ERIC
european research initiative on CLL

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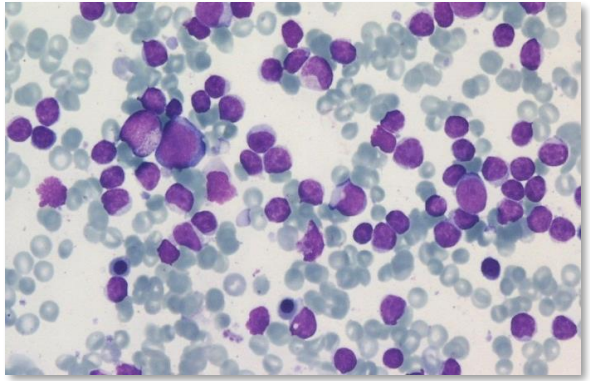
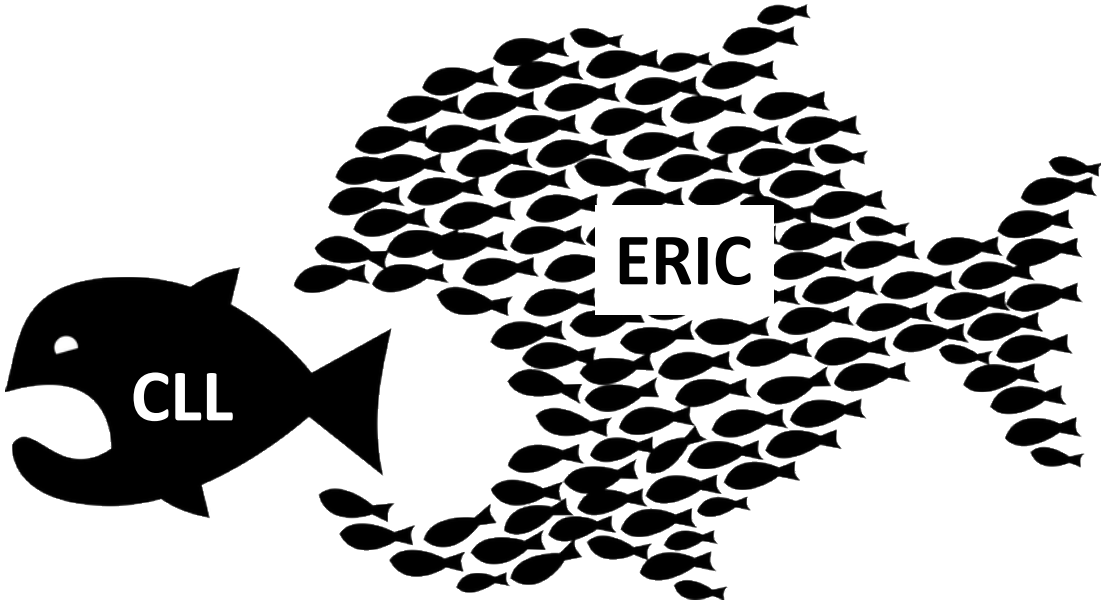
Research within ERIC: in the spirit of collaboration



Richard Rosenquist, MD, PhD
Karolinska Institutet, Stockholm, Sweden

Joining forces



New tool box for diagnostics & research

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Whole-genome

3 000 000 000 bp

Exome

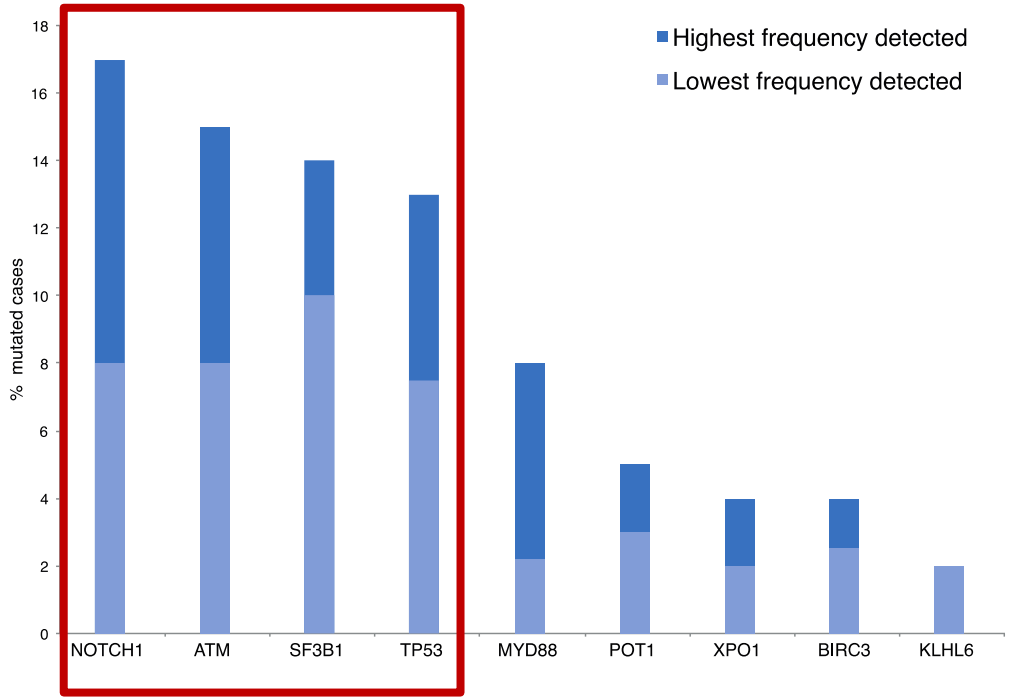
22 000 genes

Gene panels

50-500 genes

The genomic landscape in CLL

- *NOTCH1*
- *ATM*
- *SF3B1*
- *TP53*
- *MYD88*
- *POT1*
- *XPO1*
- *BIRC3*
- *KLHL6*
- *Etc*



Puente et al, Nature 2011, Fabbri et al, JEM 2011
Wang et al, NEJM 2011, Queseda et al, Nature Gen 2011

What are the frequencies of gene mutations?

What gene mutations are clinically relevant?

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ORIGINAL ARTICLE

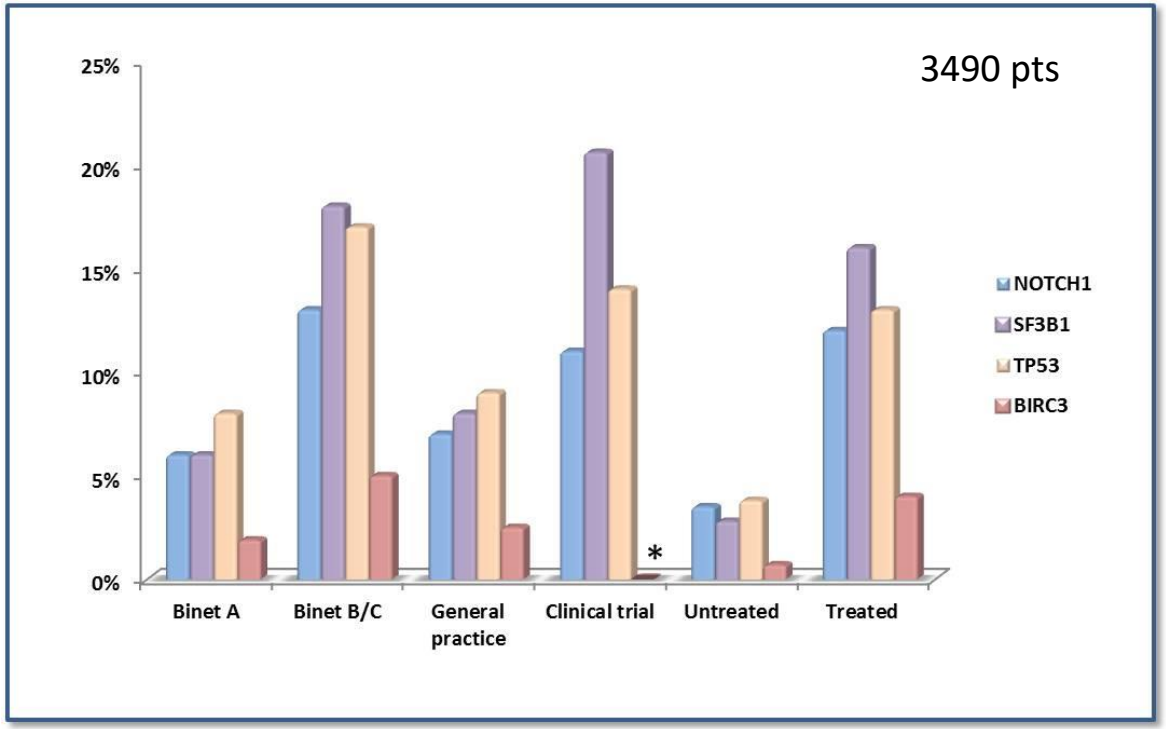
Recurrent mutations refine prognosis in chronic lymphocytic leukemia

P Baliakas[1,2], A Hadzidimitriou[1,3], L-A Sutton[1], D Rossi[4], E Minga[3], N Villamor[5], M Larrayoz[6], J Kminkova[7], A Agathangelidis[8,9], Z Davis[10], E Tausch[11], E Stalika[2], B Kantorova[7], L Mansouri[1], L Scarfò[8,9], D Cortese[1], V Navrkalova[7], MJJ Rose-Zerilli[6], KE Smedby[12], G Juliusson[13], A Anagnostopoulos[2], AM Makris[3], A Navarro[5], J Delgado[5], D Oscier[10], C Belessi[14], S Stilgenbauer[11], P Ghia[8,9], S Pospisilova[7], G Gaidano[4], E Campo[5], JC Strefford[6,15], K Stamatopoulos[1,2,3,15] and R Rosenquist[1,15] on behalf of the European Research Initiative on CLL (ERIC)

n=3490

Baliakas et al, Leukemia 2015

Increased frequency in clinically aggressive cases



Baliakas et al, Leukemia 2015

Concurrent genetic events

Baliakas et al, Leukemia 2015

Prognostic impact of novel mutations

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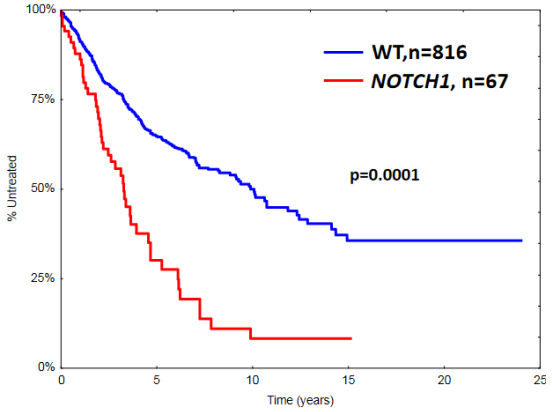
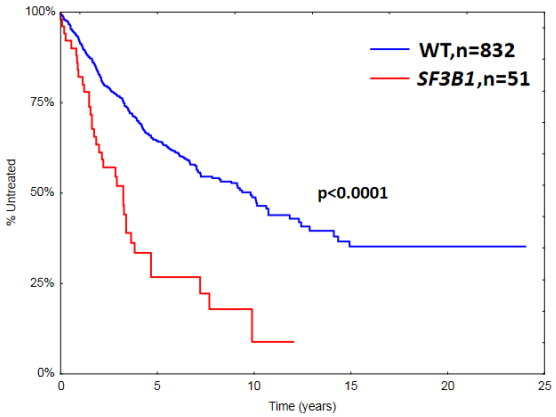
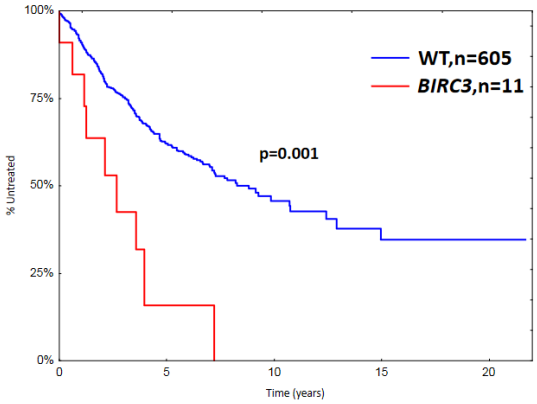
NOTCH1 **(n=816+67)**



SF3B1 **(n=832+51)**



BIRC3 **(n=605+11)**



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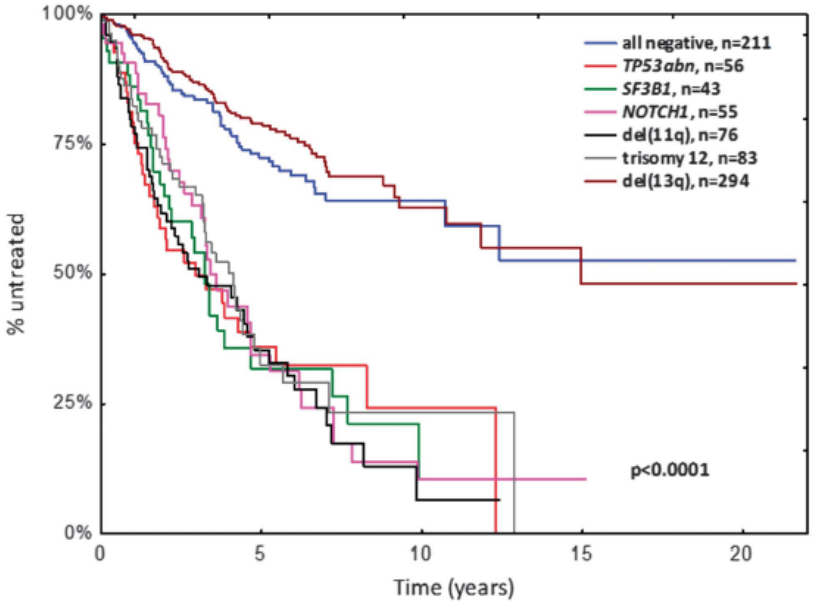
Baliakas et al. Leukemia 2015

Prognostic impact of novel mutations



	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	p
NOTCH1	2.633	1.928-3.598	<0.0001	1.305	0.923-1.847	0.128
SF3B1	2.635	1.855-3.745	<0.0001	1.644	1.134-2383	**0.008**
TP53ab	2.266	1.580-3.252	<0.0001	2.081	1.431-3.021	**0.0001**
U-CLL	4.840	3.860-6.069	<0.0001	3.701	2.833-4.434	<0.0001
del(13q)	0.524	0.413-0.663	<0.0001	0.98	0.740-1.209	0.889
del(11q)	2.924	2.229-3.849	<0.0001	1.421	1.031-1.970	0.03
+12	1.997	1.520-2.623	<0.0001	1.338	0.972-1.841	0.07

Baliakas et al, Leukemia 2015

european research initiative on CLL

Karolinska Institutet

Different spectra of recurrent gene mutations in subsets of chronic lymphocytic leukemia harboring stereotyped B-cell receptors

Lesley-Ann Sutton,[1] Emma Young,[1] Panagiotis Baliakas,[1] Anastasia Hadzidimitriou,[2] Theodoros Moysiadis,[2] Karla Plevova,[3] Davide Rossi,[4] Jana Kminkova,[3] Evangelia Stalika,[2] Lone Bredo Pedersen,[5] Jitka Malcikova,[3] Andreas Agathangelidis,[6,7] Zadie Davis,[8] Larry Mansouri,[1] Lydia Scarfò,[6,7] Myriam Boudjoghra,[9] Alba Navarro,[10] Alice F. Muggen,[11] Xiao-Jie Yan,[12] Florence Nguyen-Khac,[9] Marta Larrayoz,[13] Panagiotis Panagiotidis,[14] Nicholas Chiorazzi,[12] Carsten Utoft Niemann,[5] Chrysoula Belessi,[15] Elias Campo,[10] Jonathan C. Strefford,[13] Anton W. Langerak,[11] David Oscier,[8] Gianluca Gaidano,[4] Sarka Pospisilova,[3] Frederic Davi,[9] Paolo Ghia,[6,7] Kostas Stamatopoulos,[1,2,16*] Richard Rosenquist,[1*] and on behalf of ERIC, the European Research Initiative on CLL

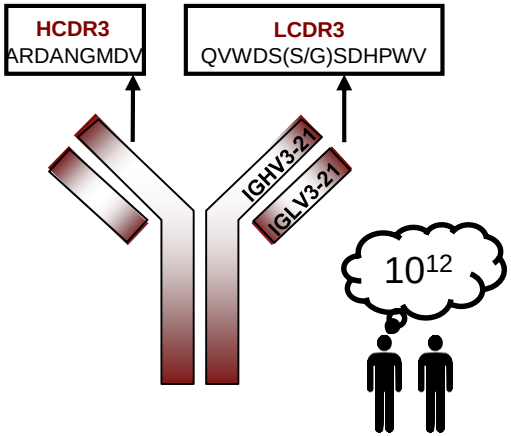
EUROPEAN HEMATOLOGY ASSOCIATION

Ferrata Storti Foundation

Haematologica 2016
Volume 101(8):959-967

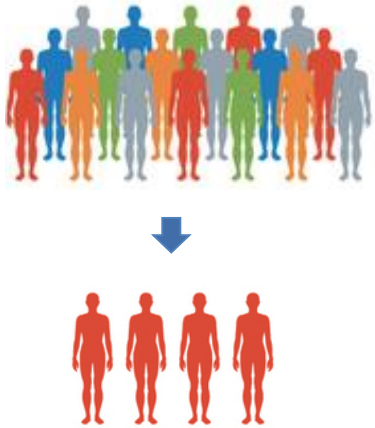
Stereotyped B-cell receptors in CLL

Highly similar B-cell receptors



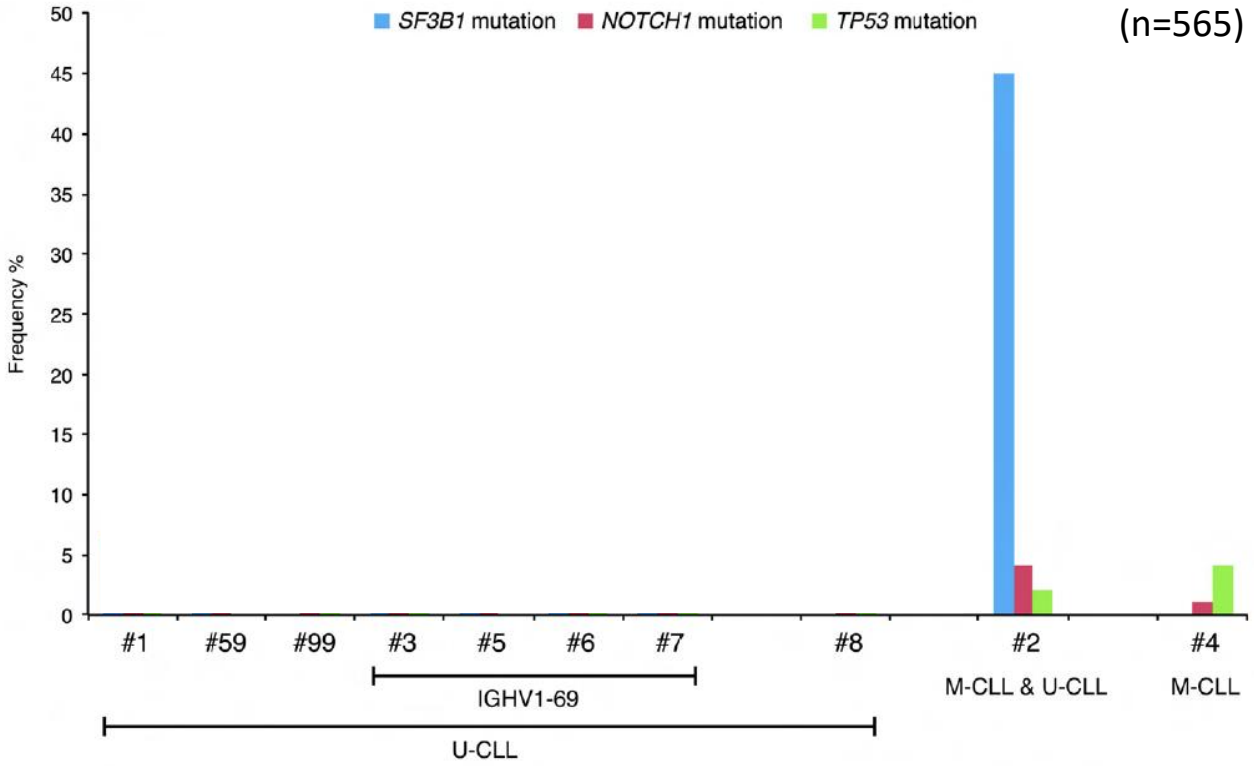
>30% of CLL patients
Recognize similar epitopes

More homogenous subgroups



Share clinical and biological profiles

Different subsets, distinct recurrent mutation profiles



Sutton et al, Haematologica 2016

european research initiative on CLL

Karolinska Institutet

Reappraising Immunoglobulin Repertoire Restrictions in Chronic Lymphocytic Leukemia: Focus on Major Stereotyped Subsets and Closely Related Satellites

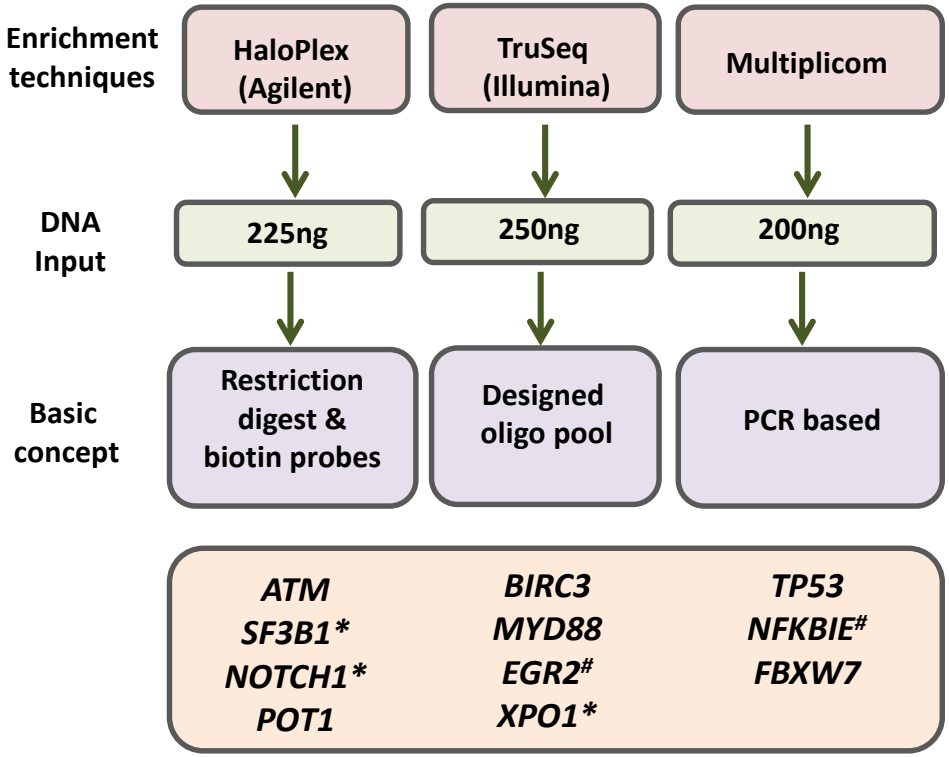
Andreas Agathangelidis[1*], Anastasia Hadzidimitriou[1*], Eva Minga[1*], Lesley-Ann Sutton[2*], Eleftheria Polychronidou[3*], Tait D. Shanafelt[4], Zadie Davis[5*], Xiao-Jie Yan[6], Karla Plevova[7*], Myriam Boudjoghra[8*], Alba Navarro[9*], Davide Rossi[10], Lone Bredo Pedersen[11*], Vasilis Bikos[7*], Panagiotis Baliakas[2*], Lydia Scarfò[12*], Mattias Mattsson[2*], Aliki Xochelli[1*], Paola Francia di Celle[13*], Krzysztof Giannopoulos[14*], Katrina Vanura[15*], Ludo Evers[16*], Silvio Veronese[17*], Monica Facco[18*], Panagiotis Moschonas[3*], Vojtech Bystry[7*], Teodora Karan-Djurasevic[19*], Maria Roumelioti[20*], Sonja Pavlovic[19*], Larry Mansouri[2*], Charles Chu[6*], Evangelia Stalika[1*], Veronique Giudicelli[21*], Panagiotis Panagiotidis[20*], Andrey Sudarikov[22*], Achilles Anagnostopoulos[23], Livio Trentin[18], Mark Catherwood[24*], Marco Montillo[25], Niki Stavroyianni[23*], Gianluca Gaidano[10], Elias Campo[26], Carsten Utoft Niemann[11], Anton W. Langerak[27*], Sarka Pospisilova[7*], Marie-Paule Lefranc[21*], Ulrich Jaeger[15], Arnon Kater[16], Christiane Pott[28*], Nicholas Chiorazzi[6], David Oscier[5], Diane F. Jelinek[29*], Stephan Stilgenbauer[30], Michael Hallek, MD[31], Dimitrios Tzovaras[3*], Nikos Darzentas[7*], Chrysoula Belessi[32], Frederic Davi[8*], Richard Rosenquist[2], Paolo Ghia[12] and Kostas Stamatopoulos[1]

21,123 cases

ASH 2016

What is the optimal strategy for gene panel analysis?

ERIC gene panel comparative study

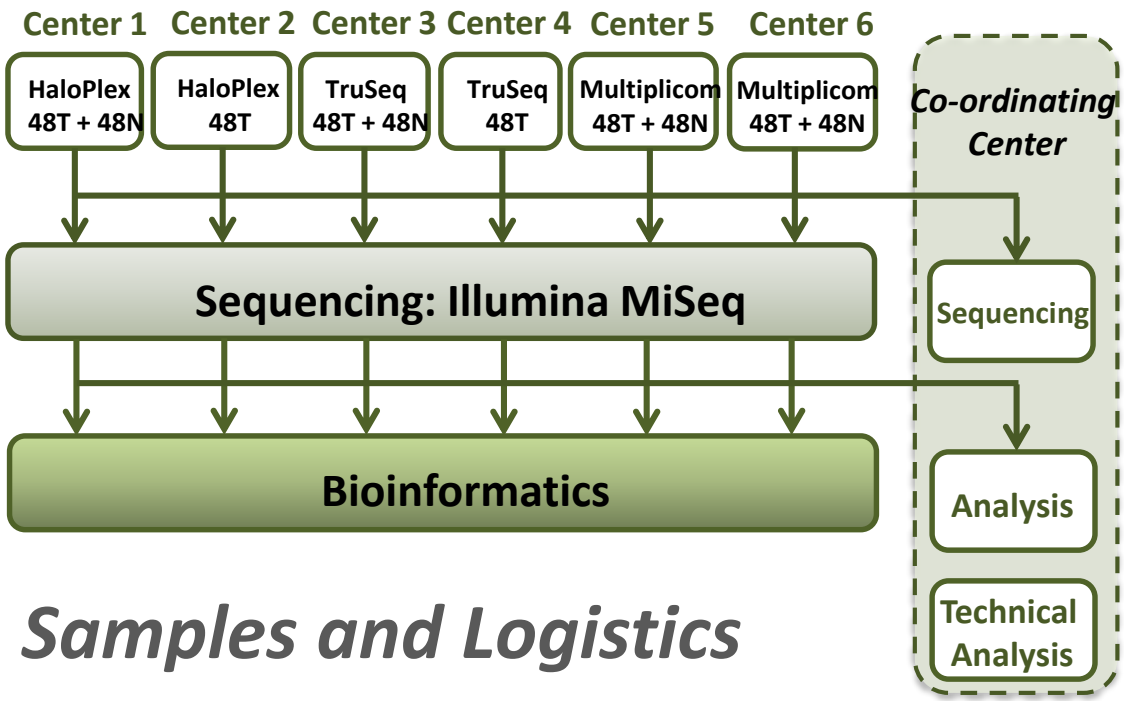


#EGR2 and NFKBIE not included in Multiplicom design

Comparison of 3 targeted enrichment custom/pre-designed technologies

11 gene panel
Full CDS or
***hotspots only**

Sutton et al, in preparation

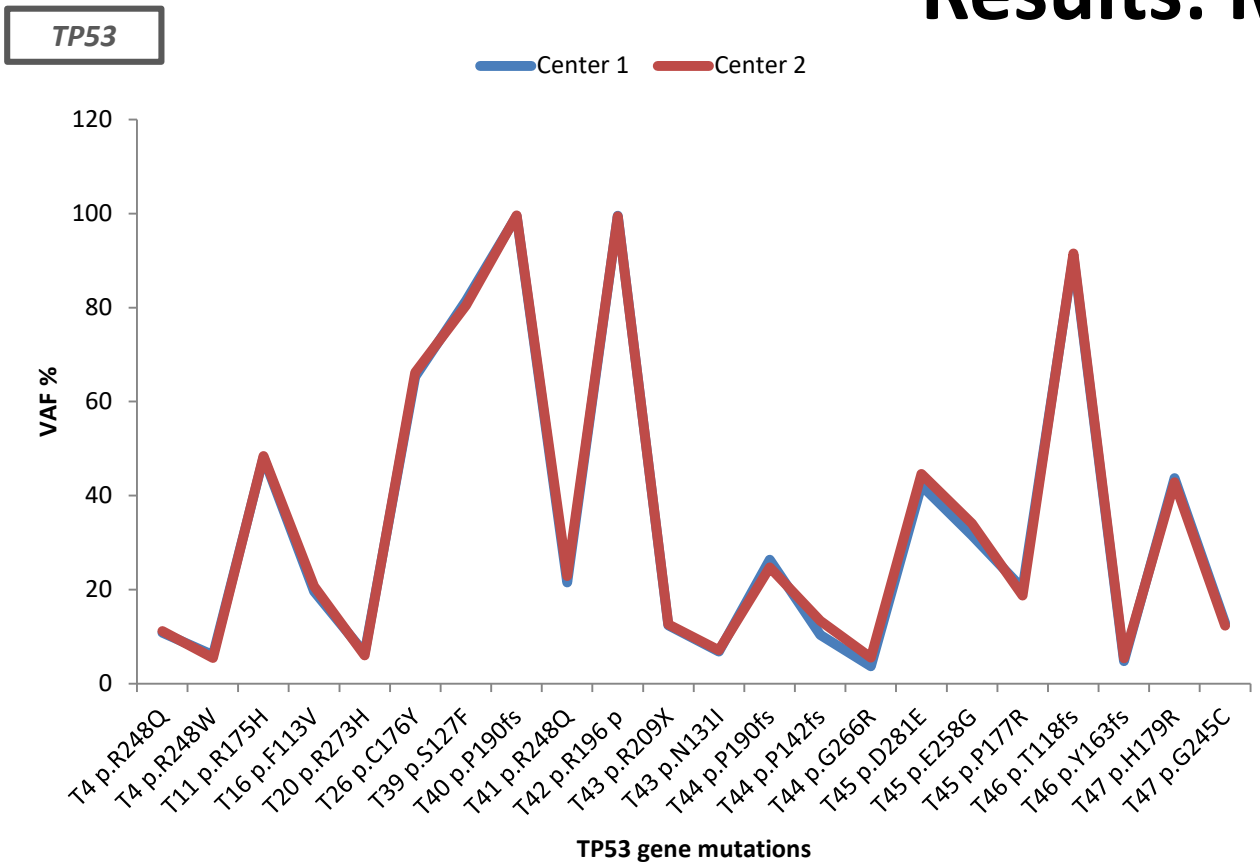


Samples and Logistics

- 48 CLL samples with matched germline DNA
- Samples previously well characterized: clinical and molecular data

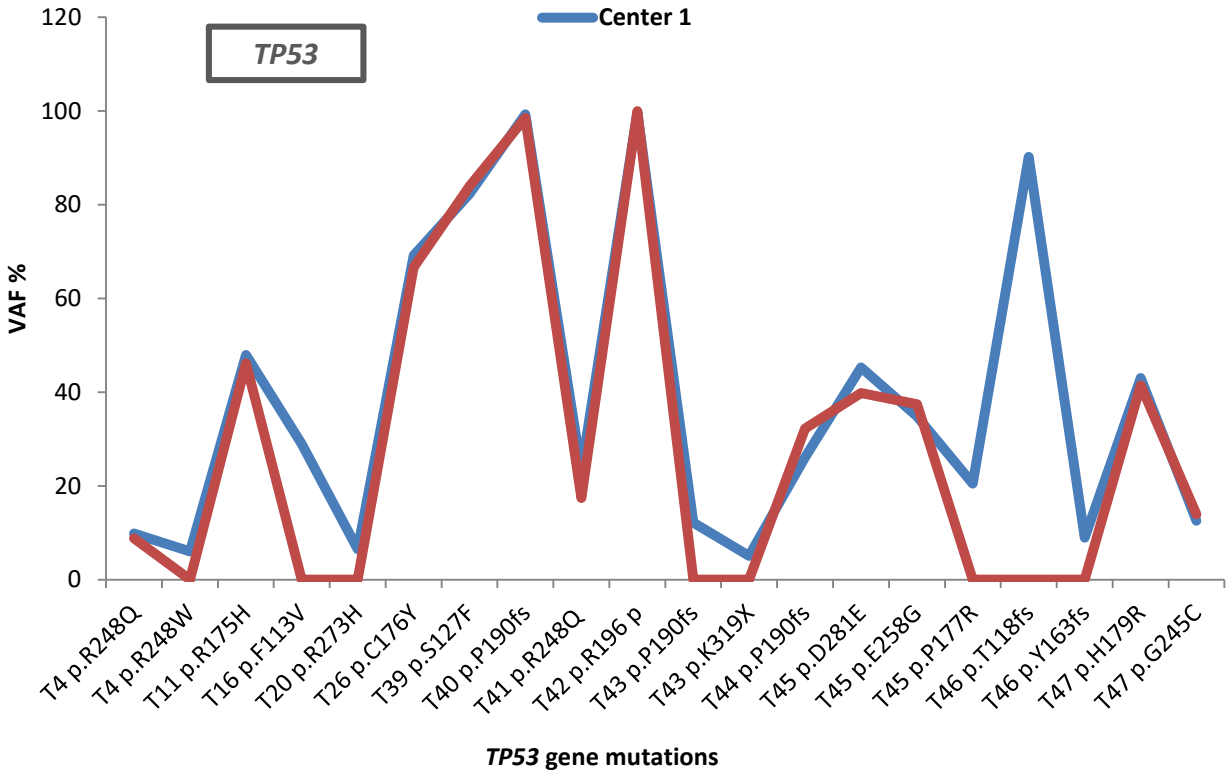
Sutton et al, in preparation

Results: Multiplicom



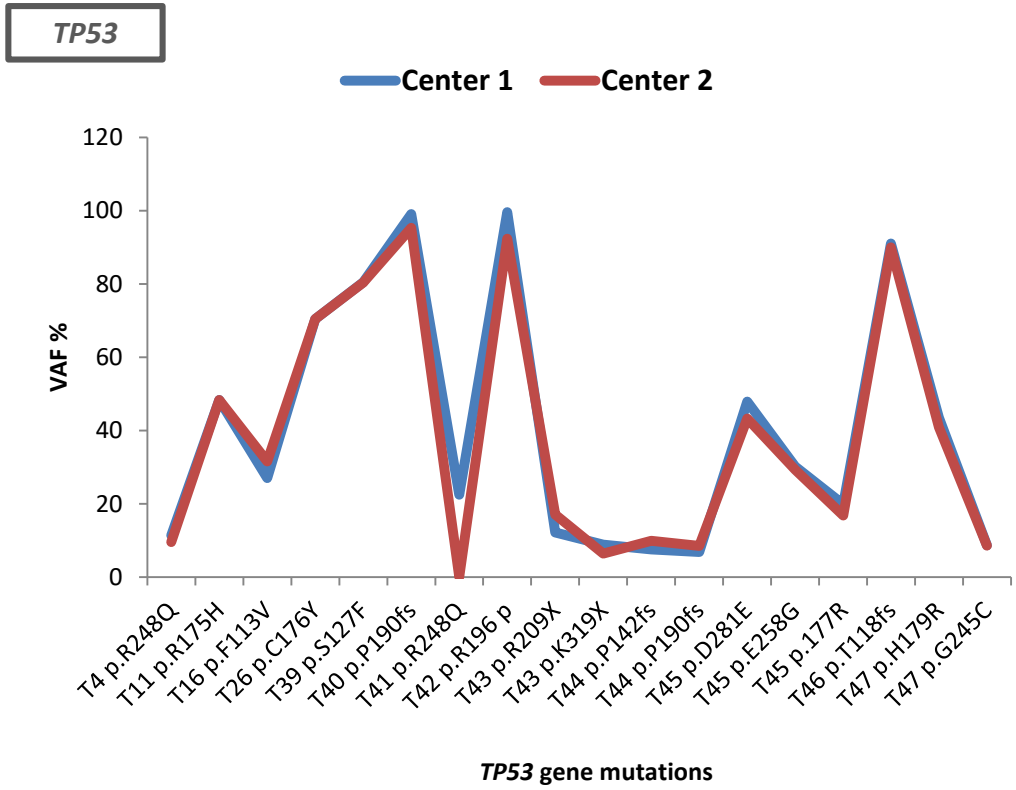
Sutton et al, in preparation

Results: Illumina TruSeq



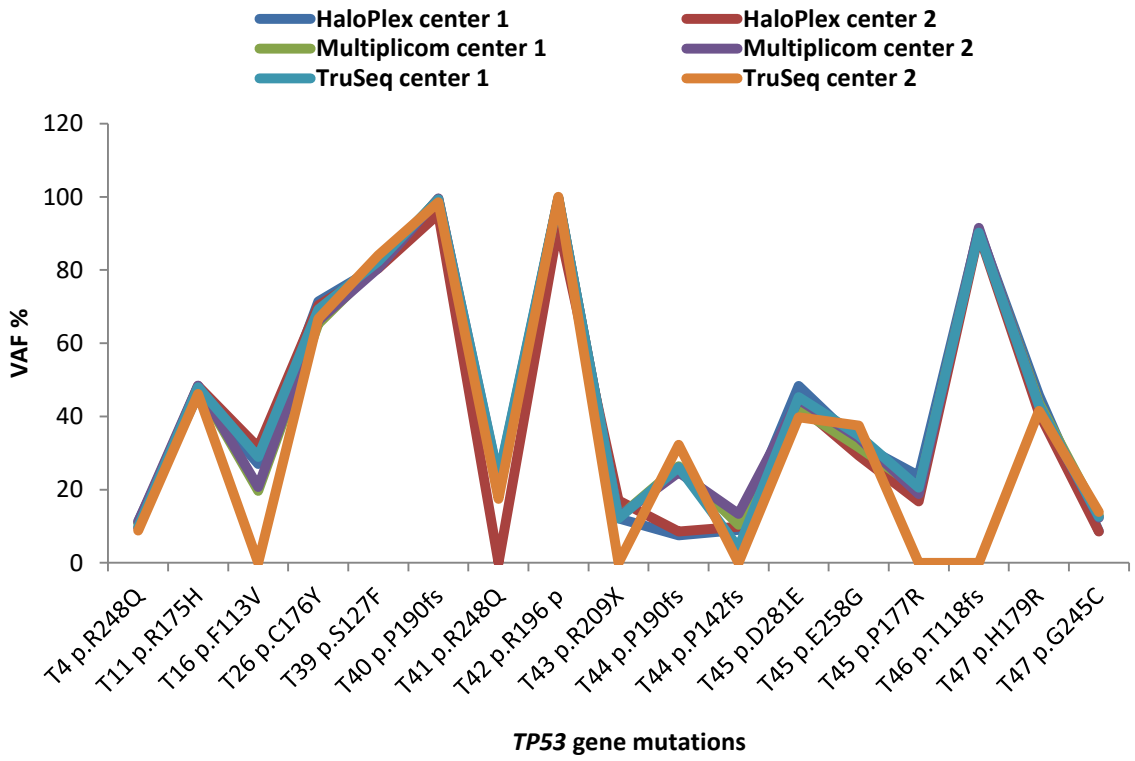
Sutton et al, in preparation

Results: HaloPlex



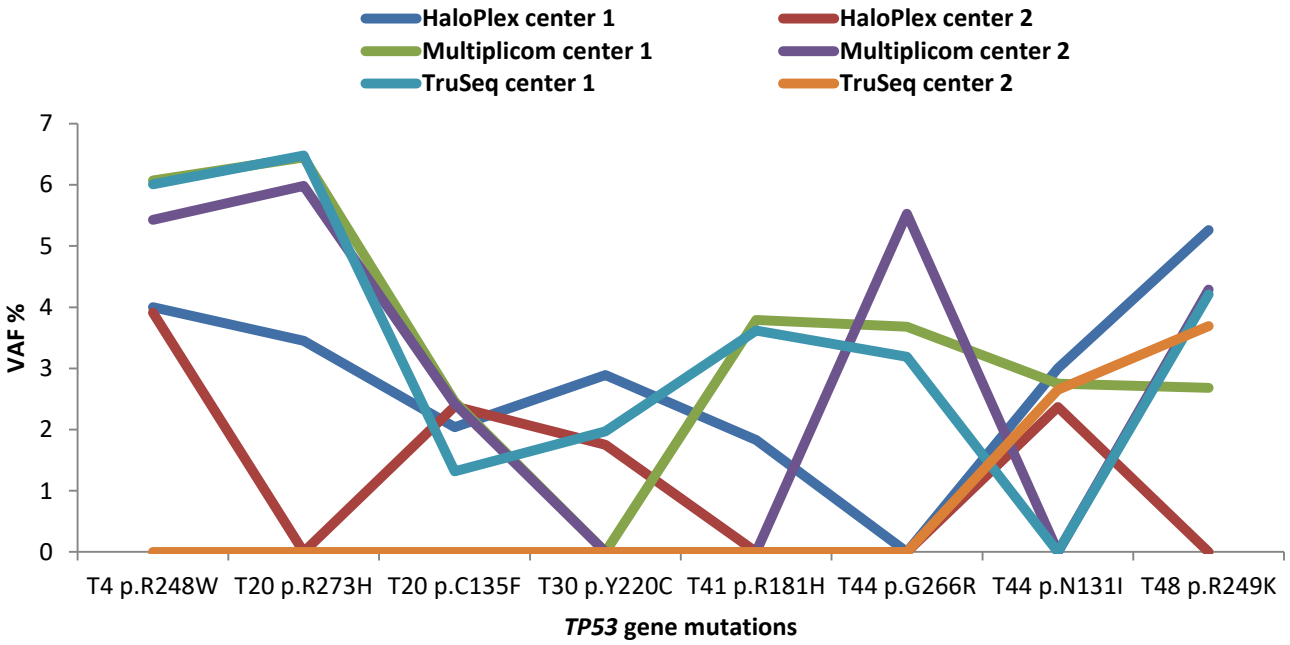
Sutton et al, in preparation

Combined *TP53* Results: *3 methodologies*



Sutton et al, in preparation

TP53 Results 1-10%



A mess!!! False positives or true low frequency variants?

Sutton et al, in preparation

MULTICENTER STUDY ON PROGNOSTIC AND PREDICTIVE IMPACT OF *TP53* VARIANTS

BELOW 10% VAF

More gene mutations detected…

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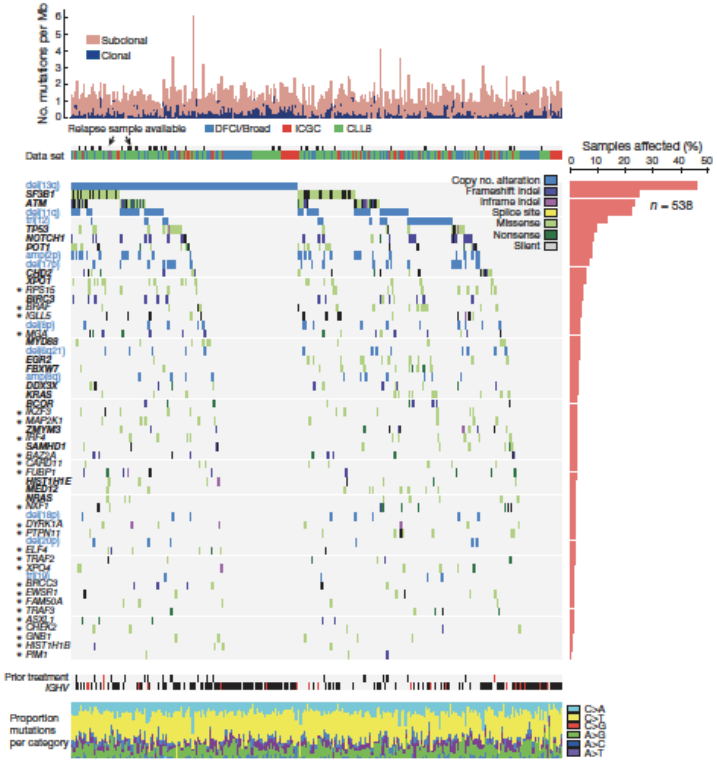

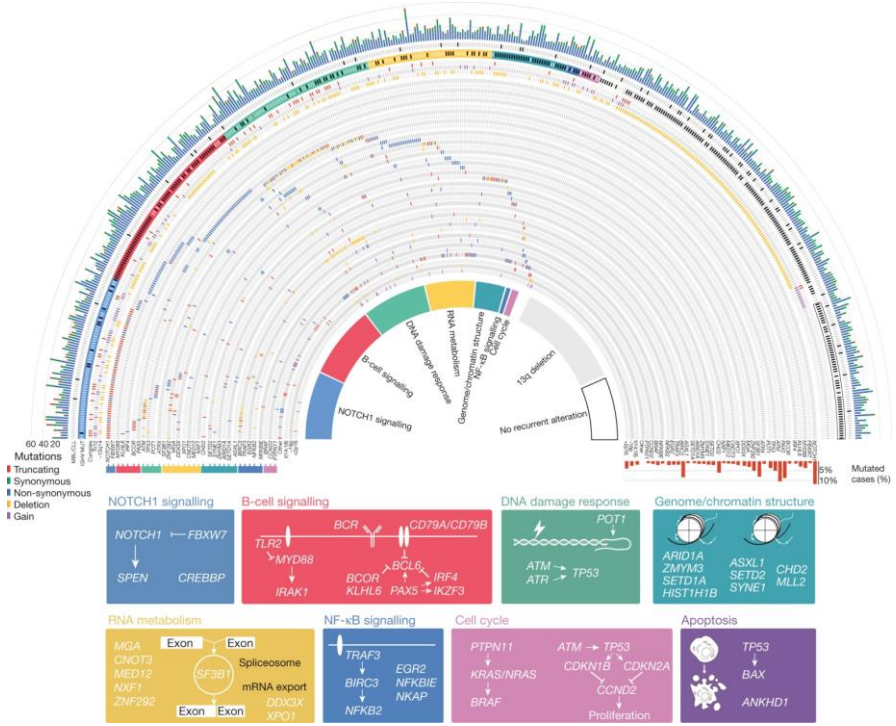

Puente et al, Nature 2015
Landau et al, Nature 2015

Large-scale project on CLL gene mutations

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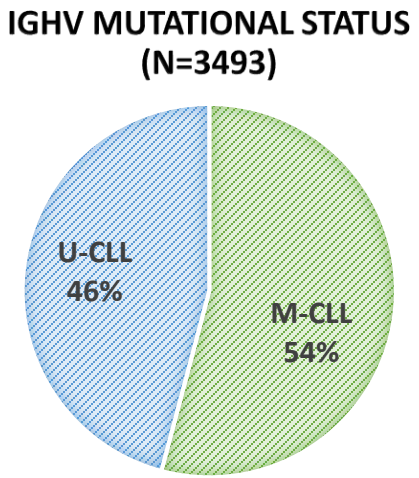
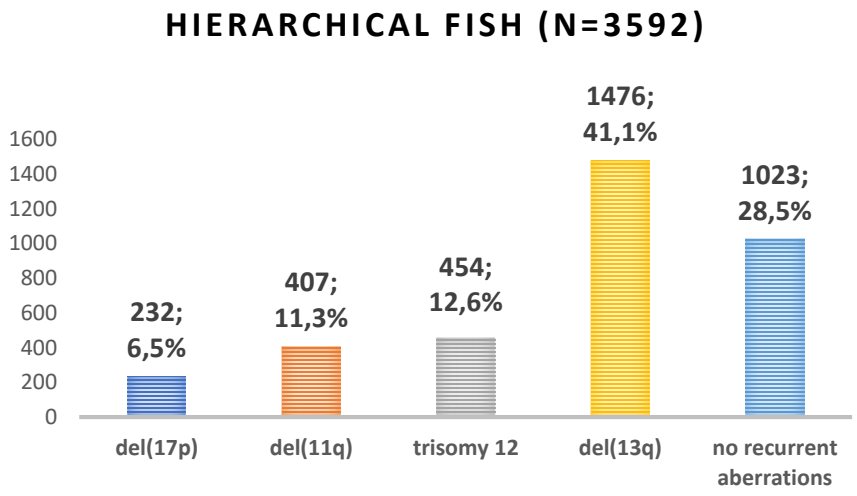
- >20 centers, 4800 pts
- 10 genes, full clinical data required

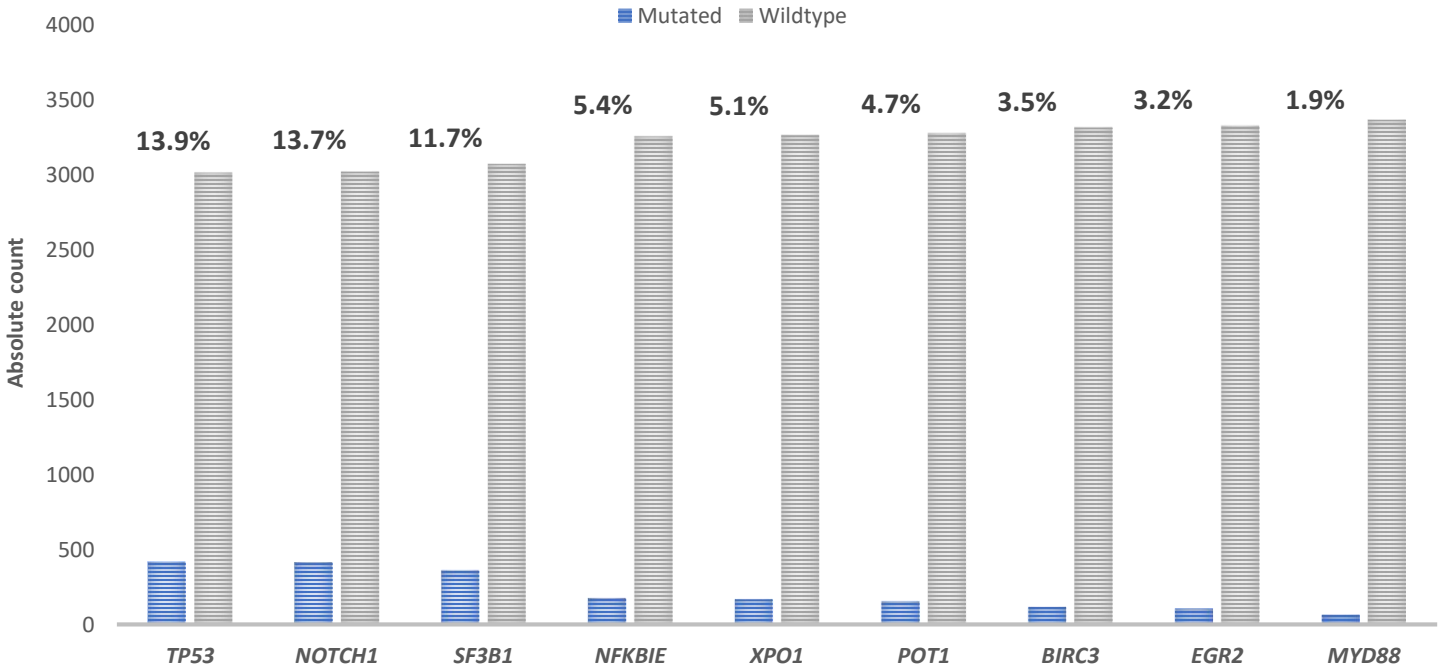




ATM
BIRC3
MYD88
NOTCH1
SF3B1
TP53
EGR2
POT1
NFKBIE
XPO1

Sutton et al.

Large-scale project on CLL gene mutations



n=3425

Sutton et al.

Relevance of genomic complexity?

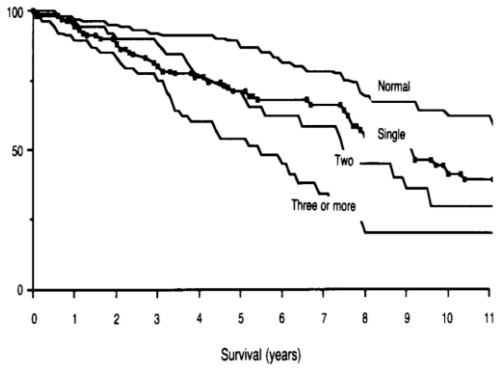
Table 1. Baseline Evaluation of Patients with CLL

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

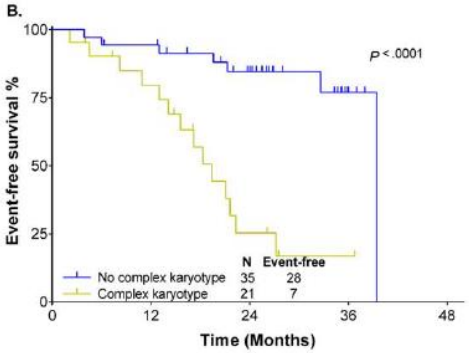
Hallek et al, Blood 2018

Complex karyotype and clinical outcome



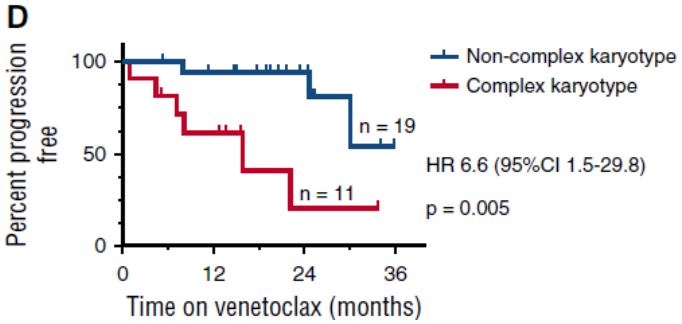
Juliusson et al, NEJM 1990

Ibrutinib



Thompson et al, Cancer 2015

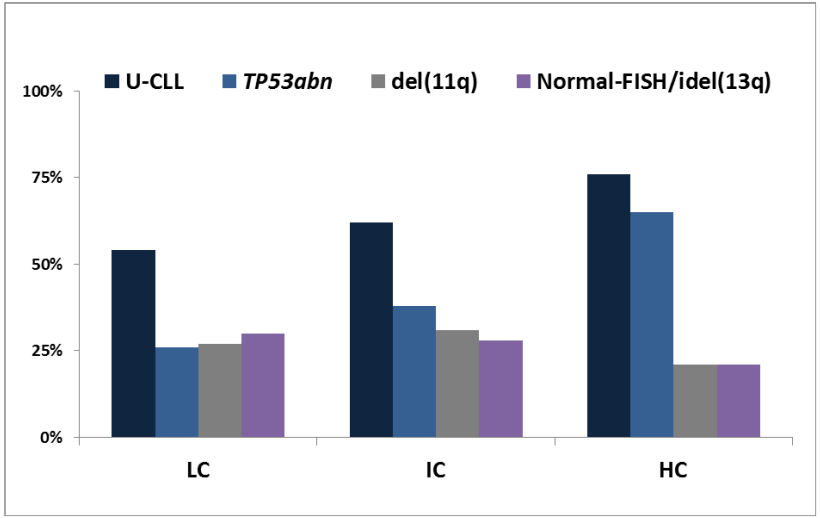
Venetoclax

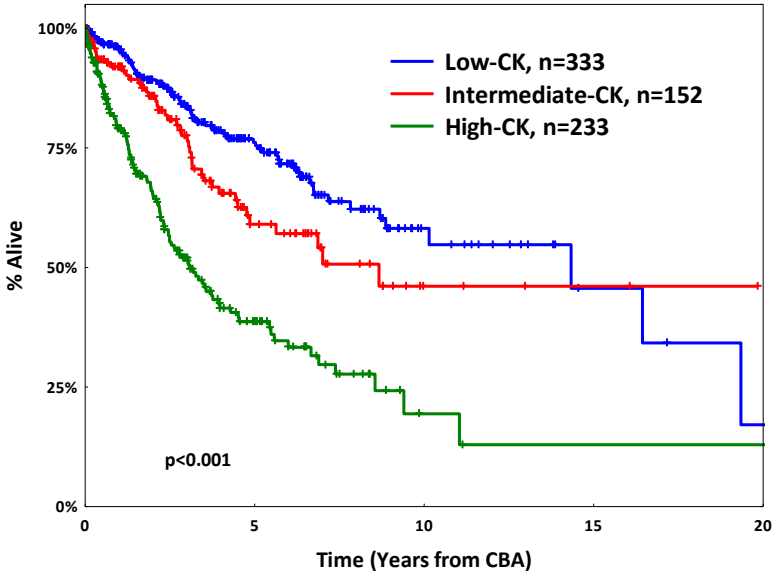


Anderson et al, Blood 2017

ERIC joining forces

n=5290



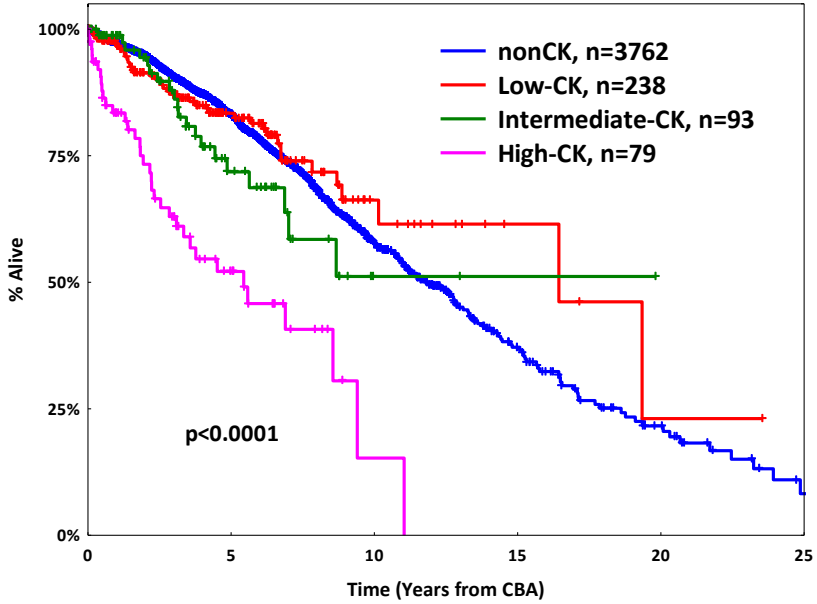


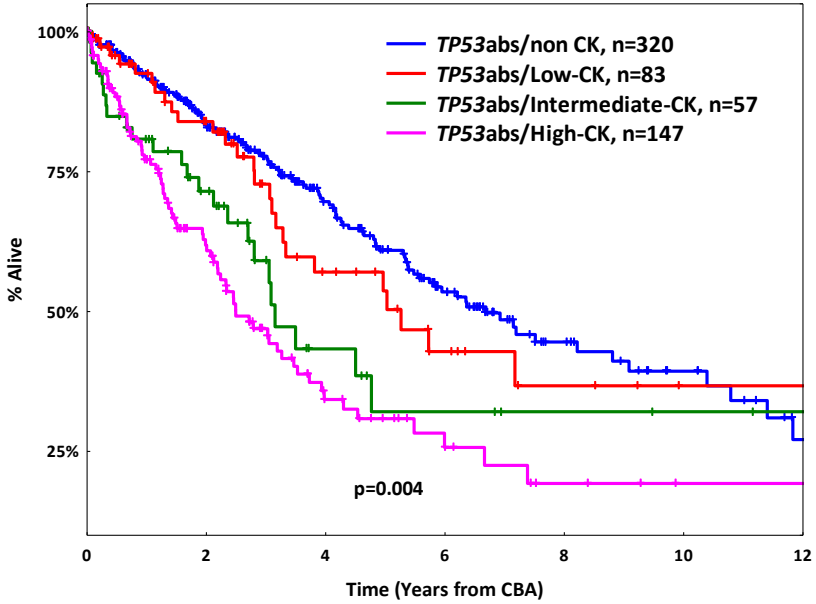
LC: 3 abn IC: 4 abn HC: 5 or more abn

Baliakas et al.

non-*TP53abn*

TP53abn





LC: 3 abn IC: 4 abn HC: 5 or more abn

Baliakas et al.

How to bring order out of this?

european research initiative on CLL

Karolinska Institutet

EUROPEAN HEMATOLOGY ASSOCIATION

Ferrata Storti Foundation

Clinical impact of recurrently mutated genes on lymphoma diagnostics: state-of-the-art and beyond

Richard Rosenquist,[1] Andreas Rosenwald,[2] Ming-Qing Du,[3] Gianluca Gaidano,[4] Patricia Groenen,[5] Andrew Wotherspoon,[6] Paolo Ghia,[7] Philippe Gaulard,[8] Elias Campo,[9] Kostas Stamatopoulos,[10] on behalf of the European Research Initiative on CLL (ERIC) and the European Association for Haematopathology (EAHP)

Haematologica 2016
Volume 101(9):1002-1009

European Expert Group on NGS Diagnostics in Lymphomas

Karolinska Institutet

- Reference group for NGS-based diagnostics
- Started 2015
- Associated with ERIC/EHA and EAHP
- Hematologists, hematopathologists and geneticists
- Aims:
  - Provide guidelines/recommendations
  - Dialogue with companies aiming for gene panel diagnostics
  - Workshops/symposiums

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EUROPEAN ASSOCIATION FOR HAEMATOPATHOLOGY

European Expert Group on NGS Diagnostics in Lymphomas

Elias Campo (Spain)	hematopathologist
Ming Du (UK)	molecular pathologist
Gianluca Gaidano (Italy)	hematologist
Philippe Gaulard (France)	hematopathologist
Patricia Groenen (The Netherlands)	clinical scientist - molecular pathology
Richard Rosenquist (Nordic countries)	clinical geneticist
Andreas Rosenwald (Germany)	hematopathologist
Kostas Stamatopoulos (Greece)	hematologist

Associated members:
Paolo Ghia (ERIC)
Andrew Wotherspoon (EAHP)

EUROPEAN ASSOCIATION FOR HAEMATOPATHOLOGY

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Great genetic heterogeneity but also common themes

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Table 1A. Mutation frequencies in different B-cell lymphoma entities.

Pathway/cellular function	CLL[a]	MCL[b]	BL[c]	FL[d]	ABC-DLBCL[e]	GCB-DLBCL[e]	SMZL[f]	HCL[g]	WM[h]
B-cell receptor signaling									
CD79A/CD79B	<1%	-	-	4%	10-20%	<5%	-	-	10%
CARD11	1%	-	-	10%	10%	5%	4%	-	-
Toll-like receptor signaling									
MYD88	3%	-	5%	-	20-30%	<5%	7%	-	>90%
NF-κB signaling pathway									
TNFPAI3	-	-	-	10%	20%	<5%	8%	-	40%*
BIRC3	<3%	5-10%	-	-	-	-	5%	-	-
TRAF3	<1%	-	-	-	-	-	5%	-	-
NFKBIE	<2%	5%	-	-	<5%	<5%	2%	-	-
Notch signaling									
NOTCH1	10%	10-15%	-	-	-	-	6%	-	-
NOTCH2	<1%	5%	-	-	-	-	15-20%	-	-
Other signaling pathways									
BRAF	3%	-	-	-	4%	-	<1%	>90%	-
CXCR4	<1%	-	-	-	<10%	-	<1%	-	25%
Transcription factors									
ID3	-	-	35-60%	-	-	-	-	-	-
TCF3	-	-	10-25%	-	-	-	-	-	-
KLF2	-	-	-	-	-	-	14%	-	-
DNA repair/genomic integrity									
ATM	11%	40-50%	-	-	-	-	6%	-	-
TP53	5%	15-20%	35%	5%	10-25%	10-20%	18%	-	-
POT1	5%	<3%	-	-	-	-	<1%	-	-
Epigenetic modifiers									
TET2	<1%	<5%	-	-	5-10%	5-10%	3%	-	-
EZH2	<1%	-	-	10-20%	-	20%	<1%	-	-
IDH2	-	-	-	-	-	-	-	-	-
CREBBP	<1%	-	-	50%	15-20%	40%	6%	-	-
EP300	<1%	-	2%	10-15%	<5%	<10%	4%	-	-

Rosenquist et al, Haematologica 2016

Clinical impact of recurrently mutated genes

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Table 2. Categorization of gene mutations based on current evidence levels.

Category	Gene mutations
1. Immediate impact on patient care	*TP53* mutations (exons 4-10) in CLL
2. Diagnostic impact	MYD88^{L265P} mutation in WM/LPL BRAF^{V600E} mutation in HCL *KLF2* mutations in SMZL *ID3* and *TCF3* mutations in BL *STAT3* mutations in LGLL *RHOA*, *TET2*, *IDH2* and DNMT3A mutations in AITL and other T_{FH}-derived PTCL
3. Prognostic impact	*CLL*: *TP53*, *ATM*, *BIRC3*, *NFKBIE*, *NOTCH1*, *SF3B1* MCL: *TP53*, *NOTCH1*, *NOTCH2* mutations SMZL: *NOTCH2*, *TP53* mutations DLBCL: *TP53* mutation & MYC translocation NKTCL: *DDX3X* mutations
4. Potential clinical impact in the near future	Therapy response to BcR inhibitors: WM: *MYD88*, *CXCR4* mutations DLBCL: *CD79B* mutations (responsive) *CARD11*, MYD88 mutations (non-responsive) Resistance to BcR inhibitors: BTK^{C481S}, PCLG2 mutations New inhibitors under development: *EZH2*, *SF3B1* & *NOTCH1*

Rosenquist et al, Haematologica 2016

Lymphoma NGS Gene Panel

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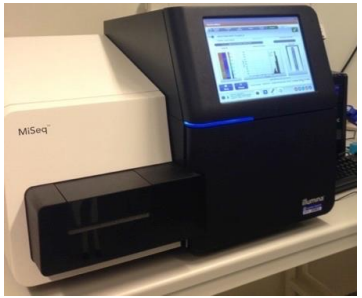
- TruSight gene panel
- 43 genes included
- Low input (10-20 ng DNA), FFPE compatible



ATM, B2M, BIRC3, BRAF, BTK, CARD11, CD58, CD79A, CD79B, CIITA, CREBBP, CXCR4 (CD184), EGR2, EZH2, GNA13, ID3, IDH2, ITPKB, JAK3, KLF2, MAP2K1, MYD88, NFKBIE, NOTCH1, NOTCH2, PLCG1, PLCG2, POT1, RHOA, RPS15, RRAGC, SF3B1, SOCS1, STAT3, STAT5B, STAT6, TCF3, TET2, TNFAIP3, TNFRSF14, TP53, TRAF3, XPO1

EUROPEAN ASSOCIATION FOR HAEMATOPATHOLOGY

ERIC european research initiative on CLL

Planned collaborative projects

EUROPEAN ASSOCIATION FOR HAEMATOPATHOLOGY

ERIC
european research initiative on CLL

A.Tumor sample

B. Library preparation

C. Sequencing

D. Analysis/reporting

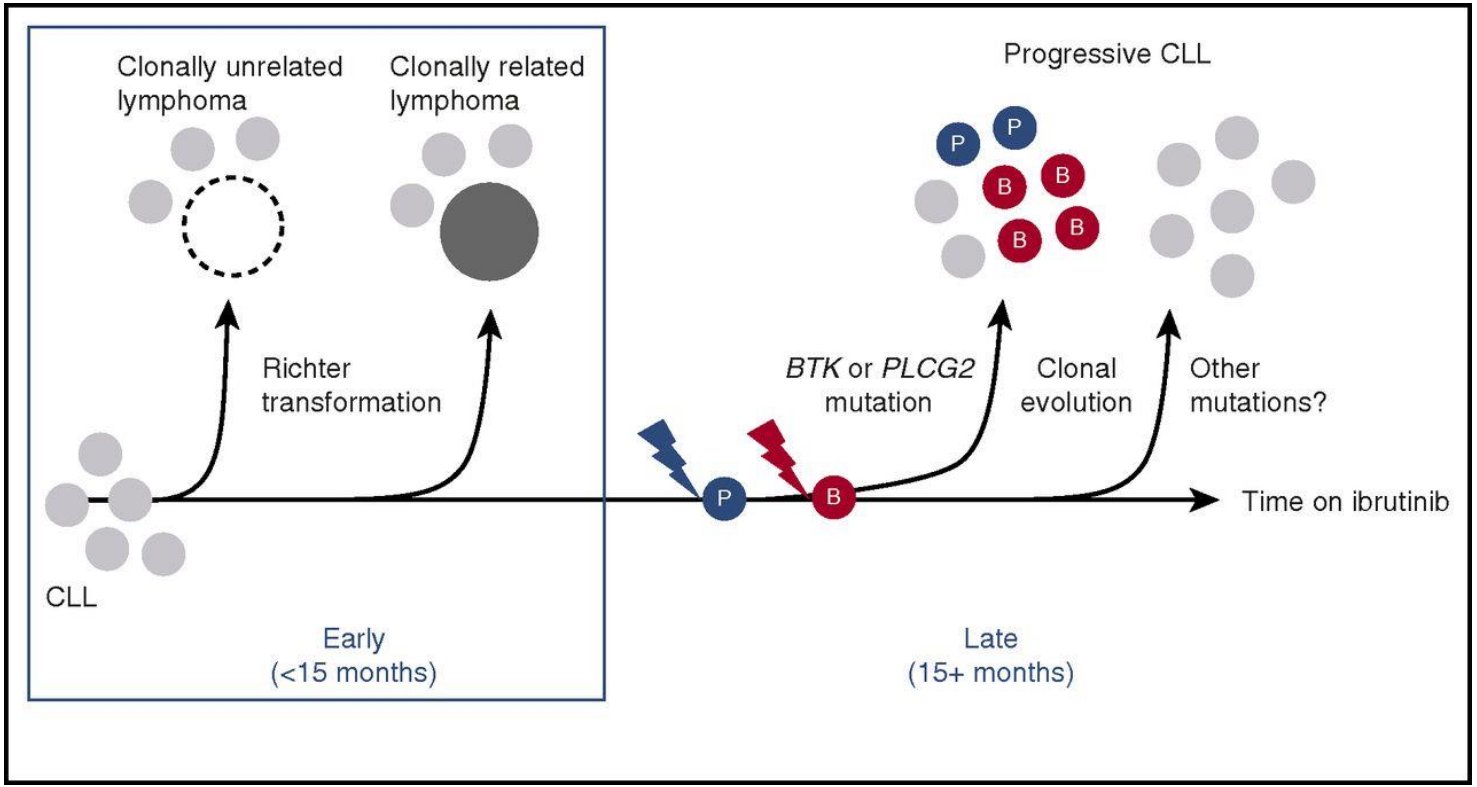
E. Knowledge DB

Lymphoid capture panel

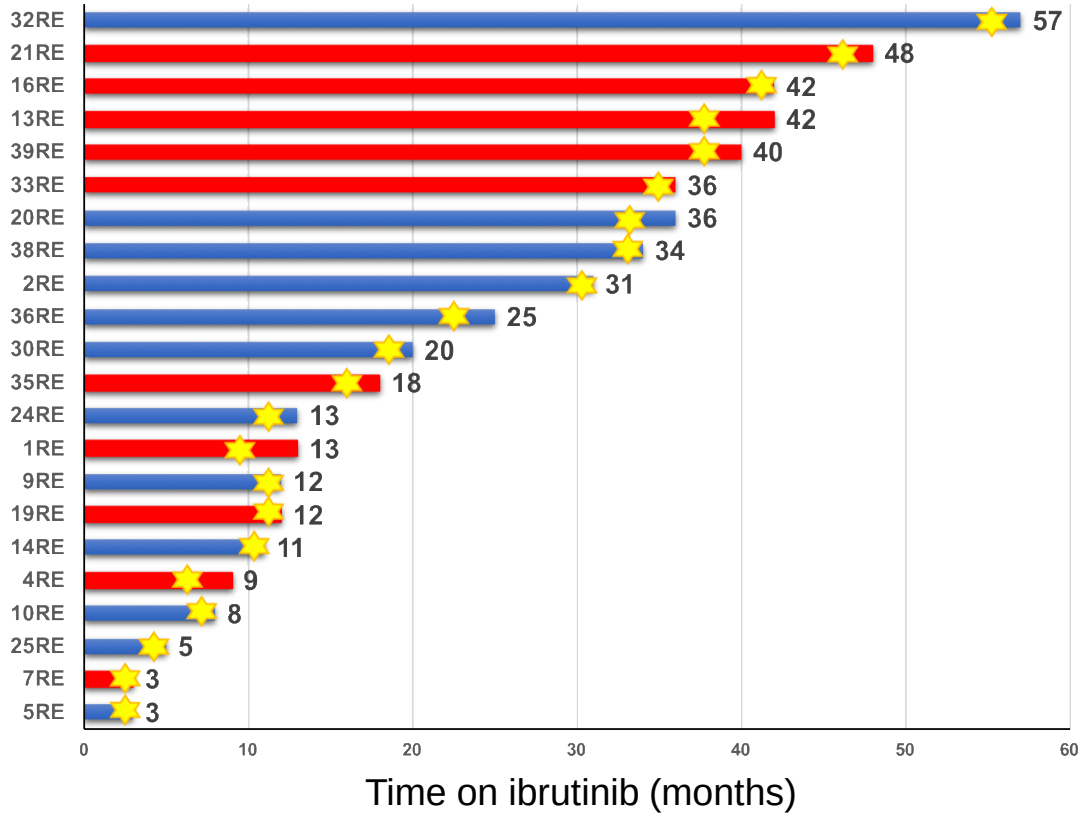
genpanel

Classification
Risk-stratification
Prediction

Detection of resistance mutations



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

european research initiative on CLL

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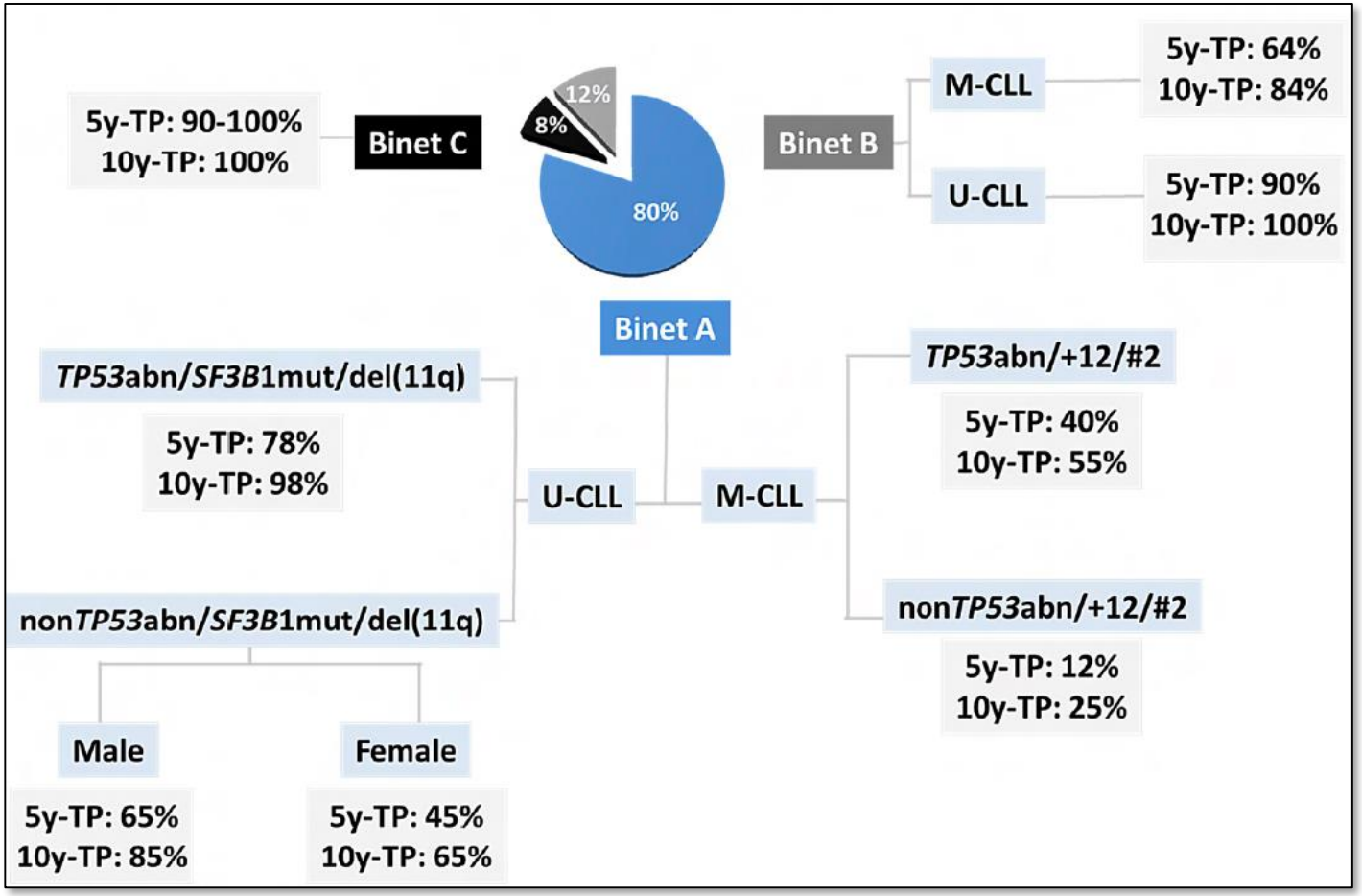
Tailored approaches grounded on immunogenetic features for refined prognostication in chronic lymphocytic leukemia

by Panagiotis Baliakas, Theodoros Moysiadis, Anastasia Hadzidimitriou, Aliki Xochelli, Sabine Jeromin, Andreas Agathangelidis, Mattias Mattsson, Lesley-Ann Sutton, Eva Minga, Lydia Scarfò, Davide Rossi, Zadie Davis, Neus Villamor, Helen Parker, Jana Kotaskova, Evangelia Stalika, Karla Plevova, Larry Mansouri, Diego Cortese, Alba Navarro, Julio Delgado, Marta Larrayoz, Emma Young, Achilles Anagnostopoulos, Karin E Smedby, Gunnar Juliusson, Oonagh Sheehy, Mark Catherwood, Jonathan C Strefford, Niki Stavroyianni, Chrysoula Belessi, Sarka Pospisilova, David Oscier, Gianluca Gaidano, Elias Campo, Claudia Haferlach, Paolo Ghia, Richard Rosenquist, and Kostas Stamatopoulos

Haematologica 2018 [Epub ahead of print]



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Baliakas et al, Haematologica 2018

Tailored approaches based on immunogenetic features for refined prognostication in CLL

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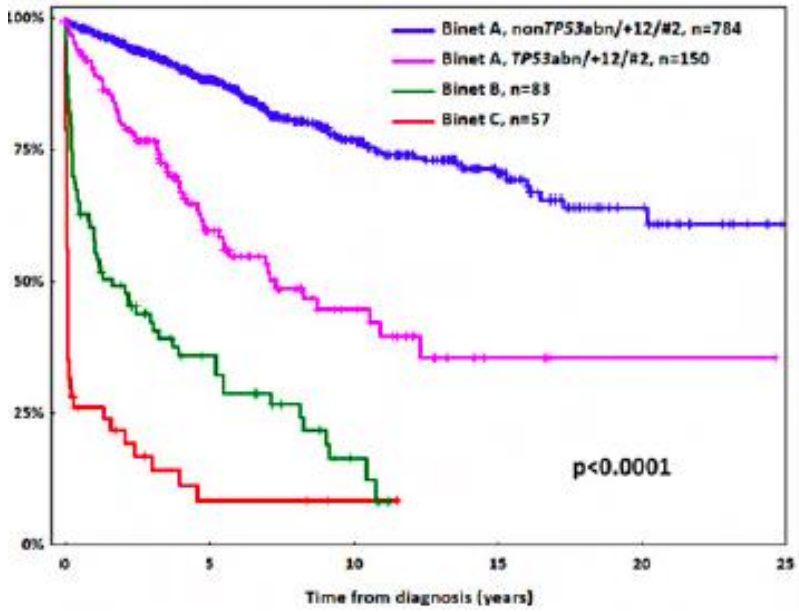
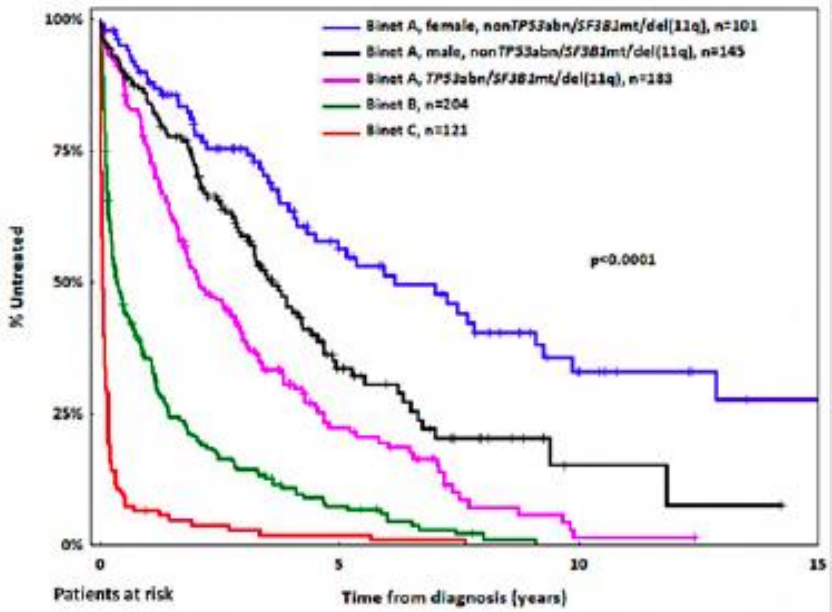
M-CLL



U-CLL



N=3015

Baliakas et al, Haematologica 2018

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We will continue!