Mantle Cell Lymphoma: Identifying Novel Molecular Targets in Growth and Survival Pathways

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Mantle cell lymphoma (MCL) remains one of the more challenging sub-types of non-Hodgkin lymphoma. This entity, which is only approximately 10 years old, is characterized by response to many different chemotherapy regimens, though the duration of those responses remains often times quite short. Re-treatment with second and third line combination regimens results in shorter and shorter durations of response, with the rapid emergence of a very drug-resistant phenotype. Despite these often frustrating clinical features, there is now a lot of new hope in managing patients with MCL. New insights into the molecular pathogenesis of MCL has revealed a plethora of new potential targets, while our continued efforts in novel targeted drug development has produced a host of agents that are already helping patients with this challenging disease. The use of proteasome inhibitors, for example, represents one example of a new strategy that has offered new hope for patients, and new opportunities for the physician treating this disease. In this review, we will put this biology into perspective, and describe how new revelations in MCL pathogenesis are leading to the identification of many exciting new drugs with promising activity.

Molecular Pathogenesis of Mantle Cell Lymphoma

Basic biological features of MCL

Mantle cell lymphoma (MCL) accounts for only about 6% of all cases of non-Hodgkin lymphoma (NHL), representing about 3000 to 4000 cases of the disease per year in the United States.[1] In addition, given the relatively short median overall survival (3 to 5 years), the prevalence of the disease is typically very low, with only about 15,000 cases of the disease being present in the population at any one time. Most pathologists recognize 4 variants of MCL based on cytologic appearance,[2] including *classic* (small- to medium-sized cells with infrequent larger neoplastic cells); *small cell* (small round lymphocytes resembling chronic lymphocytic leukemia with dense clumped chromatin); *blastic* (or blastoid) with intermediate-sized blasts, a high mitotic rate and frequent apoptotic bodies; and *pleomorphic* (with medium to large cells with large cleaved nuclei with prominent nucleoli). The immunophenotype reflects a mature B-cell arrangement ($CD45^+$, $CD19^+$, $CD20^+$, $CD22^+$, $CD24^+$ and $CD79a^+$) with strong IgM and/or IgD surface immunoglobulin. The presence of IgG or IgM in 20% of cases supports the contention that MCL can still undergo immunoglobulin isotype class-switching. Additional immunophenotypic features include $CD5^+$, $CD43^+$, $CD23^-$ and $CD10^-$.[2-4]

The immunoglobulin heavy-chain gene is known to be mutated in about 25% of all cases, which is lower than that seen with germinal center lymphomas. Unlike cases of chronic lymphocytic leukemia (CLL), unmutated MCL cases do not appear to exhibit a worse outcome than those with mutated IgH.[2] The well-known pathognomonic molecular lesion in MCL is the g(11:14)(q13;q32) translocation. This translocation juxtaposes the cyclin D1 gene downstream of the immunoglobulin heavy-chain gene promoter, leading to constitutive cyclin D1 overexpression.[5] The D-type cyclins complex with cyclin-dependent kinases 4 and 6 (CDK 4 and CDK6), which mediates the initial phosphorylation of the retinoblastoma protein (pRb) (**Figure 1**; see Color Figures, page 514). This step, followed by a similar phosphorylation reaction mediated by the cyclin E–cdk2 complexes, completes the pRb phosphorylation, which irreversibly induces progression of the cell through the $G_1 \rightarrow S$ phase transition. Subsequent progression through cell cycle is mediated by cyclin A–cdk2/1 and cyclin B–cdk1, which are responsible for the orderly S-phase progression and the $G_2 \rightarrow M$ transition.[6] This process is negatively influenced by a battery of cell-cycle–dependent kinase inhibitors belonging to the Cip/Kip (p21/p27) or INK 4 families (p16). Interestingly, it is not merely the driving force of cyclin D1 over-expression that defines MCL, but rather, the synergistic effects resulting from the simultaneous loss of CDK inhibitors (i.e., p21/p27; **Figure 2**; see Color Figures, page 514). These two cell-cycle–related events produce catastrophic cell-cycle dysregulation.

Dysregulation of cyclin D1 biology

Although translocations of cyclin D1 (CCND1) are ubiquitious in MCL, it is clear that a small percentage

of MCL cases (<10%) do not overexpress cyclin D1, and therefore lack any translocation affecting the 11 q3 locus.[7,8] A preliminary evaluation of 6 such cases suggests that these tumors exhibited a gene expression signature identical to that of cases with CCND1. In addition, in 2 of the cases, the CCND1 was replaced by CCND2 (cyclin D2), while in the other 4 cases, the CCND1 was replaced by CCND3 (cylin D3).[7,8]

Complicating this biology further is the observation that different isoforms of cyclin D1, namely D1a and D1b, can influence the rate of cellular proliferation and overall survival.[9] CCND1 contains 5 exons, which can be alternatively spliced into 2 major isoforms, cyclin D1a and cyclin D1b.[10-15] The cyclin D1a isoform is about 4.5 kb in length, and has a coding region of 882 bp. This mRNA is mostly composed of the 3′ untranslated region (3′UTR), which contains mRNA destabilizing elements. The cyclin D1b isoform is missing exon 5 but retains intron 4, which imposes a translation stop codon after 99 bp. Cyclin D1b also contains a polyadenylation signal close to the stop codon. In contrast to the cyclin D1a isoform, the D1b isoform is 1.7 kb and encodes a 274–amino acid protein (compared with the 294–amino acid protein encoded by the D1a isoform).[10-15] Interestingly, many of these studies have implicated the smaller cyclin D1b isoform as the potent transforming factor in many experimental models of human cancer.

Recently, Wiestner and colleagues[9] have provided additional insight into cyclin D1 isoform biology by demonstrating that truncated forms of cyclin D1a mRNA, which lack the 3′UTR, are more commonly associated with the most proliferative MCL tumors. The truncated version of the D1a isoform is associated with the highest levels of cyclin D1 protein expression, an observation felt to be independent of the cyclin D1b isoform. These truncated cyclin D1a isoforms are attributed to genomic deletions of the 3′UTR region and point mutations in the 3′UTR region that create alternative polyadenylation signals. These short truncated mRNAs are more stable (half-life of 3 hours) than the full-length mRNA (half-life of 30 minutes), and lack the typical mRNA-destabilizing elements.[9] Interestingly, those tumors with the truncated cyclin D1a isoform exhibit the highest proliferative rates, while those with the full-length mRNA exhibit the lowest proliferative rates. Surprisingly, this molecular finding also translated into a significant impact on survival, with those patients carrying the truncated cyclin D1a isoform having a median survival of only 1 year, compared with 3 years for those with the full-length mRNA transcript.[9] At a molecular level, it is becoming clear that the genomic deletions and point mutation, resulting in these differences in cyclin D1 isoforms, can produce profound effects on not just cell-cycle regulation, but also on the survival of patients with MCL.

Dysregulation of cyclin D1 biology alone does not produce MCL

As important as the cyclin D1 biology is in MCL, it is equally as clear that this disease is characterized by gross cell-cycle dysregulation that goes well beyond cyclin bi-

Table 1. Examples of novel targeted approaches for the treatment of mantle cell lymphoma (MCL).

Mechanism	Drugs	Rationale
Inhibition of antiapoptotic Bcl-2 family members	ABT-737/263 AT-101 GX015-oblimersen	Silence the antiapototic influence of Bcl-2, Bcl-xl, Bcl-w and Mcl-1
Modulation of proapoptotic family members and BH3-only proteins	Proteasome inhibitors (bortezomib, PR-171)	Up-regulation derepression of proapoptotoic family members will lead to induction of programmed cell death
Down-regulation of cyclin D1 and related isofroms	Cyclin D1 antisense (ASDON) Histone deacetylase inhibitors (SAHA)	Down-regulation of cyclin D1 and related isoforms will decrease the driving force for cells to transition from G_1 into S-phase, producing cell-cycle arrest
Increase cell-cycle–dependent kinase inhibitors like p27/p21	Proteasome inhibitors HDACI	A relative increase in cdk inhibitors will provide the "breaks" on cell-cycle proliferation, inducing cell cycle arrest
Inhibition of pan–cell-cycle–dependent kinases	Flavopiridol AG-024322	Induce cell-cycle arrest
Inhibition of selective cell-cycle–dependent kinases	PD-0332991 (cdk4/6) CINK4 (cdk4/6) Seliciclib (cdk2/1) BMS-387032 (cdk2/1) PNU-252808 (cdk2/1) PNU-252808 (cdk2/1) NU6102, NU6140 (cdk2/1)	Inhibit specific phase transitions of cell-cycle progression
Inhibit protein translation and signaling pathways mediated through tyrosine kinase receptors and ras	mTOR inhibitors (most derived from rapamycin, including temsirolimus), AKT inhibitor	Associated with a broad effect on cancer cell biology, including translation, NF-κB, transcription factors, and apoptosis

ology. In addition to having "gain-of-function" attributes that lead to rapid cellular proliferation (cylin D1 overexpression), tumors also exhibit "loss-of-function" attributes such as the depletion of cell-cycle–dependent kinase inhibitors (such as p21/p27), an event that synergizes with the cyclin D1 abnormalities. This point is underscored by the observation that transgenic mice overexpressing cyclin D1 in their lymphoid tissue do not develop lymphoma, but rather appear to develop lymphoid hyperplasia and sometimes breast cancer.[16,17] Collectively, these data suggest that overexpression of cyclin D1 alone is insufficient in producing MCL.

A molecular clue providing insight into this biology was provided in a couple of studies by Chiarle et al[18] and Lim et al.[19] A serial evaluation of NHL cases revealed that 91 of 112 cases of MCL and 12 of 19 cases of DLBCL had lost expression of p27.[18,19] This was in contrast to other types of "small lymphocytic lymphomas" like small lymphocytic lymphoma/chronic lymphocytic leukemia and extranodal marginal zone lymphomas, where p27 was found to be intact and ample.[19] Remarkably, the patients with MCL were found to have normal p27 mRNA expression, but demonstrated increased p27 protein degradation mediated via the ubiquitin-proteasome pathway. Clinically, patients with MCL who exhibit loss of p27 demonstrated a statistically significant reduction in overall survival.[18] While the precise mechanism of p27 loss is not entirely clear, some have suggested sequestration with cyclins D1 and D3, while others have proposed a correlation with the level of Skp2, a component of the p27Kip1 ubiquitin ligase.[19] Elevated levels of Skp2 are inversely associated with protein levels of p27, due to the fact that Skp2 levels correlate with greater E3 activity, and hence, greater proteasome-mediated degradation of the target protein (i.e., p27). These data collectively provide a very potent rational for the gross cell-cycle dysregulation of MCL. Perhaps more important, these data may provide a mechanistic basis for why proteasome inhibitors work in MCL. Bortezomib is known to produce accumulation of p27. It is possible that this inhibition of p27 degradation allows sufficient quantities of the protein to accumulate, "restoring" a more typical or wild-type state of p27 expression in those cells.

Recently, Lwin et al[20] have provided some elegant data linking the levels of p27Kip1/p21WAF1 and Skp2 in MCL, demonstrating that both proteins were markedly influenced by the cellular microenvironment. Interaction of the MCL cells with the stromal microenvironment up-regulated Chd1 (an activating subunit of anaphase-promoting complex [APC] ubiquitin ligase), which induced Skp2 accumulation and p27Kip1 degradation, implicating Cdh1 as an upstream effector of the Skp2/p27Kip1-signaling pathway. These authors demonstrated that adhesion of MCL to bone marrow stromal cells (HS-5) resulted in a reversible growth arrest and elevated p21 and p27Kip1 protein levels through down-regulation of the SCFSkp2 uniquitin ligase Skp2. This process was mediated through Cdh1, which was strongly induced by cell adhesion to stromal factors. These studies provide essential insights into the importance of cell-cell interactions in mediating cell-cycle regulation, and firmly establish a link between p27, Skp2 and basic proteasome biology.

Targeting Cell-Cycle Regulatory Pathways

Selectively targeting cyclin D1

Given the importance of cell-cycle dysregulation in MCL, it seems obvious that one of the first therapeutic strategies to consider in treating this disease is to target these aberrantly operating pathways. Strategies directed towards the reduction of cyclin D1, for example, have been studied using antisense oligodeoxynucleotide (ASODN) technologies. These approaches have the theoretical benefit of selectively targeting cyclin D1, although limitations in ASODN entry into all cells in a heterogeneously growing tumor can limit their effectiveness. Nonetheless, cyclin D1 ASODNs have been shown to decrease mRNA expression of cyclin D1, resulting in the inhibited growth and enhanced chemosensitivity of several cell lines, though the experiences in MCL specifically have been quite limited.[21,22]

Other pharmacologic strategies for modulating cyclin D1 have been recently described by Kawamata and colleagues.[23] These authors demonstrated that suberoylanilide hydroxamic acid (also called SAHA or vorinostat), a histone deacetylase inhibitor (HDACI) recently approved for the treatment of cutaneous T-cell lymphoma, dramatically decreased cyclin D1 levels by approximately 90% after only an 8-hour exposure to 5 μM of the drug in a number of MCL cell lines. Interestingly, mRNA and protein stability of cyclin D1 were minimally affected by SAHA. Metabolic labeling studies demonstrated a marked decrease in methionine incorporation into the cyclin D1 protein, implicating an effect on protein synthesis. SAHA also inhibited the kinase activity of phosphatidyl inositol (PI) kinase and decreased levels of phosphorylated AKT, mTOR, and eukaryotic initiation factor 4E binding protein (eIFE-BP). These data have opened a new and unexpected mechanism of action for the HDACIs, raising the prospect that these agents could be used with other more targeted agents affecting other aspects of the cell-cycle dysregulation in MCL.

Targeting cdk inhibitors

Another obvious strategy to consider in treating MCL would be to target the cell-cycle regulatory proteins directly. In fact, a growing number of cdk inhibitors are now rapidly moving from the laboratory into the clinic. Many of these agents are characterized as being either very specific cdk inhibitors (including for example, PD-0332991 and CINK4 targeting the cdk4/6 complex; and seliciclib and BMS-387032, targeting the cdks 2 and 1), or as pan cdk inhibitors (including for example, flavopiridol and AG-

024322).[6] In general, the principles for inhibiting these specific molecular targets is based on the following observations: (1) inhibition of the cdk 4/6 complex leads to potent Rb-dependent G_1 arrest; (2) inhibition of cdk 4/6, cdk2 and cdk1 leads to arrest at the G_1-S and G_2-M boundries; and (3) more selective inhibition of cdk2 and cdk1 leads to potent G_1 arrest and E2F-1–dependent apoptosis.[6] Flavopiridol for example, is a widely recognized potent semisynthetic flavonoid "pan-CDK inhibitor" that decreases cyclin D1 and D3 expression, resulting in cell-cycle arrest at G_1.[24] Venkataraman and colleagues[24] have shown that a 6-hour exposure to 10 nM flavopiridol induced apoptosis in a number of MCL cell lines. This induction of apoptosis was attributed to marked down regulation of key cell-cycle regulatory proteins acting at the restriction control point between G_1 and S phases. The clinical application of flavopiridol has been hampered by a number of important pharmacologic considerations. Specifically, the drug is very heavily protein bound, and requires an initial bolus infusion prior to the continuous infusion to overcome the significant protein binding.[25] Previous continuous infusion schedules failed to achieve significant steady-state concentrations of the drug in the plasma, and likely contributed to the limited activity of the compound in previously reported phase 1 and 2 studies. More recent bolus/continuous infusion studies in a number of hematologic malignancies, including CLL, highlight the drug's activity, which now includes tumor lysis syndrome.[25] Presently, a number of studies are beginning to explore the pharmacologic issues surrounding flavopiridol disposition in order to maximize its clinical effects.

A third and potentially very important strategy for targeting dysregulated cell-cycle processes is to modulate the cell-cycle–dependent kinase inhibitors. While there is no intuitively obvious strategy for accomplishing this, it has become increasing clear that proteasome inhibitors do consistently increase the levels of p21 and p27 across a number of different cell lines.[26] This observation, coupled with the fact that there may be unique pathogenetic defects in the ubiqutinating-proteasome pathway responsible for the depletion of p27 as discussed above, creates a strong rationale for these drugs in a disease grossly characterized by loss of cell-cycle inhibition. It is conceivable that the effects of bortezomib on particular cdk inhibitors is a major contributing factor in the drug's mechanism of action in MCL.

Targeting Apoptotic Pathways

Most cancers are characterized by not just their penchant for uncontrolled growth, but also by their quest to remain immortal. The targeting of those pathways that modulate elements of the programmed cell death pathway are only now being studied in the clinic. Like many other cancers, MCLs are known to be grossly dysregulated at many points in the pathways that determine their survival.[27] For example, MCLs exhibit marked down-regulation of the FAS-associated via death domain (FADD), a gene that acts downstream of the FAS cascade. In addition, certain MCLs have markedly decreased levels of the death-associated protein 6 (DAXX) gene, caspase 2 (CASP2) gene, the RIPK1 domain containing adapter with death domain (RAIDD) gene, and exhibit overexpression of Bcl-2 and Bcl-x.[27]

The cell-survival cascades are known to be regulated through the complex interplay between a number of different Bcl-2 family members. Broadly, these include: (1) antiapoptotic family members (Bcl-2, Bcl-xl, Bcl-w, Mcl-1); (2) propapoptotic family members (Bax, Bak); and (3) the BH3-only mimetics. The BH3-only family includes *activators* (Bim and tBid), which directly activate Bax/Bak, and *sensitizers/derepressors* (Bad, Bik, Bmf, Hrk, NOXA and PUMA), which do not directly activate proapoptotic family members, but rather, neutralize antiapoptotic proteins through the formation of protein-protein complexes.[28] Interestingly, this large family of proteins plays a crucial role in not just cell survival, but are well known to contribute to both intrinsic and acquired drug resistance. An imbalance of pro- and antiapoptotic influences raises the threshold required to induce apoptosis with conventional cytotoxic agents. Impairment of the cells' ability to undergo apoptosis as determined by the balance of these family members has been correlated with poor overall survival in a variety of different malignancies.

Therapeutic strategies targeting Bcl-2 represent a promising and exciting prospect for treating many types of cancers, including MCL. Given the prominent role Bcl-2 family members play in normal lymphocyte ontogeny and in lymphomagenesis, there is a strong rationale for targeting these family members in these diseases. At present, there are a number of different strategies for targeting both the intrinsic and extrinsic arms of these survival pathways, including: (1) agents that can affect the balance of pro- and antiapoptotic family members (for example, proteasome inhibitors); (2) ASODN that target Bcl-2 mRNA, leading to enhanced Bcl-2 message degradation; (3) small molecules that target specific binding sites in Bcl-2 family members; and (4) monoclonal antibodies that than act as agonists of the TRAIL-induced pathways.[29,30] Many lines of data have begun to validate that nullifying these important pathways might help overcome both acquired and intrinsic drug resistance. One of the major questions regarding the potential clinical application of these compounds however, revolves around how to exploit them in combination with conventional therapies. Practically, there is little expectation that these compounds will ever emerge as prominent single agents for the treatment of any cancer. The development path is likely to involve exploiting their ability to overcome mechanisms of drug resistance by integrating them into conventional cytotoxic chemotherapy regimens. This approach will require a detailed understanding of how Bcl-2–targeted drugs impact the relevant biology, and how basic pharmacologic considerations may affect the activ-

ity of the combination. In response to cellular damage, some BH3-only family members activate a cascade of events that leads to Bax and/or Bak activation, mitochondrial membrane permeabilization (MOMP), release of cytochrome c and other proapoptotic factors.[31,32]

For example, ABT-737 induces apoptosis by direct inhibition of Bcl-2, Bcl-X_L and Bcl-w, in a manner analogous to the proapoptotic BH3-only protein Bad. ABT-737 has shown potent single-agent efficacy against cell lines from lymphoid malignancies known to express high levels of Bcl-2 (follicular lymphoma and CLL) and small cell lung cancer.[33,34] Bad is a proapoptotic BH3-only protein that has been shown to cooperate with Noxa to induce potent killing by inducing Bax/Bak activation. Other small molecules now in development with similar effects on Bcl-2 family members include the orally bioavailable gossypol derivative AT-101 and GX15-070 (also know as obatoclax), both of which are now in late phase 1 and early phase 2 development.

While small molecules and antisense approaches offer a novel opportunity to down-regulate or inhibit those proteins responsible for their "antiapoptotic influence," it is enticing to think about strategies that might favorably alter the other arms in the apoptotic regulatory machinery. Approaches for increasing the levels of certain proteins in cancer cells pose significantly different challenges. It is clear, just as discussed earlier in the context of p27, that proteasome inhibitors, and possibly even HDACIs, may offer a pharmacologic approach for increasing protein levels, though there is little to no *a priori* rationale for knowing which proteins might be most favorably affected. While it is clear the proteasome plays an important role in regulating the intracellular availability of different proteins, it is equally unclear what all the cellular repercussions from this inhibition are likely to be.

For example, Marshansky et al[35] have demonstrated in both Jurkat (T-cell tumor) and Namala (B-cell tumor) cell lines that proteasome inhibition up-regulated the proapoptotic Bcl-2 family member Bik by decreasing its proteolytic degradation. Interestingly, other family members in this model, including Bax, Bak, and Bad, were not similarly affected. Up-regulation and accumulation of Bik was sufficient in inducing apoptosis in these leukemic cells. Additionally, these authors showed that proper functioning of the electron transport chain was dependent on proteasome activity, and that interruption of protein turnover adversely influenced the *trans*-mitochondrial membrane potential, leading to the induction of apoptosis.[35] It was shown that Bik and the antiapoptotic member Bcl-X_L coprecipitated, leading to the hypothesis that excess Bik could trap and theoretically nullify the influences of Bcl-X_L, since the level of Bcl-X_L was not changed following proteasome inhibition. This "trapping" of the Bcl-X_L offers a theoretical mechanism for overriding the antiapoptotic effects of Bcl-X_L, leading to cell death.

Interestingly, Perez-Galan et al[40] have shown that bortezomib induces apoptosis, mitochondrial depolarization, reactive oxygen species (ROS) generation, Bax and Bak conformational changes, and caspase activation in MCL lines. However, they demonstrated that bortezomib induced a marked up-regulation of Noxa independent of p53 status. The increase in Noxa led to activation of Bax, and provided an important finding that could nullify the potentially negative impact on Mcl-1 from bortezomib (**Figure 3**; see Color Figures, page 514). The effects of proteasome inhibitors on apoptosis have prompted investigators to consider rational combinations of these agents and the BH3-only mimetics. In an one example, the combination of bortezomib with the Bcl-2 antisense molecule oblimersen was evaluated as a strategy for sensitizing lymphomas to conventional cytotoxic agents. These data demonstrated that while the combination of bortezomib and oblimersen induced significant growth delay in a large B-cell lymphoma model, it markedly sensitized lymphoma in xenograft models to even nominal doses of conventional cyclophosphamide.[36] Recently, Paoluzzi et al[37] and Perez-Galan et al[38] have similarly demonstrated that the combination of a BH3 only mimetic with a proteasome inhibitor appears to be synergistic in a variety of lymphomas, including models of MCL with both wild-type and mutated p53 (**Figure 3**; see Color Figures, page 514).

The convergence of growth and survival pathways: the importance of the PI3′kinase-AKT-mTOR pathway

Cell viability and growth also depend in part on the status of the local tumor microenvironment. Activating transmembrane tyrosine kinases and activated *ras* proteins enhance the catalytic activity of the lipid kinase phosphatidylinositol-3-kinase (PI3K), an event that initiates a vast cascade of downstream second messengers (i.e., phospholipids).[39] These second messengers, which are derived from phosphorylation of phosphatidyl inositol (PI), play an important role in regulating the growth and survival of many cancer cells, including MCL. PI3K activation results in activation of AKT, a serine kinase that modulates a variety of cellular processes, including those that influence apoptosis and proliferation. It can also alter the function of downstream targets such as mTOR and other critical proteins like caspase 9, Bcl-2 family members and NF-κB.[39] For these reasons, PI3K, AKT and mTOR are all subjects of experimental therapeutics programs in MCL.

mTOR is a protein kinase that regulates mRNA translation by phosphorylating two critical substrates, namely eIF4E-BP and p70S6 kinase. These proteins play a major role in enhancing translation of proteins, including c-myc, cyclin D1 and ribosomal proteins themselves. The observation that the mTOR pathway plays a major role in MCL growth, and survival has been underscored by a recent study by Witzig et al.[40] These authors demonstrated that the potent mTOR inhibitor, temsirolimus (CCI-779), produced a 38% overall response rate in 35 patients with heavily pretreated MCL. These responses also included one complete remission. The median time to progression was approxi-

mately 6.5 months, and the duration of response among the responders was 6.9 months.[40] This study validates the importance of targeting this pathway in MCL, and provides a new and potentially complimentary approach with other targeted agents discussed above.

Conclusion

As our understanding of the pathways that regulate the growth and survival of MCL become more clear, more rational and innovative combinations that target the specific pathways of interest will begin to emerge. New platforms combining drugs that affect Bcl-2, proteasome biology, and the PI3K-AKT-mTOR pathway are establishing a biologically oriented approach for the disease. It is unlikely that these new treatment paradigms will eliminate the need for traditional chemotherapy regimens. It is more likely they will lower the threshold for induction of programmed cell death, enhancing the effectiveness of conventional chemotherapy approaches. As new more "targeted agents" emerge, it will be critical to understand how these drugs perturb the essential signaling networks in different diseases, and to validate these findings in both the laboratory and clinic. Given all the intrinsic complexities associated with these agents and the diseases we target, there will need to be a major emphasis on identifying the most appropriate pharmacodynamic endpoints and how they are modulated as a function of the observed pharmacokinetic profiles.

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