Resistance to BCL-2i

- When
- Why
- How to overcome resistance


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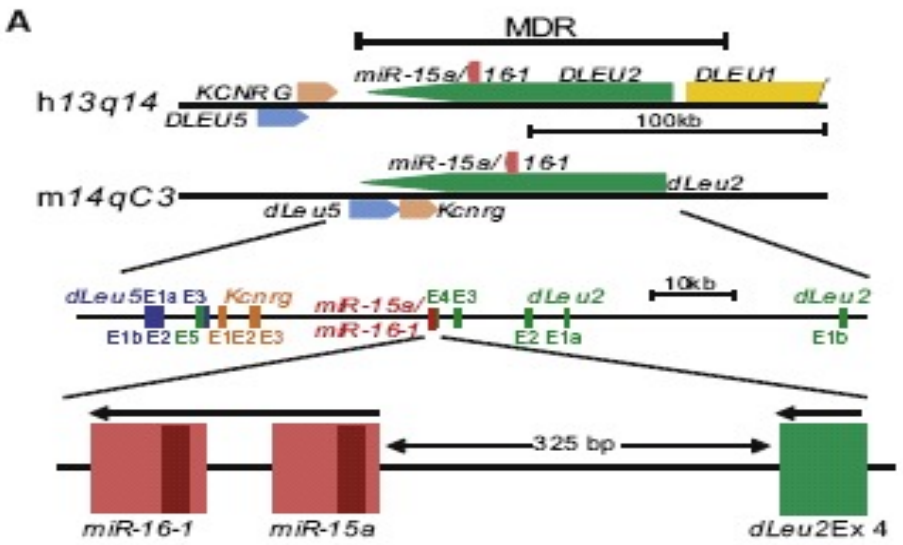
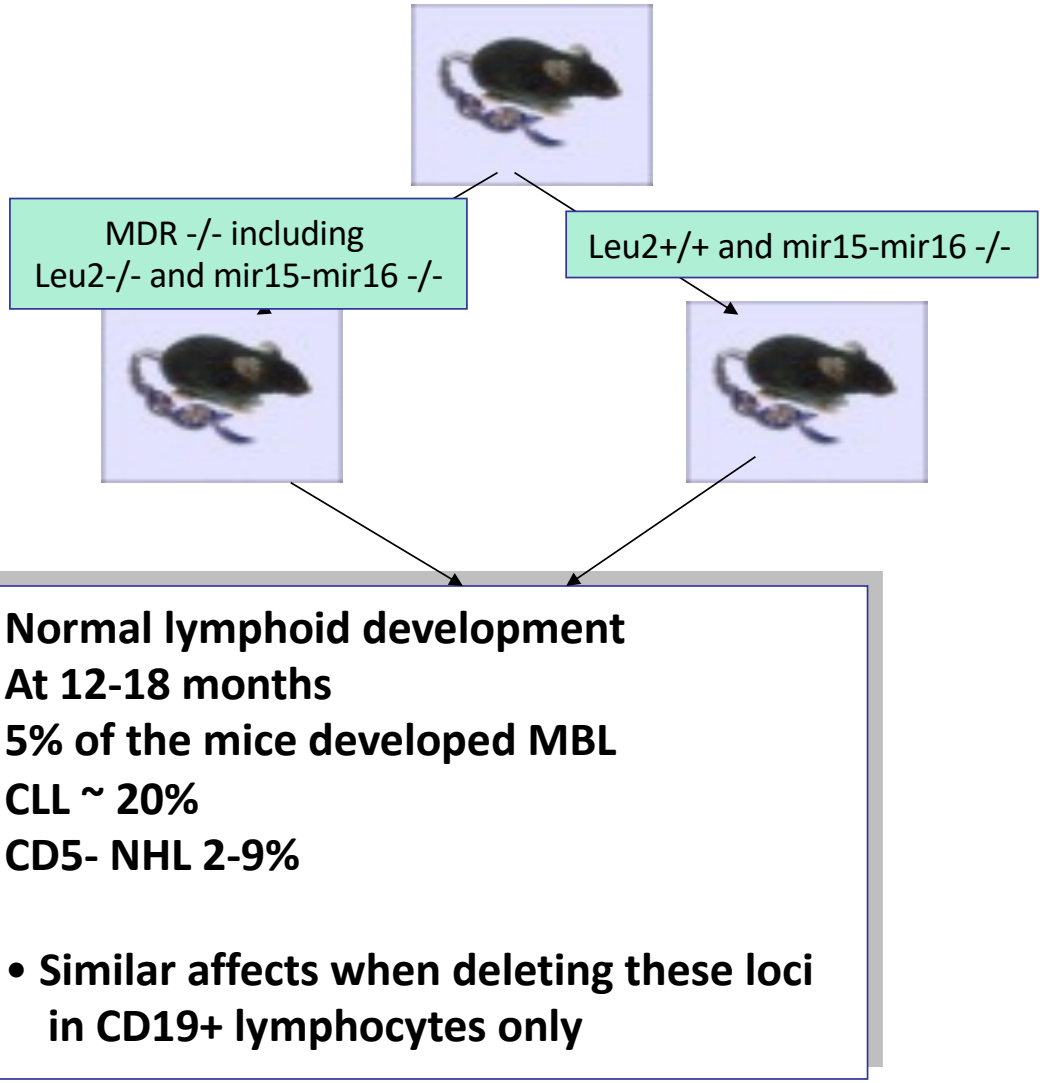
Courtesy of Dr.ssa Lydia Scarfò HSR Milan

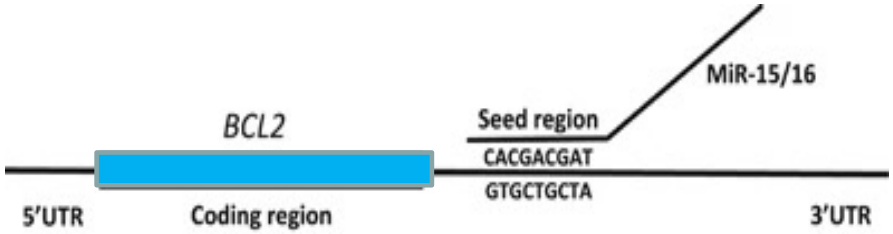
The DLEU2/Mir-15°/Mir-16-1 locus controls B-cell compartment expansion



Klein U, Cancer Cell, 2010: 17; 1-13.



miR-15/16 target BCL2 expression



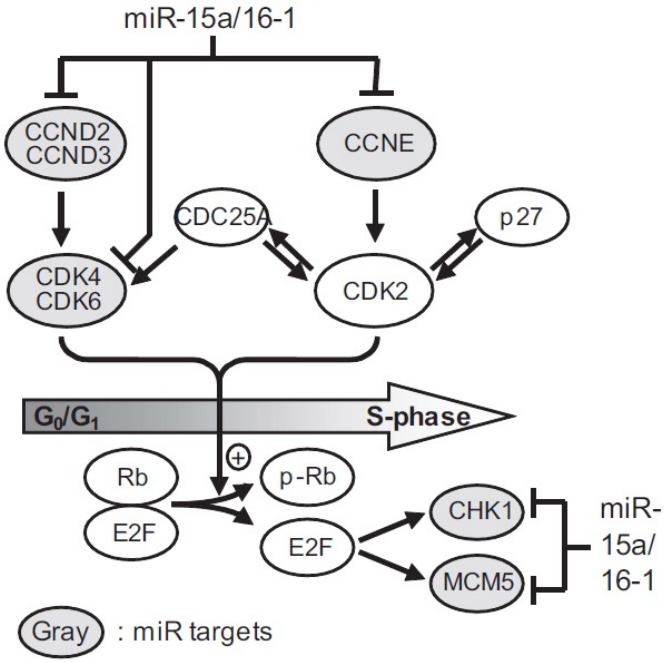
Mir-15/16 downregulation

↓

BCL2 overexpression

↓

CLL survival and progression

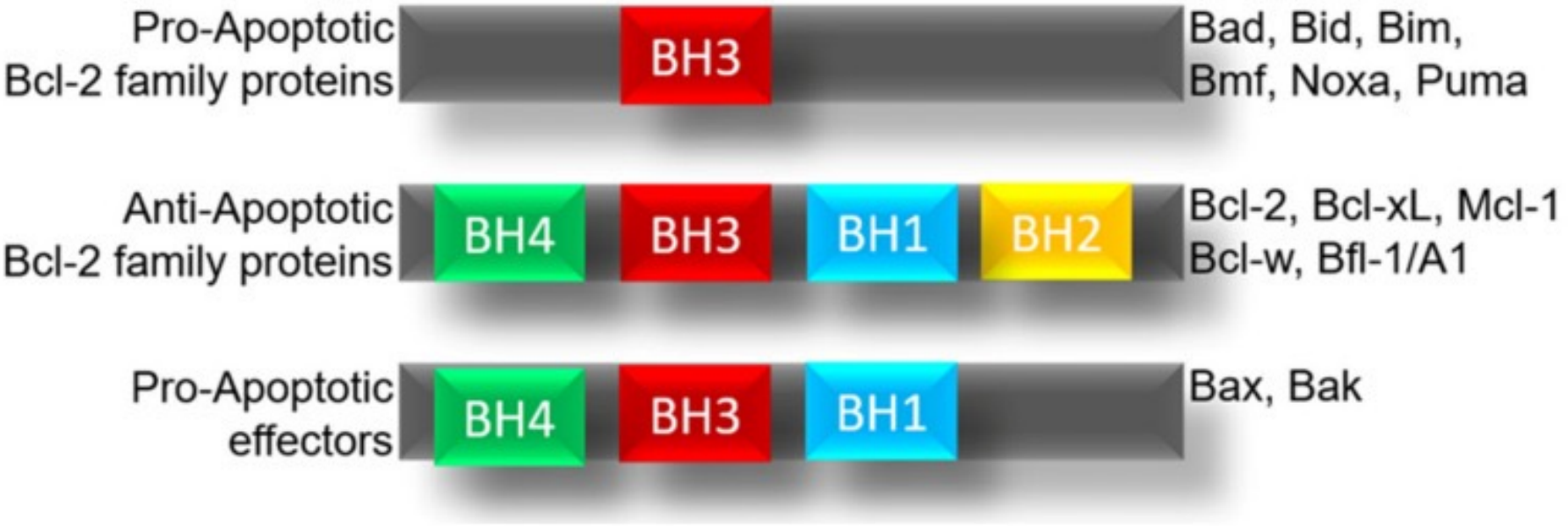


miR-15/16 deletion causes accelerated entry into cell cycle

Pekarsky Y, et al. Cell Death Differ. 2018;25:21-26.

Klein U, Cancer Cell, 2010: 17; 1-13

Members of the BCL-2 family and their role
Schematic representation of the members of BCL-2 family and his subunits

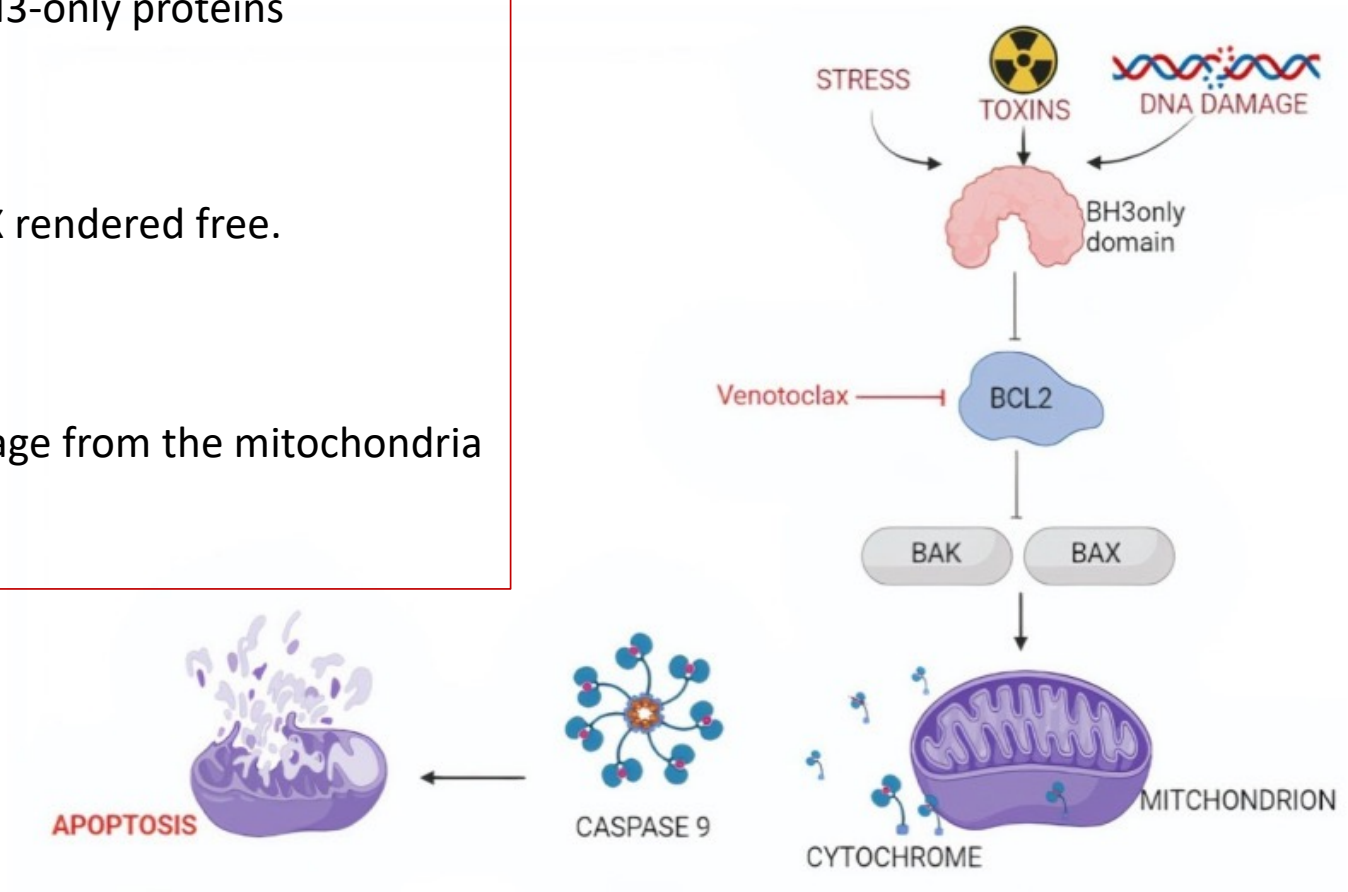


BH: Bcl-2 Homology domain, necessary for protein function

Morales-Martínez M. Int J Mol Sci. 2022 Feb; 23(4): 2193

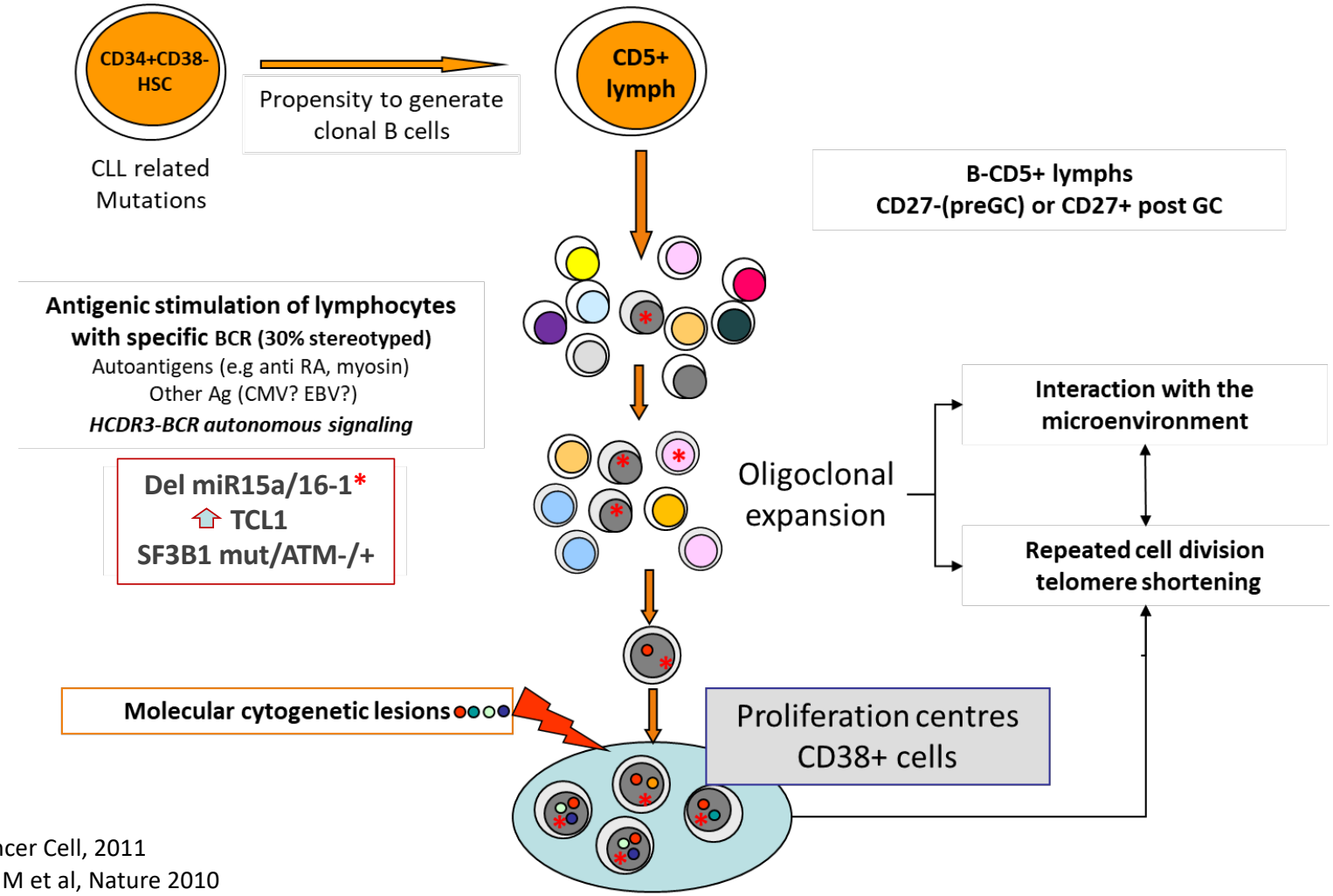
Targeting the intrinsic pathway of apoptosis

- Pro-apoptotic factors trigger BH3-only proteins
- Binding and inhibition of BCL2.
- Larger amounts of BAK and BAX rendered free.
- BAK and BAX dimerize
- Channel for Cytochrome-c leakage from the mitochondria
- Apoptosis



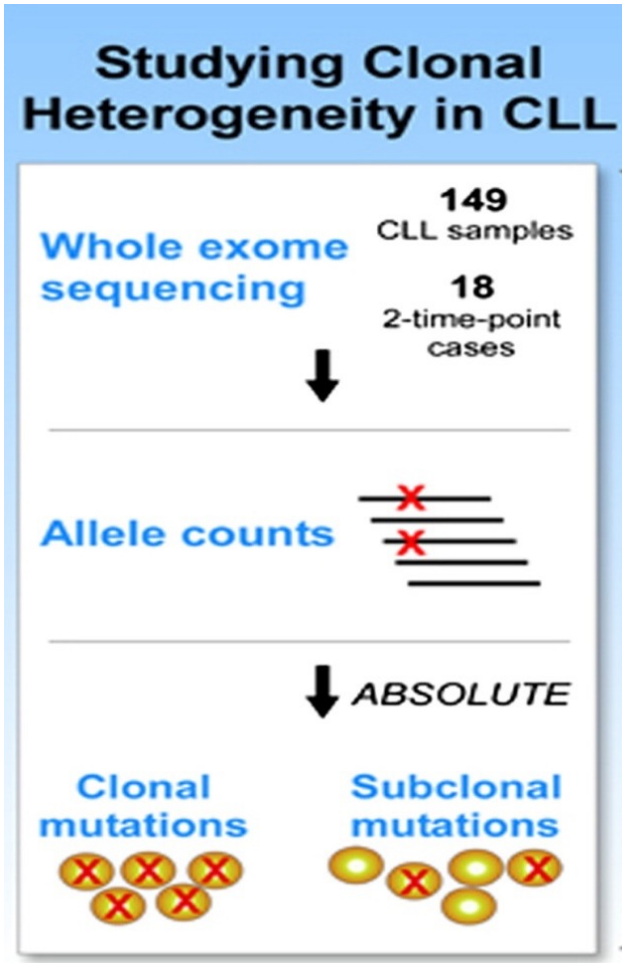
Morales-Martínez M. Int J Mol Sci. 2022 Feb; 23(4): 2193

Cell of origin, pathogenesis and genetic defects in CLL



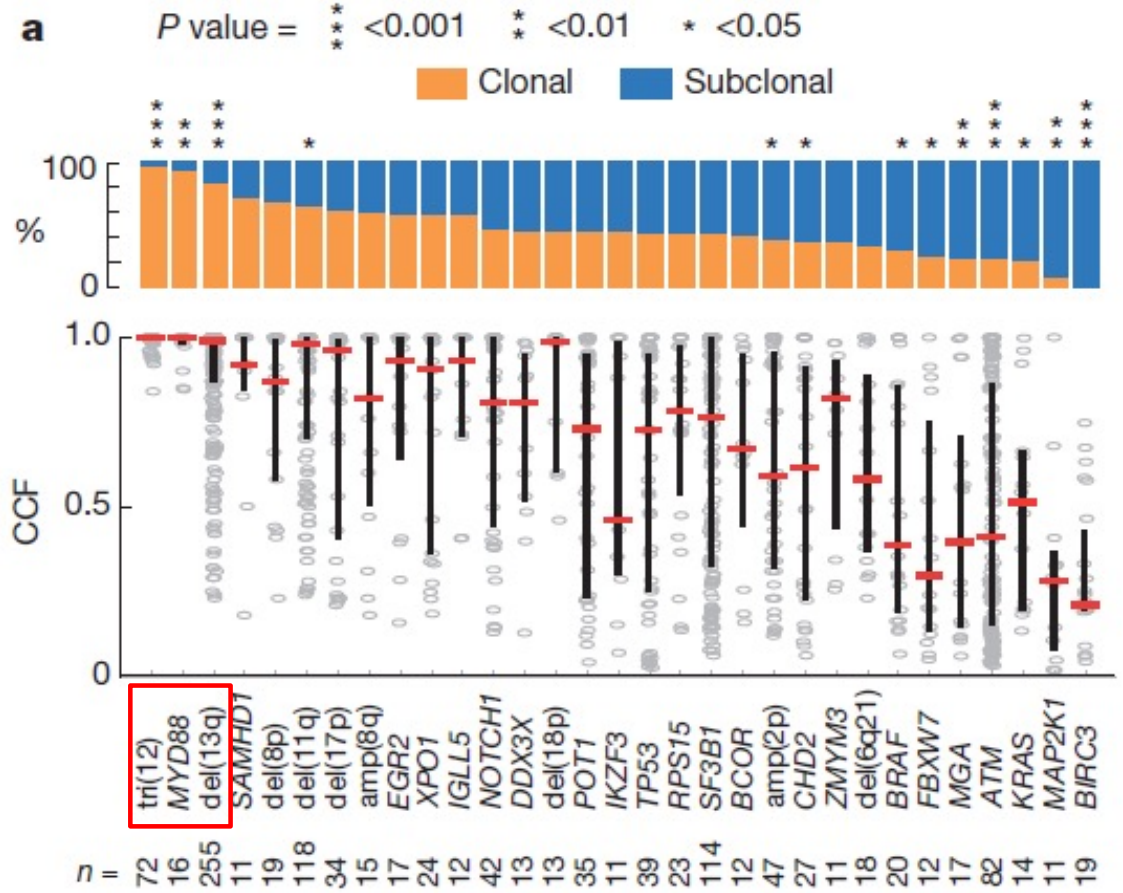
Kikushige Y et al, Cancer Cell, 2011
Dühren-von Minden M et al, Nature 2010
Seifert M, et al. J Exp Med. 2012
Yin et al., 2019, Cancer Cell 35, 1–14

Evolution and Impact of Subclonal Mutations in CLL

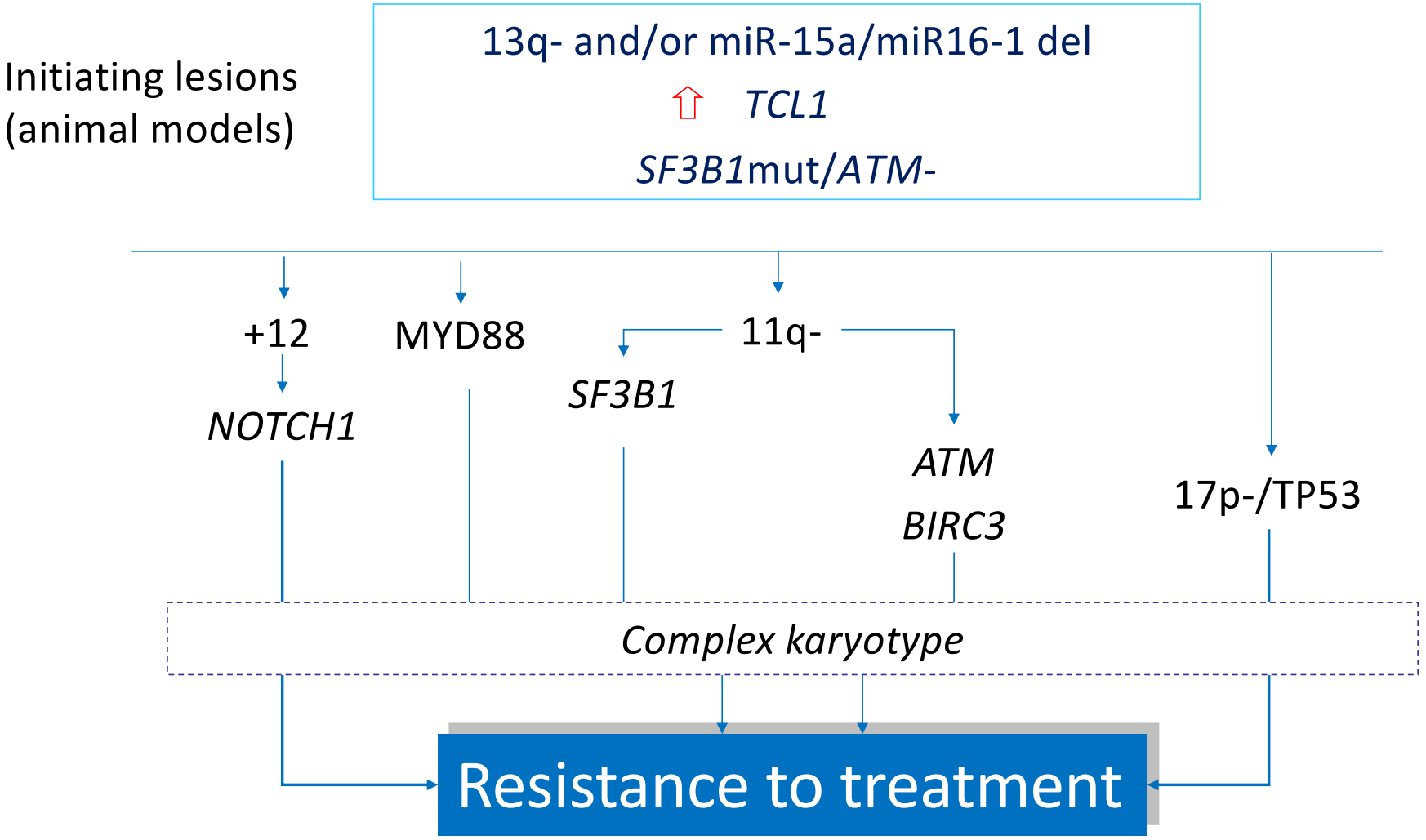


Landau D et al Cell 2013; 152: 714–726

Mutations driving CLL and their evolution in progression and relapse



Sequential development of molecular cytogenetic lesions in CLL



Risk Factors for Developing Resistance (early PD) to Venetoclax

	Relapsed CLL, continuous ven (n = 436 *[413/254]*)	Relapsed, 2 yrs ven (n = 194 *[155]*)	Frontline, 1 yr ven (n = 216 *[206]*)
• Pre-treatment variables			
– Bulky lymphadenopathy		✗	Not eval
– Resistance to BTK inhibitors		Not eval	-
– del(17p) and / or *TP53* mutation		✓	✓
– NOTCH1 mutation		✗	✗
– IGHV unmutated*		Not eval	± (@ 3yrs)
• On-treatment variables			
– Failure to achieve CR by 9 mo		✓ (best)	Not eval
– Failure to achieve uMRD by 24mo		✓ (@ 2 yrs)	✓ (@12 mo)

* IGHV status no longer significant when response variables included

Roberts *Blood* 2019, Kater *J Clin Oncol* 2020, Tausch *Blood* 2020, Fischer *Lancet Oncol* 2020

XIX IWCLL International Workshop on CLL

Courtesy of Andrew Roberts, Melbourne, Australia

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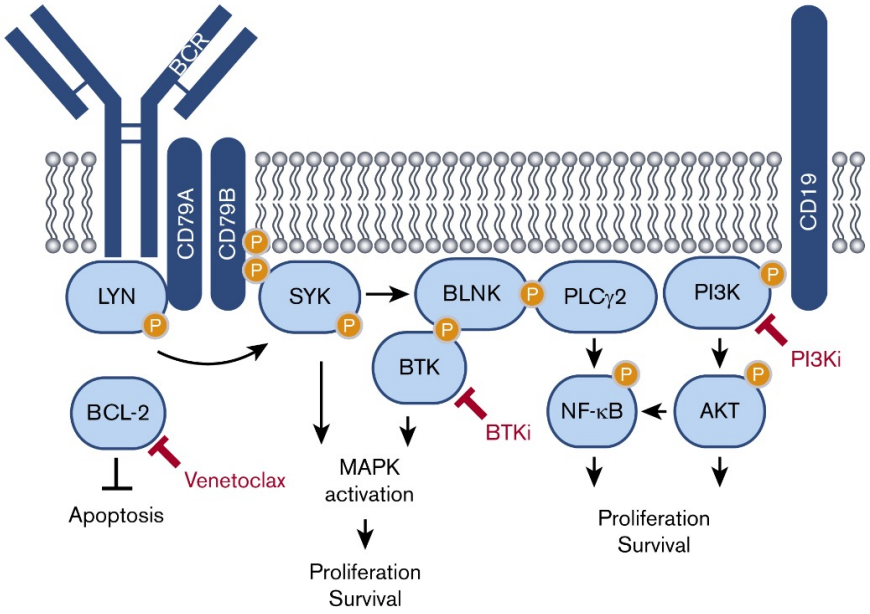
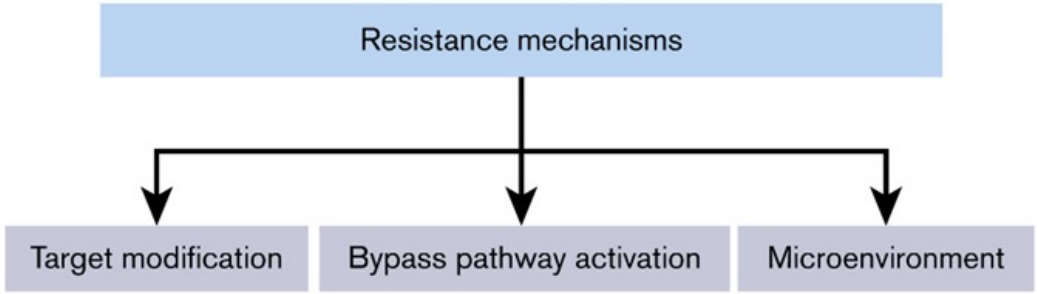
Molecular mechanisms of acquired resistance to targeted therapies





BTKi:
BTK mutations
PLCG2 mutations
BCL2i:
***BCL2* mutations**

BTKi:
Activation of PI3K-AKT pathway
BCL2i:
Overexpression of BCL-XL and MCL1

BCL2i:
CD40/CD40L stimulation

Skanland S and Mato AR. Blood Advances 2021

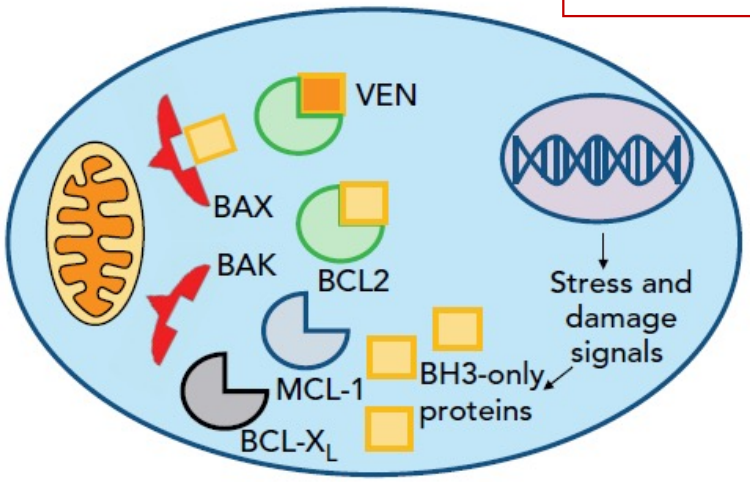
Courtesy of Dr.ssa Lydia Scarfò HSR Milan

Venetoclax Resistance

Sustained Ven exposure

BCL2 mutations
- Gly101Val/Ala
- Val156Asp
- Arg129Leu
- Ala113Gly
- Arg107_Arg110dup
- Asp103Glu/Tyr/Val
- Phe104Leu/Ser
- Leu119Val

Alternative BCL2 family proteins
- BCL-X_L upregulation
- Copy number gain *MCL-1*
- Amplification chromosome 1q23 (MCL-1)
- Microenvironment driven BCL-X_L, BLF-1, and MCL-1 upregulation
- *BRAF* mutation, potentially upregulating MCL-1



Genomic instability
- Acquisition of *TP53* aberrations
- Evolution of complex karyotype

Other
- *BTG1* mutations
- *CDKN2A/B* homozygous deletions
- Amplification of *PD-L1*
- Amplification of *PRKAB2*

Early events?

Modified from: Lew TE et al, Blood 2021

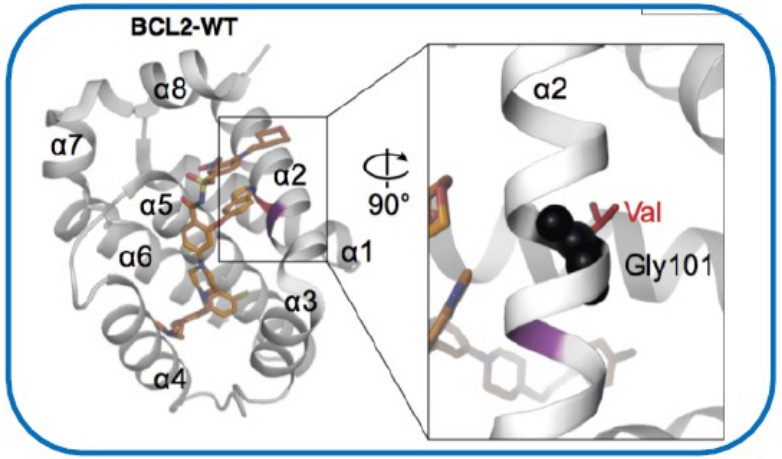
BCL2 Gly101Val is specific for CLL with resistance to Venetoclax

Population	Number assessed	*BCL2* Gly101Val detected (%)	*BCL2* Phe104Leu detected (%)
Venetoclax-naïve CLL	96	0 (0%)	0 (0%)
CLL-type progression on venetoclax	15	7 (46.7%)	0 (0%)
Other B-cell malignancies			
- Follicular lymphoma	28	0 (0%)	0 (0%)
- Mantle cell lymphoma	28	0 (0%)	0 (0%)
- Diffuse large B-cell lymphoma	47	0 (0%)	0 (0%)
- Lymphoplasmacytic lymphoma	95	0 (0%)	0 (0%)
- Multiple myeloma	103	0 (0%)	0 (0%)
Cancer database (COSMIC[a])	47,628	0 (0%)	2 (0.004%)
Population database (gnomAD[b])	30,836	0 (0%)	0 (0%)

Blombery et al. Cancer Discov 2019

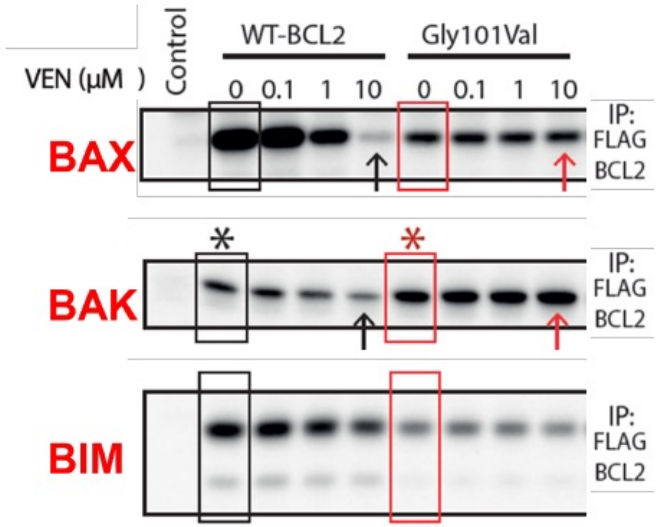
Mechanisms of Resistance to Venetoclax

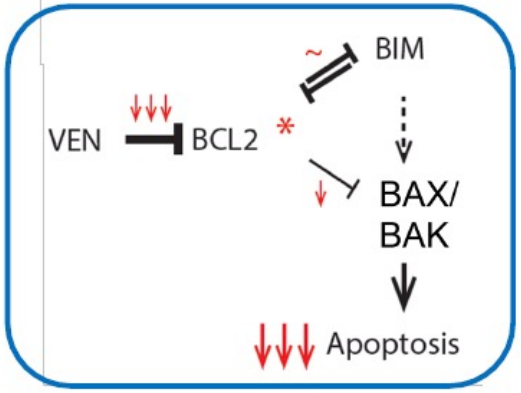
BCL2 mutations



BCL2 Protein	Venetoclax K_i (nM± SD, n=3)
Wildtype	0.018 ± 0.014
Gly101Val	3.2 ± 1.1

180-fold reduction





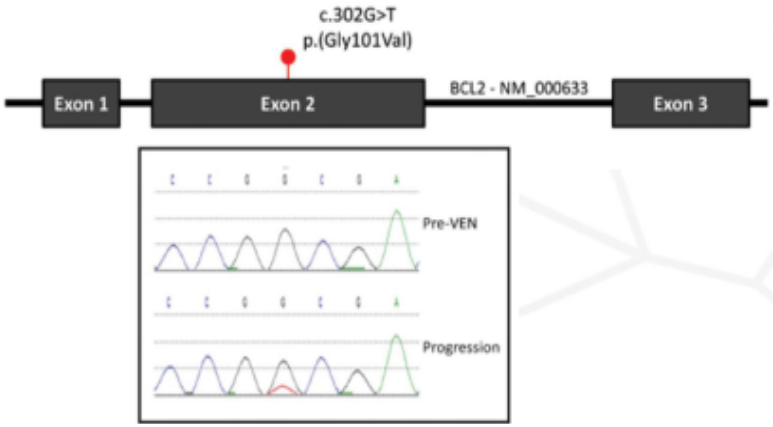
Blombery *Cancer Discovery* 2019; Birkinshaw *Nat Comms* 2019

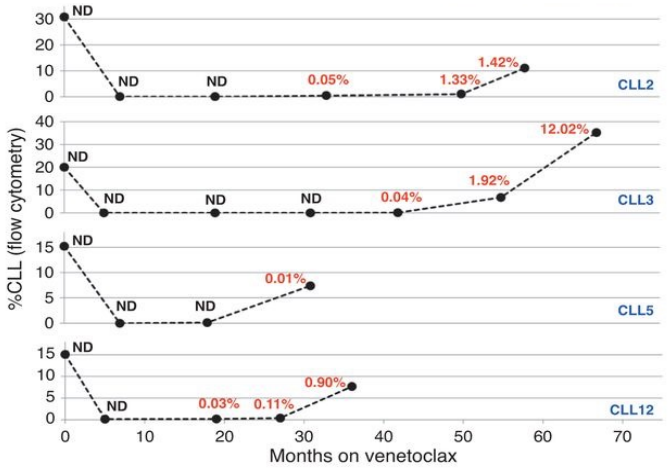
XIX IWCLL International Workshop on CLL

Courtesy of Andrew Roberts, Melbourne, Australia

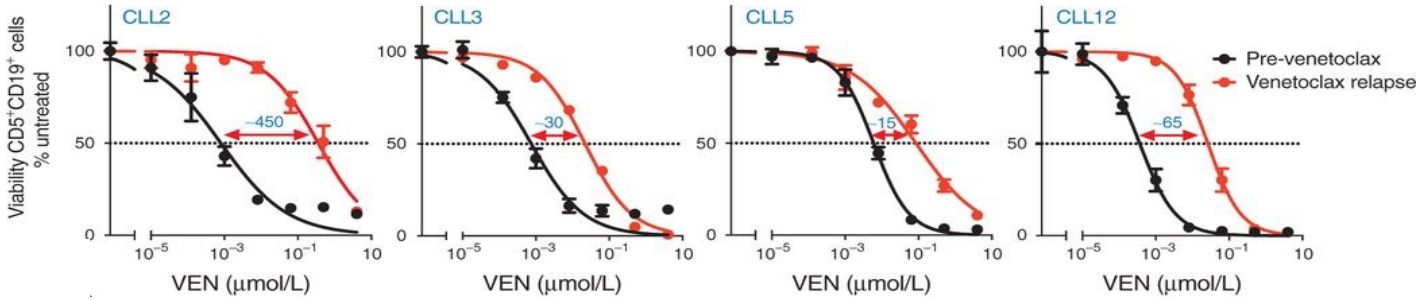
Venetoclax: mechanisms of resistance

***BCL2* c.302G>T, p.(G101V) detected in samples from 7/15 (48.7%) patients at CLL-type progression on Venetoclax**





[a]CLL cells harboring G101V at progression; calculated by adjusting the measured VAF by the % of CLL cells in the bone marrow determined by flow cytometry.



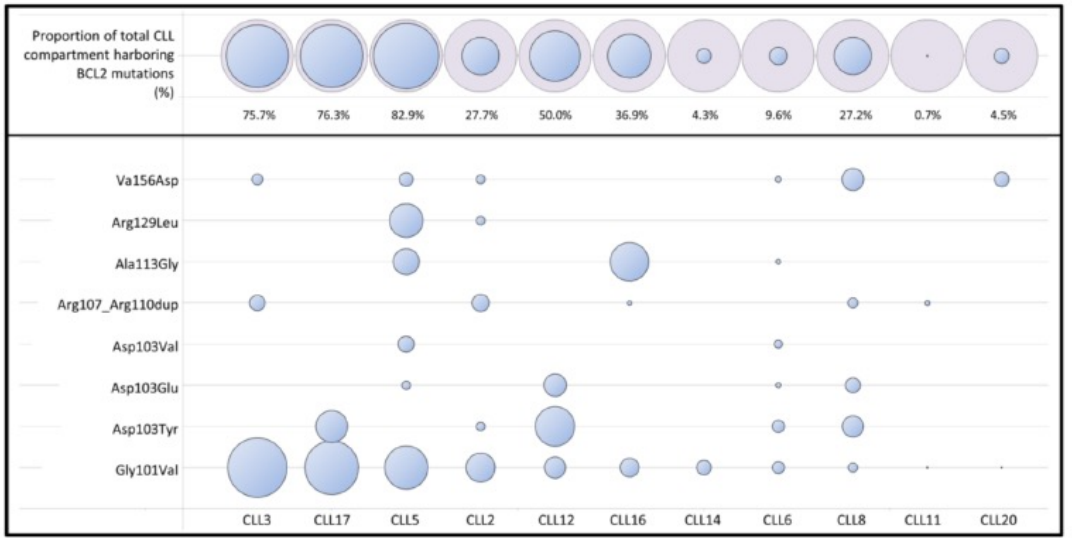
CLL cells harboring p.G101V are less sensitive to Venetoclax

Blombery et al. Cancer Discov 2019

Mechanisms of Resistance to Venetoclax

BCL2 mutations

- Almost exclusively occur in context of ven exposure
- Maintain pro-survival function
- Reduce ven binding to BCL2
- Multiple sites, multiple clones



Blombery *Cancer Discovery* 2019; Birkinshaw *Nat Comms* 2019; Tausch *Haematologica* 2019; Blombery *Blood* 2020

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Courtesy of Andrew Roberts, Melbourne, Australia

Venetoclax: insights into the clonal dynamics involved in resistance

- **Whole-exome sequencing data of eight CLL patients with *TP53* disruption that developed resistance upon BCL2-inhibition by venetoclax (4 Richter transformation)**

- BTG1 (2 patients)*
- Homozygous deletions affecting CDKN2A/B (3 patients)*
- Mutation in BRAF and a high-level focal amplification of CD274 (PD-L1)

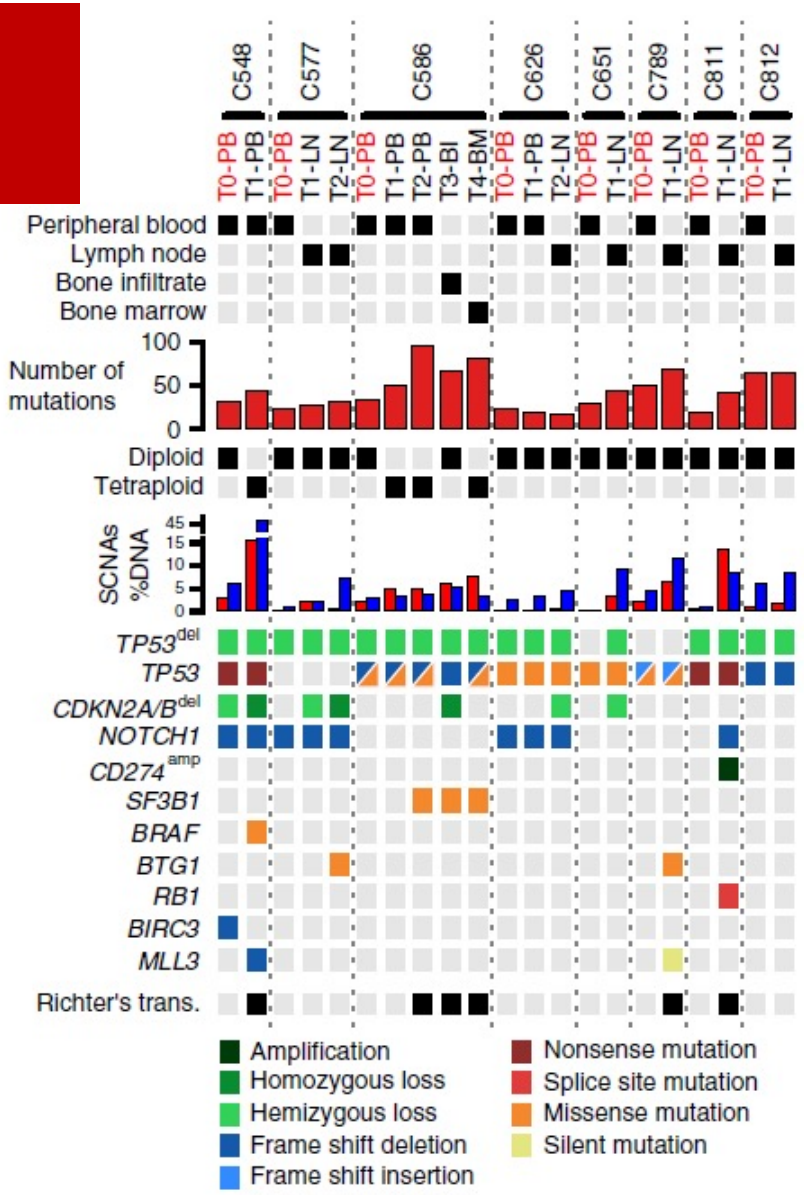
- **Pinpoint molecular aberrations offering structures for further therapeutic interventions.**

*BTG1 has been shown to counteract cell proliferation and to be regulated downstream of BCL2 and CDKN2A/B
*CDKN2A/B: cell cycle control

Herling et al. Nat comm 2018

Patients resistant to Venetoclax: whole-exome sequencing

- Number of somatic mutations
- Sample ploidy
- % of the genome undergoing copy number alterations
  - blue for losses and red for gains
- Cancer-related gene mutations with clonal dynamics during therapy



Herling et al. Nat comm 2018

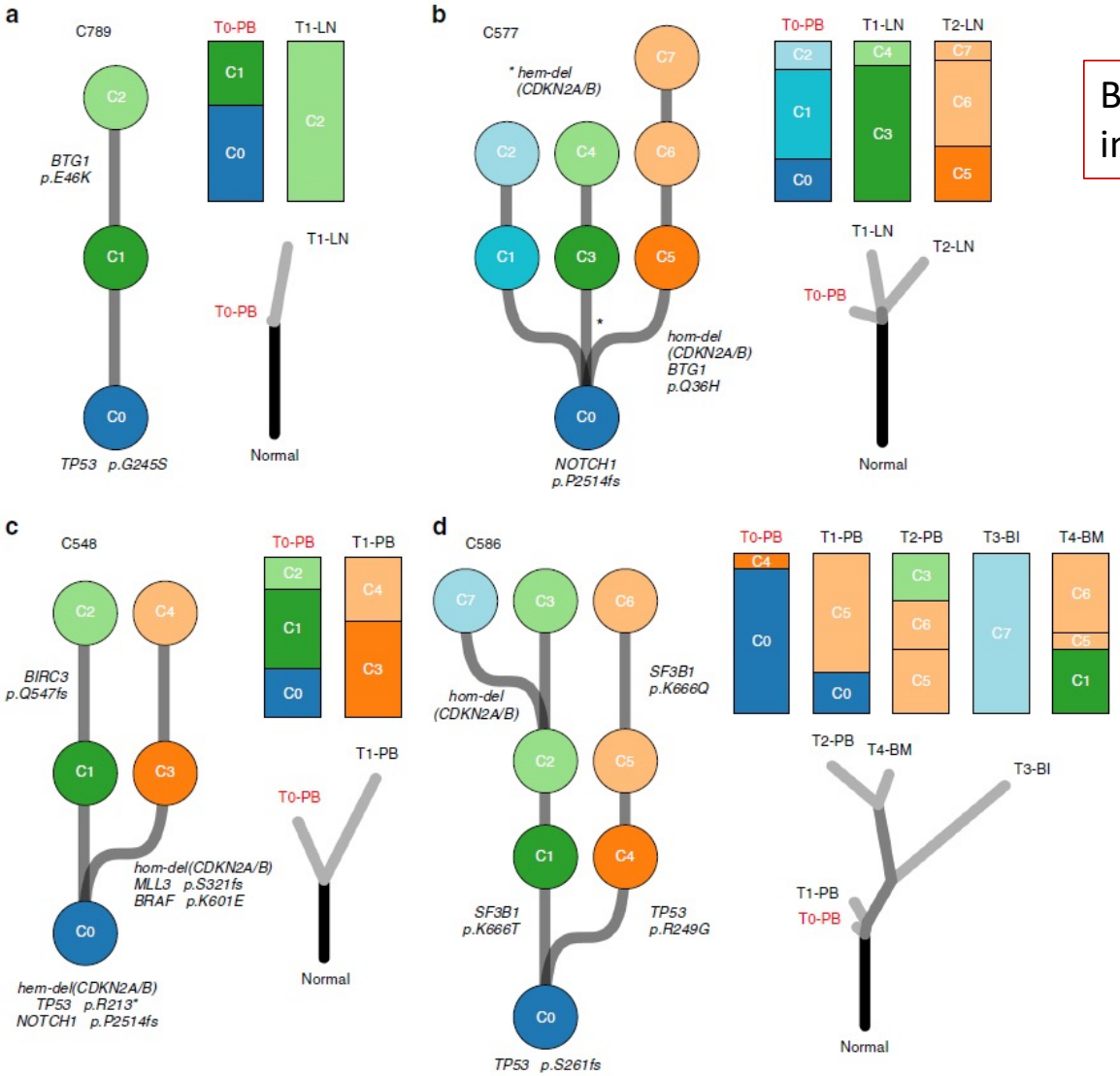
Heterogeneous clonal evolutions under venetoclax therapy

Linear evolution

Branching evolution into 3 lineages

Divergent evolution of two branches

Convergent evolution



Herling et al. Nat comm 2018

Clonal evolutions in patients with TP53 disruption who developerd resistance to venetoclax

- Alterations in cancer-related genes: BRAF, CD274, NOTCH1, RB1, SF3B1, and TP53 that evolved during venetoclax treatment
- Genetic alterations in BCL2 or functionally connected genes, such as BAX and BAK were not identified.
- Recurrent mutations in BTG1 and homozygous deletions of CDKN2A/B as recurrent genomic events at the time of relapse under venetoclax exposure
- Complete loss of CDKN2A/B alone is not sufficient to induce venetoclax resistance
- Aside the abrogation of cell cycle control by loss of CDKN2A/B, damaging mutations in BTG1 may provide a survival advantage to CLL cells under targeted BCL2-inhibition

Herling et al. Nat comm 2018

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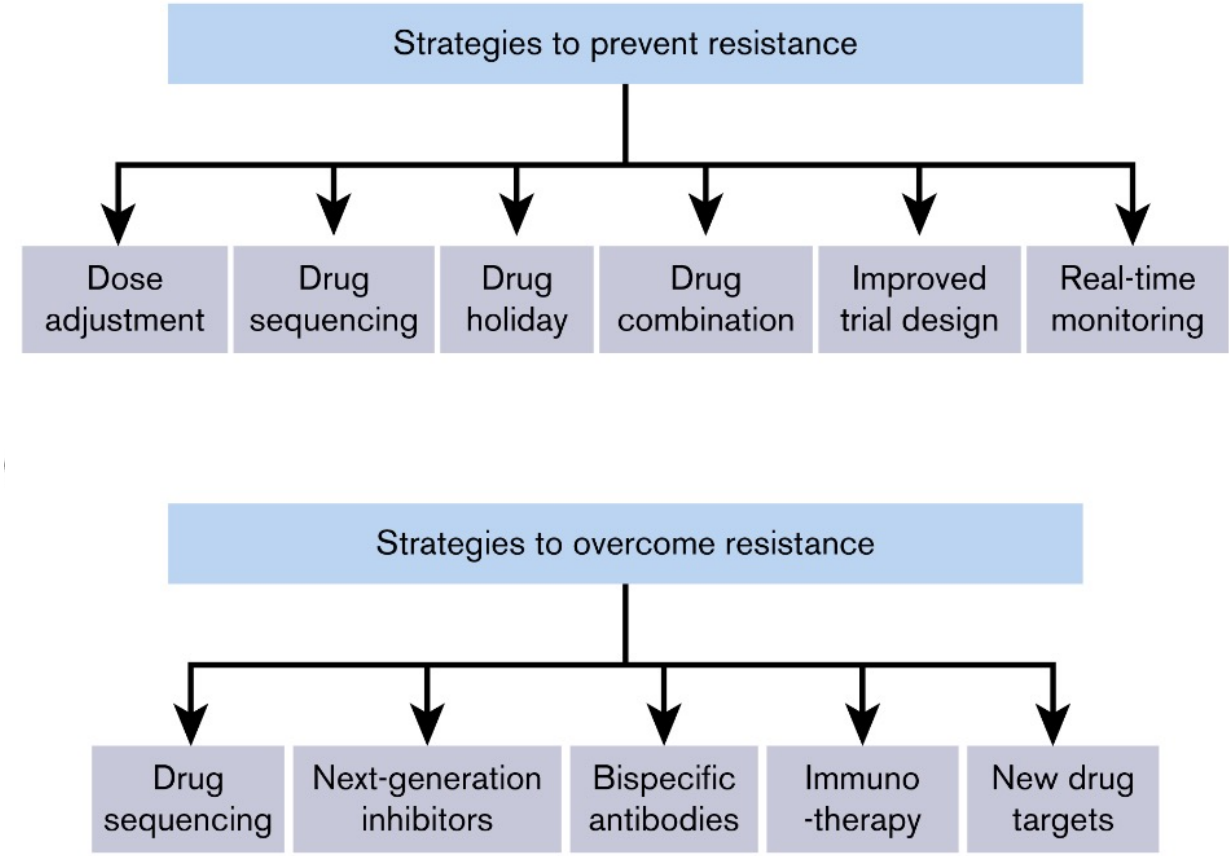
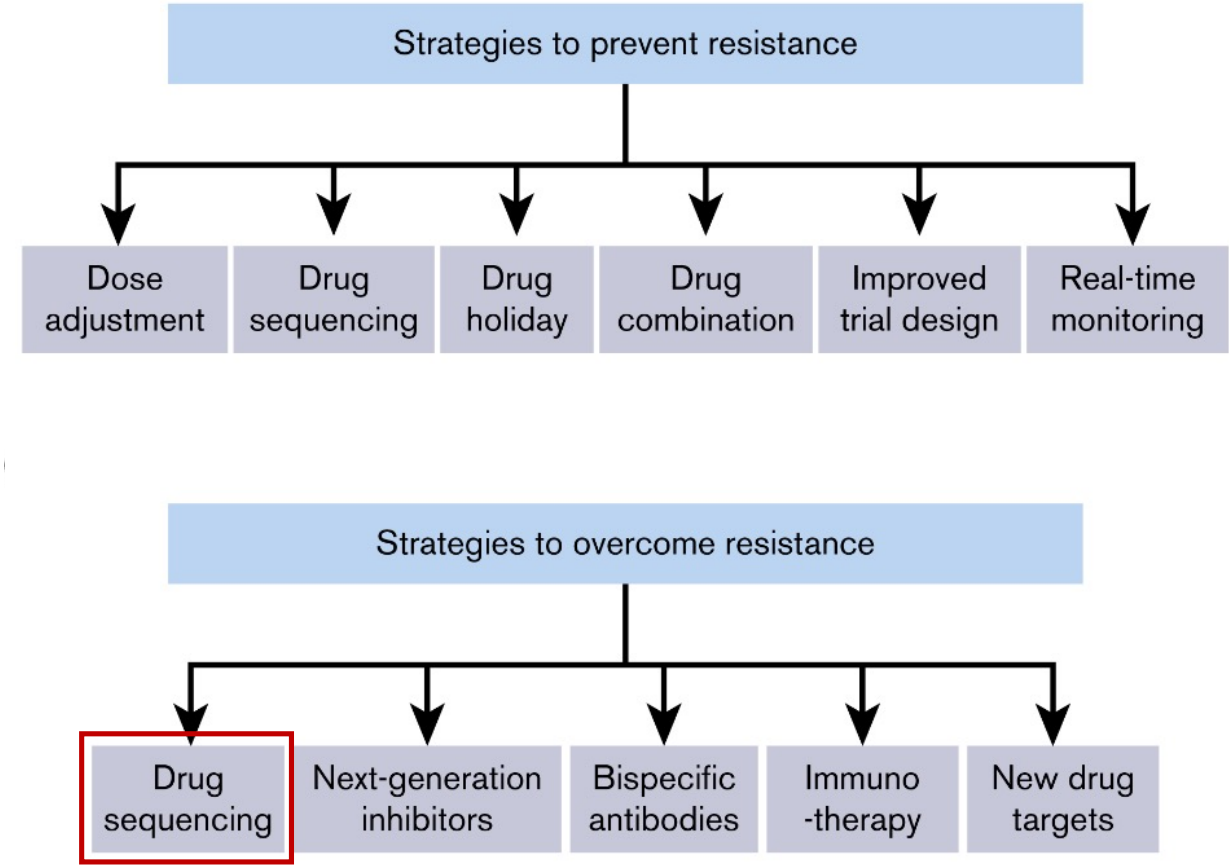
Strategies to prevent or overcome acquired resistance



Skanland S and Mato AR. Blood Advances 2021

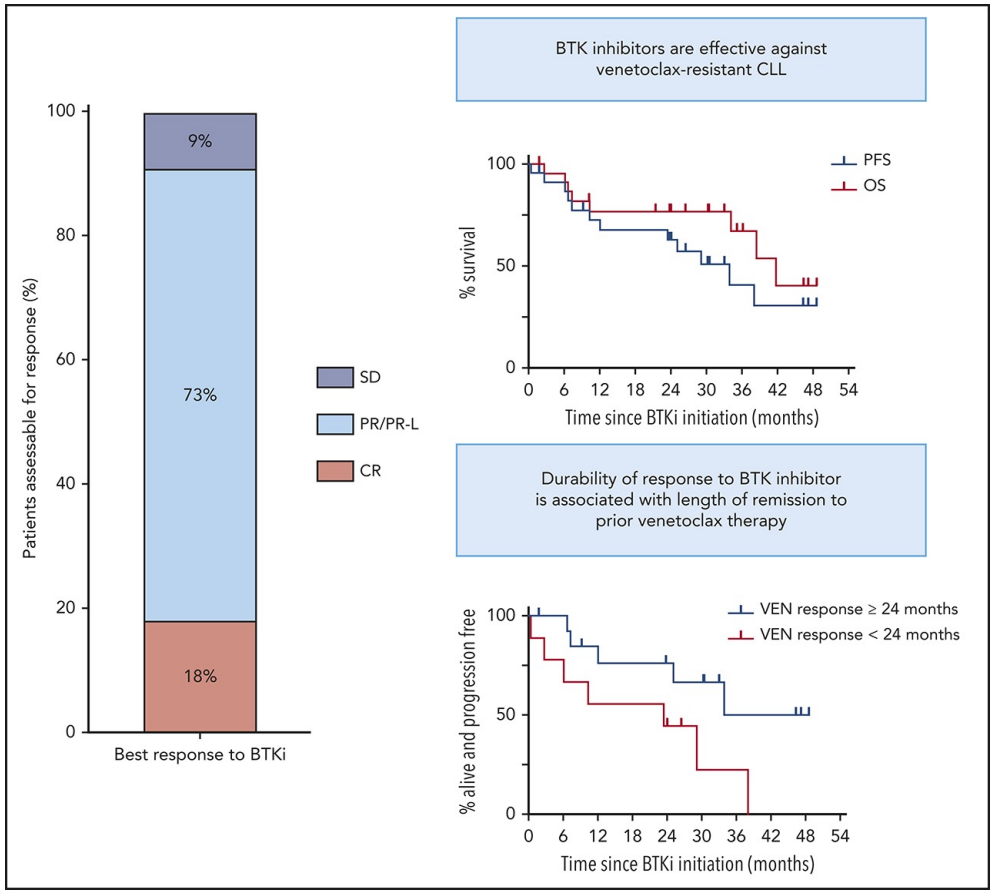
Strategies to prevent or overcome acquired resistance



Skanland S and Mato AR. Blood Advances 2021

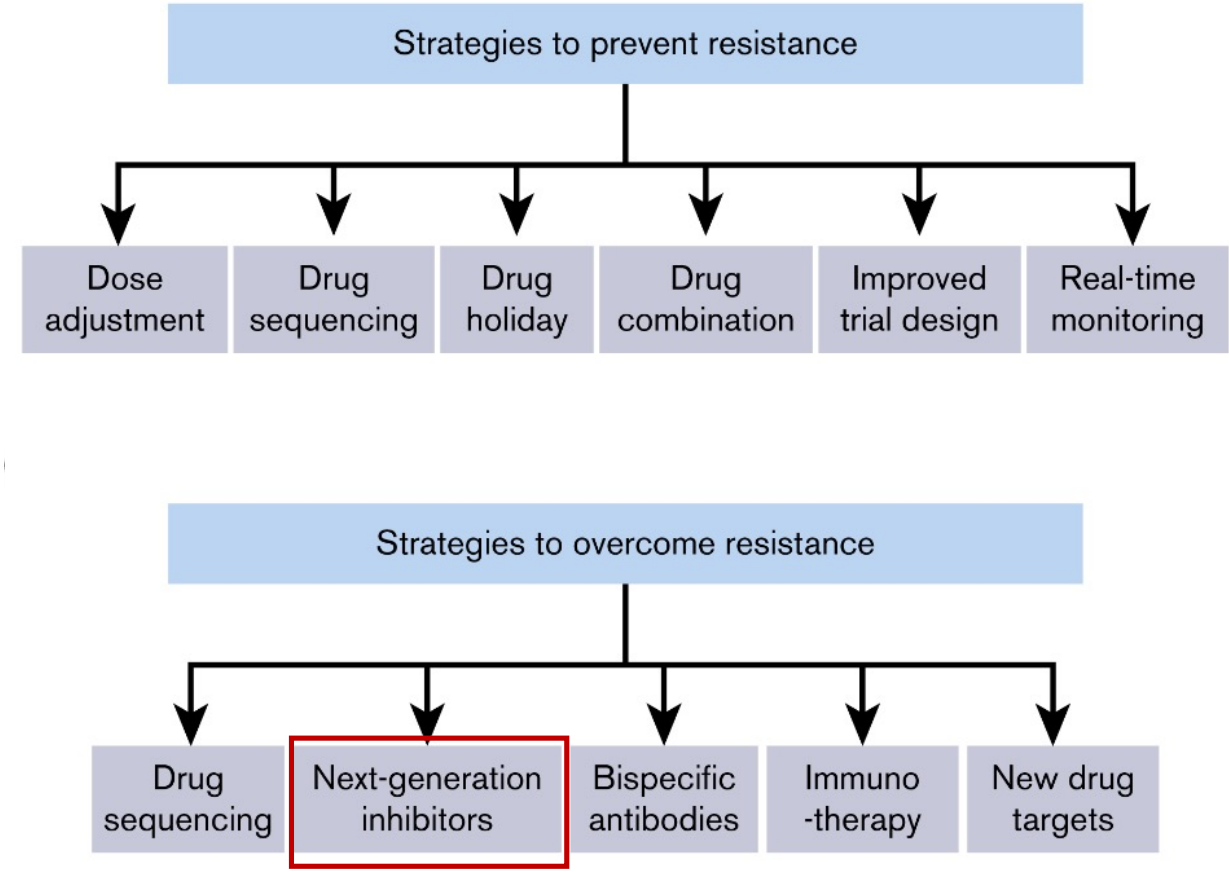
Treatment sequencing

BCL2i → BTKi



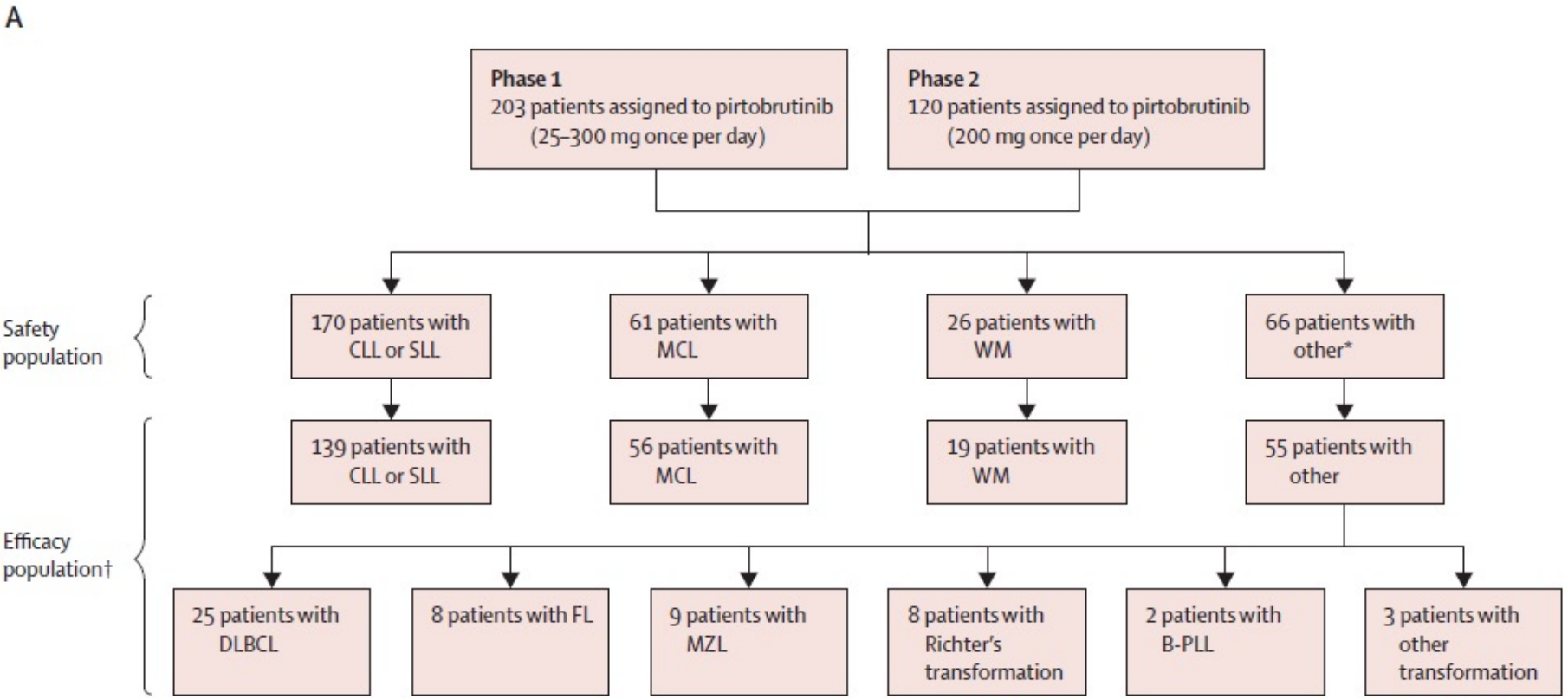
Lin VS et al, Blood 2020

Strategies to prevent or overcome acquired resistance



Skanland S and Mato AR. Blood Advances 2021

Pirtobrutinib in relapsed or refractory B-cell malignancies (BRUIN): a phase 1/2 study

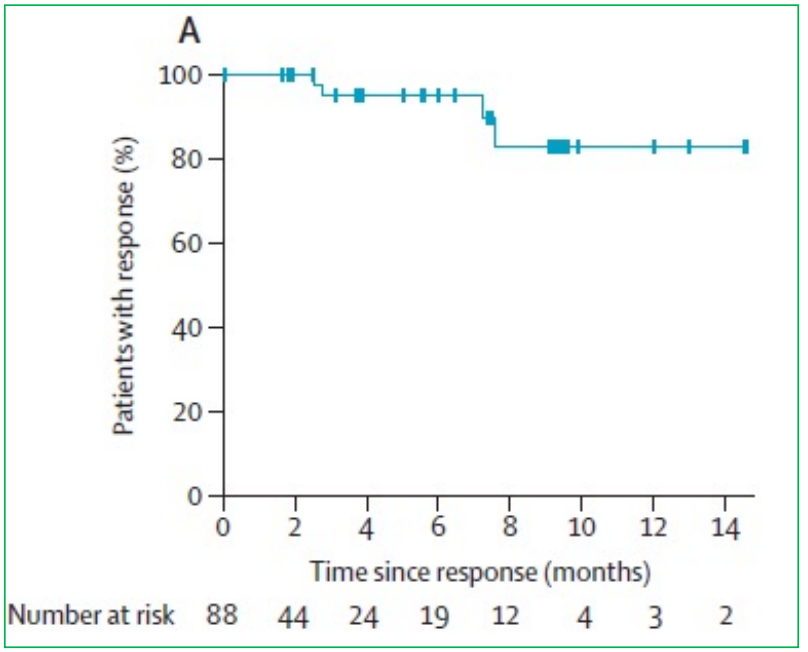


Mato A et al. Lancet 2021; 397: 892–901

Pirtobrutinib in relapsed or refractory B-cell malignancies (BRUIN): a phase 1/2 study

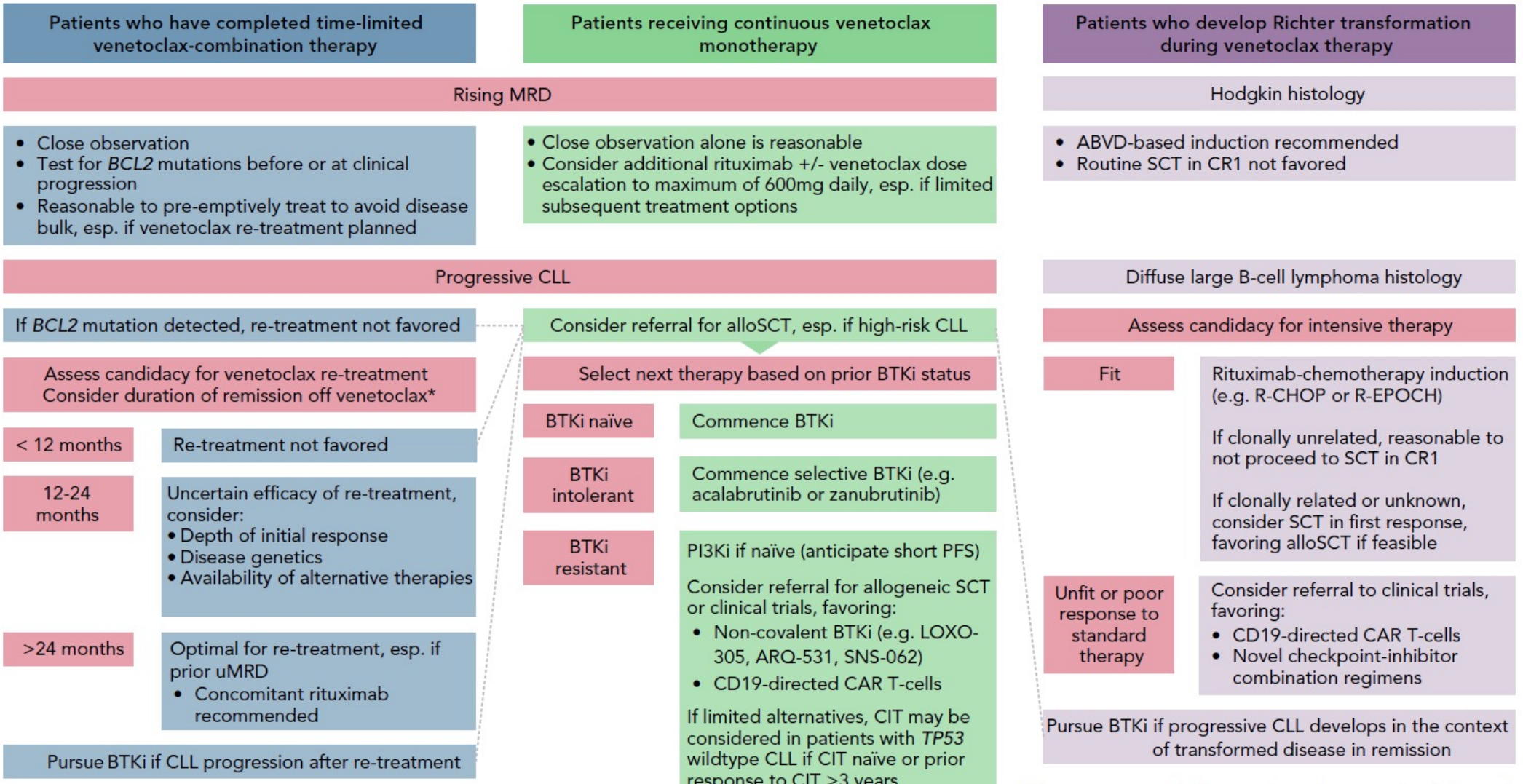
	Number of lines of previous systemic therapy	Treated	Efficacy, evaluable*	Responders	Overall response rate
Chronic lymphocytic leukaemia and small lymphocytic lymphoma					
All patients	3 (2–5)	170	139	88	63% (55–71)
Patients who had previous therapy					
With at least BTK	4 (2–5)	146	121	75	62% (53–71)
With at least BCL2	5 (4–7)	57	48	31	65% (50–78)
With at least PI3K	4 (3–6)	36	30	18	60% (41–77)
With at least BTK and BCL2	5 (4–7)	54	45	29	64% (49–78)
With at least chemotherapy, CD20, and BTK	4 (3–6)	113	93	62	67% (56–76)
With at least chemotherapy, CD20, BTK, and BCL2	5 (4–7)	48	39	27	69% (52–83)
With at least chemotherapy, CD20, BTK, BCL2, and PI3K	6 (4–9)	14	12	7	58% (28–85)
With at least CAR T-cell therapy	6 (4–9)	10	10	9	90% (56–100)
BTK mutational status†					
C481 mutant	3 (3–5)	25	24	17	71% (49–87)
Wild type	4 (2–4)	66	65	43	66% (53–77)
Reason for previous BTK discontinuation					
Progression	4 (3–6)	98	79	53	67% (56–77)
Toxicity or other	3 (2–4)	48	42	22	52% (36–68)

Duration of response in patients with CLL or SLL



Mato A et al. Lancet 2021; 397: 892–901

Treatment of progressive disease after venetoclax for CLL



Lew TE et al, Blood 2021