Section 1 History and general issues

Chapter 1

History of leukemia: historical perspectives

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Introduction

The best way to study variable human disease in variable human beings is to study variable human disease in variable human beings.

Philip Schein and Barbara Scheffler (paraphrase)[1]

The last half century has witnessed striking progress in the treatment of childhood leukemia, largely by empiric manipulation of a rather limited chemotherapeutic armamentarium through well-designed clinical studies.[2] Approximately 50 years ago, the first attempts to cure this dread disease were dismissed as futile and even cruel in providing unrealistic hopes to desperate families. However, improved supportive care has allowed patients to survive through periods of perilous pancytopenia, induced by the disease and its treatment, and cure has become routine, albeit not universal, in economically privileged countries. Outcomes remain poor for relapsed patients and for those in economically underprivileged countries where treatment may not be accessible.[3]

Appreciation of the biology of leukemia has advanced from clinical descriptions, through cytomorphology and histochemistry, to molecular genetics. The pace has quickened perceptively since the start of the twenty-first century. Genetic studies, at first focused on the single most visually obvious chromosomal abnormality by karyotype, are now expanded to genome-wide analyses[4,5] and beyond genomics to epigenomics. We are moving from a descriptive catalogue of genetic abnormalities to an appreciation of cooperating functional aberrations affecting differentiation, apoptosis, and proliferation. We not only divide leukemia into major clinical/cytomorphologic subsets such as acute and chronic, lymphoid and myeloid but also recognize the tremendous heterogeneity within each subset. Patients sharing a genetic abnormality, such as t(1;19), may have differing associated abnormalities. Emerging evidence demonstrates oligoclonality and the consequences of clonal evolution.[6,7] Some investigators feel that the limits of indiscriminate intensification of therapy have been reached. However, improved event-free survival with intensified anthracycline treatment in acute myeloid leukemia (AML)[8,9] and with "augmented" postinduction intensification in acute lymphoblastic leukemia (ALL)[10] suggests that our bag of tricks is not yet completely empty. Recent clinical advances in Philadelphia chromosome-positive ALL[11] and chronic myelogenous leukemia (CML)[12] hint at the thrilling promise of coming decades.

This chapter focuses on the history of those leukemias that affect young people, namely ALL, a predominately pediatric disease; AML, a disease that affects more adults than children; and CML, which is found in young people only rarely.

Emerging appreciation of leukemia biology

Current understanding of the molecular biology of leukemia is provided in Chs. 7 and 8. The object here is to trace its development over time.

While the first written description of cancer may be traced back to Egypt in about 1600 BC, the first report of a patient with leukemia appeared only in 1827, about three and a half millennia later (Table 1.1).[13] Examination of blood cells was not possible until the advent of the compound microscope (1665–1673) by Robert Hooke and especially by Anton van Leeuwenhoeck.[13] Jan Swammerdam and Joseph Lieutaud were the first to describe red cells and white cells, in 1668 and 1749, respectively.[13] About 20 years later, William Hewson described the lymphocyte and the lymphatic system.[13] In 1845, the category of illnesses now called leukemia was linked to white blood cells in three independent reports by Rudolf Virchow (Fig. 1.1), John Hughes Bennett, and David Craigie.[13] Virchow coined the term "leukemia" in 1847.[13,28] A French physician, Alfred Donné, had already described patients with exceedingly high white blood counts with maturation arrest and differentiated them from purulence in his 1844 textbook, *Cours de Microscopie complimentaire des Etudes médicales*. His textbook presented observations he had made in extant correspondence 6 years earlier.[29] However, credit for the first report of leukemia may best belong to a second Frenchman, Alfred Velpeau, in 1827.[14]

Henry Fuller was the first to diagnose leukemia by microscopic examination of the blood in a living patient.[13] In 1850, he described the first reported case of childhood leukemia, in a 9-year-old girl. In a series of investigations, published in 1856, Virchow[13] distinguished leukemia from leukocytosis and proposed his theory of its cellular origins – still fundamental to our understanding of the disease today. In 1868, Ernst Neumann

Childhood Leukemias, 3rd edn, ed. Ching-Hon Pui. Published by Cambridge University Press.

Table 1.1. Evolving insights and technology

1600 BC	First written description of cancer
~1670	Examination of the blood with the compound microscope
1827	First clinical description of leukemia by Velpeau[14]
1847	Term "leukemia" coined by Virchow[15]
1872	Neumann concluded that leukemia is a disease of the bone marrow
1877	Ehrlich introduced histochemical staining[16]
1913	Acute and chronic, lymphoid and myeloid leukemias
1914	Boveri proposed that leukemia arises from a single cell through chromosomal changes[17]
1934	Flow cytometry[18]
~1960	Metaphase cytogenetics; Nowell and Hungerford describe the Philadelphia chromosome[19]
1975	Monoclonal antibodies[20]
1978	Host polymorphisms (thiopurine methyltransferase)[21]
1980	Fluorescent in situ hybridization[22]
1985	Polymerase chain reaction[23]
1996	Gene expression arrays[24]
1997	Comparative genomic hybridization[25]
1998	Minimal residual disease by the polymerase chain reaction[26,27]

Fig. 1.1. Rudolf Virchow, the father of leukemia research, established leukemia as a medical entity in the years 1845 and 1856.

first reported the bone marrow changes in leukemia and by 1872 concluded that leukemia was a disease of the bone marrow, but 20 years passed before his observations found widespread acceptance.[13,28] At the time, many believed that bone was a solid, impenetrable substance and could not accept that blood cells might move back and forth between the marrow and the peripheral blood. In 1876, Friedrich Mosler introduced the antemortem bone marrow puncture.[13,28]

Introduction of histochemical staining methods by the medical student and Nobel Laureate Paul Ehrlich in 1877 allowed discrimination among leukocyte subsets. Ehrlich first identified a primitive cell, which he described as the ancestor of the various hematopoietic lineages. Leukemic marrow involvement was noted in the absence of peripheral blood involvement – so-called aleukemic leukemia.[16] The observation that specific dyes affected specific cell types differently but reproducibly gave rise to the notion that chemicals might also have differential effects on different types of cell and serve as treatment. In 1900, Otto Nägeli identified the myeloblast and the lymphoblast. By 1913, leukemia was classified as ALL, AML, chronic lymphocytic leukemia (CLL), and CML.[13,28]

In 1914, Theodor Boveri suggested that cancer might arise from a single cell with genetic instability leading to chromosomal changes, some too small to be seen by microscopy and unresponsiveness to external growth regulation.[17] Two World Wars and a Great Depression intervened.

Metaphase cytogenetics appeared in the early 1960s. Better banding techniques led to increased chromosome detail.[30] However, leukemic blasts are labile and metaphase spreads may show only residual normal metaphases.[31] Interphase fluorescence in situ hybridization and the polymerase chain reaction (PCR) later supplemented karyotyping and identified abnormalities in the leukemia cells of an increasing majority of patients. Conventionally, patients are classified by the most visually obvious chromosomal abnormality. However, we now know that most patients with leukemia harbor multiple genetic abnormalities with losses outnumbering gains of genetic material.[4] Epigenetic changes complement genetic changes. Event-free survival varies not only among cytogenetic subsets but also within each subset. Patients sharing a single abnormality such as t(1;19) may have differing associated abnormalities. The word "associated" is chosen rather than "secondary" because which abnormalities are more important than others remains to be elucidated. A single patient may harbor a variety of subclones with overlapping but distinct, evolutionarily linked constellations of cooperating abnormalities.[32] The predominating clone at relapse may differ from the predominant clone at diagnosis, being a direct descendant or sharing a common ancestor.

Flow cytometric studies examined DNA content and cell membrane expression of lineage-associated membrane and cytoplasmic proteins, starting in the mid 1970s. Typical childhood early pre-B-ALL may be distinguished from pro-B-ALL, typically seen in infants, and T-cell ALL, typically seen in older boys. Leukemias harboring translocation t(8;14)(q11;q32) and associated translocations display a mature B-cell immunophenotype.

Initial response to remission-induction treatment emerged as a consistent prognostic factor. Leukemia at levels too low for microscopic recognition is termed minimal residual disease (MRD).[26] Quantitative PCR and flow cytometric examination have supplemented and may replace morphologic evaluation of peripheral blood and bone marrow aspirates for response

evaluation.[33] Measurement of MRD is a robust prognostic indicator.[34,35] However, some patients with undetectable MRD at remission may still relapse and some patients with persistent MRD are cured, emphasizing the paramount importance of treatment after remission induction.

Host factors contribute to outcome. An intriguing report links vincristine pharmacology and outcome.[36] Polymorphisms of thiopurine methyltransferase, first identified by R. Weinshilboum,[21] nucleoside transporters MRP4 and SLC29A1, and inosine triphosphate pyrophosphatase affect mercaptopurine metabolism.[37] Genome-wide association studies are linking specific polymorphisms to variations in drug effects[38] and the development of leukemia itself.[39]

Currently, we still follow Boveri[17] and understand leukemia as a phenotype resulting from heterogeneous constellations of genetic – including microRNAs[40] – and epigenetic changes. Differences among leukemia gene expression profiles are said to exceed differences between lung adenocarcinoma or melanoma and bladder cancer.[41,42] In some cases (e.g., t(12;21)), but not in others, (e.g., t(1;19)), an initial genetic change may arise prenatally.[43] The heterogeneity in treatment outcomes within specific genetic subtypes of leukemia may be related to differences in the cooperative mutations or in host factors, currently poorly defined. In no genetic subset is cure impossible or assured.

Leukemia is oligoclonal rather than clonal. Clonal evolution ultimately results in overt leukemia in affected patients and evolution continues after clinical presentation, resulting in relapse and refractory disease in some cases.[43] Relapse or refractory disease may arise from small, covert subclones.[6] Treatment failure may arise from increased blast proliferation or decreased cell death.[44] Reaccumulation of blasts at relapse provides further opportunity for clonal evolution. However, the molecular mechanisms of treatment failure – likely varied and possibly redundant – remain to be elucidated.

Interestingly, we are able to cure a large majority of patients with rather undifferentiated therapies.[45–48] A variety of seemingly different regimens yield similar results with similar prognostic factors. Exceptions may be Burkitt leukemia, often with a t(8;14) for which a brief fractionated alkylator therapy rather than a prolonged maintenance therapy seems crucial,[49,50] and now Philadelphia chromosome-positive ALL, where addition of a tyrosine kinase inhibitor to cytotoxic chemotherapy is essential.[11]

First treatments for leukemia

Attempts to treat leukemia began soon after its recognition. In 1865, Heinrich Lissauer administered Fowler's solution, a potassium bicarbonate-based solution of arsenic trioxide (potassium arsenite)[51] to a woman with CML, achieving temporary benefit (Table 1.2). Use of arsenicals continued into the 1930s,[56] only to experience a recent rebirth as arsenic trioxide (As_2O_3).[57] Blood transfusion was first applied to leukemia by Callender in London in 1873, also with only temporary benefit.[13] Transfusions were impeded by clotting and ignorance of blood groups, not corrected until 1900 by Karl Landsteiner.[13] X-rays were discovered by William Röntgen in 1895 and quickly applied to leukemia, resulting again in only transient benefit.[13] Remissions, disappearance of leukemia with recovery of normal hematopoiesis, remained rare anecdotes. The first likely cure was reported by Gloor in an adult in 1930, following arsenic trioxide, irradiation, and blood transfusion from two siblings, perhaps presaging later bone marrow transplantation.[13] In 1948, Bruce Wiseman commented that "a fresh point of view with respect to this disease would not be undesirable."[13,28]

Military research on mustard gas during World War I and II revealed its effects on the hematopoietic system, namely anemia, leukopenia, and thrombocytopenia. Lifting of wartime censorship led to clinical trials in the USA and the UK that showed the benefit of methyl-bis-(β-chloroethyl)amine, a nitrogen mustard, in lymphoma, particularly Hodgkin disease, similar to that obtained with radiotherapy.[13] Some patients who had become resistant to irradiation responded to nitrogen mustard. Durable responses were more common in lymphoma than

Table 1.2. Emergence of conventional anti-leukemic drugs

1865	Lissauer administered potassium arsenite to a woman with chronic myelogenous leukemia[14]
1895	Radiation therapy provides only transient benefit
1930	Gloor reported a "cure" following arsenic trioxide, irradiation, and transfusion, perhaps the first successful hematopoietic stem cell transplant[13,28]
1943	Isolation of folic acid[52]
1948	Nitrogen mustard for Hodgkin disease[53] Antifols: aminopterin then methotrexate (amethopterin) for acute lymphoblastic leukemia[54]
1951	Adrenocorticotropic hormone then prednisone for acute lymphoblastic leukemia[55]
1953	Mercaptopurine, methotrexate licensed by the FDA
1955	Prednisone licensed by FDA
1958	Dexamethasone licensed by FDA
1959	Cyclophosphamide licensed by FDA
1963	Vincristine licensed by FDA
1969	Cytarabine licensed by FDA
1978	Native L-asparaginase licensed by FDA
1979	Daunorubicin licensed by FDA
1983	Etoposide licensed by FDA
1987	Mitoxantrone licensed by FDA
1994	Pegylated L-asparaginase licensed by FDA
1995	All-*trans*-retinoic acid approved for acute promyelocytic leukemia
2000	Arsenic trioxide licensed for acute promyelocytic leukemia by FDA
2001	Imatinib licensed for chronic myelogenous leukemia by FDA
2004	Clofarabine licensed by FDA
2005	Nelarabine licensed by FDA

FDA, US Food and Drug Administration.

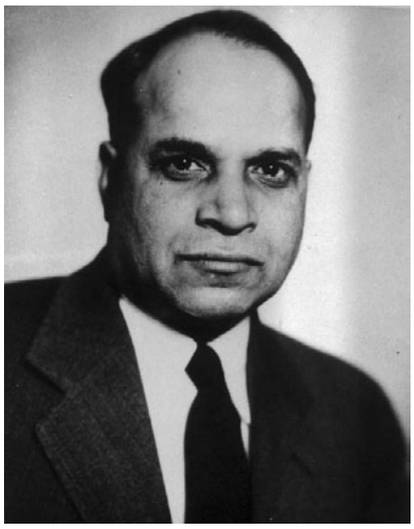

Fig. 1.2. Yellapragada Subbarao developed a method to synthesize folic acid and, with considerable input from Dr. Sidney Farber, he subsequently developed methotrexate to treat leukemia.

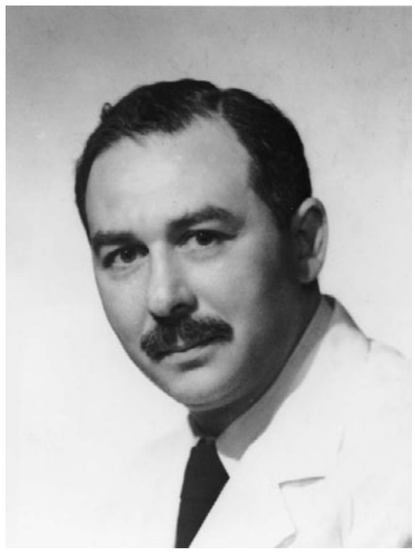

Fig. 1.3. Sidney Farber and his colleagues discovered that a synthetic anti-folate, 4-amino-pteroylglutamic acid, produced remissions of childhood leukemia, leading to antimetabolite chemotherapy and a cure for many children with leukemia.

Fig. 1.4 Gertrude Elion, recipient of the Nobel Prize in 1988, working with George Hitchings, used innovative methods to develop a number of drugs: mercaptopurine, allopurinol, pyrimethamine, trimethoprim and acyclovir.

in leukemia. In the absence of better alternatives, investigators found reason for optimism and work continued to seek more effective, less-toxic alkylating agents. Cyclophosphamide was synthesized in 1959 and still remains in use.[13,28]

Folic acid, a B vitamin, was recognized as crucial to hematopoiesis in 1943 and was chemically isolated in 1946.[13,28] Folate overcomes the maturation arrest in megaloblastic anemia and people speculated that folic acid might overcome the maturation arrest in leukemia. In some experiments, folic acid retarded cancer growth while in others it enhanced it. Heinle and Welch[58] observed that a folate-deficient diet caused a decrease in peripheral blast count. Sidney Farber at Harvard Medical School treated 90 patients with various malignancies with folic acid analogues.[54,59,60] An acceleration of leukemic growth was noted in the bone marrows of treated children that had not been seen in untreated children, leading to a hypothesis that anti-folates (antifols) might suppress leukemic growth as rapidly dividing leukemia cells might require more folic acid than normal cells.[59,60]

Yellapragada Subbarao (Fig. 1.2) and colleagues provided Sidney Farber (Fig. 1.3) and Louis Diamond with a number of anti-fols for clinical trials. A "definite benefit" was obtained for 10 of 16 patients with aminopterin.[59] Five were reported in detail.[54] The response of the medical community and particularly the pediatric housestaff, who interacted most closely with these ultimately dying children and their families, was uniformly negative, despite the cautiously worded conclusions.[61] However, the results were confirmed by others. Amethopterin (subsequently known as methotrexate), with an affinity for dihydrofolate reductase 100 000 times greater than folic acid, replaced aminopterin.[62] The lympholytic effect of adrenal corticosteroids was noted, first in mice[63] and later in people.[64,65] Prednisone rapidly replaced adrenocorticotropic hormone. Then 6-mercaptopurine was synthesized, following from the laboratory investigations of purines by Gertrude Elion (Fig. 1.4) and George Hitchings.[66]

Clinical trials methodology

Several novel, perhaps revolutionary, concepts contribute greatly to progress:

- prospective tests of candidate treatment interventions
- adherence to a written document, the experimental protocol
- a prospective analysis plan
- multicenter collaboration for adequate accrual
- concurrent controls through random treatment allocation
- complete remission rates and event-free survival as surrogates for survival
- inclusion of all eligible patients in primary analyses: intent to treat.

Leukemia treatment was dictated by rigorous adherence to a written document, an experimental protocol. The idea seems obvious today but in the 1950s protocols were often dismissed as "cookbook" medicine. A researcher complained, "I can't tell you what I will do this afternoon until I look at my mice. How can I tell you what I will do tomorrow?" By the 1970s, Glidewell and Holland wrote, "A protocol was designed which specified in detail the application and evaluation of the therapeutic programs which were to be compared."[67]

The importance of statistics and collaboration were recognized early. Analyses were guided by a prospective plan. Cancer is fortunately rare in young people. Useful clinical trials require substantial accrual for statistical power. Institutions and investigators banded together in a number of cooperative groups, such as the Children's Cancer Study Group, Cancer and Acute Leukemia Group B, and the Southwest Oncology Group. The Pediatric Oncology Group emerged from the Southwest Oncology Group and, in 2000, joined with the Children's Cancer Group to form the Children's Oncology Group. More traditional researchers, working alone or in small teams, scoffed at the notion of innovation by committee and despaired at the challenge of distributing intellectual or academic credit among the almost anonymous collaborating investigators.

Identification of a historical or concurrent comparison or control group is a critical step in clinical research. Gehan,[68] however, pointed out the critical contribution of nonrandomized trials in establishing new therapies. However, *randomization*, first employed in the negative studies of patulin for the common cold[69] and positive studies of streptomycin in tuberculosis,[70] provides concurrent controls for rigorous evaluation of candidate therapies. Some hold randomization unethical, arguing that physicians owe the patient their best treatment opinion, but most accept that mistaken opinion is frequent enough to make randomization an honest option. While an individual physician may hold a belief dearly, the uncertainty of knowledgeable colleagues provides adequate justification. Random allocation of therapies with adequate patient numbers provides the strongest test of efficacy. However, presentation of an option for randomization may leave the subject with exaggerated feelings of uncertainty and of personal responsibility for the therapy assigned. Historically, most randomized outcome trials show no difference among treatment regimens. Gains, when realized, are marginal –not all or none – and often at the expense of increased morbidity.

Remission, disappearance of all microscopically detectable leukemia and recovery of normal hematopoiesis, was found to add predictably to survival. James F. Holland, Emil Frei III, and colleagues in Acute Leukemia Group B noted that time in remission added to survival and proposed that duration of remission might serve as a measure of treatment efficacy.[67] Today, remission-induction rate, event-free survival (i.e., the time from diagnosis to induction failure, first relapse, second malignancy, or death), and disease-free survival (i.e., the time from first remission to first relapse, second malignancy, or death) remain useful determinants of treatment outcome.

All eligible patients are included in primary analyses. Naive intuition suggests that analyses might best be restricted to the patients who actually receive the treatment under study. *Intent to treat* forbids exclusion of any eligible patients, including those who receive therapy differing than that prescribed by protocol for any reason. Some may understandably wonder how a patient who does not receive prescribed therapy can contribute to its assessment. However, rules for exclusion are rarely prospective and provide an opportunity for bias. Prospectively, one cannot predict whether a patient or physician will or will not tolerate or comply with planned therapy and, therefore, the "intent to treat" strategy provides the best, least biased estimate of efficacy.

Supportive care

In describing 40 years of therapy, Freireich commented: "In the early days when children were given methotrexate in a typical leukemia ward, there was not only blood on patients and on their beds but on the walls and on the windows."[71] During the 100 years between Virchow's establishment of leukemia as an entity and the advent of alkylating agents, comforting the patient with narcotics and human empathy was the first consideration. Today, many patients are cured, but empathy and attention to symptoms remain crucial to maximizing a child's chance for recovery, by aiding a distressed family to cope with a terrifying diagnosis, to form a team with the medical personnel, and to participate knowledgeably in the child's sometimes challenging treatment (Table 1.3).

Transfusion therapy

Credit for discovery of the circulation of the blood is generally given to William Harvey in 1616.[89,90] Andrea Cesalpino, an Italian, may in fact have preceded Harvey in this discovery. The concept of transfusion soon followed and several early and usually unsuccessful attempts were documented in the mid seventeenth century. Deaths led to bans in Paris, Rome, and London, which remained in place for 150 years. In 1828, Blundell, motivated by the plight of women with postpartum hemorrhage, advocated human–human transfusion in place of the more convenient animal–human transfusion and reported a successful direct blood transfusion in a woman with postpartum hemorrhage.[89,90] However, severe unpredictable reactions discouraged further use. Landsteiner's identification of human blood groups in 1901 enabled safer blood transfusion.[73,91] During World War I, Rous and Turner discovered that a citrate dextrose solution and cold would preserve red blood cells. Robertson, an American Army surgeon who had worked with Rous, used this solution and packing boxes containing ice to preserve human red blood cells for prompt transfusion of wounded soldiers near the battle lines.[89]

For children with acute leukemia, the introduction of the hospital blood bank in 1937 was the first step in prolonging their lives.[74] By the late 1940s, blood transfusions together with the newly available antibacterial agents became generally accepted

Table 1.3. Time line: supportive care and hematopoietic stem cell transplantation

1873	Callender treated a leukemia patient with blood transfusion[72]
1901	Landsteiner described human blood groups[73]
1930	Gloor reported a "cure" following arsenic trioxide, irradiation, and transfusion, perhaps the first successful hematopoietic stem cell transplant[28]
1937	First hospital blood bank[74]
1954	Platelet transfusion[75]
1957	Successful syngeneic bone marrow transplantation[76]
1968	First successful sibling donor bone marrow transplant for immunodeficiency[77]
1972	Carbenicillin for Gram-negative infections[78] Empiric antibiotics[79] Human leukocyte antigen typing[80,81]
1972	Successful matched sibling donor marrow transplantation for aplastic anemia[82]
1974	Anthony Nolan Bone Marrow Donor Registry founded in the UK (matched unrelated donors)[83]
1977	18 survivors at >1 year among 110 patients with advanced leukemia transplanted from matched donors[84]
1979	Success >50% for matched sibling donor marrow transplantation for acute myeloid leukemia in first remission[85]
1985	US National Institutes of Health Technology Assessment Meeting concluded that a national marrow donor registry program is "premature"[86,87]
1986	National Marrow Donor Registry Program (USA)[86,87]
1989	First successful transplant using umbilical cord blood[88]

as a way of maintaining life while families tried to adapt to the prognosis and begin their grieving.

Risk of bleeding was linked to platelet count. In 1954, with the advent of plastic blood transfusion and transfer bags and the use of the refrigerated centrifuge, platelet transfusion became available to control thrombocytopenic bleeding. This resulted in a remarkable reduction in hemorrhage as a cause of death. Platelet transfusions also provided time and opportunity for emerging anti-leukemic drugs to produce remission, and this led to increased rates of remission induction.[75,92] Comparing 1954–1959 and 1960–1963, the percentage of leukemia deaths attributed to hemorrhage fell from 67 to 37%.[93] The routine availability of platelet transfusions allowed administration of higher and more prolonged dosages of myelosuppressive agents because one could support patients through periods of drug-induced thrombocytopenia.

Tumor lysis syndrome

When effective chemotherapy was first employed in acute leukemia, rapid lysis of leukemia cells often resulted in serious and occasionally fatal metabolic disturbances, particularly in patients with high white blood cell counts and/or massive organ involvement. Hyperuricosuria leads to precipitation of uric acid crystals in the renal tubules, resulting in renal impairment and exacerbating hyperkalemia, hyperphosphatemia, and hypocalcemia. The introduction of allopurinol in the early 1960s, a synthetic inhibitor of xanthine oxidase developed by Gertrude Elion and George Hitchings, along with skillful fluid and electrolyte therapy did much to ameliorate this problem.[94] The recent development of aspergillus-derived urate oxidase provides a means to break down existing blood uric acid to highly soluble allantoin.[95] Neither agent affects existing uric acid crystals in the renal tubules.

Neutropenia and fever

Awareness of the association of acute leukemia and infection dates back to at least 1845. Prior to the era of effective chemotherapy, infection drew little attention, not meriting even a mention in a 1958 textbook on acute leukemia. Early in the era of combination chemotherapy, severe and often fatal infection, particularly secondary infection or superinfection, particularly with Gram-positive bacteria such as *Staphylococcus aureus*, Gram-negative bacteria such as *Pseudomonas aeruginosa*, and fungi, posed a major challenge.[96] Boggs[97] established the link between neutropenia and infection but argued that fever alone was insufficient reason to start antibiotics. Bodey and associates[98] found the risk of infection at 5/1000 days of neutropenia for neutrophil count between 0.5×10^9 and $<1 \times 10^9$/L and 43/1000 days for neutrophil count of $<0.5 \times 10^9$/L. The risk for infection increased with duration of neutropenia. Neutropenia diminished the signs and symptoms of infection. Patients with overt leukemia were at greater risk for infection, independent of neutrophil count. Better outcomes were associated with neutrophil recovery. In 1960, Raab *et al.*[99] allowed severely ill patients to receive antibiotics prior to any positive culture but argued that antibiotics might be withdrawn after 5 days in the absence of positive cultures. Between 1954 and 1964, infection remained a cause of death in two-thirds of leukemia patients, despite effective treatment of *S. aureus* with methicillin. Both *P. aeruginosa* infection and invasive fungal infection increased. Autopsy studies showed that most major infections had escaped identification antemortem.[100] While methicillin decreased the mortality from *S. aureus*, carbenicillin, not gentamicin, had the first substantial effect on *P. aeruginosa*.[101,102] Infections with resistant Gram-positive cocci have become a problem in the past 20 years, as reliance on indwelling catheters for central venous access has increased, prompting the greater use of vancomycin in patients with staphylococcal or enterococcal infections and neutropenia.[103]

The current practice of using empiric antibiotics only emerged in the early 1970s. In the early 1980s, Pizzo and colleagues at the National Cancer Institute[79,104] established the need for continued antibiotics until neutrophil recovery and introduced the notion of empiric antifungal therapy. Development of less-toxic formulations of amphotericin B in the 1990s, effective anti-*Aspergillus* azoles (e.g., voriconazole and posaconazole) and echinocandins have markedly improved the treatment of disseminated fungal infection.[105]

Viral infection

As children survived longer, the profound immunosuppression caused by chemotherapy even with neutrophil recovery became more evident. Measles vaccination prior to diagnosis prevented infection but varicella remained a major problem. In the 1970s, children with leukemia in remission commonly died of disseminated varicella, and others had long periods of treatment interruption with consequently increased risk of relapse.[106] At first, plasma from adults convalescing from varicella zoster was used for varicella prevention or modulation in recently exposed children. After convalescent plasma was found useful, a varicella zoster immunoglobulin (VZIG) was produced.[107] The access to VZIG and education of parents and teachers about the hazards of varicella zoster exposure were a major advance in reducing mortality, morbidity, and treatment interruptions. However, the third contribution of Gertrude Elion to children with leukemia, the development of acyclovir in 1980, is perhaps yet more important.[108–110] Although patients with leukemia in remission may be immunized for varicella, debate continues regarding the need for this procedure because of the current negligible rate of fatal varicella, the need to interrupt chemotherapy for the vaccination, and any complications of vaccination. On October 27, 2004, the Advisory Committee on Immunization Practices (ACIP) was informed by the only US-licensed manufacturer of VZIG (Massachusetts Public Health Biologic Laboratories, Boston, MA) that the company had discontinued production of VZIG.[111]

Pneumocystis pneumonia

Shortly after intensive multiagent therapy was introduced for acute leukemia at St. Jude Children's Research Hospital, a peculiar pneumonia began to appear in many of the children. At first it was called "St. Jude pneumonia" and was thought to be related to drug toxicity, a viral infection, or both. However, autopsy study of the lungs and methenamine silver nitrate staining of pulmonary needle aspirates from patients revealed *Pneumocystis jiroveci* (previously known as *Pneumocystis carinii*) organisms,[112] now known to be a fungus.[113] An institutional epidemiologic study performed in collaboration with the Federal Communicable Disease Center (CDC) indicated that the disease was solely related to immunosuppression and not contagion.[114] Again, this disease became a major limiting factor in treating children with acute leukemia because of its occurrence during remission, its mortality and morbidity, and the consequent interruption of chemotherapy, particularly in critical early months of treatment. Pentamidine isethionate was used as treatment initially.[115] Finally, the brilliant studies of Hughes and colleagues, first in mice and then in patients, demonstrated the value of trimethoprim (yet another contribution of Gertrude Elion and George Hitchings) in combination with sulfamethoxazole (cotrimoxazole) not only in treatment but, more importantly, in prevention.[116,117]

Bone marrow transplantation

In 1957, Barnes *et al.*[118] administered usually lethal doses of total body irradiation to leukemic mice with or without subsequent homologous bone marrow transplants. The mice that received marrow homografts tended to survive without leukemia but died of a wasting disease; those that did not receive grafts had recurrence of leukemia. This led the investigators to suggest that the grafts had an anti-leukemic effect and this stimulated similar experiments in humans. With the introduction of human leukocyte antigen (HLA) typing and matching, from the work of Nobel Laureate Jean Dausset,[119] Nobel Laureate Thomas and colleagues achieved successful treatment of leukemia by myeloablation with total body irradiation and chemotherapy and subsequent marrow transplantation from an HLA-compatible sibling.[120,121] Over the years, success has been achieved with matched unrelated marrow, matched related and unrelated peripheral blood hematopoietic stem cells, and cord blood.[122] The name of the procedure has evolved from bone marrow transplantation to hematopoietic stem cell transplant (HSCT) as stem cell sources have expanded.

Definition of the proper role of HSCT in leukemia has been hindered by patient selection and lack of randomized comparative studies, and confounded by continuing improvements in conventional leukemia therapy.[123,124] The sequelae in children, such as chronic graft-versus-host disease, multiorgan impairment, and growth failure,[125] often preclude true cure (i.e., restoration of the capacity for normal growth, development, and health, as well as freedom from leukemia). However, some patients for whom cure was unlikely with conventional chemotherapy survive without leukemia and lead largely normal lives. Use of HSCT offered cure in CML that otherwise was only palliated by chemotherapy with busulfan or hydroxyurea in the pre-imatinib era. The same is true for juvenile myelomonocytic leukemia, myelodysplasia/myeloid leukemia associated with chromosomal monosomy 7,[126] and AML that fails to respond to or relapses after intensive chemotherapy.[127] Most agree that evidence from non-randomized comparisons suggest an advantage for transplantation for children with ALL in second remission after an early marrow relapse.[128] The role of HSCT is evolving. Introduction of tyrosine kinase inhibitors has decreased the role of transplant in CML.[129] In AML, improved outcomes with chemotherapy have limited the role of transplantation in acute promyelocytic leukemia (APL) and core-binding factor leukemias. In fact, most European pediatric AML groups are severely limiting stem cell transplantation in first complete remission.[130,131]

The use of HSCT is presented in detail in Ch. 22.

Cure

New psychosocial issues emerged as children survived longer. Farber and associates recognized early the need for "total care" for children with acute leukemia.[132] In 1964, Vernick and Karon advocated truthfulness in communication with children.[133]

Table 1.4. Landmarks: childhood acute lymphoblastic leukemia (ALL)

1850	First likely reported case of pediatric ALL by Fuller[135]
1913	Acute and chronic, lymphoid and myeloid leukemias[13,28]
1943	Folic acid in normal hematopoiesis[52]
1948	Transient "remissions" with aminopterin[54]
1956	20% 1-year survival[136]
1965	Vincristine and prednisone for remission induction[137]
1967	Effective presymptomatic CNS therapy with craniospinal irradiation cures 50% of children at St. Jude Children's Research Hospital[138]
1967	High-dose methotrexate and leucovorin rescue[139,140]
1973	Prognostic significance of time to remission[141]
1975	Immunophenotype[142,143]
1976	Berlin Frankfurt Münster Group introduces protocol II or delayed intensification (publication 1981)[144] Dana Farber Cancer Institute introduces extended weekly asparaginase (publication 1983)[145]
1981	Common ALL antigen[146]
1986	Treatment allocation by day 8 peripheral blood response by the Berlin Frankfurt Münster Group[147]
1988	Treatment allocation by day 8 marrow response by the Children's Cancer Group[148]
1993	*MLL* rearrangements in infant ALL[149,150]
1997	Presence of t(12;21) in umbilical cord blood[151,152]
1998	Prognostic significance of submicroscopic minimal residual disease
1999	Deletion or sequence mutation of *Ikaros* in *BCR-ABL*-negative ALL[153,154]
2000	Treatment allocation by day 29/85 minimal residual disease by the Berlin Frankfurt Münster Group[47]
2007	Imatinib + cytotoxic chemotherapy for Philadelphia chromosome-positive ALL[11]
2009	Elimination of cranial irradiation[155] Early T-cell precursor subset[156]

Anticipating the significance of survival quality, Soni and colleagues[134] pioneered longitudinal studies of neuropsychologic consequences of acute leukemia and its treatment.[134] Other late effects have also been studied extensively with the goal of defining the human cost–benefit ratio for each element of leukemia therapy.

Childhood acute lymphoblastic leukemia

By 1953, three drugs with recognized anti-leukemic activity were available: methotrexate, prednisone, and 6-mercaptopurine. In the 1940s, patulin provided the first example of a randomized trial.[69] Success with treatment of tuberculosis, requiring multiple agents and prolonged therapy, provided guidance in the early treatment of ALL (Table 1.4).

Vincristine appeared in 1962, an anti-tubulin plant alkaloid with substantial activity in otherwise resistant disease.[157,158] Stimulated by the work of Skipper *et al.*[159–161] and Goldin *et al.*[162–164] in mouse leukemia and the success of multiagent

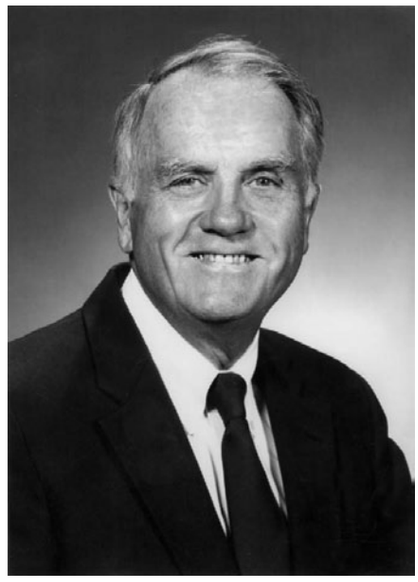

Fig. 1.5 Donald Pinkel, first director of St. Jude Children's Research Hospital, developed "total therapy studies" and led to a 50% cure rate of childhood acute lymphoblastic leukemia in late 1960s.

therapy in Hodgkin disease[165] and tuberculosis, drugs were used in two-drug and four-drug combinations and therapy evolved from sequential single agent to multiagent combinations.[166]

Multiple institutions banded together in pediatric and adult cooperative groups, such as the Children's Cancer Study Group (later the Children's Cancer Group and now in alliance with the Pediatric Oncology Group, to become the Children's Oncology Group) and the Acute Leukemia Group B in the 1950s to obtain patient numbers sufficient for useful clinical trials. In the early 1960s, 5-year "cure" rates were still in the 3–5% range for children. Crowther[167] asserted that "treatment" might actually shorten survival in adults. Pessimism prevailed and those who pursued anything beyond palliation were met with skepticism, if not scorn.

The 1-year survival was less than 20% in initial efforts in 1956 and 1957.[136] Remissions were obtained with vincristine and prednisone after 1966, and intensified with short courses of cyclophosphamide, 6-mercaptopurine, or methotrexate. Methotrexate was identified as the most active postinduction agent. Additional benefit was shown for 6-mercaptopurine and intrathecal methotrexate. The 3-year survivals – with or without leukemia – reached 50%.[136] Asparaginase[168,169] emerged from observations on the effects of guinea pig serum on lymphoblasts.[170] However, cure rates remained low. In 1965, Sutow *et al.*[171] concluded, "the present score shows no cures induced, minimal success with long-term palliation in most patients, and no information on preventative approaches."

St. Jude Children's Research Hospital was founded in Memphis, Tennessee in 1962 with the mandate to find an answer to childhood leukemia. Investigators, led by Donald Pinkel (Fig. 1.5), identified four main obstacles to cure. (1) Drug resistance as primary resistance, a substantial number of patients failed to achieve remission on single-agent therapy, and as

secondary resistance, most patients relapsed despite continued once-effective therapy. (2) Isolated central nervous system (CNS) relapse: as marrow control improved, a growing percentage of remissions ended with the appearance of leukemic blasts in the cerebrospinal fluid despite continued marrow remission. (3) The overlapping toxicities of anti-cancer drugs, particularly pancytopenia and immunosuppression. (4) A prevailing belief that cures were impossible.[138] Physicians cure patients in their minds before they cure them in their clinics.

Four components of therapy emerged and are still employed today, namely, remission induction, presymptomatic CNS therapy, postinduction intensification, and prolonged continuation therapy. Early trials had demonstrated the inadequacy of lower dose (5–12 Gy) craniospinal irradiation and the advantage of full-dose over half-dose continuation therapy. Event-free survival improved and an increasing number of children achieved cure. In addition to relapse, *P. jiroveci* pneumonia and herpesvirus posed obstacles to cure. In 1967, introduction of higher-dose craniospinal irradiation (24 Gy) led to cure of approximately one-half of patients.[172] This finding was confirmed in a later randomized trial and then in multiple centers around the world.[138]

Many have built on this work and cure rates now surpass 80%. Several strategies of postinduction intensification have been employed: intensive parenteral methotrexate by St. Jude, the Pediatric Oncology Group (now Children's Oncology Group), and the Nordic Pediatric Hematology Oncology Group; prolonged weekly asparaginase by the Dana Farber Cancer Institute,[141] based on early work by Barbara Jones and the Cancer and Leukemia Group B;[173] and reinduction/reconsolidation (i.e., protocol II or delayed intensification) first by Hansjorg Riehm and the Berlin Frankfurt Münster Group[47,144] and later by the Children's Cancer Group (now Children's Oncology Group)[45,148,174] and the Medical Research Council of Great Britain.[175] Better postinduction intensification and intrathecal methotrexate have allowed reduction or elimination of neuraxis irradiation for most or perhaps all patients.[155] With few exceptions such as imatinib, gains have been won through better use of agents available prior to 1970 – forty years ago. Attempts to shorten therapy to less than 2 years have been unsuccessful.[147]

Therapy is generally assigned on the basis of estimated risk of relapse – determined by age, white blood count, immunophenotype, and extramedullary disease, with increasing attention to genetics and initial response to therapy.[26,33] Early response to therapy may be determined by flow cytometry and quantitative PCR. Preliminary success has been reported in defining risk of relapse by gene expression arrays.[41,176]

The quest for "species-specific" therapy within ALL has proved elusive. Differences between ALL, which responds to methotrexate and glucocorticoids, and AML, which generally does not, were apparent early.

Patients with lower risk ALL have enjoyed gains similar to those enjoyed by patients with higher risk disease. Patients with T-cell ALL benefit from interventions found helpful in those with precursor B-cell disease. Identification of patients that share a therapeutic vulnerability is key. Candidate agents such as nelarabine in T-cell disease[177,178] and inotuzumab ozogamicin in precursor B-cell disease[179] present new possibilities for species specificity. The recent success of the combination of a tyrosine kinase inhibitor, imatinib, with cytotoxic chemotherapy provides the first example of true species specificity and a paradigm for the future. New international collaborations, such as the Ponte di Legno Working Group, which was founded in 1995, brings together national study committees to enhance communication and lay the groundwork for the greater collaboration necessary for the international studies required to accrue adequate numbers of patient subsets such as infants and those with Philadelphia chromosome-positive ALL.

Cure has become routine, albeit not universal, in the economically privileged countries. With better outcomes has come increasing recognition of the long-term effects of treatment on cognitive function and bone health. Issues of psychosocial adaptation have become apparent. Therapy may be unavailable to many children in the developing world, where a large majority of the world's children live. Outcomes after relapse, particularly marrow relapse within 3 years of diagnosis, remain dismal despite frequent second and subsequent remissions and increasing use of HSCT.[180]

Acute myeloid leukemia

During the first twenty years of the last century, the diagnosis of AML was made by morphology and histochemistry. Cytogenetics in the 1960s first identified recurring chromosomal abnormalities. The translocation of chromosomes 8 and 21 or inversion of chromosome 16 was associated with a favorable prognosis. In contrast, those individuals who have loss of one chromosome 7, or three or more clonal chromosomal changes have a poor prognosis.[28] In 1999, the World Health Organization (WHO) classification adopted the use of cytogenetics to differentiate between the subtypes of AML.[181] Patients with normal karyotype and abnormal nucleophosmin have a better prognosis and those with internal tandem duplication of *FLT3* have an inferior prognosis.[182] Young patients with Down syndrome or patients with AML characterized by t(8;21), t(15;17), or inv(16) cytogenetic abnormalities and rapid early response to induction therapy are generally classified as having favorable AML. Early response is measured either by MRD or more often by bone marrow response during or after the first course of chemotherapy. Unfavorable features include high white blood cell count, monosomy 7/del(7q) (-7/7q-), monosomy 5/del(5q) (-5/5q-), complex cytogenetics, and slow or no early response.[183–186] The emerging consensus is that patients with favorable AML do not benefit from HSCT in first remission.

During the past 25 years, the importance of describing the leukemia host has also become more apparent. In 1957, Krivit and Good[187] reported that children with trisomy 21 (Down) syndrome have a high incidence of leukemia, particularly acute

Table 1.5. Landmarks: acute myeloid leukemia (AML)

1968	Cytosine arabinoside for AML[190]
1969	Daunorubicin for AML[191]
1975	Combination therapy with cytosine arabinoside and daunorubicin[192]
1977	t(15;17) in acute promyelocytic leukemia[193]
1978	In vitro differentiation of AML cell lines[194]
1983	Core-binding factor mutations in AML: t(8;21) and inv(16)[195]
1985	All-*trans*-retinoic acid (ATRA) for acute promyelocytic leukemia[57]
1990	Altered retinoic acid receptor in acute promyelocytic leukemia[196]
1992	Molecular relapse in acute promyelocytic leukemia[197]
1994	Postinduction intensification with high-dose cytarabine[198]
1995	GATA-1 abnormalities in M7 AML in younger children with Down syndrome
1995	Uniparental disomy in acute leukemia in Down syndrome[199]
1999	Prognostic significance of *FLT3* internal tandem duplication in AML[200]
2002	Detection of t(8;21) translocation in cord blood of teenagers with AML[201]
2002	Cooperating class 1 and class 2 genetic or epigenetic alterations[202]
2005	Uniparental disomy common in AML[203]
2007	Benefit for detection of molecular relapse and pre-emptive therapy in acute promyelocytic leukemia[204]
2009	High-dose anthracycline in induction[8,9]

megakaryocytic leukemia. It was not until 1992 that the Pediatric Oncology Group discovered that patients with Down syndrome have twice the cure rate of other children with AML when treated with chemotherapy.[188]

Treatment of acute myeloid leukemia

While the majority of patients with ALL are children and adolescents, the large majority of patients with AML are adults. From the beginning of leukemia therapy, the morphologic differences in response to therapy were apparent: AML rarely responded to the corticosteroids and methotrexate, which are active in ALL.

The discovery of a natural supply of uracil arabinoside in extracts from sponges led to investigation of a number of pyrimidine analogues.[189] Cytosine arabinoside emerged as the most promising (Table 1.5). A variety of different doses and schedules were explored but even with the best regimens, complete remission rates were no better than 25%. In a large study involving 16 centers and 180 adult patients, the overall remission rate was 16%.[205] The use of the antibiotic daunorubicin was first reported in the early 1960s, with a 25% remission rate.[191]

Gee *et al.*[206] first reported a combination of cytosine arabinoside and thioguanine in 38 adults patients with AML, achieving a 38% complete remission and 10% partial response. A combination of cyclophosphamide, vincristine, cytosine arabinoside, and prednisone (COAP) provided a 53% remission rate.[207] Carey and associates from the Acute Leukaemia Group B[192] reported remission in approximately half of 227 evaluable cases in a large trial of combination chemotherapy. Various combinations of daunorubicin and cytosine arabinoside yielded complete remission rates approaching 60%.[208] By 1980, approximately 25–30% of unselected children with AML might be cured by intensive application of these drugs with skilled supportive care.[209]

Over the next 30 years, outcomes improved with increasing dose intensity and the cumulative dose limited as made feasible by improving supportive care.[130,131,210,211] The 5-year survival rates surpass 50% for children and younger adults. The Medical Research Council AML 10 and AML 12 report survivals of 68% with a relatively high cumulative exposure to cardiotoxic anthracycline and anthracenedione (roughly 450–550 mg/m^2).[130,212,213] Five cycles of therapy were no better than four cycles.[214] Others have found advantage for higher-dose anthracycline[8,9] or gemtuzumab ozogamicin in induction[215] and high-dose cytosine arabinoside in postinduction therapy.[198] Curiously, these interventions primarily benefit patients with the favorable core-binding factor leukemias.[216]

All-*trans*-retinoic acid (ATRA) and arsenic trioxide in APL deserves special comment. Hillestad[217] described this type of leukemia in three patients as one with a rapidly fatal course. Acute promyelocytic leukemia accounts for 10–15% of new cases of adults with AML in the USA. Chemotherapy was first employed against APL in 1967. Anthracyclines were introduced in 1973.[57] In 1977, Rowley and colleagues[193] first linked APL with the translocation t(15;17)(q22;q21). In 1978, Sachs[194] reported the ability of certain agents to induce differentiation in a number of AML cell lines. His work was furthered by others[218] and attention focused on retinoic acid in APL. Wang, Chen and co-workers at the Shanghai Rui Jin Hospital (now Shanghai Jiao Tong University School of Medicine)[219] began to screen potential differentiating agents against cell lines and patient samples in 1980. All-*trans*-retinoic acid had just been approved in Shanghai for treatment of acne and psoriasis and was found superior to *cis*-retinoic acid as a differentiating agent. In 1985, a first patient, a 5-year-old girl, was treated after failure of conventional therapy and achieved a remission. She received subsequent ATRA and conventional chemotherapy for a year and has remained in remission for 26 years at last report.[219] Six additional patients were treated, four at diagnosis and two after failure of initial therapy. All achieved complete remission. In 1988, Chen and co-workers reported 24 additional cases, 16 at diagnosis and 8 after failure of conventional cytotoxic therapy; 23 achieved remissions and the resistant patient achieved remission after addition of low-dose cytosine arabinoside.[219] In 1991, the t(15;17)(q24;q21) translocation *PML-RARA* (the promyelocytic leukemia gene and the gene for retinoic acid receptor-α) was cloned, and was shown to be the target of ATRA only in 1996.