The main BH3-only proteins in BCL-2 family

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Abstract. BCL-2 family protein is an important molecular marker of programmed cell death. The form of apoptosis exists in the balance between anti-apoptotic protein and apoptotic protein. The special small molecular protein of apoptotic protein is a window to create small molecular drugs. BH3-only protein is the main simulation form of BH3 drugs. Therefore, this review summarizes the basic structure of BH3-only protein and the related role of protein in apoptosis, which can be widely used in the combined treatment of apoptosis. Besides, this review also updates the relevant latest research news from venetoclax, which was approved for listing by the FDA. The review will contribute to BH3 drugs manufacturing, and will support models to other drugs that could easily treat with different cancers.

Keywords: BCL-2 gene, BH3-only proteins, venetoclax.

1. Introduction

During the occurrence of tumors and cancers, they spread and survive by avoiding the programmatic death of cells. Since the emergence of tumors, according to Darwin's theory of evolution, the mechanisms of tumors and cancer against apoptosis have also been constantly evolving and innovating. [1] They can resist apoptosis by creating various pathways. There are only two main pathways for apoptosis: internal and external apoptosis pathways. In the process of apoptosis regulation, the BCL-2 gene is the most common and widely studied apoptosis gene. The occurrence of apoptosis is mainly regulated by BCL-2, which is also an important reason for the wide range of research results of BCL-2 genes. The BCL-2 family has a wide range of proteins and varieties. [2] The general classification can divide BCL-2 proteins into two categories: anti-apoptotic proteins and apoptotic proteins [3]. Among them, the most noteworthy research on apoptotic proteins, which are key steps to freely control the programmatic death of cells. For example, apoptotic proteins can be divided into two categories: effector (BAX/BAK) and proteins containing only BH3 domains. In previous studies, BAX/BAK, as a effector, can only complete oligomerization after receiving activation signals, form a channel-form protein, and complete the direct bridge between the mitochondria and the cytoplasm. [4] Only after the apoptosis signal is transmitted to the mitochondria and the relative movement occurs on the mitochondrial membrane and the active substances released can the cascade reaction be completed to form apoptotic bodies. Apoptotic bodies form cellular programmatic death through structural changes.

The way to active BAX/BAK is the key factor in the apoptosis pathway in mitochondria, so their activation is regulated by the BH3-only protein. However, research shows that during the activation process, direct activation mode and indirect activation mode and the coexistence of both activation modes exist. [2] Then, after activating according to this pattern, the topology of BH3-only protein and BAK and other proteins changes [5]. After the BH3-only protein binds the four hydrophobic bonds of BAK, the structure of the a-helix changes occur, making the binding of the two stronger and closer. However, recent research shows that their binding is not absolute. There are also many forms of topology, and the existence of multiple forms is also sorted and distinguished according to the degree of bonding. This also provides a new idea for drug design. The BH3-simulator is no longer a single form of existence, and it may be better to be used in combination with other drugs. [1]

In this review, paper summarizes the important role played by the hottest BH3-only protein in cancer, discusses the basic principles of the drug model of BH3-only protein and the latest drug update

information. In this field, research on drug resistance and non-usual forms of treatment of drugs is under way.

2. Targeting BCL-2 with BH3 mimetics

When looking at the breakpoints of chromosome 18, biologists established in 1985 that B-cell lymphomas 2 serves a significant role in the development of follicular lymphoma. BCL-2 expression is dramatically increased, specifically in follicular lymphomas, because of the promoter on chromosome 14 that is immunoglobulin heavy of the proteins. Stranger still, BCL-2-expressing cells persistently remain in the G0 phase rather than proliferating, which actually distinguishes them from other oncogenes. In this manner, the Bcl-2 gene is given enough time to complete the inevitable malignant transition while also having enough time to experience cellular stressors.[1]

2.1. The regulations of apoptosis in the essential gene BCL-2

When certain stress signals cause pro-apoptotic BH3-only proteins to become active, the intrinsic route is activated. These pro-apoptotic BH3-only proteins carry out their pro-apoptotic role by inhibiting pro-survival BCL2 family proteins. Furthermore, BAX, BAK, and perhaps BOK are activated directly when these anti-apoptosis proteins are depleted or missing, resulting in permeabilization of the mitochondrial outer membrane (MOMP). [3] BAX and BAK coexist in healthy cells as inactive monomer proteins that can easily move between the cytoplasm and mitochondria.[3] In actuality, if we intend to differentiate between BAX and BAK, BAX is associated to cytoplasm permeability, whereas BAK is related to mitochondrial activities.[6] Theoretically, these two proteins entered the mitochondrial outer membrane in response to the apoptotic signal, causing the membrane's oligomerization and conformational rearrangement. Scientists hypothesised that these two proteins entered mitochondrial outer membranes in response to the signal of death, causing mitochondrial outer membrane conformational rearrangement and oligomerization to produce pores that released cytokines like cytochrome c and SMAC. [7]The precise processes of MOMP, the synthesis and breakdown of intermediates, and the precise mechanism of outer membrane opening in BAX-mediated MOMP movement are still unknown.[6] They associate with the adapter molecule Apaf-1 and the inactive promoter caspase, pro caspase 9, in the cytoplasm to form a complex of proteins known as apoptotic bodies. The first step of the activation cascade is the activation of caspase 9, which is done from other caspases.

When death receptor ligands bind to their corresponding death receptors on the plasma membrane, caspase 8 is activated by FAS-associated death domain protein, which is belong to extinctic apoptosis pathway, with or without TNFR-associated death domain protein. This launches the extrinsic process of apoptosis. Subsequently trigger caspase 8, which sets off caspase 3 and causes cell death. Each caspase cleaves a different substrate, triggering DNase and coordinating cell death. The endoplasmic reticulum and perinuclear membrane of hematopoietic cells contain Bcl-2 family proteins as well, but their primary location is the mitochondria.[2]

In other words, once the body experiences stress, pro-apoptotic protein expressions increase and the dynamic equilibrium is upset, which causes the intrinsic apoptosis signal pathway to be activated. In addition, the presence of oncogenes makes normal cells more susceptible to pro-apoptotic components. For instance, malignant cells will up their expression of the protein BCL-2 to thwart apoptosis. Overexpression of BCL-2 inhibits cell death, yet pro-apoptotic proteins have the unique capacity to isolate BCL-2 and launch cell death. The majority of studies show that the average BCL-2 proteins inhibit apoptosis. However, the BCL-2 family, which includes pro-apoptotic proteins and anti-apoptotic proteins, maintains a dynamic balance in the cells.[7]

In the other words, once the body felt the stress, the expressions of pro-apoptotic proteins will rise, and the dynamic balance is broken, leading and activating the intrinsic apoptosis signal pathway. Even more, with the different conditions of normal cells, the existing of oncogenes makes cell more sensitive to the pro-apoptotic members, for example, malignant cells will increase the level of BCL-

2 expression proteins to prevent apoptosis. The overexpressing BCL-2 stop the procedure of the cell death, but the pro-apoptotic proteins obtain the special ability to isolate BCL-2, stimulating the cell death. The majority of studies showed that BCL-2 proteins prevent apoptosis; however, the BCL-2 family, which includes pro-apoptosis proteins and anti-apoptotic proteins, maintains a dynamic equilibrium in cells. As a result, pro-apoptotic proteins are able to determine whether or not a cell will survive. In this way, the main mimetic drugs are obviously created, as well as the interesting notion is the fundamental fossil to BH3-only proteins.

2.2. The structure and functional mechanisms of BCL-2 family proteins.

Bcl-2 proteins are identified by the presence of up to four conserved linear sequence motifs or domains comprising about 20 residues and known as BCl-2 Homology (BH) motifs.[2] The BCL-2 protein fold has a unique structure---the a5 hydrophobic helix bundle, which is centered around a hollow scaffold structure that can accommodate up to eight A-helix structures. In many organisms, BCL-2 protein fold assumes the BH3 sequence into the BH-3 binding groove when BH3 mimics are binding, which is a common interaction mechanism. However, this is not the only interaction mechanism, and even the NF-kb pathway is not mediated by this mechanism of assembly into the groove. The BH3 motif is an important part of the structure of BCl-2 like proteins. In addition to this motif, BCl-2 family proteins generally possess a transmembrane domain as well for attaching to organelles, the most common of which are mitochondria. [4]According to the different domains, it depends on the different functions. And tripartite BCL2's apoptotic transition is brought on by: initiators, guardians and effectors, respectively. The initiators contains BIM, BAD, NOXA, PUMA, BIK and tBID, with the obvious feature comprising only BH3 domains on the BCL-2 chomosome. According to the muti-BH domains on the homologs, there are 6 human pro-survival family members that are clearly reported, BCL-2, BCL-XL, BCL-W, MCL1, A1and BCL-B, which are called pro-survival proteins. On the other hand, the pro-apoptotic proteins BAX, BAK and BOK share four BH domains, which play opposite roles in the body[5].

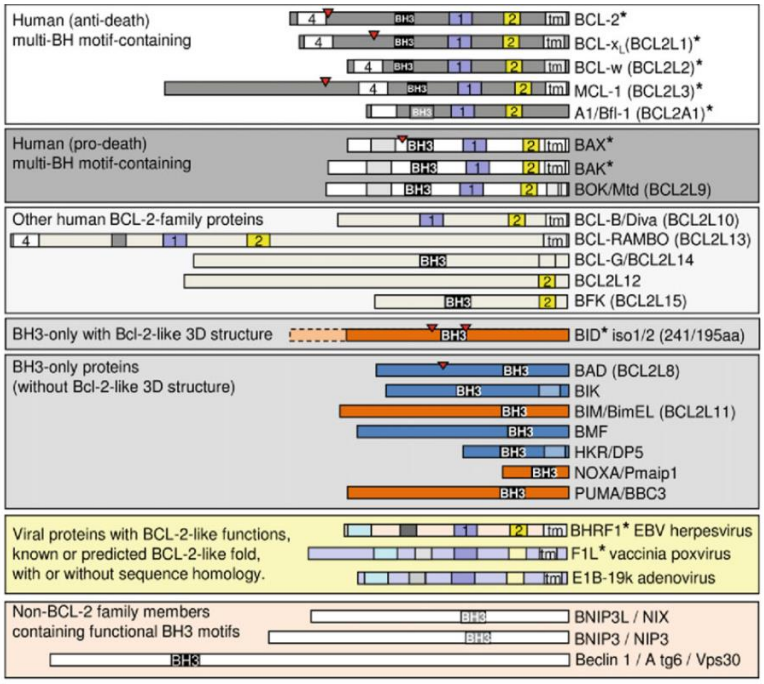

Figure 1. The structure of BCL-2 like proteins. (Adapt from Huska JD, LAMB HM, Hardwick JM, 2019)

2.3. The significant pro-apoptosis initiators: BH3-only proteins

BH3 only protein mediates apoptosis of abnormal cells through two mechanisms: neutralizing BCl-2 family proteins that promote survival or directly activating apoptosis effectors BAX and BAK.

2.3.1 Neutralizing pathway

The pro-survival proteins have been thoroughly defined, both physically and functionally, and thus maintain a close relationship with the structure of the neutralization route with pro-apoptosis proteins. For the amphipathic helix BH3 of the BH3 proteins only to bind to the hydrophobic groove of the pro-survival proteins, four residues along one side of the groove must be inserted from the hydrophobic pockets of the furrow. And one side of the sulcus should be inserted sulcus hydrophobic pockets. [2] Furthermore, a concentration of sodium must be formed between a conserved BH3 Asp residue and a conserved Arg residue in the BH1 domain of the pro-survival proteins. The complex structure is helpful for binding to BH3-only proteins.[1] However, it is desirable to bind to BH3-only proteins due to the complex structure. Because pro-survival proteins have minor differences in their BH3 domains and grooves, some BH3-only proteins, like BAD and NOXA, are selective for particular subsets of their pro-survival cousins, whereas other BH3-only proteins, like BIM, tBID, and PUMA, likely neutralise all of the pro-survival proteins. The BAK proteins, of course, shut down the apoptotic pathways completely. [8]

2.3.2 Directly acting with effectors

The activation of the BAX and BAK pathway also shares a relationship with the conserved structure of BCL-2 family proteins. Based on the crystal structure of a BID BH3 domain interacting with BAX's canonical groove and a BIM SAHB interacting with BAX's rear pocket, two distinct locations on BAX have been postulated for the engagement and activation of certain BH3-only proteins. These locations are the canonical hydrophobic internal groove and surface groove of BAX.[2]

During apoptosis, BAX and BAK insert monomers with folds mimicking the pro-survival proteins into oligomers of unknown structure that can permeabilize the mitochondrial outer membrane. Activator BH3-only proteins, such as BIM or tBID, as well as their BH3 peptides, have been shown in studies to trigger this transition. It has been suggested that BAX has two unique activation sites: the typical hydrophobic groove, which is comparable to that of the pro-survival proteins, and an alternate location on the opposite side of BAX.[1]

Focusing on NMR studies using full-length BAX, whose transmembrane domain is located in its typical hydrophobic groove, and activation Coiled-coil peptide modified with a hydrocarbon staple, the BAX "rear" activation site, which involves helices 1 and 6, was hypothesized.[2]The basal migration of BAX between the cytosol and the outer mitochondrial membrane may be sped up by the rear activation site acting as a stimulant to push the transmembrane domain of BAX out of the groove. The groove activation site may then trigger additional changes, according to some molecular data. Apoptosis could still be triggered even though changes to the back activation site may not have been necessary for BAX activation. Numerous research teams have determined that the canonical surface groove of BAK is the only location where activator BH3 domains may interact with it. Similar to BAK, BAX also contains a bound BID BH3 peptide as well as a bound stapled BID BH3 domain.

3. The main BH3-only proteins in BCL-2 family

BH3-only proteins are subfamily of BCL-2, which are pro-apoptosis proteins. As for pro-apoptosis proteins, it is clearly classified in two parts depending on functioning modes, directly and indirectly. In this article, we will demonstrate main BH3-only proteins that directly trigger the caspases responds in the apoptosis.

3.1. BID

The only protein from the BH3 family that really penetrates mitochondria is tBID. For caspase 8 to cause mitochondria to undergo full apoptosis, BID is a crucial medium. By cleaving the BID protein in vitro, caspase 8 produces the stage product BID, also known as tBID, which is then transferred to mitochondria and triggers a caspase response.In addition, in terms of immunology, a

different BID product called gtBID works in tandem with cytotoxic T lymphocytes (mechanism), and in this procedure, BID is hydrolyzed and cleaved by granzyme to produce gtBID. [4]

Additionally, BID can be cleaved into jBID by active JNK, a crucial component of the MAPK pathway, which mediates caspase-8 independent BID cleavage and results in the tBID and gtBID discussed above as well as initiating the related cascade reaction. Specific amino acid positions on BID can be phosphorylation by Jnk1-3, preventing caspase-8 from cleaving the protein and enabling improved aggregation and transport to the mitochondria. Full-length BID can also enter mitochondria, according to studies. [9]As a result, BID under JNK mechanism still has the capacity to trigger apoptosis; however, full-length BID's ability to do so is inferior to that of JNK-induced jBID. Because of the buildup of entire field proteins, the JNK-mediated phosphorylation of BID prevented the onset of apoptosis. When cells experience external stress, such as DNA damage, ATM protein kinase removes BID and transports it to the nucleus where it takes part in general cell transduction. Additionally, BID accumulation and cell growth are linked. [4] In addition, casein kinase I and II inhibited the ability of the protein to trigger apoptosis by activating murine BID at Thr58, Ser61, and Ser64 proximal to the site of caspase-8 cleavage. Additionally, it was shown that BID's loss inhibited T cell tumorigenesis, which suggested that BID had a pro-survival function. Overall, the pro-apoptotic and pro-survival properties of BID can be switched on and off by phosphorylation.[9]

3.2. BIM

The immune system and the homoeostasis of hematopoietic cells are substantially regulated by BIM, which is broadly dispersed in normal tissue cells. According to certain research, leukemias and autoimmune disorders may result from the deletion of the BIM gene. Furthermore, the apoptosis of tumour cells will be inhibited as the level of BIM expression in malignant tumors cells declines. In breast cancer, overexpression of HER2 can boost ERK and Akt activation, decrease BIM expression, block tumour cell death, and promote the growth of tumour cells. Additionally, colorectal cancer, liver cancer, and gastric cancer all have downregulated BIM expression levels. [10]The efficacy of chemotherapy and TKIs treatment will be decreased and the prognosis of postoperative patients will be impacted by the BIM gene expression level decline or the presence of BIM deletion polymorphism in NSCLC. These factors may become significant contributors to treatment failure.[10]

3.3. PUMA

In the BCL-2 family, PUMA was found to be a BH-only protein. It is believed to be a protein that the p53 signaling pathway addresses downstream of itself. To counteract the inhibitory effect of anti-apoptotic proteins on the permeability of pro-apoptotic proteins on the outer membrane of mitochondria, during conditions of acute cellular stress, p53 transcriptional activity activates BH3-only proteins such as BIM, PUMA, or NOXA. The amount of p53 expressed in a cell depends on the stress it is under, and the amount of p53 expresses the size of apoptosis. In addition to its structural functional characteristics that allow it to bind to pro-apoptosis proteins, PUMA also possesses a functional domain on its structure that is responsible for its localisation in the mitochondria.[11] P53 activates the expression of PUMA while inhibiting the transcription of ARC. PUMA and caspase-8 compete for ARC's binding, and caspase-8 cleaves BID to tBID.Because PUMA has to finish post-translational modifications, its expression is relatively modest in comparison to that of BID and BIM in the BH-3 only protein. The likelihood of post-translational alterations is also raised by high levels of PUMA, which results in higher protein expression in a number of malignancies. Additionally, PUMA can bind apoptotic inhibitors to the caspase recruitment domain (ARC) to activate caspase-8, which lyses BID to tBID. As a result, even though the rise of PUMA protein cannot cause apoptosis, it may aid in the development of some malignancies.

3.4. NOXA

p53-dependent and/or p53-independent pathways (Hif1, PKC, and p73) are both used by NOXA to cause apoptosis. Using BAK's H1 and BH3 domains, NOXA can bind to BAK directly. In addition,

NOXA can bind to BAX directly. This allows it to target Mcl-1 either directly or indirectly by ubiquitination, releasing the BIM and PUMA that Mcl-1 had previously isolated and having a pro-apoptosis effect. Additionally, BAD and NOXA can cooperate to cause apoptosis. Bcl-xL can stop apoptosis brought about by NOXA. Through the use of intramolecular disulphide bridges, NOXA can also engage covalently with Bfl-1/A1. The image also demonstrates how the direct transfer of p53 to mitochondria can change the permeability of those cells. The possibility of p53 being transferred to mitochondria first and then to the nucleus should also be noted.[11]

4. The latest tumor therapy related to BH3-only proteins

4.1. Venetoclax is most successful drugs in CLL

Venetoclax is the first small molecule inhibitor targeting protein-protein interaction in the world, with a development history of 20 years. It is also a typical successful example of fragment based drug discovery, which is the most successful drugs that is approved by FDA. Especially, venetoclax is the oral small molecular to regulate apoptosis in combination therapy with obinutuzumab in small lymphocytic lymphoma and in combination therapy chronic lymphocytic leukaemia that has relapsed or is resistant. On the other hand, venetoclax also can treat with acute myeloid leukemia, which is also can treat with azacitidine, decitabine, or little cytarabine in some particular population.[12]

CLL is a blood disorder in which tumour cells accumulate and proliferate in the blood and the treatment process for the chronic disease is quite difficult. Chemotherapy has long been the most effective treatment for CLL, but the discovery of the BCL-2 target has brought a new light to the treatment of CLL. It is important to note that tumour lysis syndrome is the most common symptom in patients with CLL and can often lead to death. When patients start taking anti-tumour drugs, the tumours begin to apoptosis and their numbers decrease rapidly, which is a sign that the drugs are starting to work. Reports have shown that taking venetoclax can lead to the development of tumour lysis syndrome when patients are under high tumors burden or have reduced kidney function. Scientists tried to solve this dilemma by using a combination of venetoclax and obinutuzumab for CLL, and the final results showed that the symptoms of tumors lysis syndrome were reduced in cll patients treated with the combination. However, in the end, the combination was not judged to be clinically effective and experts recommend that patients should be examined to determine their tumour load level before using venetoclax to ensure their health is safe.[13]

For patients with early stage CLL cancer, the clinical recommendation is to use rituximab and venetoclax in combination with real-time monitoring of tumour growth, and also in combination with physical adjuvant methods such as chemo-immunotherapy and radiotherapy. However, the treatment may be associated with adverse effects such as neutropenia, diarrhea and nausea. For patients with advanced cancer with high tumour load, the combination of rituximab and venetoclax for CLL and small lymphocytic lymphoma, rituximab has a synergistic effect on venetoclax, rituximab increases the efficacy of venetoclax and prolongs PFS. [14]

4.2. Venetoclax in AML

The combination of venetoclax and HMA (azacitidine and decitabine) has demonstrated a high safety profile in clinical testing. venetoclax and HMA have been associated with nausea and neutropenia in patients with advanced AML. the FDA-approved combination treatment modality is primarily for AML patients older than 75 years of age. It is not possible to determine whether the combination of HMA and venetoclax is the single most effective treatment modality. Based on this, current research is focused on the optimal treatment of venetoclax in combination with other drugs in younger AML patients and in relapsed/refractory AML patients.[15]

5. Conclusions

In summary, the response of BH3 mimicked medicines is transformative in some cancer types, although difficulties still exist. More precise preclinical disease models can be utilised to examine the therapeutic potential of combination therapies that may overcome resistance to BH3 simulated drug monotherapies, which will enhance the clinical application of BH3 simulated pharmaceuticals. This will increase the long-term response rate of patients with drug-resistant or recurrent diseases. The primary barrier to the widespread administration of BH3 simulated medicines is the focused toxicity linked with the essential survival-promoting proteins required to impair numerous physiological processes that are part of normal cellular function. This is crucial because preclinical studies have demonstrated that long-lasting responses can be obtained by targeting two or more survival-promoting BCL-2 like family proteins (for instance, BCL-2 and MCL-1) with various BH3 simulation medicines. In the future, it will be crucial to deliver BH3 simulated drugs to cancer cells precisely and/or promote their activation in order to realise the treatment window.

References

[1] Y. Xu, H. Ye, Progress in understanding the mechanisms of resistance to BCL-2 inhibitors 2022, Experimental Hematology & Oncology 11(1) 1-11.

[2] V. Sora, E. Papaleo, Structural Details of BH3 Motifs and BH3-Mediated Interactions: an Updated Perspective 2022, Frontiers in Molecular Biosciences 9.

[3] P. Klener, D. Sovilj, N. Renesova, et al., BH3 Mimetics in Hematologic Malignancies 2021, International Journal of Molecular Sciences 22(18) 10157.

[4] G. Sekar, A. Ojoawo, T. Moldoveanu, Protein–protein and protein–lipid interactions of pore-forming BCL-2 family proteins in apoptosis initiation 2022, Biochemical Society Transactions.

[5] P.A. Townsend, M.V. Kozhevnikova, O.N. Cexus, et al., BH3-mimetics: recent developments in cancer therapy 2021, Journal of Experimental & Clinical Cancer Research 40(1) 1-33.

[6] A.Z. Spitz, E. Gavathiotis, Physiological and pharmacological modulation of BAX 2021, Trends in Pharmacological Sciences.

[7] J.J. Montero Boronat, R. Haq, Adapted to Survive: Targeting Cancer Cells with BH3 Mimetics 2022, Cancer Discovery, 2022, vol. 12, num. 5, p. 1217-1232.

[8] F. Hagenbourger, S. Bohler, M. Erlacher, No time to die? Intrinsic apoptosis signaling in hematopoietic stem and progenitor cells and therapeutic implications 2022, Current Opinion in Hematology 29(4) 181-187.

[9] A. Wagner, C. Tittes, C. Dehio, Versatility of the BID domain: Conserved function as type-IV-secretion-signal and secondarily evolved effector functions within Bartonella-infected host cells 2019, Frontiers in Microbiology 10 921.

[10] Z. Wu, C. Chen, Y. Cai, et al., BIM-based visualization research in the construction industry: A network analysis 2019, International journal of environmental research and public health 16(18) 3473.

[11] R. Roufayel, K. Younes, A. Al-Sabi, et al., BH3-Only Proteins Noxa and Puma Are Key Regulators of Induced Apoptosis 2022, Life 12(2) 256.

[12] M. Waggoner, J. Katsetos, E. Thomas, et al., Practical Management of the Venetoclax-Treated Patient in Chronic Lymphocytic Leukemia and Acute Myeloid Leukemia 2022, Journal of the Advanced Practitioner in Oncology 13(4) 400.

[13] K. Fischer, O. Al-Sawaf, J. Bahlo, et al., Venetoclax and obinutuzumab in patients with CLL and coexisting conditions 2019, New England Journal of Medicine 380(23) 2225-2236.

[14] J.F. Seymour, T.J. Kipps, B. Eichhorst, et al., Venetoclax–rituximab in relapsed or refractory chronic lymphocytic leukemia 2018, New England Journal of Medicine 378(12) 1107-1120.

[15] S. Kayser, M.J. Levis, Updates on targeted therapies for acute myeloid leukaemia 2022, British journal of haematology 196(2) 316-328.