Genomic and Gene Expression Profiling Defines Indolent Forms of Mantle Cell Lymphoma

Verònica Fernàndez, Olga Salamero, Blanca Espinet, Francesc Solé, Cristina Royo, Alba Navarro, Francisca Camacho, Silvia Beà, Elena Hartmann, Virginia Amador, Luis Hernández, Claudio Agostinelli, Rachel L. Sargent, Maria Rozman, Marta Aymerich, Dolors Colomer, Neus Villamor, Steven H. Swerdlow, Stefano A. Pileri, Francesc Bosch, Miguel A. Piris, Emili Montserrat, German Ott, Andreas Rosenwald, Armando López-Guillermo, Pedro Jares, Sergi Serrano, and Elías Campo

Abstract

Mantle cell lymphoma (MCL) is typically a very aggressive disease with poor outcomes, but some cases display an indolent behavior that might not necessitate treatment at diagnosis. To define molecular criteria that might permit recognition of such cases, we compared the clinicopathologic features, gene expression, and genomic profile of patients who had indolent or conventional disease (iMCL or cMCL). Patients with iMCL displayed nonnodal leukemic disease with predominantly hypermutated IGHV and noncomplex karyotypes. iMCL and cMCL shared a common gene expression profile that differed from other leukemic lymphoid neoplasms. However, we identified a signature of 13 genes that was highly expressed in cMCL but underexpressed in iMCL. SOX11 was notable in this signature and we confirmed a restriction of SOX11 protein expression to cMCL. To validate the potential use of SOX11 as a biomarker for cMCL, we evaluated SOX11 protein expression in an independent series of 112 cases of MCL. Fifteen patients with SOX11-negative tumors exhibited more frequent nonnodal presentation and better survival compared with 97 patients with SOX11-positive MCL (5-year overall survival of 78% versus 36%, respectively; \( P = 0.001 \)). In conclusion, we defined nonnodal presentation, predominantly hypermutated \( IGHV \), lack of genomic complexity, and absence of SOX11 expression as qualities of a specific subtype of iMCL with excellent outcomes that might be managed more conservatively than cMCL. Cancer Res; 70(4); 1408–18. ©2010 AACR.

Introduction

Mantle cell lymphoma (MCL) is an aggressive lymphoid neoplasm with a rapid clinical evolution, short responses to therapy, frequent relapses, and a median survival of 3 to 4 years (1). The aggressive biological behavior of this lymphoma has been attributed to the genetic and molecular mechanisms involved in its pathogenesis that combine the constitutive deregulation of cell proliferation due to the \( t(11;14)(q13;q32) \) and cyclin D1 overexpression, a high level of chromosomal instability related to the disruption of the DNA damage response pathway, and activation of cell survival mechanisms (2). Patients with MCL are usually treated aggressively at diagnosis with intensive chemotherapy regimens, including hematopoietic stem cell transplantation (3–6).

Intriguingly, recent studies have identified a group of patients diagnosed with MCL that show an indolent clinical course and a long survival of more than 7 to 10 years, some of them not even requiring chemotherapy for long periods (7–11). This particular clinical evolution raises the question whether these patients represent the favorable end of the spectrum in the clinical evolution of the disease or if they correspond to a particular subtype of MCL with specific biological characteristics that may benefit from more conservative clinical management. The identification of patients with an indolent variant of the disease would allow a risk-adapted therapy. Unfortunately, diagnostic criteria to establish this distinction are not available.

We hypothesized that the analysis of the genetic and expression features of the tumors may help to identify patients with an indolent clinical evolution and provide biomarkers that could be used in the clinical setting.
Materials and Methods

Study population. Twelve patients diagnosed with MCL (from May 1994 to August 2005) who showed an indolent clinical course for more than 2 y (median, 6.4 y; range, 2.5–10.4) without chemotherapy were selected for the study. These cases were called indolent MCL (iMCL) and were compared with 15 conventional MCLs (cMCL) that required chemotherapy at diagnosis and had peripheral blood tumor cells available for the study. All the cases had the t(11;14)(q13;q32) translocation and expressed cyclin D1. Ten of the 12 iMCLs had been initially diagnosed as splenic marginal zone lymphoma (SMZL; 4 cases), chronic lymphocytic leukemia (CLL; 4 cases), or leukemic lymphoid neoplasm, not otherwise specified (2 cases) and reclassified as MCL during the evolution of the disease when a t(11;14)(q13;q32) translocation and cyclin D1 overexpression were identified. The two additional patients were diagnosed with an incidental in situ MCL detected in a lymph node biopsy in which the cyclin D1–positive cells were restricted to the mantle zone of otherwise reactive follicles. In spite of the diagnosis of MCL, none of these 12 patients received chemotherapy because of the evidence of an indolent stable disease from the moment of the initial diagnosis to the reclassification or because of limited disease. The characteristics of the 12 iMCLs and 15 cMCLs are summarized in Table 1.

To validate the significance of SOX11 expression, a gene of the differentially expressed signature between cMCL and iMCL, we studied an independent series of 112 patients diagnosed with MCL from 1986 to 2007 at the Hospital Clinic (Barcelona, Spain), Centro Nacional de Investigaciones Oncológicas (Madrid, Spain), and Institute of Pathology, University of Würzburg (Würzburg, Germany). These cases had available formalin-fixed, paraffin-embedded tissue sections and were obtained with the GeneChip Operating Software (GCOS; Affymetrix). The single-nucleotide polymorphism (SNP) allele calls were determined with the GeneChip Genotyping Analysis Software (GTTYPE; Affymetrix), and the copy number and loss of heterozygosity (LOH) data analysis was performed with the Chromosome Copy Number Analysis Tool (CNAT 4.0, Affymetrix), applying a 0.5-Mb genome smoothing filter. Gains of chromosomal material were defined as contiguous regions where a determined number of adjacent SNPs (>10) had genome-smoothed analysis of the copy number (GSA) and single point analysis of the copy number (SPA) −log10P values of ≥3. Losses were determined as regions with GSA and SPA log10P values of ≤−3 together with a LOH −log10P value of ≥18. Partial uniparental disomy (pUPD) was defined as a region spanning at least 50 SNPs with homozygous allele calls and a −log10P value for LOH >18 in the absence of a deletion.

Affymetrix GeneChip Human Genome U133 Plus 2.0 hybridization and analysis. Total RNA was extracted with the Trizol reagent following the recommendations of the manufacturer (Invitrogen Life Technologies). RNA integrity was examined with the Agilent 2100 Bioanalyzer (Agilent Technologies), and only high-quality RNA samples were hybridized to Affymetrix GeneChip Human Genome U133 Plus 2.0 (Affymetrix) arrays, according to Affymetrix standard protocols. The analysis of the scanned images and the determination of the signal value for each probe set of the array were obtained with the GCOS. Data normalization was performed by the global scaling method with a target intensity set at 150. The data analysis was performed with the DNA-Chip Analyzer 2007 software package (dChip; ref. 12) and BBArray Tools v.3.6.0 software (13). To perform an unsupervised analysis of all B-cell lymphoid neoplasms, genes were filtered according to the SD across samples (1 < SD < 1,000) and the expression level (log2 expression ≥7 in ≥10% samples). In total, 3,644 probe sets were retained after applying the filtering criteria described above and used for the clustering analysis using centered correlation as distance metric and average linkage. The robustness of the clusters was evaluated using the R (reproducibility) measure described by McShane and colleagues (14) and implemented in the BB Array Tools. An R of 1 means perfect reproducibility of that cluster, and an R of 0 means no reproducibility of the cluster. For the supervised analysis of MCL samples, gene detection calls were determined with the dChip software and only the probe sets that were present in at least 75% of either iMCL or cMCL cases were considered for further analysis. In addition, an arbitrary value of 10 was assigned to the probe sets with an expression <10 before log2 transformation. In total, 22,732 probe sets were used for differential gene expression analysis using the significance analysis of microarray data (SAM) method implemented in BB Array Tools (13). Two levels of significance were used for detecting differentially expressed genes, considering a median false discovery rate (FDR) of <0.1 and a 90th percentile FDR of <0.1.

The molecular proliferation signature value of the tumors was calculated as described by Rosenwald and colleagues (15) using the 18 of the 20 genes of the proliferation signature that were present in the Affymetrix GeneChip Human Genome.
When more than one probe set was present for a specific gene, we have used the mean as the gene expression value for that gene. Gene expression values for each gene were standardized across samples, and the mean of all 18 genes was reported as a proliferation signature value for each sample.

The primary array data are available from the Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information (NCBI) through GEO accession number GSE16455 (NCBI GEO).

### Molecular studies

The mutational analysis of the IGHV and TP53 (exons 4–8) genes was performed as previously described (16, 17). Gene expression analysis of SOX11, HDGFRP3, and CNN3 genes, selected from the differentially expressed signature between iMCL and cMCL, was performed by real-time quantitative reverse-transcription PCR (qRT-PCR) using predeveloped assays (SOX11, Hs00846583_s1; HDGFRP3, Hs01016437_m1; CNN3, Hs00156565_m1; Applied Biosystems; Supplementary Appendix). Total RNA (1 μg) from the 7 iMCLs and 15 cMCLs cases was treated with Turbo DNase (Ambion, Inc.). cDNA was then synthesized using the SuperScript III system and following the manufacturer’s instructions. Expression levels were calculated with the 2^ΔΔCt method using human β-glucuronidase as endogenous control and Jurkat cell line as mathematical calibrator (18).

The correlation between microarrays and qRT-PCR measures as well as the differences of expression between iMCL and cMCL were assessed by using the Wilcoxon signed-rank test.

### Table 1. Clinical and pathologic characteristics of the 12 iMCLs and 15 cMCLs

<table>
<thead>
<tr>
<th></th>
<th>iMCL (n = 12)</th>
<th>cMCL (n = 15)</th>
<th>P</th>
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<tbody>
<tr>
<td>Clinical and pathologic data*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Median age (range)</td>
<td>58 (41–75)</td>
<td>67 (30–83)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>9/3</td>
<td>11/4</td>
<td>NS</td>
</tr>
<tr>
<td>B symptoms (%)</td>
<td>0</td>
<td>33</td>
<td>0.03</td>
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<tr>
<td>ECOG ≥2 (%)</td>
<td>0</td>
<td>70</td>
<td>0.01</td>
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<td>Nodal presentation (lymph nodes &gt;1 cm), %†</td>
<td>17</td>
<td>93</td>
<td>&lt;0.001</td>
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<tr>
<td>Palpable splenomegaly (%)</td>
<td>50</td>
<td>60</td>
<td>NS</td>
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<tr>
<td>Gastrointestinal involvement (%)*</td>
<td>100</td>
<td>50</td>
<td>NS</td>
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<tr>
<td>Bone marrow involvement (%)*</td>
<td>92</td>
<td>91</td>
<td>NS</td>
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<tr>
<td>WBC count &gt;10^9/L (%)*</td>
<td>33</td>
<td>82</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocyte count &gt;5 x 10^9/L (%)*</td>
<td>44</td>
<td>82</td>
<td>NS</td>
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<tr>
<td>Atypical lymphocytes (%)</td>
<td>92</td>
<td>91</td>
<td>NS</td>
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<td>High serum LDH (%)†</td>
<td>0</td>
<td>46</td>
<td>0.03</td>
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<tr>
<td>High serum β2-microglobulin (%)*</td>
<td>20</td>
<td>80</td>
<td>NS</td>
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<td>Intermediate or high-risk MIPI (%)*</td>
<td>0</td>
<td>46</td>
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<td>Morphology</td>
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<td>Small cell (%)</td>
<td>67</td>
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<tr>
<td>CD5* (%)†</td>
<td>64</td>
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<td>NS</td>
</tr>
<tr>
<td>IGHV gene hypermutations (&gt;5%)*</td>
<td>70</td>
<td>20</td>
<td>&lt;0.04</td>
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<td>Genomic profile*</td>
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<td>0–1 imbalance</td>
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<td>≥2 imbalances</td>
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<tr>
<td>Splenectomy (%)</td>
<td>42</td>
<td>20</td>
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<tr>
<td>Chemotherapy at any time (%)</td>
<td>17</td>
<td>100</td>
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<tr>
<td>Median follow-up, y (range)‡</td>
<td>6.4 (2.5–10.4)</td>
<td>3.3 (1.5–5.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Dead patients (%)</td>
<td>0</td>
<td>47</td>
<td>&lt;0.001</td>
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<tr>
<td>5-y OS (%)</td>
<td>100</td>
<td>49</td>
<td>0.03</td>
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Abbreviations: NS, not significant; MIPI, MCL IPI.

*Gastrointestinal involvement was assessed in 8 cMCLs and 4 iMCLs patients; MCL IPI (41) was determined in 13 cMCLs and 8 iMCLs; IGHV mutations and SNP array were studied in 10 and 7 iMCLs, respectively, and all cMCLs; peripheral blood counts, LDH, β2-microglobulin, bone marrow involvement, and CD5 expression could be assessed in 23, 21, 18, 20, and 25 cases, respectively.

†The two IMCL patients with nodal involvement had an isolated lymph node in the cervical region.

‡Follow-up of surviving patients.

U133 Plus 2.0. When more than one probe set was present for a specific gene, we have used the mean as the gene expression value for that gene. Gene expression values for each gene were standardized across samples, and the mean of all 18 genes was reported as a proliferation signature value for each sample.

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Fernàndez et al. Cancer Res; 70(4) February 15, 2010

Cancer Research

1410 Cancer Res; 70(4) February 15, 2010

Cancer Research

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cMCL were analyzed with the Pearson correlation test and a $t$ test, respectively.

**Immunohistochemistry.** Immunohistochemistry was performed on formalin-fixed, paraffin-embedded material with the EnVision+System Peroxidase (3,3′-diaminobenzidine) method (DAKO). The antigen retrieval was done by heating samples in EDTA buffer in a microwave oven. Primary antibody against SOX11 (HPA000536, Atlas Antibodies) was used at a 1:100 dilution. The immunohistochemical expression of SOX11 was only detected in formalin-fixed tissue sections. Bone marrow biopsies and B-5–fixed tissues were not assessable. Suitable tissue samples for immunohistochemical studies were available from 12 cMCLs and 11 iMCLs. According to the original description, SOX11 protein expression was identified as a strong nuclear staining of the lymphoid cells. A dot-like cytoplasmic staining was observed in some cases. This staining is not considered specific, as previous studies have shown the lack of correlation of this staining and the SOX11 expression (19–21).

**Statistical analysis.** Conventional descriptive measures were used. Categorical data were compared using the Fisher’s exact test, two-sided $P$ value, whereas nonparametric tests were used for ordinal data. Overall survival (OS) was calculated from the day of diagnosis until death or until the end of follow-up. The Cox proportional hazards model was used to analyze prognostic factors.

**Results**

**Patients and tumors.** Patients with iMCL had leukemic disease (11 patients), no evidence of lymphadenopathy (10 patients), or only one enlarged (>1 cm) lymph node (2 patients). The characteristics of the patients and tumors are listed in Table 1. Splenectomy was performed in five patients and none received chemotherapy at diagnosis. After a median follow-up of 6.4 years (range, 2.5–10.4 years), only two patients showed disease progression at 5 and 7 years after diagnosis. One patient received chlorambucil and eventually underwent splenectomy. The other developed peripheral lymphadenopathy and gastrointestinal involvement and was treated with rituximab-chlorambucil, with achievement of a complete response. Only 4 of the 12 iMCLs had classic histologic morphology, whereas 8 tumors (67%) showed a small cell variant (1). All tumors expressed CD20 and cyclin D1 and were negative for CD3. However, 4 of the 11 (36%) iMCLs, in contrast, were negative for this marker.

**IGHV genes.** The IGHV mutational status could be examined in 10 iMCLs and 15 cMCLs (Supplementary Table S1). Seven (70%) iMCLs had highly mutated genes (>5%), whereas this high load of mutations was only seen in three (20%) cMCLs ($P < 0.04$). The identified $V_{H}$ rearrangements in the iMCL were $V_{H}13$ (3 of 10), $V_{H}4$ (5 of 10), $V_{H}1$ (1 of 10), and $V_{H}12$ (1 of 10), whereas in cMCL they were $V_{H}13$ (5 of 15), $V_{H}4$ (5 of 15), $V_{H}3$ (3 of 15), and $V_{H}5$ (2 of 15) families.

**Genetic alterations.** The genomic profile of 7 iMCLs and 15 cMCLs was examined using SNP arrays. Five iMCLs had no chromosomal imbalances and two carried only one small chromosomal loss at 3p26.2-p25.3 and 17pter-p12, respectively. No $TP53$ mutations were detected in this latter case. In contrast, 13 of the 15 (87%) cMCLs had chromosomal gain/amplifications (total, 29) or losses (total, 47) and all these tumors had two or more chromosomal aberrations. The most frequent imbalances were gains of chromosome 3q (53%) and 8q (27%) and losses of chromosome 11q (40%), 13q (27%), and 9p (20%). Minimal common altered chromosomal regions were concordant with previous descriptions in MCL (Fig. 1).

The SNP array platform allows the identification of chromosomal regions with pUPD. Three of 7 (43%) iMCLs and 7 of the 15 (47%) cMCLs had pUPD and, when present, they were the sole alteration found in the given chromosome (Fig. 1). To determine whether these pUPDs were somatically acquired in the tumor or were already present in the germline of the patients, we studied constitutional DNA of the three patients with iMCLs and four of the seven cMCLs carrying pUPD. Interestingly, most pUPDs were also present in the germline of the patients, except the pUPD at 4q32.3-qter in one iMCL and 17pter-p13.1 in one cMCL and 20q11.22-qter in two cMCLs (Supplementary Table S2).

$TP53$ gene mutations were detected only in two cMCLs with pUPD at 17p (Supplementary Table S2). $ATM$ and $CDKN2A$ allelic deletions, two common oncogenic events in MCL, were observed in five (33%) and three (20%) cMCLs, respectively, but in none of the iMCL.

**Gene expression profiling.** To determine the molecular relationship of iMCL to cMCL and other leukemic lymphoid neoplasms, we compared the gene expression profile of 7 iMCLs, 15 cMCLs, 17 CLLs, 7 FLs, 4 SMZLs, 3 HCLs, and 2 HCLvs. The unsupervised hierarchical clustering analysis revealed that all samples were grouped according to their diagnosis in four main clusters corresponding to SMZL/HCL/HCLv, CLL, MCL, and FL (Fig. 2). iMCL and cMCL were grouped together in a robust cluster ($R$ index = 0.997), showing their high similarity in the overall gene expression profile.

The MCL cluster showed an asymmetrical distribution of the cMCL and imMCL in two subclusters. We performed a supervised analysis to identify differentially expressed genes between the two subtypes using the SAM algorithm. When we considered a median FDR of <0.1, 569 probe sets were selected as differentially expressed (Fig. 3). A more stringent analysis (90th percentile FDR of <0.1) identified 23 probe sets corresponding to 13 annotated genes: $RN7TT$, $HDGFRP3$, $FARP1$, $CSNK1E$, $SETMAR$, $HMGB3$, $LGALS3BP$, $PON2$, $CDK2AP1$, $DBN1$, $CNR1$, $CNN3$, and $SOX11$ (Fig. 3; Supplementary Table S3). These genes were strongly expressed in 13 of the 15 cMCLs and significantly underexpressed in all iMCLs.

iMCL had lower values of the microarray-based proliferation signature (mean, −0.157; SD, 0.369; ref. 15) than cMCL cases (mean, 0.105; SD, 0.785). However, this difference was not statistically significant. Similarly, no significant differences in the cyclin D1 levels between iMCL and cMCL were detected (Fig. 3).

To validate the results of the microarray expression data, we reexamined by qRT-PCR the mRNA levels of three of...
the genes differentially expressed between iMCL and cMCL, including the two genes with the highest fold change ratios (SOX11 and HDGFRP3) and a third gene (CNN3) with a fold change ratio just below the 50th percentile of the fold change ratios of the 13 genes differentially expressed. The qRT-PCR data showed a high significant correlation with microarray gene expression values (SOX11, \( R^2 = 0.89; \) HDGFRP3, \( R^2 = 0.93; \) CNN3, \( R^2 = 0.97 \)). Consequently, the three genes analyzed by qRT-PCR were highly expressed in 13 of the 15 cMCLs and virtually negative in all iMCLs (\( P < 0.001 \)).

**SOX11 protein expression.** We searched for reliable antibodies to assess the protein expression of the differentially expressed genes between iMCL and cMCL on routinely processed biopsies and found only one antibody against SOX11 (19–21). Tissue sections from 12 cMCLs (9 lymph nodes and 3 spleens) and 11 iMCLs (5 spleens, 3 lymph nodes, and 3 gastrointestinal biopsies) could be investigated by immunohistochemistry. Similarly to the previous immunohistochemical studies (19–21), cases expressing SOX11 mRNA by microarrays showed a very strong nuclear protein expression that was detected in most of the cells. Cases that did not express SOX11 mRNA showed a completely negative nuclear staining, but some of them had a weak granular cytoplasmic staining. Concordantly with the array data, the strong SOX11 nuclear expression was detected in most of the tumor cells in 11 of the 12 cMCLs. The negative cMCL was also negative by mRNA (Fig. 3B). The six iMCLs examined by microarrays were negative for SOX11 in the nucleus. A very weak nuclear staining in occasional cells (<20%) was observed in one case that also had detectable low levels of mRNA in the microarray analysis (Fig. 3B). The five additional iMCLs that could not be studied by microarrays were also negative for the nuclear SOX11 protein. Weak nuclear expression in occasional cells was observed in one case. Thus, SOX11 was absent or significantly underexpressed in all iMCLs based on mRNA and/or protein expression studies, whereas it was highly expressed in 13 of the 15 cMCLs.

**SOX11 protein expression in an independent MCL series.** To confirm whether SOX11 could be a biomarker to recognize two subtypes of MCLs with different clinicopathologic features and outcome, we investigated the protein expression in an independent series of 112 patients diagnosed and managed as standard MCL regardless of the SOX11 status. SOX11 nuclear expression was detected in 97 (87%) tumors and was absent or only weakly detected in occasional cells in 15 (13%). The clinical and biological features of the patients according to SOX11 expression in the tumors are summarized in Table 2. Patients with SOX11-negative MCL had more frequently nonnodal presentation, splenomegaly, and higher WBC and lymphocyte counts than patients with SOX11-positive tumors. Ki-67 proliferative index was 22.3 ± 18 and 35.5 ± 24 in SOX11-negative and SOX11-positive tumors, respectively (\( P > 0.1 \)). The proportion of tumors with a high proliferation index (Ki-67 > 50%) was similar in SOX11-negative (20%) and SOX11-positive (28%) tumors (Table 2).

After a median follow-up of 4 years (range, 1–12.2) for surviving patients, 72 patients had died, with a 5-year OS of 41% [95% confidence interval (CI), 31–51]. Patients with SOX11-negative tumors had a longer OS than those with SOX11-positive MCL (5-year OS, 78% [95% CI, 56–100] versus 36% [95% CI, 25–47], respectively; \( P = 0.001; \) Fig. 4A). In this series of patients, variables predicting poor OS in the univariate analysis were age >60 years (\( P = 0.03 \)), nodal presentation (\( P = 0.05 \)), presence of B symptoms (\( P = 0.002 \)), poor performance...
status [Eastern Cooperative Oncology Group (ECOG) of >1; 
$P = 0.006$], high serum lactate dehydrogenase (LDH; $P = 0.004$), high-risk international prognostic index (IPI; $P = 0.009$), and high Ki-67 expression ($P = 0.016$).

Because MCL nonnodal presentation has been associated with a more indolent evolution of this tumor (7, 8, 10, 11) and, on the other hand, a good correlation was found between nonnodal presentation and negative SOX11 expression, we examined whether the most important variable for OS was nodal/nonnodal presentation or SOX11 expression. The OS curves according to the combination of these variables are depicted in Fig. 4B: patients with nonnodal presentation and SOX11-negative expression ($n = 7$; 2 dead; 5-year OS, 75%) versus nodal presentation and SOX11-negative ($n = 8$; 2 dead; 5-year OS, 75%) versus nodal presentation and SOX11-positive ($n = 96$; 67 dead; 5-year OS, 35%; $P = 0.05$). The only patient with nonnodal presentation and SOX11-positive expression, who died of lymphoma 6.6 years after diagnosis, was not included in this figure. To investigate whether the most important variable for OS was nodal/nonnodal presentation or SOX11 expression, a Cox analysis was performed with 112 assessable cases. Only SOX11 expression maintained its predictive value for OS in this model [$P < 0.001$; relative risk (RR), 5.8].

Finally, a multivariate analysis was performed including SOX11 expression, nodal presentation, and proliferative index as assessed by Ki-67 expression. In the final model with 99 cases, SOX11 expression ($P = 0.006$; RR, 3.8) and Ki-67 ($P = 0.02$; RR, 1.9) were the most important variables for OS.

**Discussion**

In this study, we have recognized that MCL with an indolent clinical evolution may correspond to a distinctive clinical and biological subtype of the disease and the patients may not require chemotherapy for a long period of time. These patients usually had an asymptomatic, nonnodal, and leukemic disease, and the tumors carried predominantly hypermutated $\text{IGVH}$ genes and noncomplex karyotypes. On the contrary, patients with cMCL had generalized lymphadenopathy and required antineoplastic treatment at diagnosis, whereas the tumors carried predominantly low or
Figure 3. A, characterization of iMCL according to their genetic and molecular features. The genomic complexity is illustrated in the bar plots at the top of the panels, reflecting the number of alterations for each case. The plot below indicates the diagnosis of the cases. Violet, iMCL; blue, cMCL. IGVH gene status of all MCL cases is indicated by color (red, >5% mutations; pink, 3–5% mutations; blue, 0–2%). Cyclin D1 gene expression was performed with the DNA-Chip Analyzer 2007 tool (dChip). Bright red, high expression; green, low expression. About the proliferation signature score, bright red represents a high score, whereas green represents a low score. The status of CDKN2a, ATM, and TP53 genes according to the SNP array data is indicated by color (black, not altered; green, loss; red, gain; blue, pUPD). Cases with mutations in the TP53 gene are displayed in yellow. The gene expression cluster was performed with the DNA-Chip Analyzer 2007 tool (dChip) using the probe sets considered differentially expressed with a median FDR of <0.1.

B, differential signature between iMCL and cMCL. Probe sets that showed a highly significant differential expression between iMCL and cMCL (90th percentile FDR of <0.1) are highlighted. *, cMCL was negative for SOX11 nuclear protein expression by immunohistochemistry.

C, immunohistochemical analysis of SOX11 gene in MCL. The cMCL (top, c1, c2, and c3) shows H&E staining (c1), cyclin D1 expression (c2), and a strong nuclear SOX11 immunostaining (c3), whereas the iMCL (bottom, c4, c5, and c6) is also cyclin D1 positive (c5) but the nuclei of the tumor cells are negative for SOX11 (c6).
unmutated IGVH genes and complex karyotypes. iMCL had a small cell variant morphology and were CD5 negative more frequently than cMCL. Our subset of patients with an indolent clinical presentation and evolution is relatively similar to the nonnodal MCL initially recognized by Orchard and colleagues (10). Our genomic and expression profiling observations suggest that these tumors may correspond to a specific biological subtype of MCL.

Despite the differences in the clinical and genetic characteristics, iMCL and cMCL share a similar global gene expression profile that is markedly different from that of other leukemic lymphoid neoplasms. These findings support the model that the t(11;14)(q13;q32) translocation and cyclin D1 overexpression present in all iMCLs and cMCLs are common pathogenetic events that determine a specific global expression profile and define a distinctive disease entity. However, our findings suggest that MCL cells carrying the same chromosomal translocation may follow two different genetic and molecular pathways associated with different clinical features. This situation may be similar to the two forms of unmutated and mutated CLL that, in spite of the dramatic clinical differences, share a characteristic global expression profile and differ only in a small subset of genes (22, 23).

The supervised comparison of the iMCL and cMCL gene expression profiles revealed 13 genes with highly significant differences between cMCL and iMCL. These genes were overexpressed in 13 of the 15 cMCLs and underexpressed in all iMCLs. Revisiting the MCL gene expression profile studies, we have confirmed that some of these genes had been found previously as overexpressed in MCL and absent in normal B cells or other B-cell neoplasm (15, 19, 24, 25). SOX11, one of the genes in the signature, was found to be expressed in MCL but not in other lymphoid neoplasm or nonneoplastic lymphoid tissues (19). Our findings indicate that the expression of this signature may be very specific for cMCL because these genes were not expressed in any iMCL or other leukemic lymphoid neoplasms. The function of these genes is currently unknown, but the silencing of HDGFRP3 induces a proliferative arrest of MCL cells, supporting a pathogenetic role in these tumors (25). Interestingly, three genes of this differential signature—SOX11, HDGFRP3, and HMG3—belong to the high-mobility group of transcription factors that bind to DNA and induce large conformational changes facilitating the binding of other transcription factors (26, 27). The genes regulated by these groups of transcription factors are not well characterized.

### Table 2. Main clinical and pathologic features of 112 patients with MCL according to SOX11 expression in the validation set

<table>
<thead>
<tr>
<th>Clinical and pathologic data</th>
<th>SOX11 negative (n = 15)</th>
<th>SOX11 positive (n = 97)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>57 (42–77)</td>
<td>63 (31–83)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>11/4</td>
<td>71/26</td>
<td>NS</td>
</tr>
<tr>
<td>B symptoms (%)</td>
<td>22</td>
<td>48</td>
<td>NS</td>
</tr>
<tr>
<td>ECOG ≥2 (%)</td>
<td>20</td>
<td>28</td>
<td>NS</td>
</tr>
<tr>
<td>Nodal presentation (%)</td>
<td>53</td>
<td>99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Palpable splenomegaly (%)</td>
<td>92</td>
<td>48</td>
<td>0.005</td>
</tr>
<tr>
<td>Bone marrow involvement (%)</td>
<td>83</td>
<td>87</td>
<td>NS</td>
</tr>
<tr>
<td>Ann Arbor stage IV (%)</td>
<td>92</td>
<td>74</td>
<td>NS</td>
</tr>
<tr>
<td>WBC count &gt;10 × 10⁹/L (%)</td>
<td>57</td>
<td>18</td>
<td>0.04</td>
</tr>
<tr>
<td>Lymphocyte count &gt;5 × 10⁹/L (%)</td>
<td>83</td>
<td>24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High serum LDH (%)</td>
<td>22</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>High serum β₂-microglobulin (%)</td>
<td>75</td>
<td>55</td>
<td>NS</td>
</tr>
<tr>
<td>IPI intermediate/high or high risk (%)</td>
<td>37</td>
<td>48</td>
<td>NS</td>
</tr>
<tr>
<td>MIPI high risk (%)</td>
<td>33</td>
<td>46</td>
<td>NS</td>
</tr>
<tr>
<td>Ki-67 high (&gt;50%), %</td>
<td>20</td>
<td>28</td>
<td>NS</td>
</tr>
<tr>
<td>Median follow-up, y (range)</td>
<td>8.3 (1.3–12.2)</td>
<td>3.8 (1–11.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Front-line Adriamycin-containing polychemotherapy (%)</td>
<td>67</td>
<td>72</td>
<td>NS</td>
</tr>
<tr>
<td>Complete response (%)</td>
<td>40</td>
<td>54</td>
<td>NS</td>
</tr>
<tr>
<td>5-y event-free survival (%)</td>
<td>75</td>
<td>23</td>
<td>0.04</td>
</tr>
<tr>
<td>5-y OS (%)</td>
<td>78</td>
<td>36</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*The number of assessable cases for bone marrow involvement, IPI, MCL IPI, peripheral blood counts, LDH, and β₂-microglobulin was 82, 62, 71, 104, 61, and 50, respectively.

†Surviving patients.

‡In 78 patients with this information available.
The high expression of these genes in more aggressive cMCL suggests that they may be pathogenetically relevant for the behavior of the tumors.

Most iMCL carried hypermutated IGVH genes. The clinical significance of IGVH mutations in MCL is controversial. Most studies have shown no relationship to the outcome of the patients (28–30), whereas a tendency to longer survival of the hypermutated MCL has been observed in some series (30–33). Most of these studies have used a cutoff of 2% to consider the tumor as hypermutated. The higher number of mutations found in most iMCLs suggests that the biological effect of IGVH hypermutations in MCL may need to be revisited under this perspective.

MCL is genetically characterized by a high chromosomal instability associated with inactivation of DNA repair pathways (2, 34, 35). Interestingly, the iMCL had noncomplex karyotypes, a finding also concordant with the lack of alterations in ATM, CDKN2A, and TP53 genes, frequently inactivated in cMCL (35–39), and the previous identification of a low-complex karyotype as a favorable prognostic factor in MCL (34, 40). These findings suggest that chromosomal instability may be a biological feature of cMCL, but may not be present in the iMCL subtype.

Two patients in the cMCL group had an expression signature similar to iMCL, with lack of expression of the 13 genes overrepresented in the other cMCL (Fig. 3B). We included these two patients in the group of cMCL because they required treatment at diagnosis. One of them presented with a leukemic and nonnodal disease but had evidence of clinical progression with rapid increase of the lymphocyte counts, B symptoms, and poor performance status. The second patient had a generalized lymphadenopathy at diagnosis. Curiously, these two tumors had hypermutated (>5%) IGVH genes and no or very low number (only two) of chromosomal aberrations. No p53 mutations or ATM and CDKN2a deletions were observed in these cases. These molecular and genetic findings are similar to those observed in iMCL and correlate well with expression signature lacking SOX11, HDGFPRP3, HMG3, and other genes. The similarities in the expression profile and genetic features between these two tumors and the iMCL suggest that they may correspond to the same biological subtype. The nodal dissemination of one of the patients and rapid clinical evolution of the second indicate that they may correspond to a progressive phase of these tumors. Wang and colleagues have recently reported that 5 patients with SOX11-negative MCL had a worse survival than 49 patients with SOX11-positive tumors (42). However, the lack of additional information on the clinical presentation and biological features in this series makes difficult to interpret this observation.

To confirm whether SOX11 expression could be useful to recognize MCL with a more indolent behavior, we studied the protein expression of this gene in an independent series of tumors, all expressing cyclin D1 and managed as standard MCL. The lack of SOX11 expression identified a subset of patients with predominant nonnodal presentation, higher blood lymphocyte counts, and longer survival similar to that of our initial iMCL. On the contrary, SOX11-positive MCL had a conventional nodal presentation and worse outcome. Of note, SOX11 expression was able to distinguish favorable MCL cases more consistently than the nodal presentation and independently of the Ki-67 index. These findings strongly suggest that the lack of SOX11 in a cyclin D1–positive lymphoma may identify a subset of MCL with a different biological behavior than conventional SOX11-positive MCL.

In conclusion, we have identified a specific subtype of MCL with a constellation of clinicobiological features that include a predominant nonnodal and asymptomatic presentation, stable disease, high rate of IGVH gene mutations, lack of genomic complexity, and absence of expression of several genes, including SOX11 and other transcription factor of the high-mobility group family. This clinical presentation and SOX11 negativity identify patients with MCL that do well without aggressive chemotherapy and may benefit from management strategies more adjusted to the biology of the disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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