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Clinical implications of cytogenetic heterogeneity in multiple myeloma patients with *TP53* deletion

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_TP53_ deletion (Δ_TP53_) in myeloma is known to be a high-risk finding associated with poorer prognosis. The prognostic impact of underlying cytogenetic heterogeneity in patients with myeloma associated with Δ_TP53_ is unknown. We studied 90 patients with myeloma associated with Δ_TP53_ identified by interphase fluorescence _in situ_ hybridization and assessed the impact of karyotype and coexisting alterations of _IGH_, _RB1_, and _CKS1B_. There were 54 men and 36 women with a median age of 59 years (range 38–84); 14 patients had a normal karyotype (NK/Δ_TP53_), 73 had a complex karyotype (CK/Δ_TP53_), and 3 had a non-complex abnormal karyotype. Patients with CK/Δ_TP53_ showed a significantly poorer overall survival compared with patients with NK/Δ_TP53_ ($P=0.0243$). Furthermore, in the CK/Δ_TP53_ group, patients with _IGH_ rearrangement other than t(11;14)(q13;q32)/_CCND1-IGH_, designated as adverse-_IGH_, had an even worse outcome ($P=0.0045$). In contrast, _RB1_ deletion, _CKS1B_ gain, ploidy, additional chromosome 17 abnormalities, or Δ_TP53_ clone size did not impact prognosis. Stem cell transplant did not improve overall survival in either the NK/Δ_TP53_ or CK/Δ_TP53_ ($P=0.8810$ and $P=0.1006$) groups, but tandem stem cell transplant did improve the overall survival of patients with CK/Δ_TP53_ ($P=0.0067$). Multivariate analysis confirmed in this cohort that complex karyotype (hazard ratio 1.976, 95% CI 1.022–3.821, $P=0.043$), adverse-_IGH_ (hazard ratio 3.126, 95% CI 1.192–8.196, $P=0.020$), and tandem stem cell transplant independently correlate with overall survival (hazard ratio 0.281, 95% CI 0.091–0.866, $P=0.027$). We conclude that comprehensive genetic assessment adds to _TP53_ status in the risk stratification of myeloma patients.
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Multiple myeloma is a clinically and molecularly heterogeneous disease with an overall survival ranging from <1 year to over 20 years.[1,2] Reliable risk stratification is a key to predicting outcome and guiding therapy. In 2015, the International Myeloma Working Group proposed the Revised International Staging System (R-ISS) for myeloma patients.[3] The system incorporates chromosomal abnormalities detected by interphase fluorescence *in situ* hybridization (FISH) and serum lactate dehydrogenase levels into the preexisting International Staging System (ISS), which was based on serum albumin and beta-2 microglobulin levels.[4] *TP53* deletion (Δ*TP53*) is also a known negative prognostic factor[5–7] and is considered a high-risk chromosomal abnormality in the R-ISS. Δ*TP53* usually results from deletion of chromosome 17p and is likely a secondary event that is often associated with disease progression.[1]

It is unclear if the negative prognostic impact of Δ*TP53* is influenced by background chromosomal aberrations such as ploidy, karyotypic complexity, or other recurrent changes that have been reported in myeloma.[8] For example, does Δ*TP53* detected by

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FISH carry the same prognostic impact as 17p deletion detected by conventional karyotypic analysis? Does clone size of Δ*TP53* as determined by FISH impact outcome? Does the presence of Δ*TP53* trump other known adverse factors, such as t(4;14) or t(14;16)? Lastly, might the combination of Δ*TP53* and t(4;14)/t(14;16) have a synergistic poorer effect on the prognosis of myeloma patients?

In this study, we investigated the prognostic impact of underlying cytogenetic heterogeneity in a group of patients with myeloma associated with Δ*TP53*, focusing on well-defined cytogenetic risk subgroups, with the goal of refining the prognostic impact of Δ*TP53*. Our data suggest that comprehensive assessment of genetic abnormalities in myeloma adds value to *TP53* status alone in the risk stratification of patients with myeloma.

Materials and methods

Study Group

We retrospectively reviewed multiple myeloma cases assessed by conventional cytogenetics and tested for *TP53* by interphase FISH at The University of Texas MD Anderson Cancer Center between 1 December 2007 and 31 December 2014. Clinical and laboratory data including hemoglobin level and serum beta-2 microglobulin and lactate dehydrogenase levels were obtained from a review of the electronic medical record. Disease stage was assessed using ISS and R-ISS. The study was approved by the Institutional Review Board.

In our laboratory, we did not routinely performed plasma cell enrichment on bone marrow aspirate specimens prior to culture until about 4 years ago. To ensure that all cases of myeloma in this study had a sufficient number of cells to avoid false-negative results, we required that all myeloma cases included in this study had 30% or more plasma cells on aspirate smears.

Cytogenetics and FISH

Conventional karyotyping was performed on cultured (unstimulated 24 and 48 h) bone marrow aspirate samples as part of the routine clinical workup and following laboratory standard procedures as have been reported.[9] At least 20 metaphases were fully analyzed whenever possible for the identification of clonal cytogenetic aberrations. The karyotypic results were reported according to the International System for Human Cytogenetic Nomenclature (ISCN) 2009 and 2013. In this system, a clone is defined as chromosomal changes in two or more metaphases and a complex karyotype (CK) is defined as ≥3 chromosome abnormalities.

FISH analyses for *TP53/CEN17*, *MYEOV/CCND1-IGH*/t(11;14), *RB1* (Vysis-Abbott Molecular, Downers Grove, IL, USA) and *CKS1B* (CytoCell, Cambridge, UK) were performed on cultured (unstimulated 24 and 48 h) bone marrow samples. Following laboratory standard protocols, all FISH cutoff values were established statistically based on the testing results on peripheral blood or bone marrow specimens from at least 20 normal healthy individuals. For *TP53*, we used a centromeric region of chromosome 17 as a control and the cutoff for a 200 interphase cell count was 4.7%. A clone size, that is, for the *TP53* deletion was determined by the number or the percentage of positive cells in 200 interphase cells analyzed. The clone size for *TP53* deletion was also further assessed by comparing it with results of *RB1* and/or *IGH-CCND1* tested concurrently and with the plasma cell percentage reported on the bone marrow aspirate differential count. FISH for *FGFR3-IGH*/t(4;14), *MAF-IGH*/t(14;16) were often performed (as a part of reflex testing) when FISH for *MYEOV/CCND1-IGH*/t(11;14)(q13;q32) was negative but with evidence of *IGH* rearrangement.

Patients with Δ*TP53* were divided initially into two major subgroups based on karyotype: normal karyotype (NK/Δ*TP53*) or complex karyotype (CK/Δ*TP53*). The CK/Δ*TP53* group, the main focus of this study, was also divided into subgroups (Figure 1) based on the presence or absence of *IGH* translocations. Among the patients with *IGH* translocations, there were three small subsets: t(4;14)/t(14;16), t(11;14), and other translocations with variable (v) loci designated here as t(v;14). Cytogenetically visible chromosome 17 aberrations, such as monosomy 17 (−17) or chromosome 17p deletion (17p−), as well as chromosome ploidy, including hypodiploidy (hypo) (chromosome count <44), hyperdiploidy (hyper) (chromosome count ≥48), and near-diploidy (chromosome count 44–47) were further analyzed in cases without *IGH* translocations and cases with t(11;14).

In addition, two independent groups of myeloma patients lacking Δ*TP53*, one with a normal karyotype

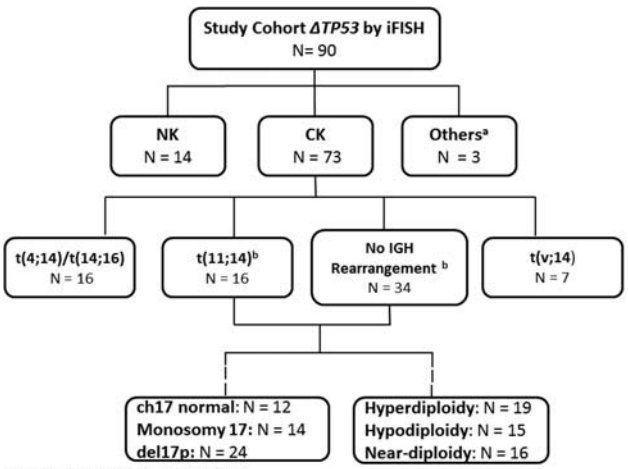

Figure 1 Study design: subgroups defined by chromosome and/or FISH analysis.

Table 1 Demographic and laboratory data of myeloma patients with *TP53* deletion and either normal karyotype (NK) or complex karyotype (CK)

	*Normal karyotype, (*N = *14)* Median (range)	*Complex karyotype, (*N = *73)* Median (range)	P-*value**
Age	57 (38–68)	58 (38–84)	0.31
M:F ratio	1.3	1.5	NA
Hemoglobin (g/dl)	11 (7.5–13.8)	10 (6.0–14.2)	0.11
Plasma cells in bone marrow aspirate (%)	54 (30–90)	78 (30–96)	*0.003*
Albumin (g/dl)	3.7 (2.7–4.3)	4.0 (2–5.0)	0.89
Beta-2 microglobulin (mg/l)	3.3 (1.6–9.9)	5.0 (2.0–90.4)	*< 0.001*
Creatinine	1.2 (0.5–2.3)	1.0 (0.3–7.4)	0.443
Lactate dehydrogenase (IU/l)	495 (265–786)	564 (243–12766)	*0.015*
TP53 deletion clone size (%) by FISH	12.8 (8–40)	30 (5–95)	*< 0.001*

*Statistically significant *P* values are in italics.

Table 2 Comparing stage, treatment and survival between patients with either normal karyotype or complex karyotype

Groups	*Normal karyotype, (*N = *14) (%)*	*Complex karyotype, (*N = *73) (%)*	P-*value**
Stages			0.058
International Staging System I	4 (29)	11 (15)	
International Staging System II	7 (50)	25 (34)	
International Staging System III	2 (14)	35 (48)	
Stages not available	1 (7)	2 (3)	
			0.038
Revised- International Staging System I	0 (0)	0 (0)	
Revised- International Staging System II	11 (79)	36 (49)	
Revised- International Staging System III	2 (14)	35 (48)	
Stages not available	1 (7)	2 (3)	
Diagnosis			0.326
New	4 (29)	11 (15)	
Relapsed/persistent	10 (71)	56 (77)	
Unknown	0 (0)	6 (8)	
Proteasome inhibitors			1.0
Yes	14 (100)	66 (90)	
No	0 (0)	4 (6)	
Unknown	0 (0)	3 (4)	
Stem cell transplant			0.234
None	4 (29)	27 (37)	
One	10 (71)	36 (49)	
Two or more	0 (0)	10 (14)	
Fatality rate	6 (43)	55 (75)	*0.015*
Follow-up months: median (range)	50 (14–97)	32 (3–101)	*0.029*
Median overall survival: median (range)	62 (14–97)	35 (3–101)	*0.024*

*Statistically significant *P* values are in italics.

(NK), and the other with a complex karyotype (CK), designated as NK/*TP53*nl and CK/*TP53*nl, respectively, were included as control groups to compare with the NK/Δ*TP53* and CK/Δ*TP53* study groups.

Survival Analysis

The follow-up interval and overall survival were calculated from the time of initial diagnosis until time of last follow-up or death. Statistical analyses were performed with the GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA). Kaplan–Meyer curves for overall survival were plotted and the log-rank test was applied with a *P*-value < 0.05 being considered as statistically significant. Multivariate analysis was performed using SPSS version 9.3 (SPSS Institute, Chicago, IL, USA). A cutoff of 50% for plasma cells and a clone size of 50% for *TP53* deletion (Δ*TP53*) were applied to assess the impact on overall survival in the multivariate analysis.

Results

Clinicopathologic Data

From a total of 1036 myeloma patients assessed during the study interval, 90 (9%) patients had Δ*TP53*, including 54 men and 36 women with a

median age of 59 years (range, 38–84) at the time of diagnosis. Fourteen (16%) patients had a normal karyotype, 73 (81%) had a complex karyotype, and 3 (3%) had a single chromosomal aberration (Figure 1). Demographic and clinical characteristics for patients in the NK/Δ*TP53* and CK/Δ*TP53* groups showed no significant differences for age, gender, or serum levels of hemoglobin, albumin, or creatinine. However, in the CK/Δ*TP53* group, patients had higher serum beta-2 microglobulin ($P < 0.001$) and lactate dehydrogenase ($P = 0.015$) levels and higher percentage of bone marrow plasma cells ($P = 0.003$) when compared with patients in the NK/Δ*TP53* group, respectively (Table 1).

In the NK/Δ*TP53* group, 29% of patients had stage I disease, 50% stage II, and 14% stage III compared with the CK/Δ*TP53* group in which 15% of patients had stage I disease, 34% stage II, and 48% stage III (Table 2). When the R-ISS was applied to the study cohort there was a significant difference in patients with stage II disease: 79% of NK/Δ*TP53* and 49% of the CK/Δ*TP53* patients ($P = 0.038$). No patients in either group had stage I disease and the percentage of patients with stage III disease was similar in both groups.

Of the 14 patients in the NK/Δ*TP53* group, 4 (29%) were newly diagnosed and 10 (71%) had either persistent or relapsed disease. In the CK/Δ*TP53* group, 11 (15%) patients were newly diagnosed and 56 (77%) had either persistent or relapsed disease; disease status in the six patients is unknown (Table 2).

Cytogenetic Heterogeneity in NK/Δ*TP53* and CK/Δ*TP53* Patients Groups

Among the 73 patients with myeloma associated with CK/Δ*TP53*, 39 had *IGH* translocations, including 16 with t(11;14), 16 with t(4;14)/t(14;16), and 7 with t(v;14). The other 34 patients had no *IGH* translocations. Combining patients with t(11;14) and patients without *IGH* rearrangement, that is, the group considered to be favorable ($n = 50$), we found 19 hyperdiploid, 15 hypodiploid, and 16 near-diploid cases. The chromosome 17 findings in these 50 cases showed 24 with 17p deletion (17p−), 14 monosomy 17 (−17), and 12 with no gross abnormalities (Figure 1).

Overall, *RB1* deletion in myeloma was detected in 9 of 14 (64%) NK/Δ*TP53* and 55 of 73 (75%) CK/Δ*TP53* cases. *MYEOV/CCND1-IGH*/t(11;14) was positive in 2 of 14 (14%) NK/Δ*TP53* and 16 of 73 (22%) CK/Δ*TP53* myeloma groups. *FGFR3-IGH*/t(4;14) or *MAF-IGH*/t(14;16) was identified in 16 cases in the CK/Δ*TP53*, but not in the NK/Δ*TP53* myeloma groups; however, t(v;14) was detected in 2 NK/Δ*TP53* and 7 CK/Δ*TP53* cases. *CKS1B* gain was observed in 2 of 4 (50%) tested in the NK/Δ*TP53* group and 26 of 32 (81%) cases tested in the CK/Δ*TP53* group. There were no significant differences shown by FISH between the NK/Δ*TP53* and CK/Δ*TP53* groups with regard to the frequency of aberrancies (Table 3). The median *TP53* deletion clone size in the NK/Δ*TP53* group was 13% (range 8–40%) *vs* 30% (range 5–95%) in the CK/Δ*TP53* group ($P < 0.001$) (Table 1).

Table 3 Summary of interphase fluorescence *in situ* hybridization (iFISH) data between patients with a normal karyotype or a complex karyotype

	Normal karyotype (N = 14)	*Complex karyotype (N = 73)*	P-*value**
RB1 deletion	9/14	55/73	0.39
CCND1-IGH	2/14	16/73	0.52
FGFR1-IGH; *MAF-IGH*	0/14	16/73	0.052
Other-*IGH* rearrangement	2/14	7/73	0.59
CKS1B	2/4	26/32	0.17

*Statistically significant *P* values are in italics.

Cytogenetic Heterogeneity and Clinical Implications

All patients received standard clinical management with immunomodulatory drugs, proteasome inhibitors, and/or stem cell transplant (SCT). Among the patients with detailed treatment data available, all 14 (100%) patients in the NK/Δ*TP53* group, 66 (90%) in the CK/Δ*TP53* group, 22 (73%) in the NK/*TP53*nl group, and all 16 (100%) in the CK/*TP53*nl group ($P = 0.019$) received proteasome inhibitors (Table 2 and Supplementary Table S1). The NK/*TP53*nl patient group was least often treated with proteasome inhibitors; there were no statistical differences between the CK/Δ*TP53* and the CK/*TP53*nl patient groups. In addition, 10 (71%) patients in the NK/Δ*TP53* group, 46 (63%) in the CK/Δ*TP53* group, 22 (73%) in NK/*TP53*nl group, and 14 (88%) patients in the CK/*TP53*nl group received an SCT, respectively ($P = 0.258$) (Table 2 and Supplementary Table S1). During the follow-up interval, 6 (43%) NK/Δ*TP53* patients and 55 (75%) CK/Δ*TP53* patients died, with the latter group showing a higher mortality rate ($P = 0.015$) (Table 2).

The median follow-up interval for all patients in the study was 32 months (range, 3–101 months) with the NK/Δ*TP53* group showing a longer follow-up interval ($P = 0.029$) (Table 2). The overall survival was 62 months for patients in the NK/Δ*TP53* group and 35 months for patients in the CK/Δ*TP53* group ($P = 0.024$) (Figure 2a).

To further assess overall survival , we included patients without Δ*TP53* and either a normal or complex karyotype designated as NK/*TP53*nl ($n = 35$) and CK/*TP53*nl ($n = 21$) from the same time interval to serve as the cytogenetic control groups. Median overall survival was 150 and 55 months for the

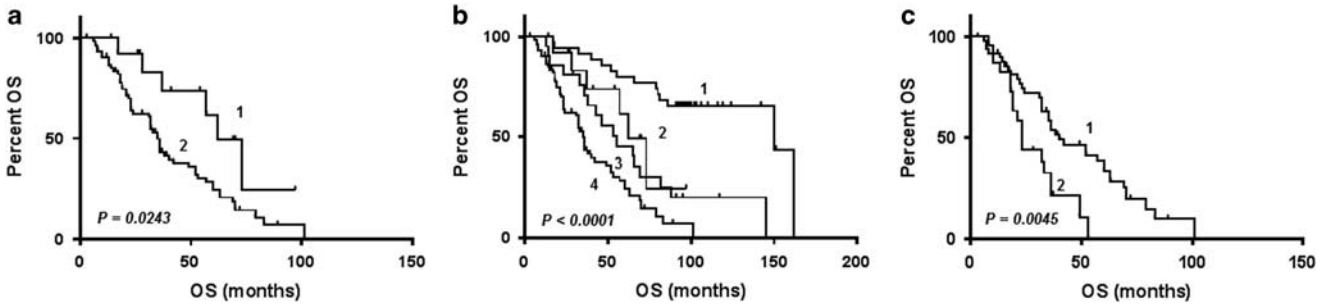

Figure 2 (**a**) Comparison of overall survival between NK/Δ*TP53* (curve 1) and CK/Δ*TP53* (curve 2) groups. (**b**) Comparison of overall survival (OS) among four subgroups: NK/TP53nl (curve 1), NK/Δ*TP53* (curve 2), CK/*TP53*nl (curve 3), and CK/Δ*TP53* (curve 4). (**c**) Comparison of overall survival between cases without (curve 1) and with (curve 2) adverse-*IGH* rearrangements in the CK/Δ*TP53* group.

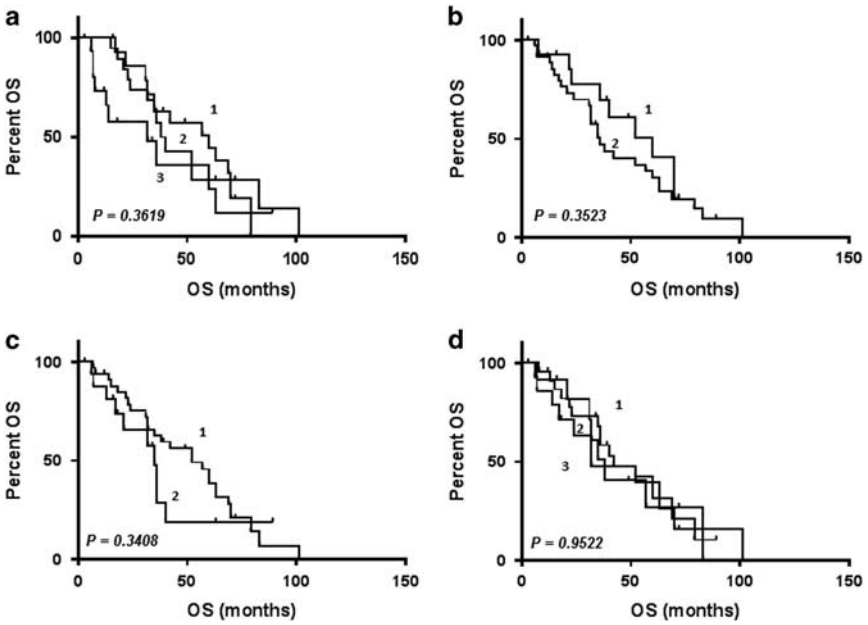

Figure 3 (**a**) Overall survival of patients stratified according to chromosome ploidy status in the CK/Δ*TP53* group (curve 1, hyperdiploidy without adverse-*IGH*; curve 2, near-diploidy without adverse-*IGH*; curve 3, hypodiploidy without adverse-*IGH*). (**b**) Comparison of overall survival between cases without (curve 1) and with (curve 2) *RB1* deletion/without adverse-*IGH* in the CK/Δ*TP53* group. (**c**) Comparison of overall survival between cases without *IGH* rearrangement (curve 1) and with t(11;14) (curve 2) in the CK/Δ*TP53* group. (**d**) Comparison of overall survival among all non-adverse-*IGH* cases without chromosome 17 aberrations (curve 1), with 17p deletion (curve 2) and monosomy 17 (curve 3).

NK/*TP53*nl and CK/*TP53*nl patient groups, respectively. When all four groups were compared, patients in the CK/Δ*TP53* group showed the poorest overall survival whereas patients in the NK/*TP53*nl group had the best outcome ($P < 0.0001$) (Figure 2b). Patients with myeloma associated with NK/Δ*TP53* or CK/*TP53*nl had similar overall survival (62 *vs* 55 months), showing that a complex karyotype alone has an independent adverse impact and suggesting that a complex karyotype has an impact comparable to that of *TP53* deletion.

In the CK/Δ*TP53* group, the overall survival of 16 patients with t(4;14)/t(14;16) *vs* 7 patients with t(v;14) was similar, 23 and 33 months, respectively ($P = 0.3289$). Combining these patients into an adverse-*IGH* rearrangement subgroup, the median overall survival was 23 months for those with adverse-*IGH* rearrangement *vs* 40 months for patients without adverse-*IGH* rearrangement ($P = 0.0045$) (Figure 2c), suggesting an additional negative impact of adverse-*IGH* rearrangement within the CK/Δ*TP53* subgroup.

We correlated ploidy and overall survival in 50 patients who had no adverse-*IGH* rearrangement, that is, patients with *CCND1-IGH* or no *IGH* rearrangement. The median overall survival was 60 *vs* 39 *vs* 32 months for hyperdiploid, near-diploid, and hypodiploid subgroups, respectively. Although the hyperdiploid subgroup showed the best overall survival, this difference did not reach statistical significance ($P = 0.3619$) (Figure 3a). *RB1* deletion or t(11;14)/*CCND1-IGH* had no independent impact on overall survival for patients in the CK/Δ*TP53* group (Figures 3b and c).

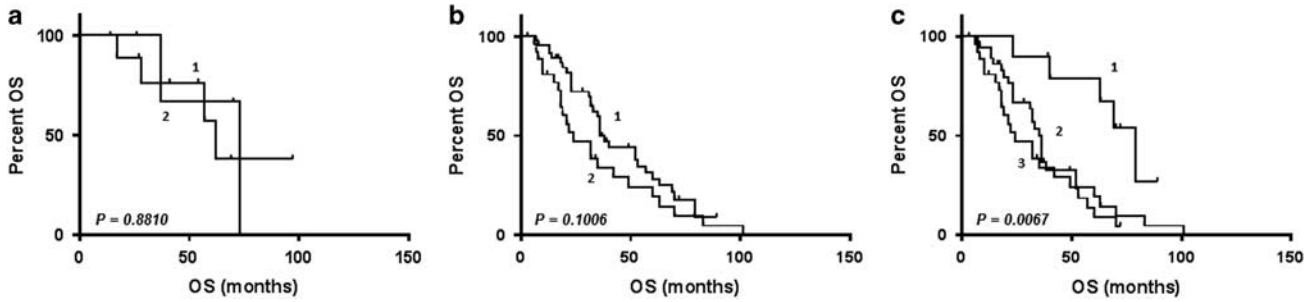

Figure 4 **a**) Comparison of overall survival between cases with (curve 1) and without (curve 2) stem cell transplant in the NK/Δ*TP53* group. (**b**) Comparison of overall survival in cases with (curve 1) and without (curve 2) stem cell transplant in the CK/Δ*TP53* group. (**c**) Comparison of overall survival among cases with tandem or more stem cell transplant (curve 1), one stem cell transplant (curve 2), and without stem cell transplant (curve 3) in the CK/Δ*TP53* group.

To investigate the possible prognostic impact of cytogenetically visible chromosome 17 abnormalities, we divided the 50 cases without adverse-*IGH* rearrangements in the CK/Δ*TP53* group into three subsets: (1) cases with apparent 17p deletions ($n=24$); (2) cases with monosomy 17 ($n=14$); and (3) cases with an apparently normal 17 ($n=12$). The median overall survival was 42 *vs* 32 *vs* 38 months among these three subsets, respectively ($P=0.9522$) (Figure 3d).

Additionally, within the CK/Δ*TP53* group, the median clone size of Δ*TP53* was 30% (range 5–95%). Patients with a clone size below or above 30% showed no significant difference in overall survival ($P=0.3759$). The median plasma cell count was 78% in the CK/Δ*TP53* group, and there was also no overall survival differences between patients with ≤78% and >78% plasma cells. In the CK/Δ*TP53* group, we also compared the Δ*TP53* clone size with the percentage of plasma cells and clone size of other altered genes including *RB1, IGH,* and *CKS1B*. We estimate that Δ*TP53* likely a part of a primary (or major) clone in 53 (73%) patients whereas it was more likely a part of a secondary clone (or subclone) in 20 (27%) patients. There was no significant difference in overall survival between patients with Δ*TP53* as a primary *vs* secondary clone.

Impact of SCT on Overall Survival

Ten (71%) patients in the NK/Δ*TP53* group and 46 (63%) patients in the CK/Δ*TP53* group received an SCT. In the NK/Δ*TP53* group, patients who received a transplant showed no survival advantage over patients who did not receive transplant ($P=0.8810$) (Figure 4a). By contrast, in the CK/Δ*TP53* group, although SCT did not improve the median overall survival (46 months with SCT *vs* 24 months without SCT; $P=0.1006$) (Figure 4b), patients who received a second (or tandem) SCT showed a significantly better overall survival (79 months, $P=0.0067$) (Figure 4c).

Table 4 Multivariate analysis of prognostic factors

	P value*	*Hazard ratio*	*95.0% CI*	
			Lower	*Upper*
Age (≥60 *vs* <60)	0.667	0.872	0.468	1.624
Sex (male *vs* female)	0.153	0.618	0.319	1.195
Normal (*vs* complex karyotype)	*0.043*	1.976	1.022	3.821
Non-adverse-*IGH* (*vs* Adverse-*IGH)*	*0.020*	3.126	1.192	8.196
New diagnosis (*vs* persistent/relapsed)	0.075	0.475	0.209	1.078
R-ISS II (*vs* III)	0.925	0.97	0.519	1.814
SCT × 1 (*vs* non-SCT)	0.969	1.013	0.534	1.922
SCT × 2 (*vs* non-SCT)	*0.027*	0.281	0.091	0.866

Abbreviations: R-ISS, Revised International staging system; SCT, stem cell transplant.
*Statistically significant p values are in italics.

Multivariate Analysis of Prognostic Factors

Multivariate analysis confirmed that a complex karyotype (hazard ratio 1.976, 95% CI 1.022–3.821, $P=0.043$) and adverse *IGH* rearrangement (hazard ratio 3.126, 95% CI 1.192–8.196, $P=0.020$) showed an independent negative impact on overall survival. In contrast, tandem SCT correlated with improved overall survival (hazard ratio 0.281, 95% CI 0.091–0.866, $P=0.027$) (Table 4).

Discussion

The widely disparate survival outcomes observed in patients with myeloma can be attributed to tumor genetic heterogeneity and host factors. Mounting evidence supports the idea that the genetic features of myeloma can be used to predict the outcome of these patients. To better understand the clinical implications of background cytogenetic heterogeneity in patients with myeloma associated with *TP53* deletion (Δ*TP53*), we studied the clinicopathologic features of patients with a plasma cell count ≥30% and Δ*TP53* identified by FISH in the context of a

normal karyotype (NK) *vs* complex karyotype (CK). In patients with myeloma associated with Δ*TP53*, we show that patients with a normal karyotype have a significantly longer overall survival than patients with a complex karyotype. Patients with myeloma associated with a complex karyotype but without *TP53* deletion also had a significantly worse overall survival than patients with NK/Δ*TP53* myeloma, suggesting that a complex karyotype is an important adverse risk factor in myeloma patients.

The frequency of *TP53* deletion in this cohort was approximately 9%, which is consistent with what has been reported previously.[1] Although others in some studies have reported a much higher frequency of *TP53* deletion, ranging from 39%[6] to 55%,[7] we believe that these studies were likely focused on patients with advanced stage III disease, plasma cell leukemia, or relapsed disease. In contrast, this study cohort was derived from a pool of patients with myeloma at various stages of disease, including both newly diagnosed and relapsed disease, with very few plasma cell leukemia patients. Although our data were generated on non-CD138-enriched plasma cells, we only included patients with a plasma cell count 30% or higher in this study. By doing the study in this manner, we have overcome some of the known technical limitations of FISH in the analysis of non-enriched samples. In keeping with the latest recommendation by the International Myeloma Working Group, future FISH studies should be performed on plasma cell-enriched samples.

The R-ISS proposed recently by the International Myeloma Working Group for multiple myeloma considers chromosome abnormalities detected by FISH a key element in defining the biological features of myeloma. However, the role of chromosomal abnormalities detected by conventional karyotyping in the staging of myeloma patients was not clearly described. The results presented here show that knowledge of the karyotype in which Δ*TP53* is detected is important: deletion detected by FISH associated with a normal karyotype does not have the same prognostic impact as does Δ*TP53* associated with a complex karyotype; patients in the latter group have significantly shorter survival.

An interesting finding in this study is that patients with myeloma associated with Δ*TP53* can have a normal or complex (≥3 abnormalities) karyotype, with very few patients having only one or two cytogenetic abnormalities. The reason for this apparent dichotomy is unknown, but the data suggest that Δ*TP53* may occur in one of two settings: (1) an early and a likely primary event in pathogenesis; and (2) a late and/or secondary event. Most likely, a complex karyotype would be more frequent in the second group. Further studies with larger study groups may help elucidate the underlying mechanisms.[9] By limiting our study cases to those with 30% of plasma cells, we aim to eliminate the possibility of a false normal karyotype result due to insufficient tumor cells.

Previous studies of myeloma patients have shown that different cytogenetic aberrations are associated with different clinical outcomes.[10–18] Myeloma patients with t(4;14)/t(14;16) or other adverse *IGH* rearrangements were also found to have shorter overall survival and therefore considered to be of high-risk,[19,20] whereas myeloma patients with t(11;14) are considered low risk with a more favorable prognosis.[21] In this study, we show that Δ*TP53* combined with t(4;14) or t(14;16) conferred an even worse outcome, suggesting a 'double hit' effect. Furthermore, a complex karyotype in the context of Δ*TP53* and adverse *IGH* rearrangement likely further contributes an even worse clinical outcome in myeloma patients. The finding is in keeping with results reported by others. A possible explanation is that the myeloma may carry multiple drug resistant clones or that the combination of multiple cytogenetic aberrations further enhances drug resistance.

Additional examination of chromosomal aberrations in myeloma cases without adverse *IGH* in the CK/Δ*TP53* group did not show independent prognostic effects for monosomy 17 or 17p−, unlike cytogenetically visible chromosome 13q deletions in other myeloma studies.[22] The median clone size in the CK/Δ*TP53* group was 30% and the median plasma cell count was 78%. Although our data showed that larger clone size of Δ*TP53* or higher plasma cell count did not have independent clinical impact, both findings were highly associated with a complex karyotype, which is an independent negative prognostic factor by multivariate analysis.

Myeloma can be broadly subdivided into three subsets at the chromosomal level based on ploidy: hyperdiploid, hypodiploid, and near-diploid. In general, hyperdiploid myeloma has a lower prevalence of *IGH* translocations compared with non-hyperdiploid myeloma.[23–26] Others[27] have shown that hypodiploid myeloma is associated with a higher prevalence of genetic alterations and inferior outcome compared with non-hypodiploid myeloma. In the current study, although patients with hyperdiploidy in the CK/Δ*TP53* group did have better overall survival, there was no statistical difference between patients in the hypodiploid or near-diploid groups, likely due to Δ*TP53* and a complex karyotype overwhelming the prognostic impact of ploidy.

Although our data showed that SCTs did not improve overall survival for the overall study cohort, tandem SCT did correlate with improved overall survival, particularly in patients with CK/Δ*TP53*. This finding further underscores the critical role of a complex karyotype in the risk assessment and management of myeloma patients.[28]

In conclusion, we assessed the impact of underlying cytogenetic heterogeneity in patients with myeloma associated with Δ*TP53* detected by conventional karyotype and FISH. The data presented show that conventional chromosomal analysis remains a powerful tool for risk assessment which supplements FISH data, particularly if high-resolution genomic

analysis such as microarray-based testing is not available. For patients with Δ*TP53* detected by FISH, those patients with a normal karyotype had a significantly longer overall survival than patients with a complex karyotype. In addition, combined *TP53* deletion and adverse *IGH* conferred a worse clinical outcome. We therefore suggest that conventional cytogenetic analysis has a role in the work up of myeloma patients because it facilitates comprehensive assessment of molecular abnormalities in myeloma, adding value to *TP53* status, in the risk stratification of patients with myeloma.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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