Original Study

Gain(1)(q21) is an Unfavorable Genetic Prognostic Factor for Patients With Relapsed Multiple Myeloma Treated With Thalidomide but Not for Those Treated With Bortezomib

Jan Smetana,[1,2] Kristina Berankova,[2] Romana Zaoralova,[2] Pavel Nemec,[1,2] Henrieta Greslikova,[1] Renata Kupska,[1] Aneta Mikulasova,[1,2] Jan Frohlich,[2] Sabina Sevcikova,[1] Lucie Zahradova,[3] Marta Krejci,[3] Viera Sandecka,[3] Martina Almasi,[1,4] Petra Kaisarova,[5] Hana Melicharova,[5] Zdenek Adam,[3] Miroslav Penka,[4] Jiri Jarkovsky,[5] Arthur Jurczyszyn,[6] Roman Hajek,[1,3,4] Petr Kuglik[2]

Abstract

Chromosomal aberrations are important prognostic factors in multiple myeloma diagnosis. We evaluated the effect common high-risk chromosomal aberrations in a cohort of 102 patients with relapsed disease treated with bortezomib or thalidomide. Our results showed that patients treated with thalidomide with a gain(1)(q21) had inferior survival compared with the bortezomib group. Therefore, bortezomib-based regiments are more effective for patients with relapsed multiple myeloma with an incidence of gain in the gain(1)(q21).

Background: Prognostic impact of specific chromosomal aberrations in patients with relapsed multiple myeloma (MM) treated with the novel agents is briefly described. **Patients and Methods:** We analyzed the prognostic value of an extended panel of chromosomal aberrations [del(13)(q14), del(17)(p13), t(4;14)(p16;q32), gain(1)(q21), and hyperdiploidy] by using the technique of interphase fluorescence in situ hybridization in a cohort of 102 patients with relapsed MM treated with thalidomide- or bortezomib-based protocols. **Results:** The gain(1)(q21) had a negative impact on overall survival for patients with MM treated with thalidomide (15.7 vs. 41.3 months; $P = .004$). Moreover, we confirmed the negative impact of the cumulative effect of 2 or more cytogenetic changes that occur simultaneously on the overall survival in the thalidomide group (20.3 months vs. not yet reached; $P = .039$). We did not find any significant impact of the aberrations studied on overall survival in the bortezomib cohort of patients. **Conclusion:** We conclude that bortezomib-based protocols are able to partially overcome the negative prognostic impact of the tested chromosomal abnormalities in patients with relapsed MM.

Clinical Lymphoma, Myeloma & Leukemia, Vol. xx, No. x, xxx
Keywords: Multiple myeloma, Thalidomide, Bortezomib, Chromosomal aberrations, FISH, Survival

[1]Babak Myeloma Group, Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3]Department of Internal Hematooncology, University Hospital, Brno, Czech Republic
[4]Department of Clinical Hematology, University Hospital, Brno, Czech Republic
[5]Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[6]Department of Hematology, University Hospital, Krakow, Poland

Submitted: Sep 19, 2012; Revised Nov 19, 2012; Accepted Nov 20, 2012

Address for correspondence: Petr Kuglik, MD, Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno-Bohunice, Czech Republic
E-mail contact: kugl@sci.muni.cz

2152-2650/$ - see frontmatter
http://dx.doi.org/10.1016/j.clml.2012.11.012

Introduction

Multiple myeloma (MM) is an incurable malignant disease of the terminal developmental stage of B lymphocytes. MM represents approximately 10% to 15% of all hematologic malignancies and in 1% to 2% of all cancers.[1] Despite recent progress in treatment management, survival of patients with myeloma is highly variable, ranging from a few months to more than 10 years.[2] The heterogeneity relates mainly to prognostic factors associated with specific characteristics of the tumors. During the past decade, considerable progress has been made in understanding the molecular basis and biology of MM.[3] Chromosomal abnormalities in plasma-cell dyscrasias are common and highly complex. Cytogenetic analyses and gene-expression profiling have contributed to the recognition of distinct subtypes of MM with different prognoses.[4,5] There are well-known correlations between prognosis and several chromosomal aberrations detected by cytogenetic analyses, including interphase fluorescence in situ hybridization (I-FISH). Deletion of *TP53* in 17p13 loci, translocations t(4;14)(p16;q32) and t(14;16)(q32;q23) detected by I-FISH are known to be associated with a poor prognosis.[6-8] The gain(1)(q21) as well as increased expression of *CKS1B* were suggested as key markers of poor prognosis.[9-11] These data are conclusive but valid only for patients with newly diagnosed MM who undergo conventional chemotherapy or autologous transplantation.[12,13] Nevertheless, there is limited knowledge about prognostic and predictive (resistance vs. sensitivity) features of chromosomal abnormalities when new agents, such thalidomide or bortezomib, are used. Thalidomide is a drug with immunomodulatory and antiangiogenic effects, and is known to induce responses in up to one-third of patients with refractory disease.[14,15] Moreover, the activity of this oral agent was demonstrated in patients who had failed high-dose therapy.[16,17] Effects of bortezomib, the first proteasome inhibitor used in human therapy, combined with other agents, were reported in various groups of patients.[18-24] Recent results have shown that bortezomib induction improves the outcome of patients with newly diagnosed with t(4;14)(p16;q32) but not the outcome of patients with del(17)(p13) and that it may overcome the negative prognostic impact of del(13)(q14) and t(4;14)(p16;q32) in patients with relapsed MM.[25-29] In this article, we evaluated the clinical and biologic impact of an extended panel of chromosomal abnormalities: [del(13)(q14), del(17)(p13), t(4;14)(p16;q32), gain(1)(q21) and hyperdiploidy/non-hyperdiploidy] detected by I-FISH in a group of 102 patients with relapsed MM treated with either thalidomide- or bortezomib-based protocols.

Patients and Methods

Patients Characteristics

Between April 2004 and December 2009, 528 patients with relapsed MM were treated with either thalidomide-based protocols or with bortezomib-based protocols at the Faculty Hospital, Brno, Czech Republic. All patients were included in this study only after they signed the informed consent form approved by the ethical committee of the hospital.

Patients recruited into the study were chosen according to the following criteria: a minimum of 1 previous line of therapy, combination therapy with glucocorticoids and/or alkylating agents (not monotherapy), and no transplantation in the bortezomib or thalidomide line of therapy. In addition, the patients who received fewer than 2 cycles of therapy were not included in our analyses. After selection, a total of 127 patients (thalidomide group, n = 60; bortezomib group, n = 67) were eligible for further analyses. In the thalidomide group, 70% (42/60) of patients received 1 previous therapy and 30% (18/60) received ≥ 2 previous therapies. Whereas, in the bortezomib group, 43% (29/67) received 1 previous therapy, and 57% (38/67) received ≥ 2 previous therapies. Patient characteristics and disease features are shown in Table 1.

In total, 91% (55/60) of the patients were treated with thalidomide in combination with glucocorticoids and alkylating agents (cyclophosphamide, thalidomide, and dexamethasone), 4 (6.3%) patients were treated by using thalidomide in combination with glucocorticoids only, and 1 (1.5%) patient was treated by using thalidomide with alkylating agents only. In total, 73% (49/67) of the patients in the bortezomib group were treated with bortezomib in combination with glucocorticoids and alkylating agents (cyclophosphamide or melphalan, bortezomib, dexamethasone), 23% (18/67) received a combination of bortezomib with pegylated liposomal doxorubicin or dexamethasone.

Plasma Cell Detection and Cell Sorting

For detection of clonal plasma cells (PC) in bone marrow samples, in 78 cases, we used immunofluorescent labeling of cytoplasmic light chain as previously reported by Ahmann et al.[30] In the remaining 49 cases, we used cell-sorting techniques. The detailed protocol of cell sorting used in our center is described elsewhere.[31] In brief, a cut-off level of 5% for $CD138^+$ PC infiltration in bone marrow was established, thus either the magnetic-activated cell sorting (MACS) (MiltenyiBiotec [Miltenyi Biotec GmbH, Bergisch Gladbach, Germany]) or the fluorescent-activated cell sorting (FACS) (BD Biosciences, San Jose, CA) technique (<5% FACS, >5% MACS, respectively) was used according to the manufacturer's instructions. Only samples with purity over 90% were included in the I-FISH evaluations.

I-FISH

The following commercial DNA probes were used for I-FISH: LSI 13q14 (RB1) Spectrum Green Probe, LSI p53 (17p13.1) Spectrum Orange Probe, LSI IGHC/IGHV Dual Color Probe, LSI IGH/FGFR3 Dual Color Probe, LSI IGH/CCND1 Dual Color Probe and for hyperdiploidy LSI D5S23/D5S721, CEP 9, CEP 15 multicolor Probe Panel (Abbott Molecular Inc, Des Plaines, IL). Hyperdiploidy was defined as a gain of at least 2 of 3 evaluated chromosomes in a single cell. The gain(1)(q21) was assessed by using in-house fluorescent probes. Bacterial artificial chromosome (clone RP11-205M9) and control probe 1p36 (RP11-62M23) were purchased, and protocols for bacterial artificial chromosome isolation and labeling were followed from the online resources of the University in Bari, Italy (http://www.uniba.it). Slide preparation and FISH analyses were performed according to the manufacturer's protocols (Abbott-Vysis [Abbott Molecular Inc, Des Plaines, IL]). We used cut-off values recommended by the European Myeloma Network,[32] 20% cut-off for deletions and numerical aberrations and 10% cutoff for translocations and *IgH* rearrangements. A minimum of 100 cells were scored in each sample. Digital image analysis was performed by using the fluorescent microscope Olympus BX-61 (Olympus Inc,

Table 1 Clinical and Biologic Characteristics of 127 Relapsed MM Patients With MM Treated With Thalidomide- or Bortezomib-Based Regimens

Characteristics	All Patients	Thalidomide	Bortezomib	*P* Value
Sex, No. (%)				.174
Men	61/127 (48)	25/60 (42)	36/67 (54)	
Women	66/127 (52)	35/60 (58)	31/67 (46)	
Age at the Time of Therapy, Median (Range), y	64 (42-87)	65 (48-87)	64 (42-85)	.139
Duration From Diagnosis to Therapy, Median (Range) mo	30.7 (1.3-227.9)	26.0 (3.7-179.9)	32.7 (1.3-227.9)	.235
Follow-Up From Therapy, Median (Range), mo	19.9 (1.5-55.4)	21.4 (2.6-55.4)	18.3 (1.5-54.2)	.340
Durie-Salmon Stage From Therapy, No. (%)				.277
I	1/127 (0.8)	1/60 (1.6)	0/67	
II	23/127 (18.1)	13/60 (21.6)	10/67 (14.9)	
III	103/127 (81.1)	46/60 (76.8)	57/67 (85.1)	
Stage A-B From Therapy, No. (%)				.088
A	107/127 (84)	54/60 (90)	53/67 (79)	
B	20/127 (16)	6/60 (10)	14/67 (21)	
ISS Stage (From Therapy), No. (%)				.929
1	52/123 (42.3)	26/60 (43.3)	26/63 (41.3)	
2	41/123 (33.3)	19/60 (31.7)	22/63 (34.9)	
3	30/123 (24.4)	15/60 (25)	15/63 (23.8)	
Ig Isotype, No. (%)				.412
IgG	74/127 (58.2)	36 (60)	38 (56.7)	
IgA	30/127 (23.6)	11 (18.3)	19 (28.3)	
B-J	12/127 (9.5)	8 (13.3)	4 (6.0)	
IgD	7/127 (5.5)	4 (6.7)	3 (4.5)	
IgM	2/127 (1.6)	1 (1.7)	1 (1.5)	
Nonsecretory	1/127 (0.8)	0	1 (1.5)	
IgG + IgM + Biclone	1/127 (0.8)	0	1 (1.5)	
No. Previous Lines of Therapy, No. (%)				<.001
1	71/127 (56)	42 (70)	29 (43.3)	
2	47/127 (37)	18 (30)	29 (43.3)	
3	9/127 (7)	0	9 (13.4)	

Abbreviations: Ig = immunoglobulin; ISS = International Staging System; MM = multiple myeloma.

Tokyo, Japan) equipped with a charge-coupled device (CCD) Camera Vosskuhler 1300 (ALRAD, Newbury, UK) and Lucia KARYO/FISH/CGH imaging system (Laboratory Imaging s.r.o., Prague, Czech Republic).

Statistical Analysis

Outcome and treatment response were assessed according to the International Myeloma Working Group criteria.[33] Overall response rate (ORR) comprised complete stringent remission (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR). In univariate analysis, overall survival (OS), time to progression (TTP), progression-free survival (PFS), and duration of response (DOR) distributions were estimated by using the Kaplan-Meier method; differences among survival curves were analyzed by using the log-rank test. In multivariate analysis, the International Staging System stage and monoclonal immunoglobulin isotype level were analyzed by using the Cox regression. Associations between chromosomal abnormalities were estimated by the Fisher exact test, with a P value $< .050$ considered significant. All statistical analyses were performed by using Statistica 7.1 software (StatSoft Inc, Tulsa, OK).

Results

Incidence of Chromosomal Abnormalities in Patients With Relapsed MM

Bone marrow samples from 127 patients were available for cytogenetic analyses. FISH analysis was successful in 80% (102/127) of the bone marrow samples. Monosomy 13/del(13)(q14) was detected in 57 (56%) of 102, del(17)(p13) was found in 13 of 86 (15%), gain(1)(q21) occurred in 50 (56%) of 89 of patients. Translocation t(4;14)(p16;q32) was observed in 25 (28%) of 89 cases. Ploidy status was evaluated in 52 cases. Hyperdiploidy was found in nearly half of all patients (45% [23/

Table 2 Summary of Cytogenetic Findings in Cohort of 102 Patients With MM Treated With Thalidomide- or Bortezomib-based Regimens

Chromosomal Aberrations	All Patients, % (No./Total)	Thalidomide, % (No./Total)	Bortezomib, % (No./Total)	*P* Value
del(13)(q14) positive	56 (57/102)	48 (21/44)	62 (36/58)	.164
del(17)(p13) positive	15 (13/86)	10 (4/41)	20 (9/45)	.235
t(4;14)(p16;q32) positive	28 (25/89)	23 (10/43)	32 (15/46)	.355
gain(1)(q21) positive	56 (50/89)	56 (50/89)	63 (31/49)	.194
Hyperdiploidy	45 (23/51)	39 (7/18)	48 (16/33)	
Non-hyperdiploidy	55 (28/51)	61 (11/18)	52 (17/33)	.567
0-1 aberration	26 (22/86)	36 (13/36)	18 (9/50)	.0797
2+ aberrations	74 (64/86)	64 (23/36)	82 (41/50)	

Abbreviations: del = deletion; MM = multiple myeloma.

52]), whereas 55% (29/52) of cases were nonhyperdiploid. Both del(13)(q14) and t(4;14)(p16;q32) were more often found in patients who were non-hyperdiploid (10/23 vs. 19/28, $P = .09$; 2/23 vs. 7/28, $P = 07$; respectively). A summary of FISH results is shown in Table 2.

FISH analysis of the coexistence of structural aberrations [del(13)(q14), del(17)(p13), t(4;14)(p16;q32), gain(1)(q21)] in a single patient was performed in 86 patients. In 22 (26%) of 86 cases, we found 0 to 1 chromosomal aberrations, a simultaneous incidence of 2 or more changes was found in 64/86 (74%) of cases. We found a strong association between simultaneous incidence of del(13)(q14) and t(4;14)(p16;q32) in our data set ($P = .005$). In addition, del(13)(q14) was frequently observed in patients with del(17)(p13) and gain(1)(q21) ($P = .0170$, $P = .0101$; respectively).

Prognostic Relevance of Evaluated Chromosomal Aberrations

In the thalidomide group, the treatment response was evaluated in 97% (58/60) of the patients. The response rate could not be evaluated in 2 patients due to early death without progression. The ORR in this cohort of patients was reached by 50% (29/58) of patients, including sCR in 3.4% (2/58), CR in 5.2% (3/29), VGPR in 10.3% (6/58), and PR in 31.0% (18/58) of patients. Progression of the disease was observed in 36.2% (21/58) of cases. In the bortezomib group, the treatment response was evaluated in 94% (63/67) of patients. The response rate could not be evaluated in 3 patients due to early death without progression, 1 patient had nonsecretory MM. The ORR was reached by 41.3% (26/63) of cases, including sCR in 1.6% (1/63), CR in 11.1% (7/63), VGPR in 11.1% (7/26), and PR in 17.4% (11/63) of patients. Disease progression was observed in 34.9% (22/63) of cases. The presence or absence of any evaluated chromosomal aberration did not have any significant impact on the treatment response, neither in the thalidomide group nor in the bortezomib group.

The median follow-up in the thalidomide cohort was 21.4 months, and the median TTP and OS were 15.3 and 39.8 months, respectively. In the bortezomib cohort, the median follow-up was 18.3 months and the median TTP and OS were 13.9 and 31.2 months, respectively. No significant difference was found in TTP, PFS, and DOR when subgroups of patients with and without any selected aberrations were compared.

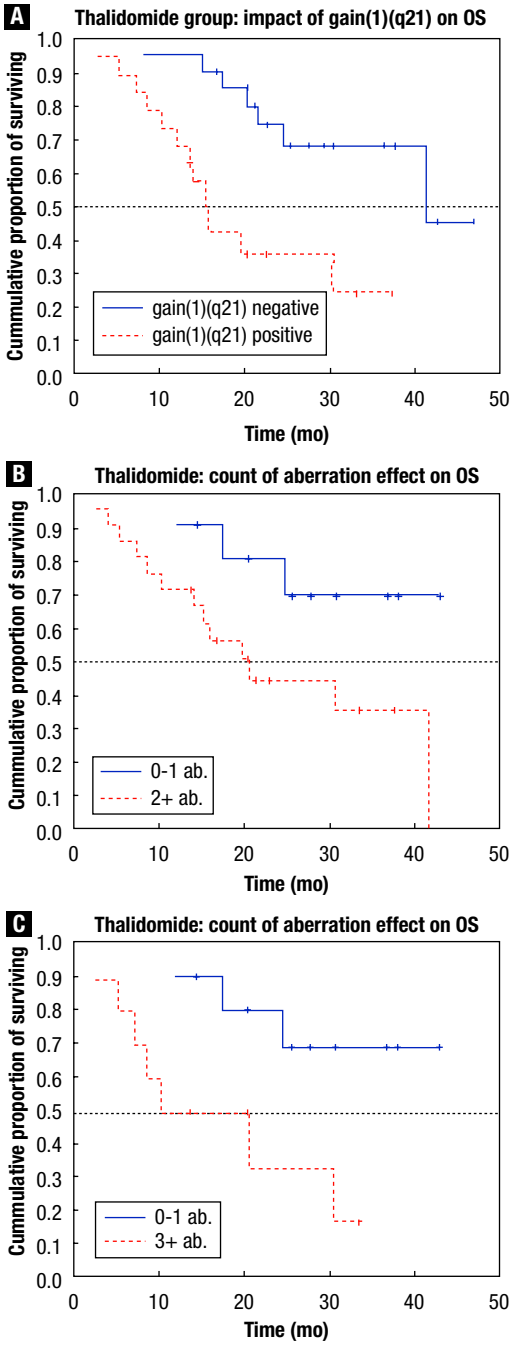
We observed adverse effect of the chromosomal abnormalities in the thalidomide cohort. The patients with gain(1)(q21) and del(17)(p13) had significantly shorter OS compared with those without these abnormalities (15.7 vs. 41.3 months, $P = .004$; 8.5 months vs. 41.3, $P = .020$). No differences in OS were found between patients with and without other evaluated aberrations in this group. Analysis of the prognostic impact of simultaneous incidence of structural chromosomal aberrations with a negative prognostic impact in the thalidomide group showed that patients with 2 and more cytogenetic changes (20.3 months vs. not yet reached; $P = .039$) and also patients with 3 changes (10.1 months vs. not yet reached; $P = .027$) had statistically significant shorter OS (Figure 1).

In the bortezomib cohort, we did not find any significant impact of studied aberrations with OS. In patients with del(13)(q14), del(17)(p13) and gain(1)(q21), we observed a trend to shorter OS (18.3 vs. 37.2 months, $P = .097$; 18.3 vs. 37.2 months, $P = .109$; 29.0 vs. 37.1 months; $P = .146$; respectively), however, the differences were not statistically significant. No significant difference was found in TTP, PFS, and DOR when subgroups of patients with and without any selected aberrations were compared. The results are summarized in Table 3.

Discussion

In our study, we sought to investigate the prognostic impact of the following chromosomal abnormalities: [del(13)(q14), del(17)(p13), t(4;14)(p16;q32), gain(1)(q21) and hyperdiploidy/non-hyperdiploidy] by using I-FISH in a cohort of patients with MM treated either with thalidomide- or bortezomib-based protocols in the relapse setting. The findings of clonal chromosomal aberrations in PC are considered one of the most important prognostic factors in patients with MM.[34] Although metaphase cytogenetic analysis is often limited by the low proliferative activity of PC and is successfully obtained in only 30% of cases,[35] molecular cytogenetic analyses by using I-FISH can identify chromosomal abnormalities in up to 90% of patients with MM.[36] Even though the outcome of patients with MM has dramatically improved in the past decade due to the introduction of

Figure 1 Effect of Chromosomal Abnormalities on Overall Survival (OS) of Patients Treated With Thalidomide-Based Regimens. Impact of gain(1)(q21) and Cumulative Impact of Coincidence of Structural Chromosomal Abnormalities on OS. (A) Impact of gain(1)(q21) on OS; n = 44, 21/44 Patients; P = .004. (B) Impact of 2 or More Chromosomal Abnormalities on OS; n = 49; 21 vs. 28 Patients; P = .027. (C) Impact of 3 or More Chromosomal Abnormalities on OS; n = 49; 21 vs. 17 Patients; P = .039



new, more effective treatments and better appreciation of potential complications and their management, the prognostic impact of high-risk chromosomal abnormalities in MM in the era of novel therapies has not yet been clearly defined.[37-39] Although the impact of chromosomal changes on the outcome of patients treated with bortezomib and with relapsed and/or refractory MM is widely accepted in patients newly diagnosed, there are limited data available on the efficacy of bortezomib and thalidomide regimens in the relapse setting.[15,16,40]

It is known that the malignant clone in MM differs from other hematologic malignancies due to a high fraction of low-proliferating malignant PC.[30] This study confirmed our previous results, which pointed out that purification or identification of these malignant cells is an essential step for standardization of FISH analyses.[31] Of 127 patients included in our study, 25 failed to pass European Myeloma Network criteria for FISH investigation.[32] The sorting techniques, for example, MACS, provide a generally higher yield of malignant PCs compared with immunofluorescent identification of (23/78 vs. 2/49; $P < .001$) because, in almost 30%, we failed to score at least 100 malignant PCs on the slide. Thus, according to our results, FISH experiments are more effective when cell sorting is used in the MM diagnosis.

We confirmed that patients with relapsed MM can be divided into 2 homogenous genetic subgroups according to ploidy status. Hyperdiploidy was found in 45% (23/51) of patients, 55% of cases were non-hyperdiploid. We found del(13)(q14) in 57% (57/102) of cases, gain(1)(q21) was observed in 63% (56/89), del(17)(p13) occurred in 15% (13/86). The translocation t(4;14)(p16;q32) was found in 28% (25/89). Even though the incidence of evaluated chromosomal changes was in agreement with previously published data,[25-28] the incidence of t(4;14)(p16;q32) was higher when compared with previous reports.[4,7,13] This was probably caused by the selection of a group of patients with progression, in which a higher incidence of chromosomal changes with negative prognostic impact could be expected.

Several studies evaluated the influence of chromosomal abnormalities on the response to bortezomib-based treatment protocols. Analysis of recent data confirmed that both del(13)(q14) and t(4;14)(p16;q32) an impact on OS[25,26] and that the simultaneous incidence of these aberrations could be partially overcome by bortezomib-based regimens in patients with relapsed and/or refractory MM.[41] However, bortezomib seems to be ineffective for patients with relapsed and/or refractory MM with gain(1)(q21) and del(17)(p13).[42]

In accordance with published data, patients in bortezomib cohort with the incidence of del(13)(q14) and t(4;14)(p16;q32) had a trend toward a shorter OS (18.3 vs. 37.2 months, $P = .097$; 15.8 vs. 31.2, $P = .196$; respectively). This result may be caused mainly by the effect of the *IgH* translocation, which was often found together with heterozygous deletion *RB1*. This finding is also supported by our previous results obtained from patients with newly diagnosed MM and also by other reports.[42,43] A trend toward a shorter OS was also found in patients with del(17)(p13) and gain(1)(q21) (18.3 vs. 37.2 months, $P = .10$; 29 vs. 37.1 months; $P = .146$); despite that difference, 1.5 year is clinically important due to the low number of

Table 3 Correlation Between Cytogenetic Aberrations and Response Rate, Time to Progression and Overall Survival in Patients Treated With Thalidomide- and Bortezomib-Based Regimen

Chromosomal Abnormality	ORR				TTP, mo				OS, mo			
	Thalidomide, no. (%)	*P*	Bortezomib, no. (%)	*P*	Thalidomide	*P*	Bortezomib	*P*	Thalidomide	*P*	Bortezomib	*P*
del(13)(q14)		.232		.244		.803		.443		.180		.097
Positive	7/20 (35.0)		15/34 (44.1)		11.7		13.9		20.3		18.3	
Negative	12/22 (54.5)		5/20 (25.0)		13.5		12.5		41.3		37.2	
del(17)(p13)		.998		.994		NA		NA		**.020**		.109
Positive	2/4 (50.0)		3/8 (37.5)		NA		NA		8.5		18.3	
Negative	19/35 (54.3)		13/34 (38.2)		16.5		12.9		41.3		37.2	
t(4;14)(p16;q32)		.999		.349		.752		.544		.779		.196
Positive	5/10 (50.0)		5/15 (33.3)		15.3		14		NA		15.8	
Negative	17/32 (53.1)		14/28 (50.0)		16.5		12.2		32.4		31.2	
gain(1)(q21)		.334		.996		.935		.983		**.004**		.146
Positive	6/18 (33.3)		13/29 (44.8)		15.3		12.9		15.7		29.0	
Negative	11/21 (52.4)		8/18/(44.4)		12.8		13.9		41.3		37.1	
Ploidy Status		.997		.716		NA		.926		.386		.390
Hyperdiploidy	3/7 (42.9)		7/16 (43.8)		NA		NA		41.3		31.2	
Non-hyperdiploidy	5/11 (45.5)		5/16 (31.3)		11.7		NA		30.4		18.3	
Aberration[a]		.996		.171		.414		NA		**.039**		.330
0-1	5/11 (45.5)		2/11 (18.2)		11.5		NA		NA		37.1	
2+	8/20 (40.0)		16/37 (43.2)		19.7		NA		20.3		29.0	
Aberration[b]		.999		.419		.896		NA		**.027**		.149
0-1	5/11 (45.5)		2/11 (18.2)		11.5		NA		NA		37.1	
3+	4/9 (44.5)		7/19 (36.8)		11.7		NA		10.1		14.7	

P values < .05 in bold.
Abbreviations: del = deletion; NA = not available due to small number of patients; ORR = overall response rate; OS = overall survival; TTP = time to progression.
[a] Comparison of patients with 0-1 aberration vs. 2 and more aberrations.
[b] Comparison of patients with 0-1 aberration vs. 3 and more aberrations.

positive cases, the differences did not reached statistical significance for both chromosomal abnormalities.

There is limited knowledge about the impact of chromosomal abnormalities on thalidomide-based regimens used in patients with MM. Attal et al[44] reported shorter event-free survival (EFS) in patients with del(13)(q14) treated with thalidomide. In our thalidomide cohort, we did not observe that patients who lack deletion of chromosome 13 would have significant benefit from thalidomide treatment. On the contrary, gain(1)(q21) was associated with adverse prognosis (15.7 vs. 41.3 months; $P = .004$) in agreement with previous results.[45] Moreover, multivariate analysis showed that, in our thalidomide cohort, gain(1)(q21) may be considered as an independent prognostic factor (Table 4). Similar to data reported by Reece et al,[46] we observed a worse OS in patients with del(17)(p13) (19.5 vs. 41.3 months; $P = .020$). Our findings suggest that, in patients with relapsed MM, thalidomide was unable to overcome the negative prognostic impact of the deletion of *TP53* in 17p13 loci, even though there was a limited number of del(17)(p13)–positive cases. In the hyperdiploid group of patients, we did not observe any significant impact on OS, TTP, PFS, or DOR. When taken together, the thalidomide-

Table 4 Multivariate Model for Overall Survival in Thalidomide-Treated Group

	HR (95% CI)[a]	*P*
gain(1)(q21) positive	4.16 (1.33-13.07)	**.015**
Men	1.15 (0.39-3.41)	.796
Age	1.03 (0.98-1.09)	.267
Durie-salmon stage III	1.43 (0.32-6.31)	.638
Stage B	1.59 (0.27-9.32)	.604
ISS stage 3	2.32 (0.76-7.14)	.141
Other than IgG isotype	0.39 (0.12-1.27)	.118
More than 1 line of previous therapy	0.52 (0.12-2.30)	.385
Duration from diagnosis to therapy	1.00 (0.99-1.02)	.859

Value in bold refer to statistical significant difference between the hazard ratio for gain(1)(q21) positive and negative patients.
Abbreviations: HR = hazard ratio; Ig = immunoglobulin; ISS = International Staging System.
[a] Based on Cox proportional hazards model.

and the bortezomib-based protocols are both effective approaches for treatment of patients with myeloma and induce durable responses in patients who relapsed.

The comparison of both cohorts with exhaustive statistical analysis of different groups showed the positive effect on OS of bortezomib- vs. thalidomide-based regimens with respect to all statistic limitation. Patients with gain(1)(q21) treated with bortezomib had a trend toward significantly longer OS than in thalidomide group (29.0 vs. 15.7 months; $P = .091$), even though the patients in bortezomib cohort were more pretreated (second relapse, 43% vs. 30%). Multivariate analysis also showed that the gain(1)(q21) detected by the FISH technique could be used as an independent prognostic factor for patients treated with thalidomide-based regimens.

No other monitored aberration had any impact on efficacy of treatment. Similar to our previous results,[42,47] in the thalidomide cohort, we observed the negative cumulative effect of 2 and more cytogenetic changes that occurred simultaneously in a single patient. These results confirmed the cytogenetic heterogeneity of an individual patient, which reflected the existence of cytogenetically defined clones with high-risk complex karyotype. In the bortezomib group, we observed only a very weak trend toward a shorter OS in patients with 3 or more aberrations detected by I-FISH.

In conclusion, analysis of our results suggests that thalidomide-based regimens are not able to overcome the unfavorable impact of gain(1)(q21) in the relapse setting. Moreover, when compared with thalidomide, analysis of our data showed that, even though bortezomib-based regimens are able to improve survival of patients with relapsed MM, they are still unable to completely abrogate the poor prognosis in patients with gain(1)(q21). The same trend was observed in patients with del(17)(p13). According to our results, bortezomib should be preferred to thalidomide in patients with relapsed and/or refractory MM with the incidence of gain(1)(q21) and also with 2 and more cytogenetic changes. Further studies that use the combination of novel agents, such as proteasome inhibitors, and immunomodulatory agents are more potent and probably can more effectively abrogate the poor prognosis of patients with high-risk chromosomal abnormalities, such as gain(1)(q21).

Clinical Practice Points

- The incidence of chromosomal aberrations in the genome of malignant PCs is considered as an important prognostic factor in the diagnosis of MM (*Fonseca et al, Blood, 2003*). "High-risk" features, including loss of *TP53* [del(17)(p13)], gain(1)(q21), and translocation t(4;14)(p16;q32) have adverse effects on OS of patients with newly diagnosed patients with MM (*Fonseca et al, Leukemia, 2009*). However, the introduction of therapies based on novel drugs such as immunomodulatory agents (thalidomide, lenalidomide) or the proteasome inhibitor bortezomib (Velcade) significantly improved OS survival of patients with MM (*Mateos et al, Blood, 2011*).
- Although the positive effect of novel agents is widely accepted for patients with newly diagnosed MM, little is known about impact of high-risk chromosomal aberrations on the prognosis in patients with relapsed MM treated by novel agents (*Sawyer JR, Cancer Genet, 2011*).
- In our study, we showed the positive effect of bortezomib-based regiments for patients with an incidence of gain(1)(q21) and more than 2 chromosomal aberrations. Patients treated with bortezomib had significantly lower OS than those treated by thalidomide. A similar trend was also observed for patients with a loss of *TP53*. Therefore, we conclude that thalidomide-based regiments seems to be ineffective for patients with MM in the relapse setting, with an incidence of gain(1)(q21), 2 or more chromosomal aberrations, and most probably even for those with del(17)(p13).

Acknowledgments

We thank all participating members of the Czech Myeloma Group, especially all patients and their caregivers for making this work possible. This study was supported by research grant MSM0021622434 from the Ministry of Education, Youth and Sports, Czech Republic, grant of the Czech Science Foundation GAP304/10/1395 and by grants NS10207, NT11154, NT12130NT, 13,190 and NT13492 from the Internal Grant Agency of the Ministry of Health. The research leading to these results has received funding from the European Union Seventh Framework Program (FP7/2007-13) under grant agreement nr278570.

Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Moreau P, Hullin C, Garban F, et al. Tandem autologous stem cell transplantation in high-risk de novo multiple myeloma: final results of the prospective and randomized IFM 99-04 protocol. *Blood* 2006; 107:397-403.
2. Barlogie B, Bolejack V, Schell M, et al. Prognostic factor analyses of myeloma survival with intergroup trial S9321 (INT 0141): examining whether different variables govern different time segments of survival. *Ann Hematol* 2011; 90:423-8.
3. Kumar SK, Uno H, Jacobus SJ, et al. Impact of gene expression profiling-based risk stratification in patients with myeloma receiving initial therapy with lenalidomide and dexamethasone. *Blood* 2011; 118:4359-62.
4. Fonseca R, Barlogie B, Bataille R, et al. Genetics and cytogenetics of multiple myeloma: a workshop report. *Cancer Res* 2004; 64:1546-58.
5. Zhou Y, Barlogie B, Shaughnessy JD Jr. The molecular characterization and clinical management of multiple myeloma in the post-genome era. *Leukemia* 2009; 23: 1941-56.
6. Smadja NV, Bastard C, Brigaudeau D, et al. Hypodiploidy is a major prognostic factor in multiple myeloma. *Blood* 2001; 98:2229-38.
7. Fonseca R, Blood E, Rue M, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood* 2003; 101:4569-75.
8. Fonseca R, Harrington D, Oken MM, et al. Biological and prognostic significance of interphase fluorescence in situ hybridization detection of chromosome 13 abnormalities (delta13) in multiple myeloma: an Eastern Cooperative Oncology Group Study. *Cancer Res* 2002; 62:715-20.
9. Shaughnessy J. Amplification and overexpression of CKS1B at chromosome band 1q21 is associated with reduced levels of $p27^{Kip1}$ and an aggressive clinical course in multiple myeloma. *Hematology* 2005; 10(suppl):117-26.
10. Zhan F, Colla S, Wu X, et al. CKS1B, overexpressed in aggressive disease, regulates multiple myeloma growth and survival through SKP2- and p27Kip1-dependent and -independent mechanisms. *Blood* 2007; 109:4995-5001.
11. Nemec P, Zemanova Z, Greslikova H, et al. Gain of 1q21 is an unfavorable genetic prognostic factor for multiple myeloma patients treated with high-dose chemotherapy. *Biol Blood Marrow Transplant* 2010; 16:548-54.
12. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. *Blood* 2006; 108:2020-202.
13. Avet-Loiseau H, Attal M, Moreau P, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe francophone du Myelome. *Blood* 2007; 109:3489-95.
14. Singhal S, Mehta J, Desikan R, et al. Antitumor activity of thalidomide in refractory multiple myeloma. *N Engl J Med* 1999; 341:1565-71.
15. Dimopoulos MA, Zervas K, Kouvatseas G, et al. Thalidomide and dexamethasone combination for refractory multiple myeloma. *Ann Oncol* 2001; 12:991-5.
16. Barlogie B, Zangari M, Spencer T, et al. Thalidomide in the management of multiple myeloma. *Semin Hematol* 2001; 38:250-9.
17. Barlogie B. Thalidomide and CC-5013 in multiple myeloma: the university of Arkansas experience. *Semin Hematol* 2003; 40;(suppl 4):33-8.

18. Rajkumar SV, Richardson PG, Hideshima T, et al. Proteasome inhibition as a novel therapeutic target in human cancer. *J Clin Oncol* 2005; 23:630-9.
19. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med* 2003; 348:2609-17.
20. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. *N Engl J Med* 2008; 359:906-17.
21. Jagannath S, Durie BG, Wolf J, et al. Bortezomib therapy alone and in combination with dexamethasone for previously untreated symptomatic multiple myeloma. *Br J Haematol* 2005; 129:776-83.
22. Richardson PG, Chanan-Khan A, Schlossman RL, et al. Phase II trial of single agent bortezomib (Velcade) in patients with previously untreated multiple myeloma (MM). *Blood* 2004; 104(suppl):100a.
23. Harousseau JL, Attal M, Coiteaux V, et al. Bortezomib plus dexamethasone as induction treatment prior to autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: preliminary results of an IFM phase II study. *J Clin Oncol* 2005; 23(suppl):598s.
24. Mateos MV, Blade J, Diaz Mediavilla J, et al. A phase I/II national, multicenter, open-label study of bortezomib plus melphalan and prednisone (VMP) in elderly untreated multiple myeloma patients. *Blood* 2004; 104(suppl):943a.
25. 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; 28:4630-4.
26. Mateos MV, Gutiérrez NC, Martín-Ramos ML, et al. Outcome according to cytogenetic abnormalities and DNA ploidy in myeloma patients receiving short induction with weekly bortezomib followed by maintenance. *Blood* 2011; 118:4547-53.
27. Jagannath S, Richardson PG, Sonneveld P, et al. Bortezomib appears to overcome the poor prognosis conferred by chromosome 13 deletion in phase 2 and 3 trials. *Leukemia* 2007; 21:151-7.
28. Sagaster V, Ludwig H, Kaufmann H, et al. Bortezomib in relapsed multiple myeloma: response rates and duration of response are independent of a chromosome 13q-deletion. *Leukemia* 2007; 21:164-8.
29. Chang H, Trieu Y, Qi X, et al. Impact of cytogenetics in patients with relapsed or refractory multiple myeloma treated with bortezomib: adverse effect of 1q21 gains. *Leuk Res* 2011; 35:95-8.
30. Ahmann GJ, Jalal SM, Juneau AL, et al. A novel three-color, clone-specific fluorescence *in situ* hybridization procedure for monoclonal gammopathies. *Cancer Genet Cytogenet* 1998; 101:7-11.
31. Cumova J, Kovarova L, Potacova A, et al. Optimization of immunomagnetic selection of myeloma cells from bone marrow using magnetic activated cell sorting. *Int J Hematol* 2010; 92:314-9.
32. Ross FM, Avet-Loiseau H, Drach J, et al. European Myeloma Network recommendations for FISH in myeloma. *Haematologica* 2007; 92:abstract 114.
33. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. *Leukemia* 2006; 20:1467-73.
34. Fonseca R, Bergsagel PL, Drach J, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. *Leukemia* 2009; 23:2210-21.
35. Sawyer JR, Waldron JA, Jagannath S, et al. Cytogenetic findings in 200 patients with multiple myeloma. *Cancer Genet Cytogenet* 1995; 82:41-9.
36. Stewart AK, Fonseca R. Prognostic and therapeutic significance of myeloma genetics and gene expression profiling. *J Clin Oncol* 2005; 23:6339-44.
37. Sawyer JR, Tricot G, Mattox S, et al. Jumping translocations of chromosome 1q in multiple myeloma: evidence for a mechanism involving decondensation of pericentromeric heterochromatin. *Blood* 1998; 91:1732-41.
38. Fonseca R, Van Wier SA, Chng WJ, et al. Prognostic value of chromosome 1q21 gain by fluorescent in situ hybridization and increase CKS1B expression in myeloma. *Leukemia* 2006; 20:2034-40.
39. Sawyer JR. The prognostic significance of cytogenetics and molecular profiling in multiple myeloma. *Cancer Genet* 2011; 204:3-12.
40. Sonneveld P, Schmidt-Wolf IG, van der Holt B, et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/GMMG-HD4 trial. *J Clin Oncol* 2012; 30:2946-55.
41. Chang H, Trieu Y, Qi X, et al. Bortezomib therapy response is independent of cytogenetic abnormalities in relapsed/refractory multiple myeloma. *Leuk Res* 2007; 31:779-82.
42. Dimopoulos MA, Kastritis E, Christoulas D, et al. Treatment of patients with relapsed/refractory multiple myeloma with lenalidomide and dexamethasone with or without bortezomib: prospective evaluation of the impact of cytogenetic abnormalities and of previous therapies. *Leukemia* 2010; 24:1769-78.
43. Nemec P, Zemanova Z, Kuglik P, et al. Complex karyotype and translocation t(4;14) define patients with high-risk newly diagnosed multiple myeloma: results of CMG2002 trial. *Leuk Lymphoma* 2012; 53:920-7.
44. Attal M, Harousseau JL, Leyvraz S, et al. Maintenance therapy with thalidomide improves survival in patients with multiple myeloma. *Blood* 2006; 108:3289-94.
45. Hanamura I, Stewart JP, Huang Y, et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. *Blood* 2006; 108:1724-32.
46. Reece D, Trieu Y, Chang H, et al. Treatment of relapsed and refractory multiple myeloma in patients with p53 deletion. *Blood* 2008; abstract 1724.
47. Greslikova H, Zaoralova R, Filkova H, et al. Negative prognostic significance of two or more cytogenetic abnormalities in multiple myeloma patients treated with autologous stem cell transplantation. *Neoplasma* 2010; 57:111-7.