A 50-Year Journey to Cure Childhood Acute Lymphoblastic Leukemia

Ching-Hon Pui and William E. Evans

The 50th anniversary of Seminars in Hematology coincides with the 50th anniversary of St. Jude Children’s Research Hospital, and both milestones are inexorably linked to studies contributing to the cure of childhood acute lymphoblastic leukemia (ALL). We thought it fitting, therefore, to mark these events by traveling back in time to point out some of the achievements, institutions, study groups, and individuals that have made cure of childhood ALL a reality. In many instances, progress was driven by new ideas, while in others it was driven by new experimental tools that allowed more precise assessment of the biology of leukemic blasts and their utility in selecting therapy. We also discuss a number of contemporary advances that point the way to exciting future directions. Whatever pathways are taken, a clear challenge will be to use emerging genome-based or immunologic-based treatment options in ways that will enhance, rather than duplicate or compromise, recent gains in outcome with classic cytotoxic chemotherapy. The theme of this journey serves as a reminder of the chief ingredient of any research directed to a catastrophic disease such as ALL. It is the audacity of a small group of investigators who confronted a childhood cancer with the goal of cure, not palliation, as their mindset.

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EDITOR’S NOTE

This is a “special” article celebrating the 50th anniversary of Seminars in Hematology. Drs Ching-Hon Pui and William E. Evans from St. Jude Children’s Research Hospital and Colleges of Medicine and Pharmacy, University of Tennessee, Memphis TN, offer us a “50-year journey to cure childhood acute lymphoblastic leukemia.” In this paper the reader will find a complete history of therapeutic advances in ALL in childhood, but also recent research developments in biology and treatment. Flow cytometry and genome-wide analyses allowed the recognition of novel leukemia subtypes with different prognosis. Pharmacokinetics, pharmacodynamics, and pharmacogenetics further helped in the choice of the best treatment at the best dosage, what the authors call “optimizing risk-directed therapy.” In 1961, the survival rate was approximately 20%; today 5-year survival (see Table 3), is 93.5% ± 1.9%. The effort to cure all patients continues, as the reader will discover in the “future directions.” We are proud to offer to our readers this special article and we thank the authors for their excellent work.

Since the inauguration of the Seminars of Hematology and the opening of St. Jude Children’s Research Hospital approximately half a century ago, there has been remarkable progress in the study of childhood acute lymphoblastic leukemia (ALL). This once uniformly fatal cancer has been transformed to one with a cure rate approaching 90% in many developed countries (Figure 1), and its dissection in the laboratory has uncovered many of the principles underlying our current knowledge of tumor cell biology—advances that are often considered one of the pivotal success stories of modern medicine. In this review, we summarize the historical perspective for these achievements and point to recent developments in the biology and treatment of ALL that will shape future directions for research and treatment advances.

HISTORICAL PERSPECTIVE

Therapeutic Advances

In 1948, Farber et al described “temporary remissions” induced by aminopterin, a folic acid antagonist, in five children with acute leukemia, opening the era of chemotherapy for this disease. This landmark demonstration was reinforced in 1961, when Frei et al achieved a
complete remission rate of 59% and a 2-year survival rate of approximately 20% in 39 pediatric patients, using a combination of mercaptopurine and methotrexate. Nonetheless, despite the introduction of several new antileukemic drugs, ALL continued to be fatal in the vast majority of patients. To meet this challenge, Pinkel and colleagues at the newly opened St. Jude Children’s Research Hospital initiated a novel curative approach (“total therapy”) to ALL treatment in 1962 that featured multiple components of therapy—remission induction, central nervous system (CNS)-directed therapy with cranial irradiation and intrathecal methotrexate, intensification (consolidation) therapy, and continuation treatment—four components that still form the backbone of ALL treatments today.3

Responses to one of these regimens (Total Therapy Study V, 1967–68) were remarkable, leading to cures in approximately half of the 35 patients who were enrolled.4 This success stimulated the conduct of similar clinical trials worldwide, with two pivotal studies in the 1970s showing that intensification therapy relatively soon after remission induction could boost cure rates to near 70%.5,6 In one study, Riehm et al5 of the Berlin-Frankfurt-Münster group introduced so-called Protocol II, treatment which specified a re-induction phase (essentially repetition of the initial remission induction therapy), while in the other, Sallan et al6 at the Dana-Farber Cancer Center incorporated weekly high-dose asparaginase into their multiagent protocol. During the same period, concern over radiation-induced complications prompted the development of triple intrathecal therapy with methotrexate, hydrocortisone, and cytarabine,7 and intermediate-dose intravenous methotrexate8 to replace prophylactic cranial irradiation. These pioneering studies demonstrated the importance of effective systemic chemotherapy when using triple intrathecal therapy as CNS-directed therapy and the effectiveness of higher doses of intravenous methotrexate to reduce systemic and testicular relapse. A subsequent trial showed that dexamethasone was more effective than prednisone in preventing CNS relapse.9 Taken together, these advances opened the way for successful elimination of prophylactic cranial irradiation in all patients with ALL who are treated with effective systemic and intrathecal therapy.10

Another turning point in the development of ALL therapy came with the finding that the systemic exposure to methotrexate (ie, steady-state serum concentration) correlated with treatment outcome.11 This discovery gave impetus to a randomized study showing that individualized doses of high-dose methotrexate, teniposide, and cytarabine based on the ability of individual patients to clear the drugs, could improve outcome,12 providing proof-of-principle for the “personalized dosing” in cancer treatment. These data also established that it is possible that some patients were not being cured because their leukemia cells are exposed to suboptimal concentrations of antileukemic agents, and not because their leukemia cells are resistant to chemotherapy. Indeed, as reviewed later, this early work laid the groundwork for our use of different doses of methotrexate in individual patients based on the phenotype and genotype of the leukemic cells, and different doses of mercaptopurine based on the patient’s inherited pharmacogenetic traits.13 Finally, the marked improvement in treatment outcome among patients with Philadelphia chromosome–positive ALL who receive an ABL tyrosine kinase inhibitor (imatinib), together with intensive chemotherapy,14 illustrates the potential of oncogenic pathway-directed therapy in ALL and provides a paradigm for the future design of targeted treatments in this disease (Table 1).

**Biologic Advances**

Cytogenetic studies of ALL began in 1958,15 and ultimately dramatically altered perceptions of ALL pathobiology. Discovery of the Philadelphia chromosome first in

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**Figure 1.** Kaplan-Meier analysis of survival for 2852 children with newly diagnosed ALL who were enrolled in 15 consecutive Total Therapy studies at St. Jude Children’s Research Hospital from 1962–2007. Ten-year survival estimates are shown. The results demonstrate steady improvement in outcome over the past half century.
chronic myelogenous leukemia by Nowell and Hungerford and then in ALL by Propp and Lizzi, the clinical significance of modal chromosomal number (ploidy) by Secker-Walker et al. and immunophenotype-specific chromosomal translocations by Williams et al. left little doubt that ALL is a disease involving DNA abnormalities. Moreover, the demonstration of T-cell markers on leukemic lymphoblasts by Borella and Sen taught us that ALL can arise in the T- or the B- lymphoid compartment of the immune system.

The early hypothesis that consistent translocations pinpoint chromosomal segments containing genes critically involved in malignant transformation of ALL resulted in identification of numerous oncogenes by molecular genetic studies. Not surprisingly, the earliest genes identified as partners of these reciprocal translocations were immunoglobulin genes or T-antigen receptor chain genes, the price paid for immunological diversity. With the availability of antibodies for leukocyte differentiation antigens, early studies showed that T-cell ALL expresses terminal deoxynucleotidyl transferase and other T-cell markers, a finding that allowed the first minimal residual disease (MRD) studies in patients with ALL. With completion of the first draft of the human genome in the early 1990s and the advent of molecular genetic technology, genome-wide studies of ALL became feasible with global gene expression profiling. This advance, together with the interrogation of changes in DNA copy number using genome-wide SNP analyses and other high-throughput methods and more recently the use of whole genome sequencing has

Table 1. Landmark Advances in the Evolution of Therapy for Childhood Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>Year</th>
<th>Therapeutic Advance</th>
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<tbody>
<tr>
<td>1948</td>
<td>“Transient remissions” induced by aminopterin.</td>
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<tr>
<td>1971</td>
<td>Combination chemotherapy and effective CNS-directed therapy cure approximately 50% of patients.</td>
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<tr>
<td>1981</td>
<td>Re-induction treatment improves outcome.</td>
</tr>
<tr>
<td>1982</td>
<td>Triple intrathecal therapy with methotrexate, hydrocortisone, and cytarabine may effectively substitute for prophylactic cranial irradiation in some patients.</td>
</tr>
<tr>
<td>1983</td>
<td>Postremission weekly high-dose asparaginase improves outcome.</td>
</tr>
<tr>
<td>1983</td>
<td>Intermediate-dose methotrexate with leucovorin rescue decreases systemic and testicular relapses.</td>
</tr>
<tr>
<td>1991</td>
<td>Dexamethasone is more effective than prednisone in preventing CNS relapse.</td>
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<tr>
<td>1995</td>
<td>Inherited genetic polymorphisms in gene encoding thiopurine methyltransferase influence mercaptopurine toxicity.</td>
</tr>
<tr>
<td>1998</td>
<td>Individualized methotrexate dose improves outcome.</td>
</tr>
<tr>
<td>2009</td>
<td>Effective systemic and intrathecal chemotherapy can eliminate the need for prophylactic cranial irradiation in all patients.</td>
</tr>
<tr>
<td>2009</td>
<td>Imatinib improves early treatment outcome in Philadelphia chromosome–positive ALL.</td>
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</table>

Table 2. Landmarks in Understanding the Biology of Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>Year</th>
<th>Biologic Advance</th>
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<tbody>
<tr>
<td>1958</td>
<td>First cytogenetic study in ALL</td>
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<tr>
<td>1970</td>
<td>First report of Philadelphia chromosome-positive ALL</td>
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<tr>
<td>1973</td>
<td>First identification of T-cell ALL by spontaneous rosette formation with sheep erythrocytes</td>
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<tr>
<td>1978</td>
<td>Classification of ALL by chromosome number &gt; 50 (hyperdiploidy) is associated with prolonged remission duration</td>
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<tr>
<td>1981</td>
<td>Immunologic monitoring of residual leukemia</td>
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<tr>
<td>1984</td>
<td>First identification of immunophenotype-specific chromosomal translocations: t(11;14) in T-cell ALL and t(1;19) in pre-B ALL</td>
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<tr>
<td>2002</td>
<td>First genome-wide profiling of gene expression</td>
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<tr>
<td>2007</td>
<td>First genome-wide study of changes in DNA copy number</td>
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<tr>
<td>2009</td>
<td>Germline genetic variants associated with the development of ALL</td>
</tr>
<tr>
<td>2012</td>
<td>First whole-genome sequencing study to identify driver mutations in early T-cell precursor ALL</td>
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</tbody>
</table>
improved our ability to define the spectrum of genomic alterations that contribute to ALL pathogenesis. Parallel studies of germline DNA from normal leukocytes has led to the detection of inherited germline single-nucleotide polymorphisms (SNPs) in genes that increase susceptibility to ALL.\textsuperscript{26,27} (Table 2).

## RECENT RESEARCH DEVELOPMENT IN BIOLOGY AND TREATMENT

The optimal use of antileukemic agents, improved supportive care, and precise risk assessment have improved 5-year event-free survival rates to more than 85\% and 5-year overall survival rates to more than 90\% in several contemporary clinical trials (Table 3).\textsuperscript{10,28–35} Paralleling these advances has been the improved understanding of ALL pathobiology, the mechanisms of drug resistance, and the disposition of antileukemic drugs in the host. With the advent of high-resolution whole-genome and transcriptome sequencing, virtually all cases of ALL can now be classified according to their specific genetic abnormalities,\textsuperscript{36,37} opening the way for new drug discovery and targeted treatment of increasing numbers of patients.

### Novel Leukemia Subtypes

ALL is broadly classified into B-lymphoblastic and T-lymphoblastic leukemias, which can be further subclassified according to specific genetic abnormalities.\textsuperscript{37} Among T-ALL cases, it is only important to distinguish a high-risk subgroup, now termed early T-cell precursor ALL,\textsuperscript{38} whose immunologic markers, gene expression profile, and mutational spectrum are reminiscent of myeloid leukemia, suggesting that this is a stem cell disease.\textsuperscript{25} The prevalence of mutations in genes regulating RAS signaling, cytokine receptor expression, and chromatin modification suggests that myeloid-directed or epigenetic therapy may improve the clinical outcome for this ALL variant.\textsuperscript{25}

Traditionally, about 70\%–80\% of B-ALL cases were classified by modal chromosomal number (ploidy) and specific genetic rearrangements into prognostically relevant subgroups.\textsuperscript{39,40} Now, with the advent of genome-wide analyses, virtually all B-ALL cases can be classified genetically.\textsuperscript{36,37} One of the newly discovered subtypes is characterized by CRLF2 expression and affects 5\%–7\% of children with B-ALL and, remarkably, about 50\% of Down syndrome patients with ALL.\textsuperscript{41} Many of these cases have cryptic translocations involving a tyrosine kinase gene (eg, JAK), and probably require intensive chemotherapy.\textsuperscript{42} Another subtype, termed Philadelphia chromosome-like (or BCR-ABL-like) ALL, accounts for nearly 10\% of B-ALL cases and exhibits a gene expression profile similar to that of Philadelphia chromosome (BCR-ABL)–positive ALL with IZKF1 alteration.\textsuperscript{43,44} A recent study with whole-genome sequencing identified alterations and mutations activating kinase and cytokine receptor signaling in all 12 cases studied.\textsuperscript{45} Importantly, several cases had
genetic abnormalities that responded to AB1 tyrosine kinase inhibitors (eg, NUP214-ABL1, EBF1-PDGFBRB) or to JAK inhibitors (eg, BCR-JAK2, mutated IL7R). Although hypodiploid ALL is known to identify a high-risk subgroup and can be subdivided cytogenetically into near-hypodiploid cases with 24–31 chromosomes, low-hypodiploid cases with 32–39 chromosomes, and rarely high-hypodiploid cases with 40–43 chromosomes, the genetic bases of these novel variants were only recently uncovered by a genome-wide study. Near-haploid cases the genetic bases of these novel variants were only recently uncovered by a genome-wide study.46 Near-haploid cases have alterations involving receptor tyrosine kinase signaling and Ras signaling, while low-hypodiploid cases are characterized by alterations in TP53 and RB1. Interestingly, both subgroups are sensitive to phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) inhibitors, suggesting that these agents might be useful as a new strategy of targeted treatment.46

**Host Pharmacokinetics, Pharmacodynamics, and Pharmacogenetics**

Early studies at St. Jude Children’s Research Hospital showed that pharmacokinetic variability can influence treatment outcome in ALL, with fast clearance of certain medications conferring an inferior treatment response.11,12 Subsequent studies found many sources of variation, some environmental (eg, hydration status, drug interactions) and others genetic.13 The classic example is the relation between inherited polymorphisms in the gene encoding thiopurine methyltransferase (TPMT) and the metabolism of mercaptopurine. Patients with inherited or two variant alleles that encode unstable and/or nonfunctional TPMT proteins are at increased risk of hematopoietic toxicity47 and the development of therapy-related leukemia 45 but can be safely treated with a reduced dose of mercaptopurine.13 Based on these data, we have been pre-emptively genotyping all patients for this enzyme and adjusting mercaptopurine dose accordingly (eg, a 90% dose reduction in patients with two nonfunctional TPMT alleles). This has resulted in markedly lower hematopoietic toxicity, without compromising the efficacy of ALL treatment.48,49

Genome-wide SNP analyses have identified several germline genetic variations that affect treatment outcome. In our early study, we identified 102 germline SNPs associated with MRD, antileukemic drug disposition, and risk of relapse; one of the strongest signals came from five SNPs located within the IL15 gene locus, which encodes a proliferation-stimulating cytokine.50 Subsequent studies showed that germline SNPs of the organic anion transporter gene SLC01B1 are associated with methotrexate clearance23 and those of ARID5B with greater methotrexate polyglutamate accumulation, offering a plausible mechanism by which this genetic variant is linked to treatment outcome.26,52

Genome-wide SNP analyses have also identified polymorphisms of several genes (ARID5B, IKZF1, CEBPE, BMI1-PIP4K2A) associated with the development of ALL in children.26,27,52,53 Interestingly, the frequency of ARID5B and BMI1-PIP4K2A risk alleles increased in the order of African Americans, European Americans, and Hispanic Americans, corresponding to the increasing incidence of ALL in these racial/ethnic groups.52,53 That patients with hyperdiploid >50 ALL have a greater probability of carrying germline ARID5B variants than do those with other genotypes also suggests an interaction between inherited and acquired genetic variations during leukemogenesis.26,27

**Precise Risk Stratification**

As the arsenal of effective antileukemic agents and the understanding of ALL pathobiology grow, so does the need for new systems of risk assessment to avoid over- or undertreatment of individual patients. Of the many variables that influence prognosis in ALL, age at diagnosis, initial leukocyte count, leukemic cell genetics, and especially the initial response to treatment are perhaps the most useful for risk stratification.37 Variable treatment responses may be evident even among cases of the same genetic subtype, partly because of differences in the target cell that underwent malignant transformation and partly because of differences in cooperating driver mutations.36,54 Such differences also may be related to host differences in drug metabolism and pharmacokinetics. Even so, it is well recognized that effective treatment can abolish the adverse prognostic impact of many clinical or biologic features. In one recent example (St. Jude Total Therapy Study XV), early intensive treatment with dexamethasone, vincristine, asparaginase, and triple intrathecal therapy, as well as high-dose methotrexate, not only yielded high cure rates for older adolescents and black patients, once considered high-risk subgroups,55,56 but also has eliminated the prognostic significance of a high initial white blood cell count.10 Similarly, the poor outcome associated with genetic evidence of >10% Native American ancestry for patients with Hispanic ethnicity or for those self-reported as white with more than 10% genetic Native American ancestry was abrogated by an additional course of delayed intensification therapy in a Children’s Oncology Group (COG) study.57 Nonetheless, some features still have prognostic implications in the context of effective treatment and warrant modifications in treatment strategy. Thus, intensive regimens are used for T-cell ALL in most clinical trials, and for patients with intrachromosomal amplification of chromosome 21 in a ALL trial from the United Kingdom.58

Not surprisingly, response to treatment in vivo as determined by MRD measurement has become a highly reliable prognostic indicator because it not only reflects intrinsic drug sensitivity, but also host pharmacodynamics and pharmacogenomics, treatment adherence, and treatment efficacy.58 Notably, this variable assessed at a later time in the clinical course (eg, at day 78 of extended...
remission induction of a Berlin-Frankfurt-Münster protocol is more predictive of ultimate outcome because it reflects the drug sensitivity or resistance to more drugs that the patients have received. Thus, in one study, remission induction failure at day 28 to day 42 did not predict a dismal prognosis for children with B-ALL who did not have other adverse prognostic features and especially for those with hyperdiploidy, which confers a favorable response to high-dose methotrexate and mercaptopurine, drugs used only after 4–6 weeks of remission induction. However, assessment of treatment response early during remission induction (eg, at day 15) is still useful in gauging the intensity of subsequent remission induction therapy to avoid overtreatment of patients with a favorable prognosis, especially those treated in developing countries with limited resources and insufficient supportive care.

The usual methods of monitoring MRD include flow cytometric detection of aberrant immunophenotypes and allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) amplification of immunoglobulin and T-cell receptor genes. The flow cytometric method requires a high level of expertise to interpret results, while ASO-PCR amplification is both time-consuming and laborious. Moreover, both strategies have a limited capacity to monitor clonal evolution during treatment, with the potential pitfall of false-negative results. New deep-sequencing methods can overcome these limitations, are more precise, and can detect very low levels of leukemia (below 0.01%). In this regard, very low levels of MRD at the end of induction therapy may have prognostic significance.

Optimizing Risk-Directed Therapy

Although an overall complete remission rate of 98% or 99% can be achieved in most study cohorts with improved supportive care and chemotherapy, there is no consensus on the optimal regimen or duration of remission induction therapy. Although dexamethasone is used in virtually all protocols because of its superior CNS control compared to results with prednisone or prednisolone, it is not often included during remission induction because of concern over high rates of toxicity and toxic death, especially at high doses (eg, 10 mg/m²) in patients 10 years of age or older, a finding partly related to slower clearance of dexamethasone in this age group. In the COG protocols, dexamethasone is used during remission induction for children less than 10 years old with high-risk B-ALL because it yielded superior event-free survival than prednisone for this age group in a randomized study, and for patients with T-cell ALL based on promising results of two European studies for this subtype.

Infant or adolescent cases and patients with Down syndrome continue to have lower induction rates, partly because they have more high-risk leukemia and partly because they are more susceptible to fatal infection, a risk that extends into the post-remission phase. Hence, remission induction should be moderate in intensity for these patients as well as those with a low- or standard-risk of relapse, reserving intensive chemotherapy for the consolidation phase of treatment, when normal hematopoiesis has been restored. Although immunoglobulin replacement therapy and prophylactic antibiotic and antifungal treatment have been used in some centers when these patients become severely immunosuppressed and myelosuppressed, prospective studies are required to establish the efficacy of this approach. As mentioned earlier, we measure MRD levels after 2 weeks of remission induction therapy, and add more asparaginase for patients with a high level of residual leukemia (ie, 1% or more leukemic cells in the bone marrow). A simple and inexpensive flow cytometric assay, based on the property of exquisite sensitivity of normal lymphoid progenitors (CD19⁺, CD10⁺, and/or CD34⁺) to corticosteroids, can be used to measure MRD for B-ALL at this early interval.

Intensification of treatment after remission induction is essential for all patients, but again there is no consensus on the best regimen or duration of treatment. Consolidation treatment is generally given soon after remission induction and consists of high-dose methotrexate and daily mercaptopurine. To achieve an adequate response in patients with T-cell ALL or perhaps B-ALL with the TCF3-PBX1 fusion, both of which accumulate methotrexate polyglutamates less avidly than other subtypes of ALL, methotrexate should be given at higher dose (~5 g/m²) over 24 hours. While it is debatable whether high-dose methotrexate is necessary for low-risk ALL, delayed intensification (also termed re-induction), given within 2–3 months post-remission is clearly beneficial to all patients. This phase of treatment consists of asparaginase, dexamethasone, and vincristine, which act synergistically, with or without an anthracycline, mercaptopurine and methotrexate. It should be noted that the intensity of delayed intensification is more important than its duration. Indeed, intensification therapy for 6 months was as effective as 10 months of the same treatment, and in three randomized trials, two re-induction courses or one re-induction course yielded the same event-free survival in standard- or high-risk patients who had a rapid early response to remission induction. Because the second re-induction course was started rather late in each of the three randomized studies (week 32 to week 48), it remains to be determined whether a second re-induction introduced earlier in the treatment course will improve outcome.

Intensive use of asparaginase, dexamethasone, and vincristine accounts for much of the recent improvement in treatment outcome in patients with ALL. Despite its higher cost, the pegylated form of *Escherichia coli* asparaginase (PEG-asparaginase), which is less allergenic than the native *E coli* product, has become the first-line asparaginase treatment in the United States, and is used increasingly in the other countries. The preparation derived from *Erwinia chrysanthemia* does not cross-react with the *E coli* preparation and is used as a second-line
therapy for patients with hypersensitivity reaction or "silent inactivation" (due to antibodies) to native *E coli* asparaginase or PEG-asparaginase. Depending on the preparation used, the treatment schedule and the concomitant immunosuppressive agent, 10%–60% of patients will develop hypersensitivity reactions and 10%–30% of those without clinical hypersensitivity may develop silent inactivation. Importantly, the presence of anti-asparaginase not only affects the efficacy of asparaginase but also may increase systemic clearance of dexamethasone, leading to increased risk of bone marrow and CNS relapse.In a recent study, a very low rate of thasone, leading to increased risk of bone marrow and nasse but also may increase systemic clearance of dexamethasone, leading to increased risk of bone marrow and CNS relapse. In another recent study, a very low rate of asparaginase hypersensitivity reaction (1.7% overall and 6.4% in high-risk cases) may be attributed to the optimal use of PEG-asparaginase, which was either preceded by a dexamethasone pulse or administered without interruption (in high-risk cases). In another recent study, patients randomized to receive individualized doses of native *E coli* asparaginase based on nadir serum asparaginase activity and, in the presence of silent inactivation, to receive *Erwinia* or PEG-asparaginase, despite receiving a lower median dose of asparaginase, had a significantly better event-free survival rate than did the controls, who were treated with a fixed dose of asparaginase. Hence, prospective identification of patients with silent inactivation of asparaginase could be an important strategy to justify a switch to alternative forms of asparaginase. In this regard, commercial tests to measure asparaginase levels are now available, but guidelines for modifying therapy based on such measurements have yet to be developed.

During continuation treatment with weekly low-dose methotrexate and daily mercaptopurine, tailoring the dosages of these drugs to the limits of tolerance has been associated with a better outcome. The preponderance of evidence indicates that the time has come to customize the dosage of mercaptopurine based on pre-emptive testing for TPMT status (eg, genotype) to reduce acute hematopoietic toxicities and the late development of mercaptopurine-induced myeloid malignancy in patients with an inherited deficiency of this enzyme, particularly if they receive high-dose mercaptopurine (eg, 75 mg/m² per day). To this end, the international Clinical Pharmacogenetics Implementation Consortium has developed guidelines for *TPMT* genotyping and dosing of thiopurines (updates at http://www.pharmgkb.org). In most clinical trials, weekly methotrexate is given orally for convenience and cost savings, but we prefer to give it intravenously to partly circumvent the problems of variable bioavailability and to ensure better treatment adherence (hence, avoiding an increased risk of relapse) as shown in a recent COG study. This approach might have been partly responsible for the improved prognosis of older adolescents treated in our Total Therapy XV study. Additional studies are needed to determine the optimal dosage of methotrexate for continuation therapy, which ranges from 20 mg/m² orally to 40 mg/m² intravenously per week, and the optimal duration of continuation treatment, which ranges from 2–3 years in various protocols.

**Omission of Prophylactic Cranial Irradiation**

Despite the well-recognized devastating complications associated with prophylactic cranial irradiation, including second cancers, neurocognitive impairment, and multiple endocrinopathy, this treatment is still used for up to 20% of patients judged to be at high risk of CNS relapse (eg, T-cell ALL with hyperleukocytosis or the presence of overt CNS leukemia [CNS3 status]). Historically, the reluctance to omit cranial irradiation in such cases could be attributed to concern not only over the risk of CNS relapse, but also the potential seeding of the bone marrow by residual leukemic cells from the CNS, the so-called "sanctuary site." There is also an ongoing debate over the optimal form of intrathecal therapy, owing to the mixed results of the randomized CCG 152 study for standard-risk ALL, in which triple intrathecal therapy resulted in a significantly lower incidence of isolated CNS relapse but more hematologic and testicular relapses (hence poorer survival), compared to treatment with intrathecal methotrexate. Conceivably, hematologic, testicular and CNS relapses are competing events, and improved CNS control with triple intrathecal therapy in the CCG 152 study might have favored leukemic relapse in other sites. Thus, effective systemic therapy is needed to realize the full benefit of triple intrathecal therapy. To this end, a recent meta-analysis showed that adding intravenous methotrexate to regimens incorporating triple intrathecal therapy improves outcome by reducing both CNS and non-CNS relapses, whereas adding it to those treated with intrathecal methotrexate yields little benefit.

Three studies (St. Jude Study XV, Dutch Childhood Oncology Group protocol ALL-9, and UKALL 2003), featuring effective systemic therapy, early intensification of intrathecal therapy, and omission of prophylactic cranial irradiation in all but the few patients with CNS3 status in the UKALL 2003 study, resulted in excellent 5-year event-free survival rates (85.6%, 81%, and 87.2%) and low isolated CNS relapse rates (2.7%, 2.6%, and 1.9%), respectively. In St. Jude Study XV, all 11 patients with an isolated CNS relapse remained in subsequent remission for 4–11 years after retrieval therapy, and in all likelihood are cured of their leukemia. With the exception of some adverse effects on complex fine-motor function observed among patients treated in the Dutch ALL-9 study and early attention deficits among those in St. Jude Study XV, global cognitive abilities were well preserved in patients treated without cranial irradiation. These results, together with the finding of a substantial risk of a second malignancy in the field of irradiation, even with 12 Gy cranial irradiation, should encourage others to eliminate CNS irradiation while optimizing chemotherapy for all patients in future studies.

**FUTURE DIRECTIONS**

Although protocol-directed therapy remains the best option for patients with cancer, it was estimated that only
56% of children with ALL were enrolled in COG protocols between 1990 and 2005 in the United States. Indeed, the low proportion of patients in the Surveillance, Epidemiology and End Results (SEER) program who had been treated in COG protocols may partly account for their inferior outcome as compared to that in single-institution studies. Hence, efforts should be made to improve protocol enrollment rates nationwide. Given the high cure rates being achieved in patients treated on contemporary protocols, those designing and conducting new leukemia treatment protocols face challenges that come from success: a radical change in treatment for all patients could jeopardize past gains in outcome, whereas modest changes are unlikely to yield significant improvements. Hence, current effort has focused increasingly on small subsets of patients with high-risk subtypes of leukemia. Recognizing the importance of international collaboration to advance the cure rates for these subtypes, Ponte di Legno Childhood ALL Working Group was formed in 1995 and has since been joined by virtually all major study groups in North America, Europe, and Asia. The collaborative efforts of this group have been very fruitful, identifying optimal treatment for specific subtypes of ALL and facilitating design of clinical trials by sharing unpublished data, and promise to make further advances in the field.

All existing antileukemic agents have acute toxic side effects, and many are fraught with the hazard of long-term sequelae, underscoring the need to develop more effective and less toxic targeted agents. In the coming decade, the decreasing cost and increasing availability of genomic analyses will permit the entire cancer and germline genomes of every child with ALL to be sequenced and genetics and epigenetic variations interrogated at diagnosis to guide the selection of agents for the individual patient. It is also hoped that new targeted therapies will emerge from discoveries of driver mutations that can be reversed or mitigated with small molecules. In the meantime, new formulations of some existing agents (eg, PEG-asparaginase and sphingosomal vincristine) may be less toxic for patients, and new nucleoside analogues (eg, clofarabine), monoclonal antibodies (eg, rituximab, inotuzumab, and blinatumomab), genetically modified T cells or natural killer cells, and molecularly targeted agents (eg, tyrosine kinase inhibitors and proteasome inhibitors) may improve cure rates for selected groups of high-risk patients.

### Table 4. Selected Developmental Therapeutics in Childhood Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>Category and Agent</th>
<th>Properties</th>
<th>Selected References</th>
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<tbody>
<tr>
<td><strong>New formulations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pegylated asparaginase</td>
<td>Long half-life, reduced immunogenicity</td>
<td>92</td>
</tr>
<tr>
<td>Liposomal vincristine</td>
<td>Enhances target-tissue delivery, decreases neurotoxicity, non-vesicant</td>
<td>93</td>
</tr>
<tr>
<td><strong>Nucleoside analogues</strong></td>
<td></td>
<td></td>
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<tr>
<td>Clofarabine</td>
<td>Less neurotoxicity, effective for some refractory ALL cases</td>
<td>94</td>
</tr>
<tr>
<td>Nelarabine</td>
<td>Selective for T-cell ALL, neurotoxic</td>
<td>95</td>
</tr>
<tr>
<td><strong>Monoclonal antibodies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rituximab (anti-CD20)</td>
<td>Potentiates chemotherapy for CD20⁺ B-ALL</td>
<td>96</td>
</tr>
<tr>
<td>Inotuzumab (anti-CD22)</td>
<td>Potentiates chemotherapy for CD22⁺ B-ALL</td>
<td>97</td>
</tr>
<tr>
<td>Blinatumomab</td>
<td>Bispecific antibody directing CD3⁺ T-cell against CD19⁺ ALL</td>
<td>98</td>
</tr>
<tr>
<td><strong>Combotox</strong></td>
<td>Combination of anti-CD19 and anti-CD22 deglycosylated ricin A chain immunotoxin</td>
<td>99</td>
</tr>
<tr>
<td><strong>Cellular therapies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CD19 chimeric antigen receptor-modified T cells</td>
<td>Genetically modified T cells against CD19⁺ ALL</td>
<td>100</td>
</tr>
<tr>
<td>Anti-CD19 chimeric antigen receptor-modified natural killer cells</td>
<td>Genetically modified natural killer cells against CD19⁺ ALL</td>
<td>101</td>
</tr>
<tr>
<td>Molecularly targeted agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABL tyrosine kinase inhibitor (imatinib, dasatinib)</td>
<td>Targets BCR-ABL1⁺ ALL</td>
<td>14,102,103</td>
</tr>
<tr>
<td>Proteasome inhibitor (bortezomib)</td>
<td>Potentiates chemotherapy</td>
<td>104</td>
</tr>
<tr>
<td>JAK1/2 inhibitor (ruxolitinib)</td>
<td>Preclinical in vivo efficacy against ALL with JAK genomic lesions</td>
<td>105</td>
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</table>
(Table 4). Various other new agents under investigation in the relapsed setting or preclinical models were summarized in a recent review. Finally, ongoing genome-wide association studies promise to identify additional inherited polymorphisms that are not only associated with the response to treatment, but also with the risk of leukemic transformation, opening the way for the development of potential preventive measures.

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