Acquired resistance toward apoptosis represents one of the hallmarks of human cancer and a major cause of the inefficacy of most anticancer treatment regimens. Based on its ability to inhibit apoptosis, the B-cell lymphoma/leukemia 2 (Bcl-2) protein family has garnered the most attention as a promising therapeutic target in cancer. Accordingly, efforts have lately been focused on the development of drugs targeting Bcl-2 proteins with considerable therapeutic success, particularly in hematologic malignancies. Here, we review the previous studies and highlight the pivotal role of the Bcl-2 protein family in the homeostasis of hematologic tissue compartment. This knowledge provides more insight into why some cancers are more sensitive to Bcl-2 targeting than others and will foster the clinical evaluation of Bcl-2-targeting strategies in cancer by avoiding severe on-target side effects in the development of healthy tissues.

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Subject Category: Cancer

Facts
- Inefficient mitochondrial apoptosis is a key determinant in therapeutic success of a number of anticancer regimens.
- Mitochondrial apoptosis is tightly controlled by the Bcl-2 protein family.
- Bcl-2 proteins have a crucial role in the development and the homeostasis of cells of hematopoietic origin.
- Imbalanced expression of Bcl-2-family members has been readily associated with the development of hematologic malignancies such as lymphoma, leukemia or myeloma.
- Several small-molecule inhibitors of Bcl-2 proteins have been developed and are currently under clinical evaluation with a marked susceptibility to hematologic malignancies.

Open Questions
- What is the impact of Bcl-2-targeting anticancer therapy in solid tumors?
- How can hematologic side effects be avoided upon Bcl-2-targeting anticancer therapy?
- How and in combination with which additional chemotherapeutics provoke Bcl-2-antagonizing protocols for a potent anticancer effect?

Compelling studies conducted over the last 20 years established the concept that apoptosis serves as a natural barrier to cancer development, which is triggered autonomously during the process of malignant transformation or as a result of anticancer treatment. Therefore, acquired resistance toward apoptosis is a hallmark of most types of human cancer and a major cause of the inefficacy of most anticancer treatment regimens. Mitochondria represents a central regulatory node in the apoptotic machinery and the decisive event thereby is the process of mitochondrial outer membrane permeabilization (MOMP). Upon MOMP, multiple pro-apoptotic molecules are released from the mitochondrial intermembrane space to coordinate most of the hallmarks of apoptosis, like nuclear condensation and caspase activation. Inefficient MOMP has been considered to be one of the key determinants of therapeutic success of a number of anticancer regimens. Accordingly, reactivation of the mitochondrial apoptotic machinery by restoration of MOMP has been viewed as a promising strategy to combat human cancer.
three classes (Figure 1) The antiapoptotic Bcl-2-family members including Bcl-2, Bcl-xl and Mcl-1 inhibit apoptosis, whereas a second class including Bax and Bak promotes apoptosis. A third divergent class of BH3-only proteins including Bad, Bik, Bid, Bim, Noxa and Puma have a conserved BH3 domain that can bind and regulate the activity of Bcl-2 proteins. Recent evidence suggests that BH3-only proteins derepress and liberate Bax and Bak by direct binding and inhibition of anti-apoptotic family members including Bcl-2. By contrast, an opposing model postulates direct activation of Bax and Bak by BH3-only proteins including Bim, tBid and Puma (Figure 1). 1

Apoptosis Represents a Fundamental Regulatory System During Hematopoiesis

Hematopoiesis gives rise to blood cells of different lineages throughout normal life. Abnormalities in this developmental program lead to blood cell diseases including leukemia and lymphoma.3 During hematopoiesis, a complex interacting network of cytokines and adhesion molecules tightly regulates the survival of progenitor cells, both positively and negatively. Following deprivation of these survival cues apoptotic death of progenitor cells actively safeguards hematologic homeostasis and prevents malignant transformation.4 Accordingly, almost 90% of pre-T- and B-cells undergo apoptosis during maturation in the thymus or bone marrow, respectively. Furthermore, after antigen exposure T- and B-cells undergo clonal expansion, giving rise to the generation of a large number of active effector lymphocytes. Apoptosis triggers the shutdown of the immune response when an infection has been overcome.5 Importantly, key elements of the basic apoptotic signaling machinery have been first discovered in the hematopoietic system associated with diseases when aberrantly expressed (Bcl-2 and lymphoma) or mutated (CD95 and ALPS),6 underscoring the intimate association of the apoptotic machinery, in particular, Bcl-2 proteins with the homeostasis of the hematopoietic system (Figure 1).

Bcl-2 Proteins – Their Physiologic Role in Cells of Hematopoietic System and Hematologic Cancer

Imbalanced expression of Bcl-2-family members has been readily associated with the development of hematologic malignancies such as human lymphoma, leukemia or myeloma. Besides the extensive biochemical characterization, gene-targeting experiments in mice repeatedly showed that Bcl-2 proteins are essential for the development and homeostasis of the hematopoietic system. In the following we will summarize the data obtained in the previous years demonstrating the pivotal role of Bcl-2 proteins in hematologic compartment homeostasis (Figure 2), which may account for
the observed association of hematologic malignancies with imbalanced Bcl-2 expression (Figure 1) and the marked susceptibility of hematologic malignancies toward Bcl-2-targeting strategies (Figure 3 and Table 1).

Antiapoptotic Bcl-2 Proteins

The discovery of Bcl-2 family of proteins is intimately linked to many B-cell malignancies.

The bcl-2 gene was initially discovered at the t(14;18) chromosome translocation breakpoint in B-cell follicular lymphomas, where its transcription becomes excessively driven by the immunoglobulin heavy chain gene promoter and enhancer on chromosome 14. In line with the data obtained in human tumor samples, mice lacking bcl-2 have severe defects in the development of lymphoid progenitor cells from hematopoietic stem cells (HSC) and display reduced lifespan of lymphoid and myeloid cells. Conversely, early studies reported that Bcl-2 overexpression enhanced the survival of T- and B-cells. More strikingly, ectopic expression of Bcl-2 was capable of rescuing lymphopoiesis in SCID mice. The oncogenic potential of Bcl-2 was explored by showing that its overexpression facilitates the c-myc-driven proliferation of B-cell precursors and tumorigenesis.

Myeloid cell leukemia sequence 1 (Mcl-1) was identified as an immediate-early gene induced by TPA-mediated differentiation of a human myeloid leukemia cell line (ML-1). Mcl-1 is one of the most highly amplified genes in a variety of human cancers. Specifically, elevated Mcl-1 was shown in acute myeloid leukaemia (AML), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBL), non-Hodgkin's lymphoma and multiple myeloma (MM). In line with these observations, removal of Mcl-1 caused cell death of transformed AML and rescued AML-afflicted mice from disease development. Mcl-1 is unique among the antiapoptotic Bcl-2 members in being essential for early embryonic development. Deletion of mcl-1 results in lethality at embryonic day 3.5, whereas tissue-specific ablation of mcl-1 in mice demonstrated that Mcl-1 is essential for the survival and the development of B- and T-lymphocytes, germinal center formation and B-cell memory, plasma cells, neutrophils, basophils and mast cells, and has an obligate role for the survival of HSCs. Remarkably, inducible Cre-mediated deletion of even a single Mcl-1 allele substantially impaired the growth of c-myc-driven mouse

Figure 2 The Bcl-2 protein family in the development and homeostasis of the hematologic system. A summary of the current knowledge about the physiological role of Bcl-2 protein family in hematopoiesis based on the results obtained in mice. common lymphoid progenitor (CLP), common myeloid progenitor (CMP), T lymphocyte (TL), BL (B lymphocyte), NK (natural killer cells), GP (granulocyte progenitor), ?P (unknown progenitor), MKP (megakaryocyte progenitor), MP (monocyte progenitor). *Bcl-2 ablation reduces the number and the lifespan of leukocytes but presumably does not impact on lymphoid development. **Noxa impacts on the lymphocyte function upon infection but is not involved in lymphoid development.
In line with these observations, overexpression of Mcl-1 results in the development of lymphomas identifying Mcl-1 as a critical regulator of hematopoiesis and hematologic malignancies. Initial studies using low stringency hybridization assays in chicken lymphoid cells identified bcl-x, a bcl-2-related gene that can function as a Bcl-2-independent regulator of apoptosis. Alternative splicing results in two distinct bcl-x

Figure 3  Structural view of BH3 mimetics. Obatoclax (GX15-070) is a Bcl-2 homology domain-3 (BH3) mimic. It occupies a hydrophobic cleft within the BH3-binding groove of Bcl-2, antagonizing Bcl-2 and thus inducing apoptosis. Gossypol is a natural phenol derived from the cotton plant (genus: Gossypium). The phenolic aldehyde permeates cells and acts as an inhibitor for several dehydrogenase enzymes and in particular in its (R)-configuration (known as AT-101) it acts as a pan-Bcl-2-family inhibitor, capable to bind and inhibit most antiapoptotic Bcl-2-family members. ABT-737 is a small-molecule BH3 mimic developed by Abbott that binds to the hydrophobic BH3-binding groove of antiapoptotic Bcl-2-family members. ABT-737 binds with high affinity (Ki ≤ 1 nM) to Bcl-xl, Bcl-2 and Bcl-w, but not to the less-homologous proteins Bcl-b, Mcl-1 and A1. ABT-263 (Navitoclax, Abbott Laboratories) is structurally related to ABT-737. It represents an orallybioavailable small-molecule Bad-like BH3 mimic which efficiently antagonizes antiapoptotic Bcl-2-family members (Ki’s of < 1 nM for Bcl-2, Bcl-xl and Bcl-w). ABT-199, generated by Abbott is a high-affinity Bcl-2-selective small-molecule BH3 mimic. It is not interacting with Bcl-xl and thus not interfering with platelet homeostasis.
mRNAs. The protein product of the larger mRNA, Bcl-xl, was similar in size and predicted structure to Bcl-2. Similar to Bcl-2 and Mcl-1, elevated Bcl-xl expression has been frequently observed in hematologic malignancies and is implicated to have a role in disease progression.32 Bcl-xl−/− mice died at embryonic day 13 and displayed massive cell death of immature hematopoietic cells and thus severe defects in the development of the hematopoietic system, underlining the essential role of Bcl-x for the survival and development of lymphoid cells. In line with these observations, an independent approach showed that genetic ablation or pharmacological inactivation of Bcl-xl reduces platelet half-life and causes thrombocytopenia in mice. The central role of Bcl-xl in malignant transformation of hematopoietic cells was further strengthened with the fact that transgenic mice overexpressing Bcl-xl developed lymphomas.35

In contrast to Bcl-2 and Mcl-1 the described roles for A1 are more restricted. A1 is a hematopoietic tissue-specific gene,36 which is induced during myeloid differentiation,36 mast cell activation,36 emphasizing the importance of A1 in the hematopoietic system. Genetic deletions of a1 gene in mice with a highly cell type-specific expression pattern. However, mice lacking only one a1 gene, a1−/−, show hematologic defects including...

| Study description | Tumor entity | Study summary | Reference |
|-------------------|--------------|---------------|-----------|
| Phase II trial of oblimersen as a single treatment | advanced CLL | 2/26 patients achieved PR; 7/17 patients showed ≥50% reduction in splenomegaly; 2/7 patients showed complete disappearance of hepatomegaly; 7/22 patients showed ≥50% reduction of lymphadenopathy; 11/22 patients showed ≥50% reduction in circulating lymphocyte count 17% CR in group 1 versus 7% CR in group 2. Among patients with CR, response duration was significantly longer in group 1 versus group 2 (>-36 months versus 22 months); 40% of patients with CR or PR of group 1 showed a significant 5-year survival benefit CR in 23% patients, a PR in 19% patients and 28% patients showed a minimal response or stable disease 12/48 patients (25%) achieved a major response with 5 CR and 7 CR without plateau recovery. Ten of the 12 patients who achieved a major response survived >6 months compared with six of 36 nonresponders | O’Brien et al.99 |
| Phase III trial of fludarabine plus cyclophosphamide with (group 1) or without (group 2) oblimersen | Relapsed or refractory CLL | 17% CR in group 1 versus 7% CR in group 2. Among patients with CR, response duration was significantly longer in group 1 versus group 2 (>36 months versus 22 months); 40% of patients with CR or PR of group 1 showed a significant 5-year survival benefit 12/48 patients (25%) achieved a major response with 5 CR and 7 CR without plateau recovery. Ten of the 12 patients who achieved a major response survived >6 months compared with six of 36 nonresponders | O’Brien et al.128 |
| Phase II trial of oblimersen in combination with rituximab | Recurrent B-cell non-Hodgkin lymphoma | 17% CR in group 1 versus 7% CR in group 2. Among patients with CR, response duration was significantly longer in group 1 versus group 2 (>36 months versus 22 months); 40% of patients with CR or PR of group 1 showed a significant 5-year survival benefit | Pro et al.101 |
| Phase II trial of oblimersen in combination with gemtuzumab ozogamicin | AML | 17% CR in group 1 versus 7% CR in group 2. Among patients with CR, response duration was significantly longer in group 1 versus group 2 (>36 months versus 22 months); 40% of patients with CR or PR of group 1 showed a significant 5-year survival benefit | Moore et al.102 |
| Phase II trial of oblimersen in combination with dexamethasone and thalidomide | Relapsed MM | 55% of patients achieved objective responses, including CR in 2/33 patients, 4/33 near CRs, PR in 12/33 patients and 6/33 patients had minimal responses | Badros et al.103 |
| Phase II trial of dexamethasone with gossypol | Advanced MM | No significant differences between the two groups in time to tumor progression or objective-response rates | Chanan-Khan et al.105 |
| Phase II trial of dexamethasone plus prednisone in combination with gossypol | Advanced melanoma | The addition of oblimersen to dacarbazine yielded a trend toward improved survival at 24-month minimum follow-up (median, 9.0 versus 7.8 months; P = 0.77) and significant increases in progression-free survival (median, 2.6 versus 1.6 months; P<0.001), overall response (13.5 versus 7.5%; P < 0.007), complete response (2.8 versus 0.8%), and durable response (7.3 versus 3.6%; P = 0.03) | Bedikian et al.104 |
| Phase II trial of docetaxel in combination with oblimersen | Castration-resistant prostate cancer | No statistical difference in overall survival | Sternberg et al.106 |
| Phase II trial of docetaxel in combination with topotecan | Relapsed and refractory SCLC | No convincing clinical activity | Heist et al.115 |
| Phase II trial of docetaxel in combination with docetaxel | NSCLC | No convincing clinical activity | Ready et al.117 |
| Phase II trial of gossypol | Chemotherapy-sensitive recurrent extensive-stage SCLC | No observed clinical activity | Bagstrom et al.118 |
| Phase II trial of gossypol plus prednisone in combination with gossypol | Metastatic castration-resistant prostate cancer | No statistical difference in overall survival | Sonnapde et al.119 |
| Phase II trial of abt-263 | Myelofibrosis | No convincing clinical activity | Parikh et al.120 |
| Phase II trial of abt-263 | Relapsed SCLC | PR in 2.6% and stable disease in 23% patients. The most common toxicity associated with navitoclax was thrombocytopenia, which reached grade III–IV in 41% of patients. | |

Abbreviations: AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CR, complete remission; MM, multiple myeloma; NSCLC, non-small cell lung cancer; PR, partial remission; SCLC, small cell lung cancer.
BH3-only Proteins

An expression screen for proteins that bind to Bcl-2 yielded a small novel protein, denoted as Bim, whose only similarity to any known protein was the short (9-amino-acid) BH3 motif shared by most Bcl-2 homologs. Sequence analysis of murine cDNAs revealed the presence of three major isoforms (BimEL-196aa, BimL-140aa and BimS-110aa), produced by alternative splicing. Independently, the same gene was discovered in an ovarian cDNA library, using Mcl-1 as bait, that they initially termed Bod (Bcl-2-related ovarian death agonist). Bim has a critical role during hematopoiesis (Figure 1) and the in vivo function of this protein has received the most attention among the pro-apoptotic Bcl-2 members. Blocking Bim expression by gene deletion or epigenetic silencing has a central role in the pathogenesis or the response to anticancer therapeutics in a number of human hematologic malignancies including Burkitt's lymphoma (BL), MCL and various B-cell non-Hodgkin's lymphomas. Consistent with these observations, gene-targeting experiments of the bim locus in mice demonstrated a crucial role of Bim in the homeostasis of most immune cells. In particular, bim−/− mice have abnormal high numbers of T- and B-cells, macrophages and granulocytes owing to improved apoptosis resistance to several stimuli, including cytokine deprivation, abnormal calcium flux or irradiation. They also show defects in the immune response shutdown as indicated by increased numbers of antibody-secreting plasma cells and the extended survival of activated cytotoxic T-cells after infection. Furthermore, Bim seems to be essential for the apoptosis of autoreactive B- and T-cells (negative selection) resulting in systemic lupus erythematosus-like autoimmune disease in bim−/− animals. Moreover, loss of Bim accelerates lymphomagenesis in Eμ-myc transgenic mice.

Puma (p53 upregulated modulator of apoptosis) is one of the most potent killers among the BH3-only proteins, which was initially identified as an antagonist of Bcl-2, induced by p53. In view of the fact that more than half of human tumors comprise p53 mutations, Puma represents an important factor in human cancer as the induction of Puma expression in response to genotoxic anticancer therapeutics is efficiently abrogated in p53-deficient tumors. Furthermore, ~40% of primary human BL fail to express detectable levels of Puma and in some tumors this is based on the epigenetic silencing of puma. mice show no abnormalities in hematologic tissue development, whereas lymphocytes, myeloid cells and certain other cell types show resistance to apoptosis induced by growth factor withdrawal or DNA damage. Suppression of Puma by shRNAs or its genetic ablation in mice enhanced myc-driven B-cell lymphomagenesis. In cooperation with Bim, Puma was additionally shown to be involved in homeostasis of mast cells and macrophages. Furthermore, the combined deletion of bim and puma, but not in either single knockout, impaired the elimination of autoreactive T-cells and led to autoimmune reactions in various organs.

After its initial description as a novel phorbol-12-Myristate-13-acetate-responsive gene in adult T-cell leukemia, Noxa was rediscovered in a differential display approach...
using mRNA from γ-irradiated wild-type and IRF-1/p53 double-deficient mouse embryonic fibroblasts. Like Puma, Noxa was initially identified as a primary p53-response gene. Noxa has been described as an important determinant of cell death in response to chemotherapy in lymphoid malignancies including CLL, HL, MM and MCL cells. Furthermore, array-based comparative genomic hybridization and gene-expression microarray analysis showed that Noxa is mutated and preferentially silenced in DLBL. Gene-targeting experiments of noxa in mice displayed defects in T- and B-cell activation and the immune response against viral infection. Furthermore, Noxa was shown to be centrally involved in neutrophils apoptosis.

By utilizing Bcl-2 protein to screen cDNA libraries the Bcl-xl/Bcl-2-associated death promoter homolog (Bad) was identified. Bad was the first BH3-only protein to be connected to proximal signal transduction through its differential phosphorylation in response to extracellular survival factors. In particular, phosphatidylinositol-3-kinase/Akt signaling, a survival pathway frequently hyper-activated in many lymphocytic malignancies, negatively regulates Bad’s function. In line with these observations, bad−/− mice develop DLBL of the germinal center or post-germinal center, B220−CD19+ B-cells expressing the zinc finger transcription factor Bcl-6 (latency >15 months). Although DLBLs were the most frequent tumor entity observed (>40%), bad−/− mice additionally suffered a broad range of different hematologic malignancies. This is particularly intriguing, as the inactivation of Bad’s pro-apoptotic function by phosphorylation appears to have a prominent role in the survival of these lymphocyte populations.

Together, the data obtained by analyzing human tumor samples and in particular the use of transgenic mouse models conclusively support the notion that the Bcl-2 protein family represents a central regulatory node in the development of hematopoietic system.

**Bcl-2 Targeting as a Therapeutic Option in Hematologic Malignancies**

Owing to their imbalance expression levels in tumor cells and their capability to regulate MOMP, Bcl-2 proteins have been viewed as promising therapeutic targets in cancer and research efforts have lately been focusing on the development of drugs targeting Bcl-2 proteins. Accordingly, several small-molecule inhibitors of Bcl-2 proteins have been developed and are currently under clinical evaluation (Figure 3 and Table 1). The following paragraphs summarize the results obtained by surveying these small molecules as a therapeutic option with special emphasis on their activity in hematologic malignancies.

**Bcl-2 Antisense Oligodeoxynucleotide G3139/Oblimersen**

G3139 (INN, trade name Genasense; also known as Augmerosen or Oblimersen) is an antisense oligodeoxyribonucleotide, specifically targeting bcl-2 mRNA. Initial in vitro studies using an 18-base phosphorothioate oligonucleotide complementary to the first six codons of the bcl-2 mRNA (G3139) showed that this molecule selectively and specifically inhibits Bcl-2 expression in the SU-DHL-4 (t(14;18))-containing lymphoma cell lines. By performing rigorous efficacy, pharmacokinetic and toxicity studies using a number of different lymphoma mouse models the preclinical evaluation of G3139 was completed and further studies were extended into a phase I study for lymphoma patients with high Bcl-2 expression. Overall the human phase I studies with G3139 demonstrated good efficacy with low toxicity. In particular, tumor regression, improvement in the laboratory parameters, and symptom improvement together with downregulation of the target protein expression were achieved. Based on these promising results several phase II/III clinical trials were initiated (Table 1). A phase II trial of oblimersen sodium as a single agent showed only modest clinical activity in heavily pre-treated patients with advanced CLL. However, a separate phase III study of fludarabine plus cyclophosphamide with or without oblimersen showed a 5-year survival benefit in a post hoc analysis of patients with CLL who achieved complete (CR) or partial remission (PR). Oblimersen in combination with rituximab was tested in a phase II trial in non-Hodgkin lymphoma. This study revealed a CR in 23% patients, a PR in 19% patients and 28% of patients showed a minimal response or a stable disease (SD). Another phase II trial was conducted for the treatment of AML with a combination of oblimersen and gemtuzumab ozogamicin with 10% patients achieved a CR and 15% patients achieved a PR. Oblimersen was also tested in combination with dexamethasone and thalidomide in a phase II trial for the treatment of relapsed MM patients. Fifty five percent of patients had objective responses, including 2/36 CRs, 4/36 near CRs, and 12/36 PR and 6/36 patients had minimal responses. However, a randomised phase III trial of oblimersen in combination with other drugs in advanced MM, melanoma or prostate cancer did not show a statistical difference in overall survival. Based on these results oblimersen was not approved as a therapeutic option by the FDA. Though, oblimersen efficiently down-regulated Bcl-2 in cell culture and thereby effectively reduced lymphoma cell survival the lack of efficiency in primary tumors might be a result of insufficient drug delivery in patients. Furthermore, those studies demonstrated a long-term benefit for some patients after completing the study and encouraged a renewal of the drug approval, which is still ongoing.

**Gossypol**

Gossypol, a natural phenol derived from the cotton plant, was characterized as a specific antagonist of Bcl-xl. Further studies showed that gossypol acts as a pan-Bcl-2-family inhibitor, capable of binding and inhibiting most antiapoptotic Bcl-2-family members. Preclinical evaluation of gossypol revealed a potent anti-tumor activity by activating the mitochondrial apoptotic pathway in DLBL. Based on these analyses an orally-bioavailable enantiomer of gossypol, AT-101, was evaluated in phase II clinical trials for the treatment of prostate and lung cancer as single agent or in combination with conventional chemotherapy.
Obatoclax (GX15-070)

Obatoclax, a pan-Bcl-2-family inhibitor, was developed from a natural lead compound that potently disrupted the interaction of members of the Bcl-2-family in a functional screen.\textsuperscript{121} As a single agent, obatoclax possesses pronounced anticancer activity in cell lines or primary cells derived from patients suffering from different hematologic malignancies including AML,\textsuperscript{122} ALL,\textsuperscript{124} CLL,\textsuperscript{125} MM,\textsuperscript{126} and HL (Hodgkin Lymphoma).\textsuperscript{127} Albeit promising results were obtained in vitro and in vivo mouse models, phase I clinical trials for the treatment of AML, CLL, ALL, lymphoma and solid tumors as well as phase II clinical trials for the treatment of HL and myelofibrosis revealed a clinical activity as single agent only in a minority of patients (Table 1).\textsuperscript{128–132} However, further phase I trials for the combined treatment show very promising clinical activity of obatoclax in specific treatment protocols, such as the combination with bortezomib in MCL\textsuperscript{133} and MM\textsuperscript{134} with 3/12 and 4/10 patients showing a clinical response, respectively.

ABT-737/ABT-263 (Navitoclax)/ABT-199 (GDC-0199)

A high-throughput NMR-based method was used to screen a chemical library to identify small molecules that bind to the hydrophobic BH3-binding groove of Bcl-xL. The resultant compound ABT-737, developed by Abbott Laboratories (North Chicago, IL, USA), bound with high affinity (Ki ≤ 1 nM) to Bcl-xL, Bcl-2 and Bcl-w, but not to the less-homologous proteins Bcl-b, Mcl-1 and A1.\textsuperscript{135} Further evaluation of its anti-tumor activity showed that ABT-737 displayed potent single-agent activity against a subset of cell lines representing lymphoid malignancies.\textsuperscript{135} In combination with conventional chemotherapeutics, ABT-737 was shown to potently induce apoptosis in HL,\textsuperscript{136} MM,\textsuperscript{137} AMC,\textsuperscript{138} and CLL\textsuperscript{139} and also solid tumors such as non-small cell lung cancer (NSCLC).\textsuperscript{135} In addition to established human tumor cell lines, ABT-737 effectively induced apoptosis in primary patient-derived lymphoma and CLL cells ex vivo.\textsuperscript{135}

However, the prospects for ABT-737 as a therapeutic agent have been hampered by its poor physiochemical and pharmaceutical properties. This compound is not orally bioavailable and its low aqueous solubility makes formulation for i.v. delivery challenging. However, the impressive biological activity of ABT-737 encouraged research to develop an orally-bioavailable compound. On the basis of various pharmacokinetic/pharmacodynamic models and animal studies ABT-263 (Navitoclax; Figure 3), an orally-bioavailable Bad-like BH3 mimetic (Kis of < 1 nM for Bcl-2, Bcl-xL, and Bcl-w) was generated. ABT-263 disrupts Bcl-2/Bcl-xL interactions with pro-death proteins (e.g. Bim), leading to the initiation of apoptosis within 2 h post treatment.\textsuperscript{140} Initial analyses showed that the oral administration of ABT-263 alone induces complete tumor regressions in xenograft models of small cell lung cancer (SCLC) and ALL. In xenograft models of aggressive B-cell lymphoma and MM ABT-263 promoted significant efficacy of clinically relevant chemotherapeutic regimens.\textsuperscript{140} A detailed activity screen revealed that ABT-263 enhances the response of multiple chemotherapeutic regimens, for example, rituximab, rapamycin, rituximab–cyclophosphamide-adriamycin-vincristine-prednisone, and bortezomib, in several models of hematologic malignancies.\textsuperscript{141} However, owing to the crucial role of Bcl-xL in platelet homeostasis\textsuperscript{134} the therapeutic use of ABT-263 was associated with transient thrombocytopenia in preclinical trials.\textsuperscript{142}

Based on the results obtained by ABT-737 and ABT-263, Abbott Laboratories has recently developed a high-affinity Bcl-2-selective BH3 mimetic, ABT-199 (GDC-0199), which spared human platelets in vitro and dog platelets in vivo.\textsuperscript{143} Tumor regression was achieved for xenographs of human lymphoma cell lines. ABT-199 was as effective as ABT-737 in prolonging survival of immuno-competent mice bearing aggressive progenitor cell lymphomas (derived from bitransgenic myc/bcl-2 mice) without causing thrombocytopenia.\textsuperscript{144} Furthermore, ABT-199 was identified as a promising therapeutic option for the treatment of t(11;14) MM and AML.\textsuperscript{146} More strikingly, the first clinical trial using a single dose of ABT-199 in three patients with refractory CLL resulted in a rapid tumor lysis within 24 h in 3 of 3 patients.\textsuperscript{143}

Together the data obtained by using different pharmacological inhibitors of Bcl-2 indicated a marked susceptibility of hematologic malignancies towards the Bcl-2-targeting protocols (Table 1).

Perspectives and Restrictions

There are not many anticancer therapeutics that are as exclusively characterized as Bcl-2-targeting strategies concerning their specificity toward the designated target and the mode of their interference with cellular actions and cell death. This is indeed based on our increasing knowledge about Bcl-2 protein family in the last 20 years, which finally has entered the translational stage in the last couple of years. One major aspect about Bcl-2 proteins is their crucial role in the development and the homeostasis of cells of hematopoietic origin. Previous data showed that an imbalanced Bcl-2 protein level causally determines hematologic malignant progression and accordingly targeting the Bcl-2 protein family has been proven to be successful, in particular, in hematologic malignancies. However, manipulations in their function or abundance in the healthy hematologic system may result in fatal changes in this tissue compartment.\textsuperscript{147} Accordingly, this may indicate that in non-hematologic malignancies Bcl-2-targeting strategies should be used with specific caution concerning the functionality and homeostasis of the hematologic system. Alternatively, given the cell type-specific role of some Bcl-2-family members and a more abundant role of other members a highly selective targeting strategy is necessary to avoid severe on-target side effects in the development of other tissues. However, agents targeting the Bcl-2 protein family have been generally shown to be a potent killer of tumor cells derived from hematologic malignancies.
and accumulating evidence supports the idea that the treatment of other cancer entities may strongly benefit from the Bcl-2-antagonizing protocols in combination with other chemotherapy regimens. The knowledge of how and in combination with which additional chemotherapeutics Bcl-2-antagonizing protocols provoke a potent anticancer effect will strongly foster the clinical evaluation of Bcl-2-targeting strategies.

Conflict of Interest

The authors declare no conflict of interest.

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1. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 2007; 26: 1324–1337.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646–674.
3. Sachs L. The control of hematopoiesis and leukemia: from basic biology to the clinic. Proc Natl Acad Sci USA 1996; 93: 4742–4749.
4. Wickremasinghe RG, Hoffbrand AV. Biochemical and genetic control of apoptosis: relevance to normal hematopoiesis and hematological malignancies. Blood 1999; 93: 3587–3602.
5. Renault TT, Chipuk JE. Getting away with murder: how does the BCL-2 family of proteins kill with immunity? Ann N Y Acad Sci 2013; 1258: 59–79.
6. Debash KM. Role of apoptosis in congenital hematologic disorders and bone marrow failure. Rev Clin Exp Hematol 2003; 7: 57–71.
7. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 1984; 226: 1097–1099.
8. Nakayama K, Nakayama K, Negishi I, Kuida K, Shinaki Y, Louie MC et al. Disappearance of the lymphoid system in Bcl-2 homozygous mutant chimeric mice. Science 1993; 261: 1584–1588.
9. Veis DJ, Sorensen CM, Shutter JR, Korsmeyer SJ. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycytic kidneys, and hypopigmented hair. Cell 1993; 75: 229–240.
10. Matsuzaki Y, Nakayama K, Nakayama K, Tomita T, Isoda M, Loh DY et al. Role of Bcl-2 in the development of lymphoid cells from the hematopoietic stem cell. Blood 1997; 89: 853–862.
11. Strasser A, Harris AW, Cory S. bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell 1991; 67: 889–899.
12. Strasser A, Whittingham S, Vaux DL, Bath ML, Adams JM, Cory S et al. Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. Proc Natl Acad Sci USA 1991; 88: 8661–8665.
13. Strasser A, Harris AW, Corcoran LM, Cory S. B-Cell expression promotes B- not T-lymphoid development in acid mice. Nature 1994; 368: 457–460.
14. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 1988; 335: 440–442.
15. Kozopas KM, Yang T, Buchanan HL, Zhou P, Craig RW. MCL1 a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. Proc Natl Acad Sci USA 1993; 90: 3516–3520.
16. Ohts K, Umewaka A, Fukumura M, Ando T, Ubara F, Sano M et al. Acute myeloid leukemia possessing jumping translocation is related to highly elevated levels of EAT/mci-1, a B-cd related gene with anti-apoptotic functions. Leuk Res 2000; 24: 73–77.
17. Khoury JD, Medeiros LJ, Rassidakis GZ, McDonnell TJ, Abruzzo LV, Lai R. Expression of bcl-2 related gene with anti-apoptotic functions in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. Proc Natl Acad Sci USA 1991; 88: 8661–8665.
18. Strasser A, Harris AW, Corcoran LM, Cory S. B-Cell expression promotes B- not T-lymphoid development in acid mice. Nature 1994; 368: 457–460.
19. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 1988; 335: 440–442.
20. Kozopas KM, Yang T, Buchanan HL, Zhou P, Craig RW. MCL1 a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. Proc Natl Acad Sci USA 1993; 90: 3516–3520.
21. Chittenden T, Harrington EA, O'Connor R, Flemington C, Lutz RJ, Evan GI et al. Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-xL. J Exp Med 2000; 191: 977–984.
22. Toyota Y, Wang F, Roth KA, Sawa H, Nakayama K, Nakayama K et al. Massive cell death in mature hematopoietic cells and neurons in Bcl-x-deficient mice. Science 1989; 267: 1506–1510.
23. Mason KD, Carpelini MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S et al. Programmed anuclear cell death delimits platelet life span. Cell 2007; 128: 1173–1186.
24. Kelly PN, Grabow S, Delbridge AR, Strasser A, Adams JM. Endogenous Bcl-xL is essential for Myc-driven lymphomagenesis in mice. Blood 2011; 118: 6380–6386.
25. Lin EY, Orfordf A, Berger MS, Prystowsky MB. Characterization of A1, a novel hemopoietic-specific early-response gene with sequence similarity to bcl-2. J Immunol 1993; 151: 1197–1198.
26. Nagy B, Lundan T, Larramendi ML, Aalto Y, Zhu Y, Niren T et al. Abnormal expression of apoptosis-related genes in hematological malignancies: overexpression of MYC is poor prognostic sign in mantle cell lymphoma. Br J Haematol 2003; 120: 434–441.
27. Mahadevan D, Spier C, Delta Croce K, Miller S, George B, Riley C et al. Transcript profiling in peripheral T-cell lymphoma, not otherwise specified, and diffuse large b-cell lymphoma identifies distinct tumor profile signatures. Mol Cancer Ther 2005; 4: 1867–1879.
28. Pva R, Pellegrino E, Mattoli M, Agnelli L, Lombardi L, Boccalatte F et al. Functional validation of the anaplastic lymphoma kinase signature identifies CEBPB and BCL2A1 as critical target genes. J Clin Invest 2006; 116: 3171–3182.
29. More S, Savage KJ, Kutok JL, Feuerhake F, Kurtin P, Milm M et al. Molecular profiling of diffuse large b-cell lymphomas identifies robust subtypes including one characterized by host inflammatory response. Blood 2005; 105: 1851–1861.
30. Xiang Z, Ahmed AM, Moeller C, Nakayama K, Hatakeyama S, Nilsson G. Essential role of the prosurvival bcl-2 homologue A1 in mast cell survival after allergic activation. J Exp Med 2001; 194: 1561–1569.
31. Tomoyako MM, Canco MP. Long-lived B cells are distinguished by expression of A1. J Immunol 1998; 160: 107–111.
32. Hamasaki A, Seno D, Nakayama K, Iehda N, Negishi I, Nakayama K et al. Accelerated neurotrophin apoptosis in mice lacking A1-a, a subtype of the bcl-2-related A1 gene. J Exp Med 1996; 184: 1985–1992.
33. Onderboender P, Kanellopoulou C, Heselmeyer V, Paepere C, Borowsk C, Aflantis I et al. Efficiency of RNA interference in the mouse hematopoietic system varies between cell types and developmental stages. Mol Cell Biol 2003; 23: 3896–3905.
34. Ortega E, Greppi F, Tischner D, Sorrota C, Geley S, Ploner A et al. Targeting antiapoptotic A1/BFL-1 by in vivo RNAi reveals multiple roles in leukocyte development in mice. Blood 2012; 119: 6032–6042.
35. Verschelde C, Michonneau D, Treseol-Biennet MC, Berberich I, Schimpi A, Bonnefoy-Berard N. Overexpression of the antiapoptotic protein A1 promotes the survival of double positive thymocytes awaiting positive selection. Cell Death Differ 2008; 13: 1213–1221.
36. Churban PI, Morefield LS, Liu CY, Chen S, Harlan JM, Willerford DM. Perturbation of B-cell development in mice using OX40 ligation of the B2-2 homolog A1. Blood 2002; 99: 3350–3359.
37. Oldv ZN, Millman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993; 74: 659–619.
38. Cittertten J, Harrington EA, O’Connor R, Flemington C, Lutz RJ, Egan VI et al. Induction of apoptosis by the Bcl-2 homologue Bak. Nature 1995; 374: 733–736.
39. Keifer MC, Brauer MJ, Powers VC, Wu JJ, Umannski SR, Toome LD et al. Modulation of apoptosis by the widely distributed Bcl-2 homologue Bak. Nature 1995; 374: 736–739.

Targeting mitochondrial apoptosis in cancer
K Brinkmann and H Kashkar

Cell Death and Disease
et al. 66. Pellegrini M, Belz G, Bouillet P, Strasser A. Shutdown of an acute T cell immune response. 77. Erlacher M, Labi V, Manzl C, Bock G, Tzankov A, Hacker G et al. 51. Adams JM, Cory S. Bcl-2-regulated apoptosis: mechanism and therapeutic potential. 78. Hemann MT, Zilfou JT, Zhao Z, Burgess DJ, Hannon GJ, Lowe SW. Suppression of tumorigenesis by the p53 target PUMA. Proc Natl Acad Sci USA 2004; 101: 9324–9330. 91. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death 92. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y et al. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. 93. Pocock CF, Malone M, Booth M, Evans M, Morgan G, Greil J et al. Comparison of genome profiles for identification of distinct subgroups of diffuse large B-cell lymphoma. 94. Devereux S, Cotter FE. Anti-sense and gene therapy approaches to the treatment of B-cell lymphomas. 95. Datta SR, Li G, Lu X, Tschopp J. Caspase-8 and RIP1 are required for death receptor-induced activation of the integrin alphaM. 96. Devereux S, Cotter FE. 104. Bedikian AY, Millward M, Pehamberger H, Conry R, Gore M, Trefzer U et al. 105. Badros AZ, Goloubeva O, Rapoport AP, Ratterree B, Gahres N, Meisenberg B et al. 106. Mestre-Escorihuela C, Rubio-Moscardo F, Richter JA, Siebert R, Climent J, Fresquet V et al. 107. Lindsten T, Ross AJ, King A, Zong WX, Rathmell JC, Shiels HA et al. 108. Hildeman DA, Zhu Y, Mitchell TC, Bouillet P, Strasser A, Kappler J et al. Bim: a novel 270 17. In vivo mediated by proapoptotic bcl-2 family member bim. 2013; 1181: 1180–1191. 109. Zha J, Harada H, Data SR, Hsu, Tofu, Gotzoy E et al. Akt phosphorylation of 110. Anderson JS, Wang J, Yang Y, Tewari M, Korsmeyer SJ. BH3-only proteins Noxa and 2013; 118: 1601–1615. 111. Seki, Misayashita T, Tanaka S, Reed JC. 112. Cotter FE, Johnson P, Hall P, Pocock C, Al-Mahdi N, Cowell J et al. Antisense oligonucleotides suppress B-cell lymphoma growth in a SCID-hu mouse model. Oncogene 1994; 9: 3049–3055. 113. Deureverus S, Cotter FE. Anti-sense and gene therapy approaches to the treatment of lymphomas. Baillieres Clin Haematol 1996; 9: 819–834. 114. Prokop CF, Matone M, Bondr J, Evans M, Morgan G, Grel J et al. BCL-2 expression by 115. Cotter FE, Waters J, Cunningham D. Human Bcl-2 antisense therapy for lymphomas. Biochim Biophys Acts 1999; 1449: 97–106. 116. O'Brien SM, Cunningham CC, Golenkov AK, Turina AG, Novick SC, Rai KR. Phase IIb multicenter study of oblimersen sodium, a Bcl-2 antisense oligonucleotide, in patients with advanced chronic lymphocytic leukemia. J Clin Oncol 2003; 21: 7697–7702. 117. O'Brien S, Moore JD, Boyd LM, Skotnicki AB, Koziner B et al. 5-year survival in patients with relapsed or refractory chronic lymphocytic leukemia in a randomized phase III trial of fludarabine plus cyclophosphamide with or without oblimersen. J Clin Oncol 2009; 27: 5028–5032. 118. Pro B, Leber B, Smith M, Fayed L, Romaguera J, Hagemeister F et al. Phase II multicenter study of oblimersen sodium, a Bcl-2 antisense oligonucleotide, in combination with rituximab in patients with recurrent B-cell non-Hodgkin lymphoma. Br J Haematol 2008; 143: 355–362. 119. Moore J, Seltler K, Koltz J, Stock W, Gles F, Kalichay M et al. A Phase II study of Bcl-2 antisense (oblimersen sodium) combined with gemtuzumab ozogamicin in older patients with acute myeloid leukemia in first relapse. Leuk Res 2006; 30: 777–783. 120. Badros AZ, Goloubeva O, Tewari M, Gach N, Meisenberg B et al. Phase II study of G3139, a Bcl-2 antisense oligonucleotide, in patients with dexamethasone and thalidomide in relapsed multiple myeloma patients. J Clin Oncol 2005; 23: 4089–4099. 121. Bedkian AF, Millward M, Pehamberger H, Conry R, Gore M, Tratzler U et al. Bcl-2 antisense (oblimersen sodium) plus darzoxabine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. J Clin Oncol 2006; 24: 4738–4745. 122. Chan-Khan AA, Nesvukly R, Hohl RJ, Zimmerman TM, Christiansen NP, Schiller G et al. Phase III randomised study of dexamethasone with or without oblimersen sodium for patients with advanced multiple myeloma. Leukemia 2009; 50: 559–565.
1. Sternberg CN, Dumez H, Van Poppel H, Skonecna I, Salla A, Daugaard G et al. Docetaxel plus olmisibom sodium (Bcl-2 antisense oligonucleotide): an EORTC multicenter, randomized phase II study in patients with castration-resistant prostate cancer. Ann Oncol 2009; 20: 1264–1269.
2. Advani RP, Paulus A, Masood A, Sher T, Chanarv-Khan A. Pharmacokinetic evaluation of olmisibom sodium for the treatment of chronic lymphocytic leukemia. Expert Opin Drug Metab Toxicol 2011; 7: 755–774.
3. Davies MS, Letal A. Targeting the B-cell lymphoma/leukemia 2 family in cancer. J Clin Oncol 2012; 30: 3127–3135.
4. Kitada S, Leone M, Sareth S, Zhai D, Reed JC, Pellecchia M. Discovery, characterization, and structure-activity relationship studies of proapoptotic polyphenols targeting B-cell lymphocyte/leukemia-2 proteins. J Med Chem 2003; 46: 4259–4264.
5. Zhai D, Jin C, Satterthwait AC, Reed JC. Comparison of chemical inhibitors of antiapoptotic Bcl-2 family proteins. Cell Death Differ 2006; 13: 1419–1421.
6. Mohammad RM, Wang S, Aboukameel A, Chen B, Wu X, Chen J et al. Preclinical studies of a nonpeptidic small-molecule inhibitor of Bcl-2 and Bcl-X(L) ([−]-gossypol) against diffuse large cell lymphoma. Mol Cell Ther 2010; 4: 13–21.
7. Meng Y, Li Y, Li J, Li H, Fu J, Liu Y et al. ([−]Gossypol and its combination with imatinib induce apoptosis in human chronic myeloid leukemic cells. Leuk Lymphoma 2007; 48: 2204–2212.
8. Balakrishnan K, Burger JA, Wierda WG, Gandhi V. AT-101 induces apoptosis in CLL B cells and overcomes stromal cell-mediated I-1 induction and drug resistance. Blood 2009; 113: 148–159.
9. Li ZM, Jiang WQ, Zhu ZY, Zhu XF, Zhou JM, Liu ZG et al. Synergistic cytotoxicity of Bcl-XL inhibitor, gossypol and chemotherapeutic agents in non-Hodgkin’s lymphoma cell lines. Cancer Biol Ther 2011; 9: 51–60.
10. Heist RS, Fain J, Chinnasami B, Khan W, Molina JR, Sequist LV et al. Phase I/II study of AT-101 with topotecan in relapsed and refractory small cell lung cancer. J Thorac Oncol 2010; 5: 1637–1643.
11. Liu G, Kelly WK, Wilding G, Leopold L, Brill K, Somer B. An open-label, multicenter, phase I study of the proapoptotic agent AT-101 plus docetaxel, in second-line non-small cell lung cancer. J Thorac Oncol 2011; 6: 781–785.
12. Baggstrom MO, Qi Y, Koczysw M, Agrins A, Johnson EA, Millward MJ et al. Phase II study of AT-101 (Gossypol) in chemotherapy-sensitive recurrent extensive-stage small cell lung cancer. J Thorac Oncol 2011; 6: 1757–1760.
13. Stampfve A, Akiner A, Burke JM, Caton JR, Pleming MT, Hutson TE et al. Randomized phase II trial of docetaxel plus prednisone in combination with placebo or AT-101, an oral small molecule Bcl-2 family antagonist, as first-line therapy for metastatic castration-resistant prostate cancer. J Thorac Oncol 2012; 7: 1803–1808.
14. Clinic MA. Phase III Clinical Trial of Lenalidomide in Combination With AT-101 for the Treatment of Relapsed B-cell Lymphocytic Leukemia (B-CLL). ClinicalTrials.gov 2009.
15. Shore GC, Viallet J. Modulating the bcl-2 family of apoptosis suppressors for potential therapeutic benefit in cancer. Hematol Am Soc Hematol Educ Program 2005; 6: 226–230.
16. Konoplewa M, Watt J, Contractor R, Tsao T, Samudro I, Ruivo PP et al. Kinome Atlas of AT-101 (Gossypol) in leukemia. Leukemia 2009; 23: 2285–2288.
17. Campas C, Cosialls AM, Barragan M, Iglesias-Serret D, Santidrian AF, Coll-Mulet L et al. Antitumor activity of the novel Bcl-2 homology domain-3 mimetic GX15-070 against aggressive Myc-driven mouse lymphomas without provoking thrombocytopenia. Leukemia 2014; 28: 2374–2380.
18. Konoplewa M, Contractor R, Tsao T, Samudro I, Ruivo PP, Ktata S et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 2006; 10: 375–388.
19. Kuroda J, Puthalakath H, Craig MS, Kelly PN, Boullot P, Huang DC et al. Bin and Bad mediate imatinib-induced killing of Bcr/Abl + leukemia cells, and resistance due to their loss is overcome by a BH3 mimetic. Proc Natl Acad Sci USA 2006; 103: 14907–14912.
20. Tae C, Shooseaker AR, Adickes J, Anderson MG, Chen J, Jin S et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res 2008; 68: 3423–3428.
21. Ackler S, Mitten MJ, Foster K, Oleskijw A, Reflo M, Tahir SK et al. The Bcl-2 inhibitor ABT-263 enhances the response of multiple chemotherapeutic regimens in hematologic tumors in vivo. Cancer Chemother Pharmacol 2010; 66: 869–880.
22. Wilson WH, O’Connor OA, Caucasan MS, LeCassas AS, Geretano JF, Leonard JP et al. Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumor activity. Lancet Oncol 2010; 11: 1149–1159.
23. Souers AJ, Leveson JD, Boghaert ER, Ackler SL, Caton ND, Chen J et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med 2013; 19: 202–208.
24. Vandenberg CJ, S Cory, ABT-199, a new Bcl-2-specific BH3 mimetic, has in vivo efficacy against aggressive Myc-driven mouse lymphomas without provoking thrombocytopenia. Blood 2013; 121: 2265–2288.
25. Touzeau C, Dousset C, Le Goulil S, Sampath D, Leveson JD, Souers AJ et al. The Bcl-2 splicing BH3 mimetic ABT-199: a promising targeted therapy for (1/114) multiple myeloma. Leukemia 2014; 28: 210–212.
26. Pan R, Hodgall LJ, Benito JM,ucci D, Han L, Borthakur G et al. Selective BCL-2 inhibition by ABT-199 causes on target cell death in acute myeloid leukemia. Cancer Discov 2014; e-pub ahead of print 13 February 2014; doi:10.1158/2155-2159. CD-13-0229.
27. Knaw SL, Merino D, Anderson MA, Glasser SP, Boullot P, Roberts AW et al. Both pharmacological and normal peripheral B lymphoid cells are highly sensitive to the selective pharmacological inhibition of pro-survival Bcl-2 with ABT-199. Leukemia 2014; e-pub ahead of print 9 January 2014; doi:10.1038/leu.2014.1.