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

The impact of *MYC* locus aberrations on the outcome of multiple myeloma (MM) patients is still a matter of debate. The aim of this study was to further investigate their influence on the survival of MM patients treated with high-dose chemotherapy. Our data suggest that the favorable prognosis factor of hyperdiploid MM (HDMM) contains a subgroup with poor survival that is characterized by concomitant MYC translocation (t(MYC)) 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 the outcome of MM patients has been insufficiently investigated and is still a matter of debate. The aim of this study was to further clarify the impact of t(MYC) and *MYC* locus copy number alterations on the 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 (see *Online Supplementary Material* for details).

We detected t(MYC) in 62 (23%) samples (*Online Supplementary Table S1*). 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%) cases 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 the 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 (NHDMM) (P<0.001). Cases with a t(11;14) showed the highest frequency of del(MYC) (43%). This was less frequent in cases with +1q21 (P=0.05). In 14 (36%) cases with +MYC we detected a gain of 8p12, and 25 (37%) 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).[3] We found similar trends for t(14;16) and t(4;14) (*Online Supplementary Table S1*), 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

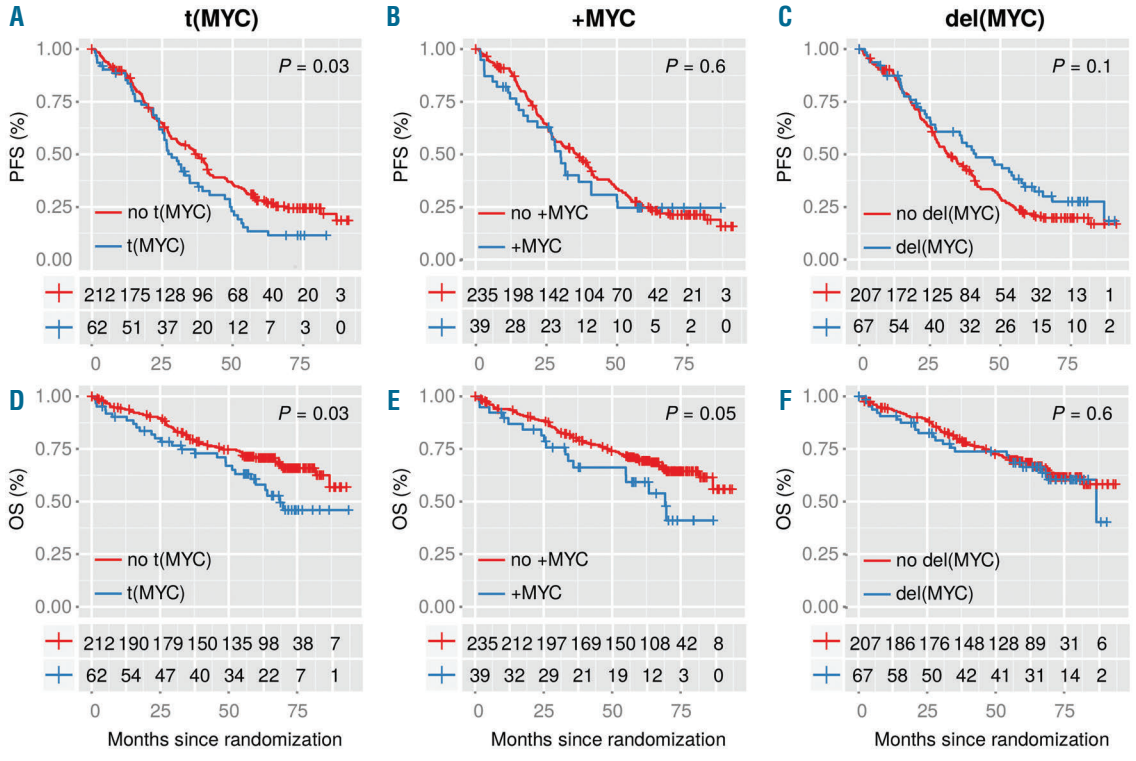

Figure 1. Impact of *MYC* aberrations on PFS and OS of MM. MM patients were stratified by the presence or absence of t(MYC) (A, D), +MYC (B, E) or del(MYC) (C, F). Patients at risk are shown below the figures.

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these discrepancies was stated by Affer *et al.*[4] Due to the high material requirements in the case of multiple molecular analyses, samples may be biased for larger tumor mass or aggressive clones. In the GMMG-HD4 trial, the processing of MM samples for FISH analyses had priority, and we included all patients in our study for whom FISH slides were available. Nevertheless, we cannot exclude sample bias.

MYC expression data were available for 172 samples (*Online Supplementary Table S1*). 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 the results of the study carried out by Walker *et al.*[3] The overexpression is due to active super-enhancers in the translocation partner loci.[3,4] 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 the results of Walker *et al.* who reported decreased PFS and OS for t(MYC) in MM patients included in the UK MRC Myeloma IX trial.[3] Sekiguchi *et al.* presented a non-significant association of MYC abnormalities with inferior PFS in MM patients treated with bortezomib and dexamethasone.[5] In contrast, Avet-Loiseau *et al.* could not detect a significant influence of t(MYC) on the survival of MM patients enrolled in the French IFM99 trials.[6] 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 *MYC* aberrations have different impacts on the outcome of molecular subgroups, we performed an analysis stratified by karyotype. Whereas 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) (Figure 2). A +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 the outcome in either of the two ploidy subgroups (*data not shown*). According to these data, t(MYC) is the only rele-

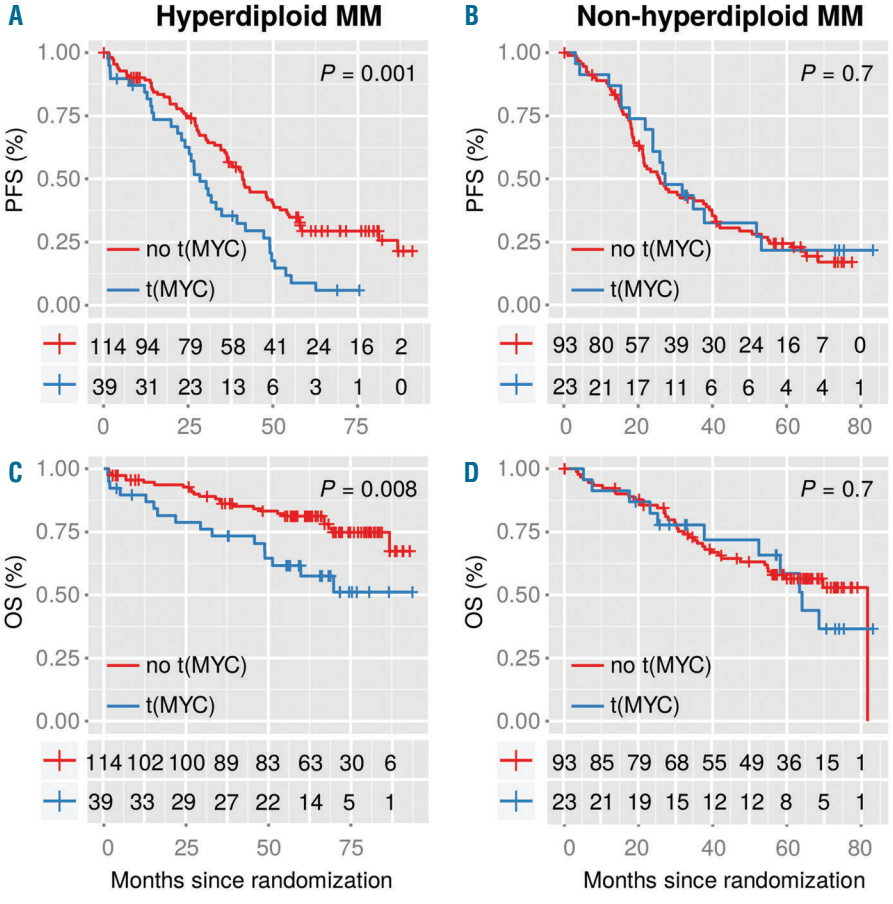

Figure 2. Impact of t(MYC) on PFS and OS of ploidy subgroups of MM. MM patients were stratified by ploidy and the presence or absence of t(MYC). The impact of t(MYC) on the outcome of hyperdiploid MM is shown in (A) and (C) The corresponding data for non-hyperdiploid MM is shown in (B) and (D). Patients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

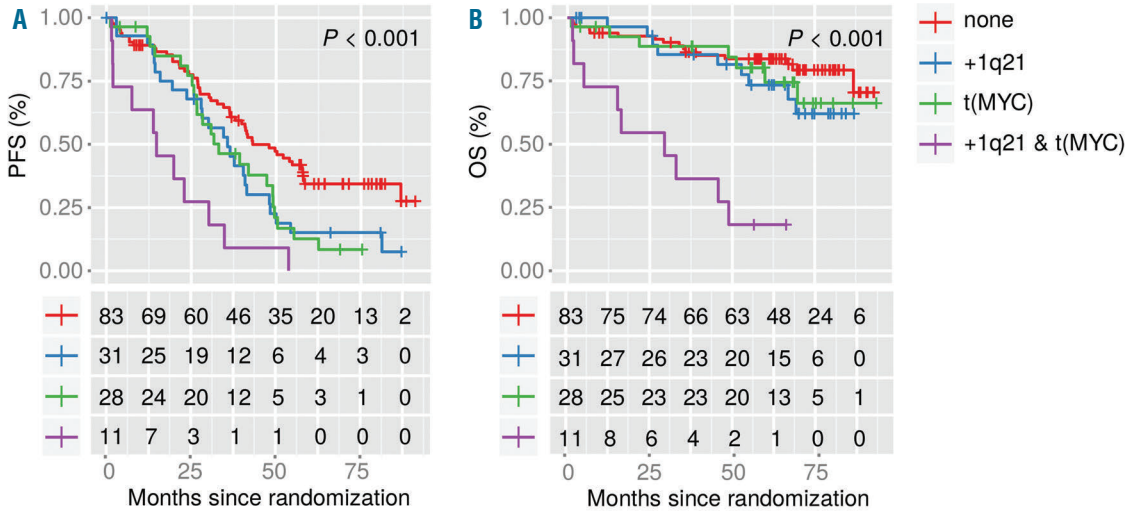

Figure 3. Impact of concomitant t(MYC) and +1q21 on PFS and OS of hyperdiploid MM. Hyperdiploid MM patients were stratified by the presence or absence of t(MYC) and +1q21. Patients at risk are shown below the figures.

vant *MYC* aberration for determining the prognosis of HDMM, but does not impact the outcome of NHDMM.

The GMMG-HD4 trial compared the effect of bortezomib-based treatment before and after ASCT (arm B) to standard treatment without this drug (arm A). HDMM with 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 t(MYC).

As the negative impact of t(MYC) was only detectable in HDMM, we focused on this subgroup in an extended analysis. We performed recursive partitioning including t(MYC) and the unfavorable aberrations +1q21 and del(17p13). As this was an exploratory study we used the univariate analysis test type. We identified HDMM with concomitant t(MYC) and +1q21 as a poor prognostic group (*Online Supplementary Figure S2*). For PFS, +1q21 and t(MYC) had a similar negative impact. The worst PFS was seen in cases with both aberrations (Figure 3A). Of note, cases with only one of these aberrations showed no difference in OS compared to cases without these aberrations (Figure 3B). In contrast, concomitant t(MYC) and +1q21 had a profound negative impact on the OS of HDMM patients (Figure 3B). The findings from the OS analysis suggested a subgroup effect of t(MYC) and +1q21. Interaction analysis using Cox regression on OS showed a significantly different prognostic effect of t(MYC) for patients depending on the presence of +1q21 (interaction *P*=0.048). We performed a multivariate analysis including t(MYC), +1q21, del(17p13), t(4;14) and ISS and identified t(MYC) as an independent predictor of PFS (HR = 1.68, *P*=0.02) but not for OS (HR=1.64, *P*=0.15) (*Online Supplementary Table S2*). The non-significant result for OS may be due to the association of t(MYC) with ISS stages II/III or to 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 what is 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 the 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.[9] 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 on HDMM may be that NHDMM 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.[7]

In conclusion, our data suggest that the favorable prognosis factor of 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.

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