Leukemic presentation in nodal mantle cell lymphoma is characterized by frequent SOX11 negativity, a poor risk genomic landscape including higher *TP53* alterations, and worse overall survival

Mantle cell lymphoma (MCL) is a mature B-cell neoplasm characterized by CCND-family rearrangements, most frequently *CCND1*. The presence of *TP53* genetic alteration is a well-known adverse event in MCL, associated with blastoid histomorphology, high Ki67 proliferation index, and aggressive behavior.[1] Conventional MCL involves lymph nodes; however, a distinct subgroup has non-nodal leukemic presentation (nnMCL) and often demonstrates an indolent clinical course. nnMCL patients typically exhibit SOX11 negativity, immunoglobulin heavy chain variable (IGHV) region gene mutated status, less complex genomic alterations, enrichment for *CCND1* mutations, and lack of *ATM* mutations.[2,3] Besides nnMCL, conventional nodal MCL also frequently demonstrates peripheral blood involvement with various levels of circulating disease. High leukocyte count has been recognized as an adverse prognostic indicator in MCL and is included in the MCL international prognostic index (MIPI), which is commonly used to guide risk-adapted treatment decisions for MCL patients.[4] However, there are limited data comparing the pathological and molecular features among nodal MCL patients with leukemic presentation (lnMCL), conventional nodal MCL patients (nMCL) without significant circulating disease burden, and nnMCL patients. This study was approved by our Institutional Review Board. We searched pathology archives from 2014 through 2023 and identified 247 primarily diagnosed MCL patients. Patients were categorized into groups with different disease presentations. nMCL were defined as patients with lymphadenopathies reported by radiologists and without significant circulating disease (<5x10^9/L circulating lymphoma cells). These included 110 patents with available peripheral blood (PB) flow cytometry data showing <5x10^9/L circulating lymphoma cells and another 83 patients with no PB flow cytometry data due to absence of lymphocytosis (<5x10^9/L lymphocytes) (total N=193, 78.1%). lnMCL were defined as patients with lymphadenopathies who also had high volume of circulating disease (>5x10^9/L lymphoma cells) either at time of diagnosis (23 patients) or during disease progression (6 patients) (total N=29, 11.7%). These included 27 patients with high circulating disease confirmed by PB flow cytometry and 2 patients with documented marked lymphocytosis (WBC counts: 322x10^9/L and 185x10^9/L). Lymphadenopathies for nMCL and lnMCL patients had a median size of 3.4 cm (range: 1.1-14 cm). nnMCL were defined as patients presenting with circulating disease and had no lymphadenopathy (N=25, 10.1%). Methods for flow cytometric study were performed as previously described.[5] Immunohistochemical expression of CyclinD1 (clone SP4, Thermo Fisher), CD5 (clone SP19, Ventata), and SOX11 (clone MRQ58, Cell Marque) were categorized as either positive or negative by using a 10% cutoff. Data for somatic alterations were collected for 236 patients from targeted next-generation sequencing (NGS)-based mutational analysis using a hybridization-capture-based panel, MSK-IMPACT, developed at the Memorial Sloan Kettering laboratory on samples at time of primary diagnosis.[6] For the remaining 11 patients, *TP53* mutational status was collected from 5 patients by MSK-ACCESS, a high coverage NGS-based assay by using cell-free DNA,[7] from another 5 patients by Archer LiquidPlex NGS assay (Invitae, CA, USA), and from one additional patient based on an external NGS report. Copy number alteration (CNA) data (available for 60 nMCL, 13 lnMCL, and 16 nnMCL patients) was collected by single nucleotide polymorphism (SNP) array testing as previously described.[8] IGHV mutational status (available for 70 nMCL, 17 lnMCL, and 21 nnMCL patients) was obtained by NGS-based LymphoTrack assay (Invivoscribe, CA, USA) by using a 2% cut-off.[9] All statistical analysis was performed by the GraphPad Prism version 10.0.0 software (Boston, MA, USA). Numerical data were compared by Student *t* test and categorical data were compared by Fisher's exact test. Data for overall survival (OS) were visualized by Kaplan-Meier curve and were analyzed by two-sided log-rank (Mantel-Cox) test. $P<0.05$ was considered statistically significant.

Clinicopathological comparisons were performed among the 3 groups (*Online Supplementary Table S1*). The nnMCL and lnMCL groups had significantly more frequent and higher volume of bone marrow (BM) involvement, greater incidence of splenomegaly, and higher circulating lymphoma cell counts than nMCL. All groups had comparable CD5 and CyclinD1 expression, while 36% of lnMCL and 75% of nnMCL were negative for SOX11, which contrasted with only 5.3% in nMCL ($P<0.001$ for both) (Figure 1A). A significantly higher number of lnMCL cases revealed high proliferation index (Ki67 >30%) (75%), in comparison to both nMCL (38.7%) ($P<0.001$) and nnMCL (33.3%) ($P=0.044$) (Figure 1B). Interestingly, both nMCL and lnMCL had even distribution of kappa and lambda light chain restriction, while 80% of nnMCL showed kappa light chain restriction ($P=0.002$ and $P=0.024$, respectively) (Figure 1C). Due to the smaller num-

bers of cases showing blastoid or pleomorphic morphology, no statistically significant difference was found amongst the 3 groups (Figure 1D).

Both nMCL and lnMCL showed similar IGHV mutational rates, which were significantly lower than nnMCL (*P*<0.001 and *P*=0.011, respectively) (Figure 1E). lnMCL were found to have the highest *TP53* mutational rates (48.3%) as compared to both nMCL (22.3%) (*P*=0.005) and nnMCL (32%) (*P*>0.05) (Figure 1F). Similarly, lnMCL showed more frequent deletion or copy-neutral loss of heterozygosity (CN-LOH) of TP53 (61.5%) than nMCL (26.7%) (*P*=0.023) and nnMCL (41.2%) (*P*>0.05) (Figure 1G). Altogether, 61.5% of lnMCL had both *TP53* mutation and deletion/CN-LOH, which was much higher than nMCL (15%) (*P*=0.001) and nnMCL (41.2%) (*P*>0.05) (Figure 1H). It is also worth noting that nnMCL showed more frequent concurrent *TP53* mutation and deletion/CN-LOH than nMCL (*P*=0.037).

The mutational frequencies for other commonly mutated genes were compared among the 236 patients with MSK-IMPACT data (Figure 2, *Online Supplementary Table S2*). In addition to *TP53*, lnMCL had significantly more frequent mutations in *NOTCH2* (10.3% *vs*. 1.6%, *P*=0.035), *SAMHD1* (10.7% *vs*. 1.7%, *P*=0.036), and *SPEN* (6.9% *vs*. 0.5%, *P*=0.050), a negative regulator for NOTCH signaling,[10] than nMCL. *NOTCH1* (17.2% *vs*. 7.1%) and *NSD2* (20.7% *vs*. 8.6%) mutations were also more frequent in lnMCL than in nMCL, although statistical significance was not reached. Both nMCL and lnMCL showed similar mutational rates of *ATM* and *CCND1*, which were distinctly different from nnMCL. More specifically, *ATM* mutations were significantly less frequent in nnMCL (4%) than in both nMCL (48.4%, *P*<0.001) and in lnMCL (44.8%, *P*=0.001), while *CCND1* mutations were significantly more frequent in nnMCL (36%) than in both nMCL (13.7%, *P*=0.009) and in lnMCL (10.3%, *P*=0.046). Also noteworthy is the absence of mutations in several genes, including *UBR5, BIRC3, NOTCH1,* and *CARD11*, in nnMCL.

Retrospective OS analysis was conducted with a median follow-up of 27 months (range: 0.27-120 months). lnMCL demonstrated the worst outcome in comparison to nMCL and nnMCL (median survival: 31.4 months *vs*. 101.9 months *vs*. not reached) (*P*<0.001) (Figure 3A). The trend for worse survival in lnMCL as compared to nMCL was retained after stratifying by different pathological parameters (Figure 3B-I), although some analyses were limited by lower case numbers. *TP53*-mutated nnMCL also appeared to show worse outcome than nnMCL with wild-type *TP53* (Figure

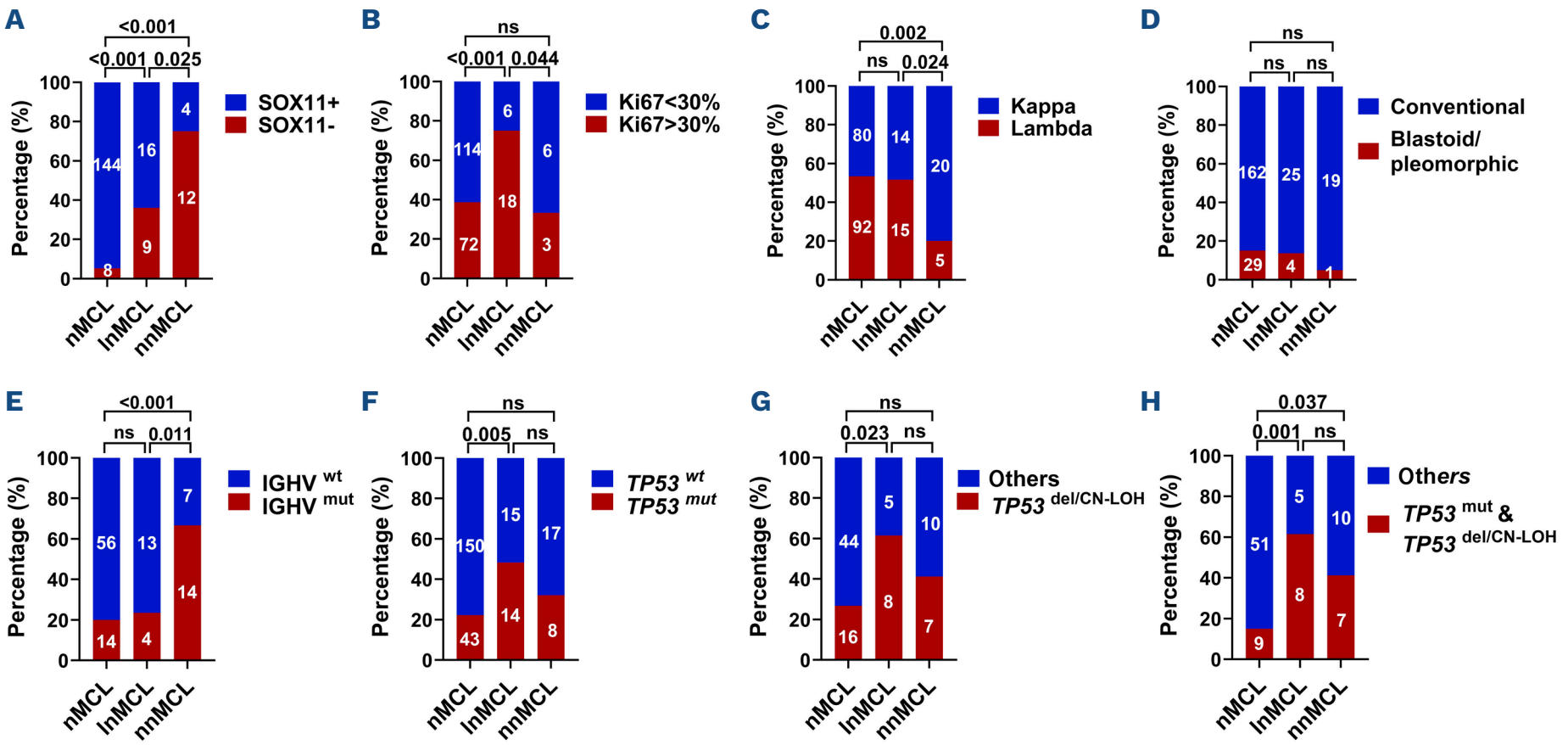

Figure 1. Comparison of various pathological features and *TP53* alterations among mantle cell lymphoma patients with different disease presentations. Comparisons of SOX11 expression (A), Ki67 index (B), light chain expression (C), histomorphology (conventional *vs*. blastoid/pleomorphic) (D), IGHV mutational status (E), *TP53* mutations (F), *TP53* copy number alterations (CNA) (G), and concurrent *TP53* mutations and CNA (H) were made among mantle cell lymphoma (MCL) patients with different disease presentations. SOX11 immunohistochemistry (IHC) for the 16 nnMCL patients was performed on bone marrow (BM) core biopsy in 15 patients and on both BM core biopsy and extranodal tissue (tonsil) in another patient with both samples showing SOX11 negativity. In addition, no discrepancy in SOX11 expression by IHC was observed in 9 lnMCL cases with SOX11 IHC performed on both BM core biopsy and nodal/extranodal tissue without decalcification. CNA data for *TP53* locus was obtained from single nucleotide polymorphism (SNP) array. Among 31 cases with CNA at 17p, 28 of them showed del(17p) (including 14 nMCL, 7 lnMCL, and 7 nnMCL) and 3 of them showed CN-LOH of 17p (including 1 lnMCL and 2 nMCL). Significant *P* values are shown on top of bars. ns: non-significant statistical comparisons. Numbers of patients in each group are indicated in each bar. Comparisons of additional clinical and pathological parameters are listed in *Online Supplementary Table S1*.

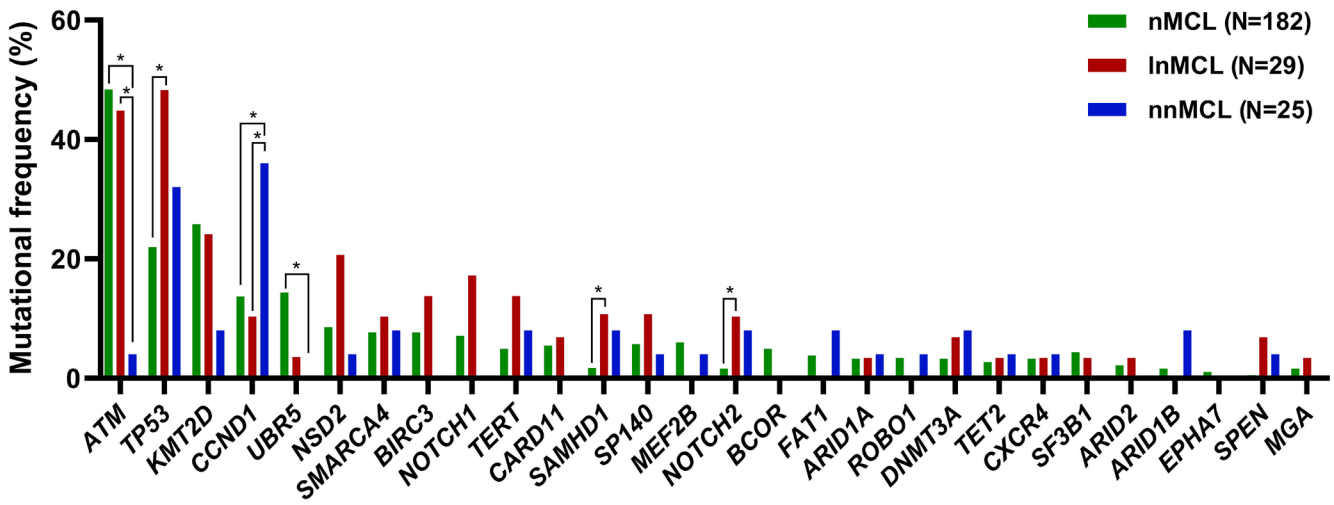

Figure 2. Comparison of mutational frequencies among mantle cell lymphoma patients with different disease presentations. Comparisons were made among 236 primary mantle cell lymphoma (MCL) patients with available Memorial Sloan Kettering (MSK)-IMPACT data. Genes mutated in more than 2% of cases in the entire cohort were included for analysis. *Statistical significance (P<0.05). Statistical comparisons between each of the two groups are also listed in *Online Supplementary Table S2*. Among the 236 patients, MSK-IMPACT was performed on lymph node tissues in 112 patients (106 nMCL, 6 lnMCL), on peripheral blood samples in 50 patients (19 nMCL, 12 lnMCL, 19 nnMCL), on bone marrow samples in 28 patients (14 nMCL, 8 lnMCL, 6 nnMCL), and on extranodal tissues in 46 patients (43 nMCL, 3 lnMCL). *TP53* mutations included 46 missense, 10 truncating, 3 splice site, 2 in frame insertion/deletion, and 4 concurrent missense and truncating mutations. Among 46 *CCND1* mutations from 37 patients, 45 mutations were located within exon 1, including 6 involving the 5'-untranslated region (UTR) and 39 involving the Cyclin_N domain; only one *CCND1* mutation was located within exon 4 and involved the Cyclin_C domain. All *TERT* mutations were located within the promotor region (10 cases with g.1295228C>T, 3 cases with g.1295250C>T, 1 case with g.1295287G>T, and 1 case with g.1295381G>C; reference genome: GRCh37).

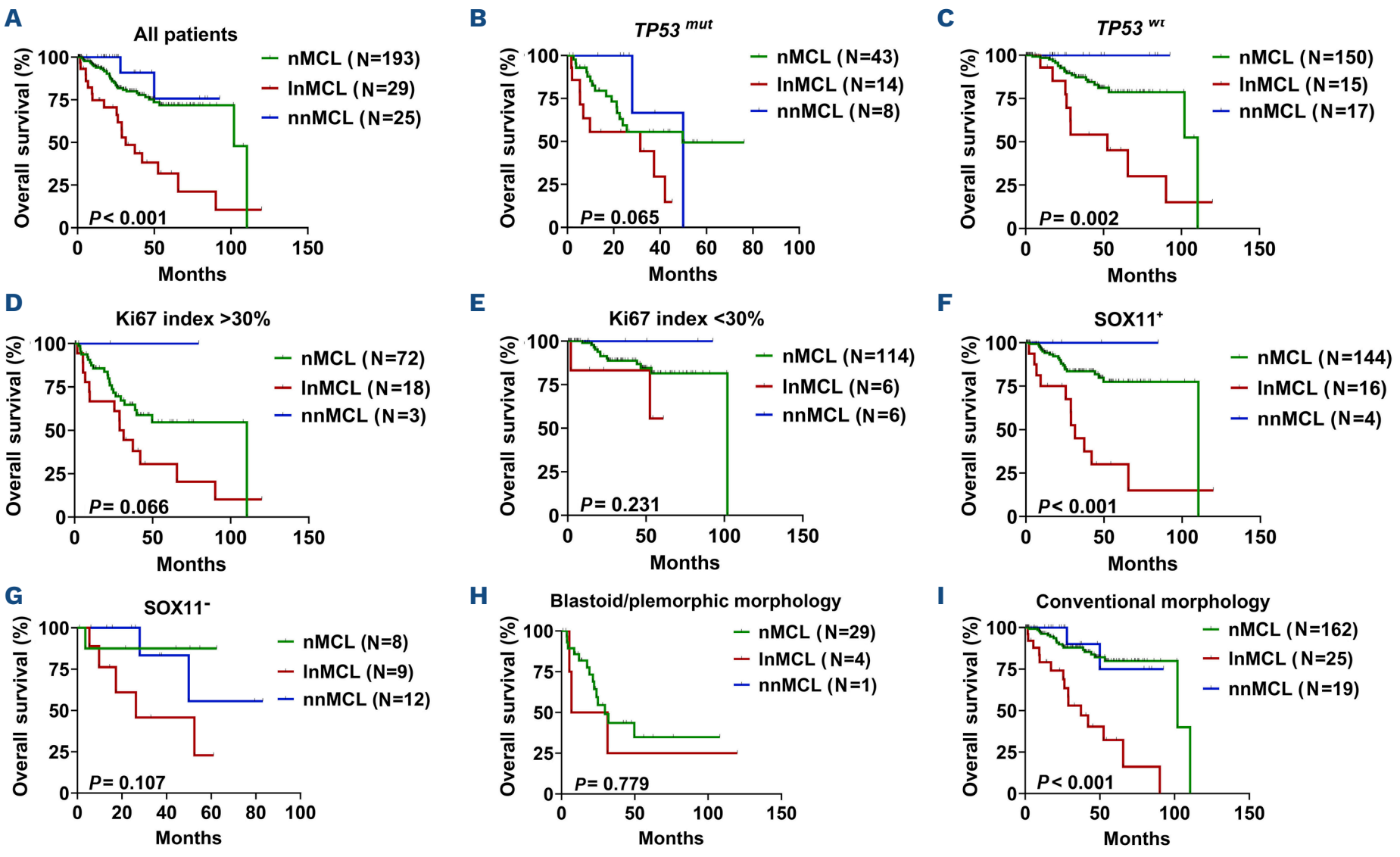

Figure 3. Overall survival analysis. (A) Comparisons were made among primary mantle cell lymphoma (MCL) patients with different disease presentations, which were further stratified by *TP53* mutations for (B) *TP53*-mutated patients and (C) *TP53* wild-type patients. Ki67 index for (D) patients with >30% Ki67 index and (E) patients with <30% Ki67 index. SOX11 expression for (F) SOX11-positive patients and (G) SOX11-negative patients. Histomorphology for (H) patients with blastoid or pleomorphic MCL and (I) patients with conventional MCL.

3B, C). Information on treatment is provided in *Online Supplementary Table S3*.

A noteworthy observation from the study is the greater number of *TP53* alterations, SOX11 negativity, and worse OS in lnMCL than in nMCL. Despite these differences, both lnMCL and nMCL share some biological features, including *ATM, CCND1*, and IGHV mutational status and light chain expression, which are all distinct from nnMCL. Therefore, lnMCL does not appear to represent a distinct biological subset of nodal MCL. The more frequent occurrence of poor-risk mutations observed in lnMCL, including *TP53* and several genes in the NOTCH signaling pathway,[11] may provide a biological basis for the association between elevated circulating disease levels and inferior prognosis in nodal MCL patients.

Although SOX11 negativity is recognized as a diagnostic feature for nnMCL, our data expand the spectrum of SOX11 negativity to a significant subset of lnMCL. SOX11 mediates tissue homing of MCL by regulating CXCR4 and FAK signaling.[12] Consequently, the frequent SOX11 negativity in lnMCL may help explain their significantly higher circulating disease levels. Additionally, low SOX11 expression has been linked to p53 overexpression in MCL patients.[13] This aligns with our findings, which show a significantly higher frequency of *TP53* mutations in SOX11-negative than in SOX11-positive nMCL patients (62.5% *vs*. 20.8%, *P*=0.016). Overall, these results suggest that negative SOX11 expression may serve as a potential biomarker for predicting *TP53* mutations in MCL patients, particularly among those with nMCL.

Among the 29 lnMCL patients, 23 exhibited a leukemic presentation at primary diagnosis, while 6 developed it during disease progression. Notably, lnMCL during disease progression were all positive for SOX11 (5/5), in contrast to the 45% of lnMCL at primary diagnosis that were SOX11-negative (9/20). However, like lnMCL at primary diagnosis, the mutational rates of *TP53, NOTCH1,* and *SAMHD1* in lnMCL during progression (33.3%, 33.3%, and 16.7%) were higher than in nMCL (22%, 7.1%, and 1.7%), suggesting a distinct genetic background between the two. Larger studies are needed to better understand the potential pathological and molecular differences between lnMCL at primary diagnosis and lnMCL during disease progression.

In summary, nodal MCL with leukemic presentation is characterized by frequent SOX11 negativity, enrichment for poor risk mutations including *TP53* and those involving NOTCH pathway, and significantly worse outcome. Such patients appear biologically distinct from nnMCL and should be distinguished from the latter due to their different clinical outcomes and therapeutic strategies.

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Contributions

MY, AD and PG are responsible for study design. MY is responsible for data collection, data analysis, and manuscript writing. SS and AK are responsible for data review. JK is responsible for interpreting molecular data. All authors reviewed and revised the manuscript. PG supervised the entire study.

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Data-sharing statement

Original data can be shared upon request by contacting the corresponding author.

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