CANCER

Interrogating B cell signaling pathways: A quest for novel therapies for mantle cell lymphoma

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Mantle cell lymphoma (MCL) is an aggressive B cell lymphoma that is largely chemoresistant. Ibrutinib, a drug that inhibits Bruton's tyrosine kinase (BTK), has improved the overall survival of patients with MCL; however, resistance to ibrutinib has emerged as a decisive, negative factor in the prognosis of MCL. Adopting a more patient-centric therapeutic approach that incorporates applied genomics and interrogation of B cell signaling pathways may offer an alternative route to reach durable remission in patients with MCL. Although targeting genetic variants in MCL is not yet feasible in the clinical setting, the identification and targeting of increasingly active B cell signaling pathways may be a viable therapeutic strategy that may improve patient outcomes. Genome-editing tools and sequencing platforms could play dominant roles in patient-centric approaches of treatment in the future, potentially improving clinical outcomes for patients with MCL.

INTRODUCTION

Mantle cell lymphoma (MCL) is a rare, aggressive, and chemoresistant form of non-Hodgkin lymphoma (NHL) that arises from a patient's B cells (*1*). MCL also metastasize to other lymphoid organs and the central nervous system (*2*). Despite the general improvement in overall response, the prognosis of MCL is still one of the worst among NHL types (*3*). With the inclusion of ibrutinib, a first-in-class oral Bruton's tyrosine kinase (BTK) inhibitor, the overall response among relapsed-refractory patients reached 68% (*4*). Unfortunately, patients on ibrutinib inevitably develop drug resistance; on average, these patients live for about 8 months after progression and respond very poorly to salvage therapies, which consist of rituximab-containing chemoimmunotherapies (*5*). One of the mechanisms underlying ibrutinib resistance, seen in another form of cancer, chronic lymphocytic leukemia (CLL), is through a mutation in the ibrutinib binding site, BTK^{C481S} (*6*). Resistance has also been reported to other active agents, most notably the U.S. Food and Drug Administration (FDA)–approved proteasome inhibitor bortezomib (*7*), further challenging the possibility of achieving durable remission in MCL.

In our quest to develop more effective and personalized therapies for patients with MCL, we envision moving away from the traditional drug-centric paradigm. In the drug-centric approach, two to three agents are usually tested in combination, and their efficacy in minimizing lymphoma progression is evaluated in clinical trials. But with the high number of targeted agents currently available and with even more in the pipeline, testing all of the promising combinations in clinical trials is impossible. To increase the likelihood of therapeutic success, patient-level genomics can be integrated into the clinical decision-making process for patients with MCL. Genome-editing tools can be used to identify target gene candidates and screen for drug resistance in tumor cells, specifying the pathways and molecular mechanisms that can be targeted in patients with MCL for therapeutic benefit. These tools have recently been implemented in a few clinical trials as therapeutic agents and have further potential in elucidating the molecular profiles of patient tumors. Incorporating a newer, patient-centric approach to treatment that uses the molecular profile of each patient's tumor may improve overall survival for patients with aggressive disease. To this end, we discuss the need for and the feasibility of a genomics-integrated therapeutic approach for MCL, focusing on interrogation of the B cell signaling pathways and their critical mutational drivers.

TRADITIONAL THERAPEUTIC APPROACH BASED ON TUMOR HISTOLOGY

For decades, the identification of histological features of cancer cells has been used to diagnose cancer. This approach as a sole method of diagnosis poses a problem as we start to better understand the magnitude of molecular aberrations in cancers. Genetic information cannot be recognized with histological diagnostic methods alone. However, next-generation sequencing, proteomics profiling, and pathway associations that could potentially identify actionable targets are not routinely performed to date. Inclusion of these genomic tools and interrogation of pathways may elevate the efficacy of diagnosis and treatment. Because of the unpredictability of cancer and the heterogeneity of MCL, chemotherapeutic outcomes have demonstrated variation and irregularity (*8*). Chemotherapy regimens, such as high-dose cytarabine with or without autologous stem cell transplant (ASCT), are effective with an overall survival of 12.7 years (*9*), but they come with the cost of high toxicity among young patients, including secondary malignancies. On the other hand, the regimen consisting of rituximab and hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD) is an active, aggressive frontline regimen with an impressive overall response (87% complete remission with an overall survival of 13.4 years) (*10*). In addition to Hyper-CVAD treatment, the rituximab, cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone or "R-CHOP" regimen (which alternates with the rituximab high-dose cytarabine with high-dose therapy consolidation) has also proven to be highly successful in targeting NHLs, including MCL (*11*). Despite the success of these approaches, it is an unfortunate reality that with time, MCL cells will acquire resistance to these agents, eventually leading to relapse. The dismal treatment outcomes in most patients with relapsed-refractory MCL necessitate that alternative strategies such as targeted therapies and genomics-based approaches need to be integrated into the clinical decision-making process.

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TARGETING GENETIC LESIONS IN MCL

The genomic landscape of most aggressive lymphomas, including the majority of MCL cases, shows tremendous variation (*12*, *13*). In MCL, frequent mutations in particular genes are reported in only a fraction of patients, thus making it difficult to target genetic lesions. We are still in the nascent stages of precision medicine in cancers, and matching a specific molecular abnormality to a therapeutic agent is especially difficult in MCL. As seen in the National Cancer Institute Molecular Analysis for Therapy Choice (MATCH) trial, it is difficult to enroll patients with lymphoma in a treatment arm, solely based on an "actionable mutation of interest." MATCH aimed to create precision medicine for a "basket" of different histological variety of cancers, including B cell lymphomas. The study was not able to match a patient with lymphoma to a treatment arm most likely because there are very few patients with aggressive B cell lymphomas who also have targetable genetic abnormalities. We should acknowledge that we currently do not have "actionable" genetic variants to target mutations in MCL. Targetable mutations may soon be identified in MCL through next-generation sequencing and/or other genomic tools; however, in the meantime, we should also explore other possibilities to affect MCL patient outcomes. One such possibility is to use newer technologies to uncover target genes for MCL proliferation and therapeutic resistance. For example, newer genomic experiments can be conducted in an FDA-mandated and compliant laboratory, signifying that the results can be used to formulate or modify treatment decisions in a clinical trial setting. A 17-gene proliferation signature was developed for patients with MCL, and the study reported statistically significantly different overall survival, separated by the genetic signature (*14*). These results pave the way for prospective risk-adapted clinical trials with MCL-specific genetic targets. Our group has already developed a genetic signature for ibrutinib resistance in MCL using RNA sequencing. We intend to validate this signature using additional technologies and potentially use the signature to identify patients who are more likely to fall into relapse while on ibrutinib therapy and offer alternative therapeutic options.

Somatic mutations in MCL

Somatic mutations, which are nonheritable genetic variations, are particularly common in MCL cells (*12*, *13*, *15*, *16*). MCL somatic mutations are associated with the activation of various B cell pathways—a phenomenon that can be explored to identify therapeutic targets (*17*). A distinctive feature of MCL is the acquisition of a high degree of molecular aberrations in the tumor genome. These genomic lesions can result in mutant clones that have a survival advantage; they can evade therapeutic intervention and circumvent the apoptotic pathway through alternative pathway activation. In addition to the mutations, epigenetic modifications also alter MCL tumor clones (*18*). Advancements have been made in high-throughput and affordable sequencing, genome-editing technologies, DNA sequencing, and RNA sequencing; studies that have examined the mutational landscape through targeted sequencing appear to have identified recurring mutations with these methods. There are several genes, namely, *ATM*, *TP53*, *MLL2*, *TRAF2*, *CARD11*, and *BIRC3* (*12*, *13*, *15*–*17*), which are reported to be frequently mutated in primary MCL cells, with the *ATM* mutation found in nearly 50% of patients with MCL (*15*). Large-scale genomic studies are using the next-generation sequencing platform to describe the frequency of these mutations, mutational burden, copy number variations, and clinical importance of the somatic variants. These data could catapult the MCL therapeutic approach to a new era of targeted therapy and facilitate the transition from absolute dependence on a traditional histology-based approach to a more comprehensive patient-specific treatment strategy that includes the identification of mutations, pathway proteomics, and interrogation of impaired signaling pathways. This is a practical and systematic approach to make precision medicine a reality in the treatment of aggressive lymphomas.

Considerations for combining MCL genomics and B cell signaling

By combining applied genomics with the interrogation of the B cell signaling pathways necessary for B cell survival, clinical benefit can be maximized by curating therapies for individual patients.

Mutation-driven biomarkers can be quickly identified and used in clinical settings, as access to high-throughput screening increases. Information about molecular signatures may be used to define a patient's response to treatment (*19*) and even predict a prognosis. The efficacy and affordability of next-generation sequencing enable physicians to use genetic testing as a preliminary method in a systematic and consistent manner, instead of only when attempting to offer a diagnosis. After generation of a relevant gene panel, DNA sequencing of patient tumors can commence, and clinically relevant genetic variants can be identified. In addition to classifying somatic variants of MCL through targeted deep sequencing, potential treatment therapies can be assessed by targeting those variants with different agents in vitro. Implementation of newly developed robotics enables high-throughput drug screening to simultaneously assess the efficacy of a library of drugs. The newly established molecular profile can aid in ranking therapeutic agents based on their efficacy. In addition to targeted sequencing, there are several functional proteomics strategies, including protein microarrays, shotgun proteomics, multidimensional protein identification, and isotope-coded affinity tags, which can allow high-throughput screening of markers of specific B cell pathways (*20*).

INTERROGATING B CELL SIGNALING PATHWAYS

Somatic mutations often lead to the constitutive activation of signaling pathways downstream of the affected gene. For instance, a point mutation in the gene encoding BTK (causing the amino acid substitution C481S in the protein) leads to persistent activation of BTK-mediated signaling and activation of the AKT (also known as protein kinase B) circuitry (*6*, *21*). Similarly, mutations involving *NOTCH1* result in increased oncogenic activity of the NOTCH signaling pathway, which is associated with poor overall survival in MCL (*16*). Activation of the alternative NF-κB (nuclear factor κ light-chain enhancer of activated B cells) pathway is linked with mutations in *BIRC3* and *TRAF2*, and activation of the classical NF-κB pathway is associated with resistance to ibrutinib (*17*). Genomic instability should be viewed as an enabling feature for MCL therapy, especially given that B cell signaling pathways can be interrogated with newer molecules. There are multiple functional proteomics platforms that can identify B cell pathways in MCL with increased activity and underlying somatic mutations. For example, proteomics strategies such as *N*-glycoproteomic profiling, can now be used to classify lymphomas according to lineage, cell of origin, and the World Health Organization subtype. Genomic analysis also provides information on the relationship between glycoproteins and RNA expression (*22*), ultimately offering a better understanding of cancer pathogenesis. Interrogating

B cell pathways in the context of drug resistance in individual patients presents a more personalized approach for MCL treatment.

When B cells interact with antigens through their receptors, such as B cell receptors (BCRs), they produce antibodies through their mature effectors, plasma cells (*23*). In addition, antigen-independent "tonic signaling" of B cells is also mediated by the BCR, underscoring the importance of B cell signaling pathways in the development and regulation of B cells (*24*). The BCR and other coreceptors and pathways ensure normal development and the correct naïve repertoire of the B cells. As a result of the interconnectedness of the B cell signaling pathways, aberrations in one pathway can affect growth and proliferation mediated by another pathway. For example, in some MCL cases, the inhibition and inactivation of phosphatase and tensin homolog (PTEN) confers constitutive activation of AKT, which may promote chemoresistance (*25*). The PI3K (phosphatidylinositol 3-kinase)–AKT–mTOR (mammalian target of rapamycin) and NF-κB pathways are unusually active in the pathogenesis of MCL (*26*, *27*). Furthermore, there is evidence that MCL tumor cells can activate alternative pathways when classical signaling is quenched (*17*). Therefore, the identification and interrogation of rogue B cell signaling networks may identify novel therapeutic strategies that overcome drug resistance and produce durable remissions in patients. This research is facilitated by a number of drugs that target B cell signaling and are either currently available or under development (Table 1).

BCR signaling is vital to the survival, function, and proliferation of MCL cells. It is a complex network of signal transduction within and cross-talk between multiple pathways, generally—and most widely understood, thus far—being the NF-κB, PI3K-AKT-mTOR, and ERK (extracellular signal–regulated kinase) pathways (*23*, *27*, *28*). Thus, there are various opportunities for developing BCR signaling–targeted therapeutics to treat patients with MCL. Here, we highlight several of the current prospects.

Table 1. B cell pathways frequently disrupted and therapeutic agents that inhibit key pathway molecules. Major B cell signaling pathways and its common mode of disruptions are shown. Within each pathway, key molecules or processes can be inhibited by various therapeutic agents, either FDA-approved or under development.

Up-regulated pathways	Mode of disruption	Possible therapeutic agents	Current settings
BCR pathway	Somatic mutation	BTK inhibitors: ibrutinib and acalabrutinib	Ibrutinib: FDA-approved Acalabrutinib: FDA-approved (NCT02029443)
NF-κB pathway	Somatic mutation	Angiogenesis inhibitor: lenalidomide Proteasome inhibitor: velcade (bortezomib)	Lenalidomide: under FDA review Bortezomib: FDA-approved
Cell cycle control	Somatic mutation; epigenetic suppression	CDK4/CDK6 inhibitor: abemaciclib	Phase 3 (FDA-approved for breast cancer)
Apoptosis/p53 pathway	Somatic mutation	Bcl-2 inhibitor: venetoclax AKT/ERK inhibitor: ONC201 MDM2 inhibitors	Venetoclax: phase 2
DNA damage repair pathway	Somatic mutation: epigenetic suppression	Poly ADP-ribose polymerase inhibitor: olaparib	Phase 1
PI3K pathway	Somatic mutation	PI3K inhibitors: idelalisib, KA-2237, and TGR-1202	Idelalisib: phase 1
Oxidative phosphorylation (OXPHOS) pathway	Metabolic disruption	Mitochondrial complex I inhibitor: IACS-010759	Phase 1

Bruton's tyrosine kinase

Aberrant BTK activity is a major event in the malignant transformation and progression of B cell lymphoproliferative disorders (*29*, *30*). In MCL, ibrutinib has been successful in relapsed-refractory cases. This orally available, first-in-class BTK inhibitor binds to the active site of the enzyme at Cys^{481} and inhibits its autophosphorylation, thereby preventing downstream signaling activities (Fig. 1) (*31*, *32*). However, identification of a BTK mutation involving substitution of serine instead of cysteine (BTK^{C481S}) has rendered ibrutinib to be a reversible inhibitor by decreasing the binding affinity to BTK. This has shown to give way to ibrutinib-resistant cells, wherein persistent phosphorylation of BTK and increased calcium influx occur after drug exposure (*33*). Advani and colleagues (*34*) conducted a clinical trial of single-agent ibrutinib in relapsed-refractory NHL patients with an overall response (OR) of 54%; for MCL the OR was 78% (nine cases). Wang *et al.* (*4*) showed a 68% OR for single-agent ibrutinib among 111 patients with relapsed-refractory MCL, with an estimated median progression-free survival (PFS) of 13.9 months, which led to the FDA approval of ibrutinib for relapsed-refractory MCL. There are multitudes of effector molecules downstream of BTK that are targets for therapeutic interventions. Newer drugs such as second-generation BTK inhibitor acalabrutinib (*35*), ONO-4059 (*36*), and BGB-3111 (*37*) have targeted BTK successfully with strong potential clinical benefit and no dose-limiting toxicity. Monitoring whether drug resistance to these other agents develops through similar mutations and/or mechanisms, as observed with ibrutinib, will provide vital insight. The synergistic effects of BTK inhibitors in combination with other pathway inhibitors, such as PI3K-AKT, may be crucial to overcoming resistance to ibrutinib.

Bringing targeted agents or intentionally combined pathway inhibitors to the frontline is the next logical step to minimize toxicity and reduce secondary malignancies. In one study among relapsed-refractory patients, treatment with ibrutinib drove the MCL cells out of organs (known as a "compartment shift" phenomenon). Once the MCL cells were circulating in the bloodstream, the patients were treated with rituximab, an antibody to the B cell surface antigen CD20, leading to an 88% overall response in this patient population (*38*). By binding to B cells, rituximab was able to trigger natural killer cell–mediated, antibody-dependent cellular cytotoxicity via CD20 capping at the surface of B cells (*39*). This process occurred independent of antibody cross-linking or intercellular contact, thus minimizing any other malignancies. On the basis of the efficacy of

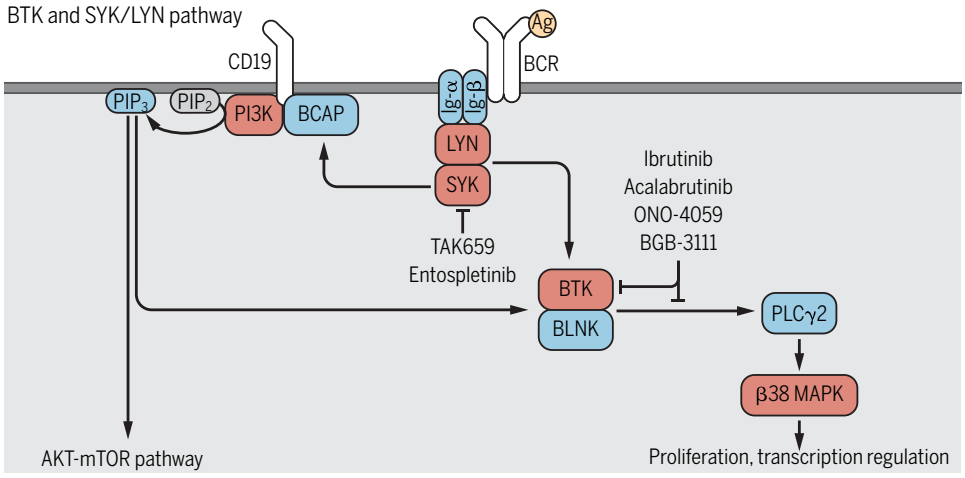

Fig. 1. Targeting the BCR-BTK signaling network in MCL. Small-molecule inhibitors of kinases involved in BCR signaling, such as those targeting SYK, are used to treat various hematopoietic malignancies. Targeting intracellular BCR signaling, such as the PI3K-AKT pathway (depicted further in Fig. 2), or by blocking the activity of BTK with ibrutinib and others has shown promising results in patients with MCL. However, the emergence of ibrutinib-resistant cells has become clinically problematic. Current research focuses on targeting molecules farther downstream to inhibit cell growth. Pathway depictions have been simplified. PIP_2, phosphatidylinositol-4,5-bisphosphate; BCAP, B cell adaptor for PI3K; BLNK, B cell linker protein; PLCγ2, phospholipase C–γ2; β38-MAPK, β p38 mitogen-activated protein kinase; Ag, antigen; Ig-α, immunoglobulin-associated alpha; Ig-β, immunoglobulin-associated beta.

this regimen, the ibrutinib and rituximab combination moved to the frontline, followed by shortened chemotherapy for young, newly diagnosed patients. Preliminary reports displayed great promise with an excellent overall response of 100% and a complete response of 82%, demonstrating unprecedented efficacy of chemo-free therapy alone (*40*) and the importance of understanding the B cell pathways. Similarly, frontline therapy with the immune modulatory compound, lenalidomide, in combination with rituximab among untreated patients showed an overall response of 92% (*41*), proving that chemo-free therapy can be more efficacious and less toxic than traditional cytotoxic combinations of four to five drugs. Ibrutinib, in combination with venetoclax, achieved 71% OR in 24 patients with relapsed-refractory MCL (Table 2) (*42*).

SYK/LYN

The BCR associates with two classes of tyrosine kinases: Src-family kinases [including LYN (Lck/Yes novel tyrosine kinase), FYN, BLK, and BTK] and spleen tyrosine kinase (SYK) (Fig. 1) (*43*). Currently, there are a handful of tyrosine kinase inhibitors emerging as drug candidates. Entospletinib, a SYK inhibitor, has persisted and moved to phase 1 and 2 clinical trials. The response rates in a phase 2 study with relapsed or refractory CLL (n = 41) or NHL (n = 145) were favorable with a PFS rate of 70.1% (*44*) at 24 weeks. Apart from entosplenitib, the SYK inhibitor TAK-659 has also entered phase 1 clinical trials in adult patients with advanced solid tumor and lymphoma malignancies (*45*). SYK/LYN inhibitors remain relevant due to the widespread capabilities of the Src-family kinases and their potential to impede BCR signaling.

PI3K-AKT-mTOR pathway

The PI3K-AKT-mTOR pathway plays a vast role in regulating cell function, growth, and proliferation in certain cancers (Fig. 2) (*46*–*48*). PI3K consists of three classes (I, II, and III) that vary in both structure and function; class IA PI3Ks are highly associated with cancers and are, therefore, the focal point of most PI3K therapies (*49*). PI3K activates AKT indirectly through the production of PIP_3 (phosphatidylinositol 3,4,5-trisphosphate) that binds AKT, facilitating its activation by other kinases. Inhibition of either step in this pathway can potentially halt cell proliferation and growth. PIP_3 compounds also activate members of the protein kinase C (PKC) family enzymes, which mediate various signals that promote cell proliferation, gene expression, and inflammatory responses. In addition, as part of the PI3K cell signaling pathway, PKC enzymes are associated with cancer and tumor growth and are thus potential targets for therapies (*50*). PI3K is recruited by CD19, a B cell–specific surface antigen that decreases the threshold for BCR signaling pathway activation. Once CD19 becomes phosphorylated upon BCR ligation, it provides a docking site for secondary effector molecules like PI3K (Figs. 1 and 2) at the plasma membrane. This facilitates BCR-induced intracellular calcium flux mediated through inositol-(1, 4, 5)-triphosphate binding to its receptors, as well as by mitogen-activated protein kinase activation. Through CD19 and adaptor protein-mediated recruitment of PI3K, BCR signals ultimately lead to the activation of the prosurvival signaling effectors AKT and NF-κB (Fig. 2) (*51*). Various PI3K-AKT inhibitors are active in preclinical studies and preliminary trials, with idelalisib emerging as one of the frontrunners (Fig. 2), specifically targeting the PI3Kδ isoform. Phase 1 trials have completed, and the overall response was 40%, with complete remission in 2 of 40 patients (5%) (*52*). Median duration of response was 2.7 months, median PFS was 3.7 months, and one-year PFS was 22%. These data provide proof of concept that targeting PI3Kδ is a viable strategy and worthy of additional study in MCL (*52*). Combination treatment with SYK inhibitors in addition to monoclonal antibody therapy used for treatment of other B cell cancers could potentially be repurposed as MCL therapies.

mTOR

The mTOR pathway acts as a downstream effector of the PI3K-AKT pathway and is important to highlight due to its specific signaling capabilities (*53*). However, mTOR may also function upstream of AKT, adding to the complexity of its pathological role in cancers (*54*). mTOR complex 1 (mTORC1) and mTORC2 (Fig. 2) both have similar but distinct regulatory properties. mTORC1 acts as a growth regulator, known for its role in cell growth and proliferation through protein synthesis (*55*). mTORC1 senses cellular stress and other signals that could potentiate abnormal mTOR signaling in tumors, due to either loss of function of upstream tumor suppressor proteins or activation of mutations within oncogenes that feed into the mTOR pathway (*55*). mTORC2 manages cell survival and cytoskeletal organization via phosphorylation of its AGC (protein kinase A, protein kinase G, and protein kinase C) kinase substrates (*56*). Enhancement of multiple elements of the mTOR pathway [gene amplification/mutation of PI3K subunits, loss of function of PTEN, and overexpression of AKT, S6 kinase (S6K1), eukaryotic translation initiation factor 4E (eIF4E), and

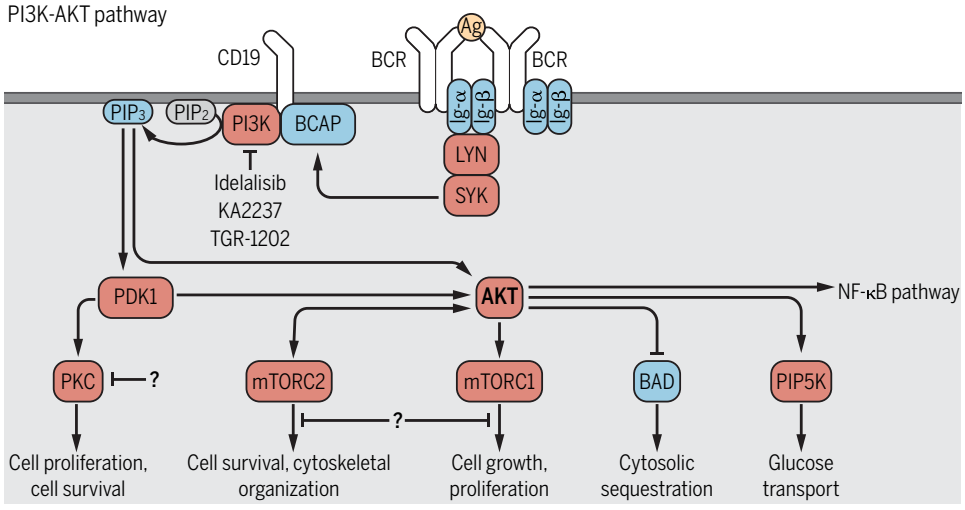

Fig. 2. The AKT network as a central therapeutic target in MCL. The AKT pathway is a major signaling network node that supports cell function, growth, and proliferation. Antigen binding to the BCR induces receptor clustering and transduction. Subsequent activation of PI3K through BCR-associated kinases (SYK or LYN) and the adaptor protein BCAP stimulates PI3K-mediated phosphorylation of the lipid PIP_2 to form PIP_3, which then recruits AKT and other proteins to the plasma membrane, thereby facilitating activation. Cell proliferation is blocked by inhibition of PI3K or the intracellular BCR mediators, SYK, or LYN (Fig. 1). By recruiting pyruvate dehydrogenase lipoamide kinase isozyme 1 (PDK1) and AKT to the plasma membrane, PIP_3 also activates PKC and mTORC1 and mTORC2, which are other potential targets for therapy. Pathway depictions have been simplified. PDK1, pyruvate dehydrogenase lipoamide kinase isozyme 1; BAD, Bcl-2–associated death promoter; PIP_5K, phosphatidylinositol-4-phosphate 5-kinase.

eIF4E-binding protein (4EBP1)] has been reported in many types of cancers (*48*). Thus, mutations within this pathway are of particular interest and manipulating or inhibiting them may uncover effective B cell cancer therapies.

NF-κB pathway

NF-κB is a family of transcription factors that controls inflammation, cell differentiation, cell survival, and cell proliferation in response to various cellular stimuli. The NF-κB family consists of five members: RelA, RelB, c-Rel, p50, and p52, which function as dimeric transcription factors (*57*). Overall, there are two main NF-κB pathways. The classical NF-κB pathway is rapidly induced mostly by physiological stimuli and is dependent on inhibitor of κB kinase β (IKKβ)–mediated activation of RelA-containing heterodimers, whereas the alternative NF-κB pathway depends on the IKKα/NF-κB–inducing kinase (NIK) complex–mediated processing of p100 to p52, which associates with RelB to induce expression of target genes (Fig. 3). IKK is an enzyme complex that is at the upstream of the NF-κB signal transduction cascade and is involved in transmitting the cellular response to inflammation (*29*). Both the classical and alternative NF-κB pathways are tightly regulated, and aberrant NF-κB activation is associated with multiple cancers (*58*). The complex consisting of caspase recruitment domain-containing protein 11 (CARD11), B cell lymphoma/leukemia (BCL10), and mucosa-associated lymphoid tissue (MALT), altogether called the CBM signaling complex, is responsible for acting as a signaling intermediate and is essential for regulation of the classical NF-κB pathway (*59*). This essential role of the CBM complex may be exploited for lymphoma; some lymphoma subtypes with gain-of-function mutant CARD11 is effectively blocked by c-Jun N-terminal kinase inhibitors (*60*).

NIK is the central player in the alternative NF-κB pathway, and its protein levels are also strictly controlled (*61*). At resting state, the endogenous abundance of NIK is low due to its constant ubiquitination-dependent proteasomal degradation by the formation of the NIK ubiquitin ligase composed of TRAF3, TRAF2, and cIAP1/2 (*61*). After being receptor-induced, TRAF3 undergoes proteasomal degradation and releases NIK. NIK then accumulates through increased protein stability and new protein synthesis (*29*).

TRAF2 and TRAF3 are negative regulators of alternative NF-κB signaling, and they interact with BIRC2 and BIRC3 to decrease NIK activity (*62*), the central player of alternative NF-κB signaling, to promote the processing of p100 to p52 (Fig. 3). Rahal and colleagues (*17*) found that NF-κB target gene activity was selectively decreased by ibrutinib in drug-sensitive but not resistant MCL cell lines. Furthermore, TRAF2 and TRAF3 were not detectable by Western blotting in ibrutinib-resistant cell lines. Because TRAF2 and TRAF3 are negative regulators of alternative NF-κB signaling, the idea that ibrutinib resistance is BTK/BCR independent and that the alternative pathway is constitutively activated in MCL pathogenesis is quite plausible and is supported by the findings of Rahal *et al. (17)*. NIK silencing reportedly blocks both classical and alternative NF-κB activation pathways (*63*) and reduces the expression of several prosurvival and antiapoptotic factors. In addition, substantial depletion of NIK from NIK-overexpressing cell lines substantially induces apoptosis and also has a more pronounced effect on cell survival than does knockdown of IKK (*63*). NIK inhibitors showed selective cytotoxicity for multiple myeloma cell lines that had NIK-dependent activation of the NF-κB (*64*). We expect NIK inhibitors to be active in BTK inhibitor–resistant MCL cases where the alternative NF-κB pathway would be activated (Fig. 3). Therefore, NIK may play an important role in conferring resistance to ibrutinib in MCL.

PROMISING THERAPEUTIC COMBINATIONS INVOLVING EFFECTIVE DISRUPTIONS OF B CELL PATHWAYS

Clinically effective disruption of B cell pathways may require agents that work in synergy. We have examined that the results of therapeutic combinations have been reported in NHL trials and preclinical studies (Table 2). High-dose chemotherapy followed by ASCT consolidation remains a standard therapy for patients newly diagnosed with MCL. For older patients with relapsed-refractory disease, ibrutinib is the drug of choice. Therapy combinations with ibrutinib-rituximab that targets BTK and CD20 (*38*), rituximab-lenalidomide that targets CD20 and ubiquitin E3 ligase (*41*), and ibrutinib-rituximab-lenalidomide that targets BTK, CD20, and ubiquitin E3 ligase have produced noteworthy clinical outcomes in patients with MCL (*65*). We anticipate that combination therapies inhibiting BTK and PI3K will exhibit synergy and strong efficacy because the PI3K-AKT pathway appears to be highly activated in the event of BTK-mediated

Table 2. Chemotherapy-free targeted regimens versus standard chemotherapy in MCL. Outcomes of chemotherapy-free regimens are shown for both newly diagnosed and relapsed patients with MCL, noting percent of patients with overall response, complete response, and toxicity. Standard chemotherapy is shown at the bottom of the table to primarily compare the toxicity effects on patients. Sources of data are cited in parentheticals, left column.

Newly diagnosed patients	Overall response	Complete response	Toxicity (grade ≥ 3)	Secondary malignancy
Therapy				
Lenalidomide + rituximab (*41*)	92% (*n* = 36)	64% (*n* = 36)	Neutropenia, 50%; rash, 29%; thrombocytopenia, 13%; tumor flare, 11%; anemia, 11%	None
Rituximab + ibrutinib (*40*)	100% (*n* = 50)	72% (*n* = 50)	Fatigue, 6%	None
Ibrutinib + lenalidomide + Rituximab (*65*)	83% (*n* = 29)	41% (*n* = 29)	Neutropenia, 38%; infections, 22%; rash, 14%	None
Relapsed-refractory patients				
Ibrutinib + rituximab (*38*)	88% (*n* = 50)	44% (*n* = 50)	Atrial fibrillation, 12%	None
Ibrutinib + venetoclax (*42*)	71% (*n* = 17)	67% (*n* = 16)	Only low grade ≤ 3 diarrhea and fatigue	None
Acalabrutinib	81% (*n* = 100)	40% (*n* = 49)	Neutropenia, 10%; anemia, 9%	None
Chemotherapy				
Rituximab-hyper-cyclophosphamide vincristine adriamycin dexamathasone (R-Hyper-CVAD) (*10*, *72*)	96% (*n* = 97)	54% (*n* = 97)	Febrile neutropenia, 73%; hematotoxicity, >50%	6.2%
Rituximab-cyclophosphamide hydroxydaunomycin oncovin prednisone (R-CHOP)/maxi-CHOP (*9*)	97% (*n* = 160)	87% (*n* = 160)	Febrile neutropenia, 75%; hematotoxicity, >50%	9.4%
Rituximab-bendamustine (R-Benda) (*73*)	100% (*n* = 20)	95% (*n* = 20)	Thrombocytopenia, 87%; neutropenia, 29%	6 to 11%

resistance. Similarly, combining BTK inhibitors with inhibitors of the mTOR pathway or of NIK could be efficacious. Moreover, synergistic cytotoxicity by combined application with venetoclax and ibrutinib was previously demonstrated in two MCL cell lines, Z138 and JVM2 (*66*), and proved very effective in the clinic for MCL (*42*). Using the informatics and genomic tools that are available today, researchers may not only uncover alternative therapies to overcome resistance but also gain further insight into the underlying mechanisms of drug resistance.

TRANSLATIONAL MODELS TO TARGET B CELL PATHWAYS

Novel human cancer cell lines, various tumor models, mouse lymphoma models, and patient-derived xenograft (PDX) models are excellent tools to gain a deeper understanding of tumor biology and response to therapeutic agents (*66*–*68*). Although human cancer cell lines are widely used for their convenience, their use has many disadvantages, particularly the inability to model the biology and responsiveness of the cancer in its natural microenvironment, given that tumor microenvironments are multifaceted and therefore not easily mimicked in a dish (*66*). The nature of any two-dimensional models is a hindrance to successful translation into in vivo settings. Three-dimensional in vitro tumor models have thus been developed to overcome these drawbacks, especially to more closely simulate the tumor microenvironment. Through either a device or matrix-assisted assembly, there is a potential for these models to provide a more accurate information regarding toxicity during the early drug testing phases. Many murine models of lymphoma have been genetically engineered to express the mutations associated with the growth of human lymphomas and hence give rise to spontaneous lymphomas (*69*, *70*). More recently, however, PDX models have gained much attention because of the closer nature of the PDXs to the patient tumor, including the complex genetic composition, and sometimes the immediate tumor microenvironment, and even the response to therapy. Molecular profiling and drug screening assays using MCL PDXs in vivo and in vitro have identified mechanisms of therapeutic resistance and drug combinations that may become clinically effective (*71*). These models can be used to interrogate B cell pathways and identify novel treatment combinations that may eventually be translated to the bedside. However, challenges remain in the use of PDX models. Because of the use of immunocompromised mice, the model cannot take into account the effects that the natural immune response has on the tumor, directly and through its effects on the microenvironment. The use of fetal bone tissue in developing these models is also a challenge, especially in states that have strict regulations against their usage. Despite these challenges, PDX models are still able to provide clinically valuable data. By using PDXs, treatment responses might be predicted for individual patients, enabling translational scientists to investigate opportunities for targeting B cell pathways in the selection of appropriate, personalized therapies.

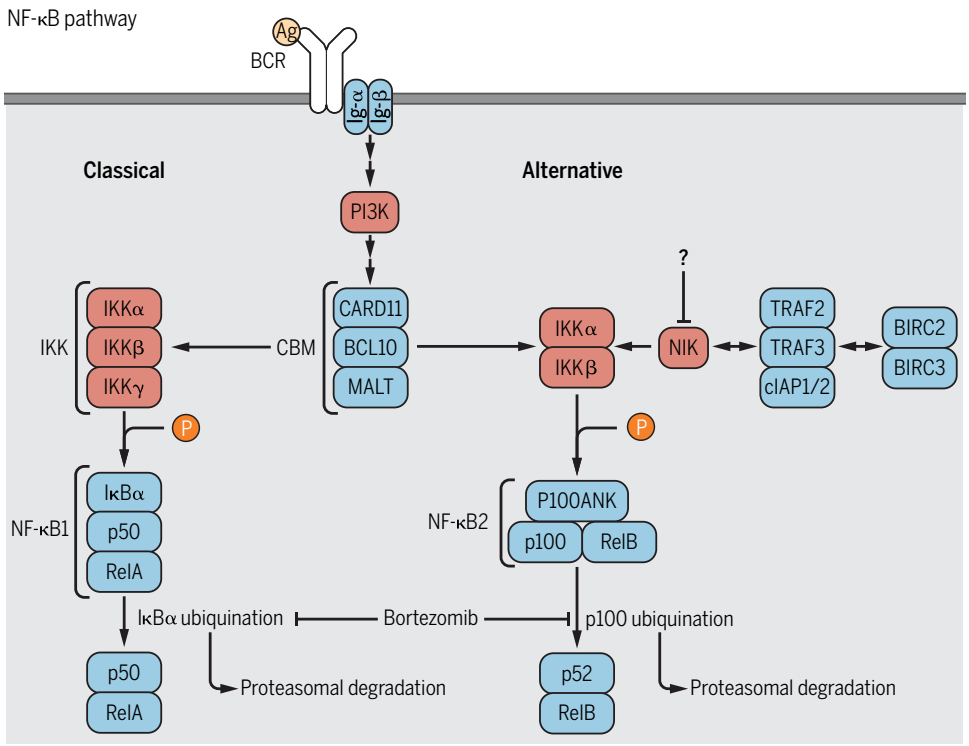

Fig. 3. Targeting NF-κB pathways in MCL. The NF-κB transcription factor family (RelA, RelB, c-Rel, p50, and p52) controls cell differentiation, survival, and proliferation. The BCR activates both the classical and alternative NF-κB pathways. Whereas the classical pathway is mediated by IKKβ-related RelA activation, the alternative pathway is mediated by IKKα/NIK complex-related RelB activation. The CBM signaling complex, comprising CARD11, BCL10, and MALT, plays a major upstream role in both pathways, as do proteasomal complexes downstream that enable the formation of transcriptionally active p50/RelA and p52/RelB complexes. Thus, NIK and the proteasome might be targeted for treating MCL. Pathway depictions have been simplified. TRAF, tumor necrosis factor receptor-associated factors; cIAP1/2, cellular inhibitors of apoptosis 1 and 2; BIRC, baculoviral IAP repeat containing gene; P, phosphorylation.

CONCLUSION AND OUTLOOK FOR THE FUTURE IN MCL THERAPY

A patient-centric approach to treating cancer is hotly anticipated to improve clinical outcomes. This review focused on the integration of genomics, informatics, and interrogation of B cell pathways to improve MCL therapies by elucidating disease pathogenesis, progression, and mechanisms of resistance. Further advancement in genomics and their interplay with multiple B cell circuitries will continue to uncover therapeutic biomarkers. Identifying dominant B cell pathways is integral to not only MCL pathology but also to all B cell malignancies and should be a focus when designing therapies, especially in the face of key drug resistance. In this evolving field of cancer trials, we anticipate more individualized approaches in targeting MCL and other B cell malignancies. For a patient suffering from aggressive metastatic cancer, the best thing we can offer is hope, a fighting chance that is grounded in rapid translational research. Translational models that can mimic tumor activity would also play crucial role in gaging the response to drug treatment and the mechanisms of evading its toxicity.

REFERENCES AND NOTES

1. I. Barista, J. E. Romaguera, F. Cabanillas, Mantle-cell lymphoma. *Lancet Oncol.* **2**, 141–148 (2001).
2. C. Y. Cheah, A. George, E. Giné, A. Chiappella, H. C. Kluin-Nelemans, W. Jurczak, K. Krawczyk, H. Mocikova, P. Klener, D. Salek, J. Walewski, M. Szymczyk, L. Smolej, R. L. Auer, D. S. Ritchie, L. Arcaini, M. E. Williams, M. Dreyling, J. F. Seymour, European Mantle Cell Lymphoma Network, Central nervous system involvement in mantle cell lymphoma: Clinical features, prognostic factors and outcomes from the European mantle cell lymphoma network. *Ann. Oncol.* **24**, 2119–2123 (2013).
3. F. Bosch, A. López-Guillermo, E. Campo, J. M. Ribera, E. Conde, M. A. Piris, T. Vallespí, S. Woessner, E. Montserrat, Mantle cell lymphoma: Presenting features, response to therapy, and prognostic factors. *Cancer* **82**, 567–575 (1998).
4. M. L. Wang, S. Rule, P. Martin, A. Goy, R. Auer, B. S. Kahl, W. Jurczak, R. H. Advani, J. E. Romaguera, M. E. Williams, J. C. Barrientos, E. Chmielowska, J. Radford, S. Stilgenbauer, M. Dreyling, W. W. Jedrzejczak, P. Johnson, S. E. Spurgeon, L. Li, L. Zhang, K. Newberry, Z. Ou, N. Cheng, B. Fang, J. McGreivy, F. Clow, J. J. Buggy, B. Y. Chang, D. M. Beaupre, L. A. Kunkel, K. A. Blum, Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N. Engl. J. Med.* **369**, 507–516 (2013).
5. C. Y. Cheah, D. Chihara, J. E. Romaguera, N. H. Fowler, J. F. Seymour, F. B. Hagemeister, R. E. Champlin, M. L. Wang, Patients with mantle cell lymphoma failing ibrutinib are unlikely to respond to salvage chemotherapy and have poor outcomes. *Ann. Oncol.* **26**, 1175–1179 (2015).
6. S. Cheng, A. Guo, P. Lu, J. Ma, M. Coleman, Y. L. Wang, Functional characterization of BTK^{C481S} mutation that confers ibrutinib resistance: Exploration of alternative kinase inhibitors. *Leukemia* **29**, 895–900 (2015).
7. P. Pérez-Galán, H. Mora-Jensen, M. A. Weniger, A. L. Shaffer III, E. G. Rizzatti, C. M. Chapman, C. C. Mo, L. S. Stennett, C. Rader, P. Liu, N. Raghavachari, M. Stetler-Stevenson, C. Yuan, S. Pittaluga, I. Maric, K. M. Dunleavy, W. H. Wilson, L. M. Staudt, A. Wiestner, Bortezomib resistance in mantle cell lymphoma is associated with plasmacytic differentiation. *Blood* **117**, 542–552 (2011).
8. E. Campo, S. Rule, Mantle cell lymphoma: Evolving management strategies. *Blood* **125**, 48–55 (2015).
9. C. W. Eskelund, A. Kolstad, M. Jerkeman, R. Räty, A. Laurell, S. Eloranta, K. E. Smedby, S. Husby, L. B. Pedersen, N. S. Andersen, M. Eriksson, E. Kimby, H. Bentzen, O. Kuittinen, G. F. Lauritzsen, H. Nilsson-Ehle, E. Ralfkiær, M. Ehinger, C. Sundström, J. Delabie, M.-L. Karjalainen-Lindsberg, C. T. Workman, C. Garde, E. Elonen, P. Brown, K. Grønbæk, C. H. Geisler, 15-year follow-up of the second nordic mantle cell lymphoma trial (MCL2): Prolonged remissions without survival plateau. *Br. J. Haematol.* **175**, 410–418 (2016).
10. D. Chihara, C. Y. Cheah, J. R. Westin, L. E. Fayad, M. A. Rodriguez, F. B. Hagemeister, B. Pro, P. McLaughlin, A. Younes, F. Samaniego, A. Goy, F. Cabanillas, H. Kantarjian, L. W. Kwak, M. L. Wang, J. E. Romaguera, Rituximab plus hyper-CVAD alternating with MTX/Ara-C in

patients with newly diagnosed mantle cell lymphoma: 15-year follow-up of a phase II study from the MD Anderson Cancer Center. *Br. J. Haematol.* **172**, 80–88 (2016).
11. C. H. Geisler, A. Kolstad, A. Laurell, N. S. Andersen, L. B. Pedersen, M. Jerkeman, M. Eriksson, M. Nordström, E. Kimby, A. M. Boesen, O. Kuittinen, G. F. Lauritzsen, H. Nilsson-Ehle, E. Ralfkiær, M. Åkerman, M. Ehinger, C. Sundstrom, R. Langholm, J. Delabie, M.-L. Karjalainen-Lindsberg, P. Brown, E. Elonen, Nordic Lymphoma Group, Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: A nonrandomized phase 2 multicenter study by the Nordic lymphoma group. *Blood* **112**, 2687–2693 (2008).
12. M. Ahmed, L. Zhang, K. Nomie, L. Lam, M. Wang, Gene mutations and actionable genetic lesions in mantle cell lymphoma. *Oncotarget* **7**, 58638–58648 (2016).
13. S. Beà, R. Valdés-Mas, A. Navarro, I. Salaverria, D. Martin-Garcia, P. Jares, E. Giné, M. Pinyol, C. Royo, F. Nadeu, L. Conde, M. Juan, G. Clot, P. Vizán, L. Di Croce, D. A. Puente, M. López-Guerra, A. Moros, G. Roue, M. Aymerich, N. Villamor, L. Colomo, A. Martínez, A. Valera, J. I. Martín-Subero, V. Amador, L. Hernández, M. Rozman, A. Enjuanes, P. Forcada, A. Muntañola, E. M. Hartmann, M. J. Calasanz, A. Rosenwald, G. Ott, J. M. Hernández-Rivas, W. Klapper, R. Siebert, A. Wiestner, W. H. Wilson, D. Colomer, A. López-Guillermo, C. López-Otin, X. S. Puente, E. Campo, Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 18250–18255 (2013).
14. D. W. Scott, P. Abrisqueta, G. W. Wright, G. W. Slack, A. Mottok, D. Villa, P. Jares, H. Rauert-Wunderlich, C. Royo, G. Clot, M. Pinyol, M. Boyle, F. C. Chan, R. M. Braziel, W. C. Chan, D. D. Weisenburger, J. R. Cook, T. C. Greiner, K. Fu, G. Ott, J. Delabie, E. B. Smeland, H. Holte, E. S. Jaffe, C. Steidl, J. M. Connors, R. D. Gascoyne, A. Rosenwald, L. M. Staudt, E. Campo, L. M. Rimsza, Lymphoma/Leukemia Molecular Profiling Project, New molecular assay for the proliferation signature in mantle cell lymphoma applicable to formalin-fixed paraffin-embedded biopsies. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **35**, 1668–1677 (2017).
15. M. Ahmed, L. Li, C. Pinnix, B. Dabaja, K. Nomie, L. Lam, M. Wang, ATM mutation and radiosensitivity: An opportunity in the therapy of mantle cell lymphoma. *Crit. Rev. Oncol. Hematol.* **107**, 14–19 (2016).
16. R. Kridel, B. Meissner, S. Rogic, M. Boyle, A. Telenius, B. Woolcock, J. Gunawardana, C. Jenkins, C. Cochrane, S. Ben-Neriah, K. Tan, R. D. Morin, S. Opat, L. H. Sehn, J. M. Connors, M. A. Marra, A. P. Weng, C. Steidl, R. D. Gascoyne, Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma. *Blood* **119**, 1963–1971 (2012).
17. R. Rahal, M. Frick, R. Romero, J. M. Korn, R. Kridel, F. Chun Chan, B. Meissner, H.-e. Bhang, D. Ruddy, A. Kauffmann, A. Farsidjani, A. Derti, D. Rakiec, T. Naylor, E. Pfister, S. Kovats, S. Kim, K. Dietze, B. Dörken, C. Steidl, A. Tzankov, M. Hummel, J. Monahan, M. P. Morrissey, C. Fritsch, W. R. Sellers, V. G. Cooke, R. D. Gascoyne, G. Lenz, F. Stegmeier, Pharmacological and genomic profiling identifies NF-κB–targeted treatment strategies for mantle cell lymphoma. *Nat. Med.* **20**, 87–92 (2014).
18. M. A. Lunning, M. R. Green, Mutation of chromatin modifiers; an emerging hallmark of germinal center B-cell lymphomas. *Blood Cancer J.* **5**, e361 (2015).
19. A. Rosenwald, G. Wright, A. Wiestner, W. C. Chan, J. M. Connors, E. Campo, R. D. Gascoyne, T. M. Grogan, H. K. Muller-Hermelink, E. B. Smeland, M. Chiorazzi, J. M. Giltnane, E. M. Hurt, H. Zhao, L. Averett, S. Henrickson, L. Yang, J. Powell, W. H. Wilson, E. S. Jaffe, R. Simon, R. D. Klausner, E. Montserrat, F. Bosch, T. C. Greiner, D. D. Weisenburger, W. G. Sanger, B. J. Dave, J. C. Lynch, J. Vose, J. O. Armitage, R. I. Fisher, T. P. Miller, M. LeBlanc, G. Ott, S. Kvaloy, H. Holte, J. Delabie, L. M. Staudt, The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. *Cancer Cell* **3**, 185–197 (2003).
20. P. Diez, S. Lorenzo, R. M. Dégano, N. Ibarrola, M. González-González, W. Nieto, J. Almeida, M. González, A. Orfao, M. Fuentes, Multipronged functional proteomics approaches for global identification of altered cell signalling pathways in B-cell chronic lymphocytic leukaemia. *Proteomics* **16**, 1193–1203 (2016).
21. D. Chiron, M. Di Liberto, P. Martin, X. Huang, J. Sharman, P. Blecua, S. Mathew, P. Vijay, K. Eng, S. Ali, A. Johnson, B. Chang, S. Ely, O. Elemento, C. E. Mason, J. P. Leonard, S. Chen-Kiang, Cell-cycle reprogramming for PI3K inhibition overrides a relapse-specific C481S BTK mutation revealed by longitudinal functional genomics in mantle cell lymphoma. *Cancer Discov.* **4**, 1022–1035 (2014).
22. D. C. M. Rolland, V. Basrur, Y.-K. Jeon, C. McNeil-Schwalm, D. Fermin, K. P. Conlon, Y. Zhou, S. Y. Ng, C.-C. Tsou, N. A. Brown, D. G. Thomas, N. G. Bailey, G. S. Omenn, A. I. Nesvizhskii, D. E. Root, D. M. Weinstock, R. B. Faryabi, M. S. Lim, K. S. J. Elenitoba-Johnson, Functional proteogenomics reveals biomarkers and therapeutic targets in lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 6581–6586 (2017).
23. M. I. Merolle, M. Ahmed, K. Nomie, M. L. Wang, The B cell receptor signaling pathway in mantle cell lymphoma. *Oncotarget* **9**, 25332–25341 (2018).
24. T. Kurosaki, H. Shinohara, Y. Baba, B cell signaling and fate decision. *Annu. Rev. Immunol.* **28**, 21–55 (2010).
25. E. Rao, C. Jiang, M. Ji, X. Huang, J. Iqbal, G. Lenz, G. Wright, L. M. Staudt, Y. Zhao, T. W. McKeithan, W. C. Chan, K. Fu, The miRNA-17~92 cluster mediates chemoresistance and enhances tumor growth in mantle cell lymphoma via PI3K/AKT pathway activation. *Leukemia* **26**, 1064–1072 (2012).
26. S. Balaji, M. Ahmed, E. Lorence, F. Yan, K. Nomie, M. Wang, NF-κB signaling and its relevance to the treatment of mantle cell lymphoma. *J. Hematol. Oncol.* **11**, 83 (2018).
27. N. S. Saba, D. Liu, S. E. M. Herman, C. Underbayev, X. Tian, D. Behrend, M. A. Weniger, M. Skarzynski, J. Gyamfi, L. Fontan, A. Melnick, C. Grant, M. Roschewski, A. Navarro, S. Beà, S. Pittaluga, K. Dunleavy, W. H. Wilson, A. Wiestner, Pathogenic role of B-cell receptor signaling and canonical NF-κB activation in mantle cell lymphoma. *Blood* **128**, 82–92 (2016).
28. J. H. Myklebust, J. Brody, H. E. Kohrt, A. Kolstad, D. K. Czerwinski, S. Wälchli, M. R. Green, G. Trøen, K. Liestøl, K. Beiske, R. Houot, J. Delabie, A. A. Alizadeh, J. M. Irish, R. Levy, Distinct patterns of B-cell receptor signaling in non-Hodgkin lymphomas identified by single-cell profiling. *Blood* **129**, 759–770 (2017).
29. J. J. Buggy, L. Elias, Bruton tyrosine kinase (BTK) and its role in B-cell malignancy. *Int. Rev. Immunol.* **31**, 119–132 (2012).
30. R. Kuppers, Mechanisms of B-cell lymphoma pathogenesis. *Nat. Rev. Cancer* **5**, 251–262 (2005).
31. A. Novero, P. M. Ravella, Y. Chen, G. Dous, D. Liu, Ibrutinib for B cell malignancies. *Exp. Hematol. Oncol.* **3**, 4 (2014).
32. B. Y. Chang, M. Francesco, M. F. M. De Rooij, P. Magadala, S. M. Steggerda, M. M. Huang, A. Kuil, S. E. M. Herman, S. Chang, S. T. Pals, W. Wilson, A. Wiestner, M. Spaargaren, J. J. Buggy, L. Elias, Egress of $CD19^+CD5^+$ cells into peripheral blood following treatment with the Bruton tyrosine kinase inhibitor ibrutinib in mantle cell lymphoma patients. *Blood* **122**, 2412–2424 (2013).
33. J. A. Woyach, A. J. Johnson, J. C. Byrd, The B cell receptor signaling pathway as a therapeutic target in CLL. *Blood* **120**, 1175–1184 (2012).
34. R. H. Advani, J. J. Buggy, J. P. Sharman, S. M. Smith, T. E. Boyd, B. Grant, K. S. Kolibaba, R. R. Furman, S. Rodriguez, B. Y. Chang, J. Sukbuntherng, R. Izumi, A. Hamdy, E. Hedrick, N. H. Fowler, Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J. Clin. Oncol.* **31**, 88–94 (2013).
35. M. Wang, S. Rule, P. L. Zinzani, A. Goy, O. Casasnovas, S. D. Smith, G. Damaj, J. Doorduijn, T. Lamy, F. Morschhauser, C. Panizo, B. Shah, A. Davies, R. Eek, J. Dupuis, E. Jacobsen, A. P. Kater, S. le Gouill, L. Oberic, T. Robak, T. Covey, R. Dua, A. Hamdy, X. Huang, R. Izumi, P. Patel, W. Rothbaum, J. G. Slatter, W. Jurczak, Acalabrutinib in relapsed or refractory mantle cell lymphoma (ACE-LY-004): A single-arm, multicentre, phase 2 trial. *Lancet* **391**, 659–667 (2018).
36. H. S. Walter, S. A. Rule, M. J. S. Dyer, L. Karlin, C. Jones, B. Cazin, P. Quittet, N. Shah, C. V. Hutchinson, H. Honda, K. Duffy, J. Birkett, V. Jamieson, N. Courtenay-Luck, T. Yoshizawa, J. Sharpe, T. Ohno, S. Abe, A. Nishimura, G. Cartron, F. Morschhauser, C. Fegan, G. Salles, A phase 1 clinical trial of the selective BTK inhibitor ONO/GS-4059 in relapsed and refractory mature B-cell malignancies. *Blood* **127**, 411–419 (2016).
37. C. Tam, A. P. Grigg, S. Opat, M. Ku, M. Gilbertson, M. A. Anderson, J. F. Seymour, D. S. Ritchie, C. Dicorleto, B. Dimovski, E. Hedrick, J. Yang, L. Wang, L. Luo, L. Xue, A. W. Roberts, The BTK inhibitor, Bgb-3111, is safe, tolerable, and highly active in patients with relapsed/refractory B-cell malignancies: Initial report of a phase 1 first-in-human trial. *Blood* **126**, 832 (2015).
38. M. L. Wang, H. Lee, H. Chuang, N. Wagner-Bartak, F. Hagemeister, J. Westin, L. Fayad, F. Samaniego, F. Turturro, Y. Oki, W. Chen, M. Badillo, K. Nomie, M. DeLa Rosa, D. Zhao, L. Lam, A. Addison, H. Zhang, K. H. Young, S. Li, D. Santos, L. J. Medeiros, R. Champlin, J. Romaguera, L. Zhang, Ibrutinib in combination with rituximab in relapsed or refractory mantle cell lymphoma: A single-Centre, open-label, phase 2 trial. *Lancet Oncol.* **17**, 48–56 (2016).
39. D. Rudnicka, A. Oszmiana, D. K. Finch, I. Strickland, D. J. Schofield, D. C. Lowe, M. A. Sleeman, D. M. Davis, Rituximab causes a polarization of B cells that augments its therapeutic function in NK-cell-mediated antibody-dependent cellular cytotoxicity. *Blood* **121**, 4694–4702 (2013).
40. M. Wang, H. J. Lee, S. Thirumurthi, H. H. Chuang, F. B. Hagemeister, J. R. Westin, L. E. Fayad, F. Samaniego, F. Turturro, W. Chen, O. Oriabure, S. Y. Huang, S. Li, L. Zhang, M. Badillo, K. H. Hartig, M. Ahmed, K. Nomie, L. T. Lam, A. A. Addison, J. E. Romaguera, Chemotherapy-free induction with ibrutinib-rituximab followed by shortened cycles of chemo-immunotherapy consolidation in young, newly diagnosed mantle cell lymphoma patients: A phase II clinical trial. *Blood* **128**, 147 (2016).
41. J. Ruan, P. Martin, B. Shah, S. J. Schuster, S. M. Smith, R. R. Furman, P. Christos, A. Rodriguez, J. Svoboda, J. Lewis, O. Katz, M. Coleman, J. P. Leonard, Lenalidomide plus rituximab as initial treatment for mantle-cell lymphoma. *N. Engl. J. Med.* **373**, 1835–1844 (2015).
42. C. S. Tam, M. A. Anderson, C. Pott, R. Agarwal, S. Handunnetti, R. J. Hicks, K. Burbury, G. Turner, J. Di Iulio, M. Bressel, D. Westerman, S. Lade, M. Dreyling, S. J. Dawson, M. A. Dawson, J. F. Seymour, A. W. Roberts, Ibrutinib plus venetoclax for the treatment of mantle-cell lymphoma. *N. Engl. J. Med.* **378**, 1211–1223 (2018).

43. T. Kurosaki, M. Takata, Y. Yamanashi, T. Inazu, T. Taniguchi, T. Yamamoto, H. Yamamura, Syk activation by the Src-family tyrosine kinase in the B cell receptor signaling. *J. Exp. Med.* **179**, 1725–1729 (1994).
44. J. Sharman, M. Hawkins, K. Kolibaba, M. Boxer, L. Klein, M. Wu, J. Hu, S. Abella, C. Yasenchak, An open-label phase 2 trial of entospletinib (GS-9973), a selective spleen tyrosine kinase inhibitor, in chronic lymphocytic leukemia. *Blood* **125**, 2336–2343 (2015).
45. J. B. Kaplan, L. I. Gordon, J. R. Infante, R. Popat, A. Rambaldi, S. Madan, M. R. Patel, G. Gritti, C.-H. Ng, I. Chau, J. A. Radford, J. P. de Oteyza, P. L. Zinzani, S. Faucette, E. Sheldon-Waniga, M. Williams, K. Stumpo, Y. Shou, C. Carpio, F. Bosch, Updated results from a phase 1 study of TAK-659, an investigational and reversible SYK inhibitor, in patients (Pts) with advanced solid tumor or lymphoma malignancies. *Blood* **128**, 624 (2016).
46. B. Hassan, A. Akcakanat, A. M. Holder, F. Meric-Bernstam, Targeting the PI3-kinase/Akt/mTOR signaling pathway. *Surg. Oncol. Clin. N. Am.* **22**, 641–664 (2013).
47. M. Laplante, D. M. Sabatini, mTOR signaling in growth control and disease. *Cell* **149**, 274–293 (2012).
48. H. Pópulo, J. M. Lopes, P. Soares, The mTOR signalling pathway in human cancer. *Int. J. Mol. Sci.* **13**, 1886–1918 (2012).
49. J. A. Engelman, Targeting PI3K signalling in cancer: Opportunities, challenges and limitations. *Nat. Rev. Cancer* **9**, 550–562 (2009).
50. C. Porta, C. Paglino, A. Mosca, Targeting PI3K/Akt/mTOR signaling in cancer. *Front. Oncol.* **4**, 64 (2014).
51. K. A. Blum, B-cell receptor pathway modulators in NHL. *Hematology Am. Soc. Hematol. Educ. Program* **2015**, 82–91 (2015).
52. B. S. Kahl, S. E. Spurgeon, R. R. Furman, I. W. Flinn, S. E. Coutre, J. R. Brown, D. M. Benson, J. C. Byrd, S. Peterman, Y. Cho, A. Yu, W. R. Godfrey, N. D. Wagner-Johnston, A phase 1 study of the PI3Kδ inhibitor idelalisib in patients with relapsed/refractory mantle cell lymphoma (MCL). *Blood* **123**, 3398–3405 (2014).
53. H. Zhou, S. Huang, Role of mTOR signaling in tumor cell motility, invasion and metastasis. *Curr. Protein Pept. Sci.* **12**, 30–42 (2011).
54. D. A. Guertin, D. M. Sabatini, Defining the role of mTOR in cancer. *Cancer Cell* **12**, 9–22 (2007).
55. E. A. Dunlop, A. R. Tee, Mammalian target of rapamycin complex 1: Signalling inputs, substrates and feedback mechanisms. *Cell. Signal.* **21**, 827–835 (2009).
56. B. Su, E. Jacinto, Mammalian TOR signaling to the AGC kinases. *Crit. Rev. Biochem. Mol. Biol.* **46**, 527–547 (2011).
57. M. S. Hayden, S. Ghosh, NF-κB, the first quarter-century: Remarkable progress and outstanding questions. *Genes Dev.* **26**, 203–234 (2012).
58. S. A. Vlahopoulos, O. Cen, N. Hengen, J. Agan, M. Moschovi, E. Critselis, M. Adamaki, F. Bacopoulou, J. A. Copland, I. Boldogh, M. Karin, G. P. Chrousos, Dynamic aberrant NF-κB spurs tumorigenesis: A new model encompassing the microenvironment. *Cytokine Growth Factor Rev.* **26**, 389–403 (2015).
59. S. E. Turvey, A. Durandy, A. Fischer, S.-Y. Fung, R. S. Geha, A. Gewies, T. Giese, J. Greil, B. Keller, M. L. McKinnon, B. Neven, J. Rozmus, J. Ruland, A. L. Snow, P. Stepensky, K. Warnatz, The *CARD11-BCL10-MALT1 (CBM)* signalosome complex: Stepping into the limelight of human primary immunodeficiency. *J. Allergy Clin. Immunol.* **134**, 276–284 (2014).
60. N. Knies, B. Alankus, A. Weilemann, A. Tzankov, K. Brunner, T. Ruff, M. Kremer, U. B. Keller, G. Lenz, J. Ruland, Lymphomagenic CARD11/BCL10/MALT1 signaling drives malignant B-cell proliferation via cooperative NF-κB and JNK activation. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E7230–E7238 (2015).
61. S.-C. Sun, Non-canonical NF-κB signaling pathway. *Cell Res.* **21**, 71–85 (2011).
62. S. Vallabhapurapu, A. Matsuzawa, W. Z. Zhang, P.-H. Tseng, J. J. Keats, H. Wang, D. A. A. Vignali, P. L. Bergsagel, M. Karin, Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-κB signaling. *Nat. Immunol.* **9**, 1364–1370 (2008).
63. L. Odqvist, M. Sánchez-Beato, S. Montes-Moreno, E. Martin-Sánchez, R. Pajares, L. Sanchez-Verde, P. L. Ortiz-Romero, J. Rodriguez, S. M. Rodríguez-Pinilla, F. Iniesta-Martínez, J. C. Solera-Arroyo, R. Ramos-Asensio, T. Flores, J. M. Palanca, F. G. Bragado, P. D. Franjo, M. A. Piris, NIK controls classical and alternative NF-κB activation and is necessary for the survival of human T-cell lymphoma cells. *Clin. Cancer Res.* **19**, 2319–2330 (2013).
64. Y. N. Demchenko, L. A. Brents, Z. Li, L. P. Bergsagel, L. R. McGee, M. W. Kuehl, Novel inhibitors are cytotoxic for myeloma cells with NFkB inducing kinase-dependent activation of NFkB. *Oncotarget* **5**, 4554–4566 (2014).
65. M. Jerkeman, C. W. Eskelund, M. Hutchings, R. Räty, K. F. Wader, A. Laurell, H. Toldbod, L. B. Pedersen, C. U. Niemann, C. Dahl, H. Kuitunen, C. H. Geisler, K. Grønbæk, A. Kolstad, Ibrutinib, lenalidomide, and rituximab in relapsed or refractory mantle cell lymphoma (PHILEMON): A multicentre, open-label, single-arm, phase 2 trial. *Lancet Haematol.* **5**, e109–e116 (2018).
66. E. C. Townsend, M. A. Murakami, A. Christodoulou, A. L. Christie, J. Köster, T. A. DeSouza, E. A. Morgan, S. P. Kallgren, H. Liu, S. C. Wu, O. Plana, J. Montero, K. E. Stevenson, P. Rao, R. Vadhi, M. Andreeff, P. Armand, K. K. Ballen, P. Barzaghi-Rinaudo, S. Cahill, R. A. Clark, V. G. Cooke, M. S. Davids, D. J. DeAngelo, D. M. Dorfman, H. Eaton, B. L. Ebert, J. Etchin, B. Firestone, D. C. Fisher, A. S. Freedman, I. A. Galinsky, H. Gao, J. S. Garcia, F. Garnache-Ottou, T. A. Graubert, A. Gutierrez, E. Halilovic, M. H. Harris, Z. T. Herbert, S. M. Horwitz, G. Inghirami, A. M. Intlekofer, M. Ito, S. Izraeli, E. D. Jacobsen, C. A. Jacobson, S. Jeay, I. Jeremias, M. A. Kelliher, R. Koch, M. Konopleva, N. Kopp, S. M. Kornblau, A. L. Kung, T. S. Kupper, N. R. LeBoeuf, A. S. LaCasce, E. Lees, L. S. Li, A. T. Look, M. Murakami, M. Muschen, D. Neuberg, S. Y. Ng, O. O. Odejide, S. H. Orkin, R. R. Paquette, A. E. Place, J. E. Roderick, J. A. Ryan, S. E. Sallan, B. Shoji, L. B. Silverman, R. J. Soiffer, D. P. Steensma, K. Stegmaier, R. M. Stone, J. Tamburini, A. R. Thorner, P. van Hummelen, M. Wadleigh, M. Wiesmann, A. P. Weng, J. U. Wuerthner, D. A. Williams, B. M. Wollison, A. A. Lane, A. Letai, M. M. Bertagnolli, J. Ritz, M. Brown, H. Long, J. C. Aster, M. A. Shipp, J. D. Griffin, D. M. Weinstock, The public repository of xenografts enables discovery and randomized phase II-like trials in mice. *Cancer Cell* **29**, 574–586 (2016).
67. L. Zhang, K. Nomie, H. Zhang, T. Bell, L. V. Pham, S. Kadri, J. Segal, S. Li, S. Zhou, D. Santos, S. Richard, S. Sharma, W. Chen, O. Oriabure, Y. Liu, S. Huang, H. Guo, Z. Chen, W. Tao, C. Li, J. Wang, B. Fang, J. Wang, L. Li, M. Badillo, M. Ahmed, S. Thirumurthi, S. Y. Huang, Y. Shao, L. Lam, Q. Yi, L. Wang, M. Wang, B-cell lymphoma patient-derived xenograft models enable drug discovery and are a platform for personalized therapy. *Clin. Cancer Res.* **23**, 4212–4223 (2017).
68. X. Xu, M. C. Farach-Carson, X. Jia, Three-dimensional in vitro tumor models for cancer research and drug evaluation. *Biotechnol. Adv.* **32**, 1256–1268 (2014).
69. K. Kersten, K. E. de Visser, M. H. van Miltenburg, J. Jonkers, Genetically engineered mouse models in oncology research and cancer medicine. *EMBO Mol. Med.* **9**, 137–153 (2017).
70. R. Kohnken, P. Porcu, A. Mishra, Overview of the use of murine models in leukemia and lymphoma research. *Front. Oncol.* **7**, 22 (2017).
71. M. Hidalgo, F. Amant, A. V. Biankin, E. Budinská, A. T. Byrne, C. Caldas, R. B. Clarke, S. de Jong, J. Jonkers, G. M. Maelandsmo, S. Roman-Roman, J. Seoane, L. Trusolino, A. Villanueva, Patient derived xenograft models: An emerging platform for translational cancer research. *Cancer Discov.* **4**, 998–1013 (2014).
72. F. Widmer, S. Balabanov, D. Soldini, P. Samaras, B. Gerber, M. G. Manz, J. S. Goede, R-hyper-CVAD versus R-CHOP/cytarabine with high-dose therapy and autologous haematopoietic stem cell support in fit patients with mantle cell lymphoma: 20 years of single-center experience. *Ann. Hematol.* **97**, 277–287 (2018).
73. A. Lipsky, P. Martin, Bendamustine–rituximab in mantle cell lymphoma. *The Lancet Haematology* **4**, e2–e3 (2017).

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