Reproducible diagnosis of CLL by flow cytometry: an ERIC & ESCCA harmonisation project

Andy C. Rawstron, Karl-Anton Kreuzer, Asha Soosapilla, Martin Spacek, Peter Gambell, Neil McIver-Brown, Katherina Psarra, Maria Arroz, Raffaella Milani, Javier de la Serna, M. Teresa Cedena, Ozren Jaksic, Josep Nomdedeu, Carol Moreno, Gian Matteo Rigolin, Antonio Cuneo, Preben Johansen, Hans Johnsen, Richard Rosenquist Brandell, Carston Utoft Niemann, David Westerman, Marek Trneny, Stephen Mulligan, Peter Hillmen, David Oscier, Michael Hallek, Paolo Ghia, Emili Montserrat.

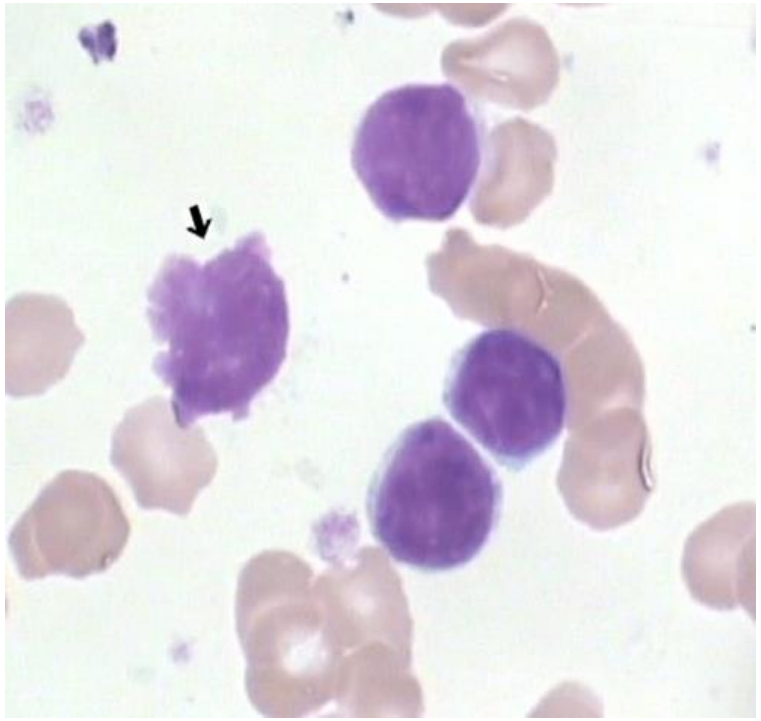
The Leeds Teaching Hospitals NHS
NHS Trust

ESCCA
European Society
for Clinical Cell Analysis

ERIC
european research initiative on CLL

Differential diagnosis of CLL

It is important to verify that the patient has CLL and not some other lymphoproliferative disease that can masquerade as CLL, such as hairy cell leukemia or leukemic manifestations of mantle cell lymphoma, marginal zone lymphoma, splenic marginal zone lymphoma with circulating villous lymphocytes, or follicular lymphoma. To achieve this, it is necessary to evaluate the blood smear, the immunophenotype, and*, in some cases, the genetic features of the circulating lymphoid cells.*



iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL

Michael Hallek,[1,2] Bruce D. Cheson,[3] Daniel Catovsky,[4] Federico Caligaris-Cappio,[5] Guillermo Dighiero,[6] Hartmut Döhner,[7] Peter Hillmen,[8] Michael Keating,[9] Emili Montserrat,[10] Nicholas Chiorazzi,[11] Stephan Stilgenbauer,[7] Kanti R. Rai,[11] John C. Byrd,[12] Barbara Eichhorst,[1] Susan O'Brien,[13] Tadeusz Robak,[14] John F. Seymour,[15] and Thomas J. Kipps[16]

Blood 2018 131:2745-2760; doi: https://doi.org/10.1182/blood-2017-09-806398

Table 1. Baseline evaluation of patients with CLL

Diagnostic test	General practice	Clinical trial
Tests to establish the diagnosis		
CBC and differential count	Always	Always
Immunophenotyping of peripheral blood lymphocytes	Always	Always
Assessment before treatment		
History and physical, performance status	Always	Always
CBC and differential count	Always	Always
Marrow aspirate and biopsy	When clinically indicated (unclear cytopenia)	Desirable
Serum chemistry, serum immunoglobulin, and direct antiglobulin test	Always	Always
Chest radiograph	Always	Always
Infectious disease status	Always	Always
Additional tests before treatment		
Molecular cytogenetics (FISH) for del(13q), del(11q), del(17p), add(12) in peripheral blood lymphocytes	Always	Always
Conventional karyotyping in peripheral blood lymphocytes (with specific stimulation)	NGI*	Desirable
TP53 mutation	Always	Always
IGHV mutational status	Always	Always
Serum β_2-microglobulin	Desirable	Always
CT scan of chest, abdomen, and pelvis	NGI	Desirable
MRI, PET scans	NGI	NGI
Abdominal ultrasound†	Possible	NGI

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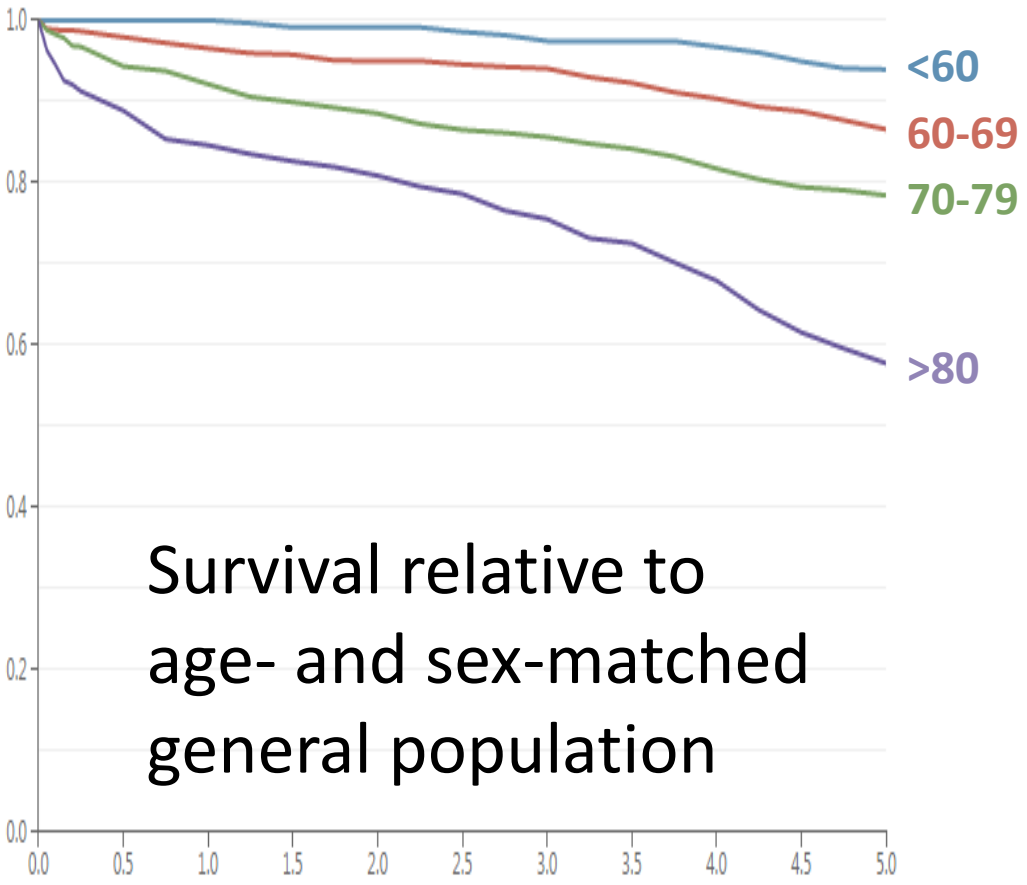
Chronic Lymphocytic Leukaemia / Small Lymphocytic Lymphoma

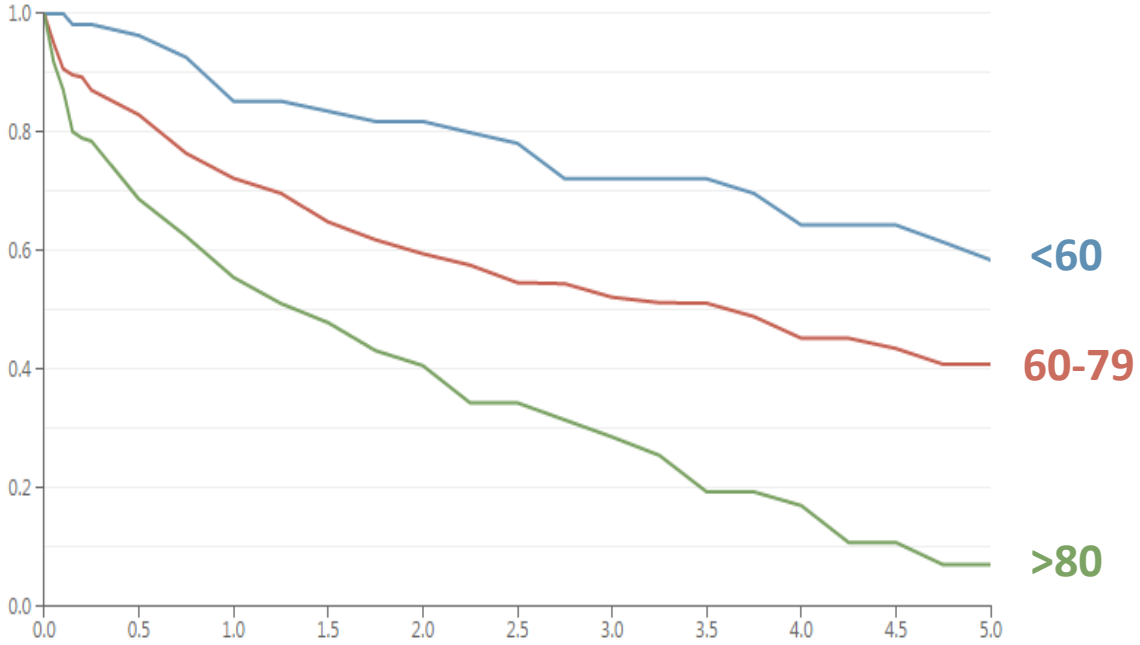
- Incidence 7.1 per 100K per year
- Abnormal B-cells in the blood (>5 x 10^9/L), bone marrow and or tissues
- **iwCLL criteria:** CLL cells the surface antigen CD5 together with the B-cell antigens CD19, CD20, and CD23. The levels of surface immunoglobulin, CD20, and CD79b are characteristically low compared to those found on normal B cells.
- >85% do not require treatment at presentation in UK
- Precursor syndrome MBL (<5 x 10^9/L) incidence 2.6/ 100K/year: ~1% progression to CLL per year
- "low-count" MBL (<0.5 x 10^9/l) – no known clinical consequences



www.hmrn.org.uk

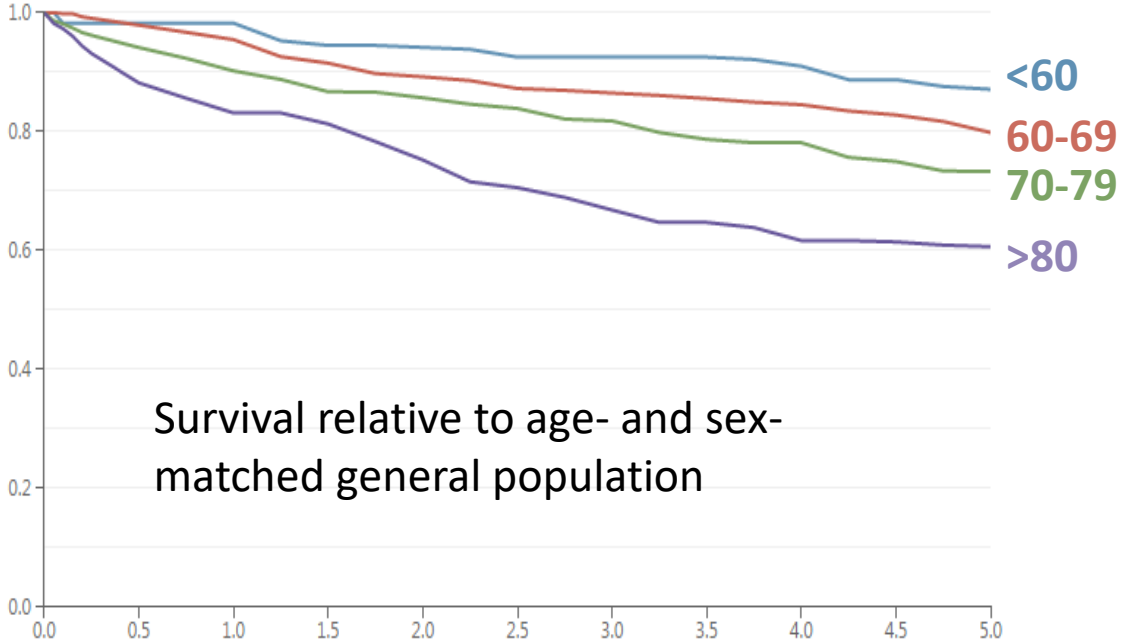
Mantle Cell Lymphoma

- Incidence 0.9 / 100K / year
  - Defined in 1991 (previously IDL/CC)
  - Molecular lesion characterised in "CLL" cases.
- Translocation of CCND1 (CyclinD1) to IGH – t(11;14)
- CD5+ CD200- &/or CD23-



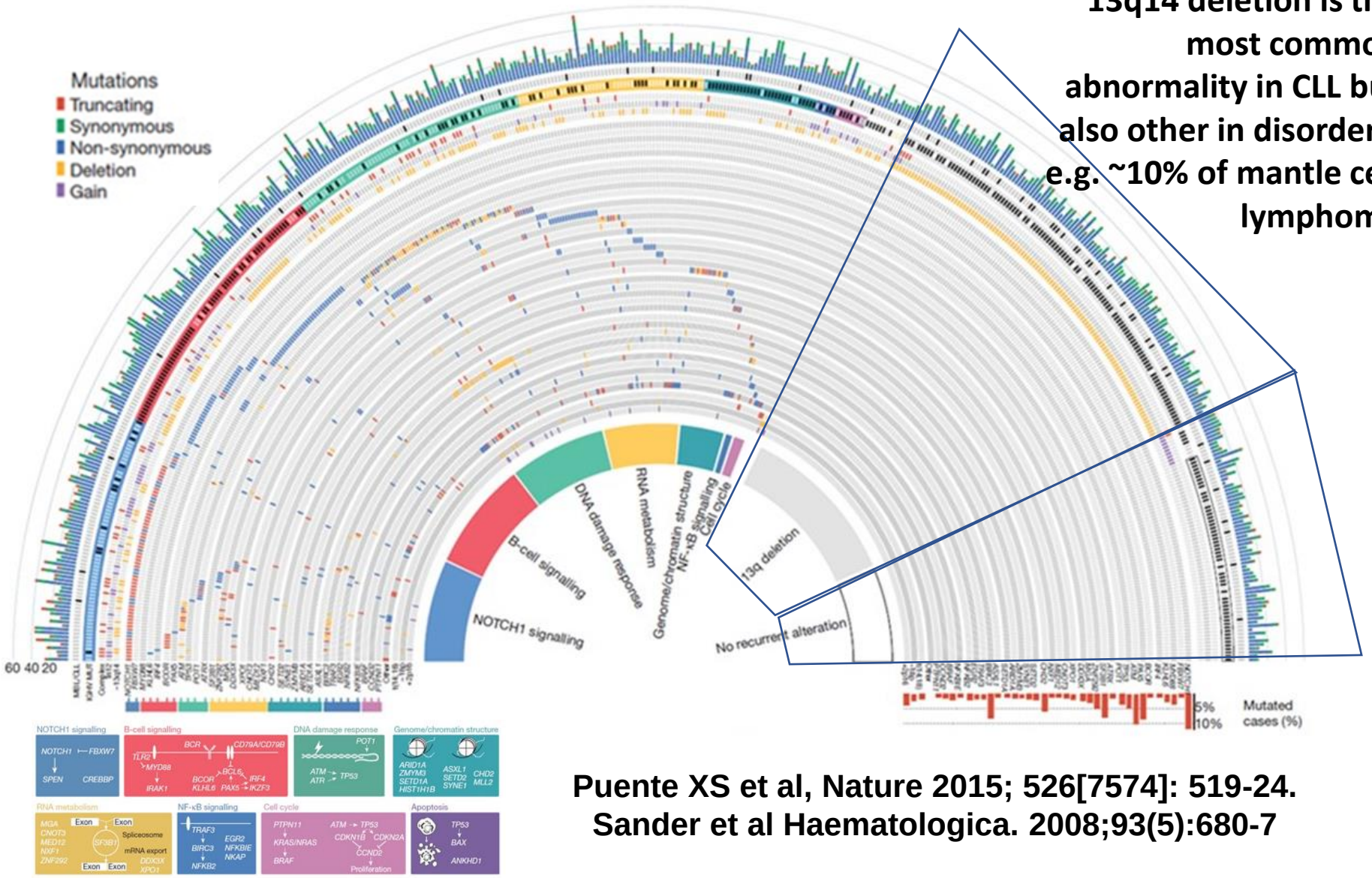
Waldenstroms / Lymphoplasmacytic Lymphoma

- Incidence 3.7 / 100K / year
- MYD88 L265P driver mutation in ~85-95% of WM/LPL (2-5% CLL)
- B-cell phenotype CD25+ with weak CD22, up to 40% may have weak CD5 expression.



The genomic landscape in CLL

13q14 deletion is the most common abnormality in CLL but also other in disorders, e.g. ~10% of mantle cell lymphoma



Puente XS et al, Nature 2015; 526[7574]: 519-24.
Sander et al Haematologica. 2008;93(5):680-7

CLL immunophenotypic score (Matutes score)

Immunophenotypic score for diagnosis of chronic lymphocytic leukemia

Flow cytometric analysis of peripheral blood or bone marrow is performed for expression of the cell surface markers listed in the table below. The scores for each marker are summed.

A score ≥ 4 is indicative of CLL. A score of ≤ 3 should prompt consideration of an alternative diagnosis.

Cell surface marker	0 points	1 point
CD79b (or CD22)	Strong	Weak
CD23	Negative	Positive
CD5	Negative	Positive
FMC7	Positive	Negative
SmIg	Strong	Weak

Adapted from Matutes et al, 1994[1] and Moreau et al, 1997.[2]

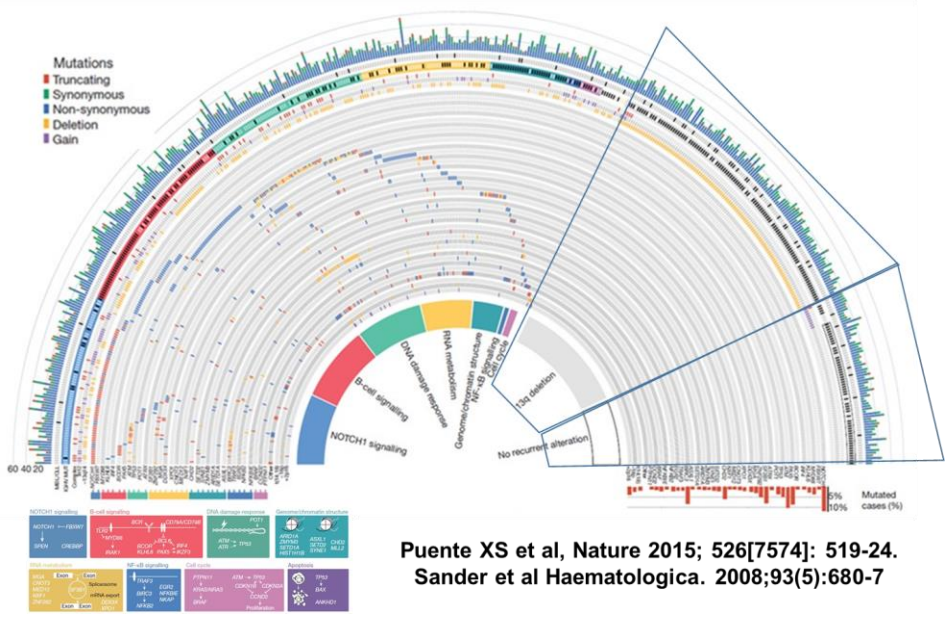
Köhnke et al, Br J Haematol. 2017 Nov;179(3):480:
"CLLflow score" is calculated by adding the percentages of CD200+ and CD23+/CD5+ B cells and then subtracting the percentages of CD79b+ as well as FMC7+B cells, resulting in the following formula:
CLLflowscore = %CD200^{+} %CD5/CD23^{+} - %CD79b^{+} - %FMC7^{+}
CLLflowscore vs. Matutes score: similar sensitivity (97·1% vs. 98·6%, P = 0·38), but higher specificity (87·2% vs. 53·8%, P < 0·001)

Mora A et al, Cytometry B Clin Cytom. 2018 Oct 16. doi: 10.1002/cyto.b.21722. [Epub ahead of print]
CD200 improved the diagnostic accuracy of Matutes score from 86.7% to 92.5% (P < .01).

- Issues:
  - Accuracy: "true" pos/neg is (at least partially) dependent on flow result
  - Reproducibility can vary by the reagents/instrumentation or definition of pos/neg/weak
  - What happens when a person with "atypical" CLL is referred?

MRD monitoring is more difficult or not possible if the pre-treatment phenotype is atypical for CLL

No pathognomonic molecular abnormality



Puente XS et al, Nature 2015; 526[7574]: 519-24.
Sander et al Haematologica. 2008;93(5):680-7

Diagnostic criteria offer guidance on marker expression but no guidance on appropriate reagents, e.g. WHO

- CLL cells usually co-express CD5 and CD23
- Using flow cytometry, the tumour cells express dim surface IgM/IgD, CD20, CD22, CD5, CD19, CD79a, CD23, CD43 and CD11c (weak). CD10 is negative and FMC& and CD79b are usually negative or weakly expressed in typical CLL.
- **Some cases may have an atypical immunophenotype (e.g. CD5- or CD23-, FMC7+ or CD11c+, strong sIg, or CD79b+).**

FCR-based CLL trials	**<2% atypical phenotype, 2% MCL**
Novel inhibitor vs. FCR	**5-10% atypical phenotype**
Novel inhibitor single arm	**5-15% [highly] atypical**

Should all CD5+ B-LPD be tested for CCND1-IGH translocation?

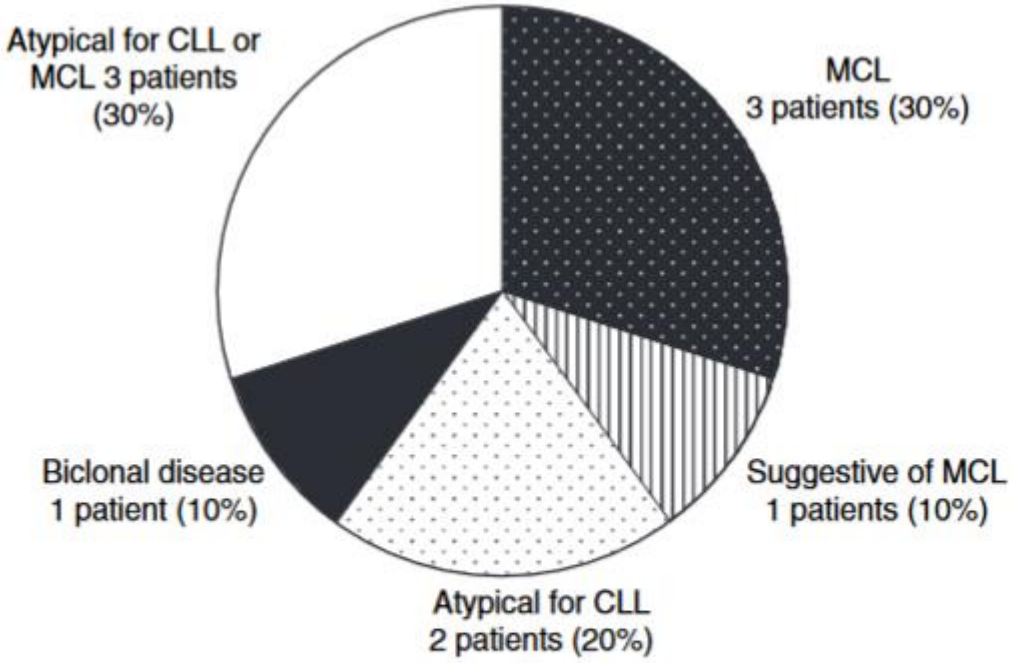


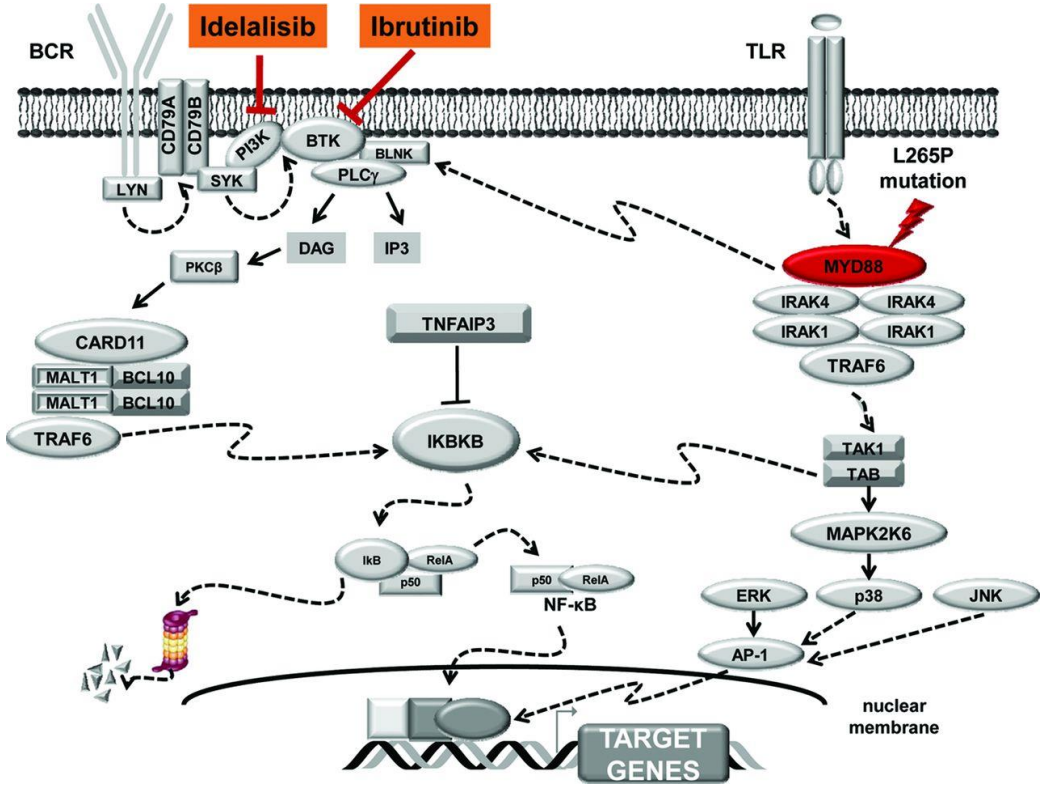
Fig 2. Immunophenotype of 10 patients with IGH/cyclin D1 fusion. The patient with biclonal disease had distinct mantle cell lymphoma and chronic lymphocytic leukaemia populations.

- 1032 patients with a presumptive diagnosis of CLL referred for Mayo Clinic CLL FISH panel
  - 10/1032 had a cyclinD1/IGH fusion
  - Phenotype with respect to CD5/CD20/CD23/sIg was atypical for CLL in 9/10 with 1/10 having biclonal disease

Nowakowski et al, Br J Haematol 2005; 130(1)

Slido.com #A470

Response to ibrutinib in Waldenstroms depends on MYD88 mutation status



Davide Rossi
Hematology 2014;2014:113-118

Table 1. Rate of Response to Ibrutinib in Patients with Waldenström's Macroglobulinemia, According to Mutation Status.*

Response Rate	Mutated *MYD88* and Wild-Type *CXCR4* (N=36)	Mutated *MYD88* and *CXCR4* WHIM (N=21)	Wild-Type *MYD88* and *CXCR4* (N=5)	P Value†
		percent		
Overall	100	85.7	60	0.005
Major	91.7	61.9	0	<0.001

N Engl J Med. 2015 Aug 6;373(6):584-6

Major response to ibrutinib in 92% with mutated MYD88 vs. 0% with wild-type MYD88

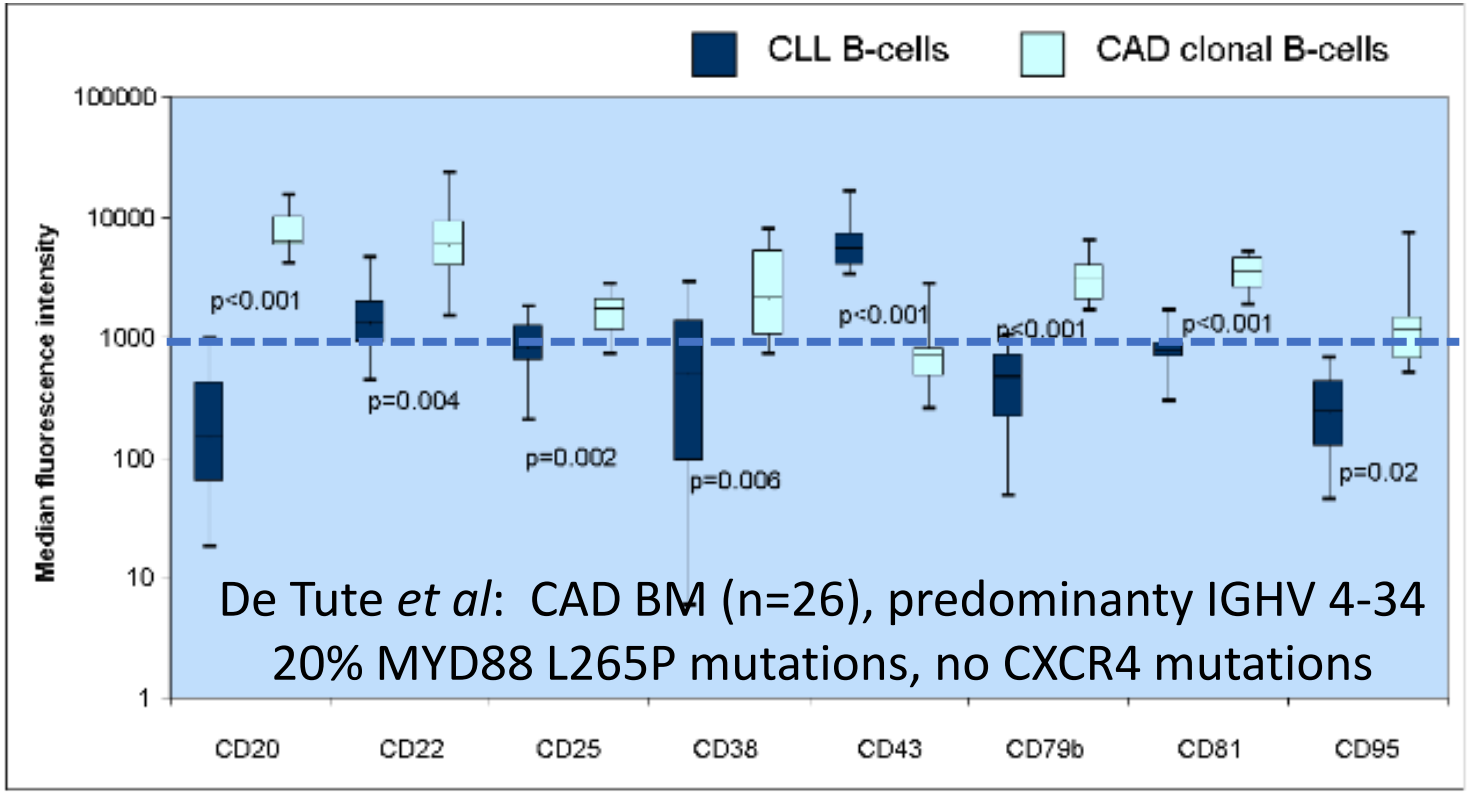
"Atypical" CLL *vs.* post-GC LPD with aberrant CD5 expression and *wild-type MYD88*

→ ? IBR non-responsive

→ ? Increased MDS rate in WM with FCR

Cold agglutinin disease

- ~80% of CAD cases have substantial CD5 expression and ~60% are CD23+ but otherwise the phenotype is distinct from CLL with moderate CD20/CD22/CD81/CD95 and weak CD43/ROR1



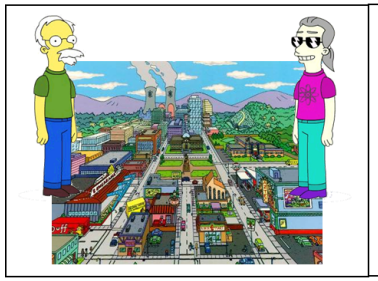
http://www.clinical-lymphoma-myeloma-leukemia.com/article/S2152-2650(15)00842-3/pdf

July 4, 2013

N Engl J Med 2013; 369:e1

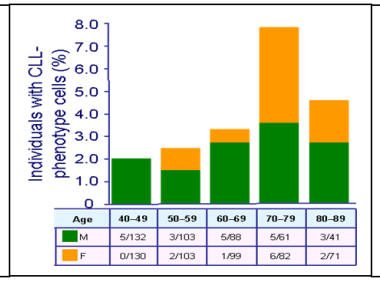
Pathogenesis of CLL:
BCL2 pathway & B-Cell Receptor (BcR) signaling

2CLR Flow
MBL in 0.6%



Vogt, Br J Haematol. 2007; 139(5): 690-700.

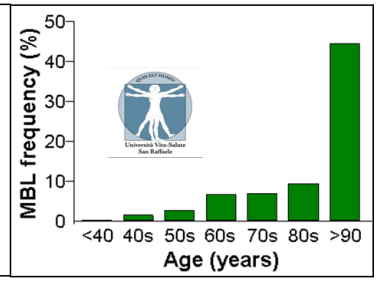
MRD-flow
MBL in 3-5%



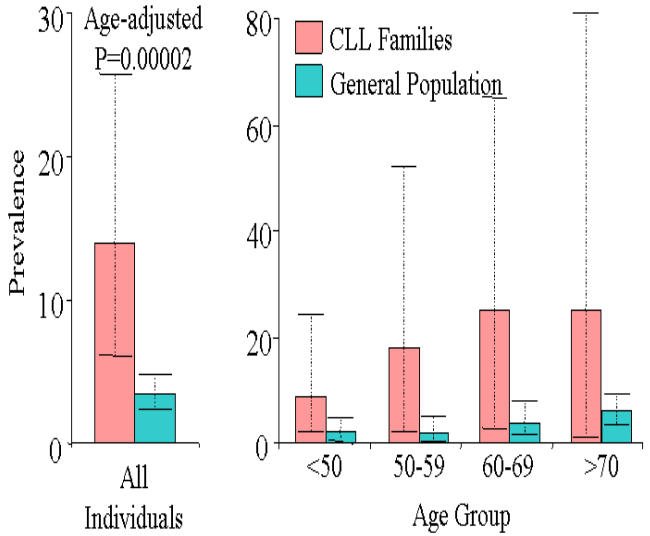
Age	40–49	50–59	60–69	70–79	80–89
M	5/132	3/103	5/88	5/61	3/41
F	0/130	2/103	1/99	6/82	2/71

Rawstron, Blood 2002; 100(2): 635-39
Ghia, Blood 2004; 103(6): 2337-42

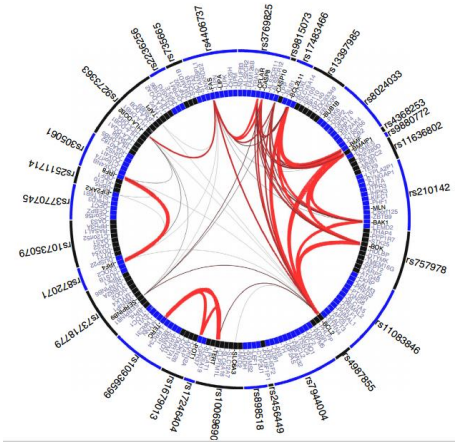
Ultra-sensitive
MBL in 10-90%



Dagklis, Blood 2009;114:26-32
Nieto Blood. 2009;114(1):33-7

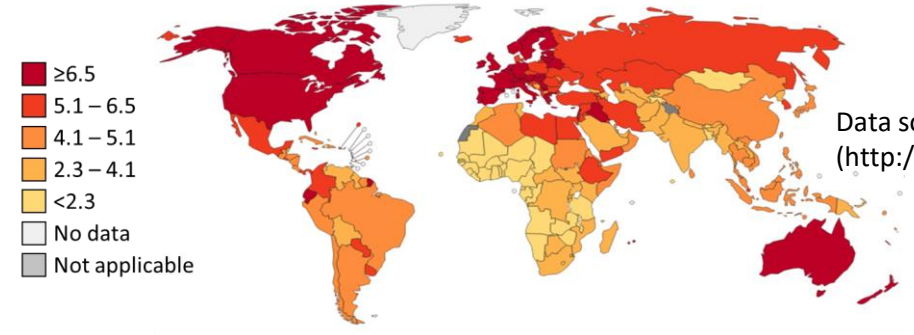


Rawstron et al Blood. 2002; 100: 2289-2290
Goldin LR et al Br J Haematol. 2010; 151(2):152-8



Susceptibility polymorphisms: BCL2-family and GC-transition

Berndt Nature Comms 2016;7:10933

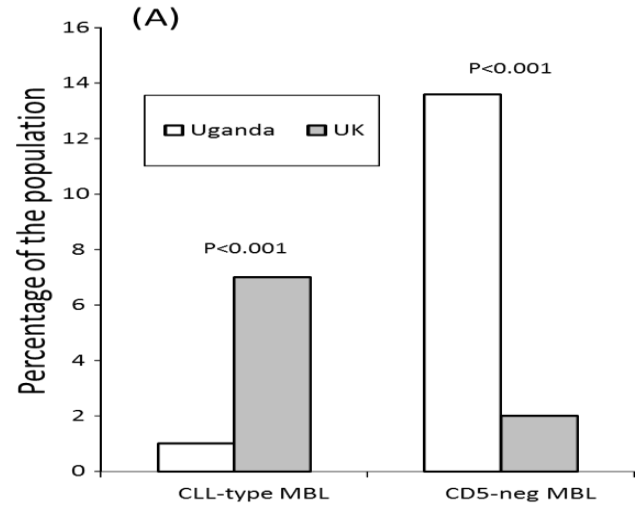


Data source: GLOBOCAN 2012 (http://gco.iarc.fr/today)

World Health Organization

CLL susceptibility polymorphisms virtually absent in African populations

CLL-type MBL absent/rare in Uganda but CD5^{neg} MBL is frequent



Rawstron, Lancet Haematology. 2017; 4(7), e334–e340

THE LANCET Haematology

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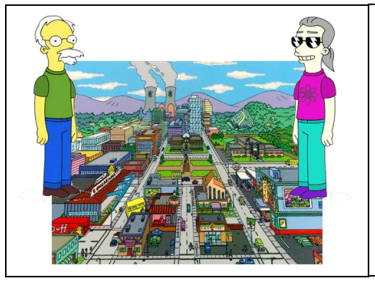

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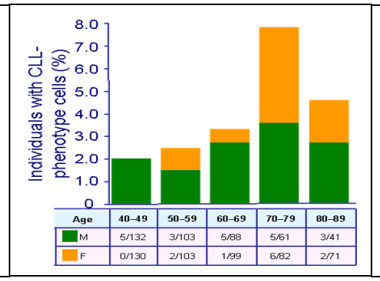

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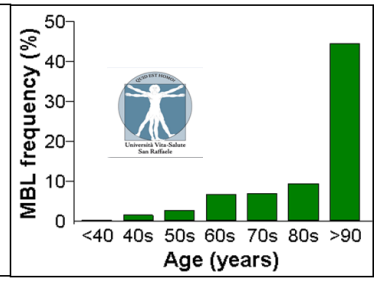

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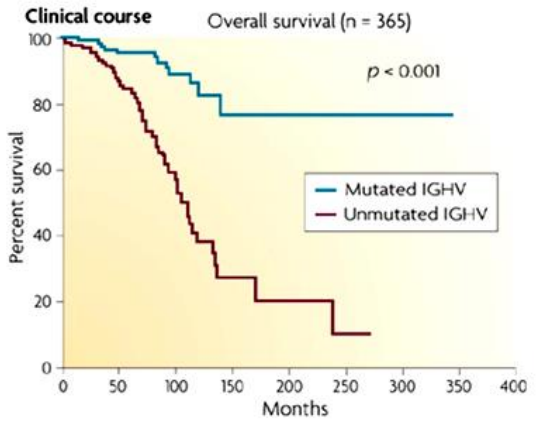


~30% of patients with CLL carry immunoglobulin receptors with highly similar primary sequence (stereotyped)

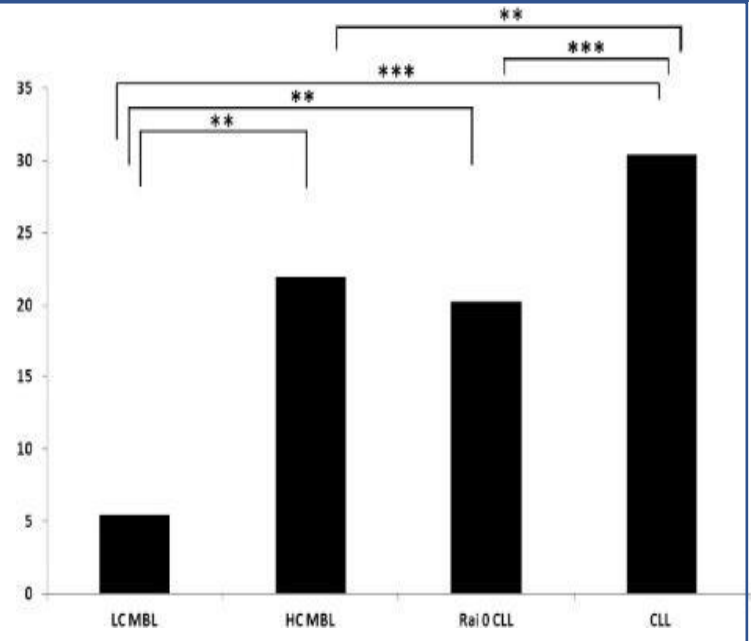
CLL phenotype independent of progression

BCL2 pathway dysregulated through:

- Inherited susceptibility polymorphisms
- 13q14 deletion: miR-15/miR-16 family downregulate BCL2 expression → ?del13q14 leads to Bcl-2 over-expression.
- Trisomy 12 → Lower bax/bcl-2 ratio
- BLC2-IGH translocation

BCR IG stereotypes infrequent in low-count MBL, but frequency (%) in high-count MBL and CLL.

**P < .01; ***P < .001.
Vardi et al, Blood 2013 121:4521-4528



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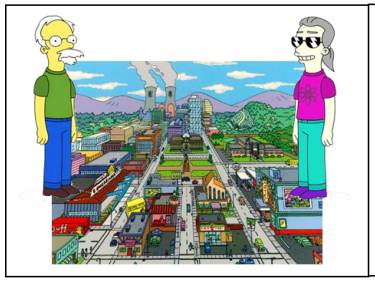

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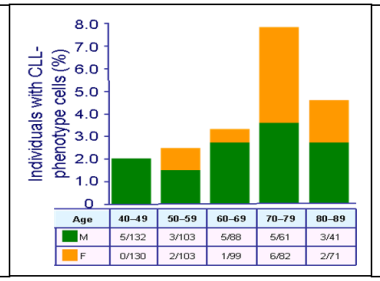

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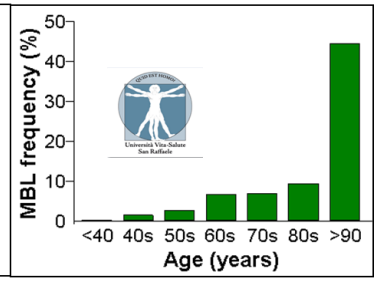

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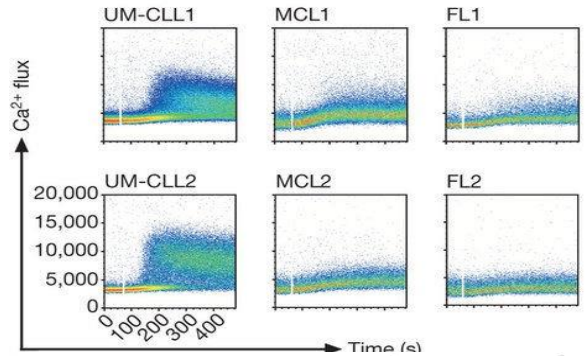
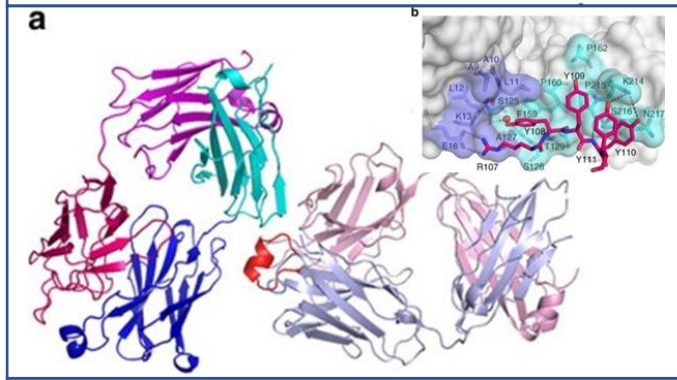


CLL-derived BCRs induce antigen-independent cell-autonomous signaling: Dühren-von Minden M et al Nature. 2012 Sep 13;489(7415):309-12.

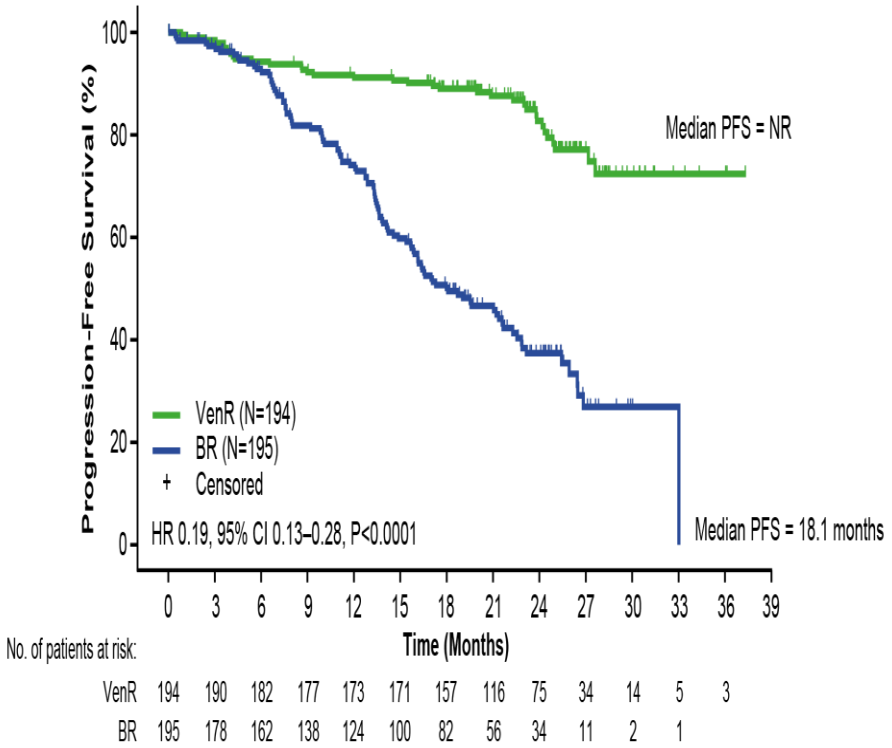


Structural basis of autonomous activation: Minici et al. Nat Commun. 2017; 8: 15746

Expansion driven through IGHV

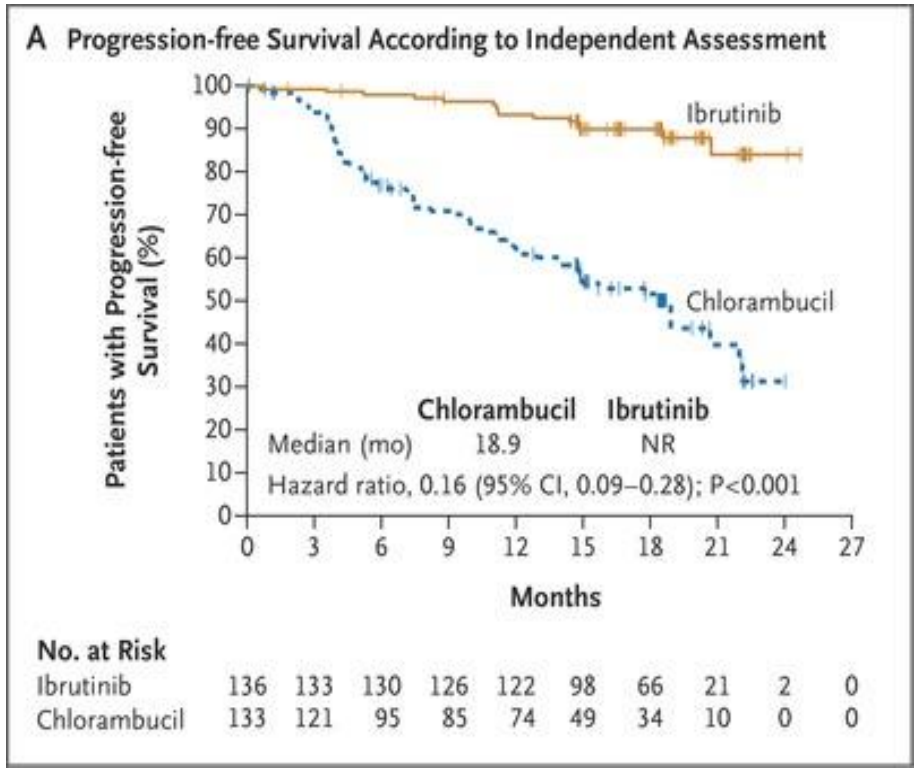
E.g. stereotype IGH4 subset 4 (IGHV4-34/D5-18/J6 and IGKV2-30/J2) binds viable memory B-cells through an epitope acquired by somatic hypermutation: Catera et al. Mol Med. 2017; 23: 1–12

Effective treatment but no effective diagnostic for BCL2 pathway & B-Cell Receptor (BcR) signaling



Venetoclax (BH3-mimetic) in R/R CLL

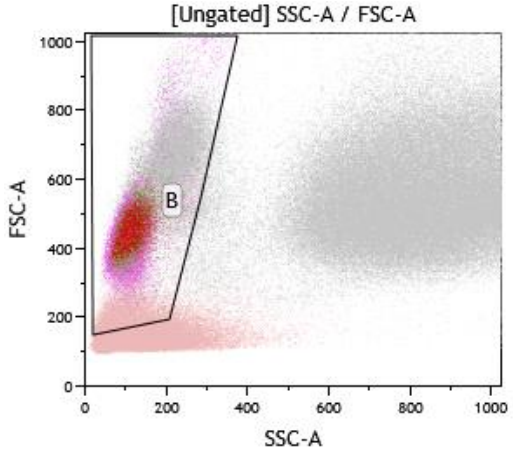
N Engl J Med 2018; 378:1107-1120
John Seymoure et al, MURANO Trial

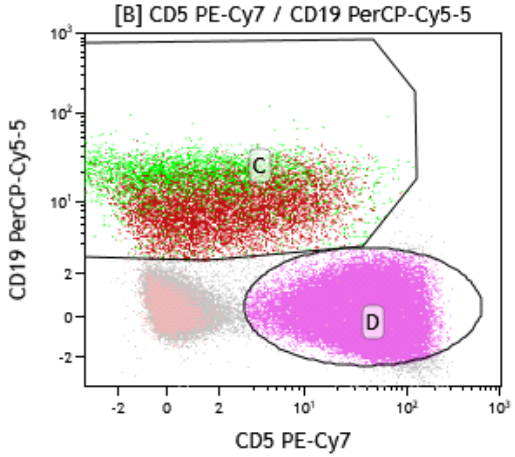


Ibrutinib (BTK-inhibitor) in frontline CLL

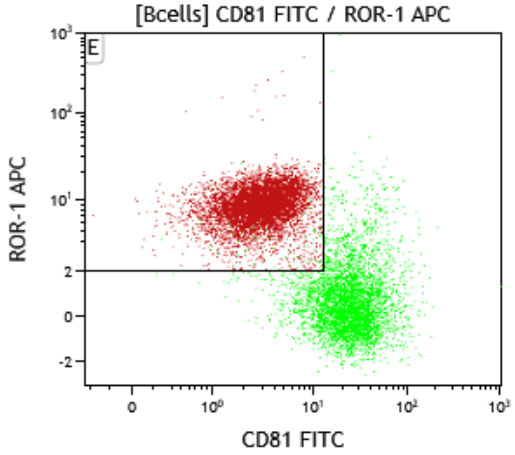
N Engl J Med 2015; 373:2425-2437
Jan Burger et al, RESONATE-2 Trial

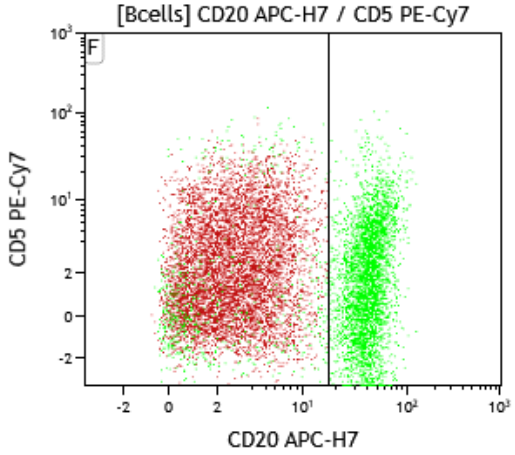
Ibrutinib and venetoclax treatment can be associated with reduced CD19 & CD5 expression on CLL cells

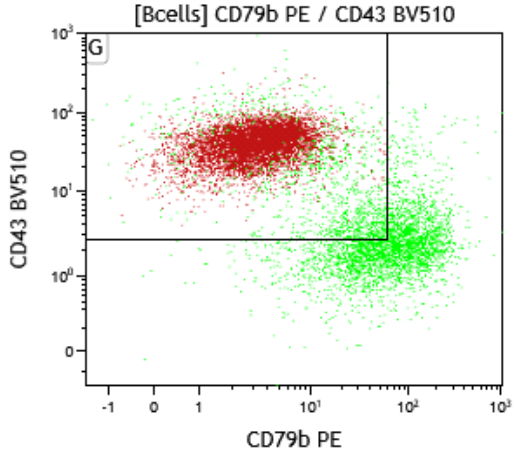




Gate	Number	%Total
All	500,000	100.000
CLL	6,562	1.312
NormB	4,654	0.931
Leucs	440,982	88.196

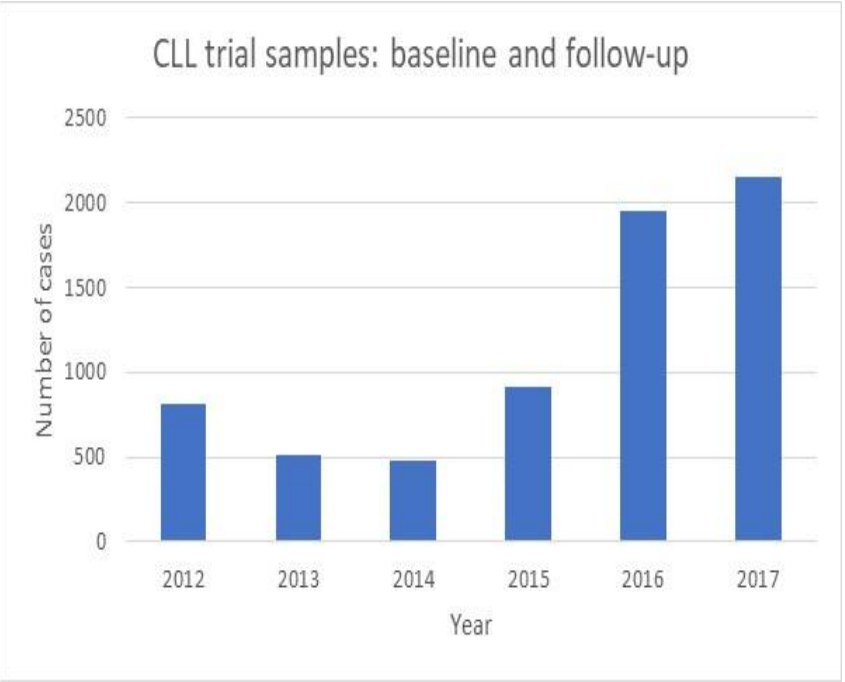






Decreases in CD19 expression usually small but substantial loss in 1-5% of patients in combination Rx

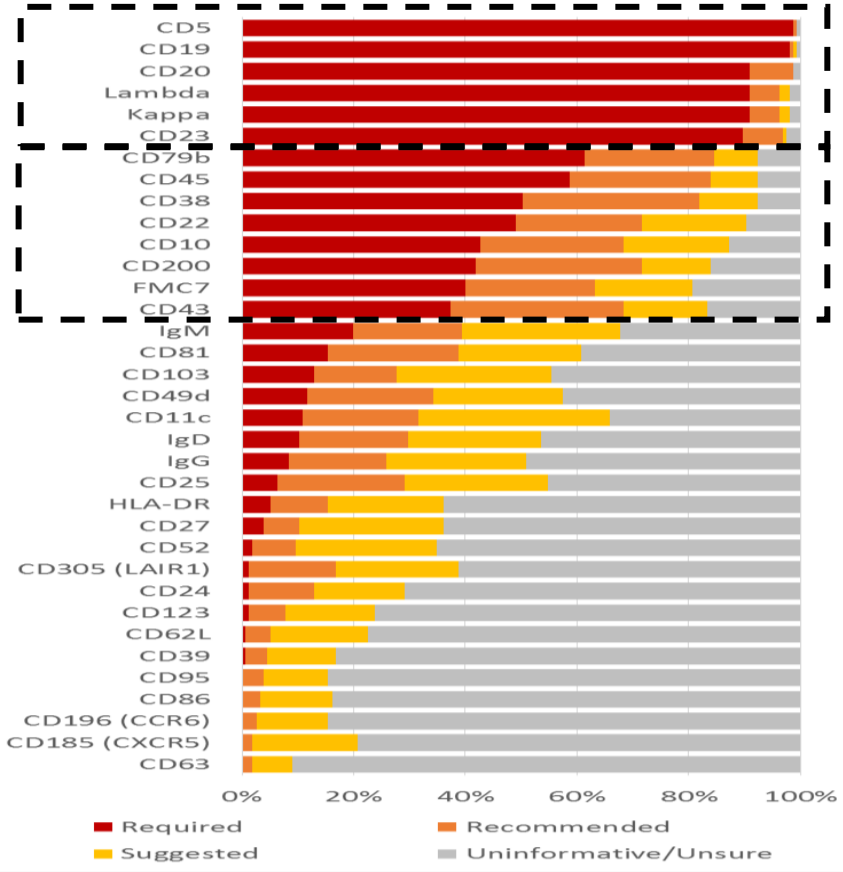
→ HLADR + ROR1 to improve gating



Diagnostic issues in CLL

- No diagnostic molecular abnormality
- Dysregulation of BCL2 pathway and BCR signaling but no/limited methods for assessment in a clinical laboratory
- Phenotypic overlap between CLL and other disorders and flexible diagnostic criteria to enable access to treatment
  - Cases with a phenotype more consistent with MCL, MZL, WM/LPL/CAD may be classified as CLL in the absence of other clinical/molecular features
- Increasing frequency of atypical cases
  - Treatment-related changes
  - Access to novel therapies

Reproducible diagnosis of CLL by flow cytometry: an ERIC & ESCCA harmonisation project



Consensus on markers "required" for diagnosis:

CD5, CD19, CD20, CD23, κ/λ

"Recommended" markers:

Consensus: *CD10, CD43, CD79b, CD200*

Steering committee:

include: *CD81, ROR1*

lab preference: *CD45, CD38, FMC7, CD22*

In WHO criteria but rarely recommended:

IgM/D, CD11c

Andy C. Rawstron, Karl-Anton Kreuzer, Asha Soosapilla, Martin Spacek, Peter Gambell, Neil McIver-Brown, Katherina Psarra, Maria Arroz, Raffaella Milani, Javier de la Serna, M. Teresa Cedena, Ozren Jaksic, Josep Nomdedeu, Carol Moreno, Gian Matteo Rigolin, Antonio Cuneo, Preben Johansen, Hans Johnsen, Richard Rosenquist Brandell, Carston Utoft Niemann, David Westerman, Marek Trneny, Stephen Mulligan, Peter Hillmen, David Oscier, Michael Hallek, Paolo Ghia, Emili Montserrat.

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Proposed minimum criteria for diagnosis

Antigen	Typical Expression (% pos vs. control)	Control Population in normal peripheral blood		Minimum Relative fluorescence intensity (preferred)
		Positive	Negative	
CD19	Positive (>95%)	B-cells	T-cells	>10 (>20)
CD5	Positive (>20%)*	T-cells	NK-cells	>30 (>65) †
CD23	Positive (>20%)*	CD23+ B-cells	CD19- Lymphocytes	>5 (>10)
CD20	Weak	CD19+ B-cells	CD3+ T-cells	>10 (>20) †
Igκ	Weak & restricted to either Igκ or Igλ	Igκ+Igλ- B-cells	Igκ-Igλ+ B-cells	>5
Igλ		Igκ-Igλ+ B-cells	Igκ+Igλ- B-cells	>5

Definition of weak: median fluorescence intensity at least 20%* lower than normal peripheral blood B-cells, range to be determined within each laboratory

* ICSH/ISLH/CLIA guidelines for stability require <20% variation, therefore reduction in fluorescence intensity less than 20% may reflect antigen/sample stability

† specifically validated (ERIC CLL MRD project) otherwise consensus

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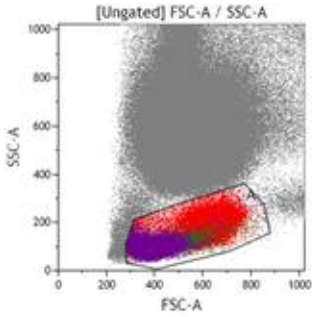
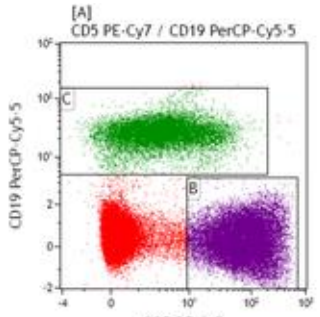
How to incorporate the minimum criteria into routine practice

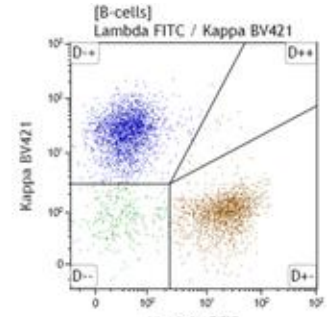
- Run the routine panel on ≥10 peripheral blood samples with only normal (polyclonal) B-cells and T-cells
- Determine the median fluorescence intensities for relevant markers
- Assess relative signal
- Determine “weak” expression threshold (80% of normal median expression)



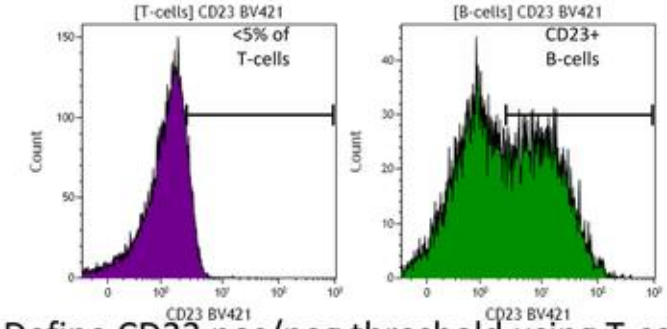
Mononuclear cell (MNC) gate



(2) Identify T-cell and B-cell gates



(3) Identify κ+λ- & κ-λ+ B-cell gates



Define CD23 pos/neg threshold using T-cell expression and identify CD23+ B-cells

Quality assessment on diagnostic panel in individual laboratories

Antigen	CD19	CD20	CD5	Kappa	Lambda	CD23
Relative signal target value	≥10	≥10	≥30	≥5	≥5	≥5
Centre 1	225 (123-479) HD37 RPE-Cy5 (Dako)	127 (51.9-183) L27 FITC (BD)	*56.3 (16.2-5892) DK23 APC (DAKO)	24.4 (12.6-87.6) Polyclonal FITC (DAKO)	100 (44.8-302) Polyclonal PE (DAKO)	11 (7.4-17.9) MHM6 FITC (DAKO)
Centre 2	5462 (4291-6393) LT19 APC (Miltenyi)	64.8 (36.6-103) 2H7 APE-eF780 (eBioscience)	*41.1 (17.7-57.2) L17F12 V450 (BD)	17.1 (4.9-37.6) G20-193 APC-H7 (BD)	**2.9 (2.1-4.9) 1-155-2 APC (eBioscience)	**4 (3.1-6.8) Tu1 FITC (Invitrogen)
Centre 3	12126 (85.1-14264) J3-119 PE-Cy7 (Coulter)	**5.4 (2.5-7.1) L27 V450 (BD)	44.2 (2.8-102) L17F12 PerCP-Cy5.5 (BD)	20.2 (7.1-55.5) Polyclonal PE (Cytognos)	35.8 (8.4-116) Polyclonal FITC (Cytognos)	43.2 (0.8-1670) MHM6 FITC (DAKO)
Centre 4	17.9 (5.6-23.5) SJ25C1 PerCP-Cy5.5 (BD)	175 (102-306) L27 FITC (BD)	237 (52.8-368) L17F12 PE (BD)	35.6 (12.6-60) TB28-2 FITC (BD)	430 (148-612) 1-155-2 PE (BD)	*49 (2.5-223) EBVCS-5 PE (BD)
Centre 5	16.5 (11.2-18.8) SJ25C1 PerCP-Cy5.5 (BD)	24.6 (16.7-30.2) L27 APC-H7 (BD)	*42.9 (15.1-56.7) L17F12 PE-Cy7 (BD)	22.6 (10.3-65.1) G20-193 BV421 (BD)	17.5 (10.3-24.2) JDC-12 FITC (BD)	18.7 (8.6-31.7) M-L233 BV421 (BD)
Centre 6	56.8 (32.8-81.9) J3-119 PE-Cy7 (Coulter)	2812 (398-5030) 2H7 PacBlue (Biolegend)	*37.2 (24.4-105) L17F12 PerCP-Cy5.5 (BD)	19.7 (11.4-65.6) Polyclonal FITC (Cytognos)	74.4 (13.6-317) Polyclonal PE (Cytognos)	15.4 (9.7-39.3) MHM6 FITC (DAKO)
Centre 7	106 (89.9-175) SJ25C1 APC (BD)	53.6 (41.2-67.4) L27 PerCP (BD)	**26.2 (17.9-39) L17F12 FITC (BD)	22.1 (6.9-45.1) TB28-2 FITC (BD)	149 (72.2-287) 1-155-2 PE (BD)	16.9 (8.6-35) EBVCS-5 PE (BD)
Centre 8	217 (130-234) J3-119 PE-Cy7 (Coulter)	82 (58.8-145) 2H7 Pacific Blue (Biolegend)	88.6 (51-123) BL1a APC (Coulter)	25.3 (10.7-80.1) Polyclonal PE (DAKO)	19.6 (7.4-74.8) Polyclonal FITC (DAKO)	10.3 (5.8-14.1) 9P25 FITC (Coulter)
Centre 9	*16.3 (5.5-130) J3-119 PE-Cy7 (Coulter)	29.9 (18.3-58.7) B-Ly1 FITC (Coulter)	**5.4 (2.4-45.6) BL1a PE (Coulter)	12.3 (4.7-29.7) Polyclonal FITC (DAKO)	46.6 (6.5-75.5) Polyclonal PE (DAKO)	19.1 (9.8-48.4) 9P25 FITC (Coulter)
Centre 10	31.6 (22.6-41.7) J3-119 ECD (Coulter)	82.1 (38.4-119) B9E9 Pacific Blue (Coulter)	**16.6 (3-31.5) BL1a APC-AF750 (Coulter)	*6.1 (1.7-11.2) Polyclonal FITC (Coulter)	18 (12.3-37) Polyclonal PE (Coulter)	9.1 (7.1-13.6) 9P25 APC-AF700 (Coulter)

Control cases meeting target signal:noise

≥80% 60-70% <50%

Proposed minimum criteria for diagnosis

Antigen	Typical Expression (% pos vs. control)	Control Population in normal peripheral blood		Minimum Relative fluorescence intensity (preferred)
		Positive	Negative	
CD19	Positive (>95%)	B-cells	T-cells	>10 (>20)
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Retrospective evaluation of the proposed criteria in 14,643 cases showed >97% concordance with current approaches in large diagnostic centres.

	Total CD5+ B-LPD diagnoses	Meeting the proposed criteria and diagnosed with CLL	Not meeting the proposed criteria	
			Other diagnosis, e.g. Mantle Cell Lymphoma	Requires MDT or trial-specific decision
Primary referral	9294	7379 (79.4%)	1025 (11%)	890 (9.6%)
Trial	2427	2267 (93.4%)	54 (2.2%)	106 (4.4%)

Excluding the 250 cases without a known final diagnosis, of the remaining 9044 primary referrals there was concordance in 97.2% (8793/9044, comprising 7379 diagnosed with CLL, 1025 diagnosed with another non-CLL B-LPD and 389 non-diagnostic with both approaches) using the reproducible criteria compared to each laboratory's current practice.

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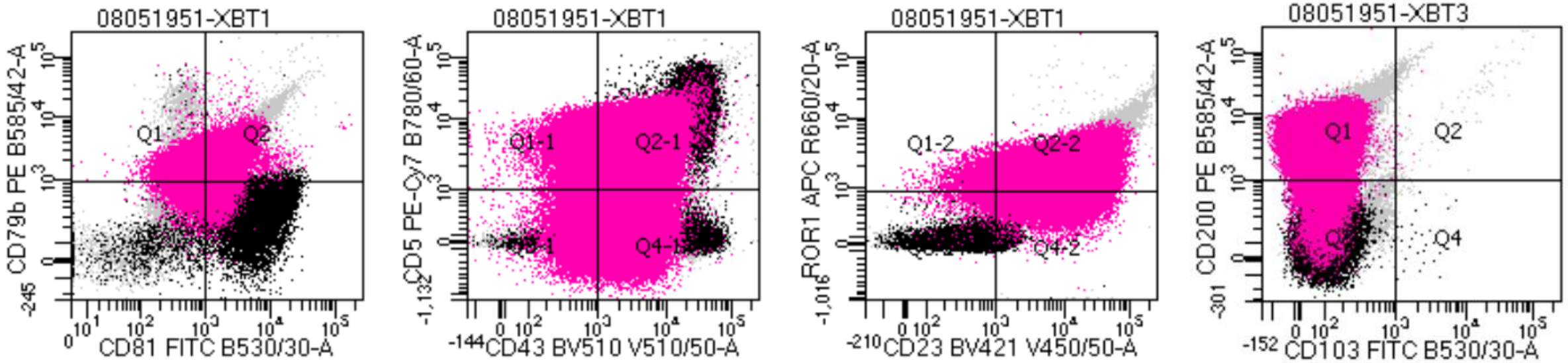
How to use the “recommended” markers: CD200, CD43 and ROR1



- CD5+CD23++, moderate CD20 expression but CD79b/sIg weak (>1 log lower than normal B-cells) and ROR1+CD43+, >90% of B-cells express CD200
  - CCND1-IGH translocation in >90% of lymphocytes, confirmed in PB & BM
- CD5+CD23+ but otherwise atypical for 1 or more marker: → 15% have CCND1-IGH translocation (usually with some ROR1/CD43 expression)
- **Which B-LPD should we test for a CCND1-IGH translocation**
  - **All CD5+ B-LPD? Only cases with an “atypical” phenotype? Only CD5+ B-LPD requiring treatment**
- NB: 17 CD5-CD200-ROR1+ cases: 3 tested for CCND1-IGH translocation, 2/3 →mantle cell lymphoma.

Experience from CLL trial baseline phenotyping:

- 782 trial baseline samples tested for deletion 13q14 (DLEU7 & RB) / ATM / TP53, trisomy 12 & CCND1-IGH translocation

- Trisomy 12 detected in 124/782
  - 76 cases with a fully typical phenotype (10% of total)
  - 48 cases atypical phenotype (6% of total), most with strong CD20 expression

- CCND1-IGH detected in 3/782
  - No translocation in cases with a fully typical phenotype
  - CCND1-IGH in 3/127 cases with an atypical phenotype (2.4%, c.f. 2.2% retrospective)
  - all atypical by minimum criteria but one CD23-CD200+, one CD23+CD200- and one CD23+CD200+

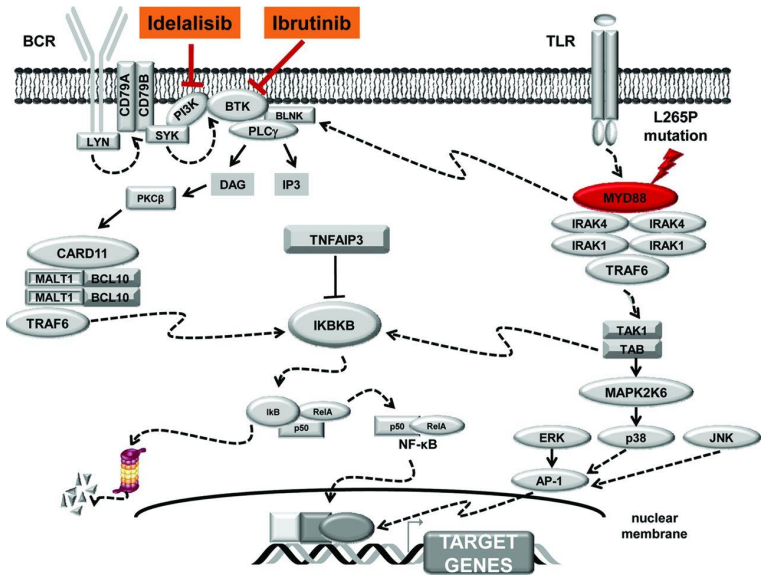
Markers ranked according to specificity for discrimination of CLL vs. MCL and CLL vs. WM/LPL/MZL

Specificity for diagnosis of CLL vs. Mantle Cell Lymphoma	
CD20 weak	**91.3%**
CD23 pos	**82.6%**
CD200 pos	**78.3%**
sIg weak	71.7%
CD81 weak	67.4%
CD43 pos	**41.3%**
ROR1 pos	**28.3%**
CD5 pos	NA

Specificity for diagnosis of CLL vs. WM/LPL/MZL	
CD20 weak	**83.0%**
ROR1 pos	**78.1%**
CD43 pos	**70.5%**
CD79b weak	67.0%
CD23 pos	**65.5%**
CD5 pos	64.0%
CD81 weak	60.2%
CD200 pos	**30.1%**

Specificity = TN/TN+FP where TN is the absence of the CLL-associated profile in another disorder (e.g. CD5-negative WM/MZL) and FP is the presence of the CLL-associated profile in another diagnosis (e.g. CD23-positive MCL).
CLL n = 658, WM/MZL n = 342, MCL n = 46.

Atypical CLL vs. post-GC B-LPD with atypical CD5 expression – lessons from MYD88 analysis



Davide Rossi Hematology 2014;2014:113-118

Table 1. Rate of Response to Ibrutinib in Patients with Waldenström's Macroglobulinemia, According to Mutation Status.*

Response Rate	Mutated *MYD88* and Wild-Type *CXCR4* (N=36)	Mutated *MYD88* and *CXCR4* WHIM (N=21)	Wild-Type *MYD88* and *CXCR4* (N=5)	P Value†
		percent		
Overall	100	85.7	60	0.005
Major	91.7	61.9	0	<0.001

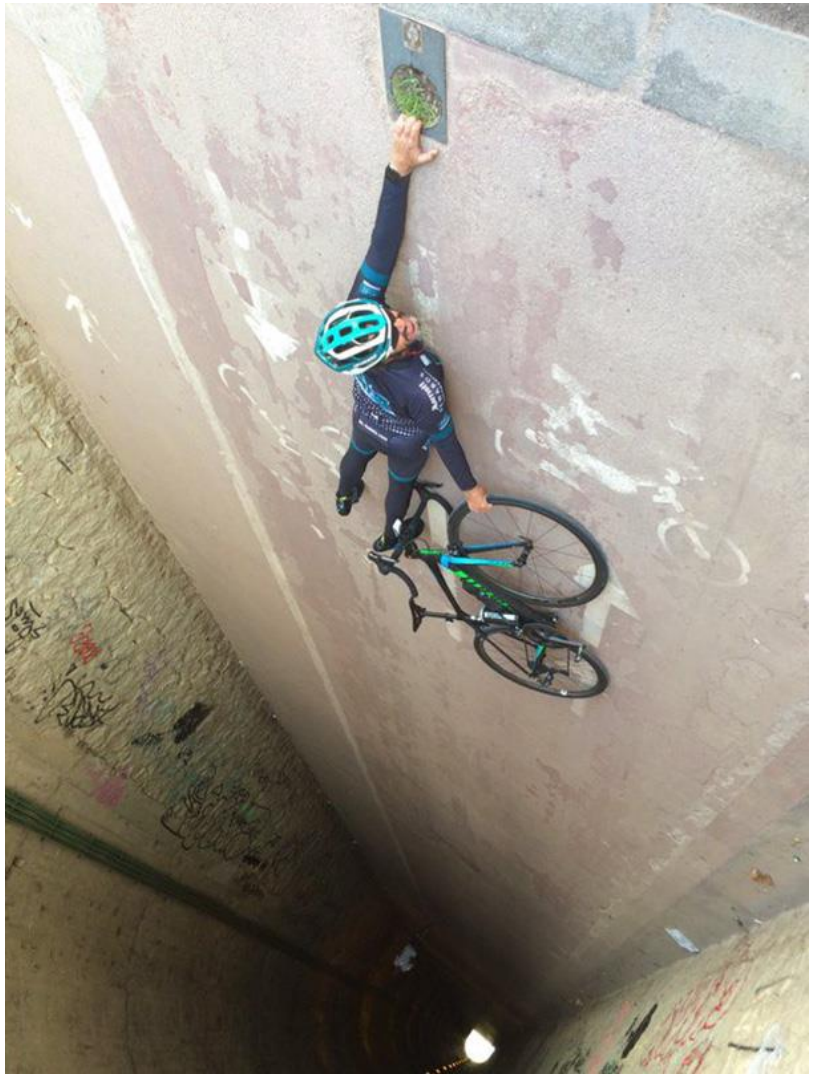
N Engl J Med. 2015 Aug 6;373(6):584-6

- **Typical CLL-phenotype:**
  - **1/286 (0.4%) MYD88 L265P**
  - **4/286 MYD88 L265P but have additional post-GC MBL (1/4 diagnosed as WM in BM)**
  - **2 suspicious, not confirmed**
- **CD5+CD23+ with ≥1 other marker atypical for CLL (usually ROR1- &/or CD43-):**
- **65/257 (25%) MYD88 L265P**

- **"Atypical" CLL *vs.* post-GC LPD with aberrant CD5 expression and *wild-type MYD88***
- **→ ? IBR non-responsive**
- **→ ? Increased MDS rate in WM with FCR**

Diagnostic issues in CLL

- Using ERIC/ESCCA criteria for CD5+ B-LPD:
  - ~65% fully typical phenotype
    - Other driver lesions extremely rare?
    - No requirement to exclude CCND1-IGH translocation ?? Only in patients requiring treatment
  - 35%: further work-up depends on clinical situation
    - 10% of total → mantle cell lymphoma
    - 10-15% of total → CLL e.g. with trisomy 12 (usually over-expression of CD20/sIg, often otherwise typical)
    - 5% of total are ROR1-CD43- MYD88 mutated ? Not CLL
    - 5-10% need better classification



Hopefully it is not as bad as it looks at first…

Acknowledgements

- Karl-Anton Kreuzer, Asha Soosapilla, Martin Spacek, Peter Gambell, Neil McIver-Brown, Katherina Psarra, Maria Arroz, Raffaella Milani, Javier de la Serna, M. Teresa Cedena, Ozren Jaksic, Josep Nomdedeu, Carol Moreno, Gian Matteo Rigolin, Antonio Cuneo, Preben Johansen, Hans Johnsen, Richard Rosenquist Brandell, Carston Utoft Niemann, David Westerman, Marek Trneny, Stephen Mulligan, David Oscier, Michael Hallek, Emili Montserrat.
- Ruth de Tute, Tal Munir and Peter Hillmen
- Paolo Ghia

ESCCA European Society for Clinical Cell Analysis

ICCS International Clinical Cytometry Society

ERIC european research initiative on CLL

Best original scientific paper Clinical Cytometry 2018

Conclusions

- CLL diagnosis and monitoring is usually easy but sometime difficult
- Potential to improve reproducibility → prospective testing required.
- Which sub-groups should be identified as atypical (variant) CLL and which are a different disorder?