BCL-2 as therapeutic target for hematological malignancies

Guilherme Fleury Perini1, Glaciano Nogueira Ribeiro2, Jorge Vaz Pinto Neto3, Laura Tojeiro Campos4 and Nelson Hamerschlak1*

Abstract
Disruption of the physiologic balance between cell proliferation and cell death is an important step of cancer development. Increased resistance to apoptosis is a key oncogenic mechanism in several hematological malignancies and, in many cases, especially in lymphoid neoplasias, has been attributed to the upregulation of BCL-2. The BCL-2 protein is the founding member of the BCL-2 family of apoptosis regulators and was the first apoptosis modulator to be associated with cancer. The recognition of the important role played by BCL-2 for cancer development and resistance to treatment made it a relevant target for therapy for many diseases, including solid tumors and hematological neoplasias. Among the different strategies that have been developed to inhibit BCL-2, BH3-mimetics have emerged as a novel class of compounds with favorable results in different clinical settings, including chronic lymphocytic leukemia (CLL). In April 2016, the first inhibitor of BCL-2, venetoclax, was approved by the US Food and Drug Administration for the treatment of patients with CLL who have 17p deletion and had received at least one prior therapy. This review focuses on the relevance of BCL-2 for apoptosis modulation at the mitochondrial level, its potential as therapeutic target for hematological malignancies, and the results obtained with selective inhibitors belonging to the BH3-mimetics, especially venetoclax used in monotherapy or in combination with other agents.

Keywords: Apoptosis, Resistance, BCL-2, BH3-mimetics, Venetoclax, Leukemia, Lymphoma, Hematological malignancies

Background
Resistance to apoptosis is an important hallmark of cancer, contributing to clonal cell development, tumor growth, and resistance to treatment [1]. In addition, blockage of apoptosis may also lead to the accumulation of other oncogenic alterations, including uncontrolled proliferation. Over the last decades, a remarkable progress in cancer research and therapy has been achieved, with a better understanding of the apoptotic machinery and the association between tumors and altered expression of proteins involved in apoptosis regulation [2, 3]. The members of the B-cell lymphoma-2 (BCL-2) family are central regulators of apoptosis triggered by environmental cues and in response to multiple stress signals [4]. In many cancers, high levels of anti-apoptotic proteins such as BCL-2 and BCL-XL were shown to contribute not only to tumor initiation and progression, but also to lack of response to chemotherapy [5, 6].

BCL-2 was the first gene shown to promote prolonged cell survival rather than increased proliferation [4, 7]. This discovery led to the concept that inhibition of apoptosis is an important step in tumorigenesis [4]. Promising results are being reported with the use of inhibitors of BCL-2 and other related molecules, especially with BH3-mimetics [8, 9]. Given that apoptosis blockage is a key oncogenic mechanism in lymphoid malignancies, and that BCL-2 overexpression is a common finding in leukemias and lymphomas, many antagonists of anti-apoptotic BCL-2 have been developed and investigated for the treatment of hematological neoplasms [2, 6].

BH3-mimetics comprise a novel class of BCL-2 inhibitors that have shown promising results in several hematological malignancies, both as single agents and in combination with other anti-cancer drugs. Among the BH3-mimetics, venetoclax (also known as ABT-199), a potent and selective inhibitor of BCL-2, was recently approved by the US Food and Drug Administration (FDA)
for the treatment of relapsed/refractory chronic lymphocytic leukemia (CLL) with 17p deletion based on its favorable safety profile and high response rates [10]. Here, we review the role of BCL-2 protein on apoptosis regulation, its importance as therapeutic target for hematological malignancies and the results obtained with BH3-mimetics drugs on preclinical and clinical trials.

The apoptosis machinery
Apoptosis is a highly complex and well-regulated form of programmed cell death. It plays an essential role in embryogenesis, tissue development, immunity, and maintenance of homeostasis. However, both excessive and insufficient cell death can lead to a wide variety of pathological conditions including neurodegenerative diseases, immunological disorders, and cancer [11–13]. In the hematopoietic system, programmed cell death exerts an important role, allowing cell turnover and rapid expansion and retraction of cell populations in response to infection [14].

Caspase activation plays a crucial role in apoptosis, with caspases being known as the central executioners of the apoptotic machinery. The proteolytic events mediated by caspases result in peculiar morphological and ultrastructural changes in dying cell that, ultimately, define the apoptotic phenotype [15]. Upon activation, caspases can often cleave and activate other procaspases, initiating a proteolytic cascade. In addition, some procaspases are also capable to form aggregates and undergo autoactivation. This proteolytic cascade, in which one caspase can activate other caspases, and in some cases, activate themselves, allows the amplification of signaling that leads to cell death [16].

Two major pathways for caspase activation and apoptosis initiation have been described in vertebrates: the extrinsic pathway and the intrinsic pathway. The extrinsic pathway involves the activation of cell death receptors located on the cell surface, such as tumor necrosis factor receptors or Fas, and whose interaction to their ligands promote the activation of caspase-8. In the intrinsic pathway, also known as the mitochondrial pathway, disruption of mitochondrial integrity is the crucial decision point [17]. Mitochondria outer membrane permeabilization (MOMP) allows proteins located in the intermembrane space of the mitochondria to be released into the cytosol, thus triggering apoptosis [4].

Both intrinsic and extrinsic pathways culminate on the activation of a caspase cascade that will activate the signaling route leading to the morphological features that characterize apoptotic cells. Cell shrinkage, plasma membrane blebbing with no loss of integrity, DNA fragmentation, and condensation of chromatin are among the characteristics exhibited by cells undergoing apoptosis. This culminates with the formation of apoptotic bodies and their subsequent rapid removal by phagocytosis and degradation by other surrounding cells [11, 16]. The apoptotic phenotype is elicited by the cleavage of cellular substrates by caspases, especially activated caspase-3 [16].

Apoptosis can be triggered by a variety of physiologic and pathologic stimuli. The mitochondrial pathway is activated by different forms of cellular stress, such as DNA damage and loss of survival signals [8]. It is also the apoptotic pathway activated by most cytokine deprivation, chemicals, and by therapeutic agents, including cancer chemotherapeutic drugs and irradiation [18]. The mitochondria play a central role in apoptosis regulation and while caspases are referred to as the executioners of apoptosis, the BCL-2 family members are known as the regulators, given their central role in controlling the integrity of the outer mitochondrial membrane [4, 17].

The BCL-2 family
The BCL-2 family comprises both pro-apoptotic and anti-apoptotic members [4]. Regarding their structure and function, members of this family can be grouped in three main categories. The anti-apoptotic subfamily is characterized by the presence of four BCL-2 homology (BH) domains (BH1, BH2, BH3, and BH4) and, in humans, includes the proteins BCL-2 (the founding member), BCL-XL, BCL-w, BCL-2-related protein A1 (BFL-1/A1), myeloid cell leukemia 1 (MCL-1), and BCL-B/Boo. The pro-apoptotic members can be divided into two subfamilies: the multi-domain pro-apoptotic ‘effectors’ (such as BAK and BAX) and those members known as ‘BH3-only proteins’ as they only have the short BH3 domain. The latter subfamily includes BAD, BID, BIK, BIM, BMF, HRK, PUMA, and NOXA [4, 19].

Activator BH3-only proteins such as BID or BIM are activated by transcriptional or post-translational modification in response to cellular damage or deregulation, and activate effectors such as BAX and BAK, which in turn are responsible for initiating the mitochondrial permeabilization and cell death processes. Although BAX and BAK were first believed to lack the BH4 domain, this domain was later identified in these proteins [6].

Anti-apoptotic members of the BCL-2 family prevent MOMP through two mechanisms: the binding and sequestration of the active forms of BAK and BAX, and/or the sequestration of the direct activators BH3-only proteins. By binding to BAX and BAK, anti-apoptotic proteins such as BCL-2 prevent them from oligomerizing [6].

BH3-only proteins modulate the activity of the other two groups of BCL-2 proteins [9, 20]. They can initiate apoptosis either by inhibiting the anti-apoptotic BCL-2 family members or by directly activating pro-apoptotic family proteins [20]. BH3-only proteins can circumvent
the inhibitory effect of the anti-apoptotic BCL-2 family members by promoting the indirect activation of BAX and BAK, as they bind through their α-helical BH3 motif to the hydrophobic groove of anti-apoptotic proteins, releasing BAX and BAK from the sequestration, and enabling BAK and BAX to oligomerize within the mitochondrial outer membrane [6, 12, 17]. Importantly, due to structural differences, interactions between BH3-only proteins and anti-apoptotic proteins occur in a selective manner and with different affinities, with each BH3-only protein being able to bind to a subset or to all anti-apoptotic members [21]. In this respect, BIM, PUMA, and tBID (formed as result of the activation of the extrinsic pathway and cleavage of BID) can bind all anti-apoptotic BCL-2 proteins, whereas other members have a more restricted affinity [6, 21].

Mitochondria permeabilization can also be promoted through the direct activation of proapoptotic effectors by molecules like BID and BIM, the so-called direct activators [9, 17].

Mitochondria disruption in response to death signals results in the release of cytochrome c and other pro-apoptotic factors into the cytosol, which leads to the activation of the caspase cascade and apoptosis [4]. De-regulation of BCL-2 family members’ expression is observed in a variety of cancers, including hematopoietic neoplasms and solid tumors [2, 22]. Studying the copy-number profiles from 3131 cancer specimens of 26 distinct histological types, Beroukhim et al. found that anti-apoptotic genes of the BCL-2 family were among the genes most commonly found in amplification peaks, while other pro-apoptotic genes were frequently found in deletion peaks [23].

The BCL-2 protein
Structure and function

Located on chromosome 18q21.33, the BCL-2 gene was first discovered by cloning the breakpoint region of the t(14;18) translocation, a chromosomal abnormality commonly found in follicular lymphomas (FL). As a result of this translocation, the BCL-2 gene at 18q21 is placed into juxtaposition with the immunoglobulin heavy chain locus region at 14q32 [5].

BCL-2 is a 26 kDa protein, located on the outer membrane of the mitochondria, but also found on the ER membrane and the nuclear envelope [4]. Although it shares structural similarities with BCL-XL, important differences were observed in healthy cells regarding the mechanisms by which BAK and BAX are restrained. BCL-XL has been shown to sequester both BAX and BAK, while BCL-2 was reported to constrain only BAX, while being unable to prevent BAK activation [24].

Despite completing the embryonic development, BCL-2-deficient mice were shown to present massive lymphoid apoptosis after birth, renal failure due to polycystic kidney disease, and hypopigmented hair [25]. Initial analyses of the distribution of BCL-2 protein revealed that BCL-2 is expressed by various normal tissues and hematopoietic lineages, including the lymphocytes at germinal centers of the follicular mantle zone, regions related to maintenance of plasma cells and memory B cells, and T cells in the thymic medulla. The BCL-2 protein is also present in non-lymphoid tissues such as the epithelium and in long-lived postmitotic cells, such as neurons [26]. Later, BCL-2 expression in the nervous system was confirmed and shown to be important for neuronal survival, preventing apoptosis induced by nerve growth factor deprivation [27].

Vaux et al. showed that BCL-2 expression induced hematopoietic cell survival even in the absence of interleukin 3 and cooperates with MYC to induce proliferation of B-cell precursors [7]. BCL-2 synergizes with MYC in tumor progression, as observed in transgenic mice [28], and this cooperation seems to involve the capacity of BCL-2 to block MYC-induced apoptosis in lymphoid cells, without affecting the mitogenic function of MYC [29].

Graninger et al. reported that BCL-2, in addition to being expressed in leukemic cells, is expressed in a variety of normal hematopoietic lineages, including T cells, with levels of expression varying according to the cell population, stage of maturation, and activation. In normal B-cells, levels of BCL-2 mRNA were found to be up-regulated in pre-B cell lines, but to decrease with differentiation towards a mature B-cell stage. Opposed to this, mature B-cell lymphomas bearing the translocation t(14;18) exhibit much higher levels of BCL-2 mRNA than matched counterparts in which the translocation is absent. As observed for other genes involved in tumorigenesis, BCL-2 levels were virtually undetectable in resting B cells, while they were augmented upon cell activation [30].

The oncogenic potential of BCL-2 was first demonstrated by Reed et al. in 1988 using gene transfer technology [31]. In the following year, BCL-2 gene rearrangements were described to be associated with poor prognosis in large-cell non-Hodgkin lymphomas (NHL) [32]. In 1990, Reed et al. showed that suppression of BCL-2 expression using antisense RNAs rendered leukemia cells susceptible to death by apoptosis [33]. As a result, over the last years, there was an explosive output of research focused on the role of BCL-2 and its family members in apoptosis’ regulation, cancer development, and resistance to treatment.

BCL-2 overexpression in hematological malignancies

High levels of BCL-2 are observed in patients with FL, CLL, mantle-cell lymphoma (MCL), and Waldenström’s macroglobulinemia. Contrasting with this, a more
heterogeneous pattern of expression of BCL-2 is reported among other hematological neoplasms, such as diffuse large B-cell lymphoma (DLBCL), for which certain subtypes present low levels of this molecule; and multiple myeloma (MM), in which BCL-2 expression is especially elevated in patients harboring t(11;14) [8].

Increased levels of BCL-2 can be driven by diverse mechanisms, including chromosomal translocations, gene amplification, and downregulation/deletion of genes encoding microRNAs (miRNAs) involved in BCL-2 RNA degradation [14]. In CLL, the most common leukemia in humans, overexpression of BCL-2 originates from the loss of repression by miRNA 15/16 [34]. Consistent with this, repression of BCL-2 at the post-transcriptional level by the microRNAs miR-15a and miR-16-1 was shown to induce apoptosis in a leukemic cell line model [34].

Cancer cells are found to be frequently primed for apoptosis [17]. Furthermore, it seems that a correlation exists between the level of mitochondrial priming and the degree of chemosensitivity. In hematological malignancies such as MM, acute myelogenous leukemia (AML), and acute lymphoblastic leukemia (ALL), a correlation was found between clinical responses to cytotoxic chemotherapy and mitochondria priming state prior to treatment; better responses to treatment were observed for patients' cells with higher pretreatment mitochondrial propensity to undergo apoptosis [35, 36].

The recognition that BH3-only proteins are key initiators of apoptosis and that BCL-2 and other BCL-2 family members play an important role in cancer development and resistance to treatment prompted the development of drugs that mimic the action of the BH3 domain as a means to restore apoptosis through the binding to one or more BCL-2 family members [9].

**BH3-mimetics that target BCL-2**

**ABT-737**

ABT-737, developed by Abbott Laboratories (North Chicago, IL, USA), is considered the prototype of BH3 mimetics as it was the ‘first-in-class’ compound developed to mimic the function of BH3-only-proteins. Discovered using a high-throughput nuclear magnetic resonance-based screening method to identify small molecules that bind to the BH3-binding groove of BCL-xL, ABT-737 binds with a much higher affinity (<1 nmol/L) than previous compounds to anti-apoptotic proteins BCL-2, BCL-XL and BCL-w, blocking their function [37]. On the other hand, it binds poorly to the other antiapoptotic BCL-2 family members sharing less similarity to BCL-XL, such as BCL-B, MCL-1, and A1 [38].

Single-agent cytotoxicity of ABT-737 was observed both against established cell lines derived from lymphoid malignancies and in ex vivo assays using primary cells derived from patients with FL and CLL [38]. Furthermore, ABT-737 was reported to synergize with radiation and chemotherapeutic drugs such as etoposide, doxorubicin, and cisplatin in various tumor cell lines [38]. Additional in vitro studies confirmed that ABT-737 displays single agent activity against multiple cancer cell lineages, including MM [39] and AML [40], without affecting normal hematopoietic cells [39, 40]. Moreover, it potentiates several chemotherapeutic and other anticancer drugs in AML, MM and chronic myelogenous leukemia (CML), and other settings [38–42]. In a cell line derived from CML (K562), co-treatment with ABT-737 circumvented the resistance to imatinib related to BCL-2 upregulation or loss of BIM or BAD [41].

Although treatment with ABT-737 induces massive apoptosis in multiple tumor cell lines derived from solid tumors, leukemia, and lymphoma, its killing activity is heterogeneous and resistance has been attributed to other members of BCL-2 family that do not interact with ABT-737, especially MCL-1 [14, 43, 44]. In AML, activity of ABT-747 was shown to be abrogated by BCL-2 phosphorylation or elevated MCL-1 [40].

Despite the favorable preclinical results, the development of ABT-737 for clinical use was constrained by its low aqueous solubility and the fact that this compound is not orally bioavailable [45]. To overcome these limitations and given the potential of this therapeutic strategy, researches invested in generating new compounds that would carry the pharmacologic properties of ABT-737, but would be orally bioavailable to optimize utility as single agent or within a combined regimen with other chemotherapeutics [42].

**Navitoclax (ABT-263)**

Navitoclax, a potent and selective inhibitor of BCL-2, is the second generation, orally bioavailable form of ABT-737.
Like its predecessor, navitoclax interacts with high affinity and abrogates BCL-2, BCL-X_L, and BCL-W, but has no activity against A1 and MCL-1. Pharmacokinetically, it exhibited low plasma clearance values and low volumes of distribution in four distinct species [42]. Navitoclax showed in vitro activity against a broad panel of tumor cell lines both as single agent and in combination with chemotherapy. In in vivo experiments, treatment with this inhibitor induced rapid and complete tumor responses in multiple xenograft models developed using small-cell lung cancer and hematologic cell lines, with responses lasting several weeks in some models. Moreover, in B-cell malignant xenograft models, combination with navitoclax significantly improved the efficacy of numerous approved anti-cancer agents. Navitoclax potentiated the activity of rituximab in the B-cell lymphoma flank xenograft model, of modified R-CHOP regimen in a flank xenograft model of MCL, and of bortezomib in a MM model [42]. Like ABT-737, navitoclax induced a rapid but reversible thrombocytopenia, reflecting apoptosis of circulating platelets as the result of blockage of BCL-2 family proteins, especially BCL-xL. Similarly to its predecessor, apoptosis induction by navitoclax also depends on neutralization of MCL-1 [42].

Regarding its mechanism of action, navitoclax-mediated cytotoxicity involves the impairment of interactions between BCL-2/BCL-X_L and pro-apoptotic members of the BCL-2 family (such as BIM), BAX translocation, and subsequent release of cytochrome c and apoptosis. This process was shown to be caspase-dependent [42].

In vivo studies conducted by Ackel et al. showed that inhibition of BCL-2 family proteins by navitoclax potentiates the response of B-cell lymphoma and MM models to distinct standard chemotherapeutic drugs and regimens such as etoposide, rituximab, bortezomib, cyclophosphamide, VAP (vincristine, Adriamycin [doxorubicin], and prednisone), CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), and R-CHOP (rituximab plus CHOP) [46].

Based on the promising results obtained in vitro and in vivo, clinical studies were designed. The anti-tumor activity and safety of two schedules of navitoclax were evaluated in a dose-escalation phase I trial conducted in patients with relapsed or refractory lymphoid malignancies. Responses were detected in distinct tumor types, with partial response being observed in 10 of 46 evaluable patients [47]. Despite the initial excitement with this agent, clinical use of navitoclax is restrained by its on target-effect on platelets. Thrombocytopenia was identified as the dose-limiting toxicity (DLT) of navitoclax in patients and is associated with the inhibition of BCL-X_L, as previous studies showed that survival of platelets largely depend on BCL-X_L expression [47].

Daily navitoclax was also evaluated in patients with relapsed or refractory CLL. Navitoclax showed anti-leukemic activity against CLL, but a more heterogeneous pattern of response was observed when compared with what was predicted by in vitro data. Partial response was achieved in 35% of patients who received a daily dose of at least 110 mg. Acute and transient reductions in platelet counts were also reported. Dose reductions were necessary in five cases, and were mostly related to severe thrombocytopenia in cycles 1 or 2. Occurrence of grade 4 thrombocytopenia during the first cycle was decreased by the application of a lead-in dosing of daily 100 mg for 7 days followed by dose escalation [48]. Diarrhea, nausea, vomiting, fatigue, thrombocytopenia, and neutropenia were the most frequent AEs. Thrombocytopenia was the main DLT and was dose-related [48].

Navitoclax has been evaluated mostly in combination with other drugs, especially in solid tumor [22]. Among the hematological neoplasms, navitoclax was evaluated in combination with rituximab in phases I and II studies conducted in patients with relapsed or refractory CD20+ lymphoid malignancies [49] and patients with B-cell CLL with no prior treatment [50], respectively. Combination of navitoclax and rituximab was well tolerated and showed an important synergistic effect in both settings [49, 50].

**Venetoclax**

**Pre-clinical development**

The development of selective inhibitors for BCL-2 and BCL-X_L is limited by the high degree of similarity shared by their BH3 domain [51]. Using reverse engineering, a new compound was developed to overcome the unfavorable effect of navitoclax on platelets as the consequence of BCL-X_L inhibition, while keeping its anti-tumor activity [52]. Venetoclax is a potent and selective inhibitor of the BCL-2 protein that has demonstrated clinical efficacy in several hematological malignancies [8, 53]. Contrasting with navitoclax, which targets BCL-2 and BCL-X_L, venetoclax has a distinct mode of action as it binds and neutralizes BCL-2 with subnanomolar affinity (Ki < 0.010 nM), while interacting only weakly with BCL-X_L and BCL-W [52]. By sparing BCL-X_L, it exerts little effect on platelet numbers. In preclinical studies, this orally bioavailable inhibitor showed cell-killing activity against a variety of cell lines, including cell lines derived from ALL, NHL, and AML. When investigated in xenografts models using hematological tumors, venetoclax promoted tumor growth inhibition in a dose-dependent fashion. For those models in which venetoclax had little effect as single-agent, improved efficacy was achieved with the combination with other drugs [52].
Additional in vitro studies demonstrated that normal and malignant human peripheral blood B-cells were highly susceptible to venetoclax, contrasting with T-cells and granulocytes that were less sensitive [54]. The lower hematological toxicity of venetoclax compared with navitoclax was also confirmed by in vitro and in vivo studies [54]. Regarding its mechanism of action, venetoclax-induced cytotoxicity in lymphoid cells significantly relies on the activation of BAX and BAK [54]. Experiments using Z-VAD, a wide-spectrum caspase inhibitor, revealed that cell-killing mediated by venetoclax is dependent on caspase activation [52]. Moreover, BIM seems to be the main initiator of apoptosis induced by this BH3-mimetic, as cell sensitivity to venetoclax is drastically abrogated in cells lacking this molecule. However, different levels of resistance due to the absence of BIM were observed across distinct subsets of lymphoid cells [54].

The efficacy of venetoclax was also investigated in childhood ALL [55] based on its better tolerability profile and the observation that ALL xenografts were sensitive to inhibition of BCL-2 by navitoclax [42]. A strong correlation between poor response to venetoclax and the expression of BCL-XL was observed. Unlike CLL cells that largely depend on the expression of BCL-2 alone, killing of most ALL xenografts required simultaneous inhibition of BCL-2 and BCL-XL explaining the contrasting results obtained with venetoclax and navitoclax. The only exception was the xenografts from mixed lineage leukemia-rearranged infant ALL, in which venetoclax had a similar effect of navitoclax, and may represent an interesting subgroup for clinical trials with venetoclax [55].

**Clinical studies with venetoclax in monotherapy in CLL**

Based on the promising preclinical results, a first-in-human phase 1 dose-escalation study was conducted to investigate the safety and efficacy of daily oral venetoclax in patients with relapsed or refractory CLL or small lymphocytic lymphoma (SLL) [56]. A total of 116 patients were included considering both the dose-escalation and the expansion cohorts. At study admission, nearly 90% of the patients had genetic or clinical characteristics known to be associated with adverse prognosis, including chromosome 17p deletions, chromosome 11q deletions, unmutated immunoglobulin heavy-chain variable region, resistance to fludarabine, and bulky adenopathy. In the majority of the cases, at least two of these features were present. Regarding exposure to previous treatments, most patients had received multiple previous therapies (median of three, ranging from 1 to 11). Activity was detected at all dose levels tested, with impressive reduction of tumor burden in all tissue compartments. Overall response rates (ORRs) in the dose-escalation and expansion cohorts were of 77 and 82%, respectively. Notably, ORRs higher than 70% were also reported for patients carrying the deletion 17p and among patients with unmutated IGHV or resistance to fludarabine, with complete response (CR) rates > 15% in all cases [56]. Patients in the dose-escalation cohort had a median progression-free survival (PFS) of 25 months (95% confidence interval [CI], 17 to 30). The median PFS was 16 months among CLL patients with deletion 17p. Due to the short follow-up at the time of publication, PFS could not be estimated for the expansion cohort. CRs were associated with longer duration of response. An overall survival rate of 84% was estimated at 2 years [56].

Venetoclax had a favorable safety profile. In the dose escalation cohort, tumor lysis syndrome was detected in 18% of the patients and represented the most relevant toxicity. Based on this, a stepwise ramp-up strategy up to 400 mg was used in the expansion cohort, together with prophylaxis and treatment for cases identified as at risk for developing this adverse event, which did not occur in the expansion cohort. Diarrhea, upper respiratory tract infection, nausea, and neutropenia were the most frequent adverse events, occurring in nearly half of the patients. Neutropenia was the most common grade 3 or 4 toxicity, with 33 patients having grade 4 neutropenia. Febrile neutropenia was reported in 6% of patients and was the most common serious adverse event. In this study, a maximum tolerated dose was not identified, and the dose of 400 mg per day used for the expansion cohort was decided based on the efficacy and safety data obtained in the dose-escalation phase [56].

The impressive high response rates reported for CLL patients with deletion 17p treated with venetoclax were confirmed in a subsequent multicentric phase 2 trial [10]. A total of 107 patients with relapsed/refractory CLL with deletion 17p and who had already undergone at least one type of treatment were included. After a weekly dose ramp-up schedule, patients received continuous venetoclax at 400 mg/day. Analysis of the ORR assessed by independent review, the study primary endpoint, revealed that venetoclax led to a response rate of 79.4% (95% CI, 70.5 to 86.6). Consistent with the phase I study, high response rates were obtained irrespective to the presence of other poor-prognosis features [8, 10]. Regarding safety, neutropenia (40%) followed by infection (20%), anemia (18%), and thrombocytopenia (15%) were the most common grade 3 or 4 events. Grade 4 neutropenia was detected in 23% of patients and responded to dose reductions or the use of granulocyte colony-stimulating factors. Fifty-nine patients had serious adverse events, with febrile neutropenia being registered in five patients [10]. Based on these promising results and to accelerate its development, venetoclax was granted the status of ‘breakthrough therapy designation’ by the FDA. In April 2016, this inhibitor was...
approved by the FDA for the treatment of patients with CLL who have 17p deletion and have received at least one prior therapy. In December 2016, venetoclax monotherapy received the approval of the European Medicines Agency for the treatment of CLL in the presence of 17p deletion or TP53 mutation in adult patients who are unsuitable for or have failed a B-cell receptor pathway inhibitor (BCRi); and for CLL in the absence of 17p deletion or TP53 mutation in adult patients who have failed both chemoimmuno-therapy and a BCRi [57].

Venetoclax as single agent is being evaluated in patients with CLL who relapsed after or are refractory to ibrutinib (Arm A) or idelalisib (Arm B), two BCRIs. Results of the phase 2 M14-032 trial (NCT02141282) demonstrated that monotherapy with venetoclax is well tolerated and effective in this setting, promoting high rates of durable response (ORR of 70 and 62% in arm A and B, respectively, as assessed by an Independent Review Committee) and of MRD-negativity [58]. A post hoc subgroup analysis of 28 patients who had received > 1 prior BCRi out of the 127 enrolled in this study showed that venetoclax safety profile in this subgroup was consistent to what was expected in relapsed/refractory CLL patients, and, although response rates were lower than in other settings, venetoclax was shown to be active in this subgroup [59]. A more recent publication of this trial reported promising results (ORR of 67%; median time to first response 2.5 months) and a well-tolerated safety profile for monotherapy with venetoclax when data of 36 patients with CLL refractory to or who relapsed after idelalisib were analyzed. Hematologic events accounted for most grade 3 or 4 adverse events reported, and tumor lysis syndrome was not observed [60]. Similarly, an interim analysis of the 91 patients (43 from the main cohort and 48 in the expansion cohort) whose disease progressed during or after discontinuation of ibrutinib, revealed an ORR of 65% and a safety profile within the expected as neutropenia, thrombocytopenia, and anemia were the most common treatment-emergent grade 3 or 4 toxicities [61].

In a pooled analysis of three trials, relapsed/refractory CLL patients with fewer number of prior lines of treatment and less disease bulk were reported to benefit the most from the venetoclax monotherapy when outcomes as CR rate, PFS, and OS were analyzed, suggesting that favorable outcomes are more likely to occur with the earlier introduction of venetoclax [62].

**Clinical studies with venetoclax in monotherapy in other hematological malignancies**

The safety and activity of venetoclax in monotherapy have also been evaluated in other clinical settings, such as relapsed or refractory lymphoma and MM [8, 63, 64]. A total of 106 patients were enrolled in a phase I dose-escalation study of venetoclax in patients with relapsed/refractory NHL. Among the different subtypes of NHL included in the dose-escalation cohort, MCL, DLBCL, and FL were the most common. Overall, venetoclax showed a tolerable safety profile. Treatment-emergent adverse events were reported by nearly all cases (97%) and were mostly grade 1 to 2 in severity. A total of 59 (56%) patients reported grade 3 to 4 events, with hematologic toxicities being the most frequent. Regarding its efficacy, an ORR of 44% was achieved; however, the activity of venetoclax as monotherapy varied among NHL subtypes. Among patients with DLBCL, the ORR was 18%, and in many patients, response was not sustained. Clinical results in FL and MCL patients were considered favorable, as the ORRs were 38 and 75%, responses had longer duration, and median PFS was 11 and 14 months, respectively. In all settings, it is possible that patients would benefit from the use of venetoclax in combination therapies to improve response intensity and duration [64].

In heavily pretreated (median of 5 prior therapies) relapsed/refractory MM, monotherapy with venetoclax also showed a tolerable safety profile. As expected, most commonly reported adverse events were mild to moderate gastrointestinal toxicities, including nausea, diarrhea, and vomiting. Two patients experienced DLTs of epigastric pain and nausea with abdominal pain. Overall response was achieved by 21% (14/66) of the patients, while 15% (10/66) achieved very good partial response (VGPR) or better. Higher response rates were reported among patients harboring t(11;14). Efficacy data of this phase I study with dose-escalation and expansion cohorts suggest that this strategy may be especially interesting in patients with t(11;14), whose disease is marked by low expression of BCL-XL and high BCL-2 [63].

The safety and efficacy of venetoclax in monotherapy are being investigated in a number of clinical trials (Table 1).

In addition to interventional trials, prospective observational studies (NCT03415035 and NCT03342144) are being conducted to assess venetoclax effectiveness regarding best response and response duration, survival endpoints, patient-reported outcomes in patients with CLL, and parameters related to treatment costs under real-life settings. In a real-world study, achievement of deep response in second-line therapy was found to be associated with improved long-term outcomes such as PFS and OS among patients with relapsed/refractory CLL [65].

**Venetoclax in combination with other anticancer drugs**

Besides its use as a single agent, venetoclax is being combined with several classes of agents, including DNA damaging chemotherapy, anti-CD20 antibodies (rituximab and obinutuzumab), proteasome inhibitors (bortezomib
and carfilzomib), hypomethylating agents (azacitidine or decitabine), kinase inhibitors (sunitinib, ibrutinib, idelalisib, dinaciclib, cobimetinib), Mdm2 inhibitor (idasanutlin), and inhibitors of anti-apoptotic molecules Bcl-X L and Mcl-1, to improve its efficacy and overcome resistance [8, 66]. The rational for these combinations is that, contrasting with CLL, FL, MCL, and Waldenström macroglobulinemia, which are marked by high levels of BCL-2 and malignant cells largely depend on this molecule for survival/resistance to death, in other hematological malignancies (including DLBCL, ALL, MM, AML, and CML), this anti-apoptotic molecule exhibits a more varied pattern of expression and its contribution to cell resistance to death is not ubiquitous. For example, resistance to venetoclax has been reported to rely on the overexpression of BCL-X L or/and MCL-1 [66].

In these cases, the sole inhibition of BCL-2 is not sufficient to achieve important results in clinical outcomes. Therefore, different combinations are being investigated in these clinical settings, and the drugs combined with venetoclax are chosen based on the molecular pathways that are altered in the particular malignancy.

Most studies on combinations of venetoclax and other agents are still ongoing. Table 2 presents selected planned studies.

### Table 1 Clinical trials investigating venetoclax as single agent in hematological malignancies

| Disease(s) | Study phase | Estimated* or actual** enrollment | Register number in clinicaltrials.gov |
|------------|-------------|-----------------------------------|---------------------------------------|
| NHL (non CLL/SLL/MCL) | I | 11** | NCT01969695 |
| R/R CLL after BCRis (ibrutinib or idelalisib) | II | 127** | NCT02141282 |
| R/R CLL/SLL and NHL | I | 211* | NCT01328626 |
| R/R AML or untreated AML unfit for intensive therapy | II | 32** | NCT01994837 |
| R/R or untreated CLL with 17p deletion | II | 158** | NCT01889186 |
| R/R CLL | III | 250* | NCT02756611 |
| R/R CLL with 17p deletion | II | 70* | NCT02966756 |
| R/R CLL | III | 200* | NCT02980731 |
| Waldenström Macroglobulinemia | II | 33** | NCT02677324 |

AMLL acute myelogenous leukemia, BCRi B-cell receptor signaling pathway inhibitors, CLL chronic lymphocytic lymphoma, NHL non-Hodgkin lymphoma, R/R relapsed/refractory, SLL small lymphocytic lymphoma, V venetoclax

Results retrieved from the Clinical Trials database (www.clinicaltrial.gov), accessed at 20 Feb 2018. *Estimated enrollment at the time of Clinical Trials database access. **Actual enrollment at the time of Clinical Trials database access

The following studies were not included in this table: study with status "withdrawn" (NCT02095574); studies on myelodysplasic syndromes, and observational studies (NCT03415035 and NCT03342144)

and carfilzomib), hypomethylating agents (azacitidine or decitabine), kinase inhibitors (sunitinib, ibrutinib, idelalisib, dinaciclib, cobimetinib), Mdm2 inhibitor (idasanutlin), and inhibitors of anti-apoptotic molecules Bcl-X L and Mcl-1, to improve its efficacy and overcome resistance [8, 66]. The rational for these combinations is that, contrasting with CLL, FL, MCL, and Waldenström macroglobulinemia, which are marked by high levels of BCL-2 and malignant cells largely depend on this molecule for survival/resistance to death, in other hematological malignancies (including DLBCL, ALL, MM, AML, and CML), this anti-apoptotic molecule exhibits a more varied pattern of expression and its contribution to cell resistance to death is not ubiquitous. For example, resistance to venetoclax has been reported to rely on the overexpression of BCL-X L or/and MCL-1 [66].

In these cases, the sole inhibition of BCL-2 is not sufficient to achieve important results in clinical outcomes. Therefore, different combinations are being investigated in these clinical settings, and the drugs combined with venetoclax are chosen based on the molecular pathways that are altered in the particular malignancy.

Most studies on combinations of venetoclax and other agents are still ongoing. Table 2 presents selected planned studies.

### Combinations for CLL

In patient with relapsed or refractory CLL, venetoclax has been combined with rituximab showing favorable efficacy results and a good safety profile [67]. Results from a phase 1b, dose escalation trial showed deep and durable responses and suggested benefit of retreatment. Overall and complete responses were achieved by 86 and 51% of the 49 patients enrolled in the study, respectively. The safety profile was considered acceptable [67].

Results from an interim analysis of the phase 3 MURANO study (NCT02005471) demonstrated that in patients with relapsed/refractory CLL, combination of venetoclax and rituximab was superior to bendamustine plus rituximab, improving responses and MRD-negativity rates and prolonging PFS. This positive effect on PFS was found to be consistent across subgroups, including among patients with and without del(17p) [68]. Promising efficacy results have also been reported for a sequential treatment with a debulking with bendamustine followed by obinutuzumab and venetoclax as induction and maintenance therapy in physically fit and unfit, treatment-naïve, and relapsed/refractory CLL patients [69].

A pooled analysis of data from 323 patients with relapsed or refractory CLL/SLL from three clinical trials evaluated the effect on PFS of venetoclax in monotherapy or co-administered with rituximab. Using a time-variant relative risk survival model, Freise et al. found that venetoclax affects PFS in a dose-dependent manner. Moreover, concomitant administration with 6 cycles rituximab provided a synergistic benefit with important prolongation of PFS median estimates (1.8 years [95% CI, 1.7–2.1] vs 3.9 years [95% CI, 2.8–5.6]) [70].

### Combinations for MM

Positive results were also obtained for the combination of venetoclax, bortezomib, and dexamethasone in patients with relapsed/refractory MM who had received a median of three prior therapies. Data from the phase 1b study conducted by Moreau et al. suggest that this combination is safe and active, with an ORR of 67% and higher response rates obtained in patients with no prior treatment [71]. VGPR or better
Table 2  Ongoing clinical trials investigating venetoclax in combination with other agents for the treatment of hematological malignancies

| Intervention                                      | Disease                    | Study phase(s) | Estimated* or actual** enrollment | Register number in clinical trials.gov |
|--------------------------------------------------|----------------------------|----------------|-----------------------------------|----------------------------------------|
| V + ibrutinib + obinutuzumab                    | MCL                        | I/II           | 48*                               | NCT02558816                            |
| V + ibrutinib                                   | MCL                        | II             | 24*                               | NCT02471391                            |
| V + ibrutinib                                   | R/R MCL                    | I              | 28*                               | NCT02419560                            |
| V + low-dose cytarabine                          | Treatment-naive AML        | I/II           | 94**                              | NCT02287233                            |
| V + rituximab                                    | Relapsed CLL and SLL       | I              | 49**                              | NCT01682616                            |
| V + bortezomib + dexamethasone                   | R/R MM                     | I              | 66**                              | NCT01794507                            |
| V + bendamustine + obinutuzumab                  | CLL                        | II             | 66**                              | NCT02401503                            |
| V + dexamethasone                                | R/R MM t(11;14)-positive MM | II            | 166*                              | NCT01794520                            |
| V + bendamustine/rituximab                       | R/R NHL                    | I              | 60**                              | NCT01594229                            |
| V + decitabine or azacitidine                    | AML                        | I              | 260*                              | NCT02203773                            |
| V + obinutuzumab                                 | Untreated or R/R CLL       | I              | 82**                              | NCT01685892                            |
| V + rituximab or obinutuzumab and standard doses of CHOP | NHL                        | I/II           | 267***                            | NCT02055820                            |
| V + rituximab vs bendamustine + rituximab        | Relapsed/resistant CLL     | III            | 389**                             | NCT02005471                            |
| V + bendamustine + rituximab vs BR alone         | R/R FL                     | II             | 164**                             | NCT02187861                            |
| V + bortezomib and dexamethasone                 | R/R MM                     | III            | 291**                             | NCT02755597                            |
| V + bendamustine + rituximab or bendamustine and obinutuzumab | R or untreated CLL       | I              | 84**                              | NCT01671904                            |
| V + obinutuzumab + ibrutinib                    | R/R or untreated CLL       | I/II           | 63**                              | NCT02427451                            |
| V + obinutuzumab + vs obinutuzumab + chlorambucil | CLL with coexisting medical conditions | III            | 445**                             | NCT02242942                            |
| V + azacitidine vs placebo + azacitidine         | Treatment-naive AML        | III            | 400*                              | NCT02993523                            |
| V + obinutuzumab                                | R/R DLBCL                  | II             | 21*                               | NCT02987400                            |
| V + carfilzomib + dexamethasone                 | R/R MM                     | II             | 40*                               | NCT02899052                            |
| V + ibrutinib                                   | R/R FL                     | I/II           | 41*                               | NCT02956382                            |
| V + ibrutinib                                   | CLL; SLL                   | II             | 160*                              | NCT02756897                            |
| V + obinutuzumab                                | Untreated FL               | I              | 25*                               | NCT02877550                            |
| V + lenalidomide + obinutuzumab                 | R/R NHL                    | I              | 60*                               | NCT02992522                            |
| V + decitabine                                  | R/R AML                    | II             | 160*                              | NCT03404193                            |
| V + standard chemotherapy                       | ALL                        | I              | 22*                               | NCT03319901                            |
| V with or without chemotherapy                  | Pediatric and young adult R/R malignancies (ALL, AML, NHL, and other non-hematological tumors) | I | 135* | NCT03236857 |
| V + ibrutinib                                   | CLL/SLL with progressive disease on ibrutinib | I | 24* | NCT03422393 |
| V + obinutuzumab and bendamustine               | FL                         | II             | 56*                               | NCT03113422                            |
| V + dose-adjusted EPOCH-R                       | Richter's syndrome         | II             | 20*                               | NCT03054896                            |
| V + navitoclax + chemotherapy                   | R/R ALL                    | I              | 42*                               | NCT03181126                            |
| V + ibrutinib                                   | High-risk CLL; SLL         | II             | 45*                               | NCT03128879                            |
| V + obinutuzumab, after different debulking regimens | Untreated CLL              |                 | 100*                              | NCT03406156                            |
| V + ublituximab + umbralisib (TGR-1202)          | R/R CLL; SLL               | I/II           | 30*                               | NCT0379051                             |
| V + TAK-659                                     | R/R NHL                    | I              | 53*                               | NCT03357627                            |
| V + R-ICE                                      | R/R DLBCL                  | I              | 18*                               | NCT03064867                            |
| V + chemotherapy (FLAG-IDA)                     | AML                        | I/II           | 56*                               | NCT03214562                            |
| Intervention                                                                 | Disease                                                                 | Study phase(s) | Estimated* or actual** enrollment | Register number |
|------------------------------------------------------------------------------|------------------------------------------------------------------------|----------------|-----------------------------------|-----------------|
| V + low-dose cytarabine vs low-dose cytarabine                               | Untreated AML                                                          | III            | 175*                              | NCT03069352     |
| V + cobimetinib vs cobimetinib vs V + cobimetinib + atezolizumab            | R/R MM                                                                | I/II           | 72*                               | NCT03312530     |
| V + rituximab vs bendamustine + rituximab                                    | R/R CLL                                                               | III            | 389**                             | NCT02005471     |
| V + bendamustine, rituximab, and ibrutinib                                    | R/R MCL                                                               | Early phase I  | 18*                               | NCT03295240     |
| V + ibrutinib                                                                | R/R CLL/SLL                                                           | II             | 20*                               | NCT03045328     |
| V + ibrutinib vs ibrutinib                                                   | MCL                                                                   | III            | 287*                               | NCT031112174    |
| V + ibrutinib + obinutuzumab                                                  | CLL patients with TP53 deletion (17p-) and/or mutation                 | II             | 40*                               | NCT02758665     |
| V + ketoconazole                                                              | R/R NHL                                                               | I              | 12**                              | NCT01969669     |
| V + atezolizumab + obinutuzumab                                              | R/R FL, R/R aggressive DLBCL, R/R indolent NHL                        | II             | 138*                              | NCT03276468     |
| V + rituximab                                                                | R/R CLL; SLL                                                          | I              | 49**                              | NCT01682616     |
| V + ixazomib citrate + dexamethasone                                          | R/R MM                                                                | I/II           | 71*                               | NCT03995539     |
| MOR00208 + V vs MOR00208 + idelalisib                                        | R/R CLL/SLL Patients pretreated with BTKI                             | II             | 24*                               | NCT02639910     |
| V + cobimetinib and V + idelalisin                                            | RR AML not eligible for cytotoxic therapy                             | I/II           | 140*                              | NCT02670044     |
| V + DA-EPOCH-R                                                                | Aggressive B-cell lymphomas (including DLBCL; HGBCL; TiNHL, and unclassifiable with intermediate features between DLBCL and BL) | I              | 18*                               | NCT03036904     |
| V + ibrutinib, with or without TP53 aberrations                              | R/R CLL with or without TP53 aberrations                              | II             | 230*                              | NCT03226301     |
| V + ibrutinib                                                                | Treatment naïve CLL/SLL                                                | II             | 289*                              | NCT02910583     |
| V + RO6870810, with or without rituximab                                     | R/R DLBCL                                                             | I              | 94*                               | NCT03255096     |
| V + chemotherapy                                                              | R/R AML or acute leukemia of ambiguous lineage                        | I              | 54*                               | NCT03194932     |
| V or V + azacitidine or V + rituximab                                         | AML, CLL, MM, NHL, SLL                                                | I/II           | 37*                               | NCT02265731***  |
| V + ibrutinib and rituximab                                                  | R/R DLBCL                                                             | I              | 30*                               | NCT03136497     |
| Obinutuzumab + idelalisin and V or rituximab + idelalisin and V              | R/R FL or R/R DLCBL                                                   | I/II           | 140*                              | NCT0315262      |
| Obinutuzumab, rituximab, polatuzumab, vedotin, and V                         | R/R FL or DLBCL                                                       | I              | 134*                              | NCT02611323     |
| V + daratumumab and dexamethasone (with and without bortezomib)             | R/R MM                                                                | II             | 90*                               | NCT03314181     |
| Obinutuzumab, rituximab, polatuzumab, vedotin, and V                         | R/R FL or DLBCL                                                       | I              | 134*                              | NCT02611323     |
| Standard chemotherapy vs rituximab + V vs obinutuzumab + V vs obinutuzumab + V | Fit patients with untreated CLL without del(17p) or TP53 mutation   | III            | 920*                              | NCT02950051     |
| V + ibrutinib + prednisone + obinutuzumab, + Revlimid                       | R/R B-cell lymphoma                                                   | I              | 38*                               | NCT03223610     |

AML: acute myelogenous leukemia; BL: Burkitt lymphoma; CLL: chronic lymphocytic lymphoma; DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; FLAG-IDA: chemotherapy includes fludarabine, cytarabine, idarubicin, and filgrastim; HGBCL: high-grade B-cell lymphoma; MCL: mantle cell lymphoma; MM: multiple myeloma; NHL: non-Hodgkin lymphoma; RICE: rituximab, ifosfamide, carboplatin, etoposide; R/R: relapsed/refractory; SLL: small lymphocytic lymphoma; TiNHL: transformed indolent NHL; V: venetoclax. Results retrieved from the Clinical Trials database (www.clinicaltrial.gov) accessed at 20 Feb 2018 *Estimated enrollment at the time of Clinical Trials database access. **Actual enrollment at the time of Clinical Trials database access. Actual recruitment status completed or active, not recruiting. ***This trial has four arms of treatment: Arm A: phase I of venetoclax in monotherapy for R/R NHL or MM; Arm B: phase I of venetoclax in monotherapy in CLL/SLL; Arm C: phase I of venetoclax with the addition of azacitidine in participants AML; and Arm D: phase 2 of venetoclax with the addition of rituximab in R/R CLL.
were achieved by 28 of 66 patients (42%); 10 patients achieved CR. Median duration of response was 9.7 months, while median time to progression was 9.5 months. Higher response rates, more durable responses and longer time to progression were observed for patients who were non-refractory to bortezomib and who had received 1 to 3 prior lines. Higher levels of BCL2 were reported for patients who achieved partial response or better. Gastrointestinal toxicities of mild severity and grade 3–4 cytopenias were the most frequent adverse events [71].

A phase 1 study is evaluating venetoclax as single agent or in combination with dexamethasone in patients with relapsed/refractory, heavily pretreated MM. Analysis of 50 patients with t(11;14) MM treated in the study (30 with venetoclax and 20 with the combination) revealed that both regimens were well tolerated. Regarding efficacy outcomes, the combination with dexamethasone was associated with higher ORR (65 vs 40%) than venetoclax monotherapy [72].

Primary plasma cell leukemia, the most aggressive form of MM, has a high prevalence of t(11;14). A very deep and impressively rapid hematologic response was achieved when a combination regimen incorporating venetoclax with daratumumab, bortezomib, and dexamethasone was used to treat a 55-year-old female patient with very refractory primary plasma cell leukemia with t(11;14) [73], reinforcing the importance of investigating new treatment options including BCL-2 inhibitors.

Combinations in AML The combination of venetoclax and hypomethylating agents seems to be an attractive therapeutic strategy for AML. Recently, a subgroup analysis of the phase III DACO-016 trial suggested that the hypomethylating agent decitabine improves response rates and prolong PFS in elderly (≥65 years) patients with newly diagnosed with AML and with monosomal karyotype [74].

The safety and efficacy of venetoclax combined with azacitidine or decitabine is under investigation in a phase 1b study conducted in elderly patients (≥65 years at diagnosis) with previously untreated AML who were not eligible to for intensive induction therapy (NCT02203773) [75]. Although four patients died within the first dose of venetoclax due to sepsis, bacteremia, lung infection, and respiratory failure, a tolerable safety profile was otherwise observed. Thrombocytopenia (47%), febrile neutropenia (42%), and neutropenia (40%) were the most common grade 3–4 treatment-emergent adverse events reported. Tumor lysis syndrome was not reported. Preliminary efficacy results were favorable, with high rates of response. Complete remission or complete remission with incomplete marrow recovery was achieved by 61% of 57 patients; similar results were obtained in either combination. The dose of 400 mg of venetoclax was found to provide the best benefit-risk balance. A phase 3 trial (NCT02993523) combining this dose of venetoclax with azacitidine vs azacitidine plus placebo is ongoing in treatment-naïve AML patients not eligible for induction.

Promising results are likely to arise for AML patients from the new combinations of venetoclax with four drugs (midostaurin, enasidenib, CPX-351, and gemtuzumab ozogamicin) that received approval for AML by the FDA in 2017 [76].

Combinations in other hematological malignancies Another group of patients that may benefit from the combination of venetoclax and rituximab is refractory cutaneous B-cell lymphoma (CBCL), patients, as upregulation of BCL-2 was suggested to be involved in treatment resistance to rituximab [77].

Additional in vitro and in vivo studies The ex vivo sensitivity of leukemic cells derived from 73 AML patients was evaluated to search for biomarkers that could be used to predict response to BCL-2 blockade. Responses were more variable among AML samples than among samples derived from CLL patients, which are known to be highly sensitive to this therapeutic approach or compared with non-sensitive cells derived from healthy donors and used as controls. An important upregulation of specific HOXA and HOXB gene transcripts was found in AML cells highly sensitive to BCL-2-inhibitors. Based on the strong correlation observed between response and HOX gene expression, the authors suggested that HOX gene expression pattern may be a useful marker to improve the selection of AML patients that are more prone to respond to BCL-2 inhibitor in clinical trials [78].

Blastic plasmacytoid dendritic cell neoplasm (BPDCN), a rare and aggressive hematologic malignancy with poor prognosis and for which no standardized therapeutic approach has been established, is another neoplasm that may potentially respond to venetoclax. Recently, Montero et al. showed that primary BPDCN cells are highly dependent on BCL-2 protein and sensitive to venetoclax. In addition, tumor regression with improved survival was achieved with venetoclax in an animal model using BPDCN patient-derived xenografts. Notably, significant disease responses were observed in two patients with relapsed/refractory BPDCN who received venetoclax off-label [79].

BCL2 and MYC are oncogenes commonly deregulated in lymphomas. DLBCL represents 30 to 40% of all NHL. It is a heterogenous disease clinically, morphologically, and molecularly. Based on studies on gene expression
profile, important subgroups for prognosis were defined for DLBCL. The chromosomal translocation t(14;18) (q32;q21) is found in nearly 20% of DLBCL cases [80, 81]. In this setting, the presence of this chromosomal abnormality was shown to define a unique subset of DLBCL within the germinal center B-cell-like subgroup [80]. In approximately 5% of DLBCL, translocations t(14;18) and t(8;14) resulting in overexpression of BCL-2 and MYC, respectively, occur concurrently [82].

The importance of BCL-2 and MYC as therapeutic targets for DLBCL with concomitant overexpression of these two proteins was investigated by Sasaki et al. using two cell lines established from chemoresistant patients. Inhibition of BCL-2 by ABT-263 had a more potent cell-killing activity than blocking MYC using a specific inhibitor (10058-F4). Moreover, combination of ABT-263 with genotoxic agents had additive and/or synergistic effect on apoptosis promotion, whereas a more modest effect was observed with the association of the MYC inhibitor and genotoxic drugs. Notably, simultaneous inhibition of BCL-2 and MYC was more effective than either agent alone [83].

More recently, Boidol et al. published the first evidence of activity of venetoclax as single agent both ex vivo and in human in T-cell prolymphocytic leukemia [84].

## Conclusion

The association between BCL-2-family proteins and hematologic malignancies is well established. Consistent with this observation, drugs that mimic key modulators of apoptosis have emerged over the last years producing favorable outcomes. Inhibitors of BCL-2, such as venetoclax and navitoclax, were shown to selectively induce apoptosis in malignant cells and have been extensively investigated as single agents and in combination with other drugs in several malignancies, including acute leukemia, lymphomas, and solid tumors. Navitoclax was shown to be an important inhibitor in clinical subgroups in which the exclusive inhibition of BCL-2 is not sufficient. Important response rates were obtained with venetoclax even in CLL patients with disease features implicated with poor outcomes with chemom immuno-therapy. Results presented so far support that BH3-mimetics may be useful components in several treatment regimens given their synergism with chemother-apy drugs and other agents. As interactions between BH3-only proteins and anti-apoptotic members of the BCL-2 family occur in a selective manner, elimination of cancer cells may require the blockage of all or some anti-apoptotic BCL-2 proteins at the same time to induce death [3]. Several studies are now focused on the identification of expression signatures that could be used to predict sensitivity to the BCL-2 inhibitors and allow the identification of subgroups of patients that would benefit more from more selective or pro-miscuous inhibitors.

## Acknowledgement

DENDRIX assisted with the preparation of the manuscript. AbbbVie participated in the interpretation of data, review, and approval of the content.

## Funding

AbbbVie funded medical writing services provided by Ana Elisa Barreiros Bueno da Silva, a DENDRIX employee.

## Authors’ contributions

All authors designed, drafted, discussed, and revised the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Competing interests

GFP has served as consultant and as speaker to AbbVie, Janssen, and Takeda; has received sponsorship for scientific events from Abbvive, Janssen, Roche, and Takeda; and is Advisory Board member of Janssen.

GNN has served as consultant to Janssen; as speaker to Janssen, Roche, and AbbVie; as investigator to Janssen; has received sponsorship for scientific events from Janssen and AbbVie; and is Advisory Board member of Janssen.

JVPN has served as consultant to AbbVie, Celgene, Janssen, Roche, and Takeda; as speaker to Janssen, Roche, and Takeda; as investigator to Roche; has received sponsorship for scientific events from Celgene, Janssen, Roche, and Takeda; and is Advisory Board member of Celgene, Janssen, Roche, and Takeda.

LTC is employee of AbbVie Brazil.

NH has served as consultant and/or as speaker to Takeda, Roche, Novartis, Amgen, and United Medical; and is Advisory Board member of AbbVie.

## Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## Author details

1. Hospital Israelita Albert Einstein, Av. Albert Einstein, 627, Sao Paulo, Sao Paulo 05652-900, Brazil.
2. Hospital das Clínicas da Universidade Federal de Minas Gerais, Av. Prof. Alfredo Balena, 110, Santa Efigênia, Belo Horizonte, Minas Gerais 30130-100, Brazil.
3. Cettro-Centro de Cáncer de Brasilia, SMHN Quadra 2, Bloco A, Edificio de Clinicas, 12 andar, Brasilia, DF 70710-904, Brazil.
4. AbbVie; Avenida Jornalista Roberto Marinho, 85-7 andar, Brooklin, Sao Paulo, Sao Paulo 04576-010, Brazil.

## Received

15 December 2017 Accepted: 26 April 2018

Published online: 11 May 2018

## References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
2. Delbridge AR, Grabow S, Strasser A, Vaux DL. Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies. Nat Rev Cancer. 2016;16(2):99–109.
3. Hainaut P, Plymoth A. Targeting the hallmarks of cancer: towards a rational approach to next-generation cancer therapy. Curr Opin Oncol. 2013;25(1):50–1.
46. Ackler S, Mitten MJ, Foster K, Oleksijew A, Refici M, Tahir SK, Xiao Y, Tse C, Frost DJ, Fesik SW, Rosenberg SH, Elmow SW, Shoemaker AR. The Bcl-2 inhibitor ABT-263 enhances the response of multiple chemotherapeutic regimens in hematologic tumors in vivo. Cancer Chemother Pharmacol. 2010;65(5):869–80.

47. Wilson WH, O'Connor OA, Czuczmar MS, LaCasce AS, Gerecitano JF, Leonard JP, Tucci A, Dunkley K, Xiong H, Chiu YL, Cui Y, Busman T, Elmow SW, Rosenberg SH, Kivoshek AP, Enschede SH, Humerichouse RA. Navitoclax, a target-high efficacy inhibitor of Bcl-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. Lancet Oncol. 2010;11(12):1149–59.

48. Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, Carney DA, He SZ, Huang DC, Xiong H, Cui Y, Busman TA, McKeean EM, Kivoshek AP, Enschede SH, Humerichouse R. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. J Clin Oncol. 2012;30(5):488–96.

49. Roberts AW, Advani RH, Kahl BS, Persky D, Sweetenham JW, Carney DA, Yang J, Busman TB, Enschede SH, Humerichouse RA. Seymour JF. Phase 1 study of the safety, pharmacokinetics, and antitumour activity of the BCL2 inhibitor navitoclax in combination with rituximab in patients with relapsed or refractory CD20+ lymphoid malignancies. Br J Haematol. 2015;170(3):369–78.

50. Kipps TJ, Eradat H, Grosseck S, Catalano J, Cosolo W, Dyagi S, Yalamanchili S, Chai A, Sahasramanam S, Punnose N, Hunt D, Polypenko H. A phase 2 study of the Bcl-3 mimetic BCL2 inhibitor navitoclax (ABT-263) with or without rituximab, in previously untreated B-cell chronic lymphocytic leukemia. Leuk Lymphoma. 2015;56(10):2826–33.

51. Petros AM, Medek A, Netteysche DG, Kim DH, Yoon HS, Swift K, Matayoshi ED, Oltersdorf T, Fesik SW. Solution structure of the antiapoptotic protein Bcl-xL. J Biol Chem. 2004;279(18):17765–72.

52. Souers AJ, Levenser JD, Boghara ET, Ackler SL, Caton ND, Chen J, Dayton BD, Ding H, Enschede SH, Fairbrother WJ, Huang DC, Hymowitz SG, Jin S, Khaw SL, Kova P, Larn LT, Lee J, Maeker HL, Marsh KC, Mason KD, Mitten MJ, Nimmer PM, Oleksijew A, Park CH, Park CM, Phillips DC, Roberts AW, Sampath D, Seymour JF, Smith ML, Sullivan GM, Tahir SK, Tse C, Wendt MD, Xiao Y, Xue JC, Zhang H, Humerichouse RA, Elmow SH, ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19(2):202–6.

53. Gang S, Kagaravapu C, Savojiyo J, Song Y, Liu D. ABT-199 (venetoclax) and BCL-2 inhibitors in clinical development. J Hematol Oncol. 2015;8:129.

54. Khaw SL, Mitten MJ, Foster K, Oleksijew A, Refici M, Tahir SK, Xiao Y, Tse C, Frost DJ, Fesik SW, Rosenberg SH, Elmow SW, Shoemaker AR. The Bcl-2 inhibitor ABT-263 enhances the response of multiple chemotherapeutic regimens in hematologic tumors in vivo. Cancer Chemother Pharmacol. 2010;65(5):869–80.
75. DiNardo CD, Pratz KW, Letai A, Jonas BA, Wei AH, Thirman M, Arellano M, Frattini MG, Kantarjian H, Popovic R, Chyla B, Xu T, Dunbar M, Agarwal SK, Humrichhouse R, Mabry M, Potluri J, Konopleva M, Pollyea DA. Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukemia: a non-randomised, open-label, phase 1b study. Lancet Oncol. 2018;19(2):216–28.

76. Wei AH, Tiong IS. Midostaurin, enasidenib, CPX-351, gemtuzumab ozogamicin, and venetoclax bring new hope to AML. Blood. 2017;130(23):2469–74.

77. Wobser M, Voigt H, Eggert AO, Houben R, Kauczok CS, Brocker EB, Becker JC. Bcl-2 expression in rituximab refractory cutaneous B-cell lymphoma. Br J Cancer. 2007;96(10):1540–3.

78. Kontro M, Kumar A, Majmundar MM, Elfdons P, Parsons A, Pernovska T, Saarela J, Yadav B, Malani D, Floresaud Y, Hoglund M, Remes K, Gjertsen BT, Kallioniemi O, Willenberg K, Heckman CA, Potkka K. HOX Gene expression predicts response to BCL-2 inhibition in acute myeloid leukemia. Leukemia. 2017;31(2):301–9.

79. Montero J, Stephansky J, Cai T, Griffin GK, Cabal-Hierro L, Togami K, Hogdal LJ, Galinsky I, Morgan EA, Aster JC, Davids MS, LeBoeuf NR, Stone RM, Konopleva M, Pemmaraju N, Letal A, Lane A. Blastic Plasmacytoid dendritic cell neoplasm is dependent on BCL-2 and sensitive to Venetoclax. Cancer Discov. 2016.

80. Huang JZ, Sanger WG, Greiner TC, Staudt LM, Weisenburger DD, Picking DL, Lynch JC, Armitage JO, Wannek RA, Alazadeh AA, Lossoi ES, Levy R, Chan WC. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-cell gene expression profile. Blood. 2002;99(7):2285–90.

81. Igual J, Sanger WG, Horsman DE, Rosenwald A, Perryon DL, Dave B, Dave S, Xiao L, Cao K, Zhu Q, Sherman S, Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Ott G, Muller-Hermellin HK, Deabie J, Baziem RL, Jaffe ES, Campo E, Lynch JC, Connors JM, Vose JM, Armitage JO, Grogan TM, Staudt LM, Chan WC. BCL2 translocation defines a unique tumor subset within the germinal center B-cell-like diffuse large B-cell lymphoma. Am J Pathol. 2004;165(1):159–66.

82. Johnson NA, Savage KJ, Ludkovski O, Ben-Neriah S, Woods R, Steidl C, Dyer MJ, Siebert R, Kuruvilla J, Klama R, Connors JM, Gascoyne RD, Horsman DE. Lymphomas with concurrent BCL2 and MYC translocations: the critical factors associated with survival. Blood. 2009;114(11):2273–9.

83. Sasaki N, Kuroda J, Nagoshi H, Yamamoto M, Kobayashi S, Tsutsumi Y, Kobaishi T, Shimizu Y, Matsumoto Y, Taki T, Nishida K, Horikue S, Ako Y, Tanikawa M. Bcl-2 is a better therapeutic target than c-Myc, but attacking both could be a more effective treatment strategy for B-cell lymphoma with concurrent Bcl-2 and c-Myc overexpression. Exp Hematol. 2011;39(8):817–28.e811.

84. Boidol B, Kornauth C, van der Kouwe E, Prutsch N, Kazianka L, Gutrekik S, Hoermann G, Mayerhoefer ME, Hopfinger G, Hauswith A, Pannin M, Aretin MB, Hilgart B, Sper WR, Valnet P, Simonitsch-Klupp I, Moriggl R, Merkel O, Kenner L, Jager U, Kubicek S, Staber PB. First-in-human response of BCL-2 inhibitor venetoclax in T-cell prolymphocytic leukemia. Blood. 2017;130(23):2499–503.