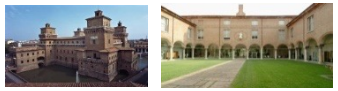
Modalità diverse di resistenza

CIT **BTKi** **BCL2i**

- Genetic lesions and pathways
- Time of appearance
- Practical consequences

Antonio Cuneo



Modalità diverse di resistenza

CIT

- Genetic lesions and pathways
- Time of appearance
- Practical consequences

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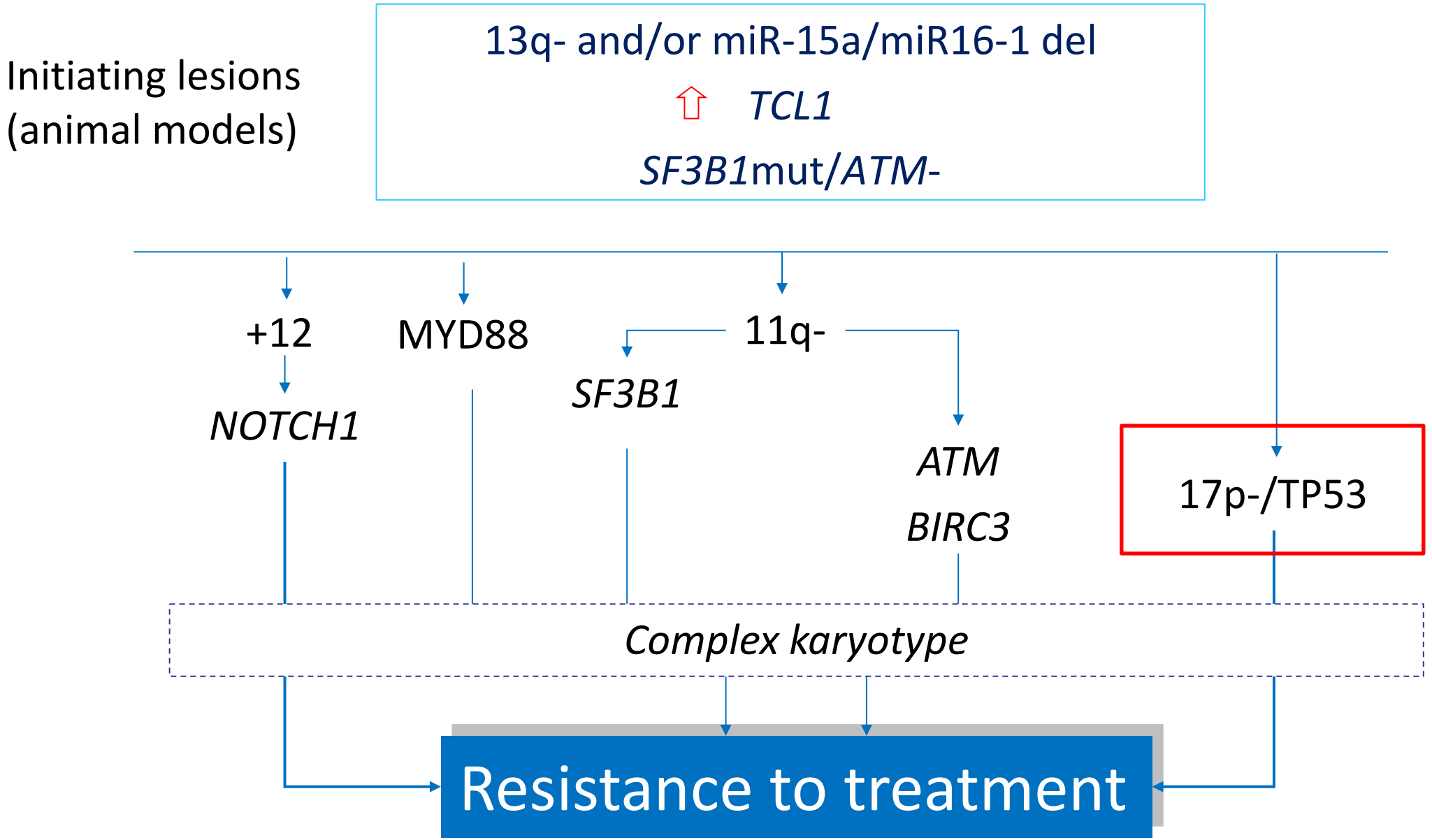
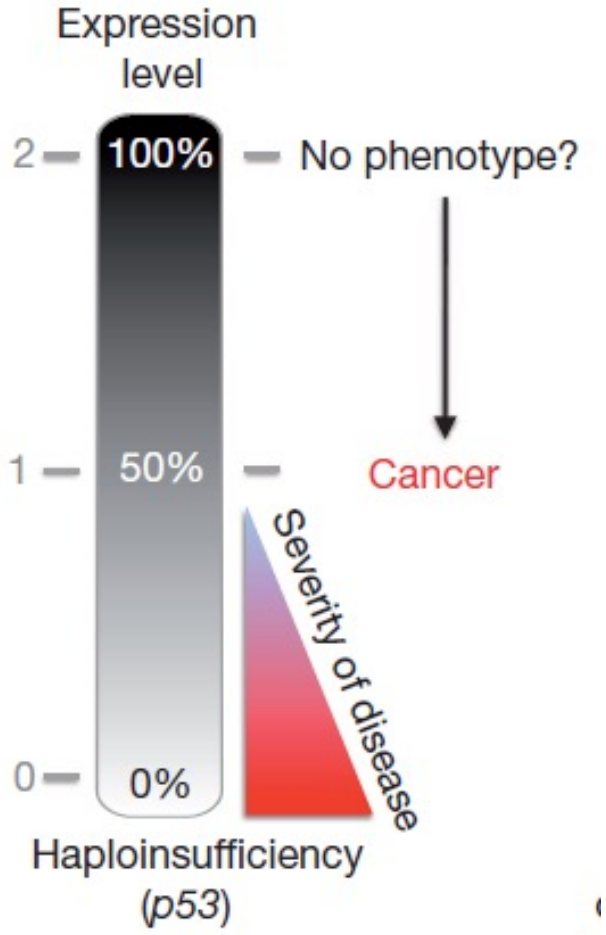
Sequential development of molecular cytogenetic lesions in CLL



Paradigms of tumour suppression: Classical haploinsufficiency

Loss of one TP53 allele is sufficient for induction of cancer.



Alice H. Berger. 11 AU G U S T 2 0 1 1 | V O L 4 7 6 | N AT U R E | 1 6 3

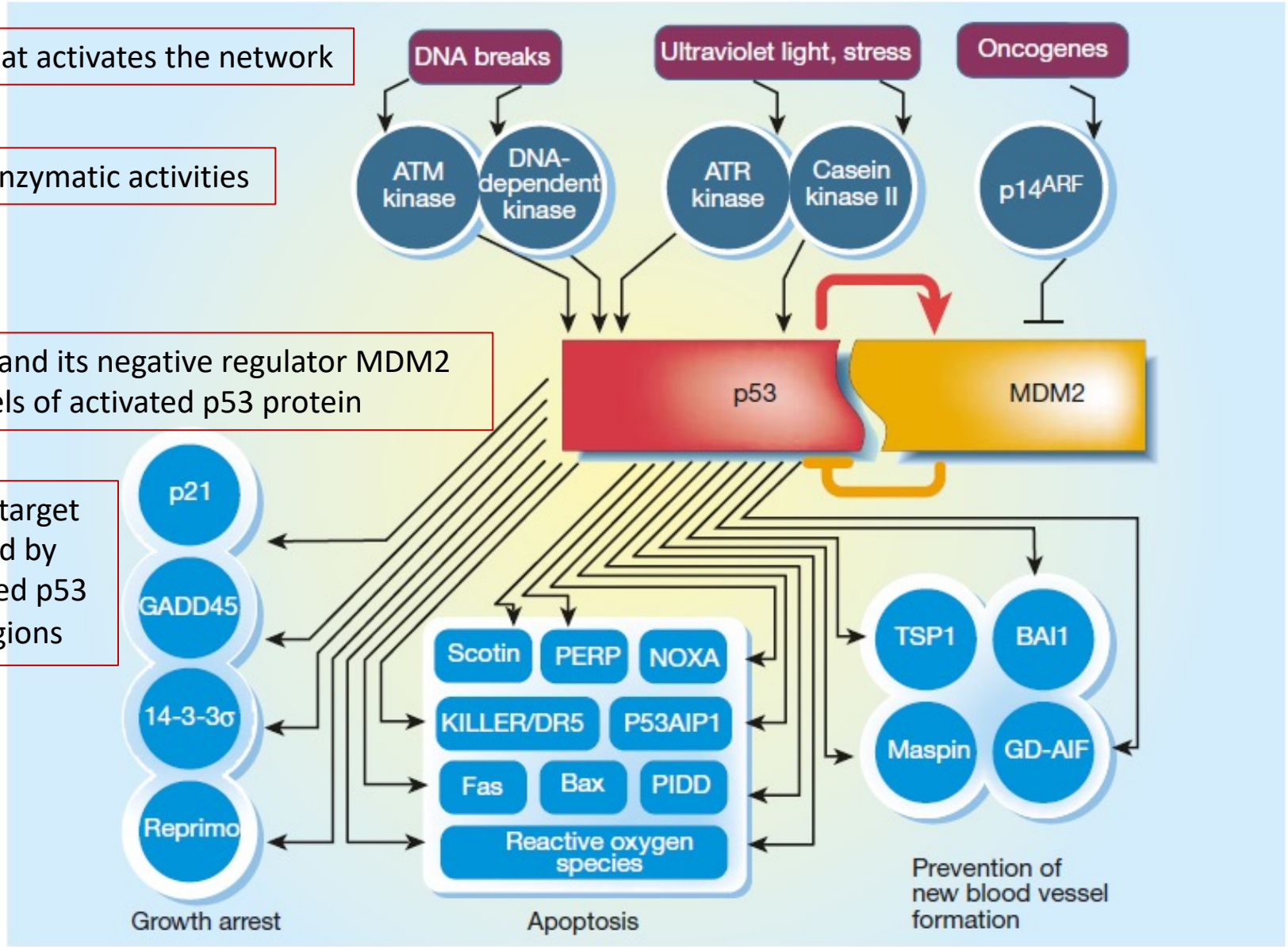
Activation of the p53 network

Stresses that activates the network

Enzymatic activities

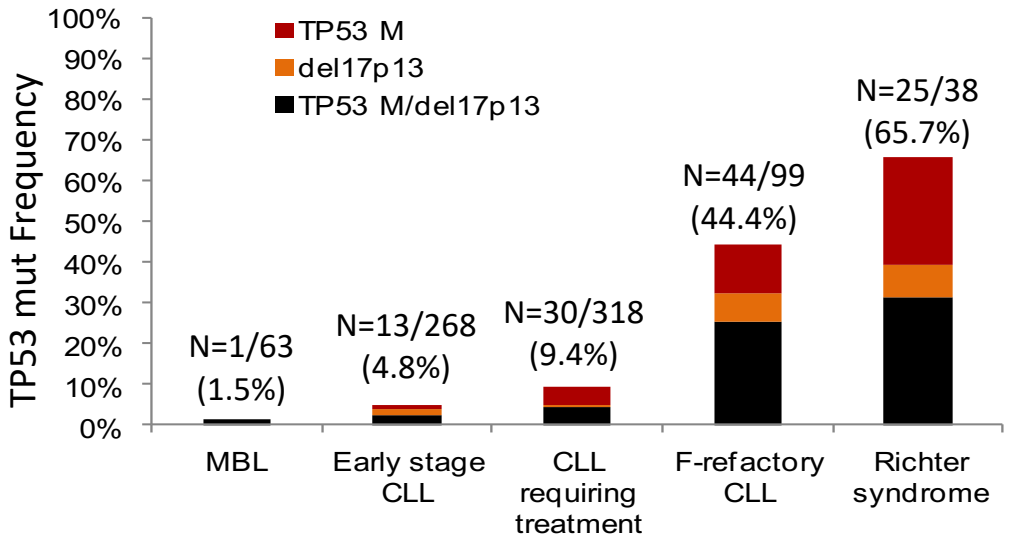
Modification of p53 and its negative regulator MDM2
Increased levels of activated p53 protein

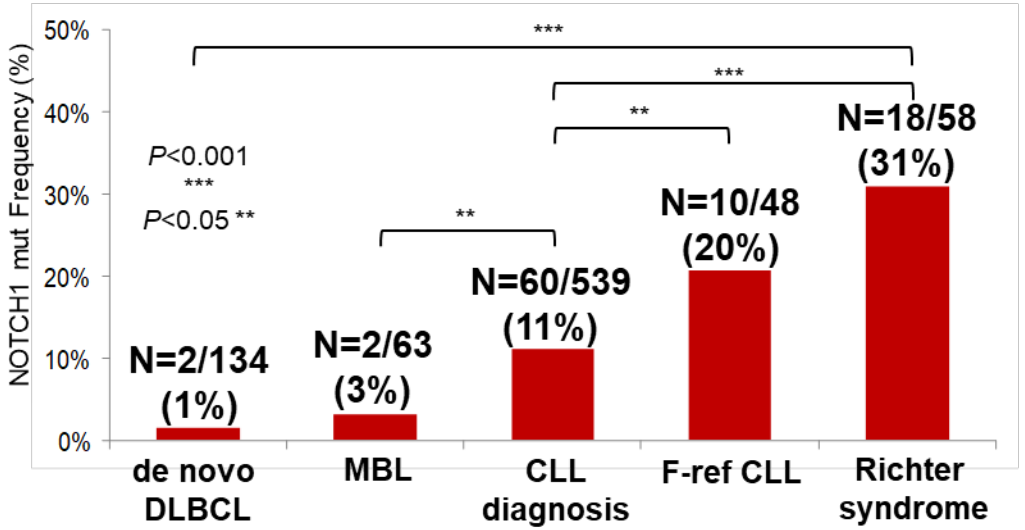
Expression of several target genes is then activated by binding of the activated p53 to their regulatory regions

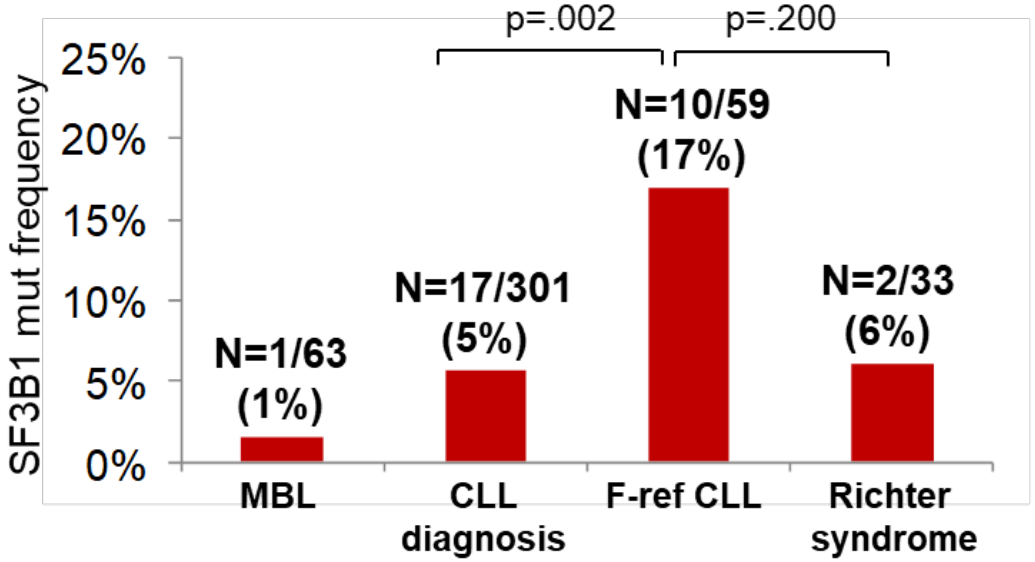


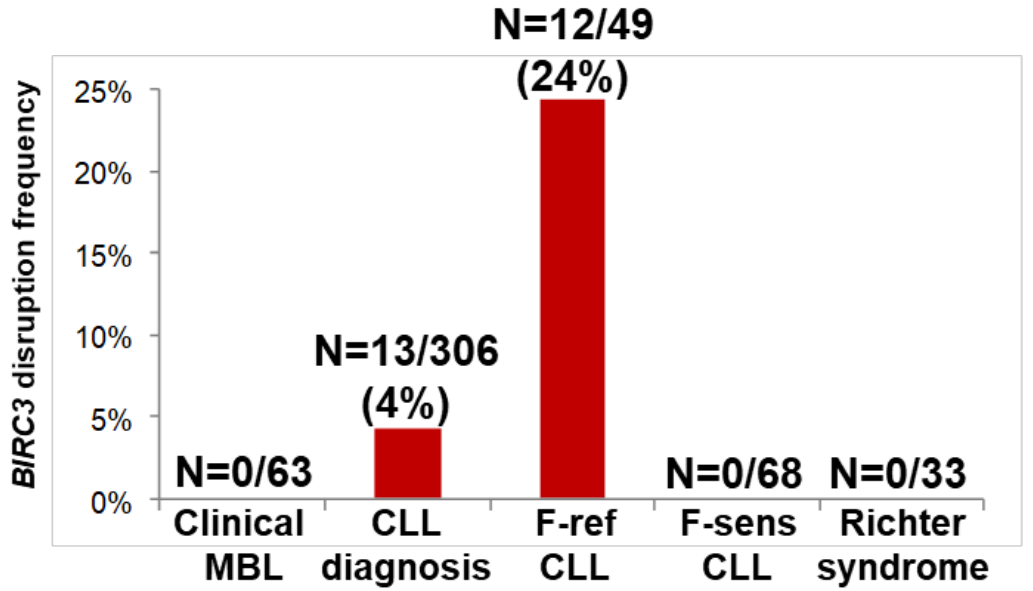
Bert Vogelstein, David Lane and Arnold J. Levine. NATURE|VOL 408 | 16 NOVEMBER 2000 – 307-310

TP53, NOTCH1, SF3B1 and BIRC3 abnormalities in CLL









Data from: Gaidano G. Educational session ASH 2017 Rossi D et al Blood 2011 ;118:6904-8; Rossi D. Blood 2012 ;119:521-9; Rossi D. Blood 2012;119:2854-62

Modalità diverse di resistenza

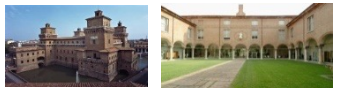
CIT

- Genetic lesions and pathways

  TP53, SF3B1, NOTCH1, BIRC3

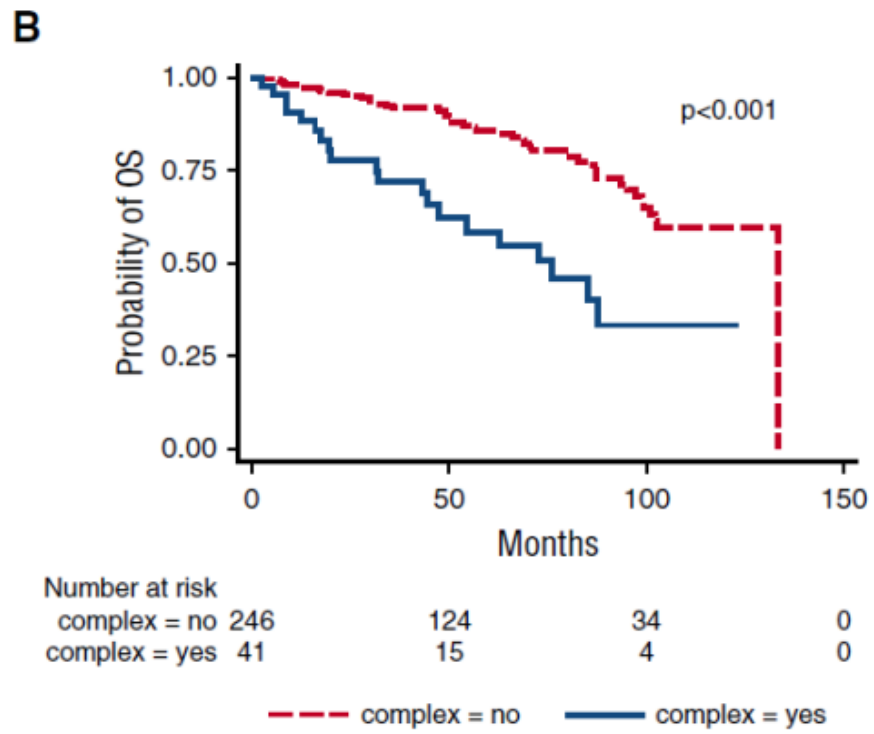
  Genomic complexity

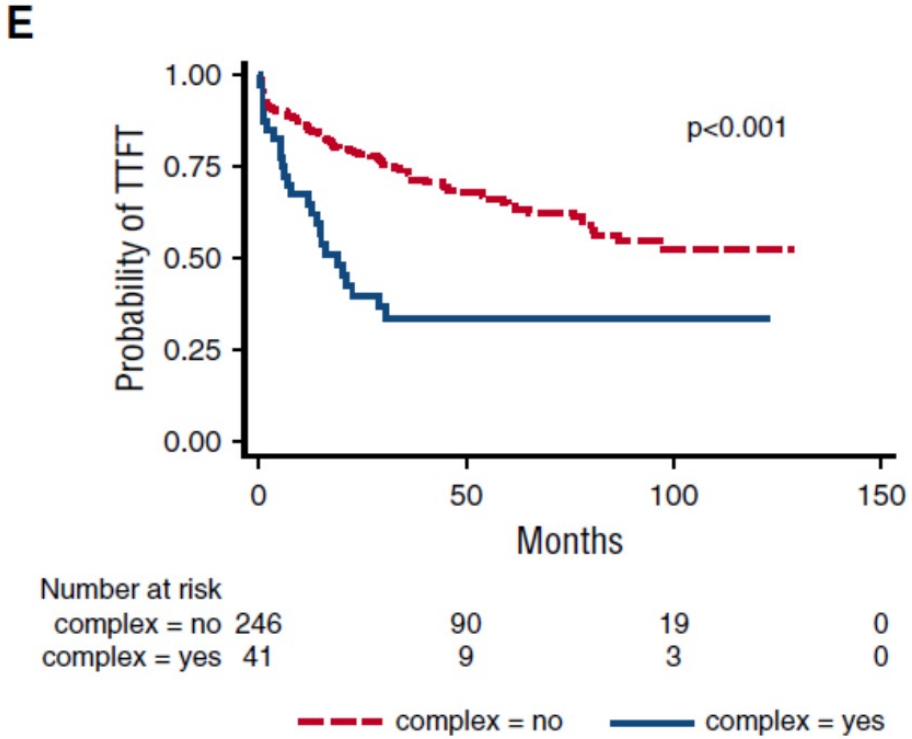
Antonio Cuneo



In CLL the complex karyotype is associated with an inferior outcome

335 untreated CLL patients diagnosed and followed at our center between 2006 and 2016





At MVA, CK confirmed its negative prognostic impact both on OS (p=0.002) and TTFT (p=0.012),
independent of CLL-IPI

Rigolin GM et al. Blood 2017;129:3495-8

Practical consenquences: options for first line treatment of CLL in 2023

no 17p-/no TP53 mutation

Finite duration

Venetoclax+O^^
(12 mos, oral and IV administration)

Ibrutinib + Venetoclax**
(15 mos., oral administration)

FCR only in «fit» and IGHV mutated
(6 mos., IV administration)

Chlor+O/BR only «unfit» and IGHV mutated
(6 mos, oral and IV administration)

Continuous treatment

Acalabrutinib
(take BID)

Ibrutinib
(take once a day)

Zanubrutinib**
(take BID)

17p- and/or TP53 mutation

Acalabrutinib
Ibrutinib
Zanubrutinib**

Ibrutinib + Venetoclax

Venetoclax+O

Idelalisib + R*

^^ In patients non-eligible to FCR in Italiy; *only in patients with contra-indications for other therapies; **not reimbursed in Italia as of june 2023
O=Obinutuzumab; Chlor: Clorambucile; R=Rituximab; F: fludarabine; C: cyclophosphamide; BID: Bis in die

Cuneo A, Rigolin G. https://www.ematologiainprogress.it/sez-leucemia-linfatica-cronica/

Modalità diverse di resistenza

BTKi

- Genetic lesions and pathways
- Time of appearance
- Practical consequences

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Select known mechanisms of BTK inhibitor resistance

Gene or chromosome region affected	Mechanism of BTK inhibitor resistance	B-cell malignancies affected
BTK	Tums covalent bond into noncovalent	CLL, WM, MCL, MZL
PLCγ2	Constitutive activation of BCR signaling pathway	CLL, MCL, MZL
Del(8p)	Downregulation of TRAIL-induced apoptosis	CLL, WM
CARD11	BTK-independent activation of BCR signaling pathway	CLL, DLBCL, FL, MCL, WM
TRAF2, TRAF3, BIRC3, MAP3K14	Constitutive activation of alternative NF-κB pathway leading to cell survival independent of BCR signaling	MCL
ARID2, SMARCA2, SMARCA4	Increased BCL-XL, an antiapoptotic protein, limiting cell death	MCL
MYD88, KLHL14	Promotes assembly of multiprotein complex that constitutively activates NF-κB pathway leading to cell survival independent of BCR signaling	DLBCL
TNFAIP3	Inactivation of negative regulator of NF-κB, which constitutively activates NF-κB pathway leading to cell survival independent of BCR signaling	DLBCL

Byrd J. Blood® 30 SEPTEMBER 2021 | VOLUME 138, NUMBER 13 1099

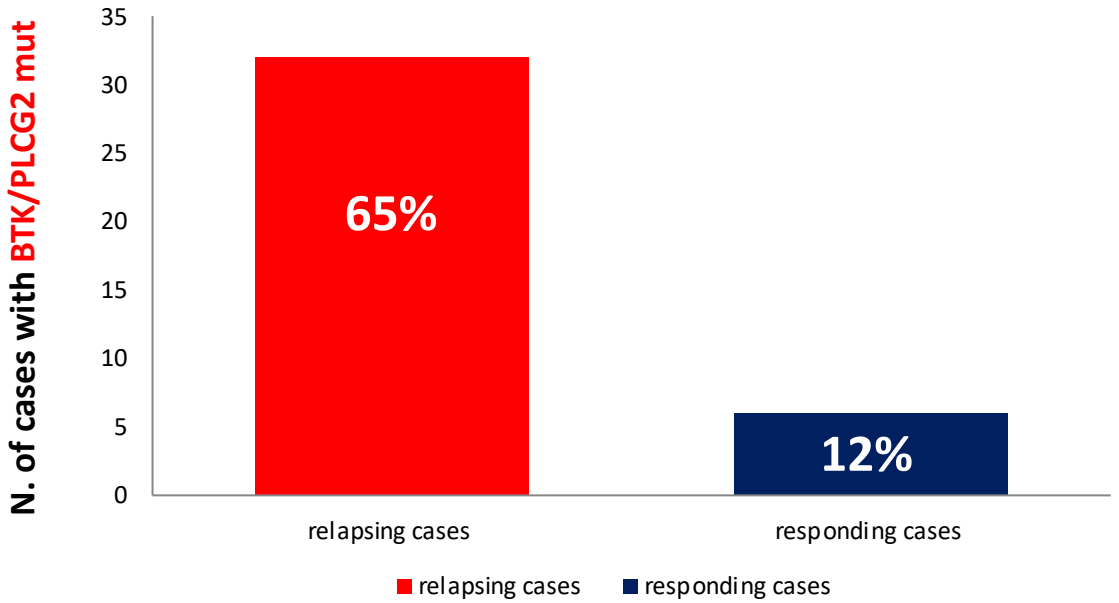
BTK and PLCG2 remain unmutated in one third of patients with CLL relapsing on ibrutinib

98 patients with CLL treated with ibrutinib

- NGS panel (1% sensitivity) comprising 13 CLL-relevant genes including BTK and PLCG2
- BTK hotspot mutations were validated by droplet digital PCR (0.1% sensitivity)

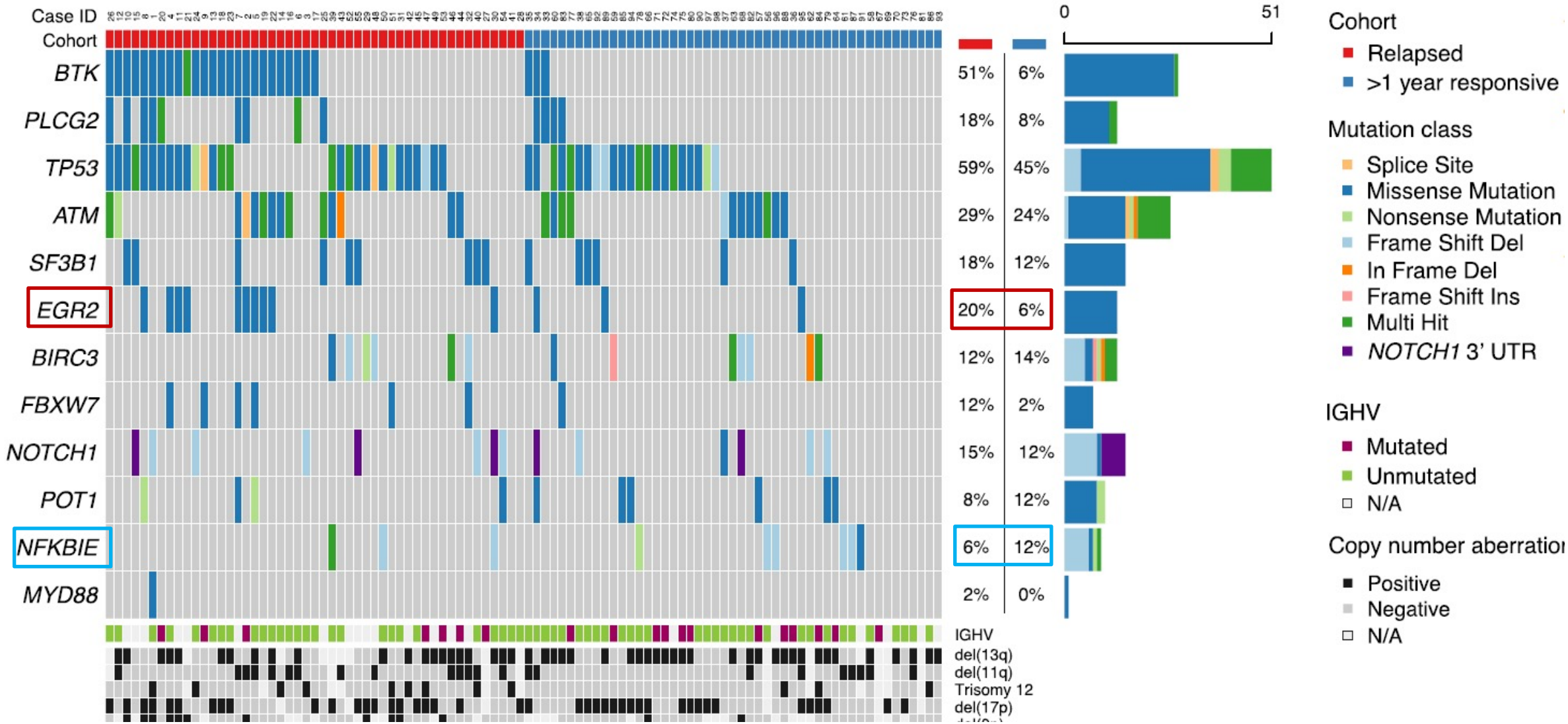
49 relapsing after an initial response

49 still responding after ≥1 year



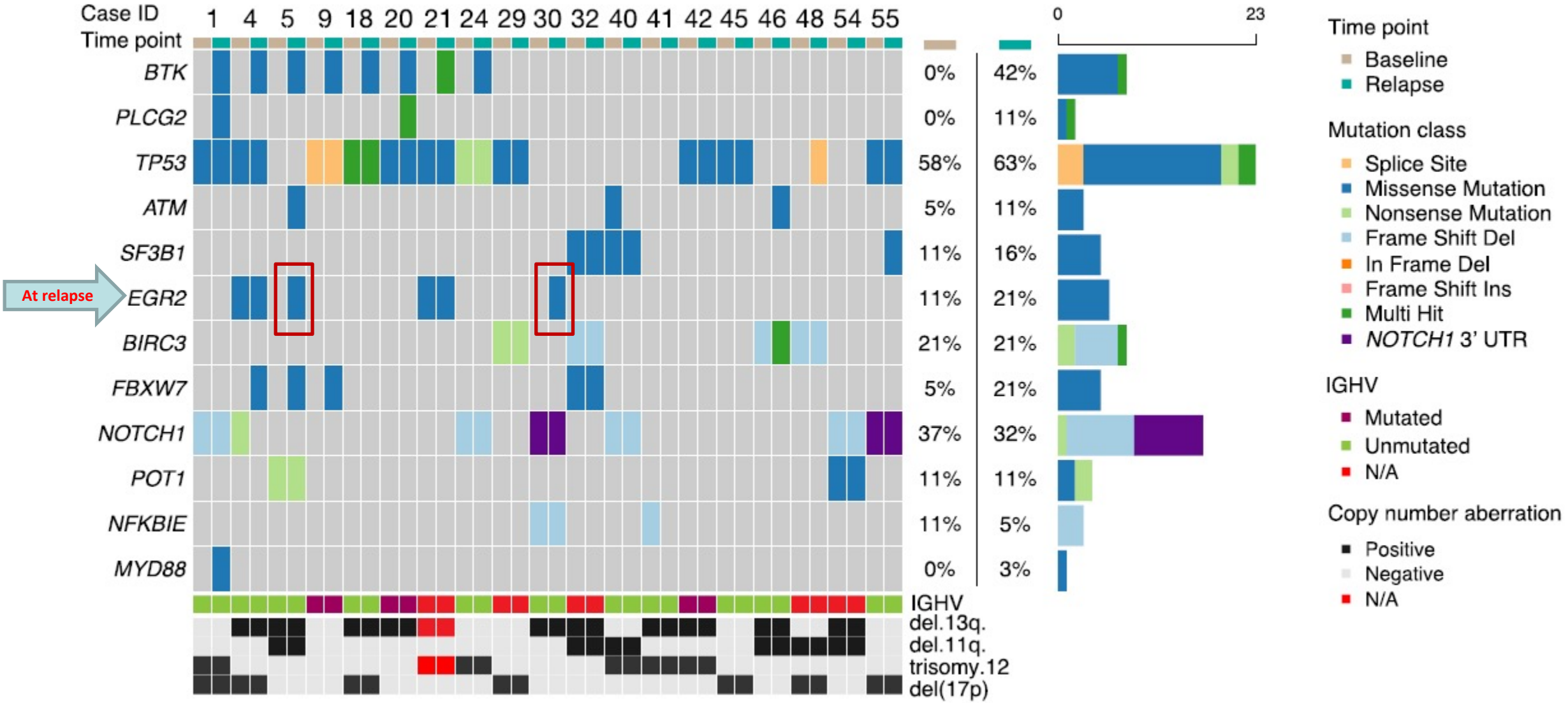
Bonfiglio S. Blood Adv. 2023 Jan 25:bloodadvances.2022008821. doi: 10.1182/bloodadvances.2022008821. Epub ahead of print. PMID: 36696464.

Somatic variants, IGHV mutational status, cytogenetic profile and the mutation frequency of each gene



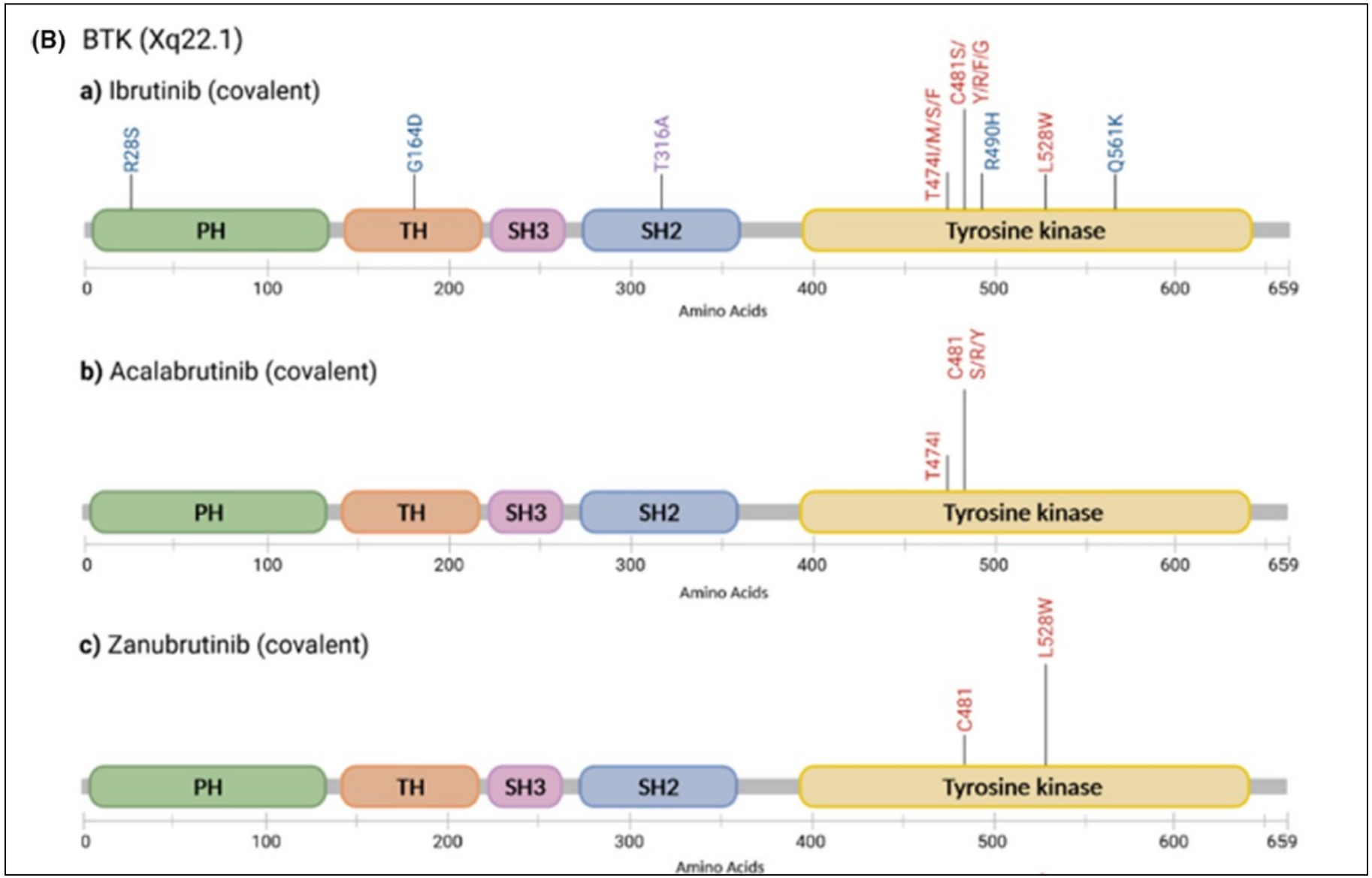
Bonfiglio S. Blood Adv. 2023 Jan 25:bloodadvances.2022008821. doi: 10.1182/bloodadvances.2022008821. Epub ahead of print. PMID: 36696464.

Somatic variants in 19 patients with paired samples (at baseline and at relapse) of the relapsed cohort



Bonfiglio S. Blood Adv. 2023 Jan 25:bloodadvances.2022008821. doi: 10.1182/bloodadvances.2022008821. Epub ahead of print. PMID: 36696464.

Clinically reported BTK mutations in patients treated with ibrutinib, acalabrutinib, zanubrutinib



Nakhoda S. Br J Haematol. 2023 Jan;200(2):137-149..

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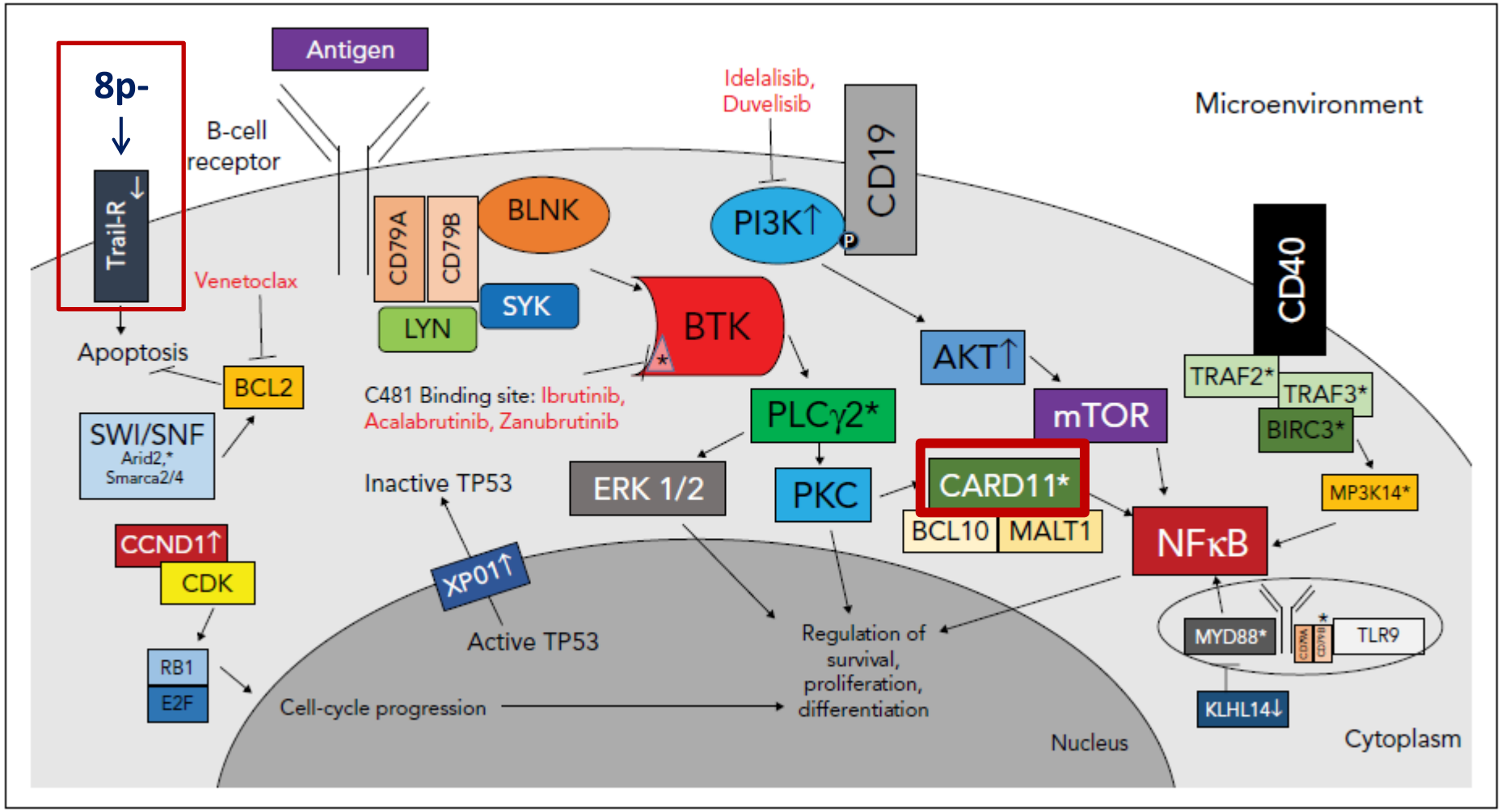
Overview of the BCR pathway with highlighted mechanisms of BTKi resistance



*Location of known BTKi resistance mutation

TRAIL: tumour necrosis factor related apoptosis-inducing ligand

Modalità diverse di resistenza

BTKi

- Genetic lesions and pathways
- Time of appearance
- Practical consequences

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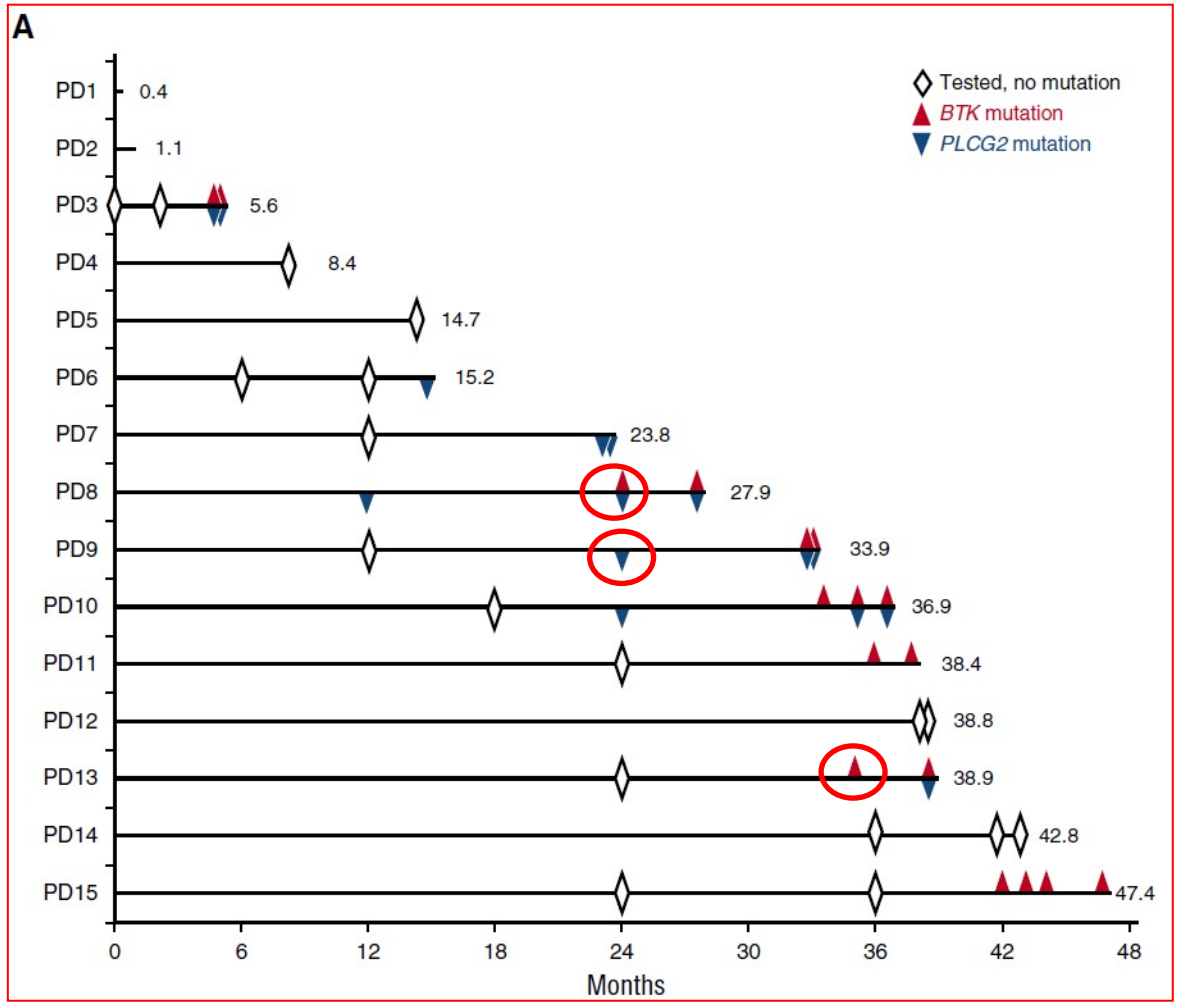
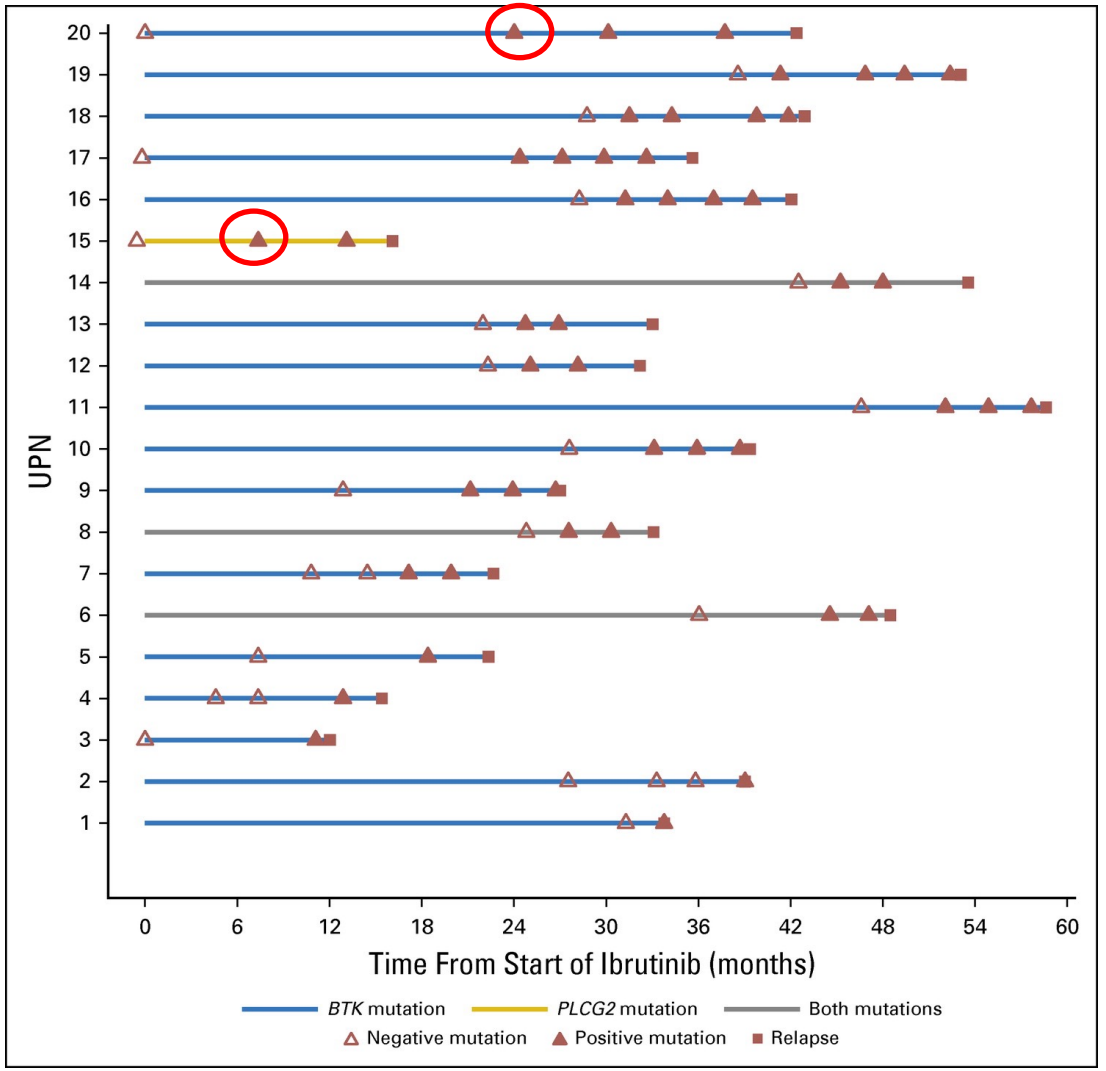
Clonal evolution during treatment with ibrutinib

15 patients who progressed on ibrutinib



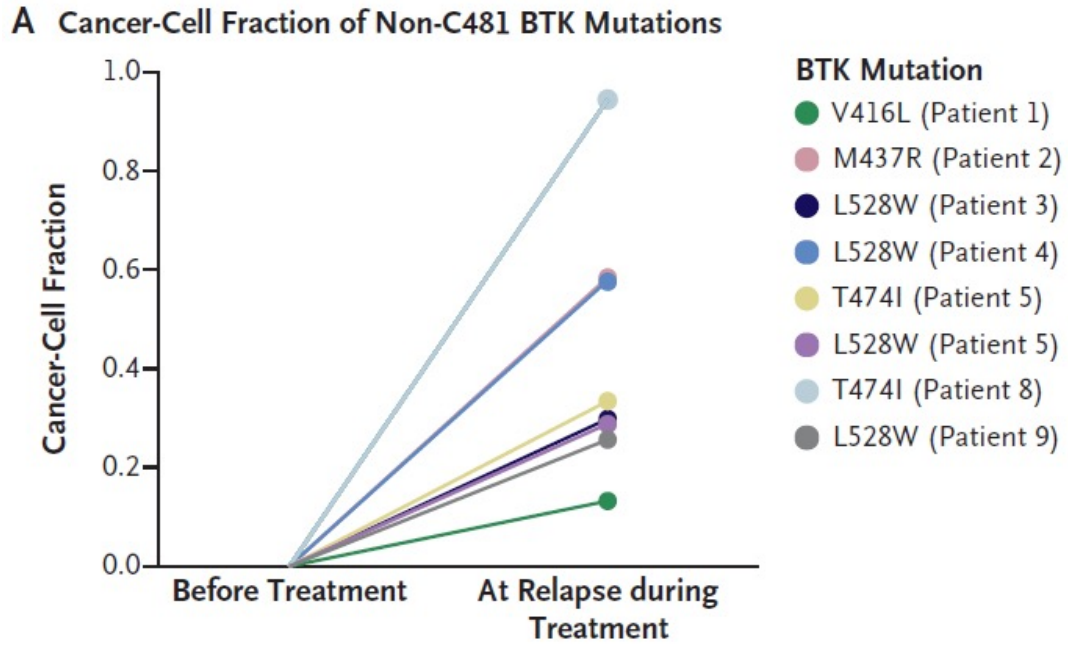
Ahn I et al. Blood. 2017;129(11):1469-1479

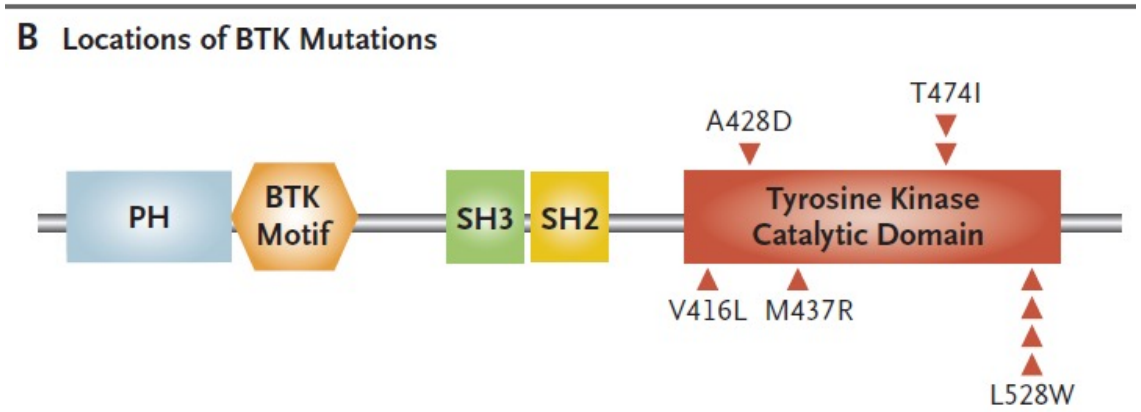
20 patients who progressed on ibrutinib

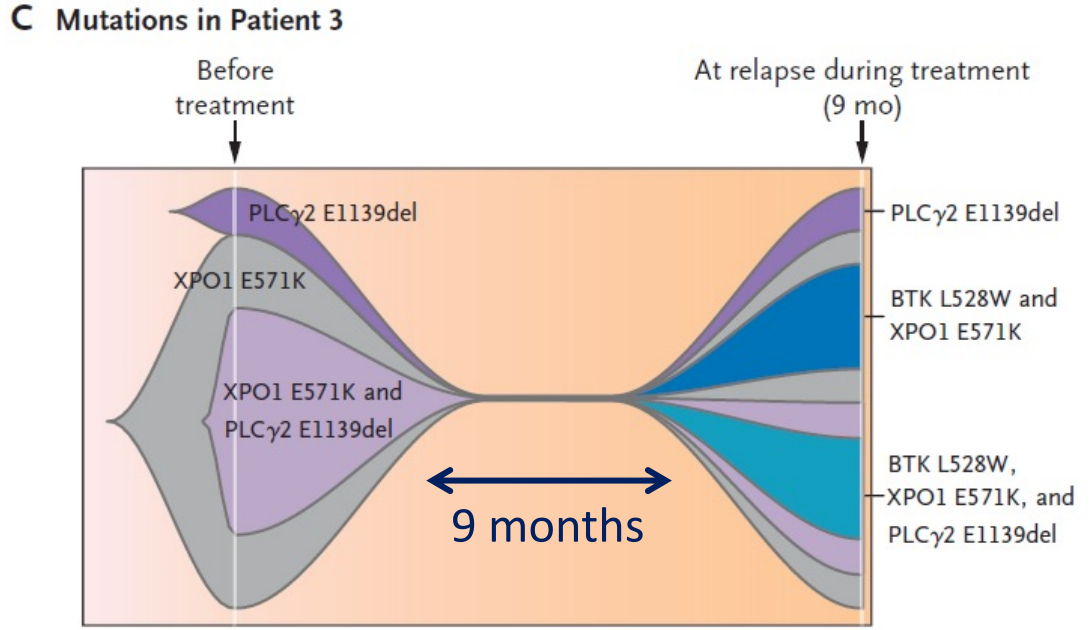


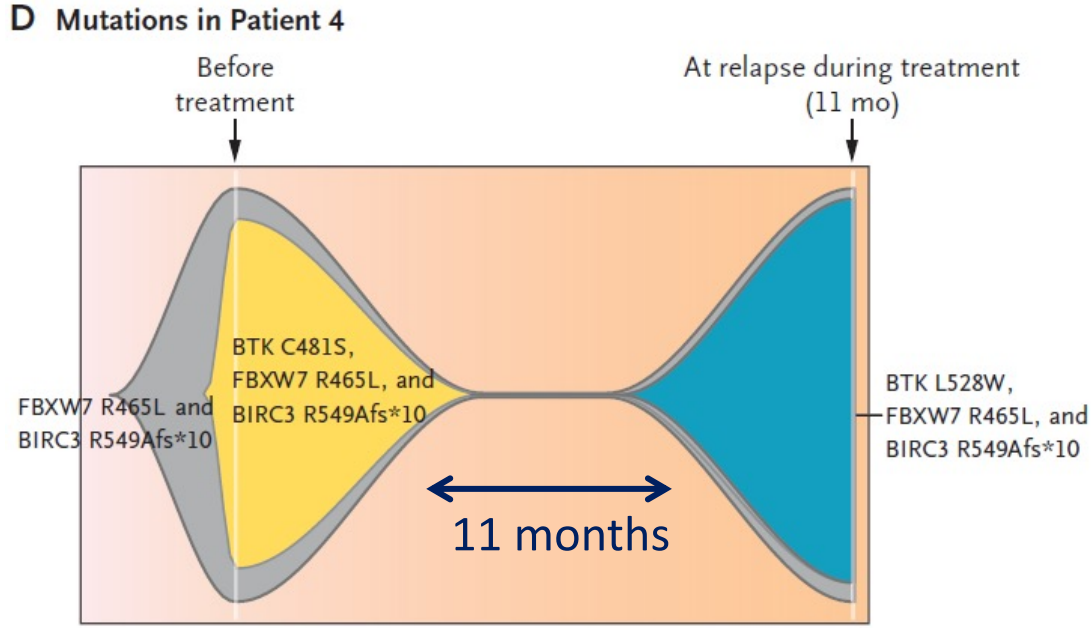
Woyach J. J Clin Oncol 35:1437-1443. © 2017

BTK Mutations in 9 CLL with Acquired Resistance to the Noncovalent BTK Inhibitor Pirtobrutinib









Wang E. N Engl J Med 2022;386:735-43

Modalità diverse di resistenza

BCL2i

- Genetic lesions and pathways
- Time of appearance
- Practical consequences

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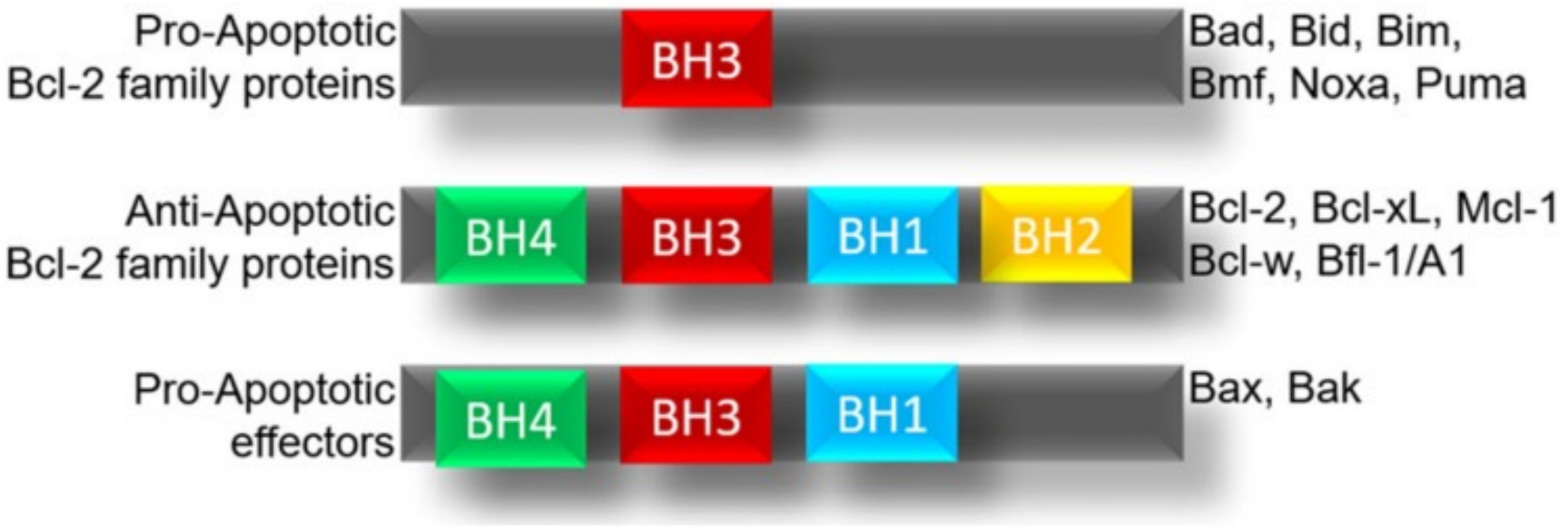
Members of the BCL-2 family and their role
Schematic representation of the members of BCL-2 family and his subunits

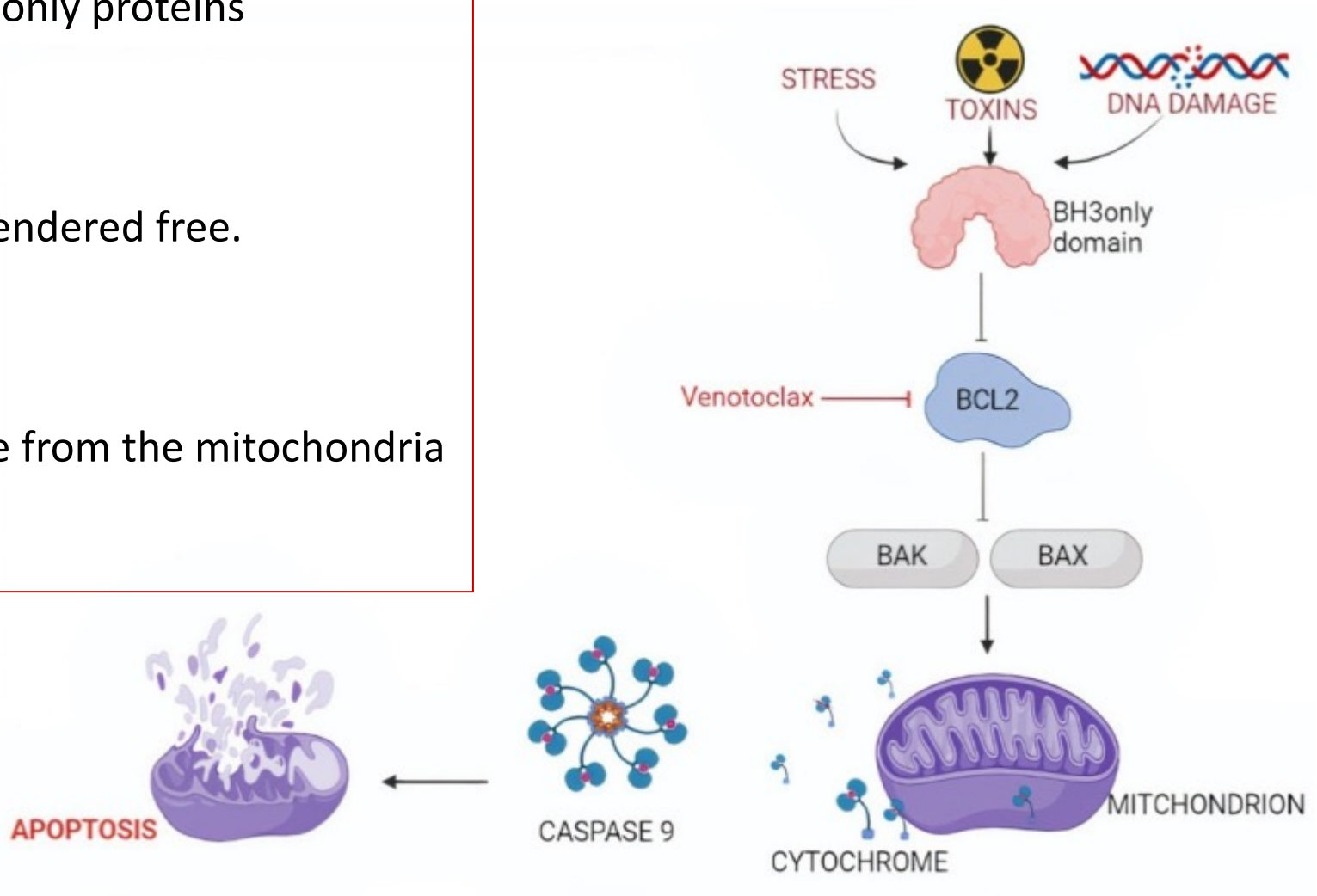


BH: Bcl-2 Homology domain, necessary for protein function

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

Targeting the intrinsic pathway of apoptosis

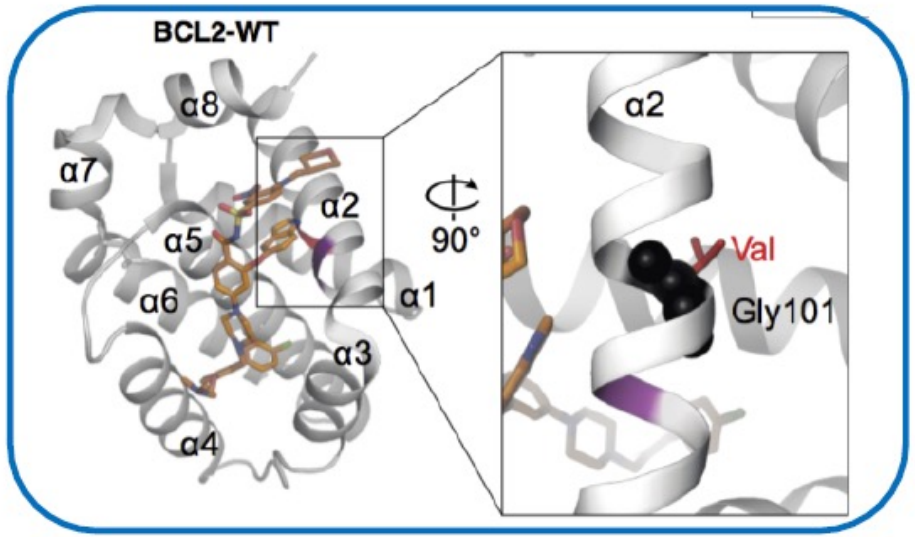
- Pro-apoptotic factors trigger BH3-only proteins
- Binding and inhibition of BCL2.
- Larger amounts of BAK and BAX rendered free.
- BAK and BAX dimerize
- Channel for Cytochrome-c leakage from the mitochondria
- Apoptosis



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

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

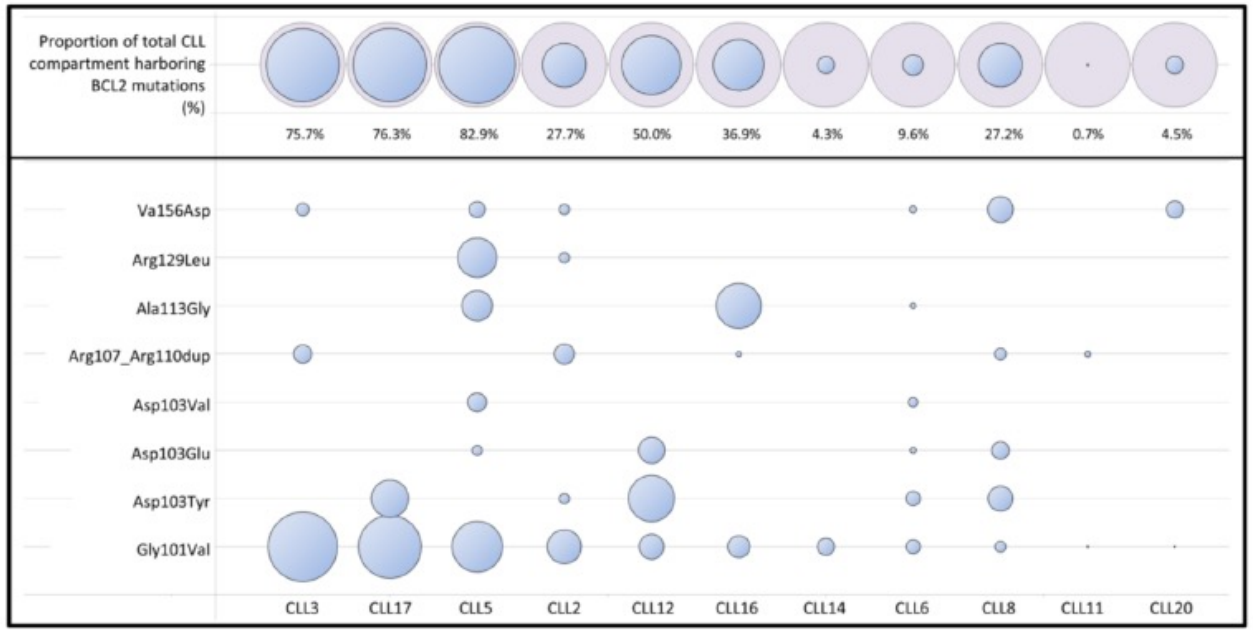
Blombery *Cancer Discovery* 2019; Birkinshaw *Nat Comms* 2019

Courtesy of Andrew Roberts, Melbourne, Australia

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

XIX IWCLL International Workshop on CLL

Courtesy of Andrew Roberts, Melbourne, Australia

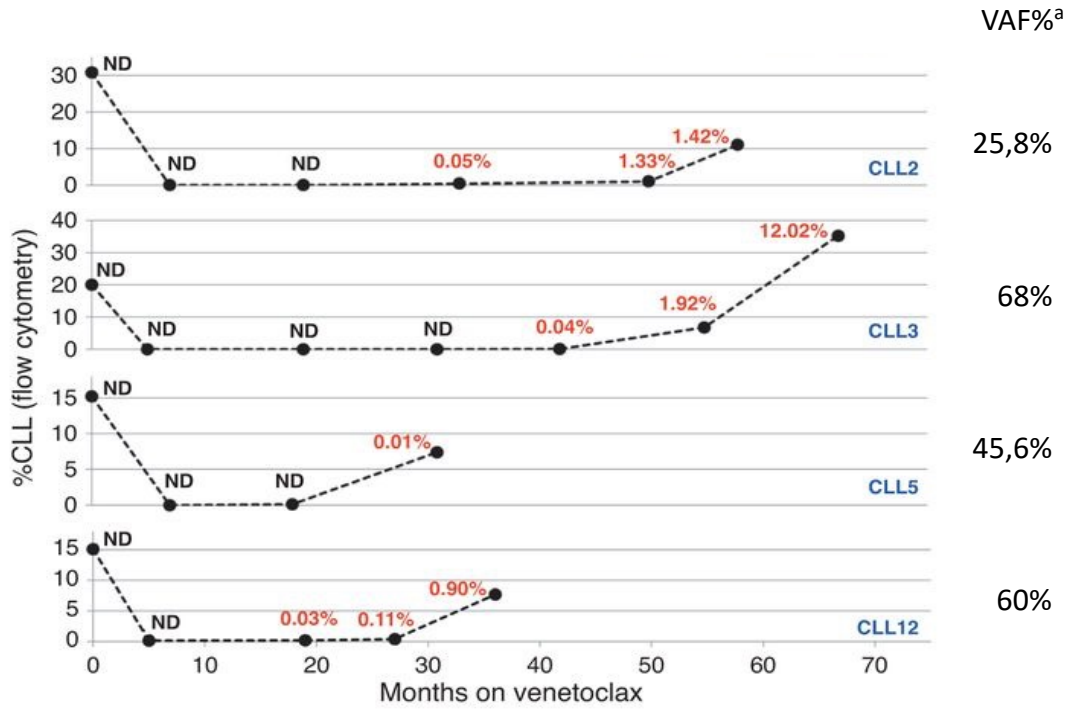
BCL2 Gly101Val is specific for CLL with resistance to Venetoclax

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

Time course of CLL response and subsequent emergence of Gly101Val mutation during venetoclax therapy

The CLL burden was measured by multi-parameter flow cytometry in serial BM aspirates from 4 patients from the initiation of venetoclax until the clinical diagnosis of progressive disease



[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.

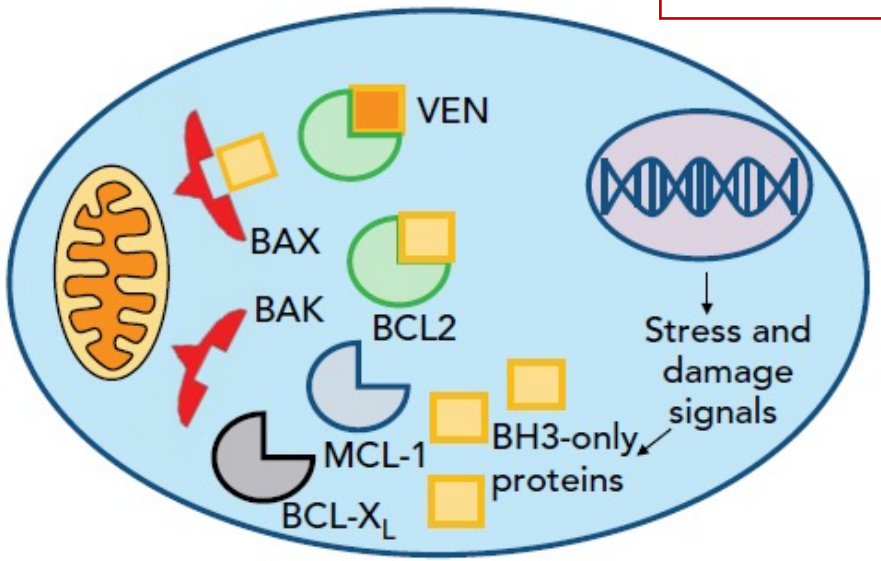
Blombery et al. Cancer Discov 2019

Different mechanisms account for VEN 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



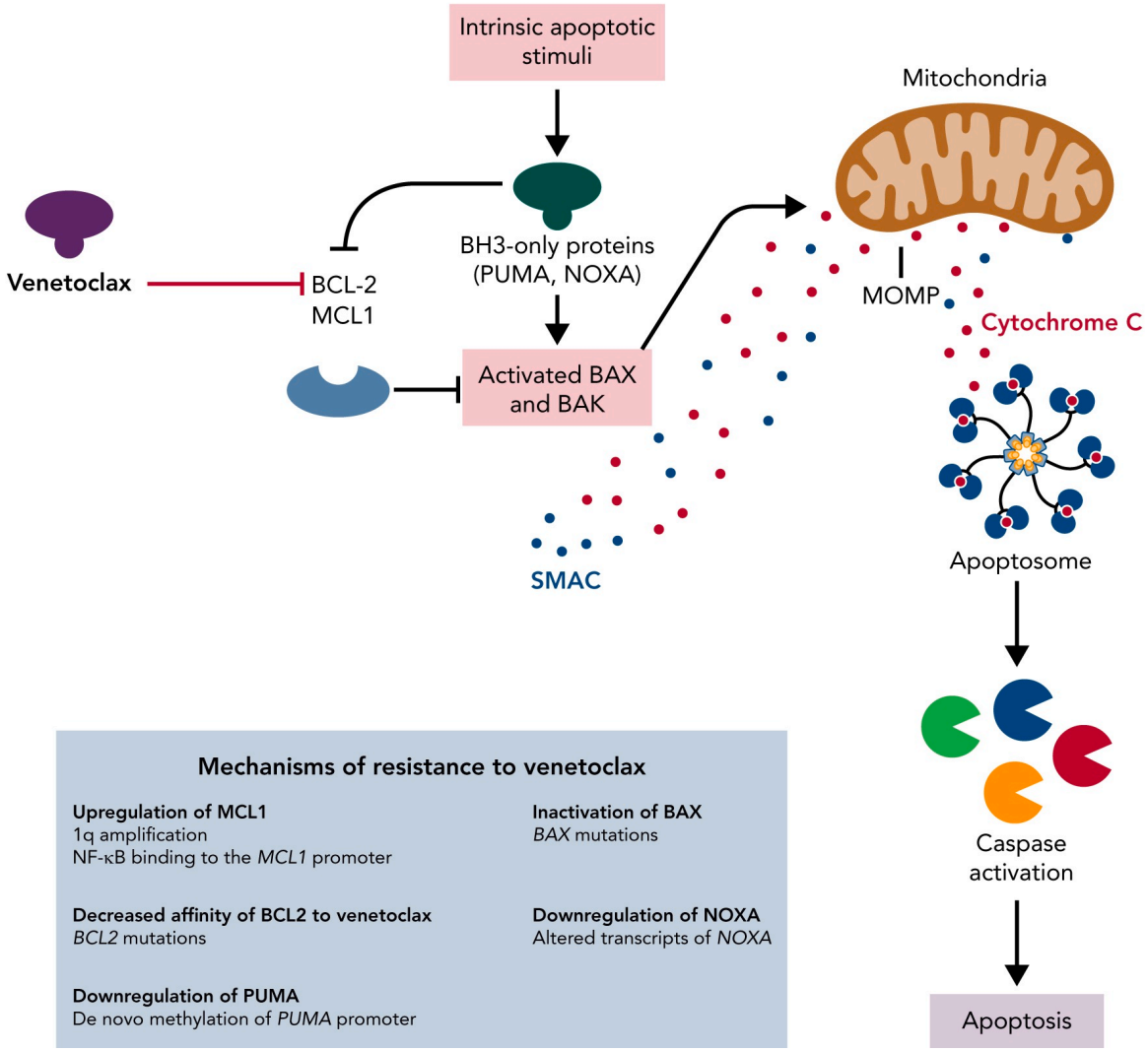
Genomic instability
- Acquisition of *TP53* aberrations
- Evolution of complex karyotype

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

Early events?

Modified from: Lew TE et al, Blood 2021

Mechanisms of resistance to venetoclax

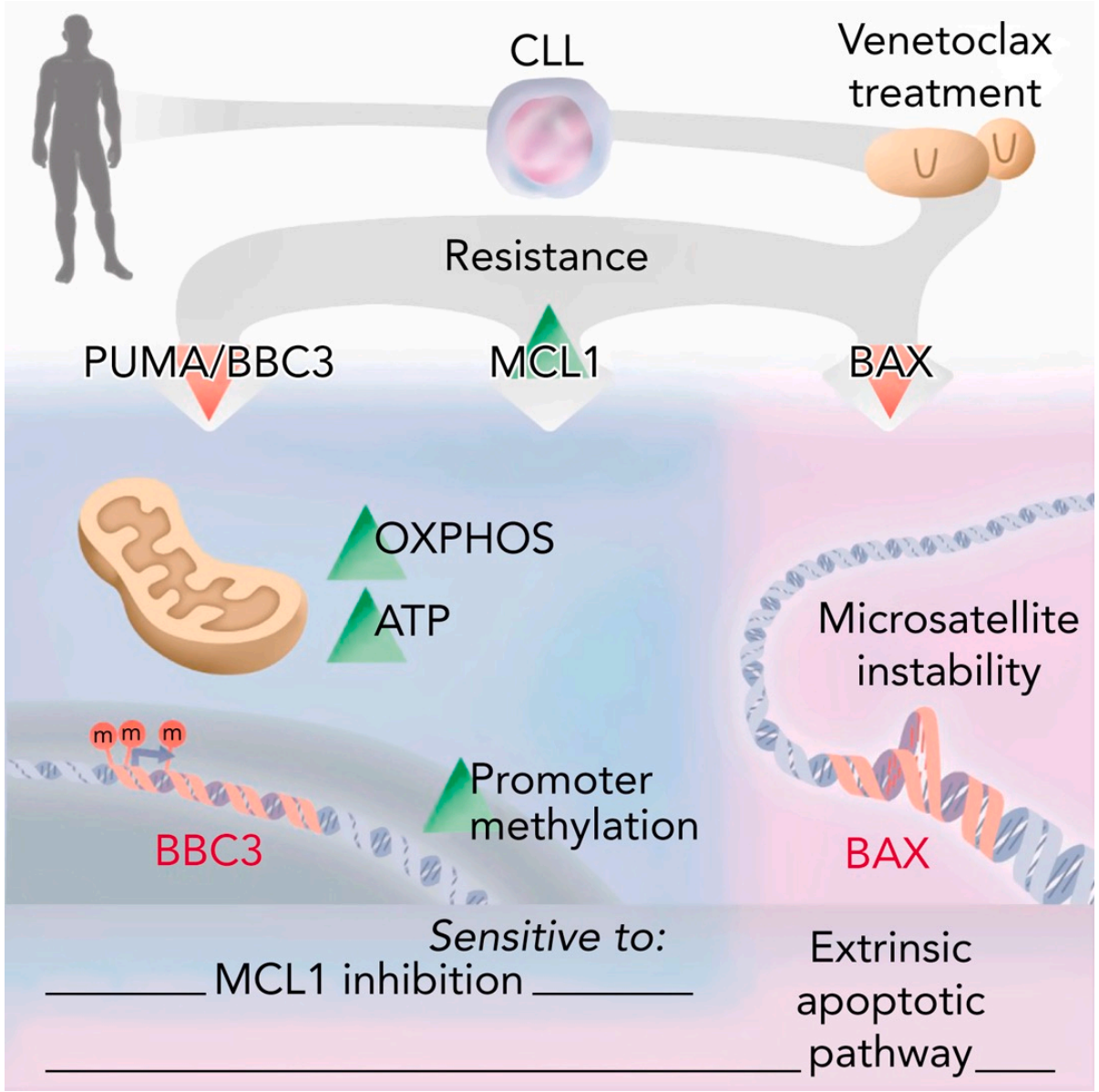


- De novo methylation of the PUMA promoter (downregulation of PUMA expression)
- MCL1 overexpression dominates in venetoclax-resistant clones
- Marked NF-κB activation and NF-κB binding to the MCL1 promoter resulted in increased MCL1 expression in relapses occurring on venetoclax therapy.
- Multiple mechanisms of escape (eg, 1q24 amplification, BCL2 mutations, BAX mutations, and altered transcript of proapoptotic NOXA) may coexist in the same tumor, usually restricted to different subclones
- Some of these escape mechanisms are unique, others are found in multiple patients

MOMP, mitochondrial outer membrane permeabilization; SMAC, second mitochondria-derived activator of caspase

Adalgisa Condoluci, Davide Rossi, Blood, 2022
Thomalla D, Blood. 2022;140(20):2113-2126.

Deregulation and epigenetic modification of BCL2-family genes cause resistance to VEN in hematologic malignancies



A CpG island within the PUMA promoter is methylated upon VEN treatment

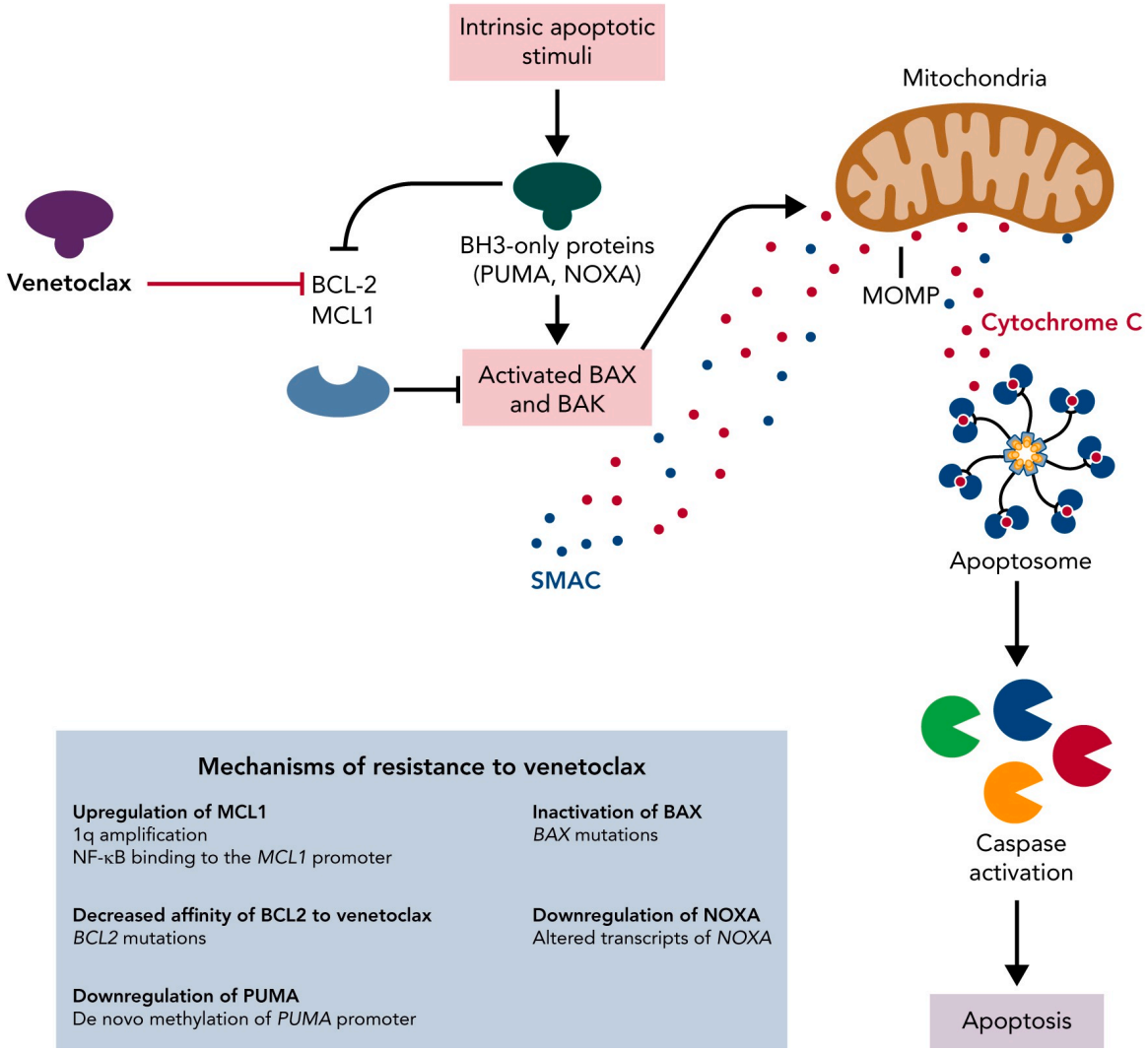
Higher oxidative phosphorylation and adenosine triphosphate production, resembling the metabolic phenotype that is seen upon VEN resistance

PUMA loss is specific for acquired VEN resistance but not for acquired MCL1 resistance

BAX is essential for sensitivity toward both venetoclax and MCL1 inhibition

Thomalla D, Blood. 2022;140(20):2113-2126.

Mechanisms of resistance to venetoclax



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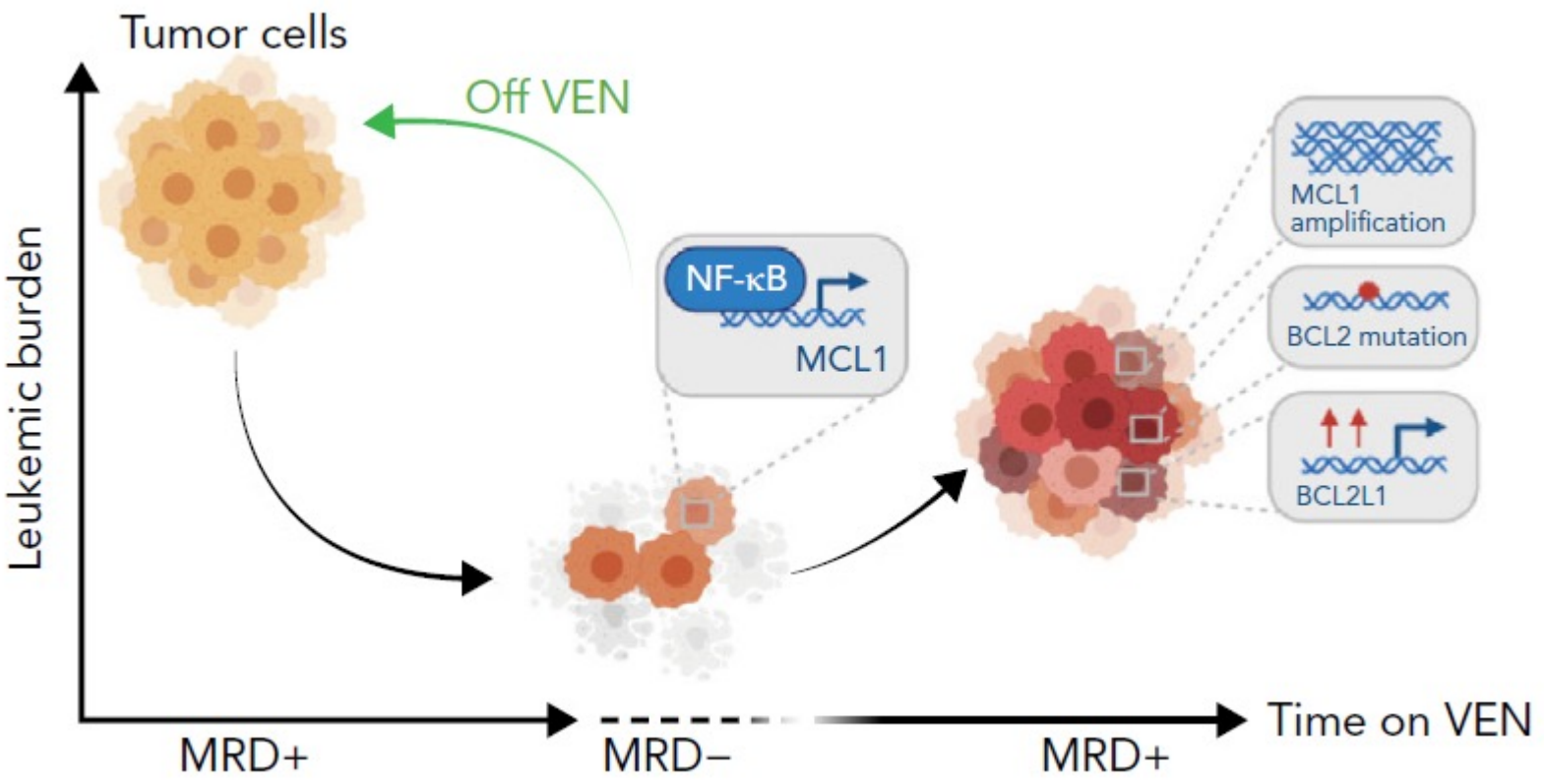
Adalgisa Condoluci, Davide Rossi, Blood, 2022

Thijssen R, Blood. 2022; 140:2127-2141.

Mechanisms of resistance to venetoclax: study highlights

Epigenetic changes of resistance (ie, NF-κB–promoted upregulation of MCL1) are driven and sustained by ongoing venetoclax therapy and they disappear once venetoclax therapy is stopped



Thijssen R, Blood. 2022; 140:2127-2141..

Take home messages

Causes of resistance	Implications
• Selection of CIT resistant clones with TP53, SF3B1, NOTCH1, BIRC3 mutations and complex karyotype	CIT is no longer an option in the vast majority of patients
• BTK and PLCG2 mutations account for ≈2/3 of acquired resistance to covalent BTKi and were detected in patients with acquired resistance to non-covalent BTKi • BTK/PCLG2 mutation may precede relapse by many months and may occurr in multiple independent clones	Sequential studies to detect mutations are not necessary The presence of BTK/PCLG2 mutations should prompt careful monitoring
• BCL2 mutations account for VEN resistance in ≈ 50% of cases and anticipate disease progression by many months • VEN resistance is multilayered and include epigenetic reprogramming	Sequential studies to detect mutations are not necessary Reversible epigenetic programming support fixed duration treatment