Gene abnormalities in multiple myeloma; the relevance of *TP53*, *MDM2*, and *CDKN2A*

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Background and Objectives. Disruption of either the $p14^{ARF}$- mdm2- p53 or $p16^{INK4A}$- Rb1 pathways produces a breakdown of regulatory mechanisms and creates a gateway for tumorigenesis. Since the incidence and clinical implications of abnormalities of *TP53*, *CDKN2A* (encoding for p16 and p14) and *MDM2* genes (chromosome 12) in multiple myeloma (MM) is not clear, we investigated allelic loss at the former two loci and gain at the latter locus in a series of 82 MM patients.

Design and Methods. Dual color fluorescence *in situ* hybridization (FISH) was applied to bone marrow samples to establish the incidence of changes at the above mentioned loci. The *CDKN2A* locus was tested using a probe which hybridizes to 9p21 and also targets the $p15^{INK4B}$ gene.

Results. FISH analysis revealed the presence of monoallelic TP53 deletions in 12% of patients. Ten percent of patients had hemizygous deletion at 9p21, while a further 8% had loss of 1 of 3 loci in the presence of trisomy 9. *MDM2* amplification in the face of chromosome 12 diploidy was seen in 8%, while another 8% had trisomy 12 with an equivalent increase in signals for *MDM2*. Clinical correlations revealed that allelic loss of *TP53* was the only factor associated with resistance to chemotherapy. The presence of 9p21 deletion was associated with an IgA isotype but none of the abnormalities had a significant influence on overall or event-free survival.

Interpretations and Conclusions. *TP53* and *CDKN2A* (9p21) allelic loss and amplifications of the *MDM2* gene are infrequent events in myeloma. The incidence of the latter two events was, however, higher than previously reported. Deletion of the *TP53* gene predicted resistance to chemotherapy, highlighting its importance in this disease process.

Key words: TP53, MDM2, 9p21 CDKN2A, fluorescence *in situ* hybridization, myeloma.

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The uncontrolled proliferation of cells in malignancies is postulated to result from a deregulation of two main pathways of cell cycle control: the $p14^{ARF}$–mdm2–p53 and $p16^{INK4A}$–Rb1 pathways.[1-4] Loss of function of the *TP53* tumor suppressor gene (TSG) by deletion and/or mutation has been implicated in several B-cell malignancies, with conflicting data concerning its influence on disease progression and resistance to treatment.[5]

Multiple myeloma (MM) is a neoplasm characterized by heterogeneity in a number of features including genetic abnormalities, clinical presentation, response to therapy and prognosis. Previous studies have been consistent in reporting the occurrence of *TP53* mutations to be an infrequent and late event in MM.[6-8] There has, however, been controversy regarding the incidence and prognostic impact of *TP53* deletions.[9-11] Loss of $p14^{ARF}$ and gain of MDM2 function have been suggested as alternative mechanisms for p53 knockout.[4] The *CDKN2A* locus at 9p21 encodes for the $p16^{INK4A}$ as well as the $p14^{ARF}$ TSG.[12] The $p15^{INK4B}$ resides adjacent to *CDKN2A* and shares with $p16^{INK4A}$ a role as a cyclin-dependent kinase inhibitor (CDKI). Loss of either of these TSGs would potentiate uncontrolled proliferation of the cell cycle.[13,14] Previous studies have not been systematic in investigating the incidence of 9p21 (locus for *CDKN2A* and *p15*) deletions or amplifications of the *MDM2* locus on chromosome 12 in MM. The aim of this study was to determine i) *TP53* deletions; ii) 9p21 deletions for loss of *CDKN2A* and *p15*; iii) *MDM2* gene amplification/increased gene copy number, and iv) the possible impact of each of these abnormalities on prognosis and response to treatment in a series of MM patients.

Design and Methods

Patients

Bone marrow samples from 82 MM patients were studied, three of whom were diagnosed as having plasma cell leukemia (PCL). These were consecutive patients attending the Myeloma Unit at the Royal Marsden Hospital between July 1997 and December 2001. The range of plasma cells in the samples was 10-95% (mean = 40%).

Clinical data were obtained for 61 patients whose samples were taken within 0-24 months (median = 3 months) from diagnosis. Diagnostic samples were available from 38 patients, and samples from a further 8 patients were obtained before the start of treatment, hence a total of 46/61 (75%) were studied prior to ther-

apy. The mean age of these patients at diagnosis was 58 years (range: 43-83), 40 were male and 21 were female. The majority of patients with known staging[15] were in stage III disease at the time of diagnosis (38/50; 76%), two patients had PCL, while the staging of 9 patients was unknown at diagnosis.

Fifty-one patients initially received chemotherapy in the form of C-VAMP as induction treatment.[16] Forty-eight of these went on to receive high-dose melphalan and peripheral blood stem cell support, while one patient died before initiation of the high-dose therapy. Three patients received high-dose melphalan as first-line treatment.

Fluorescent in situ hybridization (FISH)

Dual color interphase FISH was performed on separated mononuclear cells using a method previously described.[17] Briefly, mononuclear cells were obtained by centrifugation of heparinized bone marrow specimens over Histopaque (Sigma, Poole, United Kingdom), washed twice in Hanks balanced salt solution then fixed in methanol/ acetic acid 3:1 and stored at -20°C. Cells were dropped onto clean slides and aged by placing on a hot plate at 55°C for one hour. Slides were then placed in 2×SSC at 37°C for 30 minutes followed by dehydration in 70%, 90% and 100% ethanol series. Denaturation was carried out on a dry heat block at 72°C for one minute in the presence of 100 µL denaturing solution (70% formamide, 2×SSC and 0.05 M sodium phosphate buffer pH 7.0). Probes were prepared and denatured according to the manufacturer's instructions. Hybridization was carried out overnight in a humidified chamber at 37°C. Post-hybridization washes consisted of three washes in 1×SSC at 45°C followed by three washes in 0.1×SSC at 60°C and finally one wash in 4×SSC/Triton X-100 at room temperature. Signals were visualized under a fluorescent microscope (Zeiss, Oberkochen, Germany) equipped with dual and triple band pass filters.

The following probe combinations were employed in these experiments: to determine *TP53* allelic loss, a locus-specific p53 probe was used in conjunction with a chromosome 17 centromeric probe (Vysis) to distinguish monosomy 17 from a locus-specific loss. All 82 patients were tested with these probes. To investigate *CDKN2A*, a commercially available set consisting of a locus-specific probe hybridizing to 9p21 combined with a chromosome 9 centromere probe was used (Vysis). The role of the centromere 9 probe was to define patients with trisomy 9, which is a frequent observation in MM.

YAC 751a4, which hybridizes to the MDM2 locus (12q13-14), was obtained from the *Human Genome Mapping Project*, extracted, labeled by nick translation and used in conjunction with a commercially available chromosome 12 centromeric probe (Vysis, UK), to determine both the incidence of *MDM2* gene amplification and trisomy 12 (+12) in the patients. Forty-eight patients were tested for these two abnormalities.

Between 200-400 nuclei were scored per slide. Results were considered positive when the percentage of scored cells with the relevant abnormality exceeded the cut-off level. Cut-off levels were calculated from the mean value plus 3 standard deviations, and were obtained through testing each probe on five peripheral blood and five bone marrow controls and scoring 500 nuclei per slide. The cut-offs were as follows: 10% for TP53 deletion, 9.7% for deletion 9p21 and 5.5% for trisomy 9, 4.3% for three or more signals of YAC 751a4 (MDM2) and 3.2% for trisomy 12.

Statistical analysis

Clinical and laboratory data were statistically evaluated using Fisher's exact test and the Mann-Whitney test for trends to compare the distribution of each variable between groups of patients. The Wilcoxon rank sum test was used to compare continuous variables between groups.

Overall survival (OS) and event-free survival (EFS) were both measured from the time of diagnosis using the log rank test. The end point for OS was death from any cause, while that of EFS was relapse, disease progression or death. Statistical analysis was performed using SPSS statistical software.

Results

TP53 allelic loss by FISH

FISH analysis revealed the presence of monoallelic TP53 deletions in 10/82 (12%) patients studied and none of them had loss of the centromere 17 probe. Monoallelic deletions were detected in 12-65% of nuclei (Table 1 and Figure 1), which constituted 21-94% of the plasma cell population in the samples studied. One patient (PC2) with PCL showed two different clonal populations of malignant cells, one with monoallelic TP53 deletion and the other with biallelic deletion.

Deletions at 9p21

Five of the 48 patients (10%) tested showed hemizygous deletions of 9p21, and all 5 cases were biallelic for chromosome 9 (Figure 2). The deletion was detected in 11-32% of nuclei (Table 1), constituting 15-64% of plasma cells in the samples studied. Trisomy 9 was detected in 13/48 (27%) patients. However, four of these 13 cases (8% of all patients tested) had two signals for 9p21 instead of the expected three signals, indicating a loss of 9p21 from one of the three chromosome 9 copies (Figure 2).

Table 1. Patients with abnormalities of either TP53, MDM2, or 9p21 by FISH.

Patient no.	*Bone marrow plasma cells*	*TP53 deletion*	*MDM2 amplification/trisomy 12*	*9p21 deletion*
PC2	68%	31%*	Negative	Negative
PC3	90%	Negative	23% amplification	Negative
PC4	69%	65%	Negative	Negative
mm4	49%	22%	Negative	Negative
mm5	21%	14%	15% trisomy	Negative
mm6	15%	12%	Negative	Negative
mm11	85%	18%	Negative	Negative
mm12	31%	19%	Negative	Negative
mm15	60%	45%	Negative	Negative
mm26	21%	Negative	14% trisomy	Negative
mm27	70%	Negative	17% trisomy	Negative
mm35	25%	Negative	16% amplification	Negative
mm37	76%	Negative	Negative	11%
mm40	95%	28%	26% amplification	Negative
mm43	50%	Negative	Negative	32%
mm45	35%	15%	Negative	Negative
mm54	30%	Negative	15% trisomy	Negative
mm63	55%	Negative	14% amplification	18%
mm65	94%	Negative	Negative	19%
mm80	67%	Negative	Negative	27%

*Legend: * heterozygous deletion: 19%; homozygous deletion: 12%.*

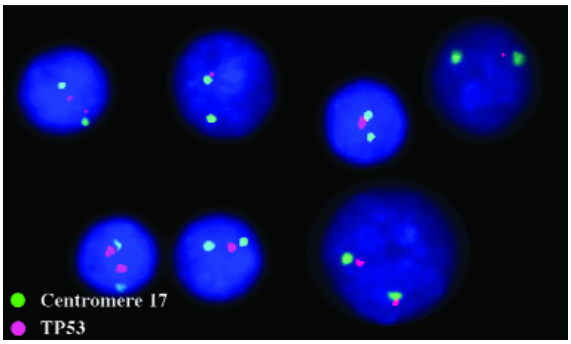

Figure 1. FISH analysis of TP53 gene copy in MM. Dual color FISH study allowed the differentiation between TP53 deletion due to a locus-specific loss and monosomy 17. Monoalleic TP53 deletion is shown in the interphase cells of a myeloma patient.

Abnormalities of the MDM2 locus

Four out of the 48 (8%) patients had amplification of this locus as shown by the presence of more than two signals for YAC 751a4 with no more than two copies of chromosome 12 (Figure 3). A further four cases showed +12 accompanied by an equivalent increase in the number of signals for the MDM2 probe (Figure 3). These abnormalities were present in 14-26% of nuclei (Table 1), which constituted 24-71% of plasma cells in the samples studied.

Clinical correlations with TP53 deletions

There were no differences between the two groups with and without TP53 deletions, regarding age, sex, paraprotein type, percentage of bone mar-

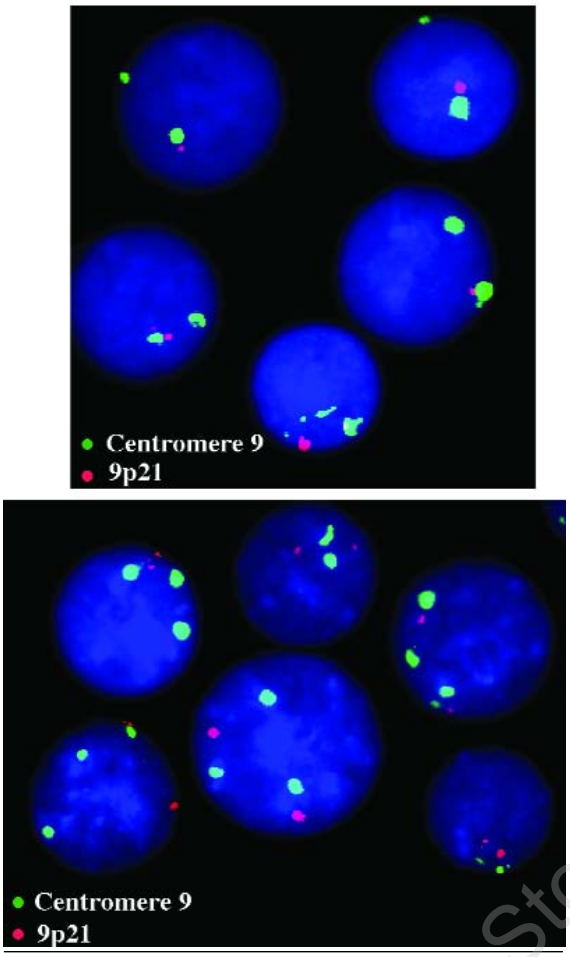

Figure 2. FISH for the 9p21 locus in MM. Dual color FISH shows: monoallelic loss of 9p21 in a sample which was disomic for chromosome 9 (above); two signals for the 9p21 locus instead of the expected three signals in cells with trisomy 9 (below).

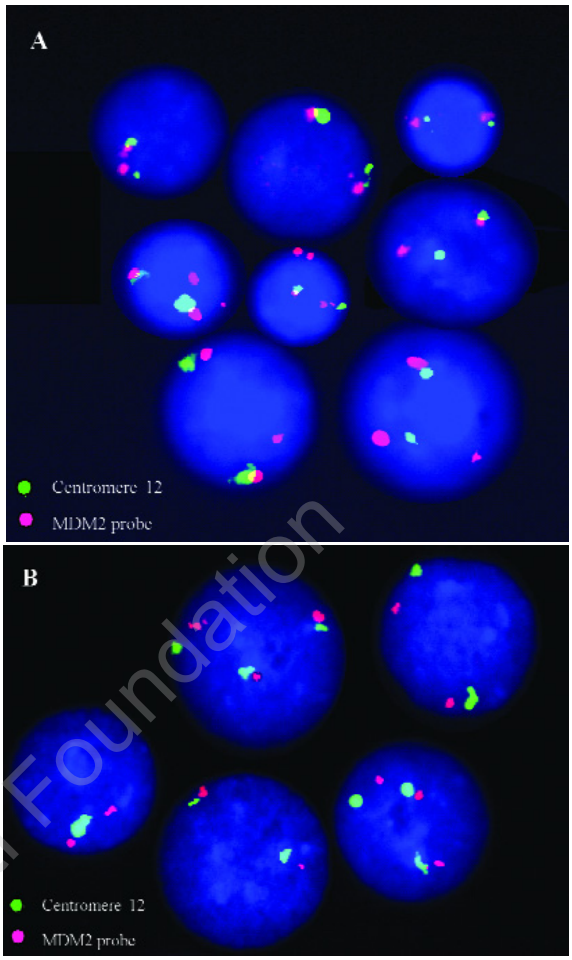

Figure 3. FISH to detect the *MDM2* gene copy number in MM. Dual color FISH study shows: A) a case with amplification of MDM2 as seen by cells with more than two signals for YAC 751a4 in the presence of disomy 12; B) a case in which some cells harbor trisomy 12 with an equivalent three signals for *MDM2*.

row plasma cells and β_2-microglobulin levels. Although most patients in both groups were in stage III at diagnosis, there was a higher proportion of patients with TP53 deletions in stage IIIb/PCL compared to the others (37.5% versus 7.5%, p = 0.096). The proportions of patients tested prior to treatment, and those who received C-VAMP and high-dose melphalan were similar in both groups. Although there was a higher percentage of patients in relapse in the deletion group (37.5% versus 9.4%), the difference was not statistically significant (p = 0.1) because of the low numbers. Lack of response to treatment was associated with the presence of TP53 gene deletion (Table 2). None of the patients with deletion achieved complete remission with high-dose therapy whereas 59% of those without a deletion did so; on the other hand 71.4% of the former group showed a partial response compared to 27.3% of the latter group (p= 0.0009). A greater proportion of those with a TP53 deletion did not respond to C-VAMP compared to those without deletion (p = 0.074). Univariate analysis of other factors such as the presence of BCL-1/ cyclin D1 abnormalities, deletion 13q (*data not shown*) deletion 9p21, β_2-microglobulin level > 3.5 mg/L, presence of an IgA isotype and stage of disease, showed that none of these factors affected response to either C-VAMP or high-dose therapy. The median OS was 31.8 months for patients with TP53 deletion, and 44.9

months for patients without deletion; however, the association between either OS or EFS and the presence of a *TP53* deletion was not statistically significant (Table 2).

Correlations with 9p21 deletions (Table 3)

There were no differences between the two groups with and without 9p21 deletions, regarding age and sex. All patients with 9p21 deletion had stage IIIa/b disease, but the difference in frequency between the groups was not significant. Likewise, the mean level of serum β_2-microglobulin was higher in the group with deletion but the difference was of no statistical significance. In contrast, a significantly higher proportion of patients with deletion had an IgA paraprotein isotype than did those without deletion (60% vs 8.6%, p = 0.017). Similar proportions of patients in each group were tested prior to treatment and received conventional and high-dose therapy and there were no differences between them regarding response. There were no significant differences between the groups regarding median OS and EFS.

Correlations with MDM2 amplification/trisomy 12

Patients with either amplification of the MDM2 gene *per se*, or with trisomy 12 were studied as one group based on the notion that both changes increase the number of copies of the MDM2 gene. There were no differences between the two groups regarding age, sex, clinical stage, bone marrow plasma cell percentage or paraprotein isotype (Table 3). The mean β_2-microglobulin level was significantly higher in the group lacking MDM2 abnormality (p <0.01). The proportions of patients tested prior to treatment and those responding to conventional and high-dose therapy were similar in both groups. Patients with MDM2 abnormalities were good responders to treatment and no significant differences in response, median OS or EFS were observed between the two groups. Overall, clinical features and outcome did not differ when considering each group with either trisomy or amplification separately.

Discussion

Inactivation of the *TP53* gene due to mutation or allelic loss has been observed in many solid tumors providing strong evidence of its association with tumor progression.[18] p53 abnormalities generally occur at a lower frequency in hematologic malignancies than in solid tumors.[19] Data in the literature, particularly on B-cell malignancies, has been conflicting regarding the influence of p53 abnormalities on disease progression.[20-22]

In this study, 12 % of MM patients were found to harbor a TP53 deletion. The incidence of TP53

Table 2. Clinical/laboratory features of MM patients with and without TP53 deletions.

Variable	Patients@ No abnormality	Patients@ With abnormality	Significance
Number of patients@	53	8	
Age (y) at diagnosis: mean, (range)	58.7 (43-79)	62.1 (46-83)	
Percentage of males	68	50	NS
Stage of MM at diagnosis (% of patients)			
Stage I or II	18.9	12.5	
Stage IIIa	56.6	50	p= 0.096
Stage IIIb/PCL	7.5*	37.5°	
Unknown	17	0	
Paraprotein type (% of patients)			
IgA	18.9	0	NS
Non-IgA	81	100	
Percentage of bone marrow plasma cells: mean, (range)	43.9 (10-95)	49.8 (15-85)	NS
β2-microglobulin (mg/L): mean, (range)	7.4 (2.1-37)	6 (5-7.1)	NS
Percentage of patients tested at diagnosis	64	50	
Percentage of patients tested prior to therapy	75.5	75	
Percentage of relapsed patients	9.4	37.5	p= 0.1
Percentage of patients who received CD therapy	83	87.5	
Response to CD (% of patients)			
NR	6.8	42.9	
PR	59.1	42.9	p = 0.074
CR	25	14.2	
Unknown	9.1	0	
Percentage of patients who received HD therapy	83	87.5	
Response to HD (% of patients)			
NR	4.5	28.6	
PR	27.3	71.4	p = 0.0009
CR	59.1	0	
Unknown	9.1	0	
Median OS (months)	44.9	31.8	p = NS
Median EFS (months)	44	31.8	p = NS

*@Patients studied within 24 months of diagnosis; *IIIb/PCL: 3/1 patients; °IIIb/PCL: 2/1 patients; CR: complete remission; PR: partial remission; NR: no response; CD: conventional dose (C-VAMP); HD: high dose (melphalan); NS: not significant; OS: overall survival; EFS: event-free survival.*

Table 3. Clinical/laboratory features of MM patients with and without 9p21 deletions and MDM2 amplifications/+12.

Variable	*9p21**		*MDM2/+12**	
	No abnormality	*With abnormality*	*No abnormality*	*With abnormality*
Number of patients	35	5	33	7°
Age (y) at diagnosis: mean, (range)	59.4 (43-83)	60.6 (52-75)	58.8 (43-83)	61.7 (51-70)
Percentage males	74.3	60	75.8	57.1
Stage of MM at diagnosis (% of patients)				
Stage I or II	14.3	0	9.1	28.5
Stage IIIa	51.4	80	57.6	42.9
Stage IIIb/PCL	14.3†	20††	15.2#	14.3##
Unknown	20	0	18.1	14.3
Paraprotein type (% of patients)				
IgA	8.6	60‡	12.1	28.6
Non-IgA	91.4	40	87.9	71.4
Percentage of bone marrow plasma cells: mean, (range)	43.9 (12- 92)	68.4 (50- 94)	51.8 (12-94)	44.6 (21-90)
β2- microglobulin (mg/L): mean, (range)	7.1 (2.2-31.2)	10.5 (3.2-37)	8.51 (2.2-37)	4.2 (2.2-6.6)@
Percentage of patients tested prior to treatment	71.4	80	72.7	71.4
Percentage of relapsed patients	14	20	12	28.6
Percentage of patients who received CD therapy	88.6	100	87.9	100
Response to CD (% of patients)				
NR	16.1	20	17.2	14.3
PR	51.6	20	51.7	14.3
CR	19.4	40	17.2	42.8
Unknown	12.9	20	13.8	28.6
Percentage of pts. who received HD therapy	82.9	100	84.8	85.7
Response to HD (% of patients)				
NR	10.3	20	10.7	6.7
PR	37.9	20	39.3	16.7
CR	41.5	40	39.3	50
Unknown	10.3	40	10.7	16.7
Median OS (months)	6.6	not reached	not reached	31.8
Median EFS (months)	6.3	not reached	not reached	30

**Patients studied within 24 months of diagnosis; °MDM2 amplification (3 patients), trisomy 12 (4 patients), † IIIb/PCL: 3/2, ††IIIb/PCL: 1/0, #IIIb/PCL: 4/1 patients; ##IIIb/PCL: 0/1 patients; CR: complete remission, PR: partial remission, NR: no response, CD: conventional dose (C-VAMP) HD: high dose (melphalan), NS: not significant, OS: overall survival, EFS: event-free survival. p values = ‡ 0.017, @p<0.01.1.*

deletions detected here is in agreement with findings by Avet-Loiseau *et al.*[10] and Schultheis *et al.*[11] who reported heterozygous TP53 deletions in 9% and 11% respectively. In contrast, deletions were reported in 32% of newly diagnosed and 55% of patients in relapse, by Drach *et al.*[9] and their findings suggested a strong correlation between TP53 deletion and survival following chemotherapy; these observations however have not been confirmed by others. *TP53* deletions were here associated with more advanced disease stages and a higher proportion of patients with stage IIIb/PCL. Unlike the findings of Drach *et al.*[9] there was no significant impact on survival in patients with *TP53* deletion. Outcome and survival were not investigated in the other studies.[10,11] The main impact of *TP53* deletion in this study was the lower response rate to treatment, as 43% of those with *TP53* deletion did not respond to C-VAMP, and none achieved complete remission after high-dose melphalan. The association between *TP53* deletion and refractoriness to therapy is similar to that observed in CLL in which *TP53* deletion is associated with non-response to fludarabine.[23]

Since many cytotoxic drugs act through induction of apoptosis, which requires a functional p53, it has been postulated that resistance to these agents arises in tumor cells with defective p53 function due to impairment of the apoptotic process.[24] Moreover, loss of p53 function can lead to a state of 'genomic instability' resulting in the development of further genetic changes which may have a cumulative effect on drug resistance. Supporting this hypothesis, 7/10 cases with TP53 deletion in the present study were also found to have 13q deletion and three cases had a BCL-1 aberration (*data not shown*).

The next question is whether additional factors compromise p53 function, such as *MDM2* amplifications or deletions of the *p14^{ARF}* gene. Overexpression of MDM2 protein has been shown in MM cell lines and was suggested to enhance the proliferative capacity of MM cells by binding to p53, E2F-1 and to p21 in cells lacking p53.[25] The mechanism of this overexpression, as well as the role of MDM2 in MM, is not well established. Amplification of MDM2 with resulting protein overexpression has been documented in sarcomas,[26,27] and breast carcinomas.[28] MDM2 amplification is infrequent in leukemias and lymphomas.[29-31] Most studies, however, employed Southern blot to look for gene amplification and the sensitivity of this technique depends on the presence of a sufficient fraction of cells with the abnormality. Fluorescent *in situ* hybridization (FISH) is a more sensitive technique for detecting aberrations present in a small population of cells. Our findings showed that the *MDM2* gene was amplified in 8% of cases, and +12 with an equivalent increase in *MDM2* signals was found in a further 8%. *TP53* deletion coincided with *MDM2* amplification in one case and in another with +12. The presence of amplification or trisomy 12 did not appear to be related to an unfavorable prognosis.

Ten percent of patients were found to harbor a monoallelic deletion at 9p21. Both p16 and p15 have similar roles, functioning as CDKIs, while p14 promotes the degradation of *MDM2* leading to stabilization of p53. Homozygous deletions of the 9p21 region containing the *p15*, *p16* and *p14* genes are found in a high proportion of acute lymphoblastic leukemias,[32-34] adult T-cell leukemia[35] and chronic myeloid leukemia.[36] Few studies have analyzed *CDKN2A* and *p15* allelic alterations in B-cell malignancies.[37-39] Homozygous deletions are infrequent and associated with tumor progression, while hemizygous loss is more prevalent, and mutations are rare.[40] Methylation of *p16* and/or *p15*, but not *p14*, has been documented in several B-cell malignancies[40] and has constituted a frequent finding in MM patients and myeloma cell lines.[41-43] On the other hand, allelic loss at 9p21 in MM may have been under-represented in previous reports. Seventeen cases were examined by Tasaka *et al.*[44] who reported a homozygous deletion of *p15* and *p16* genes in a case of PCL, and a homozygous deletion of p15 in one case of MM. Ng *et al.*[41] reported no deletions in 12 cases also studied by Southern blot.

We have shown here that monoallelic deletion of 9p21 correlated only with an IgA paraprotein isotype. This finding does, however, need to be validated on a larger cohort of patients. The significance of loss of one 9p21 locus in four cases with trisomy 9 in this study is not clear. The gene dosage in these cases should be adequate, unless either allele is afflicted with mutation or methylation. It has been suggested that hypermethylation of *p16* and *p15* are early events in MM.[41,45] In contrast, the presence of 9p21 (*CDKN2A/p15*) deletion in only a proportion of the plasma cells seen in this study points out that it may well be a secondary event which plays a role in tumor progression. It has been shown previously that both *p16* and *p14* (*p19*) null mice develop lymphomas.[46] Since the *p14* gene was also found to be disrupted in the *p16* null mice, it has been proposed that the major oncogenic event for lymphogenesis might be loss of *p14* rather than *p16*. Considering that *p14* hypermethylation is a rare event in MM, allelic loss of this gene may represent the key to enhancement of tumor progression in this disease. The role of *p14* in the stabilization of p53 has been discussed and postulations were made that loss of p14 is equivalent to that of p53 aberrations.[12] In this study, however, *TP53* and *CDKN2A/p15* deletions were not involved concomitantly in any patient, and the loss of the latter locus did not pro-

duce findings similar to those produced by loss of the former one.

In conclusion, *TP53* and *CDKN2A* allelic loss and amplifications of the *MDM2* gene are relatively infrequent events which do not seem to arise concomitantly in MM. Monoallelic deletion of TP53 was the only variable associated with resistance to chemotherapy, mainly to high-dose melphalan. Therefore its identification in MM allows the recognition of a group of patients most likely to present a therapeutic challenge.

Given the prominent clinical and genetic heterogeneity observed in MM, there is now growing interest in steering research towards a path involving global gene expression studies. Recent investigations by Zhan *et al.*[47,48] have utilized gene expression profiling technology in an attempt to study the relationship between normal plasma cell counterparts and multiple myeloma cells, as well as to establish a classification system for MM based on gene expression patterns. The final goal of such research is to integrate these results with clinical response data, which if attained will undoubtedly revolutionize clinical stratification systems in this disease.

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Pre-publication Report & Outcomes of Peer Review

Contributions

MOE: conception and design, analysis and interpretation of data, drafting the article and critical revision; AMG-W: analysis and interpretation of data, critical revision of article and final approval of version to be published; RA'H: analysis and interpretation of data and final approval of version to be published; EM: critical revision of article and final approval of version to be published; BS: analysis and interpretation of data and final approval of version to be published; RP: analysis and interpretation of data and final approval of version to be published; DC: conception and design, critical revision of article and final approval of version to be published. Order of authorship is based on the degree of contribution to the work, except the last two authors who are Heads of Departments. Primary responsibility for the paper: MOE; creator of all Tables and Figures: MOE.

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In the following paragraphs, the Editor-in-Chief summarizes the peer-review process and its outcomes.

What is already known on this topic

Various genetic abnormalities have been described in multiple myeloma and controversial findings have been reported.

What this study adds

The presence of TP53 deletion predicts resistance to chemotherapy and identifies a subgroup of therapeutically challenging myeloma patients.