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American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

Biological and Clinical Determinants Shaping Heterogeneity in Mantle Cell Lymphoma

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Cristina López (IDIBAPS, Spain) Elisabeth Silkenstedt (University Hospital, Ludwig Maximilian University, Germany) Martin Dreyling (LMU Hospital, Germany) Silvia Beà (, Spain)

Abstract:
Mantle cell lymphoma (MCL) is an uncommon mature B cell lymphoma which presents a clinical spectrum ranging from indolent to aggressive disease, with challenges in disease management and prognostication. MCL is characterized by significant genomic instability, affecting various cellular processes including cell cycle regulation, cell survival, DNA damage response and telomere maintenance, NOTCH and NF-kB/BCR pathways and chromatin modification. Recent molecular and next-generation sequencing studies unveiled a broad genetic diversity among the two molecular subsets, conventional (cMCL) and leukemic non-nodal (nnMCL), which may partially explain their clinical heterogeneity. Some asymptomatic and genetically stable nnMCL not requiring treatment at diagnosis may eventually progress clinically. Overall, high proliferation of tumor cells, blastoid morphology, TP53 and/or CDKN2A/B inactivation, and a high genetic complexity influence treatment outcome in cases treated with standard regimens. Emerging targeted and immunotherapeutic strategies are promising in refractory or relapsed cases and a few genetic and non-genetic determinants of refractoriness have been reported. This review summarizes recent advances in MCL biology, focusing on molecular insights, prognostic markers, and novel therapeutic approaches.

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Cristina López[1,2,3,4], Elisabeth Silkenstedt[5], Martin Dreyling[5], Sílvia Beà[1,2,3,4]

[1]Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain;
[2]Hematopathology Section, Pathology Department, Hospital Clínic de Barcelona, Barcelona, Spain;
[3]Centro de Investigación Biomédica en Red de Cáncer, Madrid, Spain;
[4]Universitat de Barcelona, Spain;
[5]Department of Medicine III, LMU University Hospital, Munich, Germany

Corresponding author: Sílvia Beà, Molecular Pathology of Lymphoid Neoplasms, IDIBAPS, Hematopathology Section, Hospital Clinic of Barcelona, Villarroel 170, 08036-Barcelona, Spain. E-mail: sbea@clinic.cat; Phone +34932275719

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Abstract

Mantle cell lymphoma (MCL) is an uncommon mature B cell lymphoma which presents a clinical spectrum ranging from indolent to aggressive disease, with challenges in disease management and prognostication. MCL is characterized by significant genomic instability, affecting various cellular processes including cell cycle regulation, cell survival, DNA damage response and telomere maintenance, NOTCH and NF-kB/BCR pathways and chromatin modification. Recent molecular and next-generation sequencing studies unveiled a broad genetic diversity among the two molecular subsets, conventional (cMCL) and leukemic non-nodal (nnMCL), which may partially explain their clinical heterogeneity. Some asymptomatic and genetically stable nnMCL not requiring treatment at diagnosis may eventually progress clinically. Overall, high proliferation of tumor cells, blastoid morphology, *TP53* and/or *CDKN2A/B* inactivation, and a high genetic complexity influence treatment outcome in cases treated with standard regimens. Emerging targeted and immunotherapeutic strategies are promising in refractory or relapsed cases and a few genetic and non-genetic determinants of refractoriness have been reported. This review summarizes recent advances in MCL biology, focusing on molecular insights, prognostic markers, and novel therapeutic approaches.

Introduction

Mantle cell lymphoma (MCL) is an infrequent B cell neoplasm distinguished by the proliferation of mature B cells, commonly expressing CD5. It primarily affects elderly males, with a median age of ~65 years.[1] It is usually considered aggressive but it has a very heterogeneous clinical behavior. The translocation t(11;14)(q13;q32) and cyclin D1 overexpression are MCL pathognomonic features.[1,2] Two distinct molecular subtypes with different clinico-biological features are recognized, the most frequent conventional MCL (cMCL), and the leukemic non-nodal MCL (nnMCL).[3] There are four cytological variants, the most prevalent is the classic MCL with small to medium-sized cells, the less prevalent is the small cell variant, usually associated with low proliferation and mainly found in nnMCL, and the other two variants, the blastoid and pleomorphic, are characterized by larger cells, and are associated with more aggressive clinical features. Following the t(11;14), most MCL cells acquire a high number of secondary alterations affecting genes of several pathways, but only a small number impact prognosis and response to treatment.[3,4] In this review, we summarize recent data of MCL pathogenesis, including different molecular subtypes and variants, and prognostic/predictive factors under chemoimmunotherapy or current molecular targeted approaches.

Primary Genetic Events in MCL: Cyclin D1 as a key player

The primary genetic alteration of both molecular subtypes, cMCL and nnMCL, is the translocation t(11;14)(q13;q32), which juxtaposes the enhancer of the immunoglobulin heavy chain gene (*IGH*) near the *CCND1* oncogene. In virtually all MCL patients the translocation involves *IGH*::*CCND1,* but occasional variant translocations with immunoglobulin light chain genes have been reported, the t(2;11)(p11;q13) involving *IGK*::*CCND1* and the t(11;22)(q13;q11) involving *IGL*::*CCND1* (Fig 1A).[3] Fluorescence *in situ* hybridization (FISH) using a fusion *IGH*::*CCND1* probe is widely used for diagnostic, but *CCND1* break-apart probe is recommended to recognize these variant translocations. The molecular consequence of the rearrangement is a constitutive overexpression of cyclin D1 protein. The main mechanism generating the translocation is aberrant V(D)J rearrangement, which takes place in the bone marrow precursors at the pre-B stage. Nevertheless, five cases have been reported in which the primary

rearrangement is generated via aberrant class switch recombination (CSR) or somatic hypermutation (SHM), both processes considered to occur in the germinal center (GC).[5] No clinico-biological differences had been identified in these cases.

Cyclin D1-negative MCL have been recognized,[1] and in the largest reported series 39/52 (75%) cases have *CCND2* rearrangement with immunoglobulin genes (mainly *IGK* and *IGL*, and less frequently *IGH*) and were detectable by FISH using a *CCND2* break-apart probe. The remaining 13/52 (25%) carry cryptic rearrangements, four cases with *CCND2* and nine with *CCND3*, involving the insertion of the *IGK* or *IGL* small enhancer region (~27Kb). These rearrangements are undetectable by standard FISH approaches using break-apart probes (Fig 1A).[6,7] A few cryptic immunoglobulin rearrangements with *CCND1* have also been identified.[8]

Cyclin D1, although considered a weak oncogene on its own,[9] plays a pivotal role in cell-cycle regulation by facilitating the transition from the G1 to the S phase through the CDK4-mediated phosphorylation of Rb1. Alongside the t(11;14), aggressive MCL cases often exhibit other *CCND1* alterations, such as amplification of the translocated allele,[5] and mutations or deletions in the 3' untranslated region (UTR) of CCND1 mRNA[5,10,11] which generate a truncated protein without the 3'UTR regulatory region and microRNA target sites, with increased protein half-life. All these alterations contribute to further potentiate cyclin D1's oncogenic activity. *CCND1* mutations in the 5' region and exon 1 are common in nnMCL with *IGHV* mutated genes, suggesting their acquisition in the GC microenvironment by aberrant SHM,[12] and several *CCND1* coding mutations (E36K, Y44D or C47S) have been associated with increased Cyclin D1 protein levels in cell lines.[13] Moreover, cyclin D1 functions as a transcriptional regulator, influencing the expression of genes involved in cell-cycle progression, and contributes to the DNA damage response (DDR) pathway.[14]

Differential diagnosis

The translocation t(11;14)(q13;q32) and/or cyclin D1 overexpression have been identified in other B cell lymphomas usually acquired as a secondary event at progression or relapse.[15,16] High grade B cell lymphomas with *CCND1* rearrangement as secondary event usually harbor *MYC* and *BCL2* and/or *BCL6* rearrangements. Their immunophenotypic profile differs from MCL, they lack CD5 and SOX11 expression, and

their genomic landscape is different from MCL, and more similar to DLBCL, with *BCL2*, *MYC*, *CDKN2A*, *KRAS* and *TNFRSF14* mutations.[17]

MCL molecular subtypes

MCL comprises two molecular subtypes, cMCL and nnMCL.[1,3] A leukemic presentation in the absence of lymphadenopathies, no expression of the oncogenic transcription factor SOX11, presence of *IGHV* somatic mutations, and a low genomic complexity are clinical and biological features that differentiate nnMCL from cMCL. A gene expression signature identified in peripheral blood is able to distinguish these MCL subtypes and is based in the expression of 16 genes (L-MCL16).[18] The L-MCL16 signature is stable over time. SOX11 detection by immunohistochemistry is useful for MCL diagnosis since it is expressed in MCL, and also in the cyclin D1-negative MCL but is not expressed in other B cell lymphomas, with the exception of some Burkitt and precursor cell lymphomas/leukemias.[19,20] SOX11 oncogenic roles include alterations of several pathways and altogether seem to be responsible for the more aggressive behavior of cMCL compared to nnMCL.[3,21] The primary cause of SOX11 expression is poorly understood and is related to epigenetic alterations.[22,23] cMCL is characterized by low or no mutations in *IGHV*, while nnMCL has *IGHV* mutations, consistent with a different cell of origin, a similar scenario as in chronic lymphocytic leukemia (CLL). Although a clear cut-off is difficult to define, a 97% identity with germline can predict survival.[24] *IGHV3-21* and *IGHV4-34* are the most predominant families, and MCL with an *IGHV3-21* usage almost exclusively share the same light chain (*IGLV3-19*), different copy number alterations and have better prognosis, favoring the hypothesis that *IGHV3-21* tumors may represent a distinct MCL subtype and that there is a possible role for antigens in MCL development.[25–27] In the largest MCL study, *IGHV3-21* was the most frequently rearranged gene (133/807, 17%) and was found mainly in cases with unmutated *IGHV* (97% of cases with unmutated *IGHV*). However, in this study there was no information regarding SOX11 or cMCL/nnMCL subtypes.[26] The association of *IGHV* status and *IGHV3-21* expression was also confirmed in another study, with 13 cases *IGHV3-21* and unmutated *IGHV* versus 3 with mutated *IGHV*.[24] Moreover, by WGS, we have found that MCL with *IGHV3-21* are more frequent in the cMCL subset (6/44, 14%) than in nnMCL (1/17, 6%).[5] The association of MCL *IGHV3-21* with

unmutated *IGHV* genes and better survival is interesting and further reinforces the hypothesis that MCL with *IGHV3-21* may represent a distinct MCL subset among cMCL.

Similar to CLL, another layer of information supporting the two different MCL molecular subtypes came from DNA methylation studies.[5,23] The two groups identified also reflect different cellular origin with Cluster 1 MCL, the most frequent, characterized by an imprint of B cells GC-unexperienced, with few or no *IGHV* mutations, expressing SOX11 and corresponding to cMCL; and Cluster 2 defined by an imprint of B cells that have experienced the GC and high levels of *IGHV* SHM, no expression of SOX11 and corresponding to nnMCL.[23] This study also shows that all MCL (irrespective of the subtype) show a DNA methylation profile similar to antigen-experienced cells.

Secondary Genetic Events in MCL

MCL is the lymphoma entity with the highest degree of genetic instability, only comparable to multiple myeloma.[28,29] cMCL showed very frequent complex karyotypes, whereas nnMCL at diagnosis present usually with simple karyotypes with only the t(11;14), and unexpectedly, a subset of cases also had 17p loss, frequently as isochromosome 17q.[30,31] Subsequent studies using copy number arrays and whole-genome sequencing (WGS) showed that >90% cMCL and especially all blastoid variants, display highly altered genomes, whereas nnMCL at diagnosis are genetically very stable (Fig 1B, Table 1).[5,32–34] We performed a review of copy number alterations (CNA) in diagnostic samples of two large studies.[5,34] (Fig 2A). All alterations are common in cMCL and very low or absent in nnMCL, except for deletion of 17p (*TP53)* which is more frequent in nnMCL (30%), whereas virtually exclusive alterations of cMCL are losses at 13q33-q34 (42%), 1p22 (41%), 11q22-q23 (36%), 6q (34%), 13q14 (*RB1*) (31%), 9p21 (*CDKN2A/B*) (25%), 9q22q31 (24%), 10p15-p13 (20%), and gains at 3q25-q29 (49%), 18q21-q22 (*BCL2*) (19%), and 12q13 (*CDK4*) (17%) (Fig 1B and Fig 2B). This profile of alterations, resulting from multiple intra- and interchromosomal translocations is highly specific for MCL, including the Cyclin D1-negative subset[6] and different from other B-cell lymphomas.

WGS studies unveiled that several genomic phenomena as enhancer hijacking, chromoplexy, chromothripsis and breakage-fusion-bridge cycles are relatively frequent

in cMCL compared to nnMCL and could be responsible for the activation of several oncogenes, i.e. *BMI1* at 10p12*, MIR17HG* at 13q31*, TERT* at 5p15*,* and *MYC* at 8q24. Additionally, several non-recurrent structural variants affect the regions of driver genes (Fig 1B).[5] *MYC* rearrangements with *IGH* and non-*IG* partners have been identified only occasionally in MCL, especially in pleomorphic variants.[5,35] Other previously undetected alterations identified by WGS are the non-coding mutations in the *TERT* promoter.[5] WGS studies also confirmed the high frequency of mono- and biallelic deletions of 9p21.3 locus, including always the *CDKN2A* tumor suppressor gene, and very frequently (>90%) with co-deletion of the neighboring genes *CDKN2B* and *MTAP,* which have essential roles in cell cycle/*TP53* regulation and the methionine salvage pathway, respectively. *CDKN2A* deletions are especially enriched in blastoid variants (60-92% of cases).[5,36]

In GC-derived mature B-cell lymphomas there is a propensity for aSHM and CSR potentially related to the increased genomic instability, however, in MCL only the nnMCL subtype and a minority of cMCL with *IGHV* mutations have GC-experience and aSHM/CSR, and the only known off-target aSHM gene is *CCND1*. The relationship of *CCND1* with genomic instability is still unclear. Transgenic mice with overexpression of Cyclin D1 do not develop tumors or lymphomas,[9] but when Cyclin D1 is expressed constitutively in the nucleus they develop lymphomas which overexpress *MYC* and have alterations of *TP53* or *BCL2*, two relevant genes for cell survival/apoptosis.[37] One hypothesis to explain the high genomic instability cMCL is the high prevalence of *ATM* and *TP53* alterations. In fact, MCL are the tumors with the highest frequency of *ATM* inactivation (64% of cMCL have mutation and/or deletion), and *ATM* gene is critically involved in DNA damage response and maintenance of genome integrity. *ATM* alterations in cMCL are associated to increased chromosomal instability (8 CNA vs 3 CNA).[38] Although not frequent, the downregulation of *CHEK2* and *CHEK1*, also involved in signal transduction of DNA damage response, may represent additional mechanisms leading to chromosomal instability.[39] Additional evidence is that an animal model with early B-cell-specific *ATM*-deficiency synergizes with ectopic Cyclin D1 expression and promote development of tumors morphologically similar to human MCL.[40] This suggests that the combination of high Cyclin D1 and low ATM levels accelerate and increase the incidence of pre-GC lymphomas. Furthermore, these murine lymphomas

have focal deletions of *TP53, CDKN2A, KMT2D,* and *RB1* genes (genes frequently altered in human MCL). Besides *ATM*, cMCL also harbor *TP53* alterations, which mediate several processes, including DNA damage response pathways. Nevertheless, *TP53* is not exclusively of cMCL, since nnMCL also have frequent *TP53* alterations but generally have few or no genomic alterations. Thus, it is tempting to speculate that *SOX11* expression (found in cMCL but not in nnMCL), which is a potent oncogene with multiple and diverse functions, may also contribute to the genetic instability characteristic of cMCL, although more research will be needed to elucidate this.

Different mutational profile in cMCL, nnMCL and blastoid subsets

The clinical heterogeneity observed in MCL patients could be explained, in part, by the different distribution of genetic alterations in both molecular subsets (Table 1). nnMCL have low number of driver genes per case (median <2/case) whereas cMCL accumulate alterations in several driver genes (median 7/case) affecting multiple pathways simultaneously.[5] Virtually all cases (cMCL and nnMCL) had the initial *CCDN1* alteration and cell cycle deregulation, and additional inactivation of cell cycle inhibitors, *CDKN2A/B, RB1* as well as *MIR17HG, CDK4* and *BMI1* oncogene amplifications. Besides cell cycle, the most altered pathway is DDR with very frequent and early *ATM* inactivation. *TP53*, with multiple roles, especially in cell cycle and DDR, is also frequently altered in MCL. The inactivation of *ATM* and *TP53* genes is frequently biallelic, usually by mutation and deletion.[5,34,41] Recent WGS, WES and targeted approaches allowed the identification of new alterations in cell cycle genes (*SAMHD1, CDKN1B*), and alterations in new pathways such as telomere maintenance (*TERT*), chromatin remodelers (*KMT2D/C, NSD2, SMARCA4,* and *SP140*), NF-kB signaling pathways (*BIRC3, CARD11, TRAF2,* and *TLR2*), NOTCH pathway (*NOTCH1/2*), and other genes (*HNRNPH1* and *UBR5*).[5,33,34,42–49] In 42 blastoid MCL analyzed by WES, mutations of *NOTCH2*, *NOTCH3* and *UBR5* were exclusively found, whereas *NOTCH1* mutations were enriched in blastoid compared to non-blastoid forms.[33]

We performed a systematic review of 11 recent WGS and WES studies of MCL[5,33,34,42–49] at diagnosis including a total of 432 cases and considering only single nucleotide variants (SNV) and insertions and deletions (indels). The most frequently altered (>13%) genes were *ATM*, *TP53*, *CCND1*, *KMT2D*, and *NSD2* (Fig 2C, Table 1). The

distribution of mutations varied significantly among MCL subtypes, with nnMCL characterized by *CCND1* mutations (mainly due to aSHM) found in 85% of cases and *TP53* in 25%, whereas the most recurrently mutated genes in cMCL include *ATM*, *KMT2D*, *NSD2*, and *TP53* (Fig 2D).

A recent study[34] using WES coupled with RNAseq identified four distinct MCL clusters: C1 has *IGHV* mutations, *CCND1* aSHM, and active B cell receptor (BCR) signaling, C2 is enriched with *ATM* alterations and upregulation of NF-κB and DDR pathways, C3 with *SP140, NOTCH1*, and *NSD2* mutations and downregulation of BCR signaling and *MYC* targets, and C4 with *TP53, RB1, CDKN2A/B* inactivation, active *MYC* pathway, and high proliferation. Besides the nnMCL subtype (cluster C1) with well-known different clinico-biological features, the other three clusters (C2, C3, and C4) may represent cMCL with different underlying biology, which may impact prognostic and may help personalizing therapy. C2 is enriched in *ATM* alterations, very frequent and early alterations in MCL but with no prognostic impact, despite its relationship with genome instability; in contrast C3 and C4, are enriched in multiple and concomitant cell cycle defects together with MYC deregulation, which will make the cells more difficult to eradicate. The differences of BCR functionality observed among the four clusters may also be important for the response to different targeted therapies.

Biological prognostic factors

The cMCL molecular subset exhibits a more aggressive clinical course, characterized by shorter overall survival (OS) and a significantly reduced time to treatment when compared to nnMCL. This observation has been consistently demonstrated across various studies employing diverse methodologies for stratification, including SOX11 immunohistochemistry and gene expression analyses such as microarrays, RNA sequencing (RNAseq), quantitative PCR, or the Nanostring signature L-MCL16.[5,18,34] Other measures of genomic complexity such as complex karyotype predict strongly for inferior OS and poor response to therapy,[50,51] and also increased complexity by microarray[18] or WGS and also an increased number of driver genes/regions.[5,33,34] The genomic complexity in clinical practice can be assessed by karyotype (≥3 alterations),[50] SNP- or CGH-array (≥6 alterations),[18] or WGS (>7 alterations).[5] Similar to CLL, the

genomic complexity in MCL has independent prognostic value over *TP53* alterations.[5,33,34] The presence of chromotripsis, chromoplexia and breakage-fusion-bridge detected by WGS is frequent in cMCL and enriched in blastoid forms and is associated with poor outcome.[5] The patients with blastoid histology, high proliferation levels (Ki-67≥50%) and a particular mutational profile (*CCND1, NOTCH1, TP53, SPEN, SMARCA4, KMT2C, RANBP2,* and *NOTCH2*) had very dismal outcomes.[33] The four clusters defined by WES/RNAseq present also distinct outcomes, with C1 having good prognosis, C2 and C3 intermediate, and C4 very dismal prognosis.[34]

Regarding individual genes, the key prognostic genetic alterations are *TP53* (mutations and deletions) detected in both MCL subtypes and *CDKN2A/B* deletions detected mainly in cMCL. Both alterations are especially enriched in blastoid and highly proliferative cases.[5,33] Simultaneous alterations of *TP53* and *CDKN2A/B* seem to have an additive prognostic effect and a dismal outcome despite the high-dose cytarabine received.[52] *TP53* mutations are associated with inferior OS and time to treatment both in cMCL and nnMCL subtypes.[32,53,54] Whether only *TP53* mutations or also *TP53* deletions without mutation are associated with the worse outcomes, is still unclear.[34,53] *CCND1* 3' UTR truncations are associated with aggressive behavior,[10] and several other genes like *KMT2D, NOTCH1, NOTCH2, SMARCA4*, *TRAF2,* and *SP140* and the recently described *HNRNPH1.*[5,34,43,54,55] *MYC* gains have also been associated with worse outcomes,[5,56] whereas others report that *MYC* translocations, rather than gains are independent MCL prognostic factor.[57]

In two WES studies with 11[34] and 25[42] patients with MCL samples before and after standard chemoimmunotherapy the authors noted a very high frequency of acquired *CDKN2A* deletions, *TP53* mutations/deletions, and less frequently other high-risk genetic alterations such as *KMT2D, NOTCH1, NOTCH2, SMARCA4*, and *SP140.* The resistant MCL clones detected at relapse were already present at diagnosis and were selected by therapy.[42]

Clinical prognostic factors

A prognostic score that has been confirmed in numerous series, the MIPI (MCL International Prognostic Index), was established implementing four independent prognostic factors: age, performance status, lactate dehydrogenase, and leukocyte

count.[58] To allow a simplified calculation, a s-MIPI has been defined. While these scores have been confirmed in numerous studies, it became obvious that patient age may strongly influence them, and therefore they are not appropriate to guide individual treatment strategies. Alternatively, the most important biological prognostic markers independent of clinical features are the cell proliferation rate as measured by Ki-67 expression and alterations of p53 and may even allow a more individualized risk-based therapeutic approach.[59] Accordingly, a combined score (MIPI-c) integrating clinical and biological features has been subsequently established. In fact, immunohistochemical determination of p53 expression has been prospectively confirmed as a reliable prognostic marker of p53 mutation analysis,[60] and this combined clinico-biological score, combining MIPI with p53 high expression and Ki-67>30%, together with blastoid morphology, were recently reported to define high-risk cases with significantly shorter failure-free and OS,[61] and may identify patients who may benefit from more experimental therapeutic approaches.

Furthermore, a cell proliferation gene signature (MCL35) that distinguishes patient subsets differing by more than 5 years in median survival has been identified[62] and validated in diagnostic material from patients treated in the prospective MCL Younger (NCT00209222) and MCL Elderly (NCT00209209) trials of the European MCL Network.[63,64]

Early relapses in between the first two years (POD24) are associated with a significantly worse outcome.[65–68] Most interestingly, patients with high MIPI-c and no POD24 event had the same prolonged OS as compared to patients with low or intermediate MIPI-c and no early progression (median OS not reached).[65]

Concerning the prognostic impact of minimal residual disease (MRD) status, several studies provided evidence of its strong prognostic potential predicting improved subsequent PFS for MRD-negative patients at the end of induction and before high-dose consolidation.[32,69] Furthermore, lack of molecular remission after end of currently recommended standard treatment was shown to be strongly predictive for early clinical relapse within 1-2 years.[69,70] However, use of MRD analysis in clinical routine is still limited and the impact of MRD monitoring in the context of the new targeted treatments remains unclear.

Molecular targeted therapies

Covalent BTK inhibitors

Constitutive activation of B cell receptor (BCR) signaling is a characteristic pathogenic feature of malignant B cells, including MCL. Targeting the BCR pathway with the covalent BTK inhibitor (BTKi) ibrutinib resulted in remarkable response rates leading to its approval in relapsed MCL.[71] A pooled analysis (n=370) of the results of three different trials testing ibrutinib as monotherapy revealed overall response rate (ORR) of 66% with median PFS and OS of 13 and 25 months, respectively, which was even higher when patients were treated with ibrutinib at first relapse compared to treatment at later relapses (ORR: 1 prior line 78% vs >1 prior line 67%, median PFS: 1 prior line 25 months vs >1 prior line 10 months).[72] Based on these results and the results from a randomized phase III trial comparing ibrutinib to temsirolimus monotherapy in relapsed MCL, reporting significant improvement in PFS for patients treated with ibrutinib versus temsirolimus (15 months vs 6 months),[73] treatment with BTKi monotherapy was implemented as standard of care for patients at first relapse.

Second generation BTKi acalabrutinib was approved in October 2017 by the FDA for patients with relapsed/refractory (R/R) MCL who had received at least one prior therapy based on promising results, especially regarding tolerability, of an open-label, multicenter, single-arm phase II study of acalabrutinib.[74] Final analysis of this trial including 124 patients has reported ORR and CR rates of 82% and 48% and a median PFS of 22 months.[75] Another next-generation BTKi zanubrutinib is a highly potent, selective, bioavailable, and irreversible BTKi with maximized BTK occupancy. It was approved in 2019 in the US and China for the treatment of patients with R/R MCL based on results from a phase II study enrolling 86 Chinese patients with R/R MCL reporting an ORR of 84% and a CR rate of 78%. Median PFS was 33 months, and in patients with mutated *TP53*, median PFS was 15 months.[76,77]

Treatment alternatives for patients failing covalent BTKi

Non-covalent BTKi

Pirtobrutinib, a highly selective, non-covalent BTKi, inhibits both wild type and C481-mutant BTK. This compound is very well tolerated with only 6% of patients stopping the continuous treatment due to side effects. In 90 evaluable patients with MCL,

previously treated with covalent BTKi, the ORR observed within the BRUIN phase I/II trial was 58% including 20% complete responses (CR).[78] The currently recruiting phase III BRUIN MCL-321 trial compares pirtobrutinib to investigator's choice of any approved covalent BTKi in relapsed MCL.[79]

Bcl-2 inhibitors

A monotherapy with the bcl2-inhibitor venetoclax might be a promising alternative, as a phase I trial showed response rates of 75% in patients with relapsed MCL.[80] Recently, analysis of a retrospective data collection of 20 BTKi resistant patients treated with venetoclax in a UK-wide compassionate use program showed an ORR of 53%; however, the median PFS was only 3 months.[81] A multicenter analysis of R/R MCL patients with high-risk disease features and a median of 3 prior treatments including BTKis in 91% reported similar results for venetoclax alone or in combination with an ORR of 40% and a median PFS of 3.7 months.[82] Another study evaluated the outcome of 24 multiple relapsed patients who received venetoclax-based therapies. Among them, 67% had progressed on BTKi, ORR was 50% and PFS was 8 months.[83]

Immunemodulators

Several studies confirmed a benefit of the orally available immunemodulatory drug lenalidomide in relapsed MCL, with response rates of 35-50%.[84–86] In a randomized phase II trial, this approach was superior to monochemotherapy (response rate 46% vs. 23%).[87] However, in BTKi-failures response rates at least of lenalidomide alone are only modest.[88]

CAR-T cells

The autologous CD19 CAR T-cell construct (CD28 costimulatory domain) brexucabtagene autoleucel (Tecartus, KTE-X19) was approved for R/R MCL after previous BTKi treatment, based on the results of the ZUMA-2 study.[89] After a median follow-up of 36 months, treatment with brexucabtagene autoleucel was reported to induce a durable ORR of 91% and a PFS of 26 months in patients refractory or intolerant towards BTKi treatment.[90] In a subgroup analysis of patients having relapsed in between 24 months (POD24), median PFS was substantially shorter compared to the non-POD24 group (11 vs 29 months), although ORR and CR rates were equivalent in both cohorts.[90] Another CD19-directed CAR T-cell product (lisocabtagene maraleucel)

with 4-1BB as a co-stimulatory domain was evaluated in the phase II study TRANSCEND NHL 001 (NCT02631044) for R/R MCL. Primary analysis of 83 patients evaluated reported an ORR of 83% and a CR rate of 72%. Median duration of response was 16 months and PFS was 15 months. Most common grade ≥3 treatment-emergent adverse events were neutropenia (56%), anemia (38%), and thrombocytopenia (25%). Cytokine release syndrome was reported in 61% of patients.[91] As preclinical data suggest a synergistic effect of ibrutinib on apheresis product fitness, CAR-T expansion and toxicity, the phase II TARMAC study investigated the combination of time-limited ibrutinib with tisagenlecleucel reporting a 12-months PFS of 75%.[92] Overall, results achieved with CAR T-cell treatment are very promising with long-lasting remissions even after BTKi-treatment failure. Therefore, CAR T-cell therapy should, to date, be first choice for the group of high-risk patients refractory to BTKi.

Resistance mechanisms to targeted therapies

Ibrutinib

Nearly one-third of patients receiving ibrutinib have been reported to develop primary intrinsic resistance and clinical outcome of these patients is usually poor with OS rates between 6 and 8 months.[93,94] Molecular mechanisms underlying resistance to ibrutinib have been extensively studied: sustained activation of the PI3K-AKT pathway or other genetic alterations providing an alternative activation of BCR signaling seem to play an important role.[95] Mutations in *CARD11*[47] and *CCND1*[13] were reported to mediate BTKi resistance and upregulated BIRC5/survivin expression due to 17q gain was recently reported to contribute to resistancy.[96] Sequencing of 165 samples from patients with MCL identified recurrent mutations in *TRAF2* or *BIRC3* in 15% of patients resistant to ibrutinib treatment.[97] In line with this, it was reported that MCL cell lines resistant to BTKi treatment displayed activation of the alternative NF-κB pathway, associated with genetic lesions in this pathway.[98] Another report confirmed muatations in NF-kB signaling pathways, both canonical (e.g., *TNFAIP3/A20*) and noncanonical (e.g., *BIRC2*) to be associated with ibrutinib resistance.[99] The well-described point mutation within the kinase domain of BTK at cysteine 481 position (BTK^{C481S}) and gain of function mutations in the *PLCG2* gene have been reported to confer secondary resistance to

BTKi. However, unlike in CLL, BTK^{C481S} mutation is infrequent in MCL and *PLCG2* gene mutations have not been observed.[100]

Besides the genetic changes underlying ibrutinib resistance, metabolic reprogramming to oxidative phosphorylation (OXPHOS) and glutaminolysis was shown to be associated with therapeutic resistance to ibrutinib in MCL.[101–103] RNA-seq analysis of surviving cells after ibrutinib treatment of a sensitive MCL cell line also revealed increased activity of oxidative phosphorylation and upregulated CD52 expression in the resistant cells.[104]

As covalent BTKi will be prospectively applied as part of first-line therapy, the frequency of BTKi resistant patients will rise and identification of optimal treatment alternatives for these patients are highly warranted.

Venetoclax

Venetoclax proved to be an effective alternative treatment strategy in MCL. However, many initial responders will ultimately progress and deciphering the underlying mechanisms of venetoclax resistance is of great importance. WES was performed on seven patients, including samples prior to initiation of venetoclax and after progression on venetoclax. Alterations in *SMARCA4* and *BCL2* were only observed after progression. Interestingly, clonal evolution of novel *SMARCA4* and *KMT2C/D* mutations was demonstrated in two patients with serial samples.[83]

Molecular profiling of 24 tumor samples of patients treated within the AIM trial evaluating the combination of ibrutinib and venetoclax detected chromosome 9p21.1–p24.3 (*CDKN2A/B*) loss and/or mutations in components of the SWI–SNF chromatin-remodeling complex in all patients with primary resistance and in two-thirds of patients with relapsed disease.[45]

Conclusions

Recent studies on MCL have provided novel insights into its pathogenesis, including: i) Identification of two MCL subsets with distinct cell-of-origin and clinical behavior; ii) Discovery of variant primary translocations beyond the hallmark t(11;14); iii) Recognition of a variety of mutated and altered driver genes and regions distributed differently in cMCL and nnMCL subtypes, contributing to their distinct clinical behavior;

iv) Exploration of combined molecular and clinical prognostic factors associated with poor outcomes; v) Evaluation of the efficacy of molecular targeted therapies, even in refractory MCL cases; and vi) Initial investigations into genetic and non-genetic resistance mechanisms to targeted therapies. Overall, these findings represent significant advancements in our understanding of MCL pathogenesis, with potential implications for improved clinical management and patient outcomes. Despite this huge progress, other areas related to the complex MCL biology have yet to be explored and warrant further investigation. These areas include the MCL tumor microenvironment, longitudinal clonal evolution, molecular heterogeneity across different compartments (i.e. bone marrow, peripheral blood, lymph nodes, gastrointestinal tract, plasma…) and metabolic reprogramming.

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Authorship contributions

Contribution: C.L., E.S., M.D. and S.B. wrote the review and prepared the table and figures.

Disclosure of conflicts of interest

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Table 1. Secondary genetic alterations in both molecular subsets of MCL identified by whole-exome/genome sequencing.

Feature	Gene	MCL overall (%)	cMCL (%)	nnMCL (%)	*P*-value
DNA damage response and telomere maintenance	*ATM*	38	58	0	<0.001*
	del ATM	27	36	0	<0.001*
	del TP53	23	14	30	0.171
	TP53	22	15	25	0.328
	TERT[#]	15	10	25	0.567
	SAMHD1	3	8	0	0.322
Cell cycle and survival	del 9p21 (*CDKN2A/B*)	31	25	0	0.009*
	del 13q14 *(RB1)*	25	31	0	0.004*
	3'UTR *CCND1*	18	20	15	NA
	CCND1	16	8	85	<0.001*
	gain 8q24 *(MYC)*	16	12	5	0.672
	gain 12q13 *(CDK4)*	13	17	0	0.058
	gain 18q2 *(BCL2)*	12	19	0	0.057
	gain *CCND1*	11	14	0	0.192
	gain 13q31 *(MIR17HG)*	10	12	0	0.182
	gain 10p12 (*BMI1*)	9	12	0	0.182
Chromatin remodelers	*KMT2D*	15	19	0	0.057
	NSD2	13	17	0	0.058
	SMARCA4	8	12	0	0.182
	SP140	6	8	0	0.322
	KMT2C	6	0	0	NA
BCR/NF-kB/TLR	*BIRC3*	5	5	5	1
	CARD11	1	5	5	1
	TRAF2	3	2	0	0.062
NOTCH signalling	*NOTCH1*	7	5	5	1
	NOTCH2	6	5	0	0.567
Other	*UBR5*	8	7	0	0.567
	SYNE1	3	5	10	0.596
	DLC1	3	2	0	0.062
	HNRNPH1	2	7	5	1
	MEF2B	2	7	0	0.567
	S1PR1	2	5	0	0.322
	BCOR	1	7	0	0.567
CNA	gain 3q25-q29	40	49	10	0.003*
	del 13q33-q34	30	42	5	0.002*
	del 1p22	30	10	0	<0.001*
	del 6q	20	34	5	0.017*
	del 9q22-q31	18	24	0	0.009*
	del 8p	17	10	5	0.672
	10p15-p13 del	15	20	0	0.031
	del 15q11-q13	11	12	0	0.182
	gain 15q21-q25	10	10	5	0.672
	gain 7p	9	20	0	0.031

NA: not analyzed

The number of cases used for SNV and indels (mutations) analyses is 432, based on references Karolová *et al*., 2023 Am J Hematol[42]; Yi *et al*., 2022 J Clin Invest.[34]; Nadeu *et al*., 2020 Blood[5]; Pararjalingam *et al*., 2020 Blood[43]; Jeong *et al*., 2020 Sci Rep[44]; Jain *et al*., 2020 Blood Adv[33]; Agarwal *et al*., 2019 Nat Med.[45]; Yang *et al*., 2018 Cancer Gene Ther[46]; Wu *et al*., 2016 Oncotarget[47]; Zhang *et al*., 2014 Blood[48]; Khodadoust *et al*., 2017 Nature[49]. The number of cases used for copy number alterations (CNA) is 202, based on references Yi *et al*., 2022 J Clin Invest.[34]; Nadeu *et al*., 2020 Blood[5], and the number of cases used for frequencies among cMCL and nnMCL subtypes is 59 and 20, respectively, based on Nadeu *et al*., 2020 Blood[5], after eliminating 3 post-treatment samples. Fisher Exact test was used to compare the genomic alterations within MCL subgroups. *P*-value ≤0.05 was considered significant and highlighted with asterisk. Mutations in genes of late replicating regions[105] and *IGH IGK* and *IGK* loci have been excluded.

[#]*TERT* alterations refer to promoter mutations, structural variants detected by WGS or FISH and gains or amplifications reported in Nadeu *et al*., 2020 Blood[5].

Figure legends

Figure 1. Primary and secondary MCL chromosomal alterations. (A) The left panel is a schematic representation of the *CCND1, CCND2* and *CCND3* genes and their rearrangements with *IGH, IGK* or *IGL* genes. The size of the arrows represents their frequency. The right panel indicates the molecular subtype according to *CCND* gene rearrangement and to cMCL or nnMCL. Note that still there are no reported MCL cases with *CCND2* or *CCND3* rearrangement of the nnMCL subset. (B) Circular representation of copy number alterations (CNA) and structural variants (SV) in MCL with WGS and pre-treatment (45 cMCL and 16nnMCL).[5] In the inner side, the primary SV, t(11;14) found in all cases, was represented by a thicker black line, whereas other alterations affecting (or very near) driver genes are represented by black lines (translocations) or grey lines (insertions); in the outer side, the CNA are colored (gains in blue and losses in red). Driver genes or regions frequently targeted by SV (in addition to gains and losses) are indicated. Note that no recurrent rearrangements were found.

Figure 2. Recurrent genomic MCL alterations identified using WGS/WES analysis. The frequencies displayed correspond to the studies detailed in the Table 1 legend. Panel (A) illustrates the copy number alterations (CNA) observed (overall frequency ≥2%) in 202 MCL patients. The frequency of genomic alterations highlighted with an * is based only in the Nadeu *et al.,* 2020[5] publication. (B) CNA identified in Nadeu *et al.,* 2020[5] categorized by MCL subtype. (C) single nucleotide variants and indels (overall frequency ≥2%) identified in 432 patients MCL. (D) single nucleotide variants and indels, identified in Nadeu *et al.,* 2020[5] and categorized by MCL subtype. Variants with overall frequency ≥ 2% in at least one of the MCL subtype are displayed.

Figure 1-2

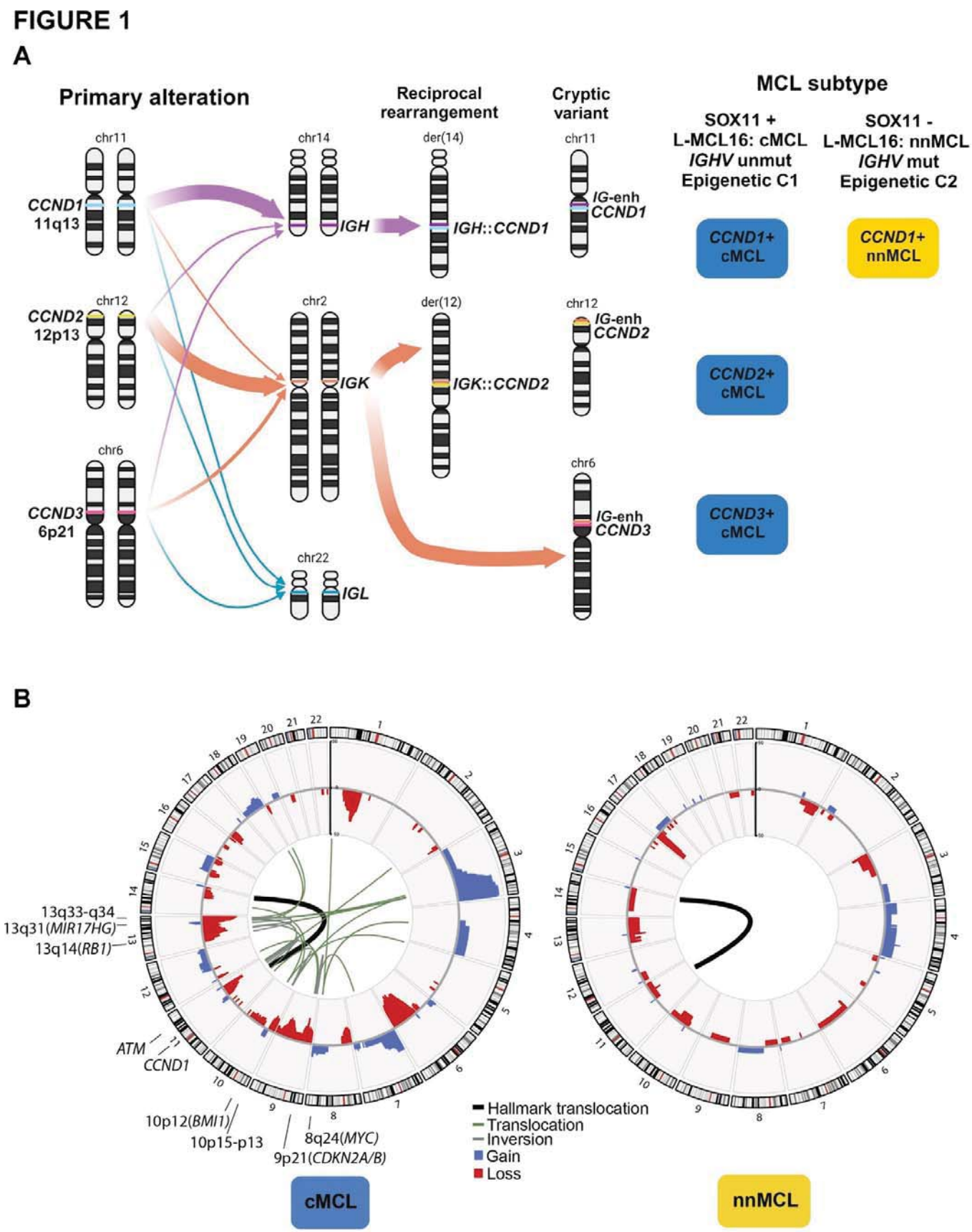

FIGURE 2