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Treatments that target B cell receptor signalling and Bcl-2

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About this resource:

- This slide deck was prepared by Elaine Vickers, PhD, director of Science Communicated Ltd.
- Elaine has over twenty years' experience providing education and resources on cancer biology and treatments
- Her book, 'A Beginner's Guide to Targeted Cancer Treatments', was highly commended by the British Medical Association
- She collaborated with AstraZeneca to create this educational resource for healthcare professionals

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"In this slide deck you will learn about treatments that target B cell receptor-controlled signalling pathways

"I will also describe treatments that block a powerful survival protein called Bcl-2

"Both of these sets of treatments are effective against B cell-derived cancers such as chronic lymphocytic leukaemia and non-Hodgkin lymphoma"

What you will learn about treatments that target B cell receptor signalling:

- What the B cell receptor is, and how it works
- Why B cell receptor signalling pathways are important to healthy B cells
- What role the B cell receptor plays in cancers that develop from faulty B cells (such as B-cell NHL and CLL)
- How cancer treatments target and block B cell receptor-controlled signalling pathways
- What happens to cancer cells when B cell receptor signalling is blocked

NHL - non-Hodgkin lymphoma
CLL - chronic lymphocytic leukaemia

What you will learn about treatments that target Bcl-2:

- What Bcl-2 is
- How Bcl-2 and other similar proteins control cell survival and cell death
- The role of Bcl-2 in various B cell cancers
- How Bcl-2 is blocked by Bcl-2 inhibitors

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Part 1:

Treatments that target B cell receptor signalling

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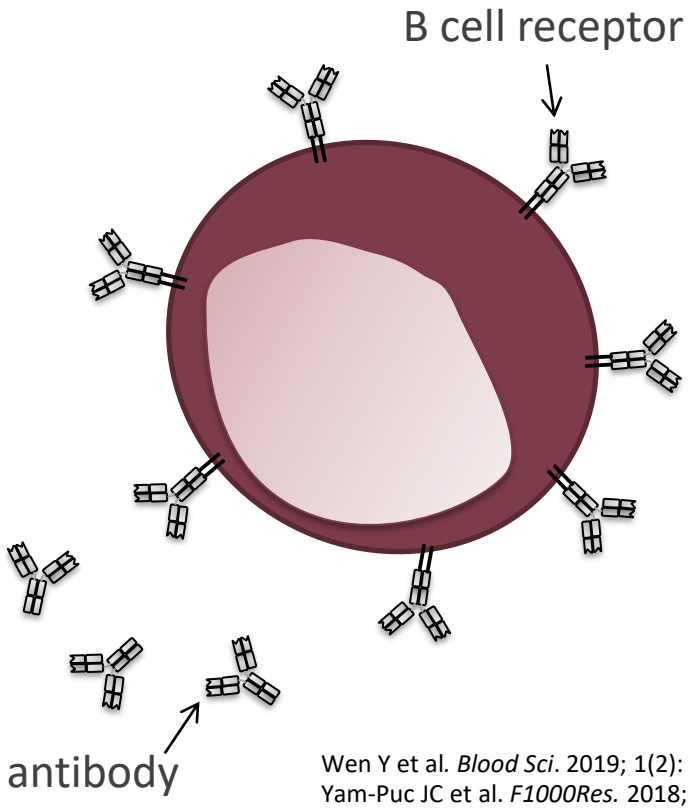
B cell receptor signalling pathways in healthy B cells

Introducing the B cell receptor

- During their development in the bone marrow, B cells cut and recombine their DNA to create a unique antibody/immunoglobulin gene
- They use this final, recombined gene to make a unique B cell receptor (BCR)
- Each healthy B cell has over 100,000 identical copies of its BCR on its surface
- B cells use their BCRs for:
  - maturation and survival
  - responding to antigens (substances that trigger immune responses)



Wen Y et al. *Blood Sci*. 2019; 1(2): 119-129
Yam-Puc JC et al. *F1000Res.* 2018; 7: 429

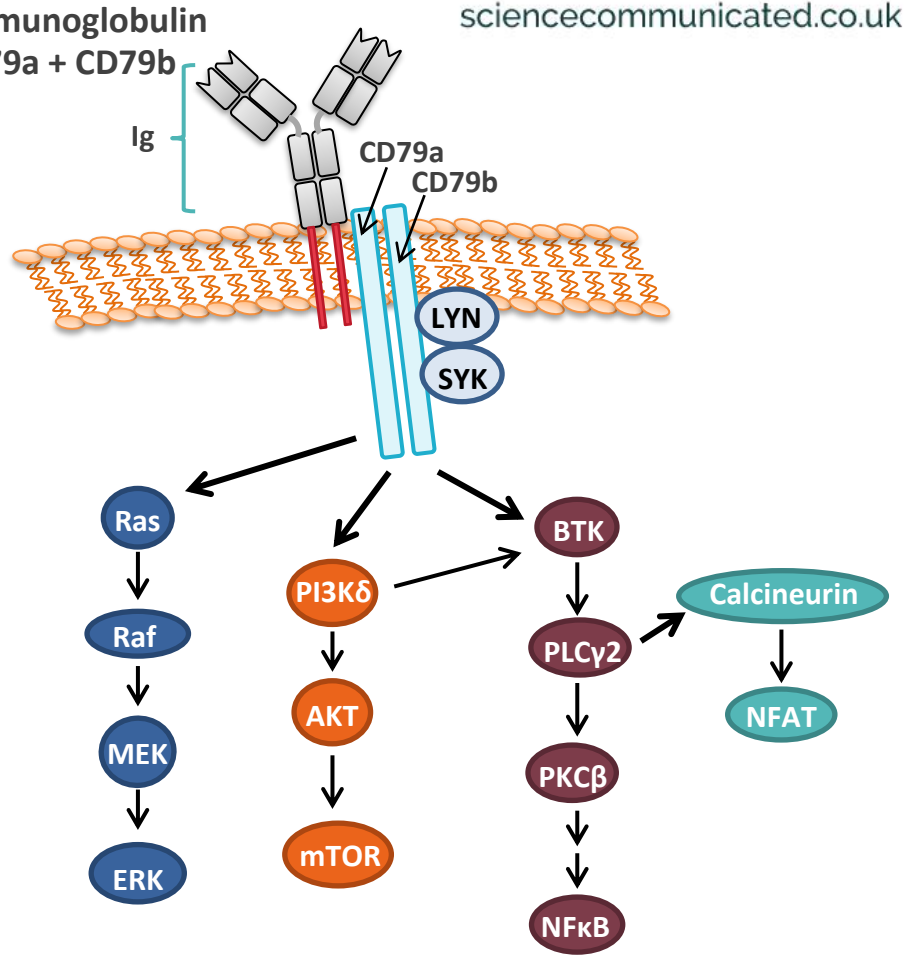
B cell receptor activation

Maximum BCR signalling takes place when a B cell's BCRs connect with a suitable antigen

This causes BCRs to cluster together in the cell membrane and recruit SRC-family kinases e.g. LYN

LYN phosphorylates CD79a and CD79b, which then recruits another kinase, SYK

SYK phosphorylates adapter proteins, which in turn activate signalling pathways

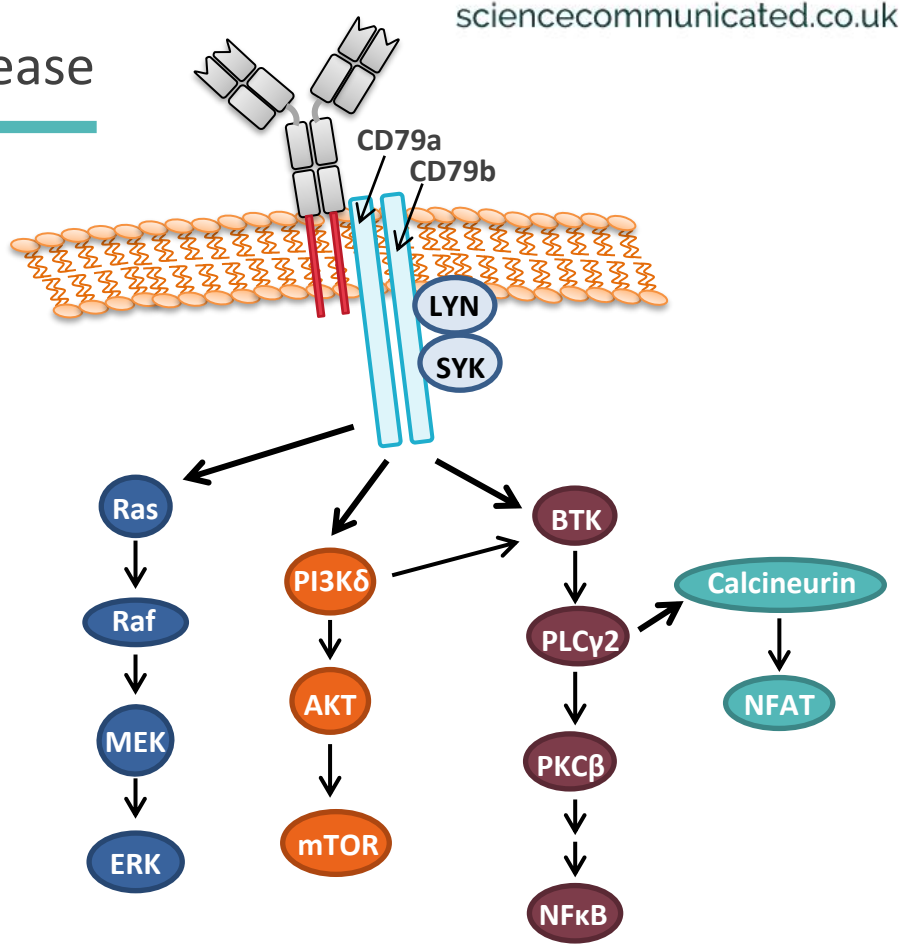


Abbreviations: BCR – B cell receptor; mTOR – mammalian target of rapamycin; PI3Kδ – phosphatidylinositol 3-kinase-delta; ERK- extracellular-signal-regulated kinase; BTK – Bruton's Tyrosine Kinase; NFκB – nuclear factor-kappa-B; NFAT – nuclear factor of activated T cells; PKCβ – protein kinase C beta; PLCγ – phospholipase C gamma

Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-167
Efremov DG et al. *Cancers*. 2020; 12(6): 1396

B cell receptor function in health and disease

- Activation of BCRs by antigen can lead to various consequences depending on the strength of the signal and the pathways activated
- Possible outcomes include cell proliferation or death
- In addition, all our mature B cells need low-level (tonic) BCR signalling to stay alive
- In many B cell cancers, the cancer cells still depend on BCR signalling
- Many of the proteins controlled by BCRs are also active in other white blood cells and in response to other receptors



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Efremov DG et al. *Cancers*. 2020; 12(6): 1396

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Understanding the role of B cell receptor signalling in CLL and B-cell NHL

CLL: chronic lymphocytic leukaemia
NHL: non-Hodgkin lymphoma

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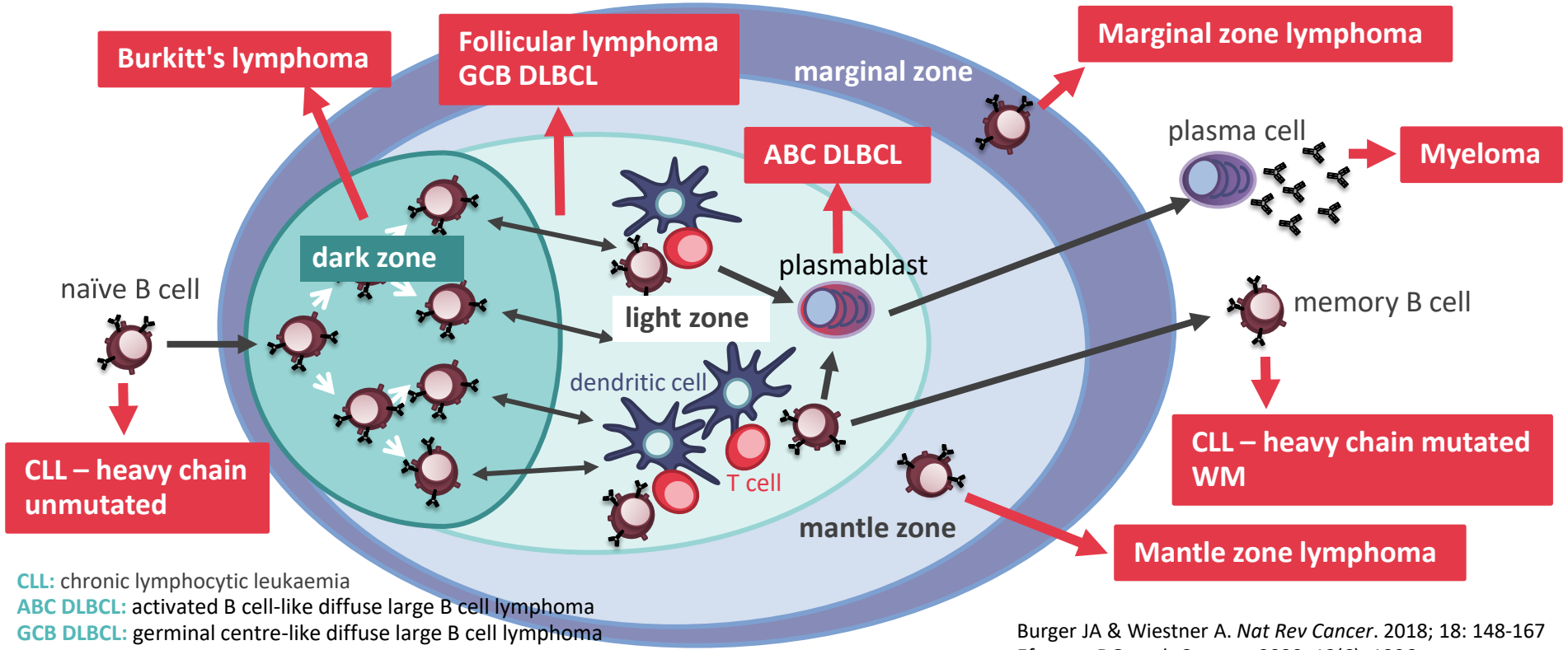
Facts about CLL and B-cell NHL and their B cell receptors:

- CLL and B-cell NHLs develop from mature B cells that have BCRs on their surface[1]
- They vary in terms of the stage the B cell had reached in its response to antigen when it became a cancer cell[1]
- They also vary in terms of their reliance on their BCRs to drive their proliferation and keep them alive[1]
- Because the degree to which they rely on their BCRs varies, so does their sensitivity to drugs that block BCR-controlled signalling proteins[2]

Abbreviations: CLL - chronic lymphocytic leukaemia; NHL - non-Hodgkin lymphoma; BCR - B cell receptor

[1] Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-167
[2] Efremov DG et al. *Cancers*. 2020; 12(6): 1396

B cell NHLs and CLL develop from B cells at different stages of activation in different locations in germinal centres of lymph nodes

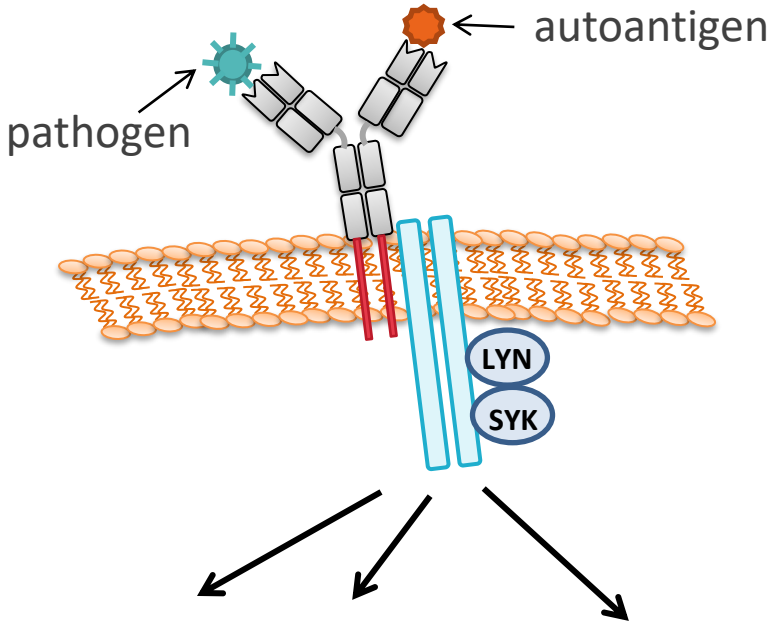


CLL: chronic lymphocytic leukaemia
ABC DLBCL: activated B cell-like diffuse large B cell lymphoma
GCB DLBCL: germinal centre-like diffuse large B cell lymphoma
WM: Waldenstrom macroglobulinaemia

Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-167
Efremov DG et al. *Cancers*. 2020; 12(6): 1396

B cell receptor signalling in CLL – part 1

- In chronic lymphocytic leukaemia (CLL), the cancer cells' BCRs are chronically active
- The antigen triggering the cells' BCRs might be a pathogen (a disease-causing invader) or an autoantigen (a normal component of the body)
- Autoantigens are often proteins or complex molecules found on the surface of macrophages or on dying cells
- Pathogens include complex molecules from bacteria, viruses and fungi
- Activation of BCRs is the main factor driving cancer cell proliferation

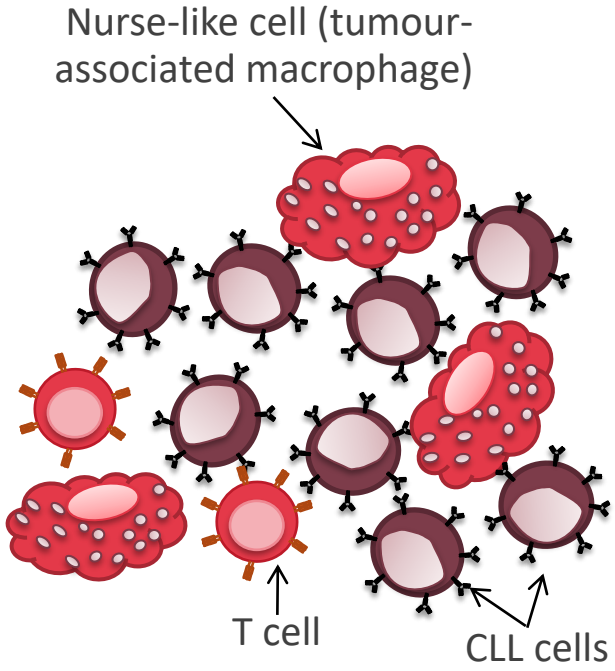


Abbreviations: BCR – B cell receptor

Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-167
Efremov DG et al. *Cancers*. 2020; 12(6): 1396
Burger JA. *N Engl J Med.* 2020; 383: 460-473

B cell receptor signalling in CLL – part 2

- CLL cells mostly proliferate in secondary lymphoid organs (SLOs) such as lymph nodes
- In SLOs, CLL cells interact with nurse-like cells and T cells, creating ‘CLL proliferation centres’
- Activation of BCRs, chemokine receptors and integrin receptors on CLL cells all activate BTK and PI3Kδ, causing CLL cells to:
  - proliferate and survive
  - migrate to lymph nodes
  - secrete CCL3 and CCL4, which attract T cells and macrophages

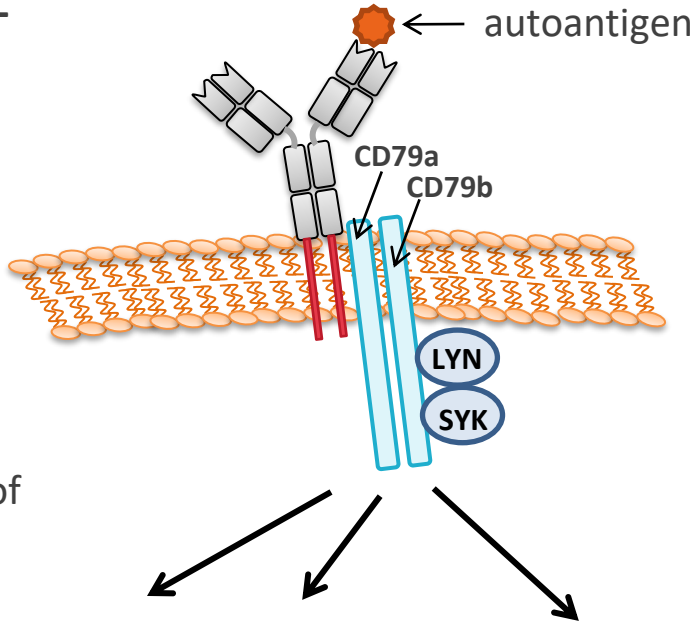


Abbreviations: BCR – B cell receptor; CCL3 – CC-chemokine ligand 3; CCL4 - CC-chemokine ligand 4

Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-167
Burger JA. *N Engl J Med.* 2020; 383: 460-473

B cell receptor signalling in B cell NHL

- In **mantle cell lymphoma**, **follicular lymphoma**, **ABC DLBCL** and **marginal zone lymphomas** cancer cells' BCRs have often reacted to an autoantigen[1]
- In **ABC DLBCL**, mutations affecting CD79b also amplify the signals generated by activated BCRs[1]
- In **marginal zone lymphomas**, infections [e.g. *H.pylori* (in **MALT lymphoma**) & *hepatitis C virus* in ***splenic marginal zone lymphoma***)] may add to BCR activity[1]
- In **GCB DLBCL** cancer cells rely on tonic, low-level, activity of BCRs, which primarily activates the PI3K-AKT-mTOR pathway but not BTK; BCRs have not responded to antigen and are not clustered together on the cell surface[2]



Abbreviations: BCR – B cell receptor

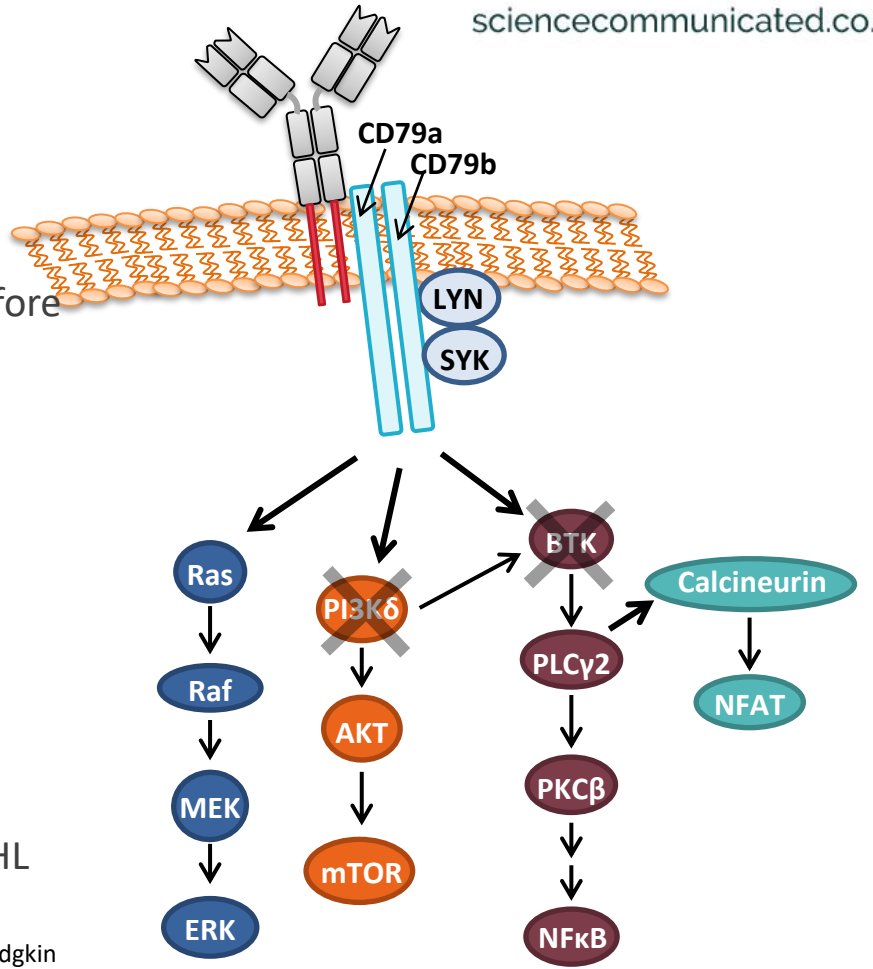
[1] Efremov DG et al. *Cancers* 2020; 12(6): 1396
[2] Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-163

ABC DLBCL: activated B cell-like diffuse large B cell lymphoma
GCB DLBCL: germinal centre-like diffuse large B cell lymphoma
MALT: mucosa-associated lymphoid tissue

Treatments that target B cell receptor-controlled signalling pathways

BCR signalling pathway blockers[1]

- BTK inhibitors and PI3Kδ inhibitors directly block BCR-controlled signalling pathways
- Both BTK and PI3Kδ are kinase enzymes, therefore drugs that block them are kinase inhibitors
- These drugs directly destroy cancer-causing B cells reliant on their BCRs to keep them alive
- They also disrupt the relationship between malignant B cells and their microenvironment, leading to the redistribution of cancer B cells into the blood and bone marrow, where they gradually die off
- BTK inhibitors and PI3Kδ inhibitors are licensed treatments for some people with CLL, B-cell NHL and Waldenström's macroglobulinaemia

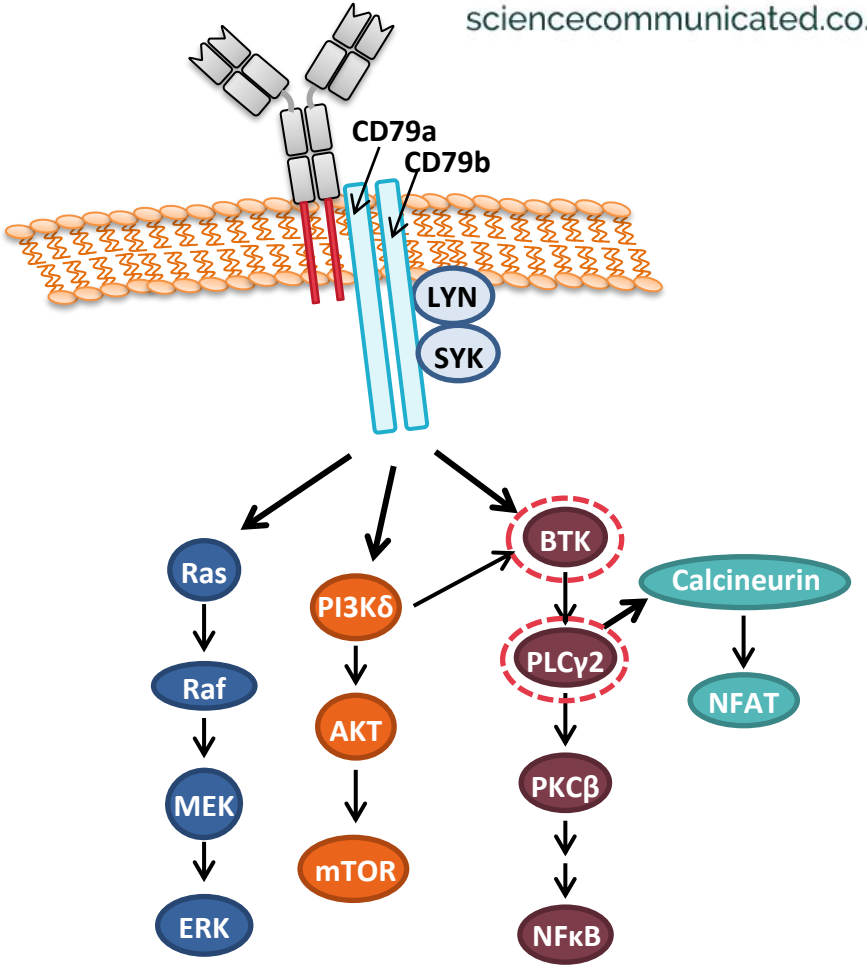


Abbreviations: BCR – B cell receptor; CLL – chronic lymphocytic leukaemia; NHL – non-Hodgkin lymphoma; mTOR – mammalian target of rapamycin; PI3Kδ – phosphatidylinositol 3-kinase-delta; ERK-extracellular-signal-regulated kinase; BTK – Bruton's Tyrosine Kinase; NFκB – nuclear factor-kappa-B; NFAT – nuclear factor of activated T cells; PKCβ – protein kinase C beta; PLCγ – phospholipase C gamma

[1] Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-163

Resistance to BTK inhibitors in CLL

- Most patients with CLL benefit from treatment with a BTK inhibitor, however resistance is common[1]
- Many BTK inhibitors chemically bond with a cysteine amino acid at position 481 in the BTK protein (they are covalent inhibitors)[1,2,3]
- CLL cells that are resistant to covalent BTK inhibitors often contain a mutant form of BTK in which the cysteine at 481 has changed to a serine[1,2,3]
- Another cause of resistance is mutations affecting PLCγ2[1,2,3]



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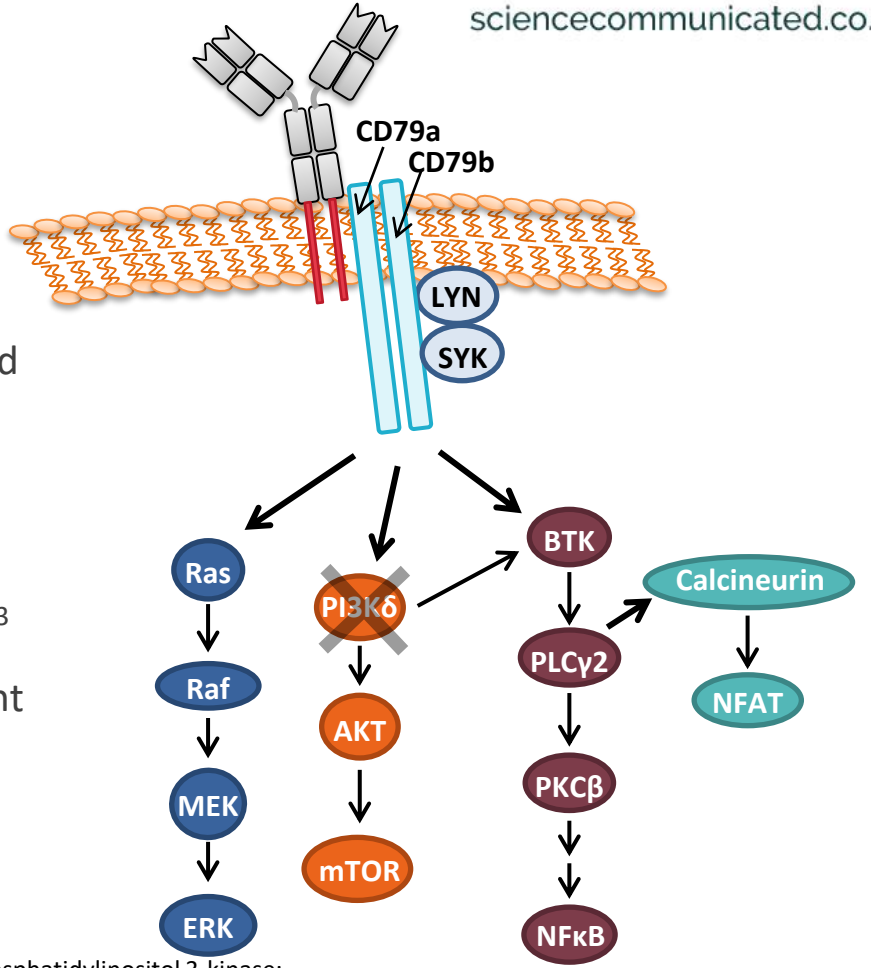
[1] Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-167
[2] Lampson BL & Brown JR. *Expert Rev Hematol*. 2018; 11(3): 185-194
[3] Gu D et al. *J Hematol Oncol.* 2021; 14:40

PI3Kδ inhibitors

- There are many different versions of PI3K in human cells, the four most well known are PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ[1]
- PI3Kδ transmits signals from B cell receptors and from other receptor proteins on B cells[1]
- PI3Kδ-selective inhibitors, as well as drugs that block both PI3Kδ and PI3Kγ, are effective for some subsets of CLL patients, such as people with relapsed and treatment-resistant disease[2,3]
- The mechanisms by which CLL becomes resistant to PI3K inhibitors are unknown[4]

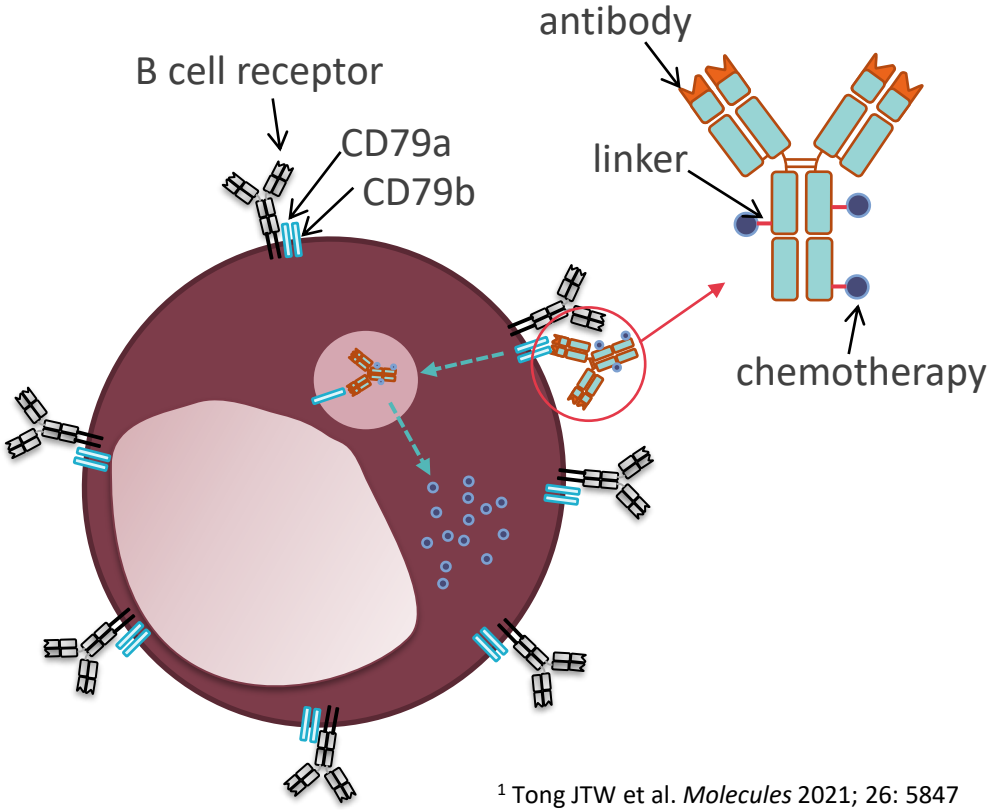


[1] Jean S & Kiger AA. *J Cell Sci.* 2014; 127(5): 923-928
[2] Burger JA & Wiestner A. *Nat Rev Cancer*. 2018; 18: 148-163
[3] Hus I et al. *Cancers (Basel)* 2022; 14(6): 1571
[4] Sedlarikova L et al. *Front Oncol.* 2020; 10: 894

Abbreviations: BCR – B cell receptor; mTOR – mammalian target of rapamycin; PI3K – phosphatidylinositol 3-kinase; ERK- extracellular-signal-regulated kinase; BTK – Bruton's Tyrosine Kinase; NFκB – nuclear factor-kappa-B; NFAT – nuclear factor of activated T cells; PKCβ – protein kinase C beta; PLCγ – phospholipase C gamma

Antibody-drug conjugates [1]

- Another way to block BCR signalling is to use an antibody-drug conjugate (ADC) that attaches to a component of the BCR complex, such as CD79b
- When the ADC attaches to its target, the cell membrane folds inwards and the ADC is drawn inside the cell
- Inside the cell, the linker attaching the drug to the antibody breaks, and the cell-killing chemotherapy component of the ADC is released into the cell



Abbreviations: BCR – B cell receptor

[1] Tong JTW et al. *Molecules* 2021; 26: 5847

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A summary of treatments that target BCR-signalling:

- Many B cell cancers rely on their B cell receptors (BCRs) to activate signalling pathways that keep them alive
- These signalling pathways can be blocked by drugs that target some of the proteins involved, such as BTK and PI3Kδ
- BTK and PI3Kδ inhibitors kill cancer cells directly and cause them to redistribute into the blood, where they die off
- It is also possible to target the BCR complex using drug-conjugated antibodies

Abbreviations: PI3Kδ – phosphatidylinositol 3-kinase-delta;
BTK – Bruton's Tyrosine Kinase

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Part 2:

Treatments that target Bcl-2

Bcl-2: B-cell lymphoma 2

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Introducing Bcl-2

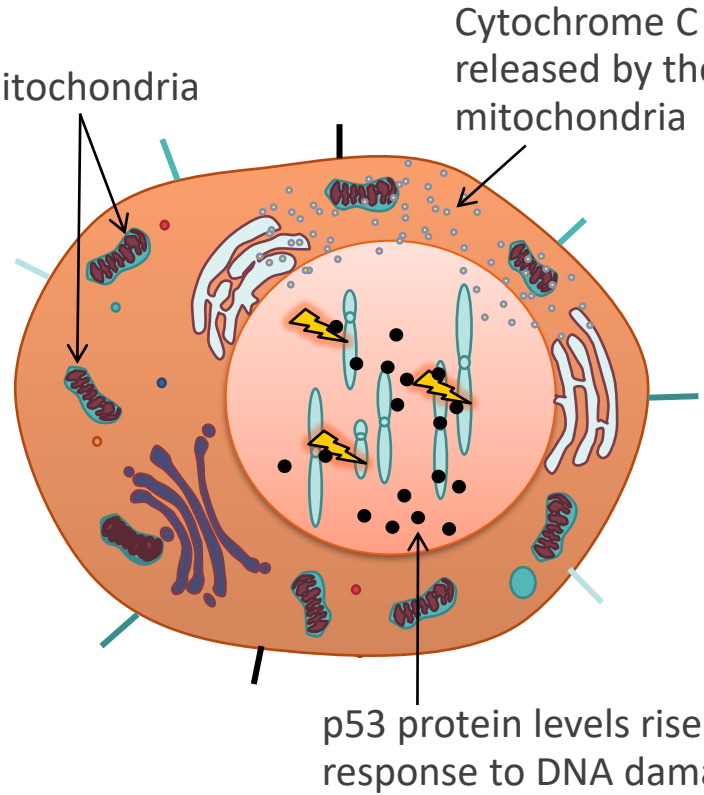
- Apoptosis is a tightly controlled, efficient, and mess-free way for cells to deliberately die
- For example, in the developing embryo many cells die by apoptosis to remove webbing between fingers and toes
- In the adult body, stressed, damaged and faulty cells also die by apoptosis and this protects us from cancer
- The Bcl-2 protein is a powerful survival protein that prevents apoptosis; it is often over-produced by cancer cells

Abbreviations: Bcl-2 – B-cell lymphoma 2

Perini GF et al. *J Hematol Oncol.* 2018; 11:65

Damaged cells die by apoptosis – part 1

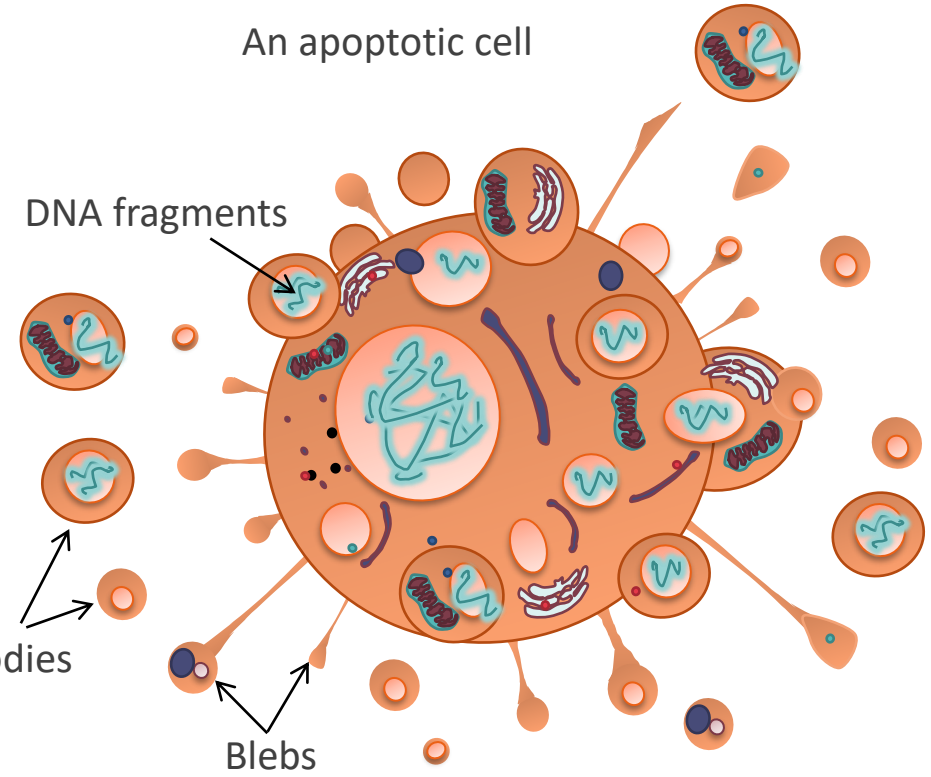
- Our cells constantly monitor their environment and check themselves for damage[1]
- A potent trigger for apoptosis is the presence of damage in a cell's DNA[1]
- This damage causes levels of a protein called p53 to increase[2]
- p53 activates DNA repair mechanisms[2]
- But, if the damage can't be repaired, p53 will cause the cell's mitochondria to release their stores of a small protein called cytochrome C[2]



[1] McIlwain DR et al. *Cold Spring Harb Perspect Biol.* 2013;5:a008656
[2] Aubrey BJ et al. *Cell Death Diff*. 2018; 25: 104-113

Damaged cells die by apoptosis – part 2

- Cytochrome C activates caspase enzymes[1]
- Caspases chop up the cell's proteins and indirectly cause the cell's DNA to fragment[2]
- The cell places these protein and DNA fragments into compartments that 'bleb' from the cell surface
- 'Apoptotic bodies' are efficiently engulfed and recycled by neighbouring cells



[1] Aubrey BJ et al. *Cell Death Diff*. 2018; 25: 104-113
[2] McIlwain DR et al. *Cold Spring Harb Perspect Biol.* 2013;5:a008656

Apoptosis is controlled by Bcl-2 family members

Bcl-2 family member	Function	Mechanism
BAX, BAK	**Cause apoptosis**	• These proteins form pores in the mitochondrial membrane and allow the release of cytochrome C • Cytochrome C activates caspase enzymes and triggers apoptosis
Bcl-2, Mcl-1, Bcl-X_L, Bcl-w	**Block apoptosis**	• These proteins interfere with BAX and BAK and prevent the release of cytochrome C
BIM, BID, PUMA, BAD, NOXA, BMF, BIK,HRK	**Cause apoptosis**	• They are referred to as '**BH3-only**' proteins • They are activated by p53 in response to DNA damage and activate BAX and BAK • They also inhibit Bcl-2, Mcl-1, Bcl-X_L, and Bcl-w

Abbreviations: Bcl-2 – B-cell lymphoma 2; Mcl-1 – myeloid cell leukaemia 1; BH3 – Bcl-2 homology

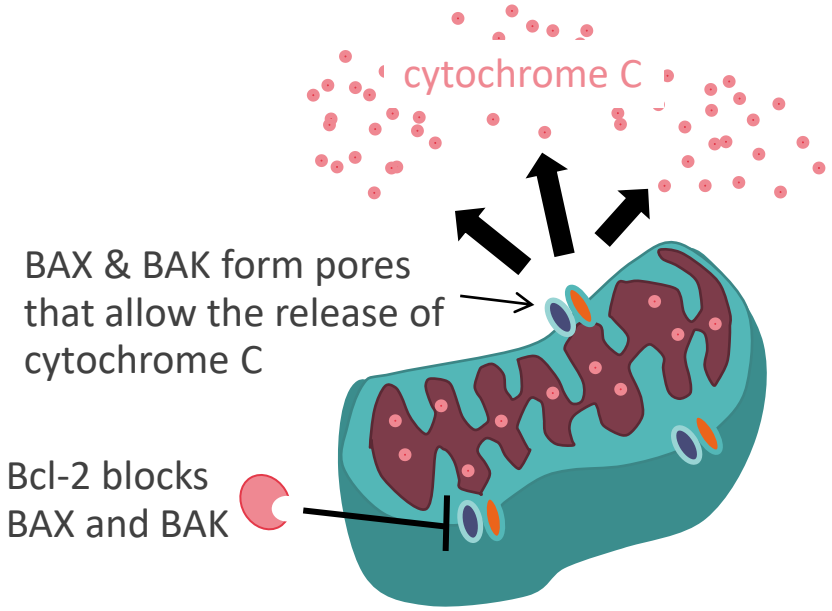
Perini GF et al. *J Hematol Oncol*. 2018; 11(1): 65
Aubrey BJ et al. *Cell Death Diff*. 2018; 25: 104-113

The role of Bcl-2 in B cell cancers

Abbreviations: Bcl-2 – B-cell lymphoma 2

Bcl-2 protects cells from apoptosis

- Bcl-2 (like BAX and BAK) is found in the outer membrane of the cell's mitochondria
- Bcl-2 protects cells from apoptosis by preventing BAX and BAK from forming pores that would allow the release of cytochrome C from mitochondria
- The cancer cells of various leukaemias and lymphomas commonly over-produce Bcl-2 and this protects them from apoptosis
- Without Bcl-2, the DNA damage found in cancer cells would trigger apoptosis



Abbreviations: Bcl-2 – B-cell lymphoma 2

Perini GF et al. *J Hematol Oncol*. 2018; 11(1): 65

Levels of Bcl-2 in a range of B cell cancers

Cancer	Levels of Bcl-2 protein
chronic lymphocytic leukaemia	Cancer cells produce high levels of Bcl-2
B-cell non-Hodgkin lymphoma:	
Follicular lymphoma	A chromosome translocation between chromosomes 14 and 18 [t(12;18)] causes over-production of Bcl-2 in most cases
Diffuse large B cell lymphoma	Bcl-2 over-production is common, particularly in the ABC (activated B cell-like) subtype
Mantle cell lymphoma	Cancer cells produce high levels of Bcl-2
Waldenström's macroglobulinaemia	Cancer cells produce high levels of Bcl-2
Myeloma	Bcl-2 is overproduced by myelomas that contain the t(11;14) translocation

Abbreviations: Bcl-2 – B-cell lymphoma 2

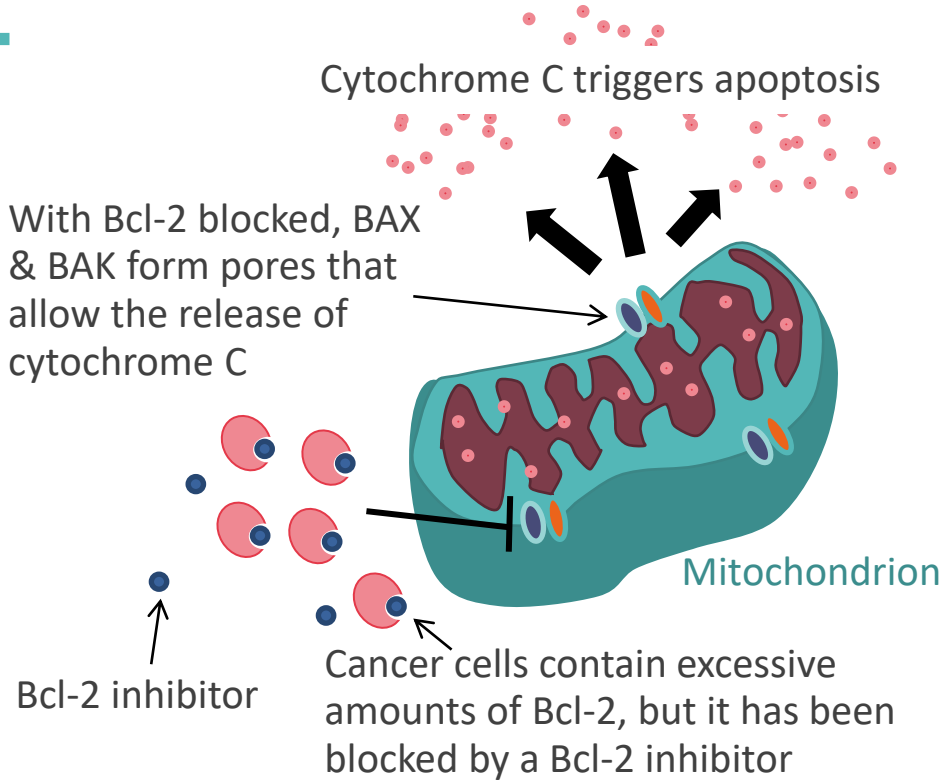
Perini GF et al. *J Hematol Oncol.* 2018; 11:65
Tomas-Roca et al. *Hemato*. 2021; 2: 281-304

Treatments that block Bcl-2

Abbreviations: Bcl-2 – B-cell lymphoma 2

Mechanism of Bcl-2 inhibitors

- ‘BH3-only’ proteins are natural proteins in our cells that disable Bcl-2 and allow apoptosis to take place
- Bcl-2 is not easy to block, but scientists have created drugs that mimic BH3-only proteins
- Early Bcl-2 inhibitors blocked Bcl-2, BCL-X_L and Mcl-1, but they were abandoned due to their poor absorption or excessive toxicity to platelets
- Newer Bcl-2 inhibitors selectively inhibit Bcl-2 and avoid toxicity to platelets



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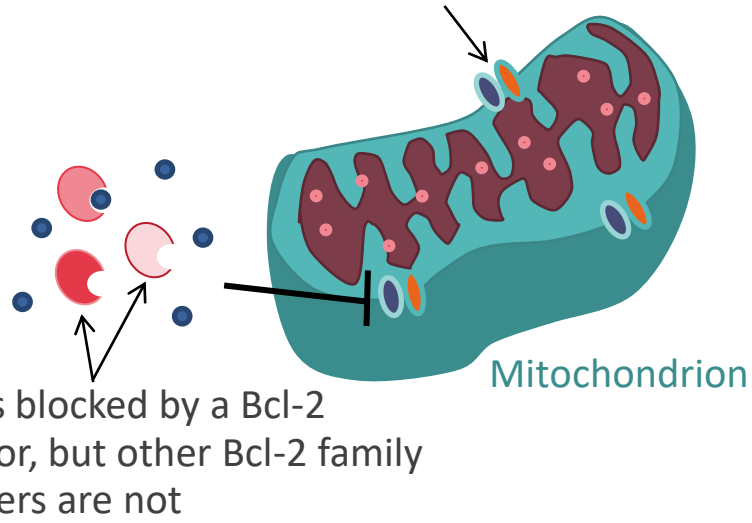
Perini GF et al. *J Hematol Oncol*. 2018; 11(1): 65

Sensitivity and resistance to Bcl-2 inhibitors

- Bcl-2 inhibitors are effective against CLL even when the disease contains aggressive features (such as loss of p53 protein) or is resistant to other treatments
- Bcl-2 inhibitors are also effective against some other haematological cancers such as multiple myeloma and acute myeloid leukaemia
- Cancer cells that are resistant to Bcl-2 inhibitors often contain high levels of other pro-survival Bcl-2 family members such as BCL-X_L and Mcl-1
- Other resistance mechanisms include mutation of the *BCL2* gene



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Perini GF et al. *J Hematol Oncol*. 2018; 11(1): 65
Yue XY et al. *Cancer Cell Int.* 2020; 20:524

A summary of treatments that target Bcl-2:

- Bcl-2 is a powerful protein that can block apoptosis
- In healthy cells DNA damage causes the activation of BH3-only proteins that block Bcl-2 and cause cell death
- Cancer cells that contain high levels of Bcl-2 are using it to protect them from the consequences of DNA damage
- Bcl-2 inhibitors remove cancer cells' protection from death; they are effective against cancers that contain high levels of Bcl-2, such as CLL

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