

## 10TH ANNIVERSARY ARTICLE

# Chromosome Aberrations in B-cell Chronic Lymphocytic Leukemia Pathogenetic and Clinical Implications

Gunnar Juliusson and Gösta Gahrton

**ABSTRACT:** Chromosome analyses were performed on leukemic cells from 102 patients with B-CLL, of whom 84 were untreated. B-cell mitogen-induced CLL cells yielded suitable metaphases in 85 patients, and 55 showed clonal chromosomal aberrations. Trisomy 12 was found in 26 patients. In nine patients the +12 was a single aberration. A 14q+ chromosome or deletions of the long arm of chromosomes 6, 11, or 13 were other recurrent aberrations. Patients with Rai stage I or more had more frequently clonal aberrations than patients with stage 0 disease ( $p < .02$ ). Patients with clonal aberrations had poorer 5-year survival than those with a normal karyotype ( $p < .05$ ). Patients with a high percentage of abnormal metaphases in the sample had poorer prognosis than patients with high admixture of normal metaphases ( $p < .01$ ). Of the specific clonal aberrations those with 14q+ or trisomy 12 tended to have slightly poorer and those with 6q- or structural aberrations involving the long arm of chromosome 13 tended to have better prognosis than patients with other chromosomal aberrations. A complex karyotype tended to be an adverse prognostic sign. Clonal evolution is rare: complex karyotypes are found at diagnosis and clones with single aberrations did not acquire additional chromosome aberrations despite progressive disease and treatment. Nine hundred and seventy-nine published cases are reviewed, and pathogenetic mechanisms, such as oncogenes and gene dosage, are discussed.

### INTRODUCTION

Specific chromosome aberrations have been found to give clinical, prognostic, and pathogenetic information in hematological malignancies [1]. The history started in 1960 with the finding of a consistent minute chromosome in chronic myelocytic leukemia (CML) [2], known as the Philadelphia chromosome, which following the advent of the chromosome banding [3] was identified to be a chromosome 22 with the long arm deleted at band q11 [4].

In chronic lymphocytic leukemia the initial chromosome studies yielded poor results [5, 6]. The reason for this was, firstly, the low mitotic index of CLL cells, and secondly, that the mitogen that was used, phytohemagglutinin [7], activates T-cells, whereas CLL in 97% of the cases is a B-cell disease. It is likely that the cells from CLL patients studied in the early and mid 1970's were normal T-cells [8, 9], with a higher rate of mitosis than the leukemia cells. This difficulty delayed chromosome studies in CLL compared with those in many other hematological malignancies. Then in 1978

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the polyclonal B-cell activating substances were found to induce mitosis in CLL cells [10], and this made it possible to perform chromosome analysis on relevant cells. Clonal chromosomal aberrations were found [11, 12], and it is noteworthy that the specific abnormalities that later were found to be among the most common in CLL, the trisomy 12 and the t(11;14), [13–15] were present also in these early cases.

During the 1980's the number of studied patients have reached a thousand. However, there are still many question marks. What is the nature of B-cell mitogen-activated CLL cells with normal chromosomes? What is the mechanism behind the occurrence of trisomy 12? Why is trisomy 12 associated with CLL? Is the pathogenesis of CLL purely a matter of gene dosage, and which genes are relevant in the malignant transformation? Are the chromosome aberrations markers for the evolution of the disease with increasing derangements of the genotype, or are they more like "phenotypic markers" of the individual leukemic clone? Are there correlations between the karyotype and the phenotype, or with stage and other clinical features? It is generally agreed that chromosome changes give prognostic information in CLL as well as in CML and in acute leukemias. Patients without clonal aberrations do better than patients in which chromosome changes are found [15–18]. However, the prognostic impact of the trisomy 12 is a matter of discussion [15–28].

Some of these problems have been elucidated by recent studies, and they will be discussed in the following. An updated review of the literature and of 102 patients studied by us will be presented.

## PATIENTS AND METHODS

### Patients

One hundred and two consecutive patients with B-CLL were studied. Sixty-eight patients were males and 34 were females, and their ages at diagnosis ranged from 35 to 89 years (median 67 years). In this report patients with prolymphocytic leukemia [29] and leukemic states of follicle center cell derived lymphomas [30] were excluded. Diagnosis was confirmed with immunological markers on blood lymphocytes, and lymph node biopsy was performed in almost every case with lymphadenopathy. Sampling for chromosome analysis was performed within 2 months from first elevated blood lymphocyte count in 30 patients, and within 1 year in 63 patients. Median time from first blood count with lymphocytosis to sampling for cytogenetic analysis was 7.4 months. Sixteen patients had received cytostatic treatment and two were splenectomized before sampling for cytogenetic analysis, whereas 84 patients were untreated. Patients were treated only if progressive disease with anaemia, thrombocytopenia, generalized lymphadenopathy, or clear B-symptoms were found. Initial treatment according to protocols of the Lymphoma Group of Central Sweden consisted of chlorambucil—prednisone, or doxorubicin, cyclophosphamide, vincristine and prednisone, or splenectomy. Patients in the different treatment arms have as yet similar survival. Survival analyses were performed by the Log rank test [31] from the date of diagnosis. Subgroup analyses were also performed excluding patients that were treated before cytogenetic analysis and patients older than 80 years at diagnosis, leaving 64 of 85 cytogenetically evaluable patients for analysis.

### Cells

Cells were obtained from heparinized blood and also from heparinized bone marrow aspirates, freshly minced lymph nodes, spleens, and pleural effusions. Lymphocytes were isolated, cultured, and activated by Epstein-Barr virus, lipopolysaccharide from

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## RESULTS

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**Table 1** Chromosome findings in 102 patients<sup>a</sup>

Karyotype	Total number	Percent of evaluable patients	Percent of abnormal karyotypes
All	102		
Evaluable	85		
Normal karyotype	30	35	
Clonal aberration	55	65	
Trisomy 12 <sup>a</sup>	27	32	49
Non-Trisomy 12	28	33	51
Single aberration	25	29	45
Trisomy 12	9	11	16
Non-Trisomy 12	16	19	29
Two aberrations	16	19	29
Trisomy 12 <sup>b</sup>	11	13	20
Non-Trisomy 12	5	6	9
≥Three aberrations	14	16	25
Trisomy 12	7	8	13
Non-Trisomy 12	7	8	13
11q (4 single)	9	11	16
6q (4 single)	7	8	13
14q+ (1 single)	7	8	13
13q (3 single)	5	6	9

<sup>a</sup> (PLL and leukemic follicle center cell derived lymphomas excluded).

<sup>b</sup> Including one patient with dup(12)

*E. coli*, tetradecanoyl-phorbol-acetate, and cytochalasin B as previously described [17]. The Q-banding technique was utilized for chromosome analysis [3]. A clonal chromosomal abnormality was defined by conventional criteria [32].

## RESULTS

### Chromosome Aberrations

Adequate numbers of metaphases evaluable for chromosomes were obtained in 85 patients, whereas in 17 patients we failed to induce metaphases despite, in most of the cases, repeated attempts with different modes of activation. Clonal chromosomal aberrations were found in cells from 55 patients. Thirty patients were considered cytogenetically normal, since no clonal chromosomal aberration was found in at least 10 evaluable metaphases. The cytogenetic pattern and correlation to clinical stage are shown in Tables 1 and 2. Extra chromosome 12 was found as the only aberration in nine patients and together with other aberrations in another 18 patients, including one patient with a dup(12) [33]. Translocations most commonly involved chromosome 14 with a breakpoint at q32 (i.e., the immunoglobulin heavy chain gene locus) resulting in 14q+ chromosomes. The donor chromosome for the additional chromosome fragment on 14q was number 11 in three patients, unidentified in one patient, and number 2, 7, and 12 in three patients, respectively. Of the three patients with a t(11;14), two had a breakpoint at 11q13, and one had a breakpoint at 11q15. Deleted chromosomes 11 were found in five patients, with breakpoints at 11q21 in two patients, 11q22 in two patients, and at 11q14 in one patient. Deletions on chromosome 6 were found in five patients, and translocations involving chromosome 6 in two

**Table 2** Chromosome findings according to Rai stage [66] at first karyotype study. In treated patients highest Rai stage during course of the disease prior to first karyotype study is indicated.

Rai stage	All patients n = 102 No.	All evaluable n = 85 No.	Normal karyotype n = 30 % of evaluable	Abnormal karyotype, non +12		Abnormal with +12	
				1 <sup>a</sup> n = 16 % of evaluable	≥2 <sup>a</sup> n = 12 % of evaluable	1 <sup>a</sup> n = 9 % of evaluable	≥2 <sup>a</sup> n = 18 % of evaluable
0	34	28	54%	14%	11%	7%	14%
I	37	31	32%	23%	10%	10%	26%
II	16	12	25%	25%	8%	25%	17%
III	4	4	0%	0%	25%	0%	75%
IV	11	10	20%	20%	40%	10%	10%

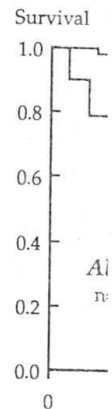
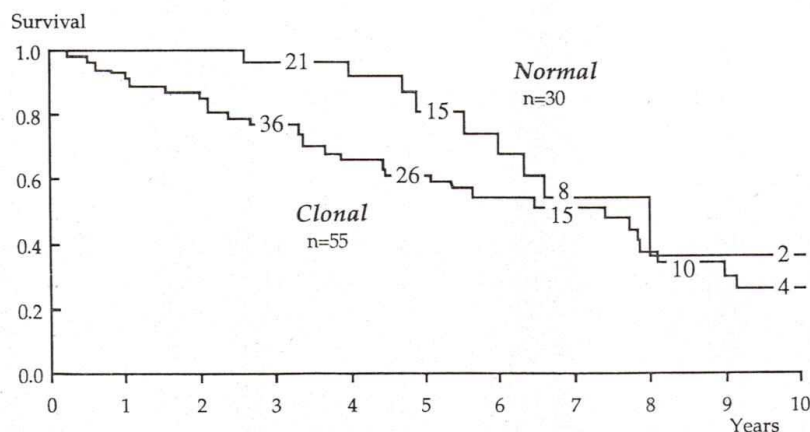
<sup>a</sup> Number of clonal aberrations: 1 = single aberration, ≥2 = two or more aberrations. Abnormal karyotypes were significantly more common in Rai stage I or more than in stage 0 ( $p < .02$ ).

patients. The breakpoints were 6q15 and 6q23 in two patients each, and 6p12, 6q21, and 6q24 in one patient each. Five patients had structural abnormalities of chromosome 13, involving band 13q13, 13q21, and band 13q34 in two patients each, and band 13q22 and 13q31 in two patients, respectively.

### Prognosis

The 5-year survival of patients with clonal chromosomal aberrations was poorer than that of patients with a normal karyotype ( $p < 0.05$ ), although the overall survival curves were not significantly different (Fig. 1). Patients with clonal abnormalities in

**Figure 1** Survival according to karyotype. Patients with clonal chromosomal aberrations versus patients without clonal aberrations in at least ten studied metaphases. Difference is significant at 5 years ( $p < .05$ ) but overall survival is not significantly different. Figures on curves indicate number of patients at risk.



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### DISCUSSION

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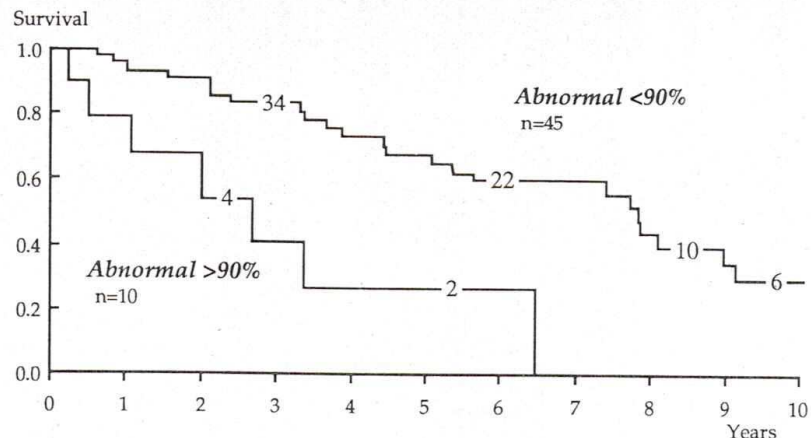
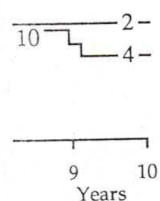
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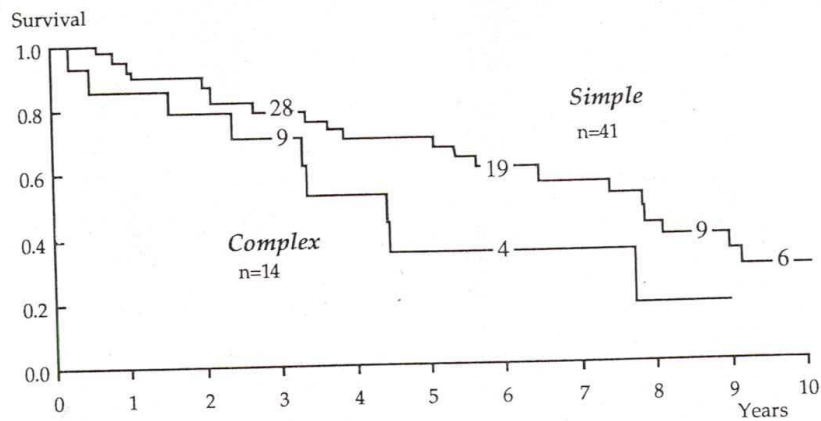
**Figure 2** Survival according to percentage of metaphases with clonal chromosomal abnormalities. Patients with more than 90% abnormal metaphases versus patients with clonal aberrations in less than 90% of studied metaphases. Difference is significant ( $p < .01$ ). Figures on curves indicate number of patients at risk.

all or nearly all metaphases had a poorer survival than patients with admixture of normal metaphases to the aberrant cells ( $p < 0.01$ , Fig. 2). The survival curves of patients with complex karyotypes (three or more aberrations within the clone) and those with non-complex abnormalities (one or two aberrations) diverged, but the difference was not significant (Fig. 3). Patients with 14q+ marker chromosomes or with trisomy 12 tended to have a slightly poorer prognosis than patients with other types of chromosomal aberrations (Fig. 4). When comparing only patients with single abnormalities, patients with trisomy 12 tended to have poorer survival than patients with single aberrations other than +12 (Fig. 5). Patients with deletions of the long arm of chromosome 6 or structural aberrations involving the long arm of chromosome 13 tended to have a better prognosis than patients with other types of clonal aberrations (Fig. 6). Patients with 11q- were also analysed separately, and their survival curve overlaid that of all patients with clonal aberrations. The survival curves of specific subgroups were similar when only untreated patients diagnosed before the age of 80 years were analysed as compared to those including all patients.

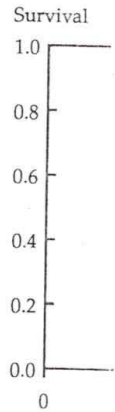
## DISCUSSION

### Incidence of Trisomy 12

Trisomy 12 is found in more than one third of CLL patients with clonal chromosomal aberrations (Table 3). However, it is also found in other B-cell malignancies, such as lymphocytic lymphoma [34], hairy cell leukemia [35], and prolymphocytic leukemia [36, 37], but only rarely in non-B cell malignancies. Recent studies on CLL-cells using the restriction fragment length polymorphism (RFLP) of genes located on the long arm of chromosome 12 show that the trisomy consists of a duplication of one of the chromosomes 12 [38-40]. In none of our studied patients with +12 did we find a

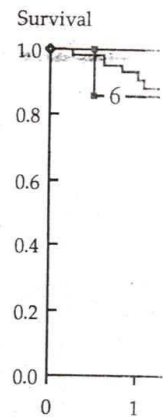
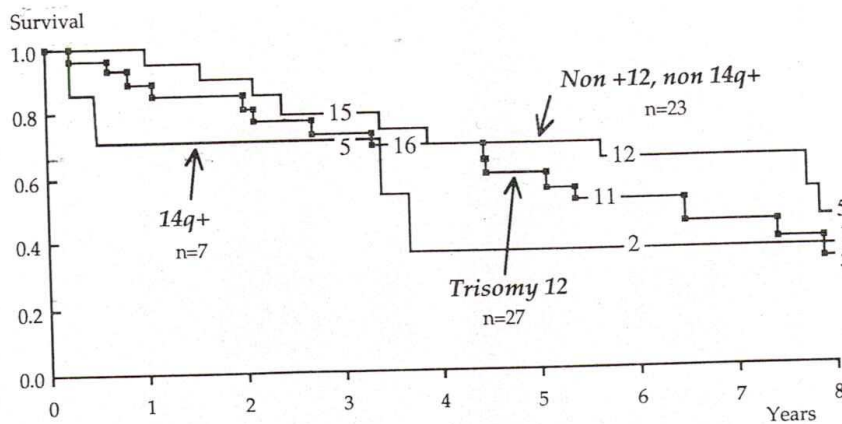


**Figure 3** Survival according to karyotype. Patients with one or two specific aberrations versus patients with three or more abnormalities. Difference not significant. Figures on curves indicate number of patients at risk.

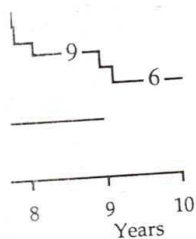


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**Figure 4** Survival according to karyotype. Patients with trisomy 12 with or without other aberrations including one patient with dup(12) and patients with 14q+ chromosomes versus patients with clonal abnormalities except trisomy 12 and 14q+. Two patients are included in both trisomy 12 and 14q+ groups. Differences are not significant. Figures on curves indicate number of patients at risk.

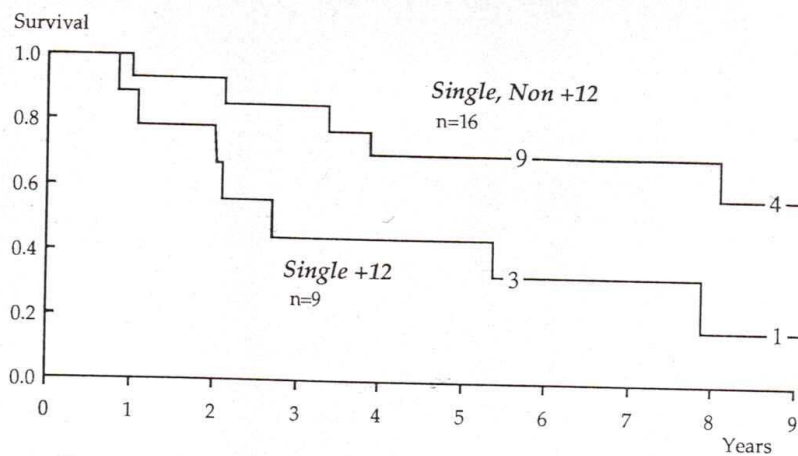
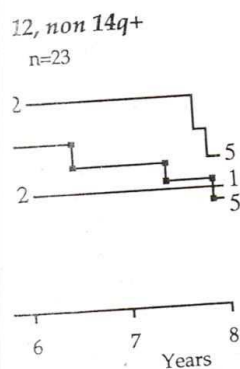


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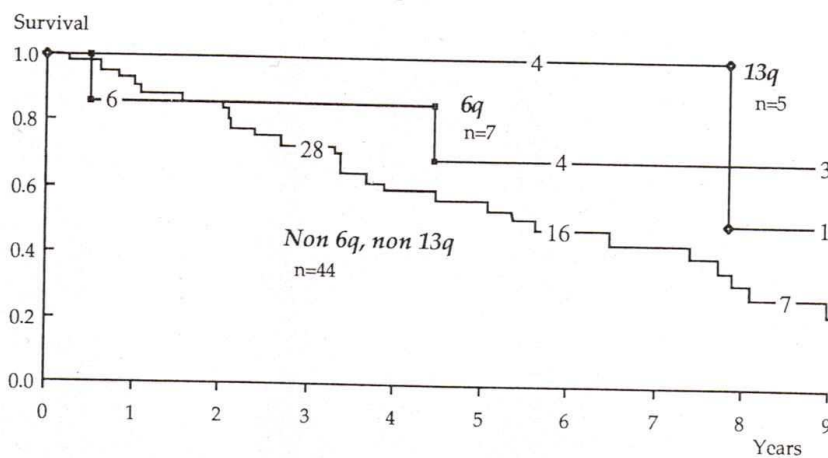
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**Figure 5** Survival according to karyotype. Patients with trisomy 12 as the sole abnormality versus patients with single abnormalities other than trisomy 12. Difference not significant. Figures on curves indicate number of patients at risk.

**Figure 6** Survival according to karyotype. Patients with deletion of 6q, and patients with structural aberrations involving 13q versus patients with clonal abnormalities excluding 6q- and 13q- aberrations. One patient is in both 6q- and 13q- groups. Differences are not significant. Figures on curves indicate number of patients at risk.





**Table 3** Published chromosome data: Number of patients with involved chromosomal abnormality (% of all) (% of abnormal)

Source [Reference]	All	Abnormal	+12	11q	13q	14q+
IWCCLL [15]	427	214 (50)	67 (31)	37 (17)	50 (23)	40 (19)
Bournemouth <sup>a</sup> [26]	141	75 (53)	22 (29)	—	24 (32)	—
Huddinge <sup>a</sup> [Table 1]	102	55 (54)	27 (49)	9 (16)	5 (18)	7 (13)
Edinburgh <sup>a</sup> [28]	138	54 (39)	15 (28)	10 (19)	14 (26)	—
Buffalo [27]	98	39 (40)	24 (62)	—	—	—
London [20]	63	33 (52)	7 (21)	4 (12)	11 (33)	17 (52)
Copenhagen [77]	110	29 (26)	8 (28)	—	—	—
Montpellier [85]	103	22 (21)	6 (27)	4 (18)	1 (5)	4 (18)
Stockholm [83]	24	16 (67)	1 (6)	5 (31)	3 (19)	0
Chicago [46]	38	15 (39)	7 (47)	4 (27)	1 (7)	4 (27)
Ferrara <sup>a</sup> [86]	23	15 (65)	10 (67)	—	—	0
Philadelphia [87]	40	14 (35)	4 (29)	—	—	5 (36)
Helsinki <sup>a</sup> [12]	14	8 (57)	3	2	1	0
Christchurch [88]	8	7 (88)	4	0	0	1
Helsinki [89]	66	5 (8)	2	1	0	1
Woodville [90]	6	5 (83)	2	2	2	0
Rochester [91]	5	2 (40)	0	0	0	0
Total	979	394 (40)	142 (36)	41 (10)	62 (16)	39 (10)

<sup>a</sup> IWCCLL, International Working Party on Chromosomes in CLL [15]. First report contains patients from Bournemouth, Edinburgh, Huddinge, Ferrara and Helsinki. IWCCLL excluded in "Total" since most patients are reported from the individual institution. Figures in "Total" might be too low because of missing data.

triplication of one chromosome 12 with the loss of the other [40]. This contrasts to a murine leukemia cell line with trisomy 15, in which triplication of one chromosome with loss of the homologue was found [41]. Furthermore, in none of our CLL patients with an apparent diploid karyotype we found a duplication of one chromosome 12 with or without loss of the other [40]. These data indicate that trisomy 12 occurs through a mitosis with nondisjunction of one of the chromosome 12 alleles, and that the incidence of trisomy 12 as found by conventional cytogenetic studies is correct, i.e., one of three patients with abnormal karyotype, or one of six to eight patients have trisomy 12.

#### Pathogenetic Mechanisms of Trisomy 12

Malignant transformation may occur through an alteration of a specific gene or by an abnormal gene dosage. The high incidence of +12 in B-CLL implicates some role of this abnormality in the pathogenesis of the disease. Why then trisomy 12, and is the duplicated chromosome normal? If so, one explanation would be that B cells randomly acquire an extra chromosome 12 copy, and the increased amount of genes localized on chromosome 12 renders the malignant characteristics to the affected B cell. Another cause might be that the malignant transformation hits and alters a specific gene on chromosome 12 that induces or facilitates a nonrandom nondisjunction leading to trisomy 12. A third possible explanation is a genetic change anywhere in the genome giving rise to CLL cells, which subsequently becomes prone to nondisjunctive mitoses with trisomy 12 occurring within a subclone.

All possibilities suggest a responsible specific gene product, produced either by the increased gene dosage alone or by gene alteration. However, there are no valid suggestions of a specific CLL gene. The *bcl-1* [42] and *bcl-2* [43, 44] genes have been identified through their localization at sites of specific recurrent chromosome

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translocations to the immunoglobulin heavy chain genes on chromosome 14. Structural aberrations involving chromosome 12 are not common and not very consistent in CLL. One of our patients had a duplication of 12(q13→q22), i.e., a triplication of the genes in this segment [33], and Ross (personal communication) found a patient with dup(12)(qter→p13::q11→qter). Another patient of ours had trisomy 12 with a subclone having a deletion of the third chromosome 12 at q22 [17]. A fourth patient had initially two clones, one with trisomy 12 and monosomy 17, and one with two normal chromosomes 12, one chromosome 17, and a marker chromosome consisting of 12q material distal to q13 translocated to a chromosome 17 deleted at p13. This patient thus had a trisomy of chromosome 12 genes from q13 to qter [45]. This specific t(12;17)(q13;p13) marker was also found in a single CLL cell by Ross (personal communication). Furthermore, two similar clonal markers, i.e., a + del(12)(q22) and a t(12;17)(q21;q11) were recently reported by Bird and coworkers [46]. Also, Mecucci and coworkers observed a patient with a duplication of a marker chromosome consisting of the short arm of chromosome 11 and the long arm of chromosome 12 [47]. Together, these karyotypes may indicate that the most important genes are located on the long arm of chromosome 12 at band q13 to q22, and that the centromeric region of chromosome 17 also may be of interest. Of defined oncogenes, two are located on chromosome 12, i.e., *k-ras-2* [48] and *int-1* [49]. However, no data have accumulated supporting a role for these oncogenes in CLL [50, 51].

The third explanation might be supported by the finding of normal metaphases in most cell samples containing CLL cells with trisomy 12. However, our RFLP data show interestingly that polymorphic genes on 12q have a clear 2:1 ratio in all samples containing some metaphases with trisomy 12 [40], indicating that the frequency of mitotic cells with normal karyotype are not equally represented in the nondividing cell population, but showing that almost all cells in such samples have trisomy 12. This supports the view of Knuutila and coworkers, who found that cells with a normal karyotype among cells with clonal aberrations are contaminating normal T cells [8, 9]. Furthermore, if explanation three was true one would expect to commonly find the development of trisomy 12 during the course of the disease. However, trisomy 12 is frequently found in very early disease [17, 45], and rarely superimposing during disease progression [45, 52].

Thus, it seems likely that the important transforming event induces changes by quality or quantity of the product of a specific gene localized on chromosome 12. However, since more than half of the patients never show changes of chromosome 12 there ought to be additional pathogenetic mechanisms, eventually using parts of the previously discussed transforming pathway. Alternatively, the trisomy 12 aberration might be a common but not obligatory result of a transforming genetic change that by itself is not possible to detect by conventional cytogenetics.

### Oncogenes

Two oncogenes, designated *bcl-1* and *bcl-2* from B-cell leukemia/lymphoma, are found to be involved in rare cases of CLL. The specific chromosomal localizations are 11q13 [52] and 18q21 [43, 44], respectively, and the recurrent chromosomal abnormalities are translocations of the oncogenes to the immunoglobulin heavy chain gene on 14q32, forming t(11;14) and t(14;18), respectively.

*Bcl-1* was cloned from CLL cells [42] but the t(11;14) is more commonly seen in lymphocytic lymphomas [34] and prolymphocytic leukemia [36, 45, 53]. In the compiled European data on 427 patients, the t(11;14) was found in 11 of 214 patients with clonal chromosomal aberrations [15]. Molecular analyses on 3 patients from Huddinge showed the classic localization of the *bcl-1* gene within the major translocation cluster (MTC) [42] in one CLL patient prior to PLL transformation, whereas

the *bcl-1* was localized 63 kb telomeric of the MTC in one patient with primary prolymphocytic leukemia [54], and the *bcl-1* gene was not detected with any of these two probes in the third patient with stage 0 CLL. No *bcl-1* involvement was found in 38 patients studied by Rechavi and coworkers [55].

*Bcl-2* is involved in most patients with follicular lymphoma [34], but also in diffuse B cell lymphomas [56]. The *bcl-2* gene products are p22-p26 proteins [57, 58] that are present in low amount in nonmalignant lymphoid tissue and abundant in t(14;18)-carrying lymphomas and cell lines [59]. The proteins have shown to be oncogenic in gene transfer assays [58]. *Bcl-2* involvement is rare in CLL; among our patients, we have found three cases of 50 by Southern blotting [60], whereas Rechavi did not find any CLL-cell clone with *bcl-2* involvement in 38 studied cases [55].

A putative *bcl-3* oncogene is also suggested from three CLL-patients with a t(14;19)(q32;q13) [62], and the gene at 19q13 has been cloned [62], but its role remains to be established.

#### Chromosomal Changes During Progressive and Indolent Disease

We and others have previously suggested from incidence and survival analyses that trisomy 12 is the primary genetic change in CLL [13], whereas other aberrations are secondary [16]. However, clonal evolution is rare in CLL [40, 45, 52]. We have analysed most patients close to diagnosis and before any treatment, and we performed sequential analysis on 41 patients with different karyotypes in indolent and in progressive states [63]. In most cases the karyotype was unchanged [45], with some patients having complex karyotypes at diagnosis and others continuously showing normal karyotype or single aberrations during long-term observation. No CLL cell clone with a single trisomy 12 aberration developed additional changes. The same finding was made by Nowell and coworkers [52]. Thus, the complexity of the karyotypic aberrations are mostly established early in the clinical disease, and disease progression is usually not accompanied by further derangement of the karyotype. This is thus in contrast to the case in CML [64] and follicular lymphoma [65, 66], and it makes it possible to use chromosomes for prognosis prediction in CLL at any time during the course of the disease.

#### Chromosomes and Clinical Stage

We and others have found that Rai [67] stage 0 patients less commonly have clonal abnormalities than patients with more advanced stages (Table 2,  $p < .02$ ). Single abnormalities are common in stage I-II, whereas complex karyotypes seem to be more common in stage III and IV (Table 2) [18, 68]. However, the proportion between +12 and non +12 karyotypes do not differ between the Rai stages.

In the studies from Buffalo [69, 70] and Chicago [46], the incidence of chromosomal abnormalities differed between untreated and treated patients. However, our longitudinal studies [45] discussed above, show that the karyotype does not change during therapy. With a random time for cytogenetic sampling and a greater likelihood of a progressive disease in patients with clonal abnormalities, patients with clonal aberrations would have a greater risk of having developed a therapy-demanding disease before sampling than patients with a normal karyotype. Thus, the karyotypic changes are not caused by treatment [45], but the individual karyotype marks the progression rate of the tumor [17].

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### Chromosomes and Phenotype

Surface membrane immunoglobulin phenotype and other cell surface markers, such as CD5, CD19, CD20 were not associated to the chromosome results. Likewise, there was no correlation of the chromosomes to the lymphocyte count or the percentage of T cells in the peripheral blood.

### Chromosomes, Prolymphocytic Leukemia and Monoclonal Proteins

Chromosome aberrations are more frequently found in prolymphocytic leukemia [27, 36, 37, 45], a disease with activated tumor cells [71], and in CLL transforming into PLL [27, 45, 46] than in CLL 'proper'. In Pittman's PLL-study, 14q+ chromosomes were the most frequent abnormalities [36], whereas Sadamori et al. found a specific translocation, t(6;12)(q15;p13), in all of 5 PLL cases [37]. This translocation has not been reported in other PLL studies. We have found clonal aberrations in 3 of 3 primary PLLs and in 3 of 3 PLL transformations of CLL. Two PLL cases had +del(3)(p13) together with other aberrations [72]. One patient with PLL and one CLL/PLL had t(11;14), one CLL/PLL had 6q- and one had +12 [45].

Furthermore, clonal chromosomal aberrations are reported to be more frequent in CLL with monoclonal serum protein bands [73]. We also found that all our seven patients with M components had clonal abnormalities, with about the same distribution of trisomy 12 and of single and complex abnormalities as the total material. Immunoglobulin-secreting cells have a distinct lymphoplasmacytic appearance, and we have subdivided our CLLs by their lymph node and bone marrow cell morphology into CLL "proper" and immunocytomas (IC) according to the Kiel classification [30]. CLL cells have a homogeneous lymphocytic appearance, whereas ICs have cells with a lymphoplasmacytoid differentiation within the malignant clone, which however is dominated by the small lymphocytes. Lymph nodes from patients with M components mostly had IC morphology, but only a small proportion of the ICs had M proteins detectable with conventional electrophoresis.

Clonal chromosomal aberrations were slightly more common in the IC group (62%) than in the CLL 'proper' group (48%) (difference not significant). One explanation might be that immunoglobulin-secreting CLL cells, due to their higher metabolic activity are more easily activated by mitogens in vitro. In fact, the mitogen-induced thymidine uptake of the malignant cells in in-vitro cultures is higher in PLL and IC than in CLL patients [25, 74]. If a greater success rate in activating and karyotyping PLL and IC cells would be the cause of the higher incidence of chromosomal aberrations, one might expect to find unrevealed karyotypic abnormalities in CLL with 'normal' karyotype. However, our RFLP studies showed no trisomy 12 in CLL cells from patients that did not yield evaluable metaphases or from those who had what we designated a "normal" karyotype [40]. Thus, there might be a true correlation between gross cytogenetic abnormalities and the immunoglobulin secretion of the CLL cells. Since the pattern of the specific chromosomal aberrations is similar in IC and CLL "proper" it seems not likely that any specific aberration is closely associated to the immunoglobulin secreting phenotype.

### Chromosomes and Prognosis—Abnormal versus Normal Karyotype

Patients with clonal aberrations have been found to have poor prognosis compared to patients with a normal karyotype [15-18]. There is an early need for therapy [17, 19, 24], and, in the study of Han et al., the survival was significantly shorter [16, 27]. In our study, the overall survival curves were not significantly different, but at 5 years the survival was significantly poorer for patients with clonal aberrations ( $p < .05$ ). In

this context it is again relevant to discuss the nature of the CLL cells with "normal" karyotype. Such a finding in chromosome analyses might frequently be the result of a low proliferation rate of the malignant cells compared to that of the normal lymphoid cells [8], and low-proliferative CLL cells are associated with good prognosis [24, 74-76].

#### Chromosomes and Prognosis—Single versus Multiple Aberrations

We have shown that the therapy-free survival is very significantly shorter in patient groups with increasing number of chromosomal aberrations within the leukemic clone [17, 24]. Also, we demonstrated that the survival curves of patients with single aberrations and complex karyotypes are separated [17, 74], and this has also been shown in studies from other groups [16, 77]. However, in studies of about 100 patients, the difference in survival has not been significant. The constant appearance of the curves in different studies although makes it likely that the poorer prognosis of complex karyotypes compared to single aberrations is true, and might be significant in larger studies, such as in future analyses of the IWCCLL [15]. This finding also corresponds well with recent studies of chromosomes and prognosis in lymphomas [78].

#### Chromosomes and Prognosis—Percentage Abnormal Metaphases

Most samples for cytogenetic analysis, with or without clonal abnormalities, contains normal metaphases. We first showed a significant association between high percentage of clonal aberrations and poor survival [24, 79], statistically analysed with Log rank test [31], as well as Cox multivariate analysis [80]. This finding has also been confirmed by Han et al. [81], and in the present study (Fig. 2), and is concordant with previous findings in acute leukemia [82]. However, the interpretation is not quite obvious. Our RFLP data [40] discussed above indicate that the normal metaphases are not representative for the cell sample since almost all cells in a sample with a trisomy 12 clone contain trisomy 12 irrespective of a low percentage of abnormal cells with the cytogenetic technique. Thus, we believe that the finding of normal metaphases mixed with the malignant clone indicates a mitotic activity of the residual nonmalignant cells, which is of benefit for the patient.

#### Chromosomes and Prognosis—Trisomy 12

Trisomy 12 is the most common aberration in CLL, and it is therefore the abnormality that has been the most suitable one for prognostic analyses. It was soon found by our group [19] that patients with trisomy 12 deteriorated faster and had an earlier need for treatment than patients with normal karyotype. The same observation was made by Sadamori et al. [70], and it was strongly confirmed by us with different statistical methods [21, 24, 25, 74]. With respect to survival there has been no statistical difference between all patients with trisomy 12 and patients with other abnormalities [16, 17, 20, 22, 26, 28, 77]. However, when low-risk patients (Rai stage 0 through II) were analysed separately, patients with trisomy 12 had a significantly poorer survival than patients with other aberrations [23]. Because of our finding of a prognostic implication of the complexity of the karyotype [17, 21], we also compared patients with trisomy 12 as the sole abnormality versus single abnormalities other than trisomy 12, showing a close to significant difference in survival (Figure 5) [18]. Such analyses have not been presented by other groups, probably because of small numbers of patients. The largest study available today is the IWCCLL compilation of 427 patients, and a

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#### Chromosomes and Prognosis—14q+ Chromosomes

Pittman and Catovsky observed a poorer survival for patients with 14q+ marker chromosomes [20]. We have analysed our patients in this respect, and the result supports this finding, although the number of patients with 14q+ chromosomes is very small (Fig. 4). The 14q+ group comprises tumors with oncogene-involvements, such as the *bcl-1* in the t(11;14) commonly found in PLL, the *bcl-2* in follicle center cell-derived lymphomas, and the *c-myc* in the (8;14)-translocation of Burkitt-like lymphomas and leukemias. CLL with such clonal aberrations might have achieved some of the distinctive character from these mostly aggressive diseases.

#### Chromosomes and Prognosis—Structural Aberrations on 13q and 6q

In the studies from Bournemouth [26], Edinburgh [28], and Stockholm [83] it was found that structural aberrations involving the long arm of chromosome 13 was, next to trisomy 12, the most common chromosomal abnormality. In the IWCCLL study [15] it was found that this group of abnormalities was associated with a better prognosis than other clonal aberrations. Figure 6 shows survival curves of patients from Hüd- dinge with 13q aberrations, and although the number of patients is very small the shape of the curve might be indicative of a better survival of patients with 13q aberrations than of patients with other chromosomal abnormalities.

Aberrations involving 13q, like trisomy 12, frequently occur as a single aberration, possibly indicating a greater pathogenetic role than aberrations that mostly are accompanied by other aberrations. The pathogenetic discussion of 13q aberrations is highly interesting, since they frequently result in the loss of an antioncogene, the retinoblastoma gene at 13q14 [84]. Future studies will tell if a deficiency of the retinoblastoma protein is involved in the pathogenesis of CLL.

Deletions of the long arm of chromosome 6 is another recurrent finding in CLL cells, and the survival of these patients also seemed to be superior to that of patients with other aberrations (Fig. 6). There is no obvious interpretation of this finding, which must be confirmed in other studies.

These data might predict that in CLL, like in acute leukemia [1], different karyotypic abnormalities will be found to be associated with different clinical behavior, eventually requiring different management of the disease.

#### DISCUSSION

Repeated analyses of patients from a number of different institutions seem to confirm the findings of clinical implications of the karyotype in CLL. Chromosomal aberrations are associated with poor prognosis compared to normal karyotype, and complex karyotypes and high percentage of abnormal metaphases are adverse prognostic factors. Trisomy 12 is the most common abnormality, and it is associated with an earlier need for treatment, and possibly poorer survival, as may be the case also with the 14q+ marker chromosome. On the other hand, structural aberration involving 13q, and possible 6q- chromosomes, might be associated with a better prognosis than other clonal aberrations. Chromosome analyses in CLL are laborious, even more than in other tumors since they require mitogenic activation and tumor cell culture. Thus only limited progress in the present knowledge is likely to be achieved from single institutions. Further studies with compiled data are necessary, and they might result in the definition of karyotypic subgroups with distinct clinical implications. Further

studies are also needed in the search for specific genes involved in the malignant transformation elucidating the pathogenetic mechanisms of CLL.

## REFERENCES

1. Yunis JJ, Brunning RD, Howe RB, Lobell M (1984): High-resolution chromosomes as an independent prognostic indicator in adult acute nonlymphocytic leukemia. *N Engl J Med* 311:812-818.
2. Nowell PC, Hungerford DA (1960): A minute chromosome in human chronic granulocytic leukemia. *Science* 132:1497 (letter).
3. Caspersson T, Lomakka G, Zech L (1971): The 24 fluorescence patterns of the human metaphase chromosomes—distinguishing characters and variability. *Hereditas* 67:89-102.
4. Caspersson T, Gahrton G, Lindsten J, Zech L (1970): Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis. *Exp Cell Res* 63:238-249.
5. Fitzgerald PH, Adams A (1965): Chromosome studies in chronic lymphocytic leukemia and lymphosarcoma. *J Natl Cancer Inst* 34:827-839.
6. Crossen PE (1975): Giemsa banding patterns in chronic lymphocytic leukaemia. *Humangenetik* 27:151-156.
7. Nowell P, Daniele R, Rowlands Jr D, Finan J (1980): Cytogenetics of chronic B-cell and T-cell leukemia. *Cancer Genet Cytogenet* 1:273-280.
8. Knuutila S, Elonen E, Teerenhovi L, Rossi L, Leskinen R, Bloomfield CD, de la Chapelle A (1986): Trisomy 12 in B cells of patients with B-cell chronic lymphocytic leukemia. *N Engl J Med* 314:865-869.
9. Autio K, Elonen E, Teerenhovi L, Knuutila S (1987): Cytogenetic and immunologic characterization of mitotic cells in chronic lymphocytic leukaemia. *Eur J Haematol* 39:289-298.
10. Robèrt K-H, Möller E, Gahrton G, Eriksson H, Nilsson B (1978): B-cell activation of peripheral blood lymphocytes from patients with chronic lymphatic leukemia. *Clin Exp Immunol* 33:302-308.
11. Gahrton G, Zech L, Robèrt K-H, Bird AG (1979): Mitogenic stimulation of leukemic cells by Epstein-Barr virus. *N Engl J Med* 301:438 (letter).
12. Autio K, Turunen O, Penttilä O, Erämaa E, de la Chapelle A, Schröder J (1979): Human chronic lymphocytic leukemia: karyotypes in different lymphocyte populations. *Cancer Genet Cytogenet* 1:147-155.
13. Gahrton G, Robèrt K-H, Friberg K, Zech L, Bird AG (1980): Nonrandom chromosomal aberrations in chronic lymphocytic leukemia revealed by polyclonal B-cell-mitogen stimulation. *Blood* 56:640-647.
14. Morita M, Minowada J, Sandberg AA (1981): Chromosomes and causation of human cancer and leukemia. XLV. Chromosome patterns in stimulated lymphocytes of chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 3:293-306.
15. Juliusson G, Oscier DG, Ross FM, Castoldi GL, Elonen E, Knuutila S, Gahrton G (1989): International workshop on chromosomes in chronic lymphocytic leukemia: First compilation of cytogenetic data on 427 patients from Scandinavia, United Kingdom and Italy. *International Society of Hematology*. Jerusalem 3-8 September 1989 (abstract).
16. Han T, Ozer H, Sadamori N, et al. (1984): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 310:288-292.
17. Juliusson G, Robèrt K-H, Öst Å, Friberg K, Biberfeld P, Nilsson B, Zech L, Gahrton G (1985): Prognostic information from cytogenetic analysis in chronic B-lymphocytic leukemia and leukemic immunocytoma. *Blood* 65:134-141.
18. Gahrton G, Juliusson G (1988): Clinical implication of chromosomal aberrations in chronic B-lymphocytic leukaemia cells. *Nouv Rev Fr Hematol* 30:389-392.
19. Robèrt K-H, Gahrton G, Friberg K, Zech L, Nilsson B (1982): Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. *Scand J Haematol* 28:163-168.
20. Pittman S, Catovsky D (1984): Prognostic significance of chromosome abnormalities in chronic lymphocytic leukemia. *Br J Haematol* 58:649-660.
21. Juliusson G (1989): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 320:151-156.
22. Han T, Ozer H, Sadamori N, et al. (1984): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 310:288-292.
23. Juliusson G (1989): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 320:151-156.
24. Juliusson G (1989): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 320:151-156.
25. Juliusson G (1989): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 320:151-156.
26. Oscier DG, Ross FM, Castoldi GL, Elonen E, Knuutila S, Gahrton G (1989): International workshop on chromosomes in chronic lymphocytic leukemia: First compilation of cytogenetic data on 427 patients from Scandinavia, United Kingdom and Italy. *International Society of Hematology*. Jerusalem 3-8 September 1989 (abstract).
27. Han T, Ozer H, Sadamori N, et al. (1984): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 310:288-292.
28. Ross FM, Castoldi GL, Elonen E, Knuutila S, Gahrton G (1989): International workshop on chromosomes in chronic lymphocytic leukemia: First compilation of cytogenetic data on 427 patients from Scandinavia, United Kingdom and Italy. *International Society of Hematology*. Jerusalem 3-8 September 1989 (abstract).
29. Gahrton G, Robèrt K-H, Friberg K, Zech L, Nilsson B (1982): Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. *Scand J Haematol* 28:163-168.
30. Lennquist G, Friberg K, Zech L, Nilsson B (1982): Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. *Scand J Haematol* 28:163-168.
31. Peto J, Peto R (1972): The analysis of failure time data. *Biometrika* 59:59-72.
32. ISCN (1978): International system for human cytogenetic nomenclature. *Geneva*.
33. Gahrton G, Robèrt K-H, Friberg K, Zech L, Nilsson B (1982): Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. *Scand J Haematol* 28:163-168.
34. Yunis JJ, Brunning RD, Howe RB, Lobell M (1984): High-resolution chromosomes as an independent prognostic indicator in adult acute nonlymphocytic leukemia. *N Engl J Med* 311:812-818.
35. Golomb D, Ozer H, Sadamori N, et al. (1984): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 310:288-292.
36. Pittman S, Catovsky D (1984): Prognostic significance of chromosome abnormalities in chronic lymphocytic leukemia. *Br J Haematol* 58:649-660.
37. Sadamori N, Ozer H, Golomb D, et al. (1984): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 310:288-292.
38. Wang J, Ozer H, Sadamori N, et al. (1984): Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 310:288-292.
39. Crossen PE (1975): Giemsa banding patterns in chronic lymphocytic leukaemia. *Humangenetik* 27:151-156.
40. Einhorn B, Zech L, Gahrton G (1982): Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. *Scand J Haematol* 28:163-168.
41. Wirsén B, Zech L, Gahrton G (1982): Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. *Scand J Haematol* 28:163-168.
42. Tsujimoto T, Zech L, Gahrton G (1982): Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. *Scand J Haematol* 28:163-168.



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21. Juliusson G, Robert K-H, Gahrton G (1984): Cytogenetic abnormalities in chronic lymphocytic leukemia. *N Engl J Med* 311:423 (letter).
22. Han T, Emrich LJ, Ozer H, Sandberg AA (1985): Prognostic implication of trisomy 12 and non-trisomy 12 karyotypes in B cell chronic lymphocytic leukemia. *Blood* 66:469-470 (letter).
23. Juliusson G, Friberg K, Gahrton G (1985): Prognostic implication of trisomy 12 and non-trisomy 12 karyotypes in B cell chronic lymphocytic leukemia. *Blood* 66:470-472 (letter).
24. Juliusson G (1986): Immunological and cytogenetic studies improve prognosis prediction in chronic B-lymphocytic leukemia: a multivariate analysis of 24 variables. *Cancer* 58:688-692.
25. Juliusson G, Gahrton G (1987): CLL-cell proliferation in vitro. Clinical implications. In: *Chronic Lymphocytic Leukemia: Recent Progress, Future Directions*. UCLA Symposia on Molecular and Cellular Biology, New Series, Volume 59, RP Gale, K Rai, eds. Alan R Liss Inc., New York, pp. 93-102.
26. Oscier DG, Fitchett M, Hamblin TJ (1988): Chromosomal abnormalities in B-CLL. *Nouv Rev Fr Hematol* 30:397-398.
27. Han T, Sadamori N, Block AMW, Xiao H, Henderson ES, Emrich L, Sandberg AA (1988): Cytogenetic studies in chronic lymphocytic leukemia, prolymphocytic leukemia and hairy cell leukemia: a progress report. *Nouv Rev Fr Hematol* 30:393-395.
28. Ross FM, Brown AG, Weir-Thomson EM, Stockdill G, Parker AC, Prescott RJ, Mackie MJ (1988): The influence of chromosome abnormalities on the survival of patients with chronic lymphocytic leukaemia (CLL). *Blood* 72(Suppl 1): 255a (abstract).
29. Galton DAG, Goldman JM, Wiltshaw E, Catovsky D, Henry K, Goldenberg GJ (1974): Prolymphocytic leukaemia. *Br J Haematol* 27:7-23.
30. Lennert K (1978): Malignant lymphomas other than Hodgkin's disease. *Handbuch der speziellen pathologischen Anatomie und Histologie*. 1 Band, Part 3B. Springer Verlag, Berlin, pp. 111-149.
31. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto H, Smith PG (1977): Design and analysis of randomized trials requiring prolonged observation of each patient. II. Analysis and examples. *Br J Cancer* 35:1-39.
32. ISCN (1978): An International System for Human Cytogenetic Nomenclature (1978). *Birth Defects: Original Article Series*, Vol. XIV, No. 8 (The National Foundation, New York, 1978); also in *Cytogenet Cell Genet* 21:309-404 (1978).
33. Gahrton G, Robert K-H, Friberg K, Juliusson G, Biberfeld P, Zech L (1982): Cytogenetic mapping of the duplicated segment of chromosome 12 in lymphoproliferative disorders. *Nature* 297:513-514.
34. Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A (1982): Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. *N Engl J Med* 307:1231-1236.
35. Golomb HM, Lindgren V, Rowley JD (1978): Chromosome abnormalities in patients with hairy cell leukemia. *Cancer* 41:1374-1380.
36. Pittman S, Catovsky D (1983): Chromosome abnormalities in B-cell prolymphocytic leukemia: a study of nine cases. *Cancer Genet Cytogenet* 9:355-365.
37. Sadamori N, Han T, Minowada J, Bloom ML, Henderson ES, Sandberg AA (1983): Possible specific chromosome change in prolymphocytic leukemia. *Blood* 62:729-736.
38. Wang LM, Block AM, Fang XE, Todd S, Naylor S, Han T, Sandberg AA, Sakaguchi AY (1985): Analysis of trisomy 12 in B cell chronic lymphocytic leukemia using polymorphic DNA probes. *Am J Hum Genet* 37 (suppl): A42.
39. Crossen PE, Horn HL (1987): Origin of trisomy 12 in B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 28:185-186.
40. Einhorn S, Burvau K, Juliusson G, Gahrton G, Meeker T (1989): Molecular analyses of chromosome 12 in chronic lymphocytic leukemia. *Leukemia* 3(12), in press.
41. Wirschubsky Z, Wiener F, Spira J, Sümegi J, Klein G (1984): Triplication of one chromosome no. 15 with an altered c-myc containing EcoRI fragment and elimination of the normal homologue in a T-cell lymphoma line of AKR origin (TIKAUT). *Int J Cancer* 33:477-481.
42. Tsujimoto Y, Yunis J, Onorato-Showe L, Erikson J, Nowell PC, Croce CM (1984): Molecular

- cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation. *Science* 224:1403-1406.
43. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM (1984): Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* 226:1097-1099.
  44. Bakhshi A, Jensen JP, Goldman P, Wright JJ, McBride OW, Epstein AL, Korsmeyer SJ (1985): Cloning the chromosomal breakpoint of t(14;18) human lymphomas: Clustering around JH on chromosome 14 and near a transcriptional unit on 18. *Cell* 41:899-906.
  45. Juliusson G, Friberg K, Gahrton G (1988): Consistency of chromosomal aberrations in chronic B-lymphocytic leukemia. A longitudinal cytogenetic study of 41 patients. *Cancer* 62:500-506.
  46. Bird ML, Ueshima Y, Rowley JD, Haren JM, Vardiman JW (1989): Chromosome abnormalities in B cell chronic lymphocytic leukemia and their clinical correlations. *Leukemia* 3:182-191.
  47. Mecucci C, Delannoy A, Van Den Berghe H (1988): The origin of trisomy 12 in B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 36:203-204.
  48. Jhanwar SC, Neel BG, Hayward WS, Chaganti RSK (1983): Localization of c-ras oncogene family on human germ-line chromosomes. *Proc Natl Acad Sci USA* 80:4794-4797.
  49. van't Veer LJ, van Kessel AG, van Heerikhuizen H, van Ooyen A, Nusse R (1984): Molecular cloning and chromosomal assignment of the human homolog of int-1, a mouse gene implicated in mammary tumorigenesis. *Molec Cell Biol* 4:2532-2534.
  50. Nusse R, Juliusson G, Gahrton G (1985): Unpublished data.
  51. Butturini A, Gale RP (1988): Oncogenes in chronic lymphocytic leukemia. *Leuk Res* 12:89-92.
  52. Nowell PC, Moreau L, Growney P, Besa EC (1988): Karyotypic stability in chronic B-cell leukemia. *Cancer Genet Cytogenet* 33:155-160.
  53. Rabbitts PH, Douglas J, Fischer P, Nacheva E, Karpas A, Catovsky D, Melo JV, Baer R, Stinson MA, Rabbitts TH (1988): Chromosome abnormalities at 11q13 in B cell tumours. *Oncogene* 3:99-103.
  54. Meeker TC, Grimaldi JC, O'Rourke R, Louie E, Juliusson G, Einhorn S (1989): An additional breakpoint region in the bcl-1 locus associated with the t(11;14)(q13;q32) translocation of B-lymphocytic malignancy. *Blood* 74:1801-6.
  55. Rechavi G, Katzir N, Brok-Simoni F, Holtzman F, Mandel M, Gurfinkel N, Givol D, Ben-Bassat I, Ramot B (1989): A search for bcl1, bcl2, and c-myc oncogene rearrangements in chronic lymphocytic leukemia. *Leukemia* 3:57-60.
  56. Aisenberg AC, Wilkes BM, Jacobson JO (1988): The bcl-2 gene is rearranged in many diffuse B-cell lymphomas. *Blood* 71:969-972.
  57. Ngan B-Y, Chen-Levy Z, Weiss LM, Warnke RA, Cleary ML (1988): Expression in non-Hodgkin's lymphoma of the bcl-2 protein associated with the t(14;18) chromosomal translocation. *N Engl J Med* 318:1638-1644.
  58. Reed JC, Cuddy M, Slabicki T, Croce CM, Nowell PC (1988): Oncogenic potential of bcl-2 demonstrated by gene transfer. *Nature* 336:259-261.
  59. Chen-Levy Z, Nourse J, Cleary ML (1989): The bcl-2 candidate proto-oncogene product is a 24-kilodalton integral-membrane protein highly expressed in lymphoid cell lines and lymphomas carrying the t(14;18) translocation. *Mol Cell Biol* 9:701-710.
  60. Juliusson G, Smith CIE, Hammarström L, Gahrton G (1989), unpublished data.
  61. Ueshima Y, Bird ML, Vardiman JW, Rowley JD (1985): A 14;19 translocation in B-cell chronic lymphocytic leukemia: a new recurring chromosome aberration. *Int J Cancer* 36:287-290.
  62. McKeithan TW, Rowley JD, Shows TB, Diaz MO (1987): Cloning of the chromosome translocation breakpoint junction of the t(14;19) in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 84:9257-9260.
  63. Juliusson G, Friberg K, Gahrton G (1988): Chromosomal aberrations in progressive and indolent chronic B-lymphocytic leukaemia. A longitudinal study. *Acta Oncologica* 27:473-477.
  64. Rowley JD (1980): Ph-positive leukaemia including chronic myelogenous leukaemia. *Clin Haematol* 9:55-86.
  65. Richardson ME, Quanguang C, Filippa DA, Offit DA, Hampton A, Koduru PRK, Jhanwar SC,

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66. Yunis  
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Dis Ch  
85. Vahdat  
phoide  
86. Castol  
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kemias with the t(11;14)  
ring of the chromosome  
translocation. Science  
251:102-104.  
L, Korsmeyer SJ (1985):  
as: Clustering around JH  
899-906.  
al aberrations in chronic  
of 41 patients. Cancer  
1985;56:102-104.  
romosome abnormalities  
ns. Leukemia 3:182-191.  
somy 12 in B-cell chronic  
leukemia. Blood 62:525-531.  
zation of c-ras oncogene  
SA 80:4794-4797.  
usse R (1984): Molecular  
nt-1, a mouse gene impli-  
cated in B-cell chronic  
lytic leukemia. Leuk Res  
1985;1:1-10.  
stability in chronic B-cell  
leukemia. Blood 62:525-531.  
, Melo JV, Baer R, Stinson  
B cell tumours. Oncogene  
1985;1:1-10.  
rn S (1989): An additional  
13;q32) translocation of B-  
cell chronic lymphocytic  
leukemia. Proc Natl Acad  
Sci USA 86:102-104.  
urfinkel N, Givol D, Ben-  
cogene rearrangements in  
B-cell chronic lymphocytic  
leukemia. Proc Natl Acad  
Sci USA 86:102-104.  
rearranged in many diffuse  
large B-cell lymphomas.  
Blood 62:525-531.  
1988): Expression in non-  
t(11;18) chromosomal translo-  
cation. Blood 62:525-531.  
ncogenic potential of bcl-2  
proto-oncogene product is  
in lymphoid cell lines and  
in B-cell chronic lymphocytic  
leukemia. Int J Cancer 36:287-290.  
of the chromosome translo-  
cation in B-cell chronic  
lymphocytic leukemia. Proc  
Natl Acad Sci USA 86:102-104.  
rations in progressive and  
terminal B-cell chronic  
lymphocytic leukemia. Acta  
Oncologica 27:1-10.  
religeneous leukaemia. Clin  
Exp Immunol 62:1-10.  
A, Koduru PRK, Jhanwar SC,

- Lieberman PH, Clarkson BD, Chaganti RSK (1987): Intermediate- to high-grade histology of lymphomas carrying t(14;18) is associated with additional nonrandom chromosome changes. *Blood* 70:444-447.
66. Yunis JJ, Frizzera G, Oken MM, McKenna J, Theologides A, Arnesen M (1987): Multiple recurrent genomic defects in follicular lymphoma. A possible model for cancer. *N Engl J Med* 316:79-84.
67. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS (1975): Clinical staging of chronic lymphocytic leukemia. *Blood* 46:219-234.
68. Han T, Sadamori N, Ozer H et al (1984): Cytogenetic studies in 77 patients with chronic lymphocytic leukemia: correlation with clinical, immunologic and phenotypic data. *J Clin Oncol* 2:1121-1132.
69. Sadamori N, Han T, Minowada J, Sandberg AA (1984): Clinical significance of cytogenetic findings in untreated patients with B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 11:45-51.
70. Sadamori N, Han T, Minowada J, Sandberg AA (1984): Chromosomes and causation of human cancer and leukemia: Chromosome findings in treated patients with B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 11:161-168.
71. Robert K-H, Juliusson G, Biberfeld P (1983): Chronic lymphocytic leukaemia cells activated in vitro reveal cellular changes that characterize B-prolymphocytic leukaemia and immunocytoma. *Scand J Immunol* 17:397-401.
72. Juliusson G, Robert K-H, Öst Å, Friberg K, Biberfeld P, Zech L, Gahrton G (1985): Del(3)(p13) in B-prolymphocytic leukemia—a new nonrandom chromosomal aberration. *Cancer Genet Cytogenet* 14:191-195.
73. Han T, Sadamori N, Takeuchi J, Ozer H, Henderson EF, Bhargava A, Fitzpatrick J, Sandberg AA (1983): Clonal chromosomal abnormalities in patients with Waldenström's and CLL-associated macroglobulinemia: Significance of trisomy 12. *Blood* 62:525-531.
74. Juliusson G (1985): Leukaemic B-lymphocytic malignancy. A clinical, immunological, and cytogenetic study, utilizing mitogens to activate and differentiate malignant B-lymphocytes in vitro. Karolinska Institute, Stockholm, pp. 1-78 (dissertation).
75. Juliusson G, Robert K-H, Nilsson B, Gahrton G (1985): Prognostic value of B-cell mitogen-induced and spontaneous thymidine uptake in vitro in chronic B-lymphocytic leukaemia cells. *Br J Haematol* 60:429-436.
76. Juliusson G, Gahrton G (1988): Clinical implications of CLL cell proliferation in vitro. *Nouv Rev Fr Hematol* 30:399-401.
77. Geisler C, Hansen MM (1989): B cell chronic lymphocytic leukaemia: recent concepts in classification and treatment. *Eur J Haematol (suppl 48)* 42:31-37.
78. Kristoffersson U, Heim S, Mandahl N, Olsson H, Ranstam J, Åkerman M, Mitelman F (1987): Prognostic implication of cytogenetic findings in 106 patients with non-Hodgkin lymphoma. *Cancer Genet Cytogenet* 25:55-64.
79. Juliusson G, Gahrton G (1985): Abnormal/normal metaphase ratio and prognosis in chronic B-lymphocytic leukemia. *Cancer Genet Cytogenet* 18:307-313.
80. Cox DR (1972): Regression model and life tables. *J Royal Stat Soc B34*:187-220.
81. Han T, Ozer H, Emrich L, Sadamori N, Ohtaki K, Gomez GA, Henderson ES, Bloom ML, Sandberg AA (1985): Prognostic importance of abnormal metaphases in chronic lymphocytic leukemia. *Proc Am Soc Clin Oncol (abstract)*.
82. Sakurai M, Sandberg AA (1976): Chromosomes and causation of human cancer and leukemia. XI Correlation of karyotypes with clinical features of acute myeloblastic leukemia. *Cancer* 37:285-299.
83. Zech L, Mellstedt H (1988): Chromosome 13—a new marker for B-cell chronic lymphocytic leukemia. *Hereditas* 108:77-84.
84. Yunis JJ, Ramsay N (1978): Retinoblastoma and subband deletion of chromosome 13. *Am J Dis Child* 132:161-63.
85. Vahdati M, Graafland H, Emberger JM (1984): Etude du caryotype de 52 leucémies lymphoïdes chroniques B. *Nouv Rev Fr Hematol* 26:189-195.
86. Castoldi GL, Lanza F, Cuneo A (1987): Cytogenetic aspects of B-cell chronic lymphocytic leukemia: their correlation with clinical stage and different polyclonal mitogens. *Cancer Genet Cytogenet* 26:75-84.

87. Nowell PC, Vonderheid EC, Besa E, Hoxie JA, Moreau L, Finan JB (1986): The most common chromosome change in 86 chronic B cell or T cell tumors: A 14q32 translocation. *Cancer Genet Cytogenet* 19:219-227.
88. Crossen PE, Godwin JM, Heaton DC, Tully SM (1987): Chromosome abnormalities in chronic lymphocytic leukemia revealed by cytochalasin B and Epstein-Barr virus. *Cancer Genet Cytogenet* 28:93-100.
89. Schröder J, Vuopio P, Autio K (1981): Chromosome changes in human chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 4:11-21.
90. Callen DF, Ford JH (1983): Chromosome abnormalities in chronic lymphocytic leukemia revealed by TPA as a mitogen. *Cancer Genet Cytogenet* 10:87-93.
91. Goh K-O (1985): Chromosomal abnormalities in chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 16:103-107.

## Cytogene

Claudia U.

**ABSTRACT:** A cytogenetic study of 100 skin, and (TSC). Incidence of and in fibroblasts of unstable and skin fibroblasts of dicentric chromosomes were established. Rearrangements, translocations, and There was a particular difference between chromosomes in the angiodysplasia, high rates of affecting chromosomes in TSC by

## INTRODUCTION

Tuberous sclerosis is an inherited, incompletely penetrant, monogenic disorder with onset below 30 years of age. It has long been considered an important visceral disease. The cutaneous symptoms, such as adenoma sebaceum, have an inheritance pattern of autosomal recessive. The rhabdomyomas of the heart, the angiomas of the retina, and the hamangiomas of the skin are identified as characteristic features. The relationship between these lesions and the underlying genetic defect is still unclear. It is hoped that i

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