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

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

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Running head: Prognostic impact of MYC abnormalities.

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Letter to the Editor

The impact of MYC locus aberrations on outcome of multiple myeloma (MM) patients is still a matter of debate. Aim of this study was to further investigate the influence of them on survival of MM patients treated with high-dose chemotherapy. Our data suggest that the prognostic favorable hyperdiploid MM (HDMM) contains a subgroup with poor survival that is characterized by concomitant MYC translocation and gain of 1q21.

The role of the transcription factor MYC in the pathogenesis of MM has been extensively studied\(^1\). In contrast, the impact of MYC locus aberrations on outcome of MM patients has been insufficiently investigated and is still a matter of debate. Aim of this study was to further clarify the impact of t(MYC) and MYC locus copy number alterations on outcome of MM patients treated with high-dose chemotherapy and autologous stem cell transplantation (ASCT). Therefore we performed FISH analysis on CD138+ plasma cells of 274 German patients enrolled in the GMMG HD4 study (Please see Supplemental Material for details).

We detected t(MYC) in 62 (23%) samples (Supplemental Table 1). This value is similar to findings of a FISH\(^2\) and a next generation sequencing based study\(^3\) which detected MYC rearrangements in 15% and 21% of newly diagnosed patients, respectively. In our study gains of MYC (+MYC) were present in 39 (14%) and deletions (del(MYC)) in 67 (25%) cases. Concomitant t(MYC) and +MYC or t(MYC) and del(MYC) were detected in 9 and 6 samples, respectively. Altogether, 153 patients (55.8%) showed MYC aberrations, confirming results of a study that used FISH and comparative genomic hybridization and detected MYC aberrations in ~50% of MM patients\(^4\). A t(MYC) (34%), +MYC (50%) and del(MYC) (83%) frequently occurred in subclones only, indicating they are often not initiating events.

The frequency of t(MYC) was significantly higher in ISS stages II/III (\(P=0.01\)). A +MYC was more often found in cases with +1q21 (\(P=0.003\)). A del(MYC) was associated with non-hyperdiploid MM (NHDM) (\(P<0.001\)). Cases with a t(11;14) showed the highest frequency of del(MYC) (43%). They were less frequent in cases with +1q21 (\(P=0.05\)). In 14 (36%) cases with +MYC we detected a gain of 8p12 and 25 (37%) of samples with a del(MYC) contained a del(8p12), indicating that a significant portion of gained or lost signals of the MYC probe were likely due to trisomies or monosomies of chromosome 8,
respectively. Recently, Walker et al. detected an enrichment for t(14;16) as well as a depletion of t(4;14) and HDMM in samples with t(MYC). We found similar trends for t(14;16) and t4;14) (Supplemental Table 1) but the sample number in these subgroups in our set were too low to draw any conclusion. In contrast, t(MYC) was not depleted in HDMM in our set but rather showed a higher frequency. One possible explanation for these discrepancies was stated by Affer et al. Due to high material requirements in case of multiple molecular analyses, samples may be biased for larger tumor mass or aggressive clones. In the HD4 trial processing of MM samples for FISH analyses had priority and we included all patients into our study for whom FISH slides were available. Nevertheless, we cannot exclude sample bias.

MYC expression data were available for 172 samples (Supplemental Figure 1). Samples with del(MYC) had a lower MYC expression than samples without MYC aberrations (mean log2 expression: 8.1 vs 9.2, P=0.04). Samples with a +MYC showed no significant MYC expression difference (mean: 8.6, P=0.39). In samples with t(MYC) MYC was overexpressed (mean: 10.2, P=0.005), confirming results of Walker’s study. The overexpression is due to active super-enhancers in the translocation partner loci. Samples with concomitant +MYC and t(MYC) showed the highest mean expression level of MYC (11.5, P<0.001) but this result was based on 6 patients only. No significant difference could be detected for three samples with concomitant del(MYC) and t(MYC) (mean: 9.8, P=0.75).

We analyzed the prognostic impact of MYC aberrations using log-rank tests. For the entire analyzed group the median progression-free survival (PFS) time was 34.7 months; the median overall survival (OS) time was not yet reached. A t(MYC) showed a negative impact on PFS (median 28.4 vs 37.5 months, HR=1.42, P=0.03) and OS (median 68.6 months vs not reached, HR=1.64, P=0.03) (Figure 1). A +MYC was associated with worse OS (median 30.1 vs 35.7 months, HR=1.7, P=0.047) but showed no impact on PFS (HR=1.12, P=0.6) (Figure 1). For del(MYC) no significant effect on outcome was detected (Figure 1). Our data support results of Walker et al. who reported decreased PFS and OS for t(MYC) in MM patients included into the UK MRC Myeloma IX trial. Sekiguchi et al. presented a non-significant association of MYC abnormalities with inferior PFS in MM patients treated with bortezomib and dexamethasone. In contrast, Avet-Loiseau et al. could not detect a significant influence of t(MYC) on survival of MM patients enrolled in the IFM99 trials. Neither the UK nor the French trial included the novel drugs bortezomib and
lenalidomide.

We and others have recently shown the importance of stratified analyses in MM\(^1,7,8\). To check whether \( \textit{MYC} \) aberrations have different impacts on outcome of molecular subgroups, we performed an analysis stratified by karyotype. Whereas \( \text{t(MYC)} \) negatively impacted PFS (median 28.4 vs 41 months, HR=1.93, \( P=0.001 \)) and OS (median not reached, HR=2.29, \( P=0.008 \)) in HDMM, no significant effect could be detected in NHDMM (PFS: HR=0.9, \( P=0.7 \); OS: HR=1.14, \( P=0.7 \)) (\textbf{Figure 2}). A +\( \text{MYC} \) showed non-significant effects on OS in HDMM (HR=1.69, \( P=0.16 \)) and NHDMM (HR=1.97, \( P=0.08 \)) (data not shown). A del(MYC) did not influence outcome in any of the two ploidy subgroups (data not shown). According to these data \( \text{t(MYC)} \) is the only relevant \( \textit{MYC} \) aberration for determining the prognosis of HDMM but does not impact outcome of NHDMM.

The HD4 compared the effect of bortezomib-based treatment before and after ASCT (arm B) to standard treatment without this drug (arm A). HDMM with \( \text{t(MYC)} \) in arm B (n=20) showed no significant difference in PFS (28.4 months vs 33.1 months, \( P=0.48 \)) or OS (median not reached vs 69.8 months, \( P=0.6 \)) compared with patients treated in arm A (n=19), indicating that bortezomib did not overcome the impact of \( \text{t(MYC)} \).

As the negative impact of \( \text{t(MYC)} \) was only detectable in HDMM, we focused on this subgroup in an extended analysis. We performed recursive partitioning including \( \text{t(MYC)} \) and the unfavorable aberrations +1q21 and del(17p13). As this was an exploratory study we used the univariate test type. We identified HDMM with concomitant \( \text{t(MYC)} \) and +1q21 as poor prognostic group (\textbf{Supplemental Figure 2}). For PFS +1q21 and \( \text{t(MYC)} \) had a similar negative impact. The worst PFS was seen in cases with both aberrations (\textbf{Figure 3A}). Of note, cases with only one of these aberrations showed no difference in OS compared to cases without these aberrations (\textbf{Figure 3B}). In contrast, concomitant \( \text{t(MYC)} \) and +1q21 had a profound negative impact on OS of HDMM (\textbf{Figure 3B}). The findings from the OS analysis suggested a subgroup effect of \( \text{t(MYC)} \) and +1q21. Interaction analysis using Cox regression on OS showed a significantly different prognostic effect of \( \text{t(MYC)} \) for patients depending on presence of +1q21 (interaction \( P=0.048 \)). We performed a multivariate analysis including \( \text{t(MYC)} \), +1q21, del(17p13), t(4;14) and ISS and identified \( \text{t(MYC)} \) as independent predictor for PFS (HR = 1.68, \( P=0.02 \)) but not for OS (HR=1.64, \( P=0.15 \)) (\textbf{Supplemental Table 2}). The non-significant result for OS may be due to the association of \( \text{t(MYC)} \) with ISS stages II/III or a lack of statistical power.
Our analysis indicates that the negative impact on outcome of t(MYC) is restricted to HDMM and is due to an interaction between t(MYC) and +1q21. But was it the functional basis of this impact on survival and why is it apparently limited to HDMM? Linear regression of MYC aberrations on MYC expression levels explained only 12% of the MYC expression variance and several cases without t(MYC) showed high expression levels. In addition, t(MYC) had no significant impact on OS of patients without +1q21, making it unlikely that increased expression of MYC by itself leads to an aggressive phenotype or resistance in HDMM. Recently, Sawyer et al. presented a possible explanation for our findings. They showed that jumping translocations of 1q12 frequently lead to the simultaneous +1q21 and t(MYC), indicating that these aberrations are based on a common mechanism. This may result in further aberrations like del(17p), finally leading to high-risk MM.

An explanation for the apparent limitation of the impact to HDMM may be that NHDMN activates mechanisms or includes aberrations with effects that are equal or even stronger than t(MYC), obscuring the impact of t(MYC). As an example, Walker et al. recently showed that cases with MAF or MAFB translocations had a tendency to acquire mutations as a consequence of APOBEC deregulation, another potential mechanism leading to high-risk MM.

In conclusion, our data suggest that the prognostic favorable HDMM contains a subgroup with poor survival that is characterized by the presence of t(MYC) and +1q21. This study shows the importance of stratified analyses in a heterogeneous cancer like MM for the detection and investigation of further biomarkers of outcome.

**AUTHOR’S CONTRIBUTIONS**
AJ and NW designed research, analyzed data and wrote the manuscript. DK and MG performed FISH analyses, interpreted data and contributed to the manuscript. TH interpreted data and participated in writing the manuscript. HG, UB, GE, HS, IWB, KW, JH, and MSR accrued patients and participated in writing the manuscript. AS and DH processed samples and performed gene expression profiling. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT
The authors declare no competing financial interests.

REFERENCES
FIGURE LEGENDS

Figure 1. Impact of MYC aberrations on PFS and OS of MM. MM patients were stratified by the presence or absence of t(MYC) (A, D), +MYC (B, E) or del(MYC) (C, F). Patients at risk are shown below the figures.

Figure 2. Impact of t(MYC) on PFS and OS of ploidy subgroups of MM. MM patients were stratified by ploidy and the presence or absence of t(MYC). The impact of t(MYC) on outcome of hyperdiploid MM is shown in A and C. The corresponding data for non-hyperdiploid MM is shown in B and D. Patients at risk are shown below the figures.

Figure 3. Impact of concomitant t(MYC) and +1q21 on PFS and OS of hyperdiploid MM. Hyperdiploid MM patients were stratified by the presence or absence of t(MYC) and +1q21. Patients at risk are shown below the figures.
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. 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


SUPPLEMENTAL TABLES

Supplemental Table 1: MYC aberrations in multiple myeloma plasma cells

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<sup>a</sup>Ploidy status and ISS stage were available for 269 and 262 cases, respectively.

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

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<th>Variable</th>
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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 hyperdiploid 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.