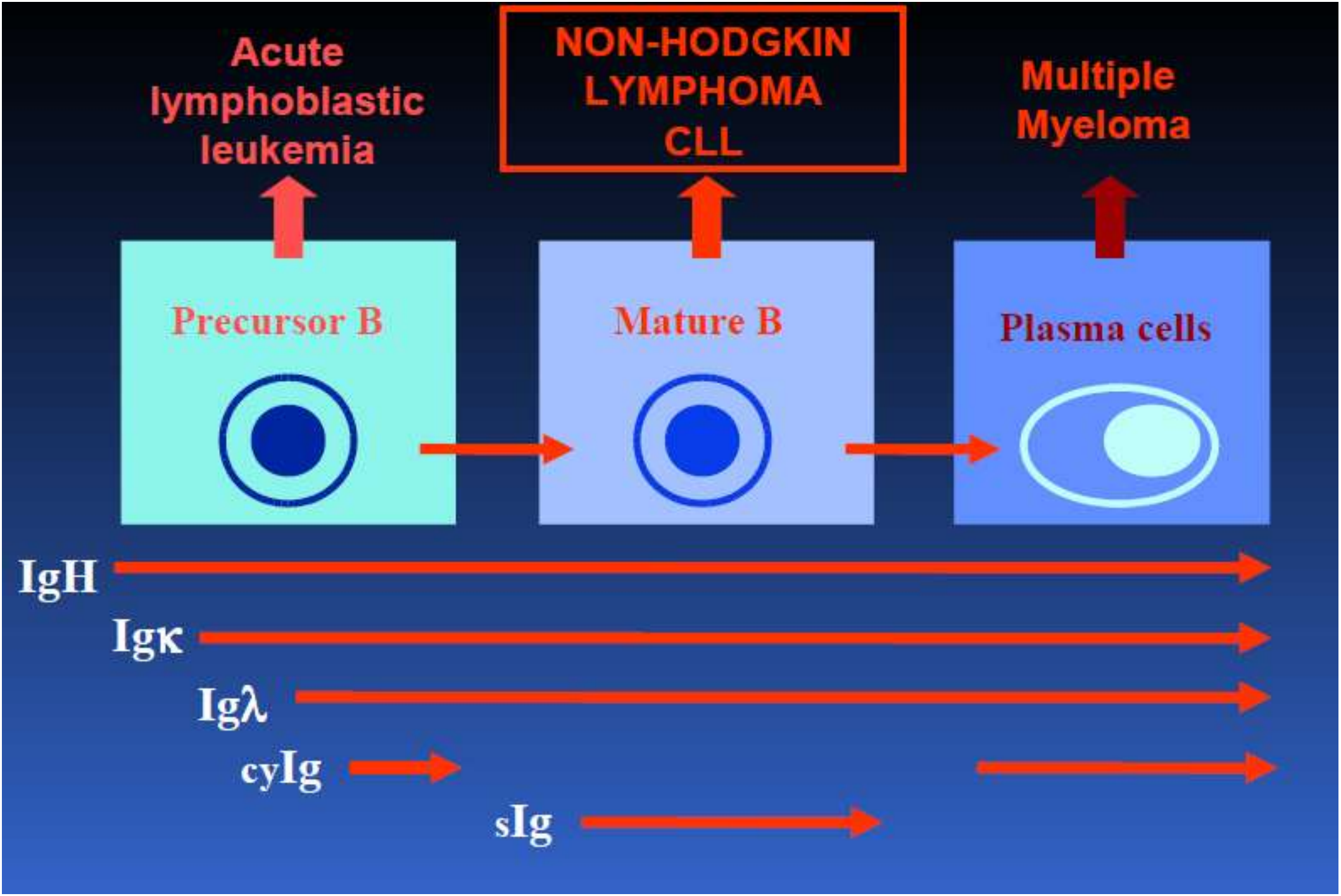
Sindromi linfoproliferative croniche

	Clinica	Morfologia	Fenotipo	Cariotipo
LLC	↑ ly- lfn- M-F	linfociti maturi	CD5+ CD23+ Sig -/+ FMC7-	13q14, 11q- +12, 17p13
aLLC	↑ ly- lfn- M-F	PLL 10-50%	CD5+ CD23+ Sig -/+ FMC7-	
PLL	↑↑ ly-M	PLL > 50%	Sig+ FMC7+ CD5-/+ CD22+	
HCL	Citopenia, ↑ M	tricoleucociti	CD103+ FMC7+ CD22+	
HCL-v	↑ ly-lfn-M	tricoleucociti at.	CD103+ FMC7+ CD22+	
SLVL	↑ ly-M	linfociti villosi	CD22+	
Marginale	LNH	Cc.	CD5- CD10-	+3, +18, 1
Immunoc.	CM,↑ M	Ly + ly plc + plc	CD5- CD23-	t(9;14)
Follicolare	LNH	CB-cc	CD10+/- Sig+ bcl-2+	t(14;18)
Mantellare	LNH	Cc	CD5+ CD23- Sig + FMC7+	t(11;14)
LGL-L	↑ ly, ↓ N	ly granulare	CD3+ CD8+ CD16+	
MF/SS	Cute, ↑ ly	nucleo cerebrif.	CD 4 +	


Acute lymphoblastic leukemia
NON-HODGKIN LYMPHOMA CLL
Multiple Myeloma
Precursor B
Mature B
Plasma cells
IgH
Igκ
Igλ
cyIg
sIg

Sindromi linfoproliferative croniche

Caratteristiche comuni

Espansione della malattia più per accumulo che per attiva replicazione

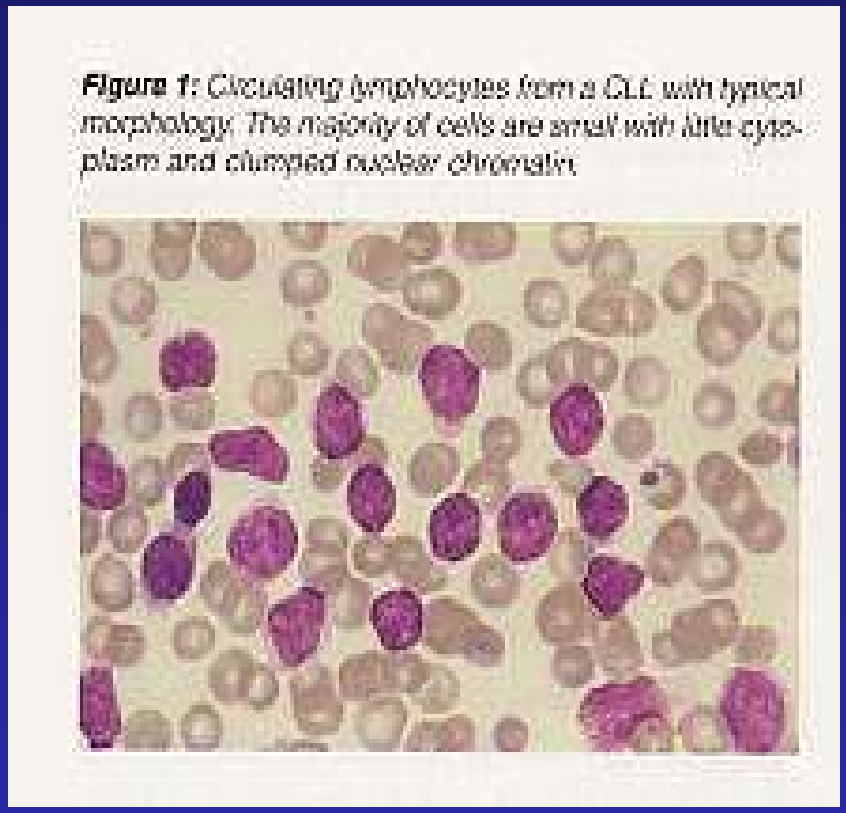
Cellule arrestate in fase G0 del ciclo cellulare per meccanismo di inibizione dell'apoptosi

Decorso spesso indolente

Difficile eradicazione

Possibilità di trasformazione verso una forma più aggressiva

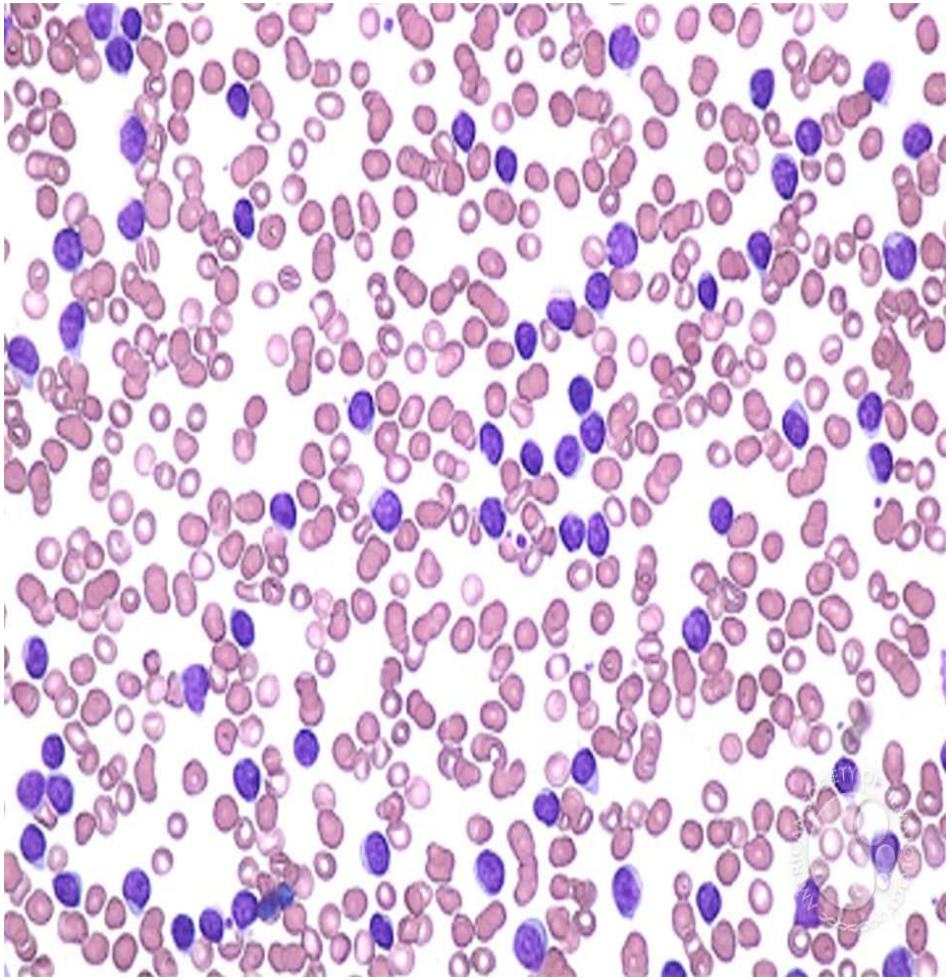
LEUCEMIA LINFATICA CRONICA

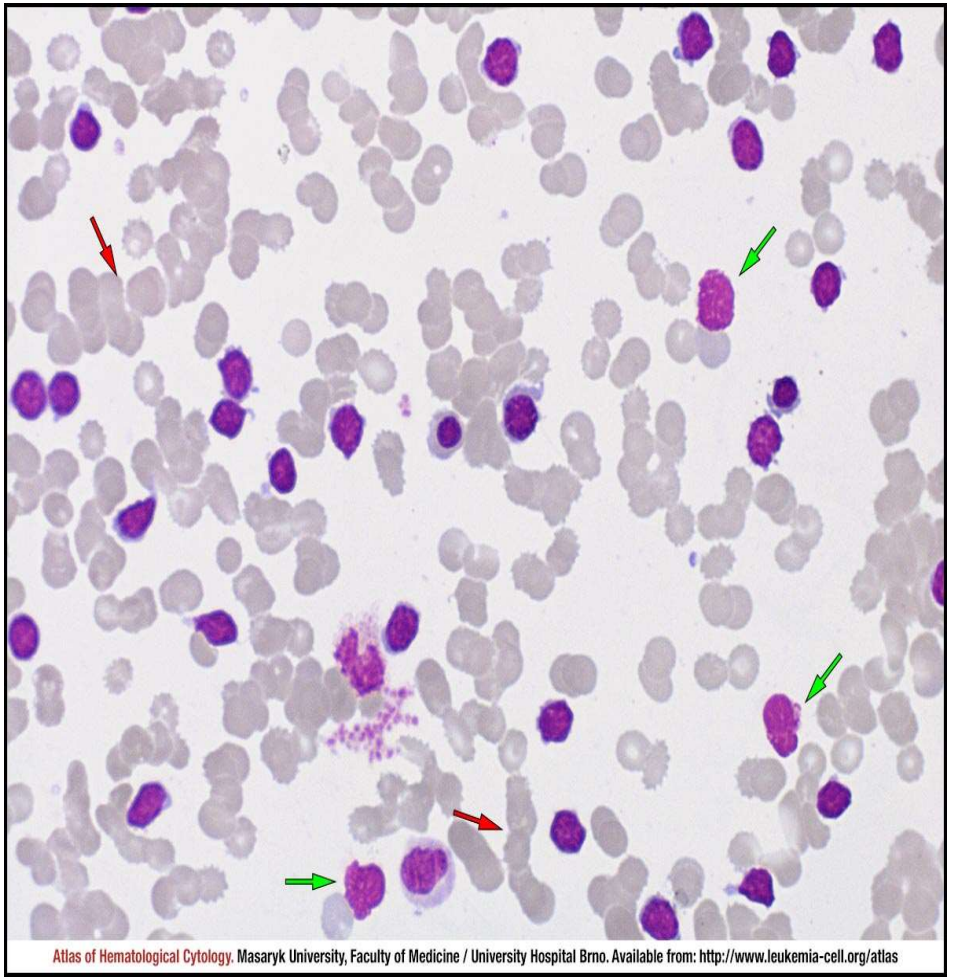


Definizione

La LLC è una neoplasia ematologica caratterizzata dalla proliferazione ed accumulo nel sangue, midollo e tessuti linfatici, di piccoli linfociti apparentemente maturi di origine B-linfocitaria.

CLL: blood smear





In blood smear an high percentage of **small**, mature **lymphocytes can be detected,** as well as, **Gumprecht** shadows

CLL EPIDEMIOLOGY

At diagnosis

Median Age 72 years

Incidence 4/100.000/year

M:F = 2:1

White:Black:Asian = 4.5:3.3:0.9

CLL – ETIOLOGY

The cause of CLL is unknown

There is increased incidence in farmers, rubber manufacturing workers, asbestos workers, and tire repair workers

Genetic factors have been postulated to play a role in high incidence of CLL in some families

Cytogenetics

- clonal chromosomal abnormalities are detected in approximately 50% of CLL patients
- the most common clonal abnormalities are:
  - trisomy 12

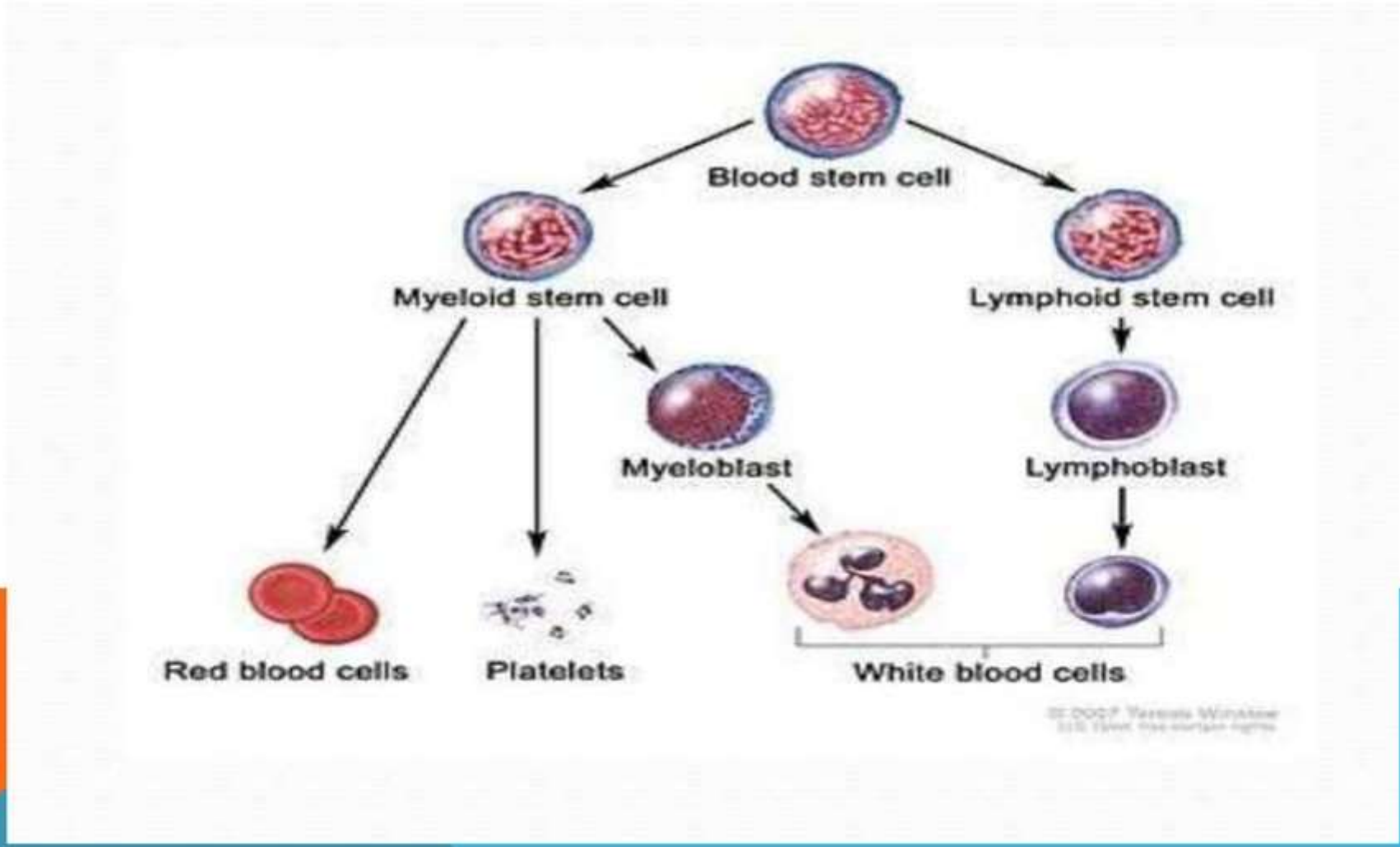
    structural abnormalities of chromosomes 13, 14 and 11

  patients with abnormal karyotypes have a worse prognosis

Oncogenes

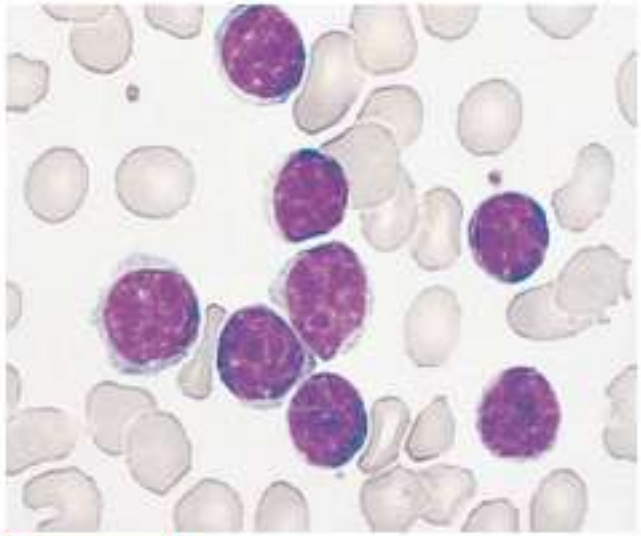
- in most cases of CLL is overexpressed the proto-oncogene c-fgr 9a member of the src gene family of tyrosine kinases

PATHOPHYSIOLOGY

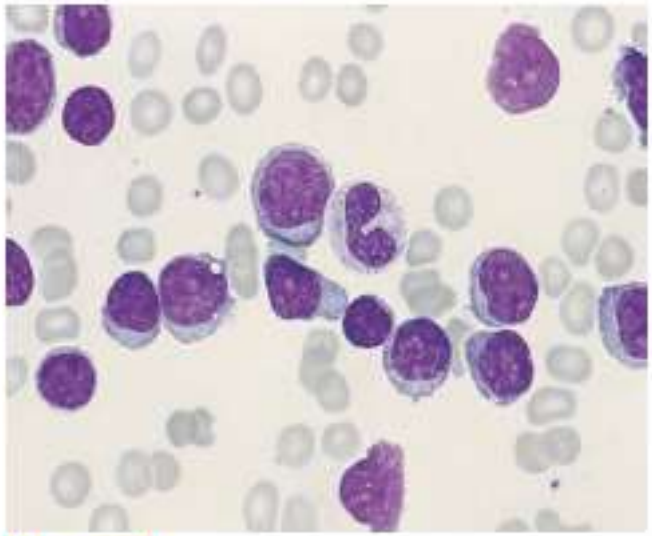


CRITERI DIAGNOSTICI PER LA DIAGNOSI DI B-LLC

- Linfociti B clonali > 5.000/mcL (se <5000/mcL in assenza di adenopatie o organomegalie: MBL)
- Presenza variabile di prolinfociti e/o di linfociti polimorfi fino al 55% e delle cosiddette "ombre di Gumprecht"
- Immunofenotipo caratteristico (CD5+/CD19+, CD23+, SmIg clonali a bassa intensità)



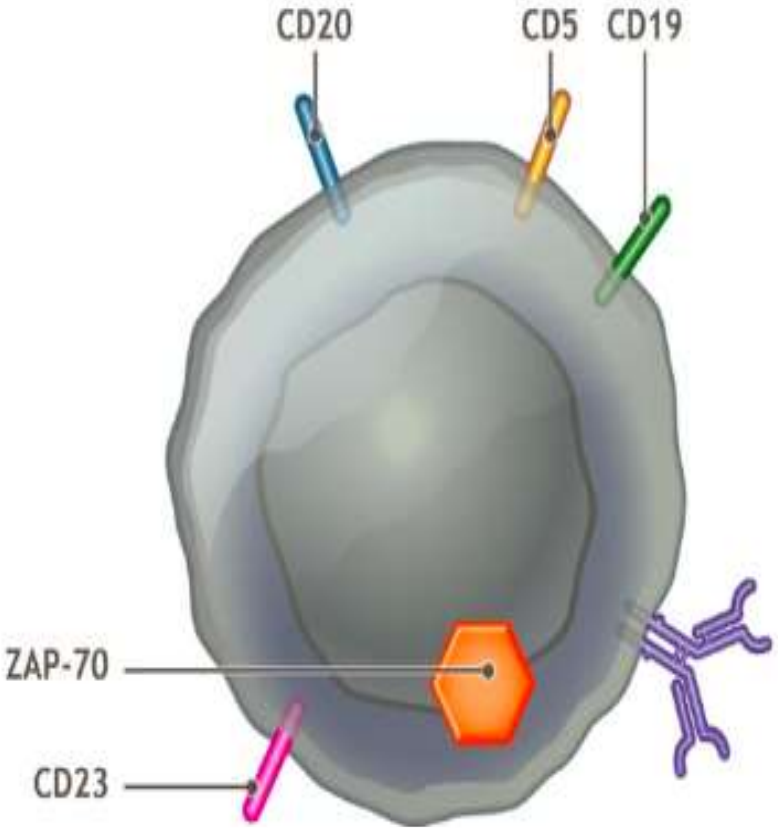
Classica



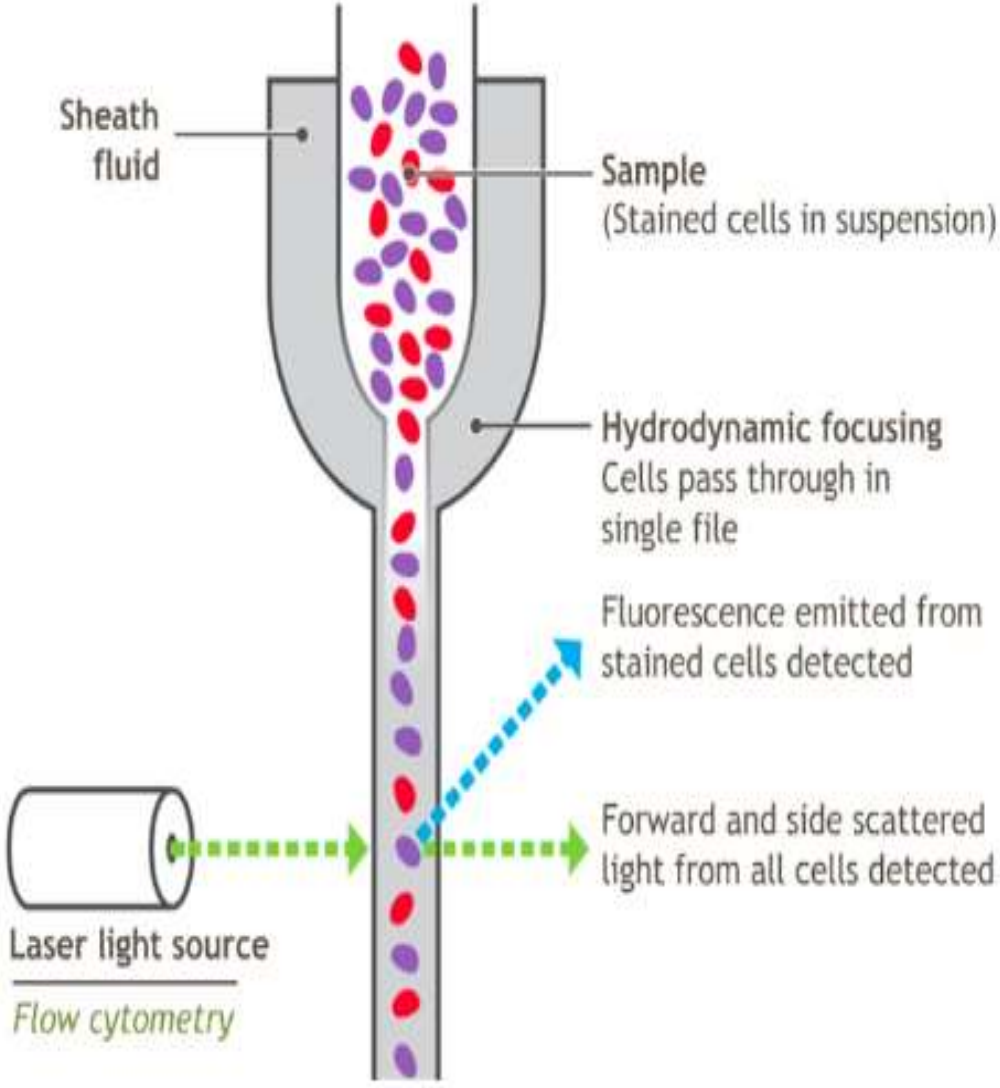
Mista

Immunophenotyping and flow-cytometry

CLL cells express the surface T-cell antigen **CD5** as well as othe B-cell antigens, including **CD19**, **CD20**, **CD23**, **ZAP70**.
Immunophenotyping allows the identification of the antigens expressed by cells

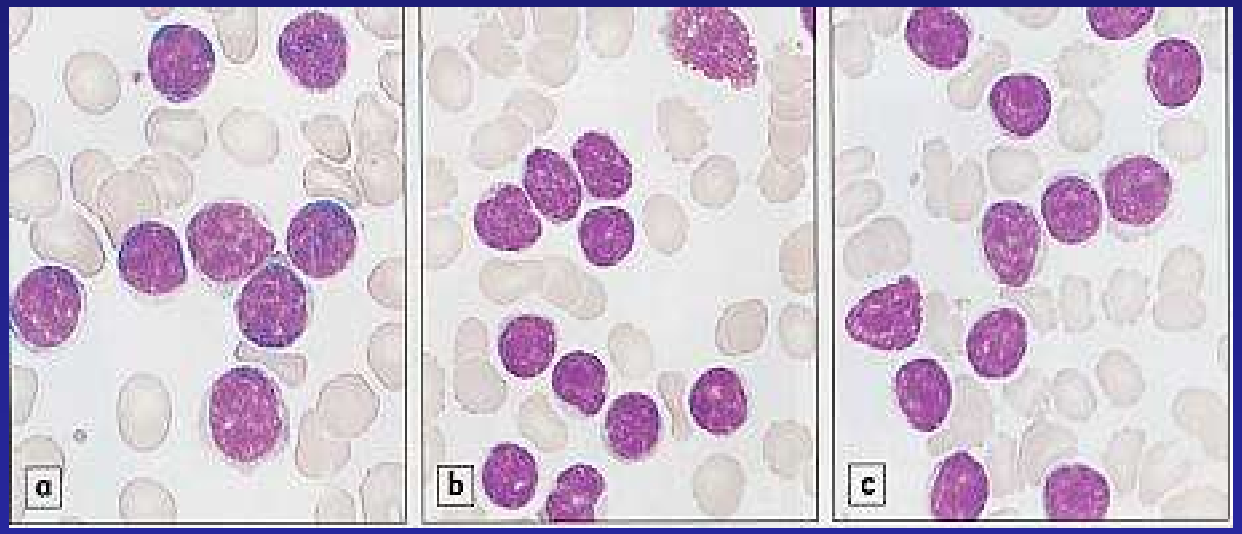


Flow-cytometry allows confirmation of the **clonality** of the B cells in peripheral blood and represent the most useful test for a definitive diagnosis of CLL

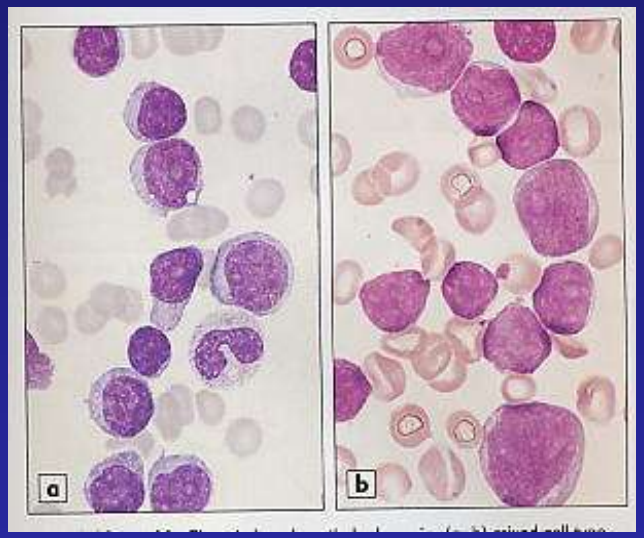


LEUCEMIA LINFATICA CRONICA

Laboratorio:sangue periferico



Morfologia tipica



Morf. atipica (PLL 10-50%)

	SIg	CD 5	CD 23	FMC7	CD 22	CD 79b
LLC	weak	+CD19 ++	++	-/+	weak/-	weak/-
aLLC	weak	++	++	-/+	weak/-	weak/-

CLL – LAB FINDINGS

a) Blood test lymphocytosis ≥ 5G/l (4 weeks)

b) Morphology monoconal population of small mature lymphocyte

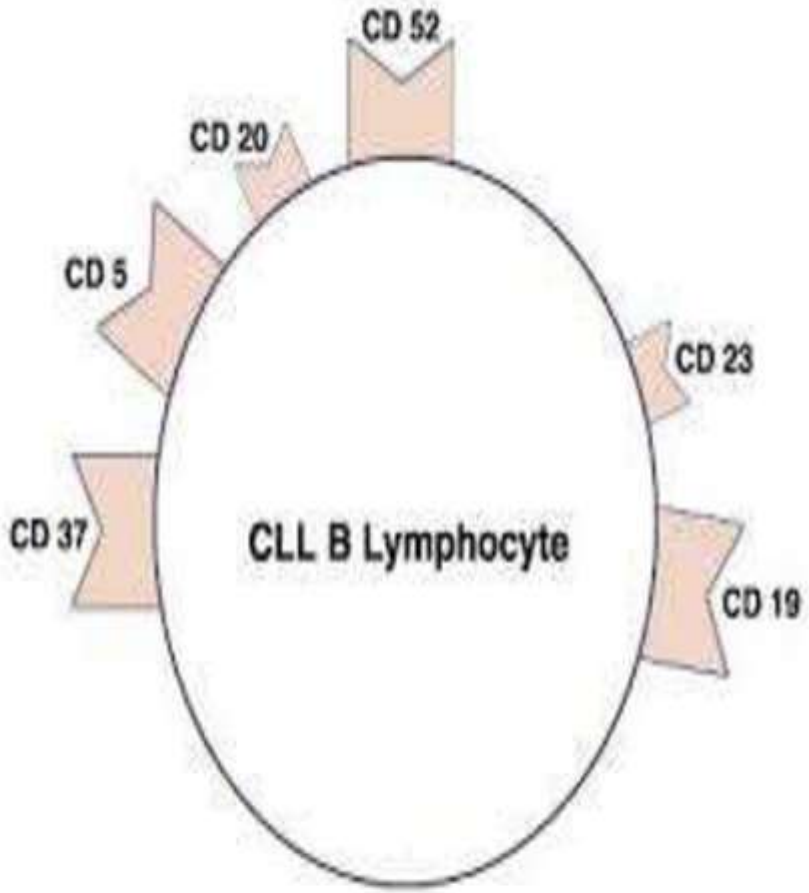
c) B-cell CLL phenotype clonal CD5+/CD19+ population

of lymphocyte

d) Markers of clonality κ/λ light chain restriction; cytogenetical abnormalities

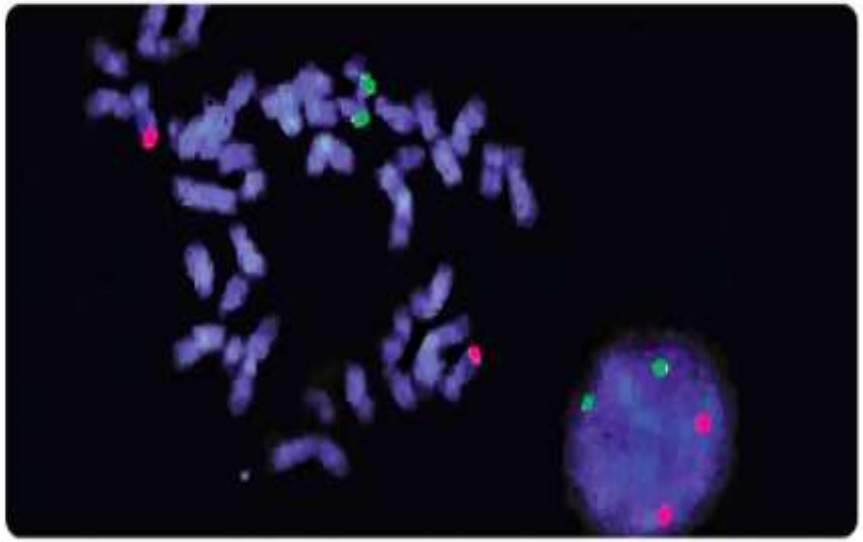
e) Bone marrow infiltrate > 30% of nuceated cells on aspirate

f) Lymph node diffuse infiltrate of small lymphocye

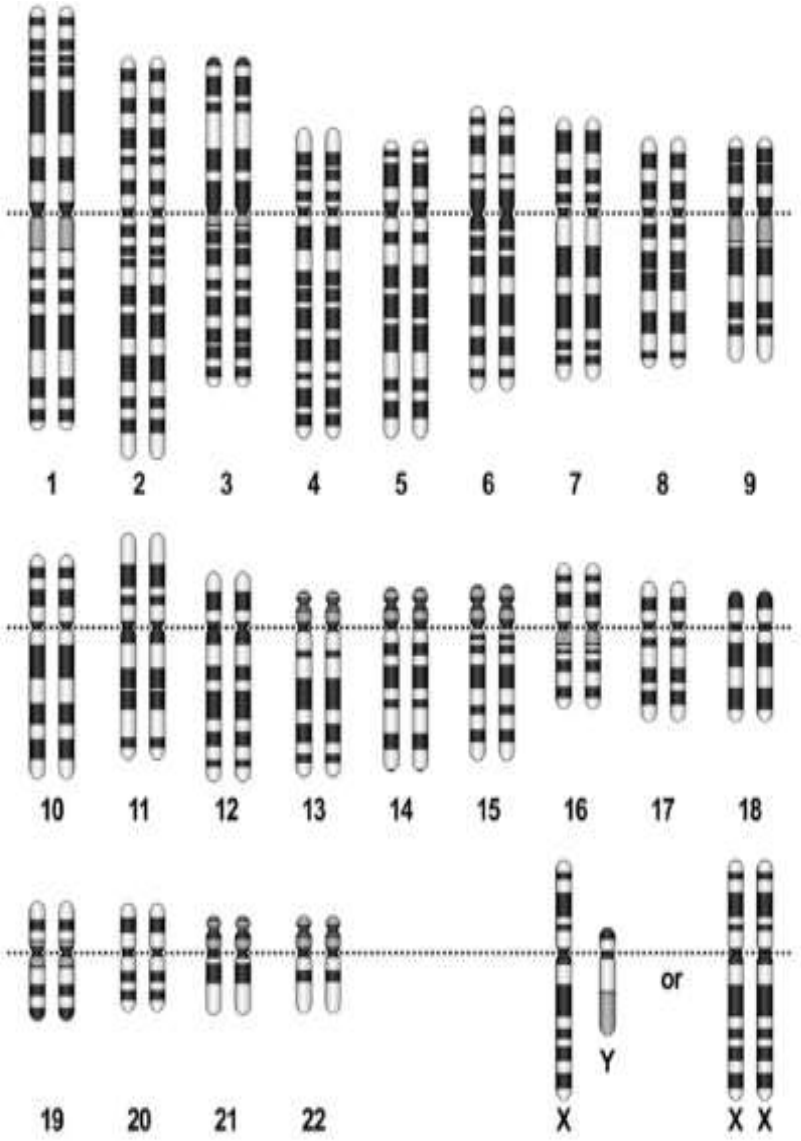


Fluorescence in situ Hybridization

FISH is a **cytogenetic technique** that uses microscopy and relies on fluorescent probes to detect the presence of specific DNA sequences on chromosome.
It does **not require** the cells to be **dividing**. This allows a more sensitive technice than chromosomal banding. **Chromosomal translocation** and **deletions** can be therefore easily identified and monitored during the course of a disease



FISH



Chromosomal banding

Incidence of cytogenetical abnormalities

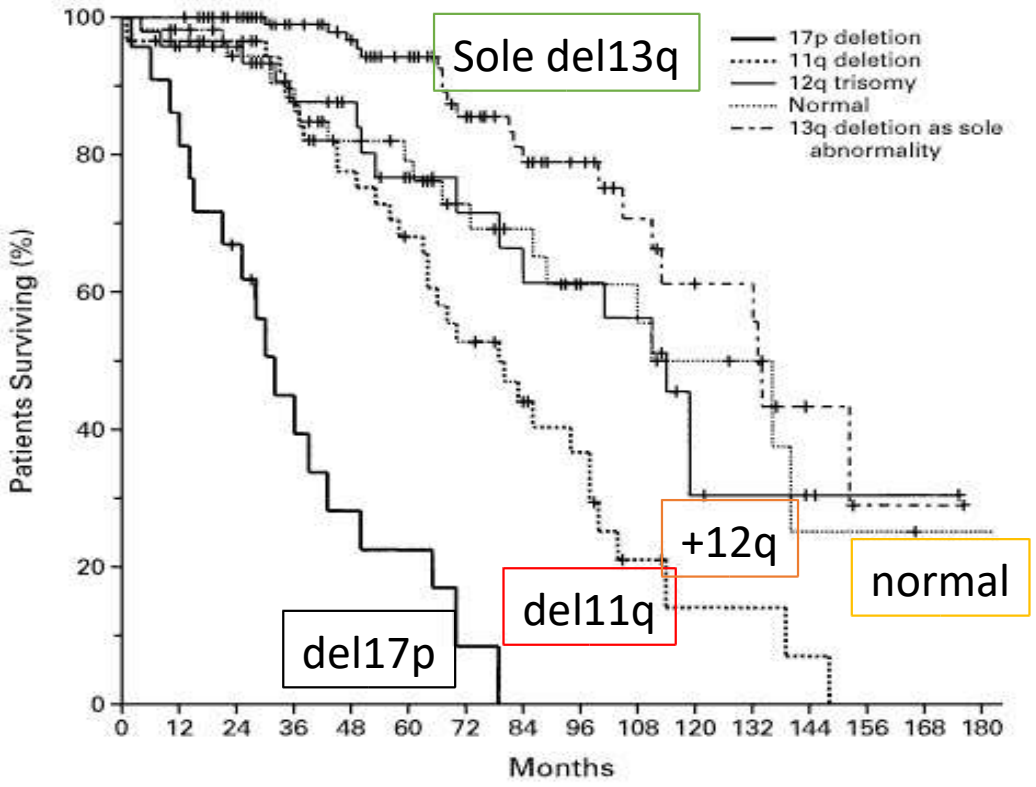
TABLE 1. INCIDENCE OF CHROMOSOMAL ABNORMALITIES IN 325 PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

ABERRATION	NO. OF PATIENTS (%)*
13q deletion	178 (55)
11q deletion	58 (18)
12q trisomy	53 (16)
17p deletion	23 (7)
6q deletion	21 (6)
8q trisomy	16 (5)
t(14q32)	12 (4)
3q trisomy	9 (3)
Clonal abnormalities	268 (82)
Normal karyotype	57 (18)

*One hundred seventy-five patients had one aberration, 67 had two aberrations, and 26 had more than two aberrations.

82% Clonal chromosomal abnormalities

35% >1 clonal alteration

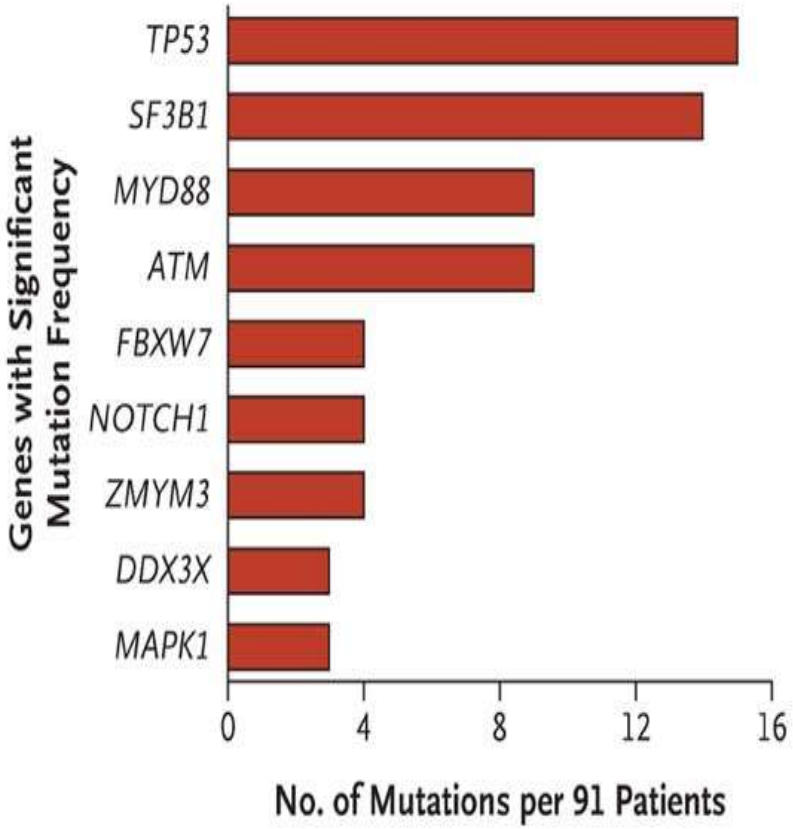


NO. AT RISK

17p deletion	23	18	13	8	5	4	1	0	0	0	0	0	0	0	0	0
11q deletion	56	53	47	43	33	27	20	15	10	4	2	2	1	0	0	0
12q trisomy	47	44	41	29	24	17	14	13	12	11	4	3	2	1	1	0
Normal	57	51	45	37	30	27	20	17	12	11	6	5	2	2	1	1
13q deletion as sole abnormality	117	117	106	91	80	63	45	36	24	16	12	11	3	1	1	0

Dohner H et al. ***N Engl J Med 2000***; 343:1910-1916

Molecular landscape in CLL



	No. of Mutations (%)	No. of Base Pairs	P Value	Q Value
TP53	15 (15)	119,041	$<1.0\times10^{-11}$	$<6.3\times10^{-8}$
SF3B1	14 (15)	359,856	$<1.0\times10^{-11}$	$<6.3\times10^{-8}$
MYD88	9 (10)	73,805	$<1.0\times10^{-11}$	$<6.3\times10^{-8}$
ATM	9 (9)	837,986	2.4×10^{-9}	1.1×10^{-5}
FBXW7	4 (4)	225,671	2.0×10^{-6}	7.4×10^{-3}
NOTCH1	4 (4)	306,968	3.3×10^{-6}	1.0×10^{-2}
ZMYM3	4 (4)	314,226	3.5×10^{-5}	7.4×10^{-2}
DDX3X	3 (3)	181,343	1.6×10^{-5}	4.3×10^{-2}
MAPK1	3 (3)	89,405	1.9×10^{-5}	4.4×10^{-2}

Wang L et al. ***N Engl J Med 2011***;365:2497-2506

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LEUCEMIA LINFATICA CRONICA

Aspetti clinici

- Linfocitosi in presenza o meno di linfoadenomegalie, epato-splenomegalia; sviluppo di anemia, piastrinopenia, ipogamma-globulinemia
- Diagnosi spesso occasionale
- Decorso spesso indolente ma progressivo
- Morbidità legata allo sviluppo di insufficienza midollare ed immunodeficit
- Principale causa di mortalità: infezioni

CLL – INITIAL SYMPTOMS

Approximately 40% are asymptomatic at diagnosis – discovered by a CBC

In symptomatic cases the most common complaint is fatigue

Well's syndrome – increase sensitivity to insects bites

B symptoms – fever, sweats, weight loss

Less often the initial complaint are enlarged nodes or the development of an infection (bacterial)

CLL - Clinical findings

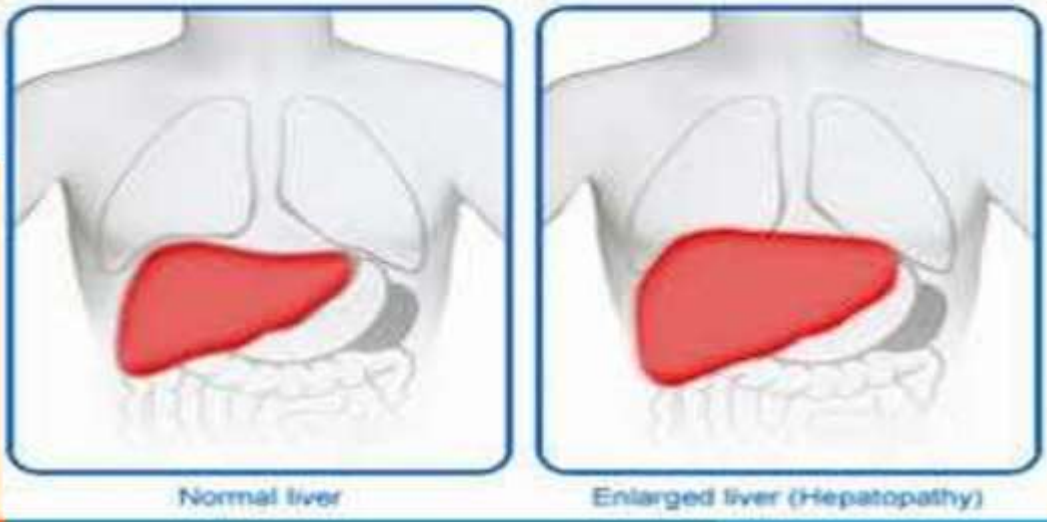
Most symptomatic patients have enlarged lymph nodes (more commonly cervical and supraclavicular) and splenomegaly

The lymph nodes are usually discrete, freely movable, and nontender

Hepatomegaly may occure

Less common manifestation are infiltration of tonsils, mesenteric or retroperitoneal lymphadenopathy, and skin infiltration

Patients rarely present with features of anemia, and bruising or bleeding



LEUCEMIA LINFATICA CRONICA

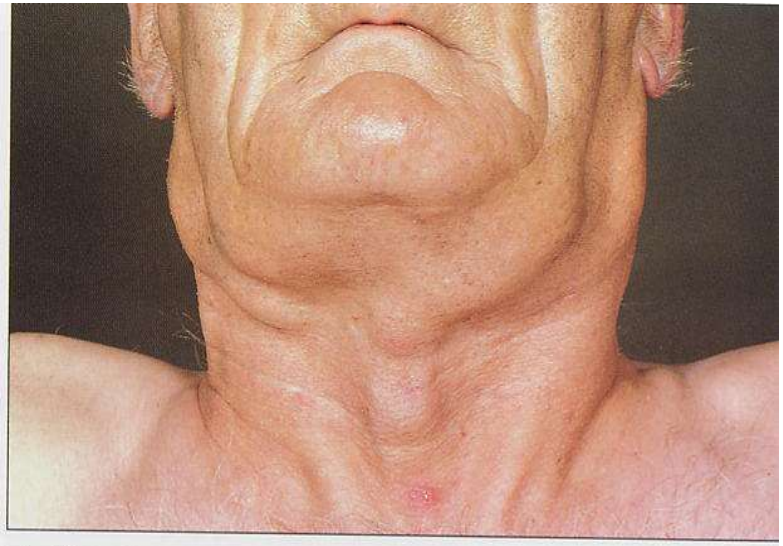
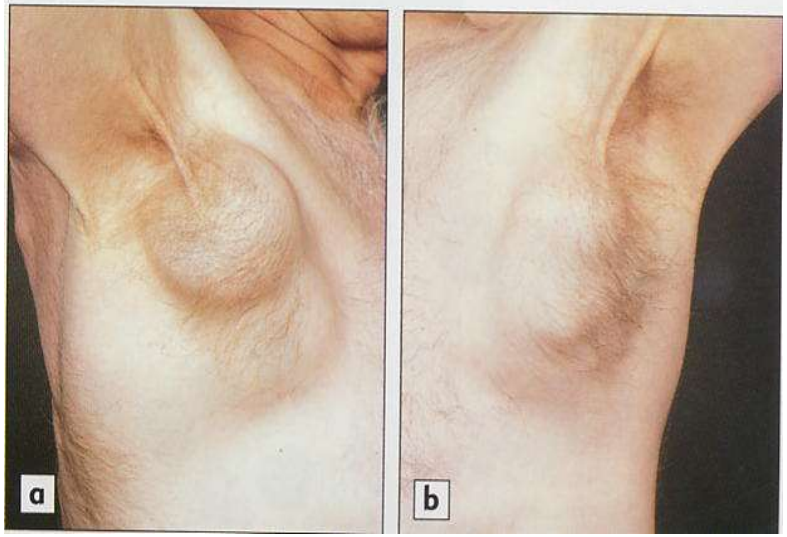
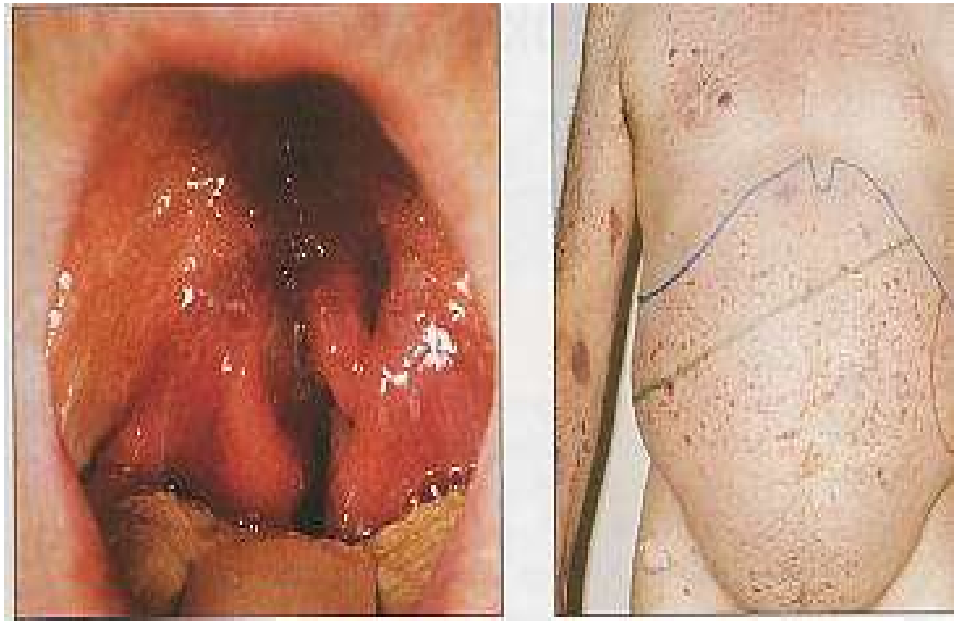
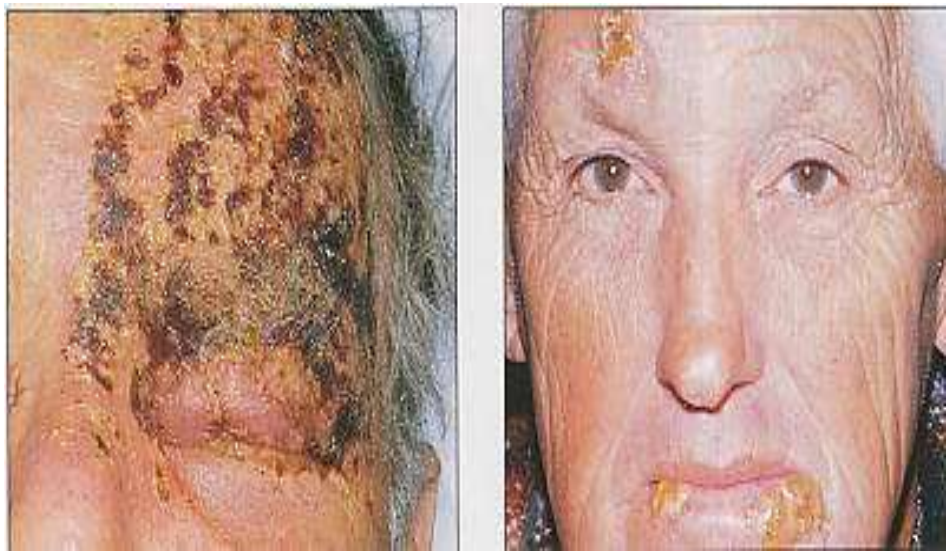


Fig. 10.2 Chronic lymphocytic leukaemia: bilateral cervical lymphadenopathy in a 65-year-old man. [Hb, 12.5 g/dl; WBC, 150 × 10^9/l (lymphocytes, 140 × 10^9/l); platelets, 120 × 10^9/l.]







CLL - COMPLICATIONS

Severe systemic infections

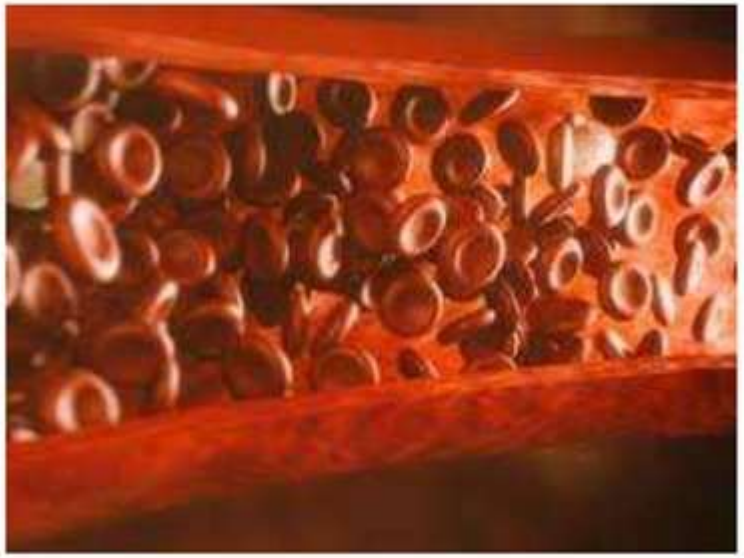
Bleeding

Richter's transformation

Prolymphocytoid transformation

Secondary malignancies

Acute myeloid leukemia



CLL

- Richter's synd. (5 %) :-
  - DLBCL (3 %), HD (O.5 %),
  - Occur in both Mut. & Unmut. variant,
  - a/w del 11q, overexpression of C-MYC gene, p53 mut,
  - Median survival 5 - 8 months,
  - MC seen in pts t/t with Purine Nucleotide Analogue

LEUCEMIA LINFATICA CRONICA

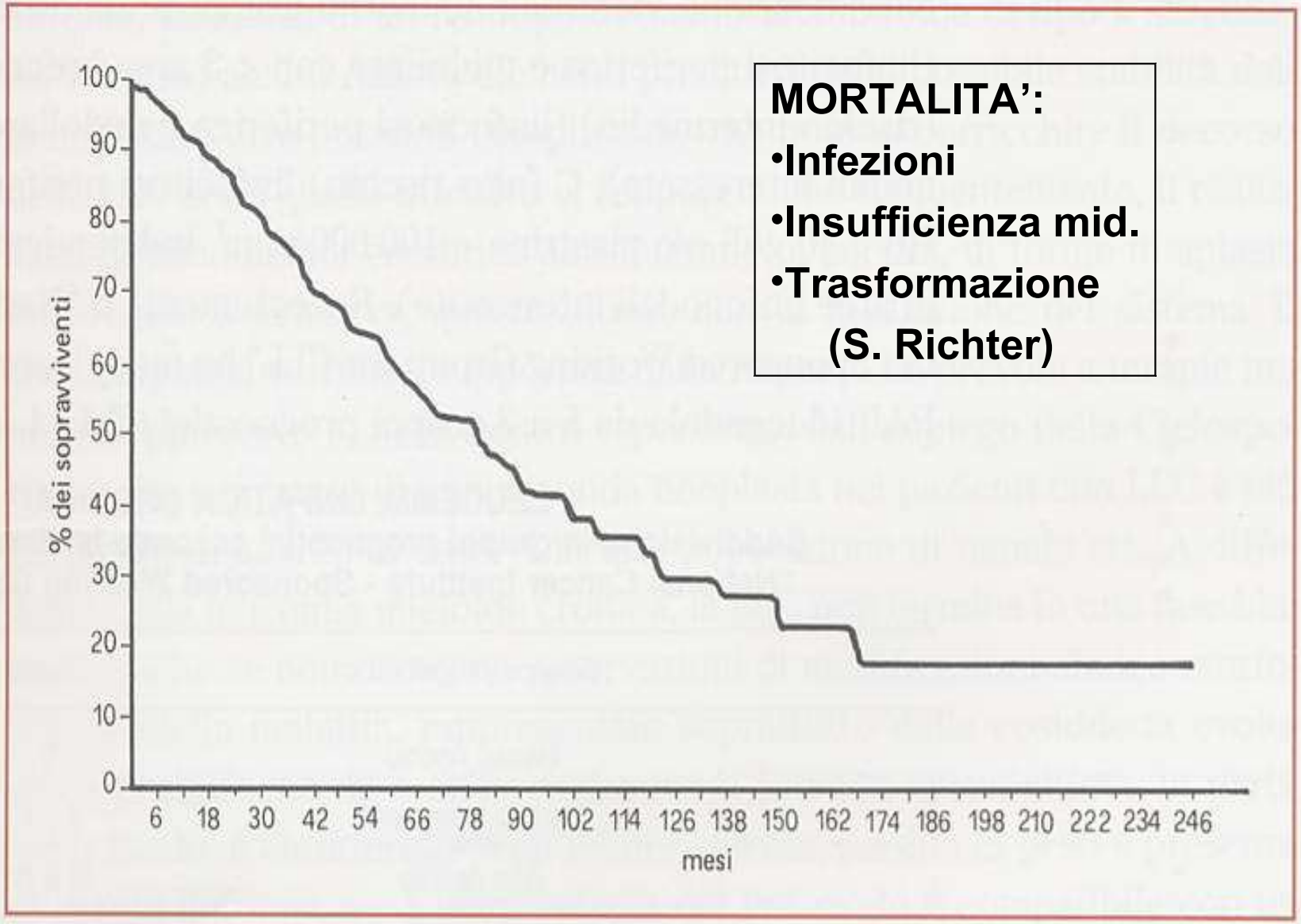


Figura 4.12 - Curva di sopravvivenza globale dei 180 pazienti con leucemia linfatica cronica.

Rai Clinical Stage (original)

Adapted from Rai et al, 1975

Stage	Clinical features
0	Lymphocytes >15 x 10s/L
I	As 0 + lymphadenopathy
II	As 0 + hepato- or splenomegaly
III	As 0 + anemia (Hb < 11 g/dL)
IV	As 0 + thrombocytopenia (platelets < 100 x10s/L)

Modified Rai Clinical Stage

Adapted from Rai et al, 1987

Risk category	Clinical features	Median Survival (y)
Low	Lymphocytes > 15 x 10s/L	>10
Inter mediate	As 0 + lymphadenopathy or hepato- or splenomegaly	7
High	Anemia (Hb ≤ 11 g/dL) or thrombocyto penia (platelets ≤ 100 x10s/L)	1.5-4

Binet Clinical Stage

* The four lymphadenopathy areas are: cervical, axillary, inguinal, spleen/liver

Adapted from Binet et al, 1981

Stage	Clinical features	Median Survival (y)
A	Lymphocytosis, does not meet criteria for stages B or C	12
B	≥ 3 areas of lymphadenopathy*, does not meet criteria for stage C	7
C	Anemia (Hb < 10 g/dL) or thrombocytopenia (platelets < 100 x10s/L)	2-4

LEUCEMIA LINFATICA CRONICA

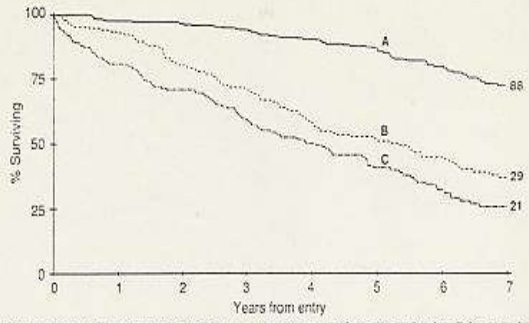


Fig 2. Outcome by International stage. Only CLL related deaths (censored at non-CLL death) were considered in all the survival curves (Figs 2–7). Numbers shown indicate number of patients alive at 7 years from entry.

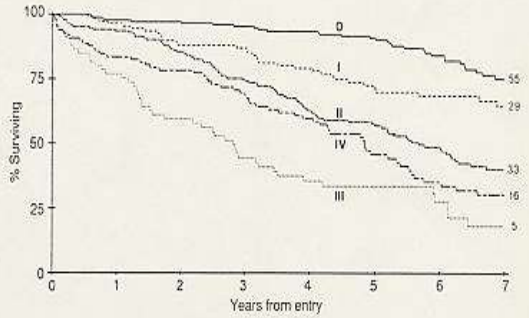


Fig 3. Outcome by Rai staging system.

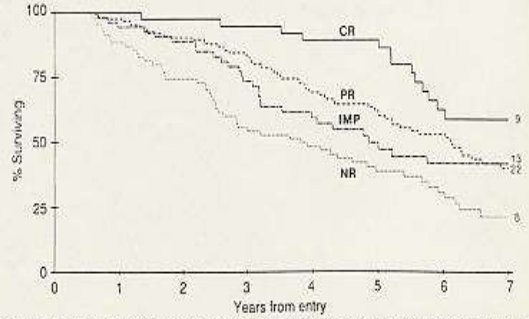


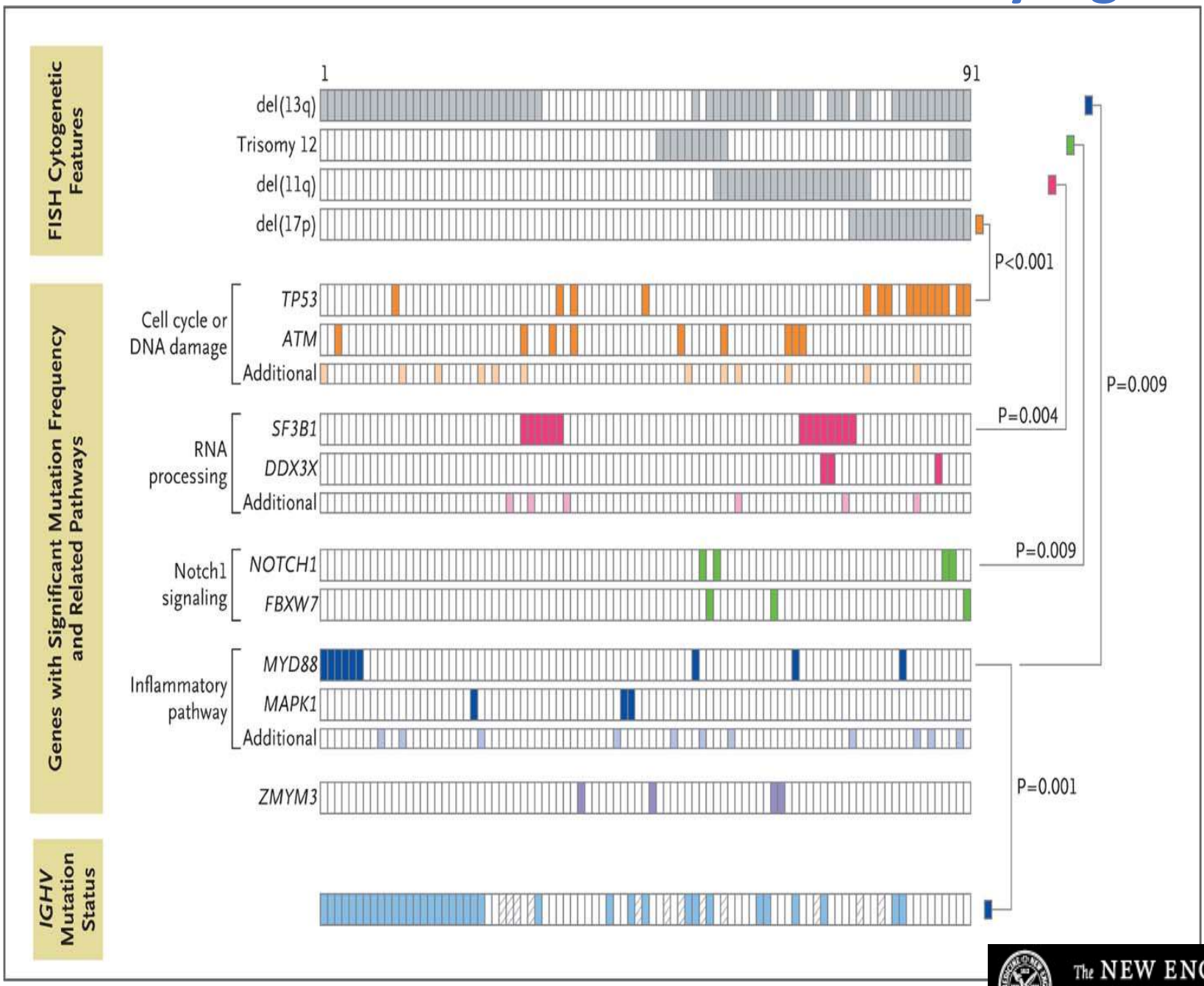
Fig 4. Outcome by response to treatment. This analysis includes all patients surviving the first 6 months of treatment. CR = complete and PR = partial response; IMP = improvement; NR = no response.

POOR PROGNOSTIC FACTORS IN CLL

Advance stage at diagnosis
Male sex
Diffuse pattern of bone marrow infiltration
Short lymphocyte doubling time
High expression of Ki67, p27, NOTCH1mut, SF3B1mut, BIRC3mut
High serum levels of B2-microglobulin, Thymidine kinase, soluble CD23, TNFa
Poor-risk cytogenetics: 17p, 11q deletions, and complex cytogenetic abnormalities
TP53 mutations
IgHV unmutated mutational status
High level of CD38
High level of zap70 expression
High expression of lipoprotein lipase
Altered microRNA
Poor response to therapy or short duration of response

Gribben. Blood, 2010

Association between molecular data and cytogenetics



Wang L et al. ***N Engl J Med 2011***;365:2497-2506

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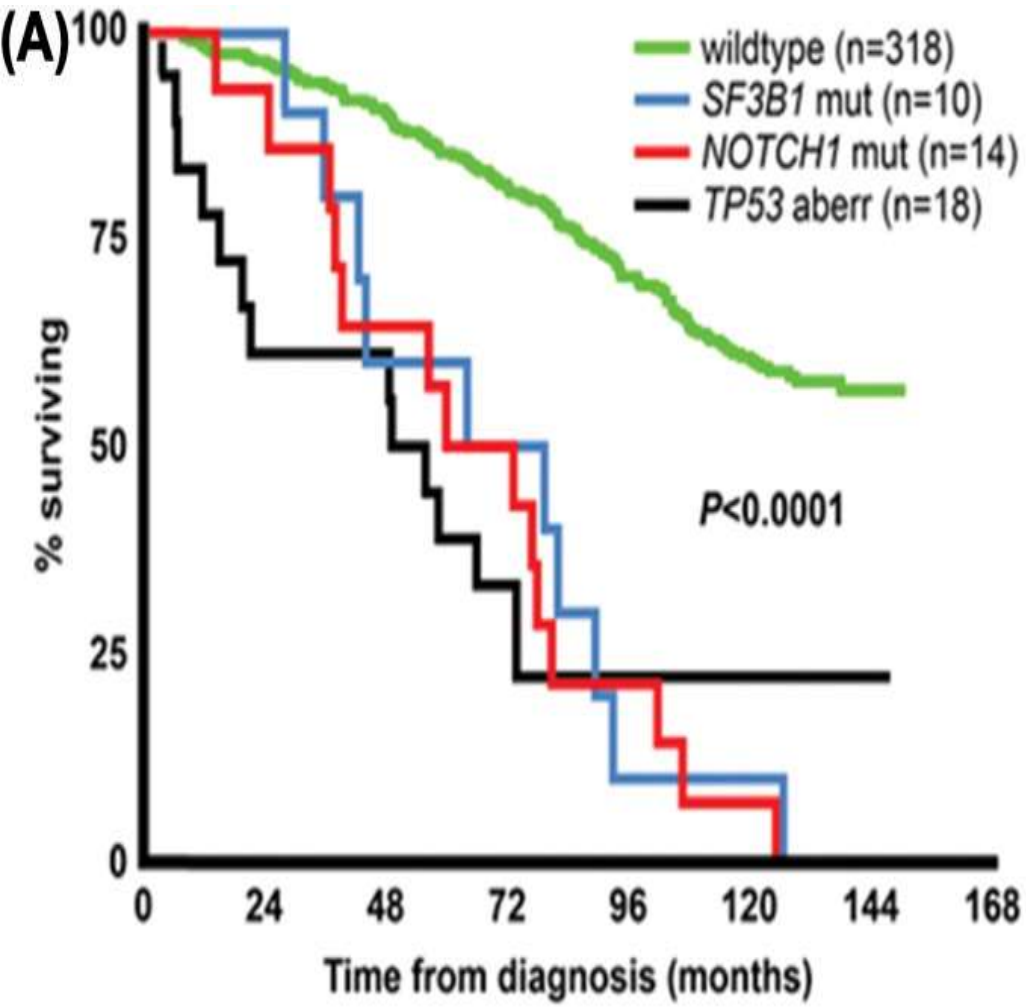
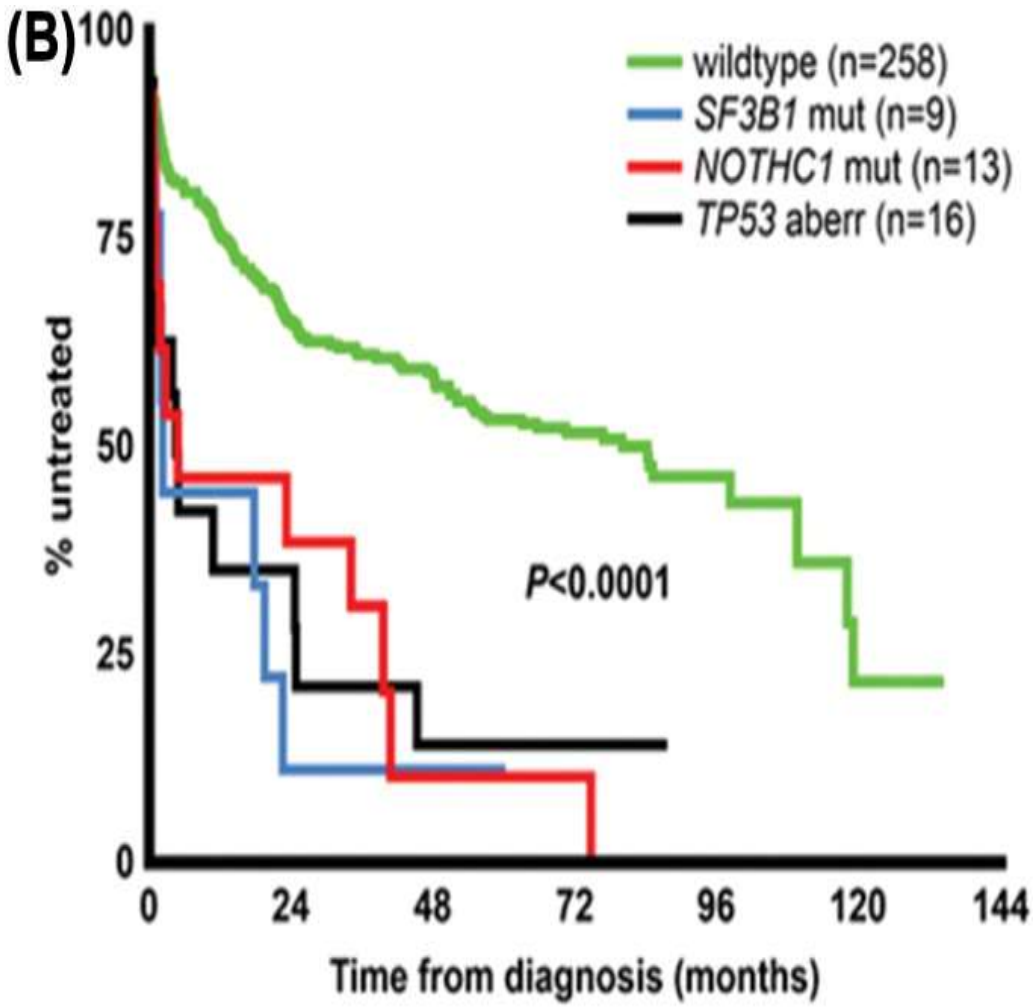
Prognostic significance of molecular data





Pre-novel agents era

Rosenqvist R et al. ***Leuk Lymphoma2013*** Nov;54(11):2351-64.

CLL IPI

	Adverse factor	Assigned risk
TP53 status	Deleted or mutated	4
IGHV mutational status	Unmutated	2
B2microglobulin concentration	>3.5 mg/L	2
Clinical stage	Rai I-IV or Binet B-C	1
Age	>65 aa	1

The International CLL-IPI working group. *Lancet Oncol*, 2016; 17:779-90.

CLL IPI

CLL-IPI category	Score	OS at 5 years (%)	Potential clinical consequence
Low risk	0-1	93.2	Do not treat
Intermediate risk	2-3	79.3	Do not treat except if disease is really symptomatic
High risk	4-6	63.3	Traetment indicated except if the disease is asymptomatic
Very high risk	7-10	23.3	If you need to treat, do not use chemotherapy but rather novel agents or treatment in clinical trials

The International CLL-IPI working group. *Lancet Oncol*, 2016; 17:779-90.

INICATIONS FOR TREATMENT

- IWCLL/NCI-WG Guidelines
- Monitoring for low risk
- Initiation of treatment for intermediate and high risk

Hallek M. et al. *Blood*, 2008. 111, 12

ACTIVE DISEASE*

- Evidence of bone marrow failure
- Massive (i.e., >6 cm below the left costal margin) or progressive or symptomatic splenomegaly
- Massive nodes (i.e., >10 cm in the longest diameter) or progressive or symptomatic lymphadenopathy
- Progressive lymphocytosis with LDT of less than 6 months
- Autoimmune anemia/thrombocytopenia poorly responsive to corticosteroids or other standard therapy
- Disease related symptoms

* At least one of the following criteria should be met

Hallek M. et al. *Blood*, 2008. 111, 12

PRE-TREATMENT EVALUATION OF CLL PATIENTS

Table 1. Pretreatment evaluation of patients with CLL

Diagnostic test	Section of guidelines	General practice*	Clinical trial
Tests to establish the diagnosis	1		
Complete blood count and differential count	1.1	Always	Always
Immunophenotyping of lymphocytes	1.2	Always	Always
Assessment before treatment	3.5.1		
History and physical, performance status	3.5.1.1, 3.5.1.2	Always	Always
Complete blood count and differential	3.5.1.3	Always	Always
Marrow aspirate and biopsy	3.5.1.4	Desirable	Desirable
Serum chemistry, serum immunoglobulin, direct antiglobulin test	3.5.1.5, 3.5.1.6, 3.5.1.7	Always	Always
Chest radiograph	3.5.1.8	Always	Always
Infectious disease status	3.3	Always	Always
Additional tests before treatment	3.5.2		
Cytogenetics (FISH) for del(13q), del(11q), del(17p), trisomy 12, del(6q) in the peripheral blood lymphocytes	3.5.2.1	Desirable	Always
IgVH mutational status, ZAP-70, and CD38	1.2	NGI	Always
CT scan of chest, abdomen, and pelvis	3.5.2.2	NGI	Desirable
MRI, lymphangiogram, gallium scan, PET scans	3.5.2.3	NGI	NGI
Abdominal ultrasound*	3.5.2.4	Possible	NGI

General practice is defined as the use of accepted treatment options for a patient with CLL who is not enrolled in a clinical trial.

mutTP53

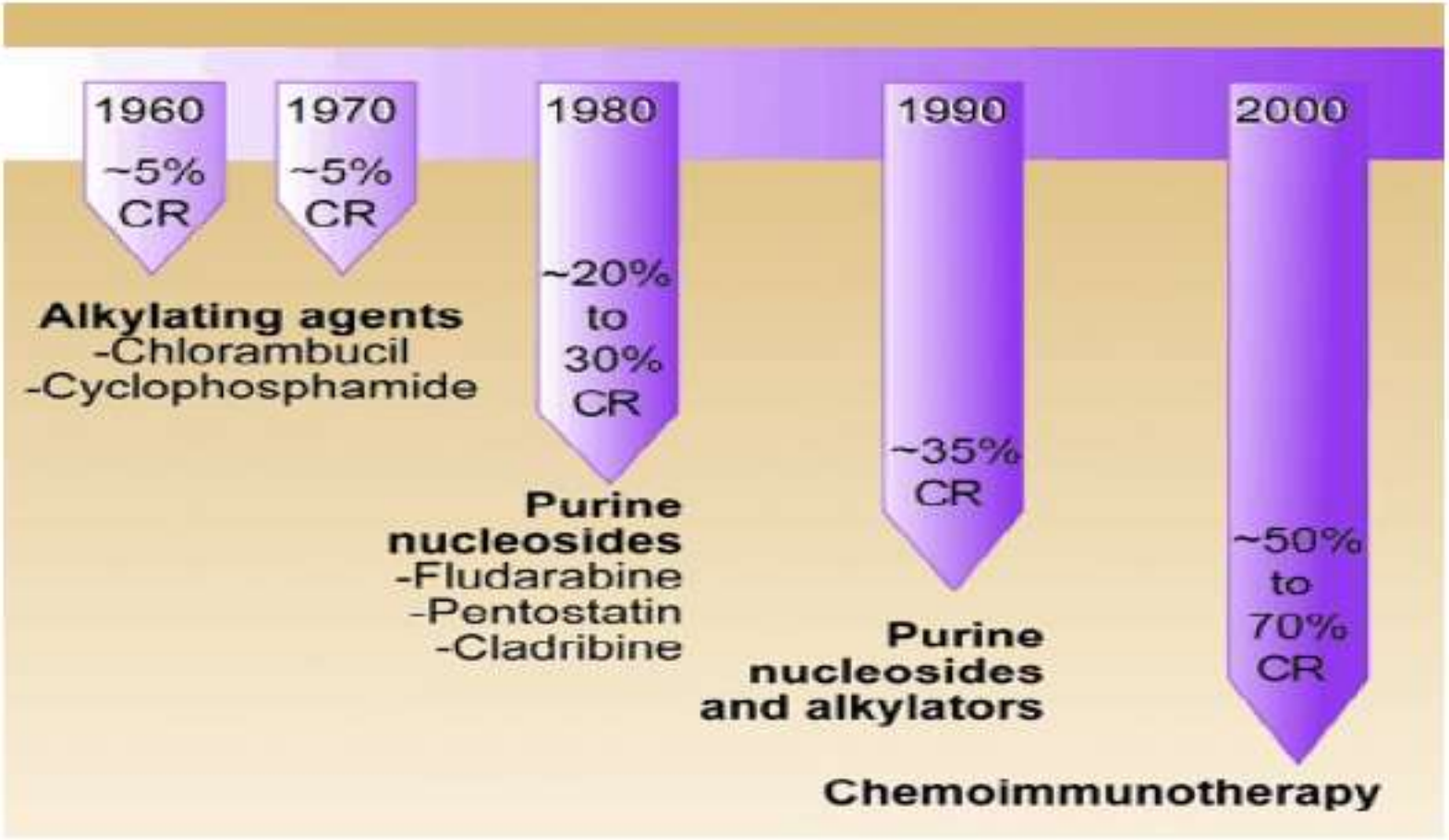
Hallek M. et al. *Blood*, 2008. 111, 12

CUMULATIVE ILLNESS RATING SCALE (CIRS)

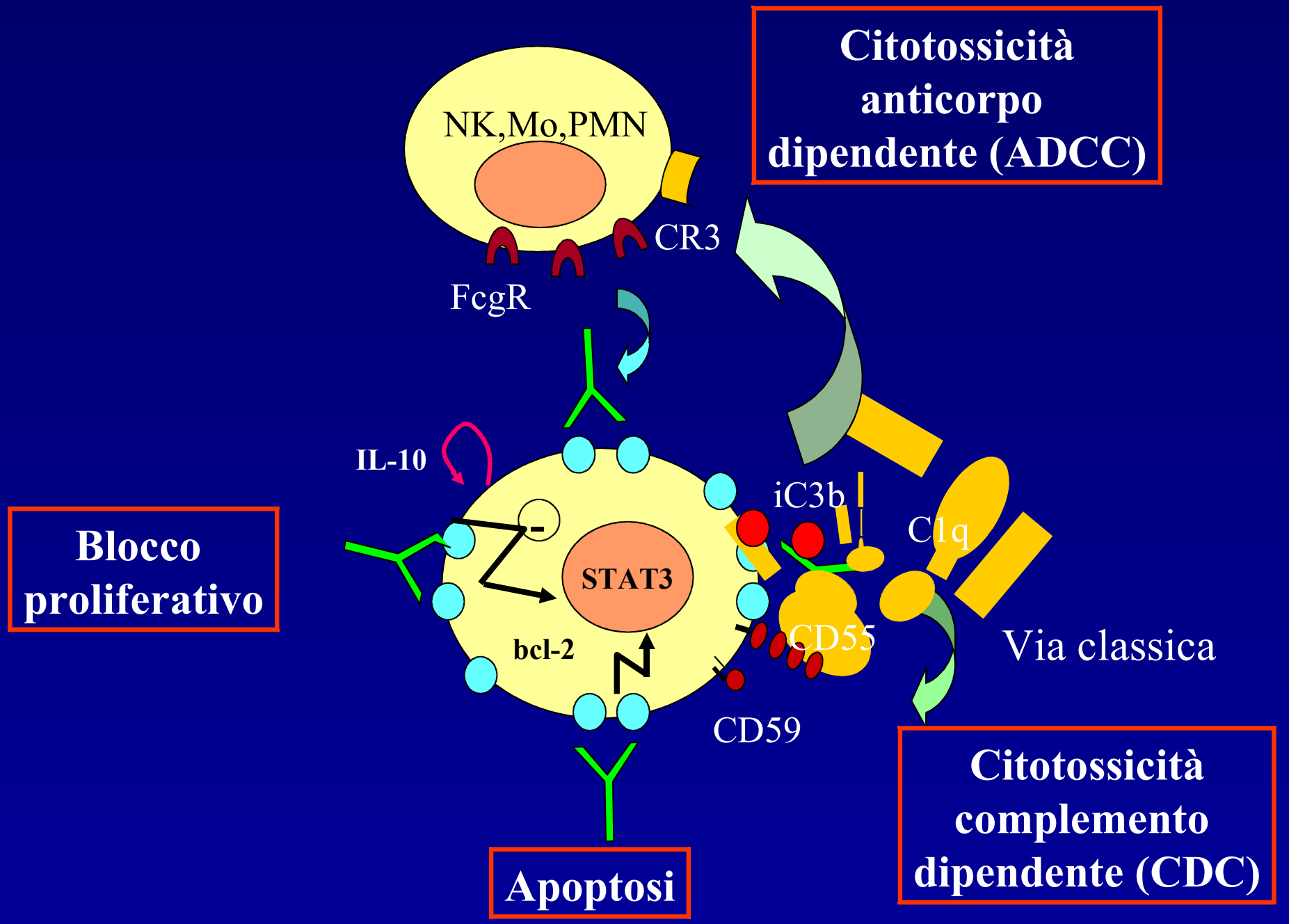
		SEVERITY				
1)	**heart diseases** (heart only)	0	1	2	3	4
2)	**hypertension** (severity should be evaluated. Involved organs should be considered separately)	0	1	2	3	4
3)	**vascular diseases** (blood, vessels, bone marrow, spleen, lymphatic system)	0	1	2	3	4
4)	**respiratory diseases** (lungs, bronchi, trachea under larynx)	0	1	2	3	4
5)	**EENT** (eyes, ear, nose throat, larynx)	0	1	2	3	4
6)	**Upper GI tract** (esophagus, stomach, duodenum, biliary tract, pancreas)	0	1	2	3	4
7)	**Lower GI tract** (bowel, hernia)	0	1	2	3	4
8)	**Liver diseases** (liver only)	0	1	2	3	4
9)	**Renal diseases** (kidney only)	0	1	2	3	4
10)	**Other genito-urinary diseases** (ureters, bladder, urethra, prostate, genitals)	0	1	2	3	4
11)	**Musculo-skeletal system and skin** (muscles, bones, teguments)	0	1	2	3	4
12)	**Nervous system diseases** (central and peripheral nervous system not including dementia)	0	1	2	3	4
13)	**Endocrine-metabolic diseases** (diabetes, infections, sepsis, toxic state)	0	1	2	3	4
14)	**Psychiatric-behavioural diseases** (dementia, depression, anxiety, agitation, psychosis)	0	1	2	3	4

FIT PATIENTS: CIRS<6, CREATININE CLEARANCE >70 ml/min

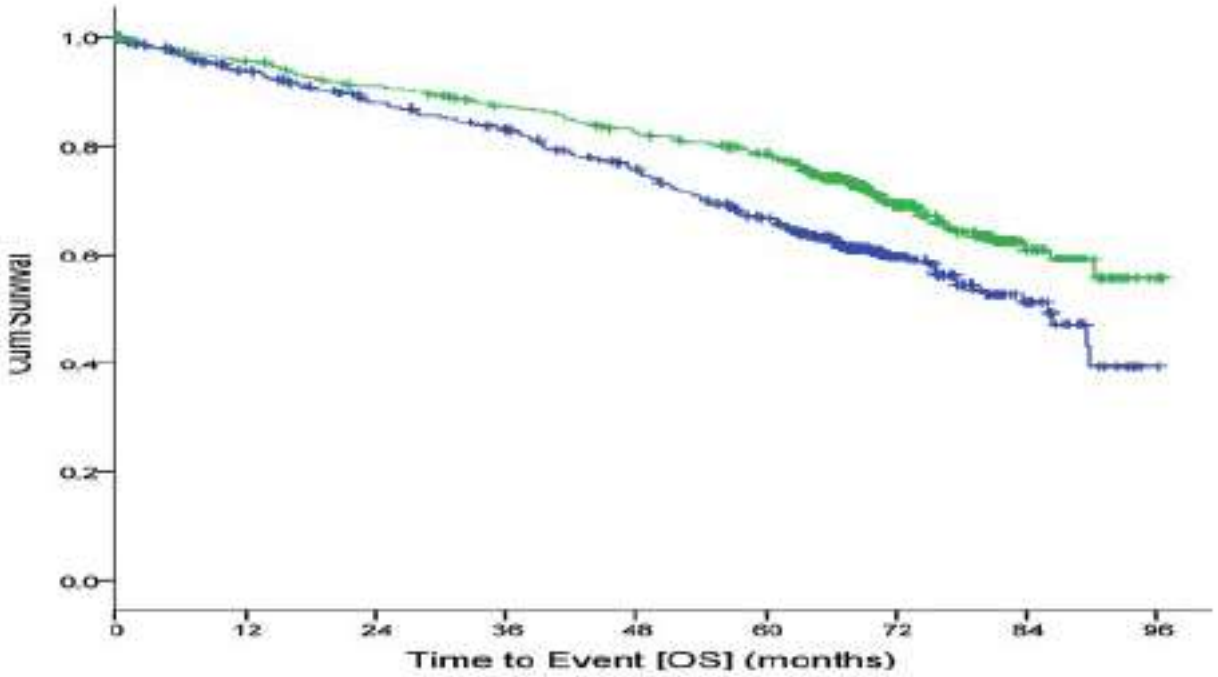
EVOLUTION TREATMENT IN B-CLL



Meccanismo di azione del Rituximab



FCR IS ESTABLISHED AS FRONTLINE TREATMENT OF CHOICE FOR FIT CLL PATIENTS AND NO del17p



Time to event [OS] months

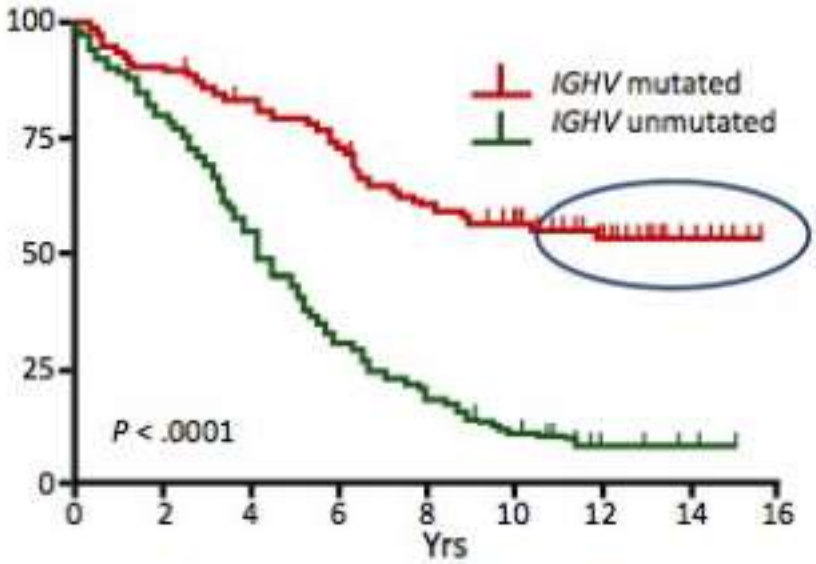
Median observation time 5.9 years

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

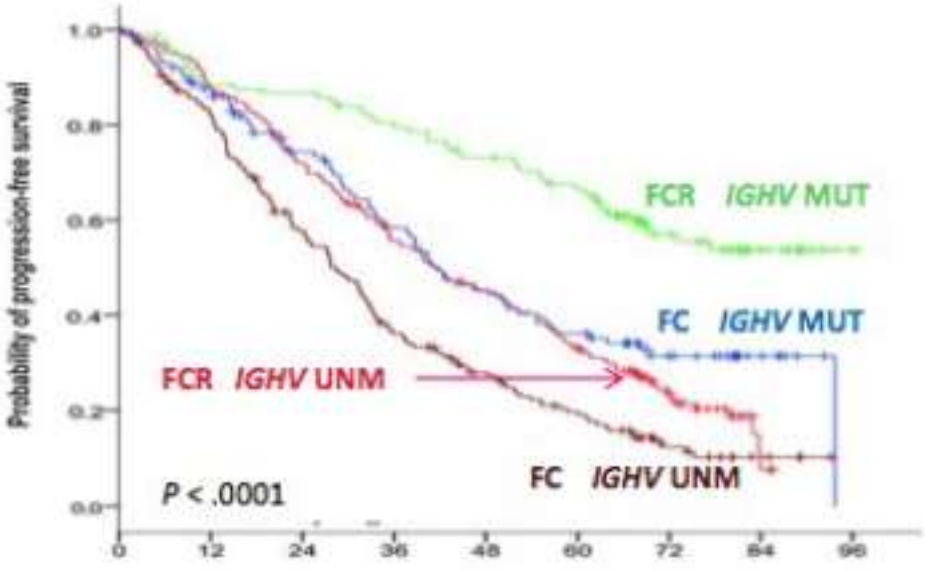
HR 0.68,
95% CI 0.535-0.858
p=0.001

Hallek et al. Lancet 2010; 376(9747):1164-74;
Fischer K et al. iwCLL 2013

IGHV mut AND del13p PATIENTS GAIN THE GREATEST BENEFIT FROM FCR

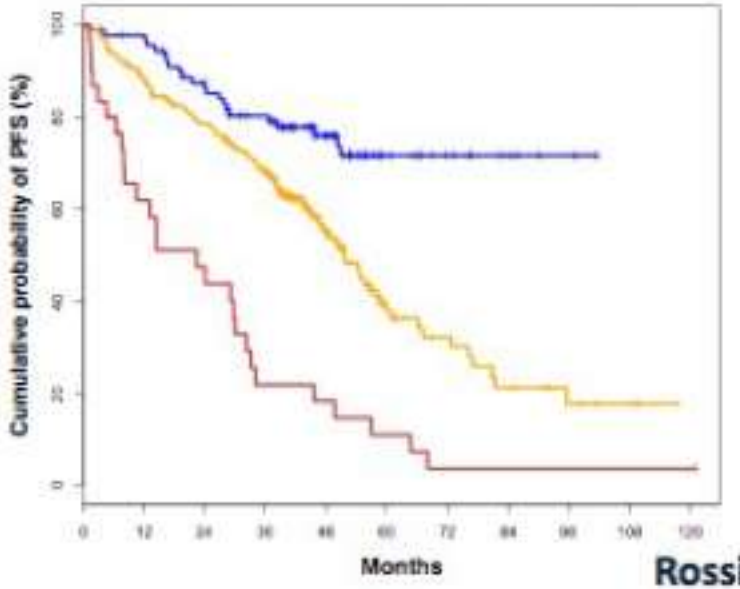


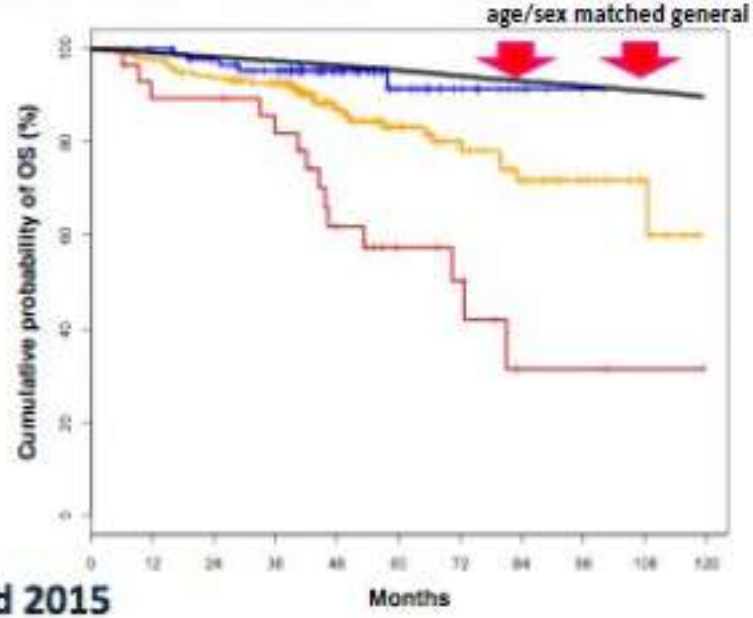
Thompson et al, Blood 2015



Fischer et al, Blood 2016

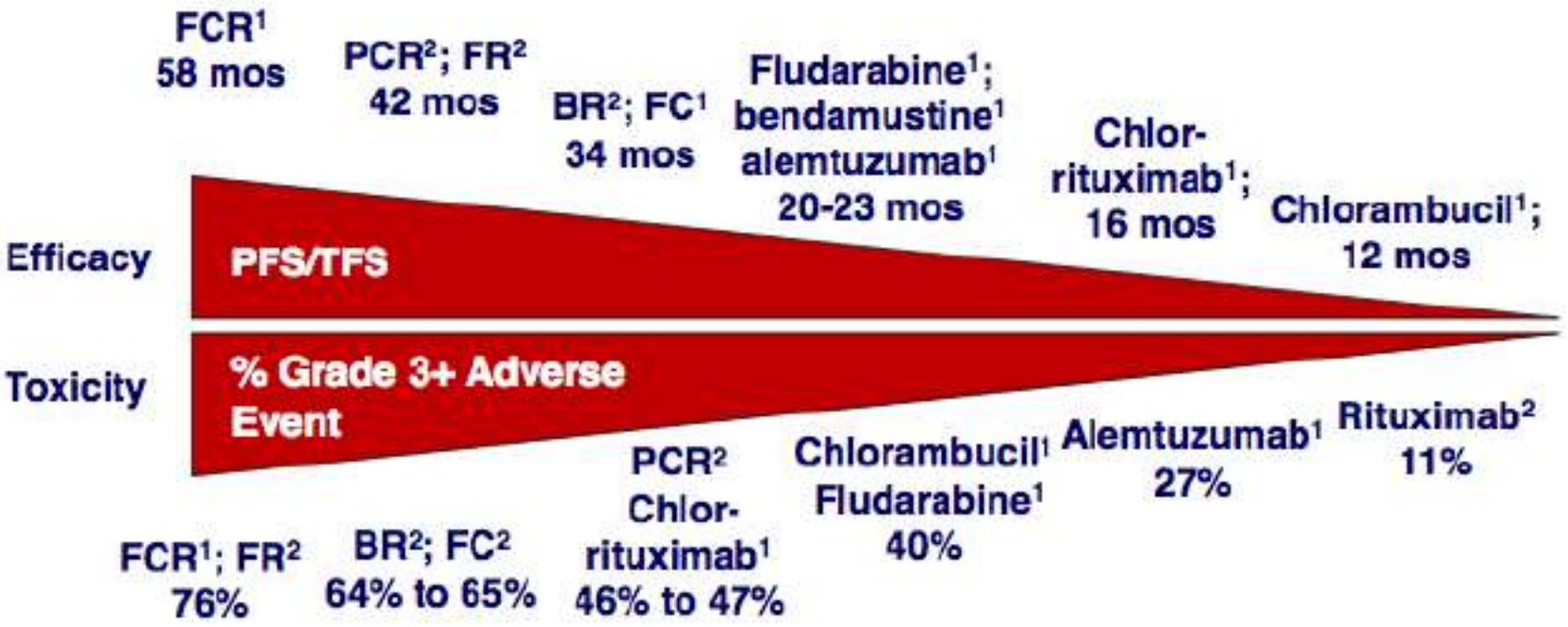
- Low-risk group (*IGHV* mutated)
- Intermediate-risk group (*IGHV* unmutated and/or 11q deletion)
- High-risk group (17p deletion)





Rossi et al, Blood 2015

EFFICACY vs TOXICITY



[1] **Phase III data.**

[2] **Phase II data.**

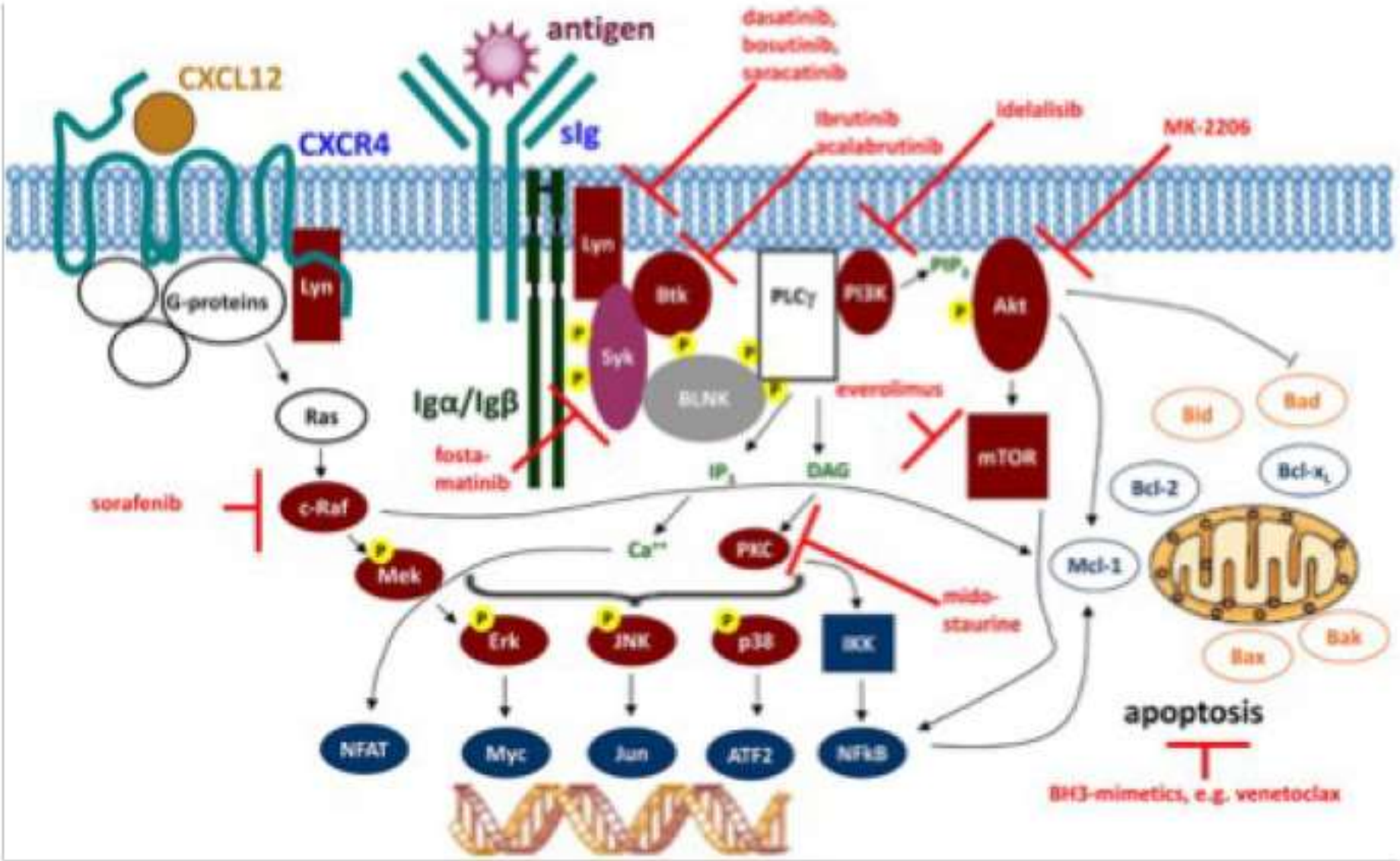
Shanafelt T. Hematology 2013;2013:158-167.

WHAT WILL THE ROLE OF CHEMOIMMUNOTHERAPY BE IN CLL PATIENTS WITH 17pDELETION OR TP53 MUTATION?

No Role
FIT/UNFIT

Novel Agents

SURVIVAL SIGNALING IN CLL: TARGETS OF NOVEL AGENTS



NEW AGENTS

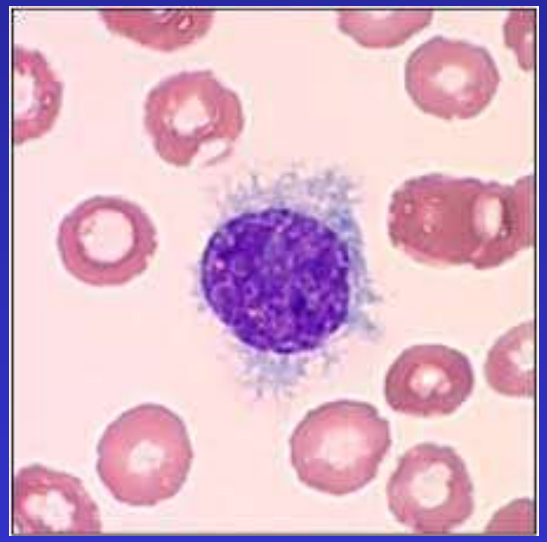
- Monoclonal Antibodies
- BTK Inhibitors
- PI3K Inhibitors
- BCL-2 Inhibitors
- Immunomodulatory Agents

HAIRY CELL LEUKEMIA

Definizione

La HCL è una neoplasia ematologica caratterizzata dalla proliferazione di linfociti B che caratteristicamente presentano lunghe e sottili protrusioni citoplasmatiche.

- Malattia rara (2% di tutte le leucemie)
- Età mediana di insorgenza 55 anni
- M/F: 5/1



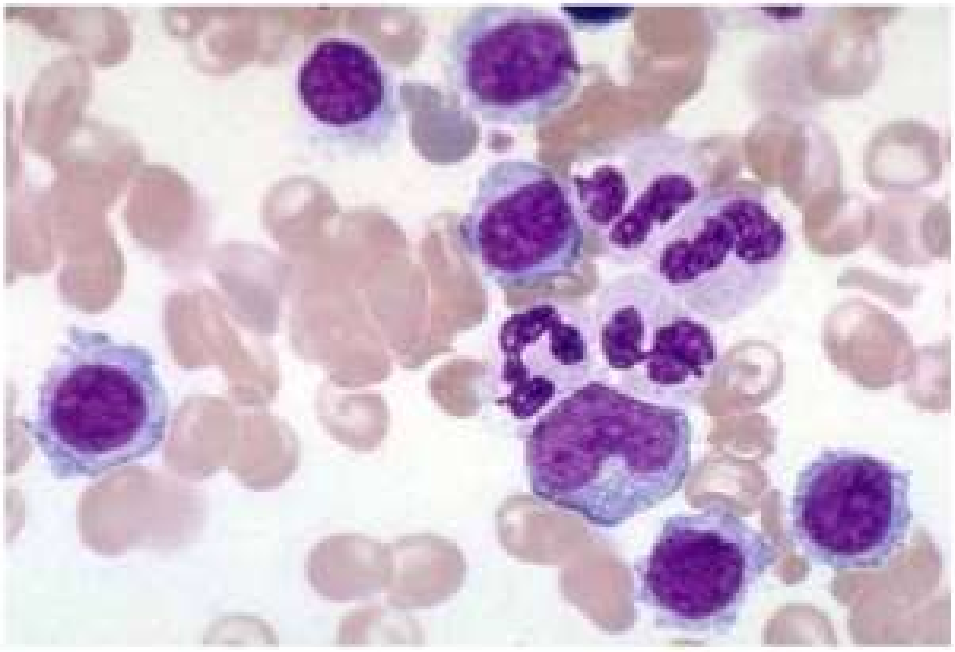
HAIRY CELL LEUKEMIA

Biology:

- In the schema of B-cell ontogeny, the hairy cell can be considered an activated, late-stage, pre-plasma cell B lymphocyte.
- Hairy cells display immunoglobulins that are light-chain restricted, but have multiple heavy-chain isotypes (IgM, IgD, IgA, and IgG)
- Hairy cells also displayed the pan-B-cell markers CD19, CD20, and CD22.

Morphology in peripheral blood films:

- approximately twice as large as normal lymphocytes
- Microvilli
- "Fluffy"
- Light basophilic cytoplasm
- Spongy chromatin
- Folded or oval nucleus
- Inconspicuous nucleoli



HAIRY CELL LEUKEMIA

Laboratorio:immunofenotipo

	Slg	CD 5	CD 23	FMC7	CD 22	CD 79b
LLC	weak	++	++	-/+	weak/-	weak/-
aLLC	weak	++	++	-/+	weak/-	weak/-
B-PLL	strong	-/+	-	++	+	++
HCL*	strong	-	-	++	++	+

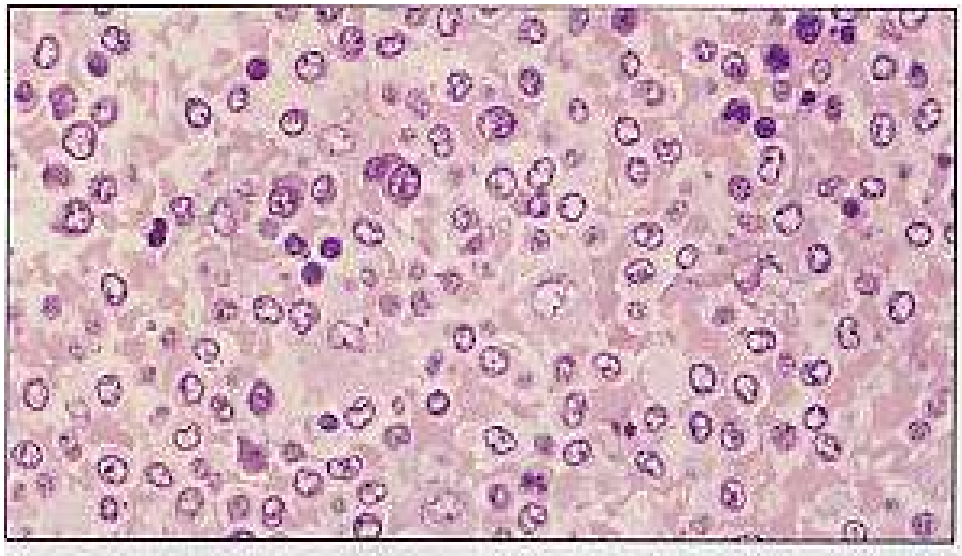
*HCL: CD20, CD25, CD11, CD103, DBA44 +

Bone marrow examination:

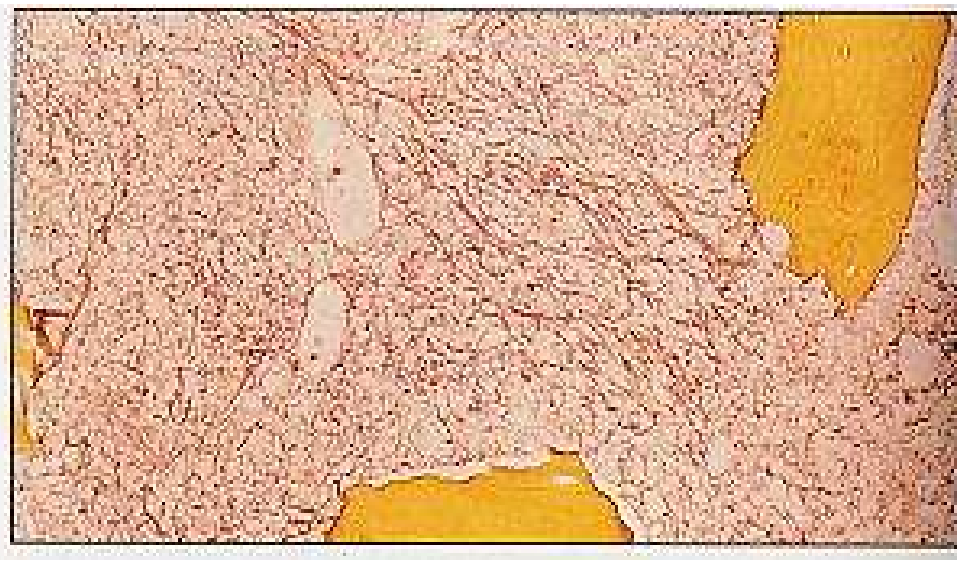
- B.M aspiration is not a valid method as it is successful in only approximately 10% of patients.
- definitive diagnosis usually requires a bone marrow trephine biopsy due to the high frequency of a dry tap on aspiration
- IHC on paraffin section: TRAP stain, CD20, CD72, Annexin A1 +, High cyclin D1

Revised guidelines for the diagnosis and management of hairy cell leukaemia and hairy cell leukaemia variant,BJH, 2011

HAIRY CELL LEUKEMIA

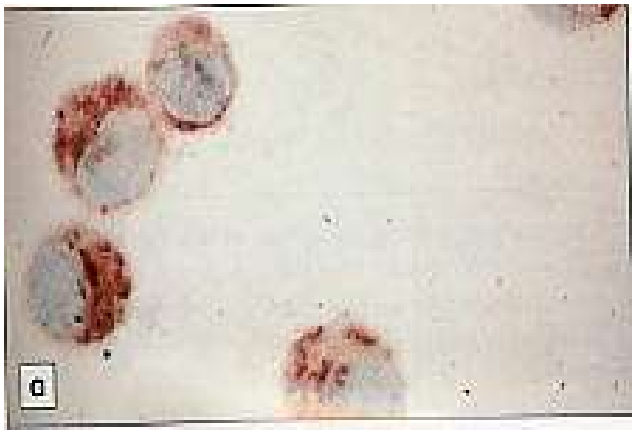


Infiltrazione mdollare. La cellularità è più spesso aumentata, ma pùo essere normale o ridotta.



Aumento della trama reticolinica alla colorazione argentofila.

Positività alla colorazione citochimica fosfatasi acida tartrato-resistente



- Patients may be asymptomatic and the disease is identified because a full blood count is taken for an unrelated reason.
- Symptoms related to Cytopenias:

Anaemia: Hb < 100 g/l	70%
Thrombocytopenia	80%
White blood cell count: $< 5 \times 10^9$/l	65%
Neutropenia: $<1 \times 10^9$/l	75%
Monocytopenia: $< 0{\cdot}1 \times 10^9$/l	90%

- Spleen, liver, and lymph nodes

Splenomegaly	60–70%
Hepatomegaly	40–50%
Abdominal lymph node enlargement*	10%

HAIRY CELL LEUKEMIA

Storia naturale della malattia

- Evoluzione cronica.
- Circa il 10% dei pazienti non necessita alcun trattamento.
- Nella maggior parte dei casi la malattia è progressiva e complicata da sintomi legati all'ingombro addominale (splenomegalia) e/o al grado di insufficienza midollare (infezioni, emorragie, astenia).
- Segnalati rari casi di remissione spontanea.

HAIRY CELL LEUKEMIA

Terapia

SPLENECTOMIA

INTERFERON - *ANNI 80*

ANALOGHI DELLE PURINE (2CDA E DCF) - *ANNI 90*

ANTICORPI MONOCLONALI (ANTI CD 20, ANTI CD 22)

PURINE ANALOGS: Cladribine

- CdA phosphylated to CdATP ➡ DNA strand breaks, inhibition of DNA synthesis, and cell death
- The largest series of cladribine in HCL, reported by Saven et al., included 358 patients,, and reported **91%** CRs and 7% PRs for an OR rate of **98%**.*

Saven A, Burian C, Koziol JA, et al. Long-term follow-up of patients with hairy cell leukemia after cladribine treatment. Blood1998

HAIRY CELL LEUKEMIA

12. 2-Clorodeossiadenosina

Zaja et al. Haematologica 1997

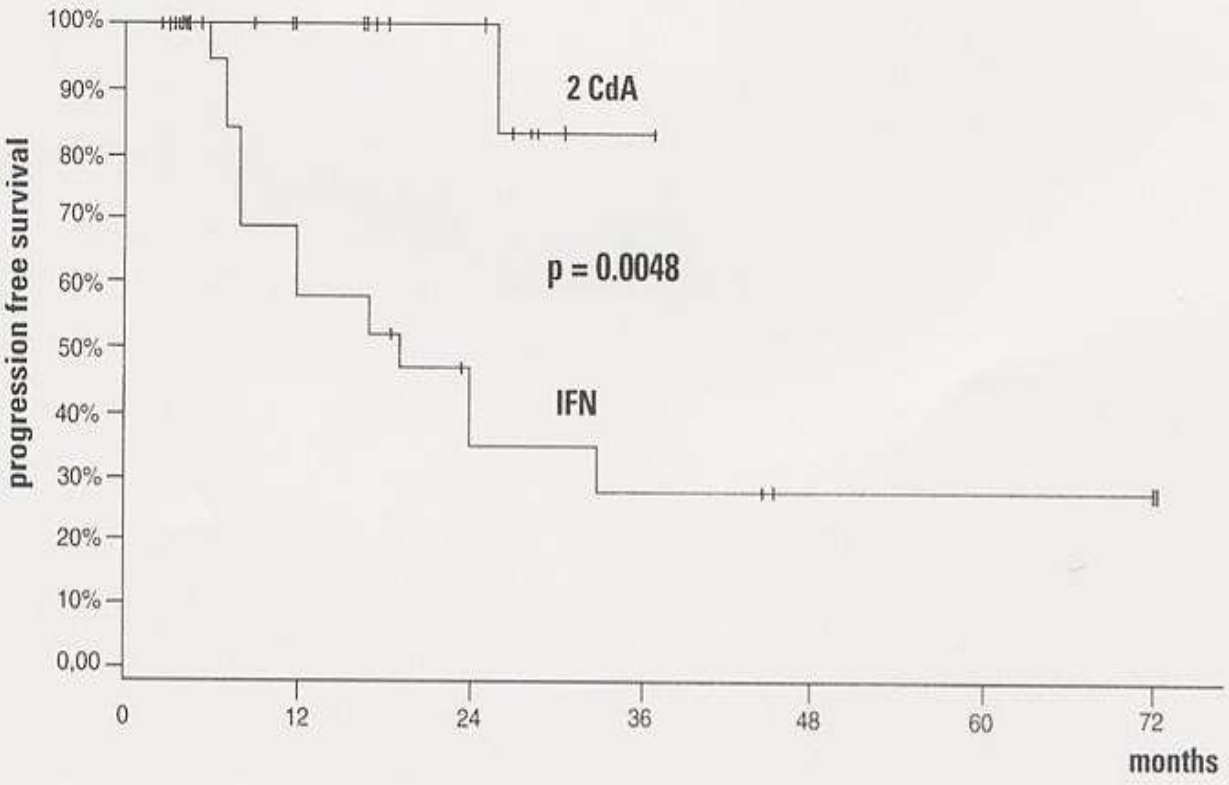


Figure 1. PFS curves of the patients treated with 2CdA and IFN-α. A significant advance was evidenced for 2CdA (p=0.0048).

PURINE ANALOGS: Pentostatin

- Irreversible ADA inhibitor
- A large cohort of previously untreated patients had **76%** CRs, 3% PRs, and **79%** OR*
- Pentostatin :4 mg/m2 every 2 weeks until maximum response plus one or two extra injections.
- Measure creatinine clearance before treatment – avoid if clearance <60 ml/min; halve dose if 40–60 ml/min.

Grever M, Kopecky K, Foucar MK, et al. Randomized comparison of pentostatin versus interferon alfa-2a in previously untreated patients with hairy cell leukemia: an intergroup study. J Clin Oncol 1995

Hairy Cell Leukemia: Definition of CR

- Recovery of cytopenias for >1 month
- No evidence of HCL in blood by morphology
- Resolution of organomegaly
- Asymptomatic from their disease
- In CR, immunohistochemistry reveals no clustering (>3 cells) of CD20-positive or DBA.44- positive cells

*** MRD may still persist…

– Presence of HCL by flow, IHC, or PCR despite above criteria

Rituximab

- Rituximab has been an effective salvage therapy for relapsed and/or refractory HCL
- Study treated 15 relapsed and/or refractory patients with rituximab 350mg/m2 for eight consecutive weeks. OR rate was 80%, including eight CRs, two CRs with minimal residual disease, and two PRs.*
- A retrospective study evaluated eight patients who had relapsed HCL to prior purine analog therapy. All eight patients received salvage therapy with rituximab in combination (either sequential or concomitant) with a purine analog. The OR rate was 100%, including 87.5% CRs**

Thomas DA, O'Brien S, Bueso-Ramo C, et al. Rituximab in relapsed or refractory hairy cell leukemia. Blood2003
Else M, Osuji N, Forconi F, et al. The role of rituximab in combination with pentostatin or cladribine for the treatment of recurrent/refractory hairy cell leukemia. Cancer 2007

BL-22 recombinant immunotoxin

- BL-22 is a recombinant immunotoxin composed of an anti- CD22 variable domain fused to a fragment of pseudomonas exotoxin.