Genomic discoveries in CLL

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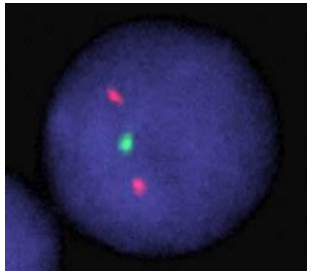
Evolution of genetic techniques

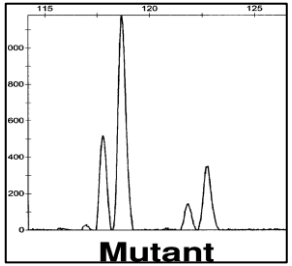
Karolinska Institutet

G-banding

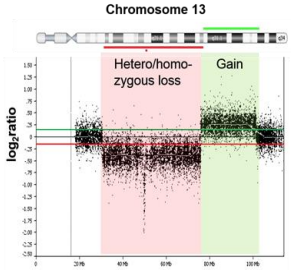
FISH



PCR



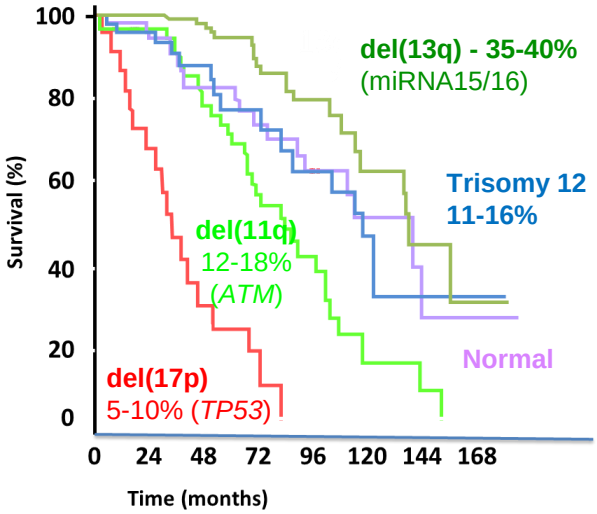
Microarrays



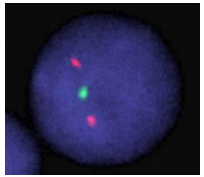
Next-generation sequencing

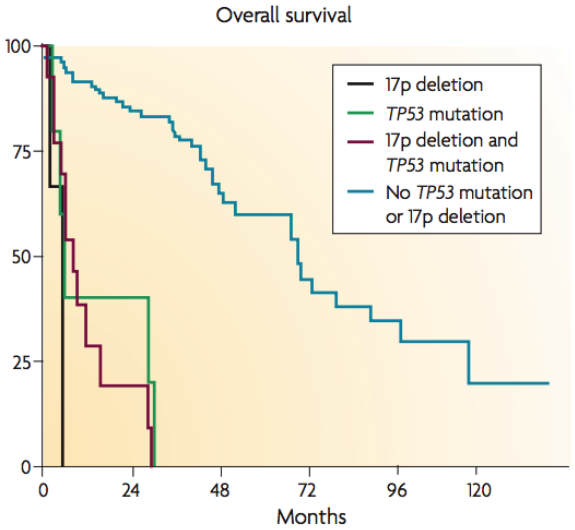
1970 → 2010

Genetic aberrations in clinical diagnostics

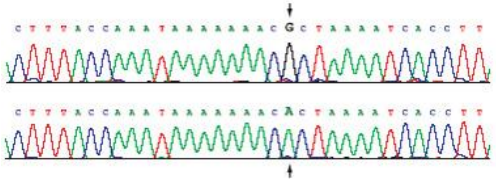


CLL-FISH





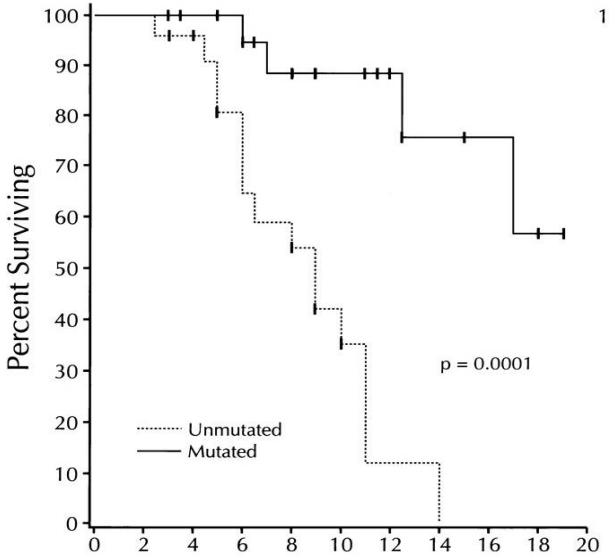
***TP53* screening (exons 2-11)**



Döhner et al, N Engl J Med 2000, Zenz et al, Nature Reviews 2010

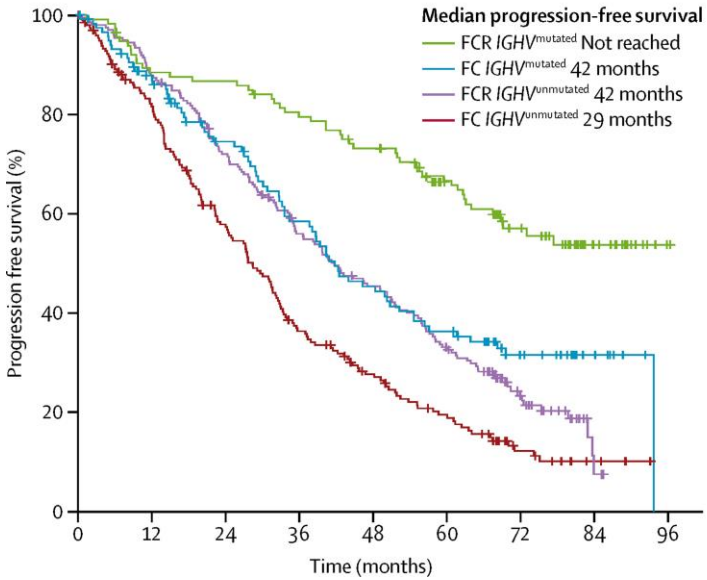
IGHV gene mutational status in CLL

Prognostic marker



Damle et al, Blood 1999

Predictive marker



Fischer et al, Blood 2016

Table 1. Baseline Evaluation of Patients with CLL

Diagnostic test	General practice	Clinical trial
Tests to establish the diagnosis		
Complete blood count and differential count	Always	Always
Immunophenotyping of peripheral blood lymphocytes	Always	Always
Assessment prior to treatment		
History and physical, performance status	Always	Always
Complete blood count and differential count	Always	Always
Marrow aspirate and biopsy	When clinically indicated (unclear cytopenia)	Desirable
Serum chemistry, serum immunoglobulin, and direct antiglobulin test	Always	Always
Chest radiograph	Always	Always
Infectious disease status	Always	Always
Additional tests prior to treatment		
Molecular cytogenetics (FISH) for del(13q), del(11q), del(17p), add(12) in peripheral blood lymphocytes	Always	Always
Conventional karyotyping in peripheral blood lymphocytes (with specific stimulation)	NGI*	Desirable
TP53 mutation	Always	Always
IGHV mutational status	Always	Always
Serum β_2-microglobulin	Desirable	Always
CT scan of chest, abdomen, and pelvis	NGI	Desirable
MRI, PET scans	NGI	NGI
Abdominal ultrasound**	Possible	NGI

New iwCLL guidelines

CLL-IPI

Hallek et al, Blood 2018

New tool box for diagnostics & research

Whole-genome

3 000 000 000 bp

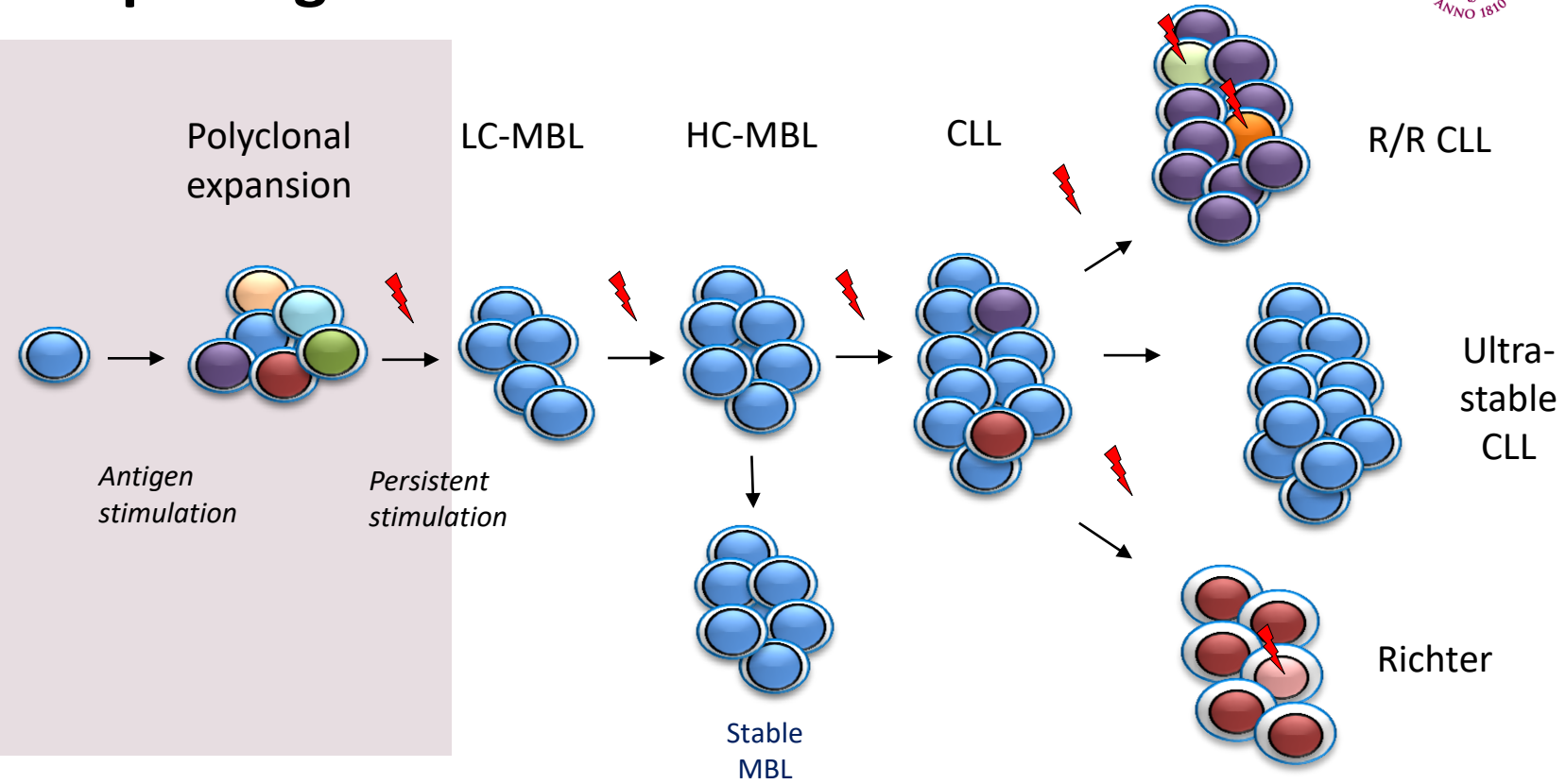
Exome

21 000 genes

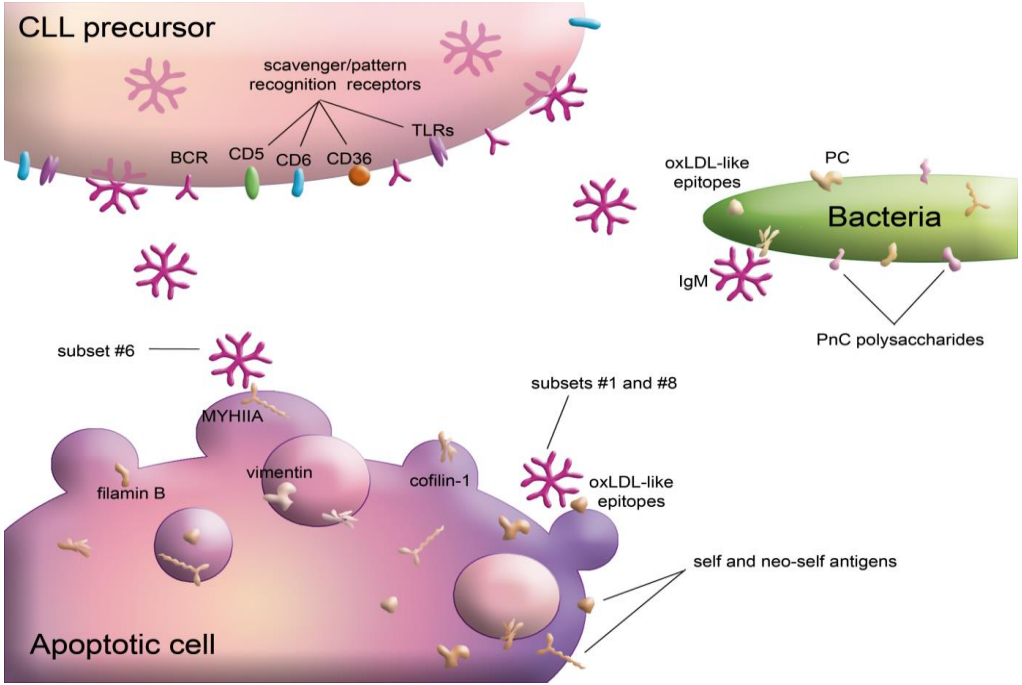
Gene panels

50-500 genes

CLL pathogenesis



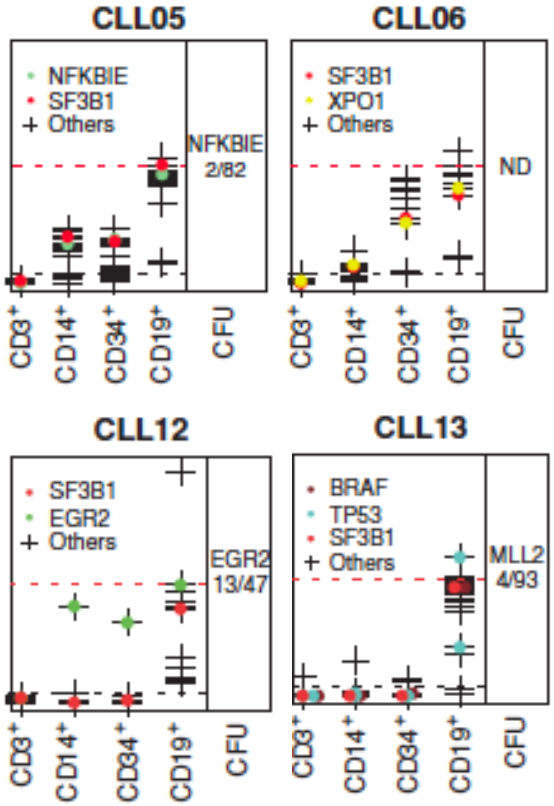
Antigens are involved in CLL development

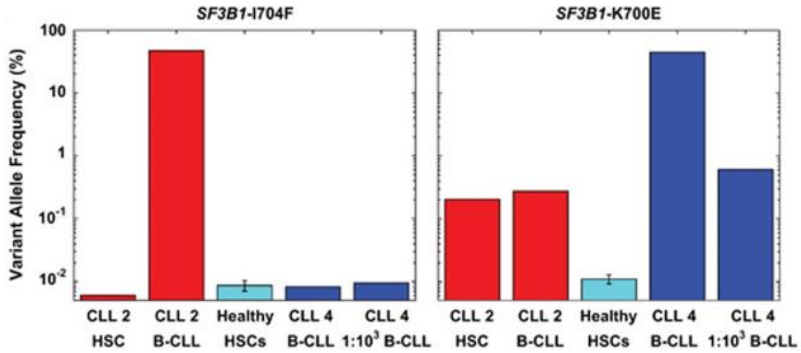


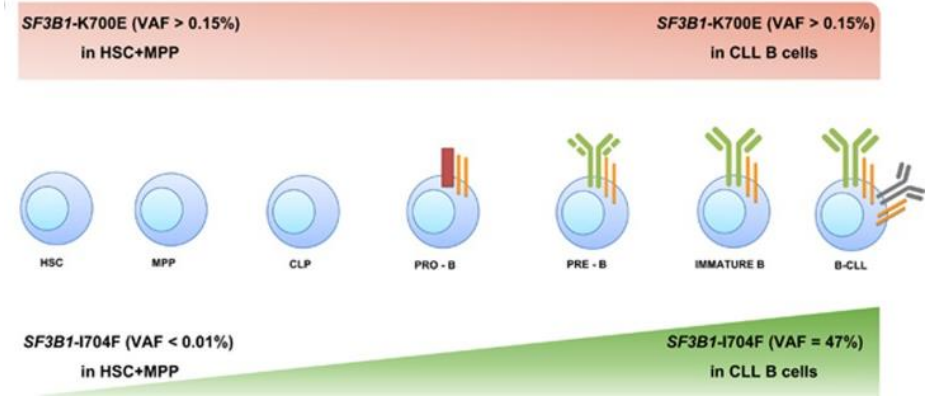
- Gene mutations can be found in hematopoietic progenitor cells
- Enrichment of specific genetic aberrations in patients carrying stereotyped B cell receptors

Rosén et al, Semin Cancer Biol 2010

Gene mutations in progenitor cells



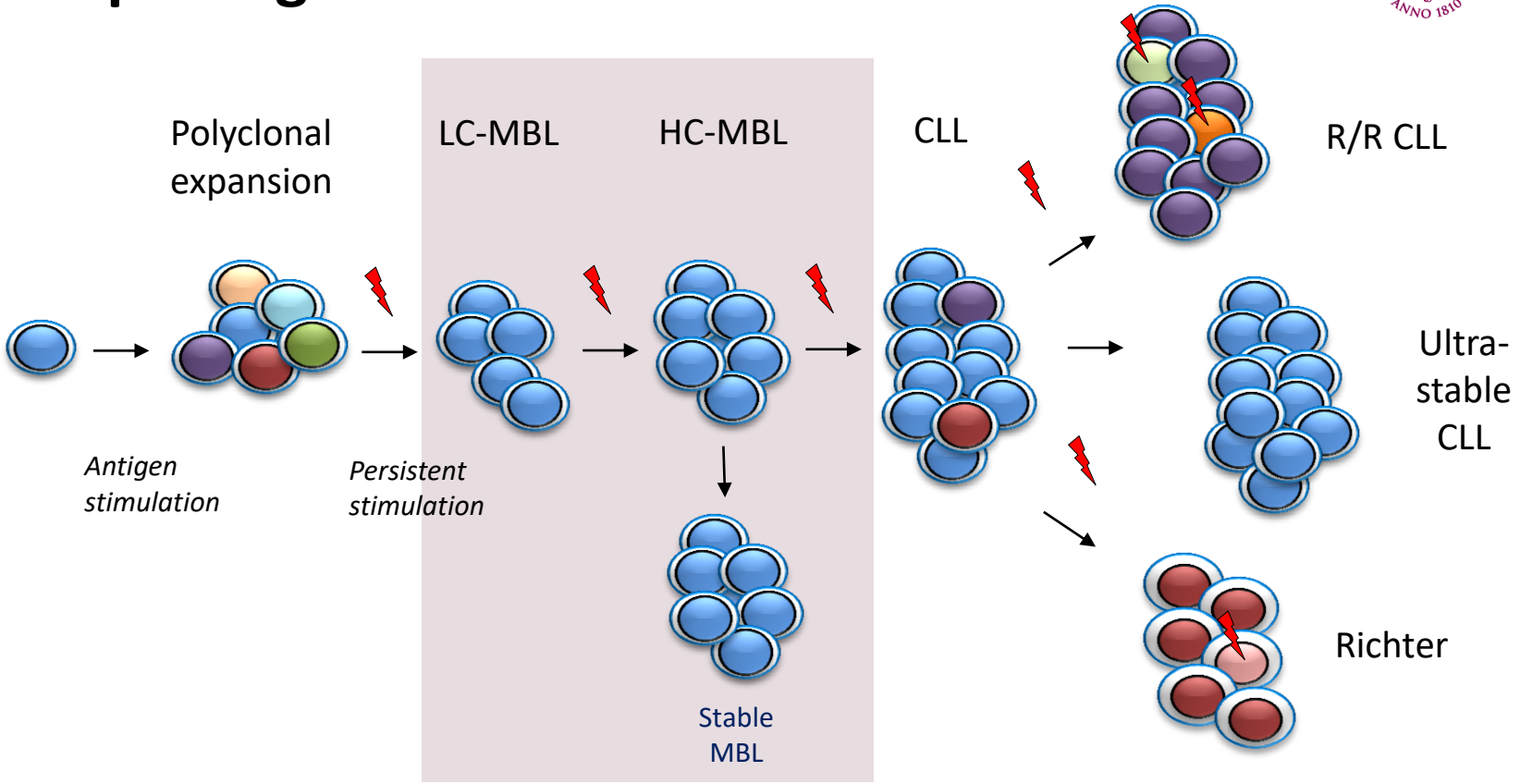




Damm et al, Cancer Discovery 2014

Marsilio et al, Leukemia 2018

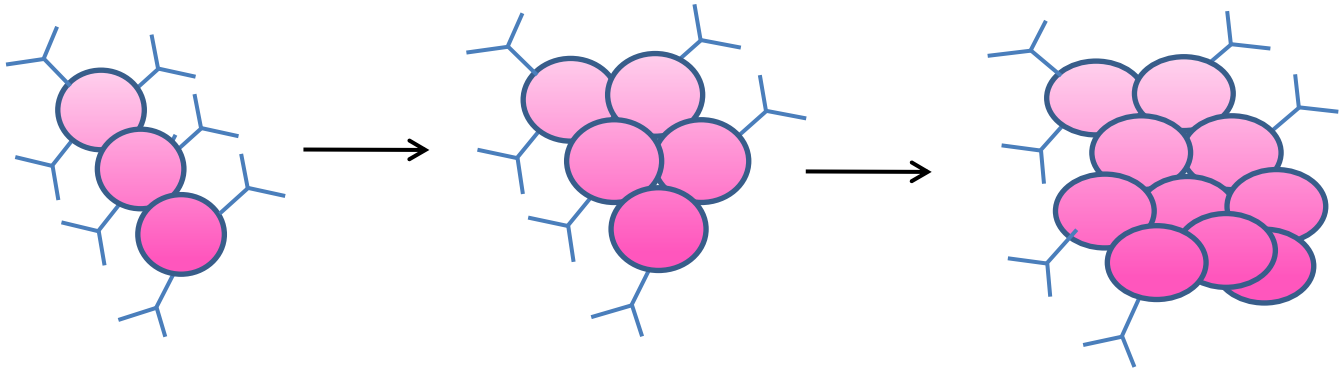
CLL pathogenesis



Whole-genome sequencing in MBL and ultra-stable CLL

Karolinska Institutet

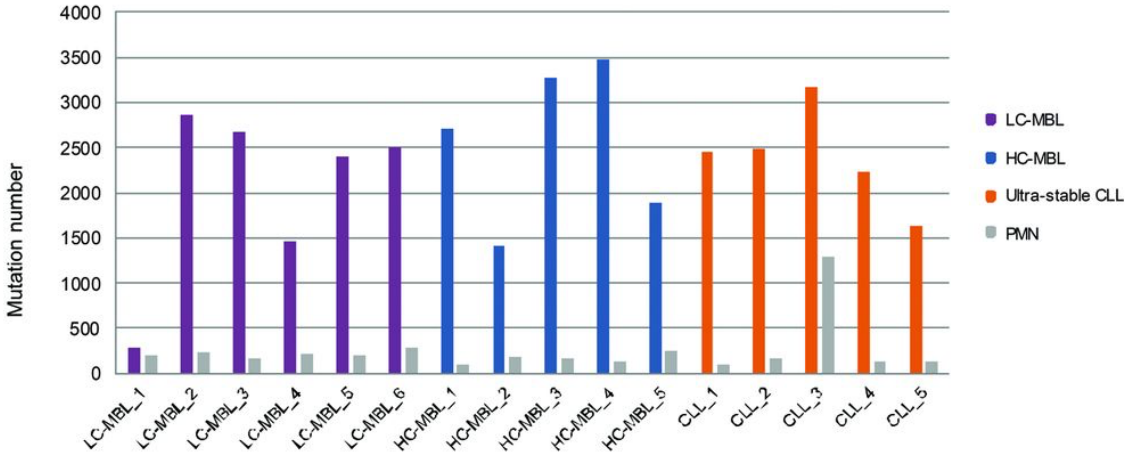
Low-count MBL (n=6) → **High-count MBL (n=5)** → **Ultra-stable CLL (n=5)**

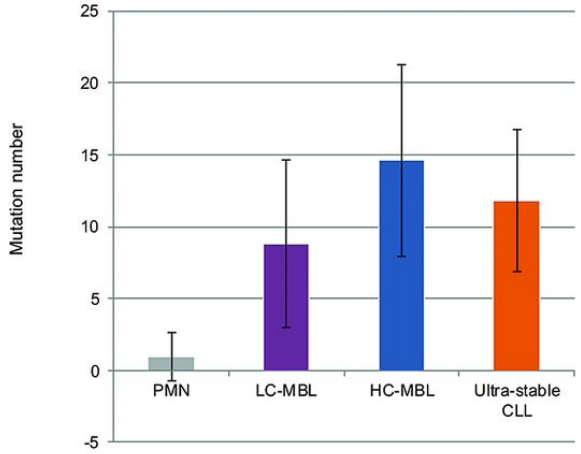


- WGS (30x) on CLL/MBL cells, PMN cells and buccal cells
- Ultradeep sequencing (11 genes) in 28 MBL/CLL cases

Agathangelidis et al, Haematologica 2018

Indistinguishable genomic landscapes in MBL and ultra-stable CLL with low frequency of driver mutations



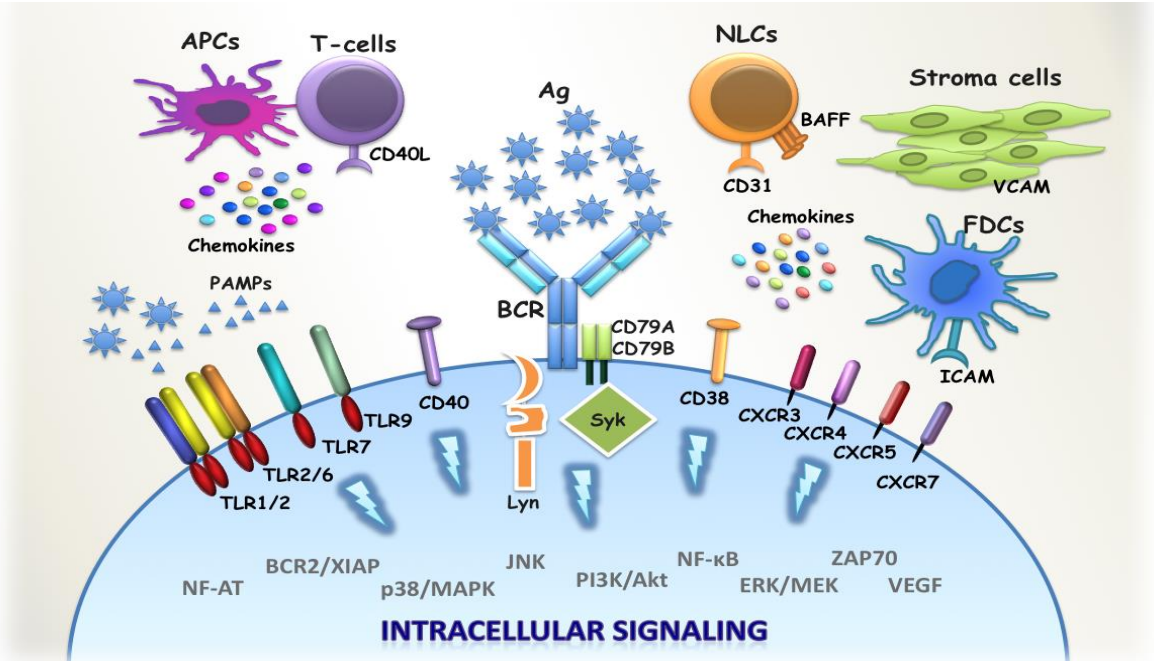


- WGS: on average 2000-2500 somatic mutations per case
- Few CLL ’driver’ mutations detected

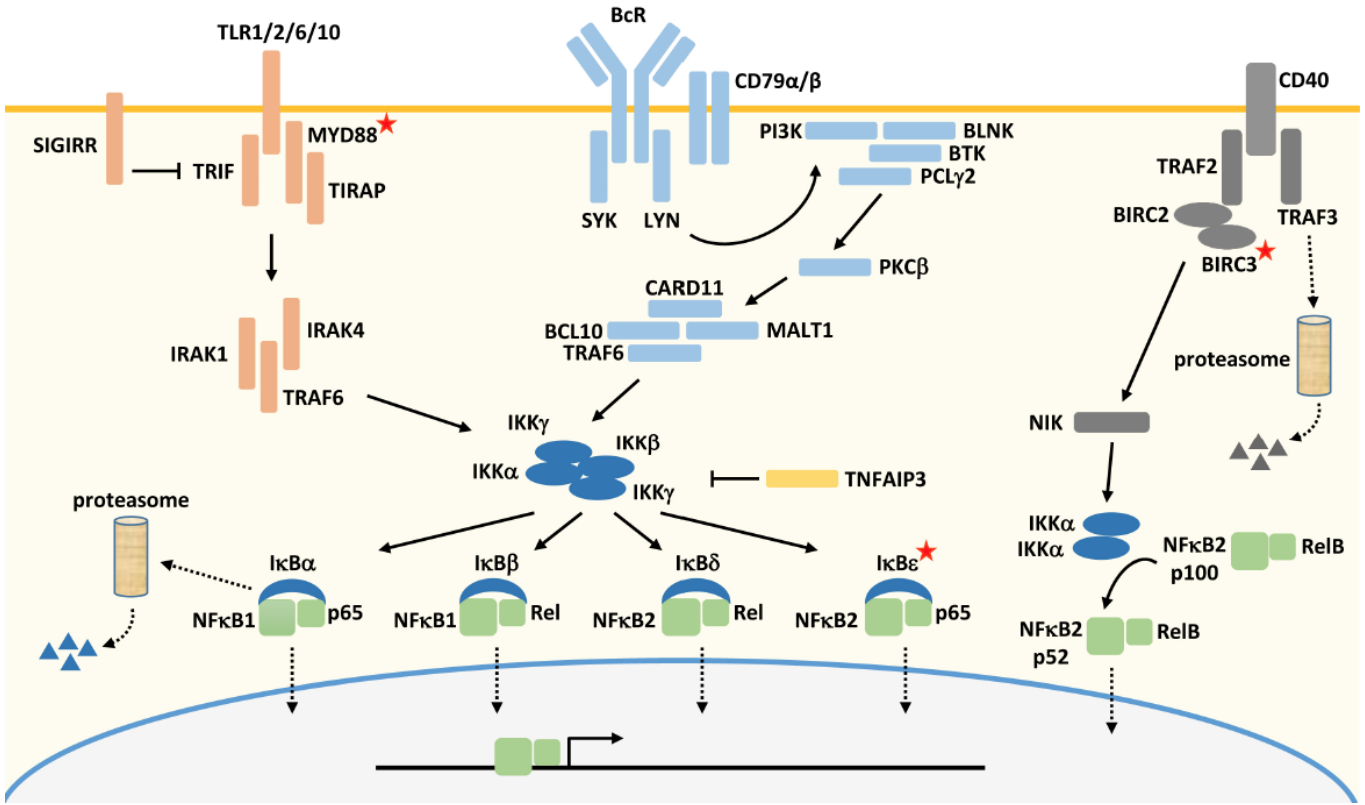
Agathangelidis et al, Haematologica 2018

B-cell receptors in CLL

Linking the outside with the inside

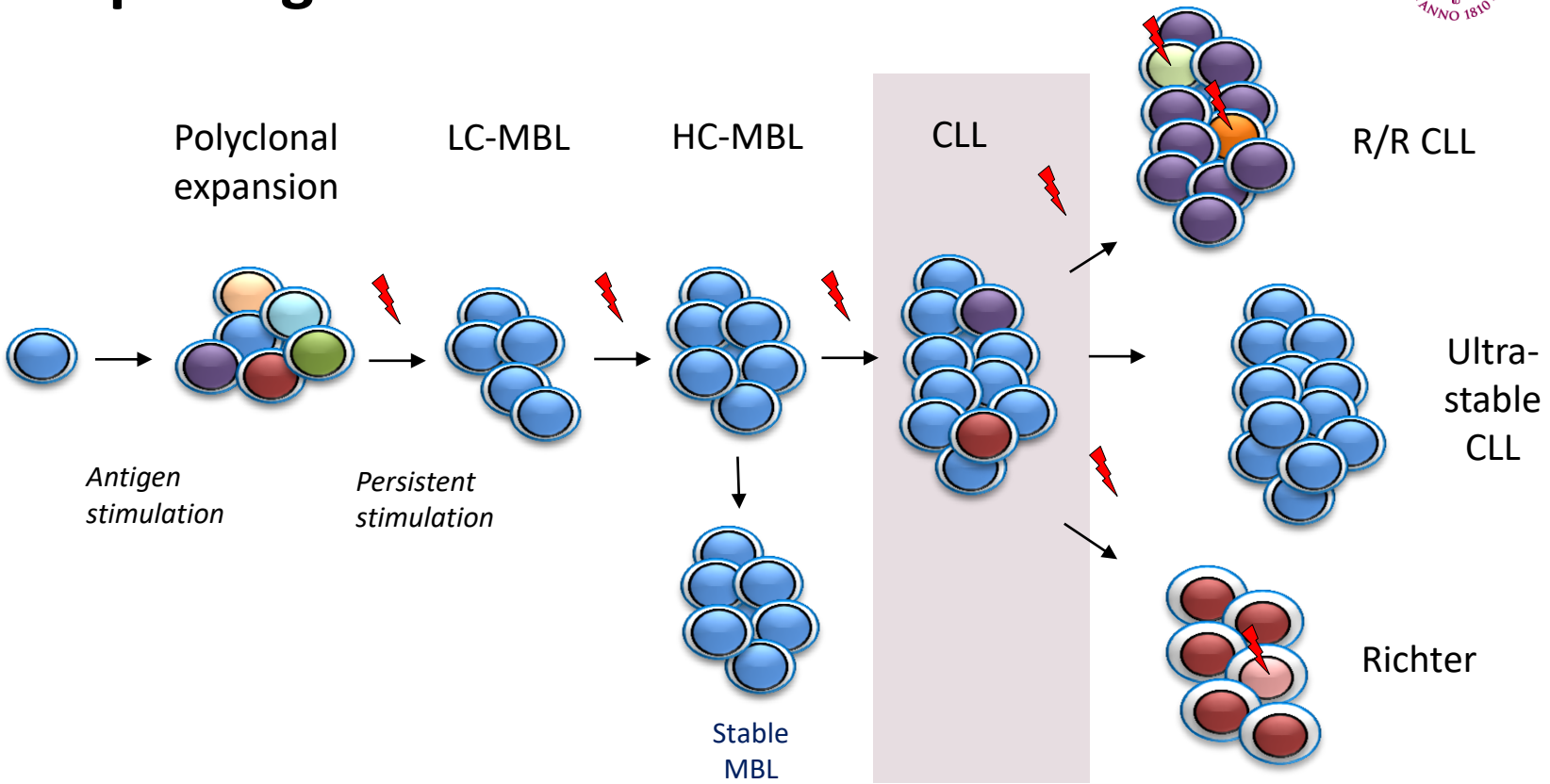


Few mutations in the B-cell receptor pathway

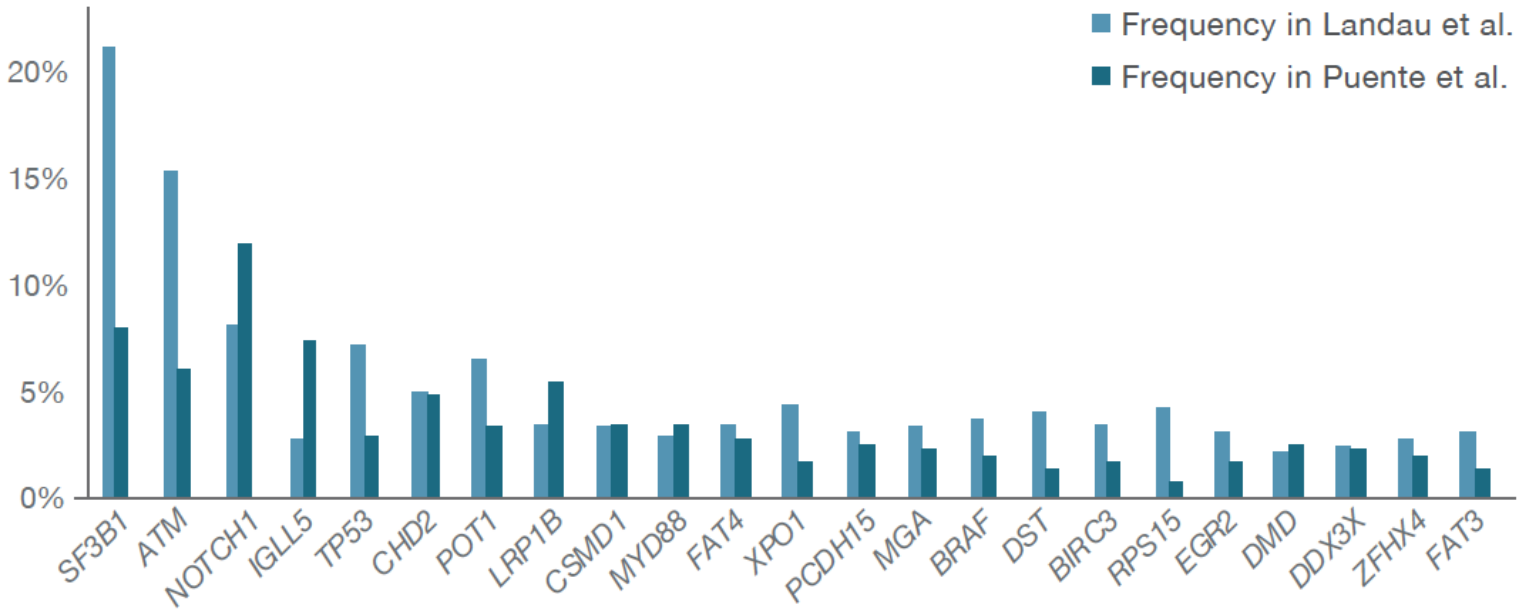


Mansouri et al, Semin Cancer Biol 2016

CLL pathogenesis

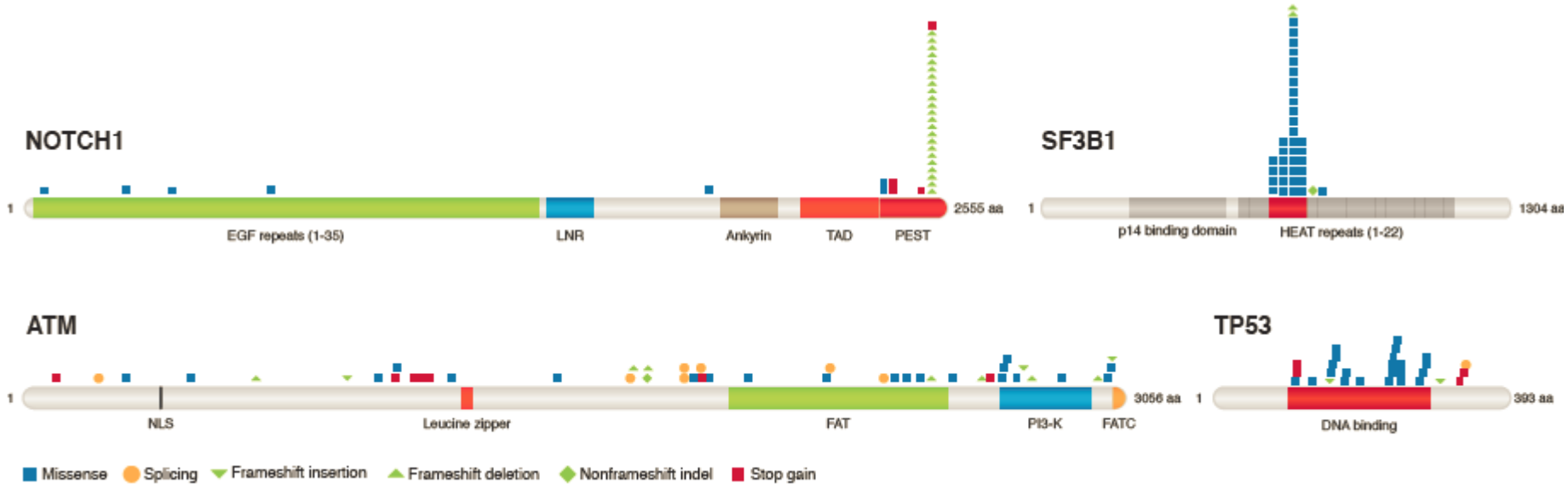


The landscape of CLL ’driver’ gene mutations



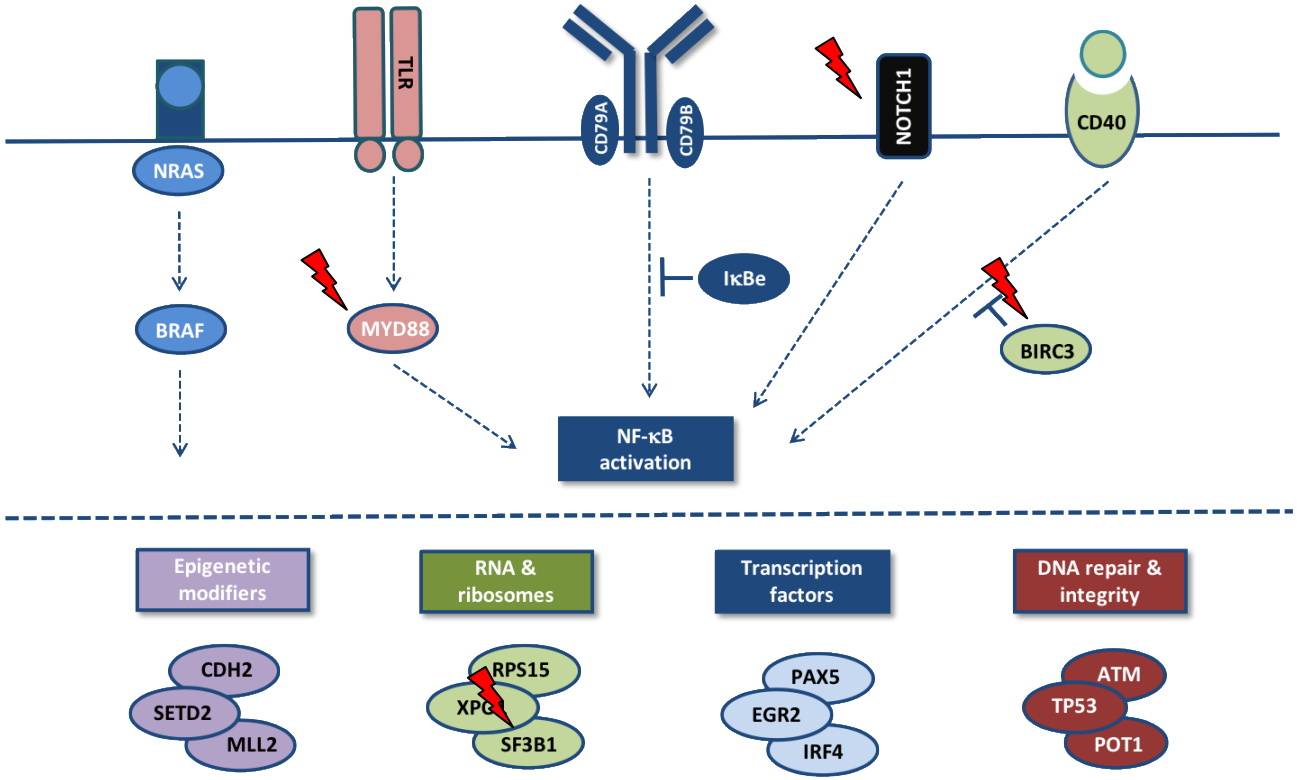
Puente et al, Nature 2015
Landau et al, Nature 2015

Peaks in the CLL landscape



Sutton et al, Haematologica 2015

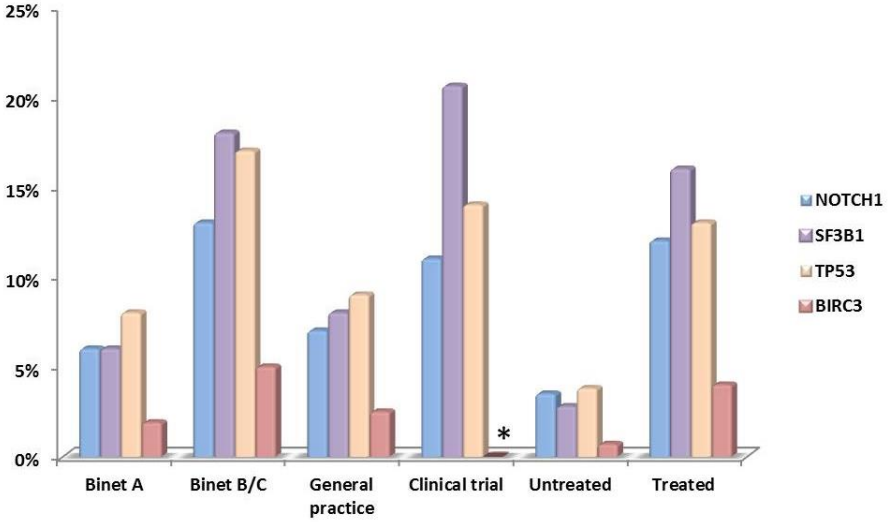
Recurrently affected pathways/processes

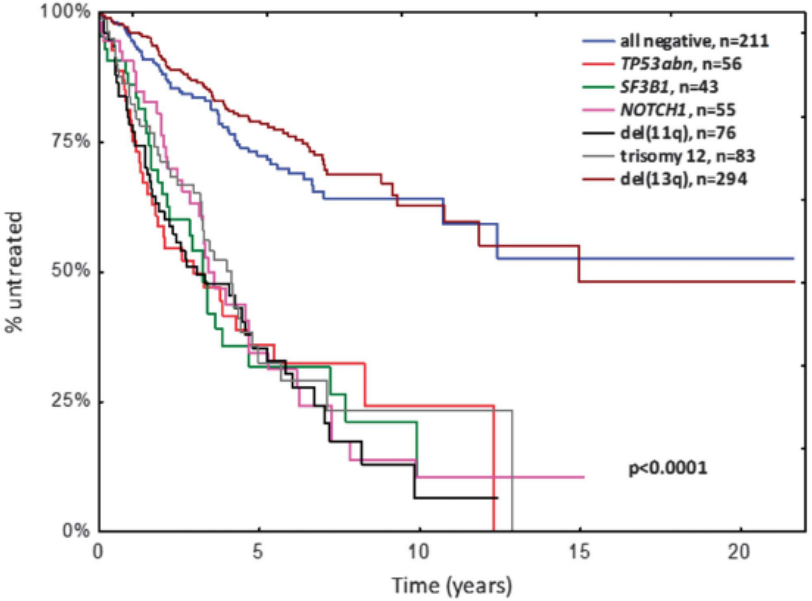


Sutton et al, Semin Cancer Biol 2015

Recurrent mutations refine prognosis in CLL

n=3490 pts

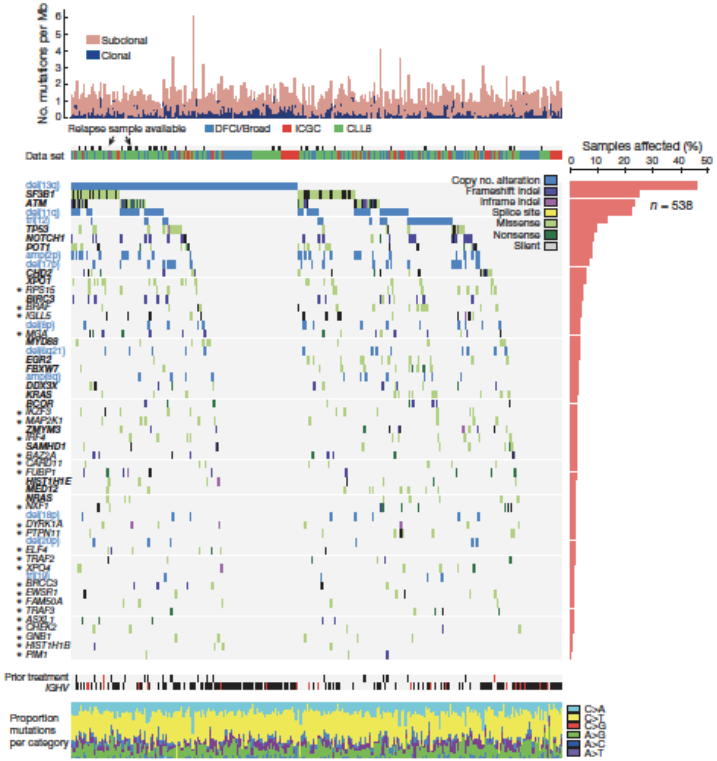


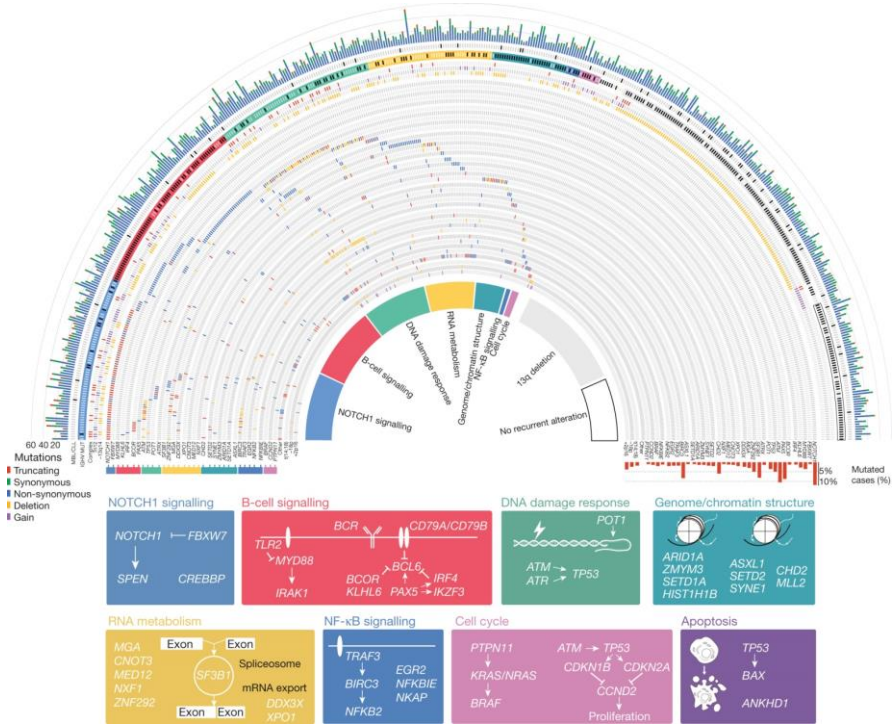


european research initiative on CLL

Baliakas et al, Leukemia 2015

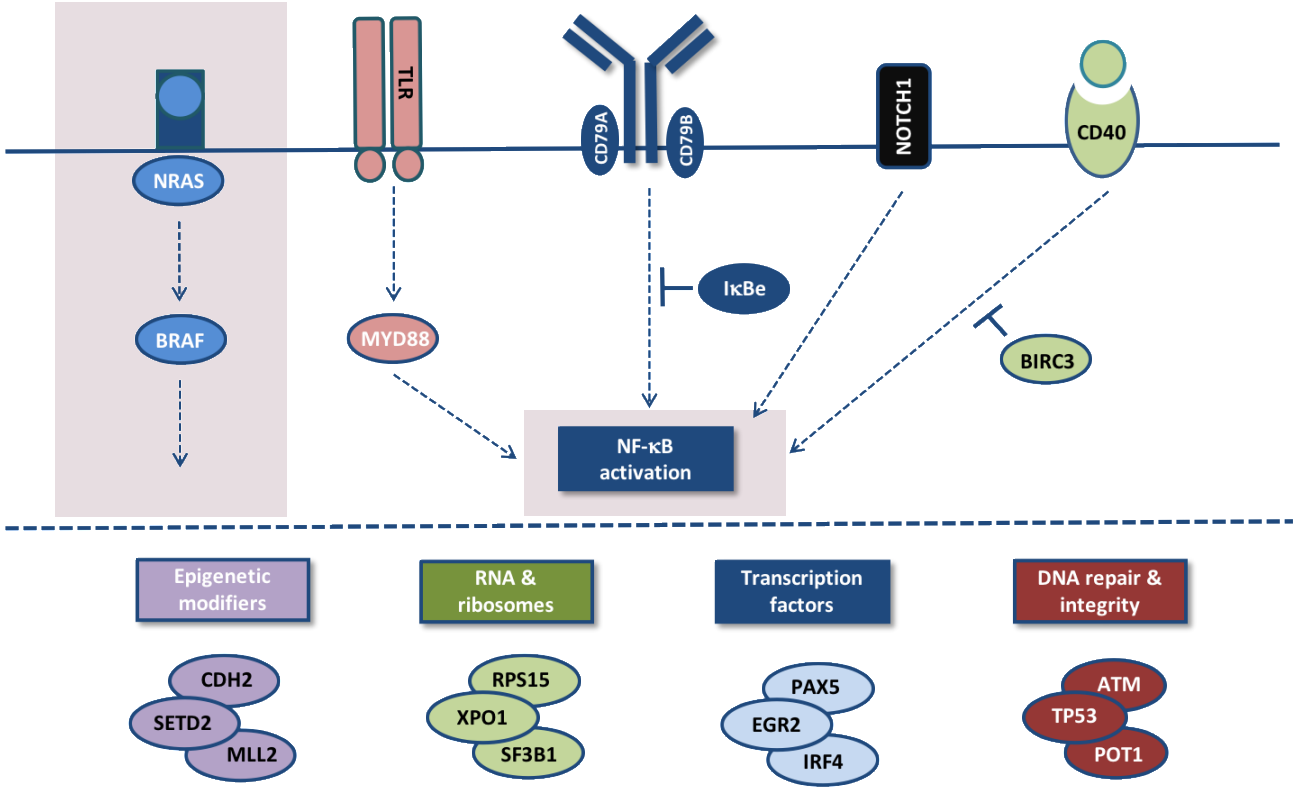
>1000 pts - reaching completion?





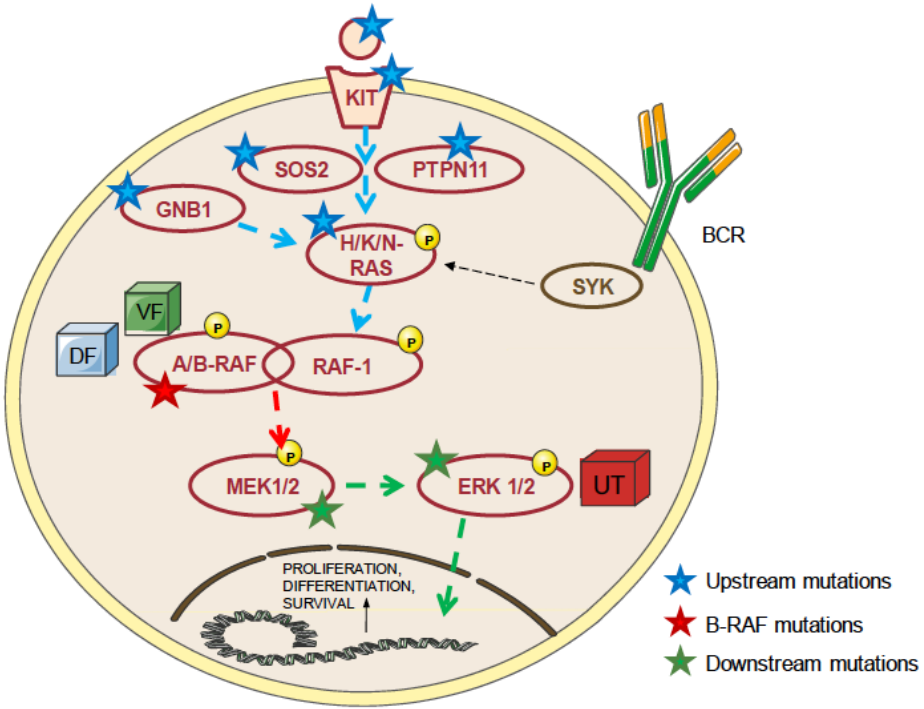
Puente et al, Nature 2015
Landau et al, Nature 2015

Relevance of ’tail genes’?



Sutton et al, Semin Cancer Biol 2015

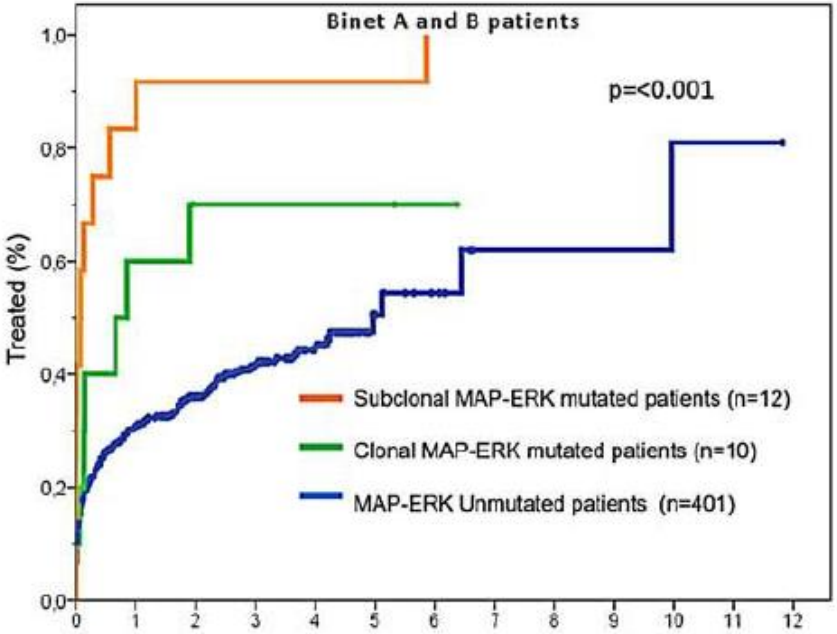
RAS-BRAF-MAPK-ERK pathway mutations define a clinically aggressive subgroup

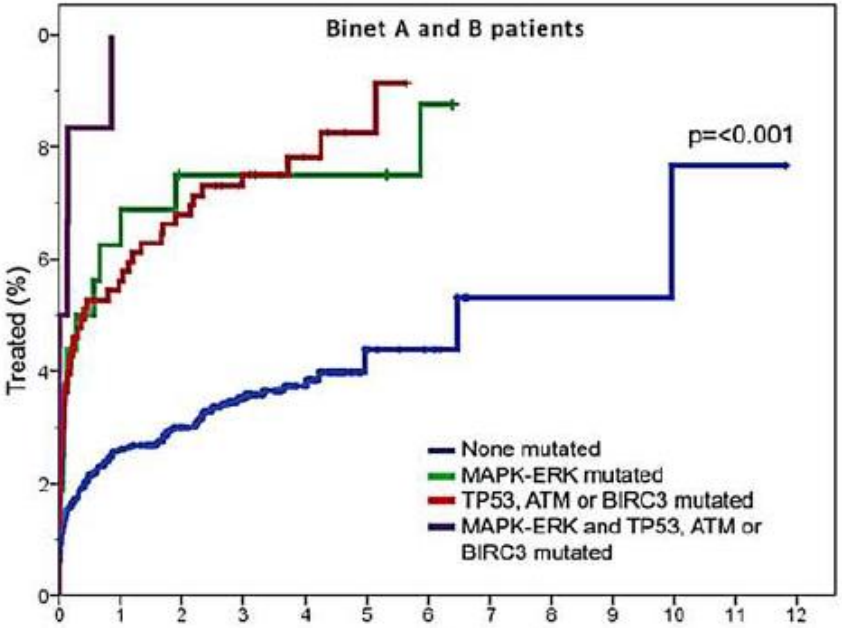


- 25 of 452 patients (5.5%)
- *BRAF* mutations in 9 patients
- Upstream: *KITLG, KIT, PTPN11, GNB1, KRAS* and *NRAS* mutations in 12 patients
- Downstream: *MAPK2K1, MAPK2K2,* and *MAPK1* mutations in 5 patients

Giménez et al, Haematologica 2018

RAS-BRAF-MAPK-ERK pathway mutations associated with short time to first treatment

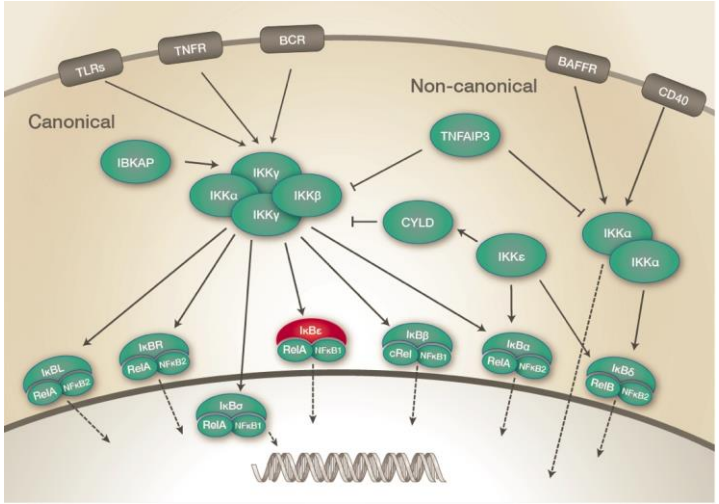


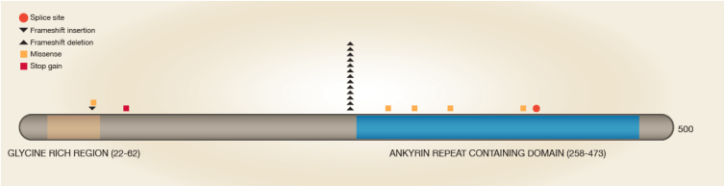


Giménez et al, Haematologica 2018

Targeted sequencing of NF-κB in CLL

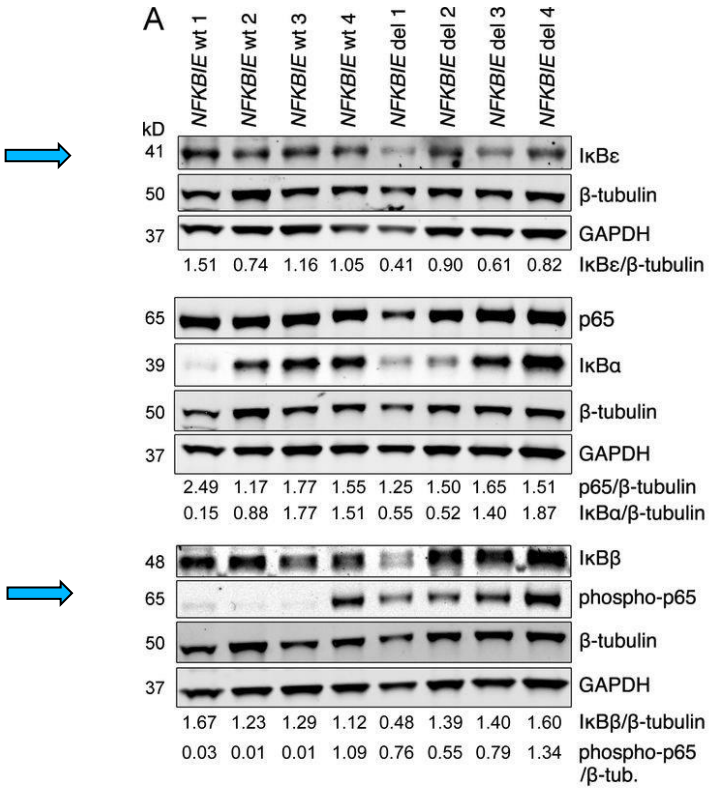
- 18 NF-κB genes
- HaloPlex gene panel
- 315 CLL patients
- **Recurrent 4-bp *NFKBIE* deletion**
- Encodes a negative regulator i.e. IκBε

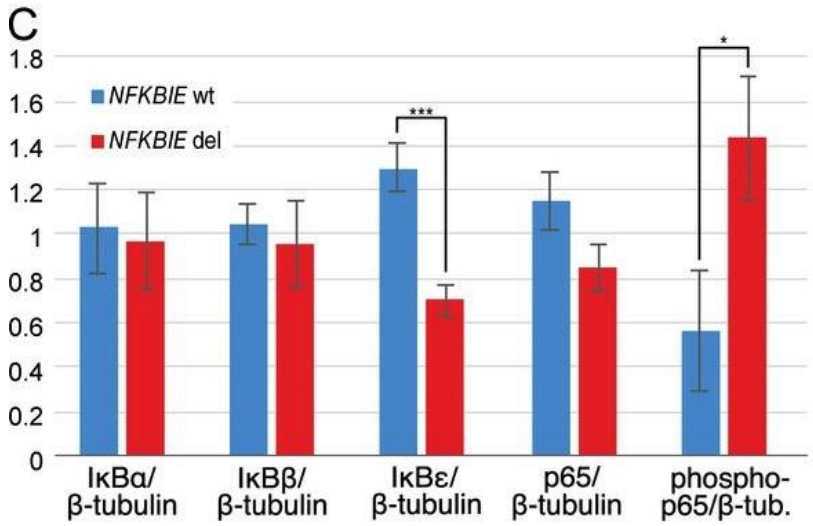




Mansouri et al, J Exp Med 2015

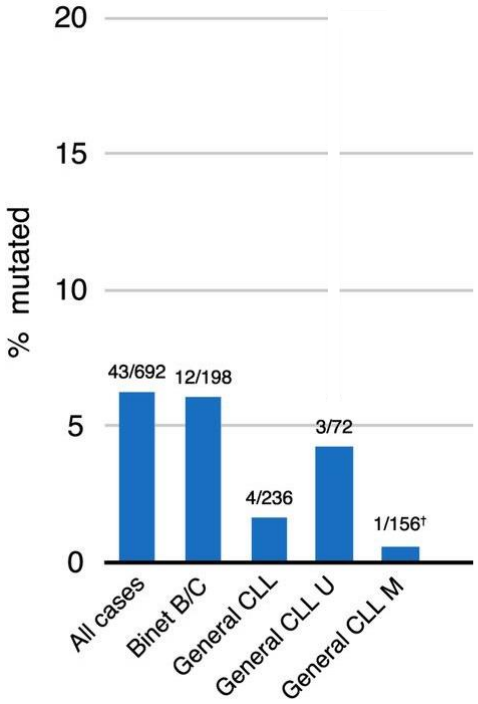
Reduced IκBε leads to increased p65





Mansouri et al, J Exp Med 2015

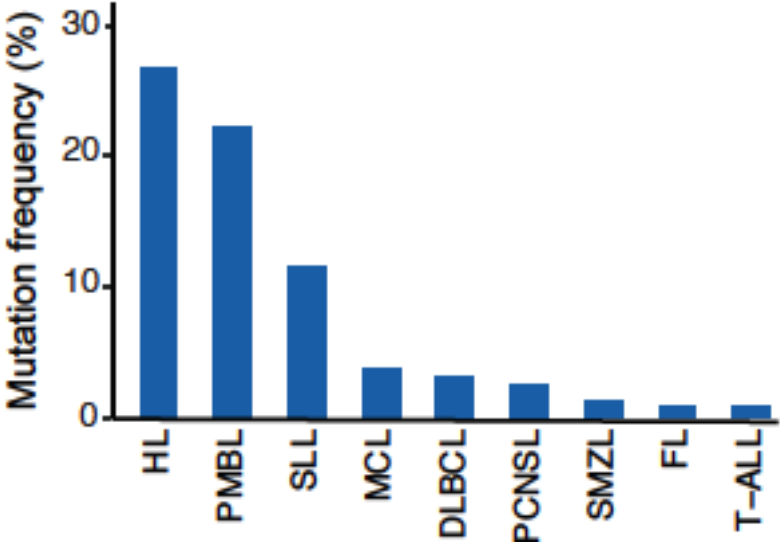
Frequency of *NFKBIE* deletion in CLL



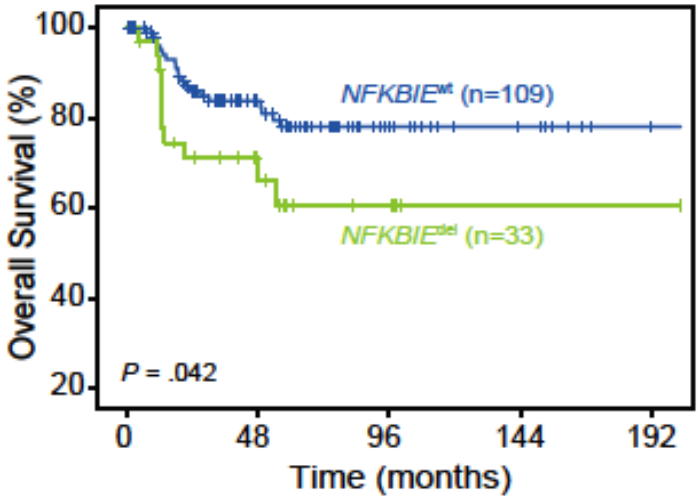
Associated with aggressive disease & enriched in patients carrying stereotyped B-cell receptors

Mansouri et al, J Exp Med 2015
Damm et al, Cancer Discovery 2014

NFKBIE deletion in lymphomas



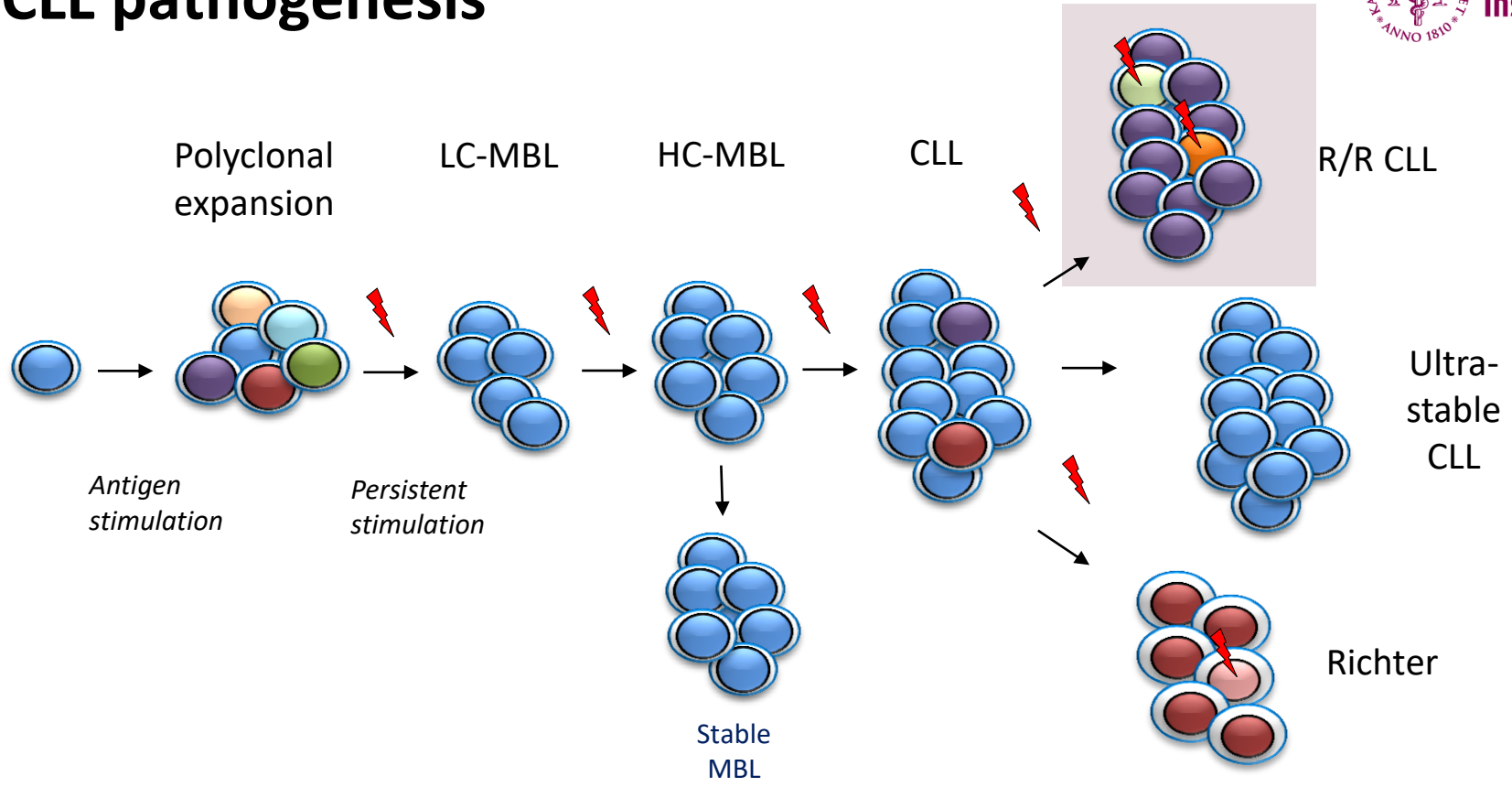
Primary mediastinal B-cell lymphomas



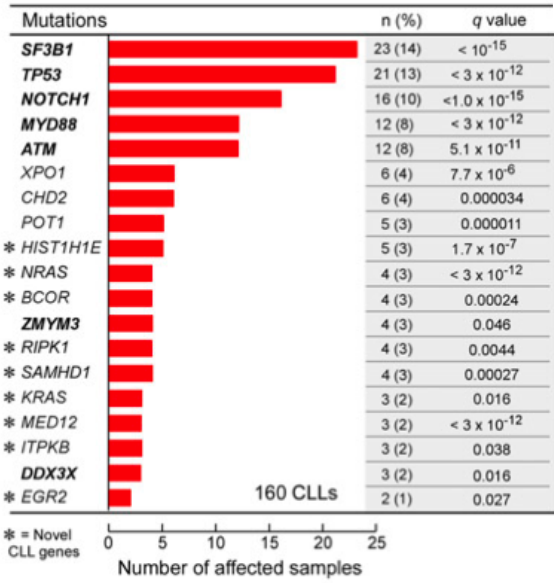
NFKBIE-deleted pts more likely to be refractory to primary chemotherapy compared to wildtype patients (25% vs. 6%, P=.022).

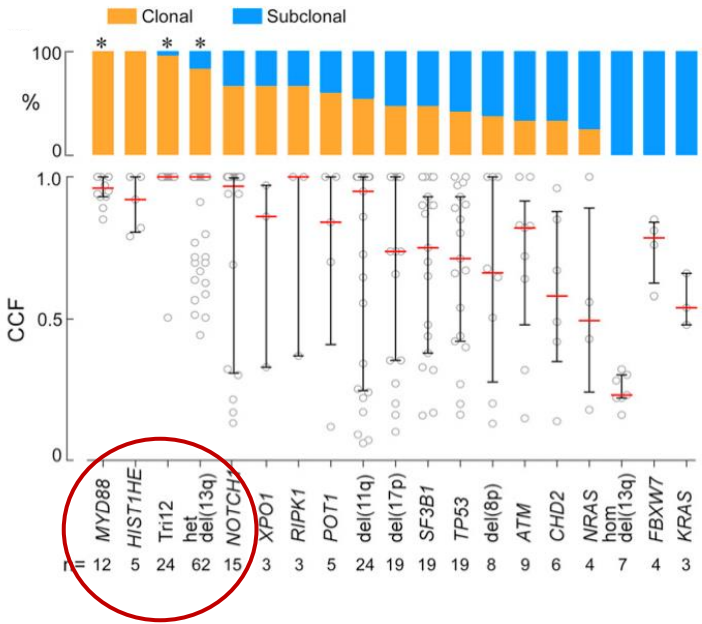
Mansouri et al, Blood 2016

CLL pathogenesis



Driver versus passenger in CLL?

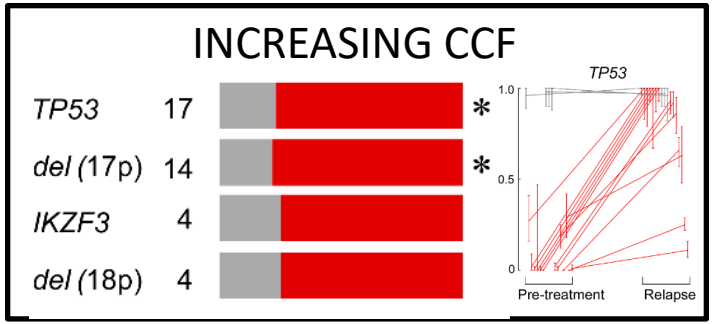


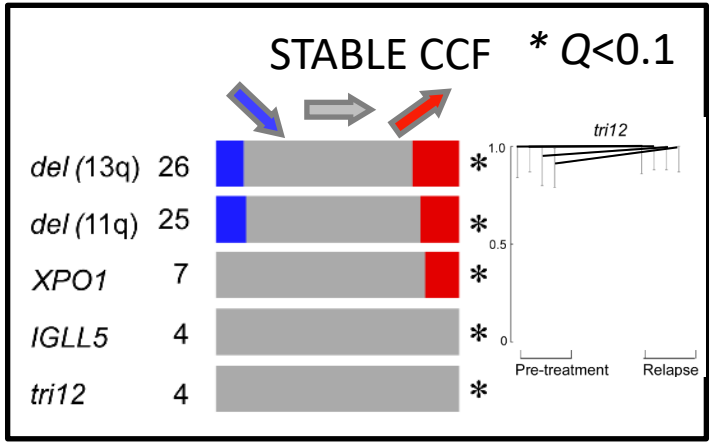


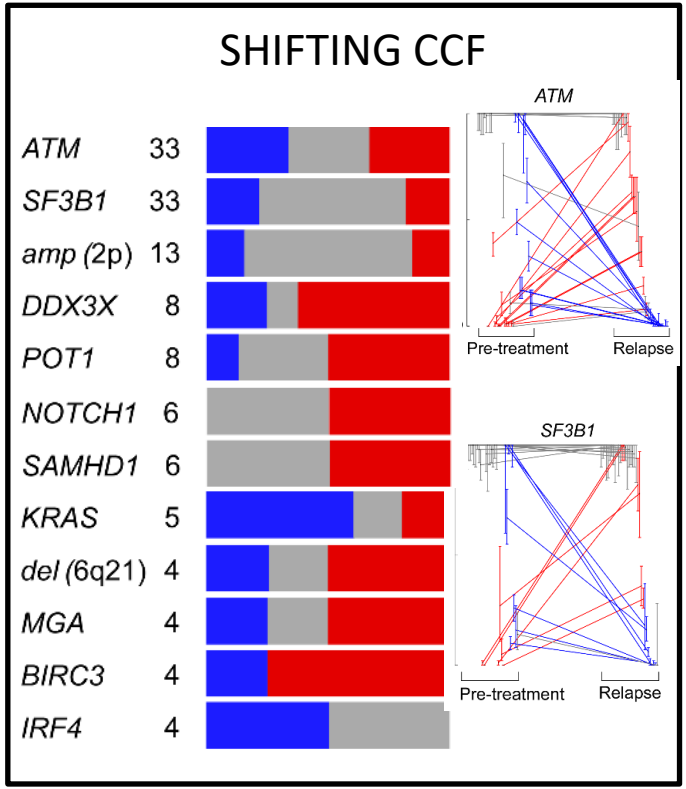
Presence of subclonal driver impacts outcome.

Landau et al, Cell 2013
Landau et al, Nature 2015

Subclonal evolution in CLL treated within the CLL8 trial

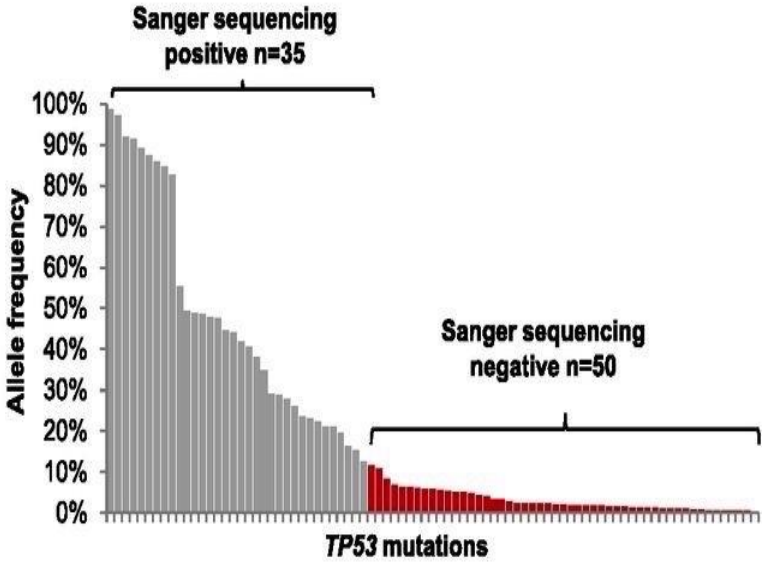


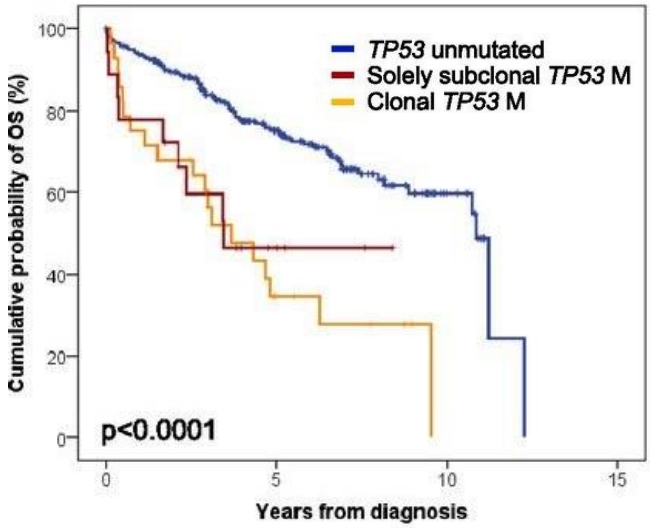




Landau, Nature 2015

Small clones matter: the case of *TP53*

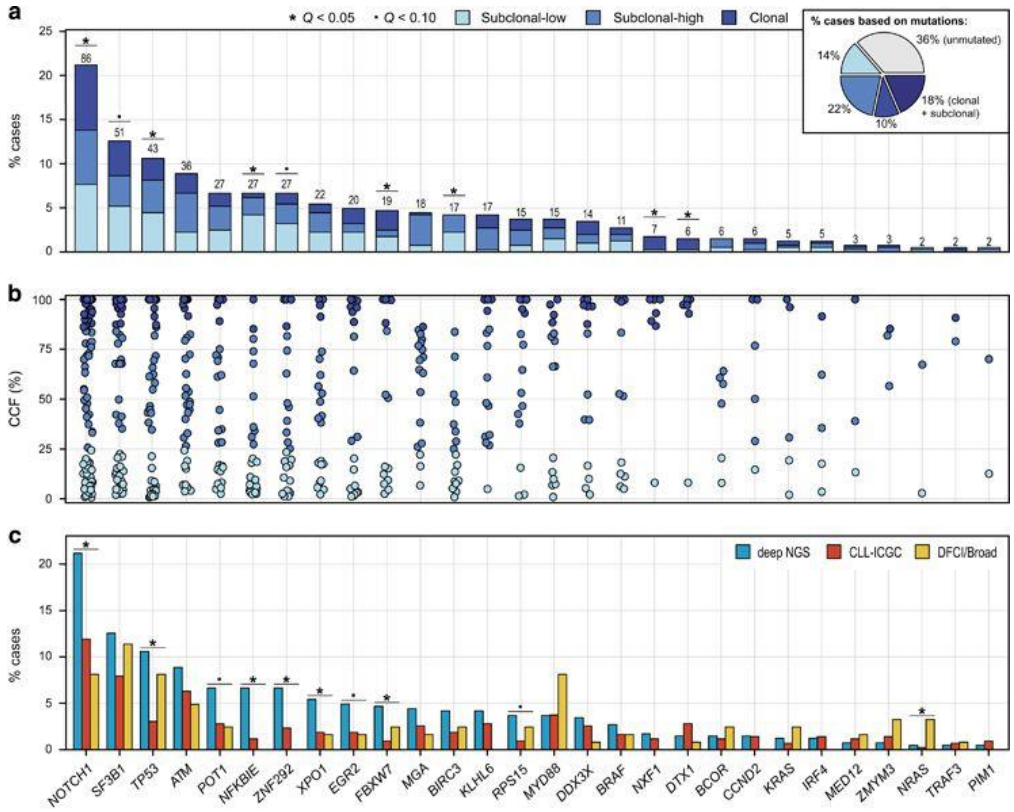


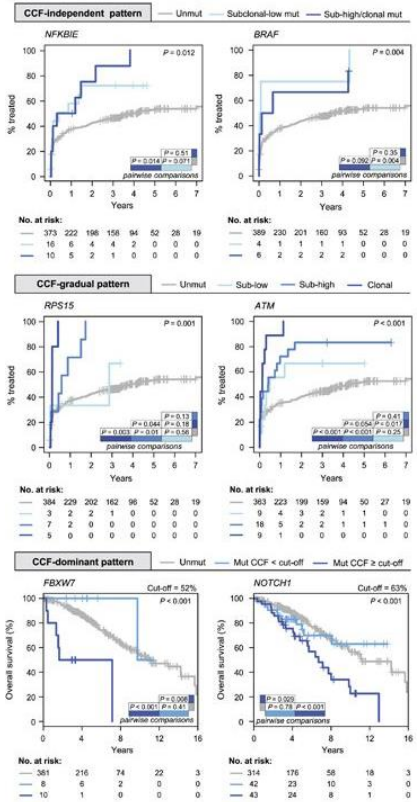


28/309 (9%) untreated CLL (median allele frequency: 2%)

Rossi et al, Blood 2014, Malcikova et al, Leukemia 2015, Nadeu et al, Blood 2016

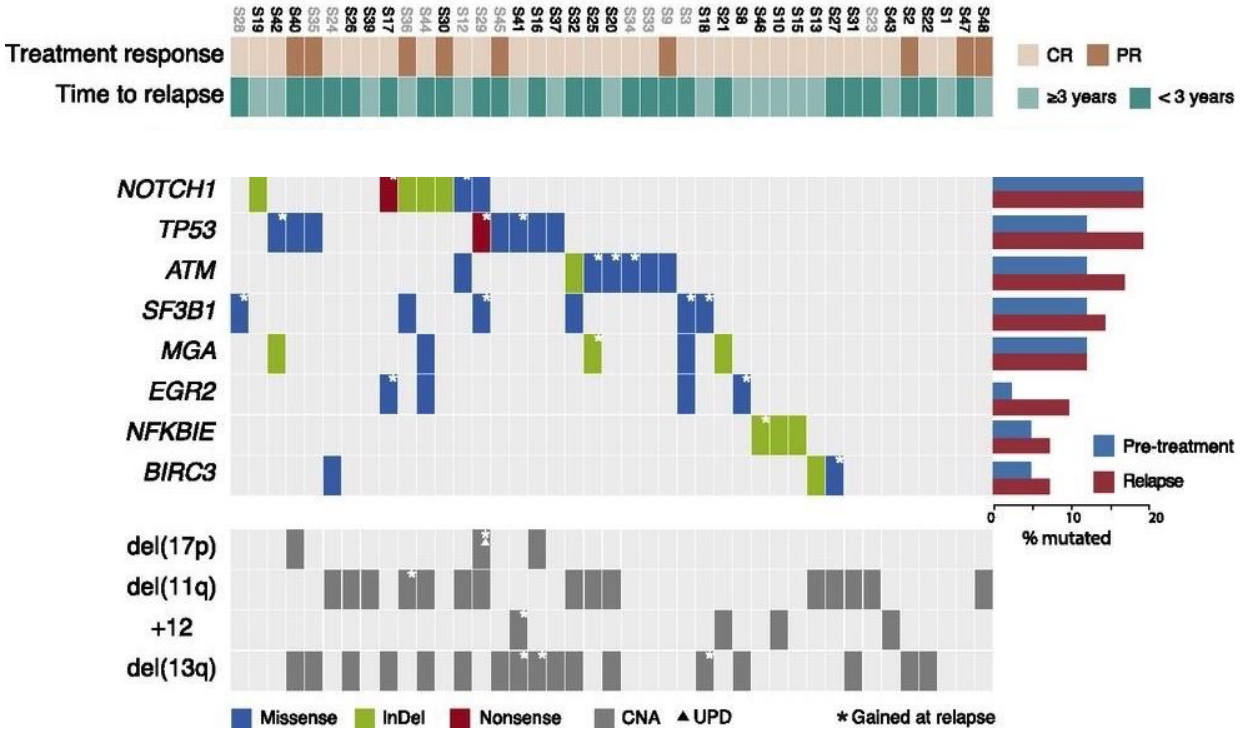
Not only *TP53*…





Nadeu et al, Leukemia 2018

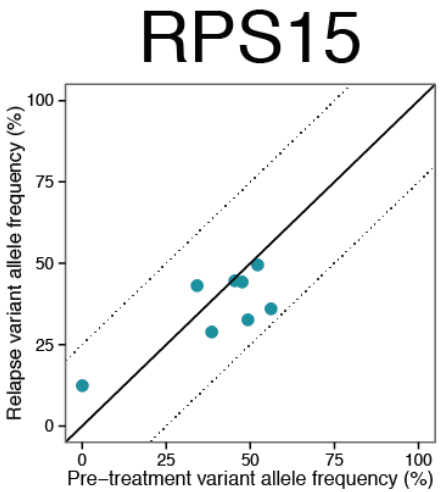
Mutation spectra in FCR-relapsing patients

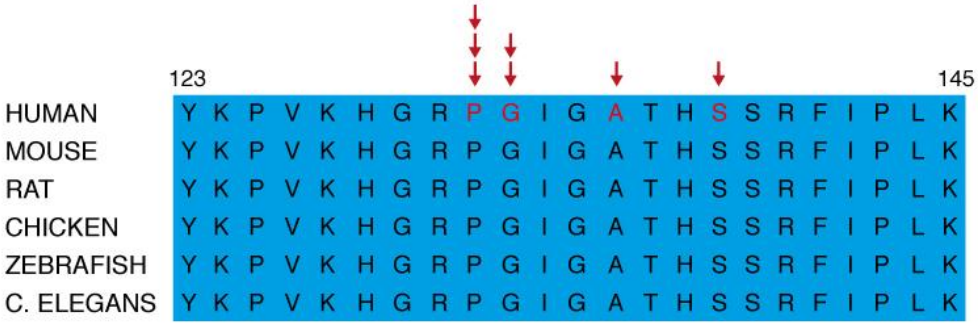


Ljungström et al, Blood 2016

RPS15 mutations in a 'hotspot' region

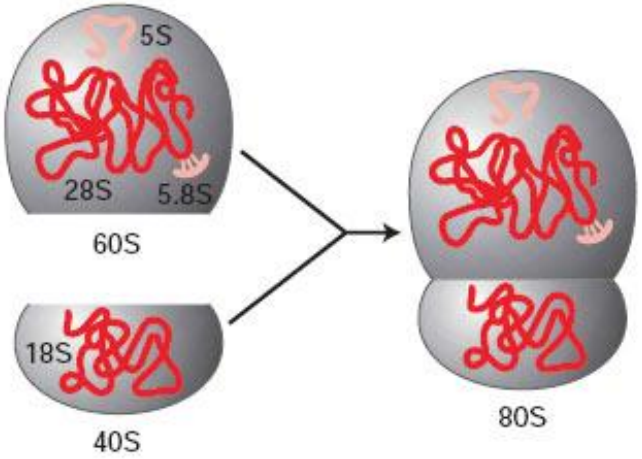
- Missense mutations, majority stable at clonal level
- 7/8 clustered to a 7 amino acid long, evolutionarily conserved region

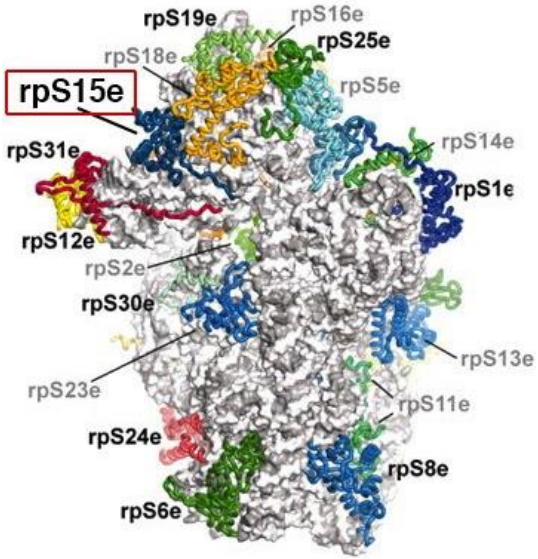




Ljungström et al, Blood 2016

RPS15 – a component of the 40s subunit

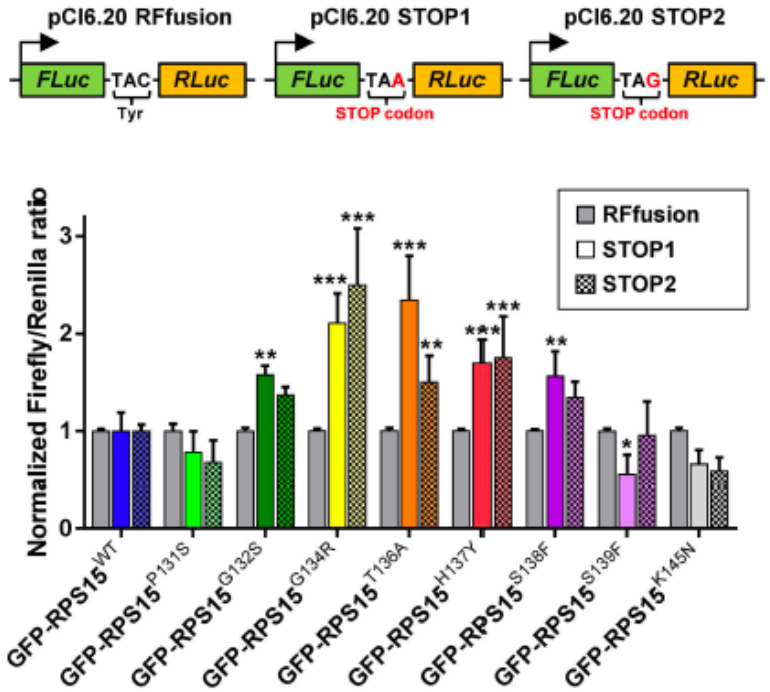


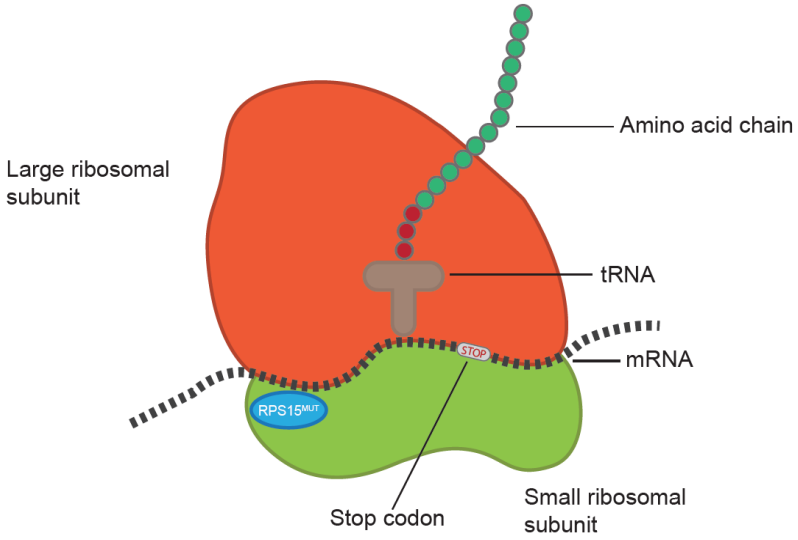


- Protein translation
- Potential role in regulating the MDM2-p53 axis

Adapted from Rabl et al, Science 2011

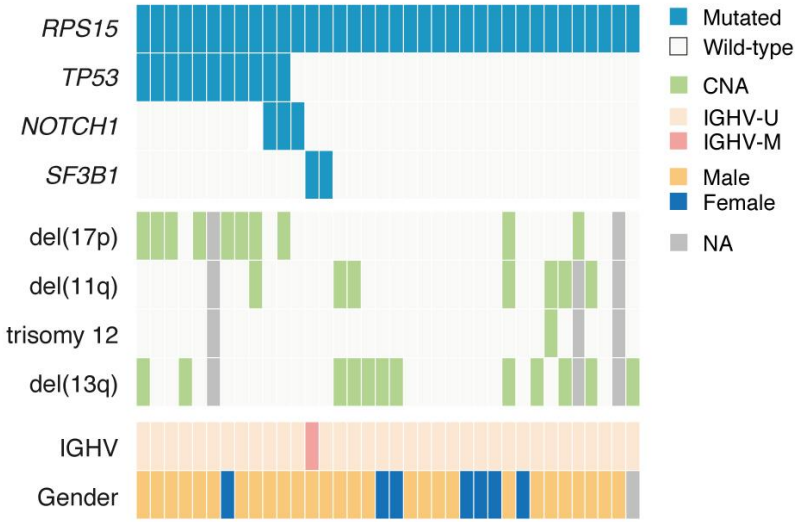
Altered ribosome activity/fidelity linked to *RPS15* mutations

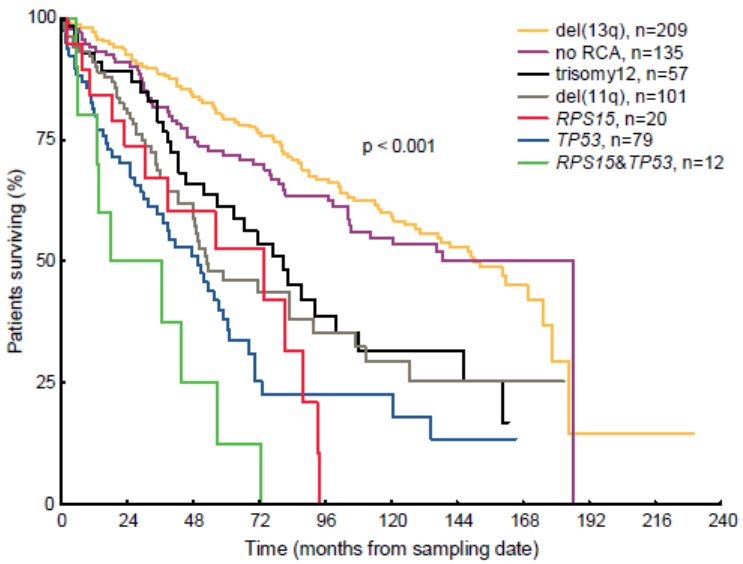




Bretones et al, Blood 2018

Clinicobiological associations - *RPS15* mutations

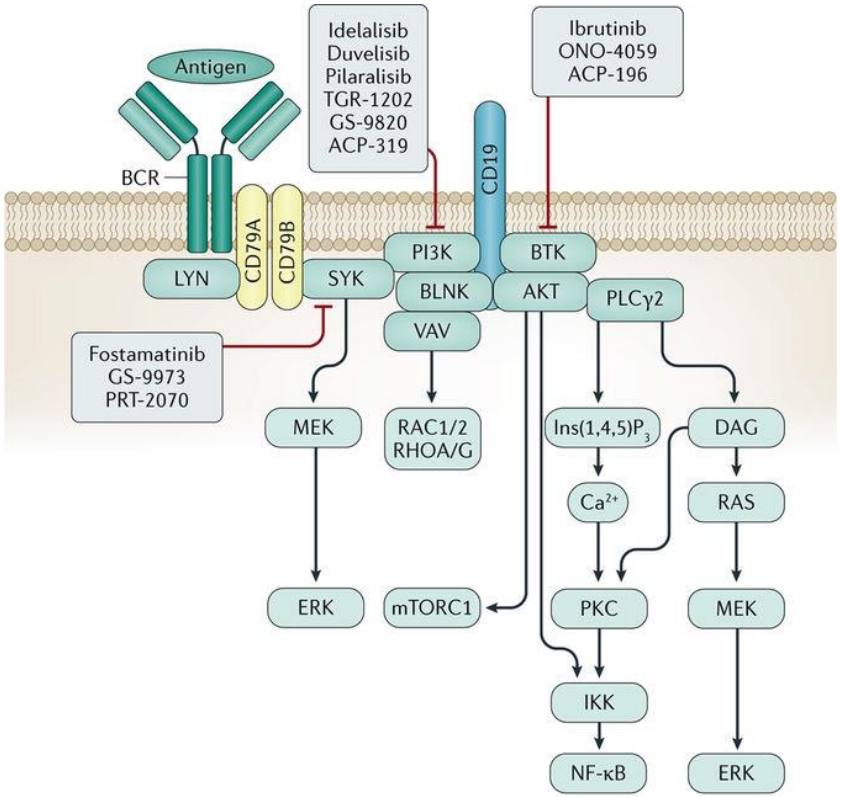




Ljungström et al, Blood 2016

The new era…

- BTK inhibitors
- PI3Kδ inhibitors
- BCL2 inhibitors



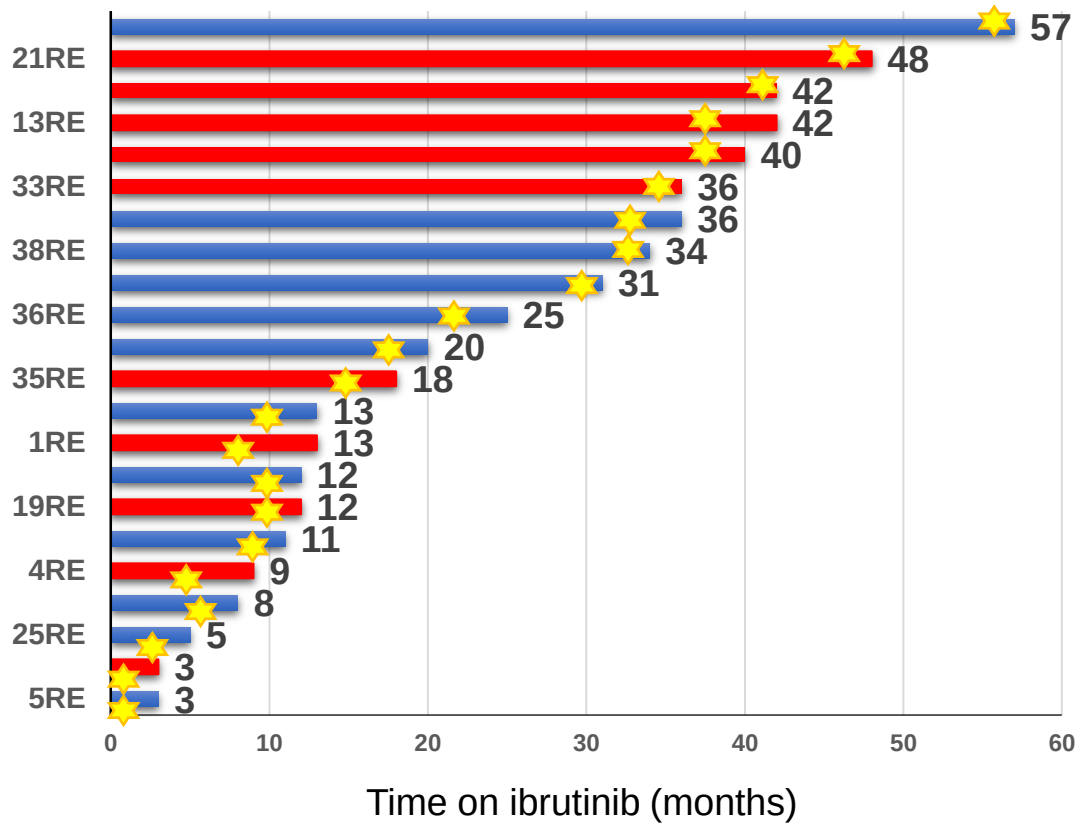
Kipps et al, Nature Reviews Disease Primers 2017

Resistance mutations on ibrutinib in relapsed CLL

Ref.	Pts with mut/total	Analytical Method	*BTK* mutations	VAF, %	PLCγ2 mutations	VAF, %
Woyach, 2014	6/6	WES	C481S	17-60	R665W; L845F S707Y	38 29 8
Ahn, 2017	9/10	High sensitivity NGS; ddPCR	C481S C481R	1.6-78.2 15.8	6nt del; R665W P664S; S707Y; L845F	0.1-18.3
Woyach, 2017	40/46 (retrospective); 8/8 (prospective)	Targeted Deep Sequencing	C481S C481R C481F C481A	0.2-94.8 18.1 1.1-100 45.3	R665W; S707P; S707F; S707Y; L845F; D993Y; L845/846del	3.6-44
Burger, 2016	2/4	WES; ddPCR	C481S	NR	M1141R; S707F; M1141K; D993H	12.0-35.0 (CCF)
Landau, 2017	5/7	Targeted Deep Sequencing	C481S	2.2-78.2	R665W; S707Y; L845F	0.2-4.7
Kadri, 2017	1/3	Targeted Deep Sequencing	C481S C481R	8.5-90.0 2.5	NR	NR

Sutton, Bonfiglio, Scarfò et al

Multicenter study on ibrutinib resistance



- Deep sequencing of *BTK* and *PLCG2*
- Relapsing (n=22) and responding pts (n=34)
- 10/22 relapsed cases showed *BTK* (10 pts) or *PLCG2* (4 pts) mutations.

Progression and sampling

BTK mutated +/-*PLCG2* mutated

BTK and *PLCG2* wildtype

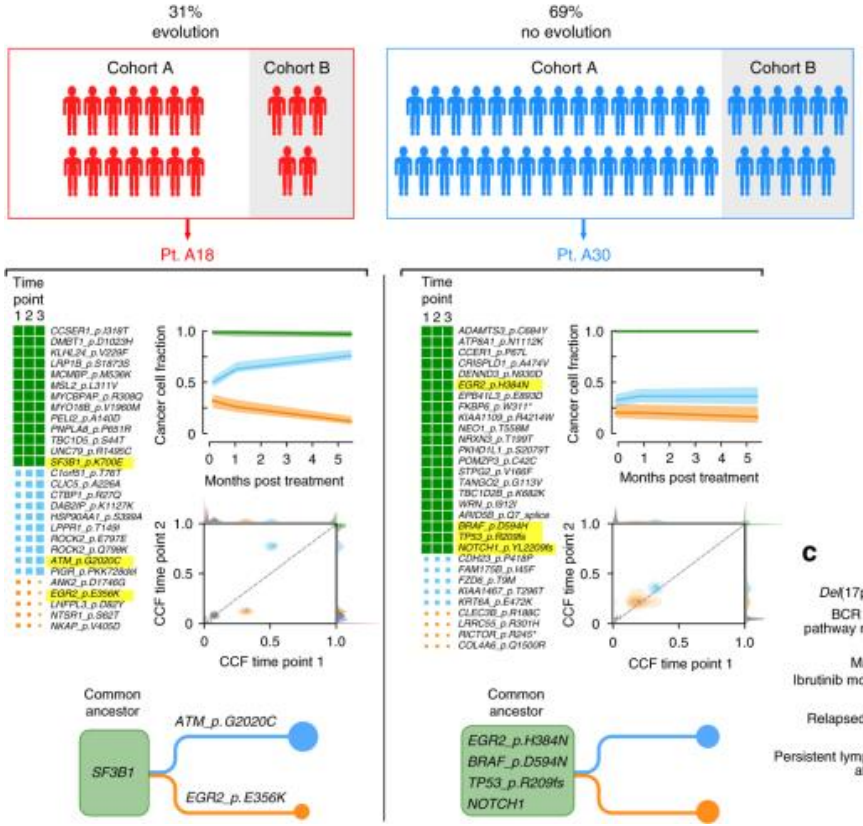
Sutton, Bonfiglio, Scarfò et al

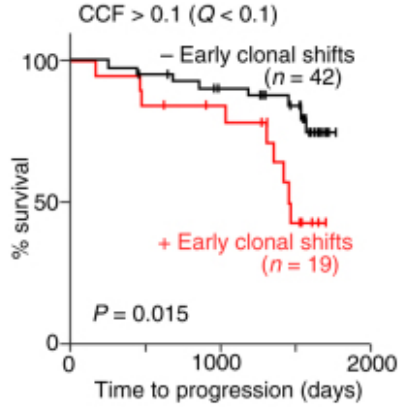
BTK mutations

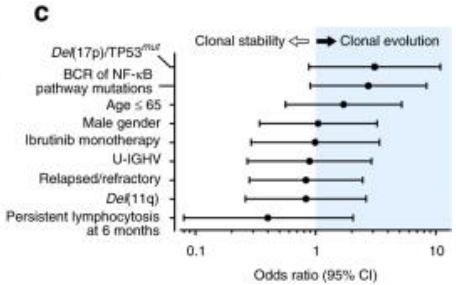
Pt #	Gene	Exon	AA change	WT/MT codon	VAF, %
35RE	*BTK*	15	C481R	c.T1441C	36.57
19RE	*BTK* *BTK*	15 15	C481S C481S	c.T1441A c.G1442C	51.80 13.20
21RE	*BTK*	15	C481S	c.G1442C	29.30
13RE	*BTK*	15	C481S	c.G1442C	34.48
1RE	*BTK*	15	C481S	c.G1442C	4.37
4RE	*BTK*	15	C481S	c.G1442C	19.29
7RE	*BTK*	15	C481S	c.G1442C	35.22
16RE	*BTK*	15	C481S	c.G1442C	33.22
39RE	*BTK*	15	C481S	c.G1442C	8.04
33RE	*BTK* *BTK*	15 15	C481S C481S	c.G1442C c.T1441A	2.67 1.98

Sutton, Bonfiglio, Scarfò et al

The evolutionary landscape of CLL treated with ibrutinib



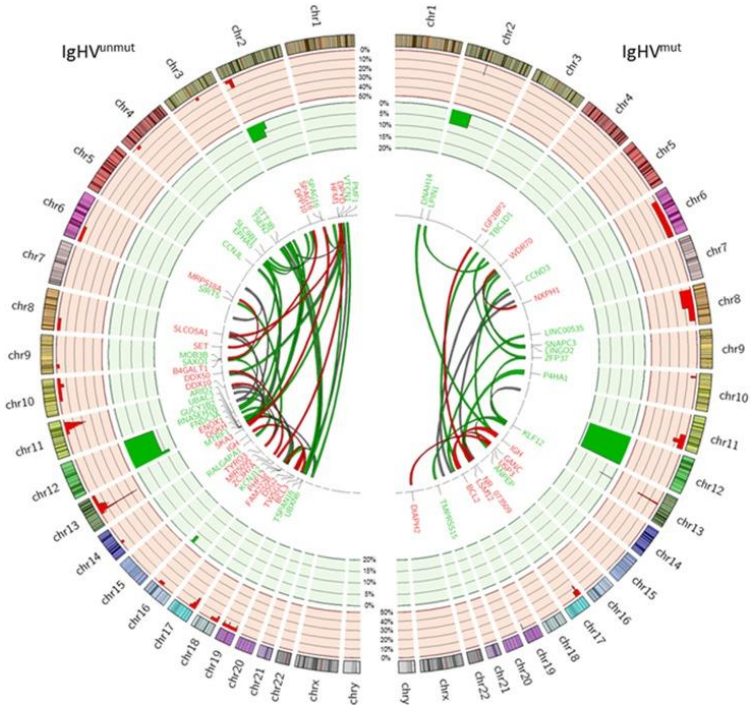


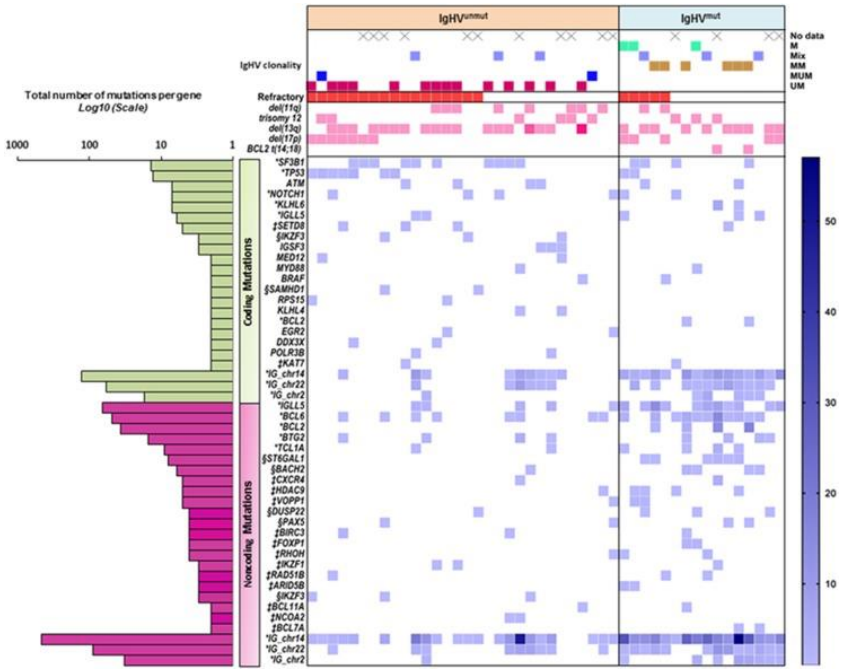


Landau et al; Nature Comm 2017

Can we subgroup patients in a better way?

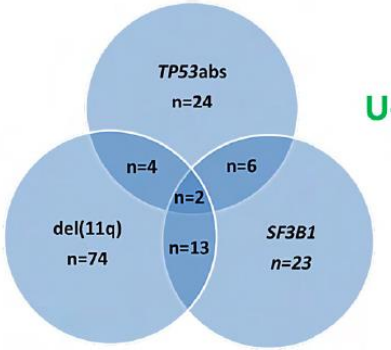
WGS reveals distinct differences in the mutational landscape between IGHV-M and IGHV-UM^{t} CLL



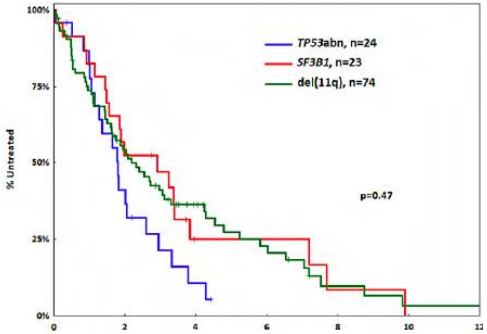


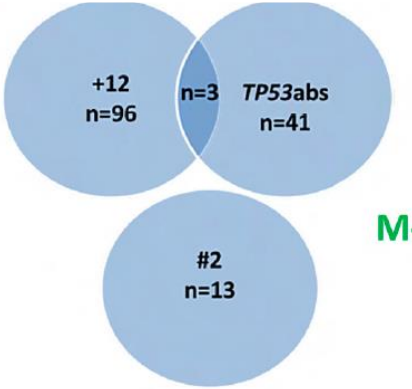
Burns et al, Leukemia 2017

Are the same factors linked to poor prognosis in IGHV-M and IGHV-U CLL?

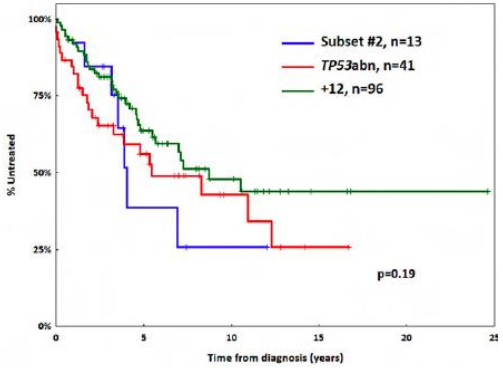


U-CLL





M-CLL

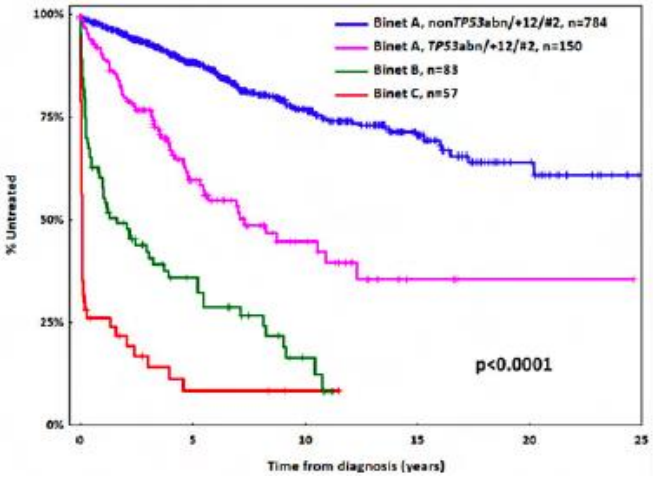


Baliakas et al, Haematologica 2018

Tailored approaches based on immunogenetic features for refined prognostication in CLL

A

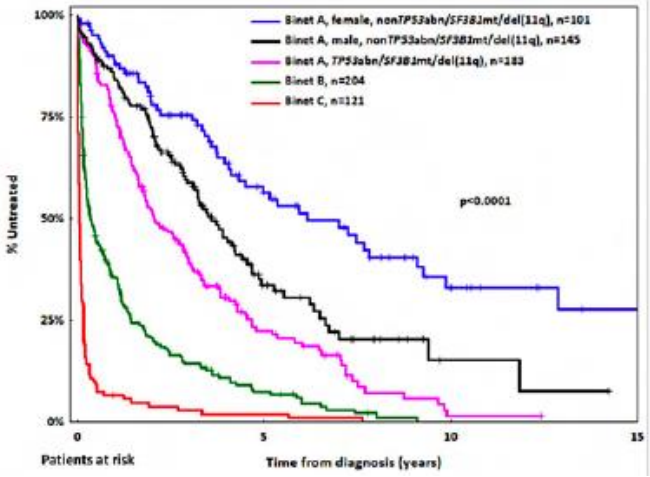
Risk group	5-yTP	10-y TP
Very high risk 5% of M-CLL mTTF: 0.1 years	92%	92%
High risk 8% of M-CLL mTTFT: 1.5 years	64%	84%
Intermediate risk 14% of M-CLL mTTFT: 8 years	40%	55%
Low risk 73% of M-CLL mTTFT: nr	12%	25%



M-CLL

B

Risk group	5-yTP	10-y TP
Very high risk 16% of U-CLL mTTF: 0.1years	100%	100%
High risk 27% of U-CLL mTTFT: 0.4 years	90%	100%
Intermediate risk 24% of U-CLL mTTFT: 2.1 years	78%	98%
Low risk 19% of U-CLL mTTFT: 3.6 years	65%	85%
Very low risk 13% of U-CLL mTTFT: 6.1 years	45%	65%



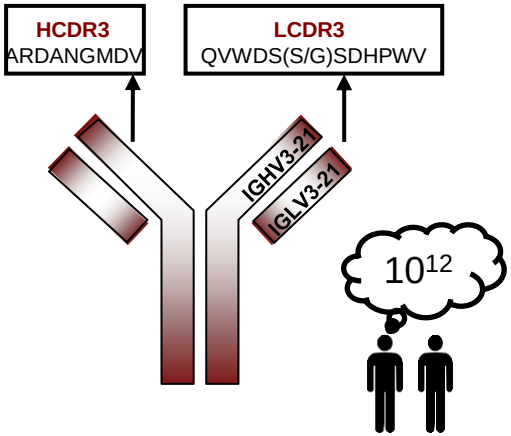
U-CLL

N=3015

Baliakas et al, Haematologica 2018

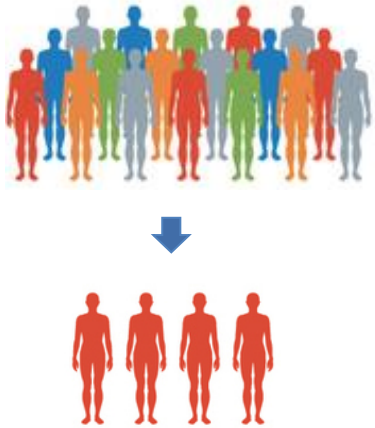
Stereotyped B-cell receptors in CLL

Highly similar B-cell receptors



>30% of CLL patients
Recognize similar epitopes

More homogenous subgroups



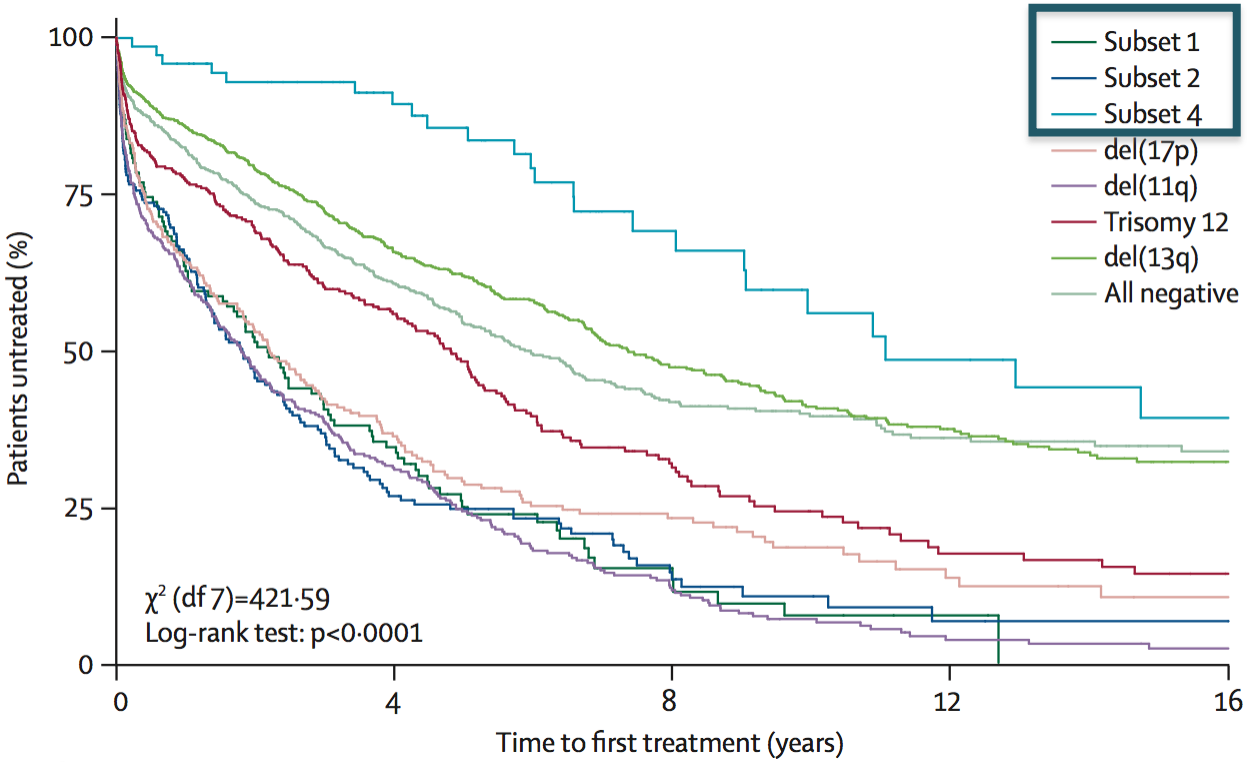
Share clinical and biological profiles

Karolinska Institutet

SUBSET #1	SUBSET #2	SUBSET #4	SUBSET #8
2.4% of all CLL	2.8 % of all CLL	1% of all CLL	0.5% of all CLL
Very aggressive (TTFT 1·9 years)	Very aggressive (TTFT 1·6 years)	Very indolent (TTFT 11 years)	Very aggressive-highest risk for RT (TTFT 1·5 years)
U-CLL	Both U-CLL and M-CLL	M-CLL	U-CLL
Significantly up-regulated EZH2 levels	High incidence of del(11)(q22q23)	Few genetic aberrations	High frequency of trisomy 12
Recurrent *NFKBIE* gene mutations	Significant enrichment of *SF3B1* mutations	Ongoing SHM	Prevalence of *NOTCH1* mutations
Pronounced BcR and TLR signaling	Low frequency of *TP53* aberrations	IG features of anti-DNA Abs	Promiscuous antigen binding reactivity
		Signature of B-cell anergy	

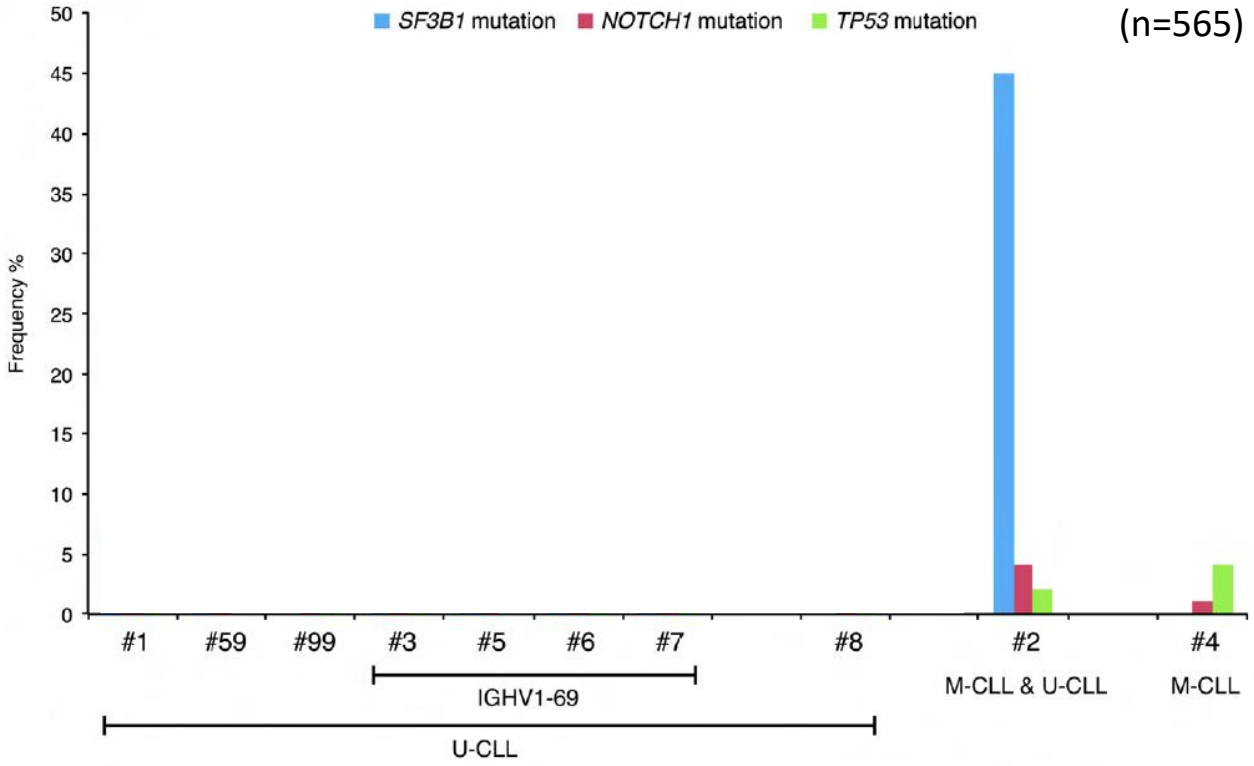
Stamatopoulos et al, Leukemia 2017

Subsets are distinct clinical entities



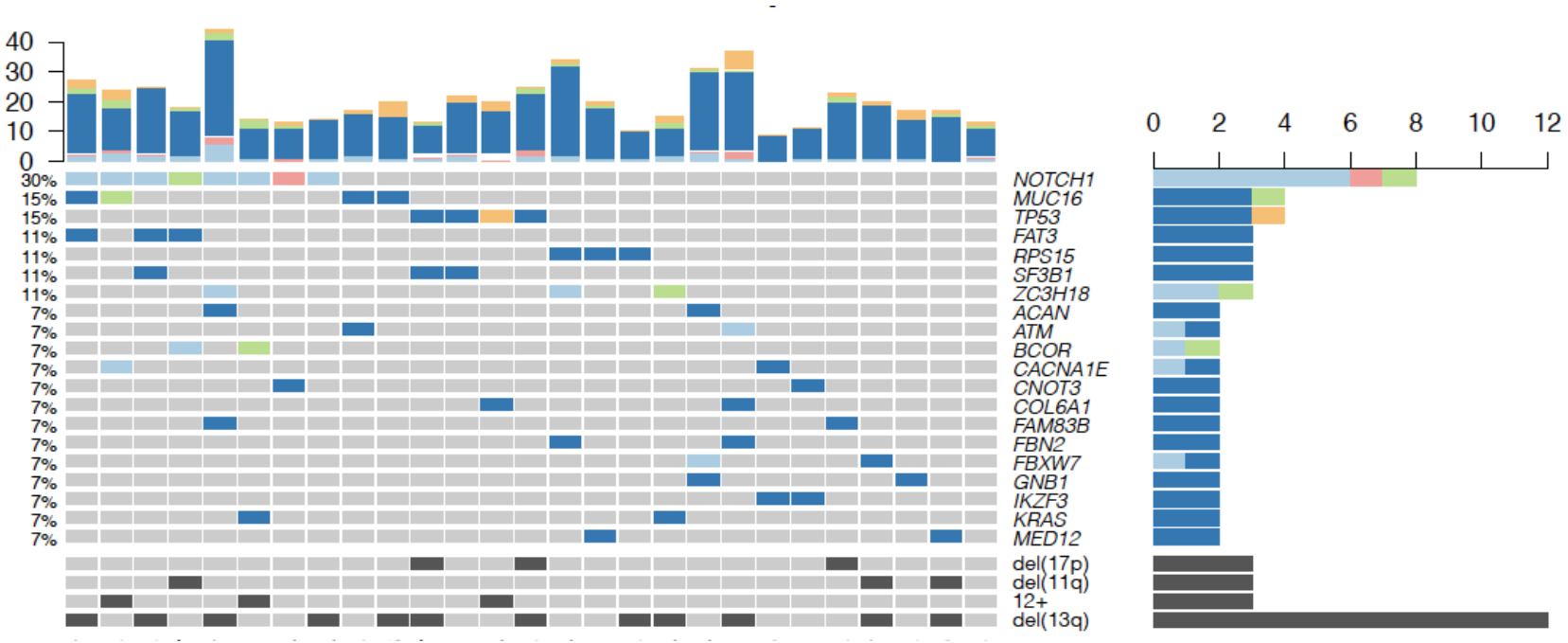
Baliakas et al. Lancet Haematol 2014

Different subsets, distinct recurrent mutation profiles



Sutton et al, Haematologica 2016

Whole-genome sequencing of subset #1



26/27 cases carry a known CLL driver gene mutation

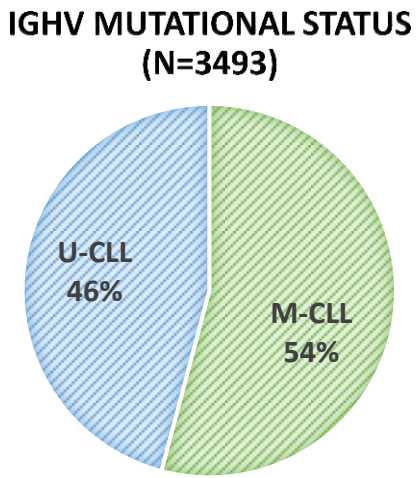
Ljungström et al

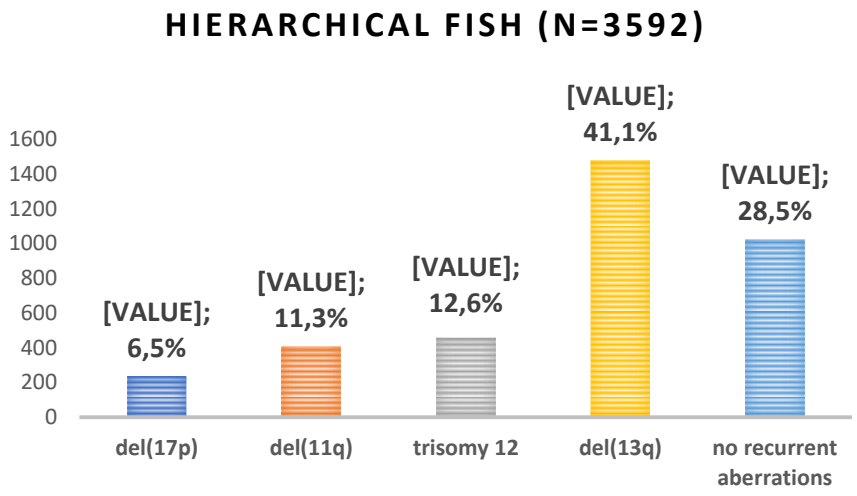
How to bring order out of this?

Large-scale project on CLL gene mutations

ERIC
european research initiative on CLL

- >20 centers, 4800 pts
- 10 genes, full clinical data required

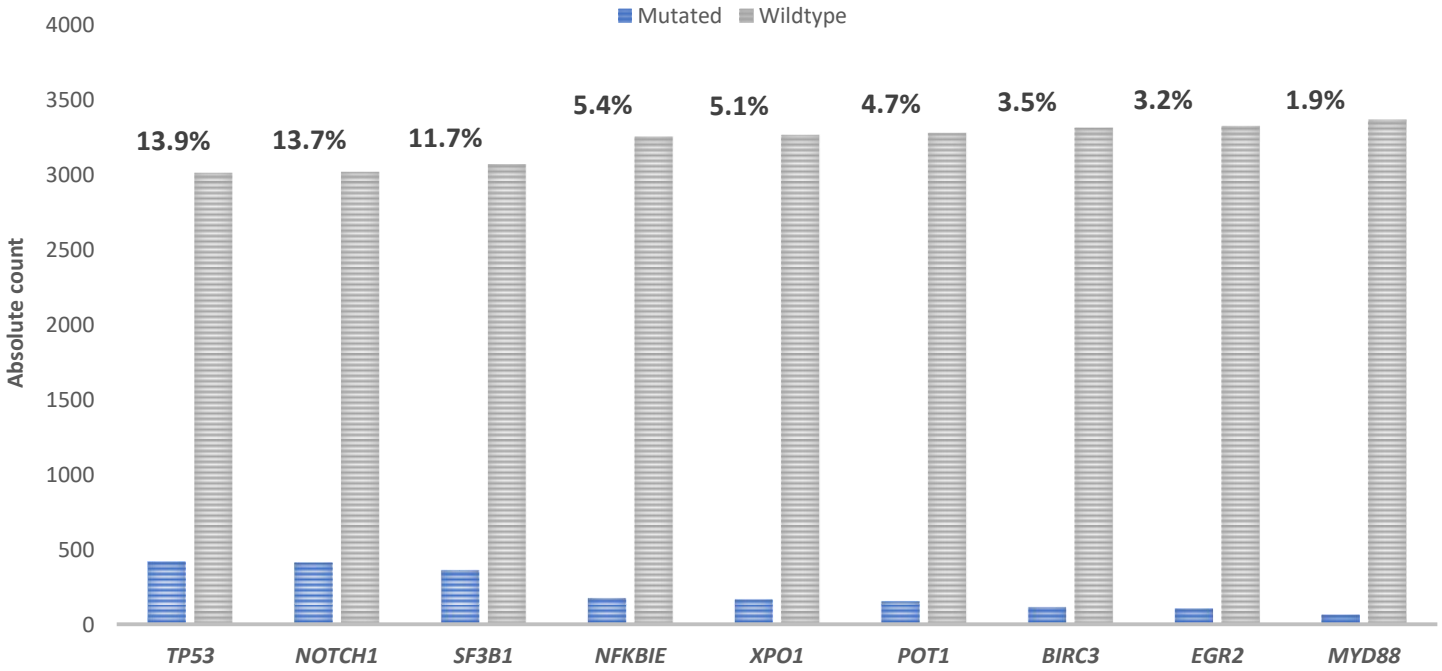




ATM
BIRC3
MYD88
NOTCH1
SF3B1
TP53
EGR2
POT1
NFKBIE
XPO1

Sutton et al.

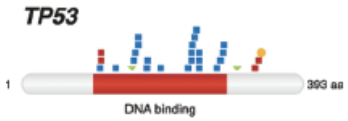
Large-scale project on CLL gene mutations

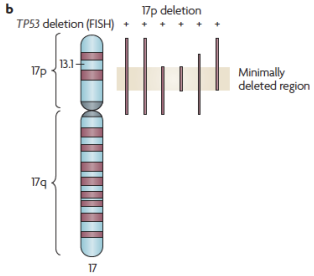


n=3425

Sutton et al.

SNV/indel	Copy-number aberrations	Structural aberrations





Today	1-2 years	3-5 years

Molecular profiling

FISH + NGS panel
Arrays/cytogenetics → Broad gene panels (IG/TR) → Whole-genome sequencing

Overtime analysis

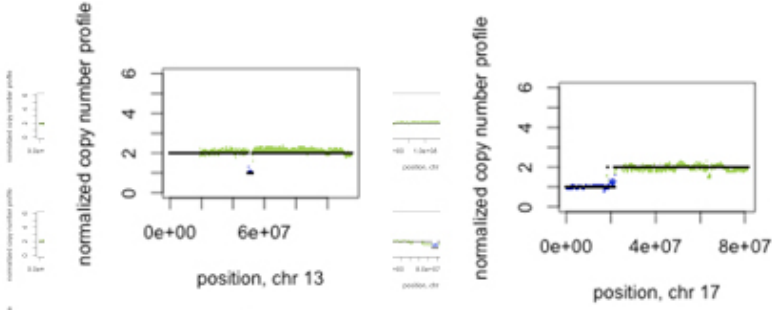
FISH
NGS panel → NGS panel
ddPCR → Whole-genome sequencing?

Extracting information from WGS in CLL

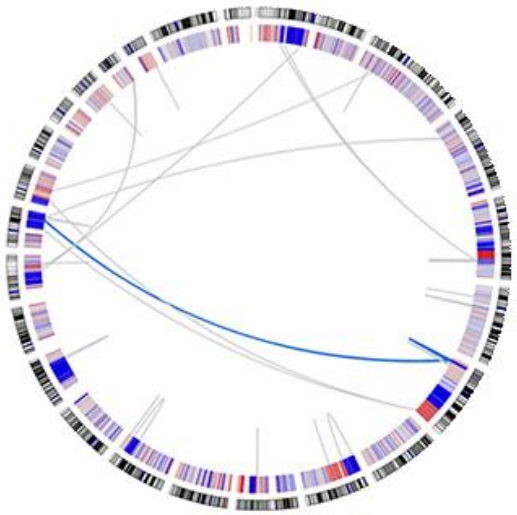
SMALL MUTATIONS

Chr	Start	End	Ref	Variant	Gene	Type	Exonic_type	VAR %
chr2	198266834	198266834	T	C	SF3B1	exonic	nonsynonymous SNV	11,45
chr11	108183167	108183167	A	G	ATM	exonic	nonsynonymous SNV	48,2
chr17	7579472	7579472	G	C	TP53	exonic	nonsynonymous SNV	46,32

COPY NUMBER ABERRATIONS

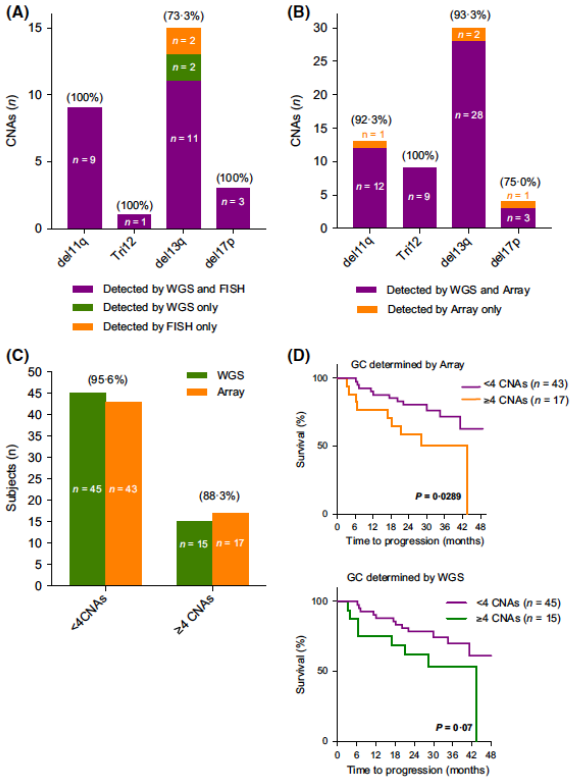


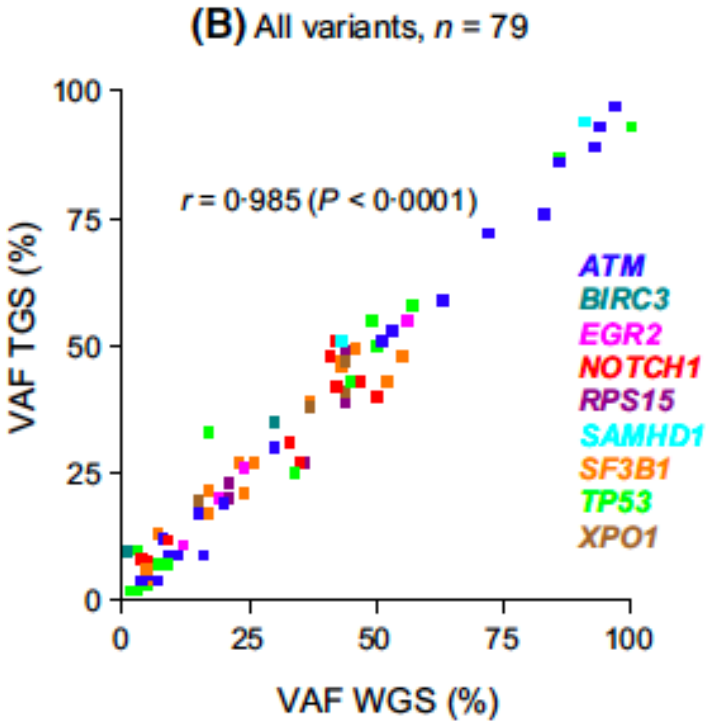
STRUCTURAL VARIANTS



Ljungström et al

Validation of WGS reveals robust detection of low-frequency variants and copy number alterations in CLL





Klintman et al BJH 2018

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