4th POSTGRADUATE

CLL Conference

Bologna
November 13-14
2023

Royal Hotel Carlton

President:
Pier Luigi Zinzani

Development of resistance to BCL2 inhibition

Paolo Sportoletti

Hematology Section
University of Perugia, Italy

STUDIUM GENERALE CIVITATIS PERUSII A.D. MCCCVIII

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Abbvie					x	x	
Beigene						x	
AstraZeneca						x	

Overview

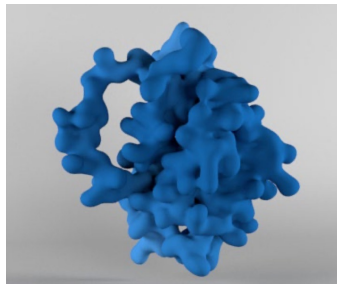
- BCL2 proteins and apoptosis

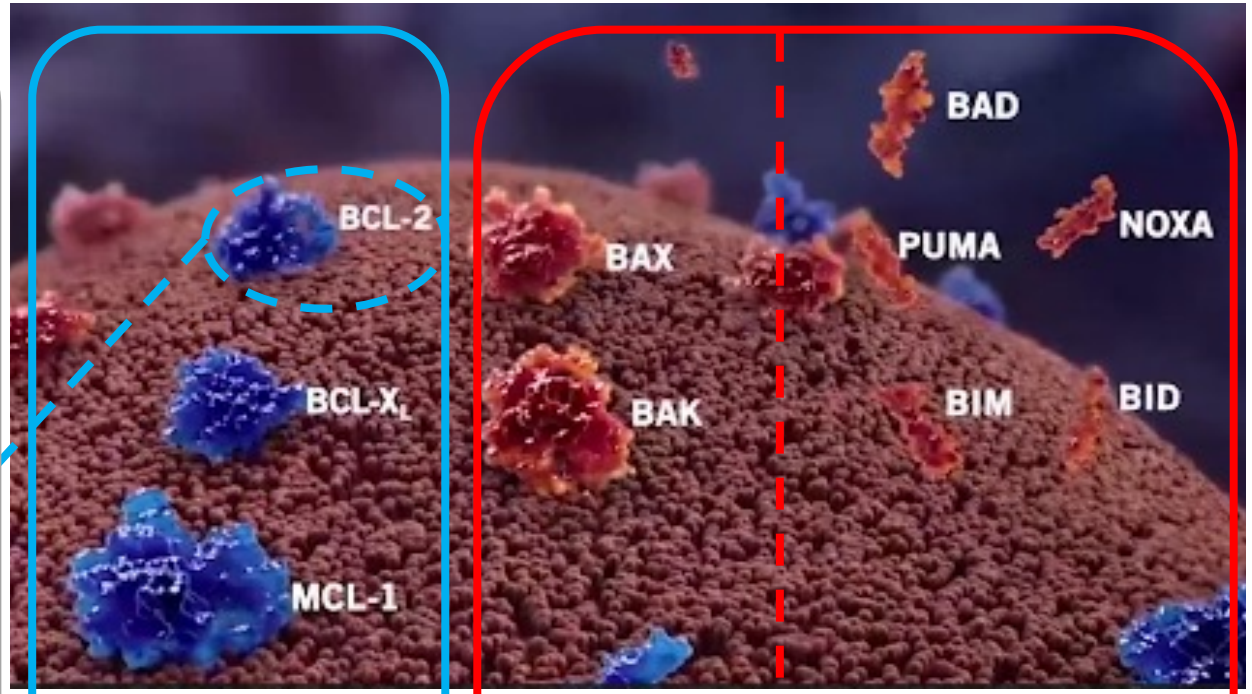
- BCL2 resistance mechanisms

- Strategies to prevent resistance

BCL-2 Family Proteins

Named after the first protein of this type to be described: B-cell lymphoma or leukemia gene 2 (*BCL-2*), first identified from human B-cell lymphomas[3]





Pro-Survival

Effectors | Initiators (BH3-Only)

Pro-Death

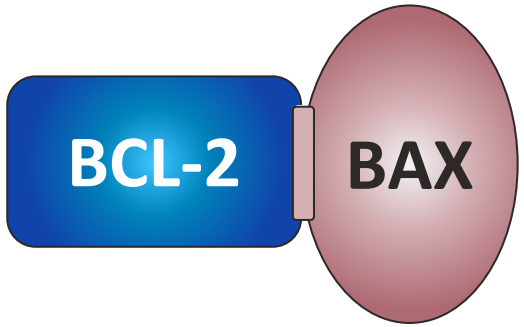
Classification is based on shared BCL-2 homology (BH) domains[1,2]

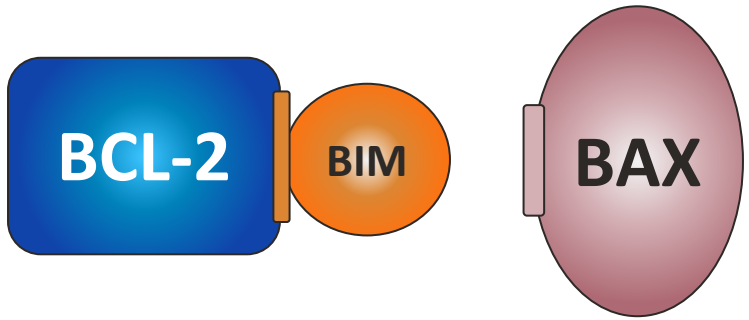
The BCL-2 apoptotic switch

Pro-Death Initiator

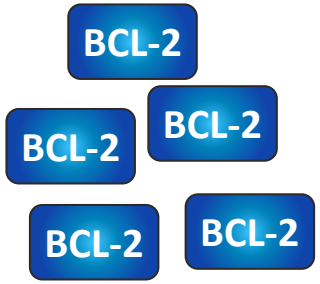
Pro-Survival

Pro-Death Effector

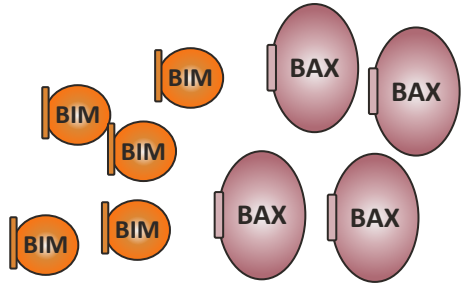


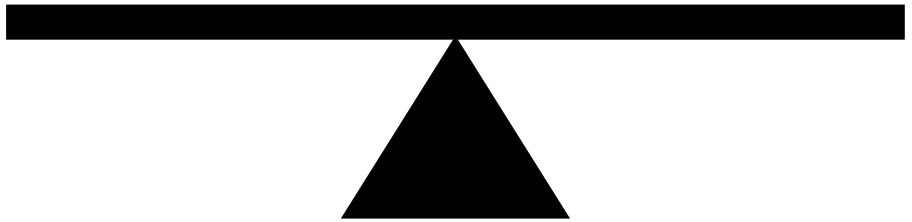


SURVIVAL



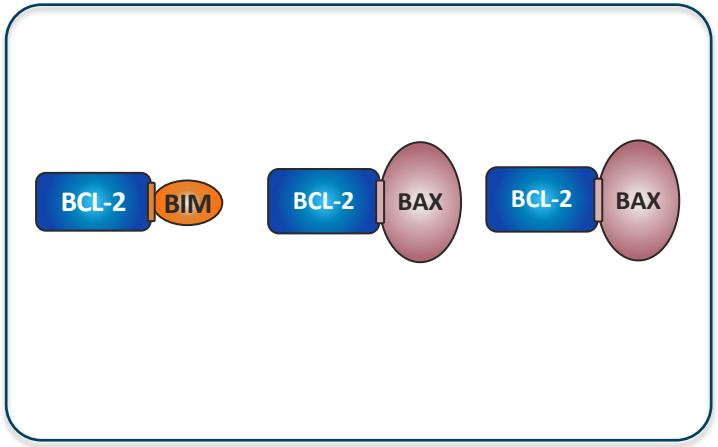
APOPTOSIS

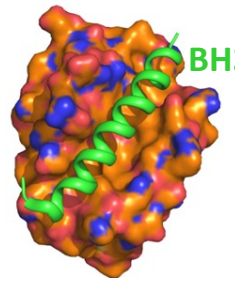




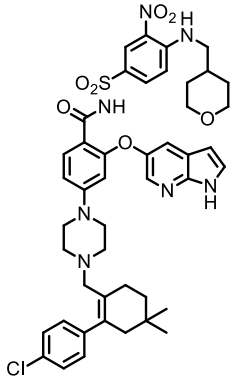
Venetoclax is a potent oral BCL-2 inhibitor

Pro-survival proteins (e.g. BCL-2) sequester pro-death proteins by binding to their BH3 motifs[1,2]

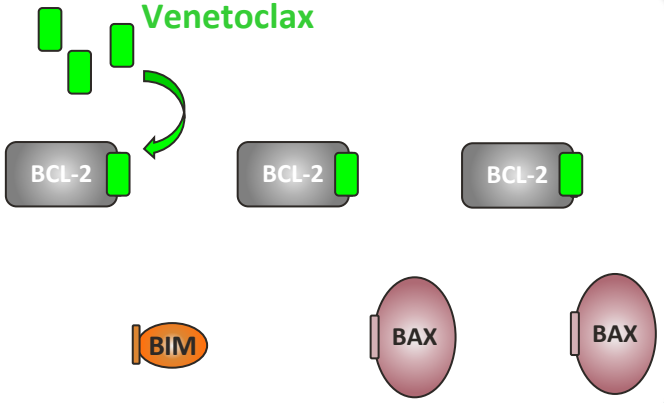


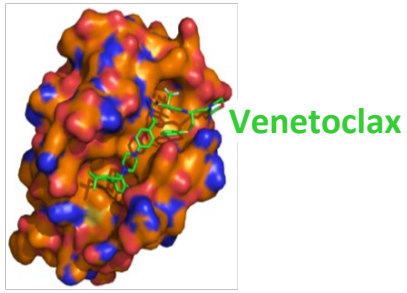


Venetoclax[3]

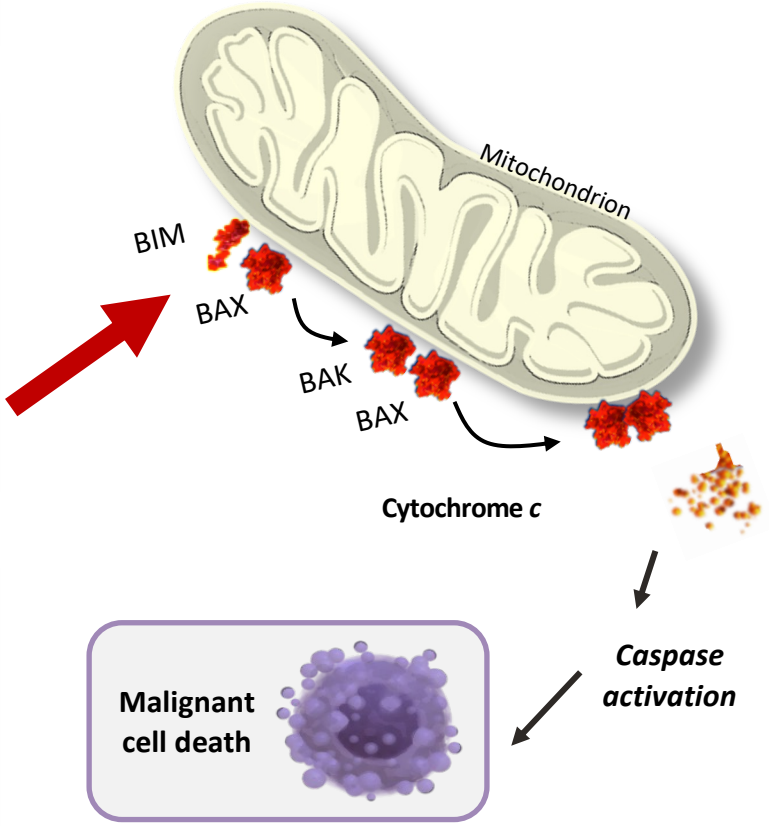


Venetoclax competes for binding to pro-survival proteins, freeing pro-death proteins[3–5]



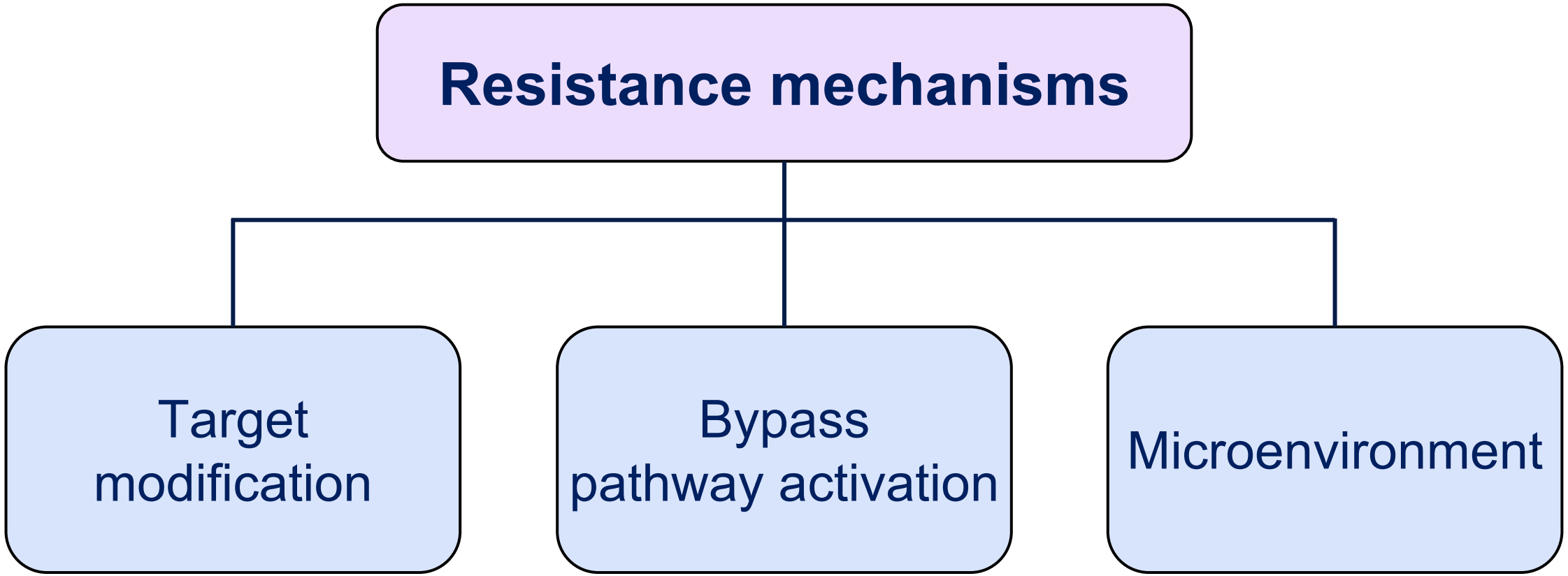


Inhibition of BCL-2 can free enough BIM and BAX to trigger apoptosis



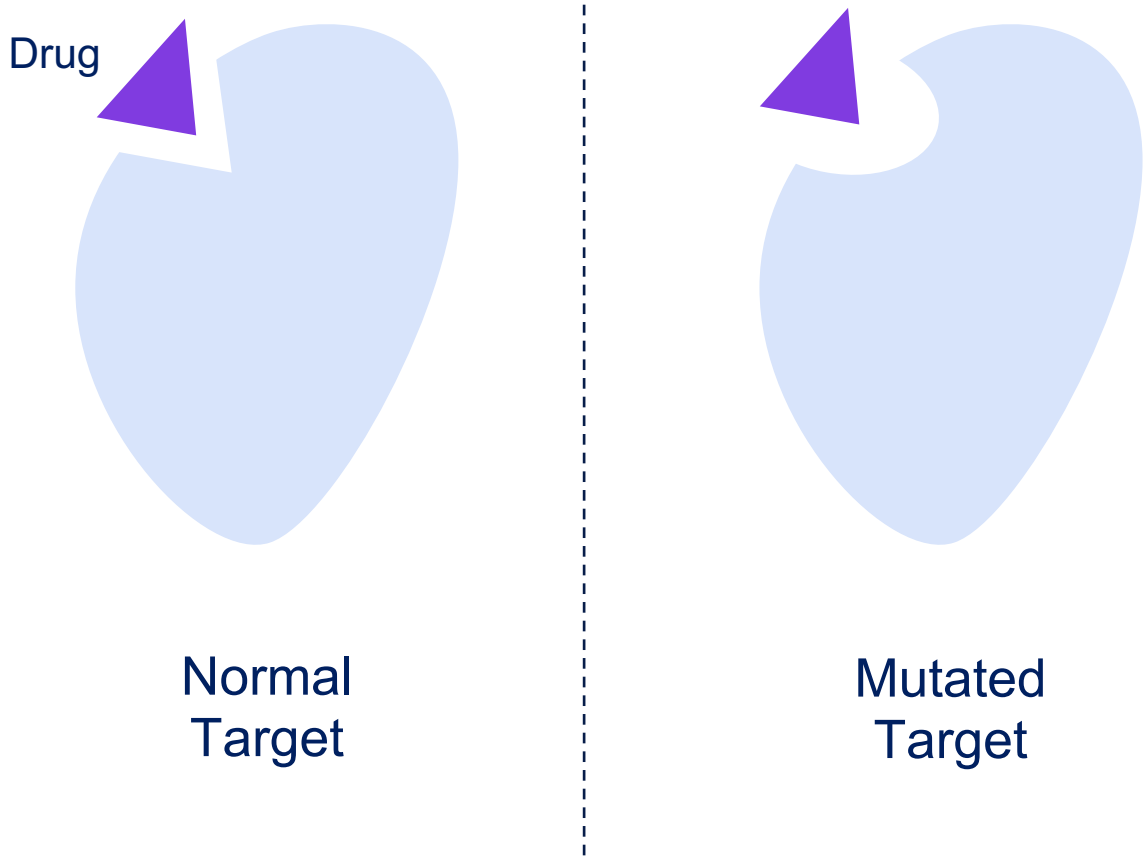
1. Plati J, *et al. Integr Biol (Camb)* 2011; **3:**279–296; 2. Czabotar PE, *et al. Nat Rev Mol Cell Biol* 2014; **15:**49–63;
3. Souers AJ, *et al. Nat Med* 2013; **19:**202–208 (incl. suppl.); 4. Oltersdorf T, *et al. Nature* 2005; **435:**677–681; 5. Tse C, *et al. Cancer Res* 2008; **68:**3421–3428.

Mechanisms of acquired resistance to targeted therapies in CLL



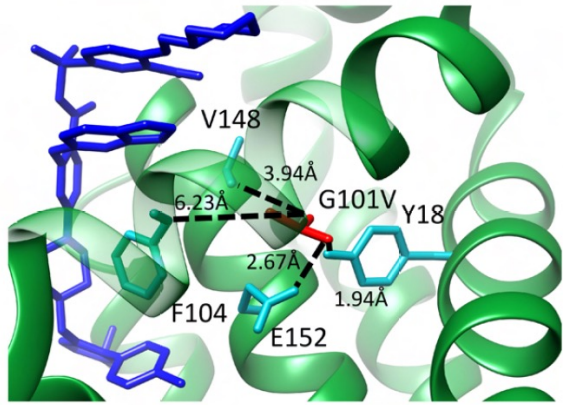
Skånland and Mato, *Blood Adv*, 2021, DOI: 10.1182/bloodadvances.2020003423

Target Modification

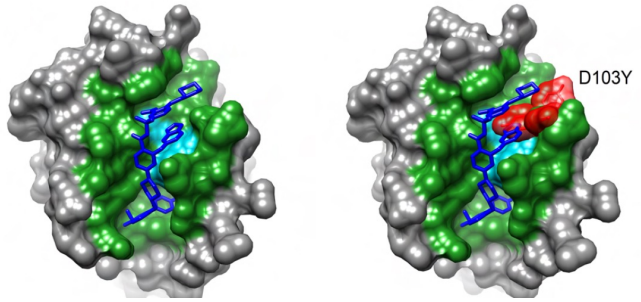


Venetoclax resistance is associated with mutations in the BCL-2 binding pocket

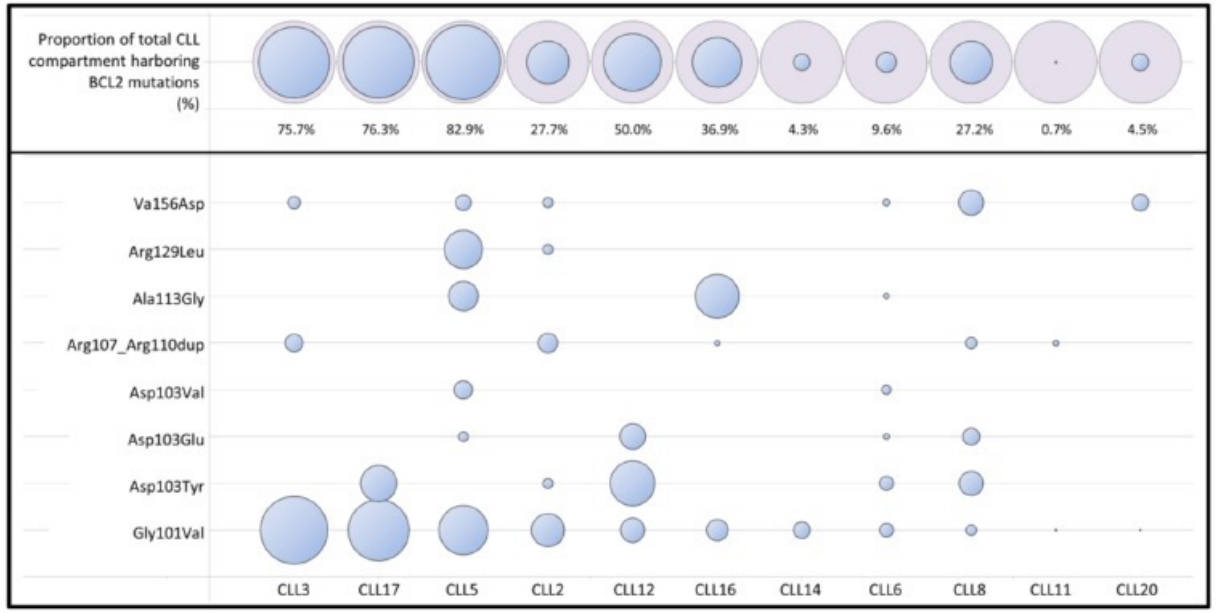
- Acquired mutations target the BH3-binding domain of BCL-2, of which G101V is the most frequent alteration [1,2]



- p.D103Y, a second mutation in *BCL2*, has also been reported in some patients.[2]



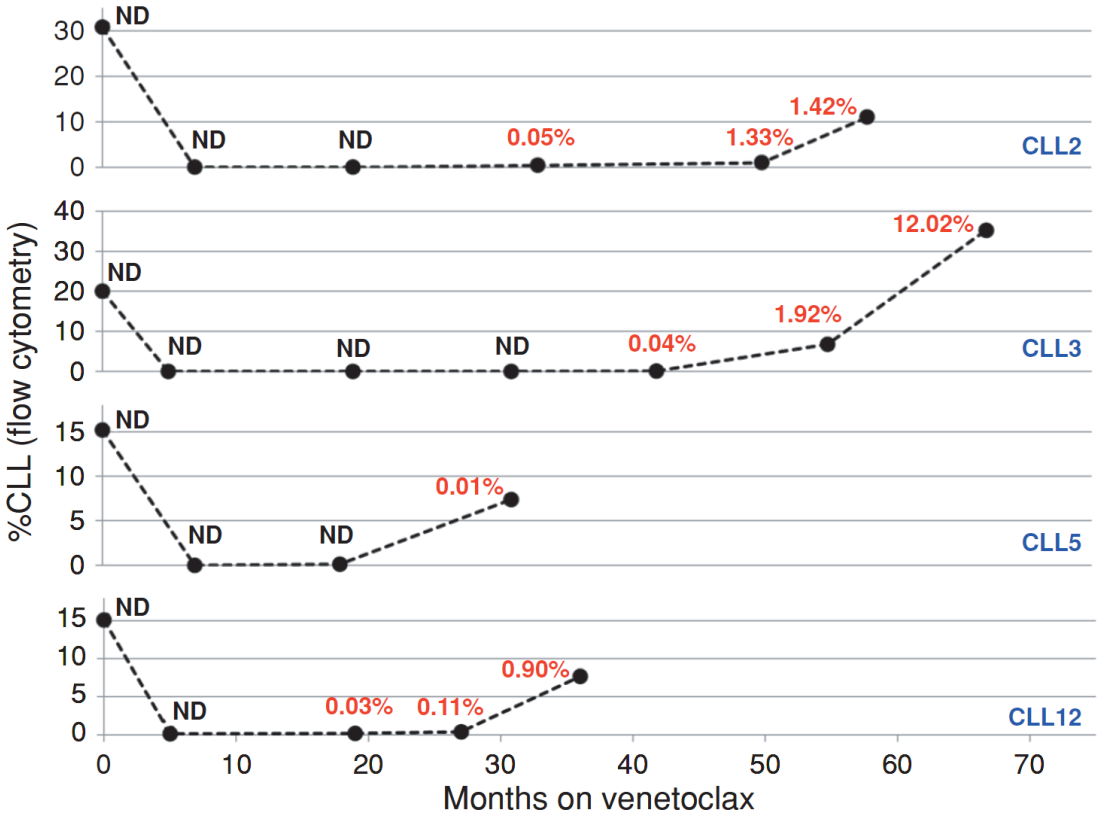
- Multiple sites, multiple clones



- *BCL2* mutations almost exclusively occur in the context of venetoclax exposure [2,3] (not detectable prior to treatment)

1. Blombery P et al.; Cancer Disov 2019; 9:342-53.;
2. Tausch E et al.; Haematologica 2019; 104(9):e434-e437
3. 3Herling CD et al.; Nat Commun 2018; 9:727.

Time course of emergence of p.G101V during Venetoclax therapy in R/R CLL

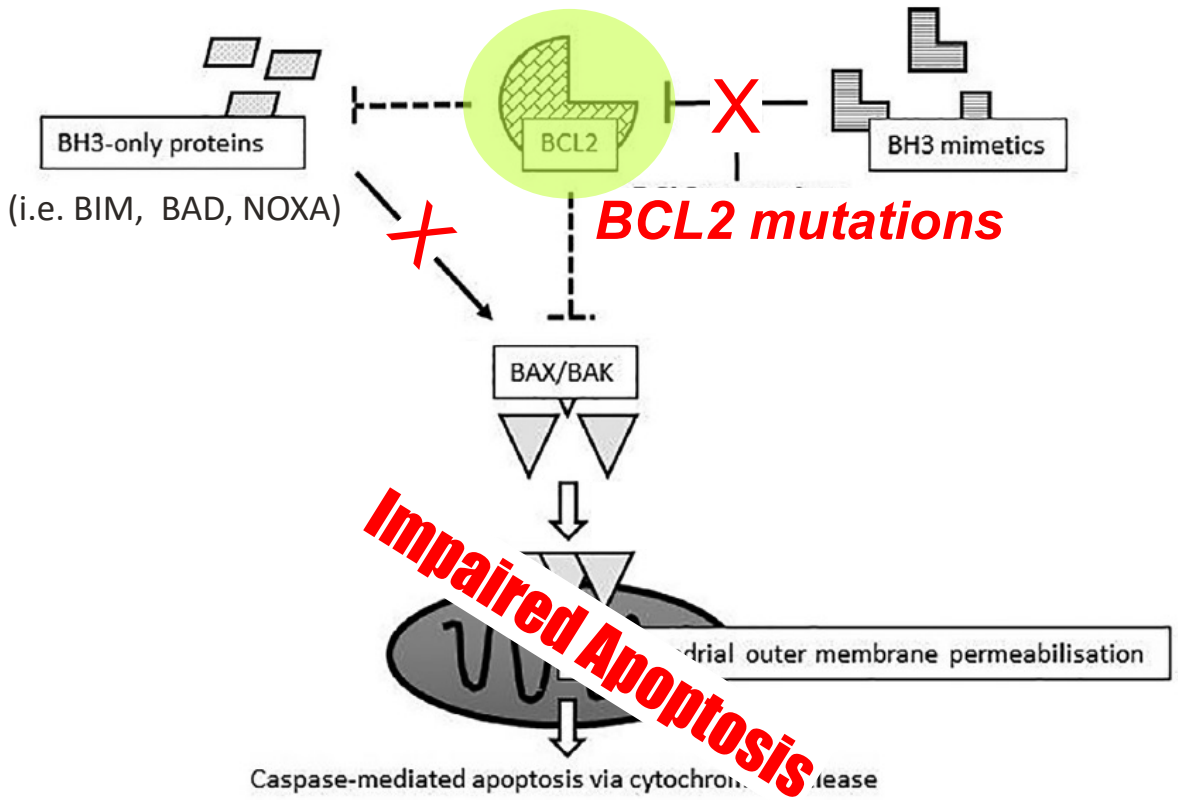


- p.G101V mutations occurred in 4/15 ***(27%)*** patients with R/R CLL on venetoclax monotherapy[1]
- Mutations detected after ***>19 months*** of therapy[1]
- ***Up to 25 months*** passed between first onset of the mutation and clinical progression

* CLL burden was measured by multiparameter flow cytometry in serial BM aspirates from 4 patients from the initiation of venetoclax until the clinical diagnosis of progressive disease. The VAF of *BCL2* p.G101V in BM samples measured by droplet digital PCR is overlaid. *BCL2* p.G101V VAF is indicated in red. BM, bone marrow; ND, not detected; PCR, polymerase chain reaction; VAF, variant allele frequency.

1. Blombery P, *et al. Cancer Disov* 2019; **9:**342–353;
2. Tausch E, *et al. Haematologica* 2019; **104:**e434–e437.

BCL2 mutations prevent binding of BH3 mimetics (Venetoclax)



Mutated BCL2 maintain prosurvival functions

⇩

Acquired **BCL2 mutations** affecting the protein binding groove impair usual binding of BH3 BCL2i, re-establishing anti-apoptotic activity as depicted by the dotted lines.

⇧

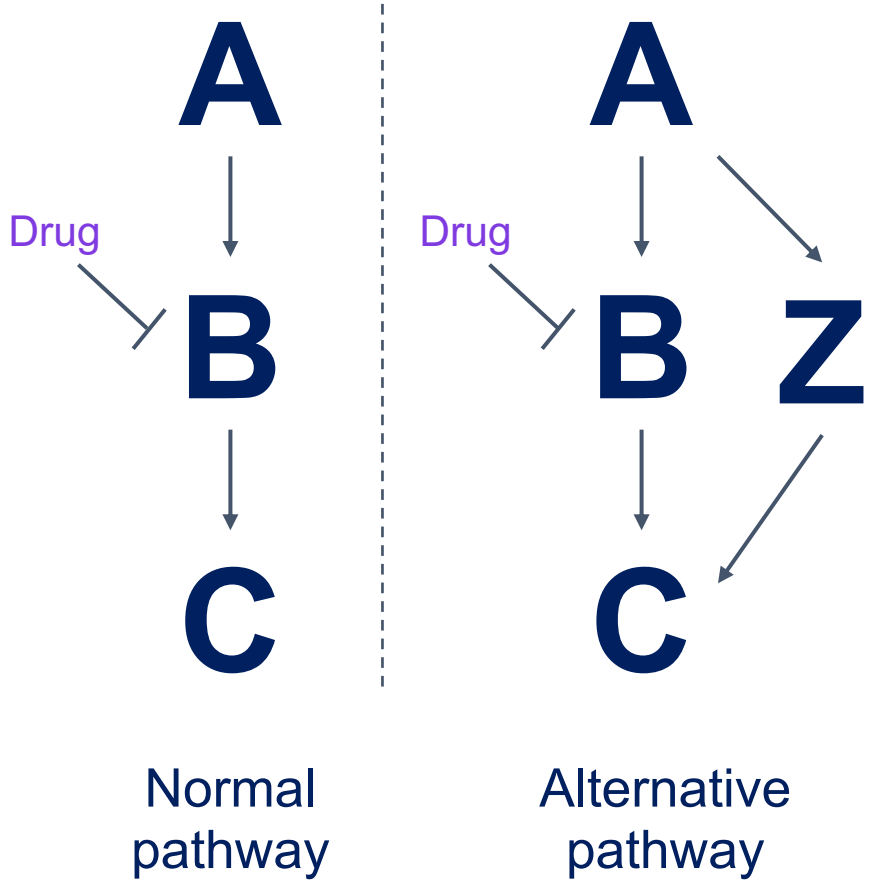
Lethal Concentration 50% increased 15–450×

Binding affinity reduced ~180×

Bennett et al., *Clinical Lymphoma, Myeloma and Leukemia*, 2022; DOI: 10.1016/j.clml.2022.07.013

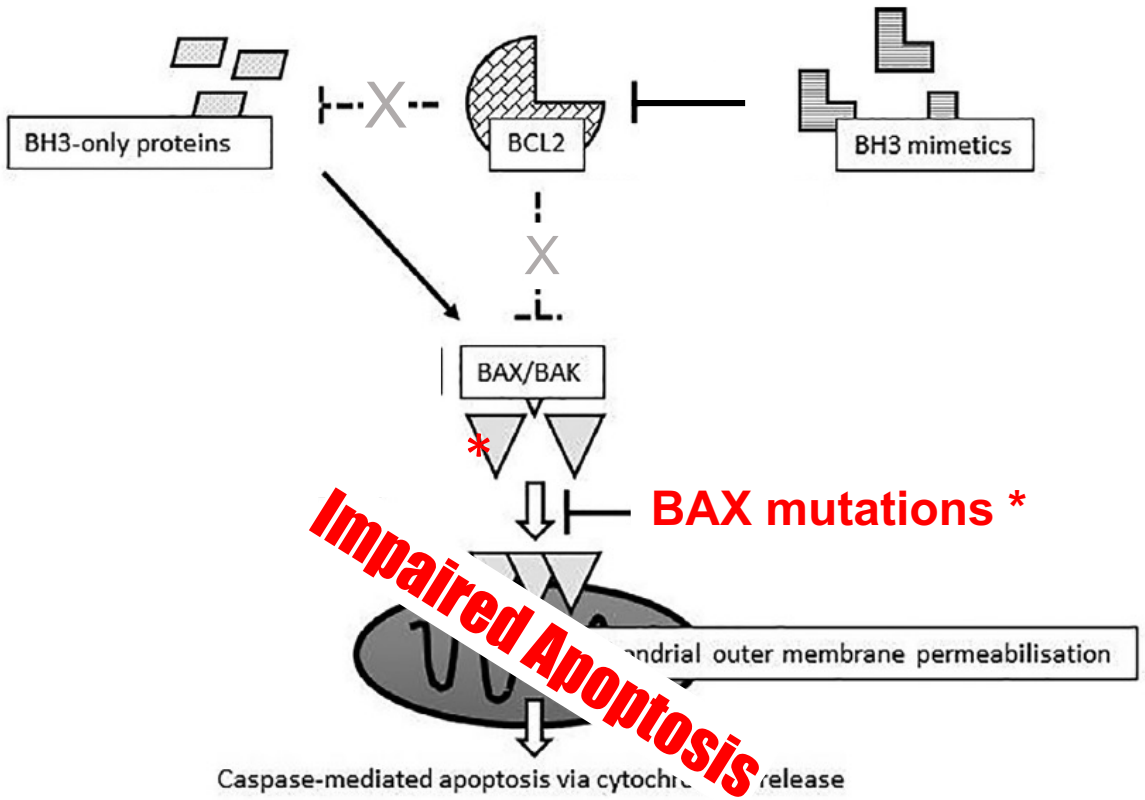
Blombery P, *et al. Cancer Disov* 2019; **9:**342–353; Tausch E, *et al. Haematologica* 2019; **104:**e434–e437.

Bypass pathway activation



Skånland and Mato, *Blood Adv*, 2021, DOI: 10.1182/bloodadvances.2020003423

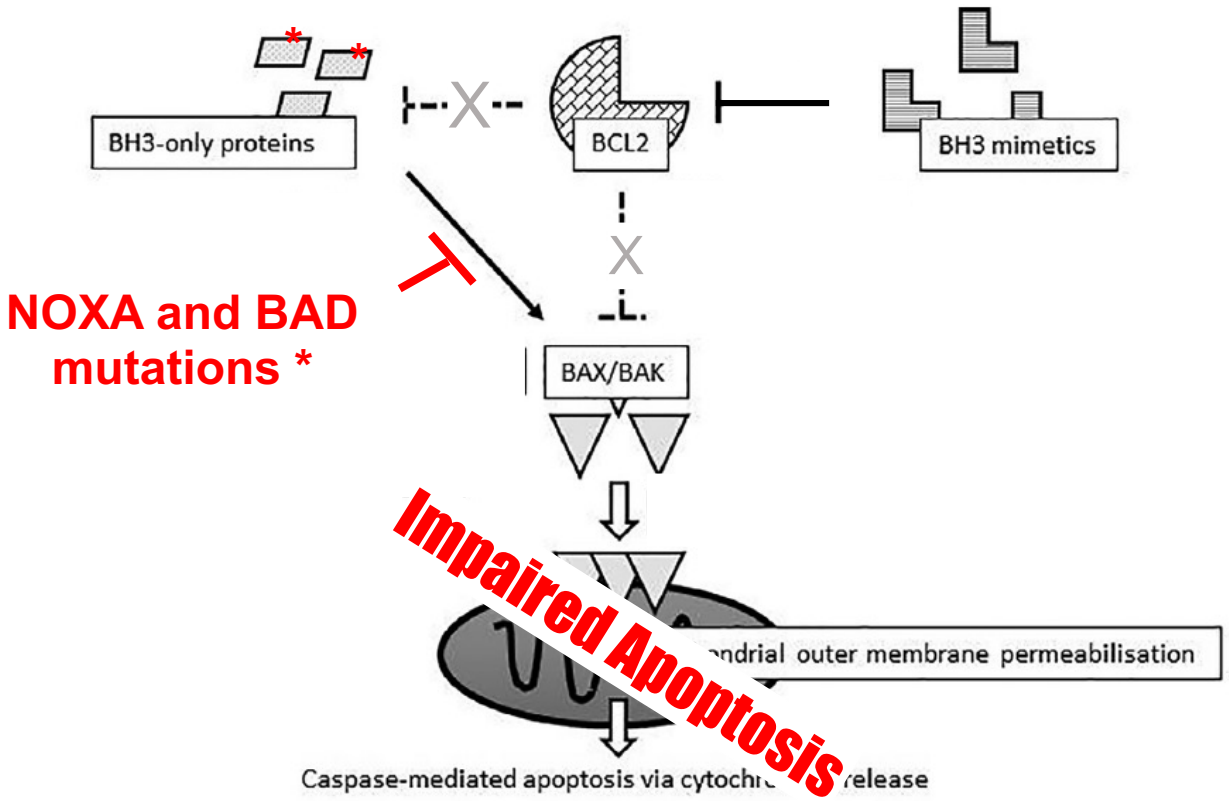
BAX mutations impair oligomerization and apoptosis



BAX mutations are thought to impair BAX oligomerization and hence mitochondrial membrane pore formation.

Bennett et al., *Clinical Lymphoma, Myeloma and Leukemia*, 2022; DOI: 10.1016/j.clml.2022.07.013

Mutations NOXA and BAD have also been reported at the time of CLL relapse

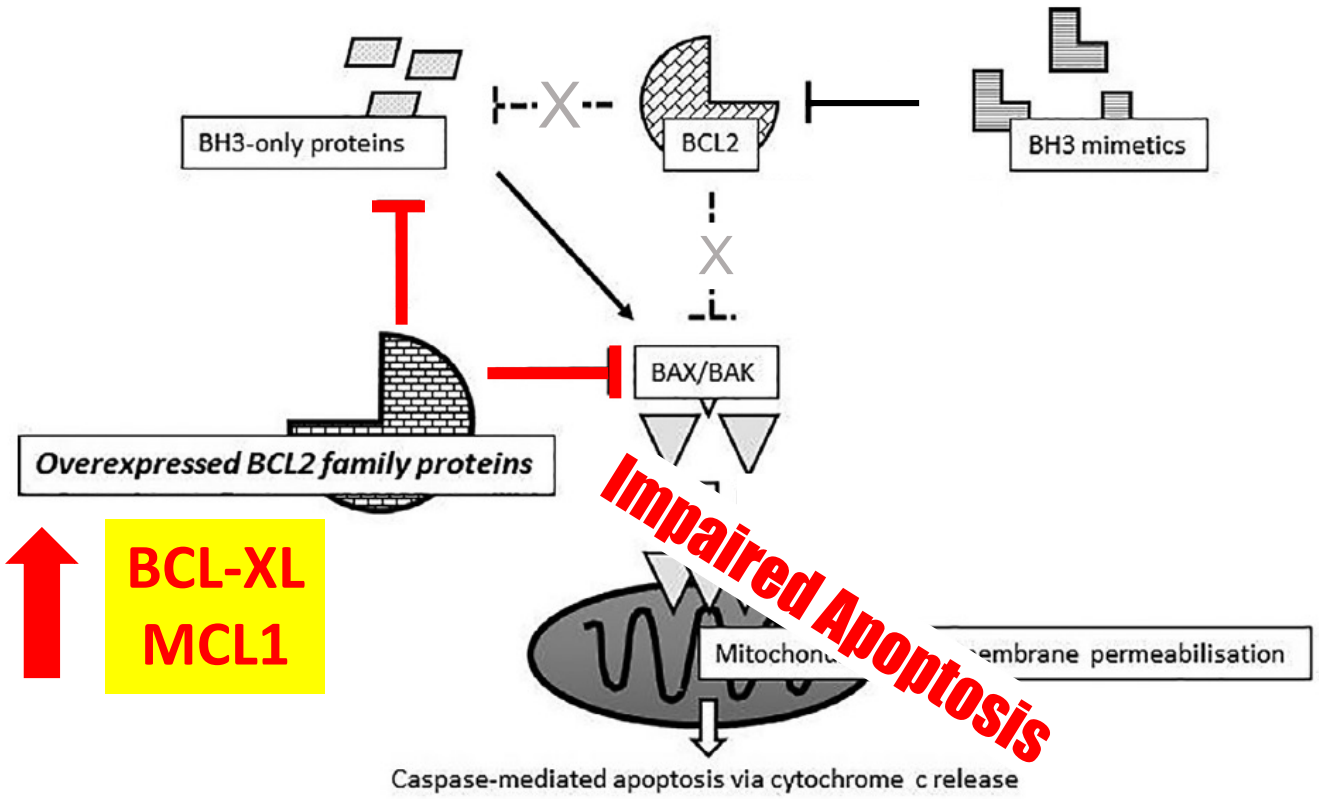


NOXA and *BAD* are BCL-2 family members that can initiate death

Mutated NOXA and BAD do not modulate the activation of the pro-deaths effector proteins BAX and BAK

Chyla BJ, *et al.* ASH 2019; Abstract 172.

Overexpression of pro-survival BCL-XL and MCL1 in BCL2i resistance



BCL-XL and MCL1 are pro-survival proteins of the BCL2 family

Overexpression of BCL-XL and MCL1 lead to increased sequestration of BH3-only initiators (BIM, BAD, NOXA) and effector proteins (BAX/BAK)

Bennett et al., *Clinical Lymphoma, Myeloma and Leukemia*, 2022; DOI: 10.1016/j.clml.2022.07.013

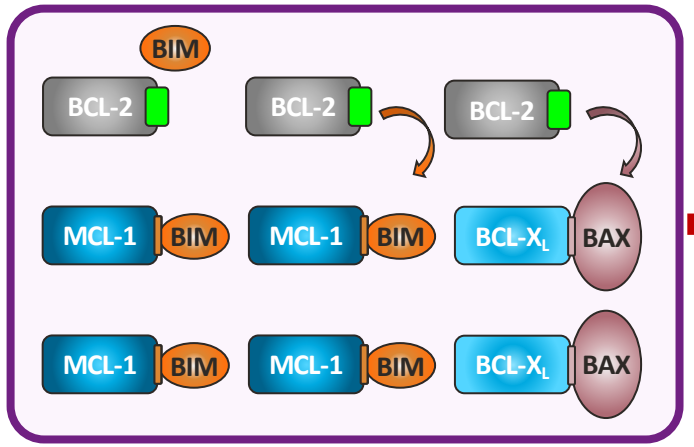
High affinity of venetoclax for BCL-2 but not for BCL-X_L or MCL-1: pros and cons

Venetoclax binding affinity by TR-FRET

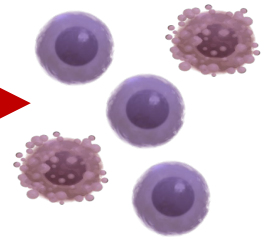
Protein	K_i (nM)
BCL-2	**<0.0100**
BCL-X_L	48
MCL-1	>444
BCL-W	245

Minimizes potential off-target effects

Inhibition of BCL-2 may not free enough BIM and BAX to trigger apoptosis in all malignant cells



Malignant cell survival

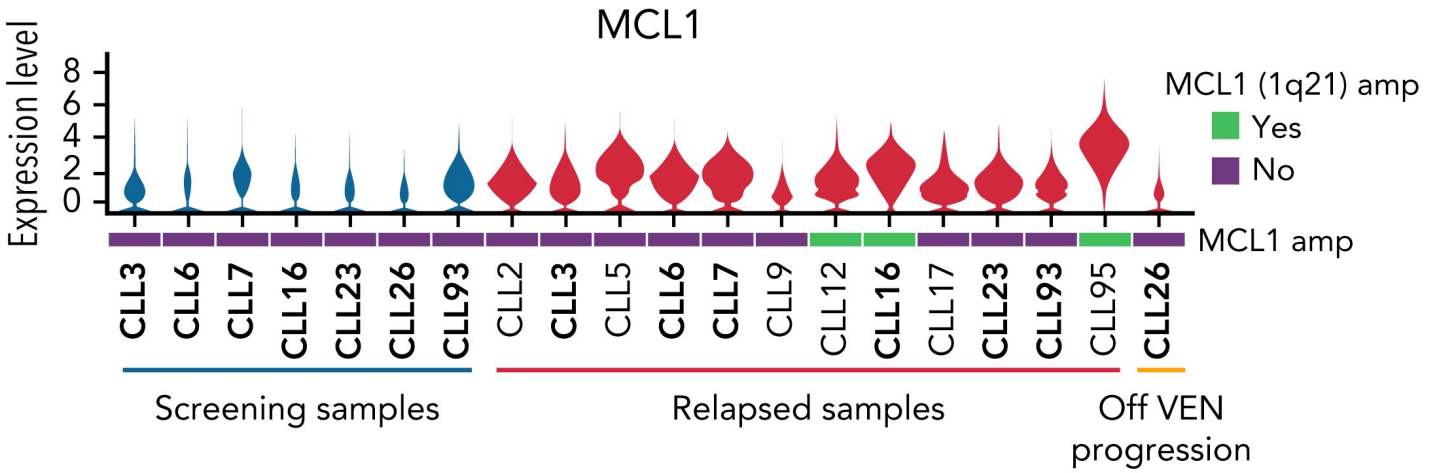


Drug resistance potential

EC_{50}, half-maximal effective concentration; K_i, inhibition constant; TR-FRET, time-resolved fluorescence energy transfer.

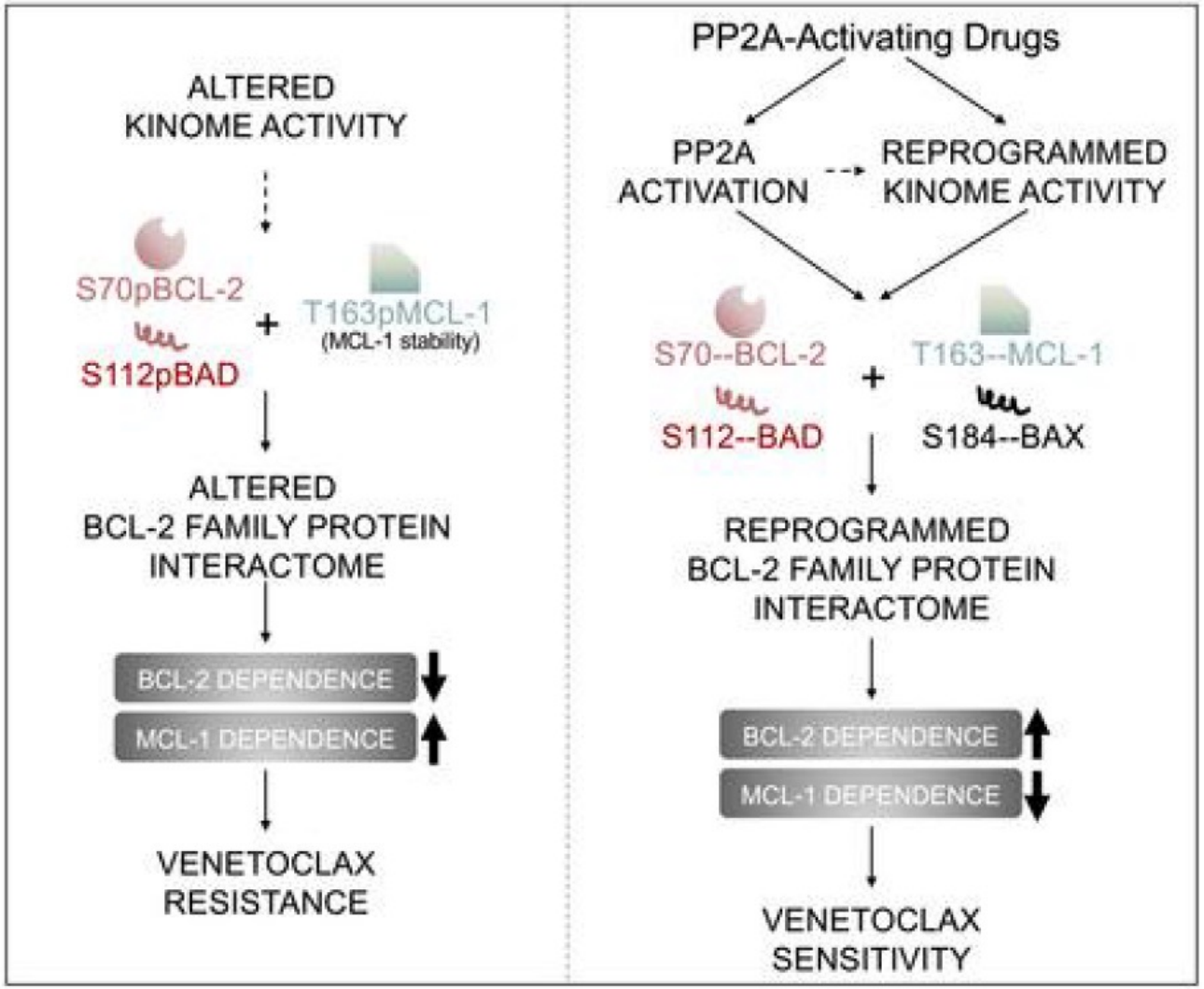
Souers AJ, *et al. Nat Med* 2013; **19:**202–208 (incl. suppl.).
Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393.

Increased *MCL1* expression at VEN relapse is partially explained by amplification of the *MCL1* gene



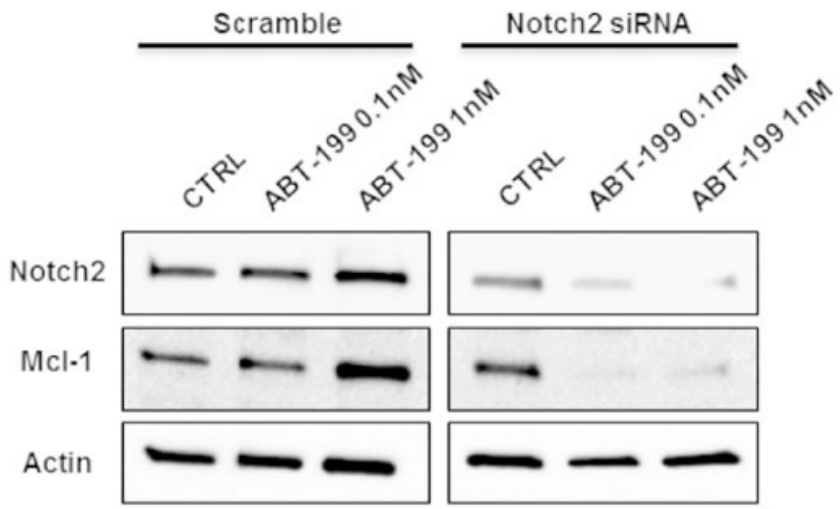
Thijssen R et al Blood 2022

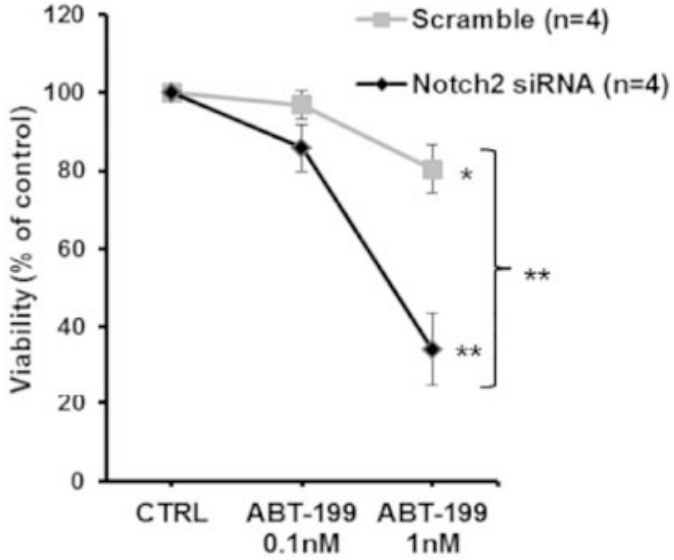
Hyperphosphorylation of BCL-2 family proteins (BCL-2, MCL-1, BAD and BAX) drives venetoclax resistance in CLL



Chong et al., *J Clin Invest*, 2023

High levels of Mcl-1 and Notch2 reduced response to venetoclax in CLL cells



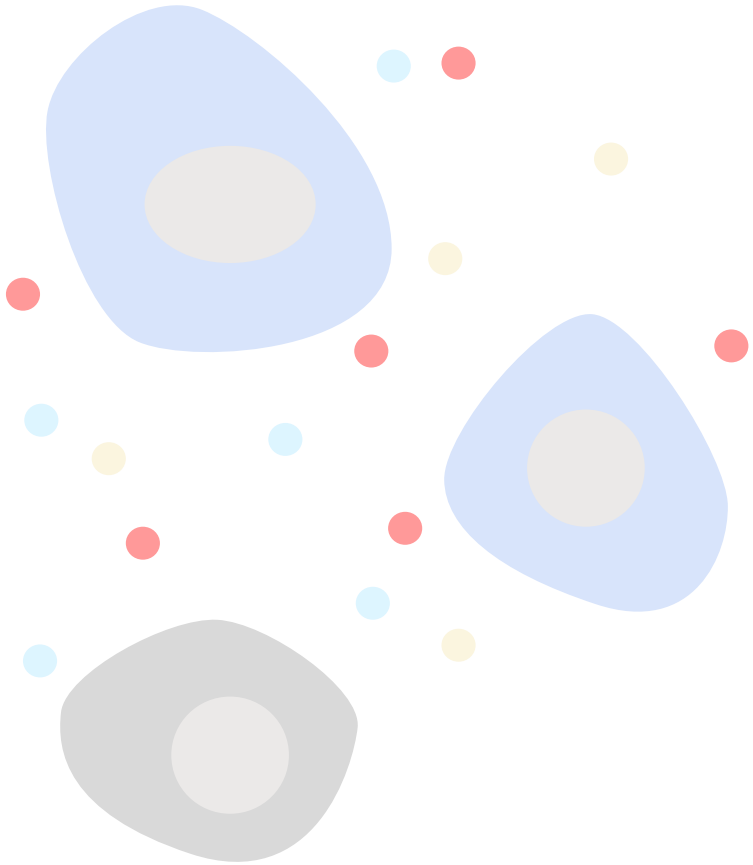


Tumor microenvironment may determine an additional support in maintaining the expression of Notch2 and Mcl-1, especially at the lymph node level.

This is in line with emerging clinical data that suggest an involvement of lymph node niches to induce resistance to proapoptotic treatments

Fiorcari S et al. Front Oncol 2022

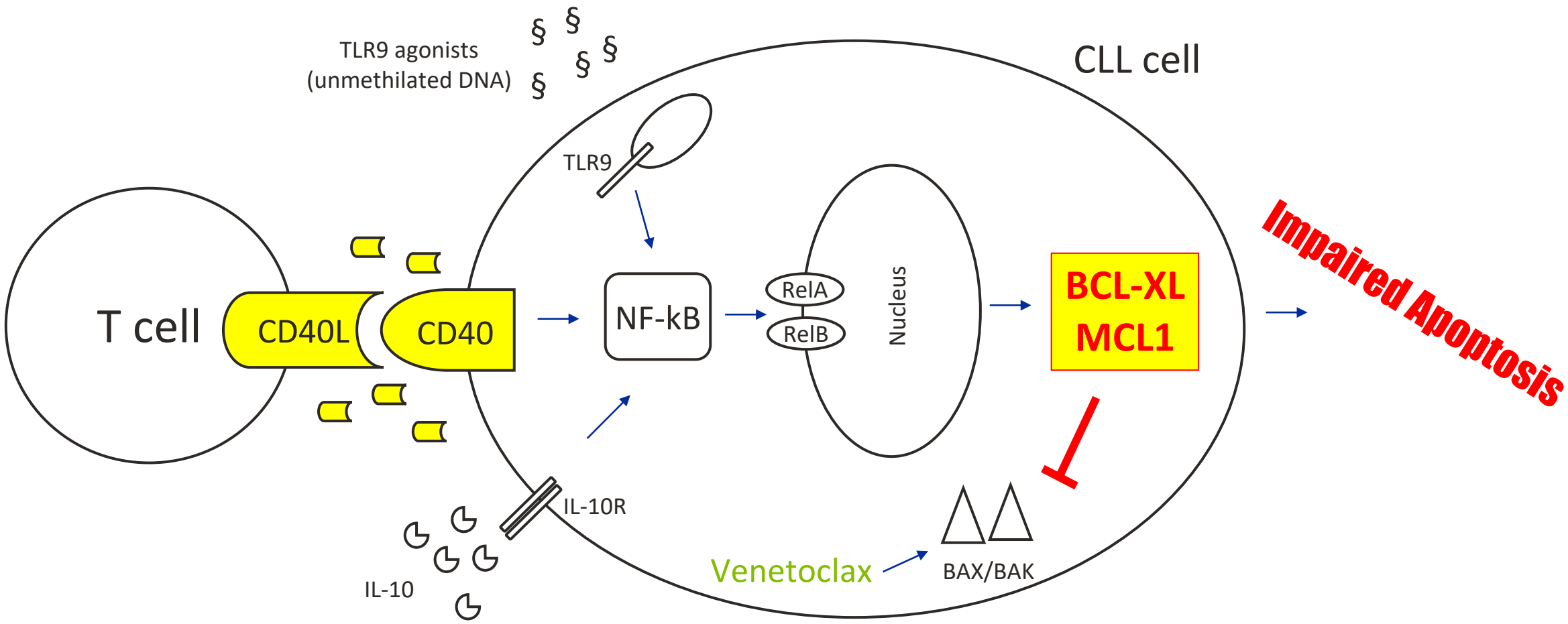
Microenvironment



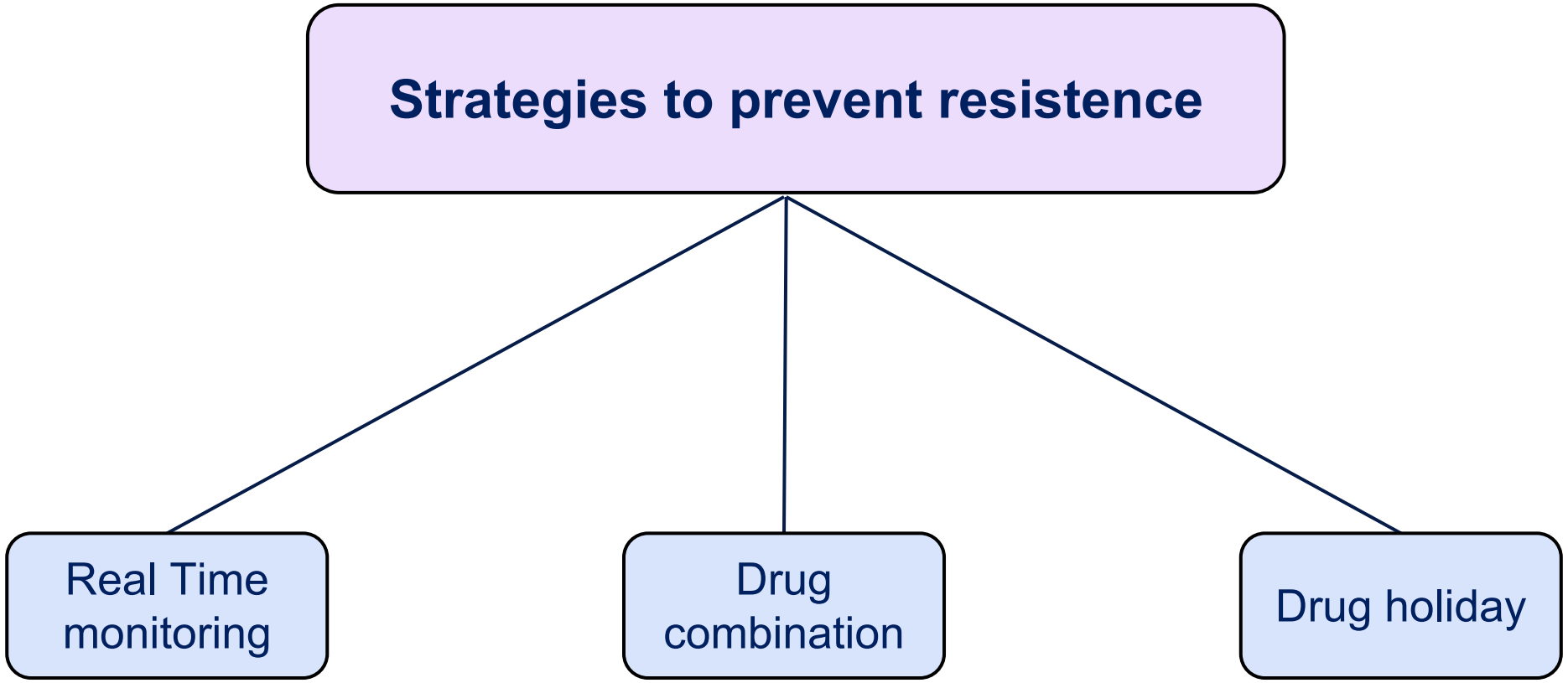
The **CLL microenvironment** within the lymph node, spleen, and bone marrow promotes **cell survival and proliferation**, as well as escape from spontaneous and drug-induced apoptosis.

Skånland and Mato, *Blood Adv*, 2021, DOI: 10.1182/bloodadvances.2020003423

Extrinsic microenvironmental agonists generate ex vivo resistance to venetoclax in CLL

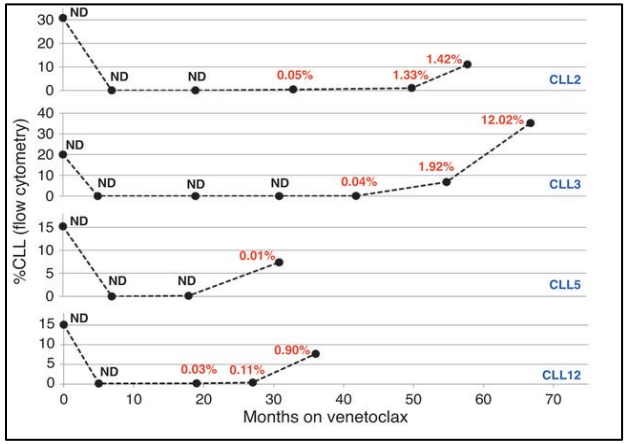


Thijssen R et al Haematologica 2015
Jayappa KD et al. Blood Adv 2017



Skånland and Mato, *Blood Adv*, 2021, DOI: 10.1182/bloodadvances.2020003423

Real Time monitoring



Venetoclax
BCL2 Gly101Val mutations
around 50%

Could be **advantageous** to monitor mutations

Important **not to discontinue** therapy immediately when mutations are detected

Ahn et al. Blood 2017
Daniel Mertens, & Stephan Stilgenbauer Blood 2017
Jain et al. Cancer 2017;
Woyach et al. J Clin Oncol 2017

Blombery et al. Cancer Discov 2019; 9(3):342-353

Drug combination

Combination Agents with Complementary MoA[2–5]

CLL Cell

Size of rectangles indicates relative dependency on specific protein for survival

Anti-CD20 mAb

obinutuzumab

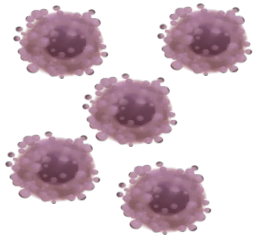
rituximab

BTKis

ibrutinib

acalabrutinib

Malignant cell death



To increase BCL-2 dependency, prevent cell proliferation, or mobilize cells away from protective lymph node niches[2–5]

1. Del Gaizo Moore V, *et al. J Clin Invest* 2007; **117:**112–121; 2. Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393; 3. Souers AJ, *et al. Nat Med* 2013; **19:**202–208; 4. Thijssen R, *et al. Haematologica* 2015; **100:**e302–306; 5. Deng J, *et al. Leukemia* 2017; **31:**2075–2084.

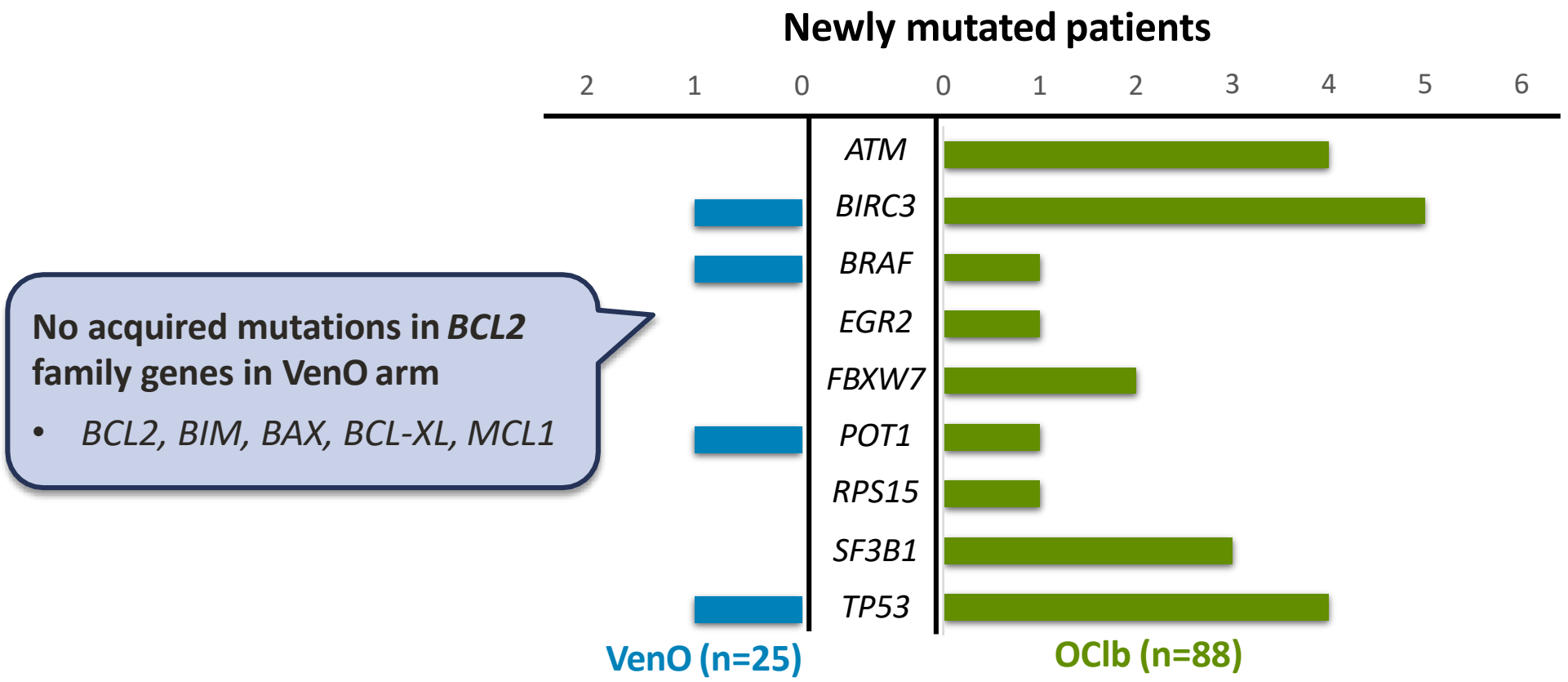
Drug holiday

- A “drug holiday” may allow re-initiation of treatment
- To allow re-initiation of treatment, the initial regimen must have a **time-limited approach:**
  - Fixed duration
  - MRD driven
- How a fixed duration approach will compare with continuous treatment is unknown, and will need to be addressed in future studies (CLL17 trial)

FTD, fixed treatment duration.

Skanland SS & Mato AR. *Blood Adv* 2021; **5:**334–343.

No acquired *BCL2* resistance mutations with fixed-duration venetoclax therapy



CLL14: Acquired mutations in previously untreated CLL patients after 12 cycles of VenO or OClb

VenO, venetoclax + obinutuzumab; OClb, obinutuzumab + chlorambucil.

Tausch E, *et al.* EHA 2021; oral presentation S144.

Summary

Dynamic interplay between pro-death and pro-survival Bcl-2 family proteins is responsible for a cell's fate

Structural mutations in BCL-2 family proteins lead to decreased affinity for venetoclax and inhibit the intrinsic apoptosis pathway.

Increased expression of the antiapoptotic proteins MCL-1 and BCL-XL plays a key role in conferring cellular resistance to venetoclax

Microenvironmental factors including nodal T-cell stimulation can influence Venetoclax resistance

Drug combination and drug holiday are valuable strategies to prevent venetoclax resistance