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Current Status of Acute Lymphoblastic Leukaemia in Children

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Abstract Acute lymphoblastic leukaemia (ALL) is the commonest form of childhood malignancy with an estimated annual incidence of 40 to 50 new cases (<15 years) in Hong Kong. The cure rate for ALL in children has improved drastically over the past 2 decades and is approaching 70% currently. Well-designed collaborative clinical trials had contributed greatly to this success in treatment outcome. The advances in new technology especially in the field of molecular biology also help to revolutionise the process of risk assessment, treatment stratification and disease monitoring. In addition to the advances in treatment, progresses had also been made in the understanding of leukaemogenesis and its associated risk factors, individual susceptibility and prognosis assessment. But while there are more and more childhood ALL children being cured, long-term therapy-related complications starts to emerge and becomes a new challenge. This review article will mainly focus on the recent advances in the areas of leukaemogenesis, prognostic assessment, current treatment design and late effect of ALL management.

Key words Childhood acute lymphoblastic leukaemia

Background

Acute lymphoblastic leukaemia (ALL) is the most common form of childhood malignancy and its annual incidence varies with different ethnic groups and geographic regions. In Hong Kong, the annual incidence is around 4.1 per million children as compared to the 6.1 among American White and 5.1 among the American Black (HKPHOSG 2002 & SEER 1988 data). During the past decade, the cure rate for childhood ALL in most developed countries reached 63-83% (Table 1). This success can be principally attributed to the effectiveness of risk-directed therapy developed through well-designed clinical trials. Chinese children who live in more affluent areas of the world have benefited from these achievements and experience a similar cure rate. However, because of a lack of access to treatment, inadequate treatment, and poor supportive care, many Chinese children with ALL still suffer a poor outcome. A simplified, tailor-made treatment approach may be more practical for Chinese patients who live in less developed regions.

Because of the remarkable advances made in the field of molecular biology during recent years, we have a better understanding of the molecular abnormalities that underlie leukaemogenesis and drug resistance. This information has led to the development of new therapeutic strategies, including immunotherapy and therapies that are

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Table 1 Summary of results from international studies of childhood ALL

Study	Year	Eligible age (years)	No. of patients	% 5-year event-free survival (±1SE) B-lineage*			
				Overall	Standard	High	T-lineage
AIEOP-91	1991-95	≤15	1194	70.8±1.3	79.9±1.5	61.5±2.9	40.4±4.1
BFM-90	1990-95	≤18	2178	78.0±0.9	87.4±1.0	66.3±2.1	61.1±2.9
CCG-1800	1989-95	≤21	5121	75±1	80±1	67±2	73±2
COALL-CLCG-92	1992-97	≤18	538	76.9±1.9	82.1±2.4	75.7±3.9	71.2±5.1
DCLSG-8	1991-96	≤18	467	73±0.2	79±2	67±5	71±6
DFCI-91-01	1991-95	≤18	377	83±2	85±2	82±4	79±8
EORTC-58881	1989-98	≤18	2065	70.9±1.1	78.4±1.3	57.3±2.4	64.4±2.9
NOPHO-III	1992-98	≤15	1143	77.6±1.4	85.2±1.5	67.9±3.3	61.3±4.9
POG	1986-94	≤21	3828	70.9±0.8	77.4±0.9	55.3±1.6	51.0±2.4
SJCRH-13A	1991-94	≤18	165	76.9±3.3	88.1±3.6	70.4±6.2	60.9±10.2
TCCSG-L92-13	1992-95	≤15	347	63.4±2.7	67.8±3.4	56.7±5.4	59.3±8.6
UKALL-XI	1990-97	≤15	2090	63±1.1	74±2.2	59±4.1	51±3.5
**TPOG		≤15	93[1]	-	72±17	-	-
			108[2]		87.2±18		
HKPHOSG-93	1993-97	≤16	145	62.6	79	61	-

AIEOP: Associazione Italiana di Ematologia ed Oncologia Pediatrica; BFM: Berlin-Frankfurt-Münster ALL Study Group; CCG: Children's Cancer Group; COALL: Cooperative ALL Study Group; DCLSG: Dutch Childhood Leukemia Study Group; DFCI: Dana-Farber Cancer Institute Consortium; EORTC-CLCG: European Organization for Research and Treatment of Cancer, Children's Leukaemia Cooperative Study Group; NOPHO: Nordic Society of Paediatric Haematology and Oncology; POG: Pediatric Oncology Group; SJCRH: St. Jude Children's Research Hospital; TCCSG: Tokyo Children's Cancer Study Group; UKALL: UK Medical Research Council Working Party on Childhood Leukaemia; TPOG: Taiwan Pediatric Oncology Group; HKPHOSG: Hong Kong Paediatric & Haemotology Oncology Study Group.

*Standard-risk group included children 1 to 9 years old with leukocyte count <50x10^9/L; **Standard risk B-lineage cases only and randomised on high[1] & low[2] dose L-asparaginase treatment.

molecularly targeted toward genetic lesions. However, because those new treatments are expected to be costly initially, it will be many years before children from underdeveloped or developing countries will benefit from them.

Another key advance in the treatment of leukaemia is the emergence of the field of pharmacogenetics. Genetic polymorphisms of certain drug-metabolising enzymes, transporters, receptors, or targets have been linked with host susceptibility to the development of de novo leukaemia or therapy-related second cancers. Furthermore, recognition of inherited differences in patients' ability to metabolise antileukaemic agents has provided rational selection criteria for optimal drug dosages and scheduling. Distinct ethnic or racial differences in the pharmacogenetic characteristics of Chinese children probably exist; however, at this time, the related data remains quite sparse. Collaborative studies that include multiple Chinese paediatric oncology centers and those abroad are currently generating more useful information in this area.

Finally, the intensity of therapy can be adjusted on the basis of treatment response. Treatment response, which is assessed by measurement of subclinical leukaemia, or minimal residual disease (MRD), has emerged as a powerful and independent prognostic indicator (Figure 1). MRD is an indicator of the sensitivity or resistance of leukaemic cells to drugs and the pharmacokinetic and pharmacogenetic properties of the host; measurement of MRD will probably become the gold standard for assessing patients' risk of relapse.

Treatment Outcome of Childhood ALL

Contemporary risk-directed therapy has resulted in 5-year event-free survival rates that have ranged between 63% and 83% in children treated for ALL in developed countries (Table 1).[1-12] Although some clinical trials resulted in a relatively low event-free survival rate, retrieval therapy boosted the overall survival to approximately 80%. A comparable treatment outcome has been reported in Chinese children treated for ALL in Taiwan and Hong Kong in recent

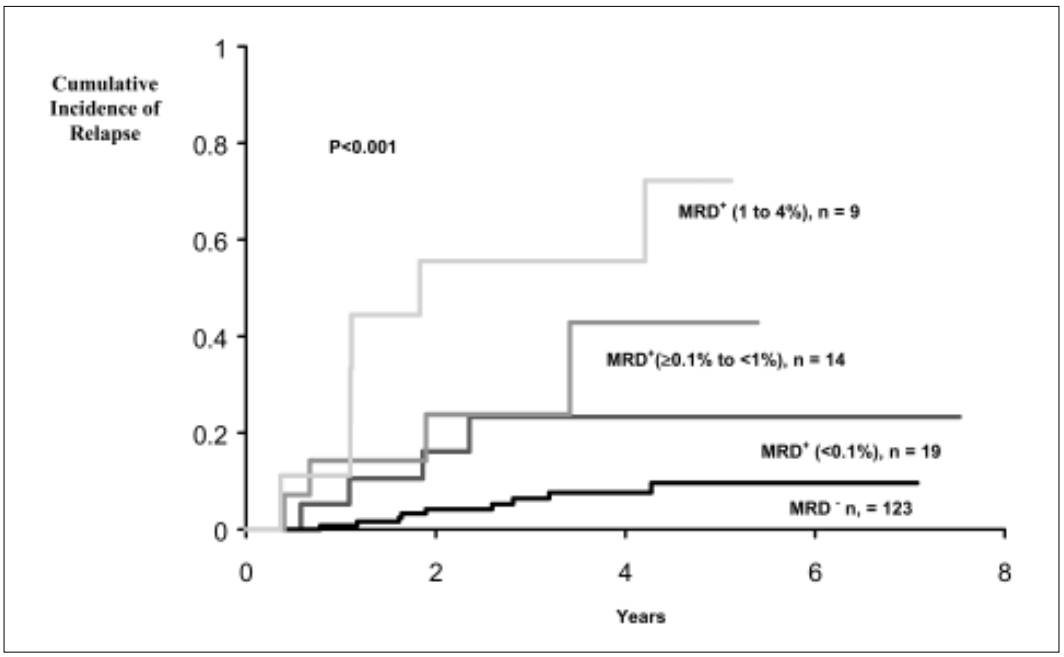

Figure 1 Cumulative incidence of relapse in children with ALL as determined by minimal residual disease (MRD) status at the end of remission-induction therapy. (Reprinted with permission from Coustan-Smith, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 2000;96: 2691-6.)

years;[13,14] however, in some regions of China and Southeast Asia, a substantial number of children with ALL still do not receive adequate treatment or supportive care. This may be due to either financial or facility constraints. A simplified, less expensive chemotherapy regimen may be useful in this setting. In addition, a collaborative, central facility may help to establish proper diagnosis and risk stratification approaches.

Leukaemogenesis in Children

Numerous investigations have examined the demographic and environmental risk factors of the development of childhood leukaemia. The factors associated with an increased risk are male sex, aged 2 to 5 years, high socioeconomic status, white race, in utero X-ray exposure, and the presence of some congenital syndromes such as Down syndrome or neurofibromatosis (Table 2).[15] Other possible risk factors include increased birth weight, maternal history of fetal loss, paternal smoking before or during pregnancy, parental occupational exposure to carcinogens, postnatal infections, and diet.

Hypothesis of Delayed Exposure to Common Infection and Leukaemogenesis

This hypothesis may explain the higher risk of ALL in children from higher socioeconomic status or developed countries. It states that delayed exposure of these children to infectious agents contributes to the etiology of childhood ALL. Descriptive epidemiologic and case-control studies conducted in developed Western countries[16] and Hong Kong[17] have provided substantial indirect support for this theory. In the Hong Kong study, roseola, fever and rash, or both experienced during the first year of life reduced the risk of ALL (odds ratio, 0.33; 95% confidence interval, 0.16 to 0.68), whereas tonsillitis during the 3 to 12-month period before reference date (date of diagnosis for cases, corresponding date for controls) increased the risk of ALL (odds ratio, 2.56; 95% confidence interval, 1.22 to 5.38). Some other indirect measures of exposure to infection at crucial times were associated with predicted patterns of risk, but day-care attendance failed to show associations.

Individual Susceptibility and Environment-Induced Genetic Change

Although traditional epidemiologic studies have yielded

Table 2 Risk factors associated with childhood ALL leukaemogenesis

Generally accepted	Suggestive	Limited evidence	Probably not associated
Males	Increased birth weight	Parental smoking prior to or during pregnancy	Ultrasound
Age (2-5 years)	Maternal history of fetal loss	Parental occupational exposures	Indoor radon
High socioeconomic status		Postnatal infections	
Race (whites>blacks)		Diet	
In utero X-ray exposure		Vitamin K prophylaxis in newborns	
Postnatal radiation (therapeutic)		Maternal alcohol consumption during pregnancy	
Down's syndrome		Electric & magnetic fields	
Neurofibromatosis type I		Postnatal use of choramphenicol	
Bloom syndrome			
Ataxic Telangiectasia			
Schwachman syndrome			

Data are from Bhatia S, et al. Epidemiology and etiology. In: Pui CH, editors. Childhood Leukemias. Cambridge University Press, 1999:38-49.[15]

a considerable amount of information about leukaemogenesis in children, the advances made in molecular biology provide a more powerful tool for further study. One current primary focus of these studies is infant ALL, which frequently involves the *MLL* gene located on the chromosome band 11q23.[18] *MLL* gene (also known the mixed lineage leukaemia gene or myeloid lymphoid leukaemia gene) serves as a homeotic gene. Translocations of *MLL* with other genes can induce regulator of leukaemia possibly via loss of wild-type *MLL* function together with gain of additional signals from sequences provided by the partner chromosomes. Many of the *MLL* translocation partners (>30) encode proteins involved in modulating gene expression. For example, the AF4 protein targeted by the t(4;11) appears to function directly as transcription factor. Approximately 75% of infants with ALL have *MLL* gene rearrangements. *MLL* rearrangements are also common in therapy-related acute myeloid or lymphoid leukaemia in all age groups. Therapy-related acute leukaemia arises shortly after treatment with topoisomerase II inhibitors such as epipodophyllotoxins [i.e. etoposide (VP16)].[19] DNA topoisomerases are nuclear enzymes that can repair the structure of DNA by both breaking and rejoining action. Topoisomerase I acts on single stranded DNA and topoisomerase II produces double stranded DNA breakage-rejoining. A topoisomerase II inhibitor acts by forming a stable tertiary DNA-topo-II-drug complex and interferes with DNA replication, repair and transcription. The similarity in the genetic abnormalities associated with infant leukaemia and topoisomerase II inhibitor-related leukaemia suggests that transplacental fetal exposure to topoisomerase II inhibitors might induce leukaemogenesis in infants.

Chemicals such as flavonoids (in food and drink), quinolone antibiotics, benzene metabolites, catechins, and estrogens can inhibit topoisomerase II in vivo and in vitro; thus, these chemicals may be potential mutagens.[20] Because dietary and environmental exposures provide much lower functional doses of topoisomerase II inhibitors than anticancer chemotherapy, infants with leukaemia or their mothers may have low activities of enzymes that detoxify carcinogens. Quinones induce topoisomerase II-mediated DNA cleavage,[21] and low activity of NAD(P)H: quinone oxidoreductase, an enzyme that converts benzoquinones to less toxic hydroxy metabolites, has been associated with infant leukaemia with *MLL-AF4* fusions.[22]

Genetic polymorphisms of other enzymes capable of detoxifying carcinogens may also affect the risk of de novo leukaemia. For example, the deficiency of glutathione S-transferases (*GST-M1* and *GST-T1*), which detoxify electrophilic metabolites by catalyzing their conjugation to glutathione, has been associated with infant leukaemias without *MLL* rearrangements[18] and with ALL in black children.[23] Another recent study related *GST-M1*-null and *cytochrome P-450 1A1*2A* genotypes to an increased risk for childhood ALL; children carrying both genotypes are at a particularly high risk.[24]

Prenatal Origin of Genetic Abnormalities Associated with Childhood Leukaemia

Genetic studies of identical twins with concordant leukaemia[25-27] and studies that backtracked leukaemia-specific translocated fusion-gene sequences (e.g., *MLL-AF4*, *TEL-AML1*, *AML1-ETO*) to neonatal blood Guthrie spots[28-30] established the prenatal origin of leukaemias in many cases. Indeed, detection of clonotypic immunoglobulin heavy-chain gene or T-cell receptor gene rearrangements at birth[31,32] suggests that most, if not all, cases of childhood ALL are fetal in origin. However, these genetic aberrations may have a different effect on the onset of leukaemia. In the t(4;11) with *MLL-AF4*, the high concordant rate in identical twins (25% to 100%), and the brief latency period (weeks to months) suggests that this fusion is sufficient to induce leukaemogenesis.[33] In contrast, childhood t(12;21) ALL with *TEL-AML* fusion, which is present in 20% to 25% of 1- to 10-year-old diagnosed with childhood ALL, and childhood T-cell ALL have a lower concordance rate in identical twins (~5%), a longer and variable postnatal latency period, diverse clinical presentations, and variable outcome of therapy among identical twins. This finding suggests that in these cases, an additional postnatal molecular event(s) is necessary for full leukaemic transformation.[25,27,33] In fact, additional genetic abnormalities were frequently found in patients who have ALL with the *TEL-AML* chimeric transcript.[34]

In a recent report of triplets, concordant leukaemia with identical *TEL-AML1* fusions was diagnosed in the monozygotic twins at 3 years of age; the remaining dizygotic co-twin was free of leukaemia and the genomic sequence.[35] In addition to the fusion transcript, the identical twins had another independent deletion of the normal *TEL* allele, a finding that suggests that a postnatal event also occurred. Interestingly, the *TEL-AML1* fusion is detected in 1% of normal neonatal blood, a rate that is 100 times that of the expected incidence and further supports the theory that a secondary transforming event(s) is necessary to induce leukaemia.

Prognostic Assessment and Disease Monitoring

Although different prognostic factors have been proposed by various study groups, there is no argument that treatment regimen is the single most important prognostic factor known currently. Many clinical and biologic variables lost predictive strength in contemporary treatment programs. Age at the time of diagnosis of ALL and leukocyte count have consistent prognostic significance in B-lineage but not T-lineage ALL.[9,10,12] Yet, these criteria are inadequate even for B-lineage leukaemia, because as many as one third of patients with standard risk (aged 1 to 9 years with a leukocyte count $<50\times10^9$/L), may relapse.

Ethnic Differences

In the U.S. collaborative group studies, African American and Hispanic children had a significantly worse outcome than did Caucasian children, even after adjusting for other prognostic features.[36,37] However, the risk factor associated with race may be abolished by a more effective treatment approach.[38] The treatment outcome of Chinese children treated for ALL in Hong Kong also appears to be dependent on the treatment protocols adopted. Replacing the UKALL regimens, which were commonly used in the 1980s, with the Berlin-Frankfurt-Münster protocols in the 1990s significantly improved the overall result (Li CK, et al. HKPHOSG Annual Scientific Workshop Report 2002).

Primary Genetic Abnormalities of Leukaemic Cells

Assigning risk on the basis of the primary genetic abnormalities of leukaemic cells is insufficient because of the clinical heterogeneity within the various genetic subgroups. For example, as many as 20% of children with favourable genetic features (i.e., *TEL-AML1* fusion and hyperdiploidy [>50 chromosomes]) eventually relapse, and approximately one third of those with high-risk abnormalities (i.e., the Philadelphia Chromosome with *BCR-ABL* fusion and the t(4;11) with *MLL-AF4* fusion) are cured with chemotherapy alone.[39]

Drug Susceptibility and Drug Interaction

Pharmacodynamic and pharmacogenetic characteristics are important determinants of treatment outcome.[40] There is a wide variability in the rate of systemic clearance of antileukaemic agents and in the absorption of orally administered chemotherapy. Low systemic exposure to methotrexate and low dose intensity of 6-mercaptopurine have each been associated with inferior treatment outcome.[41-43] These findings indicate that treatment is unsuccessful in some patients, because they received inadequate doses of drugs and not because their leukaemia was drug-resistant.

Concomitant administration of cytochrome P450 enzyme-inducing anticonvulsants (phenytoin, phenobarbital, and carbamazepine) can significantly increase the rate of systemic clearance of several

antileukaemic agents and decrease the efficacy of chemotherapy.[44] The probability of event-free survival is higher in patients who have a homozygous or heterozygous deficiency in thiopurine methyltransferase (TPMT), the enzyme that catalyzes the S-methylation (inactivation) of mercaptopurine, than it is in those who have normal TPMT activity; this difference may be caused by higher exposure to active metabolites of 6-mercaptopurine (i.e., thioguanine nucleotides).[43] The *GSTM1*-null, *GSTT1*-null, and the *GSTP1* Val_{105}/Val_{105} genotypes have also been associated with a lower risk of relapse, perhaps because patients with these genotypes experience reduced detoxification of cytotoxic chemotherapy.[45]

Treatment Responses and Monitoring of Minimal Residual Disease

As mentioned above, the most important prognostic indicator is the response to treatment, because it reflects the intrinsic drug sensitivity or resistance of leukaemic cells. Sensitivity and resistance are determined by expression of ATP-binding cassette transporters, various aberrant intracellular processes that prevent apoptosis,[46-50] and the pharmacodynamic and pharmacogenetic properties of the patient.[40] Since the early 1980s, the extent of clearance of leukaemic cells from the blood or bone marrow during the early phase of therapy has been an independent prognostic factor that is recognized by investigators of the Children's Cancer Group and the Berlin-Frankfurt-Münster consortium.[51] These investigators assessed the response to treatment by morphologic examination of the bone marrow or peripheral blood. Morphologically identifiable, persistent disease (as little as 1-4% blast cells) on day 15 of remission-induction therapy was associated with a poor prognosis, and that detected on days 22 to 25 was associated with a particularly dismal outcome. The prognostic effect of persistent morphologic disease is independent of other known risk factors, including treatment, age at the time of diagnosis, white blood cell count, DNA index, cell lineage, central nervous system status, and National Cancer Institute-Rome criteria, which is based on age and white blood cell count. Although morphologic methods can be readily applied at any center, these methods are subjective and lack precision. Approximately 20% of patients with a good response will eventually experience a relapse of disease, and a third of the patients with a poor response may become long-term survivors when treated with intensive chemotherapy alone.[52]

Several methods are currently available to measure MRD during or after initial remission-induction chemotherapy. By determining aberrant surface antigens expression (immunophenotypes) using 2 or 3 fluorescent label antibodies simultaneously, one can distinguish blast cells from normal cells by flow cytometer at cellular level.[53,54] A more sensitive but tedious method is to use semi-quantitative polymerase chain reaction (PCR) analysis of leukaemic specific clonal antigen-receptor gene rearrangements.[55-58] When applied together, these methods enable us to monitor MRD in virtually all cases of ALL. Patients who experience remission of their disease, as determined by immunologic or molecular measures (i.e., leukaemic involvement of <0.01% of nucleated bone marrow cells at the end of remission-induction therapy), are predicted to have a better clinical outcome than patients whose remission is defined solely by morphologic criteria. In studies to date, patients with MRD at a level of 1% or more at the end of remission-induction therapy have fared almost as poorly as those who experience induction failure (i.e., those with 5% or more blast cells in the bone marrow).

Sequential monitoring of MRD can further improve the clinical usefulness of risk assessment. Approximately 50% of children have fewer than 0.01% blast cells after only 2 weeks of remission-induction therapy, and these patients have a particularly favorable clinical outcome (Figure 1). The finding of comparable levels of leukaemic blast cells in bone marrow and blood of patients with T-cell ALL suggests that blood samples can be used for clinical monitoring in patients with this subtype of leukaemia.

One prerequisite for the clinical application of MRD studies is the ability to study all patients. Recent advances in real-time quantitative PCR (RT-PCR) have facilitated MRD studies and allowed successful study in up to 90% of cases.[59] In addition, comparative analyses of gene expression in normal B-cell progenitors and B-lineage leukaemic cells have identified new leukaemia-associated markers (e.g., CD58), thereby also boosting the number of cases that can be studied by flow cytometry to 90%.[60] Tandem application of flow cytometry and PCR testing resulted in a successful study in 100% of cases of ALL at St. Jude Children's Research Hospital (St. Jude).[61] This measure has, therefore, been incorporated in the risk classification schema at St. Jude (Table 3).

Evolution of Childhood ALL Treatment

The improved cure rate of ALL can be attributed mainly to the development of more effective combination

Table 3 Risk assessment used in the St. Jude total XV study (for treatment stratification)

Risk group	Estimated proportion of patients (%)	Criteria
Standard	40	B-lineage immunophenotype with a DNA index >1.16, *TEL-AML1* fusion, aged 1 to 9.9 years at the time of diagnosis with presenting WBC count $<50\times10^9$/L Must not have CNS leukaemia (CNS-3 status), overt testicular leukaemia, t(9;22) or *BCR-ABL* fusion, t(1;19) or E2A-PBX1 fusion, rearranged *MLL*, hypodiploidy (<45 chromosomes), or poor early response (>5% lymphoblasts on day 15 of remission induction or >0.01% on Day 42)
High	50	Cases not meeting the criteria for a standard or very high-risk classification (including most cases of T-cell ALL)
Very high	10	t(9;22) or *BCR-ABL* fusion, >1% leukaemic blast cells on Day 42 of remission induction, or >0.1% leukaemic blast cells 4 months after remission induction

chemotherapy regimens. Virtually all of the chemotherapeutic agents currently used to treat ALL were discovered between the early 1950s and the late 1970s. Hence, the recent improvement in treatment must be attributed to the optimal and more rational use of the existing agents and not to the discovery of new agents. In both children and adults with mature B-cell ALL (FAB-L3), a short-term (2 to 8 months), intensive chemotherapy regimen primarily based on cyclophosphamide, methotrexate, cytarabine, and intrathecal therapy resulted in a cure rate of 75-85%.[62,63] The recent development of rasburicase, a recombinant urate oxidase that is a highly effective uricolytic agent, may further improve this cure rate by reducing early morbidity and mortality caused by tumour lysis syndrome and acute renal failure.[64]

Most study groups treat infants as a unique subgroup; infants are generally treated with multiple drugs at high doses, and no cranial irradiation is administered. The prognosis for infants with ALL, especially those with 11q23/*MLL* rearrangements, remains poor. Despite current treatment regimens, the probability of event-free survival in these patients is 20-35%.[18] In several recent clinical trials, however, high-dose cytarabine, high-dose metho-trexate, and intensive consolidation-reinduction therapy appeared to result in improved clinical outcome.[65,66] Intensive systemic and intrathecal treatments without cranial irradiation appeared to provide adequate CNS protection, even in infants with CNS leukaemia at the time of diagnosis.[67]

The basic approach to treating ALL in children and adults consists of a relatively brief remission-induction phase, followed by intensification (consolidation) therapy, and then prolonged continuation treatment. All patients require treatment for subclinical CNS leukaemia; this treatment should be initiated early in the form of intrathecal therapy.

Remission-Induction Therapy

The first goal of therapy is to induce complete remission and restore normal haematopoiesis. Induction regimens invariably include a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine, and at least one other agent (asparaginase or anthracycline). Because of the improvements in supportive care and chemotherapy, the rate of complete remission of ALL now ranges from 96% to 99%.[1-12] Intensification of remission-induction therapy may not provide additional benefit to patients with standard-risk leukaemia, provided that those patients receive post-induction intensification therapy.[3,4] Highly intensive remission-induction therapy may even lead to inferior overall outcome due to increased early morbidity and mortality. The use of asparaginase and dexamethasone (instead of prednisone) during remission induction has recently been challenged;[13] these agents have demonstrated marginal clinical benefit and higher therapy-related morbidity and mortality. However, more intensive remission induction is necessary for patients with high-risk or very-high risk ALL, especially those with Philadelphia chromosome-positive ALL or T-cell ALL with a poor early response.

Intensification (Consolidation) Therapy

After restoration of normal haematopoiesis, patients in remission should receive intensification (consolidation) therapy; however, a consensus on the standard regimens or the optimal duration of this treatment has not yet been

reached. Delayed intensification (or reinduction) therapy, which was pioneered by the investigators of the Berlin-Frankfurt-Münster consortium, is the most widely adopted regimen. This regimen, which is administered 3 months after the patient enters remission, is basically a repeat of the initial remission-induction therapy and is most beneficial for patients with standard-risk ALL.[2,3] Investigators in the Children's Cancer Group have shown that double-delayed intensification (i.e. that administered 6 months after remission) improved the outcome of patients with high-risk or very high-risk leukaemia and slow early treatment response.[3]

The use of different intensification regimens in various clinical trials has led to the identification of effective treatment components for certain subtypes of leukaemia. For example, the improved outcome in patients with T-cell ALL in the clinical trials of the Dana-Farber Cancer Institute Consortium[6] and Children's Cancer Group[3] has been credited to the intensive use of asparaginase, a finding that has been confirmed in a randomised study done by the Pediatric Oncology Group.[68] Very high doses of methotrexate (5 gm/m^2) appeared to improve outcome in patients with T-cell ALL.[2] An in vitro study has also shown that T-lineage blast cells accumulate methotrexate polyglutamates, the active metabolites of methotrexate, less avidly than do B-lineage blast cells; thus, a higher serum concentration of methotrexate is needed for an adequate response.[69] High-dose methotrexate also benefits patients with B-lineage ALL, but a lower dose (2.5 g/m^2) of the agent should be adequate for most cases.[70]

Continuation Treatment

Among the various types of postremission intensification regimens, continuation treatment is the most successful.[71] High-dose pulse therapy with prolonged rest periods necessitated by myelosuppression can result in an inferior outcome,[72] perhaps because rest periods provide time for slowly proliferating tumour endothelial cells to repair and recover.[73] Continuous or frequent administration of cytotoxic drugs improves outcome by abrogating this process, a finding that is consistent with the concept of metronomic dosing.

Children with ALL, except those with mature B-cell ALL, require prolonged continuation treatment for reasons that are poorly understood. The attempt to intensify early therapy and shorten the total duration of treatment to 1 year resulted in inferior overall event-free survival.[74] Interestingly, the abbreviated therapy appeared to be adequate for a subset of patients with very high-risk ALL who responded well to prednisolone treatment. Because those patients who can be cured with abbreviated therapy cannot be identified with certainty, the general rule is to provide therapy for 2 to 2.5 years. Some investigators prefer to arbitrarily extend treatment of boys to 3 years, because boys generally experience a poorer outcome.[75-77] However, the efficacy of this approach remains to be proven.

A combination of daily 6-mercaptopurine and weekly methotrexate administration constitutes the standard regimen of continuation treatment. Individualising the doses of these agents to the patient's limit of tolerance (as indicated by low neutrophil count) has been associated with an improved clinical outcome.[78] However, overzealous use of 6-mercaptopurine is counterproductive, because this agent induces neutropenia, which necessitates the frequent interruption of chemotherapy and reduces overall dose intensity.[79]

In rare cases (1 in 300), patients are homozygous deficient for TPMT and experience extreme sensitivity to 6-mercaptopurine. Recent study showed that the 10% of patients, who are heterozygous for this deficiency and thus have intermediate levels of enzyme activity, might also require a moderate reduction in the dose of 6-mercaptopurine to avert side effects.[79] The identification of the genetic basis of this autosomal codominant trait has made the molecular diagnosis of these cases possible.[80] Studies can now be performed in patients who have poor tolerance to the combination of methotrexate and 6-mercaptopurine to identify the drug responsible for the increased myelosuppression and to selectively reduce its dosage. While undergoing antimetabolite-based therapy, patients with defective TPMT are at risk of radiation-induced brain tumour[81] and epipodophyllotoxin- or alkylating agent-induced acute myeloid leukaemia.[82] Hence, identification of these cases has important therapeutic implications.

Clinical observations have revealed that most Chinese patients with ALL cannot tolerate standard-dose mercaptopurine, despite the fact that the incidence of heterozygous and homozygous TPMT-deficiencies in these patients is comparable to that seen in Caucasian patients.[83] This finding suggests that other factors (e.g., the difference in the amount of dietary folate intake) are involved. Nonetheless, multivitamins and folate supplements should not be given during antileukaemia therapy, because these substances can reduce the efficacy of chemotherapy.

Adding intermittent pulses of vincristine and a glucocorticoid to the antimetabolite continuation regimen improves results,[84] and this approach has been widely

adopted. Many clinical trials have substituted dexamethasone for prednisone during continuation therapy, because the clinical efficacy of dexamethasone is superior. However, dexamethasone also appears to increase the frequency of avascular necrosis of bone and bone morbidity (e.g. osteoporosis);[85,86] therefore, additional studies are needed to determine the optimal dosage and duration of dexamethasone therapy during this phase of treatment.

Treatment of Subclinical CNS Leukaemia

Patients with high-risk genetic features, large leukaemic-cell burden, T-cell leukaemia, or leukaemic cells in the cerebrospinal fluid (even iatrogenic introduction during a traumatic lumbar puncture) are at increased risk of CNS relapse and require more intensive CNS-directed therapy.[87] Although high-dose methotrexate is useful for preventing haematologic or testicular relapse, this treatment has only marginal, if any, effect on the control of CNS leukaemia. In contrast, dexamethasone improves CNS control.[3,5] Whether triple intrathecal therapy (methotrexate, hydrocortisone, and cytarabine) is more efficacious than intrathecal methotrexate alone remains unknown.

Cranial irradiation is, perhaps, the most effective CNS-directed therapy, but this treatment is associated with substantial side effects such as neurotoxicity and second cancers, especially brain tumours. Currently, intensive intrathecal and systemic chemotherapy has replaced cranial irradiation in 90% or more patients. This approach, in combination with cranial irradiation for selected very high-risk cases, has lowered the rate of CNS relapse to less than 5%.[4,88,89] The dose of radiation can be lowered to 12 Gy without increasing the risk of CNS relapse, if effective systemic intensive chemotherapy is used.[90] In a recent retrospective study of T-cell ALL with either a high leukocyte count ($>50\times10^9$/L) or CNS leukaemia at the time of diagnosis, CNS irradiation reduced the rate of CNS relapse but failed to improve event-free survival. Hence, whether CNS irradiation can improve haematologic control remains controversial.[91]

Three studies that omitted cranial irradiation for all patients resulted in CNS relapse in approximately 3-4% of the patients; patients with $CD10^-$ or T-cell ALL and those with CNS leukaemia at the time of diagnosis had higher risk of CNS relapse. However, the overall rate of event-free survival in these studies was only 60-70%. More effective systemic therapy and intensification of intrathecal therapy for patients with high-risk or very high-risk leukaemia may further reduce the risk of CNS relapse. Ongoing trials at St. Jude are testing whether cranial irradiation can be omitted regardless of the patient's risk features and reserved only for retrieval therapy in those who experience CNS relapse.

Transplantation of Allogeneic Haematopoietic Stem Cells

Many advances have been made in transplantation; these include the prevention of graft-versus-host disease, expansion of the pool of suitable unrelated or related donors, acceleration of engraftment, enhancement of the graft-versus-leukaemia effort, and supportive care. Because of the high response rate to chemotherapy and the questionable efficacy of bone marrow transplantation (BMT) in patients with t(4;11), only Philadelphia chromosome-positive ALL and early haematologic relapse are clear indications for transplantation. Indications for transplantation should be subjected to constant review. Preliminary findings from the Berlin-Frankfurt-Münster consortium suggest that patients with T-cell ALL with a slow early response also benefit from this procedure (M. Schrappe, personal communication).

Late Effects of Treatment for ALL

Osteonecrosis

Most current protocols avoid the use of regimens that can induce second cancer and emphasise the use of glucocorticoids, antimetabolites, and asparaginase as the main agents of treatment. However, the increasing use of glucocorticoids during reinduction and continuation therapy has been associated with a marked increase in the occurrence of osteonecrosis. This complication is more common in older children (≥10 years) and those of female sex and white race (as compared with black race).[86,92] The increased risk in girls may be related to early pubertal development, because maturing bones with epiphyseal closure and reduced intramedullary blood flow are more susceptible to osteonecrosis. The factor(s) that contributes to the racial difference in the incidence of osteonecrosis is unknown. The difference induced by equivalent doses of dexamethasone, prednisone, and prednisolone is also unknown. Recently, several study groups started to decrease the duration of dexamethasone therapy, because the preliminary results of the Children's Cancer Group study indicated that intermittent use of dexamethasone reduces the risk of osteonecrosis (J Nachman, personal communication). Prospective monitoring and early intervention could prevent this debilitating complication, and early osteonecrotic changes may be reversible with

proper conservative management. (Pui CH, unpublished observation).

Symptomatic avascular necrosis of the hip joints was not reported in the Hong Kong childhood ALL cohorts in either the HKALL-93 protocol, which was part of UKALL-XI and included prednisone administration, or the HKALL-97 protocol, which was part of BFM-95 and included dexamethasone treatment. The recent findings cannot rule out the possibility that early asymptomatic changes occurred in Chinese patients, because this phenomenon has been described in some studies that used magnetic resonance imaging to screen for the complication.

Decrease Bone Mineral Density

Another late effect found in the bone is decreased bone mineral density, which has been attributed to cranial irradiation and intensive systemic chemotherapy, especially regimens that include high-dose antimetabolites or glucocorticoids.[93,94] Male sex and white race are significant predictors of low bone mineral density.[93] However, the incidence of low bone mineral density in Chinese children is not available. Studies are ongoing to determine whether genetic polymorphisms of the vitamin D receptor influence the severity of low bone mineral density. Although treatment of decreased bone mineral density should reduce the risk of osteoporosis and fractures later in life, intervention studies are needed to determine the optimal therapy (e.g., nutritional counseling, exercise, vitamin D, phosphate or calcium supplementation, and bisphosphonates) to prevent this late effect.

Thrombotic Effects

Thrombotic complications occur in approximately 2.4-11.5% of the patients who receive a glucocorticoid, vincristine, and asparaginase as remission induction or reinduction therapy.[95,96] Cerebral venous thrombosis accounts for half of the thrombotic complications. This high frequency can be attributed to the combined use of asparaginase and a glucocorticoid, heightened awareness of the possibility of this complication, frequent placement of central lines, and improved diagnostic imaging methods.

German investigators found that 27 of their 32 patients with thrombotic complications had one or more hereditary prothrombotic defects.[96] In fact, half of the patients with a prothrombotic defect experienced thrombosis. This finding may pave the way for effective prophylaxis; for example, during glucocorticoid-vincristine-asparaginase treatment, low-molecular weight heparin could be given to patients with hereditary prothrombotic defects. However, preliminary result of a St Jude study did not appear to support the finding (Pui CH, unpublished observation).

The incidence of thrombosis appeared to be lower in Chinese children. Only two of the 145 children on the HKALL-93 (modified UKALL-XI) protocol experienced cerebral venous thrombosis, and neither patient had the hereditary prothrombotic defect described in the literature. There may be genetic differences in the incidence of these defects among ethnic groups; the prothrombotic factor V Leidens mutation, which occurs in factor V Leiden allele, is present in about 5% of the Caucasian individuals (Europeans, Jews, Israeli Arabs, and Indians) and is virtually absent in Africans, Asians including Chinese.[97]

Cognitive Deficits

CNS-directed therapy, even that which does not include cranial irradiation, has been associated with adverse cognitive and academic late effects, particularly in girls.[98] In one study, visual and verbal short-term memory deficits were observed in children who had received approximately 20 triple intrathecal treatments (methotrexate, hydrocortisone, and cytarabine) as the sole CNS-directed therapy;[99] methotrexate was not administered intravenously. Clearly, neuropsychological function should be assessed in survivors of childhood ALL. Identification of specific therapy-induced cognitive impairments will facilitate the development of appropriate remediation programs. Currently in Hong Kong, a cross sectional functional (by IQ testing and 128 leads EEG) and structural (by MRI and DTI) assessment of the cognitive function among a cohort of childhood leukaemia and brain tumour survivors have been undergoing. By comparing different cohorts of patients who underwent high or low dose cranial irradiation, intrathecal therapy, systemic chemotherapy and a control group, we hope to find out the impact and risk factors of different treatment modalities on the cognitive function of children.

Second Malignancies

In a retrospective review of 9720 children who received the Children's Cancer Study Group ALL protocols from 1970 to 1988, 43 second cancers were identified. Among these, brain tumours (mainly in the forms of high grade glioma and meningioma) accounted for the majority (56%, 24/43). Acute myeloid leukaemia was the next commonest form of cancer (23%, 10/43).[100] Others included a variety of solid tumours such as thyroid or parotid carcinoma. The age adjusted relative risk of cancer for childhood ALL survivors were 7-fold higher than the normal childhood

population. Brain tumours were mainly found in young patients (<5 years) who received cranial irradiation[100] and also those with genetic defect in thiopurine catabolism (i.e. deficiency of thiopurine methyltransferase).[101] It can occur many years later and the prognosis was poor except for those with resectable meningioma. The risk of secondary AML appears to be closely associated with the use of epipodophyllotoxin such as VP16 as described in the previous section.[19] It has a relatively brief latency period and associated with 11q23 rearrangement in contrary to the myelodysplastic leukaemia induced by alkylating agents (i.e. cyclophosphamide). The judicious use of cranial irradiation and chemotherapy in current protocols may help to reduce this alarming complication.

Future Directions

The study of genetic polymorphisms in drug-metabolising enzymes, drug transporters, and targets of drug action has attracted intense interest over the past few years. This information can be used to individualise drug dosages (especially those with a low therapeutic index) and drug combinations (to enhance antileukaemic effects and reduce late sequelae). Efforts are also being made to identify new antileukaemic drugs and approaches to therapy.

Identification of specific oncoproteins and the elucidation of the molecular processes that regulate leukaemic-cell survival and apoptosis could also improve treatment.[102] One of the classic examples is imatinib mesylate (Gleevec), which selectively inhibits BCR-ABL tyrosine kinase. Inhibition of this kinase leads to growth inhibition and apoptosis of leukaemic cells that contain the BCR-ABL fusion product. While this agent induced an overall response rate of 82% and a complete response rate of 55% in patients with BCR-ABL$^+$ ALL or chronic myeloid leukaemia in lymphoid blast cell crisis,[103] these responses had been transient. Additional studies are needed to determine if this agent can improve outcome in patients with newly diagnosed BCR-ABL$^+$ ALL.

Recently, molecular manipulation of interleukin-4 was shown to abrogate its proinflammatory activity. This finding has provided a novel and therapeutically promising cytokine that induces apoptosis of leukaemic cells in vitro but does not affect the growth of normal haematopoietic cells.[104] Whether this cytokine is clinically useful will require additional studies.

As new information continues to emerge from the Human Genome Project, DNA microarray studies, advanced bioinformatics analyses, high-throughput DNA-screening systems, and proteomics, one can expect accelerated advances in research. DNA microarray technology already has considerable value in molecular diagnosis and identification of new leukaemia-associated markers for disease monitoring.[105] Ultimately, such advances will be used as guides for optimising and individualising therapy.

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