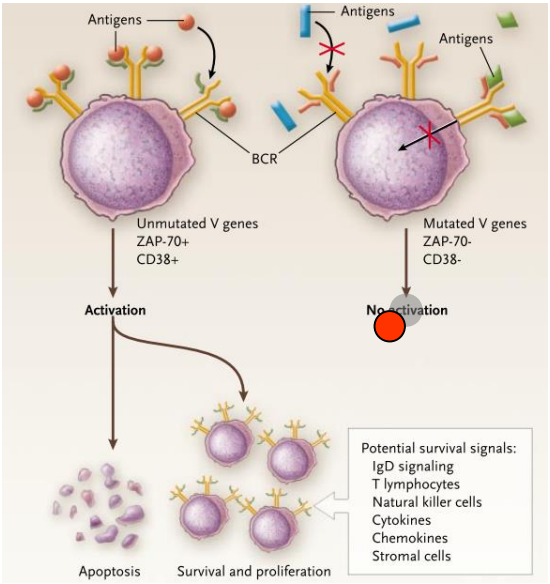
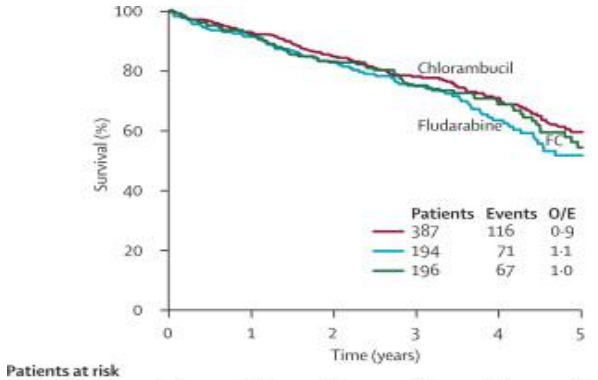
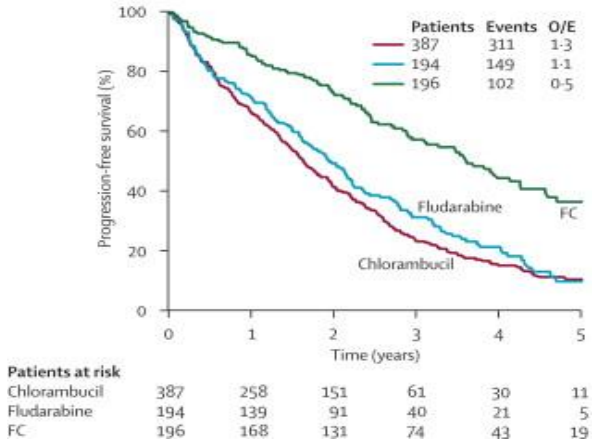
Where did we start from

Activation following BCR stimulation

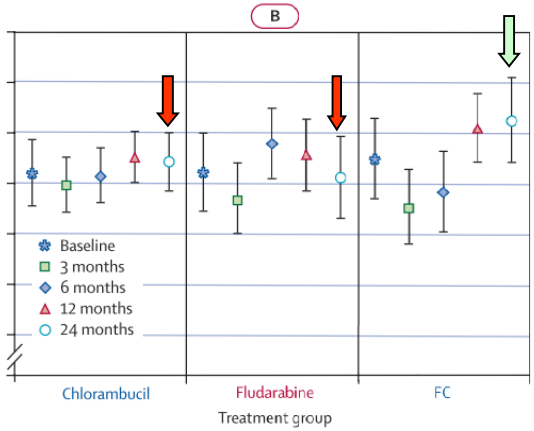


FC improves PFS, but not OS





Longer PFS = better QOL



Mechanisms of disease: Chronic Lymphocytic Leukemia
Nicholas Chiorazzi, M.D., Kanti R. Rai, M.B., B.S., and Manlio Ferrarini, M.D.
N Engl J Med 2005;352:804-15.

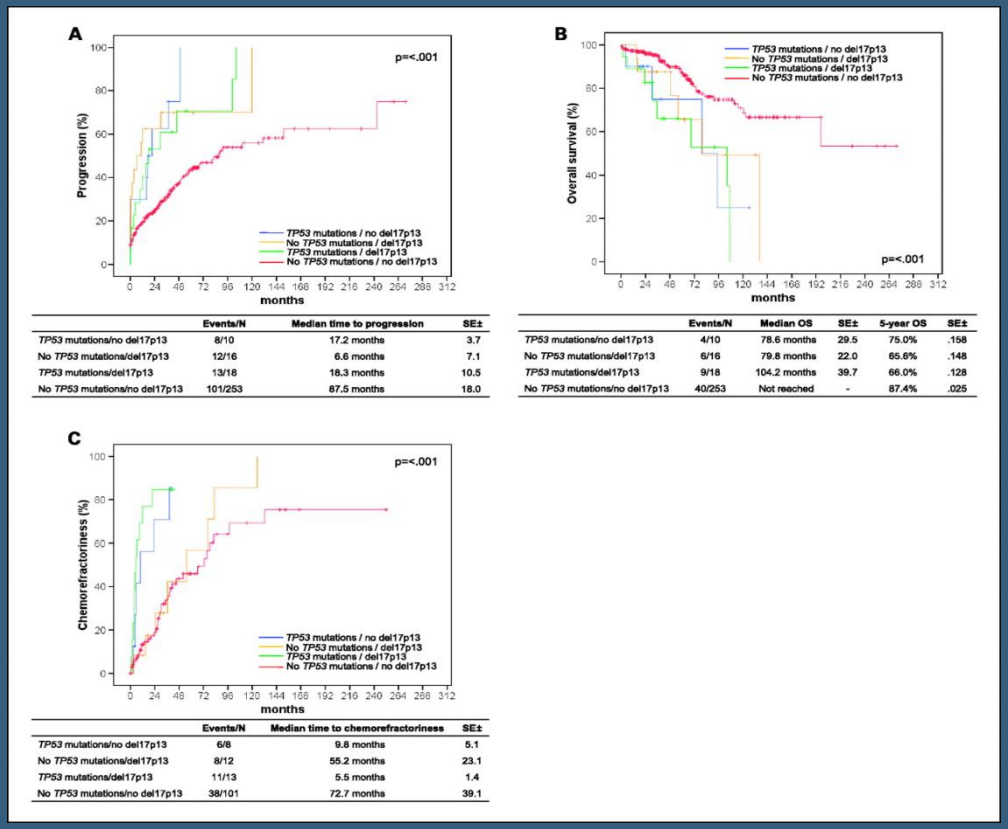
Chlor vs F vs FC in CLL LRF CLL4 trial
Catovsky et al, Lancet 2007

10 anni di EHA highlights: CLL

	Genetics					IF	Pre clinical/ Clinical studies
	TP53	telomeres	New genes	Stereotypy	Cytogenetics		
2008	As adverse as 17p-	Adverse prognosis		Prognosis (Richter)	t(14;19)	CD49d	Fluda+R Fluda+Thal
2009-2010	TP53 (trials)		prognosis in RS				FCR –
2011			WES / CE BRAF				Mo Abs R/R CAL-PIK3d BTK
2012			Cell of origin Prognosis				Genetic-driven treatment Chemoimmuno Rel/ref / BCL2
2013			Integrated classification				NOTCH1/CD20 CLL11 BCL2
2014	True predictor		mechanisms				Idela+R // Ibru vs ofa // Chlor + ofa ABT-199
2015	Small clones						FCR-IGHV mut
2016	CLL-IPI			prognosis			Acalabrutinib Obinutuzumab (green) MRD-OS / Venetoclax after BCR inhibitors
2017					Compex karyotype		FC obinu+ibru in IGHV mut // CLL-BAG Venetoclax MRD // Ibrutinib 4 y FU Ibru+venetcolax

Genetics/ cytogenetics

- >80% del17 carry TP53 mutations
- TP53 mutations as sole mutation in 4.5% CLL



	Events/N	Median time to progression	SE±
TP53 mutations/no del17p13	8/10	17.2 months	3.7
No *TP53* mutations/del17p13	12/16	6.6 months	7.1
TP53 mutations/del17p13	13/18	18.3 months	10.5
No *TP53* mutations/no del17p13	101/253	87.5 months	18.0

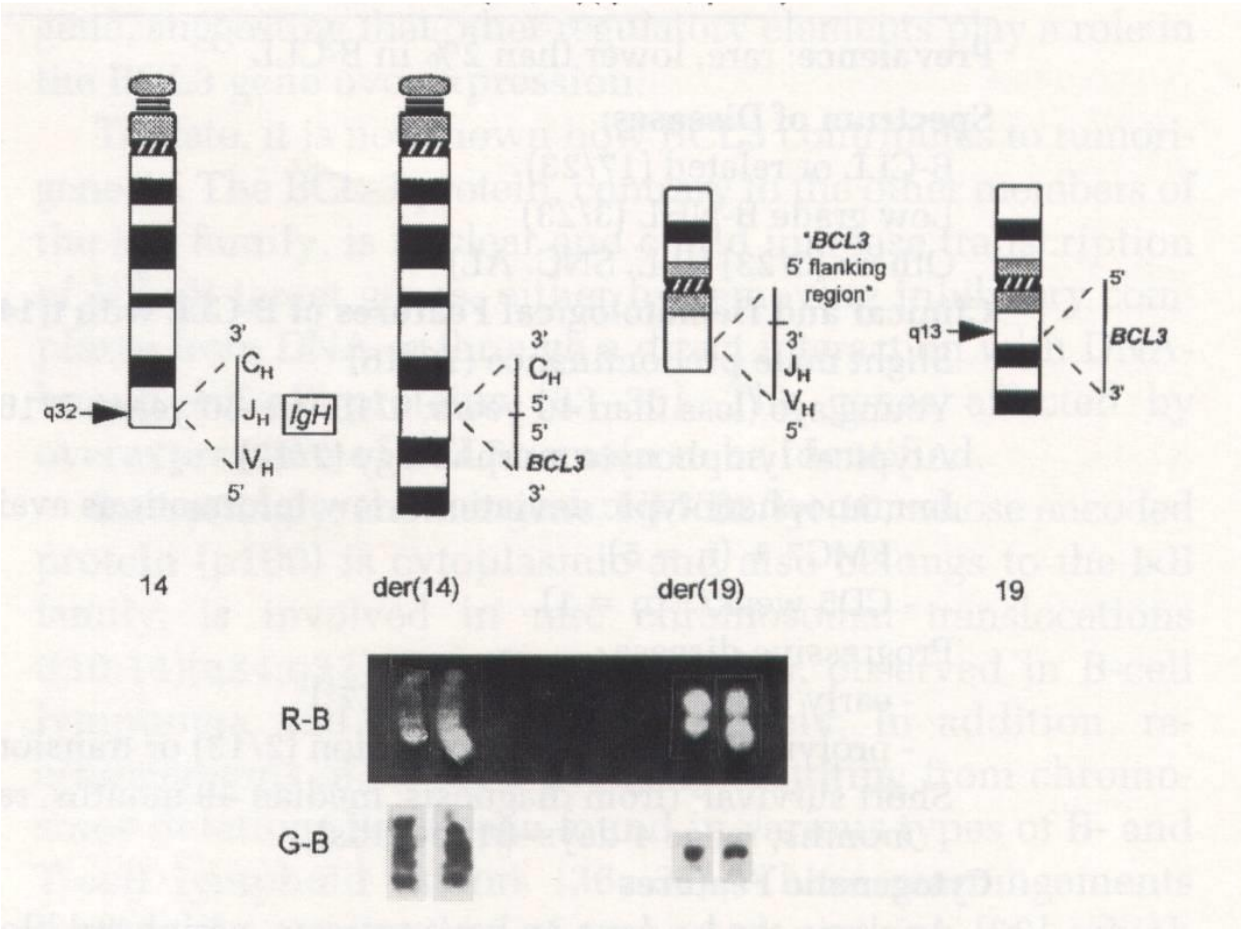
	Events/N	Median OS	SE±	5-year OS	SE±
TP53 mutations/no del17p13	4/10	78.6 months	29.5	75.0%	.158
No *TP53* mutations/del17p13	6/16	79.8 months	22.0	65.6%	.148
TP53 mutations/del17p13	9/18	104.2 months	39.7	66.0%	.128
No *TP53* mutations/no del17p13	40/253	Not reached	-	87.4%	.025

	Events/N	Median time to chemorefractoriness	SE±
TP53 mutations/no del17p13	6/8	9.8 months	5.1
No *TP53* mutations/del17p13	8/12	55.2 months	23.1
TP53 mutations/del17p13	11/13	5.5 months	1.4
No *TP53* mutations/no del17p13	38/101	72.7 months	39.1

- TP53 mutations are an independent prognostic factor

(Ghia P, adapted from Cerri et al EHA, 2008)

t(14;19)(q32;q13)/*BCL3* in CLL



t(14;19)(q32;q13)/*BCL3* in CLL

Present in 0,1-2% of CLL

Rare as single; additional trisomy 12 in 50% of the cases

Overexpression of *BCL3* (modulation of NF-kB TF)

Atypical morphology

FMC7 positive – CD5 weak positivity

Frequent in the young age

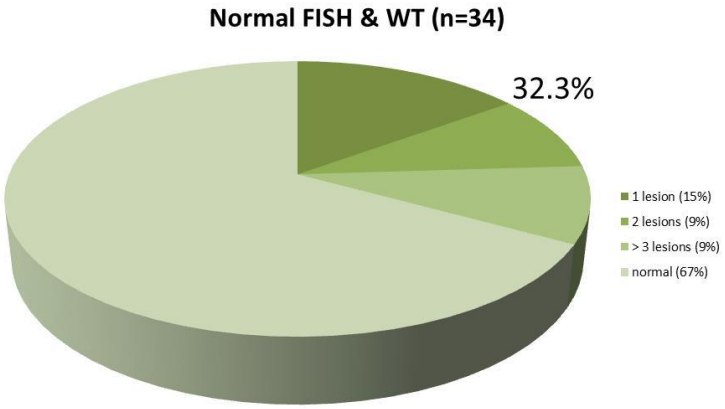
Cytologic transformation

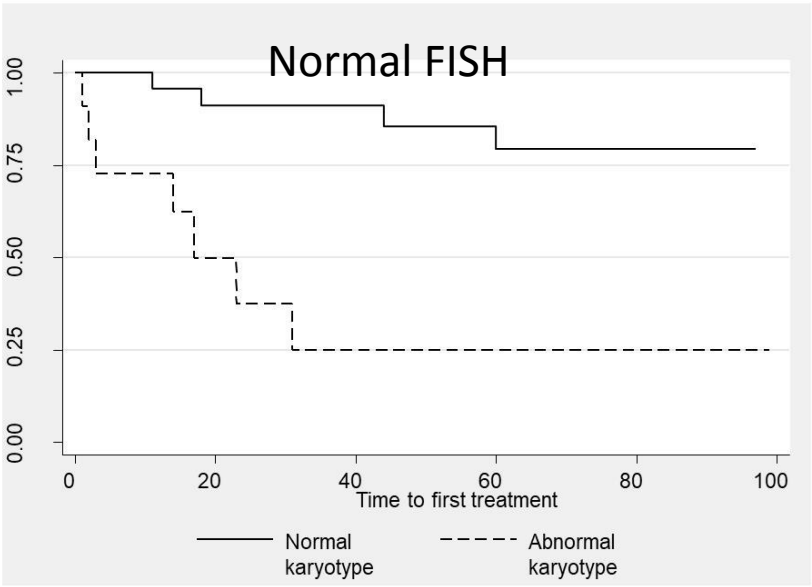
Disease progression and short survival

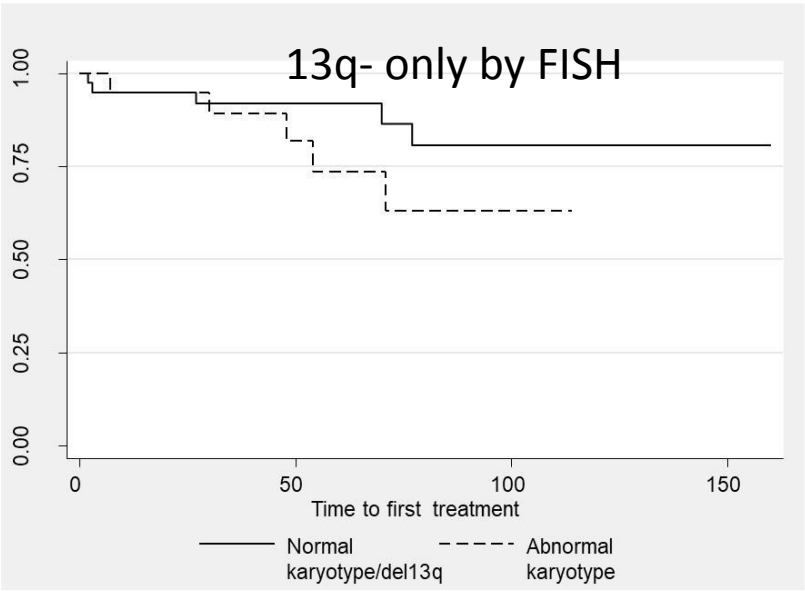
Nguyen –Kach, EHA 2008

INTEGRATION OF NOVEL GENE MUTATIONS INTO KARYOTYPE-BASED SUBGROUPS AS A PROGNOSTIC RISK STRATIFICATION TOOL IN TREATMENT-NAÏVE CLL

N = 153	n	%
17p-	5	3.3%
11q-	13	8.5%
+12	19	12.4%
Normal	46	30%
13q-	70	45.8%







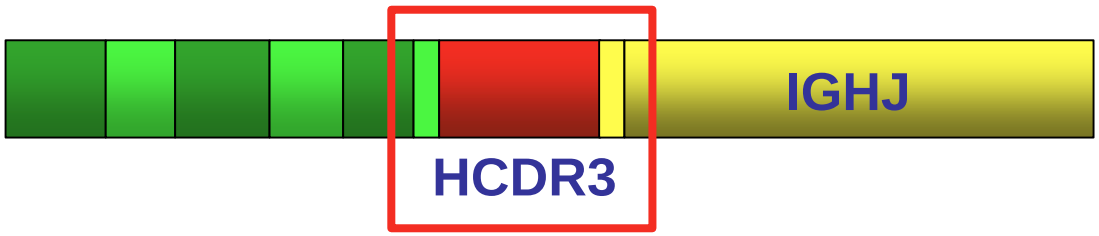
Del Giudice I et al, EHA 2014 poster

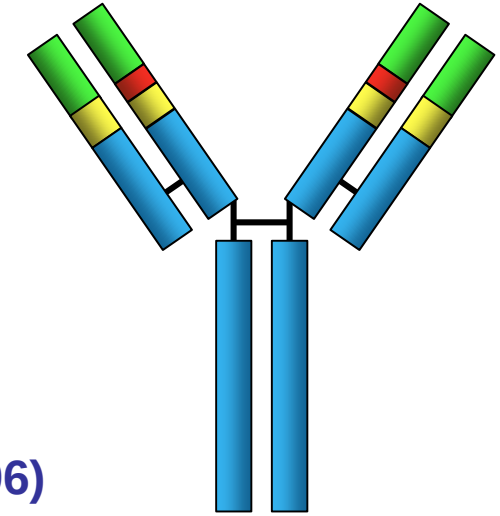
“Stereotypy” in BCR Structure

Chance that two different B-cell clones might randomly use identical B cell receptors: 10^{-10}-10^{-12} (0.00000000001%)

In CLL, this can occur once in every in 100-1000 clones

Homologous complementarity determining region





30.4% OF ALL CLL CASES (2308/7596)

˜50% of U-CLL cases

Stereotyped Receptors

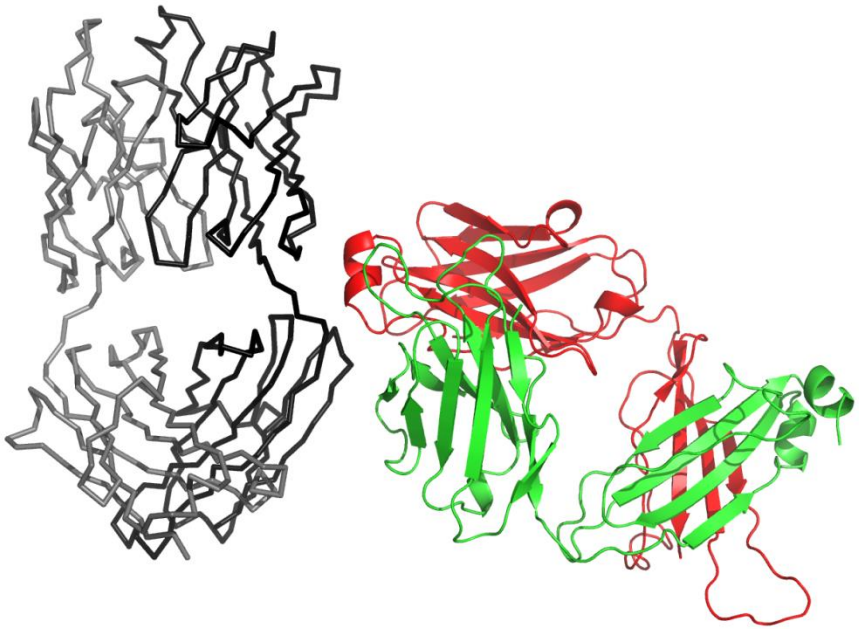
14

- Common herpesviruses and CLL: molecular evidence for a potential link with a subset of patients expressing stereotyped IGHV4-34 B cell receptors (Kostareli E., et al – Thessaloniki)
- CLL transforming to Richter's syndrome carry stereotyped HCDR3s at very high frequency (>50%) and display biased use of IGHV4-39 genes (Valeria S. et al – Novara)
- Novel molecular and clinical features of CLL expressing or not expressing stereotyped B cell receptors: results of an Italian multicentric study (Bomben R., et al – Aviano)
- Analysis of Chronic Lymphocytic Leukemis csaes with stereotypic immunoglobulin's receptors in Ukrainian cohort (Bilous N., et al – Kiev)

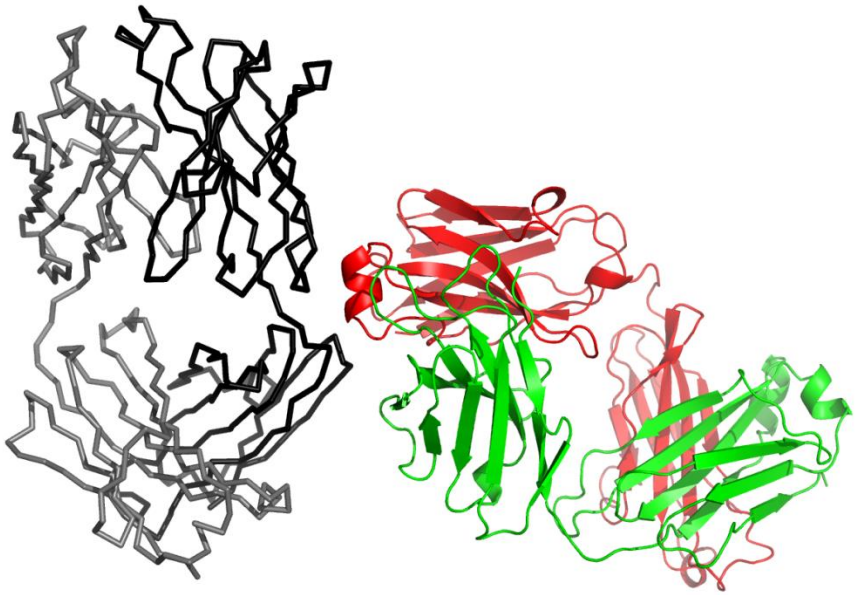
Ghia P – EHA 2008

Subset 4: self recognition of CLL Fab

CLL240



CLL183



Interaction with the V-C hinge (VH FR1 and CH1 domains)

Gounari et al, EHA-21 abstract#116, 2016

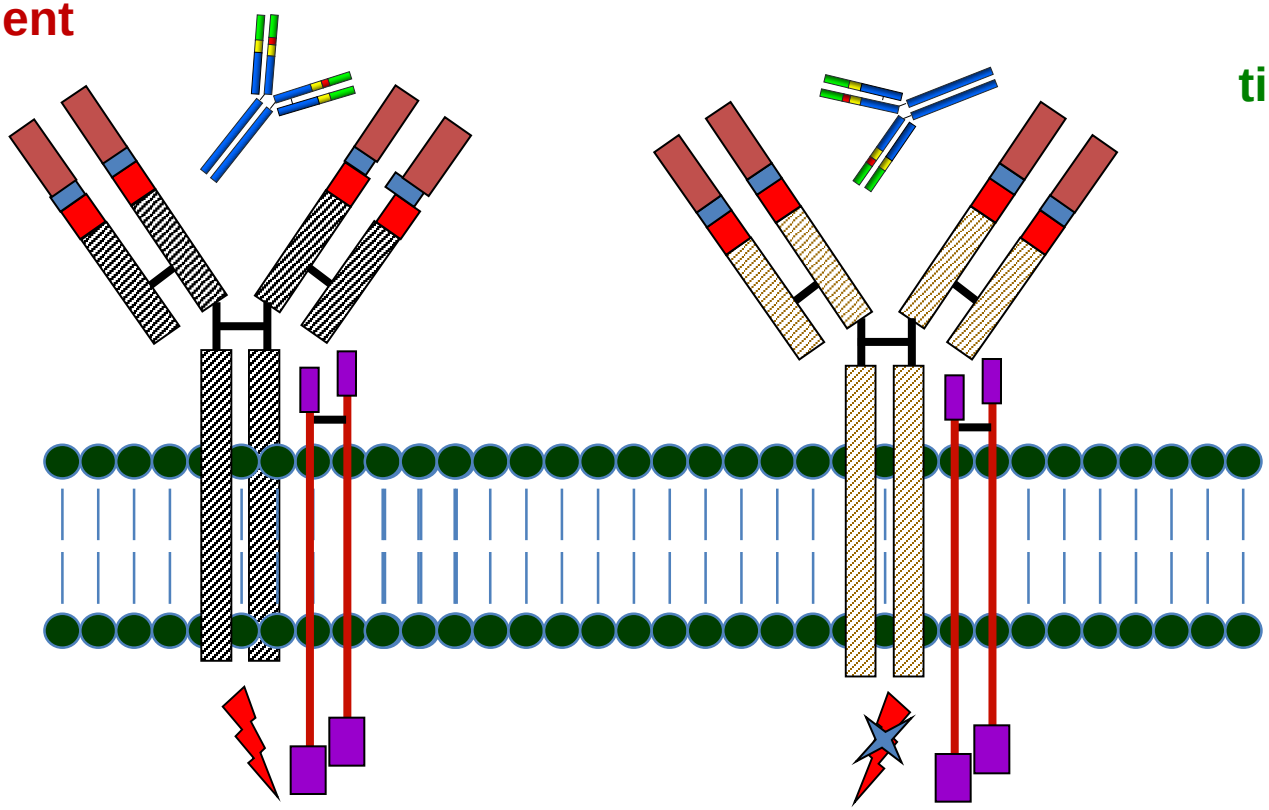
BCR signalling in CLL is heterogeneous

Aggressive

Indolent (anergic)

weak, transient binding

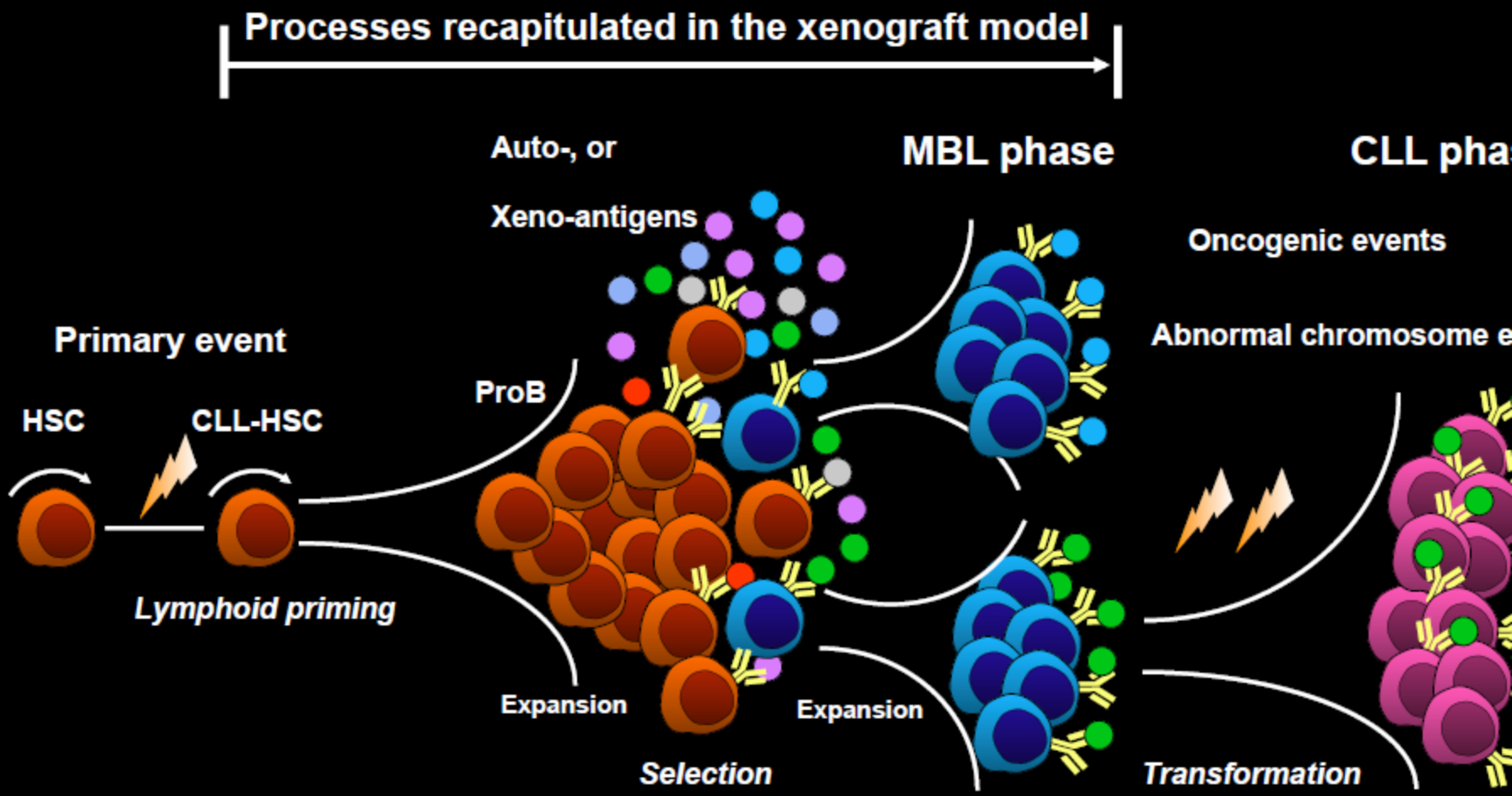
tight, stable binding



Survival
Proliferation

Courtesy of Paolo Ghia

Self-Renewing HSP is the Primary Target in the Pathogenesis of Human CLL



LETTER

doi:10.1038/nature10113

Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia

Xose S. Puente[1], Magda Pinyol[2], Víctor Quesada[1], Laura Conde[3], Gonzalo R. Ordóñez[1], Neus Villamor[3], Georgia Escaramis[4], Pedro Jares[3], Silvia Beà[3], Marcos González-Díaz[5], Laia Bassaganyas[4], Tycho Baumann[6], Manel Juan[7], Mónica López-Guerra[3], Dolors Colomer[3], José M. C. Tubio[4,8], Cristina López[3], Alba Navarro[3], Cristian Tornador[4], Marta Aymerich[3], María Rozman[3], Jesús M. Hernández[5], Diana A. Puente[1], José M. P. Freije[1], Gloria Velasco[1], Ana Gutiérrez-Fernández[1], Dolors Costa[3], Anna Carrió[3], Sara Guijarro[3], Anna Enjuanes[3], Lluís Hernández[3], Jordi Yagüe[7], Pilar Nicolás[9], Carlos M. Romeo-Casabona[9], Heinz Himmelbauer[10], Ester Castillo[10], Juliane C. Dohm[10], Silvia de Sanjosé[11], Miguel A. Piris[12], Enrique de Alava[5], Jesús San Miguel[5], Romina Royo[13], Josep L. Gelpí[13], David Torrents[13], Modesto Orozco[13], David G. Pisano[14], Alfonso Valencia[14], Roderic Guigó[15], Mónica Bayés[16], Simon Heath[16], Marta Gut[16], Peter Klatt[17], John Marshall[18], Keiran Raine[18], Lucy A. Stebbings[18], P. Andrew Futreal[18], Michael R. Stratton[18], Peter J. Campbell[18], Ivo Gut[16], Armando López-Guillermo[6], Xavier Estivill[4], Emili Montserrat[6], Carlos López-Otín[1]* & Elías Campo[3]*

Table 1 | Genes recurrently mutated in chronic lymphocytic leukaemia

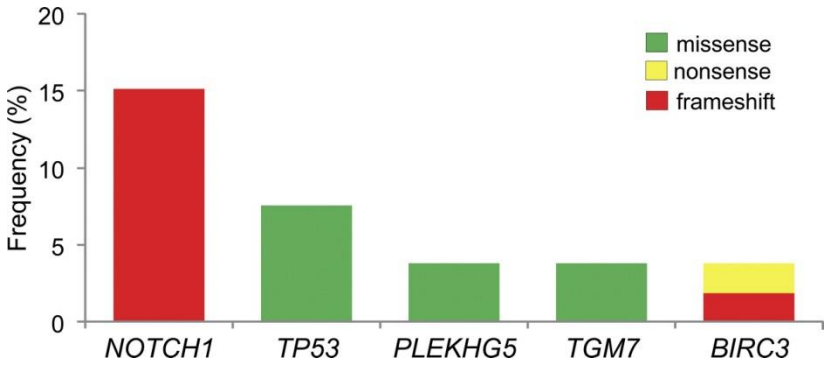
Gene	Protein	Mutation	Mutated cases / total	Overall frequency (%)	Frequency in *IGHV*-unmutated (%)	Frequency in *IGHV*- mutated (%)
NOTCH1	Notch 1	P2515Rfs*4	29/255	12.2	20.4	7
		Q2503*	1/255			
		F2482Ffs*2	1/255			
MYD88	Myeloid differentiation primary response gene 88	L265P	9/310	2.9	0.8	5.6
XPO1	Exportin 1	E571K	3/165	2.4	4.6	0
		E571G	1/165			
KLHL6	Kelch-like 6	F49L/L65P	3/160	1.8	0	4.5
		L90F				
		L58P/T64A/Q81P				

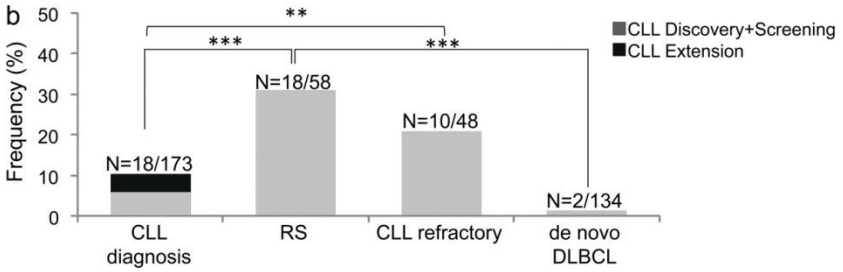
2011

Published June 13, 2011

Analysis of the chronic lymphocytic leukemia coding genome: role of *NOTCH1* mutational activation

Giulia Fabbri,[1] Silvia Rasi,[5] Davide Rossi,[5] Vladimir Trifonov,[2] Hossein Khiabanian,[2] Jing Ma,[6] Adina Grunn,[1] Marco Fangazio,[5] Daniela Capello,[5] Sara Monti,[5] Stefania Cresta,[5] Ernesto Gargiulo,[5] Francesco Forconi,[7] Anna Guarini,[8] Luca Arcaini,[9] Marco Paulli,[10] Luca Laurenti,[11] Luigi M. Larocca,[12] Roberto Marasca,[13] Valter Gattei,[14] David Oscier,[15] Francesco Bertoni,[16] Charles G. Mullighan,[6] Robin Foà,[8] Laura Pasqualucci,[1,3] Raul Rabadan,[2] Riccardo Dalla-Favera,[1,3,4] and Gianluca Gaidano[5]





Fabbri et al. J Exp Med 2011

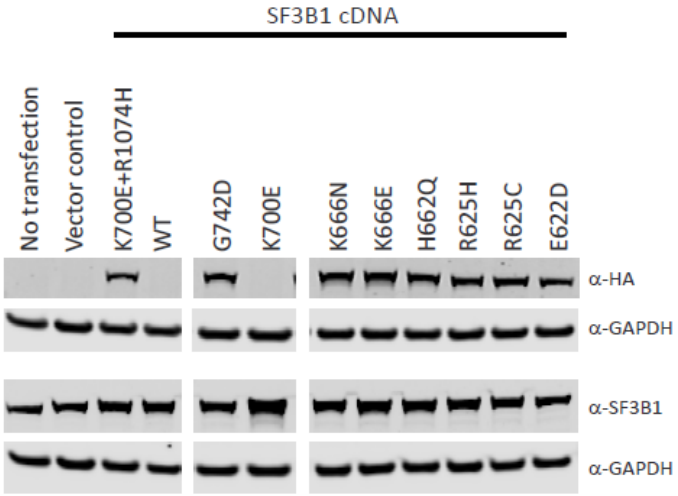
2014

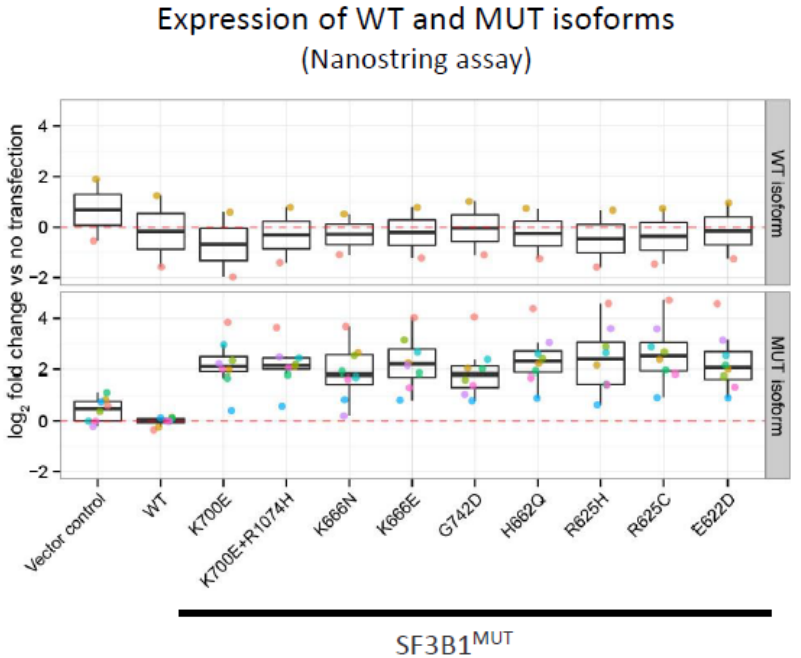
Expression of SF3B1MUT upregulates mutant associated splice isoforms

293FT cells transfected with SF3B1 cDNA

48hr

Total RNA extracted for Nanostring

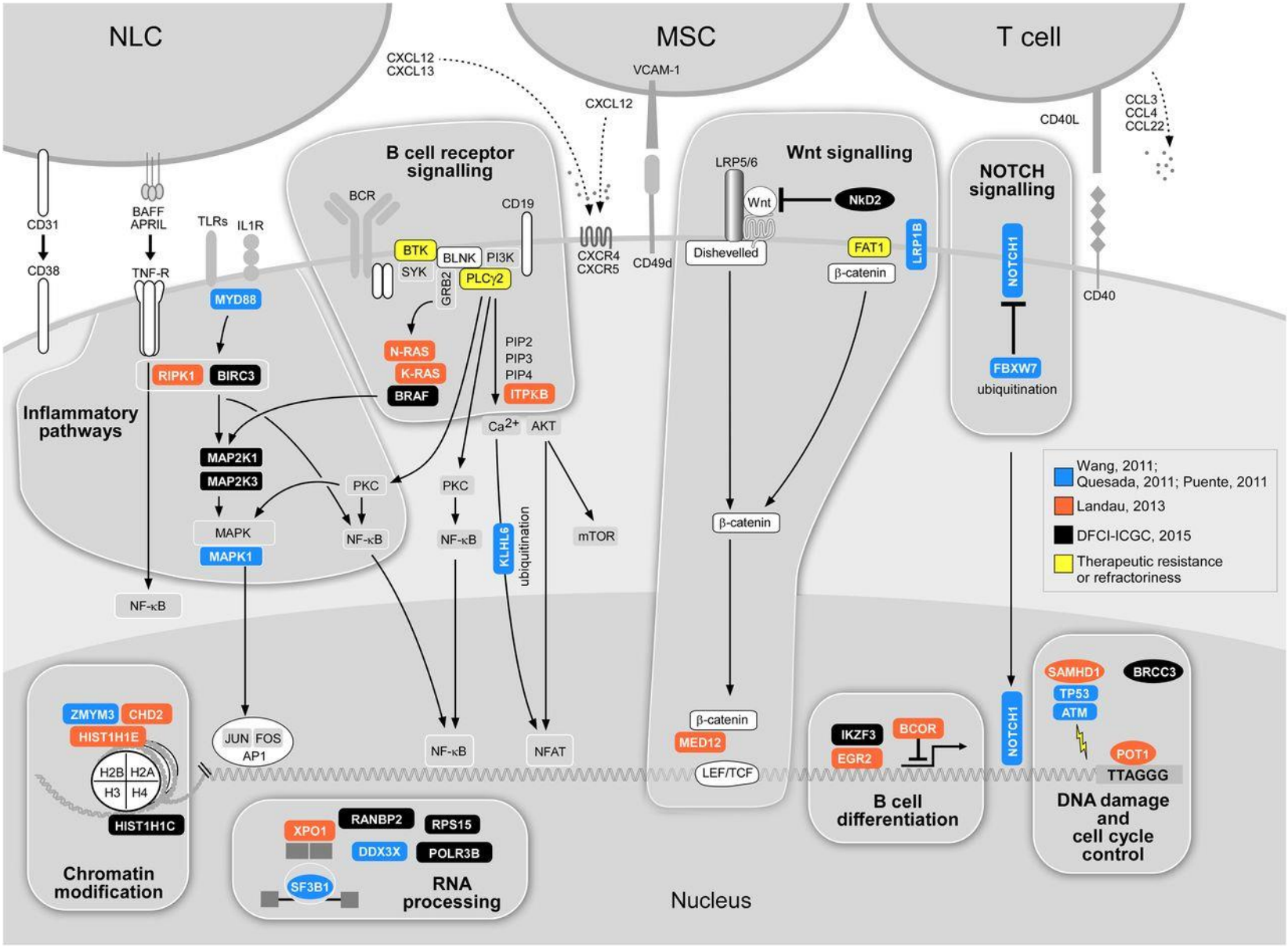




H3 BIOMEDICINE

Buonamici et al., CLL Biology Session EHA 2014

Putative core cellular pathways affected by significantly mutated genes in CLL.

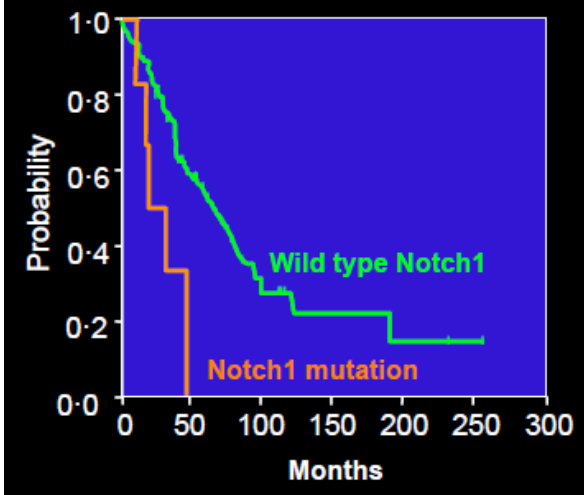


Romain **Guièze, and Catherine J. Wu Blood 2015;126:445-453**

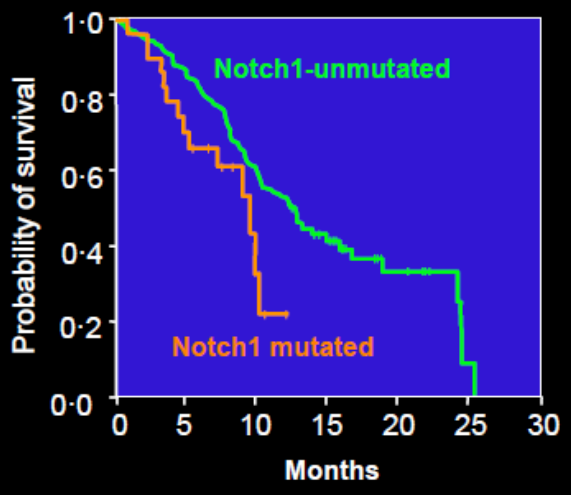
2011

Pivotal Studies Suggest a Prognostic Role of *NOTCH1* Mutations in CLL

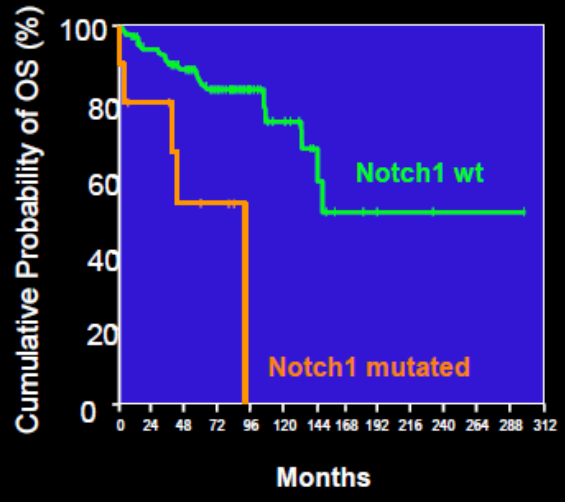
Sportoletti P. et al., Br J Haematol 2010



Puente XS. et al., Nature 2011



Fabbri G. et al., J Exp Med 2011



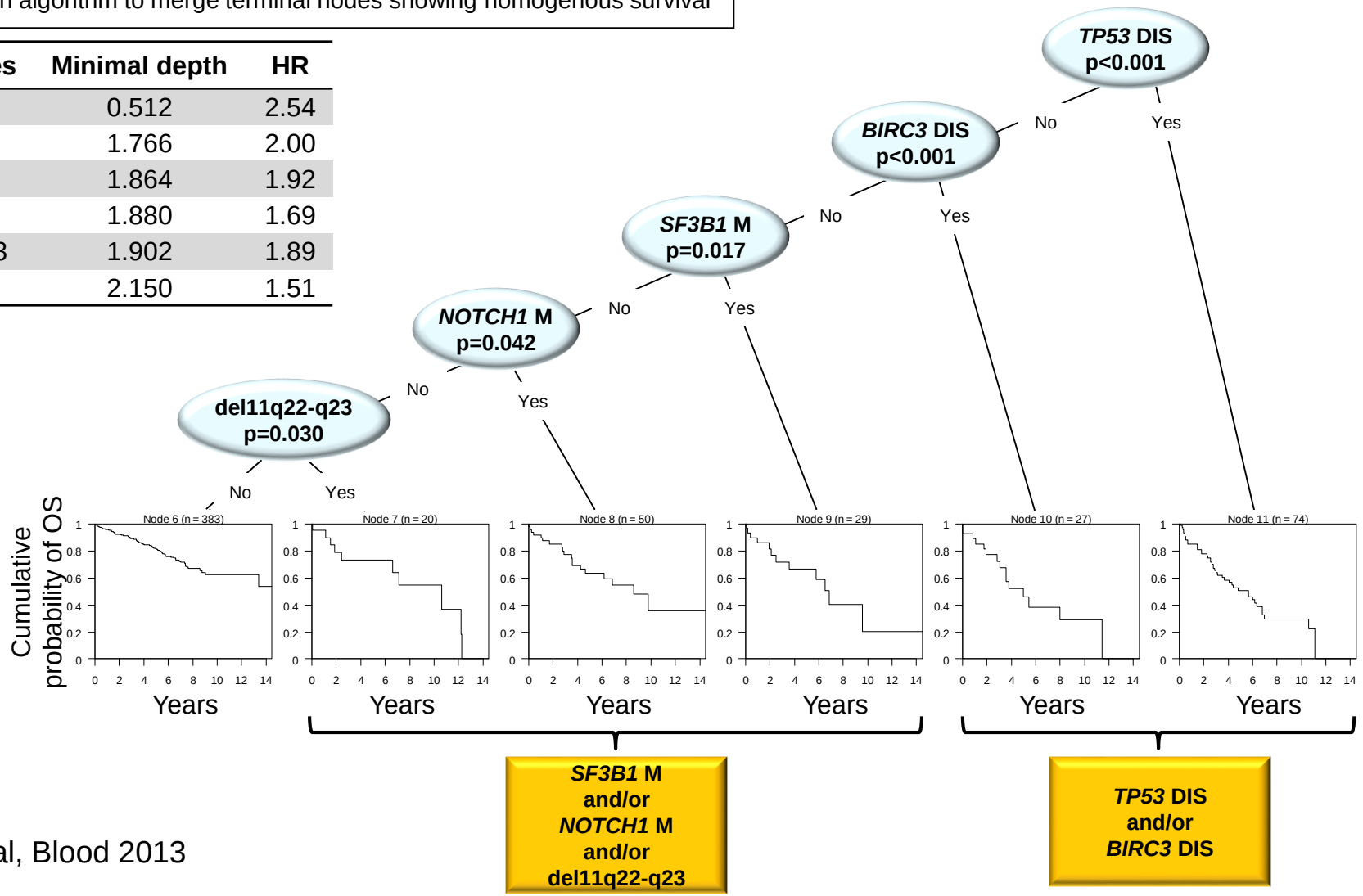
Study	N. patients	*NOTCH1* mutation	Clinical endpoint	Multivariate analysis
Sportroletti P, 2010	133	5%	TFS	No
Puente XS, 2011	255	12%	OS	No
Fabbri G, 2011	120	10%	TFS & OS	Yes

Università di Padova

Hierarchical order of relevance of genetic lesions in predicting survival in newly diagnosed CLL

- Recursive partitioning to divide patients into genetic subgroups with different OS
- Random survival forest validation of the stability of the recursive decision tree
- Amalgamation algorithm to merge terminal nodes showing homogenous survival

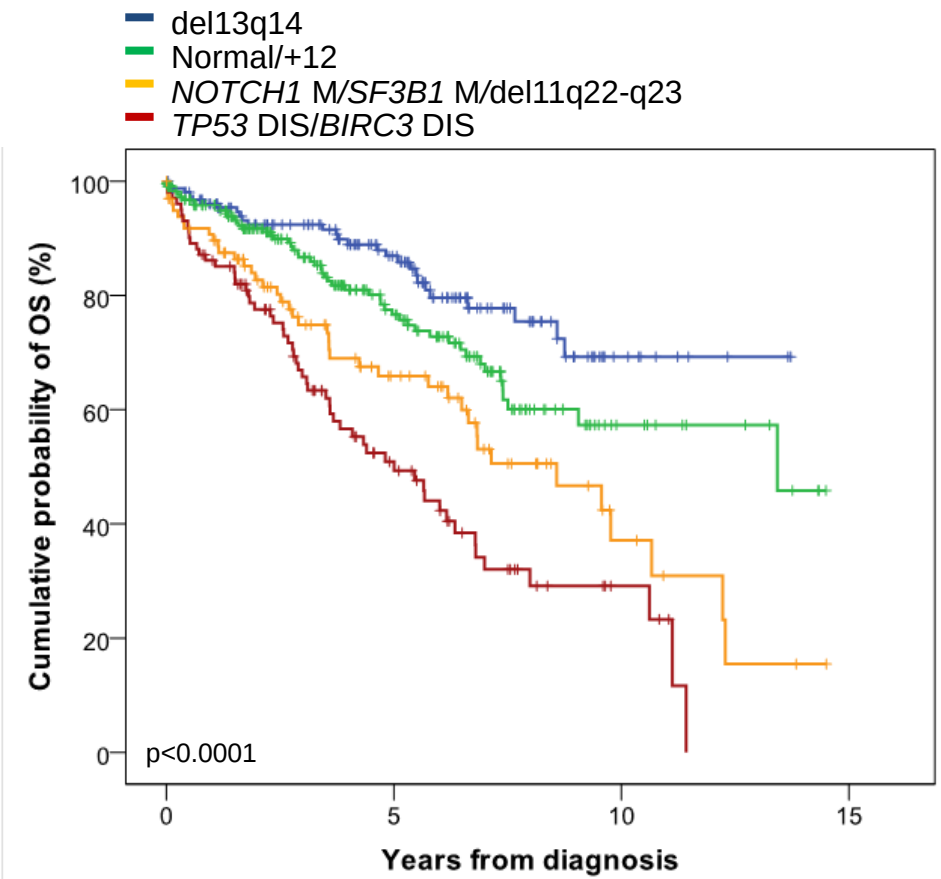
Top variables	Minimal depth	HR
TP53 DIS	0.512	2.54
BIRC3 DIS	1.766	2.00
SF3B1 M	1.864	1.92
NOTCH1 M	1.880	1.69
del11q22-q23	1.902	1.89
+12	2.150	1.51



Rossi et al, Blood 2013

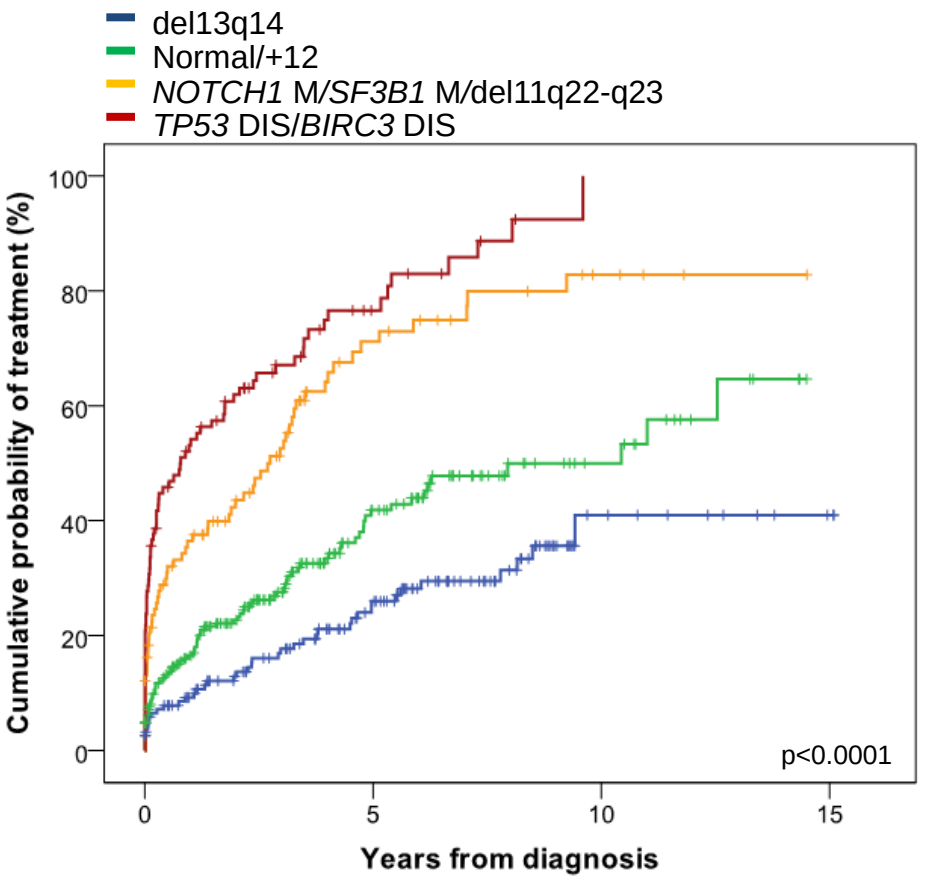
Integrated mutational and cytogenetic model for CLL prognostication *(Rossi et al, Blood 2013)*

OS



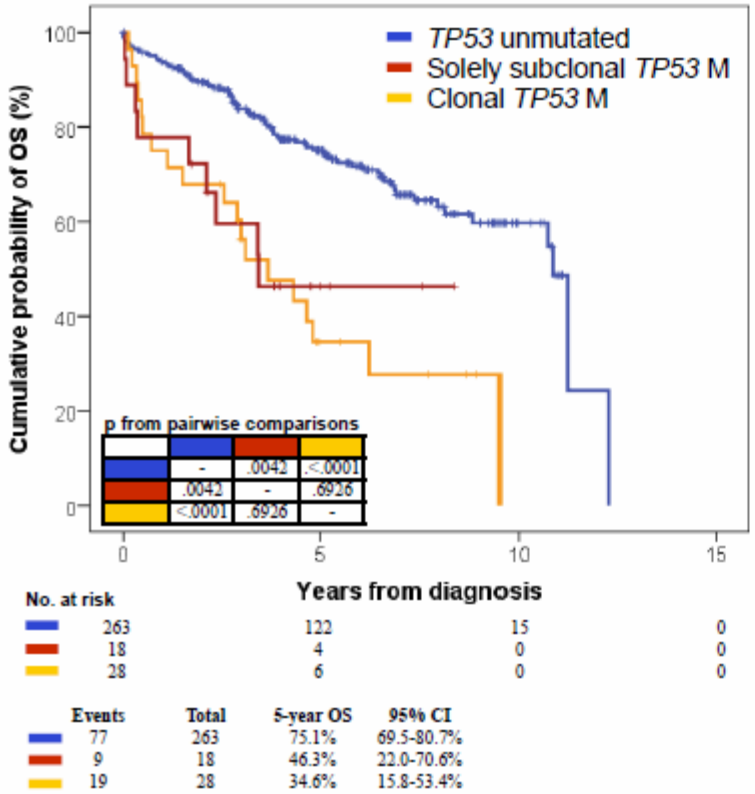
	N	%	10-years OS
del13q14	155	26%	69%
Normal/+12	228	39%	57%
NOTCH1 M/*SF3B1* M/del11q22-q23	99	17%	37%
TP53 DIS/*BIRC3* DIS	101	17%	29%

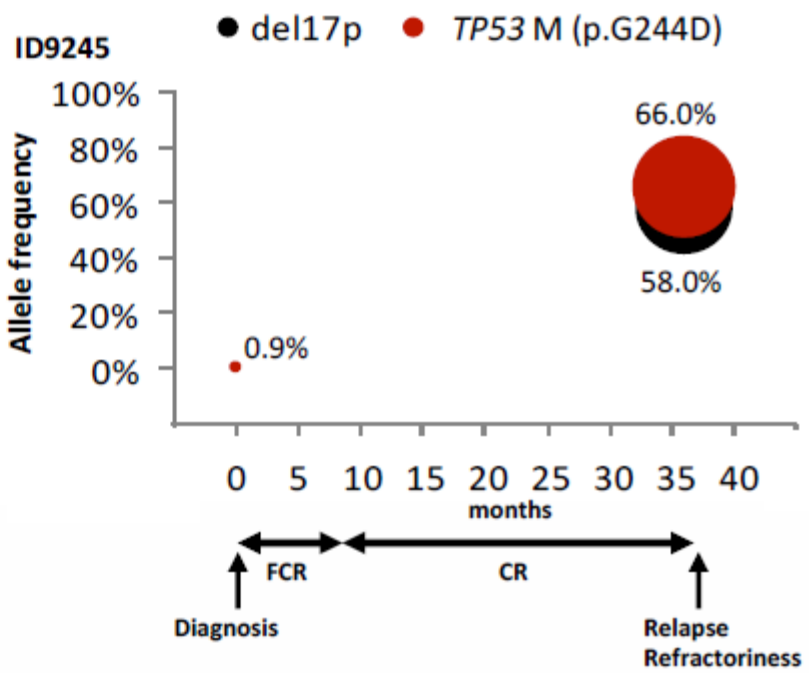
Treatment



	N	%	Treated at 10 years
del13q14	155	26%	41%
Normal/+12	228	39%	50%
NOTCH1 M/*SF3B1* M/del11q22-q23	99	17%	83%
TP53 DIS/*BIRC3* DIS	101	17%	100%

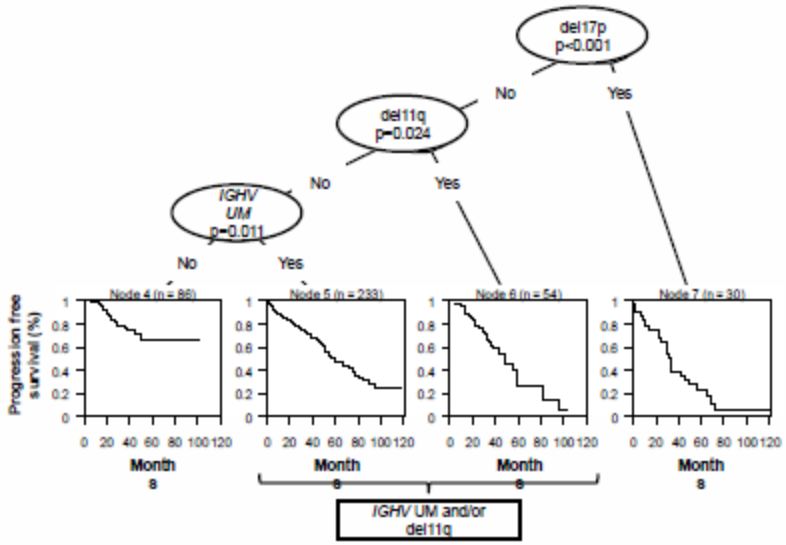
Small *TP53* mutated subclones have the same unfavorable prognostic impact as clonal *TP53* defects

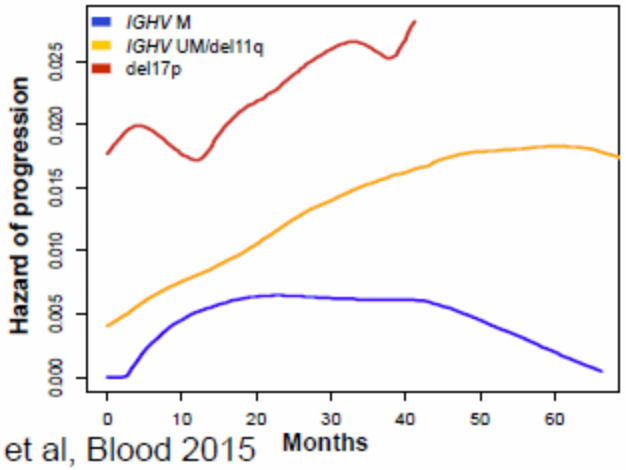


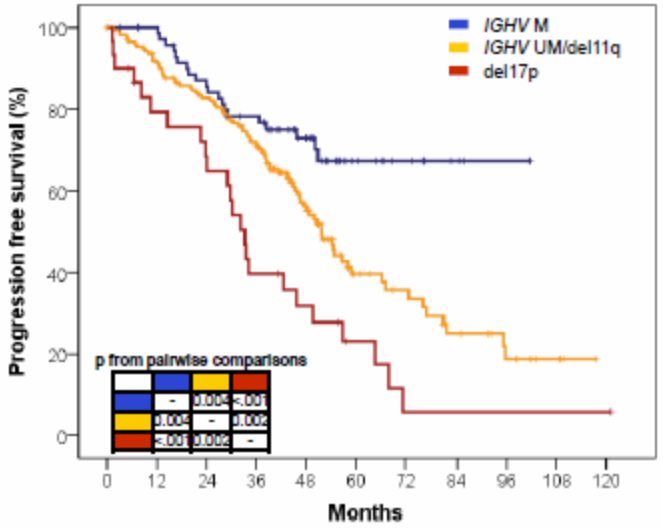


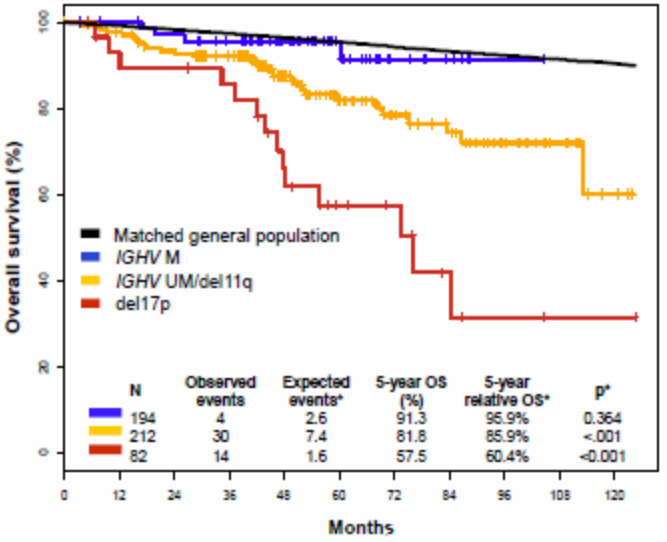
Rossi, Blood 2014

IGHV mutated patients devoid of del17p and del11q gain the greatest benefit from FCR



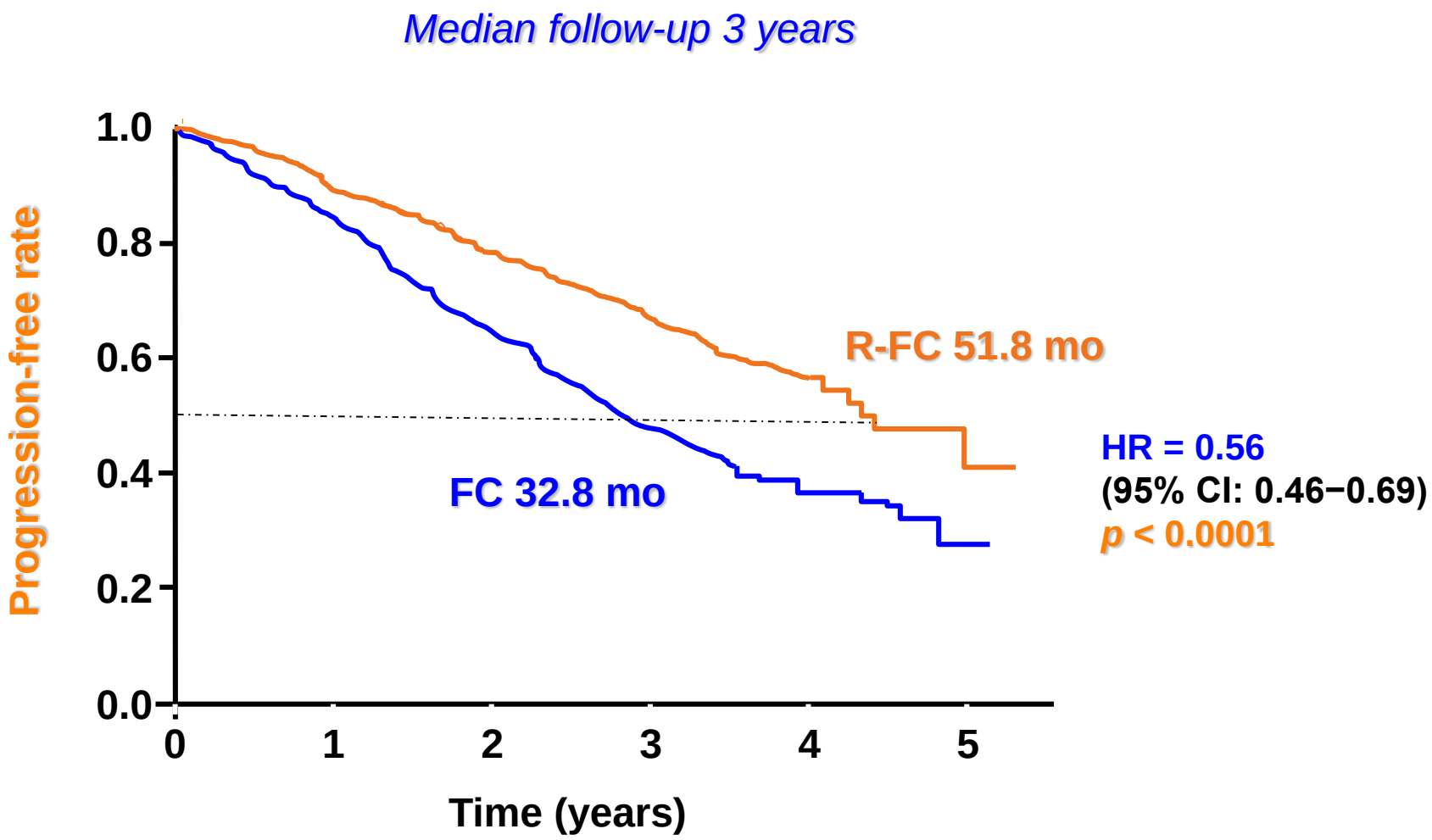






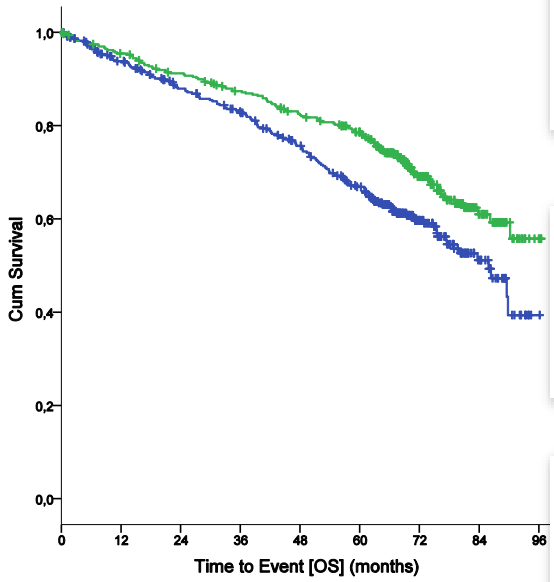
Rossi et al, Blood 2015

CLL8: Progression-free Survival



Ghia P 2010, courtesy of C. Wendtner

CLL8 trial: Overall survival, update 2012



Median observation time 5.9 years

FCR 69.4% alive
Median not reached
FC 62.3% alive
Median 86 months

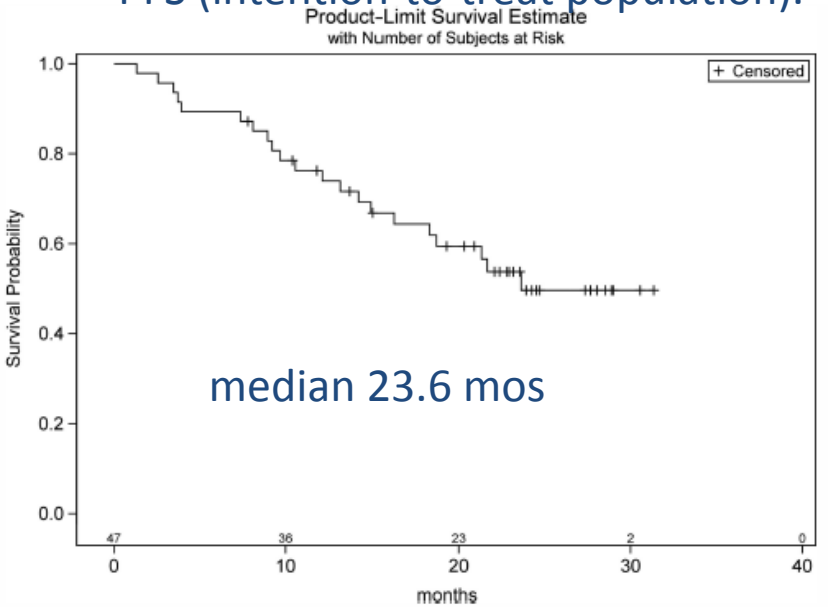
HR 0.68,
95% CI 0.535-0.858
p=0.001

A SINGLE-ARM MULTI-CENTER TRIAL OF BENDAMUSTINE GIVEN WITH OFATUMUMAB) IN PATIENTS WITH REFRACTORY OR RELAPSED CLL. GIMEMA CLL0809 PROTOCOL

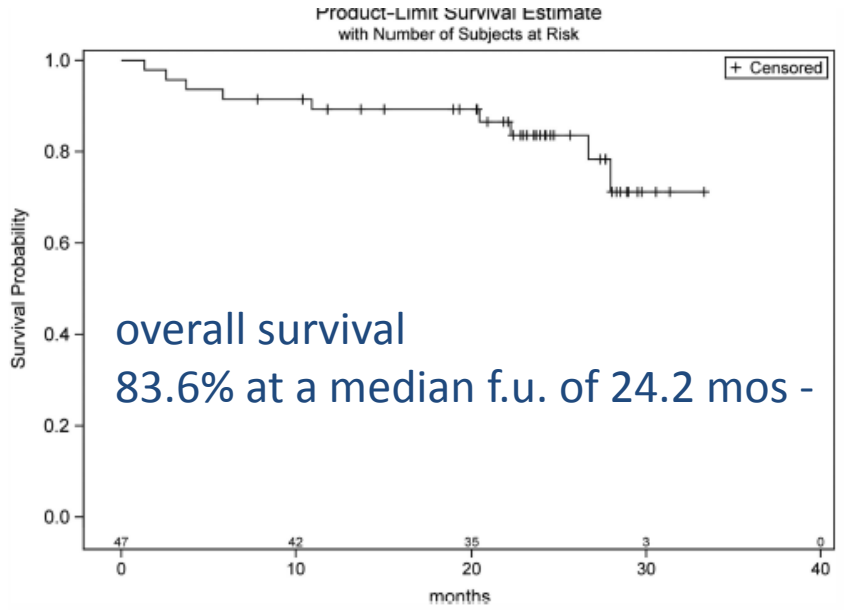
Table 1. Patient demographic and baseline clinical characteristics

Characteristic	*No. of patients*	*% Of patients*
Sex		
Male	35	71
Female	14	29
Age, years		
Median (range)	66 (46–81)	35
⩾70	17	65
<70	32	
Previous treatment lines		
1	30	61
2	19	39
Previous chemotherapy		
Fludarabine-based	37	75
Rituximab-based	27	55
Alemtuzumab-based	6	12
IGHV mutational status		
Mutated	17	35
Unmutated	32	65
Expression of ZAP-70 (>20%)	30	61
Genomic aberrations by FISH		
del(11q)[a]	6	12
del(13q)	23	47
del(17p)	9	18
+12	9	18
Expression of CD38 (>30%)	21	43
NOTCH1 mutations	8	16
BIRC3 mutations	3	6
SF3B1 mutations	11	22
TP53 mutations	9	18

PFS (intention-to-treat population).



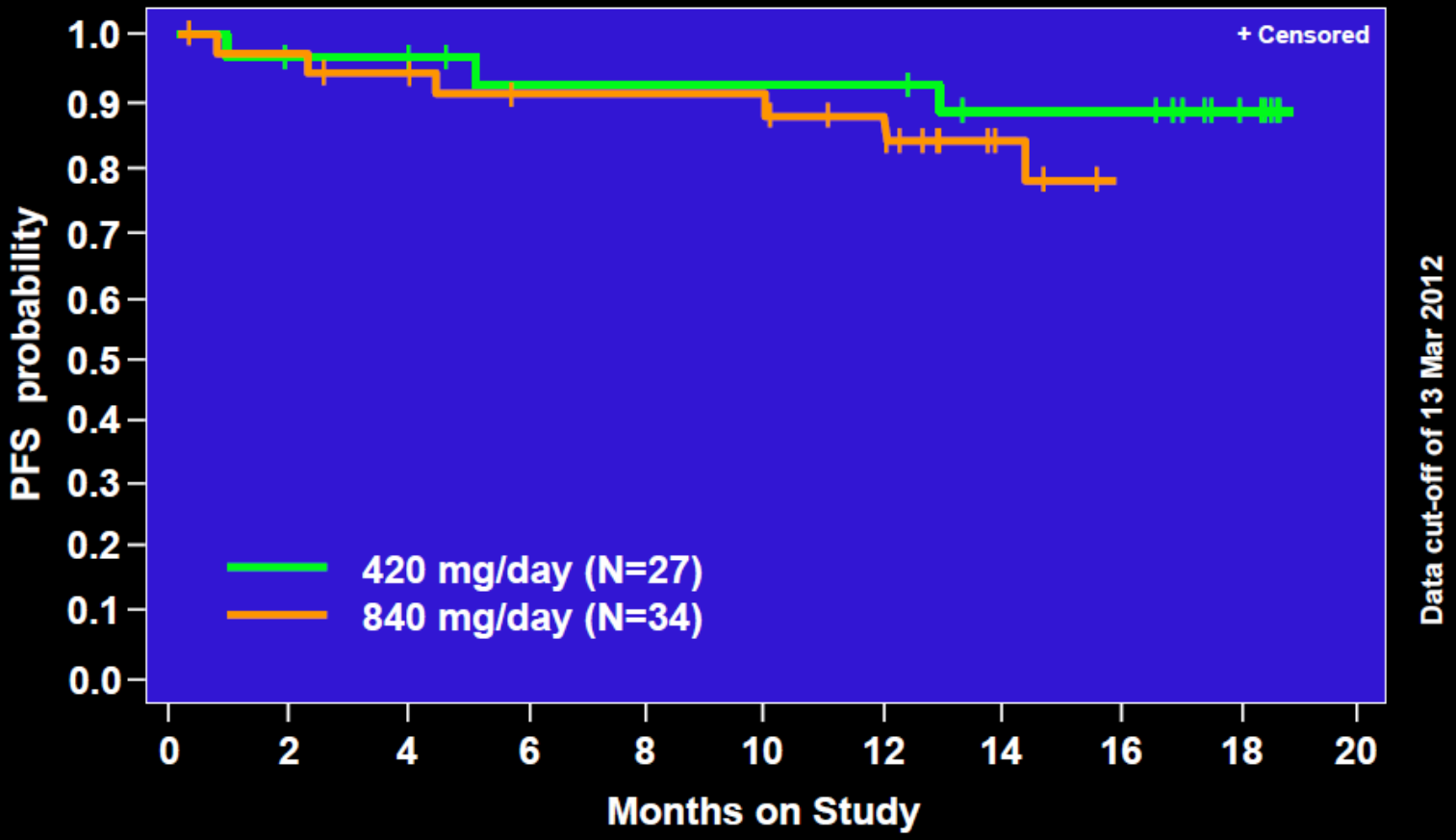
median follow-up of 24.2 months



Cortelezzi et al abs #145 -EHA 2012

PCYC-1102-CA: Progression-Free Survival (Relapsed/Refractory) by Dose

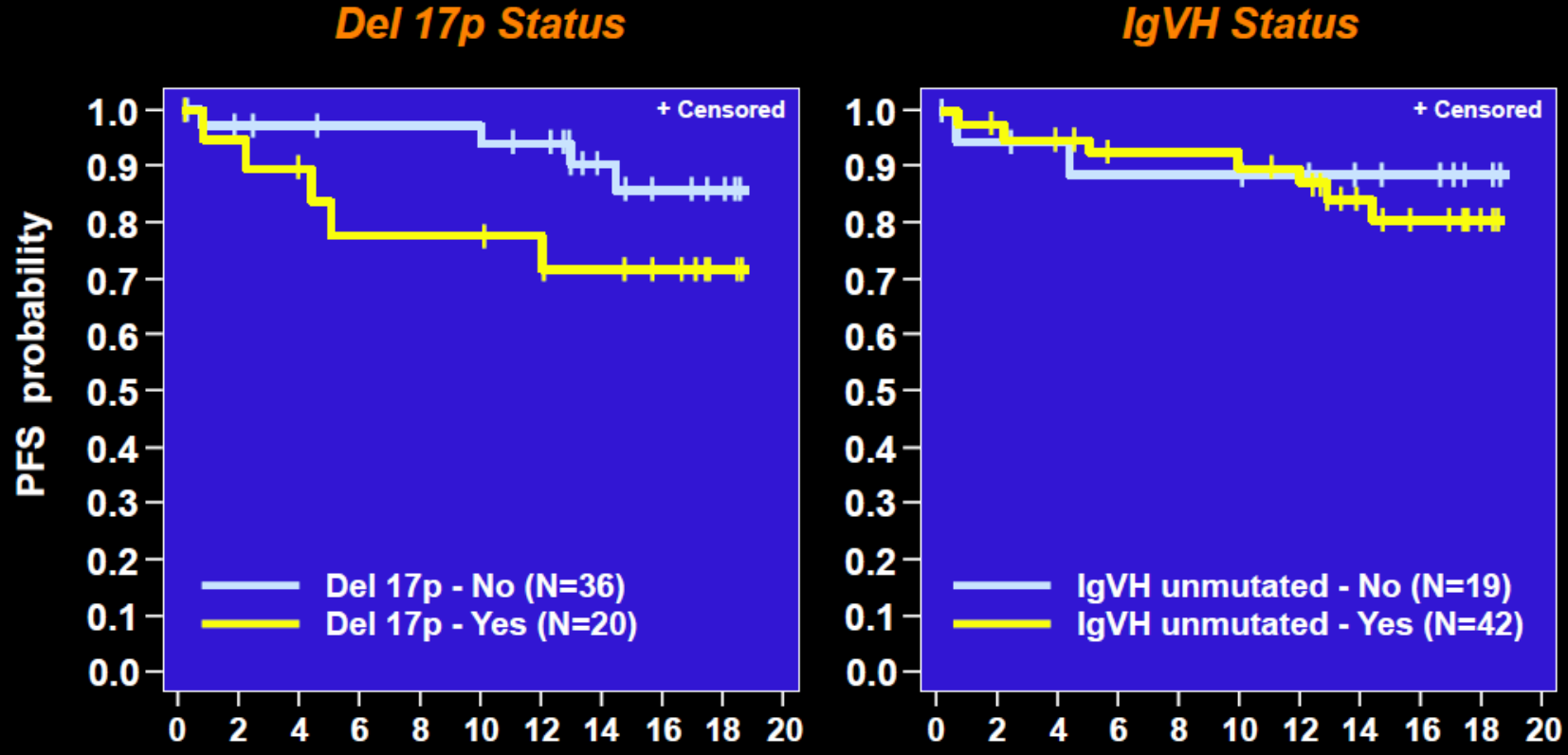
Estimated 18 mo PFS at 420 mg/d = 87.7%



S. O'Brien, EHA Amsterdam, June 14-17 (2012)

Università di Padova

PCYC-1102-CA: Progression-Free Survival (Relapsed/Refractory) by Risk Factors



S. O'Brien, EHA Amsterdam, June 14-17 (2012)

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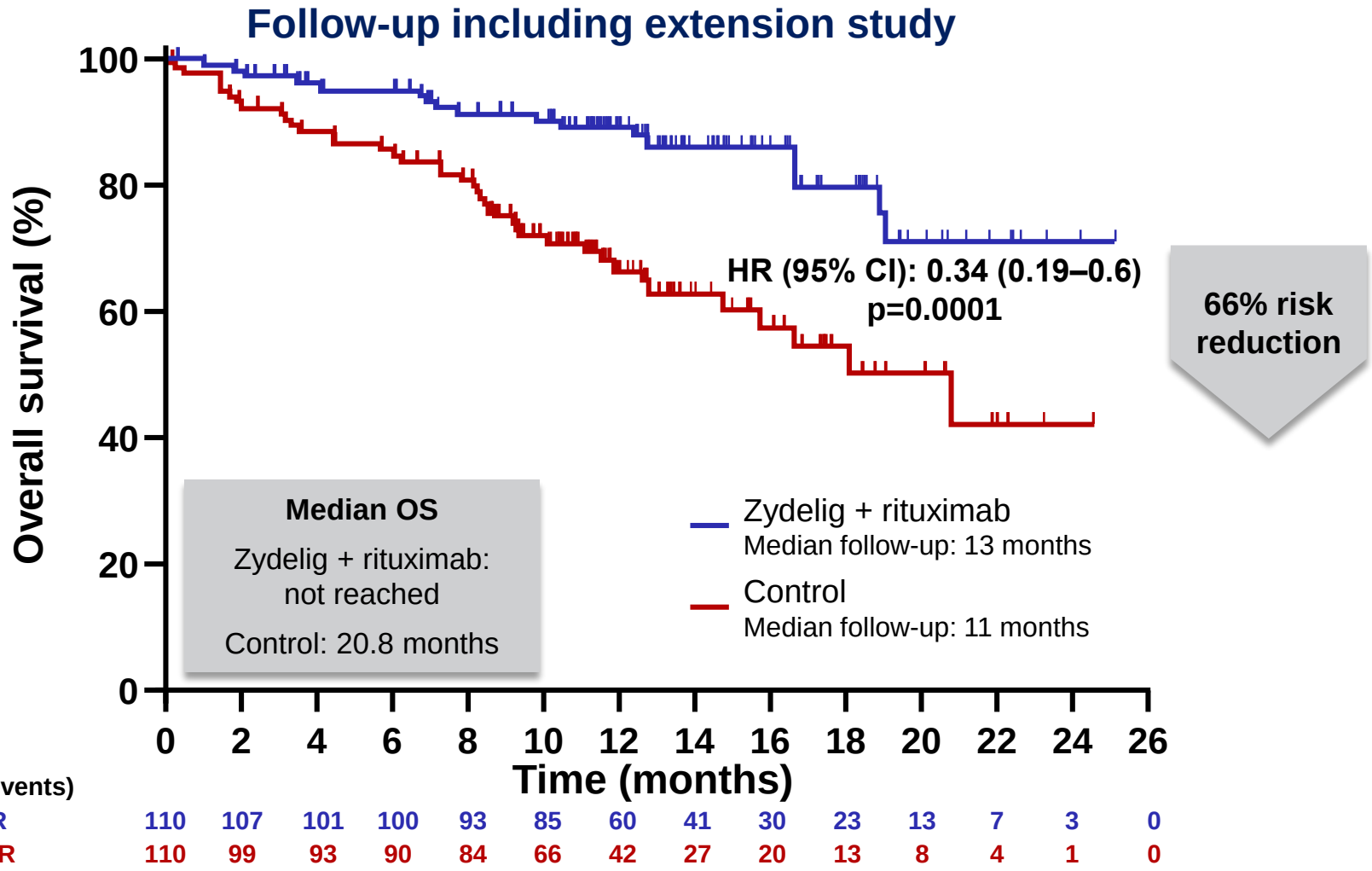
Difference in efficacy of Zydelig + rituximab maintained despite crossover in the extension study

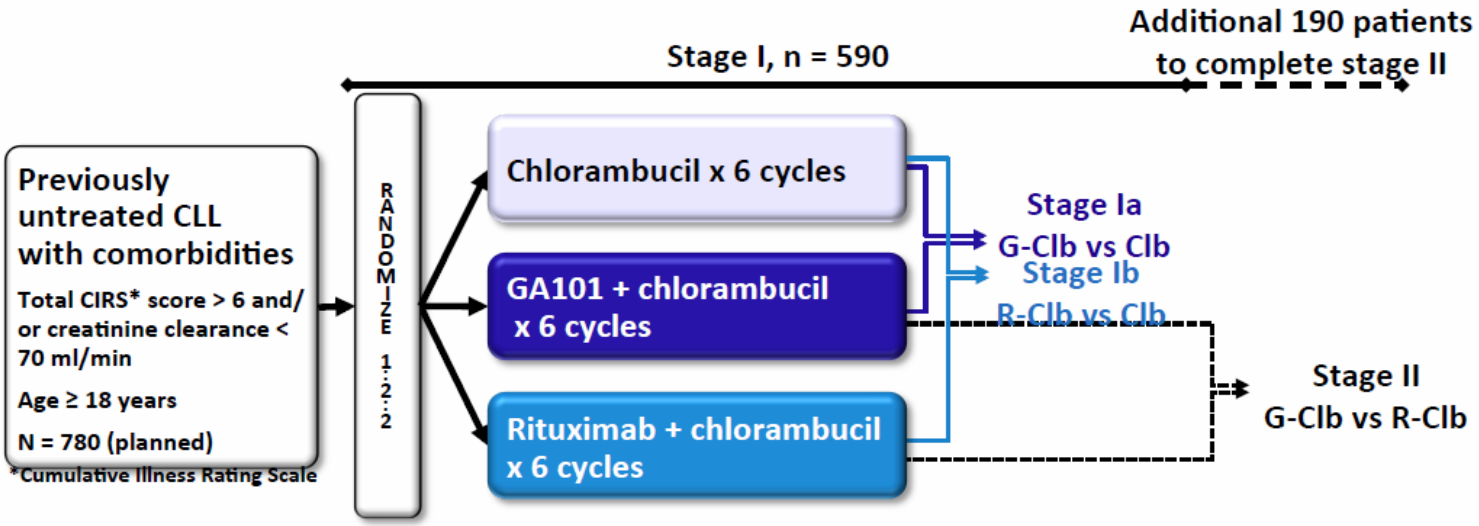


As randomised, including crossover study

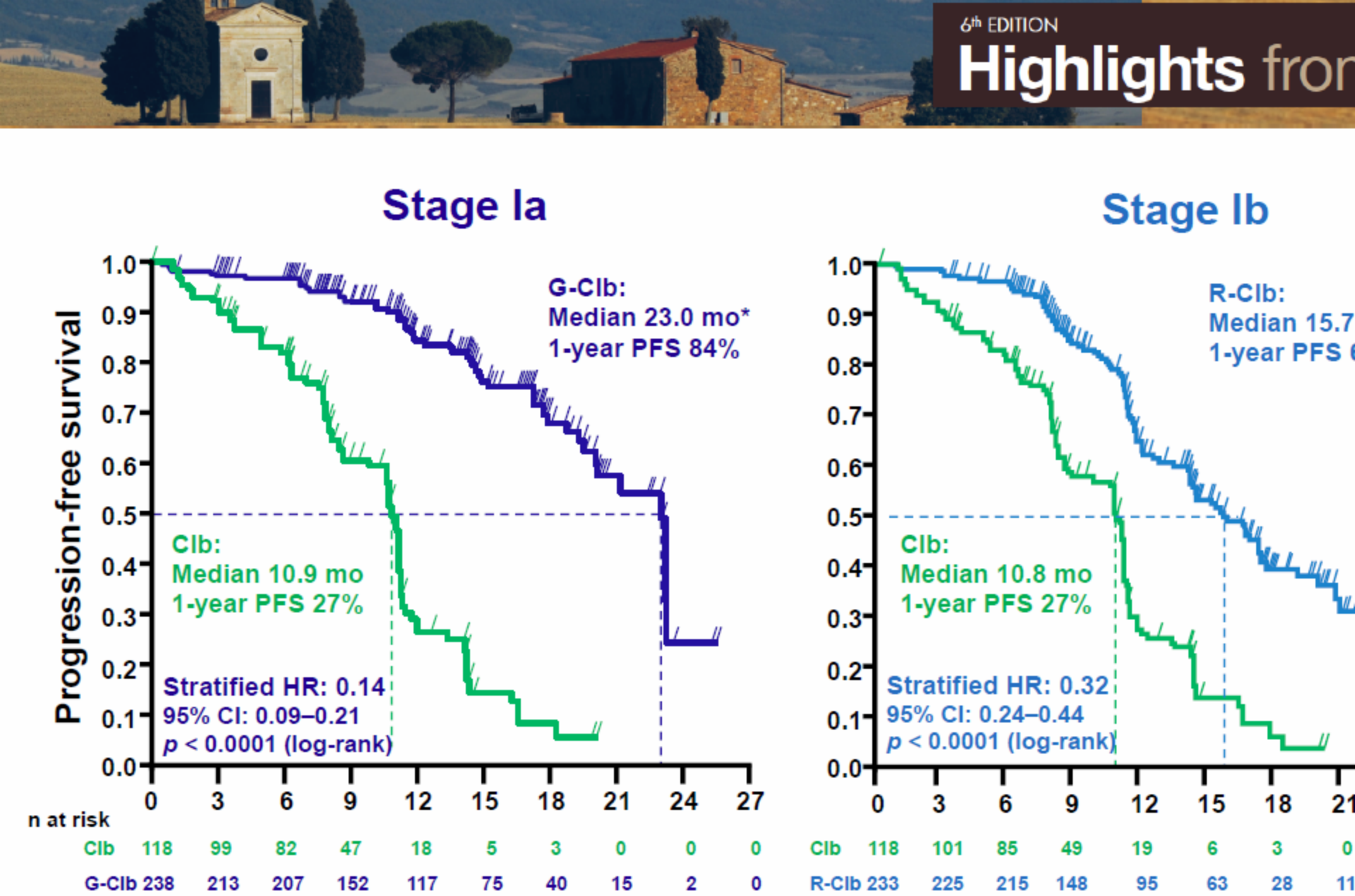
Ghia 2014, adapted from Sharman JP, *et al*. ASH 2014 (Abstract 330; oral presentation).

2013

CLL11: Study design



- GA101: 1,000 mg days 1, 8, and 15 cycle 1; day 1 cycles 2–6, every 28 days
- Rituximab: 375 mg/m^2 day 1 cycle 1, 500 mg/m^2 day 1 cycles 2–6, every 28 days
- Clb: 0.5 mg/kg day 1 and day 15 cycle 1–6, every 28 days
- Patients with progressive disease in the Clb arm were allowed to cross over to G-Clb



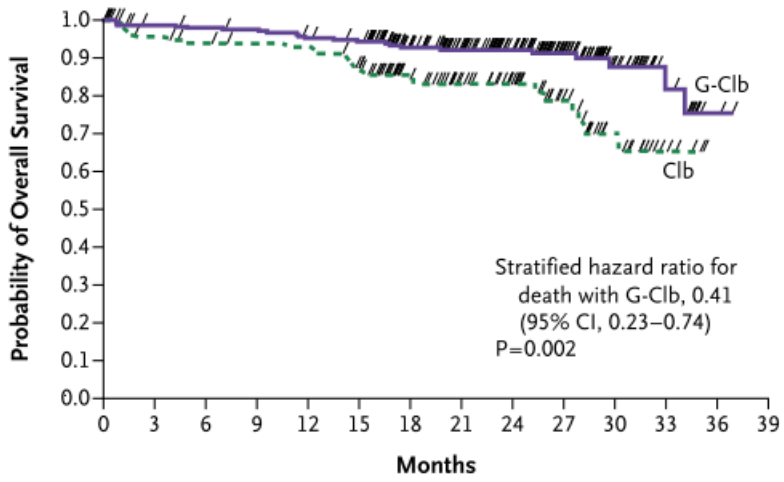
Type 1 error controlled through closed test procedure; p-value of the global test was <.0001.

* In the G-Clb arm < 10% of patients had reached the median at cutoff; therefore, in contrast to the Clb arm the G-Clb median PFS could not be reliably estimated due to the few patients at risk at time of G-Clb median.

Improved survival using Obinutuzumab plus Chlorambucil in Patients with CLL and Coexisting Conditions

The NEW ENGLAND JOURNAL of MEDICINE

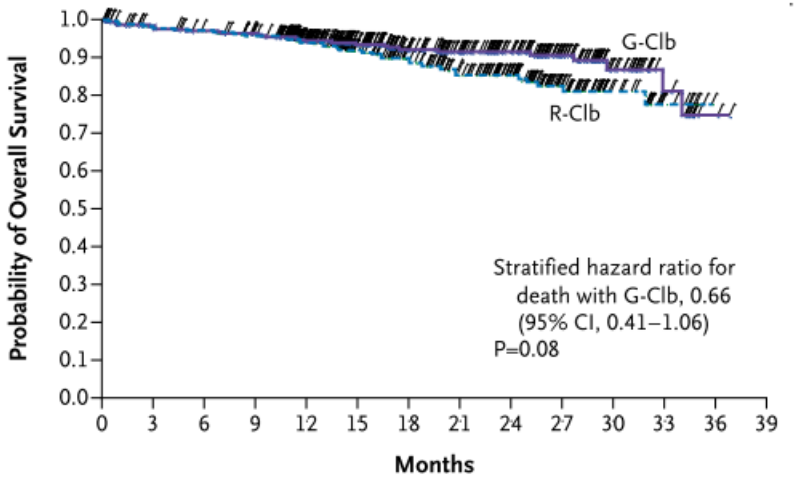
A



No. at Risk

G-Clb	238	226	223	221	215	211	170	144	115	71	34	14	2	0
Clb	118	109	105	103	102	94	70	56	44	29	15	5	0	0

C

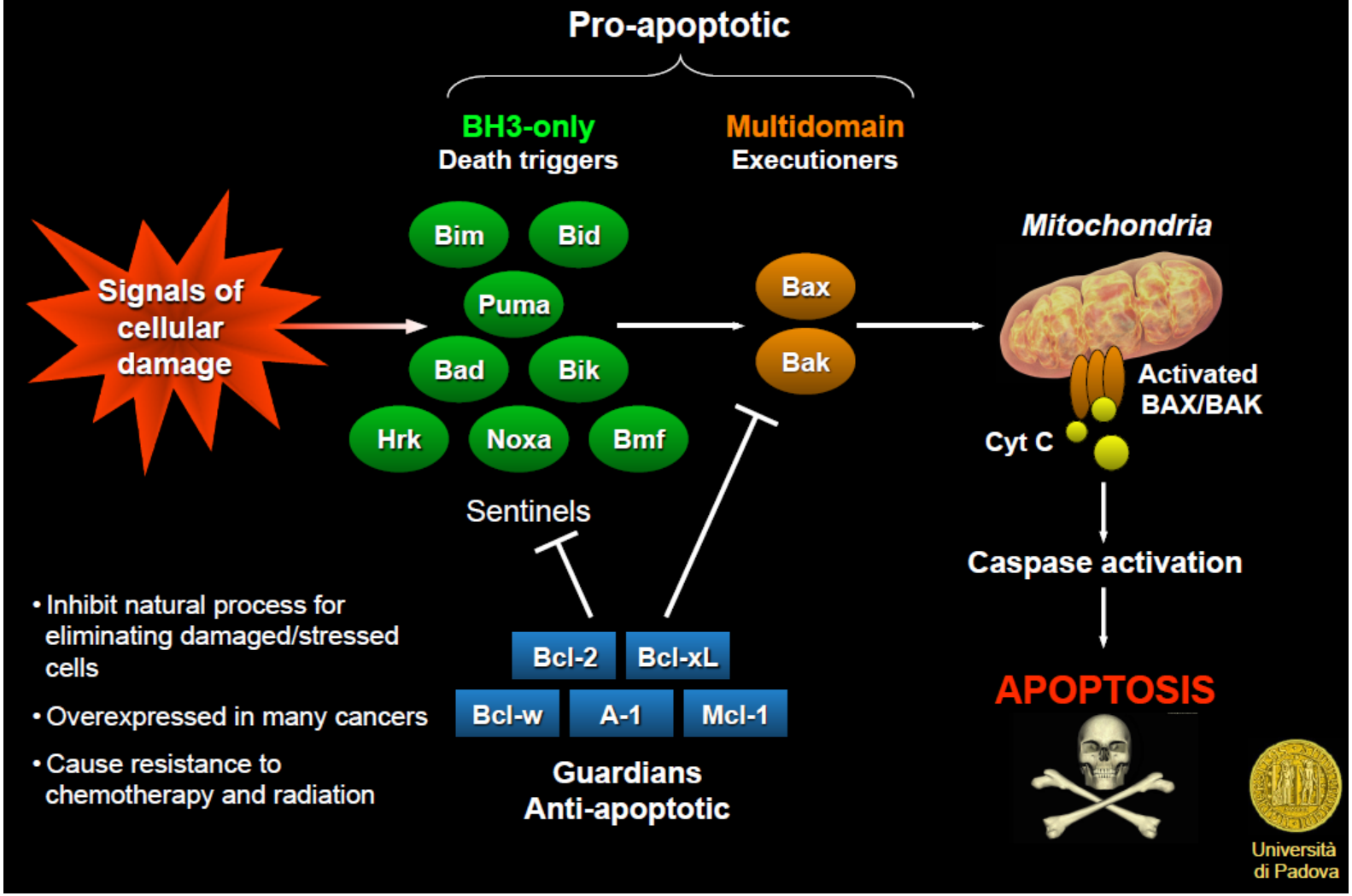


No. at Risk

G-Clb	333	316	310	303	261	214	170	144	115	71	34	14	2	0
R-Clb	330	320	314	305	255	203	169	138	105	61	27	8	0	0

Goede V, et al N Engl J Med. 2014; 370:1101-10

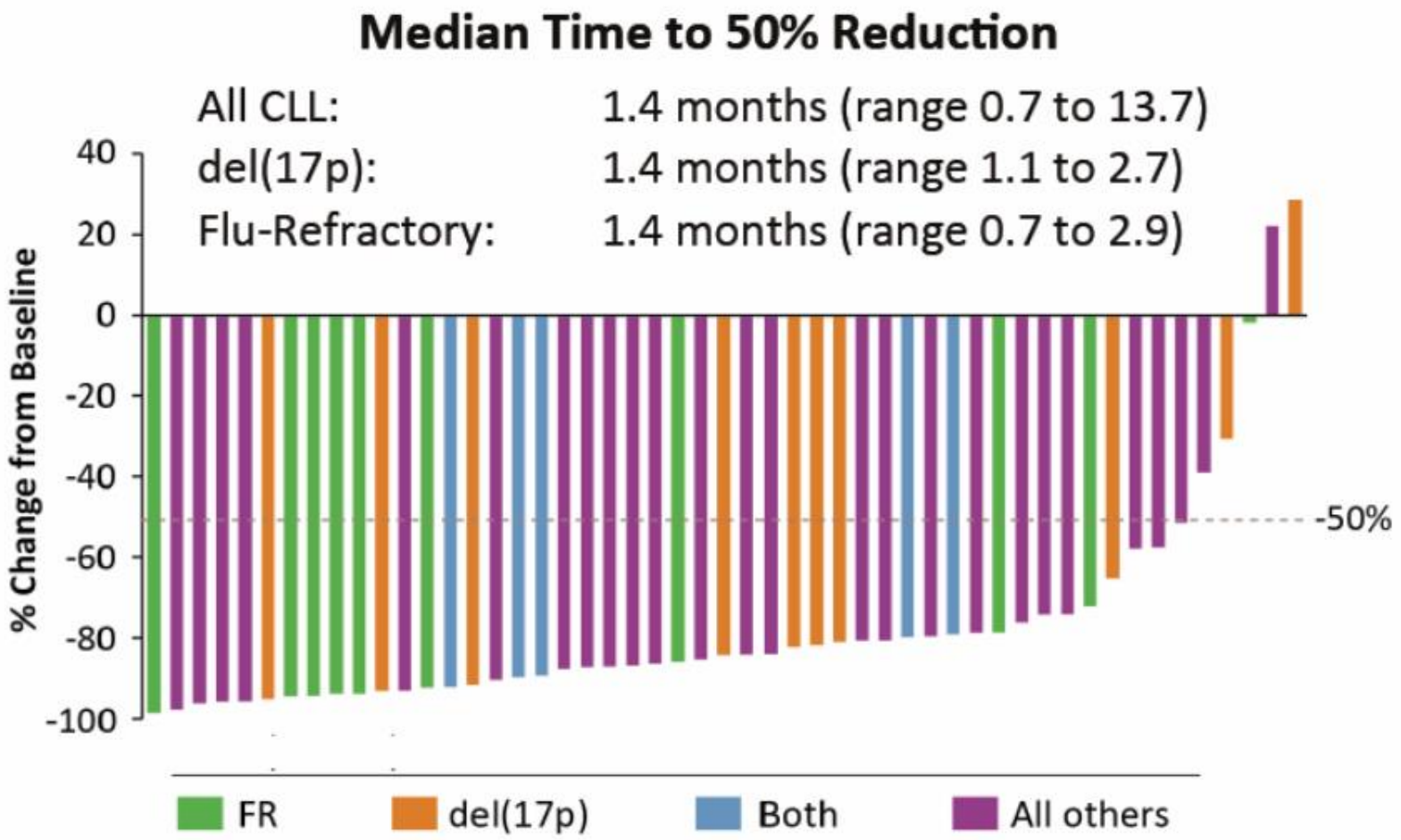
Bcl-2 family proteins regulate apoptosis



The BCL-2 inhibitor ABT-199 (GDC-0199) is active and well tolerated in ultra high risk relapsed/refractory chonic lymphocytic leukemia (CLL) (Roberts, Melbourne)

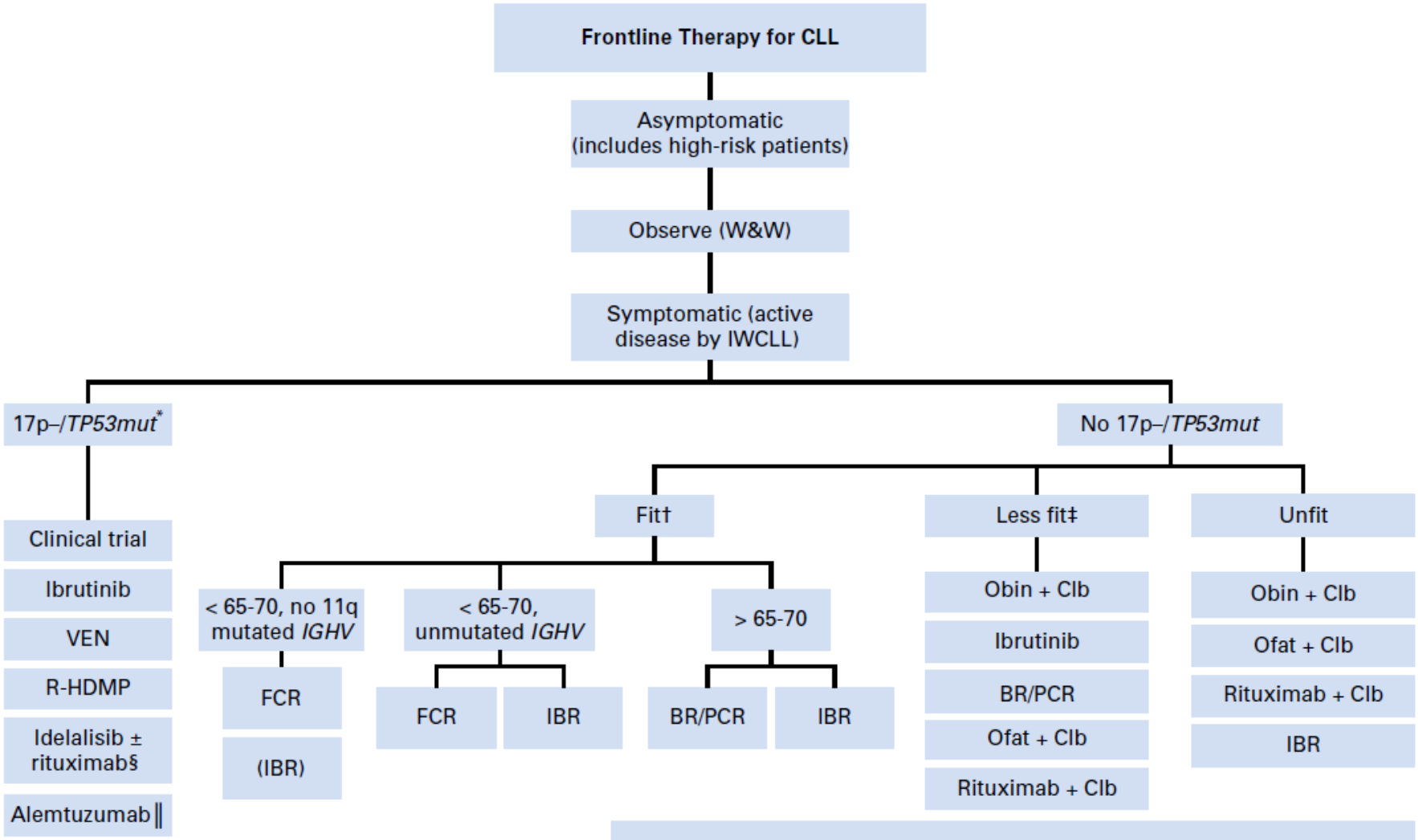
2013

Best Percent Change from Baseline in Nodal Size by CT Scan



N = 51 evaluable (at minimum, 6 week assessment).

Chemoimmunotherapy Is Not Dead Yet in CLL



Jennifer R. Brown – JCO 2017

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Domande

Cariotipo: si o no nella pratica clinica prima della terapia?

- 8/10 si, in alcuni pazienti

- Significato prognostico da validare

- Alcuni lo eseguono per studi traslazionali

- Non ha ricadute terapeutiche al momento

- Necessità di definire esattamente cosa è il cariotipo complesso

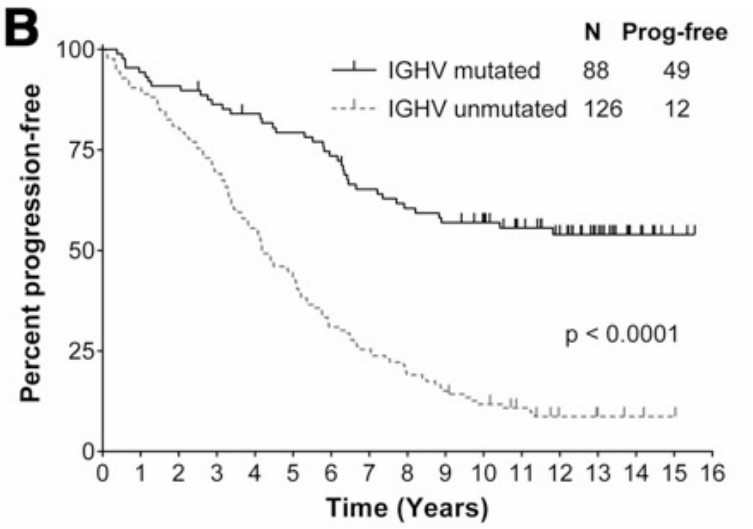
Domande

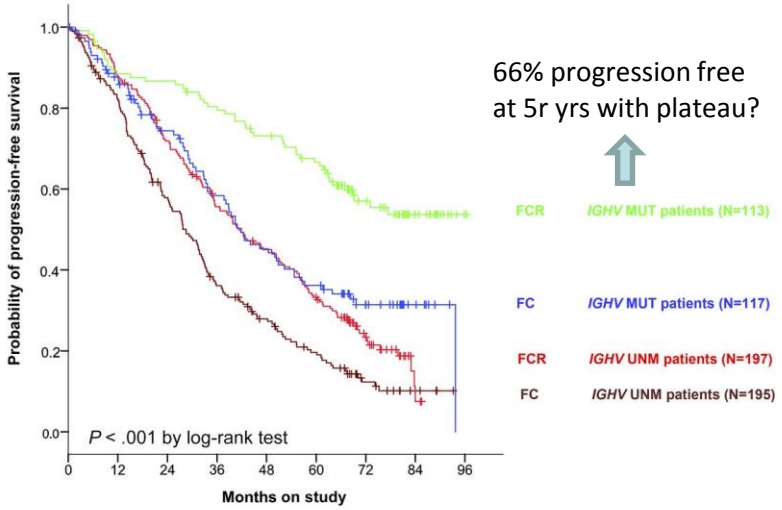
Quale è il vero «bisogno» di miglioramento nella terapia della LLC?

- 10-15% dei pazienti trattati ha l'eliggibiità a FCR ed è IGHV mutato

- Gli studi dovrebbero trovare terreno ideale nei pazienti a cattiva prognosi

Long term PFS with FCR (MDACC and GCLLSG – CLL8)





66% progression free at 5r yrs with plateau?

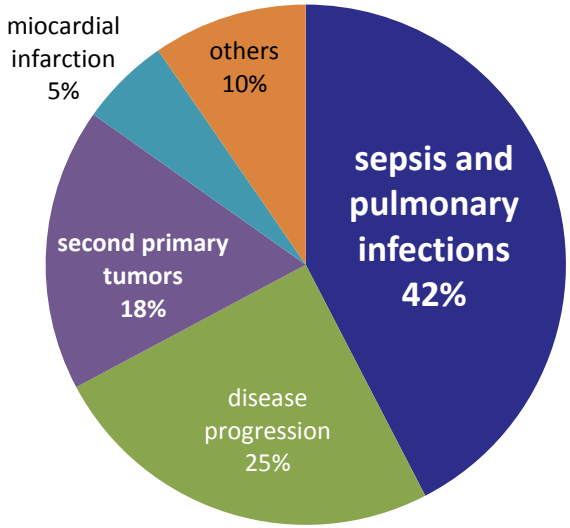
Number at risk	0	12	24	36	48	60	72	84	96
FCR *IGHV* MUT	113	99	97	89	80	71	37	15	1
FC *IGHV* MUT	117	96	75	58	45	36	21	7	0
FCR *IGHV* UNM	197	173	140	106	85	61	25	2	0
FC *IGHV* UNM	195	153	105	65	45	30	12	4	0

Thompson PA et al, Blood 2016; 127:303-9

Fischer K et al. Blood. 2016;127(2):208-215

Causes of death after FCR in the CLL8 trial

FCR arm (n.125 events / 408 patients; 5,9 yrs median f.u.)



Median time to onset (months) after last dose of study treatment	
sepsis and pulmonary infections	46
second primary tumors	27

Fischer K et al. Blood. 2016;127(2):208-215

Median PFS in high risk CLL treated by Chlor + anti CD20 (elderly/unfit)

	11q-	No 11q-	Unmutated IGHV
Treatment	**Chlor + R (UK trial)[1]**		**Chlor + R (GIMEMA trial)[2]**
Median TTP or PFS (months)	**12**	**24**	**22,8**

1. Hillmen P et al, J Clin Oncol. 2014 Apr 20;32(12):1236-41
2. Foà R et al. Am J Hematol. 2014 May;89(5):480-6

Domande

MRD- è un endpoint importante?

- Sicuramente si nei trials clinici
- In chi ha prognosi buona (IGHV mutato) FCR va bene.
- Utilizzare nuove combinazioni

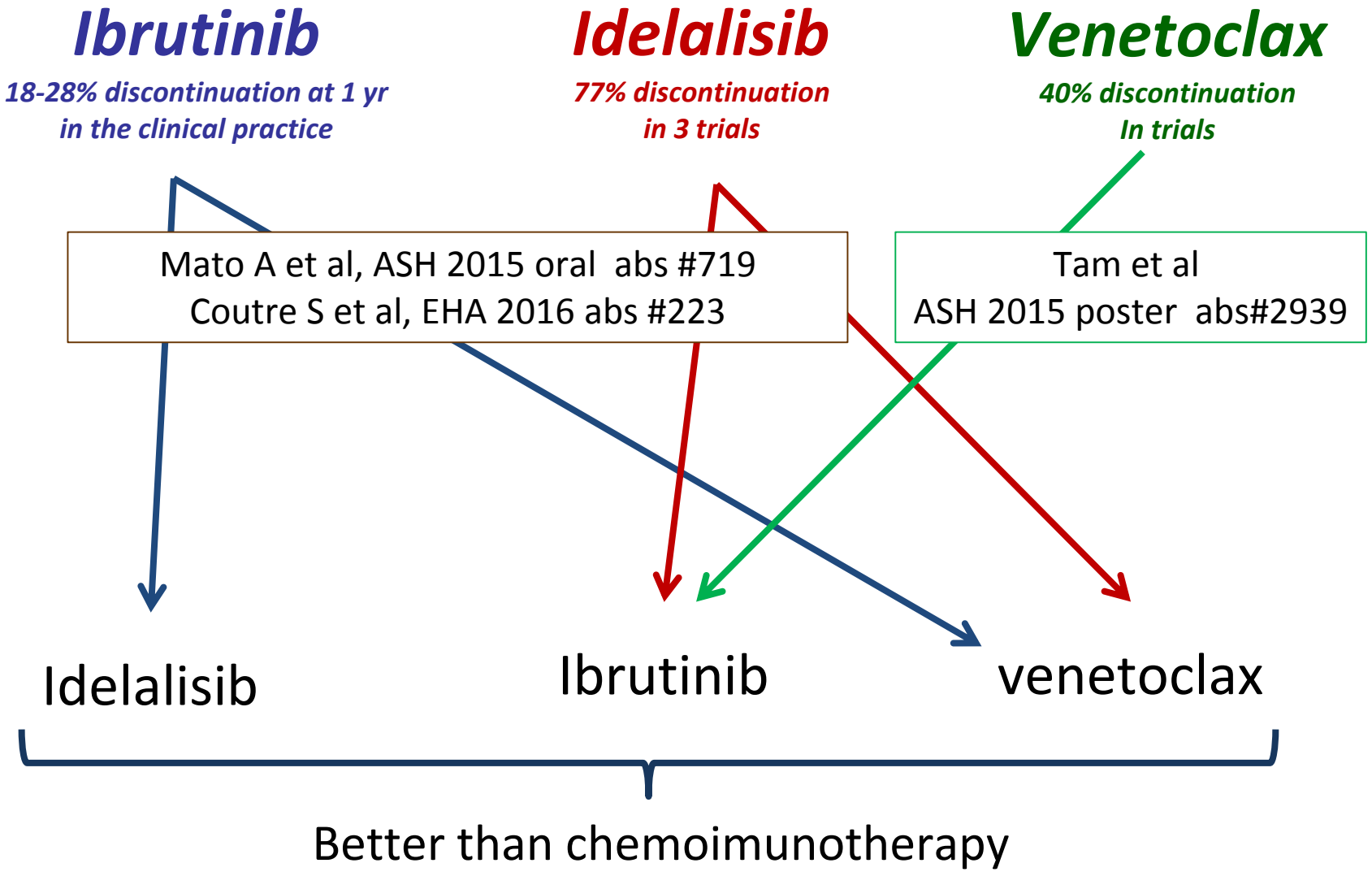
  - nel salvataggio
  - in subset a cattiva prognosi in prima linea

Domande

Ibrutinib in prima linea >65 a chi?

- Solo IGHV non mutato (risponde di più in vitro)
- Tra gli IGHV non mutati: sulla base dell'età
- Quasi mai perché funziona in seconda e terza
- <10-25% ; uno solo >50%

Possibility to cross in case of discontinuation in rel/ref CLL (toxicity or progression)



Domande

Failure of a KI

Se progressione - venetoclax (MRD-); ibrutinib, idelalisib

Se tossicità: switch ad altro KI