Haematologica 1999; 84:594-599 original paper

# Secondary chromosome changes in mantle cell lymphoma

IWONA WLODARSKA,* STEFANIA PITTALUGA,°^ ANNE HAGEMEIJER,* CHRIS DE WOLF-PEETERS,*
HERMAN VAN DEN BERGHE*

*Center for Human Genetics; °Department of Pathology, University of Leuven, Belgium
^present address: Hematopathology Section, Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, MD, USA

ABSTRACT

***Background and Objective.*** **Mantle cell lymphomas (MCLs) comprise a rare but distinct clinicopathological entity usually associated with t(11;14). This translocation is regarded as a primary event, but it has been suggested that other as yet unidentified genetic alterations are required for development and progression of MCL.**

***Design and Methods.*** **In order to identify recurrent secondary changes that might point towards specific chromosomal regions contributing to the pathogenesis of MCL we studied 43 MCL cases in which clonal chromosomal abnormalities have been found during cytogenetic analysis.**

***Results.*** **In this series 83% of cases were characterized by t(11;14) and in the majority of them the t(11;14) was associated with multiple other chromosomal aberrations. Recurrent secondary changes were found in which imbalances of genetic material prevailed, losses being more common than gains. The former involved thirteen chromosomes, especially 13, 6q, 9q, 11q, 8 / 8p, 10 / 10p, and 14, whereas recurrent gains affected 3 / 3q. Non-randomly occurring breakpoints were relatively infrequent. The identified anomalies were also involved in aberrations observed in the group of MCL not associated with t(11;14). Some of them are shared with other B-cell proliferations.**

***Interpretation and Conclusions.*** **The data presented here indicate that MCL is characterized by consistently occurring secondary chromosome changes. Their significance for the development and / or progression of MCL needs to be elucidated and confirmed by further investigations.**


Key words: mantle cell lymphoma, t(11;14), secondary abnormalities

Mantle cell lymphoma (MCL) represents up to 4-10% of all non-Hodgkin's lymphomas (NHL) and is characterized by distinctive immunophenotypic and genetic features.[1] MCL is associated with the t(11;14)(q13;q32) which is considered as the primary molecular event. The translocation results in deregulation of the BCL1 locus at 11q13 by the regulatory sequences of the immunoglobulin heavy (IgH) chain gene complex and overexpression of the cyclin D1 (CCND1/PRAD1) gene that plays an important role in cell cycle progression, particularly at the G1/S phase transition. The t(11;14)/BCL1 rearrangement and/or overexpression of cyclin D1 can be identified in nearly all MCLs indicating a crucial but as yet unidentified role of CCND1 in the pathogenesis of this neoplasm.[2-6] Experimental data suggest that CCND1 has no oncogenic potential and by itself is not sufficient for the malignant transformation of affected cells. Co-operation with other oncogenes and/or tumor suppressor genes might be critical for the generation of lymphomas.[7,8] Recent studies on the tumor suppressor genes coding for p15, p16, p53 and pRb ruled out their common involvement in the pathogenesis in MCL.[9-14] Involvement of other genes may be revealed by chromosome investigations. If consistently occurring changes are found they may be indicative of further genomic errors. However very few data concerning secondary chromosomal aberrations associated with the t(11;14) are available.[15] This situation prompted us to review the karyotypes of 43 MCL cases with clonal chromosomal abnormalities collected in our institute, in order to evaluate whether recurrent secondary changes could be identified.

*Correspondence: I. Wlodarska, Ph.D., Centre for Human Genetics, Herestraat 49, B-3000 Leuven, Belgium.
Phone: international +32-16-346057 – Fax: international +32-16-346063.*

## Design and Methods

During the past 10 years, 74 cases diagnosed as mantle cell lymphoma were investigated cytogenetically. Of these 2 investigations failed, 29 cases had a normal karyotype and 43 had clonal abnormalities. The last group is the subject of this study. Cytogenetic analysis was carried out at the time of diagnosis in 31 cases and during the course of the disease in the remaining 12.

### *Histopathology*

The histologic material was reviewed in all cases. Immunophenotyping was performed in 40/43 cases using a panel of monoclonal antibodies including CD5, CD10, CD23, IgM, IgD, κ and λ.

### *Cytogenetics*

Chromosome analysis was performed according to standard protocols. Cells from lymph node and/or spleen were cultured for 1 day without stimulation, and bone marrow and peripheral blood cells were cultured for 3 days with or without phorbol ester (TPA) stimulation. Chromosomes were G- and/or R-banded. Karyotypes were reviewed and presented in accordance with the International System for Human Cytogenetic Nomenclature (ISCN).[16] Cases included in previous studies[17-20] are indicated in Table 1, although, for most of them, a complete karyotype is described for the first time.

## Results

### *Histopathologic data*

All cases reviewed were confirmed as MCL with a typical cytomorphology in 31 cases and a *blastoid* variant with aberrant morphology, including large pleomorphic cells as well as blast-like cells, in the remaining 12 cases. Light chain restriction and IgM expression were confirmed in all cases associated with the expression of IgD in 33/40 and CD5 in 34/40 cases.

### *Cytogenetic data*

Complete karyotypes are shown in Table 1. In most of the analyzed cases (36/43) abnormal cell clones had a pseudodiploid or near diploid chromosome number ranging from 43 to 48. Polyploid cells were found in 6 cases, including one with hyperdiploid and polyploid subclones. The t(11;14)(q13;q32) was found in 36/43 (83%) cases. In two of them (# 35 and 36) with der(14)t(11;14), poor quality of the chromosomes did not allow further identification. The t(11;14) translocation occurred as the sole abnormality in 3 cases (#1-3), including one in which a subclone with a der(3)t(3;3)(p24;q13) was identified (#3). In the remaining 31 cases 1 to 2 additional chromosomal abnormalities (#4-14), or complex chromosomal rearrangements (#15-34) were present. Karyotypic abnormalities occurring in addition to a t(11;14) consisted of numerical chromosomal changes as well as structural aberrations including reciprocal and nonreciprocal translocations, inversions, isochromosomes, deletions and unidentified marker chromosomes. The following chromosomal rearrangements were found as single secondary abnormalities in addition to the t(11;14): del(2)(q31q33), trisomy 3, der(3)t(3;3)(p24;q13), monosomy 6, del(9)(q12q22), del(10)(p13), and der(11)t(3;11)(q21;p13) (cases #3-9). In Table 2, we listed the secondary chromosomal changes that were found in at least two cases among the 34 associated with t(11;14). They were subdivided into two categories: one according to recurrent chromosomal breakpoints, one with imbalances of chromosomal material (losses and gains). The latter occurred as a consequence of numerical changes, deletions and non-reciprocal translocations.

Seven patients (#37-43) with abnormal karyotypes showed no t(11;14). All these cases were characterized by complex rearrangements affecting more than 5 chromosomes. Involvement of chromosomal regions where immunoglobulin genes loci are located (IGK/2p12 and IGH/14q32) was observed in three cases (#40, 41, 43), characterized by add(14)(q32), t(2;12)(p12;p13), and dic(3;14)(q13;q32). Recurrent chromosomal abnormalities included a break in 11p11 (2x), losses of chromosomes 6q (3x), 1/1p (2x), 7q (2x), 11 (2x), 14 (2x), and complete or partial gain of chromosome 3/3q (3x).

In 6 cases of our series (#2, 16, 27, 31, 38, 41) successful cytogenetic analysis was performed on consecutive samples. In 3 cases (#16, 31, 38) the same chromosomal aberrations were found in the involved tissues analyzed after 13, 1, and 2 and 3 months, respectively. In case #2, in which t(11;14) occurred as the sole abnormality in cells from a splenectomy specimen, all analyzed metaphases from a bone marrow (BM) aspirate, taken two weeks later, showed a complex karyotype with three additional chromosomal changes. The latter clone was found in three consecutive BM samples obtained after 5, 14, and 22 months. In cases #27 and 41 lymph nodes analyzed at the time of diagnosis already showed the presence of two related subclones: a predominant main one, and a less frequent second subclone with an additional chromosome abnormality or with a polyploid chromosome number. In further consecutive samples investigated 10 and 11 months later, all analyzed cells presented the second subclone with a more complex karyotype.

Correlation between pathology and cytogenetic data showed that approximately 15% of the cases with t(11;14) lacked either CD5 or IgD expression and 2 cases were double negative (#17 and 13). Of the 12 cases with blastoid morphology, 5 had polyploid metaphases, while only 1 case with typical morphology had a similar chromosome number range. Of the eleven cases with -13/del(13)(q), 5 had *blastoid* features. In 4 cases with del(17)(*p*) only one had blastoid features reminiscent of a lymphoblastic lymphoma. In cases with consecutive biopsies (#16, 31, 41) no intercurrent changes were observed in terms of morphology or immunophenotype.

## Discussion

In this study we analyzed clonal chromosomal aberrations occurring in 43 MCL cases. Translocation t(11;14)(q13;q32), the most common cytogenetic feature of MCL (19, 21-23) was found in 83% of cases. Karyotypes of t(11;14)-positive cases showed a variable degree of complexity ranging from the translocation as the sole abnormality to karyotypes with a highly complex pattern.

Our analysis of chromosomal abnormalities associated with t(11;14) allowed for the identification of several recurrent secondary changes. The most fre-

**Table 1. Results of cytogenetic analysis.**

| *Case #* | *Source* | *Status* | *Karyotype* |
|---|---|---|---|
| 1* | LN | P/44 | 46,XY,t(11;14)(q13;q32)[17] |
| 2* | S | D | 46,XY,t(11;14)(q13;q32)[3] |
| | BM | D | 47,XY,t(1;5)(q25;p15),inv(2)(p22q21),+3,t(11;14)(q13;q32)[12]/46,XY[4] |
| | BM | P/6 | 47,XY,t(1;5)(q25;p15),inv(2)(p22q21),+3,t(11;14)(q13;q32)[1]/46,XY[14] |
| | BM | P/17 | 47,XY,t(1;5)(q25;p15),inv(2)(p22q21),+3,t(11;14)(q13;q32)[2]/46,XY[8] |
| | BM | P/22 | 47,XY,t(1;5)(q25;p15),inv(2)(p22q21),+3,t(11;14)(q13;q32)[5]/46,XY[5] |
| 3 | BM | D | 46,XY,t(11;14)(q13;q32)[2]/46,idem,der(3)t(3;3)(p24;q13)[2]/46,XY[2] |
| 4 | BM | D | 46,XY,del(2)(q31q33),t(11;14)(q13;q32)[2]/46,XY[27] |
| 5 | S | D | 47,XY,+3,t(11;14)(q13;q32)[2]/46,XY[13] |
| 6* | LN | P/73 | 45,XY,-6,t(11;14)(q13;q32)[2]/46,XY[1] |
| 7* | S | D | 46,XY,del(9)(q12q22),t(11;14)(q13;q32)[8]/46,XY[10] |
| 8* | BM | P/42 | 46,XX,del(10)(p13),t(11;14)(q13;q32)[7[/46,XX[1] |
| 9* | PBL | D | 46,XY,der(11)t(3;11)(q21;p13),t(11;14)(q13;q32)[8]/46,XY[17] |
| 10* | BM | P/28 | 45,X,-Y,del(1)(p31p13),t(11;14)(q13;q32)[1]/43,idem,add(4)(p16),-9,-12,del(13)(q13q22)[3]/46,XY[4] |
| 11* | PBL | D | 46,XY,add(8)(p11),del(9)(q21q32),t(11;14)(q13;q32)[3]/46,XY[12] |
| 12* | LN | P/104 | 46,XY,del(6)(q15),t(11;14)(q13;q32),add(15)(q26)[17]/46,XY[8] |
| 13 | S | P/23 | 45,XX,t(11;14)(q13;q32),add(11)(q22),-13[3]/46,XX[2] |
| 14 | LN | D | 46,XY,add(9)(q34),t(11;14)(q13;q32),add(15)(q26)[8]/46,XY[3] |
| 15 | LN | P/64 | 46,XX,del(3)(p21),t(11;14)(q13;q32),add(15)(p11),add(19)(q13)[7]/46,XX[1] |
| 16* | LN | P/21 | 47,XY,der(11)del(11)(q14q23)t(11;17)(q13;q21),t(11;14)(q13;q32),der(17)t(11;17)(q13;q21),+12[11]/46,XY[6] |
| | LN | P/34 | 47,XY,der(11)del(11)(q14q23)t(11;17)(q13;q21),t(11;14)(q13;q32),der(17)t(11;17)(q13;q21),+12[5] |
| 17* | LN | D | 46,XY,-3,del(4)(p14),+der(6)t(6;?;3)(q12;?;q11),t(11;14)(q13;q32),del(18)(q21),add(22)(q13)[2]/47,idem,¬ +add(14)(q32)[3]/46,XY[2] |
| 18* | PBL | P/11 | 46,XY,-8,add(9)(p24),t(11;14)(q13;q32),add(11)(q21),add(12)(q24),+mar[3]/46,idem,del(6)(q23q34)[7]/46,XY[4] |
| | BM | P/11 | 46,XY,-8,del(6)(q23q34),add(9)(p24),t(11;14)(q13;q32),add(11)(q21),add(12)(q24),+mar[4]/46,XY[7] |
| 19* | LN | D | 44,X,-Y,add(1)(q32),del(1)(p11),i(6)(p10),der(11)add(11)(q13)t(11;14)(q13;q32),-13,der(14)t(11;14)(q13;q32)¬ [11]/46,XY[2] |
| 20* | LN | D | 43,Y,-X,add(8)(p23),-9,-9,-10,t(11;14)(q13;q32),add(11)(q22),-13,+2mar[12]/46,XY[2] |
| 21* | LN | D | 48,XY,del(2)(q31q35),-5,del(6)(q21q25),+der(6)t(6;16)(p11;p11),add(10)(p15),der(11)t(11;14)(q13;q32), ¬ add(13)(q34),-14,der(14)t(14pter->14q32::11q13->q21::?::11q21->qter),-15,-6,+r,+4mar[11]/48,idem, ¬ t(1;5)(p23;q33)[2] |
| 22 | LN | D | 43,XY,+X,-10,-11,del(11)(q21),-13,der(14)t(11;14)(q13;q32),-18,-20,+mar[1]/46,XY[2] |
| 23 | LN | D | 46-47,XX,t(7;17)(p11;p11),t(11;14)(q13;q32),-13,-14,+2-3mar[cp10] |
| 24 | LN | D | 46,XY,add(1)(p36),-4,del(6)(q21),t(11;14)(q13;q32),-14,-16,+3mar[9]/46,XY[1] |
| 25* | LN | D | 88-92,XXYY,-1,-1,del(3)(p25p23),+5,+i(5)(p10),-6,-10,t(11;14)(q13;q32),+der(11)t(11;14)(q13;q32),¬ add(12)(q24), -13,der(13)t(1;13)(p13;q14),-14,-16,add(17)(p11),+19,add(20)(q13)[cp6]/46,XY[2] |
| 26* | S | D | 86-89,XXYY,-5,+6,-11,-11,-13,-14,der(14)t(11;14)(q13;q32),+15,+21,+21,inc[10] |
| 27 | LN | D | 48,XY,-1,add(2)(p25),del(6)(q23),del(7)(q22q32),t(11;14)(q13;q32),+3mar[11]/81-83,idem,¬ -11,-11,-13,-13,inc[5]/46,XY[5] |
| | BM | P/10 | 81-83,XXY,-1,add(2)(p25),del(6)(q23),del(7)(q22q32),-11,-11,t(11;14)(q13;q32),-13,-13,+3mar,inc[2]/46,XY[23] |
| 28 | LN | D | 80-89,XXY,-X,dup(3)(q12q29),t(11;14)(q13;q32),i(17)(q10),inc[8] |
| 29 | LN | D | 68-75,XX,-Y,add(11)(p15),add(11)(p11),t(11;14)(q13;q32),add(17)(q21),add(19)(q13),+8mar,inc[10] |
| 30 | BM | D | 45,XY,der(1)inv(1)(p21q42)dic(1;6)(p21;q12),-8,der(11)del(11)(q13q14)t(11;14)(q13;q32),der(14)¬ t(11;14)(q13;q32),der(19)t(8;19)(q13;p13.1), der(19)t(8;19)(q13;p13.3)[10] |
| 31 | LN | D | 45,X,-Y,del(9)(q13q34.1),t(11;14)(q13;q32),der(16)t(11;16)(q13;q24)[6] |
| | S | P/1 | 45,X,-Y,del(9)(q13q34.1),t(11;14)(q13;q32),der(16)t(11;16)(q13;q24)[1]/46,XY[1] |
| 32 | LN | D | 46,XY,der(11)del(11)(q23)t(11;14)(q13;q32),der(14)t(11;14)(q13;q32),-19,+mar[8]/46,XY[2] |
| 33* | LN | D | 46,X,-X,add(3)(q29),add(7)(p22),-9,del(10)(q22q24),t(11;14)(q13;q32),-13,der(14)t(14;17)(p11;p11),¬ -17,+r,+3mar[19]/46,XX[1] |
| 34 | LN | D | 44-47,XY,dic(3;13)(q11;q34),-8,-9,t(11;14)(q13;q32),-15,+1-3mar[cp5] |
| 35 | LN | D | 45,XY,der(14)t(11;14),inc[3]/46,XY[2] |
| 36 | LN | D | 85-91,XXY,der(14)t(11;14),inc[2] |
| 37 | LN | D | 44,XY,-1,der(5)t(3;5)(p14;p15),del(6)(q15),-9,-13,-14,-21,-22,+r,+4mar[10]/46,XY[3] |
| 38* | BM | P/19 | 44,XX,del(1)(p31p11),t(4;9)(q13;q13),add(10)(p15),-11,-12,-14,-15 ,-20,-22, +4 mar[1]/46,XX[8] |
| | BM | P/21 | 44,XX,del(1)(p31p11),t(4;9)(q13;q13),add(10)(p15),-11,-12,-14,-15 ,-20,-22, +4 mar[3]/46,XX[2] |
| | BM | P/22 | 44,XX,del(1)(p31p11),t(4;9)(q13;q13),add(10)(p15),-11,-12,-14,-15 ,-20,-22, +4 mar [1]/46,XX[12] |
| 39* | S | P/42 | 46-48,XY,add(5)(p15),-6,der(14)t(7;14)(p11;p11),+1-3mar[cp3]/46-48, ¬ idem,del(3)(p21),add(6)(q25)[cp8]/46,XY[5] |
| 40 | LN | D | 43-46,X,-Y,add(1)(q21),+dic(3;11)(q15;p15),-5,add(6)(q27),del(6)(q23),add(14)(q32),+mar[cp10]/46,XY[6] |
| 41* | LN | D | 47,XY,-2,t(2;12)(p12;p13),+3,+15,add(17)(p11)[6]/47,idem,t(2;9)(q22;p21)[5]/46,XY[1] |
| | LN | P/11 | 47,XY,-2,t(2;9)(q22;p21),t(2;12)(p12;p13),+3,+15,add(17)(p11)[6] |
| 42* | BM | D | 47,XY,del(7)(q22q32),inv(11)(p11q23),+mar[2]/46,XY[63] |
| 43* | BM | P/68 | 46,XX,t(1;11)(p21;p11),dic(3;14)(q13;q31),+der(3)t(3;3)(p25;q13),del(5)(q11q34) [2]/46,idem, ¬ del(7)(q21q31)[3]/46,XX[3] |

**Cases included in previously published studies; LN: lymph node; BM: bone marrow; PBL: peripheral blood; D: diagnosis; P: progression.*

**Table 2. Recurrent secondary chromosomal changes in MCL.**

| Type of recurrent abnormalities | Frequency t(11;14)-positive group (34 cases) | t(11;14)-negative group (7 cases) | Entire series (41 cases) |
|---|---|---|---|
| Breakpoints | | | |
| 3q11 | 2 | | 2 |
| 10p15 | 2 | 1 | 3 |
| 11q13 | 3 | | 3 |
| 11q21* | 2 | | 2 |
| 11q22 | 2 | | 2 |
| 12q24 | 2 | | 2 |
| 13q34 | 2 | | 2 |
| 15q26 | 2 | | 2 |
| 17q21 | 2 | | 2 |
| 19q13 | 2 | | 2 |
| Imbalances | | | |
| *Losses* | | | |
| 1/1p | 4 | 2 | 6 |
| 2q* | 2 | | 2 |
| 3p | 3 | | 3 |
| 6/6q* | 7 | 3 | 10 |
| 7q | 2 | 2 | 4 |
| 8/8p | 5 | | 5 |
| 9/9q* | 7 | 1 | 8 |
| 10/10p* | 5 | | 5 |
| 11q* | 7 | 2 | 9 |
| 13/13q | 10 | 1 | 11 |
| 14 | 5 | 2 | 7 |
| 17/17p | 3 | 1 | 4 |
| Y | 4 | 1 | 5 |
| *Gains* | | | |
| 3/3q* | 5 | 3 | 8 |

**Anomalies occurred as the only additional change in association with t(11;14).*

quent were losses of chromosomal material involving thirteen different chromosomes, especially chromosomes 13 (10/34), 6q (7/34), 9q (7/34), 11q (6/34), 8/8p (5/34), 10/10p (5/34), 14 (5/34), 1/1p (4/34) and Y (4/34). In the category of chromosomal gains, the only recurrent gain was that of chromosome 3 material (with the commonly over-represented region at 3q25-qter) found in 5/34 cases, including three cases in which this aberration occurred as the only additional abnormality associated with t(11;14). Trisomy 3 was previously observed in 18% of cases with a t(11;14) reviewed by Johansson *et al.*[24] and was present in karyotypes of 3/9 MCL cases published more recently by Nowotny *et al*.[25] Our cytogenetic findings of non-random chromosomal losses and gains in MCL remain in agreement with results of the CGH analysis of 27 MCL cases very recently reported by Monni *et al.*[26] The authors detected frequent gains of 3q/3q26.1-27 (52%), 8q (30%) and 15q (26%), and recurrent losses of 13q (41%), 1p (33%), 6q (30%) and 11q/11q22 (30%) material. Differences in incidence of particular DNA copy number changes observed in our cases and the MCL series reported by Monni *et al.*[26] can be explained by a higher sensitivity of CGH when compared with conventional chromosome analysis.

Monosomy 13, del(6)(q), del(9)(q), and -Y found in our series of MCL had been listed among common secondary changes occurring in t(11;14)-positive hemopathies by Johansson *et al.*[24,27] It is noteworthy that deletion 11q and 13q have been frequently found in chronic lymphocytic leukemia (CLL),[28-30] whereas del(6)(q) has been observed in different subtypes of NHL and acute leukemia.[30-33] These abnormalities have been the subject of intensive molecular studies aimed at detecting target tumor suppressor genes (TSG) involved in lymphomagenesis.[34-41] Specific loss of 13q14/D13S272 sequences representing the smallest deleted region in CLL was very recently detected by FISH in 51% of B-CLL and 70% of MCL cases by Stilgenbauer *et al.*,[38] indicating that the same putative tumor supressor gene may play a role in the pathogenesis of both these malignancies. Molecular data and the finding of a normal expression of pRb in analyzed MCL cases[13] suggest that the RB gene is not affected in this type of lymphoma. In contrast to a relatively frequent del(13)(q) in our series of MCL, loss of 17p material was observed in only 3 t(11;14)-positive cases: two cases with *blastoid* features and one with typical morphology, but with a polyploid karyotype. Similar observations of the rare del(17p) and p53 mutations in MCL usually associated with aggressive clinical course and/or *blastoid* morphology have been previously reported by other groups.[10-12]

Structural aberrations associated with t(11;14) affected almost all chromosomes. Ten recurrent breakpoints could be identified in the present series but their frequency was low. These breakpoints have been occasionally reported in NHL and only a few of them can be associated with the vicinity of known oncogenes. Overall, the most frequently affected chromosome in our series was chromosome 11. Approximately 30% (11/34) of patients with a t(11;14) showed one or two additional structural aberrations affecting either the same chromosome or the second chromosome 11.

Karyotypes of seven patients of the present series without demonstrable t(11;14) or other abnormalities of 11q13 nevertheless showed typical morphology of MCL and classical immunophenotype. Southern blot analysis demonstrated a germline configuration of BCL1 in these cases (data not shown). No common chromosomal abnormality could be detected in these non-t(11;14) lymphomas. Instead, involvement of the same chromosomes as those identified as undergoing frequent recurrent secondary changes in the group of t(11;14)-positive MCL was noted (see Table 2). These aberrations included gain of chromosome 3 and loss of chromosome 1/1p, 6q, 7q, 9, 13, 11, 14, 17p and Y. Interestingly, some similar karyotypic features were found in two t(11;14)-negative

MCLs recently reported by Argatoff *et al.*[42]

With regard to ploidy, most cases of the present series had a near diploid chromosome number and polyploid cell clones were observed in a minority of cases. Ott *et al.*[43] noted a strong correlation between *blastoid* variant of MCL and polyploidy, demonstrated by cytogenetics and/or interphase FISH. We observed polyploidy less frequently and this may be due to the fact that these cells were not identified by classical cytogenetics, because part of less numerous subclones. One group found an association between polyploidy and leukemia presentation of MCL.[44] None of our polyploid cases showed peripheral blood involvement.

Cytogenetic analysis of consecutive samples from 6 patients showed either no evolution of karyotype, or overgrowth by the pre-existing subclone with more complex chromosomal abnormalities. No sequential gain of chromosomal aberrations was detected.

In summary, our study of so-called secondary chromosome changes in 43 MCL cases shows that the most frequent recurrent abnormalities resulted in genomic imbalances. Chromosomal losses regarded as a hallmark of the site of a tumor suppressor gene were more frequent than chromosomal gains, while recurrent structural aberrations occurred infrequently. Occurrence of non-random secondary aberrations in MCL points towards specific chromosomal regions contributing to the pathogenesis of this subtype of lymphoma. Our data, supported by the very recent findings of molecular cytogenetic studies of MCL, indicate that particular attention should be focused on the gain of chromosome 3/3q and loss of chromosome 13q, 6q, 9q, 11q, 8/8p, 10/10p, and 14, that have been observed as the only additional abnormalities associated with t(11;14), and as secondary changes frequently present in complex karyotypes of MCL. The significance of these abnormalities for the development and/or progression of MCL needs to be elucidated and confirmed by further investigations.

### Contributions and Acknowledgments

*IW and SP were the principal investigators, designed the study and wrote the paper. CP, AH and HV were involved in the design of the study and in critically revising the intellectual content. The authors are grateful to Magda Dehaen and the technicians of the leukemia laboratory for cytogenetic analysis and to Rita Logist for editorial assistance.*

### Funding

*This text presents research results of the Belgian programme on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming. The scientific responsibility is assumed by its authors.*

### Disclosures

*Conflict of interest: none*

*Redundant publications: no substantial overlapping with previous papers.*

### Manuscript processing

*Manuscript received December 11, 1998; accepted March 16, 1999.*

## References

1. Weisenburger DD, Armitage JO. Mantle cell lymphoma. An entity comes of age. Blood 1996; 87:4483-94.
2. Bosch F, Jares P, Campo E, et al. PRAD1/Cyclin D1 gene overexpression in chronic lymphoproliferative disorders: a highly specific marker of mantle cell lymphoma. Blood 1994; 84:2726-32.
3. Yang W, Zukerberg L, Motokura T, Arnold A, Harris N. Cyclin D1 (BCL-1, PRAD1) protein expression in low-grade B-cell lymphomas and reactive hyperplasia. Am J Pathol 1994; 145:86-96.
4. de Boer CJ, Schuuring E, Dreef E, et al. Cyclin D1 protein analysis in the diagnosis of mantle cell lymphoma. Blood 1995; 86:2715-23.
5. de Boer C, van Krieken J, Kluin-Nelemans H, Kluin P, Schuuring E. Cyclin D1 messanger RNA overexpression as a marker for mantle cell lymphoma. Oncogene 1995; 10:1833-40.
6. Vaandrager J, Schuuring E, Zwikstra E, et al. Direct visualization of dispersed 11q13 chromosomal translocations in mantle cell lymphoma by multicolor DNA fiber fluorescence in situ hybridization. Blood 1996; 88:1177-82.
7. Bodrug SE, Warner BJ, Bath ML, Lindeman GJ, Harris AW, Adams JM. Cyclin D1 transgene impedes lymphocyte maturation and collaborates in lymphomagenesis with the myc gene. EMBO J 1994; 13:2124-30.
8. Lovec H, Grzeschiczek A, Kowalski MB, Möröy T. Cyclin D1/bcl1 cooperates with myc genes in the generation of B-cell lymphoma in transgenic mice. Embo J 1994; 13:3487-95.
9. Koduru PRK, Zariwala M, Soni M, Gong JZ, Xiong Y, Broome JD. Deletion of cyclin-dependent kinase 4 inhibitor genes p15 and p16 in non-Hodgkin's lymphoma. Blood 1995; 86:2900-5.
10. Louie DC, Offit K, Jaslow R, et al. p53 overexpression as a marker of poor prognosis in mantle cell lymphomas with t(11;14)(q13;q32). Blood 1995; 86: 2892-9.
11. Greiner TC, Moynihan MJ, Chan WC, et al. p53 mutations in mantle cell lymphoma are associated with variant cytology and predict poor prognosis. Blood 1996; 87:4302-40.
12. Hernandez L, Fest T, Cazorla M, et al. p53 mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. Blood 1996; 87:3351-9.
13. Jares P, Campo E, Pinyol M, et al. Expression of retinoblastoma gene product (pRB) in mantle cell lymphomas. Correlation with cyclin D1 (PRAD1/CCND1) mRNA levels and proliferative activity. Am J Pathol 1996; 148:1591-600.
14. Pinyol M, Hernandez L, Cazorla M, et al. Deletions and loss of expression of P16 INK4a and P21 Waf1 genes are associated with aggressive variants of mantle cell lymphomas. Blood 1997; 89:272-80.
15. Mitelman F. Catalog of chromosome aberrations in cancer. 5th ed. New York: Wiley-Liss; 1994.
16. ISCN. Guidelines for Cancer Cytogenetics. Mitelman F, ed. Supplement to: An International System for Human Cytogenetic Nomenclature. Basel: Karger; 1995.
17. Vandenberghe E, De Wolf-Peeters C, Van den Oord J,

et al. Translocation (11;14): A cytogenetic anomaly associated with B-cell lymphomas of non-follicle centre cell lineage. J Pathol 1991; 163:13-8.
18. Wlodarska I, Schoenmakers E, Kas K, et al. Molecular mapping of the chromosome 11 breakpoint of t(11;17) (q13;q21) in a t(11;14)(q13;q32)-positive B non-Hodgkin's lymphoma. Genes Chromosomes Cancer 1993; 8:224-9.
19. Pittaluga S, Wlodarska I, Stul MS, et al. Mantle cell lymphoma: a clinicopathological study of 55 cases. Histopathol 1995; 26:17-24.
20. Pittaluga S, Verhoef G, Criel A, et al. "Small" B-cell non-Hodgkin's lymphomas with splenomegaly at presentation are either mantle cell lymphoma or marginal zone cell lymphoma. A study based on histology, cytology, immunohistochemistry and cytogenetic analysis. Am J Surg Pathol 1996; 20:221-3.
21. Raffeld M, Jaffe ES. bcl-1, t(11;14), and mantle cell-derived lymphomas. Blood 1991; 78:259-63.
22. Rimokh R, Berger F, Delsol G, et al. Rearrangement and overexpression of the BCL-1/PRAD-1 gene in intermediate lymphocytic lymphomas and in t(11q13)-bearing leukemias. Blood 1993; 81:3063-7.
23. Ott MM, Helbing A, Ott G, et al. bcl-1 rearrangement and cyclin D1 protein expression in mantle cell lymphoma. J Pathol 1996; 179:238-42.
24. Johansson B, Mertens F, Mitelman F. Cytogenetic evolution patterns in non-Hodgkin's lymphoma. Blood 1995; 86:3905-14.
25. Nowotny H, Karlic H, Grüner H, et al. Cytogenetic findings in 175 patients indicate that items of the Kiel classification should not be disregarded in the REAL classification of lymphoid neoplasms. Ann Hematol 1996; 72:291-301.
26. Monni O, Oinonen R, Elonen E, et al. Genes Chromosomes Canc 1998; 21:298-307.
27. Johansson B, Mertens F, Mitelman F. Primary vs. secondary neoplasia-associated chromosomal abnormalities - Balanced rearrangements vs. genomic imbalances? Genes Chromosomes Canc 1996; 16:155-63.
28. Liu Y, Fhermanson M, Grander D, et al. 13q deletions in lymphoid malignancies. Blood 1995; 86:1911-5.
29. Döhner H, Stilgenbauer S, James MR, et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood 1997; 89:2516-22.
30. Larramendy ML, Siitonen SM, Zhu Y, et al. Optimized mitogen stimulation induces proliferation of neoplastic B-cell in chronic lymphocytic leukemia: significance for cytogenetic analysis. Cytogenet Cell Genet 1998; 82: 215-21.
31. Gaidano G, Hauptschein RS, Parsa NZ, et al. Deletions involving two distinct regions of 6q in B-cell non-Hodgkin's lymphoma. Blood 1992; 80:1781-7.
32. Offit K, Parsa NZ, Gaidano G, et al. 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. Blood 1993; 82:2157-62.
33. Hayashi Y, Raimondi SC, Look AT, et al. Abnormalities of the long arm of chromosome 6 in childhood acute lymphoblastic leukemia. Blood 1990; 76:1626-30.
34. Parsa NZ, Gaidano G, Mukherjee AB, et al. Cytogenetic and molecular analysis of 6q deletions in Burkitt's lymphoma cell lines. Genes Chromosom Canc 1994; 9:13.
35. Devilder MC, François S, Bosic C, et al. Deletion cartography around the D13S25 locus in B cell chronic lymphocytic leukemia and accurate mapping of the involved tumor suppressor gene. Cancer Res 1995; 55:1355-7.
36. Stilgenbauer S, Leupolt E, Ohl S, et al. Heterogeneity of deletions involving RB-1 and the D13S25 locus in B-cell chronic lymphocytic leukemia revealed by fluorescence in situ hybridization. Cancer Res 1995; 55: 3475-7.
37. Stilgenbauer S, Liebisch P, James MR, et al. Molecular cytogenetic delineation of a novel critical genomic region in chromosome bands 11q22.3-q23.1 in lymphoproliferative disorders. Proc Natl Acad Sci USA 1996; 93:11837-41.
38. Stilgenbauer S, Nickolenko J, Wilhelm J, et al. Expressed sequences as candidates for a novel tumor suppressor gene at band 13q14 in B-cell chronic lymphocytic leukemia and mantle cell lymphoma. Oncogene 1998; 16:1891-7.
39. Bullrich F, Veronese ML, Kitada S, et al. Minimal region of loss at 13q14 in B-cell chronic lymphocytic leukemia. Blood 1996; 88:3109-15.
40. Gèrard B, Cavé H, Guidal C, Dastugue N, Vilmer E, Grandchamp B. Delineation of a 6 cM commonly deleted region in childhood acute lymphoblastic leukemia on the 6q chromosomal arm. Leukemia 1997; 11:228-32.
41. Zhang YM, Weber-Matthiesen K, Sieber TR, Matthiesen P, Schlegelberger B. Frequent deletions of 6q23-24 in B-cell non-Hodgkins lymphomas detected by fluorescence in situ hybridization. Genes Chromosomes Canc 1997; 18:310-3.
42. Argatoff L, Connors J, Klasa R, Horsman D, Gascoyne R. Mantle cell lymphoma: a clinicopathologic study of 80 cases. Blood 1997; 89:2067-78.
43. Ott G, Kalla J, Ott MM, et al. Blastoid variants of mantle cell lymphoma: frequent bcl-1 rearrangements at the major translocation cluster region and tetraploid clones. Blood 1997; 89:1421-9.
44. Daniel M-T, Tigaud I, Flexor M-A, Nogueira, M-E, Berger R, Jonveaux P. Leukaemic non-Hodgkin's lymphomas with hyperdiploid cells and t(11;14) (q13;q32): a subtype of mantle cell lymphoma? Br J Haematol 1995; 90:77-84.