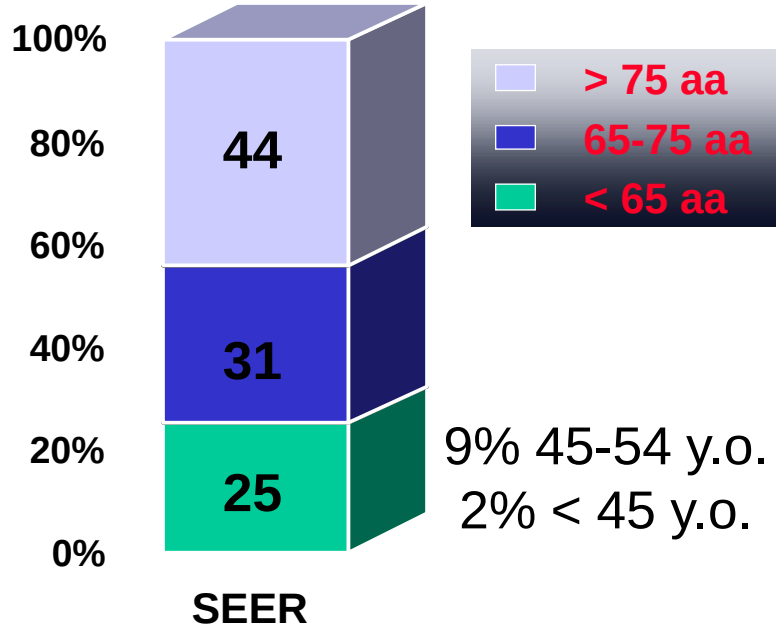
CLL: definition and nosography

- Chronic lymphoproliferative disorder
- Clonal Expansion of B lymphocytes expressing CD5
- Localization in the PB, BM, lymph nodes and spleen
- Extranodal localizations rare at diagnosis, more frequent in advanced phases
- Heterogeneous clinical course

Epidemiological data: age at diagnosis show that CLL is a disease predominantly of the elderly populatuion

Incidence 4 / 100.000
Stable incidence
M/F 2:1
White > black > asians

Risk factor
Family history of lymphoid neoplasia
Genetic predisposition



9% 45-54 y.o.
2% < 45 y.o.

CLL belongs to a wide group of leukemic chronic lymphoproliferative disorders

B-derived

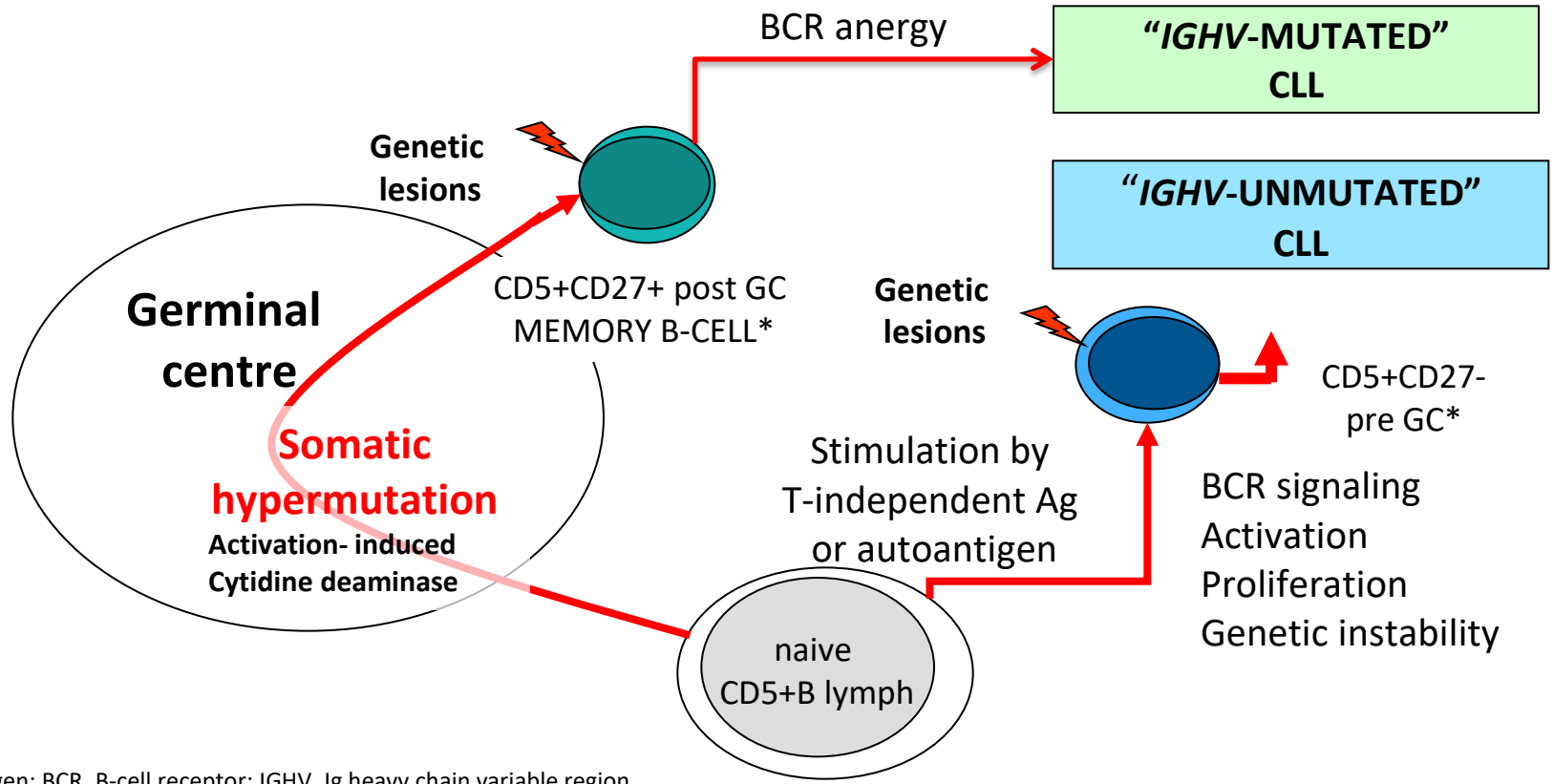
- ***CLL (typical, mixed cell type, CLL/PL)***
- PLL
- HCL
- SLVL/SMZL
- NHL in leukemic phase (LPL, FCL, MCL, others)

T-derived

- LDGL
- T-PLL
- Sezary S.
- ATLL
- PTCL in leukemic phase

The distinction of these disorders is based on morphology, histology and immunopenotyping

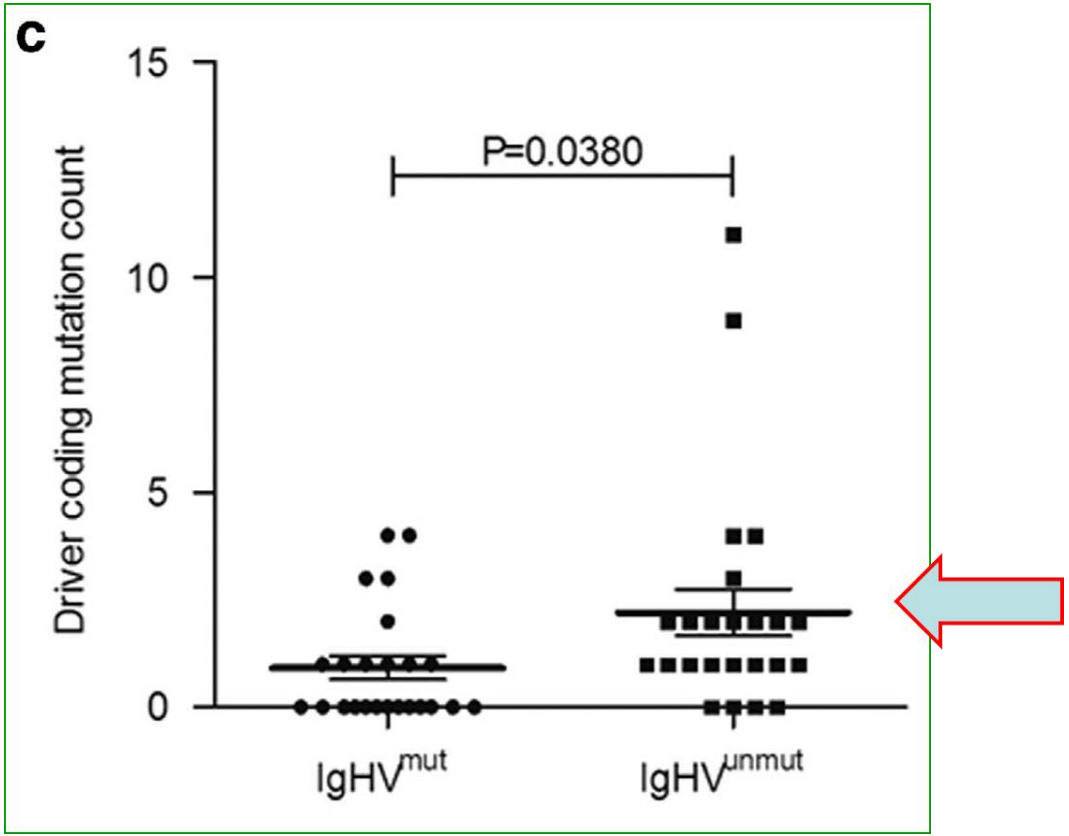
Pathogenesis
The cell of origin of CLL is a CD5+ B lymphocyte



Ag, antigen; BCR, B-cell receptor; IGHV, Ig heavy chain variable region.

*Seifert M, et al. J Exp Med. 2012;209:2183-98. doi: 10.1084/jem.20120833

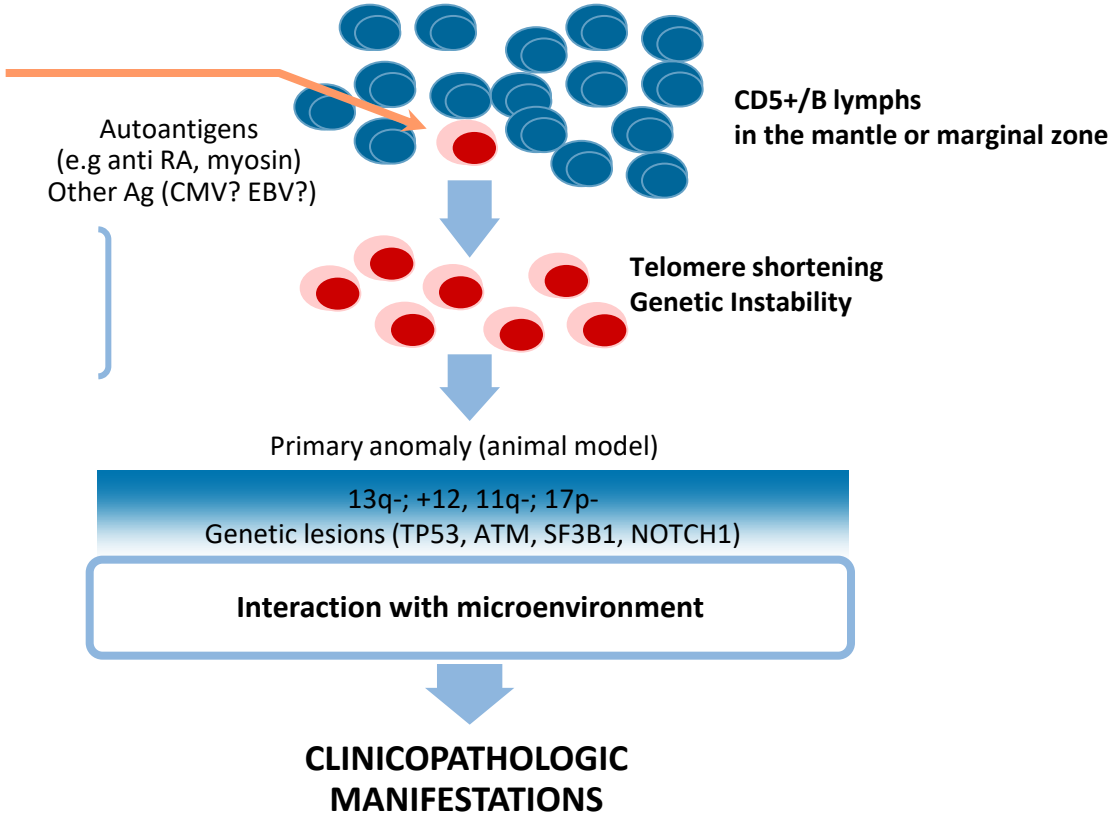
Higher load of driver gene mutations in IGHV unmutated vs IGHV mutated CLL



Burns A et al. Leukemia. 2017 Jun 6. doi: 10.1038/leu.2017.177

Four pathogenetic moments in CLL

1. Antigenic stimulation of lymphocytes with specific BCR
2. Continuous Ag stimulation
   Active BCR signalling
   Antigen driven oligoclonal expansion
3. Genetic features
4. Clinical phenotype



CLL: BCR-guided clonal expansion

Structural evidence

- Frequent expression of stereotyped BCR: response to similar antigens

Functional evidence

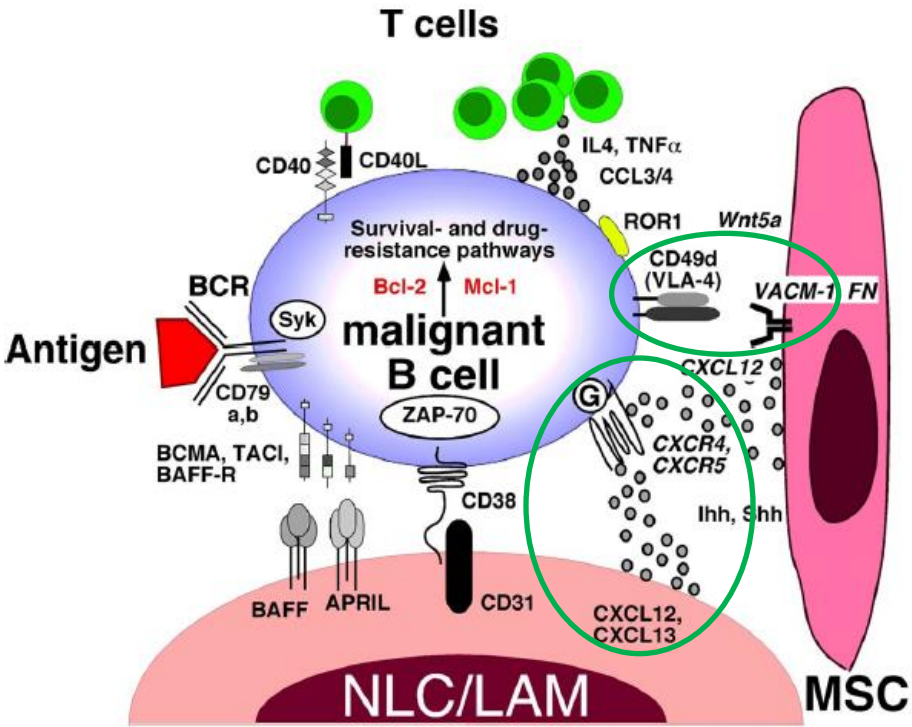
- Overexpression of BCR-target genes in CLL cells
- BCR activation supports surival in vitro of CLL lymphs

Clinical evidence

- BCR activation show a correlation with clinical course
- Response to BCR pathway inhibitors

Humblin et al, Blood 1999; Damle et al, Blood 1999
Messner et al, J Exp med 2004; Agathangelidis et al, Blood 2012
Herishanu et al, Blood 2011; Byrd et al, NEJM 2013

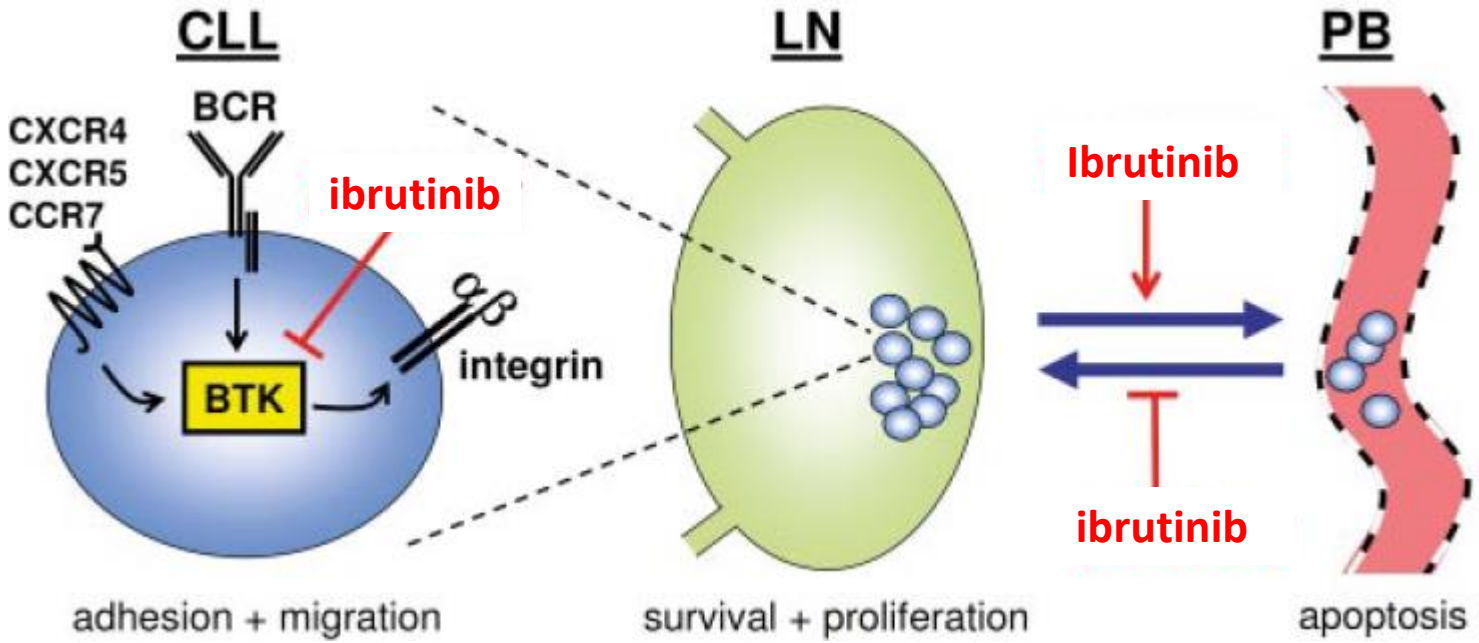
Molecular crosstalk between malignant B cells, exemplified for CLL B cells, and the microenvironment



- Integrins, particularly VLA-4 integrins (CD49d), expressed on the surface of CLL cells cooperate with chemokine receptors in establishing cell-cell adhesion through respective ligands on the stromal cells (VCAM-1 and fibronectin)
- NLCs express the chemokines CXCL12 and CXCL13, whereas BMSCs predominantly express CXCL12. The chemokine receptors CXCR3 and CCR7 are additional chemokine receptors on CLL cells that are involved in lymphatic tissue homing

Burger JA et al. Blood. 2009;114:3367-3375

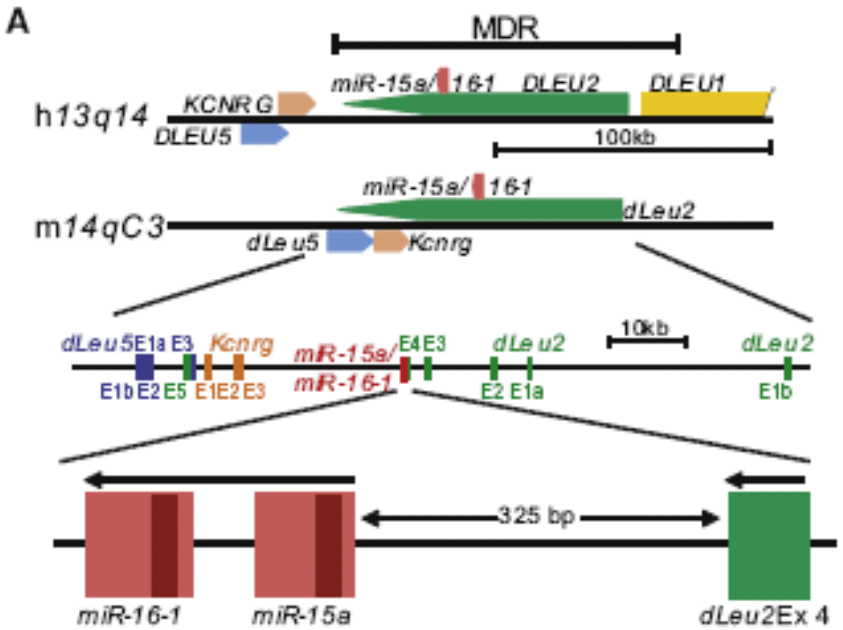
Inhibition of BTK by Ibrutinib impairs BCR-controlled integrin-mediated adhesion and chemokine (CXCL12, CXCL13, and CCL19)–induced adhesion and migration of CLL cells



de Rooij MFM et al. Blood. 2012;119:2590-2594

Genetic landscape of CLL

The DLEU2/Mir-15/Mir-16-1 locus controls B-cell compartment expansion



MDR -/- including Leu2-/- and mir15-mir16 -/-

Leu2+/+ and mir15-mir16 -/-

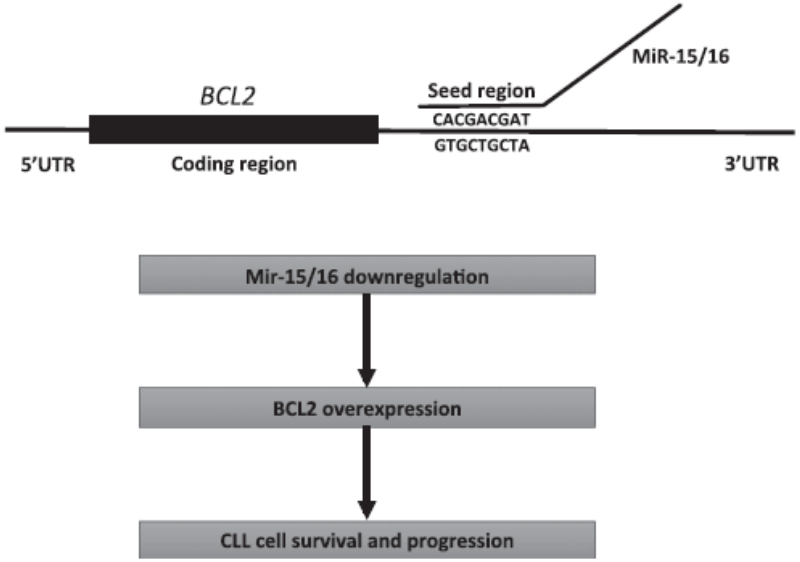
- Normal lymphoid development
- At 12-18 months

- 5% of the mice developed MBL
- CLL ~ 20%
- CD5- NHL 2-9%

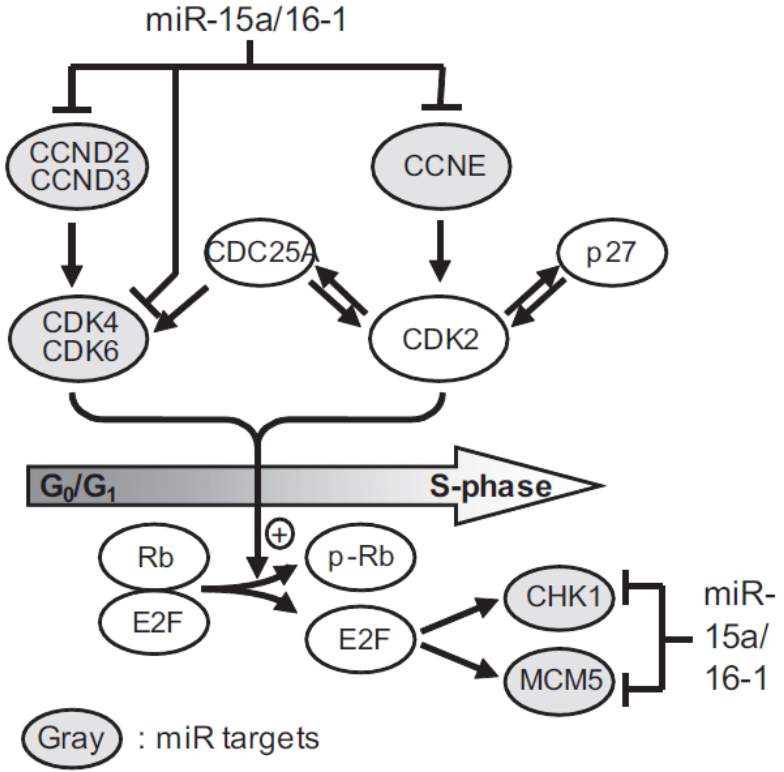
Klein U, Cancer Cell, 2010: 17; 1-13.

Identification of miR-15a/16-1 Targets in B Cells

miR-15/16 target BCL2 expression[1]



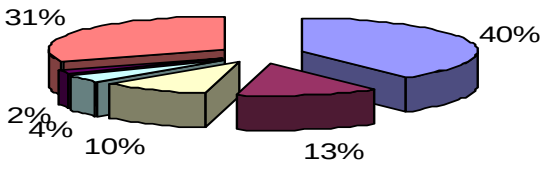
miR-15/16 deletion causes accelerated entry into cell cycle[2]



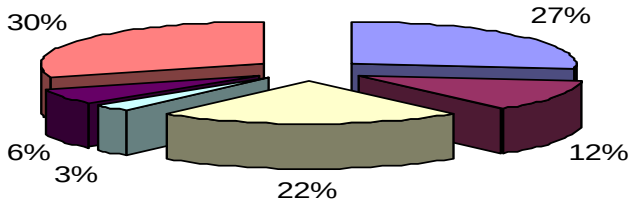
[1]Pekarsky Y et al Cell Death and Differentiation (2018) 25, 21–26
[2]Klein U, Cancer Cell, 2010: 17; 1-13

Genetic landscape of CLL

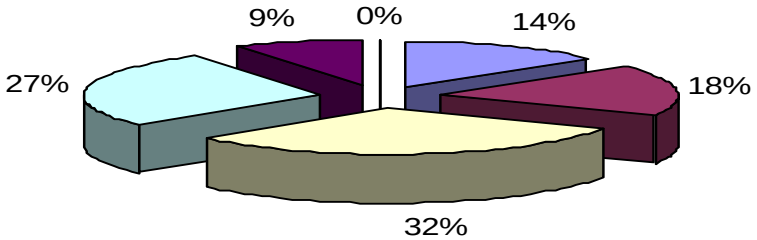
Incidence of chromosome lesions during he natural history of CLL



CLL stage A no indication for treatment

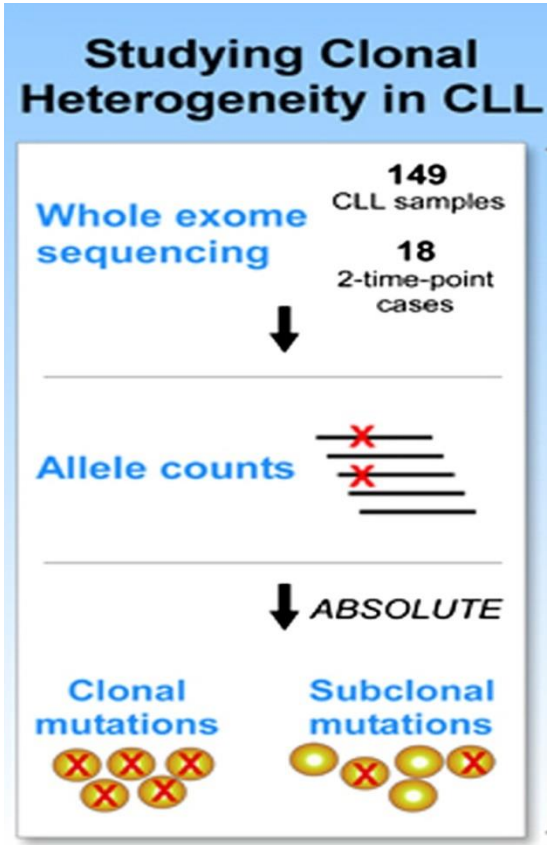


CLL stage B/C indication for treatment

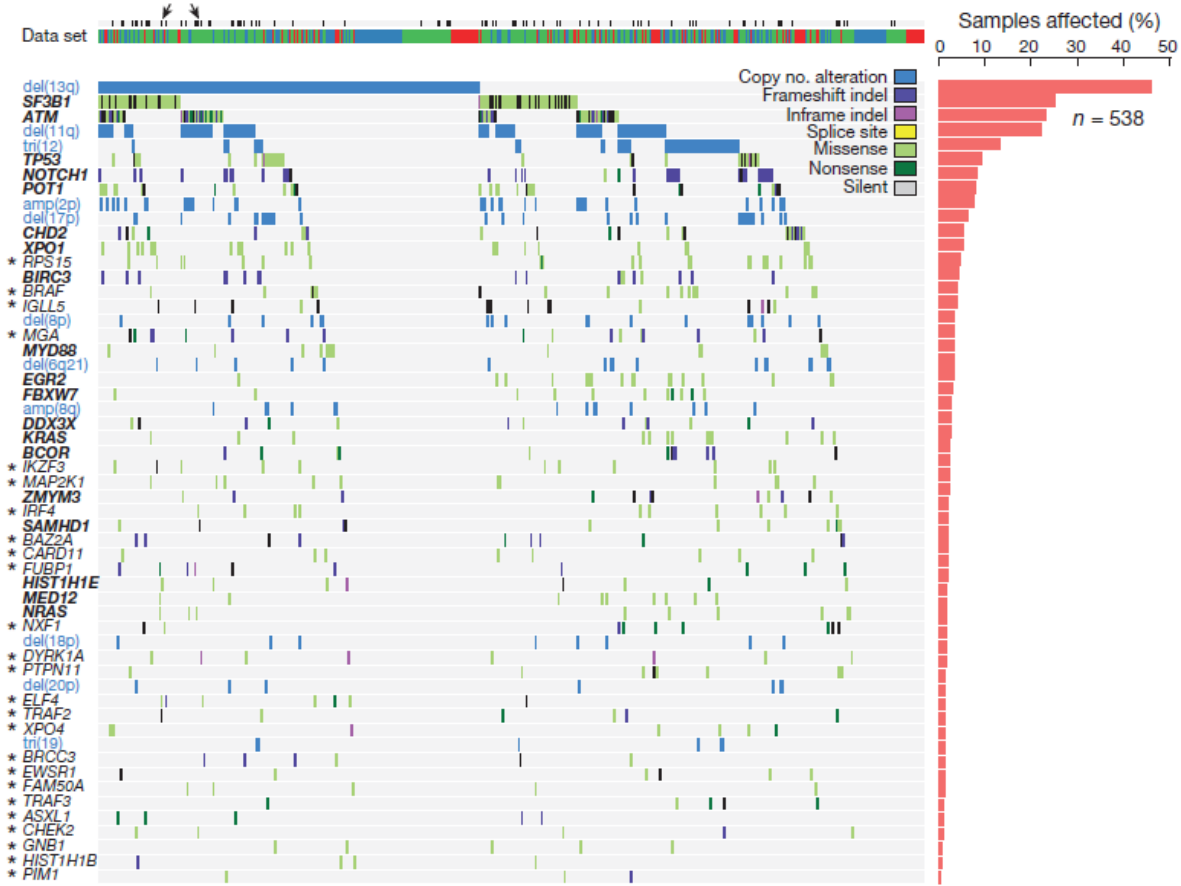


CLL fluda refractory

Recurrent mutations in CLL: The most frequent involve *SF3B1*, *ATM*, *TP53*, *NOTCH1*

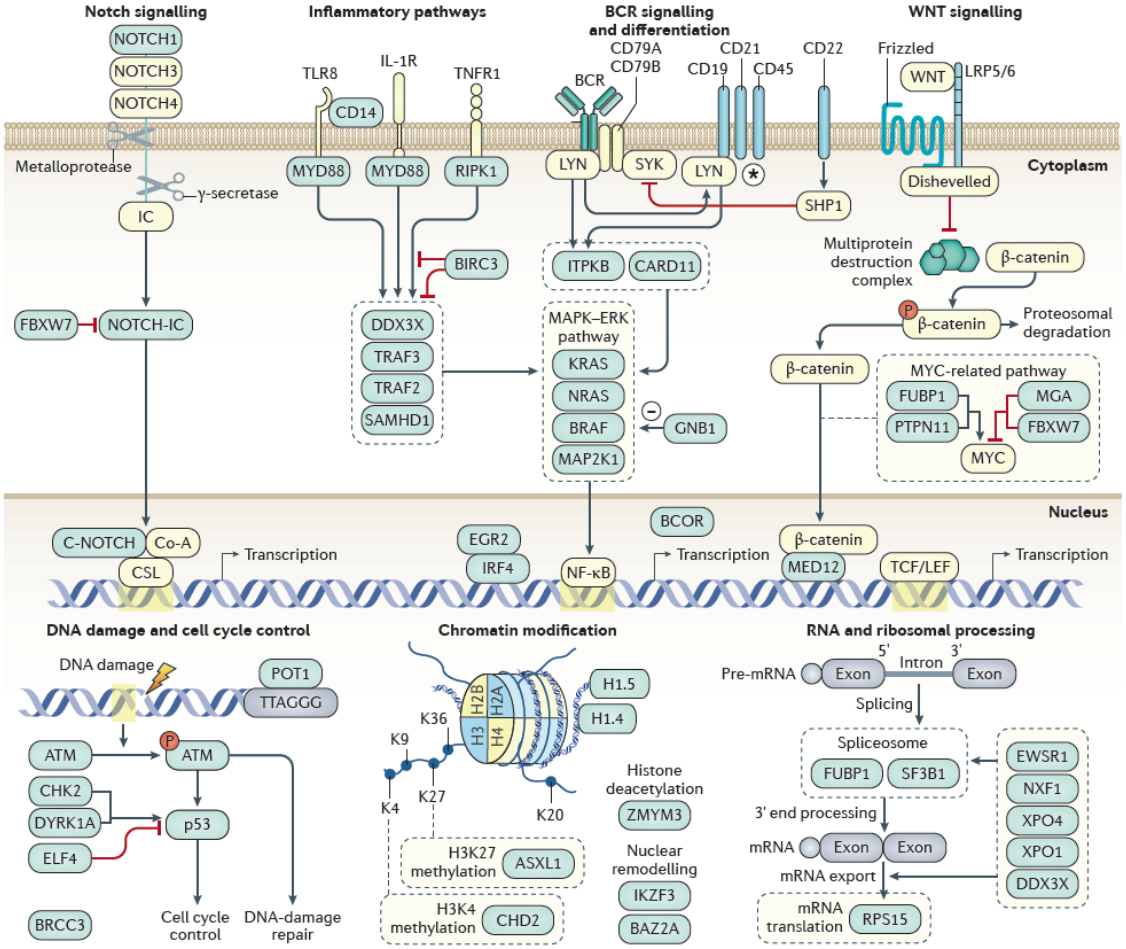


Landau D et al Cell 2013; 152: 714–726



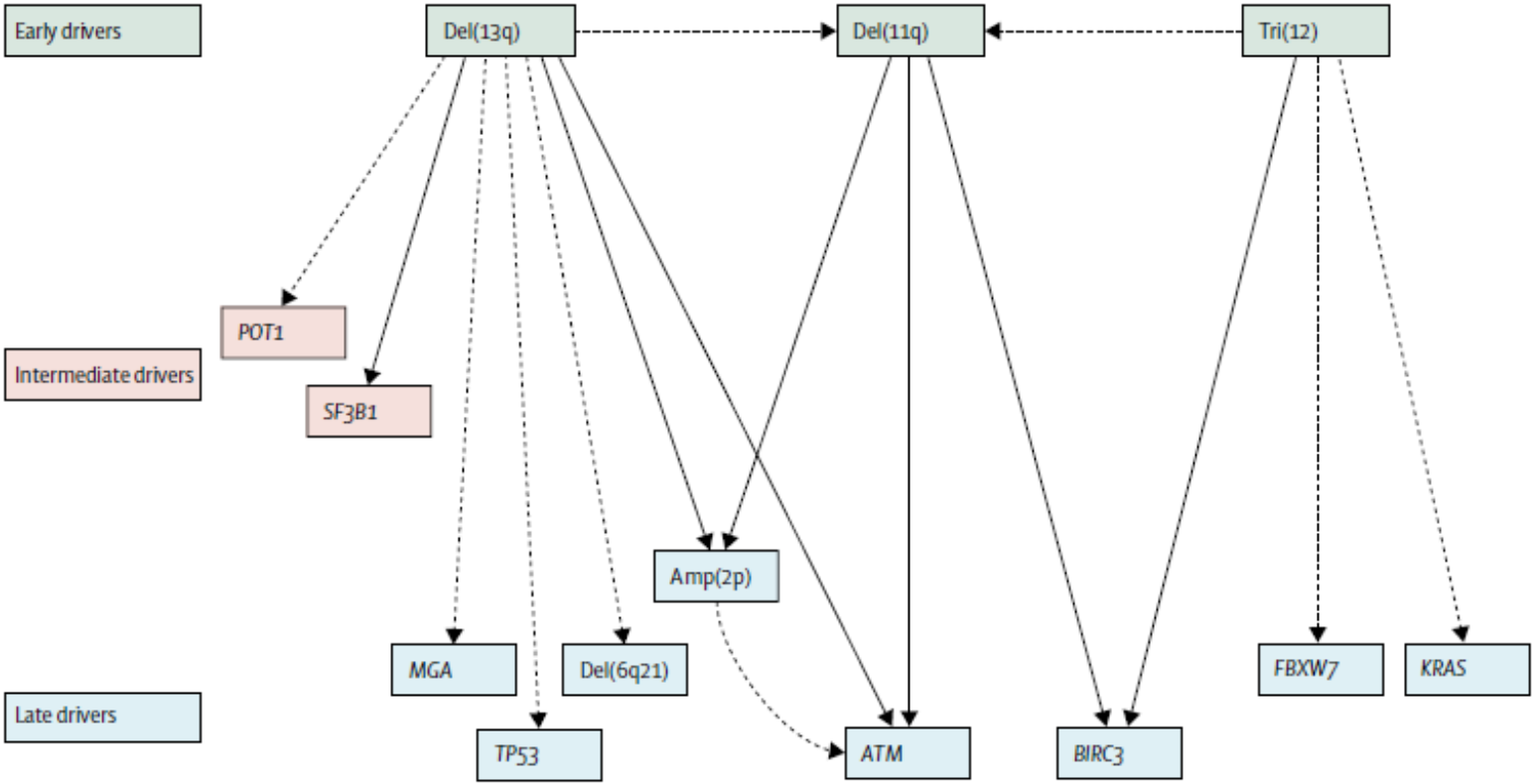
Landau D et al. Nature. 2015;526:525-30. doi: 10.1038/nature15395

Genes that are mutated in CLL are involved in several cellular pathways



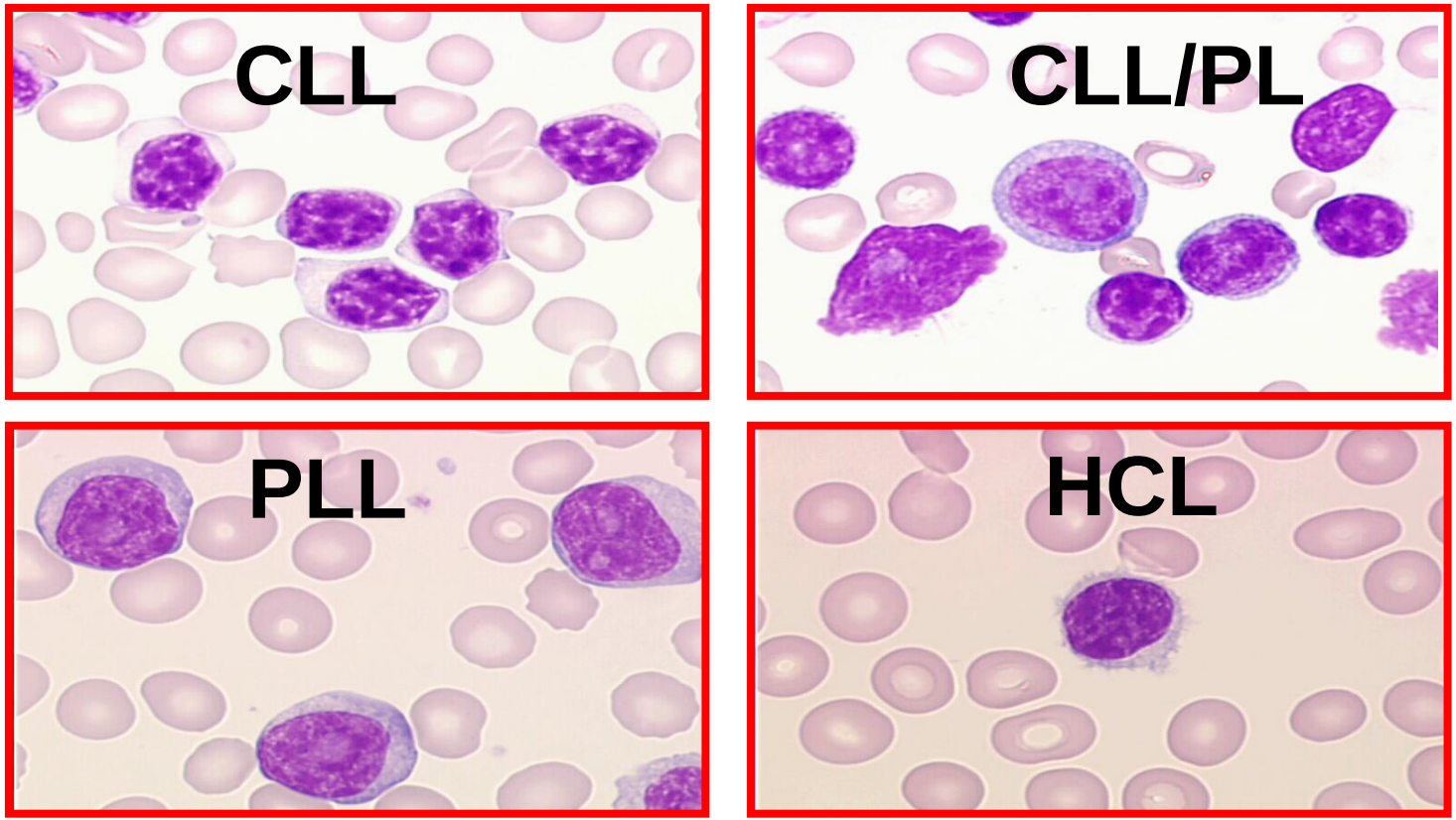
Kipps T et al. Nat Rev Dis Primers. 2017 Feb 9;3:17008. doi: 10.1038/nrdp.2017.8

Genetic drivers of chronic lymphocytic leukaemia



Adapted from Landau et al (2015)

Presentation picture: LEUKEMIC B CLD



Courtesy of Marie-Thérèse DANIEL

Quadri di presentazione più frequenti della LLC:

1) Emocromo di routine o per lieve malessere generale o per astenia.

riscontro di linfocitosi e, talora, anemia o piastrinopenia

iniziale

WBC	**12.9X10^9/L**
Hb	**13,9**
GR	**4.8X10^9/L**
MCV	**88**
MCH	**28**
Pst	**250X10^9/L**

Neu 32%
Linf 64%
Mono 4%

intermedio

WBC	**67.9X10^9/L**
Hb	**12,9**
GR	**4.5X10^9/L**
MCV	**86**
MCH	**27**
Pst	**220X10^9/L**

Neu 12%
Linf 84%
Mono 4%

avanzato

WBC	**160X10^9/L**
Hb	**9,9**
GR	**3,1X10^9/L**
MCV	**83**
MCH	**27**
Pst	**95X10^9/L**

Neu 3%
Linf 96%
Mono 1%

Altri frequenti quadri di presentazione della LLC:

2) Adenopatia (in genere multipla, di dimensioni medio-piccole (1-3 cm), di consistenza parenchimatoso-tenera, non dura

3) Anemia emolitica autoimmune

WBC	**29.9X10^9/L**
Hb	**7,9**
GR	**2,8X10^9/L**
MCV	**98**
MCH	**27**
Pst	**220X10^9/L**

Neu 12%
Linf 84%
Mono 4%

Sub-ittero o ittero franco
Test di Coombs positivo
Indici di emolisi positivi

La diagnosi differenziale è incentrata su

Morfologia delle cellule linfocitarie

Immunofenotipo delle cellule linfocitarie

Distinzione tra linfocitosi reattive e linfocitosi monoclonali

Le principali linfocitosi reattive da distinguere rispetto a quelle monoclonali sono:

- EBV
- CMV
- Brucellosi
- Toxoplasmosi

Morfologia:
Linfociti in
varie fasi di
Attivazione

Immunofenotipo:
Linfociti policlonali
Rapporto Catene K/λ normale (3/2)
Non incremento linfociti B/CD5+

Le principali linfocitosi monoclonali sono:

-LLC

-Linfomi leucemizzati (follicolare, mantellare)

-Linfoma splenico con linfociti villosi circolanti

-Leucemia a cellule capellute

La diagnosi differenziale è incentrata su

Morfologia delle cellule linfocitarie

Immunofenotipo delle cellule linfocitarie

Distinzione tra LLC e altre sindromi linfoproliferative

	morfologia	immunofenotipo
LLC	Piccoli linfociti alcuni prolinfociti alcuni grandi linfociti Ombre di Gumprecht	Linfociti monoclonali Evidente squilibrio K/λ Netto aumento linfociti B/CD5+ Intensità espressione sIg debole
Linfomi leucemizzati	Cellule clivate Irregolarità nucleo	Linfociti monoclonali Evidente squilibrio K/λ Marcatori specifici per ogni tipo di linfoma

Immunophenotype in CLL and other lymphoproliferative disorders

It is a CD5+/B-cell marker+ (CD19+) disorder

	sIg	CD5	CD23	CD79b	FMC7	CD20	CD22	CD103	CD200	CD25	CD11c	CD10	CD43	ROR1
Normal B lymphocytes	High	No	No	High	High	High	High	No	No	Low	Low	No	No	No
Chronic lymphocytic leukaemia	Low	High	High	Low	Low	Low	Low	No	Very high	Low	Low	No	Very high	High
Mantel cell lymphoma	High	High	No	High	Very high	Very high	High	No	Low	No	No	No	Very high	High
Lymphoplasmocytic lymphoma, immunocytoma	Very high	Low	Low	High	Low	High	High	No	No	Low	Low	No	Low	Not determined
Follicular lymphoma	Very high	No	No	High	Low	High	Low	No	No	No	No	Low	No	Low
Hairy cell leukaemia	Very high	No	No	Low	High	High	Very high	High	No	Very high	Very high	No	No	High
Marginal zone lymphoma	High	No	No	High	High	High	High	No	No	High	High	No	Low	Low

Hallek M. The Lancet, 2018

Diagnosi e stadiazione LLC

- Conta ematica (>5000 linfociti) ed aspirato midollare (>30% linfociti)
- Morfologia e immunofenotipo (espansione monoclonale B CD5+)
- Se adenopatia con istologia positiva per LLC, ma presenti < 5000 linfociti B nel sangue periferico la diagnosi è di linfoma linfocitico che presenta le stesse caratteristiche generali della LLC, eccetto per la localizzazione ematomidollare, che peraltro può comparire nelle fasi evolutive

Diagnosi e stadiazione LLC

- Anamnesi per condizioni generali, febbre, sudorazione, prurito, dimagramento, infezioni pregresse, pregresse anemizzazioni (emolisi)
- Visita con particolare riguardo a stazioni linfonodali, fegato e milza
- Rx torace ed eco addome
- Profilo biochimico per funzionalità renale, enzimi epatici e bilirubinemia, uricemia, LDH, beta-2-microglobulinemia. Coombs
- Elettroforesi: possibile ipogammaglobulinemia

- Valutazione dei fattori di rischio importanti per decidere il tipo di terapia nel paziente giovane (< 65 anni)
  - stadio clinico secondo Rai o Binet
  - lesioni citogenetiche
  - beta-2-microglobulinemia
  - CD38
  - profilo mutazionale geni IgVH

Sintomi e segni

Assenti nella > parte dei casi alla diagnosi

Adenopatie (raro bulky)

Splenomegalia

Sistemici per neoplasie linfoidi

Astenia ed ittero per anemia emolitica autoimmune

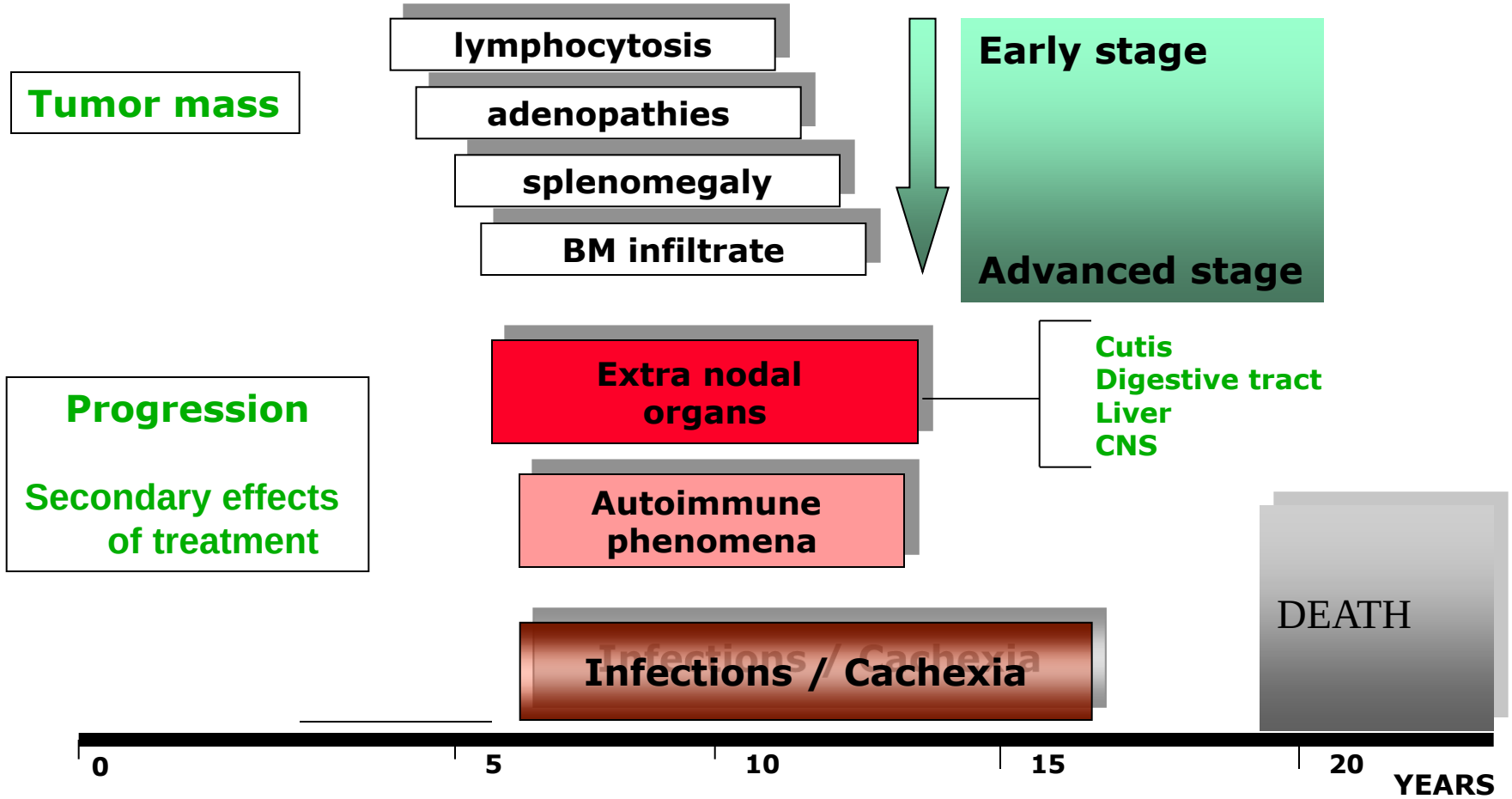
Astenia per anemia

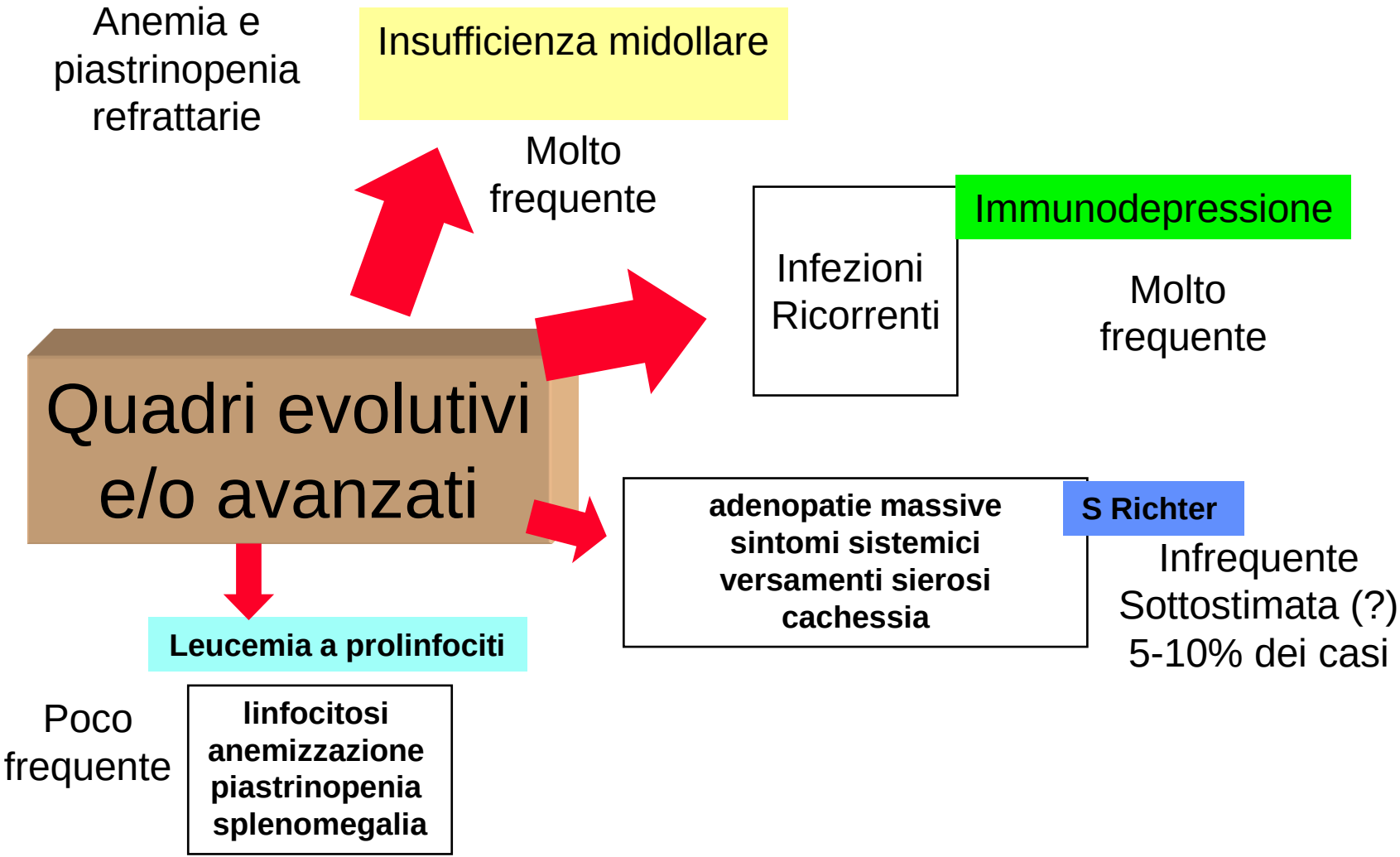
Emorragie per piastrinopenia (da insuff midollare o autoimmune)

Localizzazioni extra nodali (cute SNC, digerente)

Infezioni ricorrenti

CLL: single disease with variable clinicobiologic features




Anemia e piastrinopenia refrattarie
Insufficienza midollare
Molto frequente
Immunodepressione
Infezioni Ricorrenti
Molto frequente
Quadri evolutivi e/o avanzati
adenopatie massive
sintomi sistemici
versamenti sierosi
cachessia
S Richter
Infrequente
Sottostimata (?)
5-10% dei casi
Leucemia a prolinfociti
Poco frequente
linfocitosi
anemizzazione
piastrinopenia
splenomegalia

Stadiazione della LLC

Linfocitosi

milza

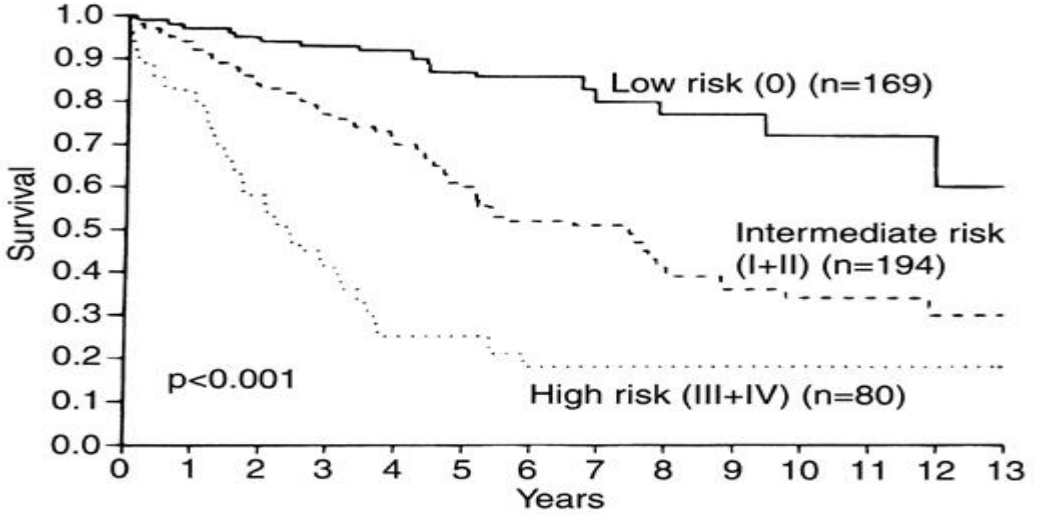
Sostituzione midollare con insufficienza funzionale

Table 79-5 Rai Clinical Staging Systems

Level of Risk	Stage	Description
Low*	0	Lymphocytosis only (in blood and marrow)
Intermediate	I	Lymphocytosis plus enlarged nodes
	II	Lymphocytosis plus enlarged spleen or liver with or without enlargement of nodes
High*	III	Lymphocytosis plus anemia (hemoglobin <110 g/L) with or without enlarged nodes, spleen, liver
	IV	Lymphocytosis plus thrombocytopenia (platelets $<100 \times 10^9$/L) with or without anemia or enlarged nodes, spleen, liver

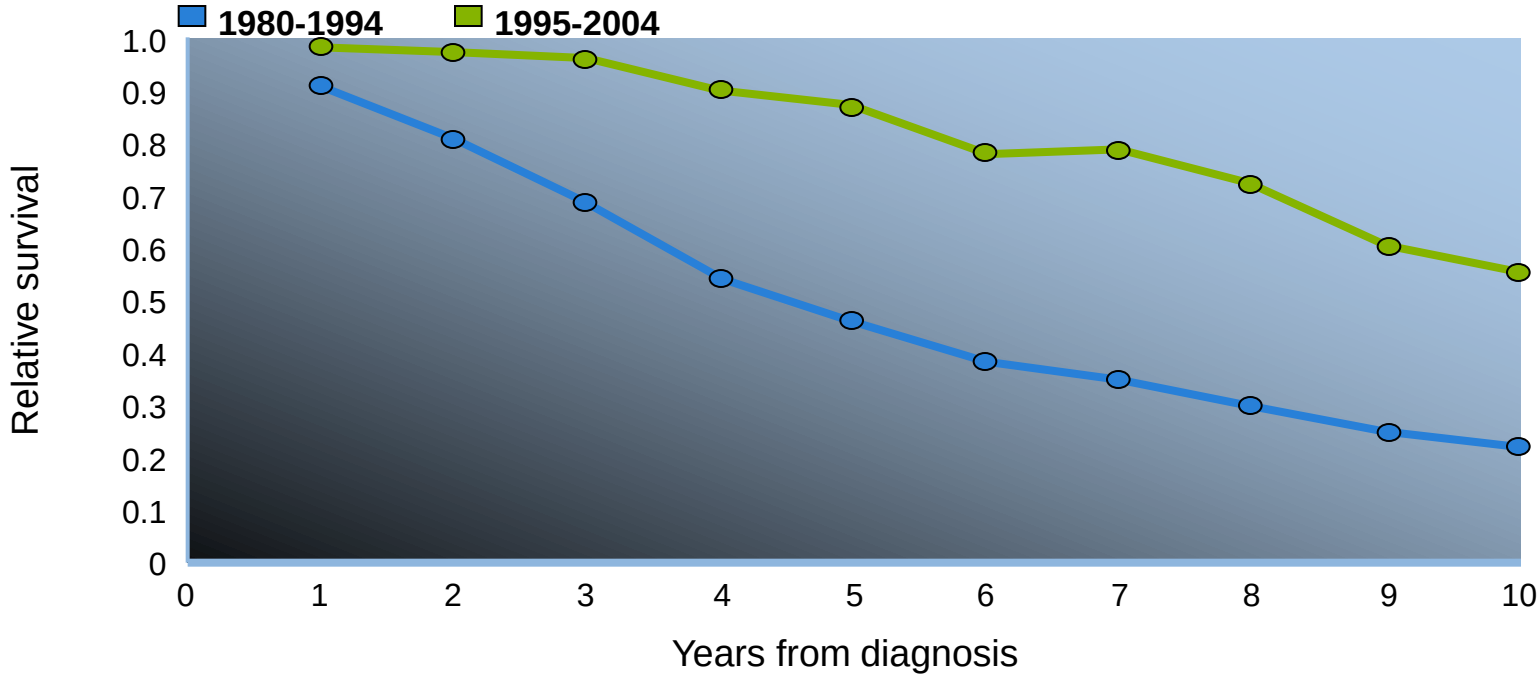
*Modified Rai system.

Survival in CLL depending on clinical stage (historical data, before the development of effective treatemnt strategies)



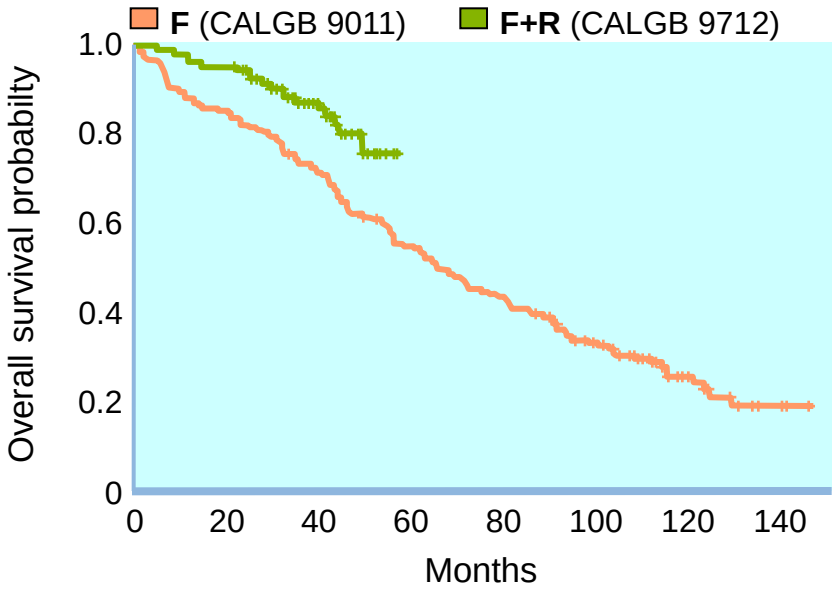
(From Montserrat E, Rozman C: Chronic lymphocytic leukaemia: Prognostic factors and natural history. Baillieres Clin Haematol 6:849, 1993.)

Ten-year relative survival curves for patients younger than 70 years in Binet stage B/C according to whether they were diagnosed in the calendar periods 1980-1994 or 1995-2004

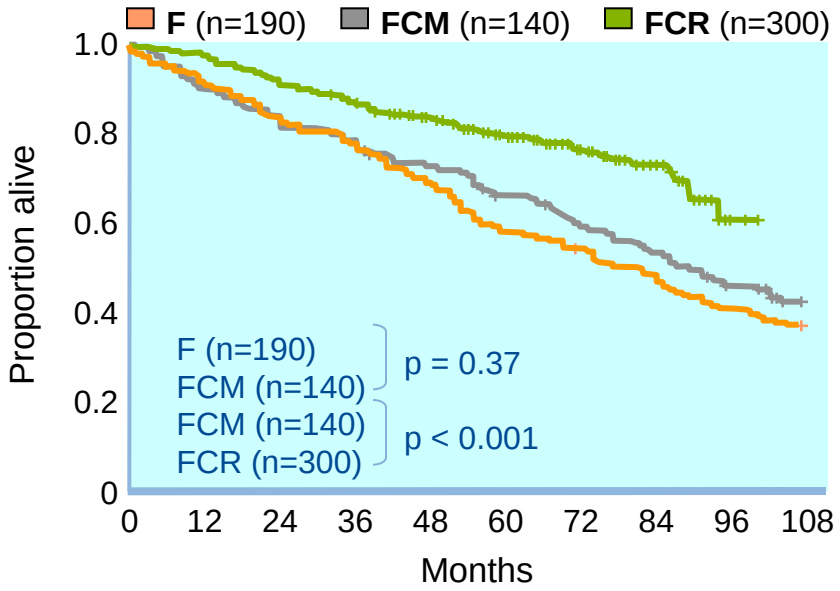


Improving survival in patients with CLL (1980-2008): the Hospital Clinic of Barcelona experience. *Blood* 2009 114: 2044-2050

Improved survival with chemoimmunotherapy

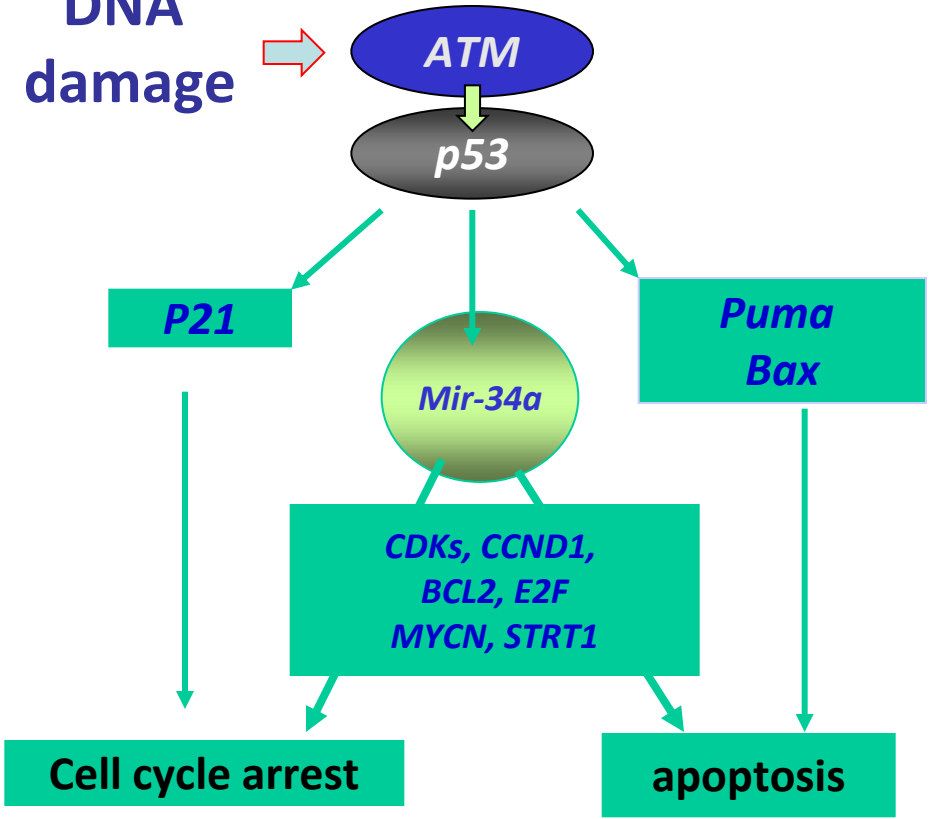


Byrd et al. *Blood*, 2005

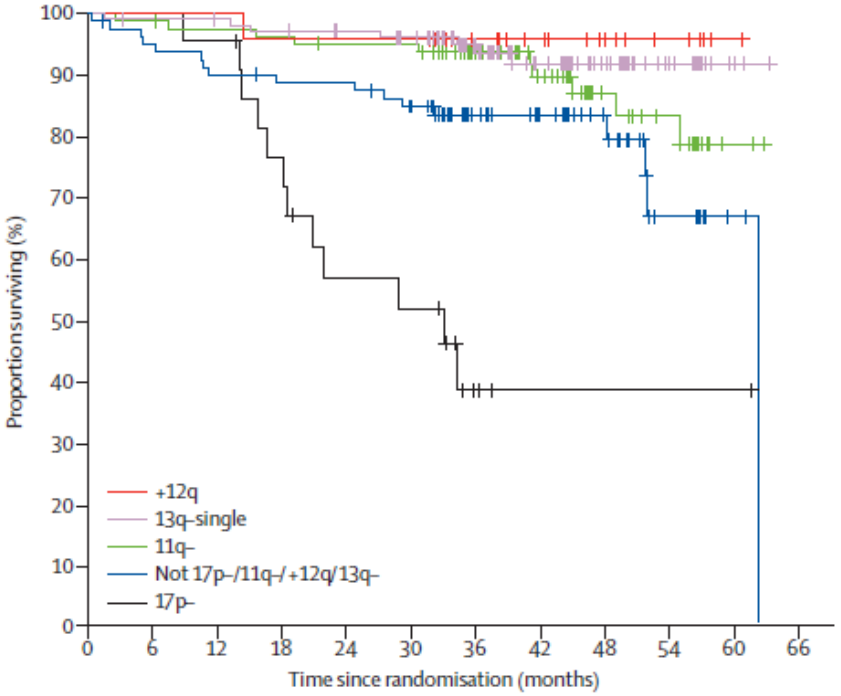


Tam, C. S. et al. *Blood* 2008;112:975-980

Chemotherapy resistance network in CLL

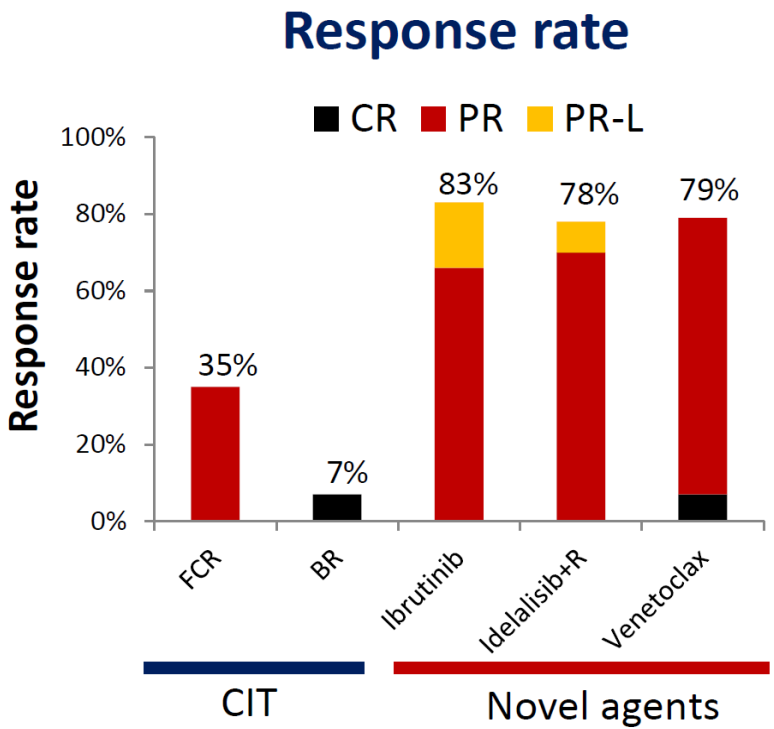


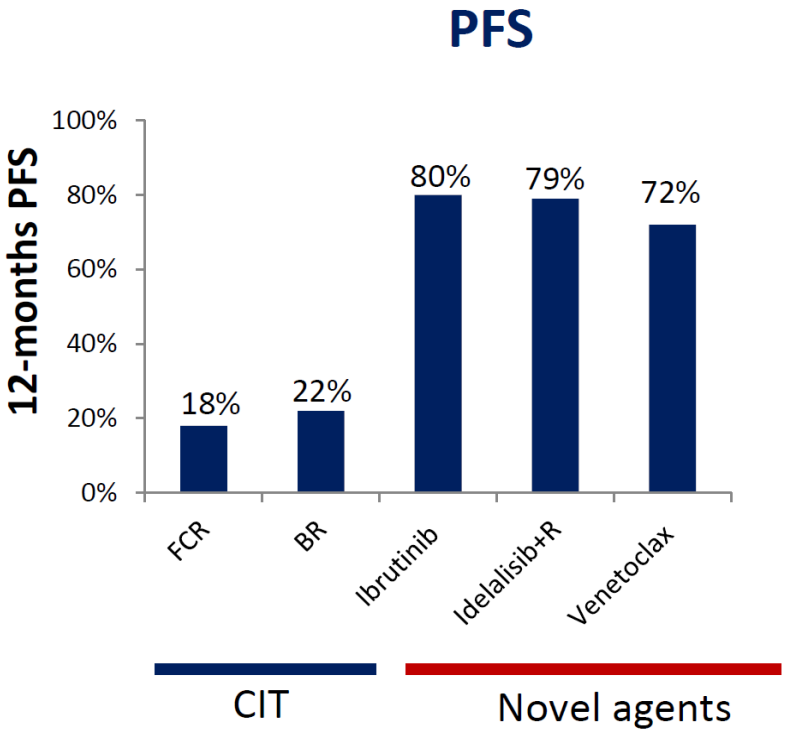
Overall survival with FCR in the CLL8 trial



Hallek M et al. Lancet 2010; 376: 1164–74

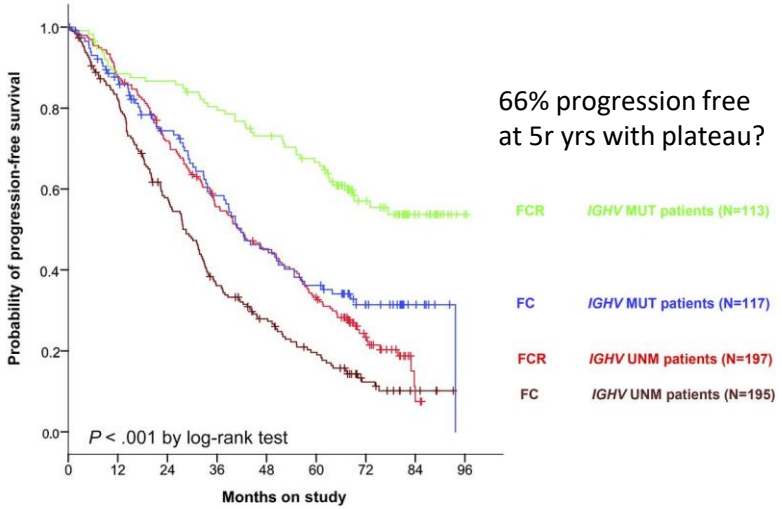
CIT versus Novel Agents in TP53 disrupted CLL



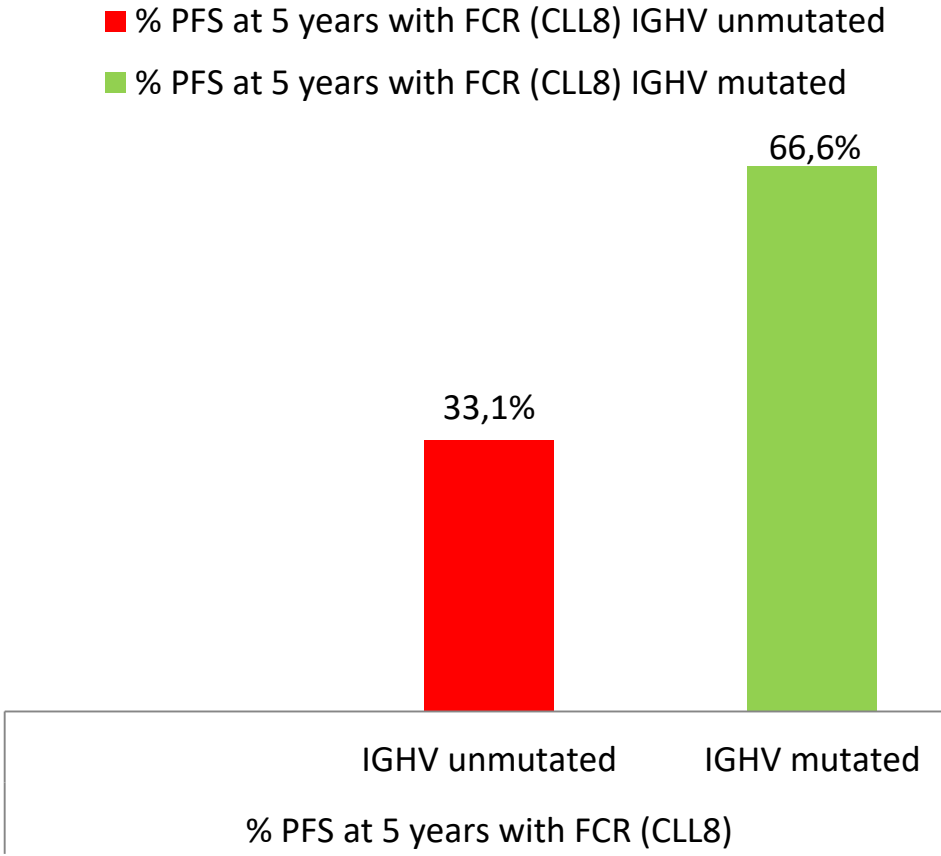


Badoux 2011, Fisher 2011, O'Brien 2016, Sharman 2014, Byrd 2015, Stilgenbauer 2016

Long term follow up in *IGHV* mutated CLL treated with FCR shows a PFS plateau: data from the CLL8 (GCLLSG) trial



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

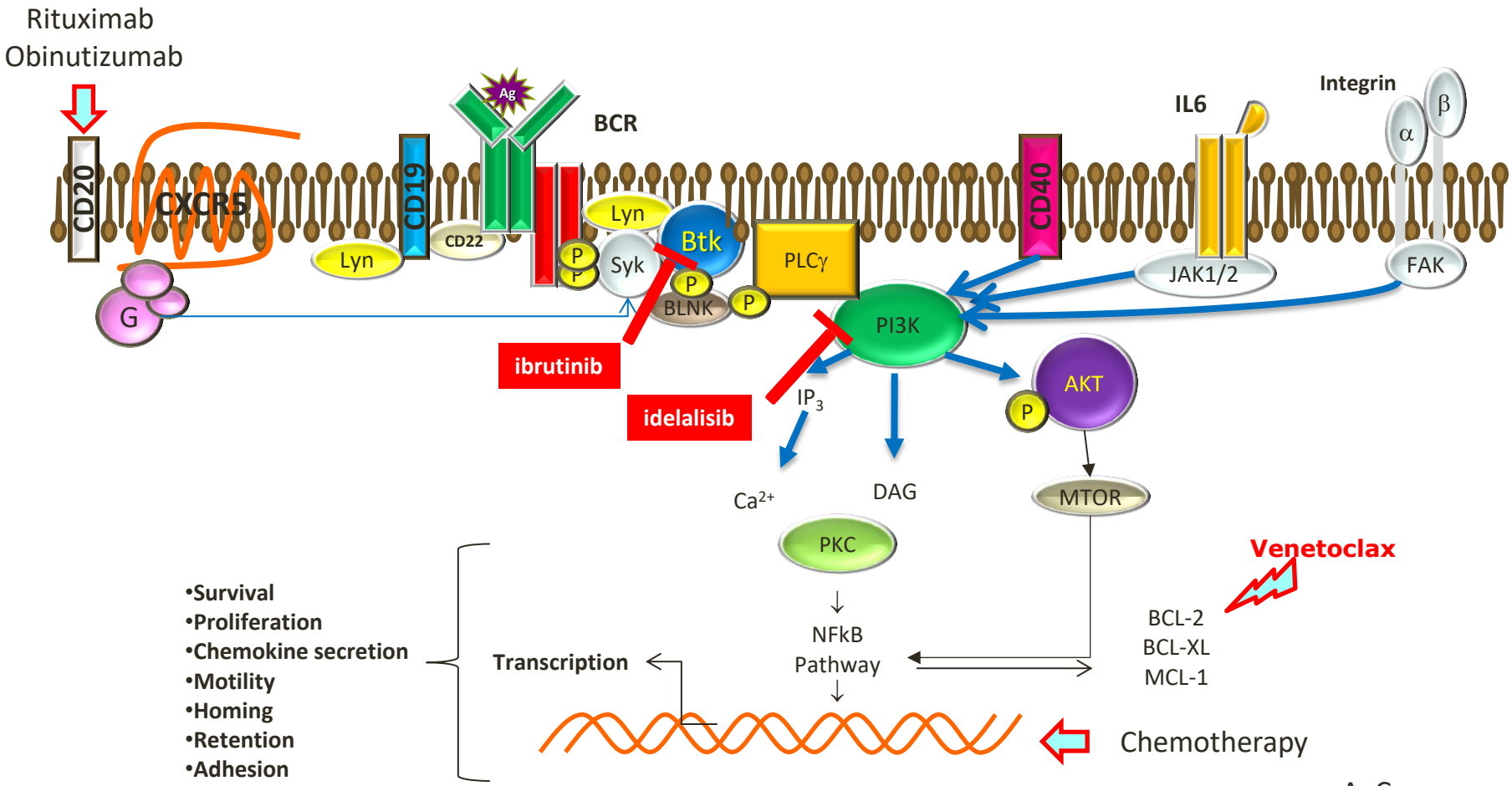


Fischer K et al. Blood. 2016;127(2):208-215

Indicazioni al trattamento

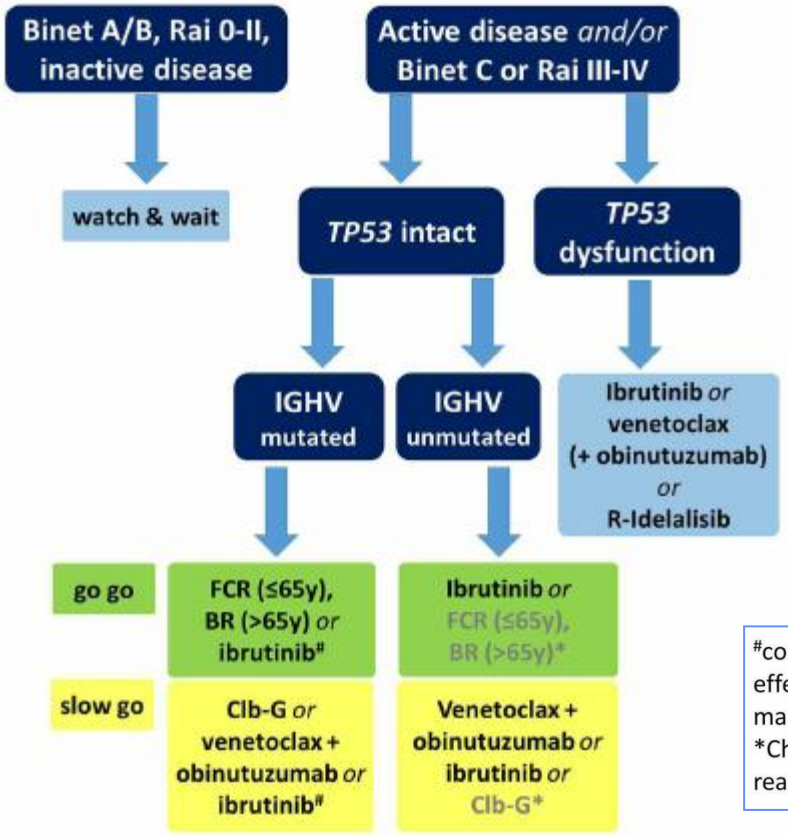
- Aumento dei GB con raddoppio < 6 mesi
- Stadio avanzato (III o IV Rai)
- Passaggio a stadio più avanzato
- Sviluppo di adenopatie o splenomegalia progressive
- Anemia emolitica autoimmune poco responsiva allo steroide
- Sintomi sistemici
- GB > 300.000 / ul

Therapeutic targets in CLL



A. Cuneo

First line treatment of CLL based on predictive factors



#consider and discuss with patient: long-term *vs.* fixed-duration therapy, specific side effects of each therapeutic option (myelosuppression, infections, secondary malignancies, cardiac toxicities, bleeding, autoimmune disorders);
*Chemoimmunotherapy as alternative treatment only if no TP53 dysfunction and reasons against continuous treatment with ibrutinib or non-availability

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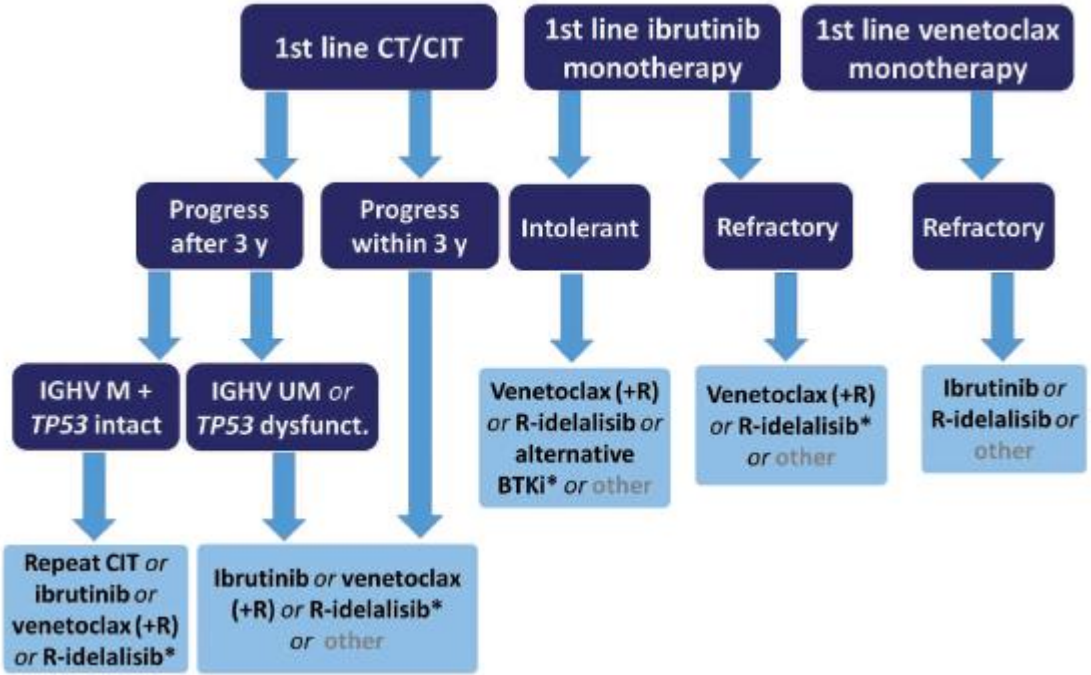
Proposed sequencing of therapy according to first-line treatment; approved options.



*consider and discuss with patient: long-term *vs.* fixed-duration (CIT: 6 months; venetoclax+R: 24 months) therapy, specific side effects of each therapeutic option (myelosuppression, infections, secondary malignancies, cardiac toxicities, bleeding, autoimmune disorders).

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