PI3K INHIBITION

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Disclosures

Roche (Advisory Board)

Janssen (Advisory Board)

Amgen (Advisory board, research support)

Gilead (Speakers' bureau)

Novartis (Advisory Board)

Morphosys (Advisory Board)

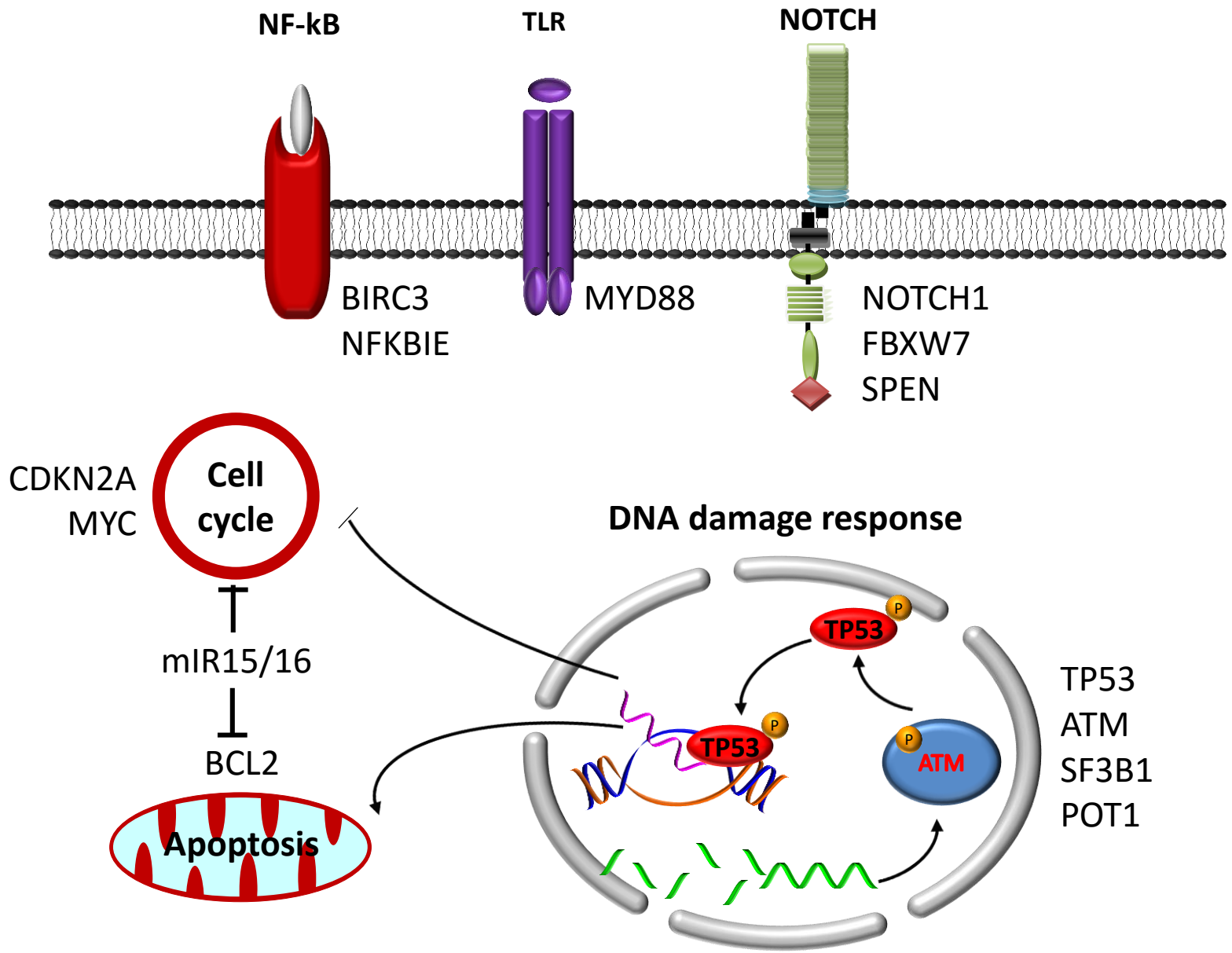
Abbvie (Advisory Board)

Karyopharm (Advisory Board)

Outline

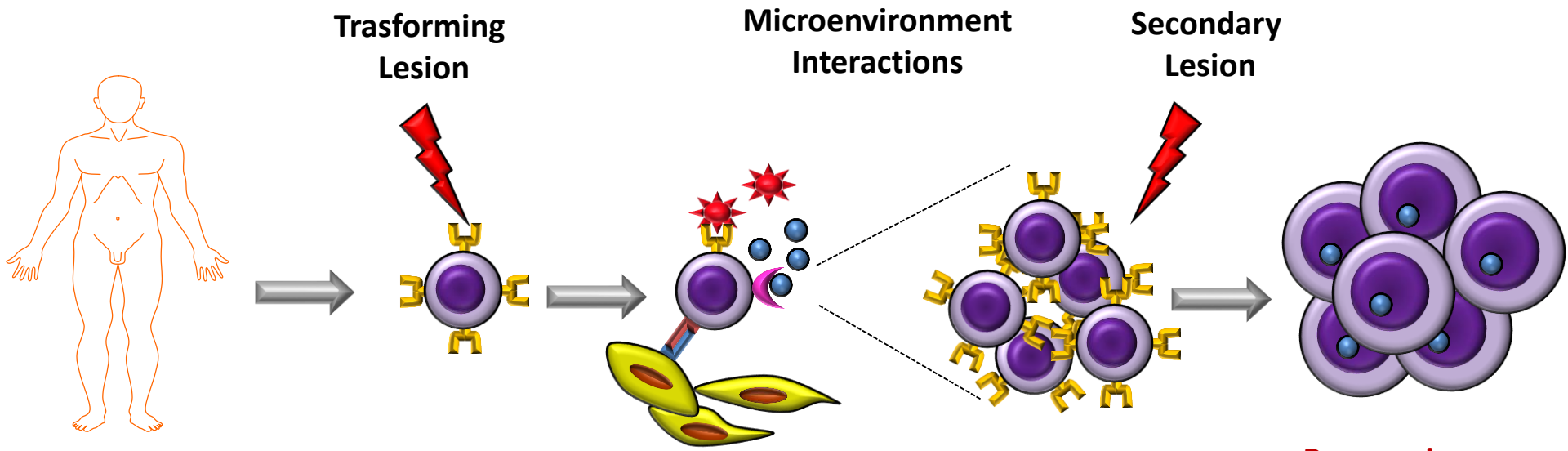
- **Rationale for the need to circumvent genotoxic refractoriness**
- The B cell receptor in B cell malignancies
- Therapeutic targets of the B cell receptor cascade: PI3K

Molecularly deregulated cellular programs in indolent B-cell malignancies



Puente, Nature 2015

Pathogenesis of CLL



Predisposition

Polygenic
IRF4
IRF8
MYC
Other

Initiation

del13q
+12

Promotion/Accumulation

Signaling pathways
BCR
NF-kB
TLR
CD38
VLA-4 integrins
NOTCH
CXCR4

Progression Chemorefractoriness Transformation

TP53
NOTCH1
SF3B1
BIRC3
ATM
MYC
CDKN2A

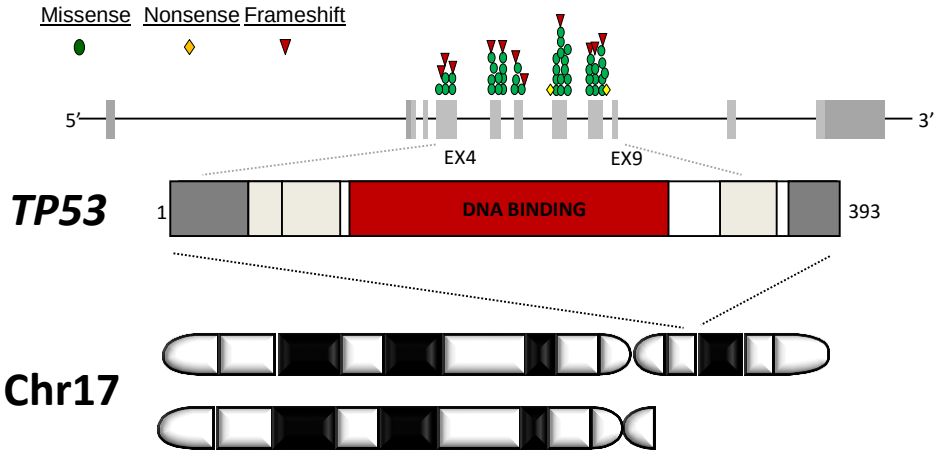
CLL mutations disclosed by NGS studies

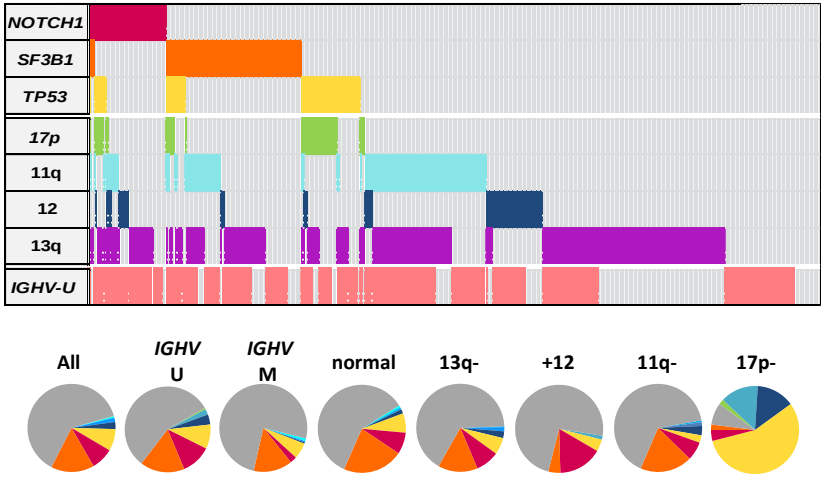
Font size according to gene mutation prevalence

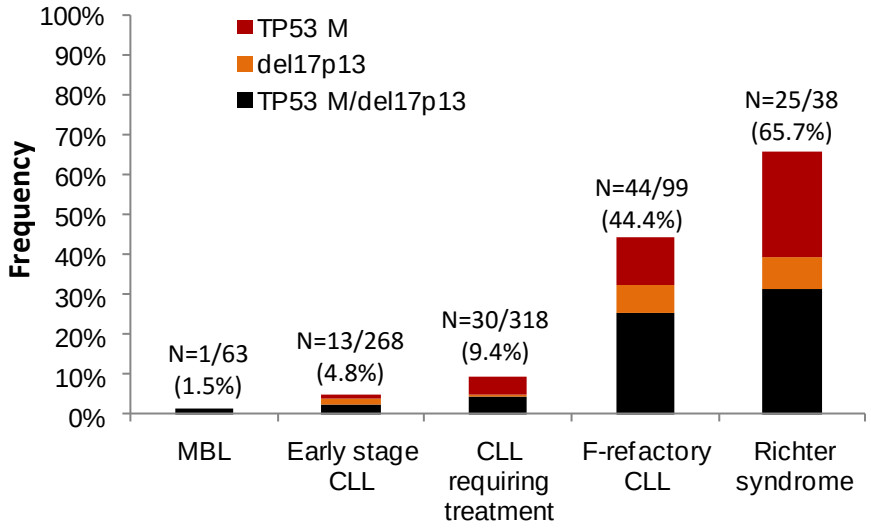
- **One of the tumor with the lowest background mutation load (0.6 per Mb)**
- **No unifying gene mutations**
- ***TP53*, *NOTCH1*, *SF3B1*, *ATM* mutated in >5% CLL**

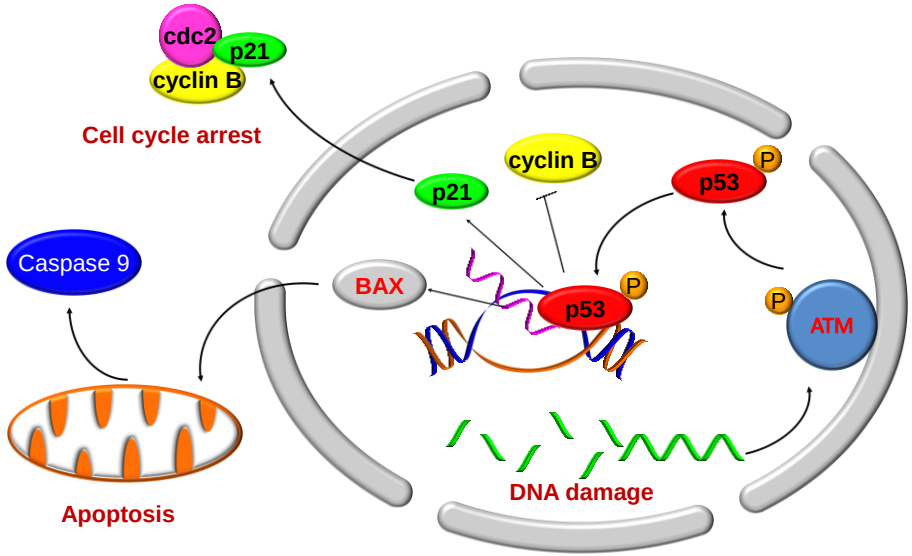
Fabbri, J Exp Med 2011; Puente, Nature 2011; Rossi, Blood 2011; Quesada, Nat Genet 2011; Wang, N Engl J Med 2011; Rossi, Blood 2012, Puente, Nature 2015

TP53 abnormalities in CLL



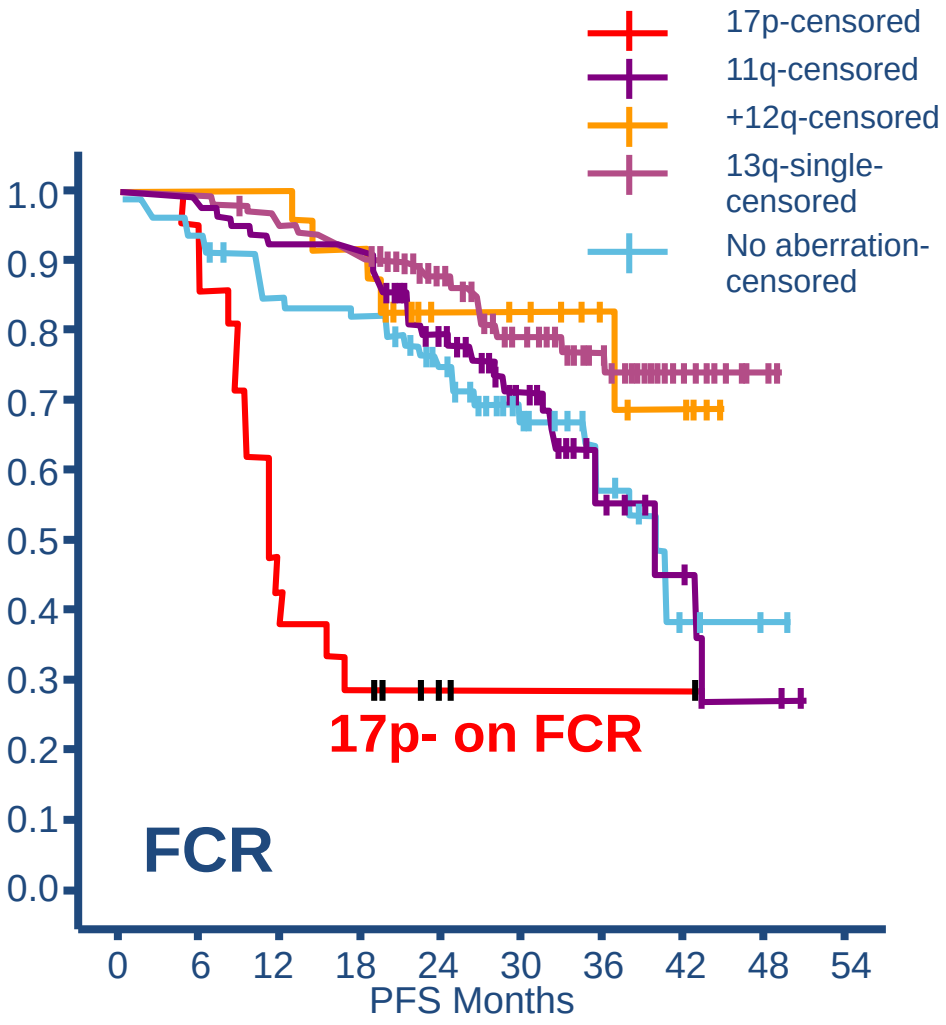




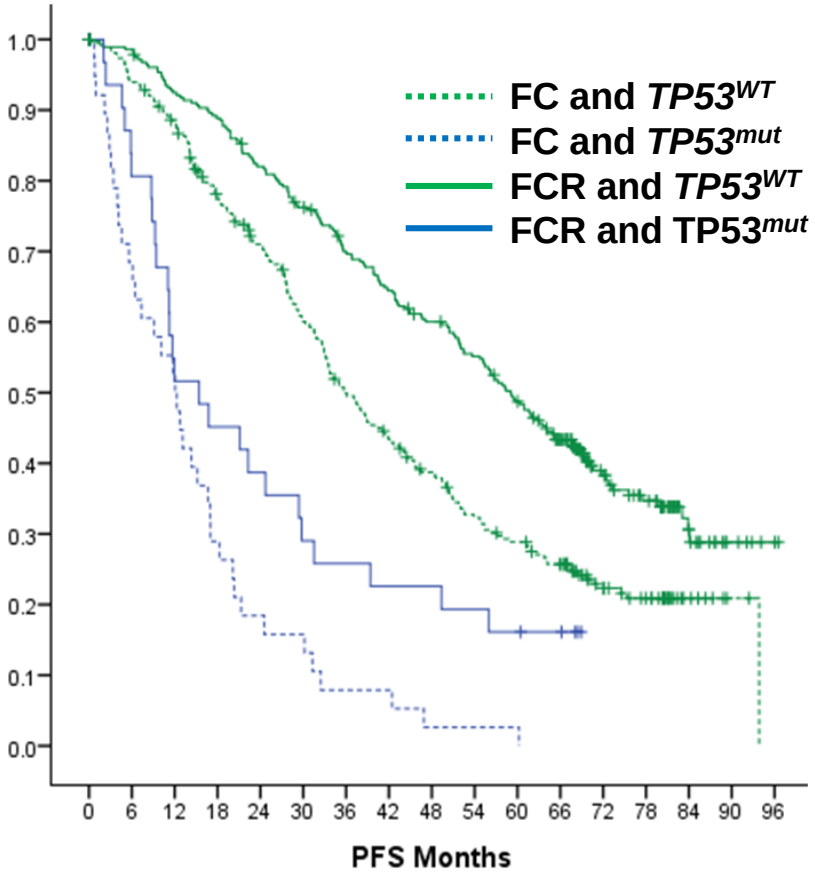


Dohner et al, New Engl J Med 2000 ; Rasi et al, Haematologica 2012; Zainuddin et al, Leuk Res 2011; Zenz et al J Clin Oncol 2010;Rossi et al Blood 2011

TP53 abnormalities in CLL



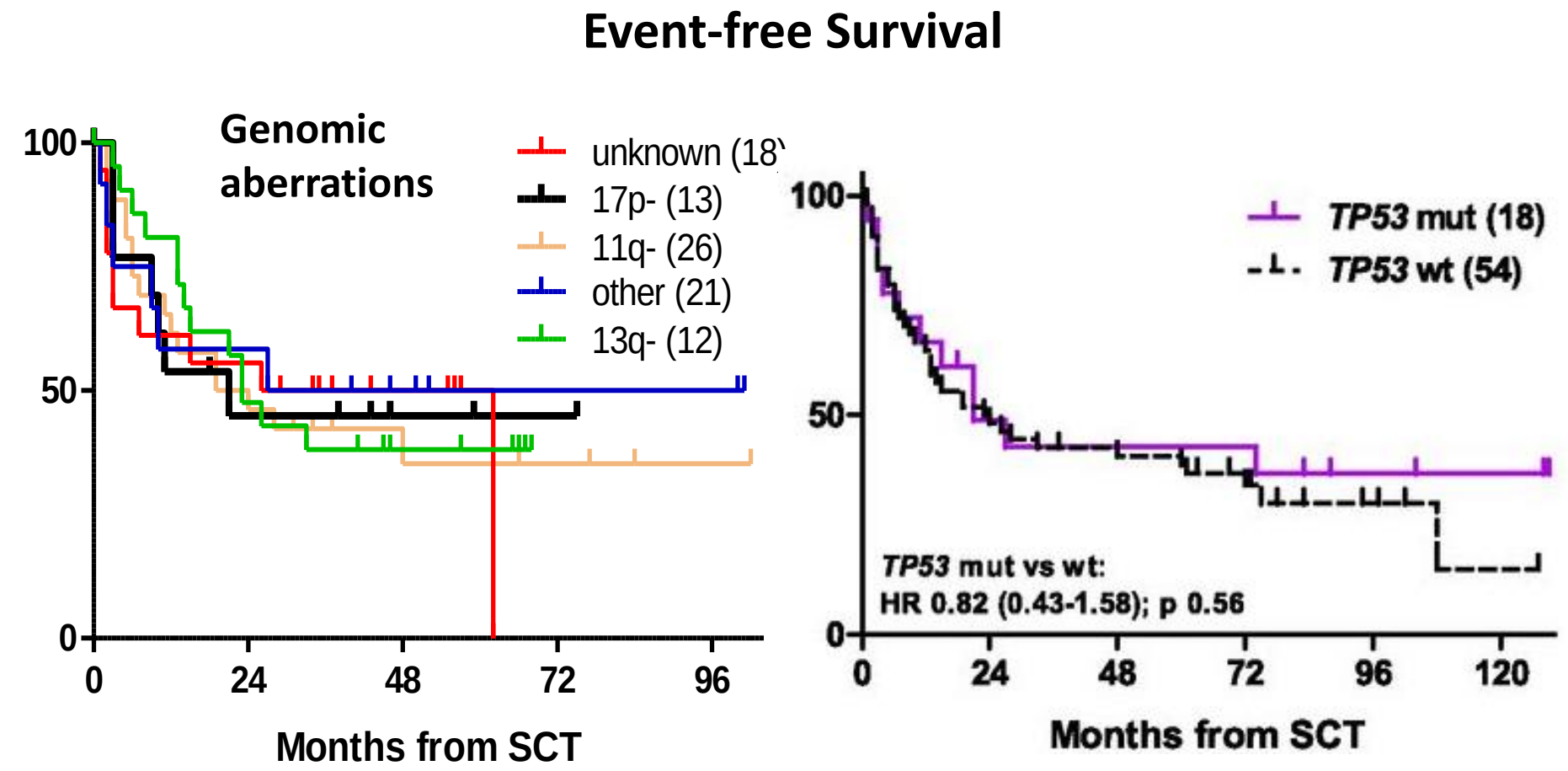
Hallek et al, ASH 2009



Stilgenbaueret al, ASH 2012

EHA-20: Ljungstrom S121; Tausch LB2070 (novel FCR prognosticators)

Allo-SCT in High-Risk CLL
CLL3X: multicenter GCLLSG



Dreger P, et al., Blood 2010; Dreger P, et al. Blood 2013

Clonal architecture of *TP53* mutated CLL

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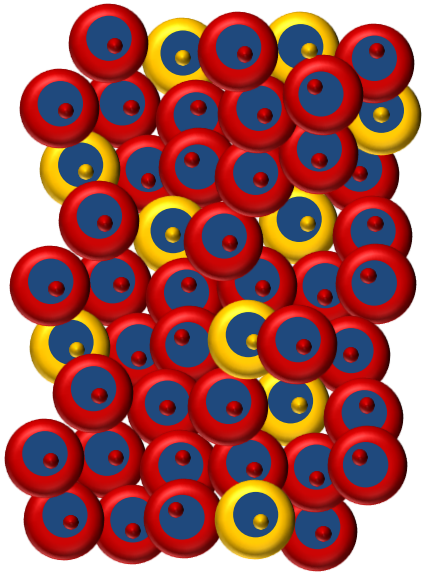
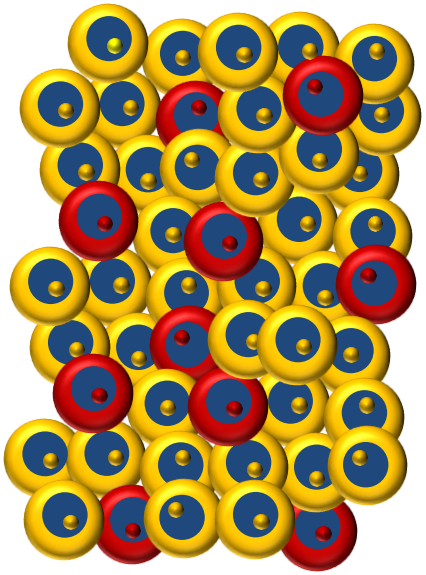
Scenario 1



TP53 mutation representation
80%

Detectable by Sanger sequencing

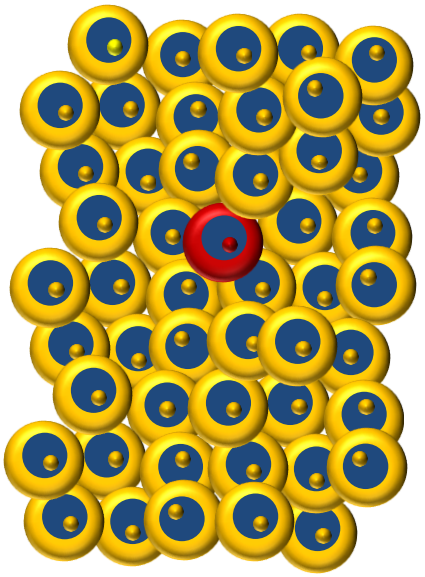
Scenario 2



TP53 mutation representation
20%

Barely detectable by Sanger sequencing

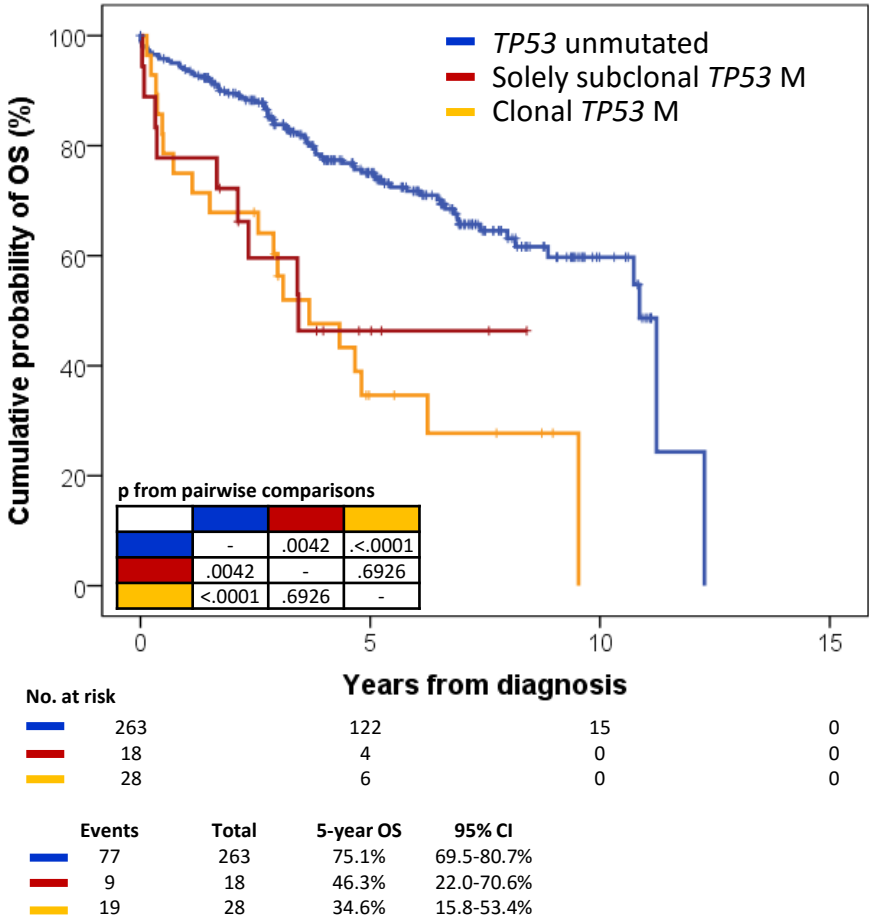
Scenario 3

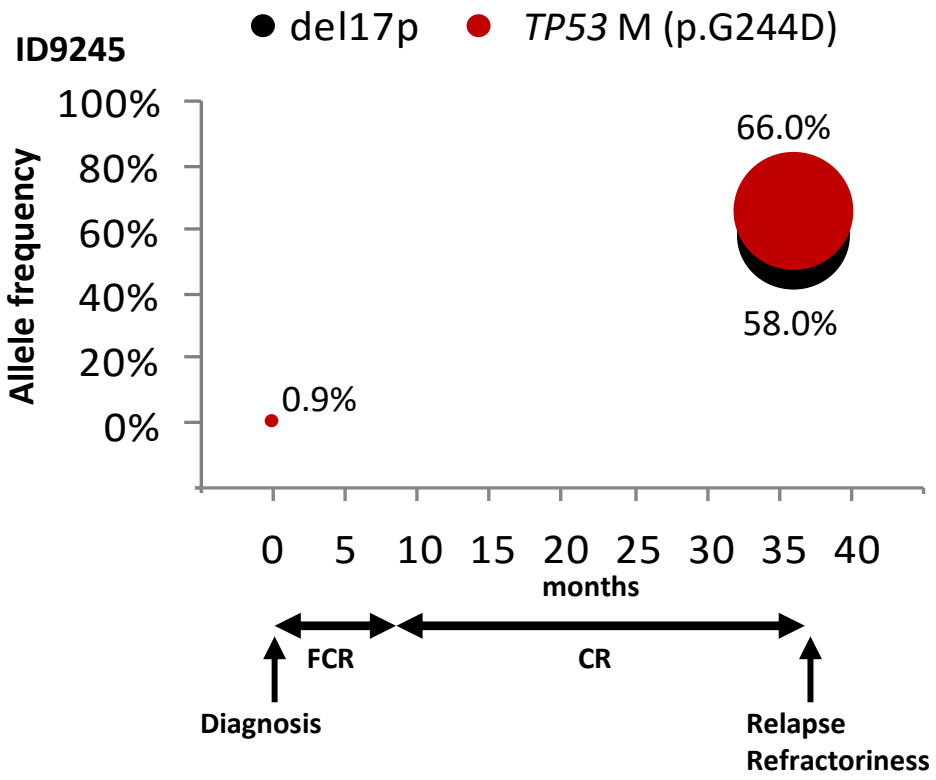


TP53 mutation representation
1%

Not detectable by Sanger sequencing

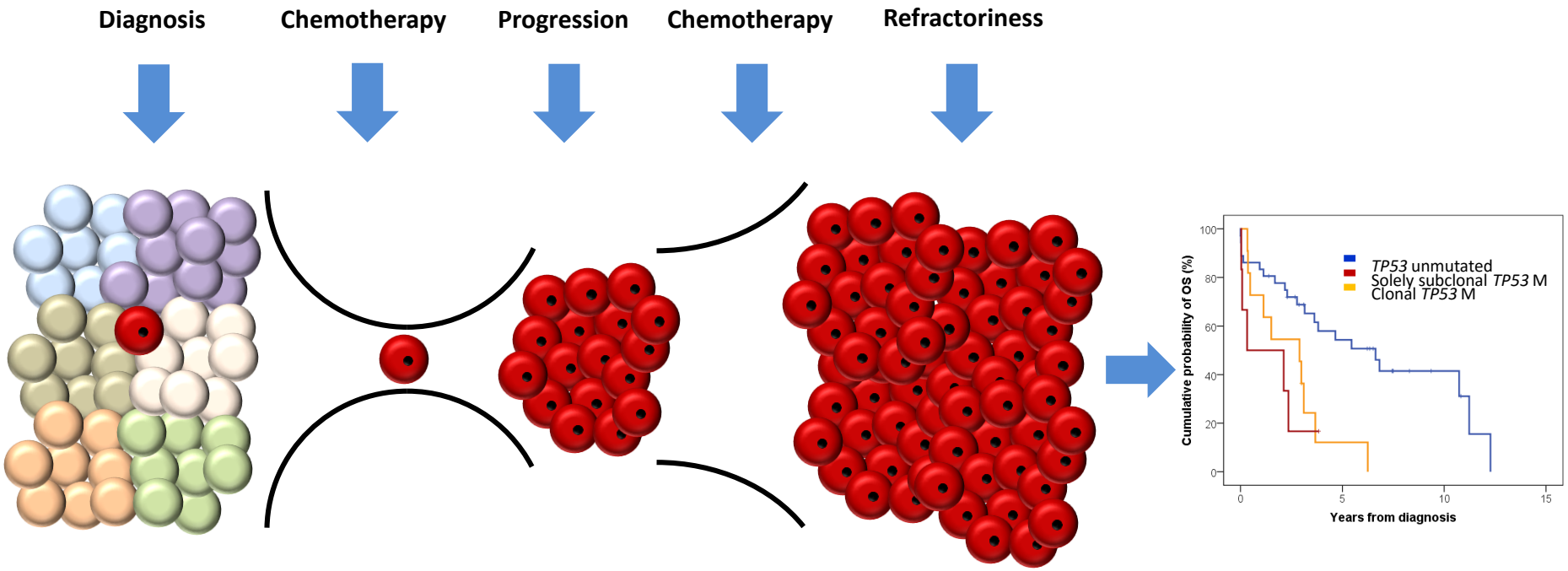
Small *TP53* mutated subclones have the same unfavorable prognostic impact as clonal *TP53* defects





Rossi, Blood 2014

Small *TP53* mutated subclones are selected by treatment because of their chemoresistance



Small *TP53* mutated subclone admixed to *TP53* wild type clones

Removal of *TP53* wild type clones and selection of the *TP53* mutated subclone

Expansion of the *TP53* mutated clone

Poor outcome

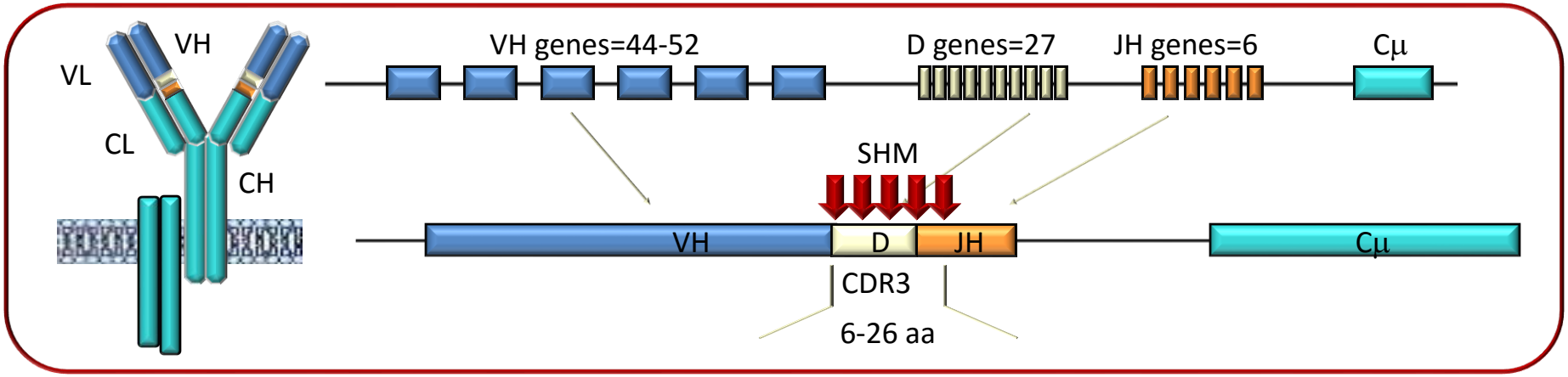
TP53 mutated CLL cell

Rossi, Blood 2014

Outline

- Rationale for the need to circumvent genotoxic refractoriness
- **The B cell receptor in B cell malignancies**
- Therapeutic targets of the B cell receptor cascade: PI3K

BCR rearrangement is the first genetic hit in CLL

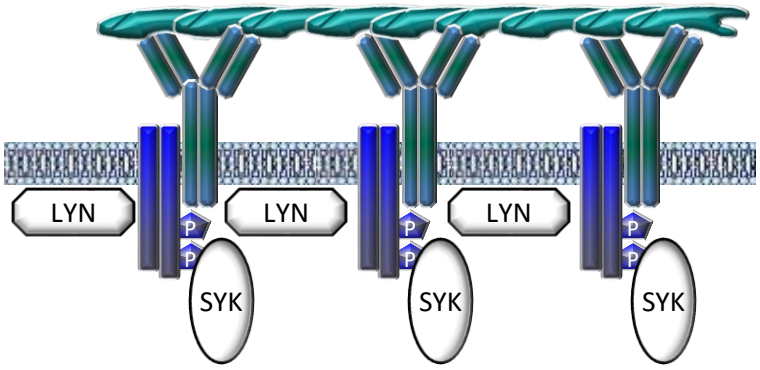


External antigens

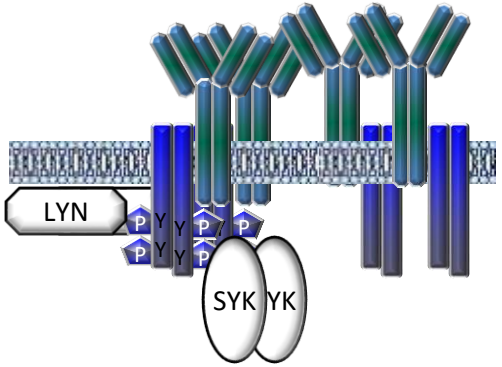
Autoantigens exposed on apoptotic cells
Microbial antigens

Cell autonomous BCR signal

Interaction between the CDR3 region of one BCR with another BCR that functions as an autoantigen



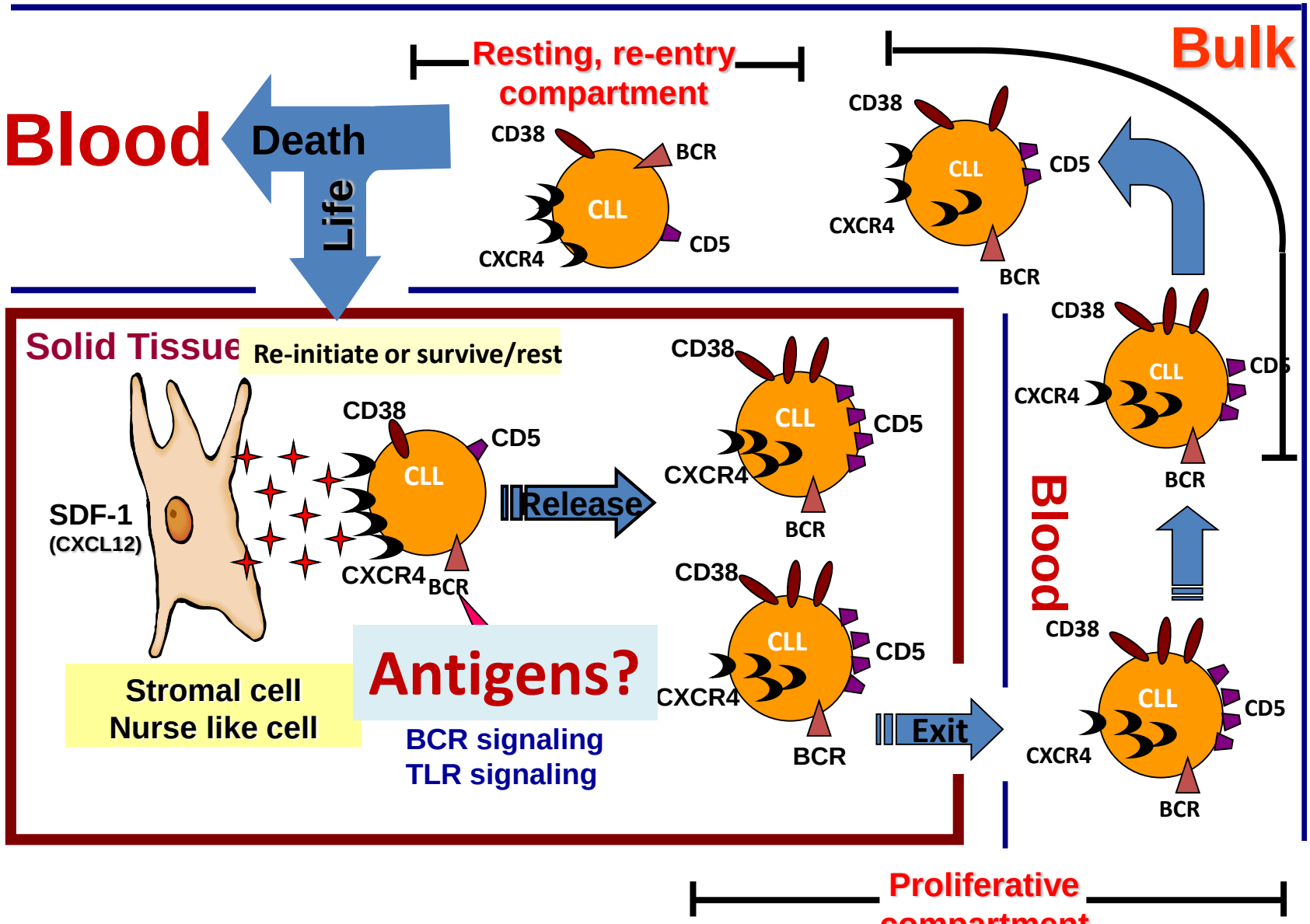
Chronic BCR signaling



Chu CC et al, Blood. 2008; Catera R et al, Mol Med. 2008; Steininger C et al, Blood. 2012

von Minden MD et al, Nature. 2012

CLL cells interact with the microenvironment through the BCR to gain proliferative advantages



Evidence that the initial expansion of the CLL clones is BCR driven

Structural evidences

- Frequent expression of **stereotyped BCRs**: recognition of common antigens

Functional evidences

- High levels of BCR target genes in CLL cells
- Expression of constitutively active BCR signaling molecules
- **BCR activation supports CLL cell survival** in vitro

Clinical evidences

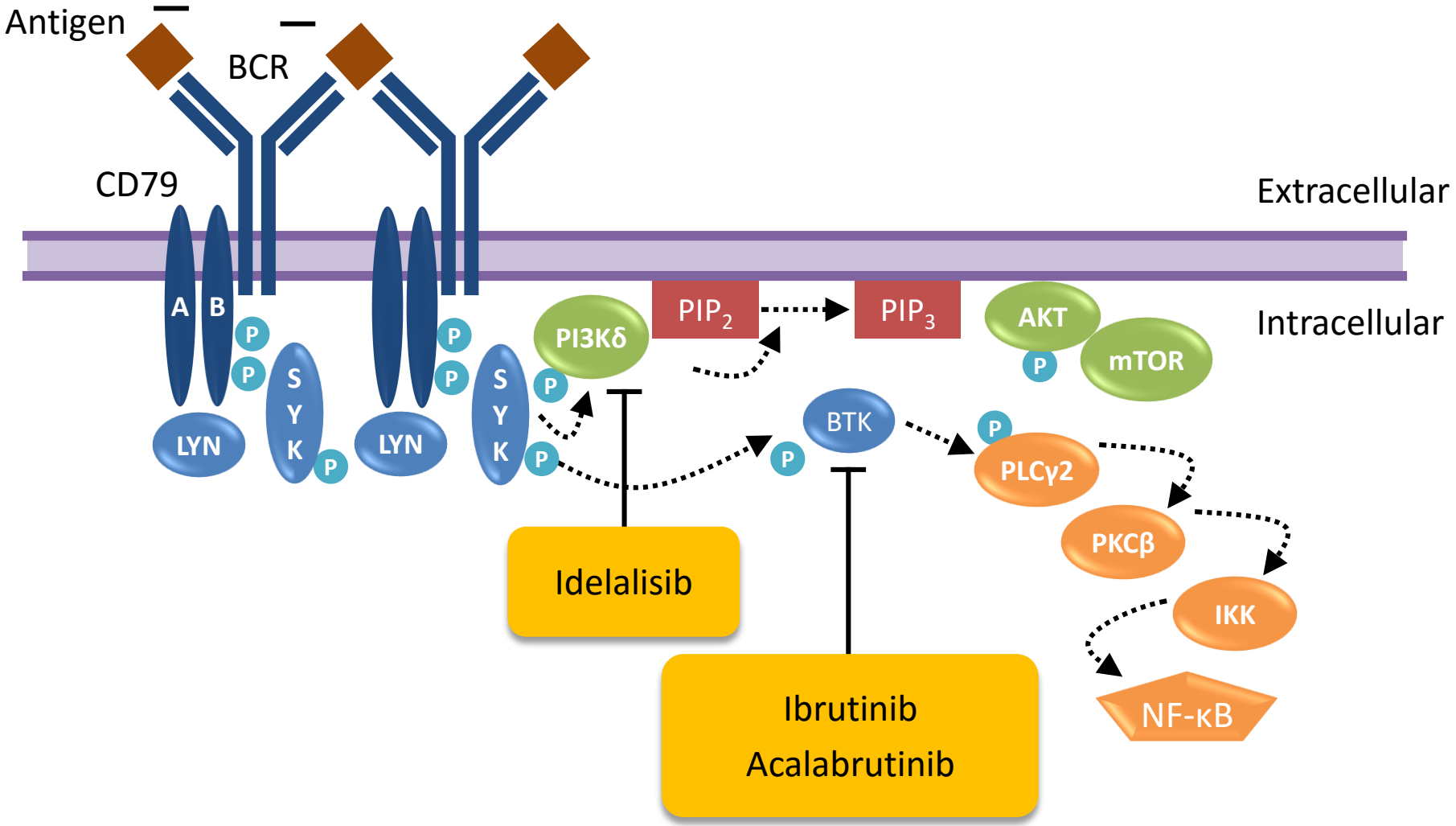
- Strong association between clinical course and *IGHV* mutation status
- BCR reactivity in vitro correlates with clinical course
- **Response to BCR inhibitors**

Hamblin et al, Blood 1999
Damle et al, Blood 1999
Messmer et al, J Exp Med. 2004
Agathangelidis A et al, Blood. 2012
Herishanu Y et al. Blood 2011
Byrd et al, NEJM 2013

Outline

- Rationale for the need to circumvent genotoxic refractoriness
- The B cell receptor in B cell malignancies
- **Therapeutic targets of the B cell receptor cascade: PI3K**

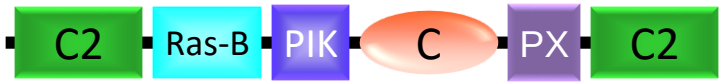
Therapeutic targeting of BCR signalling



BCR: B-cell receptor; CML: chronic myeloid leukaemia

Wiestner A. *J Clin Oncol* 2013;31:128–130.

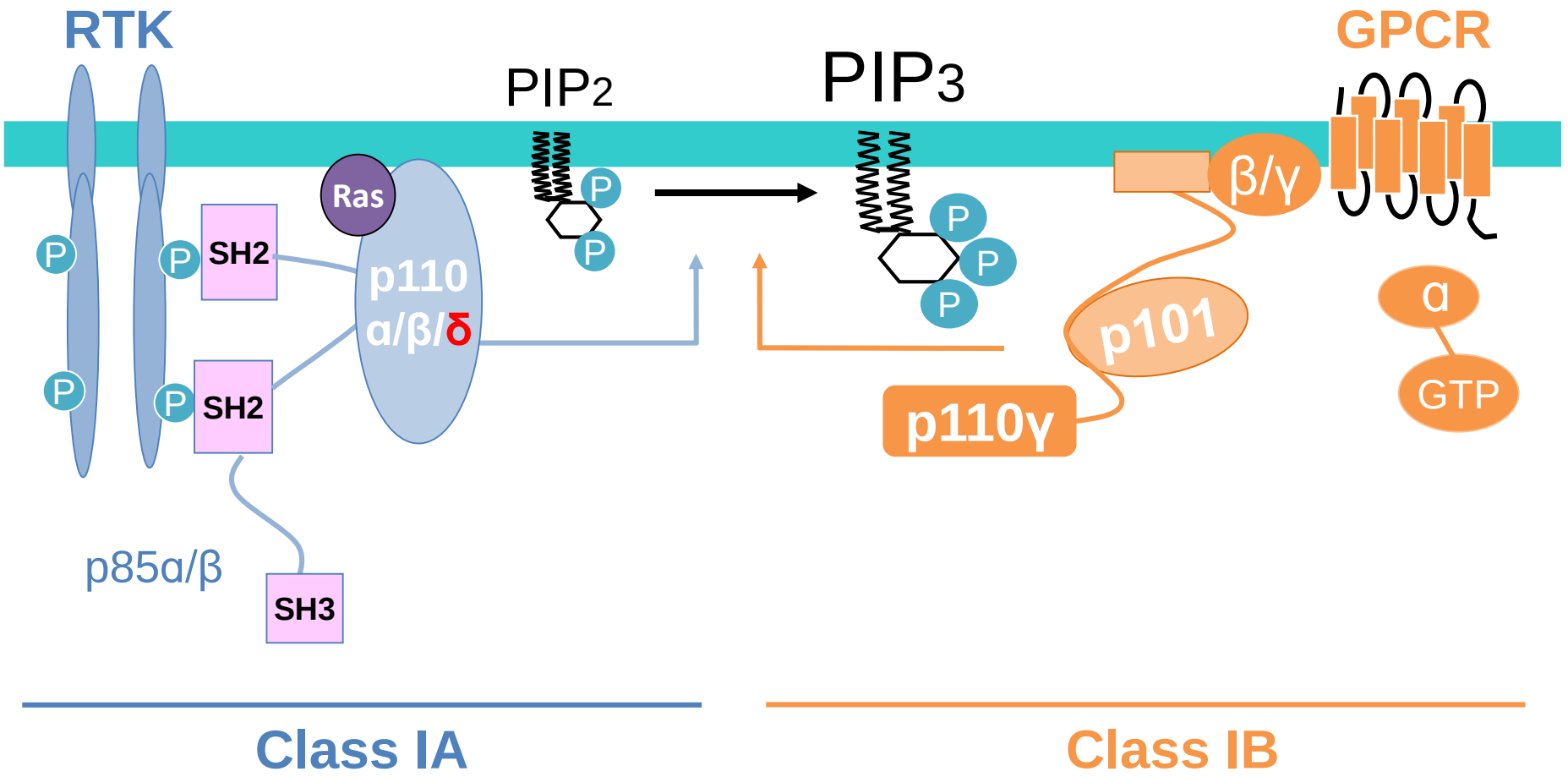
The different PI3Ks

Class	Catalytic subunit	Adaptor/Regulatory subunit	Regulation
I	PI / PI4P / $PI4,5P_2$		
A		p110α p110β p110δ	p85α,β p55α,γ p50α	Tyr kinase / associations
B		p110γ	p101	Gβγ
II	PI / PI4P		
		PI3K-C2α PI3K-C2β PI3K-C2γ	?	?
III	PI		
		hVps34p	p150	?

Ras-B: Ras binding domain

Adapted from Vanhaesebroeck B, *et al. Nat Rev Mol Cell Biol* 2012;13:195–203.

PI3Kδ activates many downstream signalling pathways and is involved in crosstalk between multiple receptors

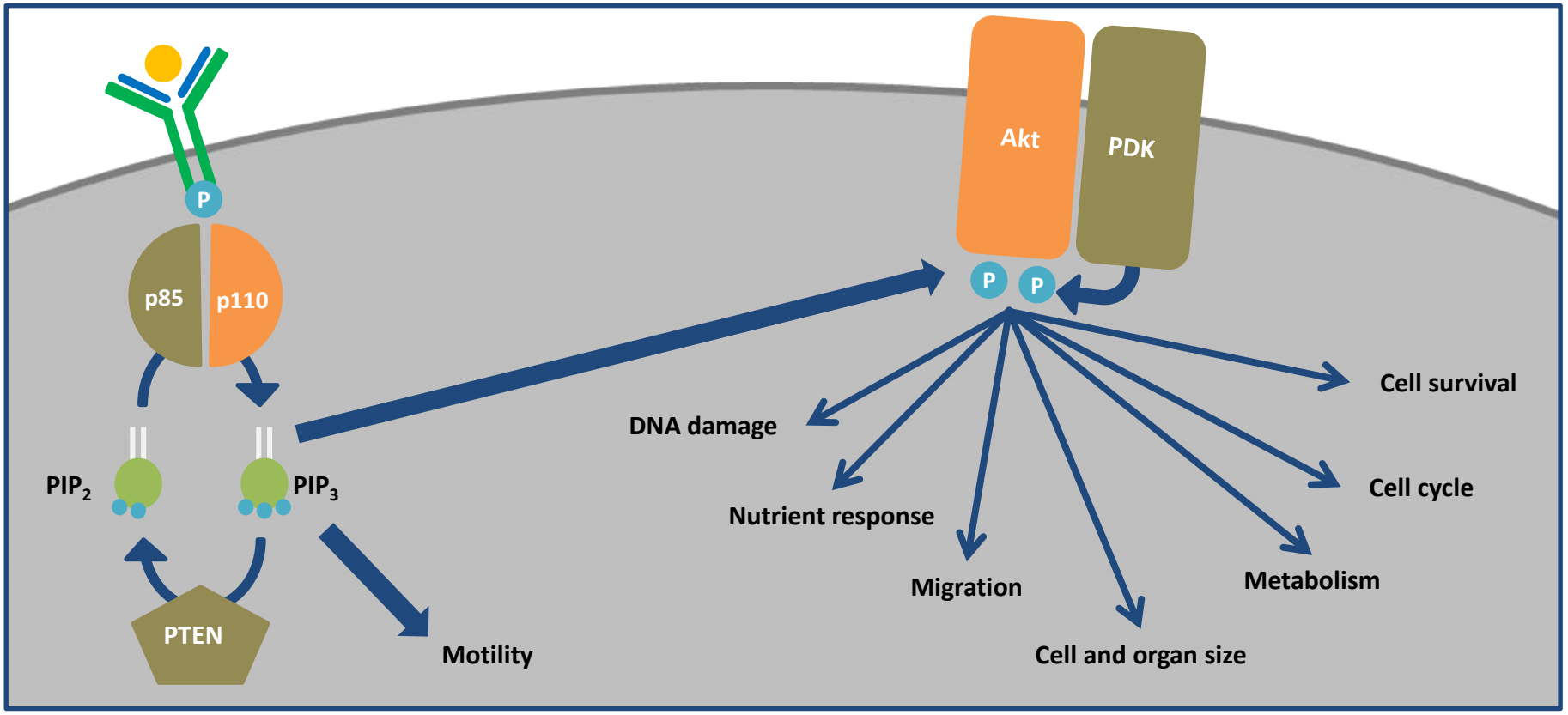


Stimulation-dependent activation of Class I PI3K

GPCR: G protein-coupled receptor:
RTK: receptor tyrosine kinase

Adapted from: Guillermet-Guibert J, *et al. Proc Natl Acad Sci USA* 2008; 105:8292–8297.
Maier U, *et al. J Biol Chem* 1999; 274:29311–29317; Kubo H, *et al. Biochem J* 2005; 392:607–614.

PI3Kδ (p110δ) catalyses conversion of PIP_2 to PIP_3, which acts as a second messenger

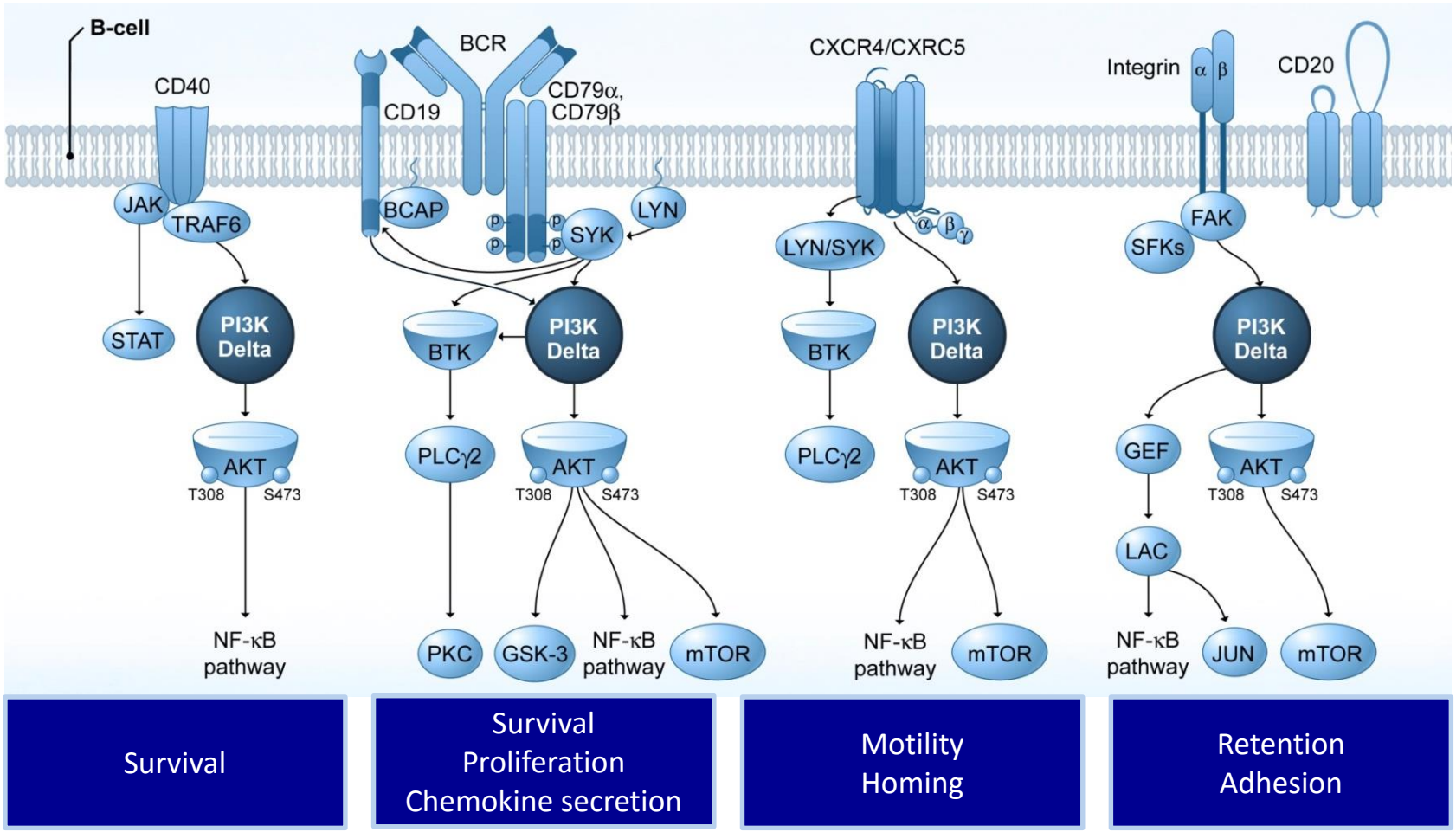


PIP_3 acts as a second messenger to activate pathways that regulate metabolism, proliferation and motility[2]

PTEN: phosphatase and tensin homologue

1. Castillo JJ, *et al*. *Onco Targets Ther* 2014; 7:333–342.
2. Somoza JR, *et al. J Biol Chem* 2015; 290:8439-8446.

PI3Kδ inhibition impacts multiple critical pathways in B-cell malignancies

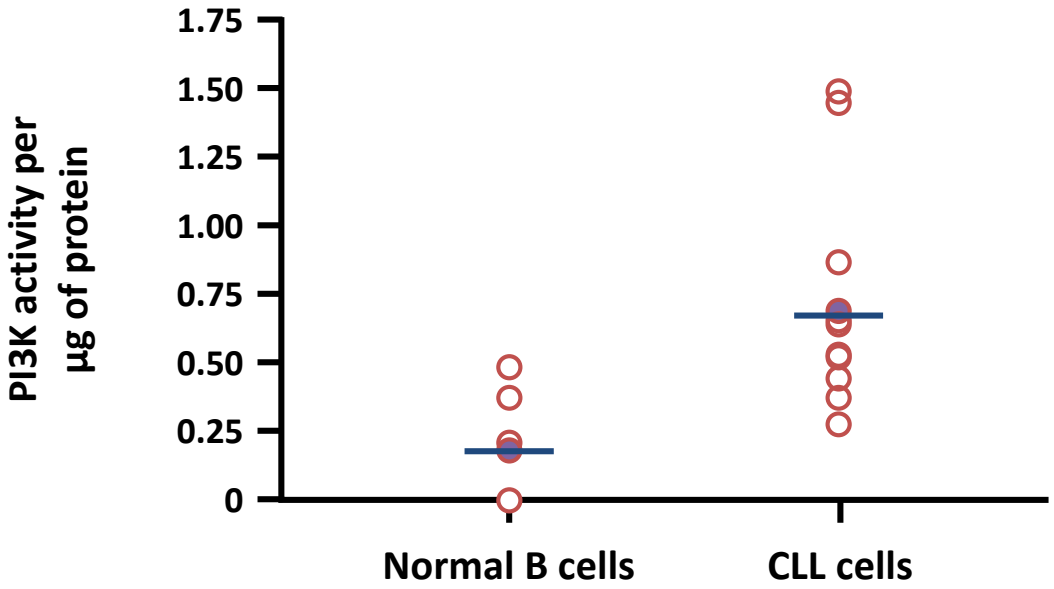


BCAP: B-cell adaptor for PI3K; BCR: B-cell receptor; BTK: Bruton's tyrosine kinase; GEF: guanine nucleotide exchange factor; mTOR: mammalian target of rapamycin; PI3K: phosphatidylinositol-3-kinase; PKC: protein kinase C; SFK: Src family kinase; SYK: spleen tyrosine kinase

Coutre S, *et al. Leuk Lymphoma* 2015; ePub ahead of print.

PI3K is constitutively activated in B-cell malignancies

CLL cells[a] have a significantly higher intrinsic PI3K activity than normal B cells (p=0.006)[1]



- PI3K pathway may be constitutively activated in some patients with FL,[2,3] WM and MZL

[a]CD19+ cells from patients with CLL
CLL: chronic lymphocytic leukaemia; FL: follicular lymphoma; PI3K: phosphatidylinositol-3-kinase; MZL: marginal zone lymphoma; WM: Waldenström macroglobulinaemia

1. Herman SE, *et al. Blood* 2010; 116:2078–88.
2. Yahiaoui OI, *et al. BMC Cancer* 2014; 14:565.
3. Leseux L, *et al. Blood* 2006; 108:4156–4162.

Idelalisib: a potent and selective inhibitor of PI3Kδ

In vitro activity[a] (IC_{50})[1] of Idelalisib and activity in cell-based assays (EC_{50})[2]

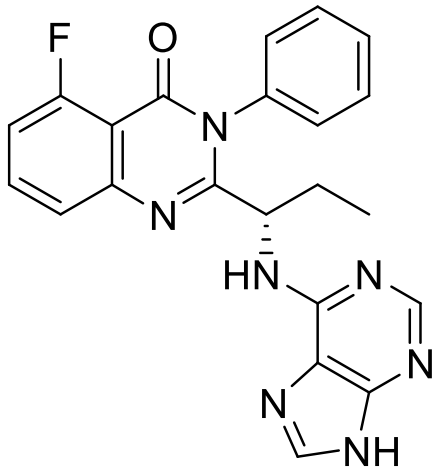
PI3K isoform	IC_{50} (nM)[1a]	IC_{50}-based PI3Kδ fold selectivity[1]	EC_{50} (nM)[2]	EC_{50}-based PI3Kδ fold selectivity[2]
δ	19	1	8.9	1
α	8600	453	>10,000	1124
β	4000	210	1419	153
γ	2100	110	2500	281

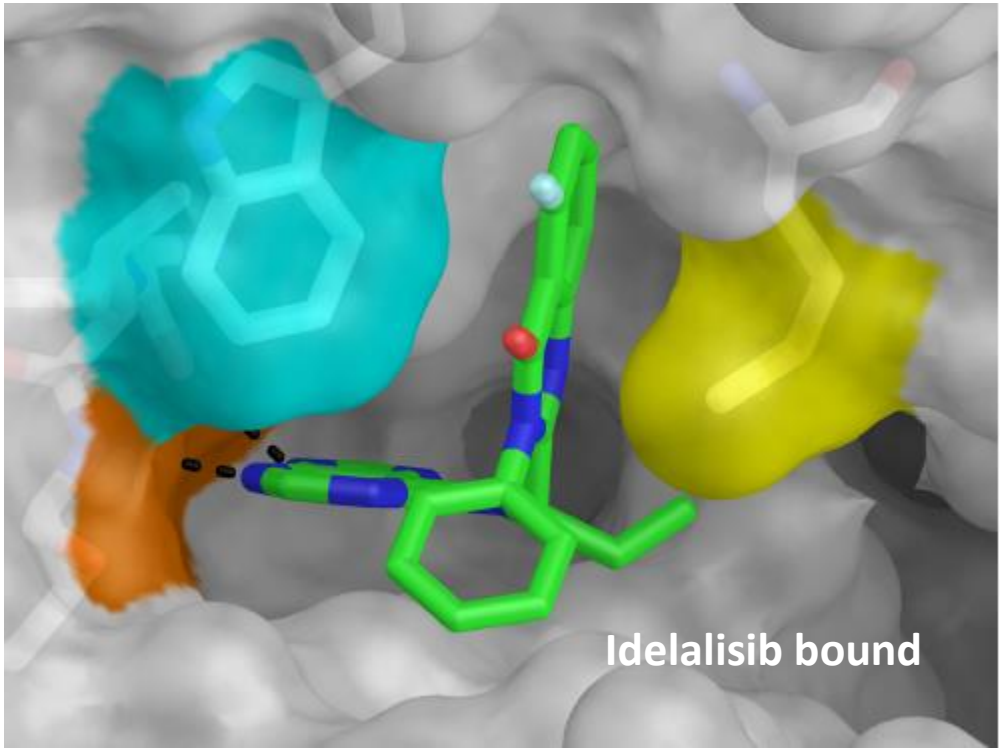
[a] In presence of $2xK_m$ adenosine triphosphate
EC_{50}: half maximal effective concentration;
IC_{50}: half maximal inhibitory concentration;
PI3K: phosphatidylinositol-3-kinase

1. Somoza JR, *et al. J Biol Chem* 2015;290:8439-8446.
2. Zydelig CHMP assessment report (Jul 2014; available at www.ema.europa.eu).

Propeller shape of Idelalisib contributes to its potency and selectivity for p110δ

Idelalisib is a first-in-class, oral, reversible inhibitor selective for PI3Kδ



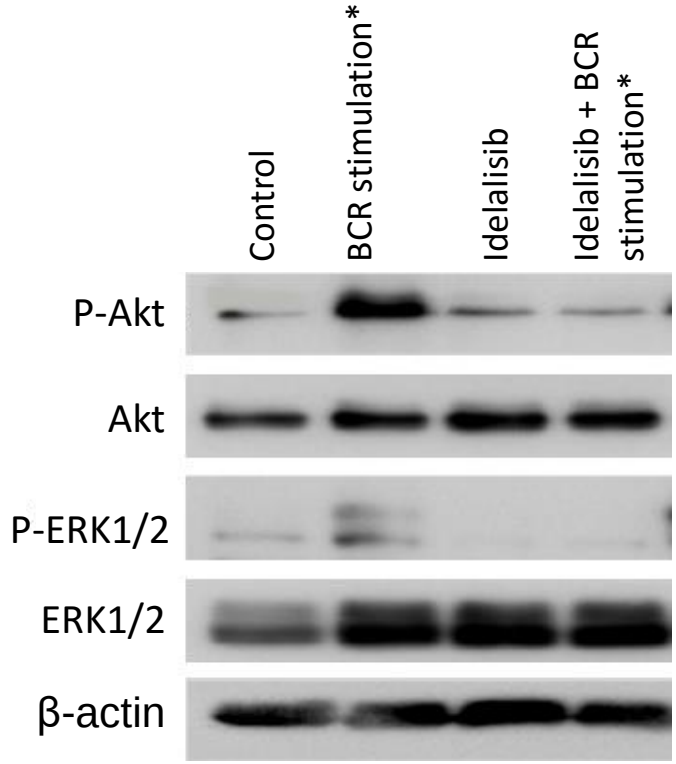


Idelalisib specifically binds to p110δ

To date, no mutations in the Idelalisib binding site have been reported

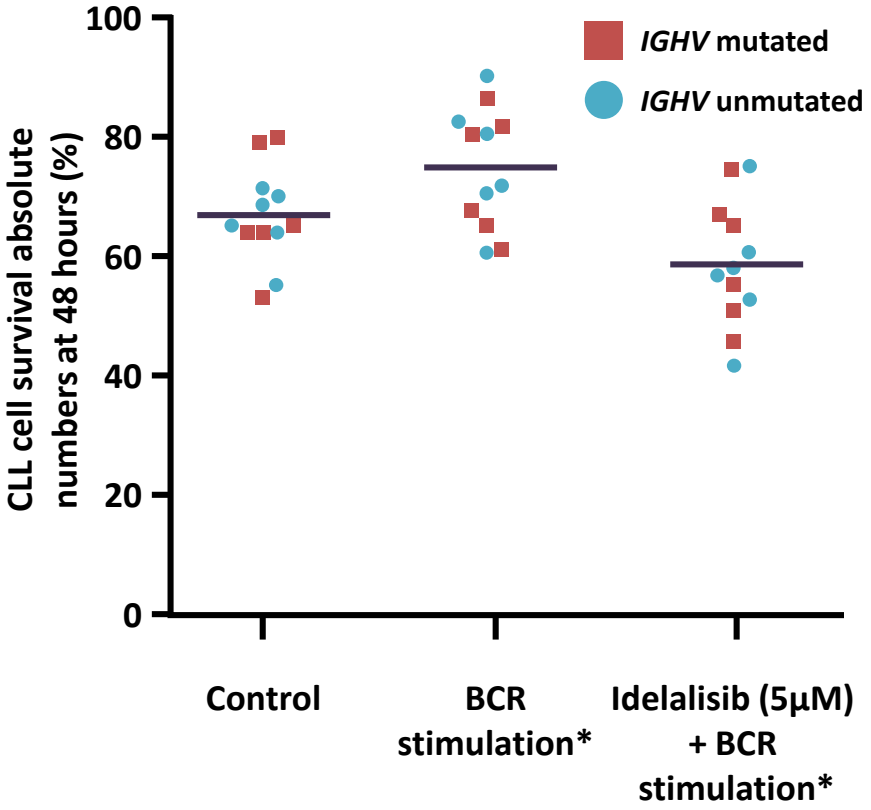
Idelalisib directly inhibits PI3Kδ activation via the BCR

Idelalisib inhibited BCR-induced AKT activation in CLL cells



Immunoblot using Ab or phospho-specific (P) Ab

Idelalisib inhibited BCR-stimulated cell survival in IGHV mutated and unmutated CLL cells

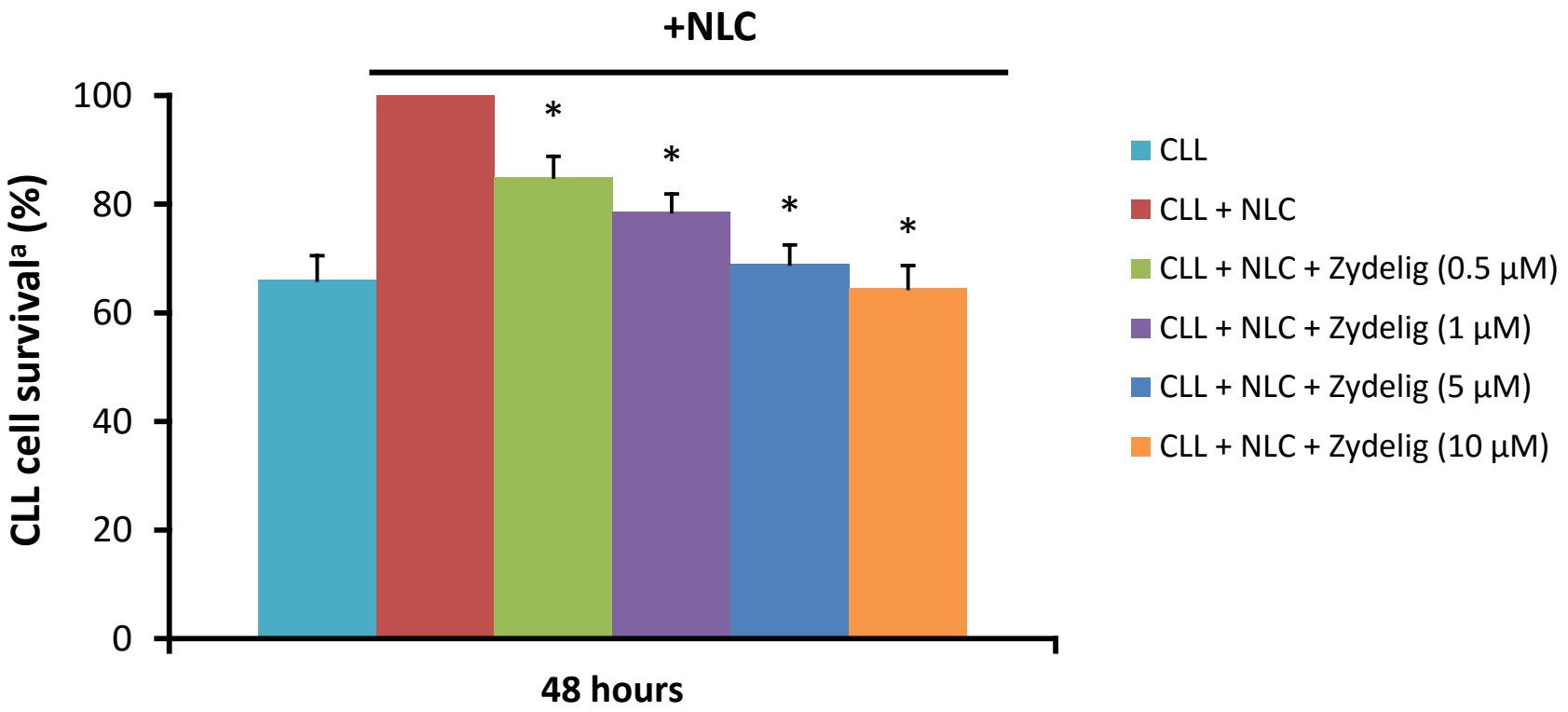


[a]Stimulated with anti-IgM antibody
BCR: B-cell receptor; CLL: chronic lymphocytic leukaemia; PI3K: phosphatidylinositol-3-kinase

Hoellenriegel J, *et al*. *Blood* 2011; 118:3603–3612

Idelalisib abrogates survival signals from the tumour microenvironment

Idelalisib significantly inhibited survival of CLL cells co-cultured with NLCs



*p<0.05 Idelalisib + CLL + NCL vs CLL + NLC

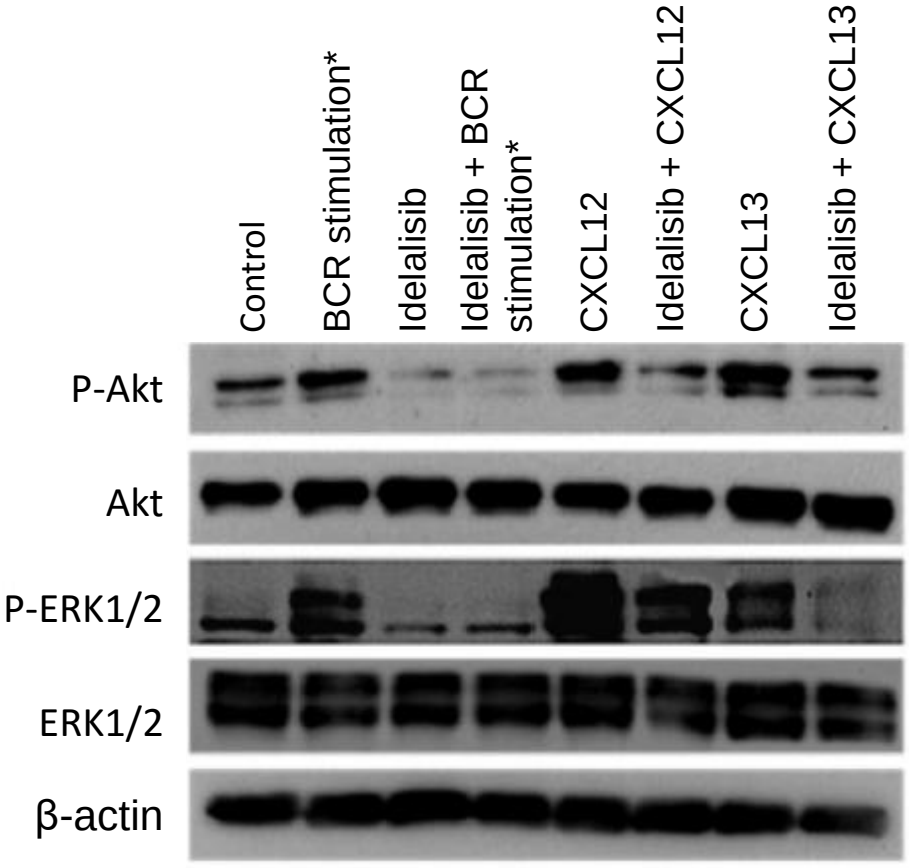
[a] Viabilities of Idelalisib-treated samples were normalised to values in CLL + NLC group

NLC: nurse-like cells

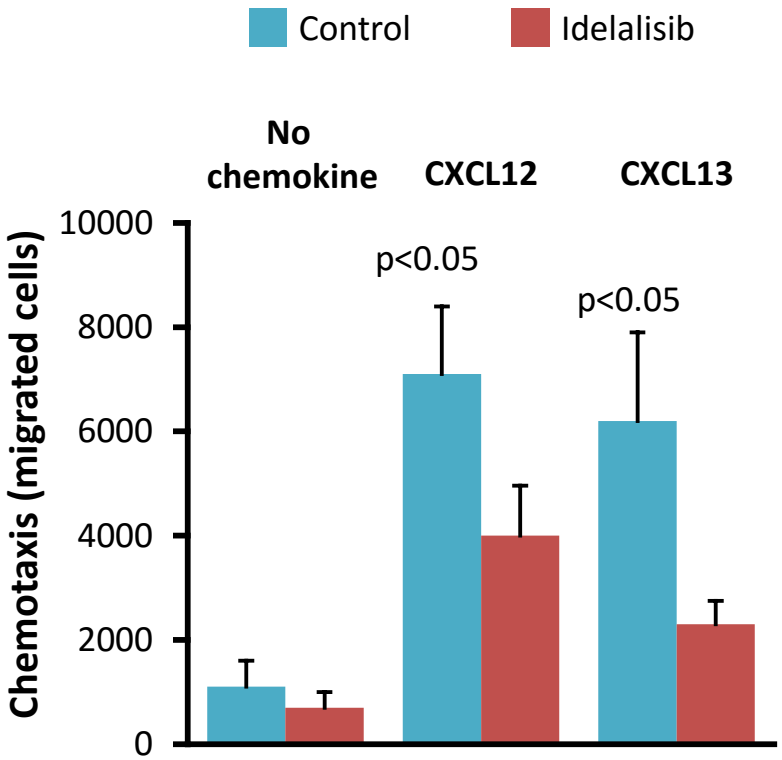
Hoellenriegel J, *et al*. *Blood* 2011; 118:3603–3612.

Idelalisib inhibits CLL cell chemotaxis and migration

Idelalisib abrogated activation of CXCR4 and CXCR5 in CLL cells



Idelalisib decreased chemotaxis of CLL cells in response to CXCL12 and CXCL13



*Stimulated with anti-IgM monoclonal antibody
CXCL: C-X-C motif chemokine ligand; PI3K: phosphatidylinositol-3-kinase

Hoellenriegel J, *et al*. *Blood* 2011; 118:3603–12

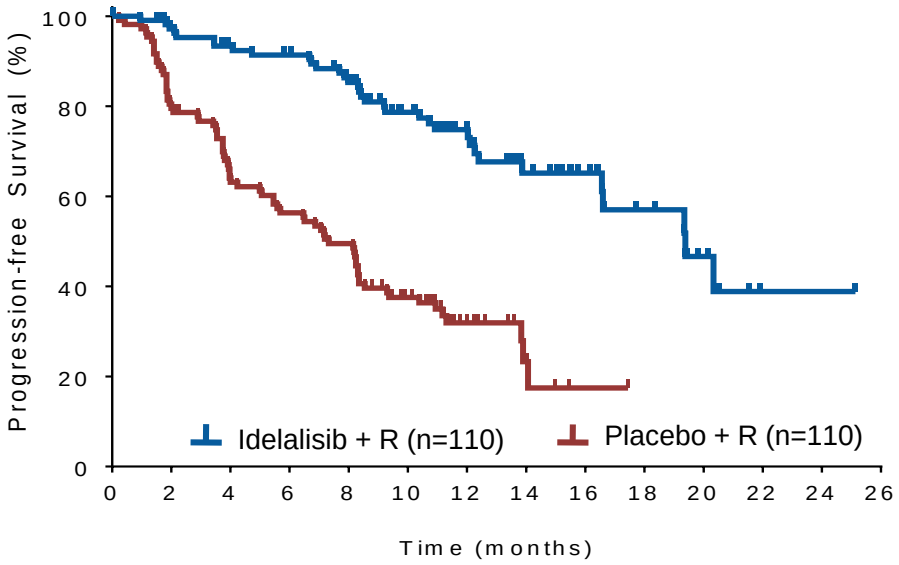
Salvage treatment: idelalisib

ORR idelalisib+R: 77%

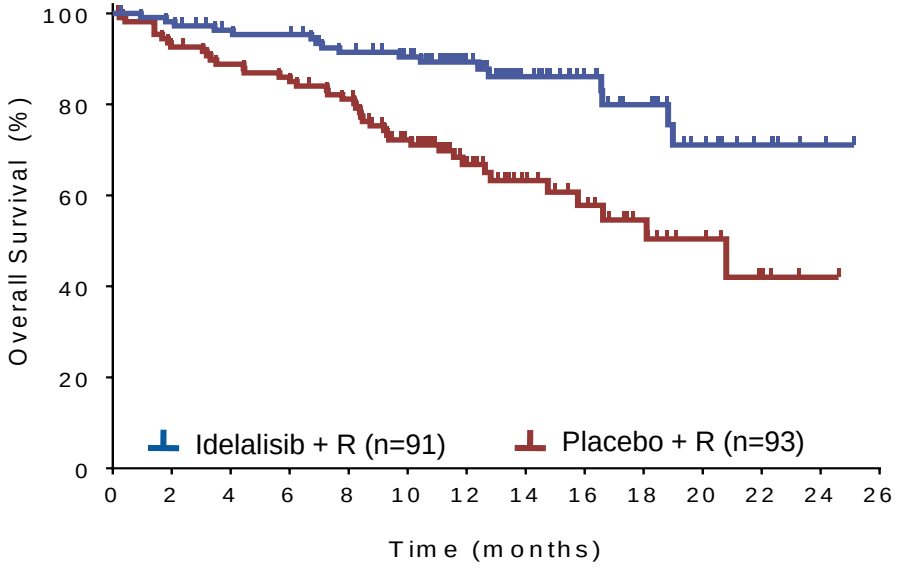
ORR placebo+R: 15%

Highly unfavourable features:
PFS <24 months after previous Tx
Appropriate for non cytotoxic treatment

- ANC <1000
- Plt <50
- CrCl <60 ml/min
- CIRS >6



Progression-free survival

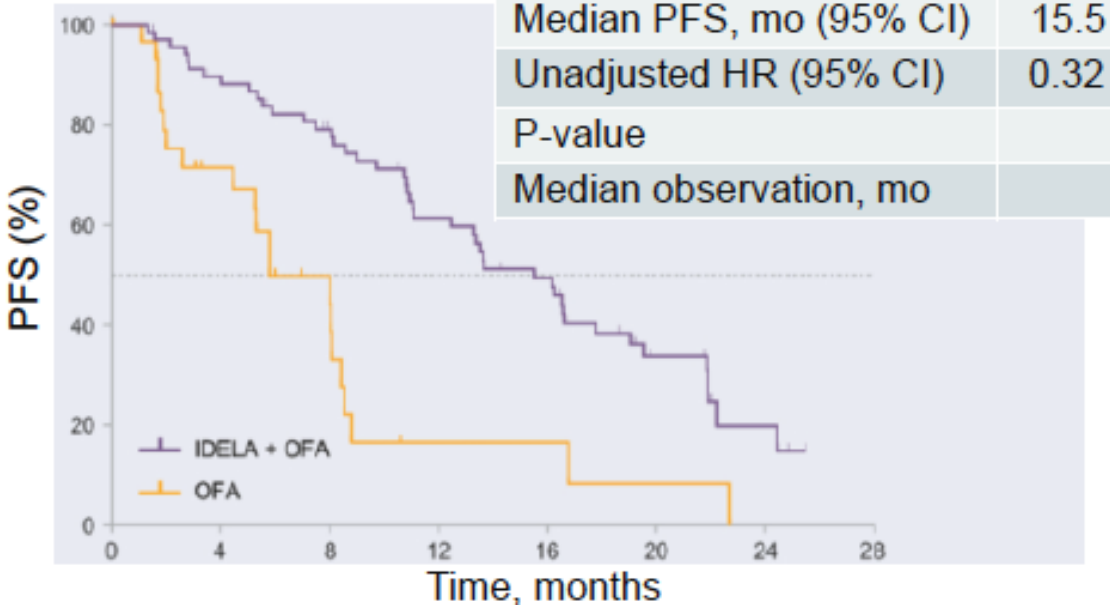


Overall survival

Sharman *et al. Blood* 2014 124:330 (ASH meeting abstracts).

Updated results from Phase 3 idelalisib and ofatumumab: PFS

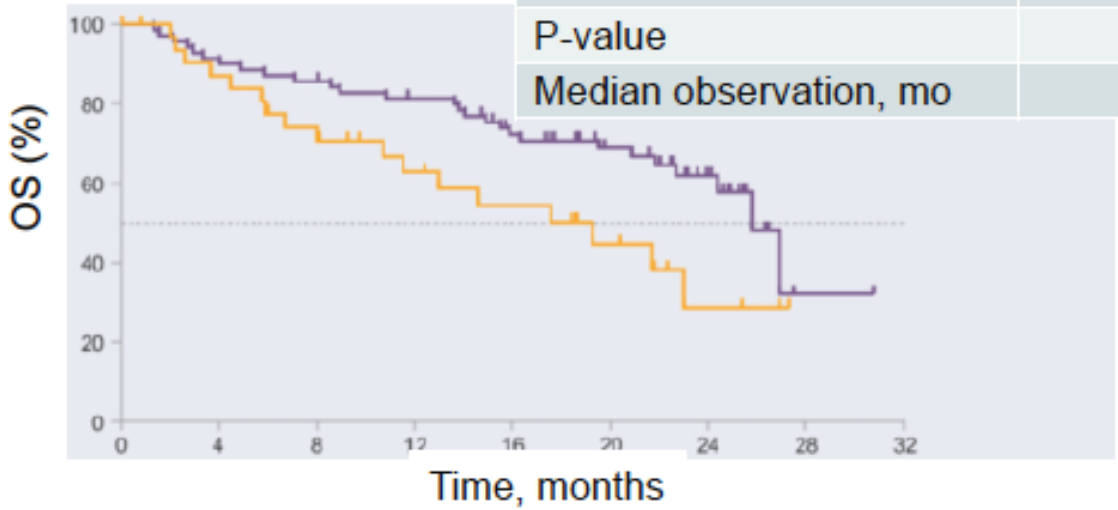
	Idelalisib (n=174)	Ofatumumab (n=87)
Events, n (%)	45 (64)	21 (64)
Median PFS, mo (95% CI)	15.5 (11.1, 19.1)	5.8 (4.5, 8.4)
Unadjusted HR (95% CI)	0.32 (0.18, 0.55)	
P-value	<0.001	
Median observation, mo	12.9	4.5



Robak et al., EHA 2016, #P213

Updated results from Phase 3 idelalisib and ofatumumab: OS

	Idelalisib (n=70)	Ofatumumab (n=33)
Deaths, n (%)	27 (39)	17 (52)
Median OS, mo (95% CI)	25.8 (22.7, NR)	19.3 (10.7, NR)
Unadjusted HR (95% CI)	0.52 (0.28, 0.96)	
P-value	<0.03	
Median observation, mo	19.7	11.5

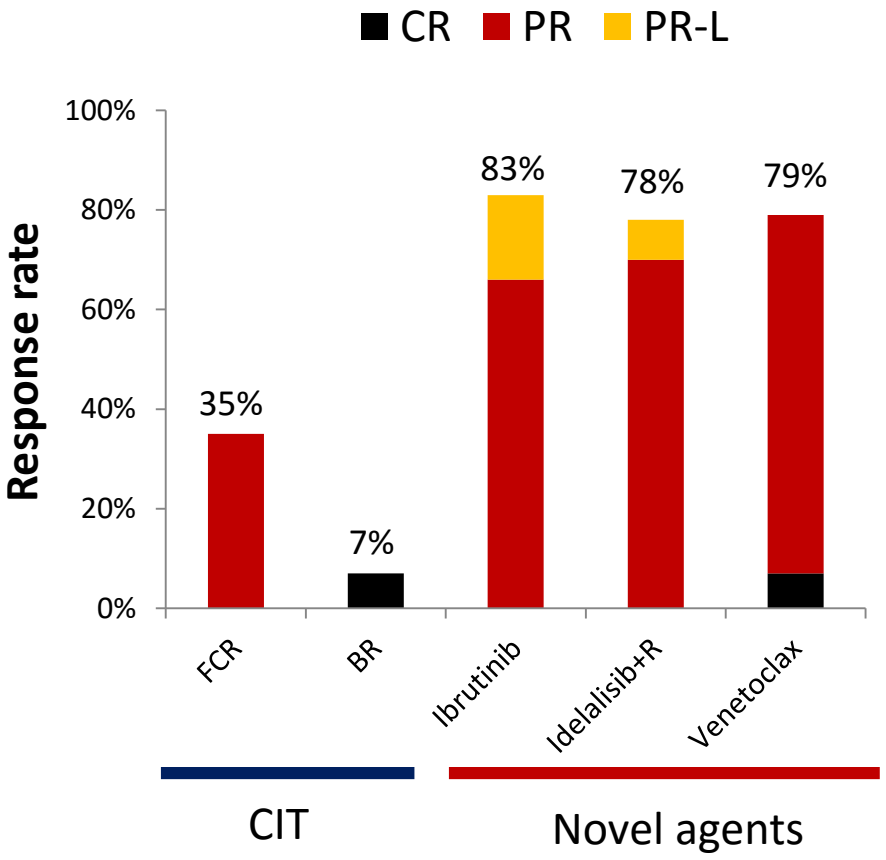


Robak et al., EHA 2016, #P213

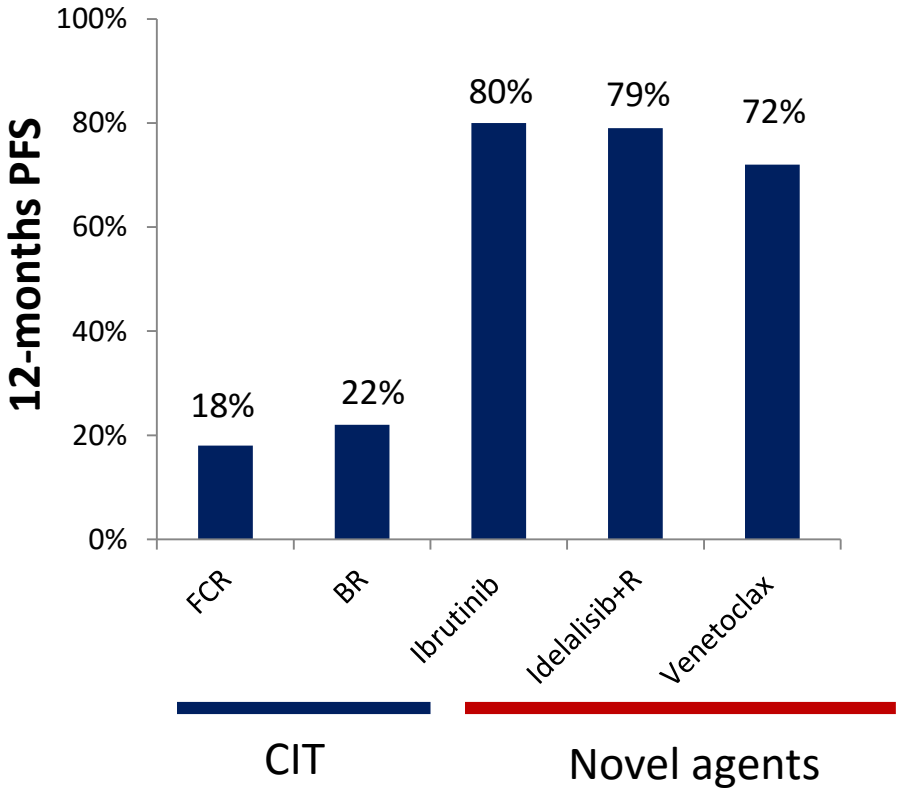
Chemoimmunotherapy (CIT) vs novel agents in *TP53* disrupted CLL

Relapsed/Refractory CLL

Response rate



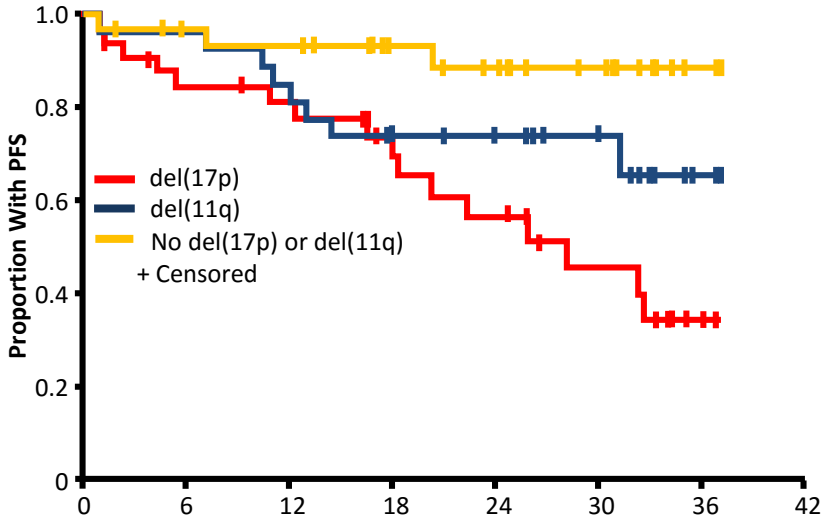
PFS



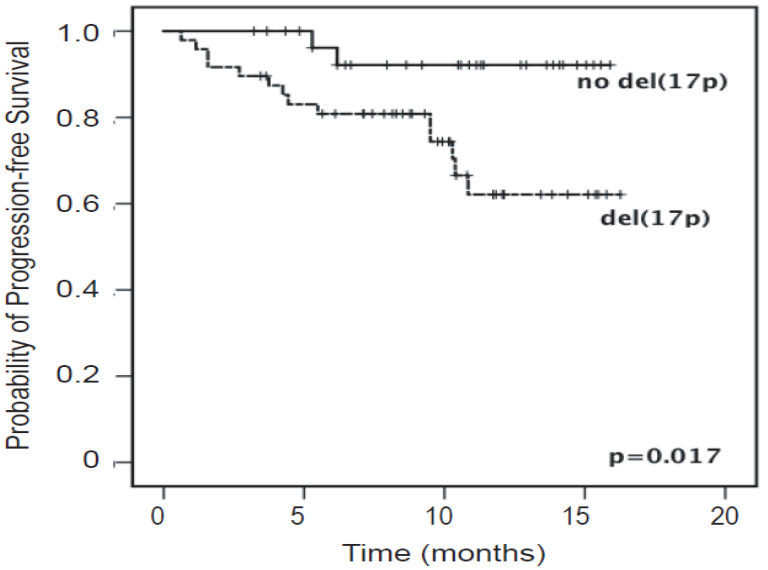
Badoux Blood 2011; Fisher J Clin Oncol 2011; O'Brien, ASH 2014; Sharman ASH 2014; Byrd ASH 2015; Stilgenbauer, ASH 2015

TP53 disruption is a prognostic biomarker in CLL treated with ibrutinib

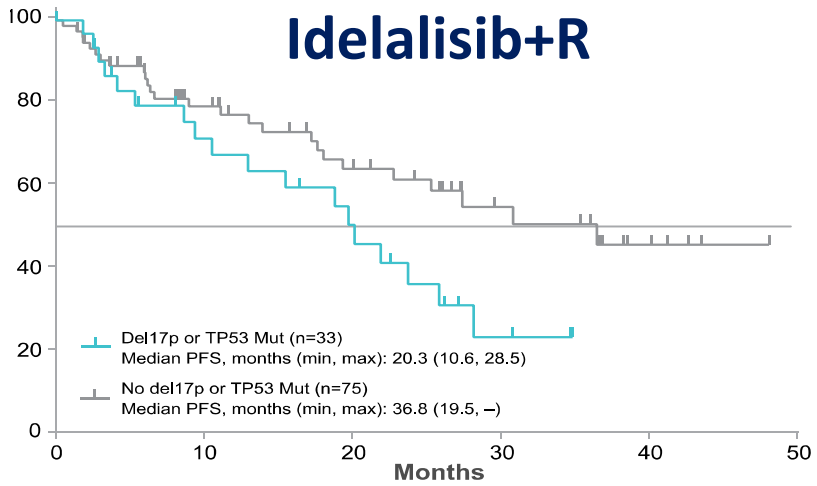
Ibrutnib in trials



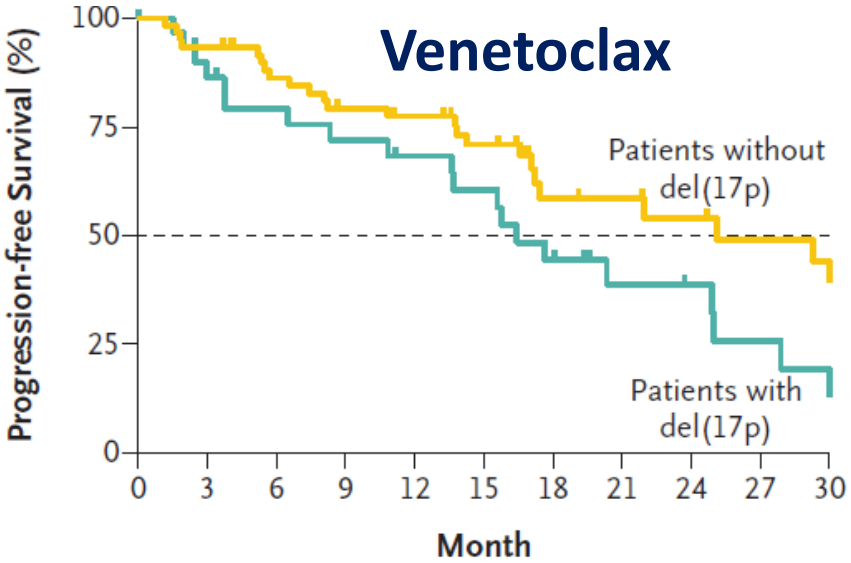
Ibrutinib in real-world practice



Idelalisib+R



Venetoclax

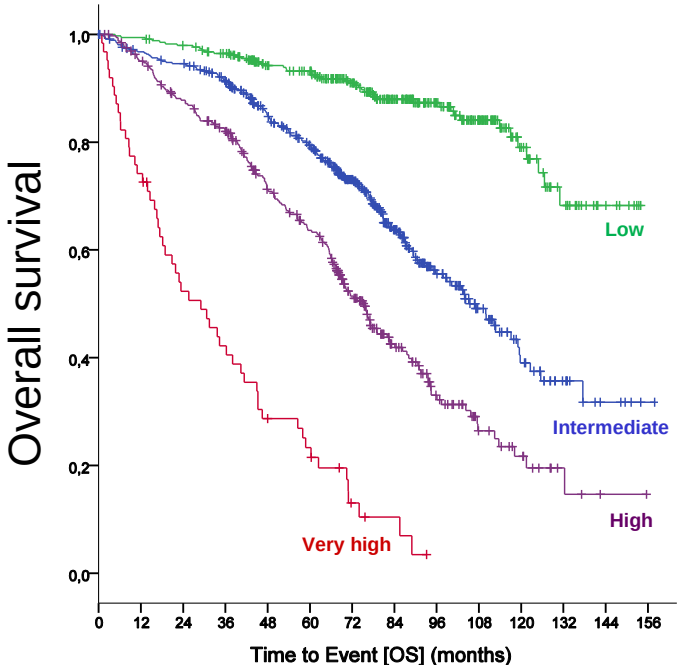


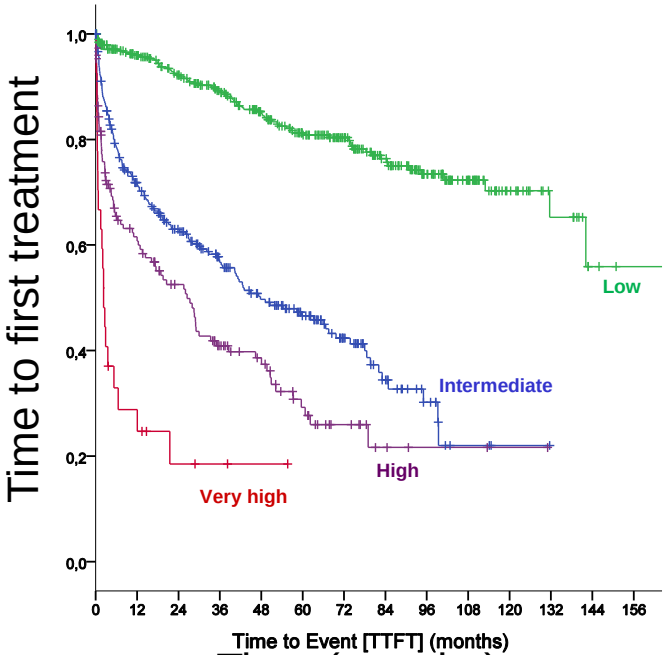
Byrd JC, Blood 2015; Thompson PA, Cancer 2015; Winqvist M, Haematologica 2016; Barrientos, ASCO,2015, 7011; Roberts, et al New Engl J Med 2016

Comprehensive approaches incorporating clinical, serum, genetic, and molecular markers into a single risk score: CLL-IPI

Variable	Adverse factor	Coeff.	HR	Grading
***TP53* (17p)**	deleted and/or mutated	**1.442**	**4.2**	**4**
***IGHV* status**	Unmutated	**0.941**	**2.6**	**2**
B2M, mg/L	> 3.5	**0.665**	**2.0**	**2**
Clinical stage	Binet B/C **or** Rai I-IV	**0.499**	**1.6**	**1**
Age	> 65 years	**0.555**	**1.7**	**1**
Prognostic Score				**0 – 10**

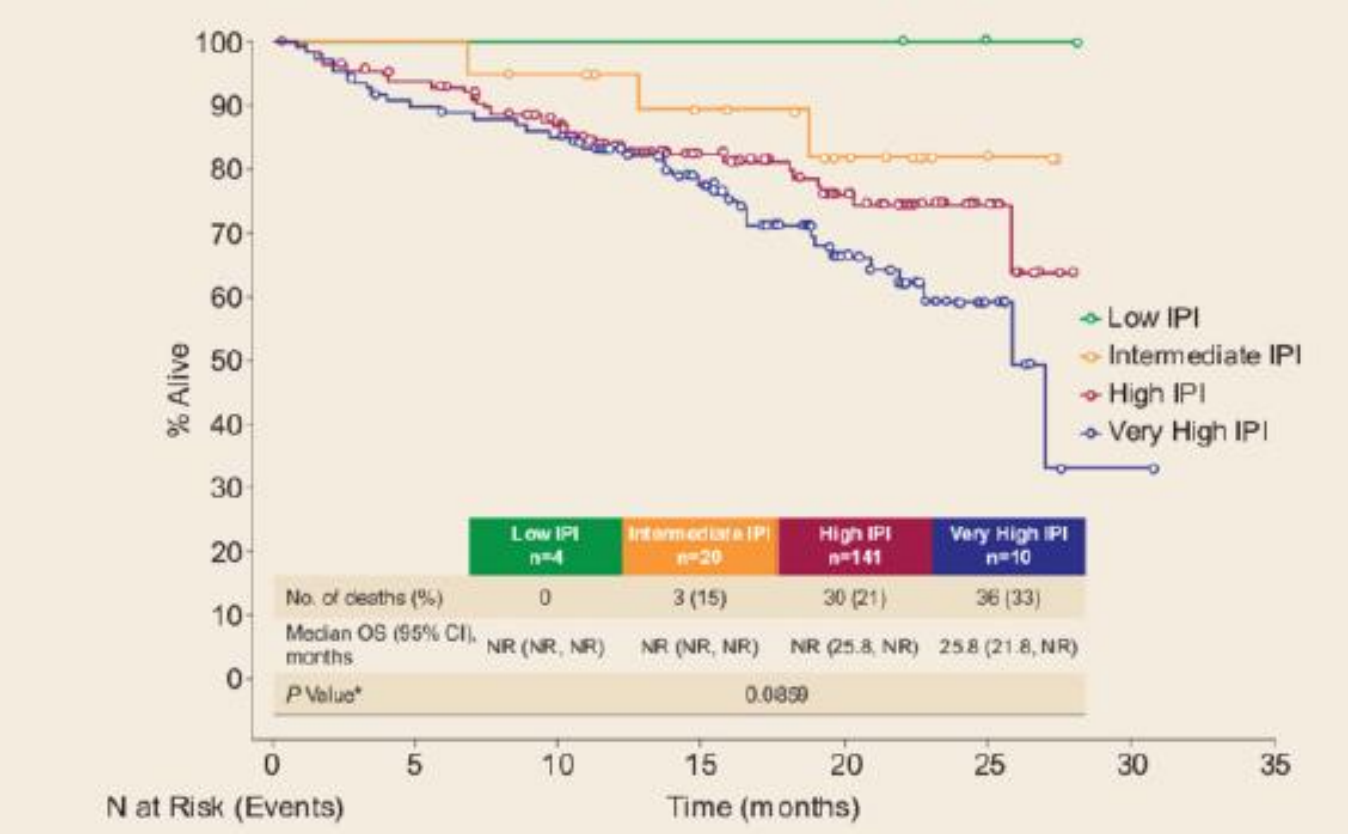
Risk group	Score	Patients N (%)	5-year OS, %
Low	**0 – 1**	340 (29)	93.2
Intermediate	**2 – 3**	464 (39)	79.4
High	**4 – 6**	326 (27)	63.6
Very High	**7 – 10**	62 (5)	23.3





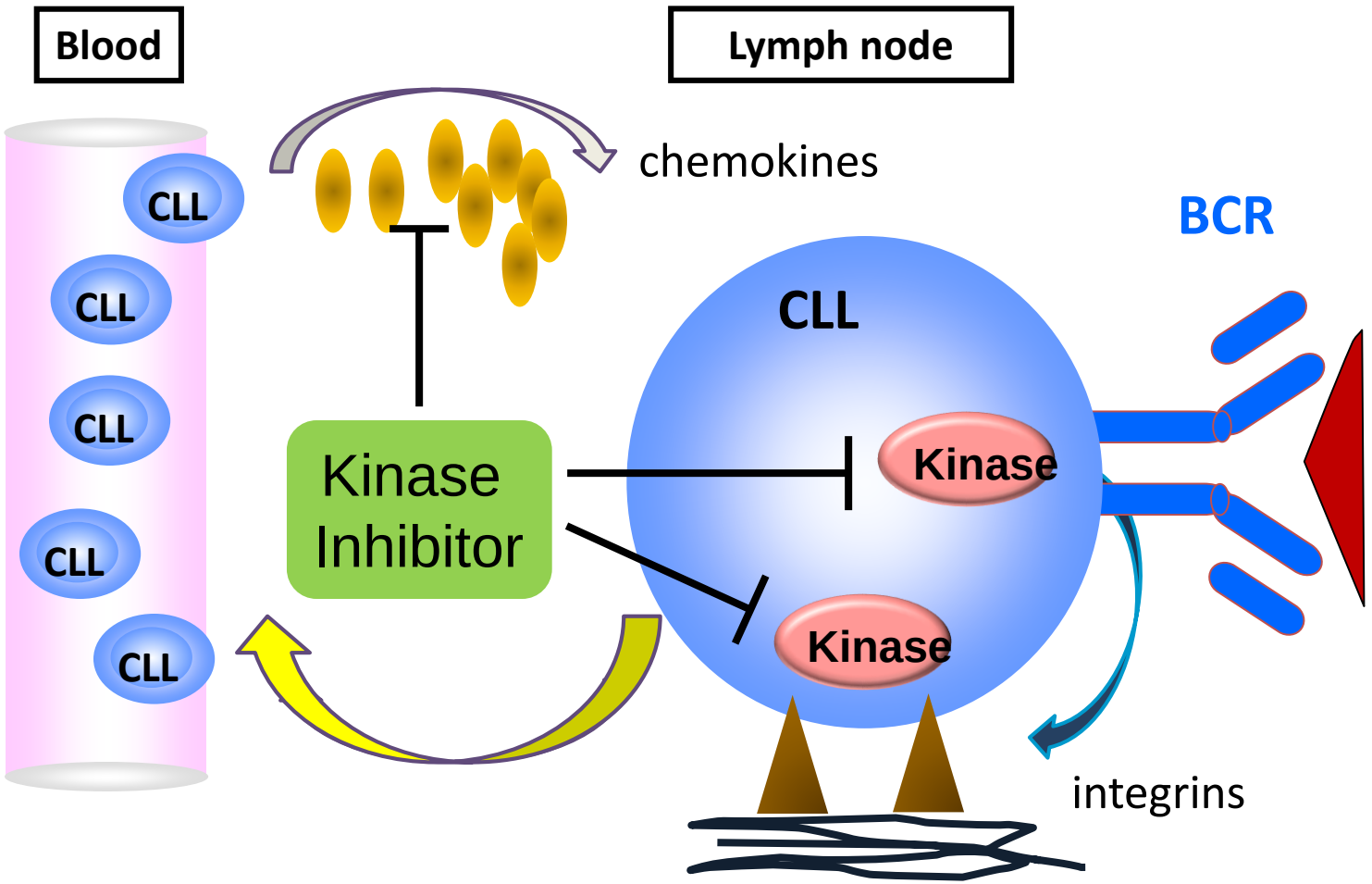
Kutsch N BJ, J Clin Oncol 2015;33(suppl). Abstract 7002; Wierda W, J Clin Oncol 2011;29:4088-4095; Pflug N, Blood 2014;124:49-62

CLL-IPI score and prognostic factor analysis in R/R CLL in patients treated with idelalisib



Soumerai et al. EHA 2016, #P214.

Redistribution lymphocytosis



De Rooij, Blood 2012; Ponader, Blood 2012; Herman, abstract #185

Toxicities of BCR inhibitors

Ibrutinib:

- Bruising, bleeding
- Atrial fibrillation
- Hypertension
- Arthralgia

- Drug interactions

CYP3A4 inducers/inhibitors affect inrutinib levels

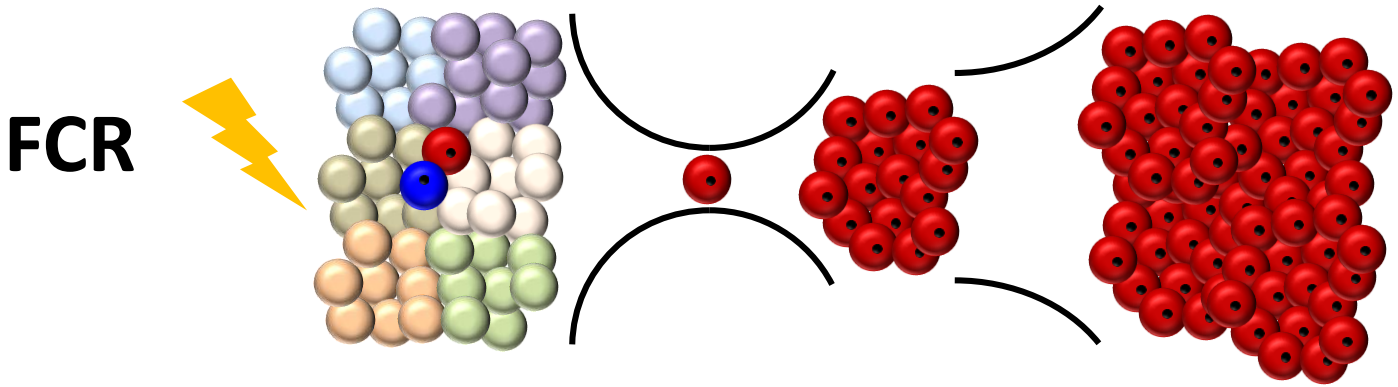
Idelalisib:

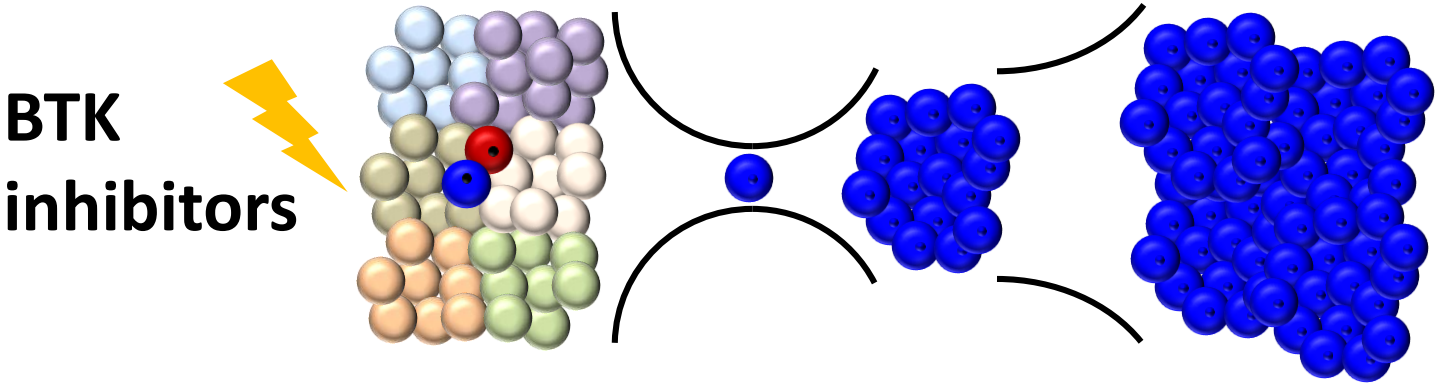
- Transaminitis
- Diarrhea/colitis
- Pneumonitis
- Infections

- Drug interactions

Idelalisib inhibits CYP3A4

Mutations that are inert under chemotherapy may become dangerous under new agents and vice versa



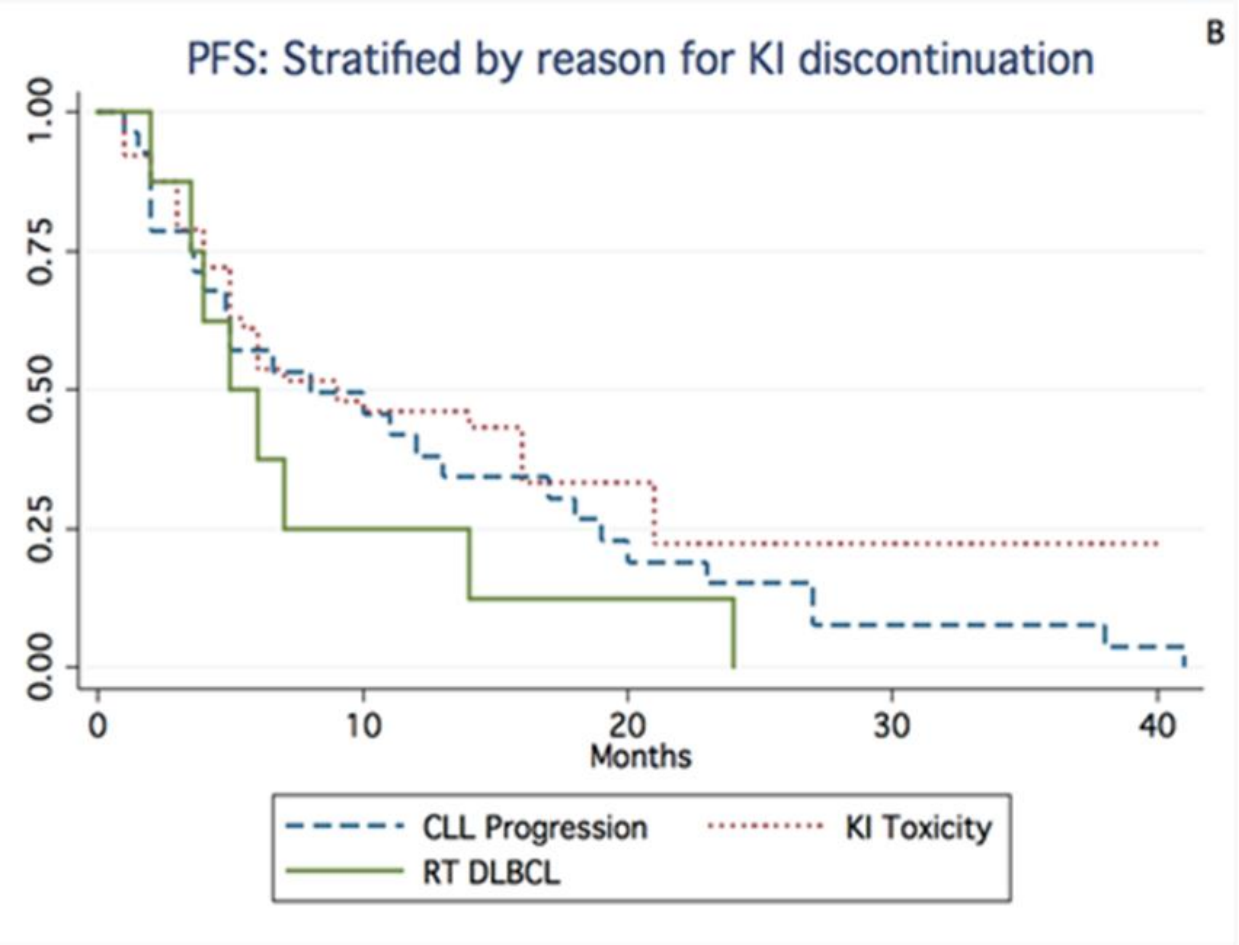


KI resistant subclone

FCR resitant subclone

Switch to another BCRi

ORR = 67%



Mato et al, ASH 2015

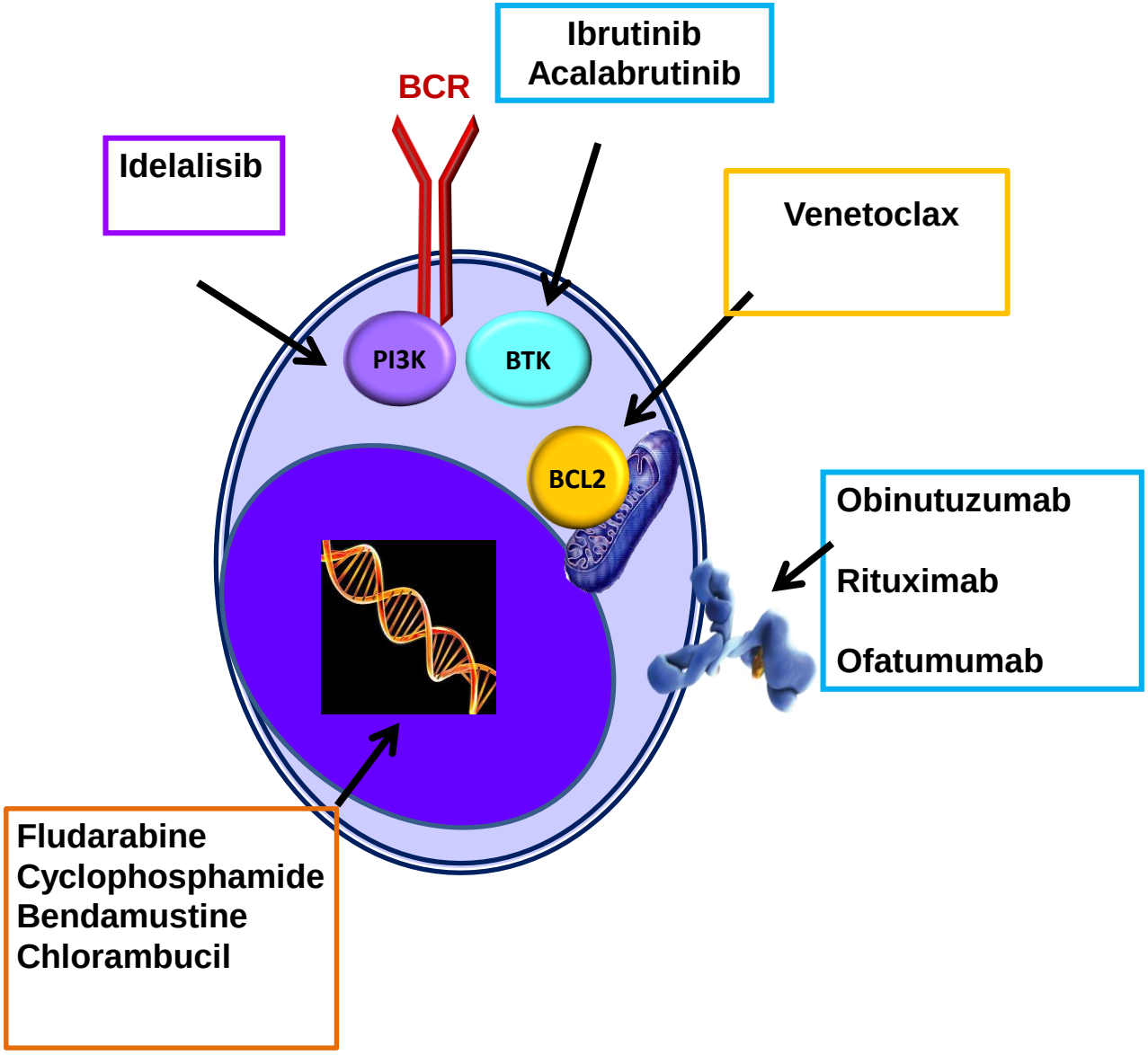
Switch to venetoclax

Phase 2 study of venetoclax in R/R CLL to ibrutinib or idelalisib

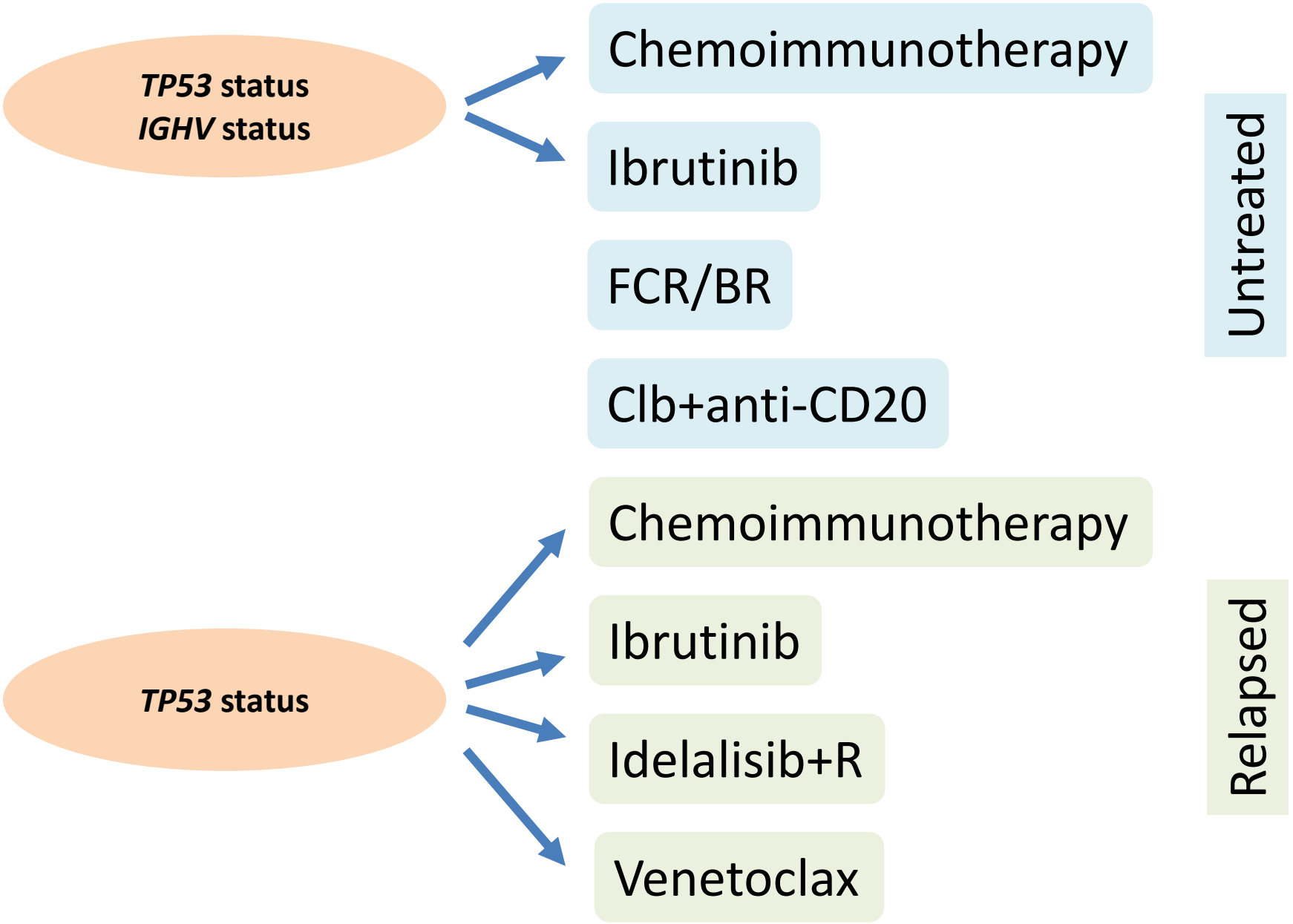
Best response, n (%)	Ibrutinib Arm (n=43)		Idelalisib Arm (n=21)	
	Investigator	IRC	Investigator	IRC
ORR	26 (61)	30 (70)	7 (33)	10 (48)
CR / CRi	2 (5) / 0	0 / 1 (2)	1 (5) / 1 (5)	0 / 0
nPR	2 (5)	0	0	0
PR	22 (51)	29 (67)	5 (24)	10 (47)
Stable disease	12 (28)	-	12 (57)	0
Disease progression	1 (2)	-	1 (5)	-
Non-responder	-	13 (30)	-	11 (52)

Coutre et al, EHA 2016

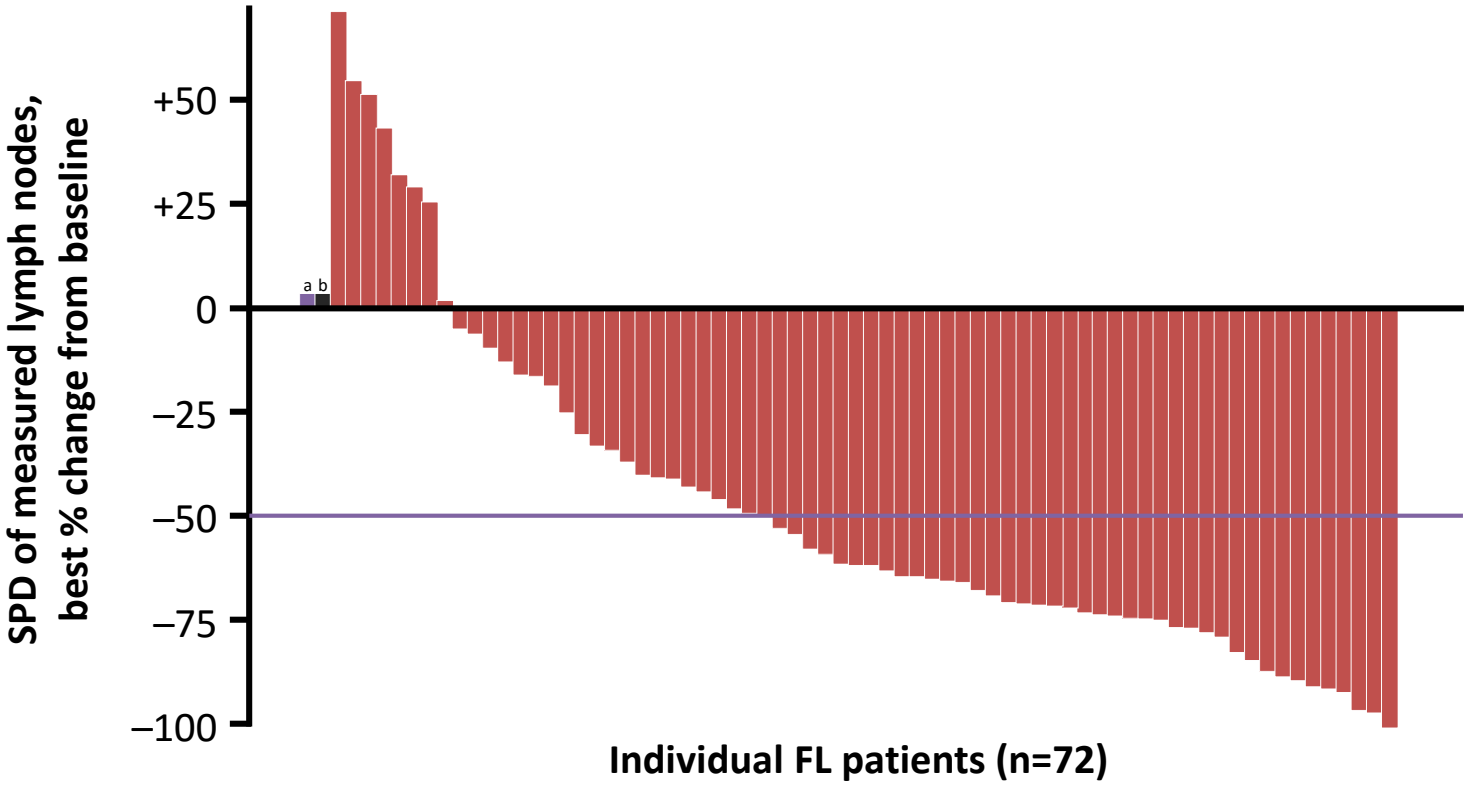
Old and new agents for CLL treatment



Can treatment decision be informed by biomarkers?



Idelalisib effectively reduced lymph node size in 89% of FL patients

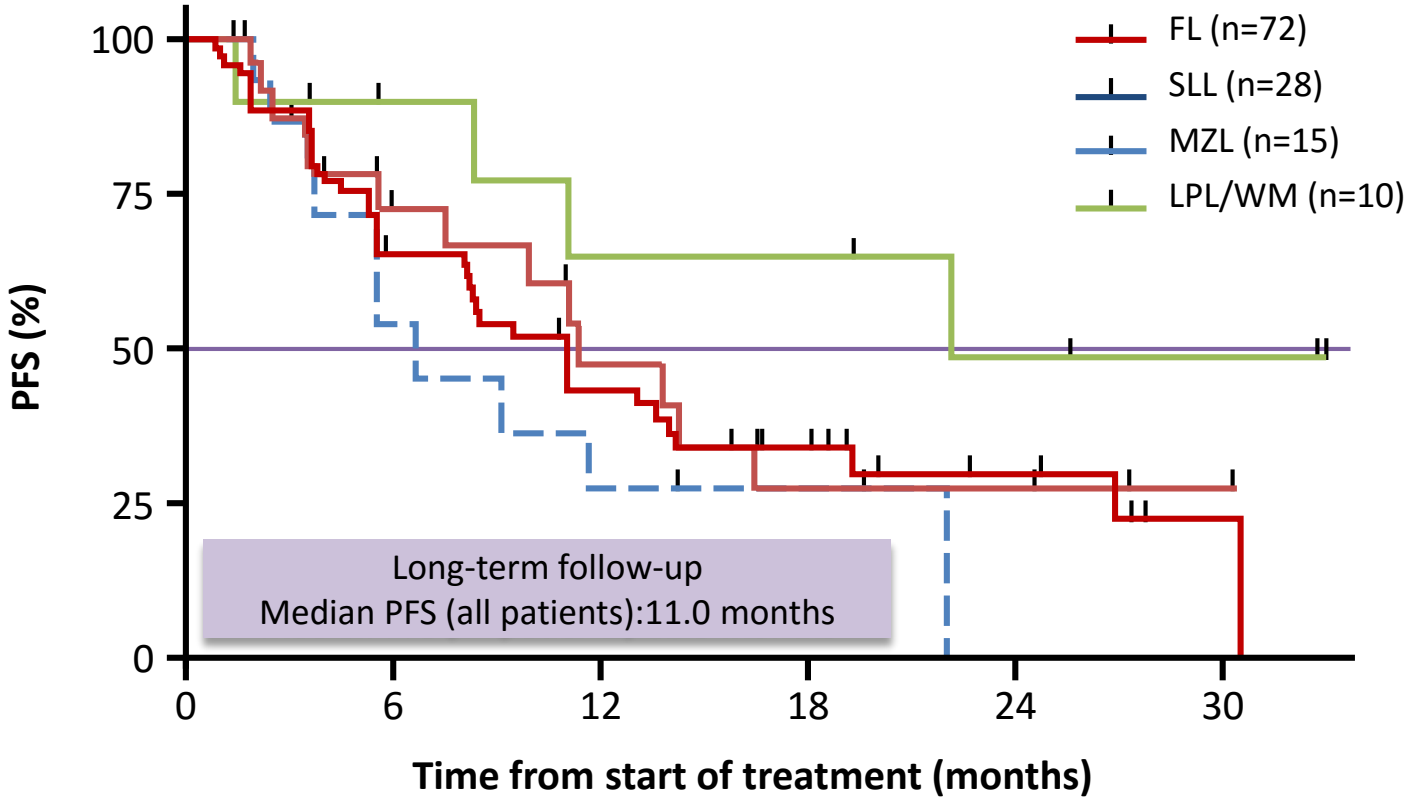


56% of patients achieved lymph node response
(long-term follow-up; June 2014 cut-off)

Two patients had no post-baseline evaluation: [a] one patient was not evaluable and
[b] one patient had disease progression on the basis of lymph node biopsy, no baseline evaluation
SPD: sums of the products of the perpendicular dimensions

Gopal AK, *et al*. ASH 2014 (Abstract 1708).

Disease progression delayed in a heavily pretreated iNHL population



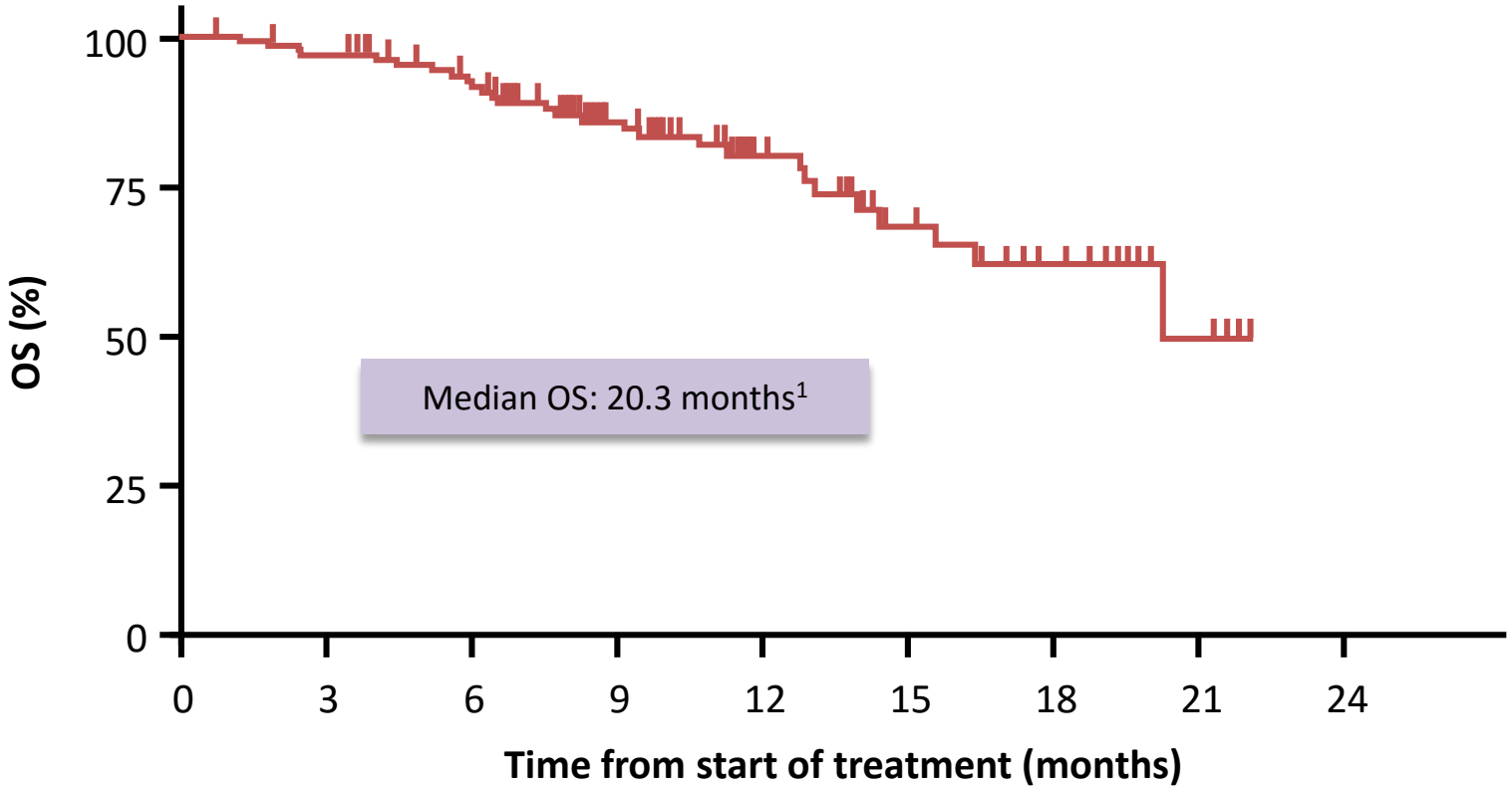
Patients at risk, n						
FL (n=72)	72	35	18	11	5	1
SLL (n=28)	28	12	7	4	4	1
MZL (n=15)	15	6	3	2	–	–
LPL/WM (n=10)	10	7	5	5	3	2

Includes patients who achieved a complete response or partial response
(or minor response for LPL/WM) according to independent review committee assessments

Gopal AK, *et al*. ASH 2014 (Abstract 1708, poster presentation).

OS prolonged in a heavily pretreated iNHL population

Updated median OS: 30.8 months[2]



1. Gopal AK, et al. *N Engl J Med* 2014; 370:1008–1018.
2. Gopal AK, *et al*. ASH 2014 (Abstract 1708, poster presentation).

Mechanism of BCR inhibitors: main clinical implications

- BCR inhibitors “circumvent” the chemorefractoriness to genotoxic agents (classical chemo)
- BCR inhibitors “circumvent” TP53 disruption
- The mechanism of BCR inhibitors is independent of acquisition of MRD negativity

The BCR undergoes genetic mutations in B cell NHL