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New Recurrent Chromosome Alterations in Patients with Multiple Myeloma and Plasma Cell Leukemia

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Key Words

Chromosome alterations · Cytogenetics · Fluorescence in situ hybridization · Multiple myeloma · Plasma cell leukemia

Abstract

Chromosome abnormalities detected in metaphases from multiple myeloma (MM) cells have a clear impact on prognosis and response to therapy. Thirteen out of 50 (26%) patients with plasma cell disorders and abnormal karyotypes (11 with MM and 2 with plasma cell leukemia (PCL)) were selected for inclusion in the present report based on the presence of karyotypes with new and/or infrequent structural aberrations. Thirty-three new rearrangements, including a novel recurrent aberration: psu dic(5;1)(q35;q10), were detected. Chromosome 1 was the most frequently involved. Gains of genetic material (57%) were noted more frequently than losses (43%). Three rearrangements that were observed only once in the literature appear to be recurrent from our data: del(16)(q13), del(5)(p13) and i(3)(q10), the latter being a single structural aberration in the karyotype. Clinical parameters from our series were compared with 2 control groups: 20 MM cases with recurrent aberrations in MM/PCL with a similar distribution of abnormalities associated with poor prognosis (group 1), and 40 with normal karyotypes and fluorescence in situ hybridization analysis (group 2). Significantly increased serum calcium levels ($p = 0.022$) in patients with new and/or infrequent chromosome changes with respect to both control groups, and a higher percentage of bone marrow plasma cell infiltration ($p = 0.005$), β_2 microglobulin, and lactate dehydrogenase levels ($p < 0.0001$) compared to group 2 were observed. Our results suggest that some of these novel rearrangements may be capable to deregulate genetic mechanisms related to the development and/or progression of the disease. The finding of new recurrent aberrations supports this hypothesis.

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Multiple myeloma (MM) is a clonal plasma-cell dyscrasia characterized by the accumulation of malignant plasma cells within the bone marrow (BM) and the presence of a monoclonal immunoglobulin in the serum and/

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or urine. Clinically, patients with MM usually present bone pain related to osteolytic lesions, frequent anemia and, less often, renal impairment [Kyle and Rajkumar, 2004]. This B-cell malignancy shows a high degree in biological and genetic heterogeneity at presentation and a great variability in clinical course, with patients evolving in a few weeks, while others may enjoy a more than 10-year survival. Plasma cell leukemia (PCL) is the most aggressive presentation of the plasma cell neoplasms and is characterized by circulating plasma cells $>2\times10^9$/l in peripheral blood or by a relative plasmacytosis >20% of blood leukocytes. It is a rare entity occurring in about 2% of plasma cell myeloma that may be classified as primary when it is the initial presenting manifestation of MM, or secondary when it is seen in the context of refractory or relapsed disease [McKenna et al., 2008].

Cytogenetic and molecular genetic studies of myeloma cells have provided evidence that virtually all cases of MM are characterized by chromosomal aberrations [Fonseca et al., 2009]. Careful analyses of large series have demonstrated 2 different cytogenetic subgroups: hyperdiploid and non-hyperdiploid [Fonseca et al., 2003; Smadja et al., 2003]. The former comprises a heterogenic group of tumors with multiple trisomies of odd numbered chromosomes and appears to have a slightly favorable outcome. In contrast, the non-hyperdiploid group is characterized by pseudo-, hypo- or tetraploidy, and a high frequency of translocations affecting the *IGH@* (immunoglobulin heavy chain) locus at 14q32, being associated with unfavorable prognosis. In MM, different studies have confirmed the clear impact of the abnormalities detected in metaphase on prognosis and response to therapy [Chiecchio et al., 2006; Zhan et al., 2006a]. These abnormal metaphases are usually very complex and include a wide variety of overrepresentations, losses and rearrangements of different chromosomal regions that may harbor target genes for the biological behavior of the tumor [Beà and Campo, 2008]. The profile of these secondary aberrations is considered to be relatively specific to each type of tumor. Among them, structural aberrations of chromosome 1, particularly 1p deletions and 1q amplifications, are the most common additional changes in plasma cell disorders, being found in up to 45% of MM and in almost all PCL patients [Hanamura et al., 2006; Chang et al., 2010].

In this context, we focused our study on describing structural rearrangements not previously reported in plasma cell disorders and their correlation with clinical characteristics of patients. New recurrent aberrations were also found.

Materials and Methods

Patients

From a total of 50 patients with plasma cell disorders and abnormal karyotypes cytogenetically studied in our laboratory, 13 cases (26%) (7 males; mean age 64.5 years; range 45–80 years) were selected for inclusion in the present report based on the presence of karyotypes with new (not previously described) or infrequent (reported only once in the literature) structural rearrangements. The diagnosis was made according to the standard criteria [Kyle and Rajkumar, 2004]. Patients were staged following the Durie and Salmon [1975] classification and the International Staging System [Greipp et al., 2005]. Eleven patients had a diagnosis of MM and 2 cases had primary PCL. Cytogenetic and FISH analysis were performed at diagnosis in 9 patients, while in the remaining 4 were at relapse. Six patients (46%) died at the moment of this study. To compare clinical parameters, we selected 2 control groups: one of them including 20 patients (8 males; mean age 59.6 years; range 33–79 years) with recurrent structural chromosome abnormalities (group 1) and another one of 40 MM cases with normal karyotype and without genomic aberrations by FISH analysis (25 males; mean age 69 years; range 46–86 years) (group 2). Patients included in group 1 had a similar distribution of abnormalities associated with poor prognosis in MM (del(17p13), t(4;14), t(14;16), t(14;20)) as cases with new or infrequent structural anomalies, except for chromosome 1q rearrangements that were more represented among novel aberrations. Age, sex, stage at diagnosis and clinical characteristics of our series and control groups are summarized in table 1. All patients provided their informed consent. The study was approved by the ethics committee of our institution.

Cytogenetic Analysis

BM cells were processed for cytogenetic analysis by direct method and/or short-term (24–48 h) culture, in F-12 medium supplemented with 20% fetal calf serum. G-, C- and DAPI-C banding techniques were used. Karyotype abnormalities were described according to the International System of Human Cytogenetic Nomenclature [ISCN, 2009].

FISH Analysis

For FISH analysis, slides were hybridized with the following locus-specific probes: LSI 13 (RB1) at 13q14 band, LSI TP53 at 17p13.1 (Vysis-Abbott) and IGH FISH DNA Probe Split Signal at 14q32 (Dako), according to the manufacturers' protocols. Four hundred interphase nuclei from patients and 10 controls were scored for each probe. The cut-off for positive values (mean of normal controls + 3 standard deviations) was: 9%, 5.5% and 2% for monosomies of RB1 and TP53, and for IGH rearrangements, respectively. CEP1 (satellite III DNA) (1q12), LSI CCND1/IGH XT Dual Color Dual Fusion Translocation Probe (11q13/14q32) and LSI IGH/FGFR3 Dual Color Dual Fusion Translocation Probe (4p16/14q32) (Vysis-Abbott), biotin-labeled whole chromosome painting (WCP) probes for different chromosomes (CAMBIO) and Spectra Vysion WCP probe (Vysis-Abbott) were also used. In each case, a minimum of 10 informative metaphases were analyzed. Image acquisition was performed using Cytovision 3.9 Software (Applied Imaging Corporation, Calif., USA).

Table 1. Clinical characteristics of patients with plasma cell disorders

Characteristics	Our series	Group 1	Our series vs. Group 1, p	Group 2	Our series vs. Group 2, p	Group 1 vs. Group 2, p
Cases, n	13	20		40		
Sex (F/M)	6/7	12/8	0.493	15/25	0.746	0.110
Median age (range), years	64.5 (45–80)	59.6 (33–79)	0.284	69 (46–86)	0.196	**0.004**
Paraprotein isotype, %						
IgG	55.6	66.7	0.697	65.8	0.508	0.413
IgA	33.3	25		31.6		
IgM	11.1	0		2.6		
IgD	0	8.3		0		
Type of light chain, %						
κ	66.7	66.7	1.000	68.4	1.000	1.000
λ	33.3	33.3		31.6		
ISS						
I	22.2	20	0.956	29	0.871	0.855
II	33.3	40		35.5		
III	44.5	40		35.5		
DS stage, %						
I	11.1	0	0.462	23.1	0.696	0.162
II	11.1	15.4		12.8		
III	77.8	84.6		64.1		
BMI, %						
<30	0	21.4	0.207	40	**0.005**	0.235
30–60	20	28.6		34.3		
>60	80	50		25.7		
Lytic bone lesions, %	82	82	1.000	50	0.086	0.086
Mean β_2 microglobulin (range), μg/ml	5.49 (0.28–9.55)	4.89 (0.2–12)	0.762	1.1 (0.11–8)	**<0.0001**	**<0.0001**
Median LDH (range), U/I	338.5 (110–536)	291 (123–834)	0.503	139 (84–363)	**<0.0001**	**<0.0001**
Mean serum calcium (range), mg/dl	10.10 (8.9–12)	8.91 (8–12)	**0.022**	8.97 (7.7–10.6)	**<0.0001**	0.841
Mean serum albumin (range), g/dl	3.22 (2.7–3.85)	3.32 (2.7–4.7)	0.675	3.65 (2.7–4.7)	0.057	0.136
Mean haemoglobin (range), g/dl	9.36 (4.6–12.6)	10.45 (6.9–15)	0.345	10.86 (5.8–15.8)	0.157	0.591
Mean creatinine (range), mg/dl	1.38 (0.8–3)	1.21(0.6–3.4)	0.561	1.92 (0.6–11.3)	0.422	0.226
Mean paraprotein (range), g	3.93 (0.3–9.4)	4.28 (0.2–8)	0.835	3.15 (0.11–9.48)	0.503	0.306

BMI = Bone marrow infiltration; DS = Durie and Salmon; ISS = International Staging System; LDH = lactate dehydrogenase. Significant values are indicated in bold.

Statistical Methods

Groupwise comparison of the distribution of clinical and laboratory variables was performed with the Student t test (for quantitative variables) and the Fisher's exact test (for categorical variables). Overall survival was estimated by the Kaplan-Meier method and compared by the log-rank test. For all tests, $p < 0.05$ was regarded as statistically significant.

Results

Cytogenetic and FISH analysis of 13 patients with MM/PCL are shown in table 2. A total of 33 chromosome structural aberrations not previously described in the literature [Mitelman Database, 2011] were found that involved 23 translocations (19 unbalanced), 2 duplications, 1 deletion, 3 pseudo-dicentric chromosomes including 2 cases with psu dic(5;1)(q35;q10), and 4 complex rearrangements, one of them as a part of a complex pseudodicentric marker (table 3). The distribution of all abnormalities is shown in figure 1. Almost all patients, except cases 6 and 11, showed complex karyotypes. In most of them (84.6%), a mixture of normal and abnormal metaphases was observed. Only cases 4 and 5 had exclusively abnormal metaphases. The modal number distribution was: hypodiploid (5 cases), diploid (1), hypo-/pseudodiploid (3) and hyperdiploid (4).

Chromosome 1 was the most frequently involved in new structural aberrations, showing 11 aberrations in 7 cases including 2 cases with the same anomaly (table 3), most of them leading to 1q gains (73%). Total chromosome 1 aberrations included 4 unbalanced translocations, 3 pseudo-dicentric chromosomes, one of them recurrent in our series: psu dic(5;1)(q35;q10), and 4 complex rearrangements showing segmental partial dupli-

Table 2. Cytogenetics and FISH results in patients with plasma cell disorders with novel or infrequent structural alterations

Case	Age/ Sex	Diag- nosis	Moment of the study	Combined karyotype (G-banding and FISH)	FISH (%)		
					IGH	RB1	TP53
1*	61/M	MM	R	45–46,XY,–1,der(1)del(1)(p36)t(1;1)(p36;q25),psu dic(5;1)(q35;q10), del(5)(q13),+10,add(11)(p15),del(11)(p11),der(13)t(2;13)(q11;p11), del(16)(q13)[cp6]/46,XY,del(16)(q13)[cp4]/46,XY[9]	1.6	**13.2**	ND
2	63/F	MM	D	43–44,X,+2,–4,–6,t(8;17)(q22;p13),t(11;14)(q13;q32),+der(14)t(13;14)(q14;p11), –18[cp9]/46,XX[9]	**5.1**	1.5	1
3	45/M	MM	D	51,XY,del(1)(p11),+der(3)t(2,3)(q21;q21),der(5)t(3;5)(q11;q33),del(6)(q23), i(7)(q10),+9,+10,–13,del(17)(p11),+20,+21,+22[cp3]/46,XY[10]	**4.7**	**25.5**	**6.6**
4*	52/M	MM	R	40–43,X,–X,der(2)t(1;2)(q25;q35),del(5)(q22),–7,–9,–16,i(17)(q10),–18,i(21)(q10), –21,–22[cp14]	0.5	6.1	**24.9**
5*	70/M	MM	R	51–53,Y,psu dic(X;1)(q24;p11),psu dic(1;11) ins inv(1;11)(q10;p11q25)dup(1p?) dup(11q?),+del(3)(p13),+del(6)(q23),der(7)t(7;7)(p22;q32),+9,+der(12)t(X;12) (q13;q13),+13,+15,–16,dup(16)(q12q22),+der(19)t(X;19)(q26;q13),+der(19)t(16;19) (q12;q11),+mar[cp27]	0.3	3.1	2.6
6	80/F	MM	D	48,XX,+X,+i(3)(q10)[cp16]/46,XX[4]	0.7	4.1	ND
7	73/M	MM	R	46,XY,der(1)t(1;4)(p12;p12) dup(1)(q25q32),+dup(5)(q13q31),+der(1;7) (p10;q10),der(8)t(4;8)(q31;p23),+del(9)(q22),der(11)t(11;13) (p15;q14),+14,+15, –16,der(20)t(1;20)(p13;p11),der(21;22)(q10;q10)[cp17]/46,XY[29]	1.7	7.4	2.3
8*	72/F	MM	D	47–52,X,der(X)t(X;1)(q28;q25),der(1)t(1;21)(q44;q11),+del(1)(p13), +3,+5,+7,+11,+15,der(16)t(16;17)(q24;q21),+19,–20[cp5]/46,XX[38]	1.1	3.3	4.3
9	64/M	MM	D	46,XY,t(6;13)(p25;q14),del(12)(p12.2),+del(12)(p12.2),–19[cp5]/46,XY[10]	1.9	5.6	**5.8**
10*	51/F	MM	D	45,X,–X,der(2)t(2;2)(p21;q21),del(3)(q13),del(3)(q25),add(4)(p12),del(5)(p12), psu dic(5;1)(q35;q10),del(6)(q21),del(11)(q23),t(11;14)(q23;q32),del(12)(q12q14), +12,–13[cp12]/46,XX[18]	**74**	**73.4**	**12.5**
11	86/F	MM	D	46,XX,t(4;14)(q32;q11)[23]/46,XX[7]	1.3	2.4	1.8
12	74/F	pPCL	D	44–45,XX,del(6)(q21q23),der(10)t(10;10)(p15;q11),–9,–11,–12,der(14)t(2;14) (p13;q32),+del(21)(q22),+r[cp6]/46,XX[7]	**16.4**	5.5	**14**
13*	53/M	pPCL	D	41–43,X,–Y,der(1;15)(q10;p11)ins(1)(q32;q12),+del(1)(p13),t(4;20)(p15.2;p13), del(5)(p13),–7,–8,del(10)(p12.2),–13,–14,t(14;16)(q32;q23),der(16)t(16;17) (q12;q21),+21,del(22)(q11.2),+mar[cp34]/46,XY[3]	**55.9**	**32.8**	**42.5**

D = Diagnosis; MM = multiple myeloma; ND = not determined; pPCL = primary plasma cell leukemia; R = relapse; * = dead patient. FISH abnormal clones are indicated in bold.

cations (fig. 2). Among them, a result of particular interest is the marker chromosome of case 5 originated from a translocation of almost the entire chromosome 11 (p13–q25) on 1q10 and subsequent tandem duplications from material of both chromosomes 1 and 11 (fig. 2a) and the aberration der(1;15)(q10;p11)ins(1)(q32;q12) found in case 13 that presented a partial duplication of the heterochromatic region 1q12 inserted at 1q32 (1q32h) (fig. 2d). The analysis of chromosome 1 imbalances showed gains/amplifications in all 7 cases; meanwhile, 2 patients (cases 1 and 5) (28.6%) also presented 1p losses as a part of complex rearrangements. A complete description of gains and losses is shown in figure 3. The smallest region of overlap was 1q25–q32. In addition, further 2 recurring cytogenetic regions of gains, 1q11–q21 and 1q10–qter, and 2 of losses, 1p36–pter and 1p13–p32, were identified.

In reference to the remaining novel anomalies, chromosome 2 showed a total of 5 rearrangements, with a recurrent region of amplification at 2q21–qter. Pairs X, 4, 5, 13 and 16 showed 4 aberrations each, chromosomes 14 and 17 with 3 rearrangements each and the remaining with 2 or 1 rearrangements. Some of the novel aberrations involving chromosomes X, 12, 16 and 19 are shown in figure 4. Recurring losses at bands 5q35–qter, 16q24–qter and 13pter–q14.1 (not affecting the RB1 locus at 13q14.2) were observed. Simultaneously, chromosome 11, which was involved in 2 rearrangements, showed a common genomic amplification at 11q22–qter (fig. 3).

Table 3. New structural chromosome abnormalities in patients with plasma cell disorders

Translocations	Other alterations
der(X)t(X;1)(q28;q25)	psu dic(X;1)(q24;p11)
der(1)t(1;21)(q44;q11)	psu dic(5;1)(q35;q10) [2 cases]
der(2)t(1;2)(q25;q35)	der(1)del(1)(p36)t(1;1)(p36;q25)
der(2)t(2;2)(p21;q21)	der(1)t(1;4)(p12;p12)dup(1)(q25q32)
der(3)t(2;3)(q21;q21)	psu dic(1;11)ins inv(1;11)(q10;p13q25)dup(1p?)dup(1q?)
t(4;14)(q32;q11)	**der(1;15)(q10;p11)ins(1)(q32;q12)**
t(4;20)(p15.2;p13)	**del(10)(p12.2)**
der(5)t(3;5)(q11;q33)	dup(5)(q31q35)
t(6;13)(p25;q14.1)	dup(16)(q12q22)
der(7)t(7;7)(p22;q32)	
der(8)t(4;8)(q31;p23)	
t(8;17)(q22;p13)	
der(10)t(10;10)(p15;q11)	
der(11)t(11;13)(p15;q14.1)	
der(12)t(X;12)(q13;q13)	
der(13)t(2;13)(q11;p11)	
der(14)t(2;14)(p13;q32)	
der(14)t(13;14)(q14.1;p11)	
der(16)t(16;17)(q24;q21)	
der(16)t(16;17)(q12;q21)	
der(19)t(X;19)(q26;q13)	
der(19)t(16;19)(q12;q11)	
der(20)t(1;20)(p13;p11)	

Alterations in PCL cases are indicated in bold.

New structural rearrangements showed a total of 70 breakpoints located at 56 different chromosome bands (fig. 5). Chromosome 1 was the most frequently affected with a total of 16 (22.9%) breakpoints distributed in both arms. The most common recurring bands were 1q25 with 4 breakpoints (5.7%), followed by 1q10, 5q35, 13q14.1 and 16q12 with 3 breakpoints (4.3% each).

Interestingly, we found 3 rearrangements that were observed only once in the literature [Mitelman Database, 2011] and appear to be recurrent in these pathologies from our data: del(16)(q13) and i(3)(q10) (cases 1 and 6, respectively) in MM patients and, del(5)(p13) found in the case 13 with PCL.

For the analysis of clinical parameters (table 1), our series with new rearrangements was compared to patients with recurrent anomalies (group 1) (table 4) and with normal karyotype and FISH analysis (group 2). Cases with new aberrations showed significant increase of bone marrow plasma cell infiltration ($p = 0.005$), β_2 microglobulin (β_2M), lactate dehydrogenase (LDH) and serum calcium ($p < 0.0001$) related to patients of group 2. The comparison with group 1 showed differences in all parameters of poor outcome with significant differences in serum calcium levels ($p = 0.022$). In addition, differences in β_2M and LDH levels between groups 1 and 2 were observed ($p < 0.0001$). The median overall survival of our series was shorter than those of group 1 (22 months and 59 months, respectively), while the curve of group 2 did not achieve the median survival.

Discussion

Cytogenetic methods to detect the presence or absence of particular chromosomal abnormalities are an important key to stratifying patients by risk groups. In plasma cell disorders, it was reported that patients with abnormal metaphases by conventional cytogenetics at diagnosis had active disease and a reduced survival rate compared with those who had only normal metaphases [Dewald et al., 1985; Fonseca et al., 2009]. This difference in prognosis would be related to the ability of myeloma cells to proliferate in vitro outside the context of their BM environment [Fassas and Tricot, 2004; Zhan et al., 2006a]. In this study,

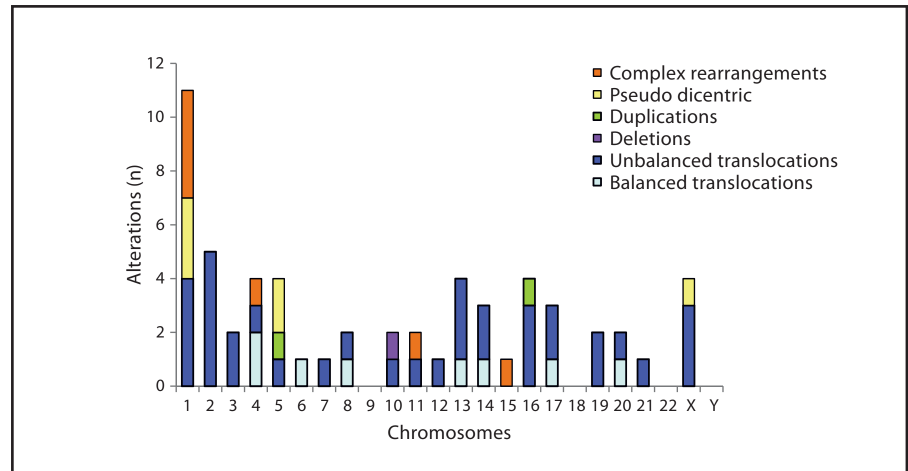

Fig. 1. Histogram showing the distribution of new chromosome rearrangements in MM/PCL patients.

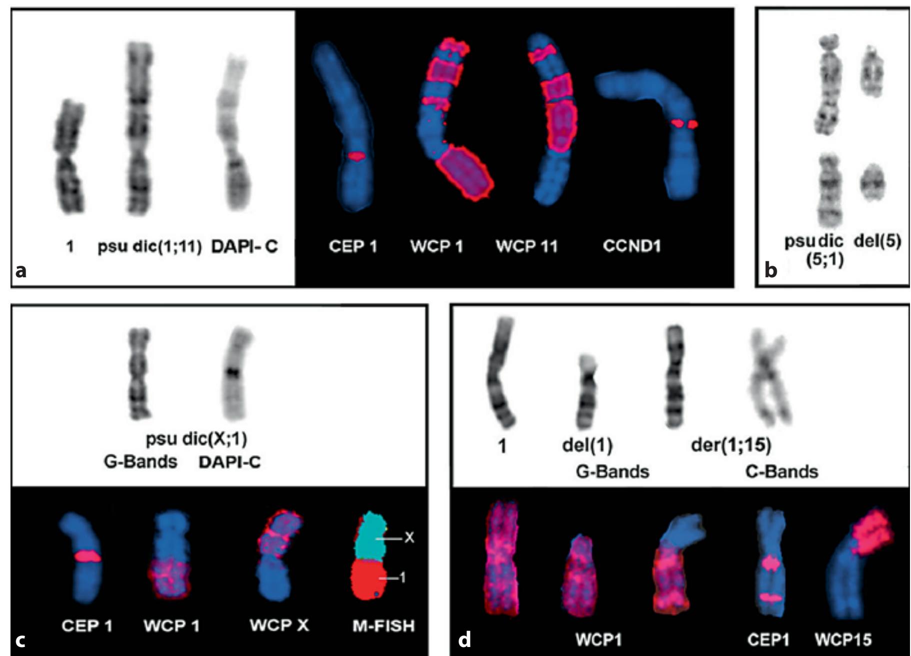

Fig. 2. Partial karyotypes of patients with MM/PCL with new rearrangements of chromosome 1. **a** G- and DAPI-C banding techniques from case 5 showing: normal chromosome 1 and the complex psu dic(1;11) and FISH analysis using CEP1 (satellite III DNA) (1p12), chromosomes 1 and 11 painting probes and CCND1 (11q13) DNA probes. **b** G-banding technique showing psu dic(5;1)(q35;q10) and del(5)(p12) from case 10. **c** G- and DAPI-C banding techniques from case 5 showing a psu dic(X;1)(q24;p11) and FISH analysis using CEP1, chromosomes X and 1 painting probes and M-FISH. **d** G- and C-banding techniques from case 13 showing normal chromosome 1, del(1)(p13) and der(1;15)(q10;p11) ins dup(1)(q32;q12) and FISH analysis with chromosomes 1 and 15 painting probes and CEP1 DNA probe.

we present novel chromosome aberrations in patients with plasma cell disorders and provide new recurrent rearrangements in MM/PCL. Chromosome 1 was the most frequently involved, with a total of 11 new chromosomal aberrations not previously described in the literature [Mitelman Database, 2011]. Interestingly, we found 4 complex rearrangements showing segmental partial duplications that are rare events associated to genomic instability scarcely reported in MM patients [Sawyer et al., 2005]. Among them, we observed a der(1;15) with duplication of 1q12 inserted at 1q32 which determines an aberrant heterochromatin/euchromatin junction. Different authors [Le Baccon et al., 2001; Itoyama et al., 2002] have proposed a role for this molecular anomaly in the pathogenesis of mature B-cell malignancies based on the influence of heterochromatin, which can affect the stability of adjacent euchromatin and genes located within them. A recent report [Sawyer et al., 2009] provides evidence for a novel breakage-fusion-bridge mechanism involving 1q12 pericentromeric heterochromatin associated to clonal evolution and gene amplification in MM. In addition, Sawyer et al. [1998] proposed that extra copies of 1q (whole arm or jumping translocations) may be associated with highly decondensed pericentromeric heterochromatin, which

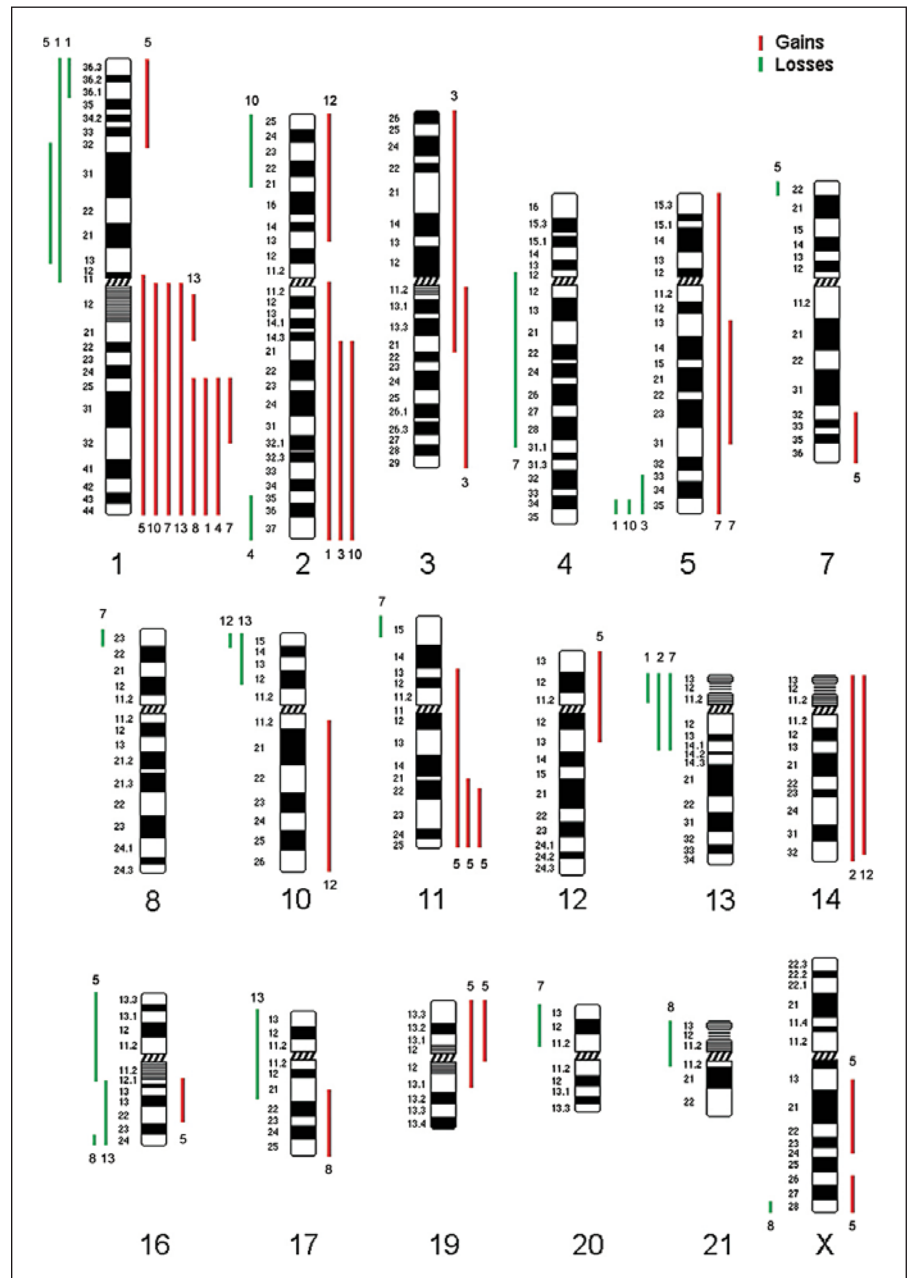

Fig. 3. Genomic imbalances observed in new structural rearrangements in MM/PCL patients. Numbers on the bars indicate the cases with chromosome gains/losses.

may permit recombination and formation of unstable translocations of chromosome 1q with other chromosome arms and/or telomeres of nonhomologous chromosomes. These rearrangements occur as secondary aberrations and are related to tumor progression and advanced disease. Such event was observed in our cases 1 and 10 that presented a novel recurrent structural aberration, psu dic(5;1)(q35;q10), in which the whole 1q arm jumped to 5q35. Both patients showed a poor outcome and died after 22 and 6 months of disease evolution, respectively. Particularly, case 10 presented a very aggressive disease and no response to treatment, being refractory to different protocols with bortezomib, cyclophosphamide, dexamethasone and lenalidomide. Finally, our patients exhibited gains involving bands 1q25–1q32, 1q11–q21 and 1q10–qter, while recurrent genomic losses were observed at 1p36–pter and 1p13–p32 regions. In concordance with these findings, different reports have associated abnormalities of both the short and long arms of chromosome 1 with shorter survival and particularly 1q21 gains, and

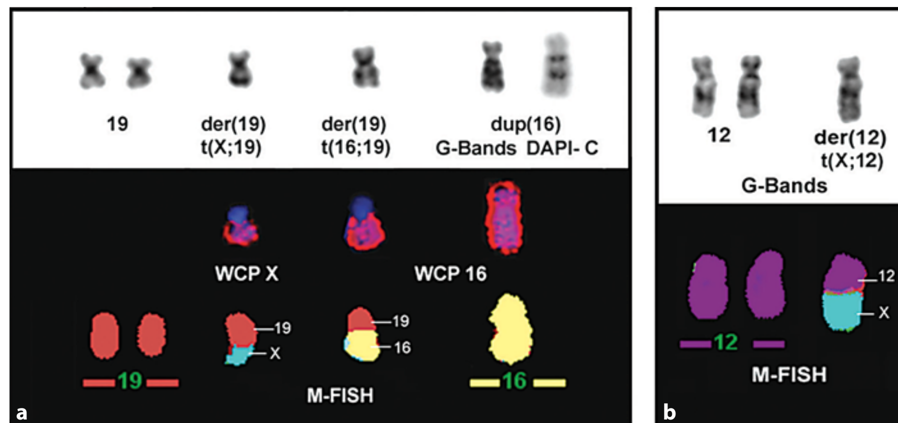

Fig. 4. Partial karyotypes from case 5 showing new rearrangements involving chromosomes X, 12, 16 and 19. **a** G- and DAPI-C banding techniques showing normal chromosomes 19, der(19)t(X;19)(q26;q13), der(19)t(16;19)(q12;q11) and dup(16)(q12q22) confirmed by FISH using chromosomes X and 16 painting probes and M-FISH. **b** G-banding technique and M-FISH showing normal chromosomes 12 and der(12)t(X;12)(q13;q13).

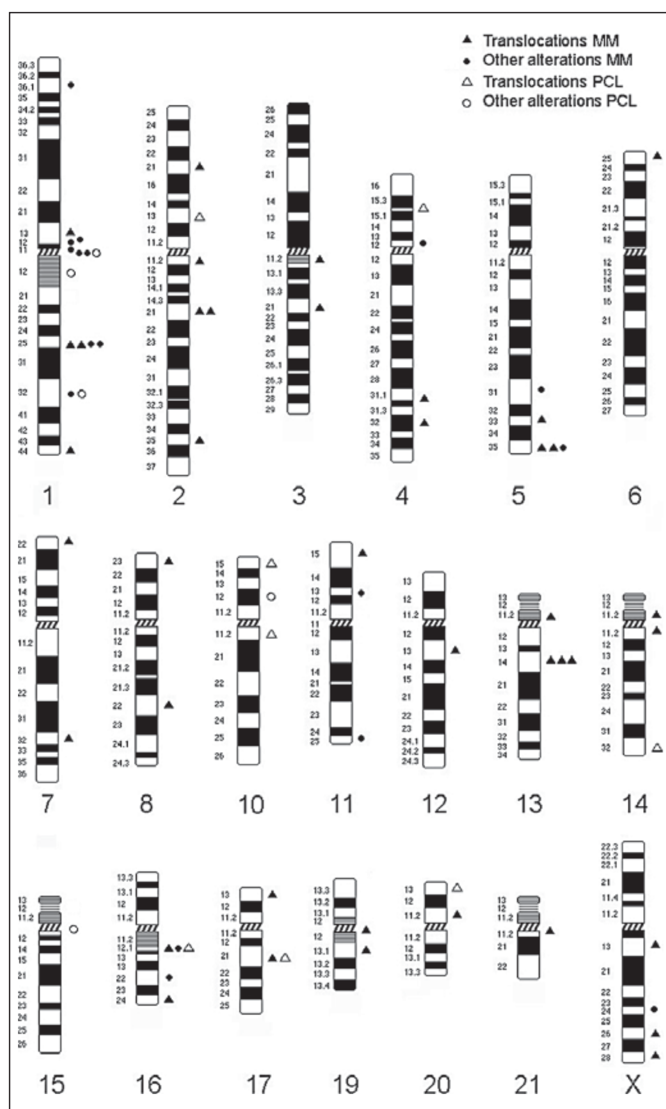

Fig. 5. Ideogram showing the breakpoint distribution detected in new structural alterations from MM/PCL patients.

1p21 and 1p31–p32 deletions were found as independent adverse prognostic factors [Shaughnessy et al., 2007; Chang et al., 2010; Chng et al., 2010].

Interestingly, chromosome 2 that is infrequently affected in plasma cell disorders [Cigudosa et al., 1998; Largo et al., 2007; Mitelman Database, 2011] was commonly involved in new unbalanced translocations in our series. Most of them leaded to 2q gains, particularly of the 2q21–qter region. These findings are of interest taking into account the capability of several genes mapping in 2q to discriminate among different MM subgroups [Zhan et al., 2006b]. More recently, *CS1* gene located at 2q31.3 was observed to be widely expressed in myeloma patients, which suggests that it could be an important therapeutic target in this pathology [Hsi et al., 2008; Tai et al., 2009].

Deletions of 13q are some of the most frequent aberrations in plasma cell disorders, corresponding to 15–20% of patients by means of conventional karyotype and in as many as 50% of cases by FISH analysis in MM patients [Shaughnessy et al., 2000; Fonseca et al., 2001] and about 80–85% of patients in PCL [Avet-Loiseau et al., 2001; Tiedemann et al., 2008]. However, translocations involving this chromosome are less frequent [Mitelman Database, 2011]. In our series, we found 4 new translocations involving chromosome 13 with different partners: 2, 6, 11 and 14. Some studies suggest that the prognostic significance of chromosome 13 anomalies depends on how they were detected. If they are observed in metaphase cells, they are associated with poor outcome, whereas patients with chromosome 13 aberrations found in their interphase nuclei had an intermediate survival rate [Dewald et al., 2005; Chiecchio et al., 2006]. Thus, our data reinforce the importance to detect chromosome abnormalities by conventional cytogenetics in MM/PCL patients and their relation to active proliferative disease.

Table 4. Cytogenetic and FISH analysis in MM patients with recurrent alterations (group 1)

Case	Age/Sex	Moment of the study	Combined karyotype (G-banding and FISH)	FISH (%)		
				IGH	RB1	TP53
1*	62/F	D	46,XX	**3.6**[a]	**36.9**	3.7
2	46/M	D	46,XY/t(4;14)(p16;q32) FISH	**4.7**	**12.9**	3.9
3*	70/M	D	46,XY	ND	**28.6**	ND
4*	65/M	D	46,XY/t(14;20)(q32;q11) FISH	**20**	5	**9**
5	61/F	D	46,XX	0.4	6.1	**6.8**
6	66/F	D	46,XX	1.4	**13.5**	**6**
7	41/F	D	ND	**7.8**[a]	1.5	**7.5**
8*	53/F	D	46,XX,del(2)(q31q33)[4]/46,XX[9]	1.4	2.6	**6.7**
9*	53/F	R	46,XX,del(5)(p11)[4]/46,XX[15]	**2.9**[a]	2.7	2.4
10*	42/M	R	46,XY,del(6)(q15q23)[7]/46,XY[6]	ND	ND	ND
11	61/M	R	46,XY,del(6)(q13q15)[2]/46,XY[8]	**2.2**[a]	2	4.7
12	47/M	R	46,XY,del(6)(q23)[4]/46,XY[13]	ND	5.1	ND
13	79/F	R	46,XX,del(6)(q25)[2]/46,XX[8]	0.5	1.7	3
14	56/M	D	46,XY,+2,del(6)(q25),–19[5]/46,XY[15]	1.5	1.98	4.3
15*	63/F	D	46,XX,del(13)(q14)[30]	ND	ND	ND
16	44/M	D	45–47,XY,+8,del(9)(q11)[cp6]/46,X[24]	0.7	3	4.2
17	75/F	D	43,X,–X,–3,del(9)(q13),t(11;14)(q13;q32),–14,–19[2]/46,XX[10]	**34.5**	1.7	2.6
18	56/F	R	89–93,XXX,del(7)(q22),–10,+12,+18,–19,+20,+22[cp5]/46,XX[39]	0.2	**95**	**94**
19	75/F	R	56–58,X,–X,+3,del(6)(q15),del(6)(q23),+7 × 2,+9 × 2,+10,+11 × 2, +14,+15x2,+20 × 2,+22,+mar [cp9]/46,XX[3]	ND	ND	ND
20*	70/F	R	62–64,X,–X,i(1)(q10),+del(1)(p11),–3,–4,add(6)(q),der(6)t(6;?)(q;?),+7,del(8) (p11),del(10)(p13),–12,–13,der(15) t(15;?)(q;?),–16,–17,–18,–20,+21,+i(21)(q10)	1	**50.2**	**97.5**

D = Diagnosis; MM = multiple myeloma; ND = not determined; R = relapse; * = dead patient. FISH abnormal clones are indicated in bold. [a] no t(11;14) or t(4;14).

Structural rearrangements involving sex chromosomes are rare events in MM patients [Mitelman Database, 2011]. On the contrary, they were frequently observed in our series, which showed 3 unbalanced translocations and a pseudodicentric chromosome involving chromosome X with different partners: 1, 12 and 19. The literature refers few aberrations involving chromosomes X and 1 [Mitelman Database, 2011], only one with chromosome 12 [Smadja et al., 2001] and no rearrangements between chromosomes X and 19. Duplications are also infrequent anomalies in plasma cell disorders. Two new duplications were observed in our series: dup(16)(q12q22) and dup(5)(q31q35). Only 1 duplication of 16q [Cuneo et al., 1996] and two of 5q involving different regions were reported [Seong et al., 1998; Smadja et al., 2001]. Different authors showed deletion and loss of heterozygosity of 16q as recurrent alterations that confer adverse prognostic impact in MM [Walker et al., 2006; Jenner et al., 2007], but no information about gains/amplifications of this chromosome was reported. More recently, a genome-wide analysis found amplifications of 5q31.3 alone associated to favorable outcome in this entity [Avet-Loiseau et al., 2009].

Interestingly, 2 rearrangements were previously reported only once in MM patients: del(16)(q13) and i(3)(q10) [Nilsson et al., 2004; Bang et al., 2006] and one in PCL: del(5)(p13) [Colović et al., 2008]. Thus, they appear to be recurrent in these pathologies from our data, and might be associated with novel gene rearrangements. Among them, i(3)(q10) was found as a single structural aberration in our series and could be a probable new primary event in plasma cell disorders. Comparative genomic hybridization (CGH) analysis showed an adverse prognostic impact for 3q gains [Gutiérrez et al., 2004] and studies using array CGH found a minimal common region of gain at 3q27.1–3q27.2 where *POLR2H* and

EIF4G1 candidate genes are located [Carrasco et al., 2006]. More recently, Hideshima et al. [2010] found that the *BCL6* gene, also located at 3q27, was upregulated in myeloma cells and involved in the growing of MM cells in BM environment.

Furthermore, the analysis of clinical characteristics in patients with chromosome aberrations (our series and group 1), compared to group 2 without cytogenetic and FISH anomalies, showed significant differences, particularly in factors related to progression of the disease like the percentage of bone marrow plasma cell infiltration, β_2M and LDH. This agrees with the literature [Stewart et al., 2007; Fonseca et al., 2009; Inamoto et al., 2009] showing that patients with abnormal karyotypes revealed by conventional cytogenetics have a poor outcome compared to those with only normal metaphases. In addition, although no significant differences were observed, the comparison between our series and group 1 showed increased levels of bone marrow plasma cell infiltration, β_2M and LDH, and lower levels of hemoglobin, albumin and overall survival in cases with novel aberrations. These findings prove to be interesting taking into account that, except for chromosome 1 abnormalities, which were more represented among novel anomalies, patients with recurrent aberrations had a similar distribution of genomic rearrangements of poor prognosis as the group with new structural aberrations. The 17p13 deletion has been identified as an indicator of very poor outcome, being considered as the most important molecular cytogenetic factor for prognostication [Avet-Loiseau et al., 2007; Fonseca et al., 2009]. The amplification of 1q is also associated with poor prognosis, but lack of consensus on any critical genes in this region determines controversies in its clinical significance [Sawyer, 2011]. Different studies support that this aberration introduces an increased level of genetic instability in MM [Sawyer et al., 2005, 2009] and suggests that perhaps 1q amplification may be a surrogate marker of more clonally advanced tumors [Chng et al., 2007]. Our patients showed a great karyotypic instability with different types of chromosome aberrations that reflect the genetic heterogeneity of this pathology. Most of the patients did not show recognized primary alterations in plasma cell disorders suggesting that new abnormalities detected in our series may be capable to deregulate genetic mechanisms related to the development and/or progression of MM/PCL. The presence of 1 new recurrent aberration in our series and 3 anomalies reported only once in the literature that became recurrent from our data supports this hypothesis.

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References

Avet-Loiseau H, Caviet A, Brigaudeau C, Callet-Bauchu E, Terre C, et al: Cytogenetic, interphase, and multicolor fluorescence in situ hybridization analyses in primary plasma cell leukemia: a study of 40 patients at diagnosis, on behalf of the Intergroupe Francophone du Myélome and the Groupe Français de Cytogénétique Hématologique. Blood 97: 822–825 (2001).

Avet-Loiseau H, Attal M, Moreau P, Charbonnel C, Garban F, et al: Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myélome. Blood 109:3489–3495 (2007).

Avet-Loiseau H, Li C, Magrangeas F, Gouraud W, Charbonnel C, et al: Prognostic significance of copy-number alterations in multiple myeloma. J Clin Oncol 27:4585–4590 (2009).

Bang SM, Kim YR, Cho HI, Chi HS, Seo EJ, et al: Identification of 13q deletion, trisomy 1q, and IgH rearrangement as the most frequent chromosomal changes found in Korean patients with multiple myeloma. Cancer Genet Cytogenet 168:124–132 (2006).

Beà S, Campo E: Secondary genomic alterations in non-Hodgkin's lymphomas: tumor-specific profiles with impact on clinical behavior. Haematologica 93:641–645 (2008).

Carrasco DR, Tonon G, Huang Y, Zhang Y, Sinha R, et al: High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. Cancer Cell 9: 313–325 (2006).

Chang H, Qi X, Jiang A, Xu W, Trieu Y, et al: 1p21 deletions are strongly associated with 1q21 gains and are an independent adverse prognostic factor for the outcome of high-dose chemotherapy in patients with multiple myeloma. Bone Marrow Transp 45:117–121 (2010).

Chiecchio L, Protheroe RK, Ibrahim AH, Cheung KL, Rudduck C, et al: Deletion of chromosome 13 detected by conventional cytogenetics is a critical prognostic factor in myeloma. Leukemia 20:1610–1617 (2006).

Chng WJ, Glebov O, Bergsagel PL, Kuehl WM: Genetic events in the pathogenesis of multiple myeloma. Best Pract Res Clin Haematol 20:571–596 (2007).

Chng WJ, Gertz MA, Chung T-H, Van Wier S, Keats JJ, et al: Correlation between array-comparative genomic hybridization-defined genomic gains and losses and survival: identification of 1p31–32 deletion as a prognostic factor in myeloma. Leukemia 24:833–842 (2010).

Cigudosa JC, Rao PH, Calasanz MJ, Odero MD, Michaeli J, et al: Characterization of nonrandom chromosomal gains and losses in multiple myeloma by comparative genomic hybridization. Blood 91:3007–3010 (1998).

Colović M, Janković G, Suvajdzić N, Milić N, Dordević V, et al: Thirty patients with primary plasma cell leukemia: a single center experience. Med Oncol 25:154–160 (2008).

Cuneo A, Balsamo R, Roberti MG, Bardi A, Piva N, et al: Interleukin-3 plus interleukin-6 may improve chromosomal analysis of multiple myeloma: cytologic and cytogenetic evidence in thirty-four patients. Cancer Genet Cytogenet 90:171–175 (1996).

Dewald GM, Kyle RA, Hicks GA, Greipp PR: The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. Blood 66:380–390 (1985).
Dewald GM, Therneau T, Larson D, Lee YK, Fink S, et al: Relationship of patient survival and chromosome anomalies detected in metaphase and/or interphase cell at diagnosis of myeloma. Blood 106:3552–3558 (2005).
Durie BG, Salmon E: A clinical staging system for multiple myeloma: correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 36:842–854 (1975).
Fassas AB, Tricot G: Chromosome 13 deletion/hypodiploidy and prognosis in multiple myeloma patients. Leuk Lymphoma 45:1083–1091 (2004).
Fonseca R, Oken MM, Harrington D, Bailey RJ, Van Wier SA, et al: Deletions of chromosome 13 in multiple myeloma identified by interphase FISH usually denote large deletions of the q arm or monosomy. Leukemia 15:981–986 (2001).
Fonseca R, Debes-Marun CS, Picken EB, Dewald GW, Bryant SC, et al: The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. Blood 102:2562–2567 (2003).
Fonseca R, Bergsagel PL, Drasch J, Shaughnessy J, Gutierrez N, et al: International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia 23:2210–2221 (2009).
Greipp P, San Miguel J, Durie BG, Crowley J, Barlogie B, et al: International staging system for multiple myeloma. J Clin Oncol 23: 3412–3420 (2005).
Gutiérrez NC, García JL, Hernández JM, Lumbreras E, Castellanos M, et al: Prognostic and biologic significance of chromosomal imbalances assessed by comparative genomic hybridization in multiple myeloma. Blood 104: 2661–2666 (2004).
Hanamura I, Stewart JP, Huang Y, Zhan F, Santra M, et al: Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. Blood 108:1724–1732 (2006).
Hideshima T, Mitsiades C, Ikeda H, Chauhan D, Raje N, et al: A proto-oncogen BCL6 is upregulated in bone marrow microenvironment in multiple myeloma cells. Blood 115: 3772–3775 (2010).
Hsi ED, Steinle R, Balasa B, Szmania S, Draksharapu A, et al: CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma. Clin Cancer Res 14:2772–2784 (2008).
Inamoto Y, Kurahashi S, Imahashi N, Fukushima N, Adachi T, et al: Combinations of cytogenetics and international scoring system can predict poor prognosis in multiple myeloma after high-dose chemotherapy and autologous stem cell transplantation. Am J Hematology 84:283–286 (2009).
ISCN: An International System for Human Cytogenetic Nomenclature: Shaffer LG, Slovak ML, Campbell LJ (eds) (Karger, Basel 2009).
Itoyama T, Nanjangud G, Chen W, Dyomin VG, Teruya-Feldstein J, et al: Molecular cytogenetic analysis of genomic instability at the 1q12–22 chromosomal site in B-cell non-Hodgkin lymphoma. Genes Chrom Cancer 35:318–328 (2002).
Jenner MW, Leone PE, Walker BA, Ross FM, Johnson DC, et al: Gene mapping and expression analysis of 16q loss of heterozygosity identifies WWOX and CYLD as being important in determining clinical outcome in multiple myeloma. Blood 110:3291–3300 (2007).
Kyle RA, Rajkumar SV: Multiple myeloma. N Eng J Med 351:1860–1873 (2004).
Largo C, Saéz B, Alvarez S, Suela J, Ferreira B, et al: Multiple myeloma primary cells show a highly rearranged unbalanced genome with amplifications and homozygous deletions irrespective of the presence of immunoglobulin-related chromosome translocations. Haematologica 92:795–802 (2007).
Le Baccon P, Laroux D, Dascalescu C, Duley S, Marais D, et al: Novel evidence of a role for chromosome 1 pericentric heterochromatin in the pathogenesis of B-cell lymphoma and multiple myeloma. Genes Chrom Cancer 32: 250–264 (2001).
McKenna RW, Kyle RA, Kuehl WM, Grogan TM, Harris NL, et al: Plasma cell neoplasms. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, et al (eds): WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues, pp 200–213 (IARC Press, Lyon 2008).
Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer: Mitelman F, Johansson B, Mertens F (eds): Available from URL: http://cgap.nci.nih.gov/Chromosomes/Mitelman (2011).
Nilsson T, Lenhoff S, Rylander L, Höglund M, Turesson I, et al: High frequencies of chromosomal aberrations in multiple myeloma and monoclonal gammopathy of undetermined significance in direct chromosome preparation. Br J Haematol 126:487–494 (2004).
Sawyer JR, Tricot G, Mattox S, Jagannath S, Barlogie B: Jumping translocations of chromosome 1q in multiple myeloma: Evidence for a mechanism involving decondensation of pericentromeric heterochromatin. Blood 91: 1732–1741 (1998).
Sawyer JR, Tricot G, Lukacs JL, Binz RL, Tian E, et al: Genomic instability in multiple myeloma: evidence for jumping segmental duplications of chromosome arm 1q. Genes Chrom Cancer 42:92–106 (2005).
Sawyer JR, Tian E, Thomas E, Koller M, Stangeby C, et al: Evidence for a novel mechanism for gene amplification in multiple myeloma: 1q12 pericentromeric heterochromatin mediates breakage-fusion-bridge cycles of a 1q12 approximately 23 amplicon. Br J Haematol 147:484–494 (2009).
Sawyer JR: The prognostic significance of cytogenetics and molecular profiling in multiple myeloma. Cancer Genet 204:3–12 (2011).
Seong C, Delasalle K, Hayes K, Weber D, Dimopoulos M, et al: Prognostic value of cytogenetics in multiple myeloma. Br J Haematol 101:189–194 (1998).
Shaughnessy J, Tian E, Sawyer J, Bumm K, Landes R, et al: High incidence of chromosome 13 deletion in multiple myeloma detected by multiprobe interphase FISH. Blood 96:1505–1511 (2000).
Shaughnessy JD Jr, Zhan F, Burington BE, Huang Y, Colla S, et al: A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood 109: 2276–2284 (2007).
Smadja NV, Bastard C Brigaudeau C, Leroux D, Fruchart C: Hypodiploidy is a major prognostic factor in multiple myeloma. Blood 98: 2229–2238 (2001).
Smadja NV, Leroux D, Soulier J, Dumont S, Arnould C, et al: Further cytogenetic characterization of multiple myeloma confirms that 14q32 translocations are a very rare event in hyperdiploid cases. Genes Chrom Cancer 38:234–239 (2003).
Stewart AK, Bergsagel PL, Greipp PR, Dispenzieri A, Gertz MA, et al: A practical guide to defininig high-risk myeloma for clinical trials, patient counseling and choice of therapy. Leukemia 21:529–534 (2007).
Tai Y, Soydan E, Song W, Fulciniti MT, Kim K, et al: CS1 promotes multiple myeloma cell adhesion, clonogenic growth, and tumorigenicity via c-maf-mediated interactions with bone marrow stromal cells. Blood 113:4309–4318 (2009).
Tiedemann RE, Gonzalez-Paz N, Kyle RA, Santana-Davila R, Price-Troska T, et al: Genetic aberrations and survival in plasma cell leukemia. Leukemia 22:1044–1052 (2008).
Walker BA, Leone PE, Jenner MW, Li C, Gonzalez D, et al: Integration of global SNP-based mapping and expression arrays reveals key regions, mechanisms, and genes important in the pathogenesis of multiple myeloma. Blood 108:1733–1743 (2006).
Zhan F, Sawyer J, Tricot G: The role of cytogenetics in myeloma. Leukemia 20:1484–1486 (2006a).
Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, et al: The molecular classification of multiple myeloma. Blood 108:2020–2028 (2006b).