

# Cytogenetic Analysis of 280 Patients With Multiple Myeloma and Related Disorders: Primary Breakpoints and Clinical Correlations

María J. Calasanz,<sup>1\*</sup> Juan C. Cigudosa,<sup>1</sup> María D. Otero,<sup>1</sup> Carmen Ferreira,<sup>1</sup> M. Teresa Ardanaz,<sup>5</sup> Aurora Fraile,<sup>4</sup> José L. Carrasco,<sup>2</sup> Francesc Solé,<sup>6</sup> Braulia Cuesta,<sup>3</sup> and Arturo Gullón<sup>1</sup>

<sup>1</sup>Department of Genetics, University of Navarra, Pamplona, Spain

<sup>2</sup>Service of Cytogenetics, La Laguna University Hospital, La Laguna, Spain

<sup>3</sup>Department of Hematology, Clínica Universitaria, Pamplona, Spain

<sup>4</sup>Department of Hematology, Hospital Ntra S de Aránzazu, San Sebastián, Spain

<sup>5</sup>Department of Hematology, Hospital Txagorritxu, Vitoria, Spain

<sup>6</sup>Laboratory of Hematological Cytology, Hospital Central L'Aliança, Barcelona, Spain

Cytogenetic analysis of unstimulated short-term bone marrow cell cultures was performed on 280 patients with multiple myeloma and related disorders. In 65% of the cases, an additional short term B-cell stimulated culture was also examined. Chromosomally abnormal clones were found in 31% of the patients, 15% in Waldenström macroglobulinemia, 25% in monoclonal gammopathies, 33% in multiple myeloma, and 50% in plasma cell leukemia. Three primary chromosomal breakpoints were recurrently involved: 14q32, 16q22, and 22q11. Structural rearrangements of chromosome 1 were the most frequent (26% of the abnormal cases), but always as a secondary change. Rearrangements of band 14q32 were found in 22% of the abnormal cases. Among the multiple myeloma patients who showed an abnormal karyotype, 33 (46%) were hyperdiploid, most frequently with 52–56 chromosomes, 29 patients (40%) were pseudodiploid, and the remaining 12 cases (14%) were hypodiploid. A highly significant relation was observed between the presence of an abnormal karyotype and the following clinical parameters: stage III ( $P = 0.0001$ ), bone marrow plasma cell infiltration greater than 30% ( $P = 0.0001$ ), presence of bone lesions ( $P = 0.0009$ ), and  $\beta 2$ -microglobulin levels greater than 4 mg/L ( $P = 0.0001$ ). *Genes Chromosom. Cancer* 18:84–93, 1997.

© 1997 Wiley-Liss, Inc.

## INTRODUCTION

The cytogenetic pattern of multiple myeloma (MM) is rather less characteristic than that of any other type of leukemia and lymphoma. So far, around 800 cases of MM have been cytogenetically reported (Cigudosa et al., 1994; Ankatil et al., 1995; Lai et al., 1995; Sawyer et al., 1995; Smadja et al., 1995) and the main chromosomic features can be summarized as follows: (1) The proportion of abnormal karyotypes is about 40%, although it varies greatly from series to series (20–60%). (2) Abnormal clones appear to be evenly divided between hypo-, pseudo-, and hyperdiploidy. (3) The most common structural chromosomal abnormality is a 14q+ marker which is either the result of a reciprocal translocation, e.g., t(11;14)(q13;32) or t(8;14)(q24;q32) or a more complex rearrangement with unidentified chromosomes. (4) Other less specific structural abnormalities frequently found have been rearrangements of chromosomes 1, 6, 13, 17, 19, and 22.

Cytogenetic analysis has become of clinical interest in MM patients, e.g., by identifying MM with

poor prognosis (those with chromosome abnormalities; Dewald et al., 1985; Lisse et al., 1988); by distinguishing patients with plasma cell proliferation who have cytopenia because of evolving therapy-associated leukemia from those with progressive bone marrow (BM) infiltration by plasma cells (Dewald et al., 1985); and by detecting patients in an advanced phase of the disease (Lewis and Mackenzie, 1984). The karyotype in these patients may be used as an independent prognostic factor because of its correlation with other clinical parameters such as production of IgG3 (Van den Berghe et al., 1984), BM plasma cell infiltration (Weh et al., 1993; Cigudosa et al., 1994), clinical stage,  $\beta 2$ -microglobulin levels greater than 4,000 mg/ml, and presence of bone lesions (Cigudosa et al., 1994).

To further clarify the cytogenetic features of MM, we have analyzed the karyotype and other hematological parameters of 280 patients with MM and related disorders.

\*Correspondence to: Dr. M.J. Calasanz, Department of Genetics, University of Navarra, Irunlarrea s/n, 31008 Pamplona, Spain.

Received 1 April 1996; Accepted 27 June 1996

TABLE I. Diagnosis and Cytogenetic Distribution of Patients<sup>a</sup>

	MM	PCL	WM	MGUS	Total
Normal	126	2	10	33	171
Abnormal	72 (33.2%)	3 (50%)	2 (15.4%)	11 (25%)	88 (31.4%)
Hyperdiploid	33	1	1	1	36
Hypodiploid	10			1	11
Pseudodiploid	29	2	1	9	41
Failure	19	1	1		21
Total	217	6	13	44	280

<sup>a</sup>MM, multiple myeloma; PCL, plasma cell leukemia; WM, Waldenström macroglobulinemia; MGUS, monoclonal gammopathy of undetermined significance.

## MATERIALS AND METHODS

The study population included 217 patients with MM, 44 patients with monoclonal gammopathies of uncertain significance (MGUS) (we excluded plasmacytoma and MGUS associated to neoplasms or to other diseases known to produce monoclonal proteins), 13 patients with Waldenström macroglobulinemia (WM), and 6 patients with plasma cell leukemia (PCL) (Table 1). MM was defined according to the diagnostic criteria of Durie and Salmon (1975). Patient samples were collected in three different Cytogenetic Services, members of the Spanish Cooperative Group of Hematological Cytogenetics. The karyotypes of 20 patients (nos. 1 through 20) have been previously reported (Cigudosa et al., 1994).

The following variables were studied at diagnosis in each patient: age, sex, hemoglobin, creatinine,  $\beta_2$ -microglobulin, type of Ig, presence of bone lesions, calcium, albumin, clinical stage, BM plasma cell infiltration, chemotherapy if any, and karyotype. Table 2 summarizes data of some clinical parameters that were found to be statistically significant when correlated with the presence of chromosome abnormalities. Statistical comparisons were made by Contingence Chi-square Test.

Cytogenetic studies were done on BM aspirates. Two BM cultures were established from each sample whenever possible (65% of the total number): one with a B-cell mitogen (pokeweed) and one without mitogens. The culture medium was McCoy's 5A, Iwakata and Grace Modification, supplemented with 20% fetal calf serum, gentamicine (50 mg/ml), L-glutamine (4 mM), and pokeweed (0.8 mg/ml) when stimulated. Unstimulated cultures were harvested within 24 hours of incubation, whereas B-cell-stimulated cultures were harvested after 48 hours of incubation. Both types of cultures were exposed to Colcemid (0.1 mg/ml) (Gibco BRL, Gaithersburg, MD) for 15 min, followed by hypotonic treatment with KCl (0.075

TABLE 2. Summarized Clinical Data on the Multiple Myeloma Patients<sup>a</sup>

Clinical parameter	No. of patients (%)
Stage (144 patients)	
I	50 (35)
II	34 (23)
III	60 (42)
Type of immunoglobulin (160 patients)	
IgG	101 (63)
IgA	38 (24)
IgD	1 (1)
Bence-Jones	15 (9)
Nonsecretory	3 (2)
Smoldering	2 (1)
$\beta_2$ -Microglobulin (mg/L; 145 patients)	
$\leq 4$	76 (52)
$> 4$	69 (48)
Bone marrow infiltration (%; 147 patients)	
$\leq 30$	70 (48)
$> 30$	77 (52)
Lytic lesions (146 patients)	
No	67 (46)
Yes	79 (54)
Previous chemotherapy (158 patients)	
No	74 (47)
Yes	84 (53)

<sup>a</sup>Sex ratio (m/f): 1.45; median age, 63.3 (33–87) year; study period, 1/1990–12/1995.

mol/L) at 37°C for 7 min, and several washes with fixative (methanol:acetic acid, 3:1). Slides were prepared by the air-drying method. G-banding with trypsin-Giemsa was used in all instances. C-banding was performed by the baric hydroxid-method when necessary. A minimum of 30 (mostly 50) well-spread metaphases were analyzed from each case (or a minimum of 25 if all metaphases showed the same chromosome aberration). Karyotypes were described according to the ISCN (1991). In order to convey the essential karyotypic features in a more easily comprehensible manner, we have illustrated the findings in a breakpoint map (see

below). This was done according to the following criteria, whose main aim was to avoid inflating any existing nonrandomness when registering the changes of clonal evolution:

1. If more than one copy of the same chromosome aberration was found in the same clone, the breakpoints involved were plotted only once.
2. If the same chromosome aberration was found in more than one related clone in any given case, the breakpoints involved were plotted only once.
3. Whenever additional copies of a derivative chromosome were further rearranged, in the same or related clones, only the additional breakpoints were plotted.
4. If the same breakpoint was involved in more than one chromosome rearrangement in related clones, it was plotted only once.
5. If the same breakpoint was involved in different aberrations in unrelated clones, it was plotted once per aberration.
6. In cases of rearrangements with uncertain breakpoint localization, e.g., del(6)(q?), the breakpoint was not included.

## RESULTS

### Cytogenetic Analysis

Table 1 shows the diagnostic distribution of the 280 patients together with the frequency of abnormal karyotypes and the proportion of failed cultures. On the whole, 7.5% of cases escaped cytogenetic analysis because of the absence or low number of suitable metaphases. The proportion of cases with chromosome alterations was 31%, but this percentage varied between 15% in Waldenström macroglobulinemia, 25% in monoclonal gammopathies, 33% in multiple myeloma, and 50% in plasma cell leukemia.

### Breakpoints and Numerical Chromosome Changes

The complete descriptions of the chromosomally abnormal clones, together with the proportion of abnormal cells, are given in Table 3, and the breakpoints are entered in the idiogram of Figure 1. We have distinguished between primary and secondary breakpoints depending on whether or not they are present as a single aberration in the karyotype. It is noteworthy that primary breakpoints clustered at 24 bands and that only 3 bands were frequently involved: 14q32, 16q22, and 22q11. Primary chromosomal rearrangements of band 14q32 were observed in 7 cases (cases 17, 30, 53, 70, 77, 83, and 87): Two of them were t(8;14)(q24;q32) and one corre-

sponded to a t(11;14)(q13;q32). The remaining were add(14)(q32) markers with unidentified chromosomal segments. Deletions at band 16q22 were found in 5 cases (cases 24, 28, 38, 80, and 82). Deletions of chromosome 22 at band 22q11 were found in 4 cases (cases 4, 12, 78, and 79). Representative partial karyotypes of these aberrations are shown in Figure 2.

Secondary breakpoints were widely distributed in all chromosomes but clustered to 81 bands (Fig. 1). The above mentioned primary breakpoints (14q32, 16q22, and 22q11) were among them, thus indicating their more specific role in the pathogenesis of MM. Adding together primary and secondary changes (Table 3), we found that 14q32 translocations were the most frequent aberration, occurring in 20 of 88 patients (23%). Less common were rearrangements of 22q11 (14 out of 88 patients), rearrangements of 16q22 (10 out of 88 cases), and deletion 6q, which was present in 7 cases (Fig. 2). Chromosome 1 structural aberrations were frequently seen (25 cases), but always as a secondary change. Breakpoints on chromosome 1 were scattered throughout its length but clustered to the centromere and bands p35-36. Less frequently, bands 17p11, 2q32, and 11q13 were also affected by secondary structural abnormalities. The reciprocal translocation t(11;14)(q13;q32) was detected, as a part of a more complex karyotype, in 2 cases (cases 32 and 37) (Fig. 2). Secondary deletions of the long arms of chromosomes 5 and 7, usually described in myelodysplastic syndromes and acute myeloid leukemia, were detected in 4 cases (cases 29, 37, 45, and 46) (Fig. 2).

Among the MM patients with abnormal karyotypes (Table 1), 33 patients (46%) were hyperdiploid, most frequently with 52–56 chromosomes, 29 cases (40%) were pseudodiploid, and the remaining 12 cases (14%) were hypodiploid, mostly with 45 chromosomes with loss of one sex chromosome. This trend towards hyperdiploidy was not observed in the monoclonal gammopathies where 80% of the abnormal cases were pseudodiploid. The most frequent numerical anomalies were gains of chromosomes 3, 5, 7, 9, 11, 15, and 21. Monosomies were less frequent than polysomies. Losses usually involved chromosomes 8, 14, 2, 13, and Y (Fig. 3). Monosomies, as the sole change in the karyotype, were noted for the sex chromosomes (cases 14, 19, and 22) and for chromosome 13 (case 11).

### Karyotype and Clinical Evolution

Six patients were cytogenetically studied during clinical management (Table 4). Three different

TABLE 3. Chromosome Abnormalities in 88 Patients With MM and Related Disorders

Case no.	Metaphases (abnormal/ total)	Classification	Karyotype (only the abnormal clone)
MM			
1 <sup>a</sup>	15/33	AN	46,XY,del(20)(q12)
2	5/32	AN	66,XXX,-1,der(1)t(1;15)(p36;p13),del(1)(p36),-2,+4,+4,+5,-8,+9,+10,+11,+11,-12,-13,-14,-17,-18,-19,-20,-20,-21,del(22)(q11)x2
3	12/33	AN	66,XXX,-1,-3,-3,-5,+6,-7,-8,+9,+11,+12,+13,-14,+15,-17,-17,+18,+19,-20,-21,del(22)(q11),+mar
4	10/32	AN	46,XY,del(22)(q11)/45,X,-Y,del(22)(q11)
5	25/25	AA	44,XX,dic(1;10)(p36;p15),-10,i(17)(q10)
6	10/30	AN	43,XY,-13,-14,-20,del(22)(q11)
7	11/28	AN	67,X,-X,der(X),-1,i(2)(q10),-4,+5,+6,add(6)(q12)x2,-8,-9,+i(11)(q10),-12,-13,+14,-16,+17,add(17)(p11)x2,+18,+18,-19,-20,+22
8 <sup>b</sup>	28/30	AN	94,XXYY,+del(22)(q11)x2
9	40/46	AN	86-88(4n),XXYY,-6,-14,del(17)(p11),-19,-19,-22/175-177(8n),XXY,-2,-2,-4,+6,+7,-9,-9,-10,-11,+12,-14,+del(17)(p11)x2,-20,-20,-22
10	11/30	AN	58,XY,-X,-4,-8,-9,-10,-11,-12,add(14)(q32),-16,-17,-18,-20,-22,+mar
11	20/26	AN	45,XY,-13
12	17/28	AN	46,XX,del(22)(q11)
13	31/31	AA	46,XX,add(17)(q25)
14	15/32	AN	45,X,-Y
15	12/30	AN	46,X,del(X)(q11)
16	20/30	AN	46,XX,add(12)(p12)
17	23/23	AA	46,XY,add(14)(q32)/46,idem,del(16)(q22)
18	23/30	AN	46,XX,t(7;12)(q32;q24)
19	10/30	AN	45,X,-Y
20	24/33	AN	46,X,-X,t(1;16)(p36;p13),-5,+7,+mar/46,X,-X,t(1;16)(p36;p13),del(2)(q11),-5,+7,del(11)(q21),del(20)(q13)
21	15/28	AN	46,X,-X,+mar/44,X,-X,add(1)(p11),del(6)(q21),-8,-13,+mar
22	26/39	AN	45,X,-X
23	10/100	AN	46,XX,del(1)(p21),inv(16)(q13q22)
24	27/40	AN	46,XX,del(16)(q22)
25	10/33	AN	46,XX,del(6)(q21),+13,-21
26	20/40	AN	47,XY,t(1;12)(p32;q24),+mar
27-1	25/50	AN	56,XY,+Y,+r(3)(p26q29),+5,+7,+8,+9,+11,+15,+17,+19
27-2	9/87	AN	55,XY,+Y,+r(3)(p26q29),+5,+8,+9,+15,+17,+19,+mar
27-3	20/100	AN	55,XY,+Y,+r(3)(p26q29),+4,+dup(7)(p21),+9,+11,+15,+17,+19
28	27/27	AA	46,XY,inv(9)(p21q13)c,del(16)(q22)
29	36/44	AN	42-44,X,add(X)(p11),+1,t(2;13)(q32;q13),del(4)(q31),del(6)(q21),del(7)(q32),-8,dup(9)(q12q34),-16,-18,-22,+2mar[cp20]/85-99,MAKA
30	19/40	AN	46,XX,add(14)(q32)
31	14/25	AN	45,XY,-C
32	21/30	AN	46,XX,t(11;14)(q13;q32),der(15)t(1;15)(q12;p11)
33	43/43	AA	46,XX,add(12)(p12)
34	20/36	AN	46,XY,der(1)t(1;1)(p36;q10),+3,i(8)(q10),der(13;15)(q10;q10)/80,XY,cx
35	6/31	AN	47,XY,+mar
36	7/30	AN	46,XY,add(14)(q32),add(16)(q22),del(22)(q11)/45,X,idem,-Y
37	36/42	AN	46,XY,del(5)(q13q22),der(10)t(1;10)(q11;p11),t(11;14)(q13;q32),der(14)t(11;14)(q13;q32)/46,XY,del(5)(q13q22),t(11;14)(q13;q32),der(12)t(1;12)(q11;p11),der(14)t(11;14)(q13;q32)
38	30/30	AA	46,XY,del(16)(q22)
39	15/40	AN	60,XY,-X,+1,del(1)(p?),t(2;7)(q22;q23),+3,del(3)(p?),-4,-6,-12,-13,-14,add(14)(q32),+15,-16,-17,-19,-20,-21,-22
40	18/30	AN	52,XY,+10,i(10q),+11,+14,+15,+16,+mar
41	25/25	AA	44,X,-X,+1,t(1;8;18)(p32;p23;q12),t(1;3)(q11;p21),-9,inv(13)(p13q22),add(14)(q32),-16
42	33/33	AA	46,XY,-2,-4,+mar1,+mar2
43	27/27	AA	49,XY,i(1)(q10),t(4;11)(q28;q24),-9,t(10;16)(q22;q22),+15,+21,+mar1,+mar2/46,XY,t(4;9)(q34;q11)/46,XY,dmin
44	37/37	AA	48,XY,+5,+6,+7,-8,-10,del(14)(q24qter),+15
45	7/30	AN	55,XX,del(5)(q21q33),del(6)(q12),+11,+11,t(11;12)(q14;p12),+13,+14,+15,+16,+17,+18,-19,+21,+2mar

TABLE 3. Chromosome Abnormalities in 88 Patients With MM and Related Disorders (continued)

Case no.	Metaphases (abnormal/ total)	Classification	Karyotype (only the abnormal clone)
46	25/25	AA	46,XY,del(7)(q36),-9,+11,del(16)(q?),add(17)(q?)/51,XY,del(7)(q36),-9,+11,del(16)(q?),add(17)(q?),+5mar
47	27/27	AA	53,XY,del(2)(q?),+3,del(4)(q?),del(6)(q?),+7,add(7)(q?),+11,+21,+22,+mar1,+mar2
48	30/30	AA	49,XY,+t(1;21)(q21;q11),+3,del(3)(q?),t(11;22)(p13;q11),+17
49	25/25	AA	46,XY,dup(1)(q11q25)
50	17/42	AN	46,XY,del(1)(p31)
51	14/31	AN	46,XX,del(1)(p?)
52	25/25	AA	57,XY,+del(3)(q?),+4,+7,+8,+9,+11,+11,+13,+20,+21,del(22)(q11),+mar
53	25/25	AA	46,XY,add(14)(q32)/38-49,XY,+der(1),add(14)(q32),-18,+19,+20,+21[cp10]
54	8/35	AN	46,XX/80,MAKA,der(1),del(22)(q11)
55	15/50	AN	52,XY,+Y,+5,del(6)(q15),+11,+14,+15,add(17)(p11),+mar
56	18/35	AN	53,XX,+2,+3,+5,+6,+11,+17,+19
57	10/30	AN	77,XXY,+Y,-2,-4,-8,+9,+11,+13,+14,+14,+17,-18,+21,+21,+3mar
58	20/40	AN	48,XY,+5,+9,add(13)(q34),add(14)(q32)
59	12/30	AN	46,XX,dmin
60	22/35	AN	48,XX,+9,+14
61	8/30	AN	92,XXXX
62	13/33	AN	56,XX,del(1)(q32),del(3)(q21),add(5)(p15),+8,+add(9)(p22),add(11)(p15),+add(11)(p15)x2,+14,+15,+18,+21,+2mar
63	10/30	AN	49,XY,+5,t(8;22)(q24;q11),+11,+19
64	11/35	AN	89,XXXX,-5,-10,-14,-15,-19,-20,-22,+4mar
65	30/30	AA	45,XX,der(13;14)(q10;q10)
66	12/50	AN	78,XXY,+Y,+1,+7,+11,+12,+14,+17,+19,+20
67	19/35	AN	46,XY,del(6)(q23)
68	13/50	AN	46,XX,+1,+der(1)t(1;15)(q10;p10)/92-123,XXXX[cp8]
69	35/35	AA	46,XY,dup(1)(q12q22)
70	30/30	AA	46,XX,t(11;14)(q13;q32)
71	17/66	AN	45,X,-Y
72	28/43	AN	46,XY/42,XY,der(1),+der(1)del(1)(p13),der(2)t(2;3)(q32;q24),-5,-8,-10,-13,der(14;21)(q10;q10),+der(15)x2,-16,der(17)t(10;17)(q23;q23),-18
<b>MGUS</b>			
73	22/75	AN	47,XX,+mar/90-95,pvz
74	15/35	AN	45,XX,-8,del(17)(p13)/45,idem,del(22)(q13)
75	24/50	AN	46,XX,der(1;4)(q10;q10),del(6)(q21),add(14)(q32),del(16)(q22)
76	28/28	AA	46,XY,add(17)(q25)
77	30/30	AA	46,XY,t(8;14)(q24;q32)
78	22/36	AN	46,XX,del(22)(q11)
79	20/35	AN	46,XX,del(22)(q11)
80	16/36	AN	46,XY,del(16)(q22)
81	28/28	AA	46,XY,inv(11)(p12p14)
82	16/30	AN	46,XY,del(16)(q22)
83	26/26	AA	46,XY,inv(9)(p11q13)c/46,idem,add(14)(q32)
<b>PCL</b>			
84	25/25	AA	46,XX,add(4)(p15),del(10)(q22),add(14)(q32)
85	26/26	AA	46,XX,del(1)(q21),+der(1),-5,-8,add(12)(q22),-14,add(14)(q32),+2mar
86	32/32	AA	46-47,XY,del(1)(q21),add(14)(q32),MAKA
<b>WM</b>			
87	30/30	AA	46,XY,t(8;14)(q24;q32)
88	27/27	AA	48,Y,-X,dup(1)(p13p22),del(2)(p12),add(2)(q32),+4,del(6)(q15q21),add(7)(p22),i(8)(q10),add(14)(q32),+add(17)(p11),+mar

<sup>a</sup>Patient with a deletion 20q-associated with previous polycythemia vera.

<sup>b</sup>Karyotype with complex rearrangements with an identifiable del(22)(q11).

types of clinical evolutions were observed. First, the karyotype demonstrated complete or partial remission during therapy (cases 15, 20, and 24). Secondly, persistence of clonal abnormalities corre-

lated with lack of response to treatment in case 27. And finally, the appearance of complex structural rearrangements in cases 37 and 46 was followed by the early death of both patients.

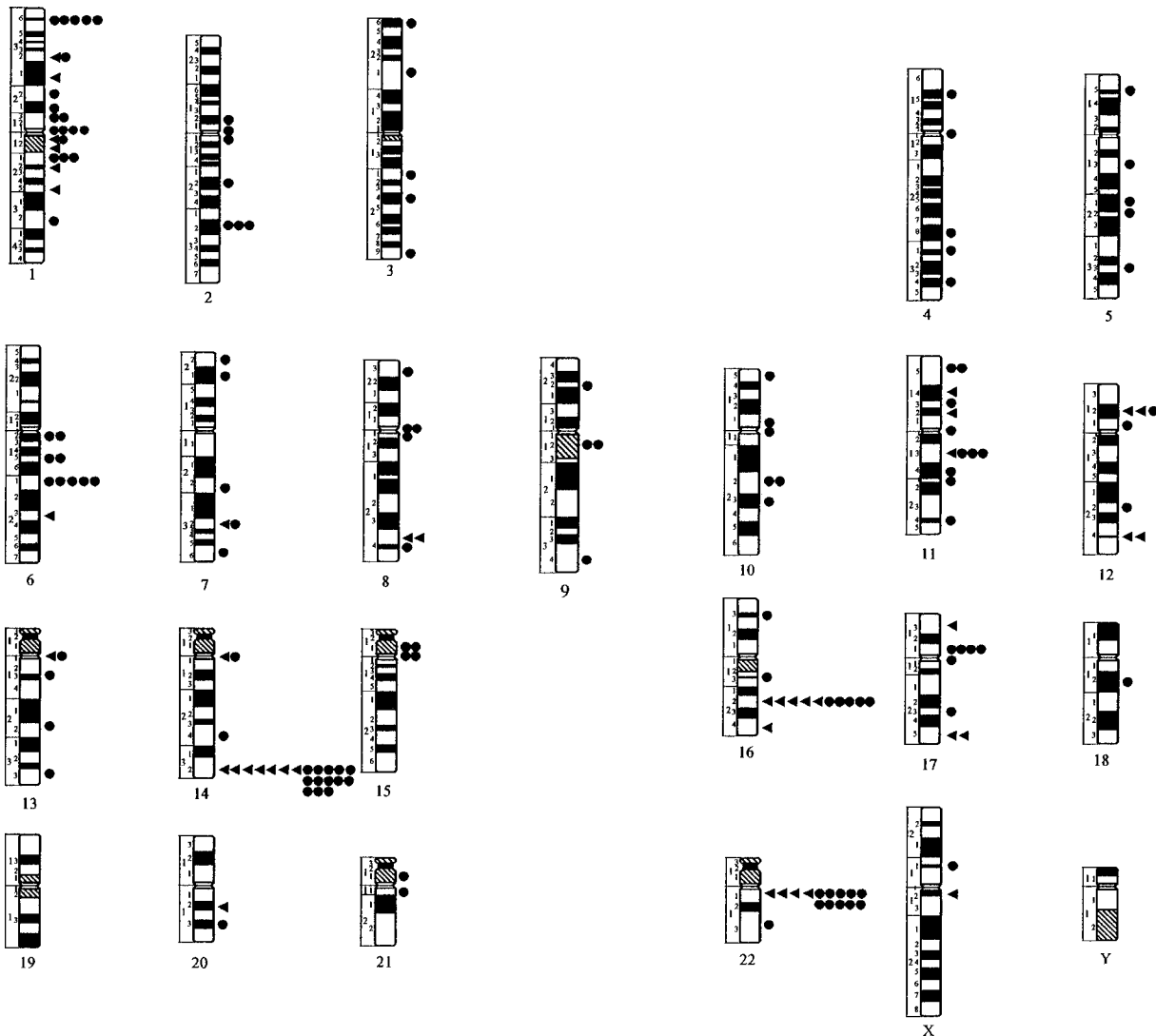


Figure 1. Diagrammatic representation of the 24 primary and 81 secondary chromosomal breakpoints involved in structural abnormalities in the 88 patients. Clustering was noted on chromosome bands 14q32, 16q22, and 22q11. Triangles represent primary breakpoints and circles represent secondary breakpoints.

### Clinical Parameters and Karyotype

In order to confirm our preliminary results, contingency tests were performed to determine the influence of the clinical parameters recorded for each patient on the chromosomal aberration rate. Stage, bone lesions,  $\beta$ 2-microglobulin level, BM plasma cell infiltration, and previous chemotherapy influenced the karyotype. The remaining parameters showed no statistically significant relationship with the abnormality rate.

As shown in Table 5, a highly significant relation existed between the presence of an abnormal karyotype and stage III, levels of  $\beta$ 2-microglobulin greater than 4 mg/L, lytic bone lesions, more than

30% of BM plasma cell infiltration, and previous chemotherapy.

### DISCUSSION

To find specific chromosome abnormalities in MM has been difficult, mostly owing to the low mitotic index of the cells implicated in the disease. Many efforts have been made to obtain an elevated number of cells suitable for cytogenetic analysis. The commonest strategies have been the use of hemopoietic growth factors such as cytokines (Facon et al., 1993; Laï et al., 1995; Smadja et al., 1995) or other known B-cell mitogens such as lipopolysaccharide (Taniwaki et al., 1994) or pokeweed (Cigu-

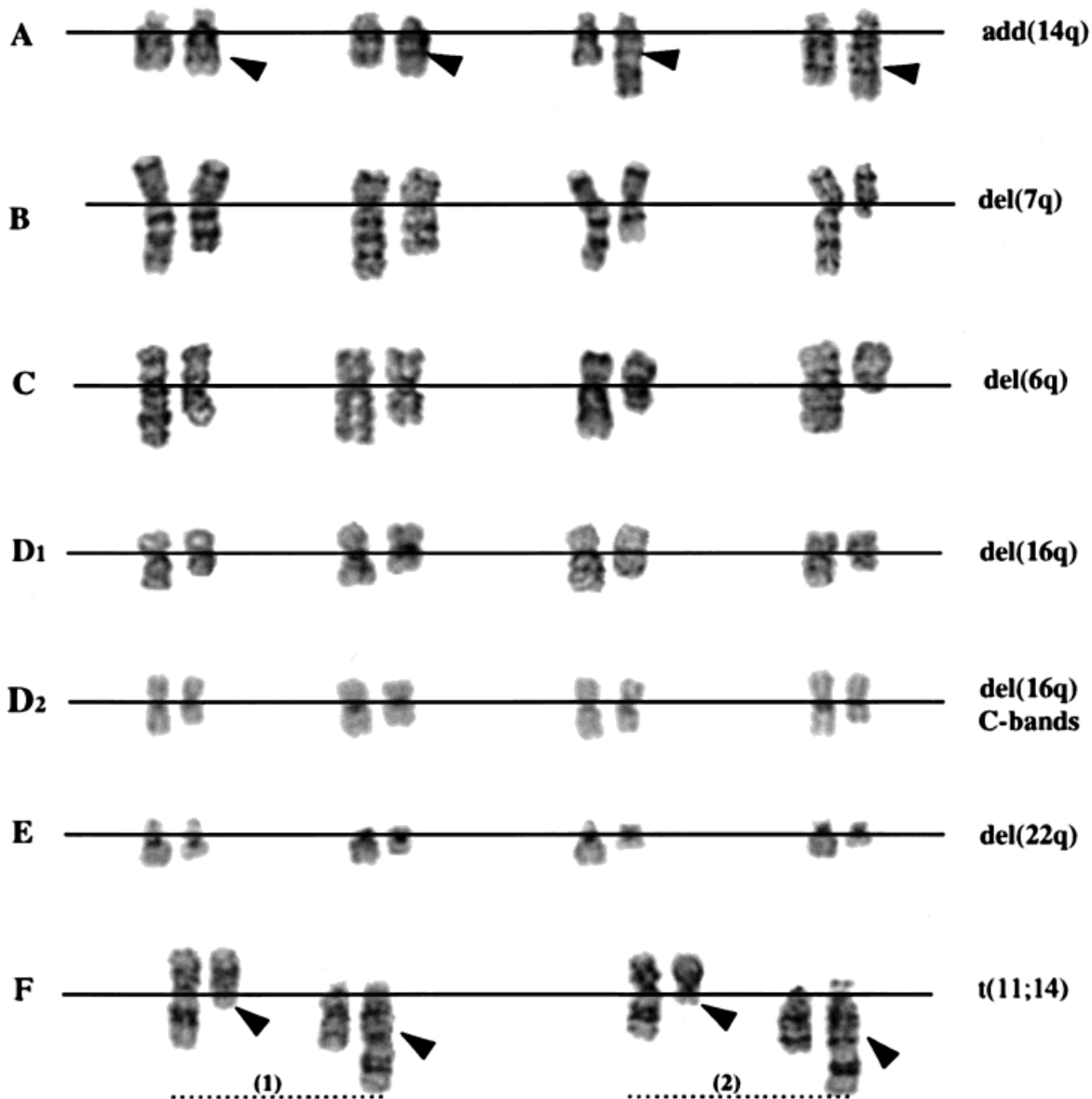


Figure 2. Structural abnormalities involving chromosomes 6, 7, 11, 14, 16, and 22. **A:** Four examples of  $\text{add}(14)(q32)$ ; **B:** four examples of  $\text{del}(6q)$ ; **C:** four examples of  $\text{del}(7q)$ ; **D1:** four examples of G-banded  $\text{del}(16q)$ ; **D2:** four examples of C-banded  $\text{del}(16q)$ ; **E:** four examples of  $\text{del}(22)(q11)$ ; **F:** two examples of the  $\text{t}(11;14)(q13;q32)$  from MM patient 37, obtained from two metaphase cells (1) and (2). Arrowheads indicate breakpoints.

dosa et al., 1994). However, controversial results have been reported when cytokine-stimulated cultures are used. Lai et al. (1995) found that the abnormality detection rate was higher when 2,000 IU/mL of interleukin 6 (IL-6) was used. On the other hand, after using the same concentration of IL-6, Smadja et al. (1995) suggested that, at least for stage III multiple myeloma at diagnosis, a 3 day culture without cytokine was the best technique to detect clonal chromosomal abnormalities. For this

study, the patient samples were collected in three different cytogenetic services which are members of the Spanish Cooperative Group of Hematological Cytogenetics. Only one of them used pokeweed as a mitogen and it was able to examine more than 30 metaphases from each patient (65% of the total number of samples). This method was reported to detect abnormal metaphases in 49% of cases (Cigudosa et al., 1994), which is very close to the proportion observed in studies where cytokines

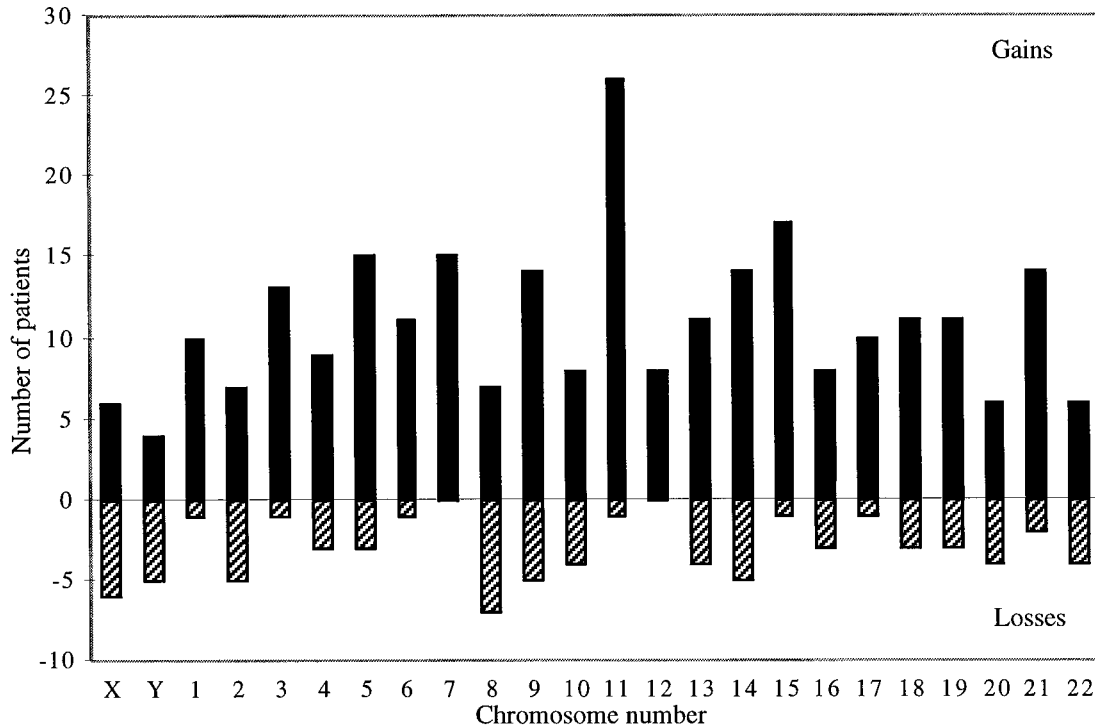


Figure 3. Frequency of numeric changes in 88 patients with abnormal karyotype.

TABLE 4. Clinical Evolution of Karyotype in Six Patients With Multiple Myeloma

Case no.	Date (month/year)	Myeloma protein type	Clinical stage	Previously treated	Metaphases (abnormal/total)	Karyotype
15	3/93 5/93	MM Bence-Jones κ idem	III-A idem	Yes Post-transplantation	(30/30) (12/30)	46,X,del(X)(q11) 46,XX/46,X,del(X)(q11)
20	10/93	MM Bence-Jones λ	II-A	Yes	(24/33)	46,XX/46,X,-X,t(1;16)(p36;p13),-5,+7,+mar/46,X,-X,t(1;16)(p36;p13),del(2)(q11),-5,+7,del(11)(q21),del(20)(q13)
27	4/94 4/94	idem MM IgA κ	idem II-A	Yes At diagnosis	(0/30) (25/50)	46,XX 46,XY/55,XY,+Y,+r(3),+5,+7,+8,+9,+11,+15,+17,+19
	12/94	idem	idem	Yes	(9/87)	46,XY/55,XY,+Y,+r(3),+5,+8,+9,+15,+17,+19,+mar
	1/95	idem	idem	Yes	(20/100)	46,XY/55,XY,+Y,+r(3)(p26q29),+4,+dup(7)(p21),+9,+11,+15,+17,+19
24	12/94 5/95	MM IgG κ idem	III-A idem	At diagnosis Yes	(30/30) (27/40)	46,XX,del(16)(q22) 46,XX/46,XX,del(16)(q22)
37	12/93 11/95	MM Bence-Jones λ idem	III-B idem	At diagnosis Yes (exitus)	(0/28) (36/42)	46,XY 46,XY,del(5)(q13q22),der(10)t(1;10)(q11;p11),t(11;14)(q13;q32),der(14)t(11;14)(q13;q32)/46,XY,del(5)(q13q22),t(11;14)(q13;q32),der(12)t(1;12)(q11;p11),der(14)t(11;14)(q13;q32)
46	1/92 8/93	MM IgG λ idem	II-B III-B	Yes Yes (exitus)	(0/50) (25/25)	46,XY 46,XY,del(7)(q36),-9,+11,del(16)(q?),add(17)(q?)/51,XY,del(7)(q36),-9,+11,del(16)(q?),add(17)(q?),+5mar



TABLE 5. Significant Dependence Between Clinical Parameters and Chromosomal Aberration Rate

Clinical parameter	No. of patients with abnormal karyotype (%)	P value
$\beta$ 2-Microglobulin (mg/L)		
≤4	18 (24)	0.0001
>4	39 (57)	
Bone marrow infiltration (%)		
≤30	13 (19)	0.0001
>30		
	48 (62)	
Stage		
I	6 (12)	0.0001
II	16 (47)	
III	39 (65)	
Lytic lesions		
No	16 (24)	0.0009
Yes	40 (51)	

were used (Lai et al., 1995). Nevertheless, when all samples here presented were taken together, the proportion of cases with chromosomal abnormalities was lower (31%) and similar to the proportion reported by other groups which did not use mitogens or growth factors (Dewald et al., 1985; Weh et al., 1993; Sawyer et al., 1995).

Only 32% of the karyotypically abnormal patients had chromosome anomalies in all metaphases; in the remaining cases, mosaicism with a varying proportion of normal cells was noted (Table 2). It has been confirmed that normal metaphases in MM originate from hematopoietic cells other than plasma cells (Weh et al., 1990; Guthensohn et al., 1992; Weh et al., 1993). For this reason, until a more accurate system of culturing plasma cells is detected, we suggest the use of any medium that allows an exhaustive analysis of a large number of metaphases to avoid misinterpretation of normal karyotypes.

The breakpoint analysis in our series points out the existence of three bands that are primarily implicated in MM (Fig. 1): 14q32, 16q22, and 22q11. The essential role played by these bands is confirmed by their persistence also as common secondary breakpoints.

As expected from previous reports, rearrangements of 14q32 were the most frequent, found in 23% of the abnormal cases. This proportion falls within the reported range for MM when larger series are analyzed (Dewald et al., 1985; Weh et al., 1993; Lai et al., 1995; Sawyer et al., 1995). The high prevalence of t(11;14)(q13;q32) was also observed in our series and identified as the most frequent rearrangement of band 14q32 (three cases out of the

five samples where the structural rearrangement of this band was fully characterized). We found rearrangements affecting 16q22 in 10% of the abnormal cases. This anomaly has been rarely reported in MM. Recently, Sawyer et al. (1995) described monosomy for chromosome 16 in 12 out of 63 abnormal patients and deletion of 16q in another 3. They noticed that this monosomy, together with monosomy 13, occurred in 7 of the 8 untreated patients of their series. Based on these data, they suggested that the loss of chromosomes or chromosome segments may have a significant role in the cytogenetic progression of MM. We fully agree with this suggestion since we have also observed monosomy or loss of 16q material in a relatively high proportion of abnormal cases. More specifically, four patients showed a deletion del(16)(q22) as the sole anomaly in their karyotype.

We detected structural rearrangements of band 22q11, mostly del(22)(q11), in 16% of the abnormal cases. This anomaly has rarely been described as a primary change in MM (Van den Berghe et al., 1979; Karpas et al., 1982; Cigudosa et al., 1994) although it was found as a secondary change in most of the reports where more than 100 patients were studied (Weh et al., 1993; Lai et al., 1995; Sawyer et al., 1995). The observed frequency of aberrations affecting 22q11 ranges from 5% found in cytokine stimulated cultures (Lai et al., 1995) to 16% in the present study, where a B-cell mitogen was used.

Numerical changes were the same as those seen in previous studies and involved gains of chromosomes 3, 5, 7, 9, 11, 15, 19, and/or 21. A similarly striking pattern has already been described for thyroid tumors (Belge et al., 1994).

Based on DNA aneuploidy (García-Sanz et al., 1995) and FISH studies (Drach et al., 1995a), it is accepted that numerical chromosomal changes are present in the majority (80–90%) of MM cases. Moreover, aneuploidy has also been detected in more than 50% of MGUS patients (Drach et al., 1995b). Thus, it seems clear that some chromosomally abnormal cases escaped cytogenetic analysis. However, it should be underlined that the DNA-ploidy and FISH approaches tell us nothing about the distinct structural aberrations or the primary breakpoints involved in the pathogenesis of this disease. It is also important to notice that, so far, no structural cytogenetic aberrations have been described for MGUS patients. We present here 11 cases of MGUS which showed chromosomal rearrangements. Their karyotypic features were similar to those described for MM with primary involve-

ments of 14q32, 16q22, and 22q11. For this reason, they have been managed as MM patients only from the cytogenetic point of view.

The clinical importance of cytogenetic analysis in MM patients is evident. As a method to monitor treatment, it can be used to detect progression or lack of response (Table 3). Of the six patients who were cytogenetically monitored during clinical management, it is noteworthy that those with 16q22 rearrangement showed complete or partial disappearance of the abnormal clone. It has been recently reported that partial or complete deletions of chromosome 13 or abnormalities involving 11q are associated with a poor prognosis (Tricot et al., 1995). For this purpose, a complete statistical survival analysis is being performed at our center to detect the prognostic value of the different chromosomal anomalies, but the study period is not yet closed and this issue will be addressed in the future. As we have previously reported in a series of 41 patients, the incidence of chromosomal anomalies was influenced by some clinical parameters (Cigudosa et al., 1994). In this report, which includes 280 patients, we confirm our results and obtain a higher statistical significance (Table 5) for the association between an abnormal karyotype and a high level of  $\beta_2$ -microglobulin, dense bone lesions, bone marrow plasma cell infiltration, and stage III, which are all characteristics of advanced disease.

## REFERENCES

- Ankantil R, Madhavan J, Gangadharan VP, Pillai GR, Nair MK (1995) Nonrandom karyotype abnormalities in 36 multiple myeloma patients. *Cancer Genet Cytogenet* 83:71-74.
- Belge G, Thode B, Rippe V, Bartnizke S, Bullerdiek K (1994) A characteristic sequence of trisomies starting with trisomy 7 in benign thyroid tumors. *Hum Genet* 94:198-202.
- Cigudosa JC, Calasanz MJ, Odero MD, Prosper F, Etxanziz A, Marin J, Rifon J, Gullon A, Rocha E (1994) Cytogenetic data in 41 patients with multiple myeloma. Karyotype and other clinical parameters. *Cancer Genet Cytogenet* 78:210-213.
- Dewald GW, Kyle RA, Hicks GA, Greipp PR (1985) The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. *Blood* 66:380-390.
- Drach J, Shcuster J, Nowotny H, Angerler J, Rosenthal F, Fielg M, Rothermundt C, Gsur A, Jäger U, Heinz R, Lechner K, Ludwig H, Huber H (1995a) Multiple myeloma: High incidence of chromosomal aneuploidy as detected by interphase fluorescence in situ hybridization. *Cancer Res* 55:3954-3959.
- Drach J, Angerler J, Shcuster J, Rothermundt C, Thalhammer R, Haas OA, Jäger U, Fielg M, Geissler K, Ludwig H, Huber H (1995b) Interphase fluorescence in situ hybridization identifies chromosomal abnormalities in plasma cells from patients with monoclonal gammopathy of undetermined significance. *Blood* 86:3915-3921.
- Durie BGM, Salmon SE (1975) A clinical staging system for multiple myeloma. *Cancer* 36:842-854.
- Facon T, Lai JL, Nataf E, Preudhomme C, Zandecki M, Hammad M, Wattel E, Jouet JP, Bauters F (1993) Improved cytogenetic analysis of bone marrow plasma cells after cytokine stimulation in multiple myeloma: a report on 46 patients. *Br J Haematol* 84:743-745.
- García-Sanz R, Orfao A, González M, Moro MJ, Hernández JM, Ortega F, Borrego D, Carnero M, Casanova F, Jiménez R, Portero JA, San Miguel JF (1995) Prognostic implications of DNA aneuploidy in 156 untreated multiple myeloma patients. *Br J Haematol* 90:106-112.
- Guthensohn K, Weh HJ, Walter TA, Hossfeld DK (1992) Cytogenetics in multiple myeloma and plasma cell leukemia: Simultaneous cytogenetic and cytologic studies in 51 patients. *Ann Hematol* 65:88-90.
- ISCN (1991): Guidelines for Cancer Cytogenetics. Supplement to an International System for Human Cytogenetic Nomenclature. Mitelman F (ed). Basel: S Karger.
- Karpas A, Fisher P, Swirsky D (1982) Human plasmacytoma cell line carrying a Philadelphia chromosome. *Science* 216:997-999.
- Lai JL, Zandecki M, Mary JY, Bernardi F, Zydzorczy KV, Flactif M, Morel P, Jouet JP, Bauters F, Facon T (1995) Improved cytogenetics in multiple myeloma: A study of 151 patients including 117 patients at diagnosis. *Blood* 85:2490-2497.
- Lewis JP, Mackenzie MR (1984) Non-random chromosomal aberrations associated with multiple myeloma. *Hematol Oncol* 2:307-317.
- Lisse IM, Drivsholm A, Christoffersen P (1988) Occurrence and type of chromosomal abnormalities in consecutive malignant monoclonal gammopathies: Correlation with survival. *Cancer Genet Cytogenet* 35:27-36.
- Sawyer JR, Waldrom JA, Jagannath S, Barlogie B (1995) Cytogenetic findings in 200 patients with multiple myeloma. *Cancer Genet Cytogenet* 82:41-49.
- Smadja NV, Louvet C, Isnard F, Dutel J-L, Grange M-J, Varette C, Krulik M (1995) Cytogenetic study in multiple myeloma at diagnosis: Comparison of two techniques. *Br J Haematol* 90:619-624.
- Taniwaki M, Nishida K, Takashima T, Nakagawa H, Fujii H, Tamaki T, Shimazaki C, Horiike S, Misawa S, Abe T, Kashima K (1994) Nonrandom chromosomal rearrangements of 14q32 and 19p13.3 and preferential deletion of 1p in 21 patients with multiple myeloma and plasma cell leukemia. *Blood* 84:2283-2290.
- Tricot G, Barlogie B, Jagannath S, Bracy D, Mattox S, Vesole DH, Naucke S, Sawyer JR (1995) Poor prognosis in multiple myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. *Blood* 86:4250-4256.
- Van den Berghe H, Louwagie A, Broeckaert-Van Orsoven A, David G, Werwilghem R, Michaus JL, Sokal G (1979) Philadelphia chromosome in human multiple myeloma. *J Natl Cancer Inst* 63:11-16.
- Van den Berghe H, Vermaelen K, Louwagie A, Criel A, Mecuci C, Vaerman JP (1984) High incidence of chromosomal abnormalities in IgG3 myeloma. *Cancer Genet Cytogenet* 11:381-387.
- Weh HJ, Fiedler W, Hossfeld DK (1990) Cytogenetics in multiple myeloma. Are we studying the "right" cell? *Eur J Haematol* 45:236-237.
- Weh HJ, Guthensohn K, Selbach J, Kruse R, Wacker-Backhaus G, Seeger D, Fiedler W, Fett W, Hossfeld DK (1993) Karyotype in multiple myeloma and plasma cell leukemia. *Eur J Cancer* 29A:1269-1273.