Genomic aberrations in mantle cell lymphoma detected by interphase fluorescence *in situ* hybridization. Incidence and clinicopathological correlations

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ABSTRACT

Background

The genetic hallmark of mantle cell lymphoma is a t(11;14)(q13;q32). However, additional genomic alterations are likely involved in the pathogenesis of this lymphoma.

Design and Methods

To determine the incidence and clinical relevance of these aberrations, we analyzed 103 well-characterized samples of mantle cell lymphoma by fluorescence *in situ* hybridization for the most common recurrent additional genomic findings.

Results

Screening 16 different regions we detected additional genomic aberrations in 92% of the cases of mantle cell lymphoma. Common gains included 3q26, 8q24, 15q23, 7p15, and common losses 13q14, 11q22-q23, 9p21, 1p22, 17p13, 6q27, and 8p22. Deletions 8p22, 9p21, 13q14, and gain of 7p15 were associated with evidence of clonal heterogeneity. While there was no correlation of additional genomic aberrations and *VH*-mutation status, gain of 15q23 and deletion 6q27 were associated with lower disease stage (p=0.01 and p=0.04, respectively). Patients with deletion 13q14 had shorter overall survival times (p=0.01), and there was a strong trend towards inferior outcome in patients with deletion 9p21 (p=0.07). In multivariable analysis, loss of 13q14 and an International Prognosis Index score ≥ 3 turned out to be significantly associated with inferior clinical outcome (p=0.002 and p<0.001, respectively).

Conclusions

The comprehensive analysis of additional genomic aberrations in mantle cell lymphoma provided further evidence for the prognostic relevance of loss of 13q14, which warrants evaluation within prospective trials. Furthermore, our analysis gave novel insights into the pathogenesis of mantle cell lymphoma with regard to the detection of clonal heterogeneity, possibly indicating clonal evolution in this type of lymphoma.

Key words: mantle cell lymphoma, fluorescence *in situ* hybridization, genomic aberrations.

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Introduction

Mantle cell lymphoma (MCL) accounts for approximately 5-10% of non-Hodgkin's lymphoma. It has an aggressive clinical course with poor response to conventional chemotherapy and the median overall survival time is only 3 to 4 years.[1] Several therapeutic developments have improved the outcome of MCL patients recently, and a number of different treatment approaches are now available which vary widely in their efficacy and toxicity.[2] There is, therefore, an increasing need for the identification of prognostic markers that may help to guide the management of patients with MCL.

The genetic hallmark of MCL is the translocation (11;14)(q13;q32), which results in the overexpression of cyclin D1 contributing to deregulated cell cycle progression at the G_1-S phase boundary. While it was believed that MCL originates from pre-germinal-center cells,[3] recent observations indicate that 20-35% of MCL cases exhibit somatic hypermutations in the immunoglobulin genes. This suggests an origin from specific subsets of B cells that have entered the follicular germinal center.[4-6] However, unlike in chronic lymphocytic leukemia, in MCL the variable heavy chain gene (*VH*) mutation status shows no association with ZAP-70 expression and its prognostic relevance is the subject of heated debate.[6-8]

Additional genetic alterations that occur in subsets of MCL appear to disturb the cell cycle machinery and to interfere with cellular responses to DNA damage. In particular, cyclin D1 upregulation, genomic amplification of cyclin-dependent kinase 4 (CDK4), deletions of the CDK inhibitor p16INK4a, and overexpression of BMI-1, a transcriptional repressor of the p16INK4a locus, are associated with deregulation of cell cycling in MCL.[9] Furthermore, the DNA damage response pathway is impaired by frequent alterations of the ataxiate-langiectasia mutated (ATM) kinase,[10,11] and p53 is often affected by alterations in MCL.[12,13]

Molecular genetic studies have demonstrated that additional clonal genomic aberrations are found in the majority of MCL.[14-17] For example, deletions of 13q14 and 11q22-q23 are common.[15,17,18] Recently, microarray-based comparative genomic hybridization (array CGH) studies have confirmed the high incidence of additional genomic aberrations.[19-22] However, the true incidence of the most frequently observed aberrations has not yet been determined within a large number of MCL cases. Furthermore, while some studies demonstrated that 17p/p53 abnormalities,[12,13] INK4a/ARF deletions,[23,24] and tetraploid chromosome clones[25] have prognostic relevance, the impact of genomic aberrations has not yet been evaluated for a large number of chromosomal regions studied simultaneously.

We, therefore, further evaluated the incidence as well as clinical correlations of additional genomic aberrations in t(11;14)-positive MCL cases. We used fluorescence *in situ* hybridization (FISH), which offers the advantage of detecting chromosomal alterations at the single interphase cell level, to screen a large set of MCL samples. Results were correlated with clinical data and discussed in the context of conventional and array CGH findings.

Design and Methods

Samples and diagnosis

One hundred and three MCL samples were collected, with informed consent, from consecutively diagnosed patients at the Universities of Würzburg (n=47), Heidelberg (n=31) and Ulm (n=25) between January 1984 and December 2001 at the time of diagnosis (n=80) or during the course of the disease (n=23).

The histological diagnosis of MCL was made according to the criteria of the WHO classification. All included cases had a t(11;14), determined by interphase FISH as previously described.[26] The blastoid variant of MCL was diagnosed in five cases.

Clinical features, treatment and VH mutation status

Clinical characteristics at the time of diagnosis are detailed in Table 1 and *Online Supplementary Table S1*. Estimated median follow-up time was 48.3 months. Treatment was heterogeneous and not within a single clinical trial.

In 57 MCL cases the *VH* mutation status was determined by multiplex polymerase chain reaction (PCR) amplification and subsequent direct sequencing as previously described.[6]

Fluorescence in situ hybridization

A set of DNA probes was developed to detect genomic aberrations by interphase cytogenetics in MCL. Chromosomal regions were selected on the basis of data from conventional chromosome banding and CGH studies.[14-16] The DNA probes allowed us to screen for partial deletions, partial trisomies, and amplifications in 1p22, 3q26-q27, 6q21, 6q27, 7p15, 8p22, 8q24, 9p21, 10p15, 11q22-q23, 12q13, 13q14, 13qter, 15q23, 17p13, and 18q21 as previously described.[26,27]

Statistical analysis

Statistical analyses were performed according to standard procedures. Details are provided in the *Online Supplementary Appendix*.

Results

Incidence and complexity of genomic aberrations in addition to the t(11;14) in MCL

FISH screening of 16 chromosomal regions in the 103 cases of MCL revealed genomic aberrations in addition to the t(11;14) in 92% (95/103) of the cases (median number of aberrations/case=4; range, 0-15). In general, genomic losses were more frequent than gains. The most common losses were detected in 13q14, 11q22-q23, 9p21, 13qter, 1p22, and 17p13 (Table 2 and *Online Supplementary Table S2*). In eight of the 35 patients (23%) with loss of 9p21 both copies were affected resulting in a biallelic deletion of the respective locus.

For 13q14 we observed a biallelic loss in three out of 44 deleted cases (7%). Furthermore, while deletion of 13q14 was observed in 44% of cases and deletion of 13qter in 33%, only 24 cases showed a deletion in both chromosomal bands, whereas in ten cases only 13q14 and in four cases the terminal region was deleted.

Recurrently gained regions included 3q26, 8q24, 15q23, 12q12-q13,7p15, and 18q21. In addition, we observed six high-level DNA amplifications in four samples (*Table 2 and Online Supplementary Table S2*). The amplified regions included chromosomal bands 7p15, 12q12-q13, 13qter, and 18q21 with 12q12-q13 and 18q21 being involved twice. All these cases showed at least seven aberrations in addition to the t(11;14).

Seriation of the MCL cases with additional genomic aberrations (n=95) revealed a group of cases characterized by gain of 3q26 without loss of the regions 9p21, 11q22-q23 or 13q14 (Figure 1; group I). Another small cohort (Figure 1; group IV) seemed to have acquired predominantly gains in addition to a gain in 3q26. On the other hand we also observed MCL cases mainly characterized by deletion of 17p13 and 6q21 (Figure 1; group II), and the largest cluster displayed deletions of 1p22, 9p21, 11q22-q23 and 13q14 (Figure 1; group III).

Correlation with CGH findings

Conventional CGH studies showed considerable heterogeneity with regard to the incidences of additional aberrations;[14-18] our observations basically confirmed these results, but revealed a greater range of aberrations (0-9 *vs.* 0-15, respectively). Furthermore, the incidence of certain deletions was higher when FISH was used for the detection rather than conventional CGH. Thus, deletions in 11q22-q23 were found in 41/94 cases (44%) *vs.* 55/206 (27%); Fisher's exact test: p=0.005), deletions of 9p21 were found in 35/101 cases (35%) *vs.* 45/206, 22%; Fisher's exact test: p=0.018), and 17p13 deletion in 26/101 (26%) *vs.* 29/206 (14%); Fisher's exact test: p=0.017).

Next, we compared the incidence of genomic aberrations in addition to the t(11;14) found in this study with the incidences in recent smaller microarray-based CGH (array CGH) studies.[19-22] While there was a good correlation of findings for the genomic regions covered by our FISH probe set (*Online Supplementary Table S3*), the data

Table 1. Clinical and treatment characteristics.

Clinical feature at diagnosis	*N. of cases/all cases (percentage)*
Median age in years (range)	61 (37-87)
Male gender	72/95 (76%)
Ann Arbor stage III/IV	79/85 (93%)
B-symptoms	33/77 (43%)
Bulk (≥10 cm)	15/61 (25%)
Leukemic manifestation	39/76 (51%)
IPI score 3-5	42/67 (63%)
Bone marrow involvement	61/79 (77%)
Splenomegaly	38/60 (63%)
Gastrointestinal involvement	16/78 (21%)
Lactate dehydrogenase elevated	25/65 (38%)
VH mutation	16/57 (28%)
Treatment/outcome	
Treated	74/79 (94%)
prior to study	23/79 (29%)
Chemotherapy	63/71 (89%)
Polychemotherapy	52/63 (83%)
Anthracyclines	28/63 (44%)
Single agent therapy	11/63 (17%)
Radiotherapy	11/79 (14%)
Splenectomy or tumor resection	7/79 (9%)
ASCT*	10/79 (13%)
CR after initial therapy	22/79 (28%)

**High dose chemotherapy followed by autologous stem cell transplantation (ASCT). IPI, International Prognostic Index; CR, complete response.*

Table 2. Additional genomic aberrations in t(11;14) positive MCL cases.

Cytoband	*Candidate genes*	*Aberration*	*Aberrations/evaluable samples*	*Incidence %*	*Median percentage of affected cells/case (range)*
1p22	*BCL10, miR-137*	del	30/95	32	74.5 (25-98)
3q26	*ETV5, TRAIL, ECT2*	+	46/102	45	64 (25-94)
6q21	*FOXO3A*	del	16/103	16	73.5 (35-98)
		+	1/103	1	36
6q27	*PDCD2*	del	21/96	22	79 (15-99)
7p15	–	del	1/100	1	89
		+[a]	15/100	15	54 (13-84)
8p22	*TNFRSF10B, TNFRSF10C*	del	20/96	21	69 (19-98)
		+	1/96	1	65
8q24	*MYC, TRIB1*	+	20/103	19	60 (10-92)
9p21	*CDKN2A, CDKN2B*	del[b]	35/101	35	72 (19-97)
10p15	–	del	12/91	13	80 (49-96)
		+	2/91	2	36.5 (20-53)
11q22-q23	*ATM*	del	39/95	41	79 (17-99)
12q12-q13	*CDK4, MDM2, GLI*	+[a]	17/101	17	74 (22-86)
13q14	*RFP2, BCMSUN, RB, miR-15, miR-16*	del[b]	43/99	43	78 (13-97)
		+	2/99	2	26.5 (11-42)
13qter	*ING1, LIG4, TNFSF13B*	del	29/87	33	74 (19-97)
		+[a]	4/87	5	60 (8-77)
15q23	*PML*	+	17/92	18	57 (33-76)
17p13	*TP53*	del	26/101	26	75 (13-99)
18q21	*BCL2*	+[a]	14/100	14	65.5 (22-92)

[a]Cases with an amplification of the respective region are included; [b]cases with a biallelic deletion of the respective region are included.

comparison also clearly demonstrated that our probe set covered the most prevalent aberrations in MCL.

Clonal heterogeneity

Clonal heterogeneity was defined as the presence of distinct subclones with different genomic aberrations indicative of a multistep process of lymphomagenesis. We observed striking differences between the percentages of aberrant cells when comparing different genomic aberrations within individual patients. For example, the percentages of cells carrying specific aberrations varied from 11% to 95% within a single case. In 40% (38/95) of the MCL cases with additional genomic aberrations there was evidence of clonal heterogeneity (Figure 1). The median number of additional genomic aberrations in cases with clonal heterogeneity was six/case (range, 2-15/case) compared to two/case (range, 0-9/case) in the other cases (*Online Supplementary Figure S1*). Within each of these cases the percentages of cells carrying additional genomic aberrations differed significantly between different genomic aberrations [a significant difference between an additional genomic aberration and the "main" clone, consisting of ≥3 aberrations, was considered if the difference was greater than three times the standard deviation of the mean of the main clone), thereby suggesting that distinct aberrations might have occurred at different time points during lymphomagenesis.

Deletions of 8p22, 9p21, and 13q14, as well as gain of 7p15, were significantly associated with evidence of clonal heterogeneity (Fisher's exact test: p=0.019, p<0.001, p<0.001, and p=0.030, respectively; Table 3). Interestingly, for all these aberrations the percentages of cells harboring the respective aberration were either significantly higher or lower than other aberrations in a given MCL case. For example, while in case #21 9p21 was lost in 94% of cells a gain of 3q26 was seen in 46% of cells, and in case #58 we observed a 9p21 loss in 38% of cells, whereas loss in 11q22-q23 and 6q27 was found in 71% and 80% of cells, respectively. However, deletions of 10p15 and 11q22-q23 were both, with one exception, exclusively seen in the clones affecting a high percentage of cells, thereby suggesting that these are early events in the case of clonal evolution.

Interestingly, 75% of cases with biallelic 9p21 deletion also showed clonal heterogeneity. In two cases a higher percentage of cells exhibited the biallelic deletion than the t(11;14) (*Online Supplementary Table S4*). Furthermore, we observed three additional MCL cases in which the proportion of cells carrying the t(11;14) was significantly lower than the percentage of cells with other aberrations (*Online Supplementary Table S4*). In one case deletion of 1p22 and 6q27 was found in a higher percentage of cells, and in two cases loss of 13q14 was more commonly seen (as well as additional gains including 7p15 in MCL#81). Interestingly, these cases were found in group III and in group IV or close to it (Figure 1, cases with discrepant t(11;14) findings are highlighted in blue). Furthermore, three of these samples were from pretreated patients, which were significantly associated with clonal heterogeneity.

Incidence of genomic aberrations with regard to VH mutation status

In a subset of MCL cases (n=57) we determined the *VH* mutation status by multiplex PCR amplification and subsequent direct sequencing, as previously described.[6] In accordance with previous findings, 29% (16/57) of the MCL cases displayed mutated *VH* using a 98% germline homology cut-off, and there was no significant correlation between additional genomic aberrations and the *VH* mutation status (*data not shown*). An analysis of the presence of *VH* mutations and evidence of clonal heterogeneity failed to reveal any significant associations [estimated correlation coefficient r=-0.13, 95% confidence interval (95% CI) -0.38 - 0.13].

Correlation with clinicopathological characteristics and prognostic impact

There was no significant association of age, extranodal involvement, performance status, lactate dehydro-

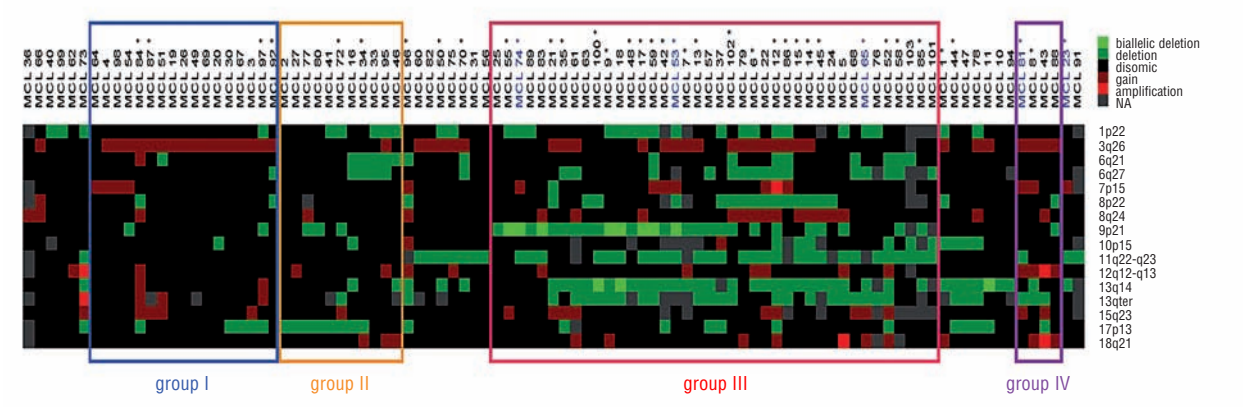

Figure 1. Seriation of MCL cases (n=95) with genomic aberrations in addition to a t(11;14). Each column represents the FISH results for a given MCL sample, each row represents the results for a given chromosomal locus. Genomic aberrations are color coded as indicated by the pseudo-color scale. Cases with evidence of clonal evolution are indicated by an asterisk (e.g. MCL 34 *). NA = data not available.

genase level, and the International Prognostic Index (IPI) score with any of the genomic aberrations. In contrast, we observed a significant association of 15q23 gains and 6q27 losses with lower stage (p=0.01 and p=0.04, respectively). All stage I/II cases exhibited a gain of 15q23 and deletion of 6q27 except for one case in which the 15q23 and 6q27 regions were not evaluable. Furthermore, deletion of 1p22 was significantly more common in pretreated MCL patients (Fisher's exact test: p=0.02). Correlation of the seriation-derived sample grouping based on the underlying additional genomic aberrations with clinical data revealed no significant differences with regard to stage and IPI, but there was a trend towards less bone marrow involvement for cases in groups III and IV (*Online Supplementary Table S5*).

As suggested by our previous array CGH findings,[19] deletion of 13q14 was significantly associated with a poorer outcome with a median overall survival of 29.0 months (95 CI% 13.8–37.7) in cases with 13q14 deletion and 48.3 (95 CI% 41.2–∞) months in cases without 13q14 deletion (log-rank test: p=0.01; Figure 2A). In addition, we observed a strong trend towards worse overall survival in patients with loss of 9p21 (log-rank test: p=0.07, Figure 2B). Evidence of clonal heterogeneity was not significantly correlated with overall survival, but the cluster-derived groups III and IV, characterized by either deletions of 1p22, 9p21, 11q22-q23 and 13q14 or multiple gains, showed a trend towards having a poorer outcome (*Online Supplementary Figure S2*). In multivariable analysis, only loss of 13q14 and an IPI score ≥3 turned out to be significantly associated with worse clinical outcome in our study (hazard ratio 3.32; 95% CI 1.55–7.13; p=0.002; and hazard ratio 3.42; 95% CI 1.79–6.53; p<0.001, respectively).

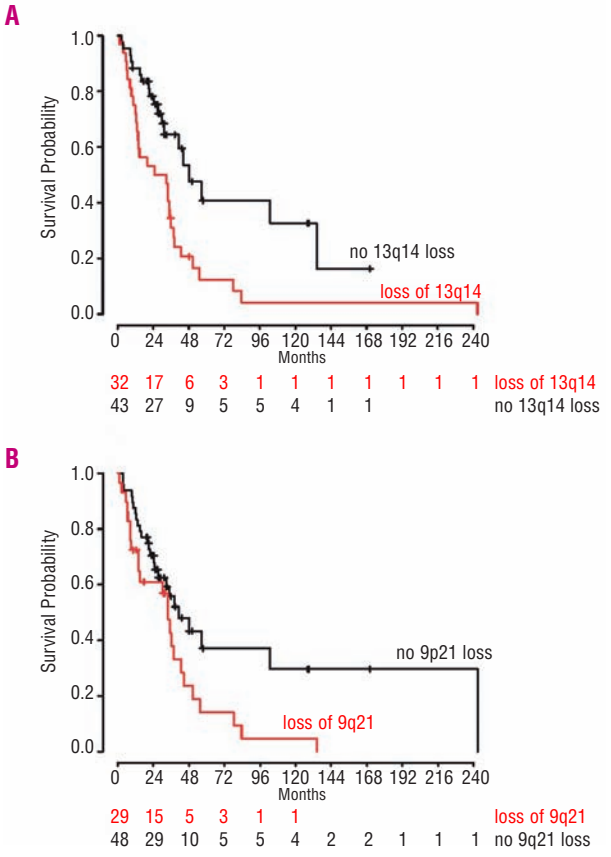

Figure 2. Kaplan-Meier survival curves comparing (A) 13q14 deleted and (B) 9p21 deleted with undeleted MCL cases, respectively. (A) Median survival times were 29 months in the subgroup with 13q14 deletion (n=32) and 48.3 months in the subgroup without 13q14 deletion (n=43; log-rank test: p=0.01). (B) Median survival times were 34 months in the subgroup with 9p21 deletion (n=29) and 41.2 months in the subgroup without 9p21 deletion (n=48; log-rank test: p=0.07).

Discussion

Although the presence of the t(11;14) is the hallmark of MCL, it is believed that the resulting overexpression of cyclin D1 is not, by itself, sufficient to cause a malignant transformation of lymphoid cells. However, additional chromosomal aberrations, which have been identified in a large proportion of MCL patients by conventional chromosome banding analyses, might be the other factors necessary for lymphomagenesis.[28,29] Approaches such as conventional CGH have already significantly contributed the characterization of recurrent genomic aberrations. In five CGH studies, which examined a total of 206 MCL cases, additional aberrations were detected in 94% of cases.[14-18] In our series, we detected a median of four additional genomic aberrations per case. However, the range of aberrations was greater, being 0-15 aberrations per case compared to 0-9 in the CGH studies,[14-18] and for the detection of deletions in 11q22-q23, 9p21, and 17p13, FISH analysis seemed to perform better, thereby confirming previous findings of smaller interphase cytogenetic studies.[30,31]

Recently, array CGH has provided a high-resolution alternative for genome-wide screening of genomic aberrations[19-22] and has indicated that genomic losses are more common than gains in MCL (median of 14 altered regions per tumor; range, 0-35).[20] There was a good correlation of findings for the genomic regions covered by our comprehensive FISH probe set, confirming the incidences found in small cohorts studied by array CGH. However, FISH analyses offer the advantage of detecting subclones of malignant cells, which are different from the bulk of lymphoma cells, and thus provide a powerful instrument to investigate clonal heterogeneity in malignant disorders.

Candidate genes located in the recurrently affected chromosomal regions known to play a pathogenic role in MCL include the tumor suppressor genes *TP53*,[12,13] *ATM*,[10,11] *CDKN2A*,[23,24] as well as the anti-apoptotic gene *BCL2*[15] and the oncogene *MYC*.[14,32] However, in many of the commonly deleted or gained regions the genes involved in the respective aberrations are still unknown. For example, in the commonly deleted region 1p21-p22 mutation analyses failed to identify mutations in the coding region of *BCL10*, a likely candidate in this region.[33] However, the recent identification of *miRNA-137* in the commonly deleted 1p region[20]

Table 3. Genomic aberrations associated with clonal heterogeneity.

Genomic aberration	All MCL cases	Aberrations/evaluable samples evidence of clonal heterogeneity	No evidence of clonal heterogeneity	Fisher's exact test Raw p value	Adjusted p-value
del 1p22	30/95	15/34	15/61	0.066	0.106
+ 3q26	46/102	22/38	24/64	0.064	0.106
del 6q21	16/103	9/38	7/65	0.096	0.140
del 6q27	21/96	12/36	9/60	0.044	0.088
+ 7p15	15/100	10/38	5/62	0.020	0.053
del 8p22	20/96	14/35	6/61	0.001	0.005
+ 8q24	20/103	9/38	11/65	0.445	0.509
del 9p21	35/101	22/38	13/63	< 0.001	0.002
del 10p15	12/89	6/29	6/60	0.194	0.259
del 11q22-q23	39/95	20/34	19/61	0.010	0.032
del 12q12-q13	17/101	7/38	10/63	0.787	0.840
del 13q14	43/99	28/38	15/61	< 0.001	< 0.001
del 13qter	29/87	17/29	12/58	0.001	0.004
+ 15q23	17/92	10/32	7/60	0.027	0.062
del 17p13	26/101	10/38	16/63	1.000	1.000
+ 18q21	14/100	7/37	7/63	0.372	0.458

suggests that deregulation and/or mutation of miRNA might play a role in MCL in analogy to cases of chronic lymphocytic leukemia (CLL) with deletions of 13q14.[34] While additional, miRNA might be involved in the pathogenesis of MCL parallel analysis of genomic aberrations and mRNA as well as miRNA expression levels will help to further narrow down the number of potential candidate genes.[17]

Interestingly, in MCL biallelic deletions were only detected in 7% of 13q14 deleted cases, a finding contrasting with the incidence of 24% in CLL.[26] This might point to an epigenetic pathomechanism similar to that recently described in CLL[35] by which the deletion of the single active copy of 13q14.3 might result in significant down-regulation of the candidate genes. However, while in CLL genomic material from chromosome band 13q14.3 is lost distal to the retinoblastoma gene (*RB*), in some MCL cases *RB* might also be a potential candidate.[36] Furthermore, additional, as yet unidentified, tumor suppressors located in 13qter might play an important role in MCL, as this genomic region is more frequently deleted in MCL than in CLL.

On the other hand, loss of 13q14 was significantly associated with clonal heterogeneity in MCL. Possibly being an early event in lymphomagenesis,[37] this aberration seems to confer a distinct genomic instability, thereby potentially resulting in clonal evolution. Interestingly, loss of 13q14 was commonly associated with deletion of 1p22, 9p21 and 11q22-q23 although no common pattern suggesting a distinct pathomechanism could be identified. However, pretreated samples more often had clonal heterogeneity and deletion of 1p22, and showed a trend towards an association with deletion of 9p21. These findings suggest treatment associated selection of distinct clones or might reflect clonal evolution occurring during the course of the disease. Furthermore, the finding that all cases with discrepant prevalence of t(11;14) and additional genomic aberrations harbored at least one clonal heterogeneity associated mutation might support the potential existence of a *MCL cancer stem cell* lacking a balanced *BCL1* rearrangement. In agreement with this hypothesis, a recent study showed that a small percentage of MCL cases, exhibiting a gene expression signature identical to that of "classical" MCL cases with *CCND1* translocation, is not characterized by elevated *CCND1* expression and lacks t(11;14).[38] While these cases definitely need to be investigated more intensively, these findings do question the global role that t(11;14) is supposed to play in the pathogenesis of MCL and future analyses should focus on the important aspect of clonal evolution in lymphomagenesis.

As previously reported, a significant proportion of MCL cases harbor somatic *VH*-mutations.[4-6] Therefore, in MCL, as in CLL, the malignant transformation might occur in a pre-germinal-center B cell with unmutated *VH* or a post-germinal-center B cell with mutated *VH*. However, in contrast to CLL, the two *VH*-mutation subtypes of MCL failed to correlate with distinct genomic aberrations. In MCL deletions 17p13 and 11q22-q23 were observed at similar incidences in *VH*-mutated and -unmutated cases. As recently published, this is of note because the inactivation of *ATM* has been believed to be a pathogenic event exclusive to pre-germinal-center B-cell-based lymphomas.[39]

Unlike in CLL, losses of 17p13 and 11q22-q23 were not associated with poorer outcome in our series of MCL, in agreement with some other recent studies.[17,40] In contrast, Rubio-Moscardo *et al*. observed a significant correlation of overall survival with loss of 9p21, 11q22-q23 and 17p13,[20] and Parry-Jones observed a trend towards poorer outcome in 11q23-mutated cases.[41] Although we did not observe the effects for 11q22-q23 and 17p13, there was a strong trend for 9p21 deletions to be associated with worse outcome in our series, as also seen by Salaverria *et al*.[17] While *TP53* mutations and not deletions might be relevant pathogenic events,[12,13] the discrepancies observed between different studies highlight the need to evaluate these factors in larger, prospective clinical trials before any risk stratification based on genomic aberrations can be used in routine clinical practice. This also applies to our observation that loss of 13q14 is significantly associated with shorter overall survival, as recently indicated

by FISH and array CGH analyses.[19,41]

In addition to the evaluation of the most relevant additional genomic aberrations within prospective trials (for which FISH provides a very sensitive, precise, fast, and cost-effective instrument), future research should comprise the correlation of genomic findings with gene expression data in order to identify candidate genes involved in the pathogenesis of MCL. The first gene expression profiling analyses based either on quantitative reverse transcriptase-PCR[32,42] or microarray analysis[38] have already provided promising novel insights into MCL biology by, for example, identifying a proliferation signature possibly underlying the prognostic impact of a high proliferation rate in MCL.[43]

Authorship and Disclosures

SSa and LB: designed and performed the research, collected and analyzed data and wrote the paper; EL, DK, TK and JK: performed research, collected and analyzed data; AB: analyzed data and wrote the paper; GO, HKM-H,TFEB and PM: contributed vital reagents or analytical tools, collected and analyzed data; PL: designed research, contributed analytical tools; HD: designed research, contributed vital reagents or analytical tools, analyzed data, wrote the paper; SSt: designed and performed research, contributed vital reagents or analytical tools, collected and analyzed data, wrote the paper. SSa and LB contributed equally. The authors reported no potential conflicts of interest.

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