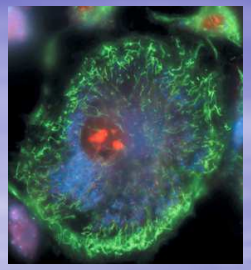


Belgische Vereniging voor Analytische Cytologie vzw.
Association Belge de Cytologie Analytique asbl.
Belgian Society for Analytical Cytology.

SCK•CEN
STUDIECENTRUM VOOR KERNENERGIE
CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE

Module n°: parallel lecture 7a (clinical)

Title : Prognosis in CLL

Lecturer : Jan Philippé

Molecular Biology and Cytometry Course, May 7-8 2009, Mol

UNIVERSITEIT GENT

Universitair Ziekenhuis Gent

Prognostic Factors in B-CLL

Jan Philippé

Mol, May 8, 2009

Clinical course & clinical response to treatment are highly variable in CLL

↓

Intensified search for biological factors predicting the clinical course & response to drugs

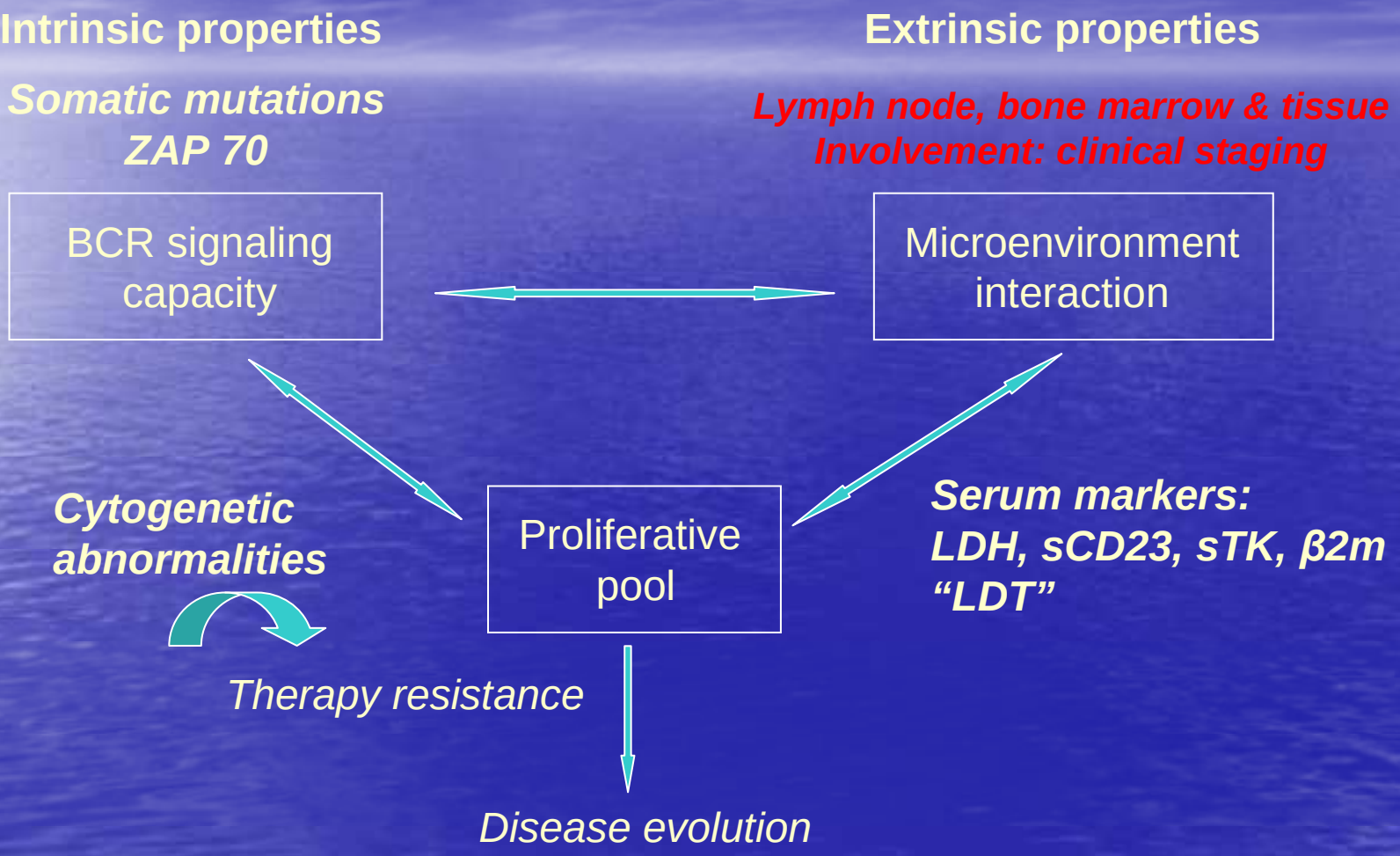
↓

Which patients will benefit from intensive therapy at an early stage?

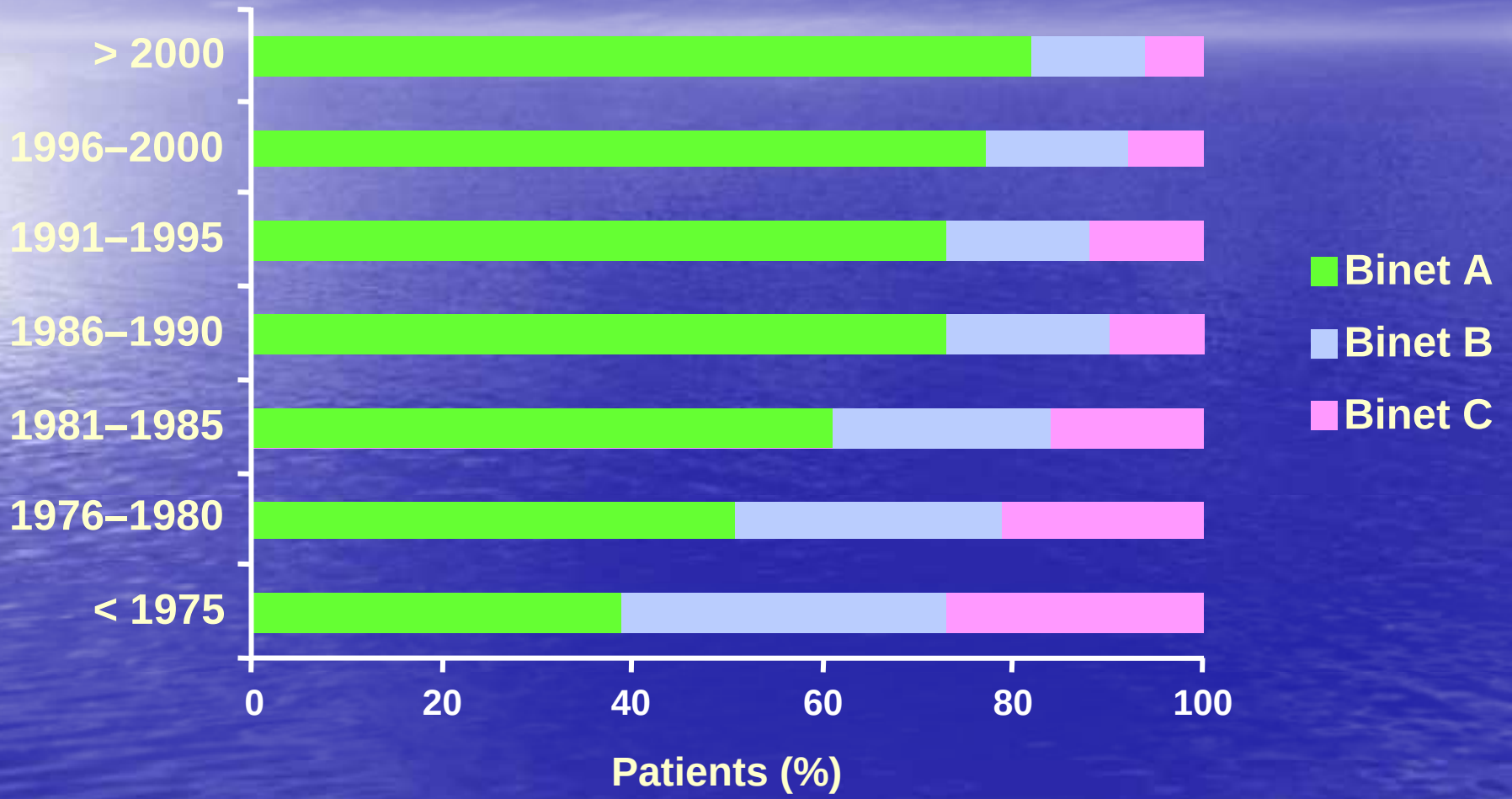
(Or 'watch & wait'?)

Which drugs should be selected in the treatment of a unique patient?

Currently used **prognostic biomarkers** & relationship with **pathophysiology**



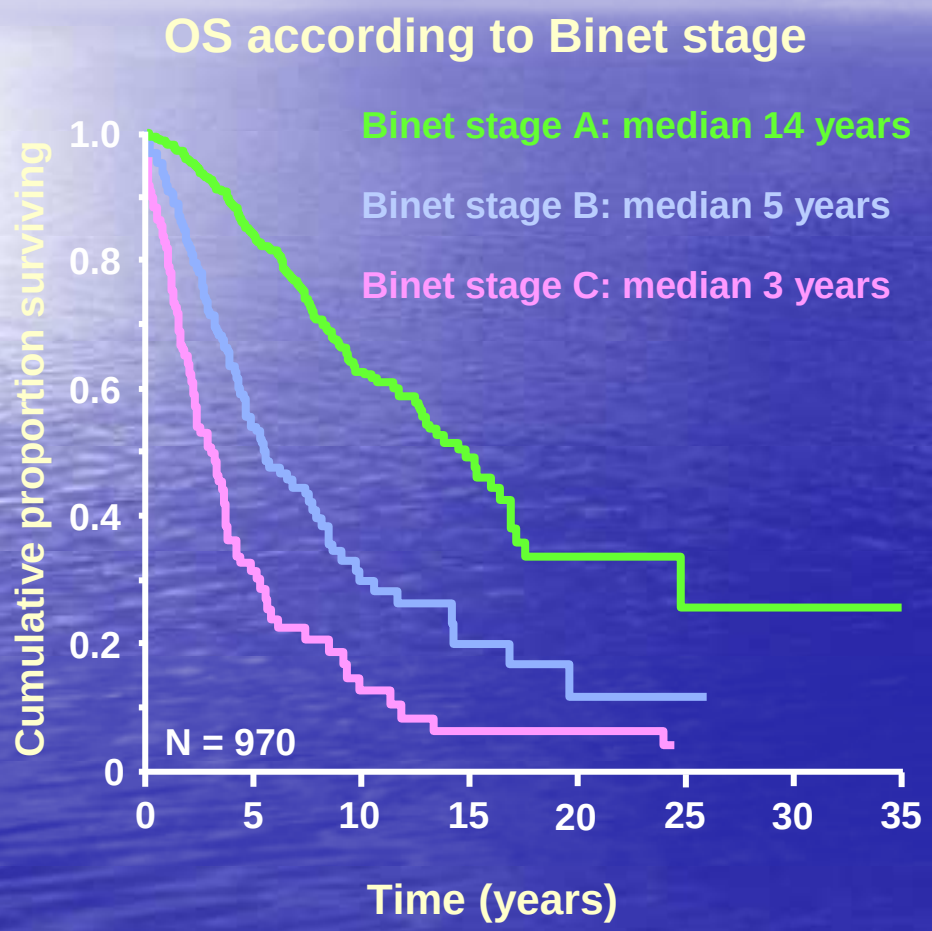
Staging systems according to Rai and Binet

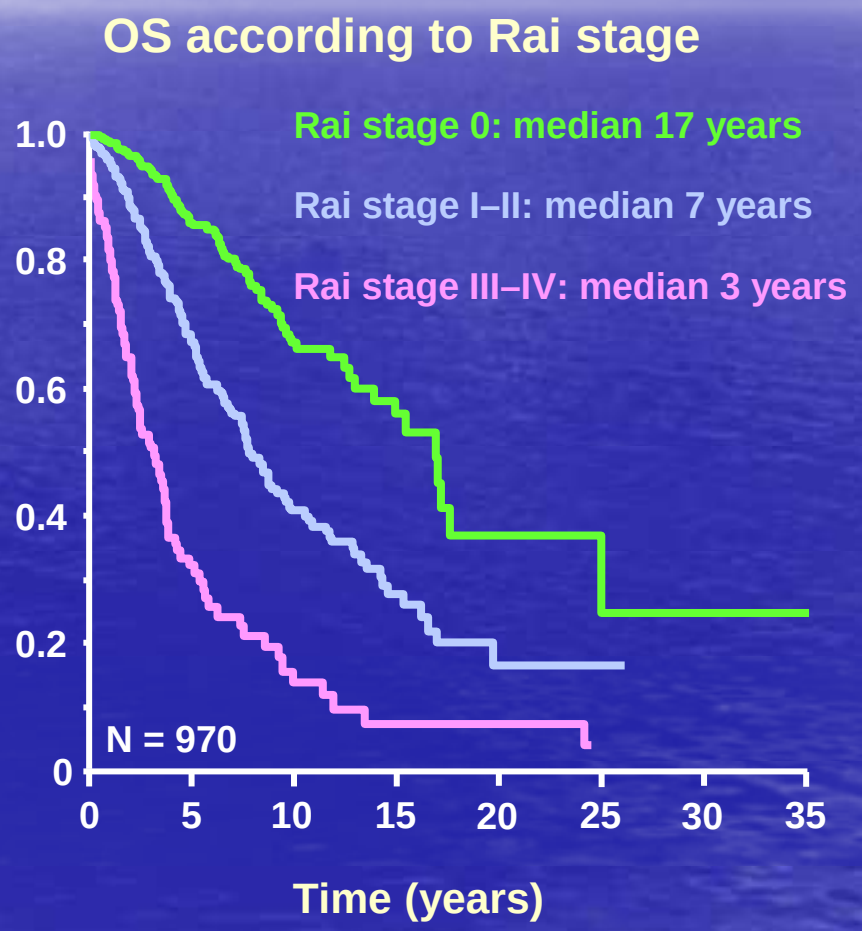


n = 1200

Hospital Clinic Provincial (HCP) Barcelona.

Staging systems according to Rai and Binet

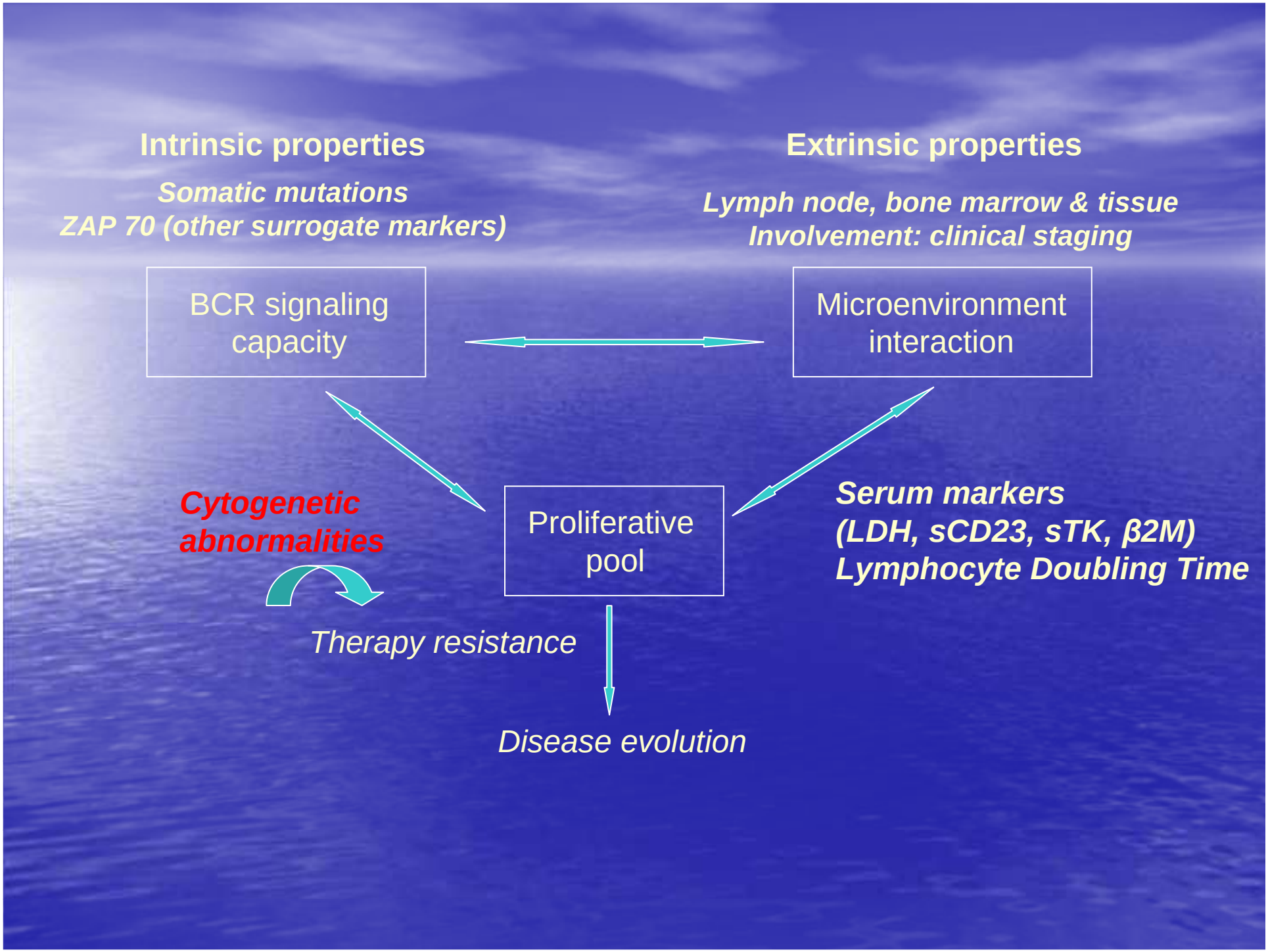




Hospital Clinic Provincial (HCP) Barcelona.

Staging systems according to Rai and Binet

- Strengths
  - Simple
  - Extensively validated
- Weaknesses
  - Too many patients in the low grade stages w/o a distinction between indolent and progressive disease
  - Lack of prediction of therapy (drug) response


Intrinsic properties
Somatic mutations
ZAP 70 (other surrogate markers)
Extrinsic properties
Lymph node, bone marrow & tissue
Involvement: clinical staging
BCR signaling capacity
Microenvironment interaction
Cytogenetic abnormalities
Proliferative pool
Serum markers
(LDH, sCD23, sTK, β2M)
Lymphocyte Doubling Time
Therapy resistance
Disease evolution

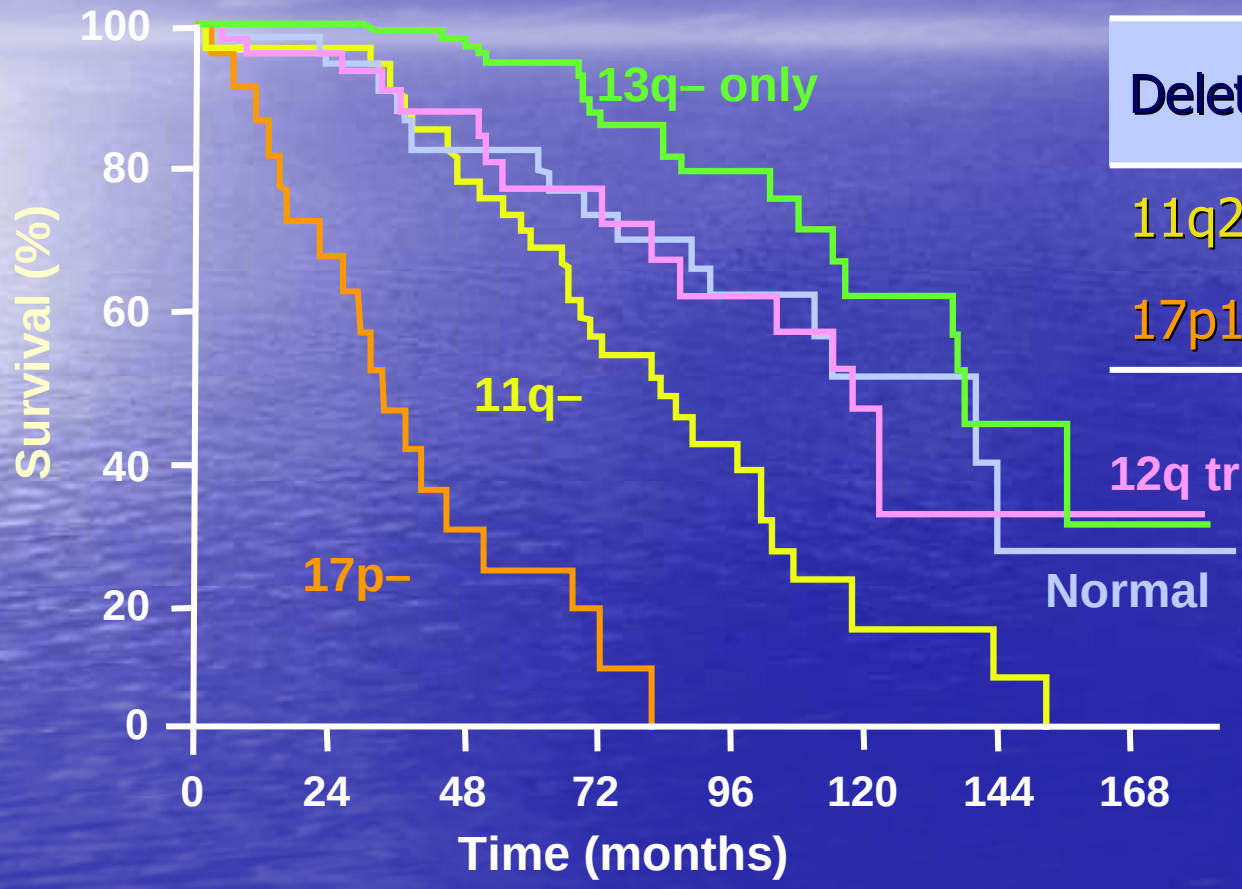
Chromosomes in B-CLL

- Conventional cytogenetics 1990
- t(12) 16%
- 13q- 13%
- 14q- 4%
- 11q- 3%

- Interphase FISH 1999
- 13q- 55%
- 11q- 18%
- del 17p 7%
- t(12) 16%
- del 6q 6%
- 14q- 4%

Stilgenbauer, IWCLL 1999

Cytogenetic aberrations and OS



Deletion	Tumour suppressor
11q22–23	↓ ATM
17p13–	↓ p53

N = 325

Döhner H, N Engl J Med 2000

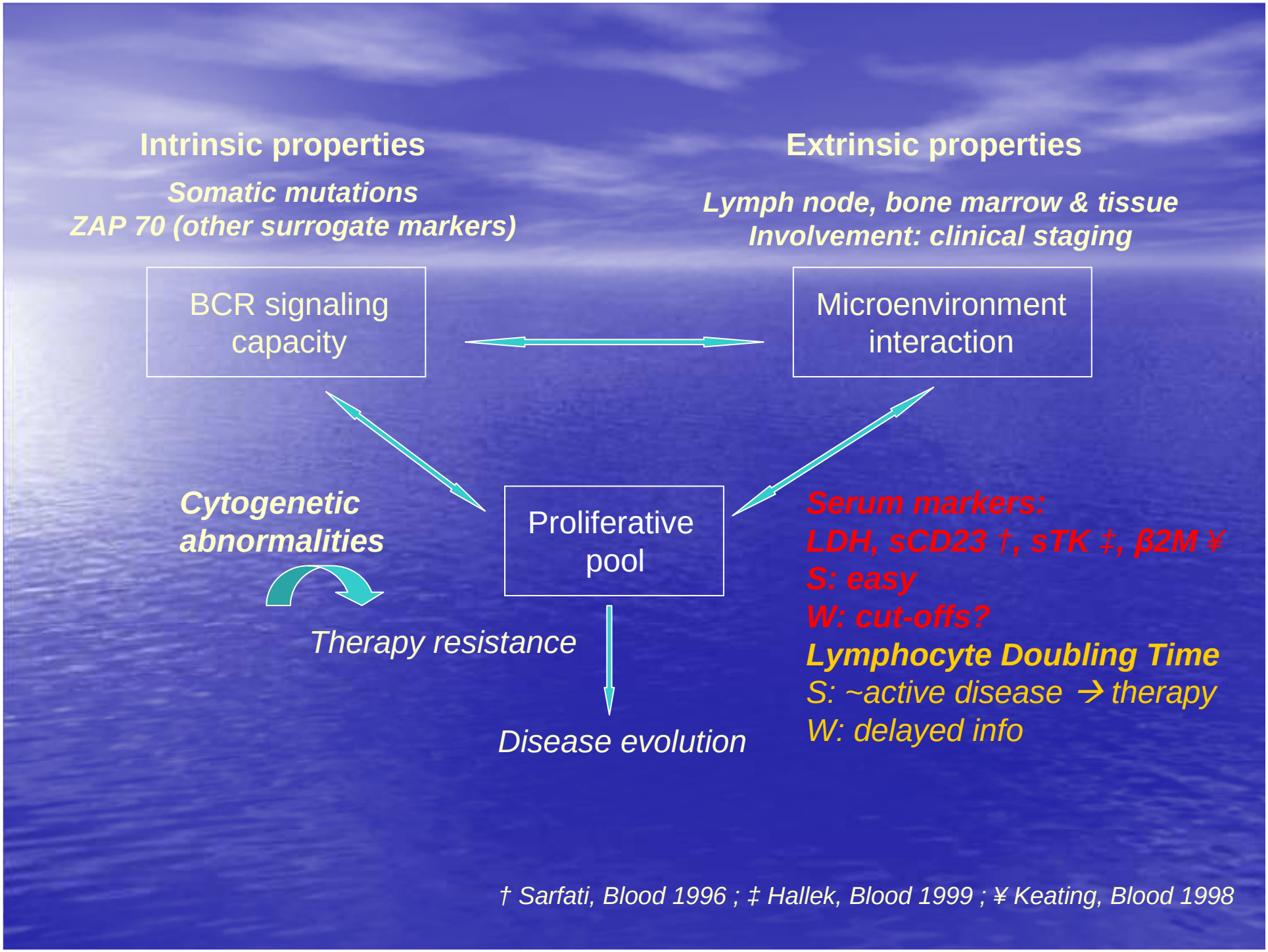
Importance of conventional cytogenetics

- Recent protocols → 97% success with CCA
- B-cell mitogens : 12-O-tetradecanoylphorbol-13-acetate (TPA)
  CD40L
  CpG-oligodeoxynucleotides
- Chromosomal aberrancies in 62% after CdA
  - 25% single
  - 17% double
  - 20% multiple
- Complex karyotype ~ TFS and OS

E. Van Den Neste, Leukemia 2007

FISH

- Strengths
  - Technical & clinical validation, however…
  - Prognostic value on top of other markers
  - Predicts therapy response/resistance
    - 17p-: fludarabin & rituximab resistance
    - 11q-: alemtuzumab resistance
- Weaknesses
  - Incongruent results if scores between 5-20% for 17p-, knowing that these scores are ~ unfavorable prognosis
  - In pretreated patients, CCA more info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Lymph node, bone marrow & tissue
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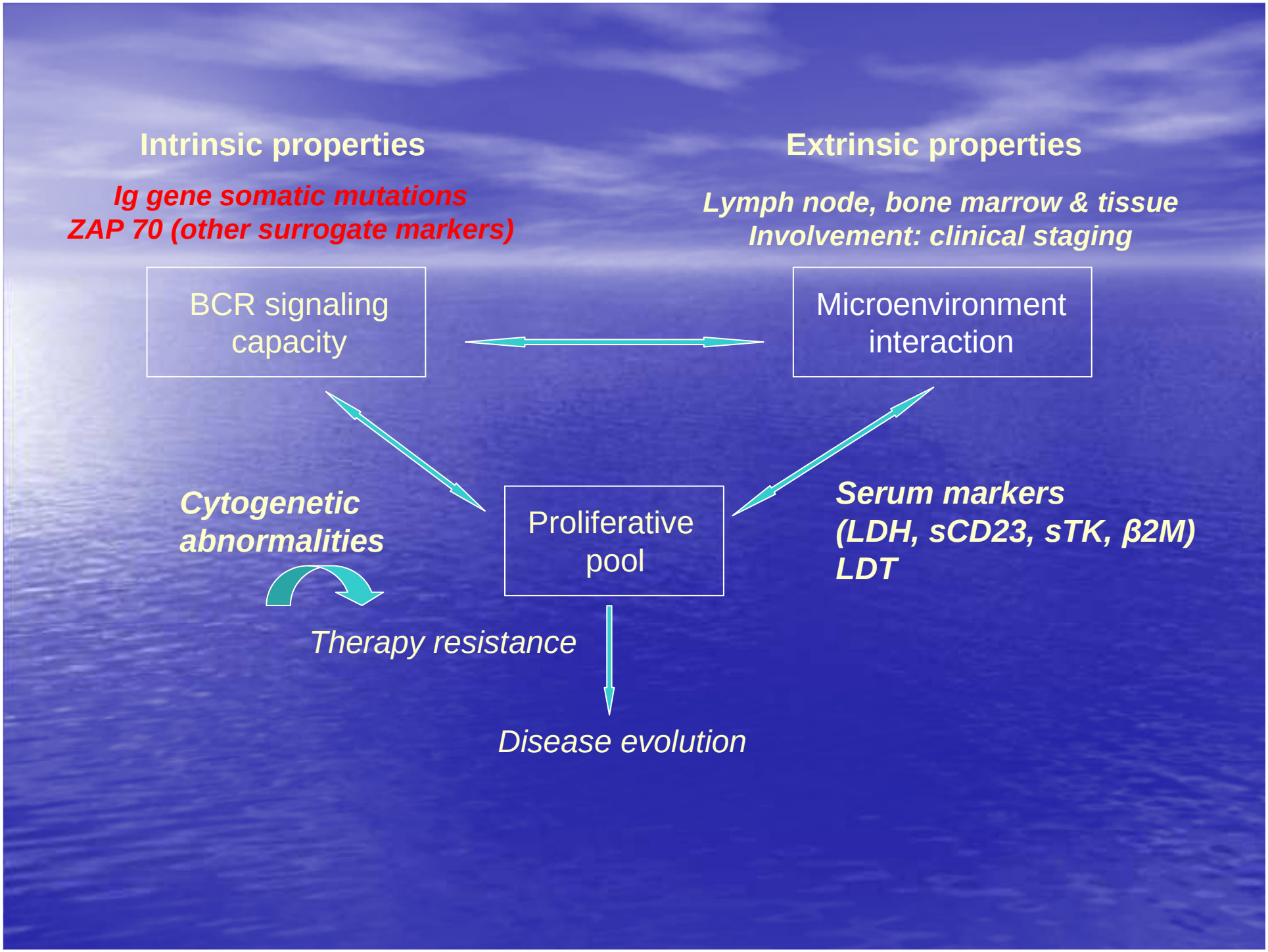
Cytogenetic abnormalities
Proliferative pool
Therapy resistance
Disease evolution
Serum markers:
LDH, sCD23 †, sTK ‡, β2M ¥
S: easy
W: cut-offs?
Lymphocyte Doubling Time
S: ~active disease → therapy
W: delayed info
† Sarfati, Blood 1996 ; ‡ Hallek, Blood 1999 ; ¥ Keating, Blood 1998

Serum markers

- Several studies have found that serum markers CD23, thymidine kinase, and ß2-microglobulin may predict survival or progression-free survival.

- Assays for these markers should be standardized and used in prospective clinical trials to validate their relative value to the management of patients with CLL


Intrinsic properties
Ig gene somatic mutations
ZAP 70 (other surrogate markers)
Extrinsic properties
Lymph node, bone marrow & tissue
Involvement: clinical staging
BCR signaling capacity
Microenvironment interaction
Cytogenetic abnormalities
Proliferative pool
Serum markers
(LDH, sCD23, sTK, β2M)
LDT
Therapy resistance
Disease evolution

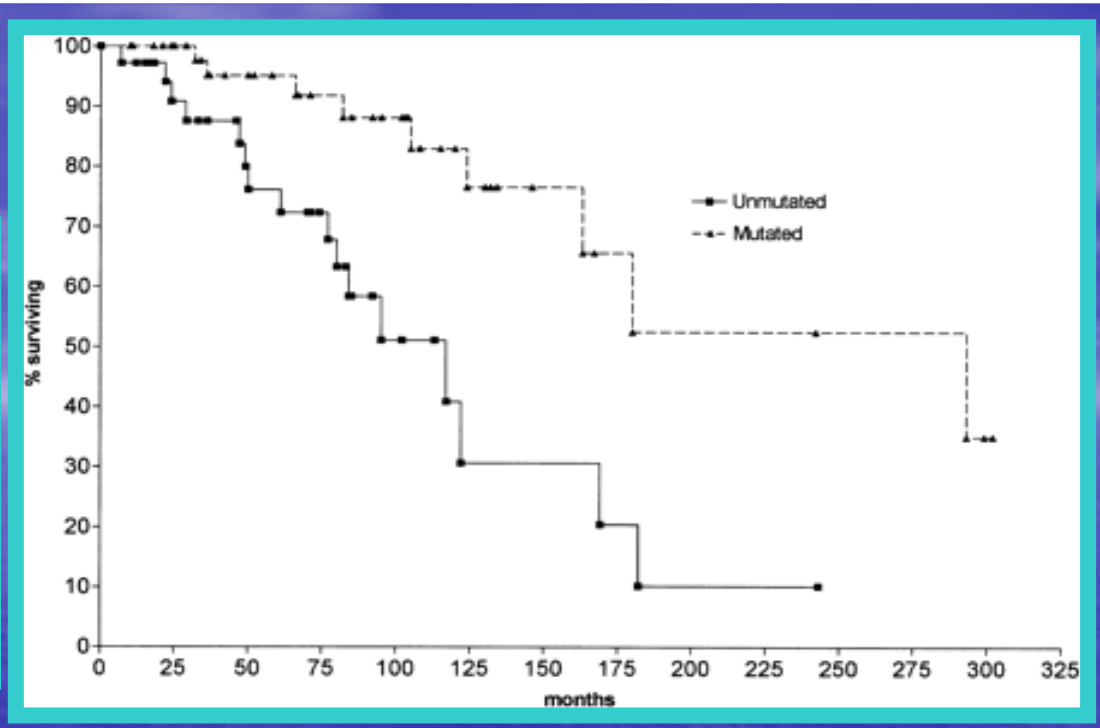
IgVH mutation-analysis

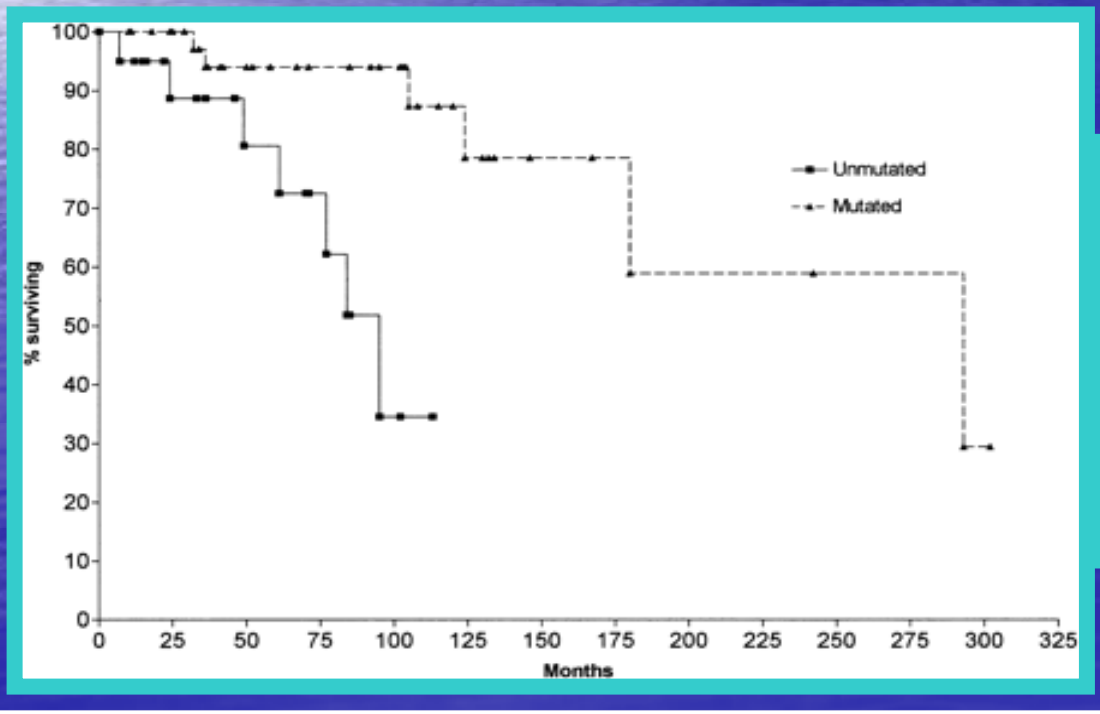
Median survival time

All stages

Mutated: 293m

Unmutated 117m





Median survival time stage A

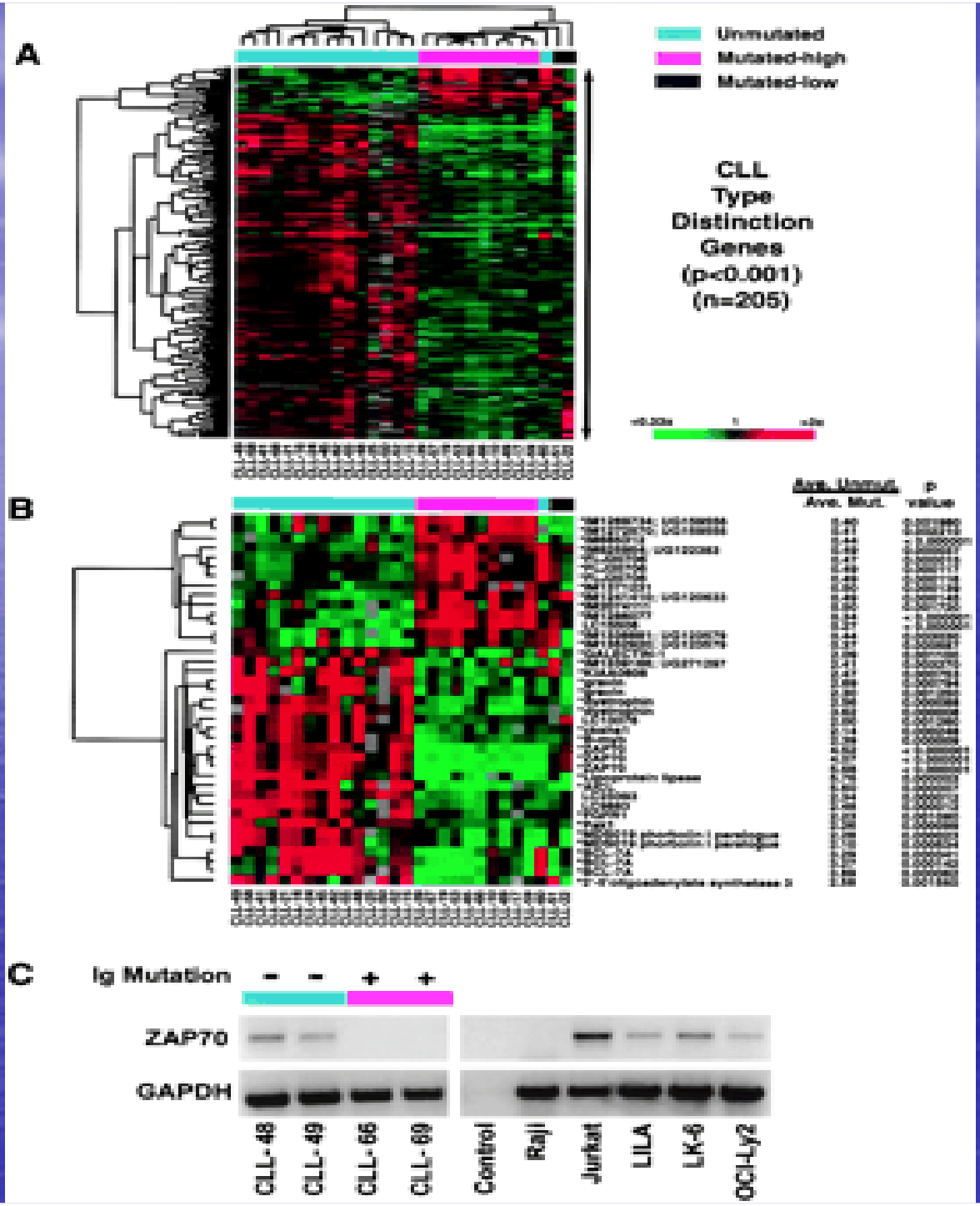
Mutated: 293m

Unmutated: 95m

Hamblin, Blood 1999

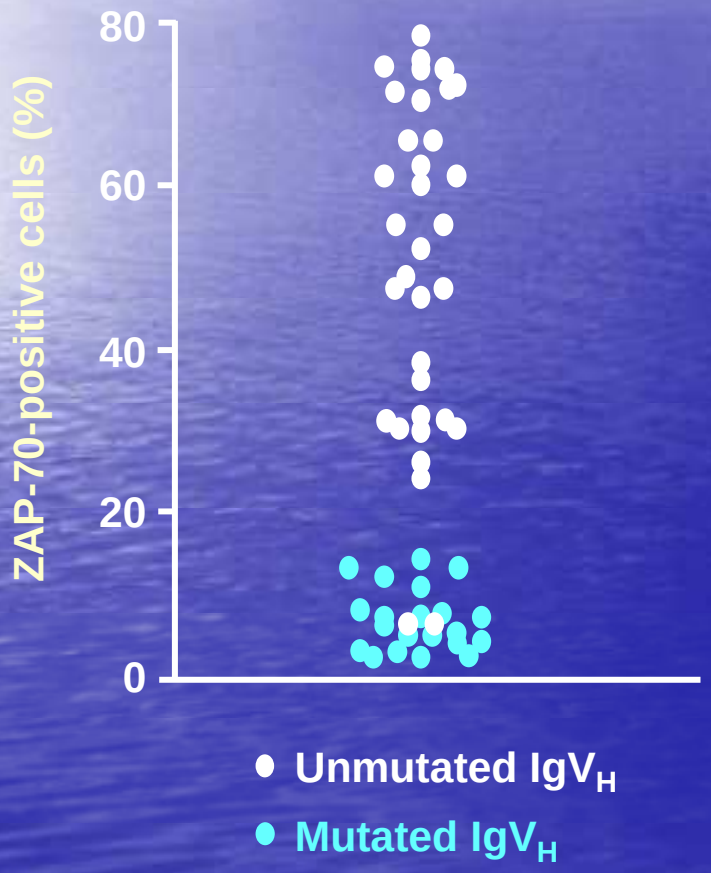
Weaknesses IgV_H mutation-analysis

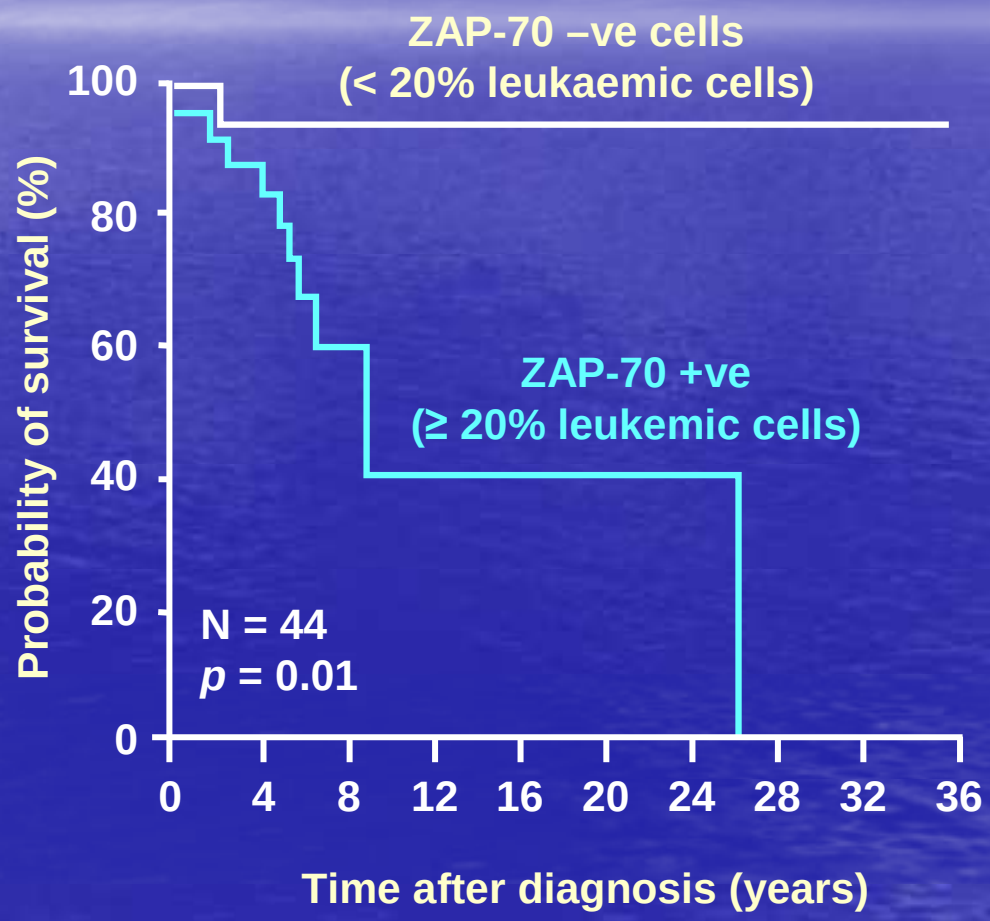
- V_H3.21 expression ~ worse prognosis
- Lack of standardisation
  - Cut-off: 2%, 3%?
  - Which reference database for germline sequencies?
    VBASE, GenBank/IgBlast, IMGT? *Davi, Leukemia 2008*
  - Educational workshops (Paris, Sept 25-26, 2008).
- Labour intensive → search for surrogate markers



Rosenwald et al.
JEM 2001

ZAP-70 as surrogate marker





Crespo M, N Engl J Med 2003

ZAP-70 expression measured by FC

Publication	Pts (n)	Endpoint	Median time to endpoint (years)		*p*-value
			ZAP-70 –ve	ZAP-70 +ve	
Bosch *et al.* 2004[1]	222	TTP	NR	3.3	< 0.001
		OS	16.3	8.5	< 0.001
Orchard *et al.* 2004[2]	167	OS	24.4	9.3	< 0.001
Rassenti *et al.* 2004[3]	307	TTT	9.2	2.9	< 0.001
Krober *et al.* 2006[4]	133	TFS	7.16	2.5	0.005
		OS	NR	8.5	0.004
del Principe *et al.* 2006[5]	289	PFS	NR	3.3	< 0.001
		OS	NR	12	< 0.001

NR = not reported

1. Bosch F, et al. Blood 2004; 104:Abstract 14; 2. Orchard JA, et al. Lancet 2004; 363:105–111; 3. Rassenti LZ, et al. N Engl J Med 2004; 351:893–901; 4. Krober A, et al. J Clin Oncol 2006; 24:969–975; 5. del Principe MI, et al. Blood 2006; 108:853–861.

ZAP-70

- Strengths
  - ZAP-70 might be better than U/M as prognostic marker

Rassenti, NEJM 2004

- Weaknesses
  - Clustering of 17p- samples within the ZAP-70- group
  - Lack of standardisation of analysis
    - Immunohistochemically
    - RT-PCR *(Van Bockstaele, Clin Cyt 2006 ; Stamatopoulos, Clin Chem 2007)*
    - Flow cytometry
      - Minimal recommendation by ERIC *(Cymbalista, Atlanta 2007)*
      - Every lab its own method?

Other surrogate markers

- **CD38** *Damle and Chiorazzi, Blood, 1999*
  - **S: relatively easy, however…**
  - **W: Lack of analytical validation**

- **LPL** *Oppezzo, Blood 2005 ; Heintel, Leukemia 2005*
  - **S: Can be analysed in whole blood** *Van Bockstaele Clin Chem 2007*
  - **W: Lack of analytical validation**

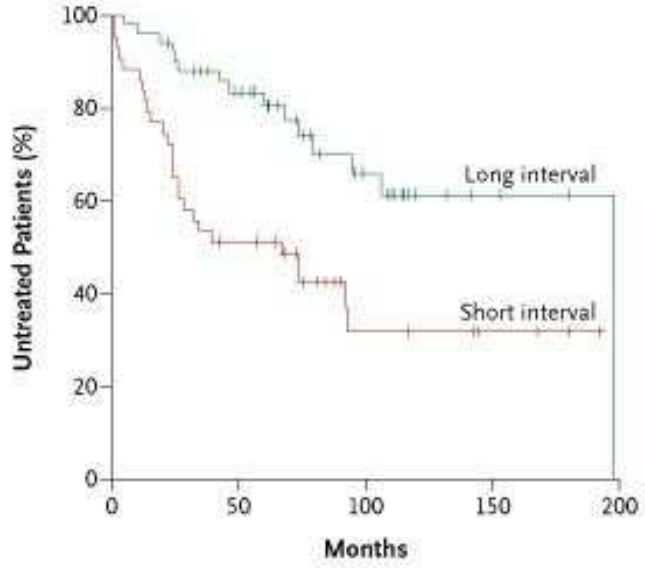
- **PEG10, Septin 10, Dystrophin, AICD, Telomerase, CLLU1, CD49d, BCL-2 polymorphism, +DAT, sVEGF, Sarcoglycan ε, L-selectin, Integrin-β2….**

Dynamic marker: MRD analysis

- A measure of outcome,
  but potentially serves to guide therapy in individual patients to tailor maintenance treatment with alemtuzumab or rituximab
  (Moreton, JCO 2005 ; Montillo JCO 2006)
- Impact on transplant decisions?
- Analytical approaches
  - Real-time quantitative ASO-PCR
  - Multicolor FC *(Rawstron, Leukemia 2007)*
    - CD5/CD19 , CD20/CD38 , CD81/CD22 , CD79b/CD43
    - Sensitivity 0.01%

MicroRNA and the Time from Diagnosis to Initial Therapy

A



B

MicroRNA	Level of Expression of MicroRNA	
	Short interval	Long interval
miR-181a	High	Low
miR-155	High	Low
miR-146	High	Low
miR-24-2	High	Low
miR-23b	High	Low
miR-23a	High	Low
miR-222	High	Low
miR-221	High	Low
miR-29c	Low	High

Calin, N Engl J Med 2005

MicroRNA Signature Associated with Prognostic Factors (ZAP-70 Expression and IgVH Mutations) and Disease Progression in Patients with CLL

Table 2. MicroRNA Signature Associated with Prognostic Factors (ZAP-70 Expression and IgV_H Mutations) and Disease Progression in Patients with CLL.*

Signature Component	MicroRNA Component	Chromosomal Location	P Value	Level of Expression in Group 1†	Putative Targets‡	Comment§
1	*miR-15a*	13q14.3	0.02	High	*BCL2, CNOT6L, USP15, PAFAH1B1, ESRRG*	Cluster *miR-15a–miR-16-1* Deleted in CLL and prostate carcinoma[10]
2	*miR-195*	17p13	0.02	High	*BCL2, CNOT6L, USP15, PAFAH1B1, ESRRG*	Deleted in hepatocellular carcinoma
3	*miR-221*	Xp11.3	0.01	High	*HECTD2, CDKN1B, NOVA1, ZFPM2, PHF2*	Cluster *miR-221–miR-222*
4	*miR-23b*	9q22.1	0.009	High	*FNBP1L, WTAP, PDE4B, SATB1, SEMA6D*	Cluster *miR-24-1–miR-23b* FRA 9D; deleted in urothelial carcinoma[13]
5	*miR-155*	21q21	0.009	High	*ZNF537, PICALM, RREB1, BDNF, QKI*	Amplified in a child with Burkitt's lymphoma[16]
6	*miR-223*	Xq12–13.3	0.007	Low	*PTBP2, SYNCRIP, WTAP, FBXW7, QKI*	Expression normally restricted to myeloid lineage[23]
7	*miR-29a-2*	7q32	0.004	Low	NA	Cluster *miR-29a-2–miR-29b-1* FRA7H; deleted in prostate carcinoma[13]
8	*miR-24-1*	9q22.1	0.003	High	*TOP1, FLJ45187, RSBN1L, RAP2C, PRPF4B*	Cluster *miR-24-1–miR-23b* FRA 9D; deleted in urothelial carcinoma[13]
9	*miR-29b-2*	1q32.2–32.3	<0.001	Low	NA	
10	*miR-146*	5q34	<0.001	High	*NOVA1, NFE2L1, C1orf16, ABL2, ZFYVE1*	
11	*miR-16-1*	13q14.3	<0.001	High	*BCL2, CNOT6L, USP15, PAFAH1B1, ESRRG*	Cluster *miR-15a–miR-16-1* Deleted in CLL and prostate carcinoma[10]
12	*miR-16-2*	3q26.1	<0.001	High	Same as for *miR-16-1*	Identical to *miR-16-1*
13	*miR-29c*	1q32.2–32.3	<0.001	Low	NA	

Calin G et al. N Engl J Med 2005;353:1793-1801

* All the members of the signature are mature microRNAs. NA denotes not available, and FRA fragile site.
† Group 1 includes patients with unmutated IgV_H and high expression of ZAP-70, both of which are predictors of poor prognosis.
‡ The top five putative targets identified with use of TargetScan at http://genes.mit.edu/targetscan[22] were included.
§ Specific gene names are available at www.ncbi.nlm.nih.gov/entrez.

Calin, N Engl J Med 2005

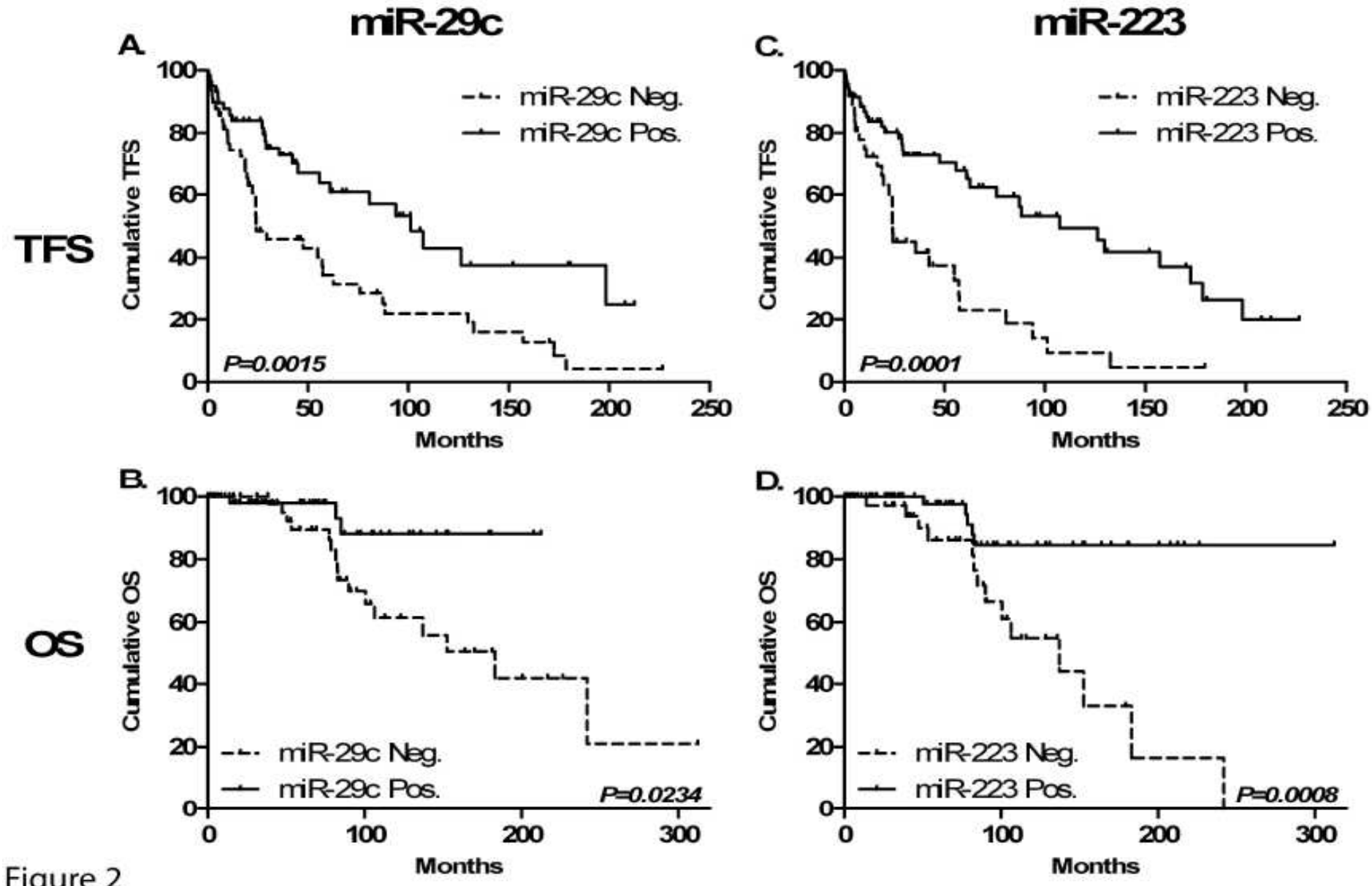


Figure 2

blood

Prepublished online Jan 14, 2009;
doi:10.1182/blood-2008-11-189407

MicroRNA-29c and microRNA-223 downregulation has in vivo significance in chronic lymphocytic leukemia and improves disease risk stratification

Basile Stamatopoulos, Nathalie Meuleman, Benjamin Haibe-Kains, Pascale Saussoy, Eric Van den Neste, Lucienne Michaux, Pierre Heimann, Philippe Martiat, Dominique Bron and Laurence Lagneaux

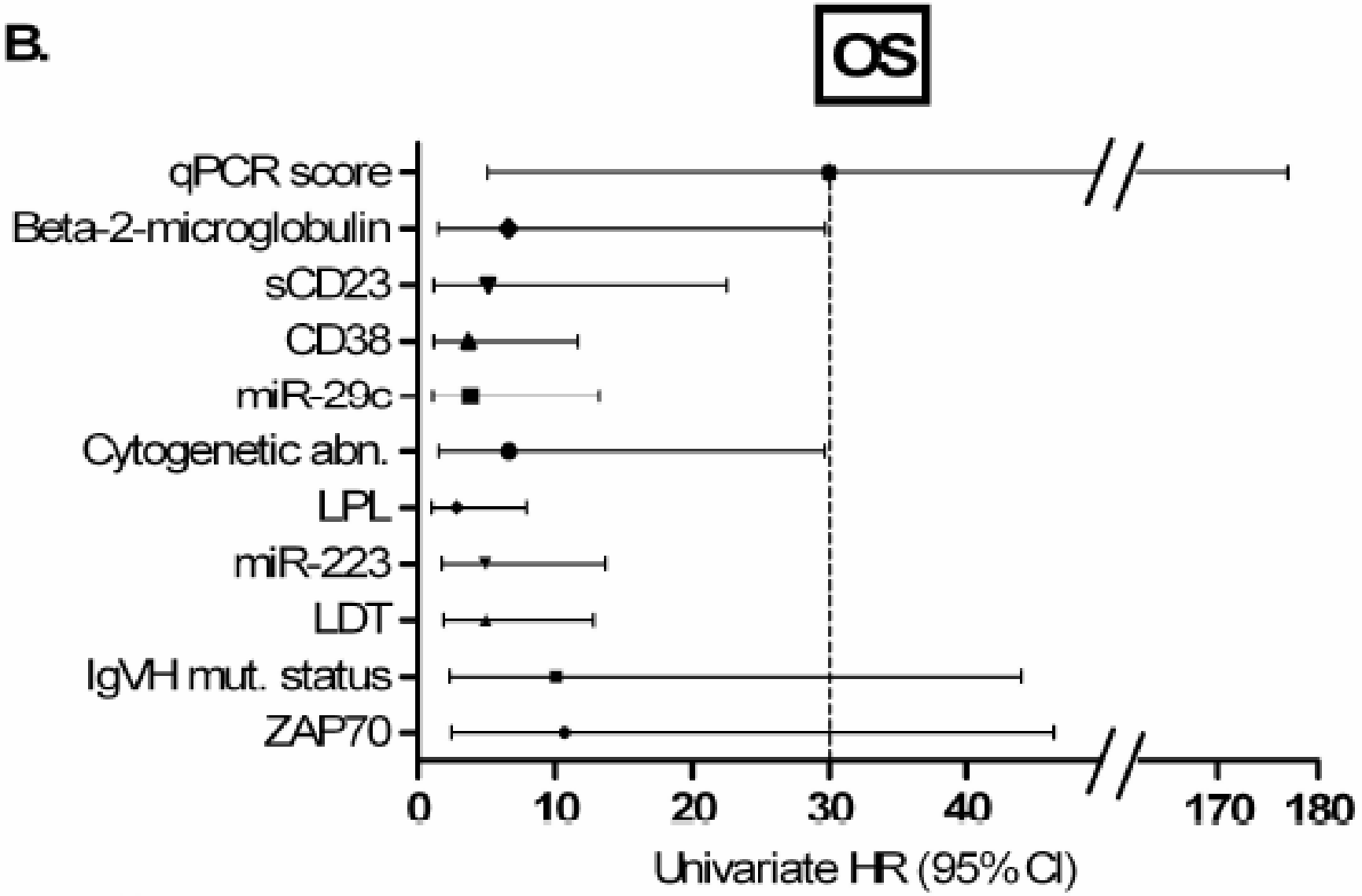


Figure 5

blood

Prepublished online Jan 14, 2009;
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MicroRNA-29c and microRNA-223 downregulation has in vivo significance in chronic lymphocytic leukemia and improves disease risk stratification

Basile Stamatopoulos, Nathalie Meuleman, Benjamin Haibe-Kains, Pascale Saussoy, Eric Van den Neste, Lucienne Michaux, Pierre Heimann, Philippe Martiat, Dominique Bron and Laurence Lagneaux

"keep it simple"

- How is CLL defined?
  - Absolute B-cell count > 5000 cells/µL (Hallek et al. Blood 2008)
  - This is an arbitrary cut-off
- What is a more relevant (predicitive) cut-off?
  - Absolute B-cell count > 11.000/µL (Shanafelt et al. Blood 2009)
  - The predicitve value of B-cell count was similar to or slightly better than FISH and CD38.
  - When analyzed together,
    - BALC + ZAP70 → best predicitor of TFS
    - BALC + FISH → best predictor of OS
- What is finally most relevant for the patient?
  - How to evaluate the likelihood of patients (CLL) or persons (MBL) for developing clinical symptoms, requiring chemotherapeutic treatment, or dying of the disorder.

Conclusions

- Numerous reports on promising CLL prognostic markers have been published
- Few have proved analytically valid
- Few have proved clinically useful
- What remains useful in clinical practice?
  - Absolute B-cell count
  - Clinical staging systems (Rai / Binet)
  - 17p deletion
- Elucidation of methodological pitfalls (NCI-EORTC Working group on Cancer Diagnostics) *McShane, J Natl Cancer Inst 2005*
- What remains to be done?
  - Systematic and complete assessment of prognostic markers in clinical trials, for the development of rational, risk-adapted treatment strategies
  - Collection of information into large international databases