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

Sandrine Sander,[1,2] Lars Bullinger,[1] Elke Leupolt,[1] Axel Benner,[3] Dirk Kienle,[1] Tiemo Katzenberger,[4] Jörg Kalla,[4] German Ott,[4] Hans Konrad Müller-Hermelink[4], Thomas F.E. Barth,[5] Peter Möller,[5] Peter Lichter,[6] Hartmut Döhner,[1] and Stephan Stilgenbauer[1]

[1]Department of Internal Medicine III, University of Ulm; [2]Department of Physiological Chemistry, University of Ulm; [3]Department of Biostatistics, DKFZ, Heidelberg; [4]Department of Pathology, University of Würzburg; [5]Department of Pathology, University of Ulm; [6]Department of Molecular Genetics, DKFZ, Heidelberg, Germany

*Citation: Sander S, Bullinger L, Lepolt E, Benner A, Kienle D, Katzenberger T, Kalla J, Ott G, Müller-Hermelink HK, Barth TFE, Möller P, Lichter P, Döhner H, and Stilgenbauer S. Genomic aberrations in mantle cell lymphoma detected by interphase fluorescence* in situ *hybridization. Incidence and clinicopathological correlations. Haematologica 2008 May; 93(5):680-687.*

*doi: 10.3324/haematol.12330*

## Supplementary Information

## Design and Methods

### Samples

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). With regard to a potential bias due to the inclusion of the 23 samples from already treated patients, these cases had been diagnosed earlier and were, in general, characterized by a lower tumor burden at the time of diagnosis (for details see *Supplementary Table S1*). However, there was no significant difference in overall survival between patients whose samples were taken at diagnosis or after treatment ($p$=0.21, log rank test). Twenty-five samples of this cohort had been previously included in an array CGH study[1] and 35 samples in a quantitative gene expression analysis.[2] Mononuclear cells were isolated from frozen lymphoid tissue blocks (n=70) and from peripheral blood or bone marrow (n=33) obtained for diagnostic procedures as previously described.[3,4]

### Diagnosis

The histological diagnosis of MCL was made according to the criteria of the WHO classification at German reference pathology institutions. Furthermore, all included cases showed the presence of a t(11;14) determined by interphase FISH, as previously described.[3] The blastoid variant of MCL was diagnosed in five cases.

### Clinical features and treatment

There were 23 women, 72 men, and in 8 cases no clinical information was available; the patients' age at the time of diagnosis ranged from 40 to 87 years (median age 61). Clinical characteristics at the time of diagnosis were available for a subset of cases as detailed in Table 1. The estimated median follow-up time was 48.3 months. Treatment was heterogeneous and not given within a single clinical trial; radiotherapy and/or chemotherapy had been administered to 23 patients prior to the study.

### VH *mutation status*

In 57 MCL cases the variable heavy chain gene (*VH*) mutation status was determined by multiplex PCR amplification and subsequent direct sequencing, as previously described.[5]

### *Fluorescence* in situ *hybridization (FISH)*

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 comparative genomic hybridization studies.[6-8] The DNA probes allowed us to screen for partial deletions, partial trisomies, and amplifications in the following regions (clone names are shown in brackets): 1p22 [yeast artificial chromosome (YAC) 968g8], 3q26-q27 (YAC 866e7), 6q21 [P1-derived artificial chromosome (PAC) 963d6], 6q27 (YAC 919h10), 7p15 (YAC 961b12), 8p22 (YAC 948d5), 8q24 (PAC c-

**Supplementary Table S1. Clinical differences between already treated patients and those investigated at diagnosis.**

| *Clinical feature at diagnosis* | *N. of cases / pretreated cases (percentage)* | *N. of cases / untreated cases (percentage)* | *p value* |
|---|---|---|---|
| Median age in years (range) | 59 (40-81) | 66 (47-87) | 0.0145 |
| Male gender | 21/23 (91%) | 39/56 (70%) | 0.0464 |
| Ann Arbor stage III/IV | 19/23 (83%) | 54/56 (96%) | 0.0562 |
| B-symptoms | 9/23 (39%) | 24/52 (46%) | 0.6216 |
| Bulk (≥10 cm) | 1/15 (6%) | 11/44 (25%) | 0.1534 |
| Leukemic manifestation | 7/21 (33%) | 29/52 (56%) | 0.1208 |
| IPI score 3-5 | 4/19 (21%) | 25/34 (74%) | 0.0004 |
| Bone marrow involvement | 14/23 (61%) | 44/53 (83%) | 0.0454 |
| Splenomegaly | 9/23 (39%) | 28/36 (78%) | 0.0052 |
| Gastrointestinal involvement | 6/23 (26%) | 10/53 (19%) | 0.545 |
| Elevated lactate dehydrogenase | 3/19 (16%) | 22/45 (49%) | 0.0233 |
| Median overall survival (days) | 1298 | 1002 | 0.2115 |
| Median year of diagnosis (range) | 1993 (1984 - 2001) | 1999 (1992 - 2001) | <0.0001 |

*IPI, International Prognostic Index*

myc), 9p21 (cosmid p16), 10p15 (YAC 813d3), 11q22-q23 (PACs 755b11 and ATM#2), 12q13 (PAC Gli#3), 13q14 (PACs D13S272/3 and 933e9#48), 13qter (PAC 933e9#56), 15q23 (YAC 954e9), 17p13 (PAC p53), and 18q21 (YAC 153a6). With this probe set, we screened all 103 MCL samples by FISH analysis as previously described.[3,4] Internal control probes to ensure a high hybridization efficiency and cut-off levels for the diagnosis of deletions were applied as described. Signal number was enumerated in 200 nuclei.

### Statistical analysis

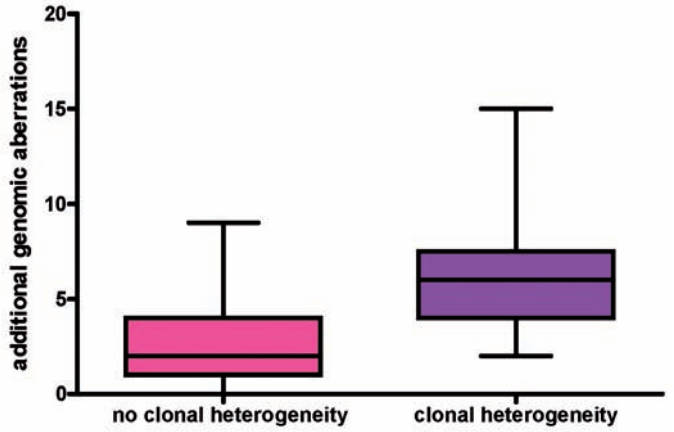
To determine clusters of similar patterns according to the observed aberrations we applied seriation by optimal leaf ordering [similarity metric: correlation (uncentered)] to group the MCL cases and visualized results using TreeView.[9] The primary clinical end-point was survival from the time of diagnosis. For 79 patients survival time data were available. All subsequent statistical analyses were performed for this subset of patients only. Survival times and censored waiting times measured from the date of diagnosis were plotted using Kaplan–Meier estimates. The median duration of follow-up was calculated according to the method of Korn.[10] The nonconcave penalized likelihood approach of the Cox proportional hazards model as proposed by Fan and Li[11] was used to identify prognostic factors for survival. Factors included in the regression model were age, stage (I/II, III or IV), serum lactate dehydrogenase (LDH) level, presence or absence of extranodular involvement, performance status, number of genomic aberrations, International Prognostic Index (IPI) score 0-2 or 3-5, and presence or absence of specific genomic aberrations in the 16 chromosomal regions analyzed. Global testing of groups of variables with respect to survival was performed as described by Goeman *et al.*[12] Univariable screening of genomic aberrations with respect to survival was done with the multiple testing procedure of Pollard and van der Laan[13] using a Cox proportional hazards model and the step-down maxT procedure

**Supplementary Table S2.** **Distribution of additional genomic aberrations among samples taken from already treated patients and those taken at diagnosis.**

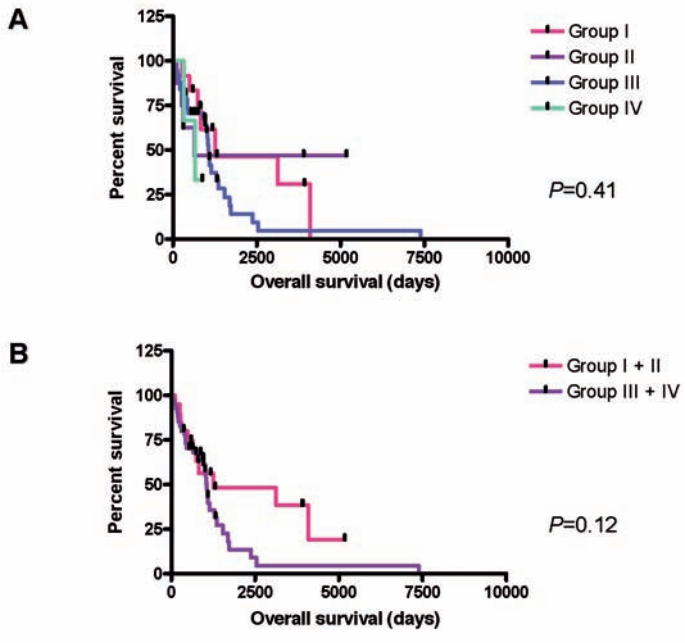
| *Genomic aberrations* | *N. of cases / pretreated cases (percentage)* | *N. of cases / untreated cases (percentage)* | *p value* |
|---|---|---|---|
| Tetraploidy | 4/23 (17%) | 8/56 (14%) | 0.738 |
| Additional genomic aberrations (range) | 4 (1-15) | 4 (0-10) | 0.1173 |
| loss 1p22 | 12/21 (57%) | 14/53 (26%) | 0.0166 |
| gain 3q26 | 12/22 (55%) | 24/56 (43%) | 0.4506 |
| loss 6q21 | 4/23 (17%) | 9/55 (16%) | 1 |
| loss 6q27 | 7/21 (33%) | 12/51 (24%) | 0.3951 |
| gain 7p15 | 5/21 (24%) | 5/54 (9%) | 0.1311 |
| loss 8p22 | 5/20 (25%) | 10/53 (19%) | 0.5369 |
| gain 8q24 | 7/23 (30%) | 9/56 (16%) | 0.2165 |
| loss 9p21 | 8/17 (47%) | 14/53 (26%) | 0.1381 |
| loss 10p15 | 4/19 (21%) | 7/50 (14%) | 0.4798 |
| loss 11q22-q23 | 10/22 (45%) | 21/49 (43%) | 1 |
| gain 12q12-q13 | 3/22 (14%) | 10/54 (19%) | 0.7453 |
| loss 13q14 | 13/23 (57%) | 19/48 (40%) | 0.2096 |
| loss 13qter | 9/20 (45%) | 16/46 (35%) | 0.5816 |
| gain 15q23 | 5/20 (25%) | 10/51 (20%) | 0.7477 |
| loss 17p13 | 5/22 (23%) | 17/56 (30%) | 0.5851 |
| gain 18q21 | 5/22 (23%) | 5/53 (9%) | 0.1464 |
| clonal heterogeneity | 14/23 (61%) | 20/56 (36%) | 0.0486 |
| VH mutation status | 5/23 (22%) | 12/43 (28%) | 0.7692 |

**Supplementary Table S3.** **Comparison of FISH and array CGH findings.**

| *Study* | *Kohlhammer et al.*[1] | *Tagawa et al.*[15] | *Schraders et al.*[16] | *Rubio-Moscardo et al.*[17] | *Sander et al.* |
|---|---|---|---|---|---|
| Patients | n=53 | n=29 | n=17 | n=68 | n=103 |
| Genomic gain % | | | | | |
| 3q26 | 55 | 48 | 50 | 46 | 45 |
| 7p15 | 13 | - | - | 16 | 15 |
| 8q24 | 28 | 24 | 36 | 19 | 19 |
| 12q12-q13 | 17 | - | - | 3 | 17 |
| 15q23 | 9 | - | - | 9 | 18 |
| 18q21 | 13 | - | - | 5 | 14 |
| Genomic loss % | | | | | |
| 1p22 | 38 | 52 | 50 | 31 | 32 |
| 6q21 | 17 | - | - | 25 | 16 |
| 6q27 | 25 | - | 36 | - | 22 |
| 8p22 | 34 | - | - | 26 | 21 |
| 9p21 | 36 | 41 | 21 | 18 | 35 |
| 10p15 | 21 | 31 | - | 18 | 13 |
| 11q22-q23 | 43 | 59 | 57 | 21 | 41 |
| 13q14 | 55 | 55 | 43 | 25 | 43 |
| 13qter | 49 | 52 | 43 | 28 | 33 |
| 17p13 | 21 | 45 | - | 22 | 26 |




**Supplementary Figure S1.** Number of additional genomic aberrations with regard to clonal heterogeneity.




**Supplementary Figure S2.** Kaplan-Meier overall survival curves comparing seriation derived grouping based on additional genomic aberrations.

**Supplementary Table S4.** MCL cases with t(11;14) not being prevalent in all subclones.

| *Genomic aberrations* | *MCL #23* | *MCL #53* | *MCL #65* | *MCL #74* | *MCL #81* |
|---|---|---|---|---|---|
| Morphology (classical/blastoid) | NA | classical | classical | NA | classical |
| t(11;14) | 27% | 60% | 73% | 39% | 17% |
| N. of additional genomic aberrations | 3 | 7 | 7 | 4 | 6 |
| loss 1p22 | di | del 82% | del 98% | del 74% | di |
| gain 3q26 | di | + 47% | di | di | + 59% |
| loss 6q21 | di | di | di | di | di |
| loss 6q27 | di | NA | del 91% | di | di |
| gain 7p15 | + 23% | + 50% | di | + 54% | + 62% |
| loss 8p22 | di | del 44% | di | di | di |
| gain 8q24 | di | di | di | di | di |
| loss 9p21 | di | bd 97% | di | bd 73% | di |
| loss 10p15 | di | NA | di | di | di |
| loss 11q22-q23 | del 17% | del* | NA | del 75% | del* |
| gain 12q12-q13 | di | di | di | di | + 58% |
| loss 13q14 | del 48% | del 88% | del 66% | di | di |
| loss 13qter | di | NA | del 54% | di | del 74% |
| gain 15q23 | di | di | + 69% | di | + 49% |
| loss 17p13 | di | di | di | di | di |
| gain 18q21 | di | di | + 63% | di | di |

** no percentage available; NA = not available*

**Supplementary Table S5.** Comparison of FISH and array CGH findings.

| *Clinical feature at diagnosis* | *Group I* | *Group II* | *Group III* | *Group IV* | *p value* |
|---|---|---|---|---|---|
| Median age in years (range) | 55 (47-73) | 60 (47-73) | 61 (40-87) | 70 (61-81) | 0.1793 |
| Ann Arbor stage III/IV | 13/13 (100%) | 9/9 (100%) | 34/39 (87%) | 3/3 (100%) | 0.3238 |
| B-symptoms | 6/12 (50%) | 2/8 (25%) | 16/37 (43%) | 1/3 (33%) | 0.7101 |
| Bulk (≥10 cm) | 3/8 (38%) | 0/6 (0%) | 5/33 (15%) | 1/1 (100%) | 0.1961 |
| Leukemic manifestation | 7/12 (58%) | 4/6 (66%) | 15/37 (41%) | 1/3 (33%) | 0.4949 |
| IPI score 3-5 | 3/7 (43%) | 2/6 (66%) | 14/14 (50%) | 1/2 (50%) | 0.7433 |
| Bone marrow involvement | 10/12 (83%) | 8/8 (100%) | 26/38 (68%) | 2/3 (66%) | 0.1265 |
| Splenomegaly | 6/8 (75%) | 2/3 (66%) | 17/32 (53%) | 1/2 (50%) | 0.508 |
| Elevated lactate dehydrogenase | 4/11 (36%) | 2/7 (29%) | 12/32 (38%) | 2/2 (100%) | 0.905 |

*IPI, International Prognostic Index*

with 1000 bootstrap iterations to control the family-wise error rate. Groupwise comparisons of the distributions of clinical and laboratory variables were performed using Fisher's exact test for binary variables and the Cochran-Armitage test for stage. We accounted for multiple testing using the false discovery rate correction according Benjamini and Hochberg.[14] All tests were two-sided. An effect was considered statistically significant if the (adjusted) *p* value was 0.05 or less. To provide quantitative information on the relevance of statistically significant results, 95 percent confidence intervals for hazard ratios were computed. The statistical analyses were performed with R, version 2.2.1 (available at *http://www.r-project.org*).

## Additional References


1. Kohlhammer H, Schwaenen C, Wessendorf S, Holzmann K, Kestler HA, Kienle D, et al. Genomic DNA-chip hybridization in t(11;14)-positive mantle cell lymphomas shows a high frequency of aberrations and allows a refined characterization of consensus regions. Blood 2004;104: 795-801.
2. Kienle D, Katzenberger T, Ott G, Saupe D, Benner A, Kohlhammer H, et al. Quantitative gene expression deregulation in mantle-cell lymphoma: correlation with clinical and biologic factors. J Clin Oncol 2007;25:2770-7.
3. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910-6.
4. Stilgenbauer S, Bullinger L, Benner A, Wildenberger K, Bentz M, Dohner K, et al. Incidence and clinical significance of 6q deletions in B cell chronic lymphocytic leukemia. Leukemia 1999;13:1331-4.
5. Kienle D, Krober A, Katzenberger T, Ott G, Leupolt E, Barth TF, et al. VH mutation status and VDJ rearrangement structure in mantle cell lymphoma: correlation with genomic aberrations, clinical characteristics, and outcome. Blood 2003;102:3003-9.
6. Bea S, Ribas M, Hernandez JM, Bosch F, Pinyol M, Hernandez L, et al. Increased number of chromosomal imbalances and high-level DNA amplifications in mantle cell lymphoma are associated with blastoid variants. Blood 1999;93:4365-74.
7. Bentz M, Plesch A, Bullinger L, Stilgenbauer S, Ott G, Muller-Hermelink HK, et al. t(11;14)-positive mantle cell lymphomas exhibit complex karyotypes and share similarities with B-cell chronic lymphocytic leukemia. Genes Chromosomes Cancer 2000;27:285-94.
8. Monni O, Oinonen R, Elonen E, Franssila K, Teerenhovi L, Joensuu H, et al. Gain of 3q and deletion of 11q22 are frequent aberrations in mantle cell lymphoma. Genes Chromosomes Cancer 1998;21:298-307.
9. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998;95:14863-8.
10. Korn EL. Censoring distributions as a measure of follow-up in survival analysis. Stat Med 1986;5:255-60.
11. Fan J, Li R. Variable selection for Cox's proportional hazards model and frailty model. Ann Statist 2002;30:74-99.
12. Goeman JJ, Oosting J, Cleton-Jansen AM, Anninga JK, van Houwelingen HC. Testing association of a pathway with survival using gene expression data. Bioinformatics 2005;21:1950-7.
13. Pollard KS, van der Laan MJ. Resampling-based multiple testing: asymptotic control of type I error and applications to gene expression data: University of California, 2003.
14. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B 1995;57:289-300.
15. Tagawa H, Karnan S, Suzuki R, Matsuo K, Zhang X, Ota A, et al. Genome-wide array-based CGH for mantle cell lymphoma: identification of homozygous deletions of the proapoptotic gene BIM. Oncogene 2005;24:1348-58.
16. Schraders M, Pfundt R, Straatman HM, Janssen IM, van Kessel AG, Schoenmakers EF, et al. Novel chromosomal imbalances in mantle cell lymphoma detected by genome-wide array-based comparative genomic hybridization. Blood 2005; 105: 1686-93.
17. Rubio-Moscardo F, Climent J, Siebert R, Piris MA, Martin-Subero JI, Nielander I, et al. Mantle-cell lymphoma genotypes identified with CGH to BAC microarrays define a leukemic subgroup of disease and predict patient outcome. Blood 2005;105:4445-54.