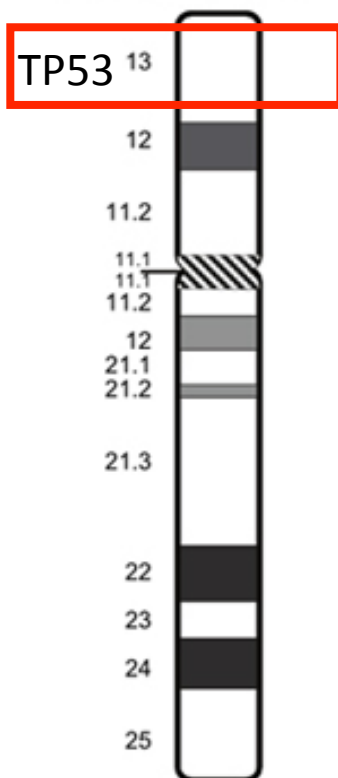


Delezione 17p13 e/o mutazioni TP53



Metodi di rilevazione: delezione 17p13 (citogenetica molecolare - FISH)
mutazione TP53 (Sanger – NGS)

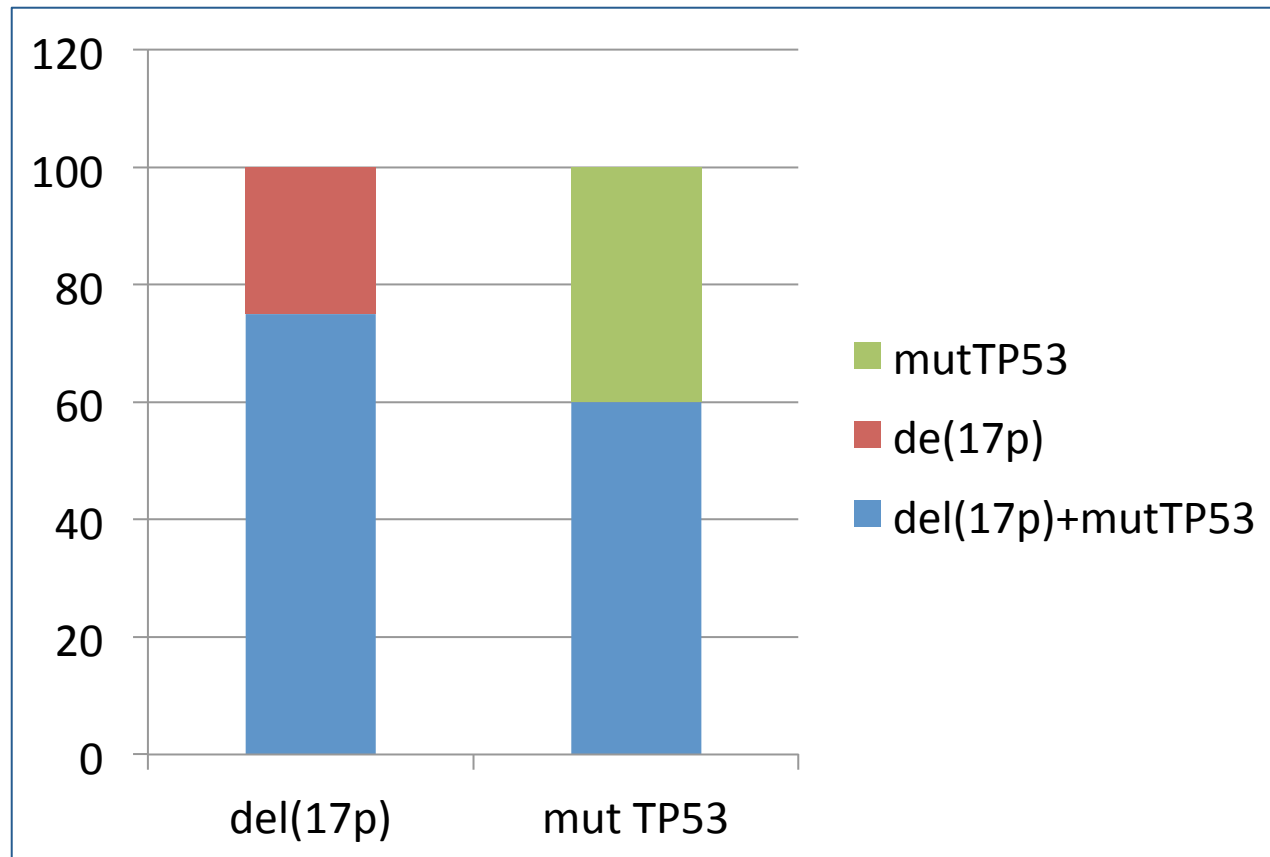
Frequenza: 5-10% dei casi alla diagnosi, fino al 40-50% dei casi
in progressione o refrattari

Alterazioni citogenetiche associate: traslocazioni della regione 17p13
cariotipo complesso

Prognosi severa

meno severa: delezione 17p13 <20% cellule
stadio iniziale
IgHV mutato

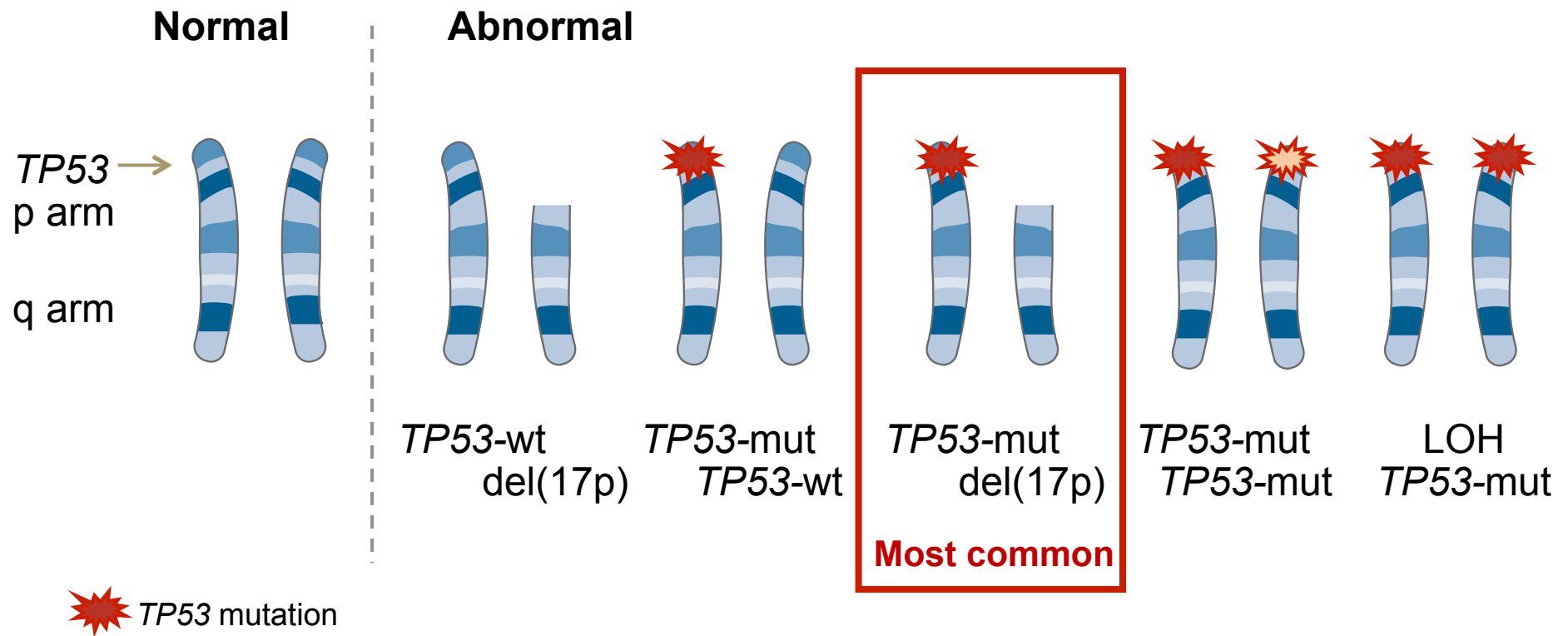
Delezione 17p13 e/o mutazioni TP53



> 70% del(17p) → mutazione TP53
circa 60% mutazione TP53 → del(17p)

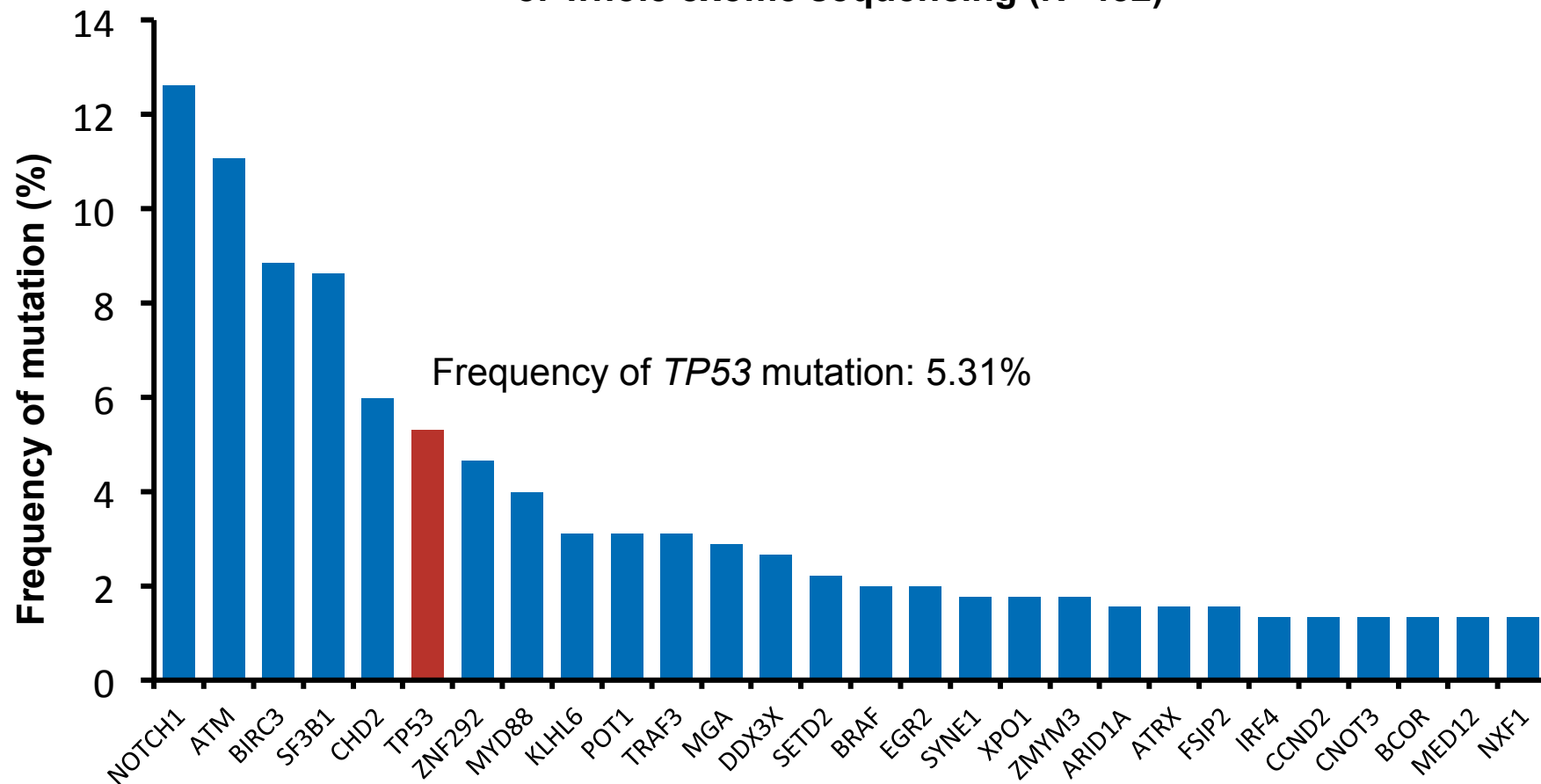
The configurations of del(17p) and *TP53* mutation associated with CLL are diverse

- *TP53* resides on the short arm of chromosome 17 at 17p13.1; del(17p) is therefore associated with loss of the *TP53* allele¹
- >70% of del(17p) patients carry a *TP53* mutation in the other allele²

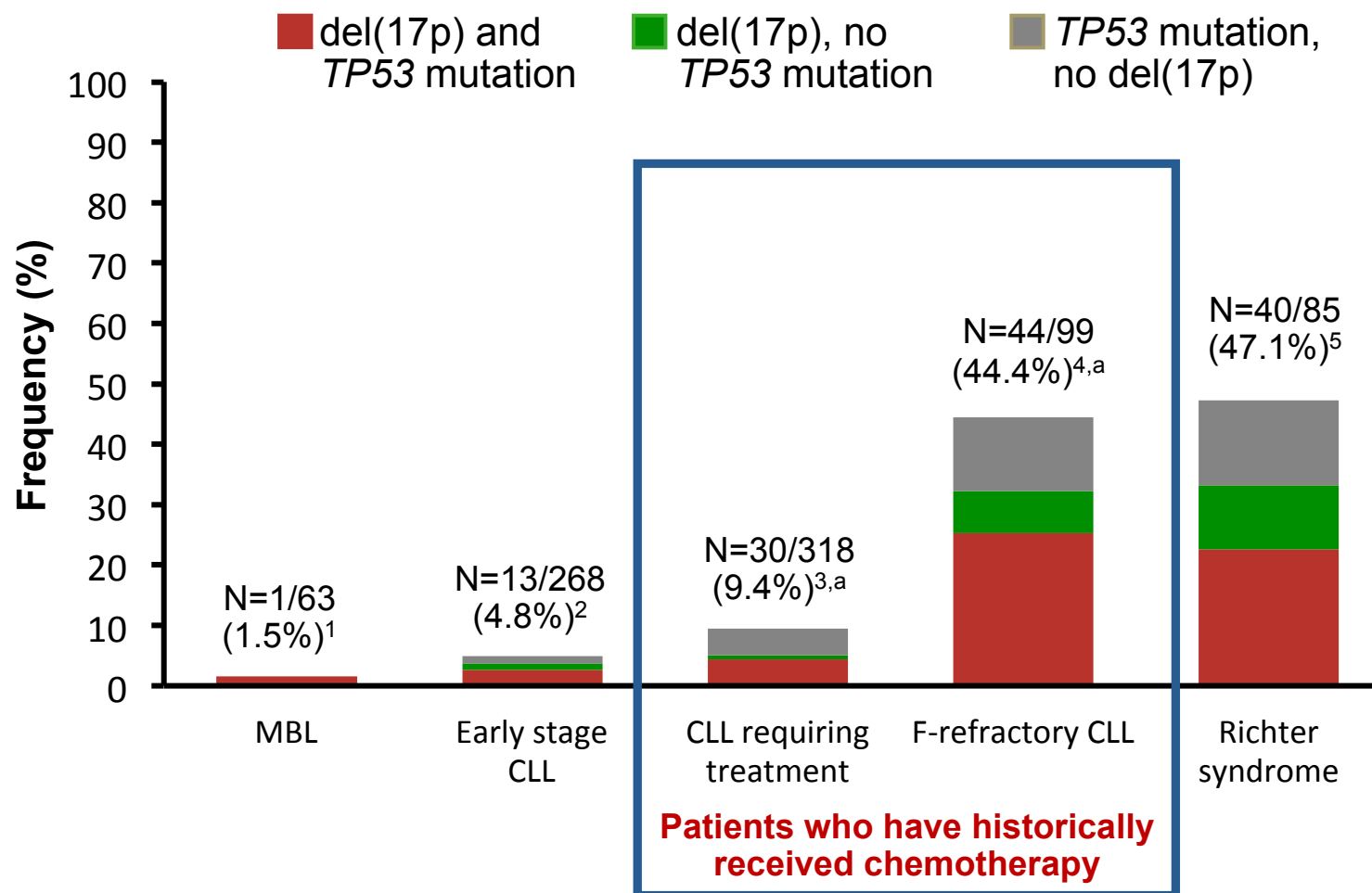


TP53 is among the most frequently mutated genes in patients with CLL

Recurrently mutated genes in untreated CLL by whole genome or whole exome sequencing (N=452)



The estimated relative incidence of *TP53* abnormalities in CLL increases during disease progression

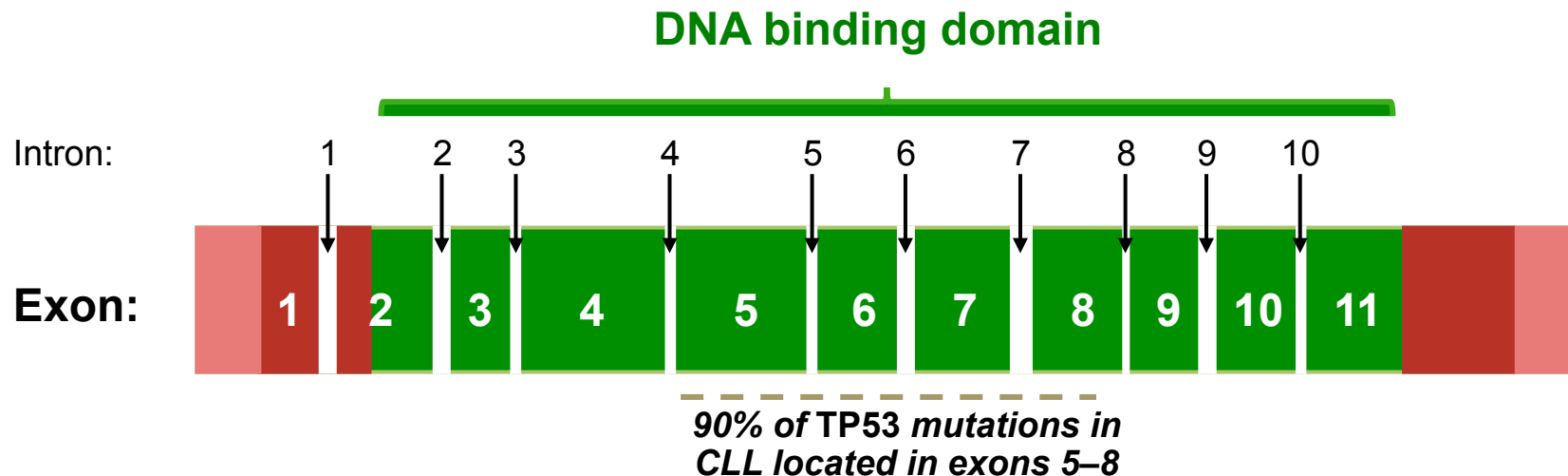


^a Estimated – reported incidences of *TP53* mutation and various configurations with del(17p) in different lines of CLL treatment vary; frequency is not clearly characterised at present time
 CLL: chronic lymphocytic leukaemia; F: fludarabine;
 MBL: monoclonal B-cell lymphocytosis

1. Rasi S, et al. *Haematologica* 2012; 97:153–154. 2. Zainuddin N, et al. *Leuk Res* 2011; 35:272–274.
 3. Zenz T, et al. *J Clin Oncol* 2010; 28:4473–4479. 4. Zenz T, et al. *Blood* 2009; 114:2589–2597.
 5. Rossi D, et al. *Blood* 2011; 117:3391–3401.

Tumour protein 53 (*TP53*) gene structure

- *TP53* is one of the most important tumour suppressor genes, and mutations are frequent in various tumours
 - Over 50% of all human cancers have mutations in the *TP53* gene
 - Most *TP53* mutations are missense^a and located within the DNA binding domain of *TP53* encoded by exons 5–8
- Human *TP53* comprises 11 exons and encodes for a 2.2 kb mRNA product; translation of the mRNA product begins at exon 2 of *TP53*



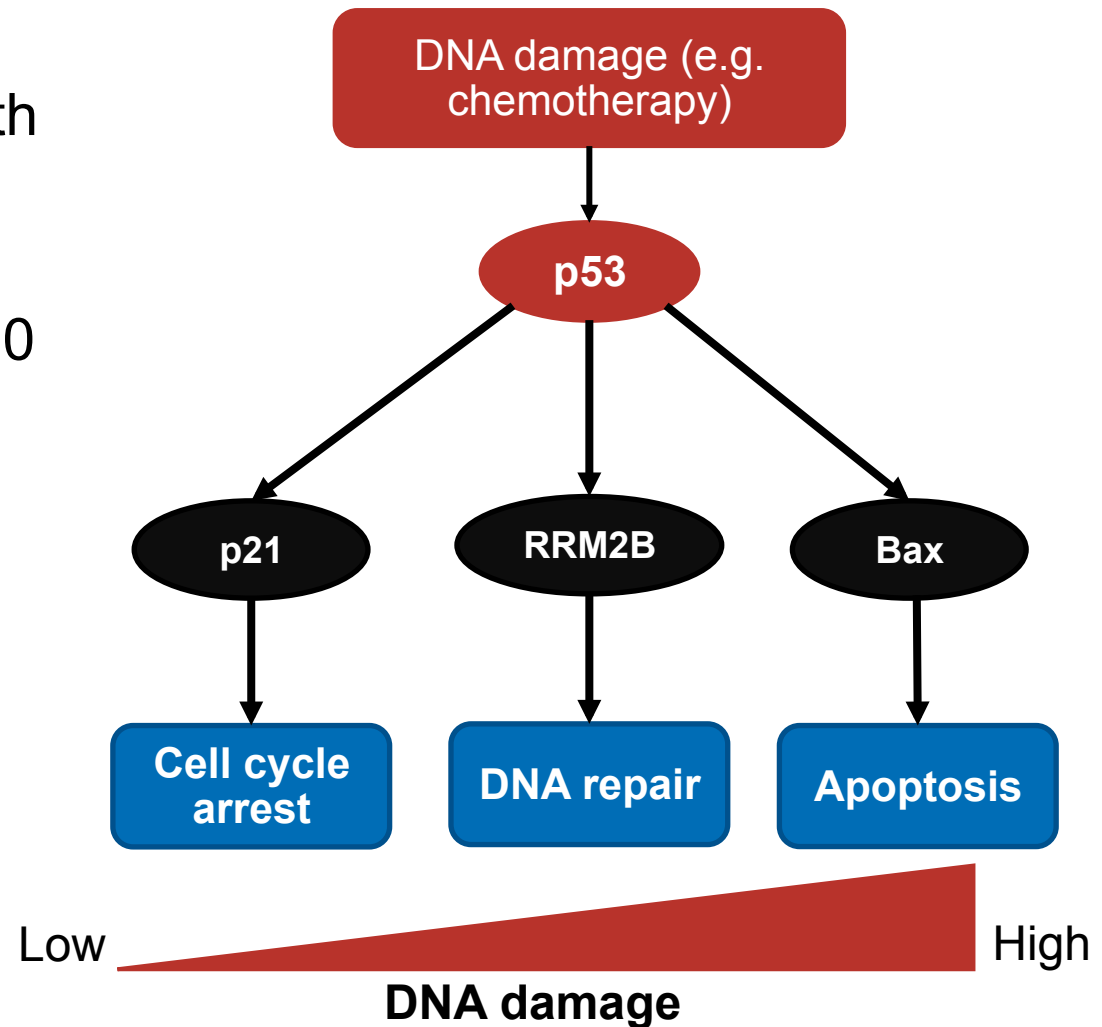
^a Missense refers to a point mutation in which a single nucleotide change results in a codon that codes for a different amino acid
CLL: chronic lymphocytic leukaemia

Strefford JC. *Br J Haematol* 2015; 169:14–31. http://p53.free.fr/p53_info/p53_gene.html

Liang SH & Clarke MF. *Eur J Biochem* 2001; 268:2779–2883.
Zenz T, et al. *Leukemia* 2010; 24:2072–2079.

The p53 protein executes distinct actions which help maintain genetic stability

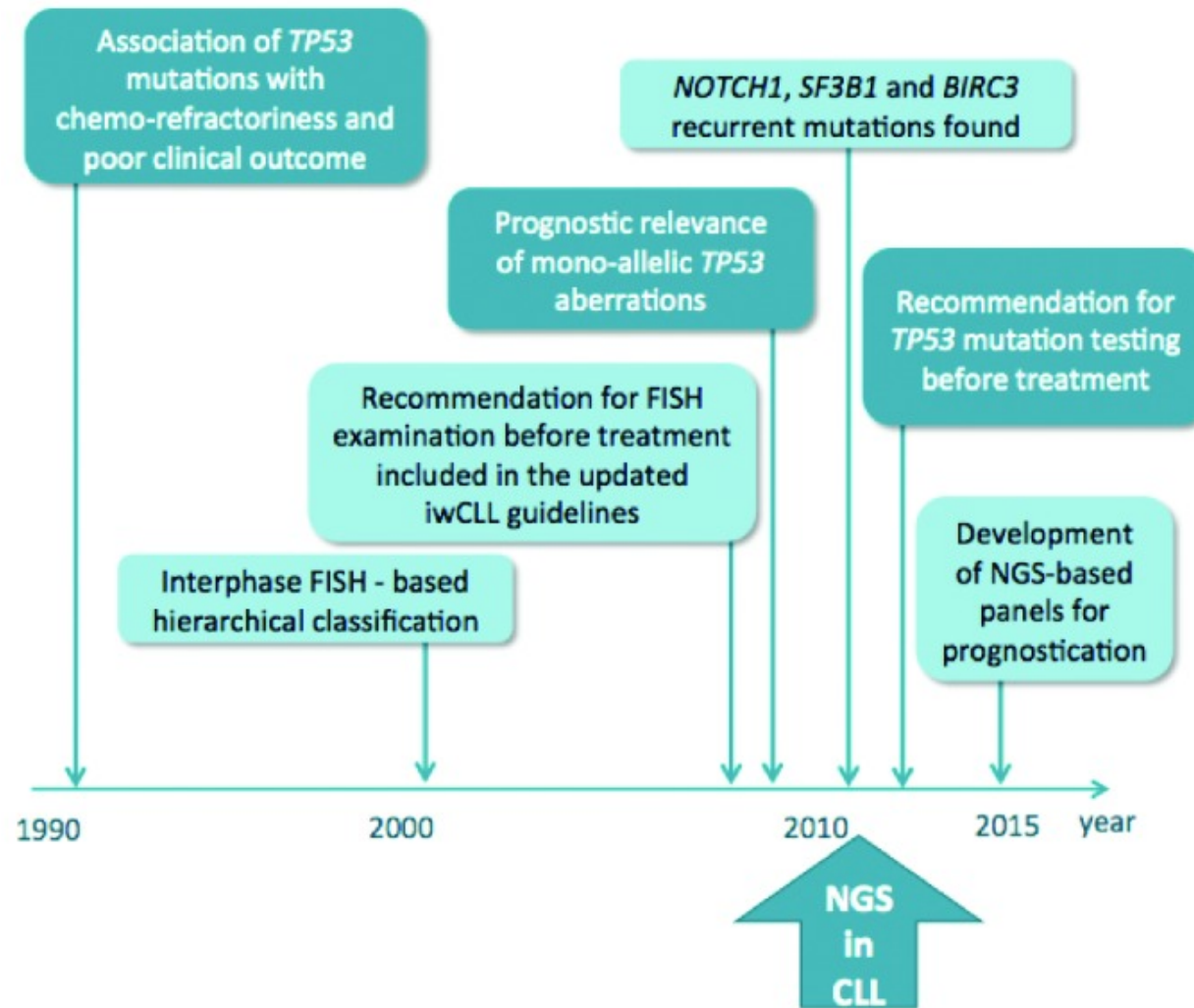
- TP53 encodes a transcription factor with a molecular weight of 53 kDa (p53)
- p53 regulates over 100 genes involved in cellular processes, including apoptosis and cell cycle arrest



Note: p21 is encoded by *CDKN1A* gene
Bax: bcl-2-like protein; RRM2B: ribonucleoside-diphosphate reductase subunit M2 B

Hanahan D & Weinberg RA. *Cell* 2000; 100:57–70.
Kamada R, *et al.* *Biopolymers* 2015; ePub ahead of print.
Liang SH & Clarke MF. *Eur J Biochem* 2001; 268:2779–2883.

analisi delle alterazioni citogenetiche e delle mutazioni geniche nella pratica clinica



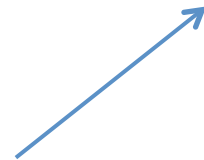
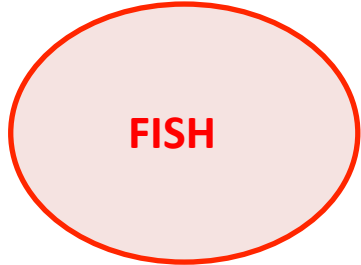
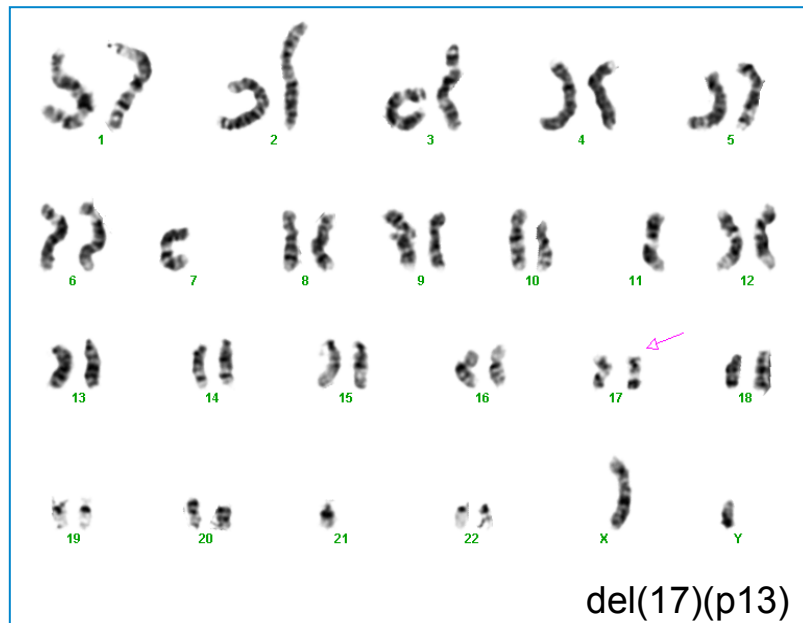
Citogenetica convenzionale

Basso indice mitotico



circa 75% metafasi analizzabili;
circa 40% alterazioni citogenetiche
+frequenti: +12 e del(13q)

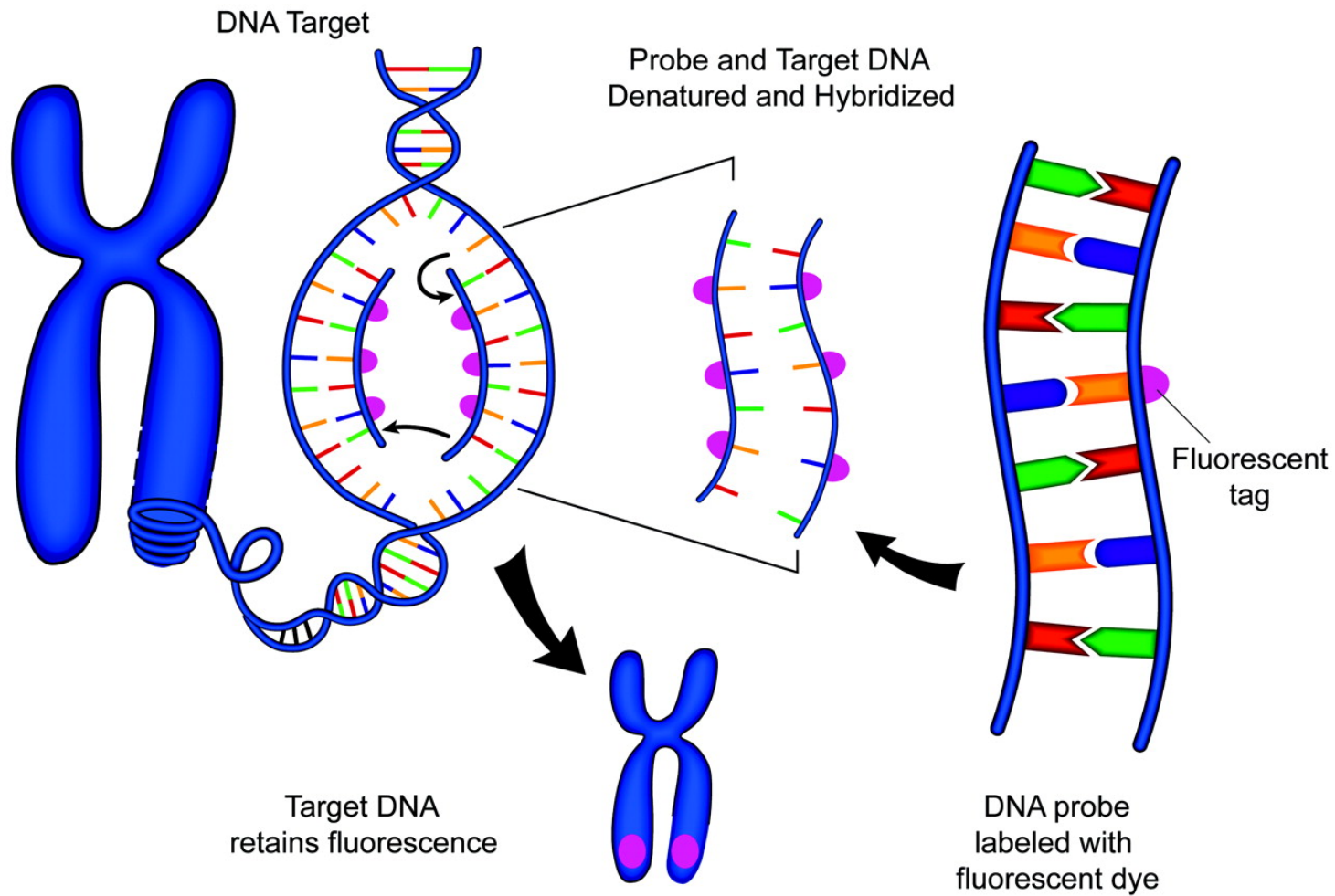
Alterazioni ad impatto prognostico criptiche in CC



Definizione di alcune alterazioni citogenetiche a forte impatto prognostico
frequente localizzazione di anomalie citogenetiche a livello dei cromosomi 6, 11, 12, 13, 14 e 17 nella LLC come già precedentemente evidenziato all'analisi del cariotipo convenzionale (Dohner et al, 2000)

FISH : maggiore risoluzione rispetto allo studio citogenetico convenzionale ed ideale nella LLC poiché rileva le alterazioni in interfase, quindi indipendente dalla capacità di divisione cellulare.

FISH (ibridizzazione in situ fluorescente)



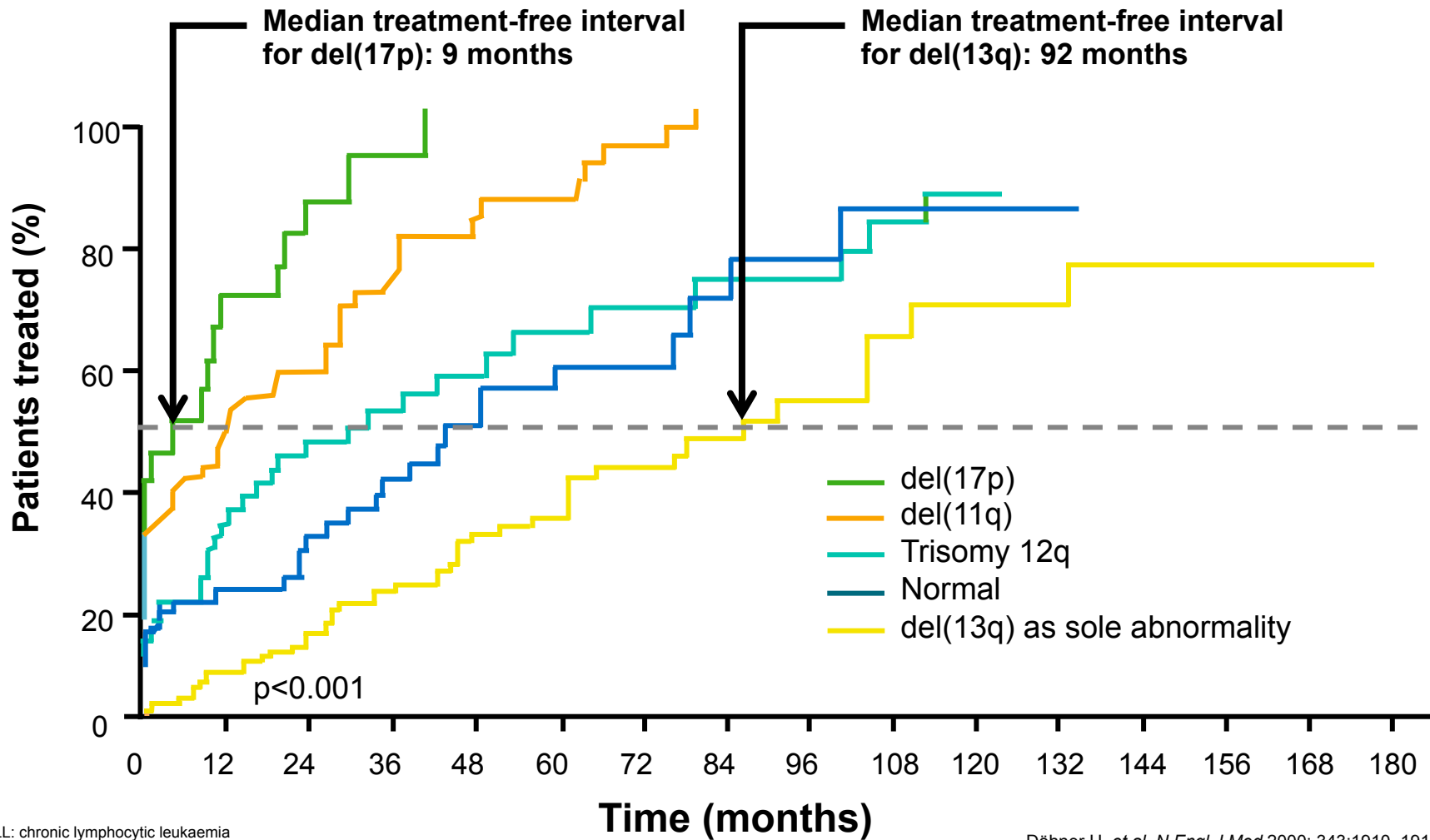
Utilizzo di sonde fluorescenti di DNA specifiche per ristrette le regioni cromosomiche

alterazioni citogenetiche

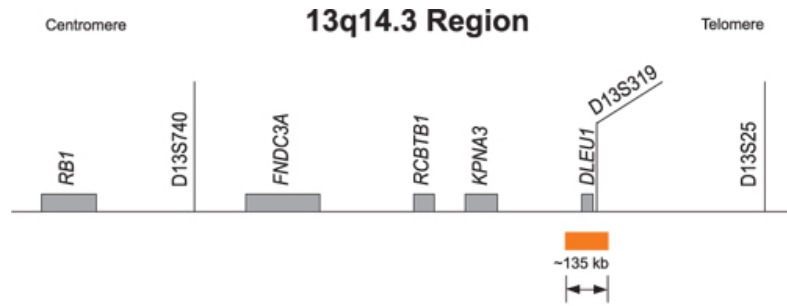
Alterazione citogenetica	Gene coinvolto	Citomorfolologia	Frequenza (FISH)	Immunofenotipo/ stato mutazionale IgVH	Prognosi
del(17p)	TP53	LLC/LP	5-8%	CD38+++/- ZAP70+++/- IgVH non mutato+++/-	Prognosi severa
del(11q)	ATM	LLC tipica	15-30%	CD38+++/- ZAP70++/- IgVH non mutato+++/-	Prognosi sfavorevole
+12	(MDM2) (CCND1)	LLC atipica	15-25%	CD38+/- ZAP70+++/- IgVH non mutato+/-	Prognosi intermedia
del(13q)	miR-15° miR-16 DLEU2	LLC tipica	50%	CD38+/- ZAP70+/- IgVH non mutato+/-	Buona prognosi (anomalia isolata)
del(6q)		LLC atipica	2-5%	CD38+++/- ZAP70++/- IgVH non mutato+/-	Prognosi intermedia
t(14q32)	IgH e differenti partners cromosomici	LLC tipica		CD38+/- ZAP70++/- IgVH non mutato+/-	Prognosi intermedia
Cariotipo complesso				CD38+++/- IgVH non mutato++++	Prognosi sfavorevole

Genomic aberrations in CLL have a clear effect on disease progression

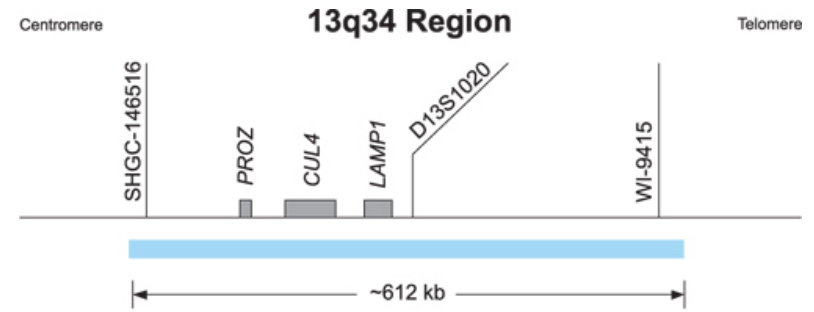
Time to first treatment from diagnosis by cytogenetic subgroup



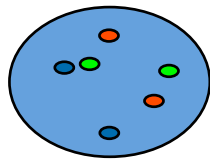
FISH



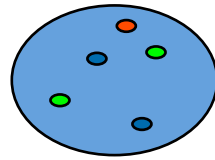
LSI D13S319 SpectrumOrange Probe



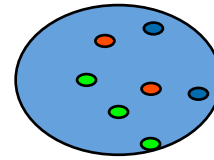
LSI 13q34 SpectrumAqua Probe



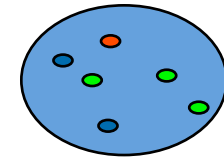
normale



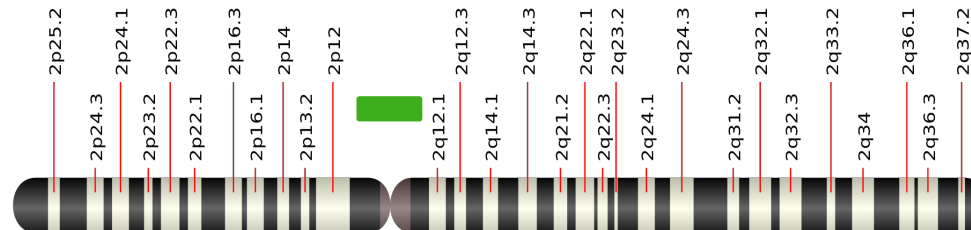
del(13)(q14)



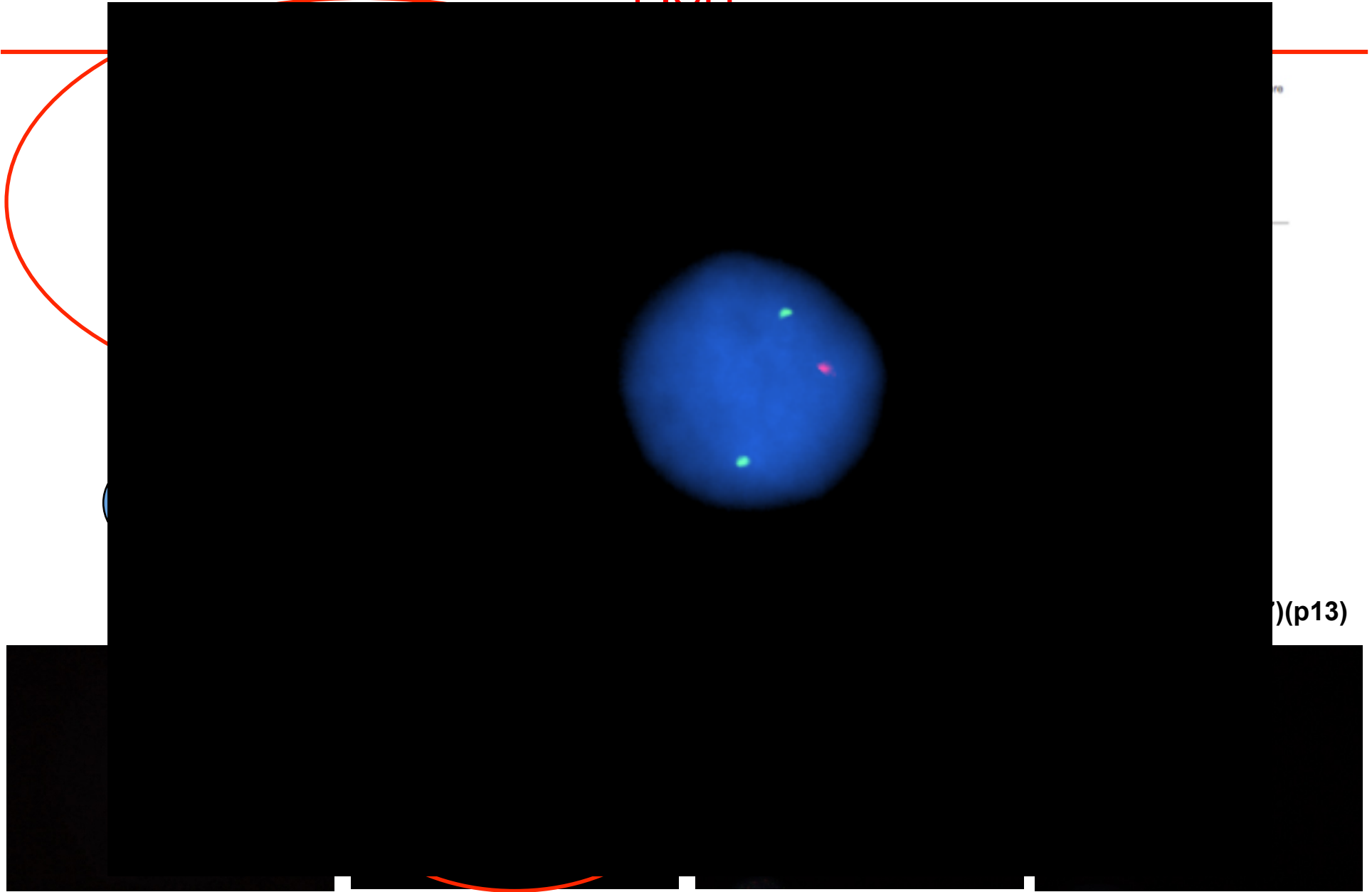
+12



+12 e del(13)(q14)

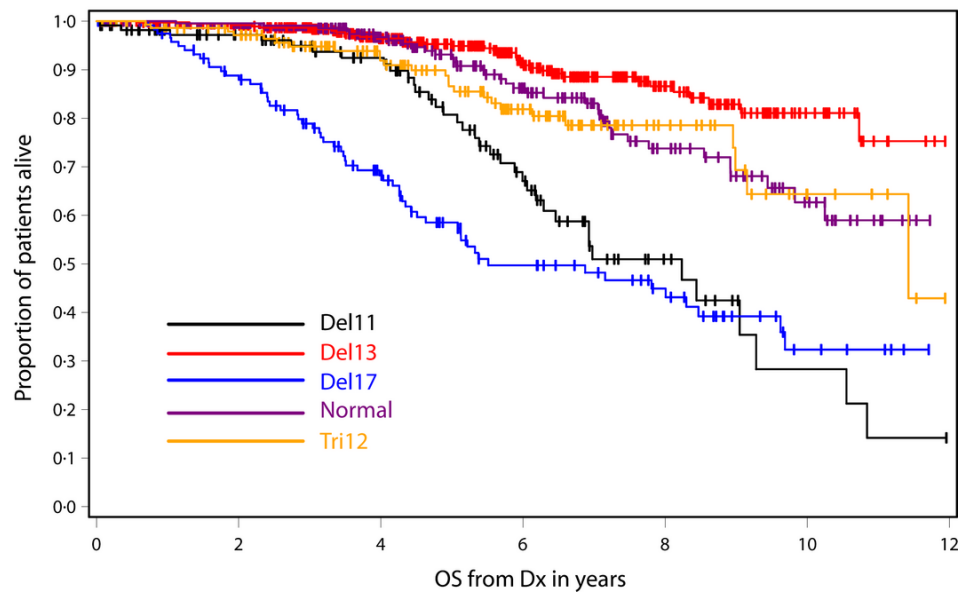
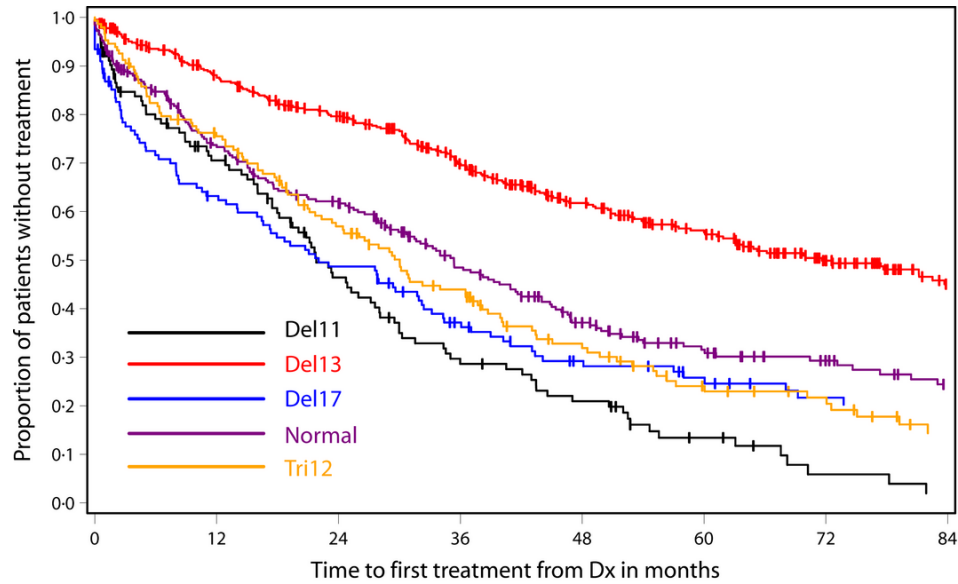


FISH



)(p13)

The Dohner fluorescence *in situ* hybridization prognostic classification of chronic lymphocytic leukaemia (CLL): the CLL Research Consortium experience



1585 pazienti

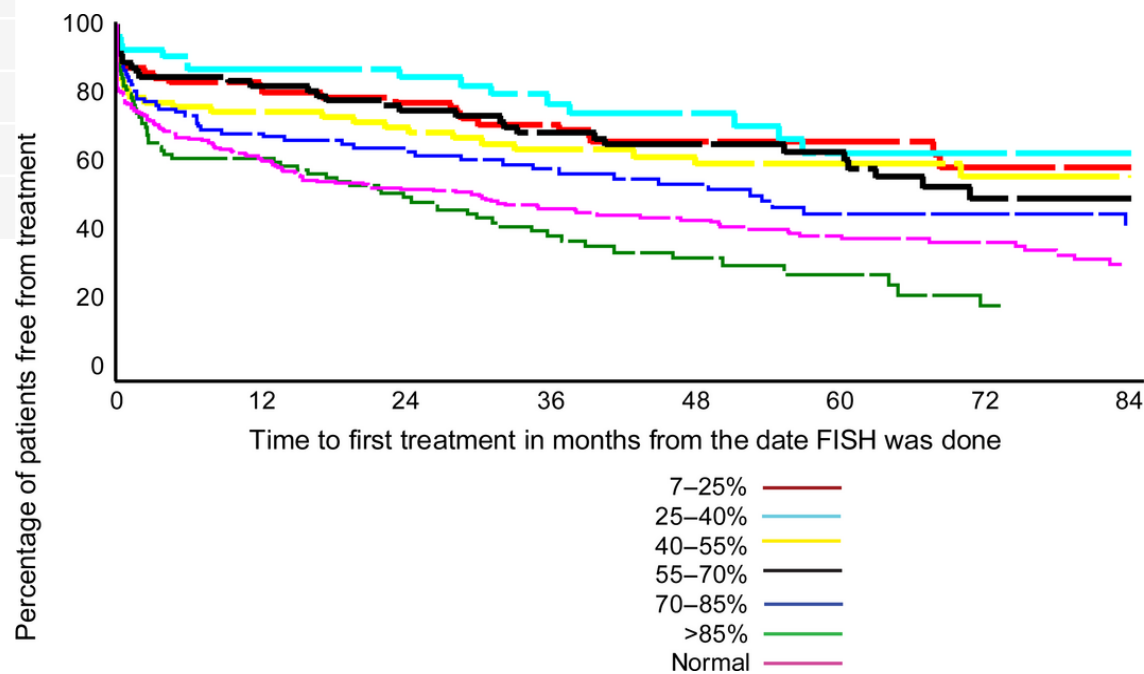
	N	Treated (n)	Median TTFT-DX (months)	P-value
FISH category				
17p deletion	122	93	22	<0.0001
11q deletion	113	92	22	
Trisomy 12	148	113	30	
Normal	252	164	35	
13q deletion	411	183	72	

	N	Death (n)	Median OS-DX (years)	P-value
FISH category				
17p deletion	122	60	5	<0.0001
11q deletion	114	36	7	
Trisomy 12	149	25	11	
Normal	252	35	Not reached	
13q deletion	411	35	Not reached	

Le percentuali delle cellule alterate sono significativamente associate a differenti TTFT per la trisomia 12 e per le delezioni 13q e 17p

	N	Median (%)	Range (%)
FISH anomaly			
17p deletion	193	38.5	5-99.5
11q deletion	187	70	6.5-100
Trisomy 12	205	55	2.5-94
13q deletion	624	64.8	7-100

	N (%)	Treated	Median TFT (months)	P value
% 13q-				
7-25%	78 (15)	26	Not reached	<0.0001
25-40%	54 (11)	17	85	
40-55%	81 (16)	31	89	
55-70%	80 (16)	32	71	
70-85%	111 (22)	53	52	
>85%	104 (20)	65	24	

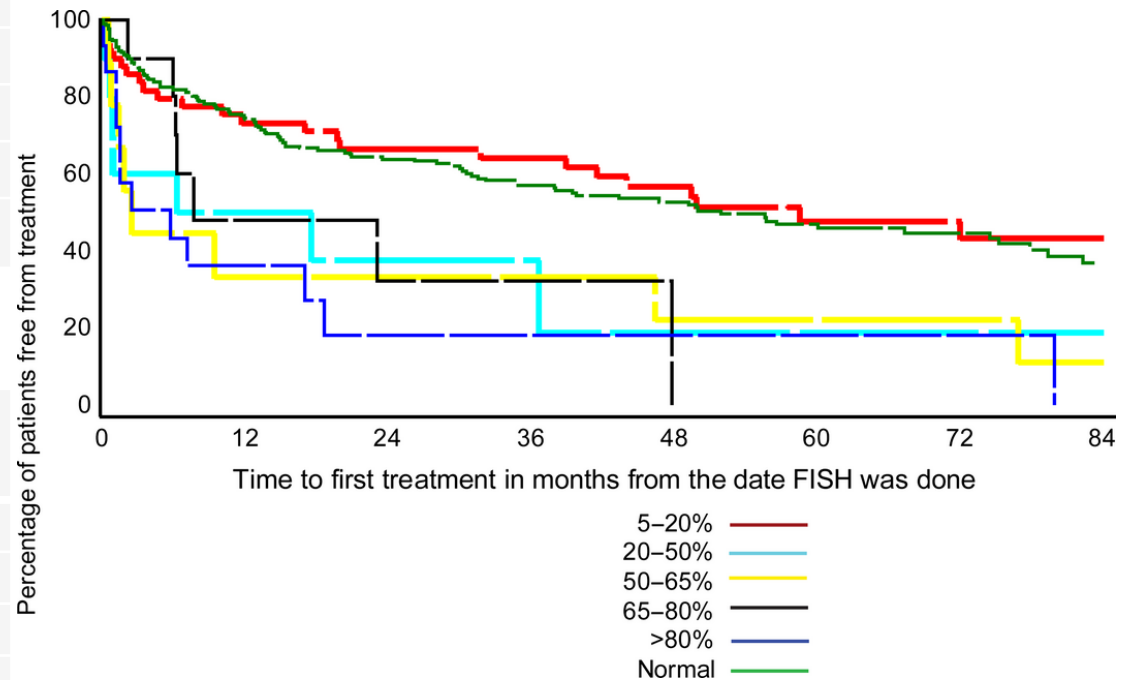


Pazienti con >85% di nuclei con delezione 13q hanno TTFT (24 mesi) mediano più breve

Le percentuali delle cellule con delezione 17p sono associate a differenti TTFT

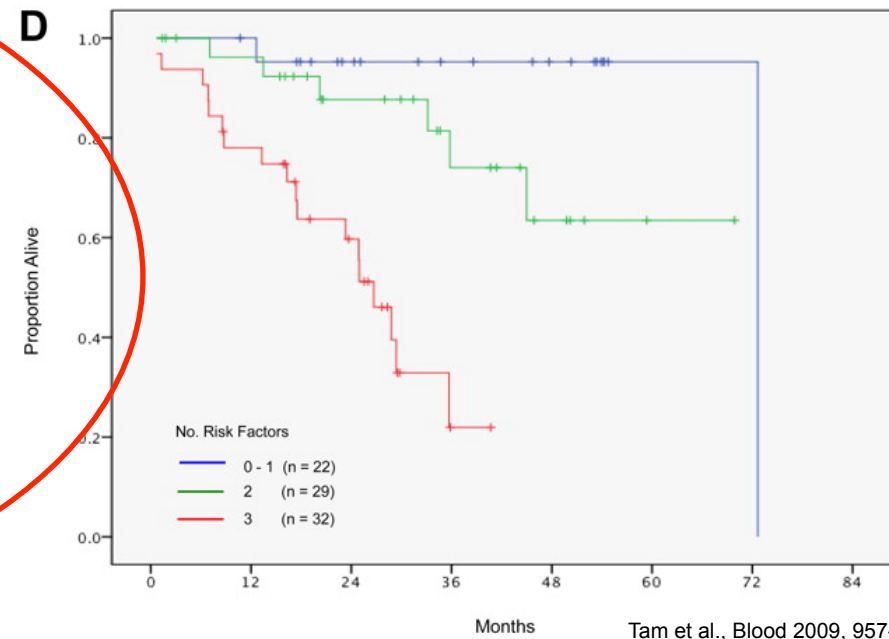
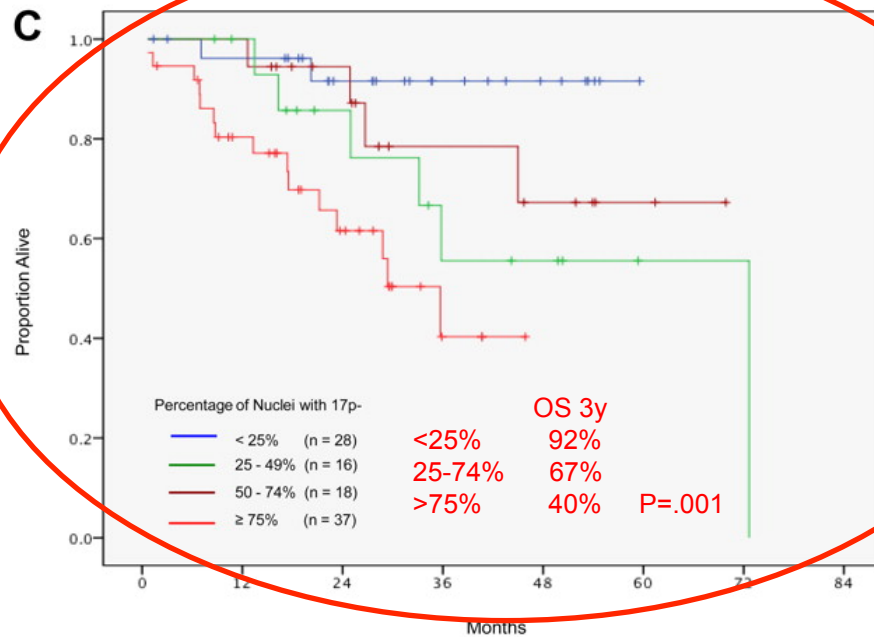
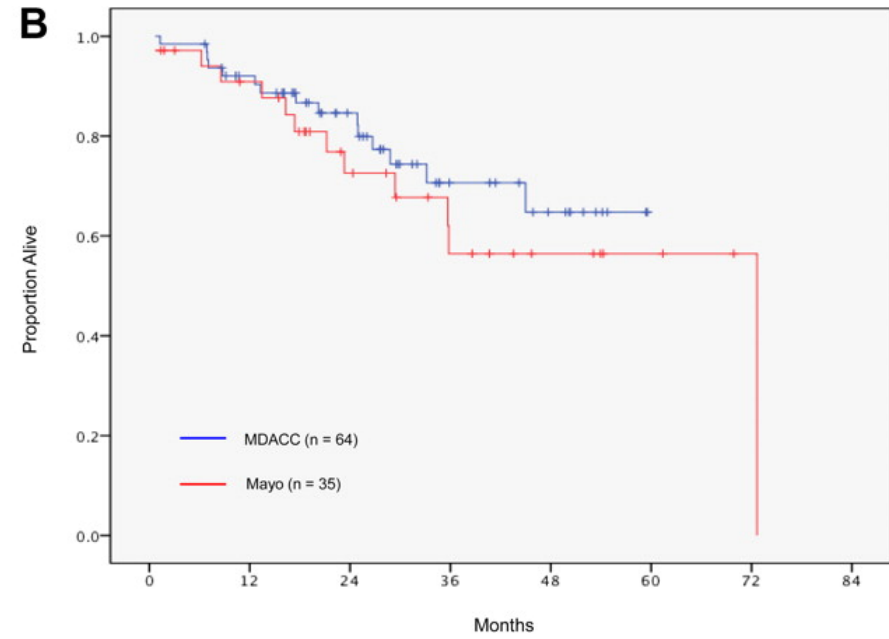
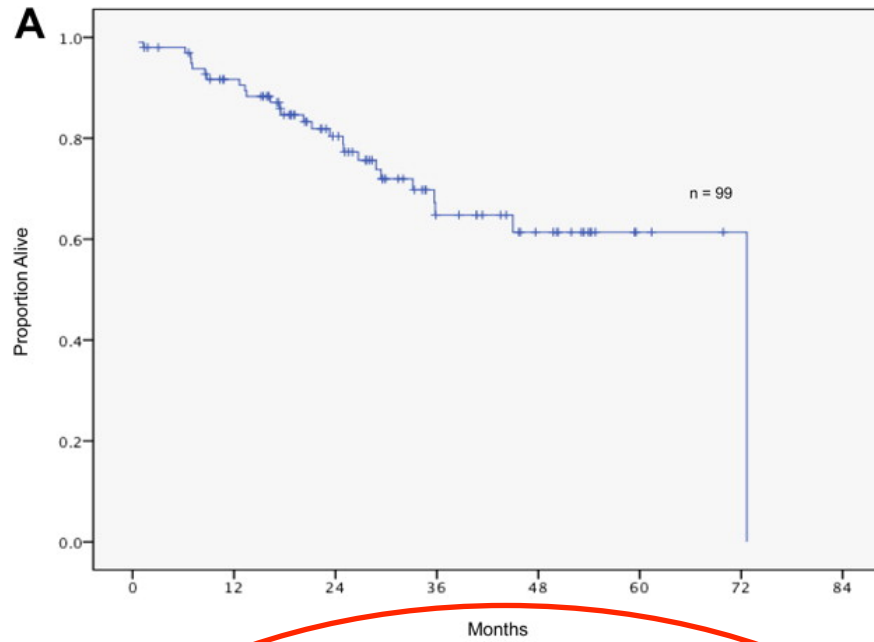
	N	Median (%)	Range (%)
FISH anomaly			
17p deletion	193	38.5	5-99.5
11q deletion	187	70	6.5-100
Trisomy 12	205	55	2.5-94
13q deletion	624	64.8	7-100

	N (%)	Treated	Median TTF (months)	P value
% 17p-				
5-20%	59 (49)	33	44	0.002
20-50%	14 (12)	10	1	
50-65%	15 (13)	12	1	
65-80%	11 (9)	8	8	
>80%	21 (18)	17	1	

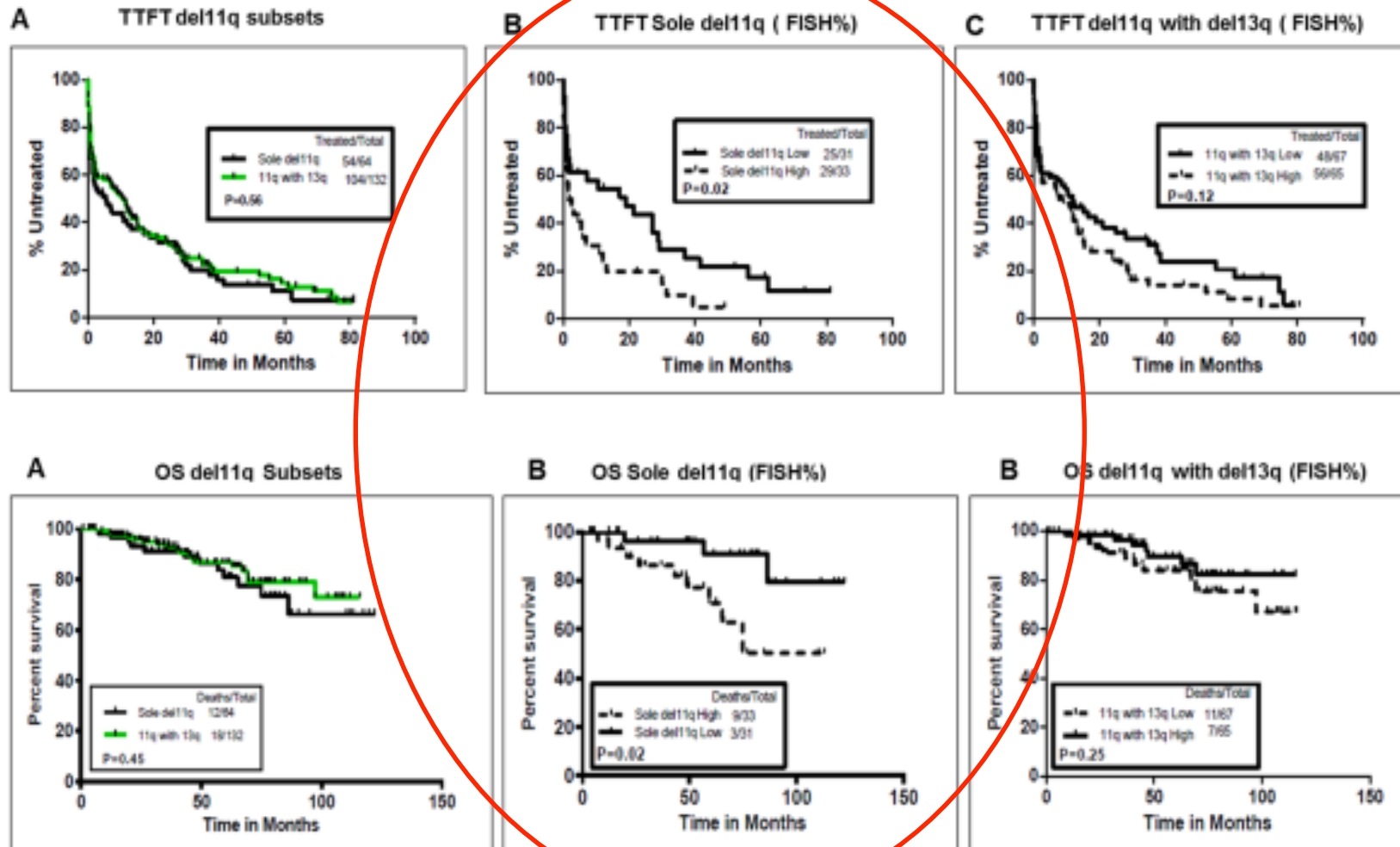


Pazienti con <20% di nuclei con delezione 17p hanno TTFT (44 mesi) e OS (11 anni) mediani più lunghi e sono più probabilmente IGHV mutati

Le percentuali delle cellule con delezione 17p sono associate a differenti OS

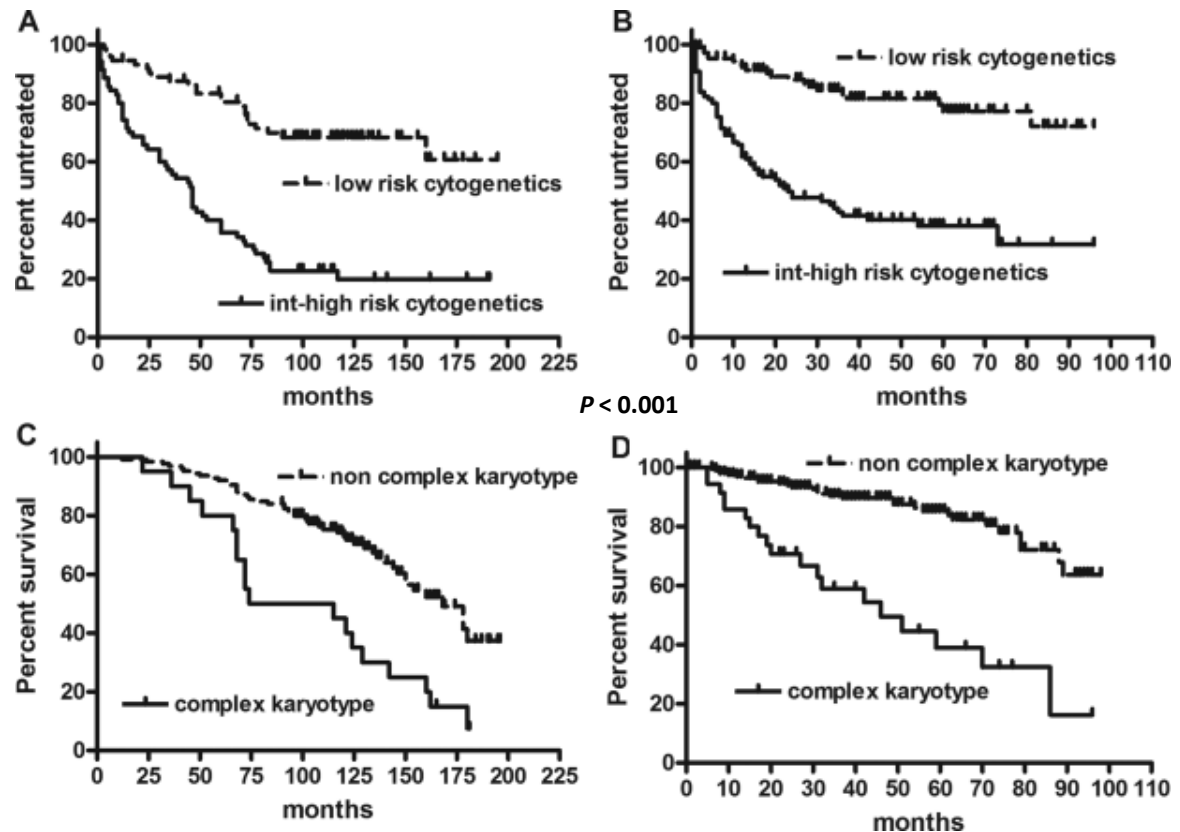


Un'alta percentuale in FISH di delezione 11q in pazienti con leucemia linfatica cronica è un fattore predittivo indipendente per prognosi sfavorevole



Analisi citogenetica con Cp-G oligonucleotide DSP30 e IL-2

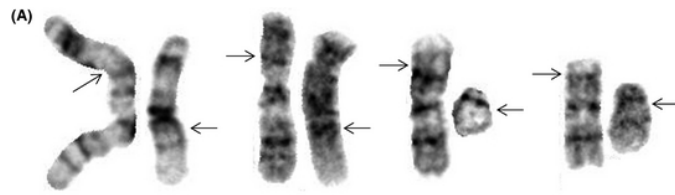
- immunostimolazione con CpG-oligonucleotide DSP30 e IL-2 del **ciclo cellulare delle cellule della LLC in vitro.**
- **informativa nel 95% delle analisi eseguite** (cut-off minimo di metafasi pari a 15).
- **anomalie cromosomiche nell'80%** dei casi, paragonabile alla FISH.
- significato prognostico e frequenza delle aberrazioni cromosomiche evidenziate con la citogenetica in casi con **FISH normale.**



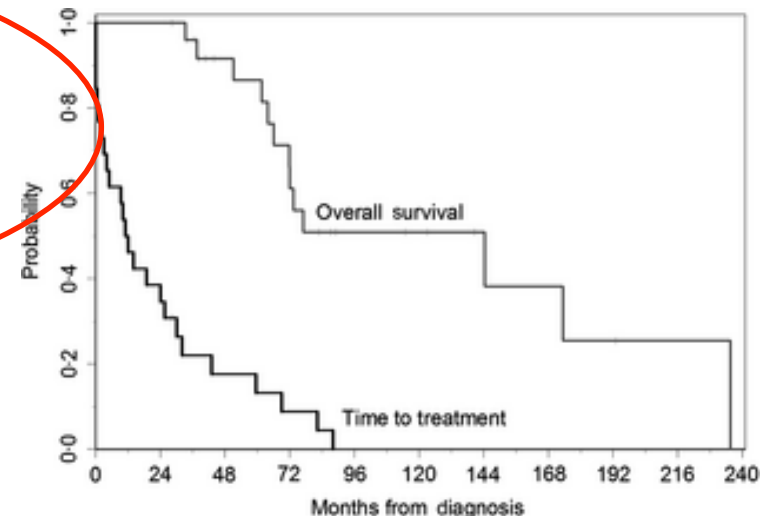
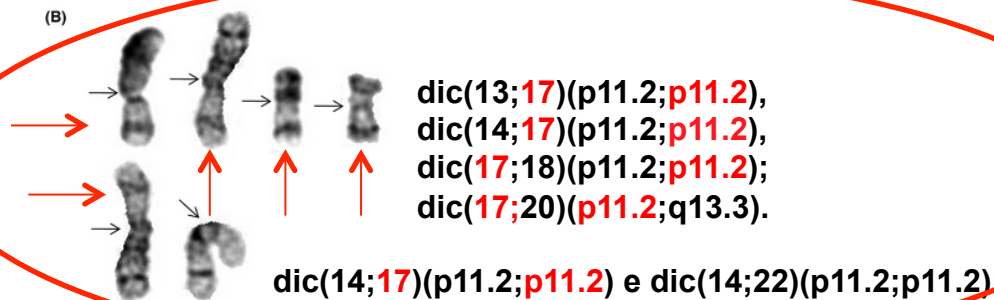
circa 1/3 dei pazienti con AC non aveva alterazioni in FISH con classico pannello di sonde

Jumping translocations (JT): meccanismo citogenetico che porta a delezione 17p13

rare alterazioni che coinvolgono la traslocazione della stessa regione cromosomica donatrice in 2 o più cromosomi riceventi, solitamente in cellule diverse

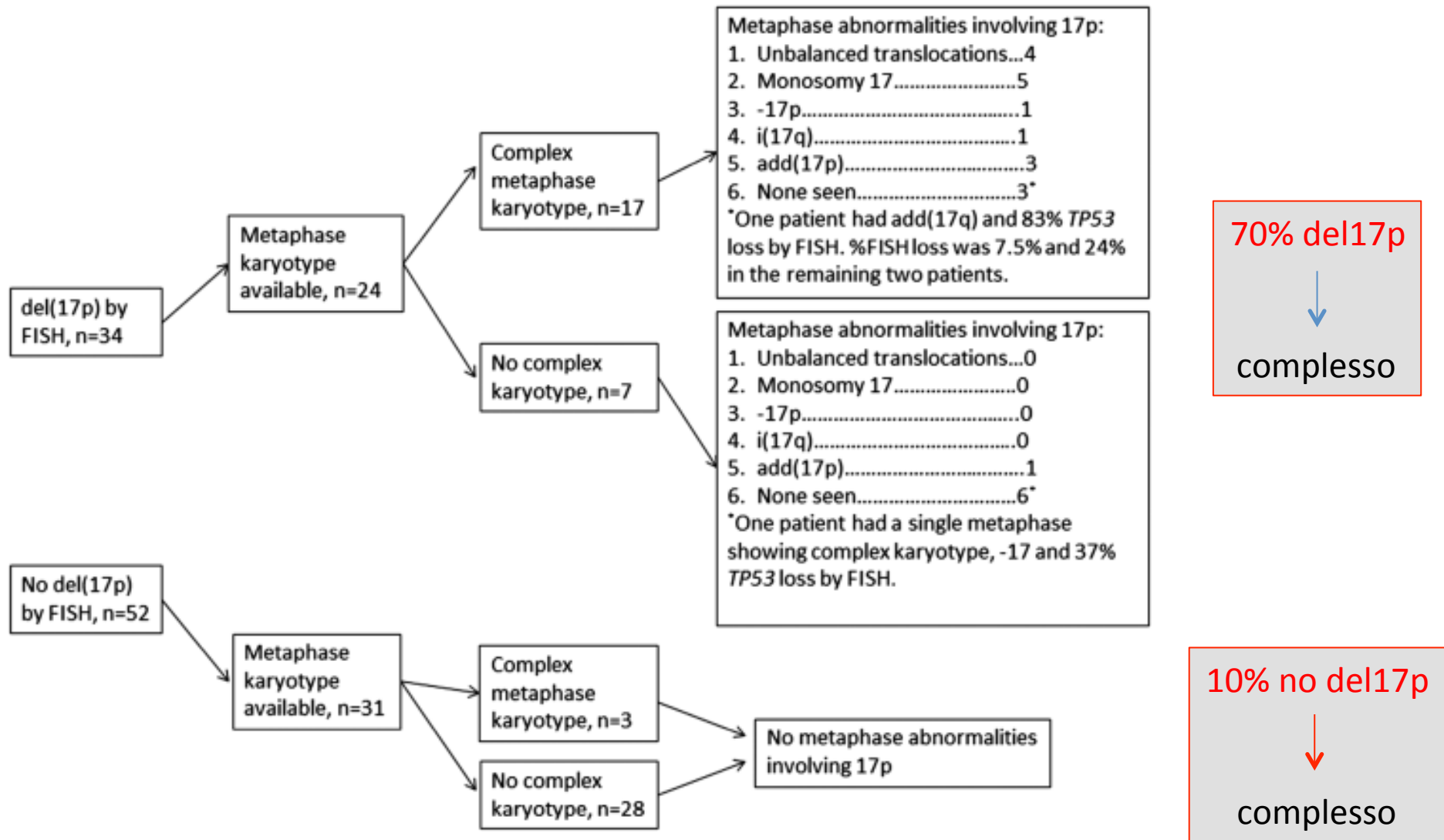


3% dei pazienti
breakpoint più frequente è 17p11.2
88% JT (23/26paz) delezione TP53

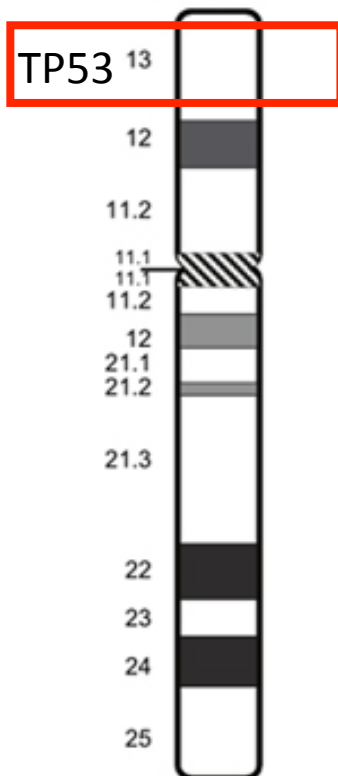


JT: alterazione ricorrente in malattia aggressiva, contribuisce al cariotipo complesso e, in molti casi, è coinvolta in riarrangiamenti cromosomici che portano a perdita di TP53

Associazione tra delezione 17p e cariotipo complesso



Delezione 17p13 e/o mutazioni TP53



Metodi di rilevazione: delezione 17p13 (citogenetica molecolare - FISH)
mutazione p53 (Sanger – NGS)

Frequenza: 5-10% dei casi alla diagnosi, fino al 40-50% dei casi in
progressione o refrattari

Alterazioni citogenetiche associate: traslocazioni della regione 17p13
cariotipo complesso

Prognosi severa

meno severa: delezione 17p13 <20% cellule

