BIOLOGIA della SINDROME di RICHTER e POTENZIALI TERAPIE INNOVATIVE

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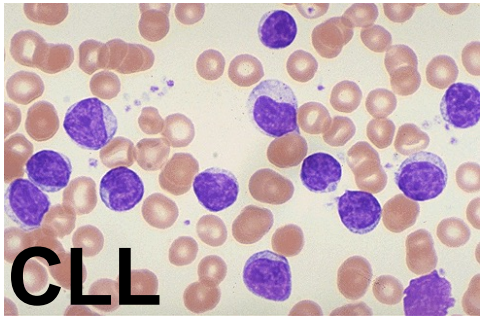
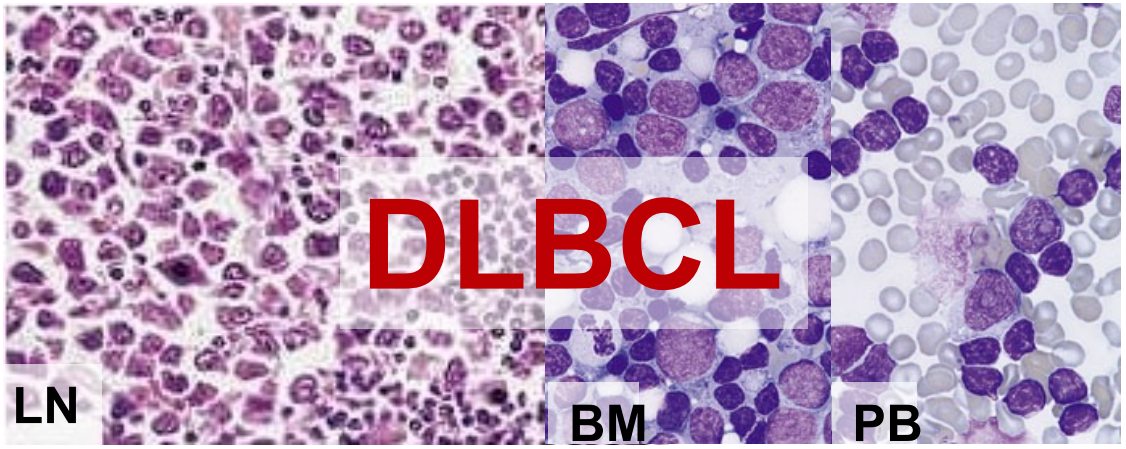
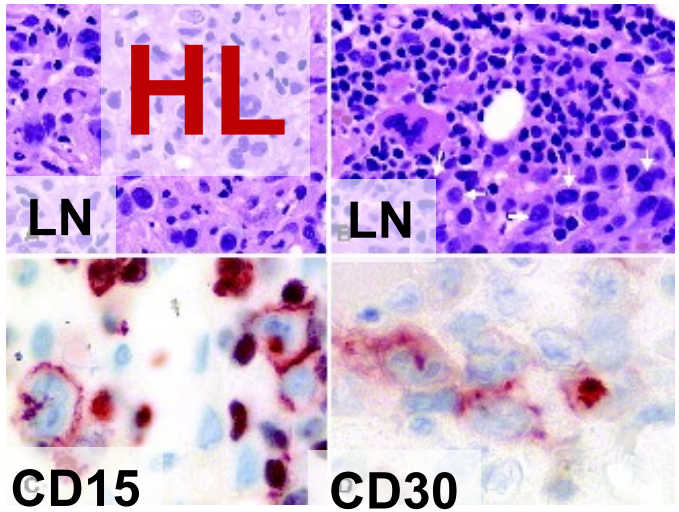
Definition of Richter syndrome



95-99%



1-5%

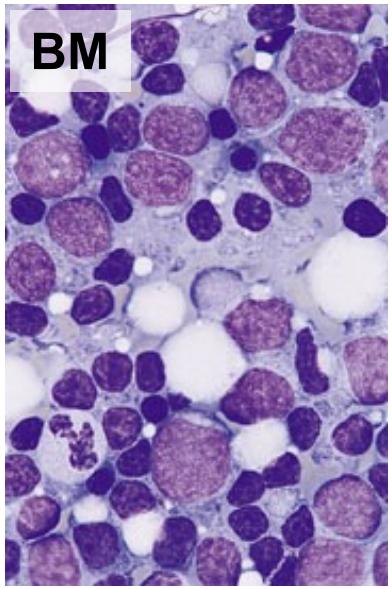


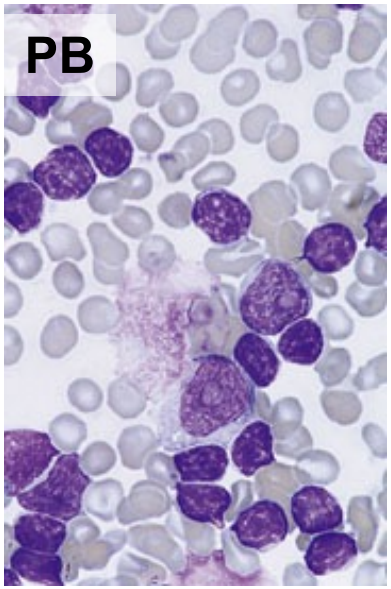
Müller-Hermelink HK, et al, WHO Classification 2008

Clinical clues of Richter transformation

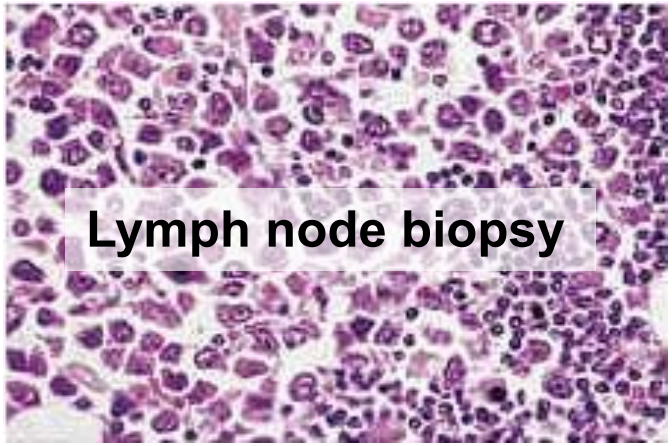
Clinical suspicion of RS

- Bulky disease
- Extranodal involvement
- B symptoms
- High LDH

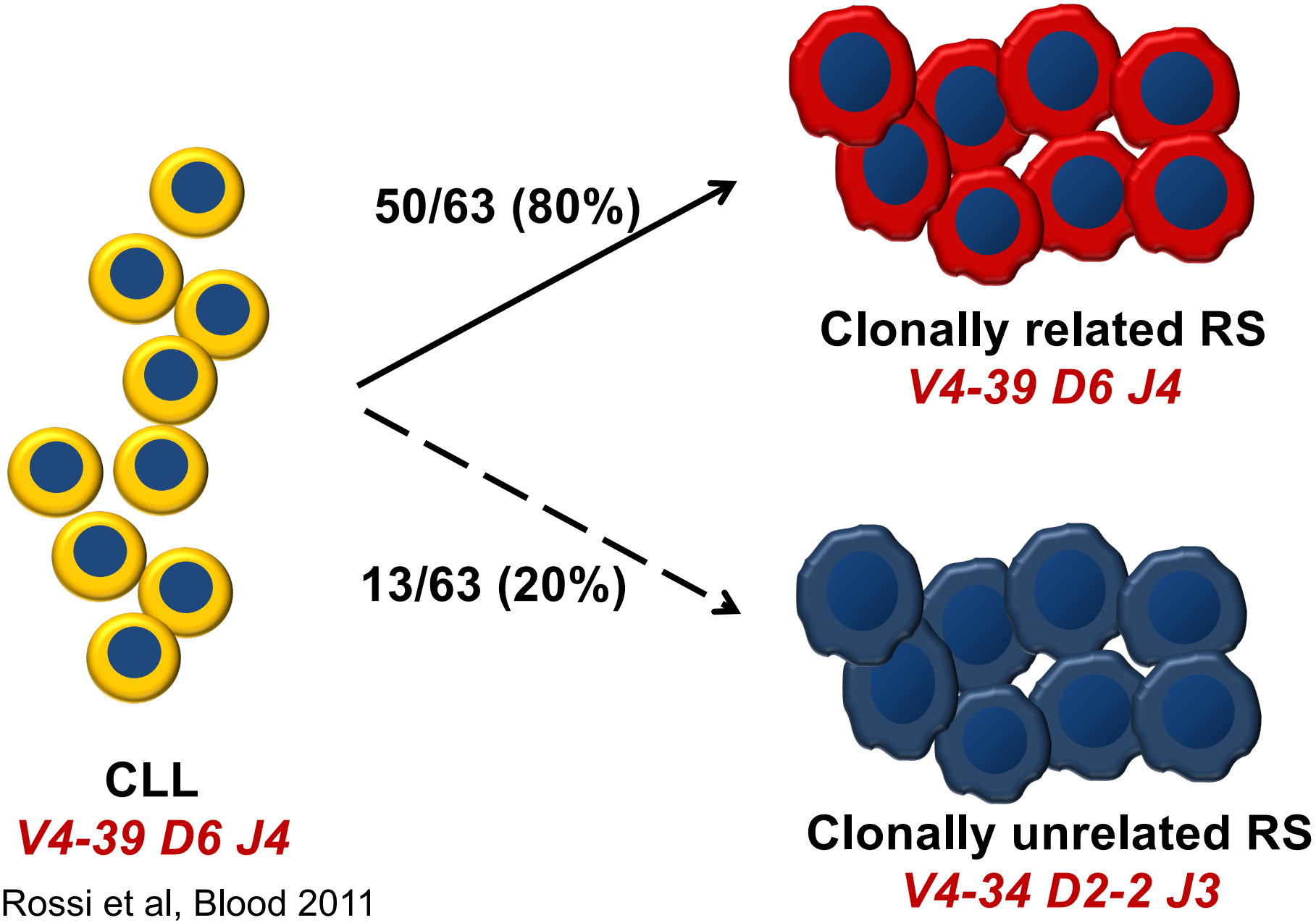




BIOPSY IS MANDATORY (PET-guided)

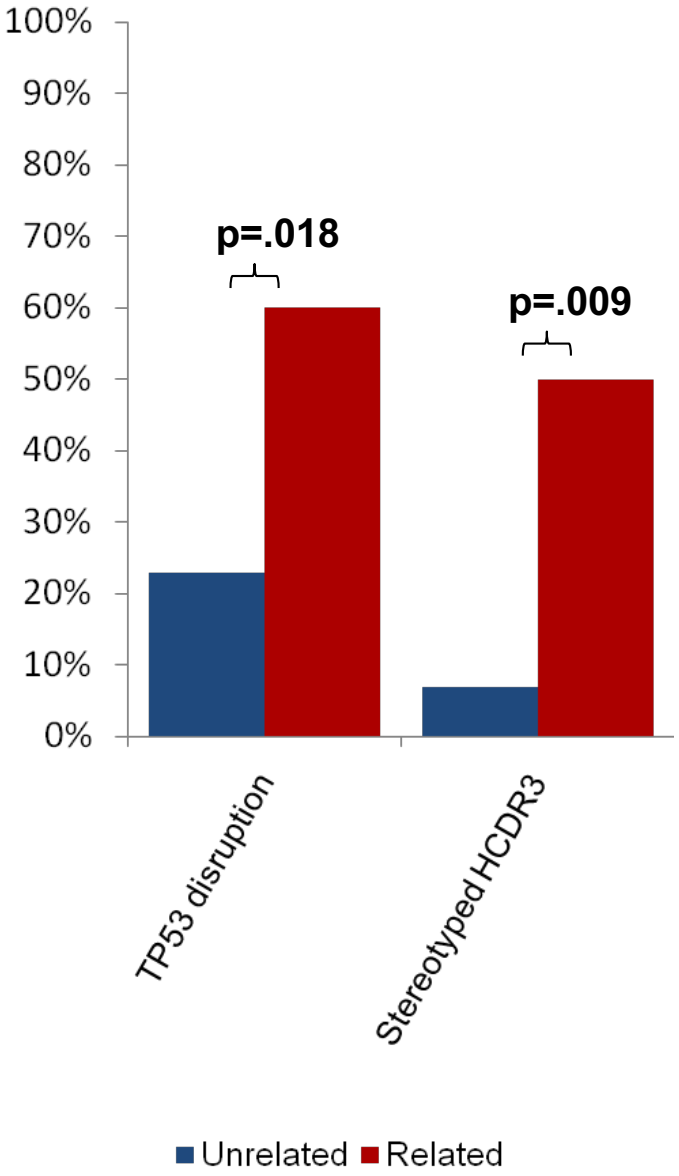


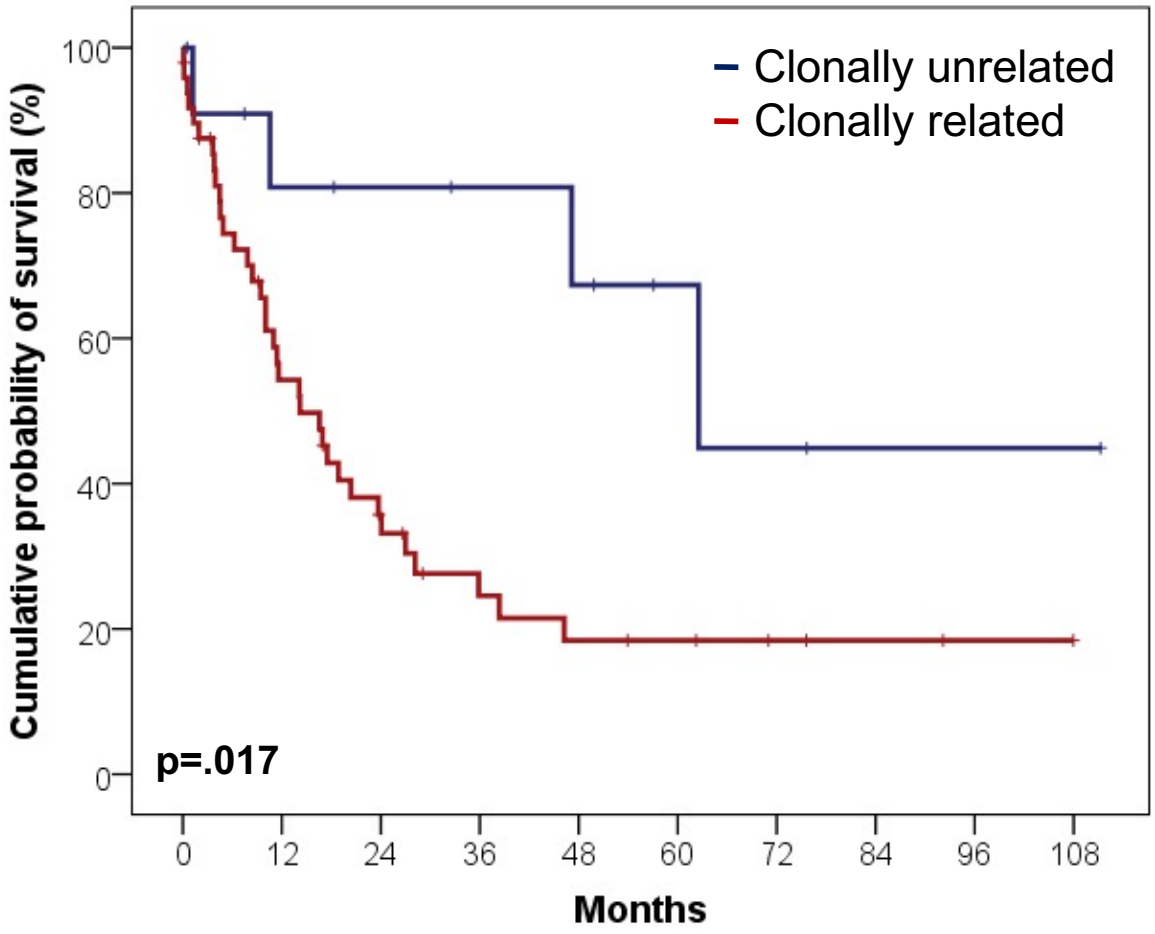
Clonally related vs unrelated Richter syndrome



Rossi et al, Blood 2011

Clonally unrelated Richter syndrome are de novo DLBCL with better outcome





Rossi et al, Blood 2011

Experimental workflow

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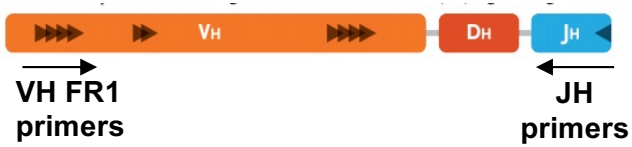
Clonally unrelated RS

Peripheral blood (CLL phase)

Tumor gDNA
2μg in order to obtain a sensitivity of 10^{-6}



LymphoTrack IGH FR1 assay

Deep NGS using Illumina MiSeq

LymphoTrack® Software

LymphoTrack Software analysis
Sequencing reads ≥10k
Top merged sequence
≥5% of the total reads

Im Muno Gene Tics Information system®
http://www.imgt.org

Bioinformatic analysis of IGHV sequence

Ultradeep NGS analysis of IGHV genes in sequential samples of clonally unrelated Richter cases

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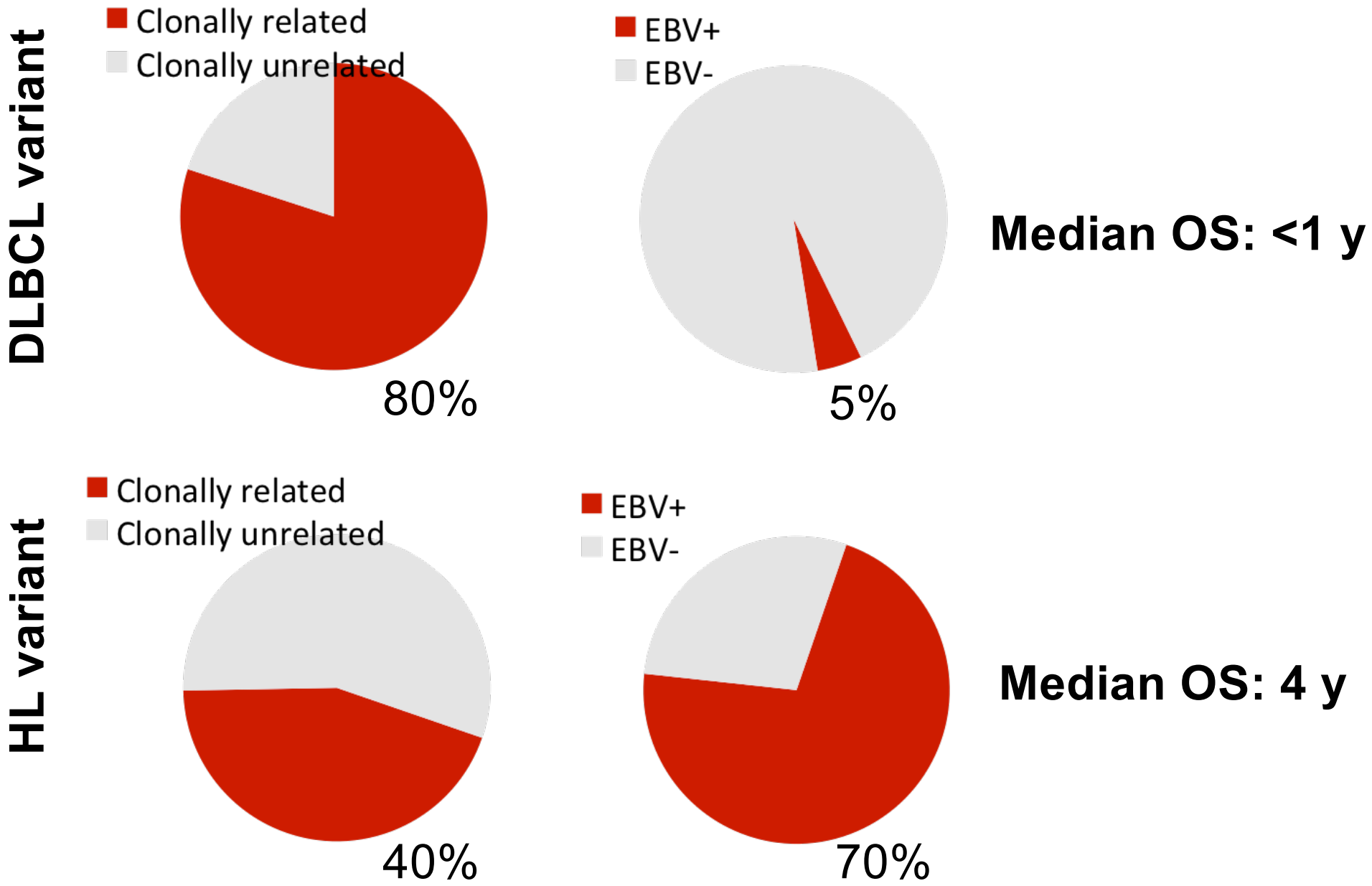
ID Sample	CLL					RS				
	IGHV	IGHD	IGHJ	Identity	CDR3	IGHV	IGHD	IGHJ	Identity	CDR3
ID1	1-69*01 or 1-69*12	3-16*02	6*02	100.00	CASKGVDDYIWGSYRYTDYYYYYGMDVW	1-69*02	3-3*01	6*02	100.00	CAREEGLTIFGVVGYYYYGMDVW
	1-69*02	3-3*01	6*02	100.00	CAREEGLTIFGVVGYYYYGMDVW					
ID2	1-3*01	6-19*01	4*02	100.00	CAREQWLGIPAFDYW	1-69*01 or 1-69*12	3-3*01	6*02	100.00	CASPTYYDFWSGYSYYYYGMDVW
ID3	4-31*03 or *04	3-3*01	6*03	100.00	CARGVYYDFWSGYYKPYYYYMDVW	1-8*01	4-17*01	4*03	95.83	CTSELRRFDYW
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

- With one exception, the RS IGHV rearrangement was not identified in the CLL phase suggesting that, in most of cases, unrelated RS does not derive from a preexisting circulating clone but stems from a different tumoral clone, probably in the lymph nodes

Most of cases unrelated Richter syndrome are truly a de novo lymphoma and do not originate from the clonal evolution of a pre-existing small CLL subclone

DLBCL vs HL variants of Richter syndrome

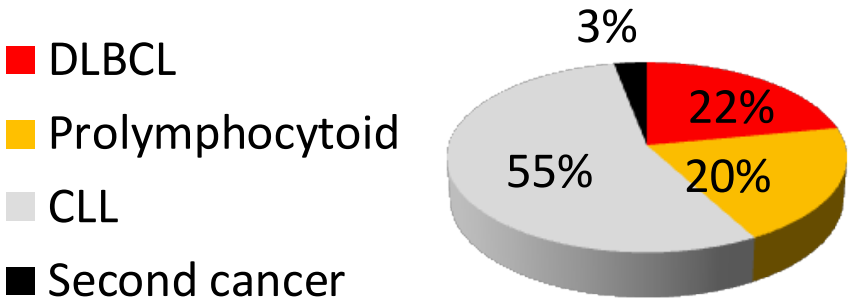


Abruzzo et al, Am J Surg Pathol 2002; 26: 630-6 O'Brien et al, Cancer 2003; 98: 2657-663 Thornton et al, Leuk Res 2005; 29: 389-95 Ammatuna et al, Leuk Lymphoma 2009; 50:; 857-8 Kanzler et al, Blood 2000; 95:1023-31 Tsimberidou et al, Cancer 2006; 107: 1294-302 Rossi D, et al, Clin Cancer Res 2009; 115: 4415-22, Xiao et al, Hum Pathol 2016;55:108-16

WHO 2016 Classification
Richter syndrome

Clinical suspicion of transformation

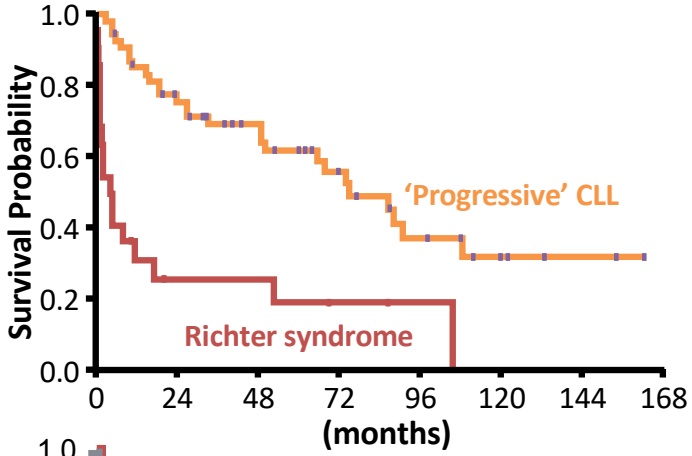
- Asymmetric growth of localized lymph nodes
- Bulky disease
- B symptoms
- Sudden and excessive rise in levels of LDH

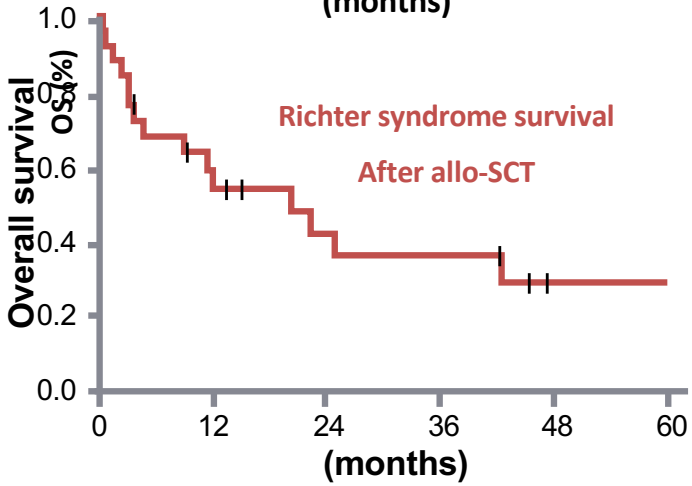


PET/CT in Richter syndrome diagnosis

	RS
Sensitivity	91%
Specificity	80%
Positive predictive value	53%
Negative predictive value	**97%**

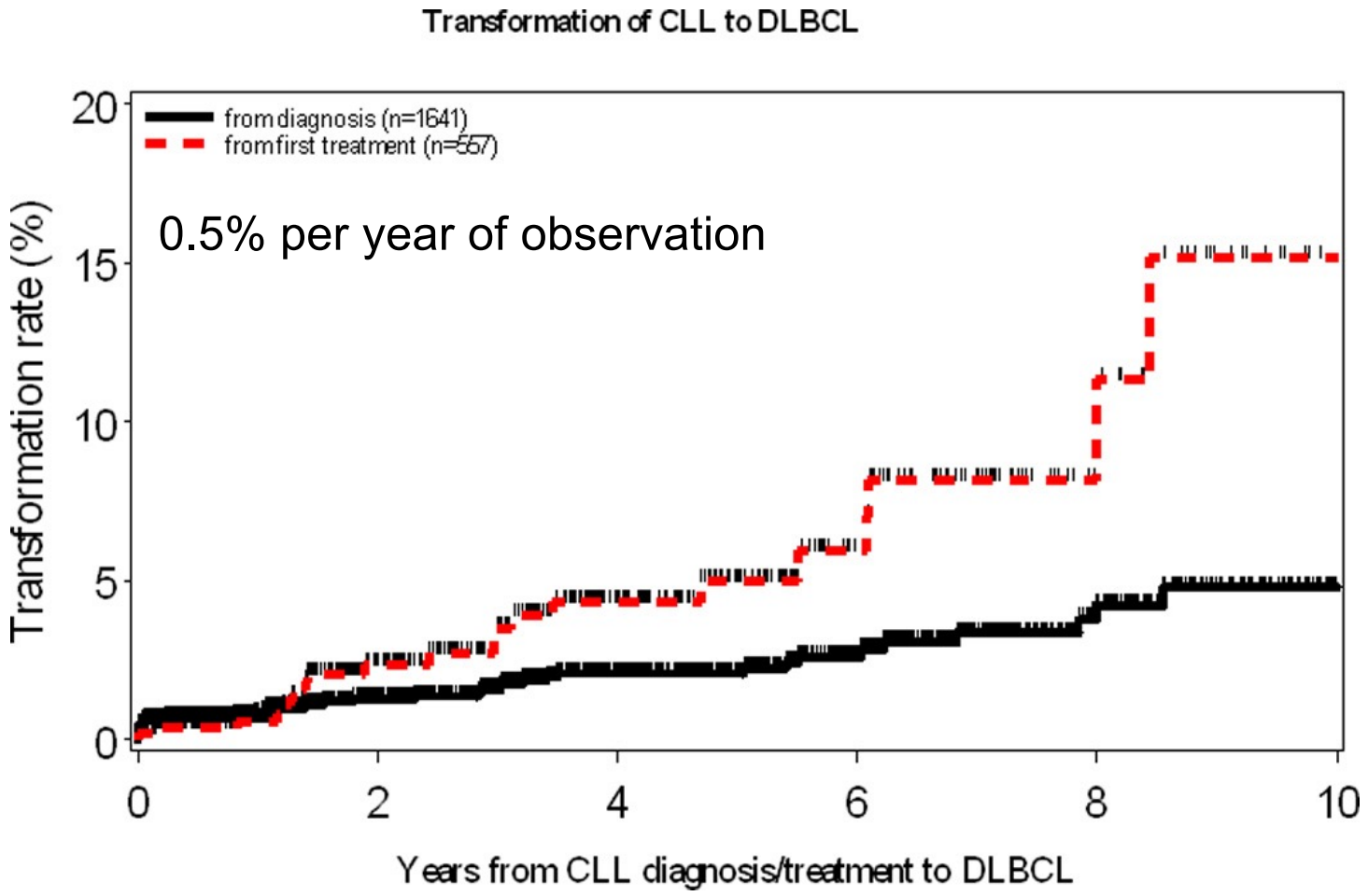
Max SUV cut off=5





Rossi D et al. Semin Oncol 2016 43:311-9
Gine' E et al. Haematologica. 2010 95:1526-33
Buzzi JF et al. J Nucl Med 2006 47:1267-73
Mauro FR et al. Leukemia 2015 29:1360-5.

Cumulative incidence of Richter syndrome “then”

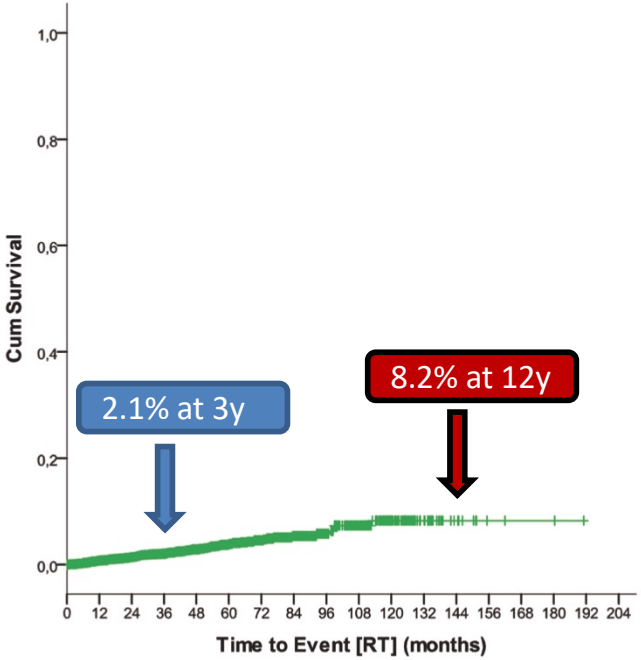


Parikh et al Br J Haematol 2013

Richter transformation in the GCLLSG trials

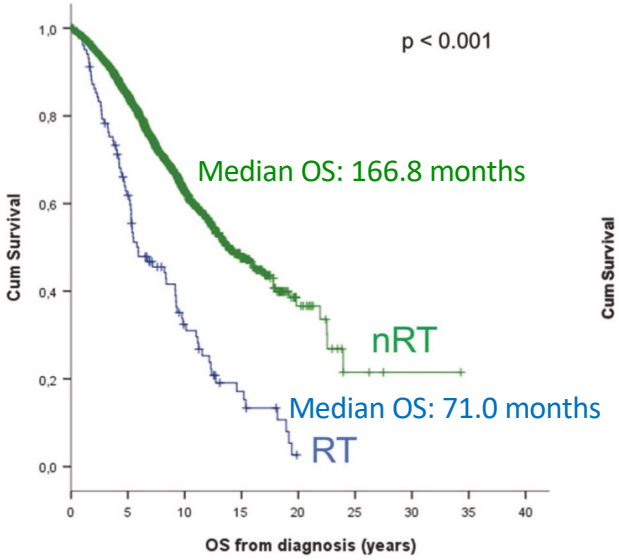
2975 patients with CLL enrolled in phase 2 and phase 3 trials of the GCLLSG
Median observation time was 53 months

Time to Richter transformation from first-line CLL treatment

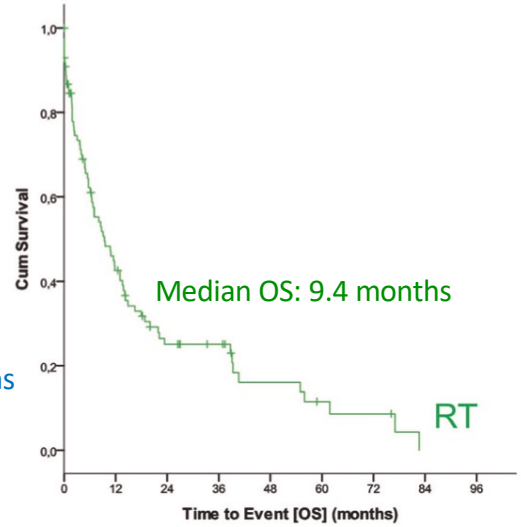


RT-free	Pts, N	Events, N	Median months	3-year Survival, %	6-year Survival, %	9-year Survival, %	12-year Survival, %
All patients	**2971**	99 (3.3)	NR	97.9	95.4	92.6	91.7

Survival from diagnosis in patients with and without Richter



Survival after Richter diagnosis



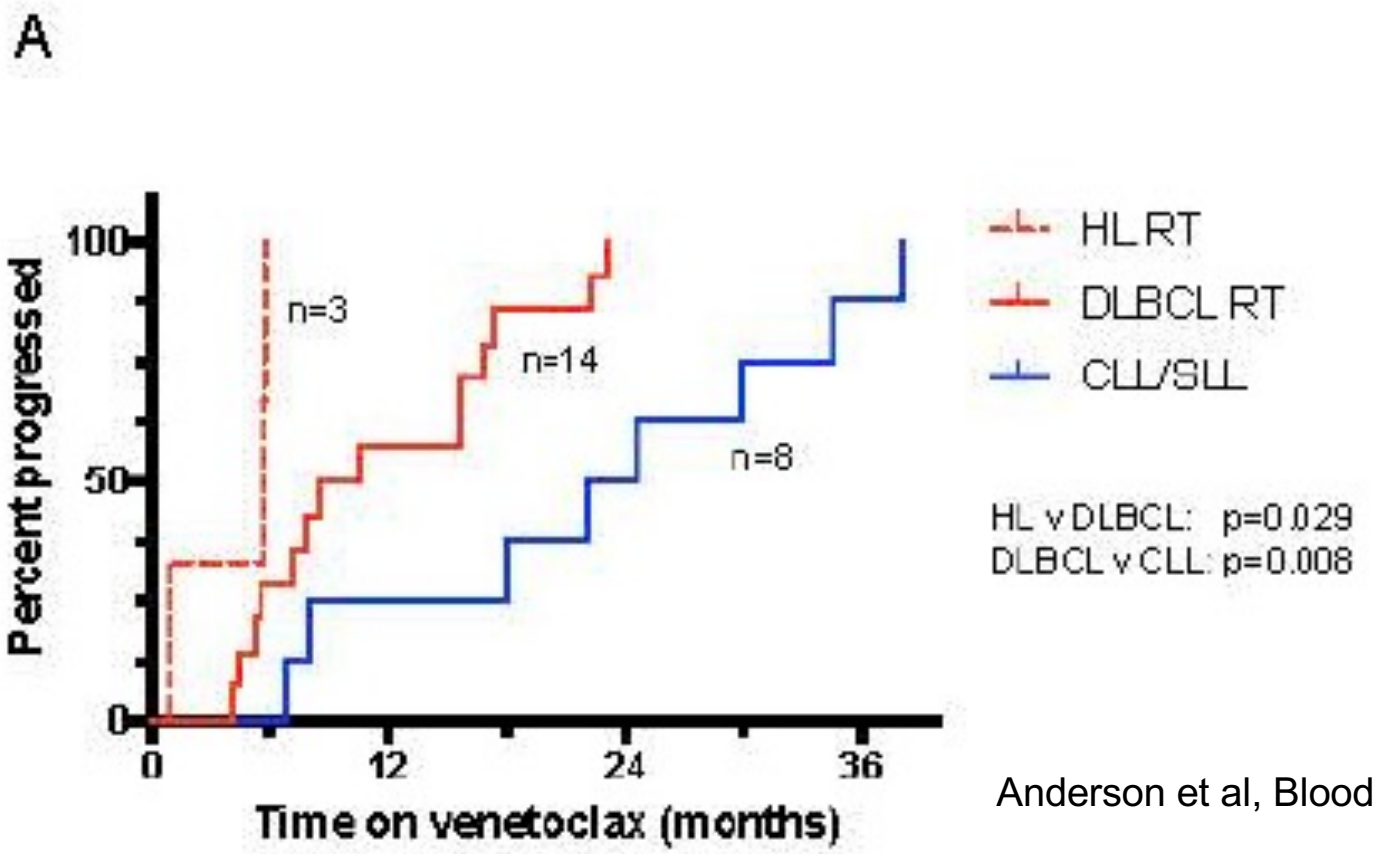
Al-Sawaf et al Leukemia 2020

Incidence of Richter syndrome “now”

Reference	Total pts	Study population	Treatment	Pts that developed RS	RS prevalence
Burger, 2015	186	Treatment naive	Ibrutinib	0	0%
Byrd, 2014	391	Relapsed	Ibrutinib	4	1%
O'Brien, 2014	29	Treatment naive	Ibrutinib	1	3%
Jain, 2015	127	Relapsed/Refractory	Ibrutinib	7	5%
Farooqui, 2015	51	17p deleted	Ibrutinib	3	6%
Mato, 2016	178	BCRi treated	Ibrutinib, idelalisib	13	7%
Byrd, 2013	85	Relapsed/Refractory	Ibrutinib	7	8%
Seymour, 2017	49	Relapsed/refractory	Venetoclax-rituximab	5	12%
Roberts, 2015	116	Relapsed/Refractory	Venetoclax	18	16%
Seymour, 2017	49	Relapsed/refractory	Venetoclax-rituximab	5	12%
Strati, 2014	63	17p deleted	Heterogeneous	15	23%

Heterogeneity conceivably due to: case mix, 1st line vs R/R, observation time

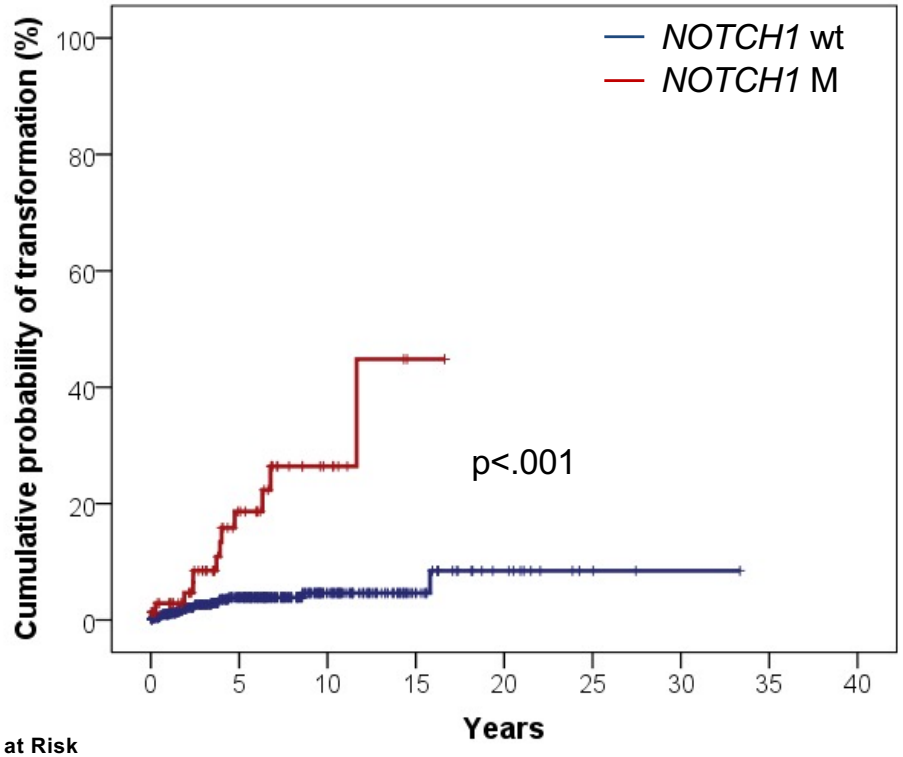
Richter syndrome in R/R CLL treated with novel agents is an early event



Anderson et al, Blood 2017

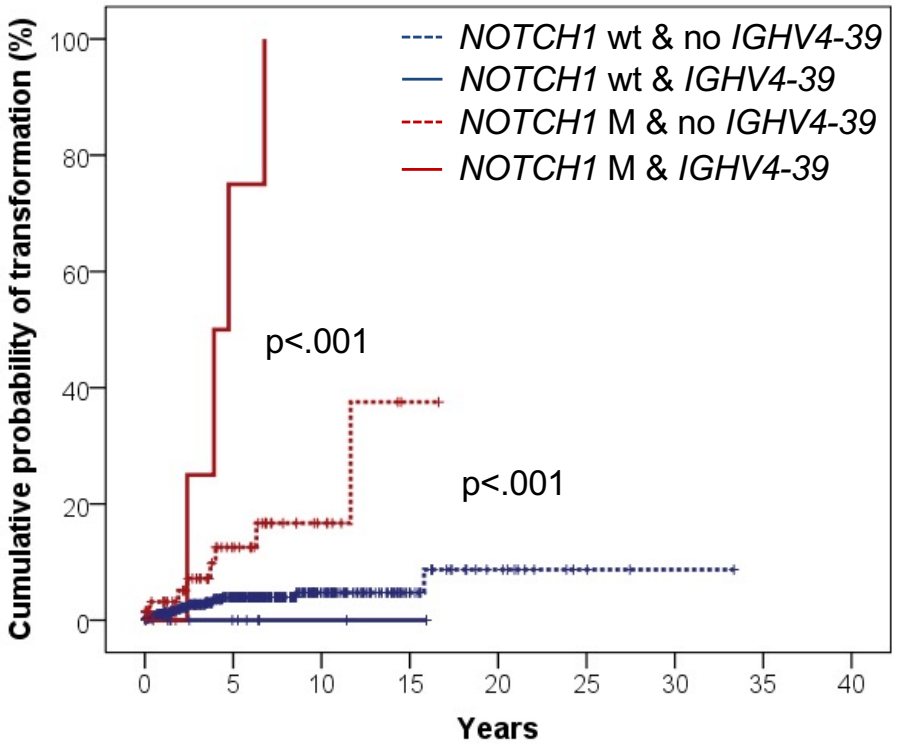
In all datasets of R/R CLL treated with novel agents (BCRi, Venetoclax), emergence of Richter syndrome is an early event, suggesting expansion of a clone that had been previously selected by chemotherapy

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

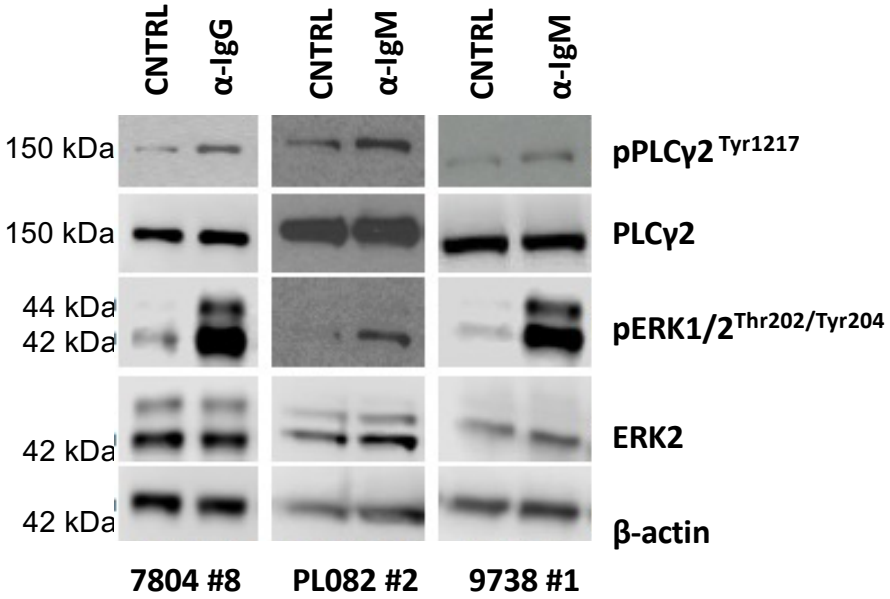
	Events	Total	5-year risk	95% CI
NOTCH1 wt	18	531	3.9%	2.0-5.8
NOTCH1 M	12	74	18.6%	7.3-29.9

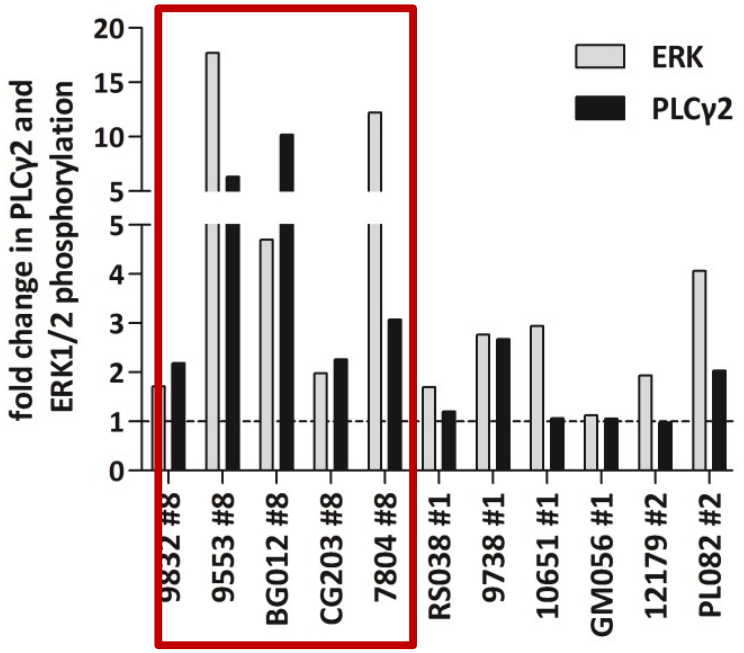


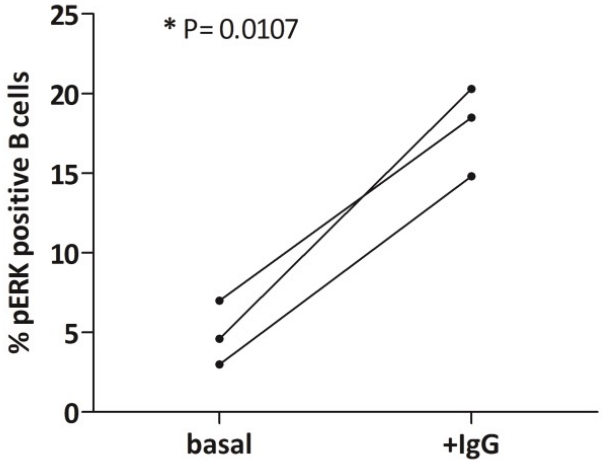
No. at Risk									
NOTCH1 wt & no *IGHV4-39*	519	273	90	30	11	3	1	0	0
NOTCH1 wt & *IGHV4-39*	12	12	12	12	0	0	0	0	0
NOTCH1 M & no *IGHV4-39*	67	27	8	1	0	0	0	0	0
NOTCH1 M & *IGHV4-39*	7	1	0	0	0	0	0	0	0

	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

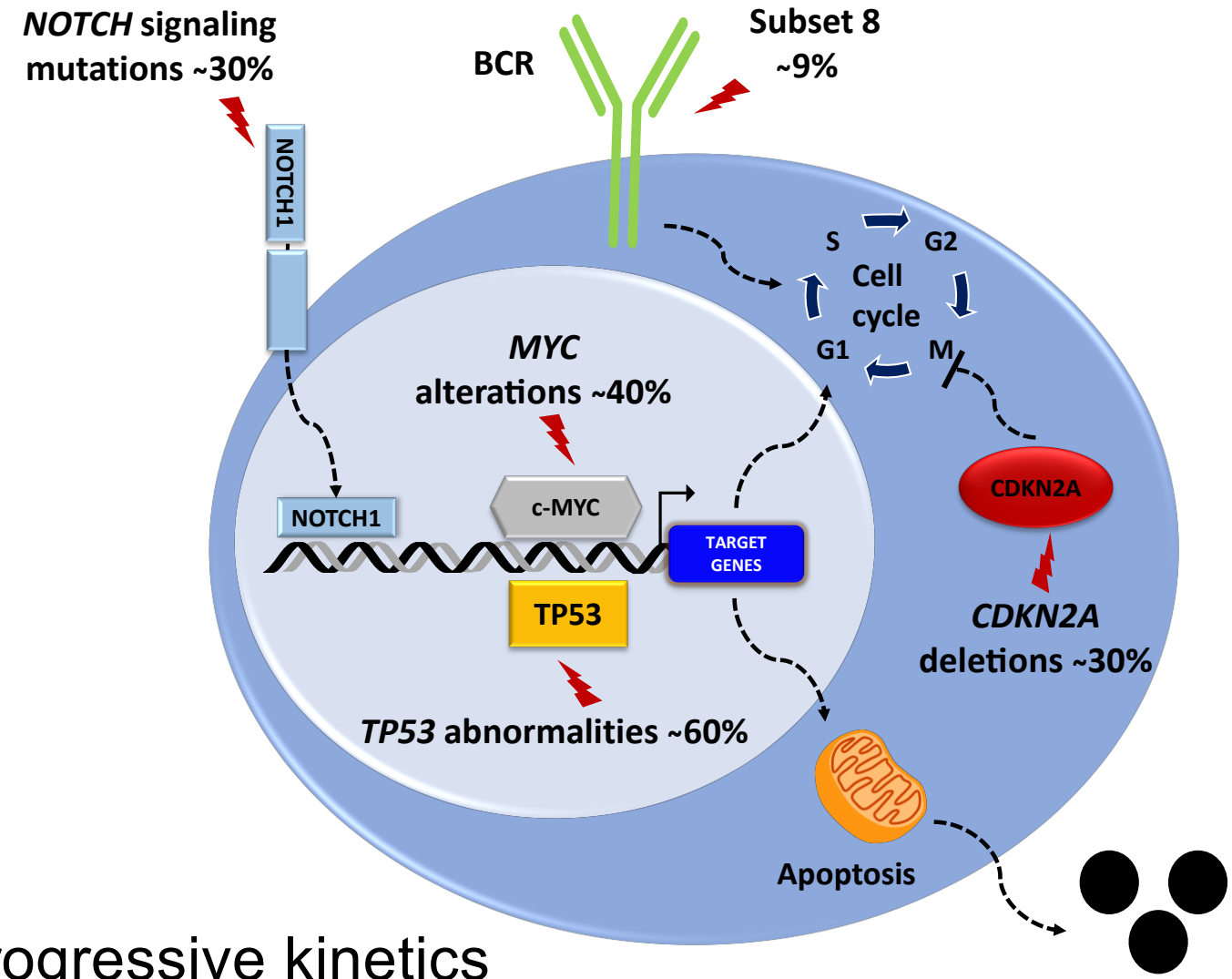






Gounari et al, Blood, 2015
Courtesy of P Ghia

Proliferation and apoptosis are the master cellular programs deregulated in Richter syndrome

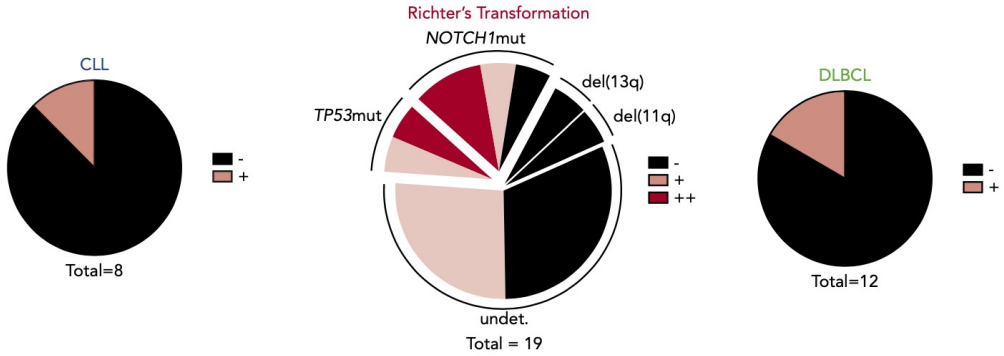


Rapidly progressive kinetics
Chemorefractoriness

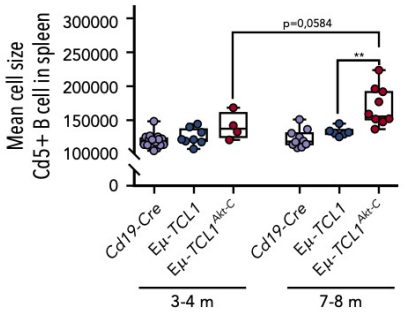
Adapted from Rossi D, *et al. Semin Oncol* 2016; 43:311–319.

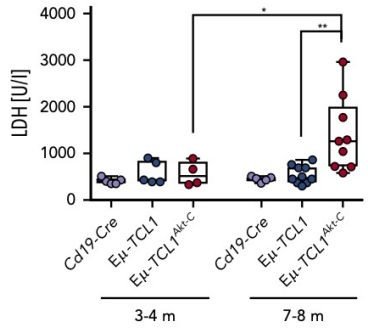
Akt signaling triggers CLL toward Richter transformation via overactivation of Notch1

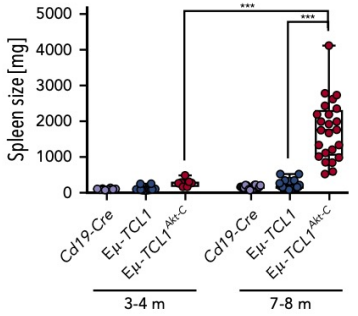
High levels of AKT phosphorylation occur both in high-risk CLL patients as well as in patients with RT

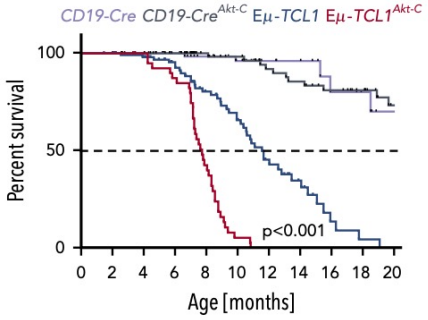


Overactivation of Akt in the murine Eμ-TCL1 CLL mouse model resulted in CLL transformation to RT with significantly reduced survival and an aggressive lymphoma phenotype

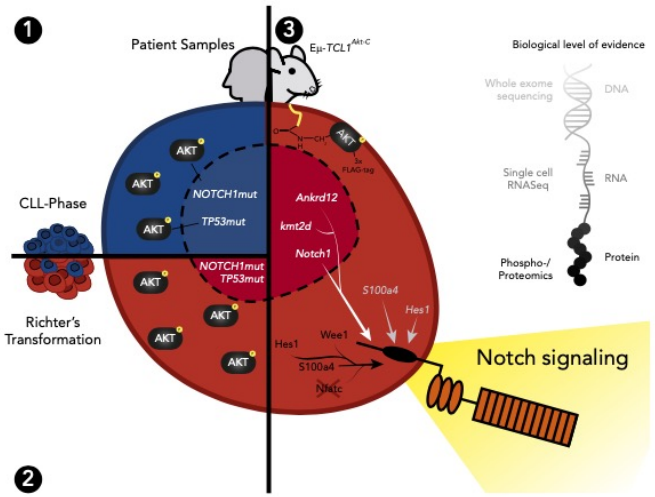


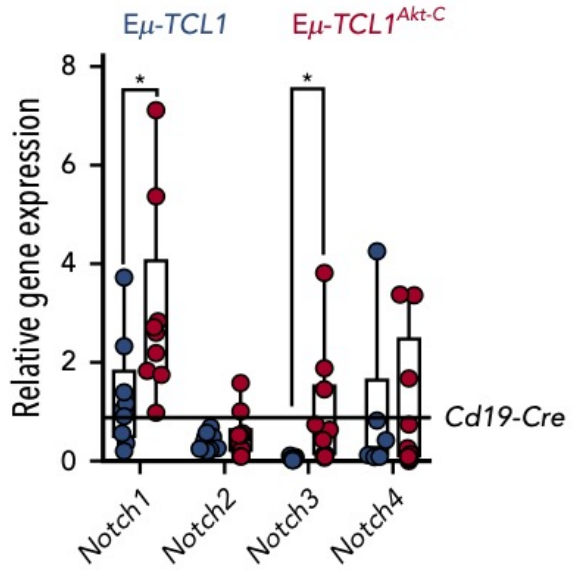






Akt activation was identified as an initiator of CLL transformation toward aggressive lymphoma by inducing Notch signalling

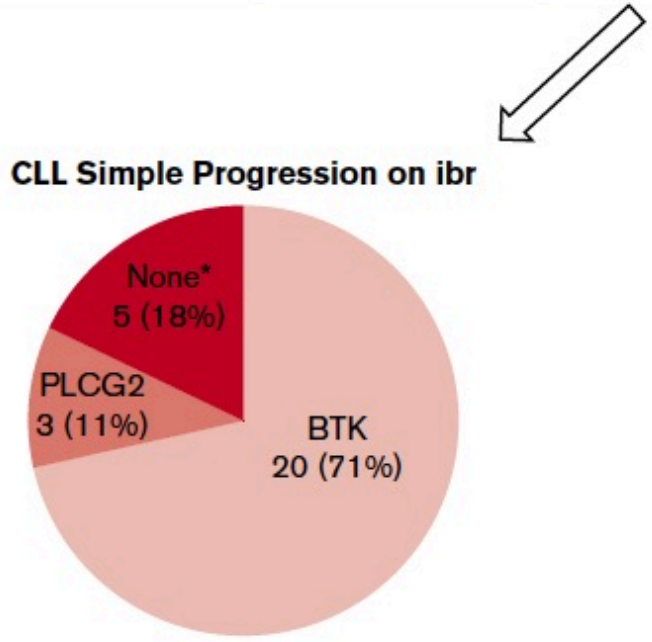




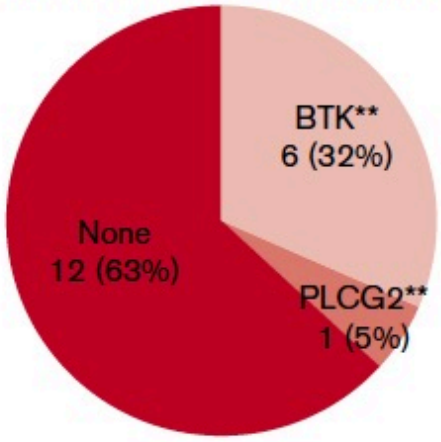
Kohlhaas *et al, Blood* 2021

BTK and PLCG2 mutations in Richter syndrome developing under Ibrutinib

	Total pt	CLLPD	RT
Maddocks[6]	20	11	9
Burger[18]	5	5	0
Ahn[43]	12	9	3
Current study	9	3	6
Total	46	28	18



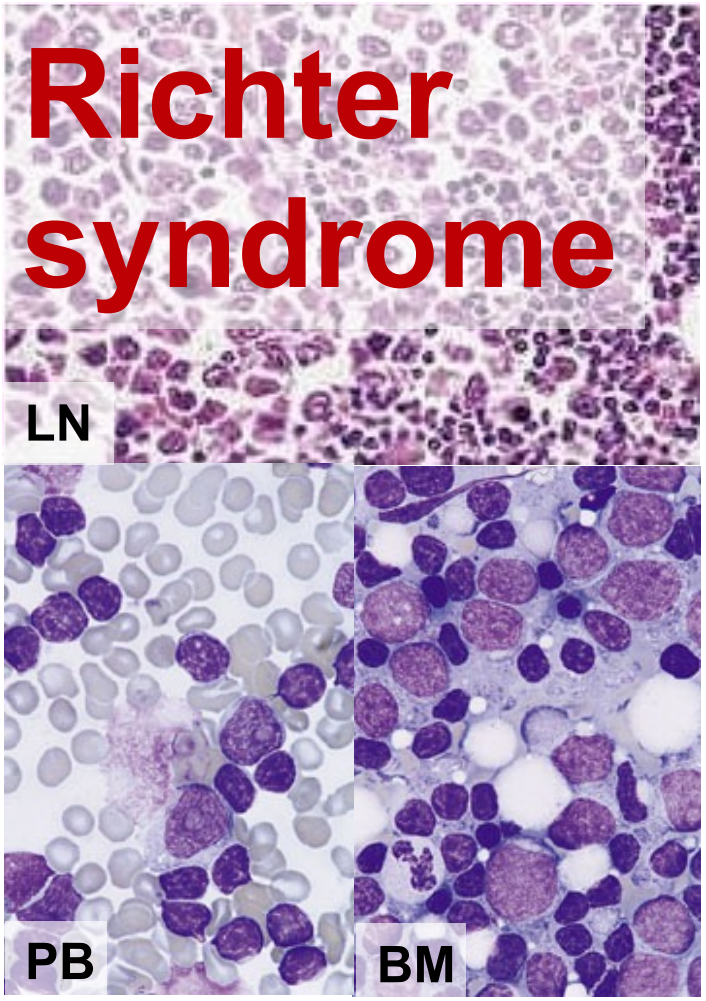
CLL Simple Progression on ibr



CLL Ricter Transformation on ibr

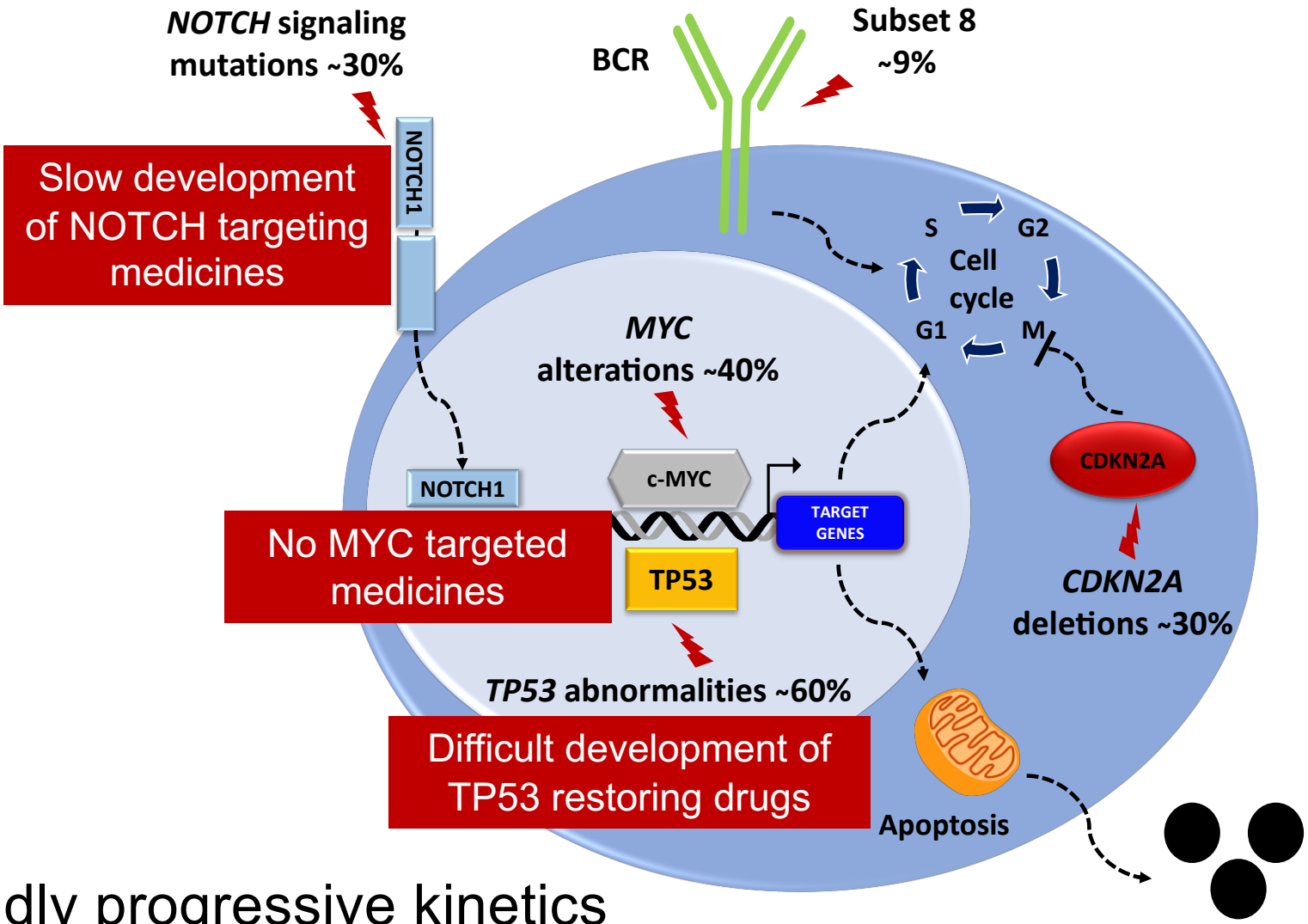
Kadri et al, Blood Adv 2017

Reasons for treatment failure in Richter syndrome



- Biology of the tumor
- Lack of dedicated treatments
- Patient frailty
- Late recognition

Issues in targeting genetic lesions of Richter syndrome



Rapidly progressive kinetics
Chemorefractoriness

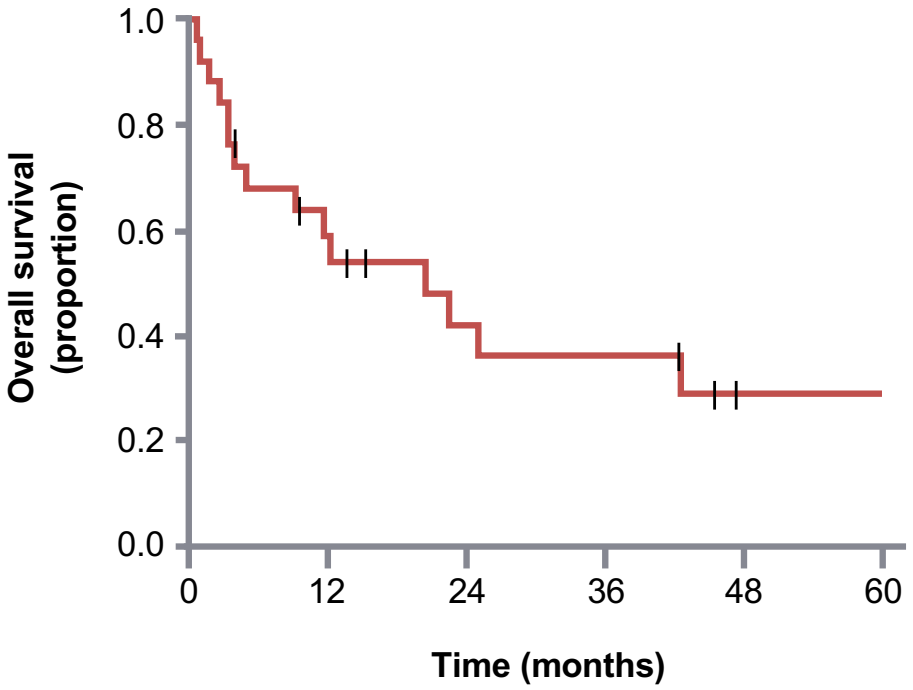
Adapted from Rossi D, *et al. Semin Oncol* 2016; 43:311–319.

Clinical trials in RS: R-CHOP and OFAR are the best available induction treatments

Regimen	Author, year	Institution	No. of Pts	Median age	CR	ORR	PFS	OS
Chemotherapy								
OFAR-2	Tsimberidou, 2013	MDACC	35	63	6	39	3	7
OFAR-1	Tsimberidou, 2008	MDACC	20	66	20	50	4	8
R-CHOP	Langerbeins, 2014	GCLLSG	15	69	7	67	10	21
O-CHOP	Eyre, 2016	UK	37	66	25	44	6	11
R-Hyper-CVAD	Tsimberidou, 2013	MDACC	35	-	-	46	6	9
R-EPOCH	Rogers, 2015	OSU	46	67	20	38	4	6
DHAP, ESHAP	Durot, 2015	France	28	63	25	43	7	8
R-Hyper-CVXD	Tsimberidou, 2003	MDACC	30	59	27	43	6	8
Hyper-CVXD	Dabaja, 2001	MDACC	29	61	38	41	-	10

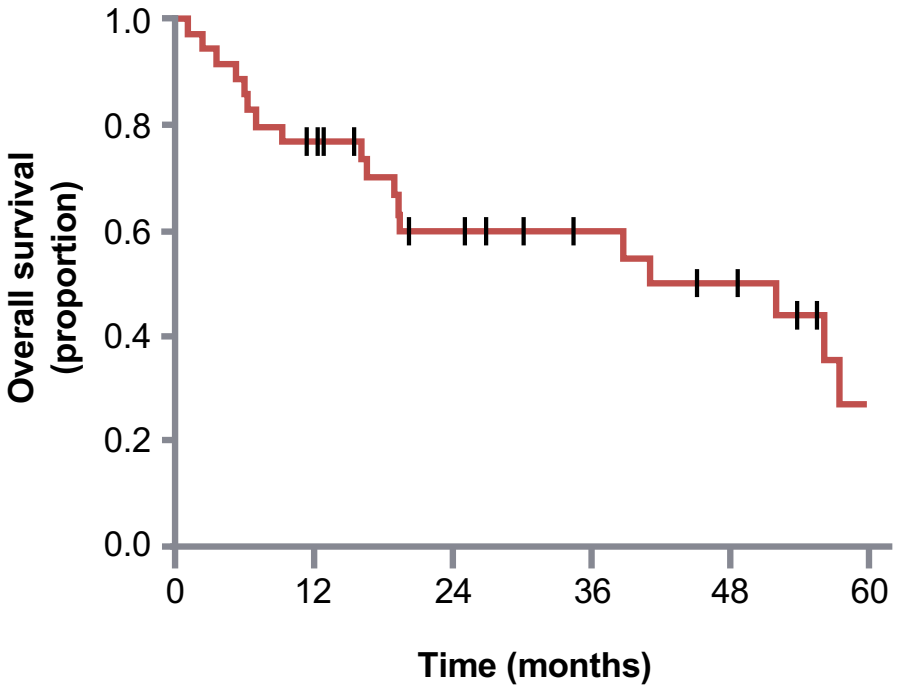
Post remission SCT is a potentially curative approach for Richter syndrome (EBMT)

Allo-SCT (n=25)



<u>Prognostic Factors</u>
Chemosensitive disease; RIC

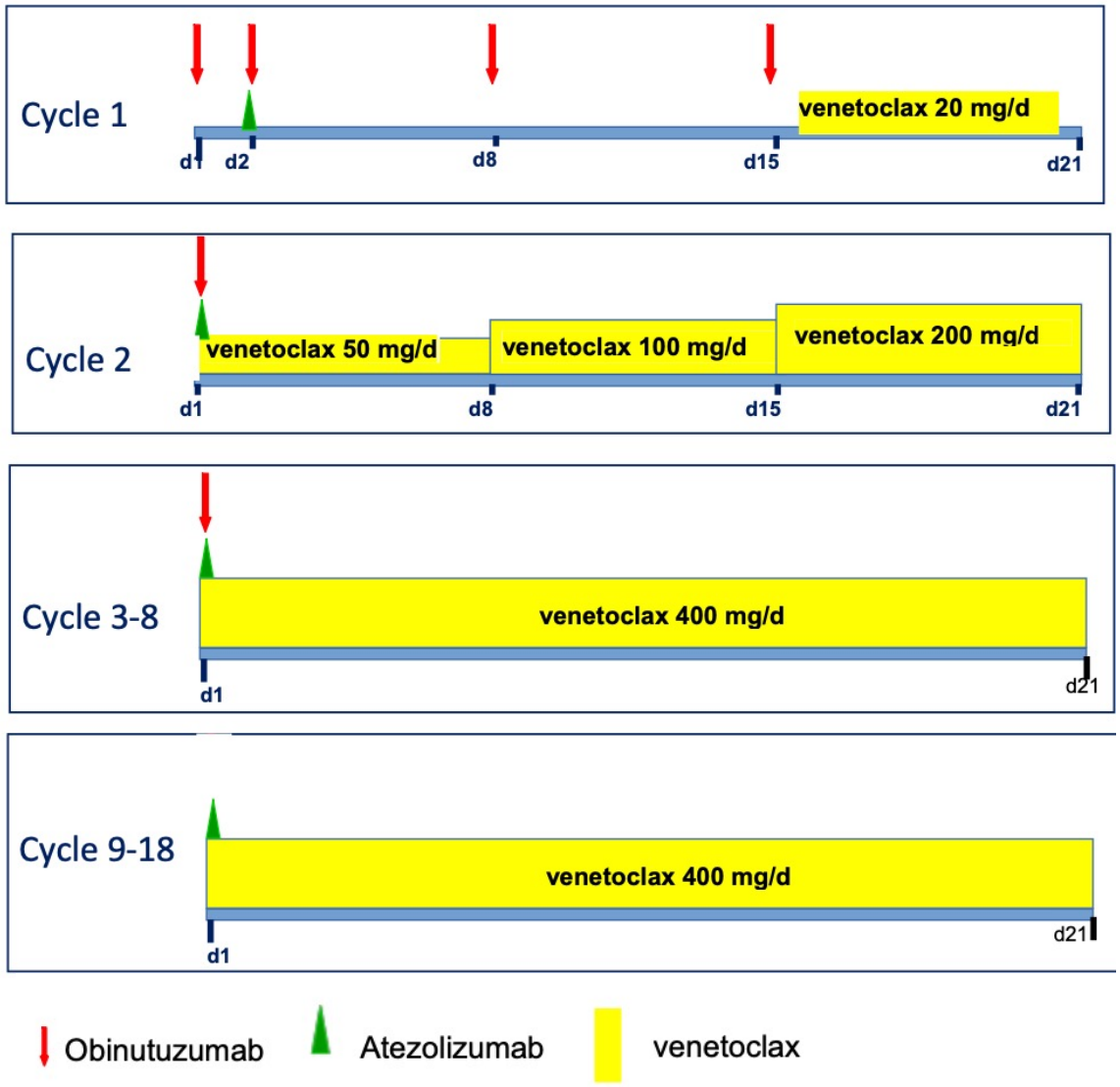
Auto-SCT (n=34)



No prognostic factors identified

Cwynarski K, *et al. J Clin Oncol* 2012; **30:** 2211–2217.

MOLTO clinical trial

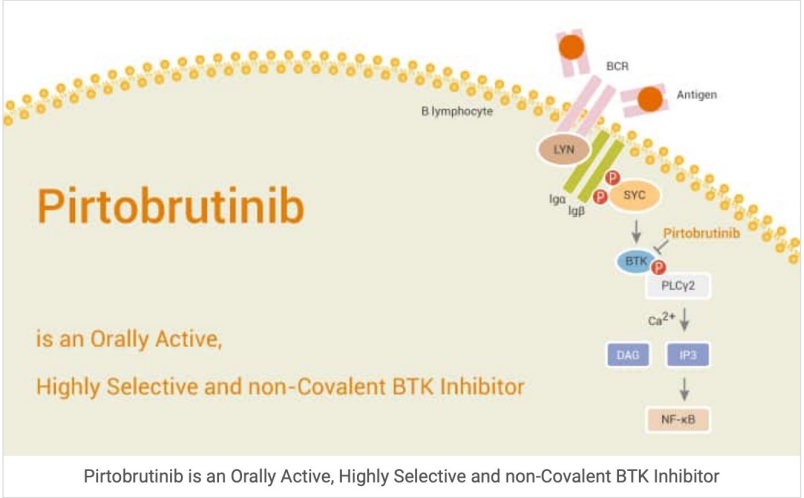


MOLTO

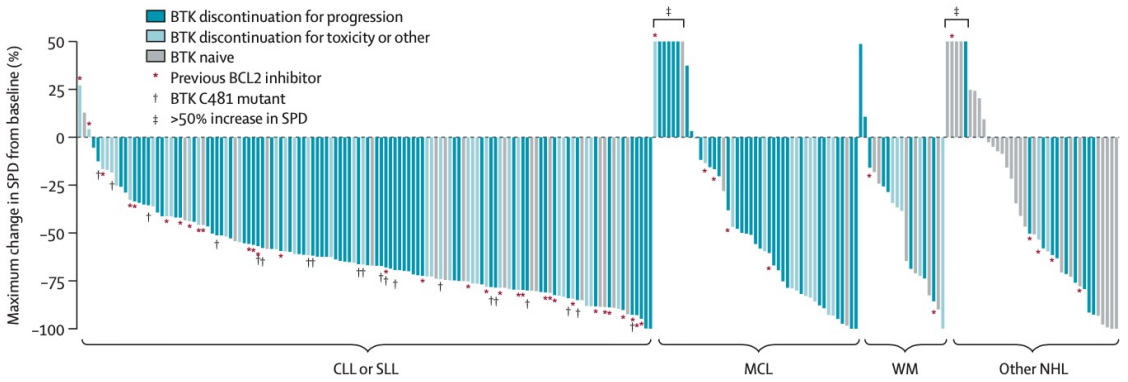
ObinutuzuMab AtezOlizumab and VenetocLax in RichTer transfOrmation

- A multi-center, open label, uncontrolled, phase II clinical trial evaluating the safety and efficacy of **atezolizumab (PD-L1 inhibitor)** in combination with **venetoclax** and **obinutuzumab** in DLBCL Richter transformation of CLL
- The primary endpoint is to asses the efficacy of the combination of venetoclax, obinutuzumab and atezolizumab in terms of overall response rate
- The planned enrolment for this study is 28 patients across Italy and Switzerland

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



Pirtobrutinib is an Orally Active, Highly Selective and non-Covalent BTK Inhibitor



ORR was 62% in CLL/SLL, and 52% in MCL

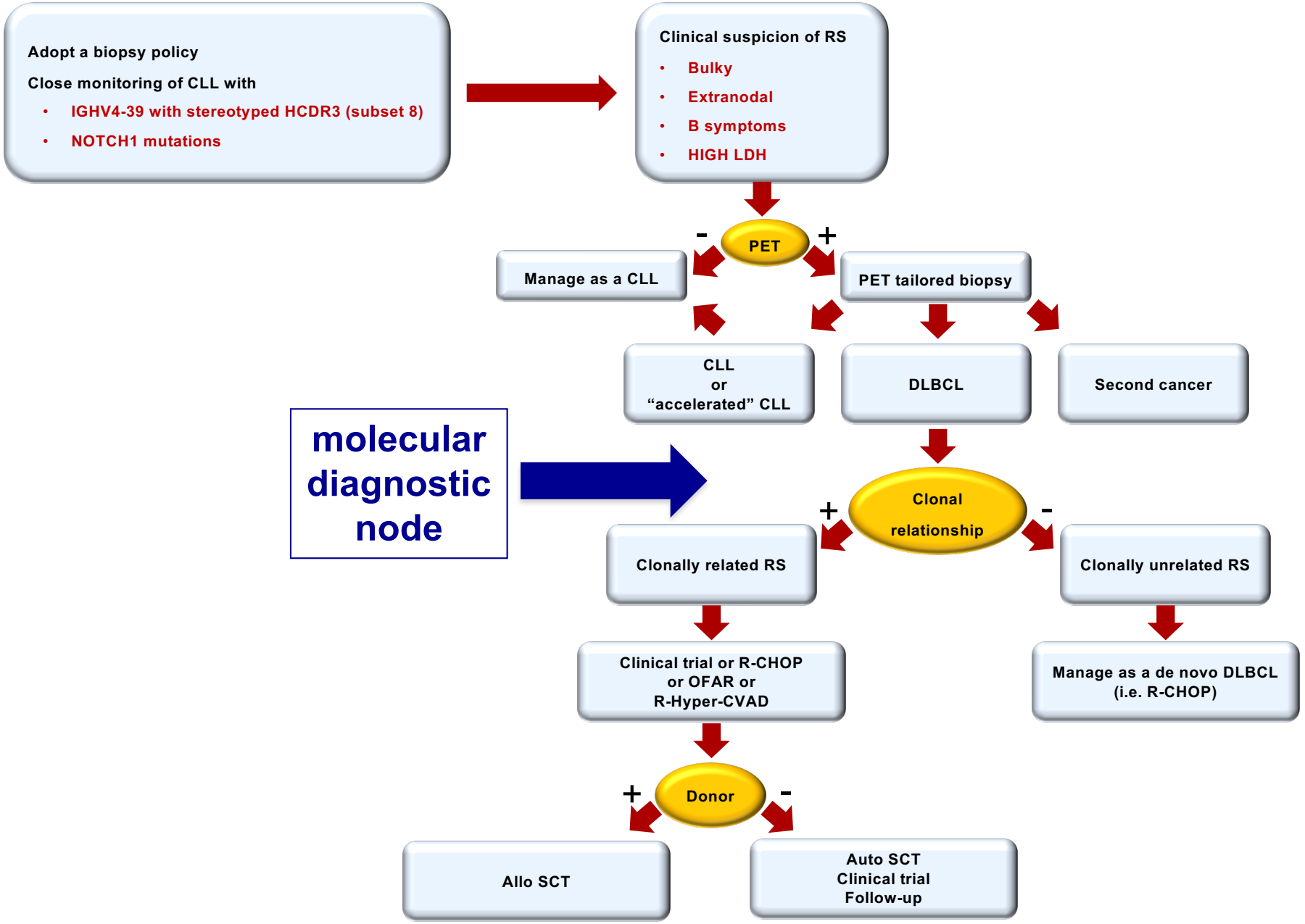
Pirtobrutinib in Richter syndrome

	Median lines of prior systemic therapy, n (IQR)	Treated, n	Efficacy-Evaluable*, n	Responders, n	ORR#, % (95% CI)	Best Response#, %
DLBCL	4 (3-5)	26	25	6	24 (9-45)	CR: 4 (16)
						PR: 2 (8)
						SD: 2 (8)
						PD: 12 (48)
						NE: 5 (20)
MZL	3 (2-5)	13	9	2	22 (3-60)	PR: 2 (22)
						SD: 7 (78)
Richter's transformation	6 (4-7)	9	8	6	75 (35-97)	PR: 6 (75)
						SD: 1 (13)
						NE: 1 (13)
B-PLL	5 (2-7)	2	2	0	0 (0-84)	SD: 1 (50)
						NE: 1 (50)
Other transformation	5 (4-8)	3	3	0	0 (0-71)	PD: 2 (67)
						NE: 1 (33)
HCL	10 (10-10)	1	0	0	0	NA

Primary Tumor Type	Prior Lines of Therapy	Prior BTK Inhibitor	Best Overall Response	Time on Treatment (months)	Treatment Status
Richter's Transformation	6	Yes	PR	2.3	Discontinued
Richter's Transformation	2	Yes	PR	7.1	Ongoing
Richter's Transformation	3	Yes	PR	6.4	Ongoing
Richter's Transformation	6	Yes	PR	2.9	Ongoing
Richter's Transformation	7	Yes	PR	3.2	Ongoing
Richter's Transformation	4	Yes	PR	2.9	Ongoing

Mato *et al, Lancet* 2021

Molecular diagnosis for the clinical management of RS



Gaidano and Rossi, Hematology 2017; Rossi and Gaidano, Blood 2018

Summing up

- The genotype of Richter syndrome sustains the clinical aggressiveness and chemorefractoriness of the disease
- A molecular workup to distinguish clonally related vs clonally unrelated cases may be useful
- In R/R CLL treated with BCR and BCL2 inhibitors, development of Richter syndrome occurs early and may reflect an aggressive clone selected by previous chemotherapy
- The outcome of Richter syndrome is still very poor and mandates the investigation of new treatment modalities
- The incidence, biology and clinical behavior of Richter syndrome in patients receiving only chemo-free regimens need to be defined

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Ministero della Salute
NOVARAIL

Richter syndrome vulnerabilities empirically probed in vivo in patients

	Patients	Regimen	ORR	CR	PFS (Mo)	TRM
Hillmen, 2016	29	Acalabrutinib	38%	14%	3	-
Tsang, 2016	4	Ibrutinib	75%	25%	-	-
Ding, 2016	9	Pembrolizumab	44%	11%	-	-
Jain, 2016	3	Nivolumab + Ibrutinib	50%		-	-
Davids, 2017	7	Venetoclax	43%	0%	-	-