Laboratory diagnosis of Lymphoma

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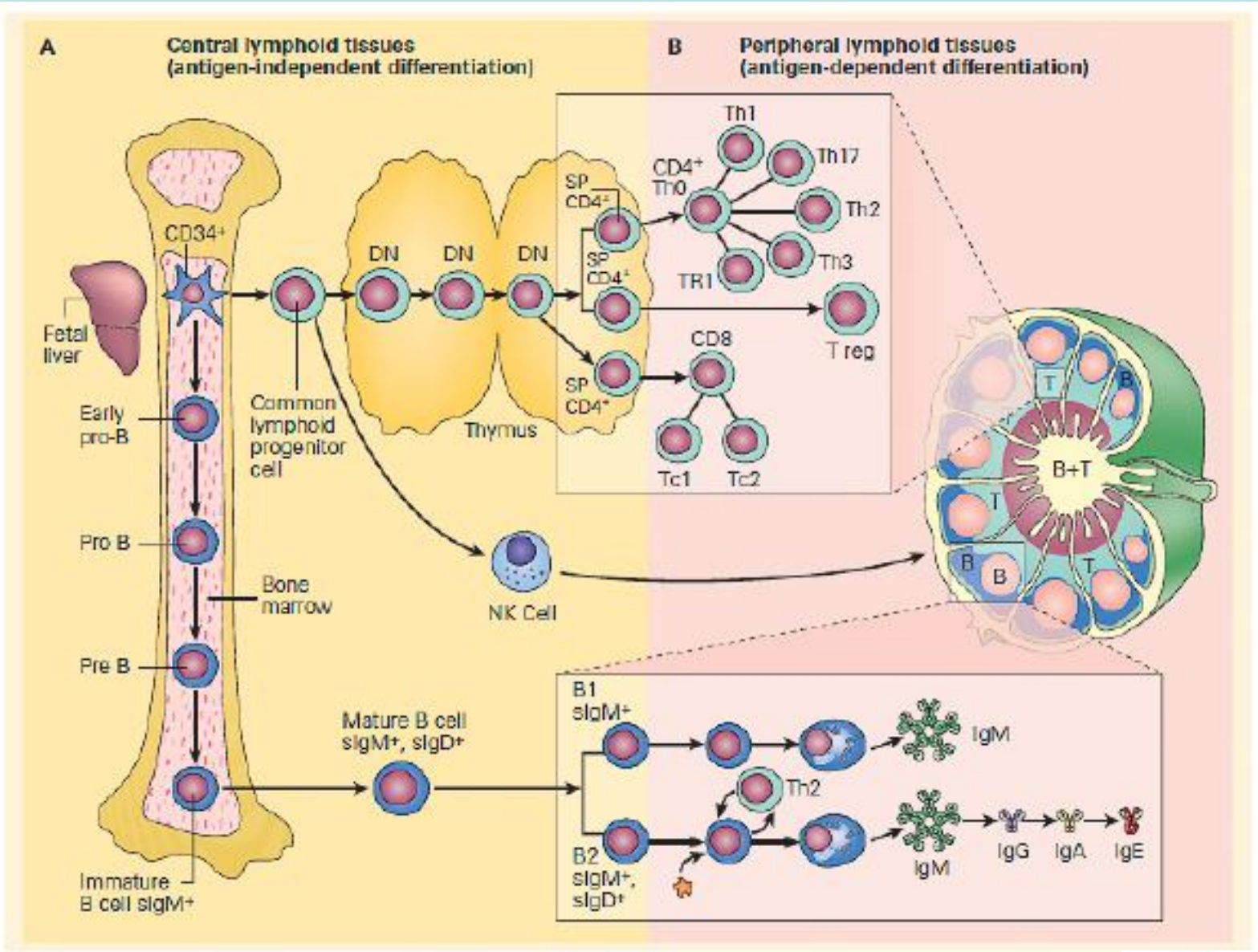
Lymphoma

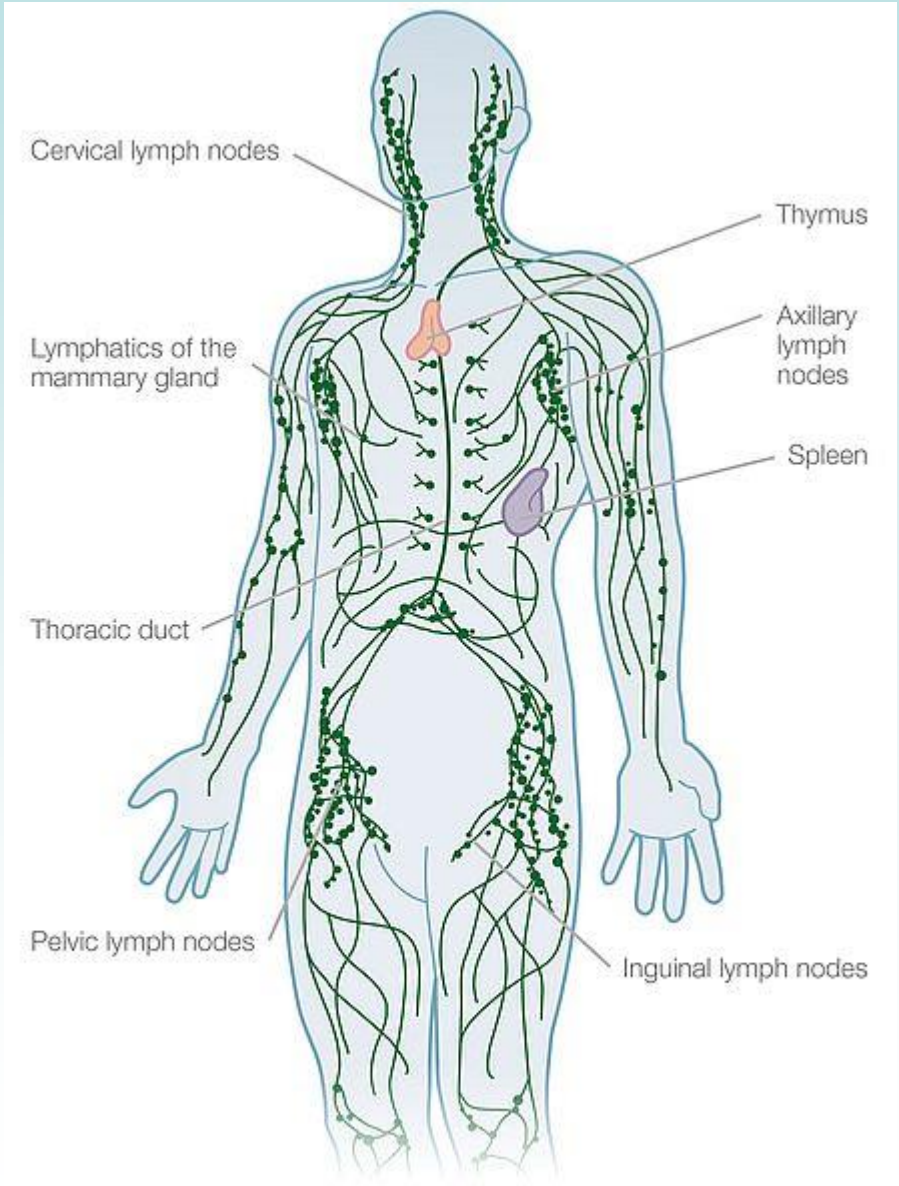
- Hodgkin lymphoma
  - Reed Sternberg cells low in number
  - Very rarely seen in bone marrow/blood

- Non-Hodgkin Lymphoma
  - Lymphoproliferative disorders
  - T, B and NK cell types
  - Often have a leukaemic phase
  - Crossover with chronic lymphoid leukaemia’s

Origin of lymphocytes



Lymphatic system



- Lymph nodes are concentrated in areas draining organs with environmental contact
- Primary (generative) lymphoid organs- bone marrow & thymus
- Secondary (peripheral) lymphoid tissues- lymph nodes, spleen, cutaneous & mucosal (eg tonsils, adenoids, lung, Peyer's patches)

Lymphoma classification

- **Rappaport classification**: 1956, revised 1966 (Cancer Res 1966;26:1082); developed before lymphocytes were classified as B and T cells; includes well differentiated lymphocytic lymphoma, poorly differentiated lymphocytic lymphoma and histiocytic lymphoma

- **Lukes and Collins classification**: 1974 (Cancer 1974;34:1488); classified non-Hodgkin lymphomas as B cell, T cell, histiocytic and unclassifiable types

- **Working Formulation**: 1982; classified as low, intermediate or high grade; nodular vs. diffuse; small, large or mixed tumor cell size (Cancer 1982;49:2112)

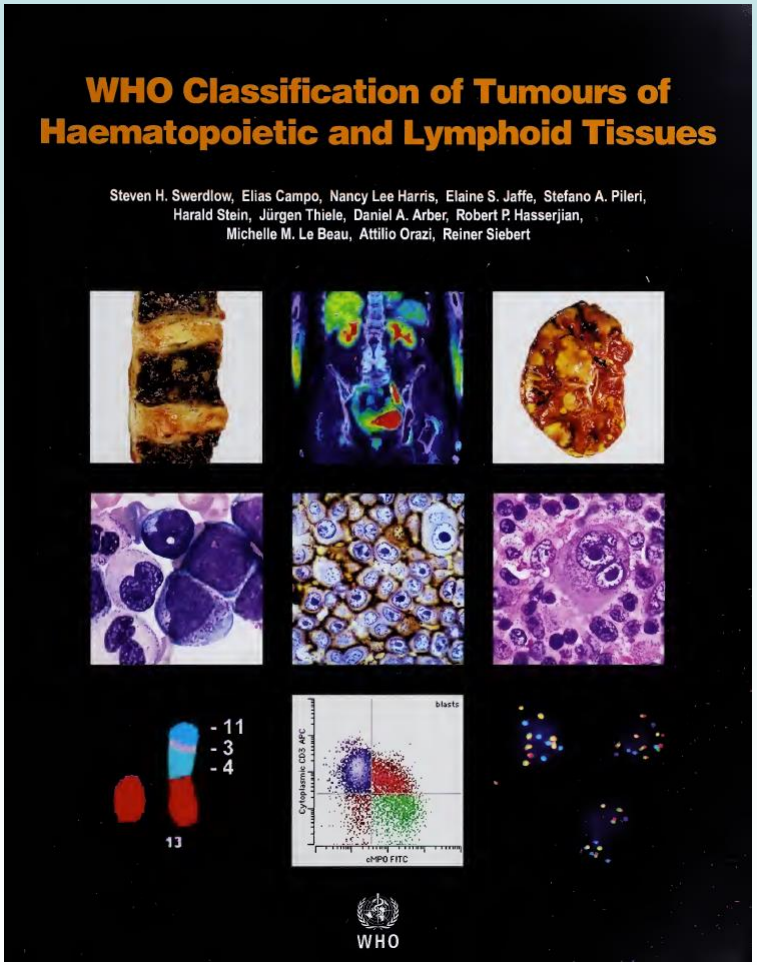
- **Kiel classification**: European system used in 1980s - 1990s, based on cellular morphology and relationship to normal lymphoid cells; proposed by Karl Lennert in 1974 (Lennert: History of the European Association for Haematopathology, 1st ed, 2006)

- **REAL (Revised European American Lymphoma)**: integrates clinical, morphologic, immunohistochemical and molecular characteristics; includes non Hodgkin's lymphoma, lymphocytic leukemias, plasma cell neoplasms; excludes histiocytic neoplasms; tumors are not classified as low grade / high grade since one entity could have both types (Blood 1994;84:1361

- **WHO**

http://www.pathologyoutlines.com/topic/lymphomabcellclassificationhistorical.html

WHO Classification of Tumours
Tumours of Haematopoietic and Lymphoid Tissue

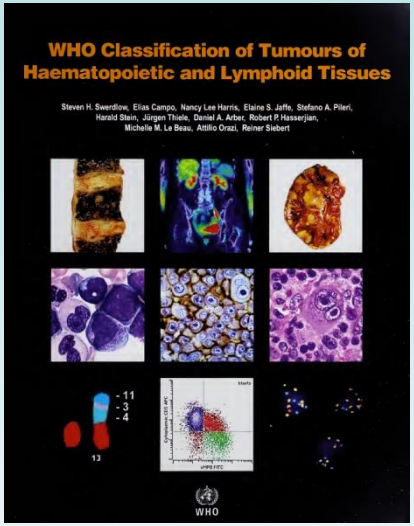


Classification incorporating

- Clinical detail
- Morphology
- Immunophenotype
- Cytogenetics
- Molecular genetics

155 separate entities

WHO Classification 2016



Lymphoid, histiocytic and dendritic neoplasms
~ 50 lymphoma

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
μ heavy-chain disease
γ heavy-chain disease
α heavy-chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma
Follicular lymphoma
In situ follicular neoplasia*
Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma*
*Large B-cell lymphoma with IRF4 rearrangement**
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
In situ mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV+ DLBCL, NOS*
*EBV+ mucocutaneous ulcer**
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK+ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
*HHV8+ DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma
Mature T and NK neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells
Aggressive NK-cell leukemia
Systemic EBV+ T-cell lymphoma of childhood*
Hydroa vacciniforme–like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK-/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma

Table 1. (continued)

Monomorphic epitheliotropic intestinal T-cell lymphoma*
*Indolent T-cell lymphoproliferative disorder of the GI tract**
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30+ T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma
Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma
*Primary cutaneous acral CD8+ T-cell lymphoma**
*Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder**
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
*Follicular T-cell lymphoma**
*Nodal peripheral T-cell lymphoma with TFH phenotype**
Anaplastic large-cell lymphoma, ALK+
Anaplastic large-cell lymphoma, ALK−*
*Breast implant–associated anaplastic large-cell lymphoma**
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma
Posttransplant lymphoproliferative disorders (PTLD)
Plasmacytic hyperplasia PTLD
Infectious mononucleosis PTLD
Florid follicular hyperplasia PTLD*
Polymorphic PTLD
Monomorphic PTLD (B- and T-/NK-cell types)
Classical Hodgkin lymphoma PTLD
Histiocytic and dendritic cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Indeterminate dendritic cell tumor
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumor
Disseminated juvenile xanthogranuloma
Erdheim-Chester disease*

Provisional entities are listed in italics.
*Changes from the 2008 classification.

Diagnosis of lymphoma

- Clinical examination
- Initial laboratory investigations
  - **Clinical details are essential**
  - Haematology

    FBC – BM function, infection, haemolysis etc

    ESR

    Retics,

    Blood film – Quantitative, qualitative, atypical cells

  - Biochemistry

    U&E, LFT's,

    Ig's

    LDH

Diagnosis of lymphoma

Additional investigations;

- Specialist Integrated Haematological Malignancy Diagnostic Services (SIHMDS)
  - Morphology
  - Flow cytometry
  - Cyto / molecular genetics
  - Histopathology & Cytology

- Ultrasound / Radiology

NICE IOG 2003

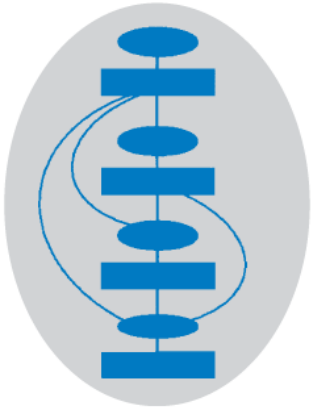
NHS

National Institute for Clinical Excellence

Guidance on Cancer Services

Improving Outcomes in Haematological Cancers

The Manual



Key recommendations

- All patients with haematological cancer should be managed by multi-disciplinary haemato-oncology teams which serve populations of 500,000 or more.
- In order to reduce errors, every diagnosis of possible haematological malignancy should be reviewed by specialists in diagnosis of haematological malignancy. Results of tests should be integrated and interpreted by experts who work with local haemato-oncology multi-disciplinary teams (MDTs) and provide a specialised service at network level. This is most easily achieved by locating all specialist haemato-pathology diagnostic services in a single laboratory.
- There should be rapid-access diagnostic services for patients with lymphadenopathy (chronically swollen lymph nodes or neck lumps).
- Clinical nurse and palliative care specialists are to have central roles in haemato-oncology teams, working closely with their medical colleagues. Clinical nurse specialists will arrange for patients and carers to receive multi-faceted support, co-ordinated care, and all the information they want, throughout the course of the illness.
- MDTs which manage patients with acute leukaemia should provide treatment intended to induce remission for sufficient new patients for the units concerned to develop and maintain expertise. Services are unlikely to be viable with five or fewer new patients per year. This treatment should be provided at a single facility within any one hospital site, in designated wards with continuous access to specialist nurses and haematologists.
- High dose therapy with progenitor cell transplantation is to be carried out only in centres which meet JACIE accreditation standards, including the minimum case-load criterion of 10 procedures per annum.

- To improve accuracy and certainty of diagnosis of haematological malignancy
- All patients with haematological malignancies must have access to a single diagnostic pathway
- Establish centralised specialist diagnostic labs
- MDT – Clinicians, CNS, Histopathologists, Radiologists and other AHP's
- Enable Cancer Networks to benefit from economies of scale.

"Improving the consistency and accuracy of diagnosis is probably the single most important aspect of improving outcomes in haematological cancer"

British Journal of Haematology, 2003, **123**, 463–468

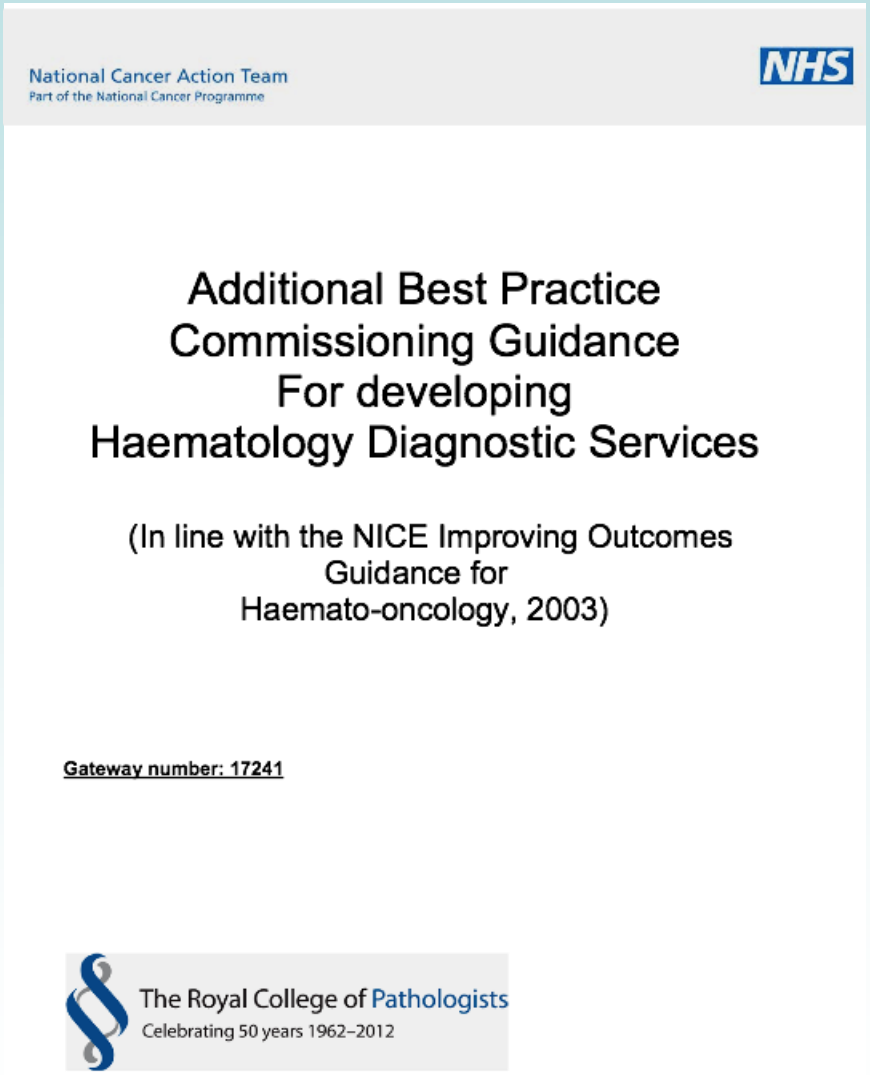
The clinical impact of expert pathological review on lymphoma management: a regional experience

JASON F. LESTER,[1] STEFAN D. DOJCINOV,[2] RICHARD L. ATTANOOS,[2] CIARAN J. O'BRIEN,[3] TIM S. MAUGHAN,[1] ELIZABETH T. TOY[1] AND CHRIS H. POYNTON[4] [1]*Velindre Hospital NHS Trust,* [2]*Department of Histopathology, Cardiff and Vale NHS Trust, Cardiff,* [3]*Departments of Histopathology and Haematology, Morriston Hospital, Swansea, and* [4]*University of Wales College of Medicine, Cardiff, UK*

125/745 cases diagnostic discrepancy – resulting in a change to the pathology diagnosis

46/99 evaluable cases had a change in management plan

National cancer action team 2012


National Cancer Action Team
Part of the National Cancer Programme

NHS

Additional Best Practice
Commissioning Guidance
For developing
Haematology Diagnostic Services

(In line with the NICE Improving Outcomes Guidance for Haemato-oncology, 2003)

Gateway number: 17241

The Royal College of Pathologists
Celebrating 50 years 1962–2012

Improving Outcomes Guidance for Haematological Oncology (IOG) was published in October 2003. This has been one of the most complex to achieve and eight years later, implementation remains incomplete[1]. Many cancer networks have been unable to work with providers and commissioners to ensure full compliance with some of the key recommendations. The most challenging recommendation has been the requirement to develop integrated laboratories for the diagnosis of haematological malignancy, commissioners will want to commission IOG compliant services to ensure accuracy and certainty of diagnosis for their populations.

Accuracy and certainty of diagnosis remains an ongoing problem, which particularly applies to lymphomas with concordance of diagnosis for lymphomas, is less than 85%[2]. There is a human and financial cost of diagnostic errors even though the financial costs of a precise diagnosis are a small fraction of treatment costs. Additionally no nationwide, validated and comparable epidemiology/population based data exist for service planning or monitoring of clinical outcomes.

In order to ensure that they commission best practice Haematology Diagnostic Services that are compliant with NICE Improving Outcomes Guidance, commissioners need to commission specialist haematological malignancy diagnostic services for their populations. Specialist Integrated Haematological Malignancy Diagnostic Services (SIHMDS) should cover a catchment population of at least 2 million. There are already existing SIHMDSs above this threshold which could support all networks, although more than half of networks continue to commission services from local non-specialist laboratories. If commissioners were to switch from using local diagnostic services to a specialist service (possibly located in a neighbouring network), the optimal scale for these services would be reached.

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ORIGINAL REPORT

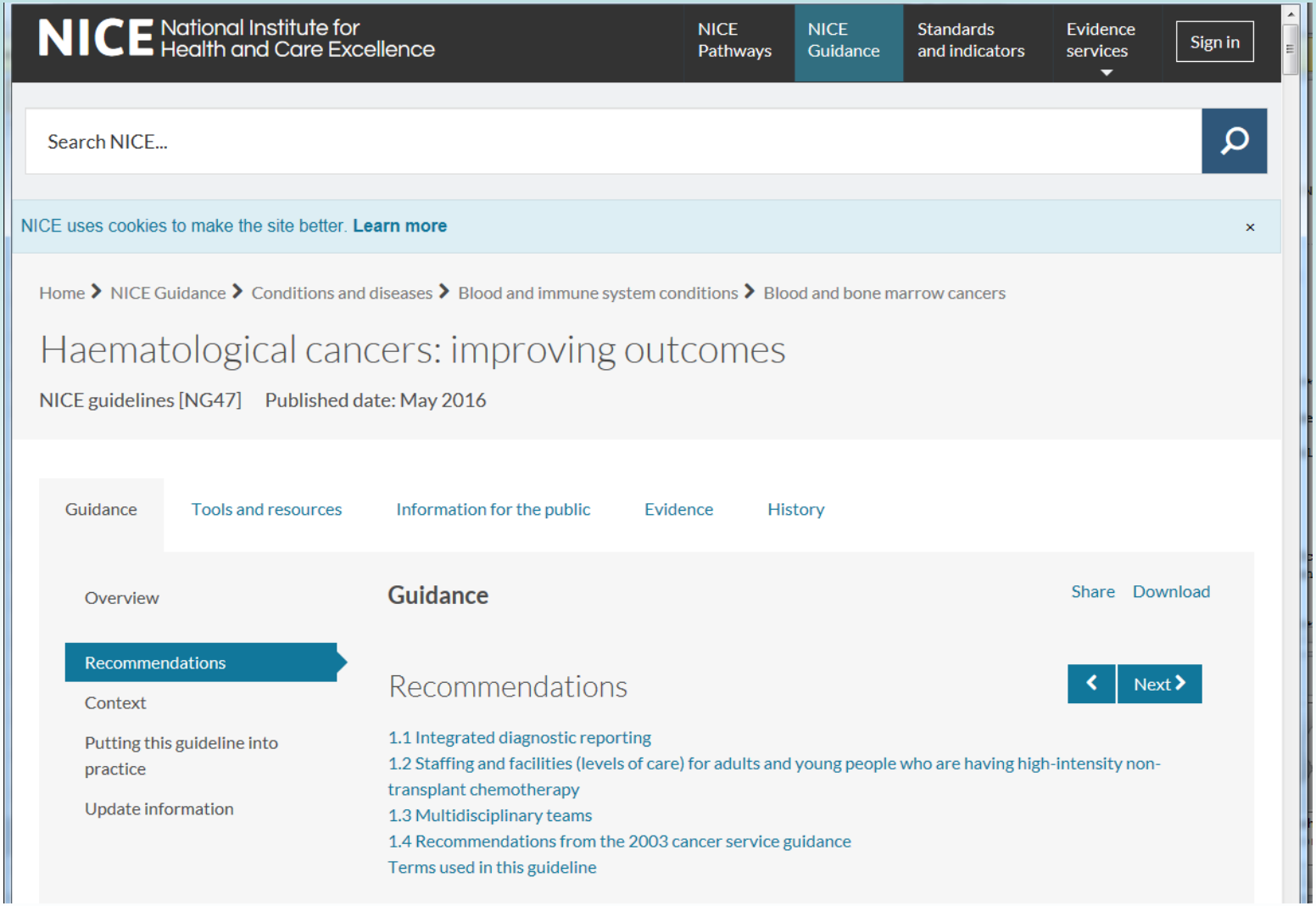
Importance of Expert Central Review in the Diagnosis of Lymphoid Malignancies in a Regional Cancer Network

Ian E. Proctor, Christopher McNamara, Manuel Rodriguez-Justo, Peter G. Isaacson, and Alan Ramsay

1949 cases reviewed (2003-2009)
27.4% discordant
9.3% delay in diagnosis
2.1% major change in management

Decrease in discordance during the 6 year period
32% to 13%

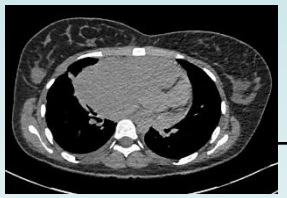
NICE 2016

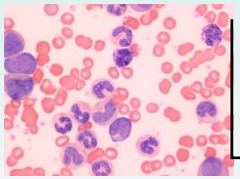


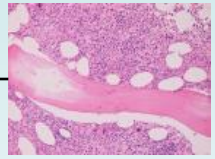
https://www.nice.org.uk/guidance/NG47/chapter/recommendations

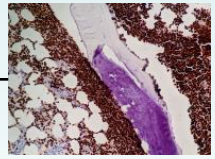
Pre IOG

Patient

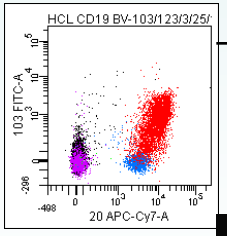


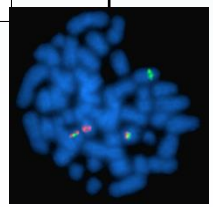


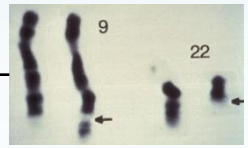


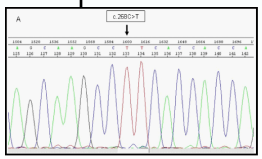


Clinician







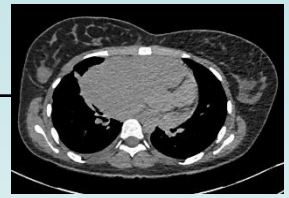


Post IOG

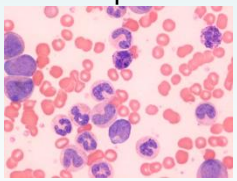
Clinician ⟺ Patient

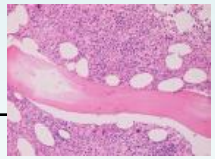
CNS
AHP's

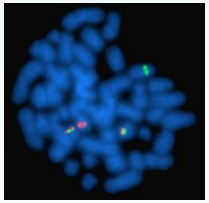
MDT

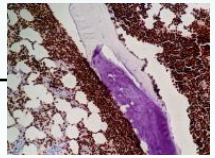


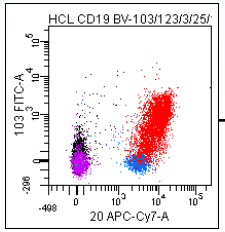
Integrated report

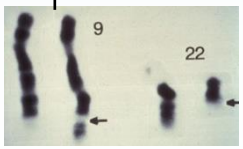


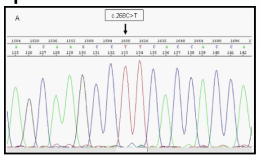












Lymphoproliferative disorders

- Low level lymphocytosis
- Most frequent flow cytometry request

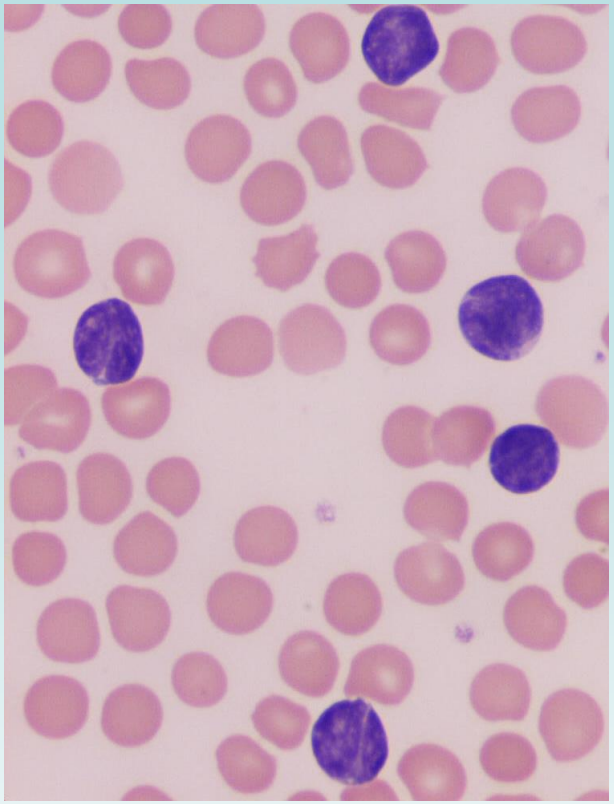
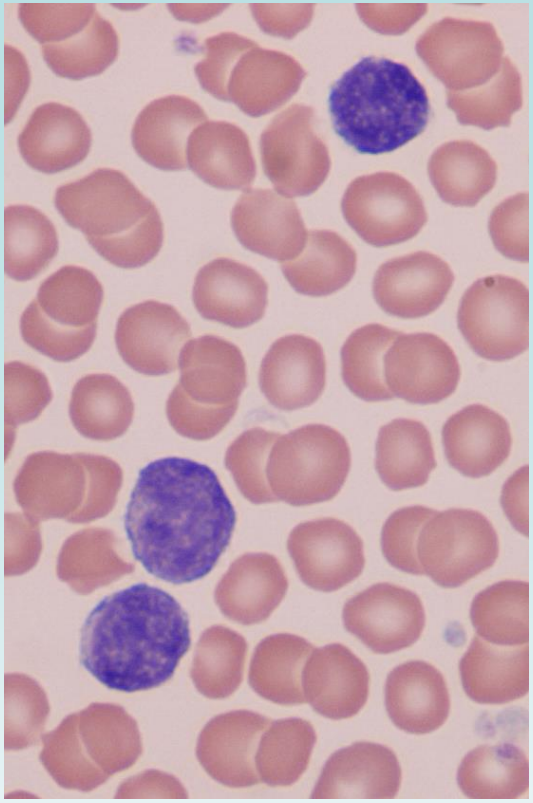
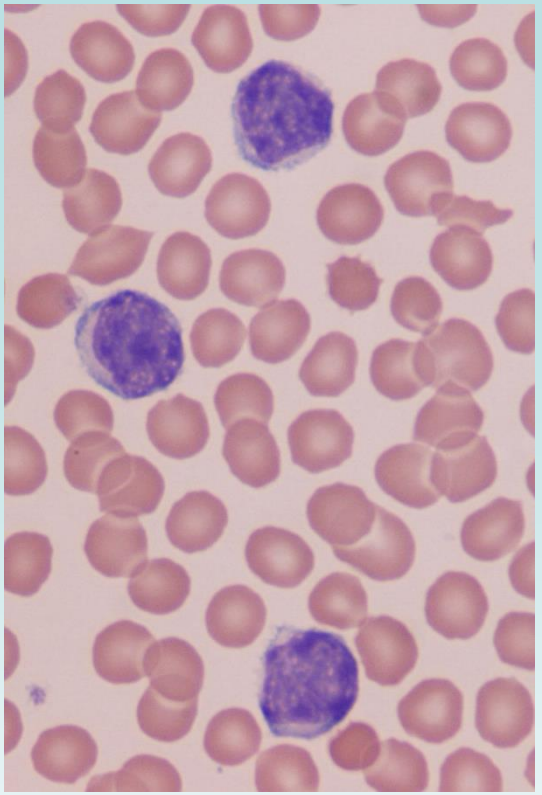
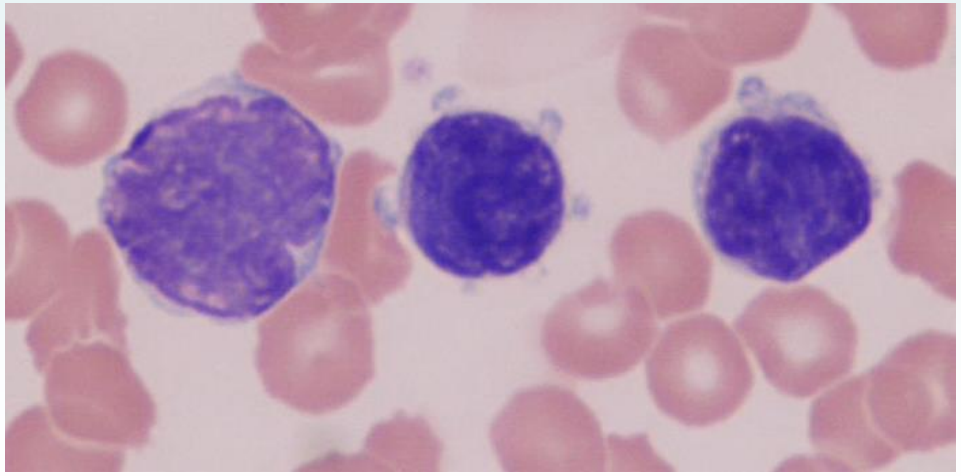
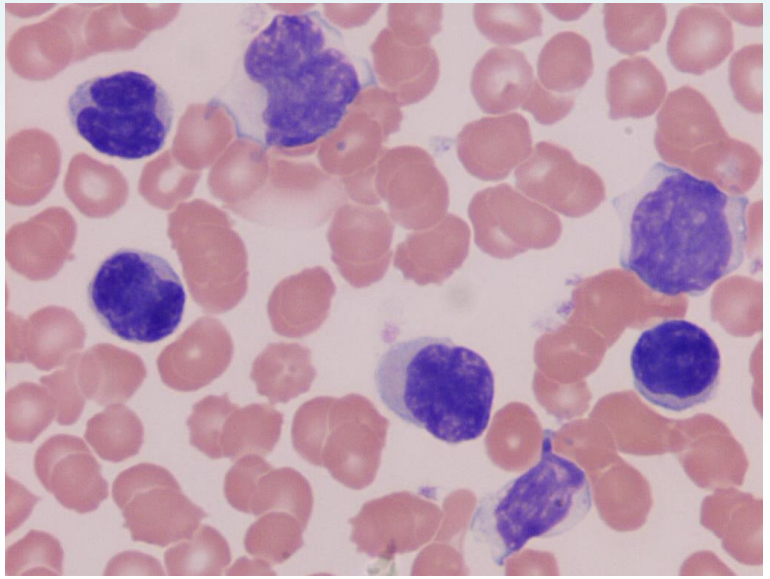
Can be reactive – often transient

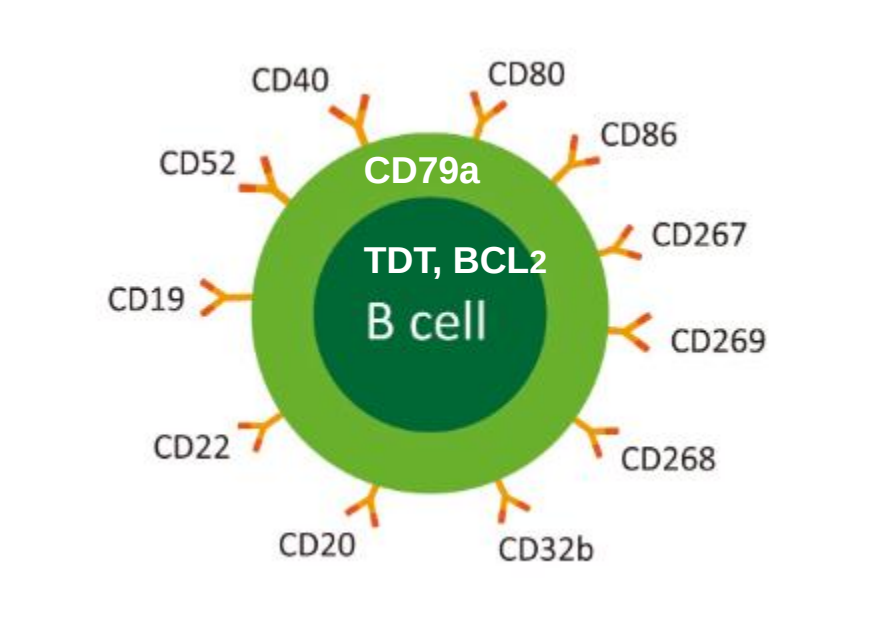
CLL/SLL/MBL

B-NHL > T-NHL

Rare entities

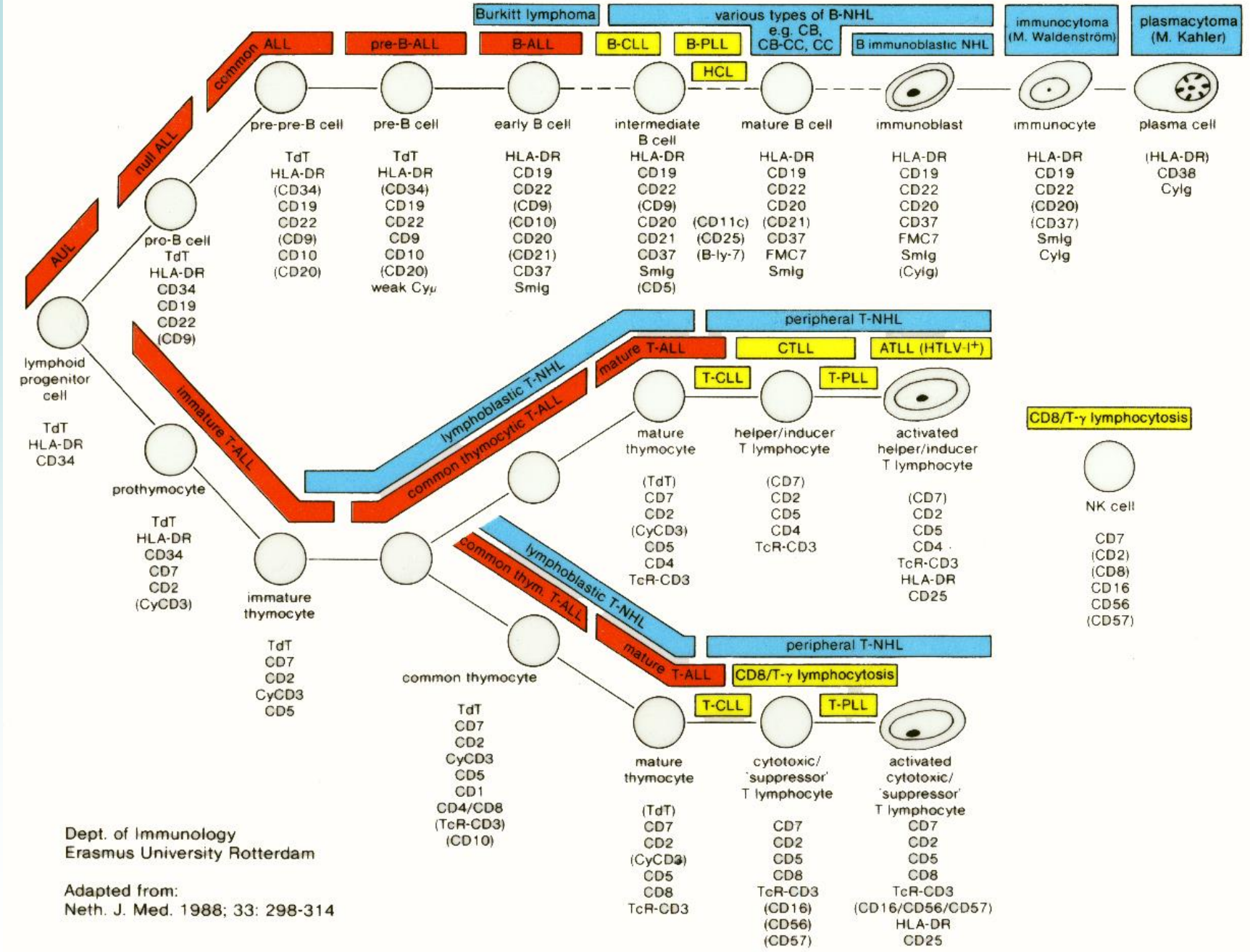
Indolent to highly aggressive



Intracellular / extracellular functional proteins
Cell signalling
Activation, cell development

CD19
CD5
CD10
CD20
CD22
CD38
CD52
CD79a
CD81
CD34
TDT



Dept. of Immunology
Erasmus University Rotterdam

Adapted from:
Neth. J. Med. 1988; 33: 298-314

The lymphoid screen

- Identify all lymphoid cells

  Lymph-sum

  T cells + B cells + NK cells = 100%

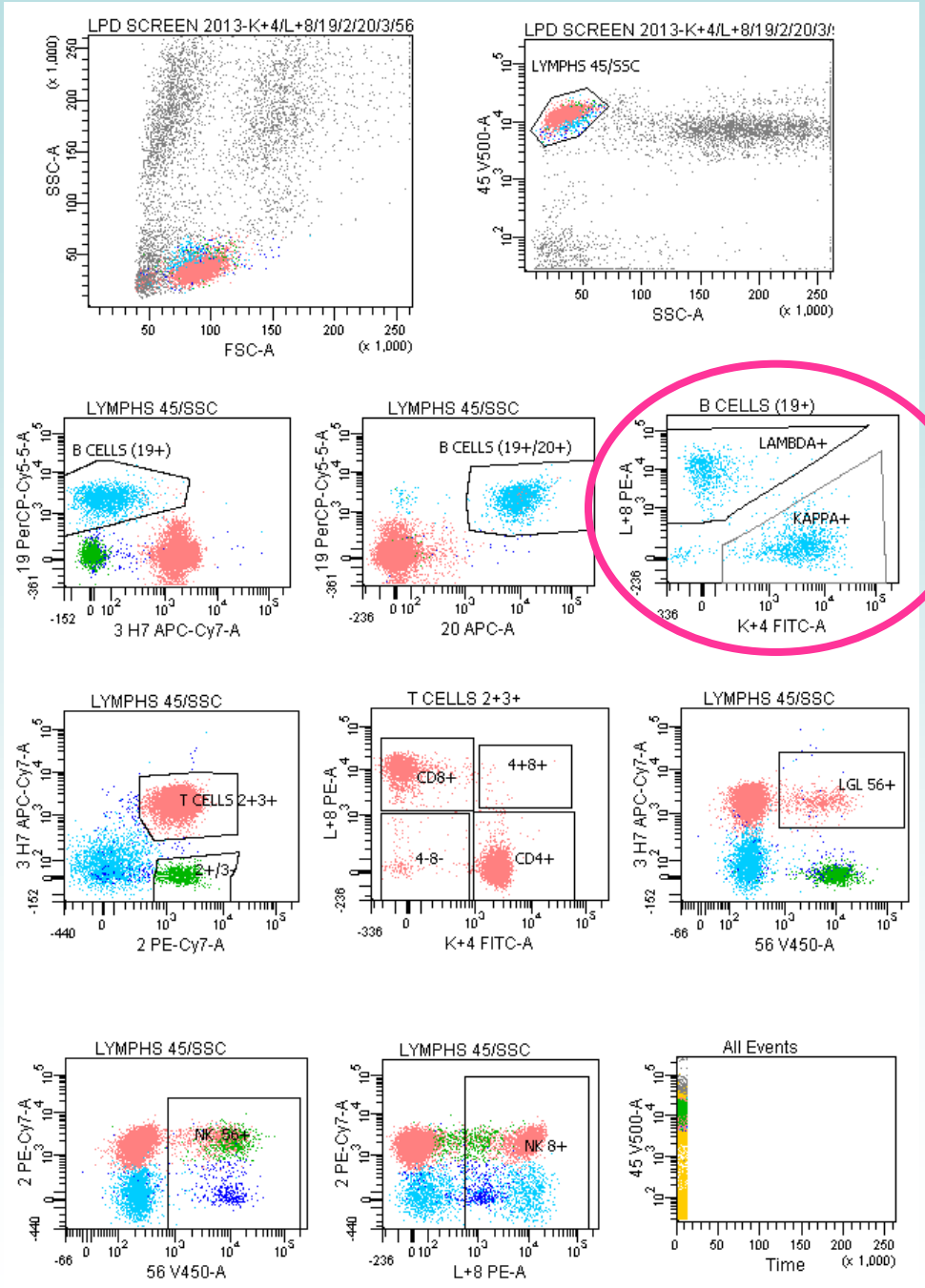
- ? Reactive or malignant

Chronic LPD screen

Single tube

- 8 colour
- 10 antigens
- 12 parameters

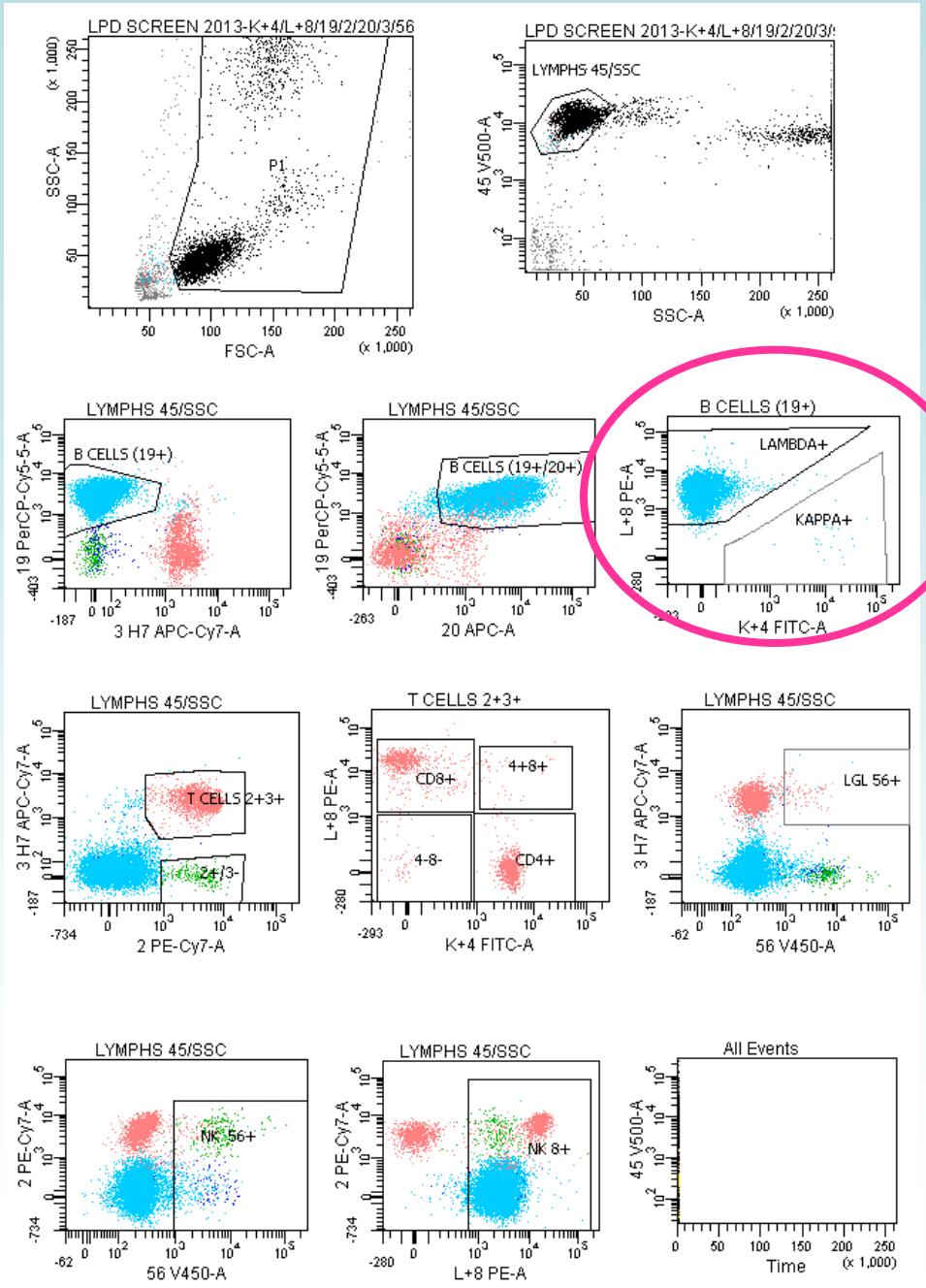
BLUE - 488				RED - 633		VIOLET - 405	
FITC	PE	PerCPCy 5.5	PE CY7	APC	APC-H7	PB/V450	V500
530	575	670	780	660	720 or 780	440	545
Kappa CD4	Lambda CD8	CD19	CD2	CD20	CD3	CD56	CD45



Tube: K+4/L+8/19/2/20/3/56/45

Population	#Events	%Parent	%Total
All Events	24,610	####	100.0
singlets	23,517	95.6	95.6
LYMPHS 45/SSC	9,965	42.4	40.5
B CELLS (19+)	1,791	18.0	7.3
KAPPA+	1,051	58.7	4.3
LAMBDA+	669	37.4	2.7
B CELLS (19+/20+)	1,763	17.7	7.2
T CELLS 2+3+	6,935	69.6	28.2
LGL 56+	511	7.4	2.1
CD4+	4,831	69.7	19.6
CD8+	1,879	27.1	7.6
4+8+	30	0.4	0.1
4-8-	167	2.4	0.7
2+/3-	729	7.3	3.0
NK 8+	304	41.7	1.2
NK 56+	718	98.5	2.9

Normal



Tube: K+4/L+8/19/2/20/3/56/45

Population	#Events	%Parent	%Total
All Events	17,318	####	100.0
singlets	16,587	95.8	95.8
LYMPHS 45/SSC	10,000	60.3	57.7
B CELLS (19+)	7,749	77.5	44.7
KAPPA+	24	0.3	0.1
LAMBDA+	7,692	99.3	44.4
B CELLS (19+/20+)	7,832	78.3	45.2
T CELLS 2+3+	1,793	17.9	10.4
LGL 56+	80	4.5	0.5
CD4+	861	48.0	5.0
CD8+	830	46.3	4.8
4+8+	40	2.2	0.2
4-8-	49	2.7	0.3
2+/3-	251	2.5	1.4
NK 8+	242	96.4	1.4
NK 56+	224	89.2	1.3
P1	15,118	87.3	87.3

Clonal B cells

Extended B cells

	BLUE - 488				RED - 633		VIOLET - 405	
	FITC	PE	PerCPCy5.5	PE CY7	APC	APC-H7	PB/V450	V500
	530	575	670	780	660	720 or 780	440	545
Screen	Kappa CD4	Lambda CD8	CD19	CD2	CD20	CD3	CD56 (v450)	45(v500)
B cell 1	CD81	CD22	CD5	CD38	CD200	CD20	CD19(v450)	45(v500)
B cell 2	CD23	CD79b		CD10	CD43	CD20	CD19(v450)	45(v500)

Often classify B disorders as
CD5+ve
CD10+ve
CD5-/CD10-ve

Further interrogation of B cells

- CD5 positive

  CLL/SLL, MCL, MZL, some DLBCL

- CD10 positive

  FL, some LPL, some DLBCL, Burkitt, LBL

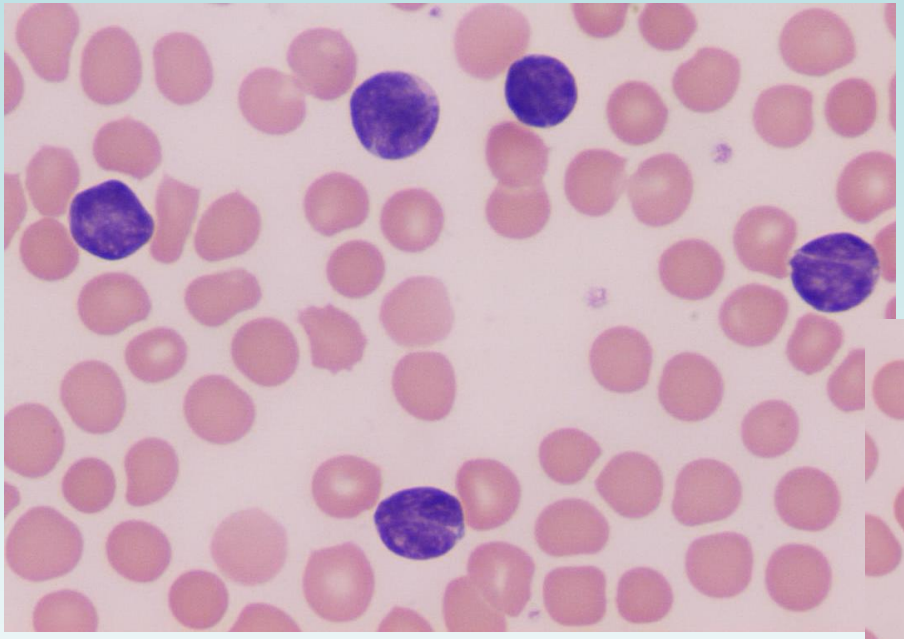
- Additional markers

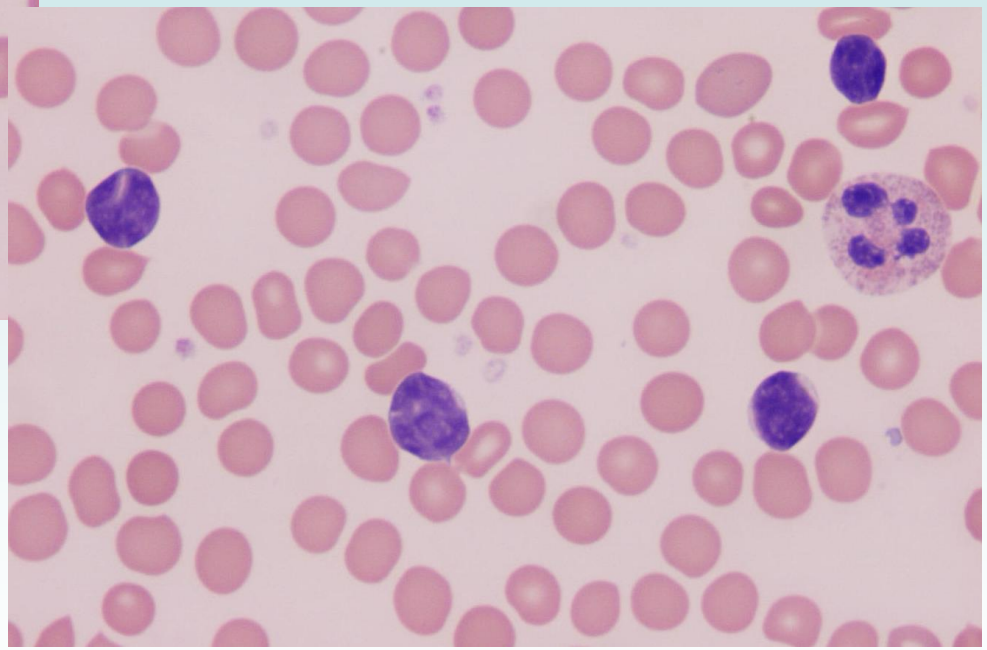
  CD20, CD22, CD23, CD43, CD79b, CD81, CD200 and CD38 (CLL prognostic marker)

  CD11c, CD25, CD81, CD103

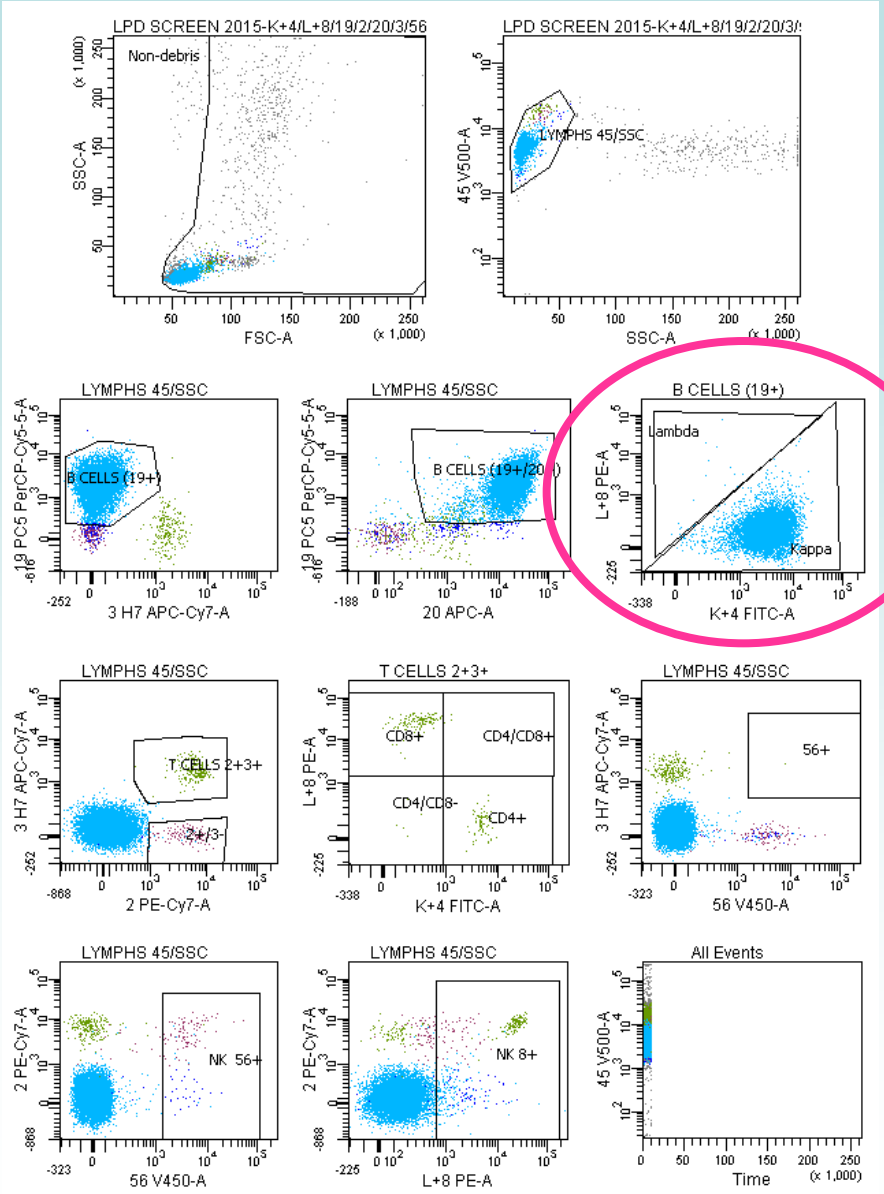
  Bcl-2, Ki-67

AH



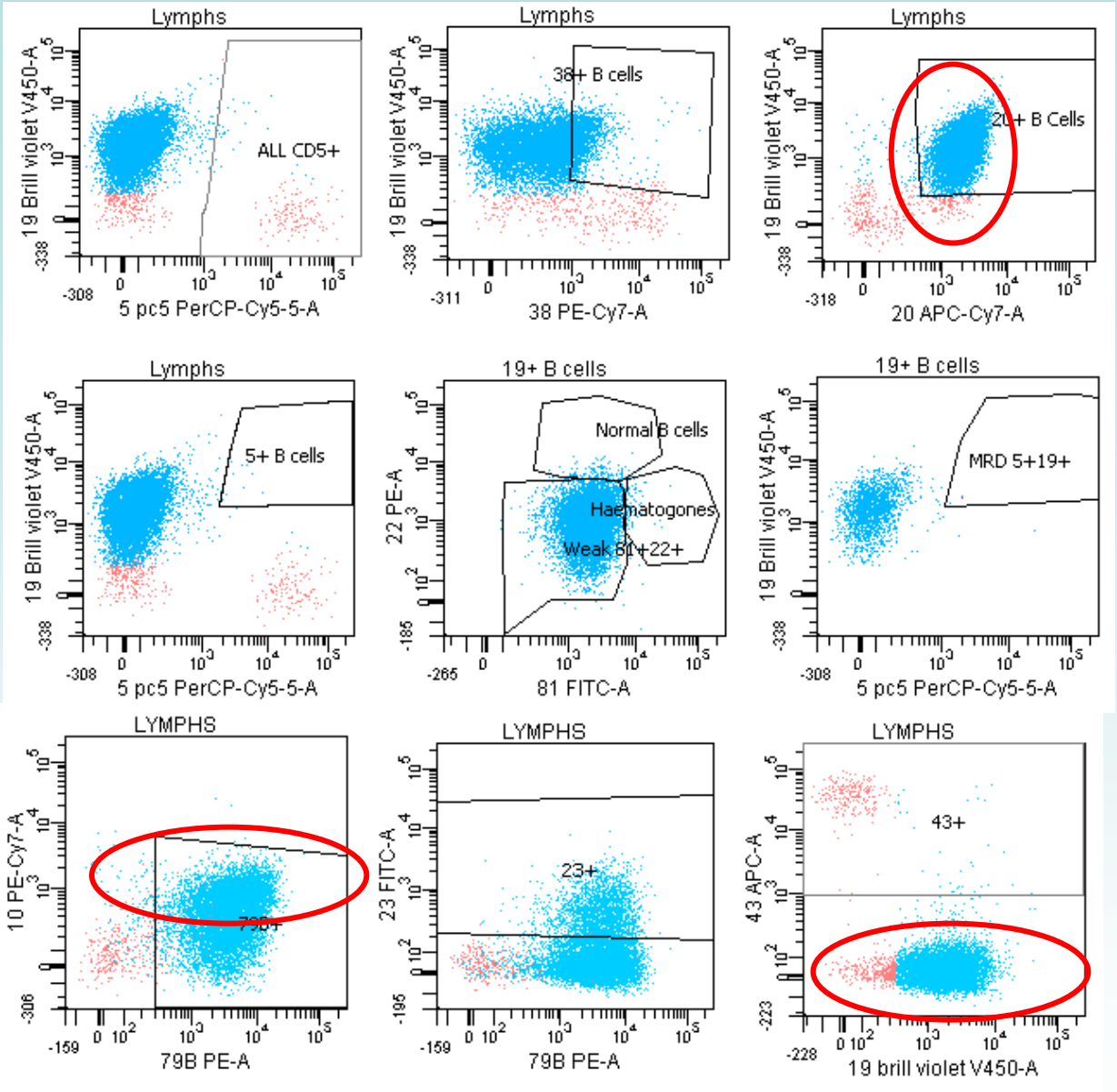


‘Peripheral blood lymphocytosis.
The lymphocytes appear morphologically mature with clumped nuclear chromatin.
Some have distinct nuclear clefting.
Flow cytometric analysis to follow.’



Tube: K+4/L+8/19/2/20/3/56/45

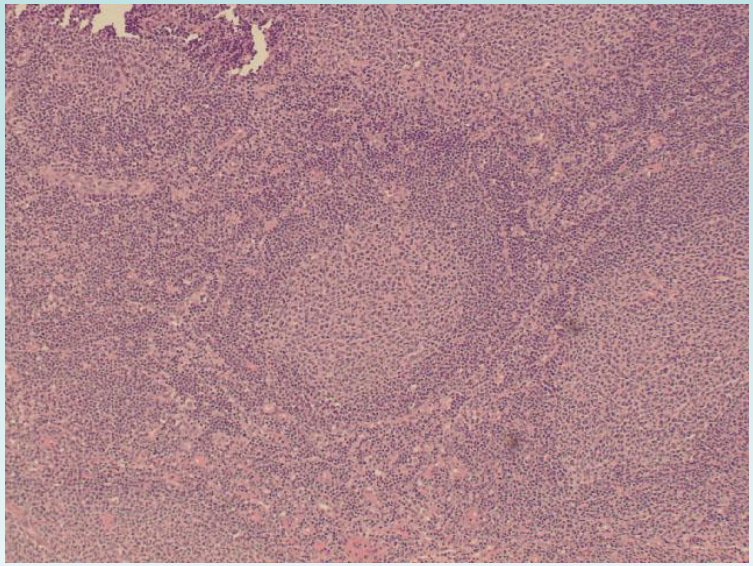
Population	#Events	%Parent	%Total
All Events	12,988	####	100.0
singlets	11,515	88.7	88.7
Non-debris	11,441	99.4	88.1
LYMPHS 45/SSC	10,014	87.5	77.1
B CELLS (19+)	9,484	94.7	73.0
Kappa	9,463	99.8	72.9
Lambda	15	0.2	0.1
B CELLS (19+/20+)	9,464	94.5	72.9
T CELLS 2+3+	216	2.2	1.7
56+	1	0.5	0.0
CD4+	77	35.6	0.6
CD8+	131	60.6	1.0
CD4/CD8-	5	2.3	0.0
CD4/CD8+	5	2.3	0.0
2+/3-	126	1.3	1.0
NK 8+	56	44.4	0.4
NK 56+	98	77.8	0.8



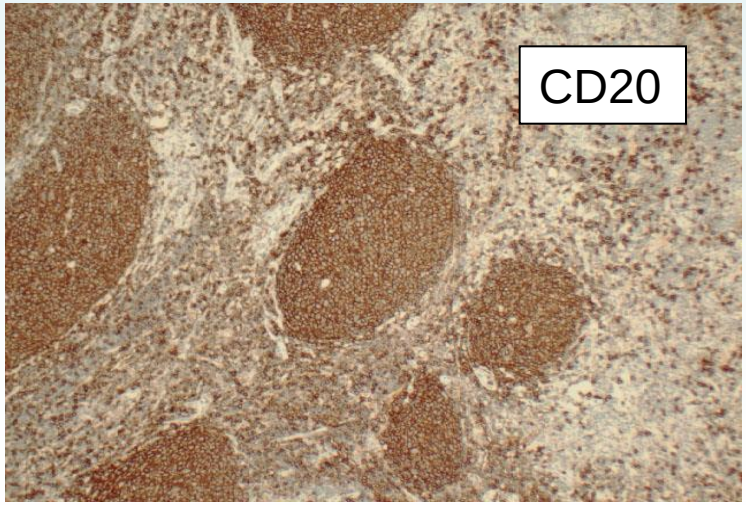
B cell phenotype : Follicular lymphoma

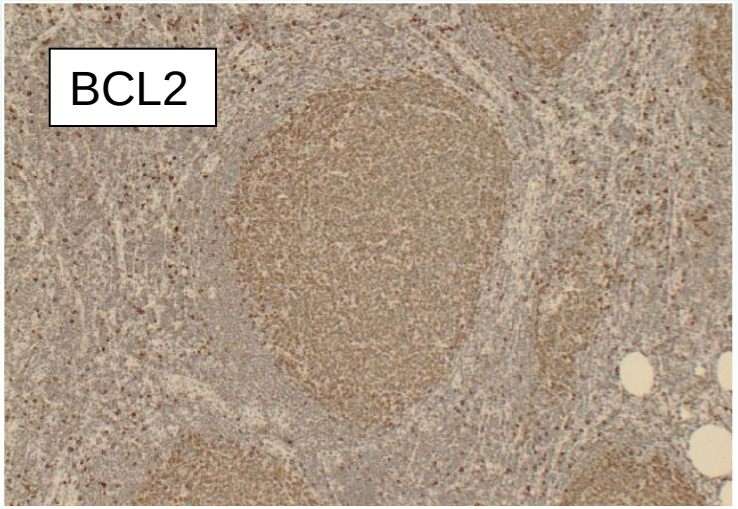
CD10+, CD19wk/+, CD20++, CD22+, CD23+/-, CD43-, CD79b++, CD81+, Kappa +

AH – Lymph node biopsy

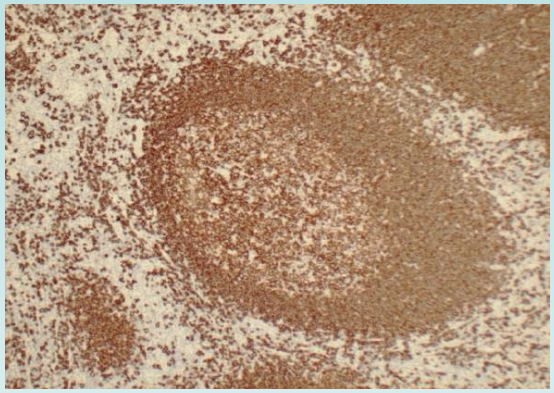


Follicular lymphoma
CD20+ CD10+ BCL2+
BCL6+
Germinal centre markers

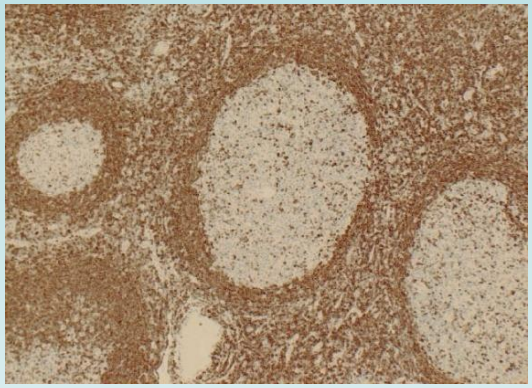




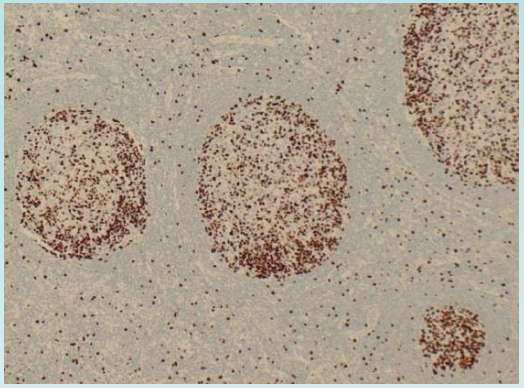
Reactive follicular hyperplasia



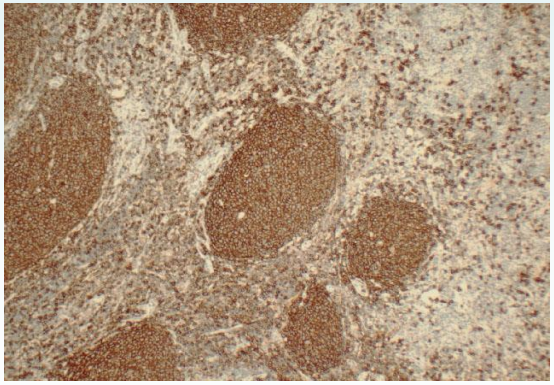
CD20

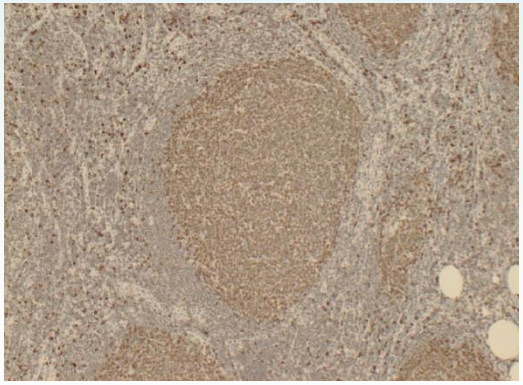


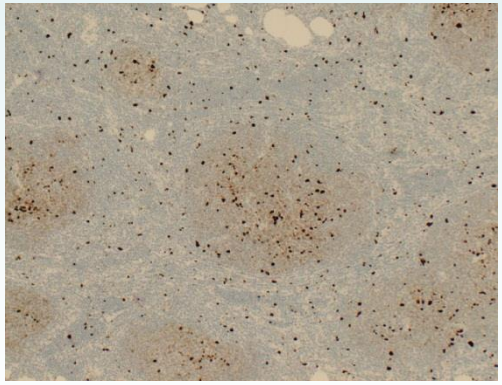
BCL2



MIB-1





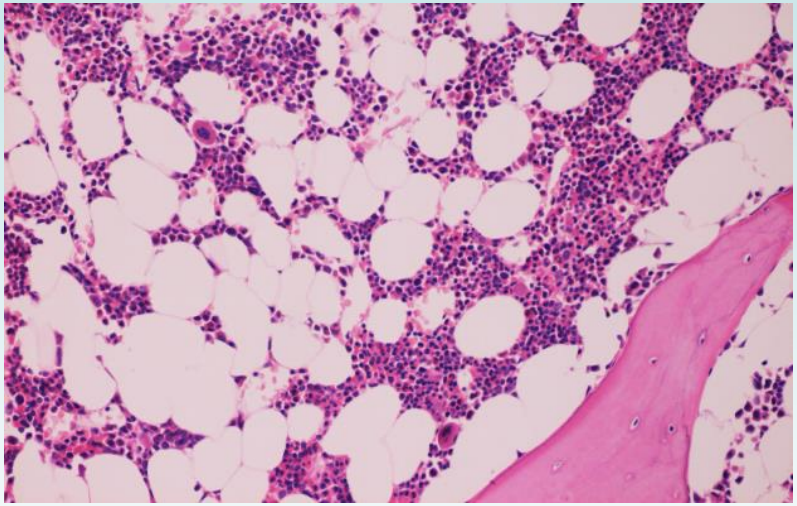


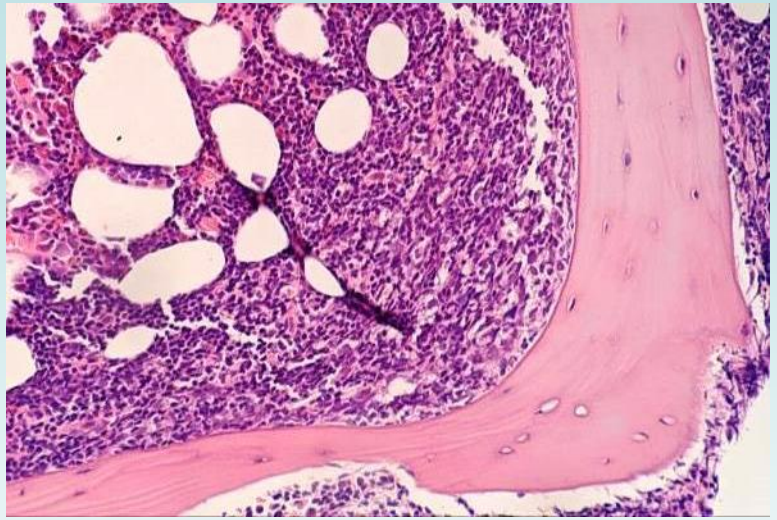
Follicular lymphoma

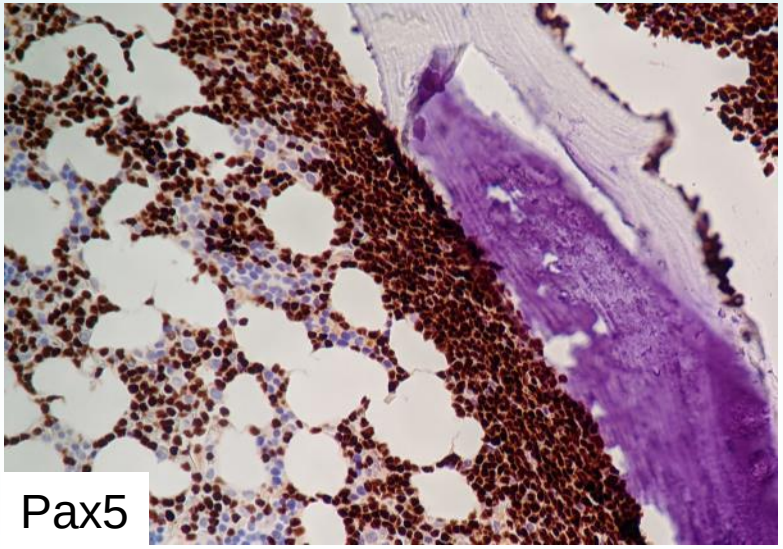
Bone marrow trephine

AH

Normal







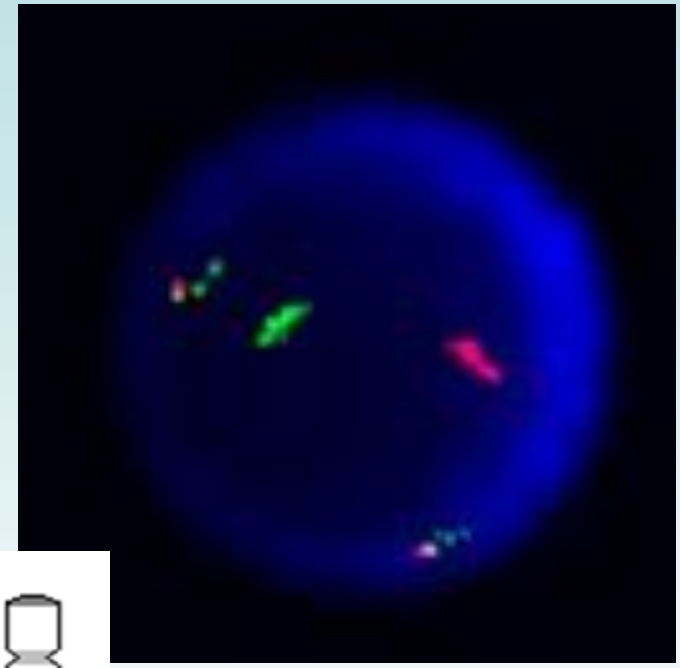
Pax5

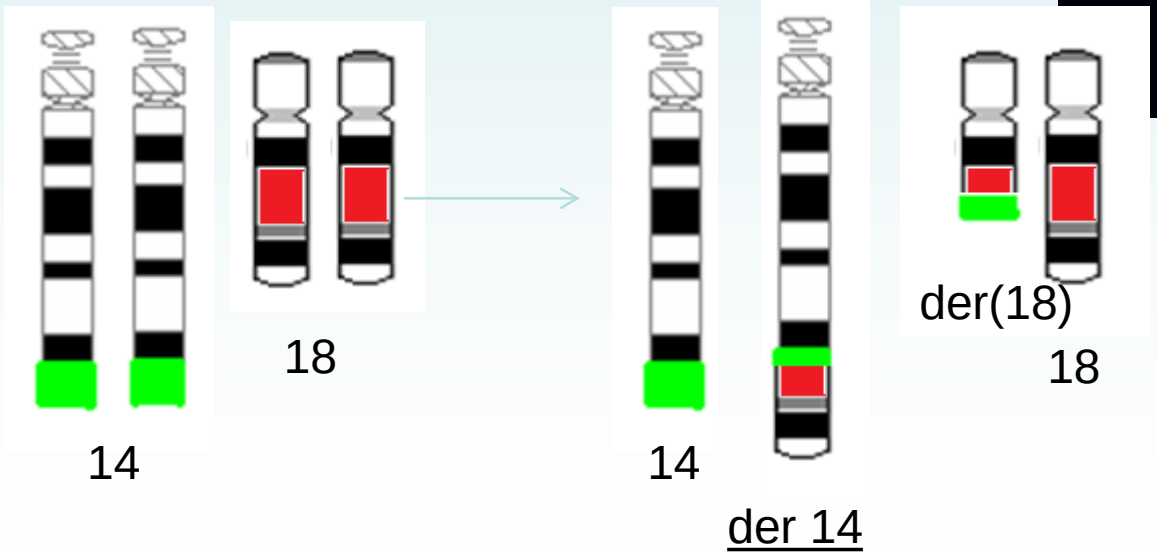
Fluorescent In-Situ hybridisation

Fluorescent DNA probes
PB, BM, fluids, tissue
Interphase nuclei
4-24hrs

IgH = Green
BCL2 = Red

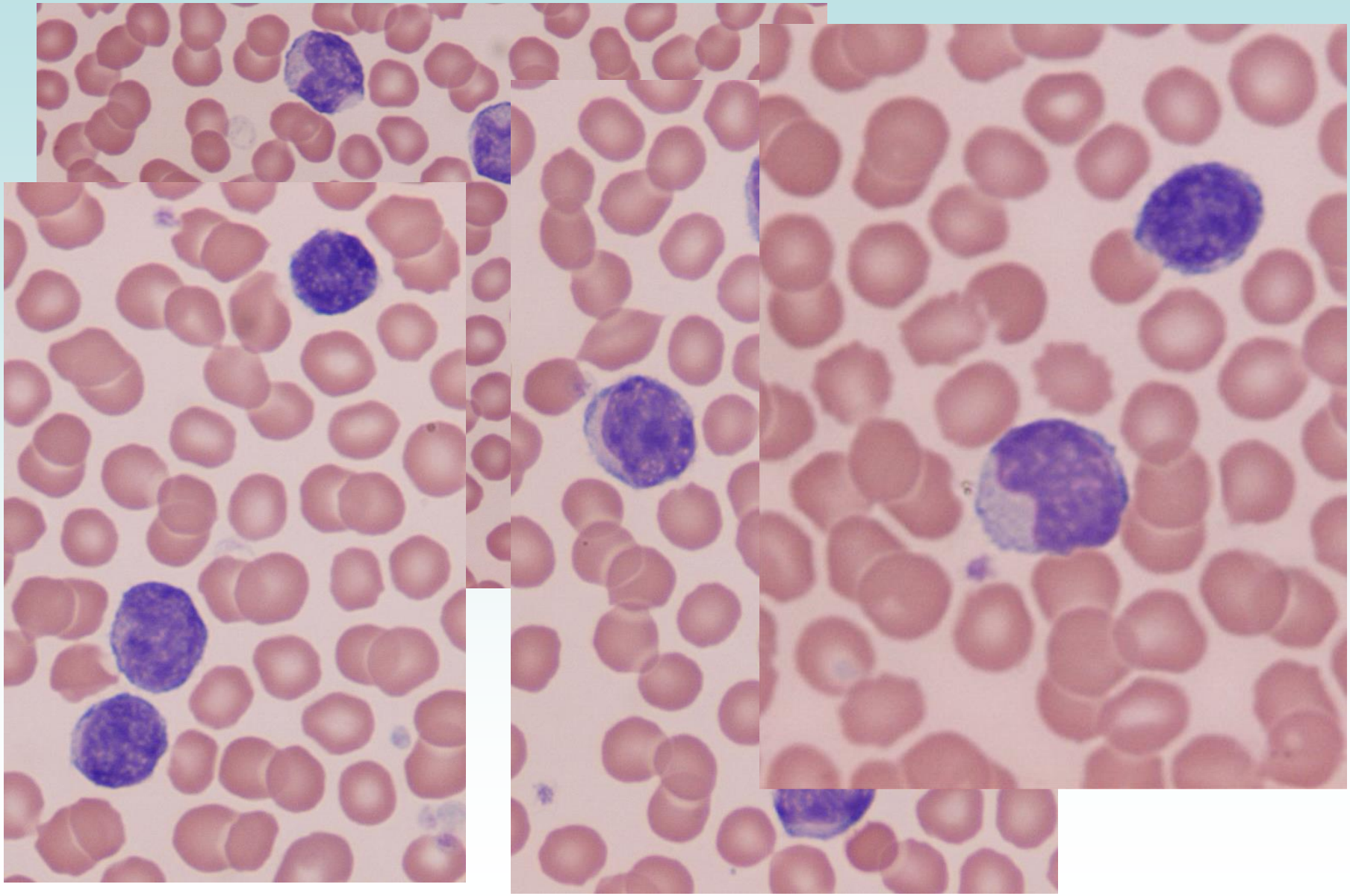
1R, 1G 2fusion

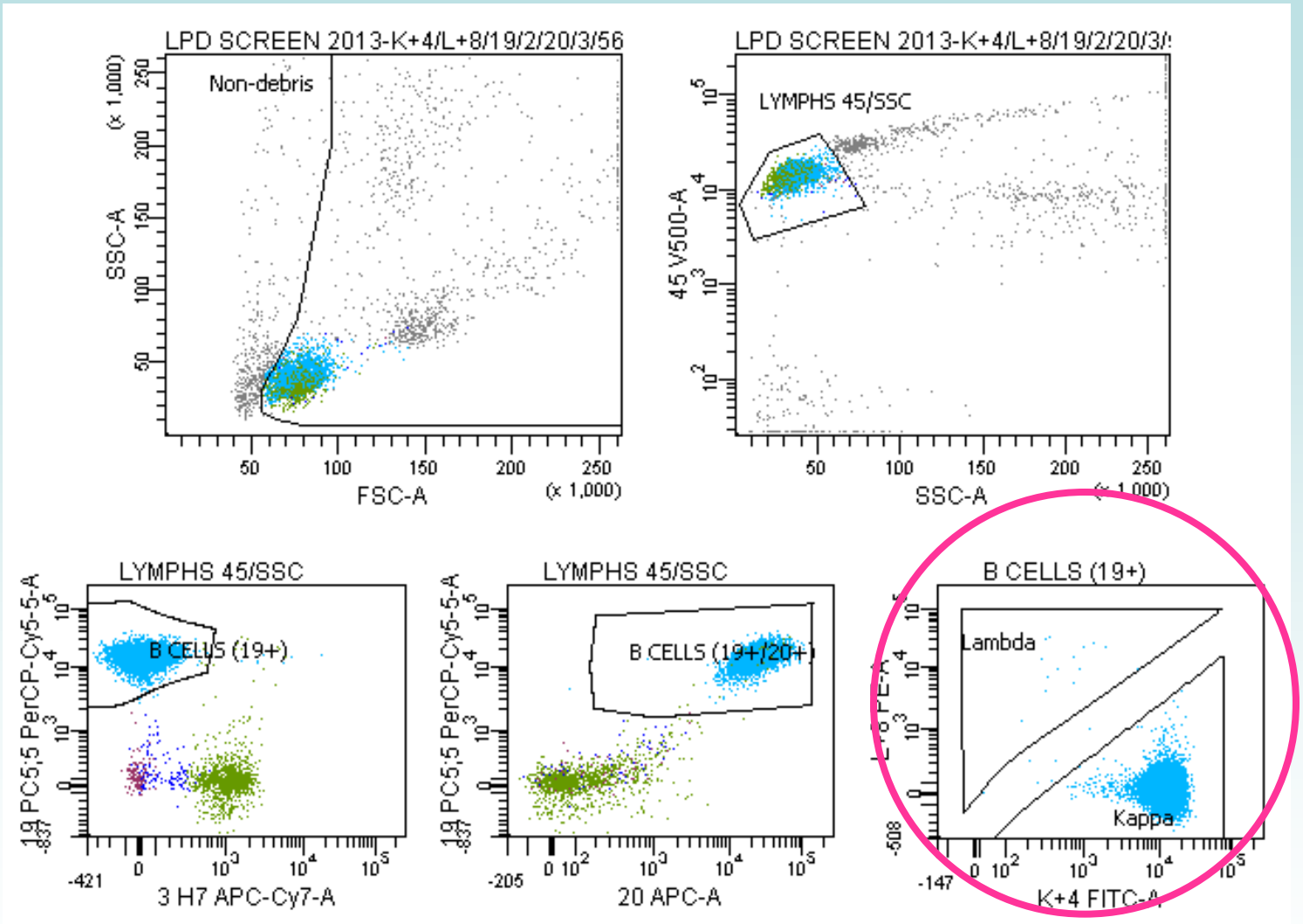


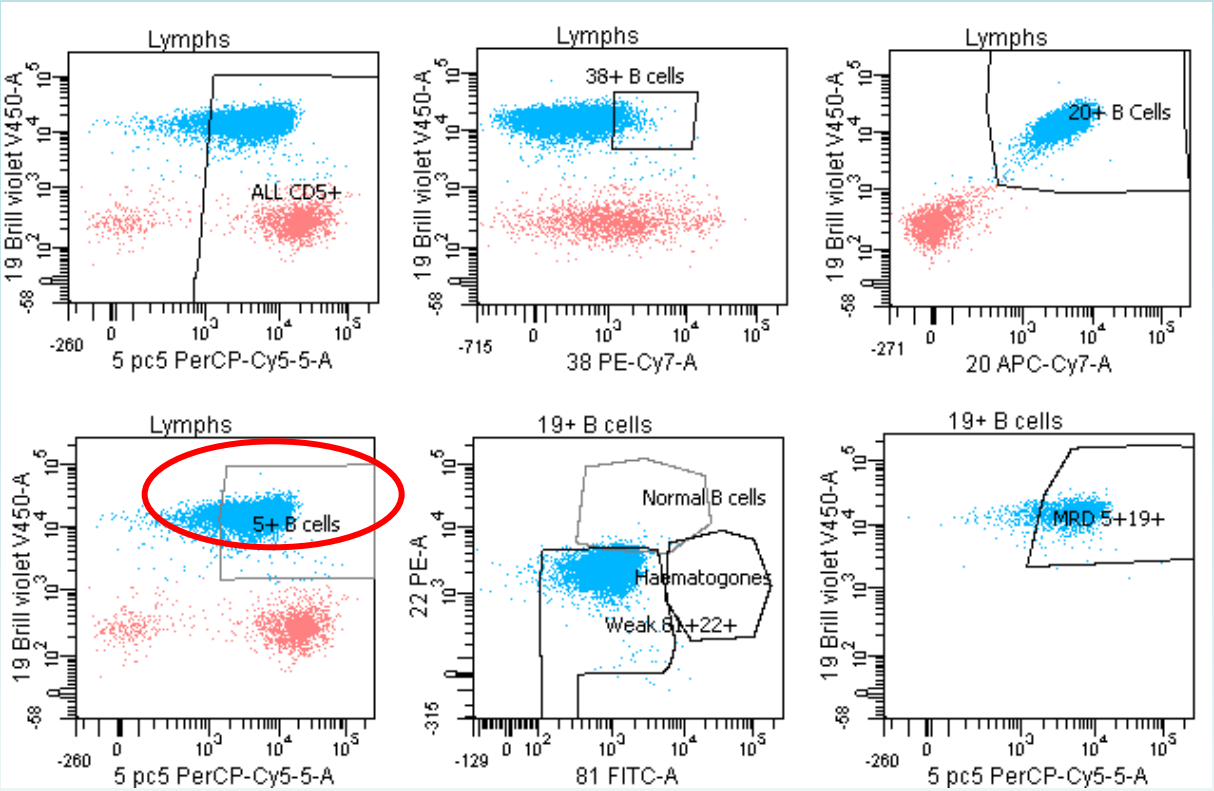


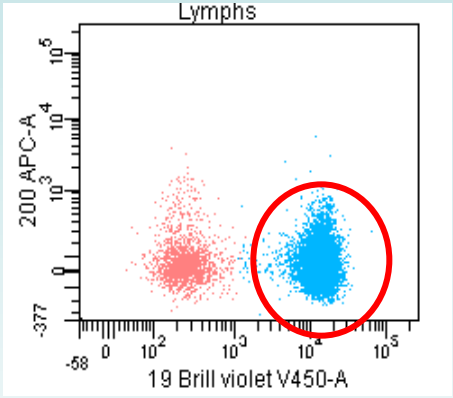
t(14;18)
IgH:BCL2

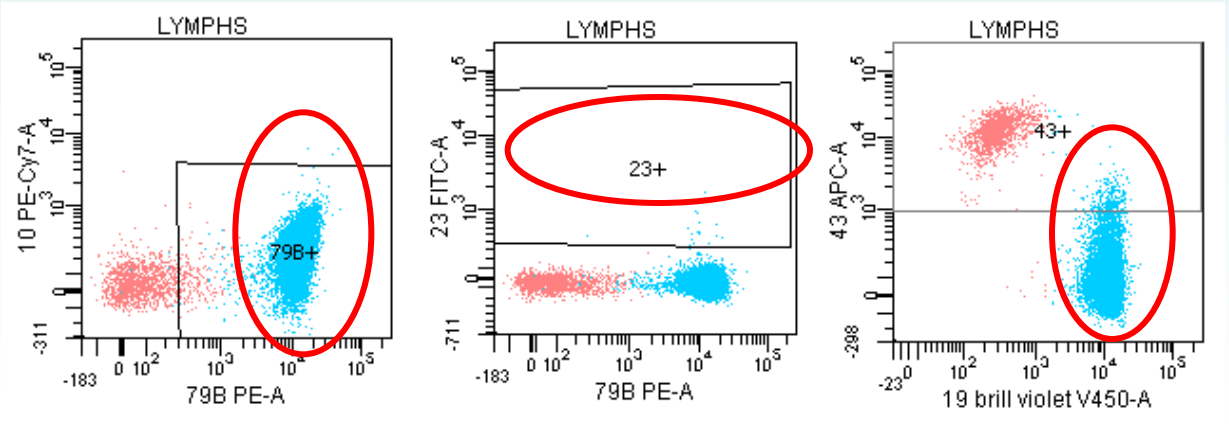
Mantle cell lymphoma




LPD SCREEN 2013-K+4/L+8/19/2/20/3/56
Non-debris
SSC-A
(x 1,000)
FSC-A
(x 1,000)
LPD SCREEN 2013-K+4/L+8/19/2/20/3/
LYMPHS 45/SSC
45 V500-A
SSC-A
LYMPHS 45/SSC
B CELLS (19+)
19 PC5;5 PerCP-Cy5-5-A
3 H7 APC-Cy7-A
LYMPHS 45/SSC
B CELLS (19+/20+)
19 PC5;5 PerCP-Cy5-5-A
20 APC-A
B CELLS (19+)
Lambda
Kappa
K+4 FITC-A

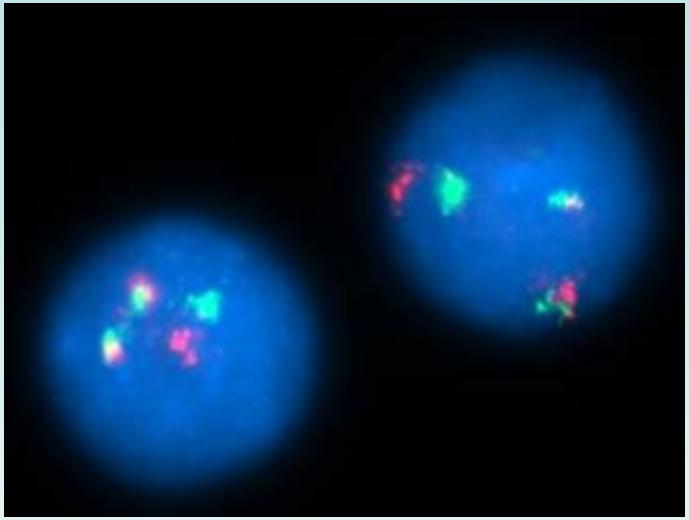

Lymphs
19 Brill violet V450-A
ALL CD5+
5 pc5 PerCP-Cy5-5-A
Lymphs
38+ B cells
38 PE-Cy7-A
Lymphs
20+ B Cells
20 APC-Cy7-A
Lymphs
5+ B cells
5 pc5 PerCP-Cy5-5-A
19+ B cells
Normal B cells
Haematogones
Weak 81+22+
22 PE-A
81 FITC-A
19+ B cells
MRD 5+19+
5 pc5 PerCP-Cy5-5-A


Lymphs
200 APC-A
19 Brill violet V450-A


LYMPHS
10 PE-Cy7-A
79B+
79B PE-A
LYMPHS
23 FITC-A
23+
79B PE-A
LYMPHS
43 APC-A
43+
19 brill violet V450-A

t(11;14) IgH:CCND1

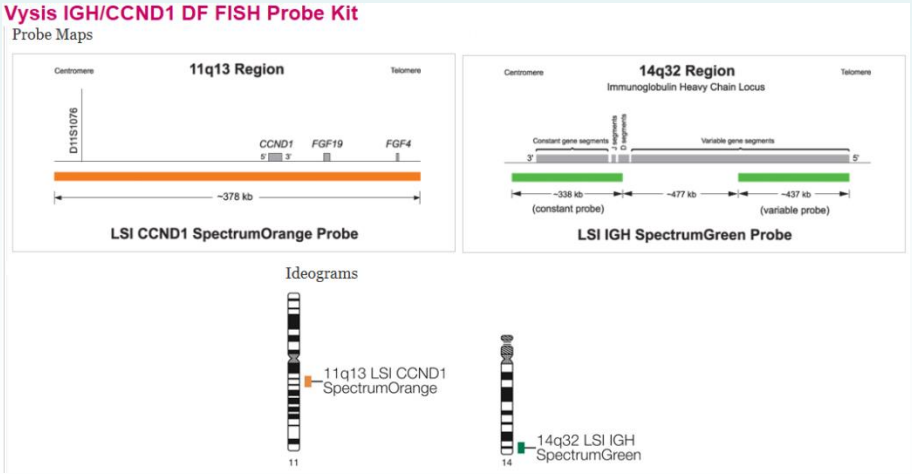
Dual Colour, Dual Fusion Probe

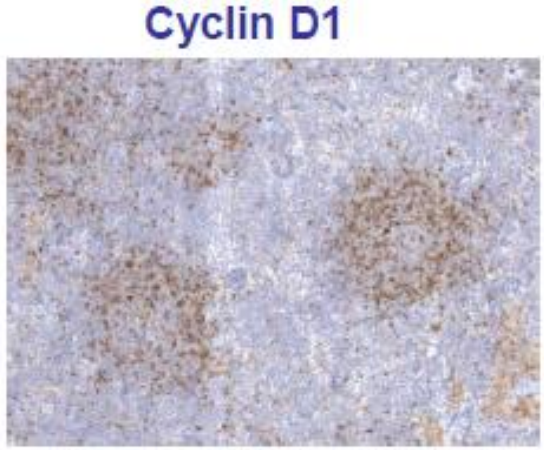


One red, one green, two fusion (1R1G2F) signal pattern.

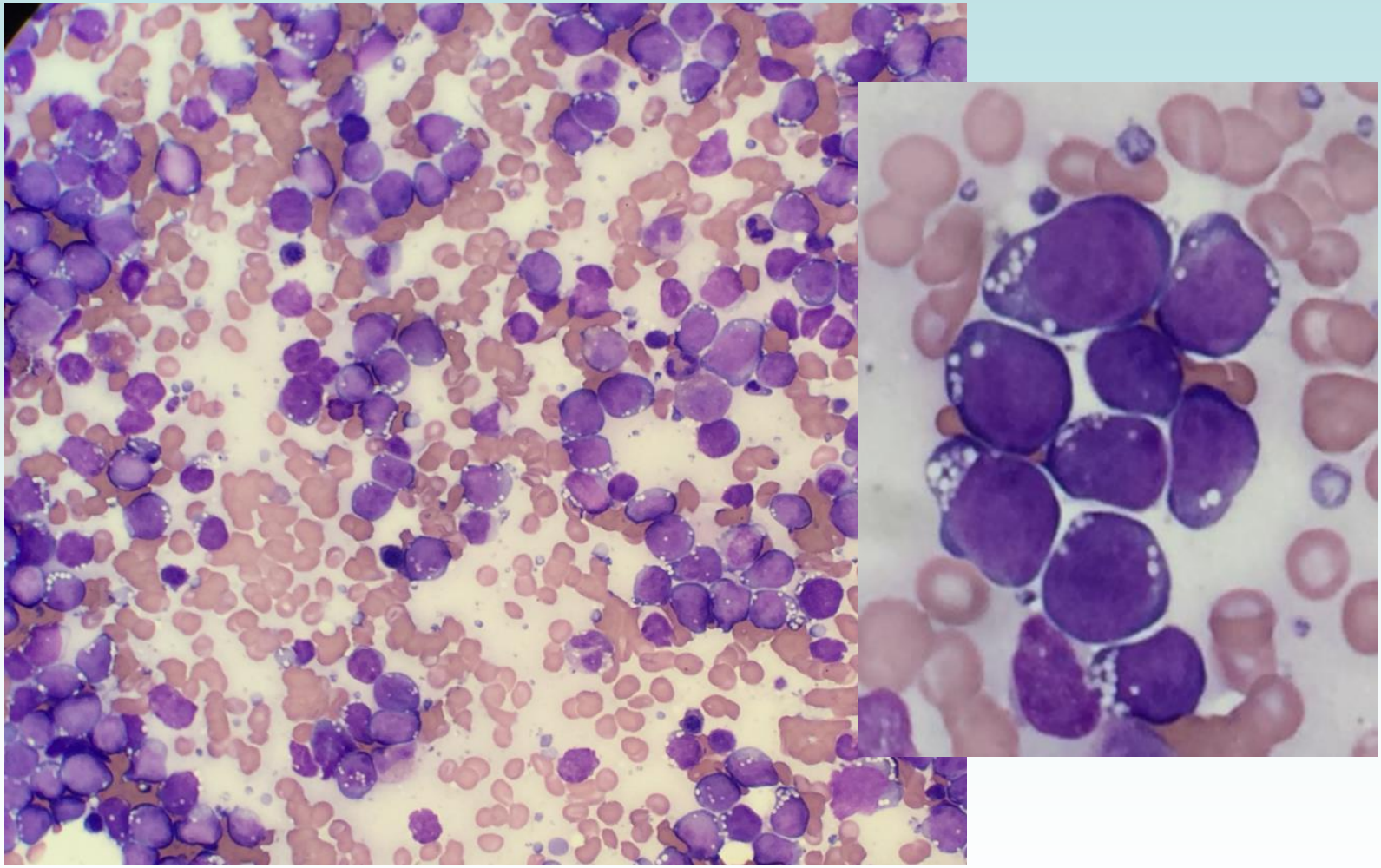
One red = normal CCND1 (11q13) signal
One green = normal IGH (14q32) signal

Two fusion = one on each derivative chromosome 11 and derivative chromosome 14.

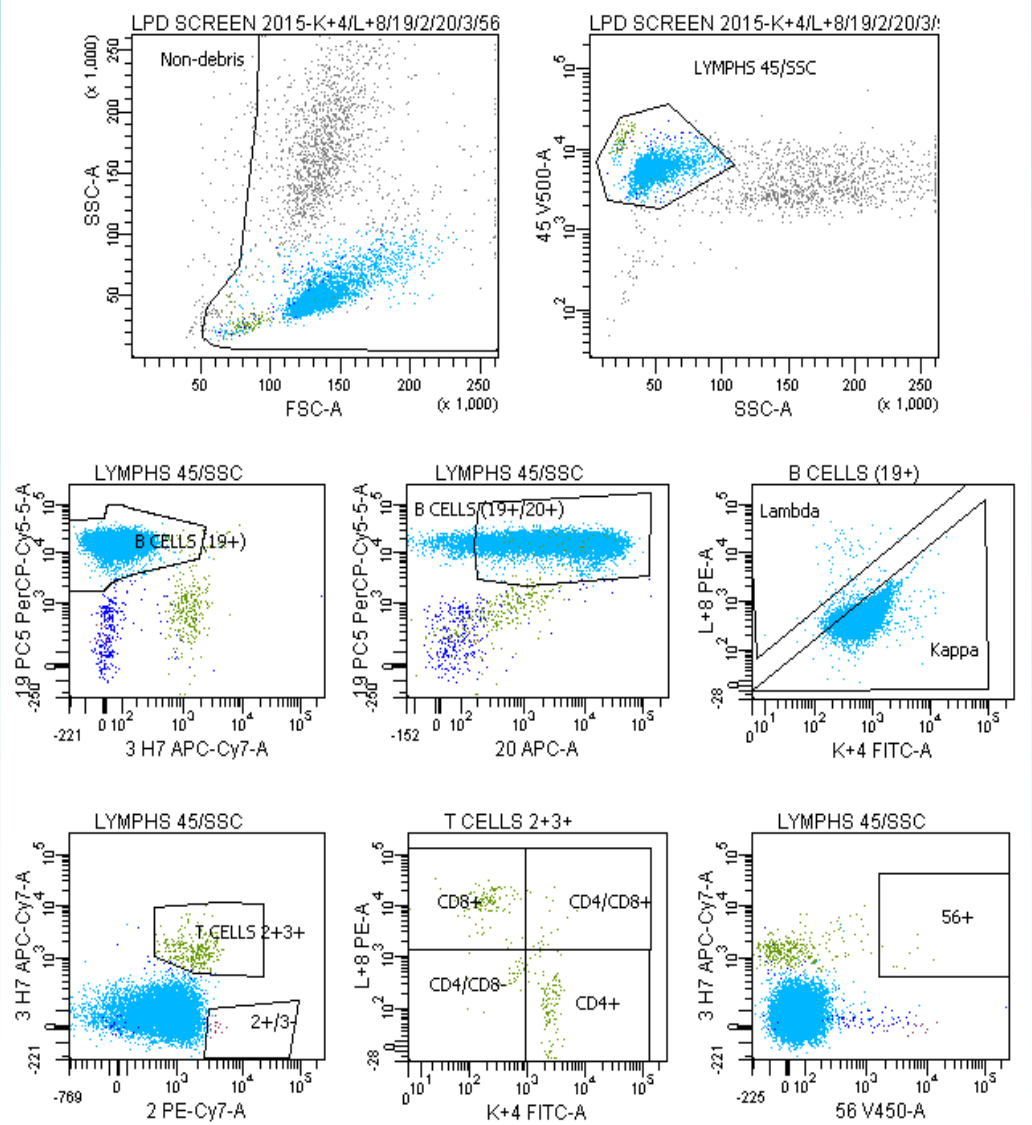


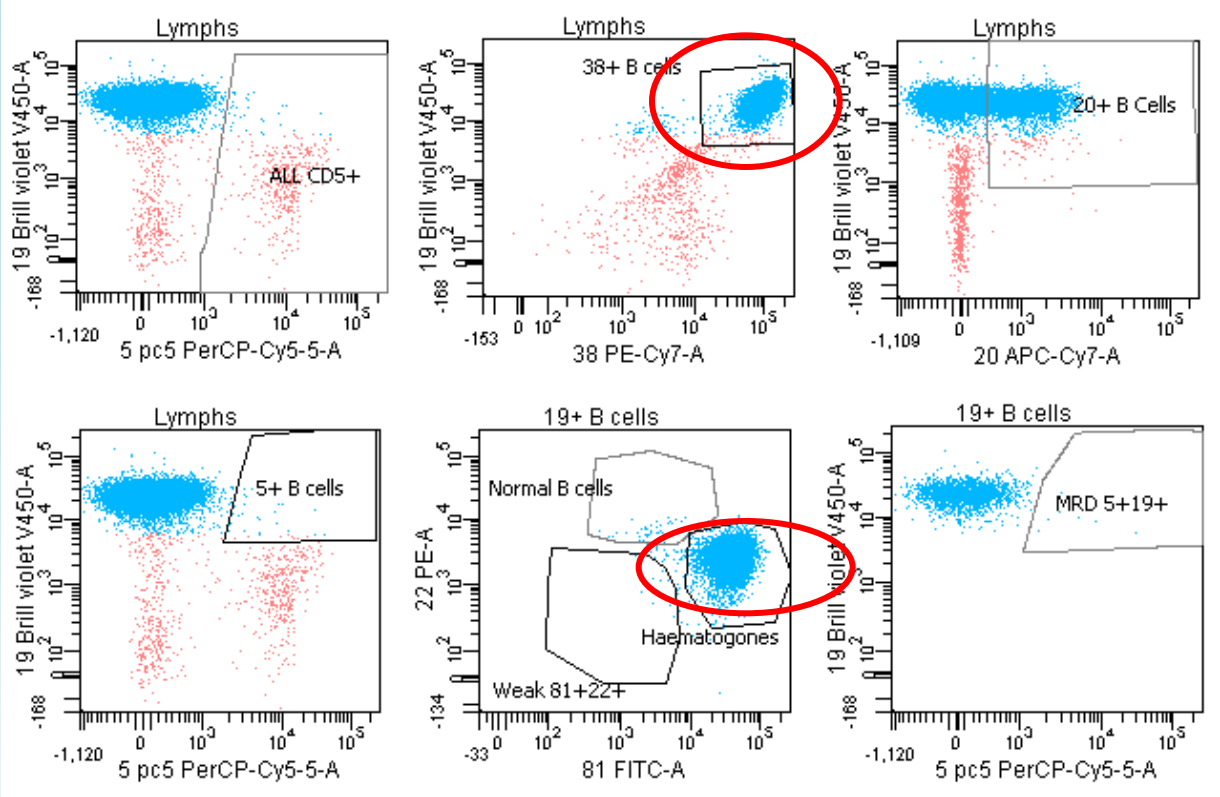


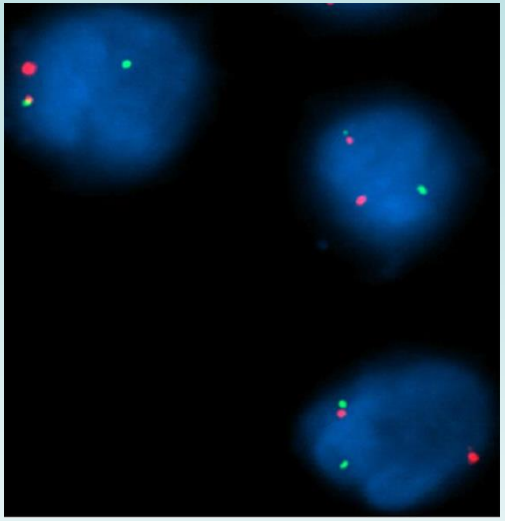
Burkitt Lymphoma



Burkitt Lymphoma

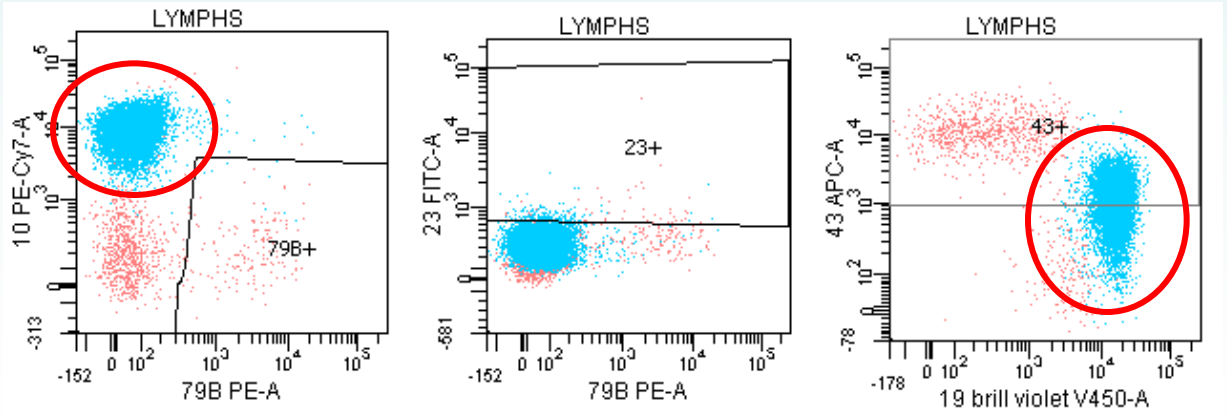






t(8,14) MYC-IgH

Rarely t(2;8) MYC-IgK
or t(8;22) MYC-IgL

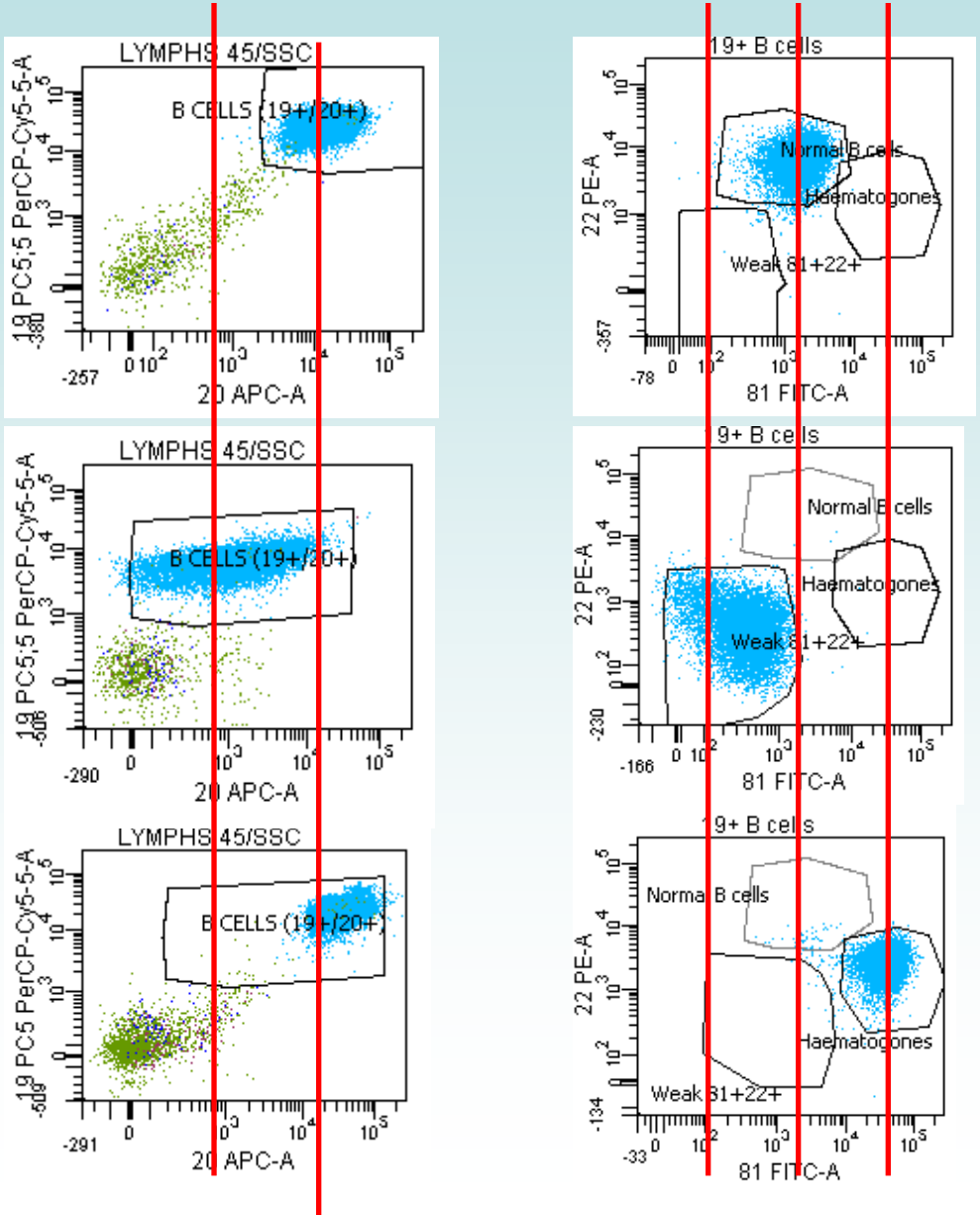


Antigen expression levels

Normal B cells

CLL /SLL

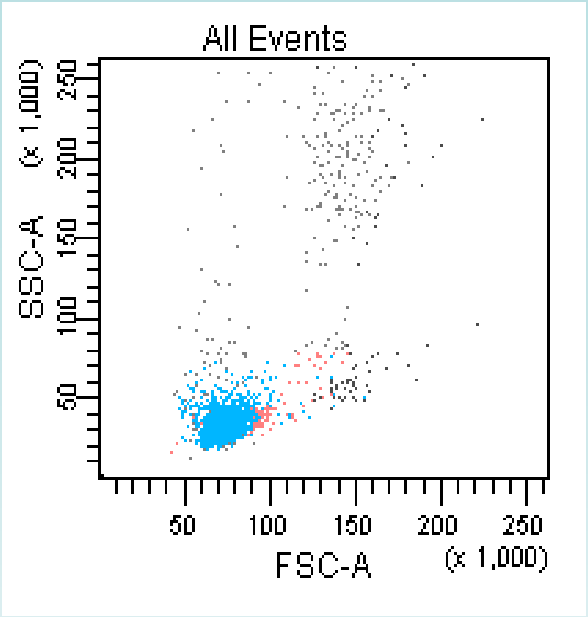
Burkitt lymphoma



Size matters

CLL / SLL

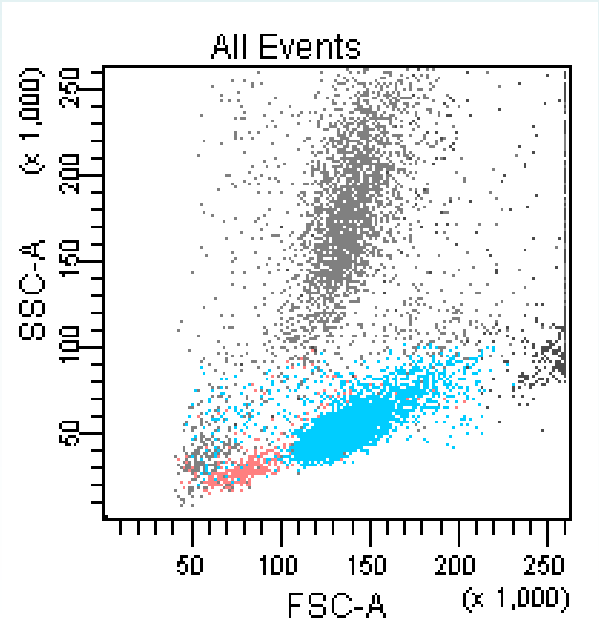
‘low grade’



SIZE

DLBCL

‘high grade’



T cell malignancies

- Lymphoid screen
- CD4:CD8 imbalance
- Loss/diminished antigens

  CD2, CD3, CD5, CD7
- Over expression of antigens

  CD7
- Others

  CD10, CD25, CD26, CD30, HLA-DR

  Cyt.CD3, TDT, TCR $\alpha\beta$ / $\gamma\delta$

Further interrogation of T cells

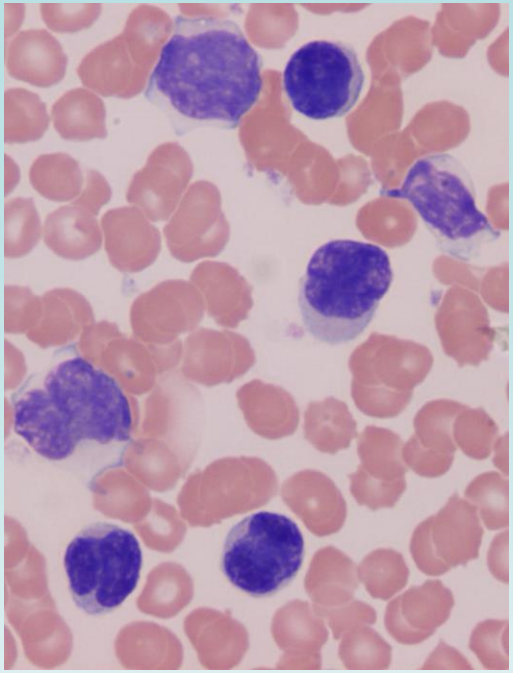
- CD4 positive

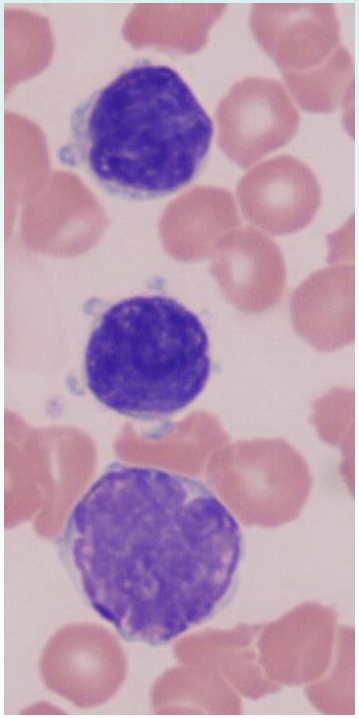
  Peripheral T, Mycosis fungoides/SS, ATLL, ALCL, T-PLL
- CD8 positive

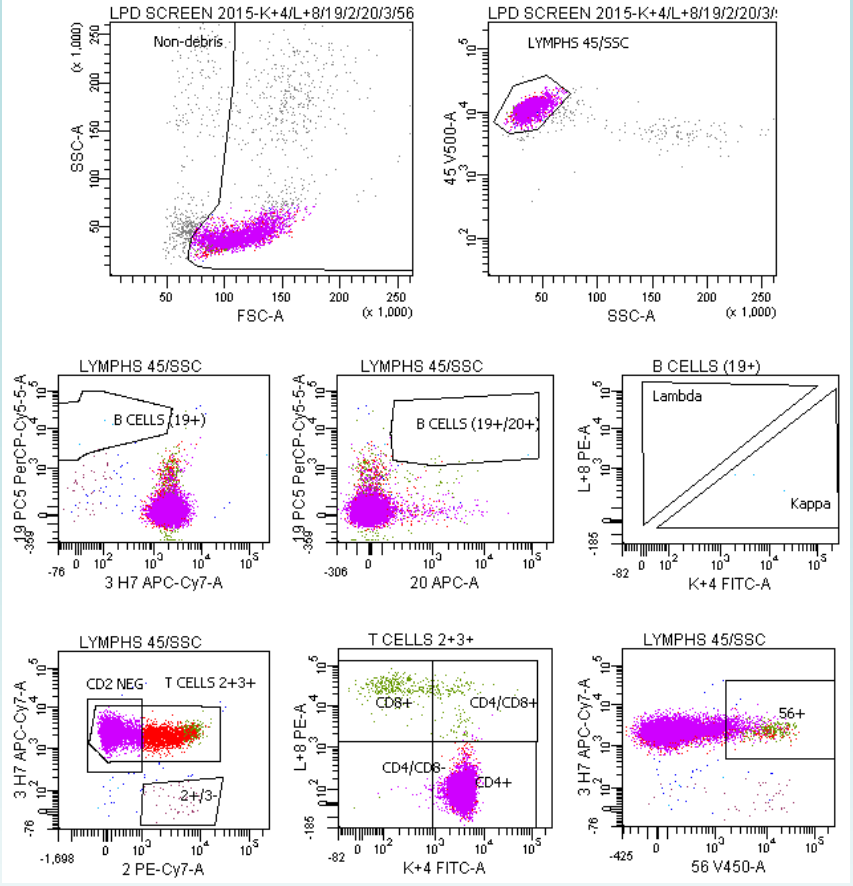
  LGL/NK, EBV+ve,
- CD4/CD8 double positive

  Lymphoblastic lymphoma, AITL, T-PLL,
- CD4/CD8 negative

  HSTCL, Lymphoblastic lymphoma



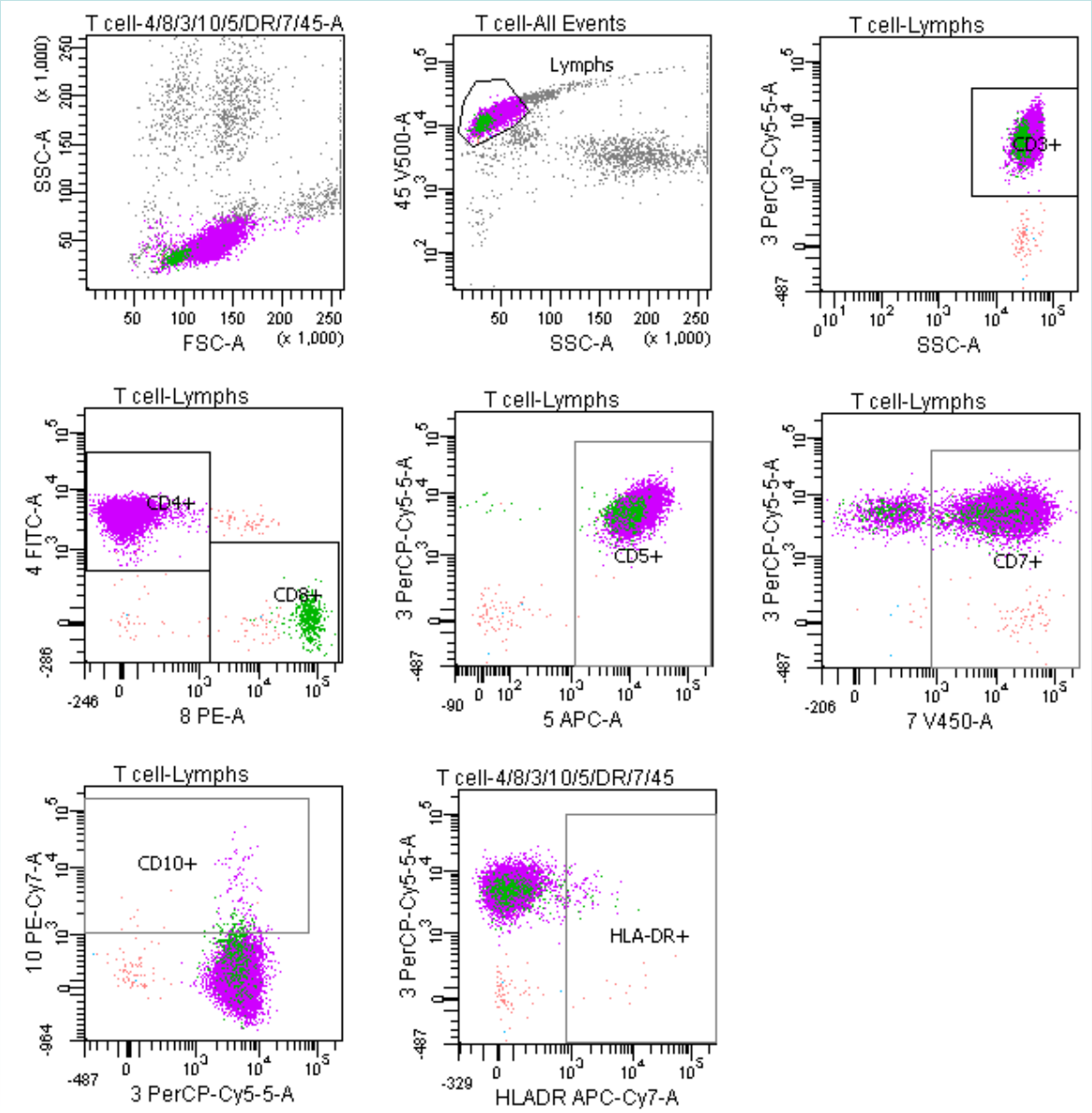


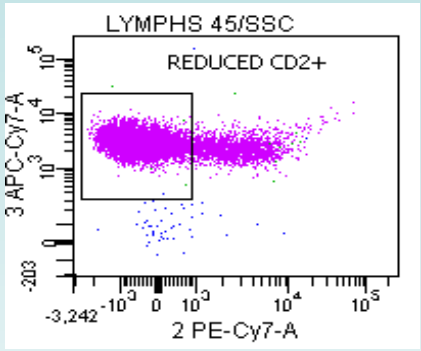


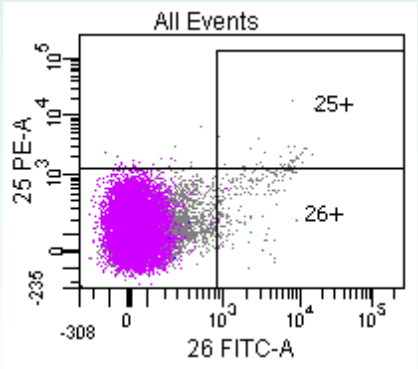
Tube: K+4/L+8/19/2/20/3/56/45

Population	#Events	%Parent	%Total
All Events	12,375	####	100.0
singlets	11,418	92.3	92.3
Non-debris	10,197	89.3	82.4
LYMPHS 45/SSC	9,359	91.8	75.6
B CELLS (19+)	3	0.0	0.0
Kappa	3	100.0	0.0
Lambda	0	0.0	0.0
B CELLS (19+/20+)	11	0.1	0.1
T CELLS 2+3+	9,277	99.1	75.0
56+	817	8.8	6.6
CD4+	8,774	94.6	70.9
CD8+	331	3.6	2.7
CD4/CD8-	28	0.3	0.2
CD4/CD8+	148	1.6	1.2
CD2 NEG	7,163	77.2	57.9
2+/3-	44	0.5	0.4
NK 8+	40	90.9	0.3
NK 56+	35	79.5	0.3

Extended T cell







Flow cytometry of fresh tissue

Aims

- Obtain a single cell suspension from a solid tissue biopsy
- Analyse by multi-parametric flow cytometry
- To provide a ‘working diagnosis’
- Provisional report <3hrs
- ‘Steer’ further investigations

Requirements

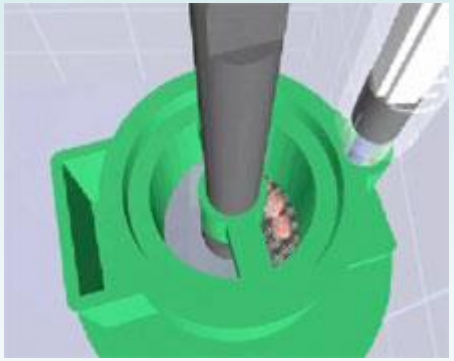
- Fresh unfixed tissue biopsy

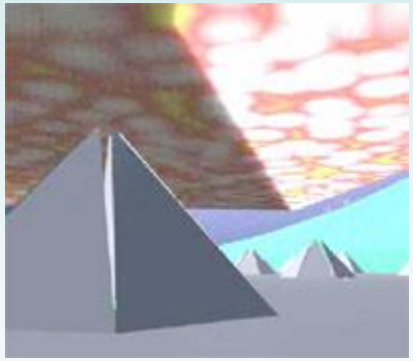
  Gross specimen, core biopsy, aspirate

  (Lymph node, Liver, Spleen, Tonsil, Testis, Skin, lumps and bumps, bone marrow trephine)
- Ideally <6hrs old but up to 24hrs if stored in PBS or culture fluid.
- If a small biopsy use culture fluid or saline to prevent drying

Processing

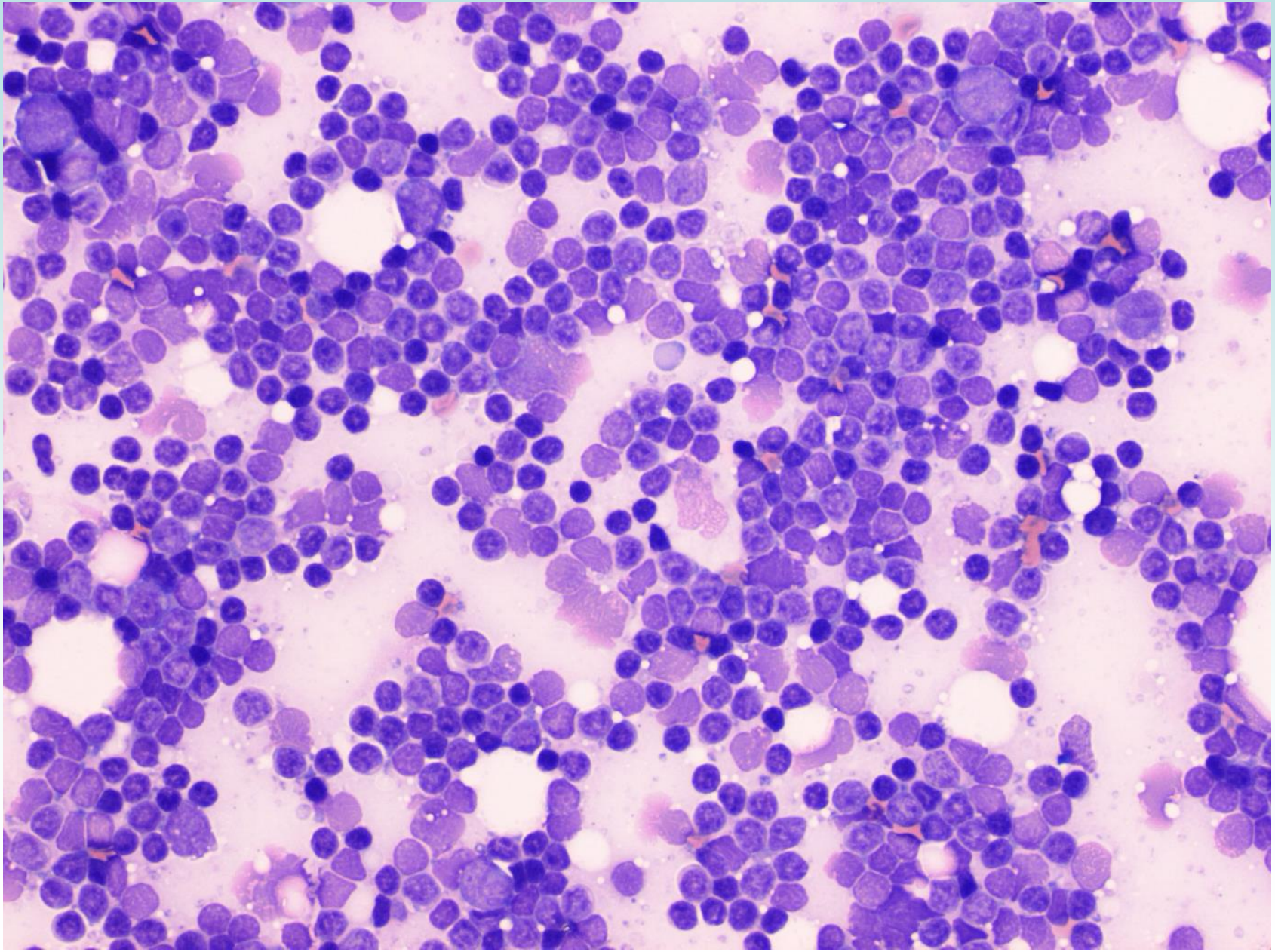
- Cut new surface & make imprint
- Metal mesh
- 21g Hyperdermic needle / Scalpel+
- Medicon – tissue grater



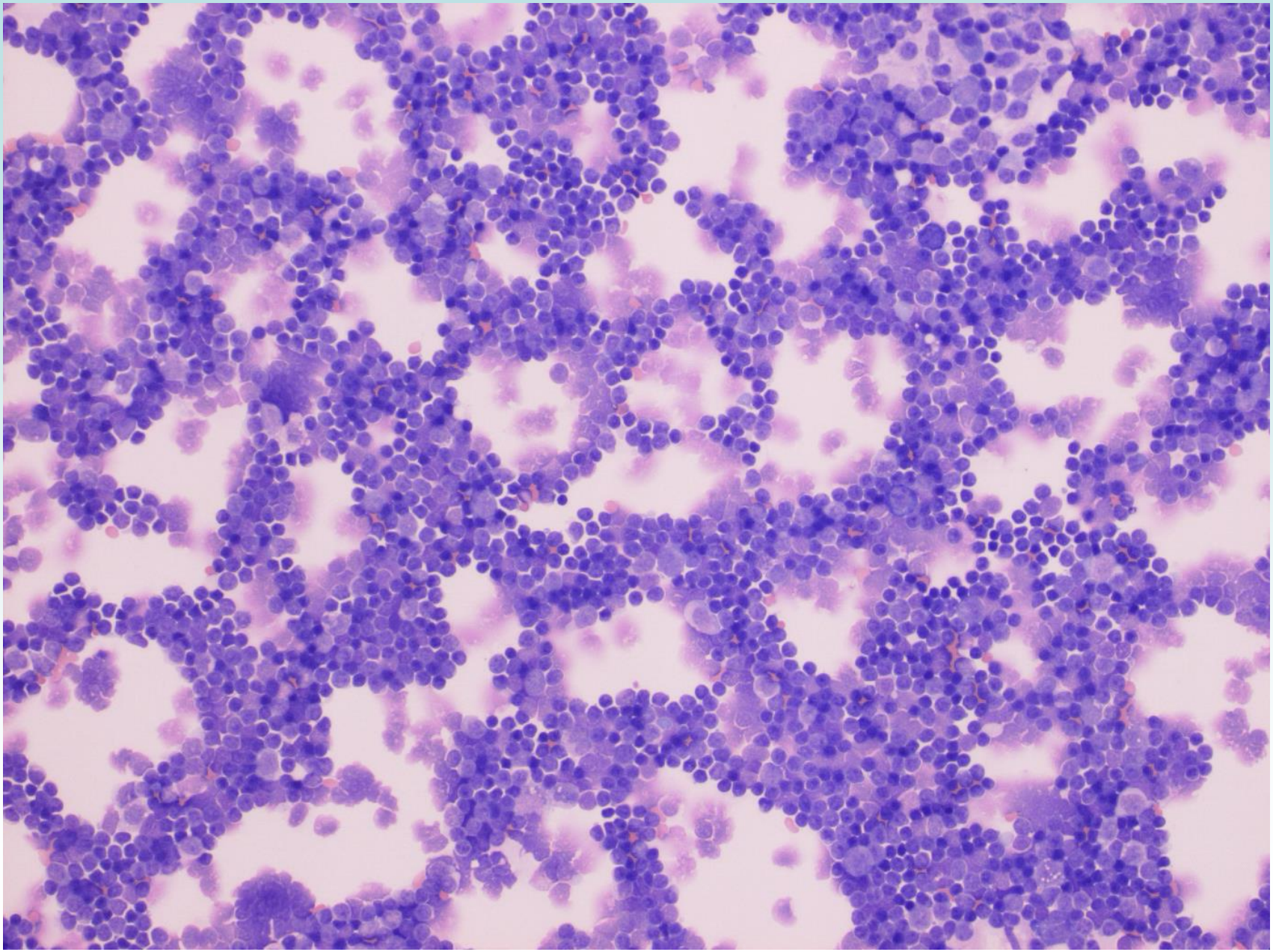


- Prepare cytospin, label, flow
- Try to avoid aerosols

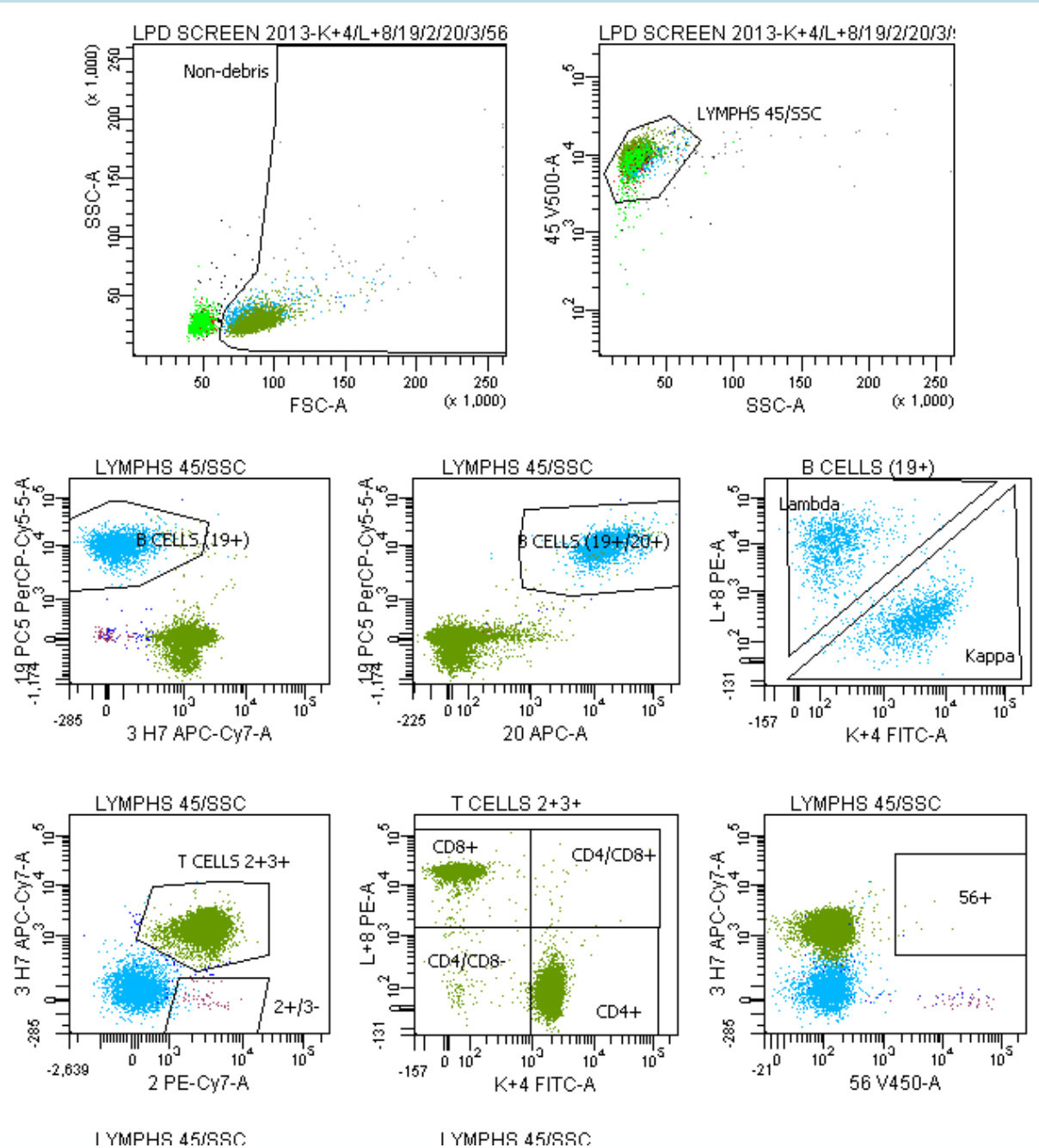
Tissue bx imprint



Cytospin prep



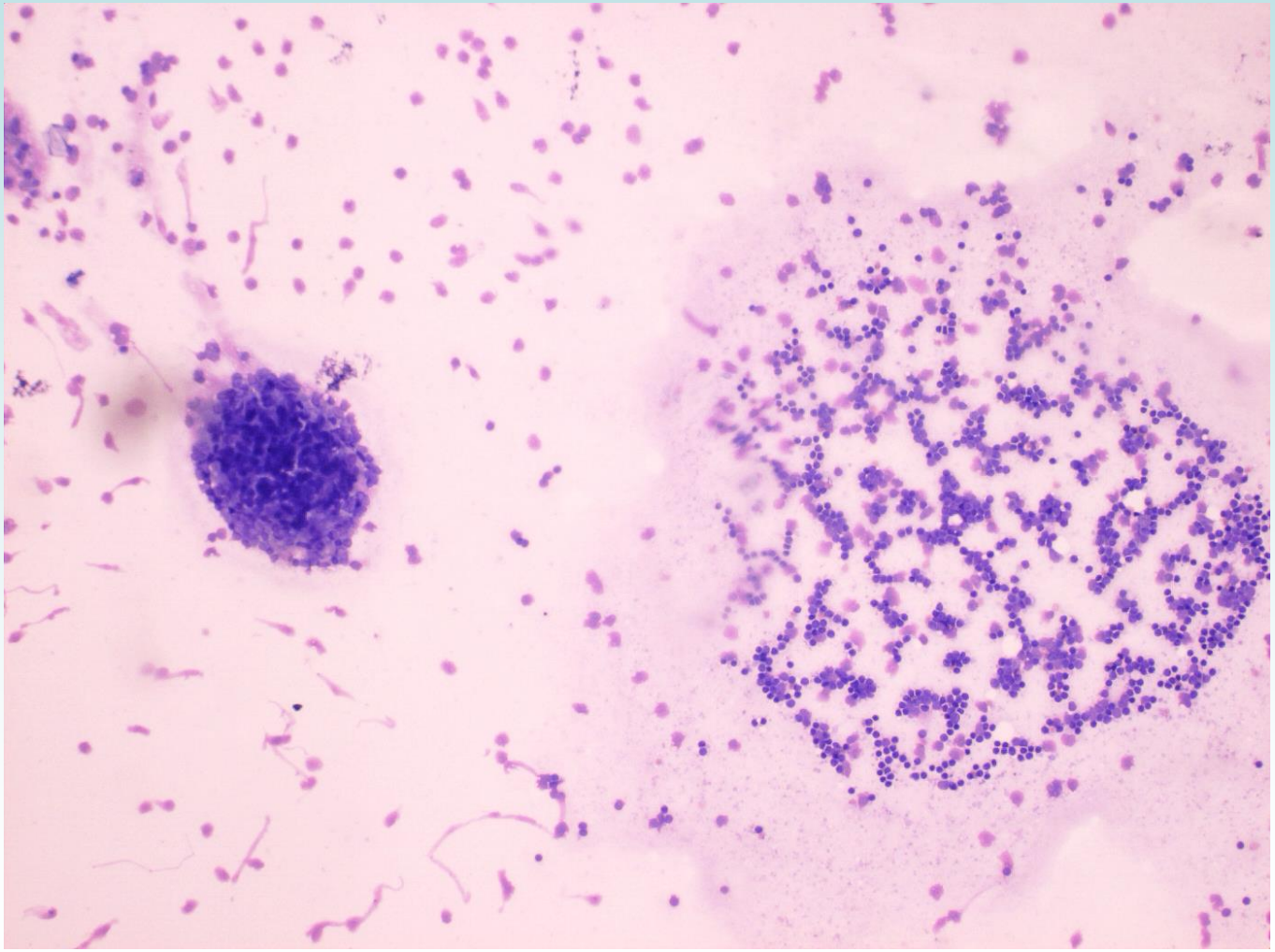
LPD screen

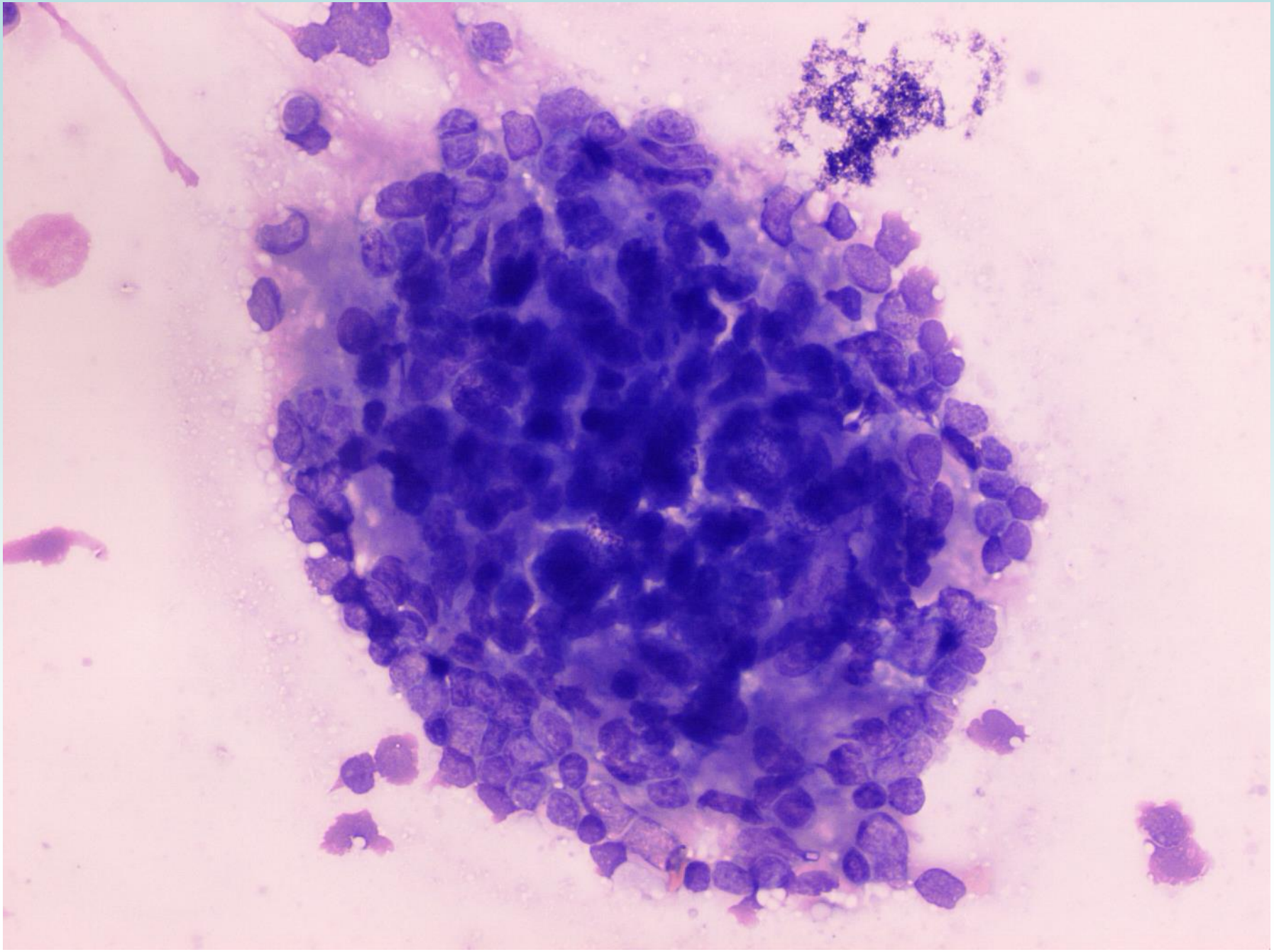


Tube: K+4/L+8/19/2/20/3/56/45

Population	#Events	%Parent	%Total
All Events	12,295	####	100.0
singlets	12,113	98.5	98.5
Non-debris	9,689	80.0	78.8
LYMPHS 45/SSC	9,617	99.3	78.2
B CELLS (19+)	2,556	26.6	20.8
Kappa	1,431	56.0	11.6
Lambda	1,094	42.8	8.9
B CELLS (19+/20+)	2,578	26.8	21.0
T CELLS 2+3+	6,949	72.3	56.5
56+	7	0.1	0.1
CD4+	5,025	72.3	40.9
CD8+	1,755	25.3	14.3
CD4/CD8-	143	2.1	1.2
CD4/CD8+	32	0.5	0.3
2+/3-	88	0.9	0.7
NK 8+	26	29.5	0.2
NK 56+	35	39.8	0.3
NOT(Non-debris)	2,424	20.0	19.7
B	604	24.9	4.9
T	1,719	70.9	14.0

A word of warning….





Summary

Comprehensive diagnosis of lymphoma requires a fully integrated approach

- Clinical & radiological assessment
- Haematology/biochemistry investigations
- Morphology (PB, BM, Tissue architecture)
- Phenotype (IHC and flow cytometry)
- Genotype (FISH & molecular analysis +/-Clonality)

- Referral to an MDT

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