Advances in Acute Lymphoblastic Leukemia

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DATA SOURCES: Current literature

DATA SYNTHESIS: Acute lymphoblastic leukemia (ALL) is a stem cell disorder characterized by an overproduction of lymphoblasts in the bone marrow that eventually spill into circulation, producing lymphocytosis. As with the other acute leukemias, the most common symptoms experienced by patients include fatigue, bleeding, and recurrent infections resulting from the suppression of normal hematopoiesis in the bone marrow by the accumulating blasts. ALL primarily affects children and exhibits the best response to standard chemotherapy as compared to acute myeloblastic leukemias (AML). Further, remission rates are highest among ALL patients, many of whom are experiencing sustained remissions suggesting cure. In light of early treatment successes, researchers began to investigate modifications of standard treatment regimens to accommodate variability in weight, age, and response to therapy among children with ALL. Individualized treatment plans were implemented where some patients received a reduced intensity course of therapy to minimize drug toxicity while others received drug intensification to maximize response. More recently, research efforts have been directed at the elucidation of leukemogenic mechanisms implicated in ALL to identify specific protein mutants that can be used to design drugs tailored to interfere with the activity of these mutant protein targets. Identification of chimeric proteins produced from chromosomal translocations and gene expression profiles from microarray analyses are the primary techniques used to identify the potential therapeutic targets.

CONCLUSION: Several reliable prognostic indicators have been identified and are being used to improve therapeutic planning and outcome prediction in ALL patients. Individualized treatment regimens have been developed based on the specific characteristics of each patient to minimize treatment related adverse events and maximize response. Through the use of cytogenetic, molecular, and microarray testing, ALL classification schemes have improved and potential therapeutic targets have been identified. It is anticipated that the next major advance in the treatment of ALL will involve the use of designer therapies developed to specifically interfere with particular molecular abnormalities producing the leukemogenic aberration to the normal signal transduction pathways.

ABBREVIATIONS: 6MP = 6-mercaptopurine; ABL = ableson oncogene found in a strain of mouse leukemia virus; ALL = acute lymphoblastic (lymphocytic) leukemia; BCR = breakpoint cluster region; CBF = core-binding factor; CCG = Children's Cancer Group; CDK = cyclin-dependent kinases; EFS = event free survival; FAB = French-American-British; HAT = histone acetyltransferase; HDAC = histone deacetylase; MLL = mixed-lineage leukemia; MRD = minimum residual disease; OPAL1 = outcome prediction for acute leukemia number 1; POG = Pediatric Oncology Group; RB = retinoblastoma; RFC = reduced folate carrier; TPMT = thiopurine methyltransferase; WHO = World Health Organization.

INDEX TERMS: acute lymphoblastic leukemia, acute lymphocytic leukemia, ALL, childhood leukemia, pediatric leukemia.

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Focus Continuing Education Credit*: see pages 247 to 249 for learning objectives, test questions, and application form.*

LEARNING OBJECTIVES

1. Briefly outline the FAB classification of acute lymphoblastic leukemia (ALL), the immunological revisions to the classification of ALL and the proposed changes to ALL classification schemes by the World Health Organization (WHO).

2. Discuss the prognostic indicators of ALL to include:
   a. clinical indicators.
   b. molecular indicators.
   c. therapeutic response indicators.
3. Briefly describe patient stratification strategies using:
   a. risk assessment (as predicted by clinical and cytogenetic data).
   b. response to therapy.
   c. microarray profiling.
4. Identify potential therapeutic targets with a focus on chimeric proteins produced from chromosomal translocation and mutations in proteins affecting tumor suppressor gene pathways.

Acute lymphoblastic (lymphocytic) leukemia (ALL) is a stem cell disorder in which the bone marrow produces an increased number of blasts that accumulate and eventually spill into circulation. It is thought that the disease begins with genetic mutations that occur in a hematopoietic stem cell. These mutations accumulate in the stem cell to produce three general functional impairments: increased rate of proliferation by increasing self-renewal and resistance to negative growth controls, loss of differentiation beyond the blast stage, and reduced apoptotic death.[1] The types of genetic mutations identified thus far in ALL can be placed into one of three general categories: aberrant expression of proto-oncogenes, chimeric protein kinases and transcription factors resulting from chromosomal translocations, and hyperdiploidy involving more than 50 chromosomes.[2] These cellular aberrations cause patients to present with many of the peripheral blood findings commonly associated with most forms of acute leukemia to include increased WBC count, blasts in circulation, neutropenia, anemia, and thrombocytopenia. The presence of blasts in the peripheral blood is due to their release from the overcrowded bone marrow accounting for the elevated WBC count. The neutropenia, anemia, and thrombocytopenia result from the inhibition of normal hematopoiesis by the accumulating bone marrow blasts that deprive the normal hematopoietic tissue of space, nutrients, and growth factors. Failure of bone marrow or other organ systems resulting from the deposition and accumulation of blasts is responsible for the morbidity and mortality experienced in ALL patients.

ALL is primarily considered a leukemia of childhood but it can occur in individuals of any age. The prevalence of ALL is bimodal with the majority of cases occurring between the ages of 2 and 10 years and the remaining minority occurring in the elderly.[3] Prior to the 1960s, treatment success was dismal in all forms of acute leukemia, to include ALL, both in children and in adults. However, the introduction of chemotherapeutic agents significantly improved outcomes for patients with ALL compared to the other acute leukemias. Today, the five-year event-free survival (EFS) rate in children with ALL is 80% while in adult ALL patients it is 40%.[4,5] Children presenting with one type of ALL known to have a good prognosis are experiencing complete remission rates of 90% with approximately 60% of those being potentially cured.[6] In contrast, adults experience a complete remission rate of 68% to 91% with an estimated cure rate of 25% to 41%.[7]

Classification of ALL has changed over the years and is still evolving. The first universally accepted classification scheme was the French-American-British (FAB) system that relied primarily on morphologic criteria. Once cytochemical staining confirmed the blast lineage as lymphoid, ALL patients could be classified into one of three categories; L1, L2, or L3, based on the morphology of the lymphoblasts. Generally, L1 lymphoblasts were small and homogeneous, L2 lymphoblasts were large and heterogeneous, and L3 lymphoblasts were large, homogeneous, dark staining, and heavily vacuolated. The L1 morphology accounted for 71% of ALL cases, L2 represented 27%, and L3 morphology was observed in the remaining 3% of patients.[3] After only a few years of using the system it became clear that the L1 and L2 morphologic groups did not predict patient age, lymphocyte subtype (T-cell vs. B-cell), response to treatment, or outcome, and it was eventually considered clinically irrelevant. Although the L3 group did prove to be a reliable predictor of a true ALL subtype (mature B-cell), another classification system was needed.

A more clinically relevant system of ALL classification was accomplished by immunologic detection of cell surface and cytoplasmic proteins known as immunophenotyping. By using a dozen or more monoclonal antibodies specific to individual protein markers, the presence or absence of the corresponding protein resulted in the identification of at least four different subtypes of ALL; early pre-B (progenitor-B), precursor-B, mature-B, and T-cell (Table 1). Two additional subclasses, common acute lymphoblastic leukemia (cALLa) and precursor-T cell have also been described. The cALLa subtype is very similar to progenitor-B cell ALL, differing by only one surface marker (CD10) causing some to fold cALLa ALL into the progenitor-B ALL category creating early pre-B ALL. Similarly, some have chosen to collapse the precursor-T cell ALL and mature T-cell ALL into one category citing no clinical relevance to the distinction.

The newest classification system was introduced by WHO and focuses the subtyping of ALL more on immunologic, cytogenetic, and molecular measures instead of morphologic features. Regarding ALL, the WHO group retained two of the immunologic groups (mature B-cell and T-cell) and subdivided the precursor-B cell groups into four distinct cytogenetic subtypes. The FAB group, L3, was found to be consistent with the immunologic group, mature-B cell ALL, and is represented in the WHO classification system as Burkitt cell (B-cell) ALL. The immunologic group, T-cell ALL, was also retained in the WHO classification system as precursor T-cell ALL. The two precursor B-cell ALL subtypes described in the immunological classification system were subdivided into four distinct subtypes based on the following cytogenetic abnormalities: t(9;22)-BCR/ABL; t(v;11q23)-MLL rearrangement; t(1;19)-E2A/PBX1; and t(12;21)-ETV/CBFα. The specifics of these four translocations will be discussed later.

Hematologists have long recognized that discussions of ALL are viewed as bitter sweet. On the one hand, treatment success and the potential for cure in ALL patients has outpaced all other acute leukemias bringing excitement and hope to the field. However, the realization that the majority of patients are children brings regret as we witness the morbidity resulting from treatment sequelae and inevitable mortality in a subset of patients who fail to respond to therapy. The focus of recent advances in ALL are directed at identifying predictors of treatment success so patients can be stratified into groups that can be offered a reduced treatment protocol to minimize sequelae and those requiring more intensified treatment regimens to improve outcomes. The future of ALL will involve the development of new therapeutic drugs tailored to target particular molecular abnormalities that produce aberrant signal transduction pathways with the hope of improving outcomes in every subtype of ALL.

Table 1. ALL classification based on immunophenotyping

Marker	Early pre-B cell	Pre-B cell	Mature-B cell	T-cell
TdT	+	+	-	+
HLA-DR	+	+	+	-
CD34	+	-	-	-
CD19	+	+	+	-
CD10	+/-	+	+/-	-
CD20	-	+	+	-
Cytoplasmic μ (cμ)	-	+	-	-
Surface Ig (sIg)	-	-	+	-
Ig gene rearrangement	+	+	+	-
CD2	-	-	-	+
CD3	-	-	-	+
CD5	-	-	-	+
CD7	-	-	-	+
CD4	-	-	-	+
CD8	-	-	-	+
TCR gene rearrangement	-	-	-	+

PROGNOSTIC INDICATORS

Both patients and physicians are most interested in disease indicators that will best predict therapeutic responses and prognostic outcomes. Drug selection, when more than one type is available for a given disorder, and appropriate dosing are of utmost concern. Selection of an inferior drug or a dosing schedule that is too low to be effective or so high as to produce adverse events are to be avoided. Above all, physicians and patients desire to be empowered with sufficient information to accurately identify the disorder, predict how well the disease will respond to the chosen therapy, and predict the patient outcome. Several prognostic indicators will be discussed to include clinical indicators, genetic and molecular indicators, and therapeutic response indicators (Table 2).

CLINICAL INDICATORS

To date, the most reliable predictors of treatment success for all forms of ALL are patient age and white blood cell count (WBC) at presentation.[8] These two variables have maintained their reliability over the past forty years even into the age of molecular biology. A threshold has been set for both variables by the National Cancer Institute (NCI)/Rome placing all patients into one of two categories for each variable. The threshold for age is ten years while the cut-off for WBC count is 50,000/uL. Patients who meet both criteria (<10 years of age and WBC <50,000/uL) are considered standard risk and those who are either ≥10 years of age or present with a WBC count of ≥50,000/uL are considered higher risk.[9] These criteria are not only reliable but are also easily obtained in almost all clinical settings. One exception to the rule involves patients less than one year of age who consistently have a poor prognosis. WBC count remains a reliable prognostic pre-

dictor because the number of WBCs reflects tumor burden and is suggestive of leukemic growth rate and aggressiveness.

Other prognostic indicators include gender, blast phenotype, and presence of disease within the central nervous system. Girls consistently show a more favorable response compared to boys regardless of treatment intensity. Historically, mixed lineage ALL blasts coexpressing myeloid markers (My+) have a less favorable prognosis: however, changes in therapy have equalized the prognosis of mixed lineage ALL to that of pure B-cell ALL.[10] Patients with a T-cell type ALL have a poorer prognosis but also express poor prognostic indicators like older age, mediastinal mass, and lymphadenopathy.[11] In addition to differences in prognosis, patients with T-cell ALL are placed into a separate category because of differences in response to certain chemotherapeutic agents. T-cell blasts are more sensitive to asparaginase and 2-amino-6-methoxypurine arabinoside (506U), while being less sensitive to methotrexate. The drug 506U is a water-soluble prodrug converted to ara-G by adenosine deaminase. Blasts in the cerebrospinal fluid (CSF) is considered a poor prognosis even when the CSF WBC count is normal.[12]

GENETIC AND MOLECULAR INDICATORS

Blast hyperdiploidy is a consistently positive prognostic indicator and is observed in about one third of patients with childhood ALL. These patients show hyperdiploidy (chromosome numbers around 50) in their blast cells that exhibit an increase in apoptosis in vitro and sensitivity to various chemotherapeutic agents. Patients with trisomies of chromosomes 4, 10, and 17 express event-free survivals (EFS) of between 75% to 90%, which improves to >90% in patients with triple trisomies.[13] In contrast, patients with hypodiploidy and trisomies of chromosome 5 have a poorer prognosis. EFS is approximately 40% (±18%) for patients with 33 to 44 chromosomes in their blasts and it drops to 25% (±22%) with chromosome numbers of less than 28.[14]

Another one-third of the ALL population exhibit one of four chromosomal translocations in the absence of increases in chromosomal number which can predict outcome. Three of the four translocations: t(1;19), mixed-lineage leukemia (MLL) translocations, and the t(9;22) Philadelphia chromosome are associated with a poor prognosis while the most common translocation, t(12;21), predicts a favorable response. The t(1;19) translocation fuses the E2A and PBX1 genes and is observed most often in pre-B ALL in which cytoplasmic μ heavy chains are identified.[15] Translocations involving the MLL gene are observed in approximately 6% of ALL patients and can involve over 40 different chromosome partners. The most common MLL translocation is the t(4;11) that predominates in the infant ALL population.[2]

The Philadelphia chromosome is present in approximately 3% to 5% of children and 20% of adults with ALL. Although Philadelphia positive ALL produces the BCR/ABL fusion gene, most noteworthy in chronic myelocytic leukemia (CML), a different breakpoint in the BCR produces a fusion protein that makes the disease more difficult to treat. As in CML, the chimeric fusion protein produced in ALL is

Table 2. Prognostic indicators for ALL

Prognostic Indicator	Favorable Response	Poor Response
WBC count	<50,000/uL	≥50,000/uL
Age at presentation	1 to 10 years old	<1 or ≥10 years old
Gender	Female	Male
Blast phenotype	B-cell	T-cell and mixed lineage
Abnormal karyotypes	Hyperdiploidy Trisomy 4, 10, 17 t(12;21) (TEL/AML1)	Hypodiploidy Trisomy 5 t(1;19) (E2A/PBX1) Mixed lineage leukemia (MLL) t(9;22) (BCR/ABL – Philadelphia)
BM blast count during induction therapy	Marked reduction at day seven	Mild reduction at day seven

a constitutive protein kinase that abnormally phosphorylates and activates multiple signaling pathways resulting in loss of controls on cell proliferation, survival and self-renewal.[16] Even therapy involving the tyrosine kinase inhibitor imatinib mesylate that has revolutionized CML treatment does not produce a favorable response in ALL.

The most common translocation observed in childhood ALL, the t(12;21), brings together the TEL and AML1 genes and is observed in approximately 25% of patients with precursor B ALL.[17] The translocation is associated with sensitivity to asparaginase and predicts an excellent prognosis.

THERAPEUTIC RESPONSE INDICATORS

Evidence is compounding that dramatically lowered blast counts achieved following seven days of conventional induction chemotherapy is predictive of five-year EFS rates. A Children's Cancer Group (CCG) study reported that at day 7 of therapy, 52% of children who achieved a markedly lowered marrow blast count (M1) had an 80% (±1%) EFS. Of the remaining patients, 23% achieved a moderate reduction (M2) in the marrow at day 7 with an EFS rate of 74% (±2%) while the remaining 25% only experienced a mild reduction (M3) in marrow blasts and an EFS rate of 68% (±2).[18,19] Another CCG study reported improved EFS rates in ALL children who were given intensified therapy regimens beginning at day 7 of conventional therapy when slow early response rates (SER) were observed. More aggressive courses with methotrexate and asparaginase produced EFS rates of 75% (±3.8%) compared to 55% (±4.5%) for those remaining on standard therapy.[19] This evidence suggests that rigorous assessment of blast counts at day 7 of conventional therapy will not only predict outcomes but it could also govern therapy decisions. Patients achieving dramatically lowered blast counts may receive a reduction in therapy, patients with a moderate reduction may remain on standard therapy, and those achieving only mild reductions may be given an intensified regimen. More sensitive methods of measuring blast counts using flow cytometry and molecular techniques are redefining minimum residual disease (MRD) for children with ALL. Patients with no detectable MRD at day 7 of therapy showed >90% EFS at three years while children with high MRD exhibited a 25% three year EFS rate.[20] These types of assessments will continue to shape therapy decisions and impact prognosis.

RISK CATEGORIES

Using the clinical, genetic, and therapeutic response indicators, children with precursor-B ALL can be grouped into one of four risk categories; low, standard, high, and very high. Low risk is defined as patients who present with the standard risk clinical criteria (<10 years of age; WBC <50,000/uL), low risk cytogenetic markers (t(12;21), TEL/AML1 or trisomies of chromosomes 4, 10, and 17) and dramatic reductions in bone marrow blasts at day 7 of induction chemotherapy. Approximately 22% of cases of precursor-B ALL meet the criteria for low risk and are eligible for a reduction in therapy intensity.

Standard risk children present in one of three ways. First, they may exhibit standard clinical risk factors but do not show either low risk or high risk cytogenetic markers. Second, standard risk children may exhibit high risk clinical indicators and low risk cytogenetic indicators. Third, patients may present with standard risk clinical indicators and low risk cytogenetic markers but exhibit slow clearance of bone marrow blasts following seven days of induction chemotherapy. The standard risk group comprises about half of the patients with precursor-B ALL and is a heterogeneous group. While some patients could achieve the same long standing outcome with reduced intensity therapy, a significant number will relapse following remission.

Another 30% of children with precursor-B ALL will fall into the high risk category in which patients present with both high risk clinical features and high risk cytogenetic indicators like t(4;11) and t(1;19). A very high risk group represents the remaining 3% of children with precursor-B ALL and is characterized by the presence of the Philadelphia chromosome [t(9;22)] or hypodiploidy (<45 chromosomes).[20]

TREATMENT GROUPS

In light of the early successes experienced with chemotherapy, physicians began to recognize that childhood ALL holds great promise in achieving dramatic outcome improvements by implementing individualized treatment strategies. While over 75% of children with ALL have been cured through medication alone, the remainder will ultimately succumb to their disease. Because there is great diversity in patient age, liver function, and physical size, the maintenance of drug levels within the narrow therapeutic range requires close attention at more frequent intervals. Drug levels that rise above therapeutic range can produce drug related adverse events while inappropriately low levels are less effective. In addition, dose escalation in slow early responders has proven successful in achieving EFS in many such patients.

A newly organized panel of experts called the Children's Oncology Group, representing a merger of the Pediatric Research Group and the Children's Cancer Group, has proposed a single treatment stratification strategy. The panel developed four

distinct treatment groups: T-cell, infant, high risk precursor-B, and standard risk precursor-B. These four treatment groups represent an amalgamation of prognostic indicators that classify patients at the time of diagnosis based on the following criteria: age, presenting WBC count, and immunophenotype. Patients less than 12 months of age are placed into the infant category, a poor prognostic indicator. Immunophenotyping will divide the remaining patients into T-cell and B-cell types. The B-cell group is further divided into high risk and standard risk based on the WBC count at presentation. The two precursor-B groups are reassessed at day eight, following the one-week induction period, and at days 15 and 29 to determine molecular abnormalities in the blasts, response to therapy, and bone marrow morphology. Quantitation of minimum residual disease is also assessed at day 29. At each assessment point, all precursor-B ALL patients are assigned to one of four prognostic groups: low risk, standard risk, high risk, and very high risk based on the definitions previously described. Treatment strategies will be adjusted appropriately as patients move among prognostic groups. The emergence of MLL mutations will prompt treatment intensification while t(12;21)-TEL/AML1 may justify relaxation of the treatment regimen.[21]

Mutations in genes that code for drug metabolizing proteins will also reduce drug clearance resulting in toxic sequelae. The chemotherapeutic agent 6-mercaptopurine (6MP) is an important drug in the treatment of ALL but requires the enzyme thiopurine methyltransferase (TPMT) to accomplish normal drug metabolism. Inherited point mutations in TPMT genes reduce the metabolism of 6MP by increasing the natural proteolytic degradation of the TPMT enzyme.[22] Approximately 1 in 300 people have a deficiency of TPMT with 10% expressing heterozygosity at the TPMT locus. Individuals expressing inactivating mutations on both TPMT alleles cannot inactivate 6MP through normal methylation, resulting in toxic accumulation of active thioguanine nucleotides. Many patients experience life-threatening toxicities from normal doses of 6MP. Studies have confirmed that dose reductions are most often indicated in homozygous TPMT mutants, required less often in heterozygous TPMT mutants, and rarely needed in wild-type patients. However, since slowed metabolism from TPMT mutations results in elevated 6MP blood levels, reduced doses still provide therapeutic plasma drug levels needed to produce the desired antileukemic effect.[23]
Point mutations in genes that code for other detoxifying enzymes can also affect antileukemic drug metabolism prompting dose modifications to produce the desired antileukemic effect without the accompanying toxicity. Polymorphisms in the number of tandem repeats in the enhancer of the thymidylate synthetase gene increase the expression of the corresponding protein resulting in excess metabolic turnover of methotrexate and a poorer outcome. Mutations in the RFC1 gene (reduced folate carrier), produces a protein that is unable to effectively transport methotrexate from the blood stream into ALL blast cells.[24]

ALL SUBTYPES USING MICROARRAYS

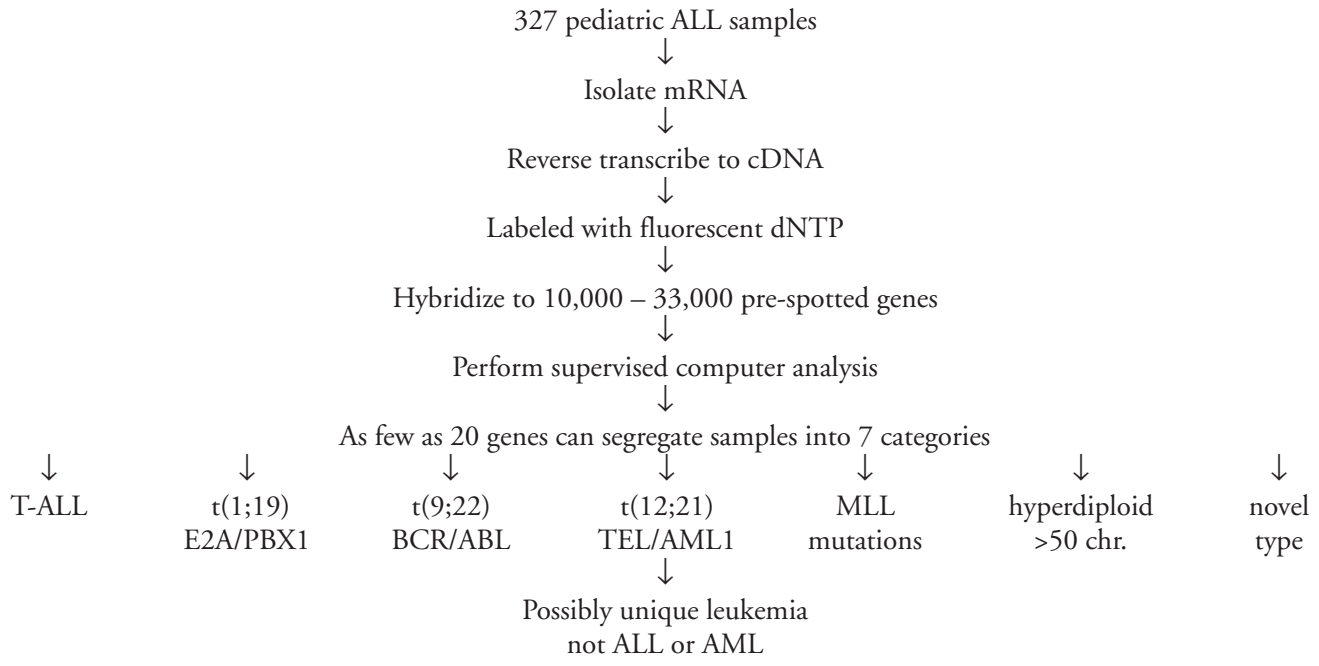
Microarray is a new and powerful technology that is used to determine gene expression profiles in cell populations. Gene expression patterns have the potential to produce a specific signature for a particular cancer type or subtype. Microarray testing slides are prepared by spotting either PCR amplified complementary DNA (cDNA) molecules or synthesized oligonucleotides complementary to specific genes onto a specially coated glass slide. Hundreds to as many as 50,000 gene sequences can be spotted onto a single microarray slide. The cells to be tested for gene expression are separated from contaminant cells and total RNA is isolated from the cell population. All mRNA species are reverse transcribed into cDNA using three regular and one fluorescently labeled deoxynucleotide triphosphate (dNTP) that bears a particular fluorescent color. In most applications this process is applied to both a population of normal and abnormal cells using two different fluorescent labels so to distinguish their respective gene expression profiles by color differences. When performing microarrays to evaluate leukemias, normal and leukemic cells found in blood or bone marrow are first separated by density gradient centrifugation. The fluorescently labeled cDNA molecules are then generated such that the normal cells emit one fluorescent color and the leukemic blasts emit another. The gene expression patterns of the leukemic blasts can be compared to the normal counterpart.

Researchers performing experiments to analyze gene expression panels in ALL patients using microarrays are proposing alternate ALL classification schemes. One report studied gene expression profiles on 327 pediatric ALL samples and proposed 7 distinct ALL subtypes: T-ALL; t(1;19) E2A-PBX1; t(9;22) BCR/ABL; t(12;21) TEL/AML1; MLL rearrangements; hyperdiploid (>50 chromosomes); and a novel subtype (Figure 1).[25] Most of the earlier analyses were performed on pre-spotted arrays containing 10,000 genes while the latter analyses were performed on similar arrays containing 33,000 genes.[26] Another investigator using microarray gene expression profile data proposed that MLL expressing leukemias be classified as a separate entity distinct from both ALL and AML citing the existence of a unique expression profile

involving FLT3 and certain HOX genes.[27] It is known that the HOX genes represent a family of transcription factors that regulate expression of genes important in both embryogenesis and in self-renewal and proliferation of hematopoietic stem cells.[28,29] The reliability of using gene expression profiles on microarrays to accurately classify or subtype leukemias is still in question. However, by using statistical approaches to identify class discriminating genes and sophisticated computer-assisted supervised learning algorithms to determine if the class discriminating genes are useful in leukemia classification, some groups are reporting an accuracy of 96% in the subtyping of various pediatric ALL patients.[29] Using this approach it is estimated that as few as 20 genes, analyzed in parallel, may be sufficient to diagnose the different ALL subtypes proposed. In addition, consistent gene expression patterns suggest potential links to leukemogenic mechanisms of transformation. However, caution must be exercised in making such assumptions because many inappropriately expressed genes from the leukemic clone may retain important diagnostic value, but prove not to be leukemogenic.

Another group, led by Mosquera-Caro, used microarrays to analyze 127 cases of infant leukemias that were classified as either ALL or AML by traditional methods (Figure 2). Infant leukemias were defined based on a patient population of less than 356 days of age which characteristically show poor survival rates of <25%. Pre-spotted microarrays containing 12,625 genes were used and expression results were analyzed using sophisticated statistical approaches and computer-assisted nonsupervised clustering analysis. In nonsupervised cluster analysis the computer algorithm will identify aggregates, or clusters, of like data and place them into groups that were not predetermined. This process is able to create categories that may not have previously existed. Following the analysis, three biologically distinct groups emerged but none clustered according to the AML vs. ALL divisions nor was clustering based on the expression of MLL rearrangements. For example, the first group contained 21 cases in which 16 were previously categorized as ALL and the remaining 5 as AML. The types of genes expressed (EPOR, AML1, KIT, CD34, FLT1, and HOX) were consistent with gene profiles found in very primitive hemato-

Figure 1. Supervised microarray analysis of pediatric ALL patients



ABL = ableson oncogene; BCR = breakpoint cluster region; cDNA = complementary DNA; dNTP = deoxynucleotide triphosphate; E2A = a.k.a. TCF3 (transcription factor 3); MLL = mixed-lineage leukemia; mRNA = messenger RNA: PBX1 = pre-B-cell transforming factor; TEL = ETS protein (oncogene from E26 erythroblastosis virus).

poietic stem cells. The second cluster contained 52 cases, 51 of which were previously categorized as ALL. This group exhibited a more homogeneous gene expression pattern: CD19, IgM, BCL7 and t(4;11) that resembled committed precursor-B cells. The last cluster consisted of 54 cases in which 42 were previously diagnosed as AML and 12 cases as ALL. This group exhibited a more heterogeneous gene expression pattern mainly involving genes in or related to the RAS family: ECGF, CCR1, MAPKAPK3. Interestingly, members in all three groups expressed MLL rearrangements, albeit different types of rearrangements, with varying levels of penetrance.[20]

A retrospective pediatric study by Mosquera-Caro, using microarrays on a large cohort of pediatric ALL patients from two Pediatric Oncology Group (POG) studies, identified nine distinct biologic clusters in ALL. The patients tested by microarray analysis were segregated into six groups: t(4;11), t(9;22), t(1;19), t(12;21), monosomy 7, and monosomy 21 based on cytogenetic abnormalities, and five additional demographic categories. The retrospective design involved patients who were past the fourth year of therapy so they could be subdivided into two treatment groups, those who failed therapy and those who achieved complete remission. A similar design as was used in the infant ALL studies previously described was also applied to the pediatric study, except the analytical approach was supervised because the patients were first segregated into groups. The supervised approach is looking for gene expression patterns that characterize previously existing categories. The microarray results exhibited similar gene expression patterns in each cluster. As expected, each cluster had in common the cytogenetic abnormality used to originally subdivide the patient cohort. However, when a full unsupervised approach (not segregated into cytogenetic groups) was performed on 147 target genes, nine distinct biologic clusters emerged. These nine biologic clusters included two T-cell clusters and seven precursor-B cell clusters. Although there were over 100 unique genes that characterized these nine clusters, surprisingly there were no cytogenetic abnormalities that defined any of the clusters. In addition, three novel genes were discovered and named G0, G1, and G2. The G0 gene showed the greatest power to divide the cohort into good and poor outcome groups. The G0 gene was cloned and named OPAL1, which stands for outcome prediction for acute leu-

Figure 2. Nonsupervised microarray analysis of infant ALL patients

127 infant ALL samples (<1 year old)
↓
Isolate mRNA
↓
Reverse transcribe to cDNA
↓
Labeled with fluorescent dNTP
↓
Hybridize to 12,625 pre-spotted genes
↓
Perform nonsupervised computer analysis

3 clusters:	Group #1 (n = 21)	Group #2 (n = 52)	Group #3 (n = 54)
Leukemia type:	ALL=16, AML=5	ALL=51, AML=1	ALL=12, AML=42
Genes:	EPOR, AML1, KIT, CD34, FLT1, HOX	CD19, IgM, BCL7, t(4;11)	ECGF, CCR1, MAPKAPK3

AML1 = acute myelocytic leukemia 1; BCL7 = B-cell leukemia/lymphoma 7; CCR1 = chemokine receptor; cDNA = complementary DNA; dNTP = deoxynucleotide triphosphate; ECGF = endothelial growth factor; EPOR = erythropoietin receptor; FLT1 = fms related tyrosine kinase 1; HOX = homeobox transcription factor; KIT = tyrosine kinase receptor for stem cell factor (feline sarcoma virus oncogene); MAPKAPK3 = mitogen-activated protein kinase-activated protein kinase 3; mRNA = messenger RNA.

kemia number 1. When OPAL1 expression is high, 87% of the cohort had a good outcome as compared to 32% when OPAL1 is low.[30] Therefore, therapy intensification may be warranted in pediatric ALL patients with low OPAL1 expression even if the other indicators suggest low risk disease. Overall, these data suggest that microarrays may produce ALL subtypes that provide physicians better therapeutic guidance and outcome prediction capabilities than the cytogenetic groups previously defined.

It seems clear that while still in the age of traditional chemotherapy, patient stratification should begin with the clinical indicators of age and WBC count. Therapy can then be adjusted based on bone marrow response at day 7, adverse drug reactions, emergence of certain karyotypes like MLL, and the identification of mutated drug metabolism genes. However, as microarrays and other molecular approaches begin to identify mutations in important proteins central to aberrant signal transduction pathways, new patient stratification systems may be developed around treatment protocols tailored to these mutants.

FUTURE THERAPEUTIC TARGETS

Although therapeutic responses in patients with ALL are generally better than other acute leukemias, the identification of new therapeutic targets has the potential of improving outcomes and reducing treatment related sequelae. Current therapeutic strategies are based largely on traditional chemotherapeutic approaches. Most chemotherapeutic drugs produce a cytotoxic effect on malignant cells by interfering with normal DNA replication or protein synthesis systems. In this way, rapidly dividing cells are targeted and eliminated. Unfortunately, some malignant cells will often escape destruction by being quiescent during courses of therapy or being physically separated from the chemotherapeutic agent. In contrast, many normal cells will also succumb to death from chemotherapy due to exposure to the chemotherapeutic agent while in the cell cycle. A therapeutic approach that is more specific to aberrations unique to the malignant clone has the potential to selectively target the leukemic cells while sparing normal cells. A better understanding of normal signal transduction pathways and the aberrant messengers produced by cancer causing mutations observed in ALL, will likely identify future therapeutic targets.

ABERRANT MESSENGERS CAUSED BY CHROMOSOMAL TRANSLOCATIONS

As stated earlier, t(12;21) is the most common translocation observed in childhood ALL, accounting for approximately 25% of patients with precursor-B ALL.[17] The translocation predicts an excellent prognosis due to its sensitivity to asparaginase. The t(12;21) translocation joins the TEL and AML1 genes from chromosomes 12 and 21, respectively. This translocation creates a chimeric gene in which the head is composed of the 5' end of the TEL gene and the tail houses the majority of the 3' end of the AML1 gene. The TEL gene product normally functions as a transcription factor providing a protein/protein interacting domain that is essential in the homing of hematopoietic stem cells to the bone marrow.[31] The normal AML1 gene codes for core-binding factor α (CBFα), the alpha subunit of the heterodimeric transcription factor core-binding factor (CBF), composed of CBFα (AML1) and CBFβ. The CBF heterodimer functions as a transcription factor by binding specific DNA sequences (core enhanced sequence) and recruiting a protein complex with histone acetyltransferase (HAT) activity. HAT proteins activate transcription by acetylating lysine residues in the core histones that unpackage chromatin allowing RNA polymerase to bind and transcribe genes. These genes are instrumental in regulating hematopoietic stem cell growth probably through the action of the HOX genes.[27,32] By virtue of the retained DNA binding domain of the AML1 moiety, the TEL/AML1 transcription factor is able to dimerize with CBFβ forming the heterodimer complex. This complex will bind the same core enhanced sequence as AML1 preventing normal AML1 from binding. Further, when the TEL/AML1/CBFβ complex binds DNA it recruits proteins with histone deacetylase activity (HDAC) that remove the acetyl groups from the lysine residues causing the chromatin to repackage, preventing transcription.[33] Therefore, the t(12;21) translocation produces a hybrid transcription factor that forms a complex preventing the transcription of proteins critical in the regulation of hematopoietic stem cells that alters both self-renewal and differentiation.[34] Small-molecular inhibitors of histone deacetylase are currently in clinical trials and are exhibiting some antileukemic activity when used alone but appear to exert a greater effect when administered in combination with other therapeutic agents.[35]

Approximately 6% of ALL patients possess translocations involving the MLL gene (mixed-lineage leukemia) that can partner with over 40 different chromosomes.[2] The t(4;11) is the most common MLL translocation that predominates in the infant ALL population. The MLL gene codes for a nuclear binding protein that is required to maintain the transcription of HOX genes, particularly HOXA7 and HOXA9, whose importance have already been described. Various translocations involving the MLL gene each result in a fusion protein

with MLL at the 5' head and another partner at the 3' tail. Translocations involving the MLL gene causes overexpression of MLL fusion proteins that enhances the activity of the downstream HOX genes affecting self-renewal, proliferation, and differentiation of stem cells and committed progenitors.[36]

The t(1;19) translocation is observed in 5% of childhood ALL but occurs in 25% of pre-B ALL in which cytoplasmic μ heavy chains are identified.[15] The translocation fuses the E2A and PBX1 genes from chromosomes 1 and 19, respectively. Both E2A and PBX1 normally function as transcription factors. PBX1 is involved in the regulation of HOX gene expression and the E2A target genes are thought to play some role in hematopoiesis. The protein product of the fusion gene is a chimeric protein that also acts as a transcription factor. The E2A/PBX1 transcription factor disrupts the normal expression of HOX genes and E2A target genes resulting in aberrant stem cell growth patterns.[37] Since the HOX genes are downstream messengers common to the t(12;21), t(4;11) and t(1;19) translocations, they have become attractive targets for the development of therapeutic interventions.

Philadelphia chromosome, caused by the t(9;22) translocation, is well established in chronic myelocytic leukemia (CML) but is also found in about 3% of childhood ALL patients. However, the breakpoint in the BCR gene from chromosome 9 usually occurs in a different position than in CML creating a larger fusion protein that is not sensitive to imatinib therapy. The presence of the Philadelphia chromosome is considered a poor prognostic indicator. Actually, three of the four chromosomal translocations discussed, those involving MLL, t(1;19) and t(9;22), are associated with a poor prognosis while the t(12;21) has a favorable prognosis.

MUTATIONS IN TUMOR SUPPRESSOR GENES

Mutations in the proteins involved in the various pathways controlled by the retinoblastoma protein (RB) are common in ALL. The retinoblastoma protein functions as a tumor suppressor by inhibiting transcriptional factors that stimulate expression of proteins necessary for cells to enter the S-phase of the cell cycle.[38] Cell surface signals designed to stimulate proliferation, induce the expression of D-type cyclins like D1, D2 and D3. In general, cyclins are proteins that regulate the cell cycle by combining with cyclin dependent kinases (CDKs) to form an enzymatic complex. These complexes phosphorylate proteins that affect how cells move between phases of the cell cycle. D-cyclins bind with cyclin-dependent kinases (CDKS), like CDK4 and CDK6, and phosphorylate the RB protein. Phosphorylation of RB removes the inhibitory activity of the RB protein which, in turn, releases transcription factors like E2F from inhibition. These transcription factors are then free to stimulate the expression of genes that synthesize proteins which allow the cell to enter S-phase of the cell cycle. Thus, lack of RB phosphorylation inhibits cell proliferation. Mutations to RB genes are rare in ALL, but mutations in proteins that affect RB phosphorylation, like p16 and p15 are common in T-cell ALL but also occur in B-cell ALL.[39]

Like the RB protein, p53 is a tumor suppressor that is rarely mutated in ALL patients but whose function is frequently altered by mutations to genes that code for proteins that regulate p53 function. Whereas RB prevents excessive proliferation by inhibiting cells from entering S-phase, p53 triggers the arrest of the cell cycle, also known as apoptosis. Activation of p53 occurs in response to cells that have acquired DNA damage that may be engaged in aberrant cell proliferation. Mutations to proteins that regulate p53 function, like HDM2, p14, and p21, are frequent findings in ALL.[40,41]

A more thorough understanding of the normal signal transduction pathways involved in the control of cell proliferation, cell differentiation, and natural cell death will provide the framework to determine the manner in which mutated proteins alter these pathways and impact cell growth. Those mutant proteins that have the greatest impact on abrogating normal cell growth patterns become favorable targets for the development of tailored drug therapy. Most experts believe that the next major advance in ALL will lie in the stratification of patients based on particular mutation or gene expression patterns and the development of drugs designed to target the products of these mutations that disrupt the pathways that control normal cell growth.

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