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BIOLOGIA ED EZIOPATOGENESI

DELLA LEUCEMIA LINFATICA CRONICA

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Disclosures

Abbvie (Advisory Board, Speakers' Bureau)

Astra-Zeneca (Advisory Board)

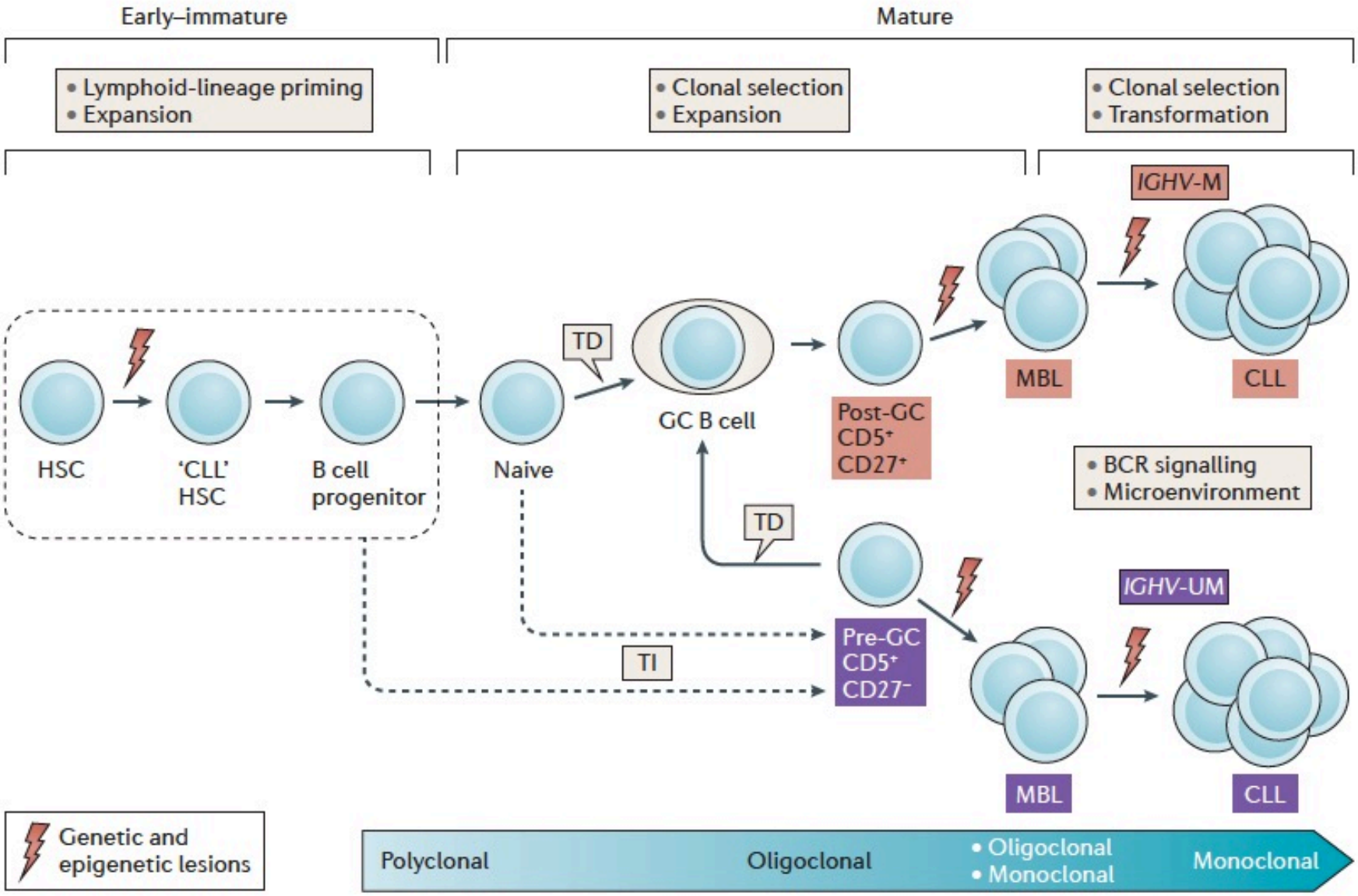
Beigene (Advisory Board)

Incyte (Advisory Board)

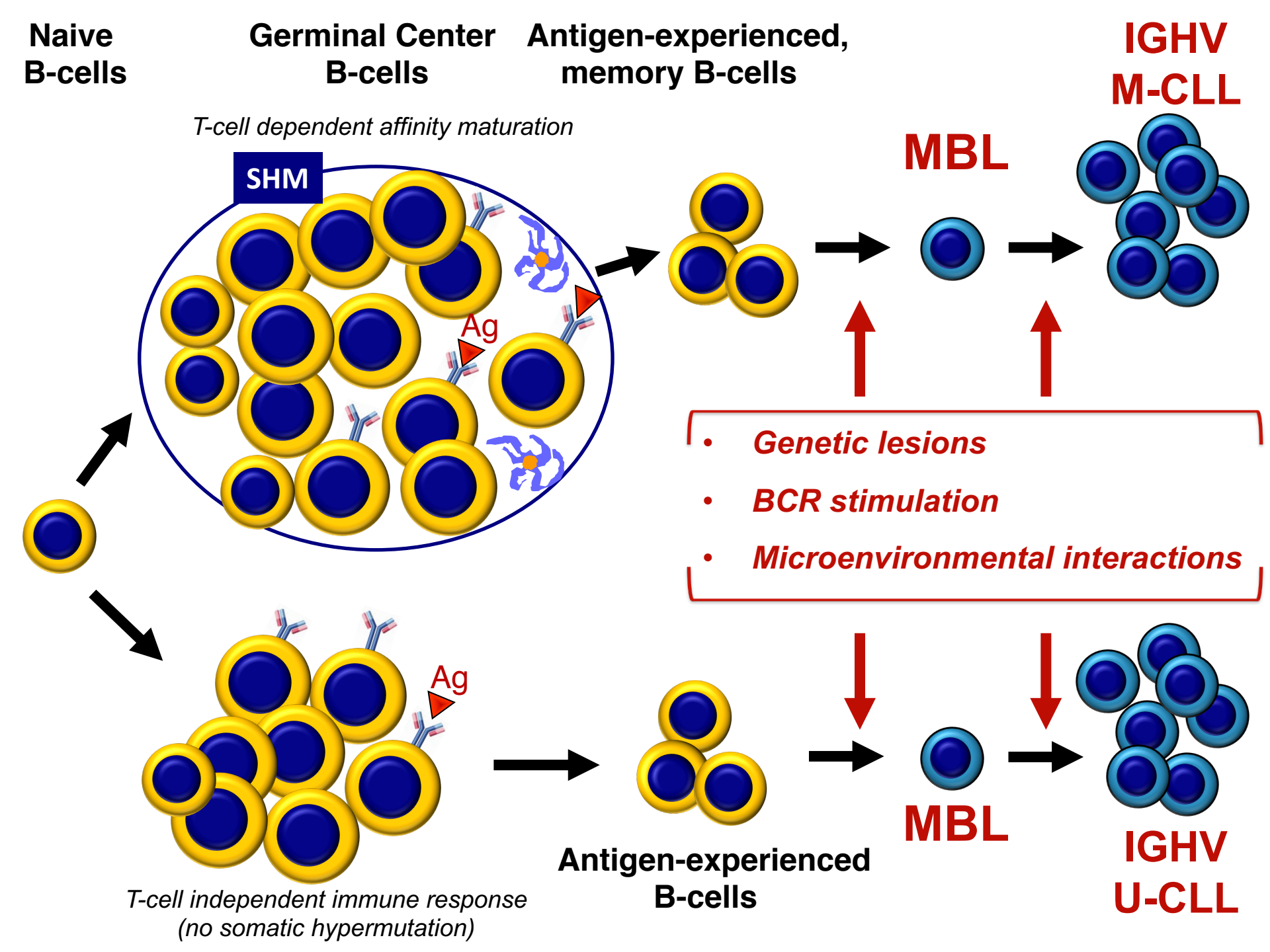
Janssen (Advisory Board, Speakers' Bureau)+

Roche (Advisory Board)

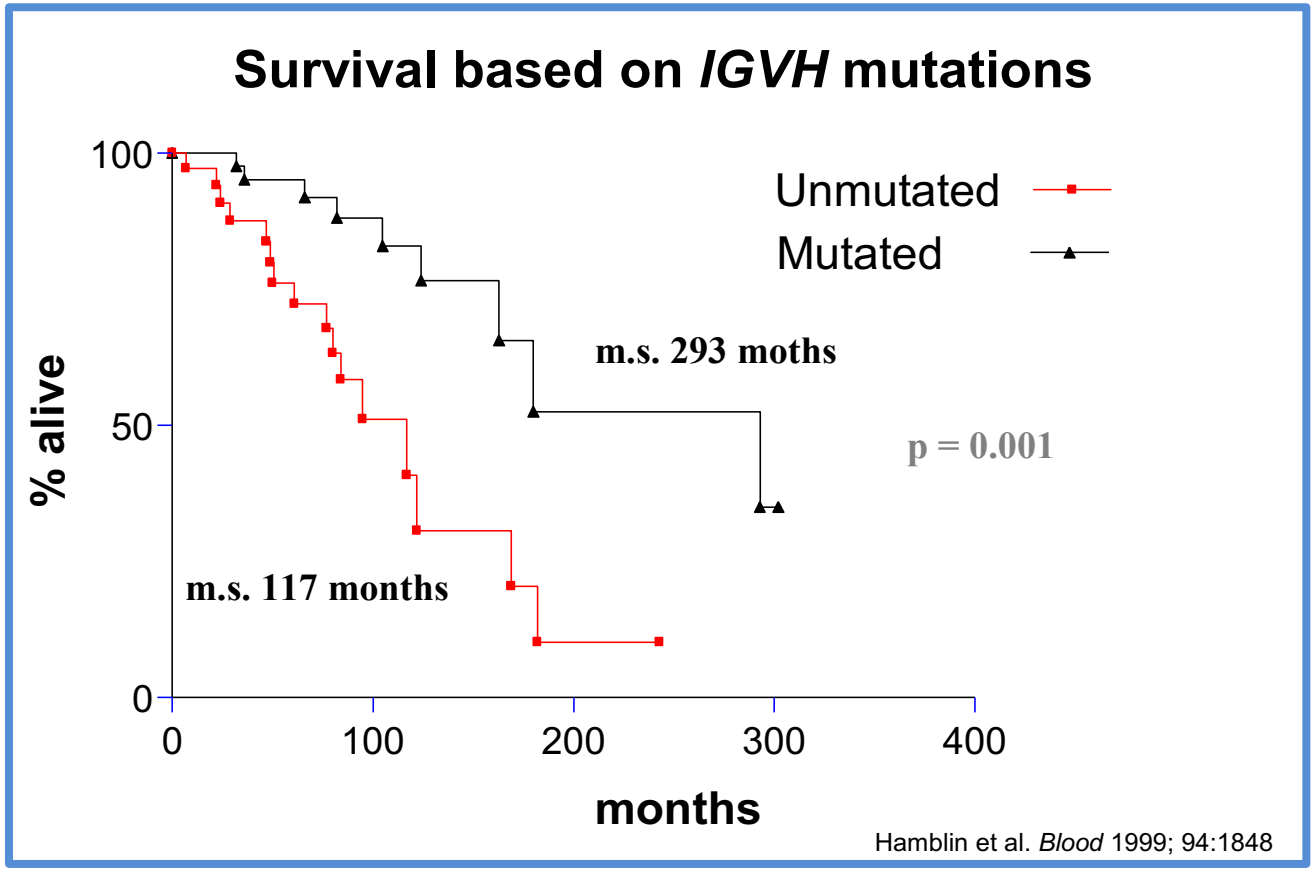
The cellular origin of CLL



Fabbri and Dalla-Favera, Nat Rev Cancer 2016


Naive B-cells
Germinal Center B-cells
Antigen-experienced, memory B-cells
IGHV M-CLL
T-cell dependent affinity maturation
SHM
Ag
MBL
• Genetic lesions
• BCR stimulation
• Microenvironmental interactions
Ag
T-cell independent immune response (no somatic hypermutation)
Antigen-experienced B-cells
MBL
IGHV U-CLL

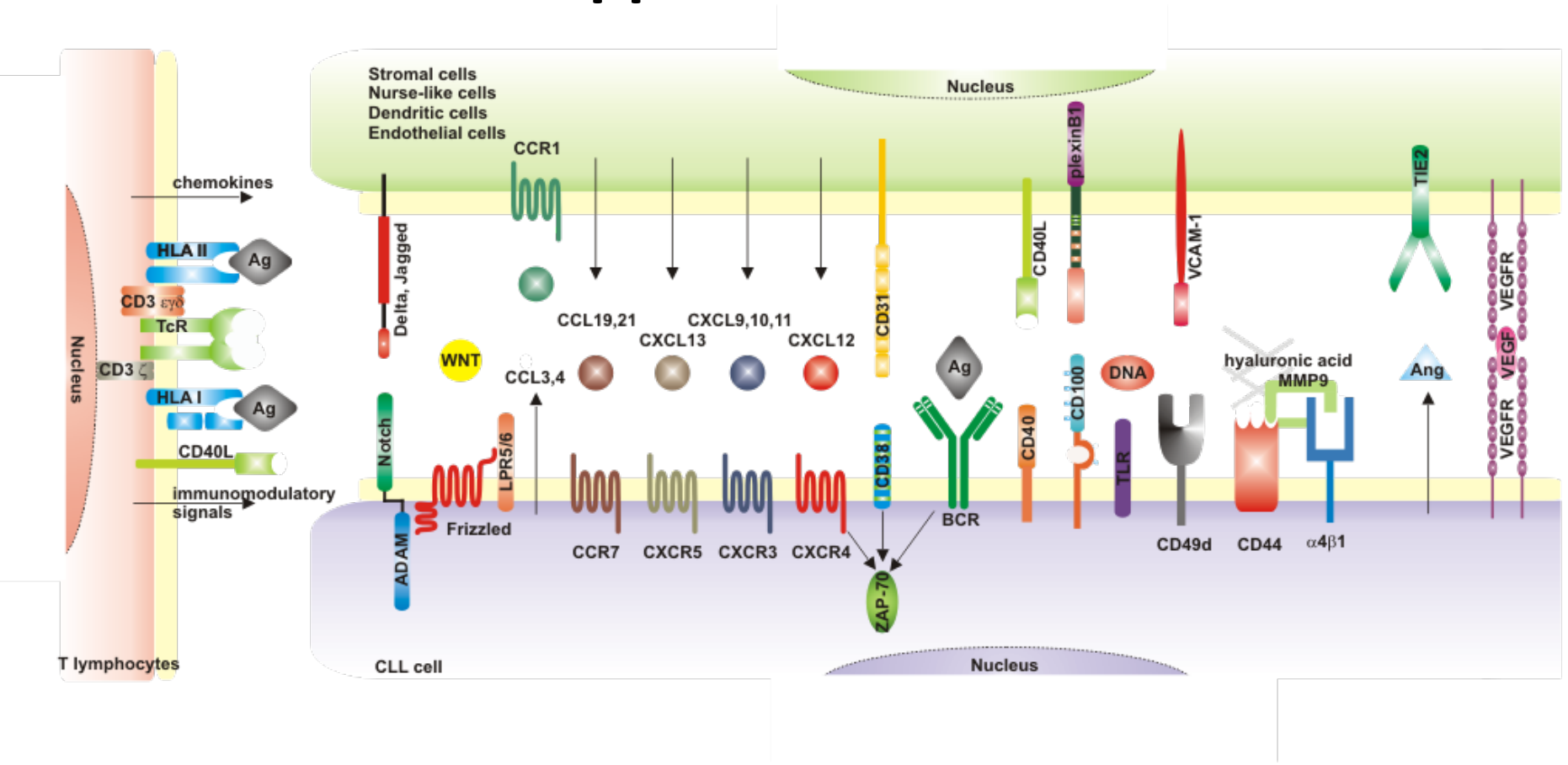
IGVH mutation status as a prognosticator: somatically unmutated IGHV genes associate with poorer survival



Hamblin et al. *Blood* 1999; 94:1848

CLL is a tumor that is “addicted to the host”

“an opportunistic tumor”



Evidence that the initial expansion of the CLL clones is B cell receptor (BCR) driven

Structural evidences

- Frequent expression of **stereotyped (= highly homologous) BCRs**: recognition of common antigens

Functional evidences

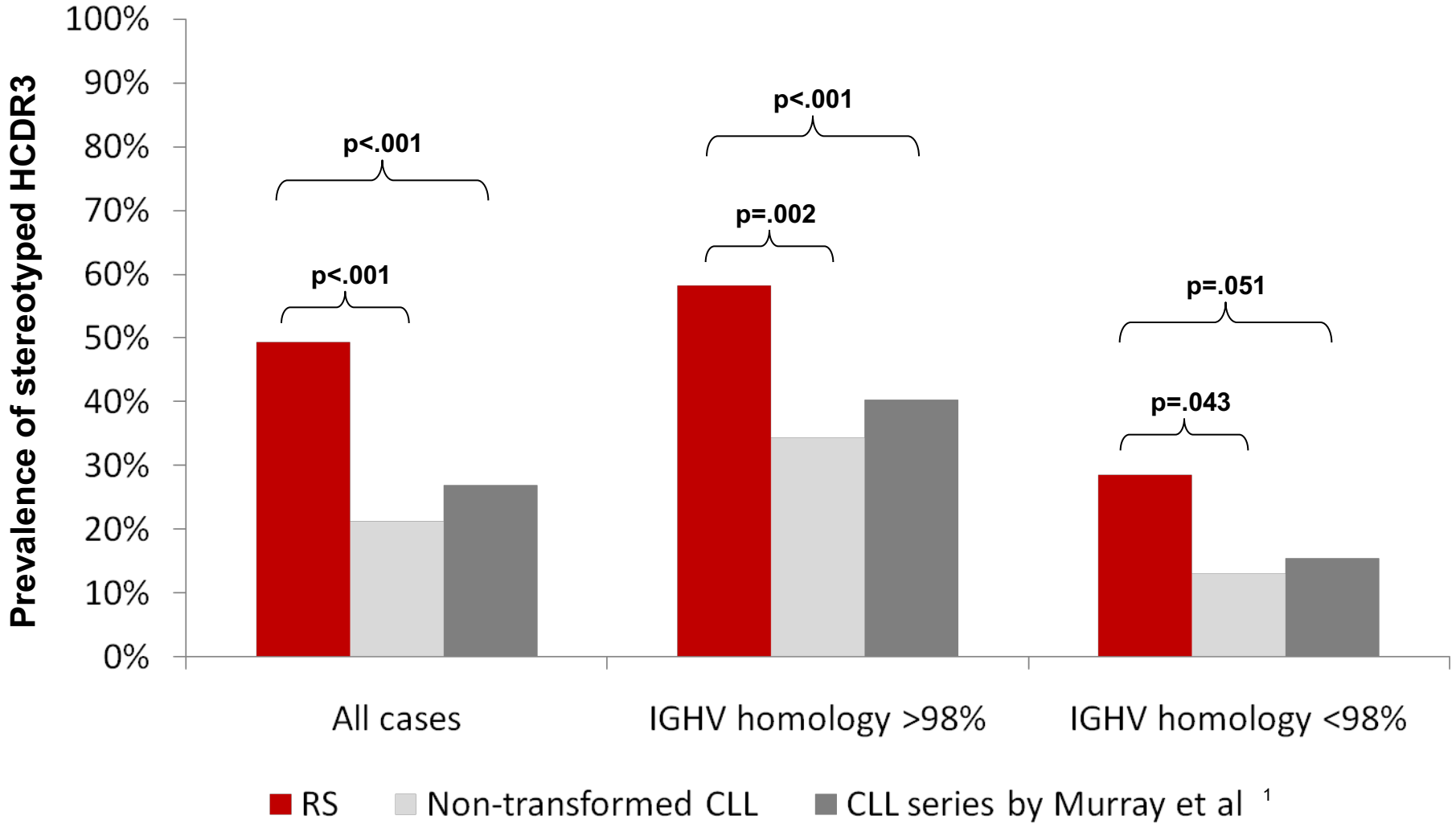
- High levels of BCR target genes in CLL cells
- Expression of constitutively active BCR signaling molecules
- **BCR activation supports CLL cell survival** in vitro

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

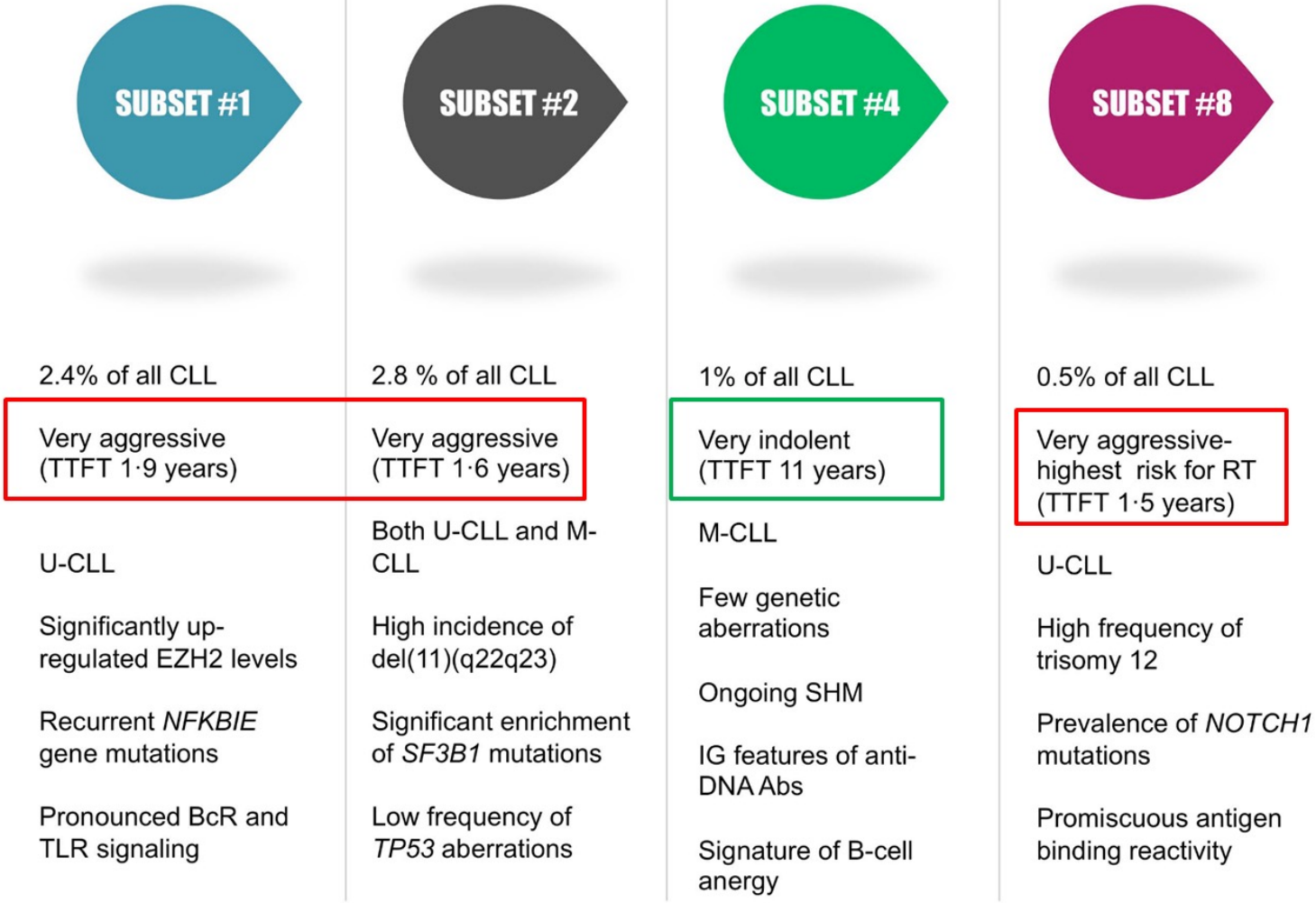
CLL carry stereotyped B cell receptors (=highly homologous) at high frequency



Rossi et al, Clin Cancer Res 2009

[1]Murray et al, Blood 2008

Stereotyped BCRs and CLL prognosis



Stamatopoulos K et al., *Leukemia*. 2017 Feb;31(2):282-291.

Subset #2 is an independent marker for unfavorable prognosis

assessment within prospective GCLLSG clinical trials of chemoimmunotherapy

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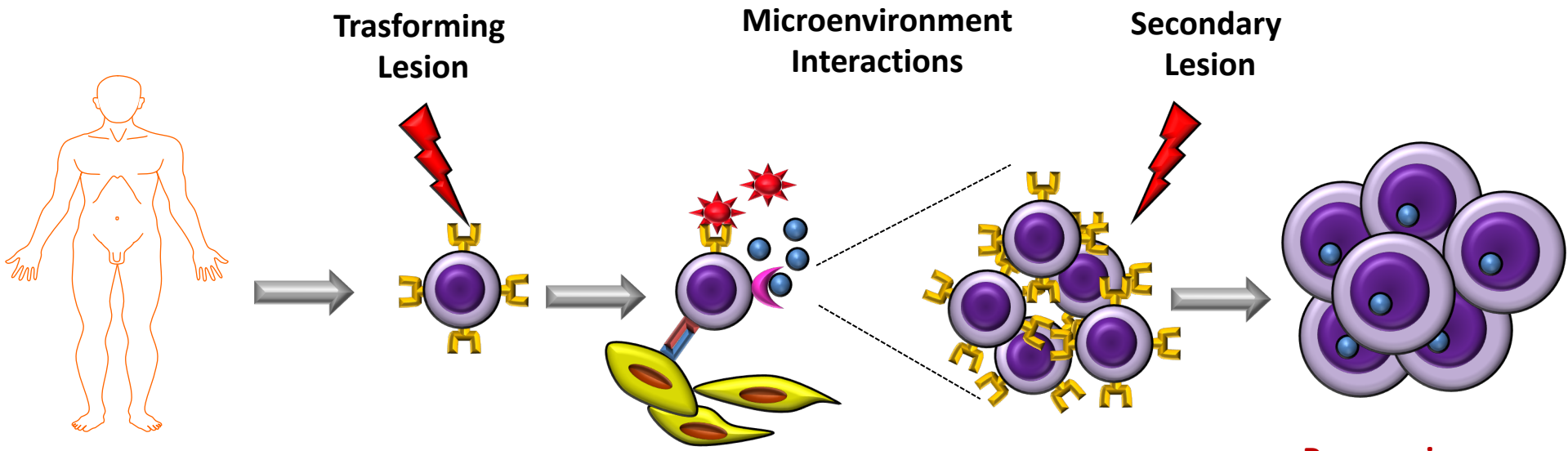
subset #2 should be proposed for risk stratification of patients

subset #2 patients do not benefit from chemoimmunotherapy

Jaramillo et al. Haematologica 2020; 105(11):2598-2607

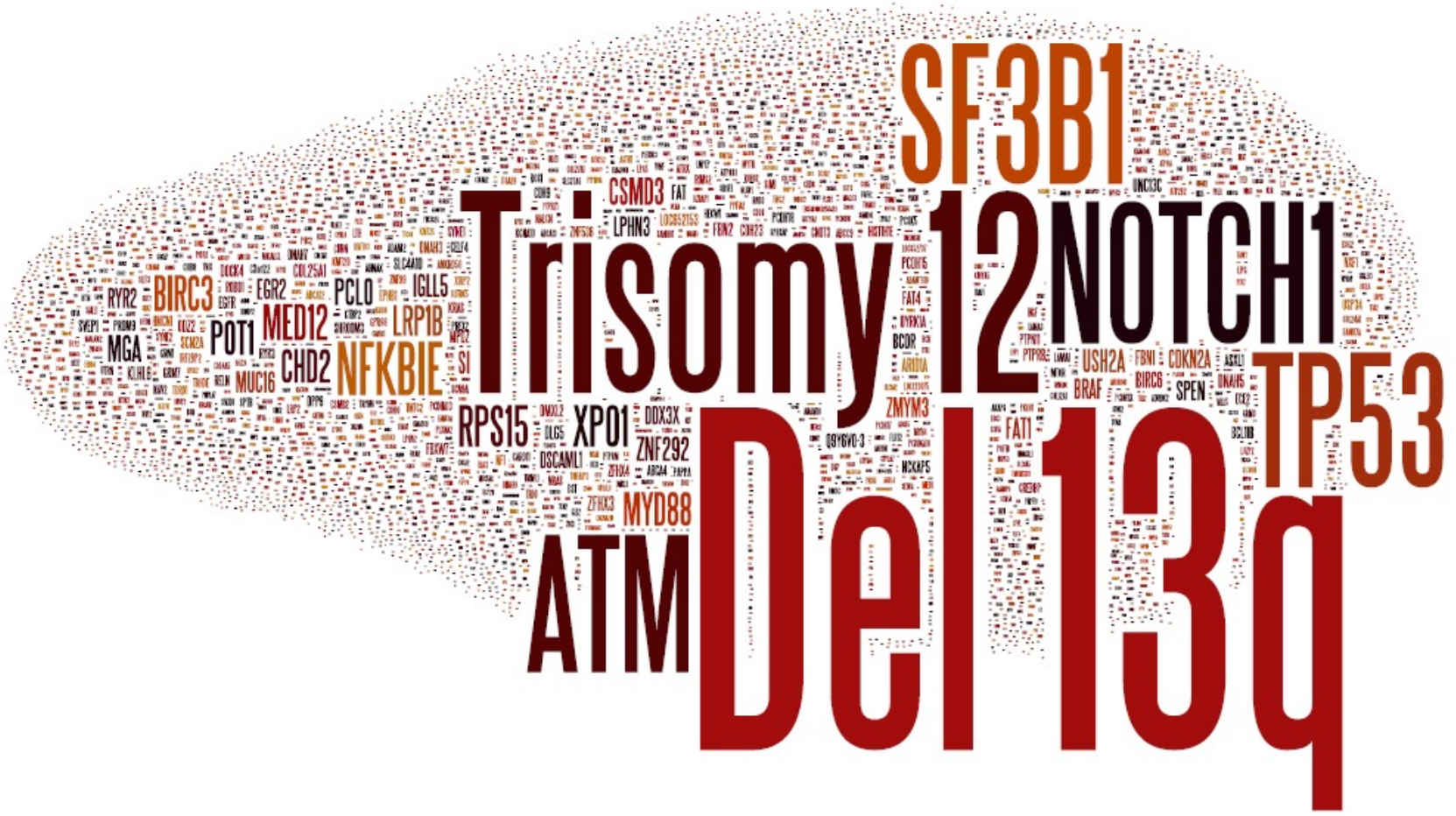
Pathogenesis of CLL



Polygenic	Del13q (BCL2)*	Signaling pathways	*TP53**
IRF4	+12	BCR*	*NOTCH1**
IRF8		NF-kB	*SF3B1*
MYC		TLR	*BIRC3**
Other		CD38	*ATM*
		VLA-4 integrins	*MYC*
		CXCR4	*CDKN2A*

**therapetic targets or predictors*

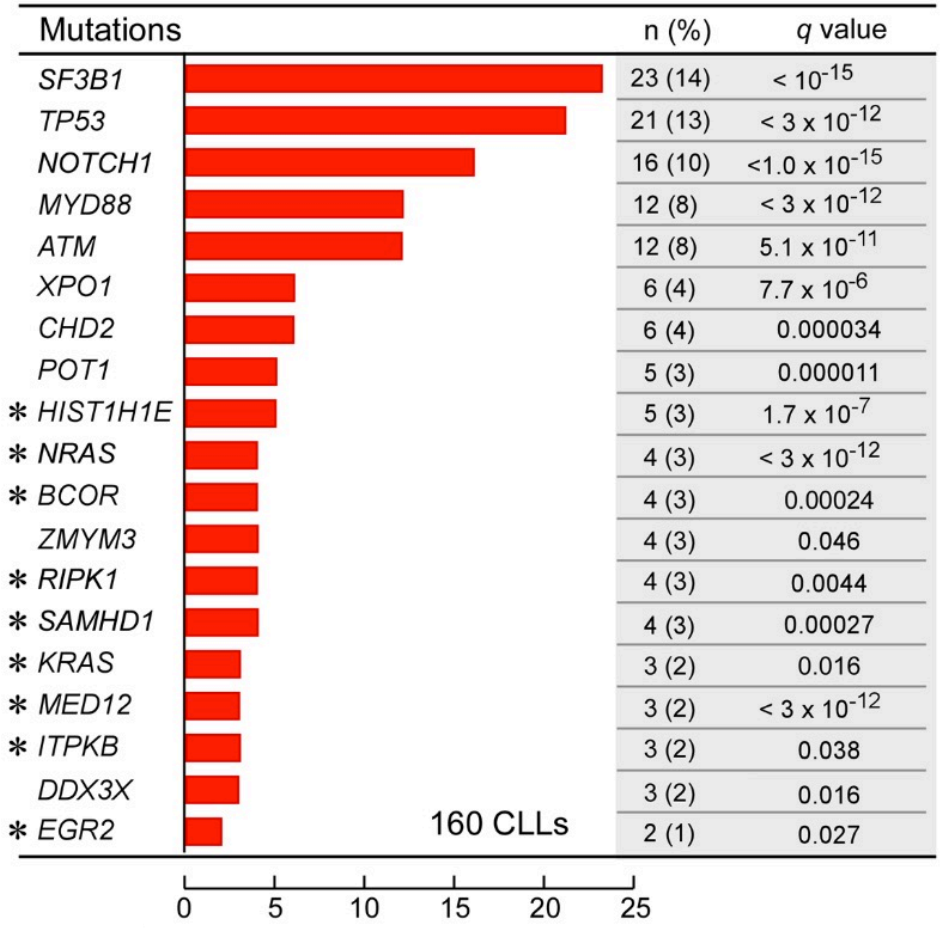
CLL is genetically heterogeneous and lacks disease defining genetic lesions

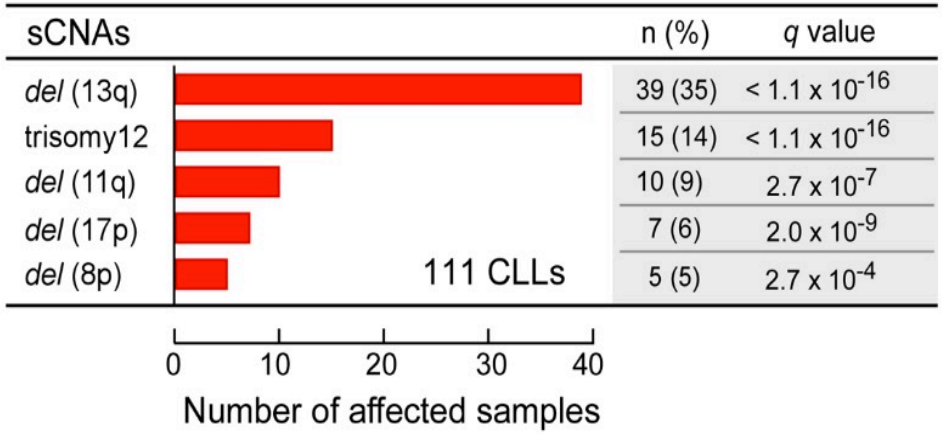


- **One of the tumor with the lowest background mutation load (0.6 per Mb)**
- **No unifying gene mutations**
- ***TP53*, *NOTCH1*, *SF3B1*, *ATM* mutated in >5% CLL**

The wordcloud shows the genes that are reported as mutated in CLL by the v77 of the Catalogue of Somatic Mutations in Cancer (COSMIC). The size of the font is proportional to the mutation frequency

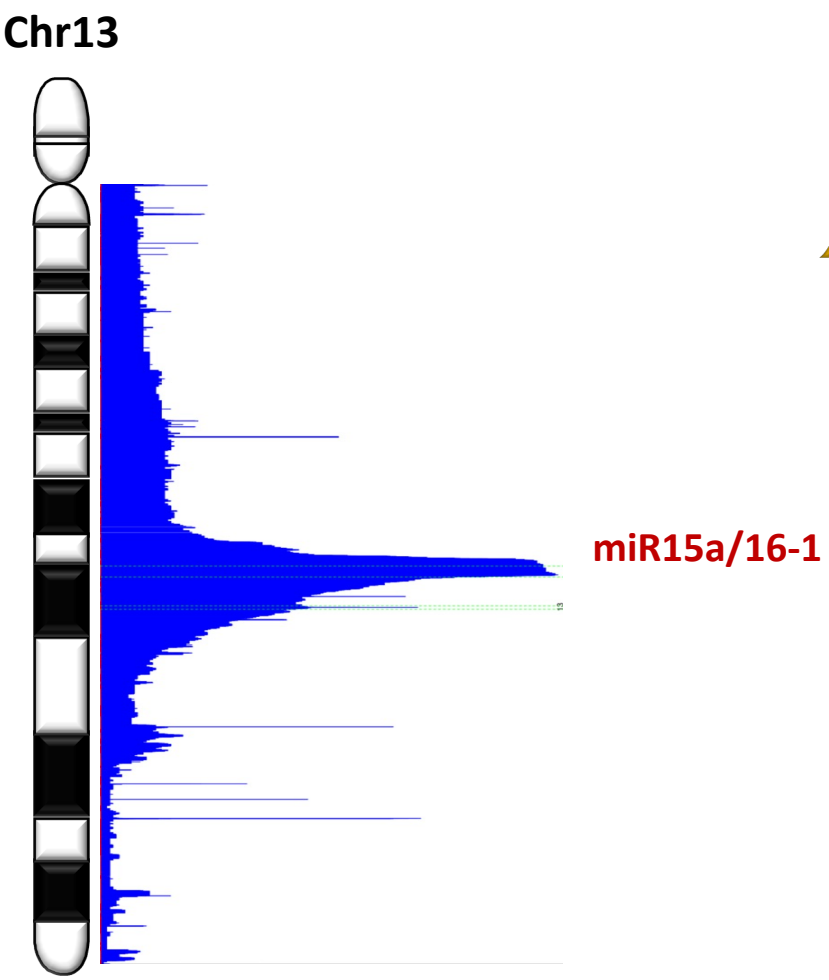
25 Recurrent Drivers in CLL



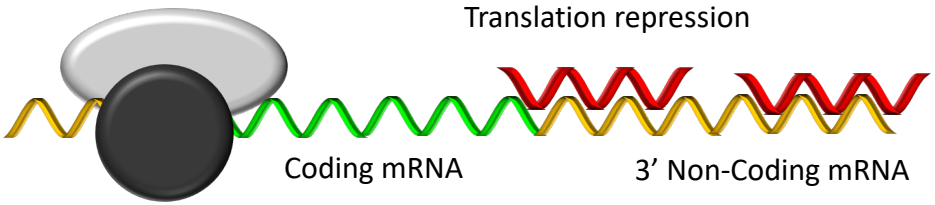


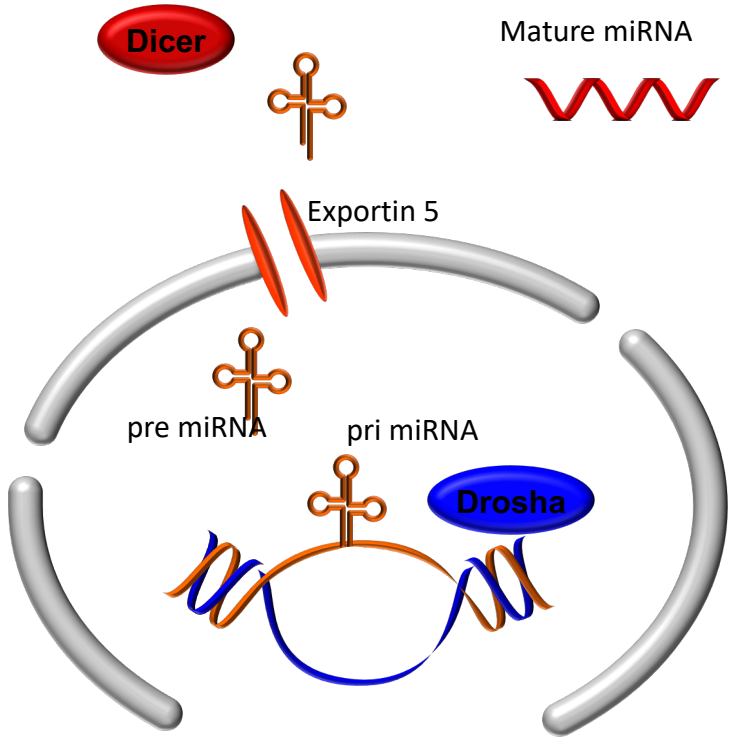
Puente et al, Nature 2011
Wang et al., *NEJM*, 2011
Quesada et al., *Nat Gen*, 2011
Fabbri et al., *JEM*, 2011
Brown et al., *Clin Can Res*, 2011
Rossi et al, Blood 2011
Rossi et al, Blood 2012
Edelmann et al., *Blood* 2012
Landau et al, Cell 2013

The minimal deleted region on 13q14 affects two microRNAs, termed miR15 and miR16

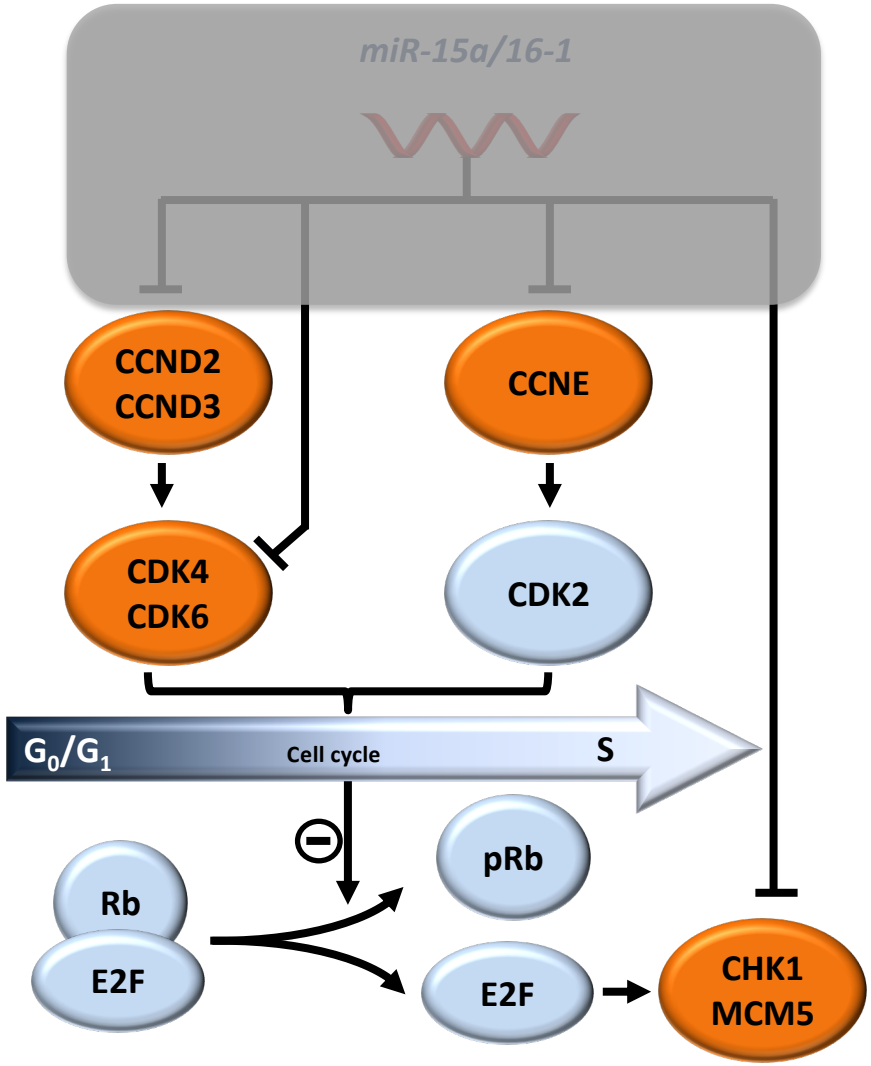


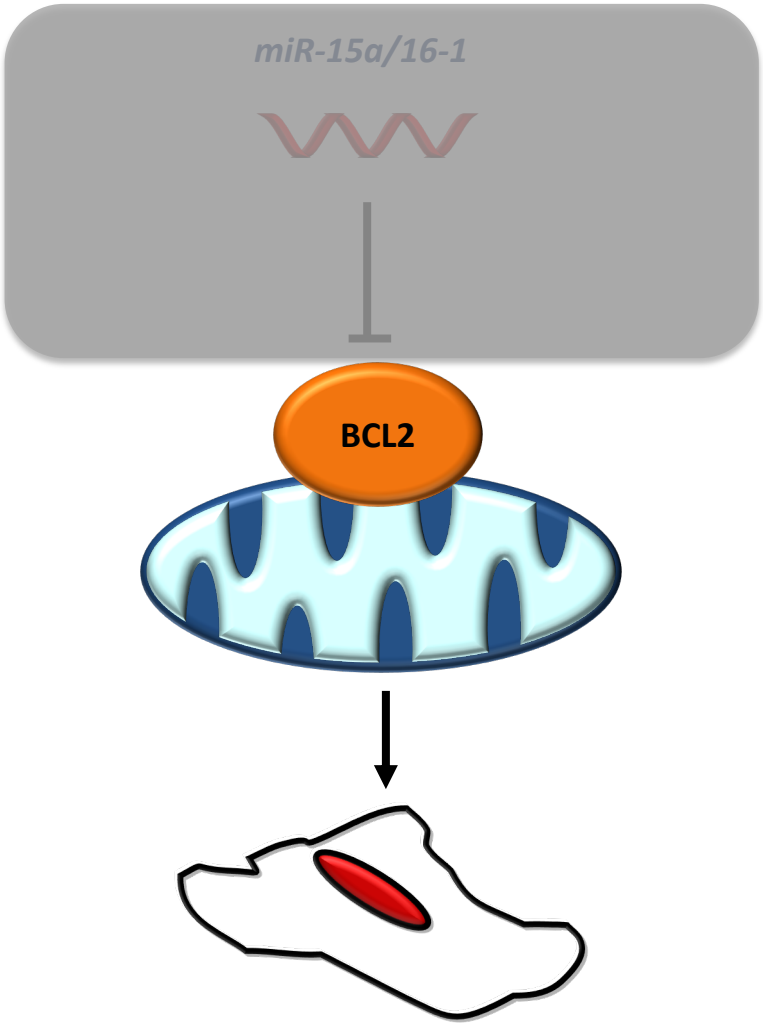
miRNAs regulate the expression of other genes at translation level





miR-15a/16-1 fine tune cell cycle progression and apoptosis

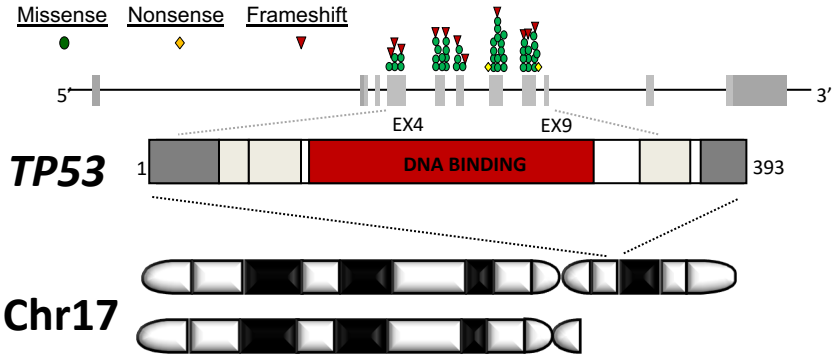


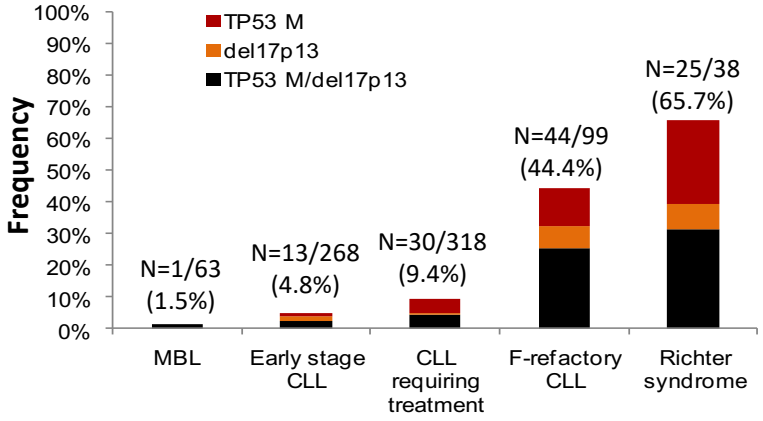


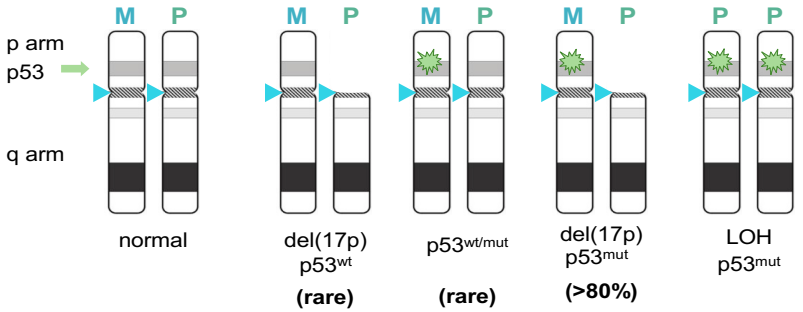
Klein et al, Cancer Cell 2010

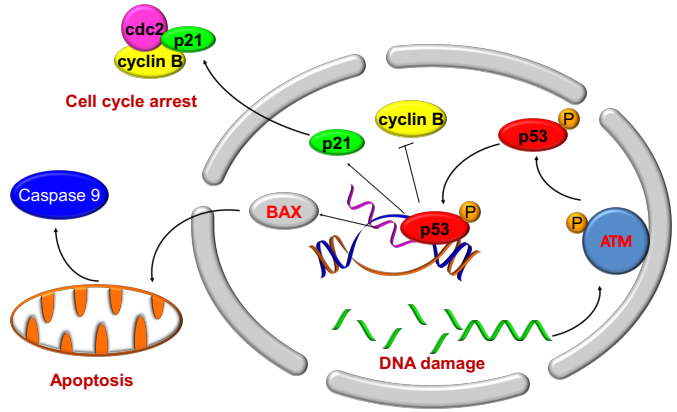
Cimmino et al, PNAS 2005

TP53 abnormalities in CLL



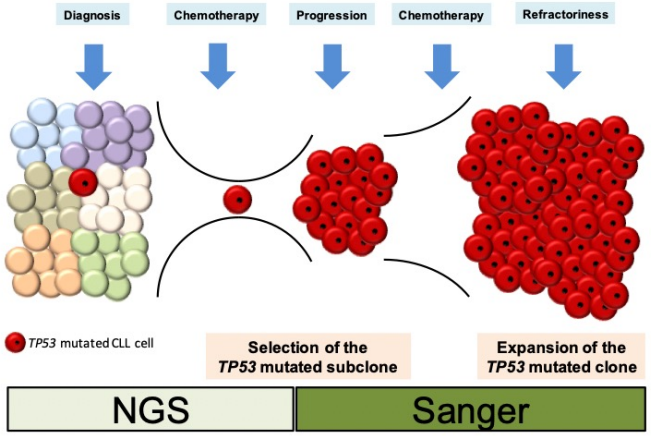


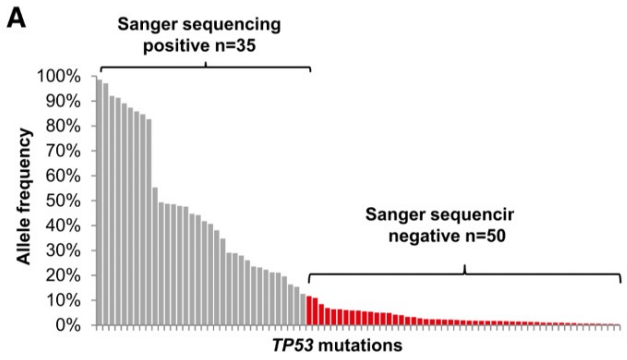


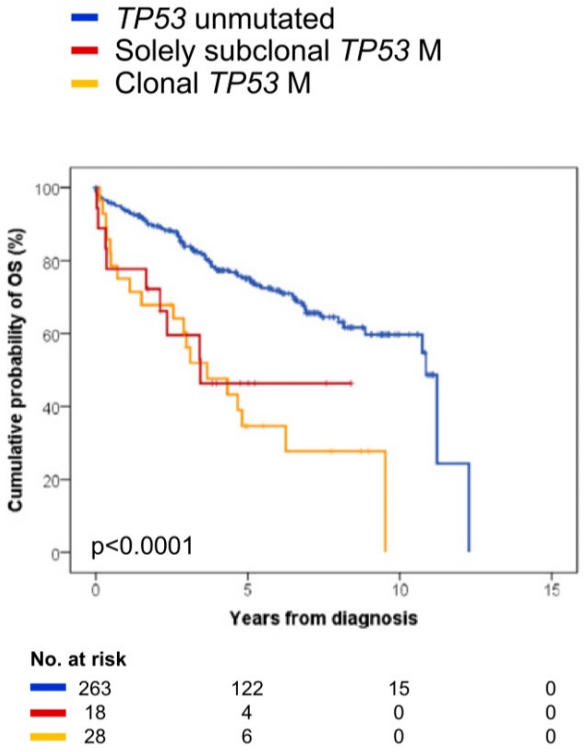


Dohner et al, New Engl J Med 2000 ; Zenz et al J Clin Oncol 2010; Rossi et al Blood 2011; Zainuddin et al, Leuk Res 2011; Rossi et al Blood 2014

Clonal evolution in CLL

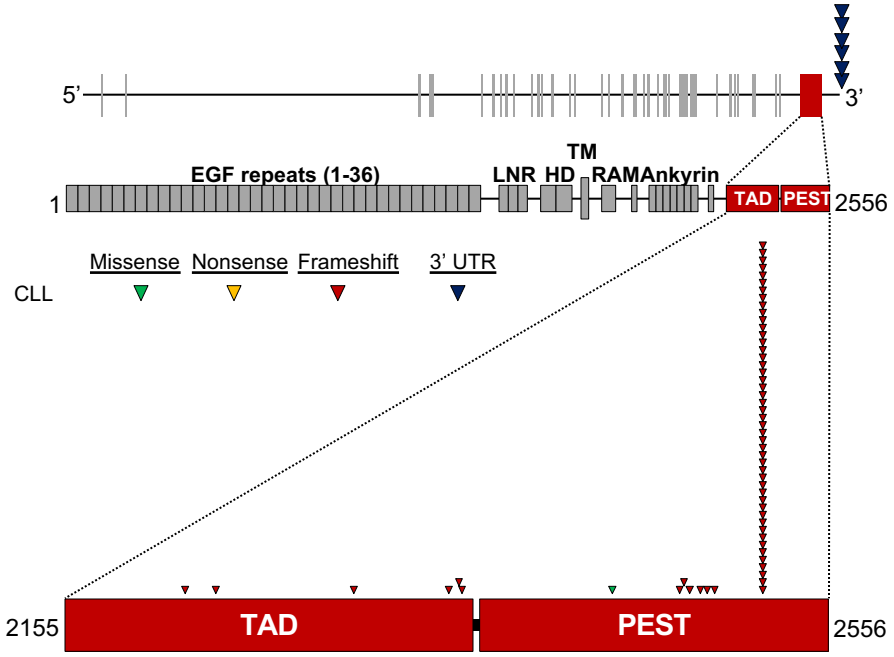


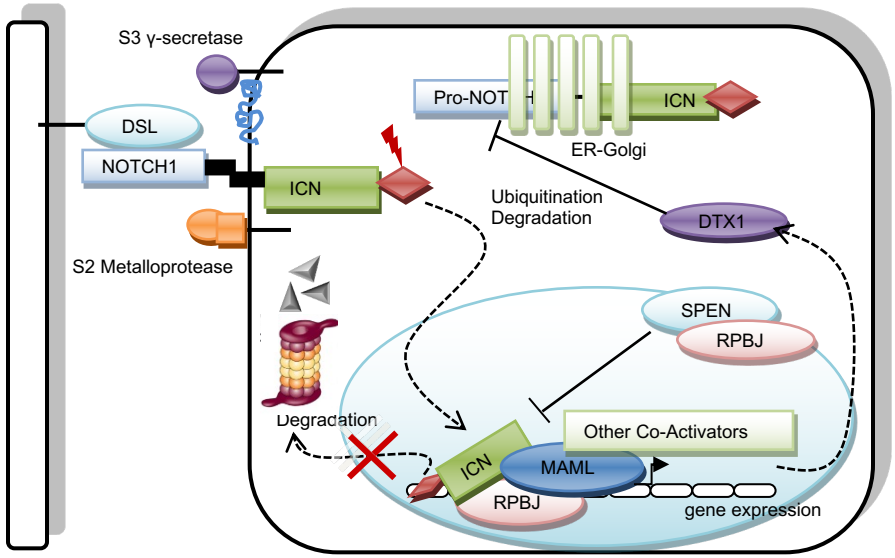




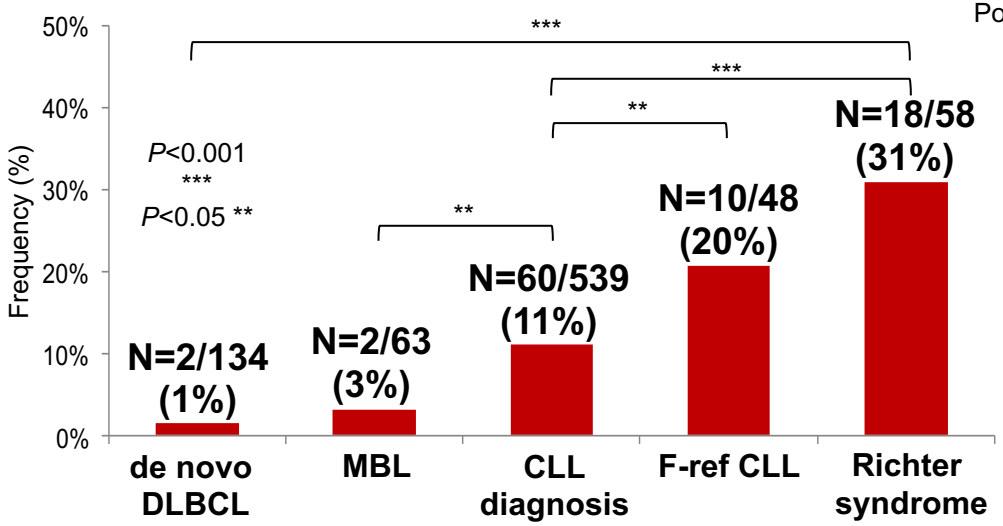
Rossi *et al, Blood.* 2014

NOTCH1 mutations in CLL





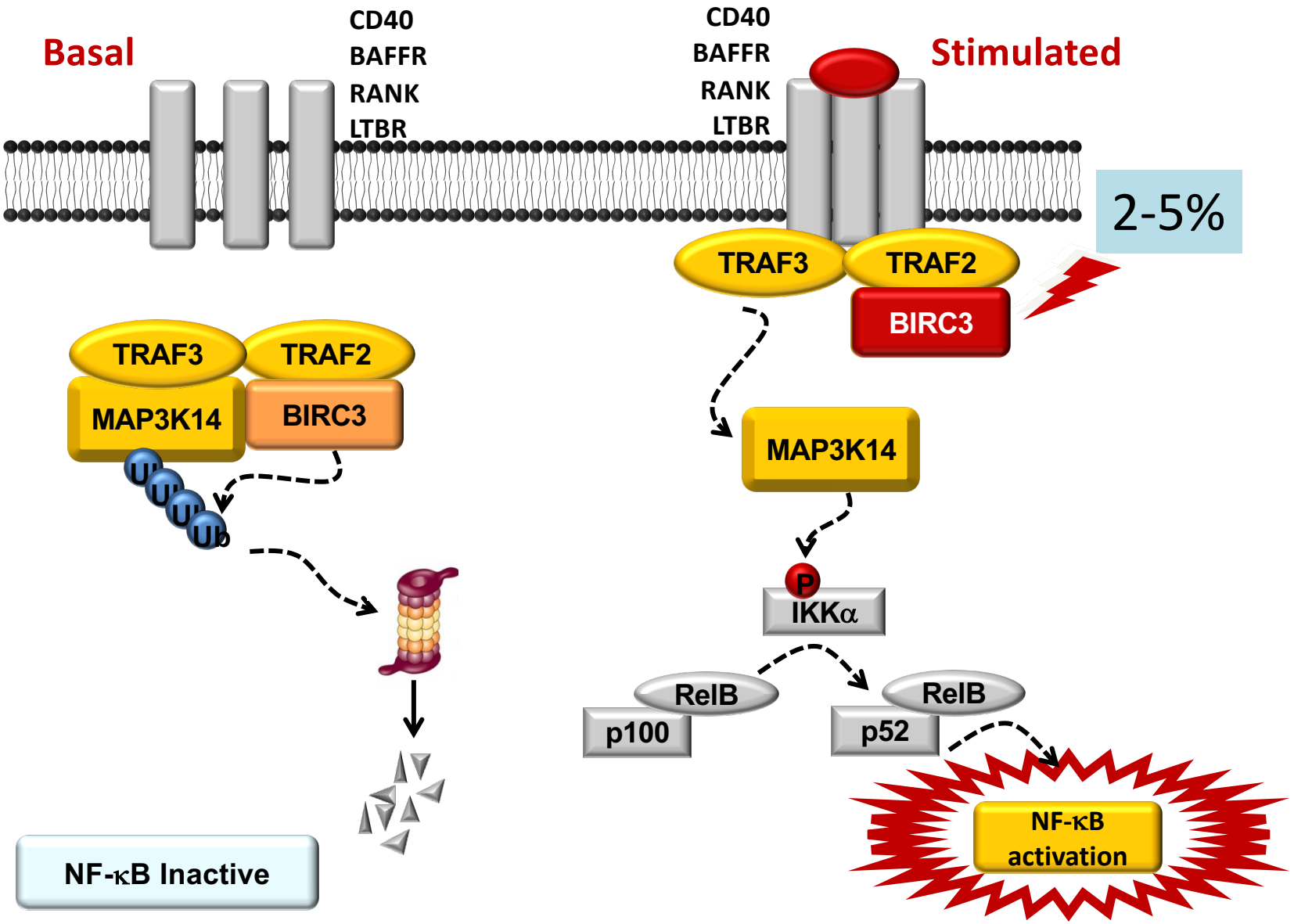
Arruga F et al. Leukemia 2013
Arruga F et al. Leukemia 2016
Fabbri G et al. PNAS 2017
Pozzo F et al. Leukemia 2017



***MYC* (proliferation)**
***DUSP22* (migration)**
CD20 (anti CD20)

Fabbri, et al. J Exp Med 2011
Puente, et al. Nature 2011
Wang, et al. New Engl J Med 2011
Rossi, et al. Blood 2012
Rasi, et al. Haematologica 2012

The non-canonical NF-κB pathway



BIRC3 mutations disrupt the RING catalytic domain and activate the non-canonical NF-κB pathway

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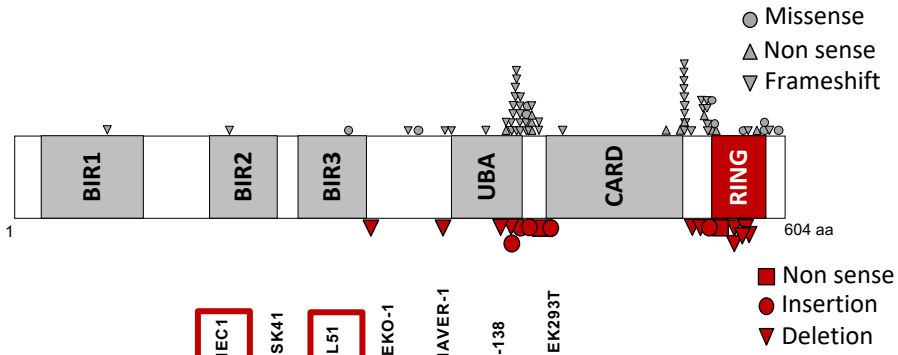
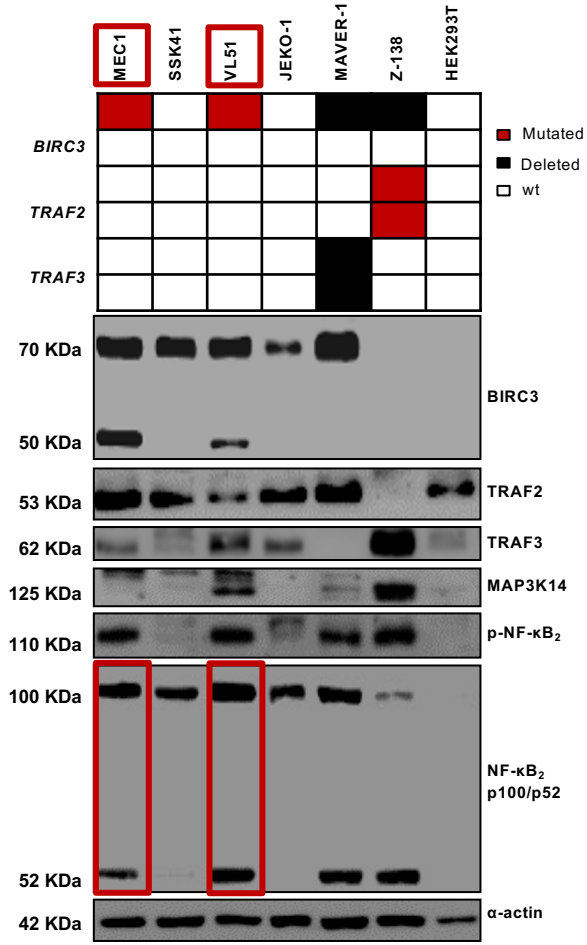
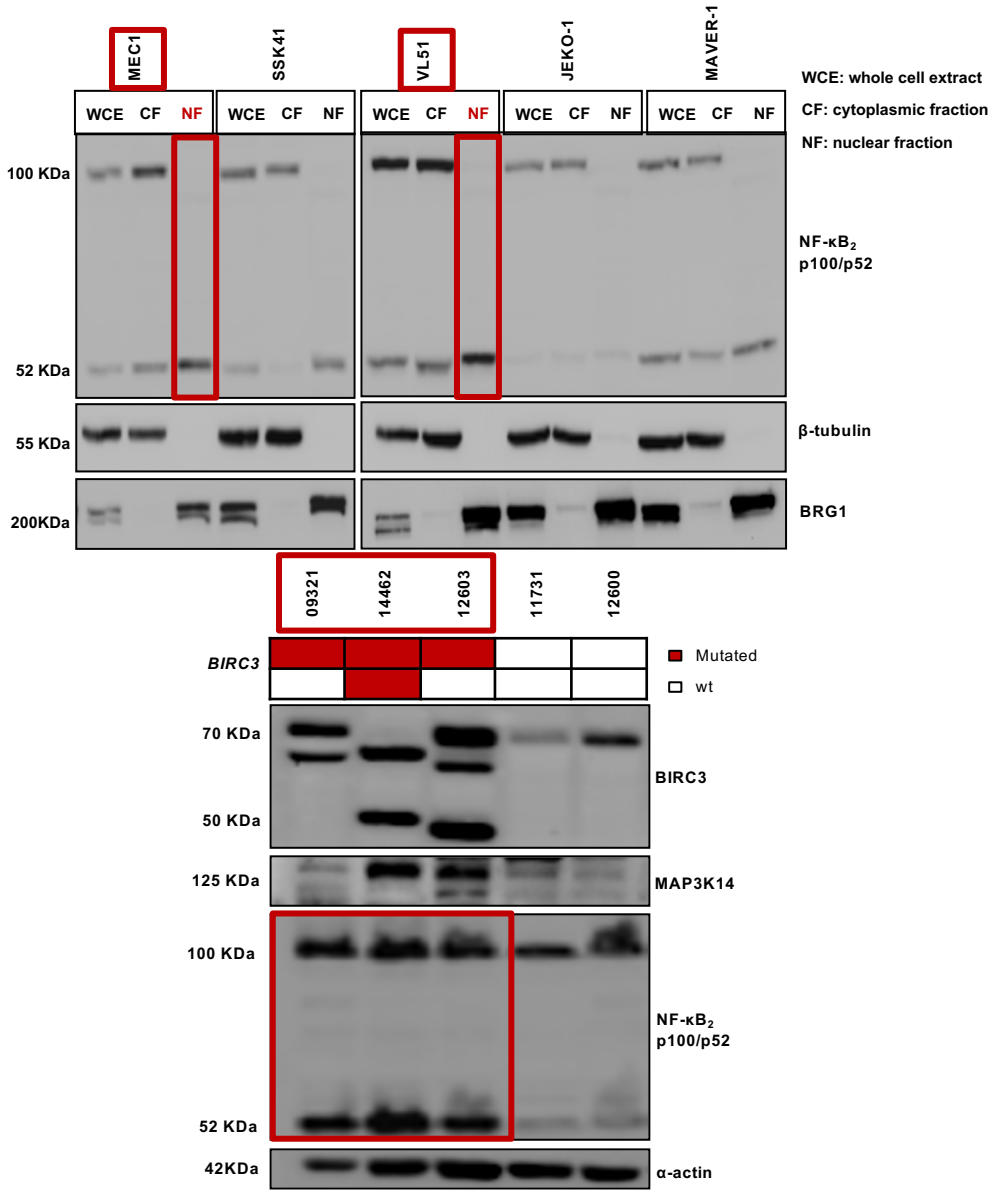
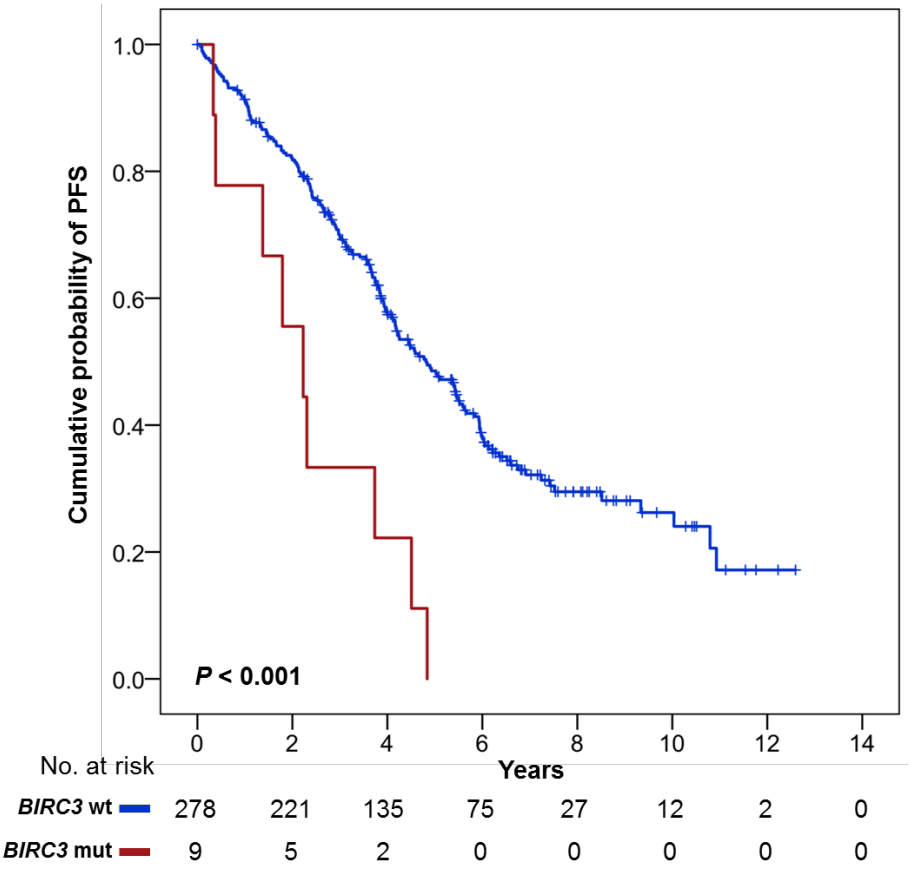


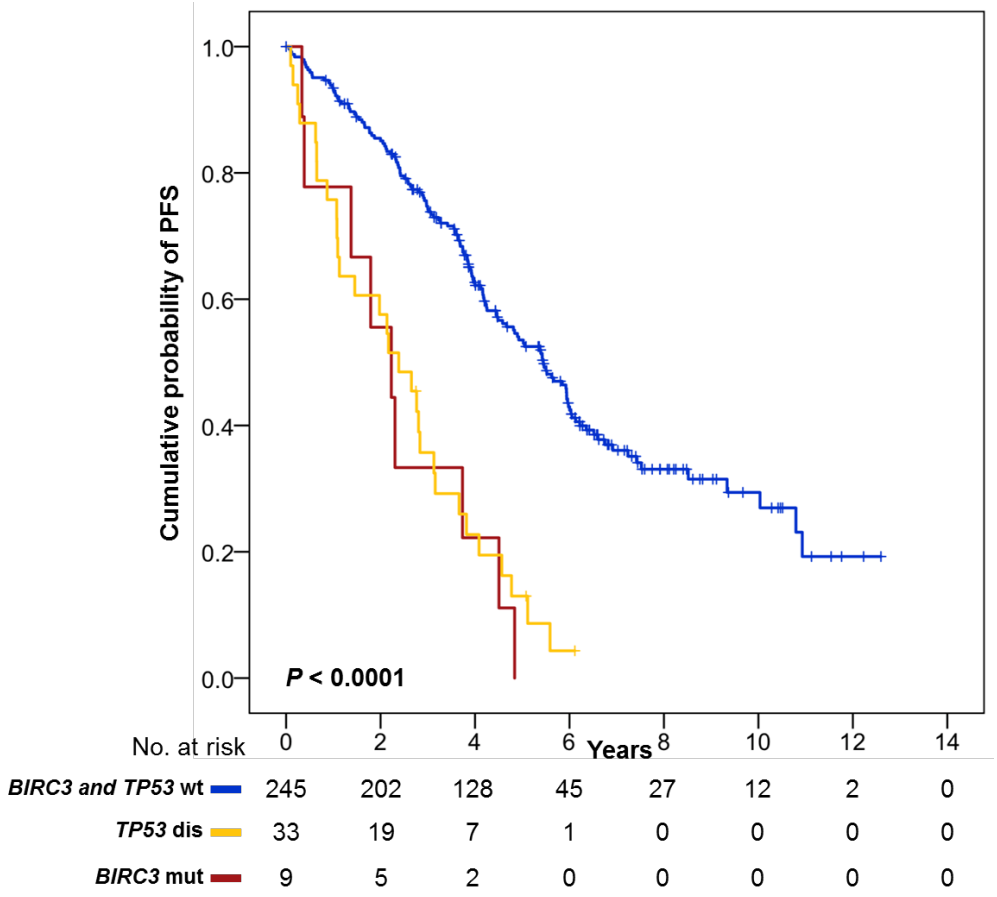




Diop, Moia *et al.*, *Haematologica.* 2019.

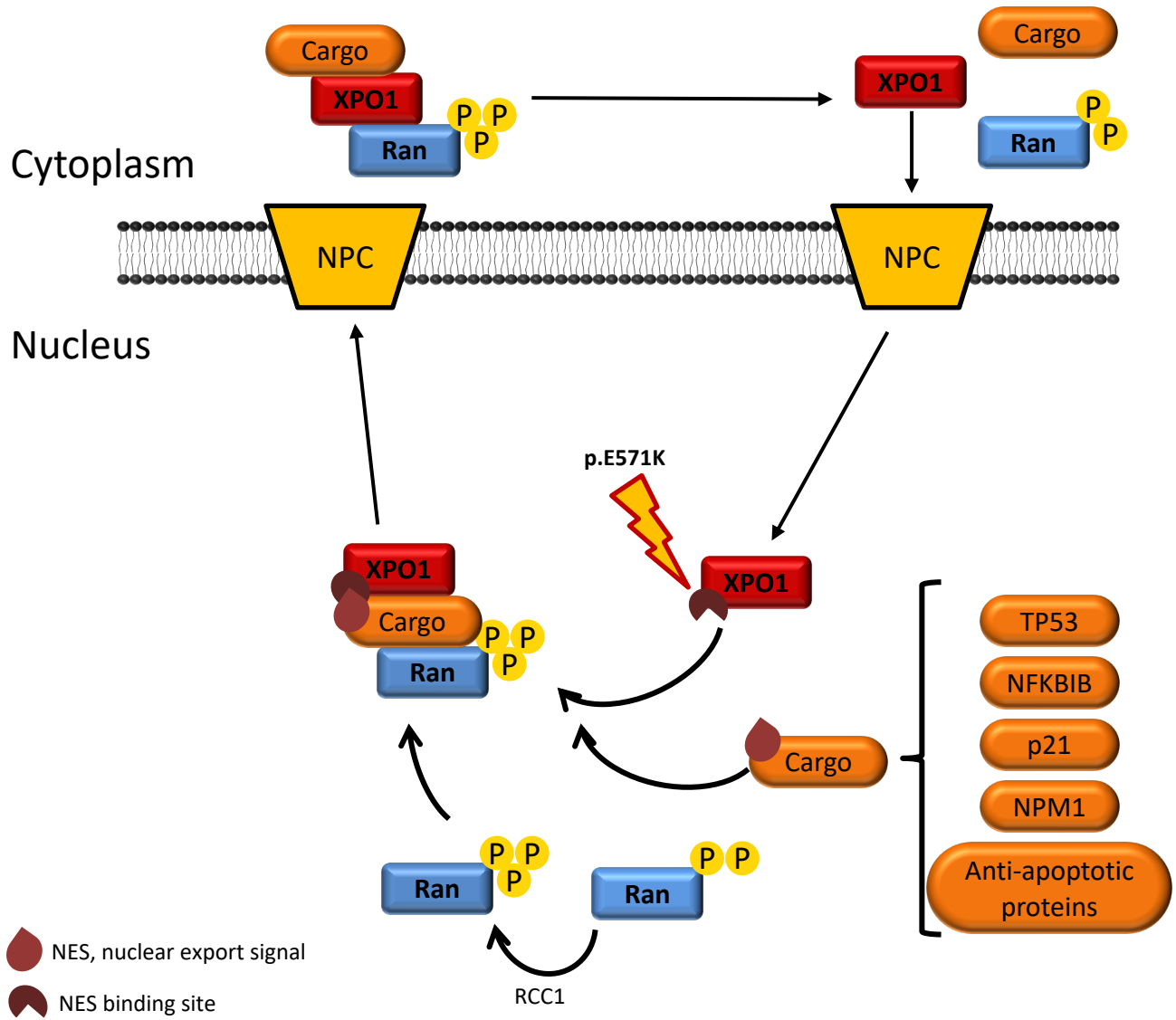
BIRC3 mutated patients have a poor outcome superimposable to *TP53* disrupted patients upon FCR





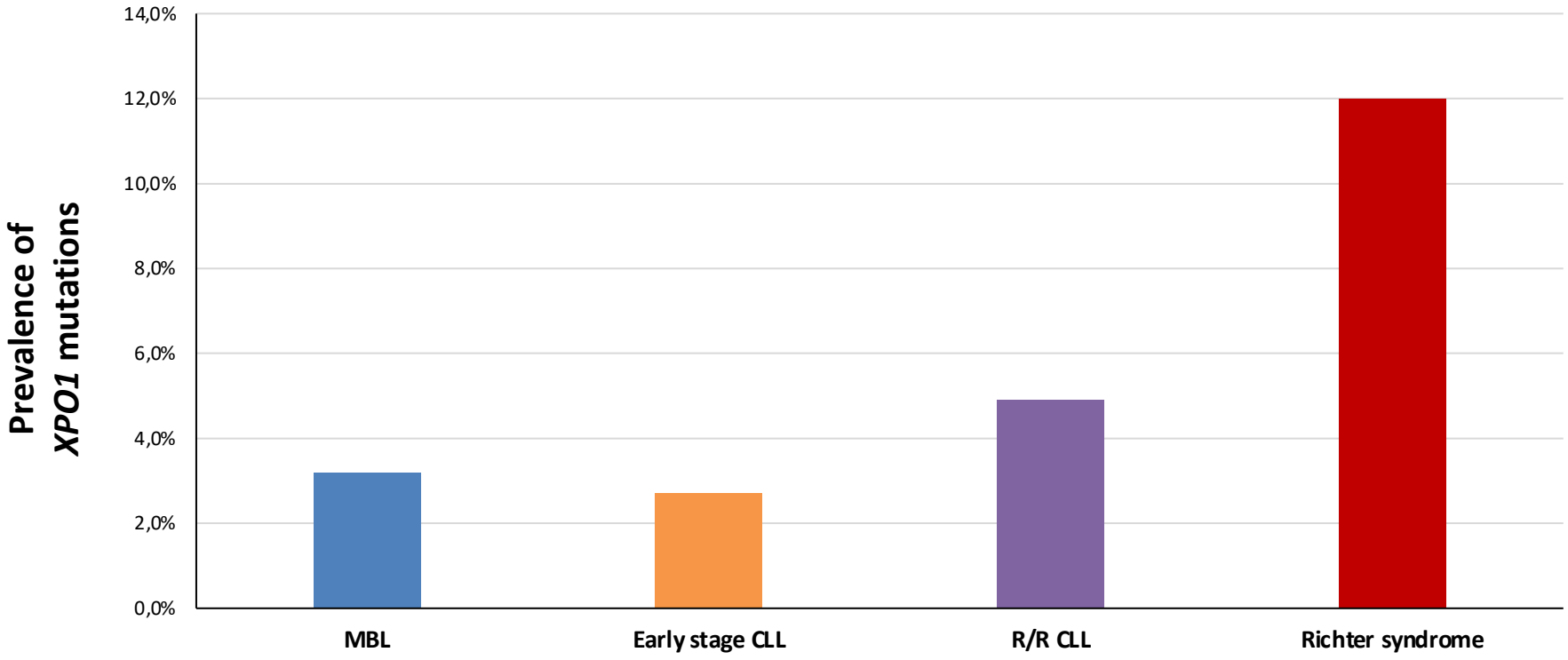
Diop *et al., Haematologica*. 2020

The *XPO1* biological pathway



- The XPO1 protein exports nuclear proteins to the cytoplasm
- **TP53, RB, p21, NPM1 and other anti-apoptotic proteins have been identified as the main Cargo proteins of XPO1**
- **XPO1 mutations increased the affinity for Cargo proteins thus enhancing their exportation outside the nucleus**
- **Selinexor and other XPO1-targeting drugs inhibit wt and mutated XPO1 and are currently in clinical studies**

XPO1 mutations are more frequent in RS than in CLL



CLL transformed into DLBCL (RS) showed a **higher prevalence of XPO1 mutations** compared the other CLL stages

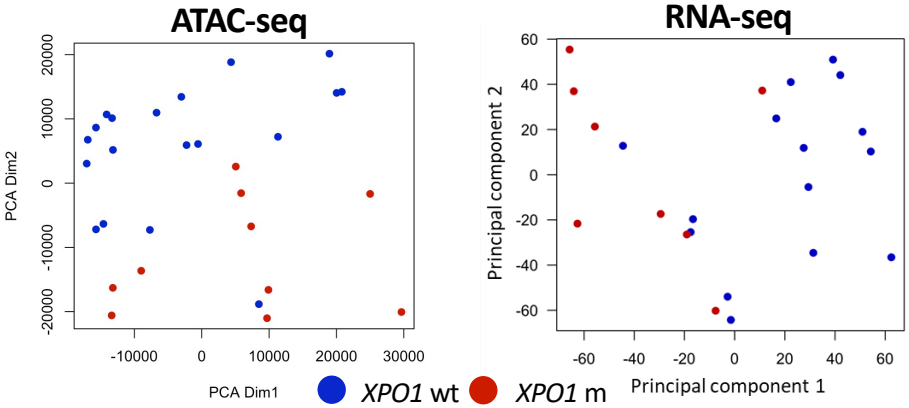
Moia *et al., submitted*

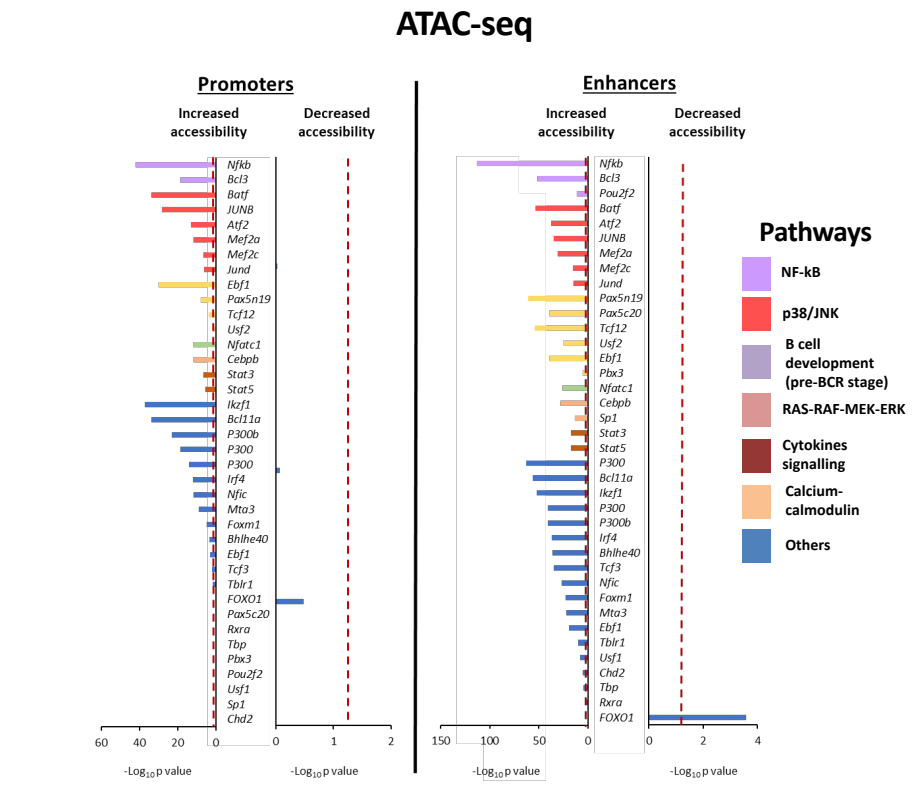
XPO1 mutated patients showed a chromatin accessibility and a transcriptomic profile distinct from wild type cases and are characterized by higher BCR activity

8 *XPO1* mutated patients

15 *XPO1* wild type patients matched for FISH karyotype, IGHV and *TP53* status

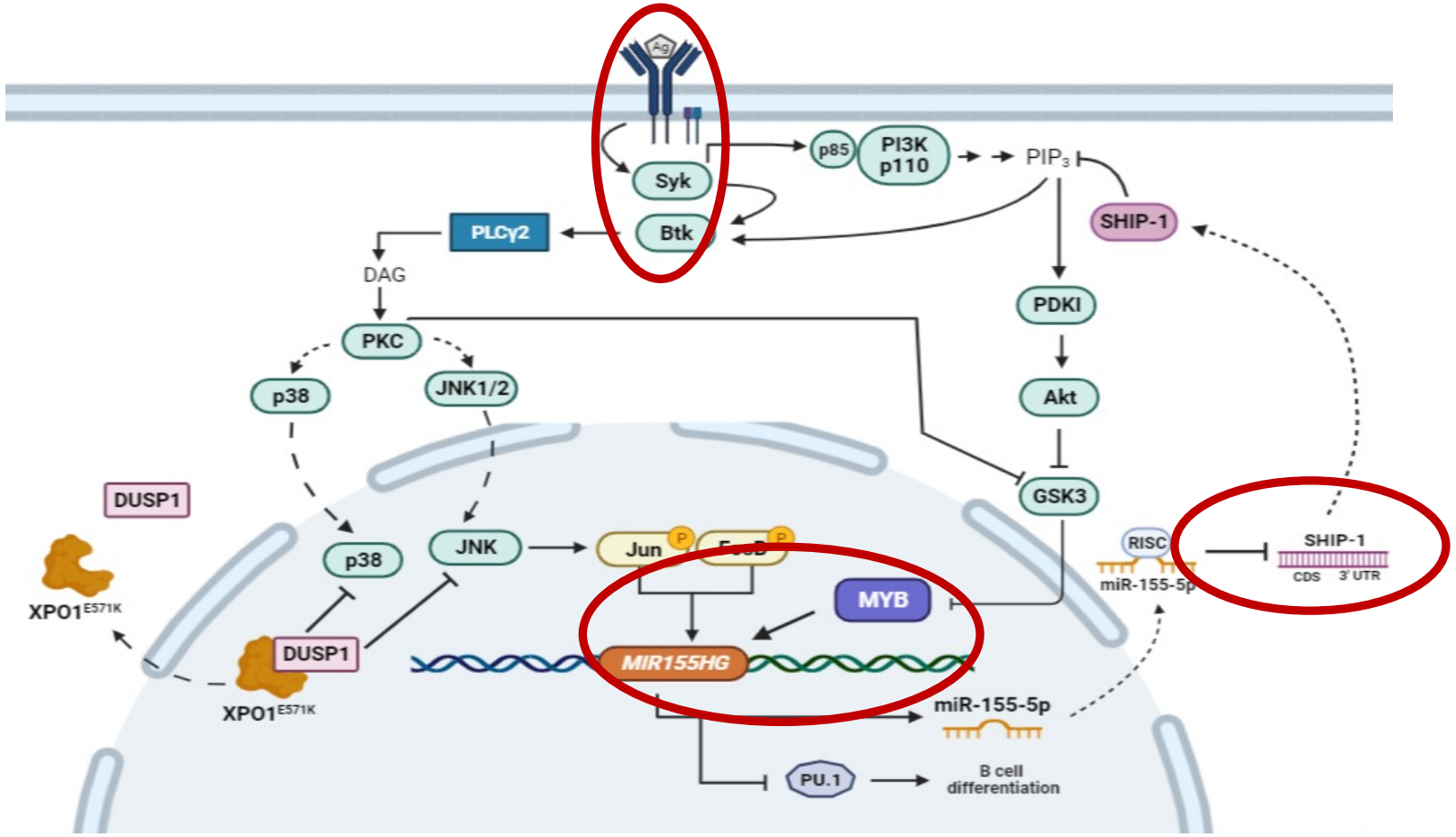
ATAC seq
RNA seq





- Chromatin regions more accessible in *XPO1* mutated CLL were enriched in binding sites for transcription factors regulated by pathways emanating from the BCR, including NF-kB, p38/JNK
- RNA seq revealed an enrichment in pathways coding for inflammation, early B-cell response and MAPK activation in *XPO1* mutated cases

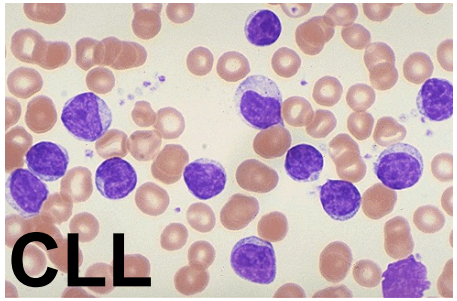
XPO1 mutations enhance BCR overactivation



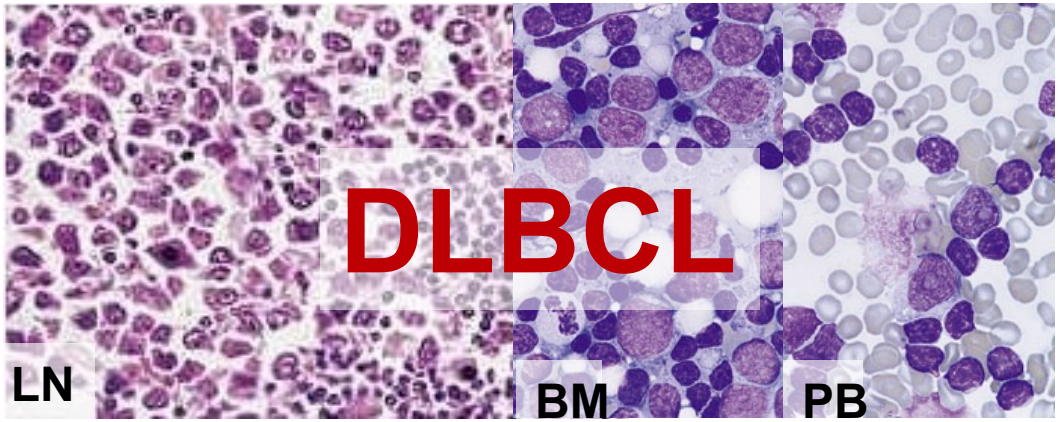
XPO1 mutations, probably through miR-155/MYB pathway stimulation, enhance BCR signaling, which is leads to CLL cells proliferation and disease progression

Moia *et al., submitted*

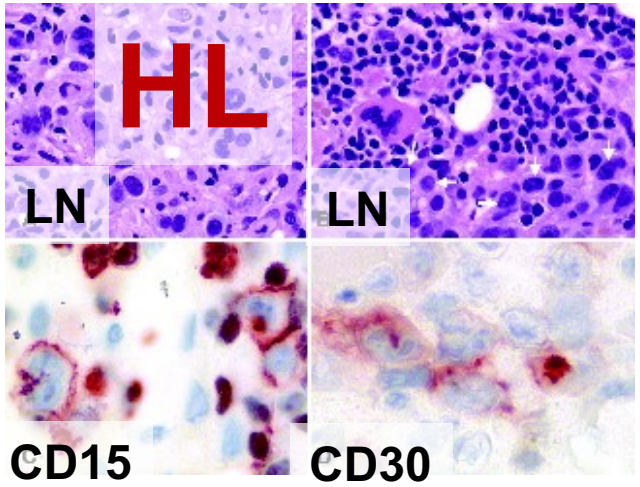
Definition of Richter syndrome



95-99%

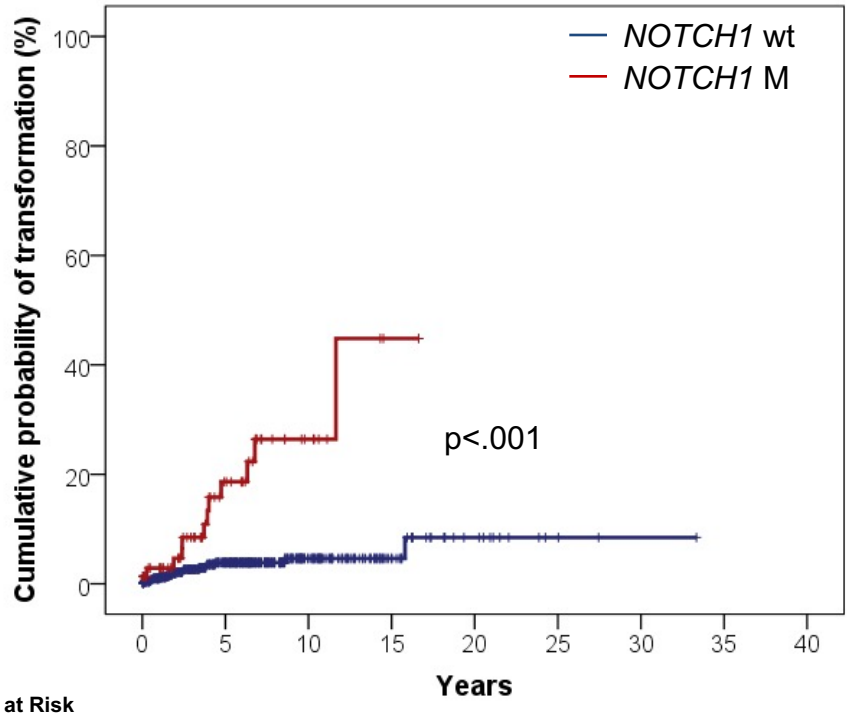


1-5%



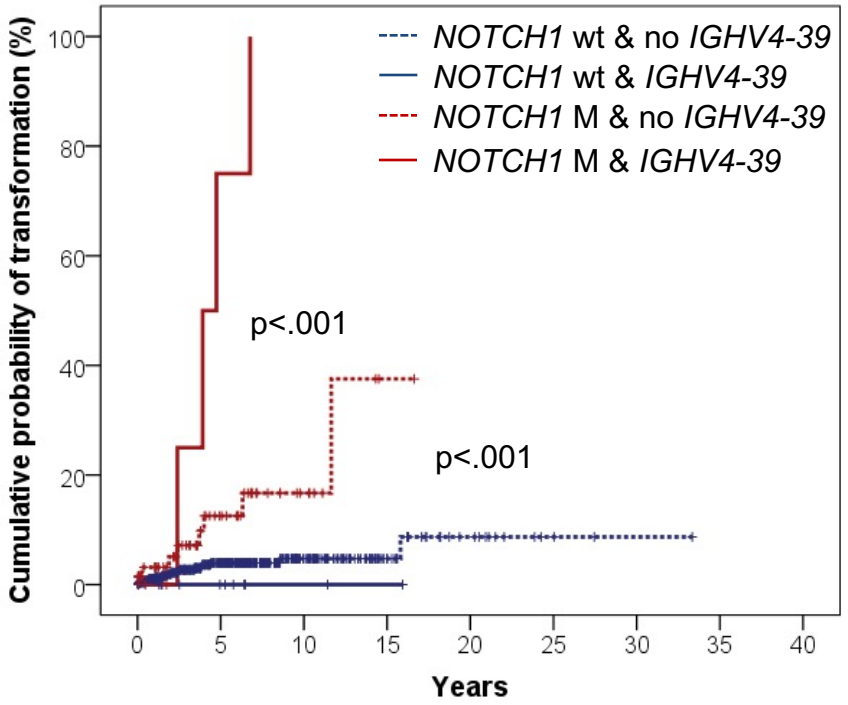
Müller-Hermelink HK, et al, WHO Classification 2008

Risk of Richter transformation according to *NOTCH1* mutation status and IGHV usage at CLL diagnosis



No. at Risk									
NOTCH1 wt	531	279	92	31	11	3	1	0	0
NOTCH1 M	74	28	8	1	0	0	0	0	0

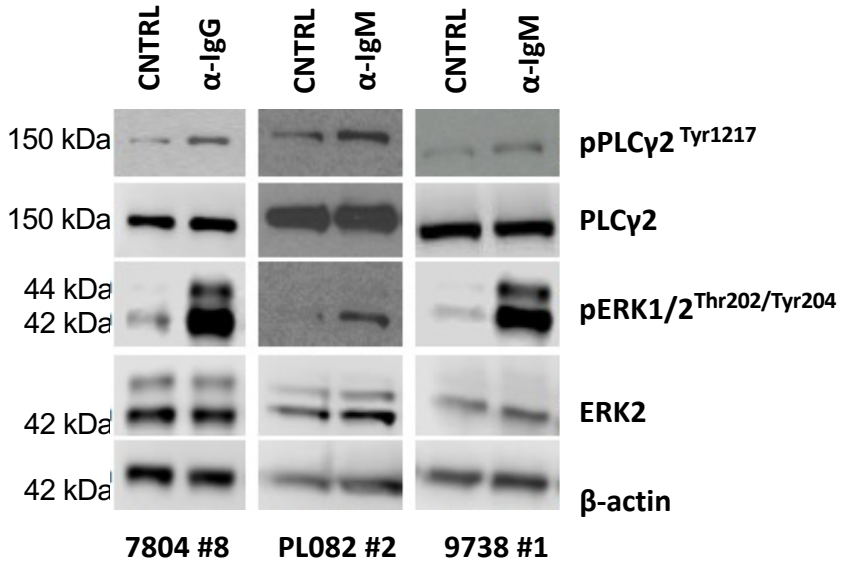
	Events	Total	5-year risk	95% CI
NOTCH1 wt	18	531	3.9%	2.0-5.8
NOTCH1 M	12	74	18.6%	7.3-29.9

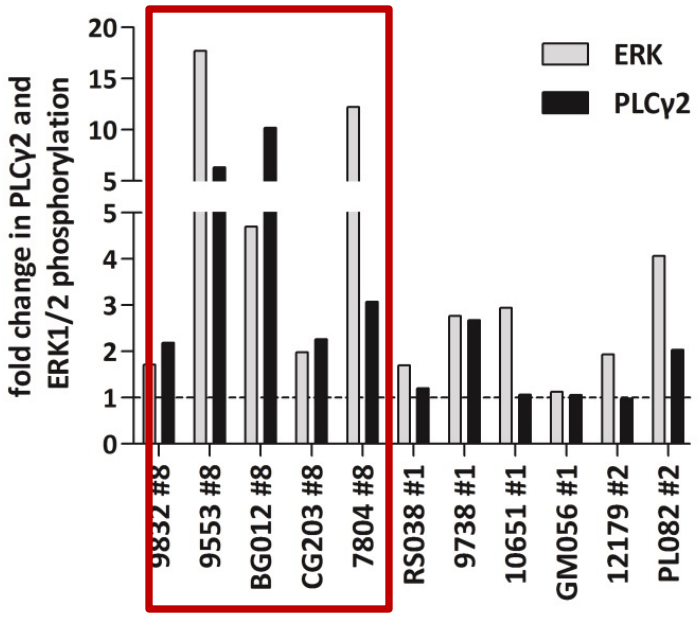


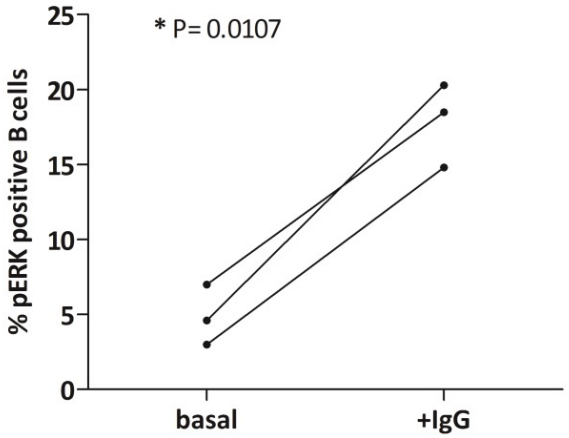
No. at Risk									
NOTCH1 wt & no *IGHV4-39*	519	273	90	30	11	3	1	0	0
NOTCH1 wt & *IGHV4-39*	12	12	12	12	0	0	0	0	0
NOTCH1 M & no *IGHV4-39*	67	27	8	1	0	0	0	0	0
NOTCH1 M & *IGHV4-39*	7	1	0	0	0	0	0	0	0

	Events	Total	5-year risk	95% CI
NOTCH1 wt & no *IGHV4-39*	18	519	4.0%	2.1-5.9
NOTCH1 wt & *IGHV4-39*	0	12	0	
NOTCH1 M & no *IGHV4-39*	8	67	12.5%	2.9-22.1
NOTCH1 M & *IGHV4-39*	4	7	75.0%	32.5-100

Subset 8 cells respond avidly through the BcR

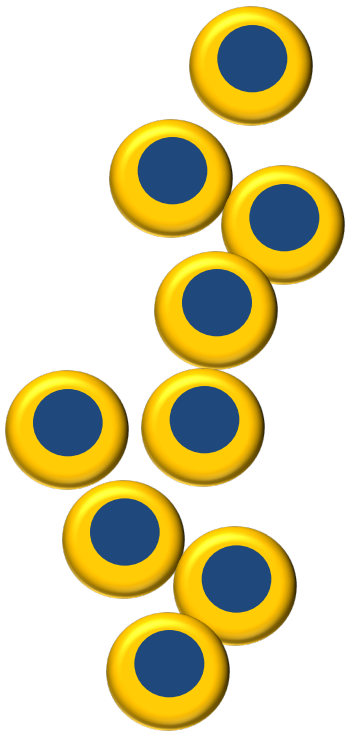






Gounari et al, Blood, 2015
Courtesy of P Ghia

Clonally related vs unrelated Richter syndrome

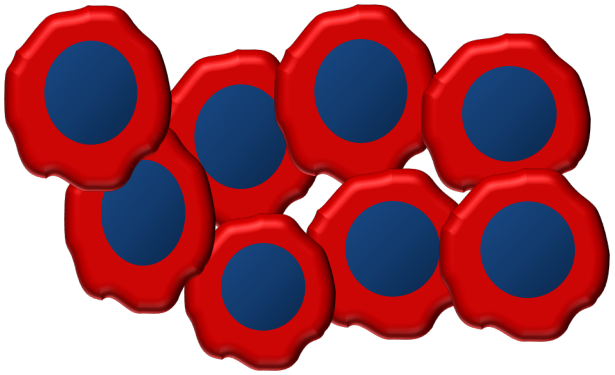


CLL

V4-39 D6 J4

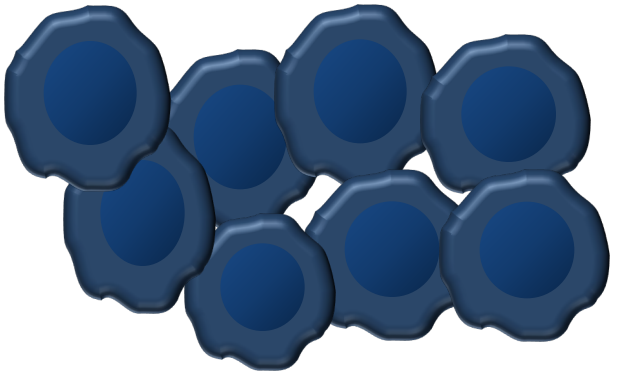
50/63 (80%)

13/63 (20%)



Clonally related RS

V4-39 D6 J4

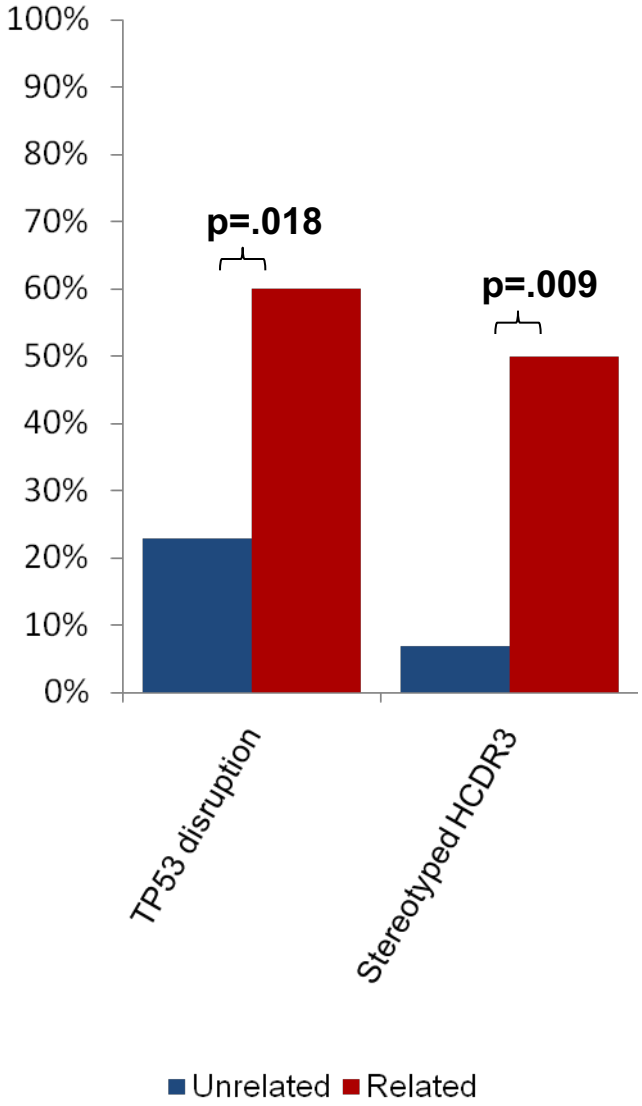


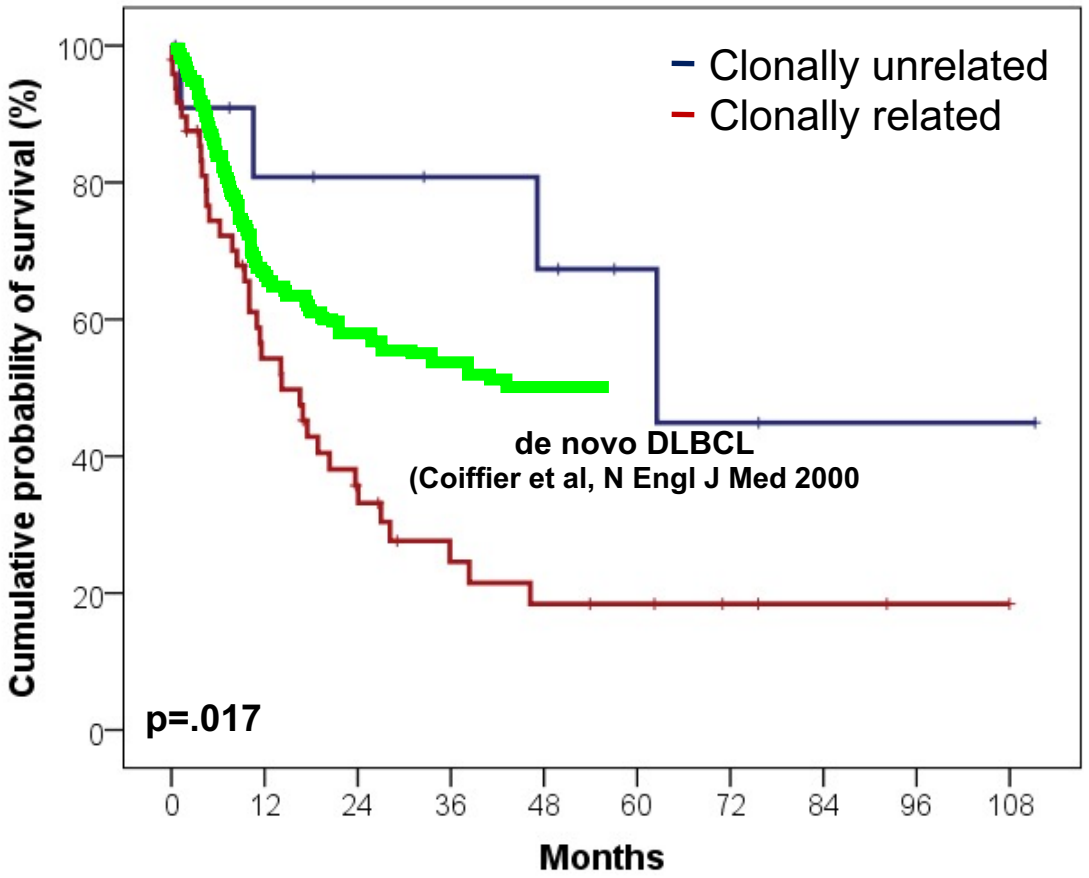
Clonally unrelated RS

V4-34 D2-2 J3

Rossi et al, Blood 2011

The genetic profile of clonally unrelated RS differs from that of clonally related RS





Rossi et al, Blood 2011

Clonally unrelated Richter syndrome are truly *de novo* DLBCL with a mutational profile reminiscent of clonally related Richter syndrome

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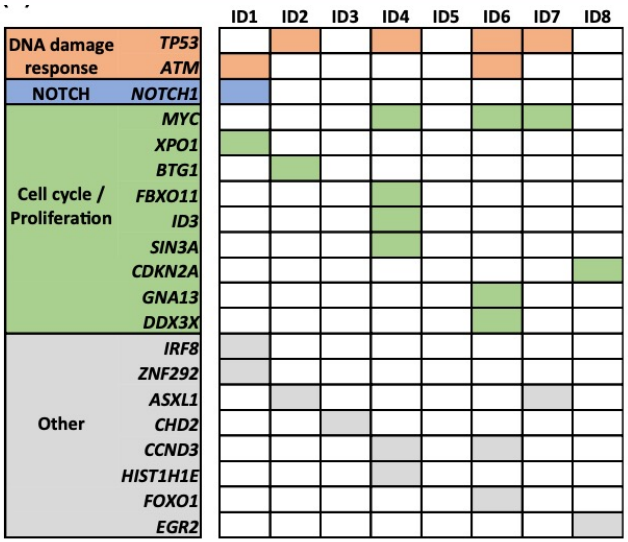
CLL cells of 8 patients with unrelated RS were subjected to an ultra-deep next-generation sequencing approach with a sensitivity of 10^{-6}

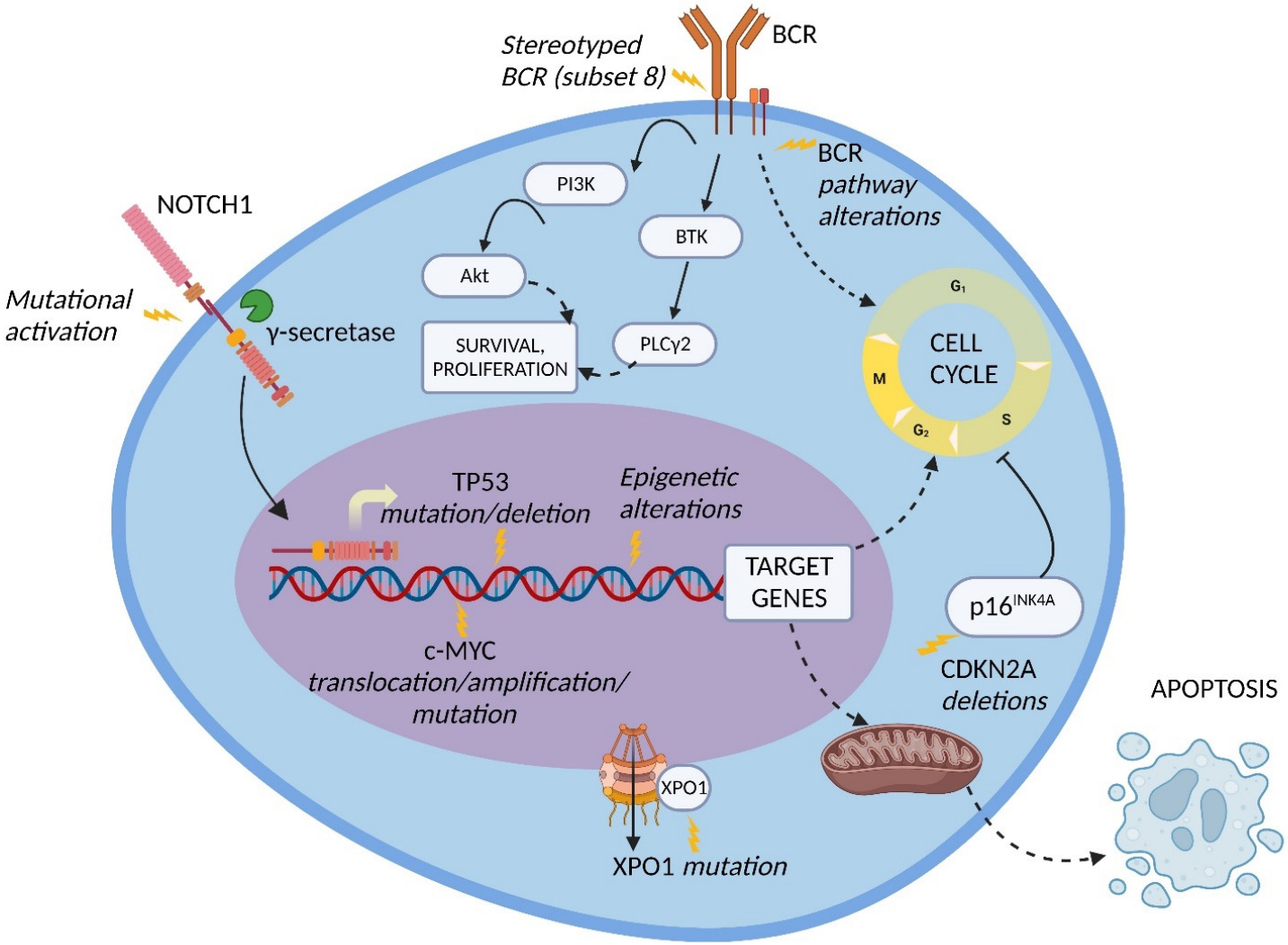
	CLL					RS				
ID sample	IGHV	IGHD	IGHJ	Identity %	CDR3	IGHV	IGHD	IGHJ	Identity %	CDR3
ID1	1–69*01 or 1–69*12	3–16*02	6*02	100	CASKGVDDYIWGSYRYTDYYYYYGMDVW	1–69*02	3–3*01	6*02	100	CAREEGLTIFGVVGYYYYGMDVW
		*3–3*01*	*6*02*	*100*	*CAREEGLTIFGVVGYYYYGMDVW*					
ID2	1–3*01	6–19*01	4*02	100	CAFEQWLMIPAFDYW	1–69*01 or 1–69*12	3–3*01	6*02	100	CASPTMYDFWSGYSYYWYGMDVW
ID3	4–31*03 or *04	3–3*01	6*03	100	CARGVYYDFWSGWYKPYMYYMDVW	1–8*01	4–17*01	4*03	95.83	CTDELRRFDWW
ID4	1–69*01	1–7*01	6*02	99.65	CAKTPPLWNSPPHYYYYYGMDVW	3–30*03 or *18 or 3–30-5*01	2–2*01	4*02	92	CAKTSCDSINCYIPFDYW
ID5	1–02*02 or 1–02*05	3–9*01	4*02	92.36	CARSSEPPRYYDSWSGHTAAW	1–02*02 or 1–02*05	3–9*01	4*02	92.36	CARSSEPPRYYDSWSGHTAAW
						3–21*01	3–22*01	3*02	87.15	CTRGPLAYESDGFDMW
ID6	1–69*01	4–17*01	6*03	100	CAGISKVGDLVDYGDRETYYYYMDVW	4–59*01	6–6*01	4*02	92.98	CARVRGRQLASDYW / CARVRGRHLASDYW
ID7	3–23*01	3–9*01	4*02	92.01	CAKDLEVENKNWLLKLDYW	3–23*01	6–19*01	4*02	98.96	CAKDEASGWYDYFDYW
ID8	6–1*01	–	6*02	98.32	CARDFYYGMDVW	6–1*01	–	6*02	96.97	CARDFYYGMDVW
						4–34*01 O *02	3–9*01	6*02	96.79	CARHLKTLRGYPGRYYYYGMDVW

Targeted resequencing was applied on tissue biopsy of clonally unrelated RS cases



Favini *et al., BJH*. 2022.

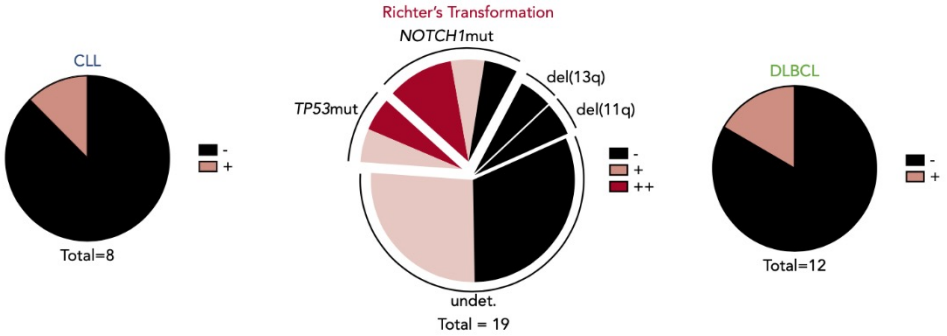
Molecular alterations in RS



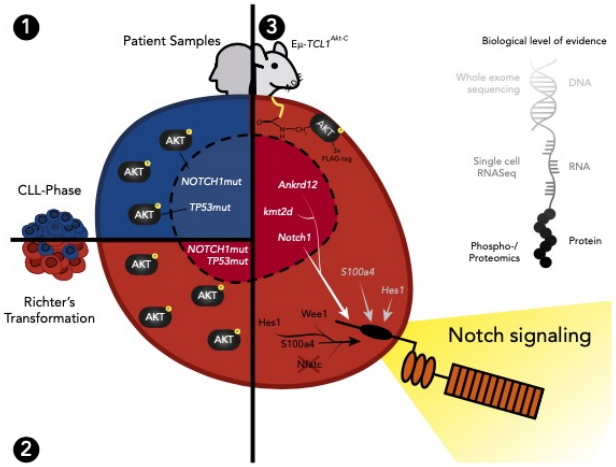
Mouhssine and Gaidano, Cancers, 2022

Akt signaling triggers CLL toward Richter transformation via overactivation of Notch1

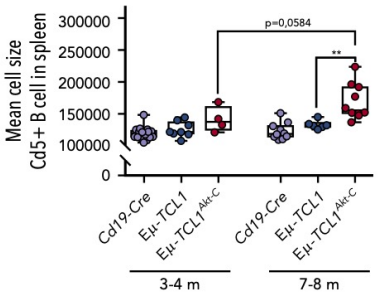
High levels of AKT phosphorylation occur both in high-risk CLL patients as well as in patients with RT

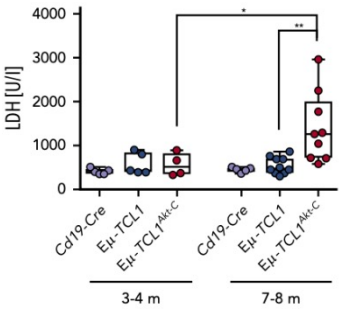


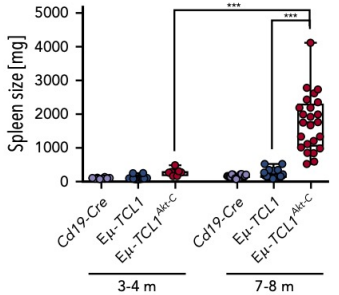
Akt activation was identified as an initiator of CLL transformation toward aggressive lymphoma by inducing Notch signalling

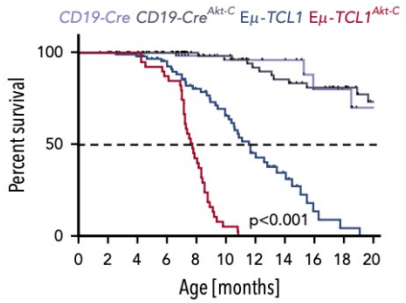


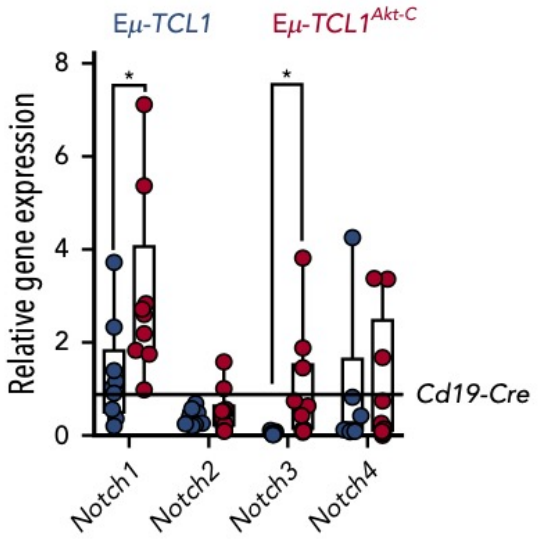
Overactivation of Akt in the murine Eμ-TCL1 CLL mouse model resulted in CLL transformation to RT with significantly reduced survival and an aggressive lymphoma phenotype





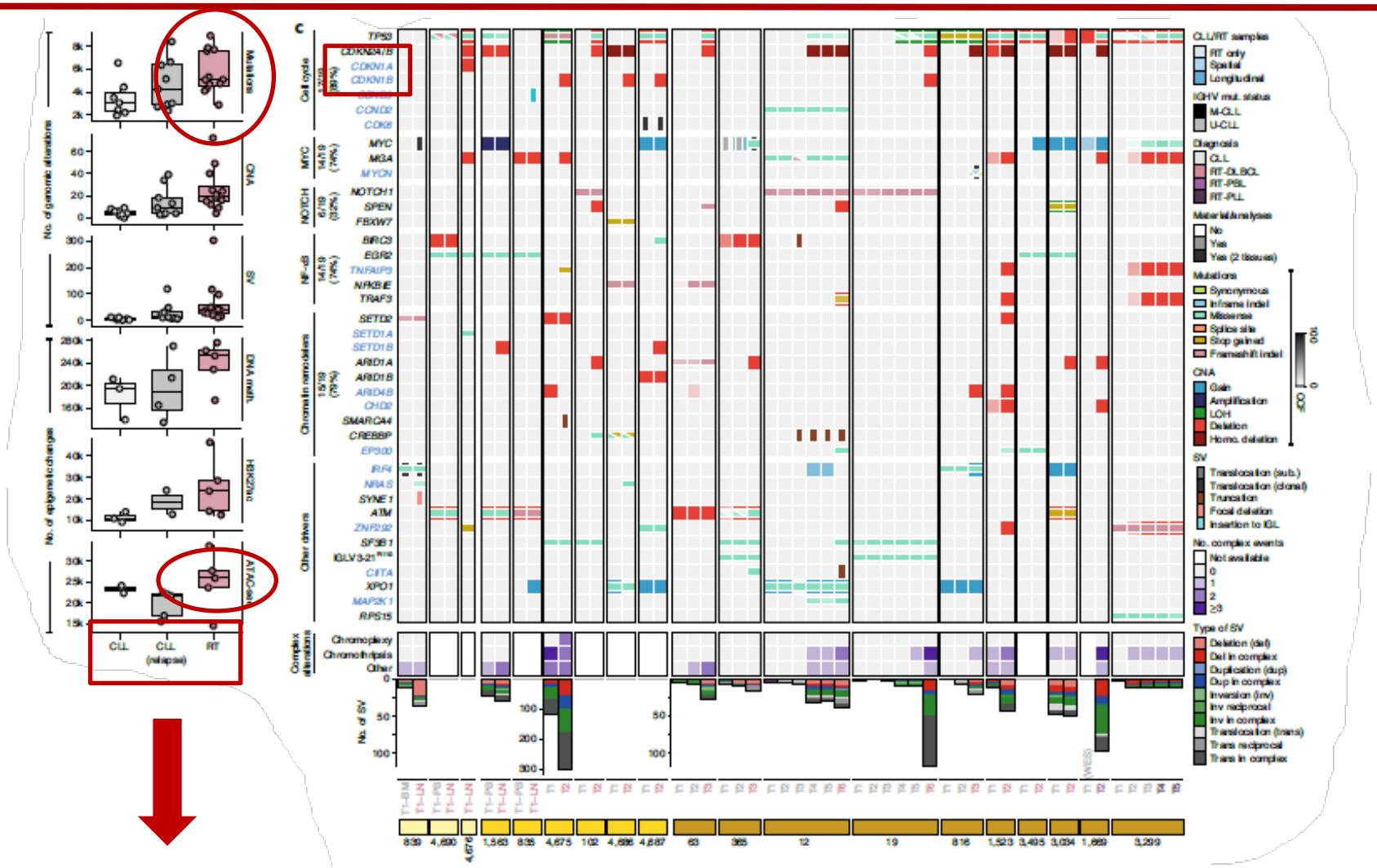






Kohlhaas *et al, Blood* 2021

Genomic complexity of Richter syndrome

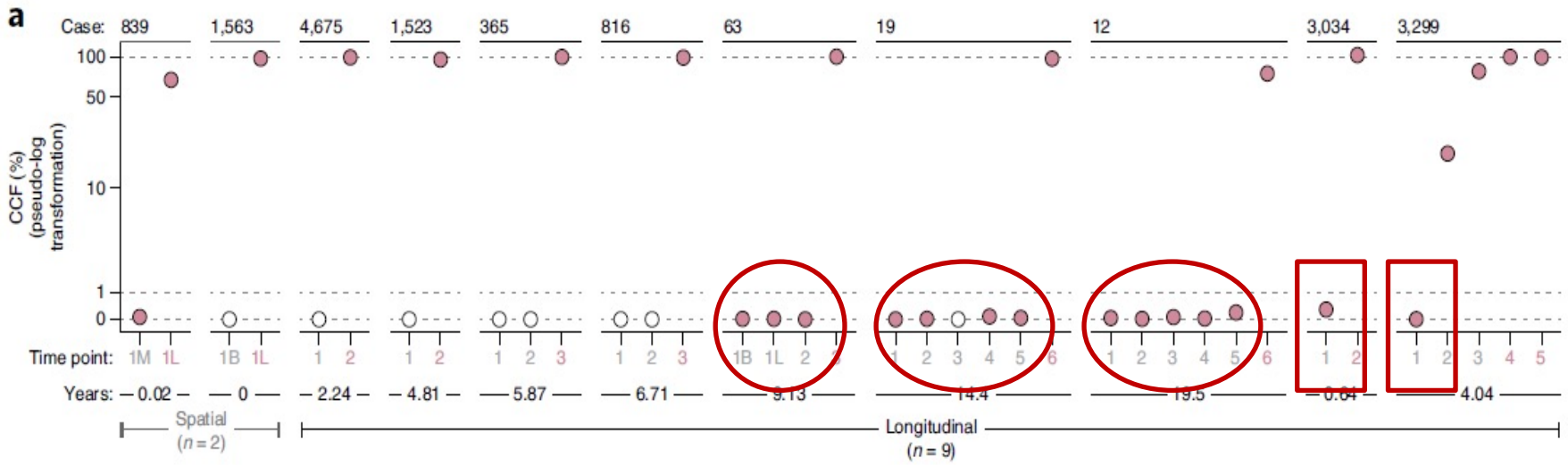


The WGS and epigenome of CLL and RT revealed a concordant **increased complexity from CLL diagnosis to relapse and RT**

New driver genes of RS transformation were identified, such as downregulation of CDKN1A and CDKN1B expression

Nadeu et al., Nat Med 2022

Detection of early seeding of RS transformation in CLL



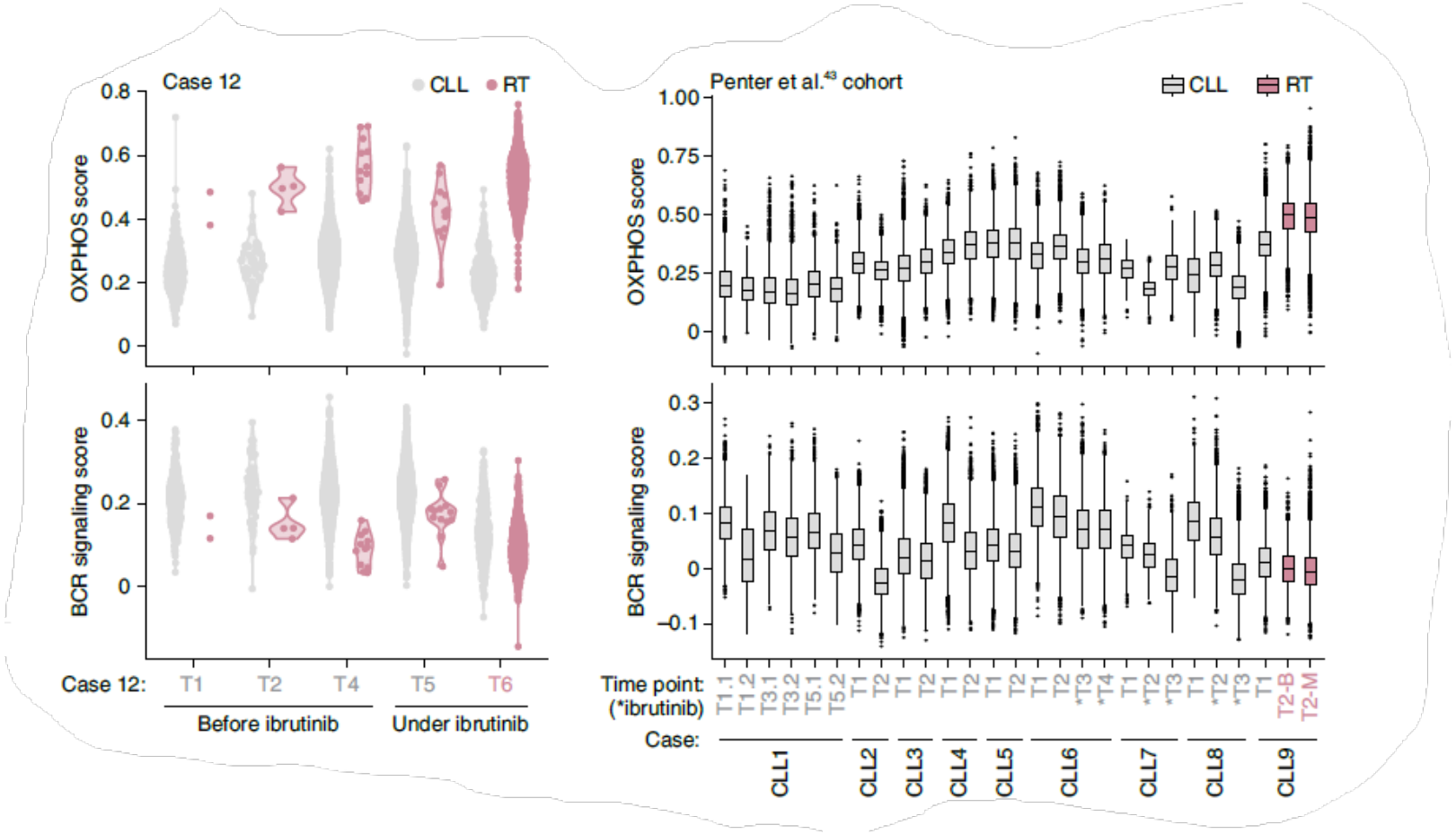
The RS subclone was present at a low cancer cell fraction in CLL samples collected before clinical manifestation in 56% of patients

In some cases, the clone remained stable for many years, in others rapidly expanded driving to clinical manifestation

RS subclones can be detectable time before clinical manifestation

Nadeu et al., Nat Med 2022

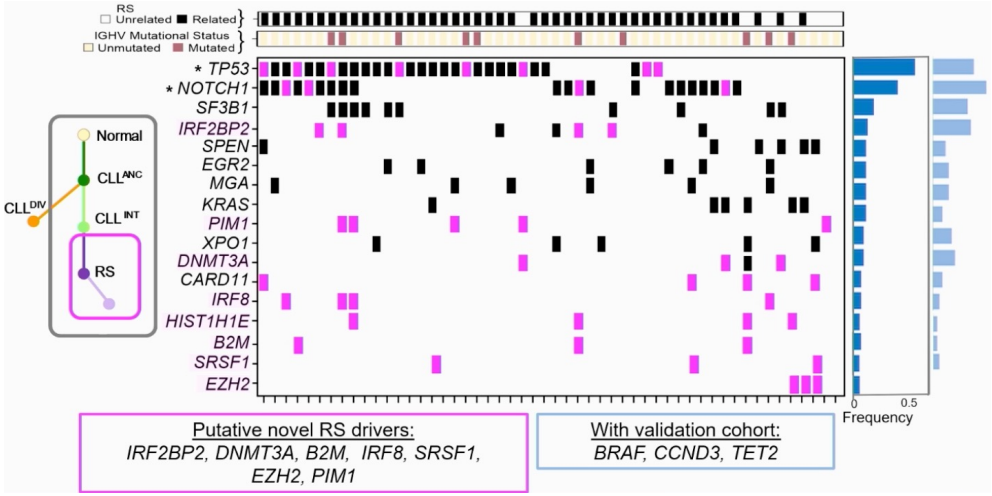
OXPHOS/BCR profiles of RT are already identified in the early dormant RT cells

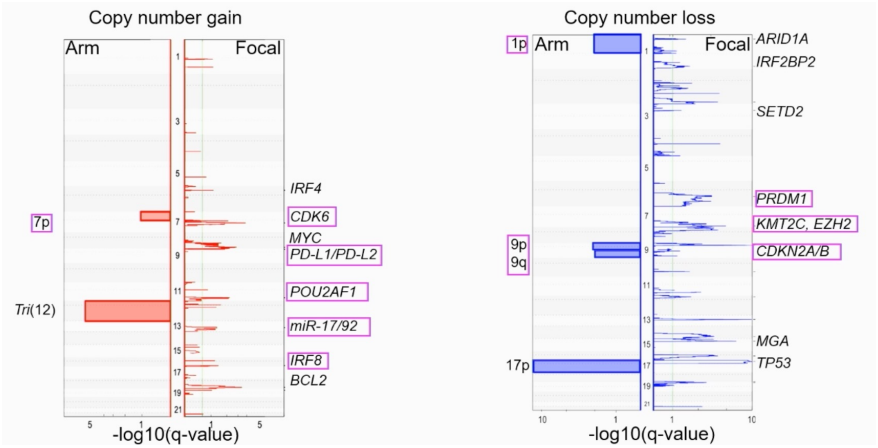


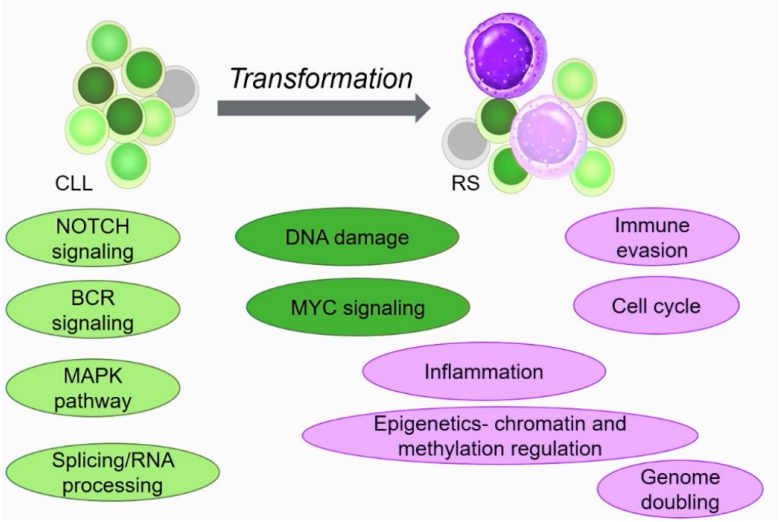
The epigenome and transcriptome of RT converge to an **OXPHOS^{high}–BCR^{low} axis**, which is detectable also before therapy with BCRi → selection and rapid expansion of small RT subclones under therapy with BCRi

Nadeu et al., Nat Med 2022

Differences in the genomic and transcriptomic between CLL and Richter

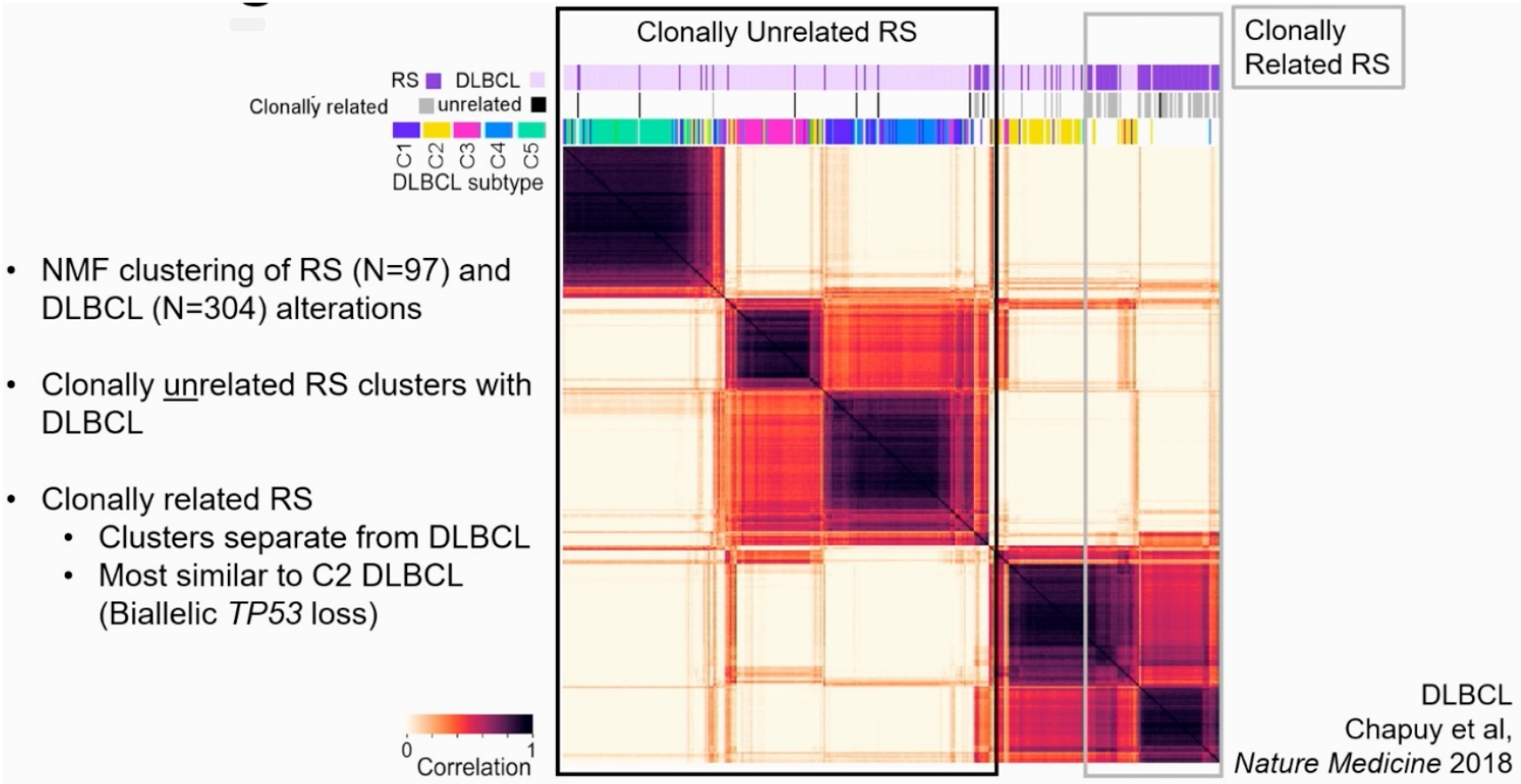






Parry *et al., ASH*#633

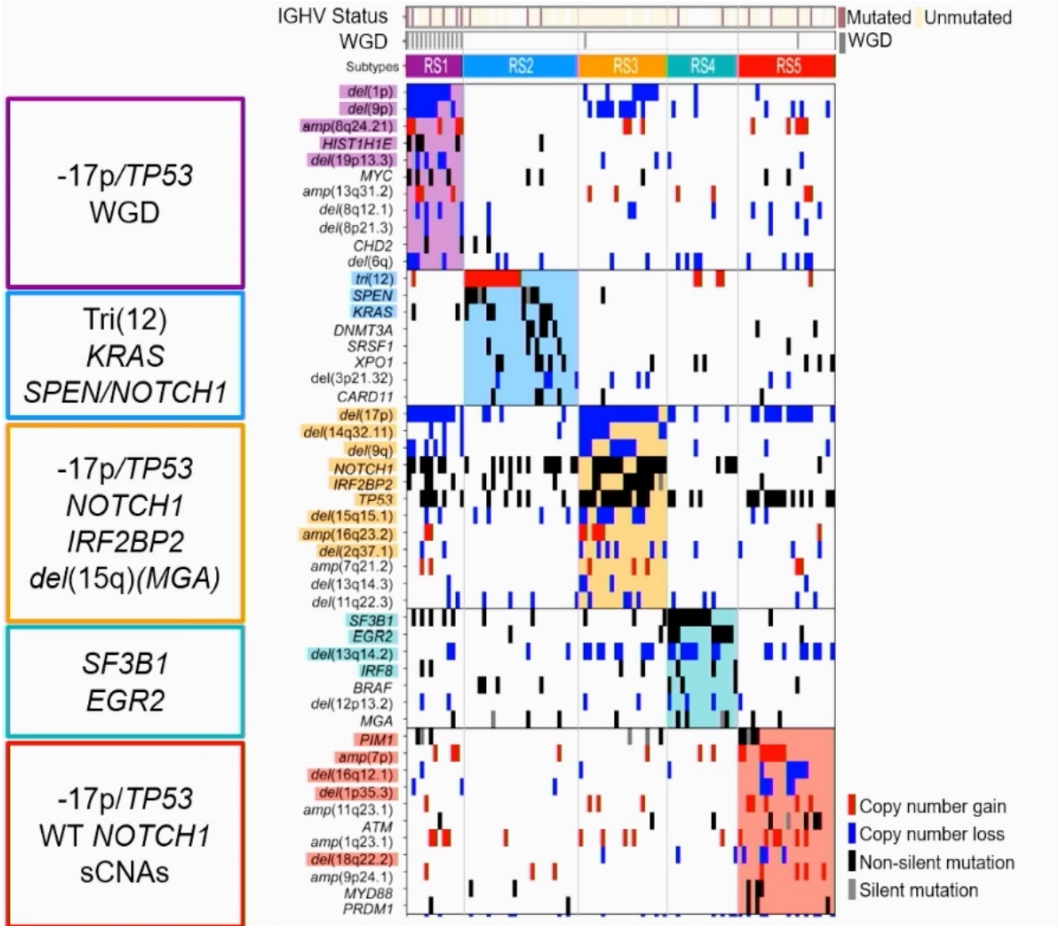
Clonally related RS cluster differently compared to *de novo* DLBCL

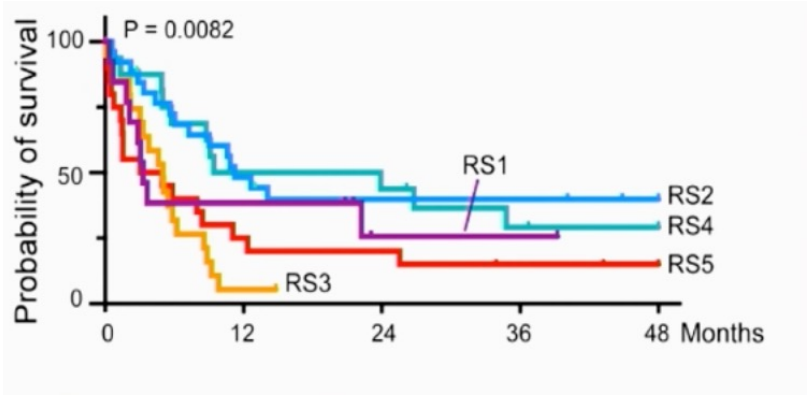


- NMF clustering of RS (N=97) and DLBCL (N=304) alterations
- Clonally unrelated RS clusters with DLBCL
- Clonally related RS
  - Clusters separate from DLBCL
  - Most similar to C2 DLBCL (Biallelic *TP53* loss)

Parry *et al., ASH*#633

Molecular composition and clinical impact of RS clusters

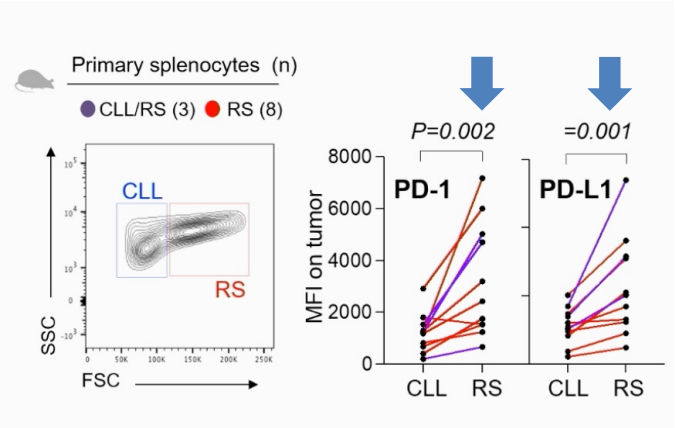




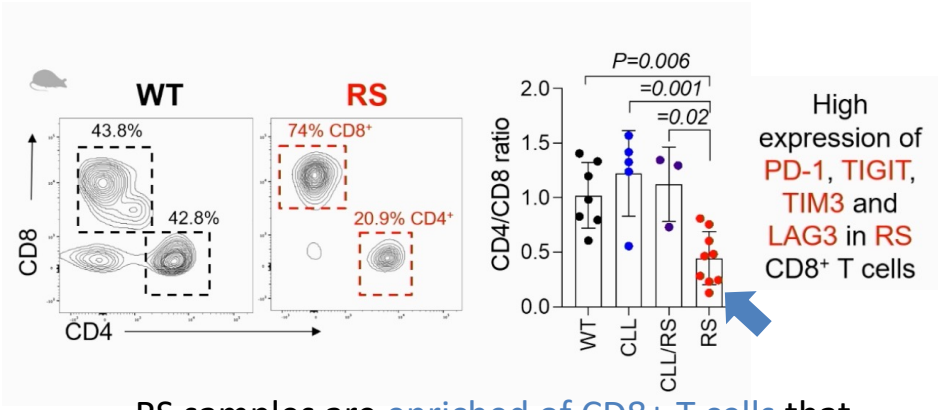
- 5 different RS subtypes have been identified
- 3 clusters are characterised by *TP53* abnormalities (RS1, RS3, RS5) and associated with worse outcome
- 2 clusters are not characterised by *TP53* abnormalities (RS2 and RS4) and associated with better prognosis

Parry *et al., ASH*#633

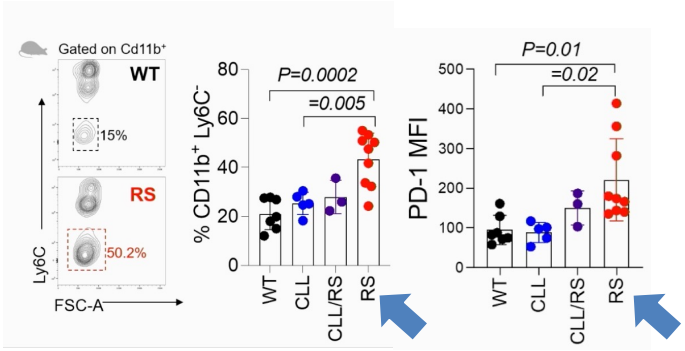
Immunological differences between RS and CLL



RS samples are enriched in PD-1 and PD-L1 expression compared to CLL samples



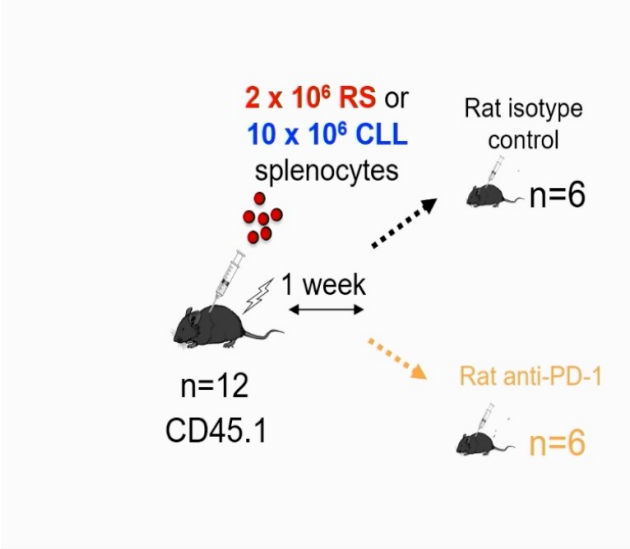
RS samples are enriched of CD8+ T cells that express markers of exhaustion compared to CLL samples

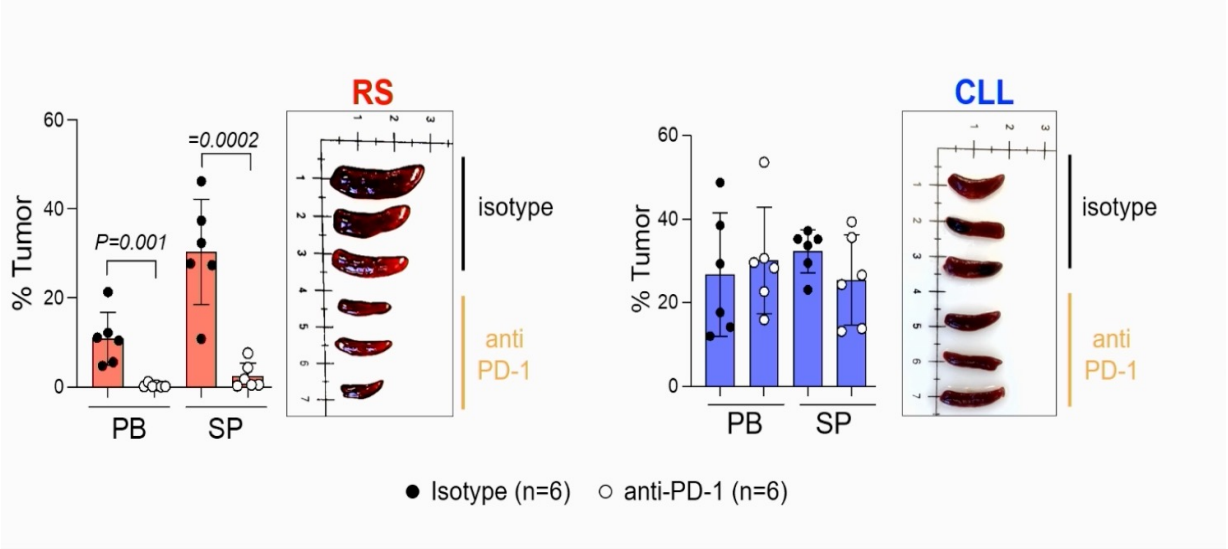


RS samples are enriched in tissue associated macrophages that express higher levels of PD-1 compared to CLL samples

Ten Hacken *et al., ASH*#636

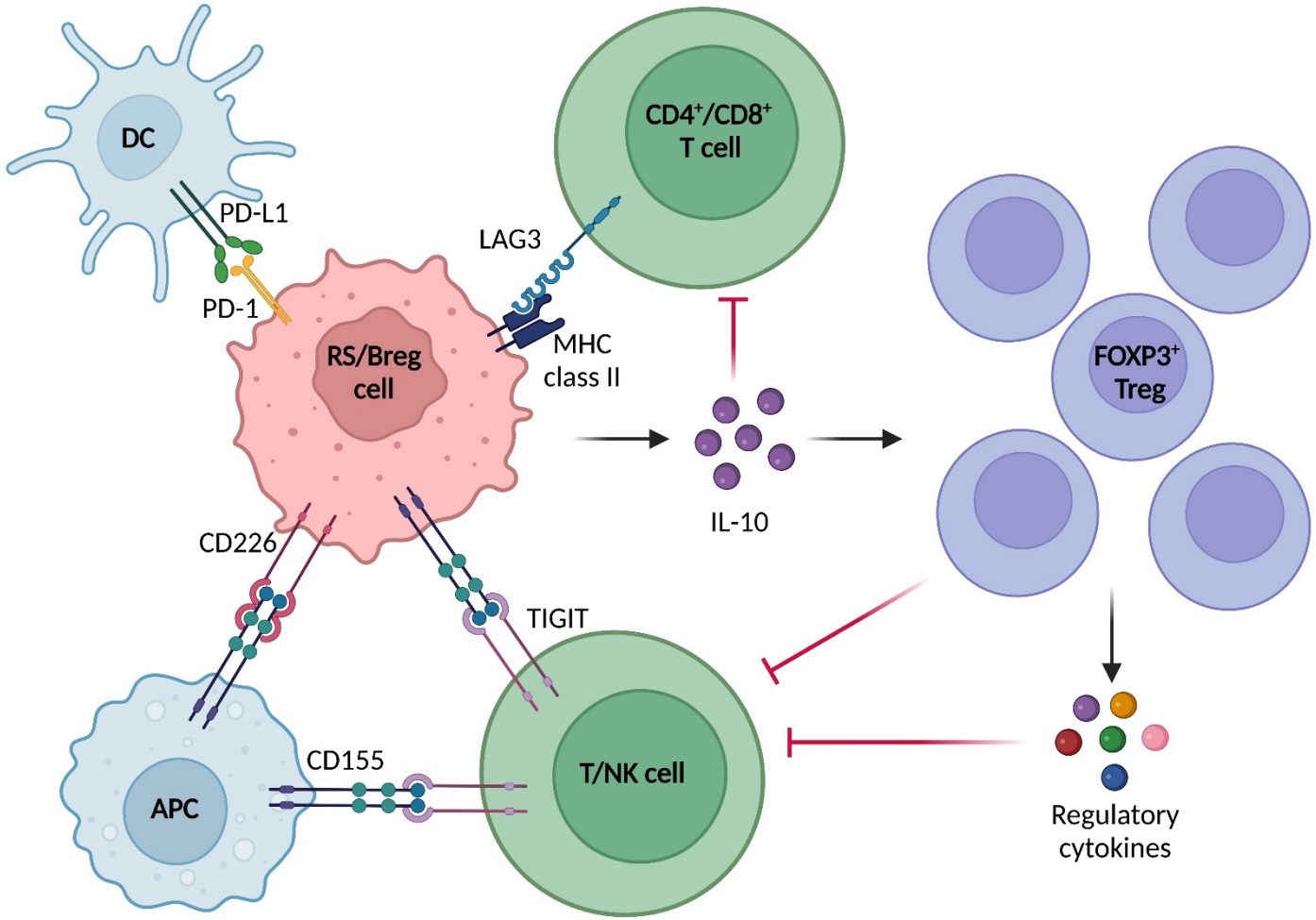
Anti PD-1 therapy is active in mice injected with RS but not with CLL





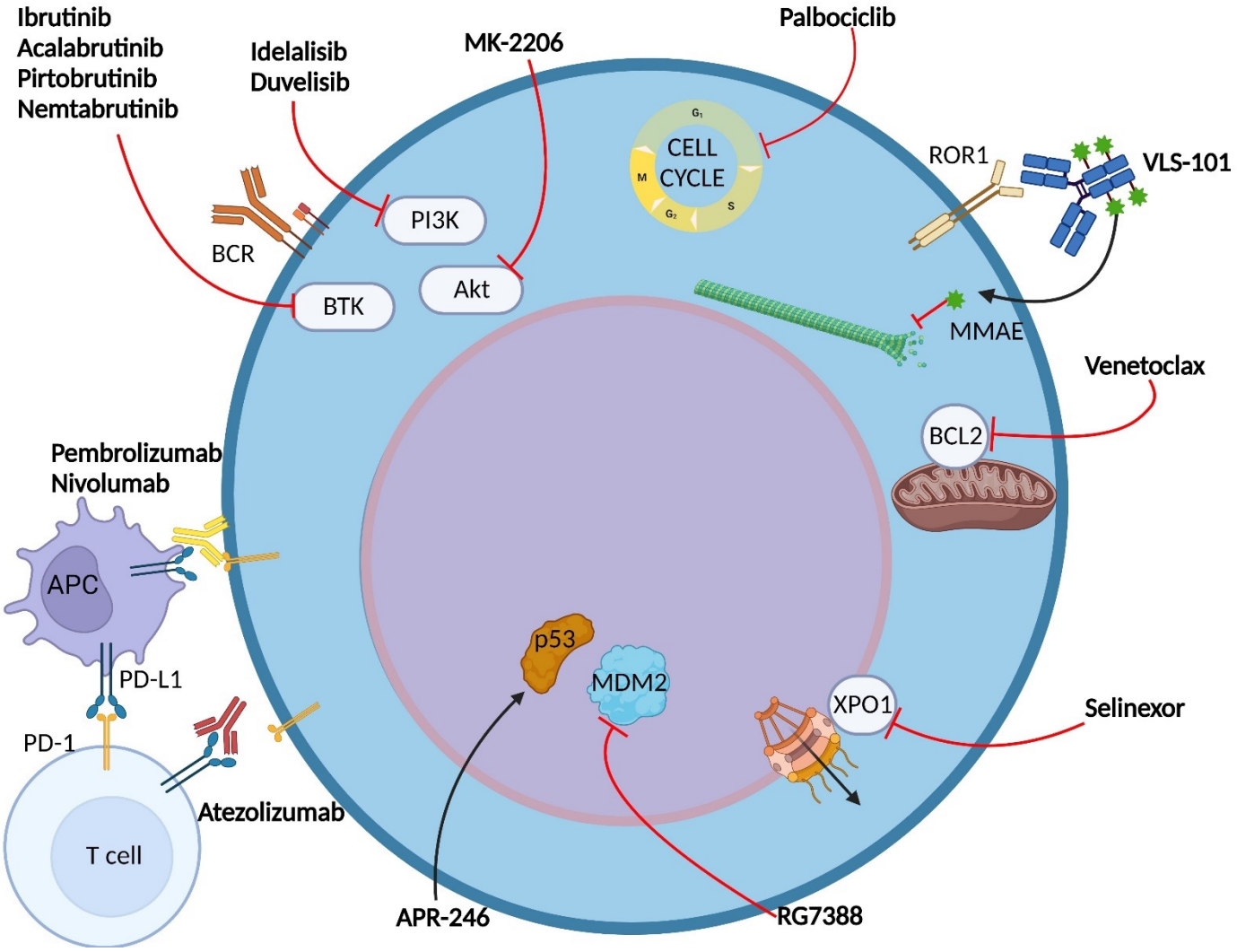
Ten Hacken *et al.*, *ASH*#636

Immune alterations in RS: an overview



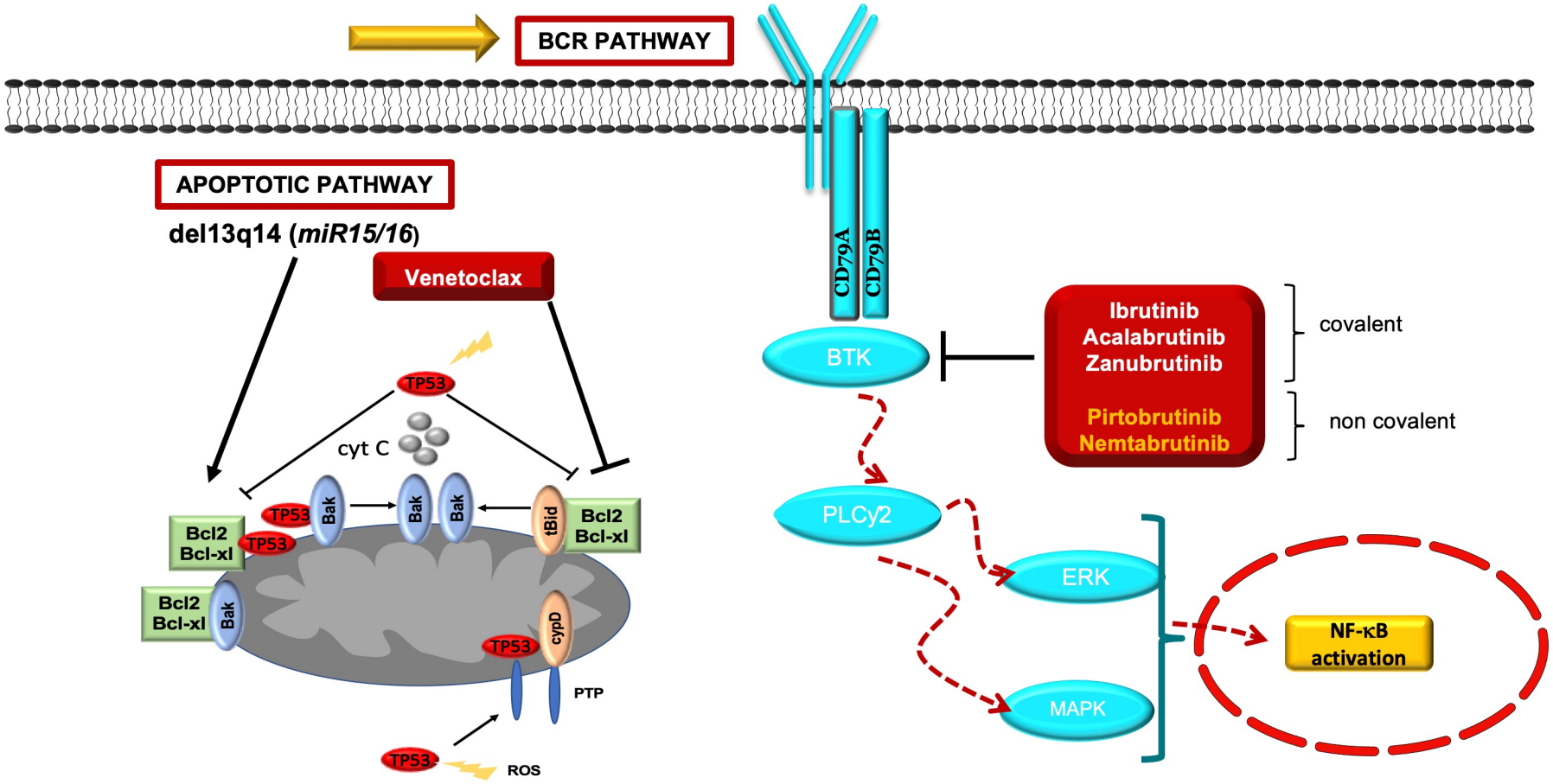
Mahmoud et al, Cancers, 2023

Molecular vulnerabilities in Richter syndrome



Mouhssine and Gaidano, Cancers, 2022

Therapeutic targets in CLL



Clinical applications of predictive and prognostic biomarkers in CLL

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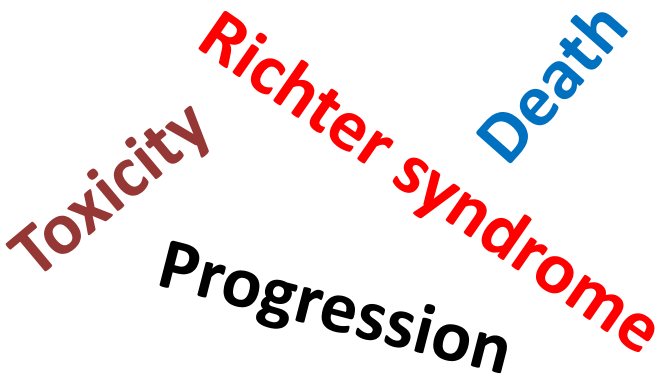
Prognostic biomarkers



Patient counseling

Frequency of follow-up

Predictive biomarkers

Treatment tailoring

Mutated SF3B1 recognizes cryptic splice sites and leads to abnormal splicing / mis-spliced mRNAs