Biology and Treatment of Acute Lymphoblastic Leukemia

Rob Pieters, MD, MSc, PhD\textsuperscript{a,*},
William L. Carroll, MD\textsuperscript{b}

\textsuperscript{a}Department of Pediatric Oncology and Hematology, Erasmus MC-Sophia Children's Hospital, Dr Molewaterplein 60, 3015GJ Rotterdam, The Netherlands

\textsuperscript{b}Division of Pediatric Hematology/Oncology, New York University Medical Center, 160 East 32nd Street, 2nd Floor, New York, NY 10016, USA

Acute lymphoblastic leukemia (ALL), the most common type of cancer in children, is a heterogeneous disease in which many genetic lesions result in the development of multiple biologic subtypes. The etiology of ALL is characterized by the acquisition of multiple consecutive genetic alterations in the (pre)leukemic cells. In the most common genetic subtypes of ALL, the first hit occurs in utero [1], as evidenced, for example, by the presence of the \textit{TEL/AML1} gene fusion or hyperdiploidy in neonatal blood spots on Guthrie cards. These first genetic abnormalities are, in fact, initiating preleukemic cells, not leukemic ones, because most children whose neonatal blood spots show a genetic defect typically associated with leukemia never develop leukemia. Also, such preleukemic cells harbor additional genetic abnormalities. T-cell acute lymphoblastic leukemia (T-ALL) is an exception, because the majority of genetic lesions described in T-ALL seem not to occur in the neonatal blood spots [2].

Today, with intensive multiagent chemotherapy, most children who have ALL are cured. The factors that account for the dramatic improvement in survival during the past 40 years include the identification of effective drugs and combination chemotherapy through large, randomized clinical trials, the recognition of sanctuary sites and the integration of presymptomatic central nervous system (CNS) prophylaxis, intensification of treatment using existing drugs, and risk-based stratification of treatment. The many national or institutional ALL therapy protocols in use tend to stratify patients in a multitude of different ways. Treatment results often are not published for the

* Corresponding author.

\textit{E-mail address: rob.pieters@erasmusmc.nl} (R. Pieters).
overall patient group but rather are reported only for selected subsets of patients. This limitation hampers the comparison of outcomes in protocols. In 2000, the results of ALL trials run in the early 1990s by the major study groups were presented in a uniform way [3–12]. The 5-year event-free survival (EFS) rates seemed not to vary widely, ranging from 71% to 83% (Table 1). Overall remission rates usually were 98% or higher.

Risk-based stratification allows the tailoring of treatment according to the predicted risk of relapse. Children who have high-risk features receive aggressive treatment to prevent disease recurrence, and patients who have a good prognosis receive effective therapy but are not exposed to unnecessary treatment with associated short- and long-term side effects. Clinical factors that predict outcome and are used for stratification of patients into treatment arms are age, gender, and white blood cell count at presentation. Biologic factors with prognostic value are the immunophenotype and genotype of the leukemia cells. Another predictive factor is the rapidity of response to early therapy, such as the decrease in peripheral blood blast count in response to a week of prednisone or the decrease in bone marrow blasts after 1 to 3 weeks of multiagent chemotherapy. More recently the determination of minimal residual disease (MRD) in the bone marrow during the first months of therapy using flow cytometry or molecular techniques has been shown to have a high prognostic value and therefore is used for stratification in many contemporary trials. The detection of MRD accurately distinguishes very good responders to therapy from those who will

<table>
<thead>
<tr>
<th>Study group</th>
<th>Years of study</th>
<th>Patient number</th>
<th>Overall 5-year event-free survival (%)</th>
<th>B-lineage ALL 5-year event-free survival (%)</th>
<th>T-lineage ALL 5-year event-free survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFCI-91-01</td>
<td>1991–1995</td>
<td>377</td>
<td>83</td>
<td>84</td>
<td>79</td>
</tr>
<tr>
<td>BFM-90</td>
<td>1990–1995</td>
<td>2178</td>
<td>78</td>
<td>80</td>
<td>61</td>
</tr>
<tr>
<td>NOPHO-ALL92</td>
<td>1992–1998</td>
<td>1143</td>
<td>78</td>
<td>79</td>
<td>61</td>
</tr>
<tr>
<td>COALL-92</td>
<td>1992–1997</td>
<td>538</td>
<td>77</td>
<td>78</td>
<td>71</td>
</tr>
<tr>
<td>SJCRH-13A</td>
<td>1991–1994</td>
<td>167</td>
<td>77</td>
<td>80</td>
<td>61</td>
</tr>
<tr>
<td>CCG-1800</td>
<td>1989–1995</td>
<td>5121</td>
<td>75</td>
<td>75</td>
<td>73</td>
</tr>
<tr>
<td>DCOG-ALL8</td>
<td>1991–1996</td>
<td>467</td>
<td>73</td>
<td>73</td>
<td>71</td>
</tr>
<tr>
<td>EORTC-58881</td>
<td>1989–1998</td>
<td>2065</td>
<td>71</td>
<td>72</td>
<td>64</td>
</tr>
<tr>
<td>AIEOP-91</td>
<td>1991–1995</td>
<td>1194</td>
<td>71</td>
<td>75</td>
<td>40</td>
</tr>
<tr>
<td>UKALL-XI</td>
<td>1990–1997</td>
<td>2090</td>
<td>63</td>
<td>63</td>
<td>59</td>
</tr>
</tbody>
</table>

Abbreviations: AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; BMF, Berlin-Frankfurt-Münster; CCG, Children’s Cancer Group; COALL, Co-operative Study Group of Childhood Acute Lymphoblastic Leukemia; DCOG, Dutch Childhood Oncology Group; DFCI, Dana Farber Cancer Institute; EORTC-CLG European Organization for the Research and Treatment of Cancer; NOPHO, Nordic Society of Pediatric Haematology and Oncology; SJCHR, St. Jude Children’s Research Hospital; UKALL United Kingdom Acute Lymphoblastic Leukemia.
respond poorly to therapy, irrespective of the biologic subtype of ALL and
the underlying mechanism of this response [13]. In several protocols, MRD
is used to stratify patients for reduction of therapy (ie, patients who are
MRD negative especially at early time points) or intensification of therapy
(ie, patients who are MRD positive at later time points).

**Age and immunophenotype**

Over the years, age has remained an independent predictor of outcome
(Table 2). Children aged 1 to 9 years have the best outcome; children and
adolescents aged 10 to 20 years have a slightly worse outcome, which is
associated in part with a higher incidence of T-cell leukemia and a lower
incidence of favorable genetic abnormalities such as TEL/AML1 and hyper-
diploidy. For adults, survival rates decrease further with increasing age.
When results are corrected for differences in immunophenotype, ALL cells
from older children and adults are more resistant to multiple antileukemic
drugs than are cells from children in the first decade of life [14,15].

Infants diagnosed at less than 1 year of age have a relatively poor out-
come that is associated with a high incidence of the unfavorable very imma-
ture proB-ALL phenotype and especially the presence of MLL gene
rearrangements [16]. The poor outcome has led physicians in the United
States, Japan, and the International Interfant collaborative group including
European and non-European countries and institutes to develop specific
protocols to treat infant ALL [13,17,18]. Biologic characteristics of infant
ALL cells are described later in the paragraph discussing the MLL gene.

T-cell ALL is detected in approximately 15% of childhood ALL. It is
detected in approximately 15% of childhood ALL. It is
characterized by a relative resistance to different classes of drugs when com-
pared with B-lineage ALL [14]. T-cell ALL cells accumulate less methotrex-
ate polyglutamates and less cytarabine triphosphate than precursor B-ALL
cells [19]. With risk-adapted therapy the outcome of T-cell ALL now ap-
proaches that of B-lineage ALL in many study groups (see Table 1).

Approximately 85% of childhood ALL is of B lineage, mainly common
or preB ALL. A very immature subtype characterized by the lack of CD10

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Clinical and biologic factors predicting clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>Favorable</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>1–9 years</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>Low (eg, &lt; 50 or &lt; 25 × 10⁹/L)</td>
</tr>
<tr>
<td>Genotype</td>
<td>Hyperdiploidy (&gt; 50 chromosomes)</td>
</tr>
<tr>
<td>t(12;21) or TEL/AML1 fusion</td>
<td></td>
</tr>
<tr>
<td>Immunophenotype</td>
<td>Common, preB</td>
</tr>
</tbody>
</table>
expression (proB ALL) is associated with a high incidence of MLL gene rearrangements and an unfavorable outcome. Mature B-lineage ALL, defined by the presence of immunoglobulins on the cell surface, has a favorable outcome only when treated with B-non-Hodgkin lymphoma protocols.

**Genetics**

*Hyperdiploidy*

Hyperdiploidy (a DNA index > 1.16 or > 50 chromosomes per leukemia cell) is found in approximately 25% of children who have B-lineage ALL. It is associated with a favorable outcome, especially when extra copies of chromosome 4, 10 or 17 are present [20]. Hyperdiploid ALL cells have an increased tendency to undergo apoptosis, accumulate high amounts of methotrexate polyglutamates, and are highly sensitive to antimetabolites and L-asparaginase [21].

**TEL/AML1**

The TEL/AML1 fusion, also found in approximately 25% of cases, is mutually exclusive with hyperdiploidy and also is associated with a favorable outcome. It is formed by a fusion of the TEL gene on chromosome 12 encoding for a nuclear phosphoprotein of the ETS family of transcription factors and the AML1 gene on chromosome 21, a transcription factor gene encoding for part of the core-binding factor. The TEL/AML1 fusion probably inhibits the transcription activity of the normal AML1 gene involved in proliferation and differentiation of hematopoietic cells. TEL/AML1 fusion is associated with a high chemosensitivity, especially for L-asparaginase [22]. The mechanism behind this asparaginase sensitivity remains unclear but is not caused by a low asparagines synthetase activity in the leukemic cells [23,24]. TEL/AML1-rearranged cells also may be more sensitive to other drugs, especially anthracyclines and etoposide [25].

Both hyperdiploidy and TEL/AML1 occur mainly in children younger than 10 years of age with common/preB ALL and are rare above this age and in other ALL immunophenotypes.

**MLL**

Abnormalities of the mixed lineage leukemia (MLL) gene on chromosome 11q23 occur in only approximately 2% of children above the age of 1 year, although it is present in approximately 80% of infants who have ALL. All types of MLL gene rearrangements, such as MLL/AF4 created by t(4;11), MLL/ENL created by t(11;19), and MLL/AF9 created by t(9;11), are associated with a poor outcome in infants who have ALL [17]; in older children this poor outcome may only hold true for the presence of MLL/AF4 [26]. The MLL/AF9 rearrangement occurs in older infants
and is characterized by a more mature pattern of immunoglobulin gene rearrangements, suggesting another pathogenesis [17,27].

The precise actions of the fusion products involving MLL are not known, but they are associated with abnormal expression of HOX genes, which may lead to abnormal growth of hematopoietic stem cells [28]. ALL cells with MLL gene abnormalities are highly resistant to glucocorticoids in vitro and in vivo and also to L-asparaginase [14,17,29]. These cells, however, show a marked sensitivity to the nucleoside analogues cytarabine and cladribine [30]. This sensitivity is related to a high expression of the membrane nucleoside transporter ENT1 [31]. MLL-rearranged ALL cells do not show a defective methotrexate polyglutamation [32] and have no overexpression of multidrug resistance proteins [33]. Methotrexate pharmacokinetics might be different in the youngest infants [34].

**BCR-ABL**

The translocation t(9;22) fuses the BCR gene on chromosome 22 to the ABL gene on chromosome 9 causing an abnormal ABL tyrosine kinase activity associated with increased proliferation and decreased apoptosis. The BCR/ABL fusion is found mainly in common and preB ALL. The incidence of BCR/ABL increases with age: it is seen in approximately 3% of children who have ALL but in approximately 25% of adults who have ALL. The presence of BCR/ABL predicts a poor outcome. Children who have BCR/ABL-rearranged ALL or MLL-rearranged ALL more often show a poor response to prednisone [29,35] and have high levels of MRD after induction therapy.

**Genetics in T-cell acute lymphoblastic leukemia**

The prognostic value of genetic abnormalities in T-ALL is less clear [36]. Ectopic expression of TAL-1 is caused by the translocation t(1;14) in only a few percent of T-ALL cases or, more often, by the SIL-TAL fusion transcript. Activation of HOX11 by the translocations t(10;14) and t(7;10) occur in approximately 10% of T-ALL cases. Two recently described abnormalities occur frequently and exclusively in T-ALL. These are the ectopic expression of HOX11L2, mainly caused by the translocation t(5;14), in approximately 25% of T-ALL cases and activating mutations of the NOTCH1 gene in 50% of T-ALL cases. NOTCH1 mutations are not associated with a poor outcome and may be associated with a favorable outcome [37].

**Others**

Many other recurrent genetic and molecular genetic lesions exist in small subsets of childhood ALL such as the translocation t(1;19) leading to an E2A-PBX1 fusion detected in less than 5% of precursor B-ALL, mainly preB ALL. Although in the past this translocation had been associated with
a poor prognosis, this is not longer true with contemporary treatment protocols. Two percent of precursor B-lineage ALL cases harbor an intra-chromosomal amplification of chromosome 21 that is associated with poor survival [38]. Hypodiploidy (< 45 chromosomes) is detected in only 1% of children who have ALL and is associated with poor outcome, particularly in the low-hypodiploid (33–39 chromosomes) or near-haploid cases (23–29 chromosomes) as shown in a recent retrospective international study [39].

A discussion of all other abnormalities is beyond the scope of this article. It should be mentioned that children who have Down syndrome and ALL do not have a better outcome and perhaps even have a worse outcome than other ALL cases because they lack favorable genetic features [40,41].

**Therapy**

The backbone of contemporary multiagent chemotherapeutic regimens is formed by four elements: induction, CNS-directed treatment and consolidation, reinduction, and maintenance.

**Induction**

The goal of induction therapy is to induce morphologic remission and to restore normal hematopoiesis. Induction therapy contains at least three systemic drugs (ie, a glucocorticoid, vincristine, and L-asparaginase) and intrathecal therapy. The addition of an anthracycline as a fourth drug is matter of debate. In some protocols all patients receive an anthracycline; in other protocols it is reserved for high-risk cases. The induction phase aims to induce complete morphologic remission in 4 to 6 weeks.

**Central nervous system–directed treatment and consolidation**

CNS-directed therapy aims to prevent CNS relapses and to reduce the systemic minimal residual leukemia burden. CNS therapy usually is achieved by weekly or biweekly intrathecal therapy along with systemically administered drugs such as high-dose methotrexate (MTX) and 6-mercaptopurine (6-MP). Some groups rely on other drugs (eg, cyclophosphamide, cytarabine) in the consolidation phase to reduce systemic tumor burden further.

**Reinduction**

Reinduction therapy or delayed (re)intensification most often uses drugs comparable to those used during induction and consolidation therapy and has clearly shown its value by reducing the risk of relapse.

**Maintenance**

Therapy for ALL is completed by prolonged maintenance therapy for a total treatment duration of 2 years, or even longer in some protocols.
Maintenance consists of daily 6-MP and weekly MTX. In some protocols additional pulsed applications of a glucocorticoid and vincristine and intrathecal therapy are administered.

A fifth element, allogeneic stem cell transplantation (SCT), is reserved for only a small number of selected patients in first complete remission. The contribution of specific parts of treatment depends on the total therapy administered to a patient. A few important topics for which new data have been produced recently are discussed in the following sections.

Anthracyclines in induction?

It is unclear if addition of an anthracycline to a three-drug induction regimen is of benefit. Regimens that do not contain anthracycline are less myelosuppressive. Studies performed by the Children’s Cancer Group, however, showed that selected patients younger than 10 years of age did not benefit from the addition of an anthracycline, whereas selected older children did [42].

Dexamethasone or prednisone?

Several recent randomized studies have shown that the substitution of prednisone (approximately 40 mg/m²) by dexamethasone (approximately 6 mg/m²) significantly decreases the risk of bone marrow and CNS relapses when used in what are thought to be equipotent dosages [43,44]. One Japanese study, however, did not confirm the advantage of using dexamethasone [45]. The benefit of dexamethasone may result from higher free plasma levels and a better CNS penetration or from the fact that the presumed equivalent antileukemic activity for prednisone/dexamethasone is not a 6:1 dose ratio but is higher, as some (but not all) in vitro experiments suggest [46,47]. At this dose ratio dexamethasone also results in more toxicity than prednisone [43]. In vitro, a strong cross-resistance to prednisone and dexamethasone exists in ALL cells.

Which dose intensity of which asparaginase?

Randomized studies have revealed that at the same dose schedules, the use of L-asparaginase derived from *Escherichia coli* resulted in significant better EFS and overall survival (OS) rates than when asparaginase derived from *Erwinia chrysanthemi* (Erwinase) was used [48,49]. This difference results from differences in the half-lives of the drugs, and the difference presumably would not be found if Erwinase were given in an adequate dose-intensity schedule. The dose-intensity schedule to achieve complete asparagine depletion is 5000 units/m² every 3 days for *E coli* asparaginase. Erwinase must be scheduled more frequently than *E coli* asparaginase to achieve the same asparagine depletion. For the pegylated type of *E coli* asparaginase (PEG-asparaginase), 2500 units/m² once every 2 weeks leads
to the same pharmacodynamic effects. Lower doses of PEG-asparaginase (1000 units/m²) also lead to complete asparagine depletion in serum but not in the cerebrospinal fluid [50].

Intensification of asparaginase in induction and reinduction has improved outcomes in different studies [51–53]. Also, asparaginase intolerance was an important factor predicting an inferior outcome [54,55]. Allergic reactions usually are responsible for the discontinuation of asparaginase. Allergic reactions occur mainly when the drug is readministered in reinduction several weeks after first exposure during induction. In addition, the presence of asparaginase antibodies may lead to inactivation of the drug. Consequently, many investigators favor the use of the less immunogenic PEG-asparaginase from therapy outset rather than using it only after allergic reactions have occurred. In the light of these data, pharmacodynamic monitoring of asparaginase administration may prove very important for individual children who have ALL.

**Which central nervous system–directed therapy?**

To clarify the role of different CNS-directed therapies, a meta-analysis was published in 2003 [56]. From this analysis it became clear that long-term intrathecal therapy leads to EFS rates comparable with those of radiotherapy. Radiotherapy seemed to be more effective than high-dose MTX in preventing CNS relapse, but intravenous MTX reduced systemic relapses, resulting in comparable EFS rates for high-dose MTX and radiotherapy. It was concluded that radiotherapy can be replaced by multiple intrathecal doses of chemotherapy and that intravenous MTX reduces systemic relapses. It is still unclear whether intrathecal triple therapy (glucocorticoid, MTX, cytarabine) has any advantage over the use of intrathecal MTX as single drug. A recent Children’s Cancer Group study suggested that intrathecal triple therapy prevented CNS relapse but did not improve OS because fewer bone marrow relapses occurred when intrathecal MTX was used as a single agent [57].

The results of CNS-directed therapy depend on the treatment used. For example, the use of systemic dexamethasone reduces the incidence of CNS relapse. The comparison of different CNS preventive regimens is hampered because results are described for heterogeneous groups of patients. In several protocols, radiotherapy is still given to selected groups of high-risk patients such as those who have T-ALL with high white cell counts and children who have CNS involvement at diagnosis. Cranial radiotherapy is specifically toxic for very young children because of its detrimental effect on cognitive function.

**What type of reinduction/intensification and maintenance?**

Maintenance therapy consists of daily oral 6-MP and weekly intravenous or oral MTX. The intravenous administration of MTX may overcome compliance problems, but there is no evidence that it is more effective than oral
MTX. Several randomized studies have shown that the use of thioguanine offers no advantage over 6-MP in maintenance therapy [58,59]. For unknown reasons, 6-MP is more effective when administered in the evening than in the morning. Continuous adaptations of the doses of MTX and 6-MP based on peripheral blood counts are necessary to reduce the risk of relapse, on the one hand, and the risk of infections, on the other [60,61]. There are large interindividual differences in the doses that are tolerated or needed to reduce cell counts. This variability reflects pharmacogenetic differences, for instance in the status of thiopurine methyltransferase, a key enzyme that inactivates thiopurines [60,62]. Allelic differences are associated with reduced activity. Also, large intraindividual differences in doses occur (eg, because of concurrent viral infections). Recently, the major ALL study groups reached consensus on how to adjust the doses of 6-MP and MTX during maintenance so that the white blood cell count remains between 1.5 and $3.0 \times 10^9/L$. Routine measurements of liver function are not necessary in patients who do not have symptoms of liver dysfunction.

A meta-analysis of 42 trials showed that both longer maintenance (3 years versus 2 years) and the use of pulses of vincristine and a glucocorticoid during maintenance result in lower relapse rates but increased death rates [63]. The most important factor that has helped reduce relapses and improve survival is the use of an intensive reinduction course at the start of maintenance therapy. Several randomized studies proved the value of reinduction therapy for childhood ALL [64,65]. Attempts to omit reinduction led to a significant increase in relapse rate [66]. More than 50% of patients who were treated without reinduction did not relapse, however, illustrating that not all patients really need this intensification element. The question, of course, is how to identify these patients early on. When an intensive reinduction course is given, neither longer maintenance nor the use of vincristine/glucocorticoid pulses may contribute significantly to a better OS [63].

The results of the meta-analysis do not exclude the possibility that subgroups of patients may benefit from a longer duration of maintenance. Several study groups use longer maintenance therapy for boys than for girls. Reduction of the duration of maintenance below 2 years in a Japanese study led to an increased risk of relapse [67]. This study, however, also demonstrates that not all patients need 2 years of maintenance therapy. Again, the important question is how to identify these patients. It might be that a long maintenance therapy is less effective in high-risk leukemias with a very aggressive behavior, such as \textit{MLL} gene–rearranged ALL, \textit{bcr-abl}–positive ALL, and T-ALL, in which relapses occur relatively early; the more smoldering types of ALL, such as hyperdiploid and \textit{TEL/AML1}–gene rearranged ALL, might benefit more from maintenance therapy.

A recent large, randomized study did not show a benefit for the use of pulses with vincristine and a glucocorticoid in a selected group of patients treated on a Berlin/Frankfurt/Münster regimen [68]. The benefit of these pulses therefore may be found only in studies that use no or a less intensive
reinduction course, such as in the Dutch Childhood Leukemia Study Group-6 study [69] or in studies in which the upfront therapy is relatively mild.

Who should (not) be transplanted?

Autologous SCT is not effective in childhood ALL and therefore should not be performed. A collaborative study of several large study groups has shown that BCR/ABL-positive ALL benefits from allogeneic SCT from a matched related donor both in terms of EFS and OS [12]. For other types of donor this benefit was not proven. A comparable analysis for children who had t(4;11) could not detect a beneficial effect of SCT from any type of donor [26]. Recently, a comparison was performed between children who had very high-risk ALL in first remission who were assigned by the availability of a compatible related donor to receive SCT or to receive chemotherapy when no donor was available [70]. “Very high risk” was defined in this study by the presence of one or more of the following criteria: failure to achieve complete remission after 5 weeks’ therapy, t(9;22) or t(4;11) positivity, a poor prednisone response associated with T-cell phenotype, or a white blood cell count higher than $100 \times 10^9/L$. The 5-year disease-free survival rate was better for the patients who received SCT from a matched related donor than for those who received chemotherapy. Only one in six of these high-risk patients had a suitable family donor, however. SCT from alternative donors resulted in an inferior outcome. Therefore the role of allogeneic SCT in first complete remission is limited in these very high-risk patients. Another recent study failed to prove a benefit for allogeneic SCT in very high-risk cases [71], whereas the Berlin/Frankfurt/Münster study group showed that high-risk T-cell ALL cases may benefit from SCT [72].

Treatment of adolescents

Four recent reports from four different countries show that outcome for adolescents who have ALL is better when these patients are treated on a pediatric rather than an adult protocol [73–76]. The 5-year EFS of patients aged 15 to 21 years was approximately 30% higher when they were treated according to a pediatric protocol (Table 3). This result could not be explained by differences in immunophenotype and genetic abnormalities, but there seemed to be large differences in the dose intensity used during treatment. The pediatric protocols contained more glucocorticoids, vincristine, L-asparaginase, MTX, and 6-MP. In addition, it is conceivable that the longer delays between different parts of treatment noted in adolescents treated according to the adult protocols might have played a role. It is possible that hematologists have a different approach in managing toxicities because they generally treat older patients who do not tolerate intensive therapy well. Also, the toxicity caused by SCT usually is accepted as part of therapy, whereas adult hematologists have less experience with glucocorticoid- and asparaginase-induced toxicities. In the Dutch study, use of the
adult ALL treatment protocol resulted in both a higher relapse rate and in a higher toxic death rate for adolescents [74].

**Side effects**

Nearly all chemotherapy side effects seen in children treated for ALL are temporary. The single most important cause of toxic death is infections: 0.5% to 1.5% of patients die from infections during induction therapy, and between 1% and 3% die from infections while in complete remission [77]. Many toxicities result from using a combination of drugs; some, however, are drug specific. Drug-specific toxicities include neuropathy and constipation caused by vincristine, mucositis caused by MTX, diabetes, behavior disturbances, Cushingoid appearance, osteoporosis, and avascular necrosis of bone caused by glucocorticoids, and allergic reactions and thrombosis caused by asparaginase [78].

Toxicity increases with patient age. For example, children older than 10 years have a higher incidence of side effects to glucocorticoids such as avascular necrosis of bone and hyperglycemia, and pancreatitis and thromboembolic complications caused by L-asparaginase [55]. About 5% to 15% of children older than 10 years of age and adolescents experience one or more of these side effects. It has been shown that short pulses of glucocorticoids (5 days) lead to fewer side effects than more continuous schedules with the same cumulative doses of glucocorticoids.

**Perspectives**

**New genomic techniques**

The recent sequencing of the human genome and technical advances in high through-put analysis of DNA copy number and mRNA expression now allow a “molecular portrait” of leukemia. Gene-expression profiling can be helpful in classifying ALL patients, in revealing new insights into the pathways involved in different genetic subtypes of ALL, and

### Table 3

Outcome of adolescents treated on a pediatric or adult acute lymphoblastic leukemia protocol

<table>
<thead>
<tr>
<th>Study group [reference]</th>
<th>Patient number</th>
<th>Age category (in years)</th>
<th>5-year event-free survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dutch: pediatric [23]</td>
<td>47</td>
<td>15–18</td>
<td>69</td>
</tr>
<tr>
<td>Dutch: adult [23]</td>
<td>44</td>
<td>15–18</td>
<td>34</td>
</tr>
<tr>
<td>French: adult [12]</td>
<td>100</td>
<td>15–20</td>
<td>41</td>
</tr>
<tr>
<td>United Kingdom: pediatric [72]</td>
<td>61</td>
<td>15–17</td>
<td>65</td>
</tr>
<tr>
<td>United Kingdom: adult [72]</td>
<td>67</td>
<td>15–17</td>
<td>49</td>
</tr>
</tbody>
</table>
in identifying new pathways involved in therapy resistance and new therapeu
tic targets [79].

The first studies using gene-expression profiling showed that known mor-
phologic, immunophenotypic, and genetic subclasses of ALL had specific
gene-expression profiles [28,80,81]. Gene-expression profiling may be even
more suitable for classifying ALL cases because it takes into consideration
the biologic state and genetic progression [82]. Gene-expression patterns
have been revealed that are related to in vitro resistance to several classes
of individual agents, to clinical outcome, and to cross-resistance to multiple
antileukemic drugs [83,84]. These studies, for example, have shown that
MCL-1 overexpression is involved in glucocorticoid resistance in ALL. Mod-
ulation of MCL-1 expression sensitizes ALL cells to glucocorticoids [85].

Bhojwani and colleagues [86] revealed that gene-expression profiles of
early relapsed ALL samples were characterized by the overexpression of
genes involved in cell-cycle regulation; this finding might identify attractive
new targets for therapy. Armstrong [87] and Stam [88] showed high levels of
wild-type FLT3 in MLL-rearranged ALL. High levels of FLT3 are related
to a poor outcome [89], and inhibition of this tyrosine kinase is very effective
in MLL-rearranged ALL cells in vitro [88] as well as in an in vivo mouse
model [87]. This finding has led to the design of two different phase I/II stud-
ies of these inhibitors in MLL-rearranged ALL.

Genome-wide techniques to screen for mutations and amplifications and
for single-nucleotide polymorphisms (SNPs) recently have revealed many re-
current genetic alterations that are important for the development of ALL
[90–93] and for the sensitivity to chemotherapy. For example, polymor-
phisms in folate-related genes are related to the MTX sensitivity of ALL
cells [94]. Mullighan and colleagues [90] used SNP arrays to reveal that
childhood ALL samples show recurrent gene deletions and amplifications
including somatic PAX5 deletion, which is present in about one third of
all ALL cases [90]. Overall deletions were more common than amplification,
specifically deletions of genes involved in B-cell differentiation, indicating
that arrested development is a key feature of leukemia transformation. In
the forthcoming years, large-scale studies will analyze the profile of micro-
RNAs in ALL subtypes [95] and the role of newly discovered genetic sub-
type-specific microRNAs in ALL.

**Targeted therapies**

Several new targeted therapies may contribute to a further improvement
in treatment results in childhood and adolescent ALL (Table 4). The ulti-
mate target of therapy is the leukemogenic fusion product. The best example
is the BCR/ABL fusion product leading to an abnormal ABL tyrosine
kinase activity. Imatinib is an effective inhibitor of this kinase [96], but re-
sistance rapidly occurs when it is used as a single agent, mainly because
of the selection or development of leukemic subclones with BCR-ABL point
mutations. It therefore seems that imatinib must be combined with standard antileukemic agents to treat \( BCR-ABL \)-positive ALL effectively. A European randomized study currently is attempting to assess the efficacy and toxicity of the addition of imatinib to all chemotherapy blocks. Resistance to imatinib is caused mainly by the outgrowth of subclones with mutations in the kinase domain of \( BCR-ABL \) that interfere with imatinib binding. For most mutations, this resistance can be overcome with dasatinib [97] or nilotinib [98]. A pediatric phase I-II study with dasatinib is underway. The very rare subset of T-ALL with \( NUP214-ABL1 \) fusion also may be a suitable group for targeted therapies using these compounds.

The recent finding that half of T-ALL cases have activating mutations of the \( NOTCH1 \) gene provides a rationale for targeted therapies of the \( NOTCH \) pathway. Cleavage of the trans-membrane receptor \( NOTCH1 \) by gamma secretase leads to release of the intracellular domain of \( NOTCH1 \) (ICN1), followed by translocation to the nucleus and transcription activation. Inhibitors of ICN1 production and activity seemed to be toxic for T-ALL cells in vitro and have led to a clinical trial of a gamma secretase inhibitor in patients who had refractory T-ALL; however, this trial was stopped because of gastrointestinal side effects. Targeting the enzyme purine nucleoside phosphorylase in T-ALL, especially by forodesine [99], is another strategy that will be tested in childhood ALL in the forthcoming years. Nelarabine is a nucleoside analogue that is converted intracellularly to cytarabine with promising activity as single agent in T-ALL [100,101].

Overexpression of wild-type \( FLT3 \), especially in \( MLL \)-rearranged ALL and hyperdiploid ALL, also provides an opportunity for targeted therapies with \( FLT3 \) inhibitors. Another opportunity may be found in the hypermethylation state of \( MLL \)-rearranged ALL, where the tumor-suppressor gene \( FHIT \) is silenced by hypermethylation. Re-expression leads to the
killing of infant MLL-rearranged ALL cells, and demethylation agents have the same effect [102].

Finally, different monoclonal antibodies, directed against different antigens (CD20, CD22, and CD52), with or without conjugated toxins, are in early clinical studies in childhood ALL.

**Host pharmacogenetics**

There is no doubt that host polymorphisms in drug-metabolizing genes alter drug levels and target engagement. The ultimate goal of host pharmacogenetic studies is to optimize drug dosing for each patient to achieve maximum treatment efficacy with a minimum toxicity. Germline SNPs determine the toxicity of different antileukemic drugs [103]. The most extensively studied is the gene encoding for thiopurine methyltransferase (TPMT) involved in the metabolism of 6-MP. Genetic polymorphisms in TPMT correlate with enzyme activity and with both 6-MP toxicity and outcome in ALL. Many other genes are subject to genetic polymorphisms, and the development of tools such as SNP arrays facilitates the studies of many of these polymorphisms simultaneously.

**Summary**

More than 80% of children who have ALL are cured with contemporary intensive chemotherapy protocols. In the forthcoming decades it will be of great importance to tailor therapy for individual patients according to early response to therapy (mainly by detecting MRD) so that the intensity of therapy can be reduced or augmented. Also, more specific therapy schedules will be developed for immunophenotypic and genetic subclasses of ALL, because it now is apparent that ALL is not a single disease entity but in fact includes different diseases with differing underlying biology and clinical courses. New genomic techniques will lead to the discovery of new molecular genetic abnormalities that will provide more insights into the biology of the different ALL subtypes. New targeted therapy approaches will be developed, and it will be important to investigate how new agents can be incorporated in existing regimens.

**References**


