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Targeting intrinsic apoptosis and other forms of cell death by BH3-mimetics in glioblastoma

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Abstract

Introduction: Novel approaches to treat malignant brain tumors are necessary since these neoplasms still display an unfavorable prognosis.

Areas covered: In this review, the authors summarize and analyze recent preclinical data that suggest that targeting intrinsic apoptosis may be a suitable strategy for the treatment of malignant gliomas. They focus on the anti-apoptotic Bcl-2 family members of proteins and the recent drug developments in that field with a special focus on BH3-mimetics. With the discovery of BH3-mimetics that interfere with anti-apoptotic Bcl-2 family members in the low nanomolar range significant excitement has been generated towards these class of inhibitors, such as ABT-737, ABT-263 and the most recent successor, ABT-199 which is most advanced with respect to clinical application. The authors discuss the more recent selective inhibitors of Bcl-xL and Mcl-1. Concerning Mcl-1, these novel classes of inhibitors have the potential to impact malignant gliomas since these tumors reveal increased levels of Mcl-1.

Expert opinion: The recent development of certain small molecules raises significant hope that intrinsic apoptosis might soon be efficiently targetable for malignancies of the central nervous system. That being said, additional studies are necessary to determine which of the BH3-mimetics might be most suitable.

1. Introduction

While there are many recent basic research-related breakthroughs reported for malignant glial tumors, patients with these neoplasms still have a poor prognosis under current treatment regimens [1, 2, 3]. With the advent and discovery of a wealth of different small

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molecules that target a key enzyme in the nanomolar range it appears obvious that in the presence of such an arsenal of compounds more efficient and less toxic drug combination therapies should be investigated, designed and proposed. In keeping with this strategy, we summarize the current and potential role of BH3-mimetics, which are small molecules that inhibit anti-apoptotic Bcl-2 family members, in the setting of malignant gliomas. BH3-mimetics elicit programmed cell death with all classical hallmarks of apoptosis in a BAX/BAK-dependent manner. Although these molecules were primarily designed for the induction of apoptosis, they also impact alternative cell death pathways, such as autophagy and necroptosis. The newly designed BH-3 mimetics interfere with their targets in the low nanomolar range, suggesting high on-target efficacy and fewer side effects. While certain cell cultures and in vivo model systems are primarily sensitive to BH3-mimetics, the vast majority is intrinsically resistant and requires “sensitizer”. For the most part, these sensitizers are other drug compounds, but also include other treatment modalities, such as radiation. Here, we review the preclinical data on BH3-mimetics either alone or in combination with other treatments in model systems of glioblastoma.

2. The role of intrinsic apoptosis

Intrinsic apoptosis is part of programmed cell death and is distinct from extrinsic apoptosis by the fact that extrinsic implies that death ligands activate a surface receptor and thereby lead to a specific cascade of caspase activation. Classical activators of extrinsic apoptosis are TNF-related apoptosis-inducing ligand (TRAIL) [4] and the Fas – ligand [5]. Upon binding of these ligands to their cognate receptors, a complex called death inducing signaling complex (DISC) forms that results in the activation of caspase-8 with subsequent activation of caspase-3 and promotion of apoptosis. In contrast, intrinsic apoptosis is activated by stress, drug compounds, such as BH3-mimetics, and radiation. Upon activation, the pro-apoptotic multi-domain proteins BAX and BAK are activated which mediate the release of cytochrome-c from the mitochondria. In turn, cytochrome-c activates the apoptosome and caspase-9 activation. Caspase-9 cleaves caspase-3 to finalize apoptosis induction. A complex interplay of a class of molecules, called the Bcl-2 family of proteins, regulate the sensitivity of cells towards intrinsic apoptotic stimuli.

3. The Bcl-2 family members.

The Bcl-2 family of proteins consists of pro- and anti-apoptotic molecules. Examples of pro-apoptotic Bcl-2 family members are BAX, BAK, BAD, BIM, PUMA and NOXA [6, 7]. BAX and BAK are the main effectors and directly affect cytochrome-c release from mitochondria. BAX is also known to be regulated by p53 and is therefore involved in p53-mediated apoptosis. The most relevant anti-apoptotic Bcl-2 family members are Bcl-2, Bcl-xL and Mcl-1. In this group of molecules, Bcl-2 represents the most prominent one. It was discovered as part of a translocation that occurs in follicular lymphoma t(14;18) [8]. Initially, it was believed that this protein is another oncogene that drives proliferation. However, subsequent studies revealed that the main function of Bcl-2 lies in its inhibition of apoptosis. The major function of the anti-apoptotic Bcl-2 family proteins is the sequestration of the pro-apoptotic multi-domain proteins BAX, BAK and BIM. While Mcl-1 is capable of binding BAK, it does not interact with BAX. Amongst the pro-apoptotic Bcl-2 family

members, there are so-called sensitizer proteins, such as BAD and NOXA [9], or activators, such as BIM and BID. The BAD protein which is also regulated by phosphorylation binds to a hydrophobic groove within Bcl-2 and Bcl-xL to facilitate the release of sequestered pro-apoptotic molecules such as BIM, BAX and BAK. BIM is capable of activating BAX directly further facilitating apoptosis. The Noxa protein is another sensitizer for apoptosis. Its interaction with Mcl-1 leads to a dissociation of BIM and BAK from Mcl-1. In glioblastoma, the anti-apoptotic Bcl-2 family members are up-regulated and therefore these molecules represent potential treatment targets in this disease. Despite their up-regulation, it is known from studies in other malignancies that the individual levels of anti-apoptotic Bcl-2 family members do not necessarily correlate with cell death dependency in a direct manner. Therefore, a technique was developed to understand the relative impact of each individual anti-apoptotic Bcl-2 family protein on apoptosis. This approach was named BH3-profiling which involves a series of pro-apoptotic peptides derived from BH3-only Bcl-2 family members such as BIM, BAD and HRK. Challenging tumor cells or mitochondria with these peptides allows to determine which anti-apoptotic Bcl-2 family protein specific cells are dependent on. The assay is based on the notion that once the mitochondrial membrane potential collapses, apoptosis will definitively proceed. In the BH3-profiling assay [10, 11, 12], the BIM peptide is used for overall priming of mitochondria without any specific selectivity to the anti-apoptotic Bcl-2 family members [13]. In contrast, the BAD peptide will bind and interfere with Bcl-2 and Bcl-xL, respectively. The HRK peptide binds to Bcl-xL, whereas the MS I peptide interferes with Mcl-1. While glioblastoma cell lines and tissues have been analyzed for the expression of pro- and anti-apoptotic Bcl-2 family members, so far no systematic BH3-profiling with the available cell lines has been performed. BH3-profiling might provide an overview of the precise dependency and therefore allow the selection of certain specific BH3-mimetics to be considered for further pre-clinical or clinical evaluation. In addition to apoptosis induction, BH3-mimetics modulate autophagy by releasing Beclin-1 from Bcl-2. However, the impact and/or importance of this phenomenon still needs to be more carefully elucidated.

4. BH3-mimetics, targeting Bcl-xL, Bcl-2 and Bcl-w

4.1 ABT-737 - the first classical BH3-mimetic

The term BH3-mimetic implies that a compound binds with high-affinity to anti-apoptotic Bcl-2 family members and in turn promotes BAX/BAK-dependent apoptosis. Off target effects such as the activation of an integrated stress response with an increase in pro-apoptotic Noxa, are not part of this mechanism. By utilizing nuclear magnetic resonance-based screening, a group of researchers has designed the bona-fide BH3-mimetic, ABT-737 [14] (Figure 2). Mechanistically, this compound was able to bind to Bcl-2, Bcl-xL and Bcl-w in the low nanomolar range [14]. In the initial publication, ABT-737 was shown to demonstrate single agent activity against acute lymphoblastic leukemia and small cell lung cancer [14]. In addition, ABT-737 was shown to act synergistically with other conventional chemotherapeutic drugs. Because ABT-737 does not bind to Mcl-1 [14], it turned out that Mcl-1 mediates resistance towards ABT-737. Therefore, modulation of Mcl-1 is pivotal to enhance the effects of BH3-mimetics. Mcl-1 is unique in that it possesses a short-half life and its levels are heavily regulated by the proteasome [15]. With respect to glioblastomas, it

was demonstrated in 2008 that ABT-737 might have therapeutic potential in malignant gliomas [16]. The initial hypothesis for utilizing ABT-737 for glioblastoma therapy was the fact that gliomas express high levels of anti-apoptotic Bcl-2 family members. Consequently, it was found that ABT-737 induced apoptosis in a range of different glioblastoma cell lines [16]. In line with other cell types and model systems Mcl-1 mediated resistance towards ABT-737-induced apoptosis in model systems of glioblastoma [16]. Given that glioma stem-like cells are an important driver for recurrence and treatment resistance, this study also showed that ABT-737 targets brain tumor initiating cells, especially when Mcl-1 levels are suppressed. Moreover, ABT-737 caused synergistic cell death when combined with vincristine, etoposide and TRAIL. Finally, it was shown that intracranial delivery of ABT-737 resulted in significant extension of overall survival in the U251 glioma model [16]. All in all, the study by Tagscherer et al. provided a strong foundation that BH3-mimetics might be feasible for glioma therapy. In addition, it was shown that ionizing radiation sensitizes glioma cells for ABT-737-mediated cell death [17]. This finding is of high relevance due to the fact that radiation is one of the mainstays for glioblastoma therapy. Unfortunately, this study did not test as to whether or not radiation and BH-3 mimetics would enhance overall survival more than each treatment modality on its own. Aside from radiation, the alkylating agent Temozolomide is another key stone for glioblastoma therapy. The combination treatment of ABT-737 and Temozolomide was successfully tested in model systems of glioblastoma [18] as well as in other tumor entities, such as melanoma. In further studies, combination therapies including ABT-737 were extended toward other targeted therapeutics. For instance, the kinase inhibitor Sorafenib was shown to enhance the pro-apoptotic effect of ABT-737 in glioblastoma [19]. Sorafenib caused a suppression of Mcl-1 protein levels through interference with STAT-3 signaling. Earlier data already suggested that Sorafenib suppressed Mcl-1 levels via the Activating Transcription Factor 5 (ATF5) [20]. It remains to be determined as to whether BH3-mimetics in combination with Sorafenib would have an impact on animal survival in orthotopic patient-derived xenograft models of glioblastoma as well. While Sorafenib is expected to cross the blood-brain barrier, the ability of ABT-737 might be impaired due to its relatively high molecular weight. Nevertheless, other BH3-mimetics might be more suitable for these approaches, such as AT-101 and Obatoclax, which based on their chemical structure, might be more efficient in crossing the blood-brain-barrier. Tagscherer et al. addressed this problem by administering ABT-737 directly into the tumor. For high-grade gliomas, this approach is considered to be feasible, albeit not necessarily the most desired one. For instance, convection enhanced delivery permits direct application of a compound to the tumor and this modality has been used in patients successfully. Other approaches to enhance the effects of ABT-737 include the usage of the proteasome inhibitor, Bortezomib. Bortezomib is an FDA-approved compound for the treatment of multiple myeloma. In preclinical model systems of glioblastoma, Bortezomib was shown to enhance the apoptotic effects of ABT-737 in PTEN wild-type cells, which involved activation of BAX and the participation of the pro-apoptotic BID protein [21]. Given that BH3-mimetics have entered clinical trials and Bortezomib is FDA-approved, this drug combination therapy is potentially applicable to patients. The survivin [22] inhibitor YM155 was also reported to be capable of enhancing the effects of ABT-737 in model systems of malignant glioma [23]. Given that YM155 is an inhibitor of survivin, one would describe this combination treatment as a strategy that dually attacks

intrinsic apoptosis by both inhibiting Bcl-2 family members and inhibitor of apoptosis proteins. However, YM155 elicited an unexpected finding since it potently suppressed Mcl-1 protein levels [23]. It was predominantly the down-regulation of Mcl-1 by YM155 that led to the potent sensitization to ABT-737 in glioblastoma cells [23]. Additional in vivo studies are of interest as to whether or not ABT-737 or related BH3-mimetics would enhance the effects of YM155 in clinically relevant models of glioblastoma.

4.2 ABT-263 in preclinical studies in malignant gliomas

After the discovery of ABT-737 it was realized that ABT-737 might not be favorable for clinical application because of certain pharmacological properties including the fact that it is not orally available. Subsequent chemical modification of ABT-737, lead to the design of ABT-263 which is orally available and still retains its ability to bind to Bcl-2 and Bcl-xL in the low nanomolar range. In model systems of glioma, ABT-263 was tested mostly in combination therapies due to the fact that single treatment with ABT-263 is not efficient in reducing proliferation of tumor cells or induction of apoptosis [24, 25]. To this end, our group has utilized several strategies to overcome primary resistance to ABT-263. In line with ABT-737, one of the most relevant factors that mediates resistance is Mcl-1. Undoubtedly, our studies have confirmed that in model systems of glioblastoma siRNA-mediated suppression of Mcl-1 broadly sensitizes glioma cells for the cytotoxic actions of ABT-263. Because of this fact, we focused our studies to identify ways of counteracting Mcl-1. One approach was to target Mcl-1 via the inhibition of PI3K signaling by GDC-0941 [26]. GDC-0941 reduced Mcl-1 levels by affecting its stability [26]. Consequently, GDC-0941 sensitized established glioblastoma and stem cell-like glioma cells to Bcl-2/Bcl-xL inhibition. Moreover, GDC-0941 affected the phosphorylation status of BAD, which regulated the sensitivity to ABT-263 as well. Another strategy we utilized was to target Mcl-1 via a broad BH3-mimetic, GX15-070 or Obatoclax [27]. Obatoclax strongly synergized with ABT-263 to cause apoptotic cell death in various model systems of glioblastoma, including stem-cell like glioma cells [27]. In addition, we made the unexpected observation that Obatoclax also reduces the protein levels of Mcl-1 and its interacting deubiquitinase Usp9X [28]. Knockdown of Usp9X itself was sufficient to sensitize glioblastoma cells to the cytotoxic effects of ABT-263, suggesting that Usp9X is implicated in the Obatoclax-mediated enhancement of the pro-apoptotic effect of ABT-263 [27]. The impact of Usp9X on BH3-mimetics-mediated apoptosis was described earlier in the setting of hematological and other solid tumors.

Earlier findings in glioblastoma models indicated that it might be worthwhile to activate extrinsic and intrinsic apoptosis simultaneously. A recent discovery in the field of apoptosis introduced a way of efficient induction of extrinsic apoptosis by a chemical compound without the need to utilize recombinant proteins, such as death ligands. TIC10/ONC201 was identified as a compound that induces the death ligand TRAIL on the level of transcription [29]. Mechanistically, TIC10/ONC201 demonstrated inhibition of two primary signaling pathways, Akt and Erk [29]. This leads to an FOXO3A-dependent increase of TRAIL expression. It was also shown that TIC10 increases the expression levels of TRAIL-receptor 2, which is also known as death receptor 5 (DR5). Independent of TRAIL-related signaling, TIC10 also activates an integrated stress response with up-regulation of activating

transcription factor 4 (ATF4) [30, 31]. In turn, ATF4 might activate cell death through enhancing the transcription of Noxa or CHOP, which are both known to positively modulate intrinsic apoptosis. As a single reagent, TIC10 affected the viability of a broad range of tumor cells, including recalcitrant glioblastoma cell lines. In an orthotopic model of glioblastoma, animals receiving TIC10 displayed an increase in overall survival. In agreement with this pre-clinical finding is the notion that TIC10 was able to penetrate the blood brain barrier. To this end, we hypothesized as to whether activation of extrinsic apoptosis, utilizing the novel TRAIL inducer TIC10/ONC201, along with Bcl-2/Bcl-xL inhibition would reduce glioblastoma cell growth in a synergistic manner [31]. Despite the fact that we observed strong synergy between ABT-263 and TIC10 [31], we found that the mechanistic basis from this observation most likely did not originate from engagement in extrinsic apoptosis since knockdown of Caspase-8 did not provide protection from ABT-263+TIC10 mediated cell death. Instead, TIC10 down-regulated the protein levels of Mcl-1 and its deubiquitinase Usp9X in glioblastoma cells [31].

Given the strong impact of Usp9X on glioblastoma survival itself, we also assessed as to whether chemical inhibition of Usp9X represents a worthwhile treatment strategy for glioblastoma [32]. In this context, we used a deubiquitinase inhibitor, WP1130 that inhibits the effects of Usp9X. While WP1130 had significant anti-glioma activity both in vitro and in vivo, its potency could be significantly enhanced in the presence of ABT-263. Moreover, extrinsic apoptosis induced by the death ligand TRAIL was enhanced as well, suggesting that interference with Usp9X broadly sensitizes for apoptosis [32]. Indeed, in a heterotopic xenograft model of glioblastoma we found that ABT-263 in combination with WP1130 led to a regression of tumors without induction of toxicity [32].

Another potential means to enhance the effects of ABT-263 is to combine it with compounds that interfere with protein metabolism. Interference with protein metabolism regulates translation and proteins, possessing short half-lives, such as Mcl-1, are usually readily affected by inhibition of translation. There are several reagents used in patients that regulate protein synthesis. To this end, we tested the FDA-approved drug, L-asparaginase, in combination with BH3-mimetics. L-asparaginase is a recombinant enzyme that depletes the serum from the amino acid asparagine. This compound has been used in patients, suffering from acute lymphoblastic leukemia, for several decades. It has also been studied in a preclinical model systems of brain tumors [33]. What makes this drug so interesting to be used in combination therapy against glioblastoma is the fact that L-asparaginase elicits its anti-tumor effects without the need to pass the blood brain barrier, which is an inherent concern for the drug treatment of glioblastoma. By its own, L-asparaginase was particularly active in a pediatric glioblastoma cell line, SF188, which is known to express high levels of c-myc [34]. Based on this notion, we were anticipating that L-asparaginase might have high anti-proliferative activity in stem cell-like glioma cells since those cell cultures are particularly dependent on c-myc. Unfortunately, single treatment with L-asparaginase was rather ineffective against these cells [34]. However, when combined with ABT-263, L-asparaginase showed strong synergism in relevant model systems of glioblastoma, including stem cell-like glioma cells. Mechanistically, L-asparaginase regulated the levels of Noxa, Mcl-1 and Usp9X, favoring a pro-apoptotic state [34]. Consistently, inhibition of Noxa by siRNA attenuated the effects of the combination treatment. Regarding the reduction of

Mcl-1, L-asparaginase appeared to regulate Mcl-1 most likely at a post-transcriptional level. In contrast, Noxa was increased at the level of transcription. The increase of Noxa might be mediated by a number of factors, such as p53, ATF3 and ATF4. Given that L-asparaginase is likely to cause a stress response on tumor cells, it seems plausible that ATF3 and ATF4 might be responsible for the L-asparaginase-mediated increase in Noxa transcript and protein levels. Recently, others have also described a Noxa-Usp9x-Mcl-1 axis [35]. The drug Pemetrexed was shown to increase Noxa levels, while at the same time it mediated a decrease in Mcl-1. The Mcl-1-mediated decrease was shown to be likely mediated by a reduction of the deubiquitinase, Usp9X, which binds and stabilizes Mcl-1.

As mentioned above, others have suggested that interference with ATF5 might enhance the effects of BH3-mimetics. In this context, we were able to show that a cell penetrating dominant negative ATF5 peptide (CP-d/n-ATF5-S1) was capable of enhancing the effects of ABT-263 by down-regulating the expression of Usp9x and Mcl-1 [36]. These effects were also confirmed in two different xenograft model systems, involving U251 glioblastoma and HCT116 colon cancer cells [36]. The regulation of Mcl-1 by ATF5 has been described before and it was determined that ATF5 elicits its anti-apoptotic effects in part through promoting the expression of Mcl-1 [20]. However, it should be noted that ATF5 regulates apoptosis through multiple means. For instance, it was also established that ATF5 regulates the expression of other anti-apoptotic Bcl-2 family members and of heat-shock-protein 70 (Hsp70) [37]. Aside from its effects on intrinsic apoptosis, ATF5 appears to regulate extrinsic apoptosis as well since CP-d/n-ATF5-S1 overcame TRAIL resistance in several different cancer cell cultures including glioblastoma.

Bromodomain and extra-terminal family proteins (BET)-inhibitors are novel means to target c-myc in malignant tumors [38, 39, 40]. Members of this group are JQ1 and a derivative OTX015 [41, 42, 43, 44]. In glioblastoma c-myc is also up-regulated and therefore represents a potential target for BET – inhibitors. However, our studies revealed that BET – inhibitors are rather ineffective on their own with the exception of stem cell-like glioma cells, which displayed a marked susceptibility to these molecules [45, 46]. The efficacy of BET– inhibitors on stem cell-like glioma cells is explained in part by their high levels and dependence on c-myc. We discovered that BET-inhibitors in combination with BH3-mimetics caused an anti-proliferative effect on established, patient-derived and stem cell-like glioma cells in a highly synergistic manner. Again, the combination treatment was particularly effective on stem cell-like glioma cells. For our model systems tested, we found that specific interference with Bcl-xL sensitizes for apoptosis induced by BET-inhibitors. These findings are in agreement with other reports showing that solid tumors, such as glioblastoma, mostly depend on Bcl-xL and less on Bcl-2. Similarly, knockdown of c-myc alone was sufficient to sensitize glioblastoma cells to the cytotoxic effects of ABT-263, suggesting that c-myc levels determine susceptibility of glioma cells to BH3-mimetics. We also found that the combination treatment of ABT-263 and OTX015 elicited an increase in Noxa, which was mediated by activating transcription factor 4 (ATF4). These findings suggest that the combination treatment engages an integrated stress response. OTX015 and ABT263 were also active in vivo and led to a partial regression of tumors.

Another recent finding is the observation that interference with mitochondrial matrix chaperone proteins (through the mitochondrial targeted Hsp90 inhibitor, Gamitrinib) enhances the killing effects of BH3-mimetics, such as ABT263, in a broad range of challenging to treat malignancies, including glioblastoma model system in vitro and in vivo [47].

Up to now, no clinical trial, involving the BH3-mimetic, ABT-263, in combination with a BET-inhibitor has been initiated in glioblastoma or malignant glial tumors. A summary of some of the pre-clinical combination therapies tested in model systems of glioma, involving ABT263 and ABT737, is provided in Table 1.

5. BH3-mimetics, targeting Bcl-2, Bcl-xL and Mcl-1

5.1 Gossypol and its derivatives

Aside from having male contraceptive properties, gossypol is an inhibitor of the anti-apoptotic Bcl-2 family members that covers a wider range than ABT-737 or ABT-263 (pan-BH3-mimetic). It was successfully tested on glioblastoma cells and demonstrated anti-proliferative effects in vitro and in vivo [48]. It was also tested in patients with recurrent gliomas and showed some minor, but still detectable, effects [49]. Later on, it was reported that (–)-gossypol induces an autophagy-like cell death in a variety of established glioblastoma cells [50]. This form of death was inhibited by knockdown of ATG5 and Beclin-1. Moreover, (–)-gossypol enhanced the effects of Temozolomide. Similarly, when radiation was combined with gossypol, cell death was enhanced as well. These results suggest that gossypol derivatives would possibly enhance the effects of radiation and Temozolomide.

Bag3 is a co-chaperone of Hsp70 and was shown to be implicated in regulating Mcl-1 levels. In turn, interference with Bag3 sensitizes cancer cells to the apoptotic effects of BH3-mimetics [27]. Recently, it was shown that knockdown or chemical interference with Bag3 sensitizes glioma cells to AT-101 mediated cell death in vivo and in vitro [51].

Notably, R-(–)-gossypol acetic acid (AT-101) was evaluated in a clinical trial (involving glioblastoma (NCT00540722) [52]. The primary endpoint for this study was survival within a time frame of up to 4.5 years. During the preparation of this article, no results for this trial have been reported according to the website (last accessed on 4/4/2017).

5.2 Obatoclax

Obatoclax or GX15–070 is a pan BH3-mimetic that binds to all three relevant anti-apoptotic Bcl-2 family members, including Bcl-2, Bcl-xL and Mcl-1 [53, 54, 55]. Because it has a less complex structure and significant smaller molecular weight than ABT-263 or ABT-737, it will likely much better penetrate the blood brain barrier. The smaller molecular weight comes along with less specificity with respect to interacting with the various anti-apoptotic Bcl-2 family members. In addition, Obatoclax bears significant off-target effects, such as the induction of a stress response [56] and formation of autophagic vacuoles [57, 58]. Along with the stress response, there is an induction of the pro-apoptotic Noxa upon treatment with GX15–070 even in model system of malignant glioma. Similarly to gossypol, Obatoclax-

mediated cell death appears to be related to the occurrence of autophagosomes. Knockdown of autophagy-related genes protects cells from death by Obatoclax. Consistently, treatment with the pan-caspase inhibitor zVAD-fmk did not protect cells from death induced by Obatoclax. Moreover, Obatoclax elicited necroptosis along with the induction of autophagy and consequently silencing of the RIP kinases protected cells from death by Obatoclax [59]. Since these mechanistic experiments were not performed in glioblastoma, it remains to be seen as to whether or not cell death mechanisms in glioblastoma will be similar. As described above, ABT263 and GX15-070 caused enhanced lethality in glioblastoma model systems in vitro and in vivo. The efficacy profile of BH3-mimetics in clinical trials will be influenced in part by the toxicity profile of these reagents. For instance, one particular toxicity related to BH3-mimetics that affect is Bcl-xL is bleeding since platelets are known to depend on Bcl-xL for their survival. Therefore, the elucidation of clear therapeutic windows is pivotal to accomplish. Certain tumors might be more dependent on a single anti-apoptotic Bcl-2 family protein and in these instances strategic selective targeting of these molecules might be accomplished by the novel selective BH3-mimetics, see below.

6. Selective BH3-mimetics

6.1 The selective Bcl-2 antagonist, ABT199

In the recent past, significant effort was taken to design selective BH3-mimetics. The foremost compound in that category is ABT-199 [60], which is a specific Bcl-2 inhibitor that does not or only weakly binds to Bcl-xL and Bcl-w. The identification of this compound should be seen as a significant breakthrough in the development of BH3-mimetics for a couple of reasons. First, the selective inhibition with high affinity is significant. Second, certain tumors, such as follicular lymphomas, highly depend on Bcl-2. Third, targeting Bcl-2 is expected to have fewer side effects concerning inhibition of blood coagulation. Fourth, ABT-199 received accelerated FDA approval for the treatment of chronic lymphocytic leukemia with a 17p loss [61]. In the context of glioblastoma, we have shown that ABT-199 and GDC-0941 caused enhanced apoptosis when compared to each reagent on its own [26]. Based on the limited amount of data with regard to ABT-199 in glioblastomas or gliomas, it remains to be determined as to whether this compound is advantageous.

6.2 The selective Bcl-xL inhibitor, WEHI-539

Recently, a selective inhibitor of Bcl-xL was developed [62]. The main purpose for the development of a selective Bcl-xL inhibitor was the reduction of toxicity. While non-solid tumors are often more dependent on Bcl-2, solid tumors, e.g. colon cancer and glioblastoma, are more often sensitive to Bcl-xL inhibition. Therefore, a selective BH3-mimetic for Bcl-xL promises to be beneficial. However, it is necessary to consider that Bcl-xL is important for platelet survival and therefore compounds, such as WEHI-539, would need to be controlled safely with respect to these side effects.

6.3 A1210477, a selective Mcl-1 inhibitor

Targeting Mcl-1 represents an appealing treatment strategy for a number of reasons. First, Mcl-1 is up regulated in glioblastomas. Second, to date several inhibitors with different specificities have been described. Third, the side effect profile with respect to blood

coagulation is more favorable as compared to targeting Bcl-xL. Fourth, Mcl-1 inhibition might prove favorable in particular in drug combination therapies. For instance, Mcl-1 levels are known to mediate resistance against Temozolomide. Recently, several inhibitors of Mcl-1 were designed, including the indole-2-carboxylic acid containing drug A-1210477 [63]. These compounds elicit a paradox reaction in cancer cells which is characterized by an increase in Mcl-1 protein levels by enhancing its stability. The precise mechanism by which Mcl-1-targeted BH3-mimetics elicit this effect remains to be determined, but it appears very much possible that it involves the ubiquitin binding status of Mcl-1. The increase of Mcl-1 may have an impact on therapeutic efficacy of these compounds. Therefore, the elucidation of the mechanisms involved might set the stage for a new combination treatment strategy that will be more powerful than single treatments. With respect to malignant gliomas, these inhibitors have not been tested in these model systems yet. Therefore, it remains to be seen how much activity they might elicit. Based on earlier evidence it is most likely that these inhibitors are most potent in the context of drug combinations. The very obvious drug combination that has been tested in other tumor entities is the combination of Bcl-2/Bcl-xL inhibition along with selective Mcl-1 inhibition. As anticipated, this strategy is efficient in pre-clinical model systems.

7. Future Outlook:

A significant amount of pre-clinical evidence suggests that malignant gliomas might benefit from treatment with BH3-mimetics. However, additional studies are necessary to determine which BH3-mimetics might be most suitable for malignant gliomas. First, it needs to be determined how efficient different classes of BH3-mimetics can penetrate the blood brain barrier. Second, it is necessary to show which Bcl-2 family member or members are the most relevant in malignant glial tumors. This might be accomplished by BH3-profiling. After having established the most efficient BH3-mimetic, the next step to take would be to determine which combination therapy, involving BH3-mimetics, is the most useful. Given the recent success of immunotherapies, it might also be useful to consider BH3-mimetics in combination with immune checkpoint inhibitors. In this regard, pan-BH3-mimetics might be most relevant because these molecules activate autophagy and modulation of autophagy has been linked to the modulation of immune checkpoint related molecules, such as PD-L1 (CD274).

8. Conclusion:

While single agent treatment with BH3-mimetics is not particularly effective in glioblastoma cell line systems, combination therapies hold, involving these compounds, hold significant promise and are efficacious even against the most resistant cell types, such as glioblastoma stem-like cells.

9. Expert Opinion:

BH3-mimetics are an impressive demonstration of how a discovery of a gene/protein went from bench to bedside over the time course of roughly three decades. This time frame exemplifies the sustainability and durability necessary in research to foster new clinical

developments. With the discovery of ABT-199 and its accelerated FDA approval for a certain type of hematological malignancy, this remarkable journey appears to be almost completed. However, additional efforts are required to fully utilize BH3-mimetics in broader oncological clinical settings. First and foremost, patient populations need to be identified who in particular benefit from BH3-mimetics. In this context, there are multiple attempts to predict sensitivity to BH3-mimetics. Most relevant in this context are the notion that high levels of Mcl-1 appear to predict sensitivity to BH3-mimetics. While in contrast, high levels of Noxa and/or BIM have been associated with an increase in susceptibility to BH3-mimetics. However, these observations are based on cell cultures and currently there is no diagnostic test that would allow prediction of sensitivity by these markers. With the rise and availability of massive deep sequencing, it would be a welcome contribution if the presence or absence of mutations were able to predict sensitivity to BH3-mimetics. This has been observed in the setting of acute myeloid leukemia which akin to gliomas often display mutation of the IDH1 or IDH2 gene (IDH1 R132H). The mutation in IDH1 and IDH2 leads to a higher dependency of myeloid leukemia cells on Bcl-2 and consequently they become more susceptible to the clinically most advanced BH3-mimetic, ABT199. Mechanistically, this effect was not associated with a modulation of the expression of either pro- or anti-apoptotic Bcl-2 family members and was assigned to a reduced efficiency of oxidative phosphorylation. However, it would be intuitive to anticipate an integrated stress response due to energy depletion mediated interference with the respiratory chain, which in turn would impact the expression levels of anti-apoptotic proteins, such as Noxa. Whether such an approach can be taken for glioblastoma or low-grade gliomas, harboring the IDH1 mutation, remains to be determined. While the findings in myeloid leukemia are intriguing, it should be noted that these glial and hematological tumors are distinct through numerous features, e.g. solid vs non-solid tumors. Another important feature is the fact that solid tumors are often more dependent on Bcl-xL than on Bcl-2. Second, a more in-depth look is necessary which role selective BH3-mimetics might have in a clinical setting. For instance, there are recently developed compounds that inhibit Bcl-xL and Mcl-1 specifically, while not affecting Bcl-2. In upcoming years, these compounds might have a higher relevance for gliomas since these neoplasms often display high levels or dependencies on Mcl-1 and Bcl-xL. Third, biomarkers need to be found that allow the design of the most efficient combination therapies, involving BH3-mimetics. With novel screening techniques, such as proteomic profiling and CRISPR/Cas9 library screen, unraveling of such markers might be more feasible. Based on the pre-clinical evidence, it appears that malignant glial tumors might benefit from the addition of BH3-mimetics to an established treatment regimen. This is supported by preclinical data that show that BH3-mimetics synergize with both Temozolomide and radiation therapy.

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Highlight Box:

- Glioblastomas are resistant to intrinsic apoptosis, which is regulated by the Bcl-2 family members.
- BH3-mimetics target intrinsic apoptosis by antagonizing anti-apoptotic Bcl-2 family members and as single agents display limited pro-apoptotic effects in glioblastoma cells.
- The anti-apoptotic Bcl-2 family member, Mcl-1, is expressed at increased levels in glioblastomas and mediates resistance towards BH3-mimetics, such as ABT263 and ABT737 that target Bcl-2 and Bcl-xL, but not Mcl-1.
- The effects of BH3-mimetics are markedly enhanced through combination treatment strategies.
- Many drug combination treatments, involving BH3-mimetics, suppress Mcl-1 protein levels, very commonly by affecting the stability of Mcl-1.

Figure 1:

Mcl-1 mediates resistance against BH3-mimetics. ABT-263 or ABT-737 inhibit Bcl-2, Bcl-xL and Bcl-w (not shown) and thereby facilitate the release of BAX (not shown), BAK and BIM. Since Mcl-1 is up-regulated in gliomas and other cancers, BAK and BIM, but not BAX, will bind to Mcl-1. Therefore means to interfere with Mcl-1 are necessary to fully leverage the effects of BH3-mimetics on glioma and/or cancer cells. Several drug compounds that inhibit Mcl-1 are provided in the figure. An intrinsic antagonist of Mcl-1 is the pro-apoptotic Bcl-2 family protein Noxa that interacts with Mcl-1 and facilitates BIM and BAK release. In turn, released BIM may activate BAX and initiate BAX/BAK-dependent apoptosis.

Figure 2:
Relevant chemical structures of discussed compounds.

Table 1:

Combination Treatments, involving BH3-mimetics in model systems of glioblastoma or other tumors

Combination treatments with ABT-737	Mechanism	Reference
ABT-737+TRAIL	In part by enhancing the mitochondrial amplification loop	16
ABT-737 + Vincristine	Not determined	16
ABT-737 + Etoposide	Not determined	16
ABT-737 + Sorafenib	Sorafenib suppresses Mcl-1 via STAT3	19
ABT-737 + Bortezomib	Activation of BID protein	21
ABT-737 + YM155	YM155 suppresses Mcl-1 levels	23
Combination treatments with ABT263 or ABT199	Mechanism	Reference
ABT263/ABT199 + GDC-0941	GDC-0941 lowers Mcl-1	26
ABT263 + Obatoclax (GX15-070)	GX15-070 lowers Usp9X and Mcl-1	27
ABT263 + TIC10	TIC10 lowers Mcl-1 levels	31
ABT263 + WP1130	WP1130 lowers Usp9X and Mcl-1 levels	32
ABT263 + CP-d/n-ATF5-S1	CP-d/n-ATF5-S1 lowers Usp9X and Mcl-1 levels	36
ABT263 + L-asparaginase	L-Asparaginase modulates Mcl-1, Noxa and Usp9X levels	34
ABT263 + BET-inhibitors	BET – inhibitors regulate Mcl-1 and Noxa.	45