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Objectives:

- Appreciate age-based normal lymphocyte parameters
- Delineate blood features for common lymphoproliferative disorders
- Review newly recognized features of lymphoid leukemias
- Review prognostic features for CLL

2

Age-Related Variations in Blood Lymphocytes

- Lymphocytes predominate in blood by 2-3 weeks of age
- Sustained lymphocyte predominance in blood throughout childhood
- Neutrophils predominate at birth and again by late childhood, early adolescence

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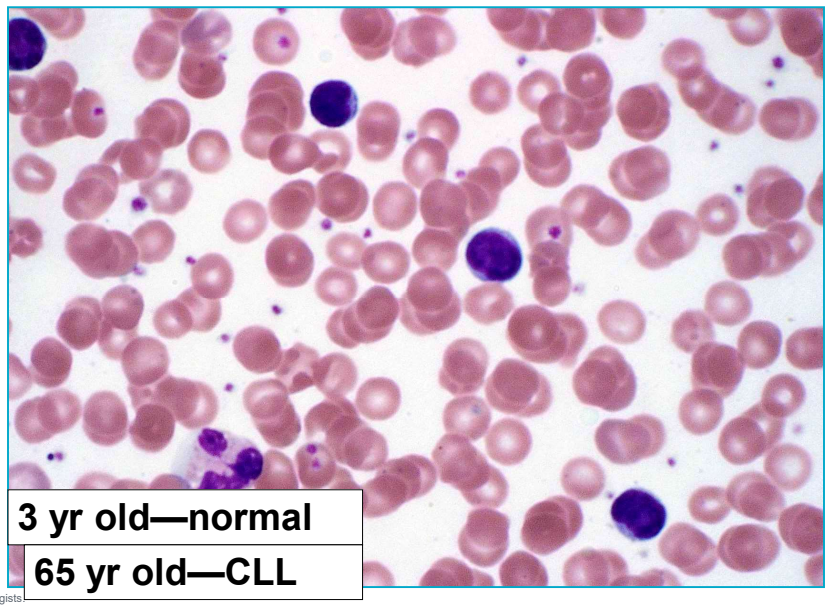
Absolute Lymphocyte Count 9,000



3 yr old—normal

65 yr old—CLL

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Absolute Lymphocyte Count by Age*

	Lymphocytosis	Lymphopenia
Newborn	> 10,000	< 2,500
< 1 year	> 9,000	< 4,000
Child	> 7,000	< 2,800
Adult	> 4,000	< 1,500

*Conventional units per µL

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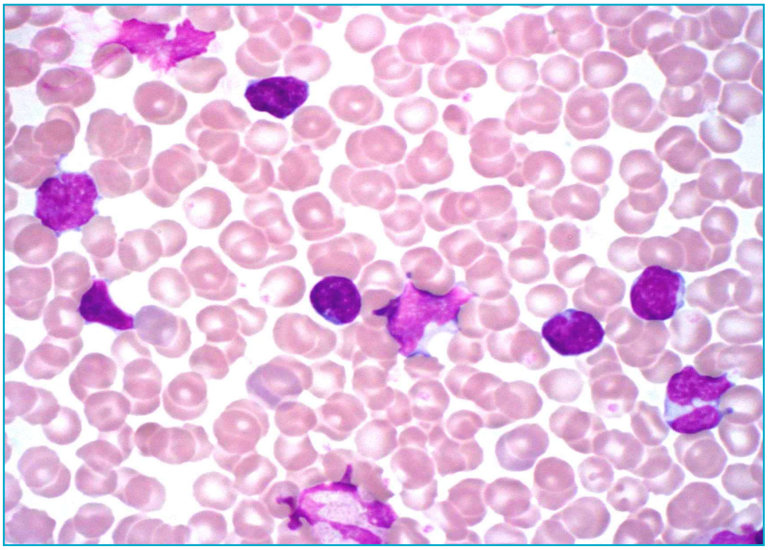
Age, Clinical Features Key

Morphology:
Non-Activated Lymphocytes



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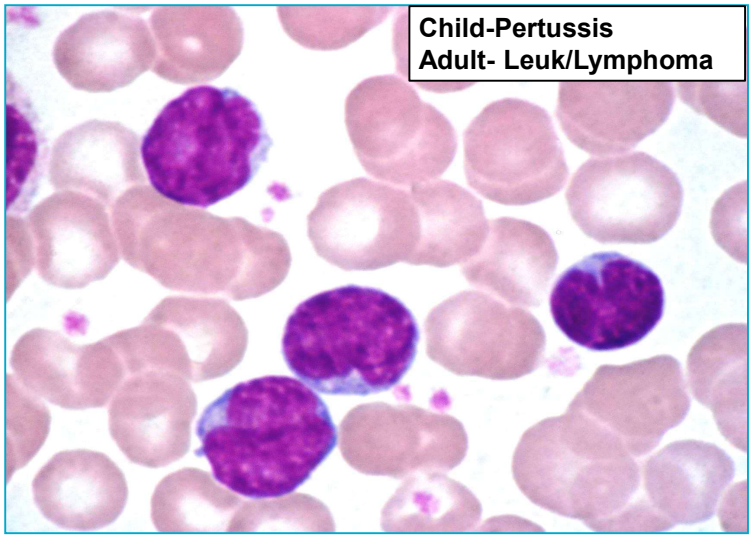
Age, Clinical Features Key

Non-Activated Lymphs: CD4 Helper T-Cells predominate



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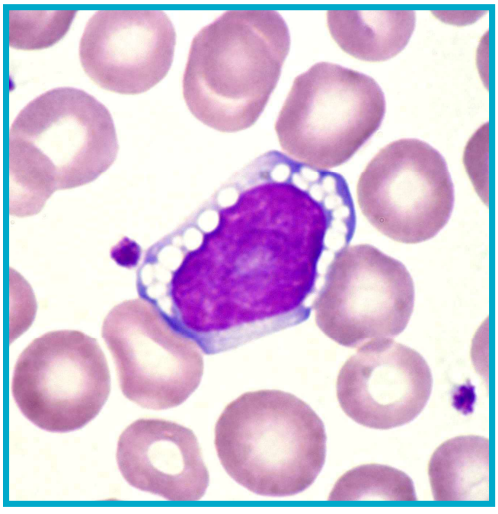
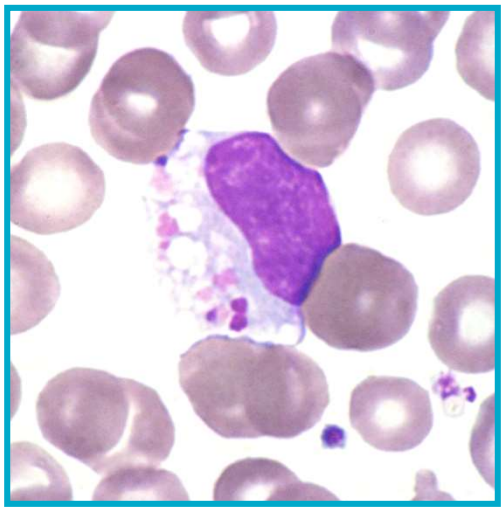
Lymphs in Young Child: Distinct Vacuoles/Granules





Sialic Acid/Storage Disease

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Lysosomal Storage Diseases with Abnormal Lymphocytes

- Sialic acid storage disease (mucolipidosis)
- Gangliosidosis
- Mucopolysaccharidosis
- Niemann-Pick disease
- Tay-Sachs

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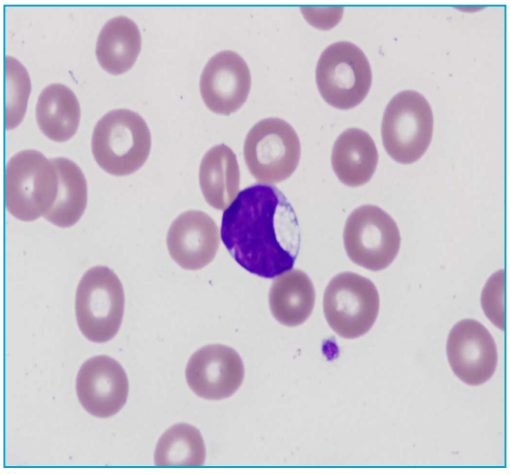
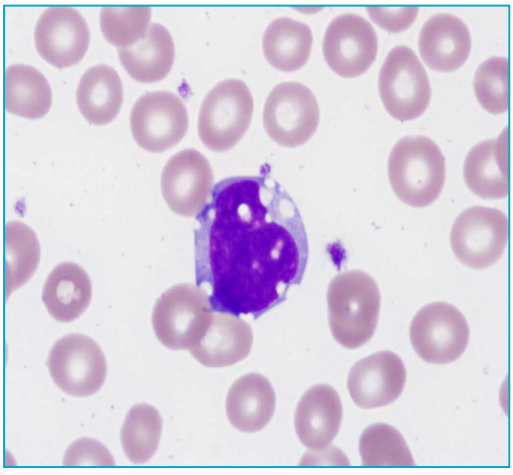
Vacuolated Lymphocytes in Adult





Mantle Cell Lymphoma

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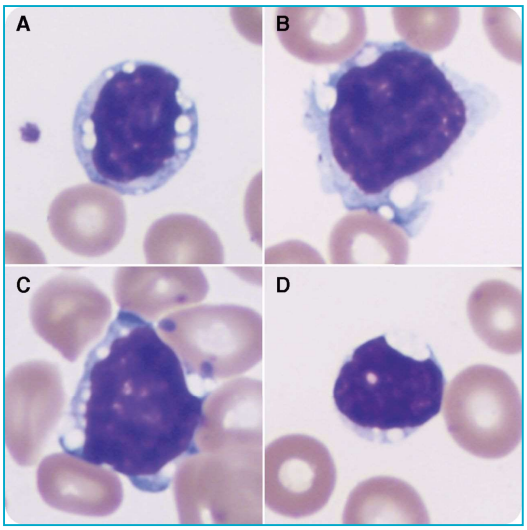
Discrete vacuoles in lymphocytes as a subtle clue to mantle cell lymphoma



Lynch DT, Foucar K. Discrete vacuoles in lymphocytes as a subtle clue to mantle cell lymphoma. *Blood*. 2016;127(25):3292. doi:10.1182/blood-2016-03-706101

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Lymphocytosis: Key Tips

- Dramatic age-related variations in normal
- Assess lymphocytes: morphologic heterogeneity (viral infection) vs. homogeneity (possible neoplasm); “kiddie” lymphs, pertussis
- Assess other lineages
- Sustained lymphocytosis in adult often neoplastic
- Isolated lymphocytosis in child more likely non-neoplastic
- Flow cytometric IP valuable on a limited basis (Flow may be misleading)

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Outline: Leukemic B,T-CLPN

B-cell Disorders

- Classification, general features
- Monoclonal B lymphocytosis
- Chronic lymphocytic leukemia
- Hairy cell leukemia
- B-cell lymphomas that mimic B-CLPN

T-cell Disorders

- Classification, IP
- T-cell large granular lymphocytic leukemia
- T-prolymphocytic leukemia
- Sézary syndrome
- Adult T-cell leukemia

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B-Chronic Lymphoproliferative Neoplasm

Definition:

- Clonal proliferation of morphologically and immunophenotypically mature B-lymphocytes. These cells are generally characterized by a low proliferation rate and prolonged cell survival.
- Prolonged cell survival often linked to BCL-2 anti-apoptotic activity

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Incidence of Leukemic Lymphoproliferative Neoplasms

Chronic Lymphocytic Leukemia	> 85%
Monoclonal B lymphocytosis (<5.0 absolute B cell count)	~10%
Hairy Cell Leukemia	< 5%
Mantle Cell, SMZL, Other Lymphomas	< 5%
Prolymphocytic Leukemia (B,T)	< 2%

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What's New for 2022

- More CLL cases reported in young patients (<40 yrs)
- Greater role of molecular genetic findings in diagnosis, risk assessment and treatment
- Trickier to distinguish CLL from MCL by flow

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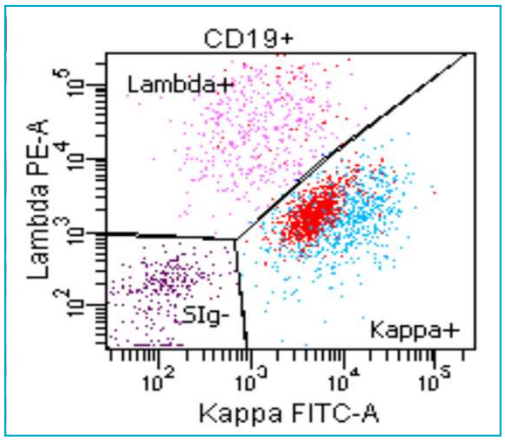
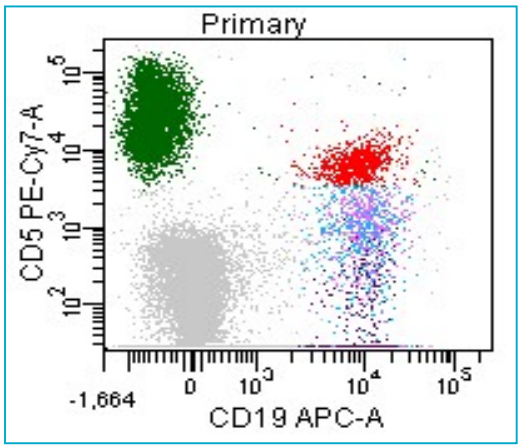
Monoclonal B Lymphocytosis (<3%)





Absolute monoclonal B cell = 300

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Monoclonal B Lymphocytosis

- High count (clinical) (≥ 500) and low count (< 500)
- Predisposes to CLL; 100 times more common
- ~12% of healthy elderly individuals
- Borderline with CLL unclear; same genetics as CLL
- Overlap with blood involvement with SLL key issue. (BM criteria for MBL not defined)
- Must know CBC data and clinical to interpret flow
- About 1% progression per year to CLL for high count MBL

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Chronic Lymphocytic Leukemia

Clinical:	Middle age to elderly
Hemogram:	**Sustained (3 mos) monoclonal lymphocytosis, (≥5,000) with CLL IP;** variable cytopenias
Morphology:	Small lymphocytes, condensed chromatin, inconspicuous nucleoli Prolymphocytes, other forms
Bone Marrow:	Pattern of infiltration nodular, mixed, diffuse
2022:	No significant revisions in diagnostic criteria

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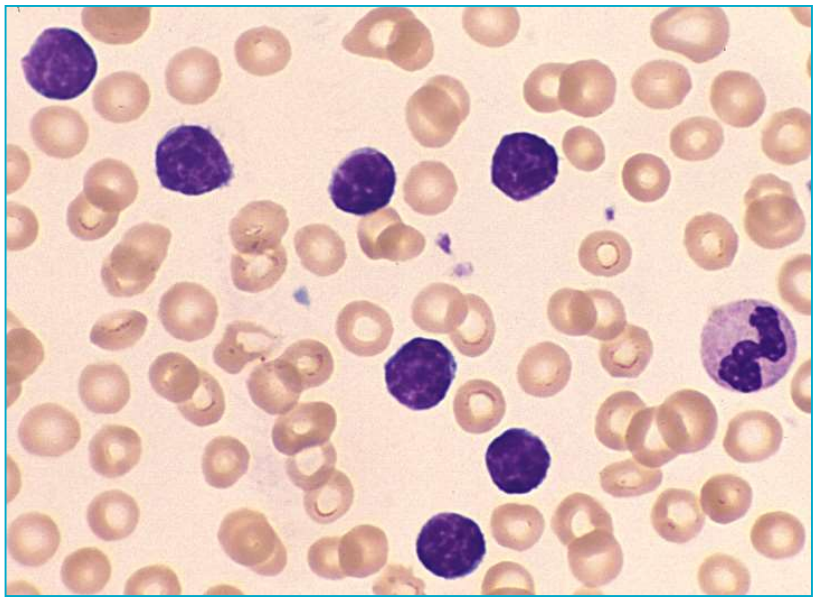
CLL: Preserved Hematopoiesis



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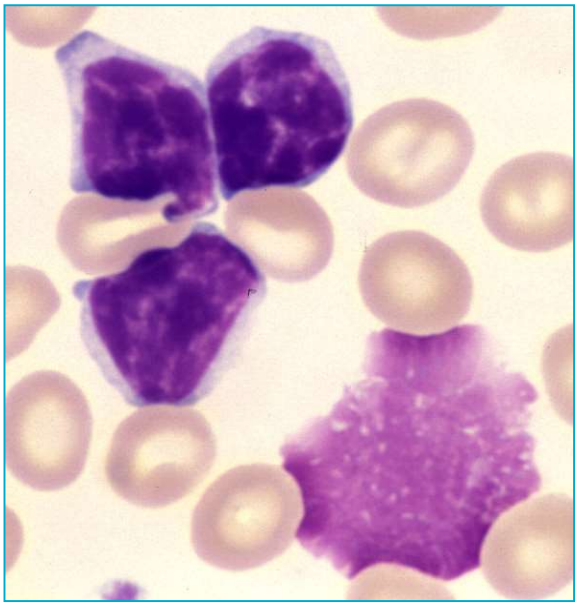
CLL, smudge cell



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CLL: Diagnostic Immunophenotypic Profile

Mature B	**Monoclonal SIg (dim)**
B-cell antigens	**CD19, dim CD20, dim CD22**
Coexpression	**CD5, CD23, CD200, LEF1**
Absent	**CD10, FMC7, CD11c (dim to neg.), CD79b, CD103**
Prognosis	**CD49d‡, CD38, ZAP 70**

‡ **CD49d** overall most significant IP feature

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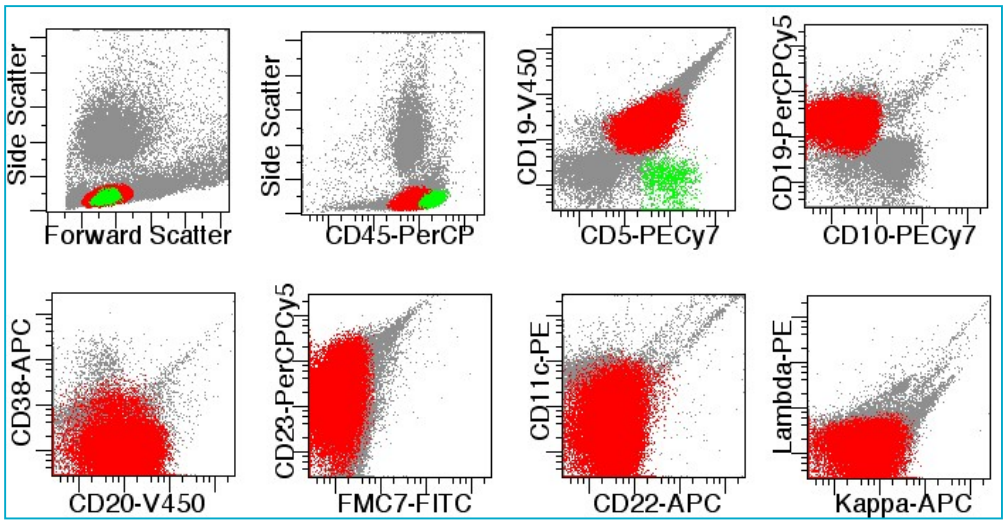
CLL: Flow Cytometry Profile



Courtesy S. Kroft

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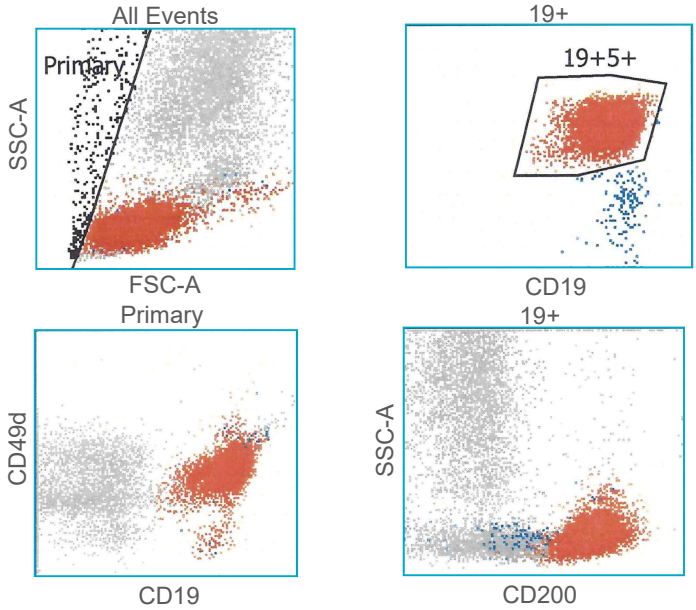
CLL: Flow Prognostic Markers: CD49d, CD38, ZAP-70

CD49d expression linked to adverse outcome: CD38$^+$, ZAP-70$^+$, also adverse



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CLL: Survival Data

- **Median survival times exceed 10-15 yrs**
- **1/3 patients never require therapy (median survival: 25 years)**
- **1/3 patients have prolonged indolent phase but eventually progress**
- **1/3 have more aggressive disease requiring upfront therapy (median survival: 8 years)**

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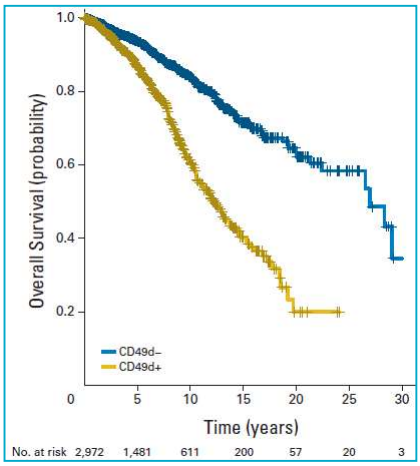
Impact of CD49d on Survival in CLL



Bullian P, et al. CD49d Is the Strongest Flow Cytometry-Based Predictor of Overall Survival in Chronic Lymphocytic Leukemia. *JCO*. 2014;32(9):897-903. doi:10.1200/JCO.2013.50.8515

- Increased hazard of death (2.3)
  - Lower OS at 5 (87% vs 90%) and 10 (62% vs 84%) years
  - **Most robust prognostic marker by flow cytometry**

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CLL: Morphology – IP – Genotype

del(13q)	linked to typical morphology and IP (key microRNA deletion)
tri 12	linked to atypical morphology, atypical IP
del(11q)	linked to high stage, aggressive disease course
del(17p)	advanced stage, chemo resistance
miRNA	deletions lead to post-transcriptional abnormalities; tumor suppressor deletion

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Genetics and Prognosis in CLL

Favorable:

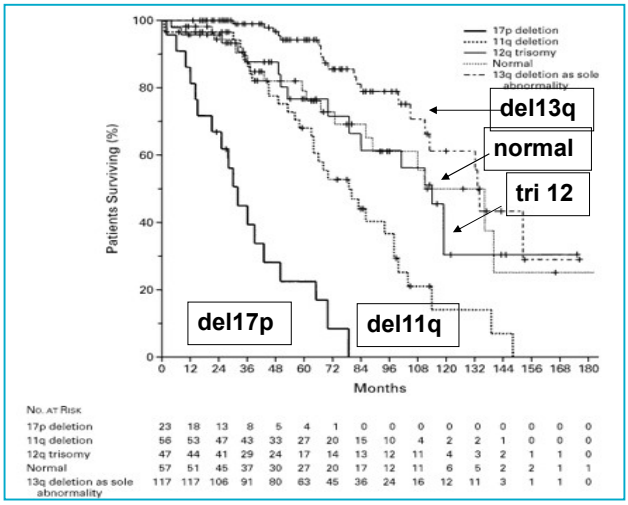
- **del13q as sole abnormality**

Intermediate:

- **Normal**
- **Trisomy 12**

Unfavorable:

- ***TP53* deletion (17p)**
- ***ATM* deletion (11q)**
- **Complex karyotype**



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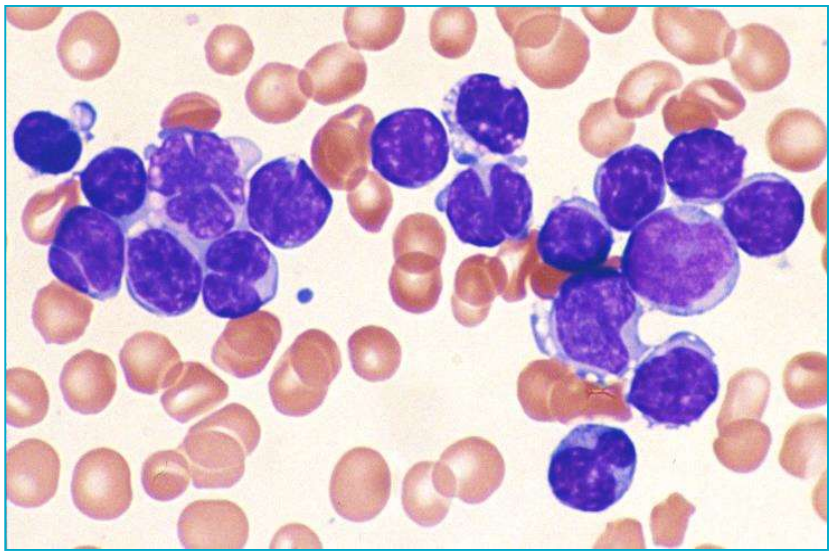
CLL with Atypical Morphology

Flow (typical):

CD5+, CD23+, dim CD20, dim SIg

FISH:
del(17p13)
(acquired late in disease course)



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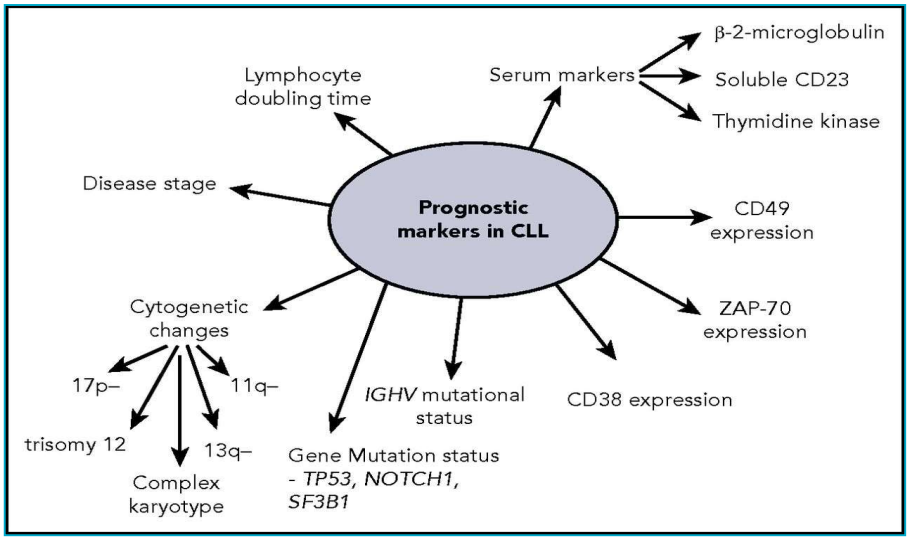
Prognostic Factors in CLL



Gribben JG. How and when I do allogeneic transplant in CLL. *Blood*. 2018;132(1):31-39. doi:10.1182/blood-2018-01-785998

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Hairy Cell Leukemia

Clinical:	Middle age to elderly, splenomegaly
Hemogram:	Pancytopenia, monocytopenia
Morphology:	Oval to round nuclei with "spongy" chromatin; abundant cytoplasm with hairy projections
Cytochemistry:	TRAP (strong) (also IHC Stain available)
Bone Marrow:	Distinct pattern of infiltration
Molecular:	*BRAF* mutation in most cases (>80%) *MAP2K1* mutation in remainder

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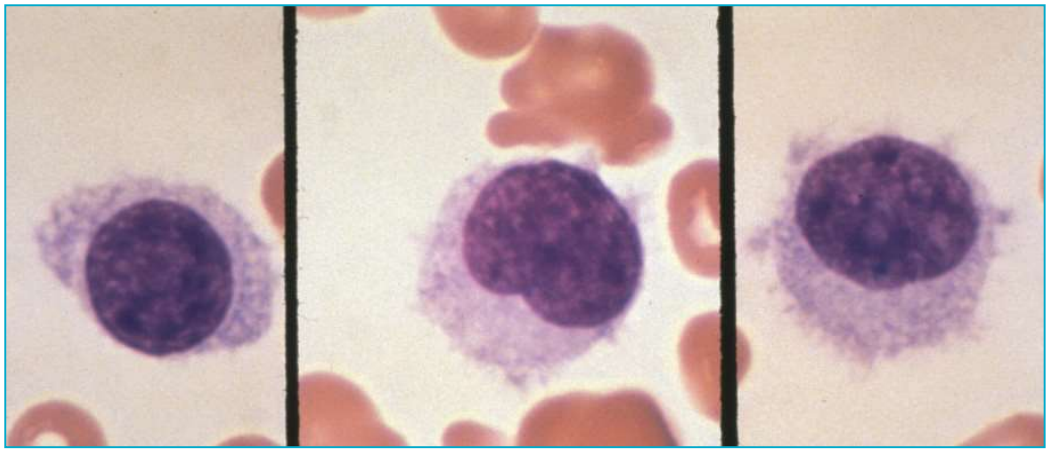
HCL: Rare cells in blood, High index of suspicion



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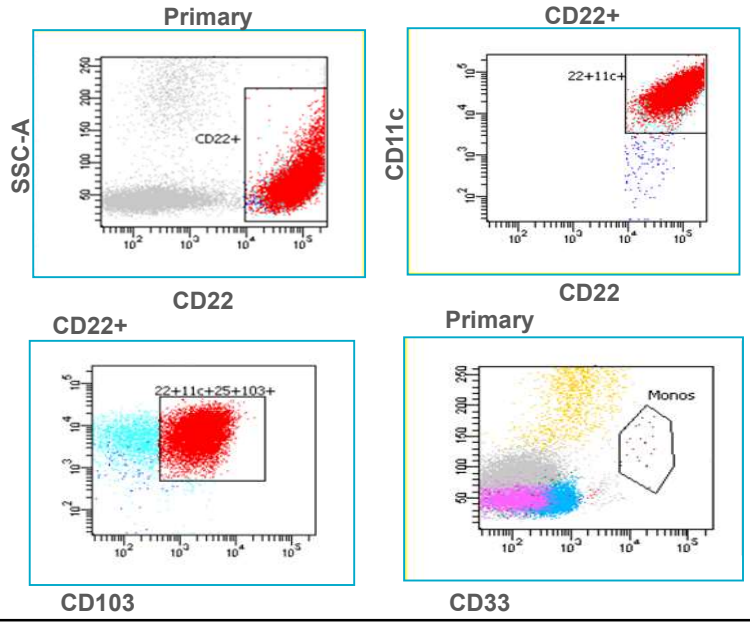
Hairy Cell Leukemia – Marked Monocytopenia

67-year-old male
with lymphocytosis



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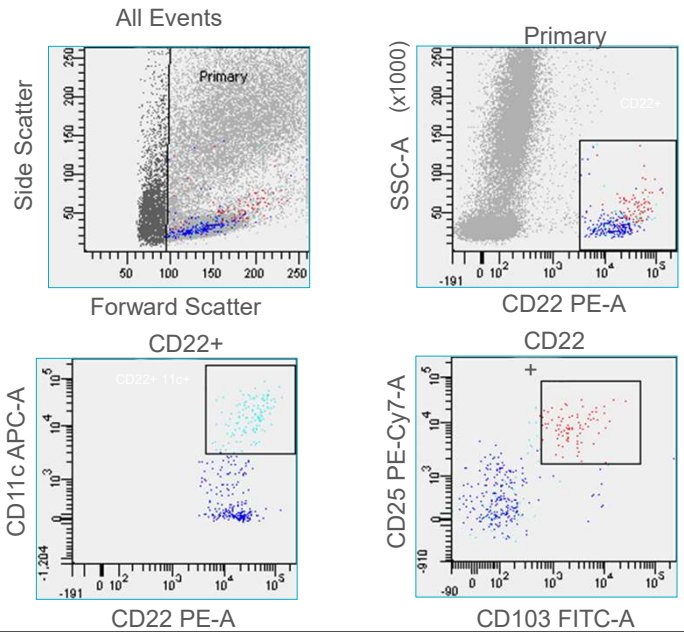
HCL: High Sensitivity and Specificity of FCI



0.3% of cells

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Splenomegalic B-CLPN with Leukemic Manifestations

HCL	Variable WBC, often low
HCLv	Leukocytosis (variable)
MCL	Leukocytosis; may be SOX-11^{-}, CD200^{+}
SMZL	Leukocytosis, villous lymphocytes
B-PLL	Leukocytosis (exclude MCL)

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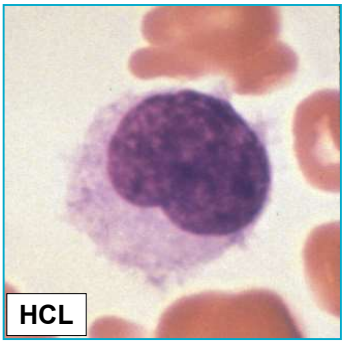
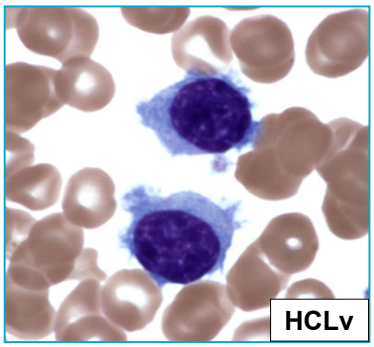
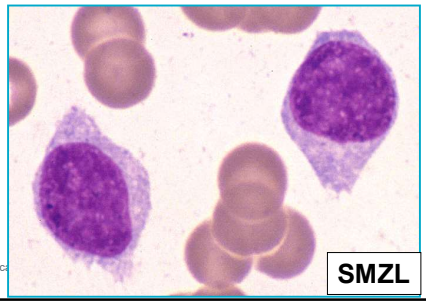
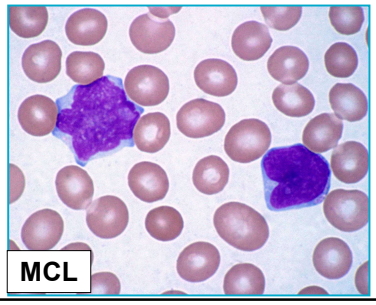
B-CLPN with Splenomegaly









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B-CLPN: Key Tips for 2022

- Recognize morphology, IP features of distinct entities
- B-CLPN have characteristic IP profile (not single marker)
- Identification of biologic subtypes of CLL important (morphology, IP, genetic clues)
- HCL: Pancytopenia (monocytopenia), very subtle BM infiltrates, Hypocellular (!)/ *BRAF* mutations
- Overlap disorders: (Atypical CLL and lymphomas), (B-PLL and MCL)
- Utility of genetics in MCL, CLL, other lymphomas

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T-CLPN/ Mature T – cell Leukemias

General Features

- Much rarer than B-CLPN
- Affect adults, wide age range
- Patients often exhibit systemic manifestations (organomegaly, skin findings, fatigue, fever)
- Key clinical and CBC "tips" to various subtypes

Subtypes

T-LGL	T-cell large granular lymphocytic leukemia
T-PLL	T-cell prolymphocytic leukemia
SS	Sézary syndrome
ATL	Adult T-cell leukemia lymphoma

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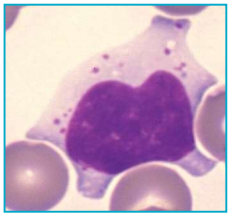
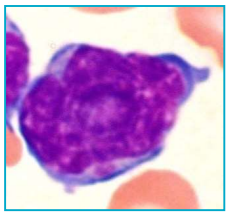
Key Tips: Mature T-cell Leukemias

T-LGL



- Cytopenias predominate; variable ALC
- Splenomegaly common
- Associations with RA, red cell aplasia, PNH
- Derived from cytotoxic/suppressor T cell ($CD8^+$)

T-PLL



- Marked leukocytosis (rapidly rising WBC)
- Marked hepatosplenomegaly
- Derived from helper T cell ($CD4^+$)
- One third coexpress CD4, CD8

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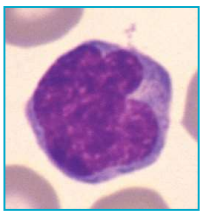
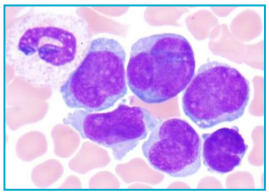
Key Tips: Mature T-cell Leukemias

SS



- Cutaneous manifestations, erythroderma
- Blood involvement by definition in SS
- Derived from helper T cell ($CD4^+$)

ATL



- Endemic disease distribution (HTLV-1-associated)
- Broad spectrum of clinical manifestations
- Leukemic subtype most aggressive
- Derived from helper T cell ($CD4^+$)

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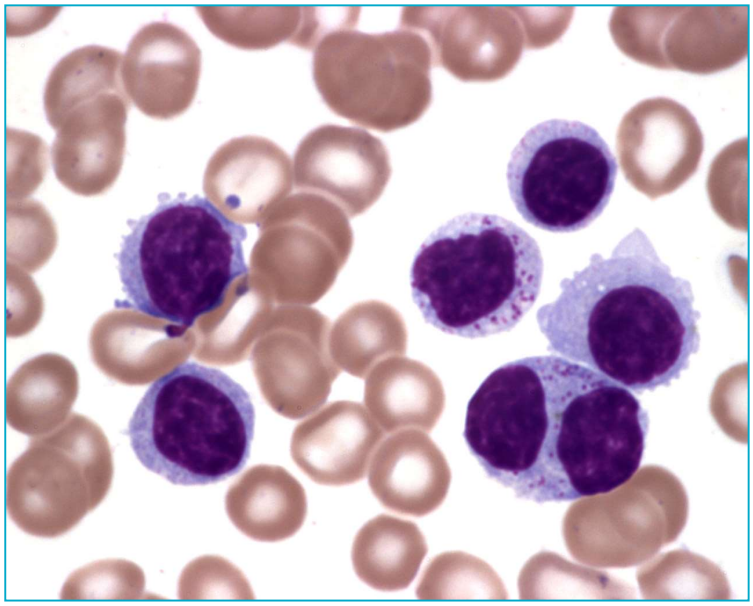
T-cell LGL

73-year-old female with Lymphocytosis



Courtesy Qian-Yun Zhang

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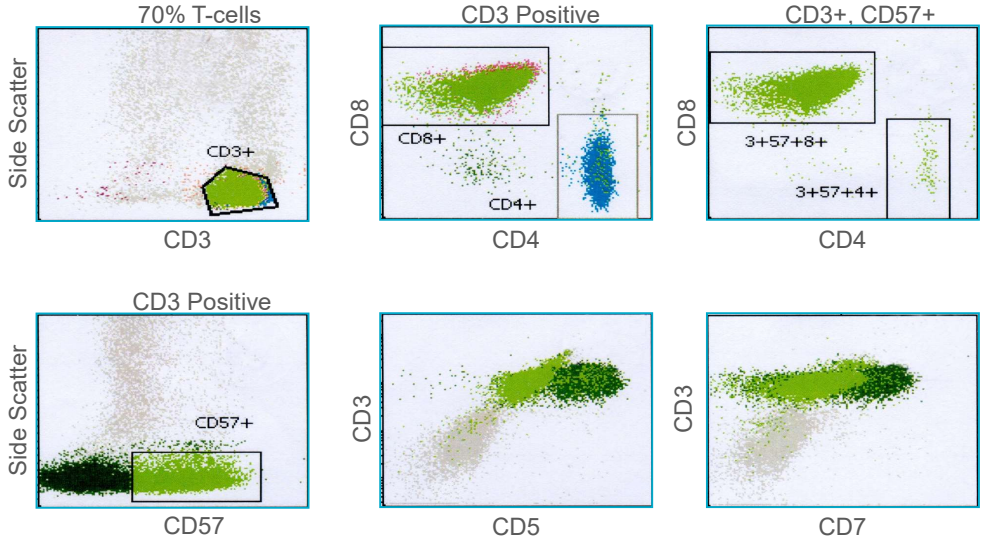
T-cell Large Granular Lymphocytic Leukemia



Diminished CD5 and CD7 on CD3+, CD57+

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Features of T-LGL

ALC:	• 2-20x 10^9 /L (mature)
IP:	• Mature cytotoxic/suppressor T-cell (CD57+, TIA-1+, CD16+) • Aberrant features common
Clonality:	• TCR gene rearrangement; Flow *TRBC1* restriction • *STAT* 3 mutations in 40%
Disease course:	• Stable for many years; spontaneous remissions

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T cell-Prolymphocytic Leukemia (T-PLL)

- **Rare (2% lymphocytic leukemias)**
- **Middle aged to elderly patients (med age 65)**
- **Marked splenomegaly, striking leukocytosis, skin 20%**
- **Notable feature: rapidly rising WBC**

Blood:	Morphologic spectrum
BM:	Diffuse pattern
IP:	Majority CD4+, one third CD4+/CD8+, *TCL-1* protooncogene +, CD52+
Genetic:	*TCL-1* gene rearrangements/inv(14)(q11q32) *STAT5 B* mutations in 40%

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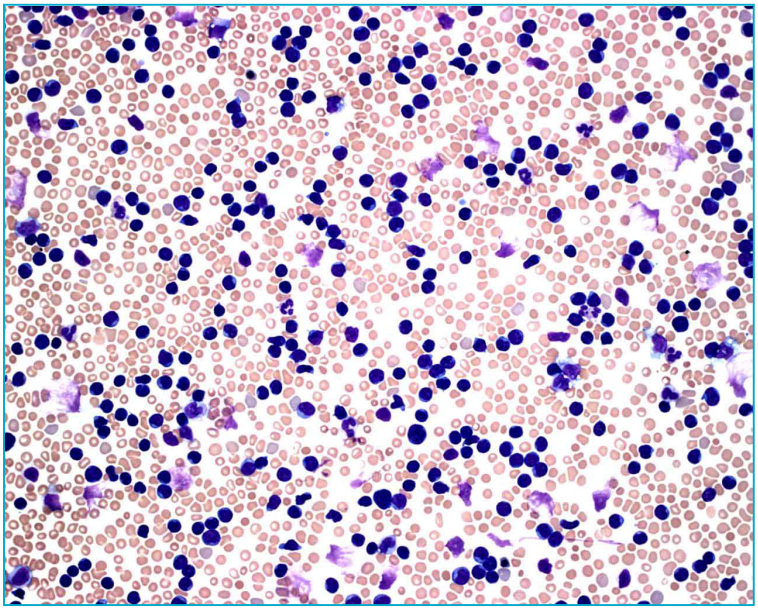
T-PLL, striking leukocytosis



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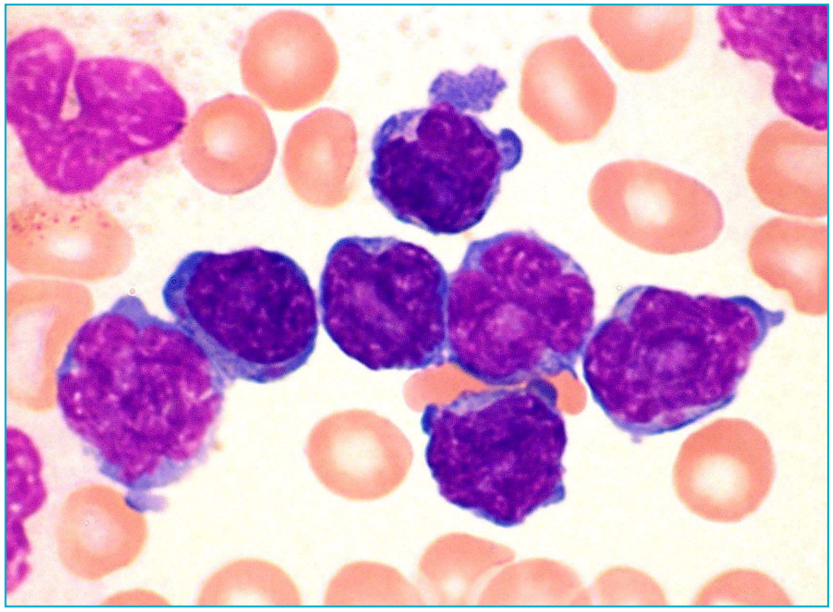
T-PLL (morphologic spectrum)



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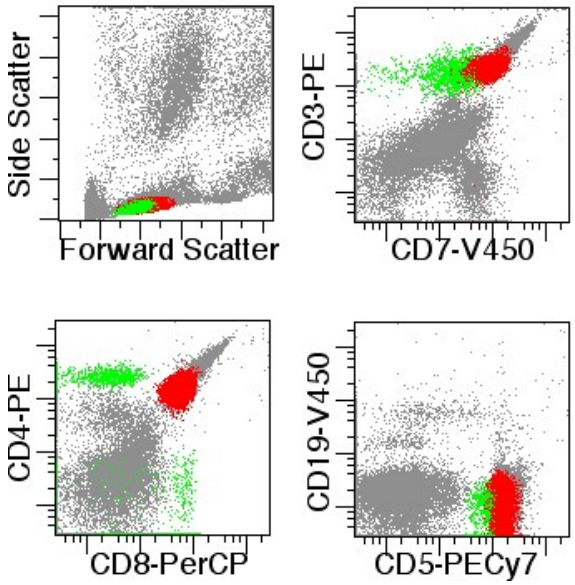
T-cell Prolymphocytic Leukemia



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Source: S. Kroft

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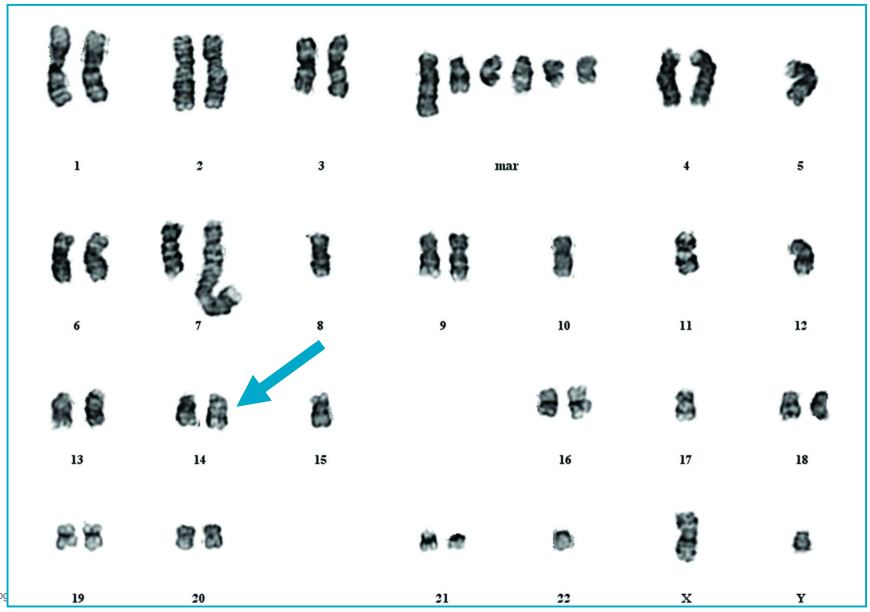
T-PLL inv (14)(q11q32)



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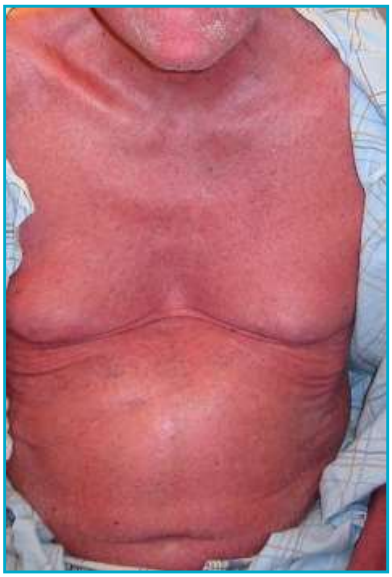
Generalized Erythroderma

72-yr-old male

(Tip: Generalized itching also occurs)



Source: Dr. Beverly Nelson

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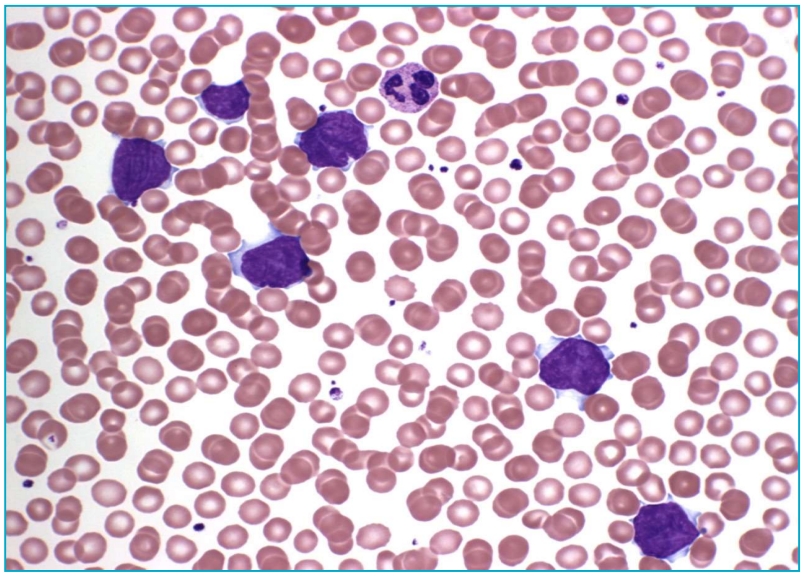
SS: marked lymphocytosis



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Bld: Sézary Syndrome: 1º and 2º

- 1º – erythroderma with initial blood involvement
- 2º –blood involvement after longstanding mycosis fungoides
- Morphology: mature nuclei with subtle convolutions, variable cell size
- IP: mature T-cell (CD3, CD5, CD4);lack CD7, lack CD26; TCR α/β
- H/S ratio >10:1
- Sezary count >1.0x10^9/L

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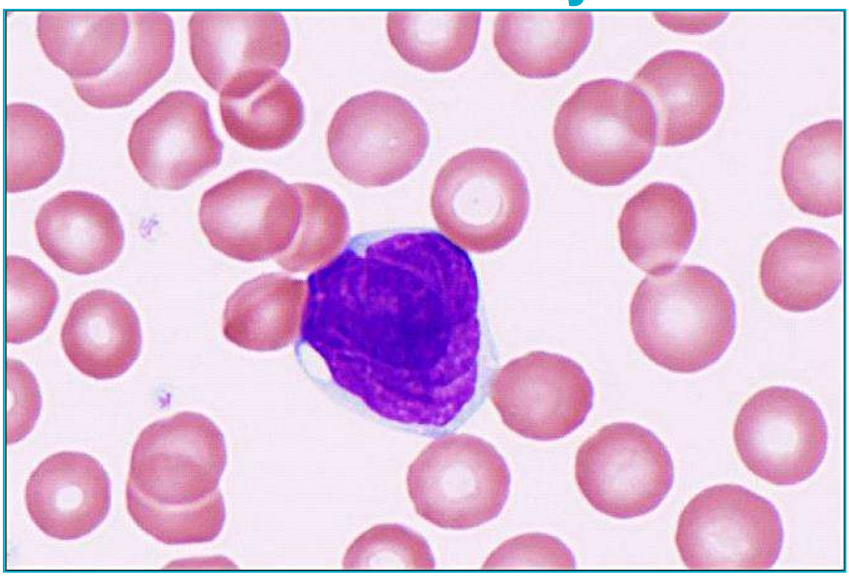
Cerebriform Sézary cell



Source: Dr. Beverly Nelson

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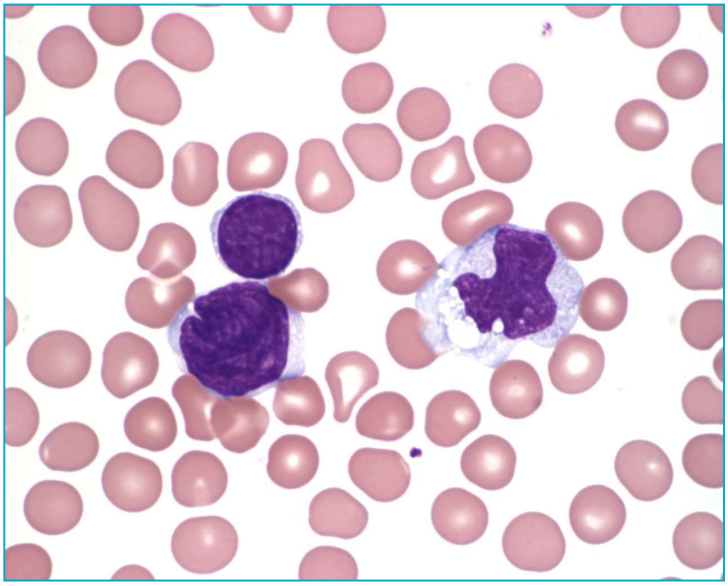
Sézary cell, monocyte, normal lymph



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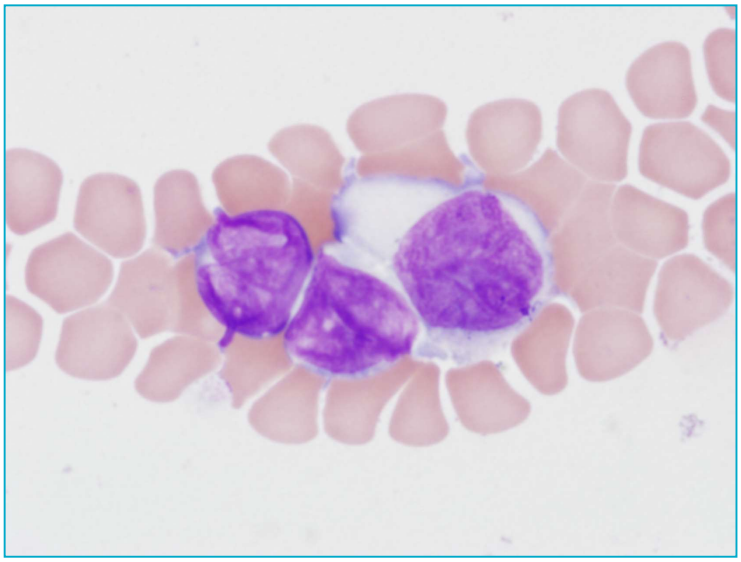
Sezary Cells at Feather Edge

Large cells enriched at feather edge and sides of blood smear



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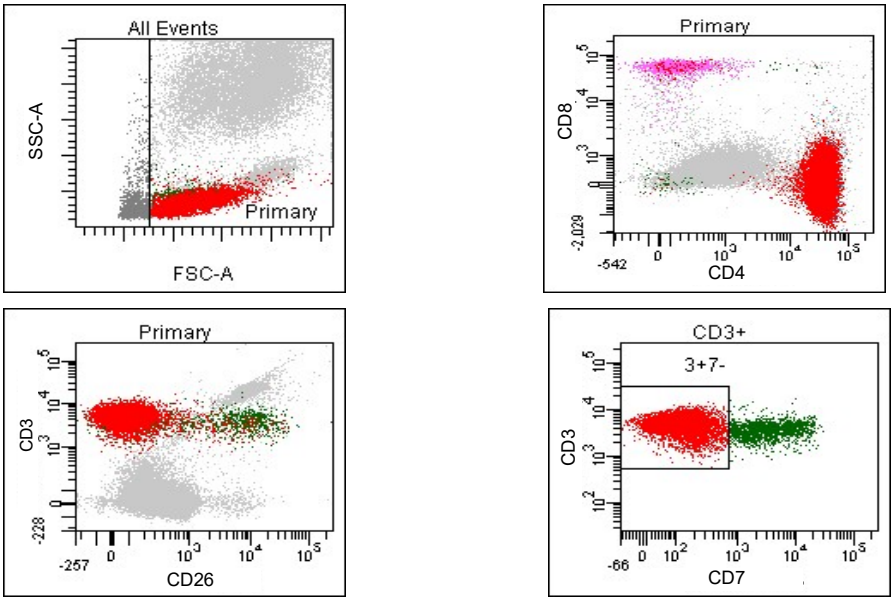
76-year-old male with eosinophilia and ALC 6.9



Immunophenotype: CD3+, CD4+, CD7-, CD26-

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Summary: T-CLPN

- Integrate morphologic, immunophenotypic, and clinical findings. Most mature T-cell leukemias can be diagnosed as specific clinicopathologic entities
- Consider T-LGL in patients with neutropenia (especially RA patients) or acquired red cell aplasia (Clonal TCR)
- New flow testing for T-cell clonality (TRCR1)
- Genetic testing in T-PLL [inv(14)]

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Case

80-year-old female with lymphocytosis

CBC			
WBC	18.0	MCV	91
ALC	14.1	MCHC	34.7
ANC	3.5	RDW	12.7
RBC	4.42	Plt	192
H/H	14/40%		

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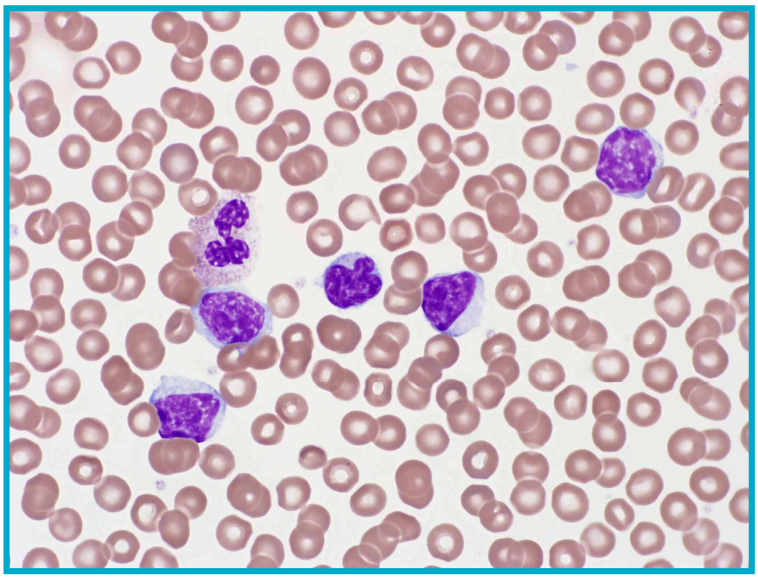
Blood: Isolated Lymphocytosis



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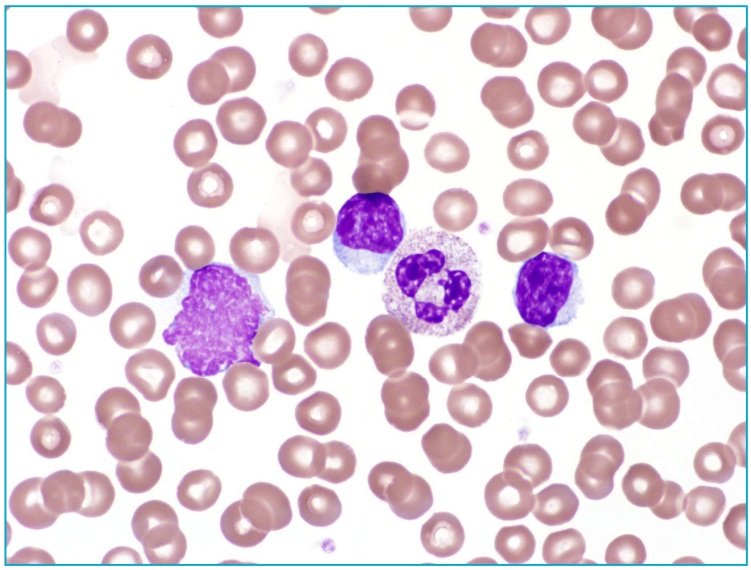
Blood: Isolated Lymphocytosis



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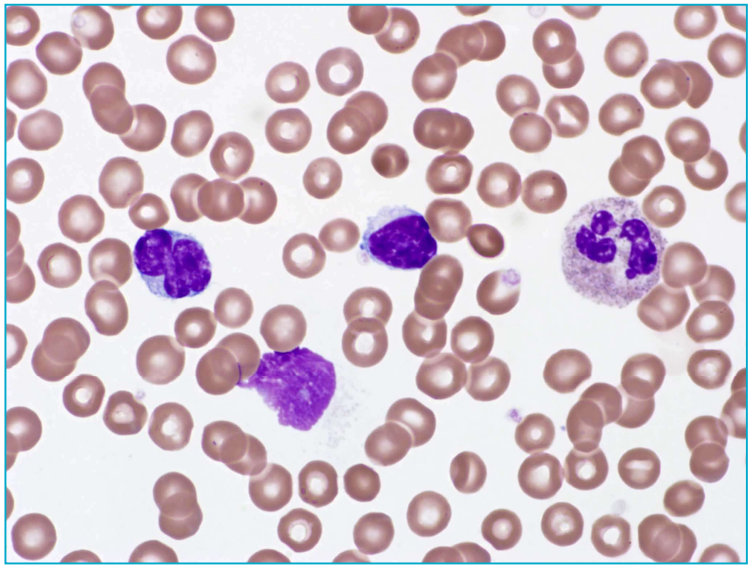
Blood: Isolated Lymphocytosis



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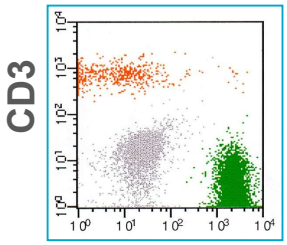
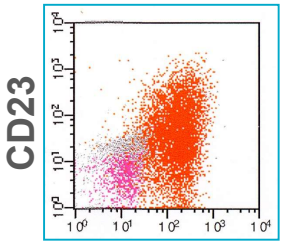
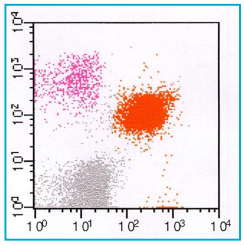
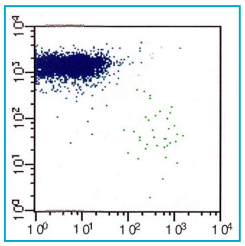
CD5+ Monoclonal B Cell Population

80 yo F
WBC 18.0
ALC 14.1
H/H 14/40
Plt 192









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Key Differential Diagnosis is CLL vs Leukemic MCL

Genetic Testing Essential

Diagnosis:

MCL with preserved hematopoiesis

FISH + for *CCND1/IGHV* [t(11;14)]

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Closing Comments & Summary

- Assess for whether HP is intact or reduced.

For lymphoid cells:

- Systematic process: Mature vs. immature, lymphoid vs. nonlymphoid, etc.
- Great utility of flow in lymphocytosis.
- CLL vs. MCL, always recommend genetics.

Questions?

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