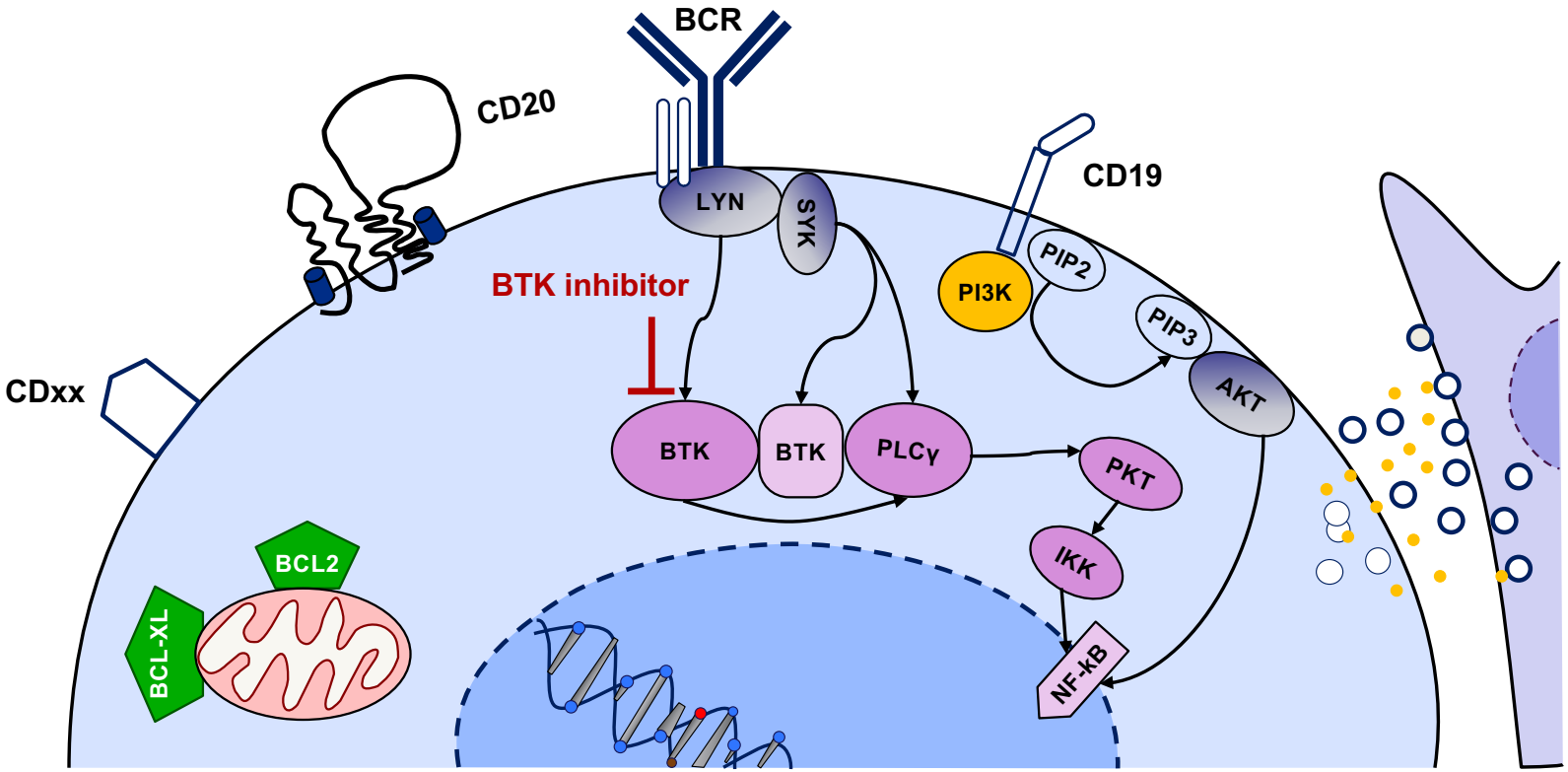
I MECCANISMI DI RESISTENZA ALLE TARGET THERAPIES

Lydia Scarfò

Strategic Research Program on CLL - Lab of B Cell Neoplasia
Division of Experimental Oncology

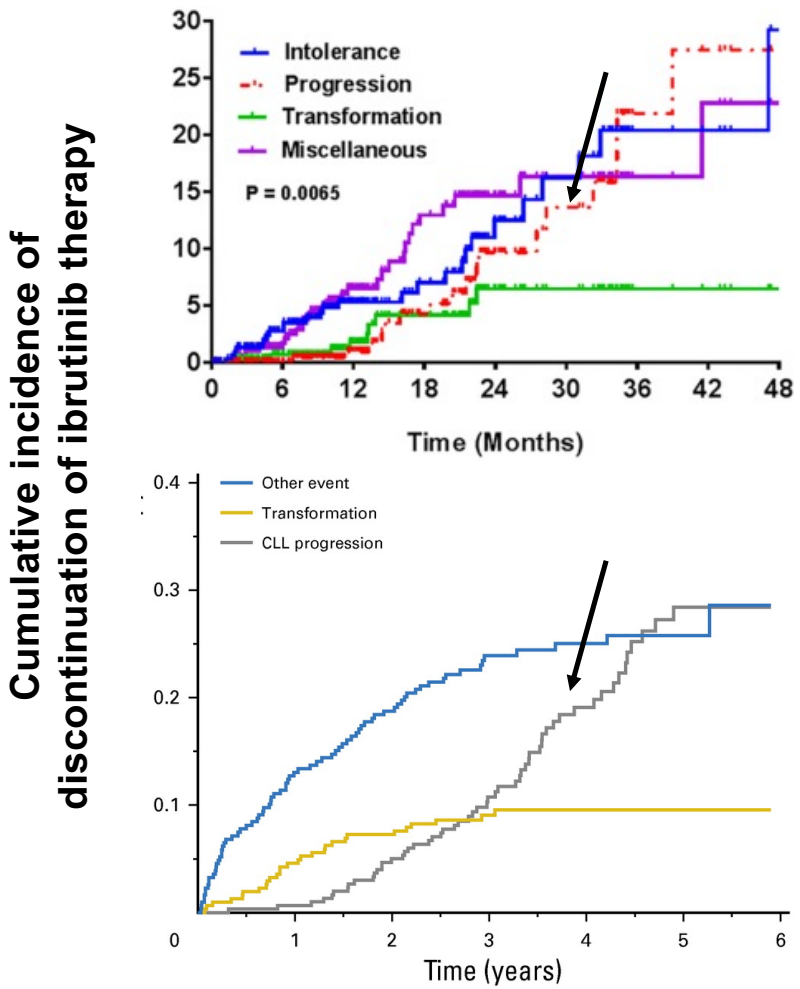
Università Vita-Salute San Raffaele - Milano
IRCCS Ospedale San Raffaele - Milano

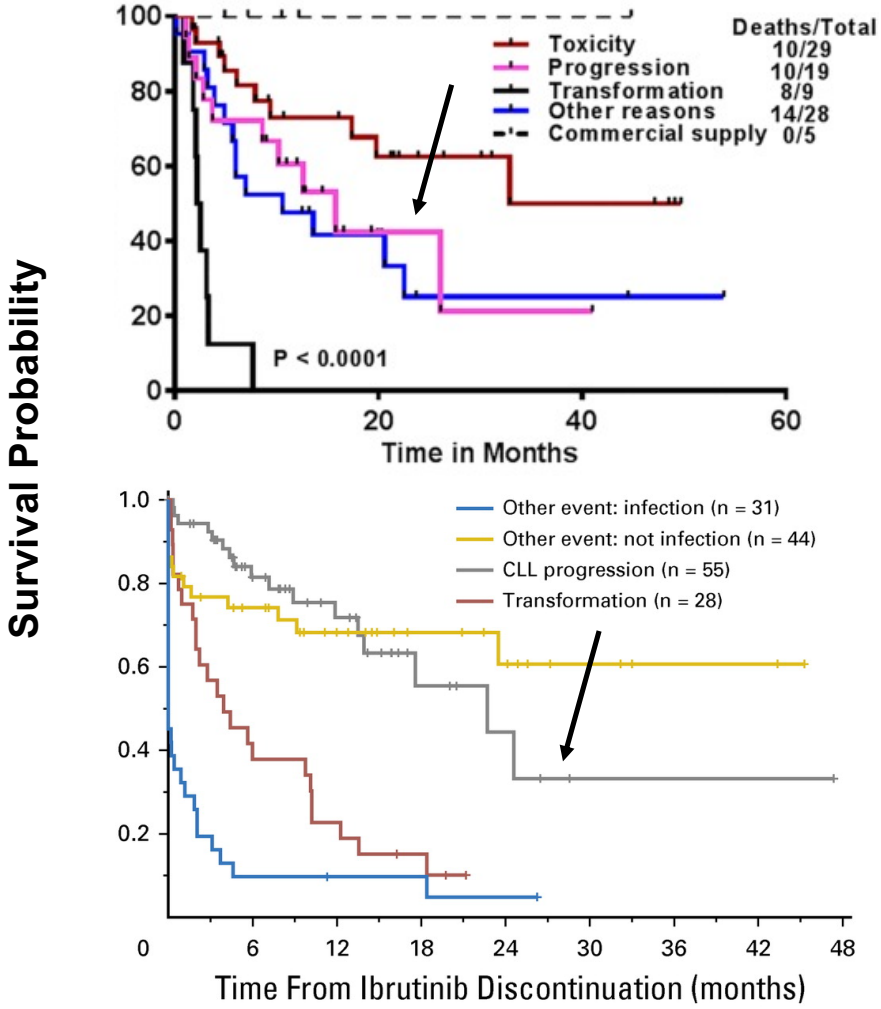
Ibrutinib Resistance



Adapted from Byrd J, et al. J Clin Oncol 2014

Ibrutinib discontinuation and outcome



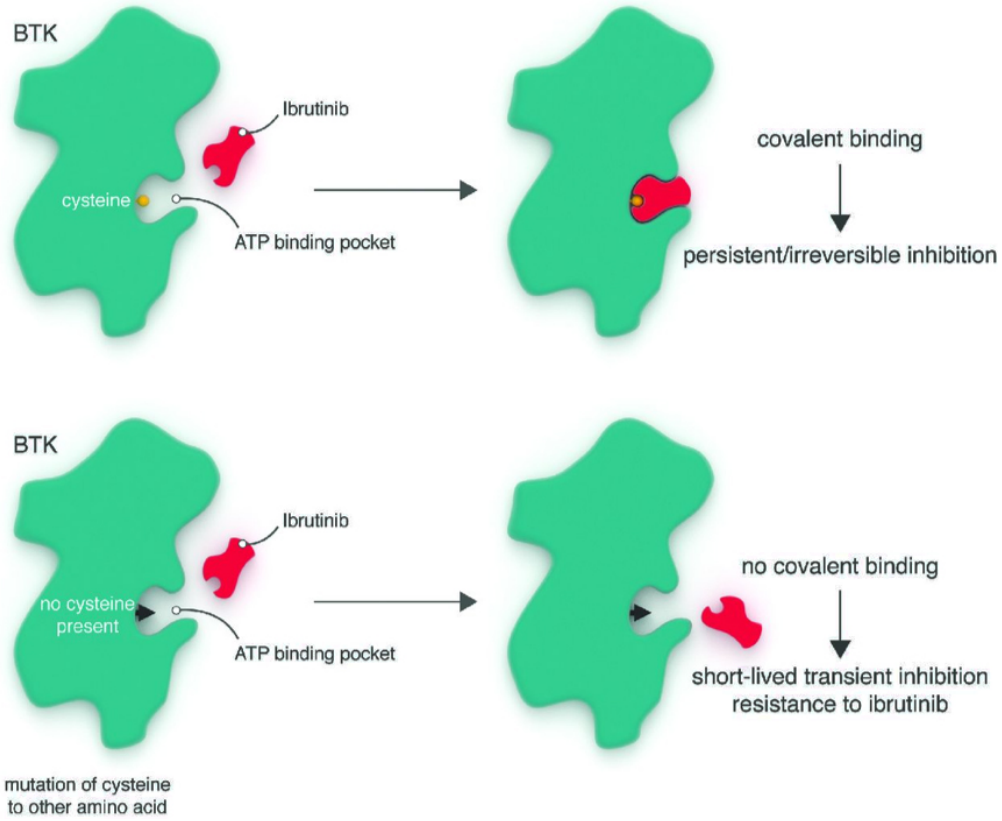


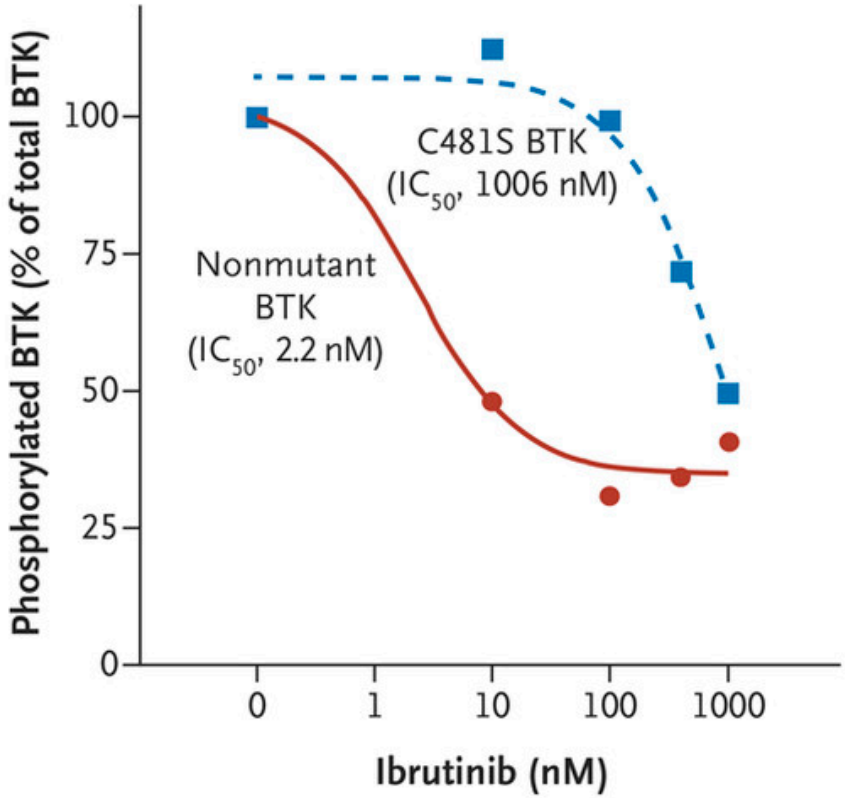
Jain et al. Cancer 2017; Woyach et al. JCO 2017

BTK and *PLCG2* mutations in CLL patients relapsed on ibrutinib

Ref.	Pts with mut/total	Analytical Method	*BTK* mutations	VAF %	*PLCG2* mutations	VAF %
Woyach, 2014	6/6	WES	C481S	17-60	R665W; L845F S707Y	8-38
Ahn, 2017	8/10	High sensitivity NGS; ddPCR	C481S C481R	1.6-78.2 15.8	6nt del; R665W P664S; S707Y; L845F	0.1-18.3
Woyach, 2017	40/46 (retrospective); 8/8 (prospective)	Targeted Deep Sequencing	C481S C481R C481F C481A	0.2-94.8 18.1 1.1-100 45.3	R665W; S707P; S707F; S707Y; L845F; D993Y; L845/846del	3.6-44
Burger, 2016	2/4	WES; ddPCR	C481S	NR	M1141R; S707F; M1141K; D993H	12.0-35.0 (CCF)
Landau, 2017	5/7	Targeted Deep Sequencing	C481S	2.2-78.2	R665W; S707Y; L845F	0.2-4.7
Kadri, 2017	1/3	Targeted Deep Sequencing	C481S C481R	8.5-90.0 2.5	NR	NR
Kanagal-Shamanna, 2019	16/23	Targeted Deep Sequencing	C481S; C481F C481R; C481Y V537I	11(1-91)	None	NA

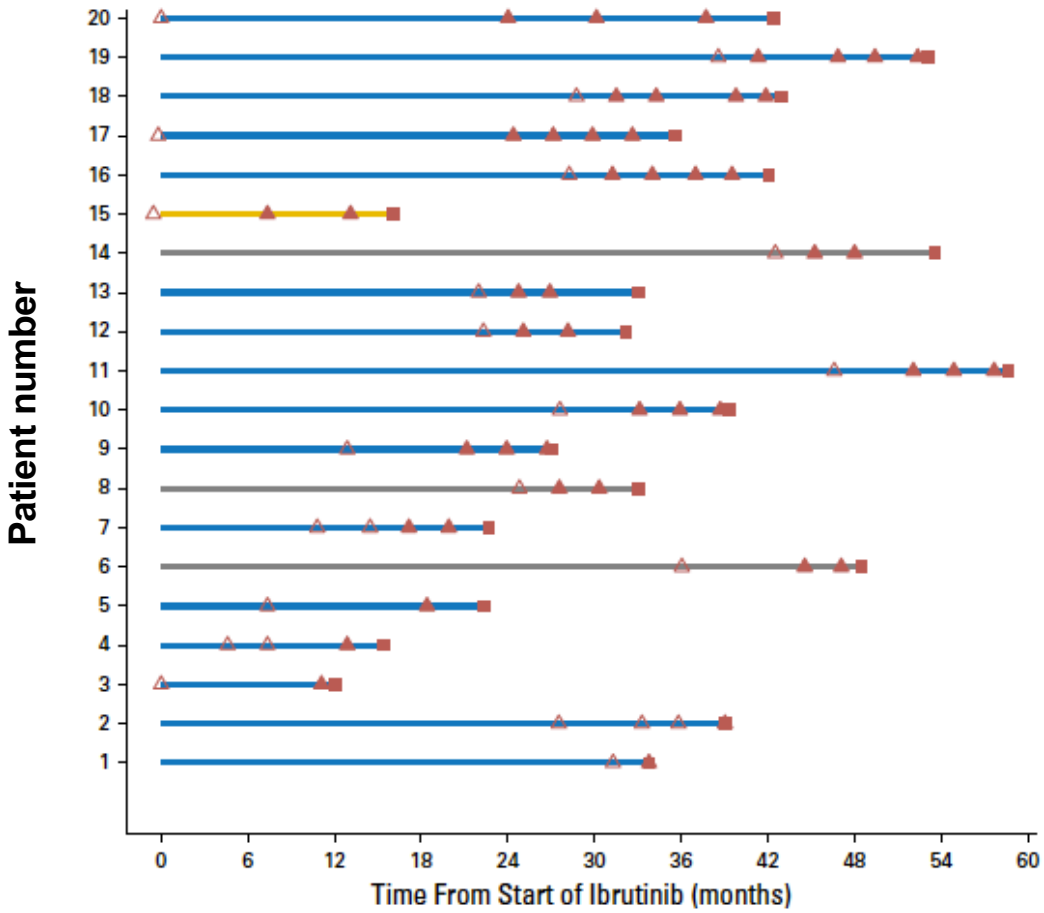
Functional characterization of *BTK* mutations





Wiestner et al. Haemtatologica, 2015 , Woyach et al. NEJM 2014

Duration of ibrutinib treatment in relapsed cases

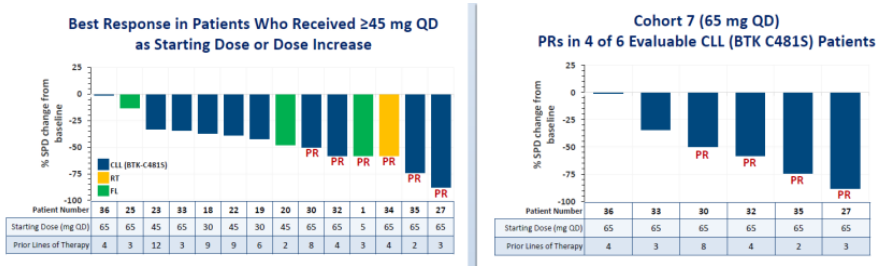


Among 20 patients with CLL progression BTKi resistance mutations could be detected 9.3 months before clinical progression

Adapted from Woyach JA et al. N Engl J Med 2014

Novel BTK inhibitors inhibit C481S BTK mutants

ARQ 531 - ArQule

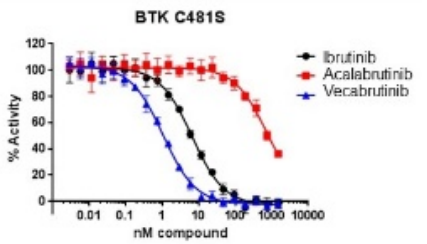


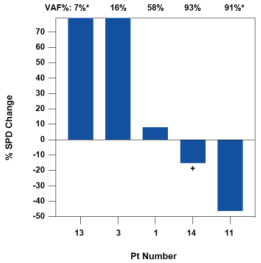
Woyach et al. EHA 2019

Vecabrutinib - Sunesis

BTK Inhibition – Kinase Assays			
IC_{50} nM	WT BTK	C481S BTK	Fold Change
Vecabrutinib	4.6	1.1	0.2
Ibrutinib	0.1	6.6	66
Acalabrutinib	4.2	707	168

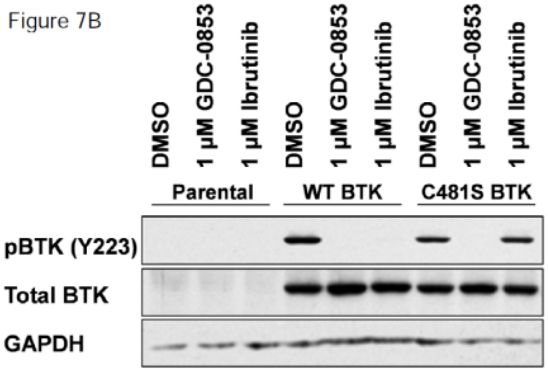
[ATP] = 50 μM.





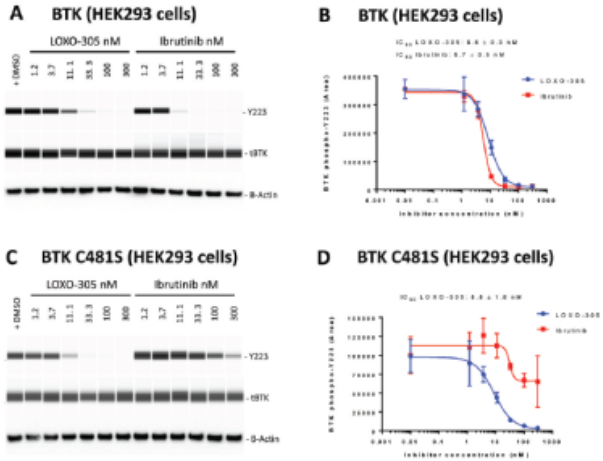
Allan et al. EHA 2019

GDC583 - Genentech



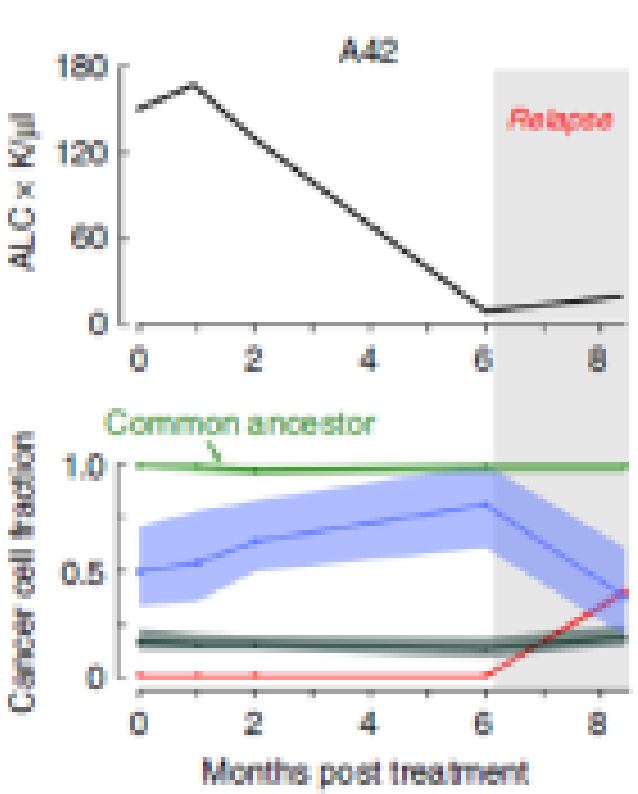
Reiff et al. Blood 2018

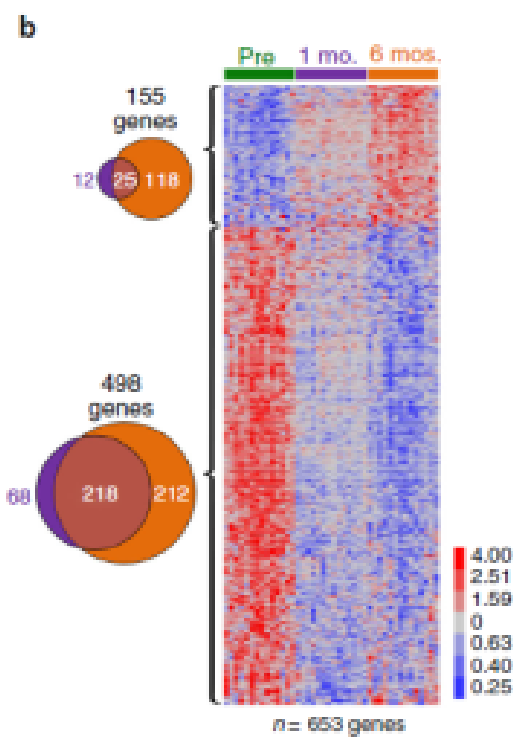
Loxo-305 - Loxo Oncology

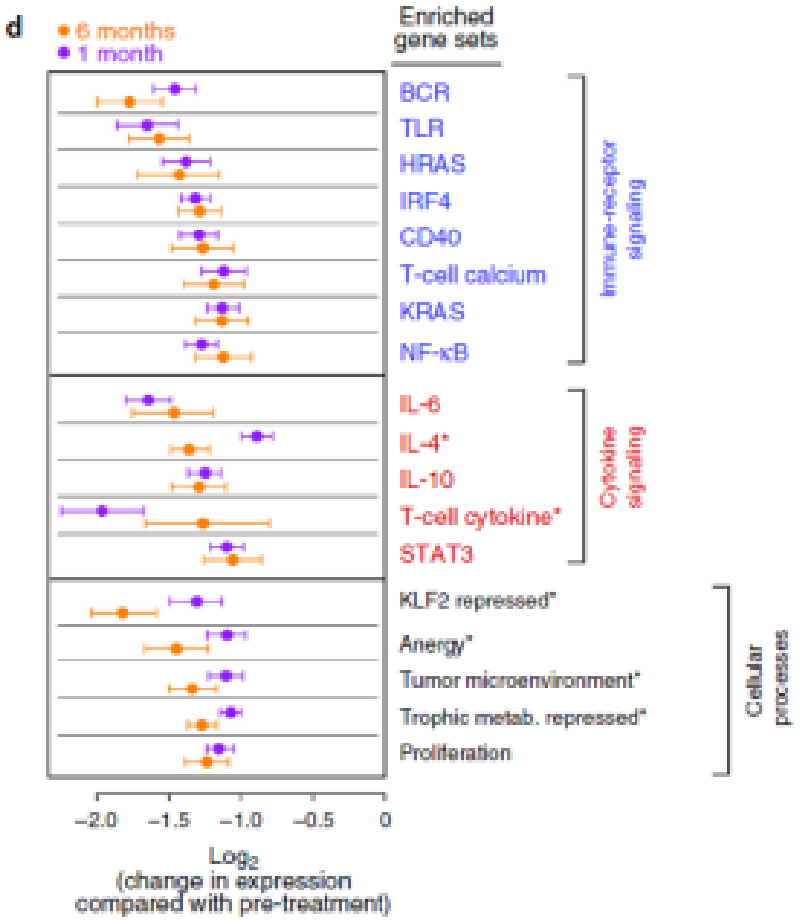


Brandhuber et al. SOHO 2018

Disease progression without BTK or PLCG2 mut

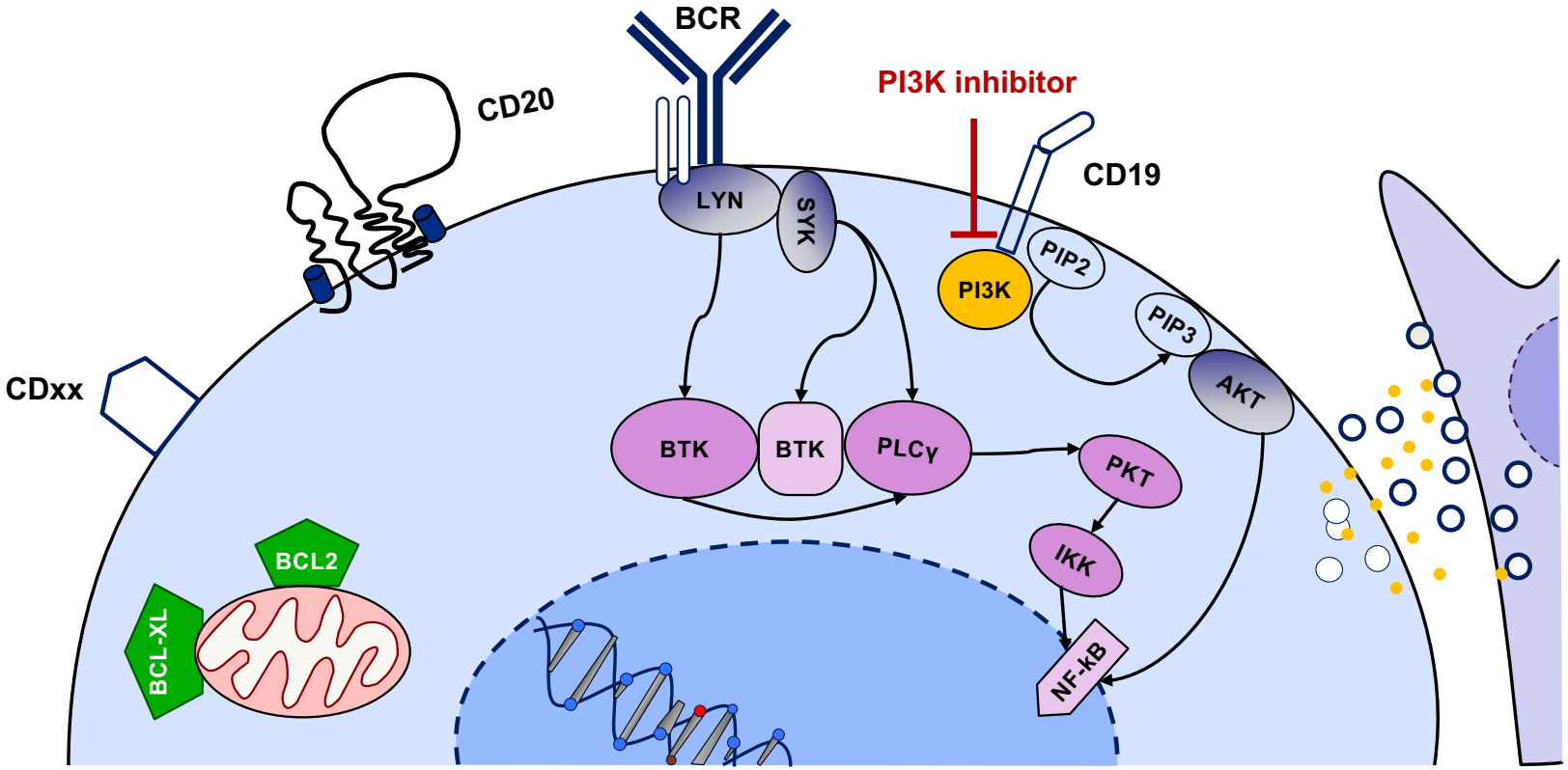






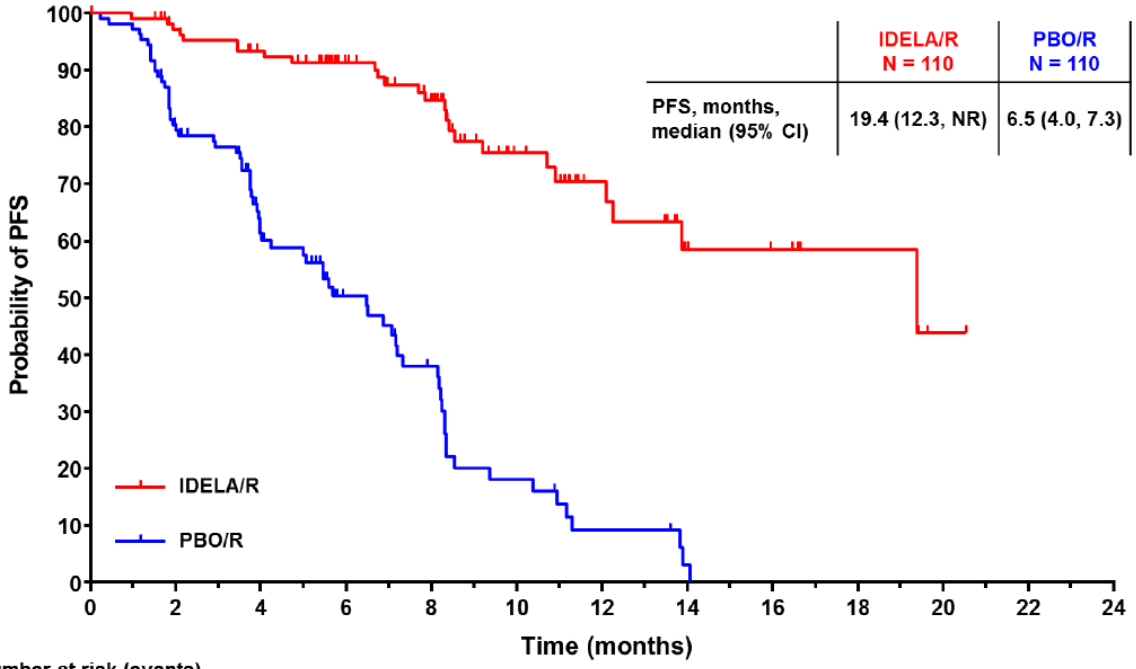
Landau et al, Nat Commun, 2017

Idelalisib Resistance

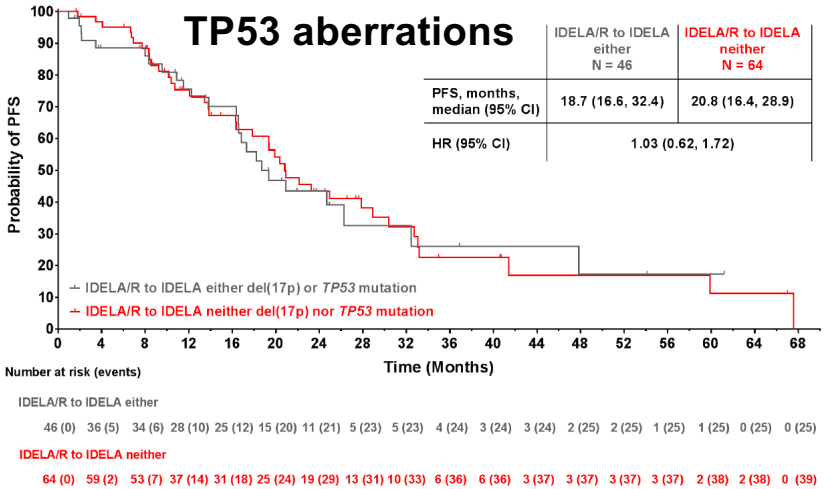


Adapted from Byrd J, et al. J Clin Oncol 2014

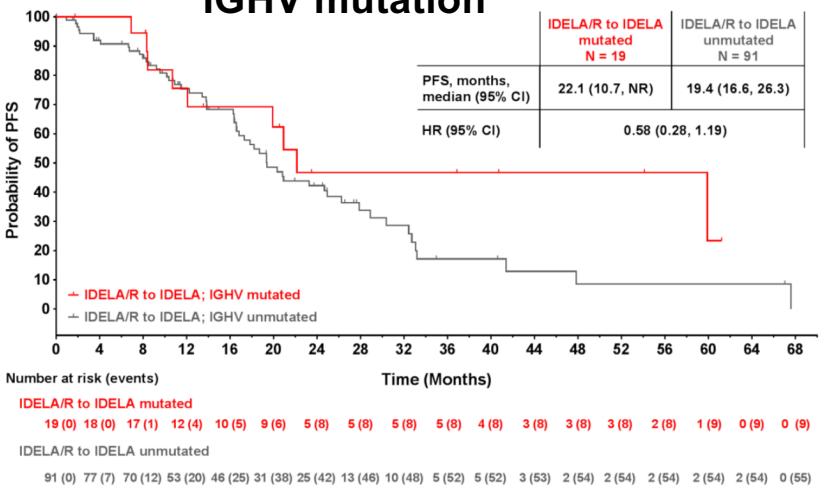
Idelalisib in Relapsed/Refractory patients with CLL



TP53 aberrations



IGHV mutation



Sharman JP et al, J Clin Onc, 2019

Idelalisib: the quest for resistance mechanisms

Study number	Patient	%CD19+/ CD5+[a]	%CD19+/ CD5+[b]	IGHV status	TP53 status	del17p status
NCT01659021 (IDELA + ofatumumab)	1	93	NA	UM	mutation	deletion
	2	94	90	UM	mutation	deletion
	3	98	NA	UM	mutation	deletion
	4	70	NA	UM	mutation	normal
	5	78	NA	UM	normal	normal
	6	83	NA	M	mutation	normal
	7	93	NA	UM	mutation	deletion
	8	97	NA	UM	normal	normal
	9	92	88	UM	normal	normal
NCT01539512 + NCT01539291 (IDELA + rituximab)	10	76	83*	UM	N/A	deletion
	11	46.2	NA	UM	normal	normal
NCT01539512 (IDELA + rituximab)	12	94	92	UM	mutation	normal
	13	83	19	UM	normal	normal

Sample selection criteria included:

- **treatment period of ≥ 180 days**
- **at least a partial nodal response followed by progression**
- **progression did not occur within a drug interruption window**
- **progression was not associated with RT**

WES identified no mutations in:

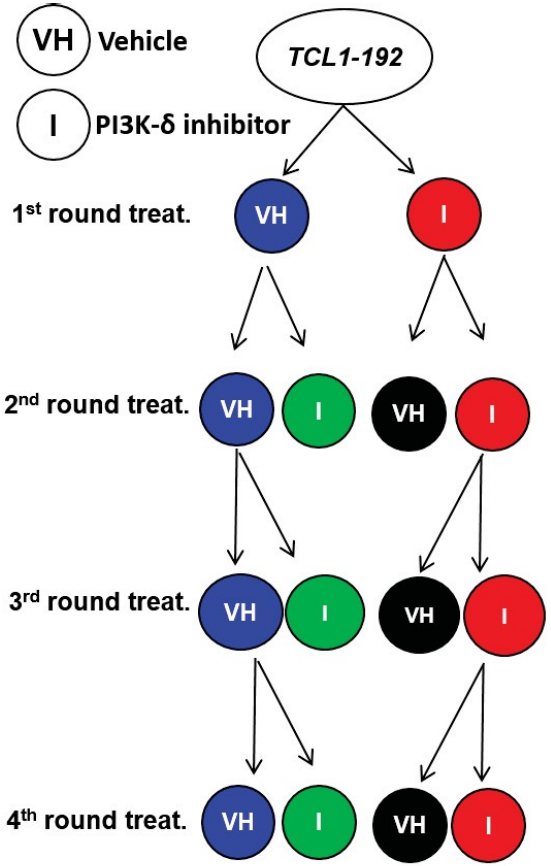
- **PI3K signaling or related pathways**
- **PI3Kδ in any sample at any time point**

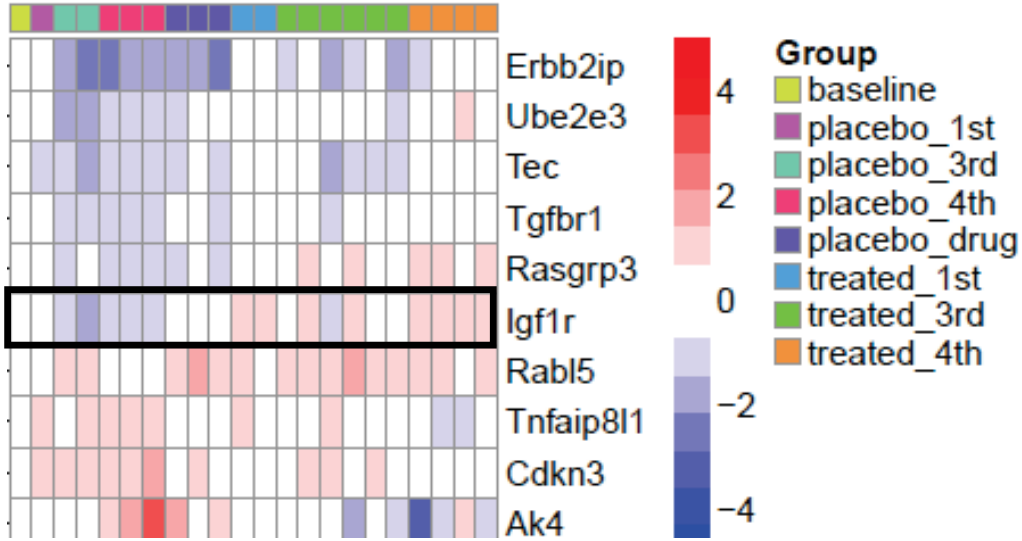
Idelalisib resistance: No common mutational mechanism

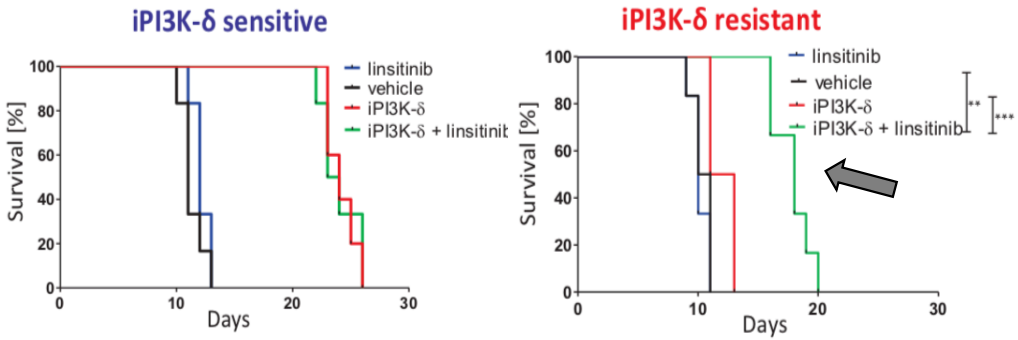
WES of Idelalisib relapsed CLL patients (n=13)

Ghia et al. ASH 2016

In vivo modeling of resistance to Idelalisib

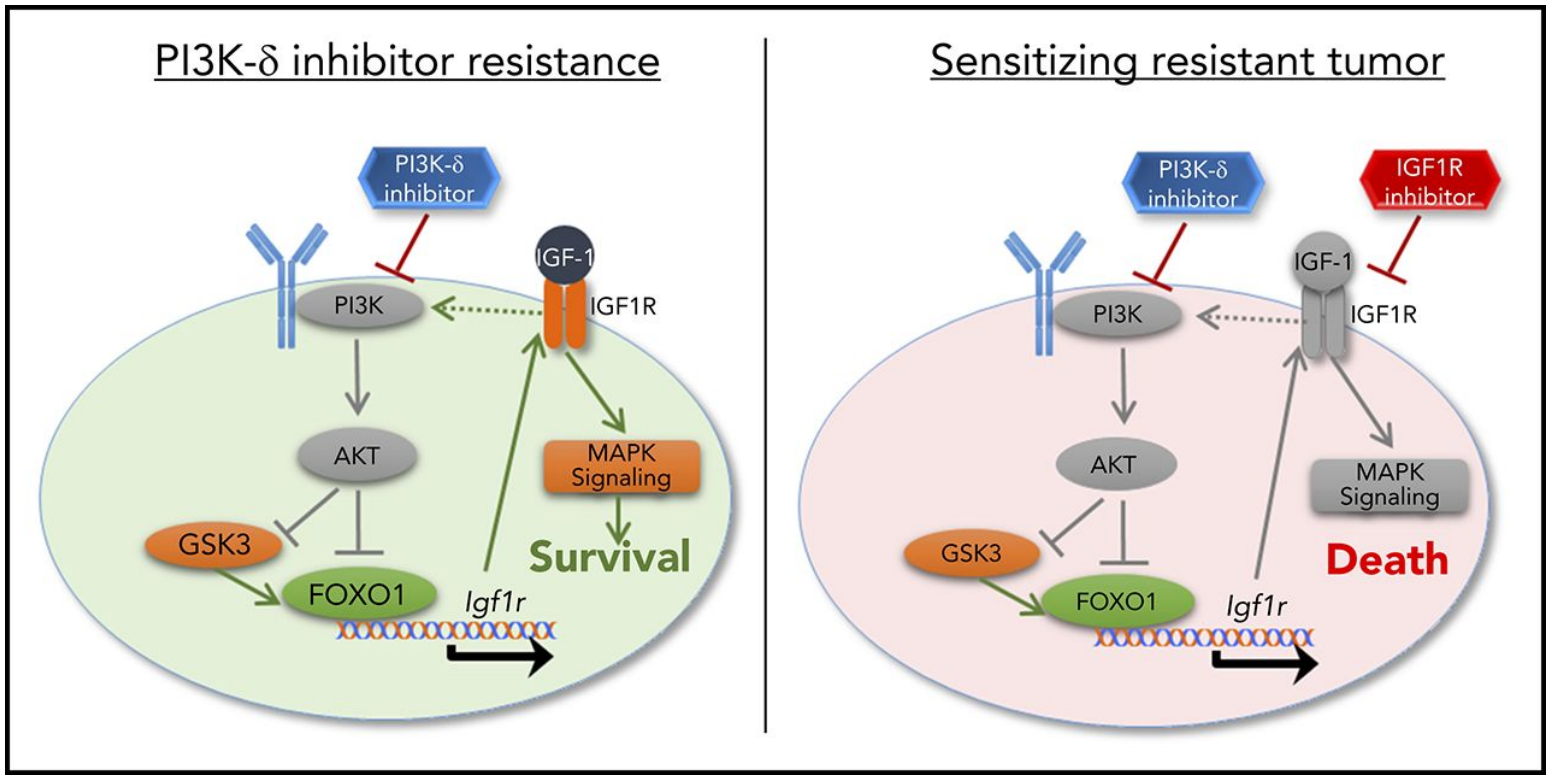






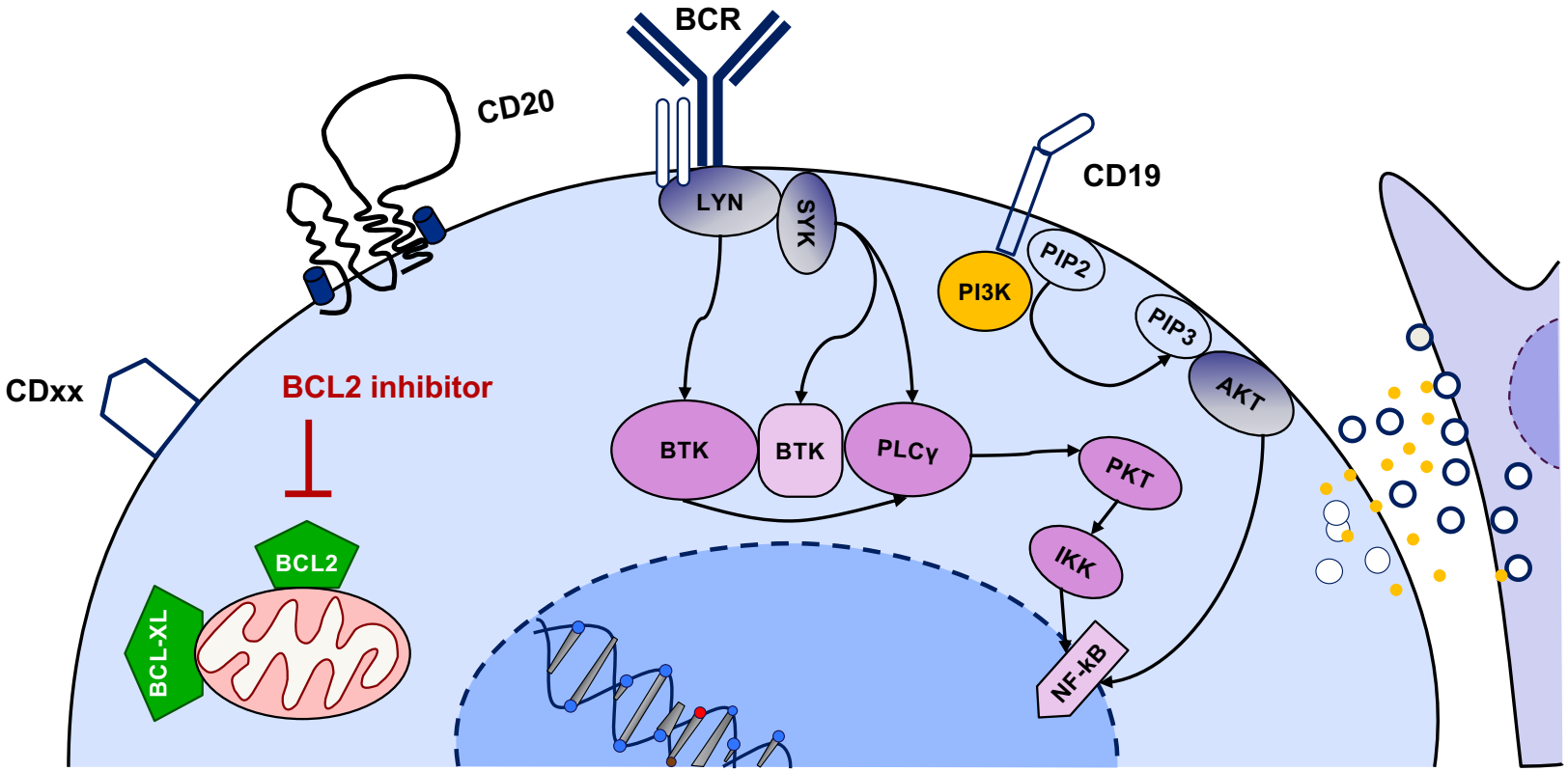
Scheffold et al. Blood 2019

In vivo modeling of resistance to Idelalisib



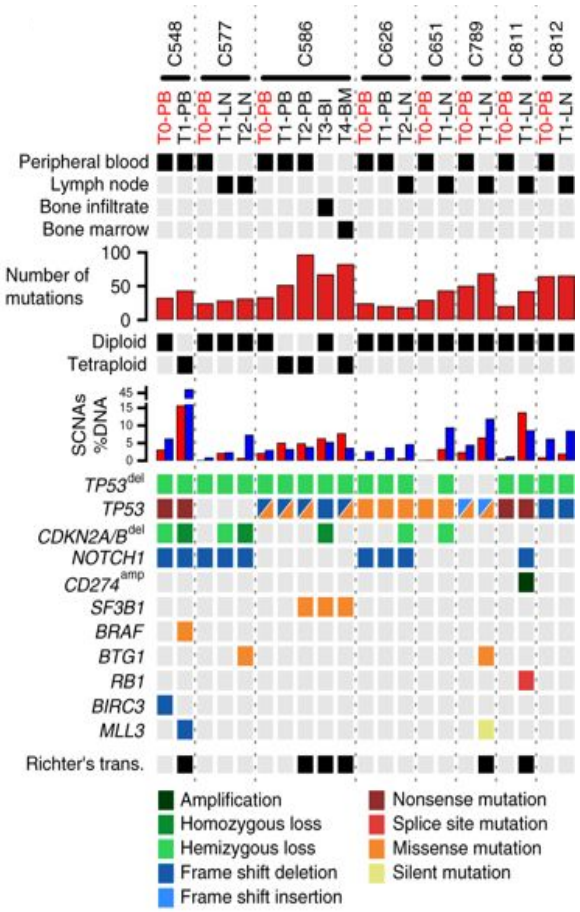
Scheffold et al. Blood 2019

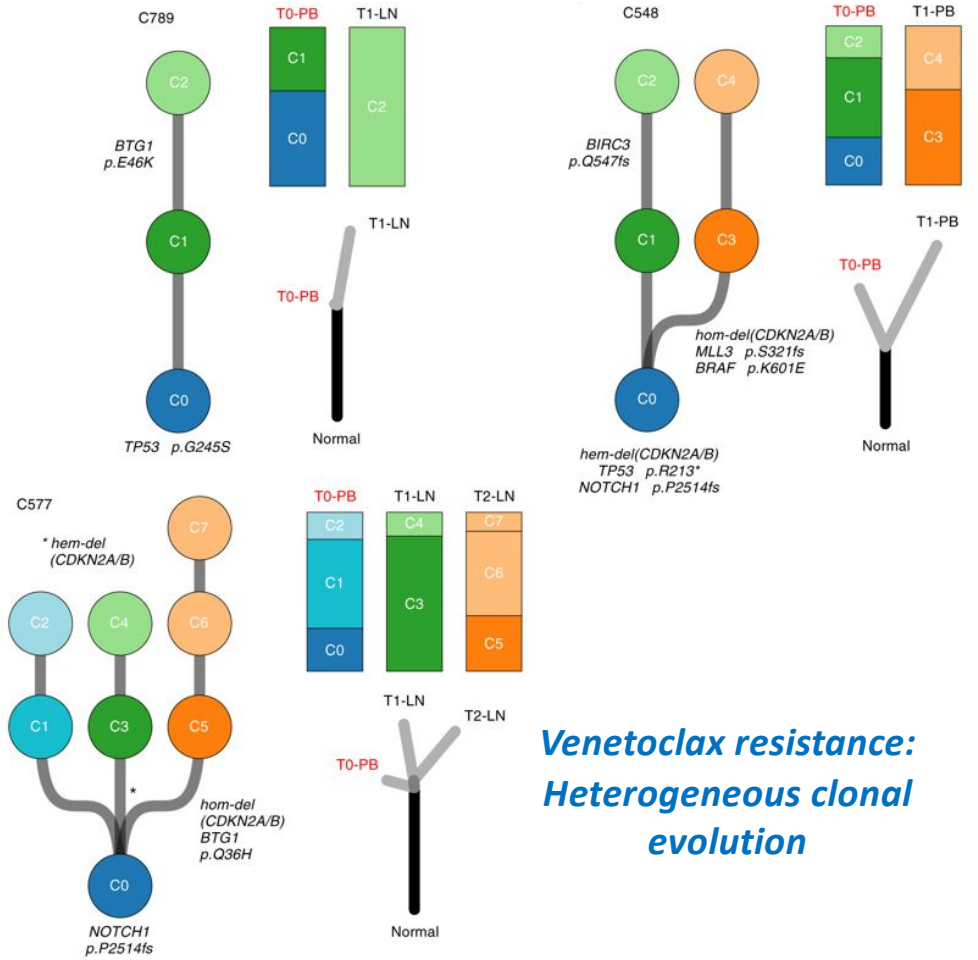
Venetoclax Resistance



Adapted from Byrd J, et al. J Clin Oncol 2014

Venetoclax: insights into the clonal dynamics involved in resistance



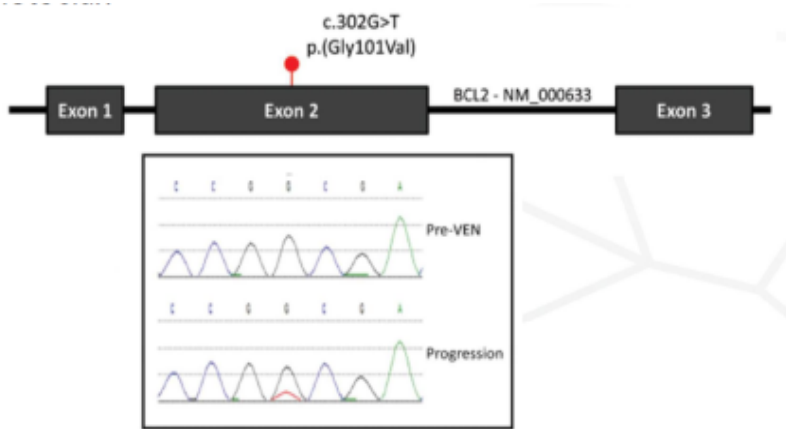


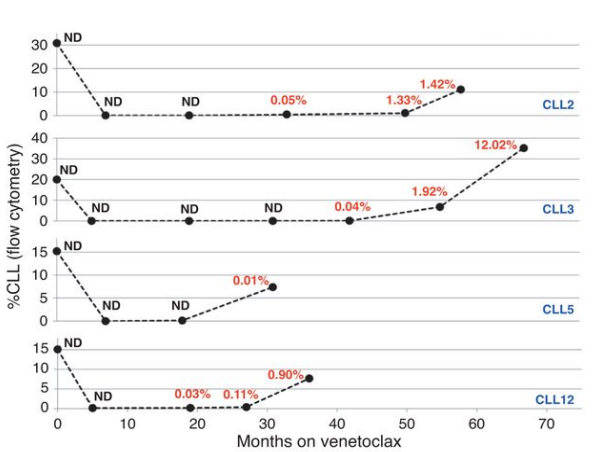
Venetoclax resistance: Heterogeneous clonal evolution

Herling et al. Nat comm 2018

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**





VAF%[a]

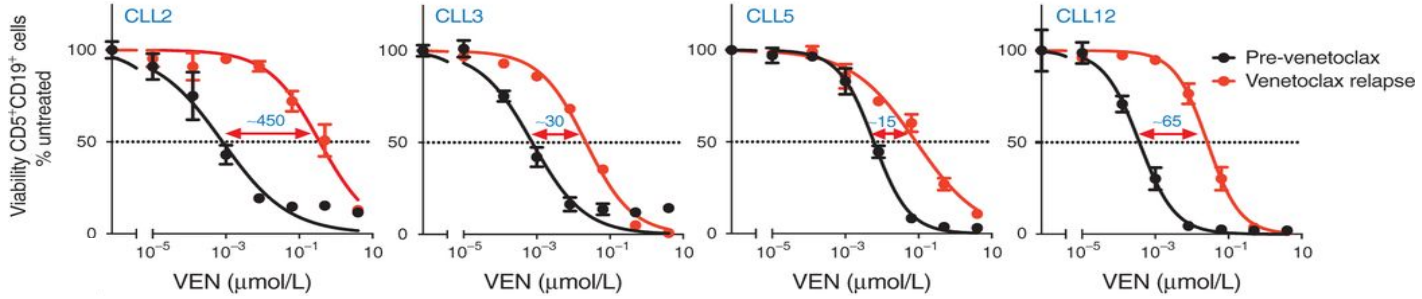
25,8%

68%

45,6%

60%

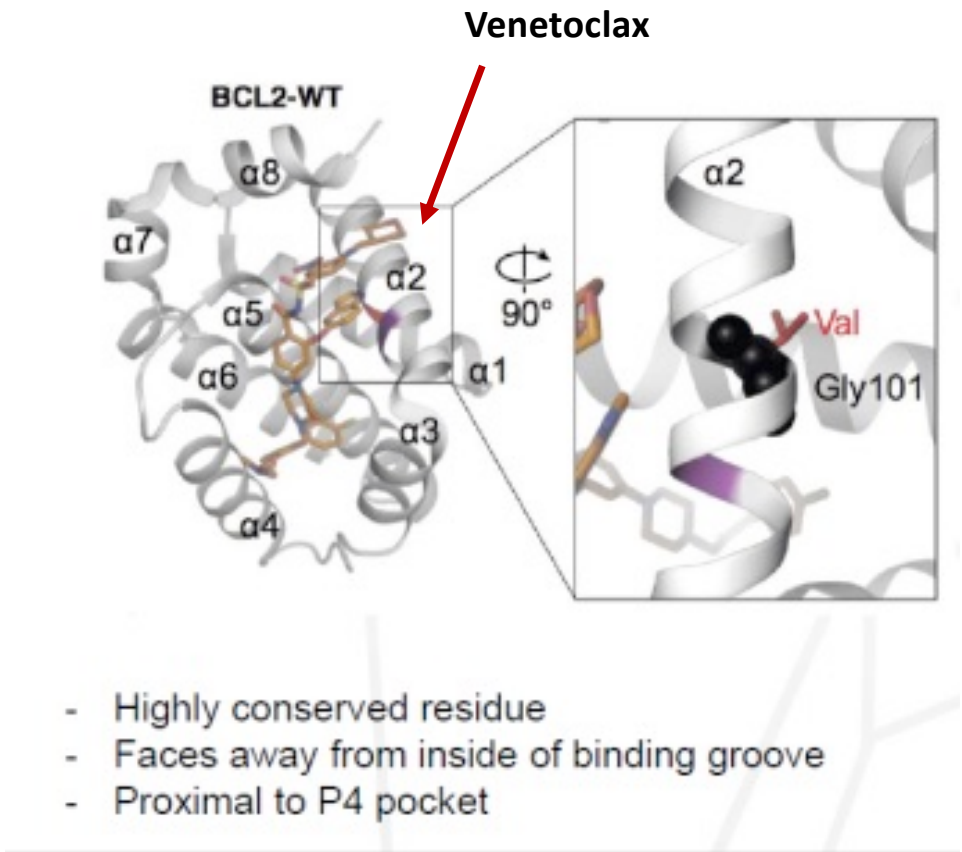
[a]CLL cells harboring G101V at progression; calculated by adjusting the measured VAF by the % of CLL cells in the bone marrow determined by flow cytometry.



CLL cells harboring p.G101V are less sensitive to Venetoclax

Blombery et al. Cancer Discov 2019

BCL2 Gly101Val occurs near the BH3-binding groove

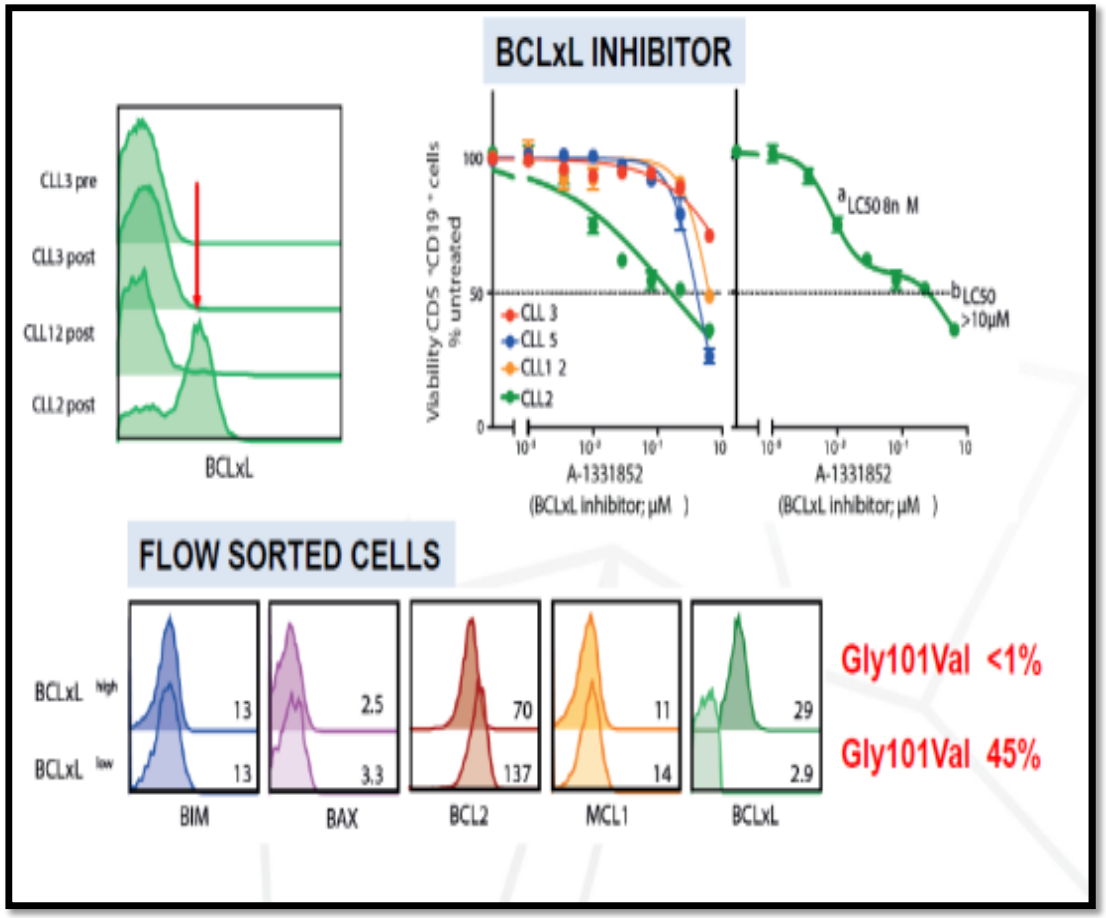


Population	Number assessed	*BCL2* Gly101Val detected (%)	*BCL2* Phe104Leu detected (%)
Venetoclax-naïve CLL	96	0 (0%)	0 (0%)
CLL-type progression on venetoclax	15	7 (46.7%)	0 (0%)
Other B-cell malignancies			
- Follicular lymphoma	28	0 (0%)	0 (0%)
- Mantle cell lymphoma	28	0 (0%)	0 (0%)
- Diffuse large 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%)

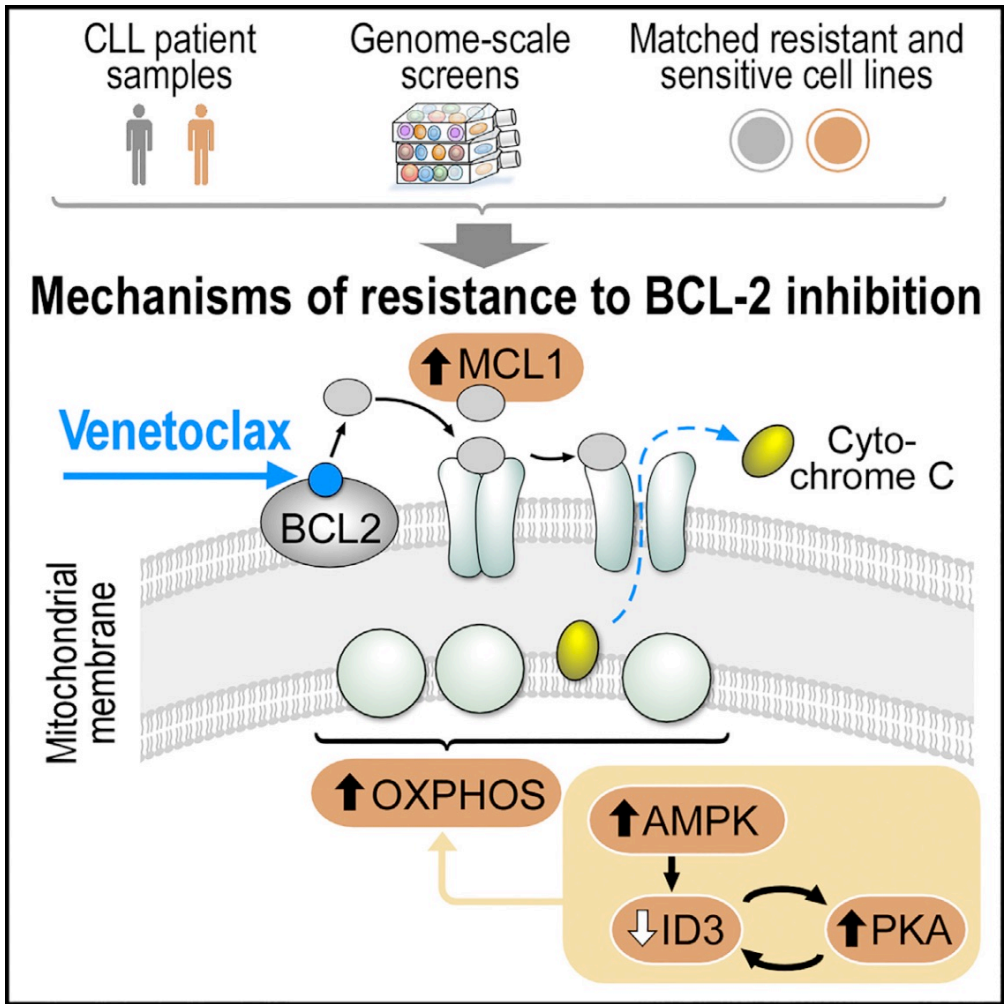
G101V reduces the affinity of BCL2 for venetoclax by 180 fold

Blombery et al. Cancer Discov 2019

Alternative resistance mechanisms can co-exist



Blombery et al, ASH, 2018



Guieze et al, Cancer Cell 2019

Resistance mechanisms to targeted agents: Outstanding questions

i. these ***mutations are not observed in all relapsing patients***;
ii. ***variant allelic frequencies*** at the time of relapse can vary considerably and are ***often less than 10%***

iii. could we use these **mutations to identify patients likely to relapse** and direct therapy toward resistant clones before patients have overt clinical relapses?
iv. what comes after **resistance has developed toward all novel drugs**?

Università Vita-Salute San Raffaele – Istituto Scientifico San Raffaele

Prof. Paolo Ghia

Laboratory of B Cell Neoplasia

Silvia Heltai, Pamela Ranghetti, Alessandra Rovida, Athanasios Pseftogkas, Daniela Belloni, Elizavet Kartomatsidou, Silvia Bonfiglio

Strategic Research Program on CLL

Luana Schiattone, Maria Colia, Virginia Sgarlato, Eloise Scarano

CERTH, Thessaloniki

Anna Vardi, Stavroula Ntoufa, Aliki Xochelli, Anastasia Hadzidimitrious, Andreas Agathangelidis, Maria Gounari, Kostas Stamatopoulos

Karolinska Institut, Stockholm

Lesley Ann Sutton, Panayotis Baliakas, Viktor Ljungstrom, Richard Rosenquist