Nonclonal Chromosomal Aberrations Induced by Anti-Tumoral Regimens in Childhood Cancer: Relationship with Cancer-Related Genes and Fragile Sites

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ABSTRACT

Cytogenetic studies were performed on 80 pediatric cancer patients to observe the chromosomal damage, both quantitative and qualitative, induced by chemotherapy. Peripheral blood lymphocytes (PBL) (n = 127) were obtained at diagnosis, during treatment, at remission, and at relapse, and chromosome analysis performed utilizing G-banding standard procedures. The results show a significant increase in the number of altered karyotypes (P = 0.03) in the samples during treatment, returning to values that were similar to those at diagnosis at 2-year remission. Most of the chromosomal aberrations (CA) detected during the chemotherapy regimens were nonclonal, unbalanced (75%), and involved chromosomes 1, 3, 5, 6, 11, 12, 16, and 17 most frequently. There was also a marked increase of CA in samples at relapse with very similar features (type and distribution) to those detected during treatment. There was a good correlation between the chromosomal breakpoints in our series and fragile sites (58%), oncogene (75%), and tumor suppressor gene (33%) loci described in the literature. The results obtained suggest that cytostatic drugs induce a transient increase in chromosome fragility occurring at several cancer-associated breakpoints.

INTRODUCTION

Analysis of chromosomal aberrations (CA) in peripheral blood lymphocytes (PBL) has been extensively used in the biomonitoring of populations exposed to genotoxic environmental agents. Various authors, based on the hypothesis that the genetic damage in PBL reflects similar events for carcinogenic processes in target tissues, have demonstrated that an increased frequency of CA is often indicative of increased cancer risk [1, 2]. Based on this evidence, chromosomal studies in cancer patients can be considered as a suitable and reliable method of monitoring the genotoxicity of chemical agents used in therapeutic regimens.

Many of the cytostatic drugs induce chromosomal damage [3] and the leukemogenic potential of antineoplasic agents is well known [4]. The number and nature of genetic changes occurring in samples obtained from patients bearing tumors, prior to any treatment, are well documented [5]. Although the increase in CA related to chemo-therapy has been reported previously [6, 7], not only the increase in frequency, but also the type and distribution of structural CA may be important. Several reports have shown that chromosome breakage induced by various mutagens and carcinogens coincides, to a great extent, with the location of cancer breakpoints [8]. A majority of mapped oncogenes are located at these breakpoints and some of them are known to be activated following chromosomal rearrangements. Fragile sites have also been implicated, because they mostly involve the same bands as cancer breakpoints or those near to oncogene and tumor suppressor gene loci [9–11].

The aim of the present study was not only to test if the CA frequency increases due to the antitumoral regimens, but also to analyze its persistence throughout clinical remission. We also intended to determine if CA preferentially occur in those bands that harbor cancer-related genes or fragile sites.

MATERIALS AND METHODS

Patients and Samples

Eighty pediatric cancer patients were included in the chromosomal analysis, and classified into four tumoral types: 22 Ewing sarcomas (ES), 32 osteosarcomas, 13 lymphomas, and 13 central nervous system (CNS) tumors.

Peripheral blood samples (n = 127) were scheduled as follows: 11 at diagnosis, 37 at the end of treatment, 38 at short-term remission (between the first and second year after the end of treatment), 31 at long-term remission (more than 2 years after treatment), and 10 at relapse.

The chemotherapy received by our patients is indicated in Table 1, in which the cytostatic agents have been classified according to their mechanism of action. Cytogenetic studies were performed on peripheral blood samples following standard procedures. A minimum of 50 well-spread metaphases, obtained from 72-hour phytohemagglutinin-stimulated cultures, were analyzed. Chromosomal abnormalities, identified by G-banding, were described according to the ISCN 1995 [12].

Statistical analyses involved the Chi-square contingency test, the Fisher's exact test, or the Kruskal-Wallis, depending on the type of variables analyzed and on the fulfillment of the normality criteria. Statistical significance was assumed if $P \leq 0.05$, and highly statistically different if $P < 0.01$ and differences between variables with $P > 0.05$ were considered not statistically different. The software used for the statistical analysis was the Statistical Package for the Social Sciences (SPSS, version 9.0).

RESULTS

We analyzed chromosomal aberrations induced by cytostatic agents used in chemotherapy regimens in 80 pediatric cancer patients. Seventy of the 127 samples (55.1%) showed altered karyotypes. There were no statistical differences in the number of samples with chromosomal aberrations between the four tumoral groups considered. Nevertheless, the number of aberrant karyotypes was higher in the lymphoma and ES groups ($P = 0.07$).

Statistical differences were detected in the number of samples with aberrant karyotypes throughout the different stages of the chemotherapy. Chromosomal aberrations most frequently appeared in the relapsed tumors (80%, $P = 0.05$) and in the samples obtained during treatment (64.8%, $P = 0.03$) (Table 2).

All CAs detected in our series were nonclonal, except in four cases: a Hodgkin lymphoma at diagnosis (46,XY, -5,+der[13]t[13;14][p10;p10]), an osteosarcoma at short term-remission [case no. 3, Table 3: 46,XY,inv(9)(p13q22)], and two CNS tumors during treatment [case no. 21 in Table 3: 46,XX,del(5)(p12),del(6)(q14),del(17)(p13); and case no. 18 in Table 3: 46,XY,t(5;9)(q11;p23)], in which clonal aberrations were detected (defined as at least two cells with the same extra chromosome or structural rearrangement or three cells with the same missing chromosome).

When considering the type of CA, we found again that there were no significant differences among tumor types; however, the distribution of unbalanced CA was different in the various stages considered; significantly increasing during treatment and at relapse (Table 4). Most patients displayed very complex karyotypes, frequently during treatment and at relapse (Fig. 1). We recorded 275 alterations in 70 aberrant karyotypes (Table 3), thus yielding a mean number of 3.9 alterations per karyotype that was statistically higher ($P = 0.02$) in the group of samples during treatment (mean 5.5) and at relapse (mean 5.26).

To convey the essential karyotypic features in a more comprehensive manner, we illustrated our findings in patients at the end of the treatment and at short-term remission in a breakpoint map (Fig. 2). We detected a total number of 134 aberrations in the samples obtained during treatment, 30 of them were balanced and 104 unbalanced. The CA did not follow a random pattern, and the most frequent aberrations were losses affecting mostly chromosomes 2, 10, and 22 (Fig. 3). The most frequently encountered chromosomal breakpoints were 1p13 and 1q25, 3p21 and 3q21–q23, exclusively found in osteosarcomas and ES, 5q11, and 5q31–q35, 6q21–q23, 11p15, 12q13, 16q22–q24, and 17p13 and 17q21. These breakpoints were correlated with the location of fragile sites (7/12, 58%), tumor suppressor genes (4/12, 33%) and especially with those of oncogenes (9/12, 75%) (Table 5).

Up to 80% of the patients who relapsed showed altered karyotypes with features very similar to those of patients undergoing chemotherapy.

DISCUSSION

The study of lymphocyte cultures has shown that healthy persons can bear CA in a very low percentage of cells, between 0–2% [13]. Our data suggest that there is a higher frequency of altered karyotypes in pediatric cancer patients in comparison to healthy children, before as well as after treatment.

Due to the heterogeneous antitumoral regimens received, it is difficult to determine the effect of individual drugs, or even that of radiotherapy, on chromosomes. This may account for the fact that no significant differences were found between the four tumoral groups analyzed, either in frequency or in the type of CA present, regardless of the combination of drugs and of whether they received radiotherapy (ES, CNS tumors, and lymphomas) or not (osteosarcomas).

In agreement with other authors [3, 6, 7], we detected a marked increase of nonclonal chromosomal alterations at the end of treatment, returning to normal values in the months following treatment. In this study, similar CA values were recorded for samples before treatment and at long-term remission, although they were still higher than those observed for the general healthy population [13].

The fact that up to 80% of relapsed patients showed altered karyotypes supports the idea that the chromosomal aberrations are the consequence of an underlying genomic damage, and therefore, their frequency would increase in periods of higher genetic instability.

Not only the increase in the frequency of structural CA, but also their type and distribution are important. We found that the aberrations induced by the mutagenic agents are mostly of the chromatid type, showing a nonrandom distribution pattern. The patients' karyotypes tended to be very complex, most often for those during treatment and at relapse, when the CA frequency doubled to that of samples at diagnosis or at long-term remission (Table 4).

Two distinct neoplasia-associated karyotypic patterns have been defined recently [14]. Primary abnormalities are believed to be essential in establishing the tumors, while secondary abnormalities occur in addition to the primary, and are important in tumor progression and maintenance. Primary abnormalities consist of balanced structural chromosomal rearrangements, mostly translocations and some inversions, and are more specific than secondary or unbalanced rearrangements, deletions, and chromosome losses. It is important to point out that in this study, a significant in-crease of unbalanced abnormalities, mostly chromosome losses, has been detected in samples at the end of treatment and at relapse, accounting for 75% of all CAs detected. Some authors [14, 15] suggest that chemotherapy is an important factor in determining which subclones with secondary aberrations will appear at remission. In contrast to the balanced abnormalities, the molecular consequences of the recurrent unbalanced abnormalities are unknown to date.

The CA induced by treatment show a nonrandom distribution pattern. Chromosome losses are the most frequently encountered aberrations in our patients, especially involving chromosomes 2, 10, and 22. The breakpoints mapped, most frequently, in chromosome bands 1p13, 1q25, 3p21, 3q21–q23, 5q11, 5q31–q35, 6q21–q23, 11p15, 12q13, 16q22–q24, 17p13, and 17q21 (Fig. 2).

Chromosomes 5 and 7 are preferentially involved in secondary AML cases following alkylating therapy and chromosome band 11q23 in topoisomerase-associated secondary AML [4, 16]. Losses of chromosomes 5 and 7 have been defined as common indicators of insults to DNA induced by radiation, alkylating agents, or by occupational exposure. In our series, loss of both chromosomes 5 and 7, as well as alterations of 5q (mostly the q31–q35 region), were among the most frequently observed CA. We did not detect rearrangements at 11q23, and the most frequent breakpoint in our patients was 11p15; we assume that it may be due to the scarce use of topoisomerase inhibitors in the chemotherapy regimens of these patients. Rearrangements of 11p15 have been reported in several childhood and adult tumors and as a nonrandom anomaly of therapy-related myeloid leukemia in children, in contrast to adults [17]. Involvement of the 11p15 has been described in osteosarcoma secondary to irradiation, suggesting that a fragile site is located at this region, and that radiation or chemotherapy (in our series) may induce chromosome breakage at this band [18]. It is also noteworthy that several oncogenes and tumor suppressor genes map to 11p15 [10, 11].

Structural abnormalities of chromosome 3 were frequently encountered in our patients. The most frequently involved bands were 3q21–q23 and 3p21, which have been reported as recurrent treatment-related breakpoints in myelodysplastic syndrome and acute myeloid leukemia [17, 19].

Rearrangements of 6q21–q23, 12q13, and 17p13 have been observed in several solid tumors, and chromosome 17 anomalies have been reported to be increased in patients receiving chemotherapy or radiotherapy [16].

It appears that the chromatin within certain bands may be especially susceptible to damage by any number of chemical agents and the fragile sites may also contribute to this special distribution of CA. There is a highly significant statistical association between the location of fragile sites and cancer breakpoints [20]. Individuals with rare fragile sites (11q13, 12q13, 16q22) in PBL have been reported in the literature who had a breakpoint apparently coincident with a fragile site present in their cancer cells [21]. In our study, a relationship possibly existed between the most frequently observed chromosomal breakpoints and fragile sites, oncogenes, and tumor suppressor gene loci described in the literature [9–11] (Table 5).

We conclude from the present study that an increased incidence of nonclonal CA is a typical finding in lymphocytes from children exposed to antitumoral regimens. This incidence tends to decrease in the months following the end of chemotherapy, reaching similar values to those at diagnosis at 2-year remission. The CAs are mostly of the chromatid type and unbalanced aberrations, and the most frequently involved bands are associated with fragile sites, oncogene and tumor suppressor gene loci.

Although there is a marked increase of CA at relapse, there is no sufficient data, however, to conclude if the chromosomal damage is predictive of relapse or secondary

tumor development at the level of the individual patient. The molecular consequences of these secondary aberrations and its putative relationship with tumoral progression are yet to be elucidated.

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Table 1. Types of treatment received by the patients included in this study

Tumor	Group A[a]	Group B[b]	Group C[c]	Group D[d]	Radiotherapy
ES	+	+	+	+	+
Osteosarcoma	+	+	+	+	—
Lymphoma	+	+	+	+	+
CNS tumor	+	—	+	+	+

Abbreviations: ES, Ewing sarcoma; CNS, central nervous system.
[a]Alkylating agents (cyclophosphamide, ifosfamide, CCNU, BCNU), cisplatin and procarbazine.
[b]Cytostatic antibiotics (actinomycin D, adriamycin, bleomycin, daunomycin).
[c]Antimetabolites (methotrexate, cytosine arabinoside).
[d]Spindle inhibitors (vincristine, vinblastine).

Table 2. Patient karyotype status before, during, and after treatment

Stage	No. of samples (*n*)	Normal karyotype	[a]Abnormal karyotype	[b]Probability (*P*)
Diagnosis	11	7	4 (36.4)	0.36
Treatment	37	12	24 (64.8)	0.03
Short remission (<2 years)	38	16	22 (57.9)	0.33
Long remission (>2 years)	31	19	12 (38.7)	0.2
Relapse	10	2	8 (80.0)	0.05
Total	127	56	70 (55.1)	0.04

[a]Numbers in parentheses refer to the percentage of altered karyotypes in each of the stages.
[b]Chi-square contingency test: not significant $P > 0.05$, statistically significant $P \leq 0.05$, highly statistically significant $P < 0.01$.

Table 3 Description of abnormal karyotypes detected in the samples obtained during chemotherapy and at short-term remission		
Case no.	**Tumor**	**Abnormal karyotype (at short-term remission)**
1	OS	46,XY,del(16)(p11.2)
2	Burkitt	48,XY,del(1)(q32),del(3)(p21),add(4)(q35),+2mar
3	OS	46,XY,inv(9)(p13q22)
4	ES	46,XX,del(17)(p13)
5	HL	46,XX,t(7;12;15)(q22;q14;q22)
6	CNS	47,XX,-2,-6,add(12)(q13),+3mar/43,X,-X,der(5)t(1;5)(q25;q22), del(9)(q22), -16, -19,del(20)(q11)/46,XX,+del(1)(p23),-6
7	ES	47,XX,+del(12)(p13)
8	OS	46,XX,del(X)(q13)/46,XX,t(1;9)(q25;q22)
9	ES	46,XY,+8,-14,+add(20)(q13),-21
10	CNS	46,XY,add(16)(p13)
11	NHL	46,XX,t(3;6)(q21;q22)/46,XX,t(5;8)(q34;q12)/46,XX,del(3)(p21)/46, XX,t(2;4)(p15;p13)
12	ES	46,XX,t(10;20)(q22;q13)
13	ES	46,XX,t(18;18)(p11.1;q11)/46,XX,del(11)(p13)
14	ES	46,XY,+8,-13,del(16)(q13)/46,XY,t(4;5)(q26;q22)
15	HL	46,XX,t(1;3)(p31;q12)
16	CNS	46,XX,t(5;13)(q11;q34)/46,XX,-2,+15
17	OS	46,XY,t(2;7)(q31;p15)
18	CNS	46,XX,t(1;14)(q43;q11),add(16)(p13)
19	ES	46,XY,inv(11)(p15q13)
20	OS	46,XX,del(2)(q23)
21	CNS	49,XY,add(2)(p24),-4, t(7;15)(p13;q11), del(10)(p12), add(16)(q24), +20,+3mar/46,XY,del(1)(q32),add(17)(p13)/46,XY, t(1;7)(p31;q34)
22	ES	45,XY,add(1)(p13),-8,-11,-22,+2mar
		Abnormal karyotype (during treatment)
1	CNS	47,XX,-18,+2mar
2	OS	48,XY,del(3)(q25),+r,+mar/47,XY,del(5)(q11q33),+r
3	ES	46,XY,del(3)(q23)/46,XY,t(3;6)(p25;p21.2)/46,XY,t(3;19)(p22;q13);46,XY,del(11)(q14)/46,XY,-8,+del(9)(q31),der(14) t(10;14)(q23;?)
4	ES	46,XX,t(4;7)(q35;q22)/46,XX,dic(3;5)(q23;q35)
5	OS	46,XY,add(8)(q13)
6	ES	44,Y,-X,-8/46,XY,del(3)(p23),i(21)(q10)
7	CNS	44,Y,-X,t(1;20)(p13;q11),-6/47,XY,+dic(1;6)(p21;q24),-2,-16,+2mar
8	OS	46,XX,del(9)(q11)

9	ES	46,XY,t(2;17)(p23;q24)/46,XY,-2,del(3)(q21),-7,add(8)(p23),+2mar/48, XY, add(1)(q21),-13,add(16)(p13),+3mar/46, XY, dup(1)(q31q32),dic(1;21) (q43;q22),del(5)(q31q33),del(8)(q21),der(11)(p15),-13,+add(15)(q24), add(16)(q24)
10	OS	48,XY,i(1)(p10),+2/46 XY,-2,+20
11	OS	46,XY,-2,add(3)t(3;?)(p13;?),add(11)(p11.2),+mar/47,XY,der(1)t(1;4)(q41;p16),-3, -4,del(7)(q21),dic(10;?)(q24;?),-10, add(14)(q32),dic(16;20)(q22;q13), +4mar/46, XY,del(6)(q22)
12	OS	46,XY,t(4;12)(q12;q13)/46,XY,t(5;12)(q31;q21)/47,XY,del(1)(p22),t(2;11)(q11;p1 5),del(6)(q23),-7,der(7)t(1;7)(q25;p15), +mar
13	CNS	45,XX,+der(1)t(1;18)(p13;q23),-2,add(11)(p15),der(16)t(2;16)(q21;q24), -18/45,XX,-12,add(17)(q21)
14	OS	45,XY,-22/44,X,-Y,del(1)(p32),+der(2),-10,-10,add(11)(p15),add(14)(q11), -15,-18,+2mar
15	ES	46,XY,del(1)(p13),-3,-16,+2mar
16	OS	45,XX,-16/46,XX,+9,-10,+11,-19
17	NHL	46,XY,t(2;17)(q24;q21)/46,XY,t(1;15)(q22;p11)
18	CNS	46,XY,t(5;9)(q11;p23)
19	ES	45,XY,-4,-5,-22,-22,+3mar
20	CNS	46, XX,del(5)(q31q33)/47,X,-X, 10,add(11)(q22),der(17)t(10;17)(p14;q24), -22,+4mar
21	CNS	46,XX,add(16)(q?)/46,XX,del(5)(p12),del(6)(q14),del(17)(p13)/46,XX,del(6)(q14q2 1)/46,X,add(X)(q28),add(2)(q24),-5, del(17)(p13),add(20)(q13),+mar
22	NHL	Complex karyotype
23	ES	46,XY,inv(11)(p15q13)
24	CNS	46,XX,del(12)(q13)
Abbreviations: OS, osteosarcoma; ES, Ewing sarcoma; HL, Hodgkin lymphoma; CNS, central nervous system tumor; NHL, Non-Hodgkin lymphoma.		

Table 4. Number and type of chromosomal alterations detected in the different stages of the evolution considered

Stage	Altered karyotypes [a]*P* = 0.04 [b]c	Total no. of aberrations *P* = 0.02 [c]n (n/c)	Balanced *P* = 0.81 n (n/c)	Unbalanced *P* = 0.05 n (n/c)	Translocations *P* = 0.80	Deletions *P* = 0.40	Losses *P* = 0.06
Diagnosis	4	9 (2.2)	3 (0.7)	6 (1.5)	3	0	3
Treatment	24	134 (5.5)	30 (1.2)	104 (4.3)	21	23	46
SR	22	62 (2.8)	16 (0.7)	46 (2.8)	15	16	16
LR	12	28 (2.3)	10 (0.8)	18 (1.5)	9	7	8
Relapse	8	42 (5.2)	9 (1.1)	33 (4.1)	8	1	13
Total	70	275 (3.9)	68 (0.9)	207 (2.9)	56	56	86

Abbreviations: SR, Short-term remission (<2 years); LR, long-term remission (>2 years).
[a]Kruskal-Wallis Test: not significant $P \leq 0.05$, statistically significant $P \leq 0.05$, highly statistically significant $P < 0.01$.
[b]Number of samples with altered karyotypes in each of the stages.
[c]Total number of aberrations detected in the altered karyotypes (n/c = mean number of aberrations per altered karyotype).

Table 5. Location of nonrandom chromosome alterations and correlation with fragile sites, oncogenes, and tumor suppressor genes

Bands	Fragile sites	Oncogenes	Tumor suppressor genes
1p13	—	NRAS	—
1q25	FRA1G	ARG, ABL2	
3p21	—	TCTA, CTNNB1, AERB2	MLH1
5q31-35	FRA5C	IL-3, PDGFRB, CFS1R, NPM, FMS	—
6q21-23	FRA6F	MYB, SYR, FYN, ROS1	
11p15	FRA11C	HRAS, RBTN1, NUP98, MTACR1	KIP2, H19
12q13	FRA12A	MDM2, INT1, GLI, CHOP, RARG, ERBB3, ATF1	—
16q22-24	FRA16B	CBFB, MAF	
17p13	FRA17A	—	TP53
17q21	—	CERBB2/NEU, RARA, AF17, NM23, NGL	BRCA1

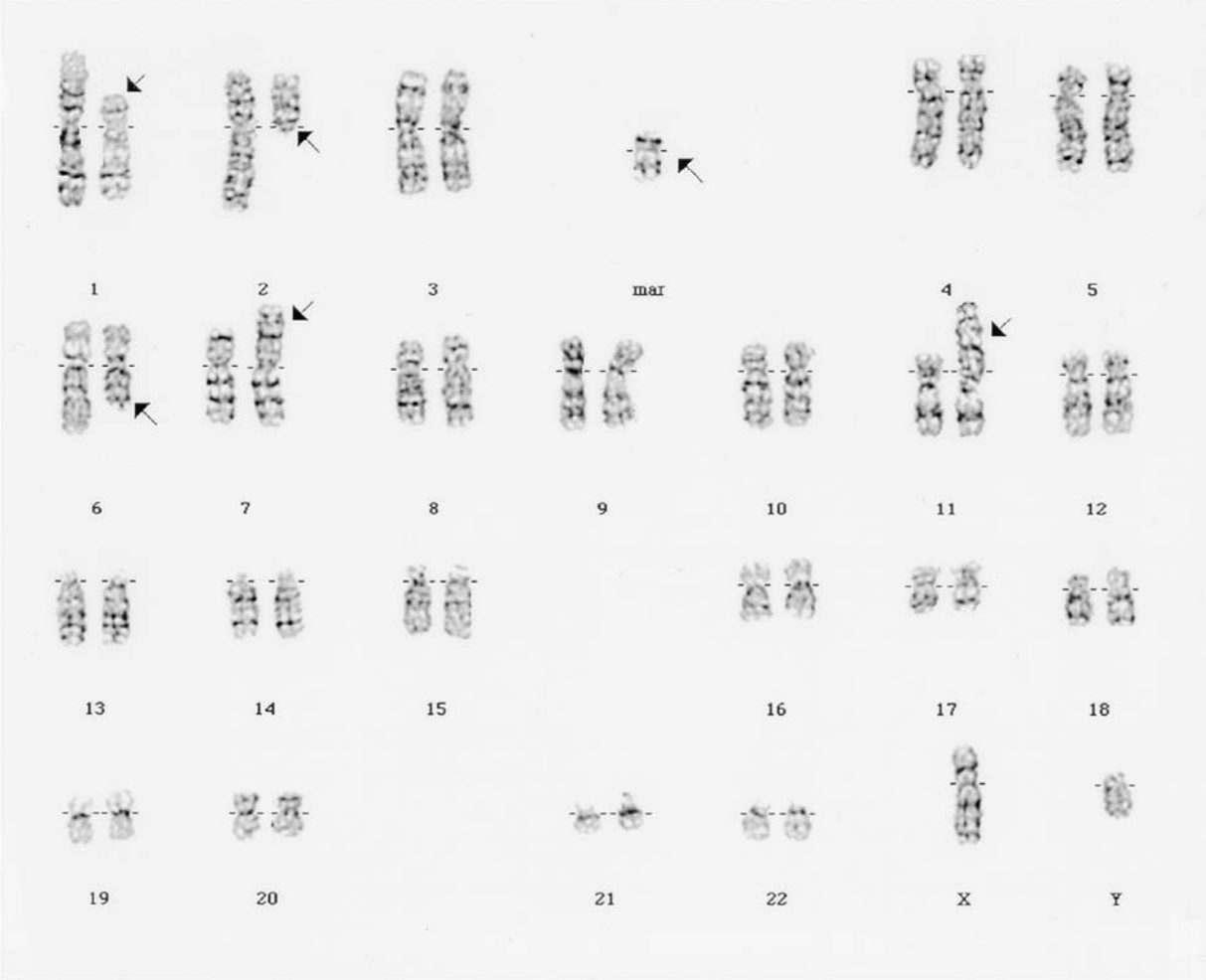

Figure 1 Example of the karyotype of peripheral blood lymphocytes of a patient with an osteosarcoma obtained during treatment showing the complex pattern of aberrations. Case number 12 during treatment, Table 3:
47,XY, del(1)(p22),t(2;11)(q11;p15),del(6)(q23),der(7)t(1;7)(q25;p15), +mar

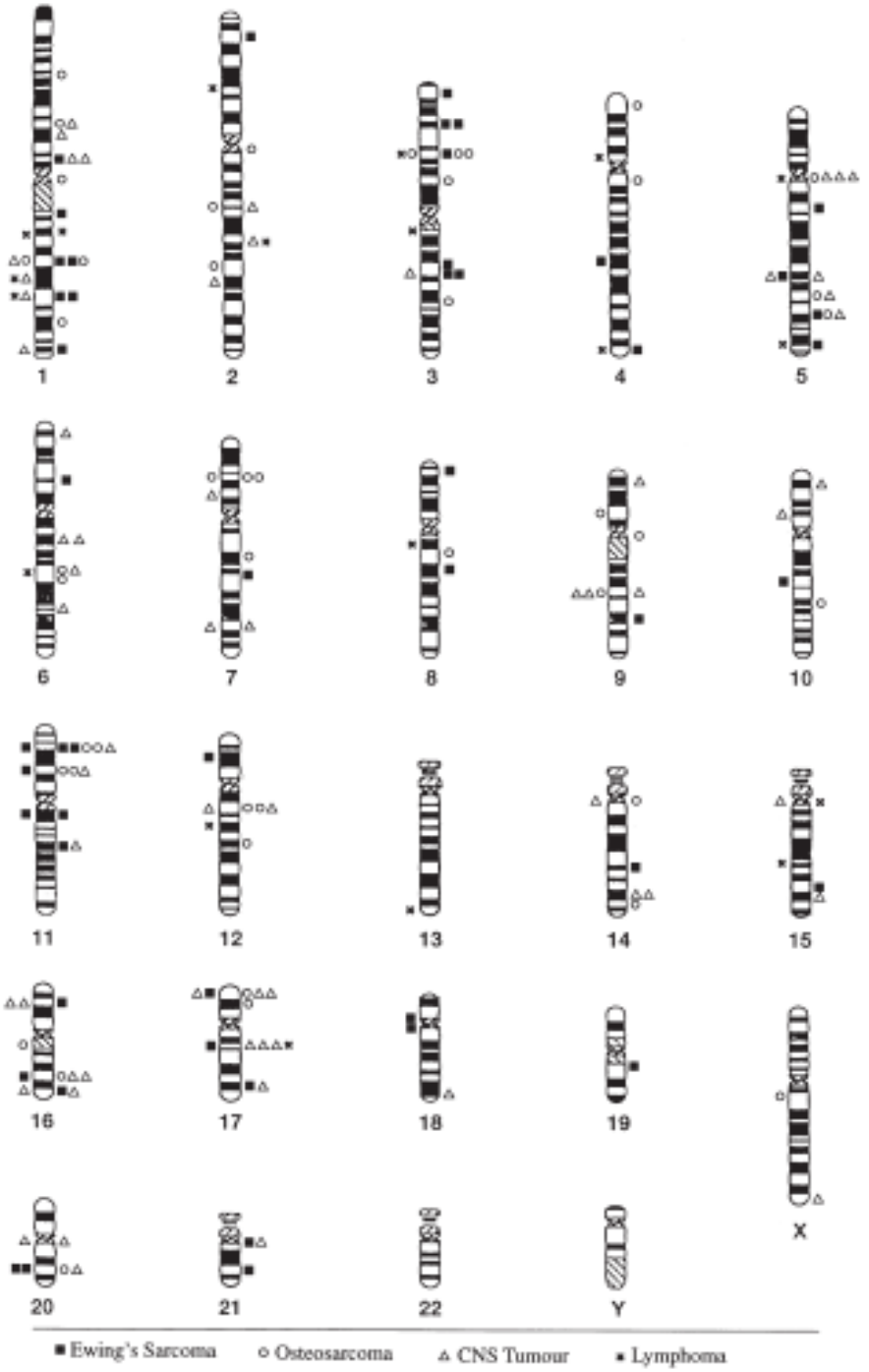

Figure 2. Ideogram showing chromosome breakpoints found in the samples at short-term remission (left) and during chemotherapy regimens (right).

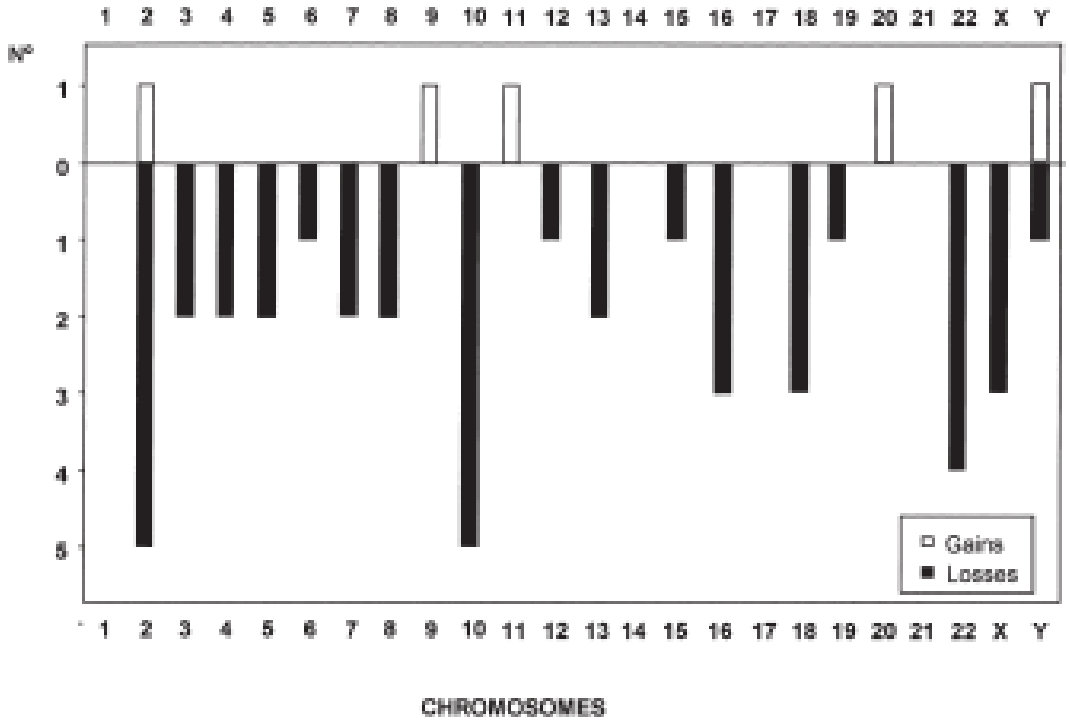

Figure 3. Histogram showing the total number of chromosome gains and/or losses in the samples obtained during treatment.