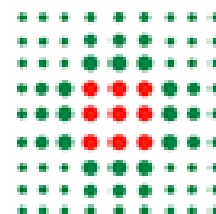


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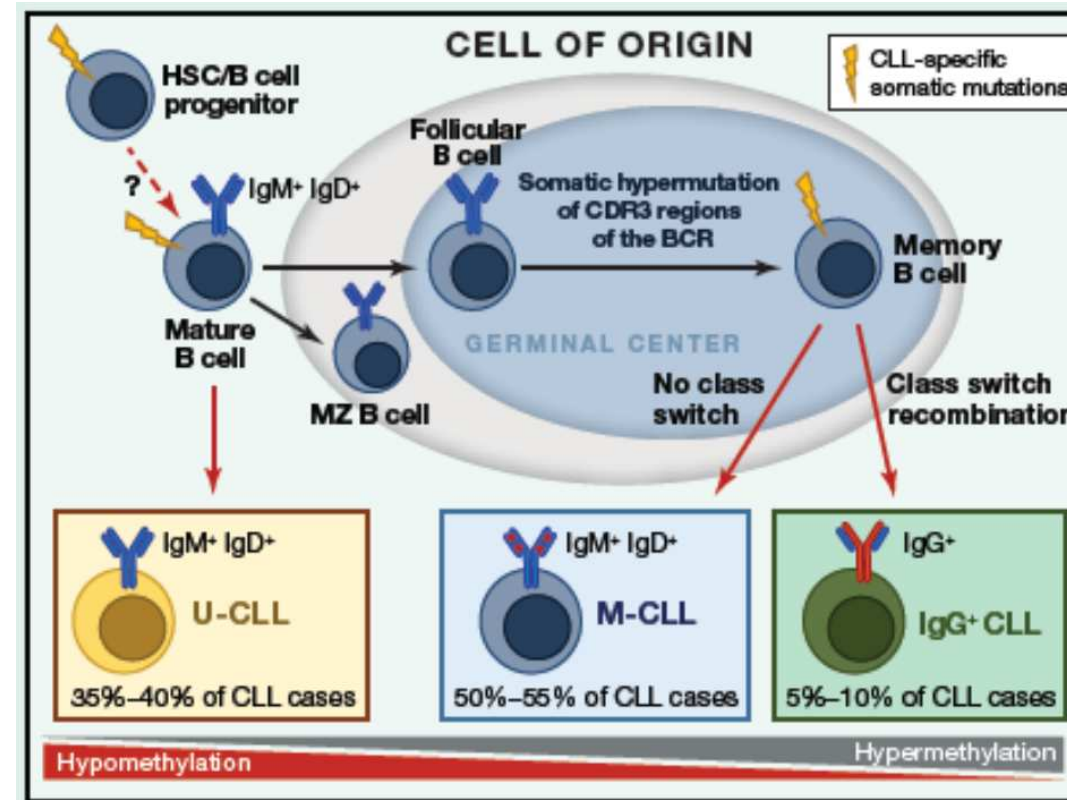
SERVIZIO SANITARIO REGIONALE  
EMILIA-ROMAGNA  
Azienda Ospedaliero - Universitaria di Ferrara

# Significato prognostico del cariotipo nella leucemia linfatica cronica

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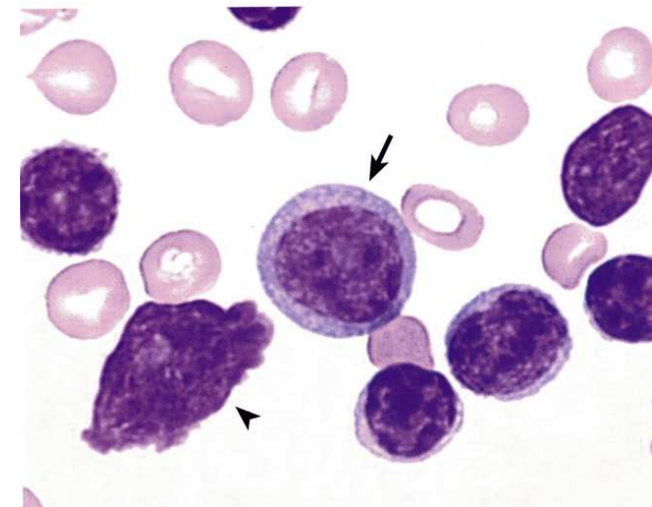
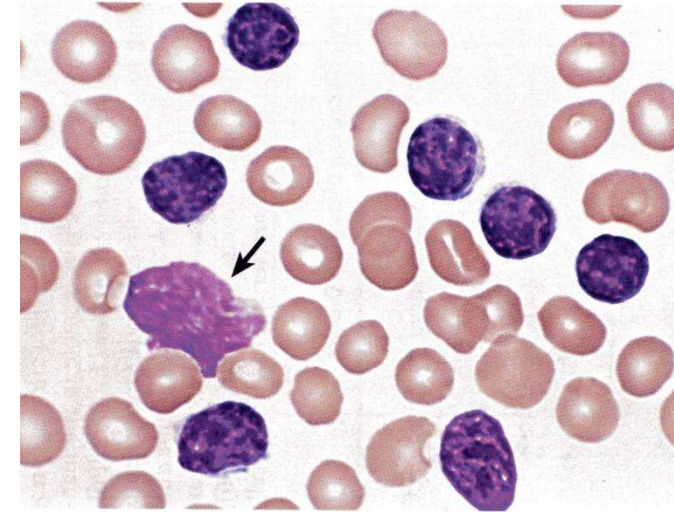
# Chronic Lymphocytic leukemia (CLL)

CLL is an indolent B-cell malignancy characterized by the clonal proliferation and accumulation of mature CD5-positive B lymphocytes within the blood, bone marrow, lymph nodes, and spleen.



# Chronic Lymphocytic leukemia

- CLL is the **most common type of leukemia** in western countries.
  - age-adjusted incidence of **4.1/100000 inhabitants**
- The median age at diagnosis **67-72 years**.
- Male-female ratio is **1.7:1**.



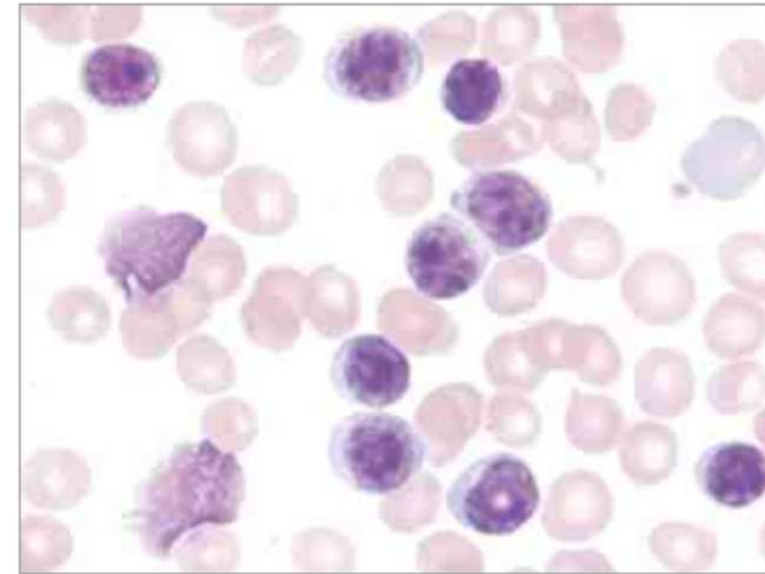
Typical and atypical CLL

Zucker-Franklin D. & Grossi CE. Atlas of blood cells. Pag 587

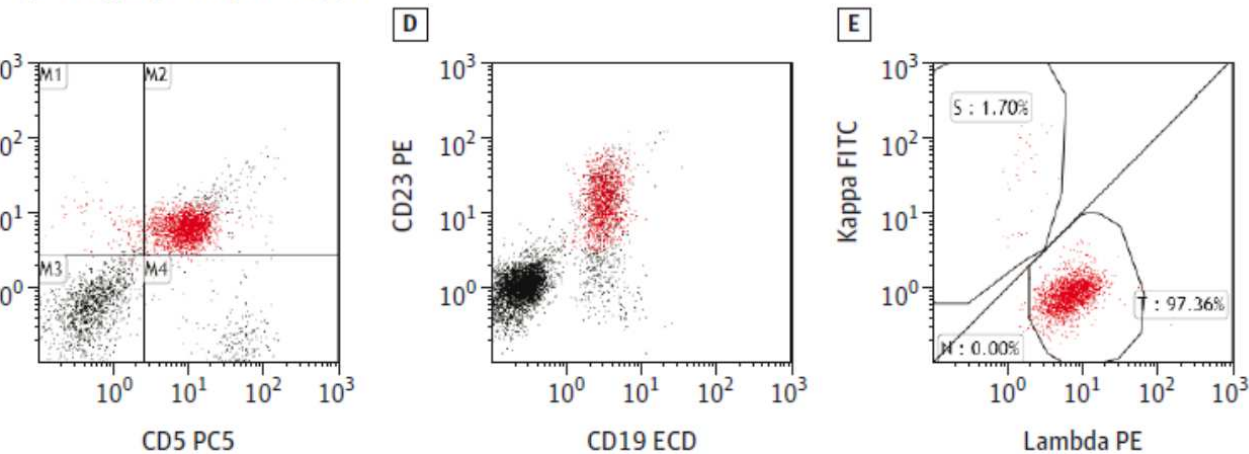
# CLL Diagnosis

presence of at least 5000 B lymphocytes/ $\mu\text{L}$  in the peripheral blood.

A Blood smear



Immunophenotyping flow cytometry plots



The clonality of the circulating B lymphocytes needs to be confirmed **by flow cytometry**.

# Clinical Presentation

- Majority of patients are **asymptomatic** at diagnosis.
- Referral is generated when **elevated WBC or lymphocyte counts, on routine blood cell counts**
- Most patients have **enlarged and palpable lymph nodes on examination**
- **Hepatosplenomegaly** (20% to 50% of patients at presentation)
- 10% of patients present **with B symptoms**
  - unexplained fevers,
  - unintentional >10% body weight loss in the preceding 6 months,
  - drenching night sweats

# CLL and clinical staging

Table 1 Rai and Binet staging systems

	Risk group	Criteria	Median survival (months)
<i>Rai stage</i> <sup>1,2</sup>			
0	Low	Lymphocytosis <sup>a</sup>	>150
I	Intermediate	Lymphocytosis + lymphadenopathy	101
II	Intermediate	Lymphocytosis + splenomegaly or hepatomegaly	71
III	High	Lymphocytosis + anemia <sup>b</sup>	19
IV	High	Lymphocytosis + thrombocytopenia <sup>c</sup>	19
<i>Binet stage</i> <sup>4</sup>			
A	Low	<3 nodal sites <sup>d</sup> involved	Not reached
B	Intermediate	≥3 nodal sites involved	84
C	High	Anemia <sup>e</sup> and/or thrombocytopenia <sup>c</sup>	24

<sup>a</sup> Absolute lymphocyte count in whole blood >5000/mm<sup>3</sup>.

<sup>b</sup> Hemoglobin <110 g/l, with or without enlargement of lymph nodes, spleen or liver.

<sup>c</sup> Platelets <100 × 10<sup>9</sup>/l, with or without anemia or enlargement of lymph nodes, spleen or liver.

<sup>d</sup> Five possible nodal sites: axillary, cervical, inguinal, spleen and liver.

<sup>e</sup> Hemoglobin <100 g/l, with or without enlargement of lymph nodes, spleen or liver.

# Prognostic vs predictive biomarkers

## Biomarker

measurement  
variable that is  
associated with disease  
outcome.

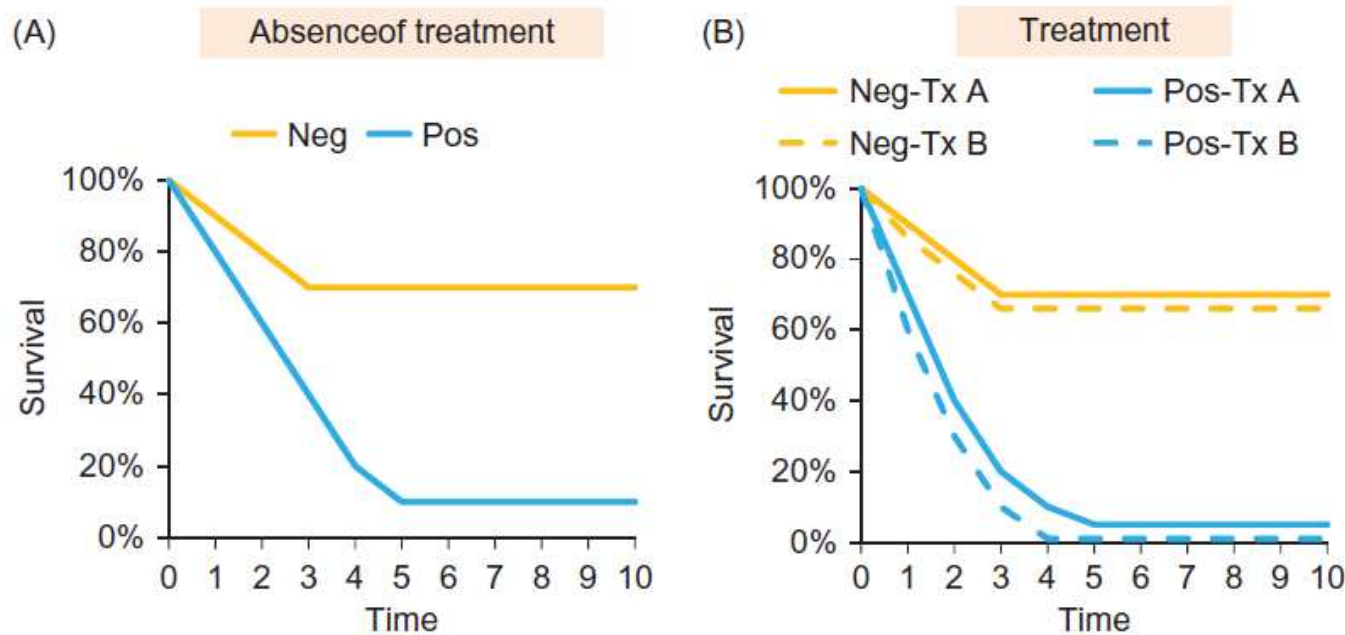
### prognostic

- If it informs about a likely cancer outcome independent of treatment received.

### predictive

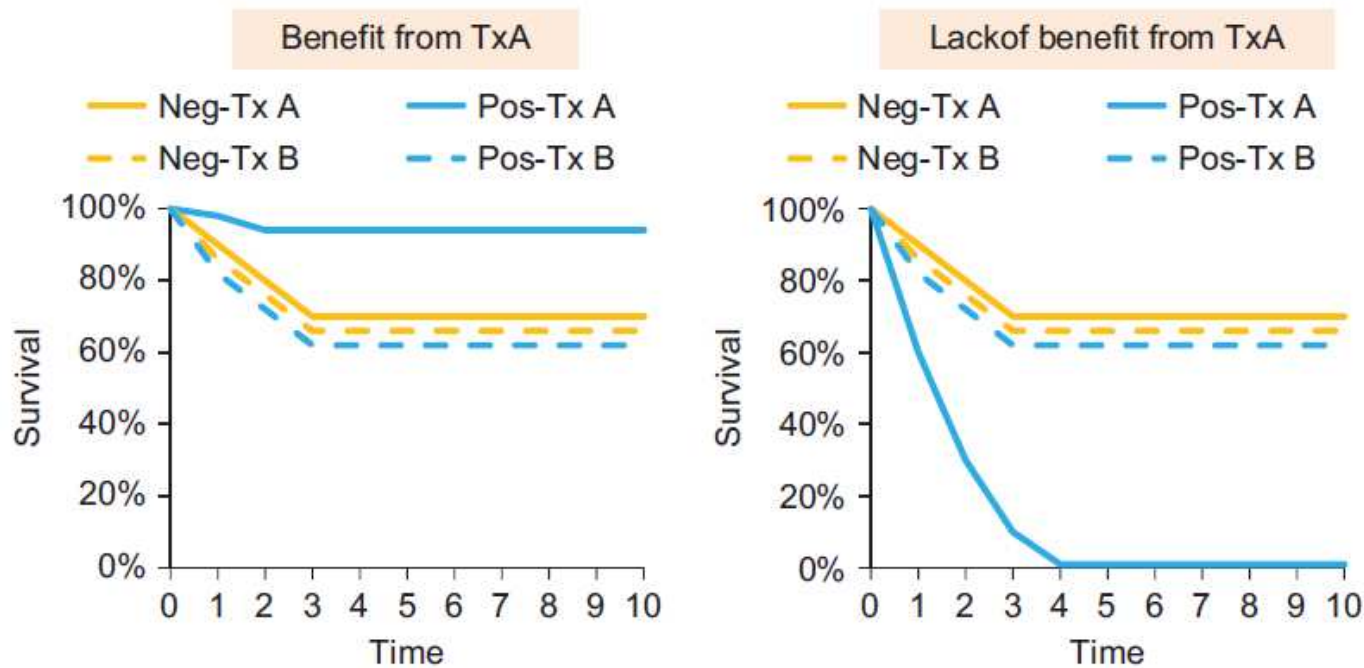
- if the treatment effect is different for biomarker-positive patients compared with biomarker-negative patients.

# Prognostic biomarker



**Figure 2.** Idealized example of a prognostic biomarker. (A) The blue line shows biomarker positive patients while the yellow line shows biomarker negative patients. (B) The dark-colored line shows biomarker positive patients while the light-colored line shows biomarker negative patients. The continuous line shows patients who received treatment (Tx) (A), while the dash line shows patients who received treatment (B). The biomarker-positive patients have a better survival than biomarker-negative patients, independent of whether they are treated (B) or not (A), and independent of the treatment group (B). The fact that the treatment effect is the same for biomarker-negative and biomarker-positive patients shows that the biomarker is not predictive.

# Predictive biomarker



**Figure 1.** Idealized example of a predictive biomarker. The solid line shows patients who received treatment (Tx) (A), while the dash line shows patients who received treatment (B). The dark-colored line shows biomarker positive (pos) patients while the light-colored line shows biomarker negative patients. There is a treatment effect only for biomarker-positive patients who received treatment (A). Hence, the treatment effect differs in quality between the biomarker-defined groups according to the type of treatment they received. This is an example of a qualitative interaction.

# Prognostic and predictive biomarkers commonly used in clinical practice.

Prognostic  
 informs about the  
 outcome independent  
 of treatment received.

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

## predictive

•if the treatment  
 effect is different  
 in biomarker-positive  
 compared with  
 biomarker-negative  
 patients.

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

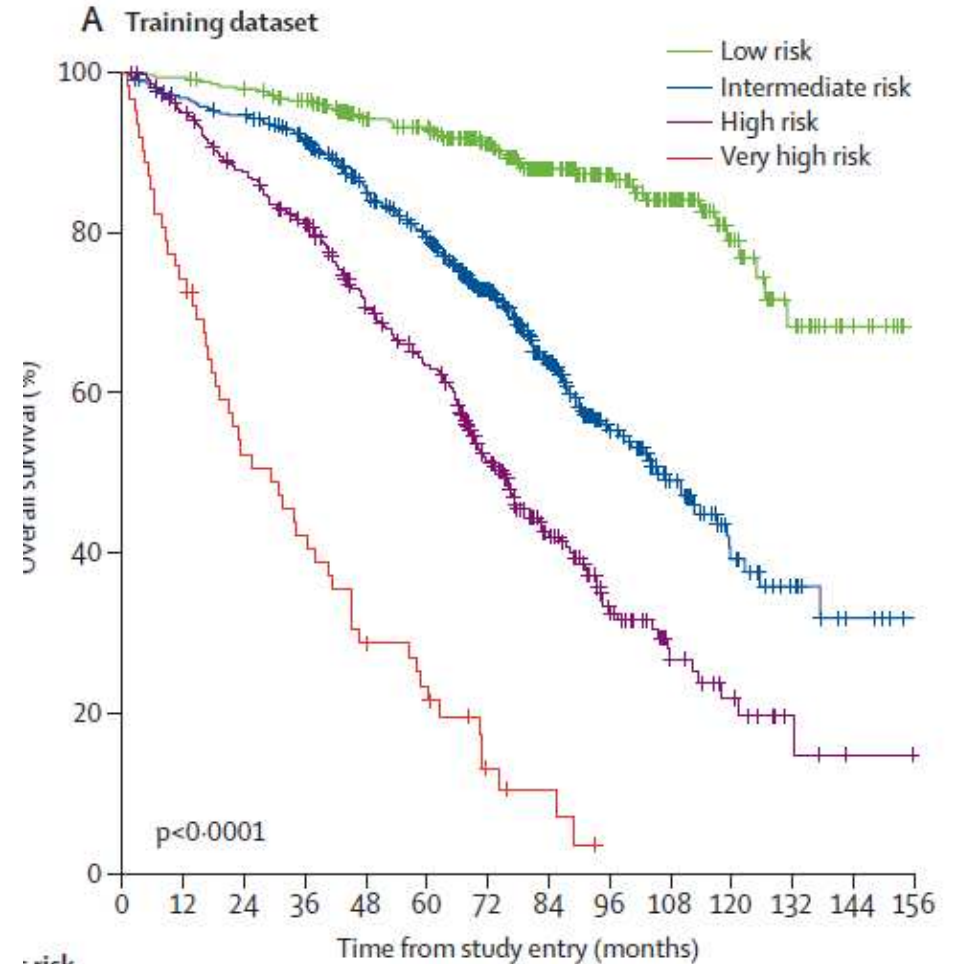
# Genetics

- **Fluorescent in situ hybridization (FISH)**
  - deletion of chromosome 13q, 11q (ATM), 17p (TP53) and trisomy 12,
- ***IGHV* mutations (different cell of origin)**
  - mutated and unmutated CLL
- **Next-generation sequencing**
  - Mutations of several genes including TP53, SF3B1, NOTCH1, ATM, BIRC3
- **Genetic and cytogenetic abnormalities identify high-risk patients and are strong biomarkers of refractory CLL**

# Comprehensive approach incorporating clinical, serum, genetic, and molecular markers into a single risk score: CLL-IPI

Variable	Adverse factor	Coeff.	HR	Grading
<i>TP53</i> (17p)	deleted and/or mutated	1.442	4.2	4
<i>IGHV</i> status	Unmutated	0.941	2.6	2
B2M, mg/L	> 3.5	0.665	2.0	2
Clinical stage	Binet B/C <u>or</u> Rai I-IV	0.499	1.6	1
Age	> 65 years	0.555	1.7	1
<b>Prognostic Score</b>				<b>0 – 10</b>

Risk group	Score	Patients N (%)	5-year OS, %
Low	0 – 1	340 (29)	93.2
Intermediate	2 – 3	464 (39)	79.4
High	4 – 6	326 (27)	63.6
Very High	7 – 10	62 (5)	23.3



The International CLL-IPI working group, *Lancet Oncol* 2016;17: 779-

# Implications of CLL-IPI

CLL-IPI may

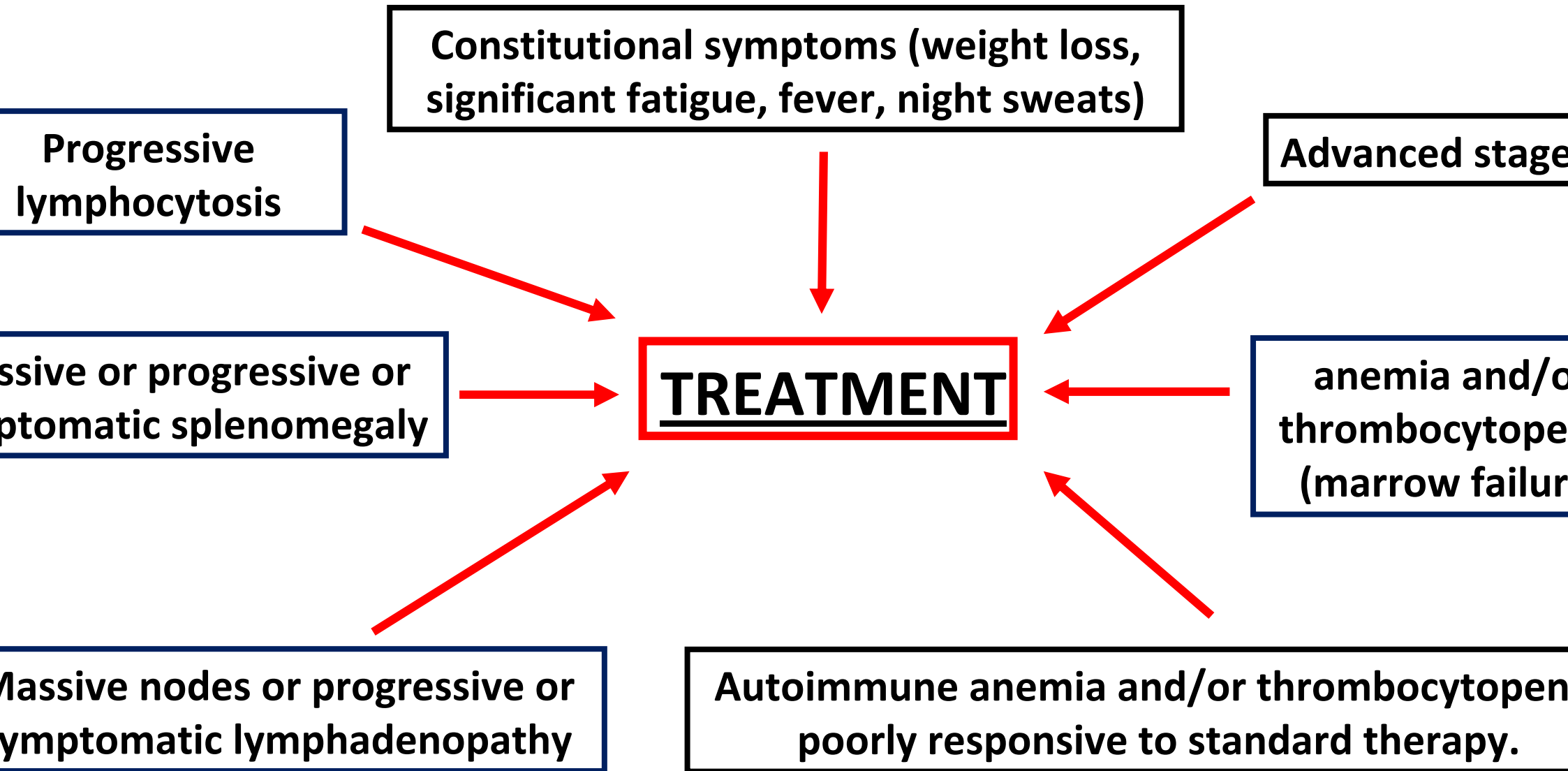
improve the precision of prognostic counselling of CLL patients.

uniform reporting of patients in clinical.

Help in the planning of treatment with more targeted and more efficacious therapies,

CLL-IPI category	OS at 5 years (%)	Potential clinical consequences
Low risk	93.2	Do not treat
Intermediate risk	79.3	Do not treat except if the disease is really symptomatic
High risk	63.3	Treatment indicated except if disease is asymptomatic
Very high risk	23.3	If you need to treat, do not use chemotherapy but rather novel agents or treatment in clinical trials.

# Indications for treatment: active disease



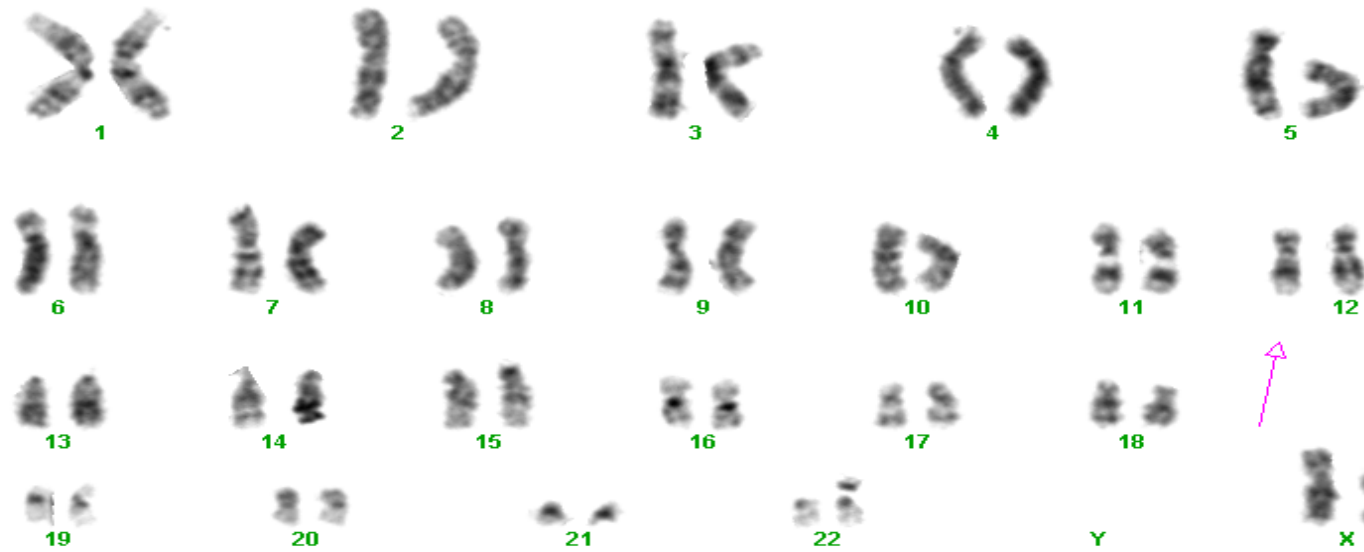
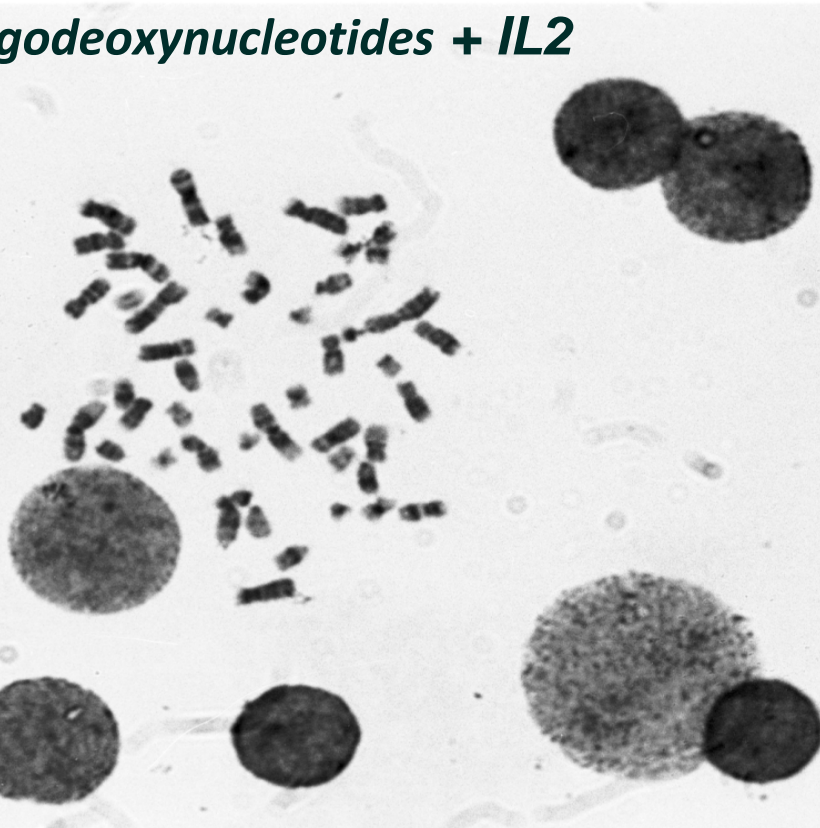
## Treatment algorithm for CLL patients

### CLL first line treatment

Stage	Fitness	del(17p) p53mut	Therapy
Binet A-B, Rai 0-II, inactive	Irrelevant	Irrelevant	None
Active disease or Binet C or Rai III-IV	Go go	No	FCR (BR above 65 years?)
		Yes	Ibrutinib, Idelalisib+Rituximab (Allogeneic SCT)
	Slow go	No	Chlorambucil + Obinutuzumab (GA-101) or + Rituximab or + Ofatumumab or Ibrutinib
		Yes	Ibrutinib, Alemtuzumab, HD Rituximab or Ofatumumab

# A second youth for conventional cytogenetic analysis

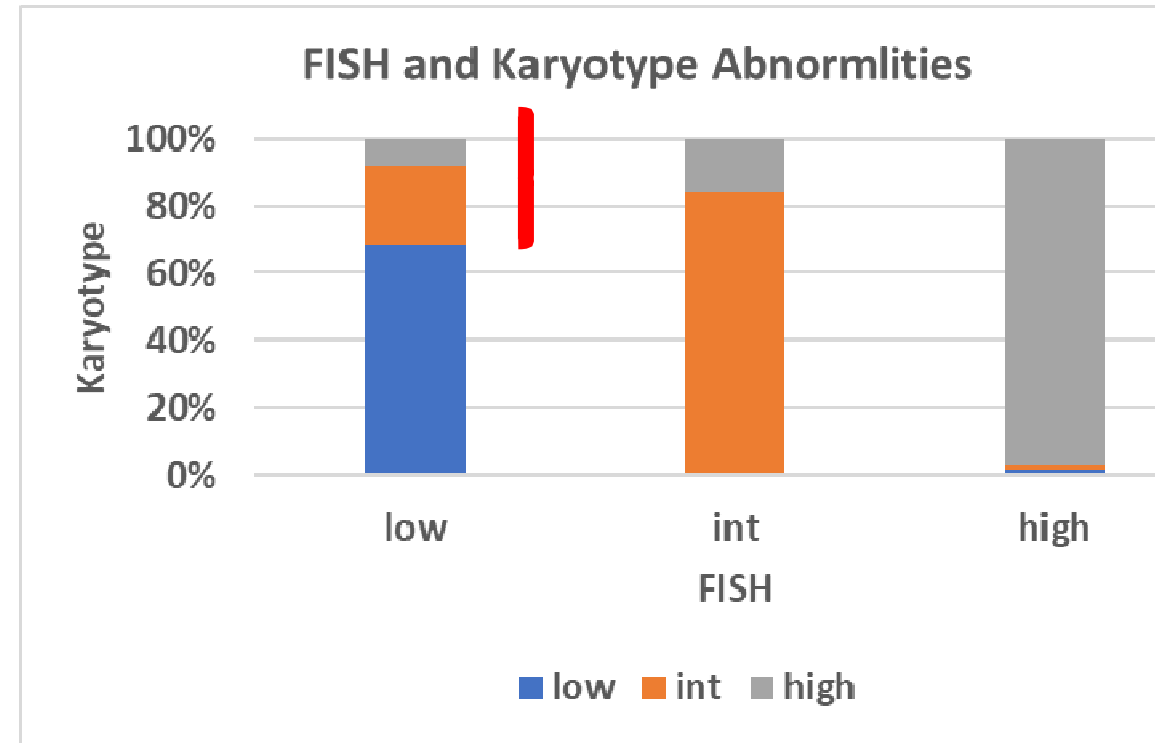
*godeoxynucleotides + IL2*



- Complete overview of chromosome lesions
- Chromosome lesions in 80% of the cases (novel effective mitogens)
- Complex Karyotype:  $\geq 3$  clonal abnormalities

# 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



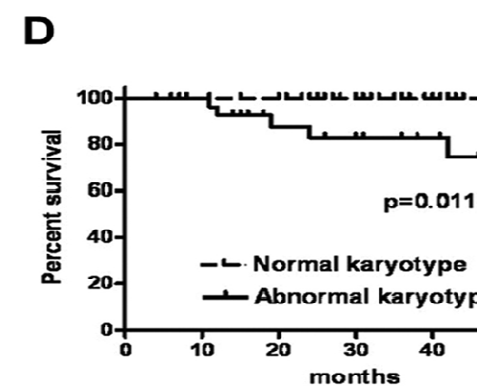
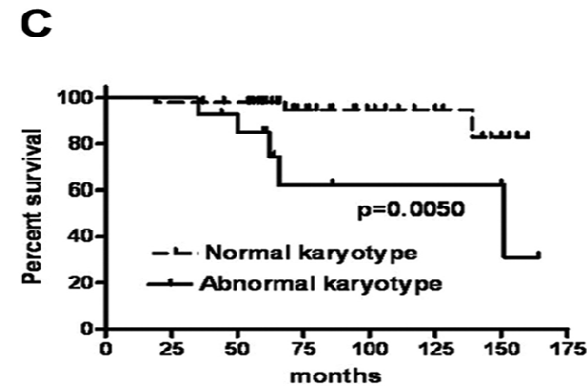
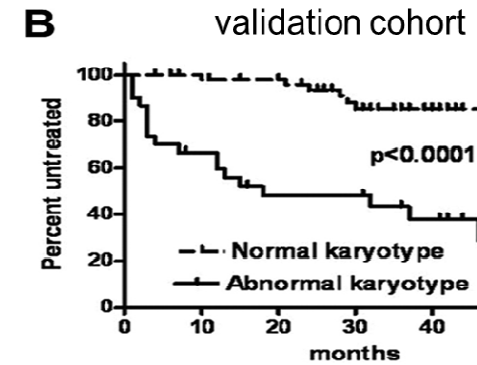
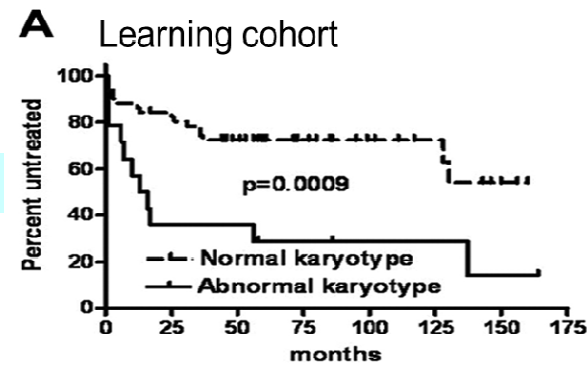
# Chromosomal abnormalities: cytogenetics vs FISH

Conventional banding analysis detected **chromosome aberrations in the karyotype in 30% of the cases without detectable aberrations by FISH**

Chromosome aberrations were associated with an **inferior outcome** at multivariate analysis

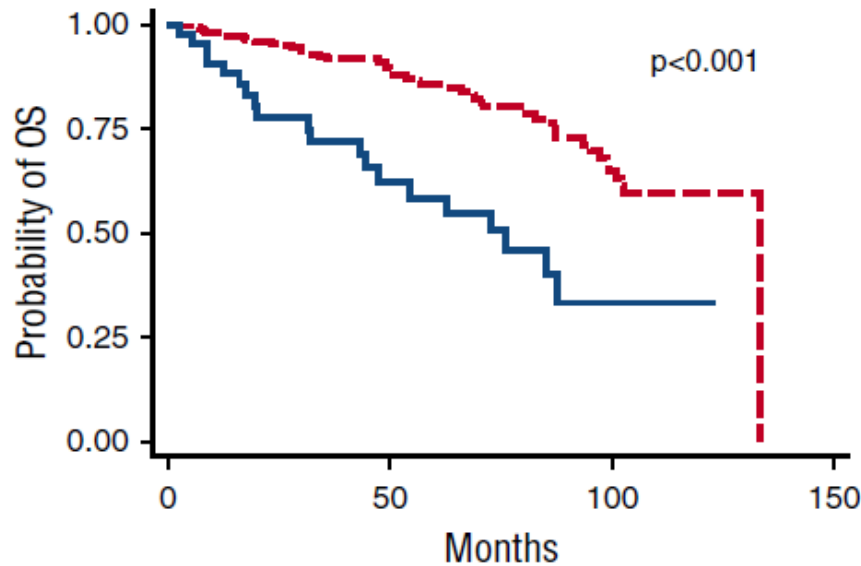
TFT

overall survival



# In CLL, the complex karyotype and comorbidities are associated with an inferior outcome independently of CLL-IPI

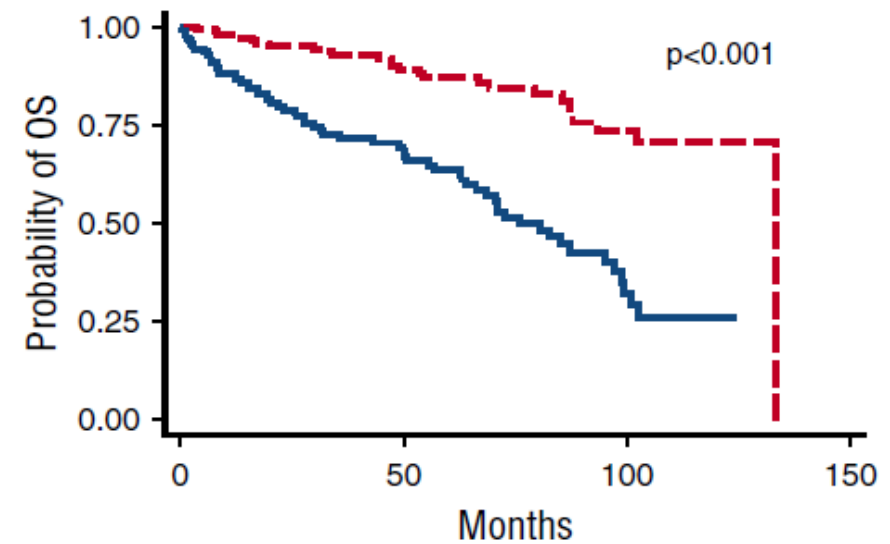
**B**



Number at risk				
	0	50	100	150
complex = no	246	124	34	0
complex = yes	41	15	4	0

--- complex = no    — complex = yes

**C**



Number at risk				
	0	50	100	150
CIRS ≤ 6	190	88	28	0
CIRS > 6	145	61	12	0

--- CIRS ≤ 6    — CIRS > 6

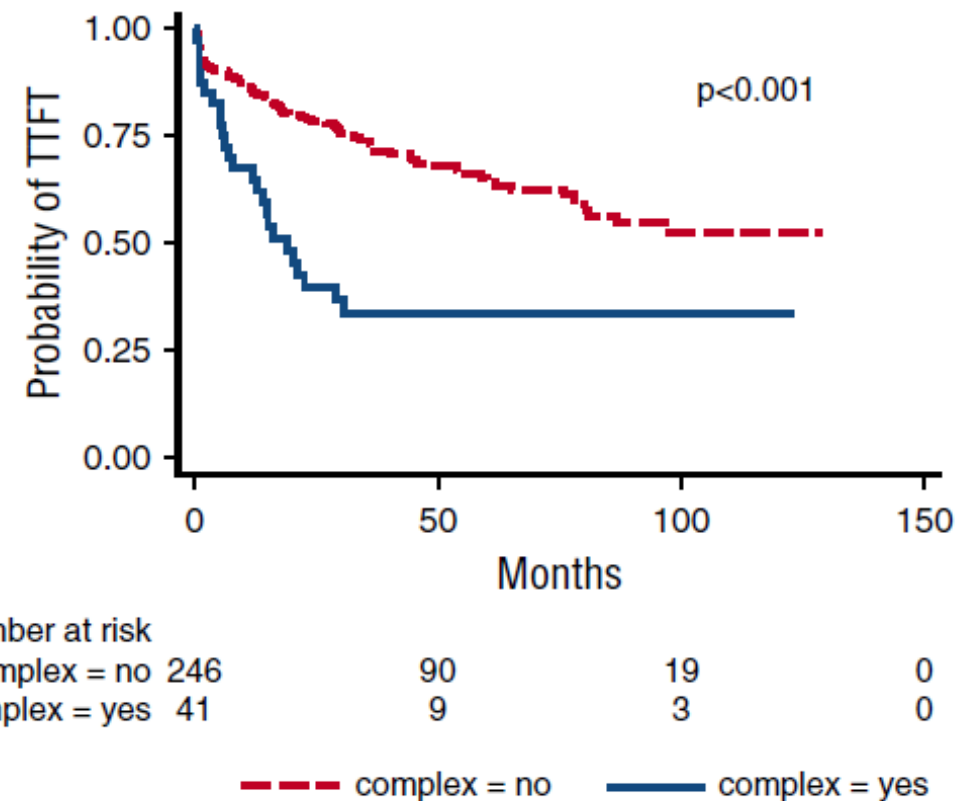
CIRS: cumulative illness rating scale

In multivariate analysis, CK ( $P = .002$ ) and CIRS score  $> 6$  ( $P = .001$ ) confirmed their negative prognostic impact on OS, independently of CLL-IPI.

## In CLL, comorbidities and the complex karyotype are associated with an inferior outcome independently of CLL-IPI

- In multivariate analysis, the CK retained its negative prognostic impact on TTFT (P =.012), independently of CLL-IPI

E



# Guideline recommendations on biomarkers

Guidelines	Diagnosis	Before treatment
EWCLL	NA	<ul style="list-style-type: none"> <li>• Stage</li> <li>• Disease activity</li> <li>• Age</li> <li>• <i>17p13</i> deletion</li> <li>• Remission duration</li> </ul>
ESMO	<ul style="list-style-type: none"> <li>• Stage</li> <li>• Disease activity</li> </ul>	<ul style="list-style-type: none"> <li>• Stage</li> <li>• Disease activity</li> <li>• Age</li> <li>• CIRS</li> <li>• <i>17p13</i> deletion</li> <li>• <i>TP53</i> mutation</li> <li>• Remission duration</li> </ul>
NCCN	<p>Essential</p> <ul style="list-style-type: none"> <li>• Stage</li> <li>• Disease activity</li> </ul> <p>Informative</p> <ul style="list-style-type: none"> <li>• <i>IGHV</i> status</li> <li>• FISH panel</li> <li>• <i>TP53</i> mutation</li> <li>• CD38</li> <li>• ZAP70</li> </ul>	<ul style="list-style-type: none"> <li>• Stage</li> <li>• Disease activity</li> <li>• Age</li> <li>• CIRS</li> <li>• <i>17p13</i> deletion</li> <li>• 11q22-23 deletion</li> <li>• <i>TP53</i> mutation</li> </ul>
ESMO	<ul style="list-style-type: none"> <li>• Stage</li> <li>• Disease activity</li> </ul>	<ul style="list-style-type: none"> <li>• Stage</li> <li>• Disease activity</li> <li>• Age</li> <li>• CIRS</li> <li>• <i>IGHV</i> status</li> <li>• <i>17p13</i> deletion</li> <li>• <i>TP53</i> mutation</li> <li>• Remission duration</li> </ul>

EWCLL: International Workshop on Chronic Lymphocytic Leukemia; ESHO: European Society of Hematology; British Committee for Standards in Hematology; NCCN: National Comprehensive Cancer Network; ESMO: European Society for Medical Oncology; CIRS: cumulative illness rating scale; *IGHV*: immunoglobulin heavy variable gene; FISH panel, 13q14 deletion, trisomy 12, 11q22-23 deletion, 17p13 deletion.

**Cytogenetic analysis with complex karyotype?**

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