Leukemia (2003) 17, 427–436

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Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma

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The nonrandom recurrent nature of chromosome abnormalities in myeloma suggests a role for them in disease pathogenesis. We performed a careful cytogenetic analysis of patients with abnormal karyotypes ($n = 254$), to discern patterns of association, search for novel abnormalities and elucidate clinical implications. Patients with karyotypic abnormalities suggestive of myelodysplasia/acute leukemia were excluded. In this study we compared survival by abnormality only between patients with abnormal karyotypes. Patients with abnormalities were more likely to have features of aggressive disease as compared to all other patients without abnormalities entered into the myeloma database (lower hemoglobin, higher β_2-microglobulin, labeling-index and plasmocytosis; all $P < 0.0001$). Several groups of patients could be readily identified; hypodiploid (22%), pseudodiploid (36%), hyperdiploid (31%) and near-tetraploid (11%). Clustering associations were seen among several trisomies and monosomy of chromosome 13 and 14. Several monosomies (–2, –3, –13, –14 and –19), 1p translocations/deletions, and hypodiploidy were associated with a significantly shorter survival. Trisomy of chromosome 13 was rare (<2%). Even among patients with abnormal karyotypes, specific chromosome abnormalities can impart biologic variability in myeloma, including several monosomies, hypodiploidy and abnormalities of 1p.
Leukemia (2003) **17,** 427–436. doi:10.1038/sj.leu.2402797
Keywords: multiple myeloma; prognosis; translocations (genetics); chromosome deletion; ploidy

Introduction

As shown by studies using interphase fluorescence *in situ* hybridization (FISH), all patients with multiple myeloma or monoclonal gammopathy of undetermined significance have chromosome abnormalities in the clonal plasma cells.[1,2] Studies using conventional chromosome studies almost never detect neoplastic metaphase cells in monoclonal gammopathy of undetermined significance, and occasionally will detect abnormalities in myeloma.[3–6] Chromosomally abnormal clones can be found in 18% to 35% of myeloma patients at diagnosis, 40% to 60% with aggressive disease and up to 85% of patients with plasma cell leukemia.[3–6] Clearly, the potential of conventional cytogenetic studies to detect an abnormal clone in myeloma is associated with aggressiveness of disease.[3–7] Studies assessing the clinical implications of chromosomal abnormalities in myeloma, detected by karyotype analysis, need to emphasize that an abnormal karyotype is more likely to be encountered in a highly proliferative clone.[8]

Several recurrent, nonrandom abnormalities have been detected in myeloma.[9,10] Among them, translocations involving the immunoglobulin heavy-chain (IgH) locus have been implicated in the origin of myeloma[11] and are seen in 50–60% of patients. Translocations at the IgH locus involve several partner chromosomes, and the most common of these translocations include t(11;14)(q13;q32), t(4;14)(p16.3;q32), and t(14;16)(q32;q23).[9,10] Aneuploidy is ubiquitous,[1,12,13] and monosomy of chromosome 13 is seen in nearly one-half of patients.[10,14] Little is known about the role of other chromosome anomalies in myeloma.

Investigations of the relationship between disease progression/pathogenesis and chromosome evolution have not been easy because the karyotype of neoplastic cells in myeloma often involves many numeric and structural abnormalities.[3,7] In addition, some chromosome abnormalities in myeloma are cryptic to conventional cytogenetic studies (for example t(4;14)(p16.3;q32)), while other chromosome abnormalities in myeloma are subtle and easily overlooked by conventional cytogenetic studies (for example t(14;16)(q32;q23)). Other abnormalities, such as those of chromosomes 1 are recurrent and further support the hypothesis that certain recurrent chromosome abnormalities in myeloma are nonrandom.[3,15] Moreover, several investigators suggest that the observed clinical heterogeneity among patients with myeloma is likely to be related to the underlying genetic and cytogenetic abnormalities[2,16] and certain specific chromosome abnormalities are known to be associated with adverse outcome.[8,17–19]

To perform a comprehensive karyotype analysis in myeloma, we studied clustering patterns of specific chromosome abnormalities detected by conventional karyotype analysis in a large cohort of patients with myeloma to look further for clues to the pathogenesis of this disorder, and to address their clinical implications. The implications of these abnormalities were only considered among patients with abnormal metaphases, thus removing the effect of comparing these patients to those in whom an abnormal karyotype cannot be detected.

Patients and methods

Patient demographics

All patients entered in the clinical cytogenetics database of Mayo and who were diagnosed with multiple myeloma were included in this study. Patients who had a previous diagnosis of myeloma and were subsequently diagnosed with myelodysplasia or acute leukemia, and who exhibited the characteristic chromosome abnormalities for these entities (eg t(1;7),pseudodiploid and –7, or –5 occurring in isolation) were excluded from further study. Our search yielded a total of 254 patients with chromosome abnormalities, which form the basis for this report. We compared results of these patients with the results of 3483 patients with various plasma cell proliferative disorders who had only normal metaphases entered into the Mayo Clinic Dysproteinemia database. Pertinent clinical and prognostic features are available for these patients including among others the plasma cell labeling index, β_2-

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Received 18 July 2002; accepted 25 September 2002

Table 1 Prevalence of chromosomal abnormalities in myeloma

	Cases	*Controls*	*P value*
Patients (*n*)	254	3483	
Age (years)	59 (31–89)	64 (17–96)	<0.0001
Males (%)	153 (60%)	2075 (60%)	0.90
Caucasian race (%)	243 (96%)	3363 (97%)	0.38
Serum M-spike (range)	3.58 (0.2–10.4)	2.90 (0.1–15.1)	<0.0001
IgG:IgA (ratio)	1.89:1	2.84:1	0.051
$\kappa\lambda$ (ratio)	1.46:1	1.77:1	0.34
Urine M-spike (present)	0.31 (0–21.13)	0.23 (0–16.99)	0.22
Hemoglobin (g/dl)	10.6 (4.5–15.8)	11.3 (2.7–19.6)	<0.0001
Calcium (mg/dl)	9.4 (6.9–17.5)	9.4 (6.6–18.1)	0.54
Creatinine (mg/dl)	1.2 (0.4–11.0)	1.1 (0.4–20.1)	0.02
β_2-microglobulin (mg/dl)	3.95 (1.08–63.8)	3.0 (0.7–82.0)	<0.0001
PCLI (%)	0.8 (0–15.6)	0.3 (0–41.0)	<0.0001
Bone marrow plasma cells (%)	55.5 (2–99.9)	30 (0–100)	<0.0001
Bone lesions (%)	157 (66%)	1862 (57%)	0.04

Numbers represent median and (range).

microglobulin, hemoglobin, serum creatinine and bone marrow plasmacytosis.

Statistical analysis

Descriptive and survival statistics: Descriptive statistics were used to characterize patients in this study. To test for association between abnormalities the Fisher's exact test[20] was used for nominal variables, and the Wilcoxon rank sum test was used[21] for continuous variables. Overall survival was estimated using the methods of Kaplan and Meier.[22] The log-rank test was used to test for differences in survival between groups.[23] Survival time was always calculated from the time of diagnosis. Patients were assigned to one of four categories; pseudodiploid for those having 45–46 chromosomes, hypodiploid if they had 44 or less chromosomes, hyperdiploid if they had 47 to 74 chromosomes, and near-tetraploid or tetraploid if they had 75 or more chromosomes.

The following clinical prognostic factors were assessed as continuous (or dichotomized) variables according to previous reports: serum calcium (>12 mg/dl), hemoglobin (>12 g/dl), plasma cell labeling index (PCLI) (<1 *vs* ≥1%), β_2-microglobulin (≥2.7, and ≥4 mg/dl), serum creatinine (>2 mg/dl), the presence or absence of bone lesions and the bone marrow plasma cell percentage.

Table 2 Prevalence of specific numerical chromosomal abnormalities

Chromosome	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Monosomy %	9	9	4	15	10	9	11	21
Trisomy %	4	4	22	4	13	6	15	4
Chromosome	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
Monosomy	5	17	12	16	43	28	7	21
Trisomy %	25	2	20	3	2	3	22	1
Chromosome	*17*	*18*	*19*	*20*	*21*	*22*	*X*	*Y*
Monosomy %	19	12	8	17	9	23	22	17
Trisomy %	2	9	22	3	11	3	3	1

Clustering analysis: We classified karyotypic abnormalities in a binomial fashion (present/not), independent of the number of times the abnormality was present. For a specific abnormality to be included it had to be present in at least seven patients (3%). Clustering analysis was performed on all data using methods of average linkage, single linkage and complete linkage using two measures of distance, Kendall's tau and Jaccard's coefficient.[24] Six cluster trees resulting from this analysis were compared.

Results

Patient population

We compared results of 254 patients with multiple myeloma who had chromosomal anomalies with the results of 3483 patients with various plasma cell proliferative disorders who had only normal metaphases. Clinical and demographic variables of these patients are summarized in Table 1. Patients with detectable chromosomal anomalies were slightly younger ($P < 0.0001$), had a higher serum monoclonal spike ($P < 0.0001$), lower hemoglobin ($P < 0.0001$), higher β_2-

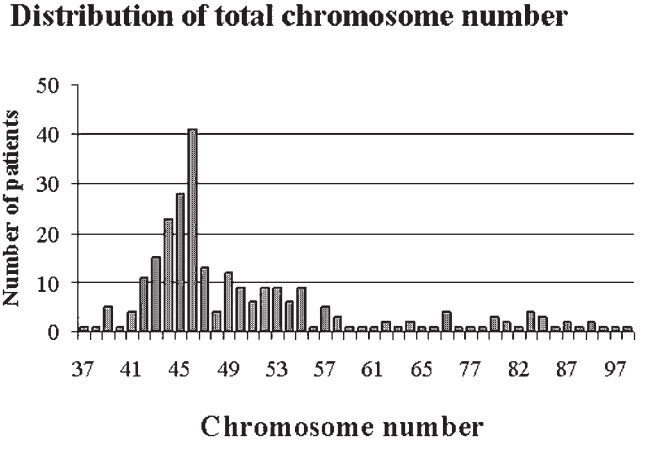

Figure 1 Distribution of total chromosome number among patients with karyotype abnormalities. Figure 1 depicts the histogram with the number of patients having a specific chromosome count.

microglobulin ($P < 0.0001$), higher PCLI ($P < 0.0001$), and had a greater extent of bone marrow involvement ($P < 0.0001$).[8] Lytic bone lesions were slightly more common among patients with chromosome abnormalities ($P = 0.04$). Median follow-up time for patients was 23.5 months (range 0–204 months).

Prevalence of chromosomal abnormalities

Patients were distributed as follows, according to the chromosome number: 55 were hypodiploid (22%), 92 were pseudodiploid (36%), 78 were hyperdiploid (31%) and 28 were near-tetraploid (11%).

Numerical chromosomal abnormalities: The most common numerical chromosomal anomalies are summarized in Table 2. Trisomies of chromosomes 5, 7, 9, 11, 15, 19 and 21 were common while trisomies of chromosomes 12 or 13 were almost never observed. Monosomy for chromosomes 8, 13, 14, 16, 17 and 22 were also common while monosomy of chromosomes 1 or 9 (Table 2) were seldom observed. Monosomy of one or more chromosomes was observed in 80% of patients, while trisomy of one or more chromosomes were only seen in about 45% of patients. The distribution of trisomies and monosomies varied according to the ploidy status of patients, and had a tendency, but not absolute, towards mutual exclusivity (Table 3).

Structural chromosomal abnormalities: Structural anomalies of chromosome 1 were frequent: 7% of patients had a deletion in the short arm, 4% had duplications in the q-arm and 19% had translocations involving the p-arm (Table 4). Other noteworthy structural anomalies included deletion of chromosome 6q in 8% of patients and translocations involving 6q in 9% of patients.

Translocations involving 14q32 (IgH locus) were observed in 10% of patients. Translocation involving16q were observed in 4% of patients. The t(11;14)(q13;q32) was observed in 29 (11%) patients. Structural chromosomal anomalies were significantly more common among patients with the hypodiploid form of myeloma ($P < 0.007$).

Distribution of chromosome abnormalities

Distribution of the total chromosome number among hyperdiploid variant myeloma followed a Chi-square distribution with a great predominance for smaller number of chromosomes (peak at 53 chromosomes) with the net chromosome number reminiscent more of that of a diploid karyotype than a tetraploid karyotype (Figure 1).

Chromosomal clustering analysis

Based on clustering analysis, chromosomal anomalies could be segregated into unique and specific patterns. In one group, cytogenetic results for patients with trisomies for chromosomes 3, 5, 7, 9, 11, 15 and 19 clustered together. In another group, cluster of trisomies between chromosomes 4 and 22 grouped together (Figure 2). However, detailed interpretation of clustering was hampered by the low number of obser-

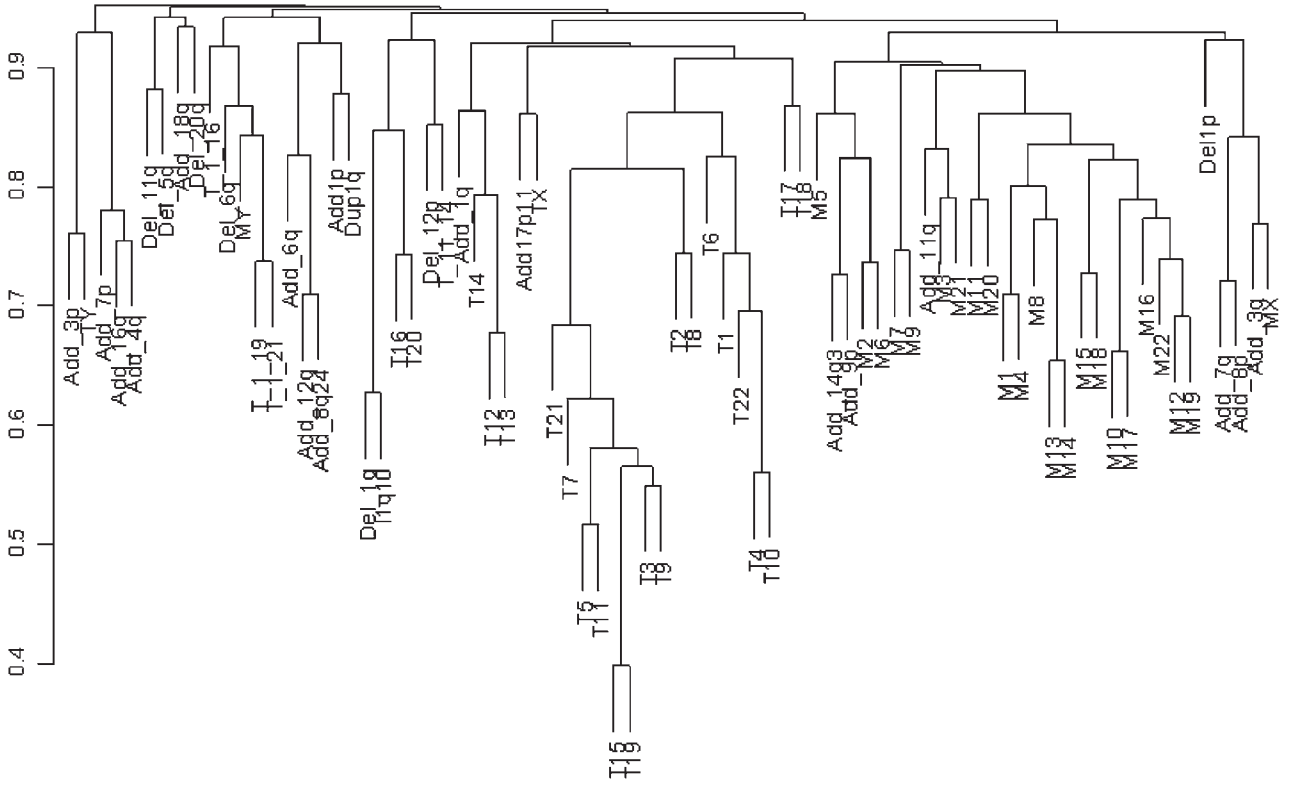

Figure 2 Average cluster tree using Kendall's tau.

Table 3 Distribution of abnormalities according to ploidy status

	+14q32	*t(11;14) (q13;q32)*	*t(1;16)*	*Any translocation*	*del 20q*	*Common trisomies*									*Any common trisomy*	*Any trisomy*	*Common monosomies*					*Any common monosomy*	*Any monosomy*
						3	*5*	*7*	*9*	*11*	*15*	*18*	*19*	*21*			*8*	*11*	*13*	*14*	*16*		
Hypodiploid (n = 55)	8	8	7	23	2	1	0	1	1	1	0	0	1	0	4	7	16	11	37	28	11	47	54
Proportion of patients (%)	15	15	13	42	4	2	0	2	2	2	0	0	2	0	8	13	29	20	64	51	20	85	98
Pseudodiploid (*n* = 92)	9	16	3	25	8	4	1	2	7	3	4	6	8	2	20	24	12	9	27	18	7	44	66
Proportion of patients (%)	10	17	3	27	9	4	1	2	8	3	4	7	9	2	22	26	13	10	29	20	8	48	72
Hypodip and pseudodip (*n* = 147)	17	24	10	48	10	5	1	3	8	4	4	6	9	2	24	31	28	20	64	46	18	91	120
Proportion of patients (%)	12	16	7	33	7	3	1	2	5	3	3	4	6	1	16	21	19	14	44	31	12	62	82
Hyperdiploid (*n* = 78)	5	2	1	8	1	43	31	32	51	46	50	14	43	24	77	77	13	2	25	11	21	47	72
Proportion of patients (%)	6	3	1	10	1	55	40	41	65	59	64	18	55	31	99	99	17	3	32	14	27	60	92
Near-tetraploid(*n* = 28)	4	3	1	8	1	7	0	4	4	2	3	2	4	3	15	18	12	9	20	13	14	26	27
Proportion of patients (%)	14	11	4	29	4	25	0	14	14	7	11	7	14	11	54	64	43	32	71	46	50	93	96
Total all	**26**	**29**	**12**	**64**	**12**	**55**	**32**	**39**	**63**	**52**	**57**	**22**	**56**	**29**	**116**	**126**	**53**	**31**	**109**	**70**	**53**	**164**	**219**
Proportion of patients (%)	**10**	**11**	**5**	**25**	**5**	**22**	**13**	**15**	**25**	**21**	**23**	**9**	**22**	**11**	**43**	**50**	**21**	**12**	**43**	**28**	**21**	**65**	**87**

Table 4 Prevalence of specific structural chromosomal abnormalities

Abnormality	*n*	*%*
Chromosome 1		
Deletions of 1p	19	7
+1p	49	19
Duplications of 1q	9	4
Deletion 1q	3	1
Add1q	12	5
Deletions		
Deletion 5q	14	6
Deletion 6q	20	8
Deletion 10q	12	5
Deletion 11q	11	4
Deletion 12p	8	3
Additions		
Add3p	8	3
Add 3q	10	4
Add4q	13	5
Add6q	22	9
Add7p	9	4
Add7q	8	3
Add8p	12	5
Add8q42	6	2
Add9p	17	7
Add11q	9	4
Add12q	7	3
Add14q32	26	10
Add16q	9	4
Add17p11.2	16	6
Add18q	8	3

vations for any given chromosome anomaly (ie trisomies of any chromosome).

Global analysis of patients classified according to the presence of trisomies and monosomies revealed two major groups; patients with predominant monosomies and rare, but occasional trisomies, and patients with predominant trisomies but also monosomies. As would be expected, the first group was composed of patients with hypodiploid and pseudodiploid myeloma, and the second group consisted mostly of hyperdiploid myeloma (Figure 3).

In addition, we observed a correlation between the presence of monosomy 13 and monosomy 14 (likely many of these patients may have either a marker chromosome or an unbalanced translocation involving chromosome 14). When all patients are taken together, patients with a t(11;14)(q13;q32) were significantly more likely to be in the hypo- or pseudodiploid group than patients without t(11;14)(q13;q32) ($P = 0.0095$).

Correlation with clinical variables

Patients with translocations involving chromosome 1p ($P = 0.004$), 6q ($P = 0.004$), 9p ($P = 0.002$), monosomy of chromosome 4 ($P = 0.008$), and females with monosomy of chromosome X ($P = 0.006$) were more likely to have an elevated PCLI (continuous). In addition, patients with a monosomy X had a significantly higher proportion of patients with PCLI >1% ($P = 0.003$). Patients with trisomy 7 ($P = 0.003$) and 21 ($P = 0.00004$) were significantly more likely to have β_2-microglobulin ≥2.7 mg/dl. Likewise patients with trisomy of chromosome 21 were significantly more likely to have a β_2-microglobulin >4 mg/dl ($P = 0.0005$). Patients with monosomy 18 were likely to have a lower creatinine ($P = 0.005$). When assessed by ploidy category, no major differences were noted in the PCLI.

Survival

The median survival for the whole cohort of patients was 23.4 months.

Univariate analysis: Negative survival associations were observed for the following chromosome abnormalities on univariate analysis; 1p deletions (5 year survival 18.3% *vs* 35.8%, $P = 0.0074$), 1p translocations (5 year survival 23.1% *vs* 37.2%, $P = 0.0013$) (Figure 4), and −2 (5 year survival 9.2% *vs* 37.5%, $P = 0.0006$), −3 (5 year survival 0% *vs* 35.9%, $P = 0.0086$), −13 (5 year survival 20.2% *vs* 44.6%, $P = 0.0003$), −14 (5 year survival 16.4% *vs* 40.1%, $P = 0.0009$), and −19 (5 year survival 12.7% *vs* 36.2%, $P = 0.0054$) (Figure 5).

When patients were classified according to ploidy status significant differences were found. Patients with hypodiploid myeloma had the worst survival (5 year survival 10%) followed by hyperdiploid and near-tetraploid (5 year survivals of 33.5% and 34.6%, respectively) and the best prognosis was observed among patients with pseudodiploid myeloma (5 year survival 49.9%) ($P = 0.0001$) (Figure 6). When patients with hypodiploid myeloma were compared to all others they had a much shorter survival (5 year survival 10% *vs* 41.4%, $P = 0.0001$) (Figure 7). Among patients with hypodiploid myeloma the presence of chromosome 13 abnormalities did not further separate them into different prognostic categories ($P > 0.2$) (Figure 7). Among patients with chromosome 13 abnormalities the presence or not of hypodiploid variant did not further separate them into different prognostic categories ($P > 0.2$).

We sought to further investigate whether any of the specific monosomies contributed for the shortened survival associated with hypodiploidy. The prognosis of hypodiploid myeloma patients could not be further divided by any of the four monosomies also associated with a shorter survival ($P > 0.2$ for monosomy 2, 3, 14 and 19). A trend was observed however for patients with hypodiploid myeloma to fare worse if they also had monosomy 14. Likewise hypodiploidy could not further discern prognosis in patients with any of the aforementioned monosomies.

The following clinical prognostic factors were associated with a shortened survival; hemoglobin <10 g/dl (5 year survival 21.9% *vs* 43.5%, $P = 0.0001$), β_2-microglobulin ≥2 mg% (5 years survival 29% *vs* 41.1%, $P = 0.0024$), PCLI ≥1% (5 year survival 16.3% *vs* 45.7%, $P = 0.0001$). A serum concentration of creatinine ≥2 mg/dl did not have prognostic significance ($P > 0.2$) among these patients.

Multivariate model: Using recursive partitioning, survival trees were created to explore the combined effects of the aforementioned abnormalities, relapse status, PCLI, β_2-microglobulin, hemoglobin, creatinine and bone marrow plasmacytosis. In this survival model the dependent variable was time to death. Using continuous variables (for the PCLI, β_2-microglobulin, hemoglobin, creatinine and bone marrow plasmacytosis), and the chromosome abnormalities as categorical variables the following factors were of prognostic importance; PCLI, hemoglobin and the presence or absence of monosomy 14. If using dichotomous (for the PCLI, β_2-

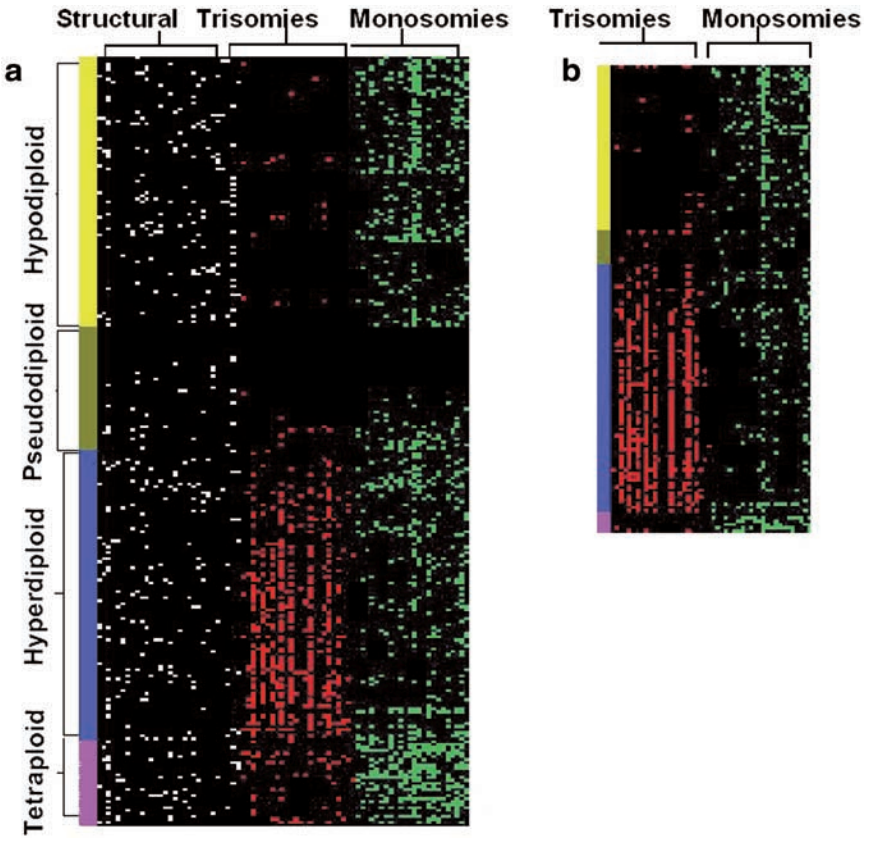

Figure 3 Distribution of chromosomal abnormalities according to ploidy. (a) Color diagram was used to provide a graphic representation of the distribution of chromosomal abnormalities in myeloma and their relation to ploidy status. Each one of the rows represents a patient ($n = 254$) and each one of the columns represents the different chromosome abnormalities. These are ordered (from left to right) as structural chromosomal abnormalities, trisomies (from left to right chromosome 1 to Y), and monosomies (from left to right chromosome 1 to Y). A square is filled with black if no abnormality was reported, white if a specific structural chromosome abnormality was present, red if a specific trisomy was present and green if monosomy for the given chromosome was present. The color bars on the left side represent the different ploidy categories; yellow, hypodiploid; brown, diploid; blue, hyperdiploid; and gray near/tetraploid. (b) Same analysis performed on the information published by Smadja and colleagues[25] (n =138 patients with karyotypic abnormalities).

Table 5 Recursive partitioning prognostic analysis

Group	*Factor 1 (% PCLI)*	*Factor 2*	*Factor 3*	*Death/ alive*	*Median survival (months)*	*HR*
Recursive partitioning using continuous variables						
1	≥3.75			18/20	5.65	4.483
2	<3.75	Hgb ≤10 g/dl		63/86	18.3	1.471
3	<3.75	Hgb >10 g/dl	−14	24/38	23.5	1.196
4	<3.75	Hgb >10 g/dl	No −14	63/108	52.5	0.619
Recursive partitioning using dichotomous variables						
1	≥1	Hgb ≥10 g/dl	Translocation 1p	9/9	12.45	2.557
2	≥1	Hgb <10 g/dl	–	43/57	10.05	2.423
3	<1	BM PC% <54%	Hypodiploid myeloma	15/18	22.98	1.261
4	< 1	BM PC% ≥54%	–	43/62	25.66	1.121
5	≥1	Hgb ≥10 g/dl	No translocation 1p	22/31	37.85	0.958
6	<1	BM PC% <54%	Not hypodiploid	36/75	71.52	0.487

microglobulin, hemoglobin, creatinine and bone marrow plasmacytosis), variables the presence of a PCLI ≥1% immediately discriminated patients into a good and poor prognostic category. The groups were further divided by the bone marrow plasmacytosis, hemoglobin level ≥10 g/dl, hypodiploid myeloma, and translocations of 1p (R^2 = 0.18) (Table 5).

Discussion

Clustering

We used clustering analysis to attempt to find biological order among the complicated cytogenetic data in myeloma, and have found significant associations between specific chromo-

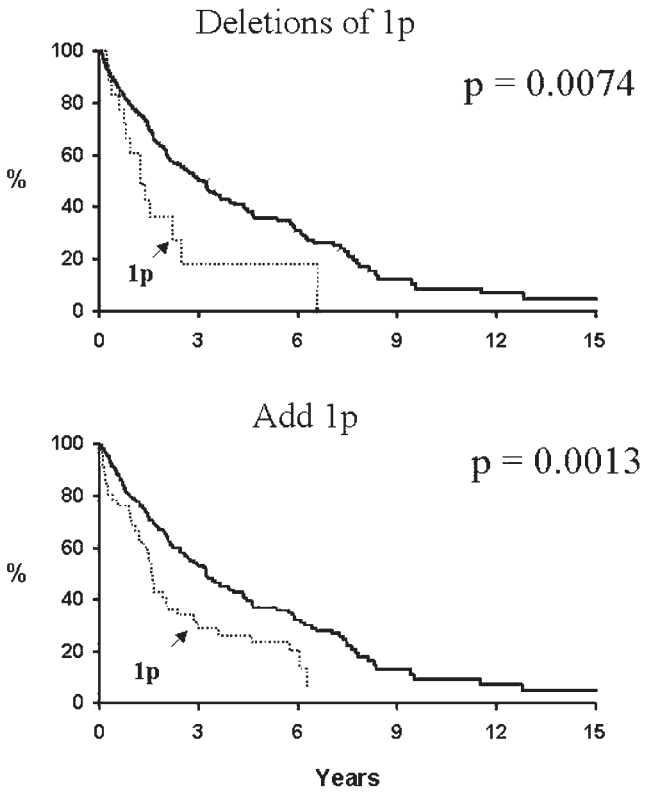

Figure 4 Overall survival of patients according to the presence of structural abnormalities of chromosome 1. Kaplan–Meier survival analysis of patients according to the presence of deletions or additions of the short (p) arm of chromosome 1. The survival since diagnosis time is presented in the x-axis in years and the *P* value is the univariate log-rank probability. The chromosomal abnormality in question is always represented by the dotted line.

somal abnormalities. Results of this detailed analysis of chromosome aberrations in myeloma suggest two major cytogenetic groups of patients with myeloma: hyperdiploid and non-hyperdiploid variant myeloma. This last group is composed of the hypodiploid, pseudodiploid and near-tetraploid variants. Each one of these subtypes can have different outcomes with the hypodiploid myeloma patients faring worse, while the pseudodiploid and hyperdiploid variants having a better outcome. These observations are consistent with other publications,[25] including studies of DNA index as measured by flow cytometry.[26] In addition, we found that structural chromosomal abnormalities were slightly more common among patients with pseudodiploid or hypodiploid myeloma.

Distribution of chromosome number

The distribution of total chromosome number is compatible with the clone following two major pathways. The first pathway is one in which chromosome gains are uncommon, or undesirable. This is a pathway in which the clone 'tolerates' occasional chromosomal losses. In this model, some chromosomes are more frequently lost than others, suggesting a survival advantage for the loss of these, or an impossibility of losing the others. This model suggests that a common genetic abnormality is capable of providing the survival advantage to the clone that allows for the loss of some chromosomes.

The second group is composed of patients whose hallmark is a broad state of genomic instability characterized both by chromosomal gains and losses, usually resulting in the net gain of genetic material. The distribution of chromosome numbers (Figure 1) in these hyperdiploid karyotypes suggest that the hyperdiploid form of myeloma is not preceded by a tetraploid state that progressively loses chromosomes, but rather results from progressive gains of some (usually specific) chromosomes.

Smadja and colleagues[25] have reported this same pattern of chromosome distribution among patients with myeloma. They also reported that the 4% of patients in their study with near-tetraploid karyotypes most likely duplicated their nuclear material. This conclusion was based on their observation that metaphases in these patients had the same abnormalities in both the 2N or 4N state.[25] At least 10% of our patients had near-tetraploid karyotypes. It is possible that some of the patients with near-tetraploid karyotypes are an artifact of cell culture and are 2N when not in division. However, DNA content analysis suggests that at least in some cases of myeloma the 4N clone co-exists at a significant level with a 2N counterpart, which is precisely what Smadja and colleagues reported.[25]

Lack of specific trisomies

Not all chromosomes are represented among the trisomic category. The most common trisomies involved chromosomes 3, 5, 7, 9, 11, 15 and 19. The prevalence of these abnormalities is in accordance to what has been reported both by conventional cytogenetics,[3,7,27,28] multicolor metaphase FISH (SKY),[4] and interphase FISH.[29–31] It is possible that some specific trisomies would have negative consequences for the survival of the clone. For instance there were very rare cases where we detected trisomy of chromosome 13. This may suggest that the presence of an extra copy of chromosome 13, or any other of the chromosomes not found to be trisomic could be deleterious for the clonal expansion. This seems more likely than to postulate that the chromosomes that are represented more often among the trisomic ones would provide a survival advantage for patients.

Prognosis by monosomy and hypodiploid state

When one assesses the global impact of abnormalities and prognosis, monosomies stand out as having a greater impact on prognosis than do other abnormalities. Overall survival disadvantage was not seen with trisomies while five monosomies (chromosome 2, 3, 13, 14 and 19) were significantly associated with an adverse outcome. However, and in conflict to previous reports, it appears that this effect is not only limited to chromosome 13, but possibly other monosomies can also have this impact. The generalization of these results is limited by the small numbers of patients for each one of the specific monosomy categories. In additions it is currently difficult to determine whether the negative outcome of patients with any monosomies is because of their higher prevalence in the hypodiploid variant or vice versa.

The negative effect on prognosis of the hypodiploid state has also been reported by Smadja, and in her series monosomy of chromosome 13 did not add prognostic significance.[25] We have confirmed that observation in this study. In fact, our study shows that even among patients with abnormal metaphases, the presence of hypodiploid myeloma is signifi-

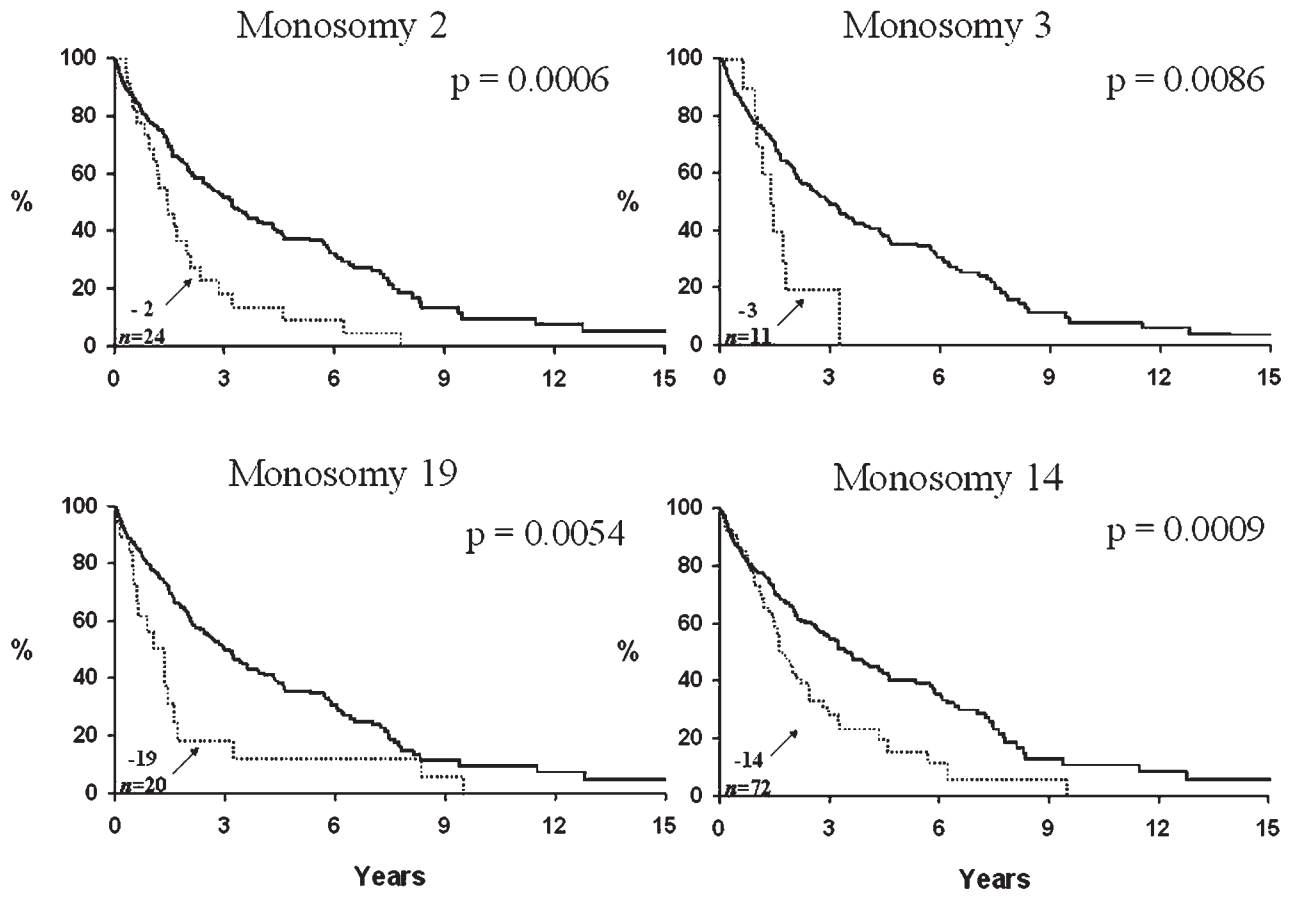

Figure 5 Prognosis of patients according to the presence of selected specific monosomies (for Δ13 see Figure 7). The time since diagnosis in years is depicted in the x-axis. The chromosomal abnormality of interest is always represented by the dotted line.

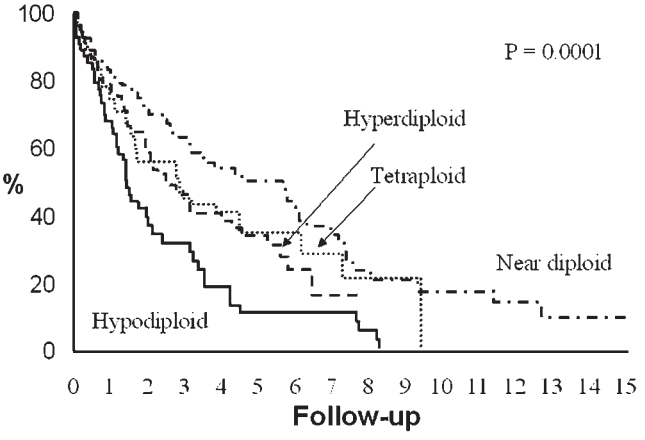

Figure 6 Overall survival of patients according to ploidy status. Kaplan–Meier survival analysis of patients according to ploidy category. The survival since diagnosis time is presented in the x-axis in years and the *P* value is the univariate log-rank probability. The chromosomal abnormality in question is always represented by the dotted line.

cantly associated with shortened survival.[25] The negative prognostic association of hypodiploidy has been previously reported by others[32] by DNA content analysis and by standard metaphase analysis,[25,33] but is highly dependent on the method of detection.[34] As mentioned previously, we speculate that patients with hypodiploid variant myeloma have underlying genetic abnormalities that favor proliferation and in fact allow the clone to continue to expand despite losses of occasional chromosomes ultimately leading to hypodiploidy ('permissive to the loss' theory).

The hyperdiploid variant of myeloma,[26,34] and trisomies,[35] are associated with a favorable prognosis compared to those with hypodiploid myeloma. It may be that two unique mechanisms are involved in the pathogenesis of myeloma; one involves a hyperdiploid mechanism and the other a hypodiploid approach.

Chromosome 1 abnormalities

We observed structural abnormalities of chromosome 1 were associated with shortened survival in myeloma, regardless of whether the p arm was translocated or deleted. This conflicts with the publication of Smadja *et al*,[25] who reported that chromosome 1 abnormalities conferred no specific prognostic information, but their sample size was smaller. No specific gene has been linked with chromosome 1 abnormalities, but it is not surprising given the wide range of breakpoints on both the p and q arm. Nevertheless, further study of genes on chromosome 1 may be fruitful in multiple myeloma. This has been recently confirmed as abnormal with a high prevalence by the study of Sawyer and colleagues.[4] Likewise an array of complex chromosomal changes has also been reported by Sawyer and colleagues, including jumping translocations at this site.[15]

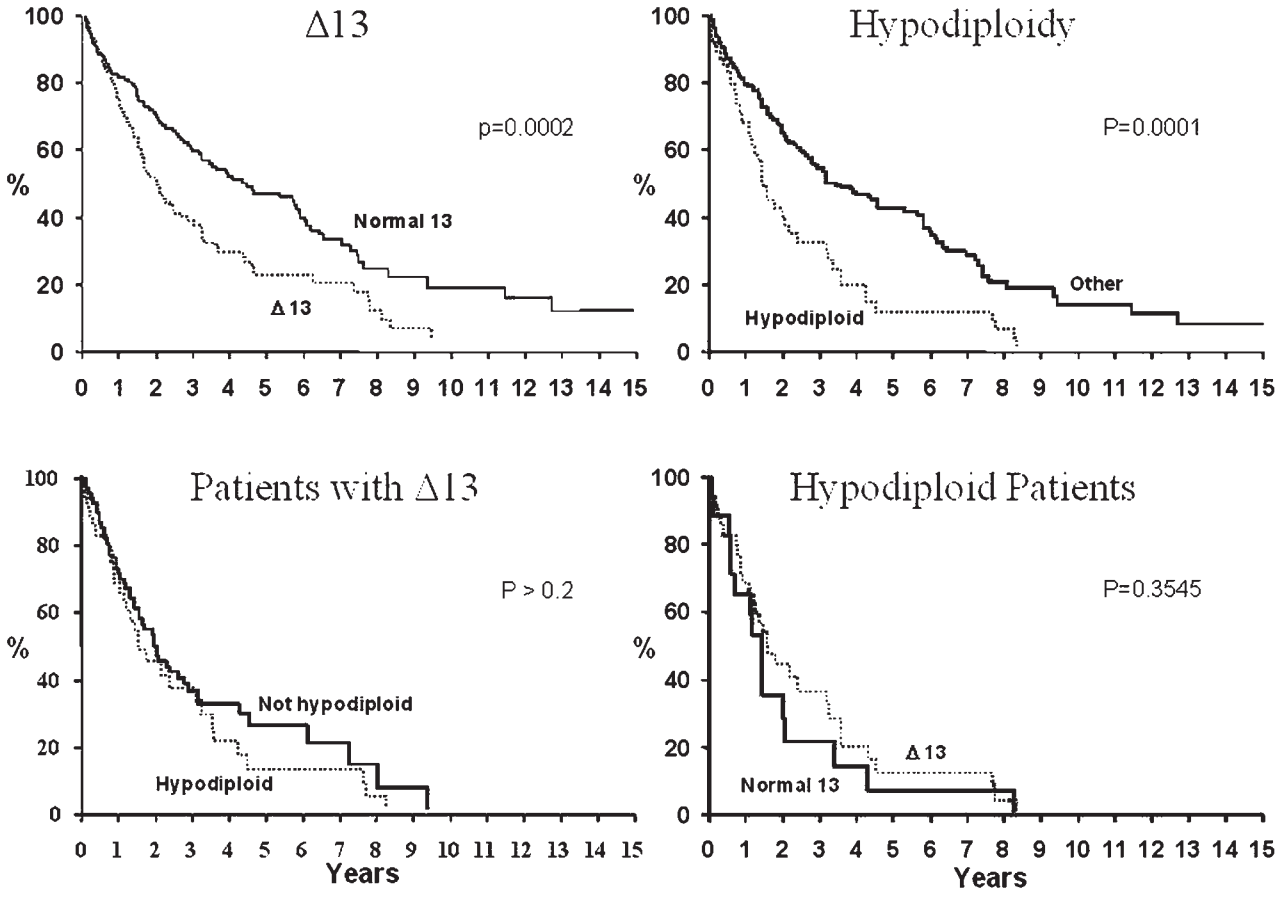

Figure 7 Relationship between hypodiploidy and Δ13. The top left figure shows the effect ofΔ13 on prognosis among patients with chromosomal abnormalities, where even within this group of patients Δ13 is of prognostic importance. The top right panel similarly shows the prognostic effect of hypodiploidy. The bottom left panel shows that among hypodiploidy on patients with Δ13 hypodiploidy did not add further prognostic information. Likewise, the bottom right panel shows that among patients with hypodiploidy Δ13 did not add further prognostic information.

Most investigators using conventional cytogenetic studies report chromosomal abnormalities in 15% to 50% of patients with myeloma, while a single study reports a prevalence of 66%.[3,7,36] Using interphase FISH studies, the incidence of chromosomal abnormalities in patients with myeloma usually exceeds 95%. Once the most important chromosome abnormalities can be elucidated by conventional cytogenetics, it will be possible to continue to use interphase FISH that can detect chromosome abnormalities in all patients with myeloma. In fact, we and others have successfully used interphase FISH for analysis and prognostication of patients.[14,18,19,37–39] A simple FISH strategy using sets of interphase FISH probes can be easily used to detect ploidy status in myeloma. We also wish to speculate that the patterns of gene expression will likely be similar between patients with these major subcategories of multiple myeloma.[40,41]

Acknowledgements

RF is a Clinical Investigator of the Damon Runyon Cancer Research Fund and a Leukemia and Lymphoma Society Translational Research Awardee. This work was supported in part by Public Health Service grant no. R01 CA83724-01 (RF) and P01 CA62242 (RAK, PRG, TEW, JAL) from the National Cancer Institute, and the 'Foundation to Cure Myeloma' supports RF and PRG. PRG is supported by the ECOG grant CA21115-25C from the National Cancer Institute.

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