GAIN(1)(q21) IS AN UNFAVORABLE GENETIC PROGNOSTIC FACTOR FOR RELAPSED MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE, BUT NOT FOR THOSE TREATED WITH BORTEZOMIB.

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ABSTRACT

Prognostic impact of specific chromosomal aberrations in relapsed multiple myeloma (MM) patients treated with the novel agents is briefly described. We analyzed the prognostic value of extended panel of chromosomal aberrations (del(13)(q14), del(17)(p13), t(4;14)(p16;q32), gain(1)(q21) and hyperdiploidy using I-FISH technique in a cohort of 127 relapsed MM patients treated with thalidomide or bortezomib-based protocols. In the thalidomide group, we found significant difference in overall survival (OS) between group of patients with and without gain(1)(q21)(15.7 versus 41.3 months; P=0.004). We confirmed negative impact of cumulative effect of two or more cytogenetic changes occurring simultaneously on OS in the thalidomide group (20.3 months vs. not yet reached; P=0.039). We did not find any

significant impact of studied aberrations on overall survival in the bortezomib cohort of patients. We conclude that bortezomib-based protocols are able to overcome the negative prognostic impact of tested chromosomal abnormalities in relapsed MM patients.

INTRODUCTION

Multiple myeloma (MM) is an incurable malignant disease of terminal developmental stages of B-lymphocytes. MM represents approximately 10-15% of all hematological malignancies and 1-2% of all cancers [1]. Despite recent progress in the treatment management, survival of myeloma patients 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 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 prognosis [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 poor prognosis [6-8]. 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 newly diagnosed MM patients 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 anti-angiogenic effects. Thalidomide 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 confirmed among patients who had failed high-dose therapy [16,17]. Effects of bortezomib, the first proteasome inhibitor used in human therapy [18], combined with other agents were reported in various groups of patients [19-24]. Recent results have shown that bortezomib induction improves outcome of newly diagnosed patients with t(4;14)(p16;q32) [25,26] but not the outcome of patients with del(17p) and that it may overcome the negative prognostic impact of del(13)(q14) and t(4;14)(p16;q32) in relapsed MM patients [27-29].

In this paper, we evaluated the clinical and biologic impact of extended panel of chromosomal abnormalities (del(13)(q14), del(17)(p13), t(4;14)(p16;q32), gain(1)(q21) and

hyper/non-hyperdiploidy) detected by FISH in a group of 127 relapsed MM patients treated with either thalidomide or bortezomib-based protocols.

MATERIALS AND METHODS

Patient's Characteristics

Between April 2004 and December 2009, 528 patients with relapsed MM were treated with either with thalidomide-based protocols or with bortezomib-based protocols in the Faculty Hospital Brno, Czech Republic. All patients were included into 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: minimum of 1 previous line of therapy, combination therapy with glucocorticoids and/or alkylating agents (not monotherapy), no transplantation in bortezomib or thalidomide line of therapy. In addition, patients who have not finished treatment and patients who received less than two cycles of therapy were not included in our analyses. After selection, a total of 127 patients (thalidomide group n = 60, bortezomib group n = 67) was eligible for further analyses. In thalidomide and bortezomib group, 68% (41/60) and 43% (29/67) resp., of patients received one previous therapy and 32% (19/60) and 57% (38/67) resp., received ≥ two previous therapy. Patients' characteristics and disease features are shown in Table 1.

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

Plasma Cell Detection And Cell Sorting

For detection of clonal plasma cells (PCs) in bone marrow samples, in 78 cases we used immunofluorescent labeling of cytoplasmic light chain (cIg-FISH) as previously reported by Ahmann *et al.* [30]. In the last 49 cases, we used cell sorting techniques. Detailed protocol of cell sorting used in our center was described elsewhere [31]. Briefly, cut off level of 5% for CD138+ PCs infiltration in bone marrow was established, thus either MACS (Miltenyi Biotecs) or FACS (BD Biosciences) technique (<5% FACS, >5% MACS, respectively) was used according to the manufacturer´s instructions.

Interphase Fluorescence *In Situ* Hybridization

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 Multi-Color Probe Panel) (Abbott Molecular Inc., Des Plaines, USA). Hyperdiploidy was defined as gain of at least two of three evaluated chromosomes in a single cell. Gain(1)(q21) was assessed using fluorescent labeled bacterial artificial chromosome (BAC) (clone RP11-205M9); protocols for BAC isolation and labeling were followed from online resources of University in Bari, Italy (http://www.uniba.it). Slide preparation and FISH analyses were performed according to manufacturer's protocols (Abbott-Vysis). We used cut-off values recommended by the European Myeloma Network [32] - 20 % cut-off for deletions and numerical aberrations and 10 % cut-off for translocations and IgH rearrangements. Minimum 100 cells were scored in each sample. Digital image analysis was assessed by fluorescent microscope Olympus BX-61 equipped with a CCD Camera Vosskuhler 1300D 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 using the Kaplan-Meier method; differences between survival curves were analyzed using the log-rank test. In multivariate analysis, International Staging System (ISS) stage, monoclonal immunoglobulin isotype, albumin, β2-microglobulin, and C-reactive protein (CRP) levels were analyzed using the Cox regression. Associations between chromosomal abnormalities were estimated by the Fisher´s exact test with P-value <0.050 was considered significant. All statistical analyses were performed using Statistica 7.1 software (St atSoft, Inc., Tulsa, OK, USA 2005).

RESULTS

Incidence Of Chromosomal Abnormalities In Relapsed Multiple Myeloma Patients

The incidence of the five chromosomal abnormalities in the group of 127 relapsed MM patients is summarized in Table 2. Hyperdiploidy was found in nearly half of all patients (45%, 23/51), while 55% cases were non-hyperdiploid. Gains of chromosomes 5, 9 and 15 in the hyperdiploid

group of patients were found in 49%, 47% and 51%, respectively. Monosomy 13/del(13)(q14) was detected in 57/102 (56%), del(17)(p13) was found in 13/86 (15%), gain(1)(q21) occurred in 50/89 (56%) of patients. Translocation t(4;14)(p16;q32) was observed in 25/89 (28%) cases. Both del(13)(q14) and t(4;14)(p16;q32) were more often found in non-hyperdiploid patients (10/23 vs.19/28, P=0.09; 2/23 vs. 7/28, P=0.07; respectively). None or one chromosomal aberration was found in 7/22 (32%), simultaneous incidence of two or more structural cytogenetic abnormalities was found in 42% of cases (24/57).

The differences in incidence of aberrations in thalidomide group vs. bortezomib group were tested by Fisher's exact test. We found no significant difference between these groups (Table 2).

Association Between Clinical Parameters And Chromosomal Abnormalities

We analyzed correlations between standard clinical parameters including beta2-microglobulin, LDH, serum calcium, CRP, hemoglobin, or serum albumin and presence of the studied chromosomal abnormalities. In the thalidomide group, we found association of del(13)(q14) with lower hemoglobin level (P=0.007). In the thalidomide group, no further statistical differences of clinical parameters correlated with presence or absence of any studied chromosomal aberration were observed.

In the bortezomib group, patients without del(13)(q14) had higher level of CRP (P=0.016). We observed a trend to higher proportion of normal CRP values in patients with del(13)(q14) (P=0.053) Patients lacking del(17)(p13) had a trend to lower level of albumin than patients with this deletion (P=0.052). Patients with pathological values of CRP mostly lacked del(17)(p13) (P=0.03). Patients lacking t(4;14)(p16;q32) had higher level of hemoglobin and lower level of B-2-microglobuline (P=0.026 and P=0.38, respectively).

Prognostic Relevance Of Evaluated Chromosomal Aberrations

In the thalidomide group, the treatment response was evaluated in 97% (58/60) of patients; two patients with unknown response rate (ORR) died without progression 60 days after end of treatment, and the response rate could not be evaluated. ORR in this cohort of patients was reached by 50% (29/58) of patients, including sCR in 3.4% (2/58), CR in 5.1% (3/29), VGPR in 10.3% (6/58) and PR in 31.1% (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) patients. Three of four patients with unknown response rate died without progression 60 days after end of treatment, one patient had non-secretory MM, and thus, the response rate could not be evaluated. ORR was reached by 41% (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.

We observed significant difference in overall survival (OS), between thalidomide- treated groups of patients with gain(1)(q21) compared to those without gain(1)(q21) (15.7 vs. 41.3 months; P=0.004). No differences in OS were found between patients with and without other evaluated aberrations in this group. We only observed worse OS in patients with del(17)(p13) (median survival 8.5 vs. 41.3 months). We have also analyzed the prognostic impact of simultaneous incidence of structural chromosomal aberrations with negative prognostic impact [del(13)(q14), del(17)(p13), gain(1)(q21) and t(4;14)(p16;q32)]. In the thalidomide group, patients with two and more cytogenetic changes (20.3 months vs. not yet reached; P=0.039) and also patients with three and more changes (10.1 months vs. not yet reached; P=0.027) had statistically significant shorter OS (Figure 1). No significant difference was found in TTP when subgroups of patients with and without any selected aberrations were compared. In the bortezomib group, we did not find any significant impact of studied aberrations on OS, TTP, PFS or DOR (Table 4).

DISCUSSION

In our study, we sought to investigate prognostic impact of an extended panel of recommended chromosomal abnormalities [del(13)(q14), del(17)(p13), t(4;14)(p16;q32), gain(1)(q21) and hyper/non-hyperdiploidy] using I-FISH [34] in a cohort of 127 relapsed myeloma patients treated either with thalidomide or bortezomib-based protocols.

Findings of clonal chromosomal aberrations in PC are considered one of the most important prognostic factors in MM patients. Unfortunately, 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 using I-FISH find chromosomal abnormalities in up to 90% of MM patients [36]. Del(13)(q14) detected by conventional metaphase analysis; del(17)(p13), and translocations t(4;14)(p16;q32) or t(14;16)(q32;q23) detected by FISH have been described as adverse risk factors in newly diagnosed MM patients who undergo conventional chemotherapy or high-dose therapy with hematopoietic stem-cell transplantation [7,8,13]. Also, gain of (1q), one of the most common recurrent chromosomal aberration in hematological malignancies, has been reported to be associated with shorter OS and with disease progression [37,38]. Nevertheless, the outcome of MM patients has dramatically improved in the past decade due to the introduction of new, more effective treatments and better appreciation of potential complications and their management. However, prognostic impact of

high-risk chromosomal abnormalities in MM in the era of novel therapies has not been clearly defined yet [39]. While the impact of chromosomal changes on the outcome of bortezomib-treated relapsed/refractory MM patients is widely accepted, there is very limited data available on the efficacy of thalidomide-based regimens.

In our cohort group of 127 relapsed MM patients, we verified that MM patients can be divided into two homogenous genetic subgroups according to presence of extra copies of odd-numbered chromosomes. Hyperdiploidy was found in 45% of all patients (23/51), 55% of cases were non-hyperdiploid. Gains of chromosomes 5, 9 and 15 were found in 49%, 47% and 51%, respectively. We found del(13)(q14) in 57% of cases (57/102), gain(1)(q21) was observed in 63% (56/89), del(17)(p13) in 15% (13/86). The t(4;14)(p16;q32) translocation was found in 28% (25/89). The incidence of evaluated chromosomal changes was in agreement with previously published data [25-28]; however, the incidence of t(4;14)(p16;q32) was higher when compared to previous reports [4,7,13]. Recently, we published results of our study with newly diagnosed MM patients [40], where incidence of t(4;14)(p16;q32) matched previously published data from other groups. However, here we report results of a selected group of patients with progression, where higher incidence of chromosomal changes with negative prognostic impact could be suspected.

Several studies evaluated the influence of chromosomal abnormalities on response to bortezomib-based treatment protocols. Recent data have confirmed that both del(13)(q14) and t(4;14)(p16;q32) have impact on OS [25,26] and that simultaneous incidence of these aberrations could be overcome by bortezomib-based regimens in relapsed/refractory MM patients [41]. However, bortezomib seems be to ineffective for relapsed/refractory MM patients with gain(1)(q21) [29].

In accordance with published data, we did not find any negative effect of del(13)(q14) and t(4;14)(p16;q32) on our bortezomib cohort. We found shorter OS (18.3 vs. 37.2 months; P=0.10) in patients with del(17)(p13); probably due to the low number of positive cases, the difference was not statistically significant. We did not find any significant negative prognostic impact of gain(1)(q21); the observed difference in survival function been deep below the limit for statistical significance, even though nearly half of patients in the bortezomib group had to undergo third line of treatment. This could be caused by the fact, that the median of follow-up is shorter than the median OS and also by positive effect of bortezomib-based treatment protocols.

There is limited knowledge about impact of chromosomal abnormalities on thalidomide-based regimens used in MM patients. Attal *et al.* reported shorter EFS in patients treated with

thalidomide with presence of del(13)(q14) compared to patients lacking del(13)(q14) [42]. In our thalidomide cohort, we did not observe that patients who lack– deletion of chromosome 13 would have significant benefit from thalidomide treatment. In accordance to previous results [43], presence of gain (1)(q21) was associated with shorter OS in relapsed patients (15.7 vs. 41.3 months; P=0.004). Similarly to data reported by Reece *et al.* [44], we observed a worse OS in patients with del(17)(p13) (median of survival 8.5 vs. 41.3 months), but due to low number of positive cases with this aberration, these results could not be statistically evaluated in this study. However, our findings suggest that in relapsed MM patients, thalidomide is unable to overcome negative prognostic impact of heterogeneous deletion of *TP53* in 17p13 loci. In the hyperdiploid group of patients, we did not observe any significant impact on OS, TTP, PFS or DOR. ORR after used treatment was not influenced by the presence or absence of any studied chromosomal aberrations in patients treated with thalidomide-based protocols.

Taking together, the thalidomide and bortezomib-based protocols are both effective approaches for treatment of myeloma patients and induce durable responses in relapsed patients. However, thalidomide appears to be ineffective in patients with gain of (1)(q21) and possibly in patients with del(17)(p13). Patients with gain(1)(q21) had worse prognosis based on OS (Figure 1) if treated by thalidomide, but this effect did not occur in the bortezomib group, where OS of patients with and without gain(1)(q21) was very similar (29 months vs. 31 months, resp., Table 3) and about twice as long as in gain(1)(q21) positive patients treated by thalidomide (15 months vs. 29 months, resp.). In our study, no other monitored aberration had any impact on efficiency of used treatment. Similarly to our previous results [45], in the thalidomide cohort we confirmed the cumulative effect of two and more cytogenetic changes occurring simultaneously; this is clearly connected with shorter OS (Figure 1). In the bortezomib group, we observed only a very weak trend to shorter OS in patients with three or more aberrations detected by I-FISH (Table 3); quite surprisingly, there was a trend to shorter OS in patients with del(13)(q14). This result may be caused by the effect of t(4;14)(p16;q32), which was often found together with del(13)(q14), thus accumulating adverse cytogenetic factors in these particular patients. This is also supported by our previous results obtained from newly diagnosed MM patients [40].

In conclusion, our results suggest that thalidomide-based regimens are not able to overcome the unfavorable impact of gain (1)(q21). We observed the same trend in patients with del(17)(p13). However, further studies are required for confirmation of effects of novel agents, such bortezomib in relapsed/refractory MM patients.

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FINANCIAL DISCLOSURE

All authors have no conflict of interest (including any financial relationship with companies/products).

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Table 1. Clinical and biological characteristics of 127 relapsed MM patients treated with thalidomide or bortezomib-based regimens

Characteristics	*All patients*	*Thalidomide*	*Bortezomib*
Sex			
Male	61/127 (48%)	25/60 (42%)	36/67 (54%)
Female	66/127 (52%)	35/60 (58%)	31/67 (46%)
Age median			
(at the time of therapy, years); range	64 (42-87)	65 (48-87)	64 (42-85)
Follow-up median			
(from therapy, months); range	19.9 (1.5-55.4)	21.4 (2.6-55.4)	18.3 (1.5-54.2)
Durie-Salmon stage (from therapy)			
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)			
A	107/127 (84%)	54/60 (90%)	53/67 (79%)
B	20/127 (16%)	6/60 (10%)	14/67 (21%)
ISS stage (from therapy)			
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			
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%)
IgD	7/127 (5.5%)	4 (6.7%)	3 (4.5%)
IgM	2/127 (1.6%)	1 (1.7%)	1 (1.5%)
Non-secretory	1/127 (0.8%)	0	1 (1.5%)
IgG+IgM+biclone	1/127 (0.8%)	0	1 (1.5%)
Number of previous lines of therapy			
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; CRP - C-reactive protein; LDH - lactate dehydrogenase.

Table 2. Summary of cytogenetic findings in cohort of 127 MM patients treated with thalidomide or bortezomib based-regimens

Chromosomal aberrations	***All patients***	***Thalidomide***	***Bortezomib***	***P-value***
del(13)(q14) positive	56% (57/102)	48%(21/44)	62%(36/58)	0.1641
del(17)(p13) positive	15% (13/86)	10%(4/41)	20%(9/45)	0.2355
t(4;14)(p16;q32)positive	28% (25/89)	23%(10/43)	32%(15/46)	0.3550
gain(1)(q21) positive	56%(50/89)	48%(19/40)	63%(31/49)	0.1974
hyperdiploidy	45%(23/51)	39%(7/18)	48%(16/33)	0.5671
non-hyperdiploidy	55%(28/51)	61%(11/18)	52%(17/33)	

Table 3. Correlation between cytogenetic aberrations and response rate, time to progression and overall survival in patients treated with thalidomide and bortezomib-based regimens.

Chromosomal Abnormality	*ORR*				*TTP (months)*				*OS (months)*			
	thalidomide	*P*	*bortezomib*	*P*	*thalidomide*	*P*	*bortezomib*	*P*	*thalidomide*	*P*	*bortezomib*	*P*
del(13)(q14) positive	7/20(35.0%)	.232	15/34(44.1%)	.244	11.7	.803	13.9	.443	20.3	.180	18.3	.097
del(13)(q14) negative	12/22(54.5%)		5/20(25.0%)		13.5		12.5		41.3		37.2	
del(17)(p13) positive	2/4(50.0%)	1.000	3/8(37.5%)	1.000	-	-	-		8.5	**-**	18.3	.109
del(17)(p13) negative	19/35(54.3%)		13/34(38.2%)		16.5		12.9	-	41.3		37.2	
t(4;14)(p16;q32) positive	5/10(50.0%)	1.000	5/15(33.3%)	.349	15.3	.752	11.2		-	.856	15.8	.196
t(4;14)(p16;q32) negative	17/32(53.1%)		14/28(50.0%)		16.5		12.9	.554	32.4		31.2	
gain(1)(q21) positive	6/18(33.3%)	.334	13/29(44.8%)	1.000	15.3	.935	14.0		15.7	**.004**	29.0	.146
gain(1)(q21) negative	11/21(52.4%)		8/18(44.4%)		12.8		12.2		41.3		37.1	
hyperdiploidy	3/7(42.9%)	1.000	7/16(43.8%)	.716	-	-	12.9	.938	41.3	.386	31.2	.390
non-hyperdiploidy	5/11(45.5%)		5/16(31.3%)		11.7		13.9		30.4		18.3	
0-1 ab.*	5/11(45.5%)	1.000	2/11(18.2%)	.171	11.5	.186	-	.926	-	**.039**	37.1	.330
2+ ab.	8/20(40.0%)		16/37(43.2%)		19.7		-		20.3		29.0	
0-1 ab.**	5/11(45.5%)	1.000	2/11(18.2%)	.419	11.5	-	-		-	**.027**	37.1	.149
3+ ab.	4/9(44.5%)		7/19(36.8%)		11.7		-	--	10.1		14.7	

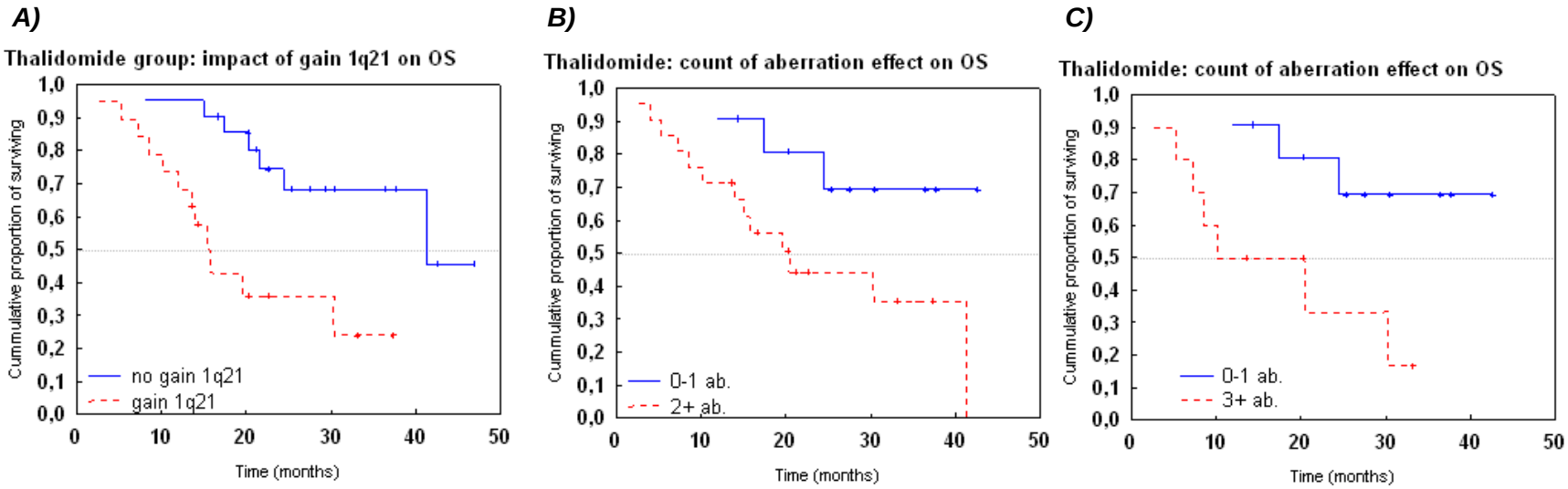
Abbreviations: ORR-overall response rate; TTP-time to progression; OS- overall survival.

* comparison of patients with 0-1 aberration vs. 2 and more aberrations

**comparison of patients with 0-1 aberration vs. 3 and more aberrations

Figure 1. Effect of chromosomal abnormalities on overall survival of patients treated by thalidomide-based regimens**.**

Impact of gain(1)(q21) and cumulative impact of simultaneous occurrence of structural chromosomal abnormalities on overall survival (OS).



A) Impact of gain(1)(q21) on OS; n=44, 21/44 pts.; *P*=.004

B) Impact of 2 or more chromosomal abnormalities on OS; n = 49; 21 vs. 28 pts., *P*=.027

C) Impact of 3 or more chromosomal abnormalities on OS: n = 49; 21 vs. 17 pts., *P*=.039