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Commentary

Bcl-2 family members as molecular targets in cancer therapy

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ABSTRACT

Escape from apoptosis is often a hallmark of cancer cells, and is associated to chemotherapy resistance or tumor relapse. Proteins from the Bcl-2 family are the key regulators of the intrinsic pathway of apoptosis, controlling the point-of no-return and setting the threshold to engage the death machinery in response to a chemical damage. Therefore, Bcl-2 proteins have emerged as an attractive target to develop novel anticancer drugs. Current pharmacological approaches are focused on the use of peptides, small inhibitory molecules or antisense oligonucleotides to neutralize antiapoptotic Bcl-2 proteins, lowering the threshold and facilitating apoptosis of cancer cells. We discuss here recent advances in the development of Bcl-2 targeted anticancer therapies.

1. Introduction

The bcl-2 oncogene was first described in 1984 in an acute B-cell leukemia cell line carrying the t(14;18) chromosome translocation [1]. In contrast with other oncogenes, bcl-2 was shown to favor cell survival through inhibition of apoptosis, rather than inducing cell proliferation [2,3]. With the completion of whole-genome sequence and progress in bioinformatics, more than 25 members of the bcl-2 gene family have been identified in humans [4]. All members of this family are involved in the regulation of cell death, with some of them being antiapoptotic, like Bcl-2 itself, and others being proapoptotic. Antiapoptotic members of the Bcl-2 family, except for Mcl-1, contain all the four Bcl-2 homology (BH) domains, whereas the proapoptotic proteins contain three (Bax and Bak, multidomain) or only the BH3 domain (the BH3-only subfamily) (Fig. 1). The Bnip subfamily is usually included into the BH3-only group based on its limited homology with BH3 domains [4]. Beclin-1 [5], a Bcl-2 binding protein that promotes autophagy, and the citosolic fragment of Erbb4 (4ICD) [6] have also been proposed to be BH3-only proteins. The antiapoptotic viral proteins F1L and M11L, could also be included in the Bcl-2 family based in its Bcl-2-like fold [7]. The antiapoptotic proteins Bcl-2, Bcl-XL and Mcl-1 are overexpressed in many tumor cells, contributing to tumorigenesis by inhibition of apoptosis. Overexpression of antiapoptotic members of the Bcl-2 family could also be implicated in chemotherapy resistance [8].

Bcl-2 family proteins are key regulators of the mitochondrial or intrinsic apoptotic pathway, inducing or preventing the release of apoptogenic proteins such as cytochrome c, apoptosis inducing factor (AIF), Smac/DIABLO, EndoG and Omi/HtrA2 that reside in the intermembrane space of mitochondria in healthy cells (Fig. 2). This event seems to determine the fate of the cell after receiving a physical or chemical insult [9,10]. Moreover, many apoptosis inducers activate one or more of the BH3-only proteins and the participation of at least a multidomain proapoptotic protein (Bak or Bak) is imperative for the intrinsic cell death pathway [11]. Because of this central role in the intrinsic pathway, mitochondria and Bcl-2 proteins are now viewed as potential drug targets for antitumor therapy. In the last years, small

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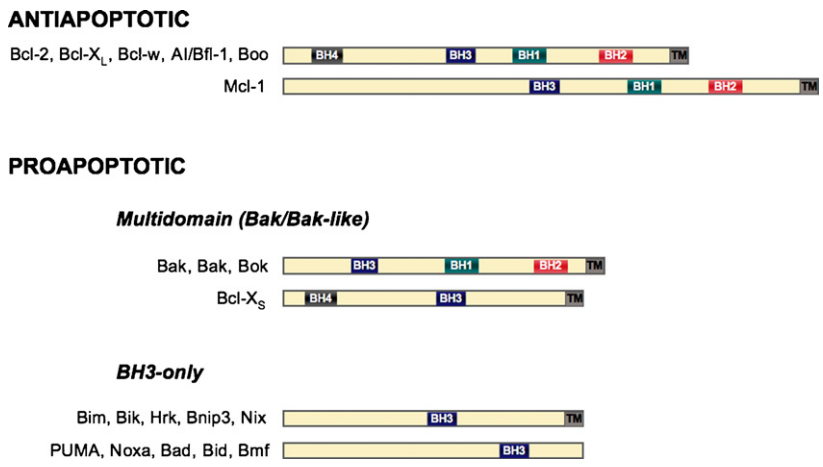

Fig. 1 – Bcl-2 family of proteins. BH, Bcl-2 homology domain; TM, transmembrane domain.

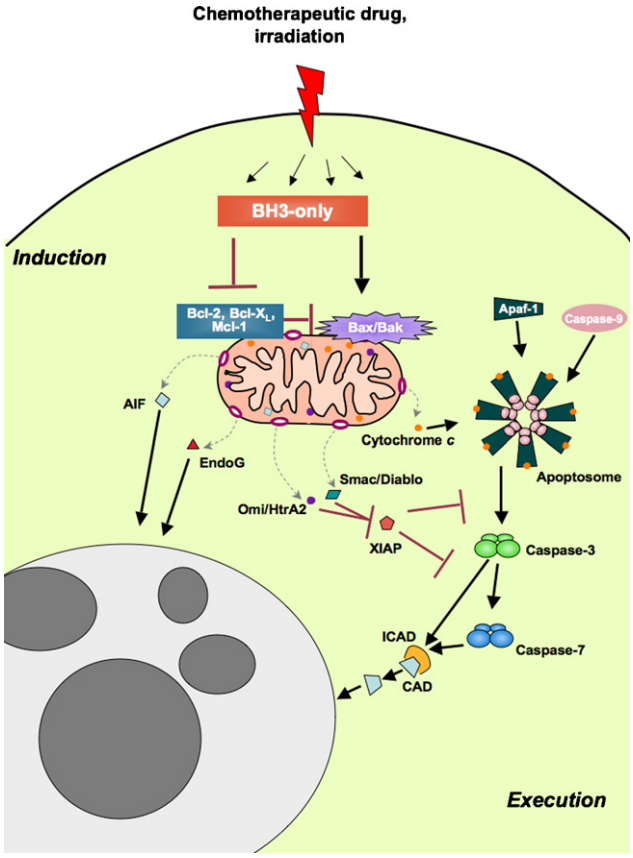

Fig. 2 – The intrinsic pathway of apoptosis. In response to a chemical insult or irradiation, BH3 proteins act as damage sensors and induce mitochondrial permeabilization either by freeing Bax/Bak from antiapoptotic Bcl-2 proteins, or by direct activation of Bax/Bak. Apoptogenic proteins cytochrome c, AIF and EndoG, Omi/HtrA2 and Smac/Diablo are released from mitochondria. Cytochrome c forms a complex with Apaf-1 and procaspase-9 leading to apoptosome formation and caspase-9 activation that triggers a caspase activation cascade. Caspases, AIF and EndoG carry on the execution of apoptosis. Bcl-2 targeted therapies seek to block the antiapoptotic members of the family, thus provoking or facilitating the mitochondrial release of apoptogenic proteins.

compounds, peptides and antisense oligodeoxynucleotides targeting Bcl-2 proteins have been evaluated in preclinical studies or even reached clinical assays.

2. Synthetic BH3-mimetics

According to the "displacement model", BH3-only members of the Bcl-2 family bind to antiapoptotic members, counteracting their protective effect by freeing multidomain proapoptotic members Bax and Bak. It has also been proposed that some BH3-only proteins can also directly activate Bax and/or Bak, although this is still a matter of controversy in the field. Irrespective of the mechanism, direct or by displacement, BH3-only proteins seem to be crucial for the intrinsic pathway of apoptosis, acting as damage sensors that determine cell fate and overexpression of BH3-only proteins induce apoptosis in different cell types [11–17].

Emulation of the proapoptotic activity of BH3-only proteins has become an attractive strategy for the development of new anticancer therapies. First, recent advances in the knowledge of the structure of complexes between BH3 domains and prosurvival members of the Bcl-2 family have opened the opportunity for rational design of anticancer drugs. Second, BH3 domains are relatively small (14–24 amino acids) making possible the synthesis of peptides or small molecules pharmacologically active that reproduce their proapoptotic function in cells.

2.1. *Peptides*

Overexpression of BH3 domains induces apoptosis and soluble BH3-peptides produce cytochrome c release from isolated mitochondria [11]. For their potential use in cancer therapy, these peptides must be modified in order to increase cell uptake and stability. Stapled BH3 peptides with enhanced stability, like the *BID-SAHB* peptide derived form Bid, have been shown to induce apoptosis in Jurkat cells and to inhibit the growth of leukemia xenografts [18]. Wang et al. synthesized a Bad-BH3 peptide with a cell permeable moiety (*cpm-1285*) that induced apoptosis in HL-60 leukemia cells in a caspase-dependent way and delayed myeloid leukemia growth in mice [19]. These reports indicate that BH3 peptides

that interfere with interactions between proteins of the Bcl-2 family might be used for antitumor treatment.

2.2. ABT-737

Small organic compounds that bind to the hydrophobic groove in the surface of antiapoptotic proteins, mimicking BH3 domains of proapoptotic proteins, have been identified through high-throughput screening and/or molecular design. One of these compounds is *ABT-737* (Abbott Laboratories, Abbott Park, IL, USA) was identified using a nuclear magnetic resonance (NMR)-based screening for Bcl-2 inhibitors [20]. *ABT-737* potently ($K(i) < 1$ nM) inhibits the antiapoptotic proteins Bcl-2, Bcl-XL and Bcl-w, but not Mcl-1 or A1/Bfl-1, according to Chen et al. [21]. In a recent study, the crystal structure of the Bcl-XL/*ABT-737* complex has been elucidated, demonstrating that *ABT-737* is a functional, but not structural, mimetic of the Bad-BH3 domain [22]. Probably due to its inability to bind Mcl-1, *ABT-737* by itself is not an efficient apoptosis inducer but shows synergy with other compounds such as the CDK inhibitor roscovitine. Other authors have reported that this compound selectively induces apoptosis in multiple myeloma cells but not in normal cells [23]. In a recent work, the activity of *ABT-737* against pediatric tumors has been tested both *in vitro* and using xenograft models [24]. Although *ABT-737* exhibited *in vitro* a toxic activity for several cell lines, its activity as a single agent *in vivo* was limited to acute lymphoblastic leukemia (ALL) xenografts. The response of cells to *ABT-737* can be predicted by "BH3 profiling", a method that allows to determine whether cells depend on Bcl-2 or Mcl-1 [25]. Using this method, Del Gaizo-Moore et al. have predicted the sensitivity of ALL cells to *ABT-737* as a single agent. In Bcr/Abl$^+$ cells, *ABT-737* cotreatment with imatinib offsets drug-resistance conferred by diverse mechanisms [26,27].

2.3. Obatoclax (GX15-070)

This synthetic indol bipyrrol derivative of bacterial prodiginines, developed by Gemin X Biotechnologies (Montreal, Canada) acts as a BH3-mimetic. In contrast to *ABT-737*, *obatoclax* seems to be a pan-Bcl-2 inhibitor that can also target the antiapoptotic protein Mcl-1. *Obatoclax* can induce apoptosis in hematological neoplasia [28–30], breast cancer [31] and even in cells that are resistant to other drugs like melphalan, *ABT-737* or bortezomib. In a melanoma cell line, *obatoclax* showed a high synergy with bortezomib [32]. At present *obatoclax* is being tested in Phase I clinical trials, alone or in combination with bortezomib, docetaxel, topotecan or rituximab in patients with relapsed or refractory mantle cell lymphoma, chronic lymphocytic leukaemia (CLL), non-small cell lung cancer and other solid tumors ([33] and http://www.geminx.com). Phase II studies are being conducted for Hodgkin's lymphoma, myelofibrosis with myeloid metaplasia, myelodysplasia and follicular lymphoma. It has been reported that *obatoclax* binds to recombinant Bcl-w, Bcl-XL and Mcl-1 [34] and disrupts the Mcl-1/Bak interaction in cells [31,32], but there are not structural data proving the mechanism of *obatoclax* as a BH3-mimetic and its binding into the hydrophobic groove of antiapoptotic proteins of the Bcl-2 family.

2.4. HA 14-1

The compound ethyl 2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate was identified by Wang et al. [35], using an *in silico* screening based in the predicted structure of Bcl-2. The interaction of *HA14-1* with Bcl-2 was verified by *in vitro* binding studies. This compound induces apoptosis in a variety of tumor cell lines and cooperates with other drugs such as flavopiridol [36], bortezomib [37], dexamethasone or doxorubicin [38]. Although these results identify *HA14-1* as a good candidate for anticancer treatment, some concerns have been recently raised about this compound [39]. First, *HA14-1* is very unstable and decomposes very rapidly under physiological conditions. Second, although the biological activity of *HA 14-1* was supposed to depend on its interaction with Bcl-2, recent data indicate that ROS generated by disintegration of *HA14-1* could be the mediators of cell death induced by this compound.

2.5. Others

In the last years some small molecules that mimic BH3 domains have been identified, although data about their activity in cells are yet scarce. A compound named *73R* (*A-385358*) was developed by NMR screening and directed parallel synthesis of molecules mimicking BH3 domains [40]. *73R* exhibits relative selectivity for Bcl-XL and efficiently kills tumor cells that depend on this protein for survival [41]. Optimization was necessary to reduce its binding to serum albumin that could hinder its possible progress to the clinical stage. Its cytotoxicity as a single agent is low against several tumor cells lines, but *73R* demonstrated ability to potentiate the activity of UV radiation *in vitro* and paclitaxel both *in vitro* and in xenograft models of human tumor growth, thus verifying the potential utility of an small molecule BH3-mimetic as an anticancer agent [40]. A recent report demonstrates that a new non-peptidic inhibitor of Bcl-2, *TW-37*, exhibits antitumor activity in a diffuse large cell lymphoma xenograft model [42]. Chemical inhibitors of Bcl-X_L based on a terephthalamide scaffold and designed to mimic the BH3 domain of Bak, disrupted Bcl-X_L/Bax interaction in HEK293 cells [43].

3. Natural products

3.1. Gossypol

Some compounds isolated from plants or microorganisms have been proposed to be ligands of proteins of the Bcl-2 family. One of these compounds is *gossypol*, a polyphenolic aldehyde derived from the cotton plant that was first proposed as a male contraceptive. Later, it has been shown that gossypol possesses antineoplastic activity against tumor cells of different origins, both *in vitro* and *in vivo* [44,45]. The (−)-enantiomer of *gossypol* (*AT-101*, Ascenta Therapeutics, San Diego, CA, USA) is a more potent inhibitor of cell growth and a new derivative of *gossypol*, *apogossypol* has demonstrated superior antitumor activity than *gossypol* in Bcl-2 transgenic mice [46]. In a clinical trial in women with refractory

Table 1 – Bcl-2 targeted therapies currently in development

	Target(s)	Stage	References
Peptides			
SAHB-BID	Bax, Bak	Preclinical	[18]
Cpm-1285 (Bad)	Bcl-2, Bcl-XL, Bcl-w	Preclinical	[19]
Small molecule BH3-mimetics			
ABT-737	Bcl-2, Bcl-XL, Bcl-w	Preclinical	[20–27]
Obatoclax	Bcl-XL, Bcl-2, Bcl-w, Mcl-1	Phase I/II	[28–34]
HA 14-1	Bcl-2	Preclinical	[35–39]
73R (A-38535)	Bcl-XL	Preclinical	[40,41]
TW-37	Bcl-2, Mcl-1	Preclinical	[42]
Terephthalamide derivatives	Bcl-XL	Preclinical	[43]
Natural products			
Gossypol (*AT-101*)	Bcl-2, Bcl-XL, Mcl-1, others.	Phase I/II	[44–51]
Chelerythrine	Bcl-XL, PKC, p38, JNK	Preclinical	[52–56]
Antisense oligonucleotides			
Genasense (*oblimersen*)	Bcl-2	Phase II/III	[57–63]
OGX-011	Clusterin (Bax inhibitor protein)	Phase II	[62,64]
4625	Bcl-2, Bcl-XL	Preclinical	[65–67]
ISIS 20408	Mcl-1	Preclinical	[68–71]

metastatic breast cancer, *gossypol* affected cell cycle but showed no clinical activity [47]. Anyway, results from several ongoing clinical trials will determine whether *gossypol* can be a suitable anticancer therapy. Although its potential in cancer treatment is clear, Bcl-2 antiapoptotic proteins could not be the only targets of *gossypol* since this compound has also been reported to bind lactate dehydrogenase [48], 5-lipoxygenase [49] and calcineurin [50,51].

3.2. *Chelerythrine*

Chelerythrine is a natural benzophenanthridine alkaloid, identified as a Bcl-XL ligand in a high-throughput screening of natural products [52]. *Chelerythrine* binds to Bcl-XL at the BH groove and not at the BH3 binding cleft [53] and induces the release of cytochrome c from isolated mitochondria and apoptosis in SH-SY5Y human neuroblastoma cells [52]. Surprisingly, *chelerythrine* shows toxicity even in Bax and Bak deficient MEFs [54] or in Bcl-XL overexpressing cells, and thus it has been proposed as a potential treatment for tumors exhibiting deficiencies in Bax/Bak or high levels of Bcl-X_L [52]. Additional work on this compound is needed to clarify whether *chelerythrine* is specific for Bcl-XL or it also acts through other mechanisms such as inhibition of protein kinase C [55] and activation of p38 and JNK [56].

4. Antisense therapy

An alternative strategy to target antiapoptotic Bcl-2 proteins is to reduce their expression through antisense or RNAi therapies. Up to now, four different antisense oligonucleotides targeting Bcl-2, Bcl-XL or related proteins have been reported (Fig. 3). *Genasense* has reached Phase III trials and *OGX-011* is now being tested in Phase II studies, but the possible clinical activity of *oligonucleotide 4625* and *ISIS 20408* remains unexplored.

4.1. *Genasense (oblimersen sodium, G3139)*

Genasense (Genta, Berkeley Heights, NJ, USA) is a 18mer phosphorothioate oligodoxyribonucleotide, which targets the initiation codon region of Bcl-2 mRNA. A Phase III trial in melanoma showed that the combination of *Genansense* plus dacarbazine improved multiple clinical outcomes when compared to dacarbazine alone [57]. Also, modest benefits of the addition of *Genasense* to therapy have been reported in patients with relapsed multiple myeloma [58], chronic lymphocytic leukemia [59], melanoma [57], and acute myeloid leukemia [60]. On the other side, some clinical trials have failed to demonstrate any advantage in the use of *Genasense* in combination with different chemotherapy drugs [61]. A possible explanation for these discrepancy between *in vitro* results and clinical effect could be a limited efficiency in reducing Bcl-2 levels in patients. Recently, *Genasense* has failed to be approved by FDA because in a Phase III trial of patients with CLL some of the endpoints missed the required statistical cutoff [59], opening a controversy about its future in the clinic [62,63].

4.2. *OGX-011*

This 2′-methoxyethyl-modified phosphorothioate antisense oligonucleotide developed by OncoGenex (Vancouver, British Columbia, Canada), blocks the expression of clusterin, a protein that inhibits the pro-apoptotic protein Bax. In prostate cancer cells caused a dose-dependent increase in cell death [64]. *OGX-011* is at present being tested in Phase II studies in combination with antitumor drugs in NSCLC, prostate and breast cancer [62].

4.3. *Oligonucleotide 4625*

An antisense oligonucleotide (*oligonucleotide 4625*), which is complementary to nucleotides 605–624 of *bcl-2* mRNA and has three mismatches to nucleotides 687–706 of *bcl-xL* mRNA, reduces levels of both proteins [65]. *Oligonucleotide 4625* induces apoptosis in cell lines of different origin and

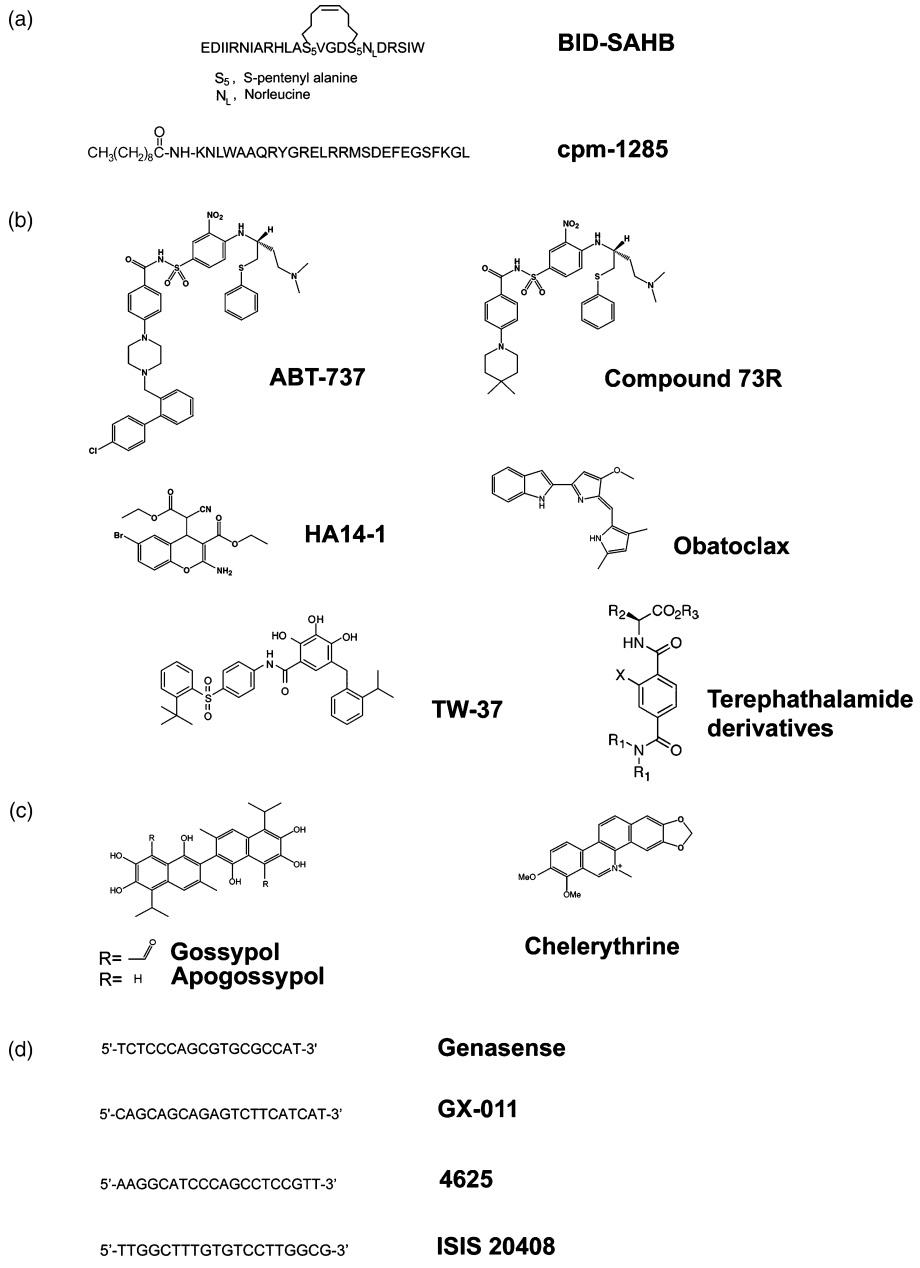

Fig. 3 – Bcl-2-based anticancer therapies. (a) Sequences of BH3 peptides. SAHB, stapled alpha helix of Bcl-2. *Cpm-1285* contains a fatty acid moiety. (b) Structure of non-peptidic BH3-mimetics. (c) Natural compounds that mimic BH3 domains. (d) Sequences of antisense oligonucleotides targeting Bcl-2 antiapoptotic proteins or clusterin. All oligonucleotides have a phosphorothioate backbone; *GX-011*, *oligonucleotide 4625* and *ISIS 20408* bear additionally 2′-O-methoxyethyl-modified nucleotides at both ends.

reduces the growth of xenografted tumors [66]. In order to improve efficacy and specificity for tumor cells, this oligonucleotide has been encapsulated in immunoliposomes targeting an epithelial adhesion molecule abundantly expressed in solid tumors [67] (Table 1).

4.4. *ISIS 20408*

An antisense oligonucleotide targeting the antiapoptotic protein Mcl-1 (ISIS Pharmaceuticals, Carlsbad, CA, USA) has *in vitro* antitumor effect in gastric cancer cells [68] and in multiple myeloma [69]. *In vivo*, simultaneous administration of *ISIS 20408* and cyclophosphamide reduced tumor weight in mice xenografted with SW872 liposarcoma cells [70]. In a melanoma growing in a SCID mouse xenotransplantation model, *ISIS 20408* potentiated the antitumor effect of dacarbazine [71].

5. Conclusion

Direct action on Bcl-2 proteins has emerged as a plausible strategy for fighting cancer. Several compounds that target

these proteins are currently under scrutiny and some have reached clinical trials (Fig. 3). Ongoing work on these drugs will hopefully lead some of them to the final stage, incorporation to treatment regimes. On the other hand, future advances in the knowledge of the molecular mechanisms that underlie the role of Bcl-2 proteins in apoptosis, will undoubtedly provide new candidate targets for the development of innovative antitumor therapies.

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