Leukemia (1998) 12, 666–674

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Multilineage involvement of Philadelphia chromosome positive acute lymphoblastic leukemia

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Acute lymphocytic leukemia (ALL) is considered a clonal disease restricted to the lymphoid compartment. The Philadelphia chromosome (Ph) is found in a subset of ALL with poor prognosis. Here we present the largest series of Ph+ ALL analyzed for involvement of the myeloid compartment. For the first time at a single cell level the presence of Ph in lineages other than lymphoid is demonstrated. Granulocytes from nine patients diagnosed with *BCR-ABL* + ALL (eight Ph+, one Ph−) were purified using two layer density gradient separation. They were further identified by the morphology of DAPI-stained nuclei and studied for the presence of the Ph by fluorescence *in situ* hybridization (FISH) using a *BCR-ABL* dual-color probe. Ph was demonstrated in 30 to 93% of granulocytes in all patients. FISH identified major and minor *BCR* gene breakpoints (M-bcr and m-bcr). In one patient, with $CD19^+/34^+/33^-/2^-/3^-/7^-/10^-$ lymphoblasts, involvement of B cells ($CD19^+$), T cells ($CD3^+$), myeloid ($CD13^+$), erythroid (glycophorin A^+) cells was found by FISH following fluorescence-activated cell sorting (FACS). The diagnosis of ALL as opposed to lymphoblastic transformation of CML was established based on clinical and laboratory data including Western blot results demonstrating the presence of p190/m-bcr in five of the nine cases studied. Results suggest that Ph+ ALL originates from a pluripotent stem cell.

Keywords: acute lymphocytic leukemia; lineage; hematopoietic progenitors; *BCR-ABL*

Introduction

Acute lymphocytic leukemia (ALL) is considered a clonal disease[1] restricted to the lymphoid compartment.[2,3] The underlying hypothesis is that a lymphoid-determined progenitor cell undergoes neoplastic transformation. In contrast, chronic myeloid leukemia (CML), which is considered to originate from a pluripotent stem cell,[4,5] shows multilineage involvement.

In 12%[6] to 43%[7] of ALL patients the translocation t(9;22)(q34;q11) is documented. It results in the Philadelphia chromosome (Ph) and is associated with the generation of a novel *BCR-ABL* fusion gene. Two common breakpoint cluster regions are localized on the long arm of chromosome 22: the major breakpoint cluster region (M-bcr) of 5.8 kb maps to an intron within exons 12 to 16 (b1–b5) and the minor breakpoint custer region (m-bcr) lies in a region of 35 kb on the first intron of the *BCR* gene. Translocations involving M-bcr and m-bcr code for different mRNAs of 8.0 and 7.0 kb, and two different *BCR-ABL* proteins of 210 kDa and 190 kDa, respectively. Because rare cases of m-bcr in CML are always associated with atypical features reminiscent of chronic myelomonocytic leukemia,[8] M-bcr is considered to be characteristic of virtually all cases of Ph+ CML[9] and of 50% of Ph+ ALL;[10] m-bcr accounts for the remaining cases and is therefore a feature specific for ALL.

While ALL in general is believed to be restricted to the lymphoid lineage, the existence of 'stem cell ALL' based on the involvement of myeloid elements in the leukemic process has been postulated.[11–13] Alternatively, it has been reasoned that multilineage involvement in Ph+ ALL represents CML in lymphoid blast crisis (CML-LBC). The two entities are sometimes not easily distinguished.

In this study, we have used molecular cytogenetics to further investigate the putative stem cell origin of Ph+ ALL. Fluorescence *in situ* hybridization (FISH) permits the visualization of the Ph in single, nondividing cells[14] whose morphology and immunologic markers can be determined. Using two-color probes, we detected Ph in the granulocytes of all patients. Multilineage involvement was demonstrated in one patient by the presence of the Ph in B- and T-lymphoid, erythroid and myeloid cells.

Materials and methods

Patient samples

Bone marrow (BM) and peripheral blood (PB) samples were obtained from nine patients diagnosed with *BCR/ABL*+ ALL, eight of them Ph+, following institutional guidelines (Table 1). Two patients were studied at diagnosis (one during the first treatment in partial remission). Remaining patients were evaluated in relapse (seven patients), one of them in addition in hematological remission. The presence of Ph had been determined by cytogenetic analysis of BM or PB metaphases cultured for 48 h (Table 2). The Ph– patient included was highly positive for the p-190 *BCR-ABL* protein by Western blot and displayed the respective fusion signals by FISH.

Granulocyte characterization by density and morphology

Leukocytes were separated using a two layer density gradient (Histopaque 1077 and 1119, Sigma Cell Culture, St Louis, MO, USA) to obtain purified granulocyte populations. Purity was 91–97%. In patient 1 a purity of 79% only was achieved because of high blast counts in the unseparated sample. FACS sorted myeloid cells, however, contained only 5% cells negative for CD13. To further exclude contaminating leukemic cells only nuclei displaying typical polymorphonuclear morphology of granulocytes were evaluated. Therefore, a triple band-pass filter (Chromatotechnology, Brattlebroro, VT, USA) was used to evaluate the presence of the fusion signal and the

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Received 12 June 1997; accepted 7 January 1998

Table 1 Patient characteristics and clinical course of disease

Patient	*Sex/ age/ race*	*Symptoms at presentation*	*Lymphadenopathy/ Hepatomegaly/ Splenomegaly*	*Response to therapy/ Time to relapse (months)/ Survival status (months)*
1	Female/ 63/ white	fatigue chest pain back pain	+/–/–	CR after first course/ 2[a] and 7[b]/ 27+
2	Male/ 20/ white	recurrent pharyngitis	+/–/+	CR during first course/ 21+/ 21+
3	Male/ 24/ white	hematomas epistaxis fatigue	+/–/–	CR during first course/ 19[c]/ 29
4	Male/ 39/ white	fatigue headache joint pain	–/+/+	CR 2 after second course/ 5[c]/ 23
5	Male/ 36/ white	fatigue chest pain	–/–/–	CR after fourth course/ 8[c]/ 17 mos
6	Male/ 65/ white	epistaxis pneumonia bone pain	–/–/–	CR after first course/ 8[a]/ 29+
7	Female/ 41/ black	back pain renal abscess	–/–/–	CR during first course/ none/ 3+
8	Female/ 60/ white	fever	–/–/–	CR after first course/ none/ 4+
9	Male/ 69/ white	fatigue bruises	+/–/–	CR after first course/ 8[c]/ 12+

CR, complete remission; course, course of cytotoxic chemotherapy.
[a]Cytogenetic relapse.
[b]Hematologic relapse.
[c]Cytogenetic and hematologic relapse.

polymorphonuclear appearance of DAPI-stained granulocytic nuclei concomitantly.

Evaluation of other lineages

For FACS analysis, performed in patient 1, erythrocytes were lysed (ammonium chloride, potassium chloride, EDTA) and cells were stained with the following directly labeled antibodies: Leu-M7/CD13, Leu-4/CD3, GlyA, and CD19 (Becton Dickinson, San Jose, CA, USA). Cell sorting was performed as described before.[15] Between 200 and 1000 cells were sorted directly on to premarked clean slides. After sorting, the slides were air dried overnight, fixed twice with 3:1 methanol:glacial acetic acid and stored at –20°C until hybridization.

FISH analysis

FISH was performed with a commercially available *BCR-ABL* dual-color kit[16] containing directly conjugated LSI bcr SpectrumGreen/abl Spectrum Orange probes (VYSIS, Downers Grove, IL, USA). Hybridization procedures were carried out following the manufacturer's instructions. Two investigators, using a Zeiss epifluorescent photomicroscope, independently evaluated fluorescence signals in 100 to 500 nuclei of granulocytic and mononuclear fractions and in cells sorted according to different lineage markers on to slides. Observations were documented using a cooled charged coupled device camera (Photometrics, Tucson, AZ, USA) and computer imaging (IP Lab Software, Signal Analytics, Vienna, VA, USA). In patient 9 who was Ph– and *BCR-ABL*+ by Western blot, in addition to FISH for *BCR-ABL* dual color FISH for evaluation of chromosomes X and Y was performed. The CEP Spectrum Orange X/Spectrum Green Y probe (VYSIS) yielded a background of 2.4% cells falsely negative for chromosome Y in normal controls.

Definition of fusion signals

Cells bearing fusion signals defined as visually inseparable red–green or yellow signals were interpreted as Ph+.[16]

M-bcr and m-bcr by FISH

Different translocation breakpoints were observed with the M-bcr not overlapping and the m-bcr lying within the green *BCR* probe. In t(9;22), M-bcr, the green probe colocalizes with the red *ABL* probe. In contrast, m-bcr splits the *BCR* probe resulting in an additional green signal large enough to be seen. It represents the *BCR* fragment which is transposed to der(9)t(9;22) (Figures 1 and 2).

The additional green signal typical of m-bcr was observed in 28.2–91.1% *vs* 0–2.5% of cells (mean ± s.d.: 63.5 ± 25 *vs*

Table 2 Laboratory data at diagnosis and breakpoint determination by FISH

Patient	*WBC, Hb, Plt/ Diff/ BM-Blasts*	*Immunocyto-chemistry (% of blasts)*	*Flow cytometry markers (% of blasts)*	*Cytogenetics*	*BCR-ABL fusion product/ BCR breakpoint by FISH*
1	13.6K, 10.6, 17K/ 93% lym 4% seg 1% bands 1% baso 1% meta/ 85%	MPO– Tdt + (83) Sudan black- After 24 months: MPO + (3) Tdt + (75)	CD19(98) CD 3(2) CD 10(13) CD33 (0.5)	46,XX t(9;22)(q34;q11) [20]	p190/ m-bcr
2	2.1K, 5.9, 273K/ 8% blasts 60% lym 26% seg 2% bands 4% mon/ 92.5%	MPO– Tdt + (81)	CD 19(82) CD 3 (16) CD 10(61) CD 13(5)	47,XY,t(9;22)(q34;q11) der(22) t(9;22) (q34;q11) [12] 47,idem, dup(1)(q31q44) [6] 46,XY [7]	p210/ M-bcr
3	4.8K, NA, 3K 12% blasts 61% lym 19% seg 8% bands/ 99%	MPO– Tdt + (95) PAS+	CD 19(83) CD 3(–) CD 10(90) CD 33(9)	52,XY,+4,+6,+8,t(9;22)(q34;q11) +14,+17 [9] 46,XY [1]	ND/ m-bcr
4	2.5K, NA, NA/ 66% blasts 11% lym 17% seg 3% bands/ 87%	MPO– Tdt + (67)	CD 19(85) CD 3(2) CD 10 (85) CD 33(35)	45,XY, t(9;22)(q34;q11),–9 [20]	p190/ NE
5	19.7K, NA, NA/ 66% blasts 30% lym 1% seg 3% mon/ 92%	MPO– Tdt + (71)	CD 19(96) CD 3(4) CD 10(95) CD 13(2) CD 14(16)	46,XY, del(7)(p15), t(9;22)(q34;q11),del(12)(p11) [20]	p190/ NE
6	33.4K, 10.2, 34K/ NA/ 28%	MPO– Tdt + (79)	CD 19(86) CD 20(90) CD 3(6.5) CD 10(84) CD 33(35) CD 13(49)	45,X,–Y, t(9;22)(q34;q11)[5] 45,idem, add(22)(q12)[3] 46,XY[17]	p210/ M-bcr
7	29K, 7.3, 10K/ 66% blasts 30% lym 1% seg 3% mon/ 92%	MPO– Tdt + (86)	NA	46,XX,–7,–8,+2mar t(9;22)(q34;q11)[3] 46,XX[1]	ND
8	92.5K, 6.4, 106K/ 26% blasts 59% lym 10% seg 2% bands 3% mon/ 79%	MPO+(2) Tdt + (80)	CD 19(91) CD 2(0.6) CD 10(96) CD 7(8)	46,XX, t(9;22)(q34;q11)[14] 46,XX[4]	p210/ M-bcr
9	70K, 8.5, 24K/ 17% blasts 80% lym 1% seg 2% mon/ 57%	MPO– Tdt + (91)	CD 19(95) CD 2(3) CD 10(94) CD 13(2) CD 13(49)	45,X,–Y,with BCR-ABL fusion signal by FISH [7] 46,XY[13]	p190 m-bcr

K, number + 10^3 in cells per μl; WBC, white blood cell counts in peripheral blood; Hb, hemoglobin count in g/dl; diff, differential cell counts; lym, lymphocytes; seg, segmented granulocytes; baso, basophilic granulocytes; meta, metamyelocytes; mon, monocytes; ND, not done; NE, not evaluable; NA, not available; MPO, myeloperoxidase; Tdt, terminal deoxynucleotidyl transferase; PAS, periodic acid-Schiff; del, deleted; dup, duplicated; add, added; der, derivative; [N], number of metaphases.

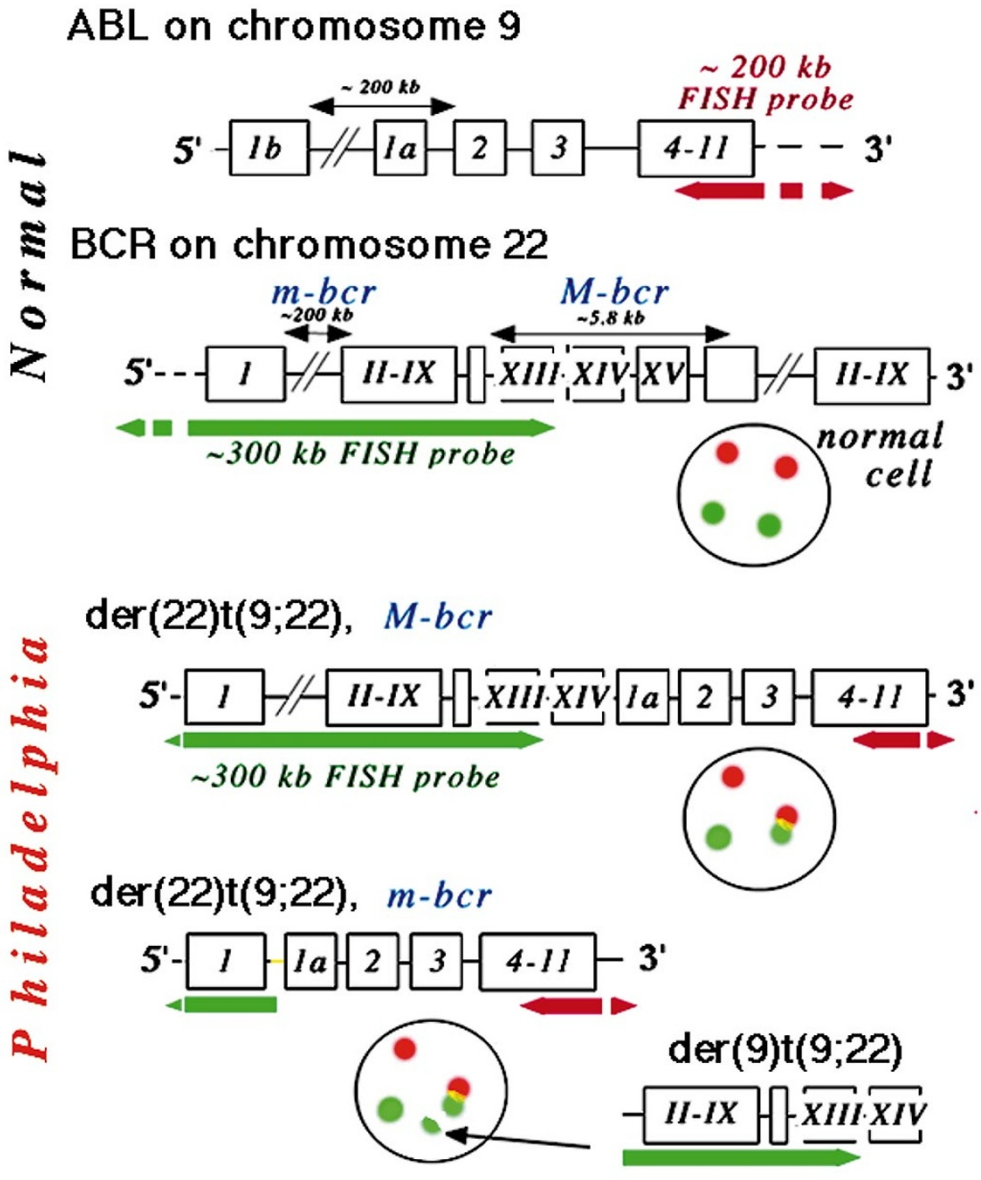

Figure 1 M-bcr, m-bcr and FISH. The two common breakpoint cluster regions on chromosome 22 are visualized by dual-color FISH.

1.5 ± 1.3) in m-bcr *vs* M-bcr cells ($n = 5$ samples of each type) confirming Western blot results.

Controls

Normal BM or PB samples ($n = 10$) and Ph-negative ALL samples ($n = 2$) were used as negative controls. They displayed percentages from 4 to 8% of cells with a fusion signal. Background (including 2 standard deviations) was calculated as 14.3%. FISH results were similar for BM and PB samples, as well as for material from healthy donors and patients with Ph-negative ALL. Patient BM samples or patient mononucleated cell fractions corresponding to the granulocytic fractions in question were used as positive controls, percentages are given in Table 3.

Western blotting test

The *BCR-ABL* Western blotting test was modified to increase the sensitivity compared to usual procedures. First, the enhanced chemiluminescent (ECL) detection system was used in place of the ^{125}I protein A detection system. Second, background was reduced by substituting powdered milk for bovine serum albumin (BSA) in the blocking solutions. The detailed procedure is as follows: Western blotting was performed with an anti-ABL (8E9) monoclonal antibody. Frozen PB cells were lysed in boiling sodium dodecyl sulfate (SDS) sample buffer for 5 to 7 min, and the lysate was clarified by centrifugation. Aliquots of the extracts corresponding to 10^7 cells were applied to each lane. Samples were electrophoresed through 6.5% polyacrylamide gels, the gels were electroblotted at 4°C overnight and transferred to Immobilon filters (Millipore, Bedford, MA, USA). The ECL Western blotting detection system was used to probe for *BCR-ABL* protein according to the

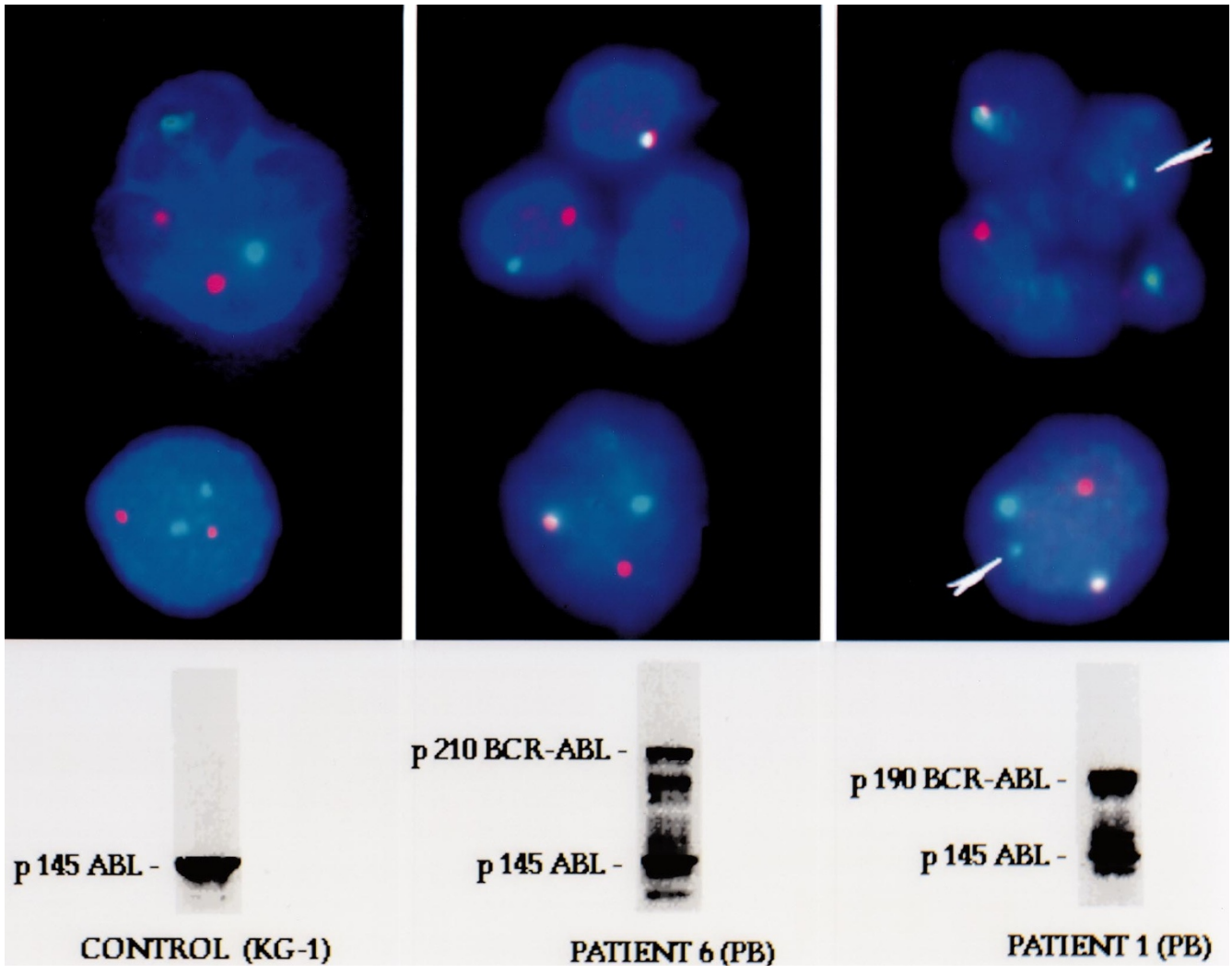

Figure 2 FISH and Western blot analyses of peripheral blood in Ph+ ALL. (Left panel) Normal polymorphonuclear (above) and mononucleated cell (below) exhibiting two green *BCR* and two red *ABL* signals each (healthy donor). Western blot in a control cell line (KG-1; lane 1) displays no *BCR-ABL* fusion protein. (Middle panel) The M-bcr type of the Philadelphia translocation (a yellow or red–green fusion signal) seen in a granulocyte (above) and in a mononuclear cell (below, patient 6). The corresponding Western blot (lane 2) reveals the presence of p210. (Right panel) m-bcr, typically found in ALL, visualized by FISH in a granulocyte (above) and in a mononucleated (below) cell. This demonstrates involvement of the myeloid lineage in addition to the lymphoid (patient 1). Note the green hybridization locus (arrow) in addition to the normal *BCR* and *ABL* signals and to the fusion signal. It represents the fraction of the *BCR* probe being translocated to der(9)t(9;22) in m-bcr. Accordingly, Western blot is positive for p190 (lane 3).

manufacturer's protocol (Amersham, Arlington Heights, IL, USA). Filters were preblocked by washing with 10% non-fat milk (NFM) in Tris-buffered saline-Tween 20 (TBS-T) buffer (20 mmol/l NaCl, 0.0038 N HCl) for 2 h and then incubated with 1:15 000 to 20 000 dilution of 8E9 in 5% NFM TBS-T buffer overnight at room temperature. The filters were then incubated with a 1:3000 to 3500 dilution of horseradish peroxidase (HRP)-labeled sheep anti-mouse IgG (Amersham, cat. No. NA 9310) for 2 h. The filters were mixed with ECL reagents and exposed to the X-ray film for 1 to 10 min. Where indicated, the intensities of p210 *BCR-ABL*, p190 and p145 *ABL* with inappropriate autoradiograms were scanned with a soft laser densitometer (Zeineh Biomed Instruments, Fullerton, CA, USA).

Results

Patient characteristics, clinical course, and laboratory data are given in Tables 1 and 2. All patients were classified as ALL and not as CML-LBC based on clinical and laboratory data at presentation. Criteria used to establish the diagnosis of ALL included lack of a preceding chronic phase in all patients, presence of significant lymphadenopathy in 4/9 patients, and Western blot or FISH results showing the presence of p190 (4/9 patients) and/or m-bcr (3/9 patients) in 5/9 patients (Table 2, Figure 2). Nine/nine patients achieved complete hematological as well as cytogenetic remission, a feature characteristic of ALL, using the Hyper-CVAD protocol.[17]

All nine patients with the diagnosis of ALL had clearly identifiable *BCR-ABL*+ granulocytes by FISH (Table 3). Among Ph+

Table 3 *BCR-ABL*-positive granulocytes in ALL samples from nine patients

Patient and sample material	*% of cytogenetically abnormal cells by FISH*	*Clinical status at time of study*
1	BCR-ABL	
Bone marrow	98	Relapse
Granulocytes	93	
2	BCR-ABL	
Mononucleated cells	31	Relapse
Granulocytes	30	
3	BCR-ABL	
Granulocytes	73	Relapse
4	BCR-ABL	
Bone marrow	82	Relapse
Granulocytes	65	
5	BCR-ABL	
Mononucleated cells	3[a]	Remission
Granulocytes	10[a]	
5	BCR-ABL	
Mononucleated cells	40	Relapse
Granulocytes	46	
6	BCR-ABL	
Mononucleated cells	84	Relapse
Granulocytes	88	
7	BCR-ABL	
Mononucleated cells	88	Diagnosis
Granulocytes	86	
8	BCR-ABL	
Mononucleated cells	80	Relapse
Granulocytes	83	
9	BCR-ABL	
	(45,X,–Y)[b]	Diagnosis
Mononucleated cells	15 (13)	(1st partial remission)
Granulocytes	18 (19)	

NE, not evaluable.
[a]FISH results are indicative of cytogenetic remission during complete hematological remission (background was 14.3%).
[b]Patient 9, being already in partial remission, had a low percentage of *BCR-ABL* positive cells. Since the percentage was close to calculated background, in addition –Y was evaluated. This clonal abnormality was clearly positive in both, mononucleated cells and granulocytes (background was 2.4%).

patients the percentage of granulocytes displaying the *BCR-ABL* fusion ranged from 30% (patient 2) to 93% (patient 1) in polymorphonuclear cells and did not differ significantly from the respective numbers of unsorted BM cells or mononucleated cell fractions. Patient 9, who was Ph– but *BCR-ABL*+ by Western blot, had a relatively small percentage of clonal cells, since he was studied in first partial remission. The *BCR-ABL* fusion in mononucleated and granulocytic fractions was positive, however, close to background which in translocation probes is relatively high. Therefore the additional clonally evolved abnormality, 45X, –Y, not seen during a later remission, was used as second marker of clonality. –Y was investigated with a FISH probe yielding a significantly lower background in normal controls (2.4%). The aberration was clearly present in 13% of mononucleated cells and 19% of granulocytes and percentages were comparable to that of *BCR-ABL* positive cells (15 and 18%).

The two *BCR-ABL* fusion proteins, p190 and p210, were documented by Western blot analysis in seven of the nine patients. FISH probes used could readily discriminate the two breakpoint regions within the *BCR* gene occurring in ALL in accordance with the manufacturers' instructions (Figures 1 and 2). These findings matched the results from Western blot analysis which demonstrated the corresponding fusion proteins.

PB samples of patient 5 were evaluated in complete remission and at relapse. Both, mononucleated cells and granulocytes, were *BCR-ABL* negative in remission and positive at relapse (Table 3).

These results suggesting leukemic multilineage involvement were confirmed in FACS-separated cell populations in one Ph+ (p-190/m-bcr) patient who had a $CD19^{+}/34^{+}/33^{-}/2^{-}/3^{-}/7^{-}/10^{-}$ immunophenotype of his lymphoblasts. Apart from cells carrying B cell markers (CD19: 71% Ph) Ph was found in cell populations that were purified according to their myeloid (CD13: 75% Ph), erythroid (glycophorin A: 47% Ph), and T cell (CD3: 36% Ph) lineages (Table 4). Of note, none of these markers were expressed in the blast cell population, therefore excluding aberrant expression of these antigens on the transformed cells.

Discussion

The existence of 'stem cell ALL', a term introduced by Secker-Walker and Craig,[11] has been postulated by several authors (Table 5).[12,13,18] Different methodologies have been used to verify this hypothesis, with the most recent work being based on FISH analysis and morphological criteria.[2] Earlier methods included lineage assignment by cytogenetic analysis of cultured hematopoietic colonies,[13] as well as cell separation and molecular analyses.[19] Points of critque were the *in vitro* conditions used to culture cells, the lack of single-cell specificity

Table 4 Multilineage involvement: B- and T-lymphocytic, erythroid, and myeloid lineages[a] carry the *BCR-ABL* (m-bcr) fusion by FISH in one patient[b]

FACS sorting	*Cells positive for the BCR-ABL fusion*[c]	*No. of cells evaluated*	*Lineage*
Unsorted (BM)	56%	560	All lineages
$CD19^+$ (BM)	71%	124	B cells
$CD13^+$ (BM)	75%	165	Myeloid progenitors
Glycophorin A^+ (BM)	47%	102	Erythroid progenitors
$CD3^+$ (PB)	36%	254	T cells

[a]Blasts did not express CD3 or 13.
[b]Patient 1.
[c]BM and PB cells were collected at different time-points, so percentages are not comparable.

Table 5 Lineage involvement in Ph+ ALL reported in the literature

Source	*CML -LBC*	*No. of cases of lymphoid restriction*	*No. of cases of stem cell ALL*	*No. of cases without clear classification*	*Method*
This report	—		8 +1 (Ph. neg, BCR-ABL +)	—	Cell separation/ FISH
Secker-Walker and Craig[11]	—	2	2	—	Cell separation/ Southern blotting
Craig *et al*[24]	—	2	—	—	Cell separation/ Southern blotting
Secker-Walker *et al*[19]	—	4	1	—	Cell separation/ Southern blotting
Kalousek *et al*[18]	—	—	—	5 "initial diagnosis of Ph+ ALL"	*In vitro* colonies/ Conventional cytogenetics
Anastasi *et al*[2]	4	4	—	—	Morphology/ FISH
Dow *et al*[13]	—	—	2	—	*In vitro* colonies/ Conventional cytogenetics
Tachibana *et al*[12]	—	—	2	—	*In vitro* colonies/ Conventional cytogenetics
Abe *et al*[3]	—	1	—	—	*In vitro* colonies/ Conventional cytogenetics
Kitano *et al*[25]	—	3	—		*In vitro* colonies/ Conventional cytogenetics
Total	4	16	15 (16)	5	

of molecular methods, and, in all cases, the inability or the lack of data to make a clear distinction between CML-LBC and ALL.

In this study, it is demonstrated that granulocytes are part of the neoplastic clone in all nine patients with the diagnosis of *BCR-ABL*+ ALL. Importantly, five of the cases investigated were m-bcr/p190 ALL. Only one patient with m-bcr Ph+ ALL has been reported as stem cell ALL so far,[13] and the view that stem cell cases are always M-bcr (and therefore possibly CML-LBC) has permeated common understanding. This represents the largest series of patients with Ph+ ALL whose cells were examined for lineage involvement. It is also the first study employing single cell analysis in ALL in which the Ph has been shown to be present in lineages other than lymphoid. Our results, which support involvement of the myeloid lineage, suggest that leukemogenesis in Ph+ ALL affects an early, noncommitted progenitor cell. Mechanisms which then regularly lead to clinical expression of the disease in only the

lymphoid compartment, like lymphoid proliferation advantage or suppression of other lineages, are possibly comparable to CML-LBC and are yet to be elucidated. Also in analogy to CML-LBC, complete cytogenetic remission in Ph+ ALL can be achieved, which points to the existence of a residual population of normal stem cells. In accordance with this suggestion we found Ph-negative cells in all samples which explains the possibility of a complete remission at a cytogenetic level. The hypothesis of 'stem cell disorder' was confirmed in one patient whose blasts lacked myeloid and T cell characteristics. FACS-sorted BM and PB myeloid, erythroid, and T cells all contained a significant fraction positive for Ph.

In this context it is important to clearly establish the differential diagnosis between CML-LBC, a stem cell disease, and ALL. Clinical features and laboratory data (Tables 1 and 2, and Results) make it likely that all nine patients had ALL and not blastic transformation of CML. In patients 1, 3, 4, 5 and 9, the diagnosis of ALL was further corroborated by the translocation breakpoint localization within the m-bcr and/or the presence of *BCR-ABL* protein p190.

A review of the literature reveals that the problem of lineage involvement in Ph+ ALL is unresolved. Anastasi *et al*[2] investigated the largest series of lymphoblastic leukemias using FISH and morphologic characteristics finding lineage restriction (four ALL patients) and reviewed an additional 15 patients published in the literature. They concluded that multilineage disease in retrospect sometimes had to be reclassified as CML-LBC, and that lymphoblast-restricted disease should be classified as Ph+ALL. The future will show whether these discrepancies can be reconciled by the finding that the target cell in Ph+ALL can be found at different levels of commitment, the 'stem cell variant' being the more common fraction. In the literature there is considerable evidence for the existence of 'stem cell ALL'. Reviewing cases 45/F and 41/F of Ph+ALL with multilineage involvement reported by Secker-Walker and Craig[11] and patient 2 in the series reported by Kalousek *et al*[18] we believe that the diagnosis of ALL is probable based on the information available. Further, m-bcr was present in patient 1, Dow *et al*,[13] and in patient 2, Kalousek *et al*,[18] lymphadenopathy was found at presentation. In at least seven cases of Ph+ALL in the literature, involvement of compartments other than the lymphoid was demonstrated contradicting the concept of a restricted lymphoproliferative disorder and in agreement with our findings.[11–13,20]

Results of the present study suggest that Ph+ALL, like CML, originates in pluripotent progenitor cells. This leads to the old question of whether they are one or two diseases.[20–22] The differential diagnostic hallmarks, such as clinical and hematologic features at presentation, clinical course, cytogenetic remission in complete hematological remission, and immunophenotype, in some cases still seem to be insufficient for distinguishing between the two disorders. The translocation breakpoint localization is a discriminator between CML-LBC and m-bcr Ph+ALL. Because the *BCR-ABL* FISH probes used here distinguish readily betweem M-bcr and m-bcr, they may be useful for the differential diagnosis in major group of Ph+ lymphoblastic leukemias. Important consequences of the results presented here will be new insights into pathomechanisms of disease and determination of the leukemic target cells in ALL for the development of selectively antileukemic therapeutic or purging strategies.

Acknowledgements

This work was supported in part by grants from the National Institutes of Health, PO1CA 55164, CA 16672 and CA 49639 and by a Max Kade Research Fellowship Grant to T Schenk.

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