Concomitant gain of 1q21 and MYC translocation define a poor prognostic subgroup of hyperdiploid multiple myeloma

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SUPPLEMENTAL MATERIAL

METHODS

Patients

We included 274 German multiple myeloma (MM) patients of the prospective, randomized phase 3 HOVON-65/ GMMG-HD4 trial into this study of whom enriched CD138+ plasma cells were available. Patient characteristics and the trial design have recently been described[1]. The HD4 compared the effect of bortezomib-based treatment before and after ASCT (arm B) to standard treatment without this drug (arm A)[1]. Of 274 patients investigated in this study 143 and 131 were treated in arm A and B, respectively. The trial was done in accordance with the Declaration of Helsinki (Version 1996) and was approved by the local ethics committees of all participating institutions. We obtained written informed consent from the patients for treatment and sample procurement.

Cytogenetic analyses

Interphase fluorescence in situ hybridization was performed as previously described[1]. A three-color MYC (8q24) break-apart probe (Kreatech, Amsterdam, Netherlands) was used to detect *MYC* aberrations. Ploidy was assessed using gains of at least two of the chromosomes 5, 9, 11, 15 and 19. A 5-chromosome combination has a sensitivity of ~90% and a specificity >90% for identification of hyperdiploid MM[2]. Subclones were defined as aberrations found in less than 60% of analyzed cells, if at least one aberration was detected in more than 80% of cells[3].

Gene expression analyses

For gene expression analysis we used the HD4 data set deposited in ArrayExpress (accession number E-MTAB-2299) collected using U133 Version 2.0 plus arrays (Affymetrix). As chip definition file (CDF) we used the Affymetrix U133 Version 2.0 plus array CDF (v17) mapping to Entrez genes[4]. Expression data were normalized using GC-RMA. Two known batch effects were corrected using Combat[5].

Statistical analysis

Fisher's exact test was used to compare the distribution of MYC abnormalities between cohorts. Group comparison of expression data was done using the Mann-Whitney Wilcoxon test. PFS and OS were calculated from the time of start of treatment and the survival rates were estimated using the method of Kaplan and Meier. The log-rank test and the Cox proportional hazards model were used to perform group comparisons and assess the impact of prognostic factors, respectively. The Bonferroni-Holm method was used if results were corrected for multiple testing. Prognostic subgroups were identified using recursive partitioning as implemented in the R package party[6]. Briefly, subgroups of patients with significantly distinct prognosis are identified based on their association with selected clinico-pathological parameters. A hierarchical tree is built top-down starting with all patients. The first split is based on the parameter with the strongest association with survival. Subgroups are split until no further significant association between survival and any parameter is found or subgroups become too small. The statistical analyses were carried out using the R software package 3.1.1.

SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL TABLES

Supplemental Table 1: MYC aberrations in multiple myeloma plasma cells

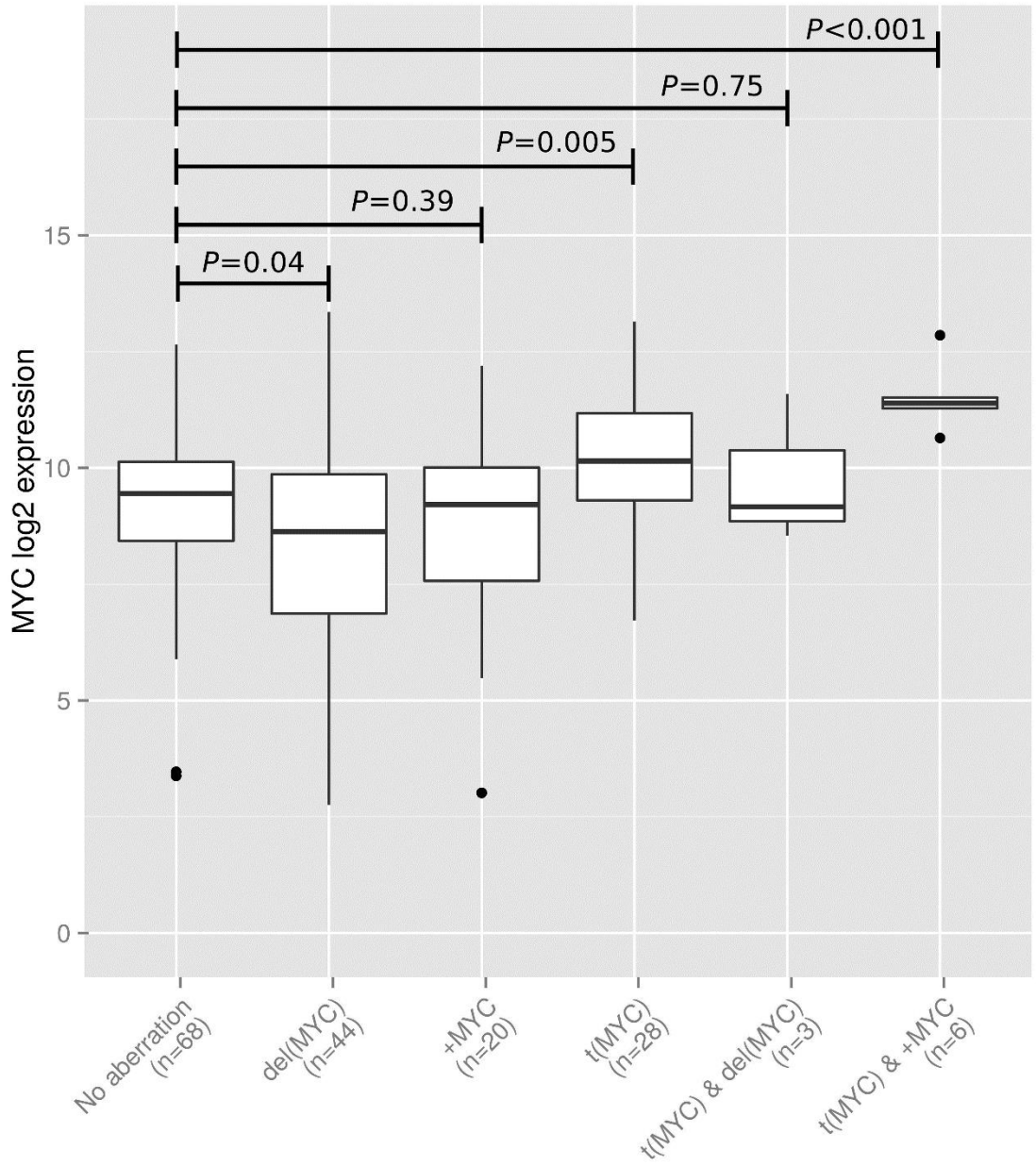
Group		N_{all}	Translocation			Gain			Deletion		
			N	%	P	N	%	P	N	%	P
Multiple myeloma		274	62	22.6		39	14.2		67	24.5	
Hyperdiploid[a]	yes	153	39	25.5		26	17.0		24	15.7	
	no	116	23	19.8	0.31	13	11.2	0.22	41	35.3	<0.001*
t(4;14)	yes	38	6	15.8		7	18.4		14	36.8	
	no	236	56	23.7	0.40	32	13.6	0.45	53	22.5	0.07
t(11;14)	yes	49	10	20.4		5	10.2		21	42.9	
	no	225	52	23.1	0.85	34	15.1	0.5	46	20.4	0.002*
t(14;16)	yes	3	1	33.3		-	-		-	-	
	no	271	61	22.5	0.54	39	14.4	-	67	24.7	-
del(17p13)	yes	32	8	25		6	18.8		10	31.2	
	no	242	54	22.3	0.82	33	13.6	0.42	57	23.6	0.38
+1q21	yes	93	25	26.9		22	23.7		16	17.2	
	no	181	37	20.4	0.23	17	9.4	0.003*	51	28.2	0.05*
ISS[a]	I	108	16	14.8		13	12		31	28.7	
	II\III	154	45	29.2	0.01*	25	16.2	0.38	33	21.4	0.19

[a]Ploidy status and ISS stage were available for 269 and 262 cases, respectively.

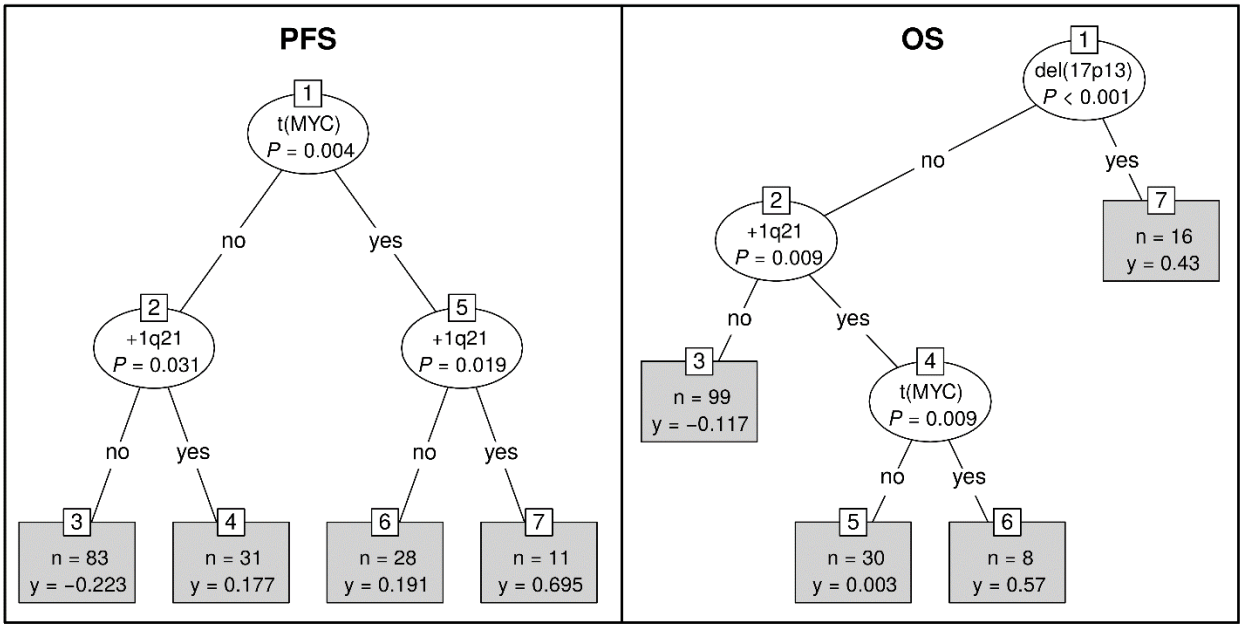
Supplemental Table 2: Multivariate analysis of prognostic impact in hyperdiploid MM

n=146	PFS		OS	
Variable	**HR**	***P***	**HR**	***P***
t(MYC)	1.68	0.02	1.64	0.15
+1q21	2.19	<0.001	3.17	<0.001
del(17p13)	1.77	0.10	3.59	0.005
t(4;14)	1.46	0.34	1.71	0.27
ISSII	1.66	0.03	2.74	0.02
ISSIII	2.27	0.002	4.36	0.002

SUPPLEMENTAL FIGURES



Supplemental Figure 1: Impact of *MYC* locus aberrations on *MYC* expression in MM PC. A three-color MYC (8q24) break-apart probe was used to detect MYC aberrations. Gene expression data of CD138+ PC was collected using Affymetrix U133 Version 2.0 plus arrays. The plot shows MYC expression in samples with no aberrations of the MYC locus at 8q24, with deletions of the locus (del(MYC)), gains (+MYC), translocations involving the locus (t(MYC)) and concomitant t(MYC) and del(MYC) or t(MYC) and +MYC.



Supplemental Figure 2: Prognostic subgroups of hyperdiplod MM. We performed recursive partitioning of hyperdiploid MM including t(MYC), +1q21 and del(17p13) as predictors. The plots show tree-structured survival models for PFS and OS. Y-values >0 in the terminal nodes of the tree indicate inferior outcome.