Adapting to technological change in haematopathology

Fine-needle aspiration and haemato-oncology diagnostics

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Issues

- Haematopathologists in the UK are generally tissue biased.

- The 'Google effect'
- Rapid *mimimally invasive diagnosis* in 'lumps & bumps' clinics.

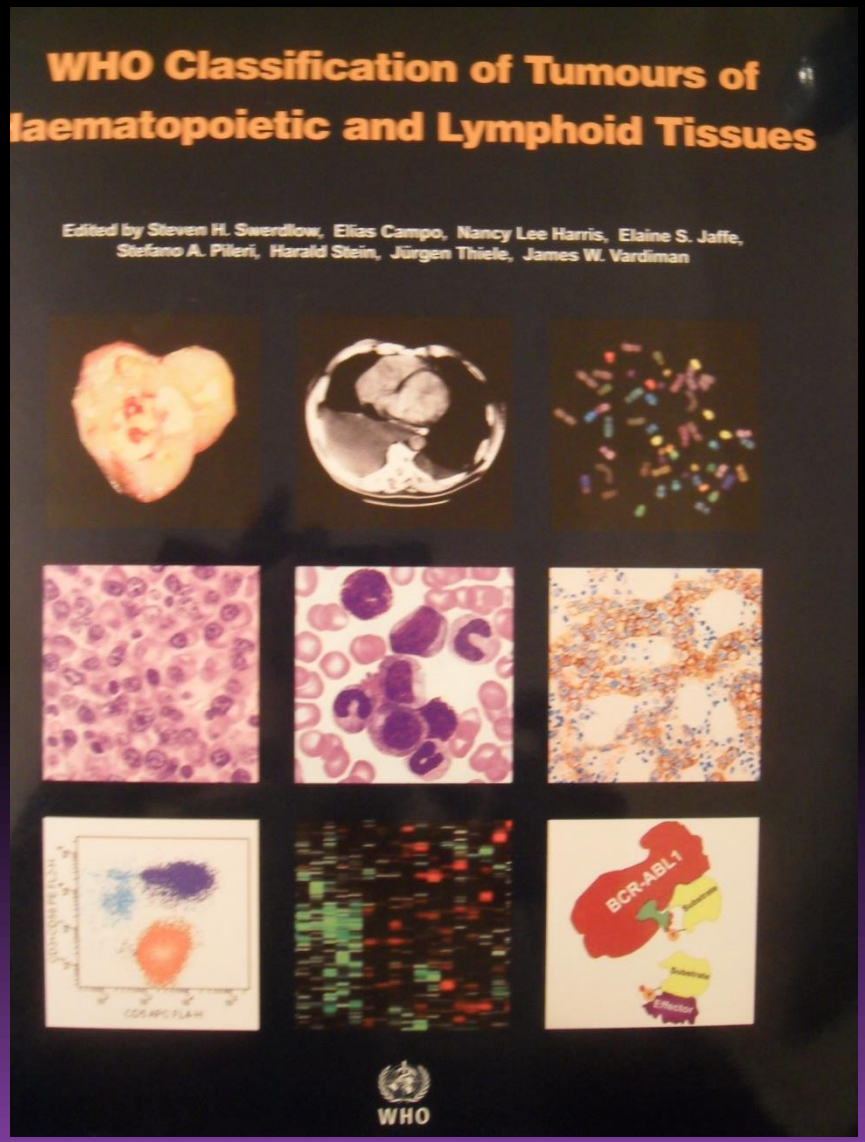
- Imaging (US/CT, esp PET) are now picking up incidental / post therapy visceral lesions.
- PET – not entirely specific, often picks up macrophage response avidly in post therapy lesions (Deauville score: >3) – requiring sampling to determine if residual disease is present.
- Morbidity / mortality associated with surgical access usually precludes utility of this modality.
- Often lesions are at sites where interventional radiologists would not dare to go.
- Therapy controls disease better, so more relapses encountered in routine clinical practise – do we have to biopsy all of them?

Cytologic material & haematolymphoid malignancy diagnosis

- Clinical
  - Can one use cytologic material to make a diagnosis of lymphoma / leukemia (akin to using a blood sample)?
  - Is this diagnosis reliable?
  - Are we able to get enough material out to do the same workup that we would be able to do on biopsy material?
  - Is the extent of sampling adequate?

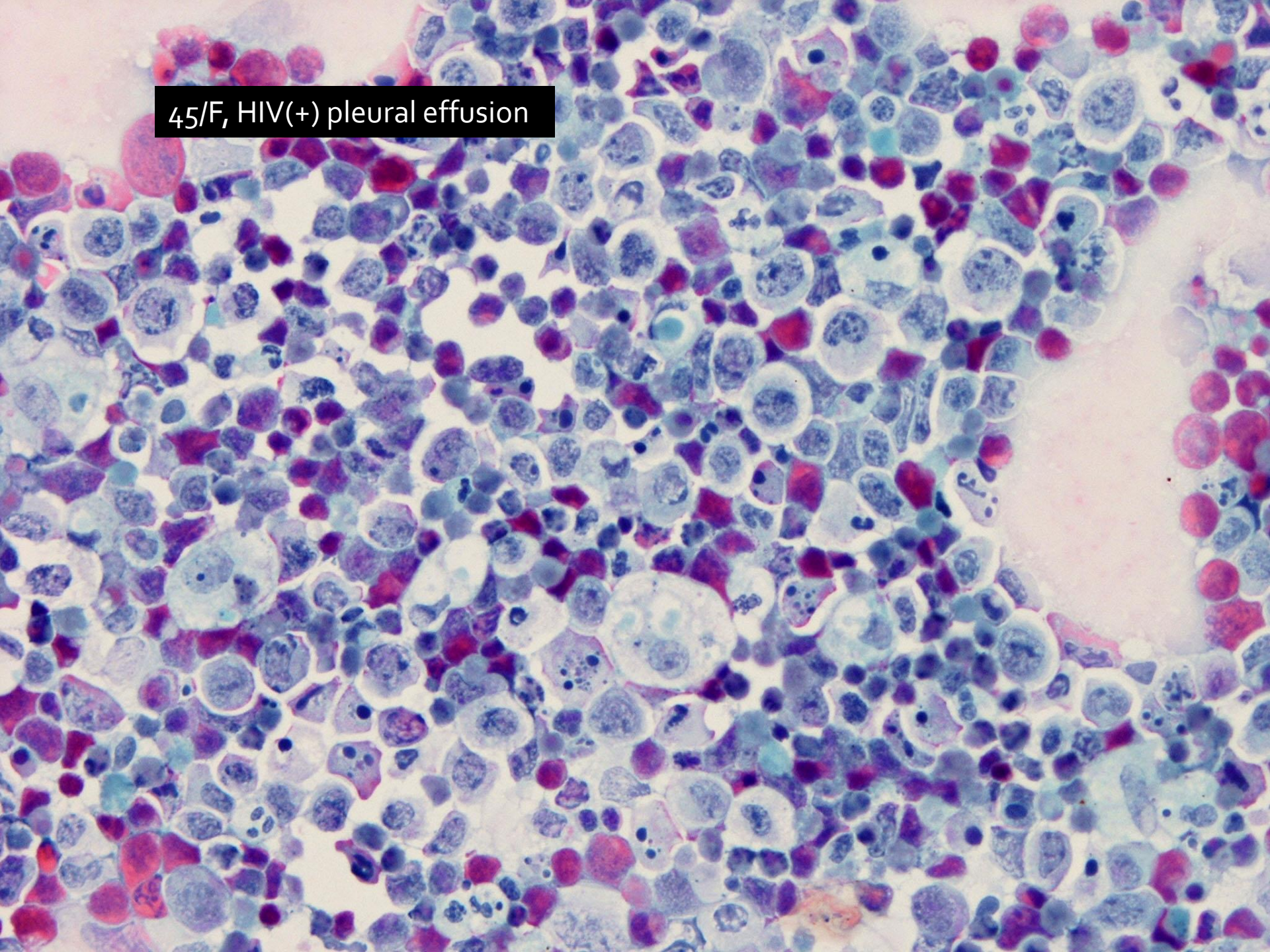
- Laboratory
  - Is it possible to perform ancillary testing on such samples – immunohistochemistry, flow cytometry, FISH, B & T-cell clonality studies?
  - Can one convert cytologic material to histologic material?

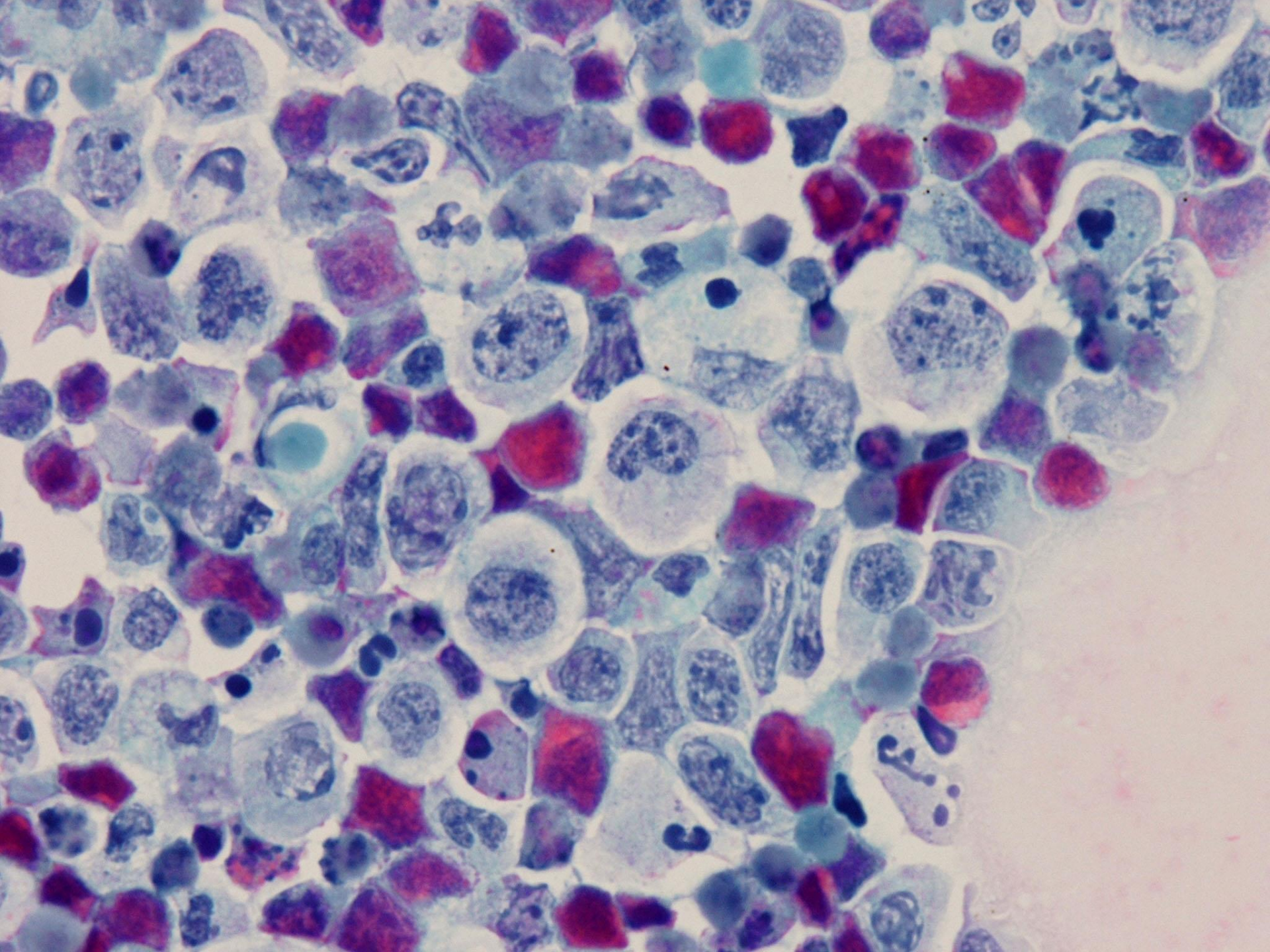
Can one use cytologic material to make a diagnosis of lymphoma / leukemia (akin to using a blood sample)?

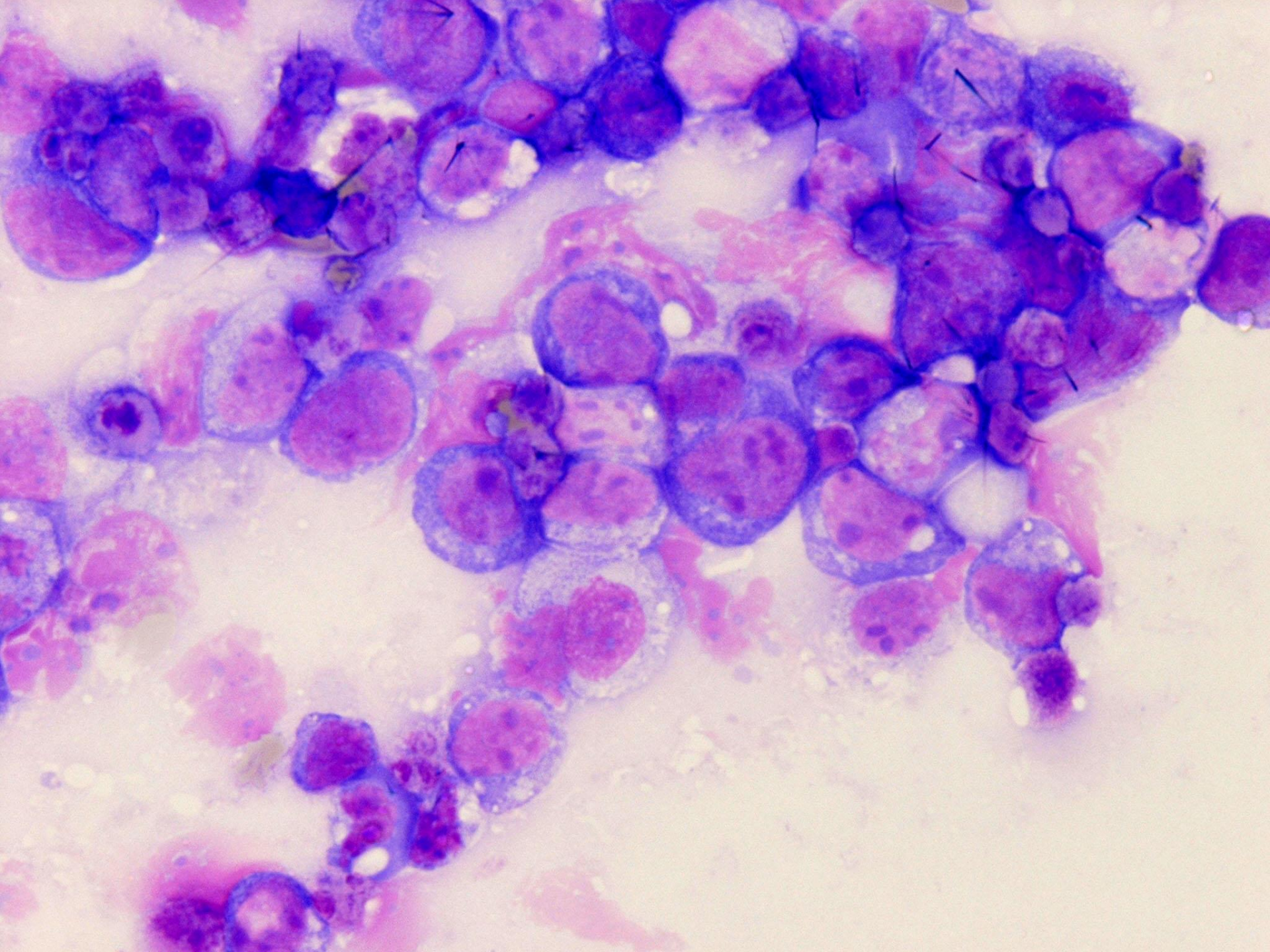


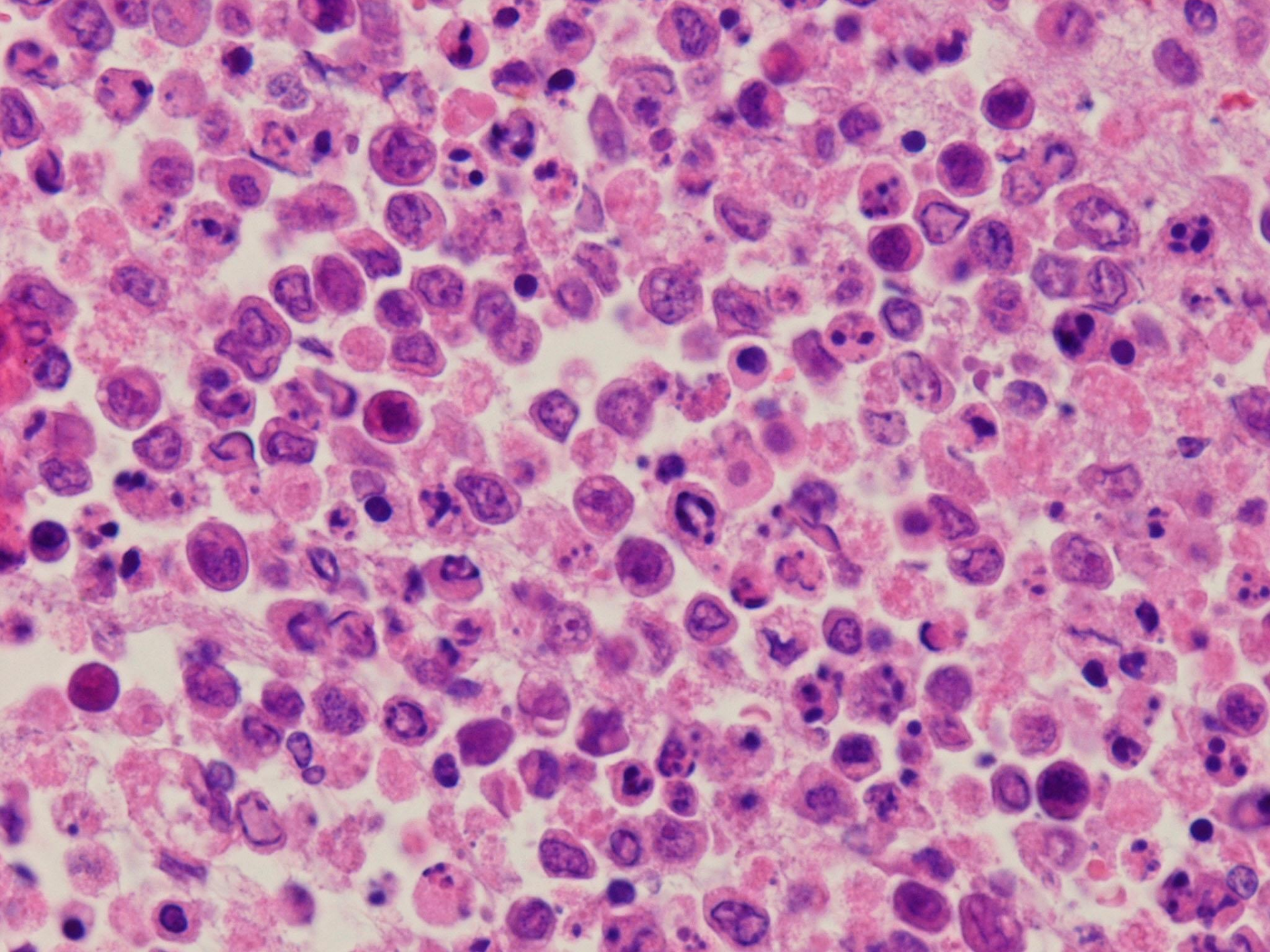
WHO 2008 – diagnosis based on:

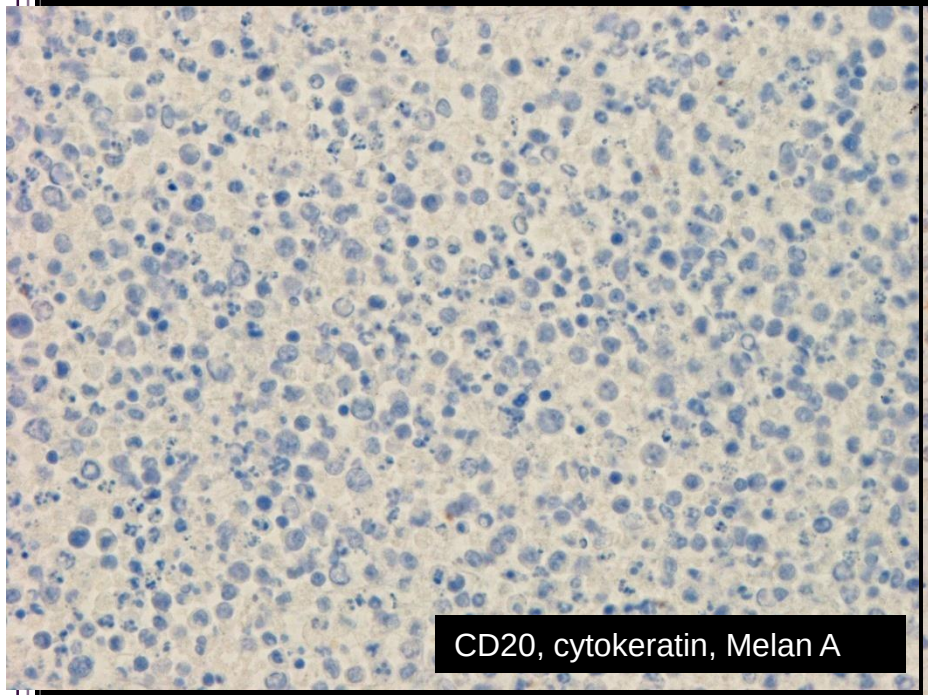
- Cytologic
- Immunophenotypic
- Genetic
- Molecular data

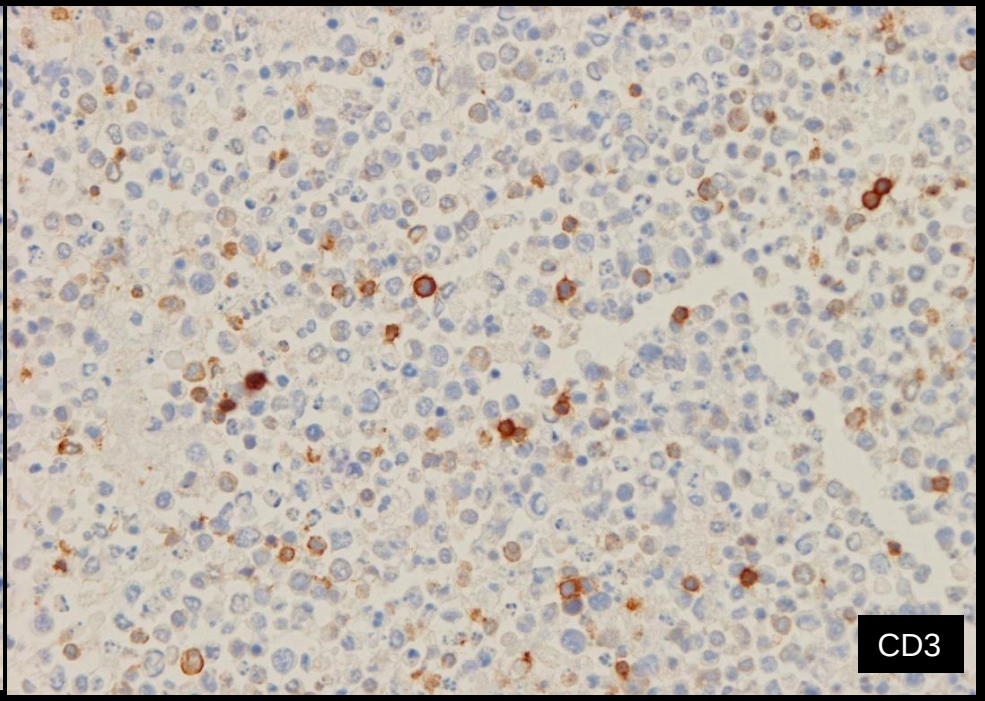

45/F, HIV(+) pleural effusion

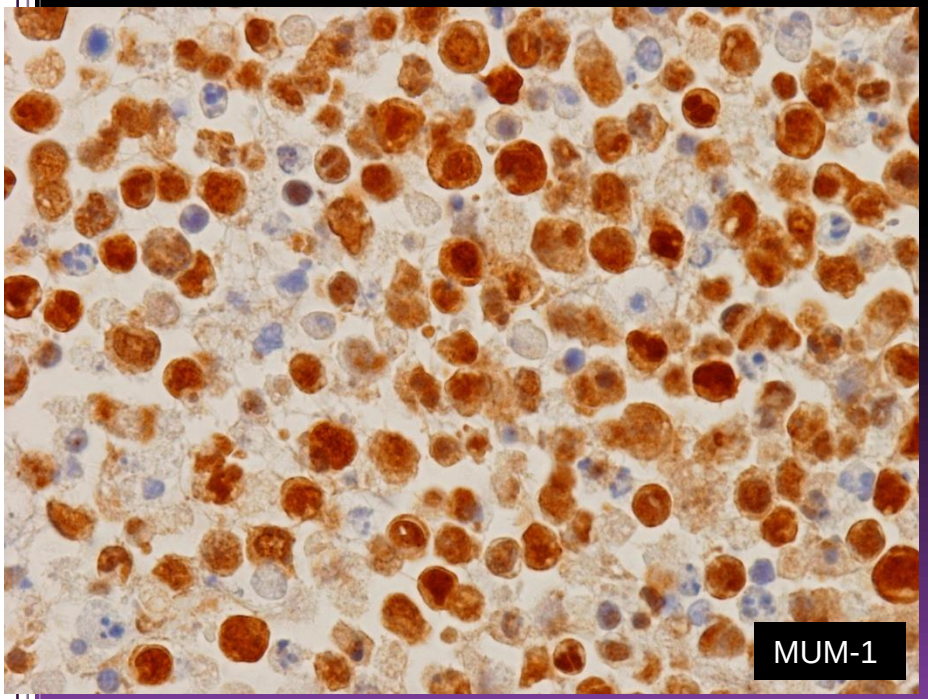


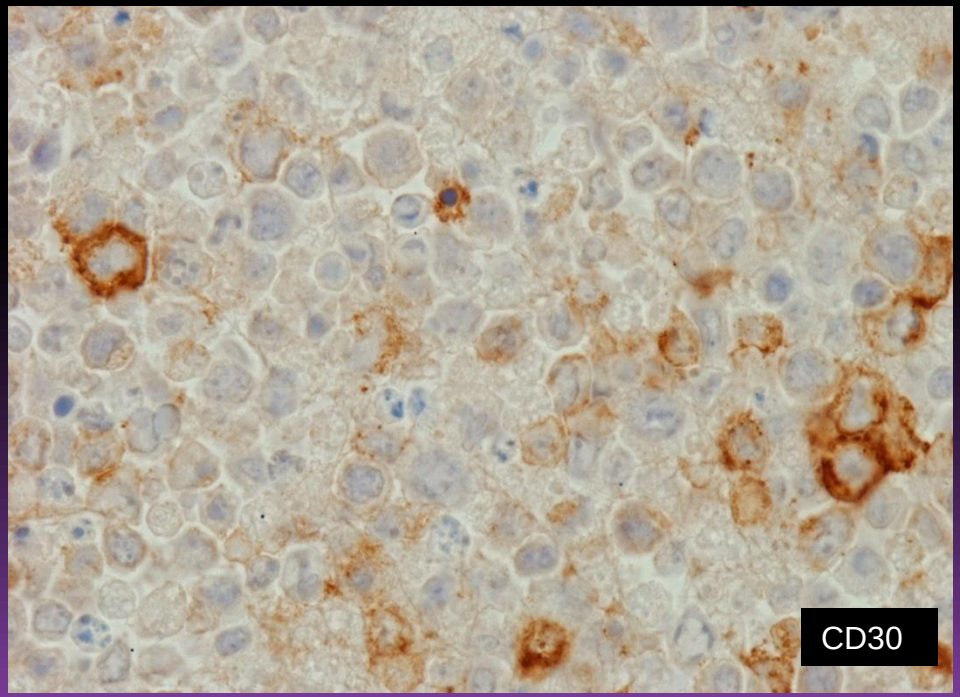


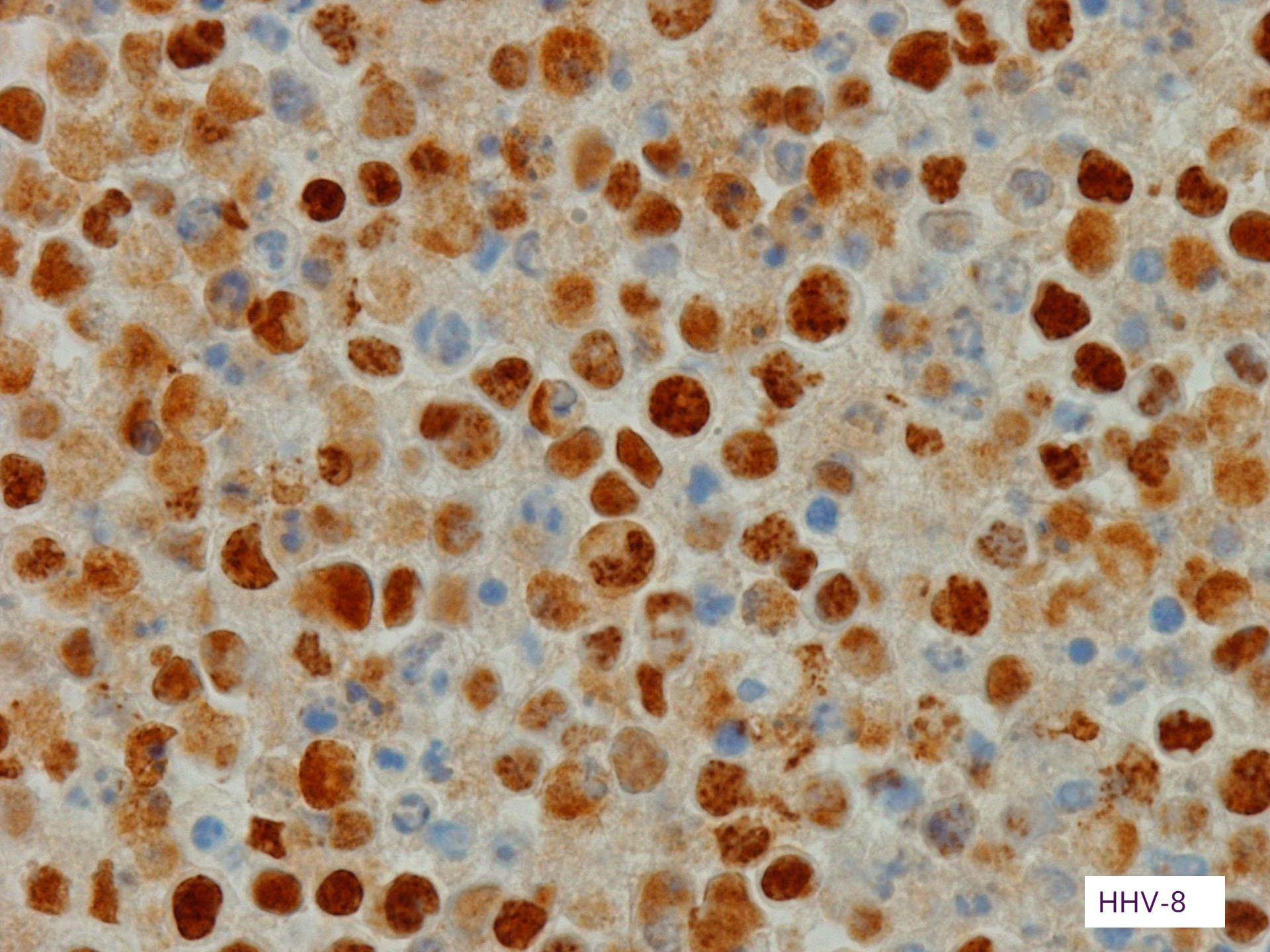



CD20, cytokeratin, Melan A


CD3


MUM-1


CD30


HHV-8

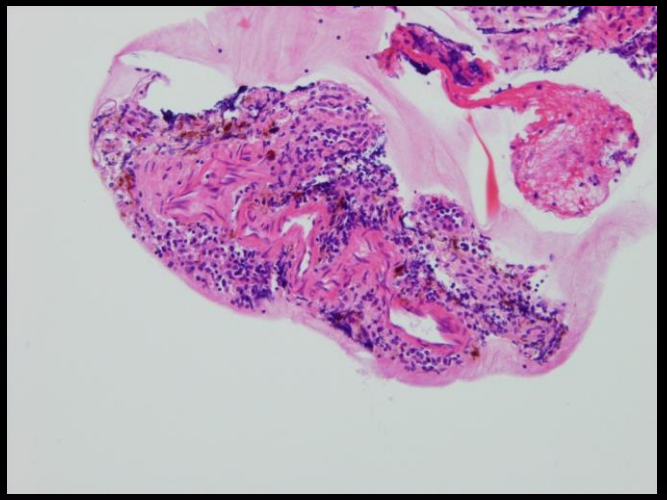
Diagnosis:

Primary Effusion lymphoma

Cell block technology

- Conversion of cytology specimens → histologic specimen
- Most cytology specimens if appropriately collected.
- Needle washings / additional passes
- Normal saline (2 ml)
- Centrifuged
- Supernatant discarded
- Sediment mixed with plasma
- Thrombin added
- Clot prepared
- Clot transferred to casettes
- Fixed & routine processing

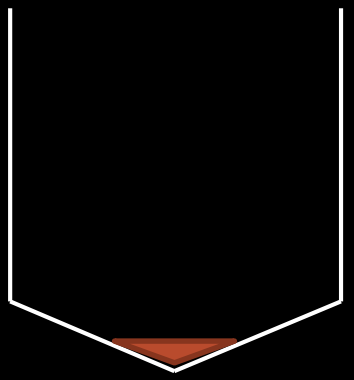
- Allows evaluation of architecture

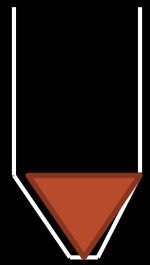


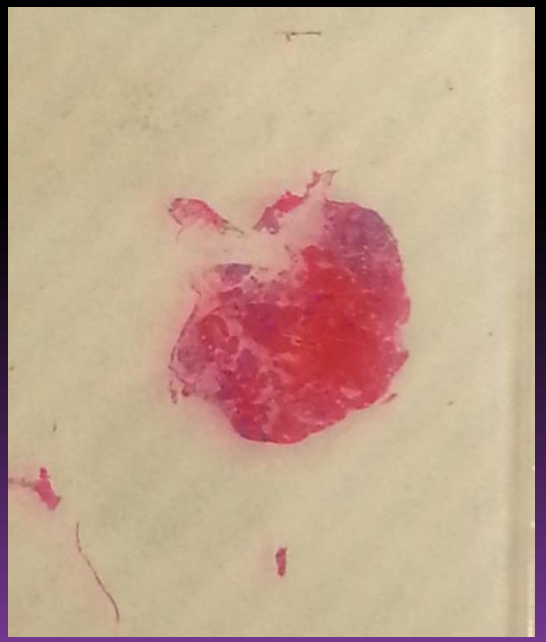
- Allows
  - Special stains
  - Immunohistochemistry
  - FISH
  - Molecular (PCR / NGS)

Cell blocks – technical issues

- Type of container used







Cell blocks – technical issues

Double the material

EBUS: **E**ndoscopic **B**ronchial **U**ltra**S**ound
EUS: Endoscopic ultrasound

Visceral lymph node / mass sampling

The EBUS story

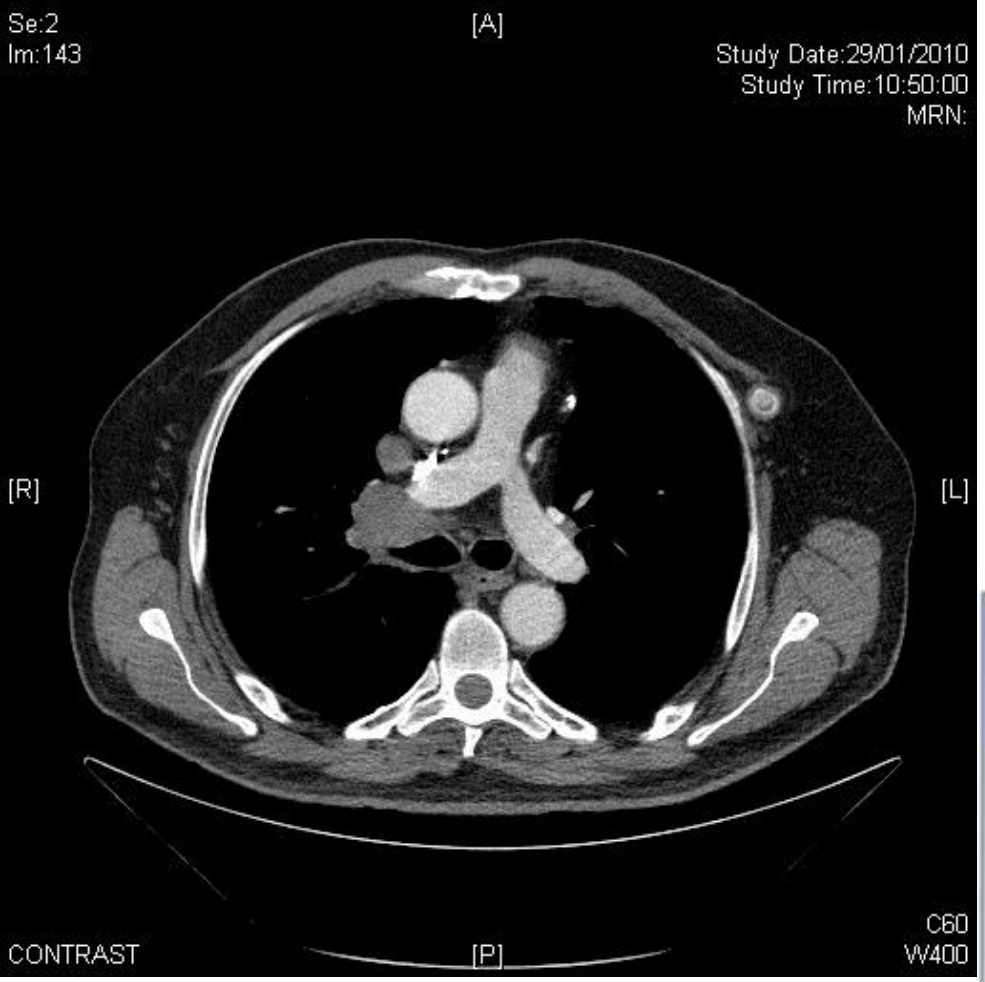


TABLE 3
Procedures Used to Sample Lymph Nodes, by Lymph Node Level

Lymph node level	Mediastinoscopy	Thoracotomy	Chamberlain/VATS	Esophageal sonography
2L, 2R	✓	✓		
4L, 4R	✓	✓		✓
5, 6		✓	✓	✓
7	✓	✓		✓
8, 9		✓		✓
10L, 10R		✓	✓	
11–14		✓		

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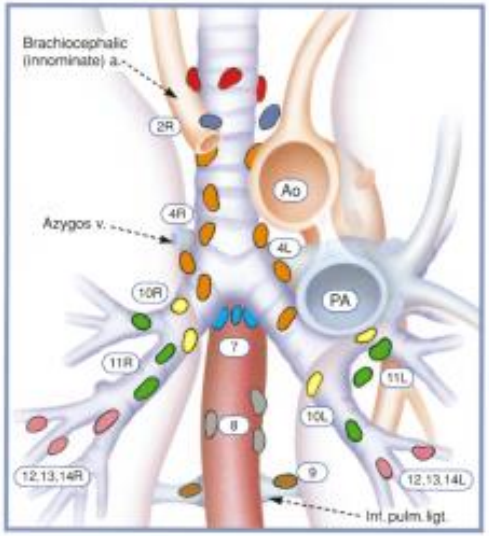

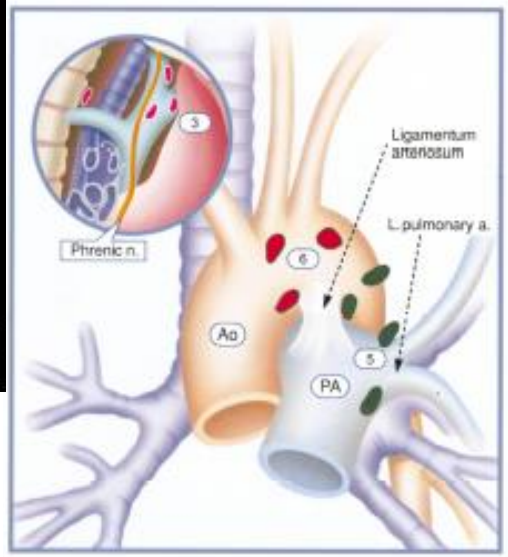

Superior Mediastinal Nodes

- 1 Highest Mediastinal
- 2 Upper Paratracheal
- 3 Pre-vascular and Retrotracheal
- 4 Lower Paratracheal (including Azygos Nodes)

N_2 = single digit, ipsilateral
N_3 = single digit, contralateral or supraclavicular

Aortic Nodes

- 5 Subaortic (A-P window)
- 6 Para-aortic (ascending aorta or phrenic)

Inferior Mediastinal Nodes

- 7 Subcarinal
- 8 Paraesophageal (below carina)
- 9 Pulmonary Ligament

N_1 Nodes

- 10 Hilar
- 11 Interlobar
- 12 Lobar
- 13 Segmental
- 14 Subsegmental

(Mountain/Dresler modifications from Naruke/ATS-LCSG Map)

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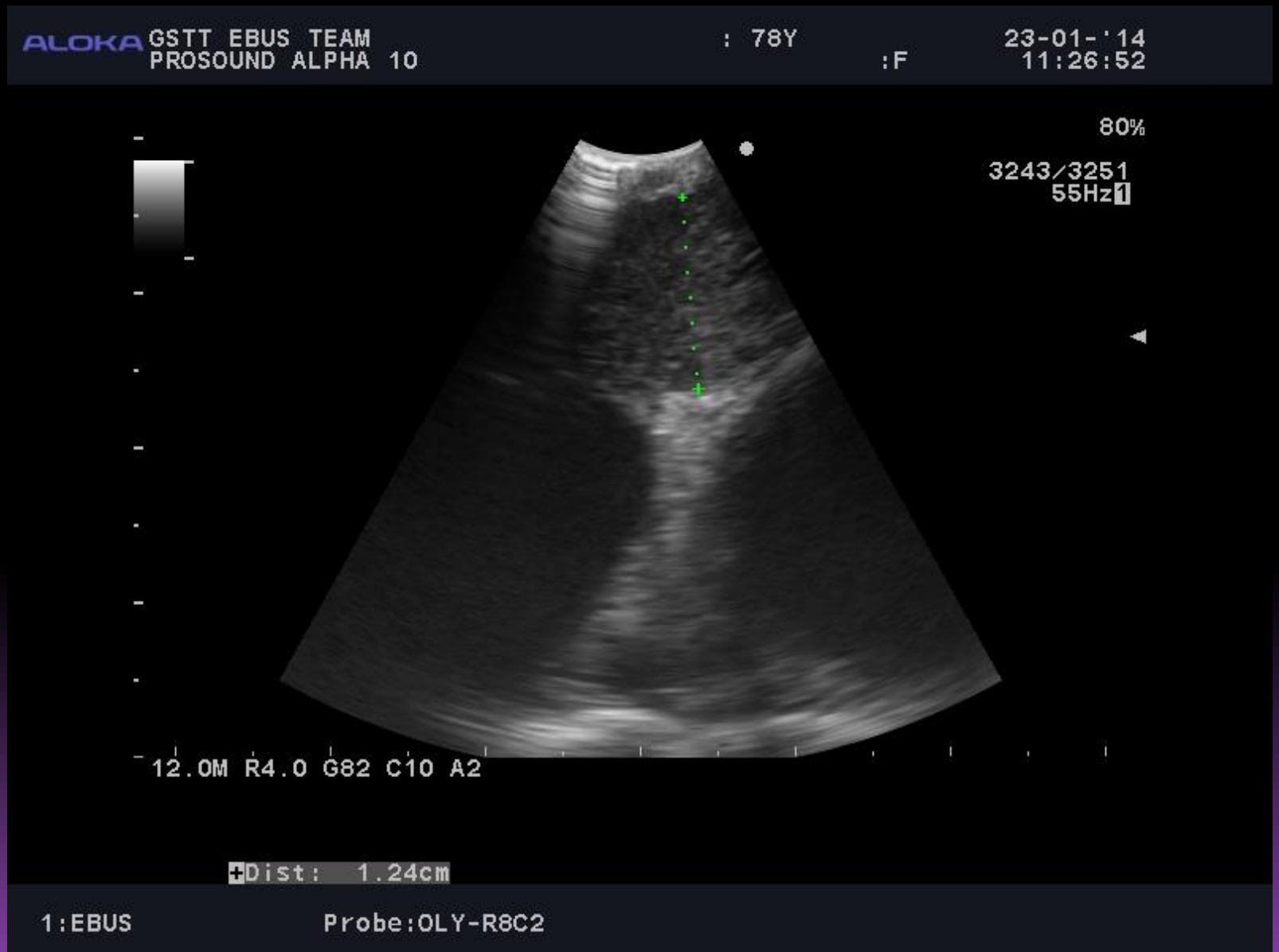
Bunyarovich T et al JNM 2006

Where radiologists do not tread....

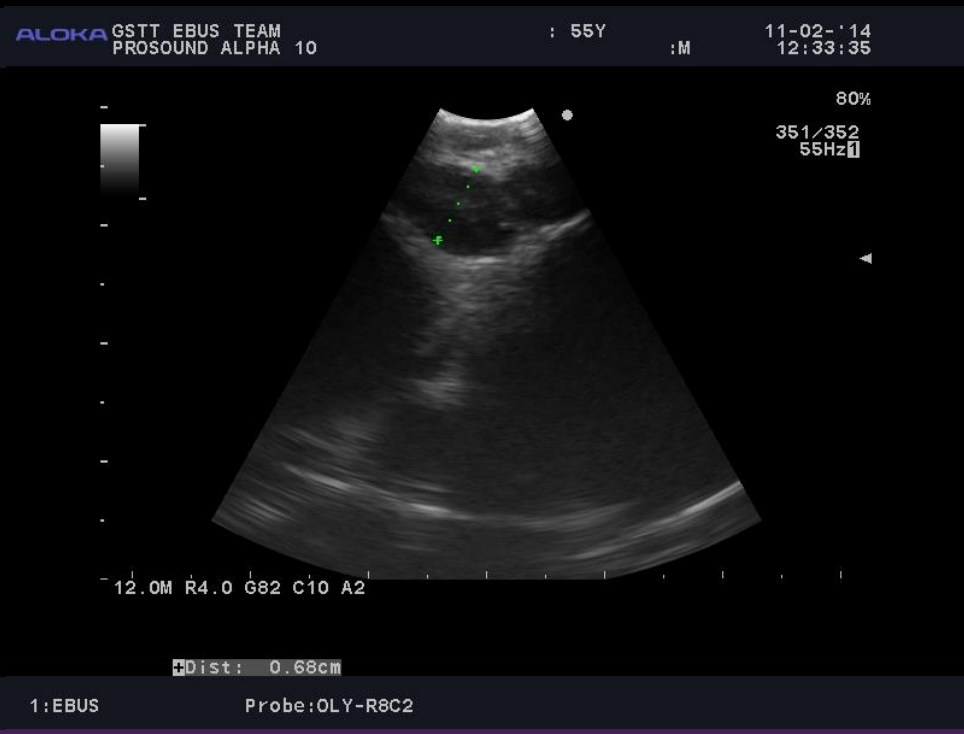


Endoscopic ultrasound setup

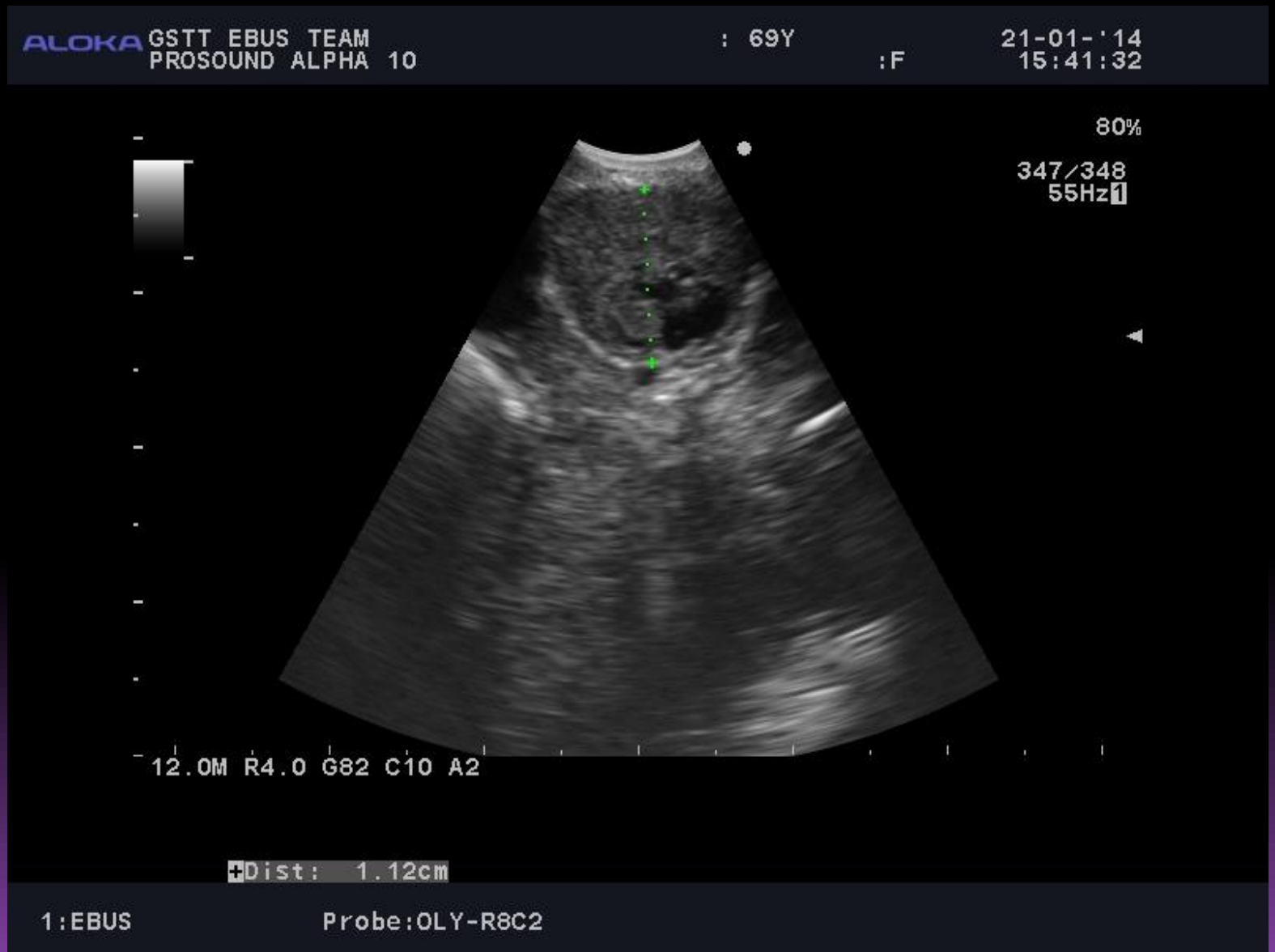
Rapid on site assessment (ROSE) setup


UPC-21L

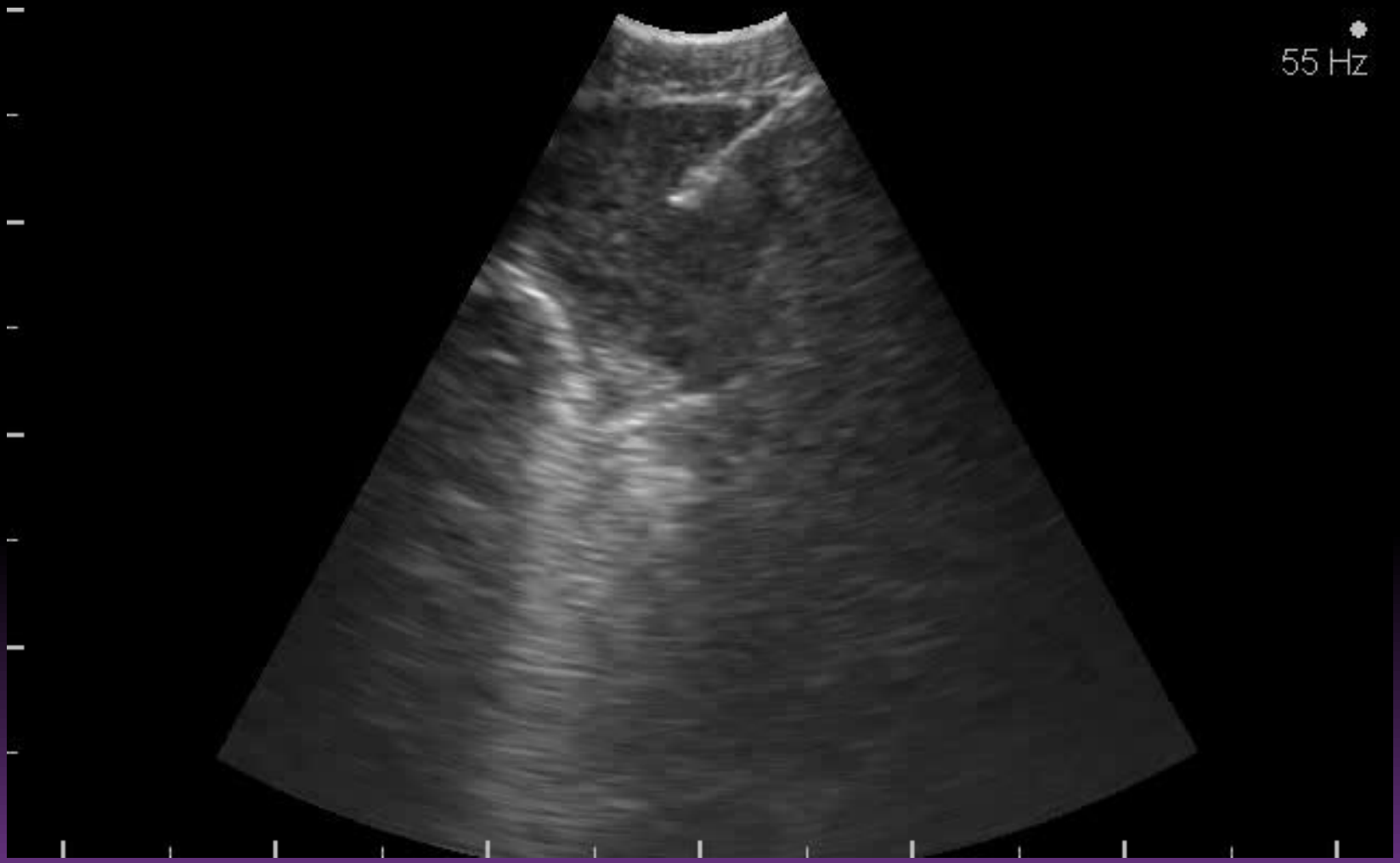
What can be targetted



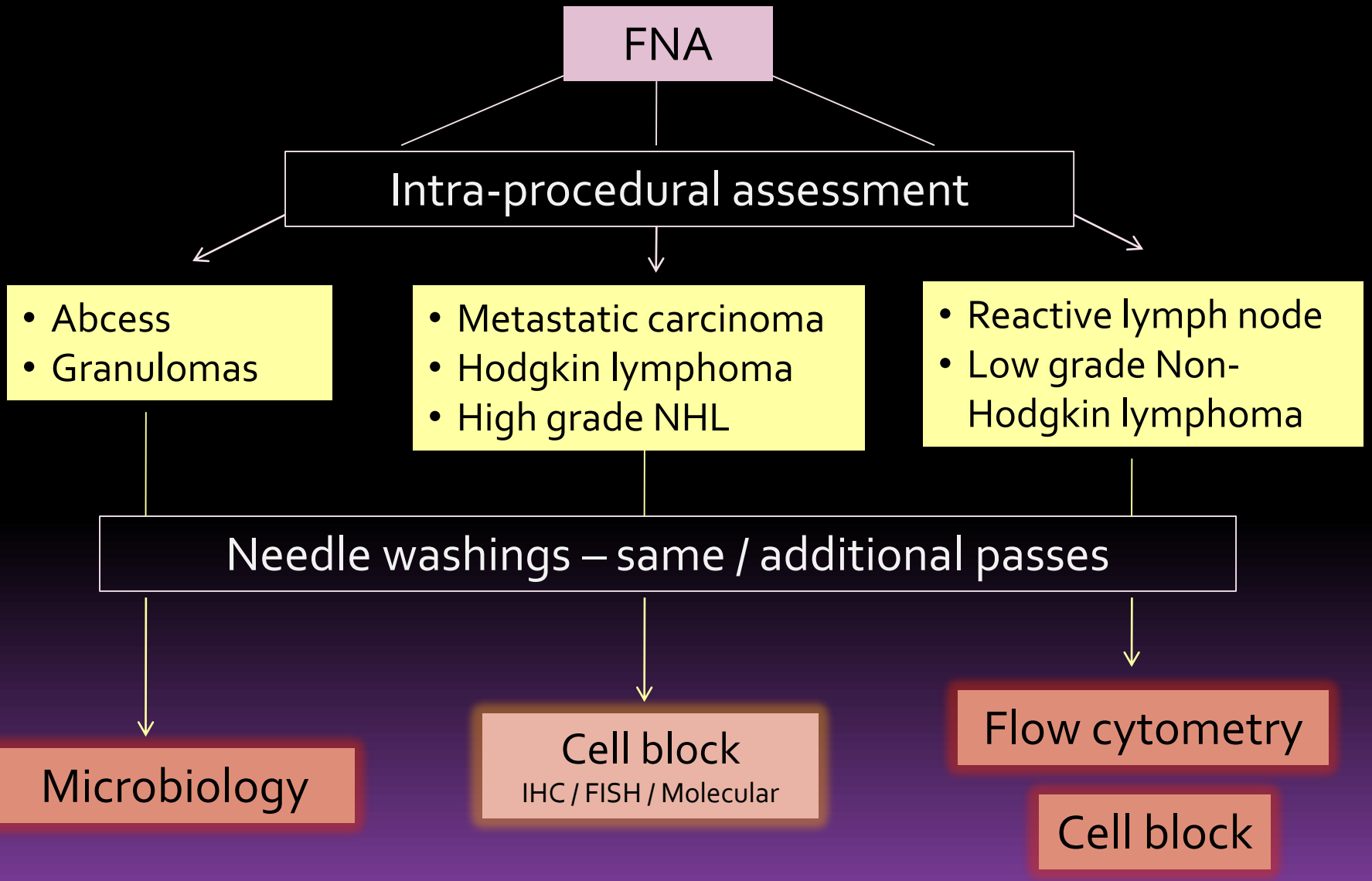
Where to sample



Is sampling adequate?



How to deal with a lymph node aspirate



Cytologic material & haematolymphoid malignancy diagnosis

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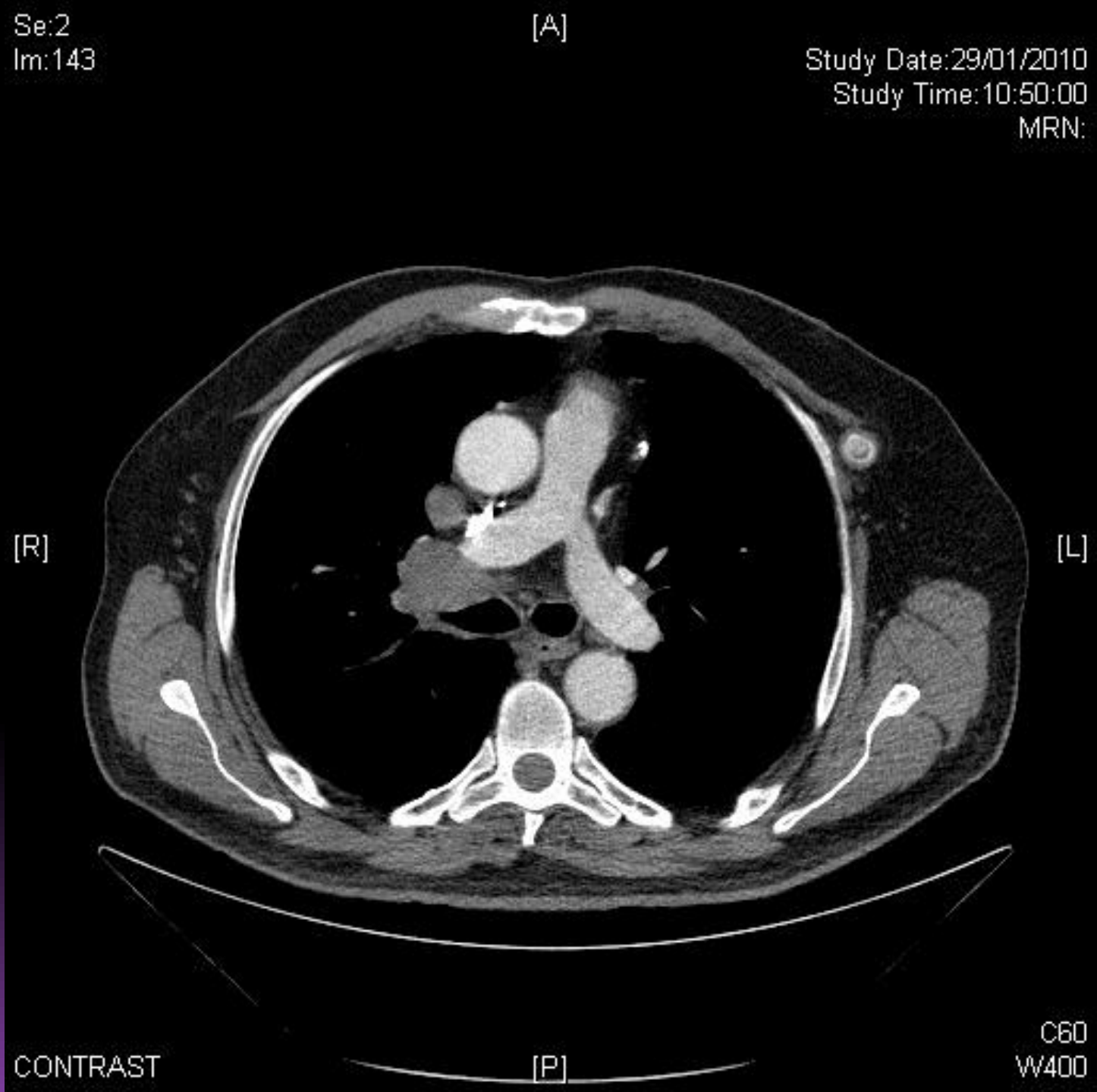
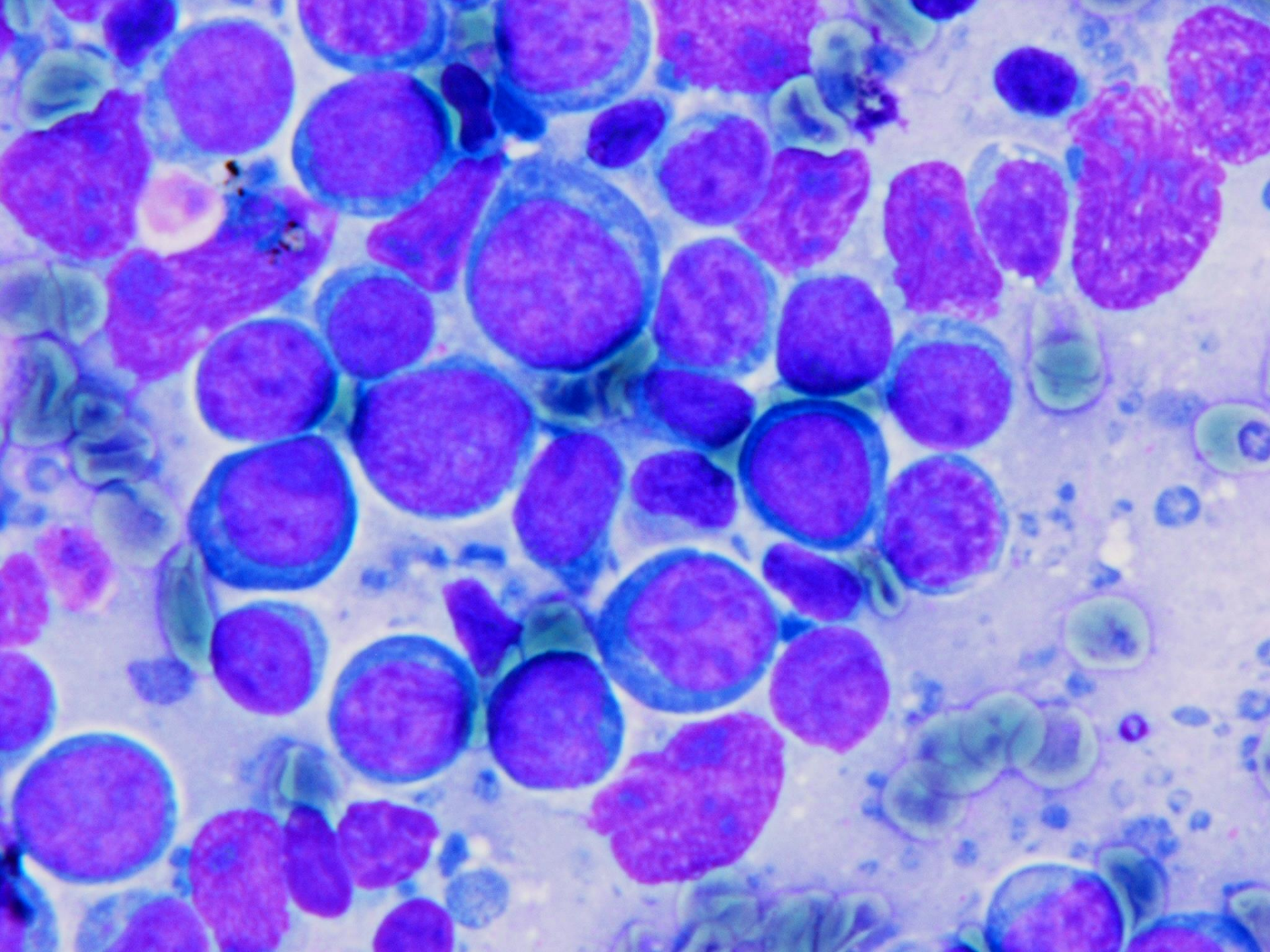
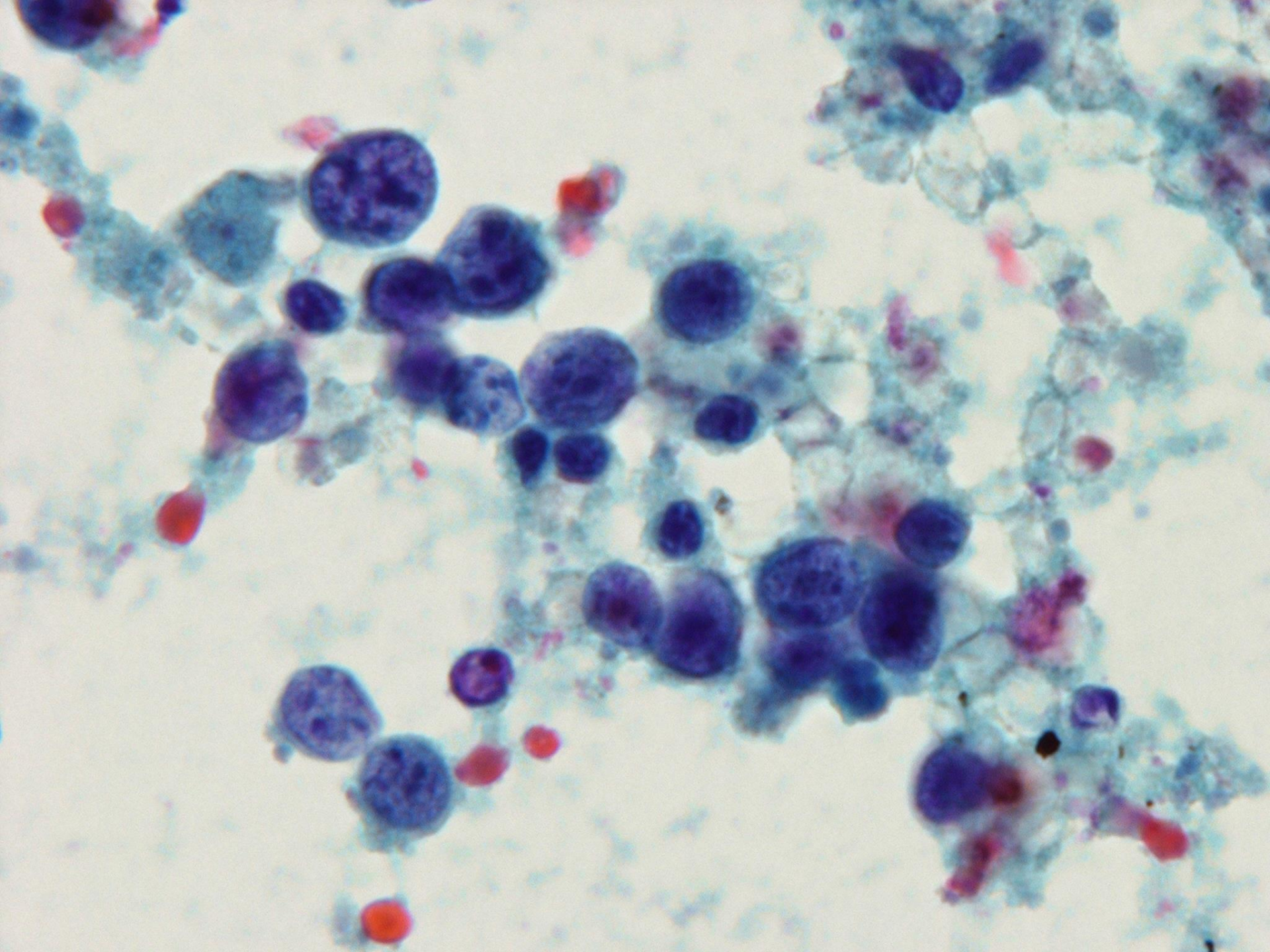
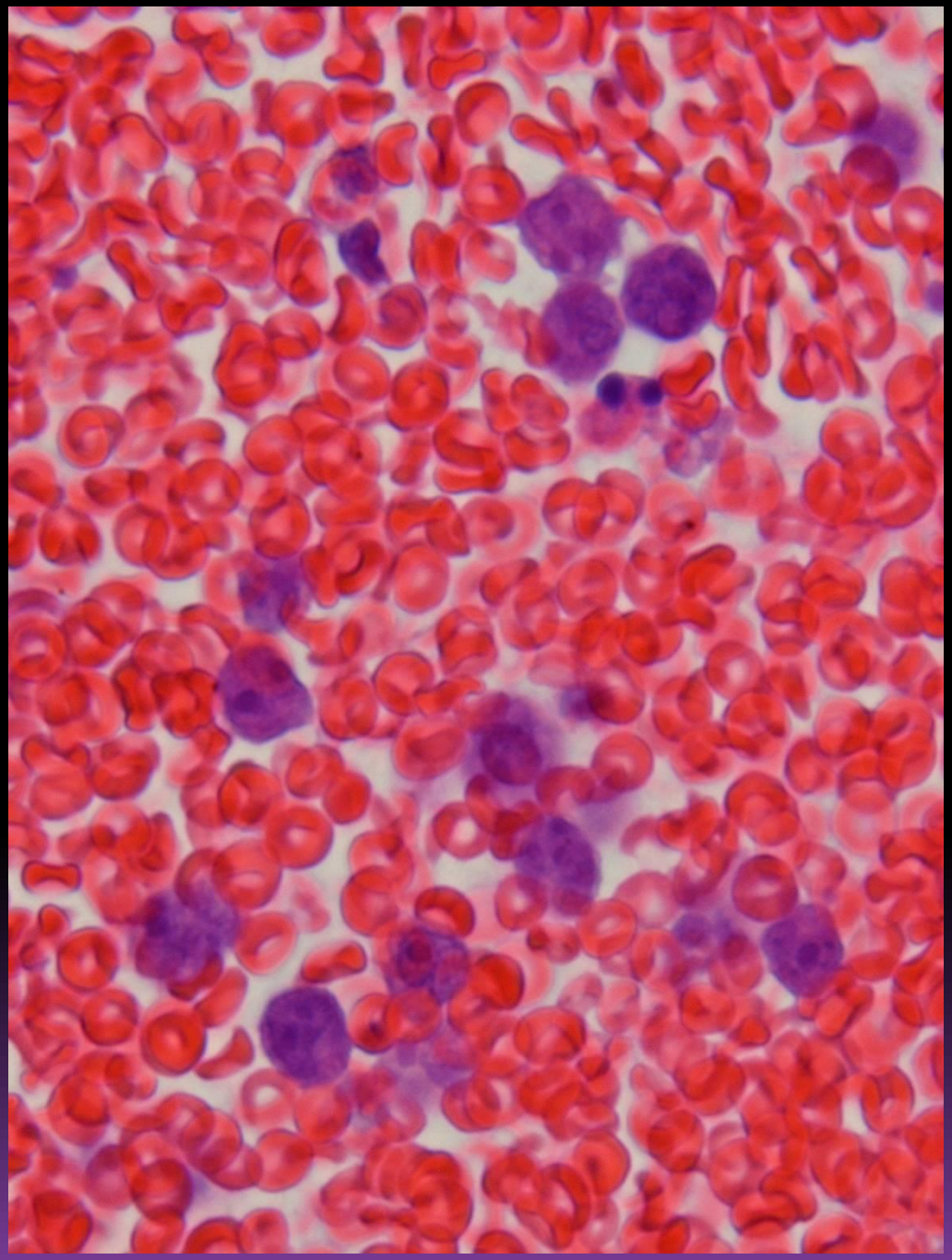
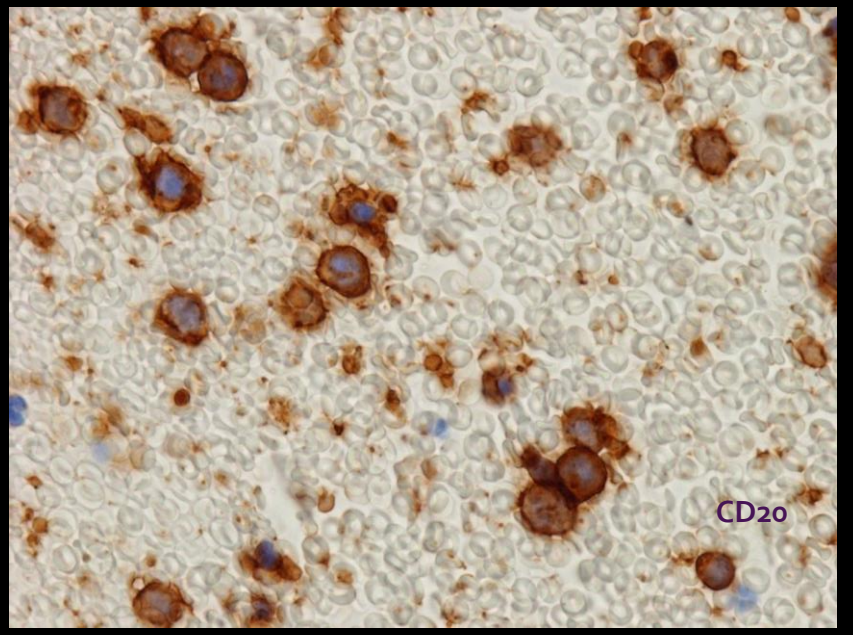
- 74 F
- Bilateral 3rd nerve palsy, deviated tongue and uvula to left
- Soft tissue mass in the anterior and superior mediastinum anterolateral to the trachea on the right measuring 4.9 x 3.8cm.
- Right pleural effusion
- LDH: 2161 IU/L

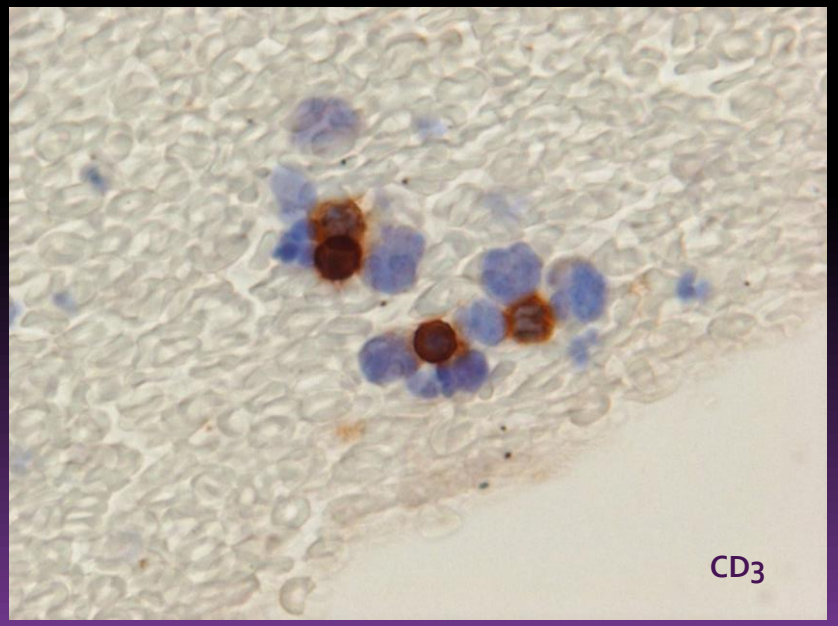


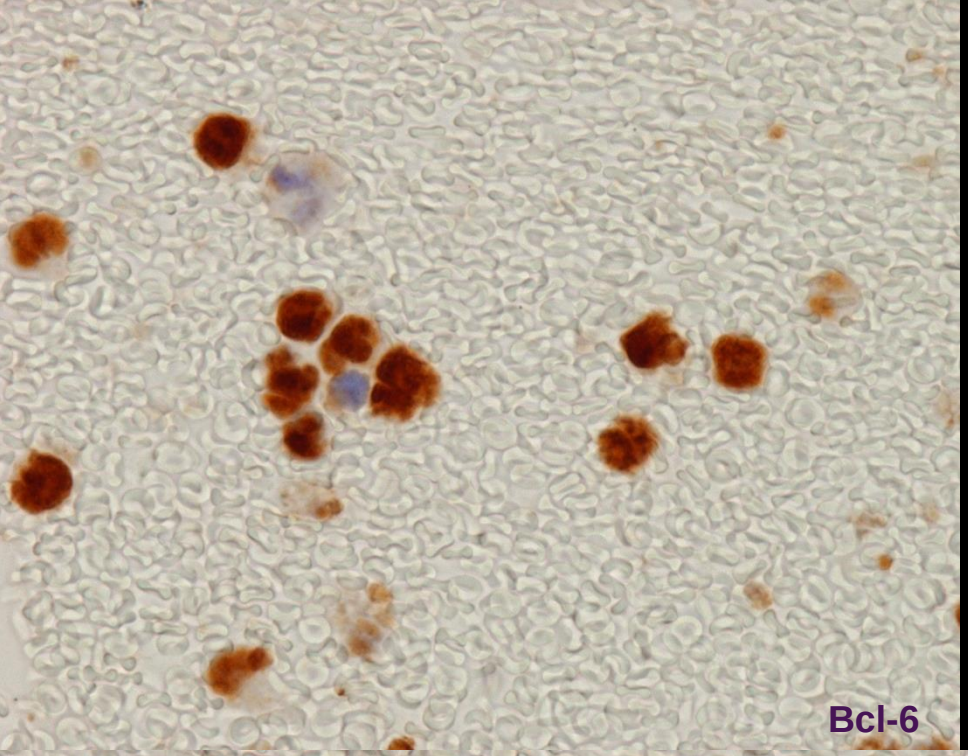






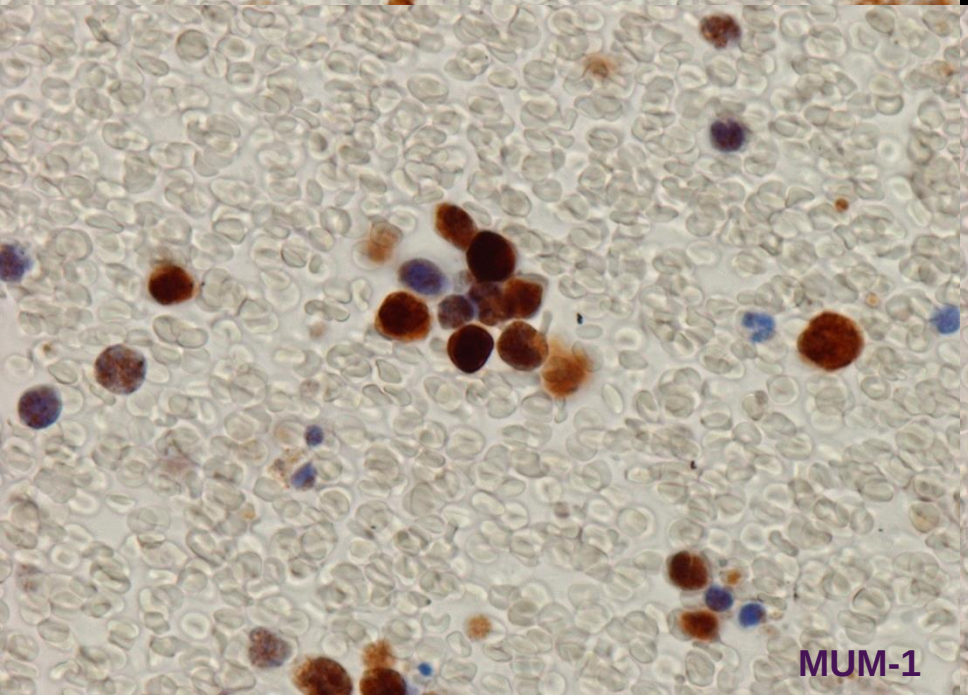

CD20

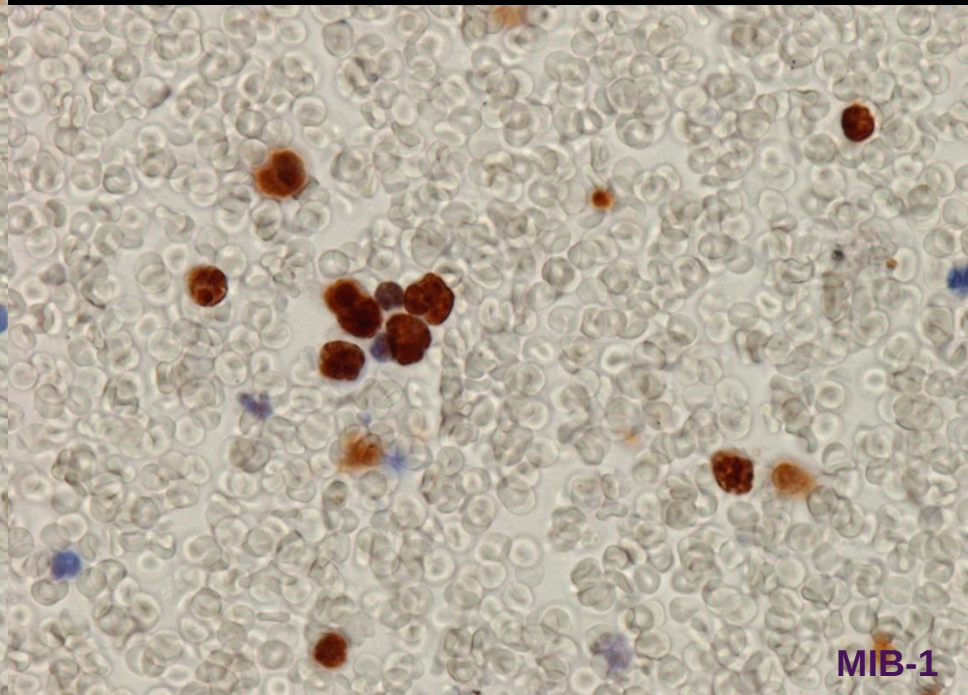

CD3



Antibody	
CD20	+
CD79a	+
Bcl-6	+
MUM-1	+
Bcl-2	+
p53	+

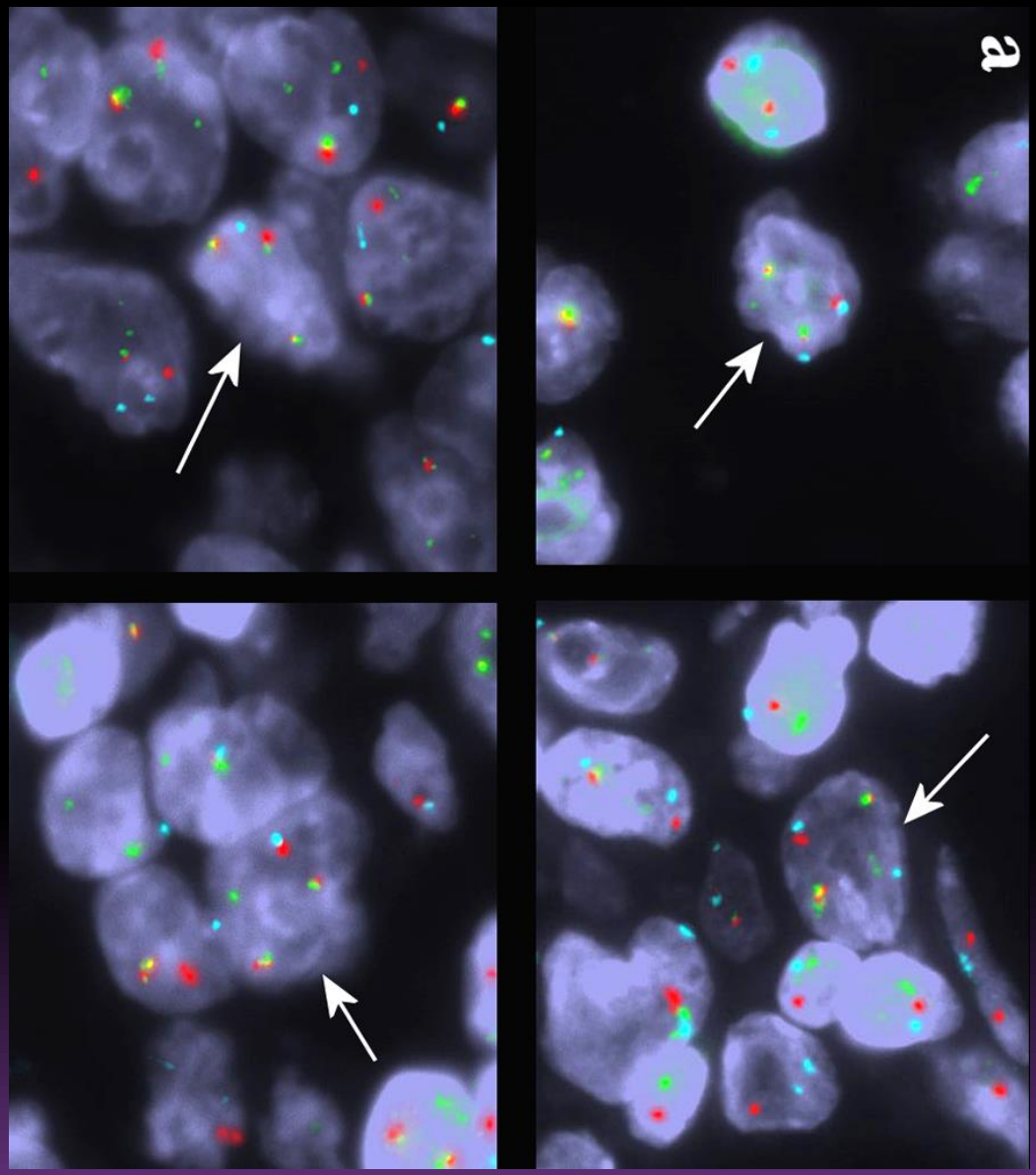
Antibody	
CD3	-
CD10	-
CD5	-
CD30	-
EBER	-
MIB-1	90%





FISH

- MYC (+)
- IGH/BCL-2 negative
- BCL6 not rearranged

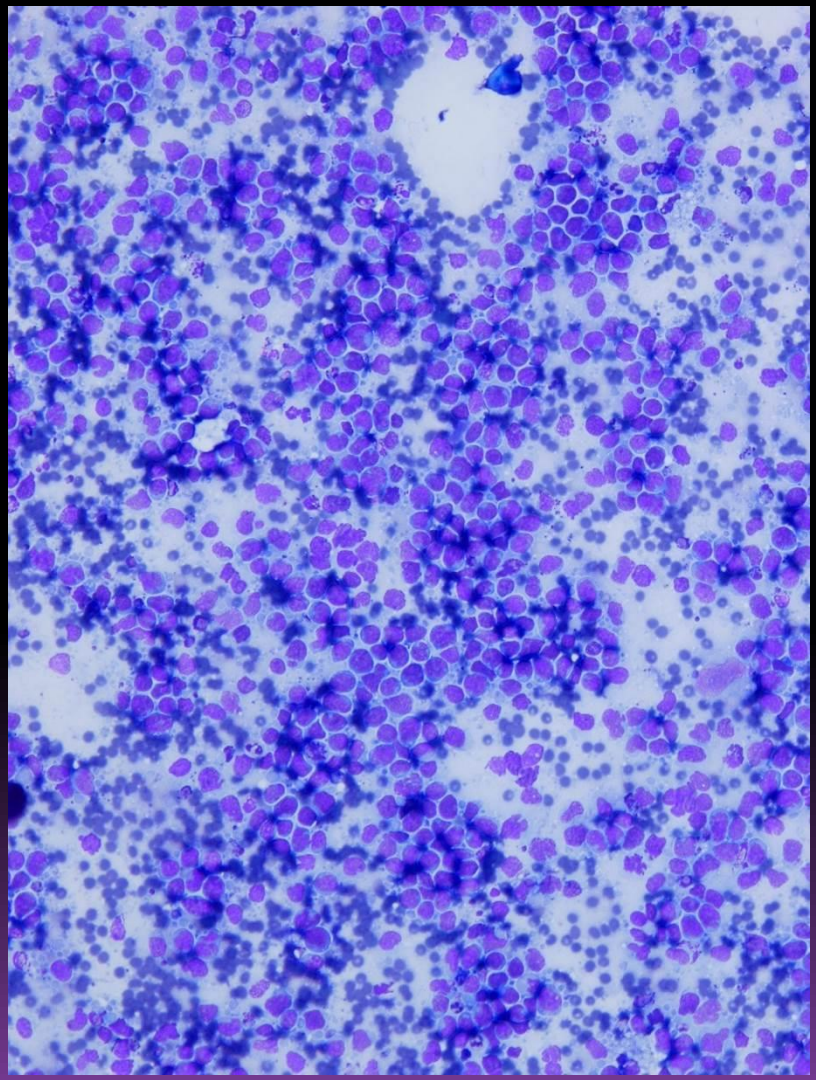


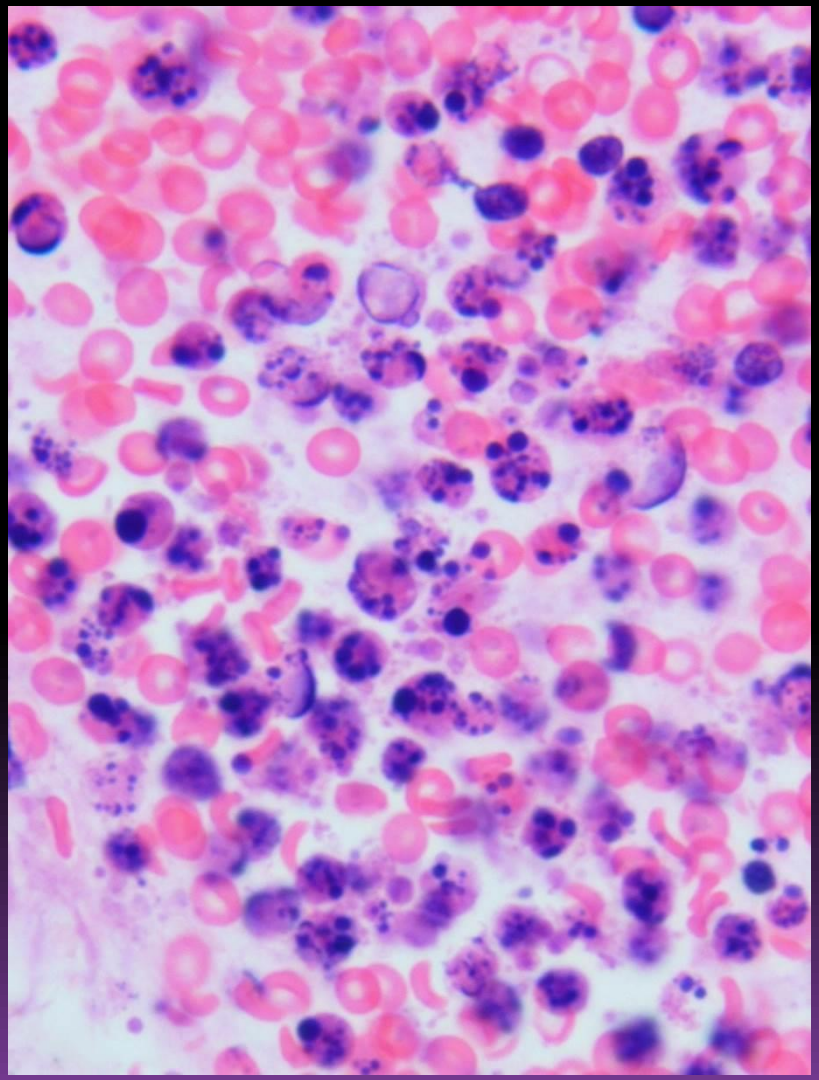
diagnosis

Non-Hodgkin lymphoma
Diffuse large B-cell lymphoma
Activated B-cell type
MYC(+)

There was still tissue left in the block if one wished to send for the REMODEL trial

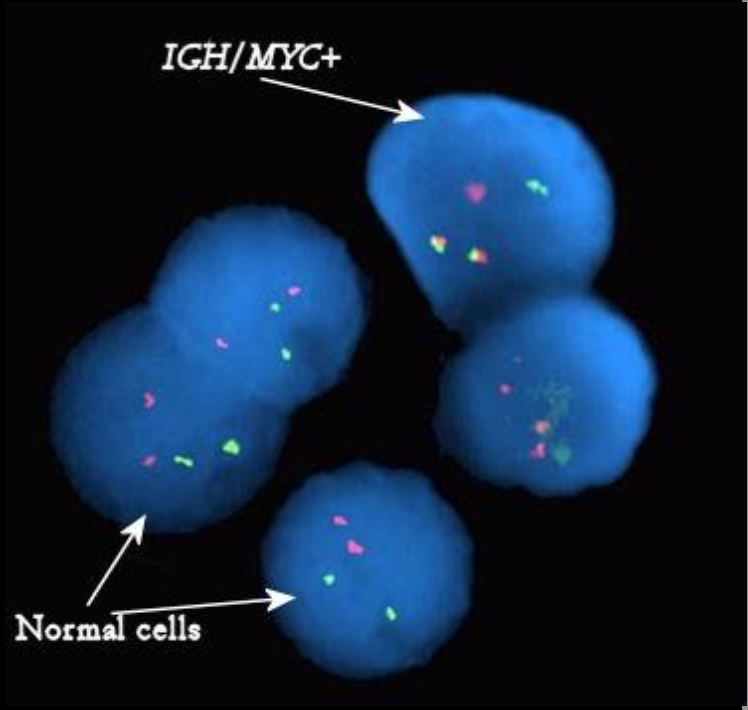
Pitfall – high proliferation tumours!

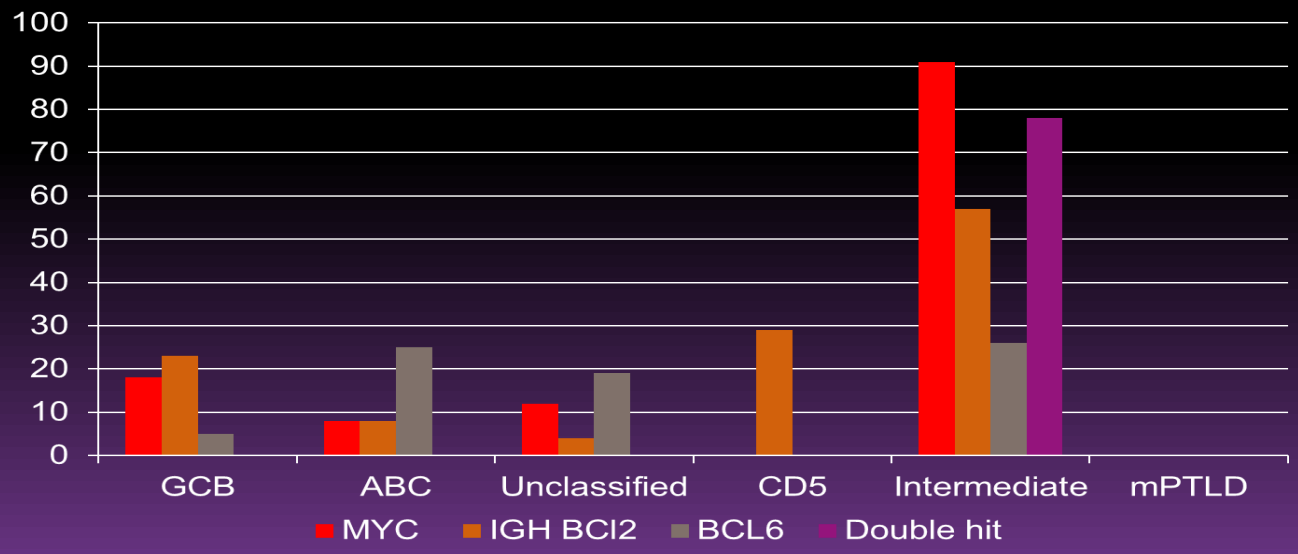




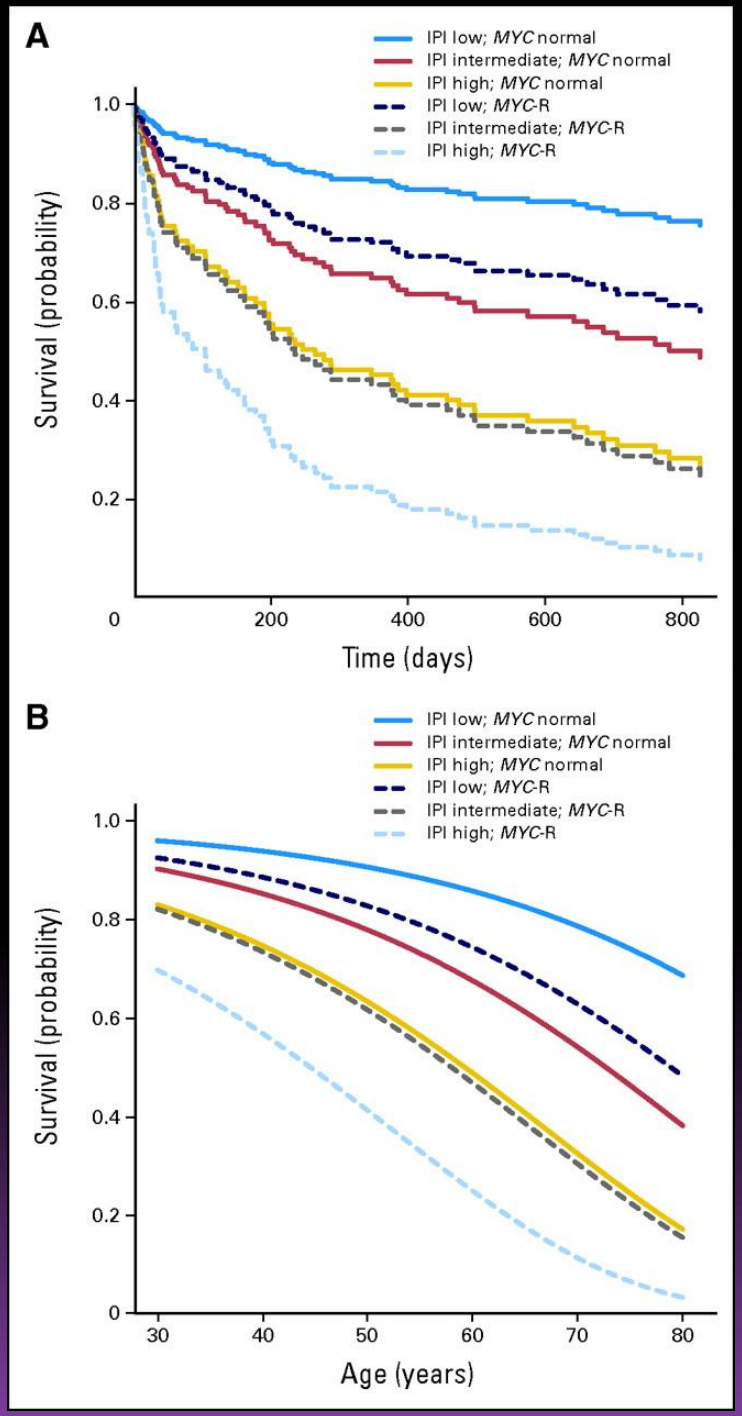
Recurrent genetic abnormalities in high grade B-cell NHL

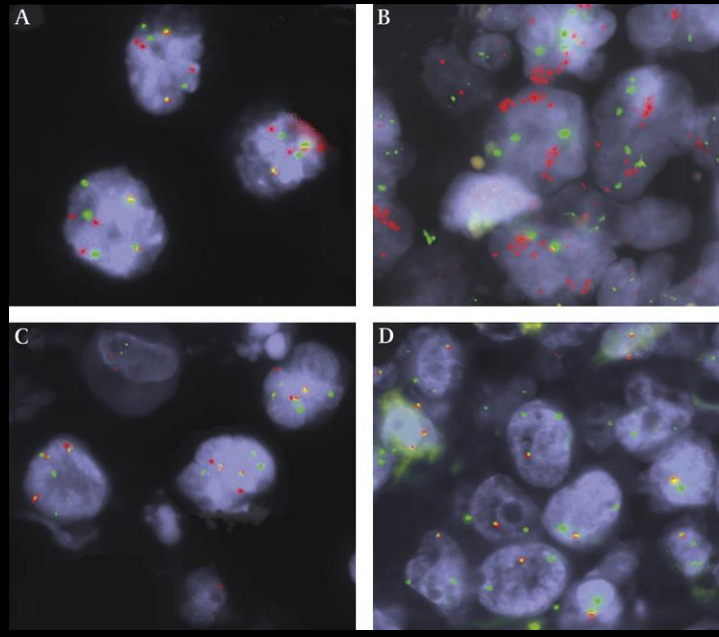
- MYC
- IGH BCL2
- BCL6

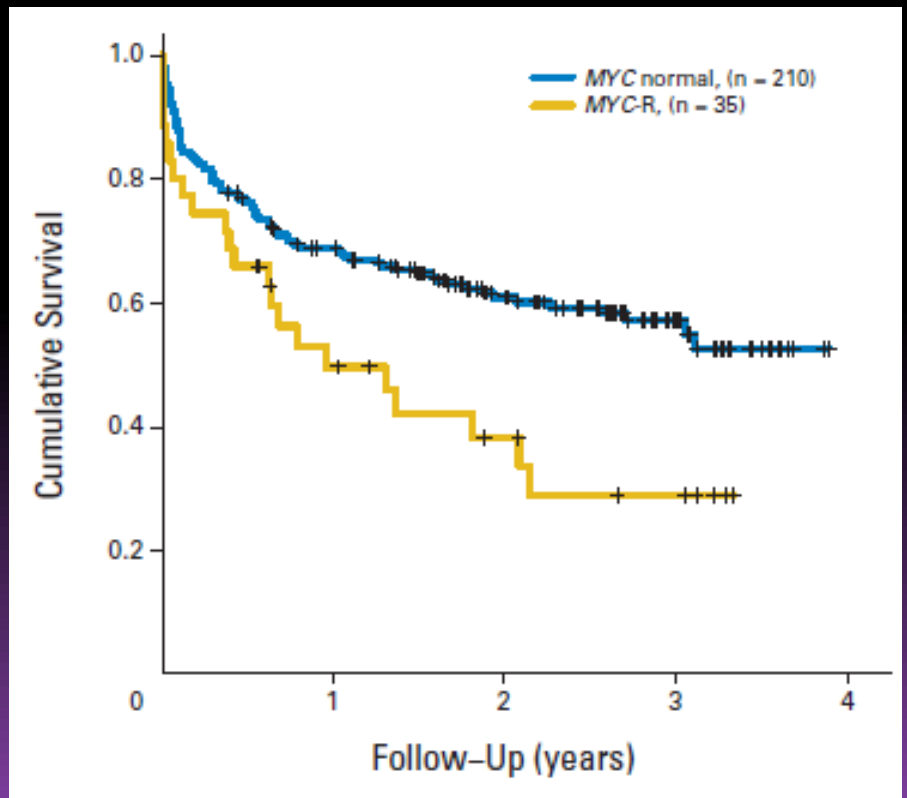




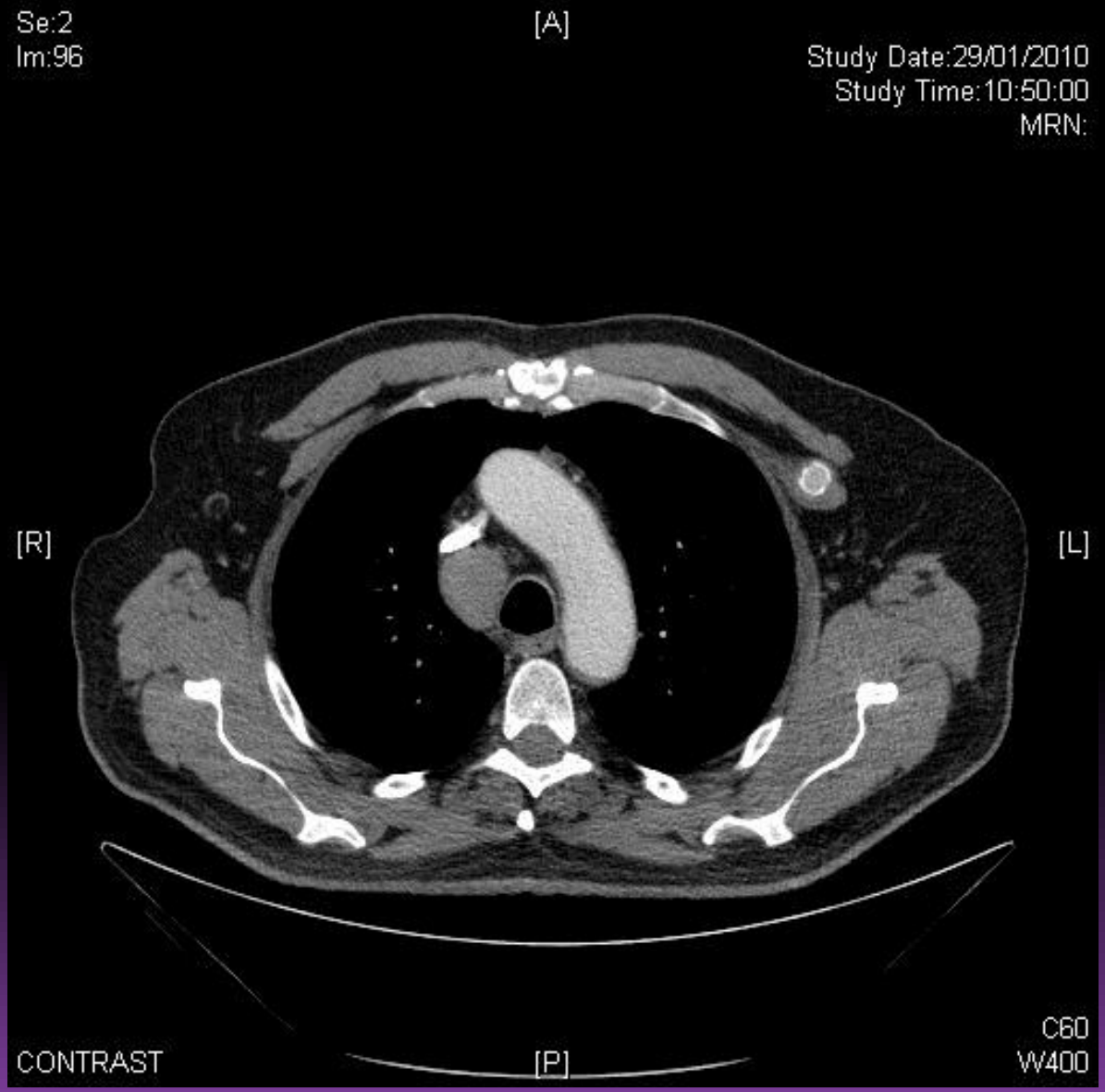
Foot N et al. J Clin Path 2011; 64: 802

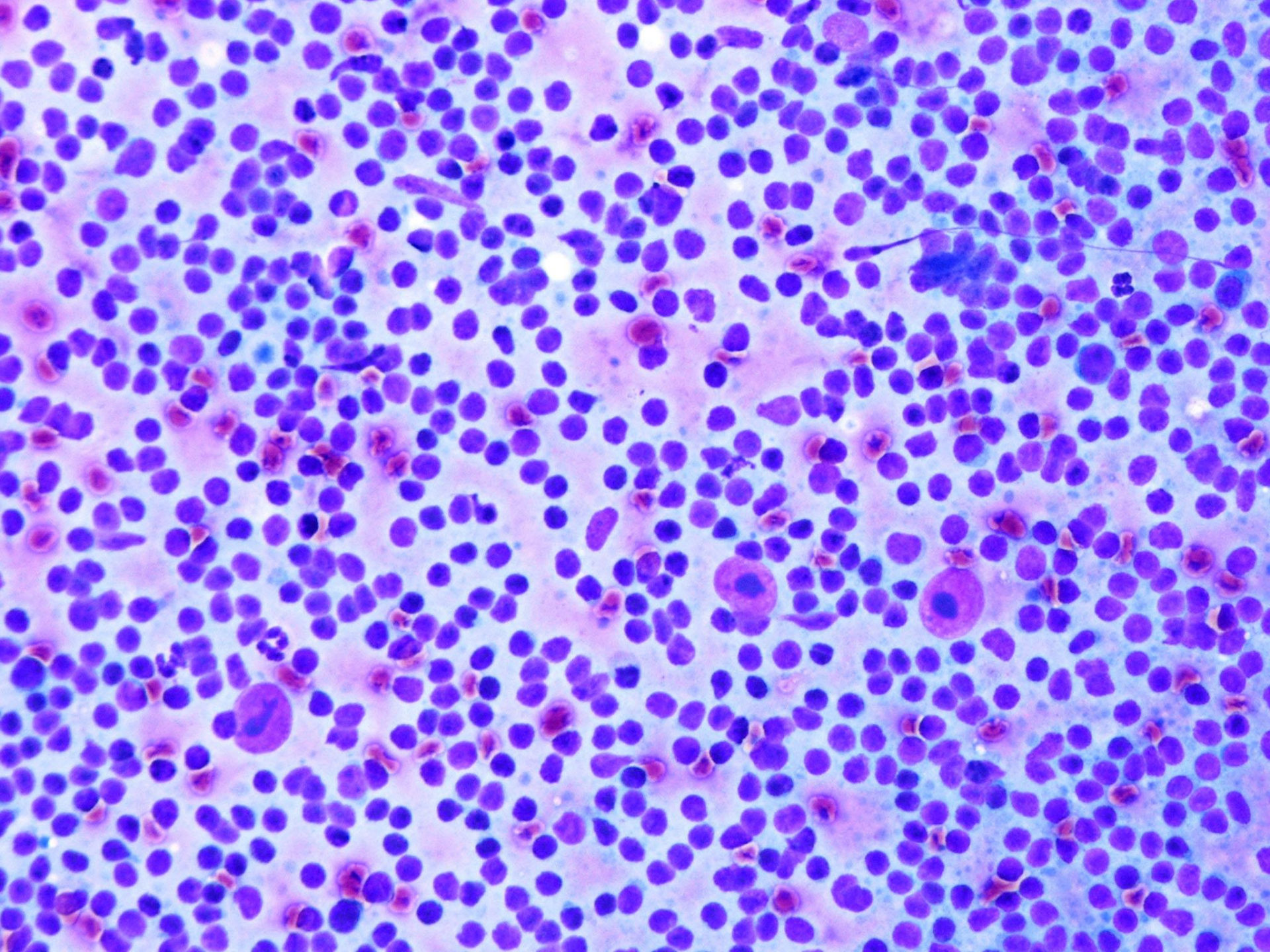

A
IPI low; MYC normal
IPI intermediate; MYC normal
IPI high; MYC normal
IPI low; MYC-R
IPI intermediate; MYC-R
IPI high; MYC-R
Survival (probability)
1.0
0.8
0.6
0.4
0.2
0
200
400
600
800
Time (days)
B
IPI low; MYC normal
IPI intermediate; MYC normal
IPI high; MYC normal
IPI low; MYC-R
IPI intermediate; MYC-R
IPI high; MYC-R
Survival (probability)
1.0
0.8
0.6
0.4
0.2
0
30
40
50
60
70
80
Age (years)

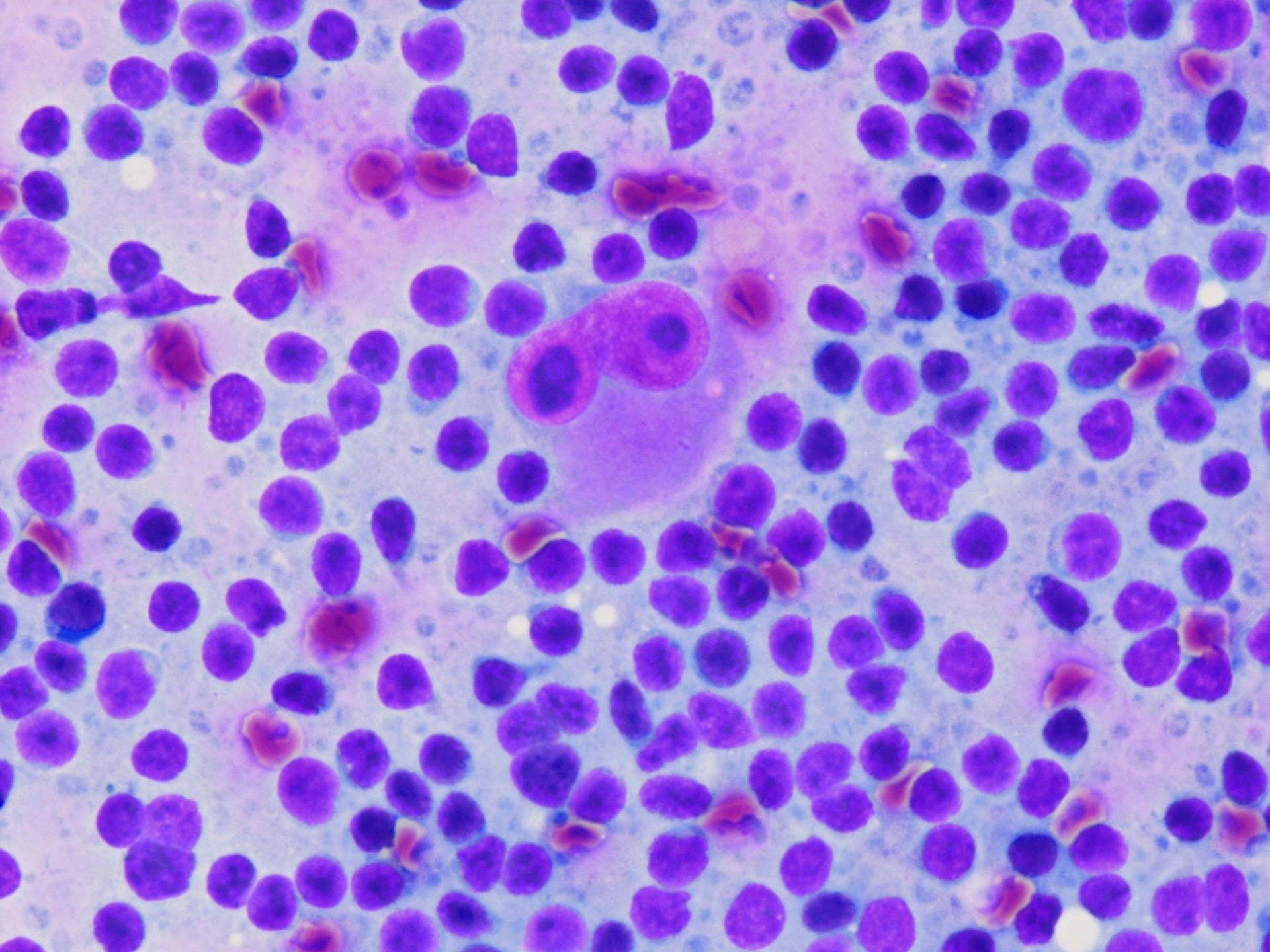

A
B
C
D

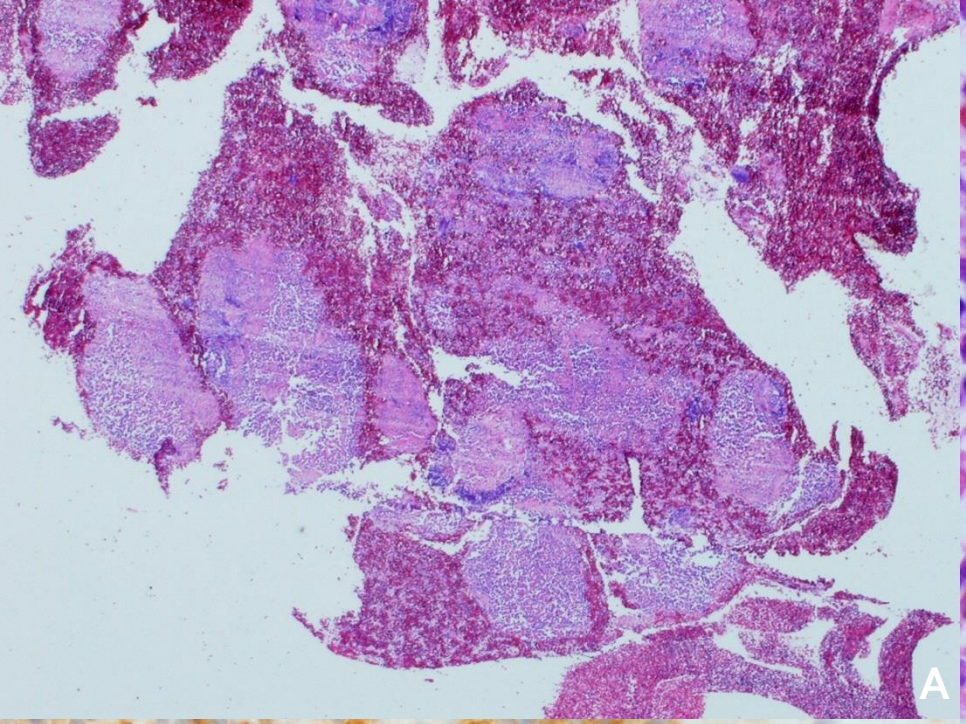

MYC normal, (n = 210)
MYC-R, (n = 35)
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0
1
2
3
4
Follow–Up (years)

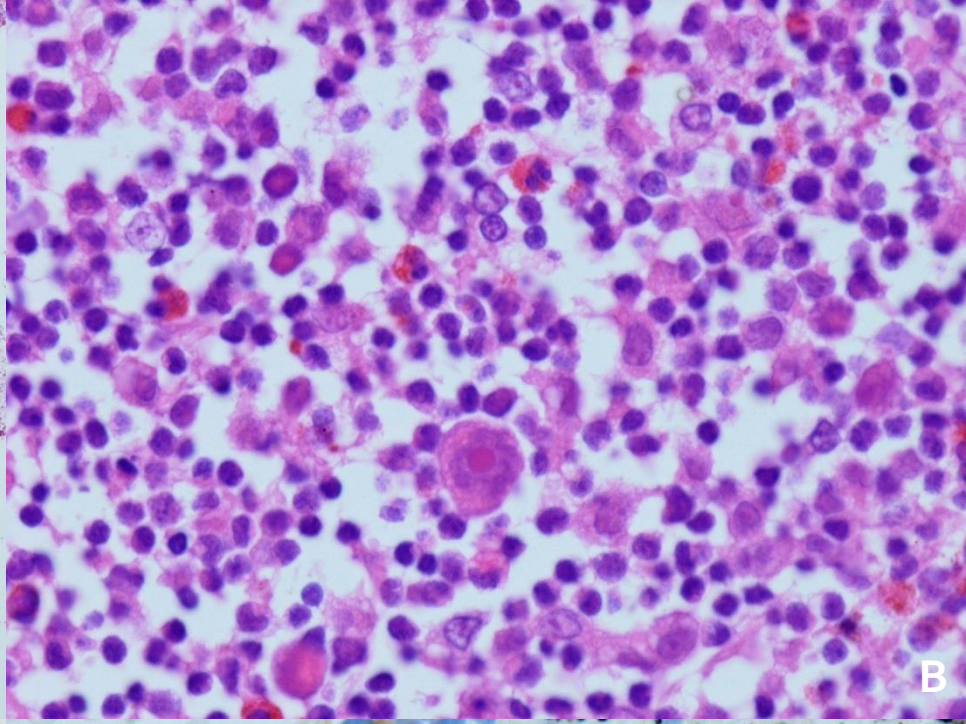
64 ♂, Fever, itching. Mediastinal mass. Subsequently, post diagnosis developed cervical masses.

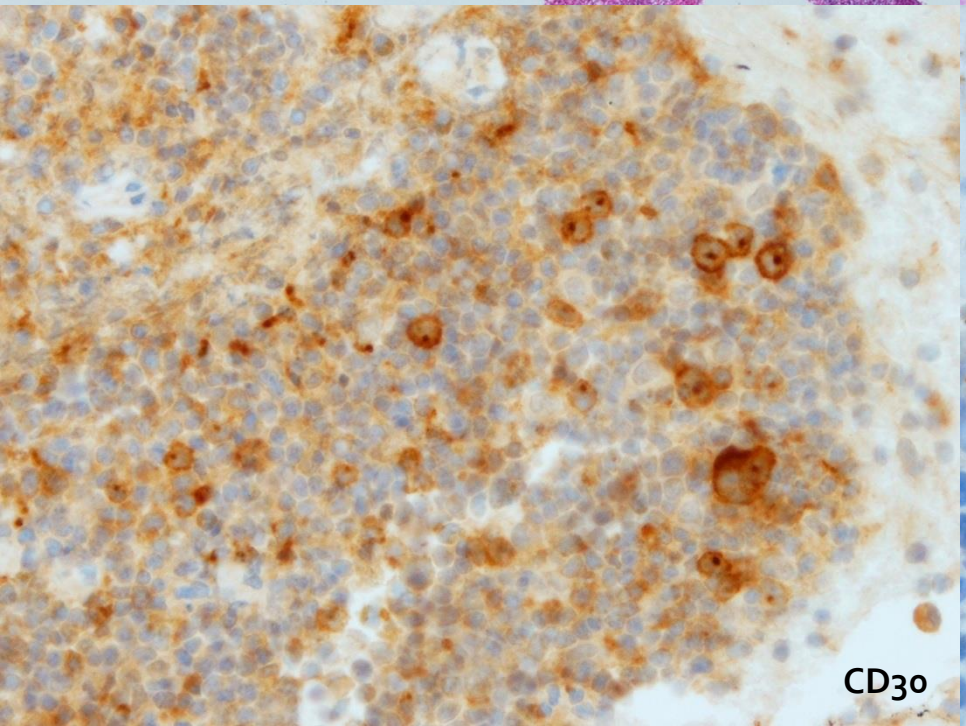


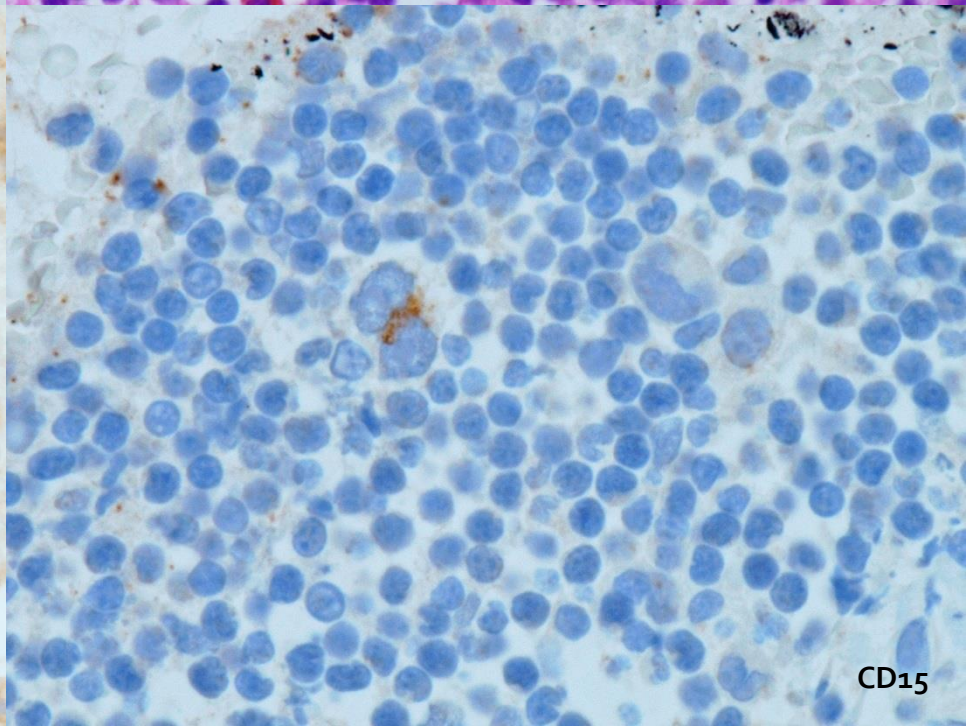





A


B

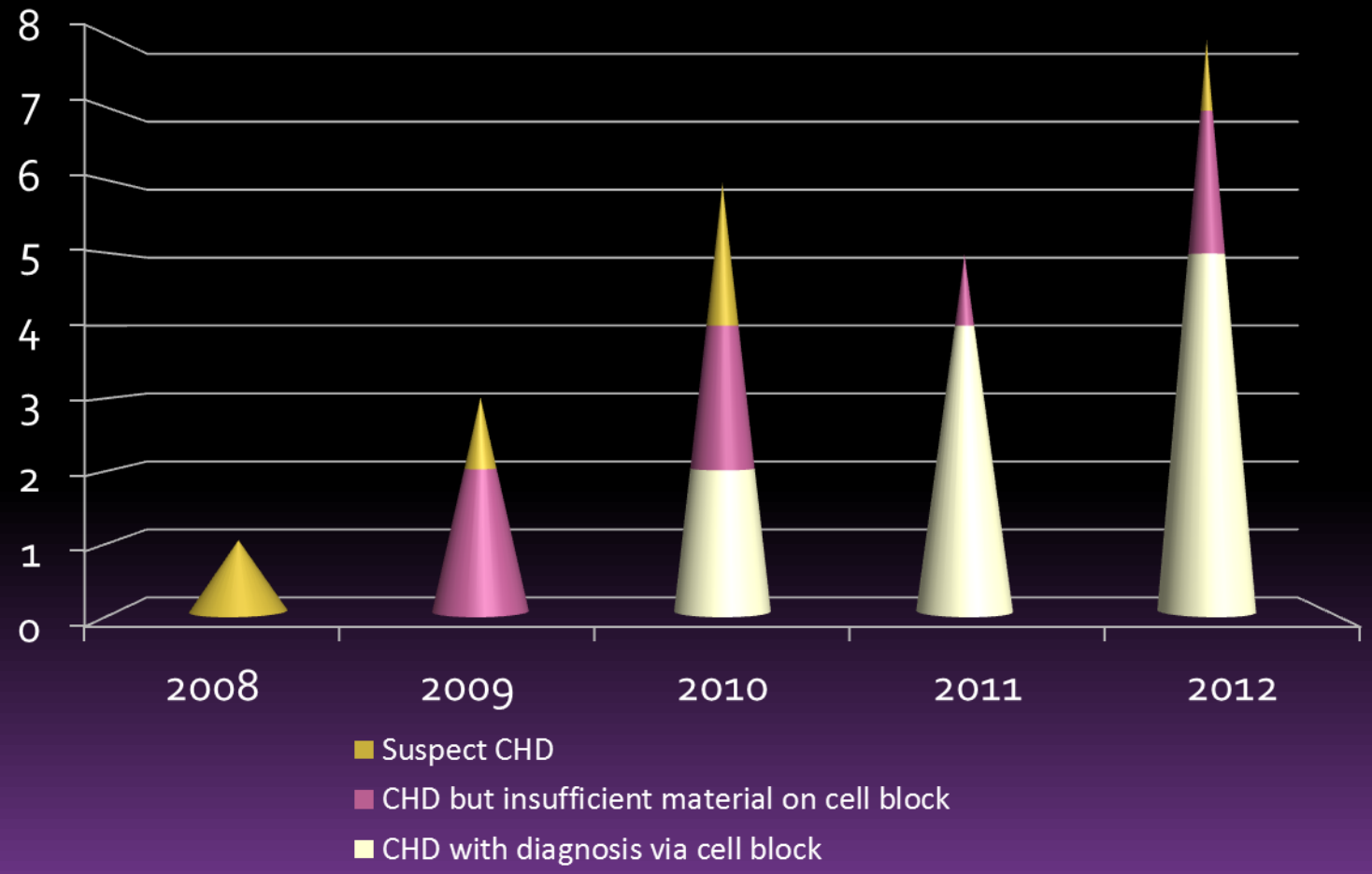

CD30


CD15

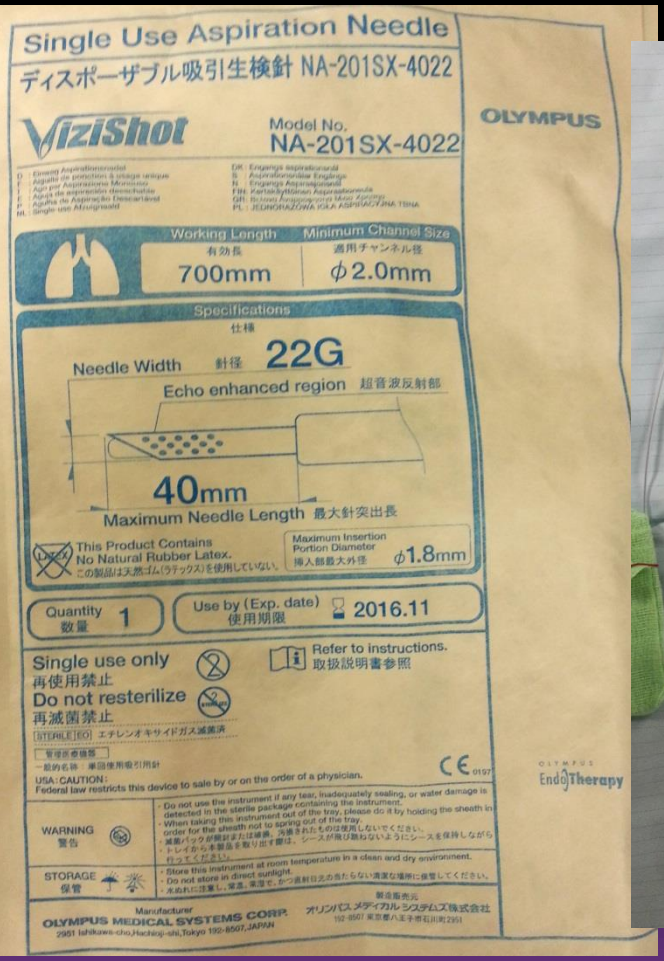
CHD diagnosed on cell blocks (11/21)

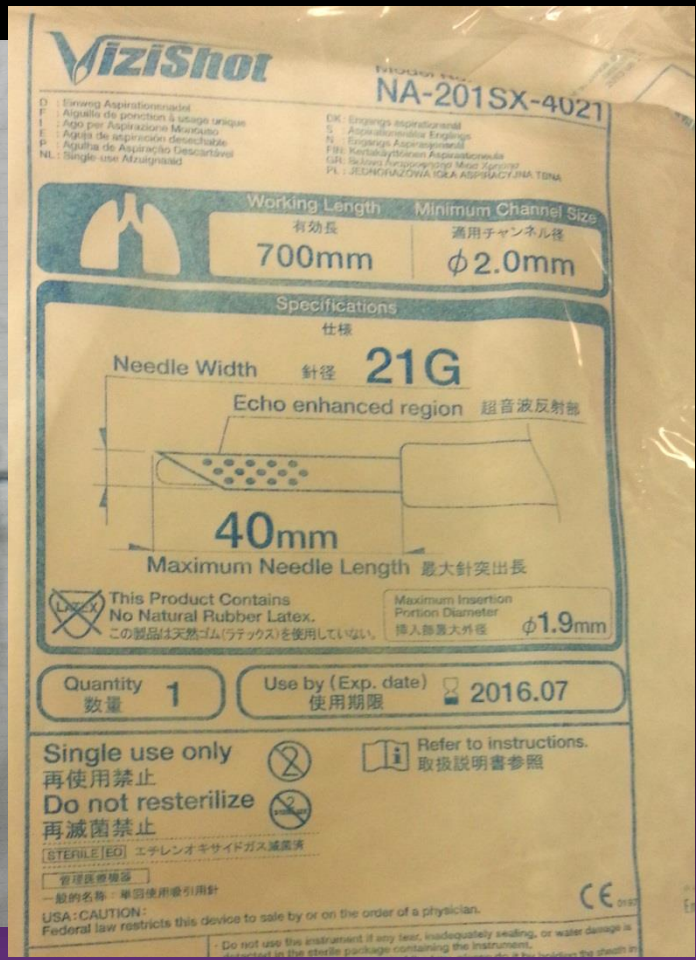
Age / Sex	EBUS site	Cell block	LCA	CD30	CD15	MUM-1	EBER	CD20	CD3
65 / F	4R, 7, 10R	Yes	-	+	+	+	+	-	-
38 / F	4R	Yes	-	+	+	+	+	-	-
39 / F	4R, 7	Yes	-	+	-	+	-	-	-
50 / M	4R	Yes	-	+	+	-	-	-	-
24 / F	4R	Yes	-	+	-	+	+	-	-
77 / M	4R, 7	Yes	-	+	+	+	+	-	-
41 / M	4I, 10	Yes	-	+	-	+	+	-	-
32 / F	4R, 4L	Yes	-	+	+	-	-	-	-
45 / F	2R, 4R	Yes	-	+	-	+	-	-	-
24 / F	4R	Yes	-	+	-	+	-	-	-
51 / F	4R+2R, 4L	Yes	-	+	+	+	+	-	-

Confidence of CHD diagnosis with development of service

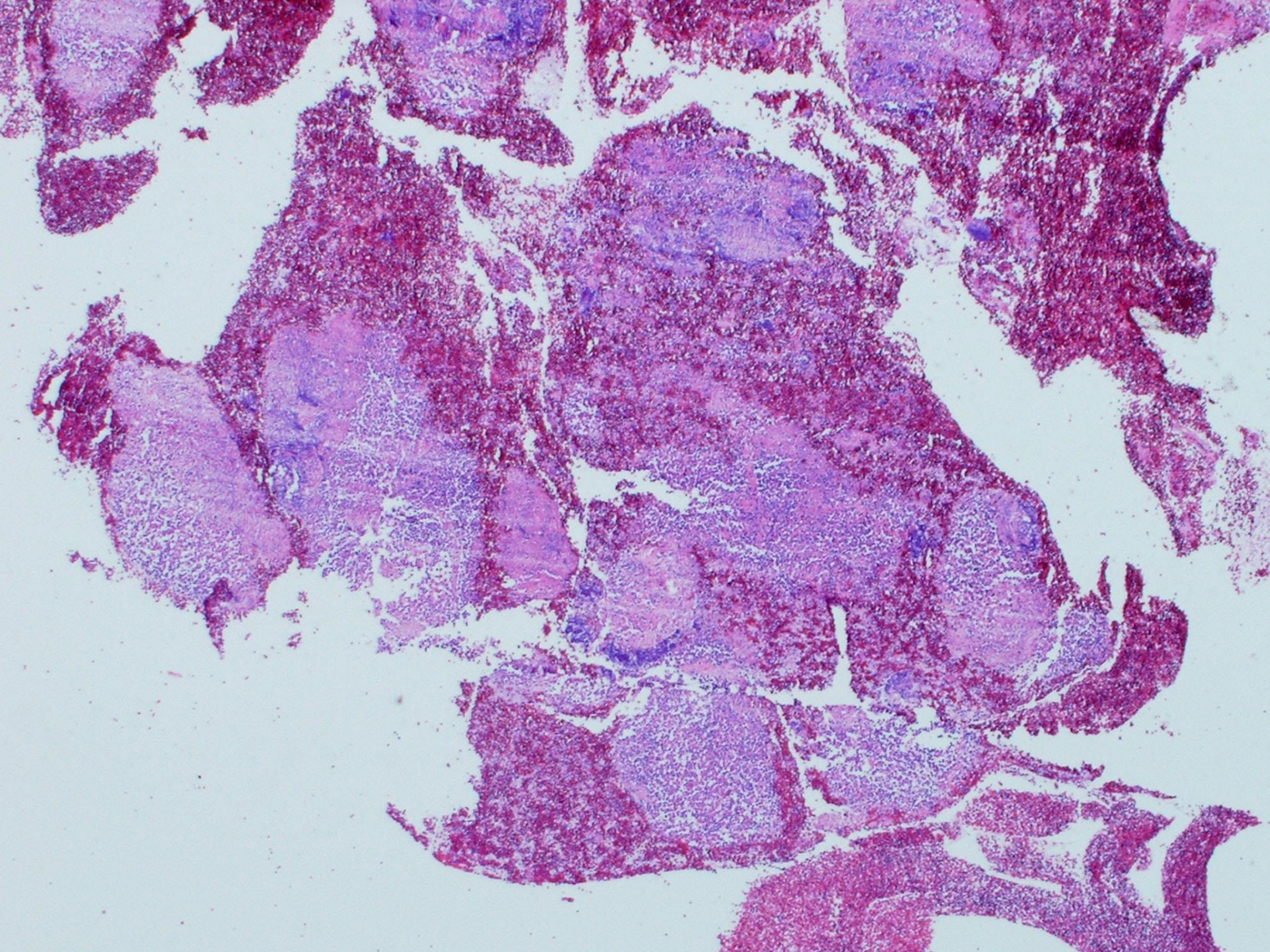


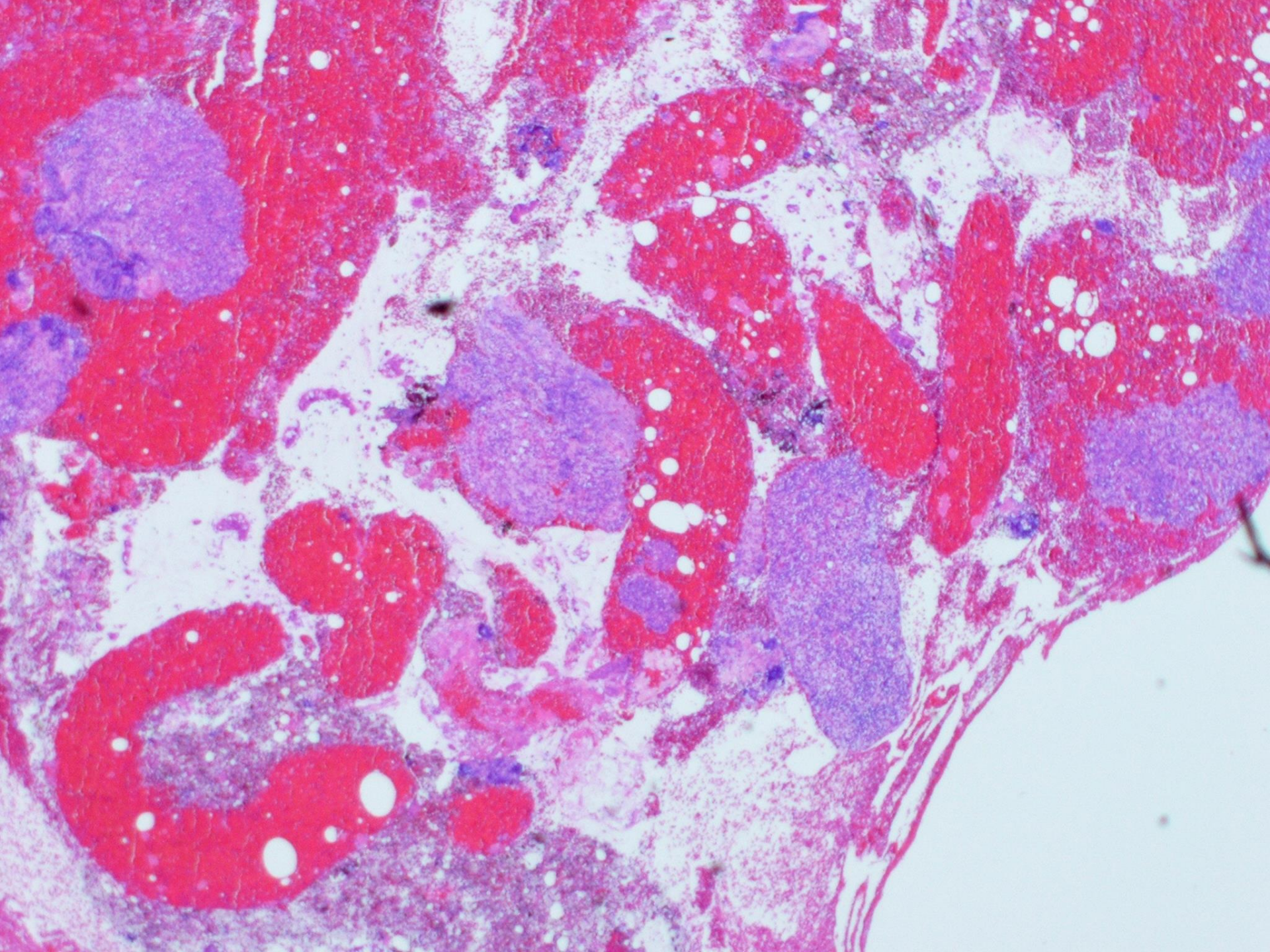
How are we able to get enough out?

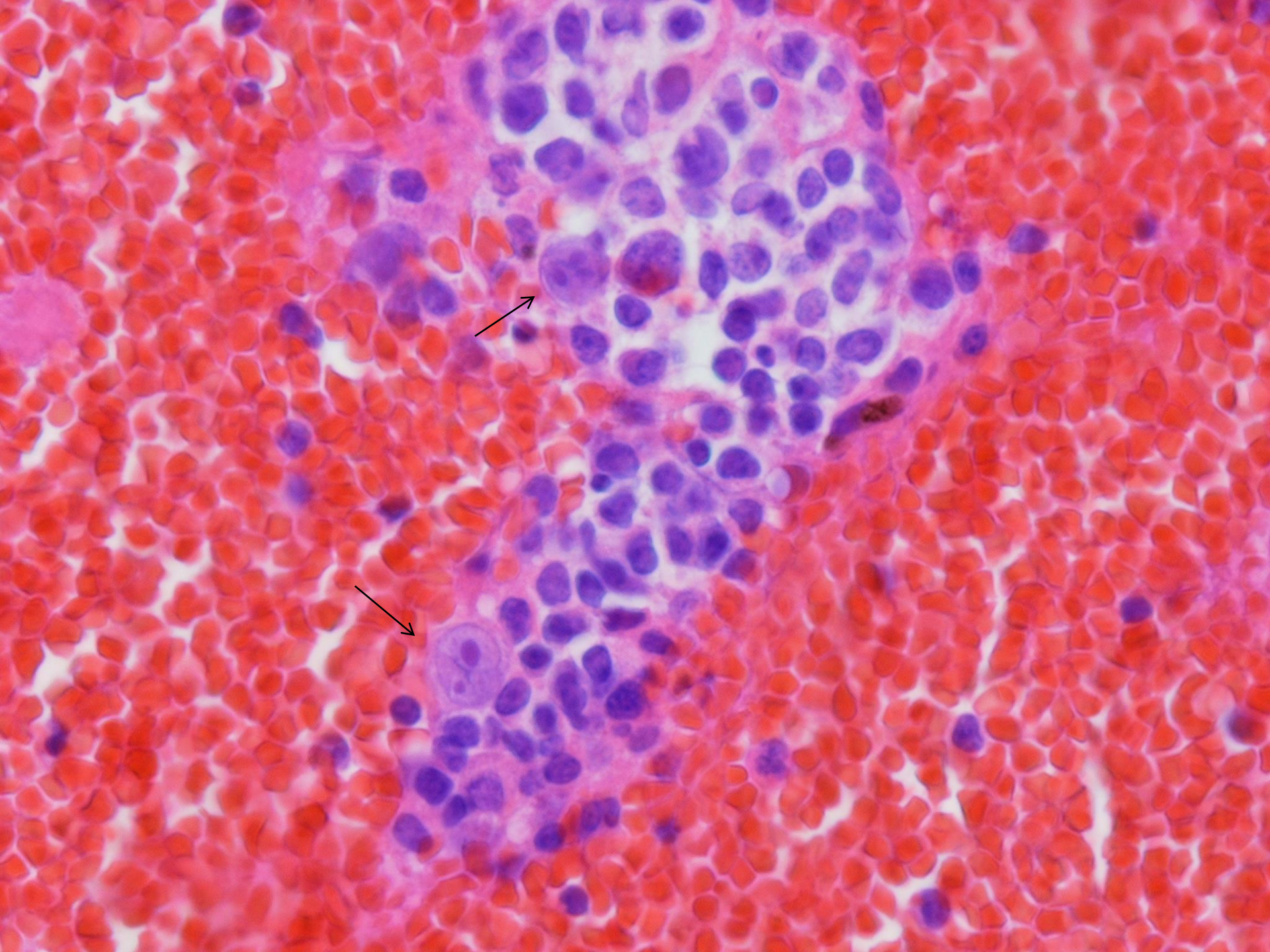




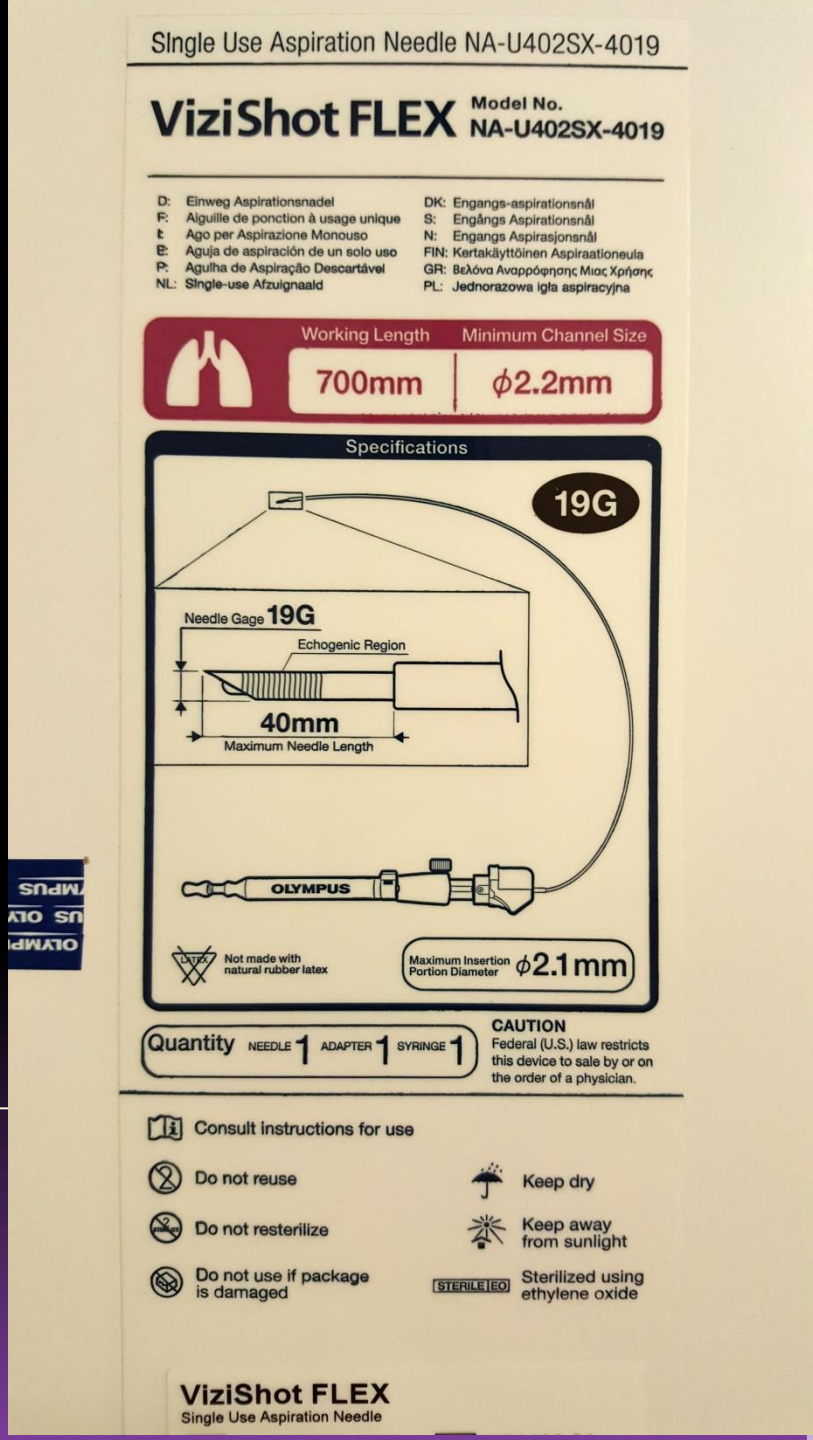
EBUS procore biopsy needles 21G

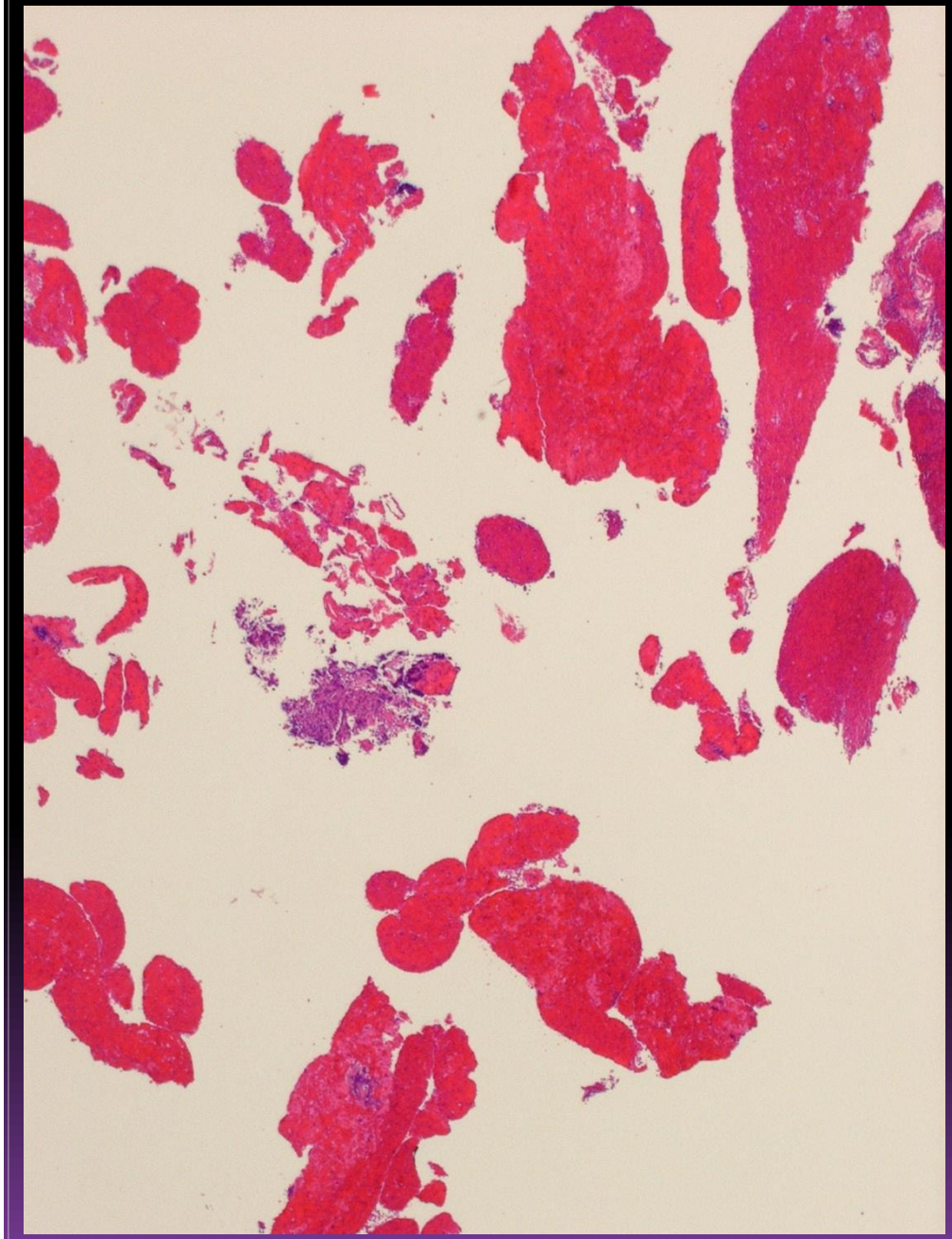


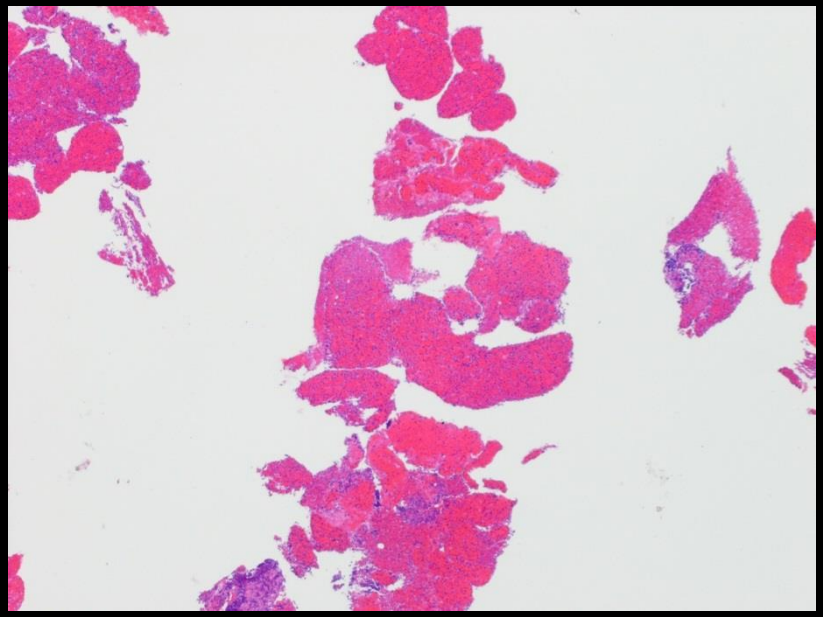


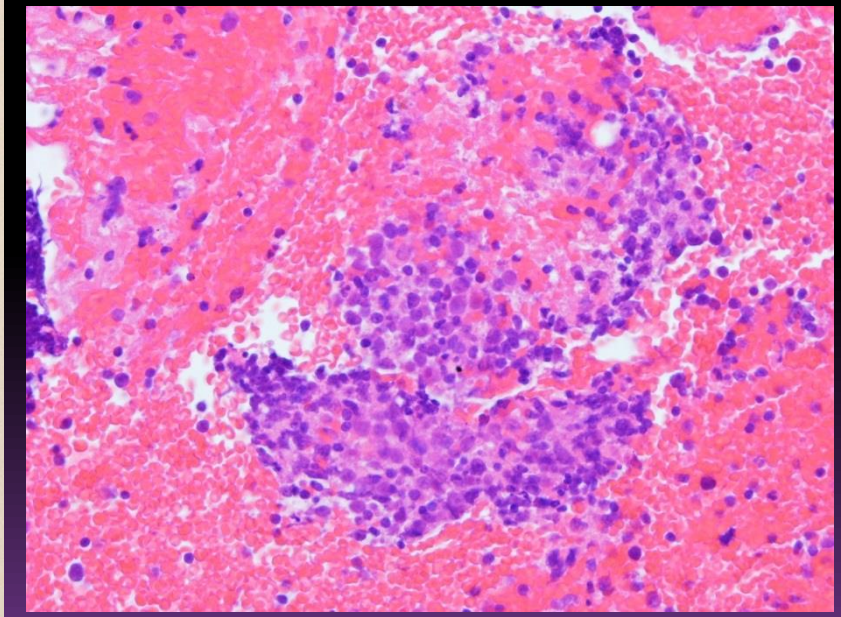


EBUS 19G needles

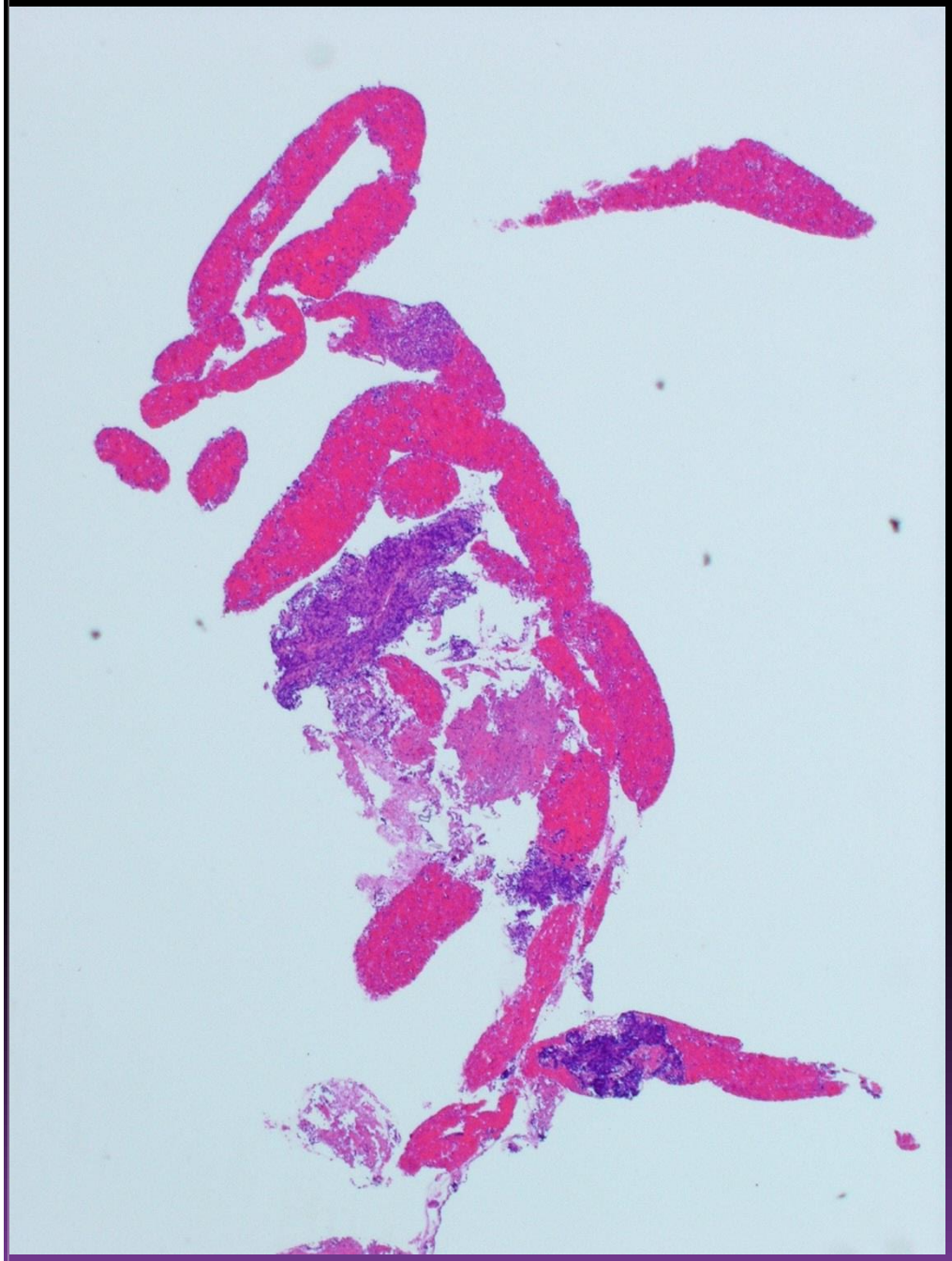


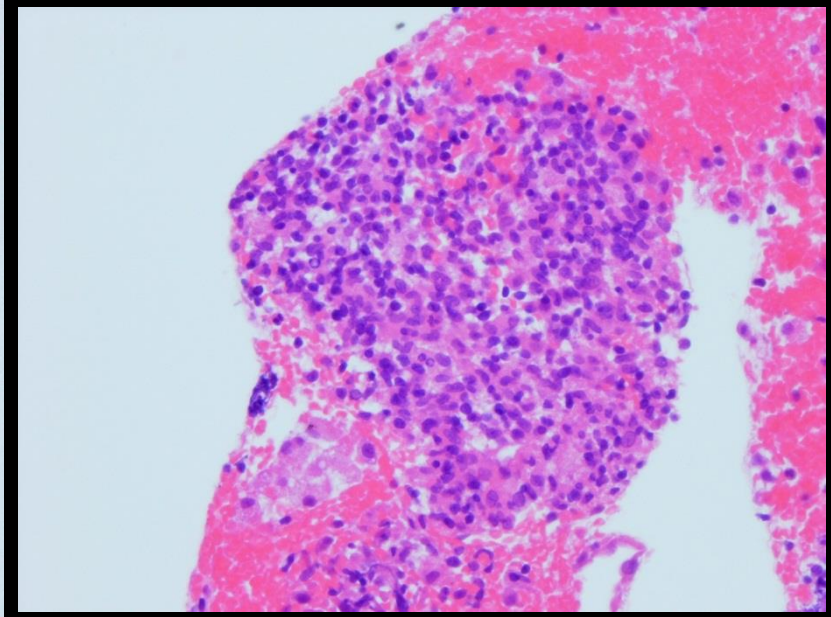


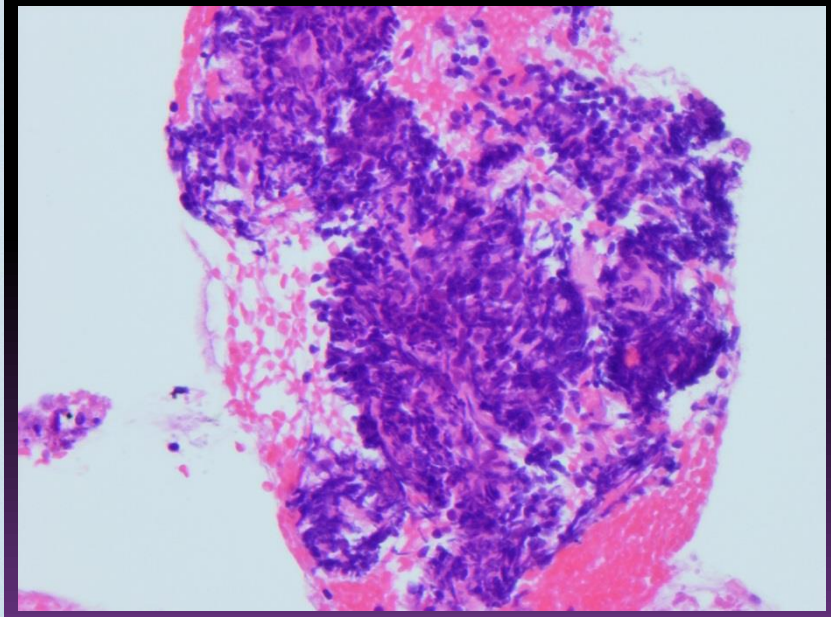




DLBCL-GC, no cytogen abn

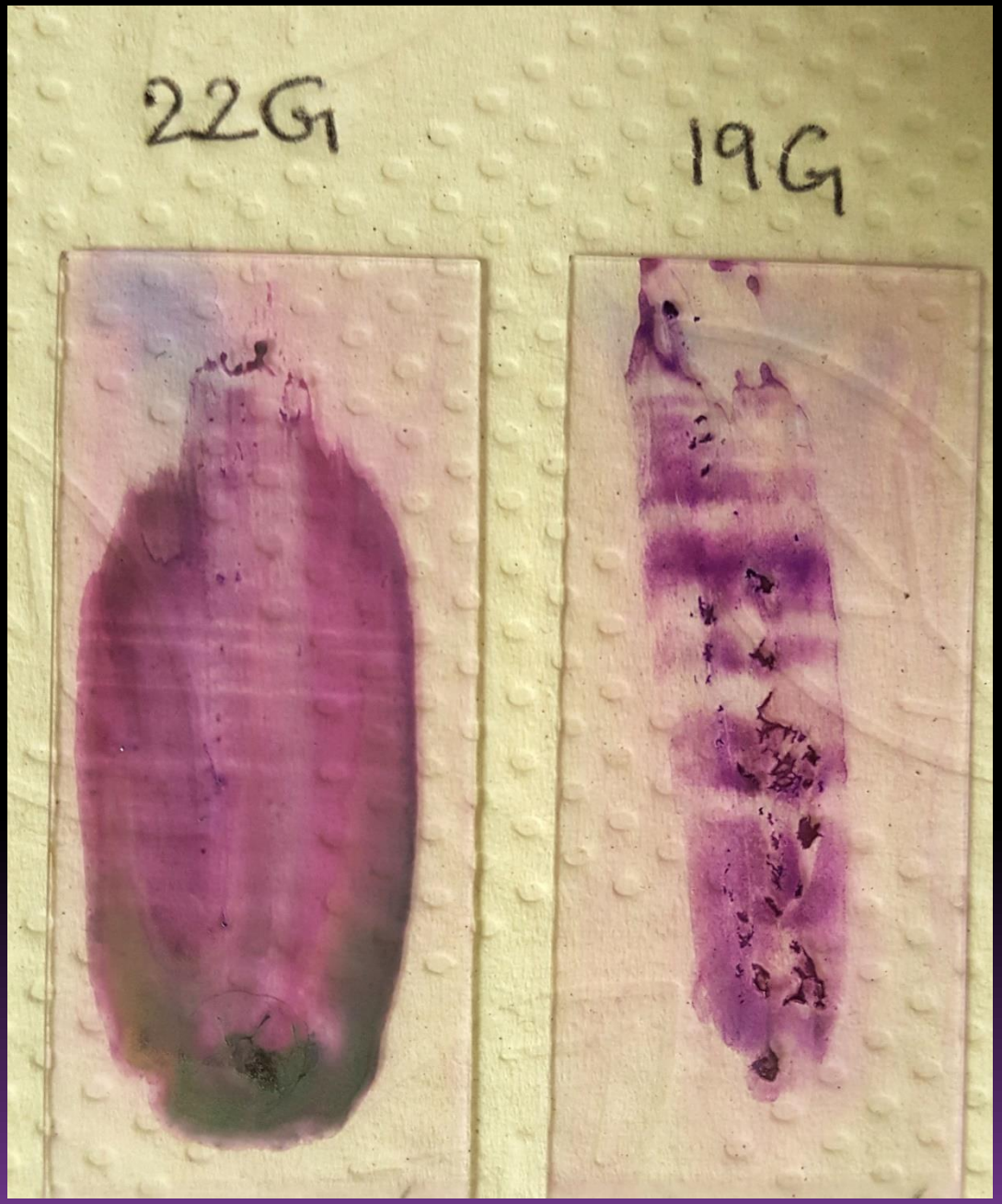


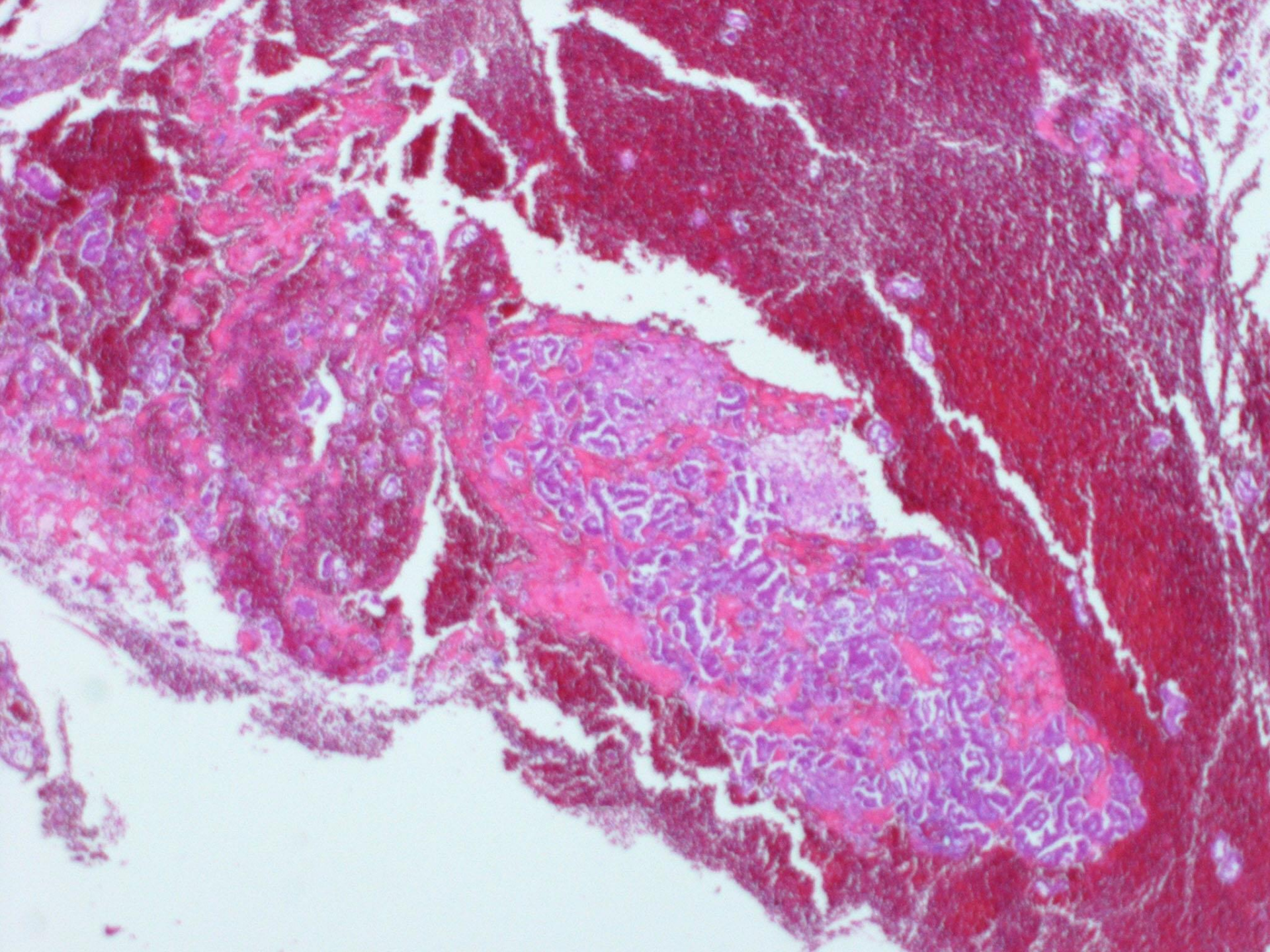


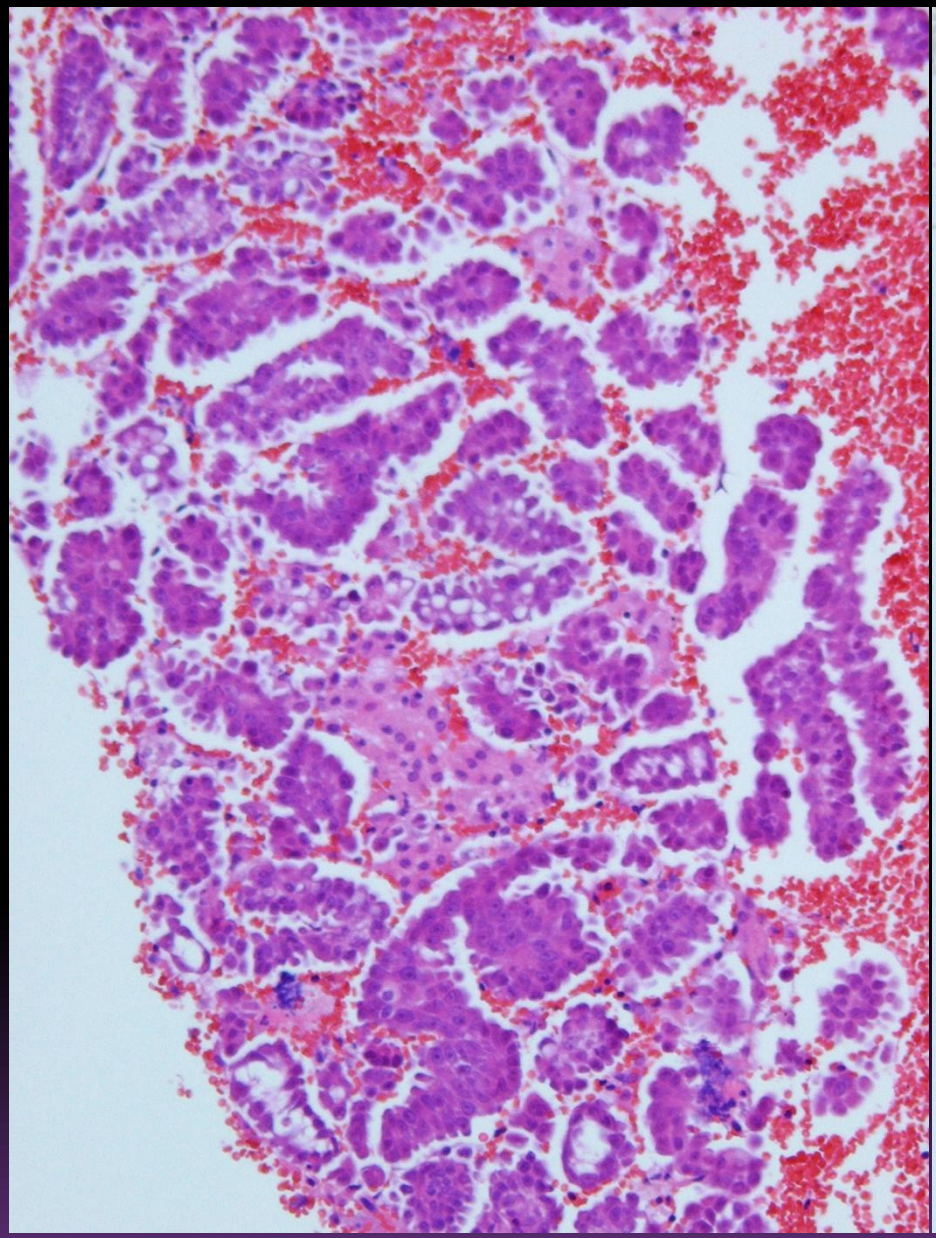


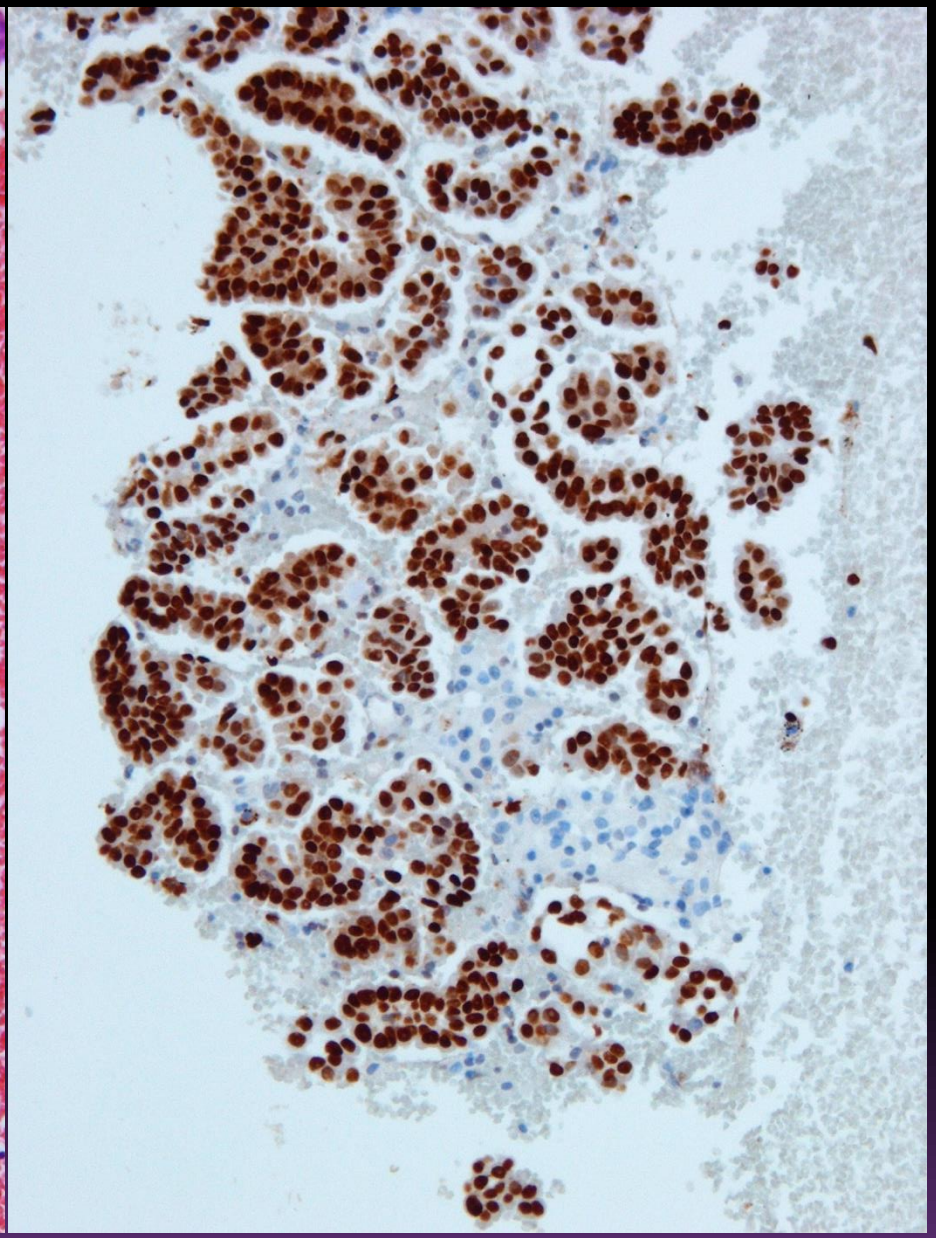
CTCL

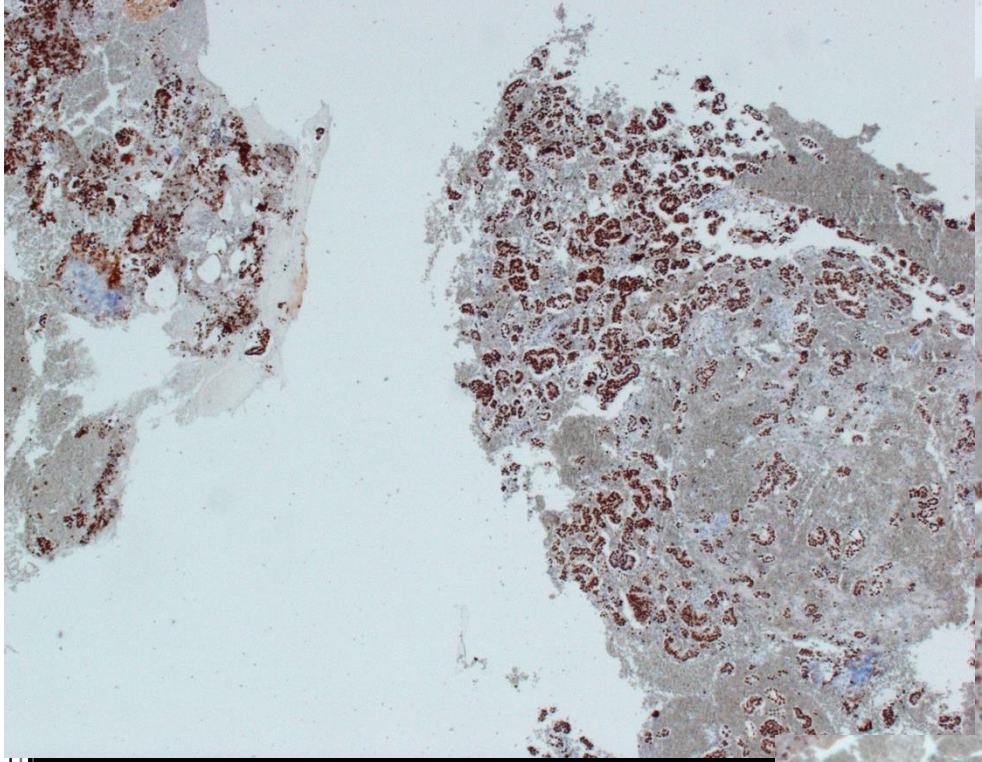
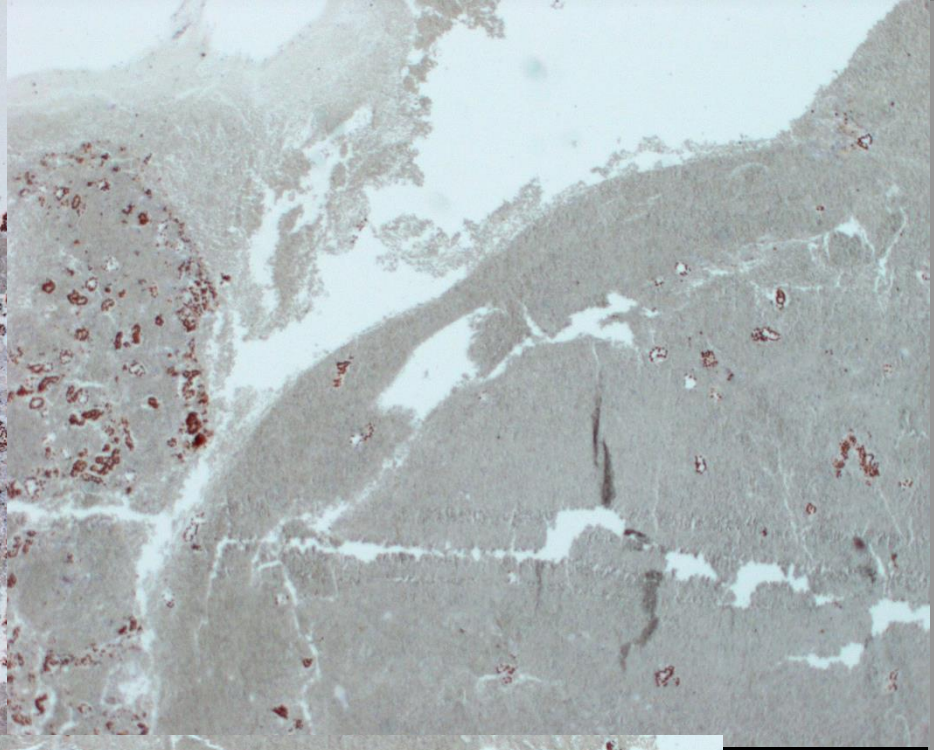
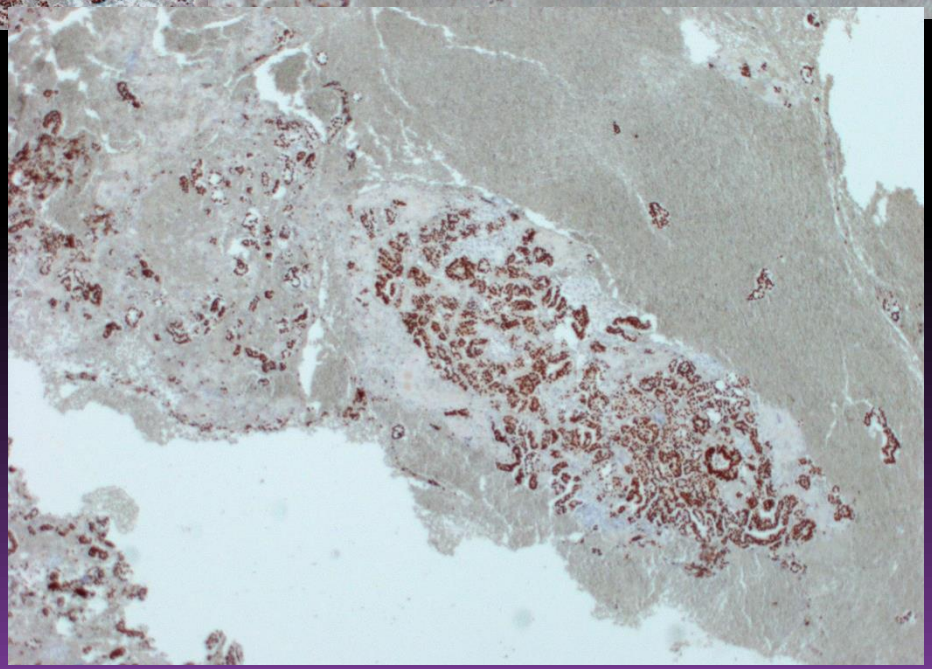

1G

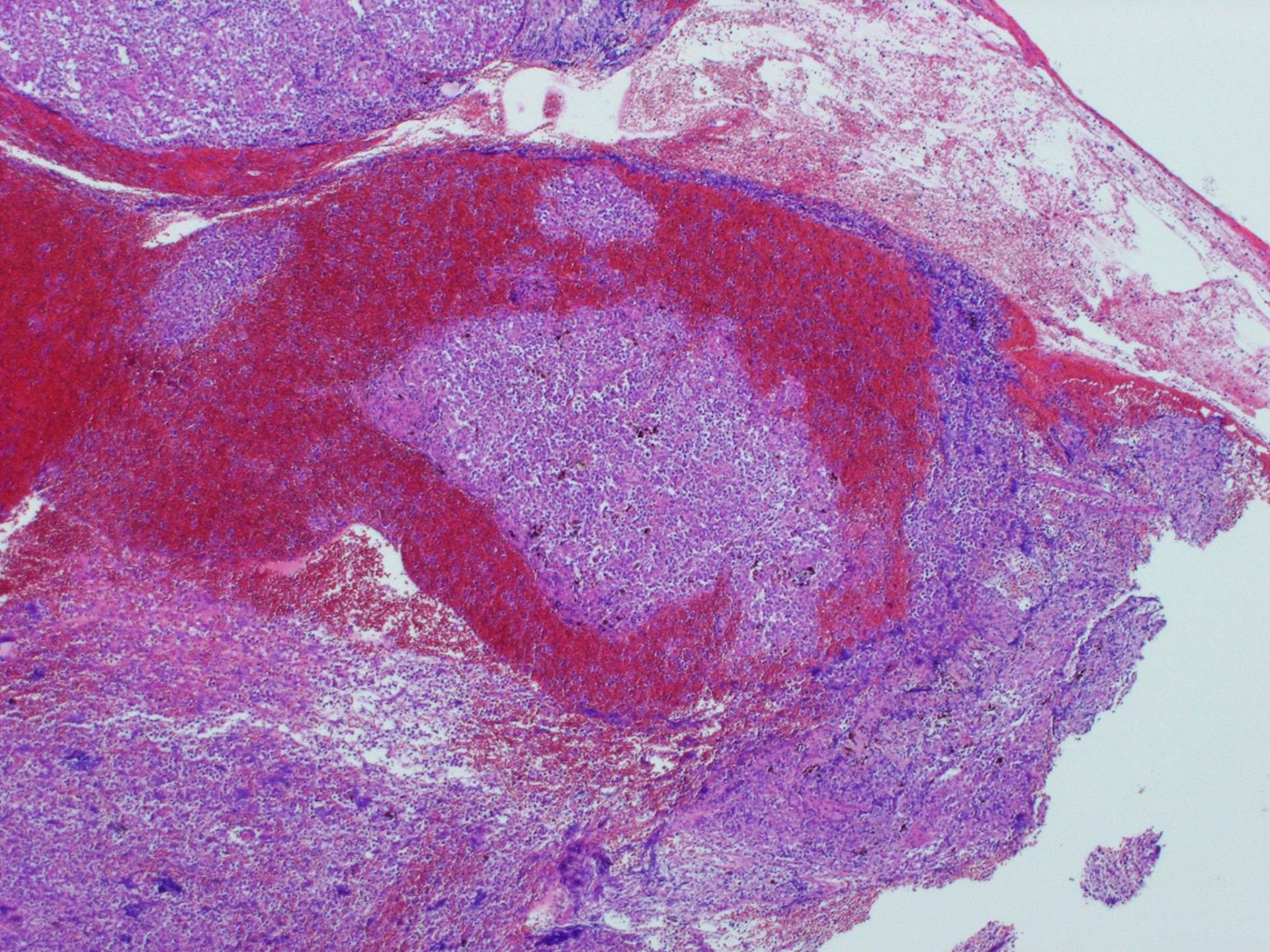

22G
19G

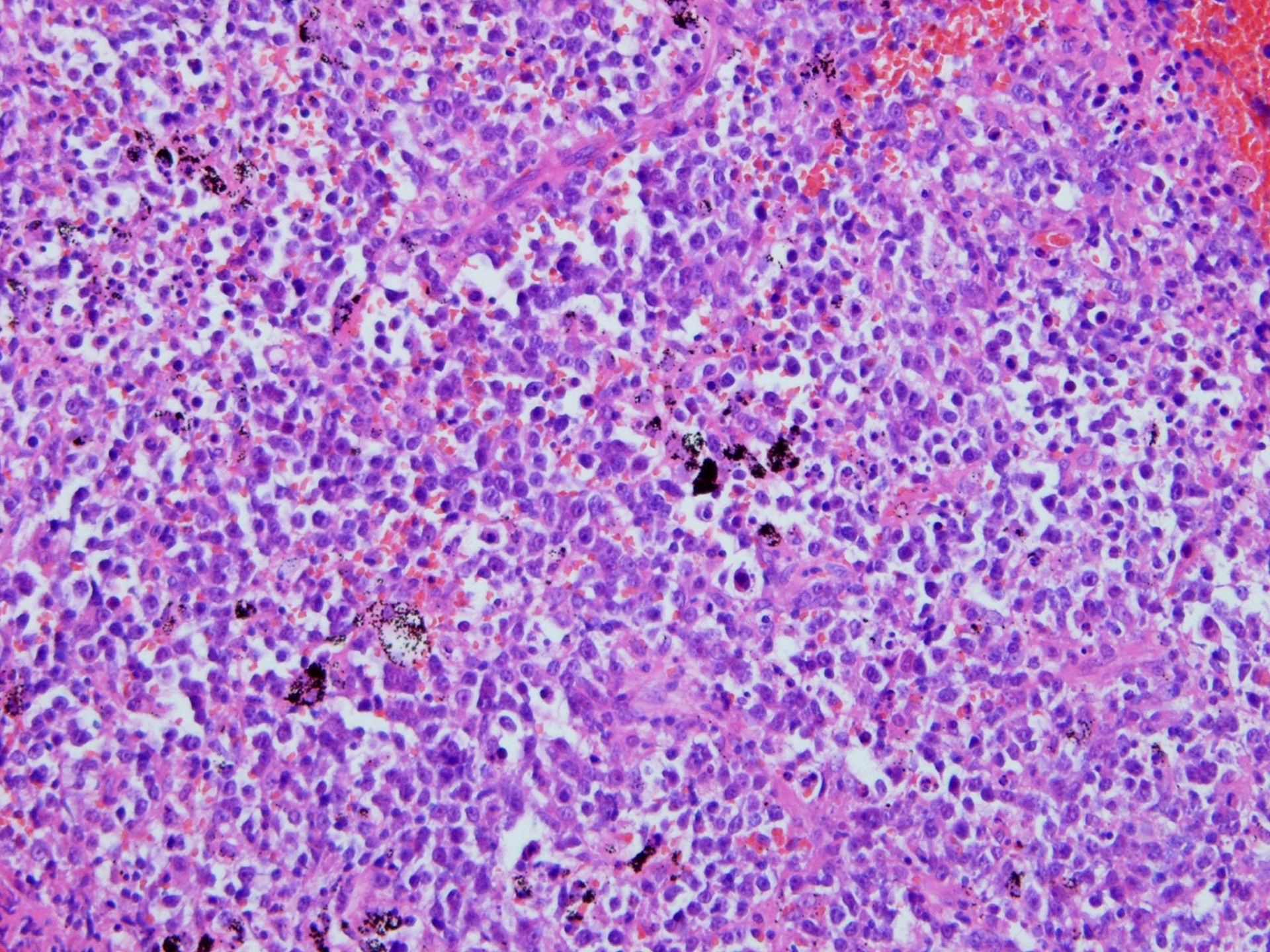


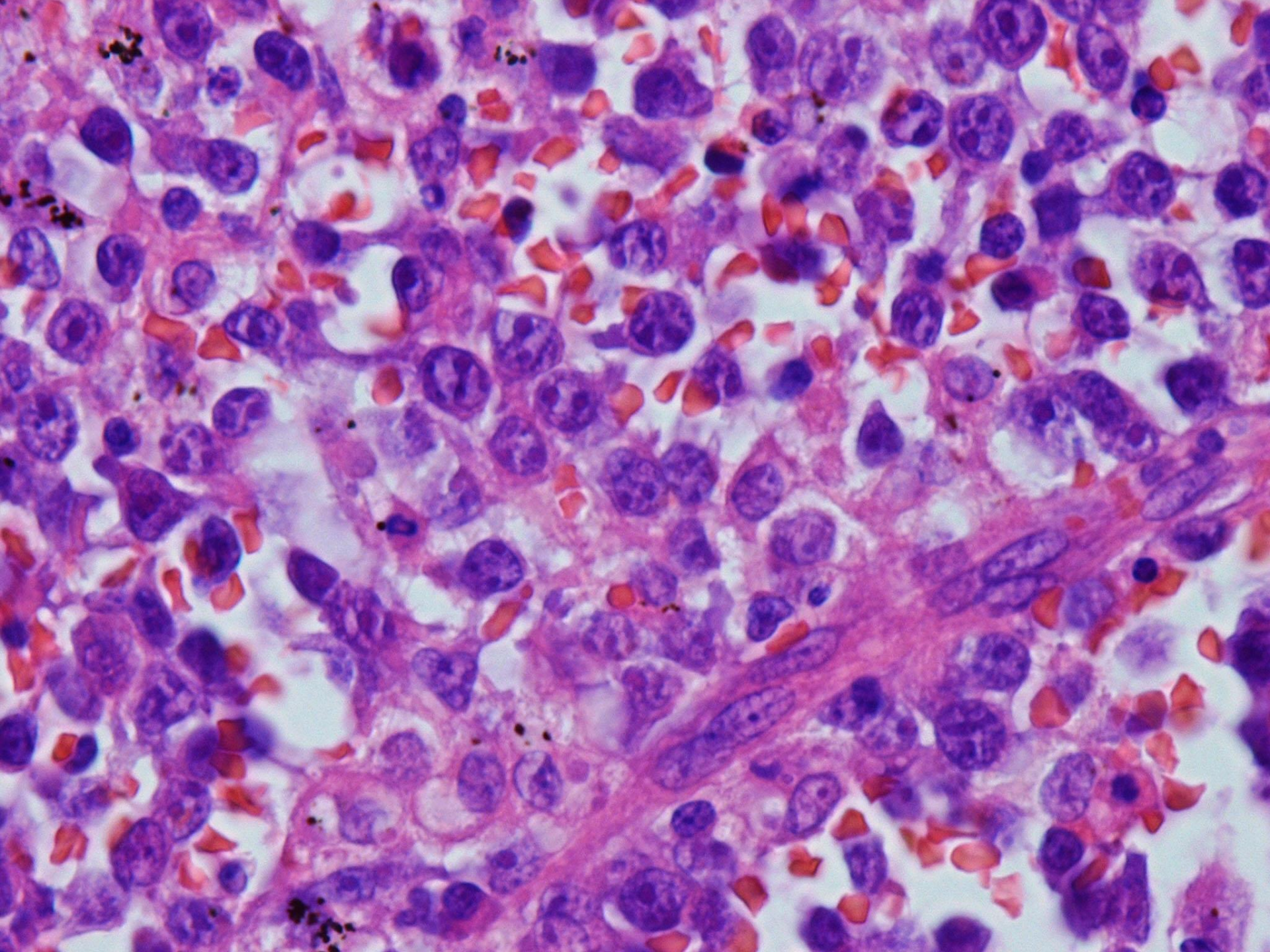






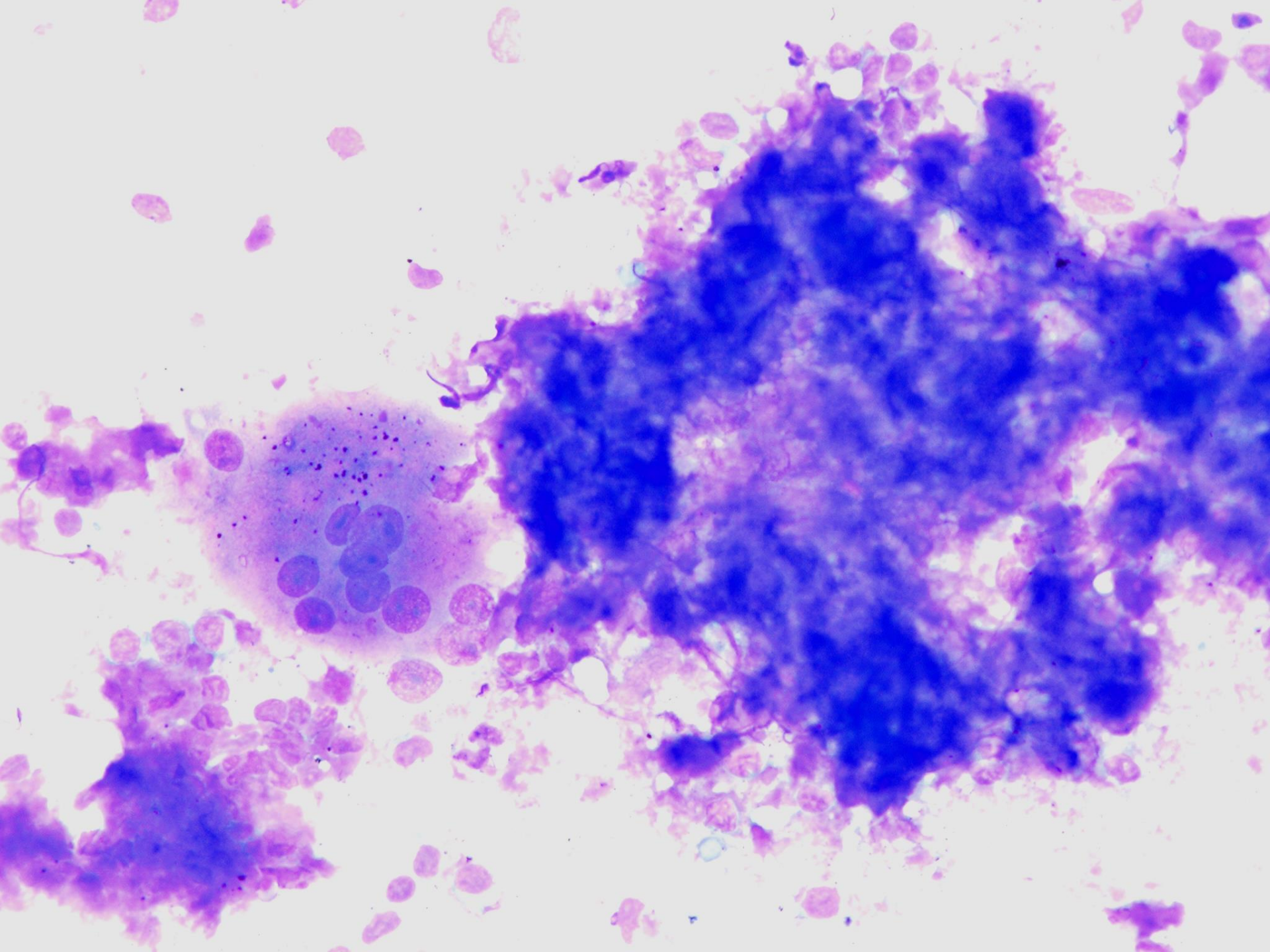


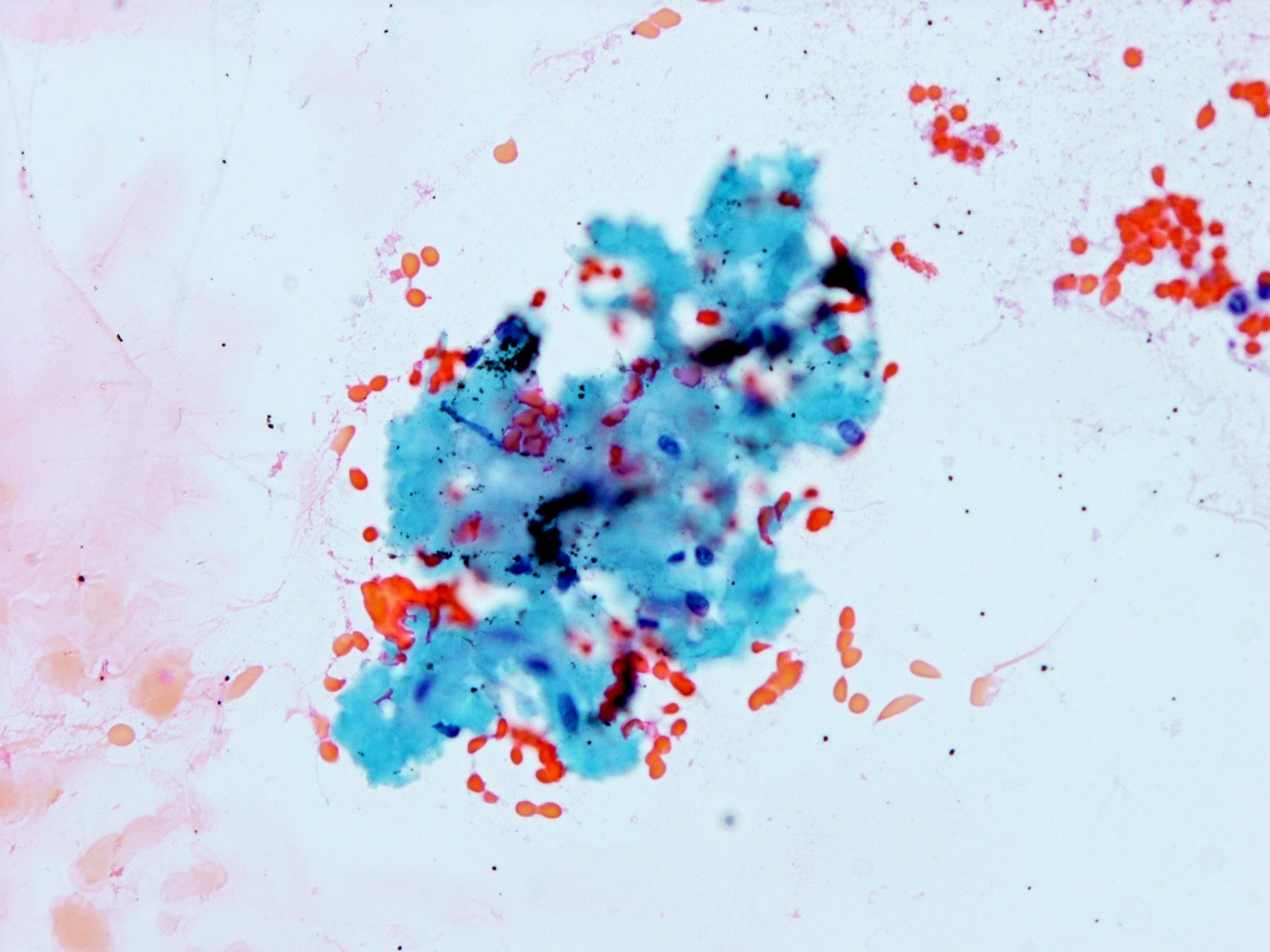


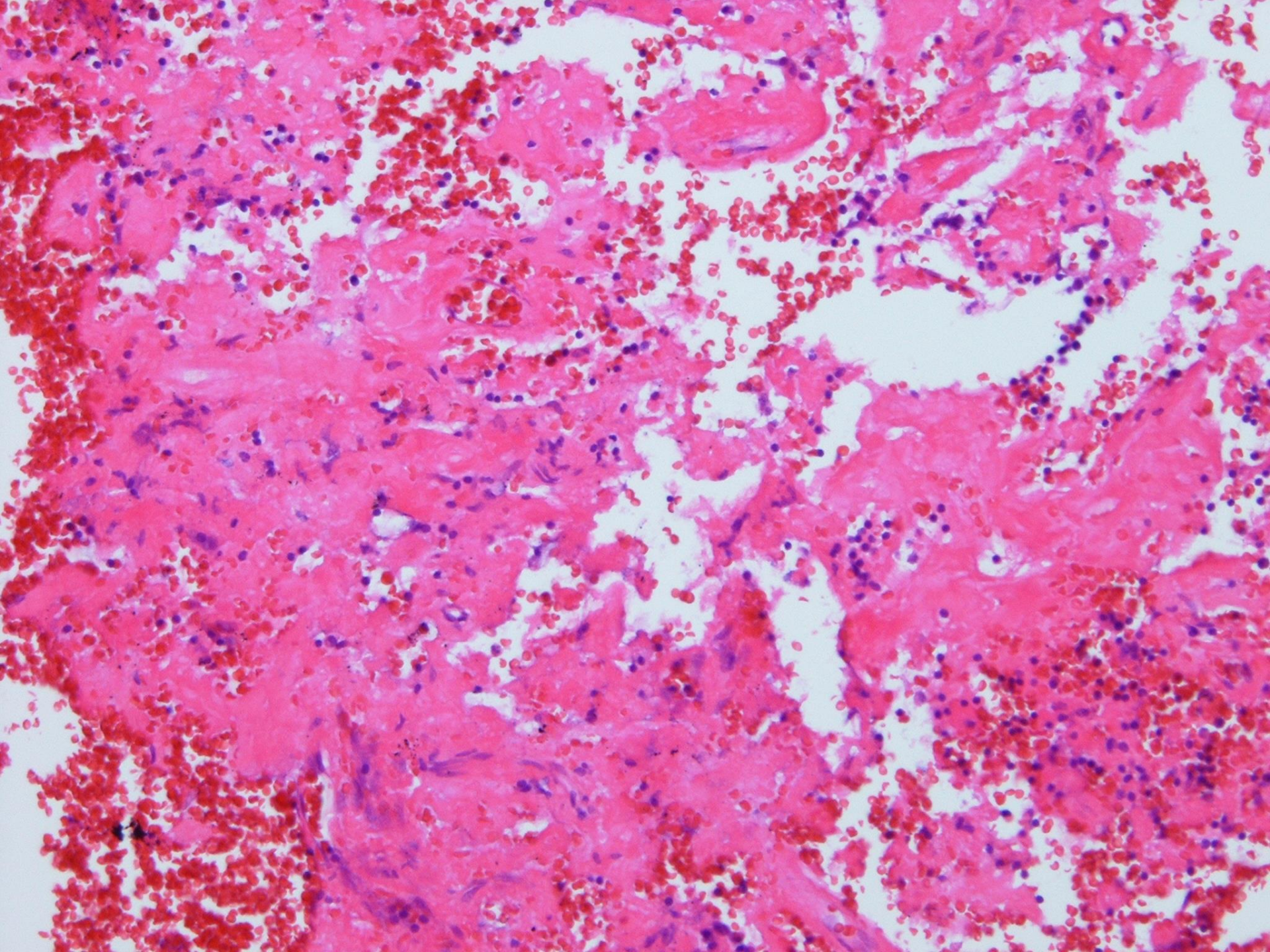
Sampling issues in Hodgkin

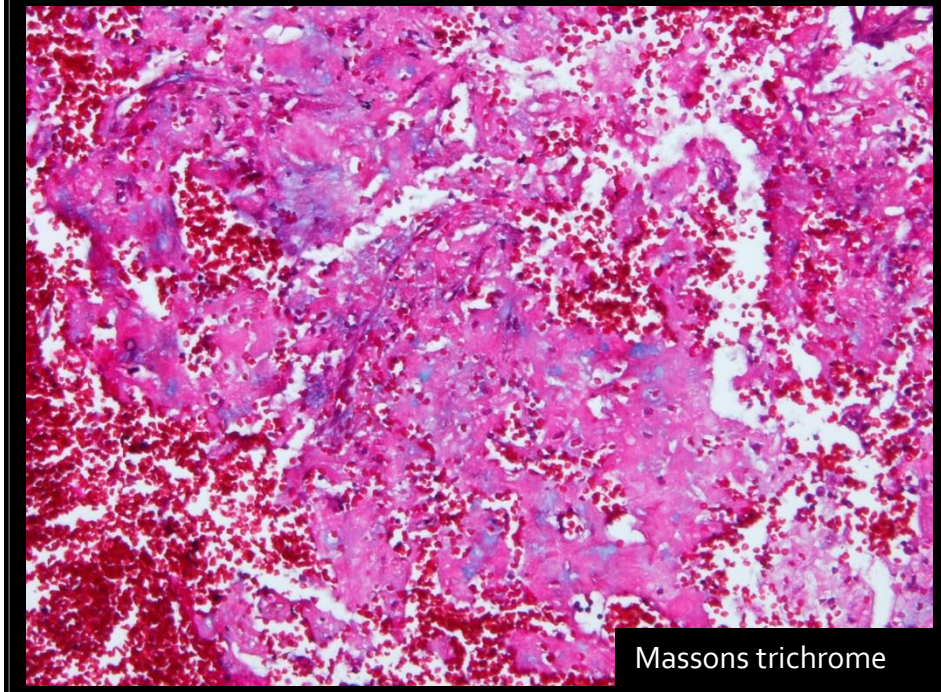
- Necrotic areas – identification on US & cytology
- Fibrosis
- Adequate time between passes for pathologist to pick up Hodgkin cells
- Once diagnosis suspected – change needles

73 M, mediastinal lymphadenopathy, small volume cervical lymphadenopathy

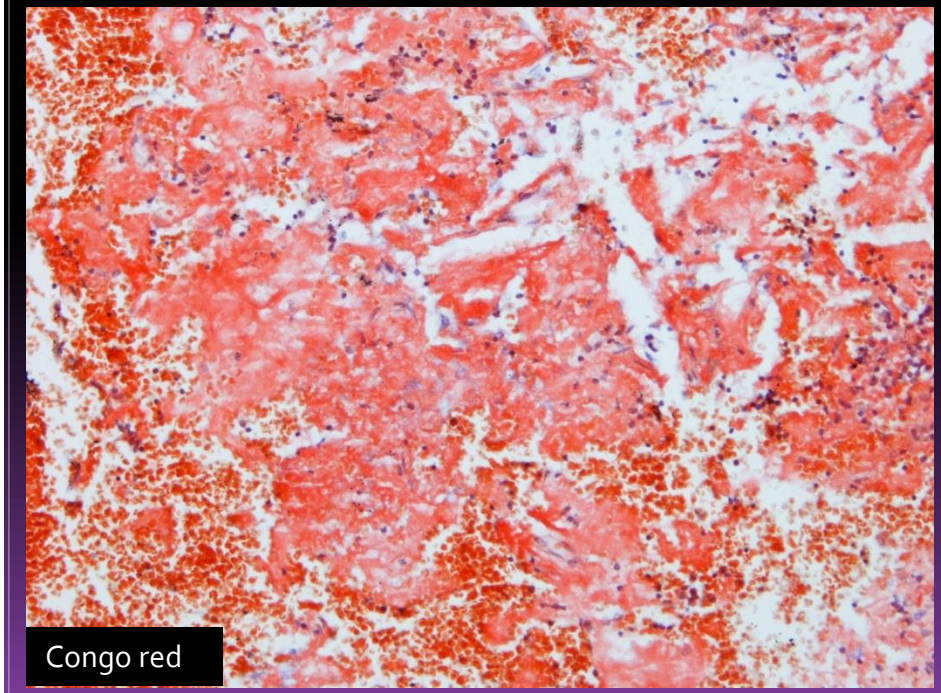




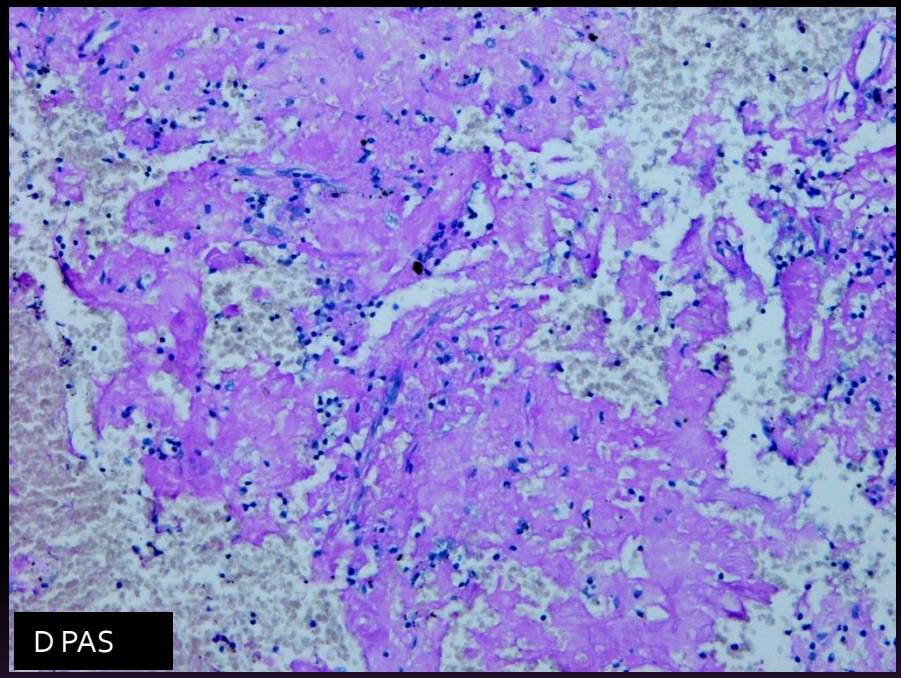




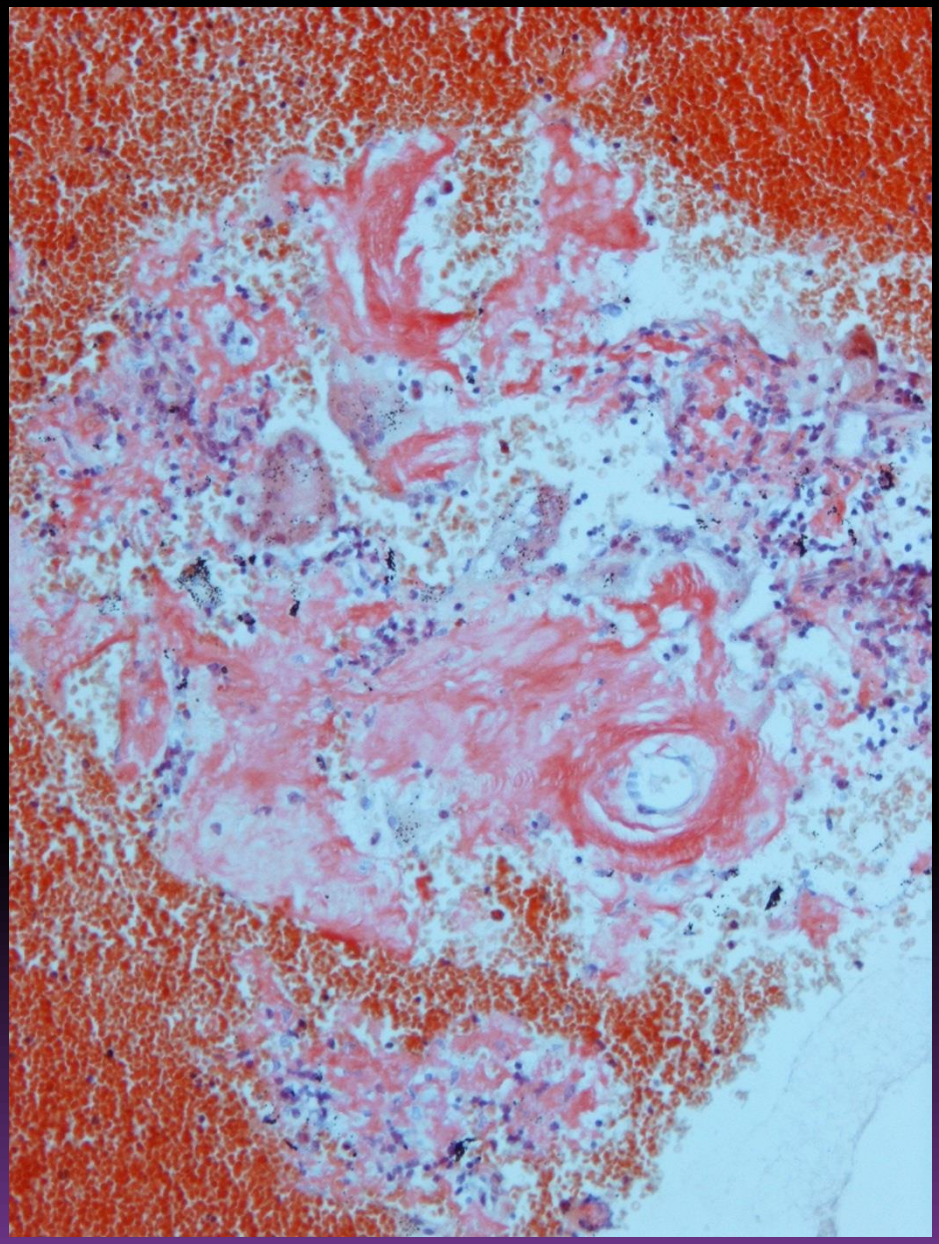
Massons trichrome

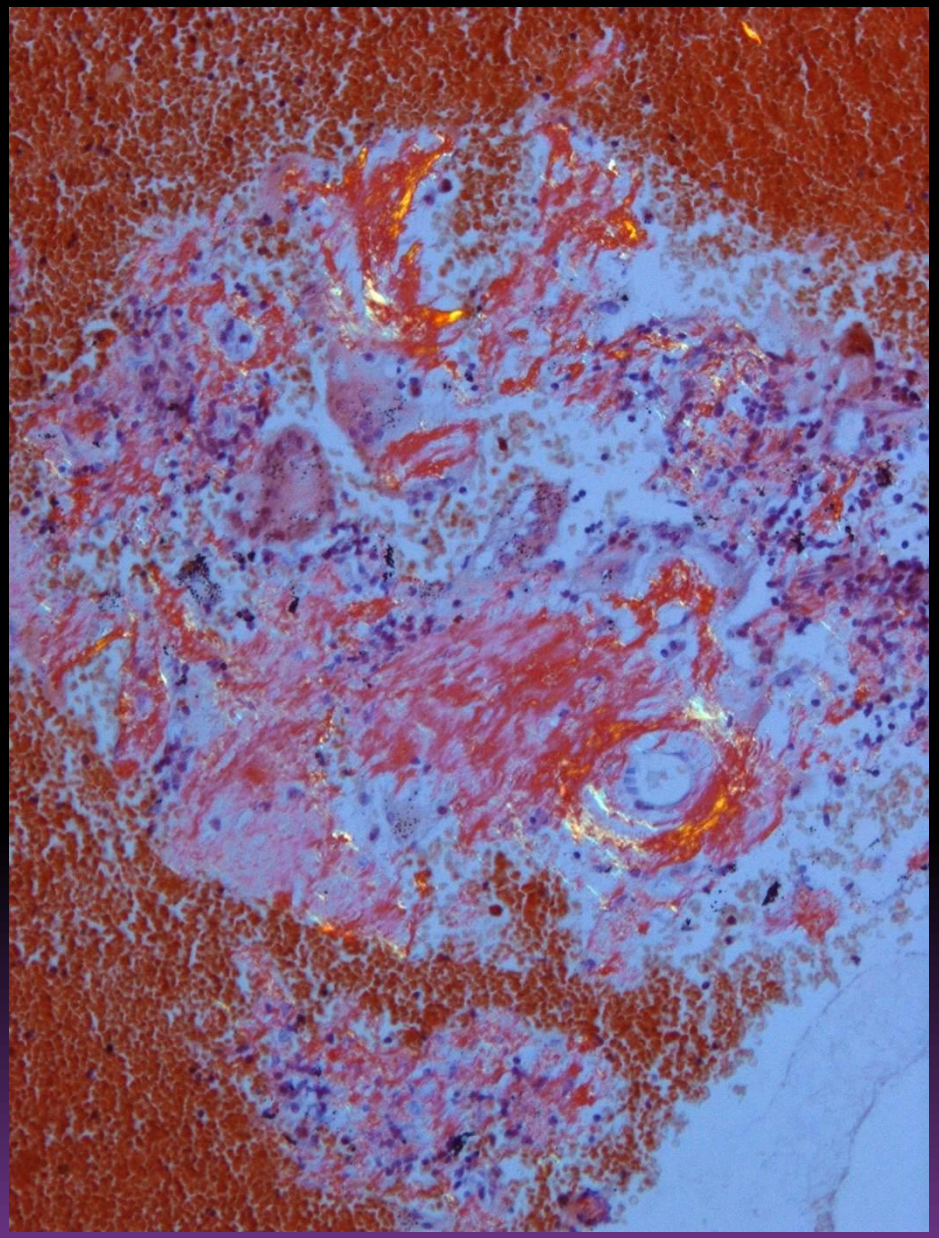


Congo red



D PAS





Negative IHC for Amyloid AA, transthyretin, kappa & lambda

Lymph node amyloidosis
(amyloid of non-AA type)

Is this diagnosis reliable?

How accurate is lymphoma diagnosis on EBUS?

	Year	Nos	Sensitivity	Specificity	PPV	NPV
Grosu	2015	75				
Senturk	2014	15	86.7	100	97	96.4
Moonim et al	2013	93	89	97	98	85
Marshall	2011	33	72	95		
Steinfort	2010	55	57	100		
Kennedy	2008	25	90.9	100		

Moonim MT et al. Diagnosis and subtyping of de novo and relapsed mediastinal lymphomas by endobronchial ultrasound needle aspiration. Am J Respir Crit Care Med 2013; 188: 216-223

Navani N, Janes S. Endobronchial ultrasound guides transbronchial needle aspiration for lymphoma: The final frontier. Am J Respir Crit Care Med 2013; 188: 1183-85

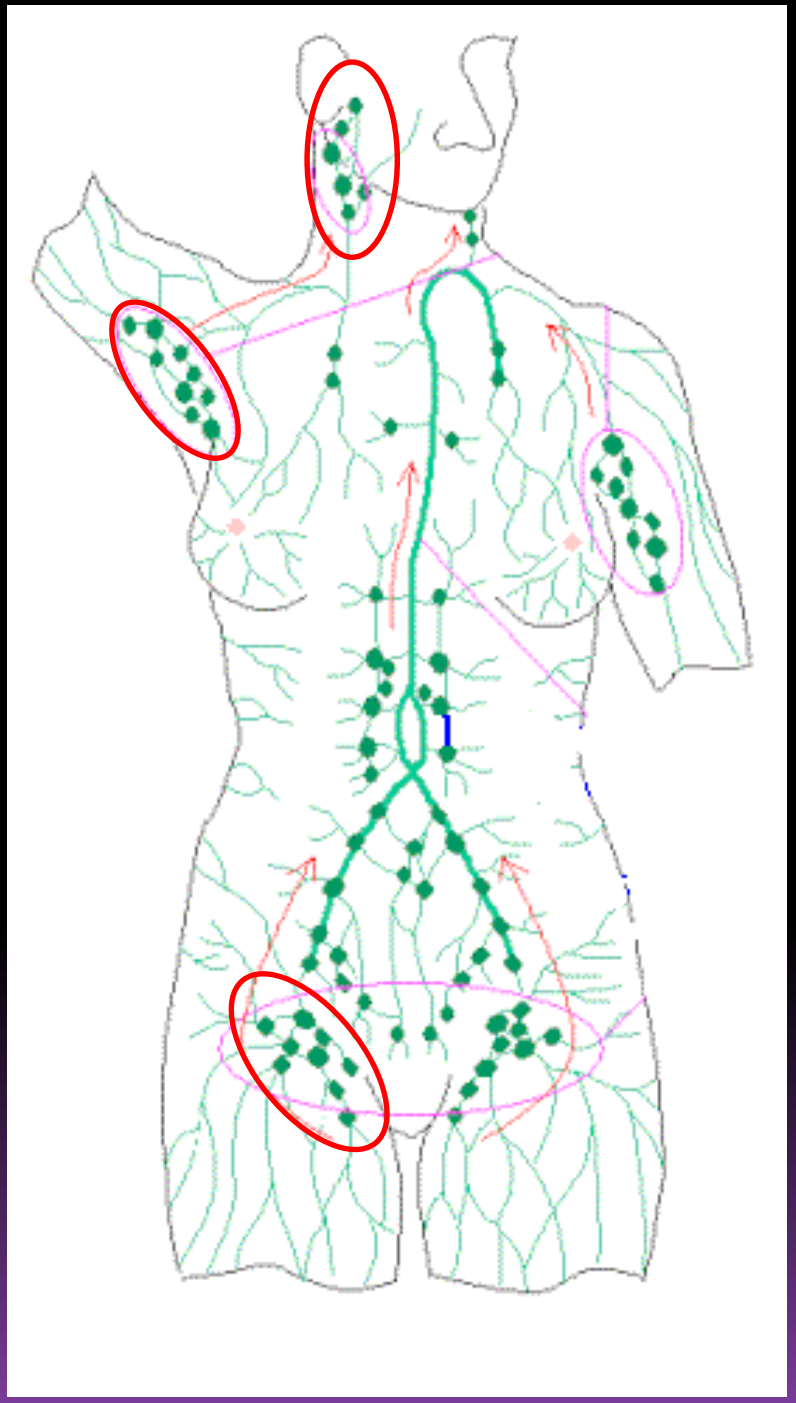
Comparison between EBUS-TBNA and final diagnoses

EBUS-TBNA diagnosis (n = 93)	Final diagnosis (n = 93)			
	High-grade NHL	Low-grade NHL	Hodgkin lymphoma	Non-lymphoma diagnosis
High-grade B/T NHL (n=9)	**9**	0	0	0
Probable high-grade NHL (n=1)	0	0	0	1
Low-grade B-NHL (n=26)	0	**26**	0	0
Hodgkin lymphoma (n=17)	0	0	**17**	0
Probable Hodgkin lymphoma (n=6)	1	0	5	0
Non- lymphoma diagnosis (n=32)	0	0	0	**32**
Inadequate (n=2)	1	0	0	1

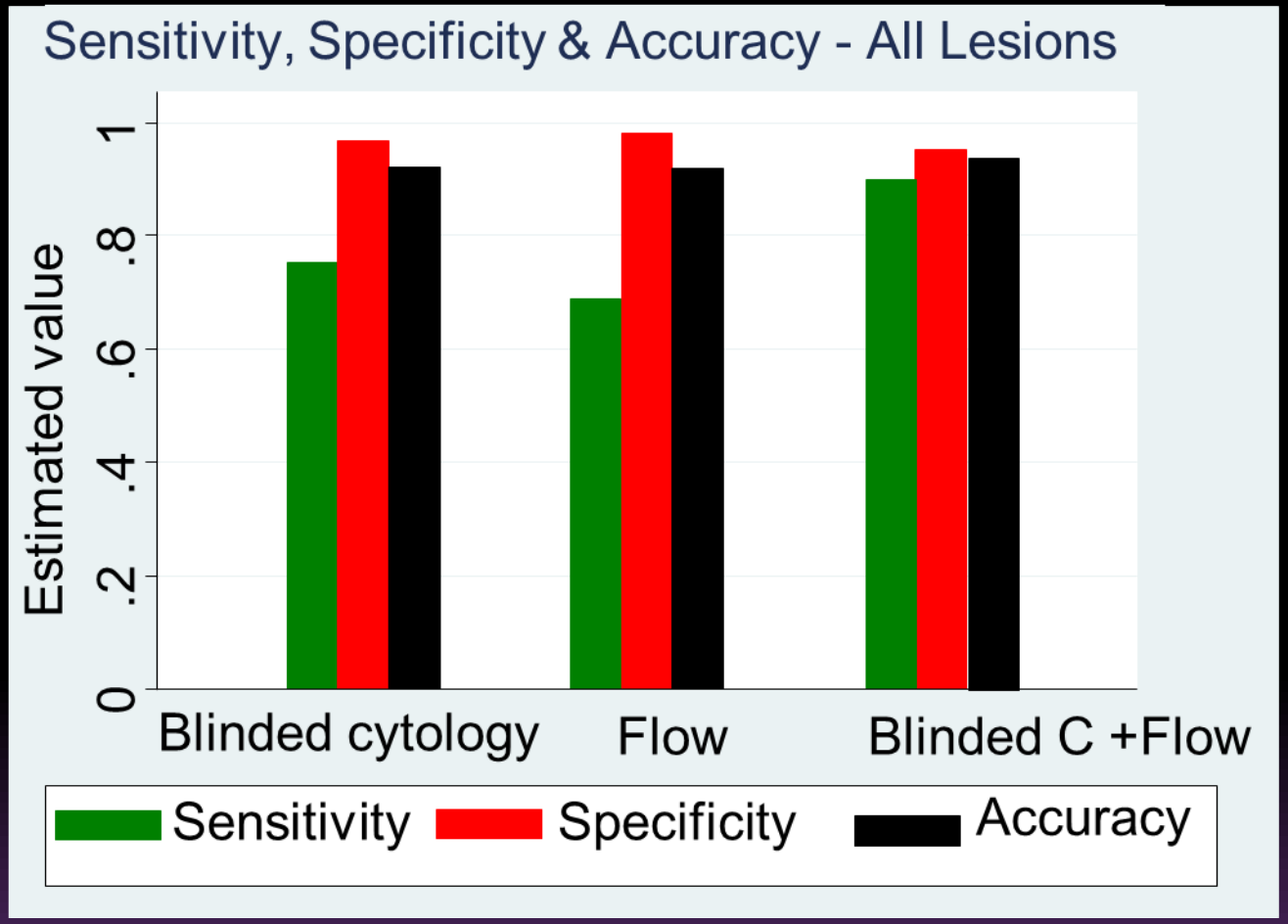
Comparison between EBUS-TBNA and final diagnoses

EBUS-TBNA diagnosis (n = 93)	Final diagnosis (n = 93)			
	High-grade NHL	Low-grade NHL	Hodgkin lymphoma	Non-lymphoma diagnosis
High-grade B/T NHL (n=9)	9	0	0	0
Probable high-grade NHL (n=1)	0	0	0	1
Low-grade B-NHL (n=26)	0	26	0	0
Hodgkin lymphoma (n=17)	0	0	17	0
Probable Hodgkin lymphoma (n=6)	1	0	5	0
Non- lymphoma diagnosis (n=32)	0	0	0	32
Inadequate (n=2)	1	0	0	1

Superficial lymph node / mass sampling



Do we need to biopsy every lump?


Sensitivity, Specificity & Accuracy - All Lesions
Estimated value
0
.2
.4
.6
.8
1
Blinded cytology
Flow
Blinded C +Flow
Sensitivity
Specificity
Accuracy

Flow cytometry of ‘normal / reactive’ lymph nodes

- Aspirates collected in normal saline.
- Add fetal calf serum for cell preservation
- Commercial flow cytometry transport media now available

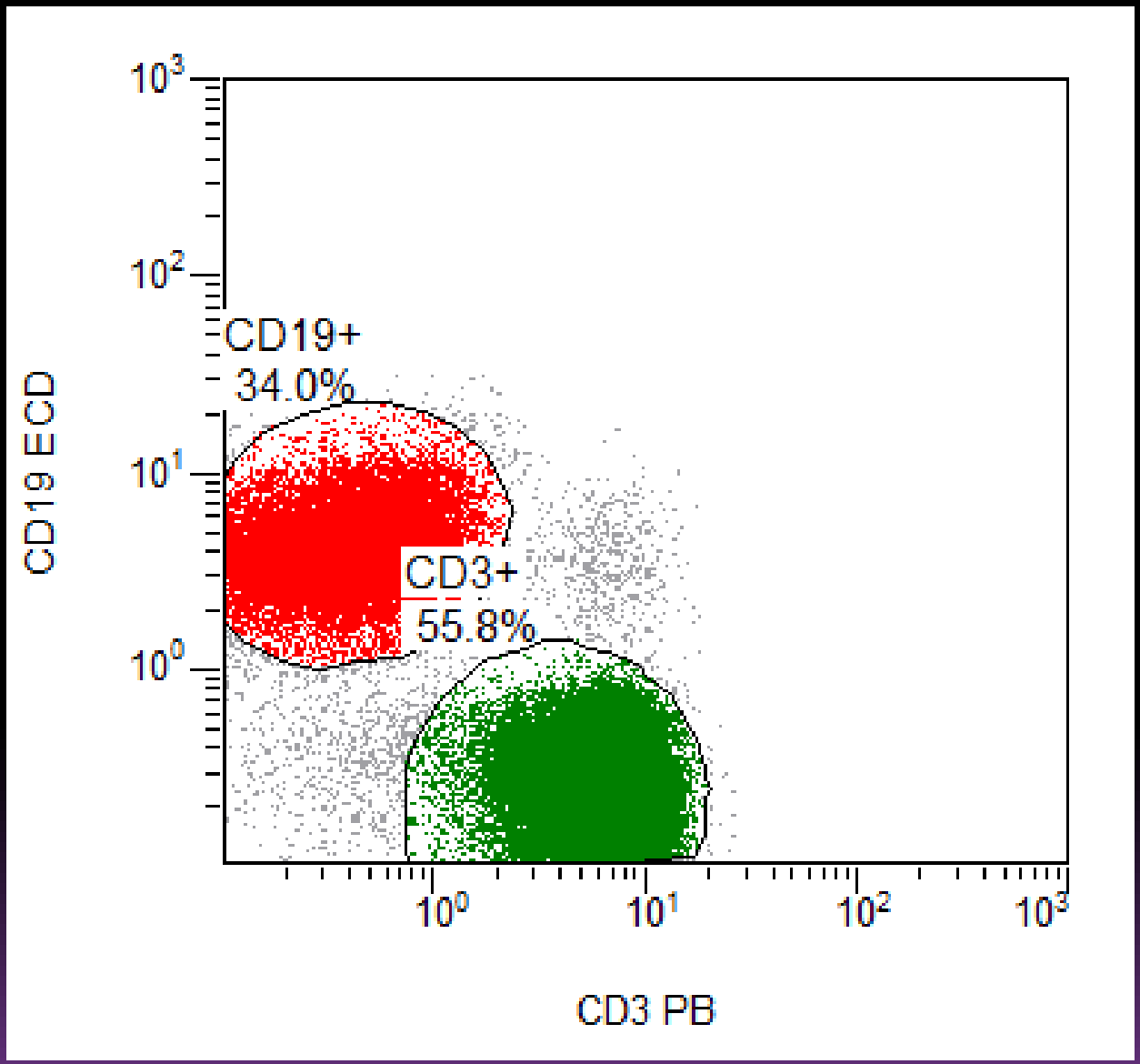
- 4 colour vs 8 colour vs 10 colour

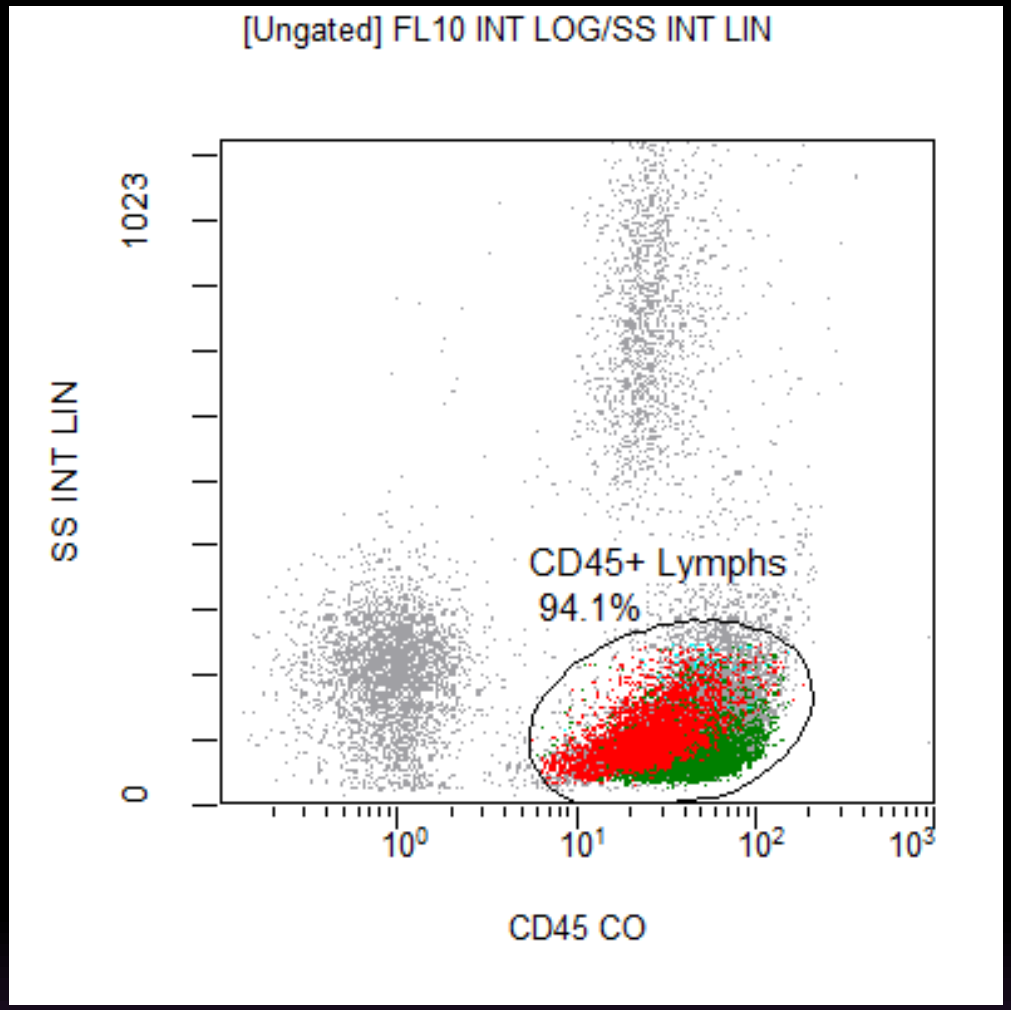
GSTT – FCM FNA panel

- 10 colour
- 2 tube
- 500 – 600 / year

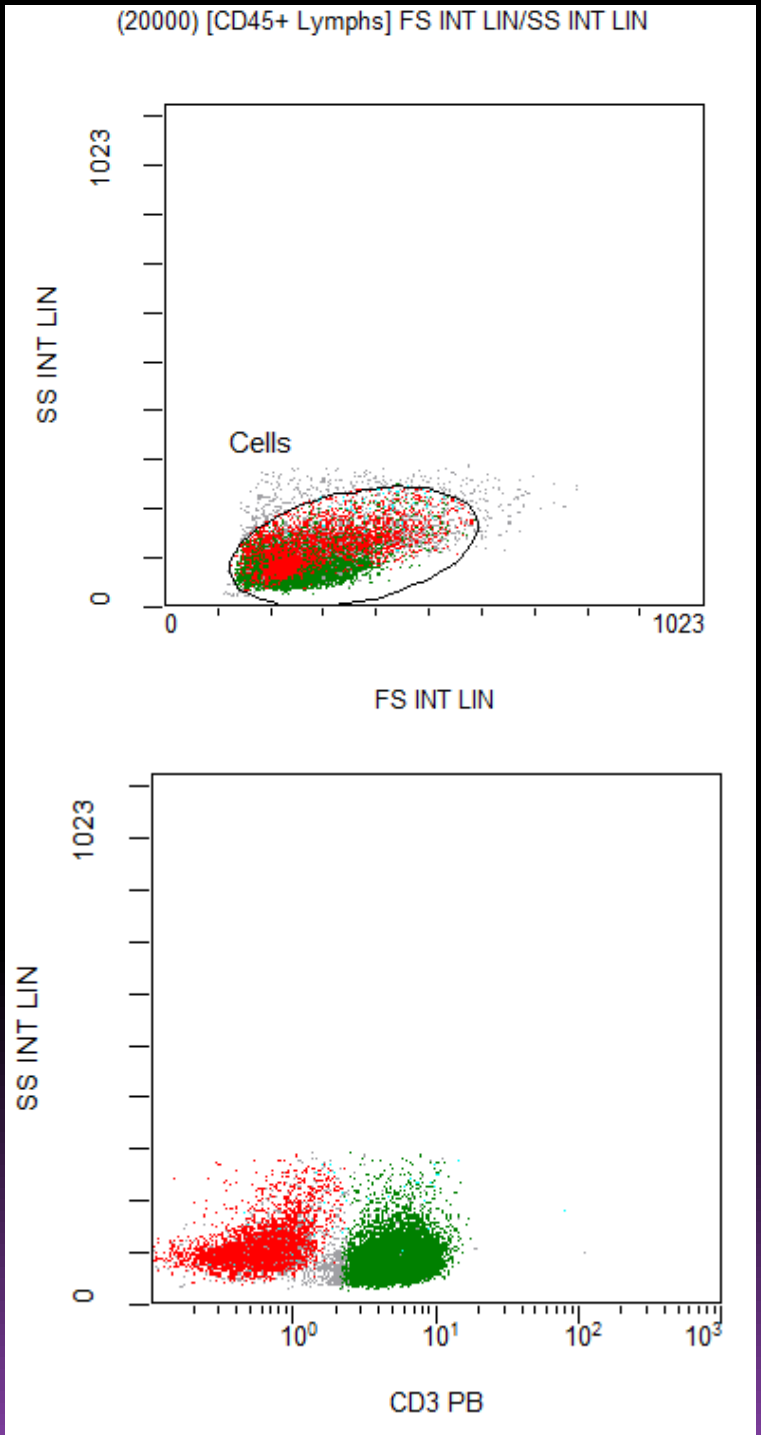
	CO	ECD	APC750	PB	PC5.5	PC7	APC	APC700	PE	FITC
Tube 1	CD45	CD19	CD20	CD3	CD5	CD7	CD4	CD8	CD14	CD43
		B-cell		T-cell					Mac/mono	
Tube 2	CD45	CD19	CD20	FMC7	CD5	CD10	CD23		Lambda	Kappa
		B-cell		B-cell NHL					Light chains	

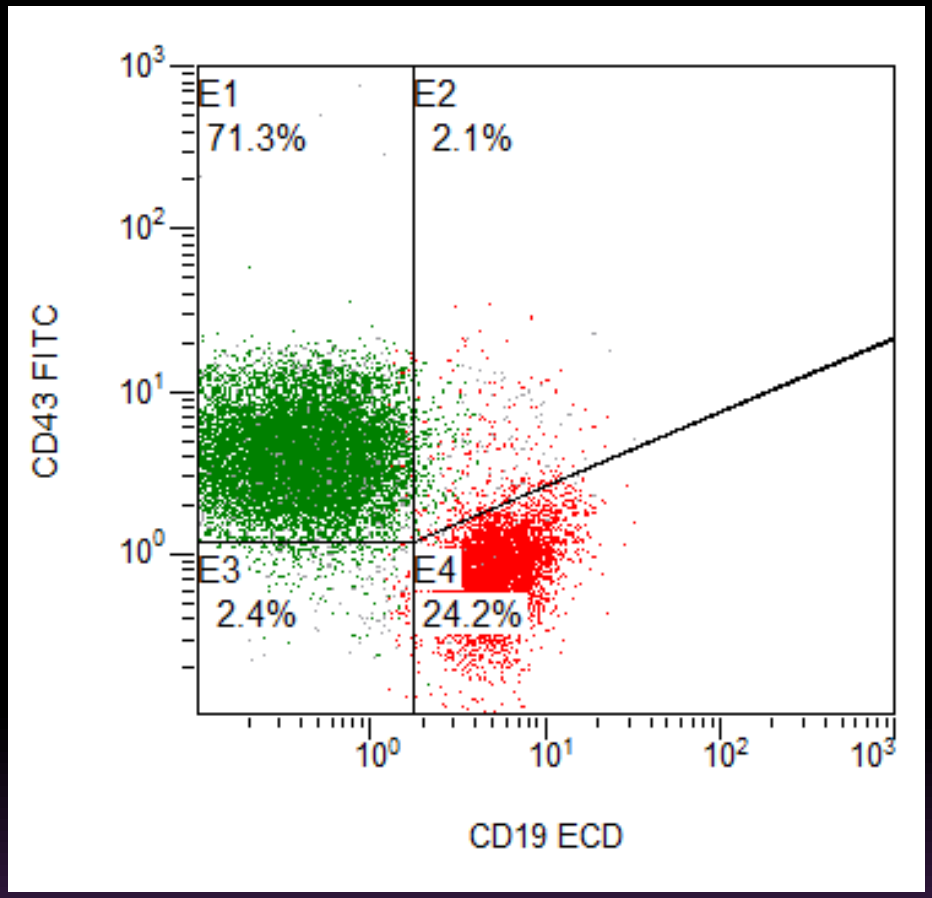
Moonim, Wilkins, Carr: FNA panel SOP

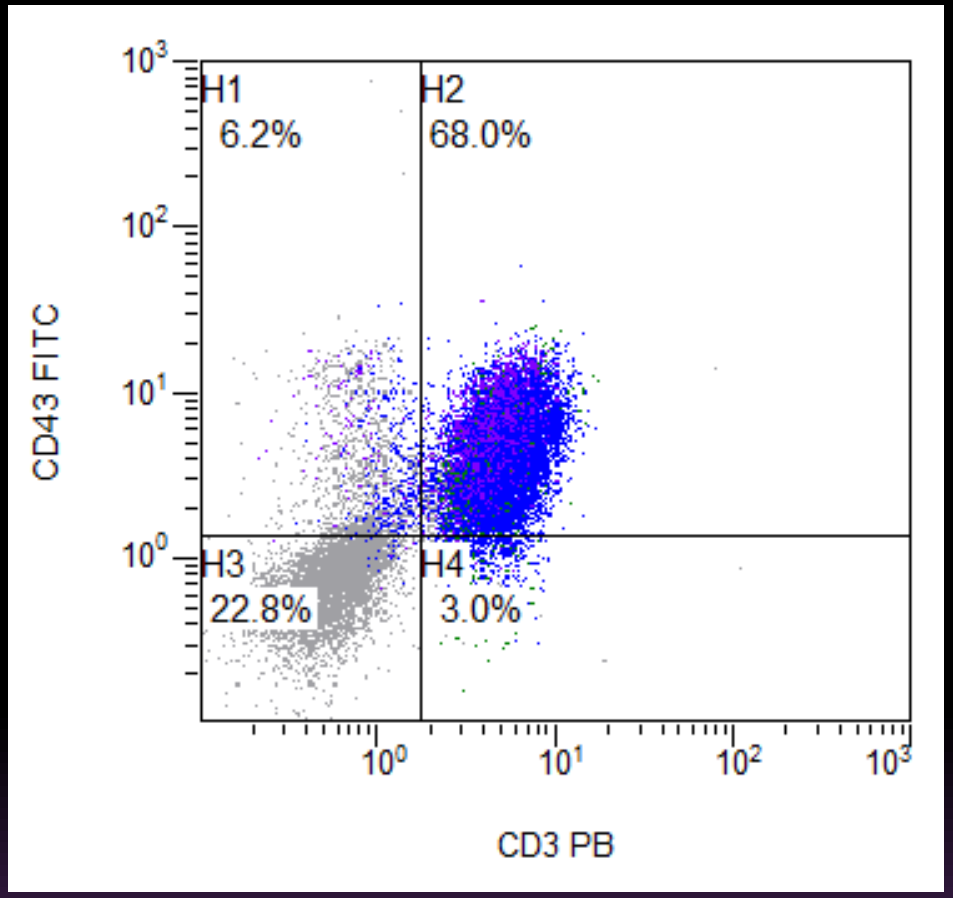

CD19+
34.0%
CD3+
55.8%
CD19 ECD
CD3 PB
10^0
10^1
10^2
10^3

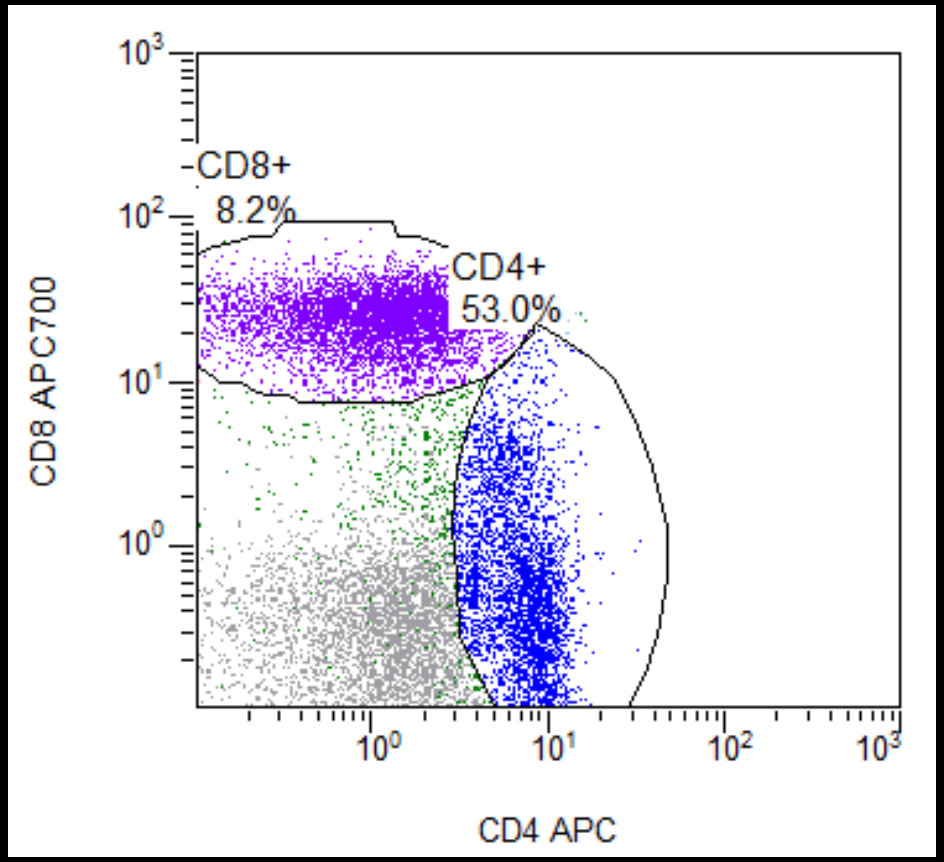


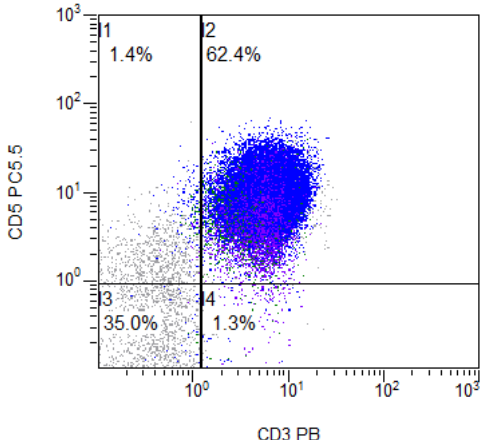
	Events / tube
Palpable mass FNA	1000 - 10000
EBUS FNA	15000 - 25000

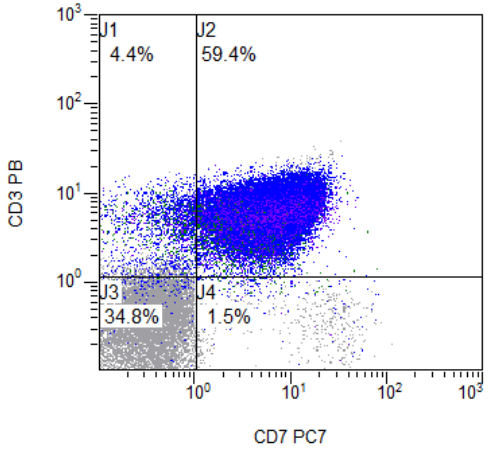


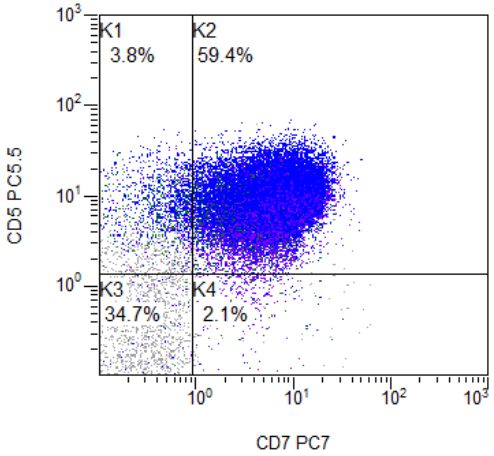

E1
71.3%
E2
2.1%
E3
2.4%
E4
24.2%
CD43 FITC
CD19 ECD
10^0
10^1
10^2
10^3

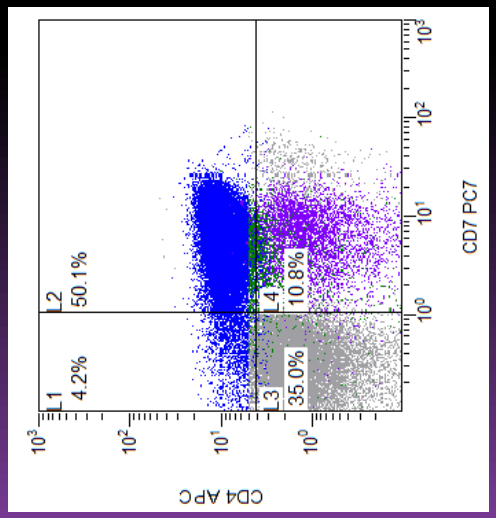

H1
6.2%
H2
68.0%
H3
22.8%
H4
3.0%
CD43 FITC
CD3 PB
10^0
10^1
10^2
10^3

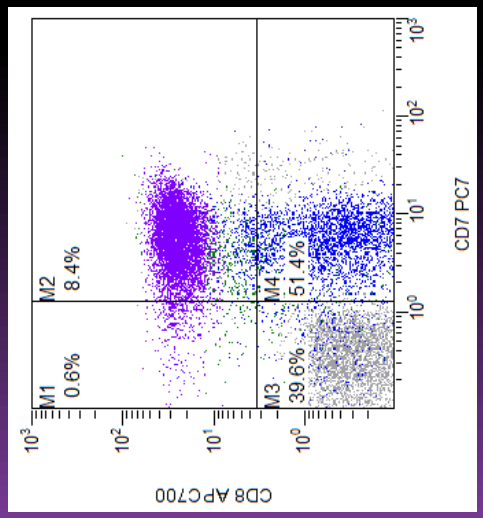

CD8+
8.2%
CD4+
53.0%
CD8 APC700
CD4 APC

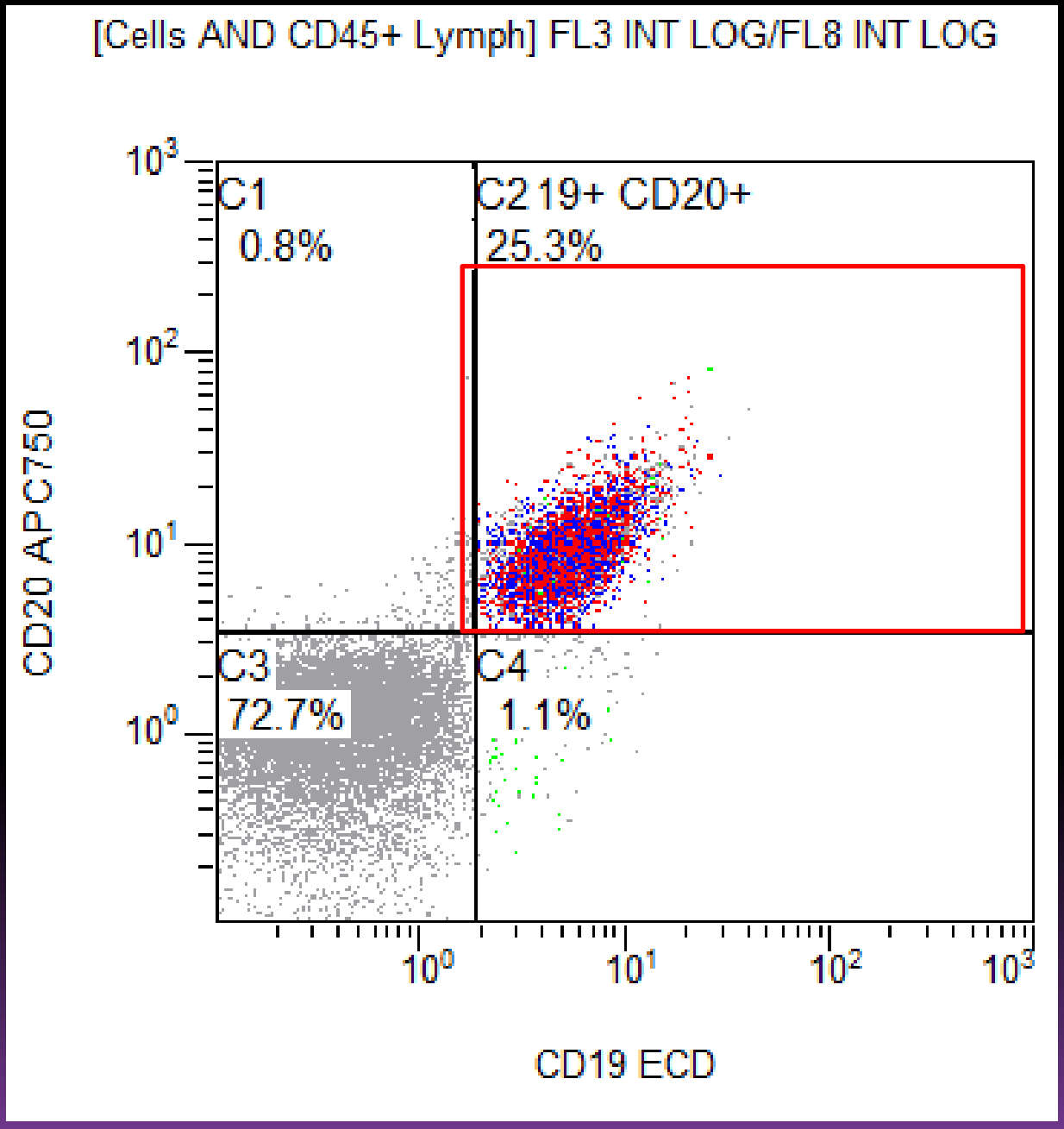

J1
1.4%
J2
62.4%
J3
35.0%
J4
1.3%
CD5 PC5.5
CD3 PB

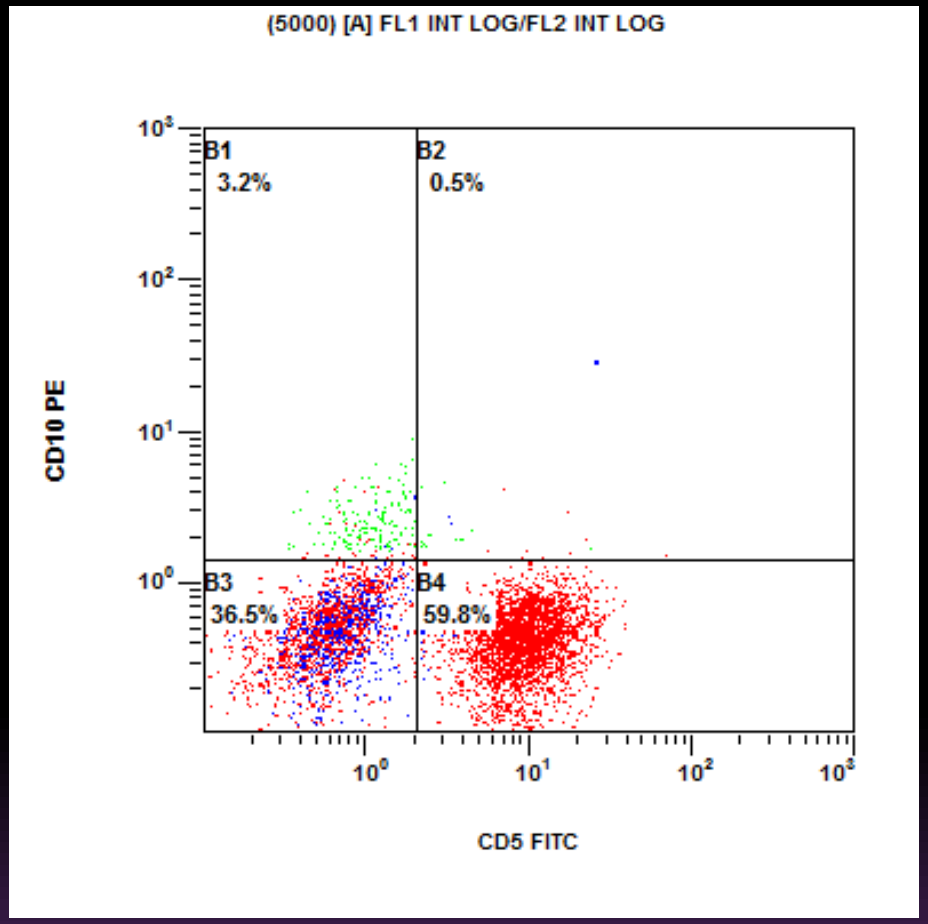

J1
4.4%
J2
59.4%
J3
34.8%
J4
1.5%
CD3 PB
CD7 PC7

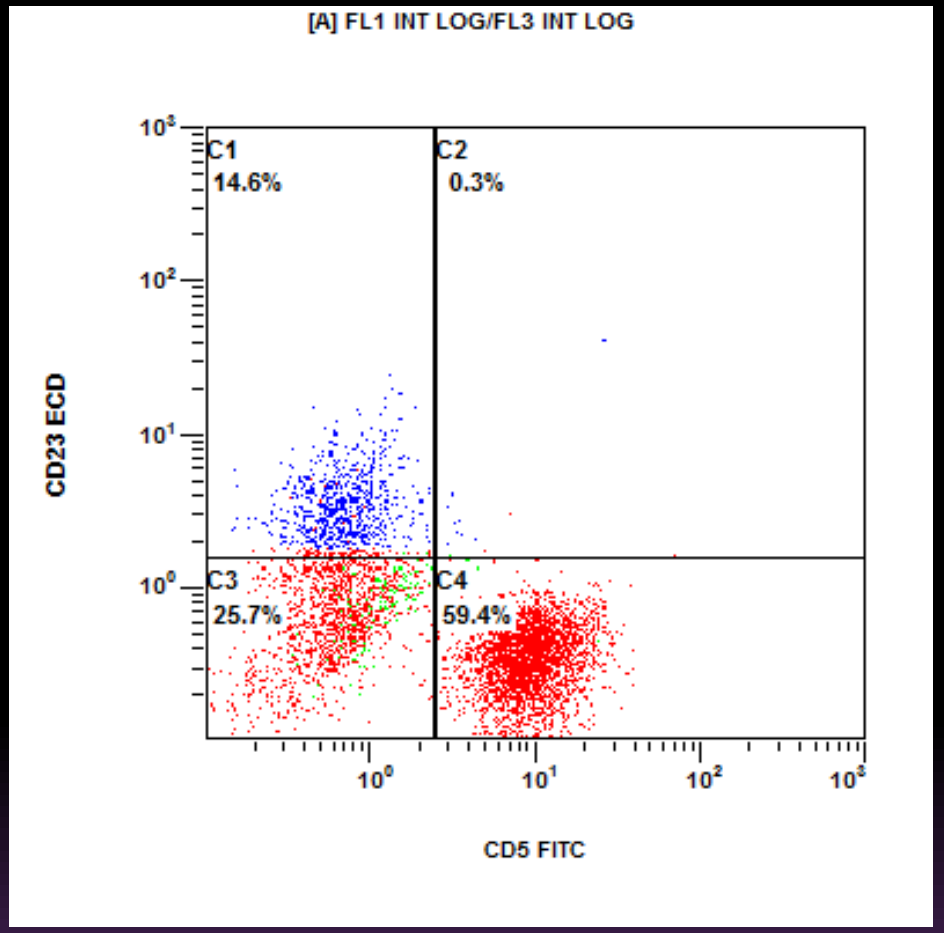

K1
3.8%
K2
59.4%
K3
34.7%
K4
2.1%
CD5 PC5.5
CD7 PC7

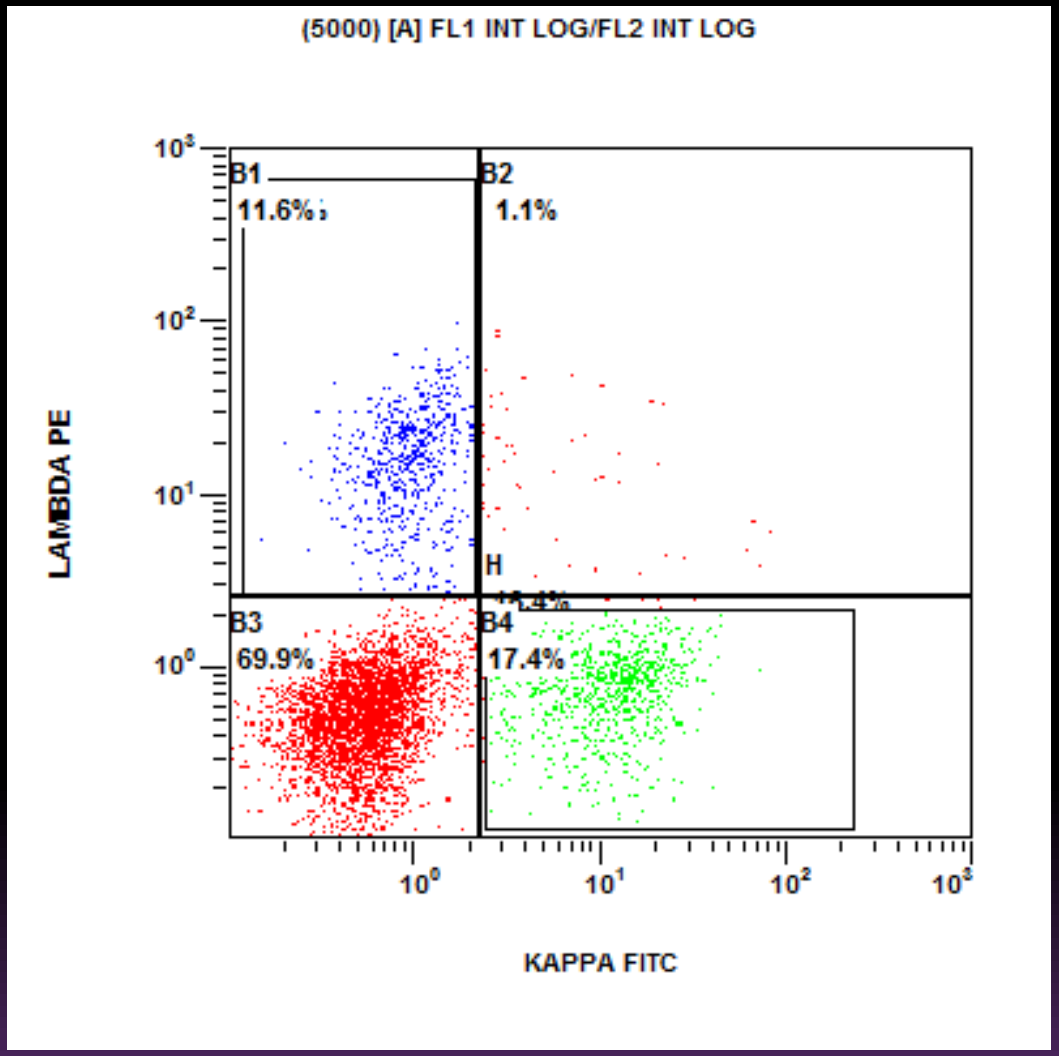

L2
50.1%
L4
10.8%
L1
4.2%
L3
35.0%
CD4 APC
CD7 PC7

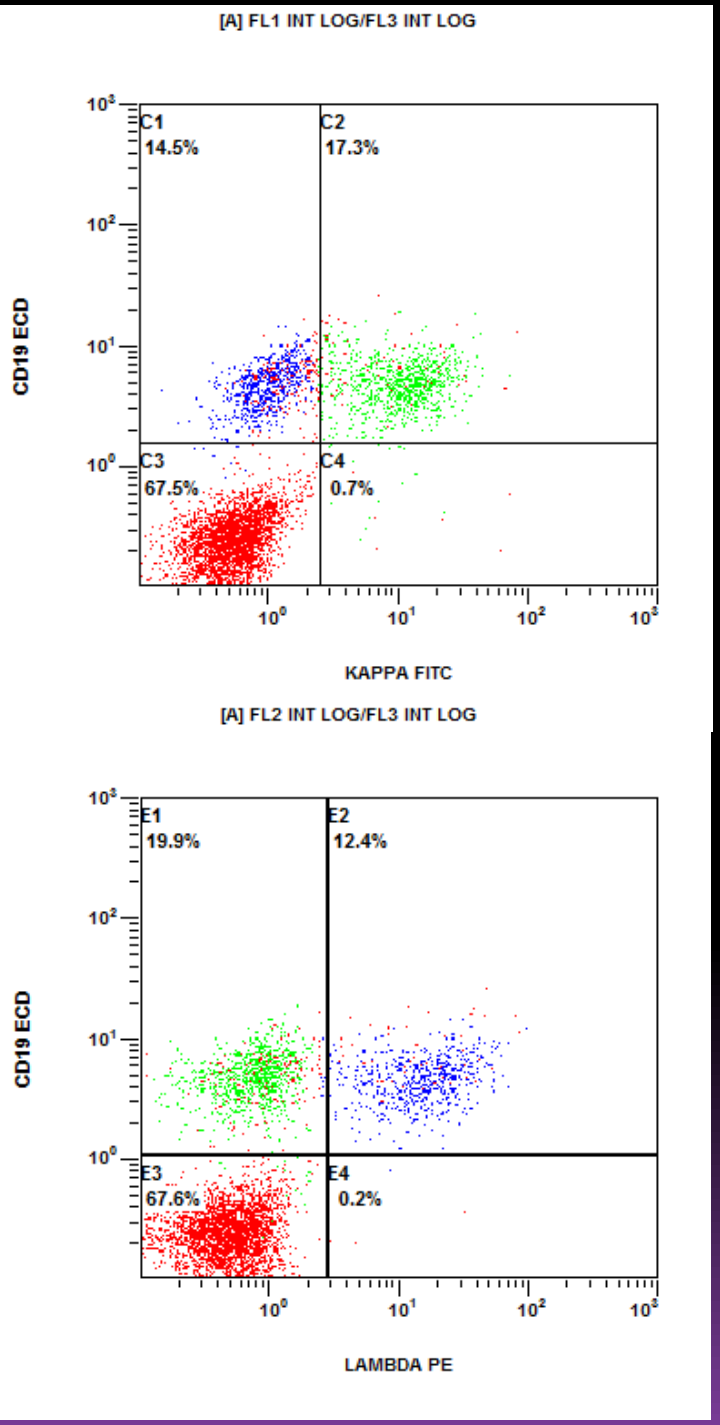

M2
8.4%
M4
51.4%
M1
0.6%
M3
39.6%
CD8 APC700
CD7 PC7

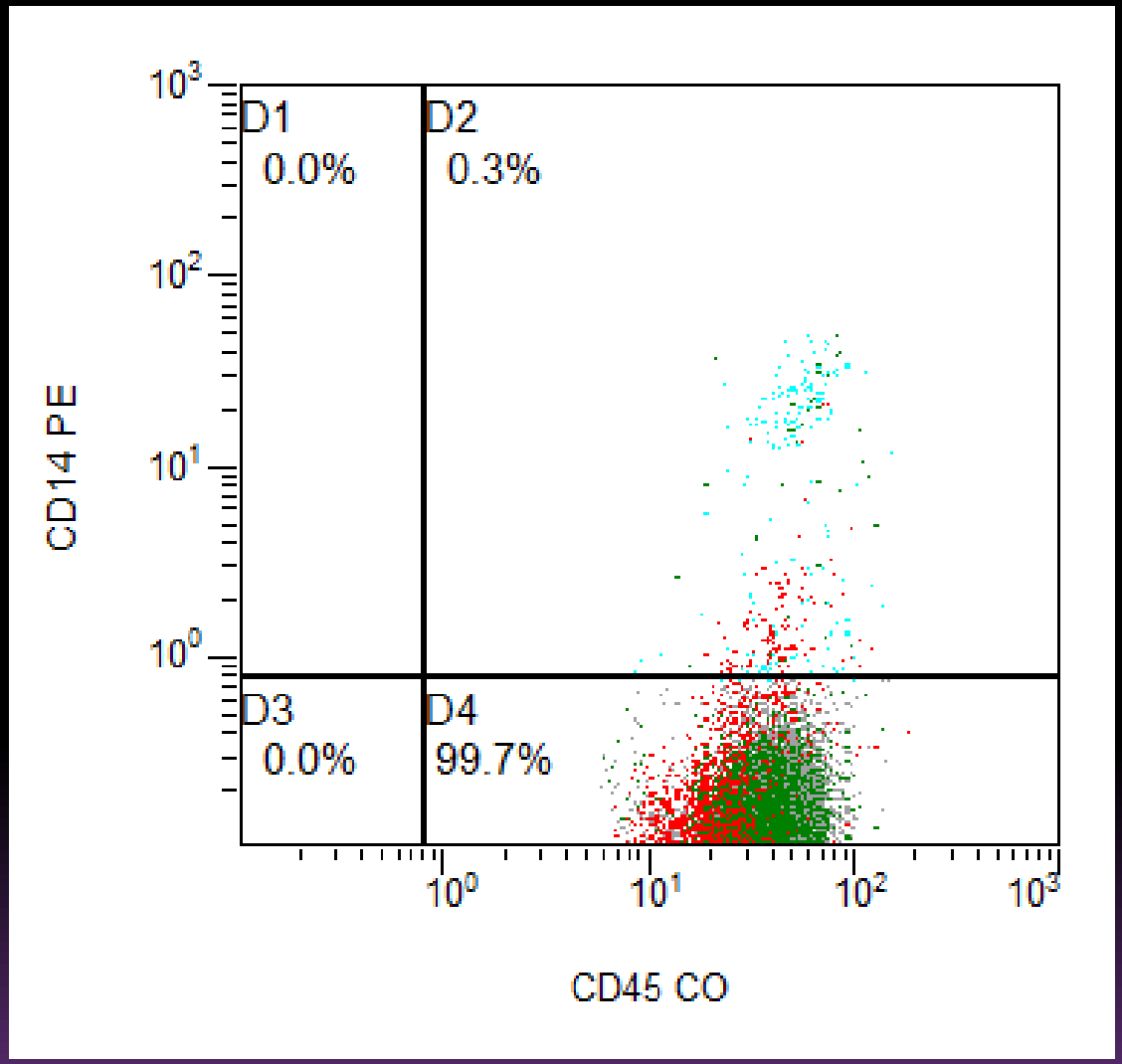

[Cells AND CD45+ Lymph] FL3 INT LOG/FL8 INT LOG
C1
0.8%
C2 19+ CD20+
25.3%
C3
72.7%
C4
1.1%
CD20 APC750
CD19 ECD
10^0
10^1
10^2
10^3


(5000) [A] FL1 INT LOG/FL2 INT LOG
CD10 PE
CD5 FITC
B1
3.2%
B2
0.5%
B3
36.5%
B4
59.8%
10^0
10^1
10^2
10^3


[A] FL1 INT LOG/FL3 INT LOG
CD23 ECD
CD5 FITC
C1
14.6%
C2
0.3%
C3
25.7%
C4
59.4%
10^0
10^1
10^2
10^3


(5000) [A] FL1 INT LOG/FL2 INT LOG
LAMBDA PE
KAPPA FITC
B1
11.6%
B2
1.1%
B3
69.9%
B4
17.4%
H
10^0
10^1
10^2
10^3


[A] FL1 INT LOG/FL3 INT LOG
CD19 ECD
KAPPA FITC
C1
14.5%
C2
17.3%
C3
67.5%
C4
0.7%
[A] FL2 INT LOG/FL3 INT LOG
CD19 ECD
LAMBDA PE
E1
19.9%
E2
12.4%
E3
67.6%
E4
0.2%
10^0
10^1
10^2
10^3


D1
0.0%
D2
0.3%
D3
0.0%
D4
99.7%
10^3
10^2
10^1
10^0
CD14 PE
10^0
10^1
10^2
10^3
CD45 CO

Patient Details

Hosp No: DoB: Lab No:	Patients name: Clinical details: **Persistent right inguinal? LN. 1.5-2cm non tender. Skin lesions on prescription drugs (GP(nature not known.**
Requesting Dr:	Date tested:
Panels to be set up:	
Previous results:	
FNA Site	**Right Groin**

Technical comments

Immunophenotyping results

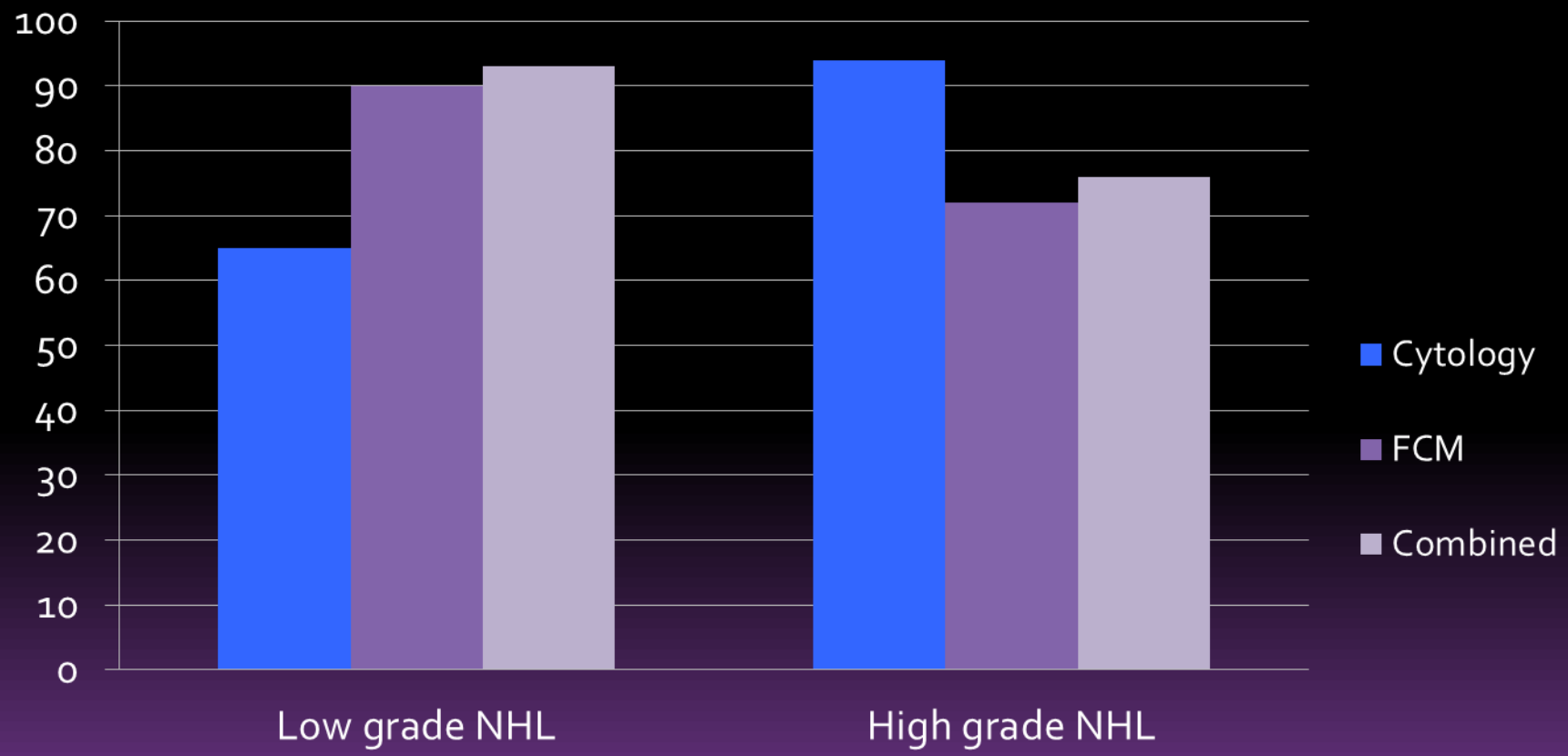
CD	% POS	CD	% POS
CD3	61	KAPPA	18
CD43	65	LAMBDA	13
CD4	50		
CD5	60		
CD7	57		
CD8	16		
CD10	4		
CD14	0		
CD19	32		
CD20	33		
CD20/5	1		
CD23	14		
CD45	97		

Clinical comment

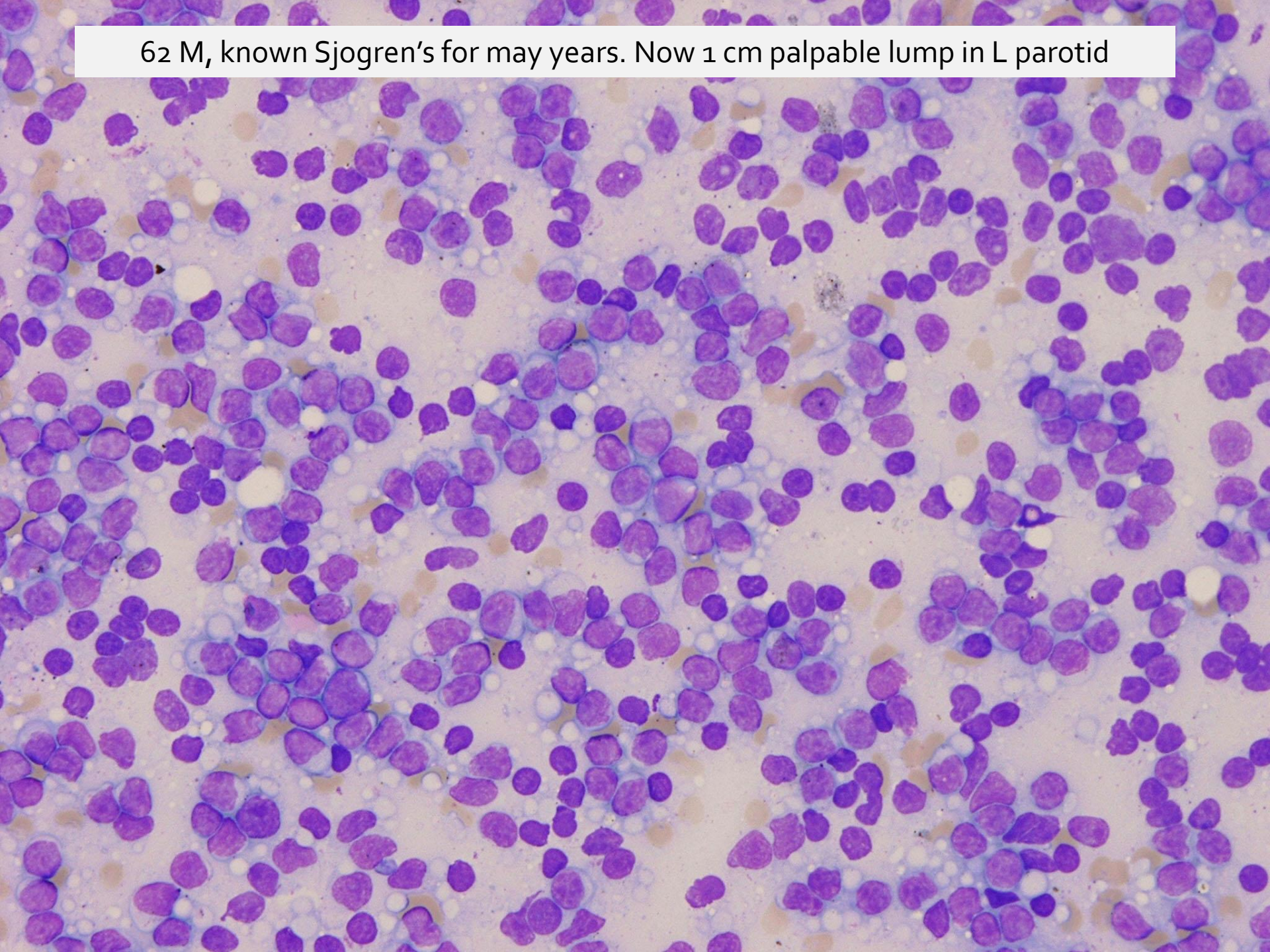
Reactive immunophenotype
No e/o B-LPD

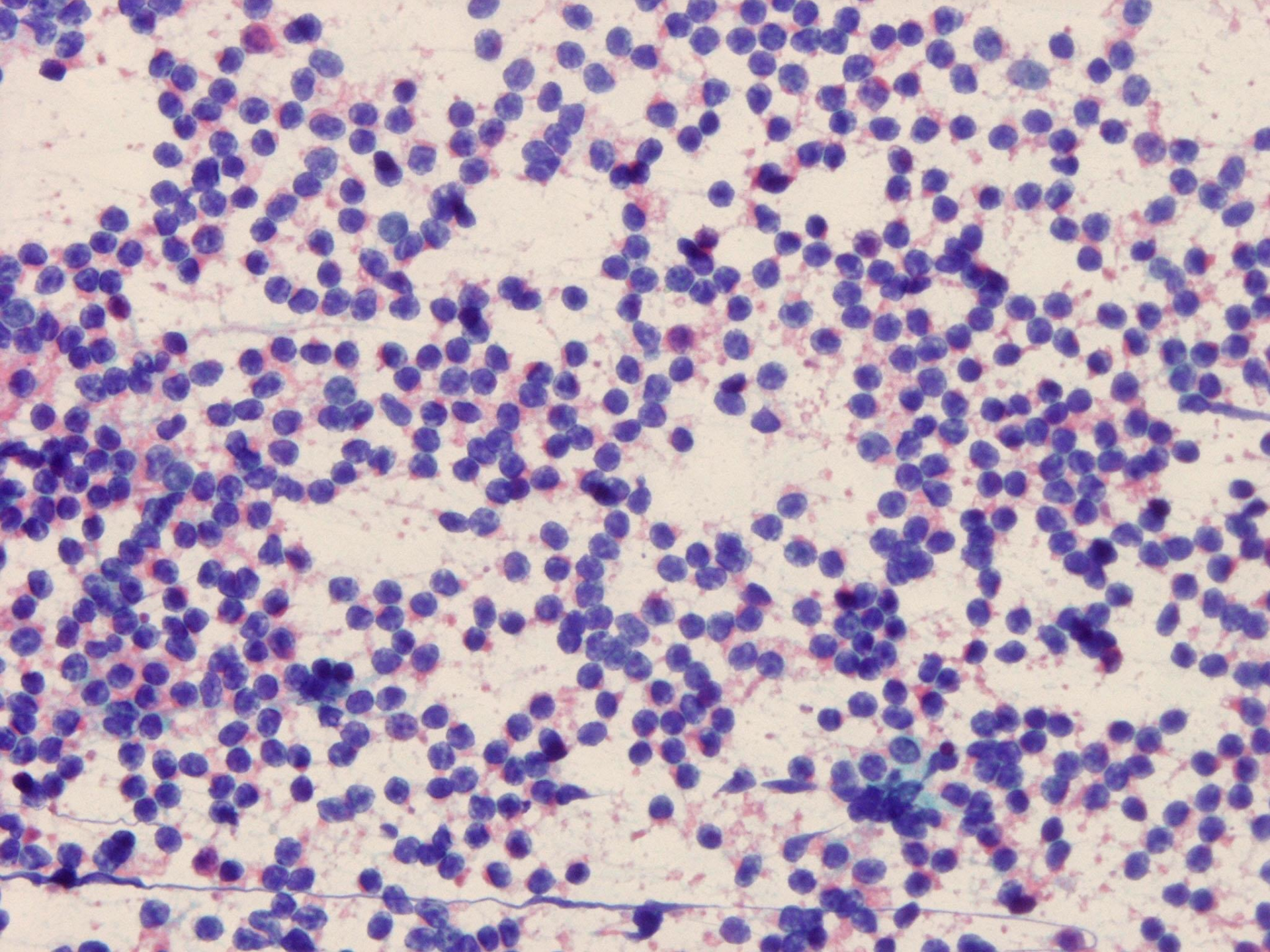
Moonim, Wilkins, Carr: FNA panel SOP

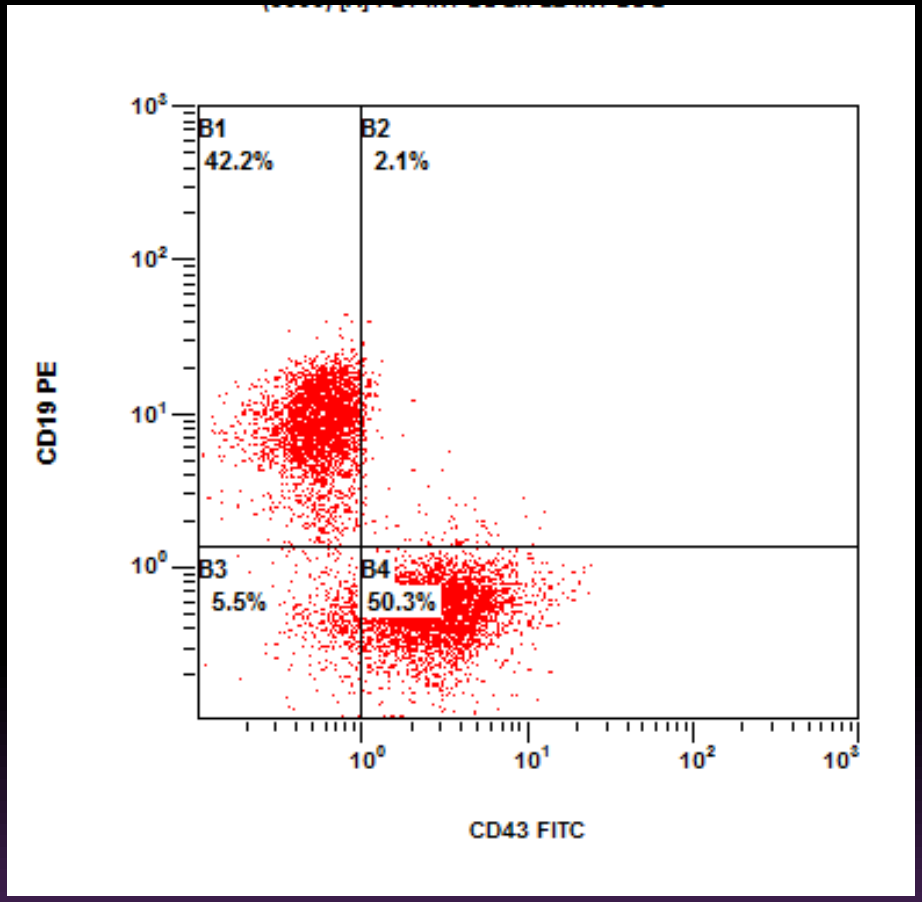
What is the actual role of flow cytometry in FNA lymphoma diagnosis?

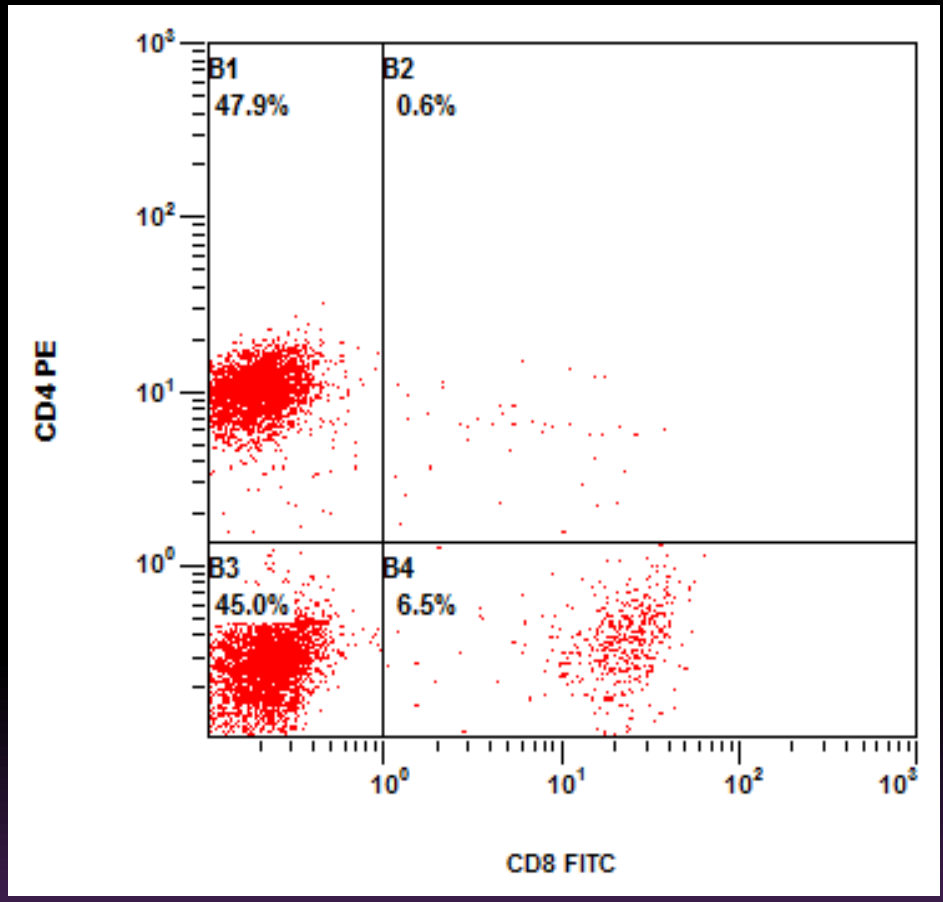


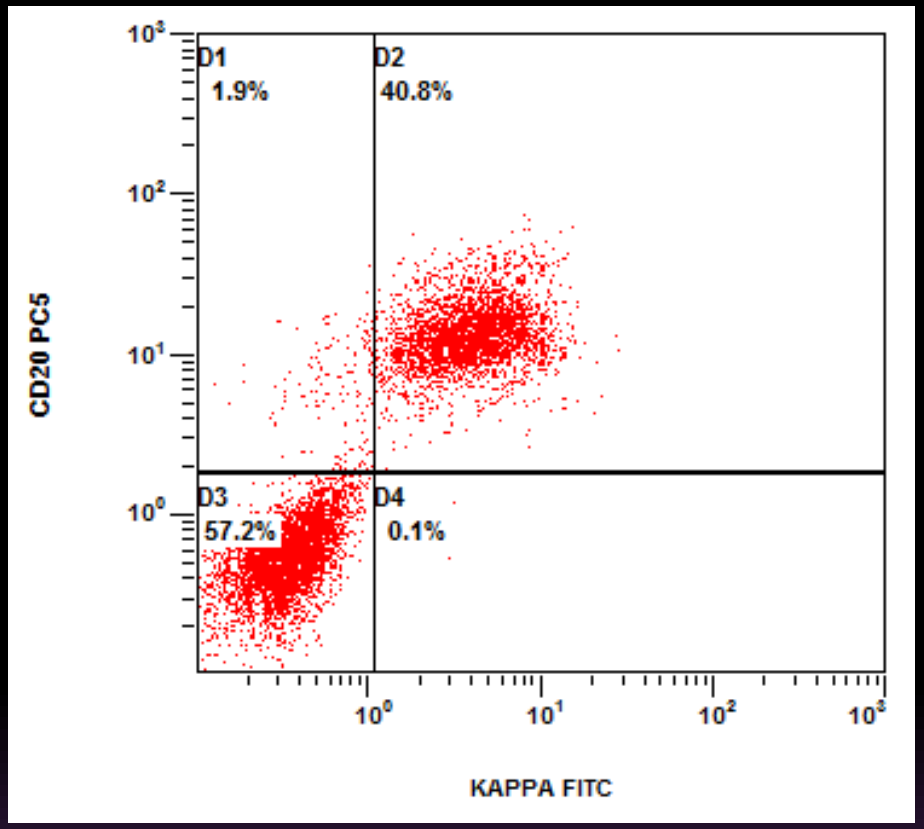
62 M, known Sjogren's for may years. Now 1 cm palpable lump in L parotid

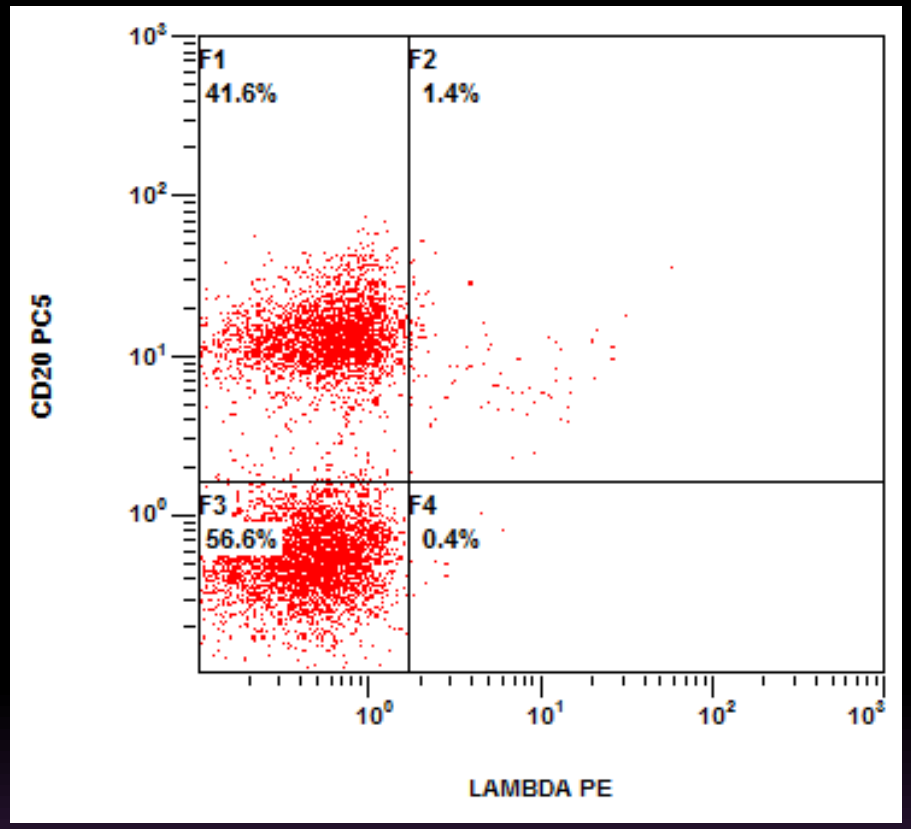


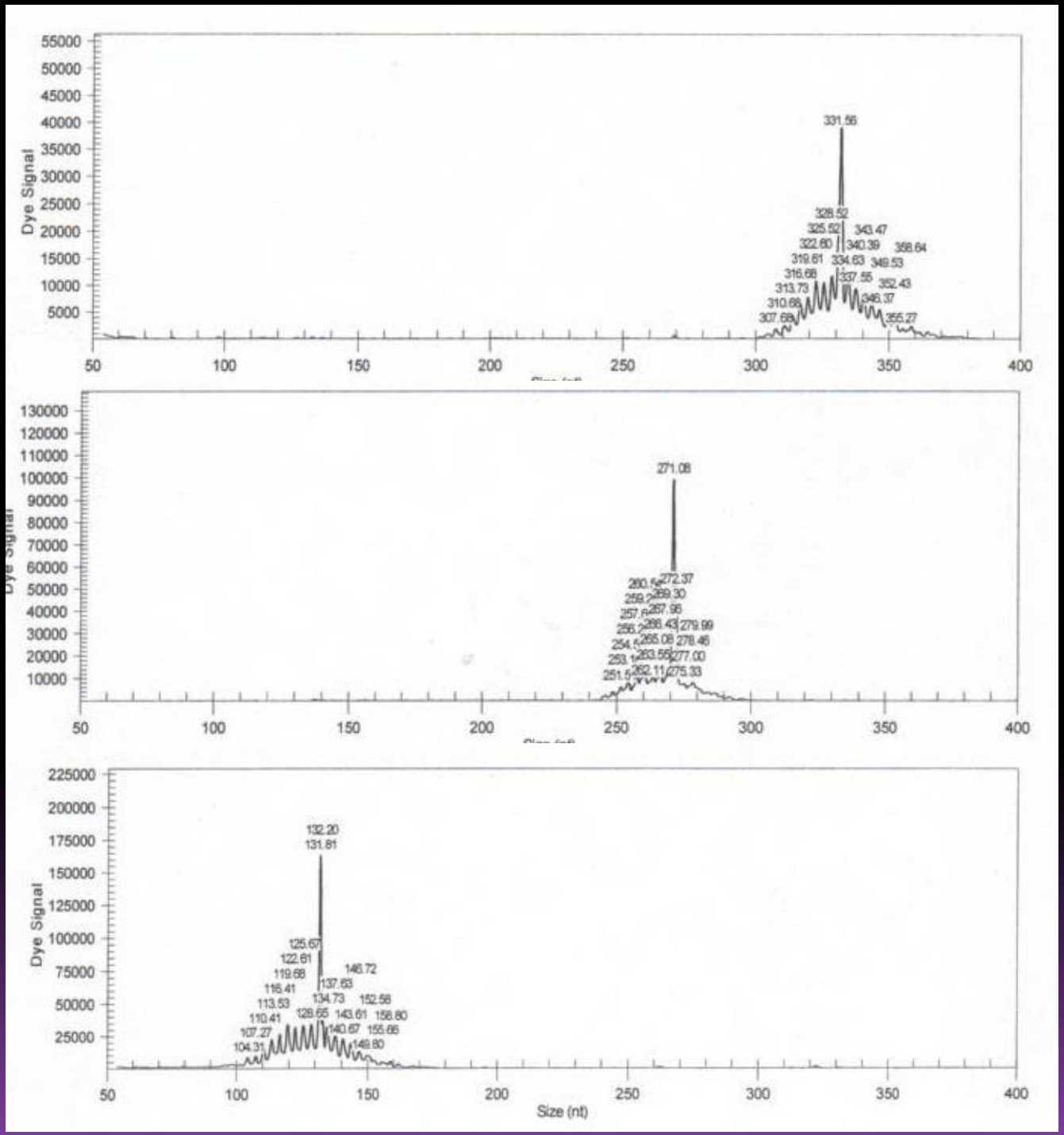



B1
42.2%
B2
2.1%
B3
5.5%
B4
50.3%
CD19 PE
CD43 FITC
10^0
10^1
10^2
10^3


B1
47.9%
B2
0.6%
B3
45.0%
B4
6.5%
CD4 PE
CD8 FITC
10^0
10^1
10^2
10^3


D1
1.9%
D2
40.8%
D3
57.2%
D4
0.1%
CD20 PC5
KAPPA FITC


F1
41.6%
F2
1.4%
F3
56.6%
F4
0.4%
CD20 PC5
LAMBDA PE


Dye Signal
331.56
328.52
325.52
343.47
322.60
340.39
358.64
319.61
334.63
349.53
316.66
337.55
352.43
313.73
346.37
310.68
355.27
307.68
271.08
272.37
259.30
267.96
268.43
279.99
255.08
278.46
263.55
277.00
252.11
275.33
132.20
131.81
125.67
122.61
146.72
119.68
137.63
116.41
134.73
152.56
113.53
128.65
143.61
156.80
110.41
140.67
155.66
107.27
149.80
104.31
Size (nt)

Parotid:

Low grade B-cell Non-Hodgkin lymphoma consistent with Marginal zone lymphoma (MALToma)

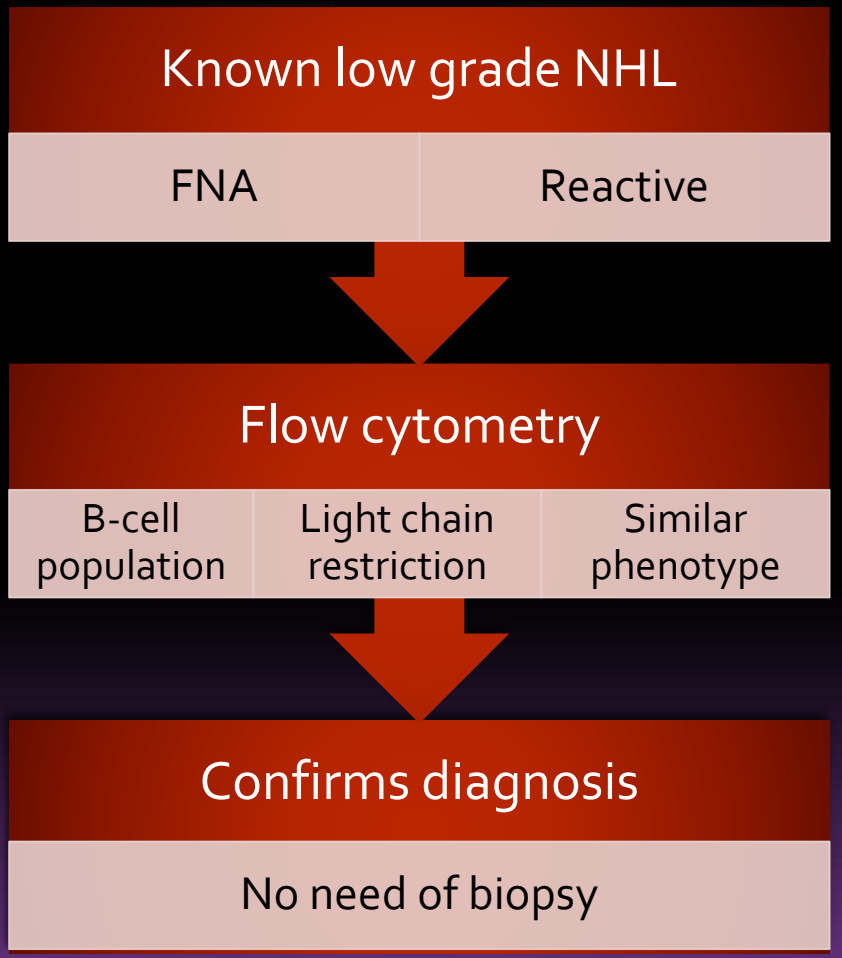
CD20(+), Kappa(+)

CD10(-), CD5(-), CD23(-)

IGH gene rearrangement studies: Clonal

Utility of FNA in low grade NHL

- Primary diagnosis: limited utility.
- Follow up / relapse / residual disease: very useful.
- If adequate material obtained – *obviates need for biopsy*.



Take home message

- Structured approach necessary
- Needle washings very useful
- Ancillary investigations often convert a consistent/suspicious report into a definitive diagnosis.
- FNA with flow cytometry is very useful in follow-up of low grade lymphoma's.

Thank you