Which patients can be monitored: diagnosis of CLL

- No universal molecular abnormality
- WHO Definition: **CLL cells *usually* co-express CD5 and CD23**
- WHO Immunophenotype: 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+)***.
- iwCLL/NCI guidelines: **CLL cells co-express the T-cell antigen CD5 and B-cell surface antigens CD19, CD20, and CD23**. The levels of surface immunoglobulin, CD20, and CD79b are ***characteristically*** low compared with those found on normal B cells. Each clone of leukemia cells is restricted to expression of either kappa or lambda immunoglobulin light chains. ***Variations of the intensity of expression of these markers may exist and do not prevent inclusion of a patient in clinical trials for CLL.***

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]

There are no reproducible criteria for the diagnosis of CLL

What is CLL – analysis in 2008

- Leeds HMDS diagnostic panel comprising 30 markers, of which 11 markers with characteristic pattern in CLL
  - CD10 negative CD20 weak CD200 positive
  - CD22 weak CD23 positive CD79b weak
  - CD43 positive CD81 weak CD5 positive
  - IgM/κ/λ weak CD95 negative
- CLL consistent in 9-11 markers, other B-LPD show CLL-like expression in ≤8 markers
  - MCL median 5 (range 3-8), GC LPD median 3 (0-6)), post-GC median 3 (0-8)
- Analysis in 909 sequential cases from 2008 of which diagnoses as 515 "CLL"
- $CD5^{POS}CD23^{POS}CD20^{wk}sIg^{wk}$ → 353 (38.8%), 347 (97%) CLL-profile
- $CD5^{NEG}CD23^{POS}CD20^{wk}sIg^{wk}$ → 38 (4.1%), 31 (84%) CLL-profile
- $CD5^{POS}CD23^{POS}CD20^{wk}sIg^{MOD}$ → 31 (3.4%), 24 (42%) CLL-profile
- $CD5^{POS}CD23^{NEG}CD20^{wk}sIg^{wk}$ → 35 (3.9%), 22 (33%) CLL-profile
- $CD5^{POS}CD23^{NEG}CD20^{MOD}sIg^{wk}$ → 16 (1.8%), 6 (68%) CLL-profile
- **CLL is a disorder of $CD23^{pos}CD20^{wk}sIg^{wk}$ B-cells, not CD5POS B-cells**

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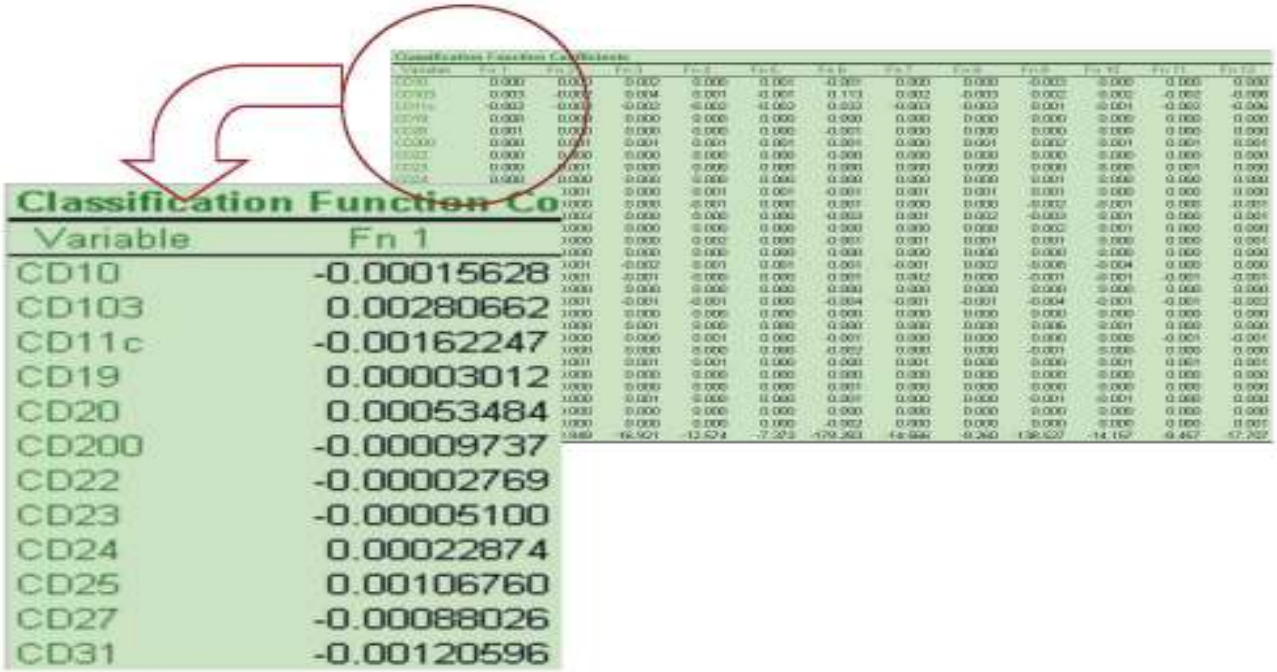
Individual markers that contribute most to identifying a consistent CLL profile

	CLL-like in	Accuracy	Negative PV	Positive PV	Specificity	Sensitivity
CD43	54	91	98	86	85	98
CD23	53	90	95	85	85	95
CD5	56	84	92	78	76	92
CD81	63	82	96	73	68	97
CD20	65	81	98	72	65	99
CD200	49	80	82	78	80	81
CD22	47	78	79	77	80	76
CD79b	72	75	99	65	52	99
IgM	67	75	92	67	58	94
CD95	72	66	83	60	45	90
CD10	88	59	100	54	23	100

- **CLL is a disorder of $CD43^{pos}CD23^{pos}$ ($CD20^{wk}sIg^{wk}$) B-cells, not CD5POS B-cells**

Will a more sophisticated approach work?



Classification Function Co

Variable	Fn 1
CD10	-0.00015628
CD103	0.00280662
CD11c	-0.00162247
CD19	0.00003012
CD20	0.00053484
CD200	-0.00009737
CD22	-0.00002769
CD23	-0.00005100
CD24	0.00022874
CD25	0.00106760
CD27	-0.00088026
CD31	-0.00120596

EHA 2009: Used linear discriminant analysis to define algorithm based on 30 markers to classify CLL and other B-LPD

Reproducibility requires using the same large antibody panel → e.g. Euroflow, but difficult to implement as a universal approach

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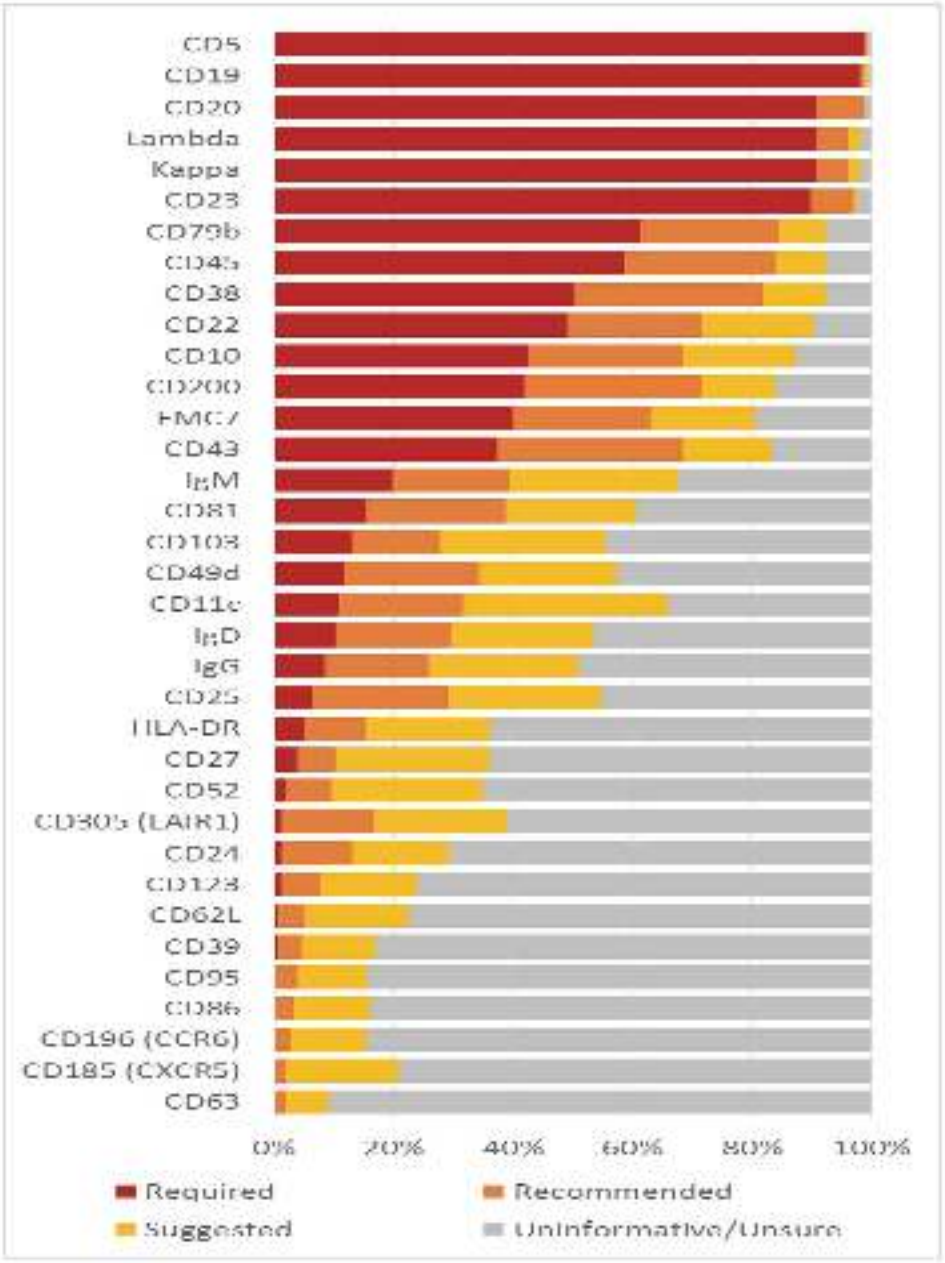
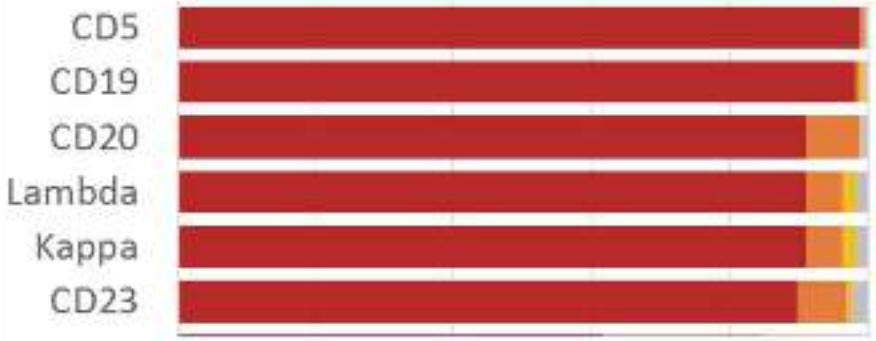
CLL Diagnostic Panel

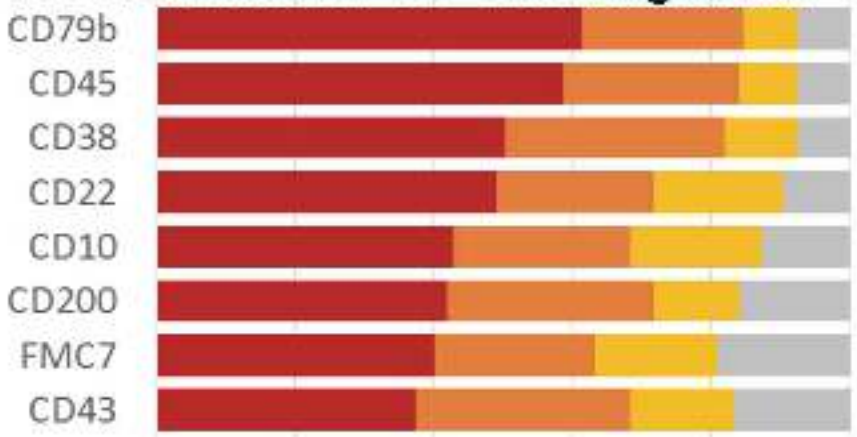
- An invitation to participate in this study was circulated among members of the ERIC and ESCCA scientific groups.
- Responses were received from 158 members of which 154 were actively involved in CLL diagnosis: 100/154 (65%) were from diagnostic laboratories, 14/154 (9.1%) were CLL clinicians and 36/154 (23%) worked in both laboratory and clinic.
- The diagnostic workload was >20 cases per week in 23/154 (15%), 5-20 per week in 82/154 (53%) and <5% in 49/154 (32%).
- Responders were invited to classify 35 antibodies selected from publications on the diagnosis of B-lymphoproliferative disorders as being required, recommended, suggested, uninformative, or of unknown value for the diagnosis of CLL.



>75% of respondents → marker is **required** for CLL diagnosis:



>50% of respondents → marker is **recommended** for CLL diagnosis:



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ESCCA European Society for Clinical Cell Analysis

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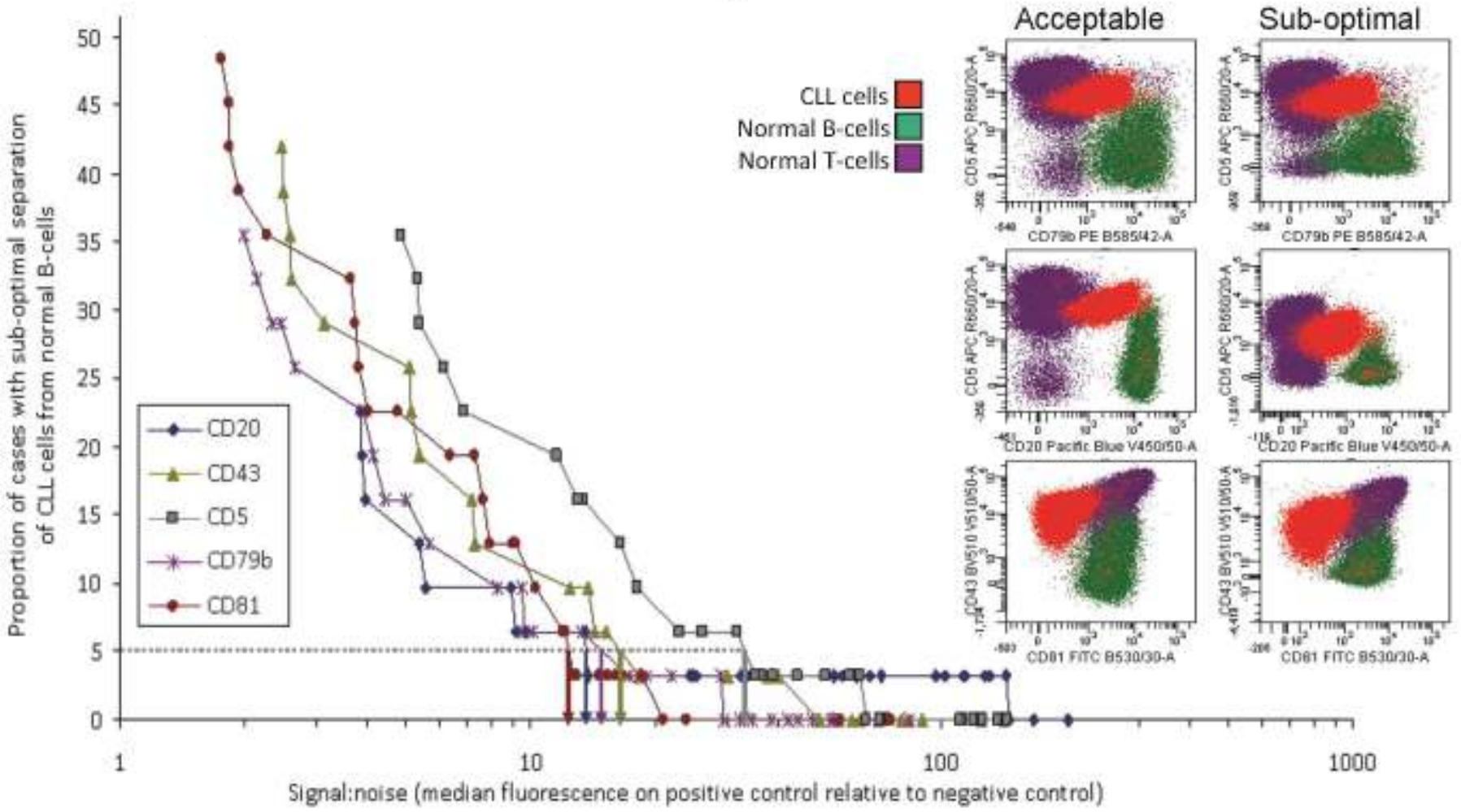
Proposal: required (minimum) and recommended panel for diagnosis

- ***Marker panel required for diagnosis:***
  - CD19 / CD5 / CD23 / CD20 / Kappa / Lambda
- ***Additional markers recommended for diagnosis and required for clinical trials:***
  - CD43 / CD79b / CD81* / CD22 / CD10
- ***Frequently recommended but not essential for diagnosis and monitoring:***
  - CD45 / CD38 / **CD200** / (FMC7)
- ***Present in current diagnostic criteria but not universally recommended:***
  - IgM/D and CD11c:

- **** CD81 was considered as a required or recommended by only 40% of participants but this marker is an essential component for the consensus MRD monitoring panels [REFS] and therefore recommended by the steering committee for clinical trials.***

Figure 3a: A platform-independent specification for MRD-flow reagents



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Antigen	Typical Expression (% pos vs. control) ‡	Control Population in normal peripheral blood		Minimum Relative fluorescence intensity (preferred)
		Positive	Negative	
CD19	Positive (>95%)	CD20+ B-cells	CD3+ T-cells	>20†
CD5	Positive (>20%)	CD3+ T-cells	CD16/56+ NK-cells	>14 (>18)
CD23	Positive (>20%)	CD20+CD27- Naïve B-cells	CD20+27+ Memory B-cells	>5†
CD20	Weak	CD19+ B-cells	CD3+ T-cells	>5 (>20)
Igκ Igλ	Weak & restricted to either Igκ or Igλ	CD20+ B-cells	CD3+ T-cells	>10†
CD43	Positive (>20%)	CD3+ T-cells	CD20+ B-cells	>7 (>50)
CD79b	Weak	CD20+ B-cells	CD3+ T-cells	>11 (>30)
CD81	Weak	CD3+ T-cells	Granulocytes	>5 (>8)
CD22	Weak	CD20+ B-cells	CD3+ T-cells	>10†
CD10	Negative (<20%)	Granulocytes	CD20+27+ Memory B-cells	>10†

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)
† consensus, not specifically validated

Application of the proposed minimum diagnostic panel

- Of 1971 samples diagnosed with B-lymphoproliferative disorder at HMDS in 2014, 1328 were CD5+ B-LPD
  - 1212 non-trial samples sent for investigation of new or relapsed B-lymphoproliferative disorder
    - Of 810 cases diagnosed with CLL, 33 did not meet the criteria
    - 1328/1971 were CD5+ B-LPD
    - 77% met the criteria for CLL
    - 13% did not and had a clear alternative diagnosis (e.g. MCL)
    - 10% not readily classified by flow cytometry
      - 53/126 (42%) B-cell count <10 x 10/9/l, no follow-up
  - 116 sent as baseline samples for CLL trial entry
    - 93% (107/116) met the proposed criteria for CLL
    - 7% (9/116) potentially excluded from trial entry

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Aims

- (1) Validate reproducible criteria for diagnosis of CLL
  - Centres with >500 cases, using the criteria based on CD19/CD5/CD20/CD23/Kappa/Lambda:
  - what proportion of cases that you currently classify as CLL would not meet the criteria?
  - what proportion of total CD5+ B-LPD meet the criteria?
  - for the CD5+ B-LPD that do not meet the criteria
    - Non-diagnostic / MBL
    - identify recurrent features (e.g. strong CD20 with trisomy 12, or weak CD5/CD23 with other feautures consistent with a post-GC B-LPD)?
- (2) Identify a diagnostic pathway for cases not meeting the criteria → David Oscier

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