Oxford University Hospitals
NHS Trust

Who, what, where, when, how?
Lymphoma classification and hot topics in lymphoma pathology

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BRITISH LYMPHOMA PATHOLOGY GROUP

Joint BLPG/ BDIAP meeting: 15th May 2014

Who reports what?

- NICE guidelines & centralisation
- Bone marrow trephines

NICE National Institute for Health and Care Excellence

Improving Outcomes Guidelines

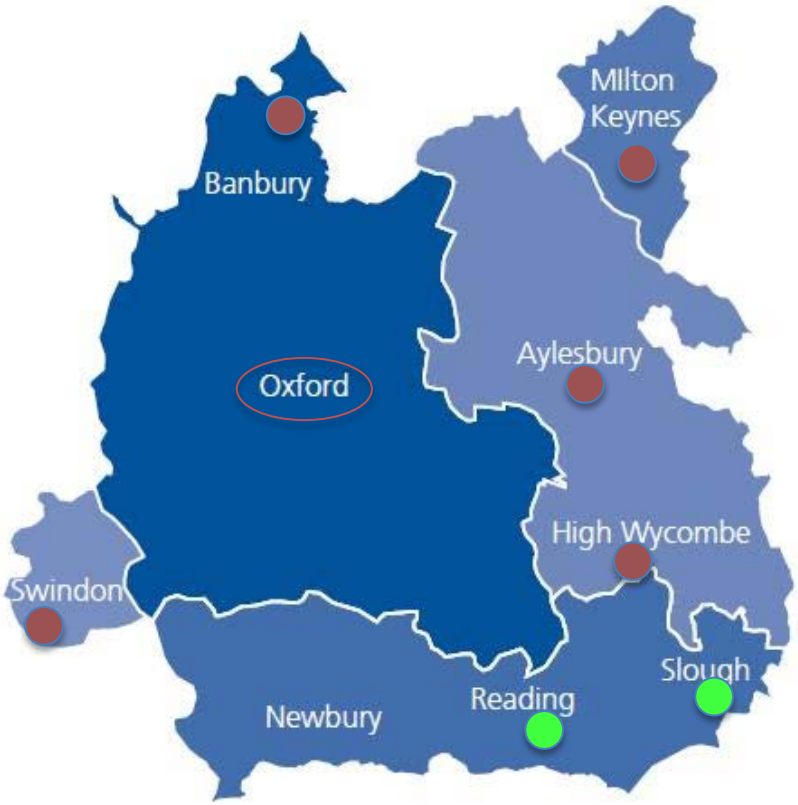
- “In order to reduce errors, every diagnosis of possible haematological malignancy should be reviewed by specialists in diagnosis of haematological malignancy.

- 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.”

The Oxford Regional Haematopathology service

- 2400 specimens per annum – increasing
- Covers 8 centres in the Thames Valley Cancer Network
- Integrated histopathological & molecular reporting
- Specialist integrated haemato-molecular diagnostic service being set up
- Weekly lymphoma & fortnightly myeloma/ myeloid videolinked regional MDTs



The Oxford Haematopathology Team

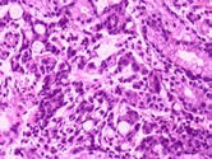
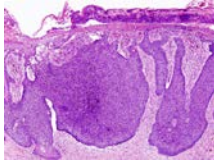
Dr Daniel Royston Professor Kevin Gatter Professor Francesco Pezzella

National progress with regional centralisation

- Wide regional variation
- Some services fully centralised
- Partly centralised services involving one or more “hubs”
- Local & specialist MDTs in some regions
- Barriers: infrastructural, financial, political

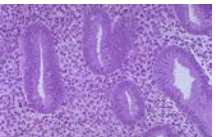
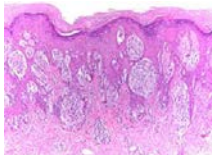
Enforcement of centralisation

- Hampered by a lack of funding
- No legal enforcement status
- Improving Outcome Guidelines are just guidelines
- External peer review can encourage the process

Role of the general pathologist

- Recognition of (potential) lymphomas
- Communication of provisional diagnosis to clinicians
- Referral of relevant material to tertiary centre for detailed classification +/- molecular analysis

The haematopathology hoop: How much should trainees know?

- FRCPath requires knowledge of common entities
- Distinguish common benign mimics from lymphoma
- Appreciate appropriate diagnostic pathways

Turf wars: Haematology vs pathology

Who should report bone marrow trephines?

Pathologists

- Good on morphology & IHC
- May not see/ report aspirate
- Variable appreciation of disease entities

"Doesn't seem to matter how carefully you put them back together you always seem to end up with pieces left over."

Haematologists

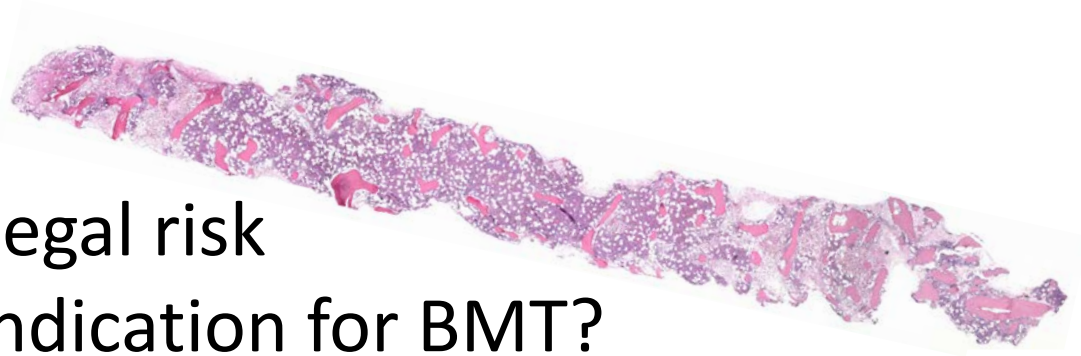
- See aspirate
- May not appreciate subtleties of histopathological artefact/ IHC
- Understand the diseases

"He's our new Palliative Specialist!"

Little structured training provided to either group

Depending on local structures, both groups may struggle to obtain cytogenetic/ molecular results

Does it matter who reports BMTs?



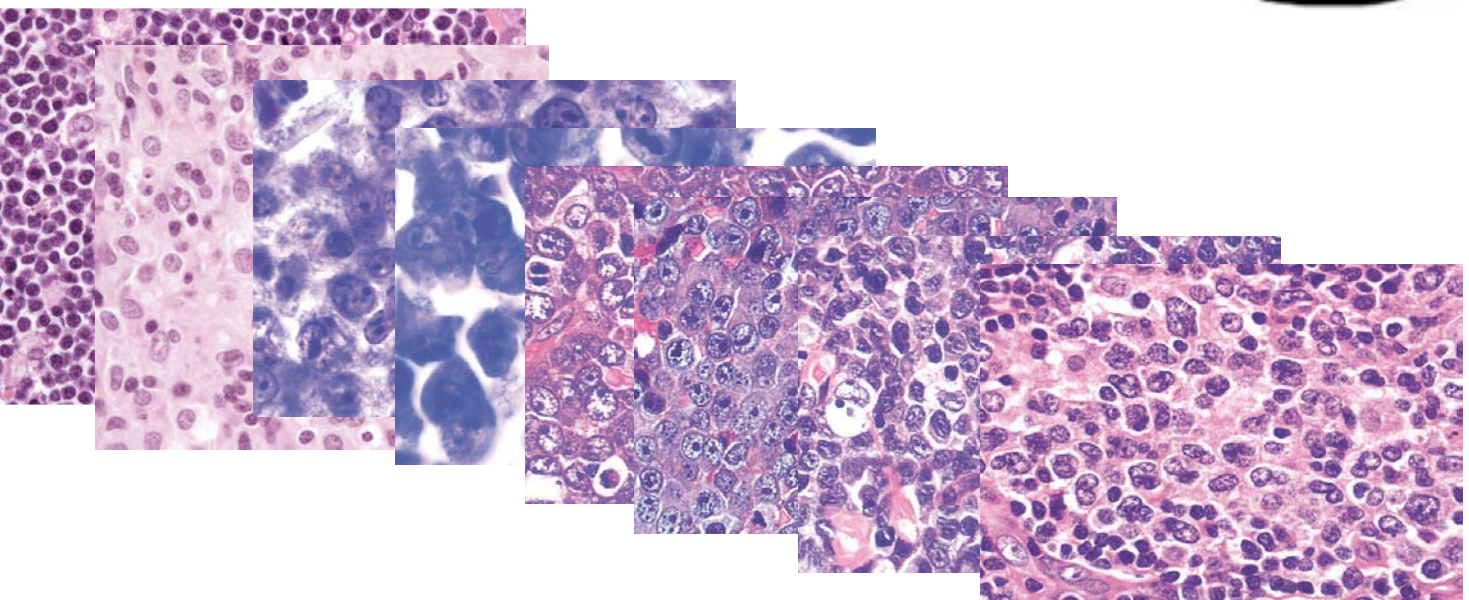
- Diagnostic accuracy
- Patient and medicolegal risk
- Does it depend on indication for BMT?
  - Lymphoma staging & myeloma diagnosis
    - Either group may report safely with appropriate IHC
  - Myeloid pathology
    - Blasts, mastocytosis often missed without immunostains
    - Reporting with aspirate may increase accuracy (haematologist), but detailed BMT morphology may be crucial if aspirate suboptimal (pathologist)
  - Carcinomas and the weird & wonderful
    - Probably the remit of the pathologist

What is it?

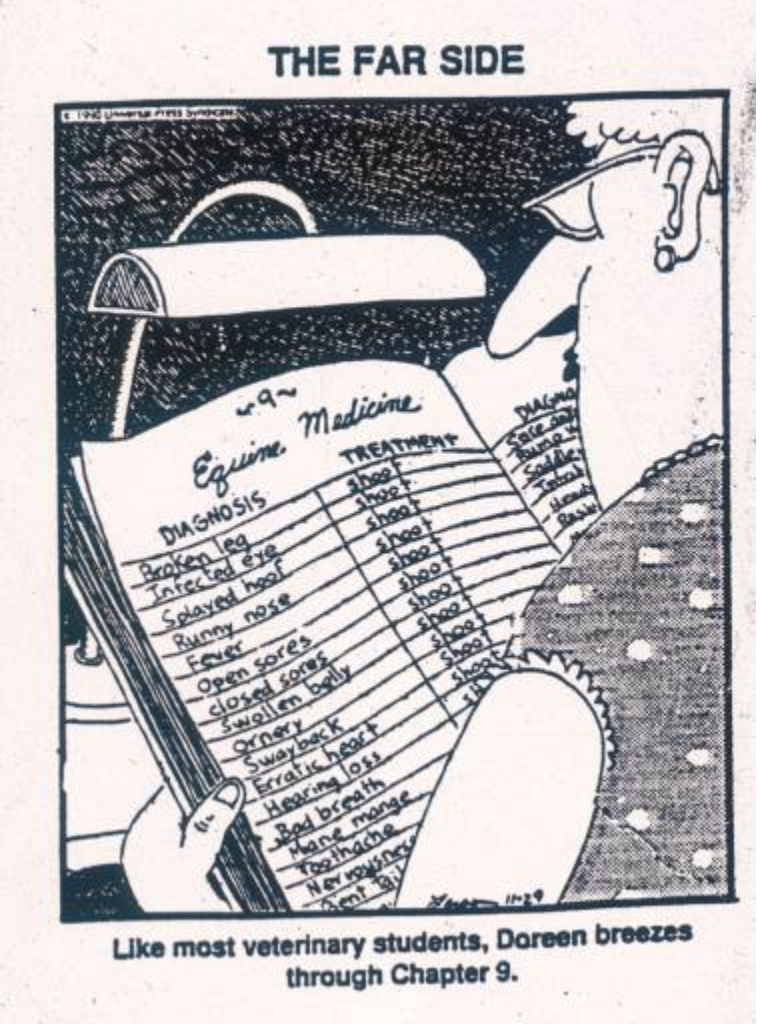
Is it “just a DLBCL”?

- “Dustbin category”

- Why bother subclassifying when everyone gets CHOP-R?



Classification is easy when the treatment is always the same!



Equine Medicine

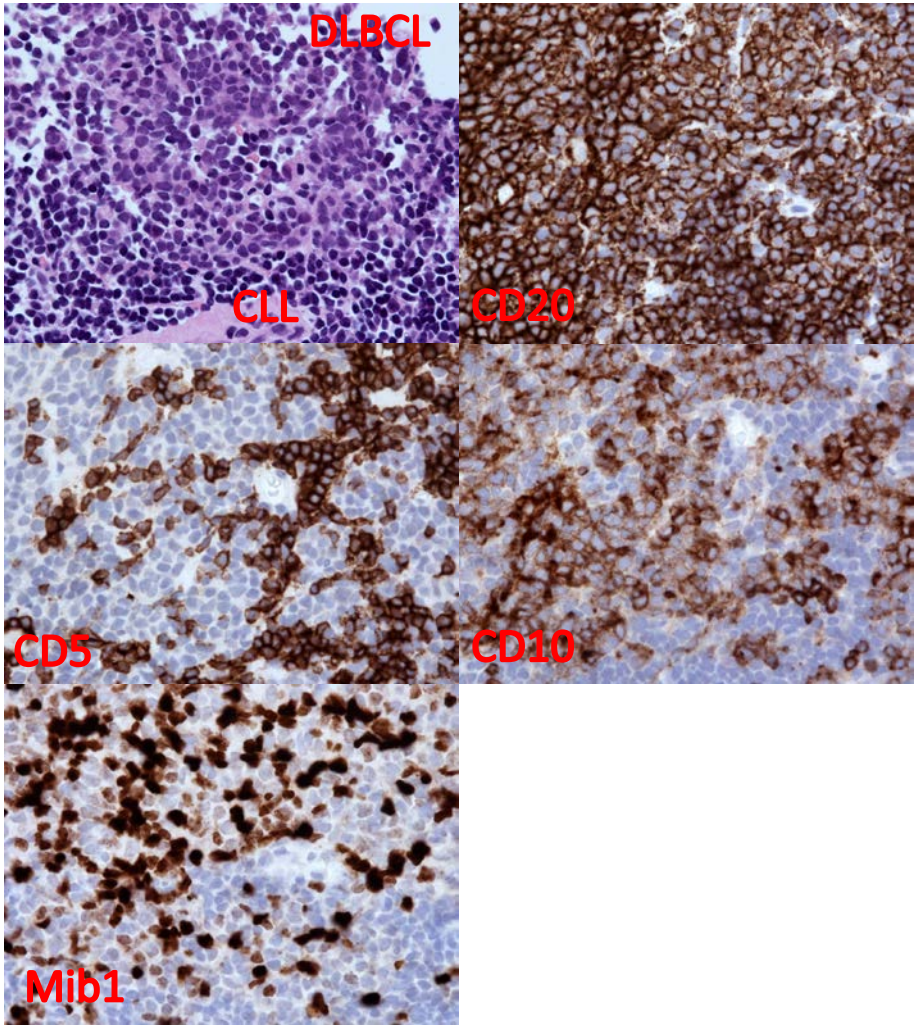
- Broken leg: shoot
- Infected eye: shoot
- Splayed hoof: shoot
- Runny nose: shoot
- Fever: shoot….

When does subtyping matter?

- Predominantly for trial entry
  - Richter's transformation of DLBCL: CHOP-OR trial
  - PHOENIX: CHOP-R with ibrutinib vs placebo (ABC-DLBCL)
  - CHOP-R with lenalidomide vs placebo (ABC-DLBCL)
  - DA-EPOCH-R vs CHOP-R: C-MYC+ DLBCL/ plasmablastic & Burkitt's
- Consideration of treatment escalation
  - MYC (+/-BCL2) rearranged

Richter's transformation of CLL

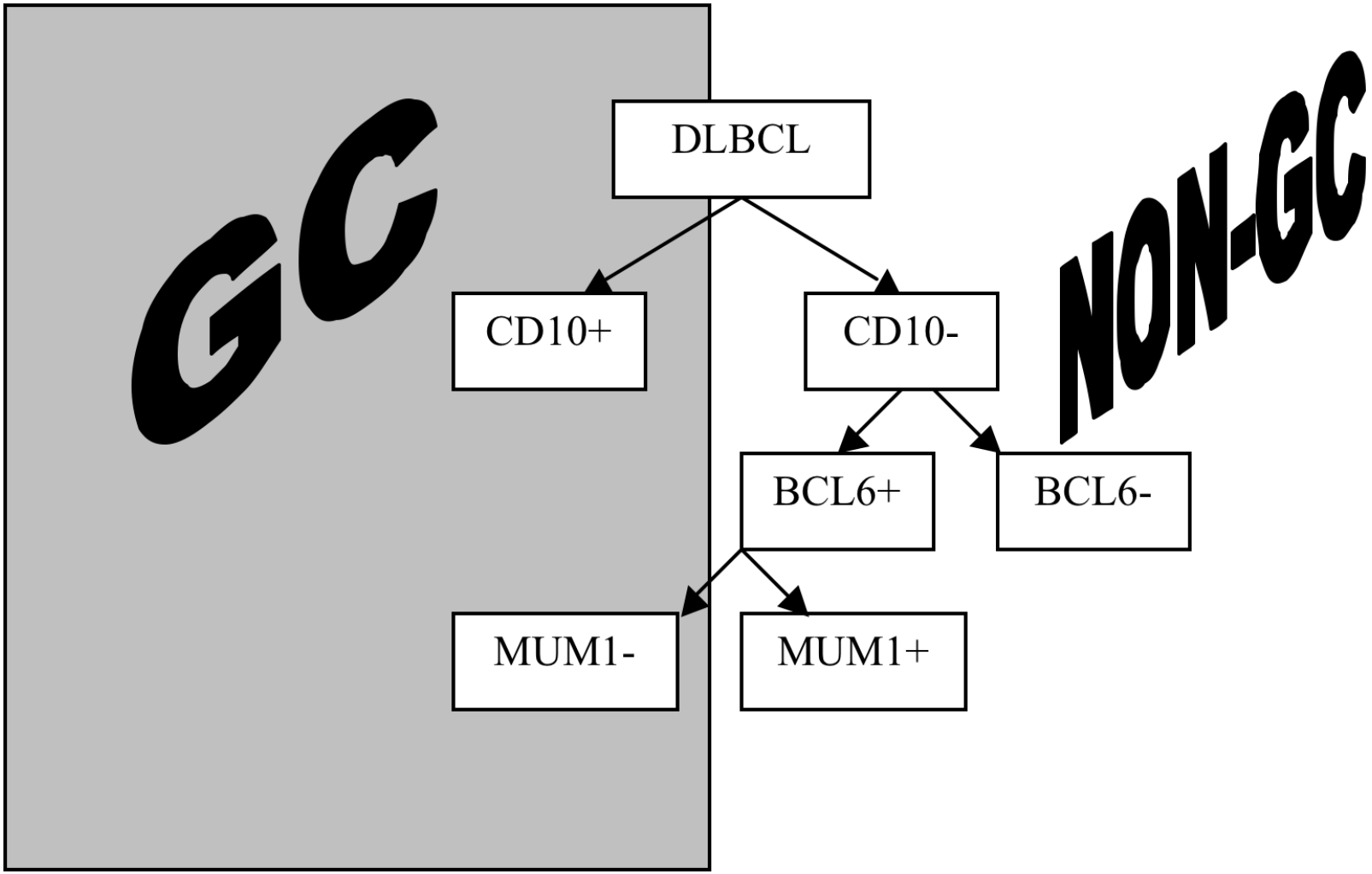
- Major decisions:
- 1. whether it's really high grade *or*
- 2. whether the DLBCL originated from the CLL
- DLBCL definition:
  - nucleus ≥2x lymphocyte/ ≥macrophage
  - Mib-1/ki-67>40% (beware of thick sections)
- Clonal origin: assumption vs comparative BCR clonality



DLBCL: ABC vs GCB

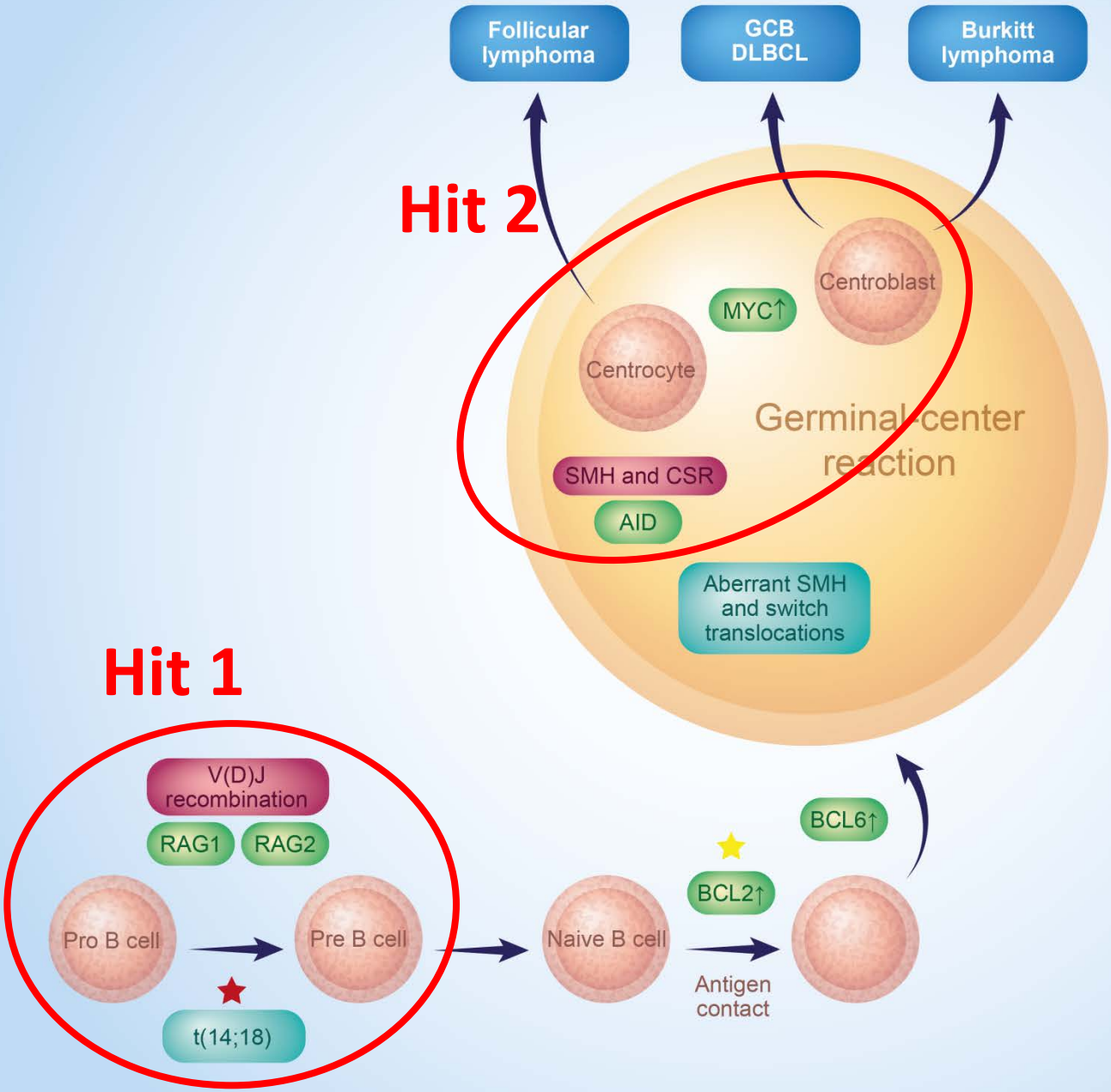
- Gene expression profile data can (roughly) divide DLBCL into 2 (or 3) groups
- Activated B-cell (ABC) group have poorer prognosis than germinal centre B-cell (GCB) group
- The “Hans algorithm”, is an imperfect immunohistochemical classifier
- Cheap & easy classifier for clinical trial entry

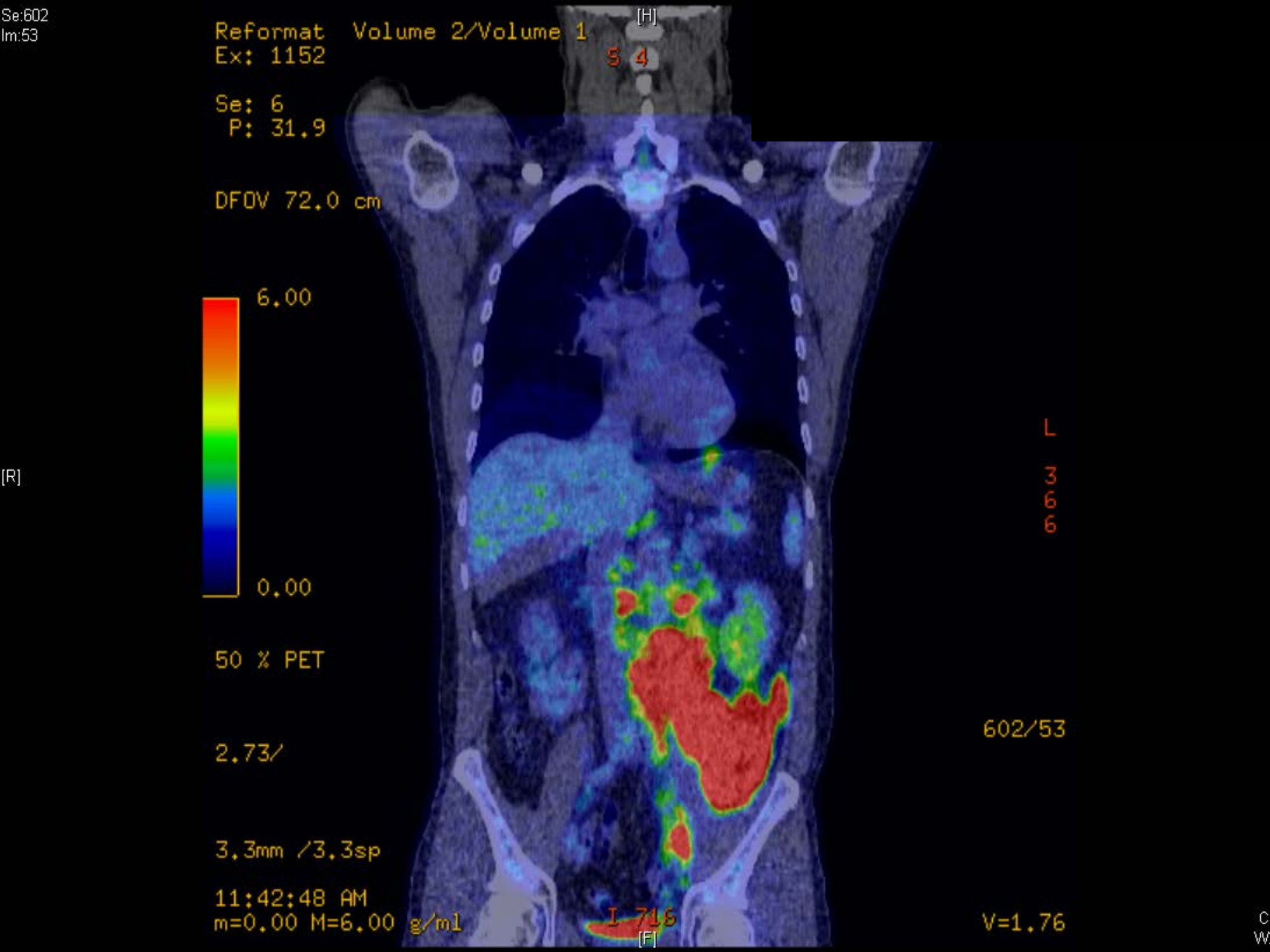
The “Hans Classifier”



“Double hit” and MYC-rearranged DLBCL

- “Double hit” = MYC and BCL2 rearranged
- Often present extranodally at advanced stage
- Tumour has high proliferation index
- May fall into BL/DLBCL overlap category
- Unknown incidence: ?5% unselected DLBCL
- Incidence increases with age
- Prognosis usually very poor


Follicular lymphoma
GCB DLBCL
Burkitt lymphoma
Hit 2
Centroblast
MYC↑
Centrocyte
Germinal-center reaction
SMH and CSR
AID
Aberrant SMH and switch translocations
Hit 1
V(D)J recombination
RAG1
RAG2
Pro B cell
Pre B cell
t(14;18)
Naive B cell
BCL2↑
Antigen contact
BCL6↑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Ex: 1152
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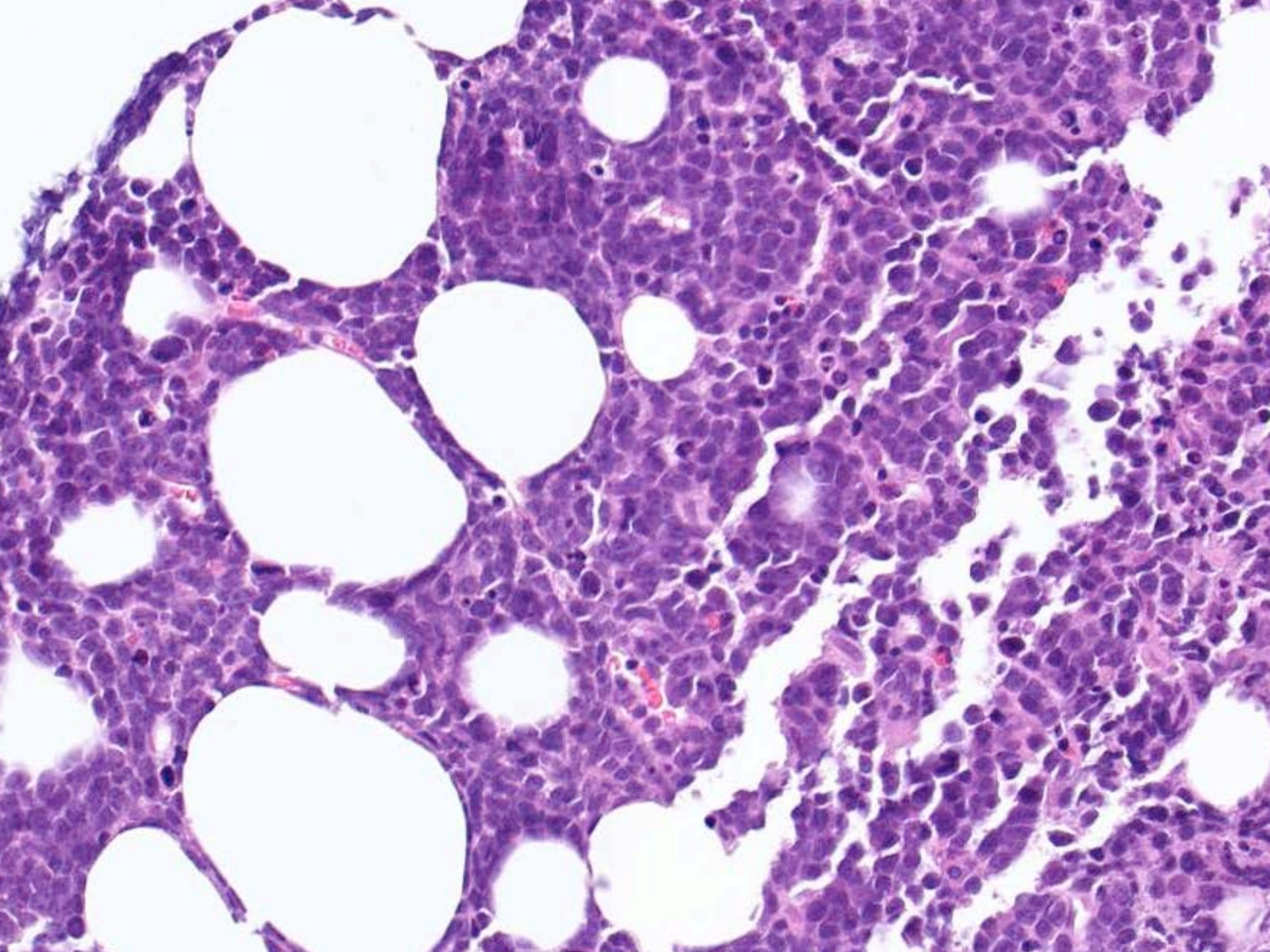
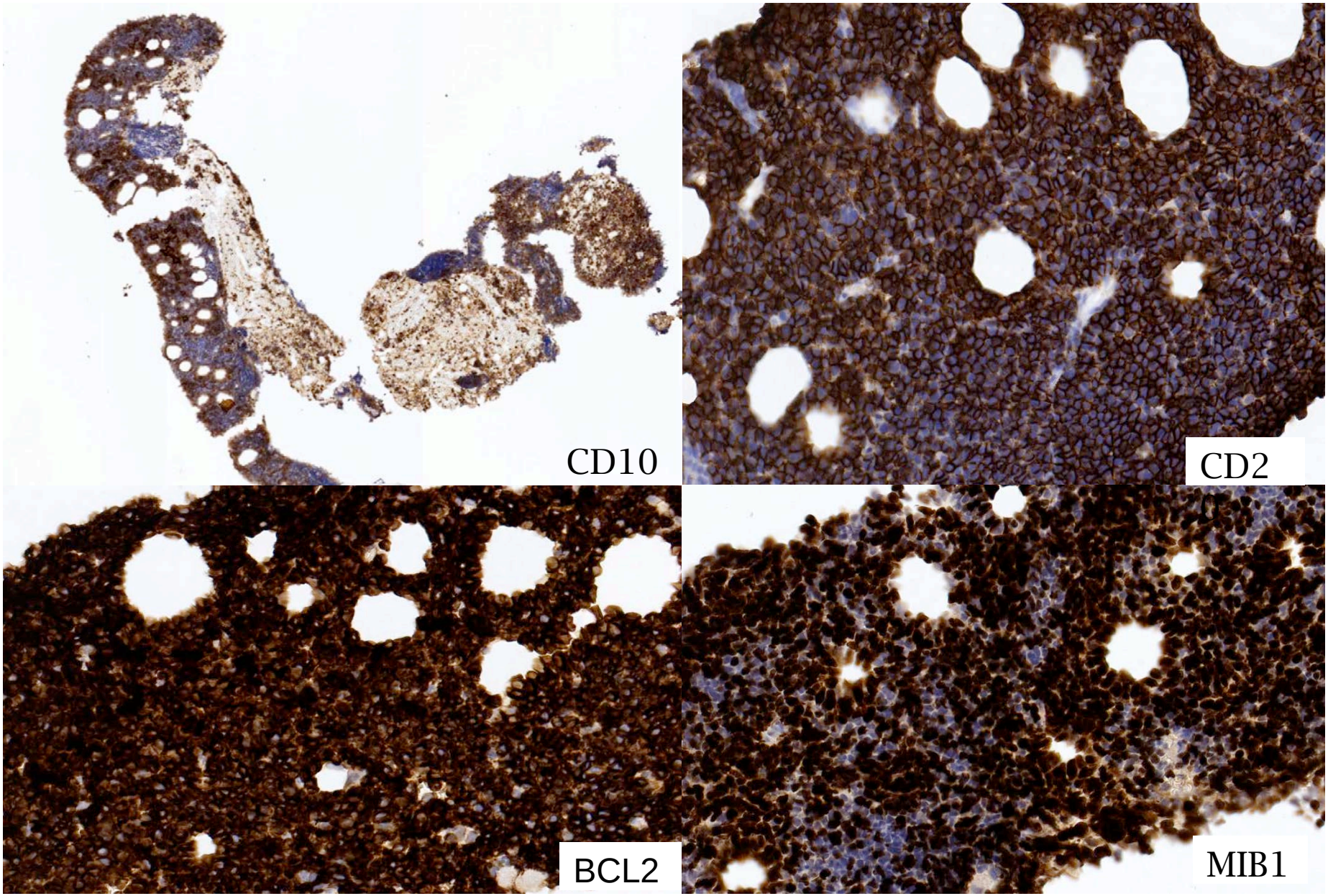
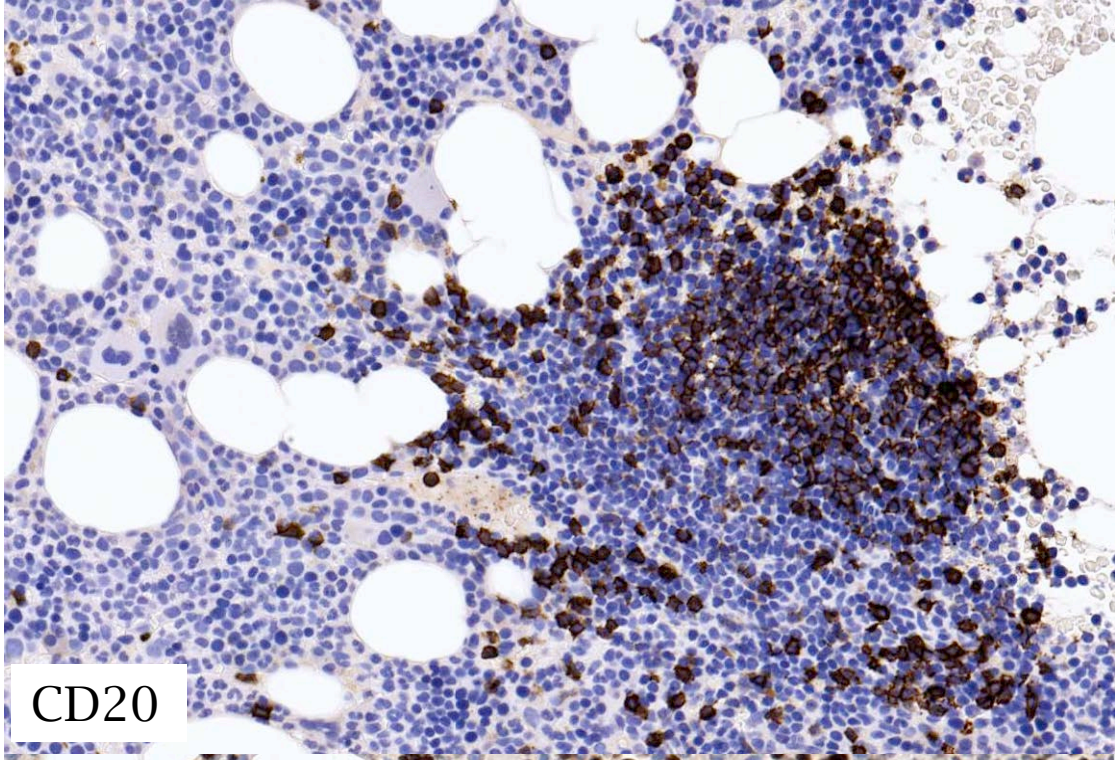


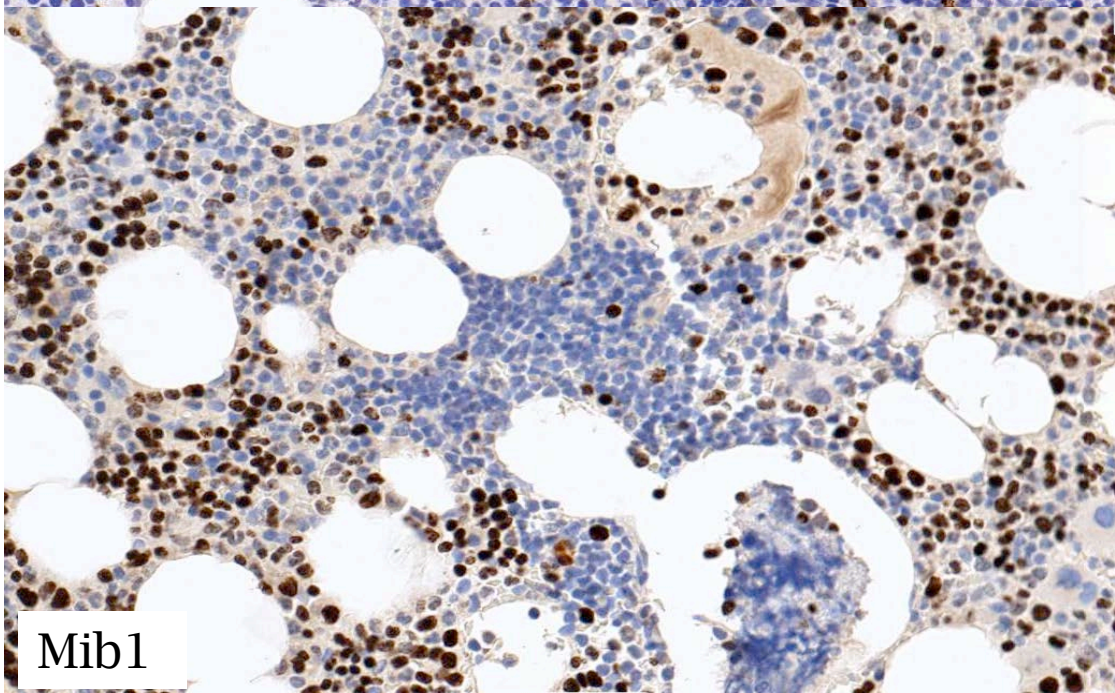

CD10
CD2
BCL2
MIB1

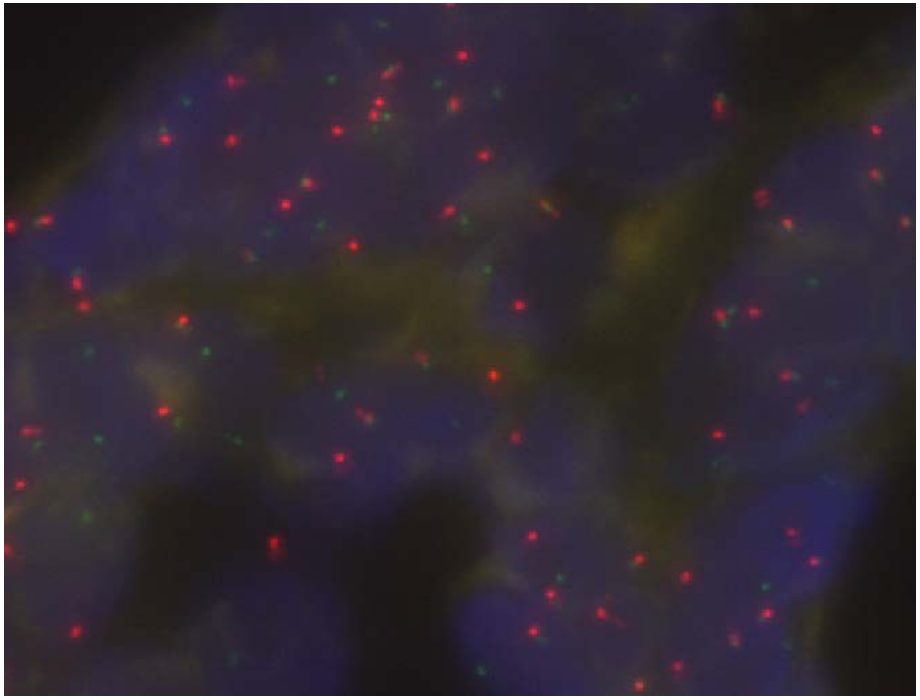
Bone marrow trephine biopsy:

minimal follicular lymphoma

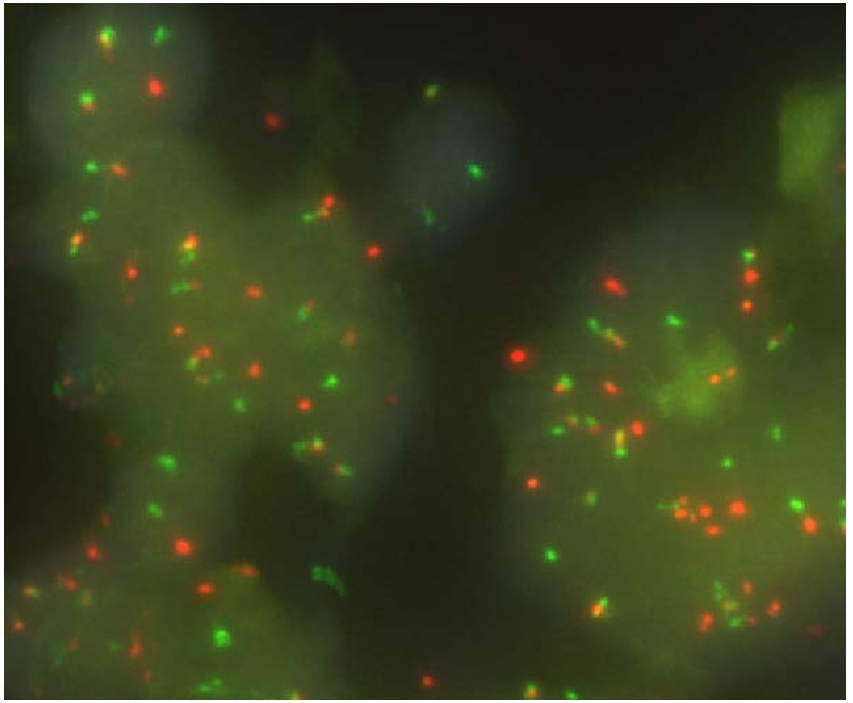
(that had presumably transformed)







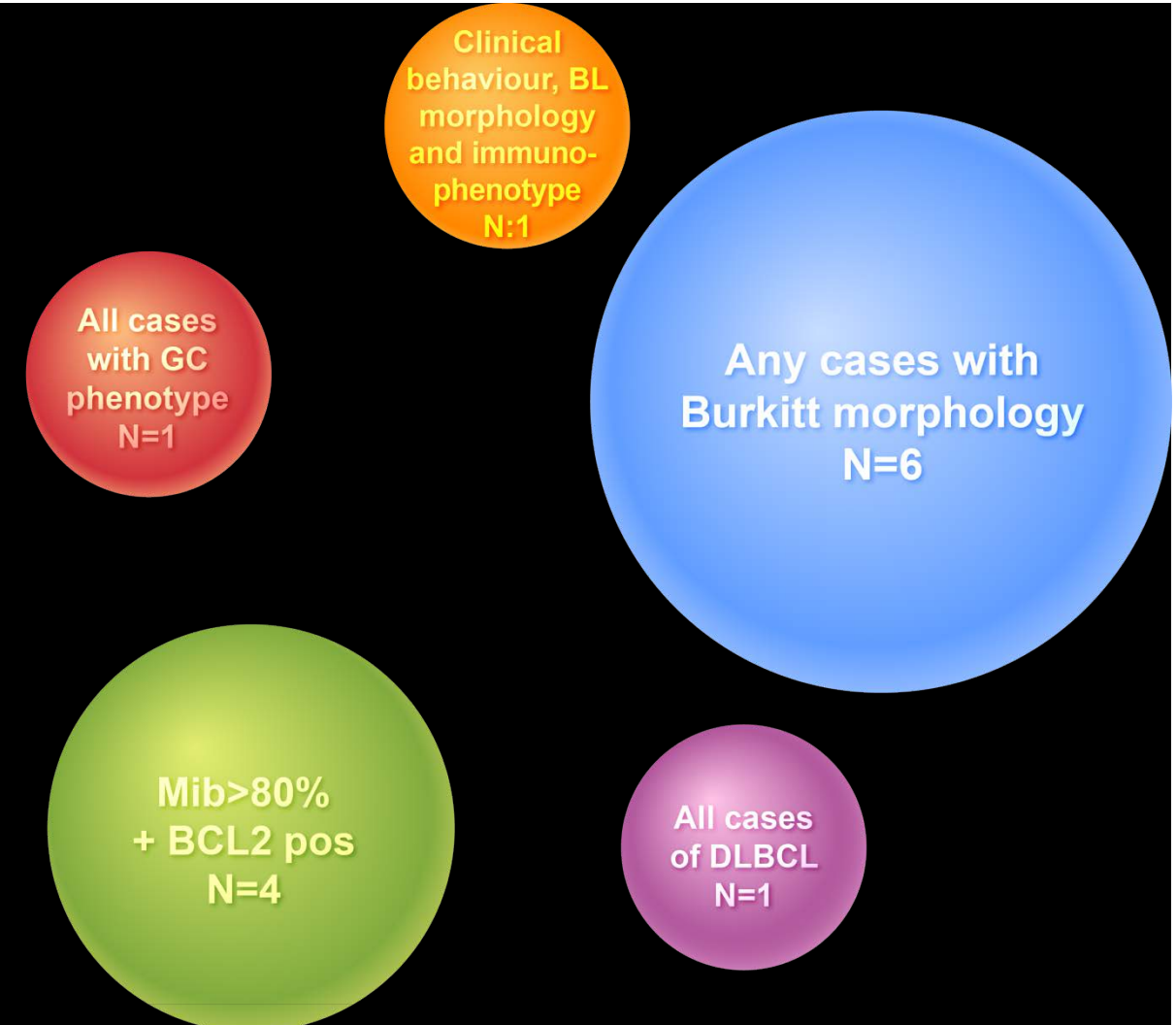
MYC rearrangement Break-Apart FISH



BCL2 rearrangement Fusion FISH

Patient currently in remission 2 years after CODOX-M chemotherapy

“Straw poll” BLPG: when do you look for MYC rearrangement in DLBCL?



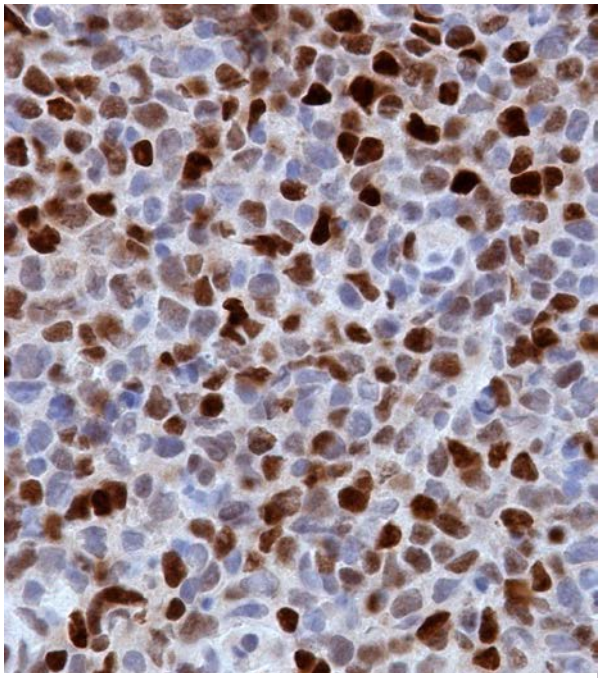
“ Sometimes our haematologists ask for C-MYC FISH for reasons only known to themselves…”

“If the patient is not fit for CODOX-M/IVAC, then we don’t look for MYC rearrangements…”

“We are moving towards doing MYC FISH for all diffuse large B’s….”

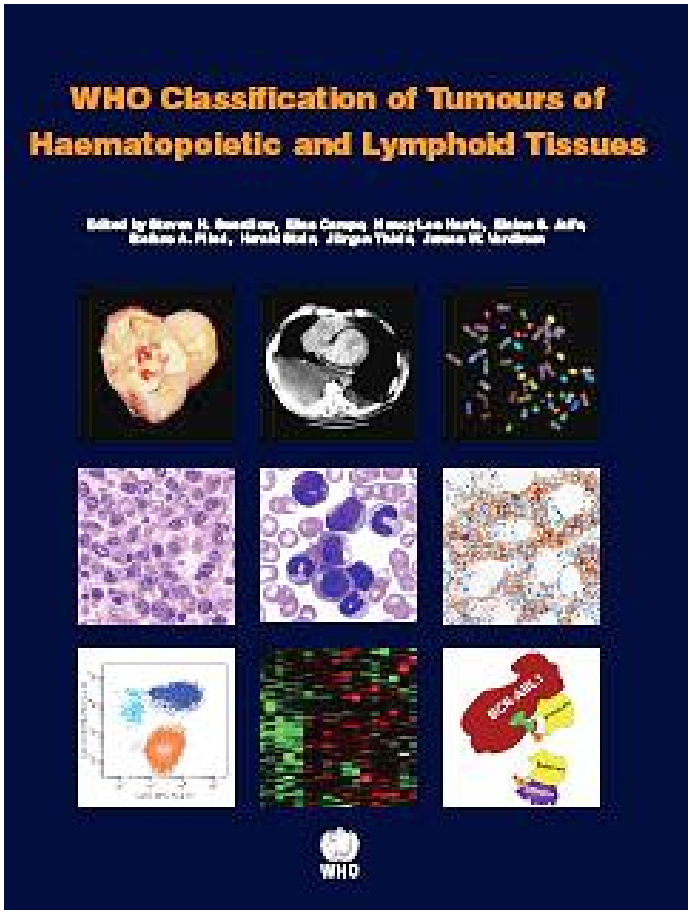
Immunohistochemical versus FISH "double hits"

- High levels of expression of bcl-2 and myc by IHC
- Larger % of cases than FISH "double hits"
- May be more biologically relevant
- Trials awaited



Myc IHC

Where is it in the WHO book now?

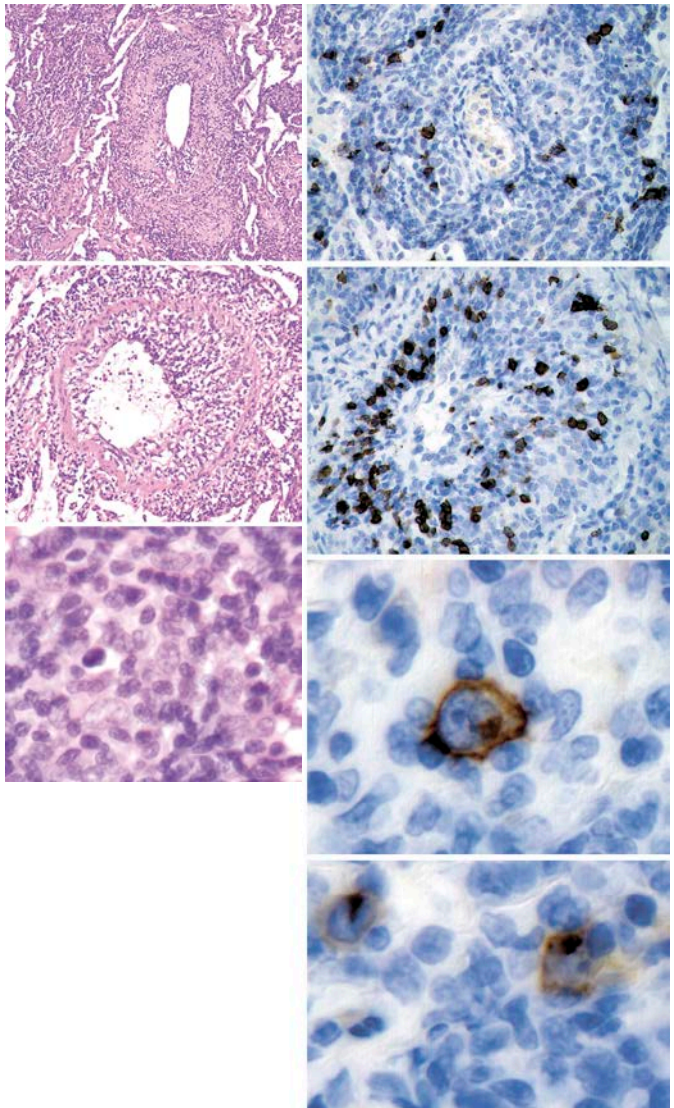


Reminders of entities that moved

- Lymphomatoid granulomatosis
- Mastocytosis
- Blastic Plasmacytoid Dendritic Cell Neoplasm
- Histiocytoses

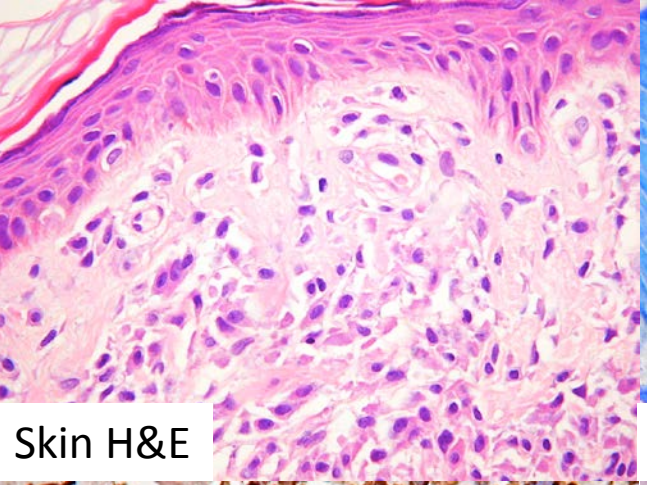
Lymphomatoid granulomatosis: now a diffuse large B-cell lymphoma subtype

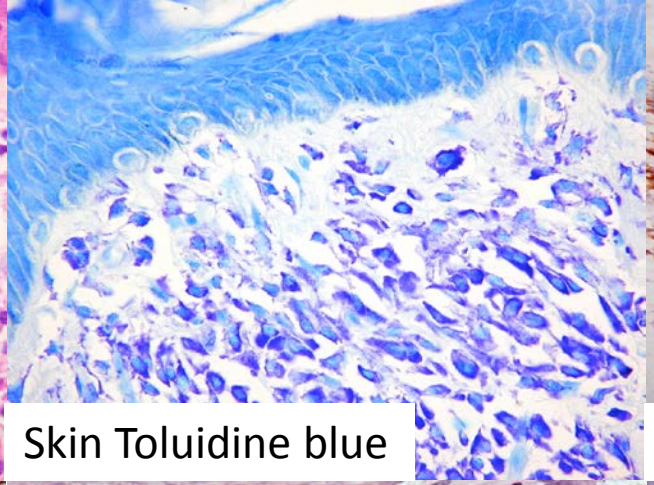
- Angioinvasive, angiodestructive, EBV-driven B-lymphoproliferation
- Adult lungs > immunodeficient children
- Graded by proportion of B-cells relative to reactive background (T-cells, plasma cells, histiocytes etc)
- Some analogies with EBV-driven PTLD

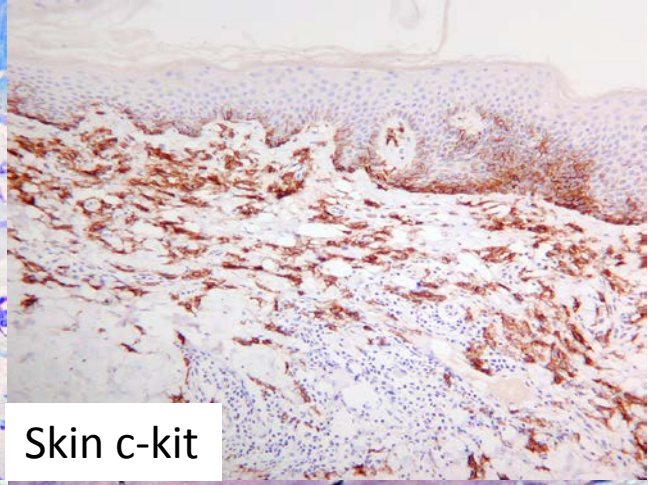


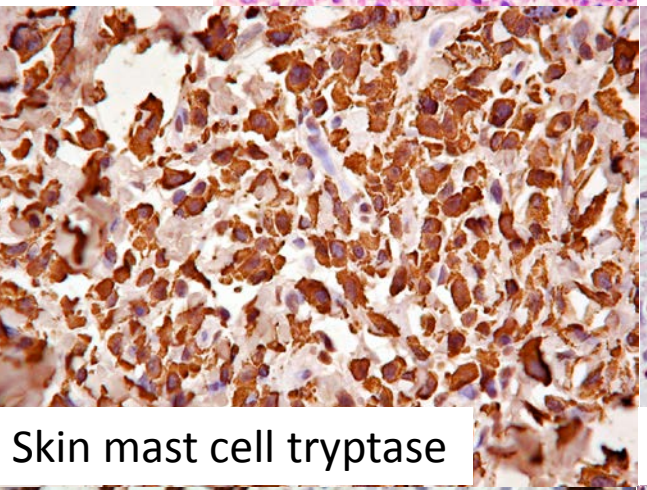
Systemic mastocytosis is a myeloproliferative neoplasm

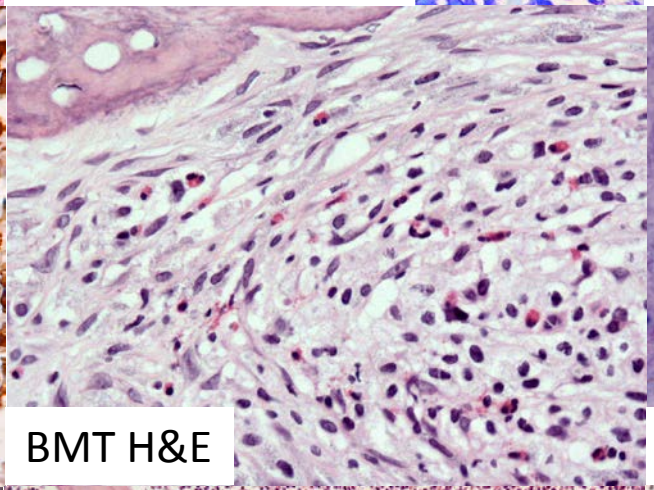
- BMT defined systemic & cutaneous forms
- C-kit+ MCT+ CD2+ CD4+ CD45+ CD68+ MITF+
- C-KIT (or other TK) mutations >95%
- Beware of association with
  - Myeloproliferative neoplasms
  - Myelodysplastic syndromes
  - Acute myeloid leukaemia

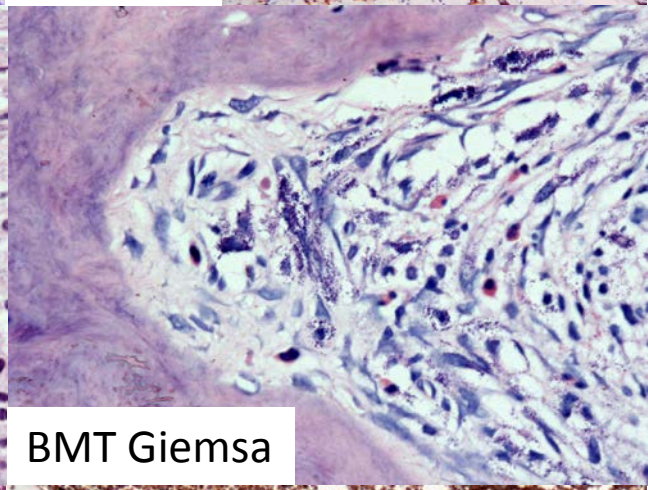

Skin H&E

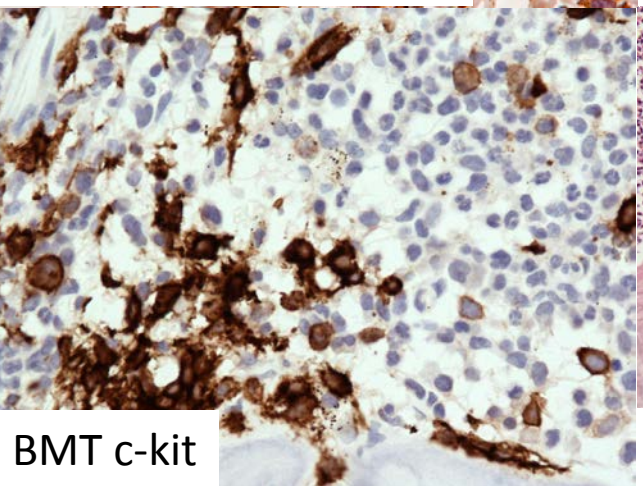

Skin Toluidine blue

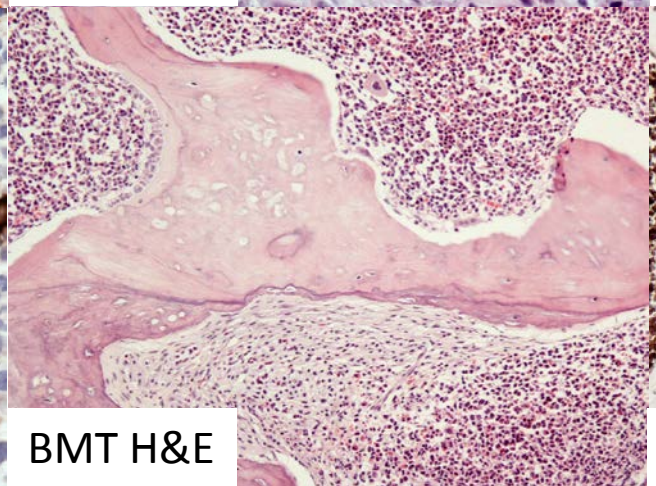

Skin c-kit

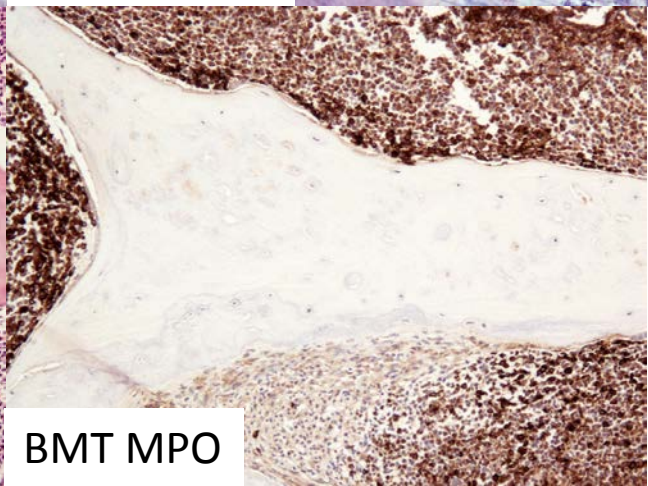

Skin mast cell tryptase


BMT H&E


BMT Giemsa

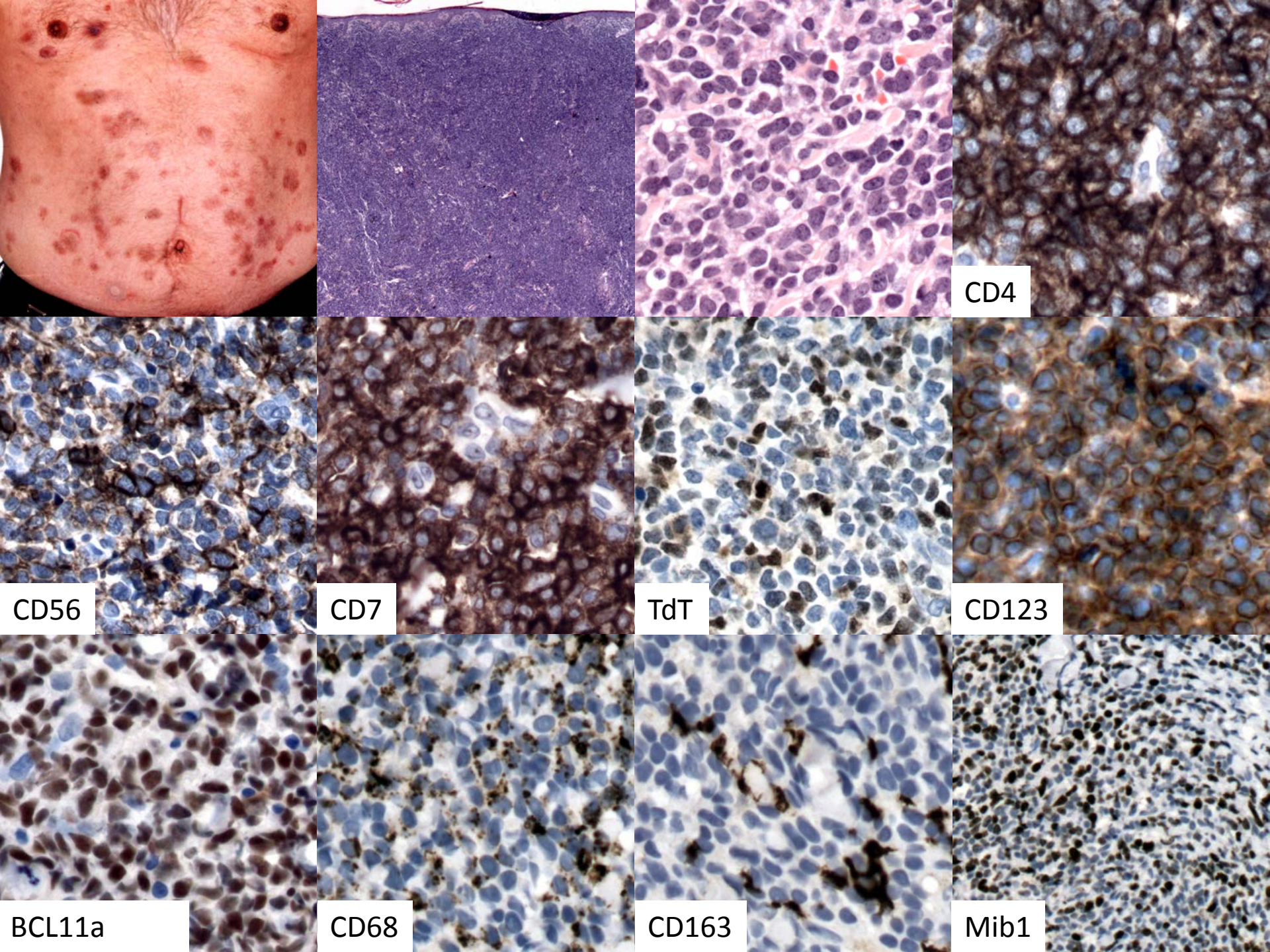

BMT c-kit


BMT H&E


BMT MPO

Blastic plasmacytoid dendritic cell neoplasm is an acute leukaemia

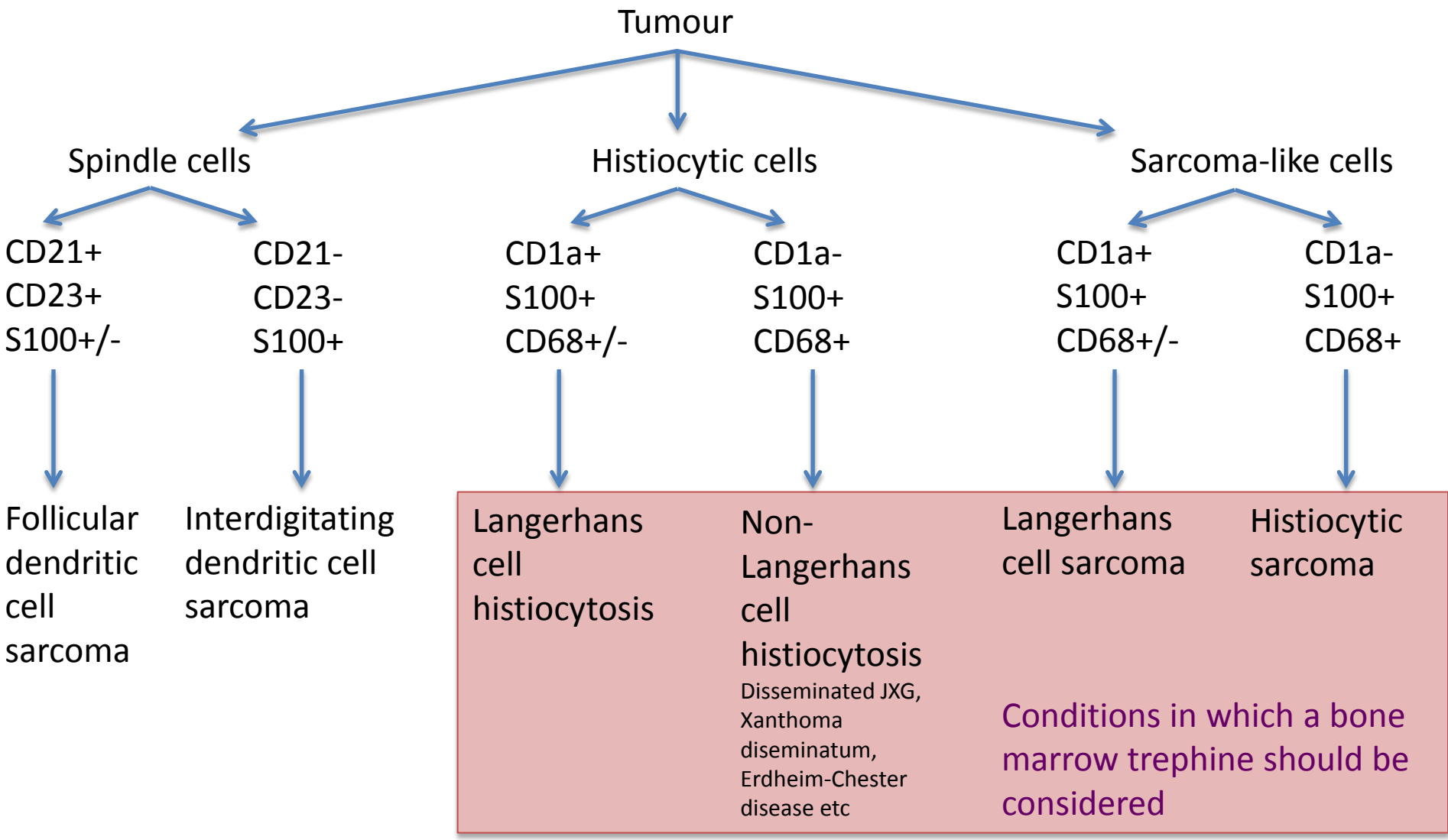
- Formerly known as “blastic NK cell lymphoma” or “haematodermic neoplasm”
- Older adults; male>female
- Often presents in skin, but can be anywhere
- Looks like lymphoblastic lymphoma
- CD45+ CD4+ CD7+ CD56+
- TdT-/+ CD3- CD20- CD79a-
- CD123+ BDCA-2+ BCL11a+


CD4
CD56
CD7
TdT
CD123
BCL11a
CD68
CD163
Mib1

The histiocytoses

- Poorly characterised, rare neoplasms of monocytes/ macrophages/ dendritic cells
- Challenge 1: neoplastic vs reactive
- Challenge 2: conflicting classifications
- Don’t forget: relationship to monocytic/ monoblastic/ myelomonocytic neoplasms (AML etc) -> patient needs a bone marrow trephine

Pragmatic classification of histiocytoses & dendritic cell sarcomas



When to undertake molecular studies?

- Clonality PCR
  - Reactive vs neoplastic
    - Caution: skin; small numbers of cells (pseudoclonality)
  - T vs B-cell
    - Disclaimer: receptor rearrangement does not always mirror lineage commitment
- FISH
  - Diagnostic
  - Prognostic

Multiplexed PCR approach to T & B-cell receptor clonality: Biomed Euroclonality for Heteroduplex Analysis or Genescanner

IgH locus

B-cell receptor

IgK locus

IgL locus

TCRB locus

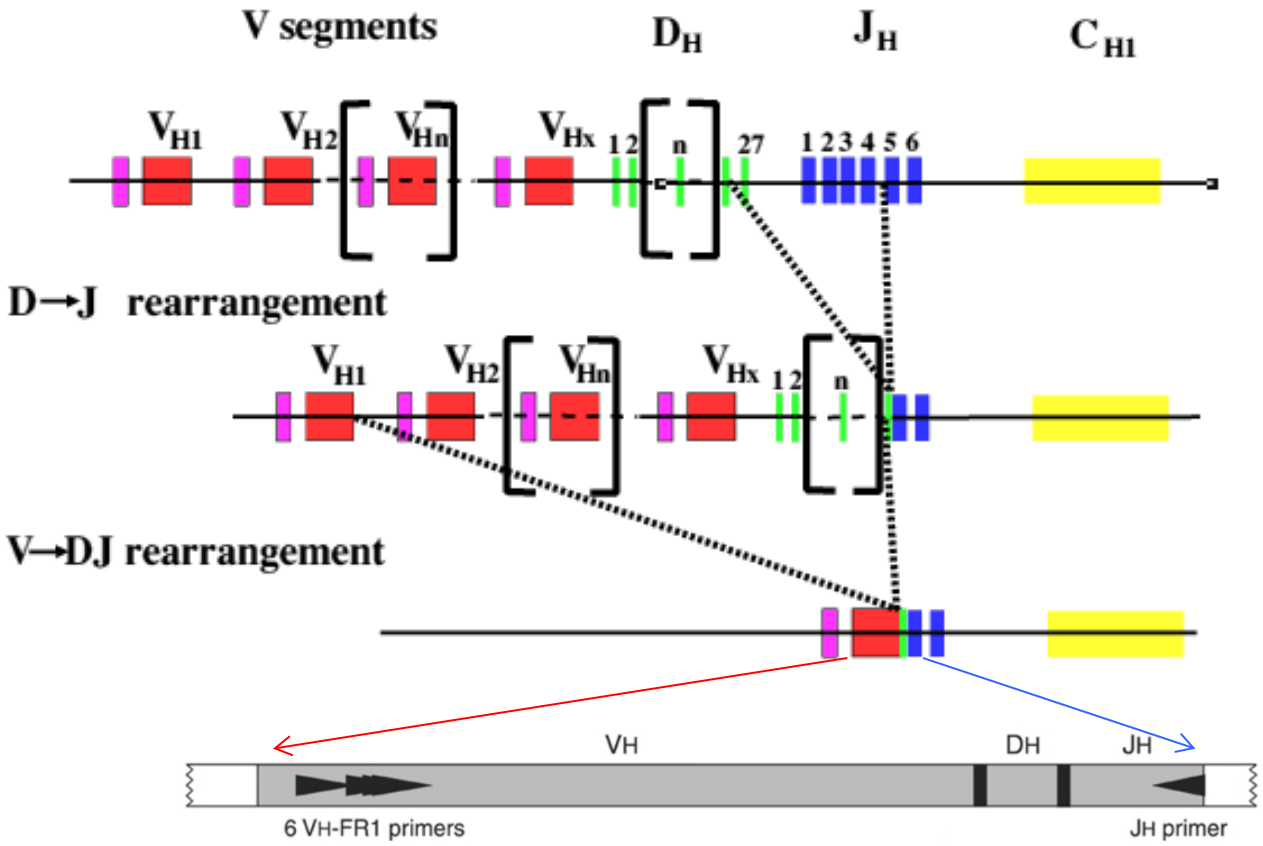
T-cell receptor

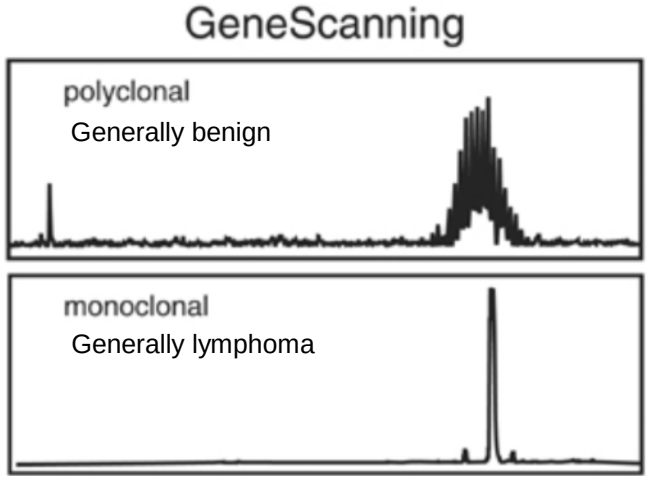
TCRG locus

TCRD locus

B and T-lymphocyte receptor clonality analysis using genomic DNA extracted from FFPE tissue (Euroclonality Biomed-2 system)

B-lymphocyte receptor variable region rearrangement at DNA level





Developments in clonality PCR

- Now moving to 1 tube per locus for next generation sequencing
- Next Generation Sequencing kit "Lymphotrack" has very competitively priced companion software for analysis
- No more Genescan analysis!

“Pre-analytical” concerns for PCR: fixation & decalcification

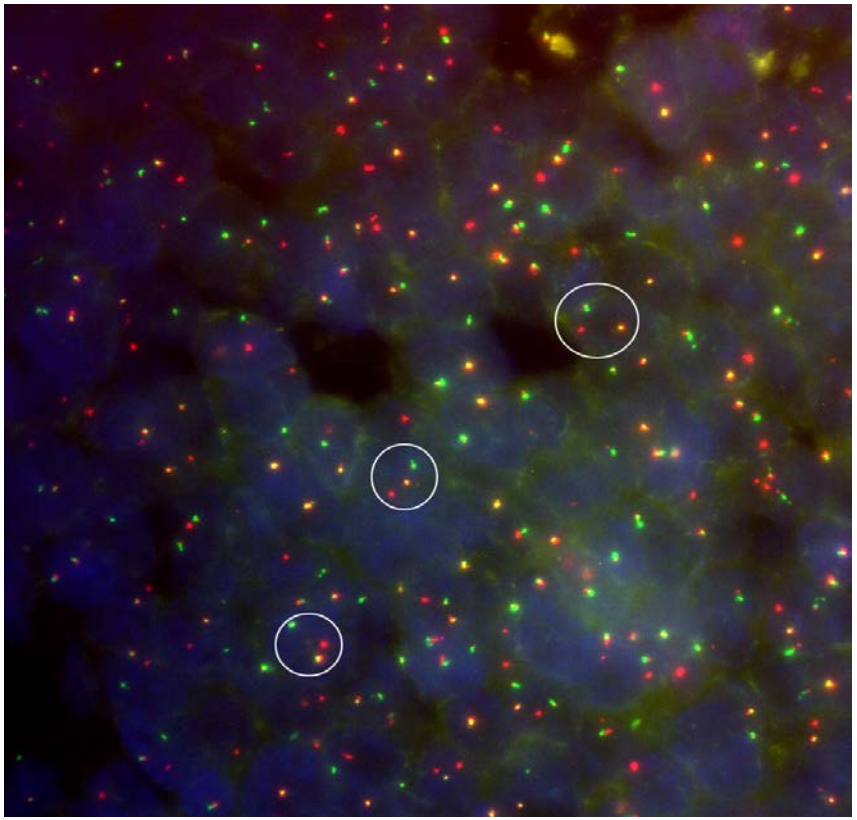
- Poorly fixed samples (e.g., spleens) contain degraded DNA -> poor PCR amplification

- The dangers of acid! Any exposure to pH < 7.0 degrades DNA

- Oxford decalcification protocol changed to 10% EDTA, pH7.4. BMT PCR 100% successful!

FISH analysis of lymphoma translocations in diagnosis

Lymphoma type	Trans-location	% + by FISH	Comments
Follicular lymphoma	t(14;18) BCL2	90% (fewer in grade 3B)	t(14;18) with MYC rearrangement also predicts very poor outcome in DLBCL
Mantle cell lymphoma	t(11;14) CYCLIN D1	>95%	
Marginal zone lymphoma (gastric/ lung)	t(11;18)	20-40%	Indicates poor response to therapy
Burkitt's lymphoma	t(8;14) MYC	90%	t(8;14) also predicts poor outcome in DLBCL
Anaplastic large cell lymphoma	t(2;5) ALK1	60-85%	ALK-negative ALCL in older patients has poorer prognosis



IgH;bcl-2 t(14;18) translocation demonstrated by FISH fusion probes in follicular lymphoma

How does the future of haematopathology look?

- The “molecular threat” ???
- Taking control of the future
  - Develop your own service
  - Be involved in molecular reporting
  - Make it chromogenic!!
- Adequate training to manage future developments

Potential chromogenic developments: Is the future golden?

- PROTEIN
  - Point-mutation-specific antibodies
- RNA
  - Kappa and lambda high sensitivity ISH
- DNA
  - Chromosomal rearrangements

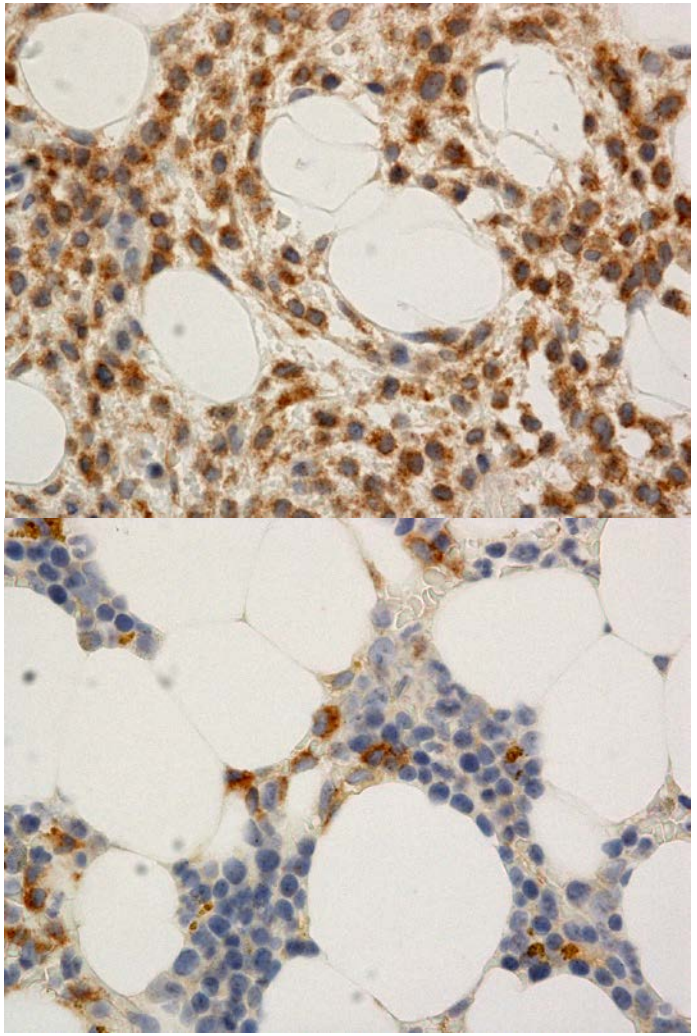
Potential chromogenic developments: Is the future

?

- PROTEIN
  - Point-mutation-specific antibodies
- RNA
  - Kappa and lambda high sensitivity ISH
- DNA
  - Chromosomal rearrangements

Immunohistochemical BRAF V600E mutation in hairy cell leukaemia

- ~90% cases V600E+
- Ventana antibody specifically detects point mutation
- Cheaper than many sequencing assays
- May replace DBA-44 & annexin A1 immunostains
- Our BRAF V600E IHC
  - Positive: 10/10 HCL cases
  - Negative: SMZL, MCL, LPL, CLL, HCL in CR, atypical HCL

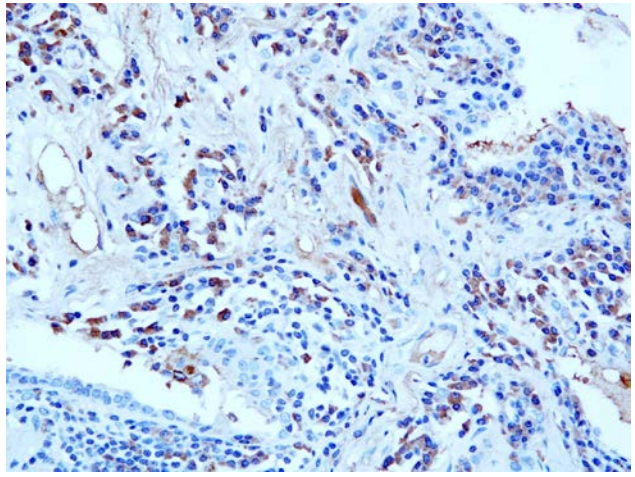


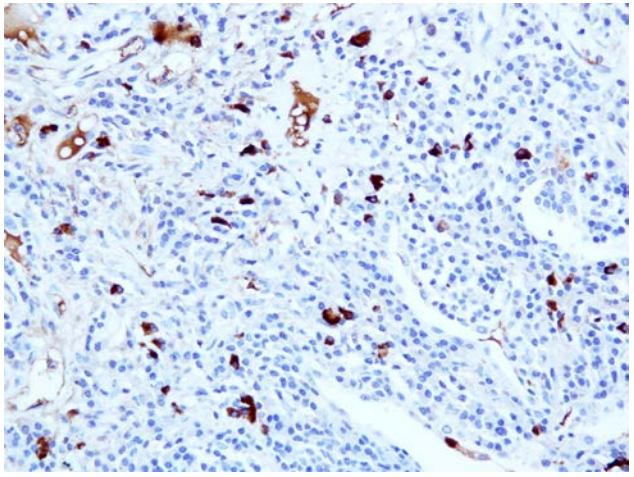
Chromogenic in situ hybridisation for RNA (CISH)

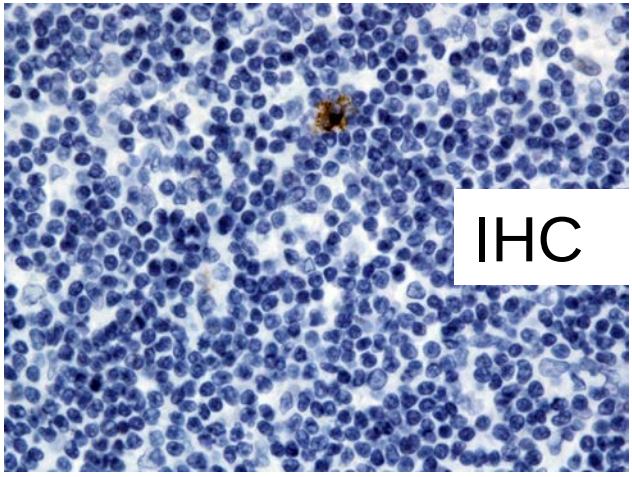
Kappa

Lambda

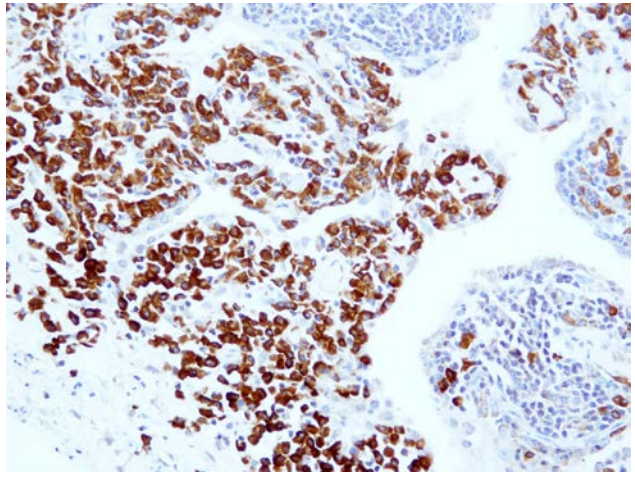
EBV

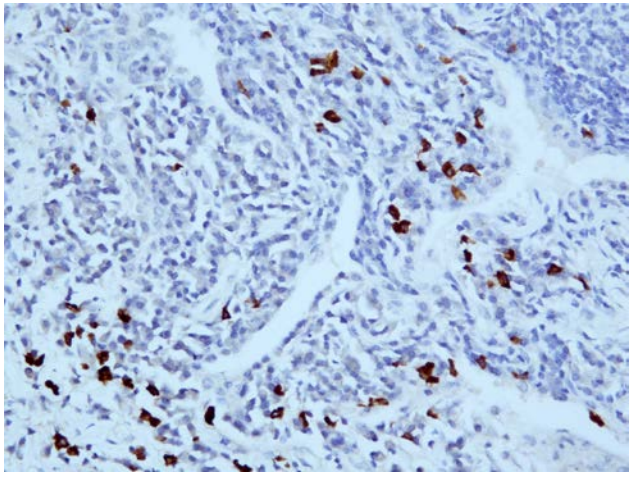


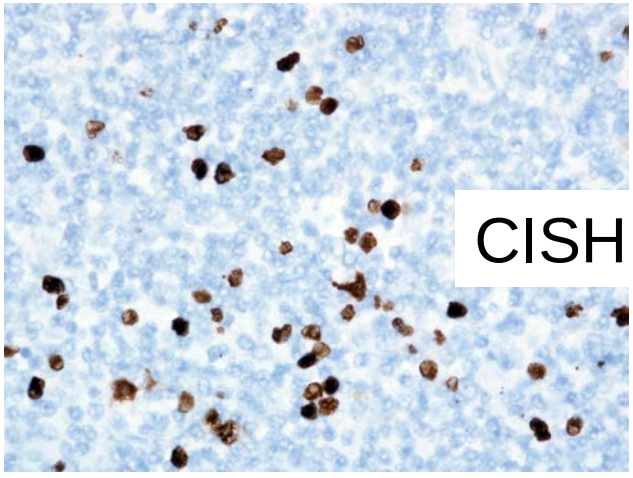




IHC

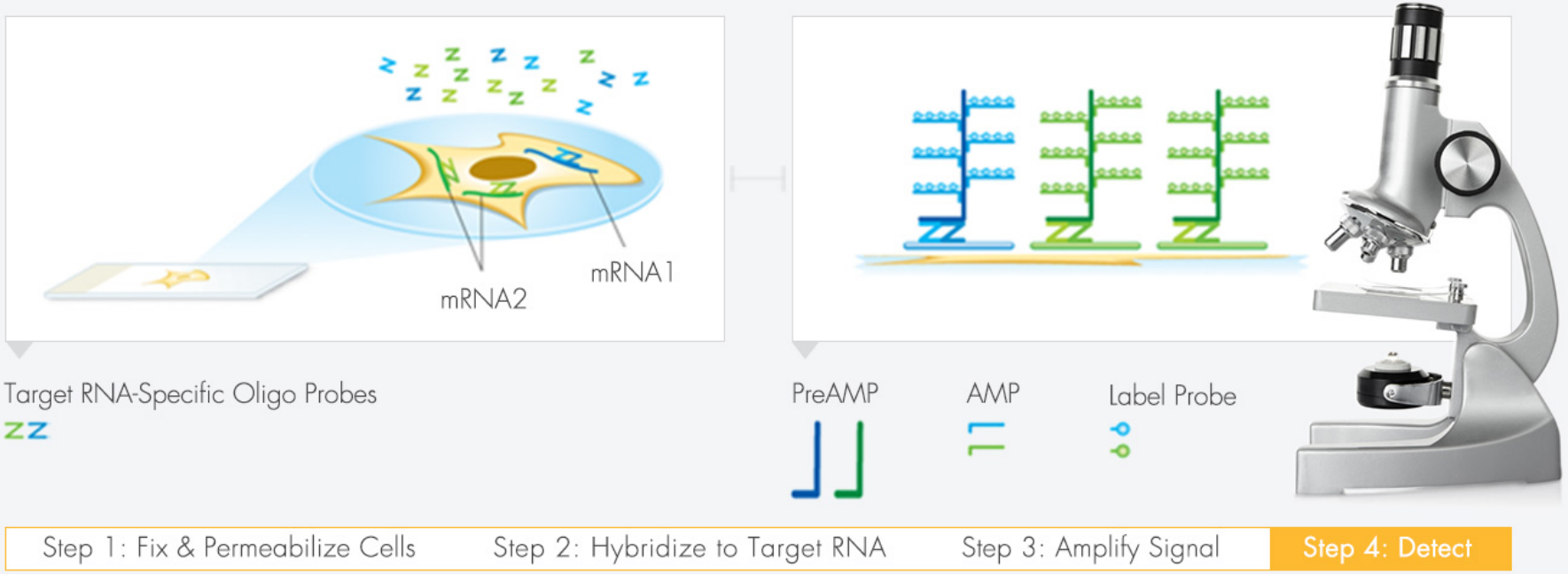






CISH

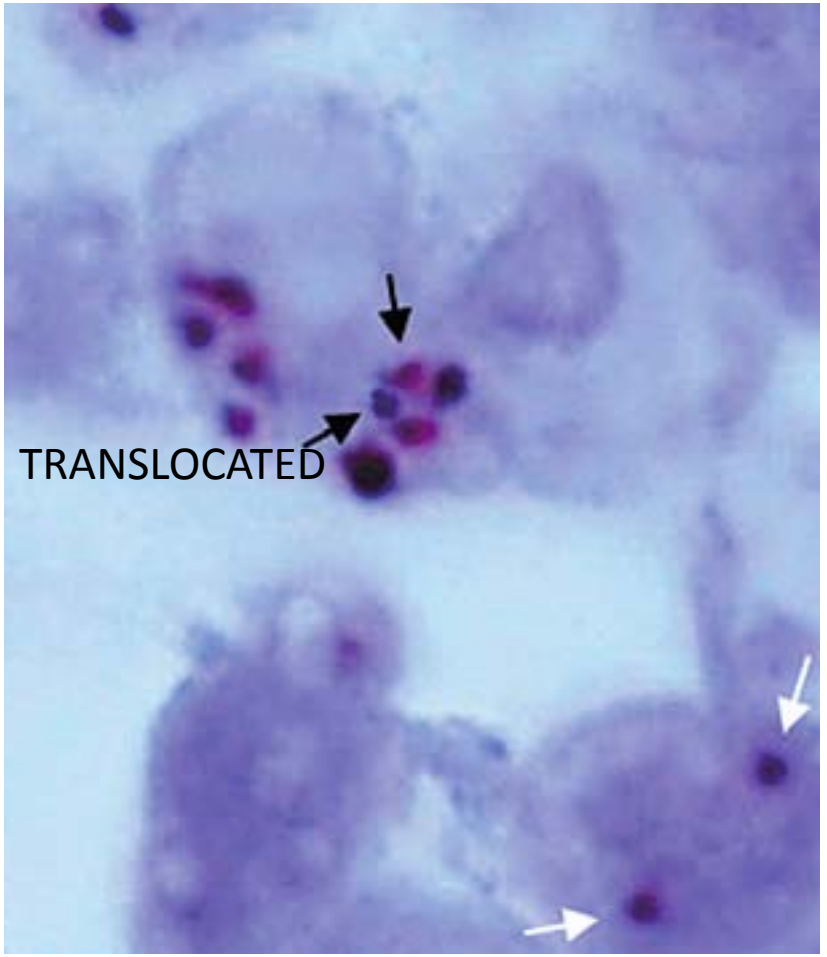
RNAScope: A high sensitivity and specificity in situ hybridisation technique



Advanced Cell Diagnostics: kappa and lambda available shortly. Can detect 1 transcript/ cell.

Chromogenic in situ hybridisation for chromosomal rearrangement

- Split-apart probes: red and blue (black when fused)
- BCL2: chromosome 18
- Japanese study: classical Hodgkin's lymphoma ex-follicular lymphoma
- Requires x 100 oil immersion lens
- Just another histological section…..
- Other colour combinations available….



Questions & Discussion

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