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Original Article

Complex karyotype determined using conventional cytogenetic analysis is a poor prognostic factor in patients with multiple myeloma

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High-risk cytogenetic abnormalities (HRCAs) influence the prognosis of multiple myeloma (MM). However, additional cytogenetic aberrations can lead to poor outcomes. This study aimed to clarify whether HRCAs and additional chromosomal abnormalities affect MM prognosis. Patients with newly diagnosed MM who were treated with novel agents were retrospectively evaluated. The primary objective was to assess the difference in progression-free survival (PFS) and overall survival (OS) between patients with/without HRCAs and between patients with/without complex karyotype (CK). The secondary objectives were to identify factors affecting PFS/OS and factors related to CK. HRCAs were defined as del(17p), t(4;14), t(14;16), and gain/amplification(1q) assessed using fluorescence in situ hybridization. CK was defined as ≥3 chromosomal abnormalities on G-banding. Among 110 patients, 40 had HRCAs and 15 had CK. In this study, survival durations between patients with/without HRCAs were similar, while the CK group had significantly poorer PFS/OS than the no-CK group (median PFS: 9 vs. 24 months and median OS: 29 vs. 97 months, respectively), and a poor prognostic impact of CK was maintained in patients with HRCAs. In multivariate analysis, CK was correlated with poor PFS/OS (hazard ratio [HR]: 2.39, 95% confidence interval [95% CI]: 1.22–4.66 and HR: 2.66, 95% CI: 1.10–6.45, respectively). Bone marrow plasma cell (BMPC) ≥60% (odds ratio [OR] = 6.40, 95% CI: 1.50–27.2) and Revised International Staging System III (OR = 7.53, 95% CI: 2.09–27.1) were associated with CK. Our study suggests that CK may contribute to the poor prognosis of MM. Aggressive disease status including high BMPC proliferation could be relevant to CK.

Keywords: additional chromosomal abnormality, complex karyotype, bone marrow plasma cell, high-risk cytogenetic abnormality, multiple myeloma

INTRODUCTION

Multiple myeloma (MM) is the second most common hematological malignancy and is considered to be incurable. With the use of novel agents in recent times, there have been notable changes in the treatment of MM. The use of combination therapy with proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), and/or monoclonal antibodies (mAbs) has substantially improved the prognosis of MM, with median progression-free survival (PFS) of more than 5 years and median overall survival (OS) of more than 7 years, regardless of eligibility for high-dose chemotherapy with autologous stem cell transplantation (HDC/ASCT).[1–5]

High-risk cytogenetic abnormalities (HRCAs) are poor prognostic factors for MM.[6,7] A report of the Revised International Staging System (R-ISS) stated that the simultaneous occurrence of ISS stage III and HRCAs defined as del(17p), t(4;14)(p16;q32) and t(14;16)(q32;q23), determined using fluorescence in situ hybridization (FISH), was a poor prognostic factor.[8] In addition, the existence of chromosome 1q abnormalities (gain or amplification) is considered an adverse cytogenetic abnormality[9–12] and has been included in the recent prognostic model.[13] However, in clinical practice, we sometimes experience disparities in outcomes among patients with these HRCAs.

Metaphase chromosomal abnormalities could influence the different prognoses of patients with MM. An American study demonstrated that two or more additional structural chromosomal changes are indicators of poor outcomes in patients with hyperdiploid MM.[14] Another study indicated

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that monosomies 13, 14, and 22 and deletions of 1p, 12p, 16q, and 17p frequently coexist with hypodiploid changes, leading to a poor survival rate.[15] Furthermore, in patients with MM who have HRCAs, the prognostic influence of additional chromosomal aberrations varies according to the type of chromosomal changes. A report from the Intergroupe Francophone du Myélome (IFM) showed that del(6q) was associated with a poor survival rate, whereas trisomy 15 and monosomy 14 had a protective effect on PFS in patients with del(17p).[16] As various cytogenetic changes could be one of the causes of prognostic heterogeneity in MM, investigating the correlation between additional chromosomal abnormalities and the clinical outcomes of patients with MM can help elucidate the causes of prognostic heterogeneity.

In this retrospective study, we aimed to evaluate whether outcomes differ according to HRCAs and whether other clinical factors, especially the existence of additional chromosomal abnormalities, influence poor outcomes in patients with MM.

MATERIALS AND METHODS

Participants

This retrospective analysis included patients newly diagnosed with MM at our institution between February 2006 and December 2020. MM was diagnosed according to the 2014 International Myeloma Working Group (IMWG) criteria.[17] We calculated the percentage of bone marrow plasma cells (BMPC) using a fresh bone marrow smear specimen at diagnosis. Patients who were diagnosed with smoldering MM, those who failed FISH or G-banding analysis, and those who were not initially treated with novel agents were excluded from the study. The participants' baseline characteristics, including demographic, clinical, and laboratory data, bone marrow surveillance results, and details of treatment and response, were reviewed using electronic medical records. This study was approved by the institutional review board of our institution and conducted in accordance with the principles of the Declaration of Helsinki.

Treatments and responses

Novel agents included PIs, IMiDs, and mAbs. HDC/ASCT was administered if patients were eligible (age <70 years and fit). Consolidation and/or maintenance therapy after induction therapy (mainly in the form of HDC/ASCT) was performed at the discretion of the physician. Treatment response was evaluated according to the IMWG uniform response criteria.[18]

Surveillance and evaluation of cytogenetic abnormalities

HRCAs were confirmed using FISH analysis, and other cytogenetic aberrations were detected using G-banding. All cytogenetic abnormalities were analyzed in fresh bone marrow samples obtained at the time of diagnosis. Two cytogenetic tests were outsourced to SRL, Inc. (Tokyo, Japan), a company that performs clinical laboratory testing. FISH was conducted in accordance with the protocol described by Inazawa *et al.*,[19] and G-banding was performed according to a conventional procedure.[20] For FISH analysis, the collected bone marrow mononuclear cells were treated with a hypotonic solution and fixed in Carnoy's solution, and air-dried slides were prepared. The target DNA in the interphase nuclei on the sample slide and the spectrum-labeled probe DNA were hybridized. We defined HRCAs as del(17p), t(4;14), t(14;16), and gain/amplification(1q). Gain(1q) was defined as three copies of chromosome 1q and amplification(1q) was defined as four or more copies of chromosome 1q. The following region-specific DNA probes were used: Vysis LSI *TP53* SpectrumOrange/*CEP 17* SpectrumGreen Probes for del(17p), Vysis LSI *IGH*/*FGFR3* Dual Color Dual Fusion Probes for t(4;14), Vysis LSI *IGH*/*MAF* Dual Color Dual Fusion Probes for t(14;16), and 1q21 *CKS1B* SpectrumOrange/1p32 *CDKN2C* SpectrumGreen FISH Probe Kit for 1q21 gain/amplification (Abbott Molecular Inc., Tokyo, Japan). A total of 200 interphase nuclei were analyzed to calculate the percentage of each HRCA. The cut-off value was set at 2% for del(17p) and gain/amplification(1q), and 1% for other HRCAs. These cutoff values were determined based on the false-positive threshold, which was set by SRL, Inc., based on the 95% confidence interval (CI) of the positivity rate using peripheral blood samples from 20 healthy individuals. For G-banding, the collected samples were added to phytohemagglutinin additive-free culture fluid, which was incubated for 24–48 h at 37°C and 5% CO_2, and air-dried slides were prepared. After G-banding, 20 metaphase nuclei were analyzed. A clone was defined as having chromosomal changes in two or more cells, and all numerical or structural abnormalities were evaluated according to the International System for Cytogenetic Nomenclature.[21] In this study, we defined a complex karyotype (CK) as a chromosomal aberration with three or more chromosomal abnormalities determined by G-banding, as frequently defined in hematological malignancies.[22]

Endpoints and statistical methods

The primary objective of our study was to determine differences in PFS and OS between patients with and without HRCAs, as well as differences in survival outcomes between patients with and without CK. The secondary objectives were to determine the factors affecting PFS and OS, as well as clinical parameters related to CK. OS was defined as the time from diagnosis to death or last follow-up. PFS was calculated as the time from the initiation of the first-line treatment to relapse, progression, or death from any cause. Nominal or continuous variables were compared using Fisher's exact test, Student's t-test, or the Mann–Whitney U test. PFS and OS were calculated using the Kaplan–Meier method, and differences between the two groups were examined using the log-rank test. Factors affecting PFS and OS were evaluated using the Cox proportional hazards model and those relevant to CK were identified using logistic regression analysis. All statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University,

Japan),[23] a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). *P* values of <0.05 were considered statistically significant.

RESULTS

Patient characteristics and cytogenetic findings

Among 126 patients with newly diagnosed MM, 16 patients who failed FISH or G-banding analysis were excluded. Thus, 110 patients were included in the study. Patient characteristics and cytogenetic findings are summarized in Table 1. The median age was 67 years (range, 32–85 years), and 61 patients (55%) were aged ≥65 years. Sixty-six patients (60%) were male. Ninety-one patients (83%) had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, 22 (20%) had elevated lactate dehydrogenase (LDH) levels, 35 (32%) had plasmacytoma, and 17 (16%) had R-ISS III. Forty patients (36%) had at least one HRCA: 17 had t(4;14), 15 had del(17p), 2 had gain(1q), 1 had amplification(1q), 1 had t(14;16), and 4 had more than one HRCA, which included 3 patients with t(4;14) and amplification(1q) and 1 patient with t(4;14), del(17p), and gain(1q). Twenty-six patients (24%) had at least one chromosomal abnormality on G-banding and 15 (14%) had CK. The patient characteristics of cytogenetic abnormalities are shown in Table 2. Patients with HRCAs tended to be male, low albumin levels (<3.5 g/dL), high β2-microglobulin levels (>5.5 mg/dL), low hemoglobin levels (<10 g/dL), and R-ISS III. Regarding characteristics between the CK and no-CK groups, all background characteristics were not significantly different, except for percentages of ISS III (47% vs. 19%, *P* = 0.040) and R-ISS III (47% vs. 11%, *P* = 0.0019).

Table 1. Baseline characteristics of the participants

Characteristics	N = 110
Age (median, years)	67 (range 32–85)
≥65 years	61 (55%)
Sex	
Male	66 (60%)
Female	44 (40%)
Isotype	
IgG	61 (55%)
BJP	24 (22%)
IgA	23 (21%)
Non-secretary	2 (2%)
ECOG PS	
0,1	91 (83%)
≥2	19 (17%)
LDH	
≤ULN	88 (80%)
>ULN	22 (20%)
Plasmacytoma	35 (32%)
Paraosseous	27 (77%)
Extraosseous	8 (23%)
R-ISS	
I	30 (27%)
II	63 (57%)
III	17 (16%)
BMPCs (median, %)	21.5 (range 1.0–86.6)
HRCAs (FISH)	40 (36%)
t(4;14)	17 (43%)
del(17p)	15 (37%)
gain/amplification(1q)	3 (8%)
t(14;16)	1 (2%)
double†	3 (8%)
triple‡	1 (2%)
Karyotype (G-banding)	
Normal	84 (76%)
Hypodiploid	16 (15%)
Hyperdiploid	10 (9%)
CK on G-banding	15 (14%)

BJP, bence jones protein; BMPCs, bone marrow plasma cells; CK, complex karyotype; ECOG PS, Eastern Cooperative Oncology Group performance status; FISH, fluorescence in situ hybridization; HRCAs, high-risk cytogenetic abnormalities; IgA, immunoglobulin A; IgG, immunoglobulin G; LDH, lactate dehydrogenase; R-ISS, Revised International Staging System; ULN, upper limit normal.
† Three cases of t(4;14) and amplification(1q).
‡ One case of t(4;14), del(17p), and gain(1q).

Treatments and responses

The details of the first-line treatments and responses are presented in Supplemental Table S1. Eighty-eight patients (80%) received triple or quadruple regimens. Thirty-three patients (30%) received HDC/ASCT and 31 (28%) received consolidation and/or maintenance therapy. Regarding the best response to treatment, 53 patients (48%) achieved very good partial response (VGPR) or better. Supplemental Table S2 compares the initial treatments and responses according to the presence or absence of HRCAs and CK. There were no significant differences in either the treatment regimen or best response between patients with and without HRCAs. Regarding differences between the CK and no-CK groups, a slightly higher proportion of patients without CK received triple/quadruple regimens than those with CK (83% vs. 60%, *P* = 0.075). Additionally, VGPR or better response rate was significantly lower in the CK group than in the no-CK group (20% vs. 53%, *P* = 0.025). The percentage of patients who underwent HDC/ASCT was slightly higher in the no-CK group than in the CK group (32% vs. 20%, *P* = 0.55).

Survival outcomes

As of December 2021, the median follow-up time was 43 months (range: 1–124 months). Of the 110 patients, 76 experienced relapse/progression after the initial treatment and 37 died. No significant differences in either PFS or OS were observed between patients with and without HRCAs (median PFS of 21 vs. 21 months, *P* = 0.88, or the median OS of not reached vs. 91 months, *P* = 0.26) (Figures 1A and 1B). Differences in survival were also absent between HRCA groups (Figures 1C and 1D). In contrast, significantly shorter PFS and OS were observed in the CK group than in the no-CK group (median PFS of 9 vs. 24 months, *P* =

Table 2. Comparison of baseline characteristics by cytogenetic abnormalities

	HRCA (n = 40)		no-HRCA (n = 70)		*P* value†	CK (n = 15)		no-CK (n = 95)		*P* value†
Characteristics	n	%	n	%		n	%	n	%	
Age ≥65 years	26	65	35	50	0.16	8	53	53	56	1.00
Male	29	73	37	53	**0.047**	10	67	56	59	0.78
ECOG PS ≥2	10	25	9	13	0.12	4	27	15	16	0.29
Alb <3.5 g/dL	23	58	19	27	**0.0022**	8	53	34	36	0.25
β2MG >5.5 mg/dL	12	30	11	16	0.091	6	40	17	18	0.081
LDH >ULN	10	25	12	17	0.33	6	40	16	17	0.075
Hb <10 g/dL	21	53	18	26	**0.0069**	8	53	31	33	0.15
Plasmacytoma	9	23	26	37	0.14	6	40	29	31	0.55
HRCA						8	53	32	34	0.16
ISS III	13	33	12	17	0.10	7	47	18	19	**0.040**
R-ISS III	13	33	4	6	**<0.001**	7	47	10	11	**0.0019**

Alb, albumin; CK, complex karyotype; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; HRCA, high-risk cytogenetic abnormality; ISS, international staging system; LDH, lactate dehydrogenase; R-ISS, Revised International Staging System; ULN, upper limit normal; β2MG, β2 microglobulin.
† Fisher's exact test.
Bold numbers indicate statistical significance.

0.0032; median OS of 29 vs. 97 months, $P < 0.001$) (Figures 2A and 2B). The CK group also showed inferior survival to the no-CK group in patients with HRCAs (median PFS of 9.5 vs. 25 months, $P = 0.019$, and median OS of 25 months vs. not reached, $P = 0.011$, respectively) and in patients without HRCAs (median PFS of 7 vs. 23 months, $P = 0.059$, and median OS of 53 vs. 91 months, $P = 0.047$, respectively) (Figures 2C–2F). We further examined the correlation between survival differences in CK and hyperdiploid/hypodiploid abnormalities. Although there was no significant difference between the CK and hyper/hypodiploid groups, patients with concurrent CK and hypodiploid tended to have the worst PFS and OS (median PFS of 2 months and median OS of 17 months, respectively) (Figures 3A–3D). The results of the Cox regression analysis, shown in Table 3, demonstrated that the CK group had a significantly increased risk of relapse or progression in both univariate (hazard ratio [HR] = 2.39, 95% CI: 1.30–4.39, $P = 0.0048$) and multivariate (HR = 2.39, 95% CI: 1.22–4.66, $P = 0.011$) analyses. Additionally, CK was found to have a negative impact on OS in multivariate analysis (HR = 2.66, 95% CI: 1.10–6.45, $P = 0.030$).

Variables correlated with CK

Patients with CK had a higher percentage of BMPCs (median 43% vs. 20%, $P = 0.0015$), higher LDH levels (median 213 IU/L vs. 178 IU/L, $P = 0.026$), and lower hemoglobin levels (mean 9.6 g/dL vs. 11.0 g/dL, $P = 0.030$) than patients without CK (Figures 4A–4C). Furthermore, CK was significantly correlated with BMPCs ≥60% (odds ratio [OR] = 6.40, 95% CI: 1.50–27.2, $P = 0.012$) and R-ISS III (OR = 7.53, 95% CI: 2.09–27.1, $P = 0.0020$) in the multivariate analysis (Table 4). The characteristics, karyotypic patterns, and outcomes of patients with CK are summarized in Supplemental Table S3. Seven patients had R-ISS III and eight had HRCAs, which included four patients with t(4;14), two with del(17p), one with t(14;16), and one with amplification(1q). As for the details of karyotypes, there was variation in terms of both numerical and structural abnormalities. The most frequent abnormal component including CK was monosomy 13 (60%), followed by trisomy 3 (53%) and trisomy 7 (53%). Notably, four patients had 8q24-related abnormalities (cases 1, 6, 11, and 14); three of them did not have HRCAs, and all three died.

DISCUSSION

This retrospective study has two key findings. First, CK was independently correlated with poor PFS and OS, and concurrent CK influenced poor outcomes in patients with HRCAs. Second, CK was associated with R-ISS III and a high percentage of BMPCs, which may reflect aggressive disease status, leading to poor outcomes. Our findings suggest that metaphase cytogenetic complexity is a key cause of poor prognosis in patients with MM.

Several retrospective studies have demonstrated the poor prognostic impact of CK in MM. A multicenter study from France demonstrated that patients with MM who had CK (n = 116) had inferior OS than patients with a normal karyotype (n = 43) (median OS 22.7 vs. 45.2 months).[24] Reports exist of poor prognosis in patients with MM who have HRCAs due to the coexistence of CK.[25–27] Concerning the complexity of chromosomal changes, a Korean study reported that more than six structural abnormalities had the strongest effect on poor prognosis.[28] In our study, although the number of patients with CK was small, each patient in the CK group showed various structural changes, which may have contributed to their dismal outcomes. Additionally, the poor outcomes in the CK group could have been partially influenced by the treatment patterns. The proportion of patients who underwent HDC/ASCT was slightly lower in the CK group than in the no-CK group, probably because of the lower

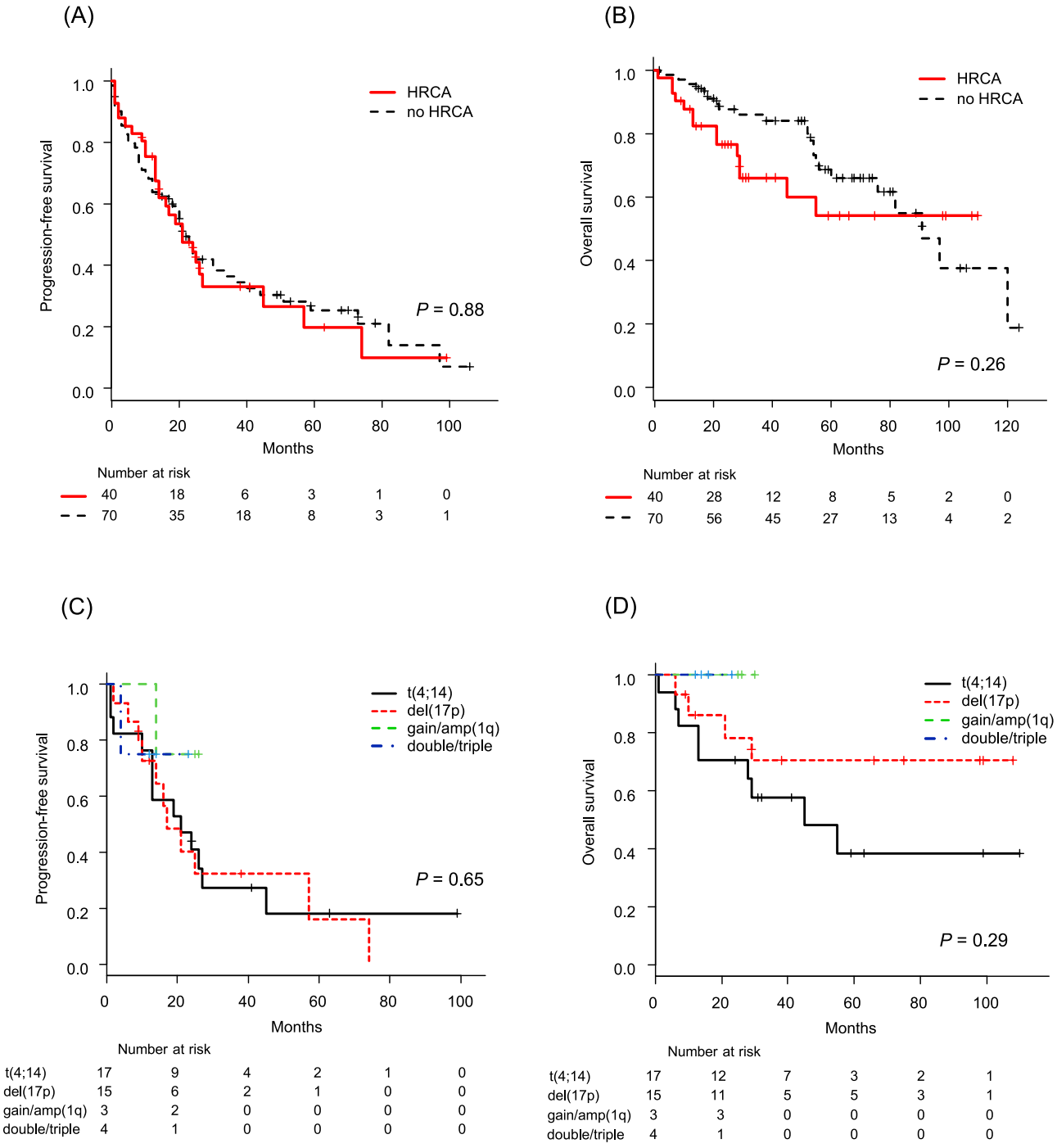


Fig. 1. Kaplan–Meier curves of patients with multiple myeloma with and without HRCAs: (***A***) progression-free survival and (***B***) overall survival. Comparison of survival curves among patients with each HRCA: (***C***) progression-free survival and (***D***) overall survival. amp, amplification; HRCA, high-risk cytogenetic abnormality

response rate to initial therapy in the CK group.

In the present study, BMPCs ≥60% was strongly associated with CK. The correlation between CK and a high percentage of BMPCs is noteworthy when considering the causes of poor outcomes in patients with CK. Patients with smoldering MM who have BMPCs ≥60% have a very high risk of early progression to symptomatic MM.[29] A connection between BMPC ≥60% and poor prognosis has also been observed in patients with symptomatic MM, regardless of the presence of HRCAs.[30–32] Moreover, several studies have revealed that metaphase cytogenetic abnormalities are related to a high plasma cell proliferation index, which has been reported to indicate aggressive disease status.[33,34] Taken together, we assume that the rapid growth of neoplastic plasma cells is due to metaphase cytogenetic complexity, resulting in adverse clinical outcomes.

This study did not show differences in survival according to the presence of HRCAs, although adverse prognostic fac-

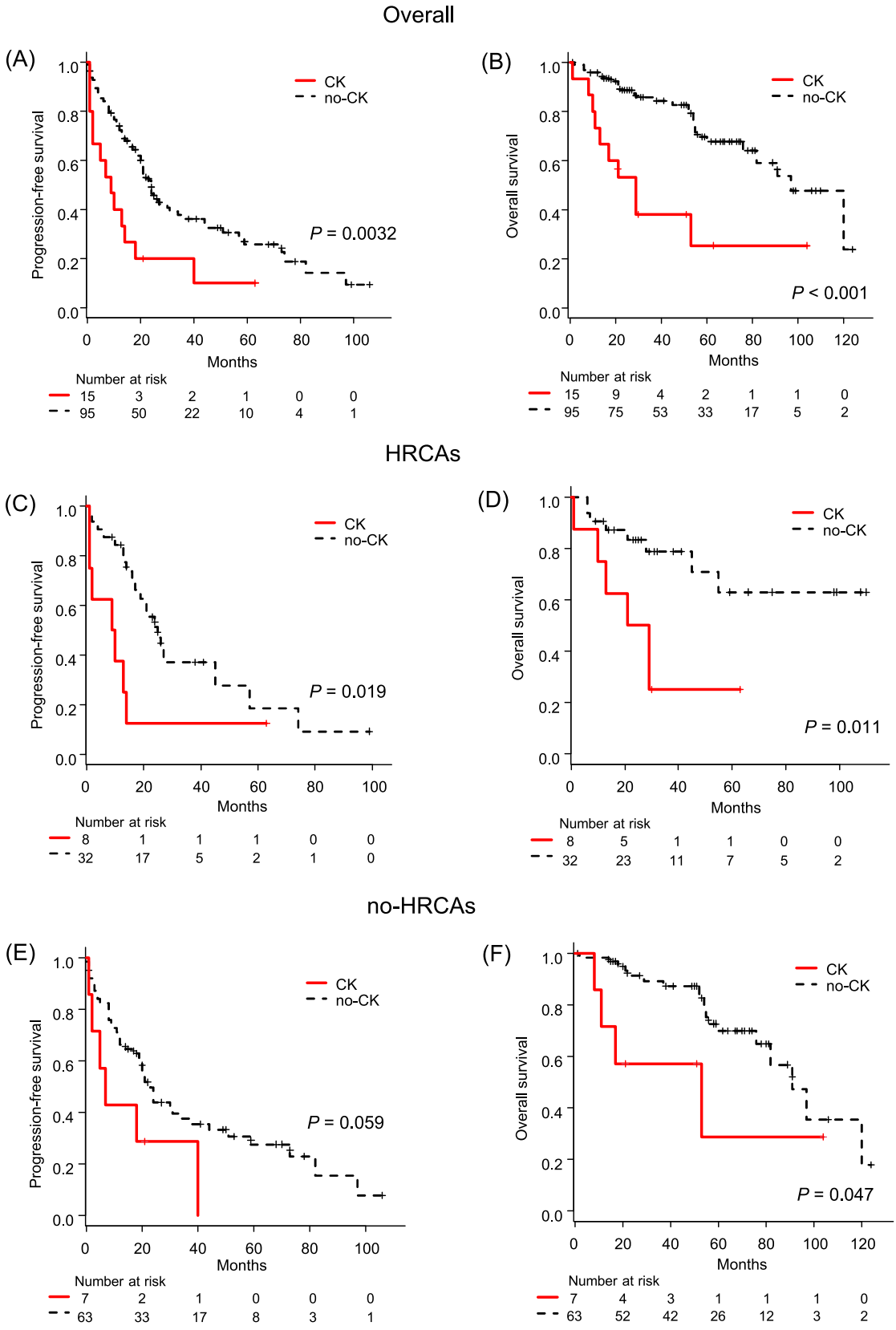


Fig. 2. Differences in PFS and OS between the CK and no-CK groups in patients with multiple myeloma: (***A***) PFS and (***B***) OS in all patients, (***C***) PFS and (***D***) OS in patients with HRCAs, and (***E***) PFS and (***F***) OS in patients with no-HRCAs.
CK, complex karyotype; HRCAs, high-risk cytogenetic abnormalities; OS, overall survival; PFS, progression-free survival

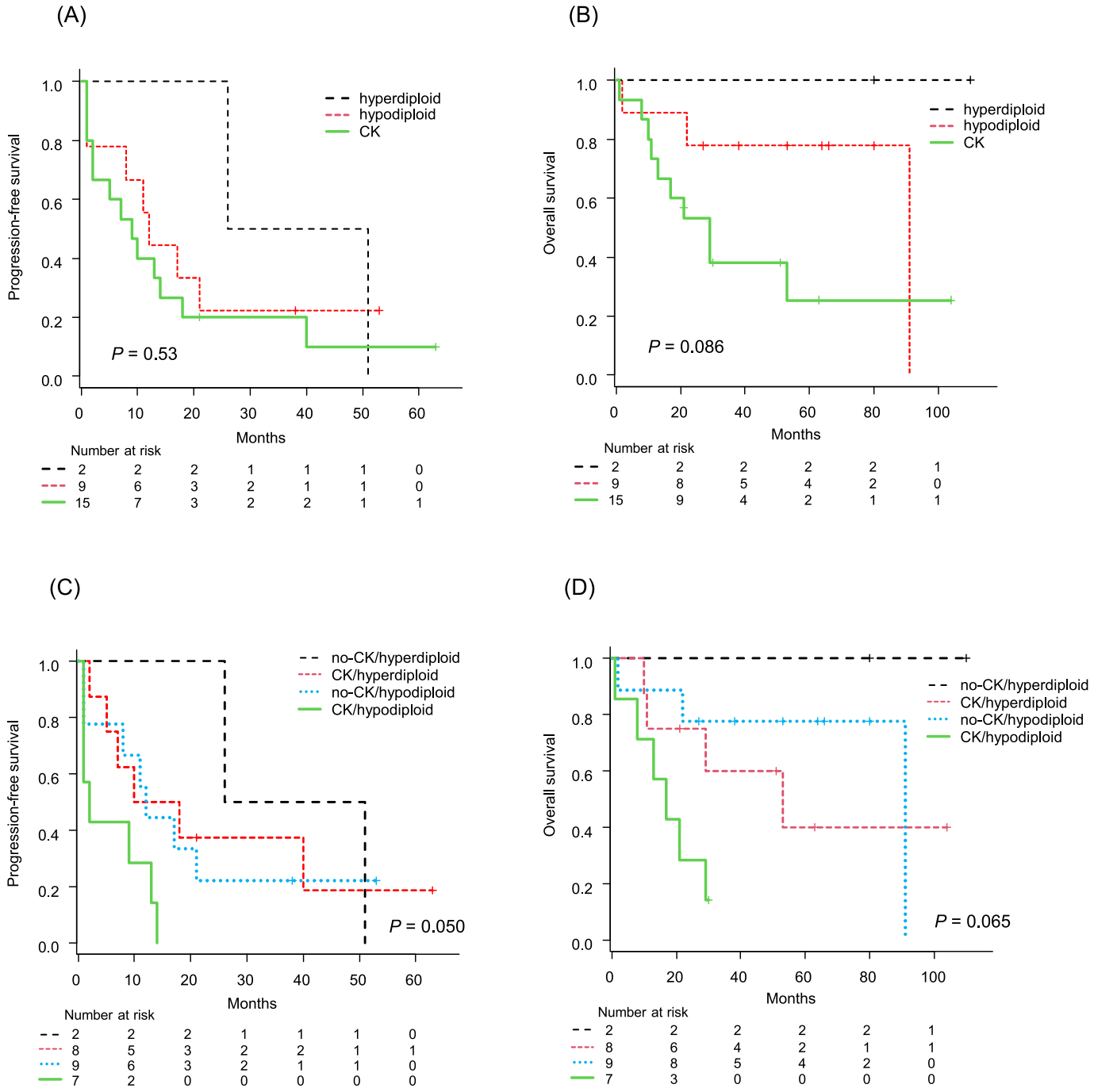


Fig. 3. Comparison of survival differences according to CK and hyperdiploid/hypodiploid abnormalities: (***A***) PFS and (***B***) OS between patients with hyperdiploid, hypodiploid, and CK; (***C***) PFS and (***D***) OS between the four subgroups: no-CK/hyperdiploid, CK/hyperdiploid, no-CK/hypodiploid, and CK/hypodiploid.
CK, complex karyotype; OS, overall survival; PFS, progression-free survival

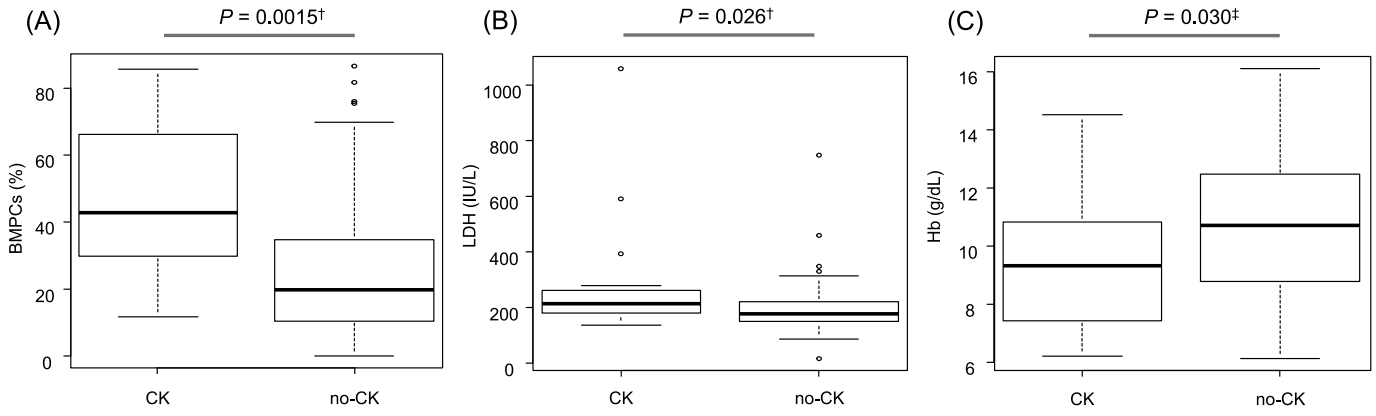
tors, such as hypoalbuminemia and high β2-microglobulin levels, were relatively frequent in patients with HRCAs. In our cohort, the median PFS in the no-HRCA group was 21 months, which was comparable to the median PFS in a group of patients with R-ISS III in a previous study.[8] Although the lack of a prognostic impact of HRCAs may be due to bias introduced by characteristics other than those investigated, our finding of poor PFS in the no-HRCA group suggests that the presence of HRCA is not the sole contributor to a poor outcome. In our study, 40% of patients in the no-HRCA group experienced relapse or progression within 18 months. A study by the IFM demonstrated that approximately two-thirds of patients who experienced early progression (≤18 months) did not have HRCAs.[35]

The results of this study did not indicate a correlation between CK and HRCAs, although the CK group had a poor prognosis. Surprisingly, none of the patients with CK had two or more high-risk FISH abnormalities, which have been

Table 3. Cox regression analysis of progression-free survival and overall survival

	PFS						OS					
	Univariate			Multivariate			Univariate			Multivariate		
Factors	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Age ≥65 years	1.14	0.72, 1.80	0.57	0.60	0.33, 1.09	0.096	1.87	0.94, 3.71	0.074	0.73	0.32, 1.69	0.47
Male	1.06	0.66, 1.70	0.80				1.71	0.84, 3.49	0.14			
ECOG PS ≥2	1.10	0.59, 2.06	0.75				1.58	0.69, 3.62	0.28			
Alb <3.5 g/dL	1.32	0.83, 2.09	0.24				2.40	1.24, 4.63	**0.0090**			
β2MG >5.5 mg/dL	1.22	0.71, 2.11	0.46				1.89	0.91, 3.94	0.089			
LDH >ULN	1.30	0.73, 2.29	0.37	0.86	0.46, 1.63	0.64	2.37	1.18, 4.77	**0.015**	1.06	0.48, 2.35	0.88
Hb <10 g/dL	1.70	1.06, 2.71	**0.027**	1.37	0.78, 2.39	0.27	2.98	1.52, 5.83	**0.0015**	1.71	0.75, 3.90	0.20
Plasmacytoma	1.02	0.63, 1.64	0.93				0.91	0.45, 1.81	0.78			
R-ISS III	1.80	0.99, 3.30	0.056	1.41	0.70, 2.85	0.34	6.40	2.97, 13.8	**<0.001**	3.47	1.29, 9.36	**0.014**
HRCA	1.04	0.65, 1.66	0.88	0.91	0.53, 1.56	0.74	1.47	0.74, 2.89	0.27	0.73	0.33, 1.65	0.46
CK	2.39	1.30, 4.39	**0.0048**	2.39	1.22, 4.66	**0.011**	3.88	1.85, 8.13	**<0.001**	2.66	1.10, 6.45	**0.030**
ASCT	0.54	0.32, 0.91	**0.022**	0.41	0.21, 0.81	**0.010**	0.28	0.11, 0.71	**0.0074**	0.28	0.09, 0.86	**0.026**

Alb, albumin; ASCT, autologous stem cell transplantation; CI, confidence interval; CK, complex karyotype; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; HR, hazard ratio; HRCA, high-risk cytogenetic abnormality; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival; R-ISS, revised-international staging system; ULN, upper limit normal; β2MG, β2 microglobulin.
Bold numbers indicate statistical significance.



† Mann–Whitney U test.
‡ Student's t-test.

Fig. 4. Clinical parameters relevant to the presence of CK: (***A***) the percentage of BMPCs; (***B***) LDH level; and (***C***) Hb level.
BMPCs, bone marrow plasma cells; CK, complex karyotype; Hb, hemoglobin; LDH, lactate dehydrogenase

reported to be an extremely poor prognostic factor in patients with MM.[36] Recently, a combination of high-risk chromosomal changes and adverse genetic abnormalities, such as biallelic inactivation of *TP53*, was shown to be a key cause of aggressive disease status, leading to early relapse or progression and poor OS.[37–39] The connection between CK and specific genetic statuses remains unclear, and further studies are warranted.

The high prevalence of monosomy 13 and the existence of *MYC*-related changes in the composition of CK might be the reasons for the poor prognosis of patients with CK. Historically, chromosome 13 abnormalities have been observed in approximately 50% of patients with MM, and have been considered poor prognostic factors,[40,41] although these abnormalities have not been included in recent prognostic models.[8,13] Meanwhile, several studies have reported that the presence of chromosome 13 abnormalities, detected as metaphase cytogenetic abnormalities, has a poor prognostic impact on MM.[42–44] *MYC* abnormalities, recognized as late progression events, are reportedly found in approximately 15% of patients with MM,[45] and *MYC* rearrangement has been shown to be an independent poor prognostic factor.[46–48] Due to the small number of patients with CK, further investigation is required to elucidate the relationship between CK and these chromosomal abnormalities.

This study had several limitations. Owing to the retrospective single-institution study, the sample size (especially the number of patients with CK) was small, and inconsistency of treatments, including HDC/ASCT, may have affected the survival outcomes. Regarding FISH surveillance, an out-

Table 4. Factors correlated with complex karyotype

	Univariate†			Multivariate‡		
Factors	OR	95% CI	*P* value	OR	95% CI	*P* value
Age ≥65 years	0.91	0.26, 3.20	1.00			
ECOG PS ≥2	1.93	0.39, 7.70	0.29			
Alb <3.5 g/dL	2.04	0.59, 7.23	0.25			
β2MG >5.5 mg/dL	3.02	0.77, 11.1	0.081			
LDH >ULN	3.25	0.83, 12.0	0.075			
Hb <10 g/dL	2.34	0.67, 8.35	0.15			
Plasmacytoma	1.51	0.40, 5.28	0.55			
HRCA	2.23	0.64, 7.95	0.16			
R-ISS III	7.23	1.83, 29.0	**0.0019**	7.53	2.09, 27.1	**0.0020**
BMPCs ≥60%	6.12	1.29, 27.8	**0.011**	6.40	1.50, 27.2	**0.012**

Alb, albumin; BMPCs, bone marrow plasma cells; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; HRCA, high-risk cytogenetic abnormality; LDH, lactate dehydrogenase; OR, odds ratio; R-ISS, Revised International Staging System; ULN, upper limit normal; β2MG, β2 microglobulin.
† Fisher's exact test.
‡ Logistic regression analysis.
Bold numbers indicate statistical significance.

sourced method (not in-house) restricts FISH surveillance depending on its availability. As surveillance of 1q gain/amplification has only been possible since February 2016, only 22 patients were surveyed for the presence of 1q abnormalities in our cohort. Furthermore, the low detectability of metaphase cytogenetic abnormalities using G-banding may limit the significance of chromosomal changes in patients with MM.

In summary, the results of this study indicate that coexisting CK could be associated with poor survival outcomes in patients with MM. The prevalence of R-ISS III was high among patients with CK, and BMPCs ≥60% was associated with CK, which may be linked with aggressive plasma cell proliferation, leading to poor outcomes. Further research in a larger population with a longer follow-up period is required to confirm the clinical importance of CK in MM. Moreover, the correlations among the presence of CK, specific chromosomal changes, and genetic mutations require further clarification.

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AUTHOR CONTRIBUTIONS

H.U. conceived of and designed the study. All authors contributed significantly to the diagnosis and treatment of patients, data collection, and analyses. The first draft of the manuscript was written by H.U. and D.M. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

H.U. received honoraria from Janssen and Ono Pharmaceuticals. Y.M. received research fees from Bristol-Myers Squibb. Y.S. received honoraria from Janssen and Sanofi. M.H. received honoraria from Celgene and Bristol-Myers Squibb. D.M. has received grants from Amgen Astellas Biopharma, Kyowa Kirin, Chugai, Takeda, Sanofi, Bristol-Myers Squibb, Eisai, Taiho, Celgene, Novartis, Ono, Janssen, Otsuka, Astellas, AbbVie, MSD, and has received honoraria from Ono, Nippon Shinyaku, Mundipharma, Chugai, MSD, Sanofi, Takeda, Bristol-Myers Squibb, Celgene, Janssen, Eisai, Kyowa Kirin, Zenyaku, SymBio, AbbVie, AstraZeneca. The remaining authors declare that they have no conflicts of interest.

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