Haematologica 1999; 84:699-706 original paper

Outcome of biphenotypic acute leukemia

SALLY KILLICK,* ESTELLA MATUTES,° RAY L. POWLES,* MIKE HAMBLIN,* JOHN SWANSBURY,°
JENNIFER G. TRELEAVEN,* ATHANASIOS ZOMAS,* AYAD ATRA,# DANIEL CATOVSKY°
**Leukemia Unit; °Academic Department of Hematology and Cytogenetics and #Pediatric Oncology Unit, The Royal Marsden NHS Trust and Institute of Cancer Research, London and Surrey, United Kingdom*

ABSTRACT

Background and Objective. **Although biphenotypic leukemia is now a defined entity, outcome of this rare form of acute leukemia has not been well documented. We present the first comprehensive study analyzing induction and consolidation therapy of biphenotypic leukemia and correlate outcome to prognostic factors.**

Design and Methods. **In this retrospective study, the incidence of biphenotypic leukemia was found to be 3.6% from 693 adult and pediatric acute leukemias referred to our center for treatment over the last 8 years. Of these, 15 were B-lymphoid / myeloid, 8 were T-lymphoid / myeloid, one was T / B lymphoid and one had trilineage differentiation.**

Results. **Induction of remission in *de novo* cases was achieved in 70% of patients and relapse of disease occurred in 15%. The use of combined lymphoid and myeloid drugs for induction resulted in a high incidence of early deaths (25%). The overall probability of survival at 2 years was 39.4%. Patients with secondary disease had a uniformly poor outcome with low remission rates and high relapse rates.**

Interpretation and Conclusions. **Prognosis was most strongly related to the presence of the Philadelphia chromosome (p=0.03) and age under 15 years (p=0.01). We conclude that patients with biphenotypic leukemia should have risk stratification with treatment tailored to their prognostic factors.**

Key words: biphenotypic leukemia, Philadelphia chromosome

Most acute leukemias are classified as lymphoid or myeloid lineage using the FAB classification[1] and a panel of immunologic markers. There is however, a minority of cases [approximately 4%[2]] which are difficult to classify using these methods because the blasts co-express myeloid and lymphoid markers. These cases have been designated as biphenotypic and have previously been described as of mixed lineage or hybrid,[3-8] myeloid antigen-positive acute lymphoblastic leukemia (My+ ALL)[9-12] and lymphoid antigen-positive acute myeloid leukemia (Ly+ AML).[10,13,14] It is likely that this miscellaneous group encompasses true biphenotypic cases along with ALL or AML with the expression of one or two aberrant markers.

The clinical significance of biphenotypic acute leukemia has not been determined and there has been a lack of uniformity in treatment. For example, there is no agreement as to whether induction therapy should be with lymphoid and/or myeloid drugs and whether this should be followed by bone marrow or peripheral blood stem cell transplantation.

The aim of this study was to correlate clinical data with treatment response in 25 patients with biphenotypic acute leukemia treated at the Royal Marsden Hospital between January 1990 and August 1997. These cases were matched with 25 AML and 25 ALL patients treated during the same period.

Design and Methods

Patient population

The case population for this retrospective study was derived from 693 adult and pediatric patients referred to our hospital for investigation and treatment between January 1990 and August 1997. They included patients with *de novo* and secondary disease. A diagnosis of AML or ALL was established using the FAB criteria.[1]

Peripheral blood and bone marrow films stained with May-Grünwald-Giemsa were reviewed and cytochemical reactions including myeloperoxidase, Sudan-black B (SBB), non-specific esterase and periodic acid-Schiff (PAS) were performed.

Clinical information including age, sex, mode of presentation (*de novo* or secondary), induction and consolidation treatment was obtained. There were 20 patients with *de novo* biphenotypic acute leukemia and 5 patients who presented after previous therapy for AML or ALL (designated secondary biphenotypic acute leukemia). The original disease in those with secondary leukemia was B lineage ALL in 2 patients, T-ALL in one and AML in 2. Complete disease remis-

Correspondence: S Killick, M.D., Current address: Department of Haematology, St George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, UK.
Phone: international +44-181-725 5448 – Fax: international +44-181-725 0245 – E-mail: skillick@btinternet.com

Table 1. Scoring system for the definition of biphenotypic acute leukemia.

Scoing points	Lineages		
	B lymphoid	*T lymphoid*	*Myeloid*
2	CD79a (mb-1) CD22 cyt IgM	CD3 anti-TCRα/β anti-TCRγ/δ	anti-MPO*
1	CD19 CD10 CD20	CD2 CD5 CD8 CD10	CD117(c-kit) CD13 CD33 CD65s
0.5	TdT CD24	TdT CD7	CD14 CD15 CD64

*Biphenotypic acute leukemia is defined when the score from two separate lineages is greater than 2; *MPO (myeloperoxidase) demonstrated by cytochemical or immunologic methods.*

sion (CR) was defined as less than 5% blasts in the bone marrow.

Flow cytometry

Immunophenotype was determined on isolated peripheral blood and/or bone marrow mononuclear cells by flow cytometry using indirect immunofluorescence with a panel of monoclonal antibodies (McAb) against lymphoid and myeloid antigens and fluorescein conjugated (FITC) goat anti-mouse immunoglobulin as second layer. Double labeling was carried out with FITC and phycoerythrin (PE) conjugated McAb and appropriate controls. Analysis was performed on a FACScan flow cytometer, gating on the blast population. Immunocytochemistry was used up to 1994 to detect nuclear TdT and cytoplasmic CD3, CD22, CD79a, IgM and anti-MPO using the immunoalkaline phosphatase anti-alkaline phosphatase (APAAP) technique. More recently, nuclear and cytoplasmic staining was carried out by flow cytometry after fixation and permeabilization of the cells.[15] All samples contained between 65 and 98% blasts. A marker was considered positive if expressed in >20% of blasts by flow cytometry or in >10% by the APAAP method.

The McAb used were as follows: CD2 (Leu5b), CD3 (UCHT1), CD7 (3A1), CD5 (UCHT2) as T-cell markers; CD10 (CALLA; J5), CD19 (HD37), CD22 (OKB22), CD79a (HM57), anti-IgM as B-cell markers; CD13 (My7), CD33 (My9), anti-MPO (MPO-7), CD14 (LeuM3), CD117 (c-kit) as myeloid markers and TdT , CD34 (HPCA-2) and anti-HLA-Dr against precursor cells. The McAb CD2, CD10, CD14, anti-HLA-Dr and CD34 were purchased from Becton-Dickinson (Mountain View, CA, USA), CD3, CD5, CD7, CD13, CD33 from Coulter (Luton, UK), CD19, CD79a, anti-MPO, anti-IgM from Dako (High Wycombe, UK) and CD117 from Immunotech (Marseille, France).

Criteria for diagnosis of biphenotypic acute leukemia

Criteria for the diagnosis of biphenotypic acute leukemia were based on the previously described scoring system[2] adopted by the *European Group of Immunological Classification of Leukemias* (EGIL).[16,17] The scoring system aims to distinguish *bona fide* biphenotypic acute leukemia from those with aberrant expression of a marker from another lineage (Ly+AML, My+ALL). It is based on the number and degree of specificity of the markers expressed by the leukemic cells.[2] Table 1 shows the markers considered most specific: i) *B-lymphoid lineage*, CD79a (mb-1), cytoplasmic immunoglobulin and CD22; ii) *T-lymphoid lineage*, CD3; iii) *myeloid lineage*, myeloperoxidase demonstrated by either cytochemical or immunologic methods.

Cytogenetic analysis

Cytogenetic analysis was performed as previously described[18] on whole heparinized unstimulated bone marrow specimens cultured for 1 to 48 hours in RPMI-1640 medium with 15% fetal calf serum using blocking with FdUr or excess thymidine, arresting with colcemid, banding with 2 x SSC and trypsin, and staining with Giemsa.

Treatment

AML induction therapy. BF12: cytosine arabinoside 2 g/m^2 twice daily, etoposide 100 mg/m^2, idarubicin 5 mg/m^2, each given for 5 days.[19] BF12/M replaces idarubicin with mitoxantrone 10 mg/m^2.

ALL induction therapy. Adults: prednisolone 40 mg/m^2 for 4 weeks, vincristine 1.5 mg/m^2 (maximum 2 mg) once weekly for 4 weeks, daunorubicin 45 mg/m^2 days 1 and 2, asparaginase 6000 units/m^2 subcutaneously (S/C) three times a week for 9 doses. For children under 15 years treatment was as above without daunorubicin. Intensification at 5, 20, ± 35 weeks with: prednisolone 40 mg/m^2 for 7 days, vincristine 1.5 mg/m^2 (maximum 2 mg) days 1 and 2, daunorubicin 45 mg/m^2 days 1 and 2, etoposide 100 mg/m^2 for 5 days, cytosine arabinoside 100 mg/m^2 for 5 days, thioguanine (oral) 80 mg/m^2 for 5 days.

AML consolidation therapy. L6: cytosine arabinoside 60 mg/m^2 twice daily S/C (3-5 days), thioguanine (oral) 80 mg twice daily (3-5 days) given as 3 courses with a 5 day gap between each cycle. Then, MACE: amsacrine 100 mg/m^2, cytosine arabinoside 200 mg/m^2, etoposide 100 mg/m^2, each given for 5 days. MidAC (mitoxantrone 10 mg/m^2 5 days, cytosine arabinoside 1 g/m^2 3 days) was used as further consolidation in the children.

Details of combined AML/ALL induction therapy are given in Table 4.

Statistical methods

The matched sets of AML and ALL patients were automatically selected from the overall population of acute leukemia patients using a computer program. This matched sex, age (±2 years), BMT (Y/N),

Table 2. Immunologic markers in patients with *de novo* (UPN 1-20) and secondary (UPN 21-25) biphenotypic acute leukemia.

UPN	TdT	CD10	CD19	CD22	CD79a	cIgM	Score	CD2	CD3	CD7	Score	CD13	CD33	MPO*	Score	FAB#
1	26	30	19	16	NT	NT	4.5	31	0	9		81	2	40	3	AML
2	61	26	64	NT	NT	neg	2.5	neg	neg	neg		61	37	14	4	AML
3	18	20	86	65	NT	0	4.5	neg	neg	neg		74	44	5	4	AML
4		64	61	80	NT	neg	4	neg	NT	neg		68	97	10	4	AML
5	76	59	89	NT	NT	neg	2.5	neg	neg	NT		76	68	7	4	AML
6	26	NT	NT	neg	NT	NT		NT	68	NT	2.5	15	NT	26	3	AML
7	31	neg	neg	NT	NT	NT		97	34	98	4	4	50	10	3	ALL
8	75	NT	NT	neg	neg	neg		NT	93	NT	2.5	99	NT	66	3	ALL
9	84	91	74	35	neg	34	6.5	48	20	22	4	59	neg	30	3	AML
10	95	87	95	67	70	neg	6.5	neg	NT	neg		82	15	70	3	ALL
11	87	98	92	72	68	18	6.5	neg	neg	neg		71	34	42	4	ALL
12	92	89	95	neg	NT	42	4.5	neg	NT	neg		75	88	53	4	ALL
13	70	38	34	61	NT	NT	4.5	85	12	71	4	53	neg	neg		ALL
14	86	58	84	81	NT	neg	4.5	neg	NT	neg		71	neg	64	3	AML
15	6	neg	neg	NT	NT	NT		85	80	77	3.5	96	30	35	4	AML
16	68	62	60	20	NT	neg	4.5	neg	NT	neg		54	neg	17	3	ALL
17	60	76	84		NT	74	neg	4.5	neg	NT	neg	95	neg	30	4	AML
18	52	10	86	31	70	33	7.5	neg	NT	53		59	75	61	4	AML
19	25	4	2	neg	neg	NT		41	88	85	5	62	0	71	3.5	ALL
20	11	17	2	NT	NT	NT		98	56	89	5	41	36	11	4	AML
21	87	63	78	92	NT	NT	4.5	81	neg	neg	64		54	9	4	
22	68	61	neg	neg	NT	NT		88	97	90	4	78	neg	28	3	
23	63	57	58	28	NT	neg	4.5	neg	neg	neg	neg	25	84	3		
24	71	neg	neg	NT	NT	NT		89	86	67	4	6	4		35	3
25	neg	neg	77	neg	neg	neg	3	neg	neg	30		96	97	17	4	

**MPO assessed by cytochemistry with Sudan-Black-B and myeloperoxidase in cases #1, 3, 5, 20. Cases #17, 19 and 20 were CD117 (c-kit)+. Cases #19 and 24 were anti-lysozyme+. Cases #19 and 20 were CD5+. #FAB classification of de novo cases based on morphology and cytochemistry. NT = not tested.*

donor type, conditioning and, from the possible matches, those patients with the nearest dates of registration. It was not possible to match chemotherapy agents in those patients who received both AML and ALL induction.

Survival was illustrated and compared using Kaplan-Meier survival curves. Differences between the curves were examined statistically using the log rank test and derivatives.

Results

Demographic details

From 693 patients presenting to our center with acute leukemia between January 1990 and August 1997, 3.6% were diagnosed as having biphenotypic disease (20 cases of *de novo* and 5 cases of secondary) according to the criteria outlined above.

In the *de novo* group, the male to female ratio was 1.5:1 and median age at diagnosis was 25.5 years (range 3-46). Eight patients were under 15 years and 12 were over 15 years.

In the secondary group, the male to female ratio was 4:1 and median age at diagnosis was 33 years (range 7-34). Median time from diagnosis of the original disease to biphenotypic acute leukemia was 24 months (range 9-177). Diagnosis was made after first CR in 4 patients and after second CR in one. One patient with Philadelphia positive B lineage ALL developed biphenotypic leukemia after a matched unrelated donor (MUD) bone marrow transplant.

Morphology

Morphologic assessment of the patients with *de novo* disease showed lymphoid features in 7, myeloid in 8 (of which 3 cases had Auer rods), undifferentiated in three and two cases showed two blast populations, one resembling lymphoblasts and the other myeloblasts. Based on morphology and cytochemistry, a total of 12 cases had the FAB diagnostic criteria for AML, and the other 8 were ALL.

Immunophenotype

The immunophenotype, biphenotypic scores and FAB classification for the patients presenting with *de novo* (#1-20) and secondary (#21-25) disease are shown in Table 2. Results of *de novo* cases show that 12 cases had a myeloid and B-lymphoid phenotype (#1-5, 10-12, 14, 16-18), 6 cases had a myeloid and T-lymphoid phenotype (#6-8, 15, 19-20), one had trilineage differentiation (#9) and another had coexpression of B and T lymphoid antigens (#13). All cases had a score of over 2 for both myeloid and lymphoid lineages. Double labeling confirmed the coexpression of lymphoid and myeloid markers in a variable proportion of blasts. Class II HLA-DR determinants were strongly expressed in 16 out of 17 cases tested and CD34 was positive in 13 out of 19. No

Table 3. Cytogenetic analysis in cases with *de novo* (UPN 1-20) and secondary (UPN 21-25) biphenotypic acute leukemia.

UPN	Karyotype
1.	46,XY,t(8;21)(q22;q22) [2] / 46,XY,idem,del(9)(q22) [7] / 45,XY,idem,-9 [3]
2.	t(9;22) (not tested at RMH at diagnosis)
3.	N/A
4.	46,XY,t(11;19)(q23;p13) [4]/49,XY,idem,+8,+12,+18 [6]
5.	46,XY,t(9;22)(q34;q11) [9] /47,XY,idem,+der(22) [2]
6.	N/A
7.	46,XY,del(6)(q15q33),del(11)(q14),del(12)(p13) [13] /46,XY [6]
8.	N/A
9.	46,XX,t(9;22)(q34;q11),i(9)(q10) [1]/46,XX,idem,-7,del(7)(p1), +mar [7]/46,XX [5]
10.	46,XX,t(9;22)(q34;q11),add(2)(q37),add(4)(q33) [7]/46,XX [6]
11.	Diagnostic failure but known to be bcr/abl negative
12.	46,XY,t(9;22)(q34;q11) [10]
13.	45,XY,der(9)t(9;17)(p11;q11),-17[16]/46,XY [9]
14.	46,XX,t(2;7)(p1?3;q3?4),add(11)(p1?5),add(16)(q2?4) [5] / 46,XX [6]
15.	46,XY
16.	46,XY,t(9;22)(q34;q11) / 50,XY,idem,+X,+Y,+4,+8
17.	45,XX,-7,t(9;22)(q34;q11) [19]/46,XX [1]
18.	46,XX,t(12;22)(p13;q11) [4]/46,XX,idem,del(20)(q11) [3] / 45,X,-X,idem,del(20)(q11) [5]/46,XX [1]
19.	46,XY,add(1)(q3),add(5)(q31),-7,del(9)(q13),del(11)(q21), +mar [5]/46,XY [20]
20.	46,XX,t(1;7)(q32;q3),add(17)(q11)/46,XX,del(9)(q12)/46,XX
21.	94,XXXX,dic(9;12)(q1;q1)x2
22.	46,XY [17] in relapse
23	At diagnosis: 46,XY,t(9;22)(q34;q11)[18]/46,XX [2] In relapse: 55-58,X,+X,-Y,+5,+6,+7,+8,t(9;22)(q34;q11),+10,+19, +20,+21,+22[9]/46,XY [8]
24.	90,XX,-Y,-Y[9]/45,X,-Y [1]/46,XY [4]
25.	N/A

N/A = Not available for review.

cases had expression of erythroid or megakaryocytic markers.

In the *de novo* group, 60% of patients had coexpression of myeloid and B lymphoid antigens. Although at last follow up 6 of the 8 patients were alive, they did not have a survival advantage over the patients with alternative phenotypes (T-myeloid, B-T lymphoid and trilineage). From our study it would appear that phenotypic characteristics alone do not predict clinical outcome.

Results of the secondary cases showed three with a myeloid and B-lymphoid phenotype (#21, 23, 25) and two cases with a myeloid and T-lymphoid phenotype (#22, 24).

Cytogenetics

Chromosome analysis was available for review in 17 of the 20 cases of *de novo* and 4 of the 5 cases of secondary biphenotypic leukemia (Table 3). Two patients had a normal karyotype (#15,22). The most frequent chromosome abnormality in the de novo

Table 4. Details of induction treatment given to *de novo* biphenotypic cases.

UPN	Age	Induction therapy (AML/ALL/ combined)	Details	CR (Y/N)	Days to CR
1	30	AML	BF12/M	Y	26
2*	46	Combined	Cytosine arabinoside 1g/m² Mitoxantrone 12 mg/m² Etoposide 100 mg/m² Vincristine 2 mg Prednisolone 60 mg/m²	N	Induction death
3	20	Combined	Cytosine arabinoside 1 g/m² Daunorubicin 45 mg/m² Etoposide 100 mg/m² Vincristine 2 mg Prednisolone 60 mg/m²	Y	49
4	13	AML	BF12	Y	35
5	36	Combined	ALL induction therapy Cytosine arabinoside 2 g/m² Etoposide 100 mg/m²	N Y	Induction death
6	43	Combined	ALL induction therapy BF12 (given 2 weeks later)	N	Induction death
7*	14	Combined	ALL induction therapy BF12 (5 weeks later)	N Y	78
8	12	ALL	ALL induction therapy	Y	28
9	45	Combined	ALL induction therapy Cytosine arabinoside 2 g/m² Etoposide 100 mg/m²	N	Induction death
10	14	ALL	ALL induction therapy	Y	85
11	26	Combined	ALL induction therapy BF12	Y	21
12	5	ALL	ALL induction therapy	Y	46
13	4	ALL	ALL induction therapy	Y	26
14	10	AML	Cytosine arabinoside 100 mg/m² Daunorubicin 50 mg/m² Etoposide 100 mg/m²	Y	34
15°	27	AML	Cytosine arabinoside 100 mg/m² Etoposide 100 mg/m² Mitoxantrone 12 mg/m²	Y	N/A
16	34	Combined	Prednisolone 60 mg/m² Vincristine 2 mg Daunorubicin 60 mg/m² Asparaginase 10,000 IU	Y	38
17*	41	AML	BF12	N	Allograft with disease
18	3	ALL	ALL induction therapy	Y	46
19	25	Combined	BF12 Vincristine 2 mg Prednisolone 40 mg/m² Asparaginase 6,000 IU/m²	Y	33
20	44	Combined	BF12 Vincristine 2 mg Prednisolone 40 mg/m²	N	Induction death

*Details of chemotherapy regimens are found in the text. *Patients receiving unsuccessful induction therapy prior to referral; °Induction therapy given by referral center. UPN 2: cytosine arabinoside, daunorubicin; UPN 7: vincristine, bleomycin, prednisolone; UPN 17: daunorubicin, cytosine arabinoside, thioguanine; N/A = Not available for review.*

Table 5. Consolidation treatment of *de novo* biphenotypic cases.

UPN	*Consolidation chemotherapy*	*PBSCT/BMT*	*Status at graft*	*Outcome*
1	L6 MACE	Autologous	1st remission	Alive
4	MACE	Autologous 6MP&MTX	1st remission	Alive
7	Allograft		1st remission	TRM
8	ALL intensification maintenance 6MP & MTX			Alive
10	ALL intensification	MUD	1st remission	Relapse Alive with disease
11	MACE	MUD Autologous rescue	1st remission	Alive
12	ADE, L6	MUD	1st remission	TRM
13	ALL intensification maintenance 6MP & MTX			Alive
14	MACE MidAC			Alive
15	MACE	Allograft	1st relapse	TRM
16		Allograft	1st remission	TRM
17		Allograft	Resistant disease	TRM
18	ALL intensification maintenance 6MP & MTX			Alive
19	MACE	Allograft	1st remission	TRM

Details of chemotherapy regimens can be found in the text. MUD = Matched unrelated transplant, PBSCT = Peripheral blood stem cell transplant, BMT = Bone marrow transplant, 6MP = 6 mercaptopurine, MTX = Methotrexate; TRM = Transplant related mortality.

group was a Philadelphia chromosome (Ph), t(9;22)(34;q11), observed in 7 cases (41%) (#2,5,9, 10, 12, 16,17), additional chromosome aberrations were found in 5 of these cases. No patient co-expressing T lymphoid and myeloid antigens was Ph positive. Structural abnormalities of chromosome 11 were seen in three patients (19%) (#4,14,19), of whom one showed rearrangement of 11q23. The translocation t(8;21)(q22;q22) was detected in one patient (#1) with AML M2 morphology.

In those patients with secondary biphenotypic leukemia, cytogenetic data on the original disease was not available in 4 out of 5 cases. Therefore it is not possible to elucidate whether these were relapsed disease or truly a secondary leukemic event. The patient for whom data were available was originally Ph+ B-ALL, t(9;22)(q34;q11); this clone was present at relapse.

Cytogenetic data on patient # 1,4,5,7,9,10,12,14, 21,22 have been previously reported by Carbonell *et al.*,[20] patient #4 also by Moorman *et al*.[21] and case #13 by Zomas *et al*.[22]

Treatment and clinical outcome

Induction chemotherapy regimens given to patients with *de novo* biphenotypic leukemia are summarized in Table 4. Fourteen of the 20 patients (70%) entered CR after induction chemotherapy. Median time to CR was 35 days (range 21-85). From the 7 patients found to be Ph positive, 3 died during induction (combined AML/ALL therapy), 3 achieved CR with induction therapy for ALL in two and combined in one, and the remaining patient was given AML induction therapy but did not achieve CR.

From those patients surviving induction therapy, 14/15 (93%) achieved CR. One patient (#3) who received combined induction therapy achieved CR but died of aspergillosis before consolidation treatment. Consolidation treatment of the 14 survivors is summarized in Table 5.

The survival of patients with *de novo* biphenotypic acute leukemia is shown in Figure 1. Median follow-up was 30.2 weeks (range 2.4-367.9) and median survival was 27.4 weeks (95% confidence intervals 27.1-69.2). Median time from diagnosis to death was 15.9 weeks (range 2.4-44.9). The probability of survival for all patients at 2 years was 39.4% (95% confidence intervals18.6%-59.7%). At last follow-up, 6 of the 8 patients under 15 years were alive compared to 2/12 adults. The probability of survival at 2 years of childhood cases (75%) was significantly better than that of adult cases (17%) (p=0.01) (Figure 2). The overall survival of *de novo* biphenotypic leukemia in both childhood and adult groups was compared to that of matched controls with AML and ALL treated in the same period of time. The childhood group showed no evidence of a difference between survival of biphenotypic leukemia and matched controls with AML or ALL. In the adult group (age > 15 years), the survival of patients with biphenotypic leukemia was worse than that in the matched controls with AML (Figure 3) or ALL (Figure 4). Test statistics, particularly those giving weight to early events, show significant differences (p=0.02 and 0.04 in Figures 3 and 4 respectively; Peto-Prentice). It must be noted, however, that the controls did not receive combination induction therapy and therefore the early induction deaths would be lower.

Although the number of patients in this study does not allow firm conclusions, 86% of patients with *de novo* disease who were Ph positive had died at last follow-up, compared to 40% of patients who were known to be Ph negative (Figure 5). There was no apparent difference in survival between patients with Ph positive biphenotypic leukemia and matched patients with Ph positive ALL, or those patients with Ph negative biphenotypic acute leukemia and those with Ph negative ALL.

Induction chemotherapy in secondary biphenotypic cases was for AML in one and for ALL in 4 patients. Only one of the five patients (#21) entered CR with ALL therapy. Induction was followed by allograft in 4 patients in whom transplantation had not previously

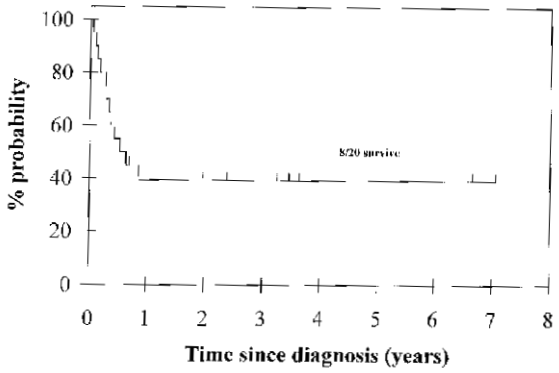

Figure 1. Overall survival of patients with biphenotypic leukemia.

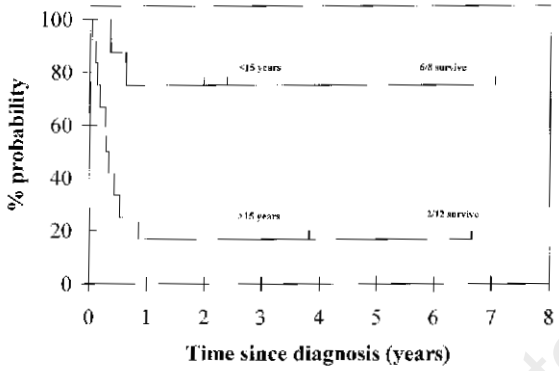

Figure 2. Survival of patients with biphenotypic leukemia against age.

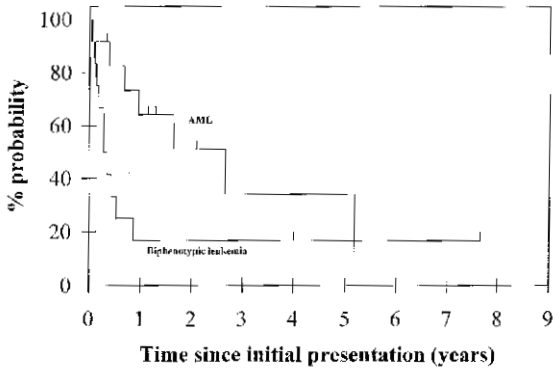

Figure 3. Survival of adult patients with biphenotypic leukemia against matched AML controls.

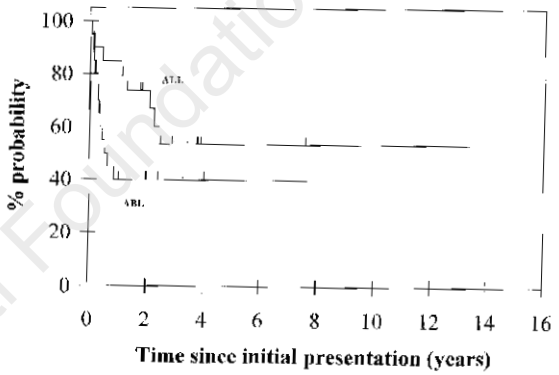

Figure 4. Survival of adult patients with biphenotypic leukemia against matched ALL controls.

been part of their treatment for the original disease. All patients in the secondary biphenotypic group died. Cause of death was transplant-related mortality in one, graft failure in one, resistant disease in two and thrombotic thrombocytopenic purpura post-allograft in one. Median time from diagnosis of biphenotypic leukemia to death was 5 months (range 2-34).

Discussion

Biphenotypic leukemia is an uncommon type of leukemia, the overall incidence in this study being 3.6% of acute leukemias. Evidence supports the involvement of a pluripotent stem cell. This is supported by cytochemistry and immunology revealing the existence of bilineal leukemias and lineage switch[23-25] and by cytogenetic studies showing that some gene rearrangements associated with biphenotypic leukemia are known to affect multiple lineages.[26,27]

The diagnosis of biphenotypic leukemia is based on immunophenotyping.[2] According to a strict scoring system considering the number and specificity of myeloid and lymphoid antigens expressed by the blasts, four groups can be identified. The most common group is that in which the blasts co-express myeloid and B-lymphoid antigens (60% in this study) and less commonly myeloid and T-lymphoid antigens (30% in this study). Co-expression of T and B- lymphoid markers and those with trilineage differentiation are rare. Most cases of biphenotypic leukemia express early hemopoietic markers such as CD34 and class II HLA DR determinants.

There is no single chromosome abnormality that is unique to biphenotypic leukemia.[20] However, our data and those of others have shown that structural abnormalities are common and that there is a high incidence of Ph positivity and rearrangements involving 11q23.[10,20,21,28,29]

Prognosis of *de novo* biphenotypic leukemia in our study was most strongly correlated to age (Figure 2; p=0.01) and Ph positivity (Figure 5; p=0.03). Childhood cases (<15 yrs), particularly those who were Ph negative, had an overall survival comparable to

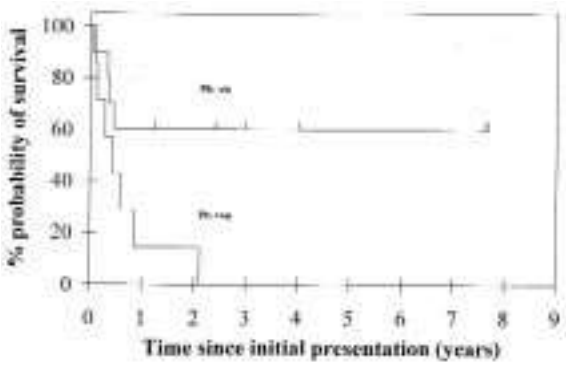

Figure 5. Disease free survival of biphenotypic leukemia : Ph+ve against Ph-ve.

matched controls with AML or ALL in the same treatment period. Adults, however, had a worse prognosis than matched controls with AML or ALL (Figures 3 and 4). There were no Ph positive disease-free survivors (adult or childhood) while 6 out of 10 of those known to be Ph negative were disease-free survivors. The only *de novo* patient in this series with a normal karyotype died after a sibling allogeneic BMT.

Relapse occurred in 3 patients (21% of those achieving CR), of whom 2 were Ph positive. Prognosis was not found to correlate with sex or immunophenotype.

There are no agreed treatment protocols for patients with *de novo* biphenotypic leukemia. In our series, induction treatment with combined AML/ALL drugs led to a high rate of early deaths. These deaths were all in adult patients of whom three of the five were known to be Ph positive, while the only child treated with combined induction therapy (Ph negative) achieved CR. Although the early death rate (25%) in *de novo* disease was similar to that in previous studies,[29] in our study there were no early deaths in patients receiving AML or ALL induction therapy alone (including three who were Ph positive). From these results we recommend that induction therapy should be with either AML or ALL drugs, the superiority of which regimen will only be determined with larger numbers of patients or randomized trials.

Consolidation therapy, especially in children, needs further study. Four out of 6 children with Ph negative disease were treated with chemotherapy alone and no BMT. All were alive and free of disease at last follow up. These results may suggest that Ph negative children should not undergo BMT in first remission.

Results of patients with secondary biphenotypic leukemia were uniformly bad. Remission induction was difficult and TRM was high.

Limited by the small number of patients, important conclusions can still be drawn from this study. Outcome-related prognostic factors are age and Ph status. For valid conclusions to be made about the treatment of biphenotypic leukemia, larger numbers of patients are needed for analysis, which due to the rarity of the disease will require multi-center collaboration. It is, however, likely that, as with other acute leukemias, treatment should be tailored to patient risk assessment based on prognostic factors.

Contributions and Acknowledgments

All authors were responsible for the concept and design of the study, analysis of results, drafting and final approval of the paper. JS specifically contributed to the cytogenetic data and I Atra to the pediatric data. We are grateful to Clive Horton for help with the statistical analysis, Toon Min, Melissa Dainton and Carol Brooker for their cytogenetic analyses.

Funding

This work was supported by Trust Funds from the Royal Marsden NHS Trust.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received January 18, 1999; accepted April 8, 1999.

References

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia: A report of the French-American-British Cooperative group. Ann Intern Med 1985; 103:620-5.
2. Matutes E, Morilla R, Farahat N, et al. Definition of acute biphenotypic leukemia. Haematologica 1997; 82:64-6.
3. Gale RP. Hybrid acute leukaemia. Br J Haematol 1987; 65:261-4.
4. Mirro J, Zipf TF, Pui CH, et al. Acute mixed lineage leukemia: Clinicopathologic correlations and prognostic significance. Blood 1985; 66:1115-23.
5. Kantarjian HM, Hirsch-Ginsberg C, Yee G, Huh Y, Freireich EJ, Stass S. Mixed-lineage leukemia revisited: Acute lymphocytic leukemia with myeloperoxidase-positive blasts by electron microscopy. Blood 1990; 76:808-13.
6. Mirro J Jr, Kitchingman GR. The morphology, cytochemistry, molecular characteristics and clinical significance of acute mixed-lineage leukaemia. In: Scott CS, ed. Leukemia cytochemistry and diagnosis: principles and practice. Chichester: Ellis Horwood 1989. p. 155-8.
7. Greaves MF, Chan LC. Mixed lineage leukemia: the implications for hematopoietic differentiation. Blood 1986; 68:598-603.
8. Pui C-H, Dahl GV, Melvin S, et al. Acute leukemia with mixed lymphoid and myeloid phenotype. Br J Haematol 1984; 56:121.
9. Drexler HG, Thiel E, Ludwig WD. Review of the incidence and clinical relevance of myeloid antigen-positive acute lymphoblastic leukemia. Leukemia 1991; 5:637-45.
10. Hanson CA, Abaza M, Sheldon S, Ross CW, Schnitzer

B, Stoolman LM. Acute biphenotypic leukaemia: Immunophenotypic and cytogenetic analysis. Br J Haematol 1993; 84:49-60.
11. 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; 324:800-8.
12. Sobol RE, Mick R, Royston I, et al. Clinical importance of myeloid antigen expression in adult acute lymphoblasti leukemia. N Engl J Med 1987; 316:1111.
13. Ball ED, Davis RB, Griffin JD, et al. Prognostic value of lymphocyte surface markers in acute myeloid leukemia. Blood 1991; 77:2242-50.
14. Cross AH, Goorha RM, Nuss R, et al. Acute myeloid leukemia with T-lymphoid features: A distinct biologic and clinical entity. Blood 1988; 72:579.
15. Faharat N, van der Plas D, Praxedes M, Morilla R, Matutes E, Catovsky D. Demonstration of cytoplasmic and nuclear antigens by flow cytometry in acute leukaemia. J Clin Pathol 1994; 47:843-9.
16. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. Leukemia 1995; 9:1783-6.
17. European Group for the Immunological Classification of Leukaemias. The value of c-kit in the diagnosis of biphenotypic acute leukemia. Leukaemia 1998; 12:2038.
18. Harrison CJ, Fitchett M, Potter AM, Swansbury GJ. A guide to cytogenetic studies in haematological disorders. Eugenics Society Occasional Papers, New Series No.1, 1987.
19. Mehta J, Powles R, Singal S, et al. Idarubicin, high-dose cytarabine, and etoposide for induction of remission in acute in leukemia. Semin Hematol. 1996; 33(4 Supp 3):18-23.
20. Carbonell F, Swansbury J, Min T, et al. Cytogenetic findings in acute biphenotypic leukaemia. Leukemia 1996; 10:1283-7.
21. Moorman AV, Hagemeijer A, Charrin C, Rieder H, Secker-Walker LM. The translocations t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3): a cytogenetic and clinical profile of 53 patients. European 11q23 workshop participants. Leukemia 1998; 12:805-10.
22. Zomas AP, Swansbury GJ, Matutes E, et al. Bilineal acute leukemia of B and T lineage with a novel translocation t(9;17)(p11;q11). Leuk Lymphoma 1997; 25:179-85.
23. Reardon DA, Hanson CA, Roth MS, Castle VP. Lineage switch in Philadelphia chromosome positive acute lymphoblastic leukemia. Cancer 1994; 73:1526-32.
24. Pane F, Frigeri F, Camera A, et al. Complete phenotypic and genotypic lineage switch in a Philadelphia chromosome-positive acute lymphoblastic leukemia. Leukemia 1996; 10:741-5.
25. Shende AC, Zaslav AL, Redner A, et al. A de novo lineage switch from B-cell acute lymphoblastic leukemia to acute myeloid leukemia: a case report. Am J Hematol 1995; 50:75-7.
26. Childs CC, Hirsch-Ginsberg C, Culbert SJ, et al. Lineage heterogeneity in acute leukemias with the t(4;11) abnormality: implications for acute mixed lineage leukemias. Hematol Pathol 1988; 2:145-57.
27. Cuneo A, Ferrant A, Michaux JL, et al. Philadelphia chromosome-positive acute myeloid leukemia: cytoimmunologic and cytogenetic features. Haematologica 1996; 81:423-7.
28. Sulak LE, ClareCN, Morale BA, Hansen KL, Montiel MM. Biphenotypic acute leukaemia in adults. Am J Clin Pathol 1990; 94:54-8.
29. 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; 100:147-55.