Genomic complexity and arrays in CLL

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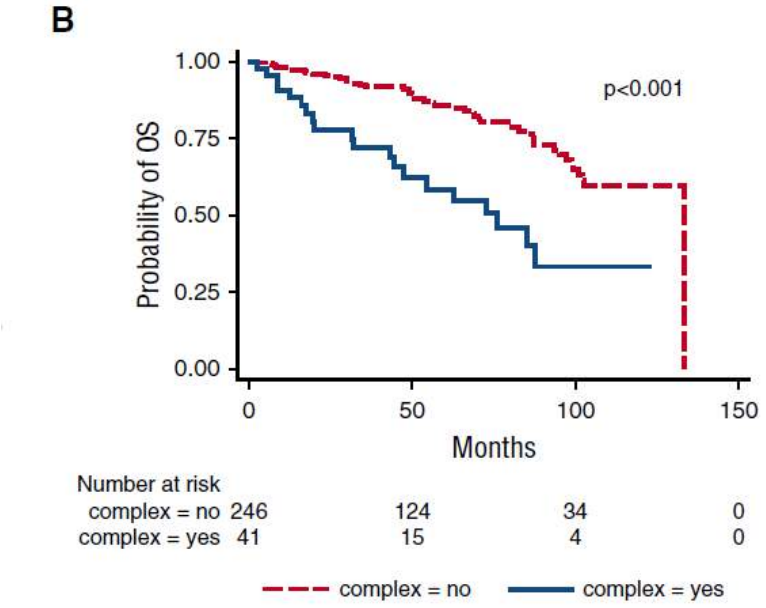
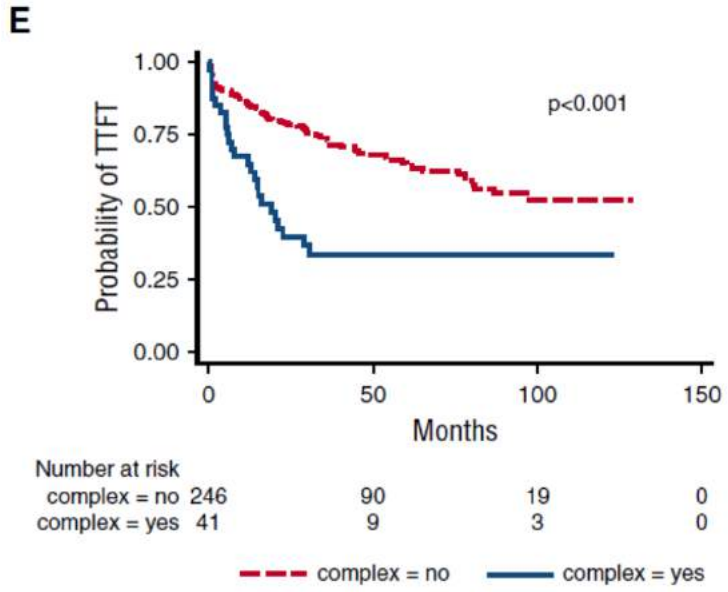
Clinical relevance of genomic complexity (GC) in CLL

- GC has been identified as a critical negative prognostic biomarker in large-scale CLL karyotyping studies.
- CK (≥3 abns) was associated with short TTFT and OS independently of CLL IPI
- CK is also emerging as a predictor of response to treatments including novel mechanism driven agents





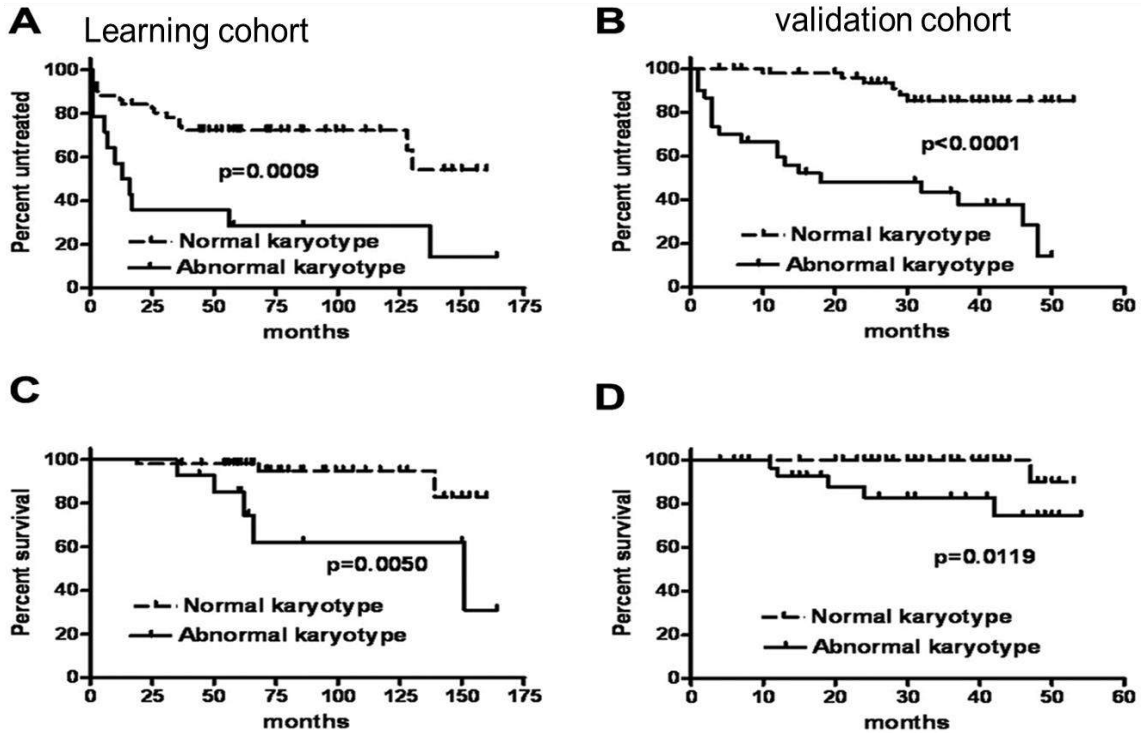
Rigolin GM et al. Blood 2017;129:3495-8

Karyotype and copy number aberrations (CNA) in CLL

- ~ 80% of cases has CNA
  - median 1.7 per case;
  - ≥2 in 44% of cases
  - ≥3 in 21% of cases.
- Karyotyping
  - Detects prognostically adverse CNAs in CLL with normal FISH.
  - Misses smaller genomic lesions

TFT

overall survival



Haferlach C, et al. Leukemia 2007; 21: 2442–2451.

Rigolin GM, et al. Blood 2012;119:2310–2313;.

CNA and arrays

- To overcome some of the **limitations** of conventional karyotyping and FISH, **arrays** have been introduced for **CNA analysis and detection of loss of heterozygosity (LOH)**

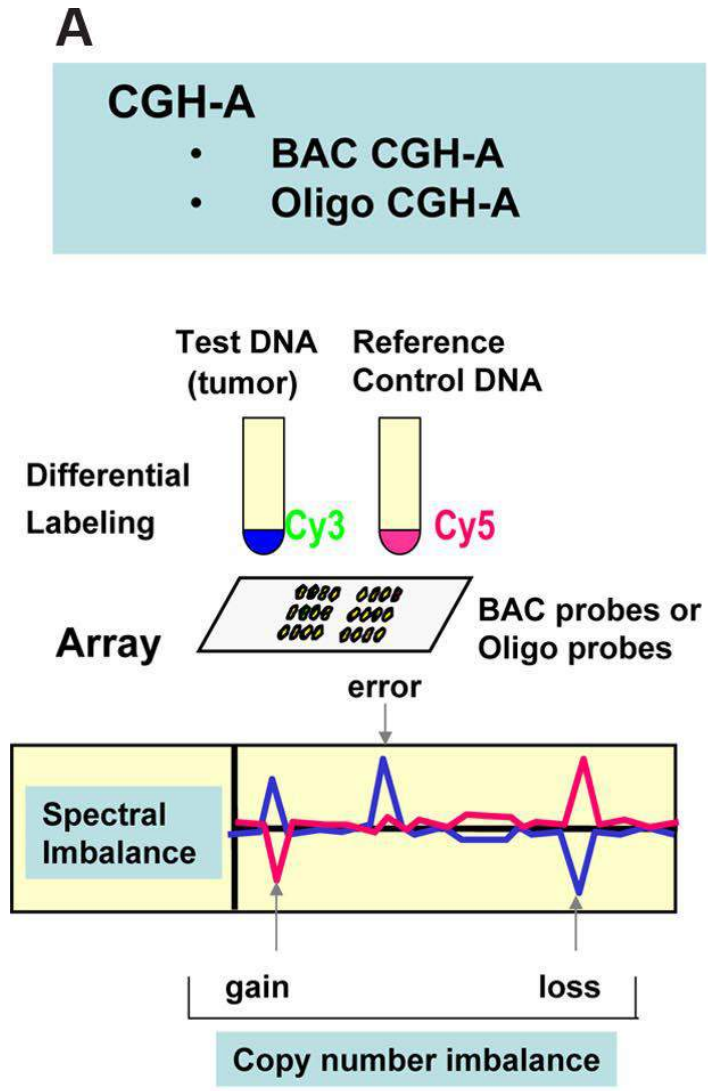
- Although it has become a valuable additional tool in research analysis, the use of arrays in the routine analysis of hematological malignancies **has yet to become a common practice**

Schoumans J et al. Genes Chromosomes Cancer. 2016;55:480-91

Comparative genomic hybridization (CGH)

- Classic CGH with metaphases chromosomes had limited resolution

- Substitution of the chromosome targets with arrays (aCGH) containing nucleic acids with defined sequences representative of whole chromosomes or chromosome arms allowed the detection of smaller gains and losses (High-resolution CGH: ~35Kb).



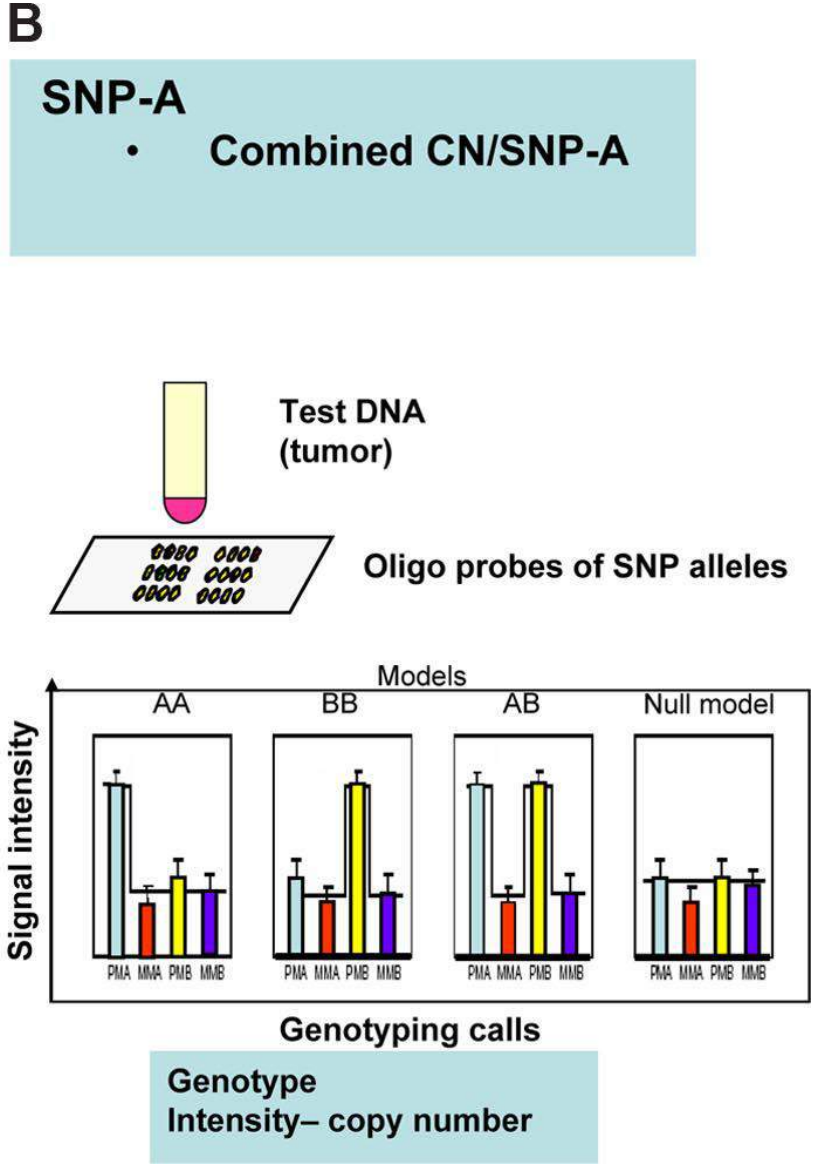
Improgo MR & Brown R. Hematol Oncol Clin N Am. 2013;27:157–171

Maciejewski JP, Mufti GJ. Blood. 2008;112:965-974

High-resolution SNP arrays

- The necessity of overcoming the resolution limits of classic CGH (10–20 Mb) and of aCGH (0.1 Mb) prompted the use of higher-resolution platforms.

- Using SNP arrays, it is possible to detect genomic alterations that would have been missed by aCGH.



Pfeifer D et al. Blood. 2007;109:1202–1210.

Improgo MR & Brown R. Hematol Oncol Clin N Am. 2013;27:157–171

Maciejewski JP, Mufti GJ. Blood. 2008;112:965-974

Technical aspects

Paired SNP array con FISH analysis

Study of 144 patients

	All CNAs	CNAs detected beyond FISH analysis
All patients (n = 144)	1.81	0.72
IGHV mutated (n = 61)	1.85	0.70
IGHV unmutated (n = 79)	1.80	0.75
No aberration (n = 28)	0.86	0.86
del(13)(q14) as sole abnormality (n = 49)	1.49	0.31
trisomy 12 (n = 19)	2.11	0.84
del(11)(q22.3) (n = 33)	2.48	0.91
del(17)(p13)/*TP53* mutated (n = 15)	2.80	1.27
Low-risk patients (n = 96)	1.40	0.55
High-risk patients (n = 48)	2.61	1.06

Genomic aberrations are grouped according to the hierarchical model.[1] High-risk is defined by the presence of del(11)(q22.3), del(17)(p13), and/or *TP53* mutation; low-risk by the absence of these alterations.

39% of pts carried CNA other than those detected by FISH

CNAs were identified in 127 pts (88%);

56 (39%) cases had 1 CNA,
33 (23%) cases had 2 CNAs,
23 (16%) cases had 3 CNAs,
15 (10%) cases had >3 CNAs, max 6

Deletions were more common than gains, with 1.44 losses versus 0.38 gains per case.

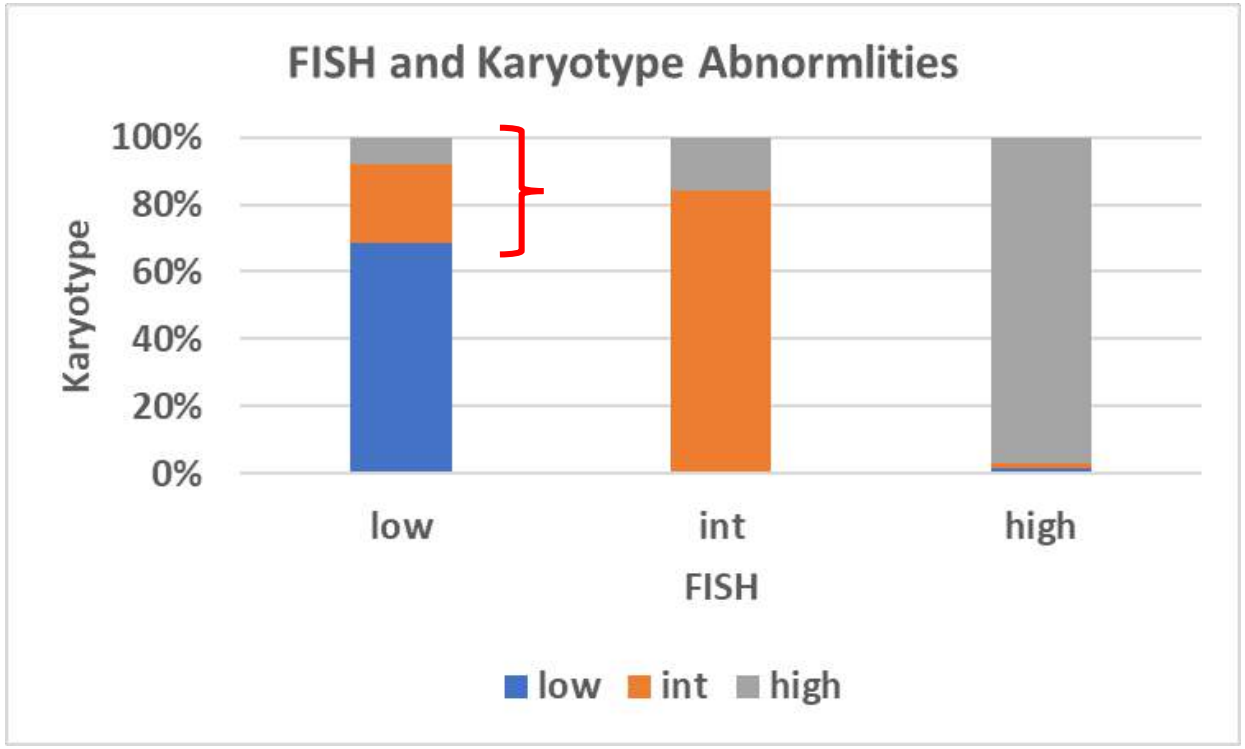
An increased number of CNAs was found in high risk genetic categories

No significant difference in CNAs per case was seen between M and UM CLL

Edelmann J et al. Blood 2012;120:4783-4794.

Chromosomal abnormalities: cytogenetics vs FISH

- Stimulation with DSP30/IL2 improved the rate of metaphase generation (95%)
- **Karyotype abnormalities undetected by FISH analysis were observed in 35% cases**
- discordant results were observed concerning risk stratification in 33/145 (22.8%) and 61/238 (25.6%) patients in the LC and VC, respectively



Rigolin et al. Genes Chrom Cancer 2015;54:818-26

Integration of microarray analysis into the clinical diagnosis of hematological malignancies: How much can we improve cytogenetic testing?

- **Purpose**:
  - To evaluate the clinical utility, diagnostic yield and rationale of integrating microarray analysis in the clinical diagnosis of hematological malignancies in comparison with classical chromosome karyotyping/FISH.

- **Methods**:
  - G-banded chromosome analysis, **FISH and microarray studies using customized CGH and CGH+SNP designs** (microarray using Agilent 4x180K CGH and CGH+SNP designs) on 27 patients with hematological malignancies.

Peterson JE. Oncotarget 2015;6:18845-62

Integration of microarray analysis into the clinical diagnosis of hematological malignancies: How much can we improve cytogenetic testing?

- **89.7% of abns identified by CCA/FISH were detectable by microarray**.

- **In 52% of cases microarrays identified additional CNA**
  - 30% of CNAs were in genomic regions of diagnostic/prognostic significance.
  - ~ 30% of novel alterations detected by microarray were >20 Mb in size.
  - 65% of genomic regions uncovered by aCGH were represented by alterations less than 10 Mb in size.

- **Balanced abnormalities were not detected by microarray;**
  - 55% (6/11) of recurrent and 13% (1/8) of nonrecurrent translocations had alterations at the breakpoints discovered by microarray.

- **~10% of cases require all the 3 methodologies to enhance interpretation.**

Peterson JE. Oncotarget 2015;6:18845-62

Performance of cytogenetic technologies for the detection of genomic lesions

Technique	Dectection ability							
	Whole chrom. aneuploidy	CNA	Polyploidy	Cell heterogeneity	Focal amplification	Balanced rearrang.	Unbalanced rearrang.	CN-LOH
Karyotyping (at low resolution)	+++	+	+++	+++	++	+++	+++	-
FISH (targeted only)	+++	++	+	+++	+++	+++	++	-
Array CGH	+++	++	–	+	+++	–	++	–
low resolution CGH+SNP array	+++	+++	+	+	+++	–	++	++
SNP array	+++	+++	++	++	+++	–	++	+++

Schoumans J et al. Genes Chromosomes Cancer. 2016;55:480-91.

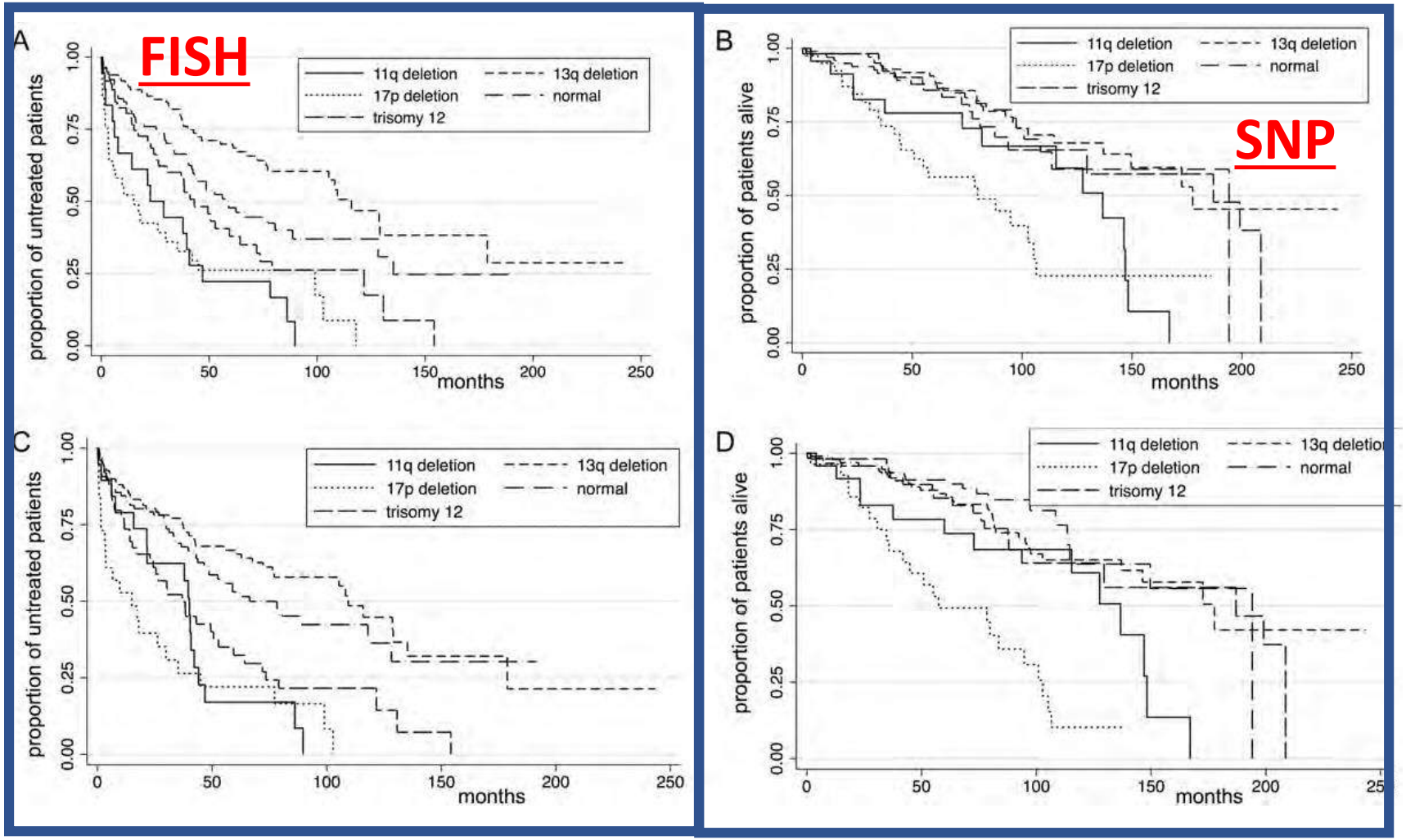
Recurrent copy number changes in CLL

Abnormalities	Frequency (%)	Associated genes	Cellular processes affected by the alteration	Prognostic significance
del(13q14)	50	*MIR15A/MIR16-1, DLEU2, RB1, DLEU7*	Regulation of BCL2 expression, cell cycle control, NFkB signalling	Good
trisomy 12	20	Unknown	Unknown	Good/intermediate
del(11q22-23)	6–20	*ATM, BIRC3*	DNA repair, NFkB signalling	Poor
del(17p13)	5–10	*TP53*	Loss of tumour suppressor	Poor
del(6q21)	5–7	*ZNF292*	Transcriptional regulation	Unknown
Gain 2p	5–28	*XPO1, REL, BCL11A, MYCN*	RNA processing, NFkB signalling, Proliferation	Poor
amp(8q24.21)	5	*MYC*	Proliferation, apoptosis	Poor
del(8p)	5	*TRAIL-R*	Apoptosis	Poor
del(15q15.1)	4	*MGA*	Transcriptional regulation	None
del(2q37)	2	*SP140/SP110*	Transcriptional regulation	None
del(3p21)	2	*SMARCC1/SETD2*	RNA splicing and DNA repair	Poor
del(10q24)	2	*NFKB2*	NFkB signalling	Unknown

Ghamlouch H et al. BJH 2017;178:852-870

genomic complexity and outcome

Large genomic aberrations detected by SNP array are independent prognosticators of a shorter TFT and OS in CLL



The concordance between FISH and SNP array varied between 84 and 97%.

OS and TTT according to the Döhner prognostic model

- by FISH (A, B; P < 0.001)
- by SNP (C, D; P < 0.001).

In MVA, independent prognosticators

- stage
- IGHV
- large genomic lesions.

Mian M et al. Ann Oncol 2013;24:378-84

Complexity in CLL Defined By Array-Based Analysis: Definitions, Associations with Other Biomarkers and Clinical Impact

- **Aims**:
  - **CK defined by the presence of ≥3 abns based on CBA may be suboptimal when analyzed by array techniques.**
  - Determine a prognostic relevant of GC by array-based analysis.
  - Comparison of the sensitivity of CBA versus array-based analysis.
- **Methods**:
  - **1895 CLL** and 16 MBL analyzed with CGH or SNP arrays (66% within the first year from diagnosis and 84% before treatment).
  - Cut off 5 Mb

Leeksma A et al. ASH 2017. 4280

Genomic Complexity in CLL Defined By Array-Based Analysis: Definitions, Associations with Other Biomarkers and Clinical Impact

Table 1. Multivariable Analysis Array-based cohort according to Schoumans criteria: GC≥3abs (left) vs GC ≥5abs (right).

N=1233	HR	HR 95% CI	P-value
Male	0.876	0.705-1.087	0.228
>70yrs	2.605	2.055-3.304	<0.001
Binet B/C	1.749	1.419-2.155	<0.001
U-CLL	3.286	2.607-4.143	<0.001
TP53abs	1.818	1.350-2.448	<0.001
Del(11q)	0.926	0.731-1.173	0.524
GC≥3abs	1.056	0.811-1.374	0.686

N=1233	HR	HR 95% CI	P-value
Male	0.882	0.711-1.095	0.255
>70yrs	2.617	2.065-3.317	<0.001
Binet B/C	1.727	1.402-2.127	<0.001
U-CLL	3.279	2.601-4.133	<0.001
TP53abs	1.623	1.223-2.152	<0.001
Del(11q)	0.884	0.701-1.113	0.294
GC≥5abs	1.797	1.271-2.542	**<0.01**

GC=Genomic complexity, HR= Hazard Ratio, yrs=years, U-CLL=IGHV unmutated CLL, TP53abs=TP53 mutation and/or del(17)p(13.1), del(11q)=del(11)(q22.3).

Analysis was performed using a size cut-off ≥5Mb

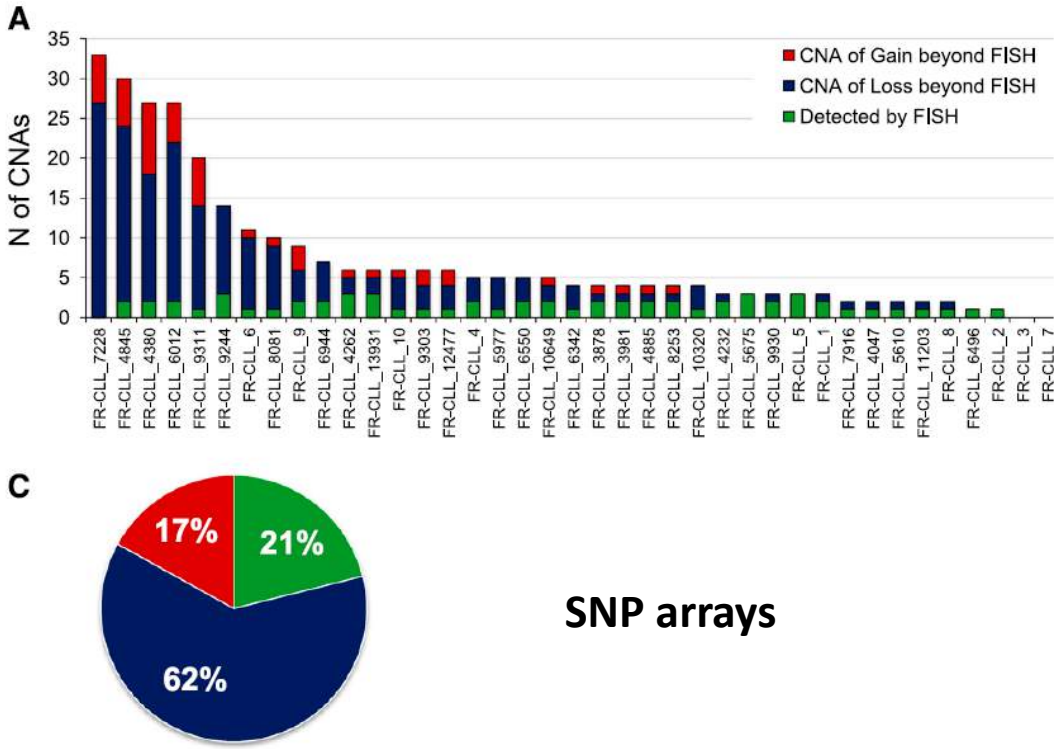
With a 1 Mb cut-off

- **a higher number of CNAs (3.04 vs 2.34) vs 5Mb.**
- **upstaging of HR GC (22 vs 12%), the median OS in the 'high risk' group was increased vs 5Mb (8.4 vs 5.5 years).**

Leeksma A et al. ASH 2017. 4280

CNA and chemorefractoriness

- **95% of patients were had CNAs,**
  - 71% >3 lesions
  - 20% >10 lesions.
- the burden of CNAs is heterogeneous across FR-CLL patients
  - range 0-33;
  - average, 7.4/case, or
  - 4.1/case if we exclude outlier.
- **Aberrations detected by FISH represented a small portion (21%) of all the CNAs and that 85% of cases displayed additional CNAs.**

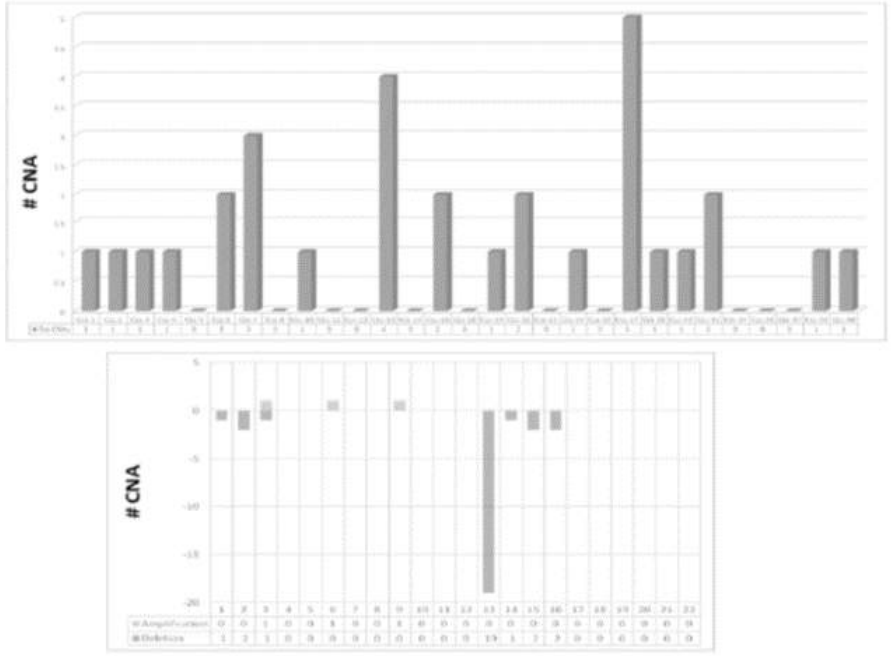


SNP arrays

Messina M et al. Blood. 2014;123:2378-2388.

Genetic landscape of ultra-stable CLL patients

- **no progression for > 10 yrs from diagnosis**
  - **no CNA with poor prognostic impact.**
    - 31 lesions (90% losses, 10% gains) in 29 cases,
    - 1 CNA/case (range: 0-5)
    - no lesions (38%) or an isolated del(13q) (31%).
    - **only 3 cases showed ≥3 lesions.**
  - No mutations of NOTCH1, BIRC3, SF3B1, ATM, TP53.
  - The expression of 6 genes allowed to build a decision-tree capable of recognizing at diagnosis US-CLL patients.



Raponi S et al. Ann Oncol 2018 in press

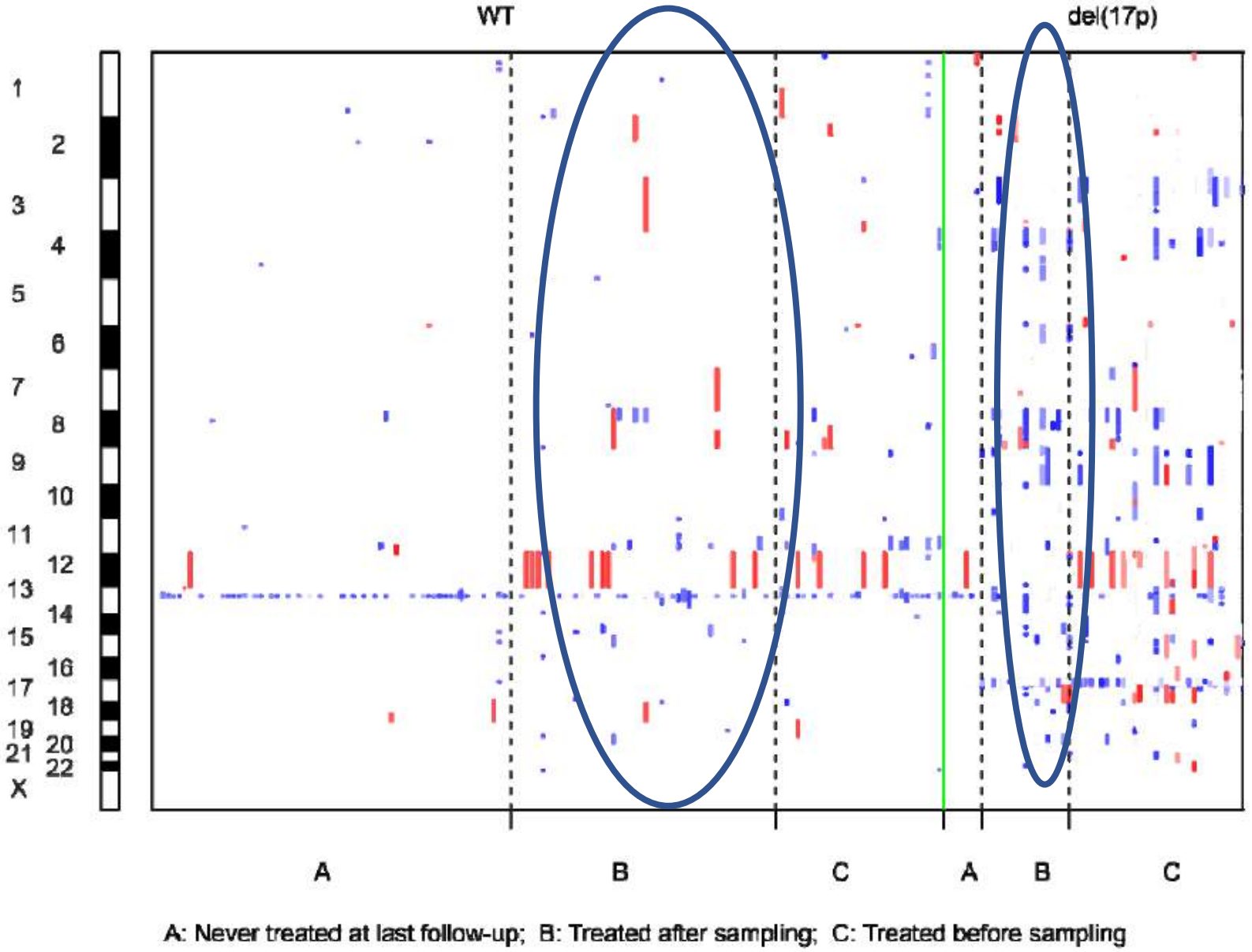
Survival of del17p CLL Depends on Genomic Complexity and Somatic Mutation

Table 1. Patient characteristics and summary of CNAs and somatic mutations

	All	wt 17p	del(17p)	*P*
N	277 (100%)	208 (75%)	69 (25%)	
Male	163 (59%)	118 (57%)	45 (65%)	0.26
Age of onset	55 (32–86)	54 (32–78)	61 (38–86)	2.2e−05
Treated before sampling	74 (27%)	39 (19%)	35 (51%)	6.4e−07
IGHV mutated	135 (52%)	125 (64%)	10 (16%)	1.2e−11
Complex karyotype	42 (32%)	20 (21%)	22 (61%)	4.03e−05
FISH cytogenetics				
13q14 loss	170 (61%)	139 (67%)	31 (45%)	1.9E−4
11q loss	37 (13%)	29 (14%)	8 (12%)	0.55
Trisomy 12	36 (13%)	25 (12%)	11 (16%)	0.54
Profiled by WES[a]	176 (100%)	123 (70%)	53 (30%)	
Total mutations	19 (0–94)	18 (0–94)	21 (7–68)	0.0048
Nonsynonymous mutations	14 (0–70)	13 (0–70)	16 (5–54)	0.0055
Synonymous mutations	4 (0–24)	4 (0–24)	4 (1–14)	0.14
Subclonal mutations	9 (0–89)	9 (0–89)	8 (2–35)	0.9
Clonal mutations	9 (0–34)	7 (0–24)	12 (0–34)	5.8E−4
Profiled by SNP[a]	200 (100%)	145 (72%)	55 (28%)	
# of CNAs	1 (0–36)	1 (0–16)	7 (0–36)	1.5e−16
# of losses	1 (0–35)	1 (0–16)	6 (0–35)	5e−15
# of gains	0 (0–19)	0 (0–3)	1 (0–19)	3.3e−08
Lost Mb	3 (0–530)	1.2 (0–88)	97 (0–530)	2.9e−18
Gained Mb	0 (0–340)	0 (0–260)	5.9 (0–340)	9.9e−6
8p loss	21 (10%)	6 (4%)	15 (27%)	1.1e−05
3p loss	15 (8%)	0 (0%)	15 (27%)	8.1e−10
4p loss	14 (7%)	2 (1%)	12 (22%)	4.1e−06
9p loss	15 (8%)	2 (1%)	13 (24%)	1.1e−06
Loss in 3p, 4p, or 9p	31 (16%)	3 (2%)	28 (51%)	8.6e−16

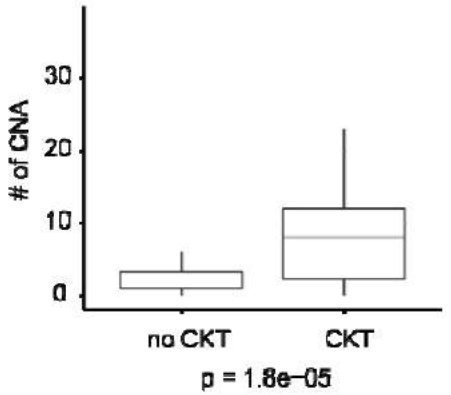
[a]Median values and ranges are presented for the WES and SNP analysis.

Yu L et al Clin Cancer Res. 2017;23;735-45.



fewer CNAs in patients who did not require therapy in the follow-up period

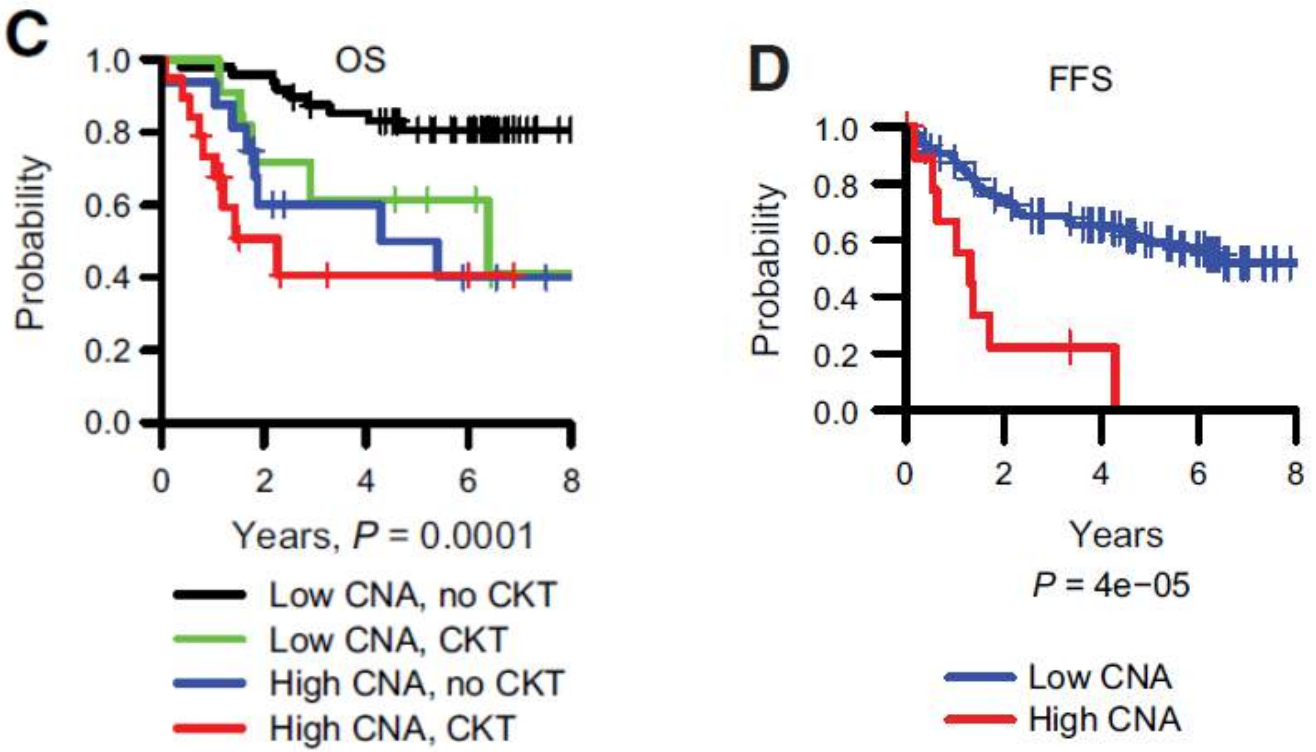
del(17p) CLL has a longer total length of deleted (97Mb vs. 1.2 Mb, P < 0.0001) and gained DNA (5.9 Mb vs. 0 Mb, P <0.0001).



Association between number of CNAs and CKT

Yu L et al Clin Cancer Res. 2017;23;735-45.

Overall Survival and Failure Free Survival



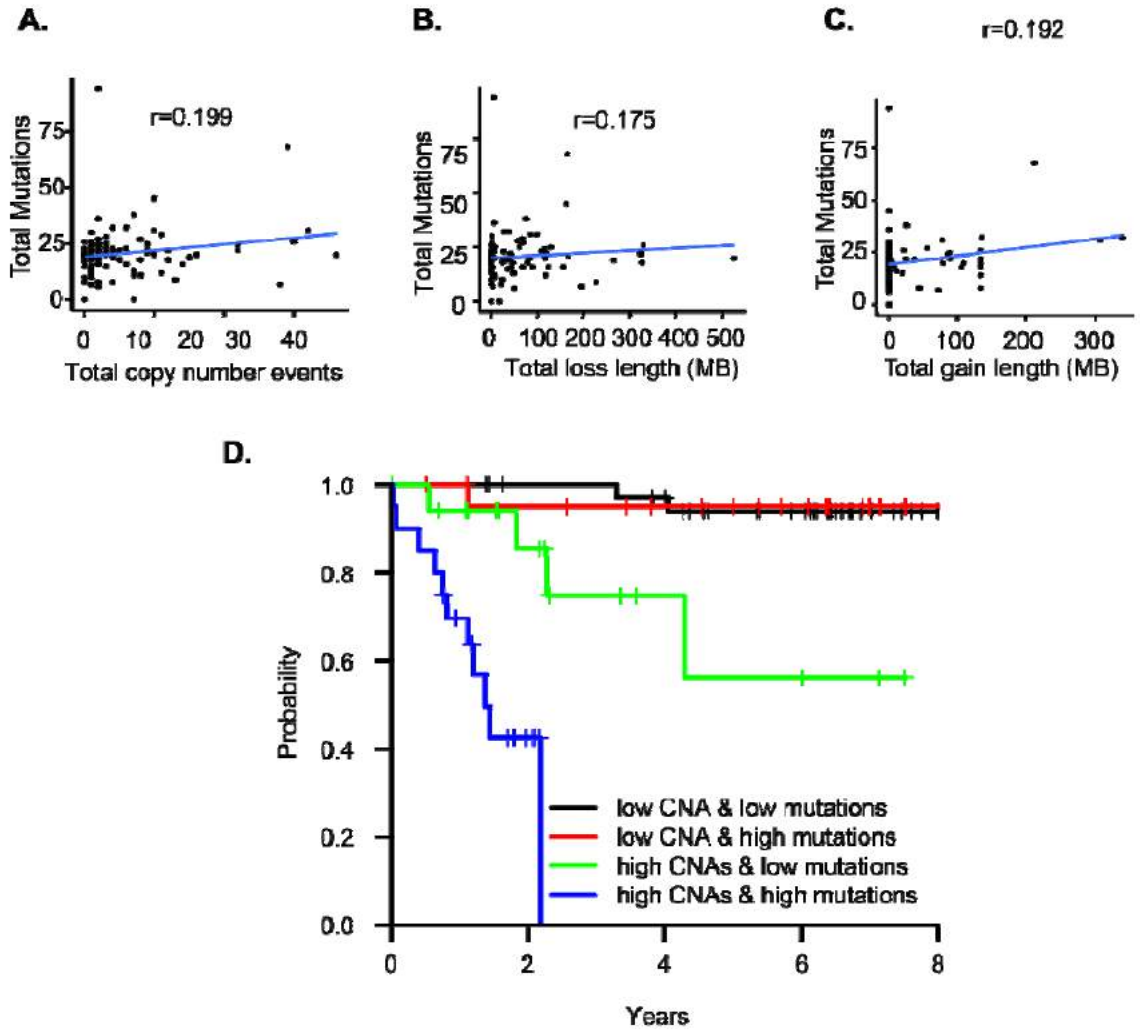
Shorter OS (UVA and MVA)

- **with mutations**
- **With a high number of CNAs, number of loss events and total length of losses**

CNAs and CK, but not somatic mutations, predicted shorter TTFT

Yu L et al Clin Cancer Res. 2017;23;735-45.

CNAs and somatic mutations in CLL



- Neither CNAs nor total length of gains or losses correlated with the number of somatic mutations

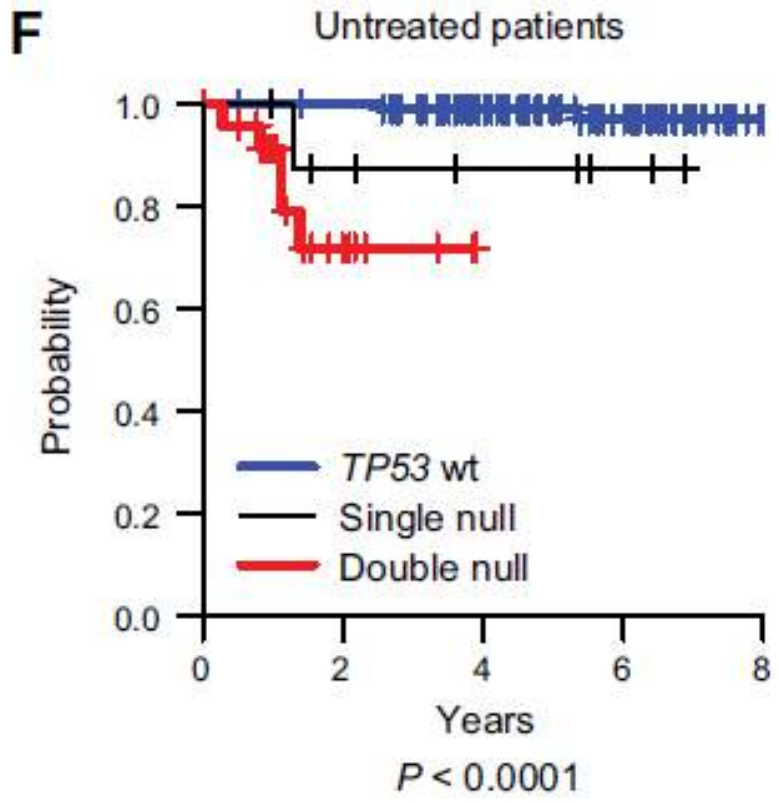
- suggesting that mutations and CNAs result from different mechanisms in CLL.

Yu L et al Clin Cancer Res. 2017;23;735-45.

del(17p) patients

- Mono- versus biallelic TP53 disruption was predictive of OS.

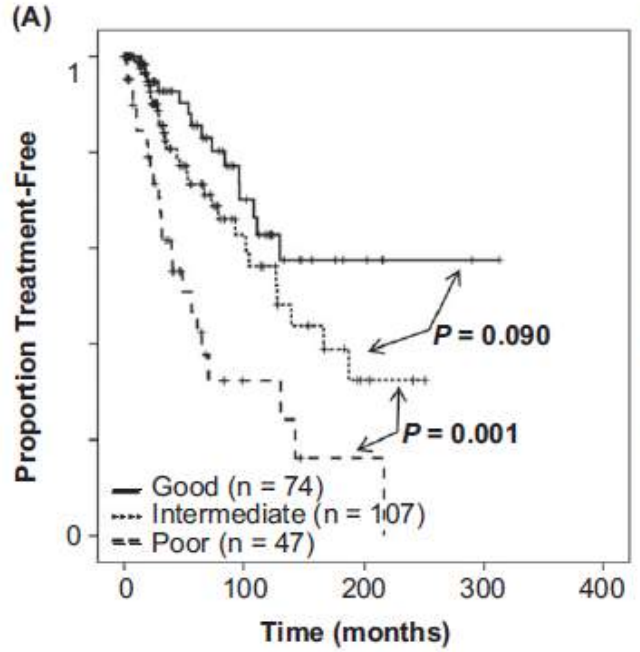
- Indolent del(17p) pts
  - absent or subclonal TP53 mutation
  - **few CNAs**
  - Overrepresentation of IGHV mutated cases (57% vs 10%)
  - no difference in somatic mutation number

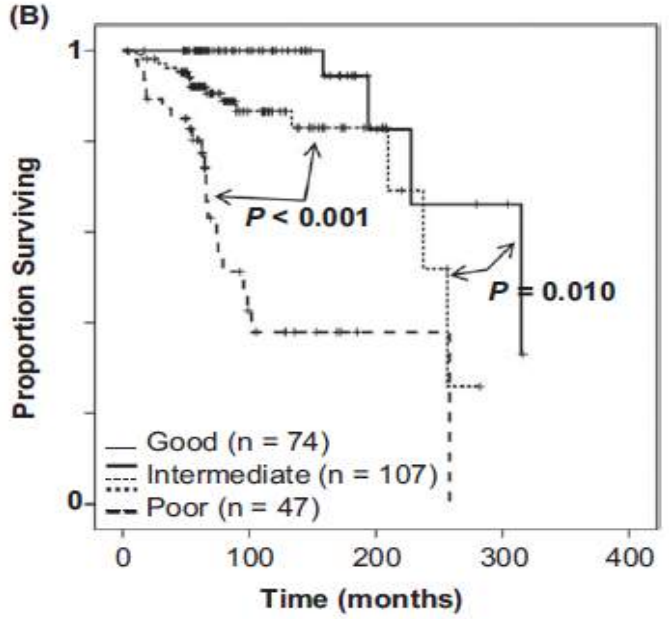


Yu L et al Clin Cancer Res. 2017;23;735-45.

CNAs: Hierarchical subclassification

- **Poor outcome** (47 cases)
  - Loss of 17p and 11q (29 cases).
  - one of the other 7 poor aCGH markers:
    - gain of 2p, 3q, 8q or loss of 7q, 8p, 17q, 18p.
- **Good outcome** (74 cases)
  - 13q14 deletions but no additional aberrations at 10 recurrent loci
    - gain: 1p, 7p, 12, 18p, 18q, 19; loss: 4p, 5p, 6q, 7p.
- **Intermediate outcome** (107 cases):
  - any aberration of the 10 loci used to define good outcome (63 cases)
  - None of the total 20 aberrations (44 cases).

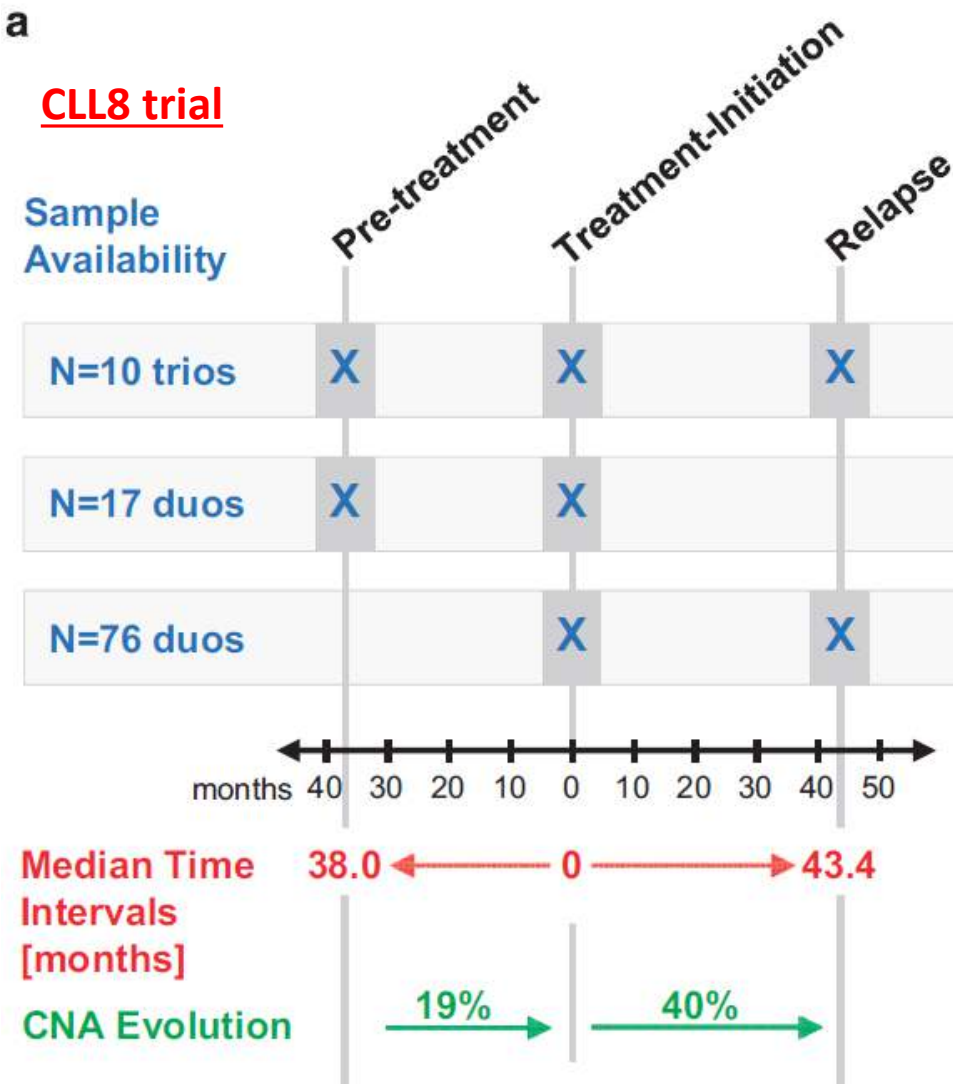




Houldsworth J et al. Leuk Lymph. 2014; 55: 920–928

Biological aspects

CNA evolution in CLL following first-line treatment with FC(R)

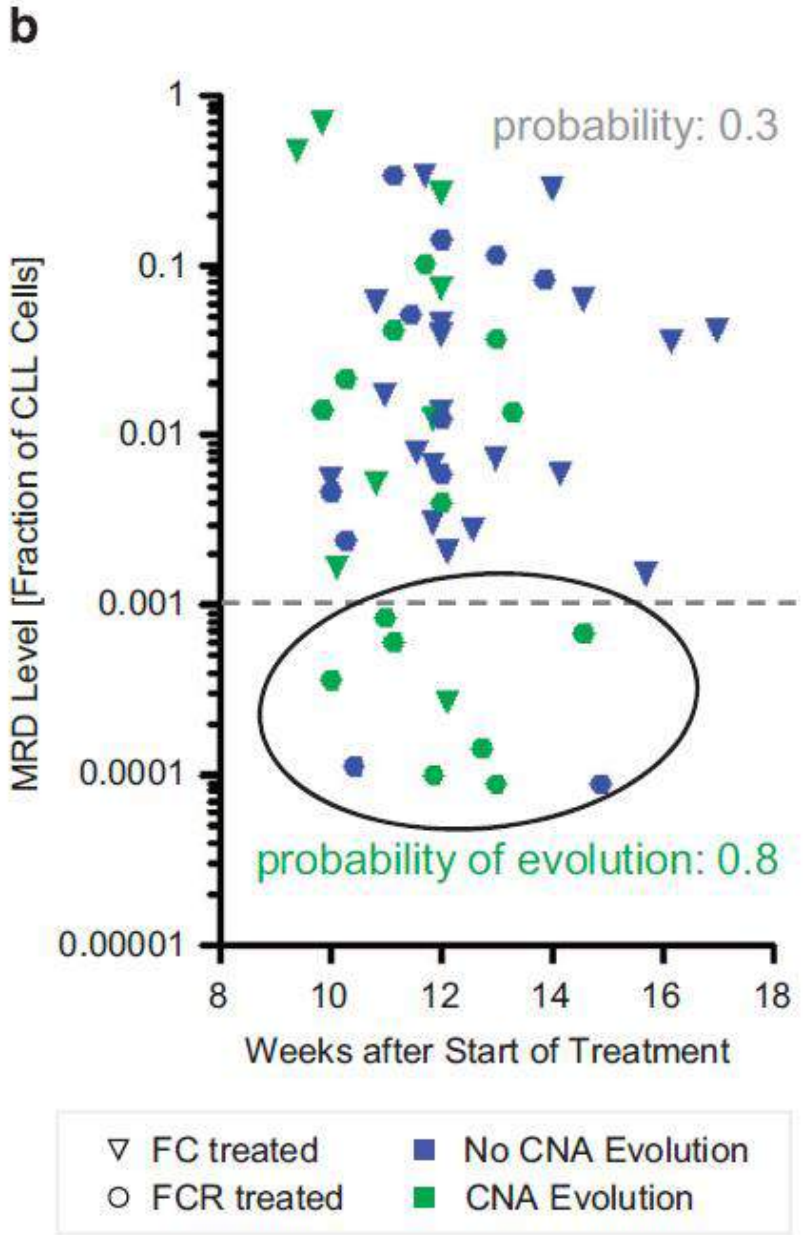


- Clonal evolution was shown to be frequent in CLL by matched sequential samples taken at treatment initiation and first relapse following CIT
- 103 individuals treated within the CLL8 trial, in which patients uniformly received FC/FCR as first-line treatment.
- The threshold for CNA detection was 10-15% of affected cells for monoallelic lesions.

Edelmann J et al. Leukemia. 2017;31:734-73

CNA evolution related to early MRD levels

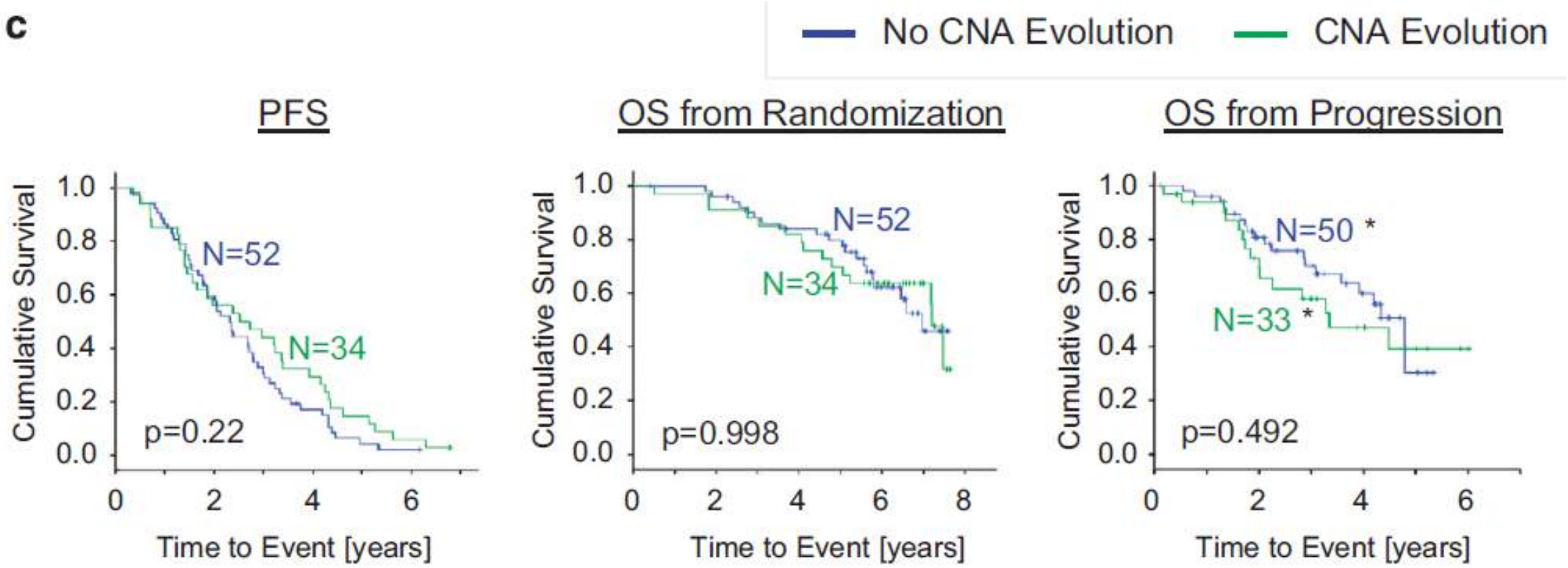
- **FCR treatment was the only variable significantly predicting for CNA clonal evolution**
- **The probability of CNA evolution was higher for cases with an MRD level <0.001 (P=0.008)**
- **This observation supports the hypothesis that clonal evolution is enhanced by a strong selection pressure imposed by CIT**



MRD by 4-color FCM after 3 cycles

Edelmann J et al. Leukemia. 2017;31:734-73

Clonal evolution and CNA: outcome



The presence of CNA evolution was not associated with survival differences

Incidences for TP53 abns was twice as high at relapse than at treatment initiation

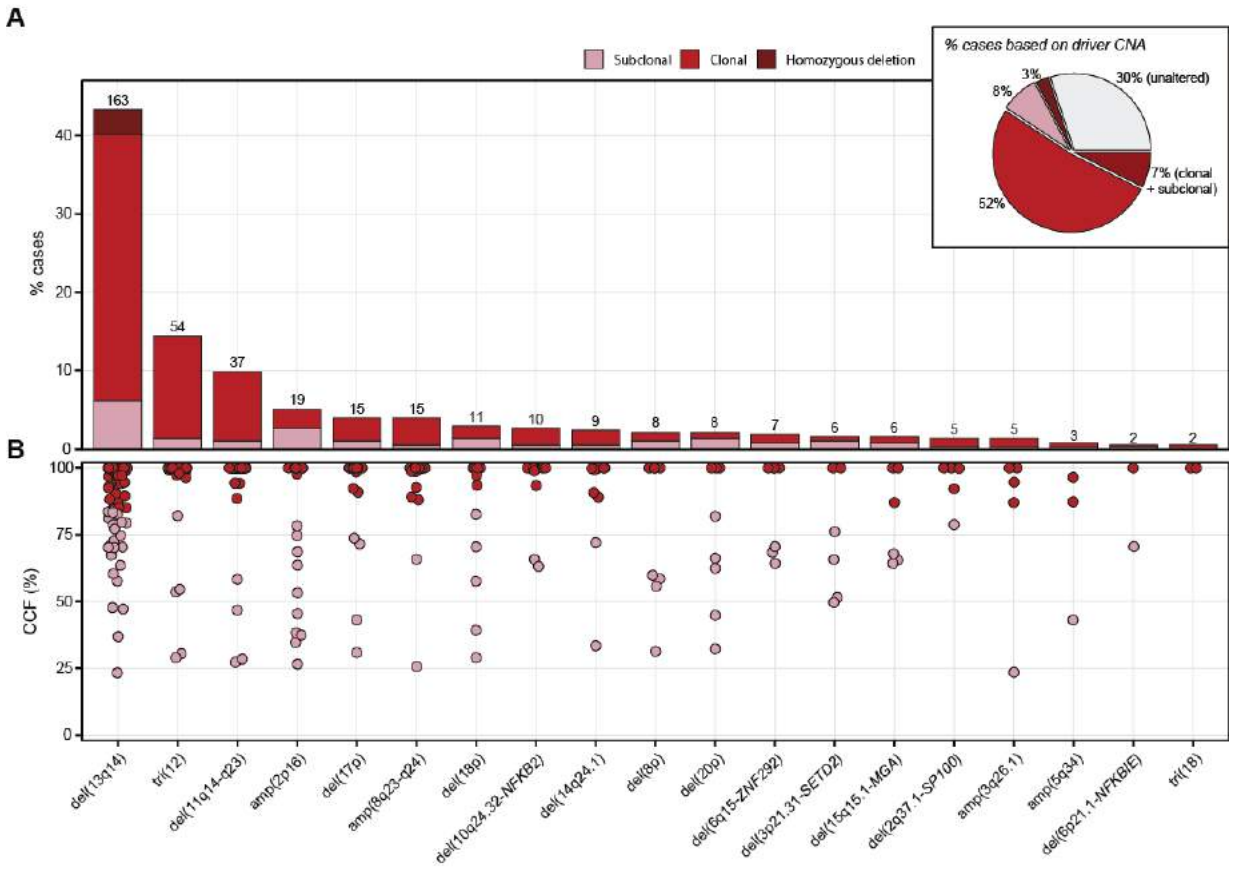
- TP53 del: from 8 to 20%; TP53 mut from 15 to 31%.

In this study the emergence of clones harboring TP53 abns was associated with a significantly shorter OS

Edelmann J et al. Leukemia. 2017;31:734-73

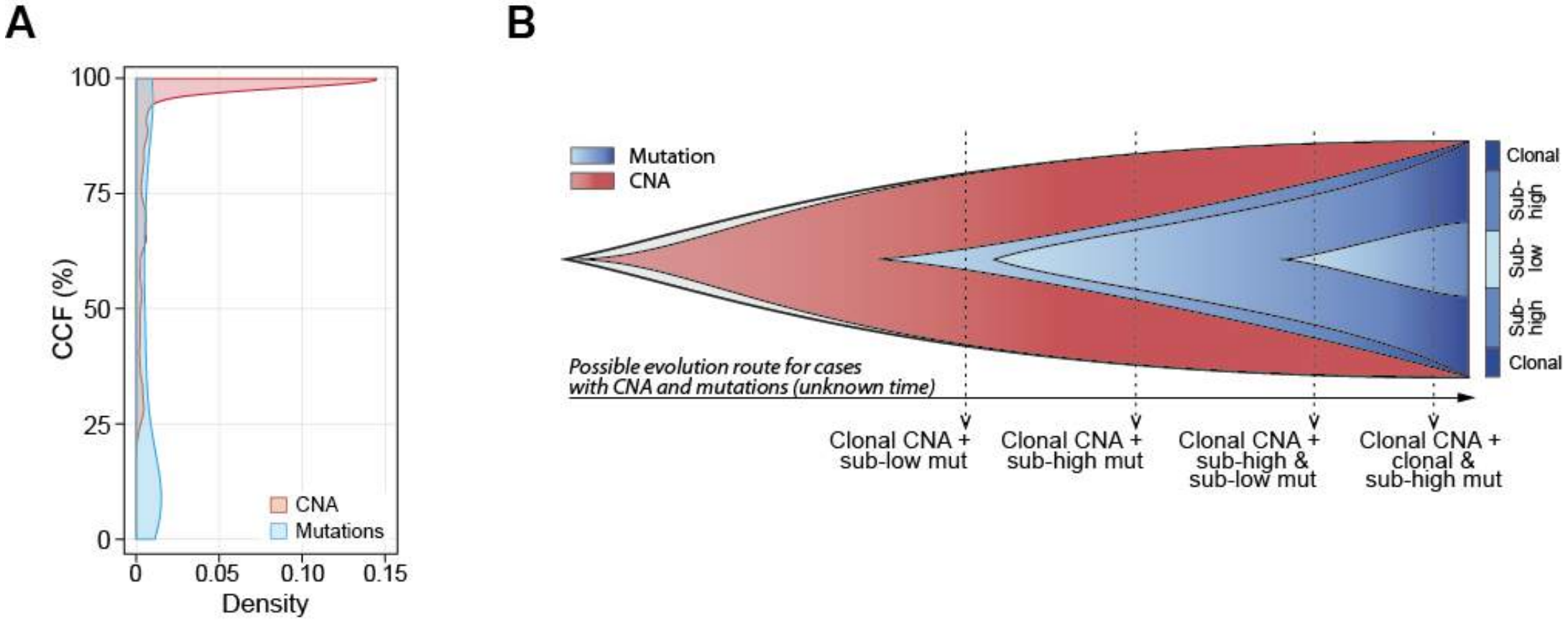
Subclonal architecture and evolutionary pathways in CLL

- **CNA identified in 295/376 (78%) cases**
  - range 1–26, median 2
- **Combining mutations and CNA, 86% (350/406) of pts carried at least one driver alteration**
  - range: 1–8, median: 2,
  - clonal in 66% (267/406) of cases.
- **No correlation between CNA and n. of mutations (ρ = 0.18).**
- Clonal driver CNA (79%) were more frequent than subclonal CNA (21%).
  - 59% of pts carried clonal driver CNA
  - 8% of pts harbored isolated subclonal alterations



Nadeu F et al. Leukemia. 2017 Sep 19. doi: 10.1038/leu.2017.291

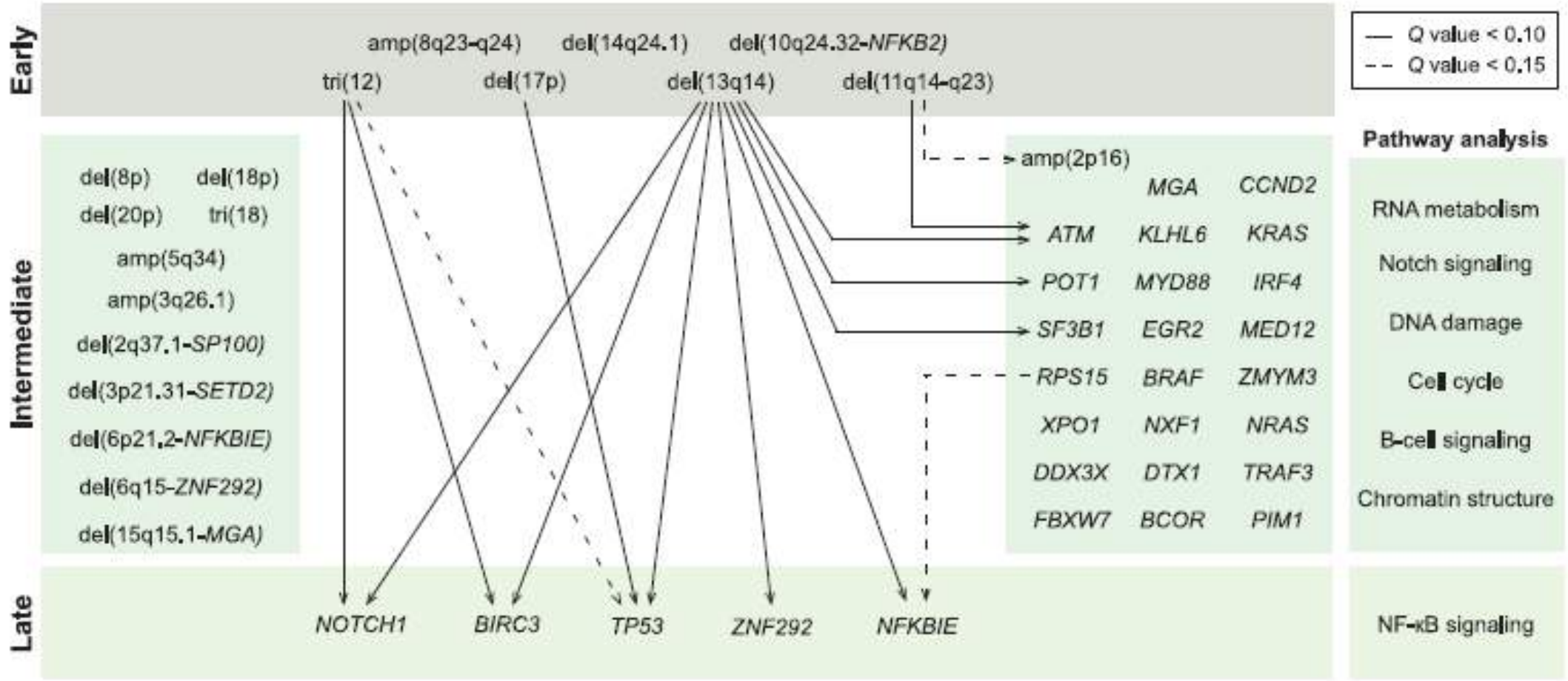
Temporal relationship and hierarchical acquisition in CLL



The distribution in CCFs of the CNA and mutations suggests a scenario in which driver CNA are acquired earlier, whereas gene mutations may be acquired at any time during CLL evolution

Nadeu F et al. Leukemia. 2017 Sep 19. doi: 10.1038/leu.2017.291

Temporal relationship and hierarchical acquisition in CLL

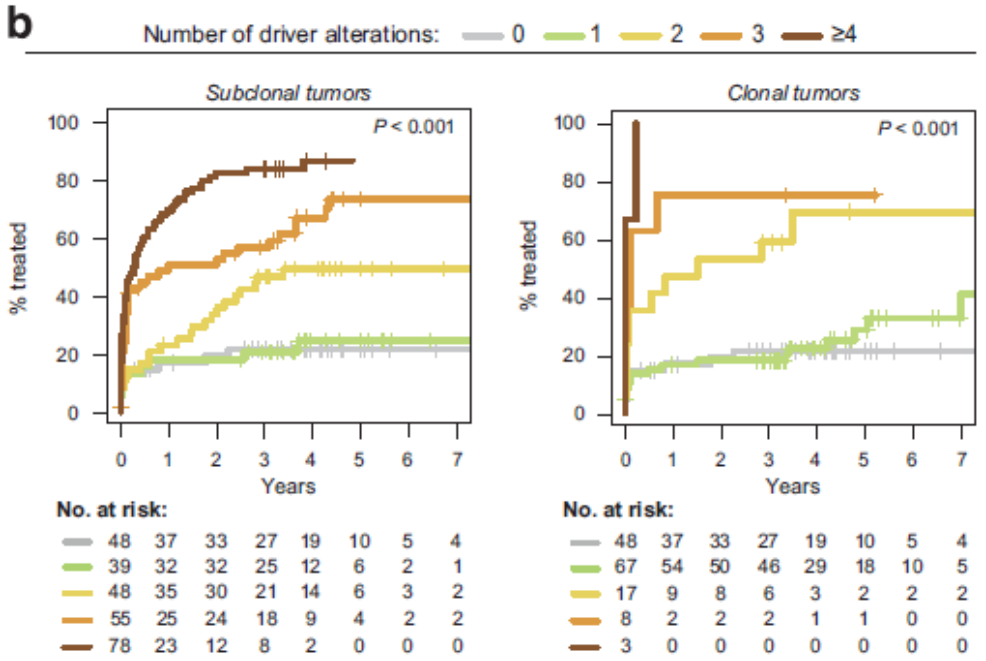


The analysis of the temporal acquisition of individual alterations suggests that CNA, particularly tri(12), del(13q), del(11q) and del(17p), but also other less recurrent CNA, are usually earlier events while most mutations may be acquired at any time in the evolution of the CLL and frequently later than CNA

The low mutational rate of CLL driver alterations as well as the much lower sensitivity for detecting subclonal CNA are limitations of this analysis. sensitivity detection of the CNA (CCF ⩾ 25%).

Nadeu F et al. Leukemia. 2017 Sep 19. doi: 10.1038/leu.2017.291

outcome



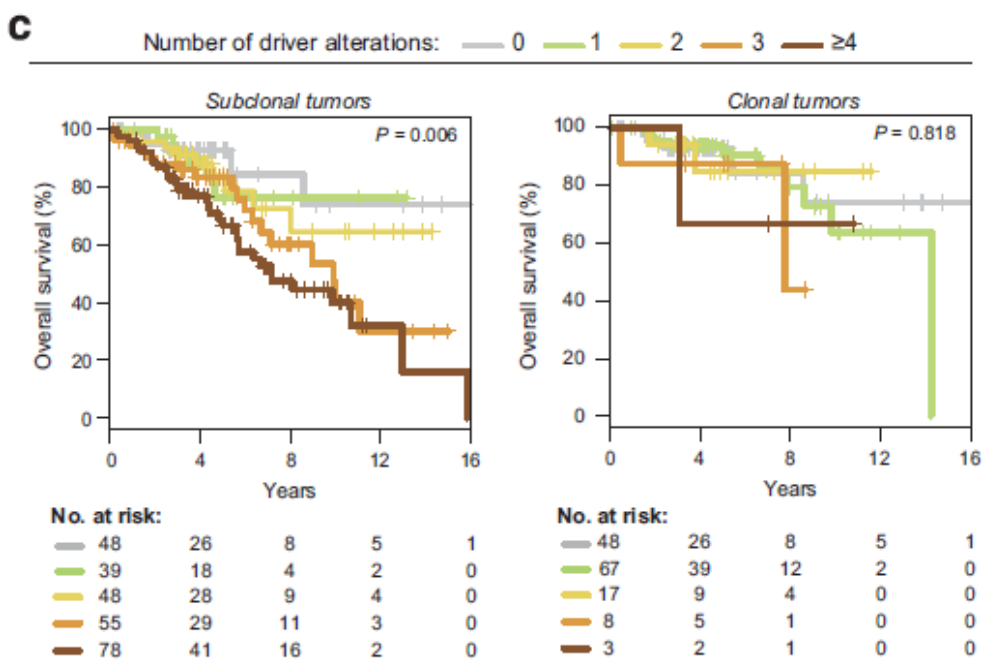


Table 3. Independent prognostic value of the accumulation of driver alterations for TTFT

Variable	*HR (95% CI)*	P-*value*
Binet stage (B/C vs A)	2.44 (1.60–3.74)	<0.001
No. drivers (0, 1, 2, 3, ⩾4)	1.44 (1.21–1.72)	<0.001
SF3B1 (mut vs unmut)	2.02 (1.39–2.94)	<0.001
IGHV (unmut vs mut)	2.07 (1.35–3.19)	0.001
ATM (mut/deletion vs wt)	1.82 (1.19–2.27)	0.006
Age at sampling (>65 vs ⩽65 years)	0.66 (0.48–0.91)	0.011

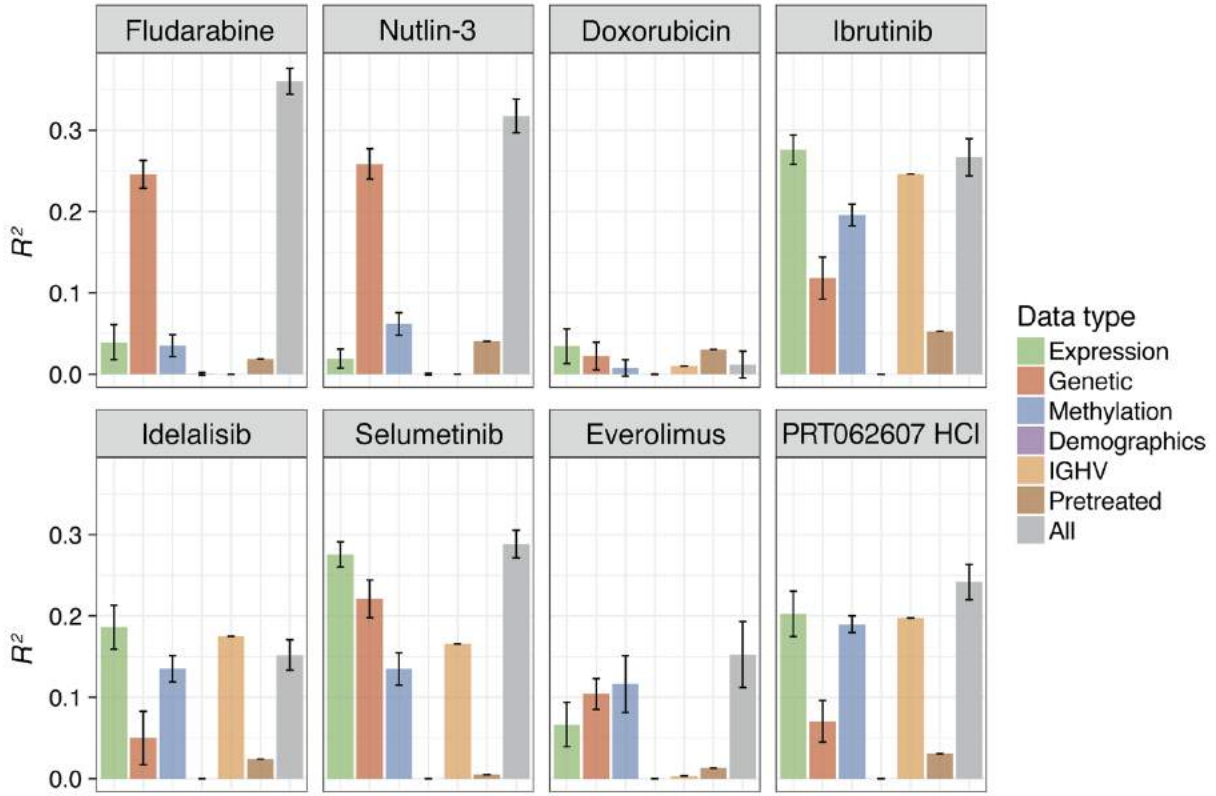
N = 307, events = 146, competing events = 27. Starting model: IGHV, Binet stage, age at sampling, gender, *TP53* aberration (mutation/deletion), *ATM* aberration (mutation/deletion), *SF3B1* mutation and number of driver alterations (not including *TP53*, *ATM* and *SF3B1* mutations, neither del(17p) nor del(11q)).

The number of drivers was associated with a worse outcome

Nadeu F et al. Leukemia. 2017 Sep 19. doi: 10.1038/leu.2017.291

Drug response prediction

- Drug responses to nutlin-3 and fludarabine were predominantly explained by genetics (gene mutations and CNA).
- In contrast, response to kinase inhibitors was best explained by IGHV status, gene expression, or methylation patterns.



Dietrich S et al. JCI. 2018;128:427-445

Conclusions

Open questions with arrays in CLL

According to the American College of Medical Genetics (ACMG) recommendations arrays should be applied **as a complementary investigation** (Cooley et al., 2013).

1. Can arrays replace conventional karyotyping and/or FISH panels?
2. What type of array (array-CGH, array-CGH+SNP, or genotyping array) is best suited to analyse and interpret the complexity of the genomes in CLL?
3. Which parts of the results are clinically relevant?
4. What is the clinical utility of identifying CNAs (< 5Mb) undetected by karyotyping?
5. What is the test sensitivity for the detection of low level clonal cell populations?
6. What information should be transmitted to the referring clinicians while keeping the report comprehensive and readable?

Schoumans J et al. Genes Chromosomes Cancer. 2016;55:480-91.

Advantages and limitations of DNA microarray analysis

advantages	• The ability to use any sample that yields DNA of sufficient quality, • Assessment of the genome at very high resolution, • Interpretation of raw data using objective biostatistical algorithms, • The ability to detect LOH with SNP-array technology, • A ready interface of the digital data with genome browsers and Web-based databases.
limitations	• Inability to detect balanced rearrangements • Inability to detect clonality with a low ratio of tumor to normal cells and to characterize clonal and subclonal populations • Inability to determine the chromosomal mechanisms of the genetic imbalance; • Inability or difficulty in detection of ploidy levels; SNP probes may facilitate detection, • results should be correlated with CCA/FISH. • not recommended for follow-up or for MRD. • not designed to detect point mutations, gene expression levels, methylation anomalies, and microRNA anomalies. • Detection is affected by platform resolution, probe spacing, gene coverage, laboratory software parameters, and sample DNA quality.

Cooley LD et al. Genet Med 2013:15:484–494

May the use of arrays in the routine analysis of CLL become a common clinical practice?

prognostic

- **If it informs about the outcome independent of treatment received.**

	Prognostic	Predictive
Age	●	●
CIRS	●	●
Stage	●	○
β2-microglobulin	●	○
CD49d	●	○
CD38	●	○
ZAP70	●	○
IGHV mutation	●	●
17p13 deletion	●	●
11q22-23 deletion	●	○
trisomy 12	●	○
13q14 deletion	●	○
TP53 mutation	●	●
SF3B1 mutation	●	○
NOTCH1 mutation	●	○

CIRS: cumulative illness rating scale; *IGHV*: immunoglobulin heavy variable gene; empty circle, yes; filled circle, no.

predictive

- **if the treatment effect is different for biomarker-positive compared with biomarker-negative patients.**

Rossi et al. Leuk Lymph. 2017;58:1548-60