Chronic Lymphocytic Leukemia

MB&C2024 UCLL - Steven Weekx - 9/2/2024

ZIEKENHUIS aan de STROOM

CLL

- 3 Cases
- CLL, the facts
- Pathogenesis
- Laboratory findings & diagnosis
- Differential diagnosis
- Prognostic and predictive markers
- Treatment strategy
- MBL, SLL, atypical CLL

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Case 1: Jos, 78y

- Yearly control GP, no complaints
- Physical examination: normal
- Ex-nicotine; occasional ethyl
- Welder
- Elevated PFOS values (Zwijndrecht)
- Laboratory results

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Case 1: Jos, 78y

HEMATOLOGIE				
Erytrocyten		4,60	x10*6/µL	4,35 - 5,61
Hemoglobine		14,3	g/dL	13,4 - 16,5
Hematocriet		41,8	%	39,7 - 49,3
MCV		90,9	fL	83,2 - 96,0
MCH		31,1	pg	27,8 - 32,5
MCHC		34,2	g/dL	32,3 - 35,7
Leukocyten	+	**14260**	/µL	3720 - 10540
Formule				
Neutrofiele segmenten	-	**22,3**	%	39,2 - 72,9
Eosinofielen		1,3	%	≤8,4
Basofielen		0,4	%	≤1,5
Lymfocyten	+	**70,5**	%	15,7 - 47,0
Monocyten	-	**5,5**	%	5,7 - 13,7
Formule (absoluut)				
Neutrofiele segmenten		3180	/µL	1760 - 7031
Eosinofielen		185	/µL	≤559
Basofielen		57	/µL	≤102
Lymfocyten	+	**10053**	/µL	964 - 3440
Monocyten		784	/µL	320 - 979
Trombocyten	-	**137**	x 1.000/µL	148 - 362
Microscopie				
		Aanwezigheid van aberrante lymfocyten		
		Enkele gelyseerde cellen		

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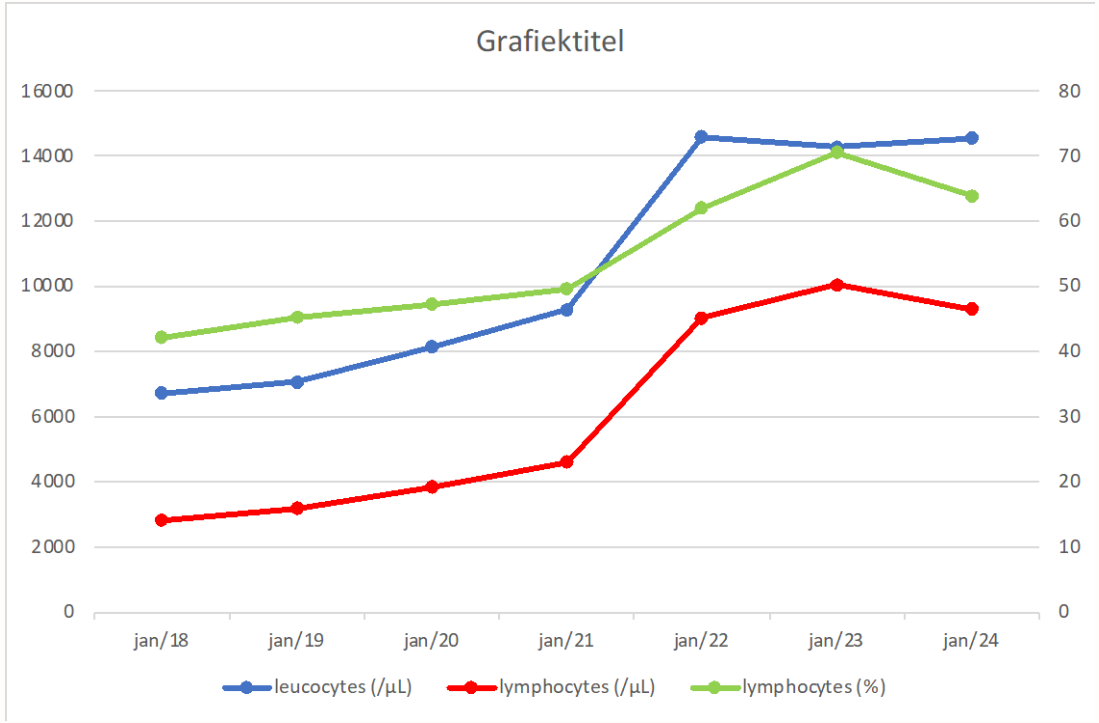
Case1: Jos, 78y

date	leucocytes (/µL)	lymphocytes (%)	lymphocytes (/µL)
nov/18	6720	42,1	2829
okt/19	7060	45,2	3191
nov/20	8130	47,2	3837
nov/21	9280	49,6	4603
dec/22	14570	62	9033
nov/23	14260	70,5	10053
jan/24	14550	63,9	9297



- CLL
- No treatment
- Follow up hematologist 6-12 months

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Case2: Frans, 75y

- Fatigue, dizziness, fainted, weight loss -5kg
- Cachectic, several lymph nodes, splenomegaly
- Nicotine 25 pack years
- 1 brother + AML; 1 brother + CLL (10 y after diagnosis)
- Lab:
  - Hyperleukocytosis: 800.000 WBC/µL → hyperviscosity
  - Macrocytic anemia, thrombopenia due to bone marrow invasion of CLL
- Treatment for CLL
- De novo diagnosis of squamous cell carcinoma (metastatic in bone and lung)

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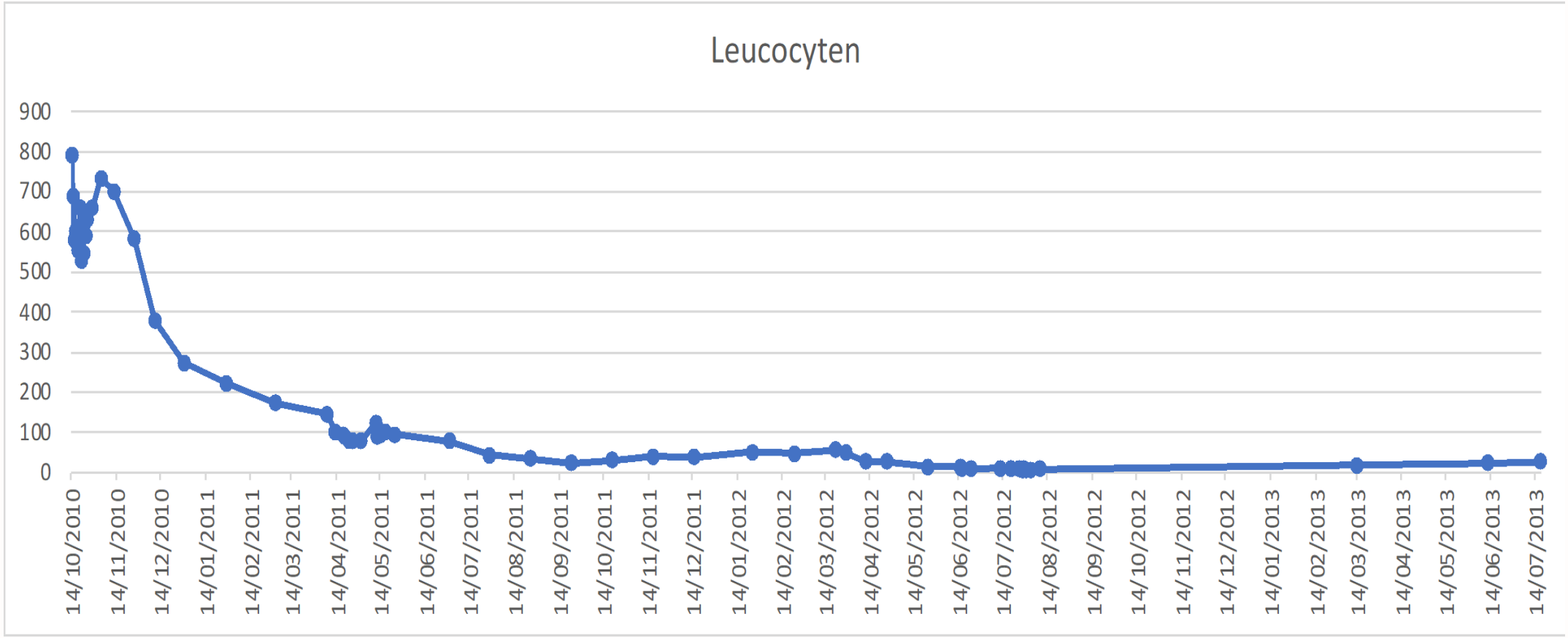
Case2: Frans, 75y



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Case3: Julien, 58y

- Consult GP: During 2 months ‘up and down’, fatigue, sore throat, cough, … Now better.
- Active sport (walking, biking, rowing, …) - no problems
- No pain, normal intake, no weight loss, no fever, no night sweating
- Dock worker
- Nicotine: stop in 1986. Ethyl: 10 – 15 U/week
- Cervical and axillar a few, very little lymphnodes
- No organomegaly, no skin laesions

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Case3: Julien, 58y

HEMATOLOGIE				
Erytrocyten	-	3,68	x10*6/µL	4,35 - 5,61
Hemoglobine	-	12,3	g/dL	13,4 - 16,5
Hematocriet	-	36,7	%	39,7 - 49,3
MCV	+	99,7	fL	83,2 - 96,0
MCH	+	33,4	pg	27,8 - 32,5
MCHC		33,5	g/dL	32,3 - 35,7
Reticulocyten	+	26,2	/1.000 RBC	7,7 - 23,0
Abs.aantal reticulocyten		96,4	x 1.000/µL	33,8 - 114,2
Leukocyten	-	3240	/µL	3720 - 10540
Formule				
Neutrofiele segmenten	-	27,2	%	39,2 - 72,9
Eosinofielen		0,6	%	≤8,4
Basofielen		0,6	%	≤1,5
Lymfocyten	+	68,8	%	15,7 - 47,0
Monocyten	-	2,8	%	5,7 - 13,7
Formule (absoluut)				
Neutrofiele segmenten	-	881	/µL	1760 - 7031
Eosinofielen		19	/µL	≤559
Basofielen		19	/µL	≤102
Lymfocyten		2229	/µL	964 - 3440
Monocyten	-	91	/µL	320 - 979
Trombocyten	-	110	x 1.000/µL	148 - 362

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Case3: Julien, 58y

Flow cytometry:

- *Presence of a population monoclonal B-lymphocytes **(231/µL)** with Kappa light chain restriction.*
- *Phenotype: CD45+, CD19+, CD20+ (dim), CD10-, CD5+, FMC7-, CD23+, CD22+.*
- *CLL-phenotype → low count-MBL*
- *very small risk of progression to CLL, no specific follow-up*

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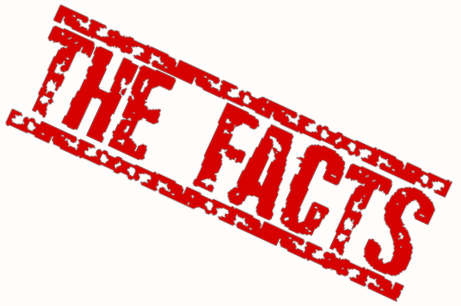
CLL epidemiology



- Incidence: 4.5 / 100 000 / year (80+ y: > 30 / 100 000 / year)
- Median age diagnosis: 70y (9% < 45y)
- M/F: 1.5/1 (1.9/1)
- Lifelong risk: 0.6%
- 1.1% of all new cancer cases
- 0.7% of all cancer deaths
- 5 year survival 65.1% (1975) → 87.2% (2021); 10 year survival 82%

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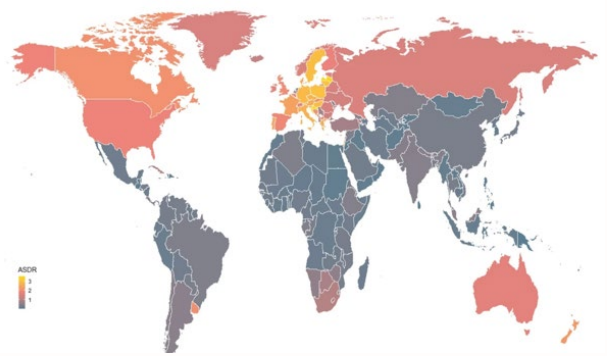
CLL geographical & etnical considerations

- Incidence varies by race and geographic location:
  - White Americans > African Americans > Asian Pacific Islanders
  - Incidence in China and Japan 10 times lower than in Western countries
- Genetic factors rather than environmental factors
  - Japanese in Hawaii no higher incidence than native Japanese
  - Frequency of CLL-associated genetic mutations is lower in African Americans



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CLL environmental considerations

- Debatable … few reports …
  - Exposure to pesticides and herbicides among farmers
  - Agent orange (Vietnam War)
  - Radon exposure
  - Ionizing radiation → all types of leukemia ↗, except for CLL
  - Respiratory tract infections, cellulitis and herpes zoster infection can presage CLL

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CLL family studies

- 6-9 ↗ risk for (first degree) relatives of CLL patients
- 17% of first degree family members of CLL patients have MBL
- Dabatable … genetic anticipation (CLL develops at an earlier age in successive generations)

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CLL pathogenesis

Complex, multistep process → accumulation of B-lymphocytes

- Monoclonal
- Mature
- Functionally incompetent
- PB, BM, LN, spleen

Sequential process, minority of cases progressing at each step

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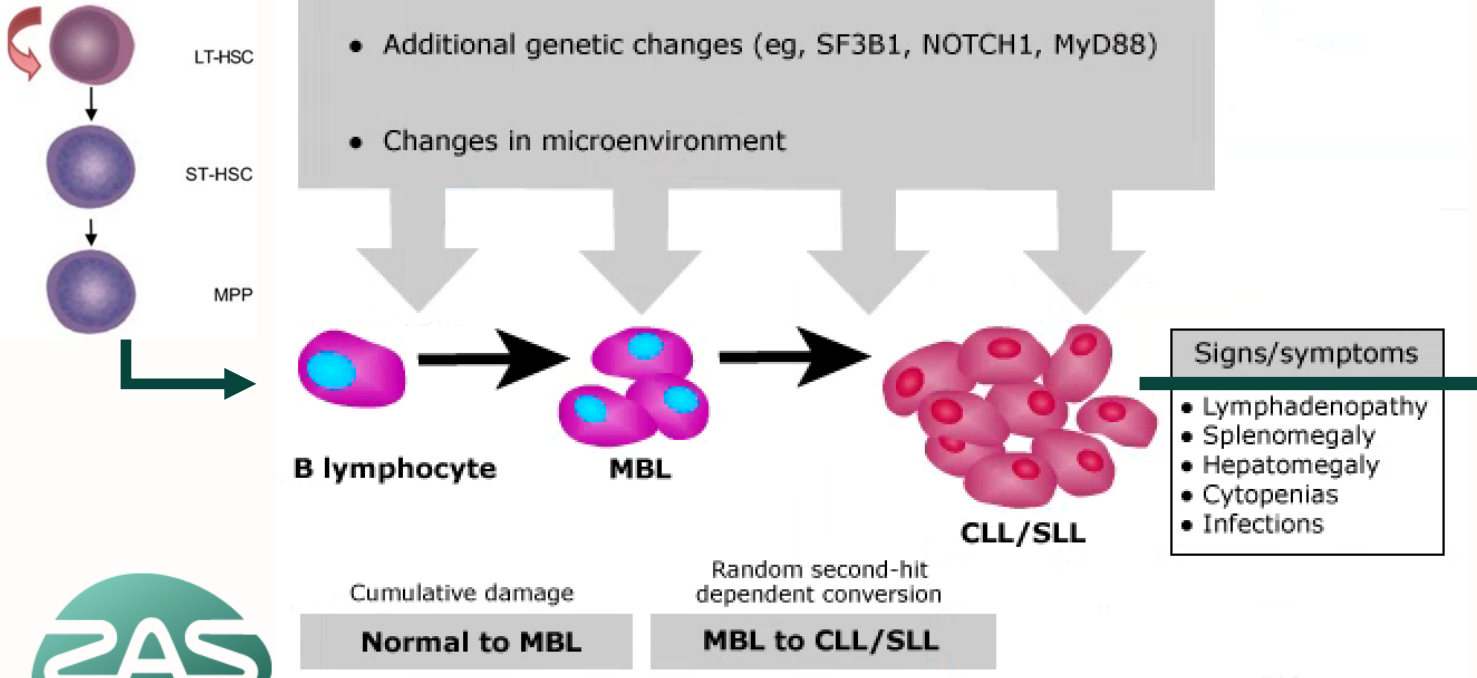
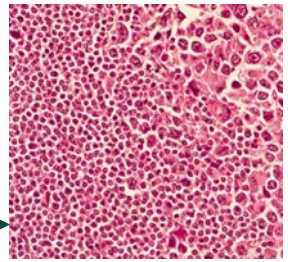
CLL pathogenesis



Multistep, sequential process →
1. Impaired apoptosis
2. Increased proliferation

Asymptomatic CLL
⇩
Symptomatic CLL



Richter transformation

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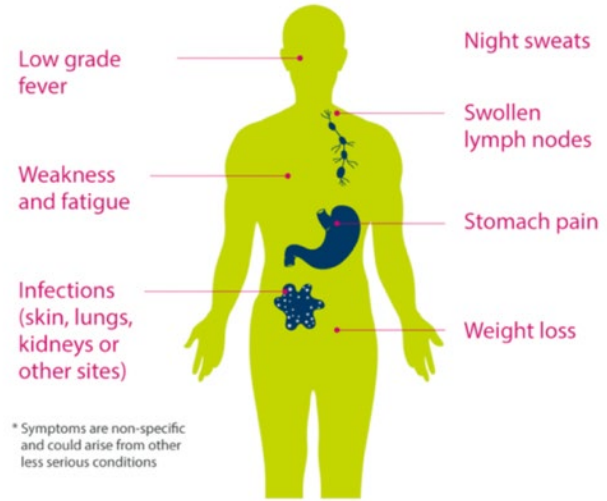
CLL clinical presentation



- NO symptoms… accidental finding during blood control
- Painless swelling of lymph nodes
- (Hepato)Splenomegaly
- B-symptoms (5 – 10%)
  - Weight loss > 10% in 6 months
  - Fevers > 38°C > 2 weeks (without evidence of infection)
  - Nights sweats (without evidence of infection)
- Fatigue
- Symptoms related to cytopenia; recurrent infections
- (Skin lesions: papules, plaques, ulcers, … < 5% / other localizations)

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CLL laboratory findings

- Lymphocytosis (PB / BM); **threshold > 5000/µL** monoclonal B-lymphocytes
- Cytopenia: neutropenia, anemia and thrombocytopenia, usually mild
- Immunoglobulin abnormalities
  - Hypogammaglobulinemia (25%), usually IgG and IgA and IgM
  - Polyclonal increase of gammaglobulins in 15%
  - Monoclonal protein in 5%
  - Sometimes detected years before CLL diagnosis
- No characteristic abnormalities in blood chemistry
  - LDH and beta-2-microglobulin levels

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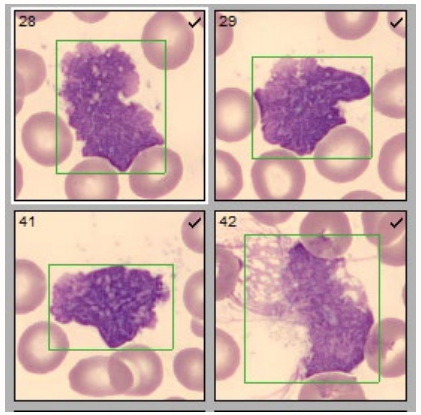
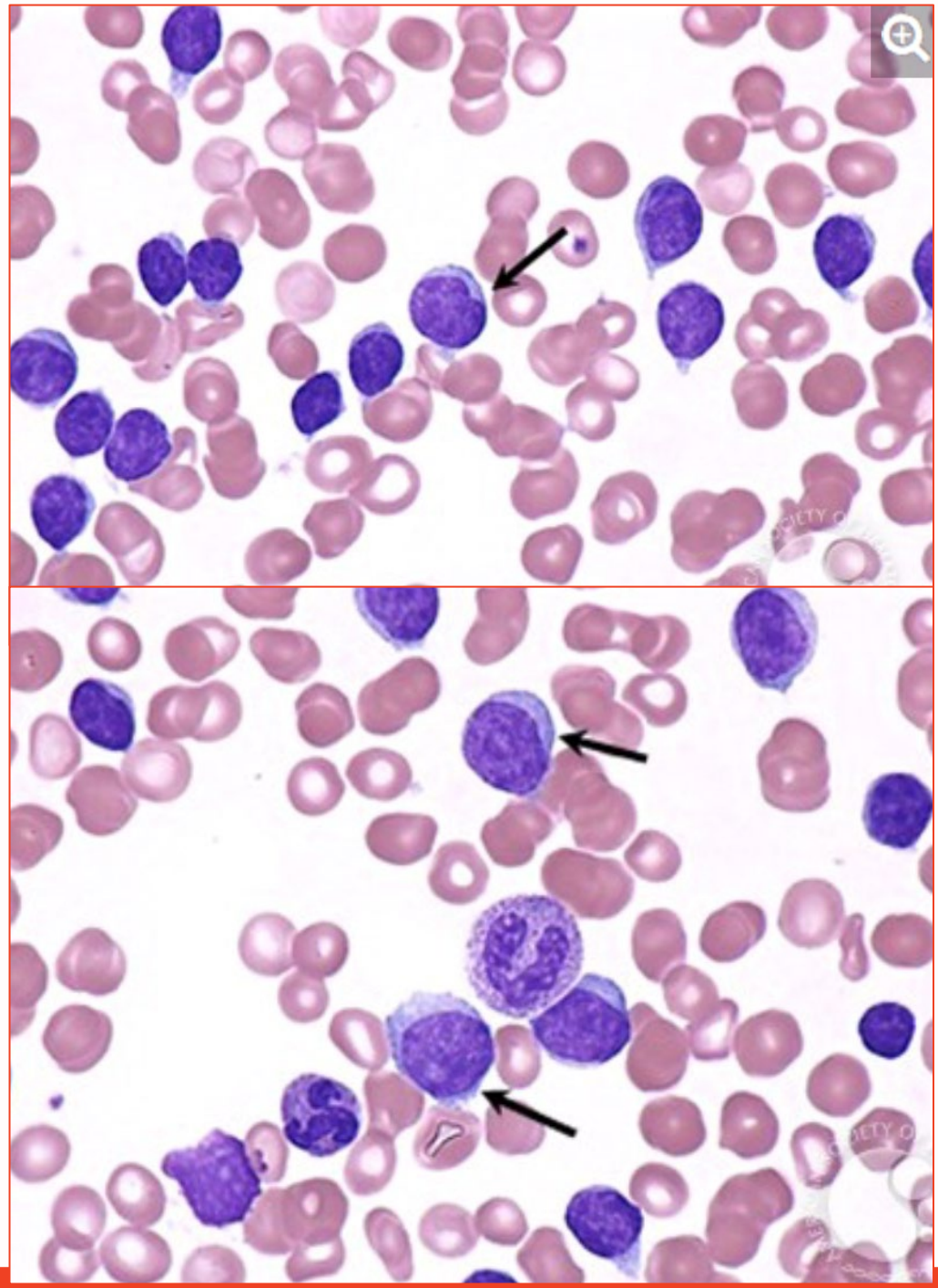
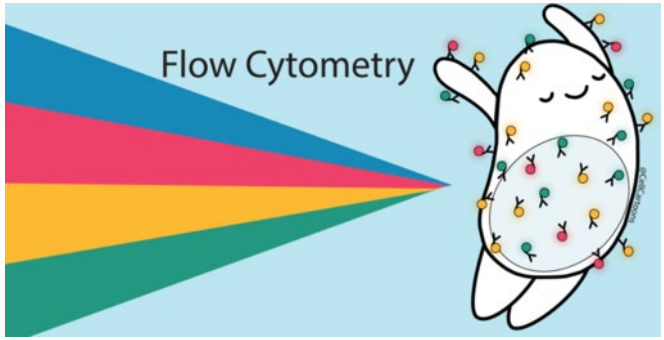
CLL laboratory findings

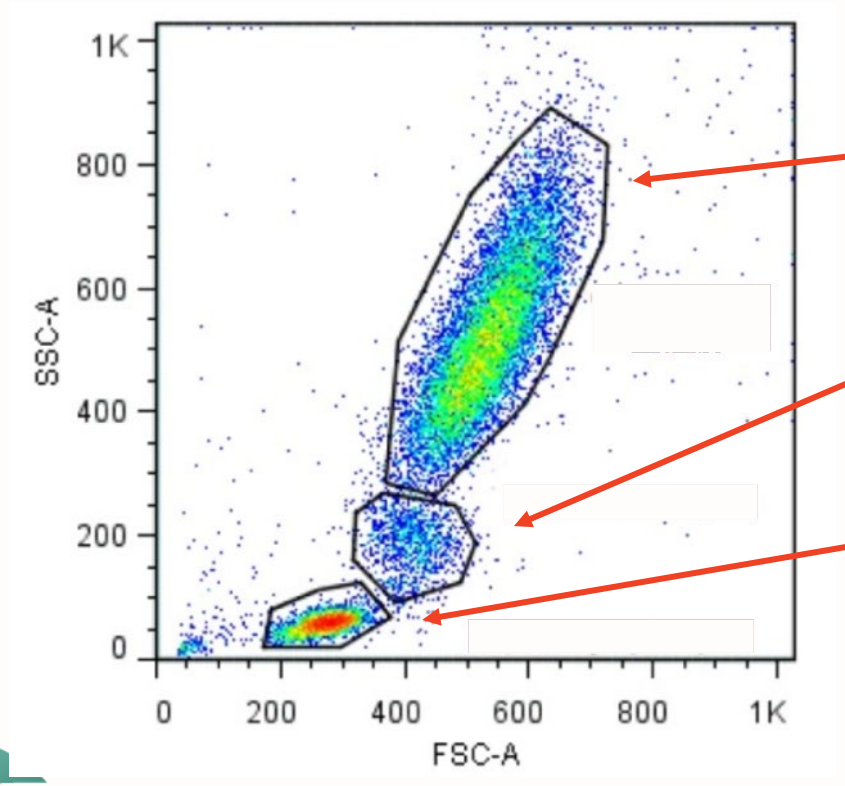


- Peripheral smear
  - Lymphocytosis
  - Small, mature lymphocytes, clumped chromatin, narrow rim cytoplasm
  - Few ‘prolymphocytes’: younger appearance, lacy chromatin, prominent nucleolus
  - Smudge / basket cells
- Bone marrow aspirate and biopsy: in se not required for diagnosis CLL
  - Increased cellularity
  - Lymphocytes > 30% of NC



CLL flow cytometry

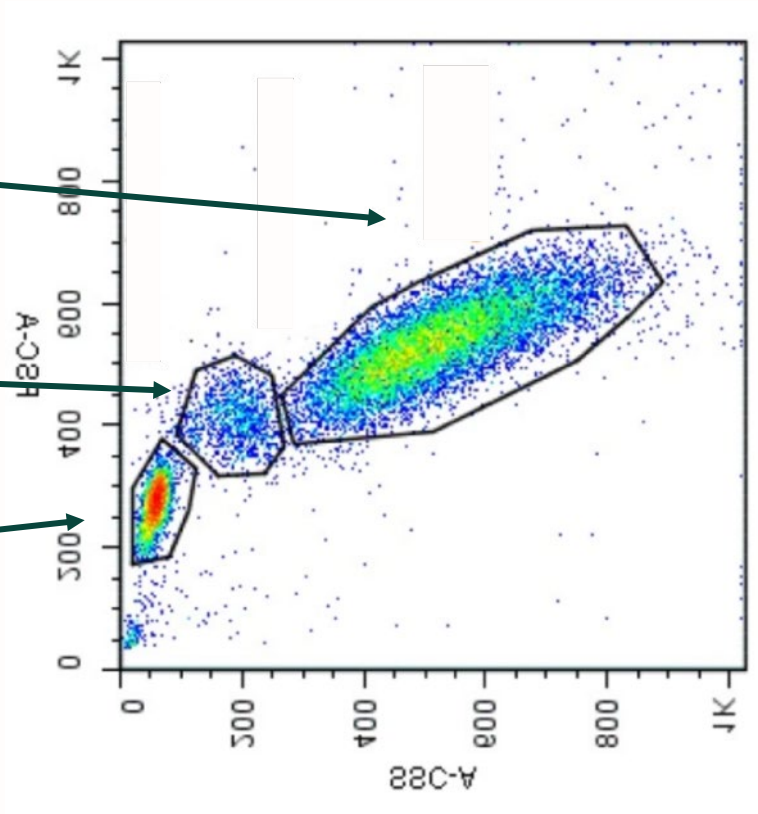




neutrophils

monocytes

lymphocytes



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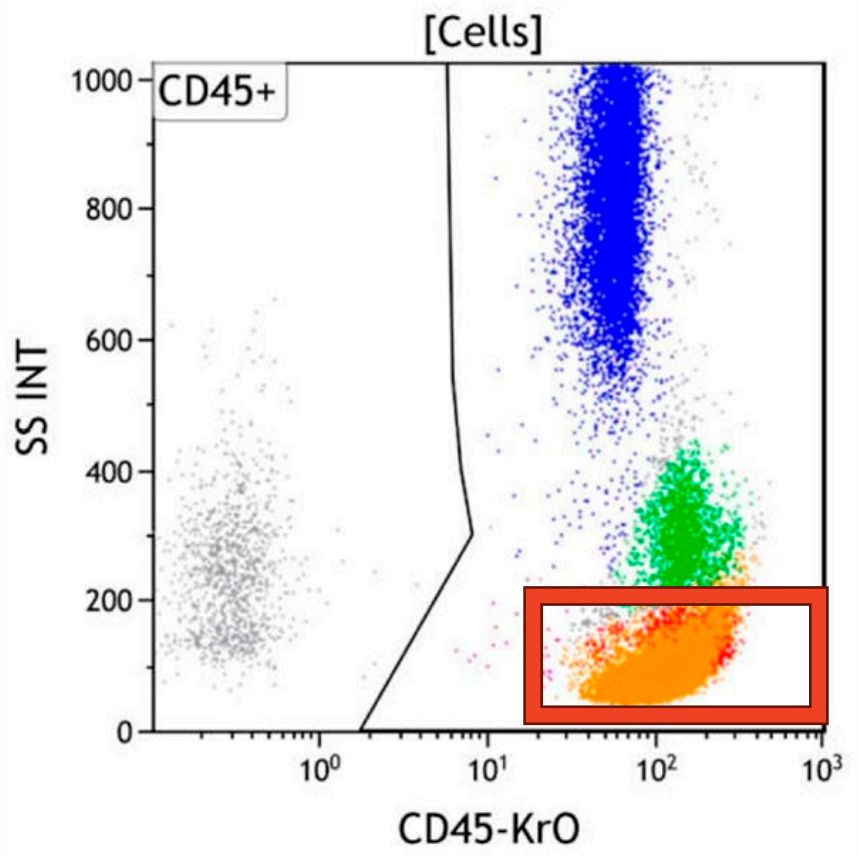
CLL FCM strategy CD45



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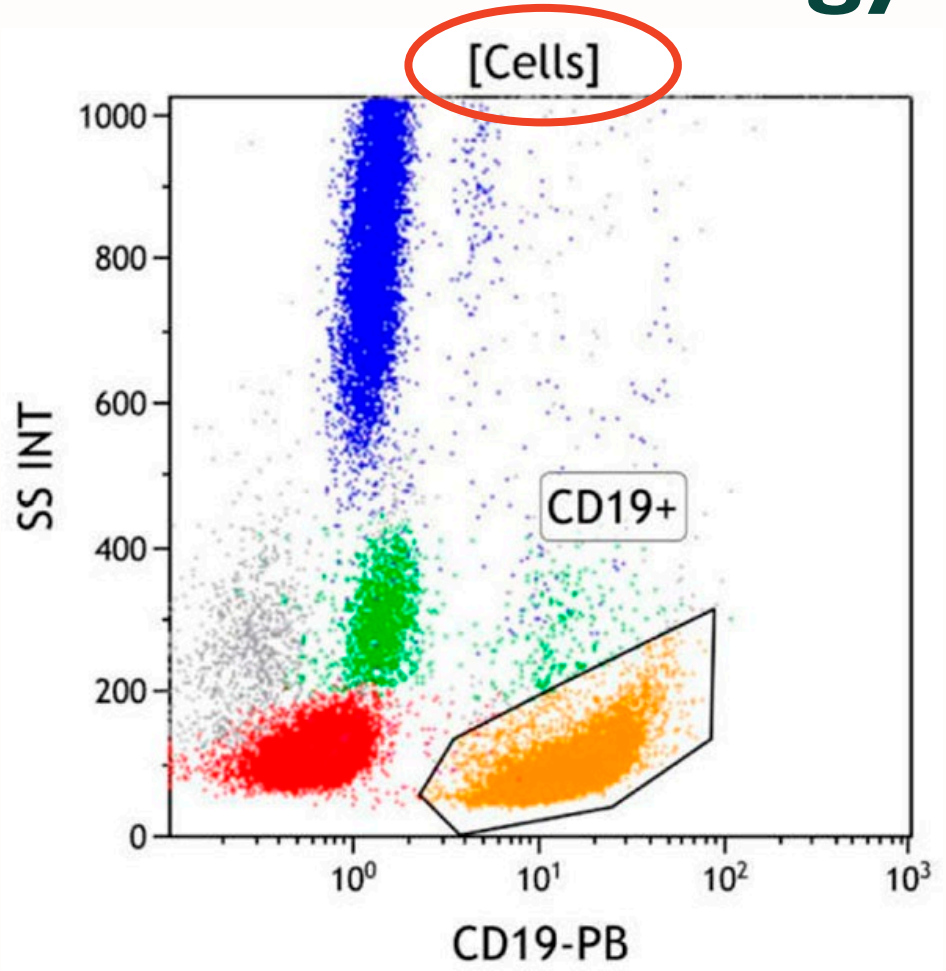
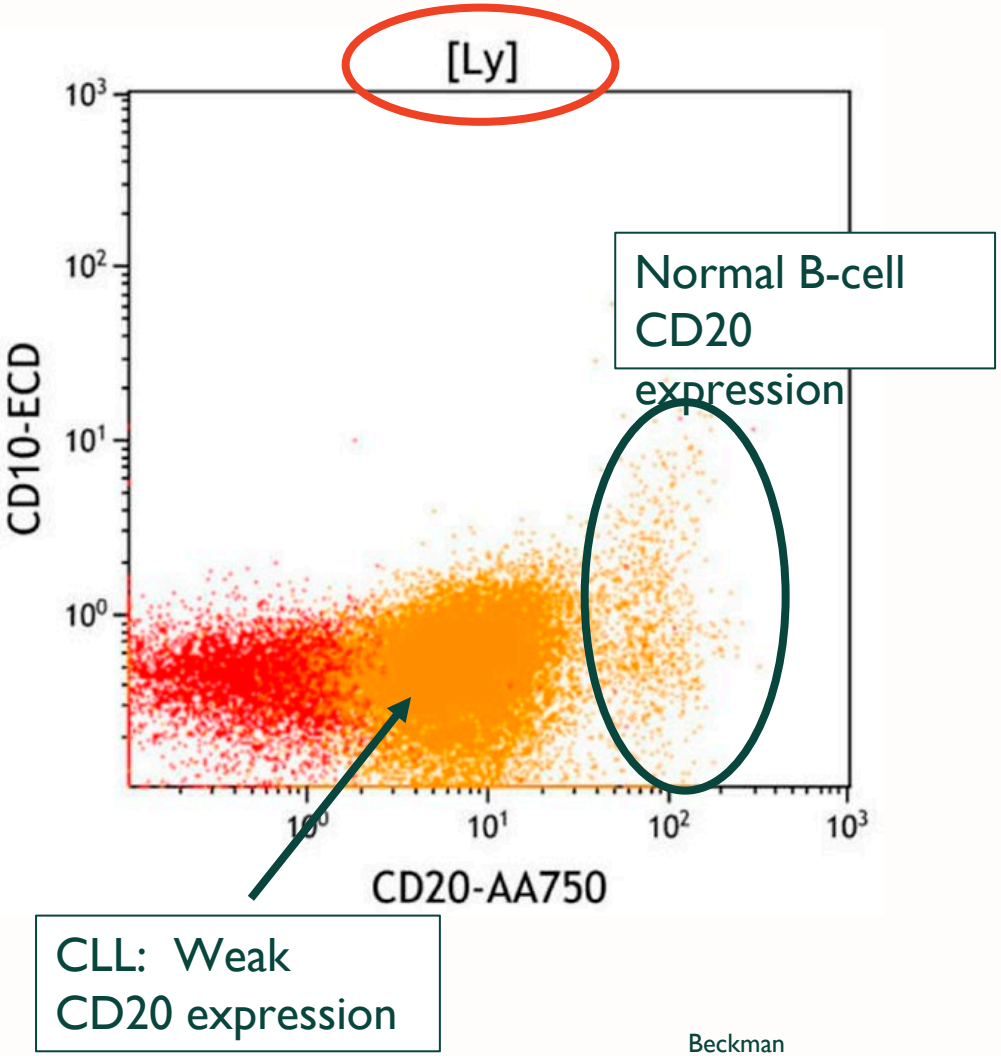
CLL FCM strategy CD19 - CD20





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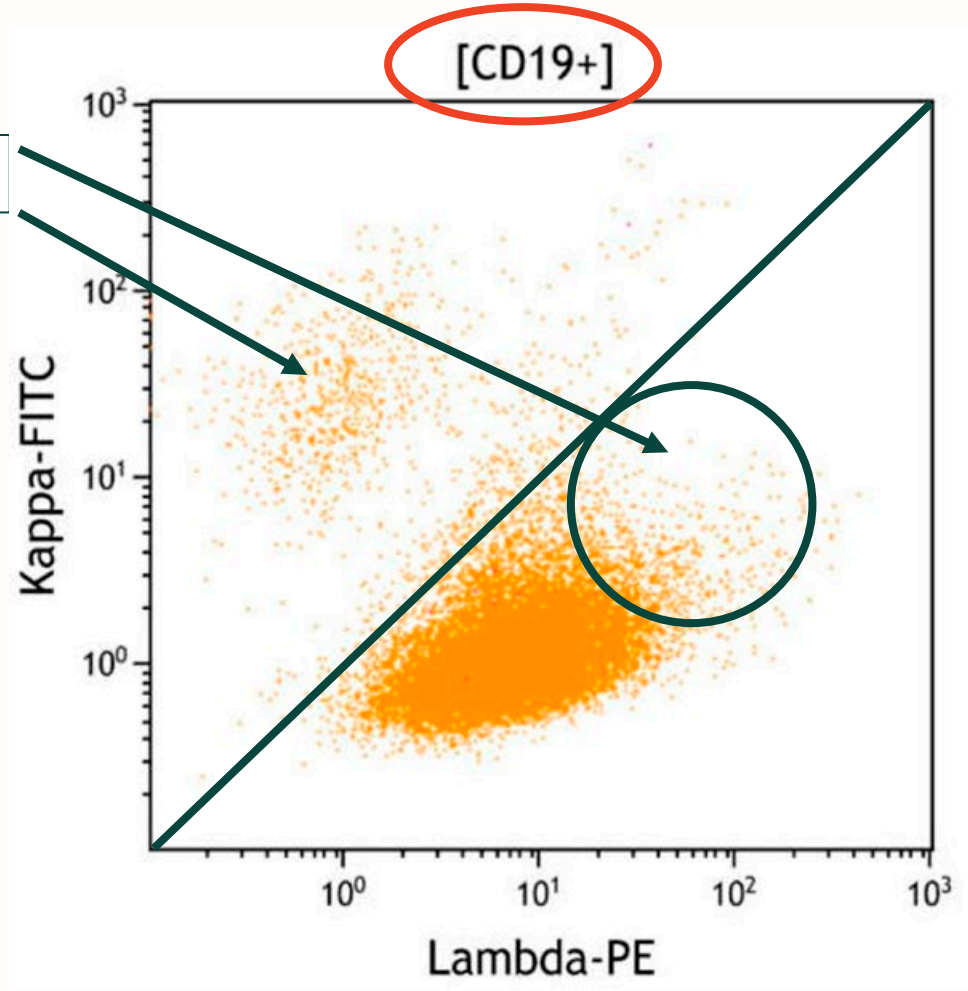
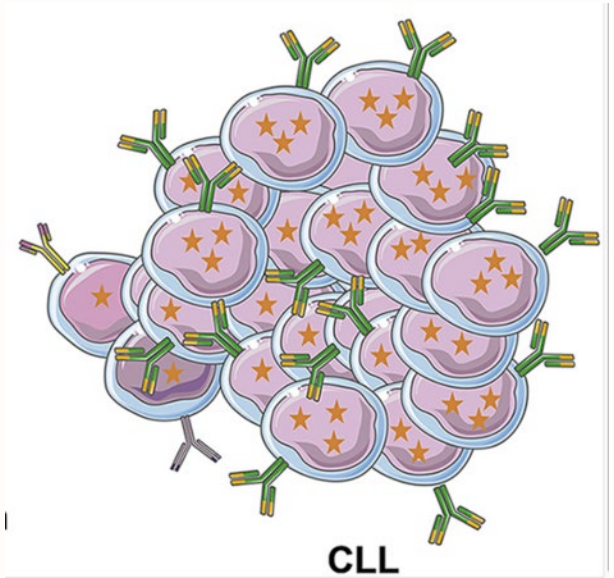
CLL FCM strategy κ/λ clonality

Polyclonal B-cells



Normal κ/λ ratio ~ 2:1

Monoclonal κ/λ ratio > 3:1 or < 0,3:1



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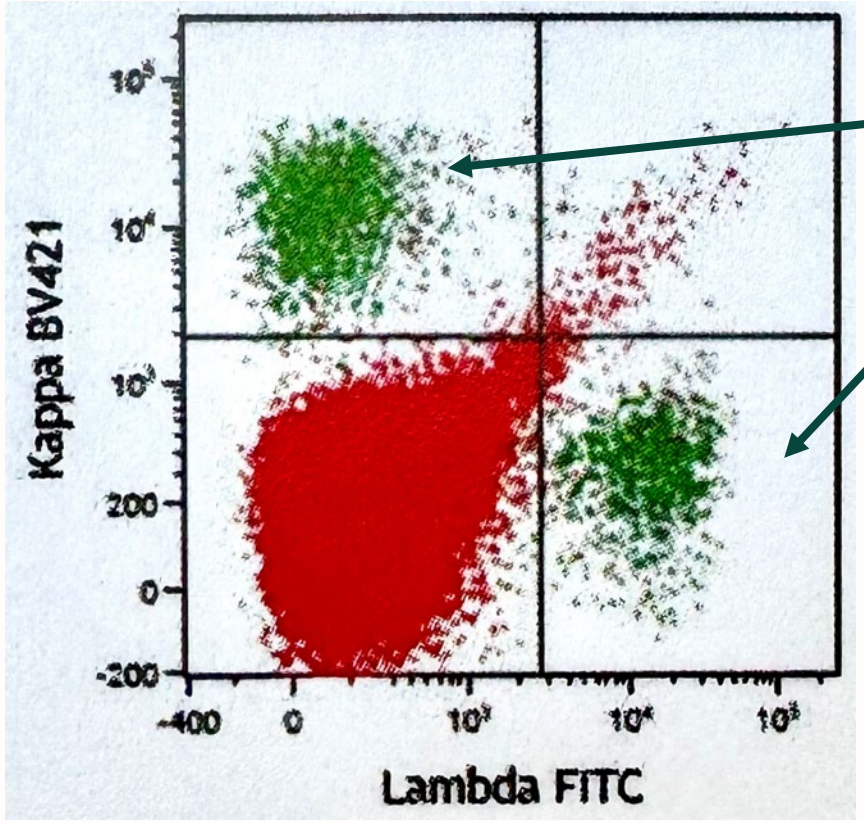
CLL FCM strategy κ/λ clonality



Polyclonal B-cells

CLL light chain expression:
Weak to Negative

When equivocal results, try cytoplasmic / intracellular staining

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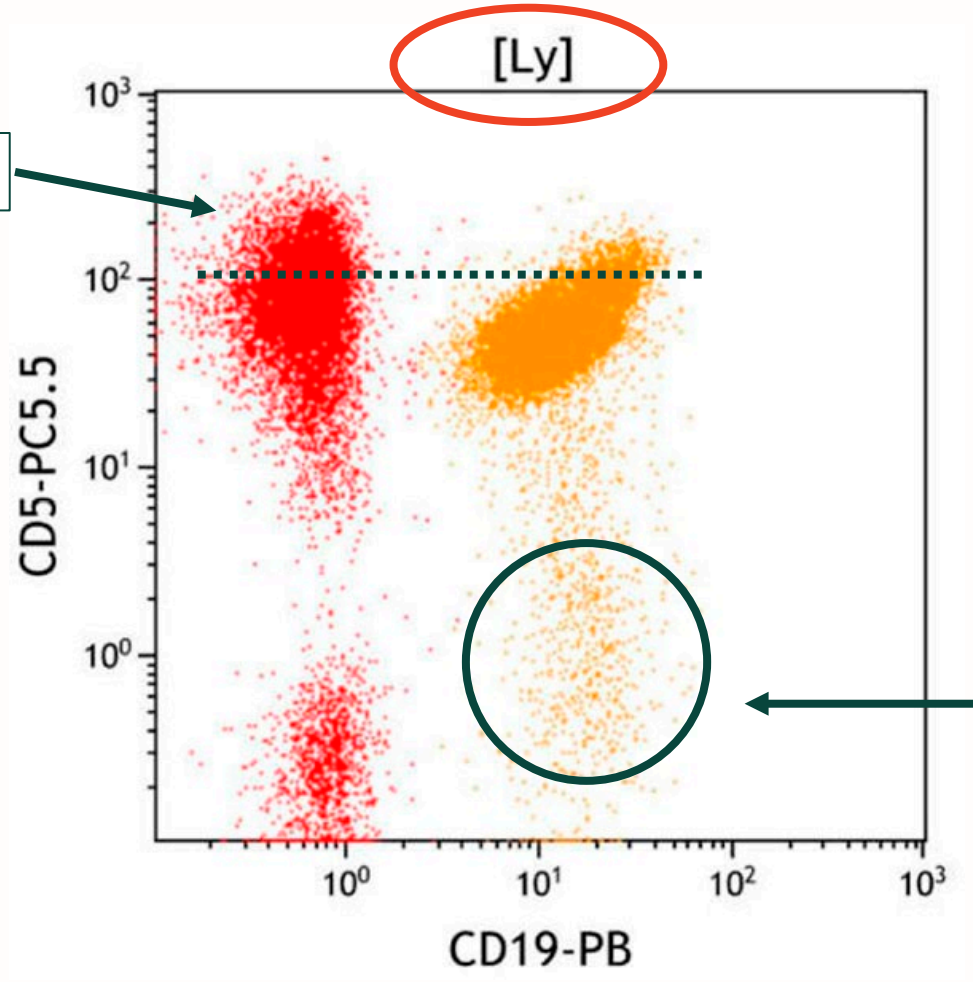
CLL FCM strategy CD5 B- vs T-cells



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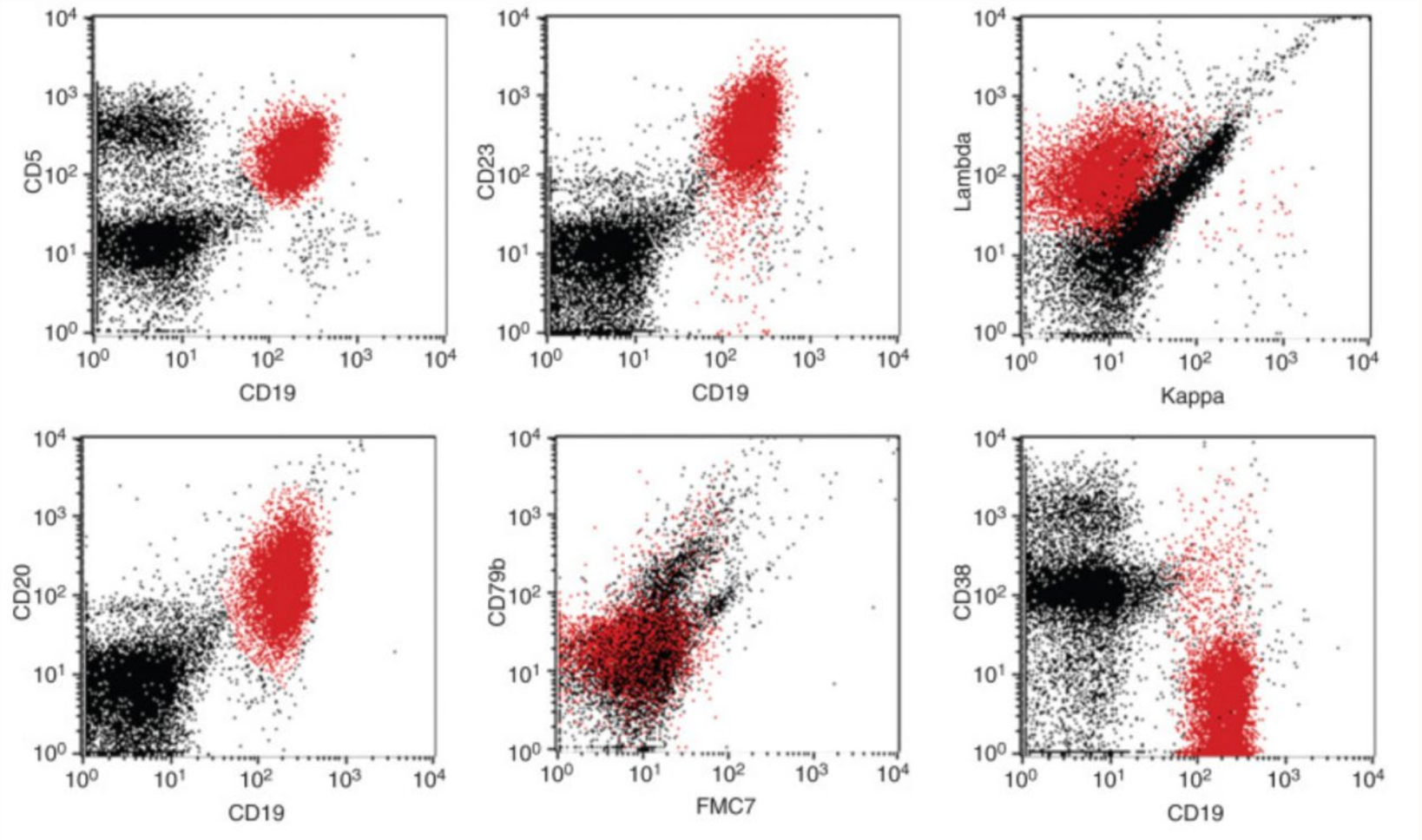
CLL FCM strategy CD23 – FMC7 – CD22



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CLL scoring system

- Matutes - Catovsky

Marker	Score points	
	1	0
SmIg	Weak	Strong
CD5	Positive	Negative
CD23	Positive	Negative
FMC7	Negative	Positive
CD22 or CD79b	Weak	Strong

Scores in CLL are >3 and in other B cell malignancies <3.
SmIg, surface immunoglobulins.

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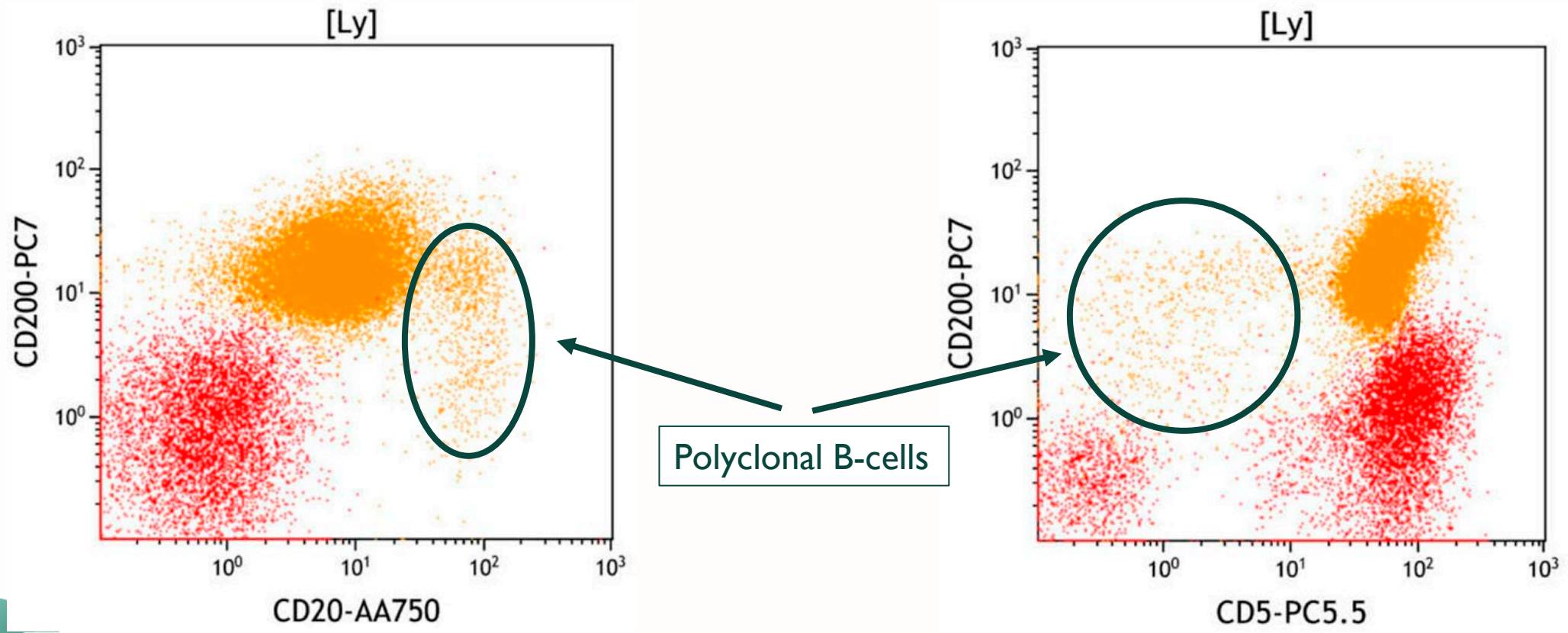
CLL extra markers CD200



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CLL scoring system

- Matutes - Catovsky

Scoring system	*Sensitivity % (95% CI)*	*Specificity % (95% CI)*	*CLL vs. non-CLL % (95% CI)*
CD5, CD23, FMC7, sIgM, CD79b	94.97 (91.0 - 97.6)	100.0 (92.3 – 100.0)	99.4 (97.4–100.0)
CD5, CD23, FMC7, sIgM, CD79b, CD200	100.0 (98.2 – 100.0)	98.04 (89.6 - 100.0)	100.0 (98.4–100.0)
CD5, CD23, sIgM, CD200	93.97 (89.7 - 96.8)	100.0 (93.0 - 100.0)	99.8 (98.1–100.0)

https://doi.org/10.1371/journal.pone.0247491.t005

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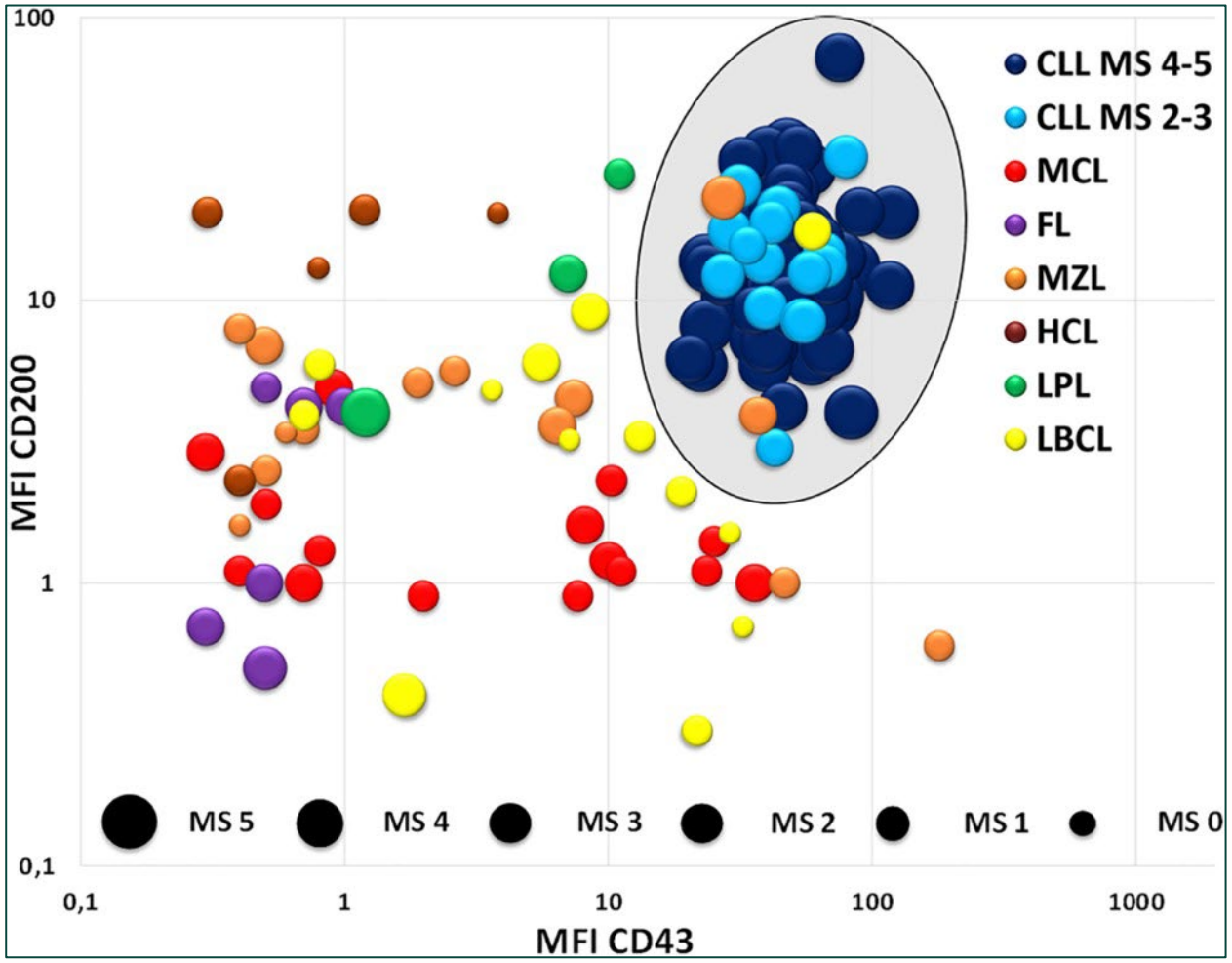
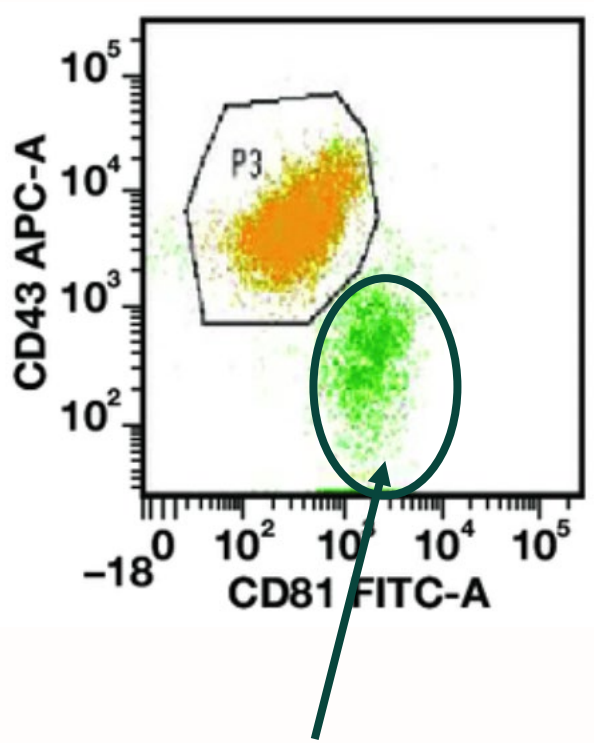
CLL extra markers CD200 / CD43 / CD81





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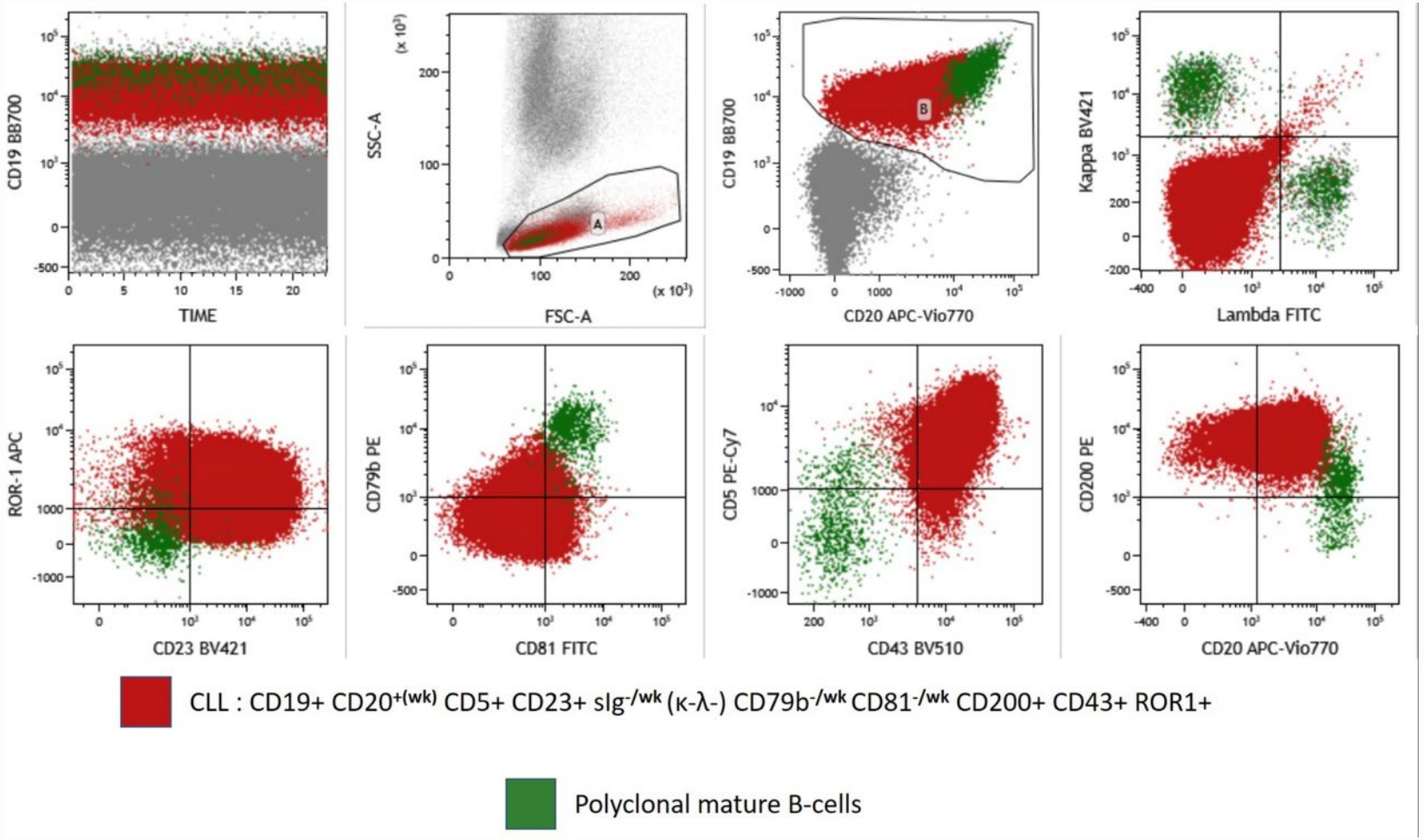
CLL examples



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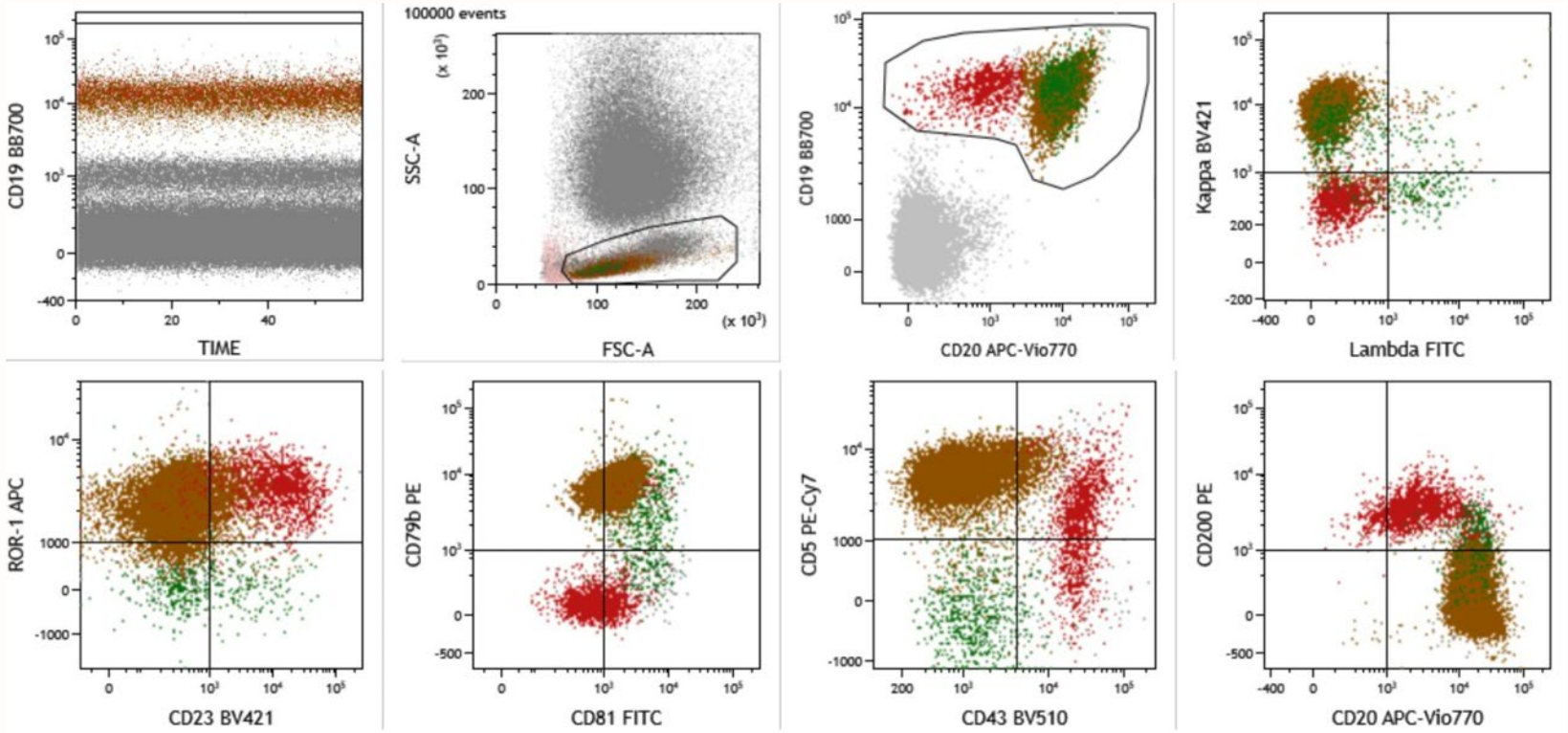
CLL examples



CLL : CD19+ CD20$^{+(wk)}$ CD5+ CD23+ sIg$^{-/wk}$ (κ-λ-) CD79b$^{-/wk}$ CD81$^{-/wk}$ CD200+ CD43+ ROR1+

Mantle cell : CD19+ CD20$^{+(mod)}$ CD5+ CD23- sIg$^{+/mod}$ (κ+λ-) CD79b+ CD81$^{wk/+}$ CD200- CD43$^{wk/+}$ ROR1$^{-/+}$

Polyclonal mature B-cells

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CLL FCM all together now

SUPPLEMENT ARTICLE

ISLH International Journal of Laboratory Hematology WILEY

Flow cytometry in the diagnosis of mature B-cell lymphoproliferative disorders

Camille Debord | Soraya Wuillème | Marion Eveillard | Olivier Theisen | Catherine Godon | Yanick Le Bris | Marie C. Béné

Major markers discriminating other B-cell lymphoproliferative malignancies

	CLL
CD19	+
CD5	+
CD23	+
sIg	low/−
CD79b	low/−
CD20	low
FMC7	−
CD22	low/−
CD10	−
CD200	++
CD43	+
CD81	low/−
CD103	−
CD123	−
CD11c	−/+
CD25	−/+
CD13	−

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Differential diagnosis of lymphocytosis

	CD5+		CD5−				Villous lymphocytes			High grade	
	CLL	MCL	LPL/WM	FL	BPLL	MZL	HCL	HCLv	SDRPL	BL	DLBCL
CD19	+	+	+	+/low	+	+	+	+	+	+	+
CD5	+	+	−/+	−	−	−/+	−	−	−	−	−/+
CD23	+	low/−	−/+	−	−	−/+	−	−	−	−	−/+
sIg	low/−	+	+	+	++	+	+	+	+	+	+/−
CD79b	low/−	++	+	+	++	++	+	low	low	low	+
CD20	low	+	+	+	++	+	++	+	+	+	+
FMC7	−	+/−	+	+	++	+	+	+	+	+	+
CD22	low/−	+	low	+	++	+	++	+	+	+	+
CD10	−	−	−	+	−	−	−	−	−	++	−/+
CD200	++	−	+/−	+/−	−/+	+	++	+	+	−	−/+
CD43	+	−/+	−/+	−	+	−	++	+	−	++	−/+
CD81	low/−	+	+	+		+	+	++	+	+	+
CD103	−	−	−	−	−	−	++	++	−	−	−
CD123	−	−	−	−	−	−	++	−	−	−	−
CD11c	−/+	−	−	−/+	−	−/+	++	+	+	−	−
CD25	−/+	−	+	−/+	−	−/+	++	−	−	−	+/−
CD13	−	−	+	−	−	−/+	−	−	−	−	−

Note: −/+ = usually negative but may be positive. +/− = usually positive but may be negative. ++ = strong positivity.

Gray cells indicate major discriminating markers.

Abbreviations: BL, Burkitt lymphoma; BPLL, B prolymphocytic leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HCL, hairy cell leukemia; HCLv, hairy cell leukemia variant; LPL/WM, lymphoplasmacytic lymphoma/Waldenström macroglobulinemia; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; SDRPL, splenic diffuse red pulp lymphoma.

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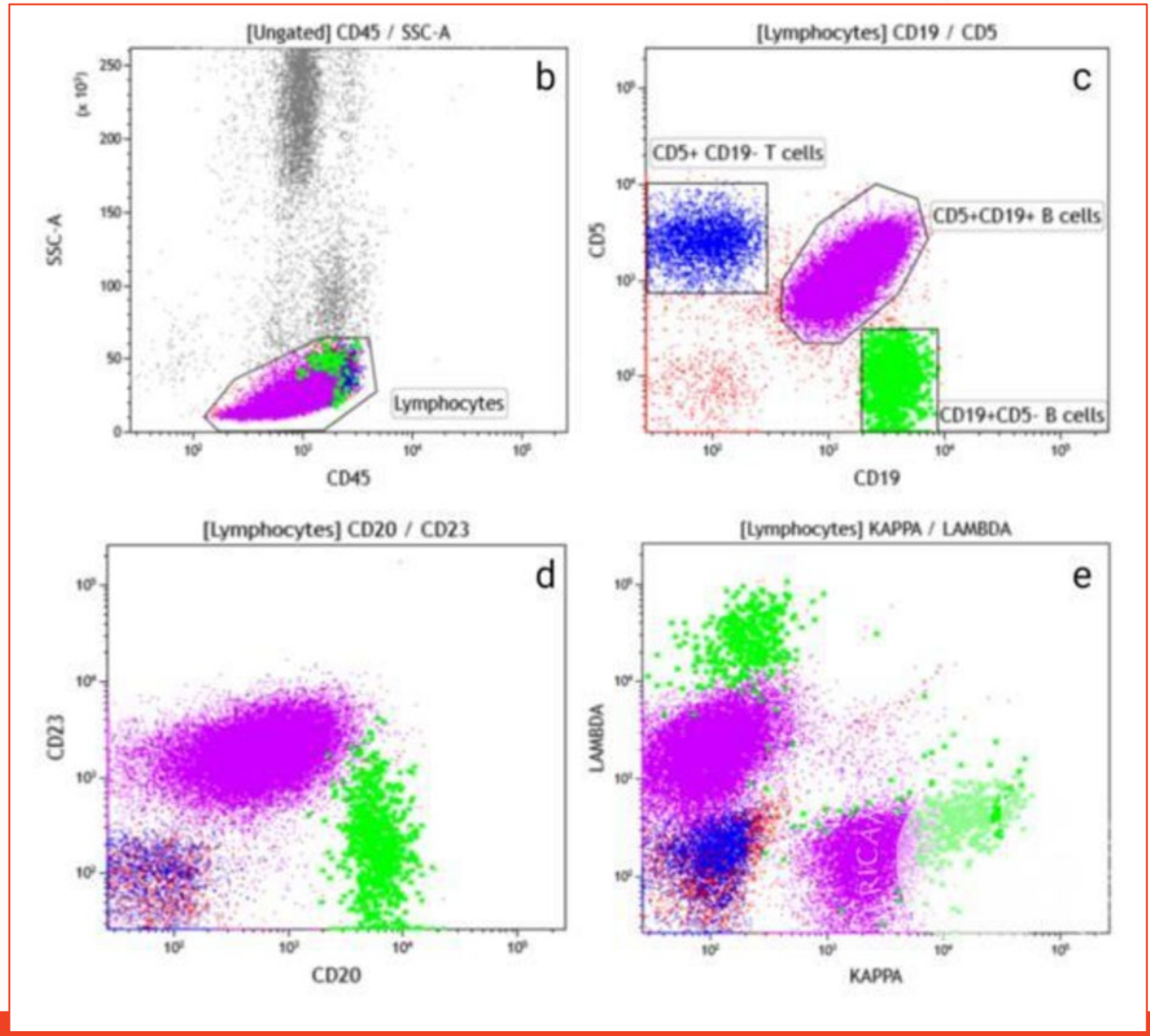
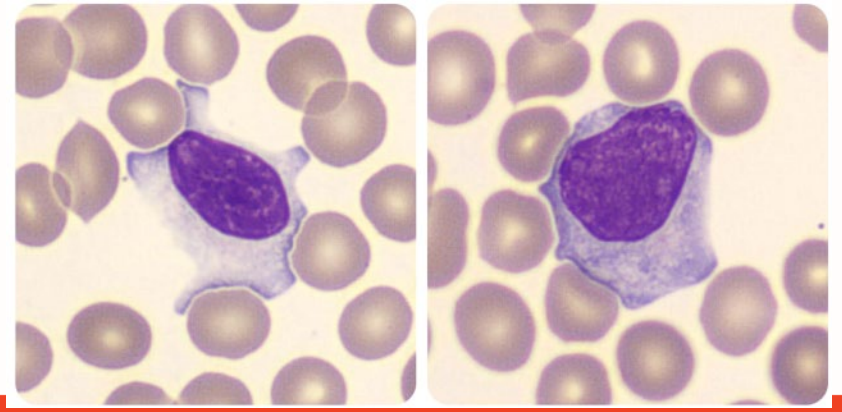
Biclonal CLL

- 1% of cases
- 2 populations with CLL phenotype CD19+, CD5+, CD23+
  - one Kappa
  - one Lambda
- PCR for immunoglobulin heavy chain gene rearrangement



Differential diagnosis of lymphocytosis

- Reactive
  - Transient due to infection: lymphocytosis < 3 months
  - Not clonal
  - No typical CLL phenotype
  - No bone marrow infiltration
  - ‘atypical lymphocytes’ (activated T-cells)
  - Viral infections, Infectious mononucleosis, pertussis, …



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Differential diagnosis of lymphocytosis

- PLL
  - > 55% prolymphocytes (> 90%)
  - Bright SmIg
  - CD5-
  - Different classifications
    - WHO-classification 5th edition (2022): new entity: "splenic B-cell leukemia with prominent nucleoli" (SBLPN)
    - International Consensus Classification - ICC (2022): B-PLL

ZIEKENHUIS aan de STROOM

CLL prognostic and predictive markers

- Prognostic (clinical outcome; PFS; OS)
  - Rai-Binet stage → higher stage, shorter survival
  - Independent of therapy received

- Predictive (likelihood of response to a treatment)
  - TP53 mutation → no response to chemoimmunotherapy → use targeted therapy

- Prognostic AND predictive (combination)
  - TP53 mutation → outcome of targeted therapy is inferior compared with patients with wild type TP53

ZIEKENHUIS aan de STROOM

CLL clinical staging

shorter survival

Stage according to Rai	Definition by Rai	Stage according to Binet	Definition by Binet
Rai 0	Lymphocytosis >5x10^9/L	Binet A	Hb≥100 g/L (6.21mmol/L), platelets ≥100x109/L , ≥3 involved lymphoid sites
Rai I	Lymphocytosis and lymphadenopathy		
Rai II	Lymphocytosis and hepatomegaly and/or splenomegaly with/without lymphadenopathy	Binet B	Hb ≥100 g/L (6.21 mmol/L), platelets ≥100x109/L, ≥3 involved lymphoid sites
Rai III	Lymphocytosis and Hb<110 g/L (6.83 mmol/L) with/without lymphadenopathy/organomegaly	Binet C	Hb<100 g/L, platelets ≥100x109/L
Rai IV	Lymphocytosis and platelets <100x109/L with/without lymphadenopathy/organomegaly		

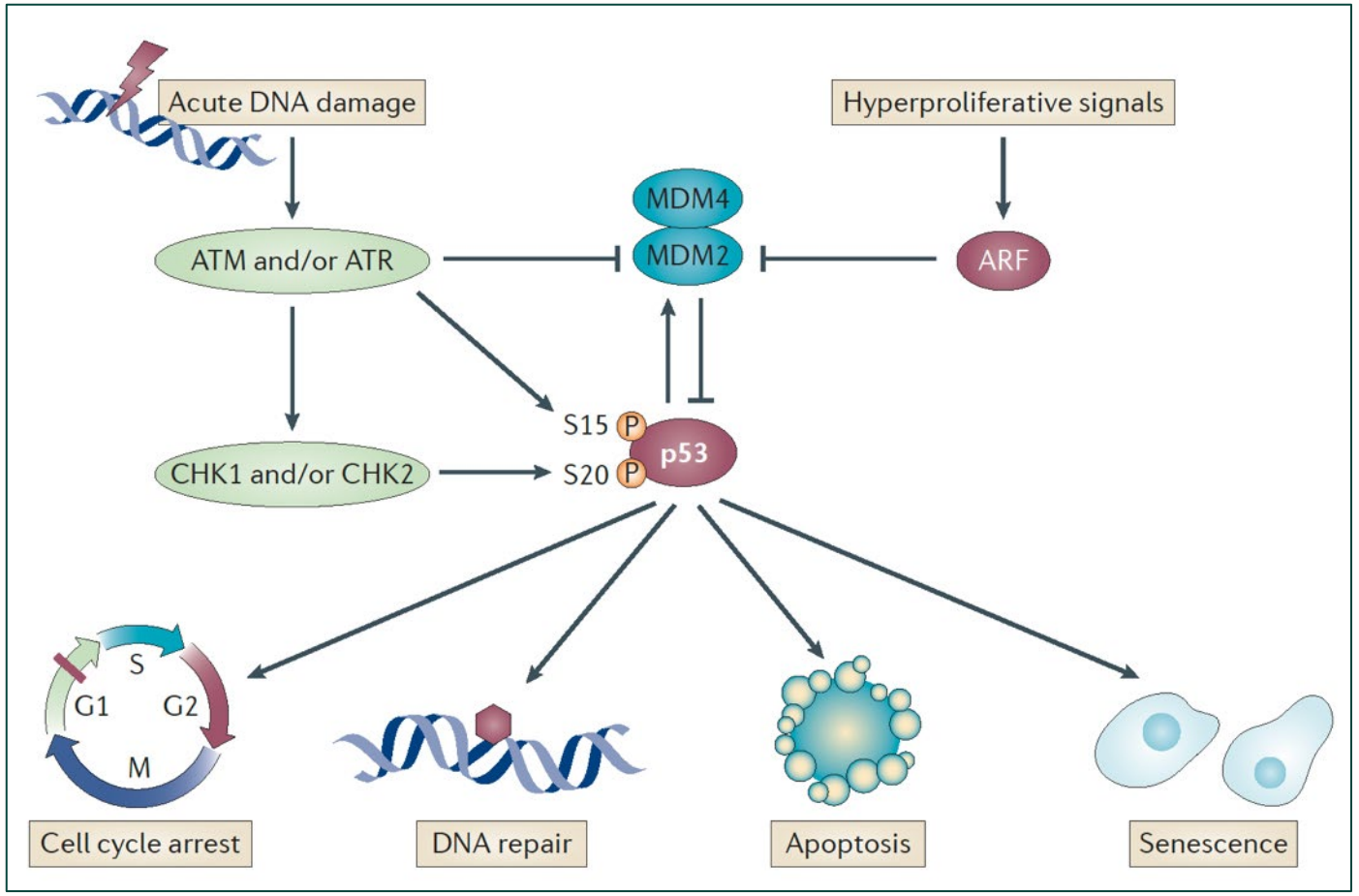
ZIEKENHUIS aan de STROOM

CLL predictive markers

- **TP53** tumor-suppressor gene on the short arm of chromosome 17
- Inactivated by del(17p) and/or point mutations
- Testing before start of therapy
  - del(17p) → FISH; 7-10% of the patients; even higher after relapse
  - TP53 mutations → Sanger sequencing or NGS; 10-47% of the patients
- Re-assessment when progressing disease in previously TP53 wild-type cases
- Impared DNA damage response → no (durable) response to chemoimmunotherapy → targeted therapy

ZIEKENHUIS aan de STROOM

CLL TP53 mutation



- Cells without normal TP53 function (Del(17p) or TP53 mutation) cannot trigger apoptosis in response to chemotherapy induced DNA damage.

ZIEKENHUIS aan de STROOM

CLL predictive markers

- Assesment the Immunoglobuline Gene Heavy Chain Variable region **IGHV** gene mutation status:
  - **IGHV-mutated** CLL (<98% germline identity). M-CLL (50% of patients) → prolonged durable remissions after chemoimmunotherapy
  - **IGHV-unmutated** CLL (≥98% germline identity). U-CLL
    - 97-97,9%: borderline mutated
    - Subset #2 configuration → IGHV-mutated with poorer prognosis
- Testing before start of therapy with Sanger sequencing
- No need to repeat the test (IGHV gene mutation status does not change over time)

ZIEKENHUIS aan de STROOM

CLL predictive markers

- NOTCH1 mutations
  - No routine
  - Predictive value still uncertain
  - ↘ CD20 expression → less effective anti-CD20 therapy

- Mutations in B-cell receptor pathway (BTK); BCL2 gene mutations
  - Uncommon at diagnosis
  - Testing at disease progression

ZIEKENHUIS aan de STROOM

CLL prognostic markers

- Rai-Binet stage
- Del(17p) and TP53 mutation status
  - → outcome of targeted therapy is inferior compared with wild type TP53
- Immunoglobuline Gene Heavy Chain Variable region **IGHV** gene mutation status
  - → U-CLL more aggressive clinical course
- Lymphocyte doubling time <1 year: more aggressive clinical course
- Beta2-microglobulin: increasing levels, poorer prognosis
- (less clear: CD38 expression; intracellular ZAP70: inferior clinical course)

ZIEKENHUIS aan de STROOM

CLL pretreatment evaluation genetics

- Very heterogeneous genetic landscape → no unique genetic lesion
- Most frequent chromosomal aberrations at diagnosis (FISH):
  - del(13q) (50-60%) → favorable outcome
  - Trisomy12 (15-20%) → unclear prognostic value
  - del(11q) (10-20%) → intermediate risk prognosis
  - del(17p) (5-10%) → worse clinical outcome

- Detection of complex karyotype (>3 or > 5 aberrations)
  → worse prognosis

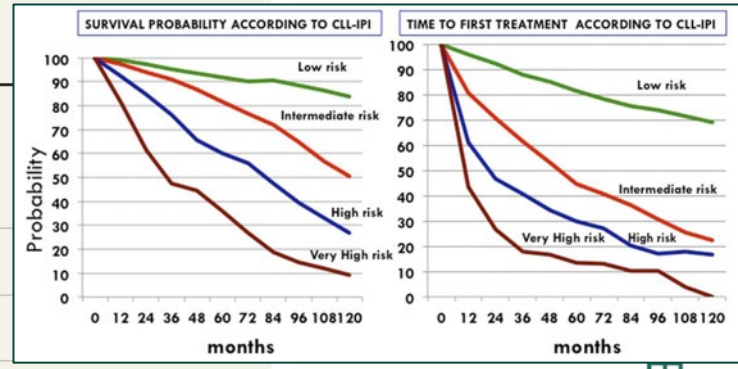
ZIEKENHUIS aan de STROOM

CLL prognostic scoring systems

Prediction of time to first treatment or prediction of estimated overall survival (OS)

Table 2. Prognostic Scoring Systems in Chronic Lymphocytic Leukemia

Variable	Points	Risk group (total points)	5-y cumulative risk of treatment start, %	
International Prognostic Score for Asymptomatic Early-stage Disease (IPS-E)				
Unmutated *IGHV*	1	Low (0)	8.4	
ALC ≥15 × 10^9/L	1	Intermediate (1)	28.4	
Palpable lymphadenopathy	1	High (2-3)	61.2	
CLL International Prognostic Index (CLL-IPI)			5-y overall survival, %	10-y overall survival, %
Del(17p12) or *TP53* variant	4	Low (0-1)	93	79
β_2 microglobulin >3.5 mg/L	2	Intermediate (2-3)	79	39
Unaltered *IGVH*	2	High (4-6)	63	22
Rai stage 1-4	1	Very high (7-10)	23	4
Age >65 y	1			



EKENHUIS aan de STROOM

CLL to treat or not?

CLL-IPI category	OS at 5 years	Potential clinical consequence
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 the disease is asymptomatic
Very high-risk	23.3%	If you need to treat, do not use chemotherapy but rather targeted agents or treatment in clinical trials

→ Most patients: no need for treatment at time of diagnosis

→ Wait and see / watchful waiting

TABLE 2 Criteria to define symptomatic or active disease according to iwCLL guidelines[5]

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia. Cut-off levels of Hb <10 g/dL or platelet counts of <100 000/μL are generally regarded as indication for treatment. However, it should be pointed out that in some patients platelet counts of <100 000/μL may remain stable over a long-period of time; this situation does not automatically require therapeutic intervention
2. Massive (i.e., ≥6 cm below the left costal margin) or progressive or symptomatic splenomegaly
3. Massive nodes (i.e., ≥10 cm in longest diameter) or progressive or symptomatic lymphadenopathy
4. Progressive lymphocytosis with an increase of ≥50% over a 2-month period, or lymphocyte doubling time (LDT) of less than 6 months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts (ALC) obtained at intervals of 2 weeks over an observation period of 2–3 months; patients with initial blood lymphocyte counts of <30 000/μL may require a longer observation period to determine the LDT. Factors contributing to lymphocytosis other than chronic lymphocytic leukemia (e.g., infections, steroid administration) should be excluded
5. Autoimmune complications including anemia or thrombocytopenia poorly responsive to corticosteroids
6. Symptomatic or functional extranodal involvement (e.g., skin, kidney, lung, spine)
7. Disease-related symptoms as defined by any of the following:
   (a) Unintentional weight loss ≥10% within the previous 6 months
   (b) Significant fatigue (i.e., ECOG PS 2 or worse; cannot work or unable to perform usual activities)
   (c) Fevers ≥100.5°F or 38.0°C for 2 or more weeks without evidence of infection
   (d) Night sweats for ≥1 month without evidence of infection

CLL treatment strategy

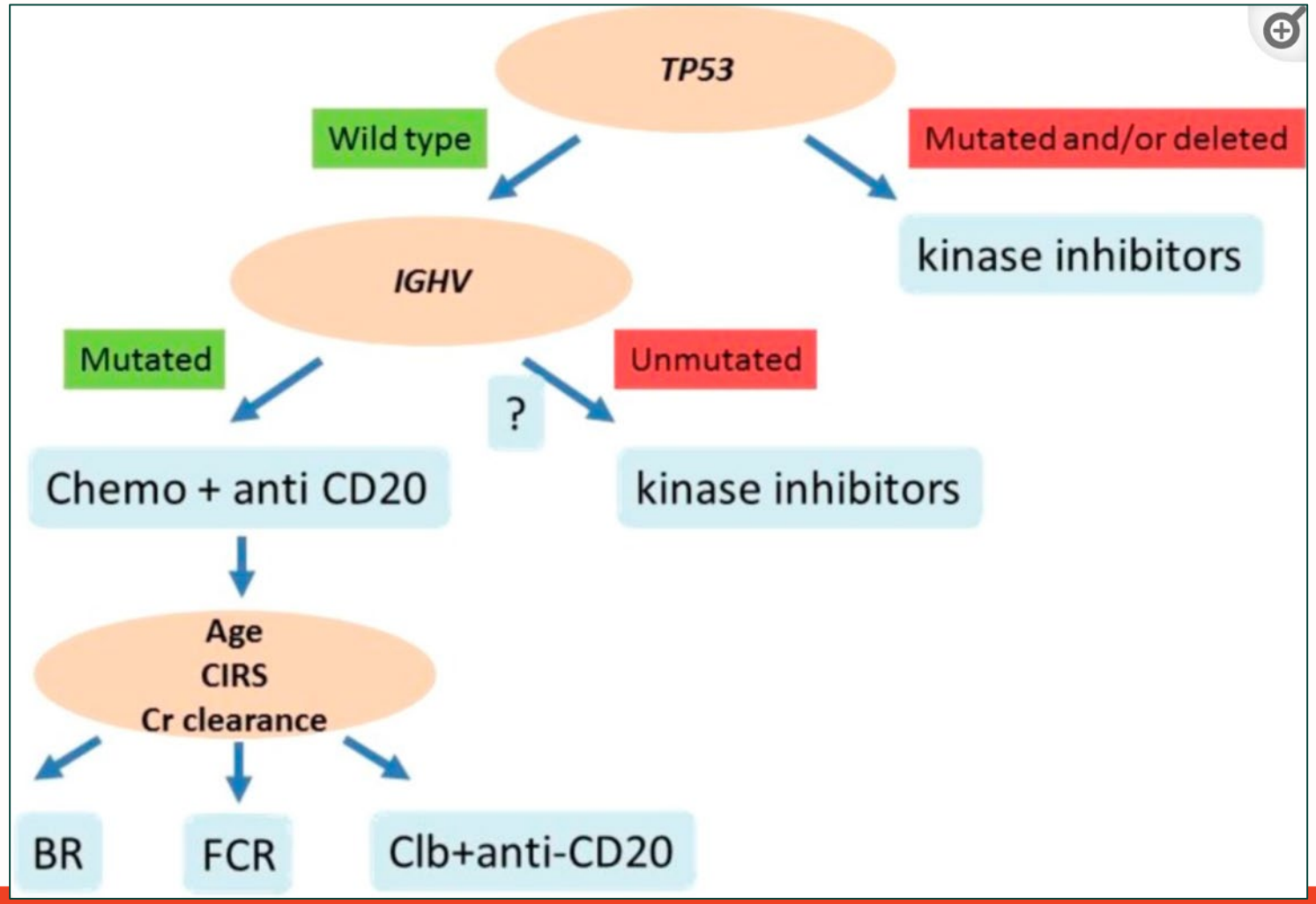


Figure 1. The Treatment Approach to Chronic Lymphocytic Leukemia

Treatment protocol for chronic lymphocytic leukemia

1 First-line treatment

Normal *TP53*

Fixed-duration treatment:
- Venetoclax + obinutuzumab

Indefinite treatment:
- Covalent BTK inhibitors acalabrutinib[a] or zanubrutinib[a] or ibrutinib

Aberrant *TP53*

Indefinite treatment:
- Covalent BTK inhibitors (preferred)[b] acalabrutinib[a] or zanubrutinib[a] or ibrutinib

Fixed-duration treatment[c]:
- Venetoclax + obinutuzumab; consider continuation of venetoclax in patient with abnormal *TP53*, especially in patients with evidence of detectable disease at 12 mo

If disease progression or intolerance to first-line treatment

2 Second-line treatment

Patient previously treated with covalent BTK inhibitor

Intolerance[d]:
- Switch to other BTK inhibitor
- Venetoclax + rituximab[e]

Progression:
- Venetoclax + rituximab[e]
- Noncovalent BTK inhibitor (pirtobrutinib) when available

Patient previously treated with venetoclax

Progression while receiving treatment or early after discontinuation of venetoclax:
- Acalabrutinib[f] or zanubrutinib[f] or ibrutinib
- Noncovalent BTK inhibitor (pirtobrutinib) when available

Progression late after discontinuation of venetoclax[g]:
- Acalabrutinib[f] or zanubrutinib[f] or ibrutinib
- Consider retreatment with venetoclax
- Noncovalent BTK inhibitor (pirtobrutinib) when available

If disease progression after BTK inhibitors or venetoclax

3 Subsequent treatment

Prior failure of covalent BTK inhibitors and venetoclax:
- Noncovalent BTK inhibitor (pitobrutinib) when available (preferred)[h]
- PI3K inhibitors: idelalisib + rituximab or duvelisib

Consideration for cellular immunotherapy:
- Consider CAR-T therapy when/if available in patients with a controlled disease[i]
- Allo-HCT if no access to CAR-T or after CAR-T

ZIEKENHUIS aan

CLL MRD minimal residual disease

- MRD: small number of residual leukemic cells in PB or BM after therapy
- MRD positive: ≥ 1/10.000 or ≥ 0,01%
- Prediction of PFS (progression free survival) and OS (overall survival)

Table 1. Comparison of CLL minimal residual disease panels studied (volume per test in parentheses).

	ERIC 8-color MRD panel		Lyophilized 10-color mLST1		Liquid 10-color mLST2	
Fluorochrome	Marker	Clone	Marker	Clone	Marker	Clone
V450	CD5 (5 µL)	L17F12	CD20/CD4	L27/SK3	CD20 (5 µL)	L27
V500c	CD3 (5 µL)	SK7	CD45	2D1	CD45 (5 µL)	2D1
BV605	–	–	CD3	SK7	CD3 (5 µL)	SK7
FITC	CD81 (20 µL)	JS-81	CD8/Lambda	SK1/1-155-1	Lambda (20 µL)	1-155-2
PE	CD79b (20 µL)	SN8	CD56/Kappa	MY31/TB28-2	Kappa (20 µL)	TB28-2
PerCPcy5.5	CD22 (5 µL)	S-HCL-1	CD5	L17F12	CD5 (15 µL)	L17F12
PE-Cy7	CD19 (5 µL)	SJ25C1	CD19/TCRgd	SJ25C1/11F2	CD19 (5 µL)	SJ25C1
APC	CD43 (5 µL)	1G10	CD23	EBVCS5	CD23 (5 µL)	EBVCS5
APC-R700	–	–	CD200	OX104	CD200 (5 µL)	OX104
APC-H7	CD20 (5 µL)	L27	CD38	HB7	CD43 (5 µL)	1G10

APC: allophycocyanin; BV605: Brilliant Violet 605™; CLL: chronic lymphocytic leukemia; Cy: cyanin; ERIC: European Research Initiative on CLL; FITC: fluorescein isothiocyanate; LST: lymphoid screening tube; MRD: minimal residual disease; PE: phycoerythrin; PERCP: peridinin–chlorophyll–protein.

ZIEKENHUIS aan de STROOM

CLL Richter transformation

- Richter Syndrome
- Aggressive lymphoma
- Incidence 0,5% / year
- Rapidly enlarging lymph nodes, fatigue, fever, weight loss, LDH ↗, PET scan, …
  - DLBCL (90-95%); poor prognosis
  - Hodgkin lymphoma (10%)
  - Histiocytic/dendritic cell neoplasms (very rare)

ZIEKENHUIS aan de STROOM

~~CLL~~ MBL monoclonal B-cell lymphocytosis

- 3 subtypes
  - Low-count MBL
    - Clonal CLL/SLL phenotype
    - Count **< 500/µL**
    - No other features diagnostic of B-lymphoproliferative disorder
  - CLL/SLL-type MBL
    - Clonal CLL/SLL phenotype
    - Count ≥ **500/µL but < 5000 /µL**
    - No other features diagnostic of B-lymphoproliferative disorder
  - Non-CLL/SLL-type MBL
    - Any monoclonal non-CLL/SLL phenotype
    - No symptoms or features diagnostic of B-lymphoproliferative disorder
    - Majority: marginal zone (MZ) origin
    - **Thresholds** have yet to be formally defined

MBL WHO 5th edition (2022)

ZIEKENHUIS aan de STROOM

MBL monoclonal B-cell lymphocytosis

- Asymptomatic condition
  - no lymphadenopathy, organomegaly, other features diagnostic of B-lymphoproliferative disorder
  - frequently found incidentally
  - Initial trigger for investigation might be fatigue, weight loss, night sweats, increased infections, but these do not exclude MBL if there are no other features of a haematological malignancy (eg pancytopenia, lymphadenopathy, organomegaly, …)
- Localization: PB (detectable in BM or secondary lymphoid tissue)
- Using high sensitivity FCM, it can be found in
  - 5% 40-50y
  - 5-25% 65-80y
  - 50-75% >90y

MBL WHO 5th edition (2022)

ZIEKENHUIS aan de STROOM

MBL monoclonal B-cell lymphocytosis

- Cytomorphology ~ CLL
- FCM
  - CLL/SLL-type ~ CLL
  - Non-CLL/SLL-type ~ MZ or other B-cell lymphoma
- **Progression to CLL?**
  - CLL/SLL-type: 0,5 – 2% per year
    - Higher if > 3000/µL
    - Very low if <1500/µL
  - Low count MBL: very small risk of progression to CLL

MBL WHO 5th edition (2022)

ZIEKENHUIS aan de STROOM

~~CLL~~ SLL Small lymphocytic lymphoma

- SLL same disease as CLL
- Tissue based diagnosis:
  - organ enlargement (lymphadenopathy > 15mm)
  - Invasion of this tissue by 'CLL-cells'
- SLL is used when < 5000 circulating monoclonal B-cells AND nodal, splenic or other extramedullary involvement

ZIEKENHUIS aan de STROOM

Atypical CLL

- NOT included in WHO HAEM5 nor in ICC classifications
- Inheritance of the FAB classification (1989)

- Atypical morphology
- Atypical immunophenotype
- Atypical genotype

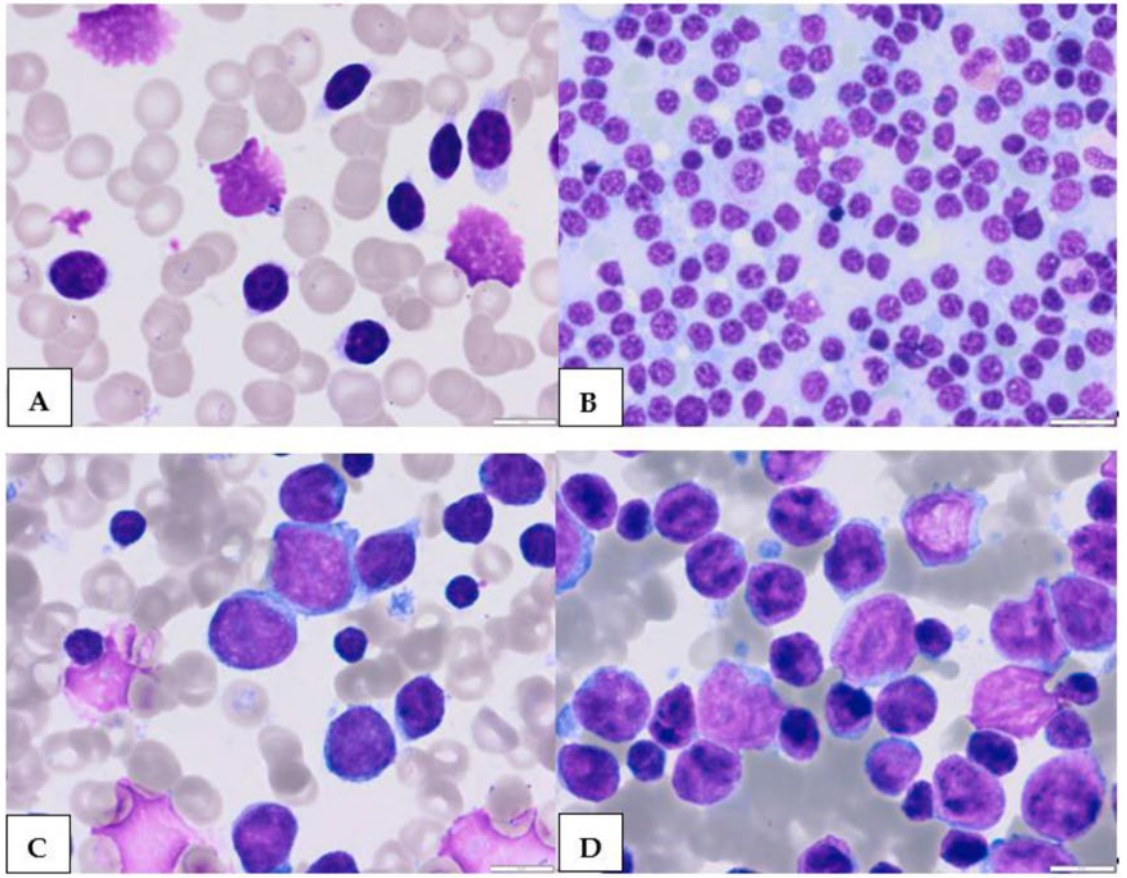


Figure 1. Morphological features of classic (**A**,**B**) and large (**C**,**D**) CLL cells. Mature CLL cells are lymphocytes with a narrow border of cytoplasm and partially aggregated chromatin in a dense nucleus ((**A**)—peripheral blood, (**B**)—bone marrow). Large atypical CLL cells ((**C**)—peripheral blood, (**D**)—bone marrow) (magnification 63×).

Atypical CLL

- Atypical morphology

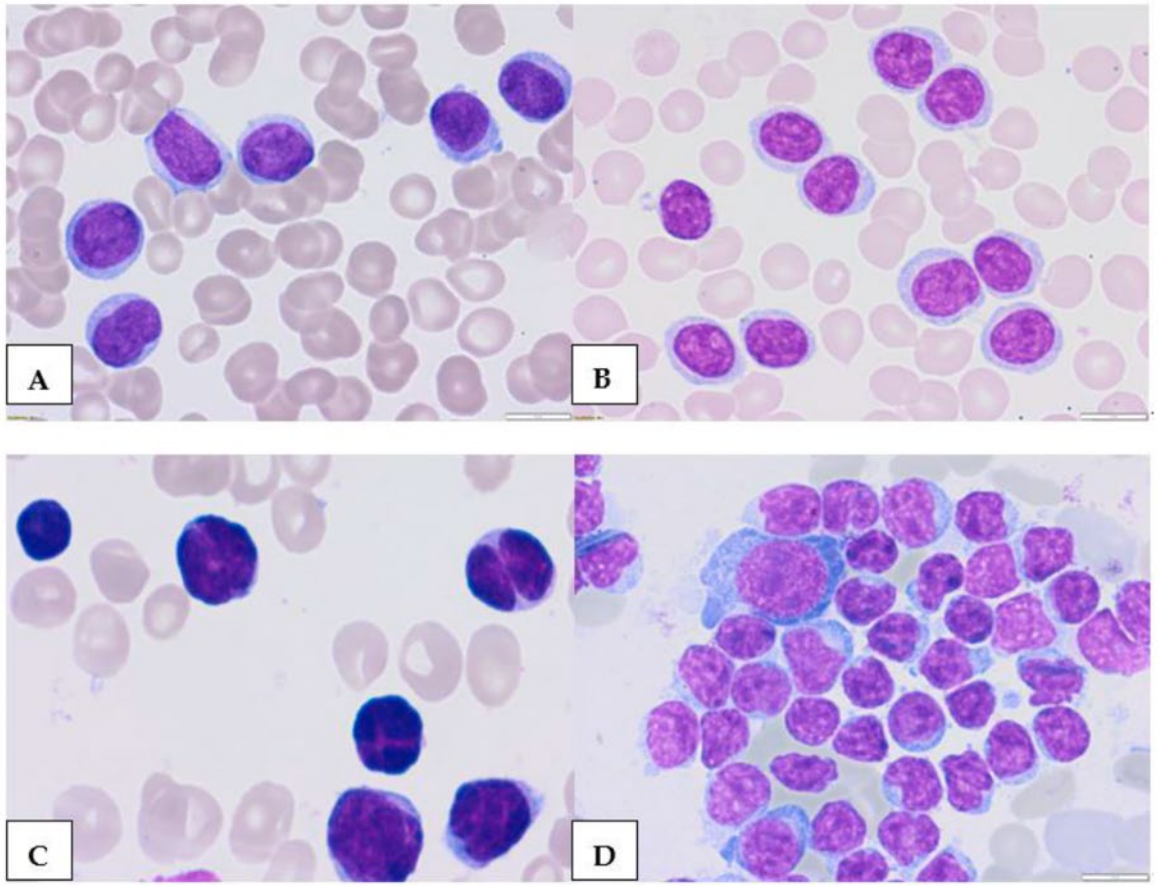
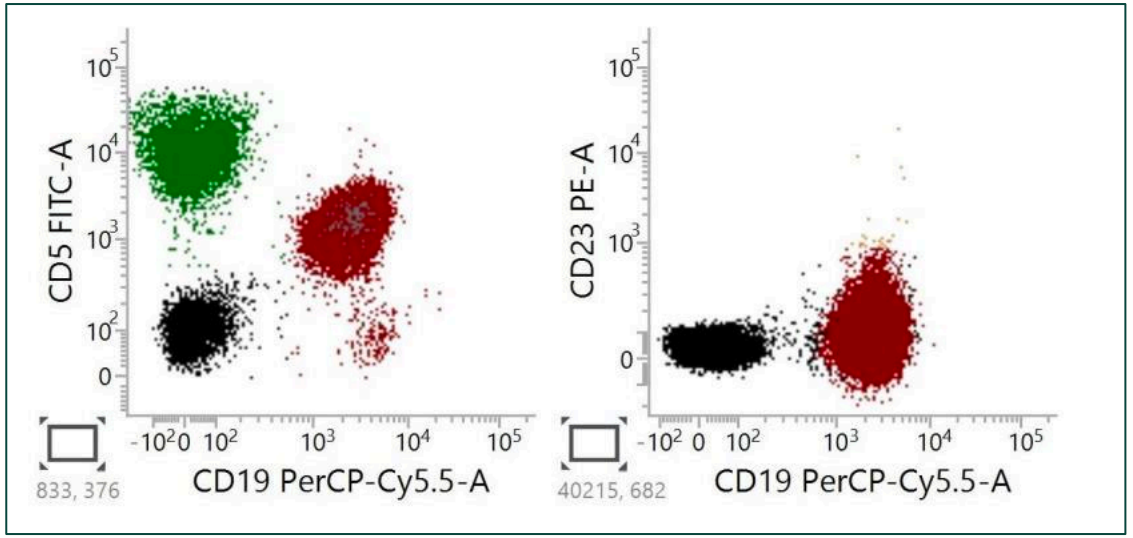


Figure 2. Morphologic features of atypical CLL cells. Prolymphocytes in peripheral blood (**A**,**B**) and cleaved cells (**C**)—in peripheral blood, (**D**)—bone marrow (magnification 63×).

Atypical CLL

- Atypical immunophenotype



Disease	Markers											
	CD19 CD20 CD22	CD5	CD23	FMC7	CD200	CD45	CD43	CD180	Cyclin D1	CD79b	LEF1	SOX11
Typical CLL	+ (dim)	+	+ (strong)	–	+ (strong)	–/+	+	+/– (week)	–	+/– (week)	+	–
Atypical CLL	+	–	–	–	+	+	+	+	–	NR	+/–	NR
MCL	+ (strong)	–	–	+/–	–	+	–/+	+ (week)	+	+/–	–	+

ZIEKENHUIS aan de STROOM

Atypical CLL

- ‘Atypical’ genotype
  - Trisomy 12 most frequently found abnormality in atypical CLL, however also found in ‘typical’ CLL …

- Atypical CLL:
  - more agressive clinical behaviour, worse prognosis
  - no therapeutic implications (treatment ~ CLL)
  - clinical significance still debated

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ZIEKENHUIS aan de STROOM

CLL according to ChatGPT

1. **Clinical Presentation**
    1. Often asymptomatic initially.
    2. Symptoms: fatigue, enlarged lymph nodes, weight loss.
2. **Diagnostic Tests**
    1. CBC with elevated lymphocytes.
    2. Peripheral Blood Smear: small mature lymphocytes.
    3. Flow Cytometry: CD5, CD23, CD19 immunophenotyping.
3. **Confirmation and Staging**
    1. Bone Marrow Aspiration/Biopsy for confirmation.
    2. Rai/Binet Staging Systems for classification.
4. **Cytogenetic and Molecular Testing**
    1. FISH for chromosomal abnormalities.
    2. PCR for mutations (e.g., TP53, NOTCH1) and IGHV status.
5. **Imaging Studies**
    1. CT/MRI for lymph node and organ assessment.
    2. PET scans for additional information.
6. **Prognostic Factors**
    1. IGHV mutation, ZAP-70, CD38 expression.
    2. Cytogenetic abnormalities, serum beta-2 microglobulin.
7. **Treatment Approaches**
    1. Watchful waiting for early-stage.
    2. Chemotherapy (fludarabine, cyclophosphamide, rituximab) for symptoms.
    3. Emerging therapies (ibrutinib, venetoclax) and immunotherapy

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Ziekenhuis aan de Stroom
[ZAS] is het netwerk van
ZNA en GZA Ziekenhuizen

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