Minor clone of del(17p) provides a reservoir for relapse in multiple myeloma

Jian Cui,[1,2] Rui Lv,[1,2] Tengteng Yu,[1,2,3] Wenqiang Yan,[1,2] Jingyu Xu,[1,2] Huishou Fan,[1,2] Lingna Li,[1,2] Yuntong Liu,[1,2] Chenxing Du,[1,2] Shuhui Deng,[1,2] Weiwei Sui,[1,2] Yan Xu,[1,2] Shuhua Yi,[1,2] Dehui Zou,[1,2] Lugui Qiu[1,2#] and Gang An[1,2#]

[1]State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Tianjin, China; [2]Tianjin Institutes of Health Science, Tianjin, China and [3]LeBow Institute for Myeloma Therapeutics and Jerome Lipper Center for Multiple Myeloma Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

Correspondence: G. An
angang@ihcams.ac.cn

L. Qiu
qiulg@ihcams.ac.cn

Received: May 14, 2023.
Accepted: July 27, 2023.
Early view: August 3, 2023.

https://doi.org/10.3324/haematol.2023.283533

Abstract

The deletion of chromosome 17p (del(17p)) is considered a crucial prognostic factor at the time of diagnosis in patients with multiple myeloma (MM). However, the impact of del(17p) on survival at different clonal sizes at relapse, as well as the patterns of clonal evolution between diagnosis and relapse and their prognostic value, has not been well described. To address these issues, we analyzed the interphase fluorescence *in situ* hybridization (iFISH) results of 995 newly diagnosed MM (NDMM) patients and 293 patients with MM at their first relapse. Among these patients, 197 had paired iFISH data at diagnosis and first relapse. Our analysis of paired iFISH revealed that a minor clone of del(17p) at relapse but not at diagnosis was associated with poor prognosis in MM (hazard ratio for median overall survival 1.64 *vs*. 1.44). Fifty-six and 12 patients developed one or more new cytogenetic abnormalities at relapse, mainly del(17p) and gain/amp(1q), respectively. We classified the patients into six groups based on the change patterns in the clonal size of del(17p) between the two time points. Patients who did not have del(17p) during follow-up showed the best outcomes, whereas those who acquired del(17p) during their disease course, experienced compromised survival (median overall survival: 61.3 *vs*. 49.4 months; hazard ratio =1.64; 95% confidence interval: 1.06-2.56; $P<0.05$). In conclusion, our data confirmed the adverse impact of a minor clone of del(17p) at relapse and highlighted the importance of designing optimal therapeutic strategies to eliminate high-risk cytogenetic abnormalities (*clinicaltrials gov. identifier: NCT04645199*).

Introduction

Although there have been significant improvements in the survival of patients with multiple myeloma (MM) over the past decade, patient outcomes still vary, and high-risk patients do not fully benefit from novel drugs.[1-3] This can be attributed, in part, to intra-tumor heterogeneity within MM, where treatment only targets sensitive clones, and chemo-resistant clones cannot be eliminated.[4,5] In addition, clonal evolution induced by therapy or disease progression is a crucial determinant of patient outcomes in MM.[6,7] Recent single-cell studies have further revealed that subclonal secondary genetic events, which are previously undetectable at baseline, may become detectable during follow-up.[8,9] Cytogenetic abnormalities (CA), particularly those detected by interphase fluorescence *in situ* hybridization (iFISH) at diagnosis, have become a crucial aspect of risk stratification in MM.[10,11] However, iFISH-based risk stratification is often used as a static prognostic indicator, and little attention has been paid to examining dynamic changes in the genetic status, such as the number of CA and risk status, from diagnosis to relapse in MM.

As a secondary high-risk CA, deletion of chromosome 17p (del(17p)), especially in the high subclonal fraction, is associated with poor prognosis in MM.[12,13] Although del(17p) is detected in approximately 5-10% of newly diagnosed MM (NDMM) patients,[8] its prevalence increases to more than 10% in patients at relapse,[13] mainly due to the emergence of new clones with acquired del(17p) during follow-up.[14,15] However, the impact of del(17p) at relapse on survival at different clonal sizes remains unclear, despite the fact that patients who acquire del(17p) during follow-up have significantly shorter overall survival (OS) than controls.[8,14,16] Furthermore, the patterns of clonal evolution of del(17p)

between diagnosis and relapse, and its prognostic value are not fully understood.

In order to address these questions, we analyzed the paired genetic profiles of 197 patients with MM at diagnosis and first relapse, characterizing the impact of risk status, number of CA, and clonal evolution on their prognostic significance between the two time points. We also assessed the prognostic value of del(17p) at different clonal sizes, both at baseline and relapse. In addition, we identified different patterns of clonal evolution of del(17p) and evaluated their influence on patient outcomes.

Methods

Patient database and study population

The patients included in this study were sourced from the MM database of the National Longitudinal Cohort of Hematological Diseases (NICHE, *clinicaltrials gov. Identifier: NCT04645199*). The inclusion criteria required patients to have MM, as defined by the International Myeloma Working Group consensus[17] and to have the necessary iFISH data, including testing for gain/amp(1q), del(17p), del(13q), and immunoglobulin heavy chain (IgH) rearrangement. MM patients diagnosed between January 2014 and June 2021 were included in this study. A total of 995 patients with NDMM and 293 patients with their first relapse were identified, with median follow-up times of 76 and 85 months from diagnosis, respectively. For acquired CA, we identified 197 patients with paired iFISH results at diagnosis and first relapse. Patients who did not experience relapse by the end of the follow-up period were excluded from the paired dataset. Patients were allocated to either immunomodulating drug-based or proteasome inhibitor-based induction, as previously described.[18] After at least four cycles of induction with a minimum partial response, patients underwent either first-line autologous stem cell transplant (ASCT) or two additional cycles of consolidation treatment. Response assessments were performed according to International Myeloma Working Group consensus criteria.[19] Post-induction minimal residual disease (MRD) was assessed by multiparameter flow cytometry as previously reported.[7,18] MRD sensitivity threshold was between 10^{-4} to 10^{-5}. All the patients provided informed consent in compliance with the Declaration of Helsinki. This study was approved by the local Institutional Ethics Committees of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Science & Peking Union Medical College (certificate: IIT2020023-EC-1).

Interphase fluorescence *in situ* hybridization testing at diagnosis and relapse

The iFISH technique used in this study has been previously described. Bone marrow (BM) aspirate samples anticoagulated with EDTA were collected, and CD138$^+$ plasma cells (PC) were isolated using CD138$^+$ magnetic beads (Miltenyi Biotec, Paris, France). iFISH analysis for CA included del(13q), del(17p), gain/amp(1q), t(11;14)(q13;q32), t(4;14)(p16.3;q32), and t(14;16)(q32;q23) in 200 interphase nuclei. The cut-off values for del(17p), gain/amp(1q), del(13q), and translocations were previously reported to be 50%, 20%, 10%, and 10%, respectively.[13] Patients with del(17p), t(4;14), or t(14;16) were categorized as having high-risk CA,[10] whereas those without these CA were considered standard-risk.

Statistical analysis

This study aimed to investigate the association between CA and survival outcomes in patients with MM. We defined progression-free survival (PFS) as the duration from diagnosis to the date of death, first progression, or last follow-up. OS was calculated from diagnosis to the date of death or last follow-up. In order to account for time bias, we conducted post-relapse landmark PFS and OS analysis. PFS2, the time from diagnosis to progression of second-line treatment, was defined based on previous studies.[20] We used the Kaplan-Meier method to analyze survival data, and differences in survival were evaluated using the log-rank test. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using the Cox regression model. Multivariable Cox stepwise proportional models were developed to assess the variables with significant impact on survival in the univariable analyses, including age, post-induction response, International Staging System (ISS) stage, post-induction MRD status, transplantation, and del(17p) at relapse. Continuous variables were compared using either Student's *t* test or Mann-Whitney U test based on the variables' distributional statistics. The χ^2 test or Fisher's exact test was used to assess the statistical significance of categorical variables between the different groups. A two-sided *P* value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS (version 26.0; IBM, Chicago, IL, USA) and R (version 4.2.0; R Foundation, Vienna, Austria).

Results

The presence of high-risk cytogenetic abnormalities at relapse exerts a greater adverse impact on multiple myeloma compared with their presence at diagnosis

This study enrolled 995 NDMM patients and 293 MM patients experienced their first relapse. Patients in these two cohorts are shown in the *Online Supplementary Table S1*. All patients underwent iFISH testing for gain/amp(1q), del(17p), del(13q), and IgH rearrangements. Gain/amp(1q) was observed in ≥20% of malignant PC in nearly half of the patients at diagnosis (457/995, 46%), making it the second most frequent cytogenetic event (Figure 1A). However, at relapse, gain/amp(1q) was observed in 63% of the patients and was the most frequent event (Figure 1B). The distri-

bution of each cytogenetic event in the two datasets is summarized in Table 1. We also observed del(17p) (present in at least 50% of malignant PC) in 6% (63/995) of patients at the time of diagnosis (Figure 1A), but this percentage increased to 17% at relapse (Figure 1B). However, the frequency of del(13q) and IgH translocations was comparable between the two groups (Table 2).

In order to further investigate this, we compared the number of CA detected by iFISH in each patient. The results demonstrated that patients at relapse carried more CA than those at diagnosis, especially for two or more CA detected using iFISH (69% *vs*. 54%; *P*<0.001; Figure 1C). Additionally, when comparing patients with fewer than two CA to those carrying more than two CA, it was observed that patients with fewer than two CA exhibited a longer OS from the time of diagnosis (at diagnosis: 68.5 *vs*. 41.0 months, HR =1.83; 95% CI: 1.44-2.32; *P*<0.001; at relapse: 62.3 *vs*. 38.7 months, HR=1.60; 95% CI: 1.15-2.23; *P*=0.005) (Figure 1D).

In order to gain a better understanding of the prognostic relevance of high-risk CA at relapse, we examined 197 patients with paired iFISH results at both diagnosis and first relapse (Figure 2A). The baseline characteristics of the patients are presented in the *Online Supplementary Table S2*. In summary, 45% of the patients had ISS stage 3, 88% had at least one CA detected by iFISH, and 33% of patients in this cohort exhibited high-risk aberrations at baseline. Additionally, del(17p) was observed in 7% (14/197) of patients at diagnosis and in 18% (36/197) of the patients at first relapse, using a cutoff value of 50% (*Online Supplementary Table S2*). Consistent with our previous findings,[6] patients with high-risk CA, whether detected early at diagnosis or later at relapse, experienced inferior outcomes (Figure 2B). Moreover, the presence of high-risk aberrations at relapse had a greater adverse effect on MM than those present at diagnosis (first OS: HR=1.79; 95% CI: 1.25-2.57 *vs*. HR=1.56; 95% CI: 1.08-2.25).

Longitudinal interphase fluorescence *in situ* hybridization reveals a clonal selection of secondary cytogenetic abnormalities

The paired iFISH results of each patient at diagnosis and

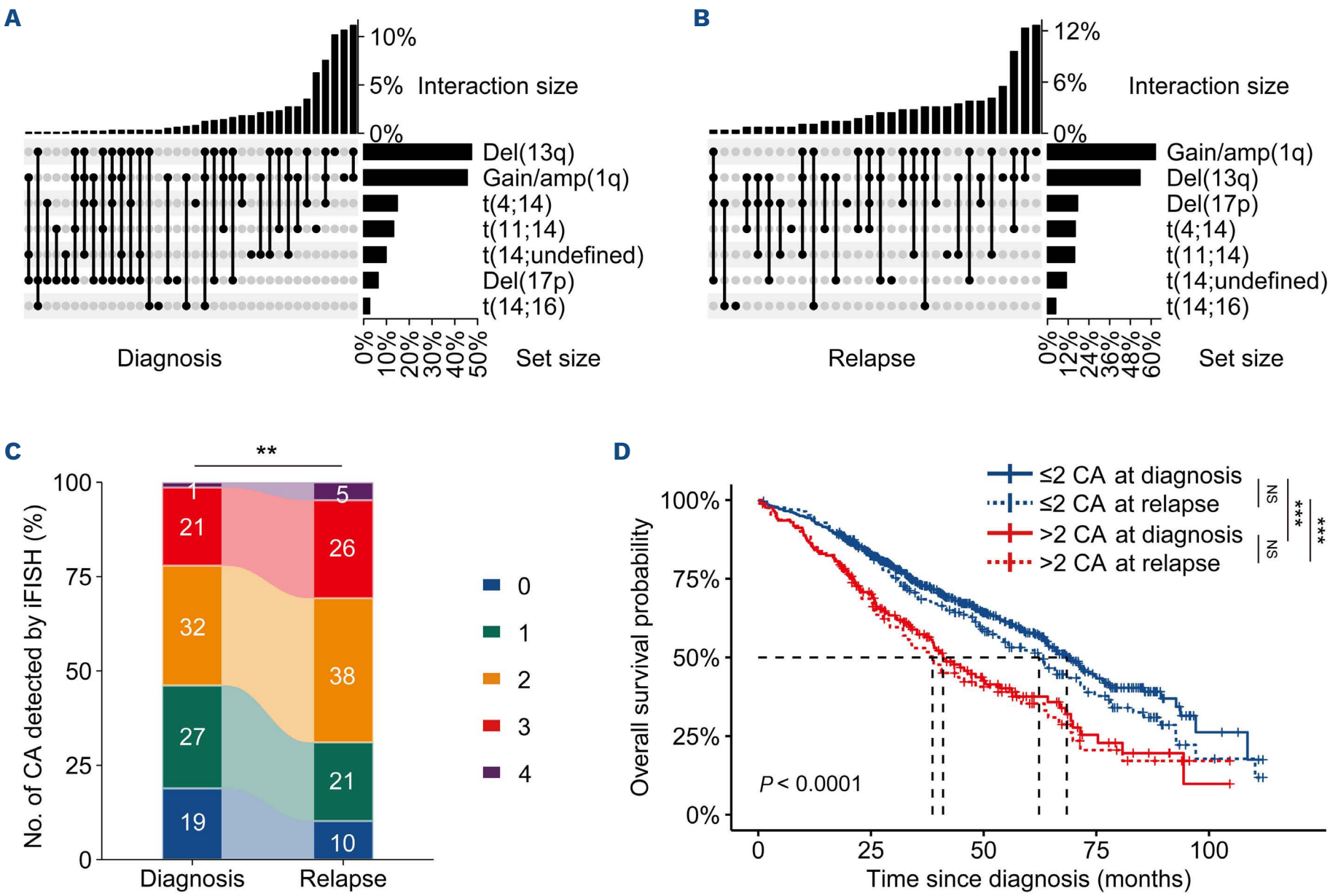


Figure 1. The prognostic significance of high-risk cytogenetic abnormalities that are present at diagnosis or at relapse. (A) Upset plots of cytogenetic abnormalites (CA) detected by interphase fluorescence *in situ* hybridization (iFISH) at diagnosis. (B) Upset plots of CA detected by iFISH at relapse. (C) Rates of the number of CA in multiple myeloma (MM) patients detected at diagnosis and relapse. ***P<0.001, by two-sided χ^2 test. (D) Kaplan-Meier analysis of overall survival (OS) for patients with ≤2 CA or >2 CA at diagnosis or at relapse. NS: not significant; ***P<0.001, by two-sided log-rank test.

relapse were evaluated, and our findings demonstrated that the newly acquired CA at relapse were primarily secondary cytogenetic events, including del(17p) and gain/amp(1q), whereas no obvious changes in the clonal architecture of del(13q) were observed (Figure 3A). Compared with the number of CA detected by iFISH at diagnosis, MM showed an increased number of CA at relapse (Figure 3B). While most patients had the same number of CA between the two time points, few patients (56, 11, and 1) developed one, two, and three new CA, respectively, at relapse (Figure 3C).

Table 1. The distributions of cytogenetic abnormalities by probes of 995 patients in the diagnosis dataset and 293 patients in the relapse dataset.

CA, N/N (%)	At diagnosis N=995				At relapse N=293			
	0-10%	10.5-20%	20.5-50%	>50.5%	0-10%	10.5-20%	20.5-50%	>50.5%
Del(13q)	517/995 (52.0)	39/995 (3.9)	81/995 (8.1)	358/995 (36.0)	136/293 (46.4)	12/293 (4.1)	30/293 (10.2)	115/293 (39.2)
Gain/amp(1q)	382/995 (48.8)	52/995 (5.2)	75/995 (7.5)	382/995 (38.4)	94/293 (32.1)	15/293 (5.1)	25/293 (8.5)	159/293 (54.3)
Del(17p)	884/995 (88.8)	17/995 (1.7)	31/995 (3.1)	63/995 (6.3)	215/293 (73.4)	12/293 (4.1)	15/293 (5.1)	51/293 (17.4)
IgH rearrangement	416/995 (41.8)	21/995 (2.1)	53/995 (5.3)	505/995 (50.8)	116/293 (39.6)	4/293 (1.4)	15/293 (5.1)	158/293 (53.9)
t(4;14)	687/835 (82.2)	4/835 (0.4)	13/835 (1.6)	131/835 (15.7)	212/259 (81.9)	0/259 (0)	4/259 (1.5)	43/259 (16.6)
t(11;14)	694/827 (83.9)	8/827 (1.0)	10/827 (1.2)	115/827 (13.9)	211/257 (82.1)	2/257 (0.8)	4/257 (1.6)	40/257 (15.6)
t(14;16)	806/832 (96.8)	0/832 (0)	3/832 (0.4)	23/832 (2.8)	245/259 (94.6)	0/259 (0)	1/259 (0.4)	13/259 (5.0)
t(14; undefined)[a]	726/824 (88.1)	4/824 0.5)	7/824 (0.8)	87/824 (10.6)	225/257 (87.6)	0/257 (0)	3/257 (1.2)	29/257 (11.3)

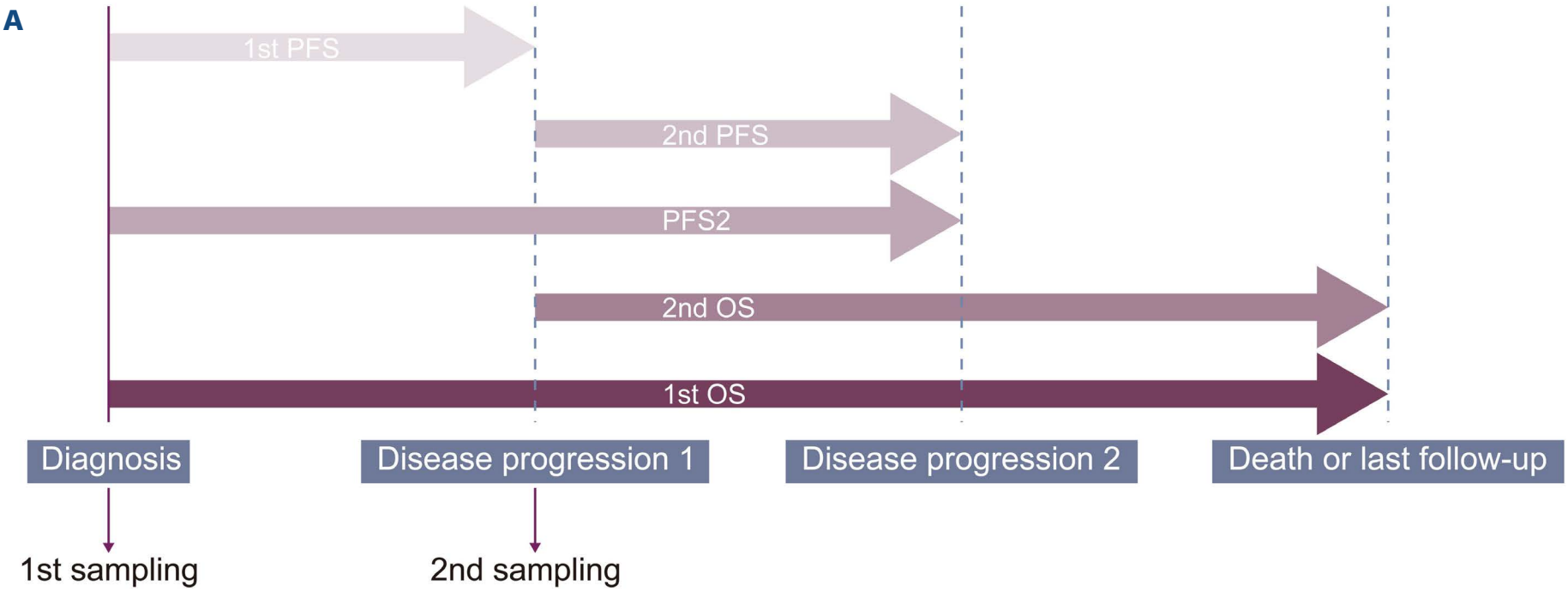
[a]t(14; undefined): patients with an undefined abnormality of the 14q32 loci not corresponding to one of the above 3 described common translocations. CA: cytogenetic abnormality; Del: deletion, amp: amplification; IgH: immunoglogulin heavy chain.

Table 2. Patient characteristics of 995 patients in the diagnosis dataset and 293 patients in the relapse dataset.

Characteristics	NDMM N=995	RRMM N=293	*P*
Male, N (%)	587 (59)	167 (57)	0.587
Age in years, median (range)	60 (29-83)	57 (34-77)	0.687
ISS stage III, N (%)	390 (39)	132 (45)	0.084
Cytogenetics, N/N (%)			
Del(13q)[a]	478/995 (48)	157/293 (54)	0.109
Gain/amp(1q)[b]	457/995 (46)	184/293 (63)	< 0.001
Del(17p)[c]	63/995 (6)	51/293 (17)	< 0.001
IgH rearrangement[d]	579/995 (58)	177/293 (60)	0.542
t(4;14)	148/835 (18)	47/259 (18)	0.950
t(11;14)	133/827 (16)	46/257 (18)	0.556
t(14;16)	26/832 (3)	14/259 (5)	0.130
t(14;undefined)[e]	98/824 (12)	32/257 (12)	0.896
At least 1 CA by iFISH	807/995 (81)	263/293 (90)	0.004
High-risk CA	231/836 (28)	105/266 (39)	<0.001

[a,b,c,d]The cutoff value for del(17p), gain/amp(1q), del(13q), and IgH translocations were set at 50%, 20%, 10% and 10%, respectively. [e]t(14; undefined): patients with an undefined abnormality of the 14q32 locus that did not correspond to 1 of the above 3 described common translocations. [f]High-risk CA: presence of t (4;14), t(14;16), and/or del(17p). CA: cytogenetic abnormality; ISS: International Staging System; Del: deletion; amp: amplification; IgH: immunoglogulin heavy chain; iFISH: interphase fluorescence *in situ* hybridization; NDMM: newly diagnosed multiple myeloma; RRMM: relapsed/refractory multiple myeloma.

A



B

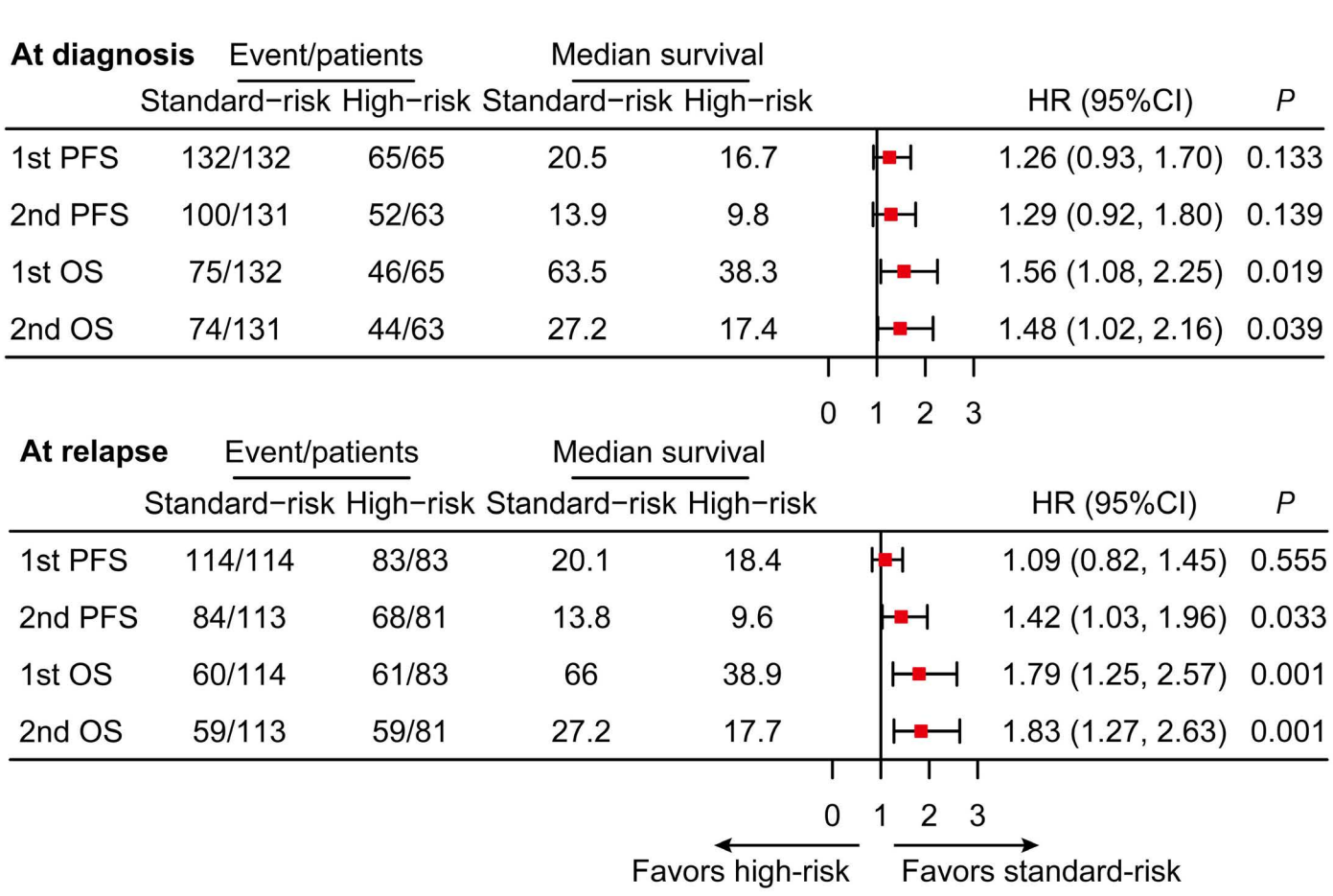
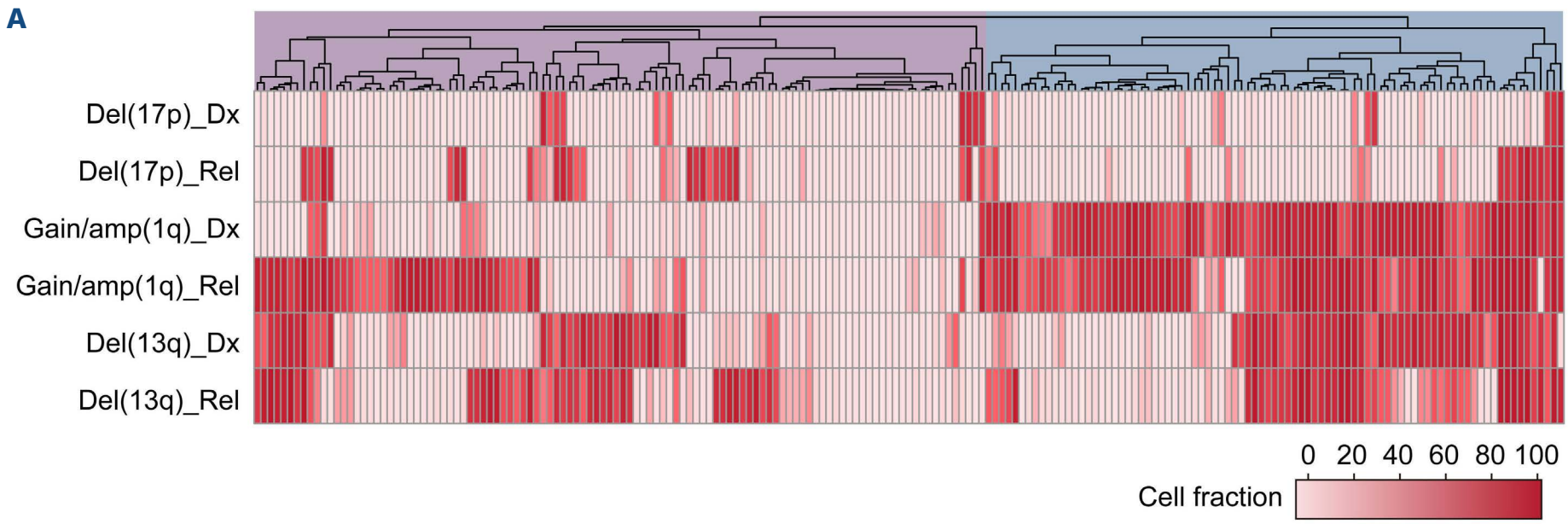
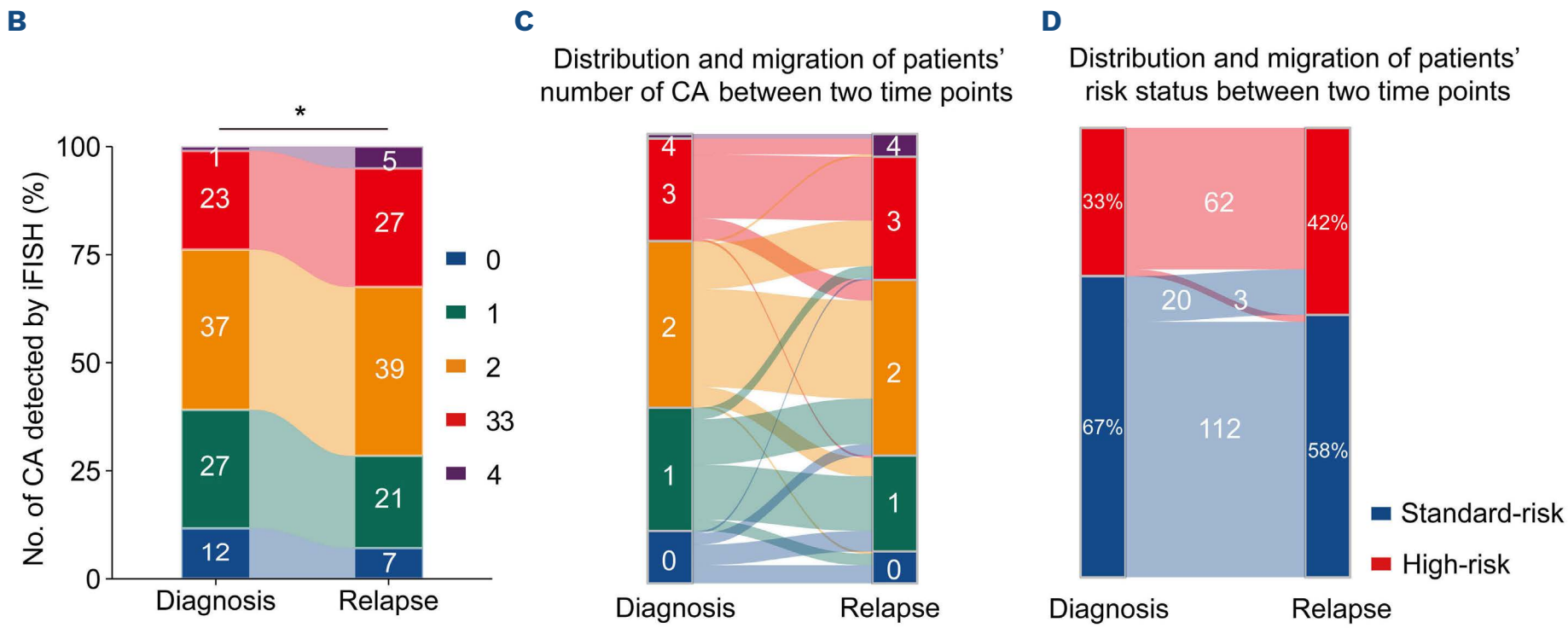


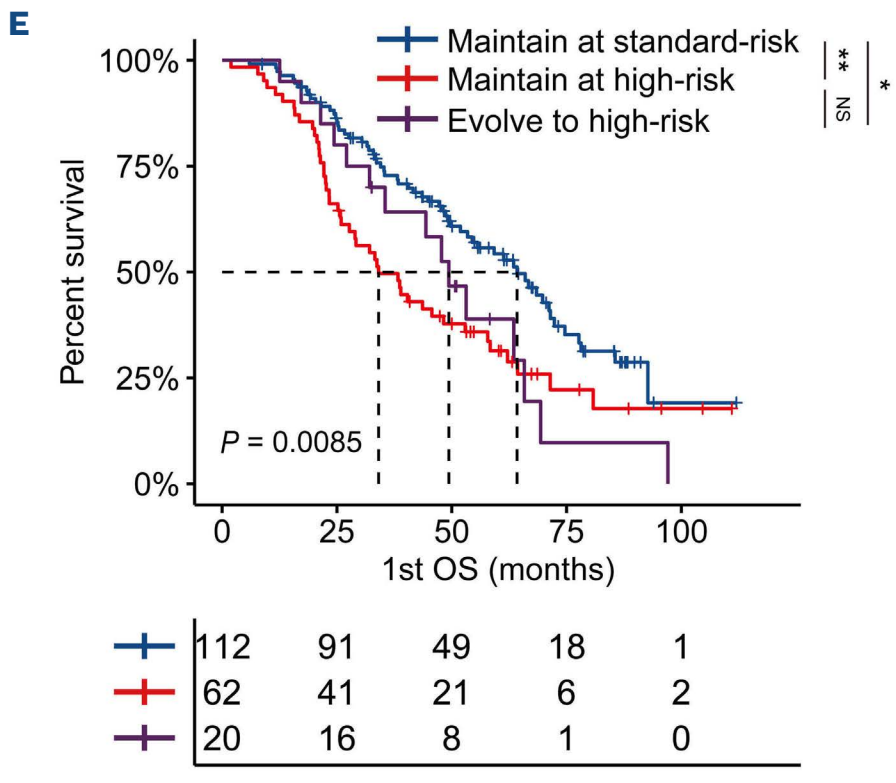
Figure 2. Survival outcomes in patients with different risk statuses identified by paired interphase fluorescence *in situ* hybridization examinations at diagnosis and relapse. (A) Diagram of different clinical endpoints used in the study. (B) Forest plots of hazard ratio (HR) for median survival in patients with standard-risk *versus* high-risk cytogenetic abnormalites (CA) at diagnosis (upper) and relapse (lower). PFS: progression-free survival; OS: overall survival; CI: confidence interval.

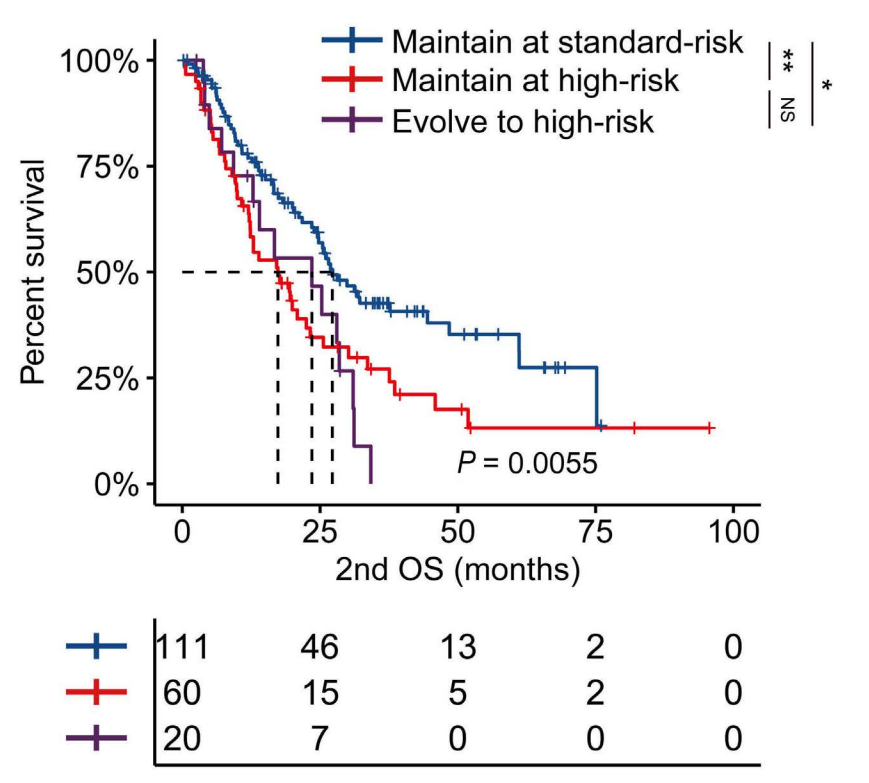
Furthermore, IgH rearrangement and chromosomal translocations into Ig loci are the founder cytogenetic events in MM. And our results did not indicate any significant changes in IgH-related CA at relapse.

We identified a total of 62 patients who maintained at standard-risk, 20 patients who evolved to high-risk, and 112 patients who maintained at high-risk during follow-up, respectively (Figure 3D). When comparing the survival outcomes, the median OS for the three patient groups was as follows: 64.2 months for those who maintained at standard-risk, 49.4 months for those who evolved to high-risk, and 34.1 months for those who maintained at high-risk (Figure 3E). Additionally, the median second OS for the three groups was 27.2 months, 23.5 months, and 17.4 months, respectively (Figure 3F). Although the log-rank test did not show statistical differences in survival between patients who maintained at high-risk and those who evolved to high-risk, our result showed that patients who evolved to high-risk experienced a relatively longer survival (1st OS: HR=0.91, 95% CI: 0.51-1.63, *P*=0.751; 2nd OS: HR=0.85, 95%









Continued on following page.

Figure 3. The cytogenetic abnormality profiles between two time points and their prognostic relevance. (A) Heatmap of cell fraction of del(17p), gain/amp(1q) and del(13q) detected by interphase fluorescence *in situ* hybridization (iFISH) at diagnosis and relapse. Each row represents a specific cytogenetic abnormalites (CA), and each column represents a patient, color coded according to the fraction of plasma cells (PC) detected with a specific CA. (B) Rates of the number of CA in multiple myeloma (MM) patients detected at diagnosis and relapse. $^{*}P<0.05$, by two-sided χ^2 test. (C) Sankey diagram showing the distribution and migration of patients' number of CA between 2 time points. (D) Sankey diagram showing the distribution and migration of patients' risk status between 2 time points. (E, F) Kaplan-Meier curves in patients with different risk statuses and evolutionary patterns identified by iFISH. Different landmarks are used: overall survival (OS) from diagnosis (E) and OS from relapse (F). NS: not significant; $^{*}P<0.05$, $^{**}P< 0.01$, by two-sided log-rank test. Del: deletion; amp: amplification.

CI: 0.47-1.56, *P*=0.61). Further analysis revealed that all patients who evolved to high-risk at relapse exhibited the acquisition of del(17p) (*Online Supplementary Table S3*).

Minor clone of del(17p) at relapse is associated with poor prognosis in multiple myeloma

In order to evaluate the prognostic impact of del(17p) at different clonal sizes, we divided the patients with this CA into three clusters based on the percentage of PC involved: 0-10%, 10-20%, 20-50%, and ≥50%. Using cutoffs ranging from 10% to 50%, the median OS at diagnosis ranged from 34.1 to 29.1 months, and at first relapse, it ranged from 38.7 to 35.5 months (Figure 4A). This highlights the additional prognostic significance of the clonal size of del(17p). Furthermore, there was no significant difference in the first PFS between patients with and without del(17p) at relapse at different clonal sizes (*Online Supplementary Figure S1A*), suggesting that poor outcomes associated with del(17p) at relapse were mainly due to reduced survival after the first relapse.

We then classified the clonal size of del(17p) into three groups: ≤10% (no del(17p)), 10-50% (minor clone of del(17p)), and >50% (major clone of del(17p)). Survival analyses revealed that patients with a minor clone of del(17p) (10-50%) at relapse experienced significantly shorter survival compared to those without del(17p) (≤10%) (1st OS: 43.9 months *vs*. 63.5 months, HR=1.64, 95% CI: 1.03-2.81, *P*=0.044; 2nd OS: 28.1 months *vs*. 17.1 months, HR=1.98, 95% CI: 1.15-3.41, *P*=0.008). Moreover, our findings indicated no significant difference in survival between patients carrying a major or a minor clone of del(17p) at relapse (Figure 4D, E; *Online Supplementary Figure S1D, E*).

In order to investigate whether a minor clone of del(17p) at relapse remained an independent predictor of outcome when taking account of other prognostic markers including age ≥65 years, post-induction response, ISS stage, post-induction MRD status, transplantation and del(17p) at relapse, we included del(17p) in a multivariable analysis. After univariable analysis, age ≥65 *versus* <65 years and del(17p) were included in multivariable analysis. Using multivariable Cox stepwise proportional model, the presence of a minor clone of del(17p) at relapse predicted shorter second OS with a hazard ratio of 1.90 (95% CI: 1.10-3.29; *P*=0.021) (*Online Supplementary Tables S4* and *S5*). Therefore, 10% may be the proper cutoff value for del(17p) at relapse.

Clonal evolution of del(17p)

Subsequently, we classified the patients into six groups according to the change patterns in the clonal size of del(17p) between the two time points (Figure 5A). Patients in group A, who experienced the loss of del(17p) at relapse, those in group B, who had a decreasing clonal size from the major to the minor clone at relapse, and those in group C, who did not have del(17p) at both time points, had similar superior outcomes (with a second OS of 50.3 months, 16.6 months, and 26.9 months, respectively). In contrast, patients in group D, who had newly acquired del(17p) at relapse, had a relatively worse survival (with a second OS of 20.2 months). Of the remaining 16 patients, those with a stable clone of del(17p) between the two time points (group E) and those with an obvious increase in clonal size of del(17p) (group F) had the poorest outcomes (with a second OS of 12.5 months and 12.8 months, respectively; Figure 5B). These six del(17p) evolutionary groups were subsequently combined into three patterns, based on the survival curve. Although there was no significant difference in the sampling time between the two time points (Figure 5C), survival analysis revealed that the different evolutionary patterns of del(17p) were able to distinguish the survival curves of OS from diagnosis and post-relapse survival (Figure 5D-F; *Online Supplementary Figure S2A*).

Longitudinal analyses were conducted to investigate the minor clone of del(17p) at diagnosis, with a focus on patients in groups D and F. For patients in group D, nine and 27 patients without del(17p) at diagnosis evolved into a minor clone or major clone of del(17p) at relapse, respectively (Figure 6A; *Online Supplementary Table S6*). Within our cohort, we also observed two patients who had del(17p) present in less than 10% of PC at baseline, but who subsequently acquired a major clone of del(17p) during follow-up (Figure 6B). Despite the relatively low incidence of del(17p) at diagnosis (14% at the 10% cutoff value), we observed that 18% (36/197) of cases acquired del(17p) during follow-up and 3% (5/197) had a significant increase in clonal size at relapse (*Online Supplementary Table S6*). Our findings suggest that clonal selection might occur on minor clones of del(17p), which is indicative of poor prognosis in MM.

Discussion

This retrospective analysis involved the examination of 995

A

CF of del(17p) at diagnosis	1st OS No. of patients	1st OS HR (95%CI)	1st OS *P*	2nd OS No. of patients	2nd OS HR (95%CI)	2nd OS *P*
>10 *vs.* ≤10	27 *vs.* 170	1.39 (0.72–2.66)	0.324	26 *vs.* 168	1.3 (0.66–2.57)	0.45
>20 *vs.* ≤20	22 *vs.* 175	1.49 (0.88–2.53)	0.137	21 *vs.* 173	1.56 (0.91–2.69)	0.107
>50 *vs.* ≤50	14 *vs.* 183	1.59 (0.98–2.58)	0.058	13 *vs.* 181	1.58 (0.96–2.58)	0.07

CF of del(17p) at relapse	1st OS No. of patients	1st OS HR (95%CI)	1st OS *P*	2nd OS No. of patients	2nd OS HR (95%CI)	2nd OS *P*
>10 *vs.* ≤10	56 *vs.* 141	1.86 (1.28–2.7)	0.001	55 *vs.* 139	2.23 (1.52–3.28)	<0.001
>20 *vs.* ≤20	49 *vs.* 148	1.86 (1.26–2.75)	0.002	48 *vs.* 146	2.1 (1.41–3.12)	< 0.001
>50 *vs.* ≤50	36 *vs.* 161	1.88 (1.23–2.89)	0.004	35 *vs.* 159	2.19 (1.41–3.4)	< 0.001

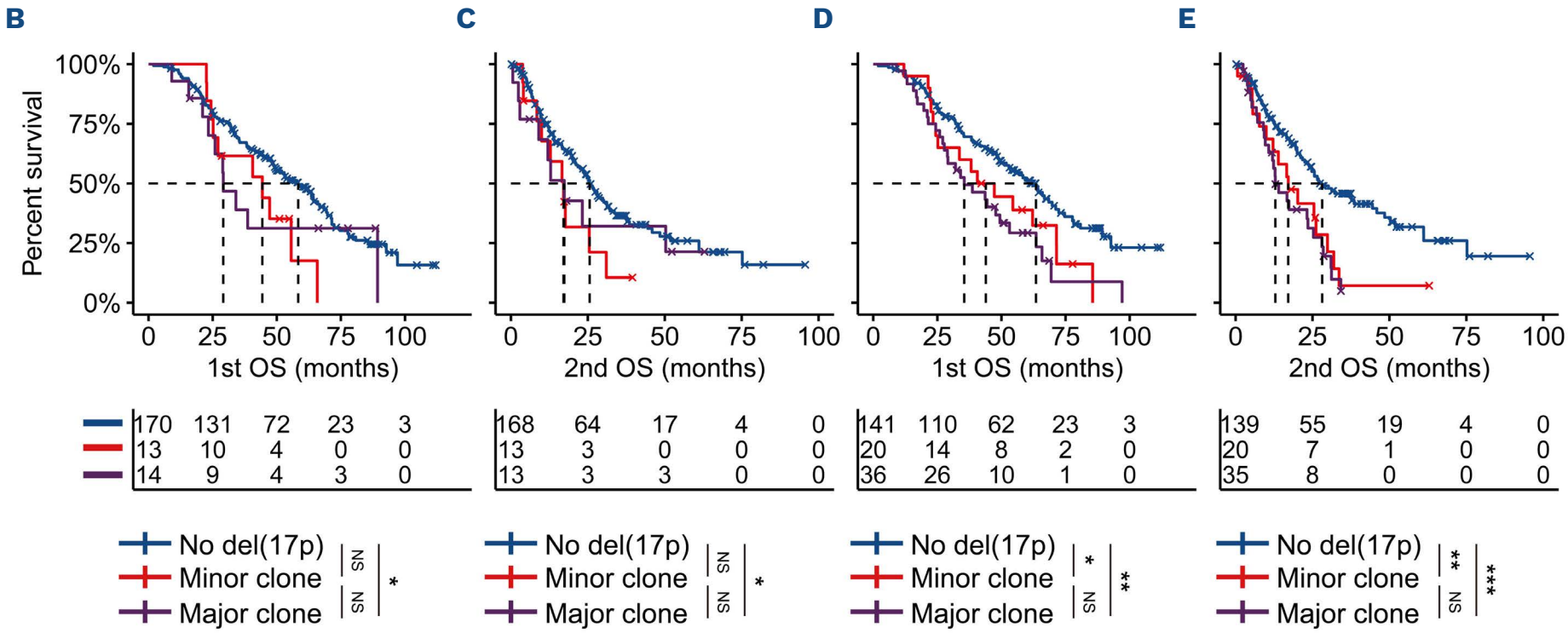
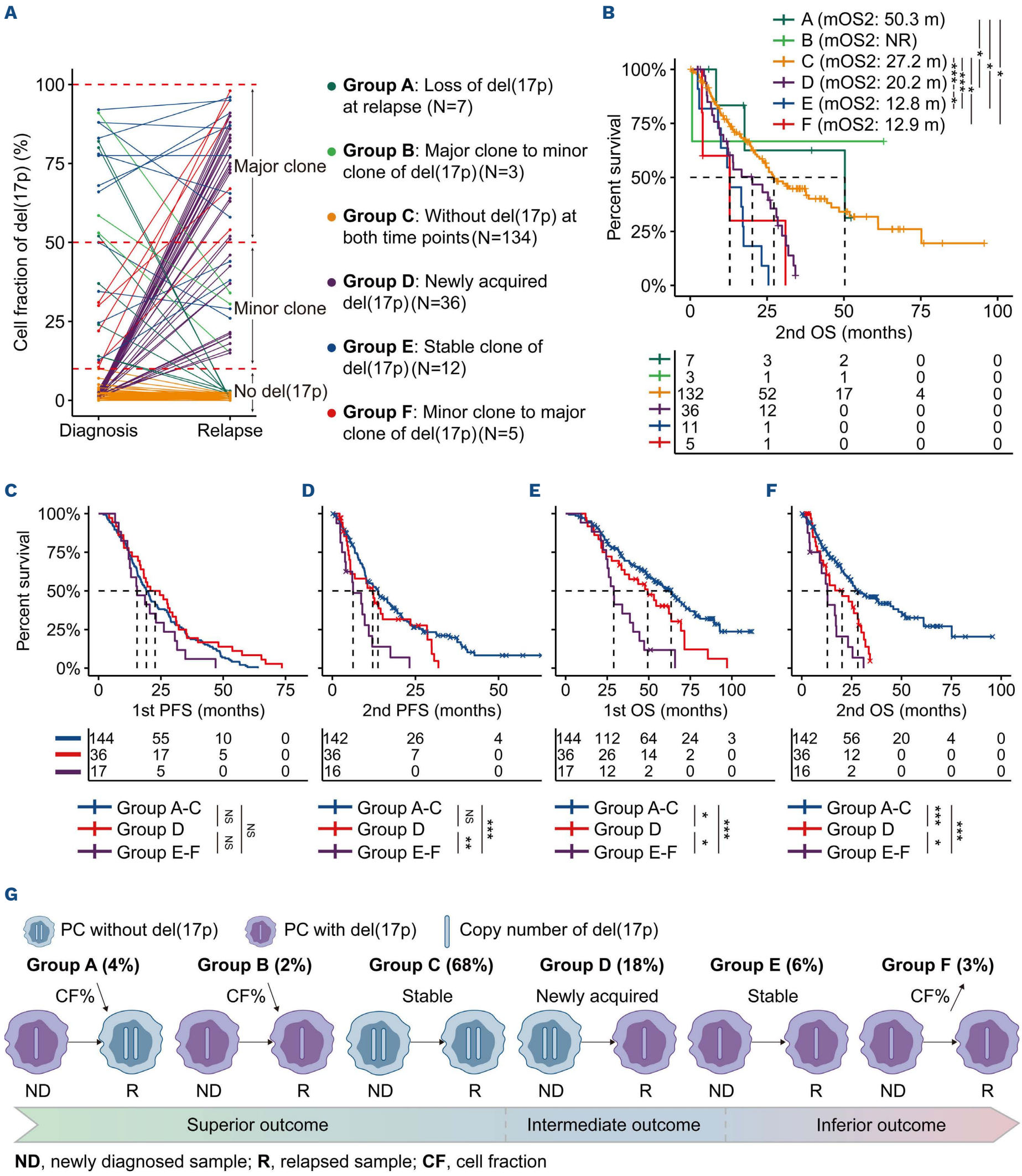


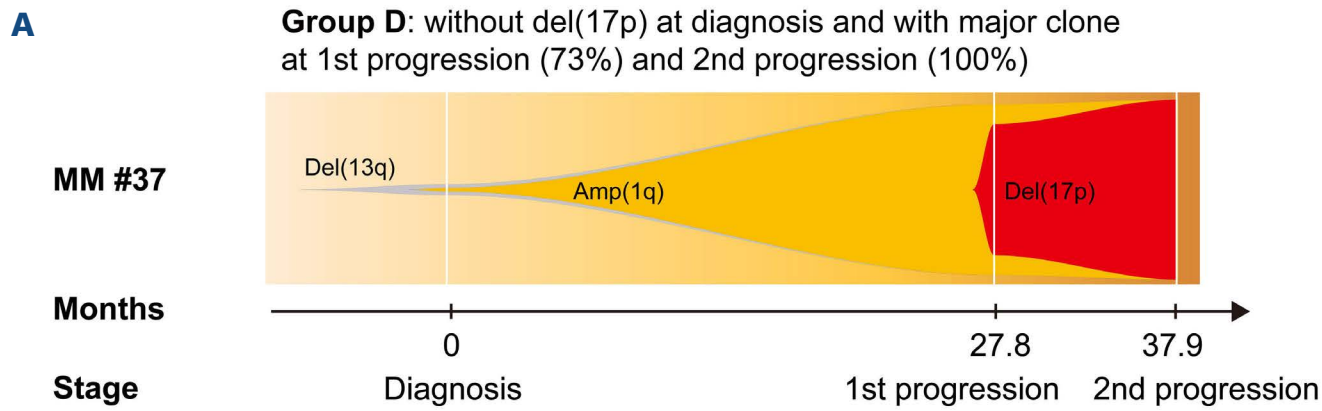
Figure 4. The prognostic significance of del(17p) that are present at diagnosis or at relapse. (A) Forest plots of hazard ratio (HR) for median survival in patients with different cell fractions of del(17p) at diagnosis (upper) or at relapse (lower). (B, C) Kaplan-Meier curves in patients at diagnosis with no del(17p), a minor clone of del(17p) or a major clone of del(17p). Different landmarks are used: overall survival (OS) from diagnosis (B) and OS from relapse (C). NS: not significant; $^{*}P<0.05$, by two-sided log-rank test. (D, E) Kaplan-Meier curves in patients at relapse with no del(17p), a minor clone of del(17p) or a major clone of del(17p). Different landmarks are used: OS from diagnosis (D) and OS from relapse (E). NS: not significant; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, by two-sided log-rank test. CF: cell fraction. CI: confidence interval.

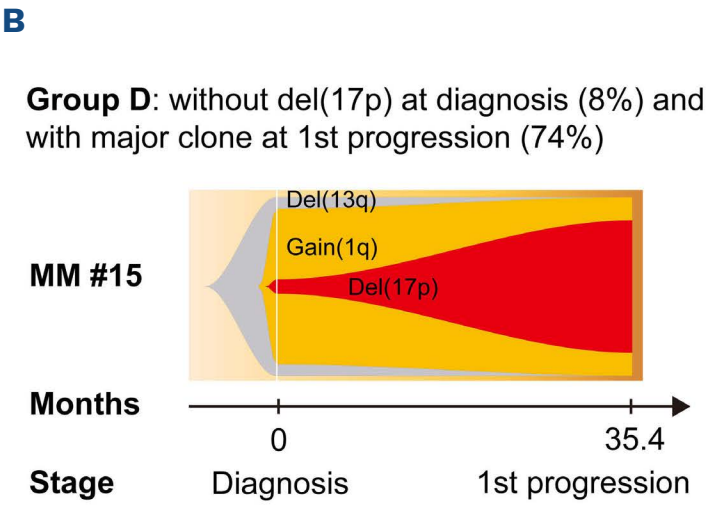
patients with NDMM and 293 patients with first-relapse MM, all of whom had cytogenetic data available. Among them, 197 patients had paired iFISH results at both diagnosis and the first relapse. Our study led to five main conclusions. First, risk status was dynamic, and routine iFISH should be performed at the first relapse to re-evaluate patients' risk statuses. Second, clonal evolution caused by disease progression resulted in a higher incidence of secondary CA, specifically del(17p) and gain/amp(1q), at relapse than at diagnosis. Third, our findings demonstrated that patients who experienced changes in risk status or acquired new CA during follow-up had poorer survival rates, both from diagnosis and post-relapse, compared to patients who maintained standard risk status or the same number of CA between the two time points. Fourth, a minor clone of del(17p) at relapse, but not at diagnosis, was associated with poor prognosis in MM. Finally, patients who never had del(17p) during follow-up had the best outcomes, whereas

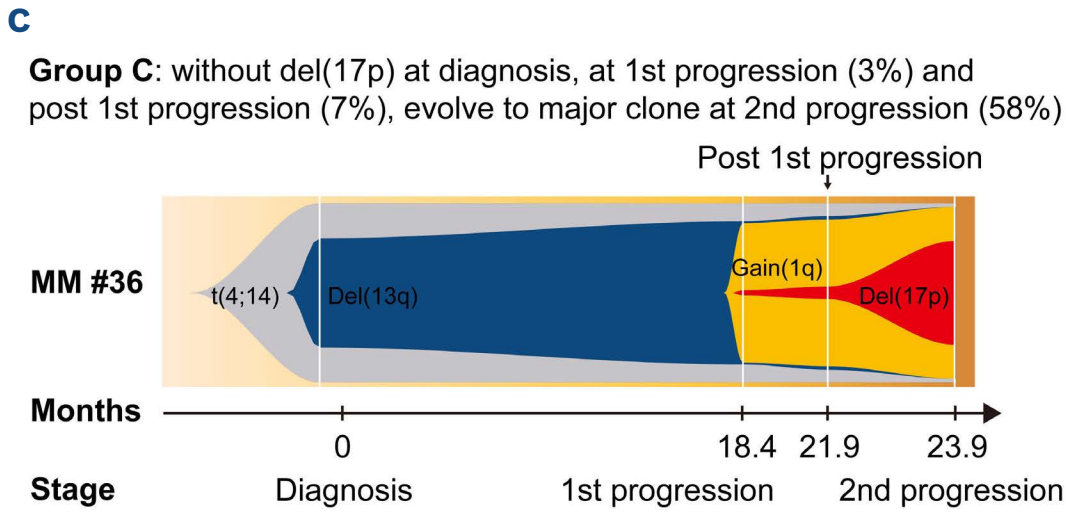


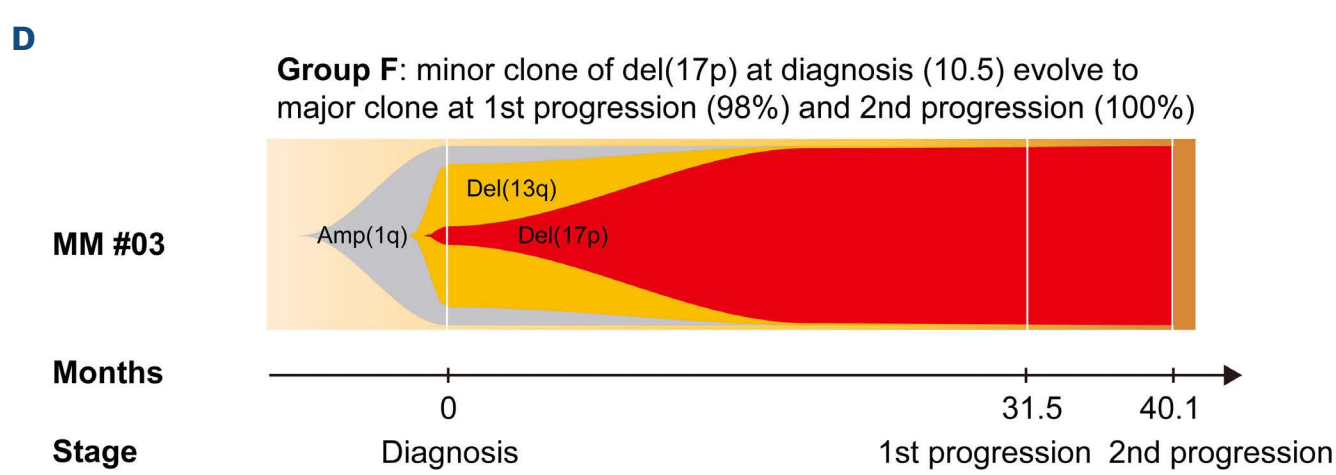
ND, newly diagnosed sample; **R**, relapsed sample; **CF**, cell fraction

Figure 5. Clonal evolution of del(17p) in 197 patients with paired interphase fluorescence *in situ* hybridization results. (A) The change in cell fraction (CF) of del(17p) between 2 time points. Different colors demonstrate 6 different evolutionary patterns. (B) Overall survival (OS) from second sampling among patients with different del(17p) evolutionary patterns. (C-F) Six del(17p) evolutionary patterns are merged into 3 groups according to the survival curves in (B). Kaplan-Meier curves for the first progression-free survival (PFS) (C), second PFS (D), first OS (E), and second OS (F) are presented. NS: not significant; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, by two-sided log-rank test. (G) Diagram of 6 different evolutionary patterns of del(17p) between 2 time points. mOS: median OS.









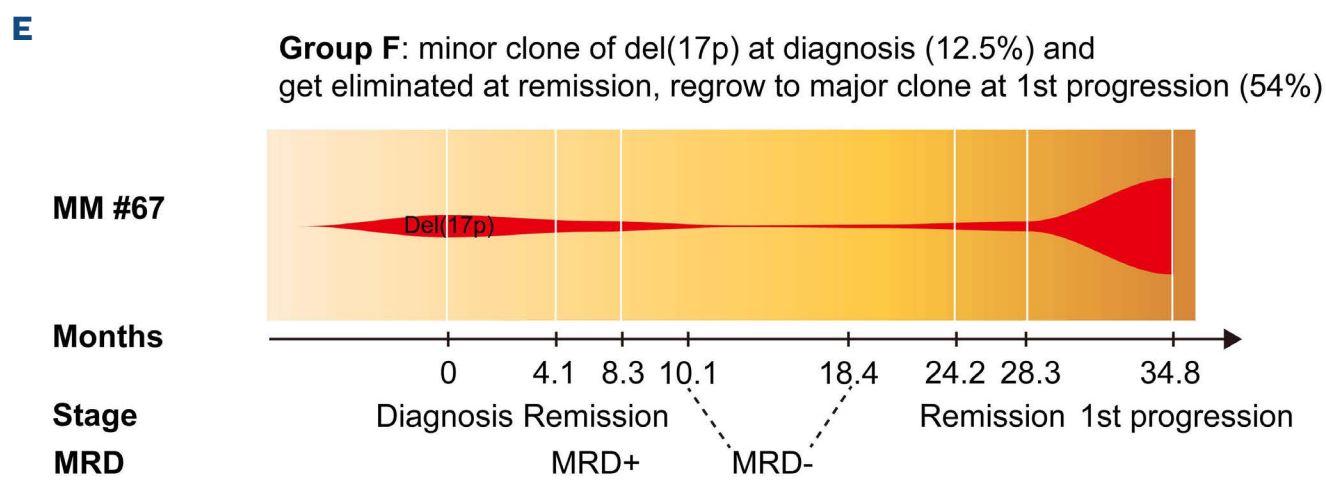


Figure 6. Representative patterns of clonal evolution of del(17p) in relapsed patients. Fish plots visualizing 5 representative patterns of clonal evolution of del(17p) in relapse patients according to the cell fraction of cytogenetic abnormalities (CA) detected using interphase fluorescence *in situ* hybridization (iFISH) at diagnosis. The vertical line highlights sampling points at diagnosis, post-induction, and relapse. (A) Without del(17p) at diagnosis and with major clones at the first progression (73%) and second progression (100%). (B) Without del(17p) at diagnosis (8%) and with a major clone at first progression (74%). (C) Without del(17p) at diagnosis, at first progression (3%) and post-first progression (7%), evolved into a major clone at the second progression (58%). (D) A minor clone of del(17p) at diagnosis (10.5) evolved into a major clone at the first progression (98%) and second progression (100%). (E) Minor clone of del(17p) at diagnosis (12.5%), eliminated at remission, regrew to major clone at first progression (54%). MM: multiple myeloma; MRD: minimal residual disease.

those who had newly acquired del(17p) had compromised survival.

The survival analyses in our study revealed that high-risk CA were associated with reduced survival compared to standard-risk CA at diagnosis for all endpoints examined. These findings remained consistent for patients with high-risk CA at first relapse, which was in line with the results of previous studies.[21,22] Additionally, patient outcomes were more significantly affected by the presence of high-risk CA at the time of relapse than at diagnosis. Clonal evolution has been widely recognized as inherent mechanism driving the progression of MM,[23,24] and extensive research has investigated various patterns of clonal evolution from diagnosis to the first relapse.[6,16] In our study, patients who evolved to high-risk experienced relatively longer survival compared to those maintained at high-risk during follow-up, while patients who maintained a standard-risk status demonstrated the best survival outcome. However, the elimination of high-risk CA during follow-up was infrequent, as demonstrated by only three (2%) patients in our cohort. These findings further supported the cumulative nature of CA, especially high-risk CA, which had a negative impact on the prognosis of MM.

While there is no universally agreed upon definition for high-risk myeloma,[25,26] previous studies have consistently demonstrated that del(17p) is a strong predictor of poor prognosis in patients.[27-29] Depending on the specific cut-off value employed, iFISH-based detection of del(17p) has been reported in 5-20% of NDMM patients,[27-33] with those with aggressive forms of the disease, such as PC leukemia, having significantly higher incidence rates of del(17p).[34] In previous reports on NDMM patients treated with bortezomib and dexamethasone, the 4-year OS rates were 50% and 79% for patients with and without del(17p), respectively.[33] A phase III trial of ixazomib or placebo, in combination with lenalidomide and dexamethasone, for relapsed/refractory multiple myeloma (RRMM), has shown that patients without and with del(17p) (observed in ≥5% of malignant PC) have a median PFS of 21.4 and 9.7 months, respectively.[35] These results highlight the prognostic value of del(17p) in both NDMM and RRMM patients.

In our study, a cutoff of 50% was established for del(17p) at diagnosis, based on the findings from our previous study.[13] Del(17p) was detected in 7% (14/197) of the patients at diagnosis and 18% (36/197) of the patients at first relapse, using the cutoff value of 50%, respectively. Additionally, our further analysis revealed that a minor clone of del(17p) at relapse, but not at diagnosis, was associated with a poor prognosis in MM. Therefore, a cutoff value of 10% may be appropriate for del(17p) at relapse. However, laboratories often prefer to use the mean + standard deviation from normal BM controls as the cutoff value. And the choice of the cutoff value for del(17p) at diagnosis remains a topic of debate, with ongoing discussions on how conservative it should be. Hence, further validation of our results is necessary to determine whether a lower cutoff value should be at relapse.

Several factors may contribute to the poor prognosis of patients with a minor clone of del(17p) at relapse. Firstly, studies have shown that therapy-induced clonal evolution can occur as early as the post-induction stage.[7,36,37] The residual PC not only undergo clonal evolution at the cytogenetic level, but also adapt to treatment at the transcriptional level. The upregulation of antioxidative genes,[36] and protein-folding response genes[37] has been observed in residual PC. Consequently, despite the small number of remaining tumor cells after treatment, their adaptation to therapy makes it difficult to eliminate these cells. From this perspective, minor clones of del(17p) can be considered "smart" tumor cells that possess an adaptive response to treatment. Secondly, a previous study reported that inflammation in the BM of MM patients persists after anti-tumor therapy.[38] And the abundances of tumor-associated macrophages, natural killer cells, and inflammatory classical dendritic cells has been linked to subclonal (10-80%) or dominant (>80%) gain/amp(1q).[9] The interactions between tumor cells and the MM tumor microenvironment contribute to the immune escape of tumor cells. It can be hypothesized that both tumor-intrinsic factors and external microenvironmental factors simultaneously contribute to the drug resistance observed in the minor clone of del(17p), which ultimately resulting in a poor prognosis for these patients.

The acquisition of del(17p) during follow-up is considered a rare event in MM, as recently reported in a study of 52 patients with MM who underwent paired targeted sequencing at diagnosis and first relapse. In this study, only 3.8% (2/52) of patients acquired del(17p).[16] In a more recent study of 76 patients who acquired del(17p) later during the disease course, the median PFS was 30.1 and 23.0 months (P=0.032), and the median OS was 106.1 and 68.2 months (P<0.001) for controls and patients with acquired del(17p), respectively.[14] In another study of 956 patients who were tested for CA by iFISH at diagnosis and first relapse, acquired del(17p) was observed in 38 patients.[8] In our cohort, 36 patients had newly acquired del(17p) at the time of relapse. Among these patients, nine and 27 patients without del(17p) at diagnosis developed minor or major clones of del(17p) at relapse, respectively. Consistent with previous studies,[8,14] patients with acquired del(17p) had significantly shorter OS than those without del(17p) at both time points (49.4 *vs*. 63.5 months; P<0.05).

Our study had some limitations owing to its retrospective nature. Data on post-relapse survival were not available for all the patients. As patients were not enrolled in a prospectively designed trial, iFISH was not performed at regular time intervals or at every relapse. Additionally, although patients received a relatively homogeneous induction treatment, there was considerable heterogeneity in their post-relapse treatment. Additionally, as the incidence of del(17p) at relapse was low (<10%), and the number of patients with del(17p) in each group was limited, this result

should be validated in future studies in larger cohorts of patients. Furthermore, recent data have shown that some patients carry micro-subclones of secondary CA that may be missed by bulk analyses such as iFISH.[8,14] Therefore, advanced techniques such as next-generation sequencing and single-cell RNA sequencing should be used to monitor the clonal evolution of MM with higher resolution. Besides, the lack of next-generation sequencing data also results in our inability to assess the *TP53* allelic state. Finally, survival analysis, other than PFS1, depends on possibly high-risk-enriched patients since they are all selected as relapse patients, thus the interpretation of the results of our study needs to take into account that the populations of interest in our study are for relapsed patients.

In conclusion, our data confirmed the poor prognosis of MM associated with high-risk CA. Our findings suggest that even a small subclone of del(17p) at diagnosis should be treated as a high-risk MM. Acquisition of del(17p) or a significant increase in the clonal size of del(17p) during disease progression, though rare events in MM, were associated with a marked reduction in patients' survival outcomes.

We recommend that prospectively designed clinical studies be conducted to regularly monitor the clonal evolution of MM and develop optimal therapeutic strategies to eliminate high-risk CA.

Disclosures

No conflicts of interest to disclose.

Contributions

JC, LQ, and GA developed the concept of the project. GA developed the methodology. RL, TY, WY, JX, HF, LL, YL, SD, CD, WS and YX acquired data, recruited and managed patients, and provided facilities. JC and GA analyzed and interpreted data (i.e., statistical and computational analyses). JC, LQ, and GA reviewed the data, wrote and revised the manuscript. JC, WY, JX, HF, and GA provided administrative, technical, or material support (i.e., reporting or organizing data and constructing databases). SY, DZ, LQ, and GA supervised the study.

Acknowledgments

We thank all MM patients who participated in this study.

Funding

This research was supported by the CAMS Innovation Fund for Medical Sciences (CIFMS) (grants 2022-I2M-1-022, to LQ; grants 2021-I2M-1-041), the National Natural Science Foundation (grants 82270218, 81670202, to GA; grants 81900214, to SD; grants 81630007, to LQ), the International Cooperation Projects of National Natural Science Foundation (grant 81920108006, to LQ).

Data-sharing statement:

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. Moreau P, Attal M, Hulin C, et al. Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): a randomised, open-label, phase 3 study. Lancet. 2019;394(10192):29-38.
2. Voorhees PM, Kaufman JL, Laubach J, et al. Daratumumab, lenalidomide, bortezomib, and dexamethasone for transplant-eligible newly diagnosed multiple myeloma: the GRIFFIN trial. Blood. 2020;136(8):936-945.
3. Attal M, Lauwers-Cances V, Hulin C, et al. Lenalidomide, bortezomib, and dexamethasone with transplantation for myeloma. N Engl J Med. 2017;376(14):1311-1320.
4. Miething CC. Clonal evolution in myeloma: a narrow road to remission. Haematologica. 2019;104(7):1292-1293.
5. Adashek JJ, Subbiah V, Westphalen CB, Naing A, Kato S, Kurzrock R. Cancer: slaying the 9-headed hydra. Ann Oncol. 2022;34(1):61-69.
6. Yan Y, Qin X, Liu J, et al. Clonal phylogeny and evolution of critical cytogenetic aberrations in multiple myeloma at single-cell level by QM-FISH. Blood Adv. 2022;6(2):441-451.
7. An G, Yan Y, Xu Y, et al. Monitoring the cytogenetic architecture of minimal residual plasma cells indicates therapy-induced clonal selection in multiple myeloma. Leukemia. 2020;34(2):578-588.
8. Lannes R, Samur M, Perrot A, et al. In multiple myeloma, high-risk secondary genetic events observed at relapse are present from diagnosis in tiny, undetectable subclonal populations. J Clin Oncol. 2022;41(9):1695-1702.
9. Tirier SM, Mallm JP, Steiger S, et al. Subclone-specific microenvironmental impact and drug response in refractory multiple myeloma revealed by single-cell transcriptomics. Nat Commun. 2021;12(1):6960.
10. Palumbo A, Avet-Loiseau H, Oliva S, et al. Revised International Staging System for multiple myeloma: a report from International Myeloma Working Group. J Clin Oncol. 2015;33(26):2863-2869.
11. Costa LJ, Usmani SZ. Defining and managing high-risk multiple myeloma: current concepts. J Natl Compr Canc Netw. 2020;18(12):1730-1737.
12. Thakurta A, Ortiz M, Blecua P, et al. High subclonal fraction of 17p deletion is associated with poor prognosis in multiple myeloma. Blood. 2019;133(11):1217-1221.
13. An G, Li Z, Tai YT, et al. The impact of clone size on the prognostic value of chromosome aberrations by fluorescence in situ hybridization in multiple myeloma. Clin Cancer Res. 2015;21(9):2148-2156.
14. Lakshman A, Painuly U, Rajkumar SV, et al. Impact of acquired del(17p) in multiple myeloma. Blood Adv. 2019;3(13):1930-1938.
15. Walker BA, Mavrommatis K, Wardell CP, et al. A high-risk, double-hit, group of newly diagnosed myeloma identified by genomic analysis. Leukemia. 2019;33(1):159-170.
16. Corre J, Cleynen A, Robiou du Pont S, et al. Multiple myeloma

clonal evolution in homogeneously treated patients. Leukemia. 2018;32(12):2636-2647.
17. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15(12):e538-e548.
18. Yan Y, Mao X, Liu J, et al. The impact of response kinetics for multiple myeloma in the era of novel agents. Blood Adv. 2019;3(19):2895-2904.
19. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17(8):e328-e346.
20. Mbanya Z, Chadda S. Time to second objective disease progression (PFS2): an emerging clinical trial endpoint with regulatory and reimbursement implications. Blood. 2014;124(21):6005-6005.
21. Lonial S, Dimopoulos M, Palumbo A, et al. Elotuzumab therapy for relapsed or refractory multiple myeloma. N Engl J Med. 2015;373(7):621-631.
22. Dimopoulos MA, Moreau P, Palumbo A, et al. Carfilzomib and dexamethasone versus bortezomib and dexamethasone for patients with relapsed or refractory multiple myeloma (ENDEAVOR): a randomised, phase 3, open-label, multicentre study. Lancet Oncol. 2016;17(1):27-38.
23. Dutta AK, Alberge J, Sklavenitis-Pistofidis R, Lightbody ED, Getz G, Ghobrial IM. Single-cell profiling of tumour evolution in multiple myeloma - opportunities for precision medicine. Nat Rev Clin Oncol. 2022;19(4):223-236.
24. van Nieuwenhuijzen N, Spaan I, Raymakers R, Peperzak V. From MGUS to multiple myeloma, a paradigm for clonal evolution of premalignant cells. Cancer Res. 2018;78(10):2449-2456.
25. Zamagni E, Barbato S, Cavo M. How I treat high-risk multiple myeloma. Blood. 2022;139(19):2889-2903.
26. Corre J, Munshi NC, Avet-Loiseau H. Risk factors in multiple myeloma: is it time for a revision. Blood. 2021;137(1):16-19.
27. Avet-Loiseau H, Attal M, Moreau P, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myélome. Blood. 2007;109(8):3489-3495.
28. Avet-Loiseau H, Attal M, Campion L, et al. Long-term analysis of the IFM 99 trials for myeloma: cytogenetic abnormalities [t(4;14), del(17p), 1q gains] play a major role in defining long-term survival. J Clin Oncol. 2012;30(16):1949-1952.
29. Lakshman A, Painuly U, Rajkumar SV, et al. Natural history of multiple myeloma with de novo del(17p). Blood Cancer J. 2019;9(3):32.
30. Avet-Loiseau H, Li JY, Godon C, et al. P53 deletion is not a frequent event in multiple myeloma. Br J Haematol. 1999;106(3):717-719.
31. Thanendrarajan S, Tian E, Qu P, et al. The level of deletion 17p and bi-allelic inactivation of TP53 has a significant impact on clinical outcome in multiple myeloma. Haematologica. 2017;102(9):e364-e367.
32. Cohen YC, Saranga A, Gatt ME, et al. Treatment patterns and clinical outcomes in high-risk newly diagnosed multiple myeloma patients carrying the 17p deletion: An observational multi-center retrospective study. Am J Hematol. 2018;93(6):810-815.
33. Avet-Loiseau H, Leleu X, Roussel M, et al. Bortezomib plus dexamethasone induction improves outcome of patients with t(4;14) myeloma but not outcome of patients with del(17p). J Clin Oncol. 2010;28(30):4630-4634.
34. Schinke C, Boyle EM, Ashby C, et al. Genomic analysis of primary plasma cell leukemia reveals complex structural alterations and high-risk mutational patterns. Blood Cancer J. 2020;10(6):70.
35. Avet-Loiseau H, Bahlis NJ, Chng WJ, et al. Ixazomib significantly prolongs progression-free survival in high-risk relapsed/refractory myeloma patients. Blood. 2017;130(24):2610-2618.
36. Goicoechea I, Puig N, Cedena M, et al. Deep MRD profiling defines outcome and unveils different modes of treatment resistance in standard- and high-risk myeloma. Blood. 2021;137(1):49-60.
37. Cohen YC, Zada M, Wang SY, et al. Identification of resistance pathways and therapeutic targets in relapsed multiple myeloma patients through single-cell sequencing. Nat Med. 2021;27(3):491-503.
38. de Jong M, Kellermayer Z, Papazian N, et al. The multiple myeloma microenvironment is defined by an inflammatory stromal cell landscape. Nat Immunol. 2021;22(6):769-780.