CLINICAL TRIALS AND OBSERVATIONS

Acute mixed lineage leukemia in children: the experience of St Jude Children's Research Hospital

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To characterize the biology and optimal therapy of acute mixed-lineage leukemia in children, we reviewed the pathologic and clinical features, including response to therapy, of 35 patients with mixed-lineage leukemia. The majority of cases (91%) had blasts cells that simultaneously expressed either T-lineage plus myeloid markers (T/myeloid, n = 20) or B-lineage plus myeloid markers (B/myeloid, n = 12). Overall survival rates for the B/myeloid and T/myeloid subgroups were not significantly different from each other or from the rate for acute myeloid leukemia (AML) but were inferior to the outcome in children with acute lymphoblastic leukemia (ALL). Patients who failed to achieve complete remission with AML-directed therapy could often be induced with a regimen of prednisone, vincristine, and L-asparaginase. Analysis of gene-expression patterns identified a subset of biphenotypic leukemias that did not cluster with T-cell ALL, B-progenitor ALL, or AML. We propose that treatment for biphenotypic leukemia begin with one course of AML-type induction therapy, with a provision for a switch to lymphoid-type induction therapy with a glucocorticoid, vincristine, and L-asparaginase if the patient responds poorly. We also suggest that hematopoietic stem cell transplantation is often not required for cure of these patients. (Blood. 2009;113:5083-5089)

Introduction

Leukemic blasts from patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) commonly express cell markers of more than one lineage while retaining characteristics that demonstrate a strong commitment to a single lineage. Acute leukemias with this type of aberrant antigen expression include cases of ALL that express myeloid-associated antigens (My[+] ALL) and cases of AML that express lymphoid-associated antigens (Ly[+] AML). Large studies of patients with My[+] ALL and Ly[+] AML have demonstrated that lineage infidelity does not have prognostic significance.[1-8]

By contrast, mixed-lineage acute leukemias (or acute leukemias of ambiguous lineage) represent a heterogeneous category of rare, poorly differentiated acute leukemias that possess characteristics of both lymphoid and myeloid precursor cells.[3,9-16] These divergent morphologic and immunophenotypic features may be uniformly present in one blast population (biphenotypic leukemia) or may be seen on distinct blast populations in a single patient (bilineal leukemia). Leukemias that switch their lineage of origin during therapy or show poorly differentiated or undifferentiated features are also included in this category. Mixed-lineage leukemias should therefore be distinguished from cases of ALL or AML that demonstrate expression of a few myeloid-associated or lymphoid-association antigens, respectively. Numerical scoring criteria have been generated for the immunophenotypic diagnosis of biphenotypic leukemias to aid in this distinction.[10,11] There are no well-defined quantitative cutoff points for the diagnosis of the less common acute bilineal leukemias.

As mixed-lineage leukemias represent only 3% to 5% of acute leukemias occurring in patients of all ages and comprise several different subtypes (biphenotypic, bilineal, and lineage switch), the optimal therapeutic approach to these cases, especially in pediatric patients, has not been defined. For example, it is still unclear whether patients with biphenotypic leukemia fare batter on induction regimens designed for ALL or AML, or whether biphenotypic and bilineal leukemias respond similarly. This question assumes increased importance in light of several reports suggesting that patients with biphenotypic leukemia have poor treatment outcomes.[17-21] Another unresolved issue is whether the immunophenotypic characteristics or gene-expression patterns of mixed-lineage leukemia might differ sufficiently among themselves and from those of typical ALL and AML to warrant consideration of a distinct leukemia subtype. Here we assess the clinical and laboratory findings in 35 consecutive cases of pediatric mixed-lineage leukemia that were diagnosed and treated over 2 decades. We also analyzed the gene-expression profiles for a subset of cases to gain insight into the molecular pathogenesis of these unusual cases.

Methods

Patients

Thirty-five patients with mixed-lineage leukemia were identified by database review of all patients with acute leukemia treated at St Jude Children's

The online version of this article contains a data supplement.

Research Hospital from 1985 to 2006. Their initial treatment followed institutional protocols or treatment plans for AML (n = 23) or ALL (n = 12).[22-26] Additional therapy was selected by the primary oncologist on a patient-to-patient basis. Diagnostic microarray gene-expression profiles were available for 13 of mixed-lineage leukemia cases in this study and were compared with corresponding profiles for 106 B-progenitor ALL, 15 T-cell ALL (T-ALL), and 105 AML patients. To evaluate the prognosis of the 32 patients with biphenotypic leukemia, we compared their overall survival rates with that of 1527 ALL patients and 328 AML patients treated on St Jude protocols from 1985 to 2006.[22,27] All therapeutic studies were approved by the St Jude Institutional Review Board.

Flow cytometric (immunophenotypic) analysis and diagnostic criteria

Flow cytometric analysis (3- or 4-color) was performed on blast cell populations identified by CD45 versus light side-scatter properties, using Becton Dickinson FACSCalibur instruments (1990 to 2006 models; BD Biosciences, San Jose, CA) and standard staining and analytic methods. All cases were characterized with a panel of antibodies to leukocyte-associated markers, including surface CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD21, CD22, CD24, CD33, CD34, CD36, CD41a, CD42b, CD45, CD45RO, CD56, CD57, CD61, CD64, CD65, CD66c, CD71, CD117, CD133, cytoplasmic CD3, CD79a, myeloperoxidase (MPO), terminal deoxynucleotidyl transferase (TdT), and surface and cytoplasmic immunoglobulins μ, κ, and λ. A marker was considered positive by this method when 10% or more of the blasts reacted with antibodies to that marker with a definite intensity shift greater than a corresponding negative control.

All cases diagnosed as acute biphenotypic leukemias or acute bilineal leukemia fulfilled criteria of the European Group for the Immunological Characterization of Leukemias and World Health Organization classifications systems.[9-11] Cases excluded from this category in our retrospective search lacked coexpression of MPO and cytoplasmic CD3 or CD79a and were classified as AML or ALL. In 5 cases (cases 13, 18, 19, 26, and 31), the available morphologic and immunophenotypic information was highly suggestive of acute biphenotypic leukemia, but the numbers of markers analyzed were not sufficient because of the diagnostic era to permit a definitive contemporary classification as acute mixed-lineage leukemias. Nevertheless, because of their coexpression of several T/lymphoid (CD2, CD7, TdT, or CD10) and myeloid antigens, the dimorphic (lymphoid and myeloid) appearance of the blasts, and their response to a combination of myeloid and lymphoid therapies, they were considered relevant to this study and were included as acute biphenotypic leukemias.

Cytogenetics

Cytogenetic analysis was performed on direct preparations or overnight-unstimulated cultures of bone marrow and tissue samples, followed by banding with Wright-trypsin stain, as previously described.[28]

Gene-expression analysis

Gene expression was measured with the Affymetrix U133 array (Affymetrix, Santa Clara, CA). Laboratory procedures for RNA extraction and microarray handling are described elsewhere.[29,30] The MAS 5.0 algorithm (Affymetrix 2002) was used to determine expression signals. The Wilcoxon rank-sum test[31] was used to select probe sets with expression patterns that significantly distinguish B-progenitor ALL from AML and T-ALL, T-ALL from B-progenitor ALL and AML, and AML from B-progenitor ALL and T-ALL. The robust method[32] was used to estimate the false discovery rate for each set of rank-sum comparisons. Principal components analysis was applied to the selected probe expression data for the AML, T-ALL, and B-progenitor ALL cases, and the results were used to visualize the distributions of all cases with respect to the expression of the selected probe

sets. Microarray data have been deposited with Gene Expression Omnibus[33] under accession number GSE14286.

Statistical methods

The Kaplan-Meier method[34] was used to estimate the probability of overall survival; SEs were determined by the method of Peto et al.[35] Survival comparisons were performed with a Monte Carlo approximation (10 000 permutations) of the exact log-rank test. Survival analyses were performed with SAS software (SAS Institute, Cary, NC), Windows version 9.1, and StatXact Windows version 7.1 (Cytel, Cambridge, MA). Gene-expression analyses were performed with S-plus software Windows version 7.0 (Insightful, Seattle, WA).

Results

Pathologic features

These 35 cases were morphologically heterogeneous. Distinct dimorphic lymphoid and myeloid or monocytic blast populations were present in both cases of bilineal leukemia (cases 33 and 34), as well as in 14 of the cases of biphenotypic leukemia (cases 2-7, 9, 17, 19, 23-25, and 29-31), in which cytochemical MPO expression was either detected in both blast populations or limited to the myeloid-appearing blasts. The remaining cases had morphologic and cytochemical features of AML (French-American-British AML M1 or M2 subtypes in cases 1, 3,13, 15,18, 26, and 27), acute lymphoblastic leukemia (French-American-British ALL L1 subtype in cases 8, 10, 11, 28, and 32), mixed ALL L1 and L3 subtypes with cytochemical MPO expression in the latter subset (case 12), and large, myeloid-type blasts with moderate amounts of cytoplasm and prominent nucleoli (cases 14, 16, 20-22, and 35). Myelodysplasia was apparent in case 14.

The immunophenotypic features of all cases are summarized in Table 1. Immunophenotyping identified 2 cases with B, T, and myeloid markers, 10 with B and myeloid markers, and 20 with T and myeloid markers. Case 28 was initially diagnosed with T-cell ALL but, at the time of relapse, was found to have biphenotypic leukemia with T and myeloid markers. In addition, 2 cases had distinct lymphoid and myeloid blast populations and were classified as bilineal, whereas a single case was classified as undifferentiated. All but 3 cases demonstrated MPO positivity by cytochemical staining, with a median of 3% positive cells (range, 1%-90%), whereas 9 cases had Auer rods.

Cytogenetic abnormalities

Among the 33 cases with successful cytogenetic studies, 29 had abnormal karyotypes (Table 2). There were no recurring cytogenetic abnormalities, although cases 4, 33, 34, and 35 had *MLL* gene rearrangements. Of note, 9 cases had abnormalities of chromosomes 5 or 7, 6 cases had 12p abnormalities, and 4 cases had extra 1q material.

Gene expression

We used a gene-expression database of 226 cases of acute leukemia with definitive diagnoses (106 B-progenitor ALL, 15 T-ALL, and 105 AML cases) to select the 100 probe sets that best distinguished each lineage from the other lineages of interest, yielding a list of 284 unique probe sets (Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). The results of principal component analysis, applied to probe set data for the B-progenitor ALL, T-ALL, and AML cases, were used to visually compare the extent of

Table 1. Immunophenotypic and cytochemical findings in 35 cases of pediatric mixed-lineage leukemia

Case type/number	B-lineage markers	T-lineage markers	Lymphoid markers, NOS	Myeloid markers	MPO*	Auer rods
Biphenotypic (B + T + My)						
1	CD19, CD20	CD2, CD7		CD13, CD14, CD33	Positive†	No
2	CD19, CD22, cyCD79a	cyCD3, CD7	TdT	CD11b, CD13, CD15, MPO	5	No
Biphenotypic (B + My)						
3	CD19, CD22, cyIgM		TdT, CD10	CD11, CD13, CD15, CD33, CD36, CD14	8	No
4	CD19, CD22, cyCD79a, cyIgM		TdT	CD13, CD15, CD33, CD36, CD65, CD117	3	Yes
5	CD19, CD22		TdT, CD10	CD13, CD33	1	No
6	CD19, CD22, CD24 cyCD79a		TdT, CD10	CD11b, CD13, CD14, CD15, CD33, CD64, CD65	2	No
7	CD19, CD22		TdT	CD15, CD33	3	No
8	CD19, CD20, CD22, CD24, cyIgMu, cyCD79a		TdT, CD10	MPO	2	No
9	CD19, CD22, CD24, cyCD79a		TdT	CD11b, CD13, CD15, CD33, MPO	2	No
10	CD19, CD22, CD24, cyIgMu, cyCD79a		TdT	CD13, CD15, CD33, MPO	5	No
11	CD19, CD20, CD22, CD24, cyCD79a		CD10	CD13, CD15, CD33, MPO	2	No
12	CD19, CD24, cyCD79a		TdT	CD13, CD15, CD33, CD65	3	No
Biphenotypic (T + My)						
13		CD2,CD7	CD10	CD11, CD13, CD14, CD15, CD33	10	No
14		CD2, CD7, cyCD3	TdT	CD13, CD15, CD36, CD117, MPO	6	Yes
15		cyCD3, CD2, CD5, CD7	TdT	CD11b, CD13, CD15, CD33, CD65, CD117, MPO	46	Yes
16		cyCD3, CD4, CD5, CD7, CD56		CD11b, CD13, CD15, CD33, CD117, MPO	4	No
17	CD22, cyCD79a	cyCD3, CD2, CD7	TdT	CD11b, CD13, CD15, CD33, CD117, MPO	4.5	Yes
18		CD2, CD7	TdT	CD11, CD13, CD15, CD33	21	No
19		CD2, CD7	TdT	CD13, CD15	3	No
20	CD19	cyCD3, CD2, CD7	TdT	CD11b, CD13, CD15, CD33, CD65	3.5	No
21		CD2, CD7, cyCD3		CD13, CD15, CD36, CD65, MPO	15	No
22	CD22	cyCD3, CD7, CD56		CD13, CD15, CD33, CD65, CD117, MPO	4	No
23	CD22, cyCD79a	cyCD3, CD2, CD7	TdT	CD11b, CD13, CD15, CD33, CD65, MPO	2.3	Yes
24		cyCD3, CD2, CD7	TdT	CD11b, CD13, CD15, CD33, CD65, CD117	1	Yes
25	CD22	CD2, CD7, cyCD3	TdT	CD11b, CD13, CD15, CD33, CD36, MPO	3	Yes
26		CD2, CD7	TdT	CD13, CD33	5	No
27		cyCD3, CD2, CD4, CD5, CD7	TdT, CD10	CD13, CD33, MPO	36	No
28		cyCD3, CD5, CD7		CD13, CD33, CD117, MPO	90	No
29		CD2, CD5, CD7, cyCD3	TdT, CD10	CD11b, CD13, CD33, MPO	1	No
30	CD22	CD2, CD7, cyCD3	TdT	CD11b, CD13, CD15, CD117, MPO	2.5	Yes
31		CD2, CD7	TdT	CD11b, CD13, CD33	13	Yes
32		cyCD3, CD7, CD56		CD11b, CD13, CD15, CD33, MPO	0	No
Bilineal (B + My)						
33	CD19, CD22, cyCD79a		TdT	CD11b, CD13, CD15, CD33, CD65, CD117, MPO	14	No
34	CD19, CD22, CD24, cyCD79a	CD56	TdT	CD11b, CD13, CD14, CD33, CD36, CD64, CD65	0	No
Undifferentiated acute leukemia						
35	CD22	CD7		CD11b, CD33	0	No

*Percentage of cells that were positive for MPO by cytochemical staining.
†Percentage of MPO-positive cells not available.

Table 2. Karyotypic findings in 33 cases of pediatric mixed-lineage leukemia

Case type/number	Karyotype
Biphenotypic (B + T + My)	
1	51,XY,+Y,+4,+6,+13,+22[18]
2	46,XY,del(12)(p11.2p13)[16]/46,XY[4]
Biphenotypic (B + My)	
3	46,XX,inv(3)(p25q21),del(6)(q13q21),t(4;12)(q12;p13),add(7)(q36),del(10)(q24)[4]/47,idem,+15[3]/46,XX[12]
4	46,XY,t(10;11)(q22;q23)[20]
5	46,X,-X,+8,der(16)t(1;16)(q21;q13)[18]/46,XX[4]
6	46,XY,t(11;20)(q10;q10),del(12)(p12)[10]/46,XY[10]
7	46,XY,del(5)(p14),del(11)(p13-15)[6]/46,XY[14]
8	45,XY,dic(9;16)(p11;p11.2),i(17)(q10)[21]
9	47,XX,+8,del(16)(q13)[1]/47,idem,del(9)(q22)[11]/46,XX[8]
10	46,XY,dup(1)(q32q21)[10]/47,idem,add(5)(q31),del(6)(q13q23),+mar[7]/46,XY[3]
11	47,XX,+mar[3]/46,XX[27]
12	46,XY,t(2;11)(p11.2;p11.2),der(9)t(1;9)(q12;q34)[20]
Biphenotypic (T + My)	
13	46,XX,t(2;14)(q24;q32),t(17;19)(p13;q13.1)[12]/46,idem,del(16)(q22)[3]
14	46,XY,i(17)(q10)[1]/46,XX(Donor)[12]/46,XY[7]
15	46,XX,der(2)?inv(2)(p11.2q13)t(2;14)(14qter->14q32.1::2p?->2qter),der(14)t(2;14)(14pter->14q32.1:: 2q13->2p12->2pter)[19]/46,XX[1]
16	47,XX,t(7;12)(q36;p13),+19[19]/46,XX[1]
17	46,XY[20]
18	46,XX[29]
19	46,XY[33]
20	46,XX,t(4;11)(q21;p15)[20]
21	46,XX,der(2)t(2;14)(q21;q32),add(5)(q31),der(14)ins(14;2)(q22;q21q37)[15]/46,XX[6]
22	46,XY,inv(2)(p11.2q13)c,inv(2)(q13q35)[16]/4n,idem[2]/46,XY,inv(2)(p11.2q13)c[2]
23	NA
24	46,XY[20]
25	46,XY,inv(7)(p24q11.2)[20]
31	NA
26	46,XY[25]
27	46,XY,t(5;7)(q13.3;p13)[17]/46,XY[3]
28	47,XY,der(1)add(1)(p36.3)add(1)(q32),t(4;7)(q21;p22),del(9)(p13),der(11)add(11)(p13)add(11)(q21),add(12)(p11.2),add(18)(q23),+mar[12]/46,idem,-der(11)add(11)[4]/47,idem,-der(1)add(1),-der(11)add(11),add(19)(p13.3)[3]/46,XY[6]
29	46,XY,inv(7)(p15q34)[7]/46,XY,der(7)inv(7)t(1;7)(q31;p22)[5]/46,XY[8]
30	46,XY[25]
31	47,XY,+4[2]/46,XY[18]
32	45,XX,t(8;12)(q13;p12-13),der(15)ins(15;17)(q15;q21q21),-17[7]/46,XX[14]
Bilineal (B + My)	
33	46,XX,t(11;19)(q23;p13.1)[20]
34	46,XY,t(9;11)(p13;q23)[18]/46,XY[2]
Undifferentiated acute leukemia	
35	47,XX,der(7)inv(7)(p22q11.2)t(7;15)(p22;q15),t(9;11)(p22;q23),+10,der(15)t(7;15)[20]

NA indicates not available.

separation among cases (Figure 1; Table S2). We observed that 5 (cases 18, 21, 26, 31, and 33) of the 13 mixed-lineage cases with available microarray data clustered with known AML cases, whereas the remaining 8 (cases 4, 6, 11, 15, 16, 20, 25, and 28) formed a highly distinct cluster with few similarities to known cases of AML, T-ALL, and B-progenitor ALL (Figure 1).

Clinical features, treatment, and outcome

The 35 patients (21 boys and 14 girls) in this study had a median age of 10 years (range, 2 days to 19 years) and a median leukocyte count at diagnosis of 18×10^9/L (range, 1-150×10^9/L; Table 3). Twenty-three patients initially received standard induction therapy for AML, generally with cytarabine, daunomycin, and etoposide, whereas 12 patients received induction therapy for ALL, with prednisone, L-asparaginase, daunorubicin, and vincristine (Table 3). In the subgroup of 23 patients

initially given AML therapy, 12 (52%) achieved complete remission (CR), 2 attained partial remission (PR), 8 had no response, and 1 died of toxicity. By contrast, of the 12 patients who first received ALL therapy, 10 (83%) achieved CR and 2 had no response. Overall, 22 of the 35 (63%) patients entered CR after their initial induction therapy, with 10 remaining in remission after treatment for AML (5 of 12) or ALL (5 of 10).

Interestingly, 8 of the 10 patients who failed to respond or had only a PR to AML therapy attained a CR after receiving standard ALL induction therapy consisting only of prednisone, vincristine, and L-asparaginase. Six of these patients had a mixed T/myeloid phenotype with expression of CD2, CD7, cytoplasmic CD3, and MPO. All 4 patients who were tested for minimal residual disease after lymphoid-directed induction therapy were minimal residual disease-negative. Moreover, 7 of the 8 patients are alive and in long-term remission after further
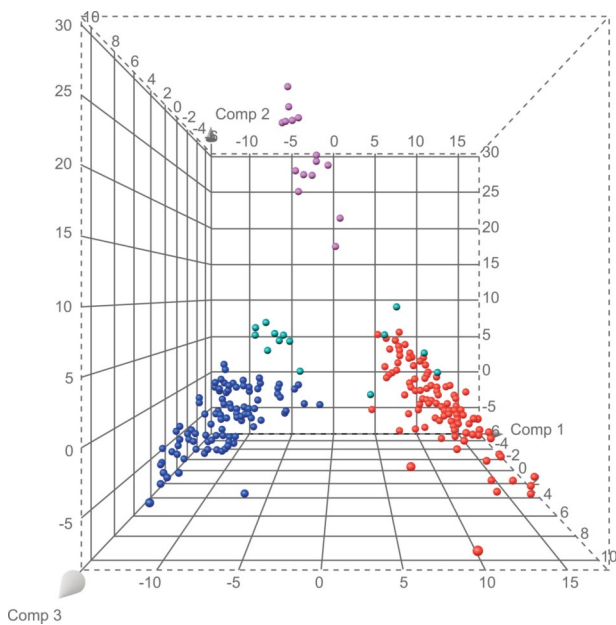
Figure 1. Principal components plot based on 284 lineage-distinguishing probe sets. Purple spheres represent cases of T-ALL; blue spheres, B-progenitor ALL; red spheres, AML; and green spheres, mixed lineage leukemia.

chemotherapy, whereas 1 died of toxicity after receiving a hematopoietic stem cell transplant. Both of the patients who were unresponsive to induction therapy for ALL entered at least partial remission (1 PR, 1 CR) after a switch to AML-directed therapy but later died of toxicity or relapse. Thus, approximately half (17 of 35) of our patients with mixed-lineage leukemia achieved long-term remissions.

Figure 2 compares the overall survival rates among patients with B/myeloid or T/myeloid biphenotypic leukemia and those with ALL or AML diagnosed by standard criteria. Outcomes were similar for the 2 subgroups of biphenotypic leukemia (5-year survival estimates, $36.0\% \pm 16.6\%$ vs $54.0\% \pm 13.8\%$, $P = .67$). The outcome of patients with B/myeloid or T/myeloid biphenotypic leukemia (5-year survival estimate, $47.8\% \pm 11.5\%$) was similar to that of patients with AML (n = 329, 5-year survival estimate, $56.5\% \pm 3.5\%$, $P = .64$) treated at St Jude Children's Research Hospital during the same time period (Figure 2). As expected, patients with ALL (n = 1527; 5-year survival estimate, $84.6\% \pm 1.1\%$) fared significantly better than either the T/myeloid or B/myeloid subgroups ($P < .001$).

There were no apparent associations between gene-expression patterns and clinical features. The 5 cases that clustered with AML cases included 4 T/myeloid biphenotypic cases and 1 B/myeloid bilineal case. Despite clustering with AML, 3 of these 5 cases had no response to AML-directed therapy. The 8 cases that formed a distinct cluster (Figure 1) included 3 B/myeloid and 5 T/myeloid biphenotypic cases. Of these, 3 achieved CR after AML-directed therapy and the other 5 achieved CR after ALL-directed therapy.

Discussion

Mixed-lineage leukemia is a rare disease entity that must be distinguished from ALL with atypical myeloid antigen expres-

sion and AML with atypical lymphoid expression.[11] Biphenotypic leukemia comprises the largest subset of cases within this general category and may be associated with a poor prognosis.[17-21] In a study of adults with acute leukemia, biphenotypic disease represented 8% of cases and conferred a poor prognosis, with a 4-year survival rate of only 8%.[18] Killick et al[20] described 20 patients with biphenotypic leukemia (8 children and 12 adults) among nearly 700 patients treated at their center. Overall, CR was achieved in 70% of patients, but the probability of survival at 2 years was only 39%. However, all 8 children with this type of leukemia achieved CR (5 after ALL induction, 2 after AML induction, and 1 after a switch to AML induction after a poor response to ALL induction). Six of these patients remained alive and in remission at 2 years of follow-up. The authors suggest that the prognosis associated with biphenotypic leukemia in children may not differ from that typically reported for childhood acute leukemia. A study of adults with ALL who were treated on the Leucemie Aigue Lymphoblastique de l'Adulte (LALA) 94 ALL protocol identified 7 patients (0.86%) with biphenotypic leukemias that expressed T-lymphoid and myeloid markers.[17] Only 2 of 7 patients entered CR after ALL induction therapy, but 4 of the other 5 attained CR after receiving AML therapy. Investigators from the M. D. Anderson Cancer Center reported that, among 31 adult patients with biphenotypic leukemia, CR rates were 78% for patients who received lymphoid-directed therapy and 57% for those who received myeloid therapy, whereas the overall survival at 2 years was 60%.[21] Acute bilineal leukemia appears to be even less common than biphenotypic leukemia, comprising approximately 1% of cases in a recent study of both adult and pediatric cases, in which CR was induced in only 6 of 16 patients, 2 of whom achieved sustained remissions.[16]

We studied 35 pediatric patients with mixed-lineage leukemia treated at this center over the past 20 years (~ 2% of all cases of leukemia). Overall, 32 patients (91%) achieved CR, with 17 (49%) remaining leukemia-free survivors. Because of the small number of patients and the heterogeneity of their clinical characteristics, immunophenotypes, and treatment regimens, we are unable to make firm recommendations regarding remission induction treatment. However, it is important that 8 of 10 patients who failed to respond to intensive AML induction therapy subsequently achieved remission after treatment with prednisone, vincristine, and L-asparaginase, and all but 1 are long-term survivors after receiving 120 weeks of rotational maintenance therapy that includes both myeloid- and lymphoid-directed components, confirming our early observation.[3] More than half (60%) of these patients had T/myeloid biphenotypic leukemia with expression of CD2, CD7, cytoplasmic CD3, and low MPO positivity, with or without Auer rods. These results, and the difficulty of predicting whether patients with mixed-lineage leukemia will respond better to ALL- or AML-type therapy, had led us to revise our treatment strategy. We now enroll both T/myeloid and B/myeloid patients in our frontline AML clinical trial. Patients who do not respond to the first course of AML-directed therapy are switched to ALL-type induction (prednisone, L-asparaginase, and vincristine) and consolidation (high-dose methotrexate and mercaptopurine), followed by maintenance treatment with rotational drug combinations that include etoposide/cyclophosphamide, methotrexate/mercaptopurine, methotrexate/L-asparaginase, and dexamethasone/vincristine/doxorubicin. Although patient numbers are small and follow-up times are short, this strategy appears to be promising. An alternative approach to induction therapy, which

Table 3. Clinical features and response to therapy in 35 children with mixed-lineage leukemia

Case and type	Age, y/sex	WBC, ×10⁹/L	Initial therapy	Response	Additional therapy and response	Duration of survival, y
Biphenotypic						
(B + T + My)						
1	17/M	15	AML	CR	Alive and in CR	20.9
2	11/M	1	ALL	NR	AML → PR → AML → hypocellular → toxic death	0.1
Biphenotypic (B + My)						
3	14/F	117	AML	CR	Relapse → ALL → CR → SCT → toxic death	1.6
4	16/M	12	AML	CR	Alive and in CR	4.1
5	9/F	10	AML	CR	Relapse → 2CDA → CR → auto SCT → relapse → ALL → toxic death	4.9
6	16/M	150	AML	PR	ALL therapy → CR	2.2
7	1/M	56	ALL	CR	Alive and in CR	12.7
8	6/M	3	ALL	CR	Relapse → ALL → CR	12.3
9	2/F	15	ALL	CR (MRD+)	SCT → toxic death	0.7
10	7/M	44	ALL	CR	Relapse → AML → CR → relapse → ALL → NR → Clo → NR	2.1
11	3/F	8	ALL	CR	Alive and in CR	2.9
12	2/M	20	ALL	CR (MRD+)	SCT → alive and in CR	0.5
Biphenotypic (T + My)						
13	2/F	11	AML	CR	Relapse → AML → multiple relapses	5.0
14	8/M	na	AML	CR	Relapse → AML → PR → AML → PR → SCT → CR	8.5
15	2/F	44	AML	CR (MRD+)	SCT → CR	2.5
16	1/F	3	AML	CR (MRD+)	SCT → CR	2.4
17	12/M	4	AML	CR	Alive and in CR	1.9
18	19/F	103	AML	CR	Relapse → AML + auto SCT → CR → toxic death	1.6
19	12/M	11	AML	CR	Relapse → AML → toxic death	4.3
20	4/F	40	AML	PR	ALL → CR	3.6
21	16/F	2	AML	NR	ALL → CR → SCT → toxic death	0.4
22	1/M	4	AML	NR	ALL → NR → ALL → NR → AML → CR → SCT → Relapse	0.9
23	12/F	79	AML	NR	ALL → CR	2.3
24	16/M	20	AML	NR	ALL → CR	5.8
25	13/M	49	AML	NR	ALL → CR	1.7
26	11/M	1	AML	NR	ALL → toxic death	0.1
27	5/M	103	ALL	CR	Relapse → AM → NR	0.1
28	10/M	2	ALL	CR	Relapse → AML → CR → SCT → relapse	2.1
29	14/M	5	ALL	CR	Alive and in CR	8.5
30	13/M	43	ALL	CR	Alive and in CR	5.7
31	10/M	41	AML	NR	ALL → CR	16.5
32	3/F	3	ALL	NR	ALL → CR → relapse → AML → CR → haploBMT → toxic death	1.4
Bilineal (B + My)						
33	12/F	26	AML	Hypocellular	Toxic death	0.1
34	2 days/M	67	AML	NR	ALL → CR (MRD+) → SCT → toxic death	0.4
Undifferentiated acute leukemia						
35	15/F	41	AML	CR	Relapse → SCT → toxic death	0.5

WBC indicates white blood cell count; AML, acute myeloid leukemia; CR, complete remission; PR, partial remission; ALL, acute lymphoblastic leukemia; NR, no response; SCT, stem cell transplantation; 2CDA, cladribine; MRD, minimal residual disease; and Clo, clofarabine.

we have not yet tested, is to administer an induction regimen that consists of both lymphoid-directed agents (prednisone, vincristine, and L-asparaginase) and myeloid-directed therapy (high-dose cytarabine, etoposide, and daunorubicin). We suggest that hematopoietic stem cell transplantation may not be required to secure long-term remissions in patients with biphenotypic leukemia, especially those who attain a molecular or immunologic remission (< 0.01% blast cells) after induction therapy. However, we do recommend hematopoietic stem cell transplantation for patients with greater than 1% blasts by flow cytometry at the end of induction. The optimal therapy for patients with low levels of disease (0.01%-1%) is uncertain.

Analysis of gene-expression patterns, available for 13 of our biphenotypic cases, suggested that a significant subset of these leukemias may represent a biologically unique entity. Indeed, we identified 8 cases whose gene-expression patterns provide a clear demarcation from B-progenitor ALL, T-ALL, and AML. If this relationship can be established in an independent cohort of patients, it may be possible to correlate the new entity with clinical features or prognosis, to identify novel mechanisms of pathogenesis, or to identify therapeutic targets.
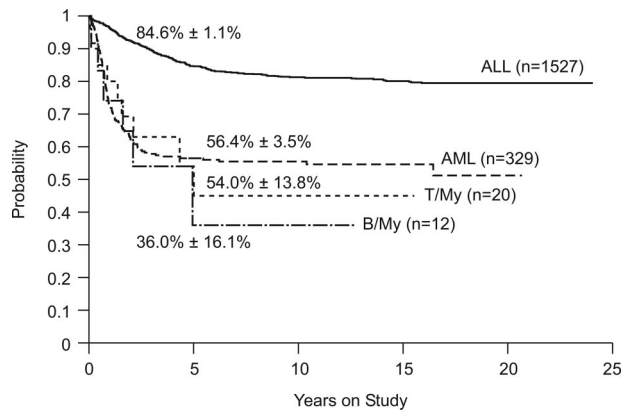
Figure 2. Kaplan-Meier plot of overall survival for patients with ALL, AML, T/myeloid, and B/myeloid biphenotypic leukemia. Five-year survival estimates are shown.

Authorship

Contribution: J.E.R. designed and performed research; collected, analyzed, and interpreted data; and wrote the manuscript; M.O. and F.G.B. performed flow cytometric analysis and pathologic analysis; S.P. and X.C. performed statistical analyses; D.C. performed minimal residual disease studies; S.S. and J.R.D. performed and analyzed gene-expression studies; S.C.R. performed cytogenetic analyses; and B.I.R., R.C.R., and C.-H.P. interpreted data. All authors critically reviewed and approved the manuscript.

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References

1. Mirro J, Zipf TF, Pui CH, et al. Acute mixed lineage leukemia: clinicopathologic correlations and prognostic significance. Blood. 1985;66:1115-1123.

2. Pui CH, Behm FG, Singh B, et al. Myeloid-associated antigen expression lacks prognostic value in childhood acute lymphoblastic leukemia treated with intensive multiagent chemotherapy. Blood. 1990;75:198-202.

3. Pui CH, Raimondi SC, Head DR, et al. Characterization of childhood acute leukemia with multiple myeloid and lymphoid markers at diagnosis and at relapse. Blood. 1991;78:1327-1337.

4. Wiersma SR, Ortega J, Sobel E, Weinberg KI. Clinical importance of myeloid-antigen expression in acute lymphoblastic leukemia of childhood. N Engl J Med. 1991;324:800-808.

5. Uckun FM, Sather HN, Gaynon PS, et al. Clinical features and treatment outcome of children with myeloid antigen positive acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood. 1997;90:28-35.

6. Pui CH, Rubnitz JE, Hancock ML, et al. Reappraisal of the clinical and biologic significance of myeloid-associated antigen expression in childhood acute lymphoblastic leukemia. J Clin Oncol. 1998;16:3768-3773.

7. Kuerbitz SJ, Civin CI, Krischer JP, et al. Expression of myeloid-associated and lymphoid-associated cell-surface antigens in acute myeloid leukemia of childhood: a Pediatric Oncology Group study. J Clin Oncol. 1992;10:1419-1429.

8. Creutzig U, Harbott J, Sperling C, et al. Clinical significance of surface antigen expression in children with acute myeloid leukemia: results of study AML-BFM-87. Blood. 1995;86:3097-3108.

9. Campana D, Behm FG. Immunophenotyping of leukemia. J Immunol Methods. 2000;243:59-75.

10. Jaffee ES, Harris NL, Stein H, Vardiman JW. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001.

11. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias: European Group for the Immunological

Characterization of Leukemias (EGIL). Leukemia. 1995;9:1783-1786.

12. Carbonell F, Swansbury J, Min T, et al. Cytogenetic findings in acute biphenotypic leukaemia. Leukemia. 1996;10:1283-1287.

13. European Group for the Immunological Classification of Leukaemias. The value of c-kit in the diagnosis of biphenotypic acute leukemia. Leukemia. 1998;12:2038.

14. Hayashi Y, Sugita K, Nakazawa S, et al. Karyotypic patterns in acute mixed lineage leukemia. Leukemia. 1990;4:121-126.

15. Matutes E, Morilla R, Farahat N, et al. Definition of acute biphenotypic leukemia. Haematologica. 1997;82:64-66.

16. Weir EG, Ii Ansari-Lari M, Batista DA, et al. Acute bilineal leukemia: a rare disease with poor outcome. Leukemia. 2007;21:2264-2270.

17. Rubio MT, Dhedin N, Boucheix C, et al. Adult T-biphenotypic acute leukaemia: clinical and biological features and outcome. Br J Haematol. 2003;123:842-849.

18. Legrand O, Perrot JY, Simonin G, et al. Adult biphenotypic acute leukaemia: an entity with poor prognosis which is related to unfavourable cytogenetics and P-glycoprotein over-expression. Br J Haematol. 1998;100:147-155.

19. Tiribelli M, Damiani D, Masolini P, et al. Biological and clinical features of T-biphenotypic acute leukaemia: report from a single centre. Br J Haematol. 2004;125:814-815.

20. Killick S, Matutes E, Powles RL, et al. Outcome of biphenotypic acute leukemia. Haematologica. 1999;84:699-706.

21. Aribi A, Bueso-Ramos C, Estey E, et al. Biphenotypic acute leukaemia: a case series. Br J Haematol. 2007;138:213-216.

22. Ribeiro RC, Razzouk BI, Pounds S, et al. Successive clinical trials for childhood acute myeloid leukemia at St Jude Children's Research Hospital, from 1980 to 2000. Leukemia. 2005;19:2125-2129.

23. Crews KR, Gandhi V, Srivastava DK, et al. Interim comparison of a continuous infusion versus a short daily infusion of cytarabine given in combi-

nation with cladribine for pediatric acute myeloid leukemia. J Clin Oncol. 2002;20:4217-4224.

24. Kishi S, Griener J, Cheng C, et al. Homocysteine, pharmacogenetics, and neurotoxicity in children with leukemia. J Clin Oncol. 2003;21:3084-3091.

25. Pui CH, Boyett JM, Rivera GK, et al. Long-term results of Total Therapy studies 11, 12 and 13A for childhood acute lymphoblastic leukemia at St Jude Children's Research Hospital. Leukemia. 2000;14:2286-2294.

26. Flotho C, Coustan-Smith E, Pei D, et al. A set of genes that regulate cell proliferation predicts treatment outcome in childhood acute lymphoblastic leukemia. Blood. 2007;110:1271-1277.

27. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354:166-178.

28. Raimondi SC, Zhou Y, Mathew S, et al. Reassessment of the prognostic significance of hypodiploidy in pediatric patients with acute lymphoblastic leukemia. Cancer. 2003;98:2715-2722.

29. Ross ME, Mahfouz R, Onciu M, et al. Gene expression profiling of pediatric acute myelogenous leukemia. Blood. 2004;104:3679-3687.

30. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell. 2002; 1:133-143.

31. Wilcoxon F. Individual comparisons by ranking methods. Biometrics. 1945;1:80-83.

32. Pounds S, Cheng C. Robust estimation of the false discovery rate. Bioinformatics. 2006;22: 1979-1987.

33. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 2002;30:207-210.

34. Kaplan EL, Meier P. Non-parametric estimation for incomplete observations. J Am Stat Assoc. 1958;53:457-481.

35. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer. 1977;35:1-39.