CLL/SLL

Updates and other things you should know

David P. Ng, MD
University of Utah, Department of Pathology
ARUP Laboratories

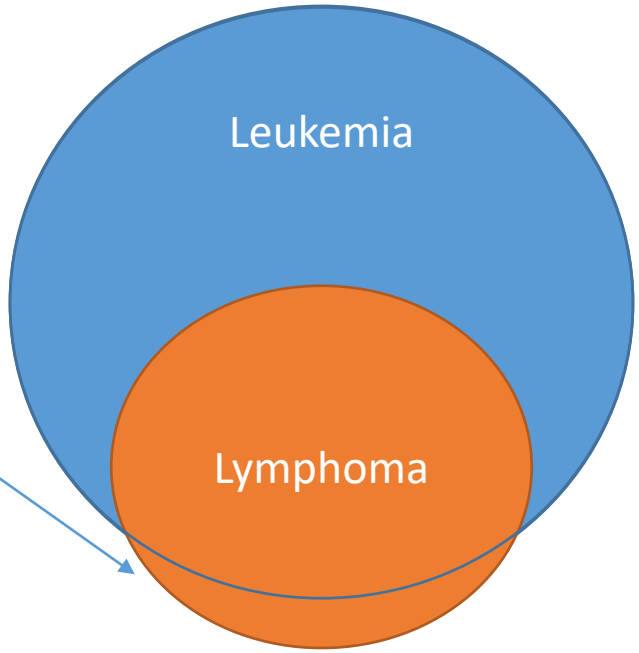
Outline

- Background
- Diagnosis
- Prognostic Testing
- Theranostic Testing

Learning Objectives

- Understand the process of diagnosing CLL/SLL
- Differentiate between diagnosis, prognosis, and theranosis.
- Recognize interference of assays in the setting of targeted therapies.

Introduction



- CLL vs SLL
  - Chronic Lymphocytic LEUKEMIA
  - Small Lymphocytic LYMPHOMA
- Same disease, different locations
- Very common
  - 21,040 new cases per year in the US

  *Does not include monoclonal B cell lymphocytosis (MBL)
- Quite Indolent
  1. 85% 5 year survival
  2. Lots of people who live with the disease (clinical or subclinical)

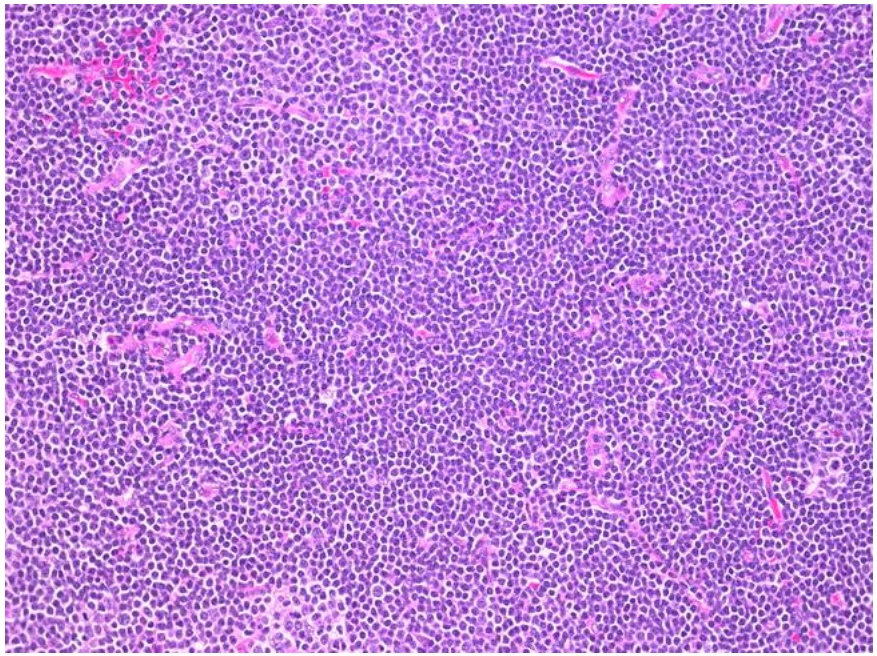
Diagnosis

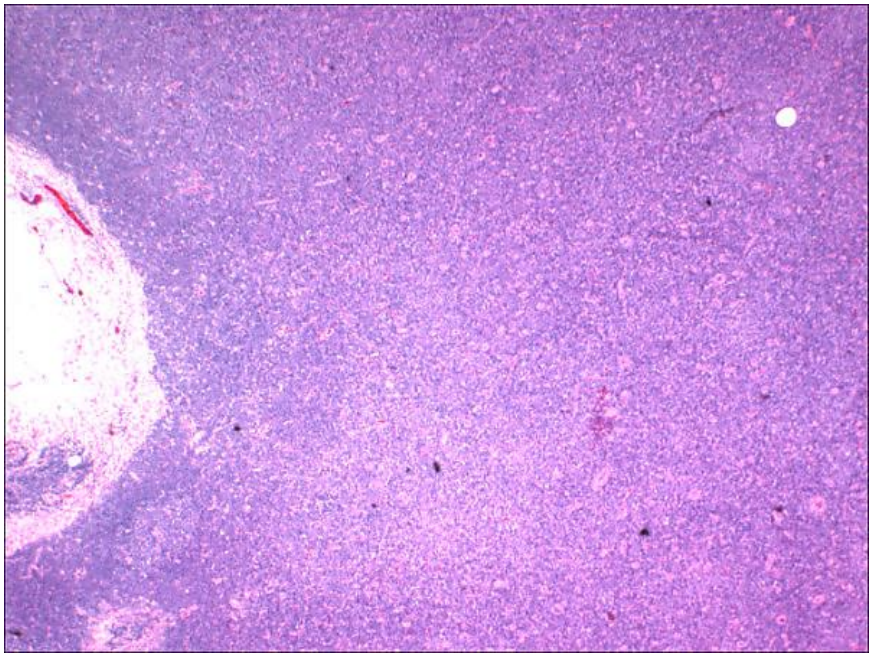
- SLL
  - Nodal involvement
- CLL
  - Bone marrow and Peripheral Blood Involvement

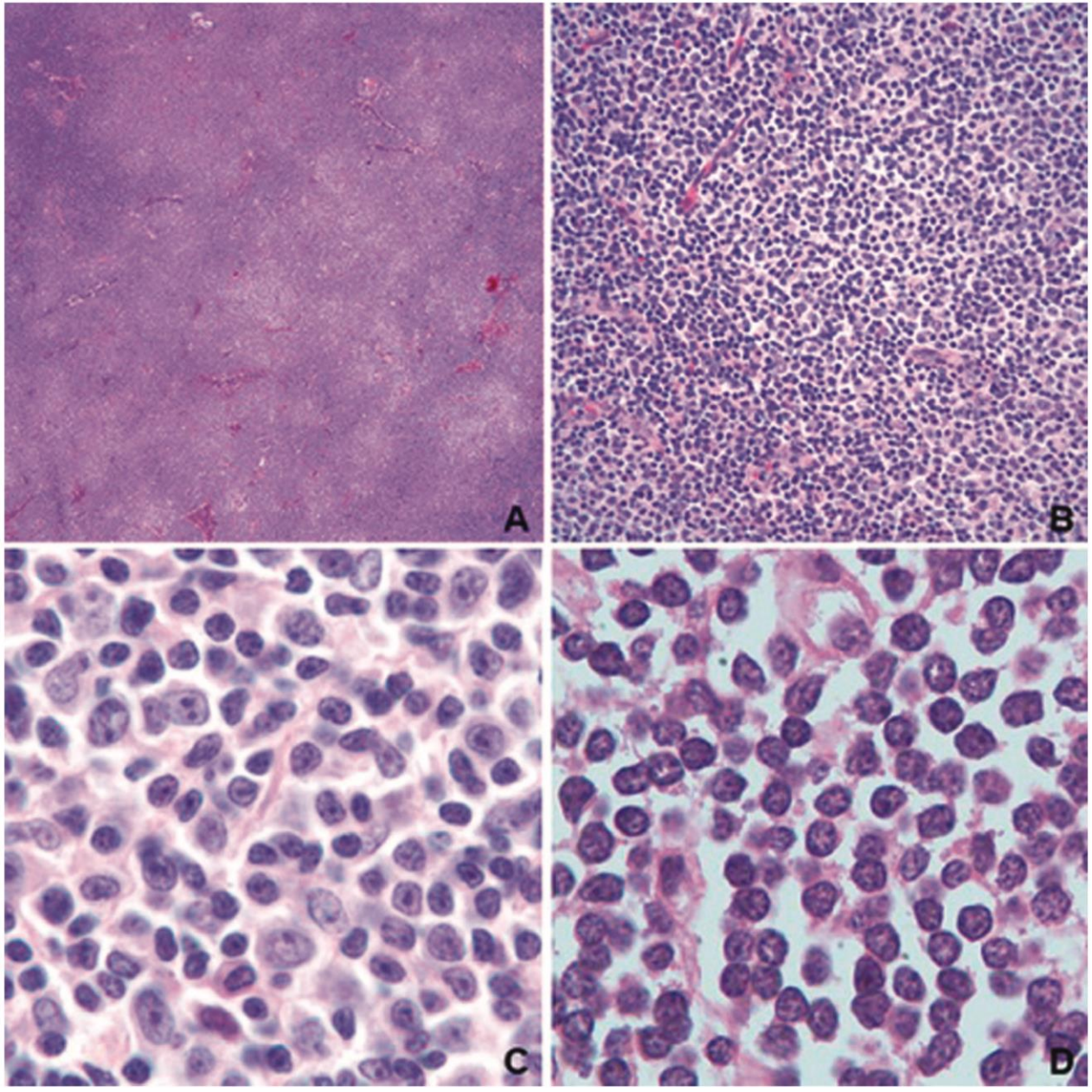
Morphology (Lymph Node)

- Effaced Architecture
  - Lighter zones with ‘pseudo’-proliferation centers
- Small cell infiltrate (small = normal resting lymphocytes)
- Soccer Ball like nuclear chromatin
- Scant Cytoplasm
- Occasional larger forms

H&E Images



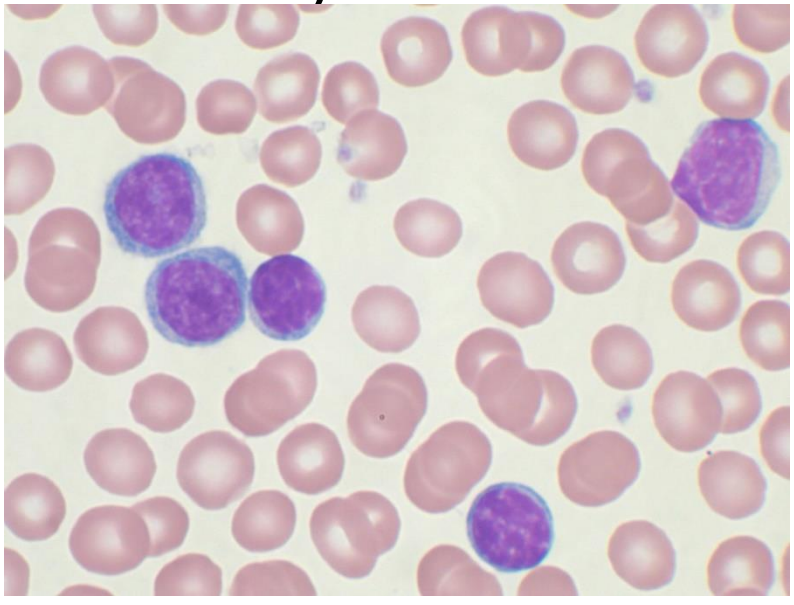


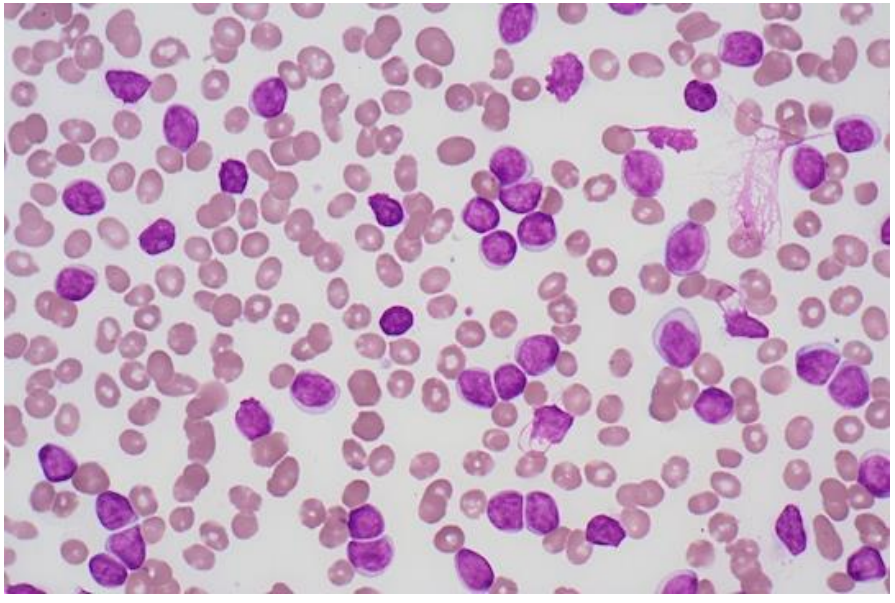

A
B
C
D

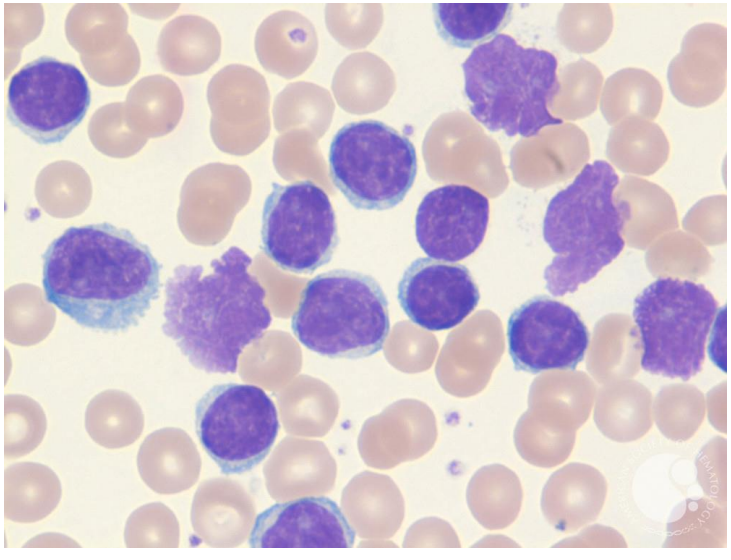
Morphology (Smear)

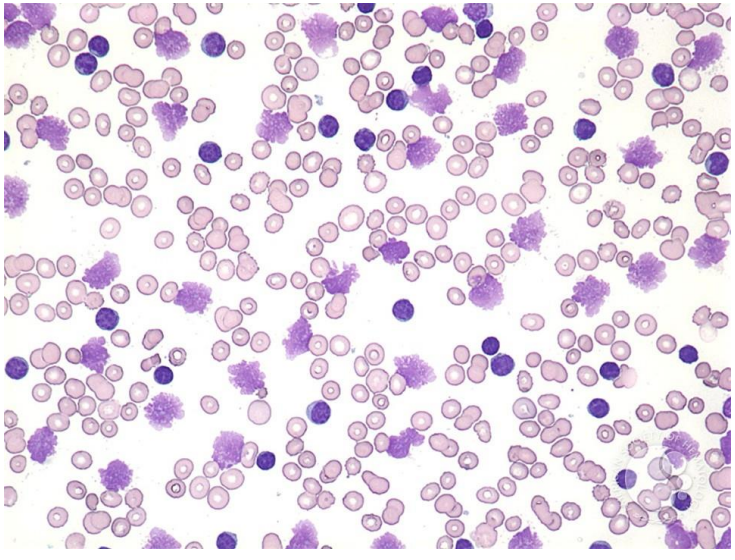
- Smudge Cells
- Albuminated slide
  - Soccer ball like nuclear chromatin

Wright Giemsa Images (ASH image bank)

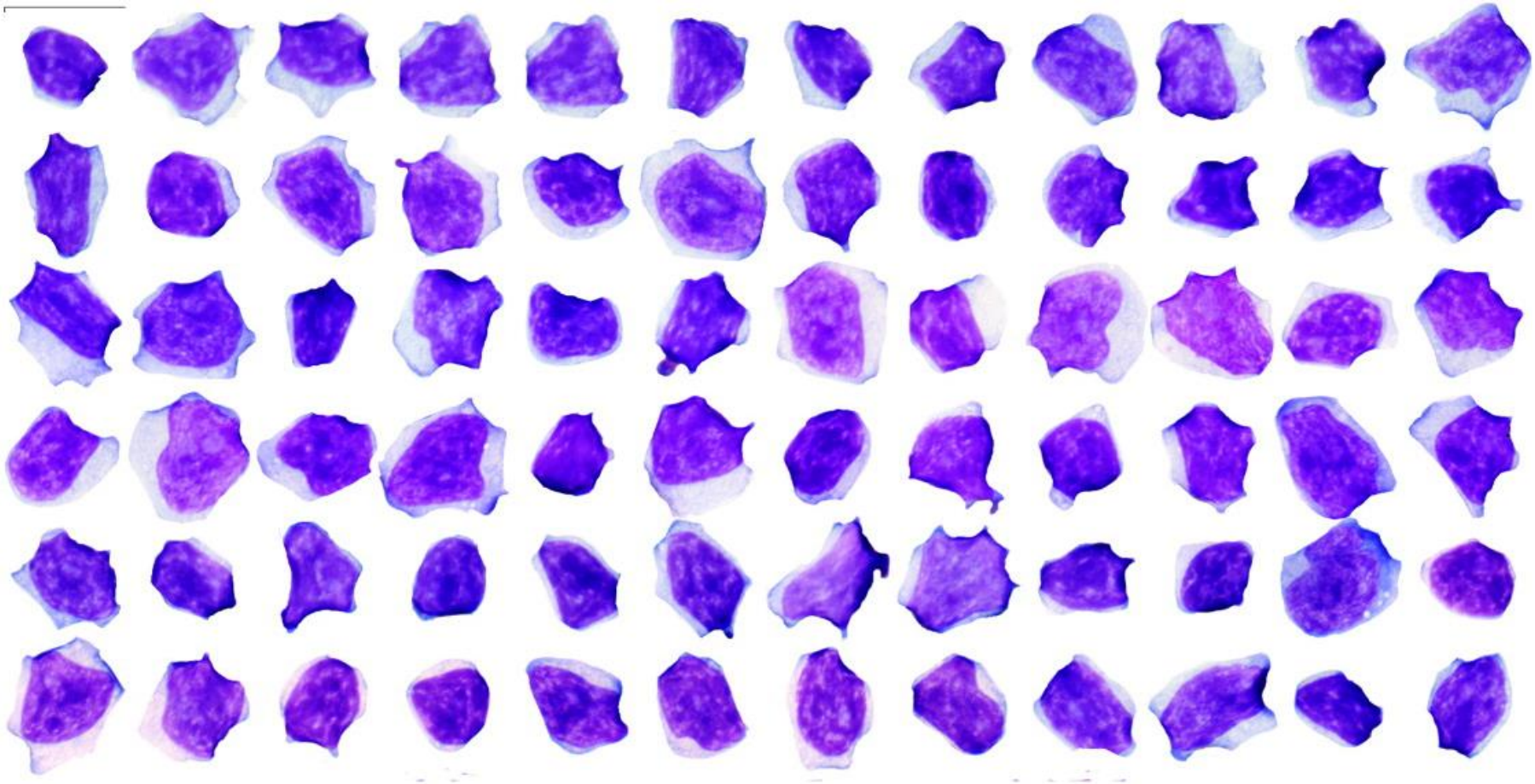






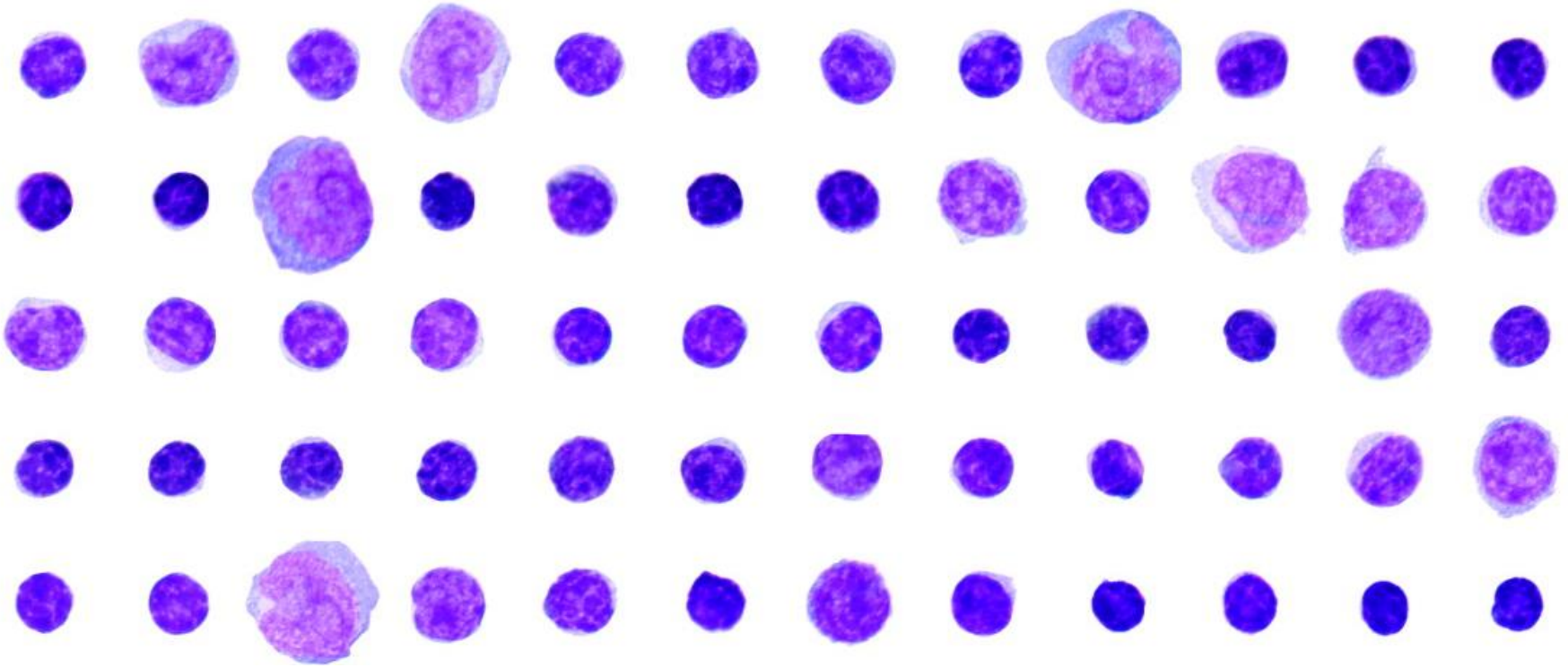


Conventional EDTA Smears



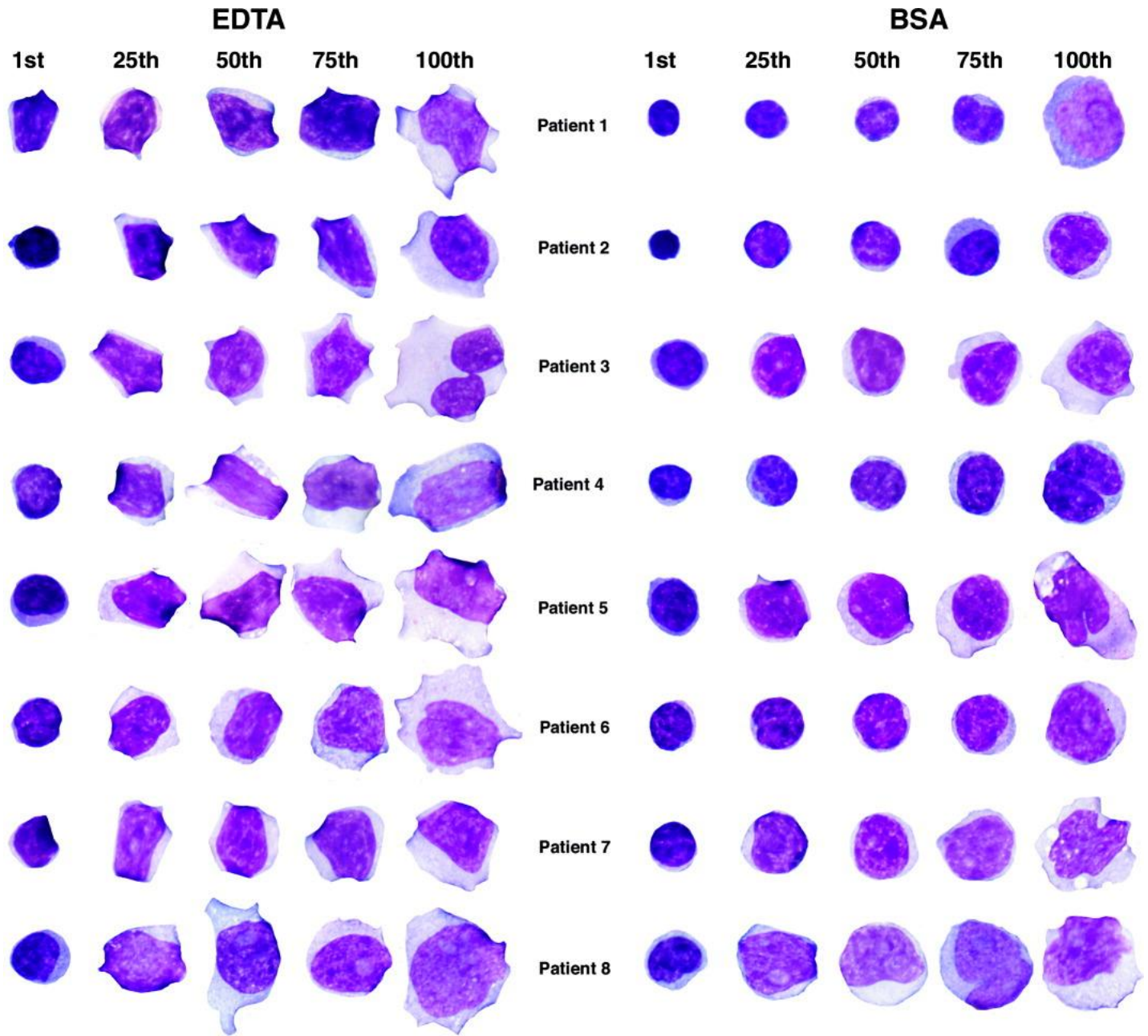
Lunning, M.A., Zenger, V.E., Dreyfuss, R., Stetler-Stevenson, M., Rick, M.E., White, T.A., Wilson, W.H. and Marti, G.E. (2004), Albumin enhanced morphometric image analysis in CLL. Cytometry, 57B: 7-14. doi:10.1002/cyto.b.10059

Albuminated Smears

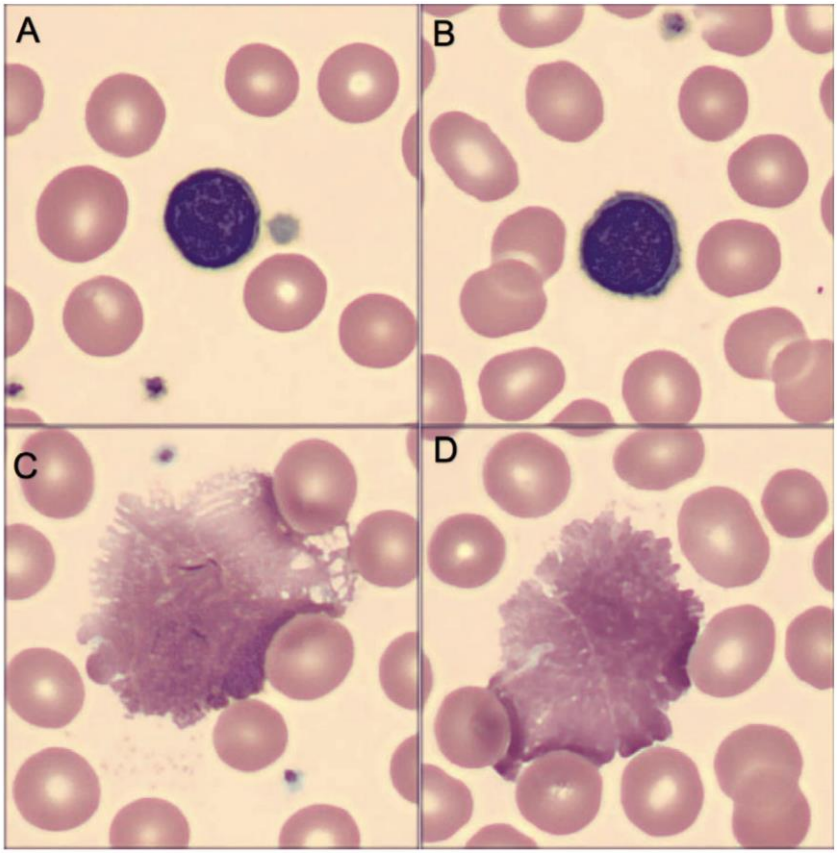


Lunning, M.A., Zenger, V.E., Dreyfuss, R., Stetler-Stevenson, M., Rick, M.E., White, T.A., Wilson, W.H. and Marti, G.E. (2004), Albumin enhanced morphometric image analysis in CLL. Cytometry, 57B: 7-14. doi:10.1002/cyto.b.10059

Albuminated vs EDTA cells



Cellavision Images



Jerez, J, Ernst, DM. High percentage of smudge cells in a patient with COVID19: rediscovering their utility. eJHaem. 2020; 1: 374–375. https://doi.org/10.1002/jha2.52

Immunophenotype

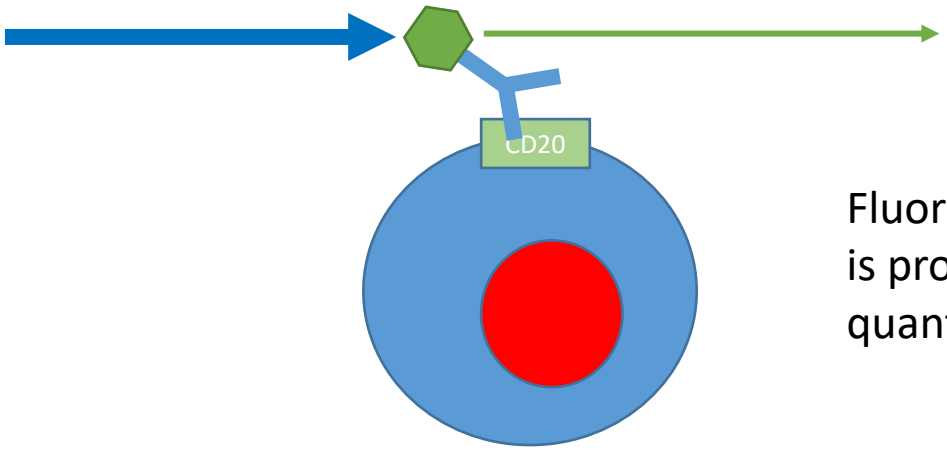
- Retained CD19
- Decreased CD20
- Low to intermediate CD5 (can be less than or equal to background T cells)
- Decreased CD22, CD79b
- Aberrant CD200, CD23, CD43, loss of FMC7, decreased CD81

Immunohistochemistry

- LEF1+
- CyclinD1-negative (rare weak cells in proliferation centers)

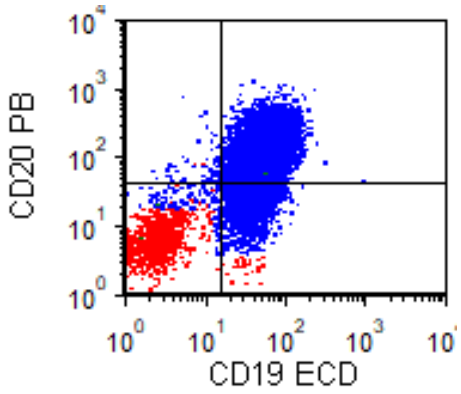
Flow Cytometry

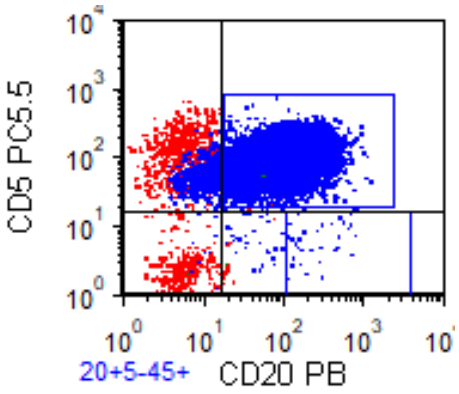
- Fastest and cheapest way to make the diagnosis of CLL
- What is flow cytometry?
  - Fluorescently labeled antibodies bound to single cells

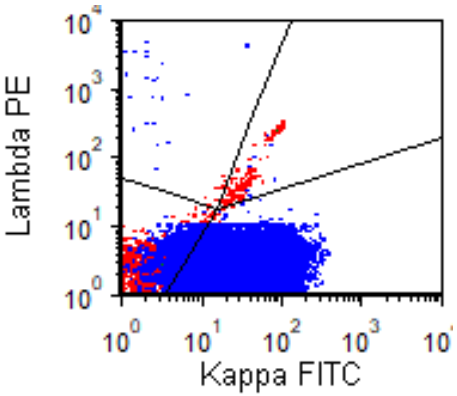


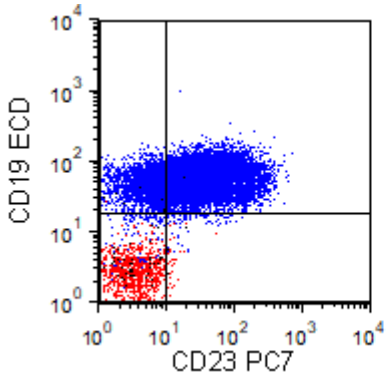
Fluorescent Signal is proportional to quantity of target

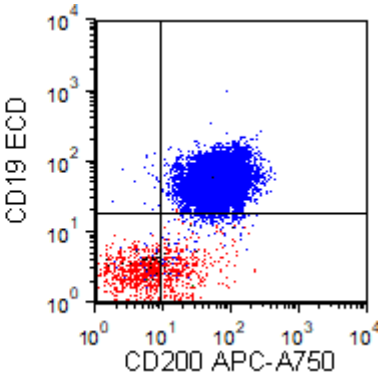
It’s a toomah

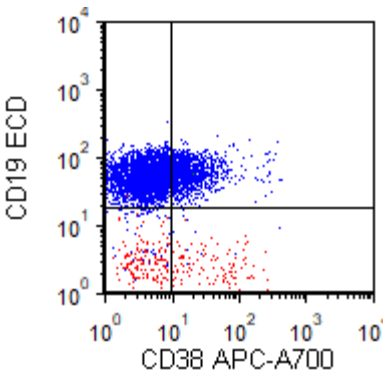

CD20 PB
CD19 ECD


CD5 PC5.5
20+5-45+
CD20 PB

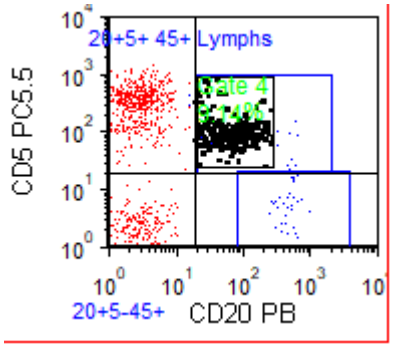

Lambda PE
Kappa FITC

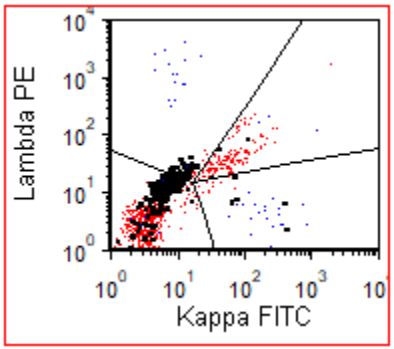

CD19 ECD
CD23 PC7

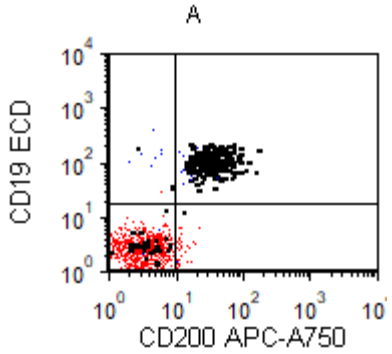

CD19 ECD
CD200 APC-A750

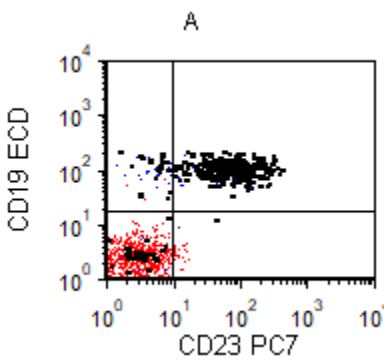

CD19 ECD
CD38 APC-A700

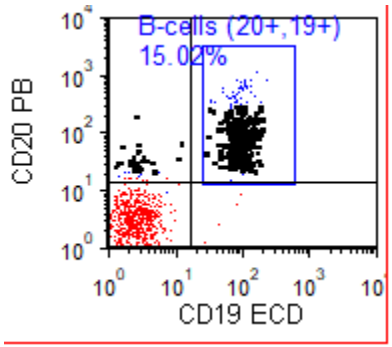
Are Light Chains useful?





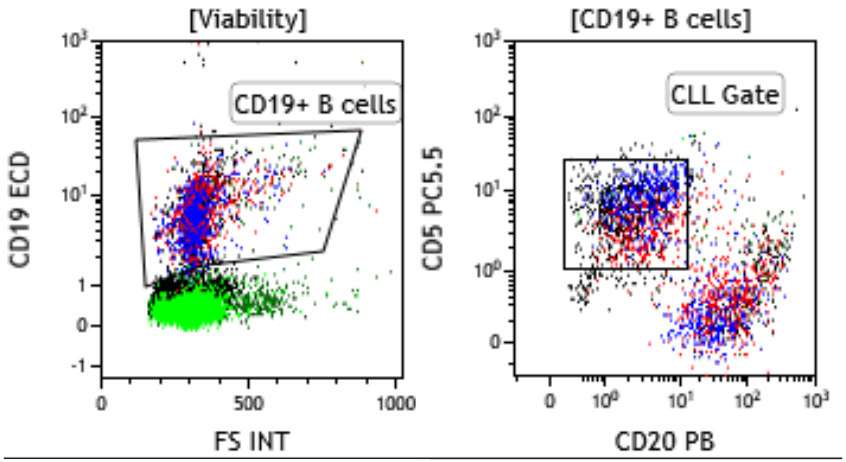


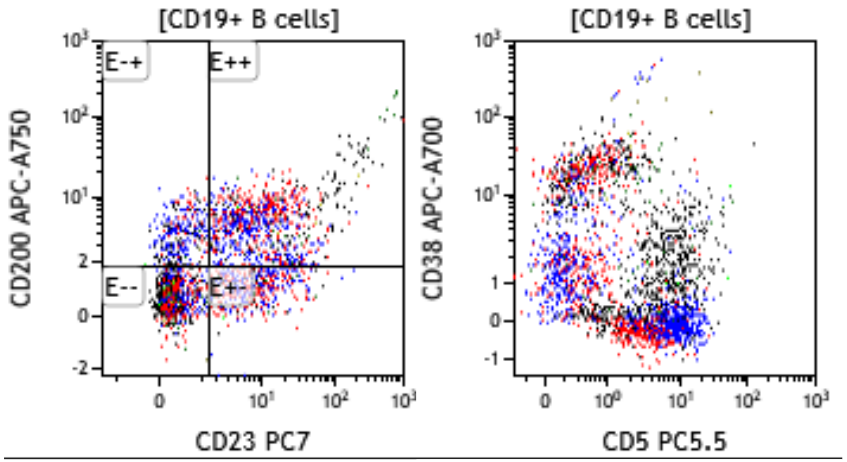


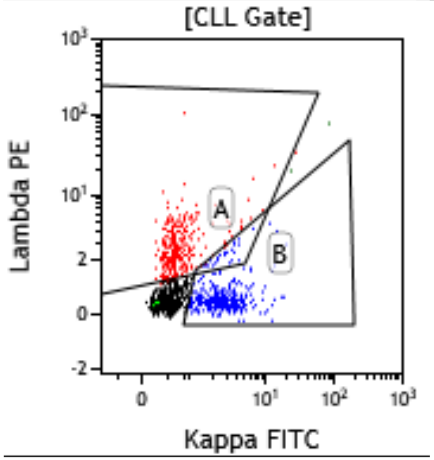


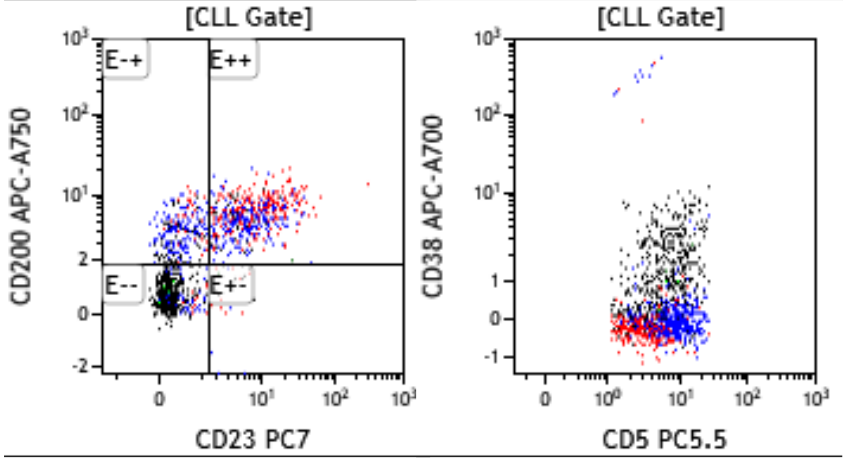
- Yes and No
- Light chains can be low to negative

Biclonal CLL









Diagnostic Criteria

- >5k/uL neoplastic lymphocytes
  - Morphology
    - Can be difficult to separate normal lymphocytes from neoplastic lymphocytes
    - Trivial if WBC is high (e.g. 30k WBC with phenotypic evidence is diagnostic for CLL)
  - Flow Cytometry
    - Accurate measurement of the % neoplastic cells/WBCs
    - Cell concentration is trickier
      - Bead Standards
      - Multiplication with WBC count from analyzer

What about cases <5k/uL

- Monoclonal B cell lymphocytosis (MBL)
  - Precursor to CLL
  - High Count >2.5k – 2% rate of transformation to CLL
  - Low Count <2.5k – <1% rate of transformation to CLL

*Some use MBL as a generic term for any small monoclonal B cell population with immunophenotypic abnormality (CD5-/CD10- B-NHLs) but for this talk, MBL means a CLL/SLL like population

Monoclonal B cell lymphocytosis

- Very Rare in young (<40 y/o)
  - 1/365 at 10^{-5} (0.01%) sensitivity
- Common as you get older
  - 20% incidence at 80 y/o
- Genetic linkage (family member with CLL -> 17x increase in incidence MBL)

Exclusion of other entities…

- CD5+ B-NHL
  - CLL/SLL
  - Mantle Cell Lymphoma
  - Marginal Zone Lymphoma (very rare)

- Exclude MCL with cyclinD1, SOX11, or t(11;14) FISH
  - NCCN Guideline
  - Misdiagnosing MCL as CLL/SLL is a bad thing

Natural History of Disease

- 20k diagnoses a year
- 5k deaths a year

Take home point: most people die *with* CLL/SLL not *of* CLL/SLL

*not to say CLL/SLL is a nice disease, there is significant morbidity (e.g. fatigue due to cytopenia)

How do things go south?

1. Marked leukocytosis >100k/uL

2. Disseminated lymphadenopathy

3. Richter transformation
   - 2-10% of CLL cases end up here

How do we predict who will die of disease?

What can we do to change that?

Prognosis

- Not all CLLs are created alike
- IgHV mutation status[1]
  - Unmutated = Bad
  - Mutated = Good
- TP53
  - Wild type = Good
  - Mutated = Bad
- Karyotype
  - >=3 abnormalities in 1 cell) = Bad
- Cytogenetics[2]
  - Del(17p) – bad
  - Del(11q) – bad
  - Del(13q) – good
  - Trisomy 12 – so-so
  - Normal – so-so

1. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94(6):1848-1854.
2. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000;343(26):1910-1916. doi:10.1056/NEJM200012283432602

National Comprehensive Cancer Network®

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)

Chronic Lymphocytic Leukemia/ Small Lymphocytic Lymphoma

Version 1.2021 — September 28, 2020

NCCN.org

NCCN Guidelines for Patients® available at www.nccn.org/patients

Continue

Stage vs Grade

- Stage
  - Degree of anatomic involvement correlated with worse outcome
- Grade
  - Morphologic Findings correlated with worse outcome

Staging Systems for CLL

Rai System[a]

Stage	Description	Modified Risk Status
0	Lymphocytosis, lymphocytes in blood >5 x 10^9/L clonal B cells and >40% lymphocytes in the bone marrow	Low
I	Stage 0 with enlarged node(s)	Intermediate
II	Stage 0–I with splenomegaly, hepatomegaly, or both	Intermediate
III[c]	Stage 0–II with hemoglobin <11.0 g/dL or hematocrit <33%	High
IV[c]	Stage 0–III with platelets <100,000/mm^3	High

Binet System[b]

Stage	Description
A	Hemoglobin ≥10 g/dL and Platelets ≥100,000/mm^3 and <3 enlarged areas
B	Hemoglobin ≥10 g/dL and Platelets ≥100,000/mm^3 and ≥3 enlarged areas
C[c]	Hemoglobin <10 g/dL and/or Platelets <100,000/mm^3 and any number of enlarged areas

[a] This research was originally published in Blood. Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. Blood 1975;46:219-234. (c) The American Society of Hematology.
[b] From: Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 1981;48:198-206.
[c] Immune-mediated cytopenias are not the basis for these stage definitions.

Continues

IgHV mutation status

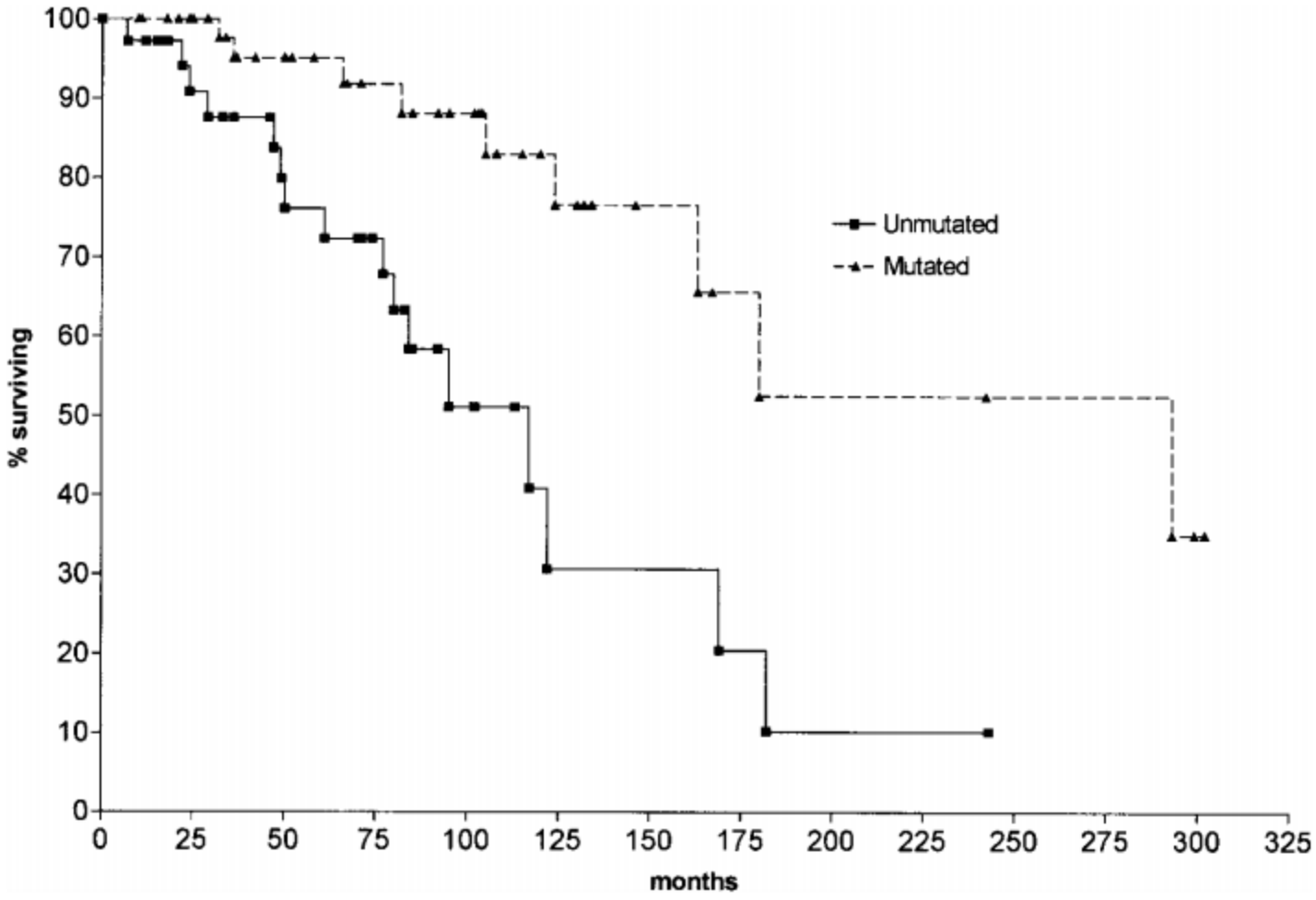
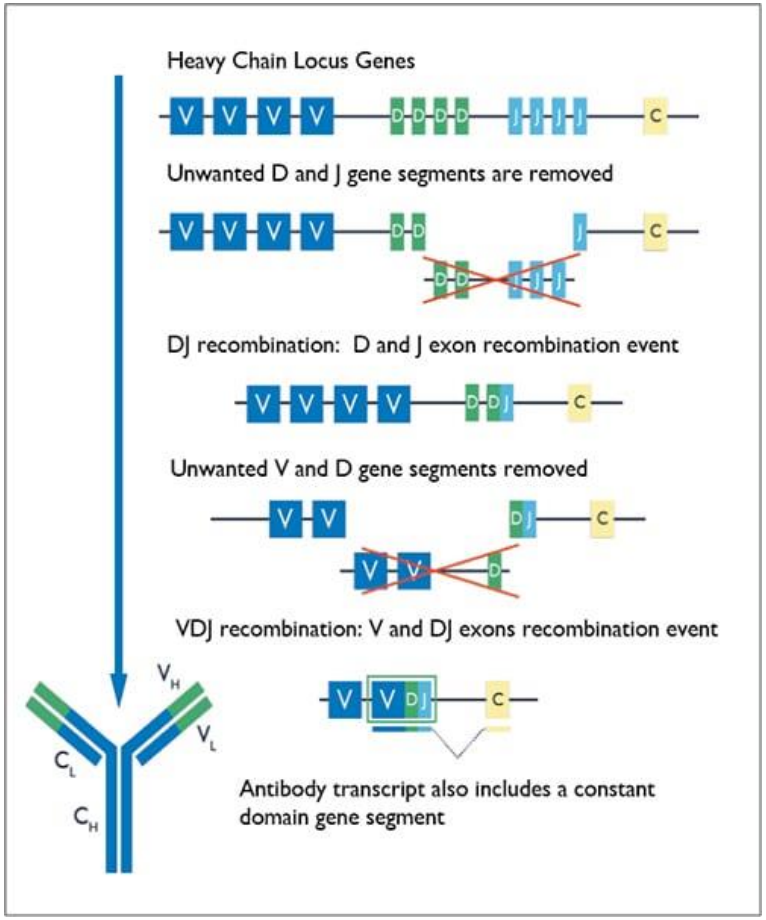


Fig 1. Kaplan-Meier survival curve comparing CLL patients with mutated and unmutated V_H genes. Median survival for unmutated CLL: 117 months; median survival for mutated CLL: 293 months. The difference is significant at the $P = .001$ level (log-rank test).

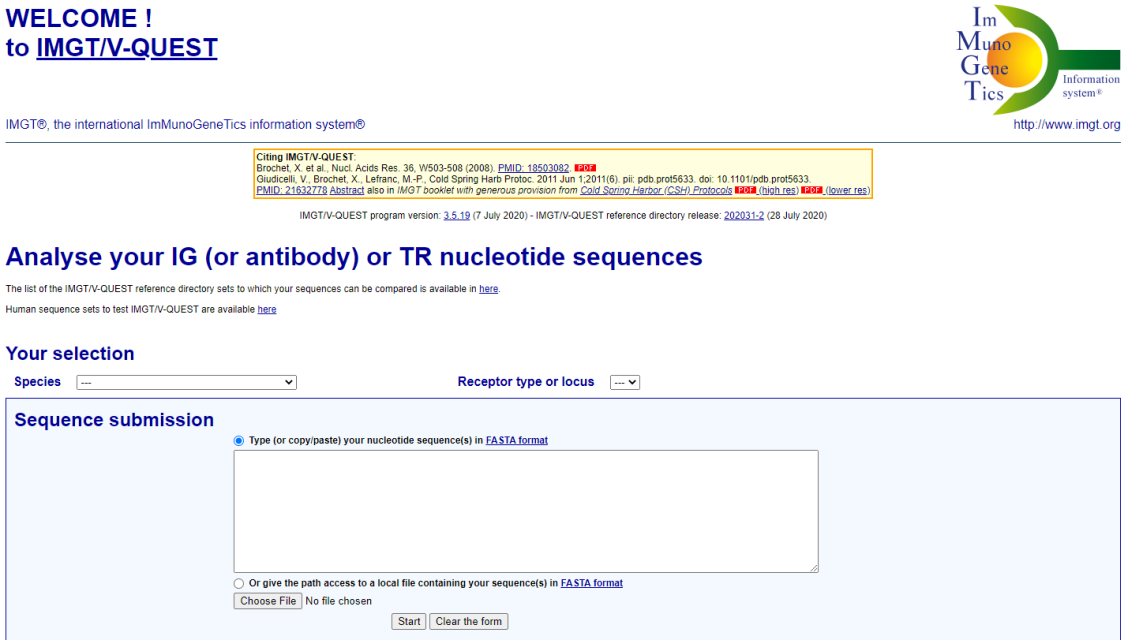
What is the Immunoglobulin Heavy Chain Variable region?



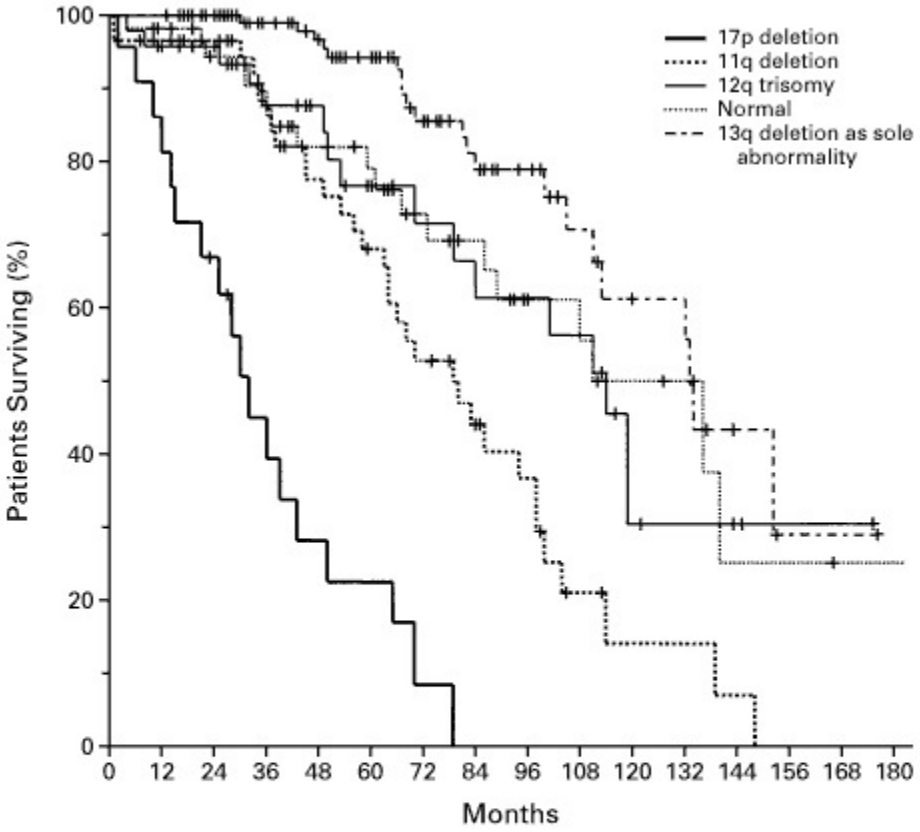
CAP today, May 2019
https://www.captodayonline.com/ighv-gene-mutation-at-heart-of-cll-treatment/

Somatic Hypermutation of the IgH locus

- Mutated
  - >2-3% difference in base pair sequence compared with germ line (of which there are many!)
- Methods
  - Sanger Sequencing
  - NGS
  - Bioinformatics



Cytogenetic Prognosis



No. at Risk																
17p deletion	23	18	13	8	5	4	1	0	0	0	0	0	0	0	0	0
11q deletion	56	53	47	43	33	27	20	15	10	4	2	2	1	0	0	0
12q trisomy	47	44	41	29	24	17	14	13	12	11	4	3	2	1	1	0
Normal	57	51	45	37	30	27	20	17	12	11	6	5	2	2	1	1
13q deletion as sole abnormality	117	117	106	91	80	63	45	36	24	16	12	11	3	1	1	0

Dohner et al.

Prognosis

- Flow Cytometry
  - ZAP-70 – associated with IgHV mutation status
    - Rarely done – difficult to do well
  - CD38 – associated with IgHV mutation status
    - Some studies suggest it may be an independent prognostic indicator
  - CD49d – independent prognostic indicator
    - Not in current guidelines

Bulian P, Shanafelt TD, Fegan C, et al. CD49d is the strongest flow cytometry-based predictor of overall survival in chronic lymphocytic leukemia. *J Clin Oncol*. 2014;32(9):897-904. doi:10.1200/JCO.2013.50.8515

Prognosis cont

- Molecular (PCR) Findings
  - TP53 mutations – Bad
  - ATM (11q)
  - SF3B1
  - NOTCH1 – Bad
  - Formerly done as single gene assays, now done as part of NGS panel (40+ genes)
- Biochemical
  - Beta-2 microglobulin

Minimal Residual Disease

- Prognostic vs Predictive
  - I know you'll do poorly but there is nothing I can do about it.
  - I know you'll do poorly and I can treat you differently with good results.

- CLL MRD certainly prognostic, studies on going regarding predictive value

MRD Modalities

- Flow Cytometry
  - 0.01% to 0.001% Sensitivity (4 to 5 log)
  - Looks for an immunophenotypically aberrant population
  - Widely applicable
  - <24h turnaround time
- NGS
  - 0.0001% sensitivity (6 log)
  - Looks for same Ig sequence as original tumor
  - Only applicable if original tumor is also sequenced
  - 1-2 week turnaround (optimistically)

CLL MRD by Flow Cytometry

- ERIC (European Research Initiative on CLL) consensus panel
- CD45, CD19, CD20, CD43, CD81, CD5, CD79b
- Platform/reagent independent panel
- Shown to be reliable down to 5 log (0.001%)
- Collecting 5 million cells for analysis
- Peripheral blood based assay

CLL MRD by NGS

- ClonoSEQ assay by Adaptive Biotechnologies
- Send out to centralized facility, 1-4 wk turn around.
- Requires a priori knowledge of sequence
- Costs $1900
- Question of whether 10^{-5} vs 10^{-6} matters

Surrogate Endpoint

- Survivals for CLL are measured in years
- Can MRD be used to predict poor outcomes of therapies? Very important for drug trials!

- FDA approval of CLL MRD as a surrogate endpoint

Targeted Therapies

- CD20 – Rituximab
  - FCR – Fludarabine, Cyclophosphamide, Rituximab
  - CR – Chlorambucil, Rituximab
- Burton Tyronsine Kinase (BTK) Inhibitors
  - Ibrutinib, Acalabrutinib
  - Useful even in high risk patients
  - Resistance mechanisms evolve in long treated patients
- Phosphoinositide 3-kinase (PI3K) inhibitors
  - Idelalisib
- BCL2 inhibitors
  - Ventoclax

What are the implication of targeted therapies?

1. Loss of target for detection of residual disease

   Do what is necessary to treat the patient!

2. Request to test for target for therapeutic purposes

   Needs to be done at the time of diagnosis or relapse!

Conclusion

- CLL is a common and usually indolent disease
- Flow cytometry is the fastest and cheapest way to make the diagnosis
- It is clinically important to separate indolent from aggressive disease (prognostication)
- Prognostication can be based on:
  - Grade, Stage, Molecular Mutations, Cytogenetic Abnormalities