

GENOMIC ABERRATIONS AND SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA

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ABSTRACT

Background Fluorescence in situ hybridization has improved the detection of genomic aberrations in chronic lymphocytic leukemia. We used this method to identify chromosomal abnormalities in patients with chronic lymphocytic leukemia and assessed their prognostic implications.

Methods Mononuclear cells from the blood of 325 patients with chronic lymphocytic leukemia were analyzed by fluorescence in situ hybridization for deletions in chromosome bands 6q21, 11q22–23, 13q14, and 17p13; trisomy of bands 3q26, 8q24, and 12q13; and translocations involving band 14q32. Molecular cytogenetic data were correlated with clinical findings.

Results Chromosomal aberrations were detected in 268 of 325 cases (82 percent). The most frequent changes were a deletion in 13q (55 percent), a deletion in 11q (18 percent), trisomy of 12q (16 percent), a deletion in 17p (7 percent), and a deletion in 6q (6 percent). Five categories were defined with a statistical model: 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as the sole abnormality; the median survival times for patients in these groups were 32, 79, 114, 111, and 133 months, respectively. Patients in the 17p- and 11q-deletion groups had more advanced disease than those in the other three groups. Patients with 17p deletions had the shortest median treatment-free interval (9 months), and those with 13q deletions had the longest (92 months). In multivariate analysis, the presence or absence of a 17p deletion, the presence or absence of an 11q deletion, age, Binet stage, the serum lactate dehydrogenase level, and the white-cell count gave significant prognostic information.

Conclusions Genomic aberrations in chronic lymphocytic leukemia are important independent predictors of disease progression and survival. These findings have implications for the design of risk-adapted treatment strategies. (N Engl J Med 2000;343:1910-6.)

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B-CELL chronic lymphocytic leukemia is the most common leukemia in adults. It has a highly variable clinical course; some patients die from the disease within a few months of the diagnosis, whereas others live for 20 years or more.¹ The clinical staging systems devised by Rai et al.² and Binet et al.³ are the most useful methods for predicting survival in chronic lymphocytic leukemia. However, these staging systems cannot be used to predict the individual risk of disease progression and survival in the early stages of chronic lymphocytic leukemia (Binet stage A or Rai stage 0 to 2 disease), when the disease is first diagnosed in most patients. The substantial heterogeneity within clinical stages has prompted searches for additional prognostic factors, but most of them have not proved useful.⁴

There is considerable interest in identifying chromosomal aberrations that could pinpoint subgroups of patients with chronic lymphocytic leukemia who have different prognoses.⁵ Conventional cytogenetic analysis has been hampered by the low mitotic activity of the leukemic cells in vitro. With the usual method, clonal chromosomal aberrations are detected in only 40 to 50 percent of cases, the most common being trisomy 12 and abnormalities of chromosome bands 13q14 and 14q32.⁶ Fluorescence in situ hybridization allows the detection of chromosomal aberrations not only in dividing cells but also in interphase nuclei, an approach referred to as interphase cytogenetics. Initial studies of chronic lymphocytic leukemia with this method demonstrated that the frequency and spectrum of chromosomal aberrations it detected differed considerably from the results obtained by conventional chromosome banding.⁷ However, in these studies only single aberrations were evaluated for their prognostic importance, and this was done mostly in small series of patients.

We designed a comprehensive set of DNA probes for evaluating genomic changes in chronic lymphocytic leukemia by interphase cytogenetics. Our objective was to assess the frequency and clinical relevance of genomic aberrations in a large group of patients with the disease.

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METHODS

Patients

Between October 1990 and August 1998, 325 consecutive patients with chronic lymphocytic leukemia from a single institution were enrolled in the study and followed with regard to survival. There were 199 men and 126 women; their ages at the time of enrollment ranged from 30 to 87 years (median, 62). The diagnosis of chronic lymphocytic leukemia required persistent lymphocytosis (>5000 lymphocytes per cubic millimeter).⁸ Immunophenotypic data, available for 314 of the 325 patients, showed that all the cases of leukemia were CD19+, 298 of 308 tested were CD5+, and 300 of 308 tested were CD23+. All these cases were therefore of the B-cell type. At the time of enrollment, 63 patients were at Rai stage 0, 48 at stage 1, 146 at stage 2, 33 at stage 3, and 34 at stage 4.² According to the Binet system, 170 patients had stage A, 102 stage B, and 52 stage C disease.³ In one patient, clinical data were incomplete. Two hundred forty-eight patients had received no previous treatment, 39 patients had received one chemotherapeutic regimen, and 38 patients had received two or more chemotherapeutic regimens before interphase cytogenetic analysis. The median time from the date of diagnosis to the date of interphase cytogenetic study was 15 months (interquartile range, 1 to 43 months).

Interphase Cytogenetic Analysis

DNA Probes

A set of DNA probes was developed to diagnose genomic aberrations by interphase cytogenetics. Chromosomal regions were selected on the basis of data from conventional chromosome-banding studies and comparative genomic hybridization.^{6,9} The DNA probes allowed us to screen for the following partial deletions, partial trisomies, and translocations (the clone designation and the gene or locus detected are shown in brackets): +(3q26) [yeast artificial chromosome 866_e_7],¹⁰ del(6q21) [963_d_6],¹¹ +(8q24) [935_a_12],¹⁰ del(11q22-q23) [755_b_11],¹² +12q13 [754_a_1],¹⁰ and del(13q14) [λ -phage clones, which recognize *RBI* (kindly provided by Dr. Thaddeus Dryja, Boston); cosmid c1325, which identifies *D13S25*],¹³ t(14q32) [cosmid cos-C_{a1/23}, which recognizes the c_{a1} and c_{a2} gene segments proximal to the J_H region; yeast artificial chromosome Y6, which identifies V_H segments telomeric to the J_H break points in the immunoglobulin heavy-chain gene (*IgH*)],¹² and del(17p13) [cosmids ICRFc105BO195-75, ICRFc105CO275-77, ICRFc105EO675-78, and ICRFc105AO144-79 for p53].¹⁴

In cases showing splitting of one fluorescence signal with the *IgH* probes, the leukemia cells were analyzed for two reciprocal translocations: t(11;14) and t(14;18). For the diagnosis of t(11;14), the *IgH* probes were combined with the differently labeled 540-kb yeast artificial chromosome 55_g_7, which recognizes DNA sequences spanning the region between the major translocation cluster and the *CCND1* gene in the *BCL1* locus at 11q13¹²; for the detection of t(14;18), the *IgH* probes were combined with yeast artificial chromosome yA153_A_6, which spans the *BCL2* proto-oncogene (kindly provided by G. Silverman, Boston).

Detection of Genomic Aberrations by Fluorescence in Situ Hybridization

DNA probe sequences from yeast artificial chromosome clones were generated by an inter-Alu polymerase-chain-reaction (PCR) protocol.¹⁵ Cosmid DNA was prepared according to the plasmid Midi Kit protocol (Qiagen, Hilden, Germany). The probes were labeled by nick translation with biotin-16-deoxyuridine triphosphate or digoxigenin-11-deoxyuridine triphosphate (Roche, Mannheim, Germany). Fluorescence in situ hybridization was performed as described previously.^{12,14}

Statistical Analysis

The primary end point was survival from the time of diagnosis. Survival times and censored waiting times measured from the date

of diagnosis were plotted with the use of Kaplan–Meier estimates. The median duration of follow-up was calculated according to the method of Korn.¹⁶ The proportional-hazards regression model of Cox was used to identify differences in survival due to prognostic factors.¹⁷ As possible prognostic factors, age, sex, Binet and Rai stages, hemoglobin level, white-cell count, platelet count, serum lactate dehydrogenase and alkaline phosphatase levels, presence or absence of splenomegaly and lymphadenopathy, extent of peripheral lymphadenopathy, greatest lymph-node diameter measured, and presence or absence of genomic aberrations (deletion in 17p, deletion in 11q, trisomy of 12q, deletion in 13q, and deletion in 6q) were included in the regression model. We estimated missing data using a multiple-imputation technique with 10 random draws. A limited backward-selection procedure was used to exclude redundant or unnecessary variables.¹⁸

Groupwise comparisons of the distributions of clinical and laboratory variables at the time of the genetic study were performed with the Kruskal–Wallis test (for quantitative variables) and Fisher’s exact test (for categorical variables). All tests were two-sided. An effect was considered statistically significant if the P value was 0.05 or less. To provide quantitative information on the relevance of statistically significant results, 95 percent confidence intervals for hazard ratios were computed. The statistical analyses were performed with the following software packages: StatXact (Cytel Software, Cambridge, Mass.), S-Plus (MathSoft, Seattle), and the Design software library.¹⁸

RESULTS

Interphase Cytogenetic Analysis

All 325 cases could be evaluated by interphase cytogenetics. Of these cases, 268 (82 percent) exhibited abnormalities. Table 1 lists these aberrations, in order of decreasing frequency. In 175 patients there was one aberration, 67 patients had two aberrations, and 26 patients had more than two aberrations. Among the 178 patients with 13q deletion, the deletion was the sole abnormality in 117 (66 percent). In the remaining 61 patients (34 percent), 13q deletion was accompanied by 11q deletion (28 patients), 12q trisomy (13 patients), 11q deletion and 12q trisomy (1 pa-

TABLE 1. INCIDENCE OF CHROMOSOMAL ABNORMALITIES IN 325 PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

ABERRATION	NO. OF PATIENTS (%)*
13q deletion	178 (55)
11q deletion	58 (18)
12q trisomy	53 (16)
17p deletion	23 (7)
6q deletion	21 (6)
8q trisomy	16 (5)
t(14q32)	12 (4)
3q trisomy	9 (3)
Clonal abnormalities	268 (82)
Normal karyotype	57 (18)

*One hundred seventy-five patients had one aberration, 67 had two aberrations, and 26 had more than two aberrations.

tient), 17p deletion (8 patients), or other abnormalities (11 patients). An 11q deletion occurred as the sole aberration in 19 of 58 patients (33 percent), 12q trisomy in 22 of 53 patients (42 percent), 17p deletion in 4 of 23 patients (17 percent), and 6q deletion in 6 of 21 patients (29 percent). All deletions were monoallelic except for the 13q14 region: in 43 of the 178 patients with 13q deletions (24 percent), there were biallelic or concomitant monoallelic and biallelic deletions. In all cases, biallelic deletion affected the *D13S25* locus, and in 2 of the 43 patients there was also biallelic *RBI* deletion. Of the 12 patients with the translocation t(14q32), 7 had t(14;18), and the rest had t(14q32) with an unidentified partner. We included patients with t(14;18) in the analysis, since they had the typical morphologic features and immunophenotype of chronic lymphocytic leukemia. No patient had t(11;14).

Correlation with Clinical and Laboratory Data

The proportional-hazards regression model with backward selection identified six significant prognostic factors: 17p deletion ($P < 0.001$), 11q deletion ($P = 0.004$), age ($P < 0.001$), Binet stage (B as compared with A, $P = 0.36$; C as compared with A, $P = 0.002$), serum lactate dehydrogenase level ($P = 0.002$), and white-cell count ($P = 0.02$). There was a statistically significant interaction effect between age and the presence or absence of an 11q deletion ($P = 0.02$): the negative prognostic effect of an 11q deletion was seen primarily in younger patients. The hazard ratios together with their 95 percent confidence limits are shown in Table 2.

On the basis of the regression analysis, we constructed a hierarchical model of genetic subgroups in which each case was allocated to one category only. Table 3 lists the five major categories to which 300 of the 325 cases could be assigned with this model.

After a median follow-up of 70 months, 112 of the 325 patients had died. The median survival time of the entire group was 108 months (95 percent confidence interval, 94 to 119). The estimated median survival times from the date of diagnosis for the five genetic categories listed in Table 3 were as follows: 17p deletion, 32 months; 11q deletion, 79 months; 12q trisomy, 114 months; normal karyotype, 111 months; and 13q deletion as the sole abnormality, 133 months (Fig. 1). The remaining 25 patients were combined into the group with various abnormalities. This heterogeneous group included patients with 3q trisomy, 6q deletion, 8q trisomy, or t(14q32). Patients in this category had a high probability of survival (the median survival time was not reached).

Table 4 shows the clinical and laboratory data for the patients in the five major categories at the time of enrollment. Patients with 17p or 11q deletions had more advanced disease than those in the other three groups ($P < 0.001$), whereas patients with 13q dele-

TABLE 2. RESULTS OF COX REGRESSION ANALYSIS OF SURVIVAL TIME FROM DIAGNOSIS (FINAL MODEL).*

VARIABLE	HAZARD RATIO FOR DEATH (95% CI)
17p deletion	8.08 (4.24–15.40)
Binet stage	
B vs. A	1.27 (0.76–2.13)
C vs. A	3.77 (1.64–8.66)
Age (10-yr increment)	
No 11q deletion	2.04 (1.56–2.67)
11q deletion	1.12 (0.74–1.69)
11q deletion	
Age 55 yr	2.89 (1.73–4.84)
Age 65 yr	1.58 (0.91–2.76)
Lactate dehydrogenase (increment of 50 IU/liter)	1.30 (1.10–1.53)
White-cell count (increment of 20,000/mm ³)	1.08 (1.01–1.15)

*Hazard ratios and confidence intervals (CIs) are computed for a 10-year increment in age, dependent on 11q deletion; for 11q deletion at the age of 55 and 65 years; for an increment of 50 IU per liter in lactate dehydrogenase; and for an increment of 20,000 per cubic millimeter in the white-cell count.

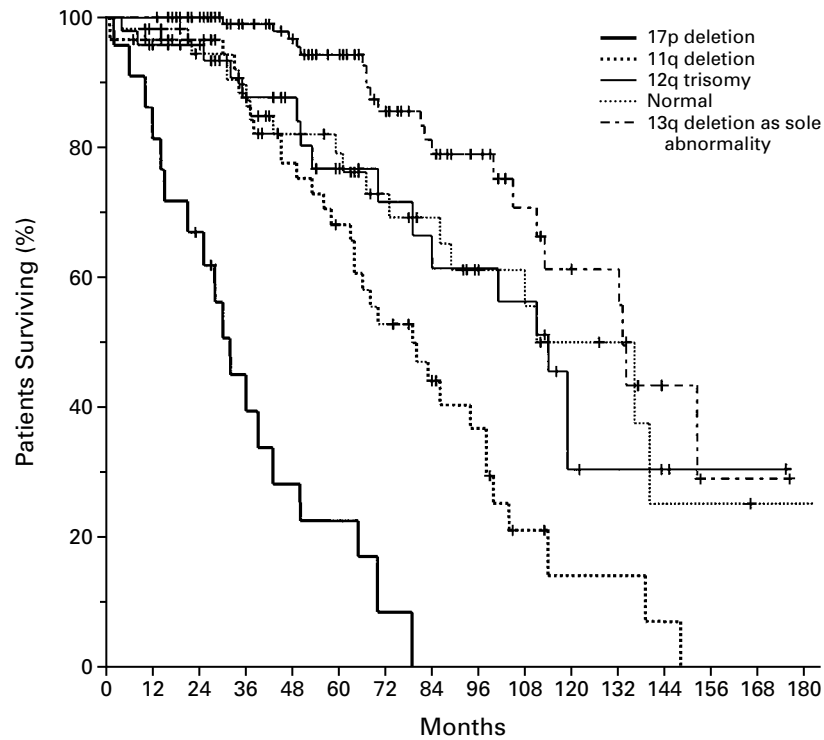
TABLE 3. HIERARCHICAL MODEL OF CHROMOSOMAL ABNORMALITIES IN CHRONIC LYMPHOCYTIC LEUKEMIA.*

KARYOTYPE†	NO. OF PATIENTS (%)
17p deletion	23 (7)
11q deletion	56 (17)
12q trisomy	47 (14)
Normal karyotype	57 (18)
13q deletion as sole abnormality	117 (36)
Various abnormalities	25 (8)

*The model was constructed on the basis of the regression analysis.

†The five major categories are defined as follows: patients with a 17p deletion; patients with an 11q deletion but not a 17p deletion; patients with 12q trisomy but not a 17p or 11q deletion; patients with a normal karyotype; and patients with a 13q deletion as the sole aberration. Twenty-five of the 325 patients with various chromosomal abnormalities could not be assigned to one of these five major categories.

tions had the highest proportion at Binet stage A (72 percent). The groups with 17p and 11q deletions were more likely to have splenomegaly, mediastinal lymphadenopathy, and abdominal lymphadenopathy and had more extensive peripheral lymphadenopathy. The extent of lymph-node involvement was particularly



No. AT RISK	0	12	24	36	48	60	72	84	96	108	120	132	144	156	168	180
17p deletion	23	18	13	8	5	4	1	0	0	0	0	0	0	0	0	0
11q deletion	56	53	47	43	33	27	20	15	10	4	2	2	1	0	0	0
12q trisomy	47	44	41	29	24	17	14	13	12	11	4	3	2	1	1	0
Normal	57	51	45	37	30	27	20	17	12	11	6	5	2	2	1	1
13q deletion as sole abnormality	117	117	106	91	80	63	45	36	24	16	12	11	3	1	1	0

Figure 1. Probability of Survival from the Date of Diagnosis among the Patients in the Five Genetic Categories. The median survival times for the groups with 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as the sole abnormality were 32, 79, 114, 111, and 133 months, respectively. Twenty-five patients with various other chromosomal abnormalities are not included in the analysis.

striking in the 11q-deletion group. Moreover, patients with 11q and 17p deletions were more likely than the others to have fever, night sweats, or weight loss (B symptoms) and had lower hemoglobin values and lower platelet counts; patients with 17p deletions had higher serum lactate dehydrogenase and alkaline phosphatase levels and lower serum albumin levels.

There were statistically significant differences in disease progression among the five genetic categories, as indicated by the treatment-free interval (Fig. 2). Patients in the groups with 17p and 11q deletions had more rapid disease progression: the median time from the date of diagnosis to the date of first treatment in these two groups was only 9 and 13 months, respectively, and eventually all these patients required therapy. The median treatment-free interval was longer

in the 12q-trisomy group (33 months) and the normal-karyotype group (49 months), and it was the longest by far in the 13q-deletion group (92 months). In the last group, nearly one third of the patients did not require therapy.

DISCUSSION

We found that molecular cytogenetic methods can detect genomic aberrations in over 80 percent of patients with chronic lymphocytic leukemia, or about twice as frequently as chromosome banding.⁵ The most frequent abnormality we found was a deletion involving chromosome band 13q14, which occurred in 55 percent of cases. This result is consistent with studies using microsatellite and quantitative Southern blot analysis.^{13,19-21} The second-most-frequent change

TABLE 4. COMPARISON OF CLINICAL AND LABORATORY DATA AMONG THE MAJOR CYTOGENETIC SUBGROUPS.*

VARIABLE	17p DELETION	11q DELETION	12q TRISOMY	NORMAL	13q DELETION	P VALUE
No. of patients	23	56	47	57	117	
Median age (yr)	63	58	63	61	63	0.11†
Male sex (%)	74	68	55	49	60	0.17‡
Disease stage at enrollment (%)						
Binet						<0.001‡
A	23	25	51	53	72	
B	41	50	34	30	21	
C	36	25	15	17	8	
Rai						<0.001‡
0	0	5	13	28	31	
1	5	20	11	11	15	
2	45	43	59	42	44	
3	23	16	13	5	5	
4	27	16	4	14	5	
White-cell count ($\times 10^{-3}/\text{mm}^3$)	48.3	43.9	32.2	30.9	27.7	0.27†
Hemoglobin (g/dl)	12.5	12.5	13.3	13.5	13.6	0.004†
Platelet count ($\times 10^{-3}/\text{mm}^3$)	148	159	192	174	191	0.004†
Lactate dehydrogenase (IU/liter)	247	188	204	169	155	<0.001†
Alkaline phosphatase (IU/liter)	152	130	124	124	115	0.004†
Albumin (g/liter)	43	45	44	45	46	0.024†
Splenomegaly (%)	86	71	66	61	53	0.004‡
Mediastinal lymphadenopathy (%)	23	28	5	4	1	<0.001‡
Abdominal lymphadenopathy (%)	64	83	53	49	23	<0.001‡
Peripheral lymphadenopathy (cm^2)§	12	17	3	3	0	<0.001†
Largest lymph-node diameter (cm)	3	5	2	2	0	<0.001†
B symptoms (%)¶	33	32	19	16	6	<0.001‡
Time from diagnosis to first treatment (mo)	9	13	33	49	92	<0.001

*Median values are given for quantitative variables. Because of rounding, percentages do not always total 100. Twenty-five patients with various other chromosomal abnormalities are not included in the analysis.

†The P value is for the overall comparison among the subgroups and was calculated by the Kruskal–Wallis test.

‡The P value is for the overall comparison among the subgroups and was calculated by Fisher's exact test.

§The values are the medians of the products of the diameters of the largest cervical, axillary, and inguinal lymph nodes in centimeters.

¶B symptoms consist of fever, night sweats, or weight loss.

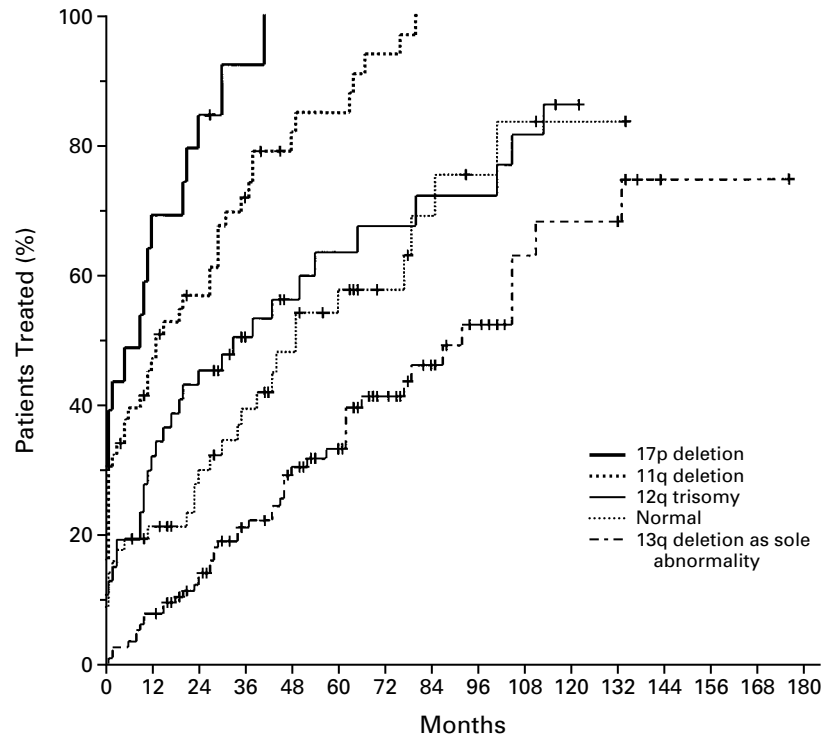
||The P value is for the overall comparison among the subgroups and was calculated by the log–rank test.

was a deletion in 11q (found in 18 percent of patients). Previous evidence from banding studies of chromosomal loss from 11q in chronic lymphocytic leukemia is inconsistent.^{5,22} Sixteen percent of our patients had 12q trisomy, which was long considered the most frequent chromosomal abnormality in chronic lymphocytic leukemia; in our study it was the third most frequent aberration.

Little is known about the molecular correlates of these chromosomal abnormalities. The tumor suppressor gene p53 is affected by 17p deletions.^{14,23} Recent studies suggest that the gene encoding the ataxia–telangiectasia mutated protein is altered in some cases of chronic lymphocytic leukemia with 11q deletion.^{24–26}

Band 13q14 probably contains a tumor-suppressor gene with a role in chronic lymphocytic leukemia.^{13,19–21} No disease-related genes have yet been associated with the other aberrations.

These aberrations are among the most important factors in predicting survival. Patients with 17p deletions had by far the worst prognosis, followed by patients with 11q deletions, those with 12q trisomy, and those with normal karyotypes, whereas patients with 13q deletions as the sole abnormality had the longest estimated survival times (Fig. 1). These observations parallel the more frequent finding of advanced disease at enrollment in patients with 17p or 11q deletions. In a smaller series of patients, extensive lym-



No. UNTREATED														
17p deletion	23	7	4	1	0	0	0	0	0	0	0	0	0	0
11q deletion	56	29	20	13	7	5	2	0	0	0	0	0	0	0
12q trisomy	47	32	26	18	12	9	7	6	6	4	1	0	0	0
Normal	57	42	33	24	17	13	8	5	3	2	1	1	0	0
13q deletion as sole abnormality	117	108	94	72	58	45	28	21	13	7	6	6	1	1

Figure 2. Probability of Disease Progression, as Indicated by the Treatment-free Interval in the Patients in the Five Genetic Categories.

The median treatment-free intervals for the groups with 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as the sole abnormality were 9, 13, 33, 49, and 92 months, respectively. The differences between the curves were significant ($P < 0.001$). Twenty-five patients with various other chromosomal abnormalities are not included in the analysis.

phadenopathy was particularly striking in patients with an 11q deletion.¹² In the multivariate analysis, both 17p deletion and 11q deletion provided statistically significant prognostic information, with 17p deletion being the strongest predictor of poor survival.

Most previous studies of chromosomal aberrations in chronic lymphocytic leukemia did not identify chromosomal abnormalities that provided independent prognostic information.⁶ The poor prognosis of patients with 17p deletion or p53 mutation has been reported in only a few studies.^{14,23,27} El Rouby et al. found that mutation of p53 was the strongest independent prognostic factor.²³ In a prospective study using chromosome banding, abnormality of chromosome 17 was associated with poor survival, and it

was the only cytogenetic finding with independent prognostic value.²⁷ Neilson et al. found that 11q deletions were associated with rapid disease progression and shorter survival times.²² The prognostic effect of 12q trisomy has been controversial^{5,6,28,29}; our data indicate that patients with 12q trisomy have shorter survival than those who have a 13q deletion as the sole aberration. The finding of a favorable outcome for patients with 13q deletions supports other data.⁶

Two recent studies further illuminate the biologic basis of the clinical variability of chronic lymphocytic leukemia.^{30,31} They indicate that chronic lymphocytic leukemia can arise at different stages of B-cell maturation, as indicated by the presence or absence of mutations of immunoglobulin variable genes: the latter

represents naive B cells before they enter the germinal center, and the former memory B cells that have passed through germinal centers. Patients with chronic lymphocytic leukemia originating from naive B cells had significantly shorter survival than patients with chronic lymphocytic leukemia arising from memory B cells. It will be necessary to assess the relative prognostic value of the currently used clinical, biochemical, and genetic markers in large, prospective trials. Our results with molecular cytogenetic techniques may already have implications for the risk-adapted clinical management of chronic lymphocytic leukemia, particularly in younger patients.

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