Combining information regarding chromosomal aberrations t(4;14) and del(17p13) with the International Staging System classification allows stratification of myeloma patients undergoing autologous stem cell transplantation

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ABSTRACT

Background
Chromosomal abnormalities have been shown to play a major role in disease evolution of multiple myeloma. Specific changes in interphase cells can be detected by fluorescent *in situ* hybridization, which overcomes the problem of the lack of dividing cells required for conventional cytogenetics.

Design and Methods
We analyzed the prognostic value of 12 frequent chromosomal abnormalities detected by fluorescent *in situ* hybridization in a series of patients (n=315) with newly diagnosed, symptomatic multiple myeloma. All patients underwent frontline autologous stem cell transplantation according to the GMMG-HD3- or GMMG-HD4-trial protocols or analogous protocols.

Results
Univariate statistical analyses revealed that the presence of del(13q14), del(17p13), t(4;14), +1q21 and non-hyperdiploidy was associated with adverse progression-free and overall survival rates independently of the International Staging System (ISS) classification. Multivariate analyses showed that only t(4;14) and del(17p13) retained prognostic value for both progression-free and overall survival. According to the presence or absence of t(4;14) and del(17p13) and the patients' International Staging System classification, the cohort could be stratified into three distinct groups: a group with a favorable prognosis [absence of t(4;14)/del(17p13) and ISS I], a group with a poor prognosis [presence of t(4;14)/del(17p13) and ISS II/III] and a group with an intermediate prognosis (all remaining patients). The probabilities of overall survival at 5 years decreased from 72% in the favorable prognostic group to 62% (hazard ratio 2.4; P=0.01) in the intermediate and 41% (hazard ratio 5.6; P<0.001) in the poor prognostic groups.

Conclusions
These results have implications for risk-adapted management for patients with multiple myeloma undergoing high-dose chemotherapy followed by autologous stem cell transplantation and suggest that new treatment concepts are urgently needed for patients who belong to the poor prognosis group. As targeted therapies evolve, different treatment options might have variable success, depending on the underlying genetic nature of the clone.

Key words: prognostic stratification, multiple myeloma chromosomal aberrations, fluorescent *in situ* hybridization, International Staging System.

Citation: Neben K, Jauch A, Bertsch U, Heiss C, Hielscher T, Seckinger A, Mors T, Müller NZ, Hillengass J, Raab MS, Ho AD, Hose D, Goldschmidt H. Combining information regarding chromosomal aberrations t(4;14) and del(17p13) with the International Staging System classification allows stratification of myeloma patients undergoing autologous stem cell transplantation. Haematologica 2010;95:1150-1157. doi:10.3324/haematol.2009.016436

KN and AJ contributed equally to this manuscript.

Acknowledgments: the authors would like to thank Katrin Heimlich, Maria Dörner and Hildegard Bethäuser for technical assistance in the enrichment of CD138 positive plasma cells and Michaela Brough, Desireé Kirn, and Stephanie Pschowski-Zuck for interphase FISH analyses.

Funding: this work was supported in part by grants from the Hopp-Foundation, Germany, the University of Heidelberg, Germany, the National Center for Tumor Diseases, Heidelberg, Germany, and the Tumorzentrum Heidelberg/Mannheim, Germany.

Manuscript received on August 31, 2009. Revised version arrived on November 17, 2009. Manuscript accepted on December 18, 2009.

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Introduction

Multiple myeloma (MM) is a malignant lymphoproliferative B-cell disease characterized by the accumulation of monoclonal plasma cells in the bone marrow. Although some progress has been achieved over time in the management of MM patients leading to improved survival, especially of younger patients, MM remains an incurable disease. The median survival after conventional treatment is 3–4 years, whereas high-dose chemotherapy followed by autologous stem cell transplantation (SCT) can extend the median survival to 5–7 years.[1,2] The course of the disease differs greatly, with some patients dying of refractory MM within a few weeks while others live for more than 10 years. For this reason several prognostic factors and staging systems have been developed to determine the disease behavior, define therapeutic strategies and to predict long-term outcome.

The combination of serum β2-microglobulin level, one of the most consistent predictors of survival in MM, with serum albumin concentration has been proposed as an outcome predictor in the new International Staging System (ISS).[3] This classification predicts outcome of patients managed with high-dose chemotherapy followed by autologous SCT as well as those treated with approaches based on novel agents and is applicable in both younger and older patients. However, the lack of inclusion of factors related to tumor biology, such as cytogenetic or molecular markers, may hamper its clinical use.

Fluorescent *in situ* hybridization (FISH) can detect specific changes in interphase cells, overcoming the problem of the lack of dividing cells required for conventional cytogenetics. Based on ploidy data, hyperdiploid and non-hyperdiploid forms of MM have been delineated, representing two major pathogenic pathways.[4,5] Hyperdiploid MM is characterized by the accumulation of extra copies of chromosomes. Multiple trisomies most frequently involve chromosomes 3, 5, 7, 9, 15, 19, and 21. By clustering analysis of chromosomal abnormalities, we recently found that non-hyperdiploid MM can be separated into three subgroups: first, the translocation t(11;14), which juxtaposes the immunoglobulin heavy chain locus (IgH) to the oncogene *cyclin D1*; second, deletion of 13q14, which is frequently associated with translocation t(4;14) and involves the oncogenes *MMSET* and *FGFR3*; and third, gain of 1q21.[6] Previous studies have identified the presence of del(13q14), del(17p13), +1q21 as well as t(4;14) and t(14;16) detected by FISH as predictors of shorter overall survival,[7-12] while t(11;14) was associated with improved survival.[13,14]

The aim of the current study was to correlate frequent recurrent chromosomal aberrations, determined by interphase FISH, with patients' outcome in 315 treated patients who received high-dose chemotherapy followed by autologous SCT in our center. We analyzed whether the presence of genomic abnormalities confers prognostic information on progression-free survival and overall survival in addition to that provided by the widely used ISS classification. Subsequently, we considered whether combinations of different chromosomal aberrations analyzed in our cohort are suitable for risk stratification.

Design and Methods

Patients

We evaluated a series of 315 consecutive patients with MM from a single institution who were tested for cytogenetic abnormalities by FISH. Approval was obtained from the institutional review board of the University of Heidelberg for this study. The subjects studied provided informed consent according to the Declaration of Helsinki. The study group consisted of 178 males and 137 females with a median age of 59 years at first transplantation (range, 25-73 years). According to the ISS, 147 patients were in stage I, 101 in stage II and 47 in stage III, while 20 patients could not be classified due to missing β2-microglobulin and/or albumin data.

All patients underwent front-line high-dose chemotherapy with melphalan 200 mg/m^2 and autologous SCT according to the protocols of the GMMG-HD3- or GMMG-HD4-trial or analogous protocols. Briefly, patients with newly diagnosed symptomatic MM in Salmon and Durie stage II or III received induction therapy with either three cycles of VAD (vincristine, adriamycin, dexamethasone) or related regimens (n=244), TAD (thalidomide, adriamycin, dexamethasone; n=29) or PAD (bortezomib, adriamycin, dexamethasone; n=42).[15,16] Stem cells were mobilized after chemotherapy with cyclophosphamide, adriamycin, and dexamethasone, applied 4-6 weeks after induction treatment, supported by granulocyte colony-stimulating factor injections until collection. After stem cell collection, all patients were treated with one (n=138) or two (n=177) courses of high dose melphalan 200 mg/m^2 followed by autologous SCT. Maintenance therapy was given to 221 patients and consisted of either α-interferon (n=118), thalidomide (n=66) or bortezomib (n=37), whereas 94 patients were not given maintenance therapy. At relapse, 47 patients were treated with a second (n=19) or third (n=28) line of high-dose chemotherapy followed by autologous SCT.

Cytogenetic analyses

Density gradient centrifugation of bone marrow aspirates over Ficoll Hypaque (Biochrom, Berlin, Germany) was performed to separate mononuclear cells using a standard protocol. CD138$^+$ plasma cells were isolated by magnetic-activated cell sorting using anti-CD138 immunobeads and an auto–magnetic-activated cell sorter (MACS) separation system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. The purity of the isolated cells was confirmed by flow cytometric analysis of CD38$^+$ and CD138$^+$ phenotypes.

Interphase FISH analysis was accomplished on CD138-purified plasma cells as previously described[6] using probes for chromosomes 1q21, 5p15/5q35, 6q21, 8p21, 9q34, 11q23, 13q14.3, 15q22, 17p13, 19q13, and 22q11, and for the translocations t(11;14)(q13;q32.3), t(4;14)(p16.3;q32.3) and t(14;16)(q32.3;q23). Hybridization was performed according to the manufacturer's instructions (Kreatech, Amsterdam, the Netherlands, and Vysis, Downers Grove, IL, USA). A total of 100 interphase nuclei per probe were evaluated using a DM RXA epifluorescence microscope (Leica, Wetzlar, Germany). Hybridization efficiency was validated on interphase nuclei obtained from the peripheral blood and bone marrow of a healthy donor. The thresholds for gains, deletions, and translocations were set at 10%. The score described by Wuilleme *et al.* was used to assess ploidy.[5] Gains of at least two of the three chromosomes 5, 9 and 15 were used for the FISH definition of hyperdiploidy.

Statistical analysis

Progression-free and overall survival were calculated from the time of the first autologous SCT and the survival rates were esti-

mated 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. For the multivariate analysis of all chromosomal aberrations multiple imputations of missing values were performed using a bootstrap approach as implemented in the aregImpute function from the R add-on package Hmisc.[17] In order to control the family-wise error rate at the two-sided level of 0.05, univariate *P* values were adjusted for multiple testing using the Bonferroni-Holm correction.[18] Kendall's tau was used to assess the correlation between chromosomal aberrations. The statistical analyses were carried out using the software package R 2.8.0.[19]

Results

Frequencies of chromosomal aberrations

Chromosomal aberrations were detected in 294 of the 315 (93%) patients. Because of the small number of purified plasma cells in many specimens and the failure of FISH in some cases, we were not able to test the full set of probes in all patients. The exact number of probes tested is shown in Table 1. Interphase FISH analysis of CD138-enriched plasma cells revealed gains of chromosomes 1q21 (36%), 9q34 (62%), 11q23 (48%), 15q22 (55%), and 19q13 (55%), as well as deletions of chromosomes 6q21 (11%), 8p21 (19%), 13q14 (46%), 17p13 (10%) and 22q11 (15%). Furthermore, the frequency of the IgH-translocations t(11;14), t(4;14), and t(14;16) was 19%, 13%, and 2%, respectively. Ploidy status was analyzed in 160 samples of which 92 (57%) were hyperdiploid and 68 (43%) were non-hyperdiploid.

Correlation of chromosomal aberrations with the patients' outcome

For the entire group, the median overall survival and progression-free survival after the first high-dose chemotherapy was 6.4 and 2.2 years, respectively. We analyzed the prognostic impact of chromosomal aberrations on progression-free and overall survival (Table 1). While del(8p21), del(13q14), del(17p13), t(4;14), +1q21, +11q23, +19q13 and ploidy status showed a significant impact on progression-free survival, del(8p21), del(13q14), del(17p13), t(4;14), +1q21 and ploidy status were of statistical significance for overall survival. When *P* values were adjusted for ISS classification, all chromosomal aberrations listed above, except del(8p21), remained of statistical significance for both progression-free and overall survival. After adjustment of *P* values for multiple testing, del(13q14) as well as +1q21 had a significant impact on progression-free survival, while del(17p13) was of statistical significance for overall survival.

For del(17p13), serial analyses of different cut-offs of plasma cells presenting the abnormality showed that the most powerful cut-offs for predicting progression-free and overall survival were between 60-70%.

The progression-free survival at 3 years for patients with del(13q14) and +1q21 was, respectively, 27% (*versus* 46% for those without; *P*=0.013) and 24% (*versus* 50% for those without; *P*=0.018). In addition, the overall survival rate at 3 years for patients carrying del(17p13) was 50% (*versus* 81% for those without the deletion; *P*=0.018). Del(6q21), del(22q11), t(11;14), +9q34, +15q22 and +19q13 were not found to have any impact on either progression-free survival or overall survival.

Correlation of +1q21 and del(13q14) with other chromosomal aberrations

Since +1q21 and del(13q14) were identified as prognostic factors for progression-free survival in the univariate analysis (Table 1), we analyzed whether these chromosomal aberrations are associated with additional chromosomal changes and the ISS score (Table 2). Deletion of 13q14 was positively linked with the presence of t(4;14), +1q21, del(17p13), del(22q11) and del(8p21), while +1q21 positively correlated with the presence of t(4;14) and the ISS score. Notably, both +1q21 and del(13q14) were linked with the presence of chromosomal aberrations t(4;14) or

Table 1. Univariate analysis of prognostic impact of chromosomal abnormalities on progression-free and overall survival.

Aberration yes vs. no	N. of patients analyzed (n)	Incidence	Progression-free survival 3-year Kaplan-Maier (%)	Log-rank *P* value	Cox PH *P* value adjusted for ISS	Overall survival 3-year Kaplan-Maier (%)	Log-rank *P* value	Cox PH *P* value adjusted for ISS
del(6q21)	165	11%	47 *vs.* 43	0.845	0.790	61 *vs.* 74	0.443	0.64
del(8p21)	236	19%	40 *vs.* 40	0.011	0.012	64 *vs.* 77	0.040	0.053
del(13q14)	312	46%	27 *vs.* 56	<0.001	<0.001	72 *vs.* 82	0.037	0.041
del(17p13)	289	10%	27 *vs.* 44	0.010	0.020	50 *vs.* 81	<0.001	<0.001
del(22q11)	166	15%	39 *vs.* 45	0.629	0.860	67 *vs.* 74	0.294	0.62
t(4;14)	299	13%	17 *vs.* 46	0.003	0.003	49 *vs.* 82	0.005	0.011
t(11;14)	302	19%	30 *vs.* 45	0.219	0.240	79 *vs.* 77	0.855	0.720
t(14;16)	210	2%	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*
+1q21	281	36%	24 *vs.* 50	<0.001	<0.001	68 *vs.* 83	0.003	0.014
+9q34	182	62%	51 *vs.* 33	0.135	0.220	77 *vs.* 72	0.877	0.840
+11q23	295	48%	51 *vs.* 36	0.021	0.019	82 *vs.* 72	0.338	0.180
+15q22	184	55%	52 *vs.* 38	0.530	0.460	77 *vs.* 73	0.936	0.930
+19q13	235	55%	53 *vs.* 26	0.013	0.013	76 *vs.* 71	0.837	0.920
HD vs. NHD	160	57% (HD)	54 *vs.* 26	0.030	0.038	77 *vs.* 51	0.041	0.043

** These numbers were not calculated because of the small sample size (n=4). HD: hyperdiploid; NHD: non-hyperdiploid. Significance level is 0.05.*

Table 2. Correlation of del(13q14) and +1q21 with other chromosomal changes and the ISS score.

	del(13q14) negative samples		del(13q14) positive samples				+1q21 negative samples		+1q21 positive samples			
	N	%	N	%	Kendall´s Tau	*P* value	N	%	N	%	Kendall´s Tau	*P* value
t(4;14)	6 of 159	4	34 of 138	25	0.30	<0.001	17 of 180	9	21 of 99		0.16	0.006
+1q21	35 of 151	23	65 of 130	50	0.28	<0.001	-	-	-	-	-	-
del(13q14)	-	-	-	-	-	-	65 of 181	36	65 of 100	65	0.28	<0.001
del(17p13)	6 of 151	4	23 of 138	17	0.21	<0.001	15 of 178	8	14 of 100	14	0.09	0.15
del(22q11)	8 of 84	10	17 of 82	21	0.16	0.04	13 of 95	14	12 of 70	17	0.05	0.54
del(8p21)	17 of 124	14	27 of 112	24	0.13	0.04	22 of 147	15	22 of 89	25	0.12	0.06
del(6q21)	6 of 85	7	12 of 80	15	0.13	0.1	13 of 95	14	5 of 70	7	-0.10	0.18
t(11;14)	30 of 160	19	25 of 140	18	-0.01	0.84	35 of 180	19	15 of 100	15	-0.06	0.35
+9q34	64 of 90	71	49 of 92	53	-0.18	0.01	64 of 104	62	47 of 74	64	0.02	0.79
+11q23	96 of 166	58	54 of 144	38	-0.20	<0.001	97 of 181	54	38 of 100	38	-0.15	0.01
+19q13	80 of 122	66	49 of 113	43	-0.22	<0.001	82 of 142	58	46 of 88	52	-0.05	0.42
hyperdiploid	64 of 82	78	27 of 78	35	-0.44	<0.001	55 of 92	60	36 of 68	53	-0.07	0.39
t(4;14) or del(17p13)	11 of 98	11	50 of 103	49	0.41	<0.001	30 of 131	23	31 of 70	44	0.22	0.002
ISS II/III	75 of 153	49	73 of 141	52	0.03	0.64	77 of 167	46	59 of 98	60	0.14	0.03

Table 3. Multivariate analysis of prognostic impact of chromosomal abnormalities and ISS score on progression-free survival (PFS) and overall survival (OS) (multivariate Cox proportional hazards analysis).

Aberration yes vs. no	PFS (n=280)		OS (n=280)	
	HR	*P* value	HR	*P* value
del(8p21)	1.46	0.26	1.39	0.45
del(13q14)*	1.49	0.16	1.16	0.74
del(17p13)	2.12	0.01	3.43	<0.001
+1q21*	1.29	0.44	1.31	0.59
+11q23	0.81	0.47	0.68	0.36
+11q13	1.19	0.54	1.40	0.42
+14q13	1.44	0.31	0.59	0.42
+19q13	0.76	0.32	1.23	0.57
t(4;14)	2.12	0.01	2.21	0.03
t(11;14)	1.22	0.53	1.23	0.67
ISS-Score II	1.42	0.10	2.18	0.016
ISS-Score III	1.45	0.17	2.93	0.003

**patients with del(13q14) or +1q21 and neither del(17p13), or t(4;14). Significance level is 0.05.*

del(17p13), which were associated with high-risk myeloma in previous studies.[10,11] Chromosomal aberrations t(4;14) or del(17p13) were observed in 49% and 44% of samples carrying del(13q14) or +1q21, respectively. In contrast, the frequency of t(4;14) or del(17p13) was significantly lower in del(13q14) or +1q21 negative samples, being 11% (P<0.001) and 23% (P=0.002), respectively.

Multivariate analysis

Since we found that del(13q14) and +1q21 were strongly correlated with the presence of high-risk aberrations such as t(4;14) or del(17p13), we tried to assess the prognostic value of del(13q14) and +1q21 independently of the high-risk aberrations. In a multivariate Cox proportional hazards analysis we considered the presence of del(13q14) and +1q21 in the absence of all high-risk aberrations (Table 3). In this model t(4;14) and del(17p13) were the only chromosomal aberrations with a statistically significant impact on progression-free survival and overall survival, whereas all other aberrations lost the significance seen in univariate analysis (Table 1). In addition the ISS score showed significant results for overall survival (Figure 1).

Development of an International Scoring System/fluorescence in situ hybridization-based prognostication scheme

In our cohort of patients the ISS score was of prognostic significance for overall survival but not for progression-free survival (Figure 1). Since ISS score, t(4;14) and del(17p13) were the most relevant variables in the multivariate Cox proportional hazards model, we analyzed whether combining the ISS score with information on the presence of high-risk aberrations such as t(4;14) or del(17p13) could improve the prognostic value with regard to patients' outcome. A combination of the presence or absence of t(4;14) and del(17p13) with the ISS classification score allowed patients to be stratified into three distinct groups (Figure 2, Table 4): a group with a favorable prognosis [absence of t(4;14)/del(17p13) and ISS I], a group with poor prognosis [presence of t(4;14)/del(17p13) and ISS II/III] and a group with intermediate prognosis (all remaining patients). Most of the patients belonged to the favorable (42%) and intermediate (44%) prognostic groups, whereas 14% were allocated to the poor prognostic group. The ISS/FISH-based prognostication scheme was able to predict both progression-free and overall survival. The median progression-free survival times for the favorable, intermediate and poor prognosis groups were 2.7 years, 2.0 years (hazard ratio 1.4; P=0.09) and 1.2 years (hazard ratio 2.9; P<0.001), respectively. The probabilities of overall survival at 5 years decreased from 72% in the favorable prognostic group to 62% (hazard ratio 2.4; P=0.01) and 41% (hazard ratio 5.6; P<0.001) in the intermediate and poor prognostic groups, respectively.

Discussion

This study includes a large series of patients with newly diagnosed myeloma, analyzed for genomic aberrations, enabling the description of definitive incidences of the most frequent chromosomal abnormalities. All interphase FISH studies were performed on CD138-enriched plasma cells, which were analyzed with a comprehensive set of 12 different DNA probes specific for the most recurrent chromosomal aberrations observed in MM. The incidence of chromosomal abnormalities detected in the present series by FISH (93%) is notably higher than that usually obtained by conventional cytogenetics[20,21] or in other FISH-based studies which were not performed on sorted plasma cells.[22] Moreover, all the patients were treated homogeneously with an intensive strategy (autologous SCT in all cases), which allows for valuable prognostic analyses. In contrast to previous studies,[11,22] survival analysis was calculated from the day of stem cell rescue and not from the day of diagnosis, because this retrospective analysis was not performed on an intention-to-treat basis. Since induction therapy was often performed in other centers, information about death or progressive disease prior to autologous SCT is not available for all of our patients.

Gains of odd chromosomes, such as 9q34 (62%), 15q22 (55%), 19q13 (55%) and 11q23 (48%), were the most frequent abnormalities, which are typically seen in hyperdiploid samples.[4,5] Gains of 1q21 (36%) are of special interest, since amplifications of the 1q and/or deletions of 1p arm have been described recently to be predictors of poor outcome in the context of high-dose chemotherapy.[12] In addition, Shaughnessy *et al.* investigated the gene expression profile of 532 newly diagnosed patients with myeloma and identified a 70-gene subset as an independent predictor of outcome end-points in a multivariate analysis.[23] Interestingly, 30% of genes included in the 70-gene predic-

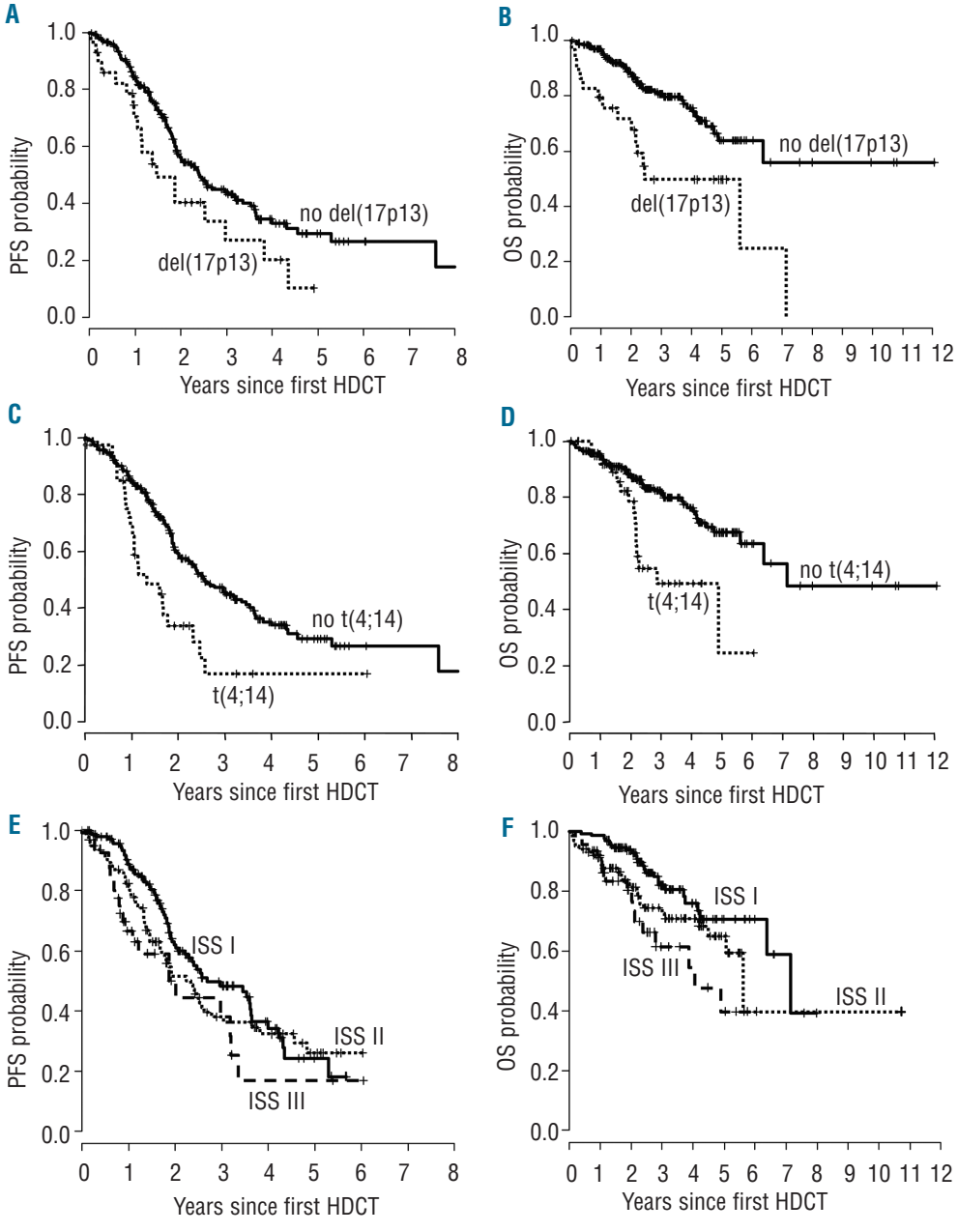

Figure 1. Impact of del(17p13), t(4;14) and the ISS score on progression-free survival (PFS) and overall survival (OS) after high-dose chemotherapy (HDCT) followed by autologous SCT. (A-D) Myeloma patients were stratified by the presence or absence of each one of the specific cytogenetic abnormalities showing statistical significance in the univariate and multivariate analyses. (E, F) Prognostic value of the ISS score, analyzed in 295 patients. The ISS score was of prognostic significance for OS but not for PFS. The probabilities for OS at 5 years decreased from 72% in the favorable prognostic group (ISS score I, n=147) to 66% (hazard ratio 2.0; *P*=0.017) in the intermediate prognostic group (ISS score II, n=101) and 39% (hazard ratio 2.8; *P*=0.002) in the poor prognostic group (ISS score III, n=47).

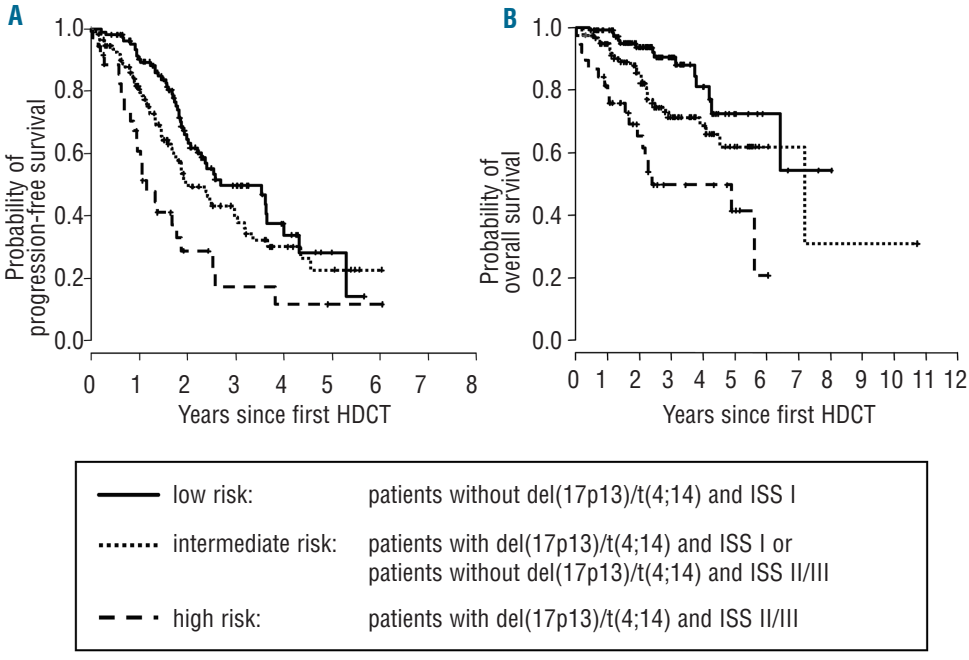

Figure 2. Combining information on chromosomal aberrations t(4;14) and del(17p13) with ISS score allows stratification of myeloma patients undergoing high-dose chemotherapy (HDCT) followed by autologous SCT. The combination of presence or absence t(4;14) and del(17p13) with the ISS score allowed stratification of patients into three distinct groups: favorable prognosis [absence of t(4;14)/del(17p13) and ISS I], poor prognosis [presence of t(4;14)/del(17p13) and ISS II/III] and intermediate prognosis (all remaining patients), representing 42%, 44% and 14% of patients, respectively.

tor map to chromosome 1, suggesting that deregulated expression of genes on chromosome 1 is of great importance for the clinical course of the disease in individual patients. In the current study, patients with +1q21 had shorter progression-free and overall survivals compared to patients without this chromosomal abnormality. However, +1q21 was not found to be an independent prognostic factor in the multivariate analysis, probably due to the fact that the presence of +1q21 was associated with other "high-risk" chromosomal abnormalities such as t(4;14).

Of all the deleted chromosomal regions analyzed in our patients, del(13q14) was the most frequent (46%), followed by deletions of chromosomal regions 8p21 (19%), 22q11 (15%), 6q21 (11%), and 17p13 (10%). According to previously published studies,[11,24] del(13q14) was predictive for both progression-free and overall survival with highly significant *P* values. However, del(13q14) was not found to be an independent prognostic factor in the multivariate analysis. In fact, most of the prognostic power of del(13q14) was related to t(4;14) and del(17p13), which are frequently associated with del(13q14). In patients lacking t(4;14) and del(17p13), del(13q14) was no longer prognostic, confirming previously published data.[11,25]

In the current study 34% of patients had translocations involving the immunoglobulin heavy chain gene on chromosome 14. The t(11;14) was found most frequently, occurring in 19% of our patients. The t(11;14) leads to up-regulation of the *CCND1* gene and was identified as a favorable prognostic factor in some recent studies.[13,14] However, in line with the results of the IFM99 trial, run by the *Intergroup Francophone du Myélome*,[11] the presence of t(11;14) had no statistical impact on outcome in the current study, assuming that outcome may be variable due to the role of other genetic or treatment-related factors. The t(4;14) was present in 13% of our myeloma patients. This translocation is known to deregulate two genes, *FGFR3* and *MMSET*, and was identified as an independent prognostic factor for progression-free and overall survival in agreement with previous studies.[11,26,27] The t(14;16) results in up-regulation of the c-maf proto-oncogene. Because of the low frequency of t(14;16) and the consecutively low number of cases (2% and n=4, respectively), we decided not to correlate this chromosomal aberration with the outcome of the patients, even though we made the clinical observation that all of the patients had an aggressive course of disease. These four patients showed progressive disease 4, 7, 18 and 24 months after autologous SCT. Notably, two patients developed a plasma cell leukemia at relapse. So far, two patients have died, only 8 and 14 months after autologous SCT.

Hyperdiploidy was of marginal prognostic significance in our cohort of patients. Most of the previous studies suggesting a favorable impact of hyperdiploidy on outcome were based on conventional cytogenetics[20,21] and thus restricted to the approximately one third of patients with an informative karyotype. The results obtained by conventional cytogenetics, in contrast to those gained by FISH, depend on the proliferation of myeloma cells. However, in line with our results, a marginal prognostic

Table 4. Prognostic impact of del(17p13) and t(4;14) in combination with the ISS score on progression-free and overall survival (Cox proportional hazards analysis).

		Progression-free survival		Overall survival	
	N	HR + 95% CI	*P* value	HR + 95% CI	*P* value
Low risk[1]	113 (42%)	1		1	
Intermediate risk[2]	119 (44%)	1.4 [0.9;2.1]	0.09	2.4 [1.2;4.8]	0.01
High risk[3]	38 (14%)	2.9 [1.8;4.8]	<0.001	5.6 [2.7;11.8]	<0.001

[1]patients without del(17p13)/t(4;14) and ISS I; [2]patients with del(17p13)/t(4;14) and ISS I or patients without del(17p13)/t(4;14) and ISS II/III; [3]patients with del(17p13)/t(4;14) and ISS II/III. The level of significance is 0.05.

impact on outcome was also observed in two large FISH-based studies including homogeneously treated patients.[11,25]

When the prognostic value of all chromosomal abnormalities was analyzed in a multivariate model, only t(4;14) and del(17p13) were associated with dismal progression-free and overall survival, which is in agreement with previous studies.[4,11,28] Therefore, both abnormalities could be considered to be the most important cytogenetic prognostic factors in MM patients treated with high-dose chemotherapy. Importantly, in our study t(4;14) and del(17p13) were prognostic for both progression-free and overall survival, whereas the ISS score failed to predict progression-free survival. The reason for this might be that the ISS score was primarily developed to predict survival only.[3] Since the presence of t(4;14) and del(17p13) was associated with an adverse outcome independently of the ISS, our results suggest that factors related to tumor biology, such as cytogenetics and molecular markers, are more informative in predicting disease behavior than the ISS classification alone.

Based on the results of the present multivariate analysis, we developed a prognostication scheme. The combination of information on the presence or absence of t(4;14) and del(17p13) together with the ISS classification allowed stratification of patients into three distinct groups: one group with a favorable prognosis [absence of t(4;14)/del(17p13) and ISS I], another group with a poor prognosis [presence of t(4;14)/del(17p13) and ISS II/III] and a third group with an intermediate prognosis (all remaining patients), representing 42%, 44% and 14% of patients, respectively. This reinforces previous results from other series of patients treated with conventional chemotherapy.[27] Fonseca and co-workers were able to stratify patients into three distinct categories: those with a poor prognosis [with t(4;14), t(14;16), or del(17p13)], those with an intermediate prognosis [with del(13q14)] and those with a good prognosis (all others), with median survivals of 24.7, 43.3 and 50.5 months, respectively (P<0.001). Although this and our prognostication scheme look quite similar, we decided to include the ISS as an additional parameter, since the ISS was identified as an independent prognostic factor with respect to overall survival in our series. In addition, del(13q14) was omitted, since we and other have shown in recent studies that del(13q14) is no longer of prognostic value in patients lacking t(4;14) and del(17p13).[11]

Based on our ISS/FISH scheme, only 14% of patients belong to the poor prognosis group. Interestingly, the gene expression study by Shaughnessy *et al.* showed similar results,[23] although a different method was applied to define high-risk patients. Kaplan-Meier estimates of overall survival in low-risk and high-risk myeloma showed lower 5-year actuarial probabilities of overall survival (28% *versus* 78%, P<0.001; HR = 4.51) in the 13.1% of patients with a high-risk signature. It thus seems that despite the fact that different treatment regimens were used in the two studies, comparable proportions of patients were found to be at high risk and it will be of interest for further analyses to compare gene expression-with ISS/FISH-based risk scores.

In our series, the ISS/FISH-based scheme predicted progression-free survival and overall survival much better than the ISS alone. Moreover, we were able to stratify our patients according to the ISS/FISH-based approach even though they were treated with different induction and maintenance therapies over time. Whether or not the adverse influence of high-risk cytogenetic abnormalities and ISS classification could be overcome by novel agents remains to be elucidated. In the current study, the impact of chromosomal aberrations with respect to different induction and maintenance therapies was not analyzed, because chromosomal aberrations with high-risk features, such as t(4;14) and del(17p13), were present in only 25% of patients and the groups would have been small for further subgroup analysis, yielding unreliable estimates. Recent reports indicate that the response rate to bortezomib is independent of cytogenetic abnormalities.[29,30] The first analysis of our HOVAN-65/GMMG-HD4 phase III trial showed that the response rate after induction therapy with bortezomib, adriamycin, and dexamethasone is independent of the presence of t(4;14) and del(13q14), whereas del(17p13) was not included in the analysis.[16]

In conclusion, our results show that at least part of the heterogeneity seen in the clinical course of MM patients after autologous SCT can be correlated with distinct chromosomal aberrations. In line with findings of the *Intergroup Francophone du Myélome*, we were able to confirm the relevance of t(4;14) and del(17p13) in an independent cohort of patients.[11] In the French study, β2-microglobulin levels (≤4 mg/L *versus* >4 mg/L) were found to give prognostic information that was independent of the influence of t(4;14) and del(17p13) on overall survival. One possible concern about this study was the use of a cut-off level of 4.0 mg/dL for defining high and low levels of β2-microglobulin, as various different cut-offs had been used in previous studies.[31-33] The ISS also comprises β2-microglobulin and was thus envisioned to incorporate the prognostic information given by the levels of this molecule, and was derived from a very large cohort of patients.[3] Since the ISS is a widely used staging system for MM patients and is consistently documented in medical reports (in contrast to its components), we feel that our results using the ISS instead of β2-microglobulin alone might lead to a broader use of our stratification scheme in daily clinical practice, even though, from a statistical point of view, the differences in outcome prediction using β2-microglobulin levels alone or the ISS might be small. In addition, our results have implications for the risk-adapted management of myeloma patients undergoing high-dose chemotherapy and suggest that new treatment concepts are urgently needed for patients who belong to the poor prognosis group, who have a median survival of only 2.4 years. As targeted therapies evolve, different treatment options might have variable success, depending on the underlying genetic nature of the clone.

Authorship and Disclosures

KN, AJ, UB, JH, MSR, ADH, DH, HG: substantial contributions to the design of the study, analysis and interpretation of the data, drafting or revising the article for intellectual content and final approval of the manuscript. KN wrote the paper; AJ, AS, TM, NZM and DH performed the laboratory work; CH and TH participated in the statistical analysis. HG is principal investigator of the GMMG HD3 and HD4 studies and takes primary responsibility for the manuscript.

The authors reported no potential conflicts of interest.

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