Landscape of somatic mutations and clonal evolution in mantle cell lymphoma

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Mantle cell lymphoma (MCL) is an aggressive tumor, but a subset of patients may follow an indolent clinical course. To understand the mechanisms underlying this biological heterogeneity, we performed whole-genome and/or whole-exome sequencing on 29 MCL cases and their respective matched normal DNA, as well as 6 MCL cell lines. Recurrently mutated genes were investigated by targeted sequencing in an independent cohort of 172 MCL patients. We identified 25 significantly mutated genes, including known drivers such as ataxia-telangiectasia mutated (ATM), cyclin D1 (CCND1), and the tumor suppressor TP53; mutated genes encoding the anti-apoptotic protein BIRC3 and Toll-like receptor 2 (TLR2); and the chromatin modifiers WHSC1, MLL2, and MEF2B. We also found NOTCH2 mutations as an alternative phenomenon to NOTCH1 mutations in aggressive tumors with a dismal prognosis. Analysis of two simultaneous or subsequent MCL samples by whole-genome/whole-exome sequencing revealed subclonal heterogeneity at diagnosis in samples from different topographic sites and modulation of the initial mutational profile at the progression of the disease. Some mutations were predominantly clonal or subclonal, indicating an early or late event in tumor evolution, respectively. Our study identifies molecular mechanisms contributing to MCL pathogenesis and offers potential targets for therapeutic intervention.

next-generation sequencing | cancer genetics | cancer heterogeneity

To gain insight into the molecular pathogenesis of MCL we performed whole-genome sequencing (WGS) and whole-exome sequencing (WES) of 29 MCL and further investigated mutated genes in an expanded series of patients. We also analyzed the subclonal heterogeneity of the tumors and their modulation during the evolution of the disease.

Results

Landscape of Mutations in MCL. We performed WGS and WES of 4 and 29 MCL, respectively. These patients were representative of the broad clinical and biological spectrum of the disease, including five patients with an indolent clinical evolution. In

Significance

This is a comprehensive whole-genome/whole-exome analysis of mantle cell lymphoma (MCL). We sequenced 29 MCL cases and validated the findings by target sequencing of 172 additional tumors. We identified recurrent mutations in genes regulating chromatin modification and genes such as NOTCH2 that have a major impact on clinical outcome. Additionally, we demonstrated the subclonal heterogeneity of the tumors already at diagnosis and the modulation of the mutational architecture in the progression of the disease. The identification of new molecular mechanisms may open perspectives for the management of MCL patients.


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addition, we performed WES of six MCL cell lines. Selected mutated genes were investigated in a validation series of 172 MCL patients (SI Appendix, Tables S1–S6). We detected about 3,700 somatic mutations per tumor (1.2 per Mb) by WGS (SI Appendix, Figs. S1A and S2A and Dataset S1). The most common substitution was the transition C > T/G > A, usually occurring in a CpG context. Two IGHV-unmutated MCL showed a higher proportion of A > C/T > G mutations than the two IGHV-unmutated cases (SI Appendix, Fig. S1B). The breakpoints of the (t(11;14) translocation differed in the four cases (13) (SI Appendix, Table S7). We next investigated the presence of regional clustering of somatic mutations by constructing "rainfall plots" (SI Appendix, Fig. S2B). Foci of hypermutation or kataegis, a phenomenon recently described in breast cancer (14), were observed in three cases. They were more frequent in the two IGHV-unmutated tumors. These clusters occurred around the 11q13 breakpoint of the (t(11;14); the Ig genes at 2p11, 14q22.32, and 22q11.22; and the deleted 9p21.3 region, but we also observed this phenomenon in regions without apparent structural alterations and lacking coding genes (SI Appendix, Table S8). The same clusters of hypermutation were observed in the sequential sample of case M003, suggesting that kataegis in MCL may occur as an early phenomenon that remains stable during the evolution of the disease.

We characterized the spectrum of mutations in 29 MCL by WES (SI Appendix, Tables S1, S3, and S9 and Dataset S2). All these cases were also analyzed by SNP array for copy number alterations (CNA) and copy number neutral loss of heterozygosity (SI Appendix, Fig. S3 and Table S10). We identified 652 protein-coding genes with somatic mutations affecting the structure of the encoded protein (nonsynonymous changes, frameshifts in the coding sequence, and mutations affecting canonical splicing sites) with a median of 20 mutations per case (range 8–47). Twenty-five of the 33 mutated genes in at least two samples were mutated at a rate significantly higher than expected by chance, and all tumors harbored mutations in at least two of these 25 genes (Fig. 1 and SI Appendix, Table S11). Similarly, five of the six MCL cell lines also had mutations in at least one of the recurrently mutated genes identified in primary tumors (Fig. 1, SI Appendix, and Dataset S3). Chromosomal CNAs were present in 26/29 (90%) cases (mean 11.1 ± 9.2 per altered case) (SI Appendix, Fig. S3 and Table S10). Tumors expressing SOX11 showed a significantly higher number of CNAs than SOX11-negative MCLs (mean 13 ± 9 versus 2 ± 2; P = 2.1 × 10−5), despite the similar number of somatic mutations (mean 24 ± 17 versus 17 ± 9; P = 0.141) (Fig. 1). Interestingly, five patients who did not need treatment (median 55 mo, range 4–147) had significantly fewer somatic protein-coding mutations (mean 11 ± 4 versus 25 ± 11, P = 3.4 × 10−5) and CNAs (mean 2 ± 3 versus 12 ± 9; P = 0.001) than patients who required treatment at diagnosis (n = 24).

Recurrent Somatic Mutations in MCL. ATM, CCND1, and TP53, previously described as drivers in MCL, were among the most frequently mutated genes in this study. ATM mutations were found in 12 of the 22 (55%) tumors expressing SOX11, but in none of the SOX11-negative MCL (P = 0.023) (Table 1, Fig. 1, and SI Appendix, Table S9). Six of the mutations were associated with deletions of the wild-type allele, whereas five cases with no 11q loss had two different ATM mutations per case and only one was a single mutation with no 11q deletion (SI Appendix, Fig. S44). These mutations mainly truncated or affected functional domains (SI Appendix, Fig. S5). CCND1 mutations were found predominantly in exon 1 (9 of 10 CCND1-mutated cases) (SI Appendix, Fig. S6) and were more frequent in SOX11-negative MCL (P = 0.03) and with IGKV-unmutated [7/12 (58%) versus 3/16 (19%), P = 0.05], suggesting their acquisition in the germinal center microenvironment (Fig. 1, Table 1, and SI Appendix, Table S9). TP53 mutations [8/29 (28%)] were associated with 17p alterations in six cases, and only one case without 17p alteration had two mutations. TP53 mutations were equally distributed in tumors regardless of SOX11 expression or IGKV mutations (Fig. 1).

We also identified recurrent mutations in WHSCI, MLL2, BIRC3, MEF2B, and TLR2 (Table 1, Fig. 1), as well as NOTCH2 in one case. WHSCI encodes a histone 3 methyltransferase of lysine-36 (H3K36) that has not been found previously mutated in lymphomas. Two missense mutations (p.E1099K and p.T1150A) were recurrently found in two cases each. Both residues are in close proximity and affect two of the most conserved domains in MLL2. We further analyzed these exons in 101 additional tumors and confirmed the presence of the same mutations in nine more cases [total 13/130 (10%) (Table 1, Fig. 2A, and SI Appendix, Table S9 and Fig. S7). Interestingly, WHSCI (also named MMSST or NSD2) is the target of the IGKH-translocation t(4;14) in plasma cell myeloma (PCM), where it is overexpressed and associated with an increase in H3K36 and a decrease in H3K27 methylation across the genome (15). Gene expression analysis of 8 WHSCI-mutated and 31 WHSCI-unmutated MCL identified 236 genes differentially expressed (false discovery rate <5%) with the majority of these genes [192/236 (81%)] up-regulated in the WHSCI-mutated cases (Fig. 3B; SI Appendix, Table S12). A gene set enrichment analysis (GSEA) using previously published lymphoid gene expression signatures (15) demonstrated that WHSCI-mutated cases displayed significant overexpression of several signatures related to proliferation and cell-cycle regulation (SI Appendix, Fig. S8) (16). Interestingly, WHSCI-mutated MCL showed a highly significant overexpression of the gene signature up-regulated in PCM with the t(4;14) translocation over-expressing WHSCI1 (17). In addition, WHSCI1-mutated MCL showed overexpression of genes up-regulated by wild-type WHSCI or the gain-of-function exon 19 mutant WHSCI in the KMS11 PCM cell line (15) (SI Appendix, Fig. S8).

In addition, the histone methyltransferase MLL2 was mutated in 4/29 primary tumors and 2/6 MCL cell lines. Four of the six mutations were truncating and two were missense changes and affected the conserved FYRN and FYRC domains (Table 1 and SI Appendix, Fig. S9). None of these cases had a deletion of the

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**Fig. 1.** WES in 29 cases and 6 MCL cell lines. Heatmap with the mutation pattern of the 25 significantly recurrent mutated genes. Each row represents a gene and each column represents a primary tumor/cell line. Vertical bar graphs show the total number of somatic mutations by WES (blue) and somatic CNAs by SNP array (green) for primary tumors, and the total number of nonpolymorphic variants and CNAs for cell lines. The plot below the case label indicates sample characteristics (SOX11 expression and IGHV gene status).
second allele. MEF2B carried the same p.K23R somatic mutation in exon 2 in 2/29 primary tumors, and a p.N49S mutation in exon 2 was found in the REC-1 cell line. We then expanded the study of exon 2 in 158 MCL cases and found the same p.K23R mutation in four additional tumors (total 6/187 (3.2%)) (Table 1 and SI Appendix, Figs. S7 and S10). Virtually all WHSC1, MLL2, and BIRC3 mutations occurred in MCL expressing SOX11.

Deletions of 11q21–q23 are common alterations in MCL. In addition to ATM, BIRC3 is also located in this region (11q22.2). We found inactivating mutations in exon 9 in two MCL cases and a splice-site mutation in an additional patient (Table 1, Fig. 1, and Fig. 3C). We expanded the study and found mutations in 11/173 (6.4%) cases, and these cases had more frequent 11q deletions than BIRC3-unmutated MCL [10/11 (91%) versus 25/87 (29%), respectively; P = 1.1 × 10−3] (Fig. 2D).

We found two mutations of TLR2 in two SOX11-negative/IGHV-unmutated MCL (Table 1). One of these mutations (p.D327V) had been previously identified in one IGHV-unmutated chronic lymphocytic leukemia (CLL) (18). To determine the potential functional activity of these mutations, we stimulated primary cells from tumors and healthy donors. Only IL-1RA had increased secretion of IL-6 and IL-1RA in MCL carrying the p.D327V mutation had extremely high basal levels of IL-6 compared with the basal or poststimulation levels of other tumors or normal B lymphocytes (Fig. 2E). Several TLR2 agonist stimuli (PGN-SA, LTA-SA, or PAM3) did not generate IL-6 increases in these cells.

Among the other mutated genes, we found mutations of the ubiquitin ligase UBR5, recently described in MCL patients (19), and an inactivating mutation affecting B2M (p.L135*10) in a MCL carrying a 15q12–q21.1 deletion, encompassing the B2M locus. However, we did not find mutations in an additional 97 MCL patients, including 3 with a similar 15q monosomic disease.

**NOTCH2 and NOTCH1 Mutations in MCL**. The finding of a NOTCH2 mutation prompted us to expand the analysis of the HD, TAD, and PEST domains in additional tumors, and we found mutations in 9/172 MCL (5.2%) (Table 1; Fig. 3A). All these mutations generate a premature stop codon within the PEST domain. Gene expression analysis of two NOTCH2-mutated and 19 NOTCH2-unmutated MCL (also wild-type for NOTCH1) showed many differentially expressed genes (n = 841) (false discovery rate < 5%); 42% of them (n = 355) were up-regulated in NOTCH2-mutated cases and were significantly enriched in cell-cycle and metabolic pathways (Fig. 3B, SI Appendix, and Dataset S4). GSEA showed that NOTCH2 mutated samples displayed a significant overexpression of genes up-regulated by NOTCH activation in lymphoid cells (20) and also had a concordant modulation of gene signatures regulated by NOTCH inhibition in MCL cell lines (21–23) (SI Appendix, Fig. S12). NOTCH2 mutations occurred more frequently in blastoid/pleomorphic MCL (66 versus 18%, P = 0.001) and conferred a dismal prognosis [3-y overall survival (OS): 0 versus 62%, hazard ratio (HR): 19.5; confidence interval: 1.3–250] (Fig. 3C) and were also associated with poor outcome. In a bi-variate analysis, both NOTCH2 mutations (hazard ratio: 3.5; 95% confidence interval: 1.3–9.5; P = 0.017) and TP53 mutations (hazard ratio: 2.4; 95% confidence interval: 1.4–4.2; P = 0.003)

**Fig. 2.** WHSC1, BIRC3, and TLR2 alterations in MCL. (A) WHSC1 mutations in MCL (above gene symbol) and PCM (below gene symbol) (41). Amino acid conservation of WHSC1 (blue plot), and multiple sequence alignment of the region containing the two mutations in residues E1099 and T1150. (B) Heatmap showing the 236 differentially expressed genes in WHSC1-mutated versus WHSC1-unmutated MCL cases. (C) BIRC3 mutations in MCL (Upper) and other hematologic neoplasms (Lower) (38, 39, 42). (D) Graphic representation of 11q deletions in BIRC3-mutated cases. Ten of 11 mutated MCL cases carried deletions encompassing BIRC3 gene (11q22.2). (E) PLOTS representing cytokine levels secreted by B cells from tumors and healthy donors. Only IL-1RA and IL-6 showed differences between TLR2-mutated and -unmutated samples. Percentage of increase or decrease of cytokine expression before and after stimulation with PGN-SA (blue bar) and PAM3 (red bar). *Significance comparing the two cases with p.D327V mutation versus TLR2-unmutated tumors (P = 0.037 and 0.026 for IL-1RA and IL-6, respectively). (F) Basal levels of IL-6 and IL-1RA in MCL, CLL, and normal B cells.

### Table 1. Frequency of recurrently mutated genes in mantle cell lymphoma

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell lines</th>
<th>MCL cases, %</th>
<th>SOX11+ MCL, %</th>
<th>IGHV-U MCL, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>41 (12/29)</td>
<td>55 (12/22)*</td>
<td>50 (8/16)</td>
<td></td>
</tr>
<tr>
<td>CND1</td>
<td>35 (10/29)</td>
<td>18 (3/22)*</td>
<td>19 (3/16)*</td>
<td></td>
</tr>
<tr>
<td>MLL2</td>
<td>2/9</td>
<td>14 (4/29)</td>
<td>18 (4/42)</td>
<td>13 (2/16)</td>
</tr>
<tr>
<td>WHSC1</td>
<td>10 (13/100)</td>
<td>15 (12/82)*</td>
<td>14 (11/80)</td>
<td></td>
</tr>
<tr>
<td>BIRC3</td>
<td>6 (11/173)</td>
<td>7 (7/95)</td>
<td>9 (9/97)</td>
<td></td>
</tr>
<tr>
<td>NOTCH1</td>
<td>5 (9/172)</td>
<td>6 (6/36)</td>
<td>6 (6/95)</td>
<td></td>
</tr>
<tr>
<td>NOTCH1R</td>
<td>5 (8/172)</td>
<td>5 (5/95)</td>
<td>7 (7/95)</td>
<td></td>
</tr>
<tr>
<td>MEF2B</td>
<td>3 (6/187)</td>
<td>5 (5/100)</td>
<td>4 (4/102)</td>
<td></td>
</tr>
<tr>
<td>TLR2</td>
<td>1 (2/171)</td>
<td>0/94</td>
<td>0/96</td>
<td></td>
</tr>
</tbody>
</table>

Data are from WES and Sanger sequencing. IGHV-U, IGHV-unmutated gene.

*Significant P values (see details in SI Appendix, Table S9).
were identified as independent risk factors for OS (SI Appendix, Fig. S13).

**NOTCH1** mutations have been recently described in MCL (20). We investigated this gene and found truncating mutations in 8/172 (4.6%) MCL cases, as well as in MINO and REC-1 cells (Table 1 and SI Appendix, Fig. S14). **NOTCH1**-mutated tumors were predominantly blastoid/pleomorphic (67 versus 19%, \( P = 0.03 \)) and showed shorter survival than **NOTCH1**-unmutated MCL (3-y OS: 33 versus 60%, \( P = 0.026 \)) (SI Appendix, Fig. S15). **NOTCH1** and **NOTCH2** mutations occurred in different subsets of tumors because only one of the 16 patients with mutations in these genes had mutations in both. Taken together, **NOTCH1/2** mutations were present in 9.5% of MCL and identified a subset of tumors with more adverse biological and clinical features including blastoid/pleomorphic morphology (67 versus 13%, \( P = 1.3 \times 10^{-5} \)) and a significant shorter survival (3-y OS: 24 versus 63%, \( P = 3.4 \times 10^{-4} \)) (Fig. 3C).

**Sequencing Simultaneous and Subsequent MCL Samples Reveals Clonal Heterogeneity.** To explore the subclonal architecture of MCL, we sequenced a second tumor sample obtained simultaneously from two different topographic sites (\( n = 6 \)) or at two time points (diagnosis and disease progression, \( n = 2 \)). Analysis of the frequency of reads supporting somatic substitution allowed the estimation of major subclonal populations (24). Four of the six patients with simultaneous samples showed the same mutations and CNAs in the peripheral blood (PB) and lymphoid tissue, suggesting the presence of a single major clone at both sites. In contrast, two cases (M023 and M026) had a subset of common mutations in both topographic sites, but they also carried a subset of mutated genes that were exclusive of the PB or tumor sample (Fig. 4A and B). This pattern of alterations is consistent with two major subpopulations derived from an initial founder clone differentially represented in the two topographic sites. In addition, we also observed a different pattern of genomic alterations at diagnosis and progression in the two cases with sequential samples (Fig. 4C and D and SI Appendix, Table S10). Patient M003 had stable disease without treatment for more than 3 y and then rapidly progressed. The major clone identified at diagnosis gained new mutations and genomic complexity at the time of clinical progression. This new clone carried the same 17p deletion and somatic mutations observed in the initial sample, but had a 16% increase in the number of whole-genome mutations (from 3,928 to 4,665) (SI Appendix, Fig. S14). These included nonsynonymous mutations in additional genes, a complex DNA copy number profile with 20 acquired CNAs, and chromothripsis involving chromosomes 4 and 12 (Fig. 4C and SI Appendix, Fig. S24). Patient M002 was treated with chemotherapy after diagnosis and relapsed 3 y later. The major clone at relapse maintained a subset of CNAs and somatic mutations present at diagnosis, but 11 of the initial mutations had disappeared and other mutated genes had emerged (Fig. 4D). This pattern of evolution is consistent with the erasure of the major initial subclone by chemotherapy and the relapse of a new subclone after treatment.

Comparison of the allele frequency of mutations in the different simultaneous or evolving subclones may help to infer the dynamic architecture of somatic events in the evolution of tumors (24, 25). Eight of the 25 recurrently mutated genes in MCL (ATM, CCND1, MLL2, KCNC2, KIAAA1671, PCSK2, TNRC6B, and TRPM6) were present at similar allelic frequency in the two subclones of different cases, suggesting that they represent early events. In contrast, four recurrently mutated genes (ABCA3, TLR2, TP53, and WHSC1) were seen in only one of the two simultaneous or emerging subclones at progression, supporting the notion that they might constitute later events. We further analyzed by Sanger sequencing 11 additional serial and 8 simultaneous tumor samples from different topographic sites (PB and lymphoid tissues) (SI Appendix, Fig. S16). Interestingly, we observed that **BIRC3** mutations were absent at diagnosis in two cases that acquired the mutation in a posttreatment sample associated with the acquisition of an 11q22.1–q24.2 deletion in one of them. Another case showed the **BIRC3** mutation in the PB but not in the simultaneous lymph node. Additionally, **NOTCH1** was mutated in only one of the synchronous samples in two cases.

**Discussion** We have conducted a comprehensive genomic study of MCL that has revealed the heterogeneous spectrum of somatic mutations of this tumor, with several molecular mechanisms contributing to the pathogenesis and the clinical progression of the disease. The genome sequencing of simultaneous and sequential tumor samples has highlighted the subclonal heterogeneity of the mutations already present at diagnosis and their dynamic evolution in the progression of the disease.

The whole-genome analysis showed a relative low number of global somatic mutations in MCL (1.2 per Mb), slightly higher to CLL (26) or acute myeloid leukemia (27), but lower than in other lymphoid or nonhematopoietic tumors (28, 29). Interestingly, we identified a distinct mutational signature characterized by \( A > C/T > G \) substitutions in a TpA context in the two MCL with **IGHV**-mutated. This signature was initially identified in CLL with **IGHV**-mutated and more recently confirmed as a unique feature of lymphoid neoplasms originating in germinal center cells (26, 29) and has been attributed to the action of the error-prone DNA polymerase \( \eta \) during the **IGHV** somatic hypermutation process (26).

The most commonly mutated genes were the previously identified MCL drivers **ATM**, **CCND1**, and **TP53**. These mutations were differentially distributed in subtypes of the disease according to the **IGHV** mutational status and SOX11 expression. Thus, **ATM** mutations were seen only in SOX11-positive tumors, whereas **CCND1** mutations were preferentially detected in MCL with **IGHV**-mutated and **TP53** mutations were equally
distributed among the different groups. The integration of the CNA and somatic mutations showed that only TP53, ATM, and BIRC3 recurrent mutations were associated with allelic losses (17p and 11q, respectively), whereas CDKN2A, the only gene targeted by recurrent homozygous deletions, did not carry somatic mutations in other cases. Interestingly, three mutated genes (WHSC1, MLL2, and MEF2B) function as chromatin modifiers, and altogether these mutations occurred in 10 of the 29 (14%) cases examined by WES. The analysis of WHSC1 and MEF2B in an expanded series confirmed the relative high frequency of these mutations in MCL (10% and 3.2%, respectively). WHSC1 mutations have not been described previously in lymphomas, but this gene is the target of the t(4;14) translocation in PCM, and the same mutation observed in exon 18 has been recently detected in an acute lymphoblastic leukemia (ALL) patient (30). The WHSC1 overexpression in PCM and the mutation in ALL seem to have an activating function because they increase the H3K36 methylation associated with a methylation decrease in H3K27 across the genome (15). Our WHSC1-mutated primary MCL had the same overexpressed gene signatures modulated by WHSC1 activation in PCM, suggesting that these mutations may also have a similar activating function in MCL. The mutations observed in MLL2 and MEF2B are similar to those detected in diffuse large B-cell lymphoma (DLBCL) and follicular lymphomas, but their functional consequences are not yet well understood (31–33). Notably, virtually all MLL2, WHSC1, and MEF2B mutations were found in MCL with IGHV-unmutated or expressing SOX11.

Recent studies have suggested a role of microenvironment stimuli in sustaining MCL (1). TLRs mediate cell responses to specific pathogen-associated molecular patterns (34). In that sense, we found mutations of TLR2 in two SOX11-negative/IGHV-mutated MCL, and these mutations were associated with increased production of IL-1RA and IL-6 by the tumor cells. Notably, high levels of IL-1RA have been associated with aggressive behavior in some lymphomas (35), and IL-6 sustains growth and survival of MCL cells (36). These findings suggest that TLR2 mutations may contribute to the pathogenesis of a subset of SOX11-negative/IGHV-mutated MCL by modulating tumor microenvironment responses.

In addition to the statistically significant mutations described above, we found a mutation in NOTCH2 in one MCL. Similar activating mutations have been recently described in splenic marginal zone lymphomas and in NOTCH1 in aggressive CLL and MCL (37, 38). These findings prompted us to expand the study of NOTCH2 and NOTCH1 mutations in MCL and found their presence in 5.2% and 4.7% of the tumors, respectively. All these mutations generated truncating and likely more active proteins and occurred in a subset of tumors with very aggressive clinical behavior. Only 1 of 16 tumors had simultaneous mutations in both genes, suggesting that they may give a similar selective advantage to the cells. All these findings indicate that NOTCH1/2 mutations in MCL activate this pathway and are a frequent mechanism involved in the aggressive behavior of the tumors.

The spectrum of mutations described in this work highlights the existence of common molecular features as well as relevant differences in the genetic alterations present in different subtypes of lymphoid neoplasms. Thus, NOTCH1 mutations have been found in CLL (18, 26) whereas NOTCH2 is mutated in splenic marginal zone lymphomas (37, 38); however, none of these tumors carry mutations in both NOTCH genes as observed in MCL. In addition, MLL2 and MEF2B mutations are shared with DLBCL (31–33) but are uncommon in CLL, whereas MCL has frequent mutations of ATM and BIRC3 that are also frequent in CLL (39) but uncommon in DLBCL. In contrast, WHSC1 mutations appear to be specific for MCL while lacking mutations in genes frequently mutated in other hematological neoplasias (e.g., MYD88, CARD11, EZH2, SF3B1).

Recent genomic studies have revealed the complex subclonal heterogeneity of different tumors and its dynamic evolution in the course of the disease (40). The sequence of two simultaneous or longitudinal samples in different topographic sites in our study has revealed that some tumors may have at least two major subclones already at diagnosis with different representation in two topographic sites, lymph nodes, and peripheral blood. In addition, the evolution of the tumors is also heterogeneous with the eradication of some clones by the chemotherapy treatment and the emergency of other new clones at the progression of the disease or at relapse after chemotherapy. The analysis of the
allelic frequency of the mutations allows the recognition of initial and acquired mutations that are probably relevant in the progression of the disease (24, 25). The recognition of this molecular heterogeneity at diagnosis and progression may have future clinical relevance for the management of patients with targeted therapies to specific mutations.

In summary, this whole-genome/whole-exome study of MCL has revealed genes and pathways recurrently mutated that may contribute to lymphomagenesis in cooperation with the t(11;14) translocation. The differential distribution of these mutations in clinical and molecular subtypes of the disease illustrates the relationship between genomic alterations and tumor heterogeneity.

We have also shown the intratumoral heterogeneity and subclonal architecture of MCL that may have relevance in the clinical evolution of the tumors. These alterations highlight mechanisms in the pathogenesis of this lymphoma and offer potential targets for therapeutic intervention.

**Materials and Methods**

**Patients and Specimens.** We sequenced the genomes of 29 patients with MCL and six MCL cell lines. Additional samples from 172 patients were obtained for clinical validation. All patients gave informed consent for sample collection and analysis. DNA and RNA were purified from tumors and normal cells. Additional details are provided in SI Appendix, SI Materials and Methods.

Whole-Genome and Whole-Exome Sequencing and Analysis. Whole-genome and whole-exome sequencing (Agilent SureSelect Human All Exon 50 MB) were performed as previously described (18, 26) using a HiSeq 2000 instrument. Sequence data analysis was performed using the Sidron mutation caller as described (18, 26). Additional details are provided in SI Appendix, SI Materials and Methods.

Microarray Experiments. Genotyping and copy number and gene expression analysis were performed using Affymetrix SNP60 arrays and Hu133+ 2.0 GeneChip (Affymetrix), respectively. Additional details are provided in SI Appendix, SI Materials and Methods.

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