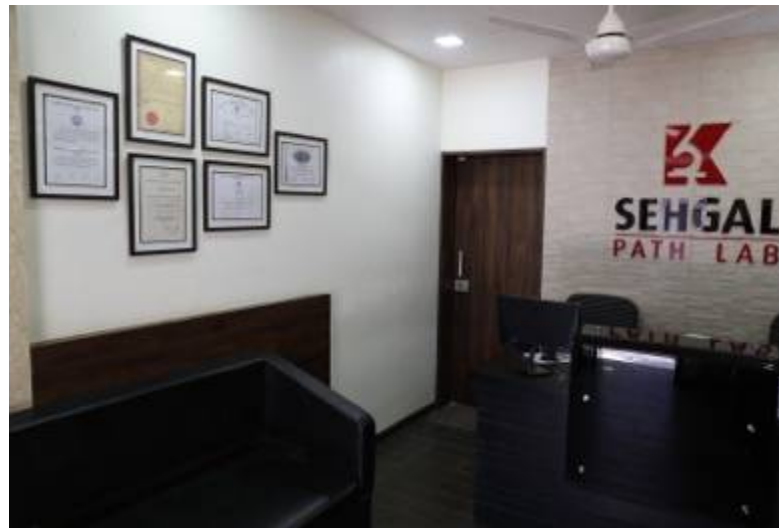


# Flow cytometry in B-Cell Chronic Lymphoproliferative Disorders A case based approach



**Dr Kunal Sehgal, M.D**

**Director, SEHGAL PATH LAB**

**Mumbai , INDIA**

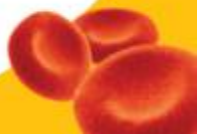
**[drkunalsehgal@gmail.com](mailto:drkunalsehgal@gmail.com)**



# Diagnosis of Lymphomas

## Multidisciplinary Approach

- Adequate Clinical History and Examination
- Laboratory work up- CBC,ESR,LDH,B<sub>2</sub> microglobulin, etc.
- Radiological Evaluation- PET Scan, CT scan , etc.
- Molecular Studies
- Morphology – H & E
- Immunophenotyping- for diagnosis, subtyping, prognosis

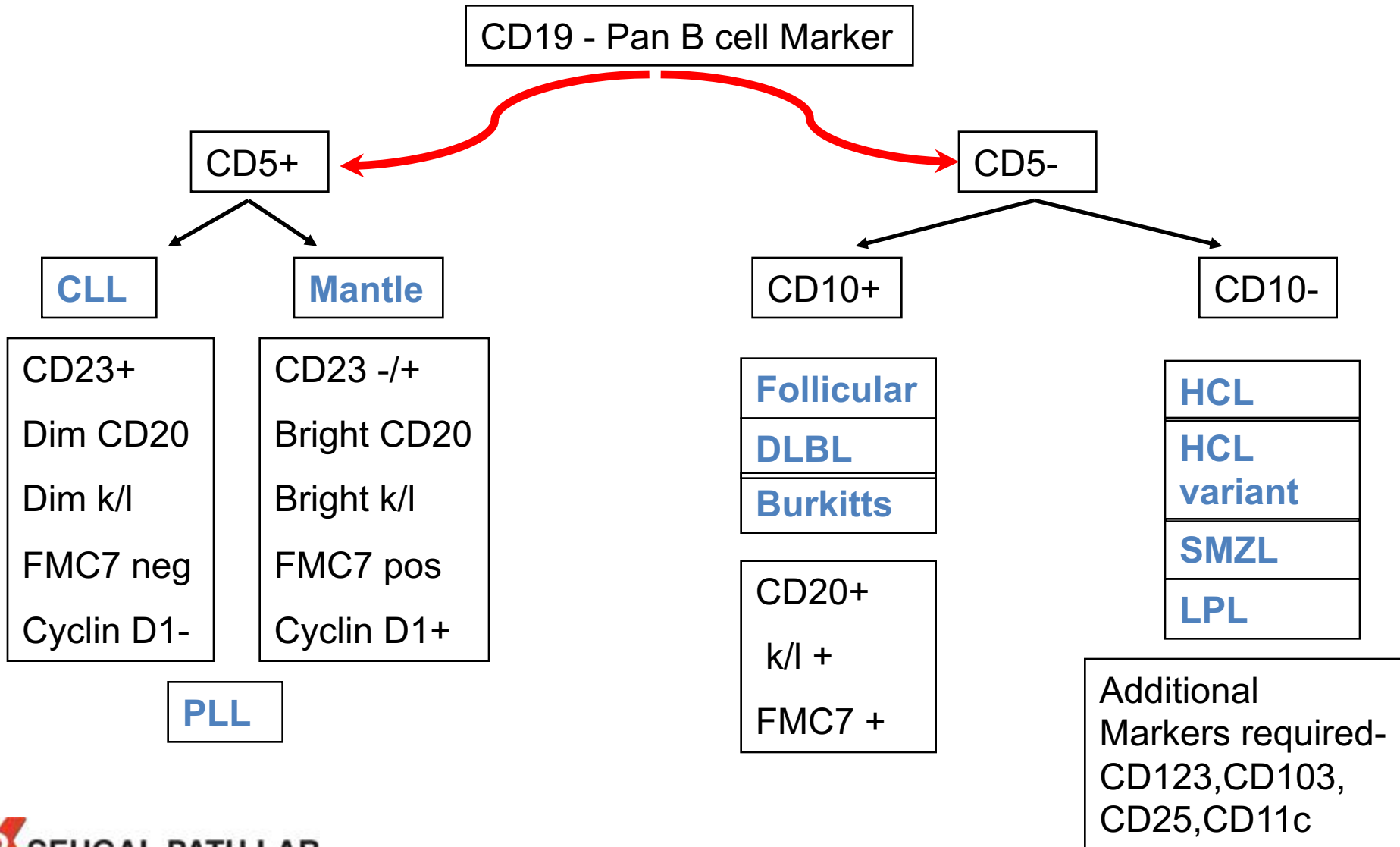


# Role of FCM in CLPD

- Diagnosis of CLPD – Reactive vs. Lymphoma
- Typing and Subclassification
  - B cell CLPD
  - T cell CLPD
  - NK cell CLPD
- Prognostic markers
  - eg. CD38 , CD49d in CLL



# Conventional B cell CLPD Classification



# Modified Matutes SCORE for CLL

Antigen	Expression	Score	Expression	Score
<b>sIgM</b>	Weak/Mod	+1	Mod/Strong	0
<b>CD5</b>	Positive	+1	Negative	0
<b>CD23</b>	Positive	+1	Negative	0
<b>CD79b</b>	Neg/Weak	+1	Negative	0
<b>FMC7</b>	Negative	+1	Strong	0

## Modified CLL scoring system

**A score of 4 or 5/5 supports a diagnosis of CLL.**



# New CLL score – Kohnke et al , 2017 BJH

This simplified score - “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:

$$\text{CLL flow score} = \%CD200+ve + \%CD5\&CD23dual+ve - \%CD79b - \%FMC7$$

If the CLL flow score is **higher than zero**, a diagnosis of CLL is likely.

**CD5/CD23, FMC7, CD79b and CD200** were included in **new CLL flow score**, which retained high sensitivity (97.1% vs. 98.6%) for the Matutes score, but showed **markedly increased specificity** (87.2% vs. 53.8%)

These results were confirmed in our validation cohort (sensitivity 97% vs. 100%) AND specificity 86.4% vs. 59.1%,



# CD200 , CD148 , CD180

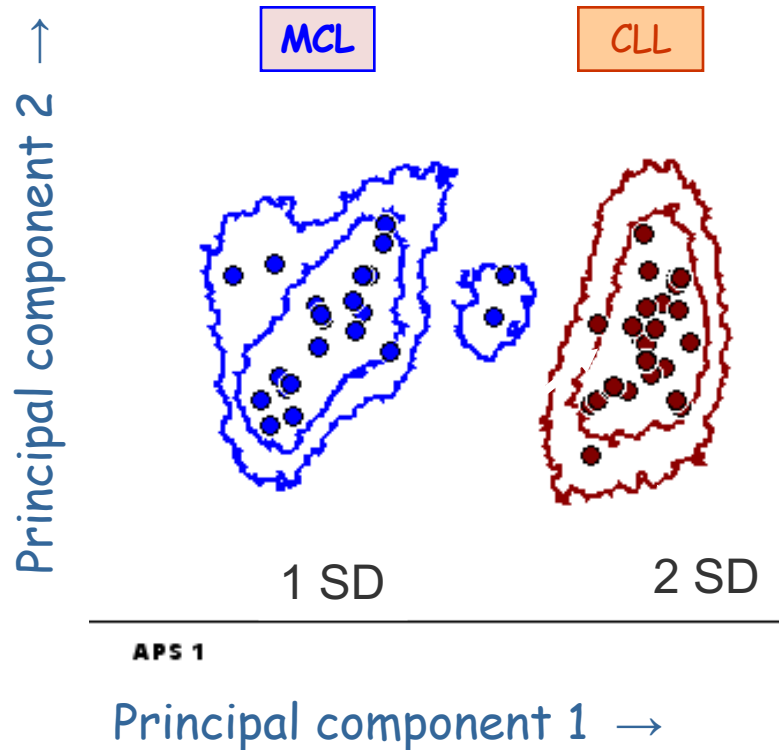
Newer Markers in CLPD for subclassification -

- **CLL** - weak expression of CD148 and CD180 coupled to a strong expression of **CD200**
- **Mantle Cell Lymphoma**- strong expression of **CD148** combined with a weak expression of CD180 and CD200.
- **SMZL** - weak expression of CD148 and CD200 coupled to a strong expression of **CD180**
- **LPL** – A moderate expression of these three markers

- Miguet et al , Blood 2014 124:5407
- Tata Memorial Hospital unpublished data



# MCL vs CLL: PCA of total immunophenotype



	PC1	
1	IgM	14.09
2	CD200	14.06
3	CD79b	13.39
4	CD23	8.60
5	CD20	6.43
	...	





# CLPD – 8 colour Antibody Panel at Sehgal Path Lab

	<b>FITC</b>	<b>PE</b>	<b>PC5.5</b>	<b>PECy7</b>	<b>APC</b>	<b>APCH7</b>	<b>BV421</b>	<b>BV510</b>
<b>B cell Tube 1</b>	<i>KAPPA</i>	<i>LAMBDA</i>	<b>CD38</b>	<b>CD19</b>	<b>CD10</b>	<b>CD45</b>	<b>CD5</b>	<b>CD20</b>
<b>B cell Tube 2</b>	Drop in	<b>CD200</b>	Drop in	<b>CD19</b>	<b>CD23</b>	<b>CD45</b>	<b>CD43</b>	<b>CD49d</b>
<b>T cell Tube 1</b>	Drop in	<b>CD7</b>	<b>CD3</b>	<b>CD34</b>	<b>CD56</b>	<b>CD45</b>	<b>CD4</b>	<b>CD8</b>
<b>Additional Tubes as per case</b>								
<b>Hairy cell tube</b>	<b>CD103</b>	<b>CD123</b>	<b>CD25</b>	<b>CD180</b>	<b>CD11c</b>	<b>CD45</b>	<b>CD19</b>	<b>CD20</b>
<b>T cell Tube 2</b>	<b>TCR AB</b>	<b>TCR GD</b>	<b>CD3</b>	<b>CD5</b>	<b>CD16</b>	<b>CD45</b>	<b>CD2</b>	Drop in

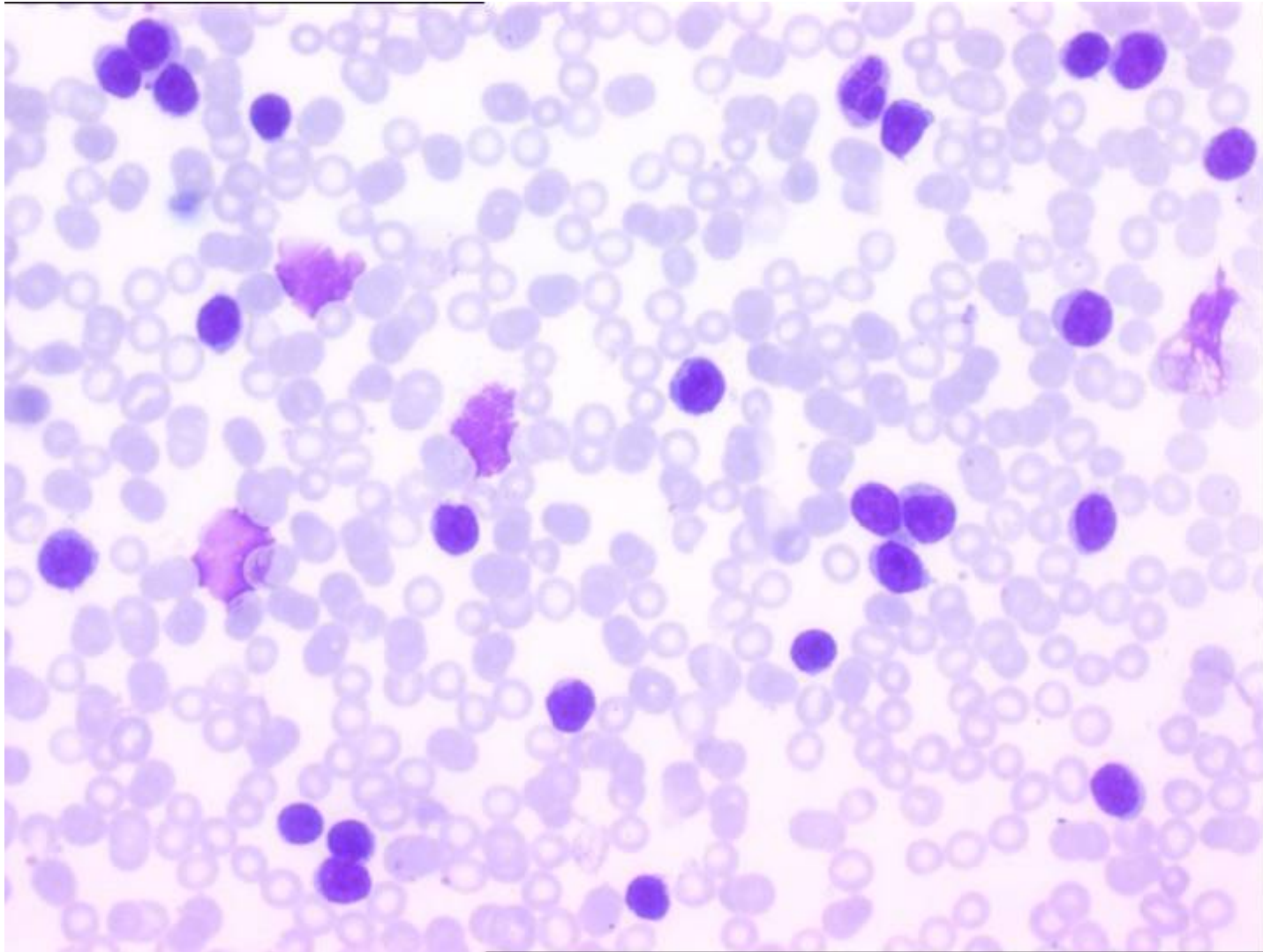


# Case

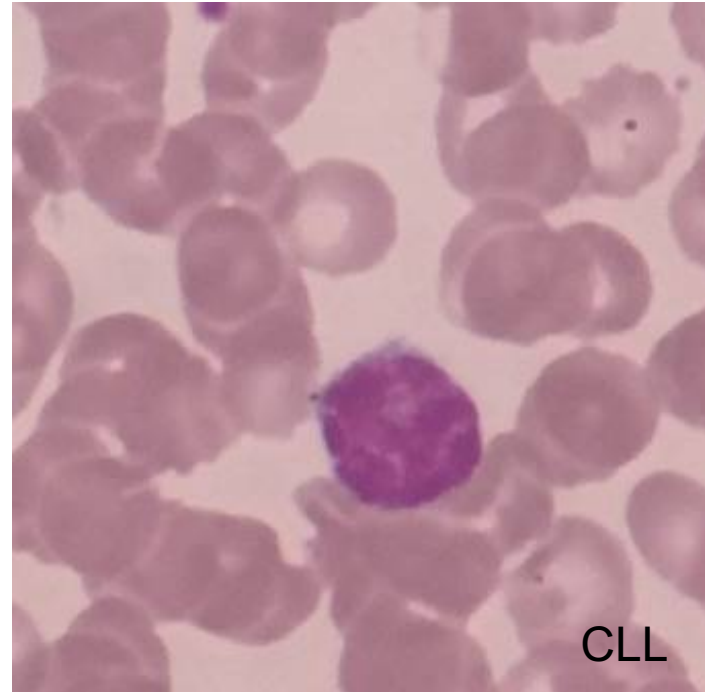
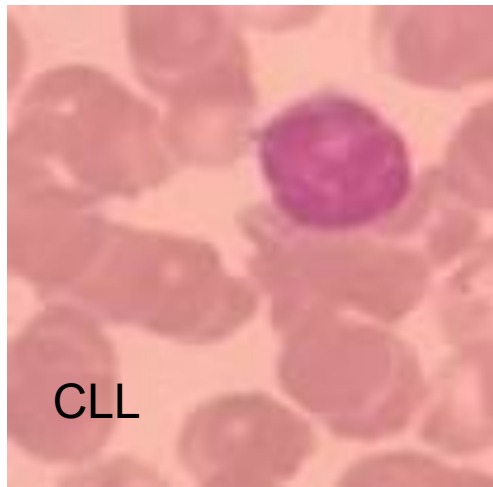
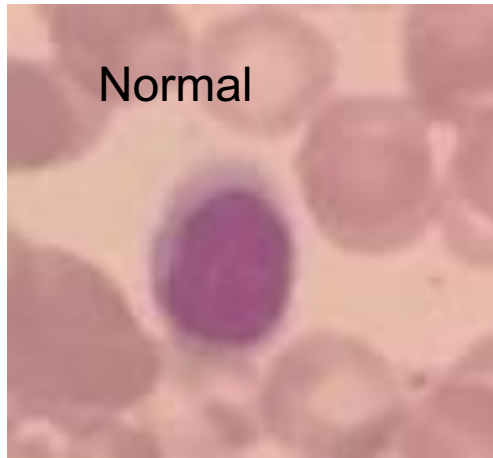
- 55/M
- Routine Health Check Up
  
- Hb -12 g/dl
- Platelets-  $2.5 \times 10^6$  /ul
- WBC -  $26 \times 10^3$  /ul
  
- WBC Differential – Absolute Lymphocytosis ,  
Lymphocytes -87%



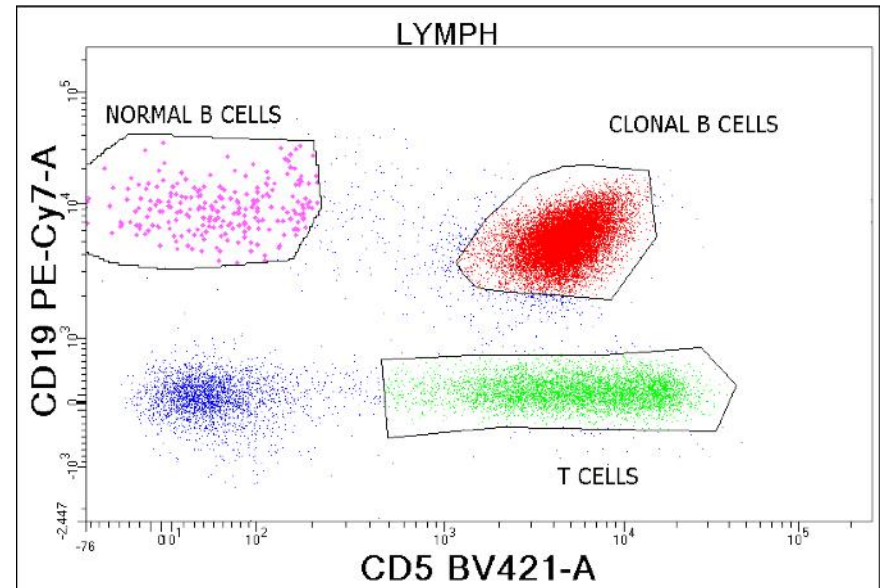
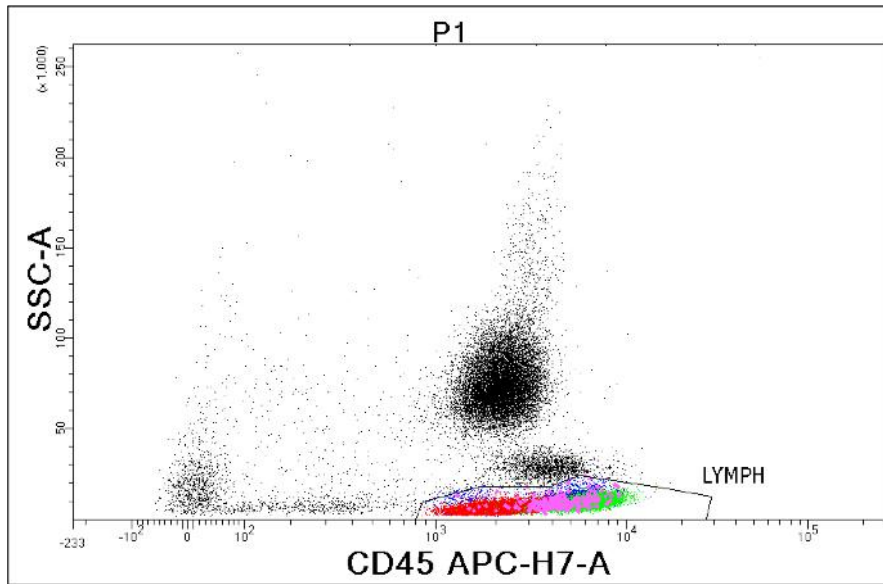
# Peripheral Smear



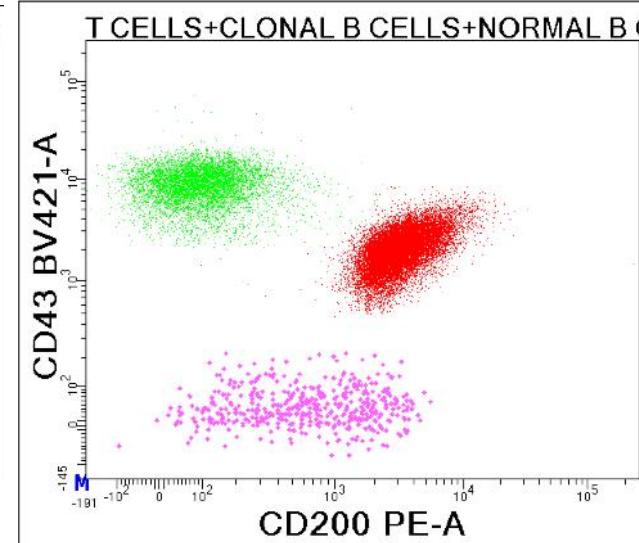
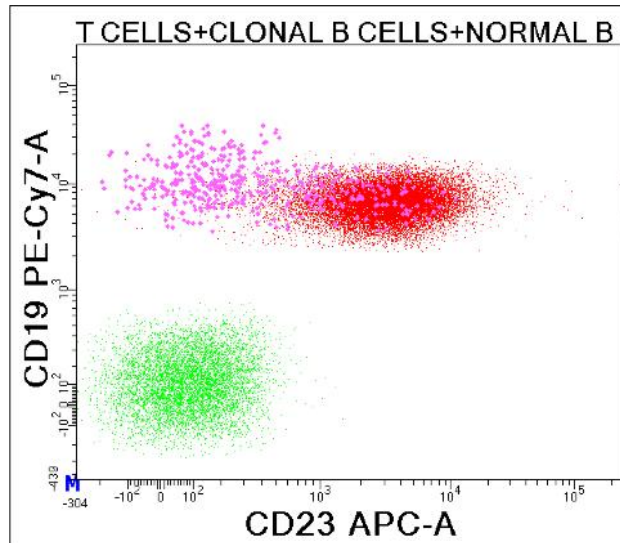
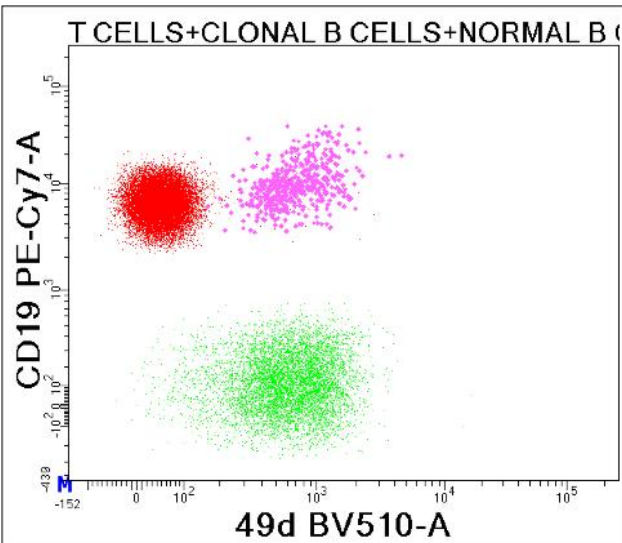
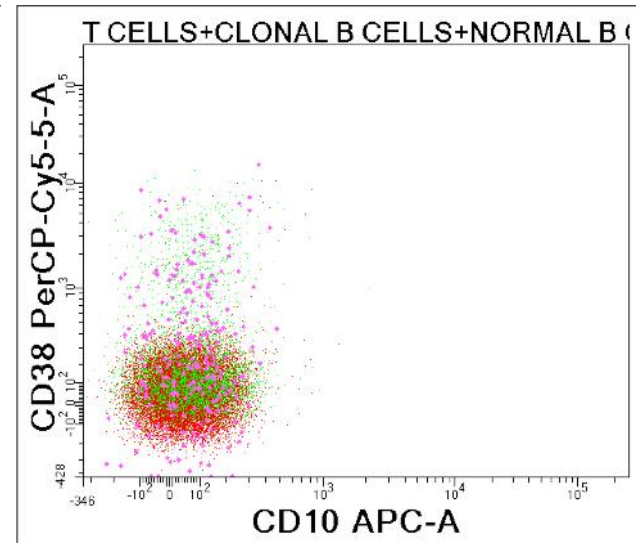
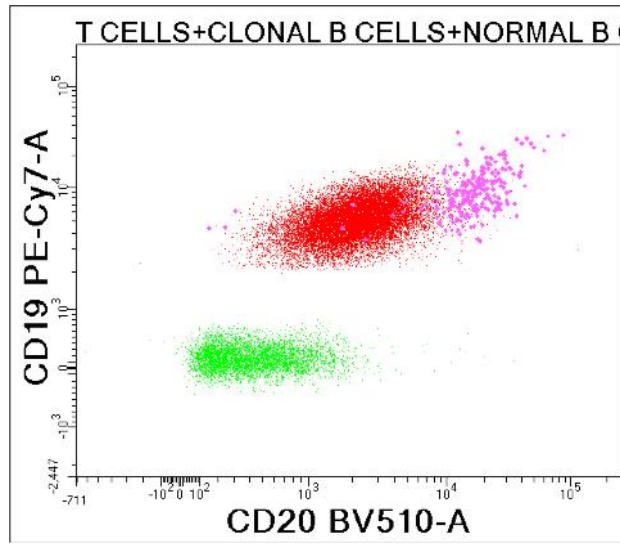
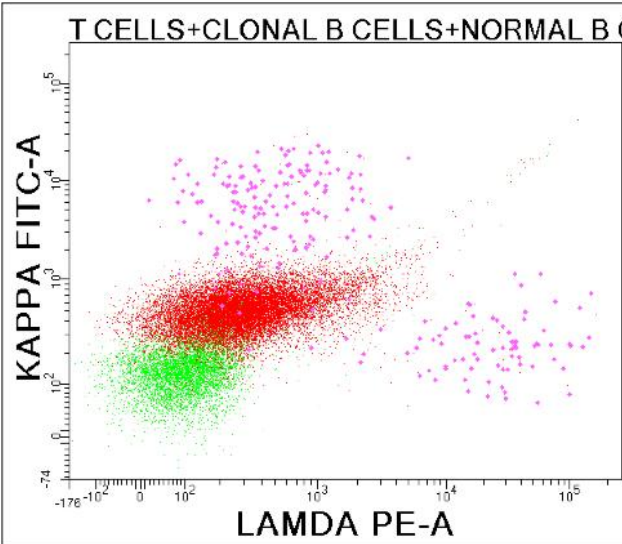
# Soccer ball Chromatin



# Flow Analysis

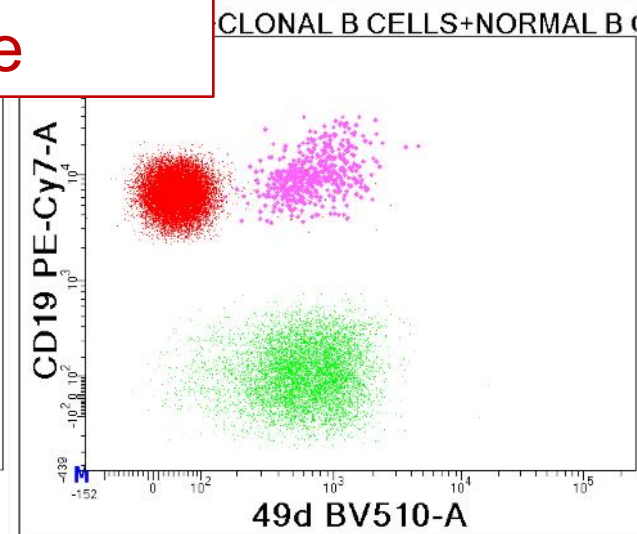
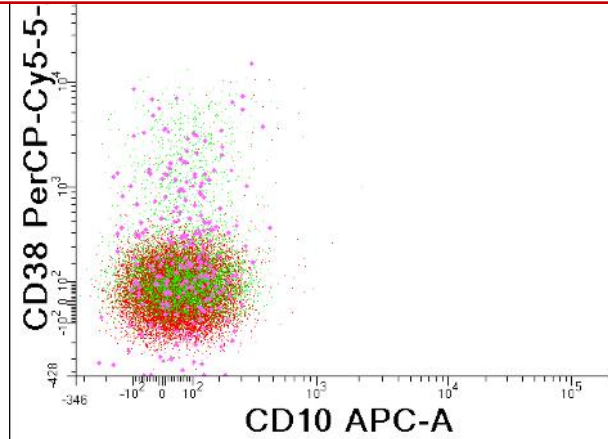
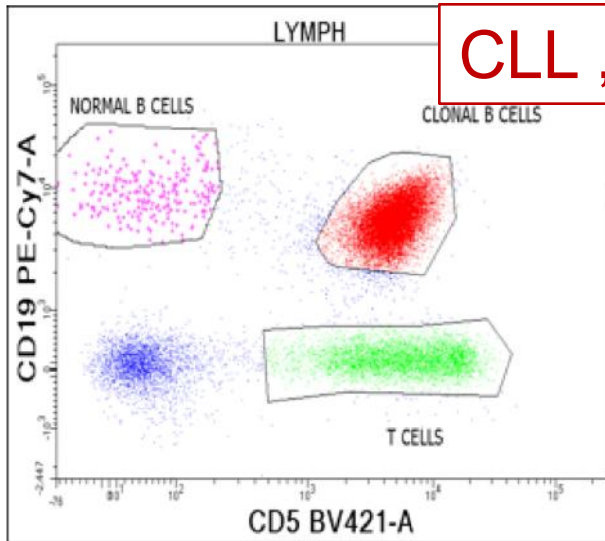


# Flow Analysis

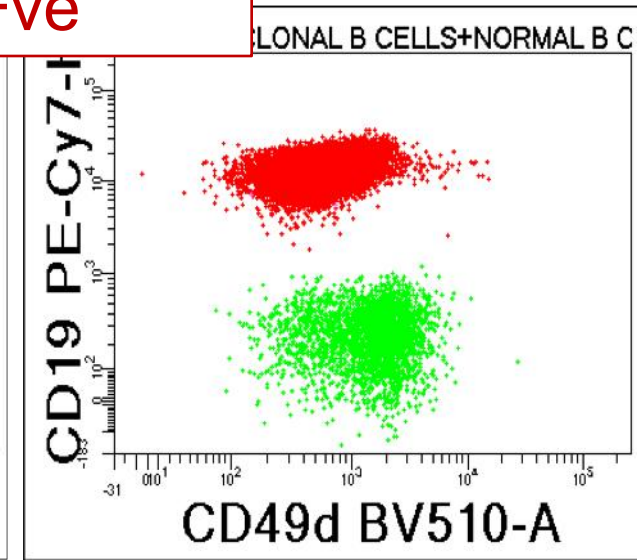
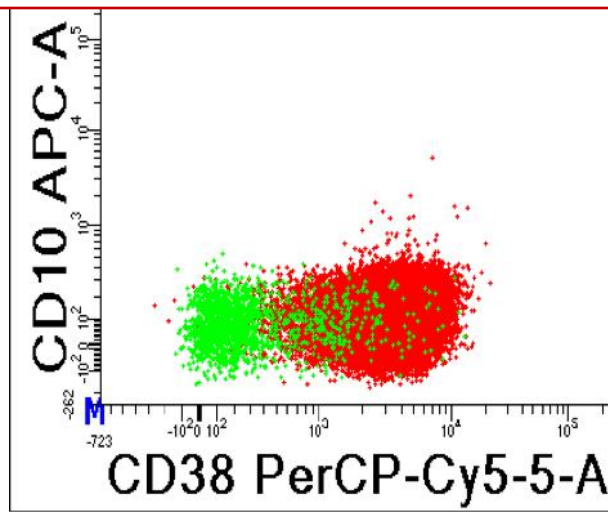
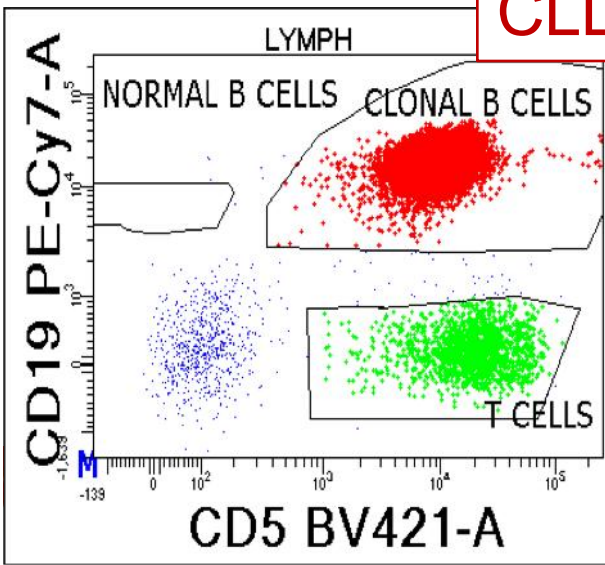


# CLL Prognostic Markers

CLL , CD38 -ve, CD49d -ve



CLL , CD38 +ve, CD49d +ve



# Follow Up

Main | Graph | **Cumulative** | Q-Flags | Service | Research

CBC

Numerical

Graph

## Cumulative Data

Date	05/10	11/05	02/06	07/06	
Time	10:22	10:51	18:30	10:45	
No.	14	10	39	19	
WBC	30.41	87.84	103.00	145.09	
RBC	4.30	4.08	3.58	3.40	
HGB	12.1	11.8	10.2	9.6	
HCT	39.1	38.2	33.5	33.0	
MCV	90.9	93.6	93.6	97.1	
MCH	28.1	28.9	28.5	28.2	
MCHC	30.9	30.9	30.4	29.1	
PLT	229	221	183	238	
RDW-SD	41.0	43.4	44.0	44.7	
RDW-CV	12.7	13.3	13.7	13.6	
PDW	13.6	12.7	13.5	13.3	
MPV	10.8	10.7	11.1	10.8	
P-LCR	31.8	31.2	32.2	30.5	



# FISH Reports

Sex/Age. : :Male /66 yrs

Category/Status. :F / Out Patient

Fax :

## **PRELIM. MOLECULAR CYTOGENETICS (FISH) REPORT**

Report Date : 13/10/2015

### **METHOD:-**

Direct Harvesting of Bone Marrow Aspirate/Peripheral Blood, Lymph Node Fluorescence in situ hybridization on interphase and metaphase cells

### **TEST:-**

Monosomy 13/13q14, TP53(17p13), ATM (11q22), MYB(6q21) deletion Analysis

### **PROBES/PROBE PANEL:-**

Kreatech LSI ATM/CEP 11, LSI 6q21/CEP 6, Zytovision LSI 13q14, TP53/CEP17 probes (Limit of detection: >=5%).

### **NUMBER OF CELLS ANALYZED:-**

200

### **RESULT:-**

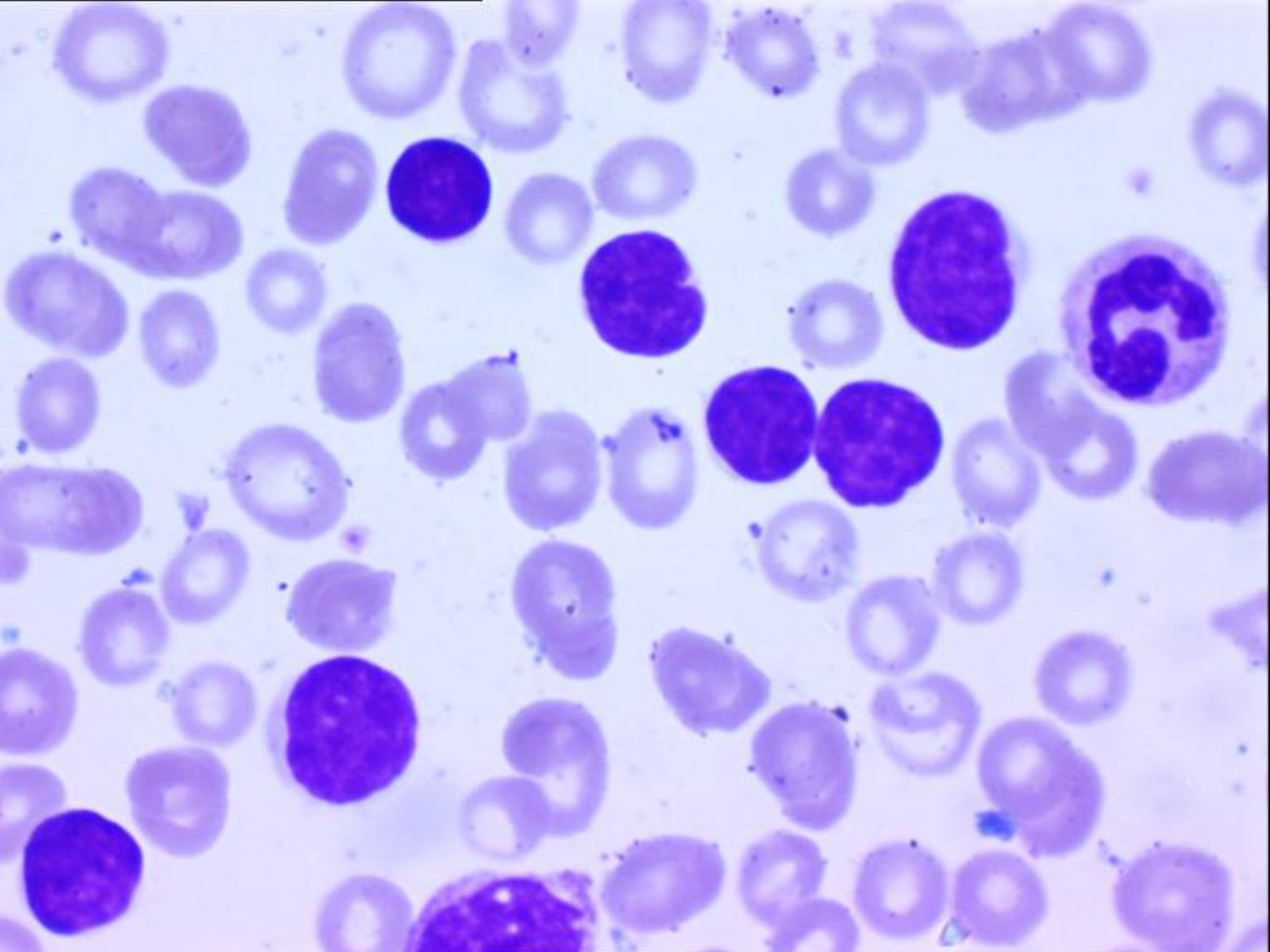
#### **Monosomy 13/13q14, TP53(17p13), ATM (11q22), MYB(6q21) deletion Analysis**

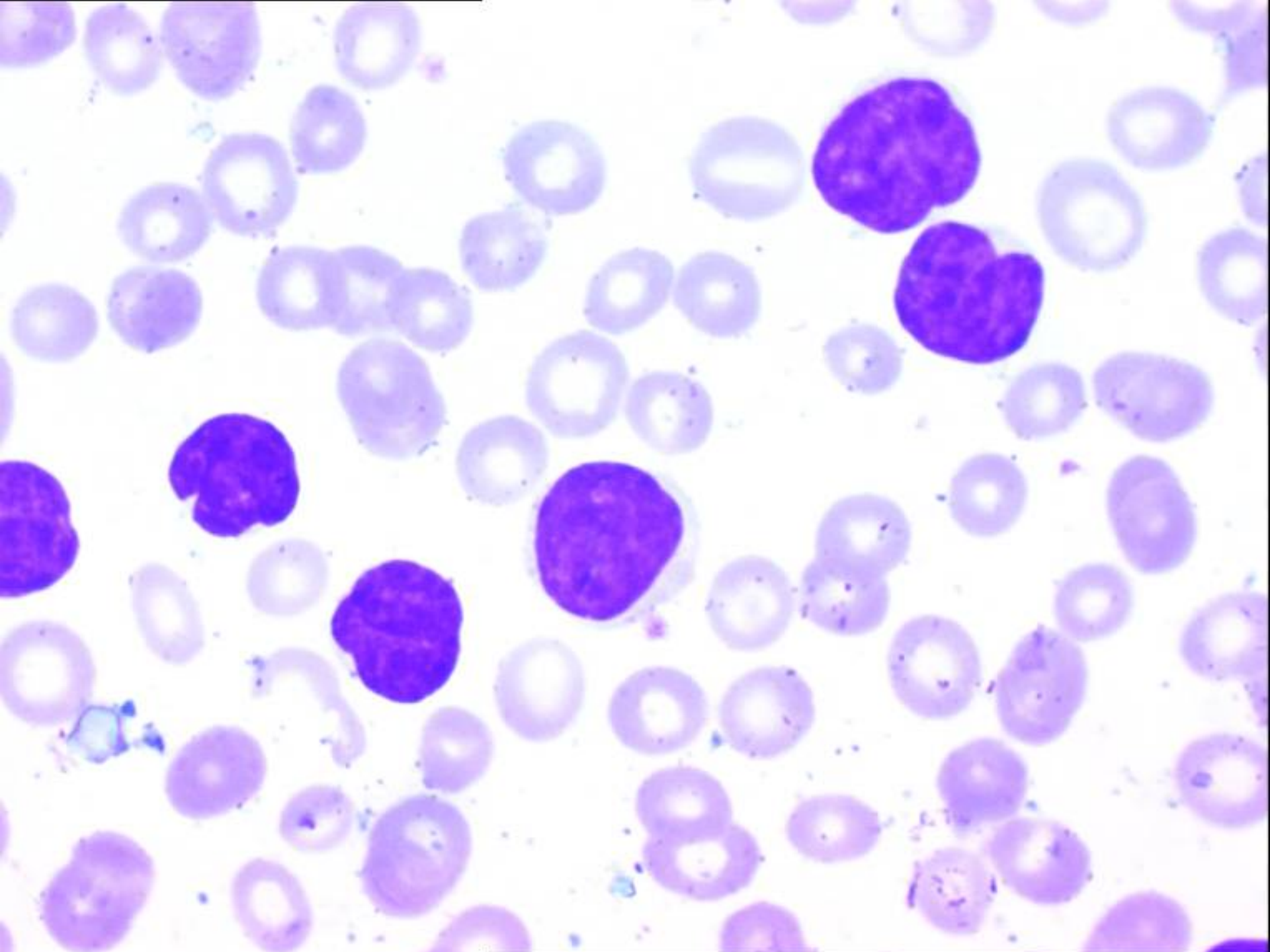
	ATM deletion	ATM(Orange)	CEP11(Green)	No. Cells
	Signal/s/Cell	1	2	180/200
	De(6q)	MYB(Orange)	CEP6(Green)	No. Cells
	Signal/s/Cell	2	2	200
Signal/s in cell	-13q deletion/ - 13	D13S319(Orange)	13q34(Green)	No. Cells
	Signal/s/Cell	2	2	200
	TP 53 deletion	TP53 (Orange)	CEP17(Green)	No. Cells
	Signal/s/Cell	2	2	200

### **IMPRESSION:-**

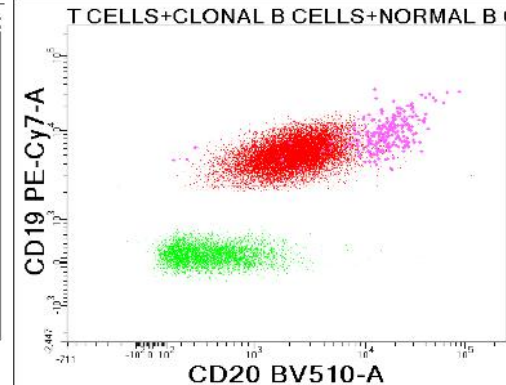
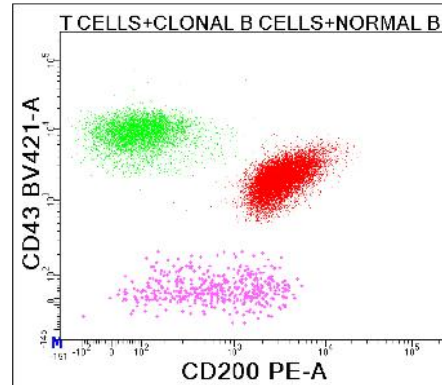
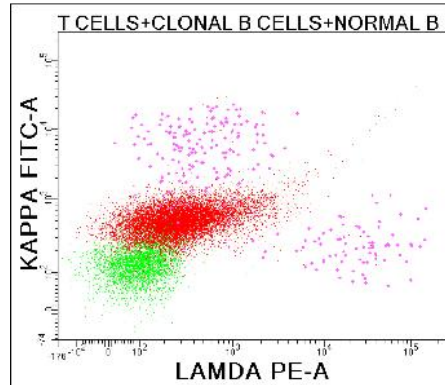
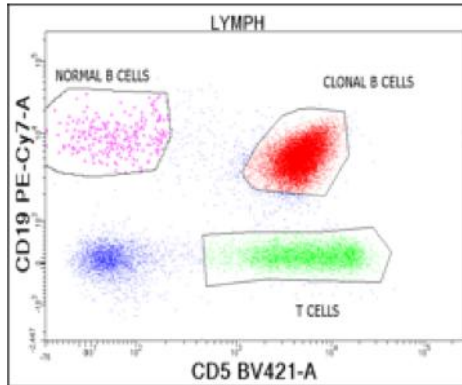
Signal pattern showed evidence of heterozygous ATM(11q) deletion in 90% cells . There was no evidence of MYB(6q) deletion , 13q deletion & TP53 deletion





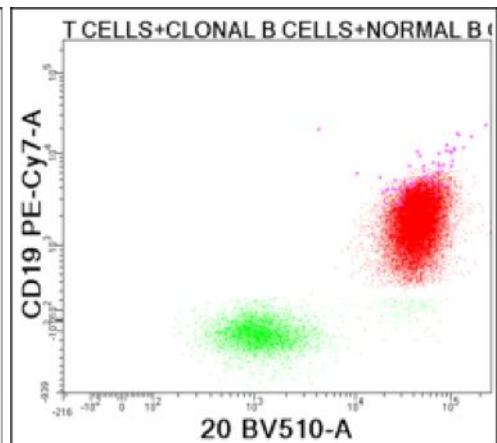
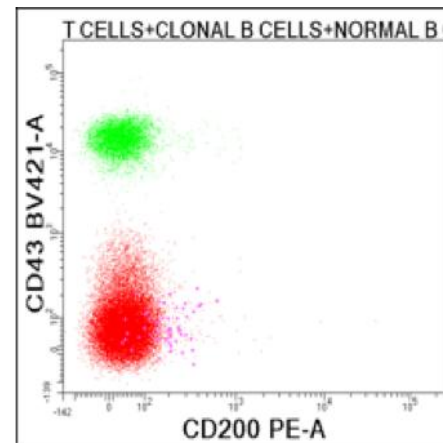
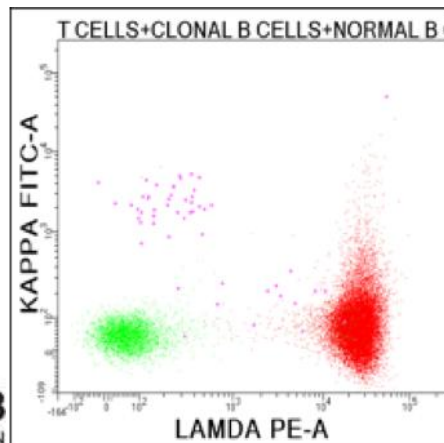
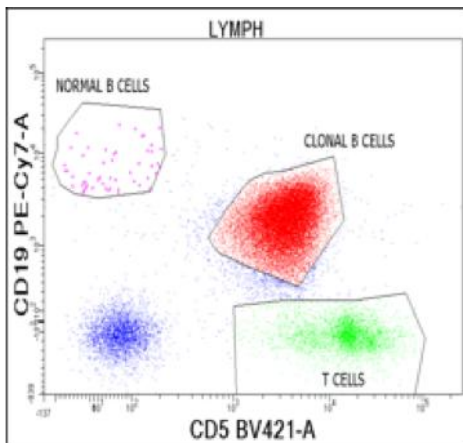


# CLL vs Mantle cell lymphoma

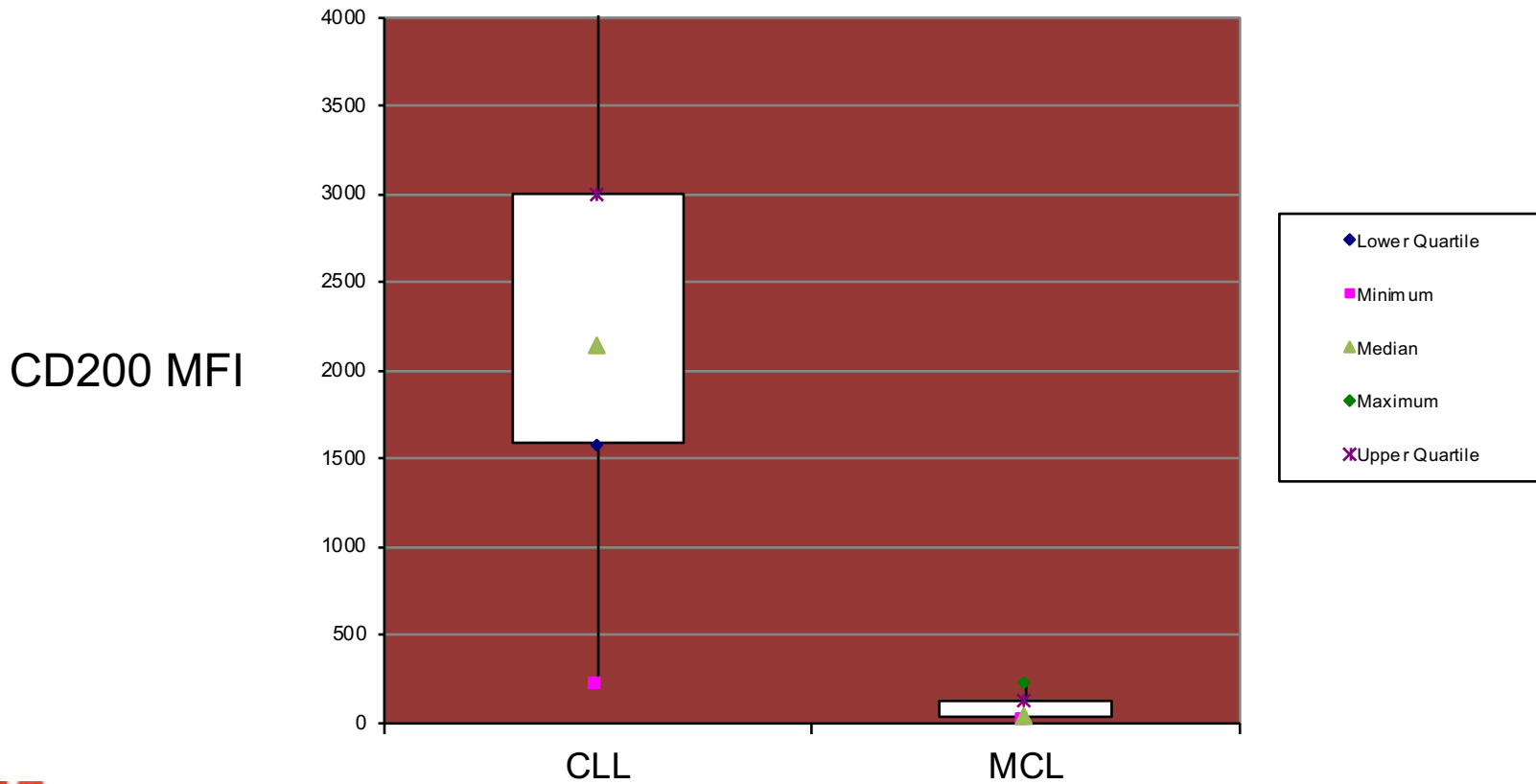


CLL

# Mantle cell lymphoma



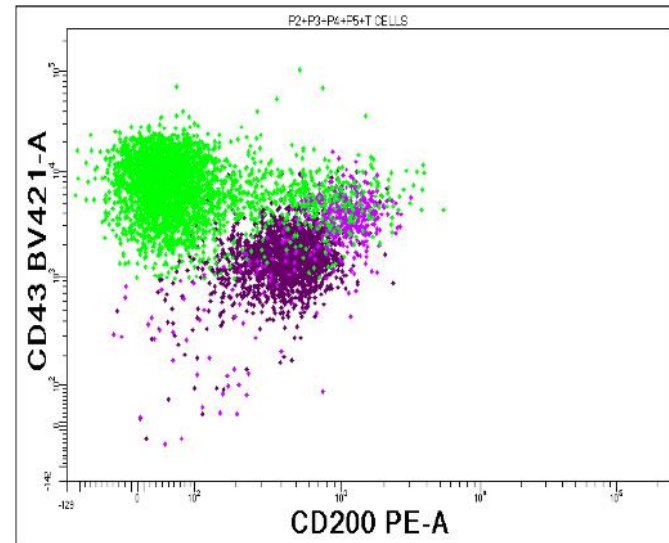
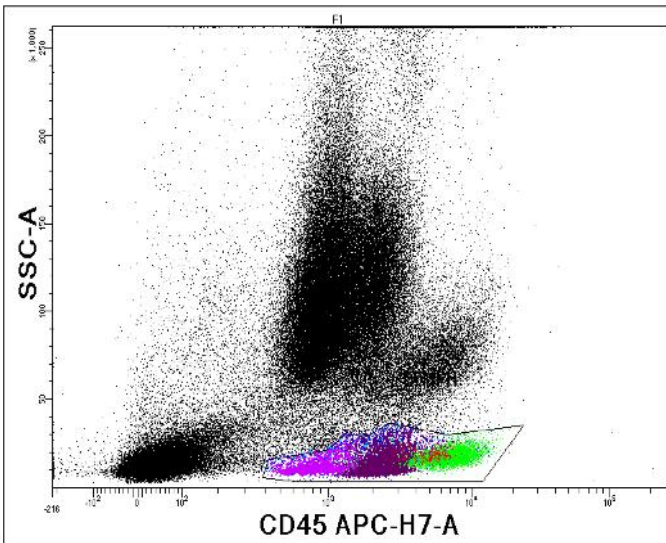
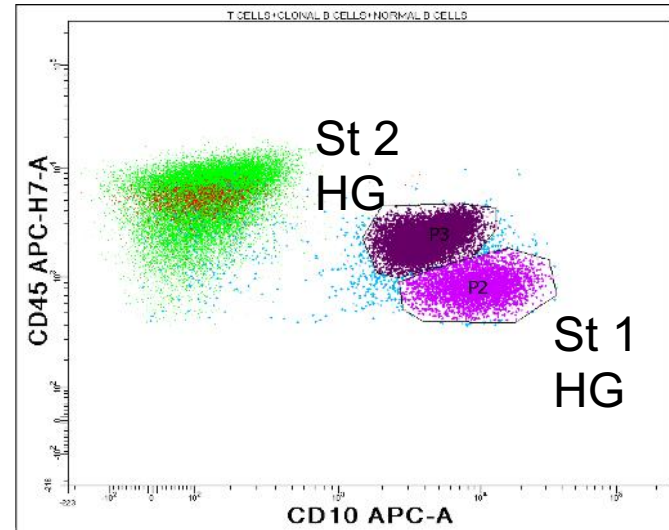
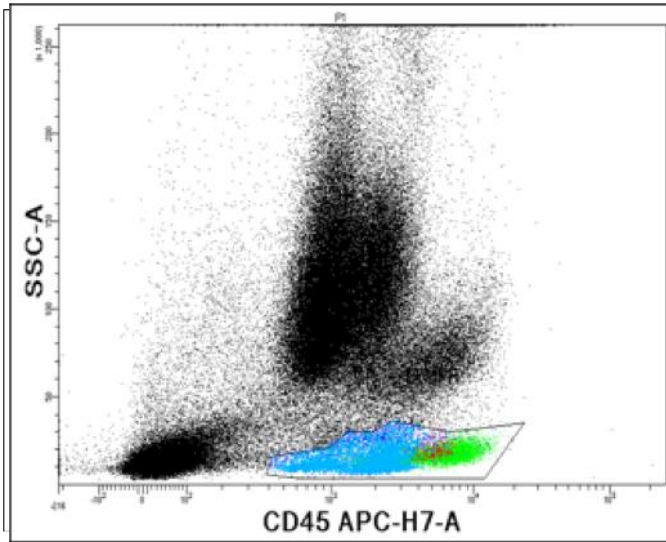
# CD200 MFI- CLL vs Mantle cell lymphoma



# CD200 MFI in CLPDs

DISEASE	TOTAL (N)	MFI MEDIAN (RANGE)	MFI NORMALIZED RATIO
<b>MBL - CLL</b>	6	<b>2035 (60 – 4503)</b>	<b>34.46</b>
<b>CLL</b>	51	<b>2148 (223 - 10043)</b>	<b>38.41</b>
MANTLE CELL LYMPHOMA	8	48 (24 – 243)	1.17
SMZL	18	338 (124 – 2377)	7.34
FOLLICULAR LYMPHOMA	2	731 (297 – 1165)	12.06
<b>HCL</b>	3	<b>3099 (3015 – 3900)</b>	<b>56.52</b>
HCL-V	2	562 (450 – 674)	8.03
LPL	8	609 (108 – 2505)	9.55

# CD200 expression in Hematogones



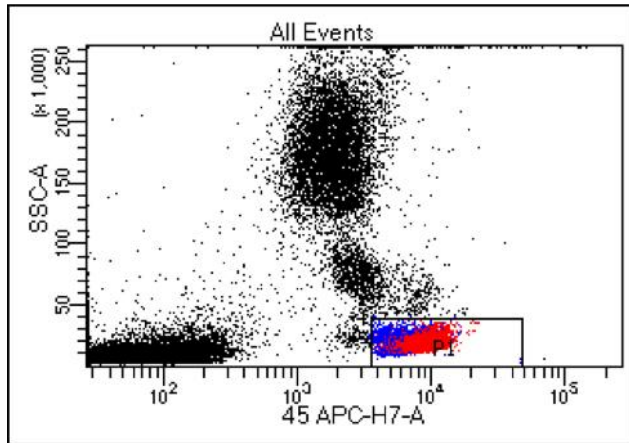
# Case

- 72/M
- Persistent lymphocytosis with mild anemia
- Hb-11.2, MCV-97
- WBC-4850/ul ( previous WBC – 4100 to 7300)
- Lymphocytes-50% ( previous Lym % – 42% to 58%)
- Platelets- 1.80 lacs
  
- Evaluated by Flow Cytometry

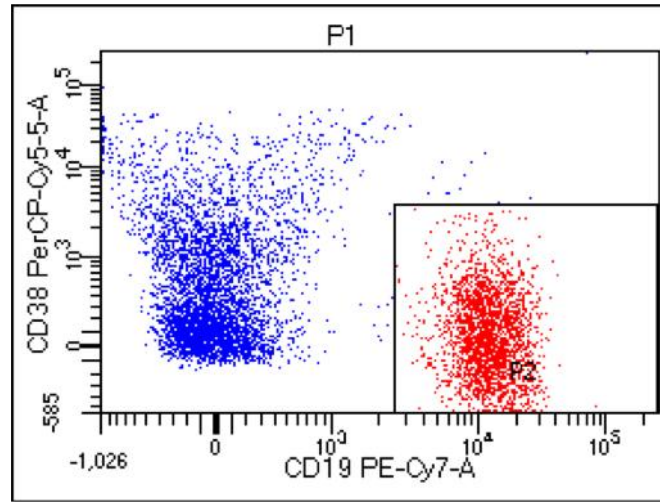




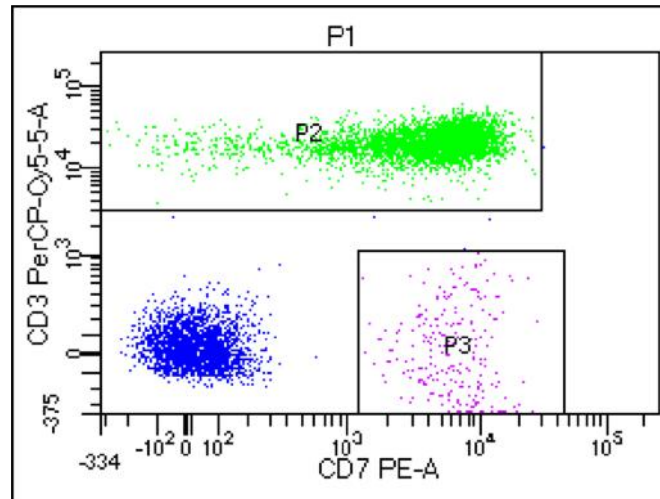
# FLOW PLOTS



T CELLS -60% OF ALL LYMPHOCYTES



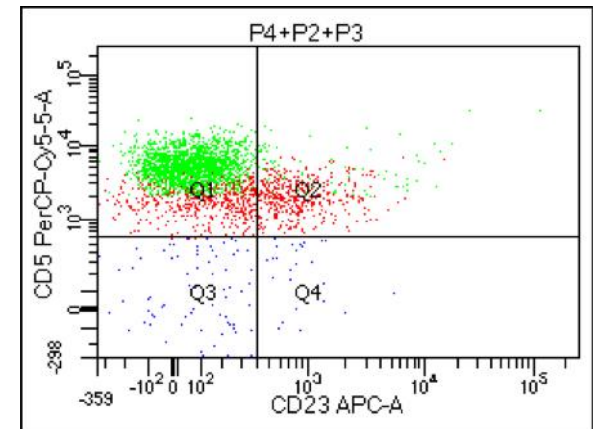
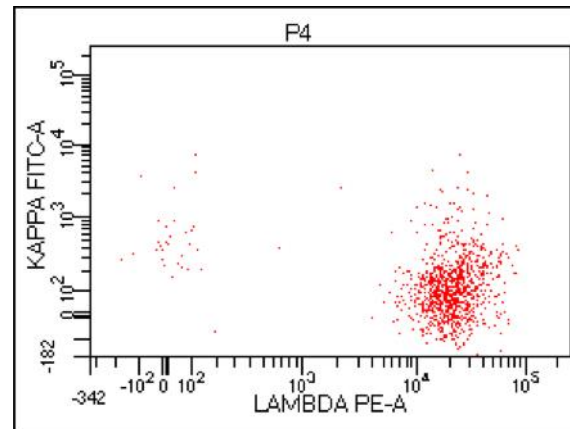
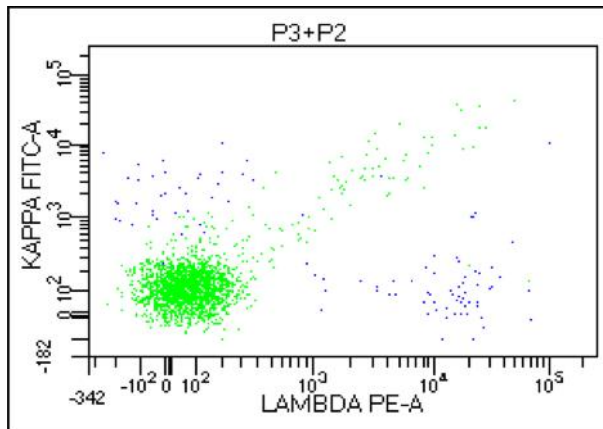
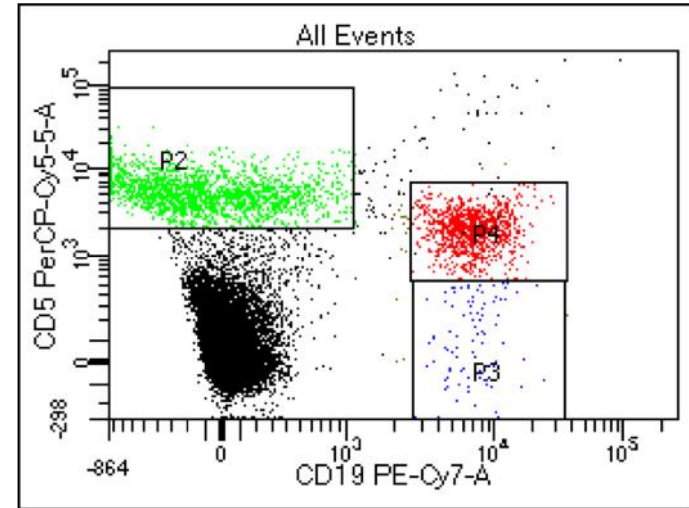
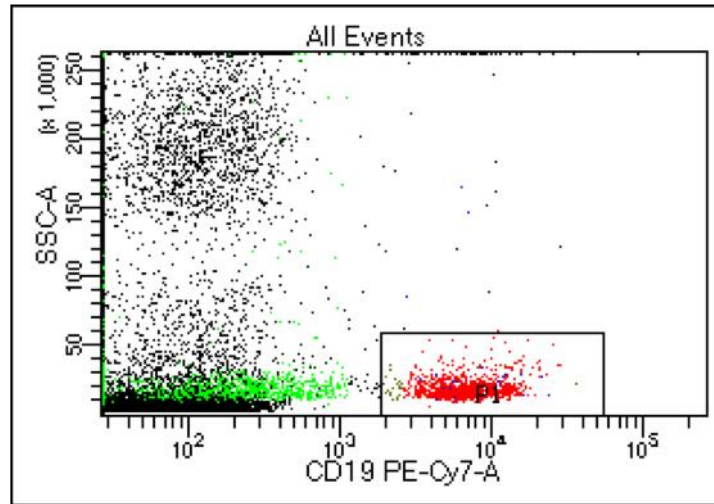
B CELLS -36% OF ALL LYMPHOCYTES



NK CELLS -04% OF ALL LYMPHOCYTES



# FLOW PLOTS



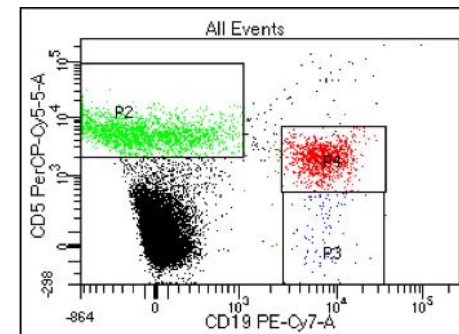
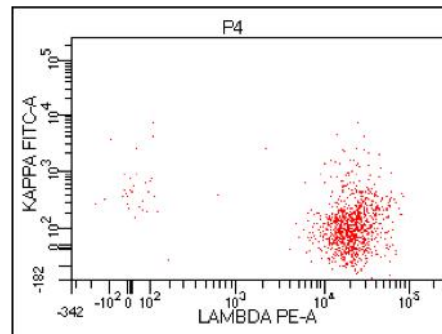
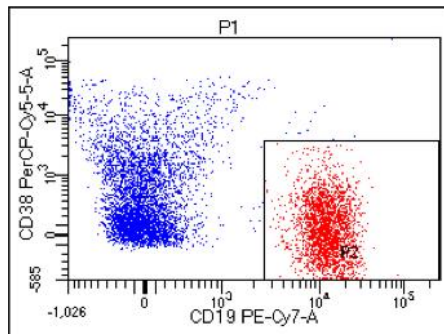
# Monoclonal B Cell Lymphocytosis

The Automated Total WBC Count - from the Analyser is **4850 cells/ul**  
Absolute lymphocyte count is 2425 cells/ul (**50% of WBCs**).

T cells are 60% of all lymphoid cells (1455 cells/ul),  
NK cells are 4% of all lymphoid cells ( 97cells/ul) and  
**B cells are 36% of all lymphoid cells (873 cells/ul).**

94% of all B cells show a lambda restricted clonal population. **The absolute clonal B cell population is 821 cells/ul.**

As per the IW-CLL criteria, with the **clonal B cell population being less than 5000cell/ul**, the above population fits into the **diagnosis of a Monoclonal B Cell (MBL) population.**



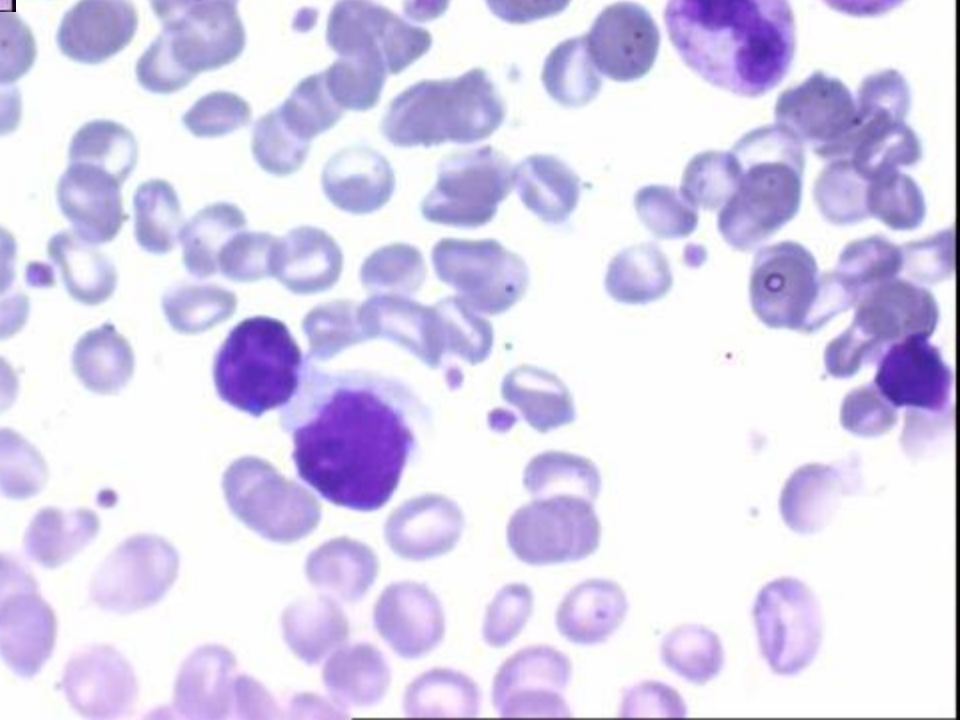
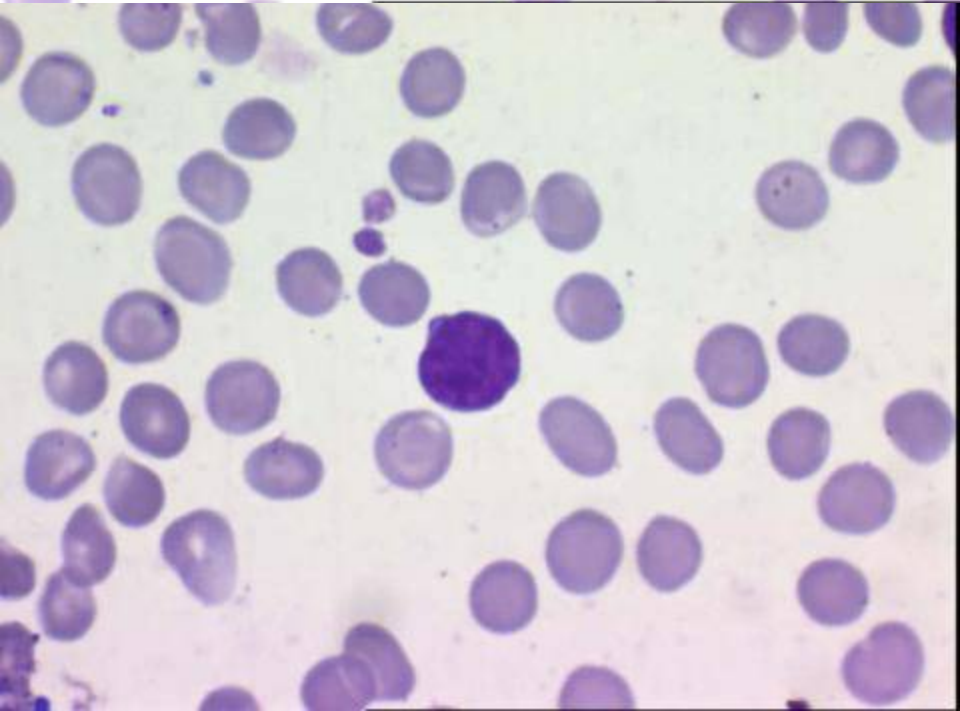
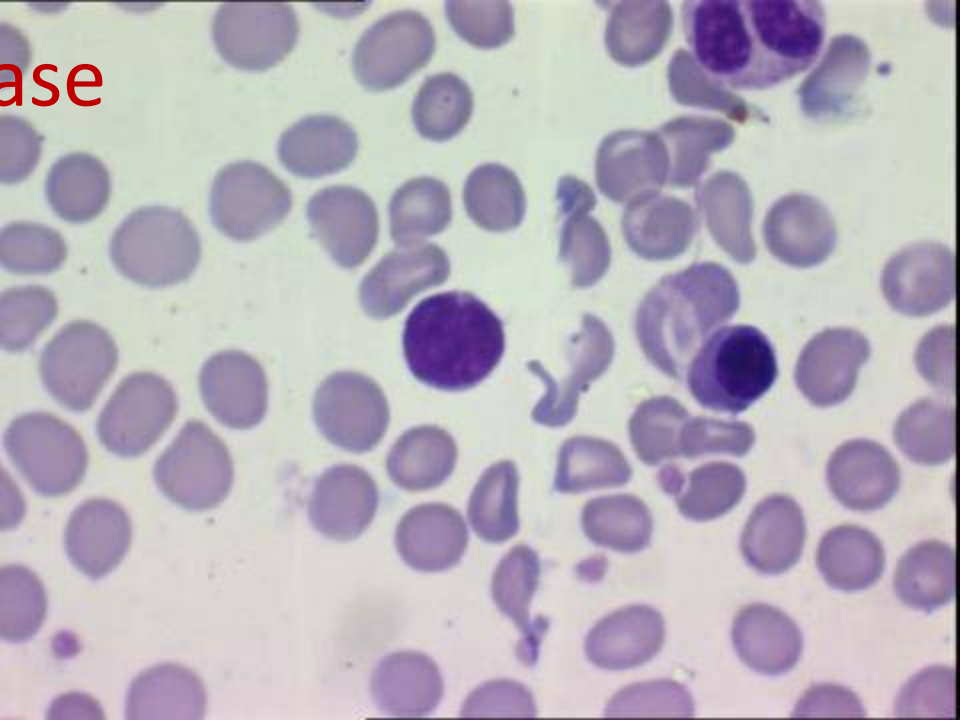
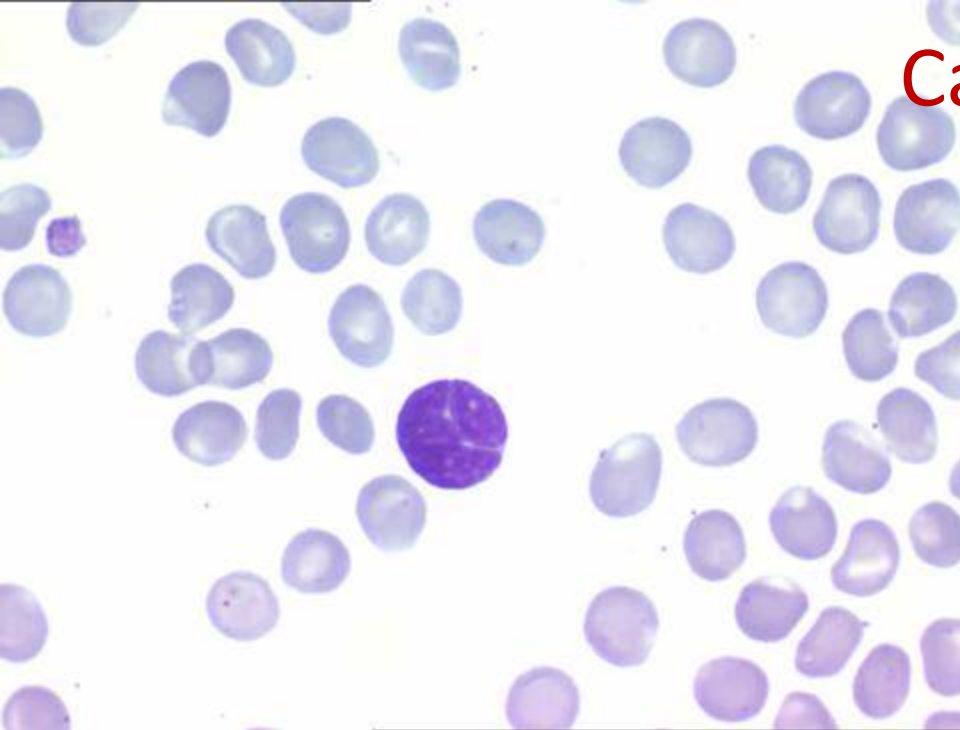
# Diagnostic criteria for MBL – (Shanafelt et al. Leukemia 2010)

- (1) Documentation of clonal B-cell population by one or more of following:
  - (a) Light chain restriction:  
Overall kappa: lambda ratio 3:1 or 0.3:1 or more than 25% of B cells lacking or expressing low-level surface Immunoglobulin
  - (b) Heavy chain monoclonal IGHV rearrangements
- (2) Presence of a disease-specific immunophenotype e.g. CLL like phenotype, atypical CLL or Non CLL phenotype
- (3) Absolute B-cell count less than  $5 \times 10^9$  cells/l
- (4) No other features of a lymphoproliferative disorder or autoimmune disease
  - (a) Normal physical exam (no lymphadenopathy or organomegaly)
  - (b) Absence of B-symptoms (for eg-, fatigue, weight loss and night sweats) attributable to NHL
  - (c) No autoimmune/infectious disease

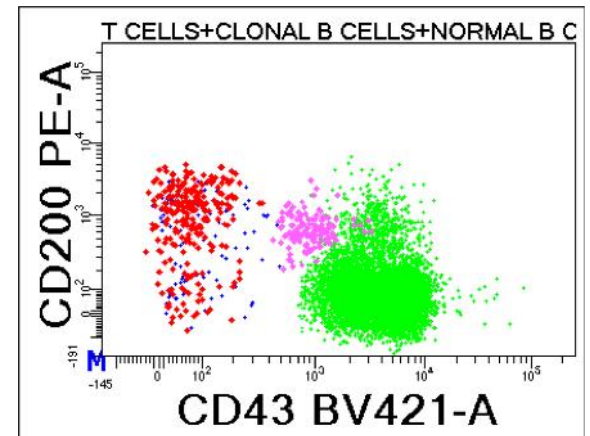
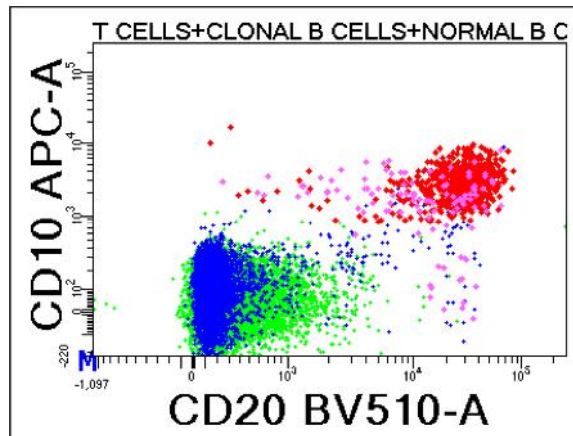
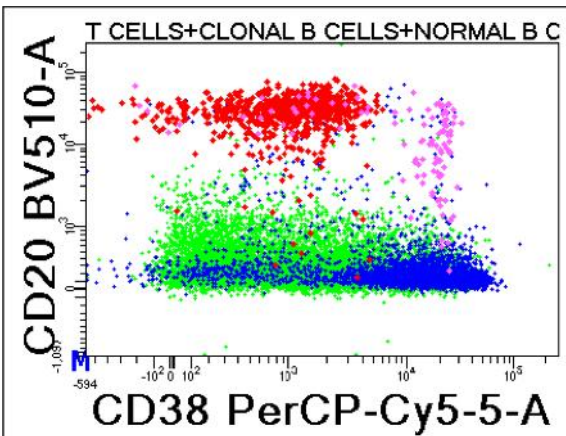
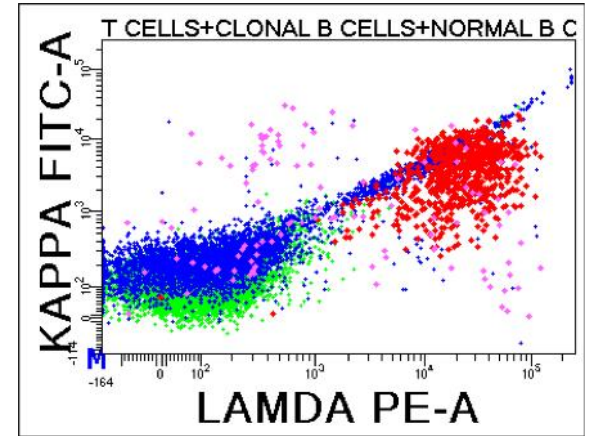
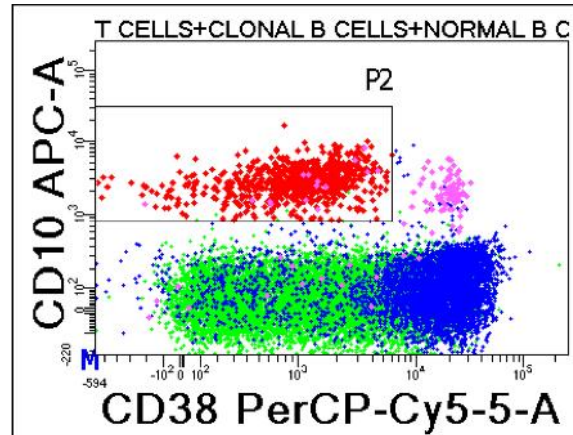
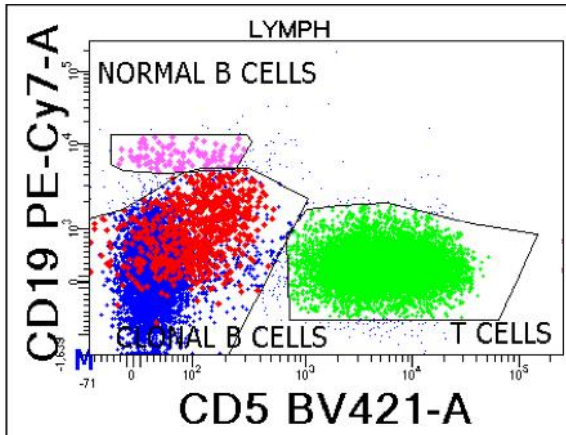
Where possible, repeat assessment should show the monoclonal B-cell population is stable over 3-month period.



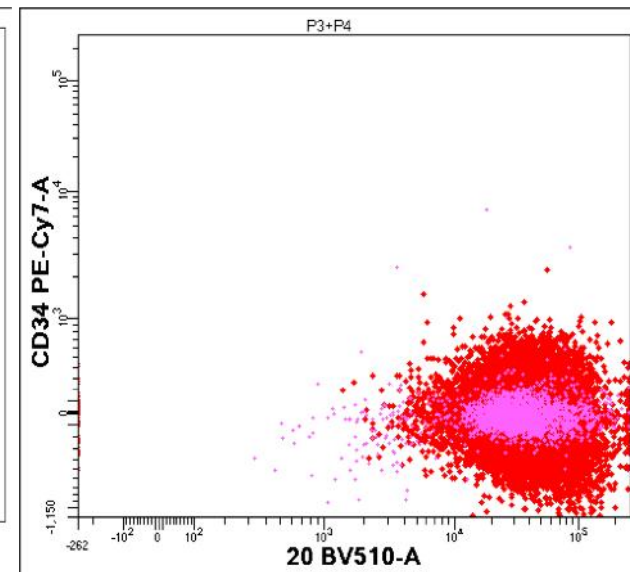
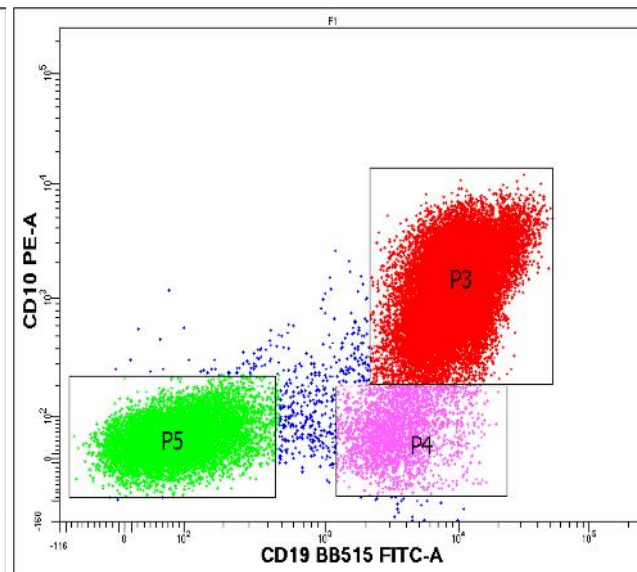
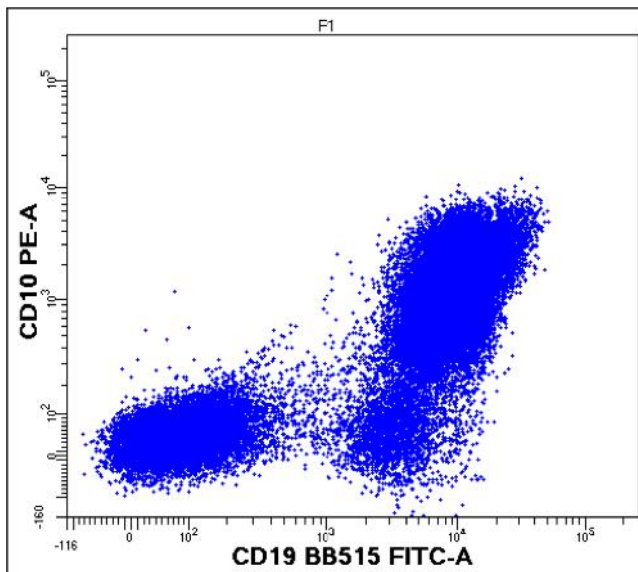
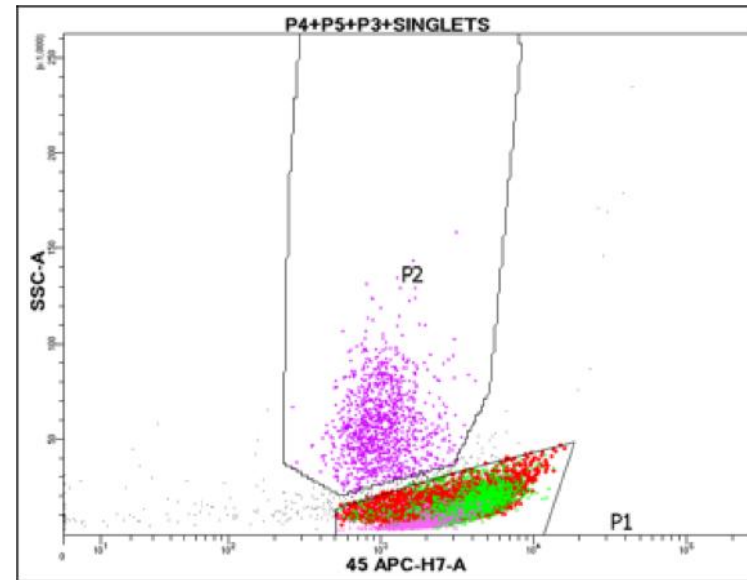
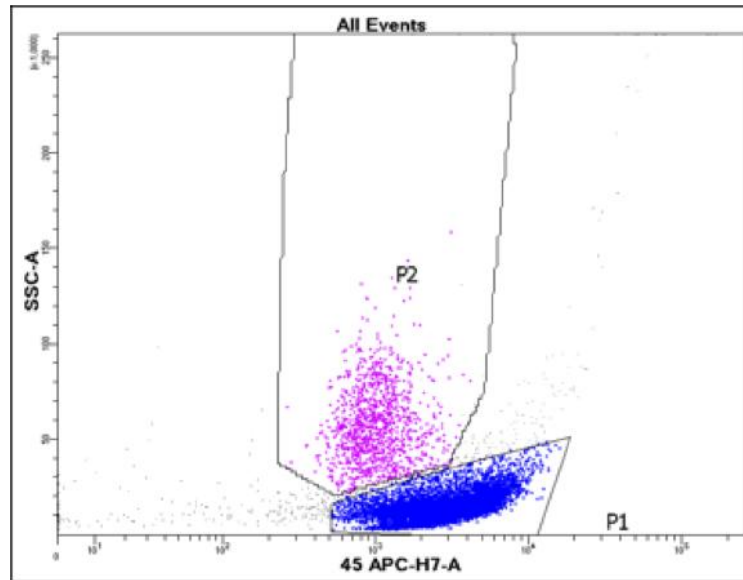
Case



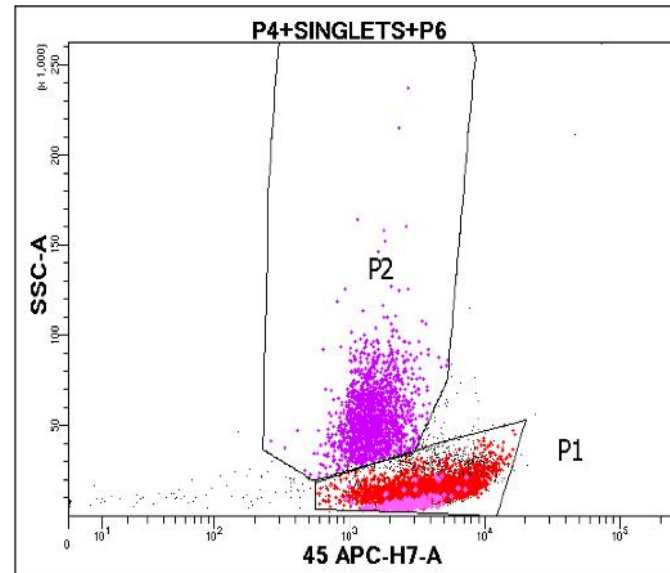
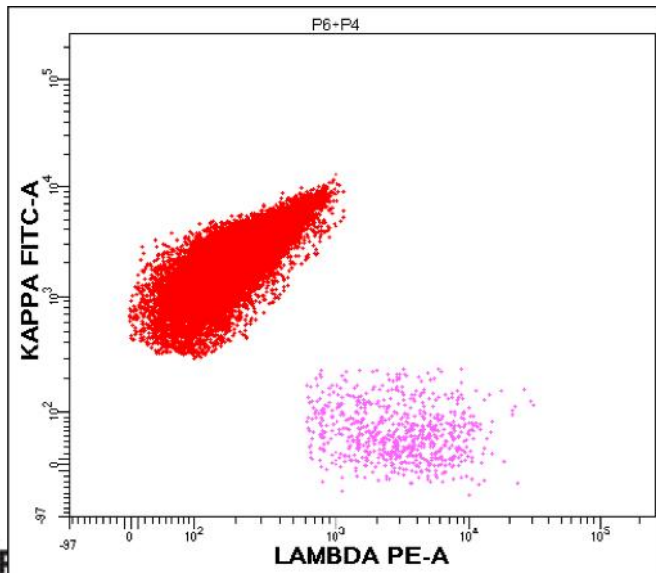
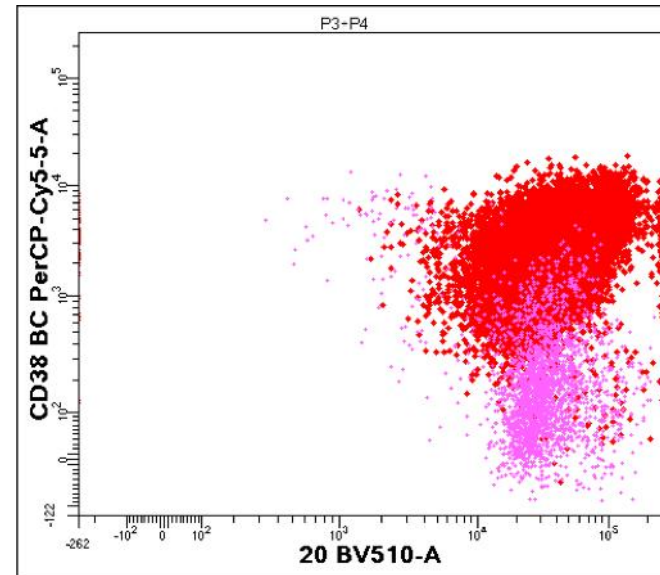
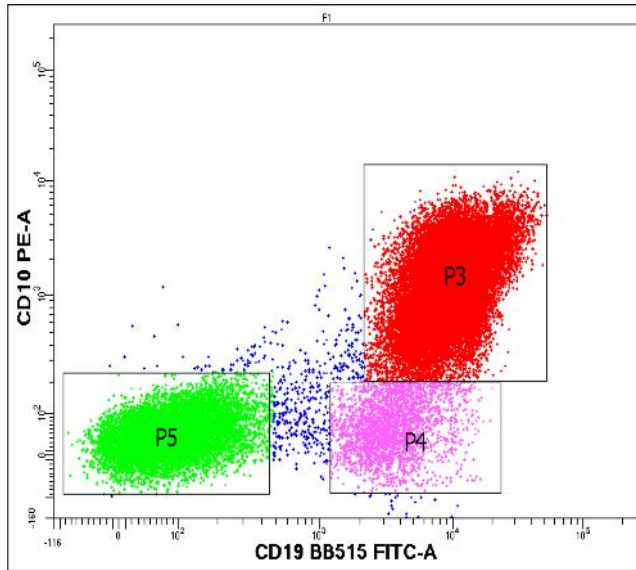
# Follicular Centre Cell Lymphoma



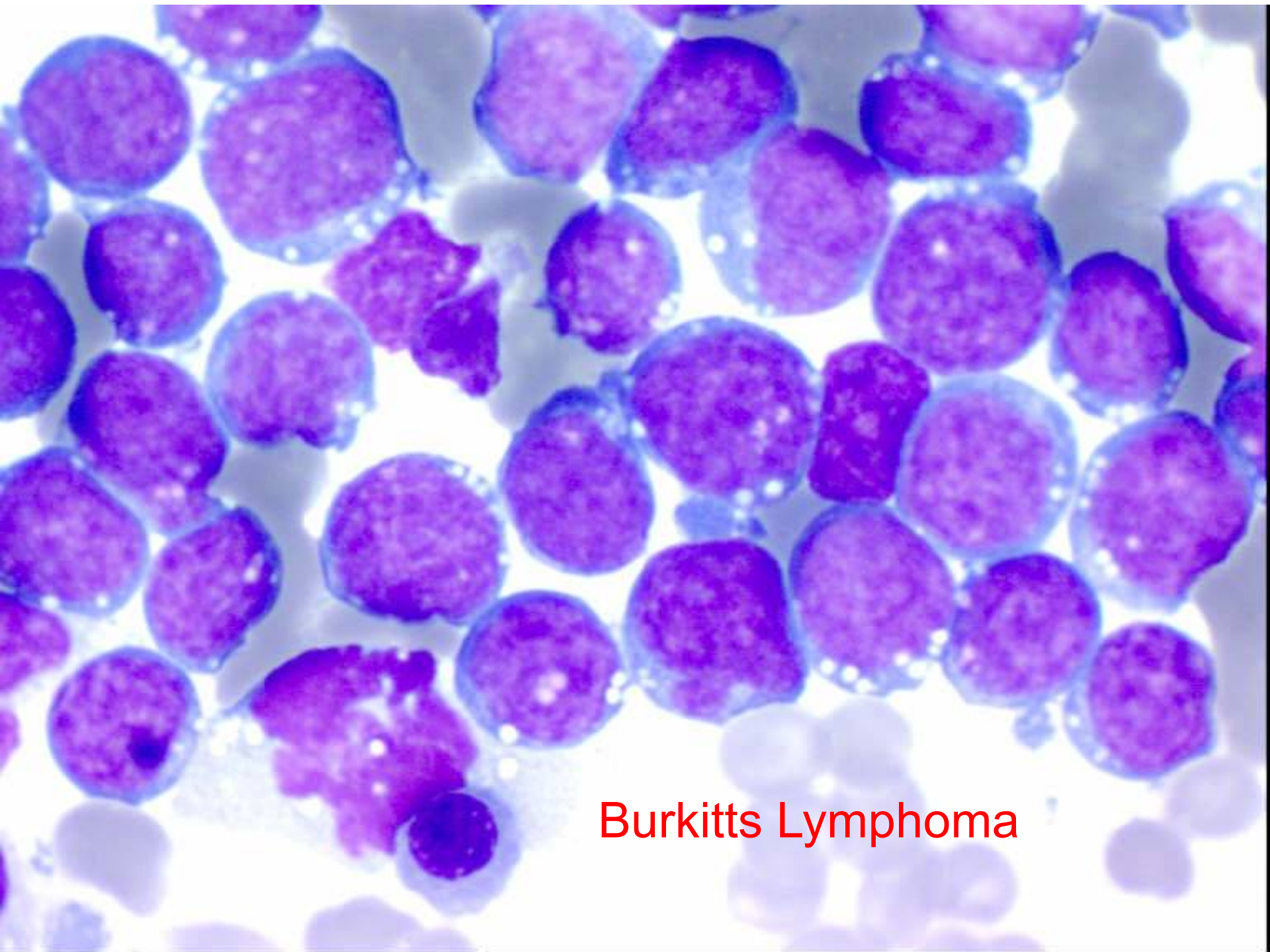
# Case – 80% blasts – AL Panel



# High Grade B cell Lymphoma

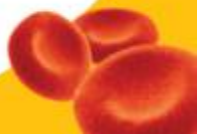
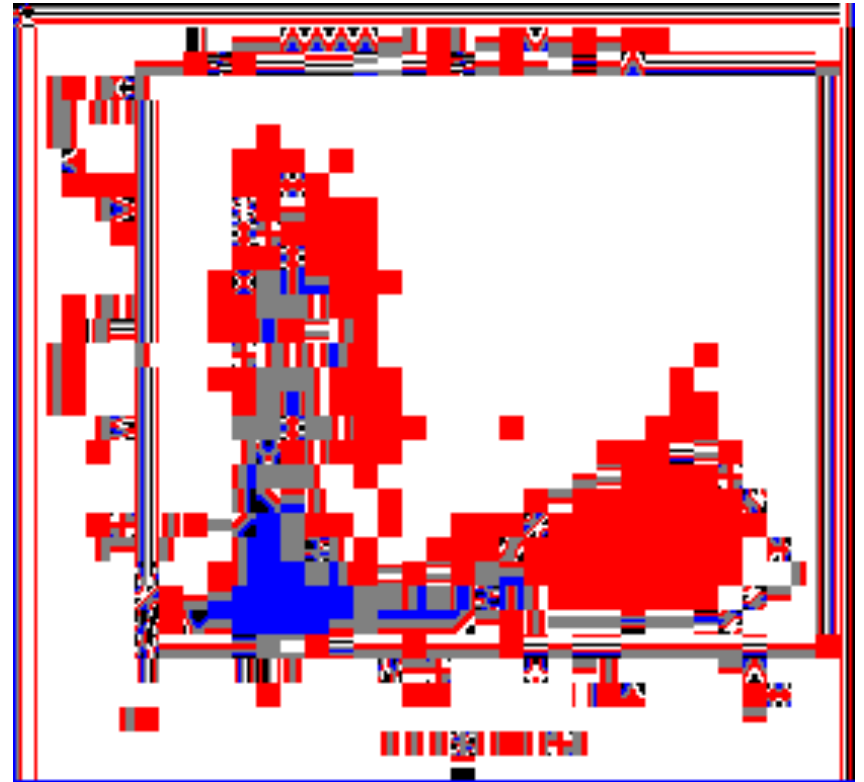
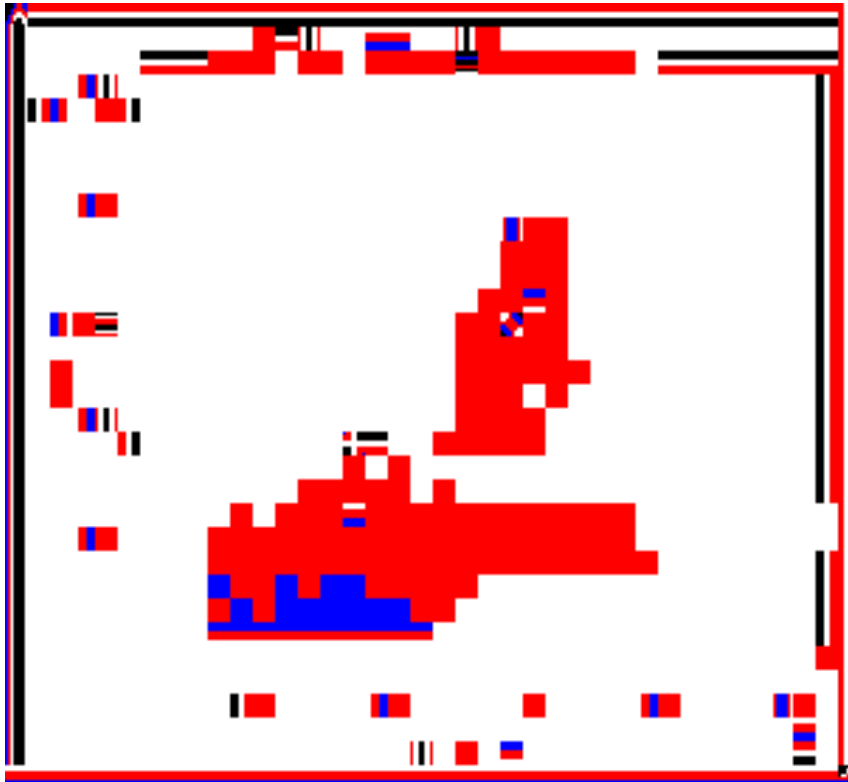


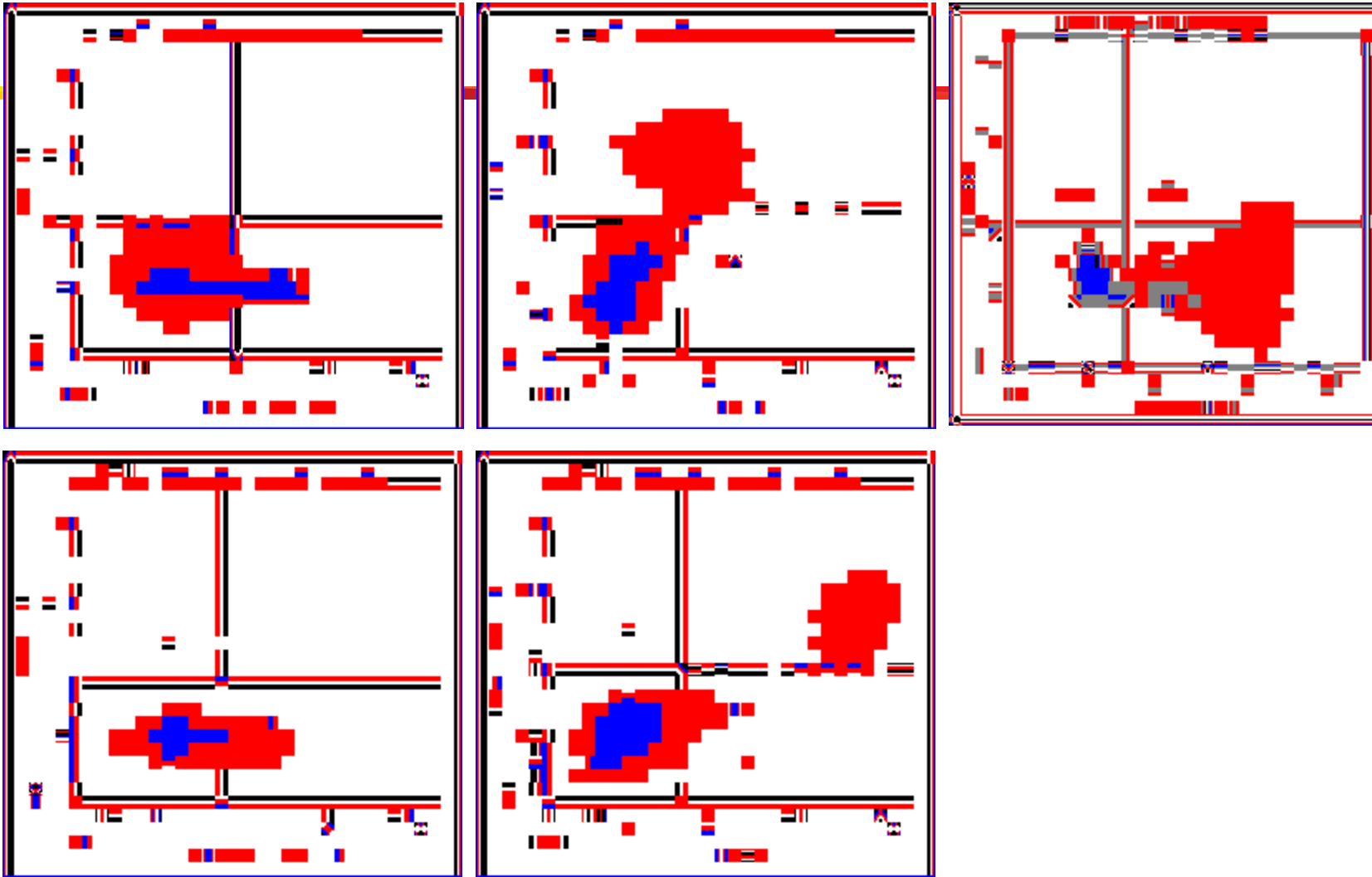




Burkitts Lymphoma

# Case

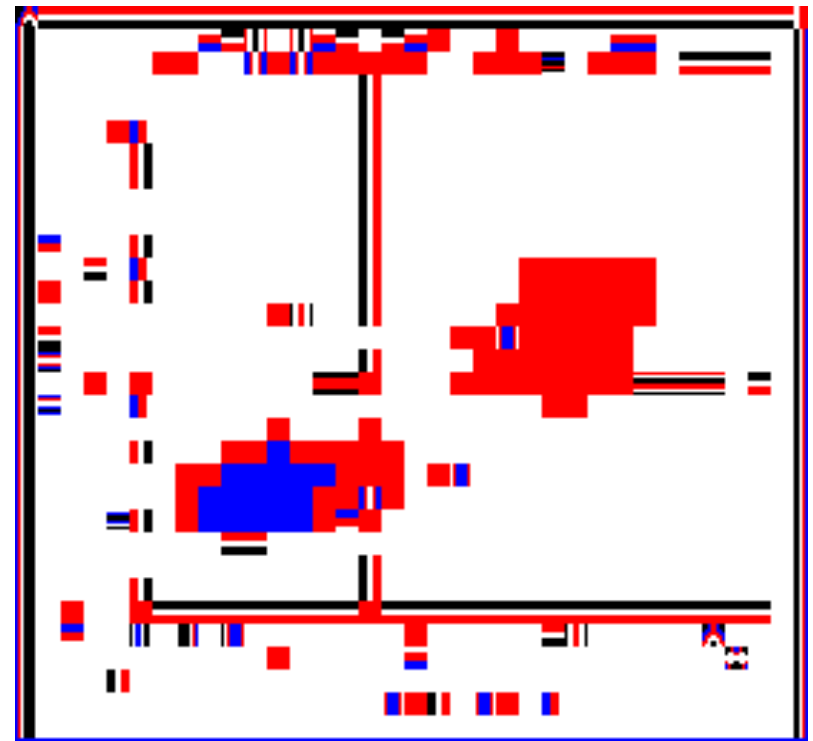
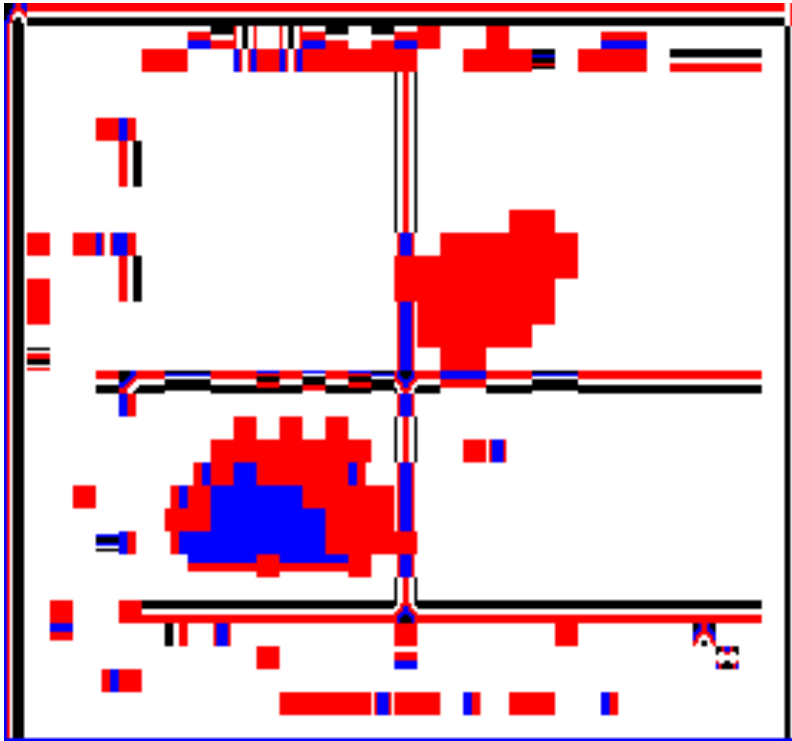


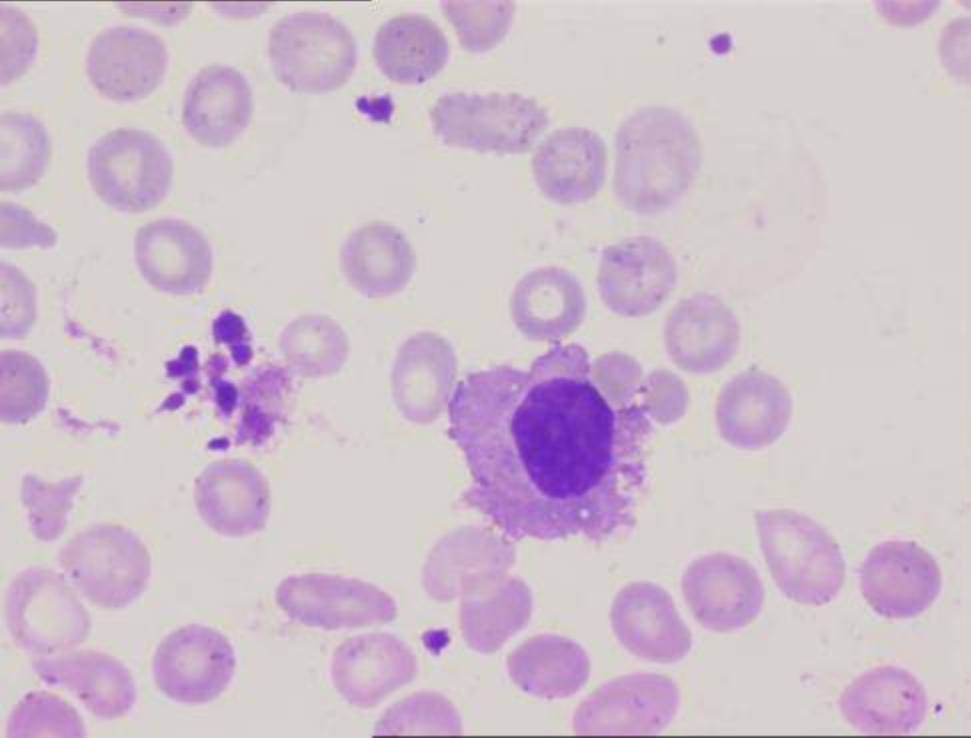


CD 5 -ve CD 10-ve B Cell NHL

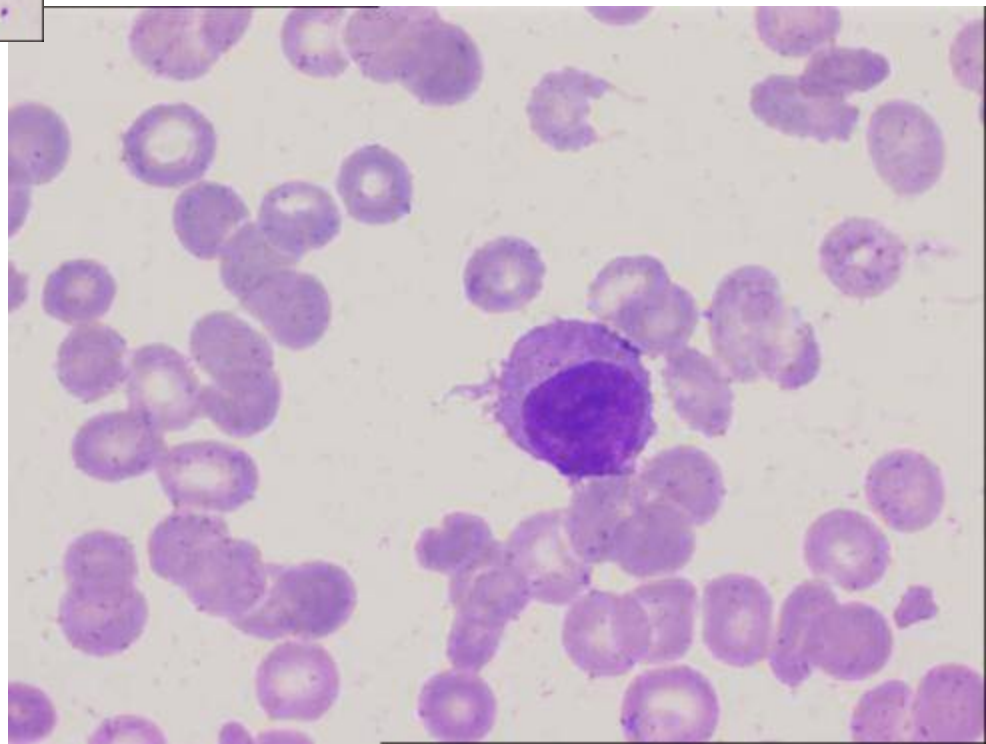


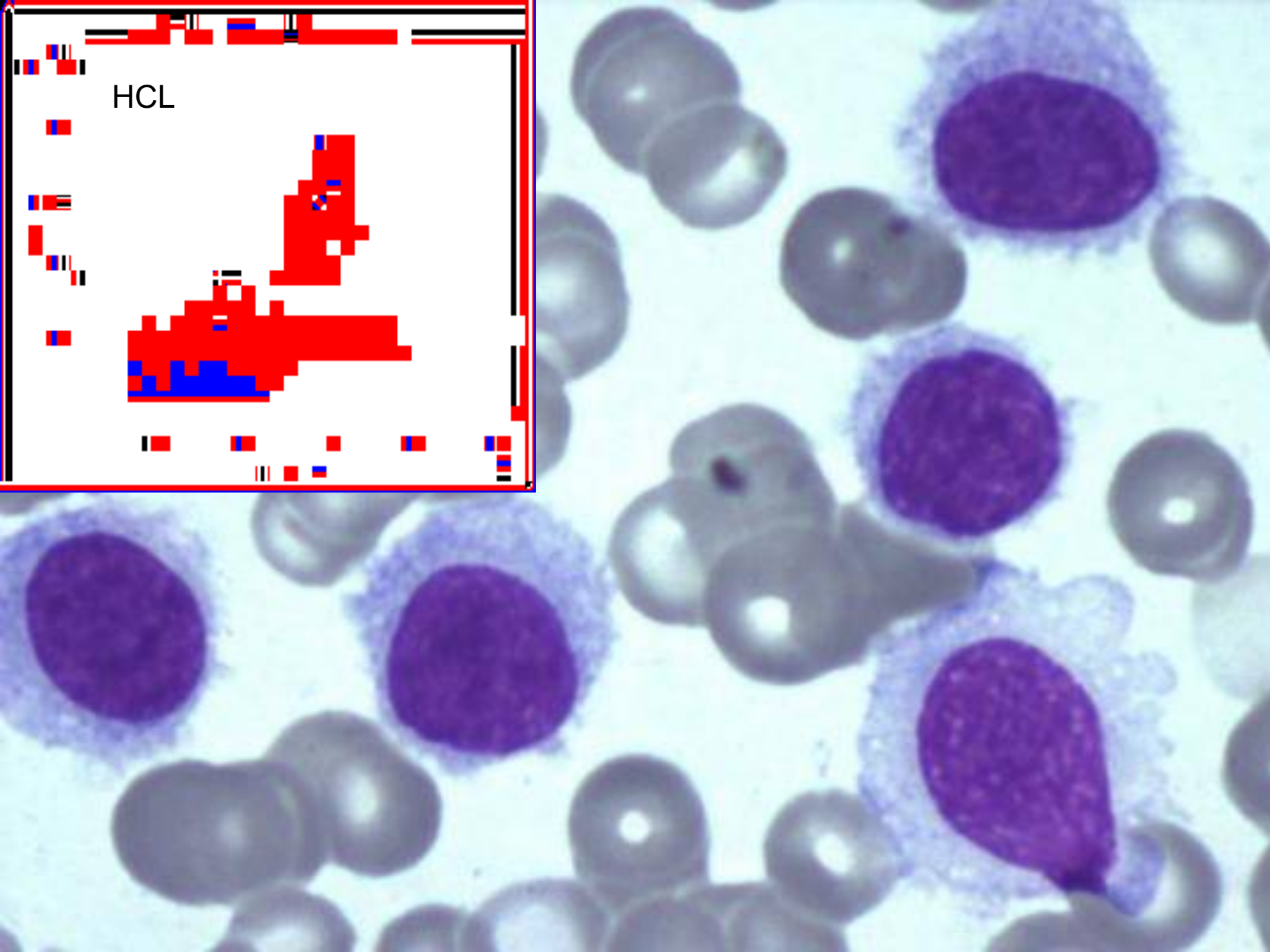
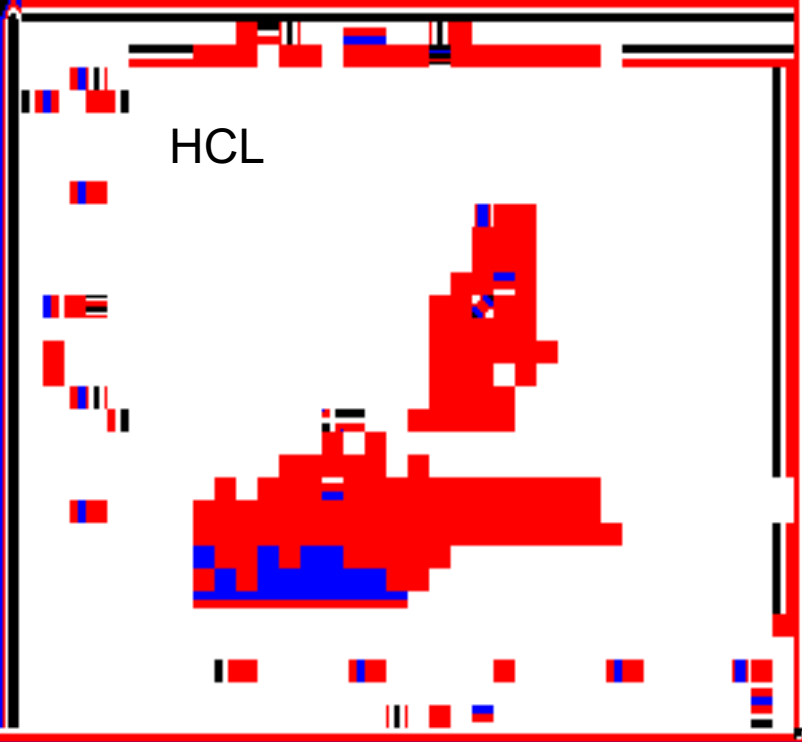
# Additional Markers





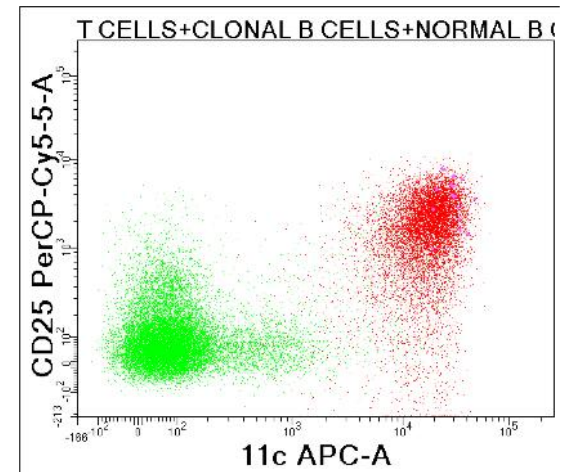
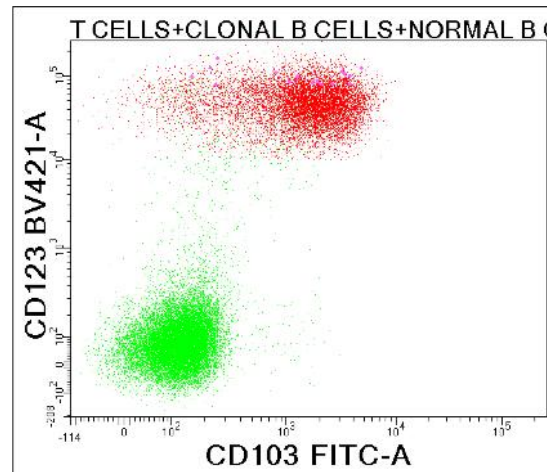
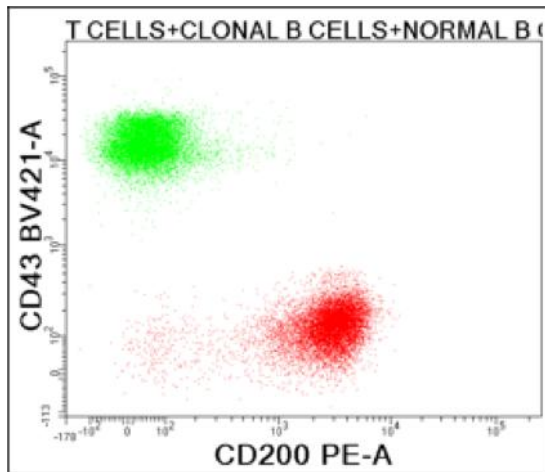
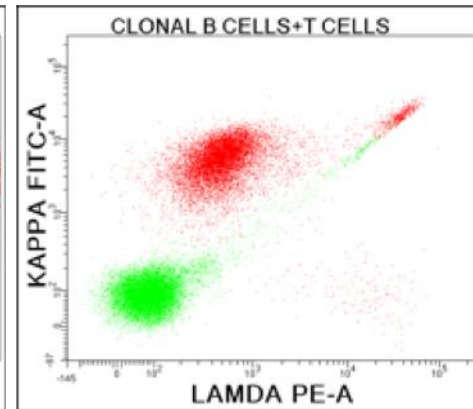
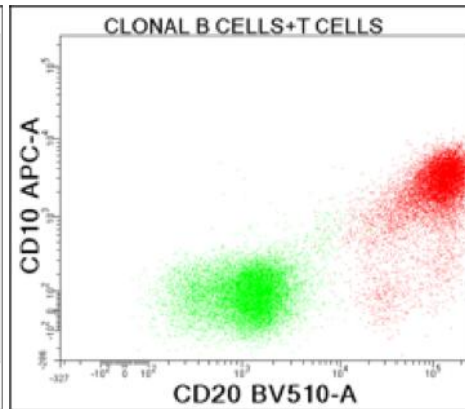
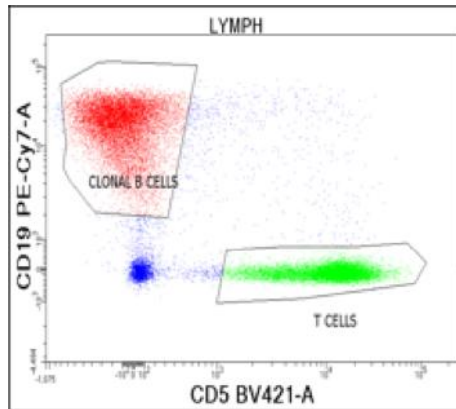
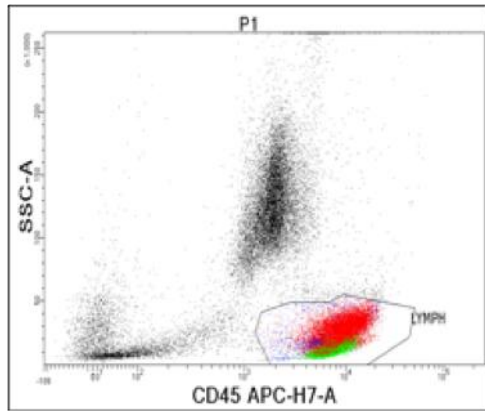
## Peripheral Smear



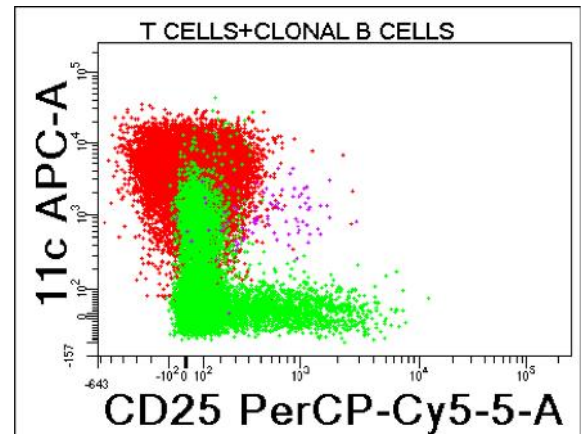
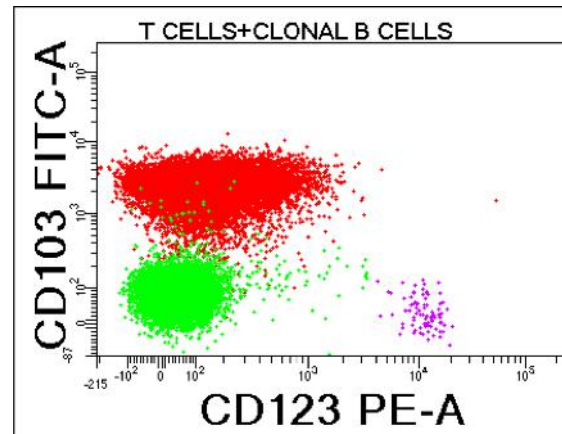
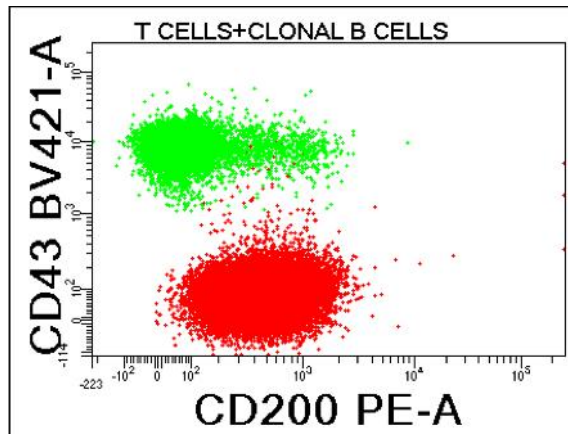
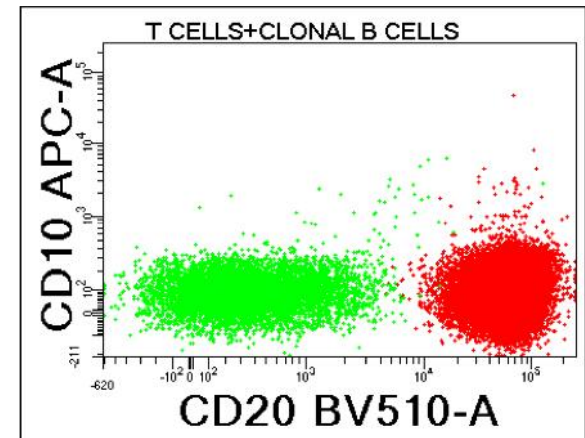
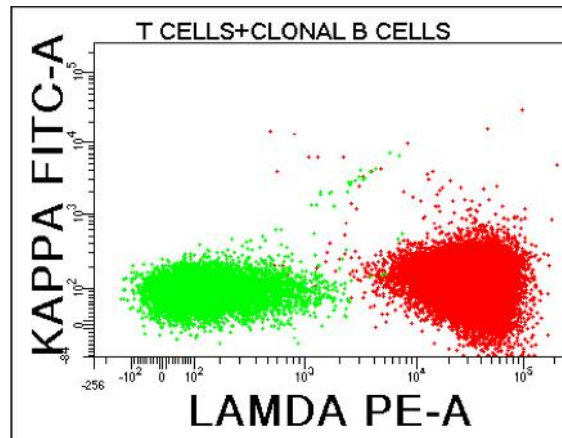
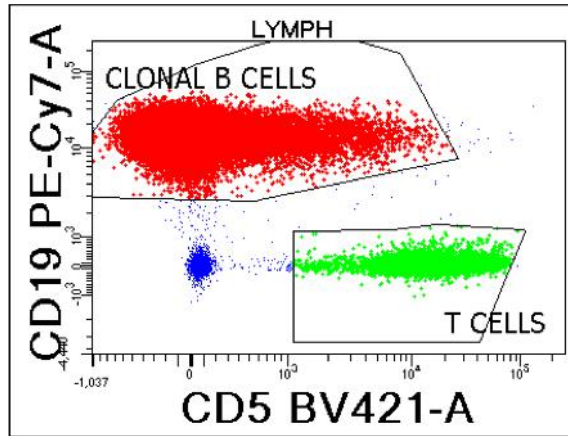


# Bright CD200 expression in Hairy Cell Leukemia Case

Case



# 50/M , Splenomegaly , Peripheral Lymphocytosis



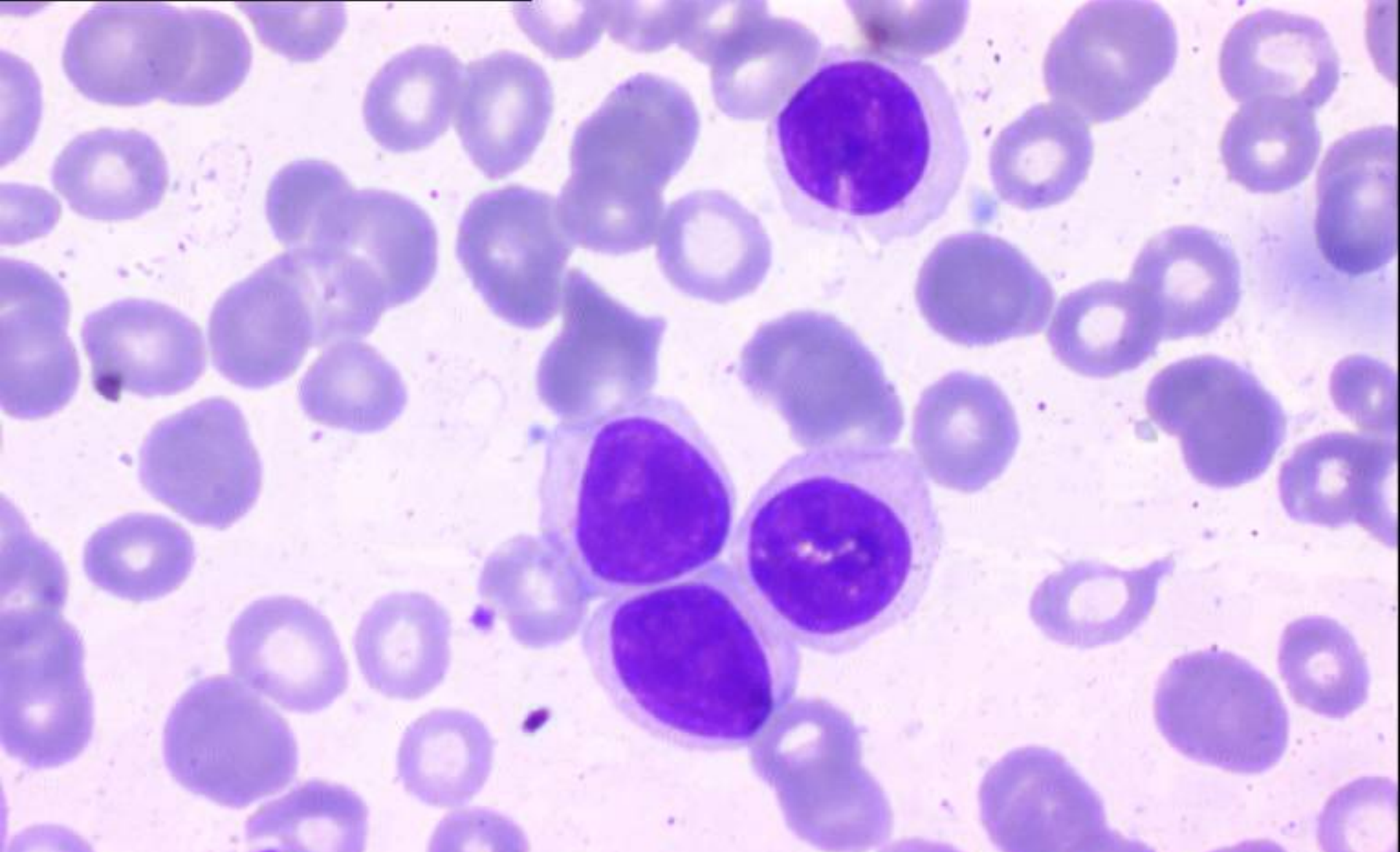


# Diagnosis ?

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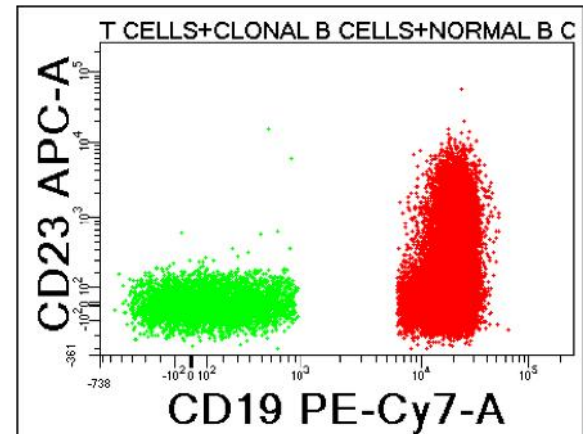
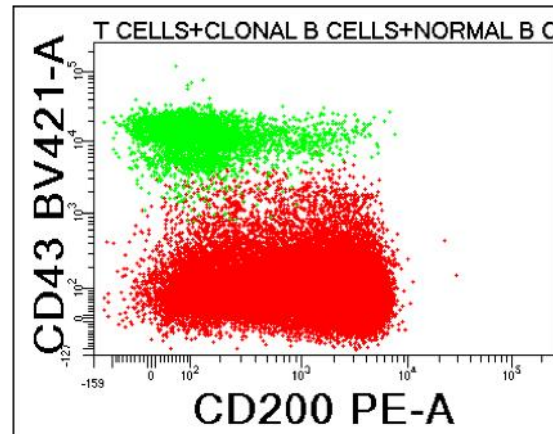
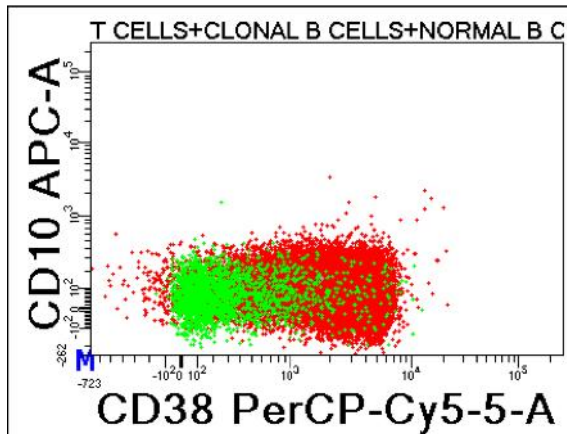
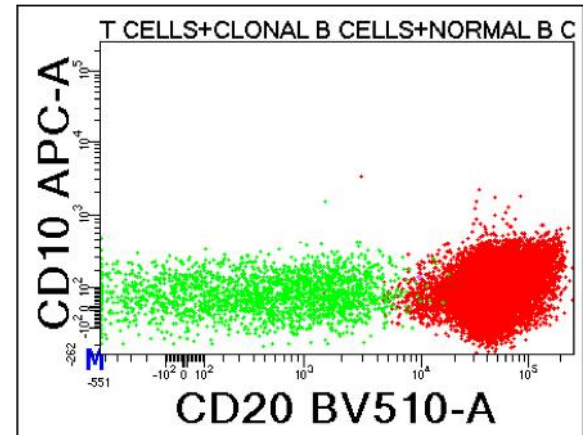
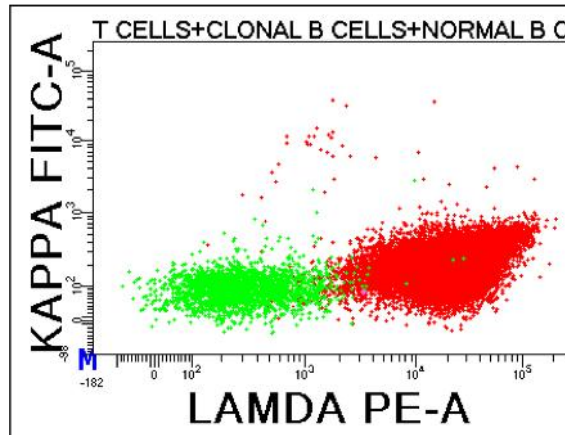
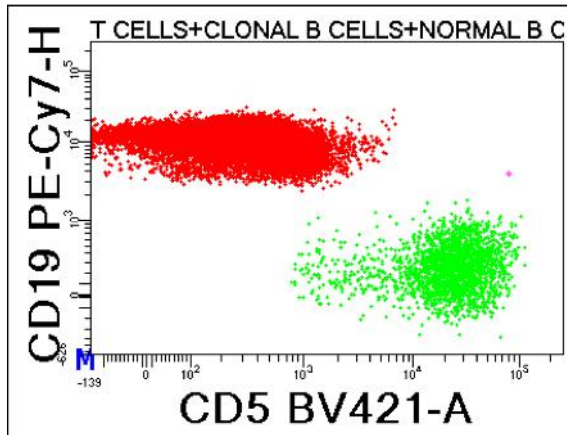
Hairy Cell Leukemia variant



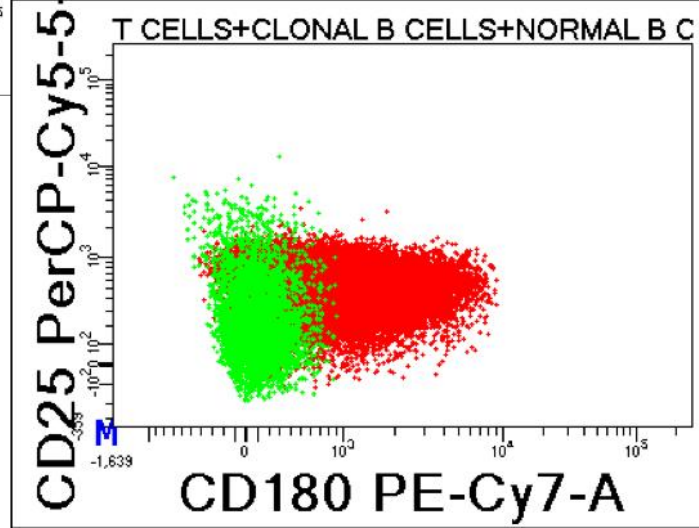
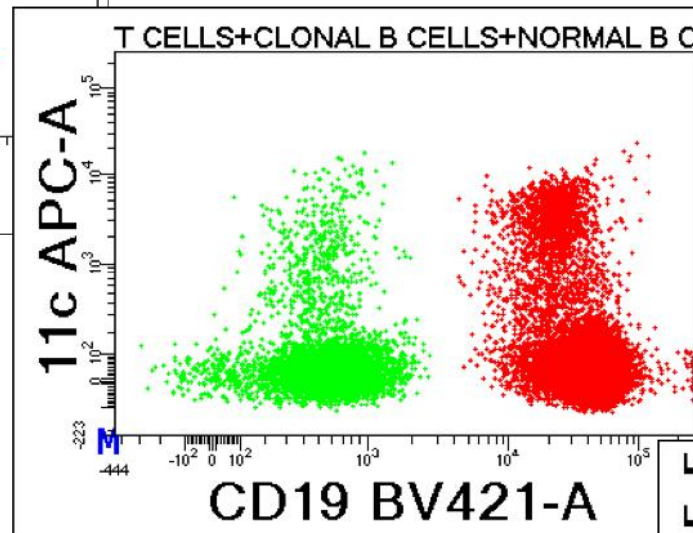
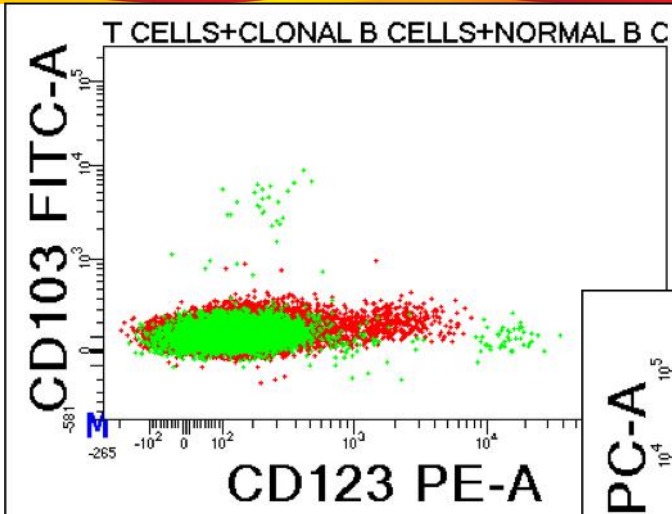


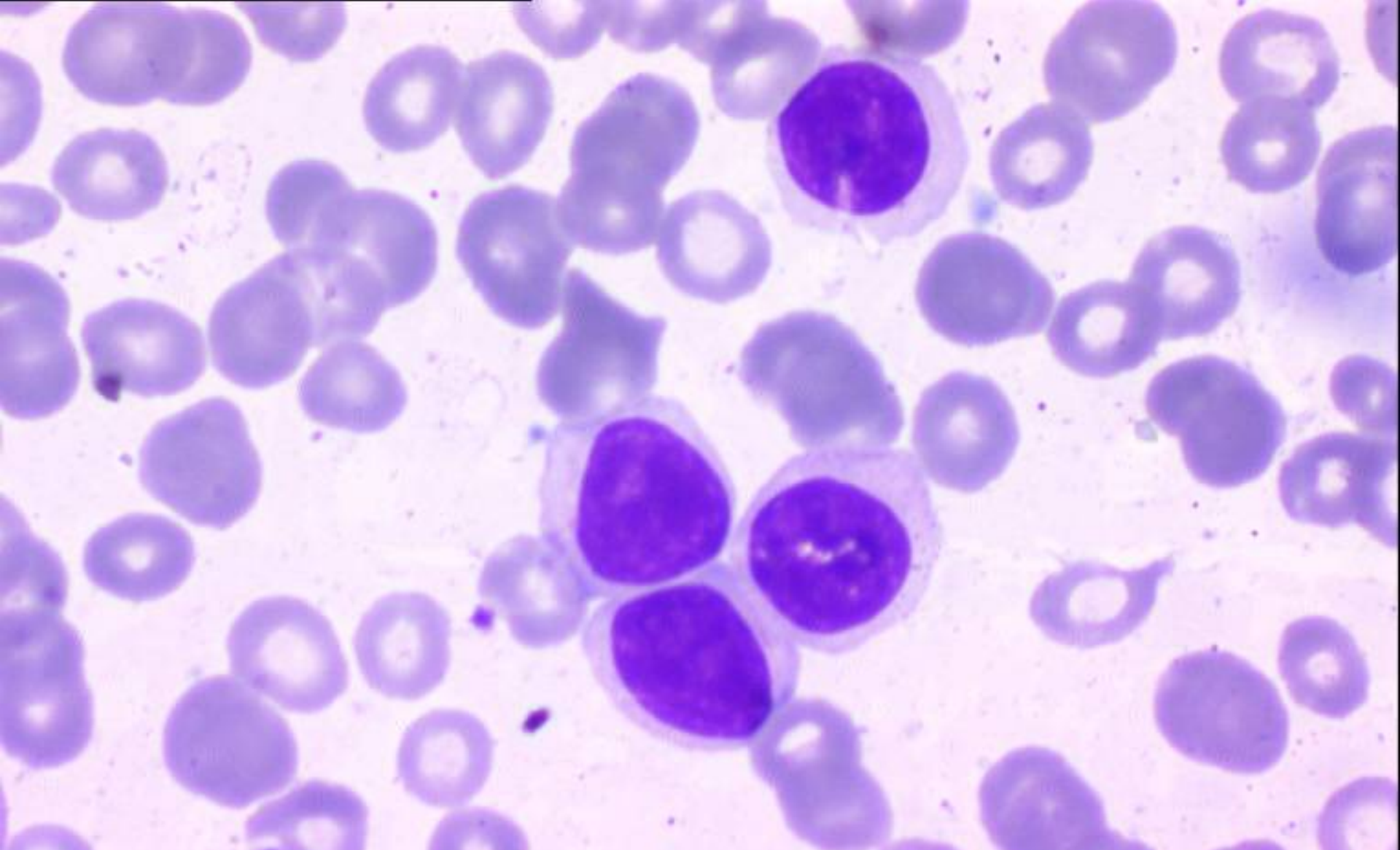
**Leucocytosis with Splenomegaly**

# Flow Analyses



# Flow Analyses





CD 5 -ve CD 10-ve B Cell NHL  
c/w a SMZL

# Thank You



[qrco.de/sehgalpathlab](http://qrco.de/sehgalpathlab)



[drkunalsehgal@gmail.com](mailto:drkunalsehgal@gmail.com)

