Objective. To study the outcome of children with acute lymphoblastic leukaemia who were treated using a protocol including one or two delayed intensifications.

Design. Prospective single-arm multicentre study.

Setting. Five designated children cancer units of the Hospital Authority of Hong Kong.

Patients. Children aged between 1 and 17.9 years with newly diagnosed acute lymphoblastic leukaemia seen from November 1997 to December 2002.

Intervention. Chemotherapy was modified from a German Berlin-Frankfurt-Muenster 95 (BFM95) protocol that included a delayed intensification similar to the induction phase repeated 5 months after diagnosis. High-risk patients were given double delayed intensification.

Main outcome measures. Overall survival and event-free survival of the whole group and the three risk groups (standard-, intermediate-, and high-risk groups), and comparison with historical controls.

Results. A total of 171 patients were recruited with a median age at diagnosis of 5.57 years (range, 1.15-17.85 years). The induction remission rate was 95.3% and non-leukaemia mortality during remission was 2.3%. At 4 years, the relapse rate of this (HKALL97) study was significantly lower than that of the HKALL93 study (15.7 vs 37.3%; P<0.001). The 4-year overall survival of HKALL97 and HKALL93 studies were 86.5% and 81.8%, respectively (P=0.51). The 4-year event-free survival for HKALL 97 and HKALL93 studies were 79% and 65%, respectively (P=0.007). Nonetheless the difference of event-free survival was most remarkable in the intermediate-risk group: 75.6% and 53.1% for HKALL97 and HKALL93 studies, respectively (P=0.06).

Conclusion. A more intensive delayed consolidation phase improved the outcome for children with acute lymphoblastic leukaemia by reducing relapses at 4 years. The early treatment complications were manageable and non-leukaemia mortality during remission remained low.
Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy. It is highly responsive to chemotherapy and induction remission rates in most clinical studies are over 95%. The major cause of treatment failure is relapse, mainly in the bone marrow and less commonly in extramedullary sites such as the central nervous system (CNS) or testes. With good supportive care, treatment-related mortality can be kept below 5%. The challenge of improving treatment outcome is to maintain continuous remission and prevent relapse. Relapses may occur in the first 2 to 3 years during chemotherapy, but are more common 1 to 2 years after stopping chemotherapy. The current trend of treatment is to increase treatment intensity in the first 6 to 9 months and continue with a milder maintenance phase for up to 2 or 3 years after diagnosis. The mode of intensification in the first 9 months of treatment nonetheless varies among collaborative groups. The HKALL93 study recruited patients from 1993 to 1997 and adopted the United Kingdom ALL XI study approach (UKALL XI). There was a high relapse rate in intermediate- and high-risk patients. The Hong Kong Paediatric Haematology and Oncology Study Group commenced a new clinical study in 1997 that adopted a German Berlin-Frankfurt-Muenster 95 (BFM95) protocol aimed at improving treatment outcome. The main difference between the German and UK protocols is the inclusion of a delayed intensification similar to induction, called re-induction, 5 months after diagnosis. This study aimed to determine the outcome of children with ALL who received a treatment protocol that included either one or two delayed intensifications.

**Patients and methods**

From November 1997 to December 2002, all children newly diagnosed with ALL in the five paediatric oncology centres under the Hospital Authority (HA) hospitals were included in the study. Patients younger than 1 year were treated under a separate infant ALL study that formed part of an international multicentre study. The age of inclusion was thus 1 year to 17.9 years. All the paediatric ALL patients in Hong Kong were treated in HA hospitals, this was therefore a population-based study. The diagnostic criteria were standardised, and morphology and immunophenotyping were centrally reviewed. Bone marrow smear was examined after Wright’s stain and standard cytochemical staining. Acute lymphoblastic leukaemia was diagnosed in the presence of more than 30% blasts in the bone marrow smear. The French-American-British morphological classification was not the essential criteria for diagnosis. Flow cytometry of marrow aspiration was performed using a batch of monoclonal antibodies with 20% as the positivity cut-off: CD10, 20, 19, 22 for B lineage, CD3, 5, 7 for T lineage, cytoplasmic and surface immunoglobulin for pre-B and mature B cell marker, respectively. The subtype was based on the scoring system from the EGIL (European Group for the Immunological Characterization of Acute Leukaemias). All bone marrow samples were also tested for cytogenetics by karyotyping, and molecular study for fusion products of BCR/ABL, TEL/AML1 and MLL/AF4 translocations were also performed.

The chemotherapy protocol was modified from the German BFM95 ALL study. The same stratification cri-

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**Table 1. Risk stratification criteria of HKALL97 and HKALL93 studies**

<table>
<thead>
<tr>
<th></th>
<th>HKALL97 (present) study</th>
<th>HKALL93 study³</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard risk</strong></td>
<td>Age: 1-5 years</td>
<td>Age: 1-9 years</td>
</tr>
<tr>
<td></td>
<td>+ WBC ≤ 20 x 10⁹ /L</td>
<td>+ WBC ≤ 20 x 10⁹ /L</td>
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<tr>
<td></td>
<td>+ Non-T cell</td>
<td>+ Non-T cell</td>
</tr>
<tr>
<td></td>
<td>+ Absence of t(9;22) or t(4;11)</td>
<td>+ Absence of t(9;22) or t(4;11)</td>
</tr>
<tr>
<td></td>
<td>+ Prednisolone good response⁡</td>
<td></td>
</tr>
<tr>
<td><strong>Intermediate risk</strong></td>
<td>Age: ≥ 6 years or WBC ≥ 20 x 10⁹ /L</td>
<td>Age: ≥10 years or WBC 20-49 x 10⁹ /L</td>
</tr>
<tr>
<td></td>
<td>+ Absence of t(9;22) or t(4;11)</td>
<td>+ Non-T cell</td>
</tr>
<tr>
<td></td>
<td>+ Prednisolone good response⁡</td>
<td>+ Absence of t(9;22) or t(4;11)</td>
</tr>
<tr>
<td><strong>High risk</strong></td>
<td>Prednisolone poor response or day 33 non-remission</td>
<td>WBC ≥50 x 10⁹ /L or T-cell</td>
</tr>
<tr>
<td></td>
<td>or presence of t(9;22) or t(4;11)</td>
<td>or day 29 non-remission or presence of t(9;22) or t(4;11)</td>
</tr>
</tbody>
</table>

⁡ WBC denotes white blood cell count
⁡ Blast <1.0 x 10⁹ /L in peripheral blood after 7 days of prednisolone pre-phase treatment
Childhood acute lymphoblastic leukaemia

Table 2. Chemotherapy protocol of HKALL97 and HKALL93 \(^5\) studies*

<table>
<thead>
<tr>
<th>Stage</th>
<th>HKALL97 (present) study</th>
<th>HKALL93 study(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Induction</strong></td>
<td>Week 1-5</td>
<td>Week 1-4</td>
</tr>
<tr>
<td>• Premdisone 60 mg/m(^2) D1-29</td>
<td>• Prednisolone 40 mg/m(^2) D1-29</td>
<td></td>
</tr>
<tr>
<td>• Vincristine 1.5 mg/m(^2) D8, 15, 22, 29</td>
<td>• Vincristine 1.5 mg/m(^2) D1, 8, 15, 22</td>
<td></td>
</tr>
<tr>
<td>• L-Asparaginase 5000 IU/m(^2) IV from D11 Q3D x 8 doses</td>
<td>• L-Asparaginase 6000 U/m(^2) SC from D4 Q3D x 9 doses</td>
<td></td>
</tr>
<tr>
<td>• Daunorubicin 30 mg/m(^2) IV weekly D8, 15, 22, 29 (\text{standard risk: only D8, D15})</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Early intensification</strong></td>
<td>Week 5-9</td>
<td>Week 6</td>
</tr>
<tr>
<td>• Cyclophosphamide 1 g/m(^2) D36, 64</td>
<td>• Prednisolone 40 mg/m(^2) D1-5</td>
<td></td>
</tr>
<tr>
<td>• Ara-C 75 mg/m(^2) IV daily on D38-41, 45-48, 52-55, 59-62</td>
<td>• Etoposide 100 mg/m(^2) IV D1-5</td>
<td></td>
</tr>
<tr>
<td>• 6-Mercaptopurine 60 mg/m(^2) po D36-62</td>
<td>• Daunorubicin 45 mg/m(^2) IV D1, 2</td>
<td></td>
</tr>
<tr>
<td><strong>Consolidation</strong></td>
<td>Week 12-20</td>
<td>Week 8-18</td>
</tr>
<tr>
<td>Standard risk</td>
<td>Standard risk</td>
<td>Standard risk</td>
</tr>
<tr>
<td>• Methotrexate 2 g/m(^2) IV Q2W x 4</td>
<td>• Methotrexate 8 g/m(^2) or 6 g/m(^2) IV Q2W x 3</td>
<td></td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>Intermediate risk</td>
<td></td>
</tr>
<tr>
<td>• Methotrexate 5 g/m(^2) IV Q2W x 4</td>
<td>• Methotrexate intrathecal methotrexate</td>
<td></td>
</tr>
<tr>
<td><strong>High risk</strong></td>
<td>High risk</td>
<td></td>
</tr>
<tr>
<td>• Three 6-day intensive blocks including CNS RT 1200 cGy</td>
<td>• 6-Mercaptopurine + CNS RT 1800 cGy</td>
<td></td>
</tr>
<tr>
<td><strong>Late intensification</strong></td>
<td>Week 22-29 (re-induction)</td>
<td>Week 20</td>
</tr>
<tr>
<td>Standard and intermediate risks</td>
<td>Standard risk</td>
<td></td>
</tr>
<tr>
<td>• Dexamethasone 10 mg/m(^2) po D1-21</td>
<td>• As early intensification</td>
<td></td>
</tr>
<tr>
<td>• Vincristine 1.5 mg/m(^2) IV D8, 15, 22, 29</td>
<td>• L-Asparaginase 10 000 units/m(^2) IV D11, 2 times/week x 4 doses</td>
<td></td>
</tr>
<tr>
<td>• L-Asparaginase 10 000 units/m(^2) IV D1, 2</td>
<td>• Cyclophosphamide 1 g/m(^2) D36</td>
<td></td>
</tr>
<tr>
<td>• Ara-C 75 mg/m(^2) IV daily on D38-41, 45-48</td>
<td>• Ara-C 75 mg/m(^2) IV D1-4 in week 39-42</td>
<td></td>
</tr>
<tr>
<td>• 6-Thioguanine 40 mg/m(^2) po D36-49</td>
<td>• Thioguanine 60 mg/m(^2) po week 39-42</td>
<td></td>
</tr>
<tr>
<td><strong>High risk</strong></td>
<td></td>
<td></td>
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<tr>
<td>• Repeat late intensification at week 37-44</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Maintenance</strong></td>
<td></td>
<td>High risk: week 35-42</td>
</tr>
<tr>
<td>• 6-Mercaptopurine 50 mg/m(^2) po daily</td>
<td>• Dexamethasone 10 mg/m(^2) po D1-10</td>
<td></td>
</tr>
<tr>
<td>• Methotrexate 20 mg/m(^2) po weekly</td>
<td>• Vincristine 1.5 mg/m(^2) IV D1, 8, 15, 22</td>
<td></td>
</tr>
<tr>
<td>• Dexamethasone 6 mg/m(^2) po D1-7 and vincristine 1.5 mg/m(^2) IV D1, 8 every 10 week</td>
<td>• L-Asparaginase 6000 unit/m(^2) IV D1 of week 39 and 41</td>
<td></td>
</tr>
<tr>
<td>• Treatment of up to 2 years from diagnosis</td>
<td>• Ara-C 75 mg/m(^2) IV D1-4 in week 39-42</td>
<td></td>
</tr>
<tr>
<td>• Methotrexate 20 mg/m(^2) po weekly</td>
<td>• Thioguanine 60 mg/m(^2) po week 39-42</td>
<td></td>
</tr>
<tr>
<td>• Prednisolone 40 mg/m(^2) po D1-5 and vincristine 1.5 mg/m(^2) IV D1 every 4 week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Treatment of up to 2 years from diagnosis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* D denotes day, IV intravenous, Q3D every 3 days, SC subcutaneous, Ara-C cytarabine arabinoside, po orally, Q12H every 12 hours, Q2W every 2 weeks, CNS RT radiation therapy of the central nervous system
Statistical analysis
The overall survival and event-free survival (EFS) were estimated by Kaplan Meier Curve, and the differences in the risk groups were tested by log-rank test. The HKALL93 and HKALL 97 (present) studies were compared: categorical variables by Chi squared test and continuous variables by Student’s t test. The survival outcome of the two groups was compared by log-rank test.

Results
During the 62-month period, 171 patients were recruited, 56.7% of whom were male. The median age at diagnosis was 5.57 years (range, 1.15-17.85 years). There were three (1.8%) patients with Down syndrome. The initial median white blood cell count (WBC) at diagnosis was 12.6 x 10^9/L (range, 0.9-999 x 10^9/L). Common ALL was the most common (56.1%) immunophenotype, and the T cell type comprised 14.0%. Karyotyping was successful in 141 (82.5%) patients. Among those with successful karyotyping, chromosome number of higher than 50 (hyperdiploidy) occurred in 21.3%; both Philadelphia chromosome and t(1;19) were present in 3.5% of the patients. The risk stratification according to HKALL97 criteria was standard risk, 33%; intermediate risk, 55%; and high risk, 11%. Patient characteristics are shown in Table 3, with the similar characteristics of HKALL93 patients included for comparison.

Response to treatment
On day 33 of induction treatment, 163 (95.3%) of 171 patients achieved remission, three patients achieved remission after further treatment. One of 171 patients died during induction because of intracranial bleeding and four did not achieve remission.

At the end of 2004, with a median follow-up period of 48 months (range, 24-86 months from diagnosis), 26 (15.7%) of 166 patients had experienced a relapse, a significantly lower proportion than that (37.3%) in the HKALL93 study (P<0.001). Among the relapers, 13 came from the intermediate-risk group, eight from the standard-risk group, and five from the high-risk group. Eleven of 26 relapses occurred within 24 months of diagnosis, ie before completion of chemotherapy. Of those patients who relapsed after completion of chemotherapy, the latest occurred 53 months after diagnosis. The sites of relapse were bone marrow (n=18), CNS (n=3), combined bone marrow and CNS (n=2), combined bone marrow and testes (n=1), testes (n=1), and combined CNS and testes (n=1). Bone marrow was the main site of relapse in both studies and was the sole affected site in 60% of relapse cases. Relapse of CNS, alone or combined with another site, occurred in six (3.6%) of 166 patients in the HKALL97 study, and 12 (8.2%) of 146 patients in the HKALL93 study. Allogeneic haematopoietic stem cell transplantation was performed in 11 patients, four in first remission and seven in second remission. Five of them survived in remission. The five patients with Philadelphia chromosome had a poor outcome: one had refractory leukaemia and died, three relapsed and two died, and one is in first remission after transplantation.

Survival outcome according to HKALL97 stratification criteria
Four patients failed to achieve remission after further chemotherapy and died from infection or bleeding 1.6 to 5.7 months after diagnosis. Four patients died from a non-leukaemic cause after achieving remission. Three deaths occurred during the re-induction phase and one following...
Childhood acute lymphoblastic leukaemia bone marrow transplantation in the first remission. The causes of non-leukaemic death were gram-negative bacte-
reria septicaemia and systemic fungal infection. The other
deaths were due to relapsed or refractory leukaemia. At the last analysis at 2 years after completion of study, 23
patients had died and 148 patients were surviving. The re-
misson status of the survivors was first remission (n=137),
second remission (n=10), and third remission (n=1). The
4-year overall survival for the whole group was 86.5%
(standard deviation [SD], 2.7%). According to the risk
groups, the 4-year overall survival for standard risk, inter-
mediate risk, and high risk were 97.9%, 89.1%, and 58.2%,
respectively (Fig 1). The 4-year EFS for the whole group
was 79% (SD, 3.3%). According to risk groups, the EFS
was 88.5%, 77.9%, and 57.1% for standard risk, interme-
tate risk, and high risk, respectively (Fig 2).

Survival outcome according to HKALL93 stratification criteria and comparison of two studies
To enable more accurate comparisons to be made, HKALL97 patients were reclassified according to
HKALL93 criteria. The HKALL93 study adopted less
strict stratification criteria, thus more patients in the
HKALL97 study were classified as standard and high
risks. Comparison of HKALL93 criteria versus HKALL97
criteria for HKALL97 patients revealed there were
47% versus 33% in standard-risk group, 23.5% versus
55% in intermediate-risk group, 29.5% versus 11% in
high-risk group, respectively. There were 24% and 36%
in the HKALL97 intermediate-risk group reclassified as
standard-risk and high-risk respectively according to
HKALL93 criteria. All the HKALL97 standard-
risk patients were also of HKALL93 standard risk,
whereas 16% and 84% of HKALL97 high-risk patients
fell into HKALL93 intermediate-risk and high-risk
groups, respectively. There was no difference in the
overall survival (81.8% for HKALL93 vs 86.5% for
HKALL97, P=0.51) but there was significantly better
EFS for HKALL97 group (65% for HKALL93 vs 79% for
HKALL97, P=0.007) [Fig 3]. According to HKALL93
criteria, the overall survival and EFS for standard risk of
the two studies were not significantly different (P=0.66
and P=0.48). There was a trend of better EFS for HKALL97
study in the intermediate-risk group, 75.6% against 53.1%
for HKALL97 and HKALL93, respectively (P=0.06).
The EFS in high-risk patients of the HKALL97 study
appeared to be better although results did not reach
statistical significance (P=0.14), probably due to the small
sample size.

Discussion
This was a multicentre trial that included all five hospitals
that treat childhood cancer in Hong Kong. It can therefore
be considered a population-based study. All data and events
were recorded prospectively. Randomisation was not
possible because of insufficient patients, thus the findings
of this study were compared with those of a previous one.
The cytogenetic and molecular data were also incomplete.
Nonetheless patient characteristics of the two studies were
similar and both studies were population-based and
included all newly diagnosed ALL children in Hong Kong.
The duration of the two studies was similar—around
5 years—so was the number of patients recruited. Isolation
facilities have improved over the time of these two studies
and Hong Kong now has dedicated children cancer
centre/ward/cubicles in the five public hospitals. Supportive
care is also much improved, with more potent anti-microbials
and cytokines (eg granulocyte colony-stimulating factor) available.

The number of non-leukaemia deaths was low in both studies, 1.3% and 2.3% for HKALL93 and HKALL97, respectively. Thus the improved outcome currently seen cannot be explained by a decrease in such deaths, but is more likely due to the decreased relapse rate (15.7%) in the HKALL97 study against 37.3% in the HKALL93 study. This decrease was a result of the increased intensity of chemotherapy in the HKALL97 study. Number of deaths due to other causes was not affected.

Overall survival in the HKALL93 and HKALL97 studies was similar (86.5% vs 81.8%), although the EFS in the HKALL97 study was superior to that of the HKALL93 study (79% vs 65%). As in our previous report,6 we observed a satisfactory overall survival but were dissatisfied with the poor EFS. The relatively good overall survival was achieved through further chemotherapy for another 2 to 3 years or bone marrow transplantation for relapsed patients. In such patients, we anticipated more late complications such as growth retardation, endocrine complications, and avascular necrosis. Secondary brain tumour is the most disastrous late complication.4 In the new HKALL97 study, the relapse rate was very much reduced and a second course of treatment was avoided in a large proportion of patients. The reduced relapse rate in our study is due to a more effective chemotherapy protocol. No new chemotherapeutic agents were administered: the reduced relapse rate was mainly due to the inclusion of a re-induction phase at around 5 months after diagnosis. The induction treatment and high-dose methotrexate during consolidation are quite similar whereas the early intensification of the HKALL93 study was toxic with pancytopenia and febrile neutropenia as common occurrences.5

Re-induction, sometimes called delayed intensification, is now recognised as an important component of treatment for ALL in children. The German BFM studies in the 1980s and 1990s demonstrated improved outcome after introduction of a more intensive delayed intensification.9 The American Children’s Cancer Group also demonstrated such an improvement for both standard- and high-risk patients after inclusion of the BFM-delayed intensification.10,11 High-dose dexamethasone may confer better CNS and systemic anti-leukaemic effects than prednisone.12 Together with other cytotoxic drugs used in this phase, the minimal residual leukaemia cells may be further cleared up. In the current study, we observed better EFS for the whole group, especially those at intermediate risk. The main difference in the chemotherapy protocols of the two studies was the use of dexamethasone and delayed intensification. Nonetheless this intensive phase is very toxic and associated with a high infection rate. The three non-leukaemia deaths in this study all occurred during this delayed intensification phase, the other patient died following transplantation.

The present (HKALL97) study included the in-vivo response to steroid as the stratification criteria. Patients who exhibited a poor response to 7 days of steroid treatment were stratified as high risk. This is a simple laboratory test that can be performed in all laboratories. Its prognostic significance has been demonstrated in many studies: it is an independent predictor for response to treatment and survival in addition to age and immunophenotyping. White blood cell count is no longer a distinguishing feature between intermediate- and high-risk patients. Thus more patients with high WBC counts were then classified as intermediate risk. The high-risk group was confined to a small subset of ‘very’ high-risk patients—only 11%. The advantage of further defining the high-risk group is the reduced number of patients subjected to cranial irradiation. The chance of second malignancy and intellectual impairment is thus further decreased.13 Whether the reduced dose of cranial radiotherapy from 1800 cGy to 1200 cGy is less damaging remains uncertain. The CNS relapse rate remained very low, 1% in standard-risk and 2.2% in intermediate-risk patients, despite a more restricted cranial irradiation approach.

Despite some modification of this study from the original BFM95 study, our preliminary results are comparable. The BFM95 study had 6-year EFS of 79% for the whole group; and 89%, 79%, and 49% for the standard-, intermediate-, and high-risk groups, respectively (unpublished data). The reduction of methotrexate dosage in the current study for standard-risk patients appeared to be safe.

Conclusion

We observed significant improvement in the survival and EFS for ALL in Hong Kong children. Cranial irradiation was avoided in most patients with consequent prevention of some late complications and improved long-term quality of life. We are now participating in a multi-national randomised study aimed at further improving EFS in intermediate-risk and high-risk patients, and also defining better methods to improve their quality of life.

Acknowledgements

Thanks to Children’s Cancer Foundation for the support of data manager and molecular study. We are grateful to doctors and nurses taking part in the clinical care of the patients. Other members participating in the study: Dr A Chiang from Queen Mary Hospital, Ds CW Luk, PW Yau, and KO Chang from Queen Elizabeth Hospital, Dr CK Li from Princess Margaret Hospital, and Dr CH Li from Tuen Mun Hospital.

References

Childhood acute lymphoblastic leukaemia


