Acute Leukemia

Bilineage acute leukemia of T-lymphoid and myeloid lineages

We report the case of a child with Philadelphia chromosome-negative *bilineage* leukemia characterized by the coexistence of T-cell leukemia and myelomonocytic leukemia. Comparative analyses of genetic markers and proliferative/differentiative potential show the possibility of malignant transformation of a T-lineage stem cell with the potential to differentiate into the myeloid lineage.

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Hybrid acute leukemia is divided into two groups, *biphenotypic* leukemia and *bilineage* or *bilineal* leukemia.[1] The extremely low incidence of *bilineage* leukemia[2] gives rise to difficulties in evaluating the clonality of this entity.

A 14-year old boy was hospitalized on April 5, 2002. Laboratory studies showed a white blood cell count of 52.3×10^9/L with 61.3% blasts, a hemoglobin level of 8.5 g/dL, and a platelet count of 89×10^9/L. There were two types of blasts in the peripheral blood (PB) and bone marrow (BM). One belonged to the myelomonocytic lineage as determined on the basis of positivity for myeloperoxidase (MPO), α-naphthyl butylate esterase, naphthol AS-D chloroacetate esterase, and CD13/CD33. The other originated from T-lymphoblasts, because it showed cytoplasmic expression of CD3 and surface expression of CD2, CD5 and CD7. The two populations did not possess each other's lineage markers. Accordingly, our case can be considered *bilineage* leukemia consisting of myeloid and T-lymphoid lineages. He was treated with etoposide, cytarabine, mitoxantrone, and L-asparaginase, but lymphoblasts persisted in his PB. On June 5, 2002, PB stem cells from his HLA-identical sister were infused after a preparative regimen of total body irradiation, cyclophosphamide and etoposide. On day 186, his PB cells were found to contain 3% of blasts. Leukemic cells were of myeloid lineage with no lymphoid markers. He died on day 280 from disease progression and pulmonary failure.

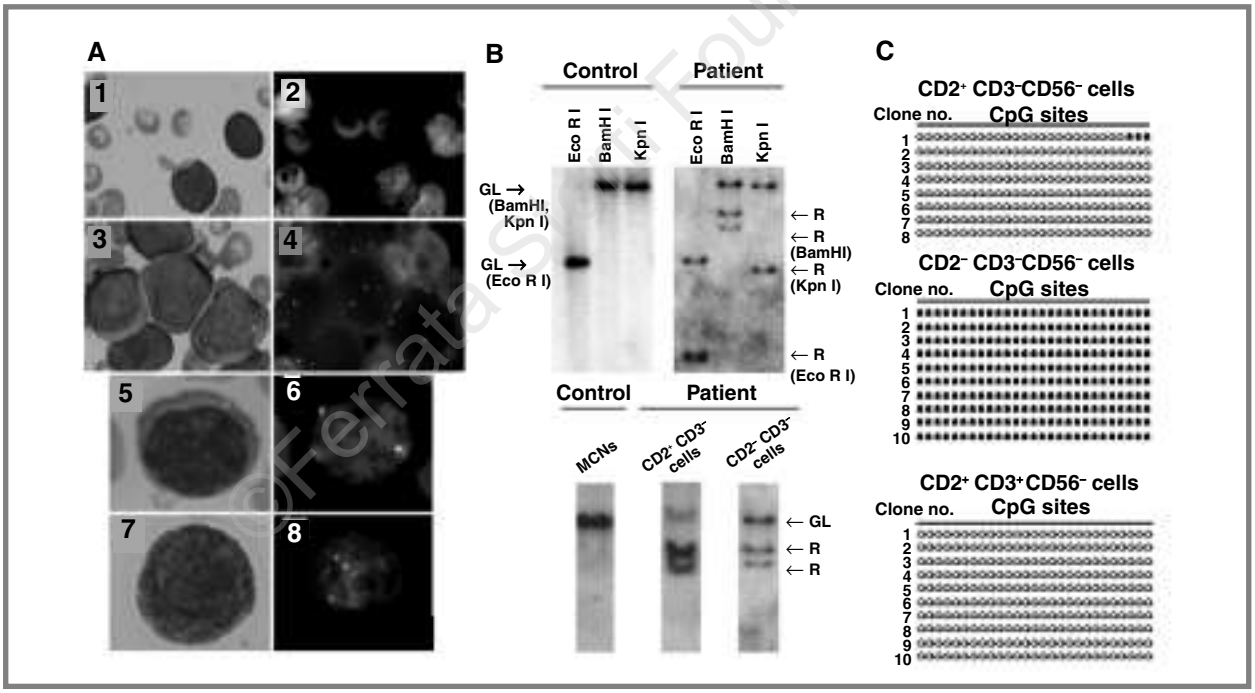

Figure 1. Evaluation of genetic markers in the two different leukemic populations (A) FISH analysis combined with May-Grünwald-Giemsa or MPO staining. A combination of May-Grünwald-Giemsa (1, 3, 5) or MPO (7) staining and FISH analysis was employed (original magnification, ×1,000). At presentation, both lymphoblasts (1, 2) and myeloid blasts (3, 4, 7, 8) showed a numerical aberration of chromosome 3, while lymphocytes (5, 6) had two spots. (B) Southern blot analysis of the TCRδ gene. DNA was prepared from PB mononuclear cells (MNC), $CD2^+CD3^-$ cells and $CD2^-CD3^-$ cells of the patient at initial diagnosis, and then digested with one of the following restriction enzymes: EcoR I, BamH I, and Kpn I. Top; Clonal rearrangement of the TCRδ gene can be seen in the patient's PB MNC. DNA from normal PB MNC was used as a control. Bottom; Identical rearrangements were observed in PB $CD2^+CD3^-$ cells and $CD2^-CD3^-$ cells from the patient (BamH I as the restriction enzyme). GL, germ line; R, rearrangement. (C) Methylation status of the p15 promoter region. We examined the methylation status of each CpG site within the promoter region of the p15 gene in $CD2^+CD3^-CD56^-$ cells, $CD2^-CD3^-CD56^-$ cells and $CD2^+CD3^+CD56^-$ cells, using bisulfite genomic sequencing. Eight to 10 clones were randomly selected and sequenced. Each row of circles corresponds to one clone. There are 27 CpG sites within the region located between positions -12 to +251 relative to the transcriptional starting site of the p15 gene. Methylated sites are marked as filled circles and unmethylated sites as open circles.

Table 1. Numerical aberration of chromosome 3 in myeloid blasts and T-lymphoblasts.

	Number of signals					
	1	*2*	*3*	*4*	*5*	*Total*
Exp. 1						
Patient PB						
MPO+ cells	1 (0.5%)	8 (4.0%)	100 (50.0%)	83 (41.5%)	8 (4.0%)	200
MPO- cells	4 (2.0%)	29 (14.5%)	99 (49.5%)	65 (32.5%)	3 (1.5%)	200
Control BM						
MPO+ cells	0 (0.0%)	150 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	150
MPO- cells	0 (0.0%)	150 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	150
Exp. 2						
Patient PB						
CD2+ cells	0 (0.0%)	27 (13.5%)	41 (20.5%)	113 (56.5%)	19 (9.5%)	200
Patient PB						
CD2+CD3-CD56- cells	0 (0.0%)	4 (2.7%)	69 (46.0%)	57 (38.0%)	20 (13.3%)	150
CD2-CD3-CD56- cells	0 (0.0%)	12 (8.0%)	67 (44.7%)	57 (38.0%)	14 (9.3%)	150
CD2+CD3+CD56- cells	0 (0.0%)	128 (85.3%)	12 (8.0%)	8 (5.3%)	2 (1.3%)	150
Control PB MNCs	0 (0.0%)	199 (99.5%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	200

Exp. 1: Combined analysis of MPO staining and FISH was used. Exp. 2: $CD2^+$ cells were prepared using immunomagnetic beads. $CD2^+CD3^-CD56^-$ cells, $CD2^-CD3^-CD56^-$ cells, and CD2+CD3+CD56- cells were sorted by flow cytometry. Then, numbers of signals for chromosome 3 were determined by FISH analysis.

We evaluated genetic markers and proliferative/differentiative potential in the two different leukemic populations. According to G-banding analysis, 18 of 20 BM cells had extra copies in two-thirds of chromosomes. In fluorescence *in situ* hybridization (FISH) using spectral karyotyping,[3] all of the abnormal cells shared five structural aberrations such as der(2)t(2;13)(q13;q14), implying a common origin for these cells. The number of copies of chromosome 3 increased to three to five in all the abnormal cells, but had no structural abnormalities. Thus, we performed FISH analysis with a probe for the centromere of chromosome 3 to examine whether lymphoblasts and myeloblasts had common cytogenetic aberrations.[4] On May-Grünwald-Giemsa staining combined with FISH,[5] all of 28 lymphoblasts and all of 22 myeloblasts displayed 3 or more signals for chromosome 3 per cell (Figure 1A). In contrast, normal lymphocytes had 2 spots. As presented in Figure 1A and Table 1, numerical aberration of chromosome 3 was found in 95.5% of MPO^+ cells, and in 86.5% of $CD2^+$ cells separated immunomagnetically.[6] Both $CD2^+CD3^-CD56^-$ cells and $CD2^-CD3^-CD56^-$ cells sorted by flow cytometry[6] expressed extra signals per cell at a frequency of higher than 90%, whereas a majority of $CD2^+CD3^+CD56^-$ cells had 2 spots. Southern blot analysis[7] showed that PB $CD2^-CD3^-$ cells as well as $CD2^+CD3^-$ cells had rearrangement of the δ chain in T-cell receptor genes (Figure 1B). In addition to the chromosomal abnormalities, the rearrangement was also found in post-relapse PB cells including 59% myeloblasts, suggesting that the disease at relapse was genetically identical to the disease at diagnosis although the phenotype differed. Taken together, T-lymphoblasts and myeloblasts might have originated from a common clone.

There are two possibilities to explain the occurrence of acute bilineage leukemia: (i) the malignant transformation of a myeloid or lymphoid stem cell with potential to differentiate into the other lineages, (ii) clonal evolution of a common stem cell that can generate both myeloid and lymphoid progeny. Herman *et al.*[8] reported that hypermethylation of the p15 gene occurs very frequently in pediatric acute myeloblastic leukemia and T-precursor acute lymphoblastic leukemia. Contrariwise, our methylation-specific polymerase chain reaction and sequencing[9] showed that $CD2^-CD3^-CD56^-$ cells were completely methylated at the p15 CpG island, whereas $CD2^+CD3^-CD56^-$ cells were nearly devoid of methylated CpG sites (Figure 1C). Thus, it is unlikely that a key set of *bilineage* mutations arose in a true stem cell.

Sorted $CD2^+$CD3-CD56- cells and $CD2^-CD3^-CD56^-$ cells were individually plated (100 cells/well) in wells containing 10 ng/mL of granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, stem cell factor, and thrombopoietin. After 14 days, $CD2^+CD3^-CD56^-$ cells generated 100-fold more MPO-positive progeny with extra signals for chromosome 3 than did $CD2^-CD3^-CD56^-$ cells, implying a hierarchical difference in the proliferative potential of T-lymphoblasts and myeloblasts. King *et al.*[10] reported that most immature thymocytes maintain a latent granulocyte/macrophage differentiation potential. Taken together, these data strongly suggest that the *bilineage* leukemia in our patient was a result of the malignant transformation of a T-lineage stem cell with potential to differentiate into myeloid lineage. Aberrant p15 gene promoter methylation might occur during and/or after myeloid development from T-lineage stem cells.

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Multiple Myeloma

Recombinant human erythropoietin and the risk of thrombosis in patients receiving thalidomide for multiple myeloma

Among 199 patients treated with thalidomide for multiple myeloma, four thromboses occurred in 49 cases during erythropoietin therapy (prevalence 8.1%; annual rate 7.25%), and another 14 events occurred in patients not on erythropoietin (9.3%; 7.56%). Thus, erythropoietin would seem not to increase the risk of thrombosis of myeloma patients receiving thalidomide.

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Since 1999, thalidomide has been increasingly used in patients with multiple myeloma (MM).[1] In relapsed/resistant MM, thalidomide alone is effective in approximately 30% of patients, and in up to 60-70% of cases when used in combination with dexamethasone or chemotherapy regimens.[2] Among the various side effects of thalidomide treatment, venous thrombosis has been frequently reported in MM patients.[3,4] When thalidomide is used alone, the prevalence of thrombosis does not exceed 5% in many studies. This figure increases to 10-15% when the drug is used with dexamethasone, and to about 30% when the thalidomide is combined with chemotherapy. Thalidomide is believed to cause thrombosis through activating endothelium injured by prior exposure to drugs, such as doxorubicin.[5] Among the drugs used in the supportive care of MM, recombinant human erythropoietin (rHuEpo) has a definite role in the treatment of anemia.

Table 1. Details of 18 thromboses during thalidomide treatment of 199 patients with MM.

Prevalence,%	9.0
Incidence rate,% patient-year	7.49
Time to event, months	
Median (range)	3.7 (1.0-18.6)
Thalidomide dosage at the time of event, mg/day	
Median (range)	100 (50-300)
Hemoglobin level at the time of event, g/dL	
Median (range)	12 (8.8-15)
Type of event	
Deep vein thrombosis	15
Intestinal ischemia	2
Myocardial infarction	1

Table 2. Thrombosis during thalidomide treatment: relationship with steroids, rHuEpo, and chemotherapy administration to 199 patients with MM.

*Concomitant treatment**	*Patients with thrombosis*			*Annual Incidence*
	Yes	*No*	*OR*	*Rate#*
	n = 18	*n = 181*	*95% CI***	*% patients*
Steroids§			2.71	
			0.86-8.56	
Yes, n = 116	14	102		7.28
No, n = 83	4	79		8.32
rHuEpo			0.86	
			0.27-2.76	
Yes, n = 49	4	45		7.78
No, n = 150	14	136		7.41
Chemotherapy°			1.00	
			0.12-8.34	
Yes, n = 11	1	10		4.37
No, n = 188	17	171		7.82

**For each treatment, we considered only the thrombotic events occurring while patients were effectively receiving the drug; §prednisone or dexamethasone; °chemotherapy was melphalan/prednisone in 10 patients, and vincristine/adriamycin/dexamethasone in one patient; **Odds ratio with 95% confidence intervals was calculated by means of contingency tables; #Rates were calculated taking into account the effective time spent on each treatment.*