

## Constitutional disorders

### *Neurofibromatosis Type 1*

The product of the *NFI* gene, neurofibromin, plays a key role in the Ras signalling pathway, which may explain its role in the development of AML. Neurofibromin is a GTPase-activating protein (GAP) that markedly increases the GTPase activity of Ras; it thus inactivates Ras by converting it to the inactive, GDP-bound form.<sup>26</sup> Mutations in the *NFI* gene therefore result in an increased amount of activated Ras because of a reduction in GTPase activity. In *NFI* patients who develop AML, there is loss of heterozygosity at the *NFI* locus, with uniform loss of the normal allele and a truncating mutation in the other.<sup>16,29</sup> In patients without *NFI*, neurofibromin activity is normal but activating Ras mutations are common.<sup>29</sup>

### *Severe congenital neutropenia*

Patients with severe congenital neutropenia (SCN; Kostmann's disease) develop severe neutropenia as infants and usually die from infection early in life unless treated with granulocyte colony-stimulating factor (G-CSF). However, with the avoidance of infection has come an increased incidence of AML in these patients. Mutations in the G-CSF receptor have been identified in some patients with SCN, and these mutations can identify the subset of SCN patients who will develop AML.<sup>30,31</sup> The mutations introduce premature stop codons into the G-CSF receptor gene, resulting in a dominant-negative receptor protein with defective internalization and sustained activation upon stimulation with G-CSF.<sup>32–34</sup> As a result of the receptor mutations, myeloid precursor cells have both an abnormally high proliferative response to G-CSF and a block in terminal differentiation.

## Chromosomal translocations

### *Core binding factor genes*

The core binding factor proteins AML1/CBF $\alpha$ 2 and CBF $\beta$  form a transcription factor heterodimer that binds to DNA via the AML1 subunit to activate expression of lineage-specific genes. The genes for both subunits are frequently involved in translocations in AML. These include t(8;21), which fuses AML1 to ETO in the French–American–British (FAB) subtype M2, and inv(16), which fuses CBF $\beta$  to MYH11 in M4Eo. The AML1-ETO fusion protein is still able to bind to AML1 target sequences in DNA, but, instead of recruiting transcriptional activators, the fusion protein interacts with nuclear co-repressors via the ETO domain to repress transcription.<sup>35</sup> The CBF $\beta$ -MYH11 fusion protein, in contrast, appears to repress transcription by sequestering normal AML1 protein in the cytoplasm and thus preventing it from binding to DNA.<sup>36,37</sup> Haploinsufficiency of the *AML1* gene has also been shown to be present in a familial syndrome of thrombocytopenia with an increased risk of developing AML.<sup>38</sup>

### *11q23 rearrangements*

The *MLL* gene, at 11q23, is rearranged in 20% of cases of paediatric AML, particularly in infants, FAB subtypes M4 and M5, and therapy-related AML.<sup>39</sup> Many partner genes for *MLL* have been identified and, in most cases, a fusion protein is created with the 5' end of *MLL* and the 3' end of the partner. In normal haematopoiesis, *MLL* regulates the

expression of different homeobox genes, and it is likely that a disruption of MLL affects haematopoiesis by altering the normal pattern of homeobox gene expression. Different MLL fusion proteins may act by different mechanisms. For example, some may affect transcriptional activity through modulation of histone acetylation, while others may alter nucleosomal structure through interaction with the SWI/SNF complex, which maintains chromatin in an open conformation.<sup>25</sup>

## PRESENTATION

Children with AML present with the signs and symptoms of leukaemic blast cells crowding out normal haematopoietic elements in the bone marrow and invading extramedullary sites. Bone marrow replacement may cause a normocytic anaemia with fatigue, pallor and, in severe cases, haemodynamic instability. Likewise, patients often present with neutropenia that places them at substantial risk for invasive bacterial infections. Finally, thrombocytopenia may cause petechiae, purpura, mucosal bleeding, or, infrequently, CNS haemorrhage. Bone pain may result from expansion of the leukaemic clone in the bone marrow.

Extramedullary disease may present with adenopathy and hepatosplenomegaly. Chloromas, peripheral collections of leukaemic blast cells, may occur in the soft tissues, skin (leukaemia cutis), gingivae, orbit, or elsewhere. Extramedullary infiltration is especially common with myelomonocytic or monoblastic variants (FAB M4 or M5). Approximately 5% of patients present with CNS disease at diagnosis. A smaller proportion of patients has CNS chloromas and present with headaches, cranial nerve palsies, focal neurological deficits, or seizures. Patients with high peripheral blast cell counts (greater than 200 000/mm<sup>3</sup>) are at risk for stroke.

Concurrent coagulopathy, which is especially common in M3 and M5 AML, increases the risk of haemorrhage. The coagulopathy may be fatal prior to or early in the course of therapy. Although the aetiology of disseminated intravascular coagulation (DIC) in M5 AML is not known, there is convincing evidence in acute promyelocytic leukaemia with t(15;17) that overexpression of annexin II on the leukaemia blast surface activates plasminogen through its interaction with tissue plasminogen activator, thereby promoting fibrinolysis.<sup>40</sup>

## DIAGNOSIS

Bone marrow should be obtained at diagnosis and suspected relapse for histochemical stains, flow cytometry, cytogenetics and, with permission, specimen banking. AML is diagnosed by the presence of more than 30% blasts on bone marrow aspirate or biopsy. The FAB classification scheme remains the standard classification system for AML.<sup>41</sup> The FAB subtypes reflect the morphology and histochemistry of the predominant AML clone. Because the FAB system correlates poorly with clinical outcome, a revised classification system for AML was recently proposed<sup>42</sup> that divides AML into two groups: myelodysplastic syndrome-related AML and true de novo AML. The former group has a poorer outcome, occurs more commonly in elderly patients and is associated with cytogenetic abnormalities, such as monosomy 7, that carry a poor prognosis. True de novo AML is more common in children and carries a better prognosis. The use of microarray analysis to characterize AML subtypes by gene expression profile may aid in classification in the future.<sup>43</sup>

Table 2. Cytogenetics in AML.		
Karyotype	Children (%)	Adults (%)
Normal	15–31	40–46
t(8;21)(q22;q22)	8–16	6–7
inv(16)(p13;q22)/t(16;16)	3–12	3–8
t(15;17)(q22;q12–21)	4–11	4–13
11q23 rearrangements	6–11	3–7
t(6;9)(p23;q24)	0–1	< 2
t(1;22)(p13;q13)	0–3	< 1
-7/del(7q)	4–6	6–8
-5/del(5q)	0–2	4–6
Abstracted from Raimondi et al. <sup>39</sup>		

Blasts from more than 90% of paediatric AML patients express myeloid associated surface antigens (CD13, CD14 or CD33). Lymphoid markers, both T and B cell, may be present in 30–60% of paediatric patients. Some correlation between immunophenotype, morphology and cytogenetics has been observed: CD19<sup>+</sup> and CD56<sup>+</sup> with FAB M2 and t(8;21), CD2<sup>+</sup> and CD7<sup>+</sup> with FAB M2 without t(8;21), and CD33<sup>+</sup> and CD34<sup>+</sup> with FAB M3 with t(15;17). Individual paediatric AML patients generally have a conserved clonotypic immunophenotype that can serve as a ‘molecular fingerprint’ of the patient’s AML. This immunophenotypic fingerprint may be helpful in disease monitoring.

While 40–50% of AML blasts in adults have a normal karyotype, only 20–30% of paediatric blasts have normal karyotypes. Approximately 60% of the abnormal karyotypes in paediatric AML blasts fall into known cytogenetic subgroups. The prevalence of these cytogenetic subgroups is summarized and compared to adult data in Table 2. Translocations or tandem duplications of the MLL gene at 11q23 are found in many cases of t-AML but are also common in infants with de novo M4 and M5 AML. Monosomy 7 and 7q- are seen less commonly in children than in adults. Because of the importance of detecting particular chromosomal rearrangements, techniques such as fluorescence in situ hybridization (FISH), Southern blotting and reverse transcriptase-polymerase chain reactions (RT-PCR) are becoming more prevalent diagnostic tools for AML.

Other paediatric malignancies may mimic AML. Alveolar rhabdomyosarcoma and neuroblastoma may resemble monoblastic (M5) AML. Undifferentiated leukaemia, L2 ALL, or metastatic alveolar rhabdomyosarcoma may look like megakaryoblastic (M7) AML. Immunophenotyping, karyotyping and PCR may aid in distinguishing AML from non-myeloid metastatic disease.

## THERAPY

### Chemotherapy

Paediatric AML treatment protocols share an overall treatment strategy but differ in the drugs, doses and schedules used. Therapy begins with remission induction and CNS prophylaxis, continues with consolidation, and ends with an intensification phase. The German Berlin–Frankfurt–Münster (BFM) co-operative group adds a maintenance phase similar to that used in paediatric ALL therapy. Induction therapy aims to reduce the leukaemic blast percentage in the bone marrow to a level below 5%, to eliminate

**Table 3.** Induction regimens in paediatric AML cooperative group trials.

Study	<i>n</i>	Ara-C	Dauno	Dauno equivalent	Other agents	CR rate (%)	Ref.
UK MRC 10	286	1000	150		6-TG Etoposide	89 93	Stevens et al <sup>78</sup>
LAME 89/91	171	5000	225	Mitox 60	Etoposide	87	Michel et al <sup>47</sup>
POG 8821	649	700	135		6-TG	85	Ravindranath et al <sup>46</sup>
CCG 2891					Etoposide		Woods et al <sup>45</sup>
Standard	294	800	80		6-TG	74	
Intensive	295	1600	60		Dex	78	
BFM 87	210	1400	180		Etoposide	78	Creutzig et al <sup>79</sup>
CCG-213 (7 & 3)	597	700	135			79	Wells et al <sup>80</sup>

Abbreviations used: Dauno, daunorubicin; CR, complete remission; Mitox, mitoxantrone; Dex, dexamethasone; 6-TG, 6-thioguanine; UK MRC, United Kingdom Medical Research Council; LAME, AML study of the French Society for Pediatric Hematology and Immunology; POG, Pediatric Oncology Group; CCG, Childrens Cancer Groups; BFM, Berlin-Frankfurt-Münster Study Group.

extramedullary disease, and to restore normal haematopoiesis. Several randomized comparisons between the standard induction regimen of '7 and 3' (7 days of cytarabine (Ara-C) at 100 mg/m<sup>2</sup>/day and 3 days of daunorubicin at 45–60 mg/m<sup>2</sup>/day) and other induction regimens have been reported (see Table 3). Current induction regimens are more intensive than the '7 and 3' and induce more durable remissions with better overall survival.

The American and French co-operative groups use matched related donor bone marrow transplantation (MRD BMT) for the intensification phase of AML therapy. If a matched related donor is not available, then consolidation and intensification chemotherapy regimens are given. All paediatric consolidation and intensification regimens rely heavily on high-dose Ara-C, but differ in the role of autologous BMT (AUTO BMT) and maintenance therapy. The Tenth Medical Research Council on Acute Myeloid Leukemia Trial (MRC AML 10) demonstrated a significant benefit in relapse-free survival with AUTO BMT versus no further therapy after four courses of intensification, but overall survival was not higher because of poorer retrieval of patients following AUTO BMT.<sup>44</sup> Children's Cancer Group (CCG), Pediatric Oncology Group (POG) and the studies of the French Society for Pediatric Hematology and Immunology (SHIP; LAME 89/91) have shown no benefit for AUTO BMT.<sup>45–47</sup> While the BFM group relies heavily on maintenance chemotherapy, CCG and LAME studies have demonstrated that maintenance chemotherapy may decrease event-free survival (EFS) and overall survival (OS). Table 4 summarizes the most recent randomized AML clinical trials. While these studies have some limitations, the use of randomized trials has led to the highest survival rates for AML in children.<sup>48</sup>

The chemotherapy agents used in paediatric AML therapy all cause substantial and often life-threatening or fatal toxicity. All induction, consolidation and BMT regimens cause mucositis, emesis and bone marrow suppression. Bone marrow suppression predisposes patients to infectious complications that are the leading cause of morbidity and mortality in paediatric AML therapy. The increased intensity of therapy in current trials raises induction mortality such that toxicity from dose and schedule

Table 4. Results of paediatric AML cooperative group trials.				
Study	Induction randomization	Consolidation randomization	Conclusion	Overall survival
UK MRC AML 10	ADE v. TDE	MRD biological randomization Early AUTO BMT v. no further therapy	No benefit to ADE v. TDE MRD beneficial Early AUTO BMT beneficial	56% at 7 years
LAME 89/91	None	MRD biological randomization	MRD beneficial	
POG 8821	None	MRD biological randomization AUTO BMT v. CT	MRD beneficial AUTO BMT and CT equal	42% at 3 years
CCG 2981	DCTER v. intensive timing DCTER	MRD biological randomization AUTO BMT v. CT	Intensive timing beneficial MRD beneficial CT superior to AUTO BMT	49% intensive timing 34% standard timing at 3 years
BFM 1987	None	XRT v. no XRT	XRT beneficial	
BFM 1993	ADE v. AIE	More intensive therapy for high risk patients	Improved survival with more intensive therapy ADE equivalent to AIE	58% at 5 years

Source: references 45–47, 78, 81, 82.  
Abbreviations used: MRD, matched related donor bone marrow transplant; AUTO BMT, autologous bone marrow transplant; CT, chemotherapy; ADE, Ara-C, daunorubicin, etoposide; AIE, Ara-C, idarubicin, etoposide; TDE, 6-thioguanine, etoposide; DCTER, 6-thioguanine, daunorubicin, etoposide; DCTER, dexamethasone, Ara-C, 6-thioguanine, etoposide, daunorubicin; XRT, cranial irradiation. For study abbreviations see Table 3.





intensification may limit future gains in remission induction and overall survival. Since durable remission induction is a pre-requisite for cure, future clinical trials need to attempt to improve remission induction outcomes.

Table 5 summarizes the most common chemotherapeutics and toxicities in paediatric AML therapy. Patients with APL receive all-*trans* retinoic acid (ATRA) and chemotherapy. While children are more likely to experience ATRA-induced pseudotumour cerebri, their rates of rapid onset pulmonary failure are similar to those of adults.<sup>49</sup> Risk assessment strategies for chemotherapy toxicity are under development. For example, children with Down's syndrome receive lower doses of chemotherapeutics since they experience substantially more side effects.<sup>20</sup> A sequential study by CCG observed an increased induction mortality (4% versus 10%) in patients lacking glutathione s-transferase (GST), an enzyme involved in drug metabolism.<sup>50</sup> This difference may be due to decreased excretion of toxic chemotherapy metabolites in GST null patients. Patients with Fanconi anaemia and AML are also unable to tolerate conventional chemotherapy or BMT regimens. Further work in determining genetic susceptibility to toxicity is needed.

## Stem cell transplantation

Allogeneic stem cell transplantation from a human leukocyte antigen (HLA)-matched sibling in first remission gives the highest disease-free survival in most biologically randomized paediatric studies. Although HLA-matched related donor BMT may have a higher regimen-related mortality compared to chemotherapy alone, it offers better treatment of leukaemia both from the use of higher, myeloablative doses of chemotherapy as well as from a graft-versus-leukaemia effect. The use of autologous stem cells eliminates the risk of graft-versus-host disease (GVHD) seen in allogeneic BMT. However, patients who receive AUTO BMT have an increased risk of relapse compared to those who receive allogeneic BMT because of the absence of a graft-versus-leukaemia effect and from contamination of the autologous stem cell product with leukaemia cells.

The risk of GVHD is less for MRD BMT than for matched unrelated donor or unmatched transplants. The risk of acute GVHD in MRD BMT is approximately 40%, but may reach 80% with alternative donor transplants. A graft-versus-leukaemia effect occurs in some patients with acute GVHD, but the high incidence of severe (grades III and IV) acute GVHD and chronic GVHD in unrelated donor transplants outweighs this benefit. Methotrexate, cyclosporine, FK506 and corticosteroids are used in various combinations for acute GVHD prophylaxis and management as well as in chronic GVHD.

The conditioning regimens for BMT expose patients to additional toxicities. The most common short-term toxicities are nephritis, pneumonitis and veno-occlusive disease, and are often fatal. The late effects of BMT include growth retardation, cardiac dysfunction, infertility, and neuropsychological deficits.<sup>51,52</sup> Both total body irradiation (TBI) and cyclophosphamide may cause infertility in males and females, although some patients maintain normal gonadal function. Females who are able to become pregnant following BMT have a 25% incidence of preterm labour.<sup>53</sup> Children under the age of 6 are at risk of cognitive decline after BMT, with the highest risk in patients under 3 years of age.<sup>54</sup> Transplant-related mortality is proportional to the extent of donor recipient mismatch, age and the extent of pre-treatment.

## Immunotherapy

The observation that patients with AML who develop acute GVHD after stem cell transplantation have a decreased rate of relapse has focused attention on the graft-versus-leukaemia (GVL) effect and the role of immune modulation in inducing or maintaining remission. Some earlier clues to the role of the immune system in inducing remission have come from rare cases of spontaneous remission. In several cases the remission occurred shortly after either a transfusion, particularly of leukocytes, or an infection.<sup>55,56</sup> A transfusion-associated remission may reflect a GVL effect of the transfused white blood cells, while infections may have an immunomodulatory effect.

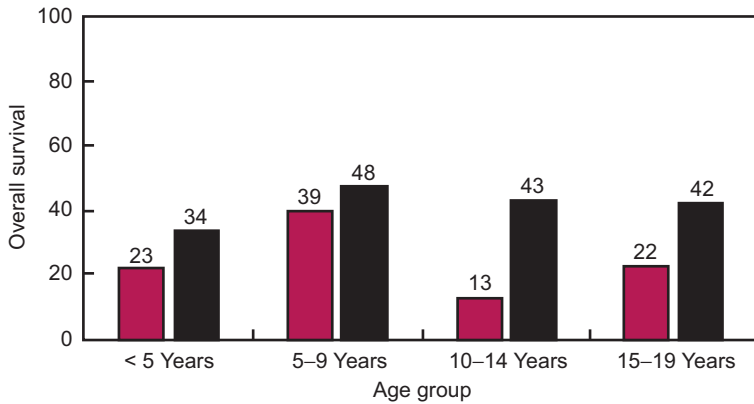
In some cases of relapse following allogeneic stem cell transplantation, remission may be induced by stopping or reducing immunosuppression to promote a GVL reaction. Interferon- $\alpha$  has also been used to augment the anti-leukaemic response.<sup>57,58</sup> Recent focus in transplantation medicine has tried to separate the GVHD and GVL effects, since some patients may develop one without the other. One approach uses T cell depletion of the allograft to prevent the development of acute GVHD followed by a combination of donor lymphocyte infusions and reductions in immunosuppressive drugs to induce GVL.<sup>59</sup> This approach may be particularly effective in cases in which there is no HLA-matched donor, and thus a high risk of severe GVHD, or in patients who cannot tolerate a myeloablative conditioning regimen. An ability to predict which patients may benefit from donor lymphocyte infusions may be aided by studies of minimal residual disease following stem cell transplantation. In addition, patients with absolute lymphocyte counts less than  $0.2 \times 10^9/\text{l}$  have a higher risk of relapse and may therefore benefit from donor lymphocyte infusions.<sup>60</sup>

A role for monoclonal antibodies in the therapy of AML is currently being studied in paediatric trials. The antibody conjugate CMA-676 consists of a humanized anti-CD33 antibody linked to a toxin, calicheamicin. The antibody is internalized upon binding to CD33, which is expressed on the majority of AML blasts, and the toxin is released intracellularly. In phase II adult studies, CMA-676 had a 43% response rate, with relatively mild toxicity consisting of fever and chills shortly after administration of the antibody, and myelosuppression, particularly thrombocytopenia. Another promising antibody is  $^{131}\text{I}$ -anti-CD45. The advantages of CD45 as a target are that it is expressed in a higher copy number on haematopoietic cells than CD33, and the antibody is not internalized after binding, thus allowing for prolonged exposure of bone marrow cells to the radionuclide.<sup>61</sup>

Other emerging options for immunotherapy include adoptive immunotherapy using T cells that have been manipulated *ex vivo* to respond to leukaemic blasts<sup>62</sup>, the development of vaccines against leukaemia-specific proteins<sup>63,64</sup>, and the generation of dendritic cells that promote an anti-leukaemic response.<sup>65</sup> These modalities are at the pre-clinical stage, or are being studied in phase I clinical trials.

## PROGNOSIS

Small sample sizes, different treatment protocols and conflicting results impede the definition of important prognostic groups. The BFM group defined a group of low risk patients who had M1 with Auer rods, M2 with less than 20 000 blast cells, M3, or M4Eo.<sup>66</sup> This risk group has not been confirmed prospectively by other co-operative groups. However, all investigators agree that white blood cell (WBC) counts greater



**Figure 1.** Paediatric AML survival rates from 1975–1994 by age group. ■, 1975–1985; ■, 1985–1994. Data are from Smith et al<sup>2</sup>.

than 100 000/mm<sup>3</sup> and monosomy 7 confer a poor prognosis, while t(8;21), t(15;17), inversion 16 and Down's syndrome confer favourable prognosis.

Patients with Down's syndrome receive relatively less intensive chemotherapy, but fare significantly better than other children with EFS ranging from 68–100%.<sup>20</sup> While all co-operative groups modify therapy for patients with acute promyelocytic leukaemia (APL) and Down's syndrome, only the BFM and the UK MRC modify therapy based on other risk assessment schemes. The UK MRC AML 12 Trial stratifies patients into three risk groups, good, standard and poor, based on FAB diagnosis (M3), karyotype and response to induction therapy. Patients in the good risk group do not receive HLA-matched unrelated donor (MUD) BMT, while patients in the poor risk group receive MUD BMT if available.<sup>67</sup> The BFM 1993 trial grouped patients into standard risk and high risk.<sup>66</sup> Standard risk patients receive chemotherapy only, while high risk patients are offered MUD BMT if available.

The overall survival from time of diagnosis ranges from 34–56% for paediatric AML. Figure 1 illustrates the gradual improvement in overall survival in paediatric AML over the past 20 years. In patients who relapse, the duration of second remission depends on the duration of first remission. In one study, patients who relapse less than 18 months after therapy have a 10% survival rate while patients relapsing more than 18 months post-therapy have a 40% survival rate. Although investigators have proposed therapy stratification based on time to relapse, such risk stratification is not currently used in paediatric AML since the therapeutic options are limited.

## SUPPORTIVE CARE

Children with AML, whether treated with stem cell transplantation or with chemotherapy alone, have a 10–20% induction mortality risk and can expect to have prolonged (> 1 month) periods of neutropenia that markedly increase the risk of serious infections. Consequently, children should be treated in centres that have expertise in the supportive care of similar patients and with easy access to intensive care unit services.

The high induction mortality of AML therapy is largely due to haemorrhage or infection. Transfusions of platelets and plasma may help to ameliorate the coagulopathy

that is frequently present at diagnosis of FAB M3 and M5. The tumour lysis syndrome is less of a problem in patients with AML than in those with lymphoid malignancies, but the use of hydration and allopurinol is recommended. Patients with high WBC counts at diagnosis are at risk of stroke from CNS leukostasis, and leukocytapheresis may be of benefit when the peripheral blast count exceeds 200 000/mm<sup>3</sup>.

With prolonged periods of neutropenia, patients are susceptible to a wide variety of bacterial and fungal infections. In particular, *Streptococcus viridans* infections are common in patients who receive high-dose Ara-C therapy.<sup>68</sup> Gram-negative organisms occur frequently at diagnosis, while both *Staphylococcus aureus* and coagulase negative *Staphylococcus* species may occur at any point in therapy in patients with central venous catheters. Prompt treatment of febrile neutropenia with broad-spectrum antibiotics with the addition of empiric amphotericin B for prolonged or recurrent fever is recommended.<sup>69</sup> The choice of antibiotics may be tailored to the predominant pathogenic organisms in the treatment centre. The role of prophylaxis for neutropenic infections is unclear. Penicillin may prevent some *S. viridans* infections, but penicillin resistance is becoming more common. Fluconazole may prevent some, but not all, *Candida* infections, and has no activity against molds such as *Aspergillus*. The use of high-efficiency particulate air (HEPA)-filter rooms may reduce the *Aspergillus* infection rate, especially in hospitals undergoing construction. Patients that undergo stem cell transplantation should receive trimethoprim-sulphamethoxazole for *Pneumocystis carinii* prophylaxis and acyclovir to prevent herpes simplex infection.

The judicious use of transfusions prevents the sequelae of anaemia and thrombocytopenia. Packed red blood cells are usually given for a haemoglobin level that is less than 8.0 g/dl.<sup>70</sup> Platelets are given for a platelet count less than 10 000/mm<sup>3</sup>, but this cut-off point is increased in the presence of active haemorrhage, DIC, the presence of dysfunctional platelets (e.g. renal failure), or at the time of a procedure.<sup>71,72</sup> There appears to be no advantage of single-donor platelets over random-donor platelets for most patients.<sup>73</sup> It is recommended that all blood products be irradiated, to prevent transfusion-associated GVHD, and leukocyte-depleted, to reduce the incidence of allo-immunization, febrile transfusion reactions, and transmission of cytomegalovirus. Finally, G-CSF-mobilized granulocytes may have some utility in the treatment of refractory infections in the neutropenic patient.<sup>74</sup>

Haematopoietic growth factors are widely used in the care of children with AML. Concerns that the growth of leukaemia cells can be promoted by G-CSF or GM-CSF have not been shown to be significant in vivo. Similarly, attempts to increase leukaemic cell death by inducing cell cycling with growth factors prior to chemotherapy have not been successful. These factors find their primary use in the treatment of neutropenia. Several studies have shown that G-CSF reduces the duration of neutropenia following chemotherapy, but in most studies the incidence of serious infections remains unchanged, probably because G-CSF does not prevent the nadir in neutrophil count.<sup>75,76</sup> GM-CSF is less widely used for the treatment of neutropenia because of a higher incidence of side-effects, but it has some use in the adjunctive treatment of fungal infections. Interleukin-11 (IL-11) is beginning to find a role in the treatment both of thrombocytopenia and of mucositis.<sup>77</sup>

## SUMMARY

The overall survival of paediatric patients with AML has improved over the last several years. This success is due to the large number of paediatric patients who are enrolled

in clinical trials that have demonstrated benefits for therapy intensification and to the use of HLA-matched sibling bone marrow transplants when available. However, about 50% of all newly diagnosed patients will not survive. The high treatment-related mortality, despite advances in supportive care, suggests that there are limits to therapy intensification. Improvements in survival will come from the development of targeted cytotoxic therapy and from a better understanding of the molecular pathogenesis of AML, which may result in therapeutic agents that induce differentiation of the malignant clone. Further advances in diagnosis and in the characterization of poor prognostic groups will allow therapy to be tailored to the disease type.

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