B-Lymphoblastic Leukemia With Aberrant CD5 Expression

Clinicopathologic Features and Outcome in 10 Cases

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Key Words: B-acute lymphoblastic leukemia; CD5; Short overall survival

Am J Clin Pathol October 2021;156:586-595

DOI: 10.1093/AJCP/AQAA269

ABSTRACT

Objectives: *B-acute lymphoblastic leukemia (B-ALL) is a neoplasm of precursor lymphoid cells committed to the B-lineage. Expression of CD5 is rare in B-ALL.*

Methods: *We studied the clinicopathologic, immunophenotypic, and molecular genetic features of 10 cases of B-ALL with aberrant CD5 expression, and compared with CD5– B-ALL.*

Results: *B-ALL with aberrant CD5 expression is rare and predominantly affects men. Patients with CD5+ B-ALL had shorter median overall survival (21 vs 45 months,* P = *.0003). Expression of CD5 imposed a challenge in the differential diagnoses between B-ALL and other CD5+ B-cell lymphomas with blastic morphology. Dim CD20 and CD45, lack of surface immunoglobulin, expression of CD34 and TdT, negative immunostain for cyclin D1, and absence of t(11;14)(q13;q32) support a diagnosis of B-ALL.*

Conclusions: *CD5 expression is rare in B-ALL and associated with poor clinical outcome. CD5+ B-ALL represents a distinct entity that needs to be considered in the differential diagnoses of CD5+ B-cell lymphoproliferative disorders.*

Key Points

- This is the largest study to describe the clinicopathologic, immunophenotypic, and molecular genetic features of B-acute lymphoblastic leukemia (B-ALL) with aberrant CD5 expression and to compare with a control group.
- CD5 expression in B-ALL predicts poor clinical outcome and may serve as a marker for predicting prognosis, monitoring minimal residual disease, and developing target therapy.
- CD5 expression in B-ALL imposes a challenge in the differential diagnoses of CD5+ B-cell lymphomas with blastic morphology.

B-acute lymphoblastic leukemia (B-ALL) is a clonal stem cell disorder of precursor lymphoid cells committed to the B-lineage, involving bone marrow (BM), peripheral blood (PB), and occasionally presenting at primary nodal or extranodal sites.[1] It is characterized by numerical and structural chromosomal alterations that include aneuploidy and rearrangements resulting in oncogene dysregulation or expression of chimeric fusion genes. Identification of these abnormalities is important for the diagnosis, risk stratification, and target therapy of B-ALL. The primary molecular genetic aberrations with poor prognostic impact include hypodiploidy, Philadelphia chromosome (Ph), Ph-like acute lymphoblastic leukemia (ALL), *KMT2A*/*MLL* rearrangement, and iAMP21, whereas hyperdiploidy and translocation t(12;21)(p13.2;q22.1)/*ETV6-RUNX1*

confer favorable prognosis.[1] The progress in the understanding of the pathogenesis of B-ALL has led to the development of target therapy, such as the BCR-ABL1 tyrosine kinase inhibitors; monoclonal antibodies to CD19, CD20, and CD22; and chimeric antigen receptor T-cell therapy.

Immunophenotypically, the lymphoblasts in B-ALL almost always express B-cell markers CD19, CD22, and cytoplasmic CD79a and are positive for CD10, CD24, dim CD45, PAX5, and TdT in most cases, with variable CD20 and CD34 expression.[1] The myeloid lineage markers CD13 and CD33 may be expressed in a subset of B-ALL. However, expression of T and natural killer (NK) antigens is rare.

CD5 is normally expressed on T cells and a small subset of B cells in cord blood, adult PB, and lymph nodes.[2] Aberrant CD5 expression is typically seen in chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL).[3] CD5 expression in B-ALL is extremely rare and has been reported only as case reports[4-9] or small case series[10,11]; the clinical and pathologic features of these cases have not been well characterized. In this study, we describe the clinicopathologic, immunophenotypic, and molecular genetic features of 10 patients with CD5-positive (CD5+) B-ALL seen at our hospital, the largest series reported to date.

Materials and Methods

Case Selection

We searched the database of our hospital from January 1, 2000, to July 31, 2020, for cases of B-ALL that were positive for CD5 as shown by flow cytometric analysis and/or immunohistochemistry. A group of 40 patients with CD5-negative (CD5–) B-ALL matched by age, sex, and blast count within the same study period were selected as a comparison group. The diagnosis was based on morphologic and immunophenotypic criteria as specified in the World Health Organization classification.[1] The clinical information was obtained by review of medical records. The study was conducted according to an institutional review board–approved laboratory protocol and in accord with the Declaration of Helsinki.

Morphologic Evaluation

We reviewed Wright-Giemsa–stained PB smears and BM aspirate smears/touch imprints, as well as H&E-stained clot and core biopsy specimens. Differential counts on 200 and 500 cells were performed manually on PB and BM smears, respectively. Cytochemical stain for myeloperoxidase was performed using a standard method.

Immunophenotypic Analysis

Flow cytometric immunophenotypic analysis was performed on BM aspirate specimens using a FACScan instrument (Becton Dickinson) as described previously.[12] The blast population was gated using right-angle side scatter and CD45 expression. The panel of monoclonal antibodies included reagents specific for CD2, CD3 (cytoplasmic and surface), CD4, CD5, CD7, CD10, CD13, CD14, CD15, CD19, CD20, CD22, CD25, CD33, CD34, CD38, CD56, CD64, CD66c, cytoplasmic CD79a, CD117, HLA-DR, cytoplasmic IgM, CRLF2, TdT, myeloperoxidase, and surface immunoglobulin κ and λ light chains (Becton Dickinson). An isotype control was used for each antibody.

Immunohistochemical studies were performed using formalin-fixed, paraffin-embedded BM core biopsy specimen or clot using the avidin-biotin-peroxidase complex method and an automated immunostainer (Ventana Biotech) as described previously.[13] All tissue sections underwent heat-induced antigen retrieval. The antibodies used were specific for CD3 and CD20 (Dako), CD5 and cyclin D1 (SP4) (Labvision/Neomarkers), PAX-5 (Transduction Labs), TdT (Leica Biosystems), and SOX11 (Cell Marque). Membranous reactivity for CD5 by the neoplastic CD20+ or PAX5+ B cells was considered positive. CD3+ T cells were used for comparison in the assessment of CD5 expression.

ERK Phosphorylation and *BRAF* Mutation Analysis by Immunohistochemistry

The highly specific antibody phospho-p44/42 MAPK (Thr202/Tyr204) p-ERK (Cell Signaling) was used to assess for the presence of nuclear and cytoplasmic phosphorylated p44 and p42 MAPK (Erk1 and Erk2). The VE-1 antibody (Spring Bioscience) was used to assess for cytoplasmic staining supportive of the presence of *BRAF* V600E mutation.

Cytogenetic Analysis

Conventional cytogenetic analysis was performed on metaphase cells prepared from BM aspirates cultured for 24 to 48 hours without mitogens, using standard techniques. Twenty Giemsa-banded metaphases were analyzed, and the results were reported using the International System for Human Cytogenetic Nomenclature, 2016.

DOI: 10.1093/ajcp/aqaa269

Fluorescence in situ hybridization (FISH) analysis for the detection of *BCR-ABL1* gene fusion was performed on interphase nuclei using the Vysis *BCR-ABL1* ES, dual-color, locus-specific probe according to the manufacturer's instructions (Vysis/Abbott Laboratories). We also performed FISH analysis for *IGH* and *CCND1* gene rearrangement using an LSI *IGH*/*CCND1* dual-color, dual-fusion translocation probe in one case (Vysis/Abbott Laboratories).

Molecular Analysis

A real-time reverse transcription polymerase chain reaction (PCR) was performed to detect *BCR-ABL1* fusion transcripts. RNA was extracted from PB or BM aspirates using Trizol reagent (Gibco-BRL) and reverse transcribed using random hexamers. The complementary DNA was amplified in a multiplex PCR that simultaneously detects b2a2, b3a2, and e1a2 fusion transcripts followed by capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).[14]

A fluorescence-based multiplex PCR was performed to detect internal tandem duplication (ITD) and D835 point mutation of the *FLT3* gene using genomic DNA extracted from BM aspirates.[15,16] For D835, the PCR products were digested with EcoRV restriction enzyme that cuts only the wild-type sequence. The PCR products were then subjected to capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer to distinguish wild-type and mutant genotypes.

In a subset of the cases, genomic DNA was PCR amplified and subject to mutation analysis using a panel of 81 genes (commonly mutated in hematopoietic neoplasms) by next-generation sequencing (NGS) on an Illumina MiSeq NGS platform (Illumina), as described previously.[17] The panel of genes included *ANKRD26*, *ASXL1*, *ASXL2*, *BCOR*, *BCORL1*, *BRAF*, *BRINP3*, *CALR*, *CBL*, *CBLB*, *CBLC*, *CEBPA*, *CREBBP*, *CRLF2*, *CSF3R*, *CUX1*, *DDX41*, *DNMT3A*, *EED*, *ELANE*, *ETNK1*, *ETV6*, *EZH2*, *FBXW7*, *FLT3*, *GATA1*, *GATA2*, *GFI1*, *GNAS*, *HNRNPK*, *HRAS*, *IDH1*, *IDH2*, *IKZF1*, *IL2RG*, *IL7R*, *JAK1*, *JAK2*, *JAK3*, *KDM6A*, *KIT*, *KMT2A*, *KRAS*, *MAP2K1*, *MPL*, *NF1*, *NOTCH1*, *NPM1*, *NRAS*, *PAX5*, *PHF6*, *PIGA*, *PML*, *PRPF40B*, *PTEN*, *PTPN11*, *RAD21*, *RARA*, *RUNX1*, *SETBP1*, *SF1*, *SF3A1*, *SF3B1*, *SH2B3*, *SMC1A*, *SMC3*, *SRSF2*, *STAG1*, *STAG2*, *STAT3*, *STAT5A*, STAT5B, *SUZ12*, *TERC*, *TERT*, *TET2*, *TP53*, *U2AF1*, *U2AF2*, *WT1*, and *ZRSR2*. For some cases, genomic DNA was PCR amplified and subject to mutation analysis for codons 12, 13, and 61 of *KRAS* and *NRAS* by pyrosequencing using a PSQ HS 96 Pyrosequencer (Biotage).[18]

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism 8 (GraphPad Software). Clinicopathologic and cytogenetic features were compared between the CD5+ and CD5– groups using the Fisher exact test. Overall survival was calculated from the date of initial diagnosis to the date of death or last follow-up. Survival was analyzed using the Kaplan-Meier method and compared using the log-rank test. A *P* value of less than .05 was considered statistically significant.

Results

Clinical Findings

We identified 10 cases of B-ALL with CD5 expression, representing less than 1% of total B-ALL cases seen during the study period of 20.5 years. There were eight men and two women, with a median age of 25 years (range, 7-68 years) at the time of initial diagnosis. The most common presenting symptoms were B-symptoms and lymphadenopathy, seen in five (100%) of five and four (80%) of five patients with initial presentation data available, respectively. Splenomegaly was noted in two (40%) of five patients. The other five patients presented to our hospital upon relapse.

Laboratory data at initial presentation were available in seven patients. All (100%) patients had leukocytosis (median WBC count, 20.9 × 10^3/μL; range, 12.2-252.9 × 10^3/μL; reference range, 4-11 × 10^3/μL). All (100%) patients had anemia (median hemoglobin, 10.6 g/dL; range, 8.5-13.3 g/dL; reference range, 14-18 g/dL for men and 12-16 g/dL for women). Five (71%) patients had thrombocytopenia (median platelet count, 49 × 10^3/μL; range, 36-253 × 10^3/μL; reference range, 140-440 × 10^3/μL). All seven (100%) patients had an elevated serum lactate dehydrogenase (LDH) (median, 823 IU/L; range, 641-6181 IU/L; reference range, 313-618 IU/L), and five (71%) of seven patients had an elevated serum β2-microglobulin (β2M) level (median, 3.9 mg/L; range, 1.7-5.3 mg/L; reference range, 0.6-2.0 mg/L). The clinical and laboratory findings are summarized in **Table 1**.

Morphologic Findings

The BM biopsy specimens were hypercellular for the patients' age (median cellularity, 95%; range, 65%-100%) with trilineage hypoplasia and significantly increased blasts (median blast count, 90%; range, 69%-92%). The blasts ranged from small to intermediate to large in size with round to slightly irregular nuclear contours, fine chromatin, occasional prominent nucleoli, and scant to

DOI: 10.1093/ajcp/aqaa269

moderate amount of basophilic cytoplasm. Cytoplasmic vacuoles were noted in three cases Figure 1.

In one case (patient 6), the patient initially had an extramedullary mass in the right lung that was shown to be B-lymphoblastic lymphoma at an outside hospital, in addition to involvement of the BM. He subsequently developed an extramedullary tumor in soft tissue in the right thigh as well as the right cheek. Two patients (patients 3 and 6) had leptomeningeal disease with lymphoblasts detected in cerebrospinal fluid.

Immunophenotypic Findings

Flow cytometric immunophenotypic analysis demonstrated that the blasts were positive for CD5, CD19, CD22, CD38, and CD45 (dim) in all cases. The intensity of CD5 expression ranged from dim partial (in <50% of the blasts, n = 5) to moderate-strong (in >50% of the blasts, n = 5) Table 2 and Figure 2. Other markers expressed in subsets of patients included cytoplasmic CD79a (8/9, 89%), cytoplasmic IgM (7/9, 78%), HLA-DR (7/9, 78%), CD123 (3/4, 75%), CD20 (7/10, 70%), TdT (6/9, 67%), CD34 (6/9, 67%), CD10 (6/10, 60%), CD33 (5/10, 50%), CD7 (2/8, 25%), CD13 (2/9, 22%), CD66c (2/9, 22%), and CD56 (1/9, 11%). All cases assessed were negative for CD2 (n = 8), CD3 (cytoplasmic and surface, n = 9), CD4 (n = 8), CD14 (n = 9), CD15 (n = 9), CD25 (n = 9), CD64 (n = 9), CD117 (n = 9), myeloperoxidase (n = 9), CRLF2 (n = 2), and surface immunoglobulin κ and λ light chains (n = 7). The immunophenotype of all cases and the level of CD5 expression are listed in Table 2.

Immunohistochemical staining was performed in two cases (patients 1 and 5) as a part of the clinical workup, which showed that the blasts were positive for CD5 (n = 2), PAX5 (n = 2), CD20 (patient 5), TdT (patient 1), and SOX11 (patient 1) and were negative for CD3 (n = 2). Immunostain for cyclin D1 was performed in three additional cases as a part of this study, and all were negative (Figure 1).

Cytogenetic and Molecular Findings

Conventional cytogenetic analysis was performed on all patients. Six patients showed complex karyotypes. No recurrent cytogenetic abnormality was detected. The most common aberrations were –13 and +18, each occurring in three cases, respectively. One patient had 47,XY,+Y,t(9;22)(q34;q11.2). Three patients had a diploid karyotype Table 3.

FISH analysis using the *BCR-ABL1* translocation probe was performed in nine cases. One patient (patient 9) showed *BCR-ABL1* gene rearrangement, and the other eight patients were negative. FISH analysis using the *IGH*/*CCND1* translocation probe was performed in one case (patient 1) and was negative for *IGH* and *CCND1* gene rearrangement.

NGS analysis for a panel of 81 genes was performed on two cases; both showed pathogenic *NRAS* mutations (patient 1, c.35G>A p.G12D; patient 2, c.38G>A p.G13D), and one case also showed a pathogenic *KRAS* mutation (patient 2, c.35G>A p.G12D). None of the rest of the genes in the panel was found to be mutated. We performed *FLT3* standalone mutation analysis for ITD and D835 point mutation on six cases; none of the six cases showed a mutation in the *FLT3* gene, including the two cases mentioned above. Standalone mutation analysis for *KRAS* and *NRAS* performed on patient 7 showed no mutation (Table 3).

BRAF and Phosphorylated ERK Immunohistochemical Assessment

In five cases with available blocks, we performed additional immunohistochemical studies for *BRAF* mutation and p-ERK expression. All cases were negative for

Table 1
Clinicopathologic Features of 10 Patients With CD5+ B-Acute Lymphoblastic Leukemia[a]

Patient No.	Age, y	Sex	WBC, × 10^3/μL	Hb, g/dL	Platelet, × 10^3/μL	LDH, IU/L	β2M, mg/L	HSM	LAD	CSF	Transplant	Follow-up, mo	Outcome
1	7	M	17.8	8.8	253	4,541	NA	Y	Y	N	N	3	CR
2	24	F	12.6	8.5	49	6,181	5.3	N	Y	N	N	34	CR
3	23	M	4.7	10.4	110	12,644	3.7	N	N	Y	N	20	Died
4	22	M	35.6	13.2	188	671	NA	N	Y	N	N	46	CR
5	68	M	252.9	10.6	44	785	1.7	N	N	N	N	30	Died
6	40	M	7.9	15.4	189	969	3.3	N	N	Y	Y	34	Died
7	25	M	20.9	12.6	137	860	4.1	Y	Y	N	N	1	Died
8	37	F	5.9	10.9	30	2,949	1.5	N	N	NA	N	5	Died
9	64	M	12.2	13.3	36	641	3.7	N	N	N	N	24	Died
10	16	M	96.2	9.3	40	NA	NA	NA	NA	N	N	8	Died

β2M, β2-microglobulin; CR, complete remission; CSF, cerebrospinal fluid; Hb, hemoglobin; HSM, hepatosplenomegaly; LAD, lymphadenopathy; LDH, lactate dehydrogenase; N, no; NA, not available; Y, yes.
[a]The CBC count for patients 3, 6, and 8 was from the date when they sought treatment at our hospital; the rest were from initial presentation.

DOI: 10.1093/ajcp/aqaa269

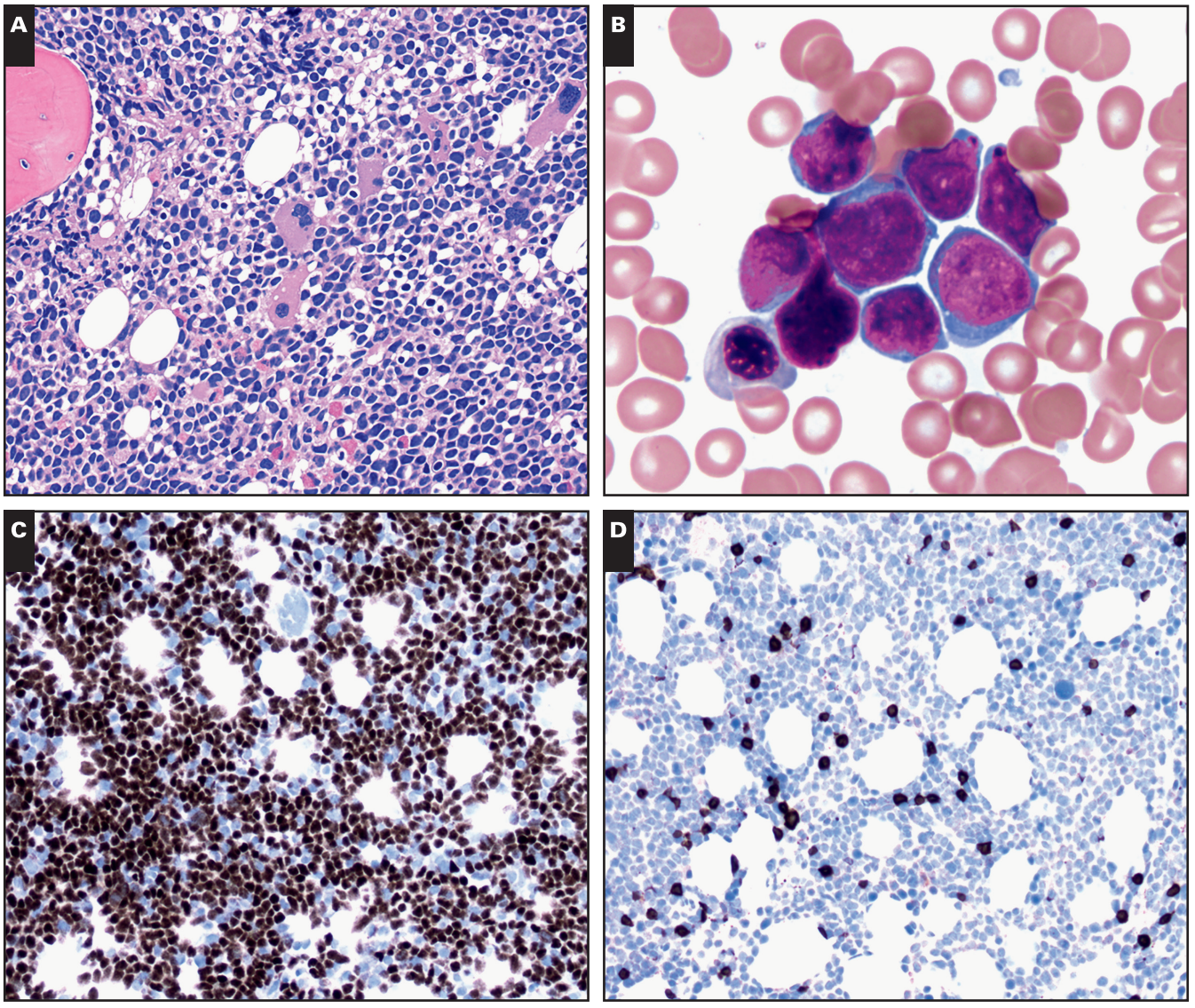

Figure 1 Morphologic features of a case of CD5+ B-acute lymphoblastic leukemia. **A**, The bone marrow is hypercellular with trilineage hypoplasia and sheets of immature cells (H&E, ×200). **B**, The blasts range from intermediate to large in size with round to slightly irregular nuclear contours, fine chromatin, occasional prominent nucleoli, and scant basophilic cytoplasm (Wright-Giemsa, ×1,000). Immunohistochemical stains show that the blasts are positive for PAX5 (**C**) and CD5 (**E**).

BRAF V600E mutant protein. p-ERK expression was observed in the nuclei and cytoplasms of blasts in three cases, including patient 2 with both *KRAS* and *NRAS* mutations, and two cases in which *KRAS*/*NRAS* mutation status was not assessed (patients 5 and 6). Patient 1 (with *NRAS* mutation) and patient 3 (mutation analysis was not performed) were negative. In all cases, stromal cells, fibroblasts, and endothelial cells were positive for p-ERK (internal control).

Clinical Outcome

All patients received multiagent chemotherapy; one patient (patient 6) also received allogeneic stem cell transplantation. With a median follow-up of 21 months (range, 1-46 months), seven patients died of disease, and three patients were in complete remission.

We compared the clinicopathologic features upon initial presentation at our hospital, karyotype, and clinical outcome between the 10 patients with CD5+ B-ALL and a group of 40 patients with CD5– B-ALL who were matched by age, sex, and blast count and were seen within the same study period. There was no statistically significant difference in CBC count, LDH, β2M, karyotype, and disease status after induction Table 4. However, patients with CD5+ B-ALL had a significantly shorter median overall survival (21 vs 45 months, P = .0003; Figure 3).

DOI: 10.1093/ajcp/aqaa269

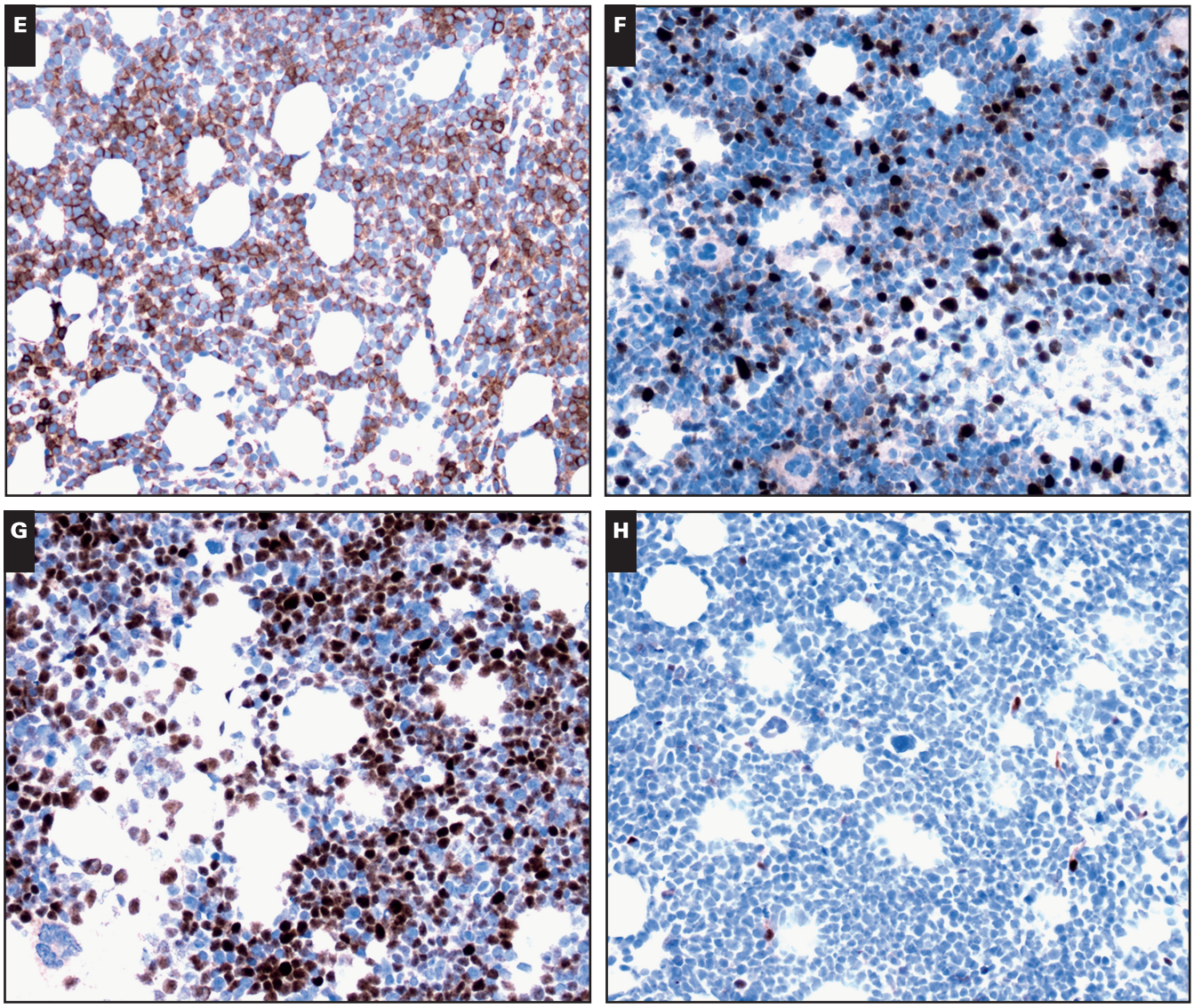

Figure 1 (cont) Blasts are positive for TdT (**F**) and SOX11 (**G**) and negative for CD3 (**D**) and cyclin D1 (**H**) (×200).

Table 2
Immunophenotype of 10 Patients With CD5+ B-Acute Lymphoblastic Leukemia

Patient No.	CD3[a]	CD4	CD5, %	CD7	CD10	CD13	CD19	CD20	CD22	CD33	CD34	CD38	CD79a	CD117	MPO	TdT	cIgM	sIg
1	N	N	96	N	P	N	P	P	P	N	P	P	P	N	N	N	N	N
2	N	N	49	N	P	N	P	P	P	N	N	P	P	N	N	P	P	N
3	N	N	92	N	N	N	P	N	P	N	P	P	N	N	N	P	N	N
4	N	N	78	P	N	N	P	N	P	P	P	P	P	N	N	N	P	N
5	N	NA	31	NA	N	N	P	P	P	N	N	P	P	N	N	N	P	N
6	N	N	16	N	P	P	P	N	P	P	P	P	P	N	N	P	P	N
7	N	N	37	N	P	N	P	P	P	P	P	P	P	N	N	P	P	N
8	N	N	37	N	P	N	P	P	P	P	N	P	P	N	N	P	P	NA
9	N	N	63	P	P	P	P	P	P	N	P	P	P	N	N	P	P	NA
10	NA	NA	67	NA	N	NA	P	P	P	P	NA	P	NA	NA	NA	NA	NA	NA

N, negative; NA, not available; P, positive.
[a]Cytoplasmic and surface.

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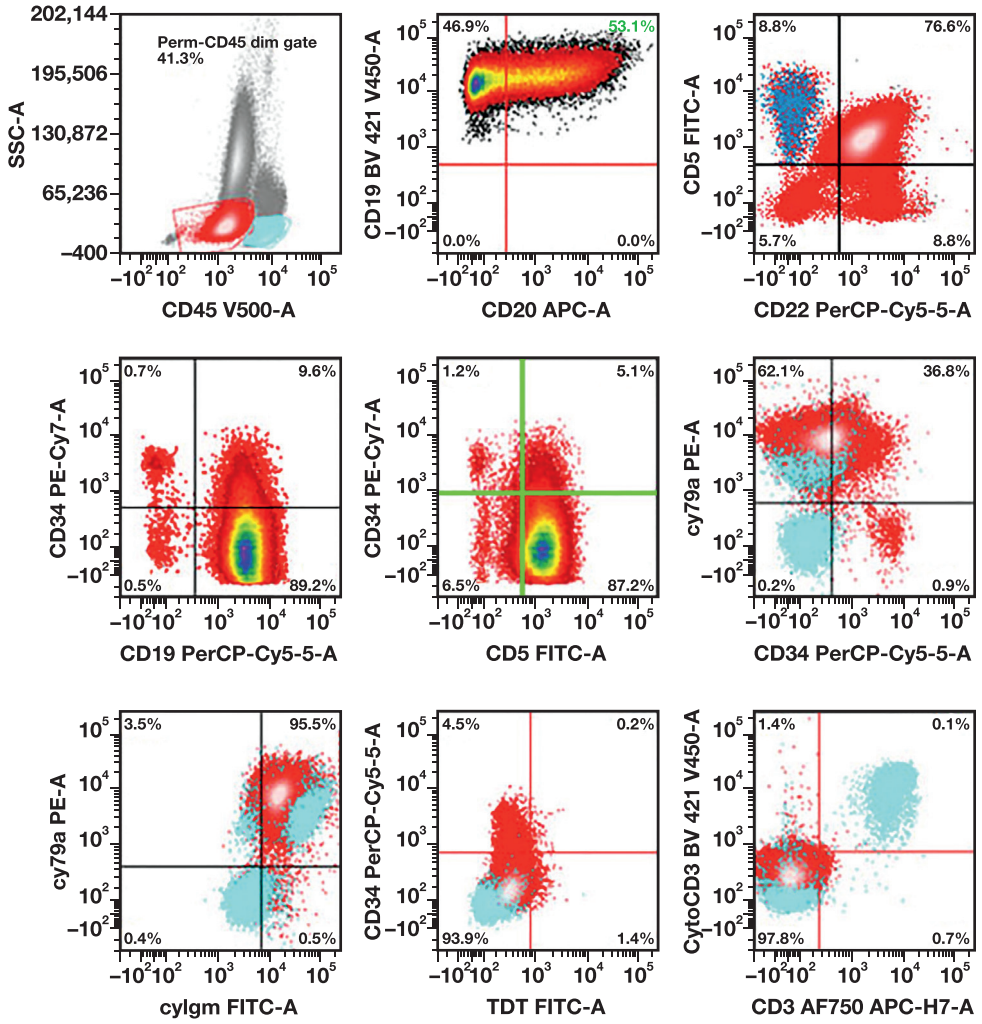

Figure 2 Flow cytometric immunophenotyping of a case of CD5+ B-acute lymphoblastic leukemia. The blasts are positive for CD5, CD19, CD20, CD22, CD34 (partial), CD45 (dim), cytoplasmic CD79a, and cytoplasmic IgM and negative for CD3 (surface and cytoplasmic) and TdT. CD5 expression on T cells is shown to provide a comparison (upper right corner).

Table 3

Cytogenetic and Molecular Data of 10 Patients With CD5+ B-Acute Lymphoblastic Leukemia

Patient No.	Karyotype	Mutation	pERK
1[a]	45~46,Y,del(X)(q22),add(1)(q31),t(3;6)(q21;p21.3),del(5)(q13q33),add(7)(q36),–8,add(9)(q34),–13,–16,+18,ins(18;?)(q21.1;?),+2~3mar[cp7]/46,XY[13]	*NRAS*+	N
2[a]	46,XX[20]	*KRAS*+, *NRAS*+	P
3	44,X,–Y,inv(5)(q13q33),inv(7)(p22q21.2),der(10;12)(q10;q10),del(13)(q12q14),add(18)(p11.2),+mar[18]	NA	N
4	46,XY,del(4)(q22q28),del(12)(p12),i(17)(q10)[13]	NA	NA
5	46-47,XY,–3,4,+add(5)(q11.2),der(6)t(1;6)(q21;q13),inv(7)(p15p22)del(14)(q21q31),add(18)(q23),+add(18),add(19)(q13.4),–22,+mar(cp16)/46,XY[4]	NA	P
6	47,XY,del(5)(q23q32),add(7)(p21),der(8)t(8;9)(q24;p13)add(9)(p24),der(9)t(8;9),del(11)(q21q25),add(15)(q21),+21[3]	*FLT3*–	P
7	46,XY[20]	*FLT3*–*KRAS*–, *NRAS*–	NA
8	46,XX[20]	*FLT3*–	NA
9	46,XY,t(9;22)(q34;q11.2)[8]/47,XY,+Y,t(9;22)(q34;q11.2)[6]	*FLT3*–	NA
10	46,XY,–9,–9,–13,+der(?)t(?;9)(?;q12),+2mar[4]/46.XY[9]	NA	NA

N, negative; NA, not available; P, positive.

[a]An 81-gene next-generation sequencing panel was done on patients 1 and 2.

DOI: 10.1093/ajcp/aqaa269

Discussion

CD5 is a 67-KDa membrane glycoprotein that is normally strongly expressed in T cells and weakly expressed in a small subset of B cells named B1 cells.[2] CD5 is associated with the B-cell receptor (BCR) and is a negative regulator of the downstream signaling pathways. Upon BCR cross-linking, CD5 mediates signals through ERK, MAPK, and STAT3 pathways and induces the production of interleukin 10, an antiapoptotic cytokine, thus contributing to B-cell survival.[19,20] CD5 expression on B cells may serve as a marker of antigen exposure.

Assessment of CD5 expression has been widely employed in the clinical workup of B-cell lymphomas. CD5 expression is typically seen in CLL and MCL and has also been reported occasionally in other B-cell lymphomas, including extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, nodal marginal zone lymphoma, lymphoplasmacytic lymphoma, follicular lymphoma, and diffuse large B-cell lymphoma (DLBCL).[21-25] In some studies, CD5 expression has been associated with certain clinicopathologic findings, a more aggressive clinical course, and resistance to therapy.[21-25] However, CD5 expression has only been rarely reported in B-ALL, mostly as case reports[4-9] or part of small case series without clinical information,[10,11] and thus its clinical and pathologic features have not been well characterized. In 2009, Seegmiller et al[11] studied immunophenotypic aberrancies in 200 patients with B-ALL and reported CD5 expression in four (2%) cases, three children and one adult. However, no other information was provided. In 2011, Hussein et al[10] assessed the frequency of T/NK-cell antigen expression in 134 cases of B-ALL and detected CD5 expression in 6 (4.5%) cases. Five were younger than 18 years, and one was a young adult aged 36 years. Although it seems that only two patients were in remission at the end of the study, the

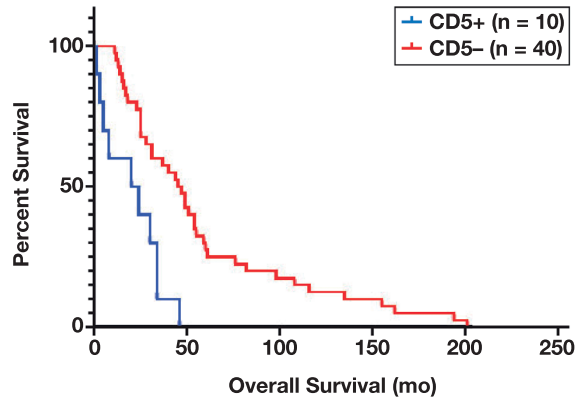

Figure 3 Overall survival of patients with CD5+ B-acute lymphoblastic leukemia (n = 10) and CD5– B-acute lymphoblastic leukemia (n = 40). *P* = .0003 by log rank test.

Table 4
Comparison Between CD5+ and CD5– B-Acute Lymphoblastic Leukemia[a]

Characteristic	CD5+ B-ALL (n = 10)	CD5– B-ALL (n = 40)	*P* Value
Age, y	25 (7-68)	25 (7-68)	
Sex, male/female, No.	8/2	32/8	
WBC, × 10^3/μL	15.2 (4.7-252.9)	6.4 (0.6-292.9)	.7753
Hb, g/dL	10.8 (8.5-15.4)	9.9 (7-15.2)	.2092
Platelet, × 10^3/μL	80 (30-253)	58 (4-376)	.8103
LDH, IU/L	969 (641-12,644)	1,110 (146-16,477)	.5077
β2M, mg/L	3.7 (1.5-5.3)	2.5 (1.1-5.9)	.1022
Blast, %	90 (69-92)	92 (52-97)	.8558
Present to us as new patient, No. (%)	5 (50)	24 (60)	.7232
Karyotype, No. (%)			
Diploid	3 (30)	16 (40)	.7222
Simple (1-2 aberrations)	1 (10)	8 (20)	.6651
Complex (≥3 aberrations)	6 (60)	16 (40)	.3023
Disease status after induction, No. (%)			
MRD negative	4 (40)	15 (38)	>.9999
MRD positive	1 (10)	11 (27)	.4155
CR (MRD not done)	4 (40)	8 (20)	.2251
Persistent B-ALL	1 (10)	6 (15)	>.9999
Transplant, No. (%)	1 (10)	4 (10)	
Follow-up, mo	21 (1-46)	45 (11-201)	
Outcome, No. (%)			
Died	7 (70)	16 (40)	
CR	3 (30)	24 (60)	

B-ALL, B-acute lymphoblastic leukemia; β2M, β2-microglobulin; CR, complete remission; Hb, hemoglobin; LDH, lactate dehydrogenase; MRD, measurable residual disease (by flow cytometry immunophenotyping).
[a]Values are presented as median (range) unless otherwise indicated.

DOI: 10.1093/ajcp/aqaa269

length of follow-up is unknown. The frequency of CD5 expression in our series is much lower, representing less than 1% of all B-ALL cases seen within the study period. Most of the previously reported cases affected children (12/17, 71%), with a male-to-female ratio of 1.6:1. However, our cases differed from previous studies in that most of the patients (8 of 10, 80%) were men, and only 2 (20%) patients were children younger than 18 years of age, 6 were young adults ranging from 22 to 40 years of age, and 2 were older than 60 years. We do not have a good explanation for this discrepancy; one possibility is that most of our patient population is adult patients.

The expression of CD5 raises a diagnostic challenge in the differential diagnoses between B-ALL and other CD5+ B-cell lymphomas with blastic morphology, such as blastic MCL, de novo CD5+ DLBCL, CD5+ high-grade B-cell lymphoma, Richter transformation of CLL, and acute mixed phenotype leukemia (MPAL). Morphologic features in combination with dim expression of CD20 and CD45, lack of surface immunoglobulin, expression of immature markers such as CD34 and/or TdT, negative immunostain for cyclin D1, and absence of t(11;14)(q13;q32) as shown by karyotyping and FISH support a diagnosis of B-ALL. Lack of other T-cell antigens, especially surface and cytoplasmic CD3, excludes a diagnosis of MPAL. Of note, SOX11 may be expressed in ALL, MCL, and Burkitt lymphoma. Thus, SOX11 expression is not useful to differentiate B-ALL and blastic MCL.[26]

Aberrant CD5 expression has been associated with poor clinical outcome in several B-cell lymphomas, such as follicular lymphoma[24] and DLBCL.[25] Study of the impact of CD5 expression on the clinical outcome of B-ALL has been limited by the small number of reported cases. It was suggested that some patients with CD5+ B-ALL had poor prognosis in several case reports,[4,5,7,9] but this was not supported by other reports.[6,8] Hussein et al[10] performed multivariate Cox regression analysis and showed that the risk of relapse was higher in cases expressing T/NK antigens overall (CD2, CD3, CD4, CD5, CD7, and CD56), suggesting that T/NK antigen expression in B-ALL may be an independent predictor for poor prognosis. However, the individual role of each marker such as CD5 expression was not assessed. We compared the clinical outcome between patients with CD5+ B-ALL and a matched group of 40 cases of CD5– B-ALL and demonstrated that patients with CD5+ B-ALL had a significantly shorter overall survival. Therefore, CD5 may serve as a marker for prognostic stratification in B-ALL, and this small subset of patients may require more aggressive treatment.

The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway is a key regulatory pathway in many essential cellular processes. Activation of the G-protein RAS triggers sequential changes in the phosphorylation status of the downstream kinases RAF, MEK, and the serine/threonine kinases ERK-1 and ERK-2. Phosphorylated and activated ERK translocates to the nucleus, where it regulates the activity of several transcription factors, which in turn induce expression of genes required for proliferation, differentiation, apoptosis, and survival.[27,28] Mutations that constitutively activate members of this pathway occur in several nonhematologic malignancies such as melanoma and glioma, as well as hematologic diseases, including hairy cell leukemia, chronic myelomonocytic leukemia, Langerhans cell histiocytosis, Erdheim-Chester disease, and Rosai-Dorfman disease.[29-32] Two of the three cases in our series that were assessed for *KRAS*/*NRAS* mutation showed mutations in *NRAS* and/or *KRAS*. None of the five cases that were assessed for *BRAF* mutation (by 81-gene NGS and/or immunohistochemistry) and of the two cases that were assessed for *MAP2K1* mutation (by 81-gene NGS) harbored *BRAF* or *MAP2K1* mutation. Nevertheless, immunohistochemistry showed p-ERK overexpression in three of five cases, supporting MAPK/ERK pathway activation in at least a subset of the cases. There was no correlation between p-ERK overexpression and *KRAS*/*NRAS* mutation. One possibility is that ERK is activated by nonmutational mechanisms induced by the microenvironment. Another possibility is that ERK activation is not a universal end point and that a proportion of cases use alternative pathways downstream of RAS, such as the PI3K-mTOR pathway, or outside the MAPK/ERK pathway axis.

In summary, our data show that CD5 expression is extremely rare in B-ALL and is associated with a poor clinical outcome. Expression of CD5 in B-ALL imposes a challenge in the differential diagnoses of CD5+ B-cell lymphomas with blastic morphology. Larger-scaled collaborative studies may be helpful to delineate whether CD5 may serve as a marker for the development of target therapy and identification of minimal residual disease.

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DOI: 10.1093/ajcp/aqaa269

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