ORIGINAL ARTICLE

The impact of cytogenetic evolution and acquisition of del(17p) on the prognosis of patients with multiple myeloma

Aleksander Salomon-Perzyński[1], Aleksandra Bluszcz[2], Agnieszka Krzywdzińska[3], Zofia Spyra-Górny[4], Natalia Jakacka[1], Joanna Barankiewicz[1], Katarzyna Borg[2], Iwona Solarska[5], Tomasz Szpila[1], Bartosz Puła[1], Sebastian Grosicki[4], Krzysztof Jamroziak[1]

1 Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland
2 Cytogenetic Laboratory, Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland
3 Immunophenotyping Laboratory, Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland
4 Department of Hematology and Cancer Prevention, Faculty od Health Sciences, Medical University of Silesia in Katowice, Katowice, Poland
5 Molecular Biology Laboratory, Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

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EDITORIAL

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ABSTRACT

INTRODUCTION Prognosis of patients with newly diagnosed multiple myeloma (MM), a third most common hematological cancer, is dependent on baseline cytogenetics. However, little is known about the prognostic significance of cytogenetic evolution (CE) at the time between the diagnosis and relapse of MM.

OBJECTIVES Here, we retrospectively analyzed the prognostic impact of CE detected in a routine interphase fluorescence in situ hybridization (FISH) test in a cohort of patients with MM.

PATIENTS AND METHODS Among 650 patients evaluated with the FISH MM panel at our center between 2014 and 2019, we identified 29 individuals with MM who had been tested twice, at the time of diagnosis and relapse. Cytogenetic evolution was defined as the acquisition or loss of at least 1 cytogenetic abnormality at relapse (FISH2) compared with the baseline test result (FISH1).

RESULTS Cytogenetic evolution was seen in 14 patients (48%), whereas 15 had stable cytogenetics. Acquired chromosome 17p deletion (del[17p]) was the most common type of CE, found in 7 patients (24%). In univariable analysis, stable cytogenetics predicted longer overall survival (median not reached vs 3.8 years; hazard ratio [HR], 0.15; $P = 0.04$; median follow-up of 3.1 years) and longer overall survival after FISH2 (median not reached vs 0.8 years; HR, 0.13; $P = 0.002$; median follow-up of 0.6 years). In multivariable analysis, acquired del(17p) predicted shorter progression-free survival and the overall survival after FISH2 (HR, 9.3 and 18.8; $P = 0.005$ and $P = 0.004$, respectively).

CONCLUSIONS Presence of CE and, particularly, the acquisition of new del(17p) at relapse, negatively affect the outcome of MM. Therefore, re-evaluation of FISH at MM relapse should be included in routine clinical practice.

Correspondence to:
Aleksander Salomon-Perzyński, MD, Department of Hematology, Institute of Hematology and Transfusion Medicine, ul. Indiry Gandhi 14, 02-776 Warszawa, Poland, phone: +48 22 349 63 21, email: salomon.perzynski@gmail.com
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INTRODUCTION Multiple myeloma (MM) is the third most common hematologic malignancy in the European Union, with approximately 33 000 new cases and 20 000 deaths annually.[1] The introduction of novel agents, such as proteasome inhibitors (PIs),[2-4] immunomodulatory drugs (IMiDs),[5-7] and anti-CD38 monoclonal antibodies[8-10] along with high-dose melphalan followed by autologous hematopoietic stem cell transplant (auto-HSCT)[11] have significantly improved the prognosis of patients with MM. However, most cases of MM are still incurable.

Recent studies using next-generation sequencing (NGS) approaches have provided evidence that MM is characterized by spatial and temporal genetic heterogeneity and is composed of multiple populations of genetically distinct subclones, which evolve over time following a pattern

WHAT'S NEW?

Prognosis of patients with newly diagnosed multiple myeloma (MM) depends on baseline cytogenetics. However, relatively little is known about the occurrence and prognostic significance of cytogenetic evolution at the time between the diagnosis and progression of MM. In this article, we showed that cytogenetic evolution and, in particular, the acquisition of deletion of chromosome 17p (del[17p]) detected in routine fluorescence in situ hybridization (FISH) testing negatively affects the outcomes of patients with relapsed and/or refractory MM. Patients with acquired del(17p) have a particularly poor prognosis. Therefore, our results indicate that FISH re-evaluation at each subsequent MM relapse should be included in routine clinical practice.

of branched, linear, or stable evolution.[12-17] In this context, the clonal selection of treatment-resistant subclones during anti-myeloma therapy is now recognized as the main cause of treatment failure.[12,13,15] Nevertheless, despite the great potential, tracking the clonal evolution of MM using NGS has not been applicable in routine clinical practice yet, and cytogenetic testing by interphase fluorescence in situ hybridization (FISH) remains the most commonly used method for determining the genetic features of MM both at the time of diagnosis and at subsequent relapses.[18]

The prognostic significance of baseline cytogenetic abnormalities (CAs) in newly diagnosed MM is well documented.[19,20] The presence of the defined panel of CAs, ie, the deletion of the short arm of chromosome 17 (del[17p]) and/or translocations involving the *IGH* locus, namely, t(4;14) and t(14;16), is one of the criteria defining high-risk disease according to the Revised International Staging System (R-ISS).[21] The group of patients bearing high-risk CAs is characterized by 5-year overall survival (OS) and 5-year progression-free survival (PFS) rates of only 40% and 24%, respectively.[21] However, the evolution of these high-risk CAs during the treatment and its potential prognostic consequences remain unclear.

In this article, we assessed the impact of cytogenetic evolution (CE) detected in a routine FISH test on the prognosis of patients with relapsed or refractory MM (RRMM).

PATIENTS AND METHODS

Study population We collected clinical and laboratory data from 650 patients who underwent baseline FISH with MM panel probes because of suspected or diagnosed MM at the Institute of Hematology and Transfusion Medicine in Warsaw (Poland) between the years 2014 and 2019. Out of this group, we selected 177 individuals with the confirmed diagnosis of MM and full clinical data available. A subpopulation of this group including 29 patients with MM who had FISH testing conducted twice during the course of the disease were involved in this retrospective analysis. The first FISH test (FISH1) needed to be performed at the time of the diagnosis of MM, and the second FISH test (FISH2) could be evaluated during any of the subsequent relapses.

The study was approved by the Ethics Committee of the Institute of Hematology and Transfusion Medicine in Warsaw (Poland) and conducted in accordance with the provisions of the 1964 Declaration of Helsinki with amendments and the International Conference on Harmonization Guidelines for Good Clinical Practice. Written informed consent to the use of their clinical and cytogenetic data was given by all study participants.

Cytogenetic evaluation The FISH panel for MM routinely used at the Institute of Hematology and Transfusion Medicine in Warsaw (Poland) consisted of the following probes: 11q22.3 (*ATM* gene), 17p13.1 (*TP53* gene), 14q32 (*IGH* gene), *FGFR3*/*IGH* t(4;14) and *IGH*/*MAF* t(14;16).[22]

Plasma cells were magnetically isolated from the bone marrow aspirates with CD138 MicroBeads (Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II, Stemcell Technologies, Vancouver, British Columbia, Canada) and evaluated with the FISH probes. A sequential FISH testing strategy was used, that is, the evaluation for t(4;14) and t(14;16) was only performed when the *IGH* rearrangement was present in the absence of the *TP53* deletion. The cutoff values established in the local laboratory for a positive FISH test results were 7% for the 17p13.1 (*TP53* gene) deletion, 4% for the 11q22.3 (*ATM* gene) deletion, and 8% for any translocation involving the 14q32 (*IGH* gene) locus.

For the purpose of this analysis, we defined the presence of CE as: 1) the acquisition of at least 1 new CA in the FISH2 test compared with the FISH1 test results (ie, a new additional copy or copies of the *TP53*, *ATM*, or *IGH* genes, new deletion of the *IGH*, *MAF*, or *TP53* genes) or 2) loss of at least 1 CA in the FISH2 test that was present in the FISH1 test results (ie, loss of an additional copy or copies of the *IGH* gene or loss of an additional copy of the *FGFR3* gene).

Definitions Patients' responses to treatment were defined according to the 2016 Revised International Myeloma Working Group Criteria.[23] Refractory disease was defined as lack of any response during the treatment or disease progression during or within 60 days after the cessation of the therapy.

The study follow-up was defined as the time from the date of diagnosis of MM to death of any cause or to the date of the last follow-up visit with the cutoff date of September 6, 2019. The follow-up after FISH2 evaluation was defined as the time from the date of the FISH2 test to death of any cause or to the date of the last follow-up visit with the cutoff date of September 6, 2019. Progression-free survival was defined as the time between the initiation of the therapy received immediately after the FISH2 evaluation and disease progression or death. The patients who did not receive antimyeloma treatment after the FISH2 evaluation were excluded from the PFS analysis. Overall survival 1 (OS1)

was defined as the time from the diagnosis of MM to death of any cause, and overall survival 2 (OS2) as the time from the FISH2 evaluation to death of any cause.

Statistical analysis The Fisher exact test was used to assess the difference in the distribution of categorical variables between the study groups. Continuous variables were compared between the groups using the nonparametric Mann–Whitney test. The values of PFS, OS1, and OS2 were estimated by the Kaplan–Meier method. The effect of prognostic factors on time-dependent variables, OS1, OS2, and PFS, was assessed with the log-rank test. The Cox proportional hazards model was used to identify factors affecting PFS and OS2 at the time of the FISH2 evaluation. Statistical analysis was performed using the MedCalc for Windows, version 19.1.3 (MedCalc Software, Ostend, Belgium), and GraphPad Prism, version 8.3 (GraphPad Software, Inc., San Diego, California, United States), software. For all statistical tests, a *P* value less than 0.05 was considered significant.

RESULTS

Patients' baseline characteristics and cytogenetic evaluation The mean time from the diagnosis of MM to the FISH2 evaluation was 2.2 years (95% CI, 1.8–2.6 years). In 19 patients (66%), the FISH2 test was evaluated at the first clinical (n = 17) or biochemical relapse (n = 2). In the remaining cases, the FISH2 test was performed at the second (n = 6 [21%]), third (n = 3 [10%]), or fourth (n = 1 [3%]) relapse. Detailed clinical characteristics of the study patients are shown in TABLE 1.

In all patients with MM, whose clinical data were available and who had the FISH test performed at the time of diagnosis (n = 177), high-risk CAs, such as t(4;14), t(14;16), and del(17p), were found in 18 (10%), 4 (2%), and 12 (7%) patients, respectively. In turn, in the group tested twice in the course of the disease, each of these abnormalities was found in a single patient (3.5%). Cytogenetic evolution between the FISH1 and FISH2 tests (eFISH) was seen in 14 patients (48%), whereas 15 patients (52%) had stable cytogenetics (sFISH) over time. New del(17p) was acquired by 7 patients: as a new isolated cytogenetic alteration in 2 individuals and as a part of complex cytogenetic changes in 5 individuals. The median (range) percentage of plasma cells with acquired del(17p) was 82% (13%–98%). No other acquired high-risk CAs, such as t(4;14) and t(14;16), were found. The overall spectrum of cytogenetic changes detected in FISH2 tests is shown in TABLE 2.

Patients with eFISH and sFISH were similar with respect to baseline clinical (age, ISS [1 to 2 vs 3] and R-ISS [1 to 2 vs 3] stage, Eastern Cooperative Oncology Group [ECOG] performance status [0 to 1 vs 2 to 3], percentage of bone marrow plasma cells, and the presence of extramedullary disease and osteolytic lesions) and laboratory investigations (hemoglobin, lactate dehydrogenase [LDH], creatinine, beta-2 microglobulin, and albumin levels; platelet, neutrophil, monocyte, and lymphocyte counts).

Patients received a median (range) number of 3 (2–6) lines of antimyeloma therapy. Importantly, all but 1 patient received PIs-based treatment combination (97%), and 26 patients (90%) were treated with IMiDs. Daratumumab as a single agent or in combination with PIs and/or IMiDs was used in 6 patients (21%). Almost half of the study patients received auto-HSCT (n = 14 [48%]). Between the diagnosis and the FISH2 test, patients with eFISH received significantly more treatment lines (median [range], 2 [1–4]) compared with those with sFISH (median [range], 1 [1–3]) (*P* = 0.04). Before the FISH2 evaluation, 11 (38%) and 12 (41%) patients were refractory to PIs (bortezomib or carfilzomib) and IMiDs (thalidomide or lenalidomide), respectively. Double refractoriness to PIs and IMiDs was observed in 9 patients (31%), whereas 11 patients (38%) were refractory to the last line of the previous therapy (TABLE 1). However, both the refractory status to PIs and/or IMiDs and refractoriness to the last treatment line before the FISH2 evaluation were not significantly associated with the occurrence of cytogenetic evolution. Similarly, there was no association between CE and the depth of response achieved to the last treatment line before the FISH2 evaluation (complete response or very good partial response versus partial response).

Prognostic significance of cytogenetic evolution on progression-free survival After the FISH2 evaluation, 3 patients did not receive any further antimyeloma treatment (1 patient died before starting the next treatment line, and 2 patients had the FISH2 test performed during biochemical relapse) and were excluded from the PFS analysis. Of the remaining 26 patients, 17 (65%) experienced disease progression. A median PFS for the entire cohort was 7.1 months (95% CI, 6.4–12). The presence of CE was shown to have a negative impact on PFS (median PFS, 3.9 months and 9.3 months for eFISH and sFISH, respectively; hazard ratio [HR], 2.9; 95% CI, 1.05–7.9; *P* = 0.04) (FIGURE 1 and TABLE 3).

Patients who acquired del(17p) during the course of the disease achieved significantly shorter PFS compared with those who did not acquire del(17p) over time (median PFS, 1.5 months and 8.9 months, respectively; HR, 18.5; 95% CI, 3.1–109.5; *P* = 0.001) (FIGURE 2 and TABLE 3).

Laboratory and clinical factors assessed at the time of the FISH2 evaluation, such as age (≥65 years or <65 years), ISS stage (3 vs 1 to 2), ECOG performance status (2 to 3 vs 0 to 1), and percentage of bone marrow plasma cells (>70% vs ≤70%), refractoriness to PIs, IMiDs, or double refractoriness to PIs and IMiDs had no significant prognostic effect on PFS. On the other hand, higher LDH serum activity (above the upper limit of normal vs below the upper limit of normal) was

TABLE 1 Patient characteristics

Parameter	Before FISH2 evaluation	At the time of or after FISH2 evaluation
Age, y, median (range)	64 (46–72)	66 (49–76)
Female sex	14 (48)	–
ECOG score		
0	2 (7)	4 (14)
1	19 (66)	18 (65)
2	6 (21)	3 (10)
3	2 (7)	1 (3.5)
Not reported	–	3 (10)
ISS stage		
I	3 (10)	6 (21)
II	13 (45)	4 (14)
III	13 (45)	12 (41)
Not reported	–	7 (24)
R-ISS stage		
I	1	Not evaluated
II	23	Not evaluated
III	2	Not evaluated
Not reported	3	–
High-risk CA		
t(4;14)	1 (3.5)	1 (3.5)
t(14;16)	1 (3.5)	1 (3.5)
del(17p)	1 (3.5)	7 (24)
Lines of the previous therapy, n, median (range)	1 (1–4)	1 (0–4)
Multiple antimyeloma therapy		
Bortezomib-based regimens[a]	26 (90)	12 (41)
Kd	1 (3.5)	3 (11)
Lenalidomide-based regimens[b]	7 (24)	11 (38)
Pomalidomide-based regimens[c]	0	3 (11)
Daratumumab-based regimens[d]	0	7 (25)
Other regimens[e]	10 (34)	9 (31)
Single auto-HSCT	9 (31)	2 (7)
Tandem auto-HSCT	4 (14)	0
Refractoriness to treatment		
PIs	11 (38)	8 (28)
IMiDs	12 (41)	11 (38)
PIs + IMiDs	9 (31)	5 (17)
Last line of the previous therapy	11 (38)	11 (38)

Data are presented as number (percentage) unless otherwise indicated.

a Bortezomib-based regimens: BVD, PAD, PanVD, VCD, VD, VMP, VTD, and VTD-PACE

b Lenalidomide-based regimens: IRd and Rd

c Pomalidomide-based regimens: Pd and PCd

d Daratumumab-based regimens: DKd, DVd, DRd, DVTD, DBP, and daratumumab monotherapy

e Other regimes: BP, DCEP, CTD, MPT, and VMBCP

Abbreviations: auto-HSCT, autologous hematopoietic stem cell transplant; BP, bendamustine, prednisone; BVD, bendamustine, bortezomib, dexamethasone; CA, cytogenetic abnormality; CTD, cyclophosphamide, thalidomide, dexamethasone; DCEP, dexamethasone, cyclophosphamide, etoposide, cisplatin; DKd, daratumumab, carfilzomib, dexamethasone; DRd, daratumumab, lenalidomide, dexamethasone; DVd, daratumumab, bortezomib, dexamethasone; ECOG, Eastern Cooperative Oncology Group; FISH2, the second fluorescence in situ hybridization test; IMiDs, immunomodulatory drugs; IRd, ixazomib, lenalidomide, dexamethasone; ISS, International Staging System; Kd, carfilzomib, dexamethasone; MPT, melphalan, prednisone, thalidomide; PAD, bortezomib, doxorubicin, dexamethasone; PanVD, panobinostat, bortezomib, dexamethasone; PCd, pomalidomide, cyclophosphamide, dexamethasone; Pd, pomalidomide, dexamethasone; PIs, proteasome inhibitors; Rd, lenalidomide, dexamethasone; R-ISS, Revised International Staging System; VCD, bortezomib, cyclophosphamide, dexamethasone; VD, bortezomib, dexamethasone; VMBCP, vincristine, carmustine, cyclophosphamide, melphalan, prednisone; VMP, bortezomib, melphalan, prednisone; VTD, bortezomib, thalidomide, dexamethasone; VTD-PACE, bortezomib, thalidomide, dexamethasone; cisplatin, doxorubicin, cyclophosphamide, etoposide

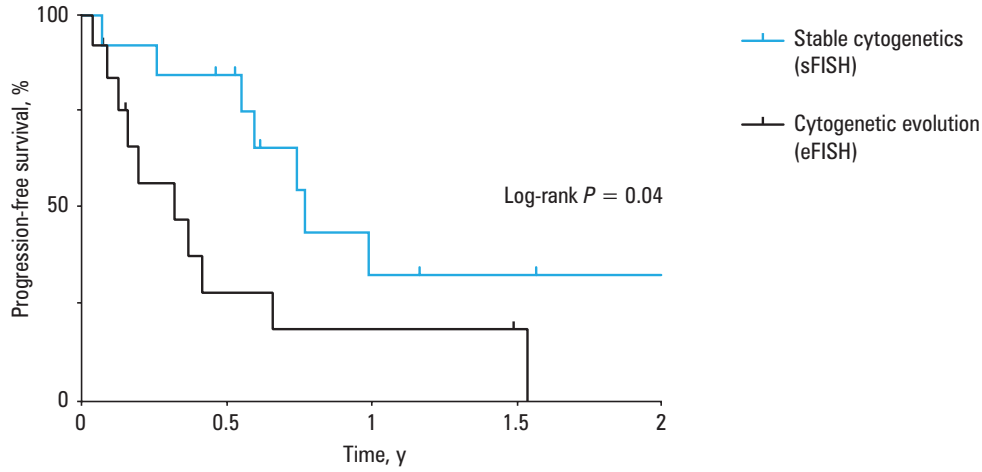

FIGURE 1 The effect of cytogenetic evolution on progression-free survival after the second fluorescence in situ hybridization test
Abbreviations: eFISH, cytogenetic evolution between fluorescence in situ hybridization tests 2 and 1; sFISH, stable cytogenetics observed after comparing the results of fluorescence in situ hybridization tests 2 and 1

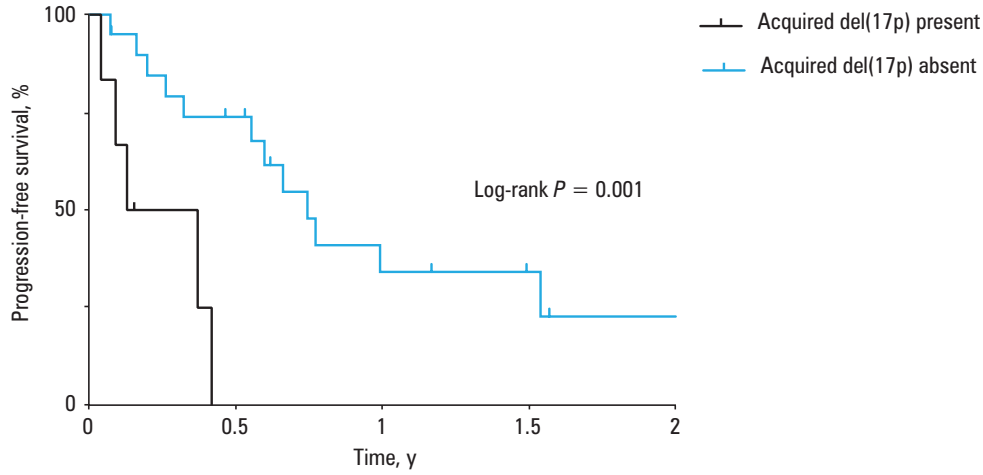

FIGURE 2 The effect of the acquisition of the chromosome 17p (del[17p]) deletion on progression-free survival after the second fluorescence in situ hybridization test

TABLE 2 The overall spectrum of cytogenetic changes detected in the second fluorescence in situ hybridization test

Cytogenetic change	n (%)
New additional copy/copies of the *TP53* gene	3 (21)
New additional copy/copies of the *ATM* gene	7 (50)
New additional copy/copies of the *IGH* gene	1 (7)
Loss of additional copy/copies of the *IGH* gene	3 (21)
Loss of additional copy/copies of the *FGFR3* gene	2 (14)
New deletion of the *IGH* gene	2 (14)
New deletion of the *TP53* gene/del(17p)	7 (50)
New deletion of the *MAF* gene	2 (14)

associated with a significant reduction in PFS (HR, 4.9; 95% CI, 1.1–21.9; $P = 0.04$).

After univariable analysis, the presence of acquired del(17p), CE seen when comparing the results of the FISH1 and FISH2 tests, bone marrow plasma cell percentage higher than 70%, and LDH serum activity above the upper limit of normal had $P < 0.1$ for predicting shorter PFS and were included in multivariable analysis (TABLE 3). In the multivariable Cox proportional model, only the presence of acquired del(17p) and a higher LDH serum activity significantly predicted shorter PFS with HR of 9.3 (95% CI, 2–44; $P = 0.005$) and 5 (95% CI, 1.3–19.7; $P = 0.02$), respectively (TABLE 3).

Prognostic significance of cytogenetic evolution on overall survival 1 and 2 In total, 10 deaths were reported during the follow-up. The median OS1 was 4.6 years (95% CI, 3.8–5.2 years). At a median (range) study follow-up of 3.1 (0.8–5.2) years, patients with sFISH had improved survival compared with those with eFISH (median OS1 not reached [NR] vs that of 3.8 years; HR, 0.15; 95% CI, 0.04–0.55; $P = 0.04$). The median OS1 in patients with acquired del(17p) was 2.95 years (95% CI, 2.16–5.2) compared with 4.7 years (95% CI, 4.1–4.8) in those who did not acquire del(17p) during the course of the disease ($P = 0.16$).

At a median (range) follow-up after the FISH2 evaluation of 0.6 (0.1–4.4) years, the median OS2 was 1.7 years (95% CI, 0.8–2.4) and was significantly longer in the sFISH group compared with the eFISH group (median OS2, NR vs 0.8 years; HR, 0.13; 95% CI, 0.04–0.45; $P = 0.002$) (FIGURE 3 and TABLE 3). The study patients with acquired del(17p) achieved a significantly shorter median OS2 compared with those who did not acquire del(17p) over time (median OS2, 0.5 years [95% CI, 0.13–0.8] and 2.4 years [95% CI, 1.12–2.4], respectively; HR, 41.6; 95% CI, 5.9–292.7; $P < 0.001$) (FIGURE 4 and TABLE 3). After excluding the group with acquired del(17p) from the analysis, the median OS2 in the sFISH group was still significantly longer compared with

TABLE 3 The Cox proportional hazards model identifying factors affecting progression-free survival and overall survival at the time of the second fluorescence in situ hybridization test

Independent variable	PFS				OS2			
	Univariable analysis		Multivariable analysis		Univariable analysis		Multivariable analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Cytogenetic evolution (eFISH)	2.9 (1.05–7.9)	0.04	1.8 (0.4–7.9)	0.42	7.8 (2.2–27.9)	0.002	2.7 (0.24–33.3)	0.44
Acquired del(17p)	18.8 (3.1–109.5)	0.001	9.3 (2.0–44)	0.005	41.6 (5.9–292.7)	<0.001	18.8 (2.6–134.9)	0.004
LDH serum activity >ULN	4.9 (1.1–21.9)	0.04	5.0 (1.3–19.7)	0.02	3.4 (0.64–18)	0.05	5.7 (0.98–33.5)	0.05
Bone marrow plasma cells >70%	2.5 (0.9–7.2)	0.09	3.3 (0.8–13.3)	0.1	–	–	–	–

Abbreviations: del(17p), deletion of the short arm of chromosome 17; HR, hazard ratio; LDH, lactate dehydrogenase; OS2, overall-survival after the second fluorescence in situ hybridization test; PFS, progression-free survival; ULN, upper limit of normal; others, see **FIGURE 1**

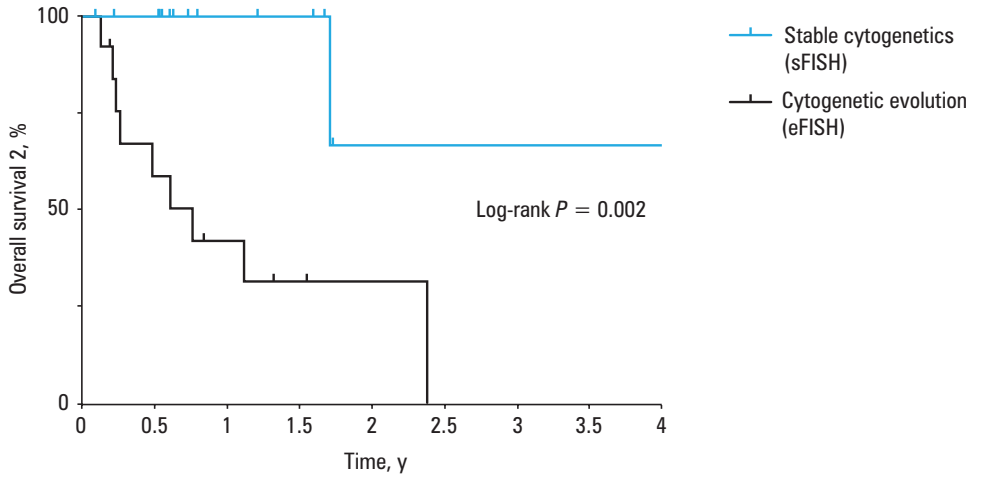

FIGURE 3 The effect of cytogenetic evolution on overall survival after the second fluorescence in situ hybridization test Abbreviations: see **FIGURE 1**

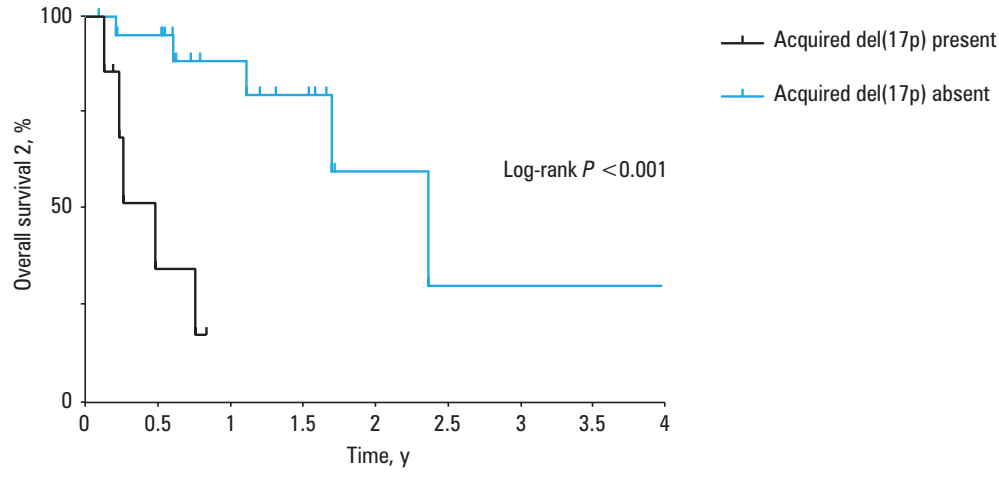

FIGURE 4 The effect of the acquisition of the chromosome 17p (del[17p]) deletion on overall survival after the second fluorescence in situ hybridization test

the eFISH group (median OS2, NR vs 1.12 years [95% CI, 1.12–2.4], respectively; HR, 0.13; 95% CI, 0.02–0.88; *P* = 0.04).

Other laboratory and clinical factors assessed at the FISH2 evaluation, such as age (≥65 years or <65 years), ISS stage (3 vs 1 to 2), ECOG performance status (2 to 3 vs 0 to 1), percentage of bone marrow plasma cells (>70% vs ≤70%), LDH serum activity (above the upper limit of normal vs below the upper limit of normal), refractoriness to PIs, IMiDs, or double refractoriness to PIs and IMiDs had no significant prognostic impact on OS2.

After univariable analysis, the presence of acquired del(17p), CE seen between the FISH1 and FISH2 tests, and LDH serum activity above the upper limit of normal showed *P* <0.1 for predicting shorter OS2 and were included in multivariable analysis (**TABLE 3**). In the multivariable Cox proportional model, only the presence of acquired del(17p) significantly predicted shorter OS2 with HR of 18.8 (95% CI, 2.6–134.9; *P* = 0.004) (**TABLE 3**).

DISCUSSION Although the survival of patients with MM has dramatically improved over the past

decades,[24] the disease still presents a relapsing clinical course, and treatment for subsequent relapses remains a major challenge in routine clinical practice.

In recent years, studies using NGS have provided a new insight into the molecular biology of MM, highlighting its intratumor genetic heterogeneity and evolutionary nature. Myeloma is now considered a mosaic of genetically distinct subclones that compete with each other over time following different patterns of clonal evolution.[12,13,15,16] Both the tumor microenvironment and the antimyeloma treatment exert selection pressure on subclones, ultimately shaping the clonal architecture of the disease in time.[12,14,17] However, the potential of NGS has not been translated into routine clinical practice yet, and FISH testing remains the basic and widely used method for assessing the genetic status of MM.[18]

On one hand, there is a general consensus that cytogenetics evaluated by FISH is essential for risk stratification and should be part of the initial diagnostic workup in all patients with newly diagnosed MM.[25-29] On the other hand, relatively little is known about the occurrence and prognostic significance of CE at the time between the diagnosis of MM and disease progression. Clinical practice guidelines differ with respect to recommendations for re-evaluation of FISH tests at MM relapse. The European Society for Medical Oncology guidelines do not recommend clinicians to reassess FISH tests during the MM relapse,[26] whereas the National Comprehensive Cancer Network clinical practice guidelines advocate FISH test re-evaluation depending on the clinical situation.[29] In contrast, the American Society of Clinical Oncology[28] and Mayo Clinic[27] guidelines recommend full cytogenetic restaging in every MM relapse. Conversely, the routine diagnostic approach in Poland is solely based on the FISH evaluation at the time of MM diagnosis,[22] which may explain a relatively low percentage of patients in our database who had 2 FISH tests evaluated during the course of the disease.

In this study, we showed that in a significant proportion of patients (48%), the signs of CE can be detected in a routine FISH MM panel using a limited number of probes. The occurrence of CE in our study cohort was independent of the ISS and R-ISS stage and other commonly used laboratory and clinical prognostic factors for survival in MM. Therefore, our findings suggested that CE is not easily predictable, and basic clinical data are not useful for reliable identification of patients who require the second FISH evaluation.

The univariable analysis revealed that the occurrence of CE between the time of the diagnosis of MM and relapse had a significant negative impact on OS and survival after the second FISH evaluation in the study patients. These findings are consistent with the results of a recent large-scale, retrospective study of 164 patients with MM who underwent serial FISH evaluations, which demonstrated that the development of additional CAs during the 3 years following the diagnosis of MM was associated with increased subsequent mortality with HR of 3.3 (P <0.001).[30]

In the era of novel antimyeloma agents, the presence of primary high-risk CAs, such as t(4;14), t(14;16), t(14;20), and, in particular, del(17p), still negatively impacts the outcomes in patients newly diagnosed with MM.[31,32] The prevalence of primary high-risk CAs is significant, with 15%, 3% to 5%, and 5% to 8% of patients with newly diagnosed MM affected by t(4;14), t(14;16), and del(17p), respectively.[33,34] In our database, the incidence of high-risk CAs at the time of the diagnosis of MM was in accordance with previously reported data with t(4;14), t(14;16), and del(17p) found in 10%, 2%, and 7% of patients, respectively. Nevertheless, in the group of patients in whom the FISH analysis was performed twice during the course of the disease, each of these primary high-risk CAs was found in a single patient (3.5%).

In contrast to the well-defined prognostic significance of primary high-risk CAs, the prognostic impact of high-risk CAs acquired during the progression of MM is less documented. In our cohort, del(17p) was the only high-risk CA acquired in the course of MM progression and no other acquired high-risk CAs, such as t(4;14) and t(14;16), were found. This could be explained by the fact that *IGH*-involving translocations are one of the initiating genetic events in the genesis of myeloma.[35] These findings are in line with the results recently reported by Merz et al.[36] In that retrospective study evaluating cytogenetic changes in 128 patients with MM who relapsed after auto-HSCT, there were no new t(4;14), t(11;14), or t(14;16) translocations found at relapse. However, in opposite to our study, *IGH* translocations with other unknown partner genes were observed.[36] In another study, the acquisition of t(4;14) during relapse was observed in 14 out of 268 patients with MM.[37] However, the detailed analysis showed that t(4;14) was already present at the time of the diagnosis of MM in a minor subclone that had not been initially detected by FISH. That subclone underwent positive selection during antimyeloma therapy and became the dominant clone during relapse.[37]

Our results indicated that the acquisition of del(17p) during the progression of MM is associated with an extremely poor prognosis in the population of patients with MM with the median OS after the gain of del(17p) of 6 months (HR, 18.8; 95% CI, 2.6–134.9) and the median PFS from the start of the next line of therapy of 1.5 months (HR, 9.3; 95% CI, 2–44). Recently, Lakshman et al[24] reported outcomes of 76 patients with MM and acquired del(17p) who were treated at Mayo Clinic. The median OS from detection of del(17p) was 18.1 months (95% CI, 11.9–25), and the median PFS from the start of the next line of therapy was 5.4 months (95% CI, 2.7–7.7).[24] These clinically significant differences in the outcomes of patients with acquired del(17p) between our

study group and the Mayo Clinic cohort may, at least partly, result from differences in access to modern antimyeloma agents,[38] as more patients were treated with daratumumab-, pomalidomide-, carfilzomib- or ixazomib-containing regimens in the Mayo Clinic cohort.

It should be emphasized that cytogenetic reassessment has had only prognostic significance so far and currently no prospective data are available indicating clinical benefits of adapting the therapeutic strategy to the current cytogenetic profile of RRMM. Nevertheless, data from the subgroup analysis of randomized clinical trials have provided some information that may prompt a clinician to choose a specific therapy depending on patient's current cytogenetics. In this context, there are data suggesting that patients with RRMM and del(17p) could benefit from pomalidomide-dexamethasone[37,39,40] or lenalidomide-dexamethasone in combination with carfilzomib[39,41] or ixazomib[2,39] or daratumumab.[9]

Admittedly, our study was limited by its retrospective design. Despite the fact that the data came from a large European hematology center, a relatively small group of patients was evaluated by FISH twice during the course of the disease. Moreover, the FISH data were limited by their routine character, which was associated with the restricted number of probes used for analysis. Finally, patients were not treated uniformly and novel antimyeloma drugs, such as carfilzomib, pomalidomide, and daratumumab, were administered relatively rarely.

In conclusion, we showed that CE and, in particular, the acquisition of del(17p) detected in routine FISH testing negatively affects the outcomes of patients with RRMM. Given the extremely poor prognosis of patients with acquired del(17p), the re-evaluation of FISH tests at each subsequent MM relapse should be included in routine clinical practice.

ARTICLE INFORMATION

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CONTRIBUTION STATEMENT ASP and KJ conceived the concept of the study. All authors were involved in data collection. ASP and NJ performed statistical analysis. ASP, AB, AK, JB, and NJ analyzed the data. ASP, JB, and KJ drafted the manuscript. KJ coordinated funding of the project. All authors edited and approved the final version of the manuscript.

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