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CHROMOSOME BANDING STUDIES IN 106 CASES OF CHRONIC MYELOGENOUS LEUKEMIA[1]

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Summary Chromosome banding studies performed on 106 cases of CML in Sapporo revealed that 101 (95.3%) were Ph[1]-positive, and 5 (4.7%) Ph[1]-negative, the latter including a case of juvenile type CML. Of the 101 Ph[1]-positive patients, 98 showed the standard type Ph[1] translocation, t(9;22)(q34;q11), while the remaining 3 had a complex Ph[1] translocation as represented by t(4;9;22), t(9;14;22), or t(9;10;15;19;22). There were 28 patients who showed other chromosome changes in addition to the Ph[1] translocation. Trisomy 8, duplication of Ph[1], isochromosome 17q, and trisomy 19 were most frequently involved in the additional changes, and 2 or more of them often participated in the major routes of karyotypic evolution. Other additional changes observed were 6 translocations, 4 partial deletions, 2 partial trisomies for 1q, trisomies 6, 7, 12, 15, and 21, a monosomy 5, a partial duplication of no. 9, a missing Y, and so on.

The present cytogenetic findings were evaluated with respect to some of the clinical and therapeutic parameters.

INTRODUCTION

Since the discovery by Rowley (1973) of a new consistent translocation, t(9q+; 22q−), in 9 cases of Philadelphia chromosome (Ph[1])-positive chronic myelogenous leukemia (CML), cytogenetic information is now available on banded karyotypes of more than 1,000 cases of CML reported from a number of laboratories in various countries. Although the overall frequencies of both the standard and variant Ph[1] translocations and other types of chromosome abnormalities in CML have been estimated from time to time (Lawler, 1977; Sonta and Sandberg, 1977;

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FIWCL, 1978; Rowley, 1980; Sandberg, 1980; Mitelman and Levan, 1981), the number of cases dealt with in most of the reported series seems to be still insufficient to allow reasonable comparison of data among different geographic areas or ethnic groups. In fact, we are aware of only 5 original reports in which more than 100 cases of CML were investigated by banding methods (Seabright and Pearson, 1978; Pasquali *et al.*, 1979; Fleischman *et al.*, 1981; Potter *et al.*, 1981; Oshimura *et al.*, 1982).

In this report, we present chromosomal findings in 106 cases of CML referred to us since 1973 to 1980.

MATERIALS AND METHODS

The 106 cases of CML referred to us from several hospitals in Sapporo consisted of 1 juvenile and 105 adult type cases, including 2 previously reported ones (Hayata and Sasaki, 1976; Tomiyasu *et al.*, 1980). Sixty-six cases were examined in the chronic phase (CP) only, 19 cases in the blastic phase (BP) only, and 20 cases in both CP and BP. Chromosome preparations were made by our routine methods on bone marrow and/or peripheral blood cells with or without short term culture. Phytohemaggultinin (PHA)-added blood cultures were made in some instances. For chromosome banding, the QFQ-staining was employed in all cases, while in some cases the GTG-, CBG-, and/or RFA-staining were also used. An abnormal cell line was defined as two or more cells with identical extra chromosomes and/or structural rearrangements, or three or more cells with identical missing chromosomes. Chromosome aberrations and karyotypes were described in accordance with ISCN (1978).

RESULTS

Of the 106 patients, 101 (95.3%) were found to be Ph^1-positive, while the remaining 5 cases including a case of juvenile type were Ph^1-negetive. The Ph^1-positive cases consisted of 63 males and 38 females. Their ages at diagnosis ranged from 8 to 72 years with the median of 40.5 years, and their survivals after diagnosis 2 to 173 months with the median of 23 months, including the updated data on 24 patients who are still alive.

Of the 101 Ph^1-positive cases 70 were found to possess the standard Ph^1 translocation, t(9;22)(q34;q11) [hereafter abbreviated as tPh^1], as a sole abnormality, though one of them had a constitutional reciprocal translocation, t(6;13)(q15;q34), in both Ph^1-positive leukemic cells and Ph^1-negative PHA-stimulated lymphocytes. Of the 70 cases with tPh^1, 56 (80%) were examined in CP only, 10 (14%) in BP only, and 4 (6%) in both CP and BP. Supplemental data on the age, sex, survival, status of therapy, and number of cells analyzed for each group of the patients are summarized in Table 1.

Table 1. Summary of data on 70 cases of CML with a standard Ph^1 translocation, t(9q+;22q−), without additional karyotypic changes.

Case No.	Sex	Therapy[a]				Stage[b]	Total no. of cells karyotyped/scanned (no. per case)		Age in years median (range)		Survival in months median (range)[c]		No. of cases alive
		−	+	−/+	?								
1-26	F	18	6	1	1	CP	277/615	(10. 7/23. 6)	34	(8-69)	23	(8-173)	14
27-56[d]	M	20	5	4	1	CP	347/936	(11. 6/31. 2)	44	(16-72)	25	(2-46)	12
Subtotal		38	11	5	2	CP	624/1, 551	(11. 1/27. 7)	41	(8-72)	24	(2-173)	26
57	F		1			BP	2/5		63		19		0
58-66	M		7	2		BP	123/350	(13. 7/38. 9)	41	(13-55)	9	(2-90)	0
Subtotal			8	2		BP	125/355	(12. 5/35. 5)	42. 5	(13-55)	13. 5	(2-90)	0
67	F			1		CP/BP	16/34		59		17		0
68-70	M		1	2		CP/BP	157/236	(52. 3/78. 7)	45	(20-50)	13	(13-50)	0
Subtotal			1	3		CP/BP	173/270	(43. 3/67. 5)	47. 5	(20-59)	15	(13-50)	0
Grand total	F	18	7	2	1								
	M	20	13	8	1		922/2, 176	(13. 2/31. 1)	42	(8-72)	21	(2-173)	0

[a] −, before therapy; +, after therapy; −/+, before and after therapy. [b] CP, chronic phase; BP, blastic phase. [c] No information for 13 cases.
[d] A case with a constitutional t(6;13)(6pter → 6q15;13pter → 13q34::6q15 → 6qter) is included here.

There were 3 cases with a complex Ph^1 translocation as represented by t(4;9;22), t(9;14;22), and t(9;10;15;19;22), respectively (Table 2, Fig. 1). These cases were examined in CP only, and had no additional chromosome abnormalities besides the Ph^1 translocation. The last case (Case 73) has been reported by Hayata and Sasaki (1976).

The remaining 28 cases had various types of chromosomal changes in addition to tPh^1 (Table 3). The additional abnormalities were found in 5 cases examined

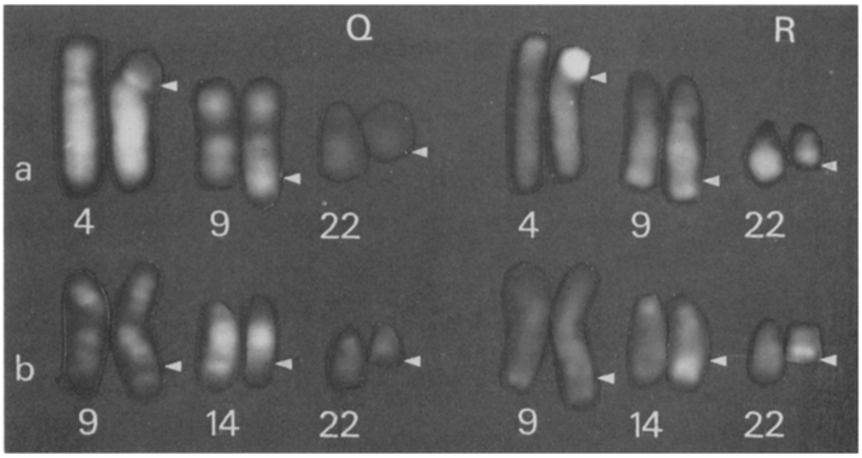

Fig. 1. Partial Q- and R-band karyotypes of Cases 71 (a) and 72 (b), shwong complex Ph^1 translocations involving chromosomes 4, 9, and 22, and chromosomes 9, 14, 22, respectively. Arrow heads indicate the break points.

Table 2. Cytogenetic and some clinical data in 3 cases of CML with a complex Ph^1 translocation.

Case No.	Patient code	Age (y)	Sex	Survival (m)	Therapy[a]	Specimen[b]	Karyotype and no. of cells analyzed (scanned)[c]
71	531,KK	61	F	43+	–	BM-d,c	46,XX,t(4;9;22)(4qter → 4p14::22q11 → 22qter;9pter → 9q34::4p14 → 4pter; 22pter → 22q11)=12(63)
					+	BM-d	The same karyotype as above=17(32)
72	753,AT	22	M	11+	–	BM-d	46,XY,t(9;14;22)(9pter → 9q34::14q24 → 14qter;14pter → 14q24::22q11 → 22qter;22pter → 22q11)=13(35)
73[d]	396,OK	23	M	?	–	BM-d,c	46,XY,t(9;10;15;19;22)(9pter → 9q34:: 10q22 → 10qter;10q22 → 10pter::19q13 → 19qter;15pter → 15q21::22q12 → 22qter;19pter → 19q13::15q21 → 15qter;22pter → 22q12)=50(50)

[a] –, before therapy; +, after therapy. [b] BM, bone marrow; d, direct preparation; c, cultured. [c] No. of cells karyotyped is indicated after the equal sign, and no. of cells scanned in the parentheses. [d] Previously reported case (Hayata and Sasaki, 1976). All cases were examined in the chronic phase only. The plus sign in survival indicates that the patient is still alive.

in CP only, 8 cases in BP only, and 15 cases in both CP and BP. Among the latter 15 cases, 13 had additional abnormalities in BP only, whereas the remaining 2 exhibited the same types of abnormalities in both CP and BP. All of the 23 cases that were studied in BP had received therapy. Out of the 20 cases that were studied in CP, 3 cases were examined after therapy, while 4 cases were studied before and after therapy, leaving 13 cases which had never been treated. The additional abnormalities in CP were found in 6 out of the former 7 cases, and in 1 of the latter 13 cases. It should be mentioned, however, that in 2 of the 4 cases studied before and after therapy in CP, the additional changes were detected even before therapy.

Allowing repetition in scoring the cases, a trisomy 8 [+8] was found in 14 cases, an extra Ph^1 [$+Ph^1$] in 13 cases, an isochromosome for the long arm of chromosome 17 [i(17q)] in 6 cases, a trisomy 19 [+19] in 6 cases, translocations in 6 cases, partial deletions in 4 cases, and partial trisomies for the long arm of chromosome 1, a trisomy 7 [+7] and a trisomy 21 [+21], each in 2 cases. Other changes that were encountered only once were monosomy 5, trisomies 6, 12, 15, and 17, a partial duplication for the long arm of chromosome 9, an additional segment on 21q [21q+], a missing Y [−Y], a marker chromosome of unknown origin [+mar], and multiple changes including a trisomy 1 and a possible isochromosome for the long arm of chromosome 2 (Table 3).

Among the 14 cases with tPh^1,+8 (Cases 74–87), Cases 74–75 were found to be accompanied with i(17q), Cases 76–78 with i(17q),$+Ph^1$, Case 79 with i(17q), +19,$+Ph^1$, Cases 80–82 with $+Ph^1$, Case 83 with +6,+19,+21,$+Ph^1$, Case 84 with +7,+12,+15,21q+, Case 85 with +17,+19,dup(9q+), and Case 86 with −5,+mar. While in the remaining one (Case 87) the +8 was the only anomaly additional to the tPh^1. Thus $+Ph^1$ was most frequent companion with +8 which occurred in 8 cases, while i(17q) and +19 coexisted with +8 in 6 and 3 cases, respectively. Other abnormalities, −5, +6, +7, +12, +15, +21, dup(9q+), 21q+, and +mar were encountered only once in association with +8. The dup(9q+) found in Case 85 was a derivative of the 9q+ marker, the regular partner of the Ph^1, in that an interstitial segment, 9q13 → 9q22, was tandemly duplicated, which was tentatively designated as tPh^1,dup(9q+)(q13 → q22). Case 79 was unique in that all of the above mentioned regular companions, *i.e.*, +8, i(17q), +19, and $+Ph^1$ were observed in cultured peripheral blood cells examined on 3 occasions in BP. The major direction in this case of the karyotypic evolution appeared to be tPh^1 → tPh^1,i(17q) → tPh^1,i(17q),+8 → tPh^1,i(17q),+8,+19, although a minor clone with tPh^1,$+Ph^1$ which was present in the 2nd BP sample disappeared thereafter A similar short-lived clone with a combination of tPh^1,i(17q),$+Ph^1$ was noted in Case 78. In CP of this case, tPh^1 was the sole abnormality, whereas 3 other cell lines with either tPh^1,i(17q); tPh^1,i(17q),+8; or tPh^1,i(17q),$+Ph^1$, were observed on 4 subsequent examinations in BP. As shown in Table 4, the cells with tPh^1, i(17q),$+Ph^1$, which were detected in the 2nd BP sample, suddenly disappeared

Table 3. Cytogenetic and some clinical data on 28 cases of Ph^1-positive CML with additional chromosome abnormalities.

Case No.	Patient code	Age (y)	Sex	Survival (m)	Therapy[a]	Stage[b]	Specimen[c]	Karyotype[d] (No. of cells analyzed/scanned)
74	454,RY	44	M	18	−	CP	BM-d+PB-c	46,XY,tPh^1=9 (9/52)
					+	BP	BM-d,c	47,XY,tPh^1,+8,−17,+i(17q)=11 (11/47)
75	280,YK	28	M	72	−	CP	BM-c	46,XY,tPh^1=4 (4/26)
					+	BP	BM-d	47,XY,tPh^1,+8,−17,+i(17q)=10/46,XY,tPh^1=5/46,XY,tPh^1,−17,+i(17q)=2 (17/22)
76	512,FK	21	F	58	+	BP	PB-c	47,XX,tPh^1,+8,−17,+i(17q)=13/48,XX,tPh^1,+8,−17,+i(17q),+Ph^1=4 (17/95)
77	245,MF	33	M	66	−	CP	BM-c+PB-c	46,XY,tPh^1=14 (14/19)
					+	BP	BM-d,c	48,XY,tPh^1,+8,−17,+i(17q),+Ph^1=33 (33/39)
78	337,KK	33	F	67	−	CP	BM-d	46,XX,tPh^1=2 (2/27)
					+	BP	BM-d×3+PB-c	46,XX,tPh^1,−17,+i(17q)=36/47,XX,tPh^1,+8,−17,+i(17q)=29/46,XX,tPh^1=23/47,XX,tPh^1,−17,+i(17q),+Ph^1=8 (96/108)
79	432,FU	28	M	?	−	CP	BM-c	46,XY,tPh^1=2 (2/4)
					+	BP	PB-c×3	46,XY,tPh^1,−17,+i(17q)=70/47,XY,tPh^1,+8,−17,+i(17q)=31/47,XY,tPh^1,+Ph^1=2/48,XY,tPh^1,+8,−17,+i(17q),+19=2 (105/111)
80	527,SS	24	M	48	+	BP	BM-d	48,XY,tPh^1,+8,+Ph^1=9 (9/18)
81	529,ZI	45	M	16	−	CP	PB-c	46,XY,tPh^1=4 (4/16)
					+	BP	BM-c	48,XY,tPh^1,+8,+Ph^1=29 (29/30)
82	671,KC	32	F	16	+	BP	PB-c	46,XX,tPh^1=16/47,XX,tPh^1,+mar=6/48,XX,tPh^1,+8,+Ph^1=2 (24/29)
83	492,TM	30	M	40	−	CP	BM-d	46,XY,tPh^1=14 (14/20)
					+	BP	BM-d	51,XY,tPh^1,+6,+8,+19,+21,+Ph^1=22/46,XY,tPh^1=8 (30/34)
84	517,HS	36	M	23	−	CP	BM-d	46,XY,tPh^1=29 (29/61)
					+	BP	BM-d×2	46,XY,tPh^1,21q+=41/46,XY,tPh^1=10/50,XY,tPh^1,+7,+8,+12,+15,21q+=3 (54/55)
85	358,SS	30	M	51	+	BP	PB-c	49,XY,tPh^1,+8,+17,+19,dup(9q+)(q13→q22)=10/48,XY,tPh^1,+8,+19,dup(9q+)(q13→q22)=5/49,XY,tPh^1,+8,+8,+19,dup(9q+)(q13q→q22)=2 (17/21)
86	510,YF	42	M	2	+	BP	BM-d,c	46,XY,tPh^1=20/47,XY,tPh^1,−5,+8,+mar=12 (32/75)
87	281,SM	42	M	35	+	CP	BM-c	47,XY,tPh^1,+8=12/46,XY,tPh^1=11 (23/23)
88	561,JY	58	M	37	+	BP	BM-d	46,XY,tPh^1=13/47,XY,tPh^1,+Ph^1=3/47,XY,tPh^1,+19=2/47,XY,tPh^1,+21=2 (20/20)

					+	BP	BM-d	47,XY,tPh^1,+Ph^1=20/46,XY,tPh^1=3 (23/46)
89	594,SO	55	F	13	−/+	CP	BM-c×2+PB-c	46,XX,tPh^1,del(13)(q12?q32?)=30/46,XX,tPh^1=3 (33/46)
					+	BP	BM-d+PB-c	48,XX,tPh^1,+19,+Ph^1,del(13)(q12?q32?)=42/46,XX,tPh^1, del(13)(q12?q32?)=9 (51/68)
90	494,YS	27	M	23	+	BP	BM-d×3	46,XY,tPh^1=66/48,XY,tPh^1,+7,+Ph^1=15/47,XY,tPh^1, +Ph^1=19 (100/126)
91	457,ES	33	F	89	+	BP	BM-d,c+PB-c	47,XX,tPh^1,−17,+t(1;17)(1qter → 1q21::17p11 → 17qter), +Ph^1=69/46,XX,tPh^1=7 (76/76)
92	621,MM	37	M	34	−	CP	BM-d,c	46,XY,tPh^1=10 (10/29)
					+	BP	BM-d	46,XY,tPh^1,t(7;11)(7pter → 7p14::11p15 → 11qter;11pter → 11p15::7p14 → 7qter)=23/47,XY,tPh^1,+Ph^1,t(7;11)(7pter → 7p14::11p15 → 11qter;11pter → 11p15::7p14 → 7qter)= 6 (29/29)
93	401,SY	43	M	32	−/+	CP	BM-d×2+PB-c	46,XY,tPh^1=36/45,tPh^1,−13,−17,+t(13;17)(13qter → 13p11::17q11 → 17qter)=20 (56/61)
94	202,TI	41	F	86	−/+	CP	BM-c×2+PB-c	46,XX,tPh^1=18 (18/39)
					+	BP	BM-c	46,XX,tPh^1,−7,+t(1;7)(1qter → 1q24::7p11 → 7qter)=22 (22/22)
95	490,YI	51	M	?	+	CP	BM-c+PB-c	46,XY,tPh^1,−3,−3,+t(3;3)(3pter → 3q29::3q22 → 3qter), +del(3)(pter → q22)=22 (22/49)
96	469,SU	34	M	39	−	CP	BM-d,c+PB-c	46,XY,tPh^1=25 (25/82)
					+	BP	BM-c	46,XY,t(9;22)(9pter → 9cen?::22cen? → 22pter;22q11 → 22cen?::9cen? → 9q34::22q11 → 22qter)=24/46,XY,tPh^1 =7 (31/47)
97	237,MI	38	F	58	+	BP	BM-c	47,XX,tPh^1,+19=11 (11/11)
98	682,SA	39	M	17	−	CP	BM-d	46,XY,tPh^1=20/45,X,−Y,tPh^1=12 (32/32)
99	546,KU	30	F	22	−	CP	BM-d	46,XX,tPh^1=18 (18/33)
					+	BP	BM-d+BM-c×2	46,XX,tPh^1,del(9q+)(qter → p21:)=41/46,XX,tPh^1= 34/92,XXXX,tPh^1,tPh^1=2 (77/103)
100[e]	514,YA	28	F	21	−/+	CP	BM-d+PB-c×2	46,XX,tPh^1,del(5)(q14q23)=51/46,XX,tPh^1=33 (84/84)
					+	BP	BM-d	46,XX,tPh^1,del(5)(q14q23)=18 (18)
101	426,SH	64	M	31	−	CP	BM-c	46,tPh^1=7 (7/11)
					+	BP	BM-c	48-73,XY,tPh^1,+1,i(2q)?,+multiple changes=38/46,XY, tPh^1=11 (49/58)

[a] −, before therapy; +, after therapy; −/+, before and after therapy. [b] CP, chronic phase; BP, blastic phase. [c] BM, bone marrow; PB, peripheral blood; d, direct preparation; c, cultured; ×2 or ×3, examined on 2 or 3 different samples. [d] tPh^1, standard type Ph^1 translocation; +Ph^1, additional 22q−. [e] Previously reported case (Tomiyasu *et al.*, 1980).

within a week or so. The major route of karyotypic evolution in this case was thought to be $tPh^1 \rightarrow tPh^1$,i(17q) $\rightarrow tPh^1$,i(17q),+8, which took over another route, possibly blanched off from tPh^1,i(17q) to tPh^1,i(17q),+Ph^1.

Table 4. Karyotype analyses in Case 78 (337,KK).

Date	Specimen [a]	Clinical phase [b]	No. of cells with			
			tPh^1 [c]	tPh^1 i(17q)	tPh^1 i(17q) +8	tPh^1 i(17q) +Ph^1 [d]
10/18/74	BM-d	CP	27			
11/24/78	BM-d	BP	18	10	2	
12/14/78	BM-d	BP		12	10	8
12/22/78	BM-d	BP	5	12	4	
2/5/79	PB-c	BP		2	13	

[a] BM, bone marrow; PB, peripheral blood; d, direct preparation; c, cultured. [b] CP, chronic phase; BP, blastic phase. [c] tPh^1,t(9;22)(q34;q11). [d] +Ph^1,+22q−.

In addition to the aforementioned 8 cases comprising +8,+Ph^1 (Cases 76–83), there were found 5 cases (Cases 88–92) which showed a +Ph^1 and some other anomalies, as represented by +19,+21; +19,del(13); +7; t(1;17); and t(7;11), respectively. In Case 88, despite the existence of 4 cell lines [tPh^1; tPh^1,+Ph^1; tPh^1,+19; and tPh^1,+21] in the 1st BP sample, the final cell population studied in the 2nd BP sample was taken over exclusively by a single cell line with tPh^1,+Ph^1. Case 89 was analyzed 3 times in CP and twice in BP. The 1st (before therapy) and the 2nd (after therapy) analyses in CP revealed 2 cell lines with either tPh^1 or tPh^1,del(13)(q12?q32?). While the latter cell line persisted throughout the subsequent studies, its derivative line showing a tPh^1,+19,+Ph^1,del(13) constitution suddenly predominated in the 4th and 5th samples.

Case 90 was studied 3 times in BP. The 1st study disclosed 2 abnormal karyotypes, 46,tPh^1 and 48,tPh^1,+7,+Ph^1, in the proportion of 2 : 1. The incidence of the latter cell type decreased to less than 1% in the 2nd sample, and all cells analyzed in the 3rd sample showed a new karyotype, 47,tPh^1,+Ph^1.

The 2 translocations, an unbalanced t(1;17) and a reciprocal t(7;11) found in Cases 91 and 92, respectively, appeared to have occurred in preexisted 46,tPh^1 cell, followed by an additional karyotypic change with +Ph^1. Four other translocations found in Cases 93–96 were t(13;17); t(1;7); t(3;3),del(3); and t(9;22) involving four-break rearrangements by which the original Ph^1 chromosome was masked (Table 3, Fig. 2). None of the break points involved in the formation of the above 6 translocations were identical, whereas the 2 unbalanced translocations, t(1;17) of Case 91 and t(1;7) of Case 94 (Fig. 3), which produced partially trisomic conditions for the long arm of chromosome 1, appeared to share a common segment of duplication from 1q24 to 1qter.

Case 97 had a tPh[1], +19 constitution in all cells examined before therapy in CP. Case 98 also examined only once in untreated CP, contained 2 cell lines with either tPh[1] or tPh[1], −Y, indicating that the missing Y was a secondary event.

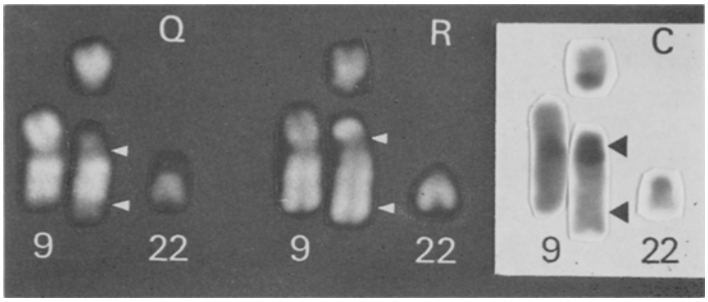

Fig. 2. Partial Q-, R-, and C-band karyotypes of Case 96, showing a masked Ph[1] translocation. Arrow heads indicate the break points.

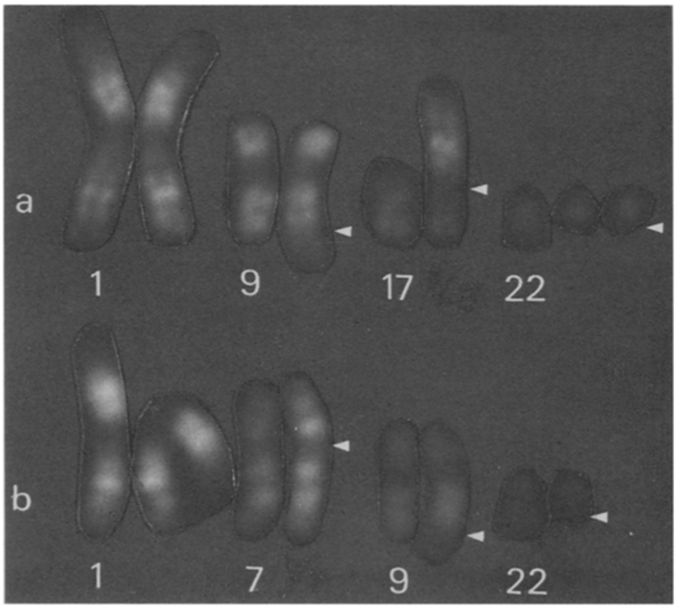

Fig. 3. Partial Q-band karyotypes of Cases 91 (a) and 94 (b), showing partial trisomies for the long arm of chromosome 1. Arrow heads indicate the break points.

Case 99 which showed a tPh[1] karyotype in the 1st BP sample taken before therapy acquired a possible terminal deletion in the tPh[1]-derived 9q+, with a break point at band 9p21. The karyotype was tentatively designated as tPh[1],

Table 5. Cytogenetic and some clinical and hematological data in 5 cases of Ph^1-negative CML.

Case No.	Patient code	Age	Sex	Survival (m)	Therapy [a]	Stage [b]	Specimen [c]	No. of cells analyzed/scanned		Karyotype	WBC ($\times 10^3/mm^3$)	Platelets ($\times 10^4/mm^3$)
102	323,YS	62y	F	70	+	CP	BM-d	9	24	46,XX	?	?
103	333,KS	8m	M	3	+	—	PB-c	4	11	46,XY	78. 0	3. 7
104	473,UK	51y	M	1	+	BP	BM-d	9	30	46,XY	9. 2	2. 5
105	506,SM	73y	M	3	+	CP	BM-d	5	35	46,XY	9. 6	18. 5
106	629,SF	48y	M	25	—	CP	BM-d	10	26	46,XY	34. 0	33. 4

[a] —, before therapy; +, after therapy. [b] CP, chronic phase; BP, blastic phase. [c] BM, bone marrow; PB, peripheral blood; d, direct preparation; c, cultured.

del(9q+)(qter→p21:). This deletion was found in about 80% of cells in the 2nd direct marrow sample taken after therapy, in coexistence with 20% of the original tPh[1] cells. However, in both the 3rd and 4th marrow samples which were studied in culture, the incidence of the del(9q+) cells decreased to 10% or less, and the tPh[1] cells became predominant. A small fraction of tetraploid cells with 2 Ph[1] chromosomes was noted in the 4th sample

Case 100 was a previously reported case (Tomiyasu *et al.*, 1980) which had an interstitial deletion of the long arm of chromosome 5, del(5)(q14q23), in addition to tPh[1].

Case 101 was started with tPh[1] in CP. In the 2nd sample, which was taken after therapy in BP and examined in culture, there were observed very dramatic chromosome changes as represented by widespread chromosome numbers ranging from 48 to 73 together with various numerical and structural variations. Among these multiple changes, an excess number of chromosome 1 and a possible isochromosome for the long arm of chromosome 2 were common features. About one-quater of cells in the 2nd sample had a 46,tPh[1] karyotype.

All of the 5 Ph[1]-negative cases including a juvenile type case of CML showed exclusively a normal karyotype without any detectable abnormality in banding patterns of individual chromosomes (Table 5).

DISCUSSION

Our data on the type and frequency of chromosome abnormalities in 106 cases of CML appeared to be somewhat different from those reported by the FIWCL (1978) and some other authors who studied relatively large numbers of CML cases (Lawler *et al.*, 1976; Engel *et al.*, 1977; Seabright and Pearson, 1978; Pasquali *et al.*, 1979; Stoll and Oberling, 1979; Bernstein *et al.*, 1980; Hagemeijer *et al.*, 1980; Kohno and Sandberg, 1980; Sadamori *et al.*, 1980; Fleischman *et al.*, 1981; Potter *et al.*, 1981; Oshimura *et al.*, 1982).

The frequency of Ph[1]-negative CML cases in our series (3.8%=4/105, excluding a case of juvenile type) seems to be lower than the usual incidence of 10–15%, even though the incidence varies among different laboratories, ranging from 0% to more than 30% (Sandberg, 1980). Relatively shorter survivals, lower WBC and platelet counts, and higher ages were noted in some of our Ph[1]-negative cases, as have been suggested to be general trends of Ph[1]-negative CMLs (Ezdinli *et al.*, 1970; Canellos *et al.*, 1976). The absence of the Ph[1] chromosome in the juvenile type CML is not unusual (Brodeur *et al.*, 1979).

The present incidence of variant Ph[1] translocations (3 of 101 Ph[1]-positive cases) seemed to be lower than the usual incidence, 8% (FIWCL, 1978), and all of the 3 variants were of complex type, the simple type being absent in our series. The hematological and clinical findings of these 3 cases did not show much deviations from those of the cases with a standard Ph[1] translocation (Sonta and Sandberg, 1977), except for rather high percentages of myeloblasts and promyelocytes in CP of Case 73. This case was unusual in that 5 different chromosomes were involved in the formation of the complex translocation. Complex Ph^1 translocations involving chromosome 4 have been reported in 7 cases (Rowley *et al.*, 1976; Geraedts *et al.*, 1977; Chessells *et al.*, 1979; Pasquali *et al.*, 1979; Fraisse *et al.*, 1980; Kessous *et al.*, 1980; Sudries *et al.*, 1980), but the break point in the affected no. 4 of t(4;9;22) in Case 71 was different from those in the reported cases. The complex Ph^1 translocation, t(9;14;22), found in Case 72 and 3 reported cases (Potter *et al.*, 1975; Borgström, 1981; Shabtai *et al.*, 1980) appeared to have the same break point at 14q24, whereas the break point in another reported case (Kolitz *et al.*, 1981) of t(9;14;22) was located at 14q32.

In the present study, the overall incidence of Ph^1-positive cases with additional changes was 27.7% (28/101); 9.5% (8/84) in CP and 62.2% (23/37) in BP. Limiting the data on 20 cases which were studied in both CP and BP, the incidence was estimated to be 15% (3/20) in CP, and 65% (13/20) in BP. These values do not seem to be much deviating from those reported from other laboratories, where the incidence varied from less than 10% (Bernstein *et al.*, 1980) to 20–30% in CP (Hayata *et al.*, 1975; Lawler *et al.*, 1976; Sonta and Sandberg, 1978; Kohno and Sandberg, 1980), and from 60% (Pasquali *et al.*, 1979) to more than 85% in BP (Sonta and Sandberg, 1978; Stoll and Oberling, 1979; Bernstein *et al.*, 1980).

The most frequent additional changes in our cases were +8, i(17q), +19, and $+Ph^1$, and two or more of them were often observed in the same cell or cell population, being in agreement with previous studies as reviewed by some authors (Lawler, 1977; Rowley, 1980; Sandberg, 1980; Mitelman and Levan, 1981). These additional changes are assumed to have occurred in preexisted Ph^1-positive cells either in CP or BP, with preferential combinations of $+8,+Ph^1$; +8,i(17q); or +8,+19, attaining further karyotypic evolution toward $+8$,i(17q),$+Ph^1$; +8,i(17q), +19; or +8,i(17q),+19,$+Ph^1$. In contrast, a single addition to tPh^1 of +8 (Case 87) or $+Ph^1$ (Case 79) was less frequent or rather short-lived, the latter being taken over by different cell lines having possibly more adaptive or grave-destined combinations, starting with tPh^1,i(17q) and ending with tPh^1,i(17q),+8,+19. Another combination in Case 78 with tPh^1,i(17q),$+Ph^1$ was also short-lived, being rapidly taken over by tPh^1,i(17q),+8, and hence rarely found; only 3 cases with this combination have been reported to date (Stoll and Oberling, 1979). It is interesting that in Case 88 which had neither +8 nor i(17q), a cell line with $tPh^1,+Ph^1$ predominated over 3 other cell lines with tPh^1; tPh^1,+19; or tPh^1,+21.

Apart from the aforementioned relatively common additional changes, all of the 6 translocations and 4 partial deletions were of different origin, in terms of the break points involved in the formation of such anomalies. However, the partially trisomic condition of 1q as resulted from the translocations in Cases 91 and 94 may merit special attention, since similar secondary changes, with a common trisomic segment of 1q25→1q32, have been suggested to occur rather frequently in various blood disorders (Gahrton *et al.*, 1978; Rowley 1978; Alimena *et al.*, 1980; Miyamoto *et al.*, 1981; Slavutsky *et al.*, 1981). The del(5) in Case 100 may bear a similar implication, as have been discussed elsewhere (Tomiyasu *et al.*, 1980; Mitelman and Levan, 1981).

It has been suggested that the observation of additional chromosome abnormalities at the time of diagnosis in CP may not have significance on the progression of the disease (Sandberg, 1978). Out of our 28 patients who showed additional abnormalities (Table 3), 17 were examined before therapy in CP. Among the latter 17, only 3 (Cases 89, 98, and 100) showed additional changes at or soon after diagnosis before therapy in CP; their survivals were 13, 17, and 21 months, respectively. In the remaining 14 patients who did not show additional changes before therapy in CP, their survivals ranged from 16 to 86 months, with the mean of 42 months and the median of 34 months. The different survivals between the above two groups were not significant statistically ($0.05<p<0.1$). On the other hand, the median survival for all of the 28 patients who developed additional changes was rather longer than that of the 70 patients without further changes; 34.5 *vs.* 21 months. However, the latter figure can not properly be compared to the contradictory findings of Sonta and Sandberg (1978), and Prigogina *et al.* (1978); since more than one third of the 70 patients are still alive, with an unusually long chronic phase, much longer median survival is expected for the latter group of our patients.

Alimena *et al.* (1979) have reported that about 85% of their 34 Ph[1]-positive

Table 6. Summary of therapeutic and cytogenetic data on 97 Ph[1]-positive CML patients.

No. of cases in which karyotype analyses were performed in			No. of cases with							
			Ph[1] translocation only[a]			+8, i(17q), +Ph[1] and/or +19			Other structural changes	
CP before therapy only	40		39	(2)[a]		1*	(1*)[b]		0	
CP after therapy only	14	[2][c]	12		[2][c]	2			0	
BP after therapy only	14	[5][c]	7		[2][c]	4	(2)[b]	[3][c]	3	(1)[b]
CP/BP before and after therapy	29	[3][c]	13	(1)[a]	[1][c]	Changes only after therapy				
						7	(1)[b]	[2][c]	7	(1)[b]
						Before and after therapy				
						0			2	
Total	97	[10][c]	71	(3)[a]	[5][c]	14	(4)[b]	[5][c]	12	(2)[b]

[a] Three cases with a complex Ph[1] translocation were shown in parentheses. [b] No. of cases with other numerical changes in addition to +8,i(17q),+Ph[1] and/or +19 was shown in parentheses, except 1 case with −Y only which was indicated by an asterisk. [c] No. of patients who received intensive chemotherapy with 6-mercaptopurine, prednisone, and/or cyclophosphamide before chromosome analyses was shown in brackets, on the basis of 77 patients with whom exact records of therapy were available. CP, chronic phase; BP, blastic phase.

CML patients had +8,i(17q), and/or $+Ph^1$, and that the frequency of each of these relatively common changes was not significantly different between the following two groups of patients: one treated with busulfan only, and the other received intensive chemotherapy with cytarabine, vincristine, daunorubicin, and/or thioguanine. Their results also indicated that other structural abnormalities of a clonal nature, especially those involving chromosome 1, were more frequent in the latter group of patients. Two of our patients (Cases 91 and 94) who showed structural abnormalities of chromosome 1 had been treated with busulfan or vercyte only. In the present study, the therapeutic records in CP were available for 97 Ph^1-positive patients. Most of them were treated with busulfan, dibromomannitol, and/or vercyte, but those who received intensive therapy prior to the chromosome examination were very few. As shown in Table 6, additional chromosome changes were less frequent in the patients whose chromosomes were examined in CP only, especially before therapy. By contrast, the incidence of additional changes were much higher in the patients examined after therapy in BP. It can not be decided, however, whether these additional changes were induced by the therapy, or they have developed simply as the result of progressive karyotypic evolution that occurred in association with the malignant growth. The frequencies of cells with structural changes other than i(17q) were almost the same as those with +8, i(17q), +19, $+Ph^1$, and/or other numerical changes. No positive evidence was obtained in favor of the relationship between structural changes and intensive therapy as suggested by Alimena *et al.* (1979), although only 10 patients were subjected to intensive chemotherapy before chromosome examinations in the present study. Similar negative results have been reported by Fleischman *et al.* (1981).

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