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Targeting BCL2 in Hematologic Malignancies

Summary

B-cell lymphoma-2 (BCL2) family proteins, key regulators of apoptosis, are widely expressed in a variety of human cancers. Hematologic malignancies, particularly chronic lymphocytic leukemia (CLL), are especially dependent on this family of proteins. Improved technologies in recent years have enabled an understanding of the structural relationships between BCL2 family members, facilitating the successful development of effective inhibitors of BCL2 proteins. The BCL2 inhibitor venetoclax first received Food and Drug Administration (FDA) approval in April 2016 for the treatment of CLL, and promising efficacy data are emerging in other types of hematologic malignancies. Investigation of venetoclax as monotherapy and in combination therapy approaches continues with anticipation that venetoclax could be added to the arsenal of therapy options available for patients across the spectrum of hematologic cancers.

Introduction

The BCL2 family of proteins are central regulators of apoptosis. Apoptosis, also known as “programmed cell death”, can occur in response to intrinsic stress signals or environmental cues. During the lifespan of any organism, proliferation must be balanced with apoptosis to ensure both appropriate development and proper mature physiological cell and organ function. This balance between proliferation and apoptosis is particularly important in highly proliferative tissues, such as the bone marrow.(Moia, 2018, p392, col1, par1, lines 1-10) Deregulation of apoptotic pathways can lead to cancer, and resistance to apoptosis was identified as a hallmark of human cancer nearly 20 years ago.(Hanahan, 2000, p61, col1, par4, lines 1-10) Over the past two decades, considerable effort has focused on developing therapies that can restore apoptosis in malignant cells.(Anderson, 2014, p219, col2, par2, lines 1-5)

Members of the BCL2 family of proteins can either inhibit or activate apoptosis. Anti-apoptotic family members include BCL2, BCL-X_L, BCL-W, BCL-B, BFL1, and myeloid cell leukemia 1 (MCL1). Pro-apoptotic family members can be subdivided into two subfamilies: the multi-domain effector proteins, BAX and BAK, and the BH3-only proteins, BID, BIK, NOXA, PUMA, BAD, and BIM.(Moia, 2018, p392, col2, par3, lines 2-9) Under normal circumstances, the anti-apoptotic and pro-apoptotic family members bind to each other in various combinations to mutually inhibit their individual functions.(Moia, 2018, p392, col2, par2, lines 11-14) The anti-apoptotic proteins BCL2 and BCL-X_L inhibit the pro-apoptotic effector proteins BAX and BAK. However, BH3-only proteins sequester and inhibit BCL2 and BCL-X_L, freeing BAX and BAK to initiate the cascade of events that lead to cell death.(Moia, 2018, p 392-393, col2, par3, lines 12-18) Therefore, strategies to develop inhibitors of anti-apoptotic BCL2 family proteins have focused on agents that can interfere with these interactions and promote the activity of the pro-apoptotic proteins. Recent advances in agents that can inhibit the anti-apoptotic BCL2 proteins have begun to validate the potential of this approach and provide new treatment options for patients with aggressive disease.

BCL2 deregulation in hematologic malignancies

In normal healthy lymphoid cells, pro-survival members of the BCL2 family restrain BAX and BAK to maintain cell viability.(Anderson, 2014, p220, col1, par3, lines 1-4) However, BCL2 overexpression is one of

the most common alterations in lymphoid malignancies, which disrupts the balance between the pro-apoptotic and anti-apoptotic proteins.(Anderson, 2014, p220, col1, par4, lines 4-18) Cells that overexpress BCL2 survive despite exposure to cell death stimuli, and the inappropriate survival of BCL2-overexpressing cells contributes to the pathogenesis of a variety of malignancies.

BCL2 overexpression is characteristic of multiple hematologic malignancies, but the mechanisms leading to overexpression differ between tumor types.(Anderson, 2014, p220, col1, par4, lines 1-18) BCL2 was first identified as a result of cloning of the t(14;18)(q32;q31) chromosomal translocation in follicular lymphoma (FL).(Tsujimoto, 1984, p1097, col1, par1, lines 3-8) This translocation places the immunoglobulin heavy chain gene enhancer in 14q32 in the region of the BCL2 promoter, leading to upregulation of BCL2 expression.(Tsujimoto, 1984, p1099, col1, par2, lines 19-24) This mechanism of elevated BCL2 expression contributes to survival in multiple hematologic malignancies; approximately 70-90% of FL, 20-30% of diffuse large B-cell lymphomas (DLBCL), and 5-10% of other less common subtypes of non-Hodgkin lymphoma (NHL) harbor this translocation.(Moia, 2018, p393, col1, par3, lines 3-10) (Chiu, 2008, p1, par1, lines 2-4)

BCL2 overexpression and impaired apoptosis are also hallmarks of chronic lymphocytic leukemia (CLL).(Moia, 2018, p391, col2, par1, lines 8-9)(Anderson, 2014, p220, col1, par4, lines 1-18) Unlike FL and other NHL malignancies, deletion of 13q14 is the most frequent genetic lesion in CLL, occurring in 50-60% of cases.(Dohner, 2000, p1911, col2, Table1) The minimal deleted region in this lesion contains the microRNAs *miR-15a* and *miR-16*, which normally inhibit BCL2 transcription.(Calin, 2002, p15528, col1, par2, lines 1-5) Deletion of these microRNAs, which has also been observed in up to 70% of mantle cell lymphomas (MCL), leads to elevated BCL2 expression and constitutive survival of tumor cells.(Stilgenbauer, 2002, p1891, col1, par1, lines 14-15)(Moia, 2018, p391, col1, par3, lines 1-9) Epigenetic silencing due to high histone deacetylase activity in CLL can also cause decreased expression of *miR-15a/16*.(Moia, 2018, p394, col1, par1, lines 3-8) In addition to chromosomal deletion and epigenetic silencing, these microRNAs can be regulated by other proteins, such as TP53. Under normal circumstances, TP53 can enhance *miR-15a/16* transcription.(Moia, 2018, p393, col2, par1, lines 22-24). However, TP53 is often altered in hematologic malignancies, including CLL.(Stengel, 2017, p705, par1, lines 3-6) Loss of TP53 function in CLL and other hematologic cancers impairs *miR-15a/16* transcription and promotes elevated BCL2 expression. In addition, TP53 normally upregulates expression of the pro-apoptotic BH3-only proteins NOXA and PUMA.(Ortiz-Maldonado, 2016, p323, col2, par1, lines 1-19) Therefore, loss of TP53 can also decrease the levels of the pro-apoptotic proteins, thus shifting the balance between the apoptotic regulators and resulting in aberrant cell survival. Additional pro-survival mechanisms observed in CLL include hypomethylation of the BCL2 gene promoter and downregulated expression of BAX and BAK, leading to an increase in the BCL2/BAX ratio.(Moia, 2018, p394, col1, par1, lines 3-8)(Ortiz-Maldonado, 2016, p323, col2, par1, lines 1-19) BCL2 overexpression has also been observed in multiple myeloma (MM), acute lymphoblastic leukemia (ALL), and some T cell lymphomas.(Anderson, 2014, p220, col1, par4, lines 1-18) Due to the numerous mechanisms leading to BCL2 overexpression, targeting anti-apoptotic BCL2 family proteins is an attractive therapeutic strategy for CLL and other hematologic malignancies.

Benefits of targeting BCL2

Inhibiting anti-apoptotic BCL2 functions may have enhanced benefits compared with other targets in hematologic malignancies. Numerous studies have demonstrated that BCL2 overexpression plays a functional role in driving malignant transformation and therapeutic resistance in CLL, FL, DLBCL, and MM.(Cory, 2002, p653, col2, par1-2)(Volger, 2017, p365, col2, par3, lines 10-12) In addition, BCL2 mutations in FL have been associated with progression to more aggressive DLBCL.(Correia, 2015, p663, col1, par3, lines 1-

[7)] Therefore, targeting a driver of tumor progression and resistance may represent an effective approach for treating these diseases.

In addition to the elevated BCL2 expression in hematologic malignancies, recurrent alterations in other pathways may provide further rationale for targeting BCL2. DNA repair pathway defects are common in CLL and contribute to reduced sensitivity or resistance to chemotherapeutic agents.(Shatnyeva, 2015, col1, par1, lines 5-8) For example, TP53 disruption is a strong predictor of resistance to chemo- or immunotherapy. However, the apoptosis pathway is downstream of the DNA repair pathway, and human B-lymphoblast cell lines or primary CLL cells with TP53 defects maintain sensitivity to BCL2 inhibitors in culture.(Moia, 2018, p394, col2, par2, lines 1-3) Because TP53 activates apoptosis by inducing BH3-only proteins, agents that antagonize BCL2 act downstream of TP53 and may be able to overcome the block to apoptosis caused by TP53 disruption.(Moia, 2018, p394, col2, par3, lines 1-16) In addition, because BCL2 overexpression can block the cell death initiated by cytotoxic therapies, targeting BCL2 may represent a novel strategy in synergistic combination approaches to overcome resistance to other therapies.(Anderson, 2014, p220, col2, par1, lines 1-7)

BCL2-targeted therapies

While more than 20 agents have been developed to target BCL2 family proteins, only a handful have been investigated in clinical trials.(Wu, 2018, p20, col2, par4, lines 10-11) Although the early agents failed to demonstrate sufficient efficacy and/or safety, recent efforts have led to the development of more effective inhibitors with promising clinical outcomes in a variety of hematologic cancers.

Anti-sense oligonucleotides: oblimersen

Initial efforts to develop anti-BLC2 therapies focused on strategies to block BCL2 synthesis. The first agent developed to block BCL2 synthesis was the anti-sense oligonucleotide oblimersen.(Ortiz-Maldonado, 2016, p324, col1, par4, lines 1-8) Anti-sense oligonucleotides can prevent translation of specific proteins by causing enzymatic cleavage of the targeted mRNA message. Oblimersen selectively hybridizes to the first 6 codons of the open reading frame for the BCL2 protein. Binding of the anti-sense oligonucleotide ultimately prevents BCL2 protein translation.(Cheson, 2007, p 856, col2, par3, lines 1-13) Oblimersen was evaluated in phase III clinical trials but was denied FDA approval in 2008 due to insufficient clinical efficacy.(Ortiz-Maldonado, 2016, p324, col1, par4, lines 1-8)

BH3 mimetics

More recent approaches to targeting BCL2 have focused on BH3 mimetics. The development of advanced technologies, including nuclear magnetic resonance (NMR)-based approaches combined with structure-activity relationships (SAR), have enabled the structural understanding of the binding between BH3-only proteins and the pro-survival BCL2 family proteins.(Reufli-Brasse, p3646, par1, lines 1-5)(Moia, 2018, p 394, col1, par4, lines 1-9) This understanding has subsequently led to the design of small molecules that can activate apoptosis by inhibiting BCL2 and BCL-X_L.(Moia, 2018, p 394, col1, par4, lines 1-9) BH3 mimetics function by competing with the pro-apoptotic proteins (BAK or BAX) for binding to BCL2. By sequestering BCL2, the BH3 mimetics enable BAX or BAK activation, leading to downstream caspase activation and cell death.(Ortiz-Maldonado, 2016, p324, col2, par1, lines 2-9)

During the development of anti-BCL2 therapies, the following four conditions are required to be considered an effective inhibitor: 1) the biological activity of the agent must depend on BAK and/or BAX; 2) the binding affinity of the compound to at least one of the anti-apoptotic BCL2 family proteins must be in the low nanomolar range; 3) the cytotoxic effects of the agent should correlate

with pro-apoptotic BCL2 protein levels in the cell and with the binding profile of the agent to BCL2 family members; and 4) in vivo treatment with the agent should result in modulation of relevant biomarkers, such as decreased platelet levels for BCL-X_L antagonists or reduced lymphocyte numbers for BCL2 antagonists.(Moia, 2018, p394, col1, par4, lines 10-20) Numerous BH3 mimetics, including obatoclax, navitoclax, and venetoclax, have been developed and tested. As described below, each agent has differing specificity for the various anti-apoptotic proteins, resulting in varying levels of efficacy and toxicities between agents.(Ortiz-Maldonado, 2016, p324, col1, par5, lines 4-12)

Obatoclax

Obatoclax (GX-15-070) inhibits all BCL2 family anti-apoptotic proteins, including BCL-2, BCL-X_L, BCL-W, and MCL-1.(Brown, 2017, p3336, col2, par2, lines 1-3) Obatoclax was investigated in a phase I clinical trial in patients with advanced CLL. Although BAX upregulation correlated with drug exposure, only one of the 26 patients treated with obatoclax achieved partial response.(O'Brien, 2009, p301, col 1, par1, lines 8-11)(O'Brien, 2009, p305, col 1, par3, lines 1-4) In addition, treatment with the drug was associated with unexplained neurological side effects, including ataxia and euphoria.(O'Brien, 2009, p301, col 1, par3, lines 1-4) Obatoclax was also evaluated in a separate phase I trial of 13 patients with relapsed CLL to assess the combination of obatoclax with fludarabine and rituximab. The overall response rate (ORR) was 85%, with 15% complete response (CR).(Brown, 2015, p3340, col1, par1, lines 18-22) Neurological toxicities, including euphoria, ataxia, and dizziness, were also observed in this study.(Brown, 2015, p3338, col2, par2, lines 8-15) Development of obatoclax was discontinued in 2013.(Volger, 2017, p368, col2, par 2, lines 10-11)

Navitoclax

Navitoclax (ABT-263), another potent, orally bioavailable BAD-like BH3 mimetic, entered clinical trials in 2006.(Moia, 2018, p394, col1, par5, lines 1-2) Navitoclax inhibits BCL2, BCL-X_L, and BCL-W and has been shown to be much more effective than obatoclax.(Anderson, 2014, p222, col2, par3, lines 2-8) In a phase I trial of navitoclax in patients with relapsed or refractory lymphoid malignancies, clinical activity was observed across all tumor types.(Wilson, 2010, p6, par4, lines 1-2) The best clinical activity was observed in CLL with a response rate of 50%; all of the 7 patients with CLL treated with navitoclax achieved at least a 50% reduction in leukemia cells.(Wilson, 2010, p6, par1, lines 4-8) Importantly, responses to navitoclax have included patients with poor prognostic features, including deletion of chromosome 17p [del(17p)], which causes loss of TP53, and bulky disease.(Anderson, 2014, p222, col2, par4, lines 1-11) Navitoclax has also been evaluated in combination with rituximab in patients with relapsed/refractory CLL and previously untreated CLL; in this study, navitoclax demonstrated improved response rates compared with rituximab alone.(Roberts, 2015, p669, par1, lines 3-8)(Kipps, 2015, p2826, col1, par1, lines 1-16) However, toxicities have been associated with navitoclax treatment. Grade 3 and 4 neutropenias have been observed in up to 29% of patients in trials of navitoclax. Because treatment of neutrophils with navitoclax in vitro does not cause apoptosis, the mechanism for this toxicity is unknown.(Anderson, 2014, p223, col1, par1, lines 14-19) In addition, significant thrombocytopenia that correlates with drug concentration in the blood has been observed upon treatment with navitoclax. Because BCL-X_L is highly expressed in platelets, this adverse effect is thought to be a result of "on-target" BCL-X_L inhibition.(Moia, 2018, p394, col2, par1, lines 5-7)(Anderson, 2014, p223, col1, par1, lines 1-7) The thrombocytopenia associated with navitoclax treatment is a limitation for the clinical use of this drug, but the efficacy observed in the early trials provided proof-of-concept for the therapeutic potential of targeting BCL2 family members.(Reufli-Brasse, p3646, par3, lines 13-16)

Venetoclax

Venetoclax (ABT-199) was developed by re-engineering navitoclax to generate a molecule that has high binding affinity for BCL2 and low or no binding affinity for BCL-X_L or BCL-W.(Souers, p204, col1, par2, lines 1-6) The affinity of venetoclax for BCL-X_L or BCL-W is more than 100-fold less than that for BCL-2, and venetoclax binds to BCL2 with greater affinity than BIM.(Reufli-Brasse, p3646, par4, lines 3-4)(Moia, 2018, p394, col2, par2, lines 7-8) Venetoclax binding to BCL2 disrupts the ability of BCL2 to inhibit BAK and BAX, allowing these pro-apoptotic mediators to initiate the cell death cascade.(Moia, 2018, p394, col2, par2, lines 12-16)

Impressive results were observed in the first clinical trial of venetoclax, which was a phase I dose-escalation study in patients with relapsed/refractory CLL or small lymphocytic lymphoma (SLL). The aim of this study was to evaluate the safety, pharmacokinetics, and efficacy of venetoclax. Among all patients with CLL, including those with del(17p) and bulky or fludarabine-refractory disease, the overall survival rate at 2 years was 84%.(Roberts, 2016, p319, col2, par1, line 9-10)(Roberts, 2016, p318, col2, par2, lines 5-17) Among the patients with del(17p), the response rate was 71%, including 16% who experienced CR. This outcome was promising because TP53 loss-of-function is generally considered a major obstacle to successful therapy in CLL.(Roberts, 2016, p320, col2, par2, lines 9-24) In addition, 5% of patients treated with venetoclax achieved minimal residual disease (MRD) negativity, which does not often occur in patients with relapsed/refractory disease.(Ortiz-Maldonado, 2016, p325, col2, par2, lines 5-8) The most significant safety finding in this trial was tumor lysis syndrome (TLS), which occurred in 18% of patients, particularly those with high tumor burden.(Roberts, 2016, p315, col2, par3, lines 1-11) Based on this early safety concern, an amendment was added to the study protocol to include TLS prophylaxis measures (including intravenous hydration, allopurinol with or without rasburicase, and strict biochemical TLS monitoring).(Moia, 2018, p395, col1, par1, lines 12-15) In addition, the venetoclax dose was increased for each patient through a stepwise ramp-up phase over several weeks, starting with 20 mg/day and increasing to a target dose of 400 mg/day. The combination of this ramp-up phase with strict adherence to prophylaxis eliminated additional cases of clinical TLS.(Roberts, 2016, p320,col1, par1,lines 1-19) Neutropenia and gastrointestinal side effects were also observed in patients treated with venetoclax. (Roberts,2016, p317, col1, par2, lines 3-11 & Table 2) A separate phase II study evaluated venetoclax in more than 100 patients with relapsed/refractory CLL with 17p deletion. In this trial, nearly 80% of patients experienced response, 40% achieved MRD negativity, and no cases of clinical TLS were recorded.(Stilgenbauer, 2016, p1,par3, lines 1-4) Together, these trials demonstrated that venetoclax monotherapy is associated with higher CR rates than other drugs or combinations of drugs in relapsed/refractory CLL and is effective in patients with del(17p) CLL, which is a very poor prognosis population.(Roberts, 2017, p12, Table 1)(Stilgenbauer, 2016, p1, par4, lines 1-3) These data provided the basis for the initial FDA approval of venetoclax in 2016 as second-line therapy for CLL associated with del(17p).(Roberts, 2017, p12, Table 1)

Venetoclax has also been evaluated in clinical trials in combination with other agents in CLL. In a Phase Ib study of venetoclax plus the anti-CD20 antibody rituximab in patients with relapsed/refractory CLL, the ORR was 86%, with CR observed in 51% of patients. In addition, 67% of patients were MRD negative after a median follow-up of almost 10 months. After 2 years, 82% of patients were progression-free, and all MRD negative patients remained in remission at 9.7 months after interruption of venetoclax. This result provided proof of concept that patients in remission do not necessarily need to receive continuous venetoclax therapy. This outcome may suggest that eradication of disease may be a realistic outcome for patients with CLL, establishing a new paradigm for CLL treatment.(Seymour, 2017, p2, par3, lines 11-16)(Moia, 2018, p398, col1, par4, lines 25-28) A phase III trial (MURANO) comparing the combination of venetoclax plus rituximab vs. bendamustine plus rituximab also demonstrated venetoclax benefit across all subgroups analyzed, with 2-year

progression-free survival rates of 84.9% for venetoclax plus rituximab vs. 36.3% for bendamustine plus rituximab.(Seymour, 2018, p1107, col1, par1, lines 5-12) The rates of progression-free survival at 2 years were similar between patients with del(17p) and those without del(17p).(Seymour, 2018, p1107, col1, par1, lines 5-12) Grade 3 or 4 TLS occurred in 3.1% of patients in the venetoclax plus rituximab group, and higher rates of grade 3 or 4 neutropenia were observed in the venetoclax plus rituximab group than in the bendamustine plus rituximab group.(Seymour, 2018, p1107, col1, par1, lines 15-19) The data from this trial led to the expanded FDA approval of venetoclax in June 2018 as second-line therapy for any patient with CLL.

Trials evaluating the combination of venetoclax plus obinutuzumab, another anti-CD20 monoclonal antibody, have also shown encouraging responses. In a phase Ib study in patients with relapsed/refractory or previously untreated CLL, all 32 previously untreated patients responded to venetoclax plus obinutuzumab therapy, with CR in 56.3% of patients. MRD negativity was observed in 100% of patients, including those with 17p deletion, and the progression-free survival rate at 1 year was 100%.(Flinn, 2017, p2, par 3, lines 1-6)

In addition to combinations with anti-CD20 antibodies, venetoclax has also been evaluated with the BTK inhibitor ibrutinib. In a phase II trial of venetoclax plus ibrutinib in patients with relapsed/refractory CLL or untreated patients with high-risk features (such as 17p and 11q deletions, TP53 mutations, unmutated IGHV, or age over 65 years), CRs were observed in more than half of the patients. Among the 14 patients with relapsed/refractory CLL, a CR rate of 64% was observed, and a CR rate of 56% was observed among the 16 high-risk, treatment naïve patients.(Jain, 2017, p2, par3-4) In a separate trial of venetoclax plus ibrutinib in 25 patients with relapsed/refractory CLL, early results have indicated an ORR of 100% and a CR rate of 60%, with 28% of patients achieving MRD negativity.(Hillmen, 2017, p2, par4, lines 11-14)(Hillmen, 2017, p3, p1, lines 1-2) Venetoclax and ibrutinib have also been combined with obinutuzumab in a phase Ib study in patients with relapsed/refractory CLL; among the 12 patients assessed, 92% experienced a response, including CR in 42% of cases and MRD negativity in 50% of cases. A phase II study with this combination of agents is ongoing in patients with relapsed/refractory or treatment-naïve CLL, and a phase III study is planned.(Rogers, 2018, p1568, par1, lines 13-18) Together, these studies have provided support for continued evaluation of venetoclax plus ibrutinib therapies as well as additional combinations of venetoclax with other agents in CLL.

Venetoclax in other hematologic malignancies

Multiple myeloma

Venetoclax has shown promising signs of efficacy in additional hematologic malignancies, including MM, NHL, and AML. In multiple myeloma, the t(11;14)(q13;q32) translocation is present in approximately 15-20% of all patients with MM, and cells with this genetic lesion are highly sensitive to venetoclax in vitro.(Valentin, 2018, p9, par3, lines 1-3) Venetoclax monotherapy has been evaluated in a phase I study in heavily pretreated patients with relapsed/refractory MM, and an ORR of 40% was observed in the subgroup of patients harboring the t(11;14) translocation.(Kumar, 2017, p4, par1, lines 12-14) Combination studies in MM have included a phase I study of venetoclax plus dexamethasone in patients with t(11;14) relapsed/refractory MM. Dexamethasone has been shown to cause greater release of BIM from BCL2 than venetoclax alone, which results in greater activation of BAX/BAK.(Valentin, 2018, p10, par1, lines 1-2) Early results have indicated an ORR of 65%, with higher ORRs in patients refractory to bortezomib (ORR of 82%) and lenalidomide (ORR of 71%).(Kaufman, 2017, p2, par2, lines 1-3)(Kaufman, 2017, p2, par2, lines 1-3) In addition, proteasome inhibitors, such as

bortezomib, have been shown to induce apoptosis through upregulation of NOXA and MCL-1, providing rationale for combining venetoclax with these agents.(Valentin, 2018, p10, par2, lines 1-2) A phase Ib study of venetoclax plus bortezomib and dexamethasone in patients with relapsed/refractory MM has also demonstrated ORR of 67% among the 66 patients enrolled, with ORR of up to of 97% in patients who were not refractory and had 1-3 prior therapies. In addition, patients whose tumor cells expressed high levels of BCL2 exhibited a higher ORR compared with patients with low BCL2 expression (94% vs. 59%, respectively).(Moreau, 2017, p4, par1, lines 12-16) The observed adverse events were mild gastrointestinal toxicities and grade 3 or 4 cytopenias, which was considered an acceptable safety profile.(Moreau, 2017, p4, par1, lines 10-11) Additional trials of venetoclax combinations in MM are ongoing, including combinations with MEK inhibitors (cobimetinib), immune checkpoint inhibitors (atezolizumab), monoclonal antibodies targeting other proteins (daratumumab, an anti-CD38 antibody), and proteasome inhibitors (bortezomib and carfilzomib).(Valentin, p10, par2, lines 9-12)

Non-Hodgkin Lymphoma

Venetoclax has also been assessed in patients with NHL. In a phase I study of 106 patients with relapsed/refractory NHL, 44% achieved an objective or partial response across all NHL tumor subtypes. Patients with MCL experienced the highest ORR of 75%; this rate is similar to the rates of response to venetoclax seen in CLL.(Davids, 2017, p876, para3, lines 8-10) However, lower response rates were observed in other NHL subtypes. The modest activity of venetoclax as a single agent across the NHL subpopulations highlights the importance of considering combination approaches. In a phase I trial, venetoclax was combined with bendamustine and rituximab in patients with relapsed/refractory NHL. Among 60 patients enrolled, the ORR was 65%, with higher responses observed in patients with FL and marginal zone lymphoma (MZL) (ORRs of 75% and 100%, respectively).(Swinnen, 2017, p90, Table 1) In addition, a phase II study evaluated venetoclax plus ibrutinib in patients with relapsed/refractory and previously untreated mantle cell lymphoma (MCL). Among the 24 patients enrolled, half harbored TP53 aberrations and 75% had a high-risk prognostic score.(Tam, 2018, p1211, par3, lines 1-4) At week 16, the CR rate was 42%, which is higher than the historical CR rate of 9% observed with ibrutinib alone. In addition, MRD negativity was observed in 67% of patients.(Tam, 2018, p1211, par3, lines 5-9) Several studies are also assessing venetoclax in combination with established chemotherapy regimens for NHL, including R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) and G-CHOP (obinutuzumab, cyclophosphamide, doxorubicin, vincristine, and prednisone).(Valentin, 2018, p9, par 2, lines 1-10)

Acute Myeloid Leukemia

Venetoclax has also been evaluated as a monotherapy and in combinations in patients with AML. A phase 2 study of 32 patients with relapsed/refractory AML or with AML unfit for intensive chemotherapy found ORRs of 19%, with a 33% CR rate in patients with IDH1/2 mutations.(Konopleva, 2017, p1107, par1, lines 5-10) The results from this study provided the rationale for combining venetoclax with other agents in patients with AML, and multiple ongoing studies are evaluating a variety of combinations.(Konopleva, 2017, p1107, par2, lines 3-4) Preclinical studies have suggested that hypomethylating agents, such as the DNA methyltransferase inhibitor 5-azacytidine, can decrease MCL-1 expression in AML cells.(Valentin, p11, par1, lines 1-3) A phase Ib study has evaluated venetoclax plus azacytidine or venetoclax plus decitabine in patients older than 65 years with treatment-naïve AML ineligible for intensive chemotherapy. While the historical CR rates for the single hypomethylating agents decitabine and azacytidine are 25.6% and 27.8%, respectively, the combination of venetoclax plus the hypomethylating agents resulted in a CR rate of 67%.(Valentin, 2018, p11, par1, lines 4-10)(DiNardo, 2018, p3, par1, lines 11-13) In addition, for patients who are ineligible for induction

chemotherapy, this combination represented a bridging strategy to allogenic stem cell transplantation. Venetoclax received its 3rd breakthrough therapy designation from the FDA for treatment-naïve AML patients unfit for standard induction chemotherapy (after receiving the first two breakthrough therapy designations in CLL).(Valentin, 2018, p11, par2, lines 7-9) An ongoing phase I study is also evaluating venetoclax in combination with the MEK inhibitor cobimetinib or the MDM2 inhibitor idasanutlin in relapsed/refractory AML. Upregulation of MCL-1 is thought to be a potential mechanism of resistance to BCL2 pathway inhibition. Because inhibition of MEK or MDM2 has been shown to downregulate MCL-1, treatment with these inhibitors may improve the efficacy of venetoclax in AML.(Daver, 2017, p1, par1, lines 3-4) The preliminary analysis of this study demonstrated an ORR of 18% in the venetoclax plus cobimetinib arm and an ORR of 38% in the arm with the highest dose of venetoclax plus idasanutlin.(Daver, 2017, p2, par5, lines 1-2)(Daver, 2017, p1, par5, line 5) Furthermore, among the 9 patients with IDH1/2 mutations, response was observed in 44% of the patients. No responses were observed among the 3 patients with known TP53 mutations.(Daver, 2017, p3, par2, lines 2-4)

Practical and Therapeutic Implications

Safety considerations

While promising efficacy has been observed in many trials of venetoclax, associated adverse events must be considered when incorporating venetoclax into therapy decisions. TLS is the most serious event observed in clinical trials of venetoclax. This side effect, which is caused by the potency of venetoclax to activate apoptosis, has been largely mitigated by the strategies that incorporate ramp-up dose escalation and TLS prophylaxis.(Moia, 2018, p397, col2, par4, lines1-6) Beyond TLS, the most common adverse events observed across the various venetoclax trials are gastrointestinal side effects and neutropenia. Although approximately 50% of all patients treated with venetoclax have experienced mild nausea or vomiting, discontinuation due to these side effects was rare across the phase I and phase II trials. The mechanisms for the gastrointestinal toxicity are currently unknown, although possibilities include an on-target effect of BCL2 inhibition or response to the chemical properties or formulation of the molecule.(Ruefli-Brasse, 2017, p3647, par3, lines 1-5) The other common adverse event associated with venetoclax treatment is neutropenia. Grade 4 neutropenia was observed in 23-28% of patients across phase I and phase II trials, and febrile neutropenia was seen in 5-6% of patients in phase I and phase II trials in CLL.(Ruefli-Brasse, 2017, p3647, par3, lines 6-10) However, neutropenia can be easily managed through treatment with growth factors or a short-term pause in venetoclax administration.(Moia, 2018, p398, col1, par2, lines 6-8) Infrequent serious infections have also been observed in 17-20% of patients in phase I and II trials, with less than 1% of cases being fatal.(Reufli-Brasse, p3647, par3, lines 1-10) Importantly, venetoclax has also been shown to be safe in combinations with other drugs. Because the adverse event profiles in combination studies have been similar to venetoclax monotherapy, venetoclax can be used at its maximum dose in combination therapy approaches. The ability to use the maximum venetoclax dose enhances the potential efficacy of this agent in combination studies.(Moia, 2018, p400, col2, par1, lines 1-4)

Resistance

In several hematologic malignancies, resistance to venetoclax can be mediated by upregulation of other anti-apoptotic BCL2 family proteins, including BCL-X_L, BFL-1, and MCL-1, which bind and sequester the pro-apoptotic BH3 proteins.(Reufli-Brasse, p3648, par3, lines 1-3) In addition, BCL2 phosphorylation can prevent venetoclax from displacing BAX and BIM, thereby reducing the efficacy of venetoclax. In CLL cells, the ratio of MCL-1 to BCL2 protein levels plus the level of

phosphorylated BCL2 can predict the cytotoxic activity of venetoclax in culture. BCL2 phosphorylation has therefore also been associated with venetoclax resistance in CLL cells.(Reufli-Brasse, p3648, par5, lines 1-6)

The upregulation of BCL2 family members leading to venetoclax resistance can be mediated by numerous mechanisms, many of which form the basis for combination therapy approaches described above. For example, sustained B cell receptor (BCR) stimulation in primary CLL cells can cause upregulation of MCL-1, leading to venetoclax resistance. Inhibitors of SYK, BTK, PI3Kdelta, MEK, and CDKs can downregulate MCL-1 and overcome this resistance.(Reufli-Brasse, p3650, par2, lines 3-5)(Reufli-Brasse, p3650, par4, lines 1-6)(Reufli-Brasse, p3650, par5, lines 1-4) As described above, numerous clinical trials across a variety of hematologic malignancies are ongoing to evaluate these agents in combination with venetoclax.

Future Directions

The FDA approvals of venetoclax since 2016 have added an important option for the treatment of patients with CLL. With promising results emerging from trials across additional tumor types, venetoclax approval may expand to other hematologic malignancies in the coming years. As development of this agent continues, further exploration of the mechanisms of resistance to venetoclax and continued evaluation of combination therapies will become increasingly important.(Reufli-Brasse, p3653, par1, lines 4-12) Because high levels of BCL-X_L or MCL-1 have been suggested to mediate resistance to venetoclax, selective inhibitors of these proteins are now under development. In addition, the variety of approaches using combination therapies to downregulate the expression of anti-apoptotic family members and promote expression of pro-apoptotic family members continues to expand. As other BCL2 family inhibitors and effective combination approaches are validated, the identification of appropriate diagnostic and biomarker strategies for risk stratification will become critical. Although evaluation of BCL2 expression levels represents one approach for identifying patients most likely to benefit from BCL2 inhibitors, the development of additional approaches based on expression profiles of additional genes may provide further predictive power. The identification of predictive biomarkers would enable individualized treatment based on the unique risk profile for each patient.(Moia, 2018, p400, col2, par7, lines 1-2) Given the promising results with the early trials of venetoclax, efforts to maximize the therapeutic potential of BCL2 inhibitors hold the potential to substantially improve outcomes for patients with hematologic malignancies.

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