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BCL-2 INHIBITORS: A POTENTIAL TARGET IN CANCER THERAPY

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Abstract
The key marker apoptotic protein, B-cell lymphoma-2 (Bcl-2), is well known for its pivotal role in cancer progression through the mitochondrial intrinsic apoptotic pathway. However, till date, the exact role and mechanism of Bcl-2 in cancer progression, and their relevance to cancer therapy remains intriguing, as it acts as a double edged sword by playing a negative regulation role on apoptosis. Overexpression of Bcl-2 protein was observed in many cancer types, wherein its expression is allied with enhanced drug resistance and tumor cell survival. This characteristic feature gains attention, as inhibition of apoptosis is a hallmark of cancer. To highlight and unveil the positive and negative role of Bcl-2 in cancer therapy, various Bcl-2 inhibitors such as venetoclax, navitoclax, ABT-199, ABT-737, BCL201, etc are in current use. These inhibitors signify an exhilarating new dimension for drugs with a unique mechanism of action either as single agents or in combination with current tumor therapies. The present review would be a key pointer in envisaging the precise role of Bcl-2 inhibitors as a potential target in tumor therapy.
Key words: Bcl-2, apoptosis, inhibitors, carcinogenesis, anti-apoptotic.

Introduction

In cancer biology, the delicate balance between the cell survival and cell death is of utmost challenging factor. Various pro-apoptotic, apoptotic and anti-apoptotic proteins were explored to unearth a promising candidate for tumor suppression. In line with this goal, the Bcl-2 family of proteins forms an attractive target, as it was the first and foremost mammalian gene product coupled with apoptosis, and discovery of its function spearheaded an extensive research on apoptosis. However, in the present scenario, its role in the balance between cell survival and death brought an array of perplexity and its function remains questionable. The controversies began when the Bcl-2 protein was discovered to protect the cells from apoptosis upon overexpression, rather than inducing proliferation (Vaux et al., 1988; McDonnell et al., 1989). In general, Bcl-2 protein through regulating the mitochondrial outer membrane permeabilization (MOMP) process, leads to the irrevocable release of intermembrane space proteins, which subsequently activates the cascade of caspase proteins, thus resulting in apoptosis (Kale et al., 2018). Hence, any significant alterations in either the level of Bcl-2 family proteins or its binding affinities towards other pro-apoptotic and/or anti-apoptotic proteins convey a phenomenal shift in the tumor suppression or progression. Therefore, in the

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recent times, a large family of Bcl-2 related proteins that regulate the mitrochondrial intrinsic pathway through non enzymatic protein:protein interactions, by either promoting or inhibiting apoptosis was explored (Cory and Adams, 2002).

Like Bcl-2, its pro-survival sub-families such as Mcl1, Bclxl (Bcl2l1), Bcl2A1 and BclB, was observed to prevent apoptosis. Following this, the other two chief cell death effector proteins such as Bax and Bak aggregate on the mitochondrial outer membrane, which upon activation, leads to the release of cytochrome C, that in turn stimulates the caspases to persuade the cell destruction. Therefore, the overall balance of activity between pro-survival Bcl-2 family proteins and BH3 pro-apoptotic proteins decides the fate of a cell whether to survive or undergo apoptosis. Collectively, this process acts as a bridge between the intracellular and extracellular signals, by promoting either cell survival (induced by growth factors, nutrients, etc.) or cell death (induced by stress, DNA damage, etc.) [Letai, 2008].

On the other hand, Bcl-2 proteins possess crucial roles in normal cell physiology such as neuronal activity, autophagy, calcium handling, mitochondrial dynamics and energetic, etc, unlike apoptosis (Hardwick and Soane, 2013). Hence, there arises a major confront in deciphering the relative significance of these normal physiological functions of Bcl-2 protein in healthy cells to apoptosis in cancer cells. An extensive research outputs and findings pertaining to the biochemical activities related to the distinctive three dimensional shape extended by Bcl-2 family members are required to arrive at a firm conclusion on the mystery of Bcl-2. A schematic representation depicting the role of Bcl-2 in normal and cancerous cells and the importance of Bcl-2 inhibitors in solving the mystery of Bcl-2 in acting as a double edged sword, i.e. apoptotic or anti-apoptotic (Figure 1).

As an interesting and alternative way for unraveling the precise role of Bcl-2, multiple highly selective inhibitors of the Bcl-2 family have been on the queue, with venetoclax being approved as the first drug in this class for the treatment of chronic lymphocytic leukemia (CLL) and acute myeloma leukemia (AML) in combination with chemotherapy or hypomethylating agents. Other commonly employed Bcl-2 inhibitors include Bcl-xl, Mcl-1, which were designed to share a common mechanism of action (Soderquist and Eastman, 2016). While other compounds such as subatoclax, obatoclax, maritoclax, gossypol, apogossypol, UMI-77, TW-37 and BDA- 366 were developed to exert a specific mechanism of action and were put in current clinical trials.

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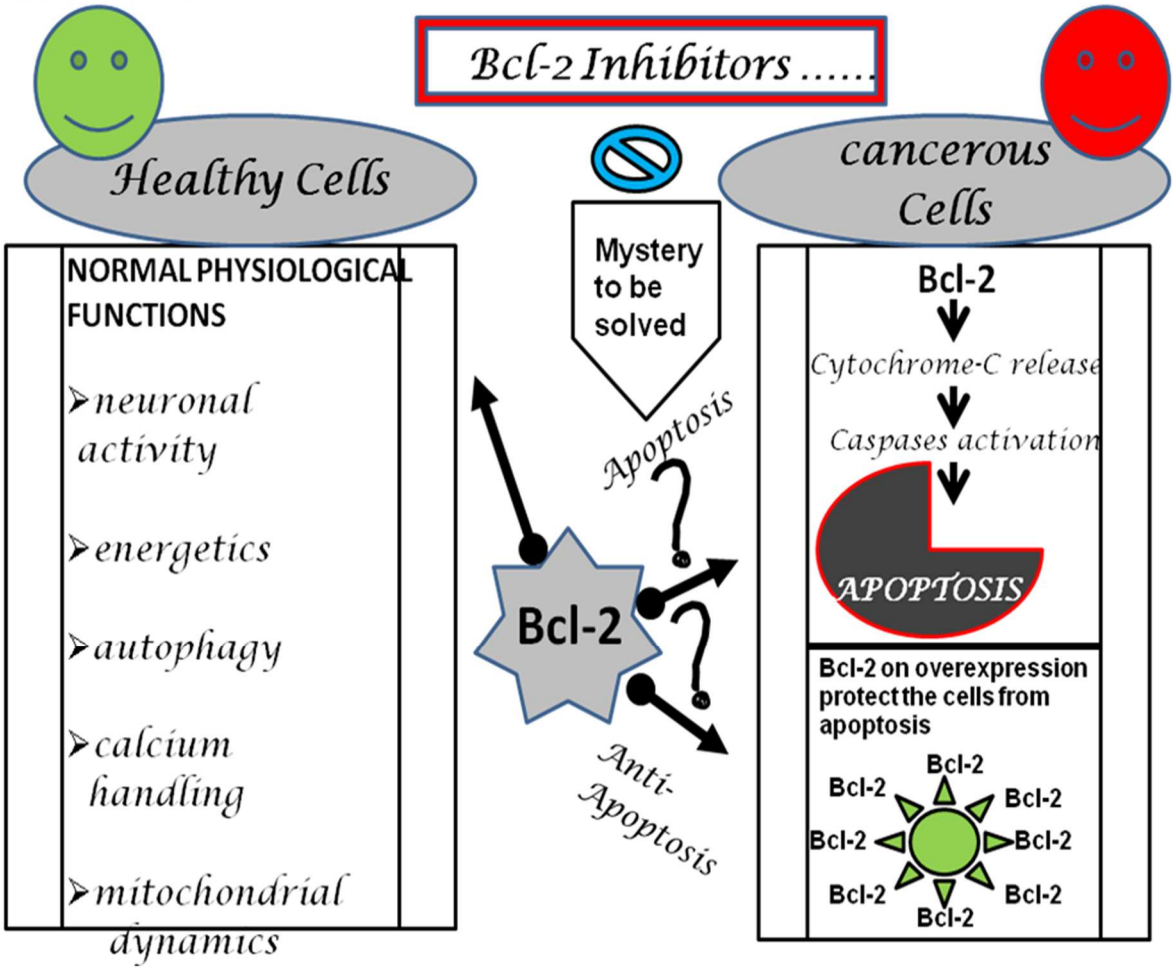

Figure 1. Schematic representation displaying the role of Bcl-2 in normal and cancerous cells

A substantial body of evidence documents that these inhibitors may influence the apoptotic process by binding to a non-specific target as well as Bcl-2 independent effects (Vogler et al., 2009). Unlike the role of BH3, these inhibitors induce cytochrome C release by binding indirectly to either mitochondrial or endoplasmic reticulum proteins, leading to the death of BAK/BAX deficient cells, thus being different from the BH3 mimetics (Villalobos-Ortiz et al., 2020). Various lists of inhibitors are charted out in Table 1. The present review widens the knowledge on the various Bcl-2 inhibitors with common and specific mechanisms of action, which would throw a spotlight on the role of Bcl-2 towards the cell survival/death balance.

BCL-2 INHIBITORS AND ITS TARGET PROTEIN		
S. No	**Inhibitor**	**Target protein**
1	Venetoclax	B-Cell Lymphoma (Bcl-2) selective inhibitor
2	Navitoclax	Bcl-2/Bcl-xL inhibitor
3	Sabutoclax	Pan Bcl-2 inhibitor

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4	Obatoclax	BH3 mimetic Bcl-2 inhibitor
5	Maritoclax	Myeloid Cell Leukemia-1 (Mcl-1) inhibitor
6	Gossypol	Pan Bcl-2 inhibitor
7	Apogossypol	Small molecule Bcl-2 inhibitor
8	UMI-77	Small molecule Mcl-1inhibitor
9	TW-37	Small molecule Bcl-2 /Mcl-1inhibitor
10	BDA- 366	A putative Bcl-2 BH4 domain inhibitor
11	A-1155463	B-Cell Lymphoma (BCL-xL)
12	A-1331852 (Re-engineered from A-1155463)	B-Cell Lymphoma (BCL-xL)
13	AZD4320	A dual inhibitor of Bcl-2 and Bcl-xL
14	AZD0466	BH3-mimetic acting as dual Bcl-2/Bcl-xL inhibitor
15	PROTAC DT2216	A selective Bcl-xL PROTAC degrader
16	ABT737	BH3-mimetic small molecule Bcl-2 inhibitor
17	Bcl201	Bcl-2 inhibitor
18	S63845	Mcl-1 inhibitor
19	Bortezomib	Mcl-1 inhibitor

Table 1. List of Bcl-2 inhibitors in current use

Bcl-2 INHIBITORS AND ITS TARGET PROTEIN

The underneath section focuses in detail about the mechanism of action of the various inhibitors, listed out in the Table. This extensive exploration about the roles of various inhibitors will deepen the understanding on the task and function of Bcl-2 protein in deciding the fate of the cell. Collectively, this review would be a cumulative data about the various inhibitors and its role at large.

1. Venetoclax:

A novel class of anticancer drug, BH3 mimetics (mimic BH3 protein), that prevent the ability of Bcl-2 to bind to either BAX or BAK would be a significant target in cancer therapy. As per the recommendation of the National Comprehensive Cancer Network and US Food and Drug Administration (FDA), venetoclax was approved as an efficient Bcl-2 inhibitor, in promoting the cancerous cell death in the patients with R/R CLL, who had been treated with ibrutinib. Venetoclax was also hailed as the second-line drug/medicine in MCL patients. ER and Bcl-2 positive metastatic breast cancer patients also revealed a positive response rate upon administration of venetoclax (Levy and Claxton, 2017). With this unique idea, inhibitor such as venetoclax was developed, which acts as a Bcl-2 specific BH3 mimetic, which induces the cell death significantly when added onto the Bcl-2 overexpressing cancer cells.

Overexpression of Bcl-2 was observed in chronic lymphocytic leukemia (CLL) cells, myeloma cells and non-Hodgkin lymphoma (NHL) cells (Qingfang et al., 2019). As per the recommendation of the National Comprehensive Cancer Network and US Food and Drug Administration (FDA), venetoclax was approved as an efficient Bcl-2 inhibitor, in promoting

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Mechanism of action of venetoclax:

Primarily**,** on the outer mitochondrial membrane, Bcl-2 (anti-apoptotic) and BIM (pro-apoptotic) exist in equilibrium. At this stage, venetoclax, inhibits the association of Bcl-2 and BIM, thereby leading to the dislocation of BIM from Bcl-2, and the involvement of the BAX/BAK in the active site of the outer mitochondrial membrane, rather than the Bcl-2 / BIM interaction. These new interaction with BAX/BAK homo-oligomerization induces the mitochondrial outer membrane permeabilization, leading to the release of cytochrome C and caspase activation, thus resulting in apoptosis (Lampson and Davids, 2017).

It has been further documented that venetoclax, under both *in vivo* and *in vitro* conditions, exerts a less impact on platelets. Under *in vitro* conditions, the growth of primary CLL cells was inhibited by venetoclax with less significant preventive effect on the NHL cell lines (Souers et al., 2013). When venetoclax is given to small lymphocytic lymphoma (SLL) or CLL bearing patients along with other anti-cancer drugs such as rituximab, they revealed around 86 % of positive response with tolerable side effects (Seymour et al., 2017). Venetoclax served as a fist and successful Bcl-2 inhibitor exerting high efficiency rats in patients bearing CLL and/or SLL, who were already in line with the first- and second-level chemotherapy treatment or 17p deletion (Levy and Claxton, 2017). As per the recommendation of the National Comprehensive Cancer Network and US Food and Drug Administration (FDA), venetoclax was approved as an efficient Bcl-2 inhibitor, in promoting the cancerous cell death in the patients with R/R CLL, who had been treated with ibrutinib. Venetoclax was also hailed as the second-line drug/medicine in MCL patients. ER and Bcl-2 positive metastatic breast cancer patients also revealed a positive response rate upon administration of venetoclax (Levy and Claxton, 2017). Nevertheless, the patients receiving venetoclax alone also exhibited an acceptable range of side effects (Stilgenbauer et al., 2016). In the first study, “M12-175,” conducted with CLL and NHL patients, the dosage of venetoclax were gradually increased to check the safety and vulnerability of the inhibitor. However, the risk of tumor lysis syndrome (TLS) was observed upon an increase in the dosage of venetoclax (Roberts et al., 2016), when administered to patients with multiple myeloma (MM) and acute myelocytic leukemia (AML). Overall, venteclax alone was found as an efficient Bcl-2 inhibitor / drug for the treatment of advanced malignancies, esp., CLL, with acceptable adverse effects. The efficiency was further escalated upon combination with the existing anti-cancer drugs. Hence, unraveling the action of venetoclax, provided a new and phenomenal insight on the influence and role of Bcl-2 in tumorogenesis.

2. Navitoclax

Navitoclax (formerly known as ABT-263), a Bcl-2 and Bcl-xL protein inhibitor was developed during the clinical generation of ABT-737, and it shares a similar structural profile as that of ABT-737 (Tse et al., 2008). Since, pre-clinical studies with ABT-737 extended a less-

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effective outcome in cancer patients due to its poor bioavailability and physicochemical properties, navitoclax (ABT-263) was designed to enhance the drug pharmacokinetics, pharmacodynamics and efficacy by altering the three important sites in ABT-737, which was the crucial factor for its high molecular weight (>800 g/mol) and poor bioavailability. These sites also stood as a reason for improper charge balance, affinity and metabolism (Park et al., 2006). Thus, modification of the active sites in ABT-737 led to a more effective ABT-263, which proved to be a better inhibitor of Bcl-2 in carcinogenesis.

Mechanism of action:

Numerous studies have documented the inhibiting potential of navitoclax on Bcl-2 family protein to cause apoptosis in SCLC together with the expression of Bcl-2, Bcl-xL, Bcl-w and Mcl-1 (Lock et al., 2008; Shoemaker et al., 2008; Tse et al., 2008). Overexpression of Mcl-1 throws a significant negative response to navitoclax, and therefore the less interaction of navitoclax with Mcl-1 protein and its subsequent protein cascade prevents the apoptotic process in tumor patients. Consequently, the effect of navitoclax was maximized by reducing the expression of Mcl-1 protein to exert its inhibiting action. In fibrosis, differentiation of fibroblast to myofibroblast is associated with the interaction of pro-apoptotic protein with the BH3 domain such as BIM in mitochondria (Lagares et al., 2017).

Importantly, the myofibroblasts induces the expression of Bcl-xL (anti-apoptotic protein) to prevent the cell death. On the contrary, navitoclax, being a highly potent BH3 mimetic drug, induced the apoptotic process by inhibiting the Bcl-xL binding with BIM in myofibroblasts (Lagares et al., 2017). As a result of setting BIM free from associating with Bcl-xL, BIM then interacts with BAX/BAK activator, leading to the apoptosis of the myofibroblast via, mitochondrial membrane permeabilization. In conclusion, navitoclax, helps in understanding the role of Bcl-2 in myofibroblast, to a greater extent.

3. Sabutoclax

Owing to the fact that dysregulation of the action of Bcl-2 protein results in inducing the survival signal, which greatly helps in withstanding the cytotoxic anticancer drugs, antagonists for preventing the anti-apoptotic protein, Bcl-2, has been likely proposed as a potent therapeutic strategy in carcinogenesis.

Mechanism of action:

As an urgent need of the hour, various lines of inhibitors with different degA potent pan-Bcl-2 inhibitor, “Sabutoclax” exert its antagonist potential by preventing the interaction of BH3 peptides with Bcl-XL, Bcl-2, Mcl-1, and Bfl-1, thus resulting in apoptosis. On the other hand, Sabutoclax inhibited the activation of Bcl-2, Mcl-1, Bcl-xL and BFL-1, leading to the activations of caspase-3/7 and -9, thus modulating the expressions of Bax, Bim, PUMA and surviving (Hu et al., 2018). Similarly, sabutoclax, via preventing the activation of IL-6/STAT3 signaling pathway, prevented the formation of drug-resistant cells. Numerous findings support the effect of sabutocalx on breast cancer cells, wherein the apoptotic process is reactivated through the prevention of various anti-apoptotic Bcl-2 family of proteins by preventing the pathways involving IL-6/STAT-3 proteins (Jackson et al., 2012). This mechanistic pathway

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acts as an underlying mechanistic rationale behind the protective effect of sabutoclax on breast cancer, prostate cancer, lung cancer and lymphoma cell patients.

4. Obatoclax

Obatoclax, also termed as GX15-070, is another potent small molecule inhibitor of Bcl-2 family by preventing the BH3-binding affinity with Bcl-2. The BH3 mimetics always reveal a specific affinity towards the Bcl-2 protein, thus inhibiting the anti-apoptotic role of Bcl-2. Obatoclax and its potent inhibiting role was documented in various cancer types, such as head and neck squamous cell carcinoma (Victor et al., 2013), colorectal cancer (Or et al., 2016).

Mechanism of action:

In a study by Or et a. (2016), the effect of obatoclax was demonstrated, wherein it was found to prevent cell proliferation, inhbits clonogenicity and activates G1-phase cell cycle arrest coupled with downregulation of cyclin-D1 protein. Obatoclax exert the downregulation of cyclin D1 protein levels, via. Inducing the cyclin D1 proteasomal degradation. In this line, an important factor for cyclin D1 proteasomal degradation, i.e., phosphorylation of threonine 286 residue in cyclin D1 protein, was also provoked by obatoclax. This study emphasizes the antagonist role of obatoclax in bringing apoptosis, in contrary toBcl-2. The main action of obatoclax was validated by targeting cyclin D1 protein through promoting its proteosomal degradation. Other than the action of obatoclax on cyclin D in colorectal cancer, it also bring its action by inducing its binding to the BH3-affinity groove of Bcl-2, Bcl-xL and Mcl-1, which as a consequent, inhibits Bcl-2 protein and BAX/BAK dependent apoptosis (Goard and Schimmer, 2013; Croce and Reed,2016; Konopleva et al., 2008). Most importantly, the unique action of obatoclax is that, other than Bcl-2, it also target Mcl-1 and induces cell death (Belec et al., 2007), thus confirming its efficacy in carcinogenesis, as a Bcl-2 inhibitor.

5. Maritoclax

Maritoclax, a Mcl-1 inhibitor, which is an anti-apoptotic protein of Bcl-2 family plays a therapeutic role in tumorigenesis. In various cancer types such as acute myeloid leukemia (AML) and multiple myeloma, maritoclax was shown to induce apoptosis by inhibiting the Bcl-2 protein.

Mechanism of action:

Maritoclax was observed to inhibit Bcl-2 protein through inducing the proteosomal degradation of Mcl-1 (Doi et al., 2012; Pandey et al., 2013), without affecting the transcription of Mcl-1. Maritoclax, through causing the proteosomal degradation of Mcl-1 induced the caspase-3 and PARP cleavage, which resulted in apoptosis through caspases activation. Overall, it has been proposed that maritoclax, unlike, other Bcl-2 inhibitors, tends to release the bound BH3 alone and multi-domain Bcl-2 family proteins that in turn activates the intrinsic apoptotic pathways (Pandey et al., 2013).

6. Gossypol

Gossypol, a Bcl-2 inhibitor, is a natural polyphenolic compound isolated from cottonseeds. It

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acts as a dual inhibitor of Bcl-2 and Bcl-xL proteins. Gossypol efficacy was tested against pancreatic cancer cell line, wherein it revealed the significant growth inhibitory effect and apoptosis of pancreatic cancer cells in a concentration dependent manner. A noteworthy factor is that gossypol did not possess any adverse effects on the normal peripheral blood lymphocytes (Mohammad et al., 2005).

Mechanism of action:

Gossypol acted as a Bcl-2 inhibitor through distracting the heterodimerizatio of Bim with Bcl-xL in BxPC-3 pancreatic cancer cells without affecting the total level Bcl-xL or Bim proteins (Mohammad et al., 2005). It has been further documented that gossypol inhibitor through downregulating NF-kappaB (NF-κB) activity, inhibits Bcl-2 and Bcl-xL, with simultaneously downregulating the levels of tumor suppressor proteins such as VEGF, MMP-9 and uPAR. In general, upregulation of EGFR, NF-κB, and Bcl-2 or Bcl-XL are observed in pancreatic cancer cells, and therefore the agents/molecule/compound/drug that could hold the potency to prevent the activity of NF-κB, might result in apoptotis through Bcl-2 or Bcl-xL inhibition. Furthermore, studies by several investigators have documented that gossypol either binds directly to Bcl-xL (Kitada et al., 2003) or inhibits Bcl-2 and its family proteins such as Bcl-xL, Mcl-1, and Bcl-w (Opydo-Chanek et al., 2017). Moreover, it has also been reported that gossypol inhibits the anti-apoptotic function of of Bcl-2, Bcl-xl, and Mcl-1 by binding to the BH3 binding groove of Bcl-2. In addition, Lian et al. (2011) have demonstrated that gossypol acts on upregulating Beclin-1 expression, which is also an apoptotic protein with simultaneously decreasing the level of Bcl-2. By altering the expressions of both apoptotic and anti-apoptotic proteins, gossypol was able to aggravate cell death through inducing Beclin-1 Atg5-a dependent autophagic pathway in cancer cells.

7. **Apogossypol**

Apogossypol, the semisynthetic derivative of gossypol, inhibits anti-apoptotic Bcl-2 proteins. The advantage of apogossypol over gossypol is that even high doses of apogossypol was tolerated by mice 2-4 times more than what accepted by gossypol doses (Kitada et al., 2008).

Mechanism of action:

The mode of action of apogossypol was almost similar to that of gossypol, wherein it imitates the role of endogenous BH3 peptide and bind with the active sites on Bcl-2, Bcl-xL, Mcl-1, Bcl-W and Bcl-B. Apogossypol, exerts a broad spectrum inhibitory role on Bcl-2, and induced cell death in various tumorigenesis, thus proving its superiority than its parent compound, gossypol with regard to its efficacy and less toxicity (Kitada et al., 2008).

8. UMI-77

UMI-77 is a small molecule Mcl-1 inhibitor, which tends to promote apoptosis by inhibiting the Bcl-2 protein. Mcl-1 was found to play a crucial role in many cancer types, especially, pancreatic cancer (Wei et al., 2008). A dose and time-dependent cell death was shown by UMI-77 through downregulating the Mcl-1 expression in pancreatic cancer cells.

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Mechanism of action:

UMI-77's inhibitory action on Bcl-2 was brought out by Bax/Bak dependent pathway, headed by preventing the Mcl-1/Bak and Mcl-1/Bax complexes, subsequently activating the Bax protein. The above signaling pathway then results in mitochondria-mediated apoptosis (Abulwerdi et al., 2021). The above reports document that UMI-77 functions as a mimetic of BH3 protein, thus revealing a most specific and mechanism-based apoptosis.

9. TW-37

TW-37 is a re-engineered form of gossypol, in other words, a second-generation benzene sulphonyl derived from gossypol, which was isolated from cotton seeds and roots. TW-37 was found to act as a potent anti-tumor agent, by attenuating Bcl-2 activation in various cancer models such as breast, prostate and lymphoma (Schniewind et al., 2004).

Mechanism of action:

Recent reports substantiate the mechanism of action of TW-37, wherein it has been reported that TW-37 by strongly binding to BH3 groove, inhibits the Bcl-2, Bcl-xL and Mcl-1 activation, finally leading to the onset of cell death (Ren et al., 2009; Wei et al., 2008). The above study highlights the action of TW-37 as a small molecule inhibitor of Bcl-2 and Mcl-1 in oral cancer cells. However, Takahashi et al. (2005) has further elaborated the mechanism of action in detail, and proved the conformational change upon Bax activation, which then inhibits Bcl-2. Wang et al. (2016) have suggested the role of TW-77 in cell growth, cell migration, invasion and angiogenesis in pancreatic cancer. It has been observed that Tw-77 acts as a downregulator of NF-κB, and thus inhibits the activation of downstream genes such as Cyclin D1, Survivin, COX-2, VEGF and MMP-9. As a consequence, TW-77 results in the prevention of cell proliferation in pancreatic cancer types. The above reports document the mechanistic role of TW-77, which ultimately helps in unraveling the role of Bcl-2 in tumor therapy.

10. BDA-366

BDA-366 is a small molecule Bcl-2 inhibitor, which targets the BH4 domain of Bcl-2. These groups of inhibitors are novel small molecules that are invented to act along with other chemotherapeutic agent in various cancer models both under *in vivo* and *in vitro* conditions.

Mechanism of action:

BDA-366 was found to bind with the domain of Bcl-2's BH4, which as a result open up the BH3 domain leading to the activation of Bax, which ultimately converts Bcl-2 into a pro-apoptotic protein. Furthermore, it has been demonstrated that BDA-366 exerts part of its cytotoxic effect by preventing the Bcl-2 phosphorylation at the amino acid residue, Ser70 (Vervloessem et al., 2020). A study by Vervloessem et al. (2020) has documented that BDA-366 apoptotic effect is by large dependent on Bax/Bak. Through the above mechanism of action of BDA-366, it was hailed as an effective anti-tumor therapy against several cancer types, influenced by the overexpression of Bcl-2. Importantly, BDA-366 was found to induce apoptosis via, caspase activation preceded by Bax activation. On the contrary, a direct relation between BDA-366 and Bcl-2 expression was not observed.

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11. A-1155463

A-1155463 is a selective Bcl-xL inhibitor, which was designed through bioinformatics tools, structure based design on a nuclear magnetic resonance fragment screening. A-1155463 revealed significant therapeutic ability against Bcl-xL dependent cells and acts as an attractive target to study the Bcl-xL mechanism. It further acts as a lead molecule for studying the Bcl-2 inhibitory mechanism (Tao et al., 2014).

Mechanism of action:

A-1155463 exhibited a strong affinity to Bcl-xL along with a weak affinity to Bcl-2 and other related proteins, Bcl-w and Mcl-1. This particular type of inhibitor was found to exert an effective anti-tumour activity by causing a platelets reduction, reversibly, thereby inhibiting the tumor growth, phenomenally (Tse et al., 2008).

12. A-1331852

A-1331852 was synthesized from its parent compound, A-1155463 through genetic engineering approach. It exerts a similar mechanism of action as that of its parent compound, which acts as a Bcl-xL inhibitor (Wang et al., 2020).

Mechanism of action

Using the structure based design technique, A-1331852 was discovered, wherein sp3-rich aminoacid moieties was introduced to the parent compound, which then was competent enough to generate a significant binding affinities within the P4 pocket of Bcl-xL, which acts as a great therapeutic tool to highlight the Bcl-2 protein biology and structure

13. AZD4320

AZD4320 is a selective dual inhibitor of Bcl-2 and Bcl-xL. Several reports have underlined the potent usage of AZD4320 as a thrombocytopenia inducer in cancer patients (Balachander et al., 2020). Downregulating the overexpressed Bcl-2 protein in cancer conditions is a prophylactic approach in cancer therapy. With an idea that a dual Bcl-2/Bcl-xL inhibitor would be more potential than the individual selective inhibitors, AZD4320 has been designed. For instance, a selective Bcl-2 or Bcl-xL inhibitor might have different targets, either targeting the resistance mechanism or successful clinical trials or affecting cell migration/invasion/proliferation, etc (Balachander et al., 2020). Hence, a multiple targeting inhibitors would be an attractive choice in tumorigenesis.

Mechanism of action

In general, the disruption of the protein-protein interactions of the Bcl-2 protein families represents a demanding mechanism, as the Bcl-2 and its family proteins consists of a long and hydrophobic binding groove, which tends to associate with the BH3 α-helix of pro-apoptotic proteins via, hydrophobic interactions (Sattler et al., 1997; Petros et al., 2009). AZD4320 was found to disrupt both the Bcl-2 and Bcl-xL binding, without affecting either Mcl-1 or Bfl-1 to Bim protein. This particular effect was found to be more significant than navitoclax, thus facilitating in exploring the mechanistic role of Bcl-2 protein in tumorigenesis.

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14. **AZD0466**

Although, the previously investigated Bcl-2/Bcl-xL dual inhibitors, such as navitoclax, revealed high potency towards several cancer types, certain adverse effects such as thrombocytopenia and dose-dependent cardiotoxicity was observed. Hence, a novel BH3 mimetic molecule has been developed to circumvent such associated dreadful effects. As a novelty, AZD4320 has been conjugated through a hydrolytically liable linker to dendrimer nanomedicine platform (Patterson et al., 2021). This newly designed inhibitor, "AZD0466" is under clinical trials currently against hemoatological and advanced solid tumor conditions. AZD0466 revealed a less thrombocytopenia with lesser/no carodiotoxic effects, as compared to that of other BH3-mimetics that acts as a Bcl-xL inhibitor (Arulananda et al., 2021).

Mechanism of action:

The unique dendrimer nanomedicine, AZD0466, was shown to be more potent similar to that of cisplatin, in inhibiting tumor growth and enhancing the survival rate. Therefore, the combinational therapy, i.e., AZD0466 with cisplatin or any other currently used chemotherapeutic drugs would be an add-on benefit in tumor therapy. Likewise, a combined treatment with AZD0466 and Mcl-1 inhibitor can also represent an ideal therapeutic strategy in carcinogenesis, by targeting both Bcl-xL and Mcl-1 simultaneously (Soderquist et al., 2018; Weeden et al., 2018; Lee et al., 2019). Nevertheless, the combination of Bcl-xL and Mcl-1 inhibitors revealed a significant anti-tumor effect, with acute hepatotoxic effects (Weeden et al., 2018).

15. PROTAC DT2216

Overexpression of the anti-apoptotic protein, Bcl-2 and its family proteins, such as Bcl-xL and Mcl-1 is a common phenomena observed in many cancer conditions, including T-Cell Lymphomas (TCLs). Exploring the BH3 profiling have disclosed the fact that cutaneous TCLs and peripheral TCLs are highly Bcl-xL dependent. The above mechanism indicates that these patients respond well to Bcl-2/Bcl-xL inhibitor, ABT263 (navitoclax) with no effect towards Bcl-2 inhibitor, ABT199 (venetoclax) (Koch et al., 2019). However, though the small molecule Bcl-xL inhibitors exerts significant anti-tumor potential, due to certain limitations such as target specificity and thrombocytopenia, design of another novel inhibitors remains in search (Schoenwaelder et al., 2011; Kaefer et al., 2014). In order to overcome these limitations, a selective Bcl-xL degrader, "DT2216" has been designed and developed. It holds the capability to act as a safe and potential anti-tumor agent, due to its ability to spare platelets (Khan et al., 2019).

Mechanism of action:

It has been proposed that DT2216 has the potential to direct Bcl-xL to target the Von Hippel Lindau (VHL) E3 ligase protein and promotes degradation by the proteasome. Due to the reason that platelets present a decreased expression of VHL, DT2216 tends to stimulate the degradation of Bcl-xL in cancer cells other than platelets (Khan et al., 2019), establishing the significance of DT2216 than navitoclax or other Bcl-xL inhibitors in platelet non-toxicity. Moreover, since DT2216 could not possess a strong interaction with Bcl-2, it could not exert proper Bcl-2 degradation. This might be attributed to the absence of lysine for ubiquitination

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and the lack of DT2216 to associate with VHL and Bcl-2 to form a ternary complex in cancerous cells. The above mechanism proves the advantages of DT2216 than the other available Bcl-xL inhibitors.

16. ABT-737

The significant and most effective small molecule Bcl-2 inhibitors are the Bad-like BH3 mimetics, till date (Zhou et al., 2012). One such potent BH3 mimetics, ABT-737 holds a strong interaction to Bcl-2, Bcl-xL, and Bclw with no effect on Mcl-1 (Oltersdorf et al., 2005). Certain studies have reported that upregulation of Mcl-1 is directly correlated to ABT-737 resistance, in which case, the agents that could inactivate or downregulate the Mcl-1 levels through overcoming the resistance to ABT-737 could be an effective therapy. In pancreatic cancer cells, Mcl-1 was observed to exert a pivotal role, owing to the fact that knockdown of Mcl-1 leads to the sensitization of cancer cells to ABT-737-induced cell death (Huang & Sinicrope, 2008).

Mechanism of action:

ABT-737 through possessing a strong interaction with Bcl-2, Bcl-xL and Bcl-w, with lesser interaction to other prosurvival proteins such as Mcl-1 and A1, promotes cell death (Oltersdorf et al., 2005). This would facilitate in the better understanding of the role of Bcl-2 in cancer therapy.

17. Bcl201

Bcl201, also known as S55746, is a selective small molecule Bcl-2 inhibitor that provokes apoptotic processes in cancer cells. A long queue of Bcl-2 inhibitors such as HA14-1, sabutoclax, S55746 (S055746, Bcl201), and gambogic acid are in the line to undergo preclinical trials, in addition to TW-37. Amongst these groups, S55746 binds strongly to Bcl-2, similar to that of ABT-737 and ABT-199 (Casara et al., 2018).

Mechanism of action:

A study by Casara et al. (2018) have suggested the role of Bcl201, wherein it was shown to attach on the hydrophobic groove of Bcl-2, with no lesser interaction with Mcl-1, Bfl-1 (Bcl2A1/A1). However, it does not have any effect on Bcl-xL as well as its dependent cells such as platelets. Additionally, Bcl201, stimulates cell death through the signaling cascade of phosphatidylserine externalization, caspase-3 activation and cleavage of PARP.

18. S63845

The Bcl-2 family proteins such as BIM, BID, PUMA and NOXA stimulate the mitochondrial apoptotic pathway through activation of the multidomain cell death proteins, such as BAX and BAK (Bhola and Letai, 2016). Sequestration of BH3-only proteins occurs through the binding of Bcl-2, Bcl-xL and Mcl-1, which helps in the prevention of Bax and Bak activation and thus favours cell survival.

Mechanism of action:

S63845 induces cell death through a specific inhibition of Mcl-1, especially in T-cell acute lymphoblastic leukemia. S63845 exhibits a lesser effect on Bcl-2 and Bcl-xL (Kotschy et al., 2016), thus exploring the role of Bcl-2 in apoptosis.

19. Bortezomib (BTZ)

The proteasome inhibitor, bortezomib (BTZ), has revealed a significant role in multiple

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myeloma (MM) therapy with a high success rate. It finds greater application in the treatment of MM either as first-line therapy or in advanced cases, as well as can be applied in those patients with organ transplantations done (Ogawa et al., 2008), despite its adverse effects like peripheral neuropathy, constipation, etc.

Mechanism of action:

Inhibition of NF-κB activity was the foremost target of BTZ in MM patients. In general, NF-κB was found to be associated with a group of Rel families, such as RelA, RelB, c-Rel, p50 (NF-κB1), and p52 (NF-κB2), which exerts a key role in tumor biology. Role of NF-κB is represented by two pathways, such as canonical and non-canonical. Translocation of RelA and p50 proteins into the nucleus and subsequent upregulation of other survival genes occurs in the canonical pathway. Phosphorylation of the protein, IκBα, following the polyubiquitination and degradation by the 26S proteasome enhances the translocation process, through the activations of cytokines like IL-6, IGF-1, and TNF-α (Hideshima et al., 2009). Nevertheless, upon BTZ administration, proteasome inhibition leads to the accumulation of phosphorylated IκB in the cytosol, which ultimately results in the inhibition of RelA and p50 complex translocation into the nucleus. The whole process ends up in the de-activation of the canonical pathway. However, the exact role of BTZ remains questionable, due to the fact that it holds the potential to activate NF-κB on the other hand. Most importantly, it also possess the ability to de-activate the non-canonical pathway simultaneously, albeit NF-κB activation. Overall, the effect of BTZ in tumor patients was observed to be associated with important tumor markers such as KLF9, Nampt, CDK5, and 3 ER stress- and UPR- associated markers (XBP1, ATF3, and AFT4), whose bioactivity and mechanistic role has to be explored (Masaki, 2016).

The overview of the role of all the Bcl-2/Bcl-xL/Mcl-1/ NF-κB inhibitors were represented in the schematic diagram underneath (Figure 2).

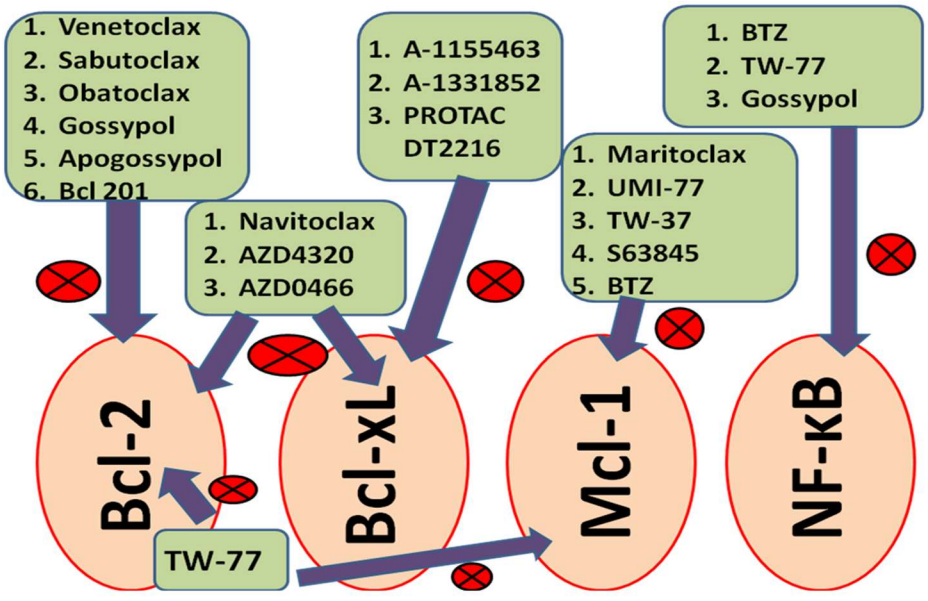

Figure2. Role of various Bcl-2 inhibitors

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Conclusion:

The present review highlights the mechanistic role of various Bcl-2 inhibitors and its target proteins and pathways. The review provides an extensive insight on the mechanism of Bcl-2 in tumor therapy, which acts as an effective therapeutic strategy in the cancer treatment. It further facilitates in exploring novel drugs/inhibitors with specific target and be an ideal candidate with lesser or acceptable side effects.

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Conflict of Interest:

The authors declare that there is no conflict of Interest.

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