head and neck) and melanomas, cyclin D1 is activated by gene amplification and is associated with poor prognosis. CCND1 overexpression have also been found in 25-50% of multiple myeloma (MM) cases. A molecular classification of MM, named TC classification, stratifies patients into five groups (TC1-TC5) based on the presence of the recurrent IgH chromosomal translocations and cyclins D expression. Patients overexpressing CCND1 can be divided into two groups: TC1, characterized by the t(11;14) or t(6;14) translocation with overexpression of CCND1 or CCND3 and a non hyperdiploid status and TC2, with low to moderate levels of CCND1, absence of any primary IGH translocation and a hyperdiploid status. *Aims*. To assess CCND1 gene and cyclin D1 protein overexpression in a series of primary MM patients, to explore its relationship to the presence of the t(11;14), and to evaluate frequency and distribution of trisomy 11 in the different TC groups. Methods. fluorescence *in situ* hybridization (FISH) analysis with specific probes for CCND1 gene amplification (probe mixture of cyclin D1 band 11q13 - CEP 11 bands 11p11-q11) and t(11;14)(q13;q32) were performed on CD138-purified plasmacells from bone marrows of thirty MM patients at diagnosis. Cyclin D1 protein expression and intensity was evaluate by immunohistochemistry. *Results.* FISH analysis revealed CCND1 overexpression in 14/30 cases (46.6%) and the presence of the t(11;14) translocation in 9/30 cases (30%) (Table 1). Patients with evidence of the t(11;14) showed strong nuclear staining for cyclin D1 (TC1 group) and 8 out 9 demonstrated CCND1 overexpression. The remaining 6 out 15 cases with increased CCND1 gene copy numbers lacked the t(11;14) and showed low to negative levels of cyclin D1 protein (TC2 group). Globally, the frequency of trisomy 11 was 40% (12/30 patients). It was demonstrated in 3 out 9 cases carrying the t(11;14) (TC1), 5 out 6 overexpressing CCND1 without the translocation (TC2) and 4 out 15 negative for both alterations (TC3-TC5). *Conclusion.* In our data, trisomy 11 don't seems to cause directly overexpression of CCND1 as it is present in 4/15 patients without overexpression of CCND1 and in 3/9 patients carrying the t(11;14). One patient belonging to the TC2 group, overexpresses CCND1 and lacks both trisomy and translocation suggesting that cyclin D1 can be dysregulated by additional mechanisms. In TC2 group trisomy 11 probably may be considered as a recurrent polysomy of the hyperdiploid status.

Table 1.

Case	Age	Sex	MC	FISH* CCND1	FISH t(11;14)	Trisomy	IHC§ Cyclin D1
1	58	F	Gk	(3-4)	t(11;14)	–	++++
2	57	M	Gk	(3-5)	t(11;14)	+11	++++
3	72	F	Ak	(3-4)	t(11;14)	+11	++++
4	49	F	k	(3-7)	t(11;14)	–	++++
5	71	M	l	(3-4)	t(11;14)	–	+++
6	80	F	Gk	–	t(11;14)	–	+++
7	60	F	Gk	(3)	t(11;14)	–	+++
8	68	M	Ak	(3-4)	t(11;14)	+11	+++
9	66	M	Al	(3-4)	t(11;14)	–	+++
10	57	F	Ak	(3)	–	+11	+
11	62	M	Gk	(3)	–	+11	+
12	40	M	Gk	(3-4)	–	+11	+
13	70	M	Gl	(3)	–	+11	++
14	60	M	Al	(3-4)	–	+11	–
15	64	F	Gk	(3-5)	–	–	–
16	56	M	l	–	–	–	++
17 63	M	Al	–	–	–	–	
18	74	F	Al	–	–	+11	–
19	62	M	Al	–	–	–	–
20	72	F	Gk	–	–	–	–
21	65	F	Ak	–	NP	+11	–
22	61	M	Gk	–	–	–	–
23	63	F	Ak	–	–	+11	–
24	64	M	Gl	–	NP	–	–
25	63	F	l	–	–	–	–
26	71	M	Gk	–	–	–	–
27	73	F	Gk	–	–	–	–
28	54	M	Ak	–	–	–	–
29	72	F	Gl	–	–	–	–
78	M	Gl	–	–	–	–	–

**In the presence of CCND1 overexpression, the number of copies for each gene is indicated. NP: not performed; MC: monoclonal component. §IHC score ++++ >75% tumor cell positive; +++ 50-75% tumor cells positive, 25-50% tumor cells positive, +10-25% tumor cells positive.*

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RAPID DETECTION OF RESPONSE TO BORTEZOMIBE-BASED REGIMEN IN MULTIPLE MYELOMA USING FREE LIGHT CHAIN ASSAYS

R.H. Hajek,[1] P. Pour,[2] H. Novotna,[2] Z. Cermakova,[2] Z. Adam,[2] M. Krejci,[2] K. Havlikova[2]

[1]*Faculty Hospital Brno, Brno, Czech Republic;* [2]*Faculty Hospital, Brno, Czech Republic*

Background. Immunoassays measuring free light chains (FLC) in serum are useful for diagnosis and monitoring of multiple myeloma (MM). FLC assay is also perspective tool as early indicator of response due to short half-life of light chains. This feature would be helpful in the clinical decision of therapy continuation. Bortezomibe (Velcade) represents powerful anti-myeloma agent with rapid clearance of monoclonal immunoglobulin (M-Ig). More than half, respectively 83% responding patients (pts.) achieved first response after second cycle, respectively after cycle 4 in APEX trial. The benefit of FLC assay in detection of responders and non-responders to bortezomibe based regimens has never been evaluated. *Aims*. To evaluate the possibility of using the FLC assay as early marker of sensitivity or resistance to bortezomibe based regimens. *Methods.* Patients with at least one relapse of MM were prospectively evaluated on day 1 and 11 of every cycle of bortezomibe based regimen during the course of their treatment. The sensitivities of serum FLC assays (lambda or kappa, index l/k) and M-Ig analysis using immunoelectrophoresis for detection of early response were compared in 3 categories: time to reduction of parameters to 25% (MR), 50% (PR) and 75% of the pretreatment value. Data of 24 patients from total of 37 pts. who underwent at least 5 cycles of therapy with median 7 (5-8) cycles were chosen for pilot analysis. *Results*. Total of 13 pts. (54%) responded to the therapy with 4% of CR, 29% of NCR (only immunofixation poz.), 21% of PR. Further 17% pts. achieved minimal response and 25% of pts. (6/24) had stable disease. The significant difference was found in the time of response detection: PR (>50%) was achieved on day 22/44/66 of the treatment in 41.7%/54.2%/54.2% (single chain), in 16.7%/16.7/33.3 (index λ/κ) and in 12.5%/37.5/54.2 (M-Ig). The difference was statistically significant when comparing single chain vs. M-Ig on day 22 (p=0.026), on day 44 (p=0.005) but not on day 66 and not when comparing the index l/k vs. M-Ig. Direct comparison of timing of response (time to reduction to 75%, 50% and 25% of pretreatment value) was done for well-correlated parameters single chains and M-Ig. Difference at the first level of response (MR - reduction to 75%) was not found (median 16.6 vs. 22 days; p=0.424) but the results were significantly better for single chains at the level of PR [50% reduction; median 16.5 (range 11-44) vs. median 44.0 (range 22-111); p<0.001] and at the level of 75% reduction. [Median 11.0 (range 11-55) vs. median 66.0 (range 33-88); p=0.054]. We did not observe sustained PR in pts. who had not responded before day 33 if FLC assay was used. *Summary/Conclusions*. In the pivotal analysis we have confirmed that FLC assays is perspective tool for detection of early responding pts. with MM treated with bortezomibe based regimens. On the contrary, the FLC assay has potential to be used as a marker of early resistance. The trial is under way and actual results will be presented.

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BORTEZOMIB THERAPY IN MULTIPLE MYELOMA EXPERIENCE OUTSIDE THE CONTEXT OF A CLINICAL TRIAL

S. Sachanas, M.C. Kyrtsonis, Z. Galanis, V. Greca, T.P. Vassilakopoulos, K. Anargyrou, S. Chrysochoou, M. Dimou, G. Panagoulias, S.I. Kokoris, E.M. Dimitriadou, M.K. Angelopoulou, N. Viniou, P. Panayiotidis, G.A. Pangalis

University of Athens, Athens, Greece

Backgrounds. Bortezomib (Velcade™) reversibly and selectively inhibits the proteasome and degrades primarily ubiquitinated proteins. Bortezomib produces high response rates in MM, as shown by two large clinical trials. Based on these results, the drug was approved for the treatment of relapsed and refractory multiple myeloma as a third or second line therapy. *Aims.* To present our experience with Bortezomib treatment in relapsed and refractory MM patients treated according to approved indications, outside the context of a clinical trial. Patients and Methods. 52 MM patients (38 males and 14 females) with a median age of 70 years were treated with Bortezomib. Immunoglobulin type was IgG in 30 (2 with secondary plasma cell leukemia), IgA in 15, BJ in 6 and IgD in 1. 36 patients had already received 2 treatment lines while 14 one. 46% had been previously treated with thalidomide At treatment ini-