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Clinical Significance of *TP53* Abnormalities in Newly Diagnosed Multiple Myeloma

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Abstract

Objective: To identify the clinical significance of *TP53* and common cytogenetic abnormalities.

Material and Methods: 114 patients with newly diagnosed MM and *TP53* abnormalities were selected from two large patient cohorts of collaborating hospitals from 2010 to 2017. The characteristics and outcomes of these patients were analyzed. *TP53* and other common mutations in MM patients were quantified by fluorescence in situ hybridization (FISH). Kaplan-Meier curves and Log-rank test were applied for survival analysis. Cox proportional hazard model for covariate analysis was used to determine the prognostic factors.

Results: By extensive data analysis, we find *TP53* amplification is a strong positive predictor for complete response (CR) to therapy and positively correlated with patient

survival. The number of simultaneous genomic abnormalities with *TP53* mutation has a modest impact on patient survival. Within these mutations, 1q21 amplification is associated with decreased CR (OR=4.209) and FGFR3 levels are positively correlated with patient progression-free and overall survival.
Conclusion: *TP53* abnormalities at the diagnosis of MM are of great clinical significance in predicting patient response to therapy and survival. Further, 1q21 and FGFR3 mutations could potentially be used in combination with *TP53* status, to better predict patient survival and guide for selecting high-risk patients to advance patient treatment strategies.
Keywords: *TP53*; multiple myeloma; genomic abnormality

Introduction

Multiple myeloma (MM) is a hematologic malignancy caused by the proliferation of plasma cells in the bone marrow. MM accounts for approximately 10% of all hematologic malignancies and 1% of all cancers (1, 2). The tumor plasma cells infiltrate bone marrow and other organs, which leads to lethal immune deficiency and organ damage (3-5). Worldwide studies indicate the incidence of MM increased by 126% globally and the 5-year survival rate is only around 50% (6).
One major factor that contributes to the low survival rate is that MM is a highly heterogeneous disease, characterized by numerous genetic alterations (7). Chromosome gain, losses and IGH translocations and mutations of specific genes are often found in MM patients (7, 8). Genetic alterations are categorized into primary and secondary changes based on when the changes being observed during the disease progression (9, 10). Cytogenetic abnormalities play a very important role in MM patient survival. For example, by FISH detection, gain (1)(q21), del(17)(p13), and t(4;14)(p16;q32), in MM patients are correlated with shorter overall survival (11, 12). The fact that the type and quantity of genomic abnormalities directly linked with MM patient's survival time and response to treatment suggest an investigation of mutations in predicting patient response and survival is of great clinical significance in MM patient management (13).
Mapped to the position of chromosome 17p13, the *TP53* gene encodes p53 protein and regulates the cell cycle. Upon discovery of p53 protein, its role in cancer has been intensively investigated. p53 is an important tumor suppressor due to its critical role in inducing cell cycle arrest and apoptosis in response to cellular stress signals (14). In MM patients, the major abnormalities of the *TP53* gene are mutation and deletion (due to deletion of 17p13 region). These abnormalities of *TP53* genes rarely occur at diagnosis, but increase in late-stage patients, suggesting the essential role of *TP53* genes in disease progression (15, 16). Many clinical reports show a strong association between a loss of *TP53* and poor prognosis in MM patients (16-19). However, due to the heterogeneity of MM, and the limited patient cases, the function of *TP53* at diagnosis as a biomarker, in different backgrounds of the major molecular cytogenetic abnormalities of MM is not well studied. Here, we

provided intensive retrospective analysis on a large cohort of newly diagnosed MM patients to identify the clinical significance of *TP53* and common cytogenetic abnormalities. We compared *TP53* loss and amplification together with common genes dysregulated in MM patients, including chromosome 1q21 amplification, translocation of 4p16.3 (fibroblast growth factor receptor 3, FGFR-3), 16q23 (MAF) to chromosome 14q32. Investigation of the risk factors of MM relapse/progression will bring insight into the development of adaptive methods for better treatment of MM patients.

Material and Methods

Patients

1046 newly diagnosed MM patients were enrolled from Beijing Chao-Yang Hospital, Multiple Myeloma Research Center of Beijing and Chuiyangliu Hospital affiliated to Tsinghua University from January 2010 to December 2017. Fluorescence in situ hybridization (FISH) was used to characterize genetic abnormalities (*TP53*, 1q21, 14q32/11q13 (CCND1 (cyclin D1 gene)), 14q32/4p16.3 (FGFR-3), 14q32/16q23 (MAF)) of these patients and diagnostic criteria were based on International Myeloma Working Group (IMWG) (20). Detailed positive criteria were provided in Table 1. Basic patient information including age, gender, habit, baseline health and disease, etc. and clinical parameters including overall survival and chemotherapy response were recorded. Patients selecting criteria is a primary diagnosis with *TP53* abnormality. Patients are excluded if they have refractory and relapsed MM. The study is approved by the ethics committee of our Hospital. All patients gave written informed consent.

FISH

Fluorescence in situ hybridization (FISH) is performed in interphase cells. CD138-expressing plasma cells were purified, then FISH was performed as previously described (21) using probes purchased from Beijing Hightrust Diagnostic Company Limited. Targets detected by FISH and threshold are included in table 1. At least 200 plasma cells were scored to determine the prevalence of each genetic abnormality.

Statistical Analysis

The primary endpoint of this study was correlated with survival from the time of diagnosis. Progression-free survival (PFS) and overall survival (OS) were evaluated according to the international uniform response criteria (22). PFS was calculated from the time of diagnosis to the date of death, progression, or the last follow-up. OS was defined as the duration from the time of diagnosis to the date of death or last follow-up. Descriptive statistics such as mean, standard deviation, median, and range were used for continuous variables, and frequency counts and percentages were used for categorical variables. An independent sample t-test was employed to evaluate the associations between genetic abnormalities and biological parameters. Chi-squared test or 2-sided Fisher exact test was performed to make the comparison of categorical variables among groups. Kaplan-Meier method was employed to plot the survival curves, with a log-rank test to assess the differences. Cox proportional hazard model for covariate analysis was used to determine the prognostic factors for PFS. All

statistical analyses were performed using SPSS version 17.0 (SPSS, Inc, Chicago, IL). The results were considered significant if the P-value was <0.05.

Results

The median follow-up time for the entire MM population was 32 months (range, 1-192 months). Among the 1046 newly diagnosed MM cases, *TP53* abnormalities were found in 153 cases. 114 of the 153 cases (male=64 cases, female=50 cases) were followed and included in the analysis, with a mean age of 59.4 years (SD=10.3 years) (Table 2). Within the 114 patients, 23 cases were stage I, 27 cases were stage II and 64 cases were stage III at the time of diagnosis based on International Staging System (ISS) (Table 2). Due to the significant effect of extramedullary disease (EMD) in survival rate reduction (23), patients' EMD status at diagnosis was recorded, with most patients (86.84%) were present with no extramedullary disease (EMD) at diagnosis (Table 2). Other patients' medical history (including hypertension, diabetes, heart disease, etc.), lifestyle (including smoking and alcohol consumption), and clinical characteristics (including neutrophil, platelet count, hemoglobin level, creatinine level, etc.), which may affect or reflect disease progression are included in Table 2 and 3. Patients were mainly received autologous hematopoietic cell transplantation and/or standard chemotherapies, including but not limited to bortezomib combined with dexamethasone (PD) or three-drug combinations of PD with liposomal doxorubicin or thalidomide (Table).

In our analysis, the overall survival of patients is mainly affected by age and chemotherapy. Younger age (<60 years old) is correlated with an increased overall survival rate than older age patients (≥60 years old) (median survival: 72 months vs 39 months, *p*-value=0.038) (Figure 1A). Chemotherapy increased the median survival time from 28 months to 77 months (*p*- value=0.029) (Figure 1B). However, the other major therapy received by our patients, autologous hematopoietic cell transplantation therapy, did not further improve patient survival rate (*p*-value=0.428, data not shown). Other factors, including gender, ISS, ECOG score, smoking, alcohol consumption, do not have a significant correlation with patient PSF and OS rate (data not shown).

Among the 114 patients with *TP53* abnormality, 54 cases showed *TP53* amplification and 60 cases showed *TP53* deletion. Compared with patients with *TP53* deletion, patients with *TP53* amplification had a higher probability to achieve complete response (Table 4, *p*-value=0.008) and had modest PFS and OS advantage (Figure 2 A-B). When 3 years survival time was used as the cutoff in analysis, the patients who survived had a higher *TP53* amplification percentage than patients who died (mean: 61.4% vs 40.27%, *p*-value=0.034). The PFS and OS rate of patients with more than 51.25% of (value calculated by ROC curve analysis; data not shown) *TP53* amplification is trending higher than patients with less *TP53* amplification (Figure 3 A-B). Together, these data suggest *TP53* amplification plays a positive role in patient survival.

The genes/chromosomes that are commonly dysregulated in MM patients were also tested in these 114 patients (chromosome 1q21 amplification, 4p16.3 (FGFR-3), 16q23 (MAF) IGH translocations, abnormal chromosome counts) to show the potential effect of the common genetic dysregulations in the background

of *TP53* abnormality. The genomic changes in these 114 patients were summarized in Table 5.

Overall, our data indicate that patients with 4 or more types of mutation in the list have a similar progression-free survival rate to patients with less than 4 types of mutation (data not shown), however, their OS rate is trending higher than the patients with less mutation burden (Figure 4A). However, when the OS analysis was done in patients separated by *TP53* status, no significant difference was found between patients with ≥4 types of mutations and patients with less than 4 types of mutations, which potentially due to low patient number in each group (Figure 4B and 4C). The effects of individual genetic abnormalities in the background of *TP53* abnormality on overall survival were also tested. In our patient cohort with *TP53* abnormality, of the five genetic abnormalities (1q21, FGFR-3, MAF, IGH and chromosome number changes), 1q21 amplification predicts the decreased probability of complete response (Table 4; OR=4.209), and the types of FGFR3 mutation is critical in predicting patient progression-free and overall survival. FGFR3 amplification yields a 5-fold increase in median survival time compared with FGFR3 deletion cases (100 months vs 19 months), and a 2-fold increase compared with FGFR3 normal cases (100 months vs 41 months) (Figure 4D). We further analyzed the median survival time between FGFR3 amplification and normal subgroups separated by their *TP53* status. Patients with FGFR3 amplification still had significantly longer median survival time in the background of *TP53* amplification (Figure 4E), but not in *TP53* loss (Figure 4F).

These data suggest that the *TP53* status, in combination with common mutations in MM, could potentially be used to predict patient survival at disease diagnosis.

Discussion

TP53 is a critical tumor suppressor and reported to correlate with MM disease progression. However, *TP53* mutation is a rare occurrence at diagnosis, which makes up around 3% of the newly diagnosed patients. The large patient cohort in our hospitals provided opportunities for us to study *TP53* mutation in early-stage MM patients, which brings insight into the clinical significance of *TP53* in newly diagnosed MM patients and in also disease progression.

In the 114 cases of newly diagnosed MM patients with *TP53* abnormalities, we found the patient age and stage of the disease are the strongest predicting factors to patient PFS and OS, with older age and later stages indicative of worse prognosis, consistent with reports from other groups (24, 25). Patients' lifestyles (smoking etc.) and pre-existing conditions (heart diseases etc.) do not have strong effects on patient survival. *TP53* deletion is more commonly found in MM patients. Here, we also reported a group of patients with *TP53* amplification, which was associated with increased PFS and OS. The mechanism of *TP53* amplification is unknown but could potentially cause by compensating of non-functional p53 protein. Within the *TP53* mutation patients, nearly half of the patients showed *TP53* amplification, and *TP53* amplification is a strong predictor for a complete response to therapy. Further, the level of *TP53* amplification (51.25) has a trend to positively correlated with patient survival rate. These data indicate that *TP53*, as a tumor

suppressor plays an important role in MM patient prognosis, and patients with *TP53* deletion at an earlier stage and older ages will potentially have decreased chance to reach complete response when treated with standard chemotherapy and autologous hematopoietic cell transplantation therapy. More advanced and intensive therapeutic strategies are potentially needed for those patients.

The common mutations in MM patients, 1q21 and *FGFR3* levels show good prediction for patient's therapy response and overall survival in our cohorts. The copy number gain of chromosome 1q21 is among the most commonly reported genetic abnormalities in MM patients. The prediction role of 1q21 amplification in MM patients in terms of chemotherapy response and patient survival is controversial. Studies have shown that 1q21 amplification strongly correlates with bortezomib resistance, but others show no response prediction or survival benefit for patients with 1q21 amplification (26-28). Our data indicate that in patients with *TP53* abnormalities, 1q21 amplification is a strong predictor for worse patient response to chemotherapy, suggesting studying 1q21's role in the context of *TP53* mutation is of great clinical importance.

On the other hand, the t(4;14) translocation is associated with upregulation of the FGFR3 amplification, which has been shown to correlate with poor patient survival (29, 30). Interestingly, contradicting to other studies, we found that in newly diagnosed MM patients with *TP53* mutation, FGFR3 levels have a strong positive correlation to patient PFS and OS. Patients with FGFR3 amplification have a nearly 2-fold increase in median survival time compared with patients with normal FGFR3 levels. These data suggest FGFR3 level is a critical prognosis indicator and a potential therapeutic target in MM patients with *TP53* mutation.

One caveat of our study is that the patient number is limited, due to the fact that *TP53* mutation is rarely presented at diagnosis. Data analysis for age, other mutation types, etc. is restricted in the total population of patients with *TP53* mutation and analysis for each feature in *TP53* amplification and deletion separately could not be performed with statistical power. Another limitation of our study is that *TP53* mutation is tested at the gene level. Whether the MM patients in our cohorts have functional p53 protein in their tumor or not is unknown, which may bring the noise to our data analysis. Addressing the functional p53 protein level in those patients in our future study could potentially help to gain more statistical power of our analysis, and a better understanding of the functional role of p53 in newly diagnosed MM patients.

Conclusion

In summary, by extensive analysis of 114 newly diagnosed MM patients with *TP53* abnormalities, we observed a positive correlation between *TP53* amplification and MM patient survival. Further investigation of *TP53* and the common mutations in MM patients will contribute to better design of biomarkers to predict MM patient therapy response and survival.

Conflict of interests: All the authors declare that they have no conflict of interest.

Statement of Ethic: The study is approved by ethics committee of Beijing Chao-

Yang Hospital, Multiple Myeloma Research Center of Beijing, and Chuiyangliu Hospital affiliated to Tsinghua University (No. SOP-CYLIRB-1.0). All patients gave written informed consent.

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Availability of data and material: The datasets used or/and analyzed during the current study are available from the corresponding author on reasonable request.

Author contribution

Fang Ye and Tongtong Wang carried out the studies, participated in collecting data, and drafted the manuscript. Aijun Liu, Yanchen Li and Ningning Li performed the statistical analysis and participated in its design. Huan Wang and Wenming Chen participated in acquisition, analysis, or interpretation of data and draft the manuscript. All authors read and approved the final manuscript.

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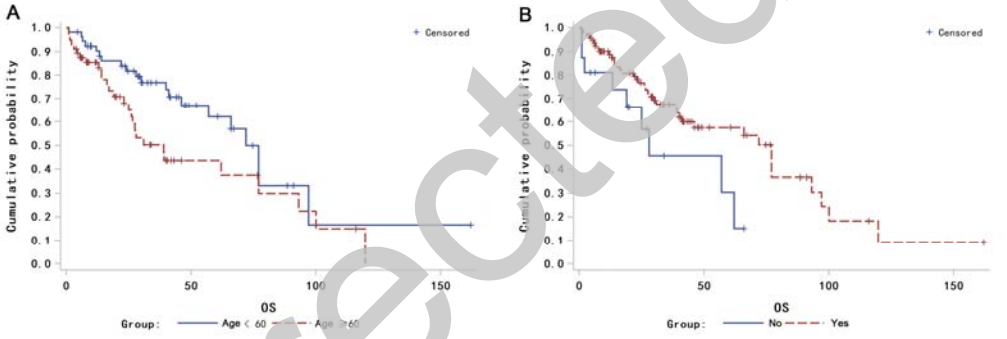
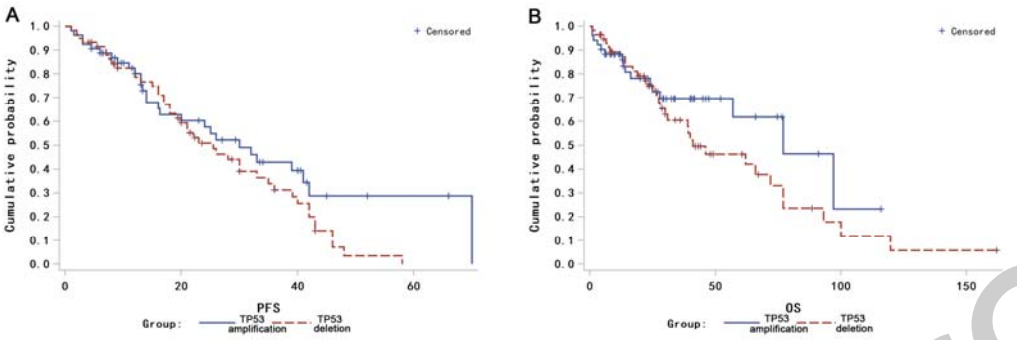
Figure 1. **Factors that influence patient survival.** Log-Rank analysis of **A**. age and **B**. chemotherapy on patient overall survival. Patient numbers are indicated in the chart. The groups of age are separated based on the mean age in our patient cohorts.



Age	n	Median survival time (Month)	Test method	p-value
<60	53	72.00	Log-Rank	0.038
≥60	57	39.00		

Chemotherapy	n	Median survival time (month)	Test method	p-value
No	16	28.00	Log-Rank	0.029
Yes	94	77.00		

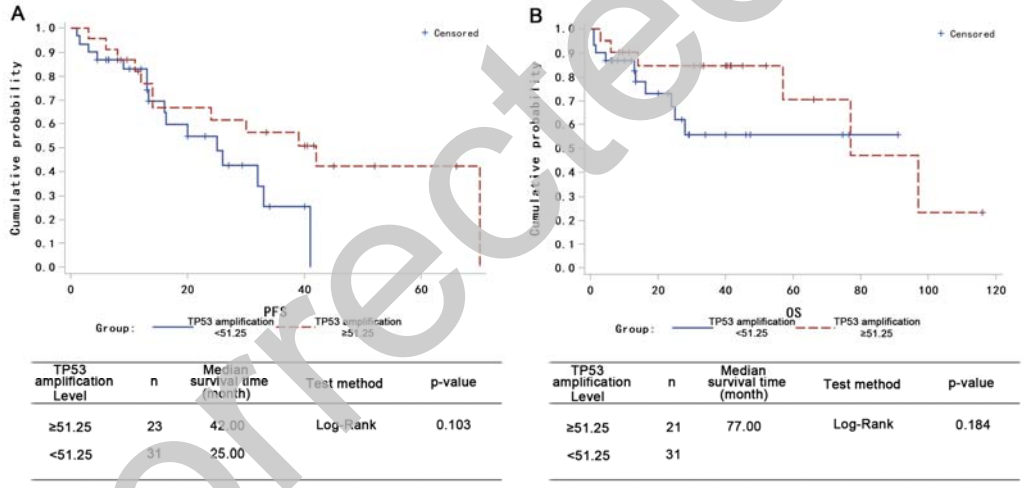
Figure 2. *TP53* level affects patient survival. Log-Rank analysis of the effect of *TP53* amplification and deletion on patient **A**. PFS and **B**. OS



TP53 Level	n	Median survival time (month)	Test method	p-value
Amplification	54	30.00	Log-Rank	0.136
Deletion	60	35.00		

TP53 Level	n	Median survival time (month)	Test method	p-value
Amplification	52	77.00	Log-Rank	0.178
Deletion	58	41.00		

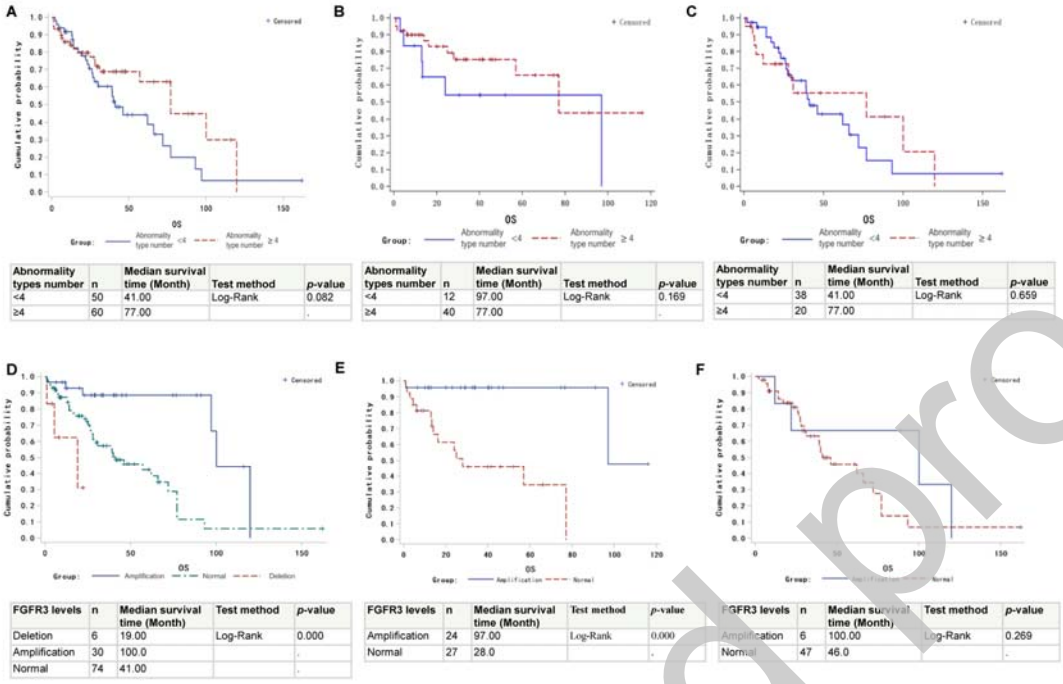
Figure 3. *TP53*amplification predicts better patient survival. Log-Rank analysis of the effect of the level of *TP53* amplification on patient **A**. PFS and **B**. OS. The cutoff threshold of *TP53* amplification is based on ROC curve analysis.



TP53 amplification Level	n	Median survival time (month)	Test method	p-value
≥51.25	23	42.00	Log-Rank	0.103
<51.25	31	25.00		

TP53 amplification Level	n	Median survival time (month)	Test method	p-value
≥51.25	21	77.00	Log-Rank	0.184
<51.25	31			

Figure 4. The effect of common mutations found in MM patients in predicting patient survival in *TP53* abnormal patients. A. The correlation between the number of genetic abnormalities and patient OS. **B.** The correlation between the number of genetic abnormalities and patient OS in the background of *TP53* amplification. **C.** The correlation between the number of genetic abnormalities and patient OS in the background of *TP53* deletion. **D.** FGFR3 level in predicting for patient median survival time. **E.** FGFR3 status in predicting for patient median survival time in the background of *TP53* amplification. **F.** FGFR3 status in predicting for patient median survival time in the background of *TP53* loss.



Abnormality types number	n	Median survival time (Month)	Test method	*p*-value
<4	50	41.00	Log-Rank	0.082
≥4	60	77.00		.

Abnormality types number	n	Median survival time (Month)	Test method	*p*-value
<4	12	97.00	Log-Rank	0.169
≥4	40	77.00		.

Abnormality types number	n	Median survival time (Month)	Test method	*p*-value
<4	38	41.00	Log-Rank	0.659
≥4	20	77.00		.

FGFR3 levels	n	Median survival time (Month)	Test method	*p*-value
Deletion	6	19.00	Log-Rank	0.000
Amplification	30	100.0		.
Normal	74	41.00		.

FGFR3 levels	n	Median survival time (Month)	Test method	*p*-value
Amplification	24	97.00	Log-Rank	0.000
Normal	27	28.0		.

FGFR3 levels	n	Median survival time (Month)	Test method	*p*-value
Amplification	6	100.00	Log-Rank	0.269
Normal	47	46.0		.

Table1. Summary FISH positive thresholds.

Probe	Test site	Positive threshold % cell tested positive
1q21	1q21	6.87
TP53	17p13.1	6.09
IGH/MAF	14q32/16q23	0.77
IGH/FGFR3	14q32/4p16.3	1.11
IGH/CCND1	14q32/11q13	4.85

Table 2. Summary of patients' general information.		
		n (%)
Gender	Male	64 (56.14)
	Female	50 (43.86)
Age	Age< 60	54 (47.37)
	Age≥60	60 (52.63)
DS	I-II	14 (12.39)
(Durie-Salmon System)	III	99 (87.61)
ISS	I	23 (20.18)
(International Staging System)	II	27 (23.68)
	III	64 (56.14)
ECOG	**0**	55 (48.25)
(Eastern cooperative oncology group performance status)	≥1	59 (51.75)
Smoking	No	81 (71.05)
	Yes	33 (28.95)
Alcohol consumption	No	90 (78.95)
	Yes	24 (21.05)
Hypertension	No	71 (62.28)
	Yes	43 (37.72)
Diabetes	No	97 (85.09)
	Yes	17 (14.91)
Heart disease/ arteriovenous thrombosis	No	103 (90.35)
	Yes	11 (9.65)
Chemotherapy	No	18 (15.79)
	Yes	96 (84.21)
Autologous hematopoietic cell transplantation	No	94 (82.46)
	Yes	20 (17.54)
EMD	No	99 (86.84)
	Yes	15 (13.16)

Table 3. Summary of patients' clinical features.						
Abb.	***Description***	***n***	***sd***	***Min***	***Max***	***Median***
NE	Neutrophils (10^9/L)	114	5.95	0.49	63.2	3.13
HGB	Hemoglobin (g/L)	114	24.2	50	152	90.8
PLT	Platelet (10^9/L)	114	89.07	20	724	165
ALB	Albumin (g/L)	114	6.75	17.6	48	34.55
CR	Creatinine (umol/L)	114	182.09	30	1004.9	77.35
LDH	Lactate dehydrogenase (U/L)	114	119.95	68	971	163.5
CA	Calcium (mmol/L)	114	1.78	1.54	20.8	2.19
BTA	β2-microglobulin (mg/L)	112*	9.73	1.42	73.5	4.34
BNP	B-type natriuretic peptide	113*	3659.42	5	35000	149.9
LVEF	Left ventricular ejection fraction	114	6.38	45	82	69
JXBP	Plasma cells % in bone marrow	114	21.48	1	93.5	36.25
*: missing values						

Table 4. Risk factors that involved in complete response to therapies.			
	Risk factors	***OR***	***p-value***
Age	Unit=1	1.045 (0.997-1.096)	0.068
DS	I-I VS. III	0.181(0.044-0.737)	0.017
Chemotherapy	NO VS. Yes	12.597(1.319-120.317)	0.028
TP53	Amplification VS. Deletion	0.225(0.075-0.677)	0.008
1q21	Amplification VS. Deletion	4.209 (1.258-14.076)	0.020

Table 5. Summary of patients' genetic abnormalities.		
		n（%）
TP53	Amplification	54 (47.37)
	Deletion	60 (52.63)
1q21	Amplification	84 (73.68)
	Deletion	1 (0.88)
	Normal	29 (25.44)
MAF	Amplification	29 (25.44)
	Deletion	24 (21.05)

	Normal	61 (53.51)
FGFR3	Amplification	32 (28.07)
	Deletion	6 (5.26)
	Normal	76 (66.67)
IGH	Amplification	25 (21.93)
	Deletion	15 (13.16)
	Normal	74 (64.91)
Chromosome	46, XY/XX	95 (83.33)
	other	19 (16.67)