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Mantle cell lymphoma (MCL) is a phenotypically and genetically heterogeneous malignancy in which the genetic alterations determining clinical indications are not fully understood. Here, we performed a comprehensive whole-exome sequencing analysis of 152 primary samples derived from 134 MCL patients, including longitudinal samples from 16 patients and matched RNA-Seq data from 48 samples. We classified MCL into 4 robust clusters (C1–C4). C1 featured mutated immunoglobulin heavy variable (IGHV), CCND1 mutation, amp(11q13), and active B cell receptor (BCR) signaling. C2 was enriched with del(11q)/ATM mutations and upregulation of NF-κB and DNA repair pathways. C3 was characterized by mutations in SPI40, NOTCH1, and NSD2, with downregulation of BCR signaling and MYC targets. C4 harbored del(17p)/TP53 mutations, del(13q), and del(9p), and active MYC pathway and hyperproliferation signatures. Patients in these 4 clusters had distinct outcomes (5-year overall survival [OS] rates for C1–C4 were 100%, 56.7%, 48.7%, and 14.2%, respectively). We also inferred the temporal order of genetic events and studied clonal evolution of 16 patients before treatment and at progression/relapse. Eleven of these samples showed drastic clonal evolution that was associated with inferior survival, while the other samples showed modest or no evolution. Our study thus identifies genetic subsets that clinically define this malignancy and delineates clonal evolution patterns and their impact on outcomes.

Introduction

Mantle cell lymphoma (MCL) is an aggressive subtype of non-Hodgkin’s B cell lymphoma that has a median overall survival (OS) of approximately 5 years (1–7). MCL can generally be grouped into 2 types based on clinical indications: aggressive conventional MCL (cMCL) and indolent leukemic nonnodal MCL (nnMCL) (2, 5). Several clinical and molecular features are used to distinguish these 2 types of MCL, including involvement of lymph nodes, expression of SOX11 (2, 5), and somatic hypermutation on the B cell receptor (BCR) immunoglobulin heavy variable (IGHV) genes (1, 5, 8, 9). Due to significant heterogeneity in the clinical outcome of patients with MCL (3, 4, 10), differentiating patients who will have poor clinical outcomes from patients who will achieve durable response with standard therapies remains a challenge. The MCL International Prognostic Index (MIPI; refs. 7, 11–14) and tumor Ki67 (11) expression are used to stratify newly diagnosed MCL patients. However, these traditional prognostic markers have not enabled tailored therapeutic strategies for MCL. In the era of novel therapies for MCL (1, 15, 16), better prognostic and predictive models that account for the biologic heterogeneity of the disease are needed to stratify patients.

In the last decade, unbiased massively parallel sequencing of whole exomes (WES) and RNA-Seq of MCL have identified recurrent mutations (TP53, ATM, NOTCH1/2, CCND1, HNRNPH1, KMT2D) associated with MCL (6, 17–22) and genetic lesions
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The ability to evaluate clonal evolution of MCL in relation of disease progression to chemoimmunotherapy.

To address these limitations, we performed WES on 152 MCL tumor samples from 134 patients (Table 1). Longitudinally collected samples were available for 16 patients, and 48 samples had matched RNA-Seq data (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI153283DS11).

Results

Mutated cancer driver genes and mutational signatures in MCL. Our samples were collected from 134 MCL patients (123 newly diagnosed...
Figure 1. Recurrent somatic genetic alterations and mutation signatures in MCL. (A) Recurrent somatic mutations and CN alterations (rows) identified following WES of 134 primary samples (columns) obtained from patients with newly diagnosed (green) and relapsed (red) MCL. Samples were annotated for prior treatment, MIPI risk, IGHV status, and Sox11 expression level when collected. Left: blue labels, recurrent CN deletion; red labels, recurrent CN amplification; black labels, somatic mutations; bold labels, novel CN alterations/mutations. Right: percentage of samples mutated. Top: total number of genetic alterations across the cohort. (B) Contributions of individual mutations to the collective WAP score of TP53. The changes in WAP score $P$ value due to removal of individual mutations are plotted as function of residue number. The radius of the circles around each point in the graphs represent the number of patients with that mutation. Color indicates Sox11 expression. (C) TP53 dimer bound to DNA fragment, PDB ID: 3IGK. One of the monomers is shown in yellow, the other in gray. DNA is shown in orange. The mutations observed in Sox11– and Sox11+ patients are shown as magenta and green, respectively. (D) $|$ $J$ Scores from genome-wide CRISPR/Cas9 screens of JeKo-1 of genes identified as having recurrent mutations.

and I1 with relapsed disease, 15 nmMCL and 119 cMCL) with a broad range of clinical characteristics, including different MIPI risk as well as IGHV unmutated and mutated (Table 1 and Supplemental Tables 1 and 2). The median follow-up time was 31.0 (range 4.5–107.3) months. The 3-year OS rate in the cohort was 69.6% (95 CI, 60.1%–78.5%).

We detected a median of 29 nonsynonymous mutations in protein-coding sequences per sample (range, 8–72), and a median mutation burden of 1.35 mutations per megabase (Mb), which is similar to that previously reported in MCL and other hematologic cancers (Supplemental Figure 3, A and B, and refs. 17,19,21,26). We identified 33 recurrently mutated genes (mutated in $>5$ samples, mutation frequency $>3\%$, Figure 1A), which included known and novel recurrent mutations ($LRP1B$, $PCLO$, $RTR2$, $PCDH10$, $OBSCN$, $TACC2$, $FAT3$, $LRP2$, $SVEP1$, $ZFHX4$, $MPDZ$, $DCDC1$, $IKKBK$, $ARID1A$; Figure 1A).

To determine which of the mutated genes are likely to contribute to lymphomagenesis, we used the clustering of mutations in protein structures (CLUMPS) algorithm (27) to identify clustering of mutations predicted to have significant impact on 3D protein structures or interference with protein’s binding partners. Mutations in $TP53$ and $CCND1$ were found with significant clustering ($P < 0.05$), whereas mutations in $ATM$, $SP40$, and $SMARCA4$ showed moderate clustering (Supplemental Figure 4A; $P < 0.1$). To determine how individual mutation affects the clustering $P$ value, we systematically removed each mutation and recalculated the weighted average proximity (WAP) score and the resulting change in $-\log_{10} (P$ value), $\Delta P$ value (Figure 1B and Supplemental Figure 4C). A positive $\Delta P$ value indicates that the mutation clusters with other mutations in the protein and that the removal of this mutation adversely affects the CLUMPS score significance. Conversely, a negative $\Delta P$ value indicates that the mutation does not cluster significantly with other mutations. In $TP53$, we found both categories of mutations with positive and negative $\Delta P$ values (Figure 1C).

For example, mutations at R248 and I195 showed negative $\Delta P$ values, while mutations at R273 had positive $\Delta P$ values, indicating that these 2 groups of $TP53$ mutations may exert their effects in different ways. Two of the mutations with negative $\Delta P$ value, R248 and S241, were at the DNA-binding interface, suggesting that these mutations may interfere with the DNA recognition by $TP53$. In contrast, mutations with positive $\Delta P$ values, such as R158, V156, and Y205, were clustered within structural domains of $TP53$ that were distant from the DNA-binding interface. These mutations may affect the function of $TP53$ through different mechanisms compared with the ones that are unclustered. We further observed that the $\Delta P$ value of the WAP score for the $TP53$ mutation was significantly different between Sox11–negative and -positive patients (Figure 1B and Supplemental Figure 4B). This implies that the Sox11–positive patients tend to have mutations that cluster together in the $TP53$ structure compared with Sox11–negative patients (Figure 1C). The hotspot mutations at the C47 and Y44 of $CCND1$ affecting the weighted average proximity (WAP) score were not in direct contact with its binding partner CDK4, but in a loop region that packs between 2 helices and maintains a half helical turn in the loop (Supplemental Figure 4E). The significance was supported by the observation that both mutations can increase $CCND1$ protein stability and promote ibritinib resistance in MCL (28).

To delineate the roles of recurrent mutations in MCL biology, we examined genome-wide CRISPR/Cas9 perturbation screen results from DepMap (https://depmap.org/portal/) for leukemia and lymphoma as well as our own CRISPR/Cas9 perturbation screen results in the MCL cell line JeKo-1. Silencing of $SP40$, $SMARCA4$, $PCLO$, $TP53$, and $TRAF2$ in JeKo-1 cells conferred a cell-growth advantage, while knockdown of these genes in other cell lines had modest or no impact on cell growth (Figure 1D and Supplemental Figure 3C), indicating that these genes may act as tumor suppressors in MCL.

Leveraging our WES data set, we identified 4 mutation signatures prevalent in MCL using the MutationalPatterns pipeline (Supplemental Figure 5, A and B, and ref. 29). This includes an age-related signature involving C-to-T transitions at CpG sites; a c-AID signature characterized by increased C $\rightarrow$ T/G mutations at a known activation-induced cytidine deaminase (AID) hotspot (SBS84); an enzyme essential for somatic hypermutation of germinal center B cells; and signatures 5 and 40, common signatures that were prevalent in most cancers and leukemia/lymphoma, respectively. The c-AID signature mainly comprised clustered mutations (Supplemental Figure 5C). Of note, most of the signatures contributed by aging-related signature and signature 40 (range: 36.2%–100%, median 68.8%, Supplemental Figure 5D).

Copy number alterations in MCL. With this data set, we identified 20 recurrent somatic copy number alterations (SCNAs) (Figure 1A and Figure 2A; $q$ value $\leq 0.1$, frequency $\geq 10\%$). Of note, the tumor-only pipeline generated highly correlated SCNA calls in the 89 paired samples, which were well correlated with FISH results (receiver operating characteristic [ROC], $P < 0.001$ for del[17p], del[13q], del[11q]) (Supplemental Figure 6, A and B, and Supplemental Figure 7). In addition to previously reported SCNAs linked to OS (del[9p], del[17p], del[13q], and del[8p23.3]; refs. 18, 30), we also identified driver SCNAs, including del[15q11-13] and amp[11q13.3] (Figure 1A).

To determine how SCNAs affect gene expression, we performed an integrative analysis in samples with WES and RNA-Seq data ($n = 48$). We focused on identifying genes that showed significant changes within the deleted or amplified regions by comparing samples with or without the lesions. Pathways that were significantly affected by amplification included DNA catabolic and translation pathways ($EIF4G1$, $RPL4$, $DDX6$, $PRL15$) and the MYC pathway.
Figure 2. Recurrent SCNAs, cooccurring genetic events, and clinical association. (A) Significant CN amplifications (left, red) and deletions (right, blue). Left sides of the mirror plots show the incidence of significant focal CNA events. Right sides of the mirror plots show q values for each region. Genes located in the peak of relevant cytobands are listed. (B) Pairwise associations between recurrent genetic alterations found in the 134 MCL samples. Low and high cooccurrence are shown in blue and red, respectively. Intensity of the color reflects the odds ratio. Statistically significant association as determined by q value is marked by asterisks. (C) Number of samples with cooccurrence of the indicated genetic events in the cohort of 134 MCL samples. Significance of Fisher’s exact test indicated by q.
MYC, NME1). Pathways that were perturbed by deletion included DNA repair and cell cycle (ATM, Cdkn1b, Pot1) and RNA splicing (Hnrnpk, Ncbp1, Srsf1) (Supplemental Figure 6, C and D).

Our WES data set revealed significant relationships between several genetic alterations (Figure 2B). In addition to known cooccurrence between TP53 mutation and del(17p), ATM mutation and del(11q), del(9p21.3) and del(17p) (Supplemental Figure 8, A–C), we also observed a high cooccurrence of del(9p21.3) with del(8p) (*p < 0.001) and del(13q) (*p = 0.004; Figure 2C). Moreover, we found low cooccurrence of genetic alterations such as mutations in TP53 and ATM or del(11q) (Supplemental Figure 8B; *p < 0.05), indicating tumor cells harboring these events may originate from a different genetic trajectory.

Association of genetic features with clinical outcomes. We examined associations between genetic alterations and key MCL features. Overall, we observed a high number of SCNAs associated with unmutated IGHV status and SOX11 expression (Supplemental Figure 9, A and B). The c-AID mutation signature was strongly associated with mutated IGHV status, while the aging signature correlated with unmutated IGHV and SOX11 expression (Supplemental Figure 9, C and D). Moreover, the number of SCNAs was able to predict clinical outcomes (Supplemental Figure 9, E and F).

We examined the prognostic significance of somatic mutations. Mutations in SP140, SMARCA4, TRAF2, and PCDH10 were predictive of poor progression-free survival (PFS) (Figure 3, A and B). SP140 mutations occurred at 8% frequency in our cohort, and 9

Figure 3. Associations of somatic mutations with clinical outcomes. (A) Lollipop diagrams of selected putative driver genes showing mutation subtype, position, and frequency. Bottom: y-axis indicates the number of identified mutations in the COSMIC database. (B) Kaplan-Meier plots (with log-rank P values) of PFS and OS associated with presence and absence of selected mutations. (C) Samples with SP140 mutations or deletions did not overlap in the cohort. (D) Deletion of SP140 affected its gene expression. SP140 expression TPM value was extracted and plotted from MCL samples with SP140 deletion, mutation, or WT. *p < 0.05. (E) Forest plots of the multivariate analysis of MIPi risk groups and individual genetic factors for PFS and OS in our MCL cohort.
out of 11 mutations were frameshift and nonsense mutations that resulted in a truncated form of SPI40 (Figure 3A), highly suggestive of loss-of-function mutations. We further identified 10 samples (7.5%) harboring SPI40 deletion (loss of 2q36.3-37.3), all of which showed downregulation of SPI40 expression compared with samples lacking the deletion (Figure 3, C and D). Mutation or deletion of SPI40 was predictive for shorter PFS and OS and associated with SOX11 expression, suggesting this gene may be a potential tumor suppressor in MCL (Supplemental Figure 9G and Supplemental Figure 10A). Consistent with previous publications (25, 31), the presence of TP53 or NOTCH1 aberrations was associated with shorter PFS (Supplemental Figure 10, B and C, and Figure 3E). Of note, TP53, NOTCH1, and PCDH10 mutations as well as the SPI40 mutation/deletion retained significance for PFS and OS when MIPI risk and IGHV mutation status were added (Figure 3E).

We next classified the WES samples with del(9) into 3 groups based on the deleted region: 9p–, 9q–, or large region (Figure 4A; z score ≥ 1). We next classified the WES samples with del(9) C2, C3, and C4 subtypes. Median PFS was not reached for C1 and with the C1 subtype had a more favorable outcome than those with P < 0.05) irrespec-

To determine the robustness of these genetic clusters, we assessed whether these genetic alterations can stratify MCL patients using a published genetically well-annotated MCL cohort for validation (26) (Barcelona cohort, Figure 6, A–D). Projection of cluster features classified patients into 4 distinct clusters, with C1 having a favorable clinical course and C2 and C3 falling in between C1 and C4. There was significant statistical difference among clusters (P = 0.001), in which C1 versus C4 (P < 0.001), C2 versus C4 (P < 0.035), and C3 versus C4 (P = 0.014) reached significance in the pairwise test.

To explore phenotypic differences among the MCL genetic subtypes, we performed an integrative analysis using matched RNA-Seq data (n = 48) across the 4 subsets (Figure 7, C1–C4, n = 12, 11, 16, and 9, respectively). We first assessed whether the recurrent mutated genes identified from WES were expressed at the RNA level and discovered most of these mutations were highly expressed (Supplemental Figure 13, A and B). Likewise, the frequent SCNAs also resulted in significant dysregulated gene expression (Figure 7A), which we further validated by reverse transcriptase PCR (RT-PCR) analysis of MCL samples containing and lacking the SCNAs (Supplemental Figure 13C). Our analysis revealed that each genetic subset has a unique gene expression pattern (Figure 7B and Supplemental Figure 14A). Consistent with the differing cellular origins for the 2 types of MCLs (1, 5), C1 was enriched for gene expression signatures of memory B cells and C2, C3, and C4 appeared to have a signature of CCR6-negative light zone B cells or naive B cells (Figure 7, B and C, and Supplemental Figure 14A). We further tested a previous reported 16-gene signature that distinguished cMCL and mmMCL (39) and found that 35 out of 36 C2–C4 patients were classified as cMCL while 9 out of 12 C1 patients were classified as mmMCL (Supplemental Figure 14B).

C1. The C1 group included 16% of samples. Most of the C1 samples were IGHV mutated and featured mutant CCND1, TP53, and amp(11q13). Most of the TP53 mutations in C1 had negative WAP scores (Supplemental Figure 4B). Patients with and without TP53 mutations had similar OS (P = 0.470, Supplemental Figure 15, A and B). Those in the C1 group had the lowest SOX11 expression (Supplemental Figure 15C). Phenotypically, C1 was enriched with a memory B cell phenotype and active BCR signaling (Figure 5A, Figure 7, B and C, and Supplemental Figure 14A). We observed enrichment of BCR signaling in the Barcelona cohort, although it was insignificant due to the small amount of available microarray gene expression data (Figure 6D).

C2. The C2 group included 23% of samples. Of 31 samples in C2, 28 harbored del(11q) (minimal deleted region contains ATM), while 19 of these 28 samples had a cooccurring ATM mutation. Consistent with these genetic lesions, genes involved in DNA replication, DNA repair, and hyperproliferation were all upregulated (Figure
Figure 4. Deletion of chromosome 9 was associated with poor survival. (A) Chromosome 9 deletion in samples from our cohort. Top: blue line indicates percentage of MCL samples with chromosome 9 deletion at the location. Known tumor suppressors and oncogenes present on chromosome 9 are color coded based on their z score in the CRISPR/Cas9 screen in JeKo-1 cells. Bottom: deletions in 9p (purple), 9q (blue), or large regions (dark red) in samples from our cohort. Homozygous minimal 9p deletions are marked in red. CCF (Supplemental Methods) of chromosome 9 deletion is shown in gray scale. (B) Unsupervised clustering analysis of gene expression in chromosome 9 distinguishes MCL samples with deletions in different region. (C) Volcano plot of genes on chromosome 9 that are differentially expressed between MCL samples that have and do not have chromosome 9 deletions. Downregulated genes that were significantly associated with shorter PFS and OS are indicated in red (Cox’s regression HR <1, P < 0.05). (D) Kaplan-Meier plots of PFS and OS according to type of chromosome 9 deletion.
In contrast with C2, we observed significant downregulation of genes implicated in TNF-α signaling via the NF-κB pathway and IFN-γ response, but with activated NOTCH signaling. Additionally, BCR signaling, MYC targets, and IL-2 STAT5 signaling were all downregulated in C3 in both discovery and validation cohorts (Figure 6D and Figure 7B). Expression of genes involved in TNF-α signaling via the NF-κB pathway and IFN-α and IFN-γ response was significantly enriched in both discovery and validation cohorts (Figure 6D and Figure 7B).

C3. The C3 group included 32% of samples. Besides enriched NOTCH1 mutations, the C3 group also harbored mutations in NSD2 (WHSC1), KMT2D, and SP140 as well as amp(13q) and del(6q). In contrast with C2, we observed significant downregulation of genes implicated in TNF-α signaling via the NF-κB pathway and IFN-γ response, but with activated NOTCH signaling. Additionally, BCR signaling, MYC targets, and IL-2 STAT5 signaling were all downregulated in C3 in both discovery and validation cohorts (Figure 6D and Figure 7B).
Figure 6. Molecular cluster and gene expression signature validated in Barcelona cohort. (A) Sample inclusion description in the validation cohort. (B) Projective nonnegative matrix factorization consensus clustering was performed using genetic alterations identified from our discovery cohort (Figure 5A). Clusters 1–4 are shown with their associated landmark genetic alterations (boxed for each cluster). Header shows cluster association (C1, black; C2, green; C3, blue; C4, red). (C) Kaplan-Meier plots of OS of patients grouped into the 4 clusters. P indicates log-rank test. Number indicates samples included in each cluster. (D) Integration of genetic and transcriptomic analyses identified gene expression signatures for each genetic subset. The heatmap was generated using normalized enrichment score (NES). Asterisks indicate the significance level of the enrichment.
Five out of 11 patients whose samples showed drastic evolution (4 cases from C1 or C3 to C4, 1 case from C1 to C2) also had a cluster change, while all 5 patients whose samples showed modest or no evolution retained the same cluster status (Supplemental Figure 17). Patients whose samples showed cluster changes had poor survival after relapse even though their relapse interval appeared to be longer (Supplemental Figure 16, E and F), showing that genetic heterogeneity drives the progression of disease.

Discussion
In the past decade, numerous studies have profiled genome-wide genetic alterations, gene expression, and epigenomic changes in MCL (17, 19, 21, 23, 24, 26, 31, 45–48). These studies not only generated insights into the molecular features (2, 30) and mechanisms of pathogenesis (21, 26), drug resistance (23, 24), and the cellular origin of subsets of MCL (26, 48), but also revealed the vast genetic complexity and phenotypic heterogeneity present within MCL, which has become a barrier to connecting genotype with disease phenotype in MCL. Here, starting with a large WES data set along with matched transcriptome data, we classified MCL into 4 clusters based on shared genetic lesions and determined their gene expression signatures as well as associations with clinical outcomes. We further studied clonal evolution patterns prevalent in MCL and inferred the order of genetic lesions in the development of MCL.

Our cluster analyses have a few implications. First, C1 is highly enriched for nnMCL and C2–C4 are mostly cMCL. Whereas cMCL may present with similar clinical symptoms, it fell into 3 distinct genetic subsets, which were all accompanied by coordinated dysregulated cellular pathways. This analysis provides clues for future biomarker-driven clinical trial designs that align particular treatments (e.g., Bruton’s tyrosine kinase inhibitors) with patients most likely to benefit (e.g., patients whose tumors were classified as C3 or C4 with downregulation of BCR pathway signaling). Based on the “Goldilocks” model of BCR singling and B cell survival, B cell survival is dependent on the tuning of BCR signaling such that it is neither overly strong nor overly weak (49). Within this context, cells with inherent increased BCR signaling would be anticipated to be less sensitive to a BTK inhibitor such as ibrutinib, as these cells do not meet the minimum threshold of BCR signaling needed for cell survival. In fact, MCL cell lines, including JeKo and Mino, which carry complex copy number (CN) variations and mutations (likely to be C3–C4; https://depmap.org/portal/), are sensitive to BTK inhibitor while JVM2 (genetic feature similar to C1) is insensitive to BTK treatment (50). Second, our results emphasize the influence of distinct genetic features on the clinical outcomes. Despite the different treatment regimens and patient population (Chinese and European descent) between our discovery and validation cohorts, all MCLs fell into 4 distinct clusters. These molecular clusters open the door to precision medicine, as they can serve as stepping-stones between genetic discovery and its application to clinical practice. Finally, selection of treatment for MCL based on individual genetic alteration may not be optimal because clinical response is determined by a cluster of genetic factors. TP53 mutations are good examples illustrating this scenario, as mutant TP53 was associated with inferior clinical courses in both previous reports (51, 52) and our analysis (Supplemental Figure 10B). In particular, C1 and C4 all harbored TP53 mutations (36% and 63%); however, the
Several studies reported the cooccurring genetic events in MCL with the implication that heterogeneous genetic alterations associated with MCL relapse (8, 23, 47). Our study revealed that branched evolution is a common feature upon chemotherapy and predictive of clinical outcomes, which suggests that intratumor heterogeneity forms the fuel for relapse and drug resistance. Although mutant TP53/del(17p) was reported to be associated with disease relapse (25), we only observed frequency of del(9p) and amp(3q) arise in response to therapies (>20% CCF changes in 50% of samples) (Supplemental Figure 16B). Large cohorts of sample analyses are needed in order for us to fully understand the genetic events and role of clonal evolution in driving MCL.

In summary, this integrative analysis provides a framework for assessing unappreciated genetic heterogeneity in the clinically defined subtypes of MCLs and forms the basis for designing precision therapies for aggressive MCL, with genetic factors and oncogenic pathways as tractable targets.

Methods

Samples and genomics studies. Diagnostic biopsy and/or blood samples representing 152 MCL tumors were obtained from 134 MCL patients. Ninety-five patients received a standard high-dose cytarabine-based aggressive regimen (Supplemental Figure 1B), while others received nonaggressive treatment (Supplemental Methods). Tumor cells were collected from bone marrow, blood, and lymph nodes (125 cryopreserved and 27 formalin-fixed, paraffin-embedded [FFPE]), with 102 (67.1%) having matched germline tissue (Supplemental Table 1). Thirty-three were longitudinal tumor samples collected from 16 patients at diagnosis (pretreatment), at progression, or at relapse following treatment (Supplemental Table 2).

WES libraries were prepared using Agilent SureSelect Human All ExonV6 (Agilent Technologies) and sequenced on the HiSeq 4000 platform (Illumina). Raw reads were aligned to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler aligner (57). Somatic single nucleotide variations (SNVs) and SCNAs were called using GATK best practice somatic mutation and somatic CN variant discovery pipelines (58, 59), respectively. A tumor-only pipeline was used to filter a panel of normal samples (16,196 normal samples, Supplemental Methods; ref. 60) from the GATK4 pipeline results for samples without matched normal tissue, which yielded comparable mutation calls in paired samples (Supplemental Figure 2). MutationalPatterns (29) was used to determine de novo mutation signatures. The CLUMPS method (27) was used to assess the significance of mutational clustering in a given 3D structure. Details of the calculation of the WAP score were described previously (27). The ABSOLUTE algorithm was used to calculate the tumor purity, ploidy, and CCF for SNV and SCNA (40). Statistical methods were adapted to infer the order of genetic alterations (43). Phylogenetic analysis was performed on longitudinally collected samples using the PhylogenyNDT package (61). Detailed methods are available in the Supplemental Methods.

CRISPR library screen. The genome-wide CRISPR library screen was carried out using the Human GeCKO v2 Library, 2-plasmid system (a gift from Feng Zhang, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; Addgene, 1000000049) and following the protocol as described (62). Briefly, the library contains 122,417 unique sgRNAs targeting the human genome with 6 sgRNAs per gene. The entire library, together with helper plasmids pMD2.g
and psPAX2, was then transfected into HEK 293T cells, and lentiviral supernatants were collected after 2 days, followed by spin infection at 1200g in 2 replicates of doxycycline-inducible Cas9-expressing JeKo-1 cells for 1 hour in the presence of 8 μg/ml polybrene. Transduced cells were selected by puromycin for 3 days, and doxycycline (1 μg/ml) was added to induce Cas9 expression, followed by culturing for an additional 14 days. Genomic DNA was harvested on days 0 (day 3 in puromycin) and 14 and subjected to high-throughput sequencing to determine sgRNA abundance. MAGeCK (63) software was used to quantify sgRNA depletion or enrichment.

Consensus clustering of genetic alterations. All recurrent mutated genes (frequency ≥5%), IGHV mutational status, and significant regions of SCNAs (GISTIC2.0, q value ≤ 0.1, and frequency ≥ 10%) were assembled into a gene matrix, and NMF consensus clustering

Figure 8. Clonal driver events associated with clinical outcomes. (A) CCF values for each sample affected by a recurrent genetic alteration across all 134 samples. Median CCF values are shown (top, bars represent the median and interquartile range for each genetic alteration). Alterations with a CCF value of greater than 0.9 were defined as a clonal event. The cumulative proportion of a recurrent genetic alteration found as clonal (blue) or subclonal (red) in the cohort is shown in bottom plot. (B) Computational inference of temporal order of genetic alterations in MCL. Arrows indicate when paired clonal and subclonal genetic alterations were found in the same sample. Dashed lines indicate the temporal order was found in 3 or more samples; solid lines that the temporal order was found in 5 or more samples. (C) Kaplan-Meier plot of PFS according to the number of clonal driver events.

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**Figure 8. Clonal driver events associated with clinical outcomes.**

(A) CCF values for each sample affected by a recurrent genetic alteration across all 134 samples. Median CCF values are shown (top, bars represent the median and interquartile range for each genetic alteration). Alterations with a CCF value of greater than 0.9 were defined as a clonal event. The cumulative proportion of a recurrent genetic alteration found as clonal (blue) or subclonal (red) in the cohort is shown in bottom plot.

(B) Computational inference of temporal order of genetic alterations in MCL. Arrows indicate when paired clonal and subclonal genetic alterations were found in the same sample. Dashed lines indicate the temporal order was found in 3 or more samples; solid lines that the temporal order was found in 5 or more samples.

(C) Kaplan-Meier plot of PFS according to the number of clonal driver events.
Figure 9. Clonal evolution pattern in MCL and its association with clinical outcome. (A–C) Depiction of tumor clonal evolution from diagnosis to relapse in a representative patient (MCL34). (A) Dynamic changes in genetic alterations during disease progression. Representative genetic alterations for each cluster are listed in the plot. (B) Clonal evolution estimated using PhylogicNDT. The mean CCF and 95% CI of each cluster are indicated. (C) Fish plot showing the clonal evolution process. The width of each time point indicates the clonal fractions of each subclone population. (D) Joint distributions of CCF values of genetic alterations across 2 (or more) time points (ND, newly diagnosed; P, progression; R, relapse; R1, first relapse; R2, second relapse) were estimated using clustering analysis. Each line corresponds to cluster of genetic alterations (range 3–33) and illustrates the dynamic changes in CCF at the different time points for clusters. We classified any CCF increase or decrease greater than 0.5 between 2 time points for any cluster as extreme evolution. CCF changes between 0.2 and 0.5 or less than 0.2 were classified as moderate evolution or no evolution, respectively. (E) Sample interval and number of clonal clusters in patients with either extreme evolution or with modest or no evolution. (F) Kaplan-Meier plot of survival from either first sampling (left) or second sampling (right).
was used to identify genetic clusters as previously reported (38). Briefly, all genetic lesions were scored based on the following attributes: nonsilent mutations, 1; IGHV mutation, 2; low-level CN deletion (1.0 ≤ CN ≤ 1.7 copies), 1; high-level deletion (CN < 1.0 copies), 2; low-level amplification (2.3 ≤ CN ≤ 3.7 copies), 1; high-level amplification (CN > 3.7 copies), 2. The NMF consensus clustering algorithm was used to assign samples into different clusters. Both cophenetic coefficient and silhouette values for K = 2 to K = 10 were calculated to determine the best solution, as shown in Supplemental Figure 12A (K = 4). Fisher’s exact test was used to identify markers for each cluster by testing whether the frequency of variants in one cluster was higher than in other clusters. P values for multiple comparisons were adjusted using the Benjamini-Hochberg correction. Genetic alterations with q < 0.1 were defined as markers. The main algorithm code can be accessed at GitHub (https://github.com/broadinstitute/DLBCL_Nat_Med_April_2018/tree/1c5dc2d7b8859fbb7839f4e1d9725e455b14df4d with commit ID 1c5dc2d7b8859fbb7839f4e1d9725e455b14df4d). The results were visualized as a heatmap using R package ComplexHeatmap 2.4.2 (64). In the Barcelona cohort, we adopted the single-cell projective nonnegative matrix factorization (scPNMF) (65) method to project features extracted from the discovery cohort. The parameter –K 15, method = KL was used and samples were assigned into a nearest cluster of discovery cohort based on UMAP. Thirty-three samples with matched gene expression profiling data available were used to validate our expression features in different clusters.

**Integrative genomics and transcriptomics pathway analysis.** RNA-Seq libraries were generated with the NEBNext UltraTM RNA Library Prep Kit (New England Bio) and sequenced on the HiSeq platform (Illumina). Raw reads were aligned to the human reference genome (GRCh38/hg38) using STAR (66), and expression levels of mRNAs were normalized to transcript per million (TPM). To directly compare pathway expression for each cluster, the log-transformed TPM values for all genes in the gene set were averaged to provide a signature value for each sample and then the value for samples assigned to each cluster was calculated as the cluster average expression of the signature. These values were linearly transformed, and the F test was used to compare each cluster.

**Statistics.** Survival curves were estimated using the Kaplan-Meier method, and log-rank test was used to assess statistical significance for PFS and OS between cohorts. Multivariate Cox’s regression analysis was used to assess the independent prognostic impact from MIPI risk, IGHV mutational status, and individual genetic factors for outcomes in the MCL cohort. Student’s t test or Mann-Whitney U test was used to evaluate differences between continuous variables.

**Study approval.** All samples were obtained from MCL patients. Written, informed consent was obtained from all participants, in accordance with the Declaration of Helsinki, and the study was approved by the Institute of Hematology and Blood Disease Hospital, the Chinese Academy of Medical Sciences, and the Peking Union Medical College Ethics Committees.

**Author contributions**

LW and LQ designed the study and wrote the manuscript. SY conceived the project and provided leadership. Y Yan performed experiments, analyzed data, and prepared figures. MJ performed computational analysis. SY, Y Yan, Jun Wang, FL, DZ, RC, QZ, TW, ZY, LL, WL, RL, WS, WH, WX, ZH, MH, Jianxiang Wang, and TC managed patients and collected samples. S Bhattacharya performed CLUMPS analysis. Y Wu, LY, ATP, and VNN provided CRISP/RCas9 screen data. Y Wang, Y Yan, and Y Yu performed clinical data annotation. QS, LC, and HW were responsible for pathologic diagnosis. AFH, CL, and AD contributed to clinical data association. EC, GC, EG, and S Bea contributed to the validation Barcelona cohort. All authors reviewed the manuscript and provided final approval for submission. The order of first authors was determined by the time that each joined the project.

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7. Vose JM. Mantle cell lymphoma: 2017 update on diagnosis, risk-stratification, and clinical manage-


