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In search of new markers in chronic lymphocytic leukemia and lymphoma

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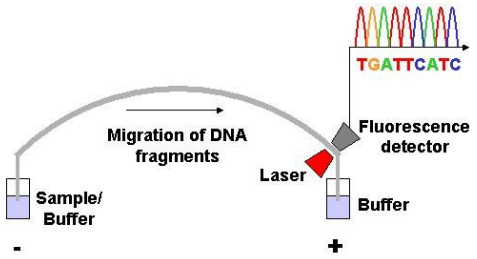
Decoding the genome of human cancers

The availability of the human genome sequence has raised the possibility that DNA sequencing could become the primary tool to explore cancer genomes

Sanger sequencing → Candiate gene approach (human bias)

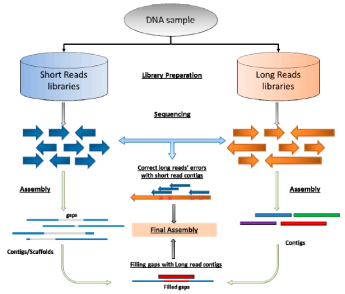


Based on amplification of the DNA fragment to be sequenced by DNA polymerase and incorporation of modified nucleotides

Allows only a *single-gene approach* and *limited sensitivity*

Since the early 1990 has dominated the "sequencing scenario"

Next Generation Sequencing (NGS) → Whole genome ("unbiased")



Is a *high-throughput* technology that parallelizes the sequencing process, producing thousands or millions of sequences at once

Many different methods have been developed

Allows a *genome-wide approach and high sensitivity*

Transformed cancer genomics

Shendure J *Nat Biotech* 2008; Meyerson M *Nat Gen Rev* 2010; Metzker M *Nat Rev Gen* 2010

NGS reveals potential new therapeutic targets in lymphoid malignancies

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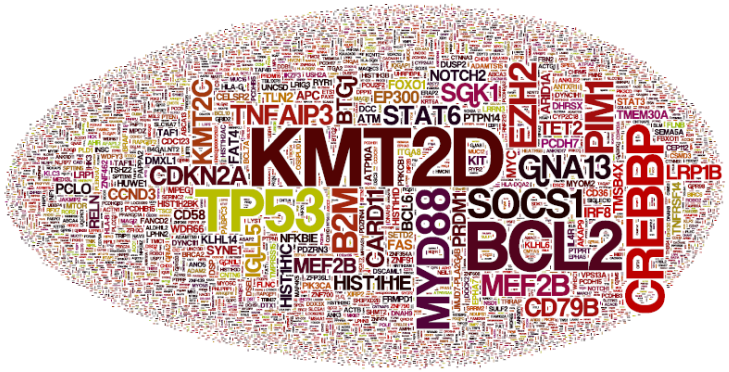
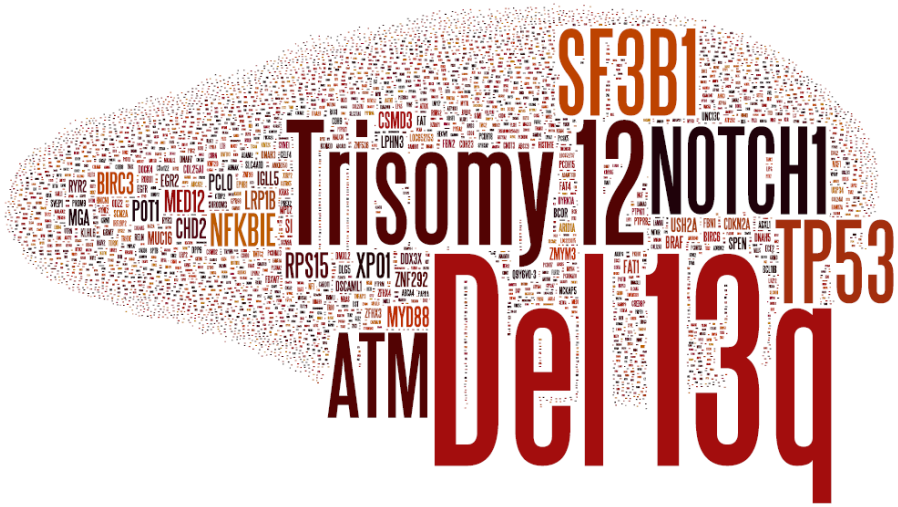
DLBCL



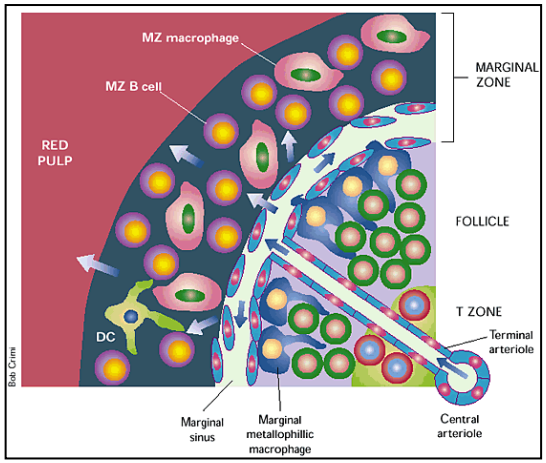
cHL

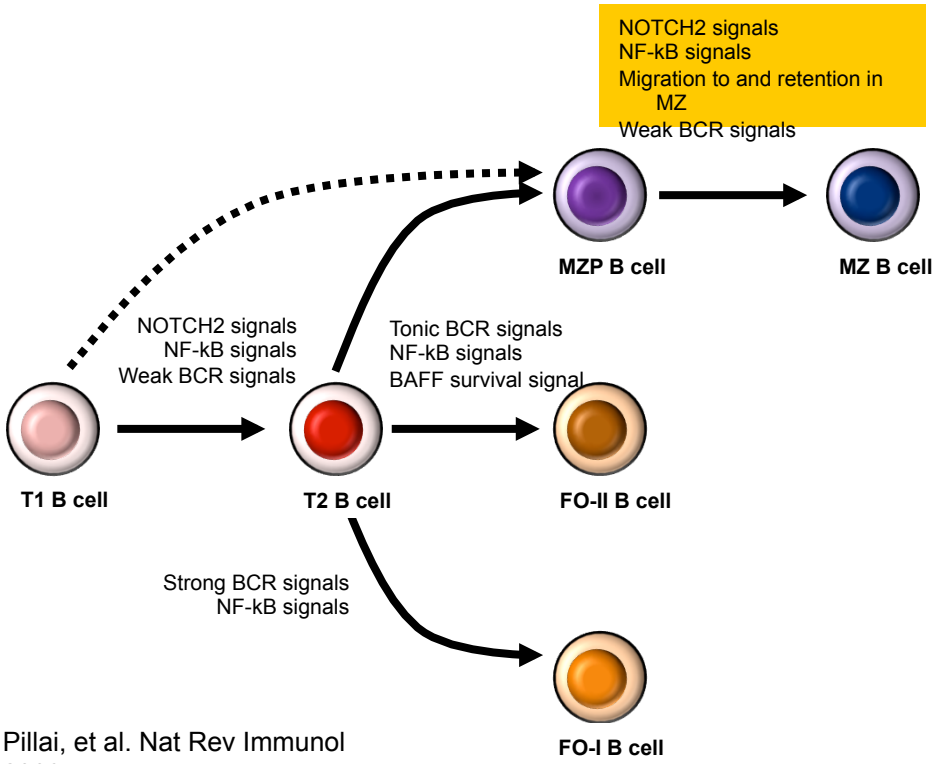
CLL



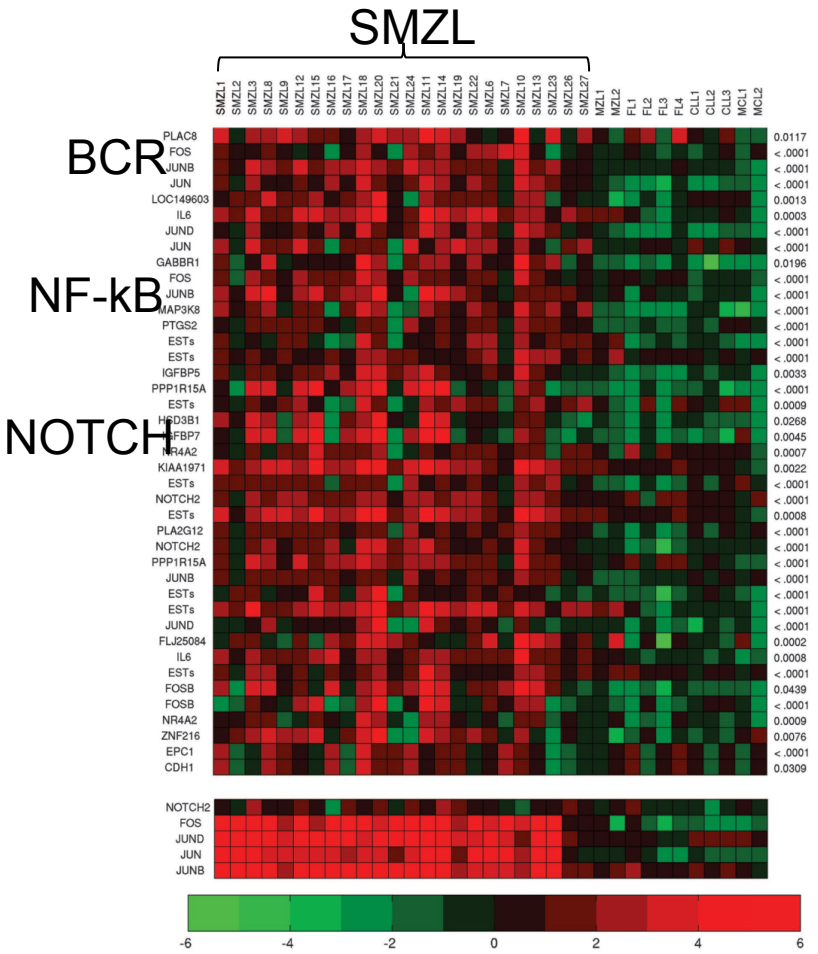
Pasqualucci L, *et al., Semin Hematol* 2015; Reichel J, *et al., Blood* 2015; Landau et al, Nature 2015; Puente et al, Nature 2015

Gene expression profiling allows the identification of potential targets not otherwise identified by candidate approaches



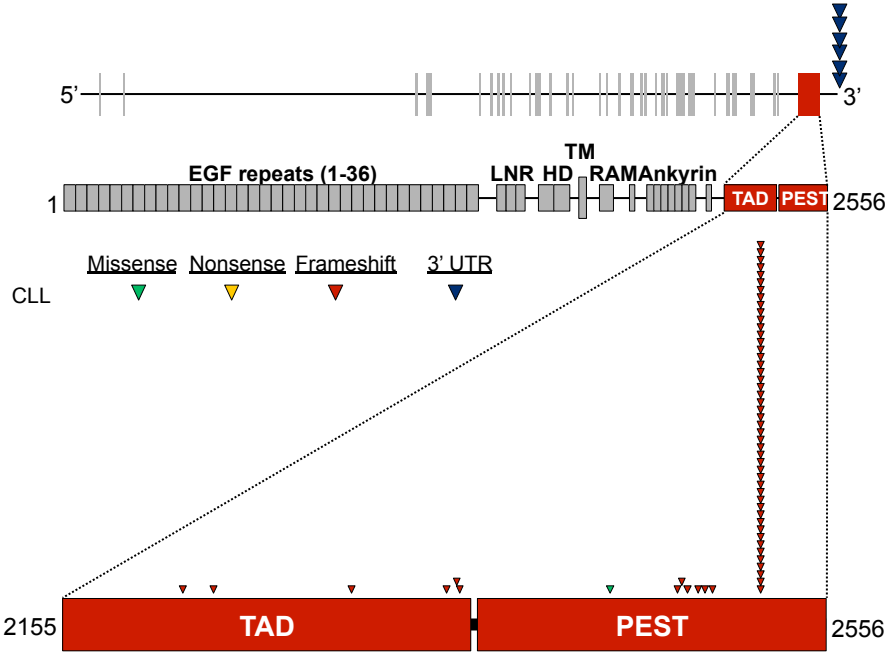


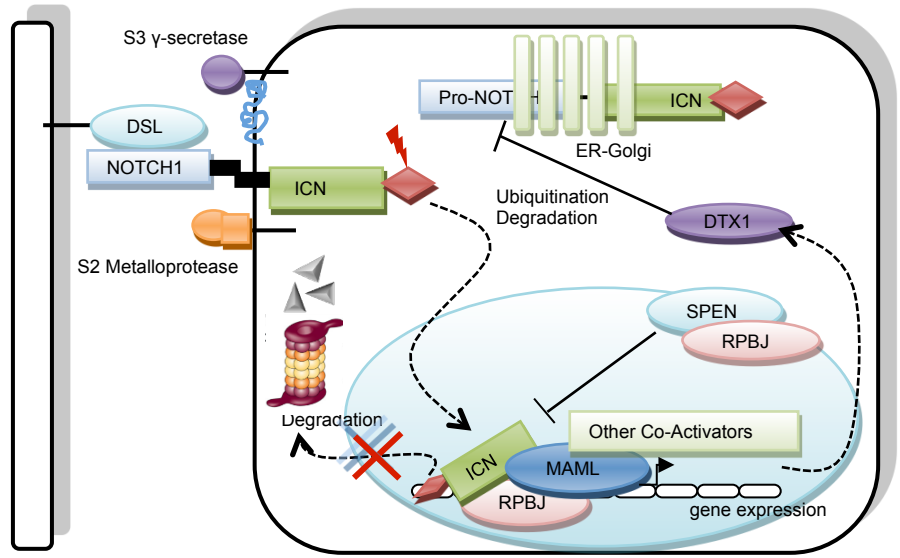
Pillai, et al. Nat Rev Immunol



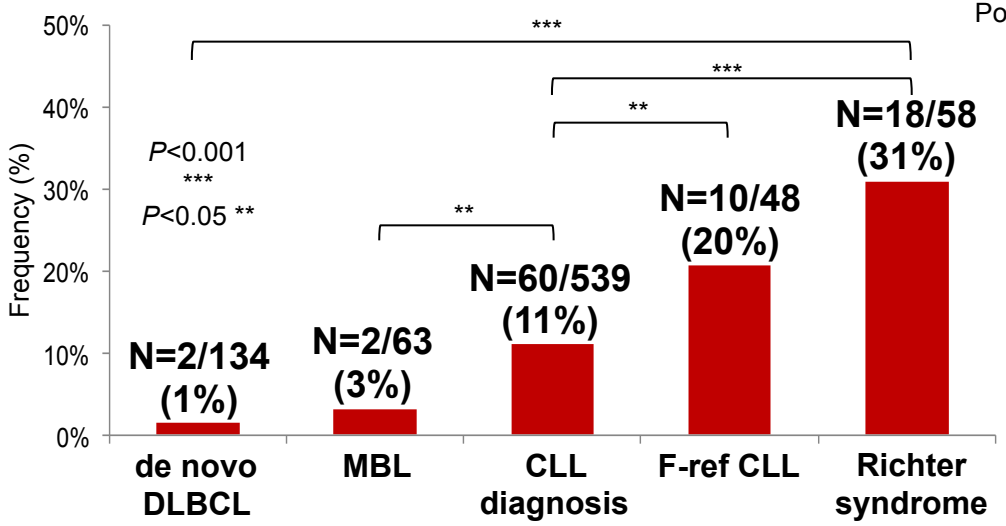
Trøen G, et al. J Mol Diag 2004
Ruiz-Ballesteros E, et al. Blood

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

Targeting NOTCH1 with Brontictuzumab

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- A phase I study for patients with previously treated CLL, MCL, DLBCL, anaplastic large cell lymphoma, transformed mycosis fungoides, Sezary Syndrome, T-cell acute lymphoblastic leukemia, or other hematologic malignancies with known NOTCH1 mutations

- 24 patients were enrolled and 23 have been treated in 4 dose escalation cohorts at doses of 0.25 mg/kg every 4 weeks (Q4W), 0.5 mg/kg Q4W, 1 mg/kg Q4W, and 1 mg/kg every 2 weeks

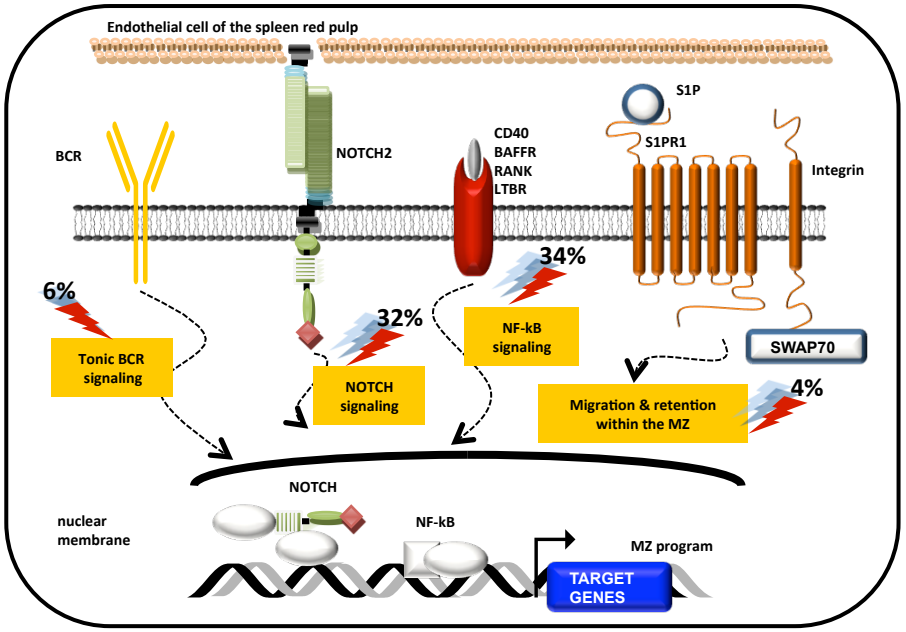
- The most frequent treatment-related adverse events of any grade were: diarrhoea (22%), fatigue (17%), anemia (13%), abdominal pain (9%), nausea (9%), vomiting (9%)

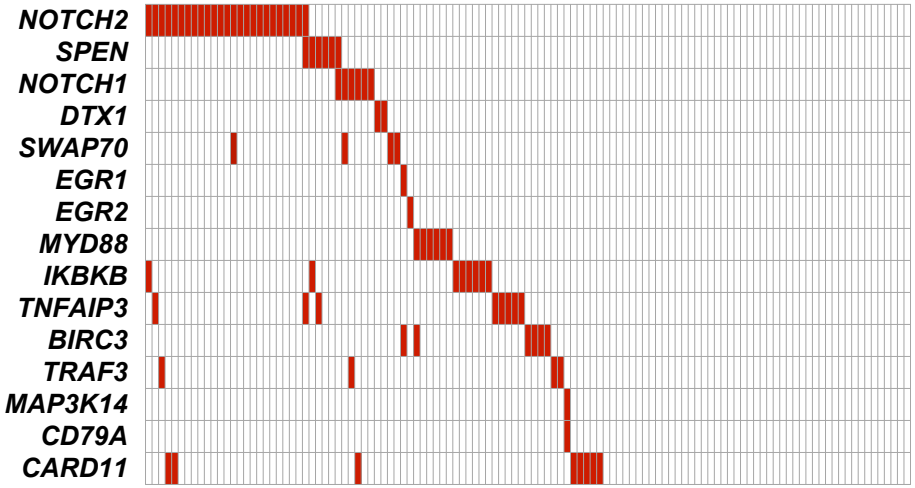
- One patients with transformed mycosis fungoides had partial response to treatment, after receiving 1 mg/kg Q2W. Two additional pts had stable disease as best overall response (1 with MCL, and 1 with TMF)

- The mAb is well tolerated and has moderate antitumor activity

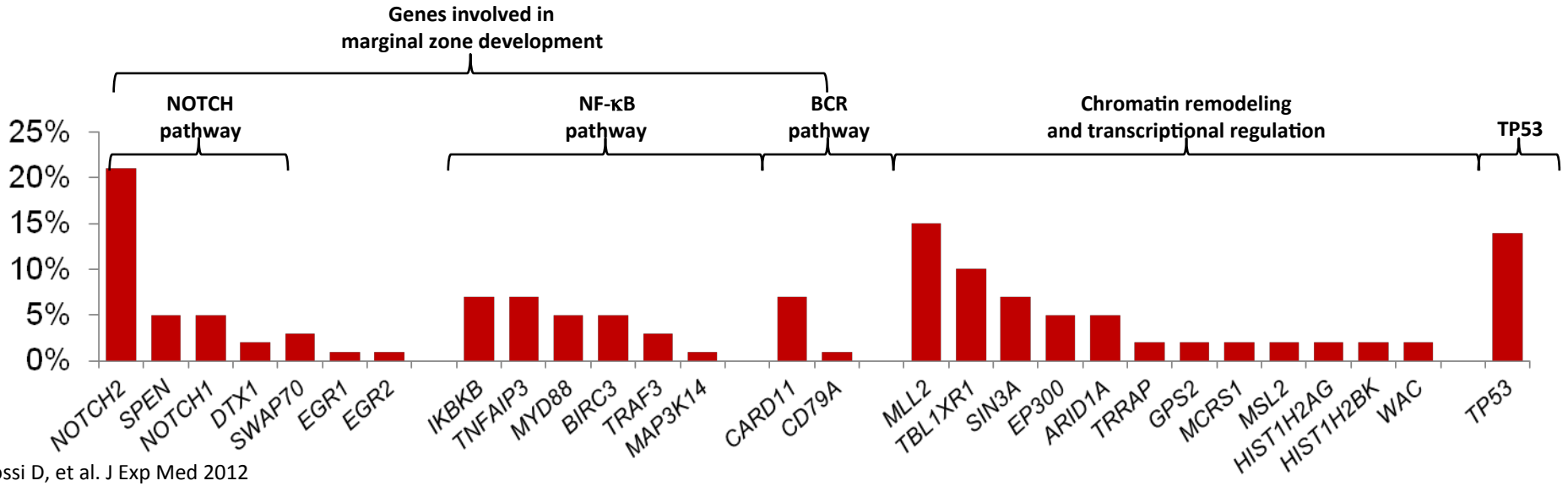
Casulo et al, ASH 2016

Mutations of genes regulating MZ development characterize ~60% SMZL



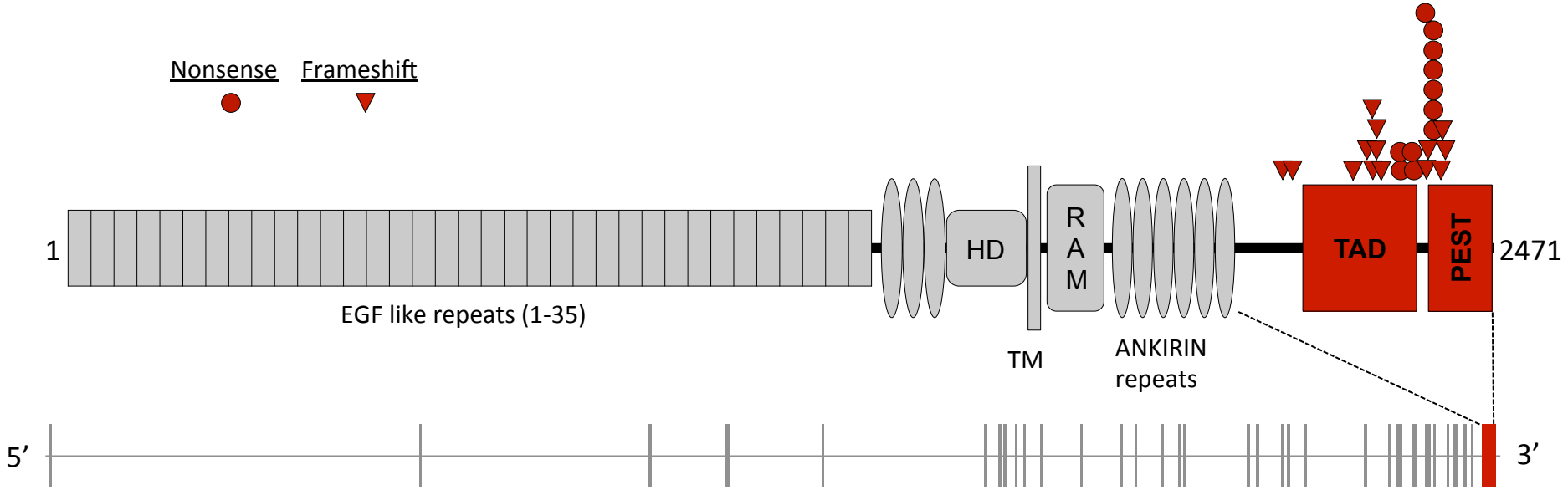


SMZL (n=117)

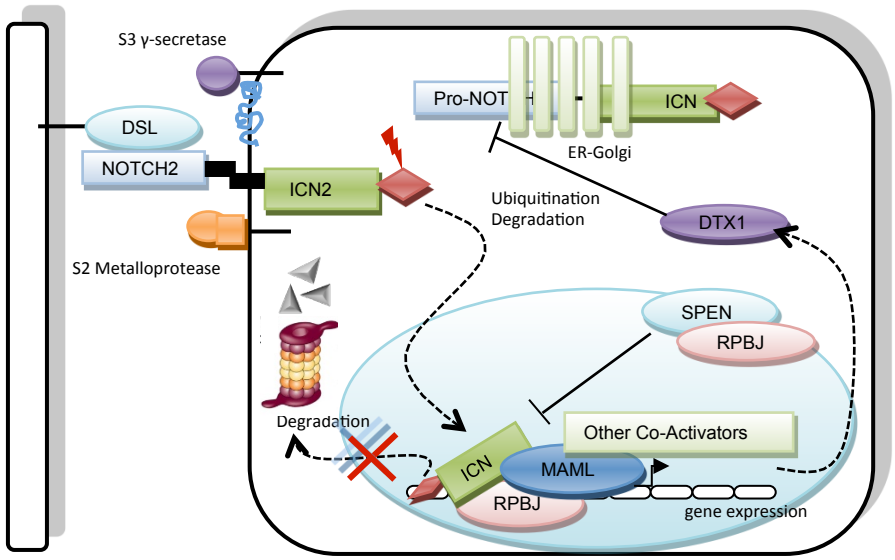


Rossi D, et al. J Exp Med 2012

NOTCH2 is the most frequently mutated gene (~20%) in SMZL



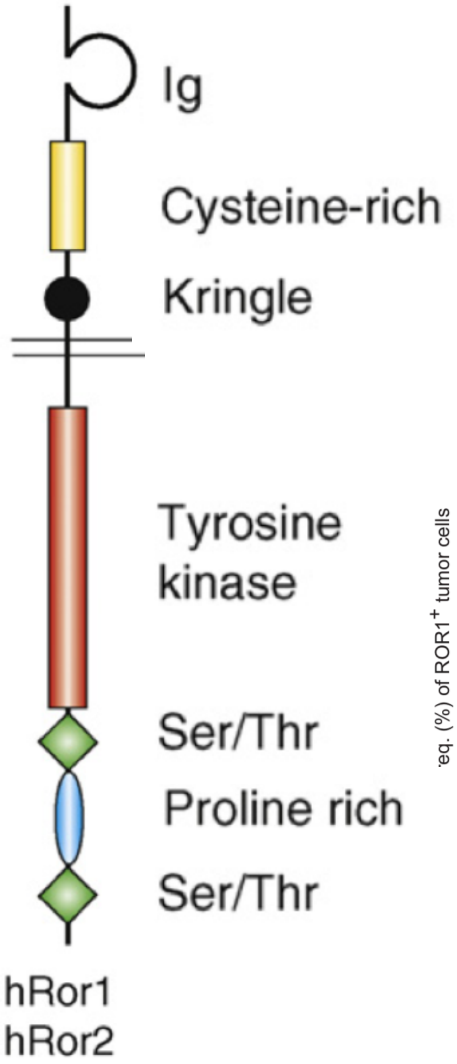
- Mutated SMZL=25/117 (21.3%)
- Hotspot in exon 34
- All truncating mutations (14 indels; 11 nonsense)
- Recurrent p.R2400* (6/25, 24% mutations)
- Somatic in all instances



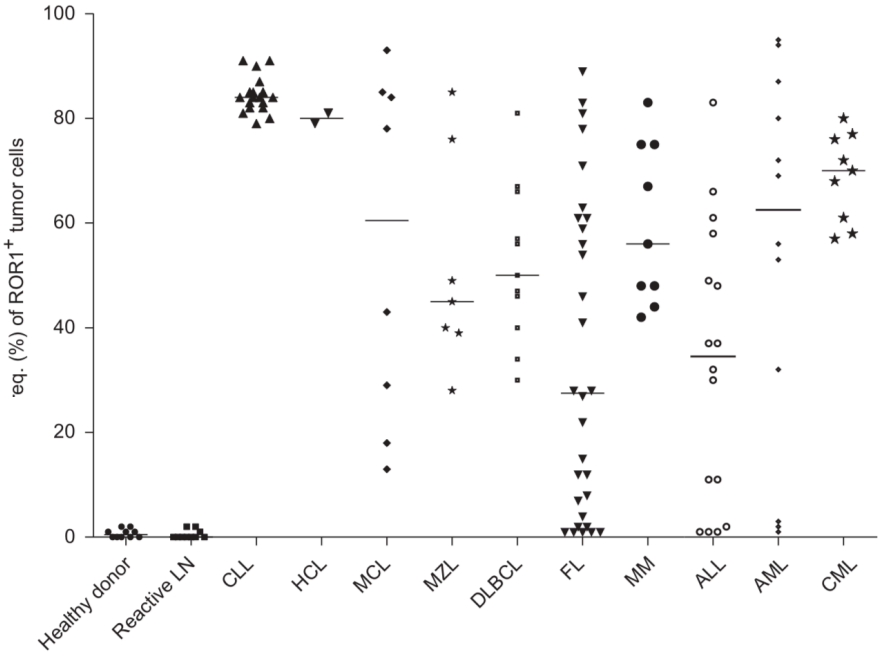
Predicted functional consequence of *NOTCH2* mutations

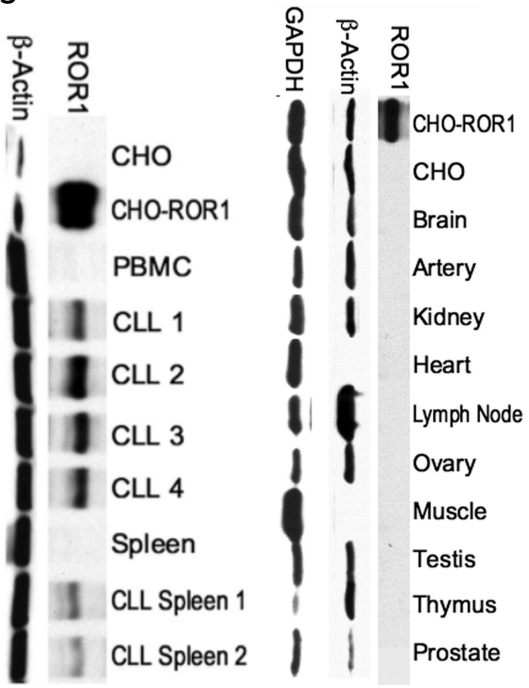
Rossi D, et al. J Exp Med. 2012

Receptor tyrosine kinase-like orphan receptor 1 (ROR1)



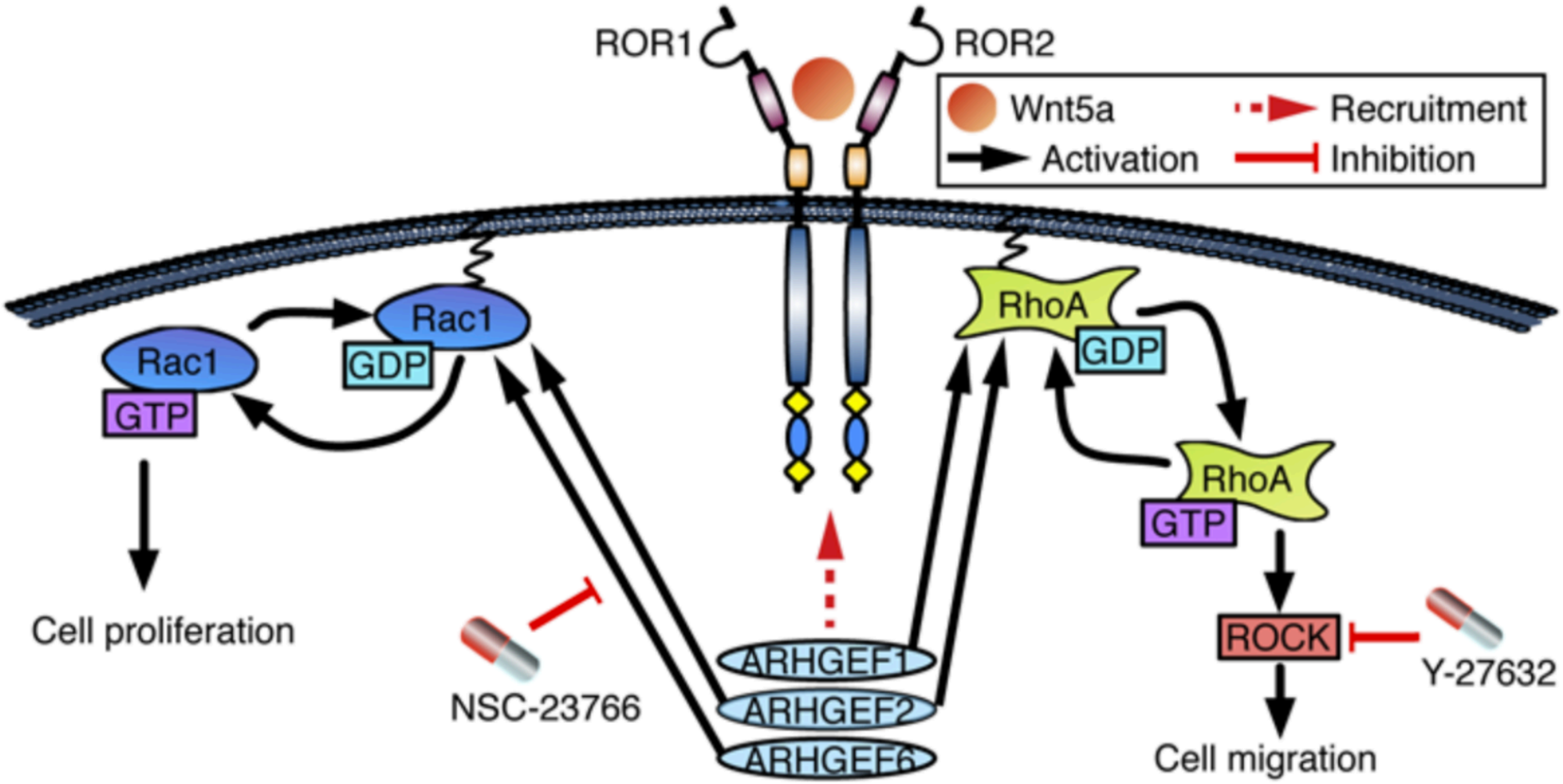
- Evolutionarily conserved, type-I membrane protein serving as receptor for Wnt5a
- Has a tyrosine-kinase-like and Ser/Thr-rich domains
- Expressed primarily during embryogenesis (oncoembryonic protein)
- ROR1 is expressed on nearly all cases of CLL
- ROR1 is NOT expressed on CD5 B cells of healthy adults, in normal adult tissues and in hematopoietic stem cells





Fukuda, *et al., PNAS*. 2014; Zhang *et al., Am J Pathol*. 2012

ROR1 pathway



Ju *et al., J Clin Invest.* 2016

ROR1 expression promotes CLL cell growth and affects CLL survival

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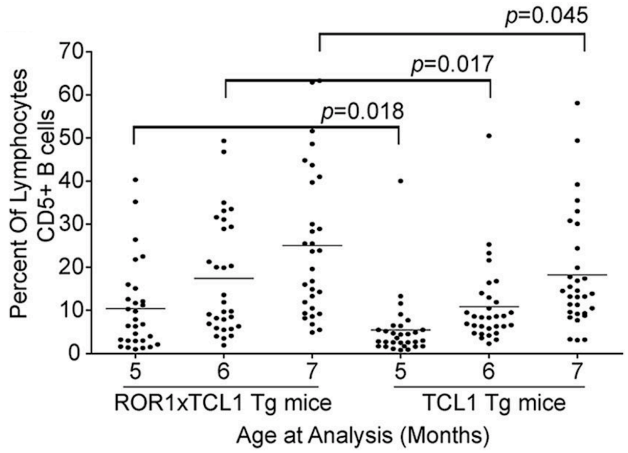
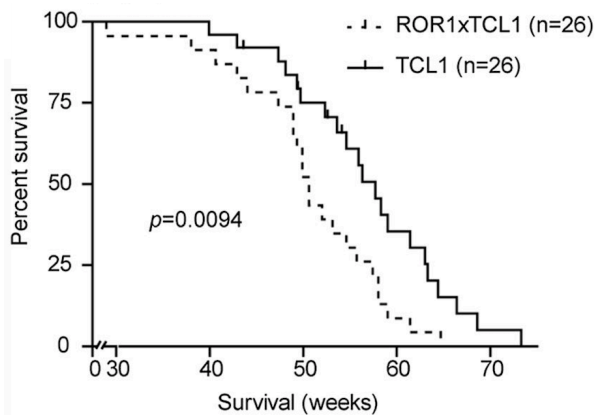
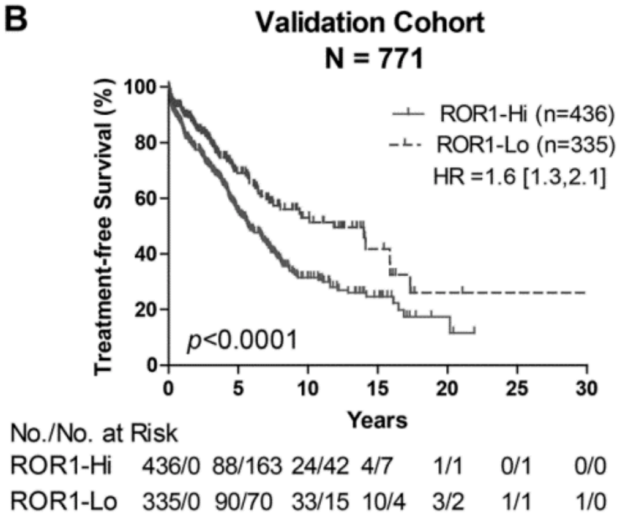
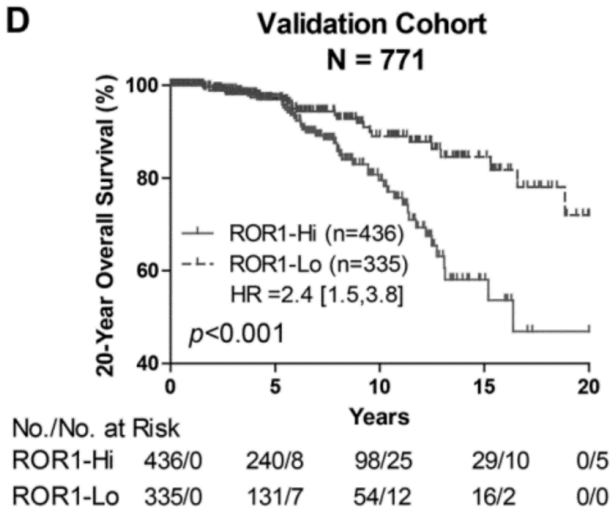
ROR1 enhances CLL growth in the TCL1 model





ROR1 expresssion affects CLL outcome





Widhopf II, *et al., PNAS*. 2014; Zhang *et al., Am J Pathol*. 2012; Cui *et al., Blood* 2016

Immunologic targeting of ROR1

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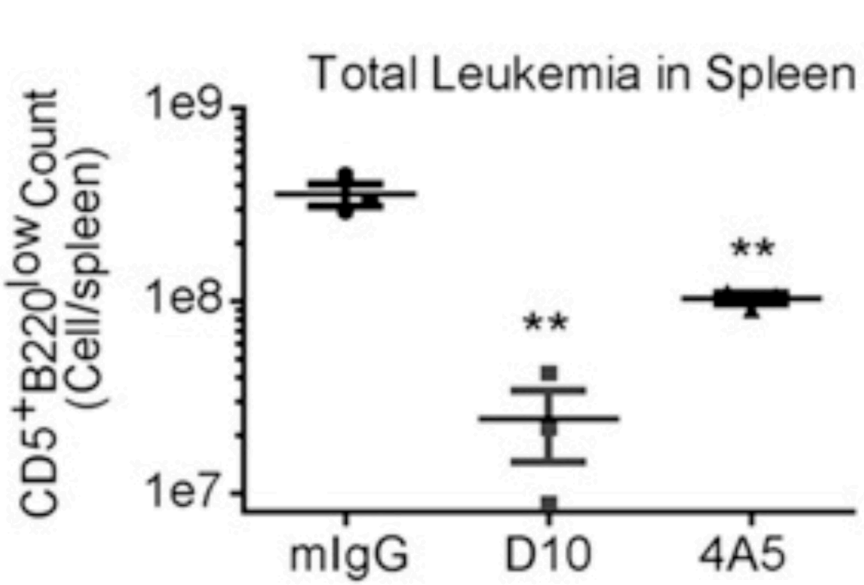
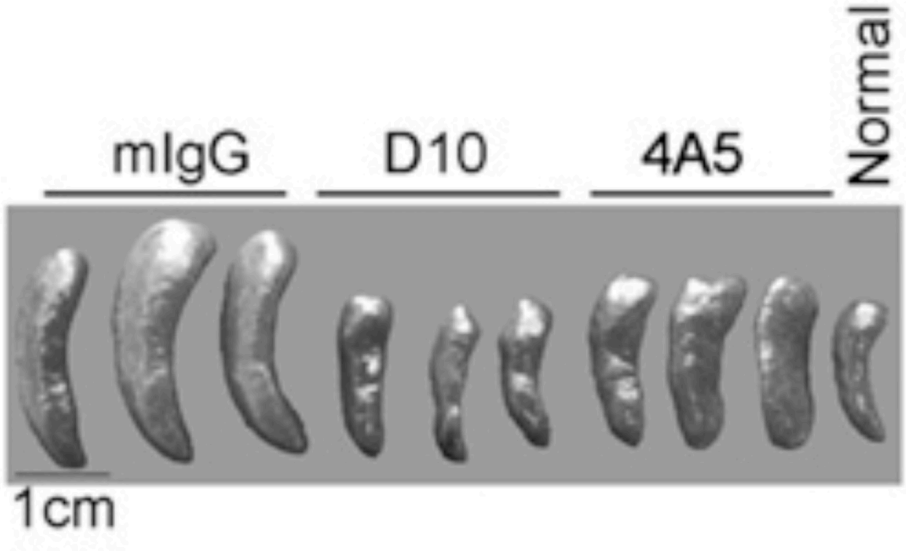
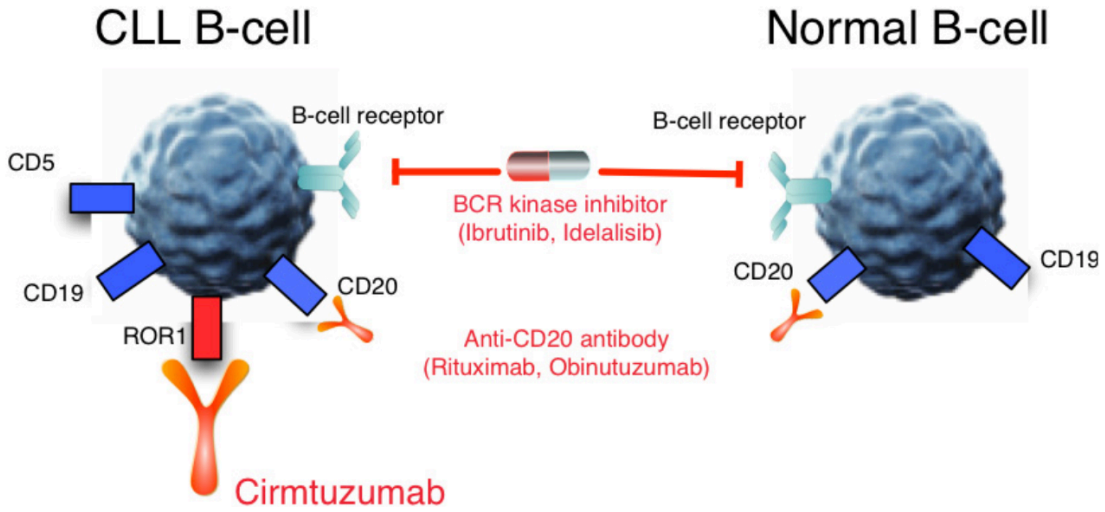
Anti-ROR1 mAbs reduce spleen size in ROR1-TCL1 animal models





Cirmtuzumab (UC-961): a humanized IgG1 monoclonal antibody able to block ROR1 signaling



Widhopf II, *et al., PNAS*. 2014

Cirmtuzumab phase 1 trial in R/R CLL

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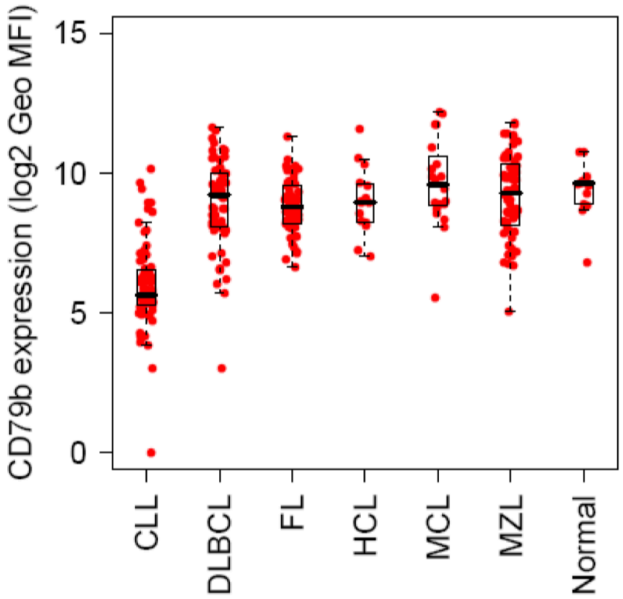
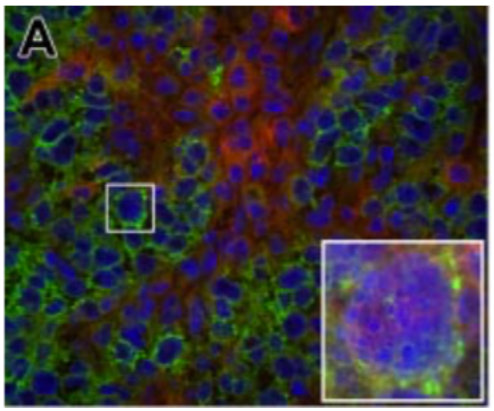
- Dose-escalation trial in patients with relapsed/refractory CLL
- 25 patients were enrolled and received four bi-weekly infusions of cirmtuzumab at doses ranging from 0.015 to 20mg/kg
- Cirmtuzumab was safe and well-tolerated. There were no drug-related SAE, or infusion-related reactions
- Pharmacokinetics studies demonstrated that at higher doses was 32.4 days (SD 1.9 days) and cirmtuzumab levels remained detectable in the plasma until approximately 3 months following the final infusion
- 16 of 19 evaluable patients had stable disease 2 months after the final infusion of the drug and the median time to requiring next treatment due to progressive disease was 259 days

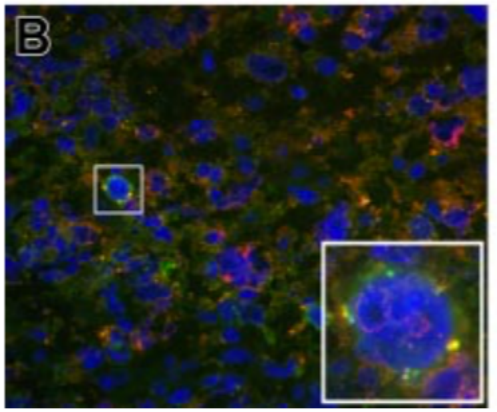
Choi *et al., Blood*. 2017

The CD79b antigen

- CD79b is a cell-surface antigen is expressed in all mature B cells except plasma cells
- It is expressed in a majority of B cell malignancies, including nearly all NHL and CLL
- Relating specifically to DLBCL, CD79b is expressed in essentially all tumor cells, enabling its use in all subtypes of DLBCL
- Antibodies bound to CD79b are rapidly internalized, which makes CD79b ideally suited for targeted delivery of cytotoxic agents

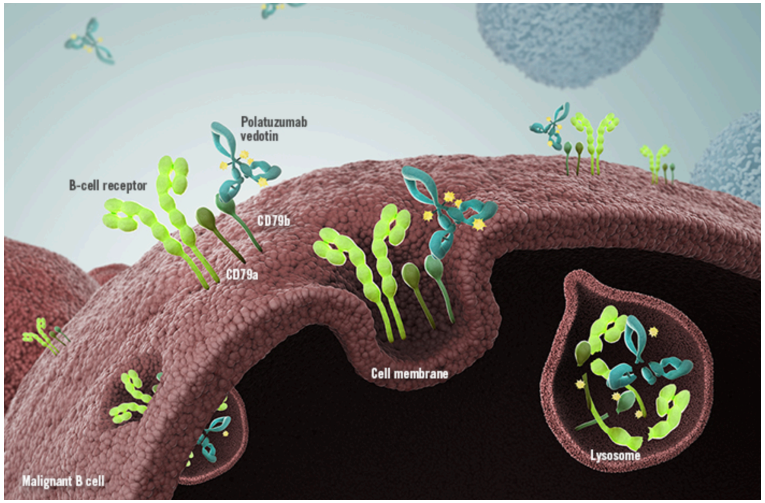




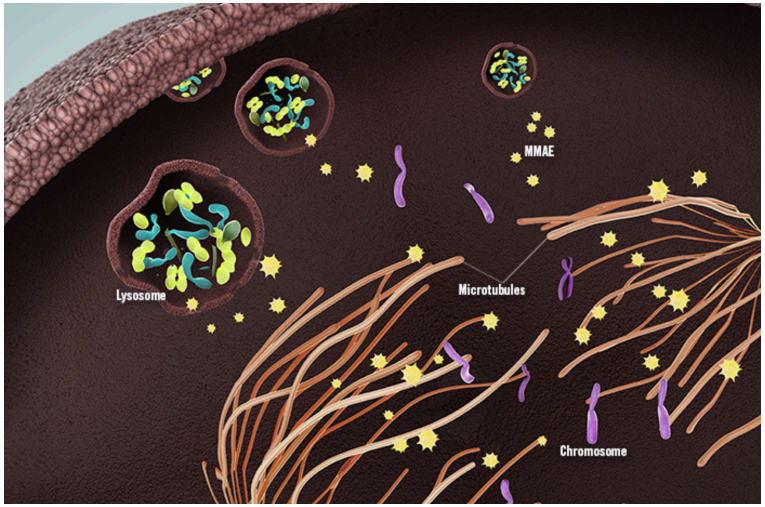


Olejniczak *et al., Immunol Invest*. 2006; Polson *et al., Blood*. 2007

Polatuzumab Vedotin



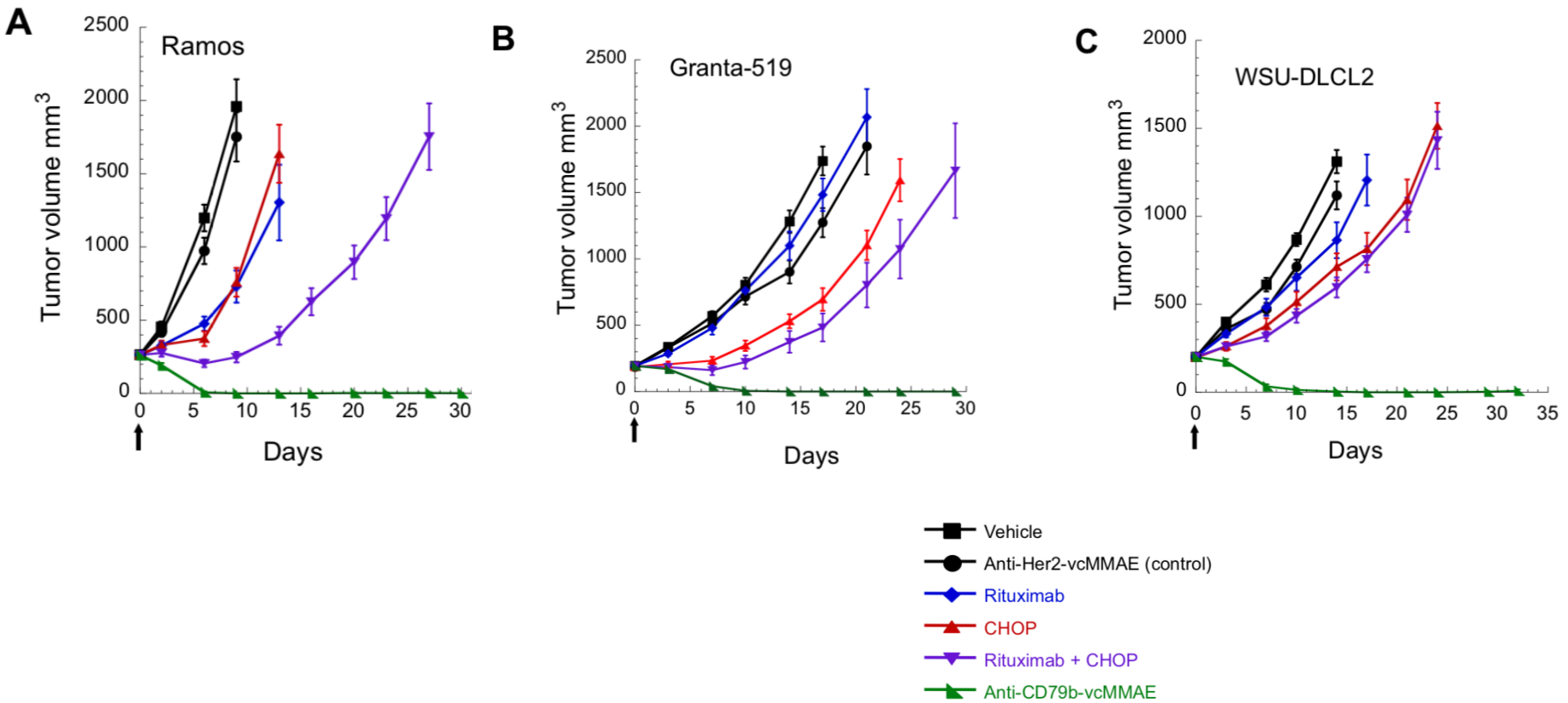
- Polatuzumab vedotin is an antibody-drug conjugate (ADC)
- It contains a humanized IgG1 anti-human CD79b monoclonal antibody and a potent anti-mitotic agent, mono-methyl auristatin E (MMAE), linked through a protease-labile linker, maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl.



- MMAE then binds to tubulin and disrupts the microtubule network, resulting in inhibition of cell division and cell growth

Polatuzumab Vedotin: preclinical activity

The administration of the anti–CD79b-vcMMAE induces sustained complete tumor remission in xenografts, whereas R-CHOP slows tumor growth or decreases tumor volumes



Dornan *et al., Blood*. 2009

Polatuzumab Vedotin phase 1 clinical trial for NHL or CLL not suitable for a curative therapy

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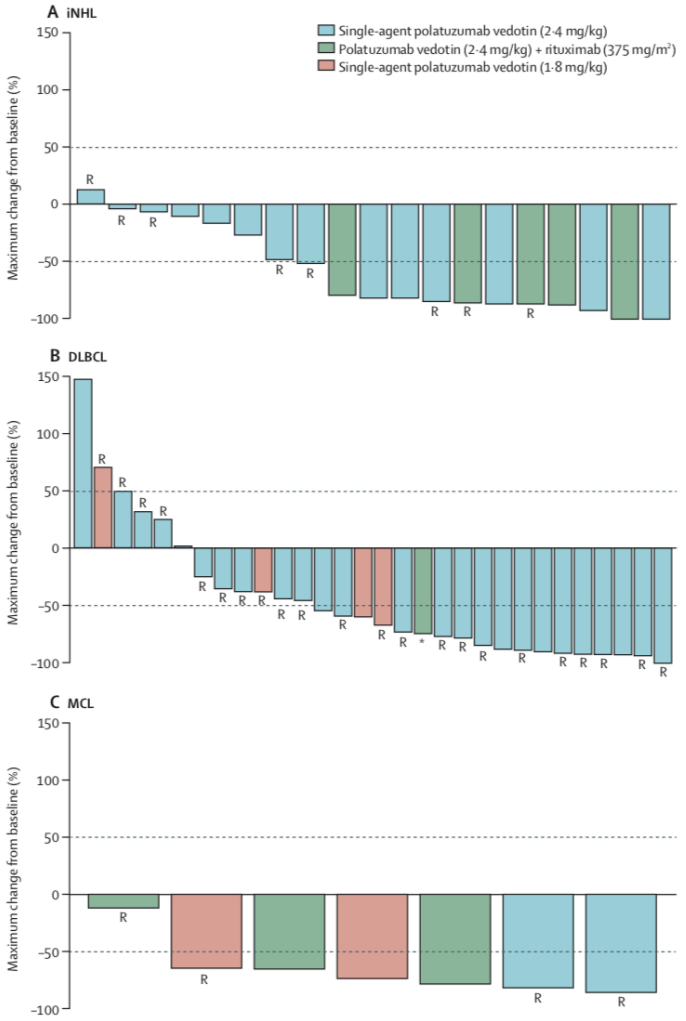
- 95 patients
- The most common grade 3-4 adverse events were neutropenia (40%), anaemia (11%), and peripheral sensory neuropathy (9%)
- Objective responses were noted in 23 of 42 activity-evaluable patients with NHL given single-agent polatuzumab vedotin (14 of 25 with DLBCL, seven of 15 with indolent NHL, and two with MC
- No objective responses were observed in patients with CLL

	NHL*			Chronic lymphocytic leukaemia		NHL treated with combination (n=9)‖
	<1·8 mg/kg (n=17)†	1·8 mg/kg (n=6)	2·4 mg/kg (n=45)§	<1·8 mg/kg (n=13)‡	1·8 mg/kg (n=5)	
Neutropenia						
Grade 1–2	0	1 (17%)	2 (4%)	1 (8%)	0	1 (11%)
Grade 3	4 (24%)	1 (17%)	11 (24%)	1 (8%)	0	1 (11%)
Grade 4	1 (6%)	2 (33%)	7 (16%)	2 (15%)	0	4 (44%)
Diarrhoea						
Grade 1–2	4 (24%)	2 (33%)	18 (40%)	2 (15%)	1 (20%)	4 (44%)
Grade 3	0	1 (17%)	1 (2%)	0	2 (40%)	0
Grade 4	0	0	1 (2%)	0	0	0
Pyrexia						
Grade 1–2	5 (29%)	2 (33%)	12 (27%)	3 (23%)	4 (80%)	4 (44%)
Grade 3	0	0	1 (2%)	0	0	1 (11%)
Nausea						
Grade 1–2	5 (29%)	3 (50%)	16 (36%)	1 (8%)	1 (20%)	6 (67%)
Peripheral sensory neuropathy						
Grade 1–2	4 (24%)	3 (50%)	12 (27%)	0	0	6 (67%)
Grade 3	1 (6%)	1 (17%)	3 (7%)	0	0	0
Grade 4	0	0	1 (2%)	0	0	0
Fatigue						
Grade 1–2	5 (29%)	2 (33%)	5 (11%)	2 (15%)	1 (20%)	2 (22%)
Grade 3	2 (12%)	1 (17%)	0	2 (15%)	0	1 (11%)
Anaemia						
Grade 1–2	2 (12%)	0	4 (9%)	3 (23%)	0	0
Grade 3	0	1 (17%)	5 (11%)	1 (8%)	0	1 (11%)
Grade 4	0	0	0	0	1 (20%)	1 (11%)



Palanca-Wessels *et al., Lancet*. 2015

Polatuzumab Vedotin phase 1b/2 clinical trial

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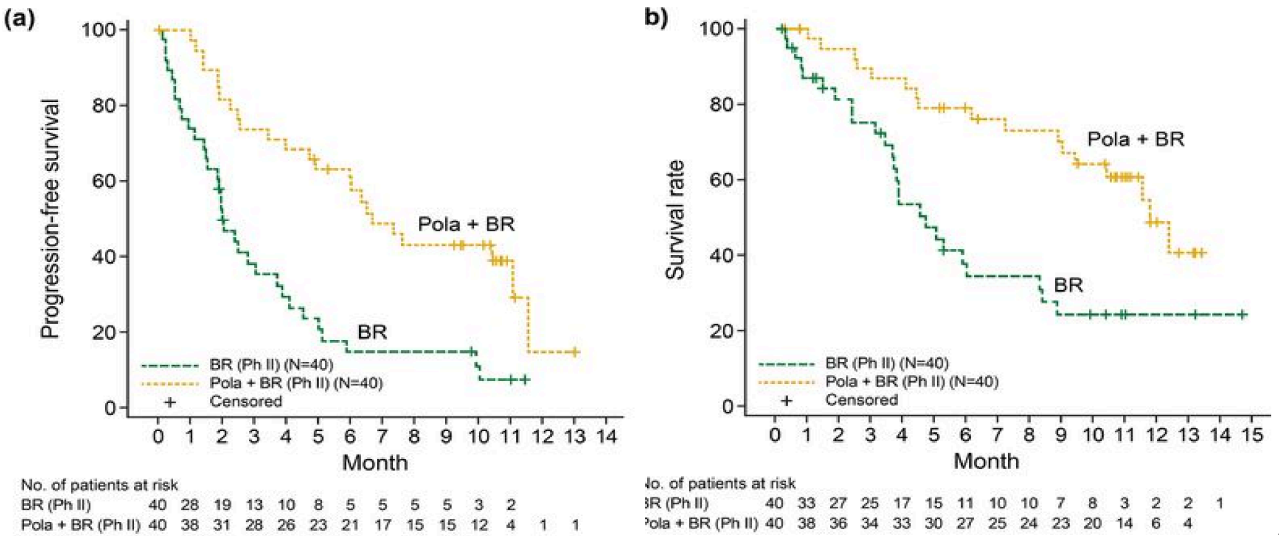
Addition of Polatuzumab Vedotin to Bendamustine and Rituximab (BR) Improves Outcomes in Transplant-Ineligible Patients with R/R DLBCL Versus BR Alone

	Pola + BR (n=40)		BR (n=40)	
	Best	PRA	Best	PRA
Overall response rate CR PR	28 (70) 23 (58) 5 (13)	19 (48) 17 (43) 2 (5)	13 (33) 8 (20) 5 (13)	7 (18) 6 (15) 1 (3)
SD	1 (3)	0	2 (5)	1 (3)
PD	7 (18)	13 (33)	22 (55)	26 (65)
Missing/UE	4 (10)	8 (20)	3 (8)	6 (15)
Median duration of response, mo (95% CI)	8.8 (4.5, NR)		3.7 (2.6, 7.8)	
Median PFS, mo (95% CI)	6.7 (4.9, 11.1)		2.0 (1.5, 3.7)	
stratified HR (95% CI)[1] p-value[2]	0.31 (0.18, 0.55) <0.0001			
Median OS, mo (95% CI)	11.8 (9.5, NR)		4.7 (3.7, 8.3)	
stratified HR (95% CI)[1] p-value[2]	0.35 (0.19, 0.67) 0.0008			



Sehn *et al., Blood*. 2017

Polatuzumab Vedotin phase 1b/2 clinical trial

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Polatuzumab Vedotin Combined with Obinutuzumab, Cyclophosphamide, Doxorubicin, and Prednisone (G-CHP) for Patients with Previously Untreated (DLBCL)

- 21 patients with previously untreated DLBCL had been enrolled to receive G-CHP + pola at 1.8 mg/kg every 21 days for a total of 6 or 8 cycles
- The most common grade 3/4 adverse events were neutropenia (38%), anemia (14%) and thrombocytopenia (14%
- Efficacy assessed at end of treatment by PET-CT, demonstrated overall response of 91% with 81% CR and 10% PR

Common Adverse Events (all grades), n (%)		Grade 3/4 Adverse Events > 1 patient, n (%)	
Fatigue	14 (67)	Neutropenia	8 (38)
Diarrhea	12 (57)	Febrile neutropenia	7 (33)
Nausea	12 (57)	Thrombocytopenia	3 (14)
Neutropenia	10 (48)	Anemia	3 (14)
Alopecia	8 (38)	Leukocytosis	3 (14)
Febrile neutropenia	7 (33)	Hypokalemia	3 (14)
		Pancytopenia	2 (10)
		Pneumonia	2 (10)

Forero-Torres *at al., Blood*. 2017

The CD37 antigen

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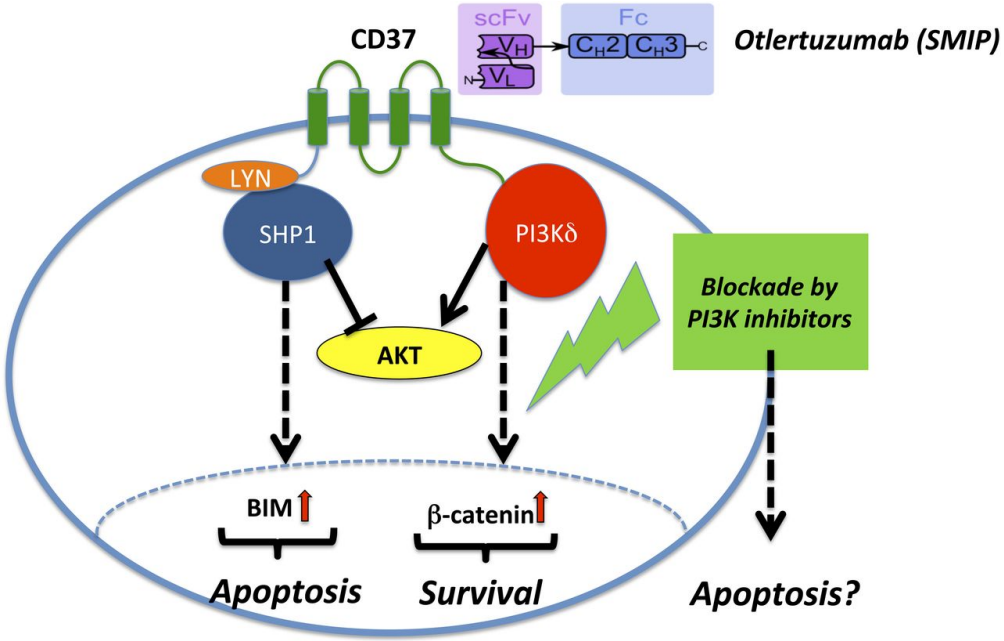
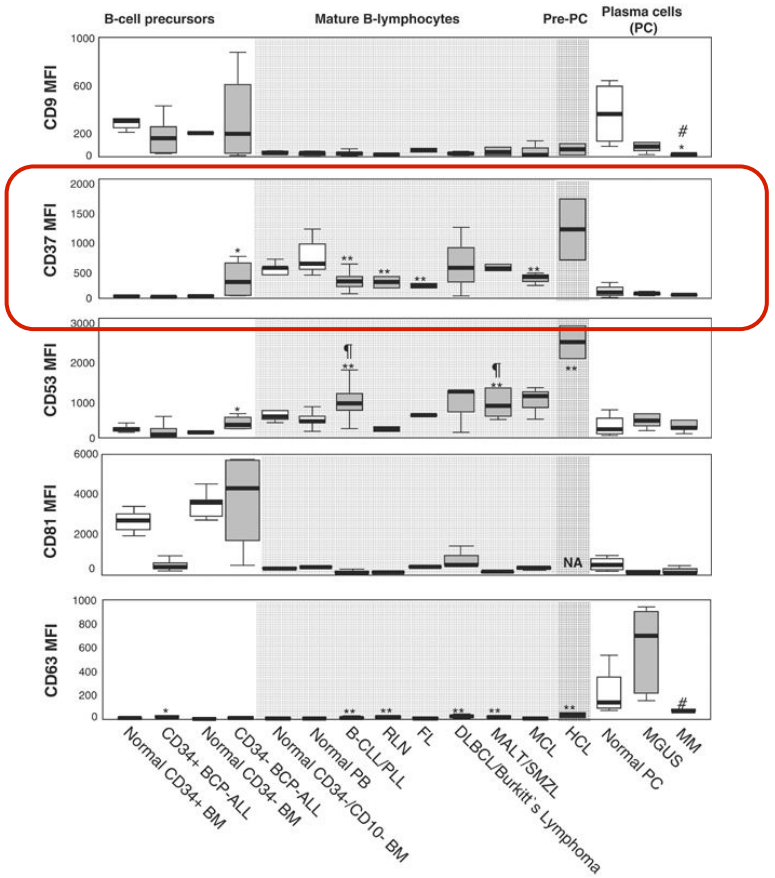
CD37 is a member of the tetraspanin proteins and is involved in cell adhesion, motility and apoptosis

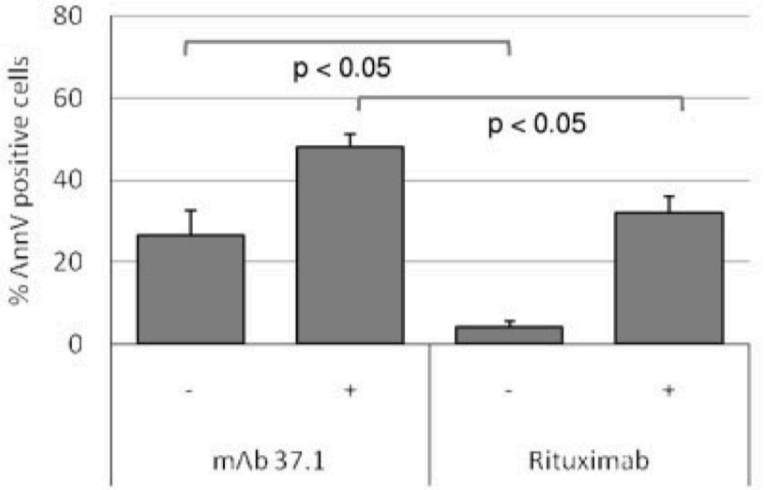


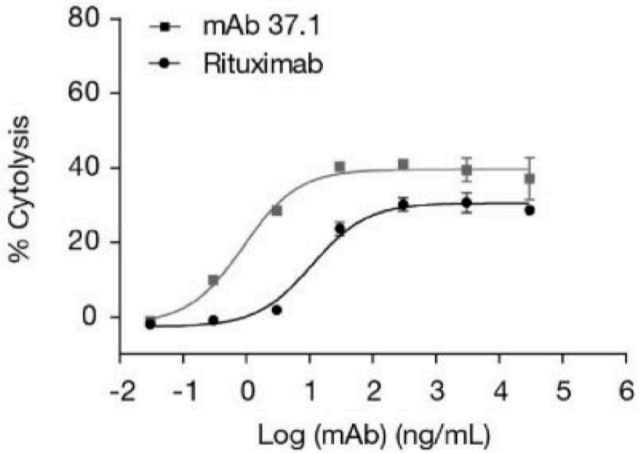
CD37 is expressed on B-cells from the precursor to mature B-cell stages, and is also expressed in NHL and CLL

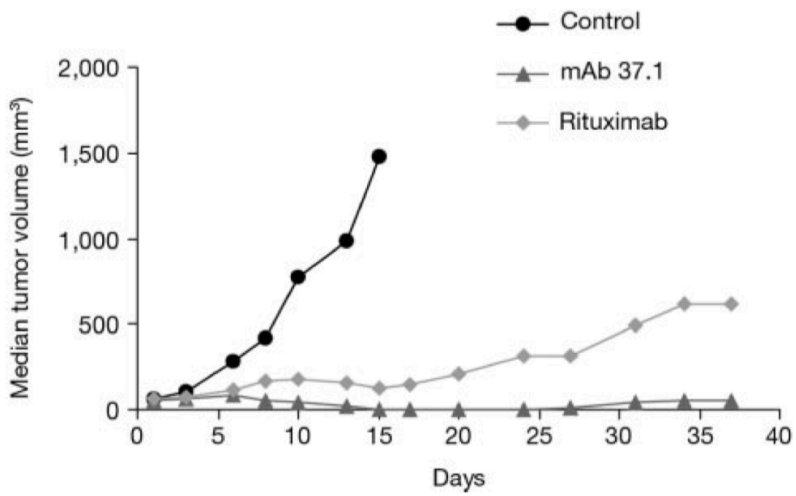


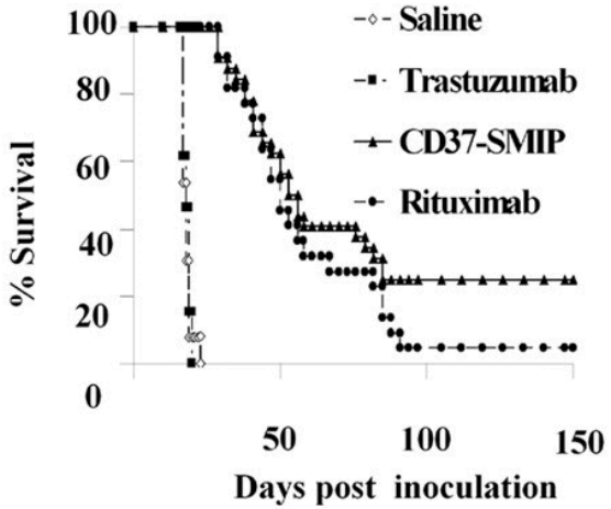
Barrena *et al.*, *Leukemia*. 2005

Targeting CD37 with specific antibodies









Heider *et al., Blood*. 2011; Zhao *et al., Blood*. 2007

Targeting CD37 with otlertuzumab in CLL

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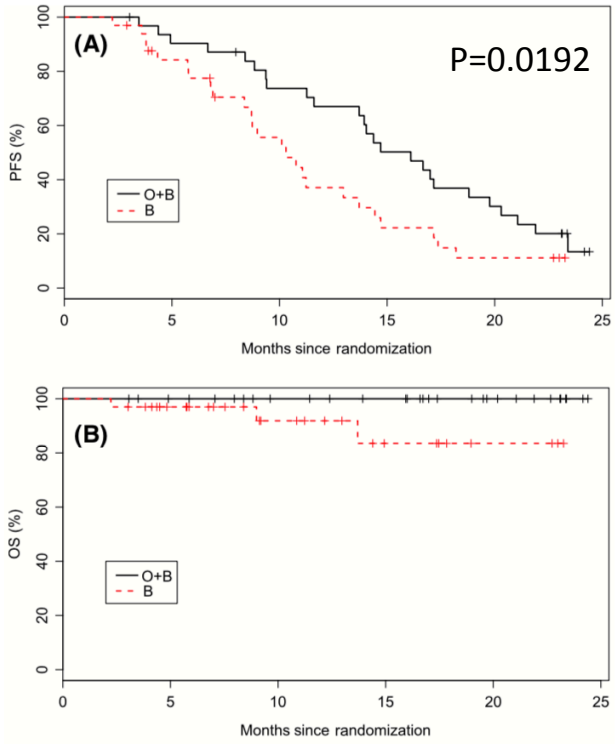
Response by IWCLL criteria (Hallek *et al*, 2008)	Otlertuzumab + bendamustine ($N = 32$)		Bendamustine only ($N = 33$)		Rate ratio (95% CI†)	*P*-value‡
	n	% (95% CI)	*n*	% (95% CI*)		
Overall response	22	68·8 (50·0–83·9)	13	39·4 (22·9–57·9)	1·75 (1·08–2·83)	0·026
Complete response	3	9·4 (2·0–25·0)	1	3·0 (0·1–15·8)		
Partial response	19	59·4 (40·6–76·3)	12	36·4 (20·4–54·9)		
Stable disease	5	15·6 (5·3–32·8)	10	30·3 (15·6–48·7)		
Progressive disease	5	15·6 (5·3–32·8)	10	30·3 (15·6–48·7)		



	Otlertuzumab + bendamustine ($N = 32$)		Bendamustine ($N = 33$)	
	All events (%)	Grade 3/4 (%)	All events (%)	Grade 3/4 (%)
Any event	91	66	100	70
Infection	59	13	61	27
Neutropenia	59	56	39	39
Thrombocytopenia	34	19	27	15
Pyrexia	34	3	12	0
Anaemia	31	13	33	15
Nausea	19	0	30	0
Diarrhoea	16	3	21	0
Fatigue	16	0	15	3
Pruritus	16	0	3	0
Cough	13	0	24	0
Vomiting	13	0	15	3
Hyperuricemia	13	0	9	3
Chills	13	0	6	0
Headache	6	0	15	0
Constipation	6	0	24	0
Upper abdominal pain	6	0	12	0
Dizziness	3	0	12	0

Robak *et al.*, *BJH*. 2016

Targeting CD37 with otlertuzumab in NHL

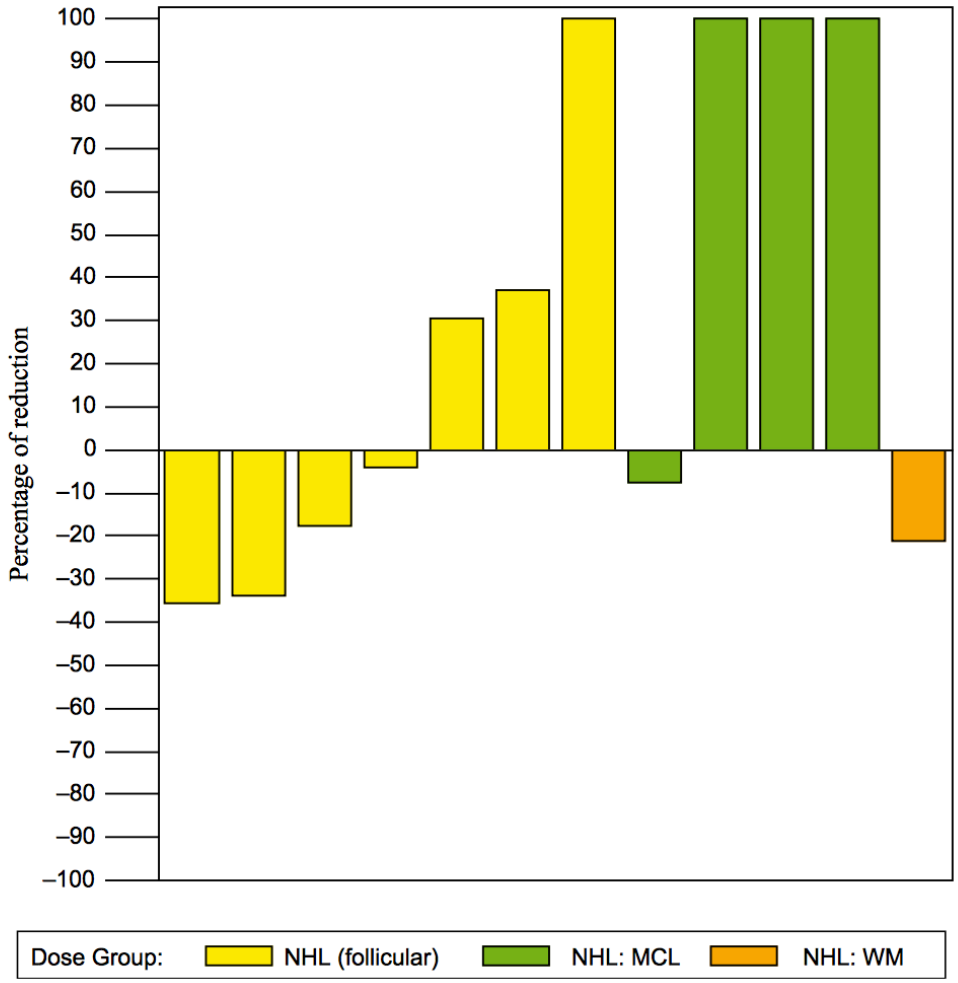


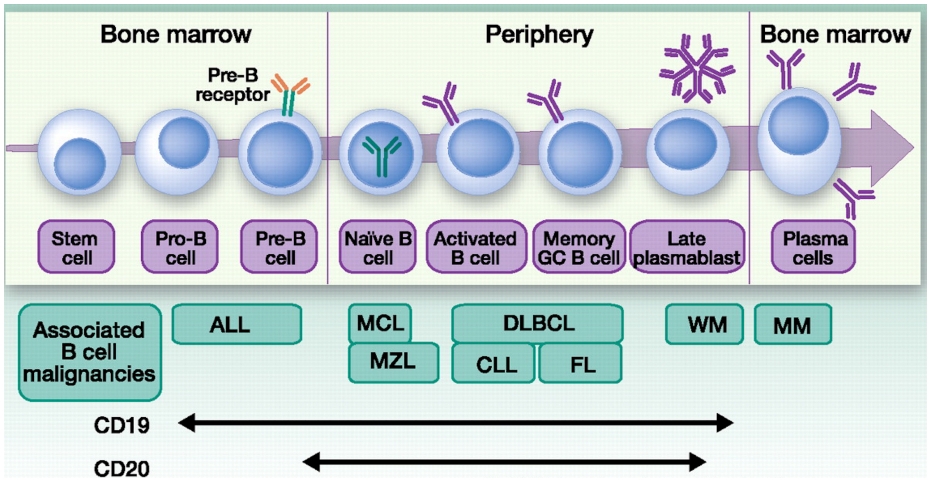
Table III. Adverse events occurring in >1 Patient (n = 16).

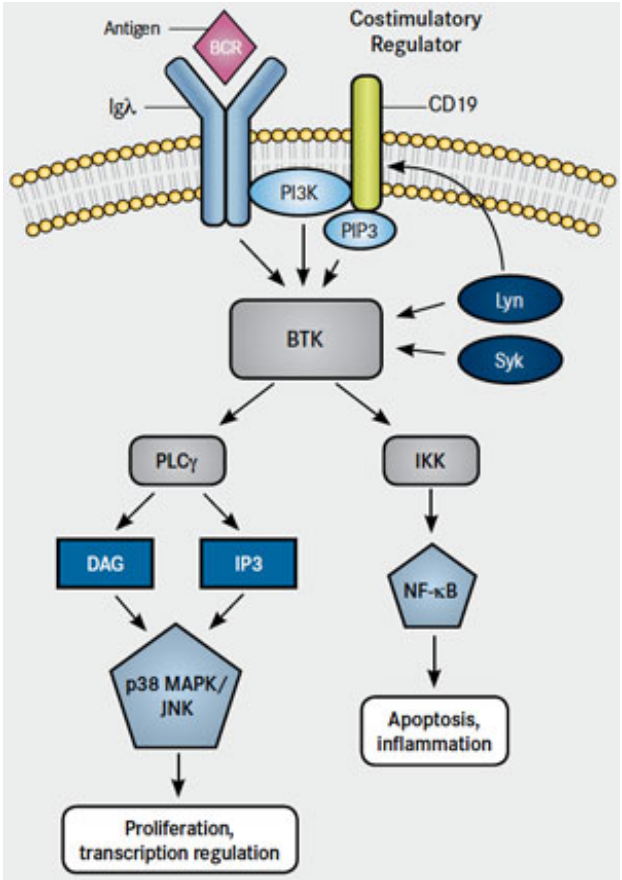
	All Events		Grade 3/4 Events	
	n	%	*n*	%
Any Event	14	87·5	9	56·3
Neutropenia	6	37·5	6	37·5
Fatigue	6	37·5	1	6·3
Nausea	5	31·3	0	0
Thrombocytopenia	4	25	1	6·3
Diarrhoea	4	25	0	0
Peripheral Oedema	4	25	0	0
Night Sweats	3	18·75	0	0
Anaemia	2	12·5	1	6·3
Lymphopenia	2	12·5	1	6·3
Constipation	2	12·5	0	0
Vomiting	2	12·5	0	0
Chest Pain	2	12·5	0	0
Non-Cardiac Chest Pain	2	12·5	0	0
Pyrexia	2	12·5	1	6·3
Anorexia	2	12·5	0	0
Hypokalaemia	2	12·5	0	0
Hypophosphataemia	2	12·5	1	6·3
Muscle Spasms	2	12·5	0	0
Headache	2	12·5	0	0
Hypotension	2	12·5	0	0

Pagel *et al., BJH*. 2014

The CD19 antigen

- CD19 is broadly and homogeneously expressed across different B cell malignancies including DLBCL and CLL
- CD19 enhances tumor cell survival and proliferation via BCR signaling
- CD19 expression is suggested to be preserved during treatment of B cell malignancies





Olejniczak *et al., Immunol Invest.* 2006; Fujimoto *et al., J Immunol.* 1999

Targeting CD19

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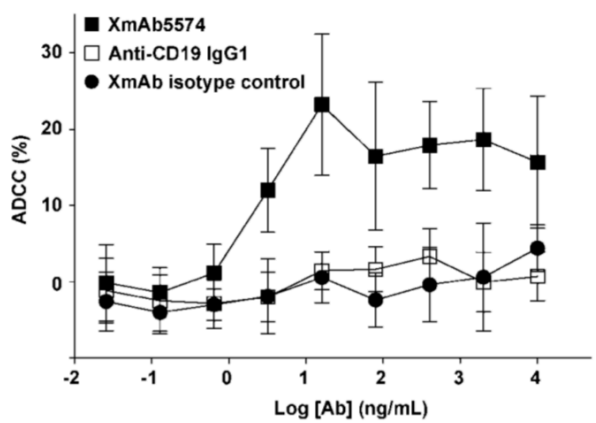
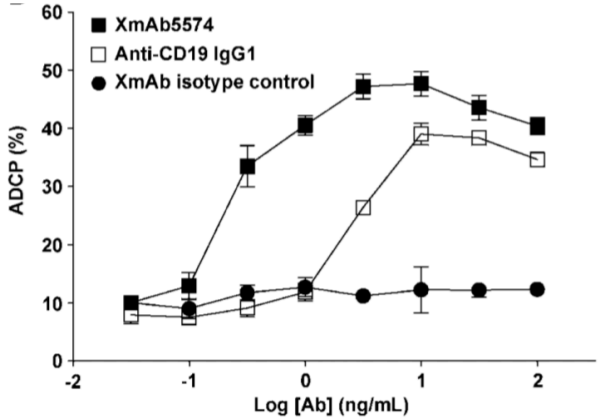
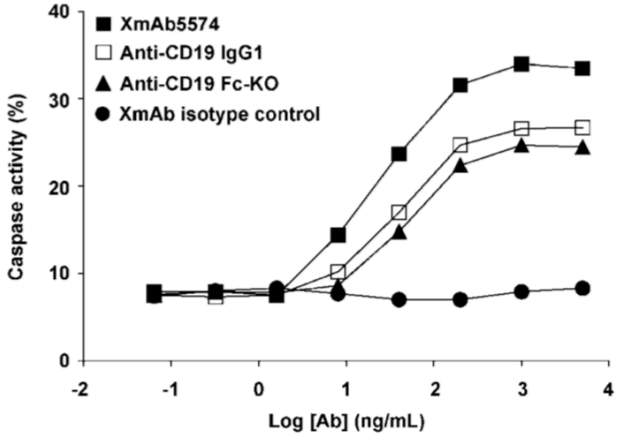
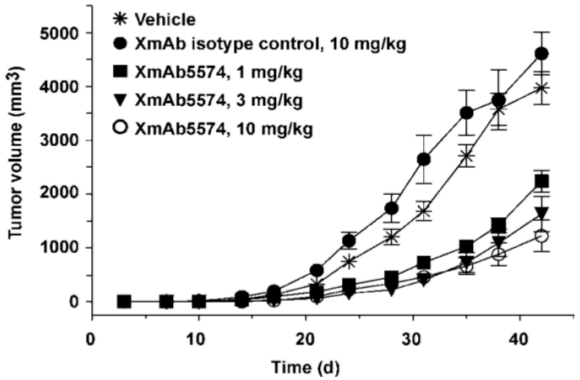
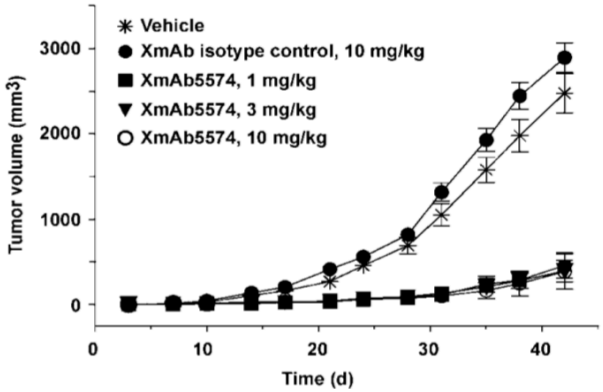
XmAb5574 (MOR208) is a humanized anti-CD19 antibody with an engineered Fc domain that increases the binding capacity to Fc receptors on immune cells and thus increase Fc-mediated effector functions

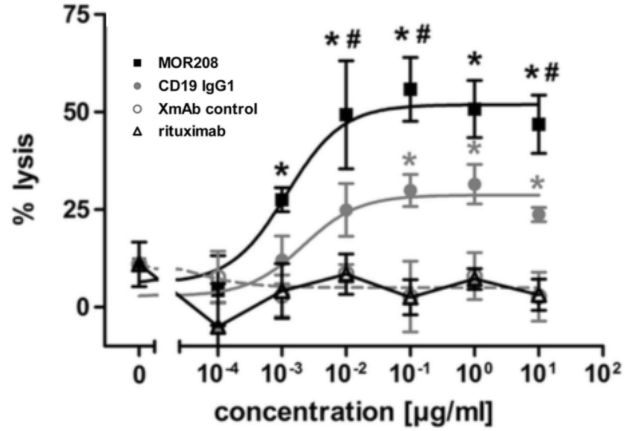












Horton *et al., Cancer Res*. 2008; Kellner *et al., Leukemia*. 2013

Targeting CD19 with MOR208 in R/R B-cell lymphomas

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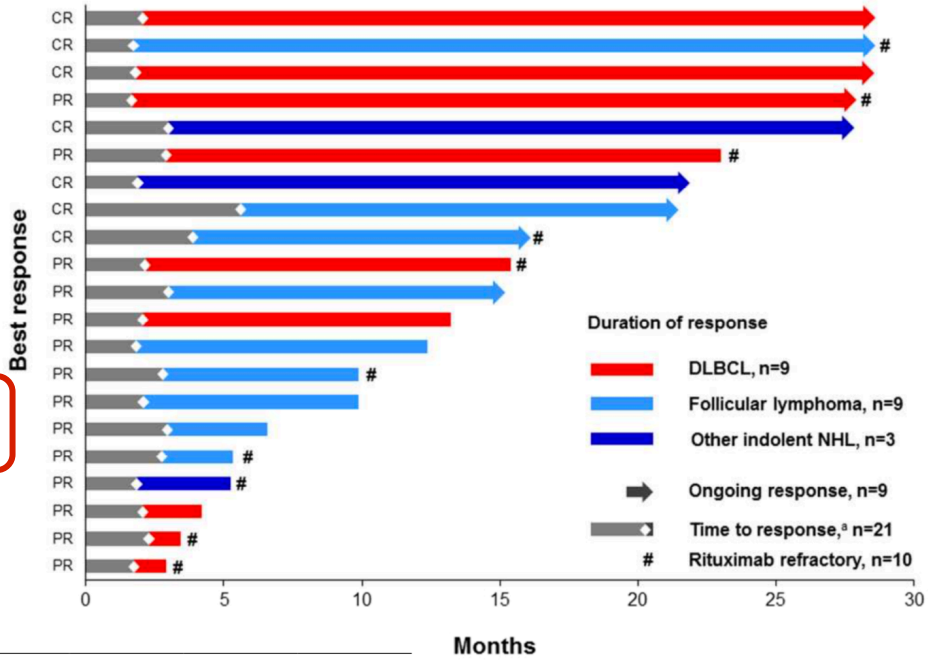
	DLBCL *N*=35	FL *N*=34	Other iNHL *N*=11	MCL *N*=12	Total *N*=92
Best overall response					
Complete response	2 (6)	3 (9)	2 (18)	0	7 (8)
Partial response	7 (20)	7 (21)	1 (9)	0	15 (16)
Stable disease	5 (14)	16 (47)	4 (36)	6 (50)	31 (34)
Progressive disease	11 (31)	4 (12)	3 (27)	5 (42)	23 (25)
Not evaluable[a]	10 (29)	4 (12)	1(9)	1 (8)	16 (17)
ORR (all patients)	9 (26)	10 (29)	3 (27)	0	22 (24)
ORR (evaluable patients only[b])	9 (36)	10 (33)	3 (30)	0	22 (29)
DCR (all patients)	14 (40)	26 (76)	7 (64)	6 (50)	53 (58)



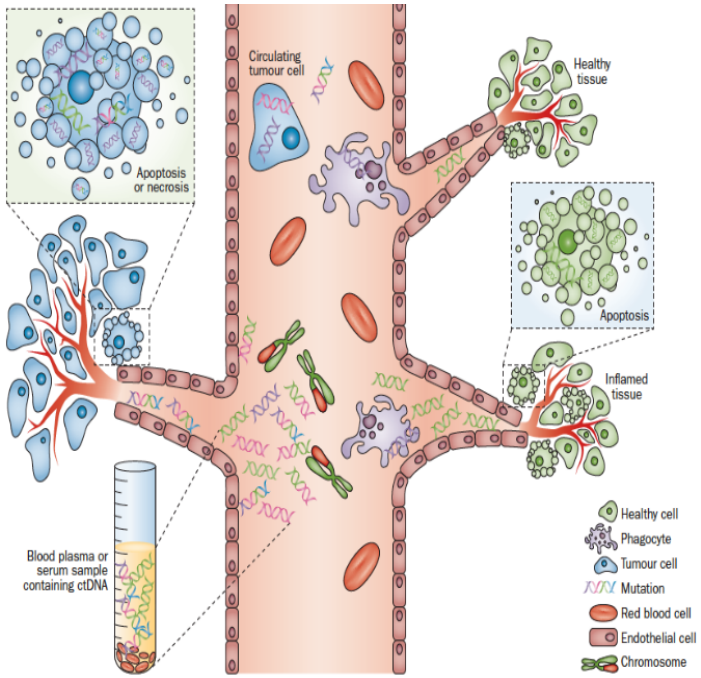
TEAEs,[a] n (%)	DLBCL *N*=35	FL *N*=34	Other iNHL *N*=11	MCL *N*=12	Total *N*=92
Any grade ≥3[b]	**19 (54)**	**9 (27)**	**5 (46)**	**4 (33)**	**37 (40)**
Hematological[c]					
Neutropenia	6 (17)	2 (6)	0	0	8 (9)
Thrombocytopenia	2 (6)	1 (3)	0	1 (8)	4 (4)
Anemia	3 (9)	0	0	0	3 (3)
Non-hematological[c]					
Dyspnea	2 (6)	1 (3)	0	1 (8)	4 (4)
Pneumonia[d]	3 (9)	0	0	0	3 (3)
Fatigue	1 (3)	1 (3)	0	0	2 (2)
Hypokalemia	1 (3)	1 (3)	0	0	2 (2)
Infusion-related reaction,[a] n (%)					
Any	**4 (11)**	**4 (12)**	**1 (9)**	**2 (17)**	**11[e] (12)**
Grade 1/2	4 (11)	3 (9)	1 (9)	2 (17)	10 (11)
Grade 4	0	1 (3)	0	0	1 (1)

Jurczak *et al., Ann Oncol*. 2018

Liquid Biopsy vs Tissue Biopsy

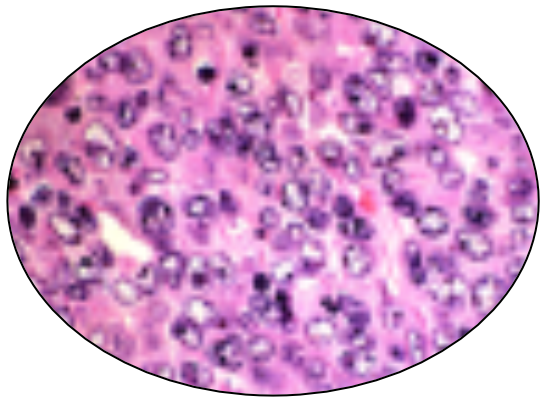
Liquid biopsy:

- allows early disease detection
- enables assessment of tumor heterogeneity and monitoring of tumor dynamics
- in solid cancers, allows evaluation of metastasis in real-time and monitoring of the treatment response



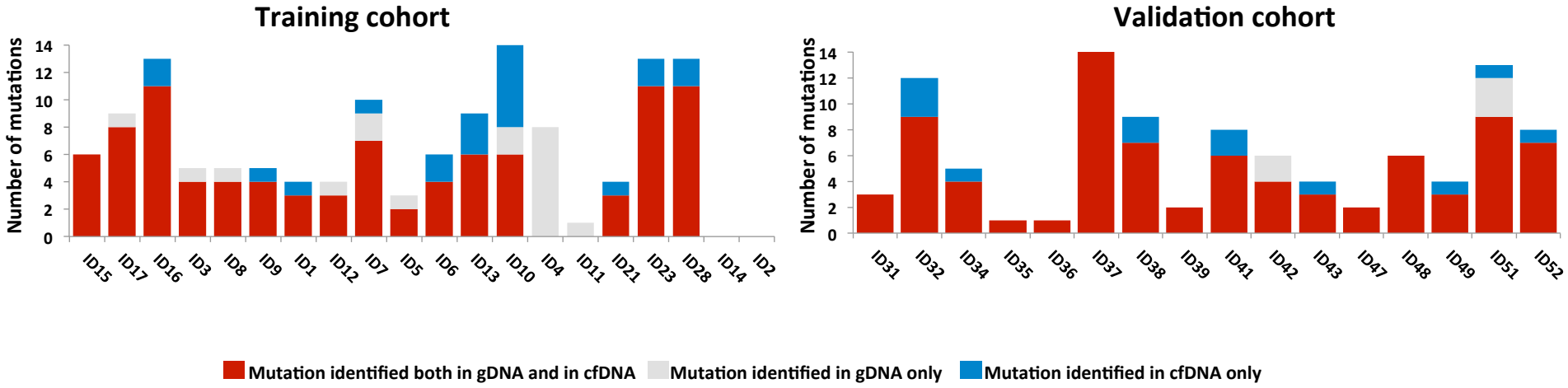
Tissue biopsy during clinical course:

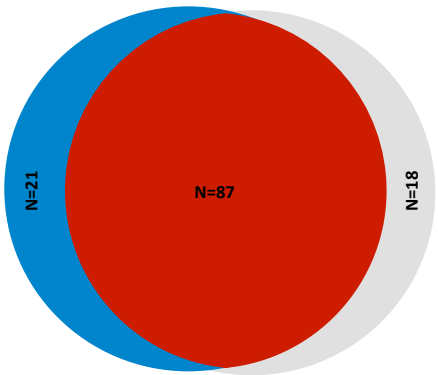
- may not reflect current disease condition
- may not be feasible based on patient conditions or tumor accessibility
- impractical for periodic monitoring for progression/recurrence

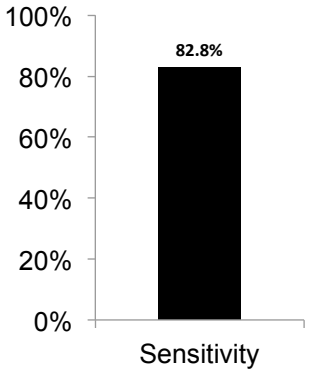


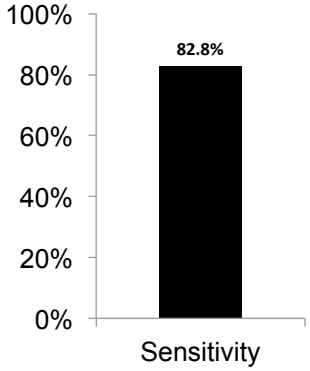
Crowley E, *et al*, *Nat Rev Clin Oncol*, 2013
Rossi D, Diop F, *et al*., *Blood*, 2017
Rossi D, Diop F, *et al*., *Blood*, 201

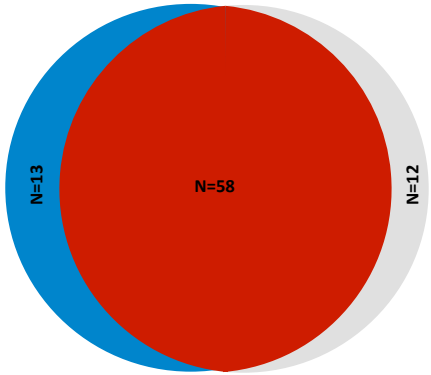
Plasma cfDNA genotyping vs tumor gDNA genotyping





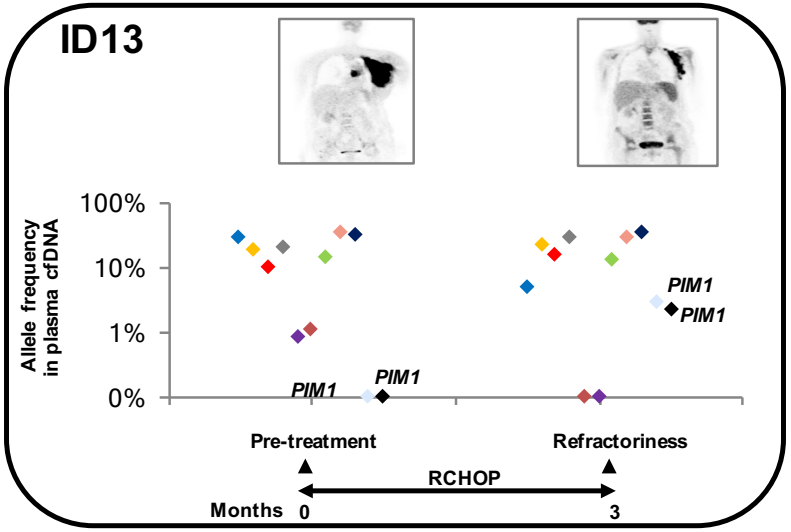


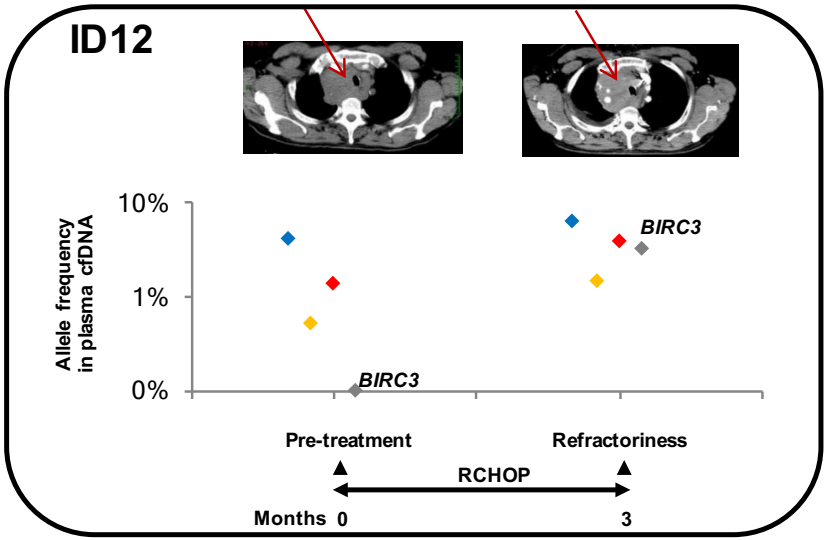


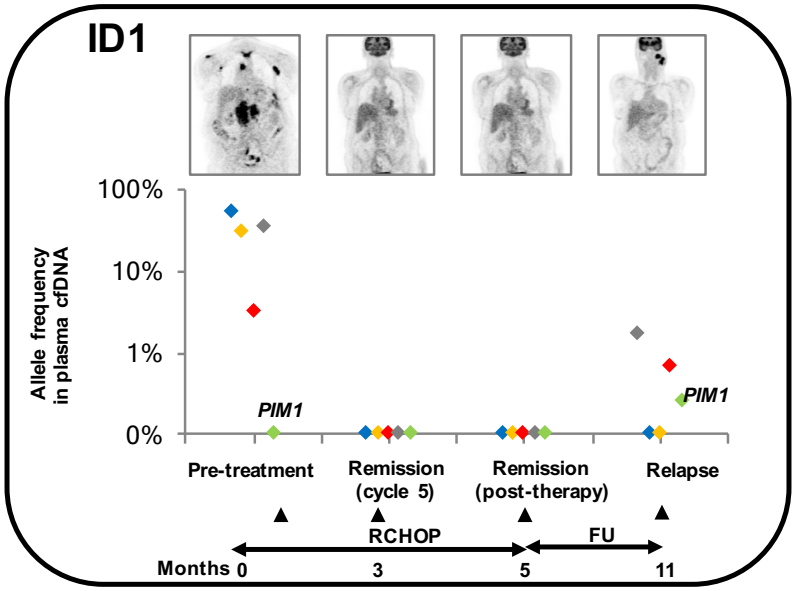


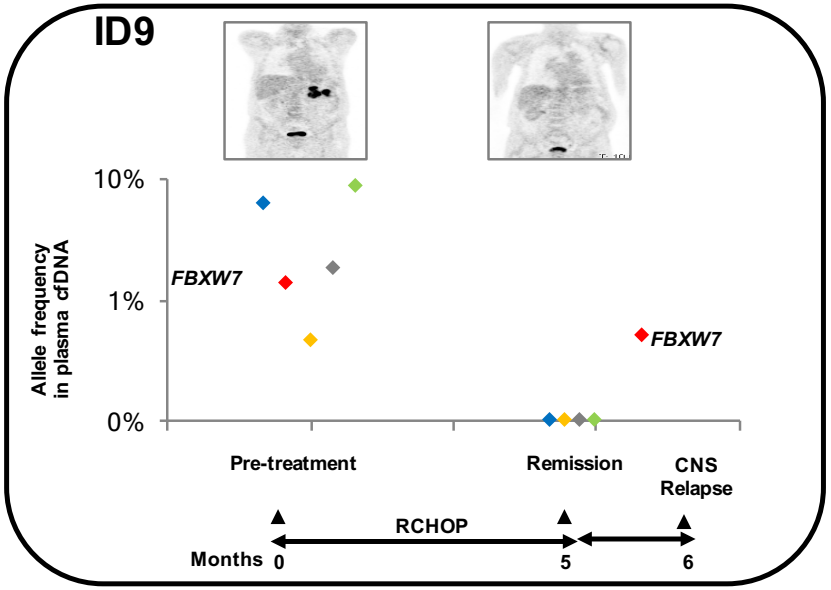
Rossi D, Diop F, *et al.*, *Blood*, 2017

Longitudinal cfDNA genotyping allows real-time monitoring of clonal evolution



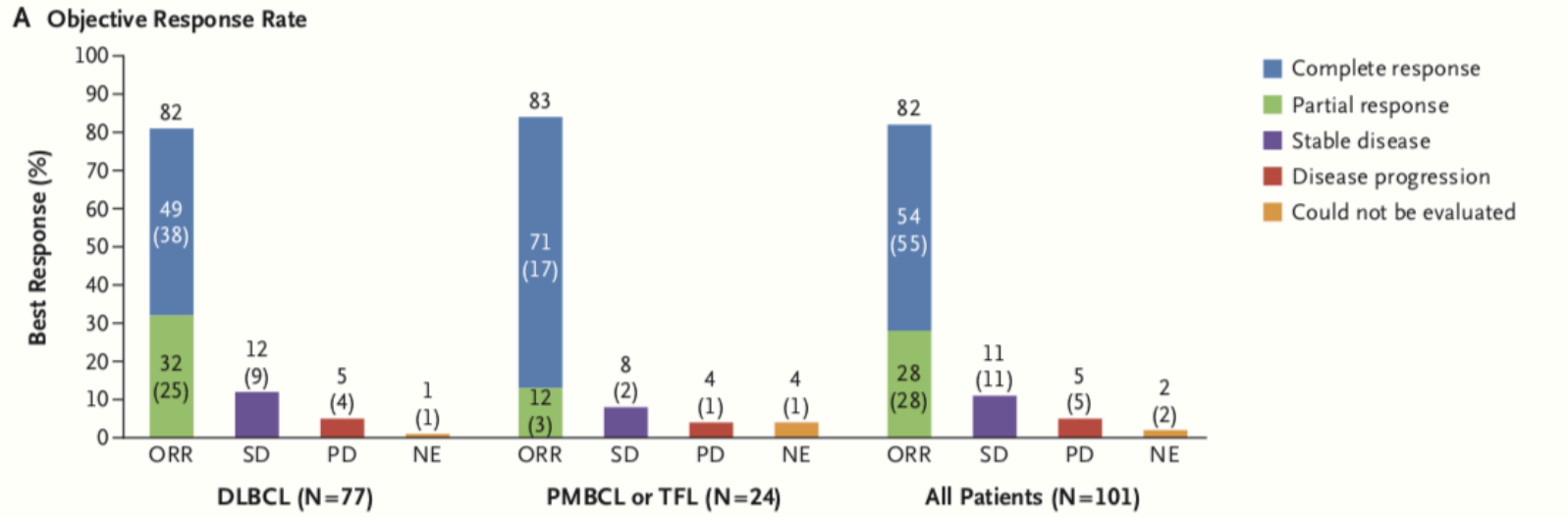


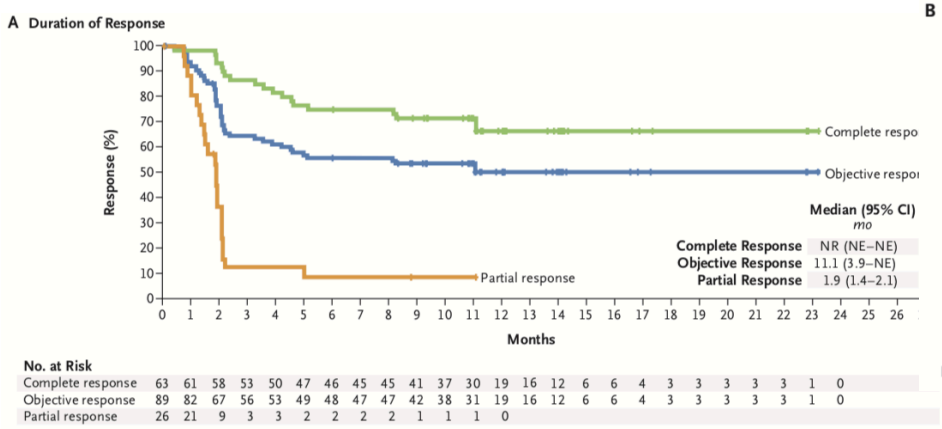


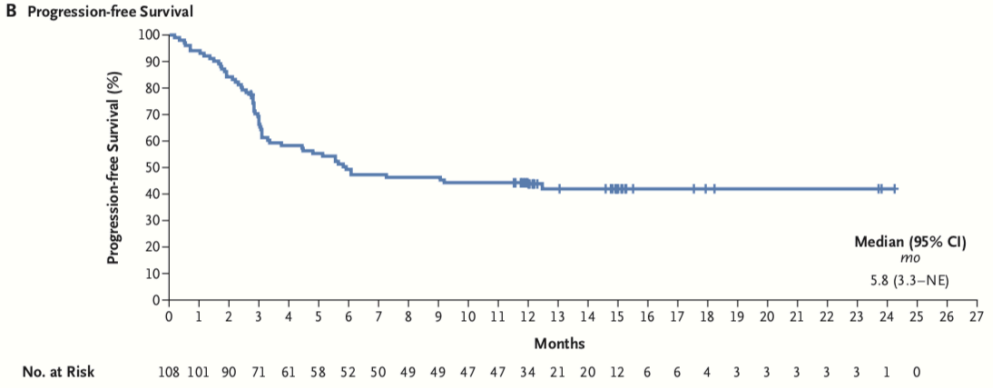


Rossi D, Diop F, *et al.*, *Blood*, 2017

CD19 CAR T-Cell trial in lymphomas





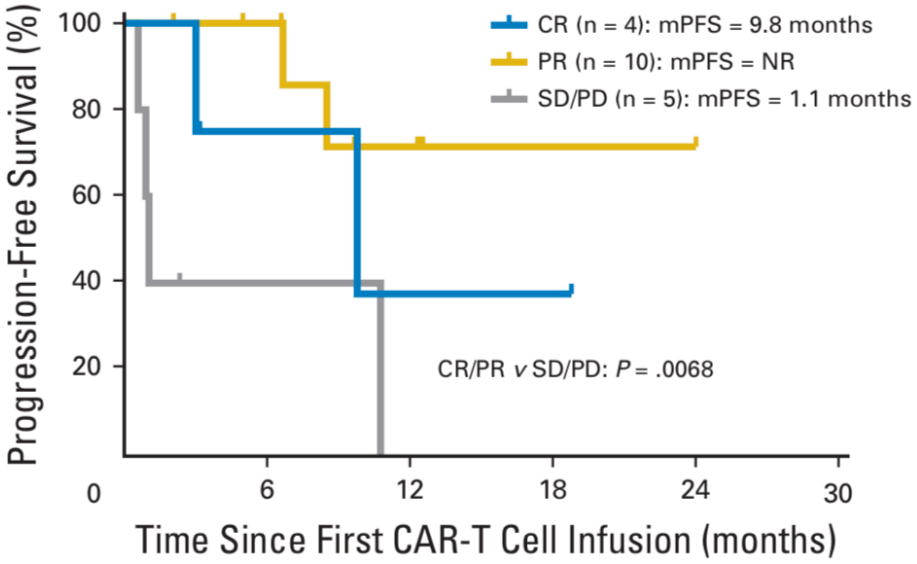


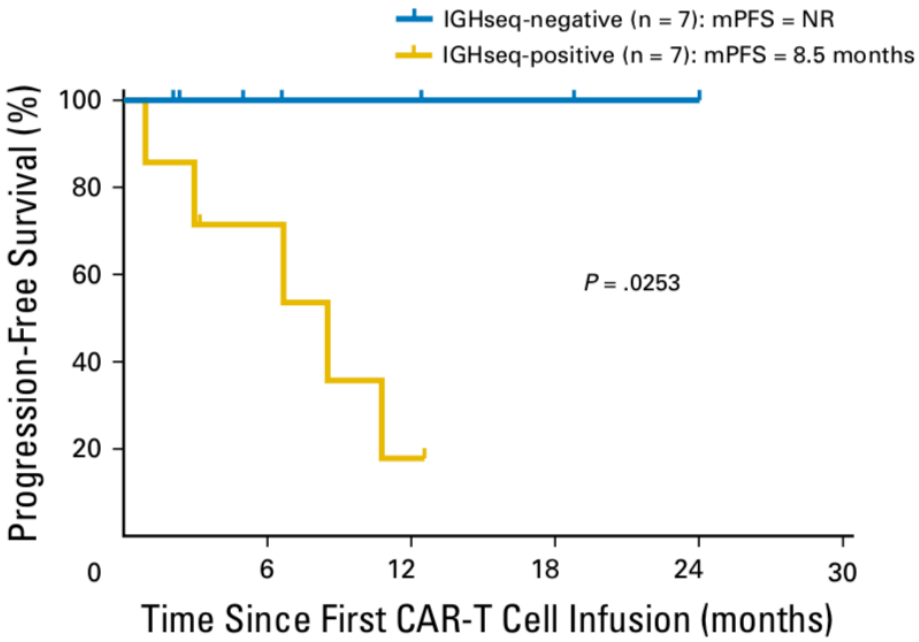
Neelapu *et al.*, *NEJM*. 2017.

CD19 CAR T-Cell trial in CLL

RESULTS:

- 24 patients CLL who had previously received ibrutinib were enrolled;
- The overall response rate by International Workshop on Chronic Lymphocytic Leukemia (IWCLL) criteria was 71% (17 out of 24);
- Twenty patients (83%) developed cytokine release syndrome, and eight (33%) developed neurotoxicity.





Turtle *et al., JCO*. 2017.