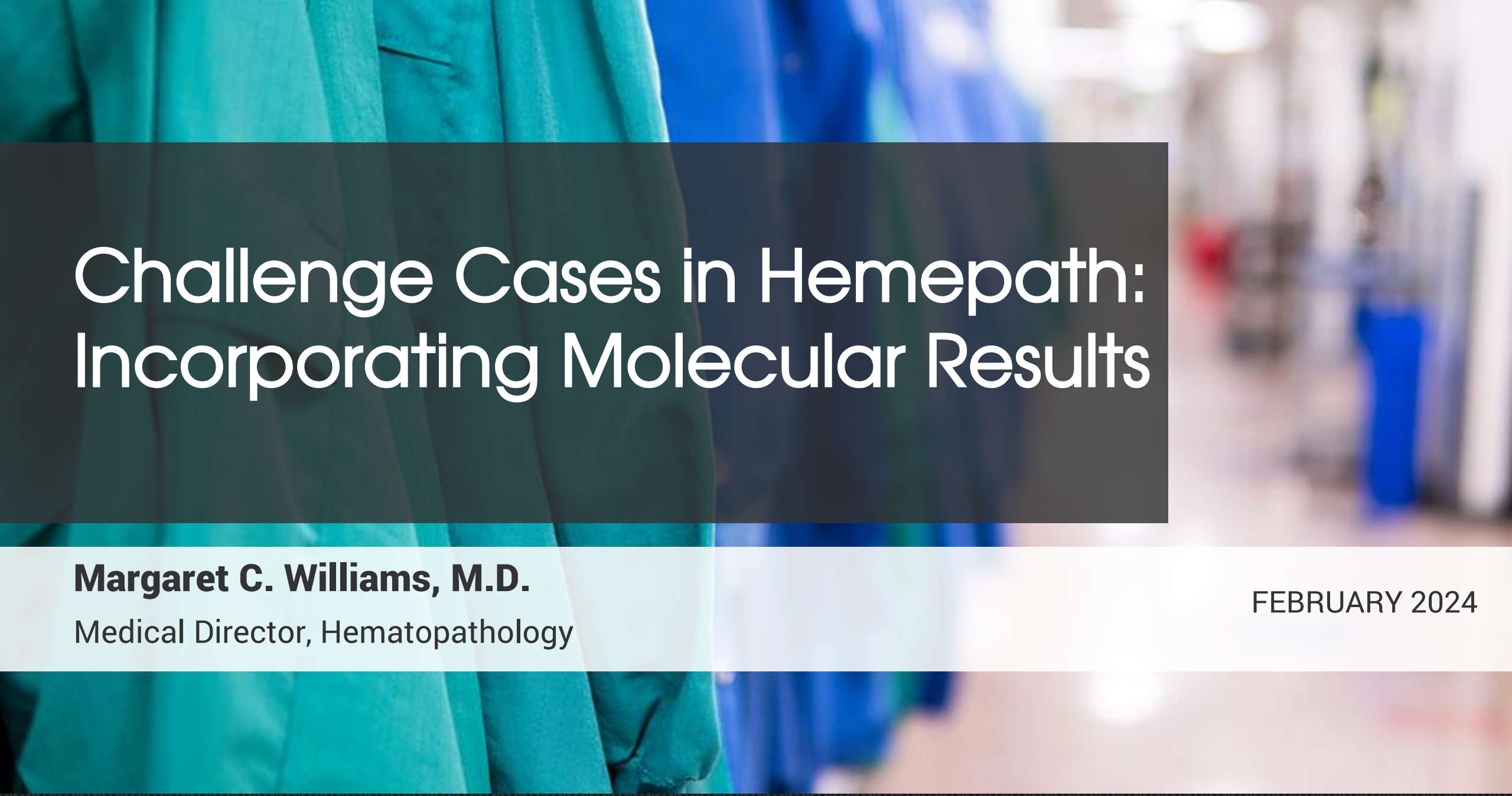
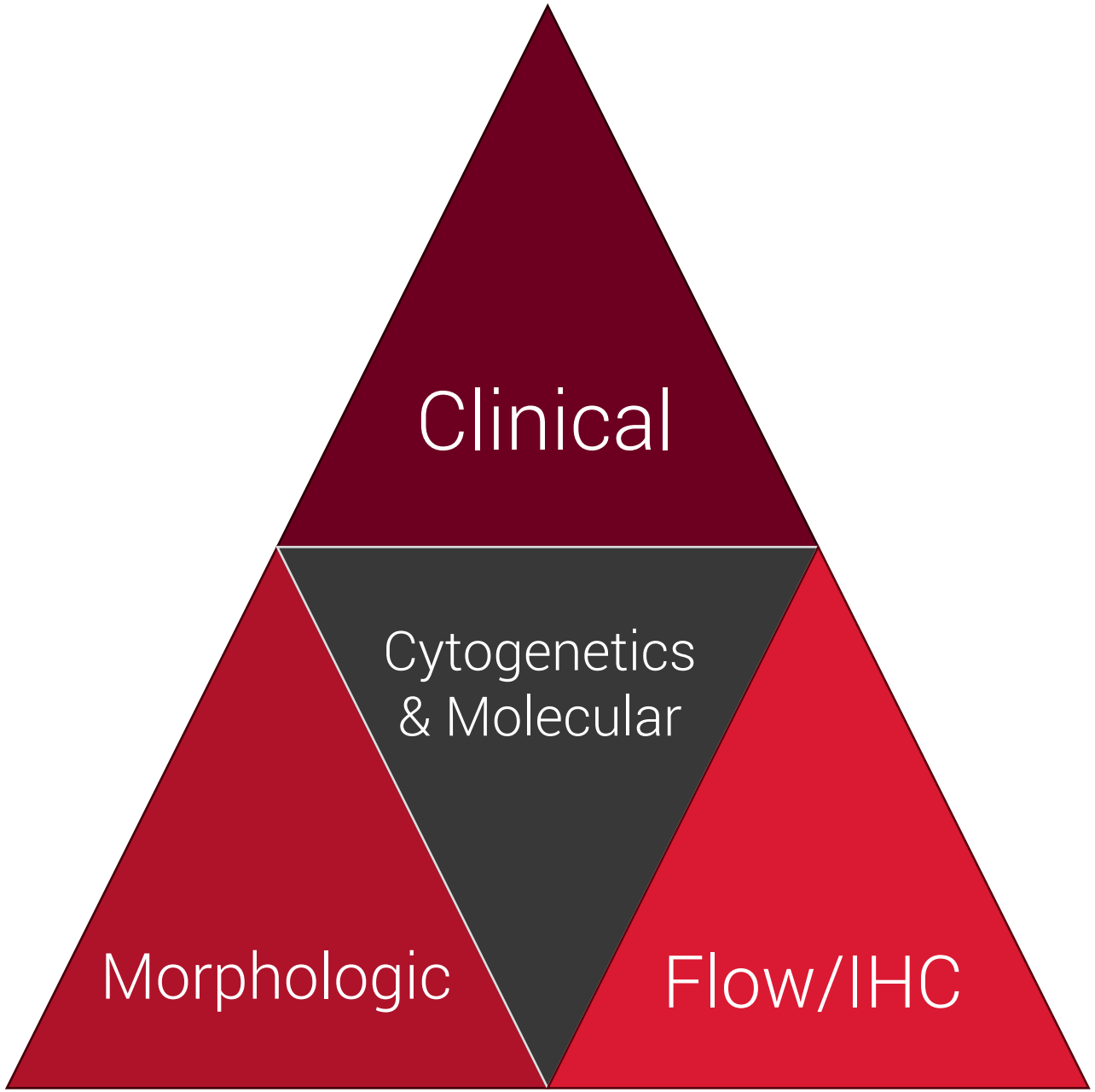


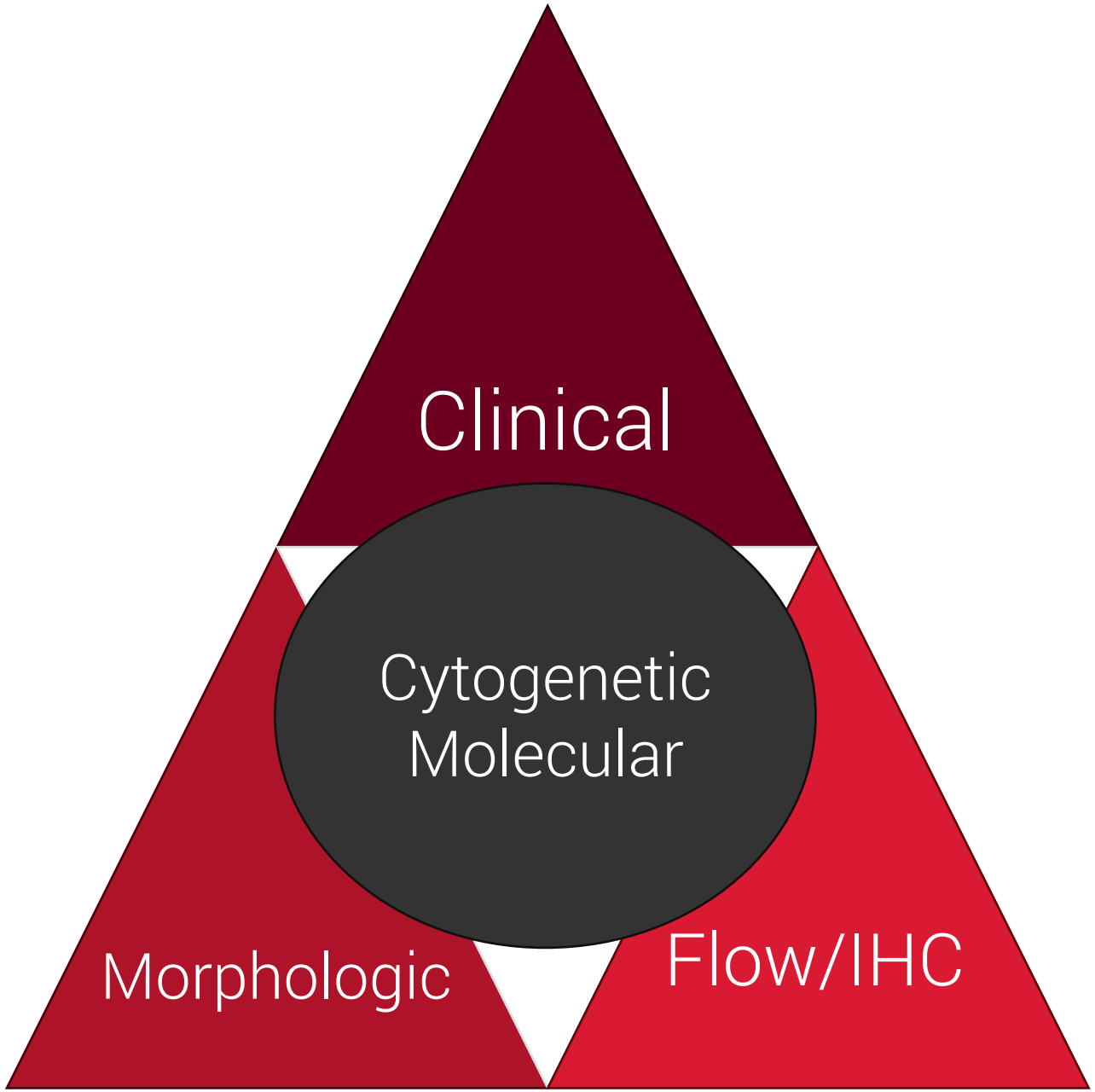
Challenge Cases in Hemepath: Incorporating Molecular Results

Margaret C. Williams, M.D.

Medical Director, Hematopathology

FEBRUARY 2024





Agenda

Three Challenge Cases

Can we develop an approach to unexpected ancillary findings?

Agenda

Three Challenge Cases

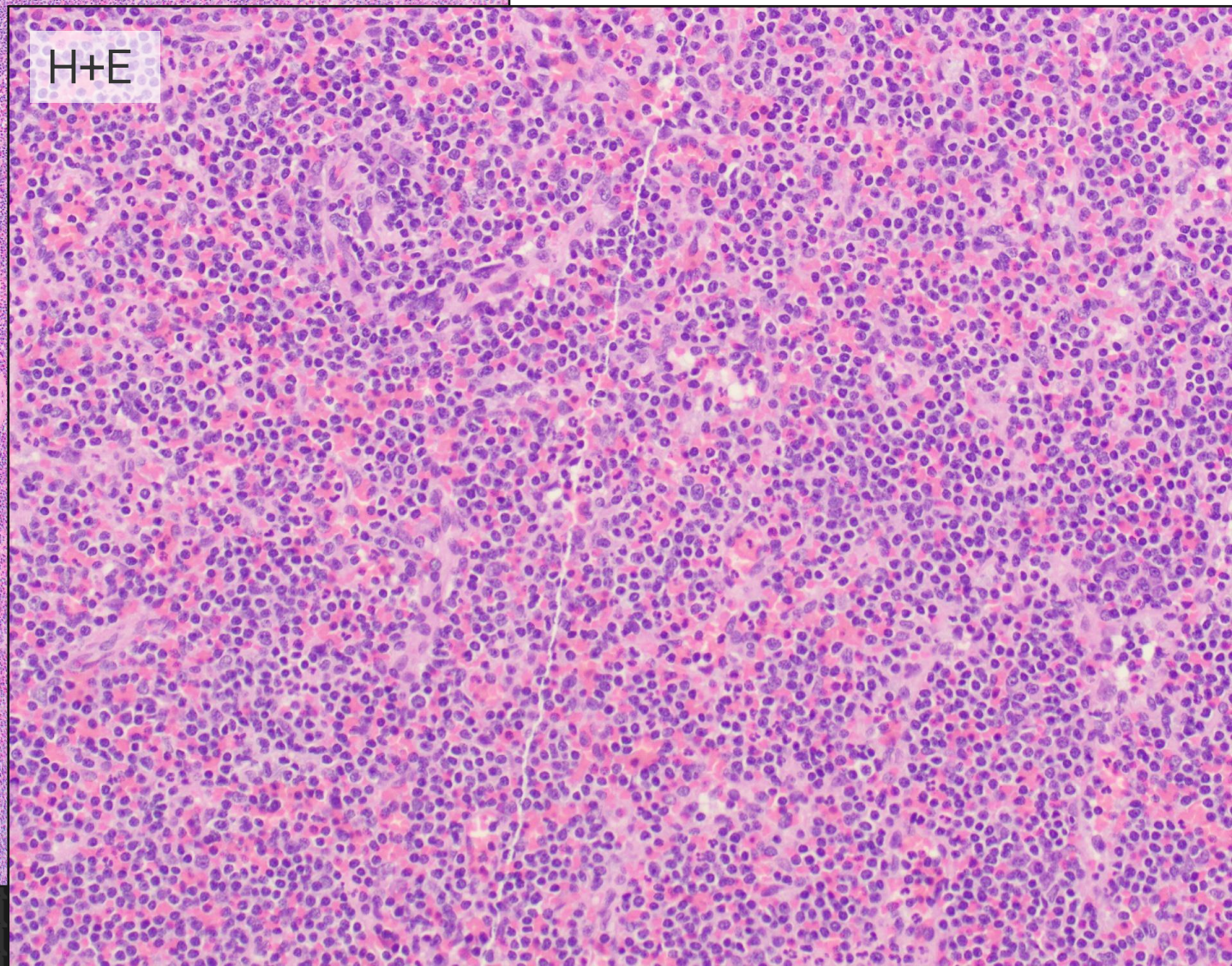
Can we develop an approach to unexpected ancillary findings?

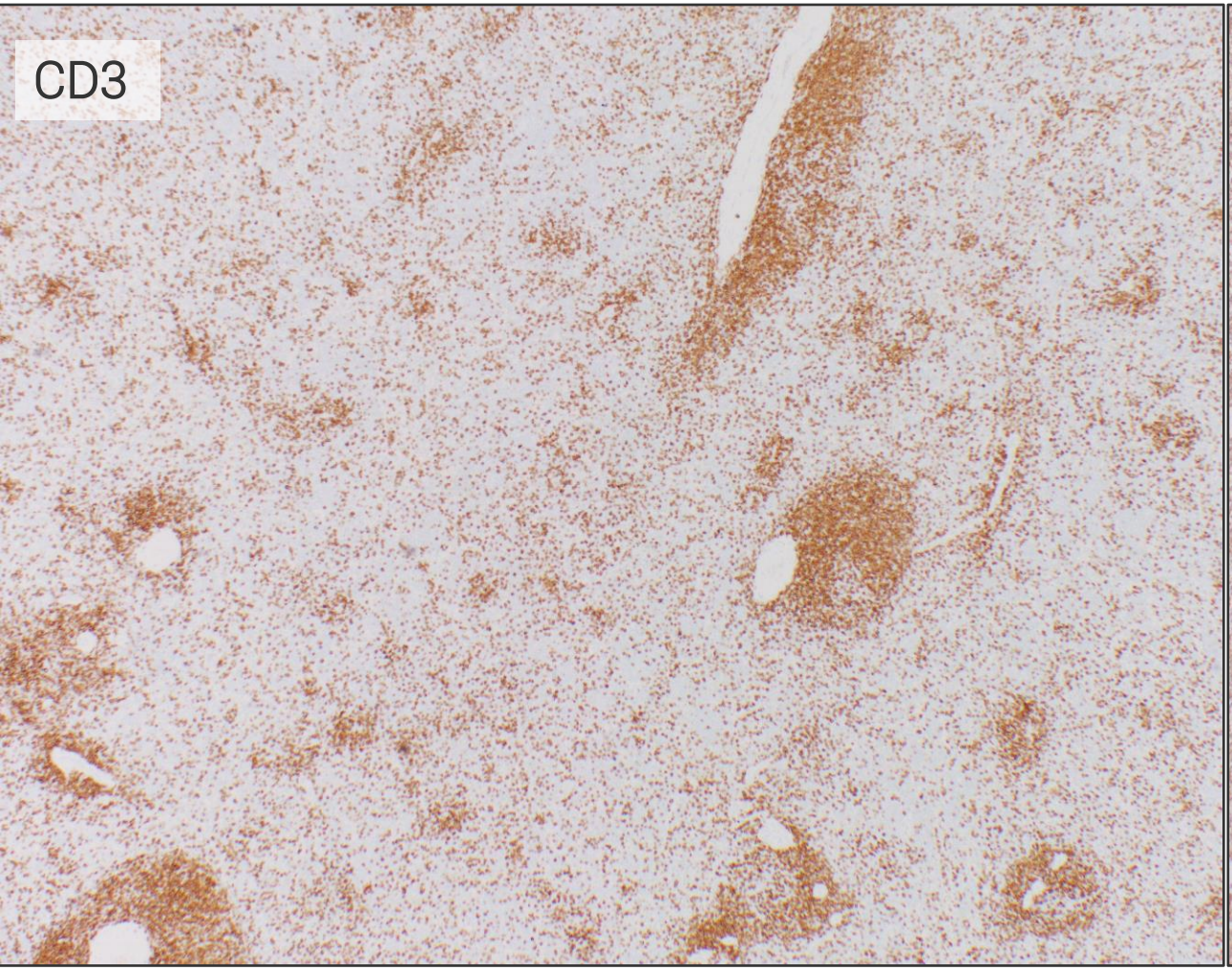
Case 1

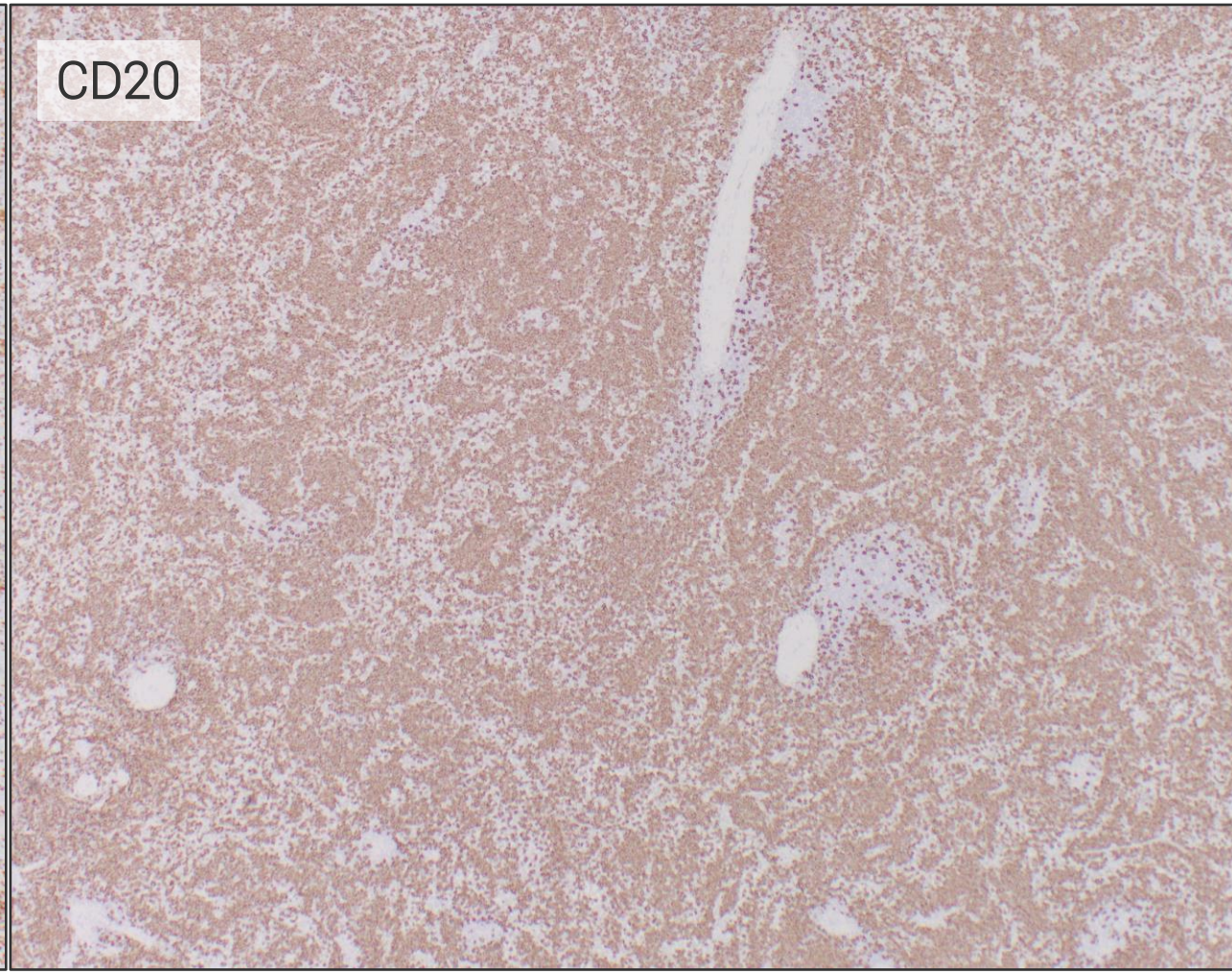
Case 1:

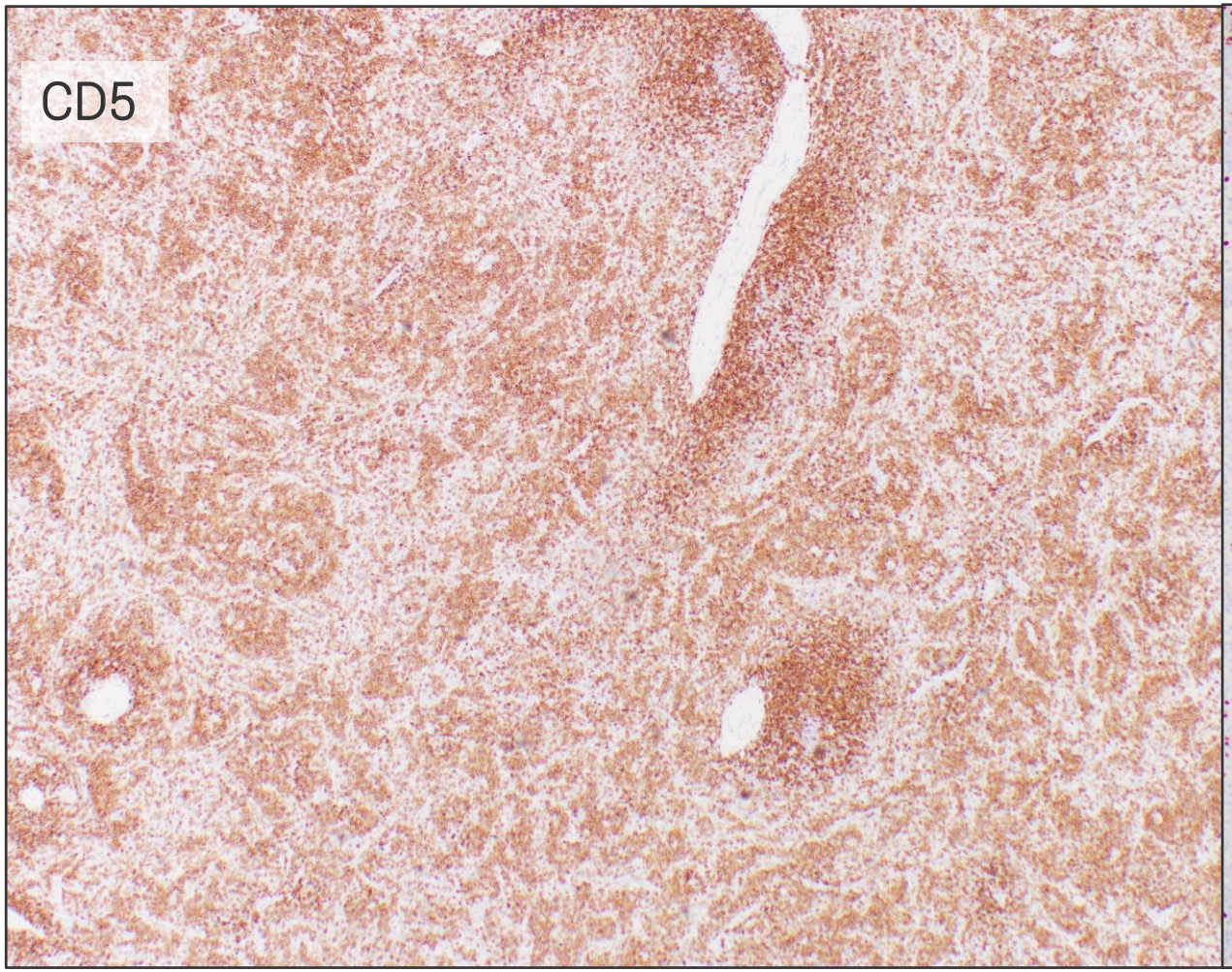
- 60-year-old man presents with hepatosplenomegaly and non-traumatic rupture of the spleen
- Imaging shows no suspicious lymphadenopathy
- Spleen is sent for evaluation

Case 1: H+E





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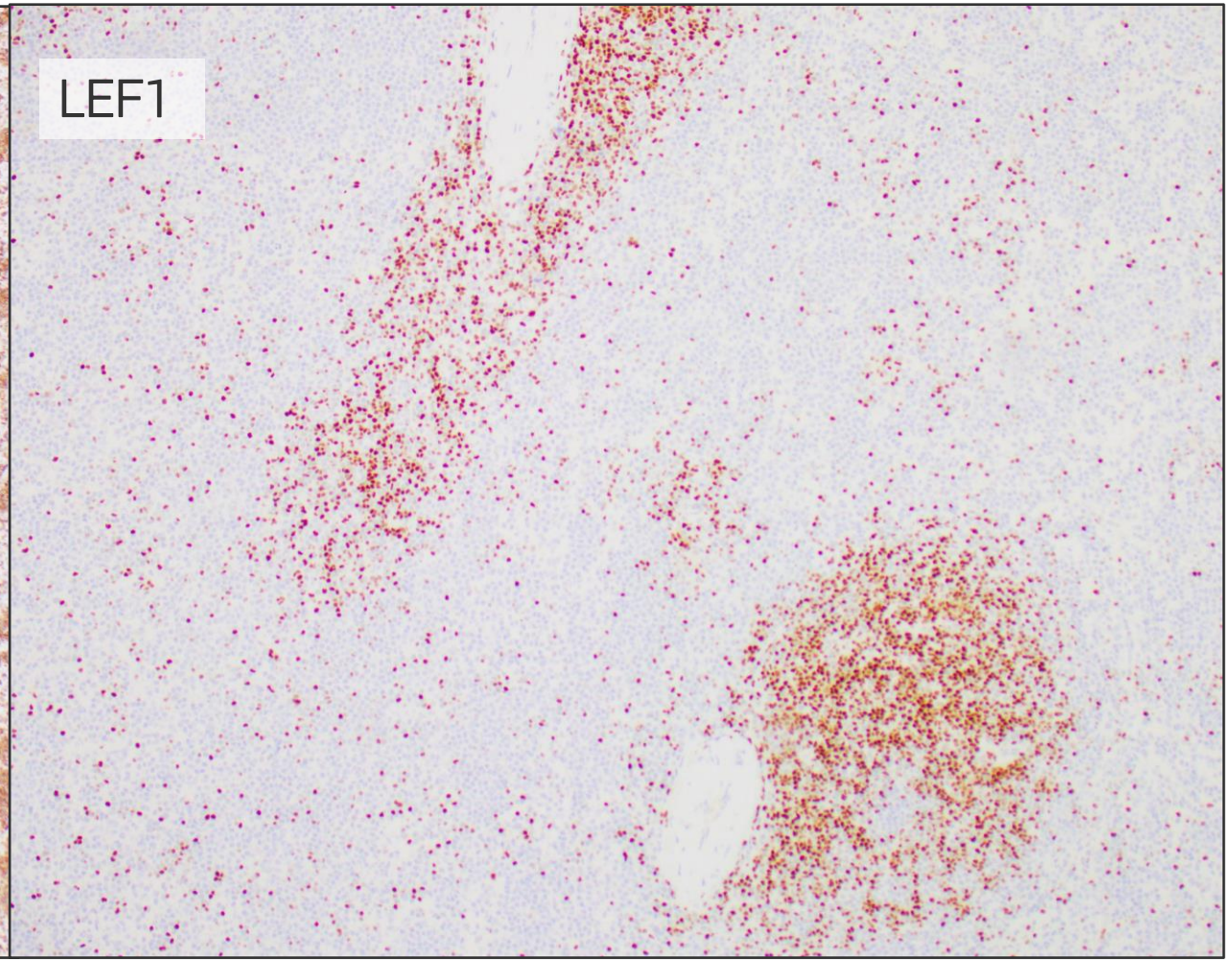
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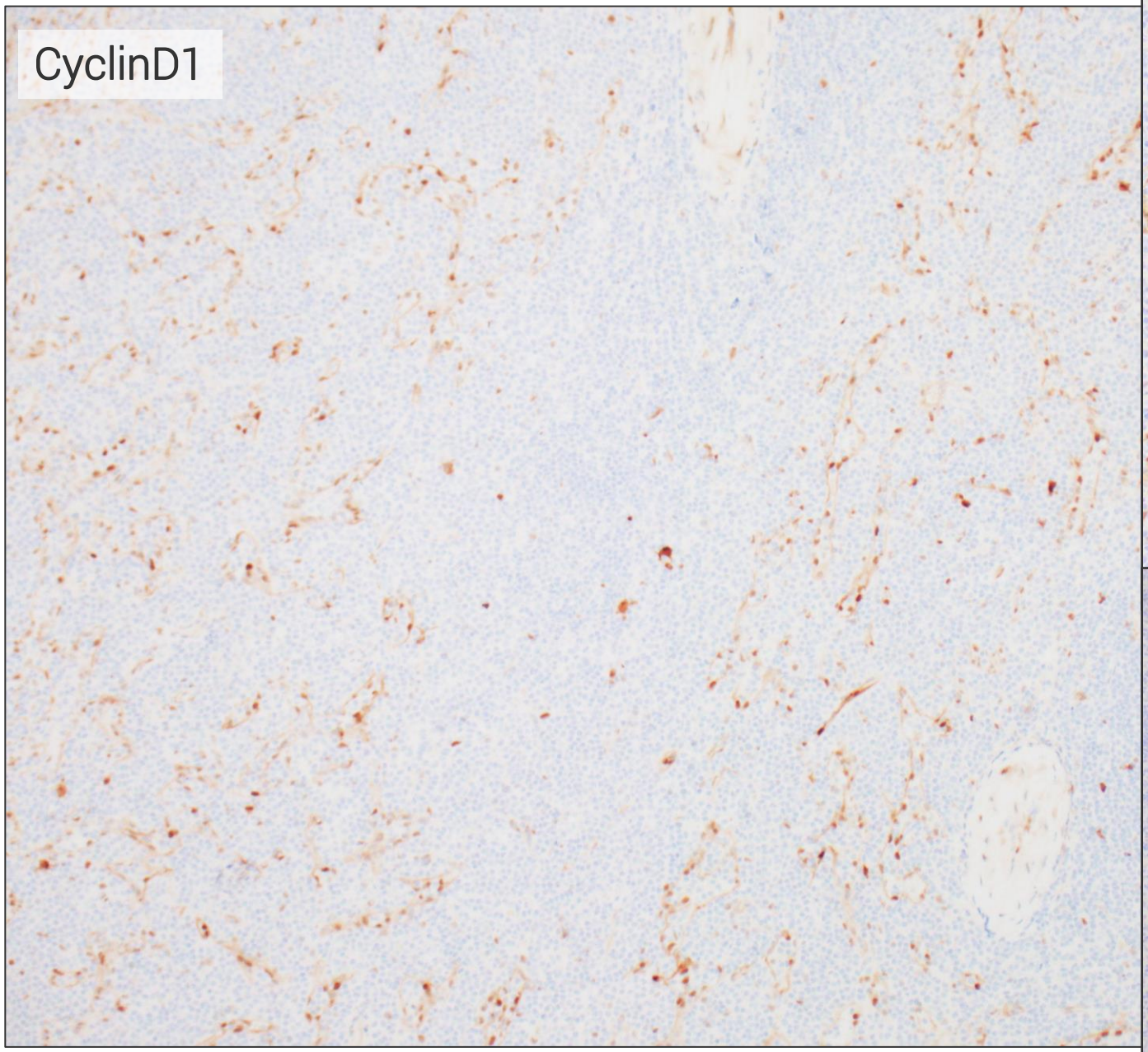
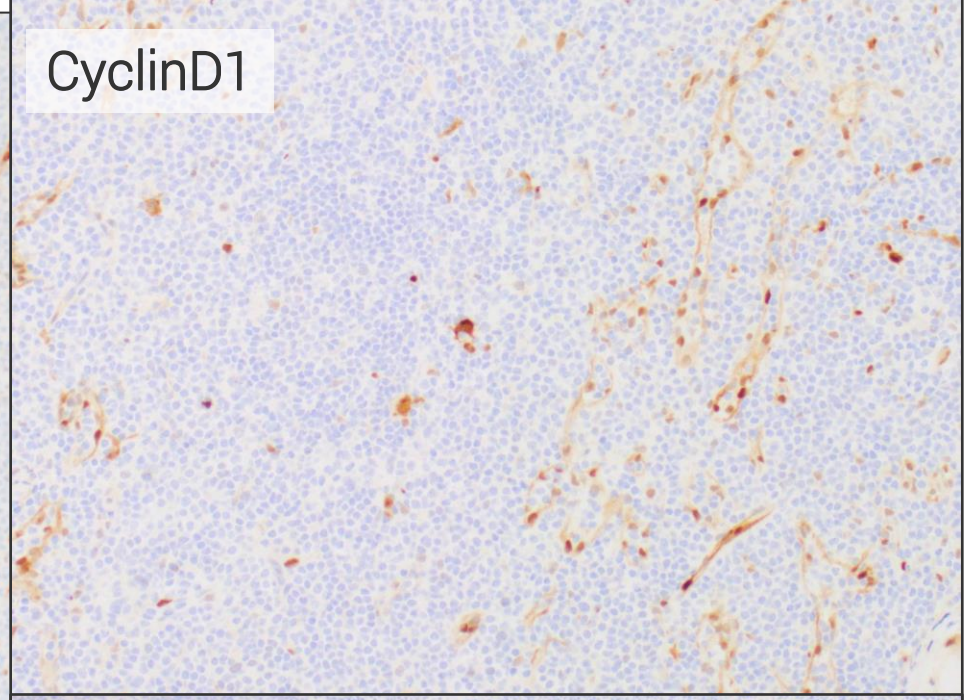
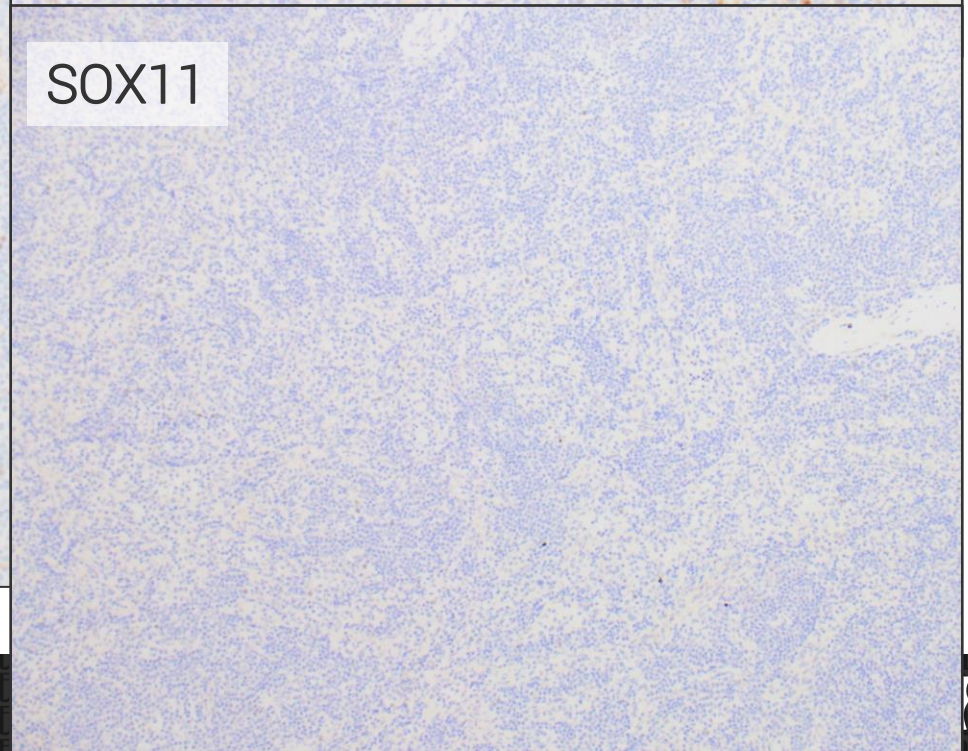


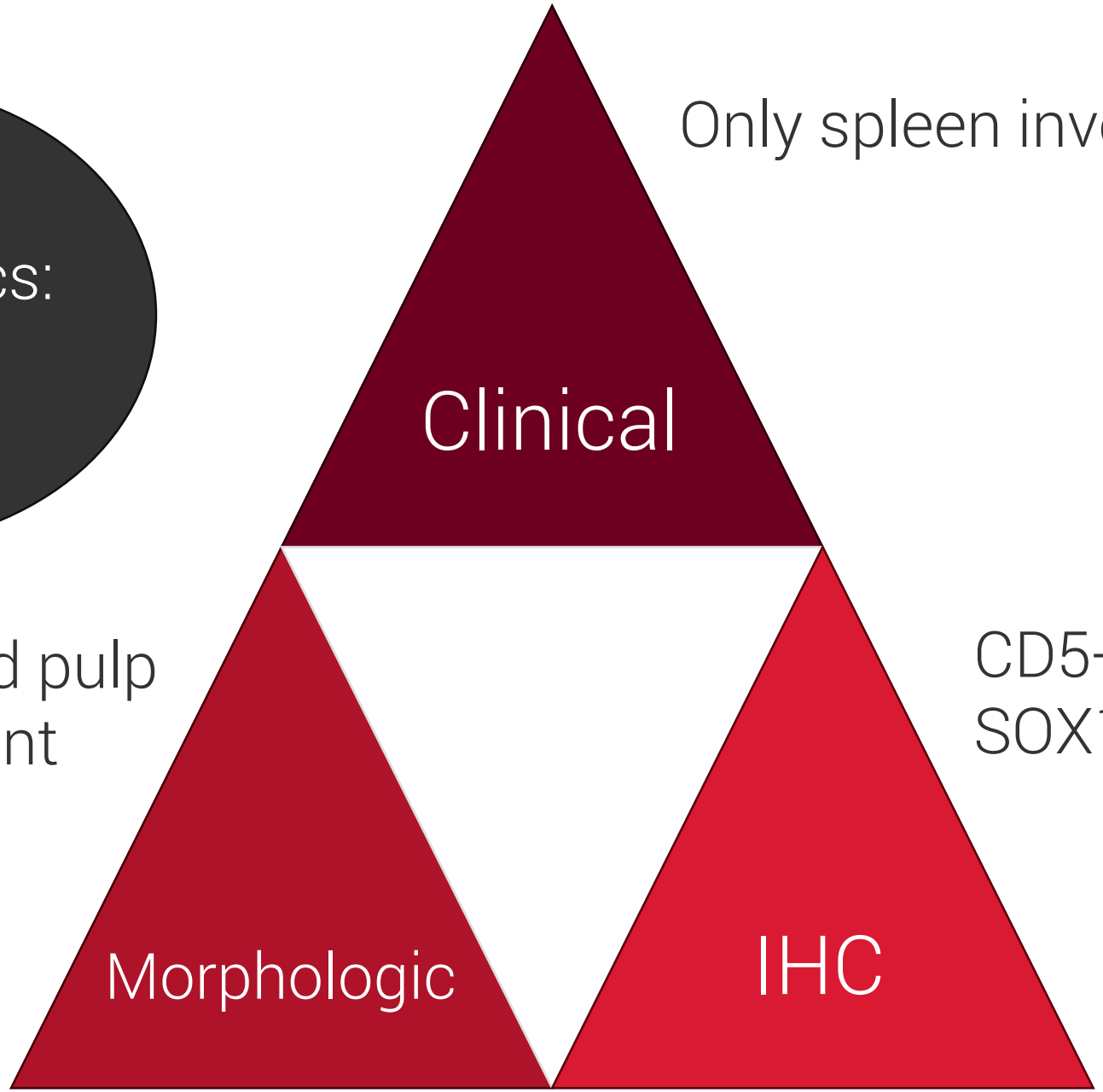




Case 1:

- IHC:
  - » Expressed CD20, CD5, CD200
  - » Negative for cyclin D1, SOX11, LEF1, CD103, CD25, CD123, Annexin A1, CD10, CD23, CD138, CD3
- Flow Cytometry:
  - » CD5+ Kappa restricted B-cells
  - » Bright CD20+, CD11c+, negative for CD23
- Cytogenetics:
  - » Positive for *CCND1::IGH* , t(11;14) by FISH

Cytogenetics: t(11;14)+



Only spleen involved

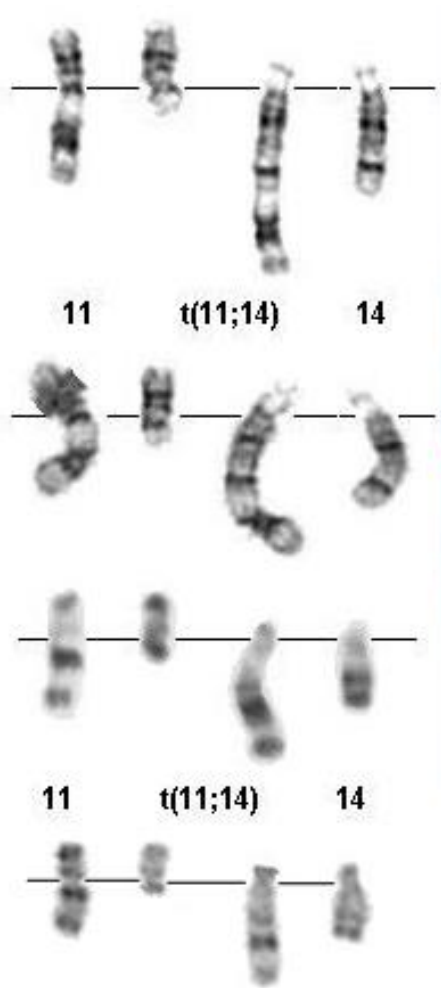
Diffuse red pulp involvement

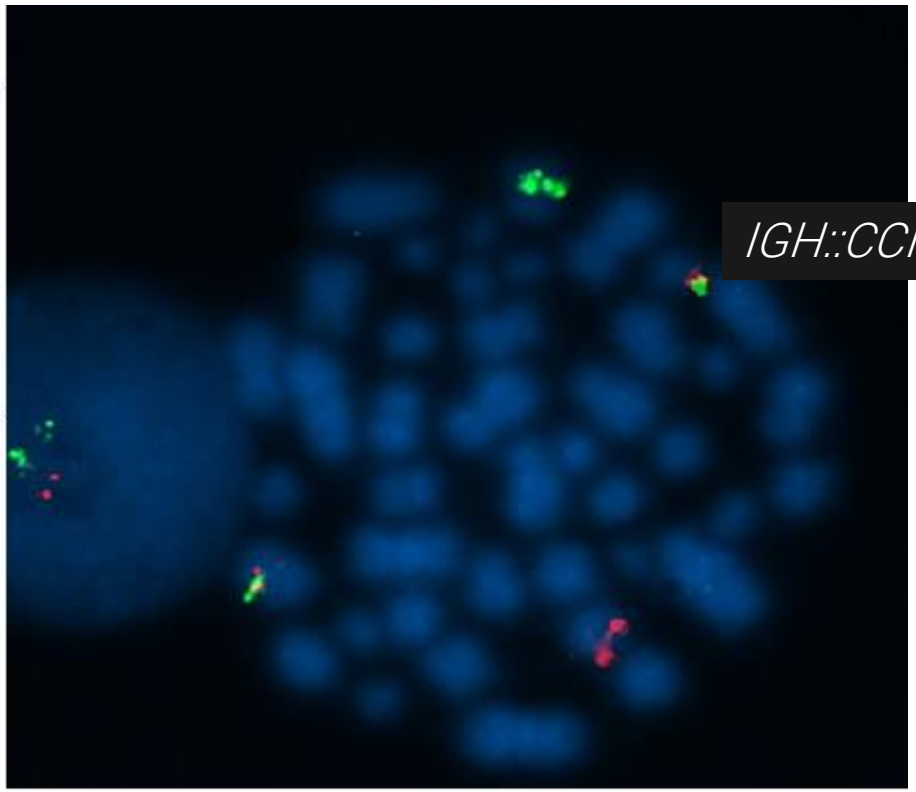
CD5+ but CyclinD1 and SOX11 negative by IHC

Causes of IHC and FISH Discordance in Mantle Cell Lymphoma?

FISH in Mantle Cell Lymphoma

- >95% of MCL have t(11;14)(q13;q32) or *IGH::CCND1*
  - » Leads to overexpression of *CCND1* and its product, cyclin D1
  - » Rare translocations reported between *CCND1* and light chain loci or *CCND2* or *CCND3* and immunoglobulin loci





t(11;14)(q13;q32)

Image from: https://atlasgeneticsoncology.org/haematological/2021/t(11;14)(q13;q32)-igh-ccnd1

IHC in Mantle Cell Lymphoma

- Cyclin D1:
  - » Expressed in >95% of MCL
  - » Also expressed in hairy cell leukemia, myelomas, endothelial cells, highly proliferative cells
  - » Rare in other B-cell lymphomas
- SOX11:
  - » Positive in 70-90% of MCL
    - less common in indolent/leukemic variants or in cases with *TP53* mutation
  - » Can be seen in a subset of other B-cell lymphomas

Causes of Discrepant Negative FISH:

- Rare alternate translocations (*CCND1* with light chain loci or *CCND2* or *CCND3*) that lead to cyclin D1 expression but aren't detectable by our t(11;14) FISH probes
- Cryptic translocations:
  - » Rearrangement of a segment smaller than FISH probe can identify

Additional steps to evaluate: *CCND1, CCND2* break apart FISH probes, karyotype

PMID: 31753927, 31015206, 37064110, 32408254, 30538135

Causes of Discrepant Negative IHC:

- Mutations in *CCND1* that prevent binding to the antibody for IHC

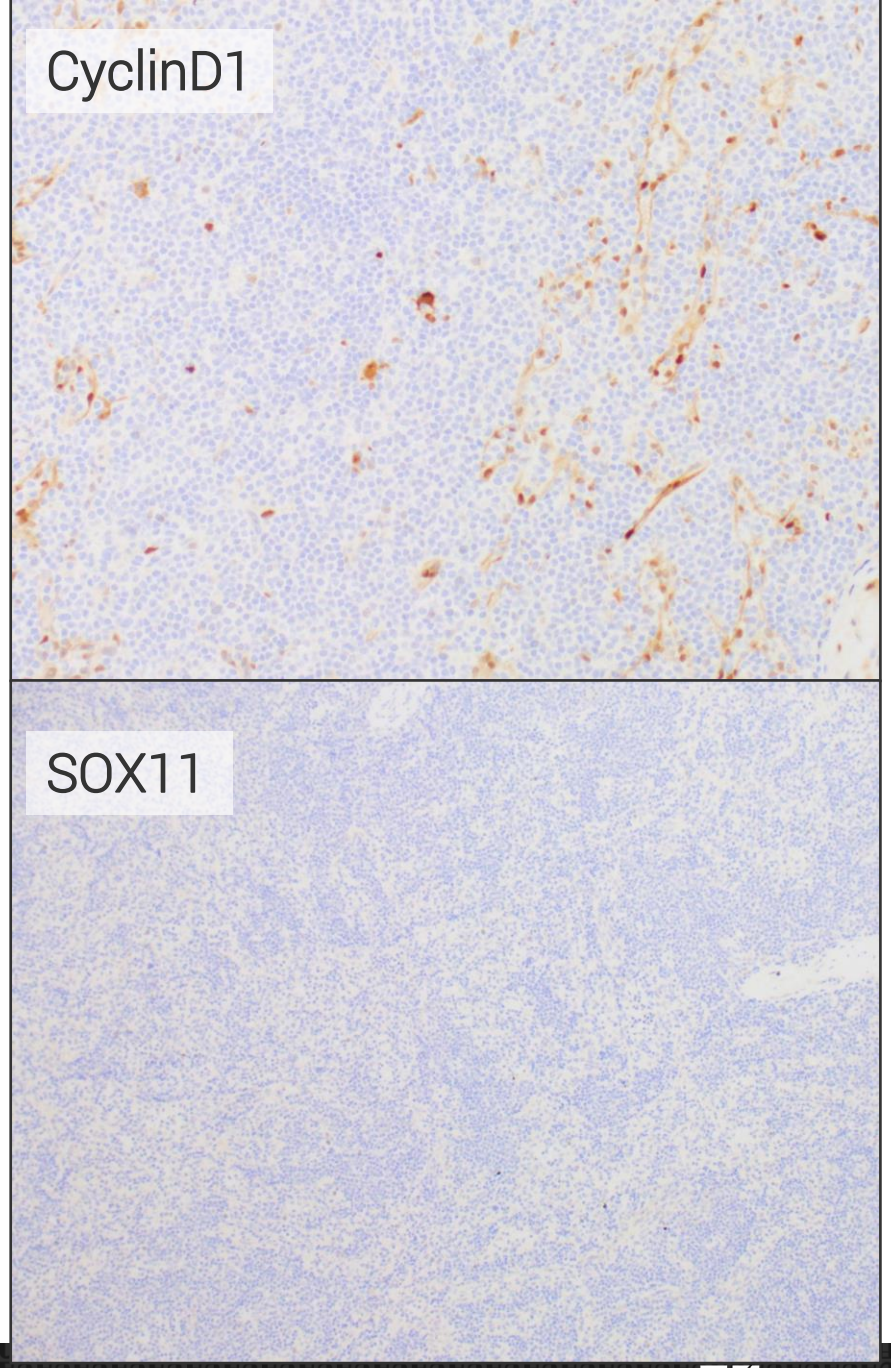
Additional steps to evaluate: SOX11 staining and t(11;14) FISH

- Rearrangements in *CCND2* and *CCND3*

Additional steps to evaluate: *CCND2* break apart FISH probes, karyotype

Case 1:

- Negative for CyclinD1 and SOX11 by IHC
- Unusual clinical and morphologic features for mantle cell lymphoma
- Outside FISH study



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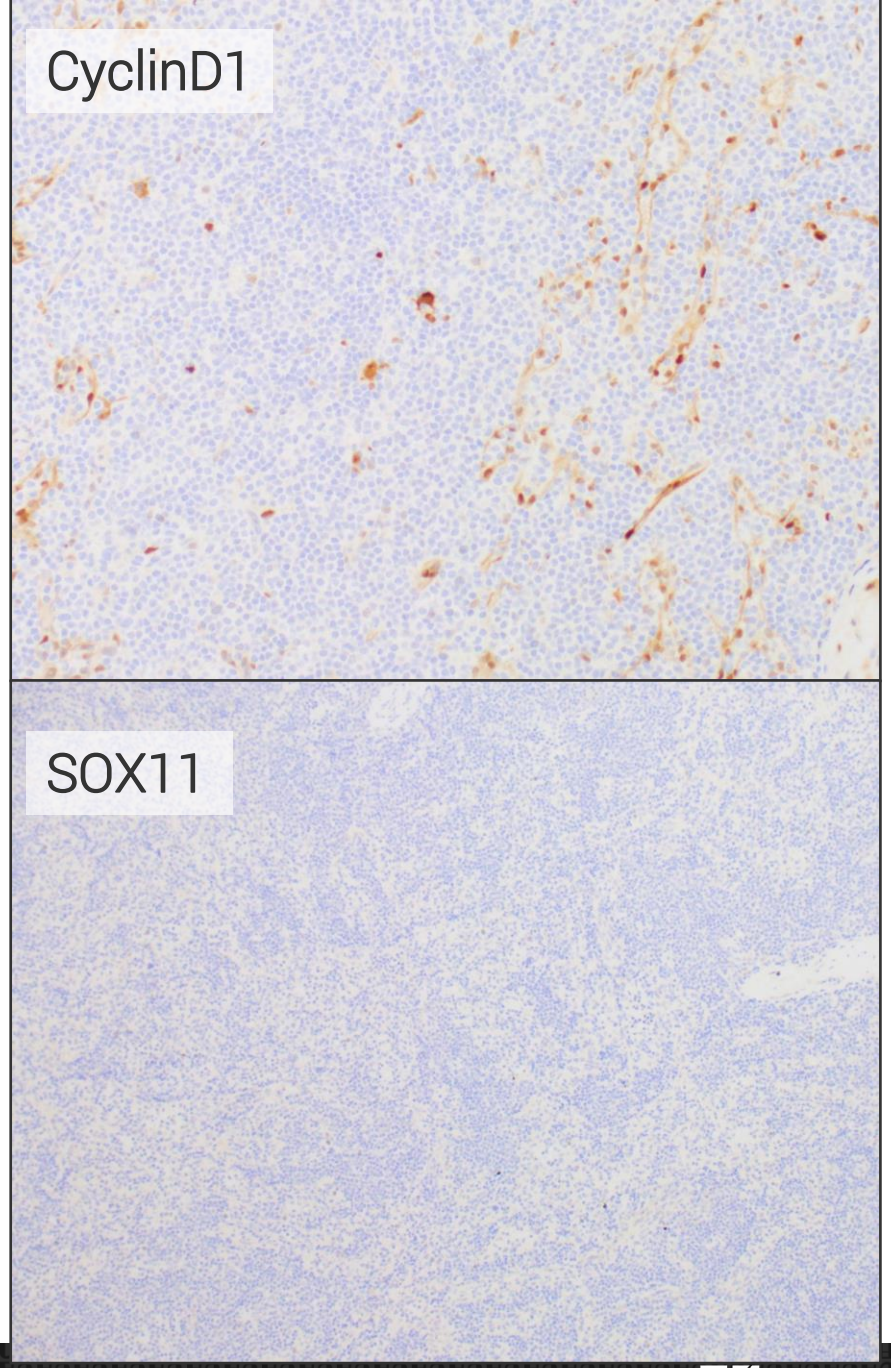


Our resolution:

- repeated t(11;14) FISH and got a negative result

Case 1: Final Diagnosis

- Essentially excluded:
  - » Mantle cell lymphoma (negative cyclin D1, SOX11, positive for CD200, and negative for t(11;14) by FISH)
  - » CLL/SLL (bright CD20 by flow, negative CD23 and LEF1 by IHC)
  - » Hairy cell leukemia (negative CD103, CD25, CD123, and Annexin A1)
  - » Splenic B-cell lymphoma with prominent nucleoli (aka HCL-v, negative CD103)

Case 1: Final Diagnosis

Mature B-cell lymphoma, favor splenic diffuse red pulp small B-cell lymphoma

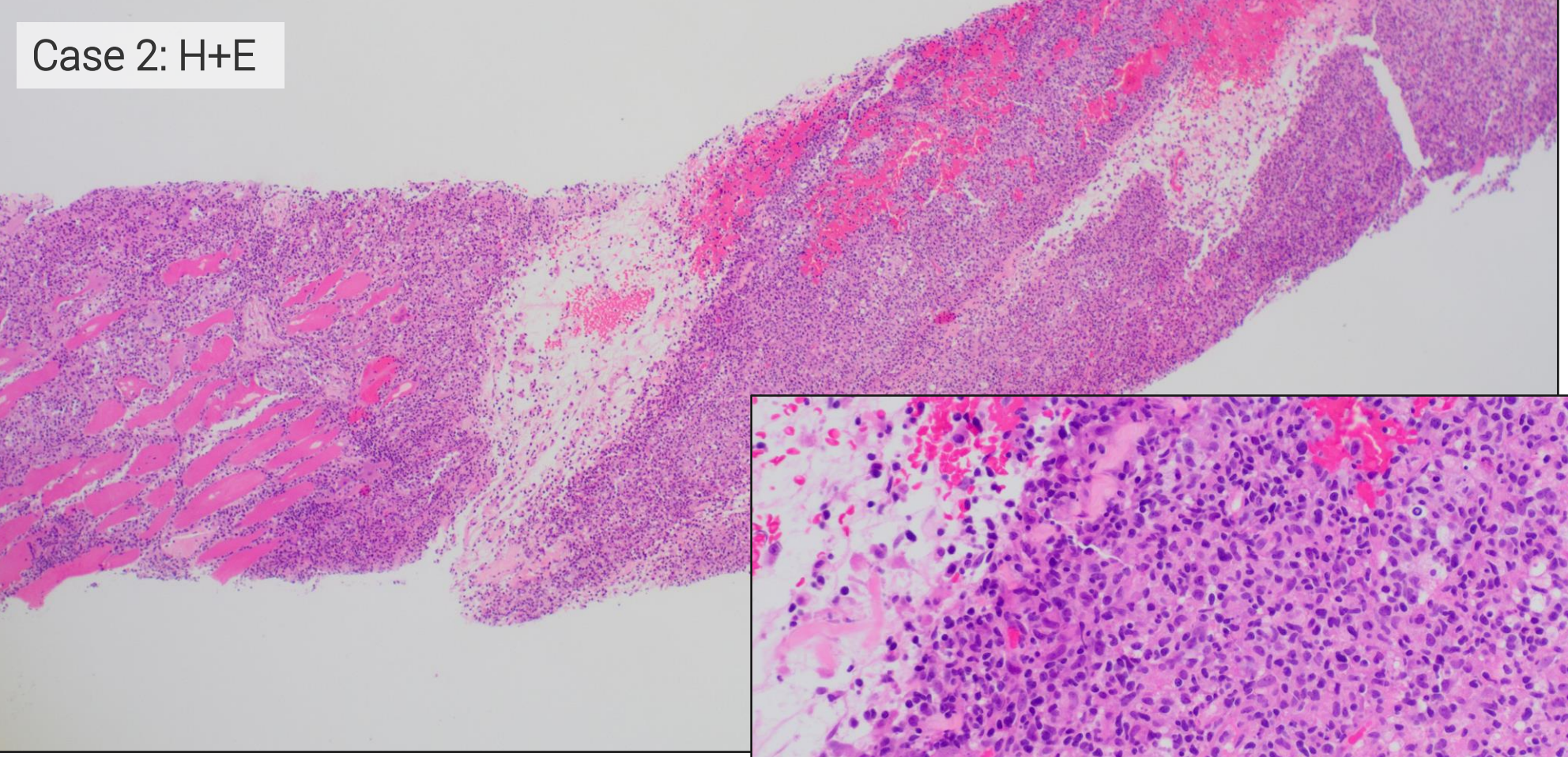
- Diffuse red pulp pattern fits this entity, even if CD5 and CD11c expression is somewhat unusual
- Differential would include a splenic marginal zone lymphoma, but the diffuse pattern doesn't fit well with this

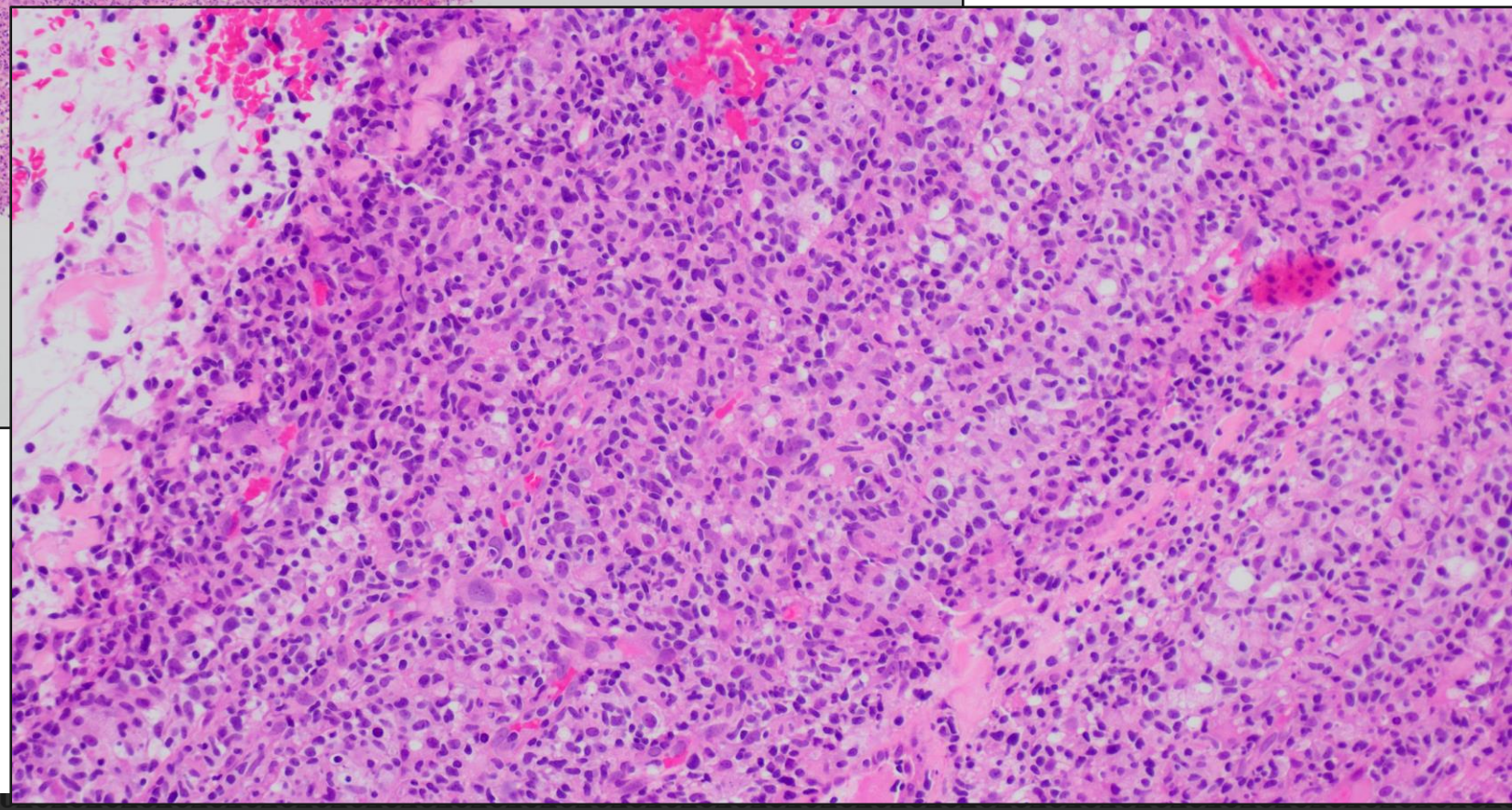
Case 2

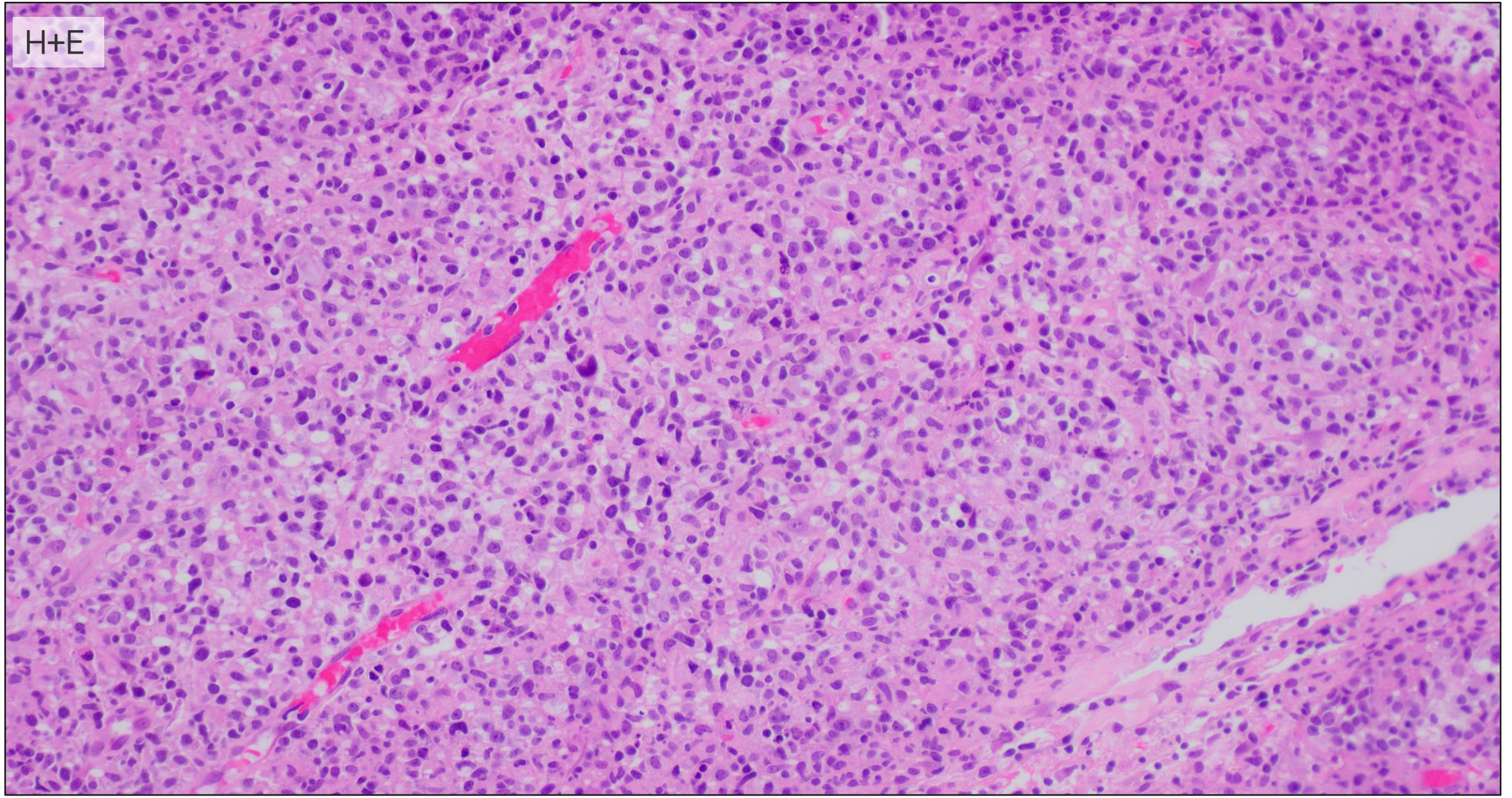
Case 2:

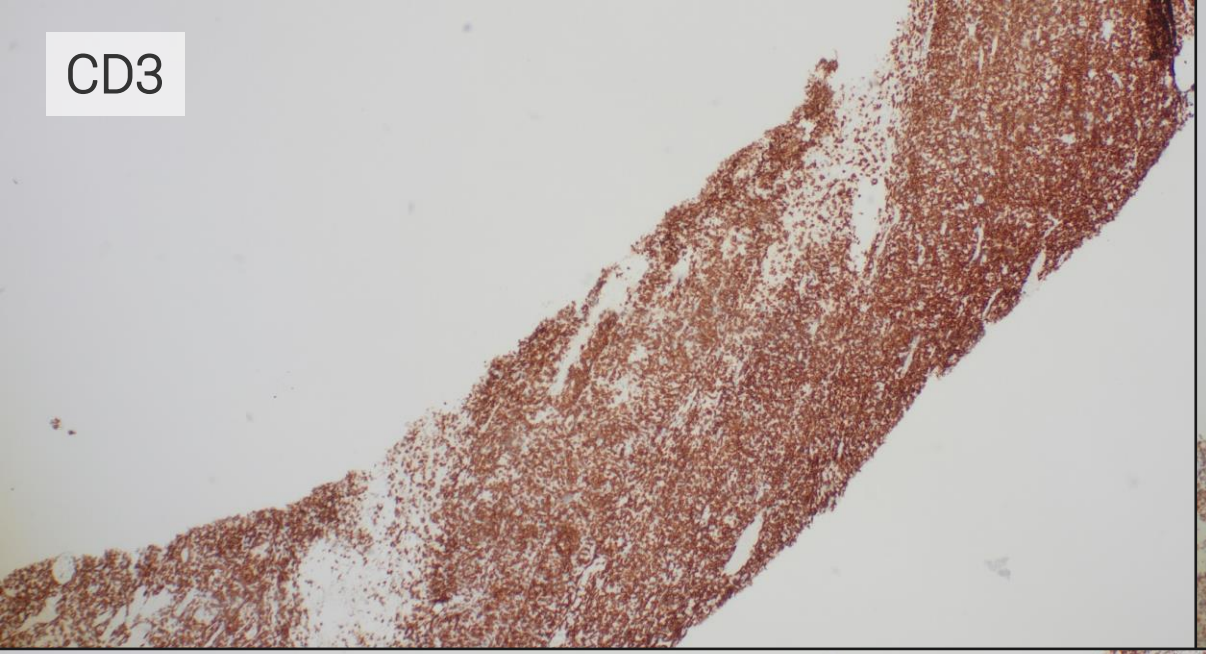
- 24-year-old man presented with a right chest lump.
- PET CT showed a hypermetabolic, ill-defined, soft tissue mass involving the pectoralis major muscle and measuring 6 cm
- No other foci of uptake on PET, no clinically described lesion in overlying skin

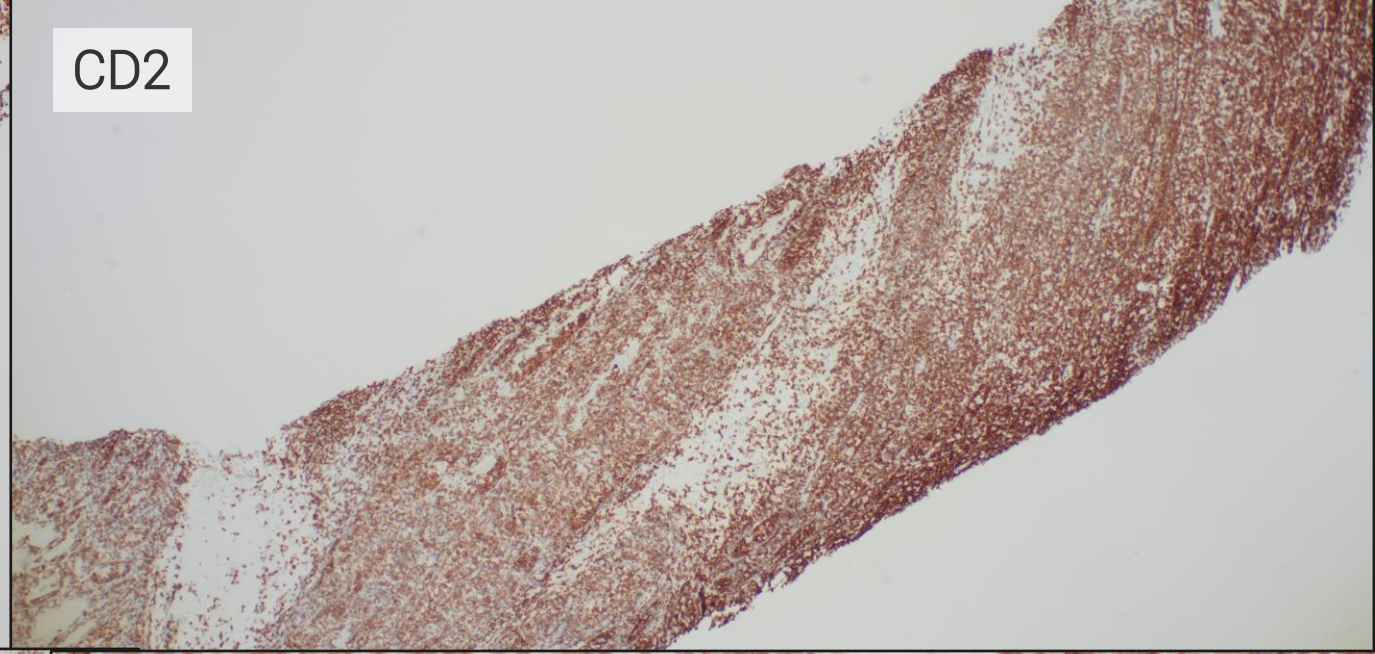
Case 2: H+E

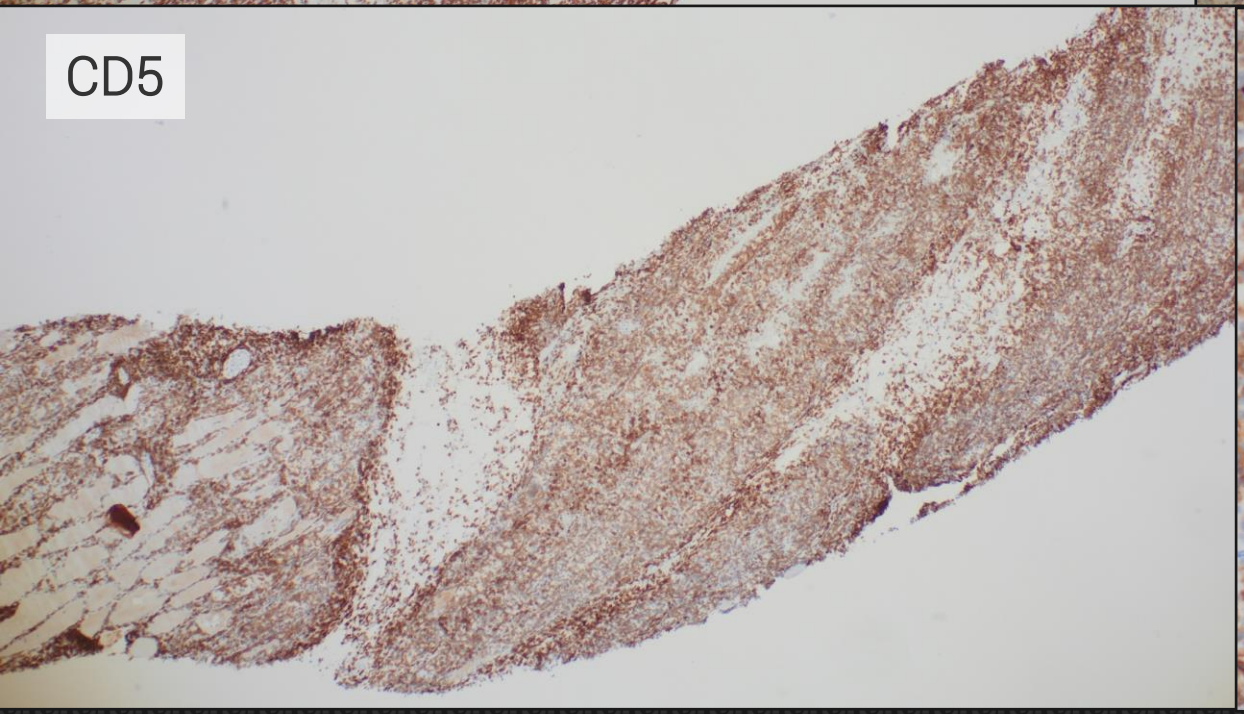


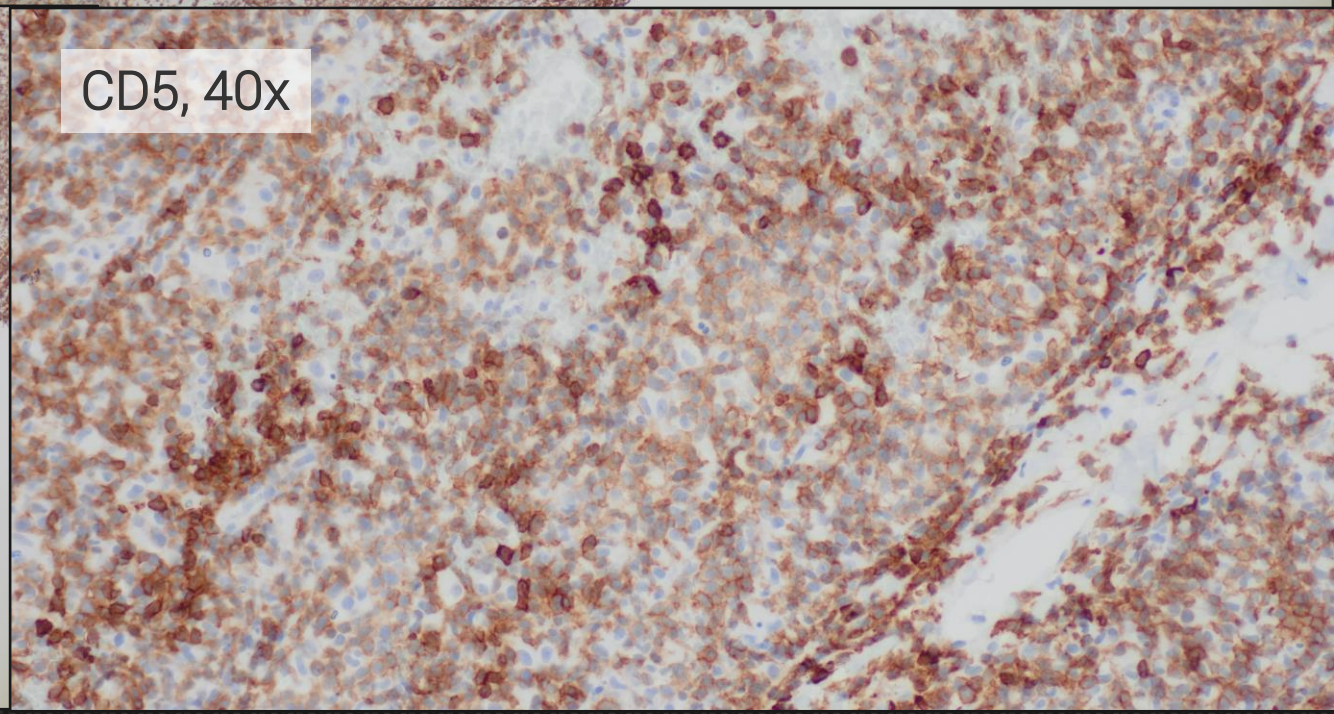


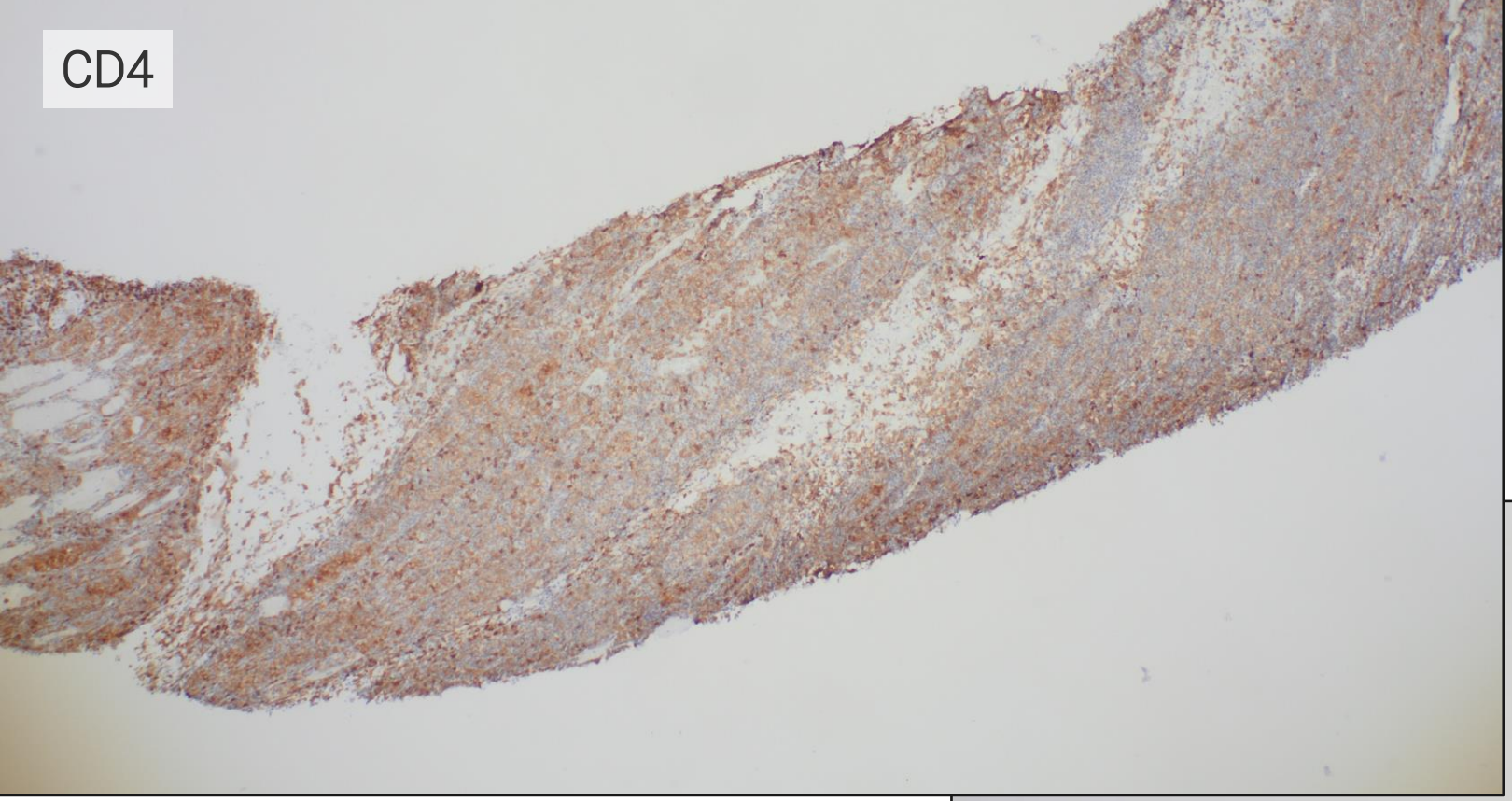


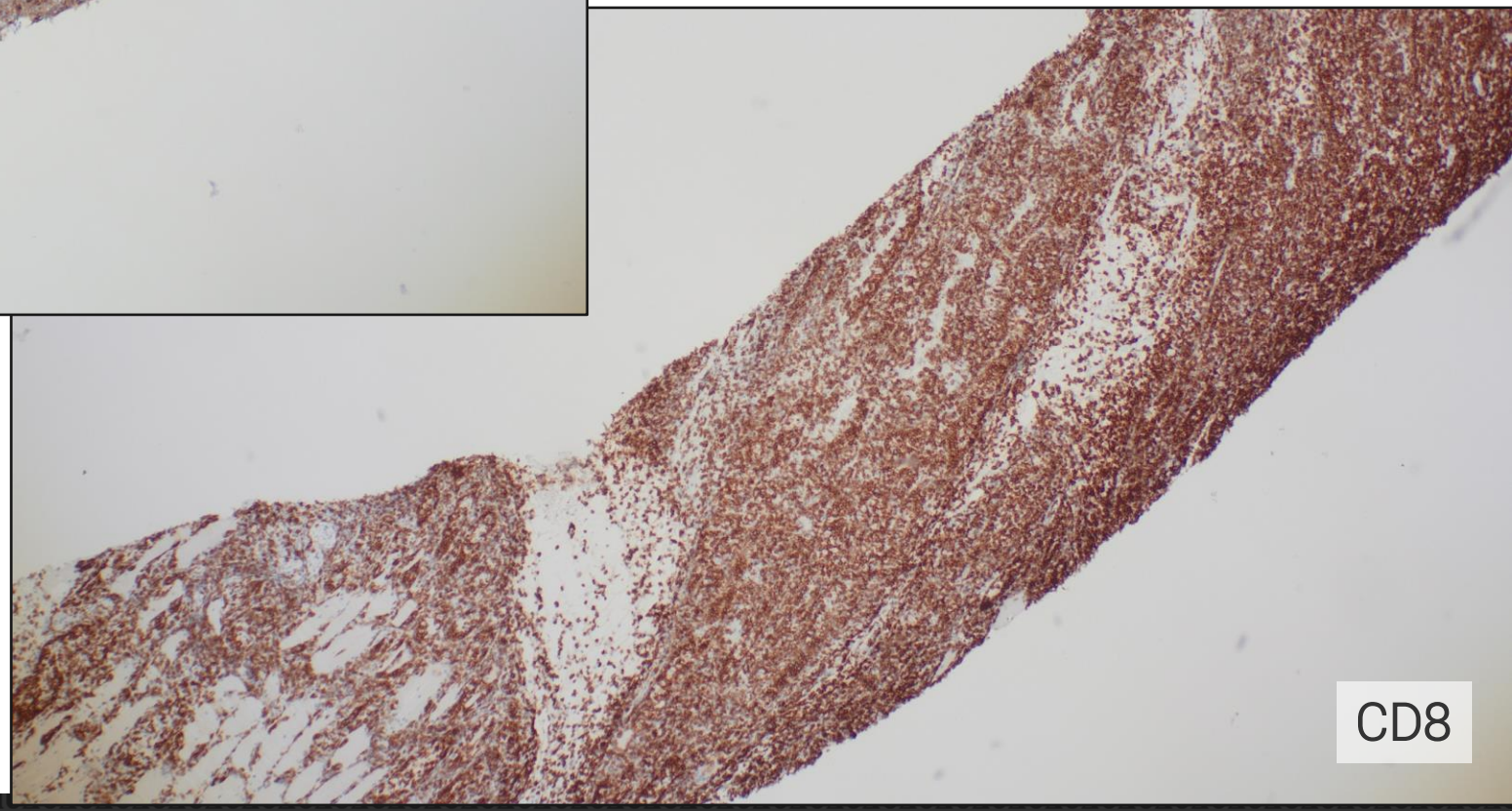


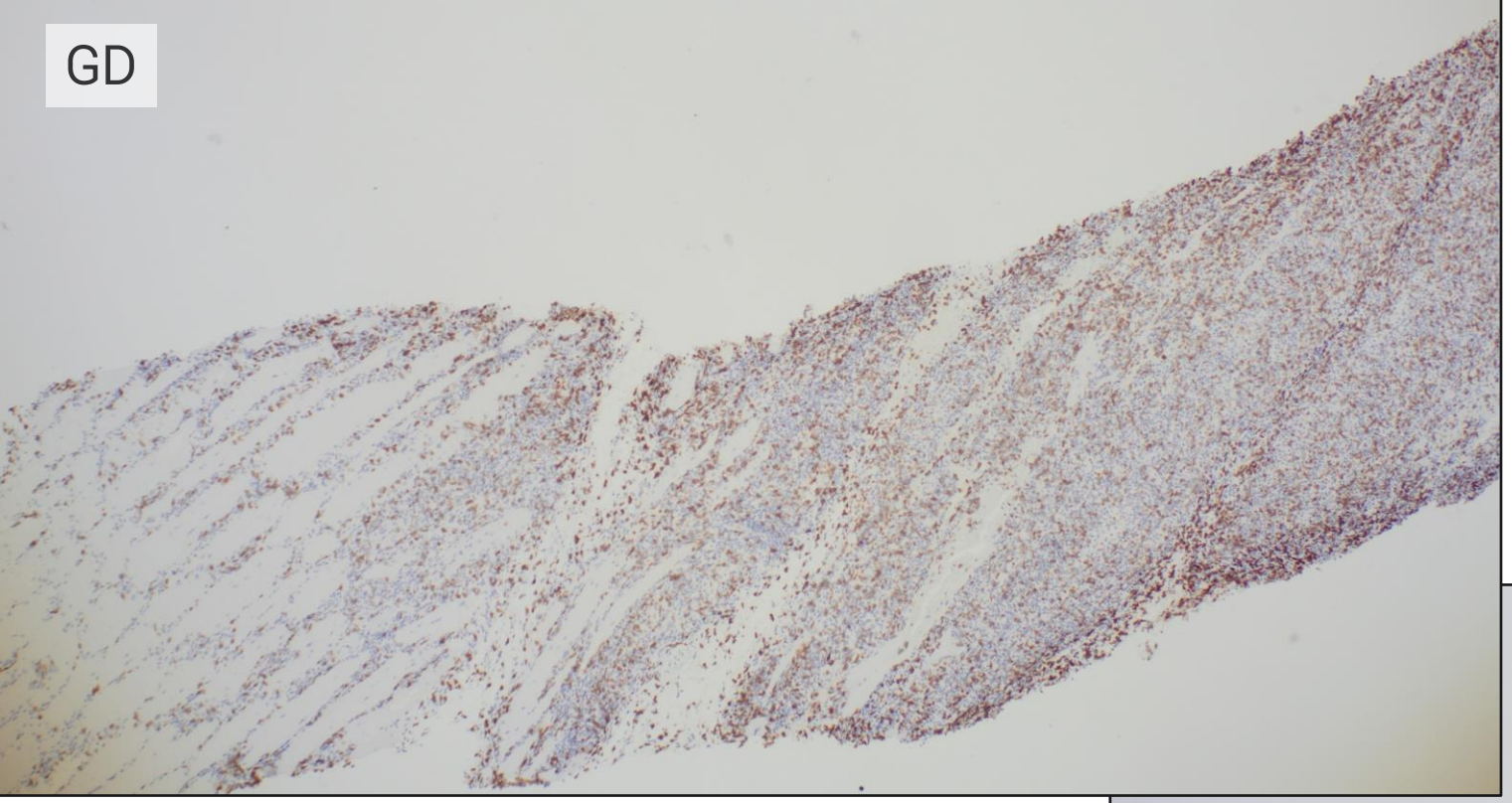


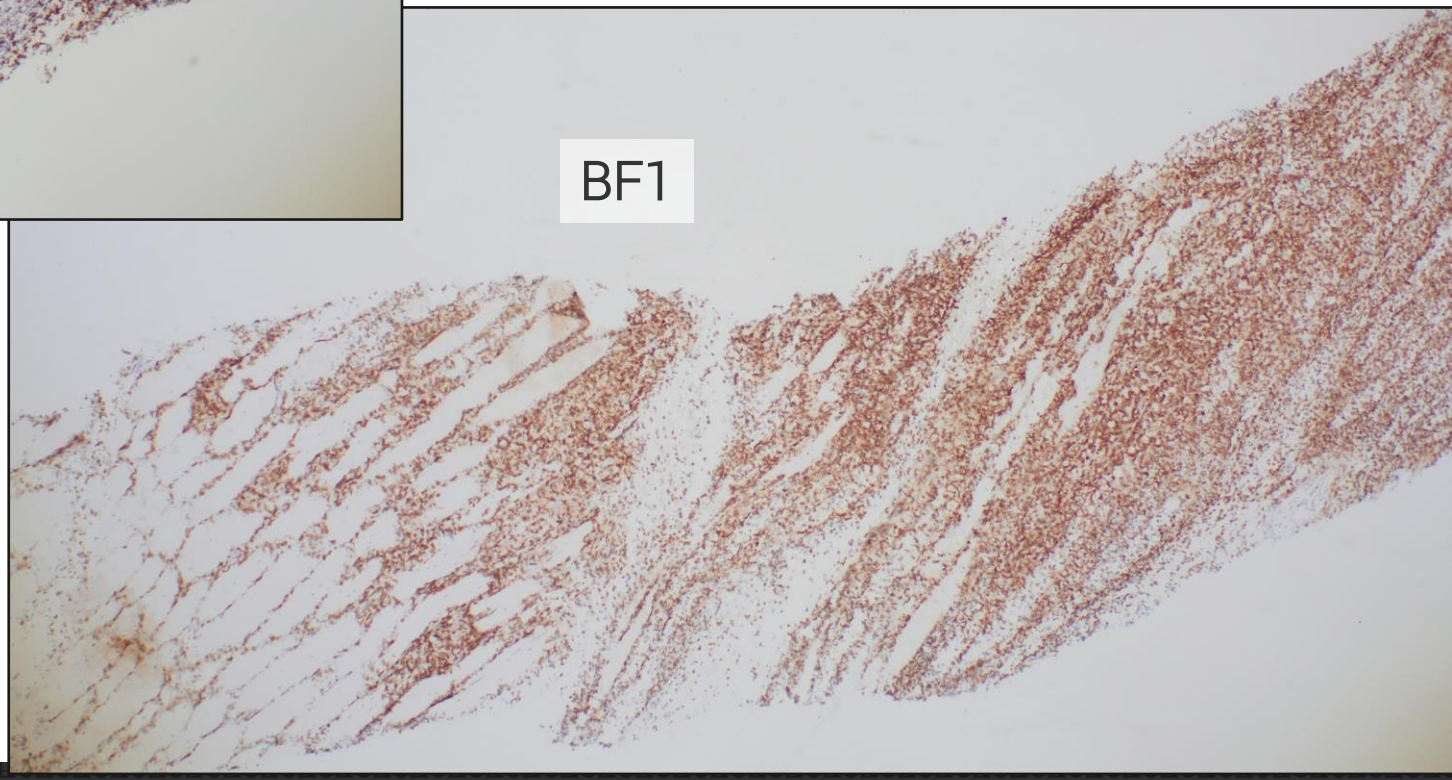






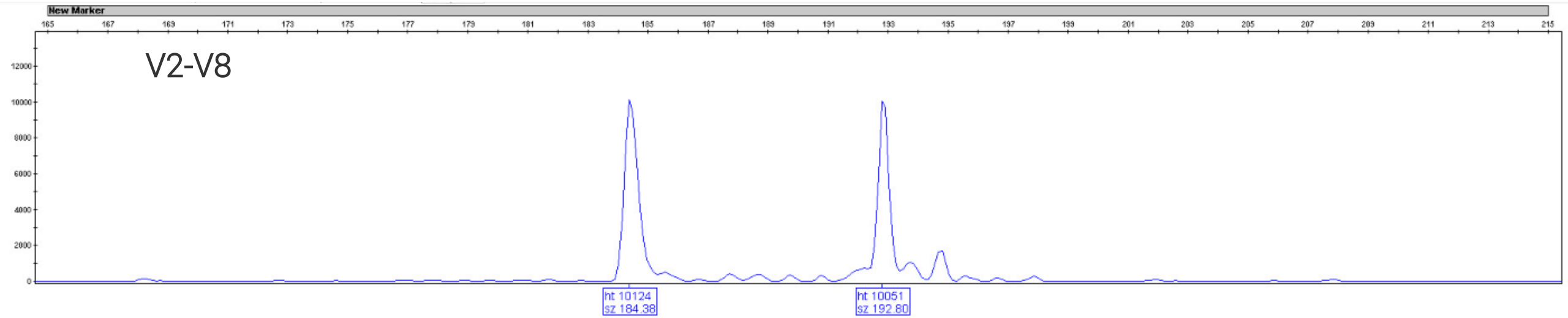


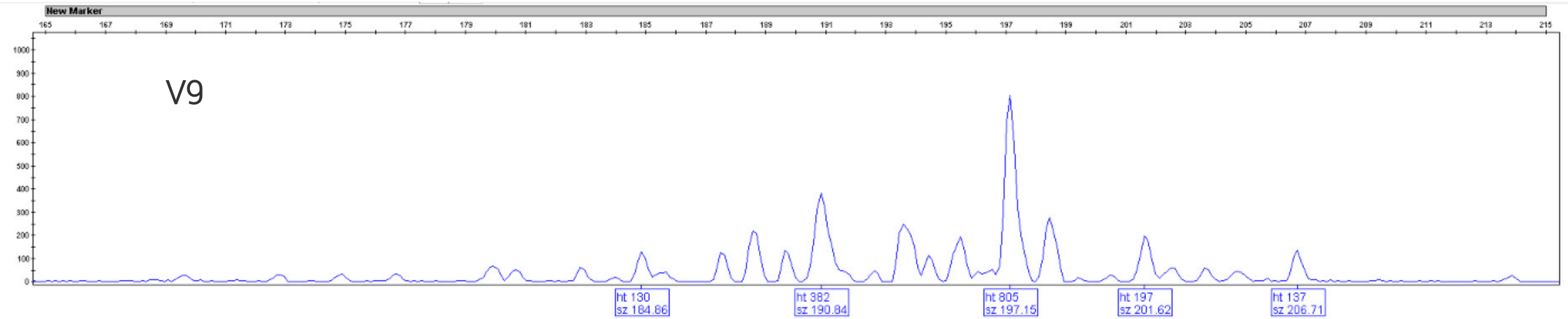


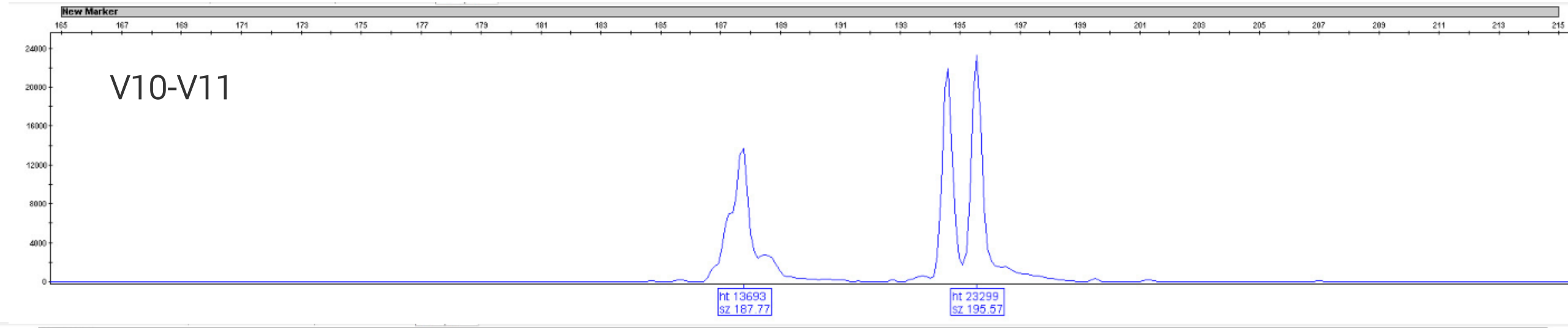


Case 2: T-cell Receptor Gamma Gene Rearrangement

Adapted from van Dongen et al 2003



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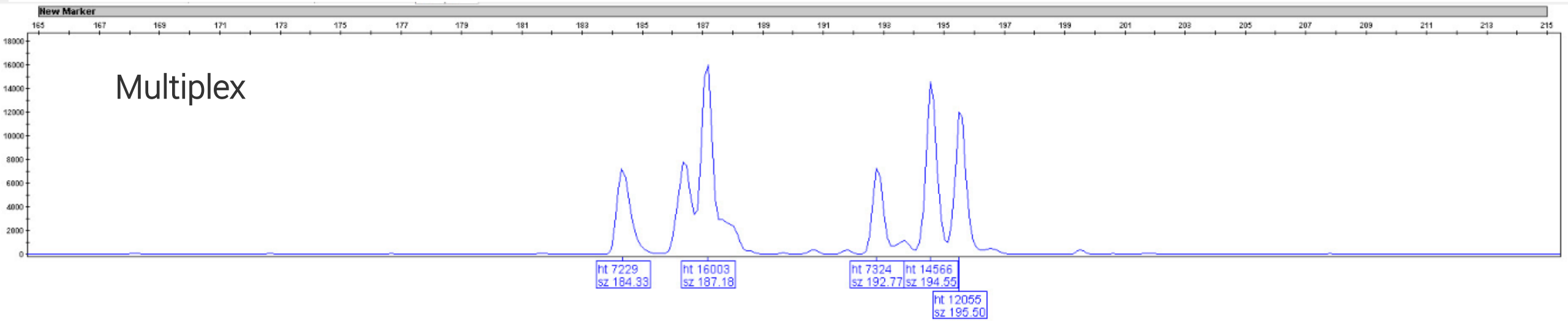
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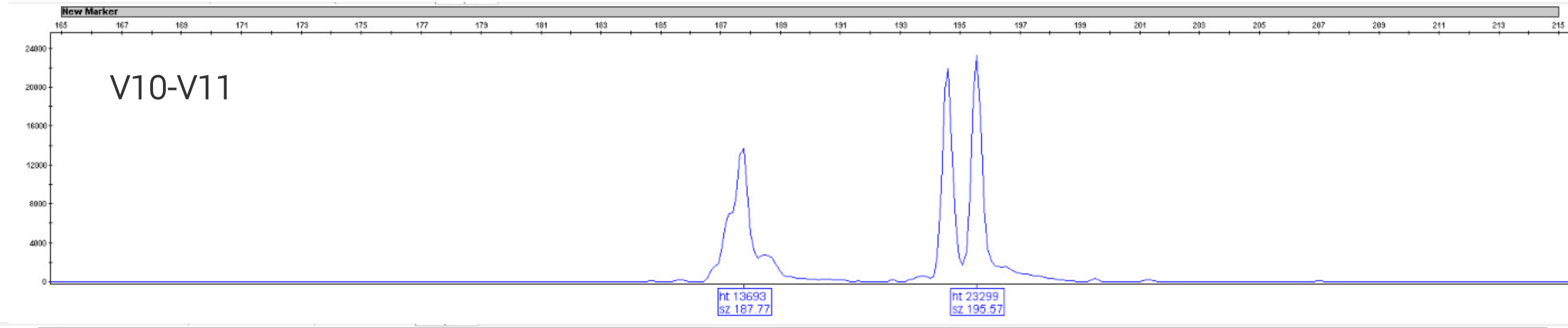
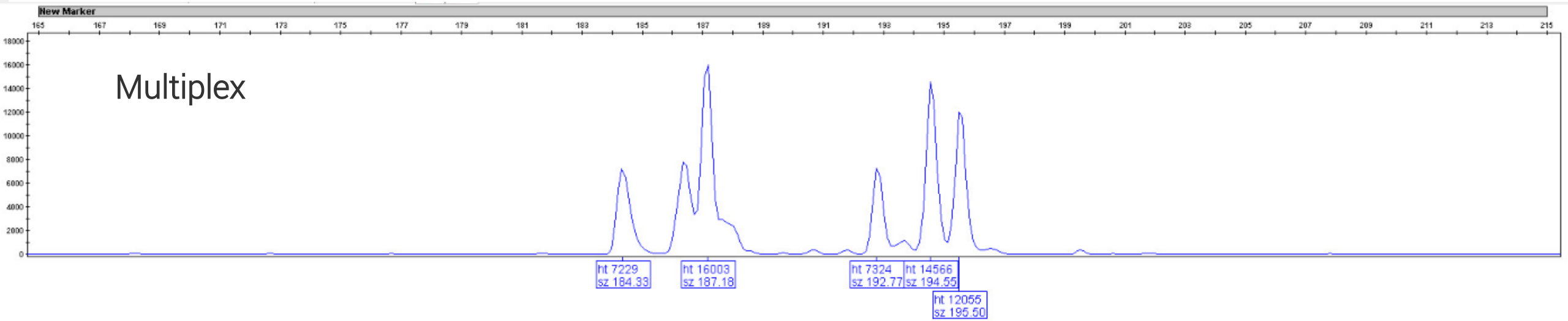
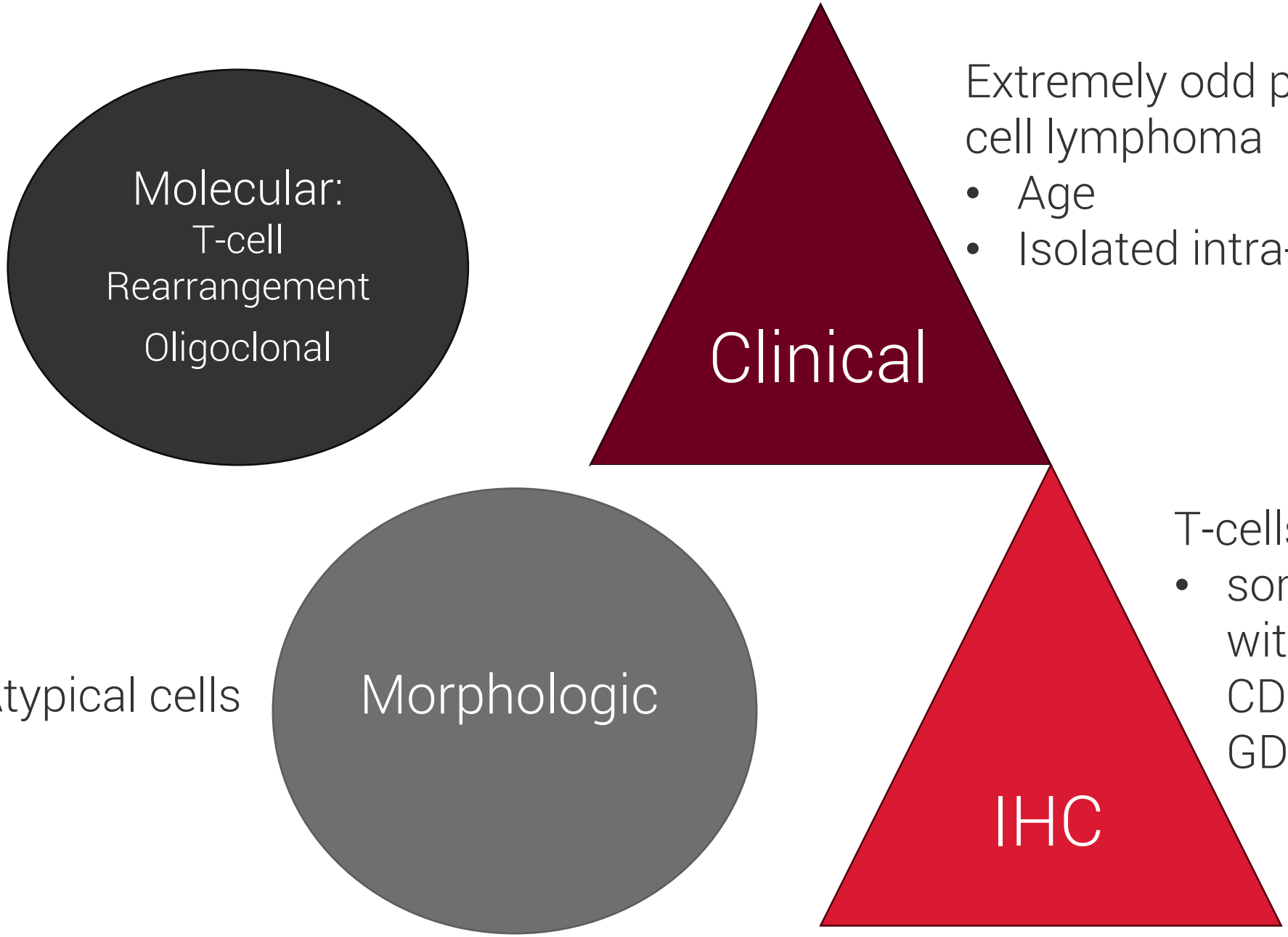
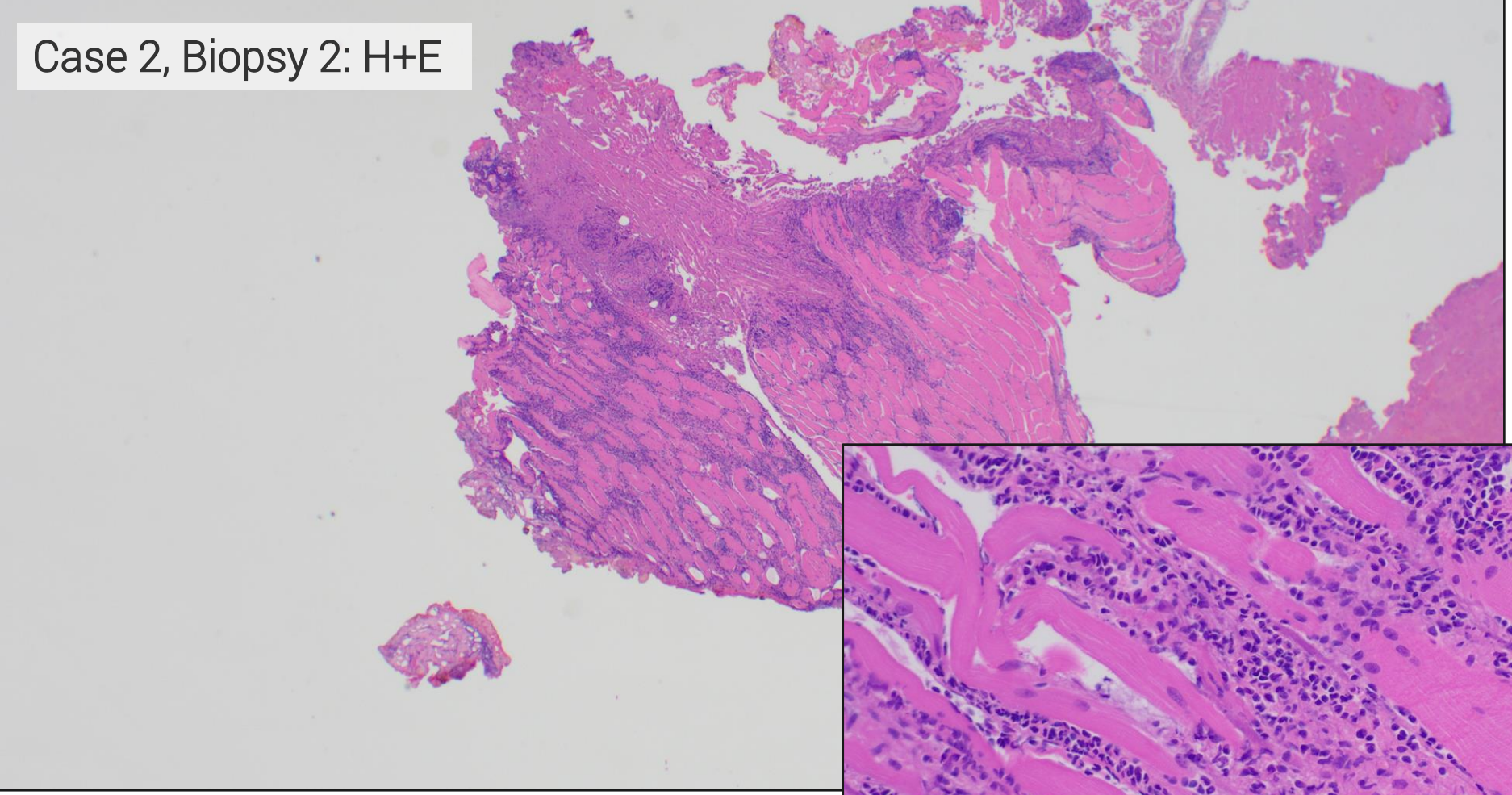


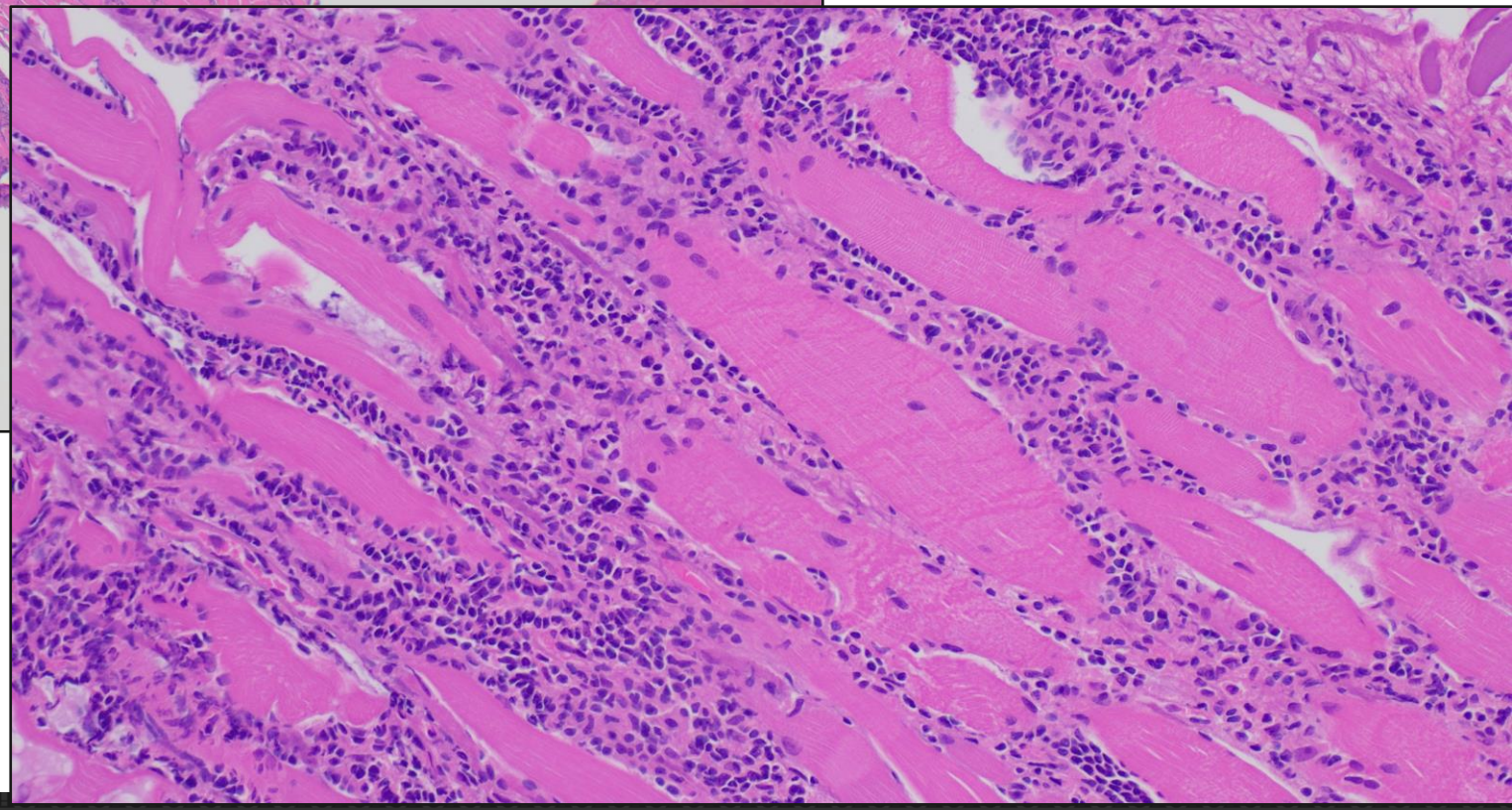


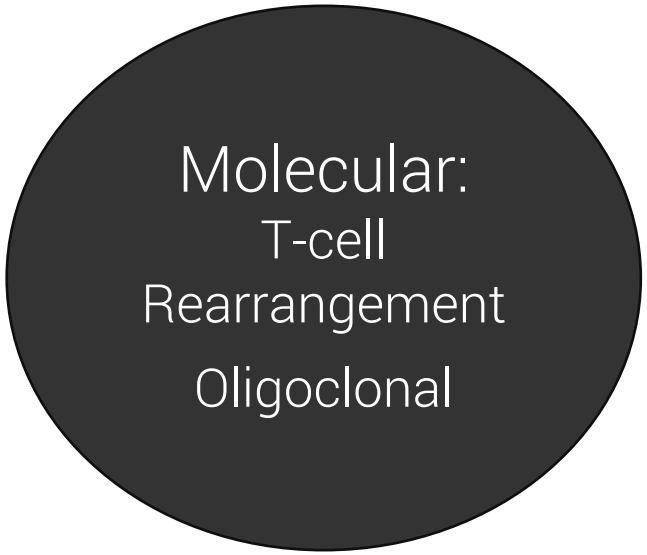
Oligoclonal pattern: 3 or more peaks that meet criteria for clonality

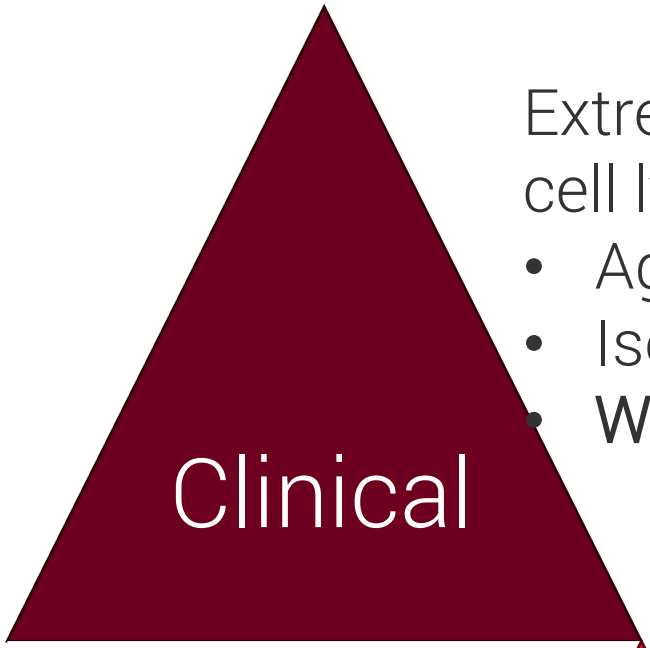


Case 2, Biopsy 2: H+E





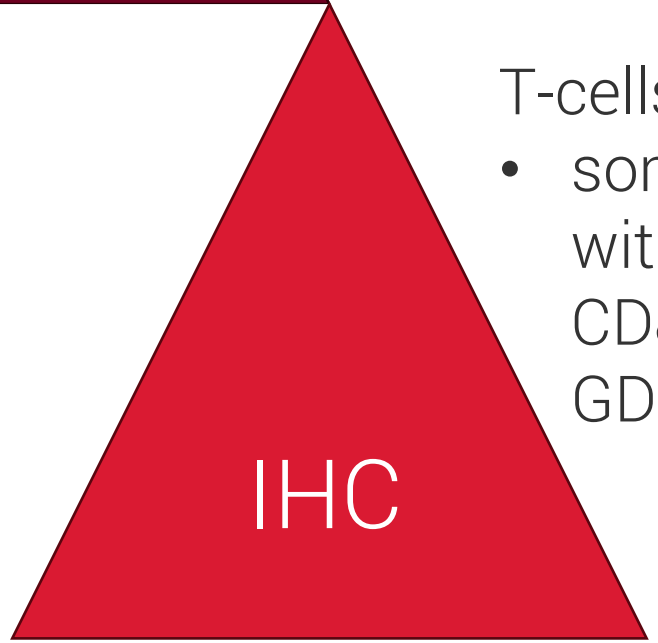




Extremely odd presentation for a T-cell lymphoma

- Age
- Isolated intra-muscular mass
- **With partial regression?**

Atypical cells



T-cells

- somewhat heterogenous with partial CD4, partial CD8, partial BF1, partial GD

Discordant Clonality Results

Scenarios with a Detectable Clone

- True Positive:
  - » Malignant clonal lymphoid population
- False Positive:
  - » Clonal population in a clinically benign condition
  - » Lineage Infidelity/Promiscuity
  - » Pseudoclone

Scenarios with an Undetectable Clone

- True Negative:
  - » Polyclonal lymphoid population
- False Negative:
  - » Malignant clonal population with somatic hypermutation affecting primer annealing
  - » Malignant cells below the level of detection
  - » Rare rearrangements not covered
- Other Sources of Failure:
  - » Failure of amplification

Clonal Populations in Benign Conditions

- Lymphoid populations in clinically benign or reactive conditions can show detectable, reproducible B and/or T cell clones:
  - » One study showed 10% of cases called reactive by morphology had detectable clones and 15% were oligoclonal
    - 2 of these on re-review actually showed partial involvement by Mycosis Fungoides or Marginal Zone Lymphoma
    - Tissue mostly comprised of germinal center cells
    - Reactive populations in skin, lymph node, or spleen

Lineage Infidelity/Promiscuity

- VDJ recombination can occur in lymphoid progenitors prior to lineage commitment
- Most commonly identified in immature lymphoid neoplasms
  - » TCR rearrangements in 1/3 of B-ALL patients
  - » IGH/IGK rearrangements in 10-15% of T-ALL patients
  - » Can also be detected in some myeloid and histiocytic neoplasms

Secondary Clonal Population

- In mature lymphoid neoplasms, a cross-lineage clonal population is more likely to be a secondary clone rather than true lineage infidelity
- Common example: angioimmunoblastic T-cell lymphoma where the clonal B-cell population usually reflects EBV-infected B-cells but doesn't define a second malignancy

Pseudoclone

- Cases with a too few B or T cells can produce pseudoclonal bands
  - » These bands should be non-reproducible on repeat testing
  - » Pitfall is mitigated by performing reactions in duplicate up front
- For B-cell clonality, recommended about >7,000 cells with >10% B-cells

Discordant Clonality Results

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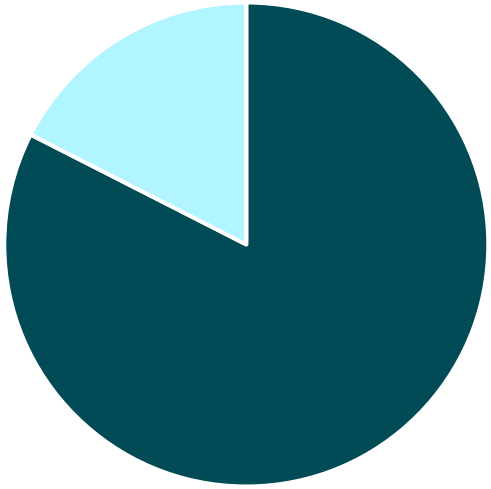
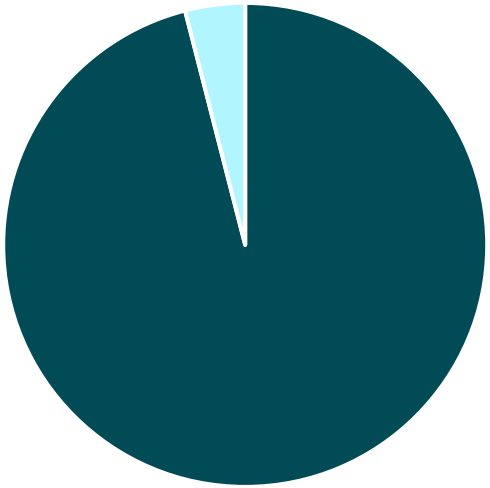
Somatic Hypermutation

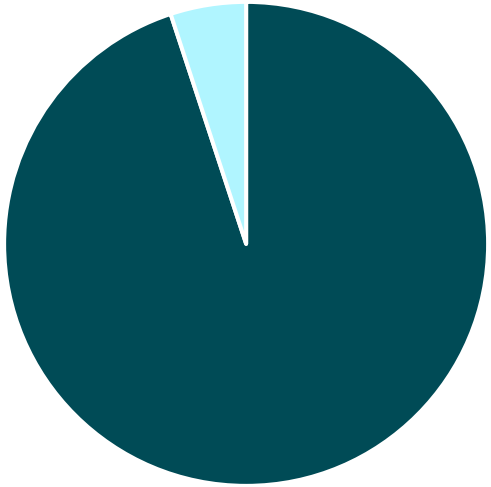
Somatic hypermutation affects the entire length of the VDJ segment, including our primer sites



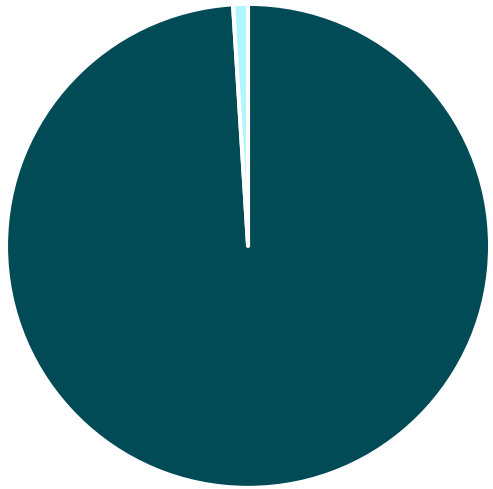
80-85% FL and DLBCL detected by IGH or IGK alone



96% FL and DLBCL detected by either IGH or IGK



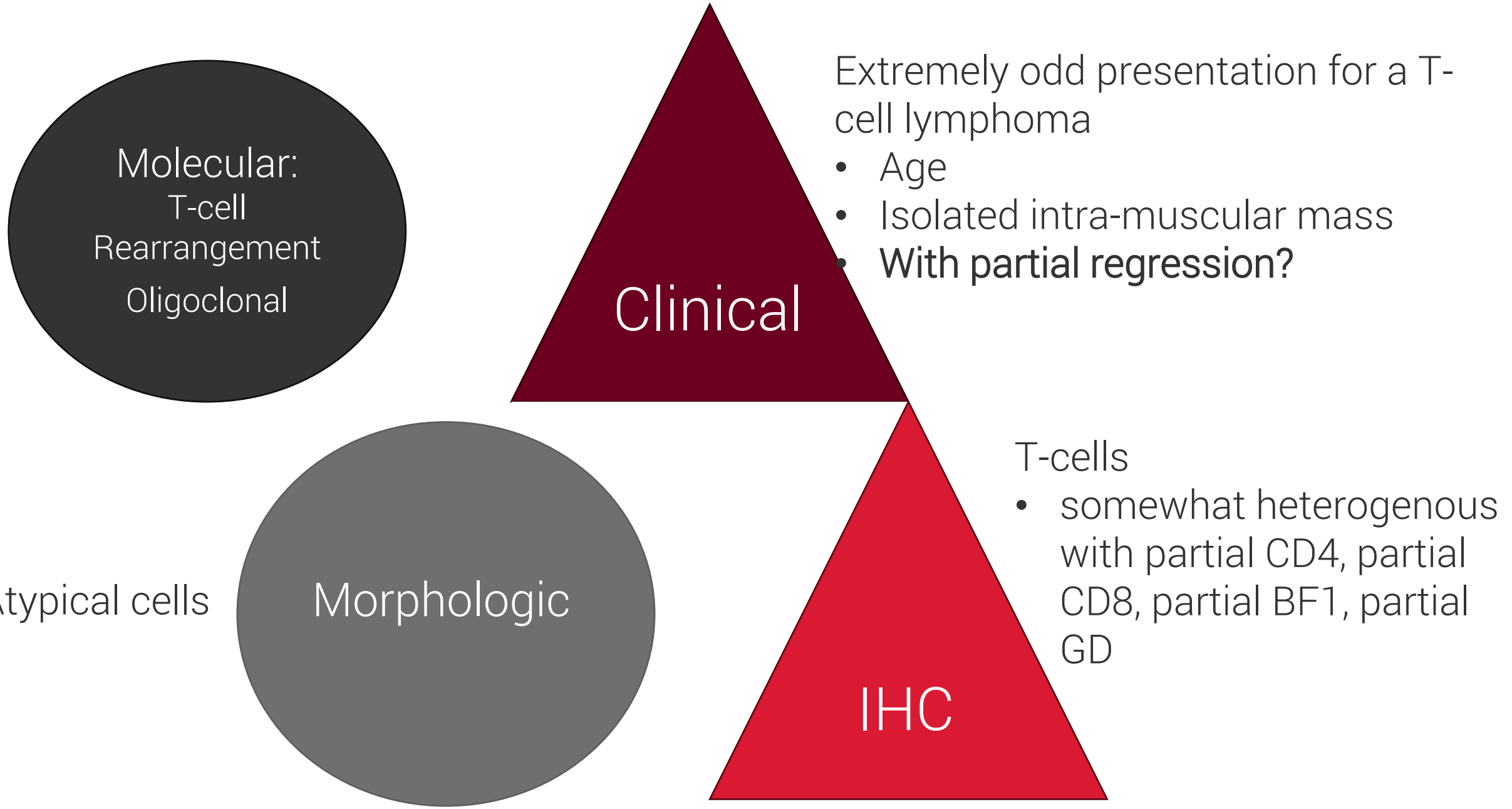
94% extranodal MZL detected by either IGH or IGK



100% MCL, CLL/SLL, nodal MZL detected by either IGH or IGK

Few Neoplastic Cells within Reactive Background

- Relatively low rates of B-cell clone detection in:
  - » Hodgkin Lymphomas (~25-50% CHL, 0% in NLPHL)
  - » T-cell Histiocyte Rich Large B-cell Lymphoma



Case 2: Final Diagnosis

Atypical CD8+ T-cell infiltrate, see comment.

- Morphologic atypia and extent of the CD8+ T-cell infiltrate raise concern for a T-cell lymphoproliferative disorder
- Oligoclonal by T-cell gene rearrangement studies
- BUT
- Age of the patient
- Presentation as an isolated intramuscular mass
- Lack definitive phenotypic atypia (no loss of pan-T-cell antigen expression)
- More limited in extent on 2nd biopsy

Case 2: Clinical Follow-up

- Repeat imaging 2 months later showed that the mass was significantly decreased in size with only focal uptake on PET CT
- Patient is clinically followed for any recurrent mass or lymphadenopathy

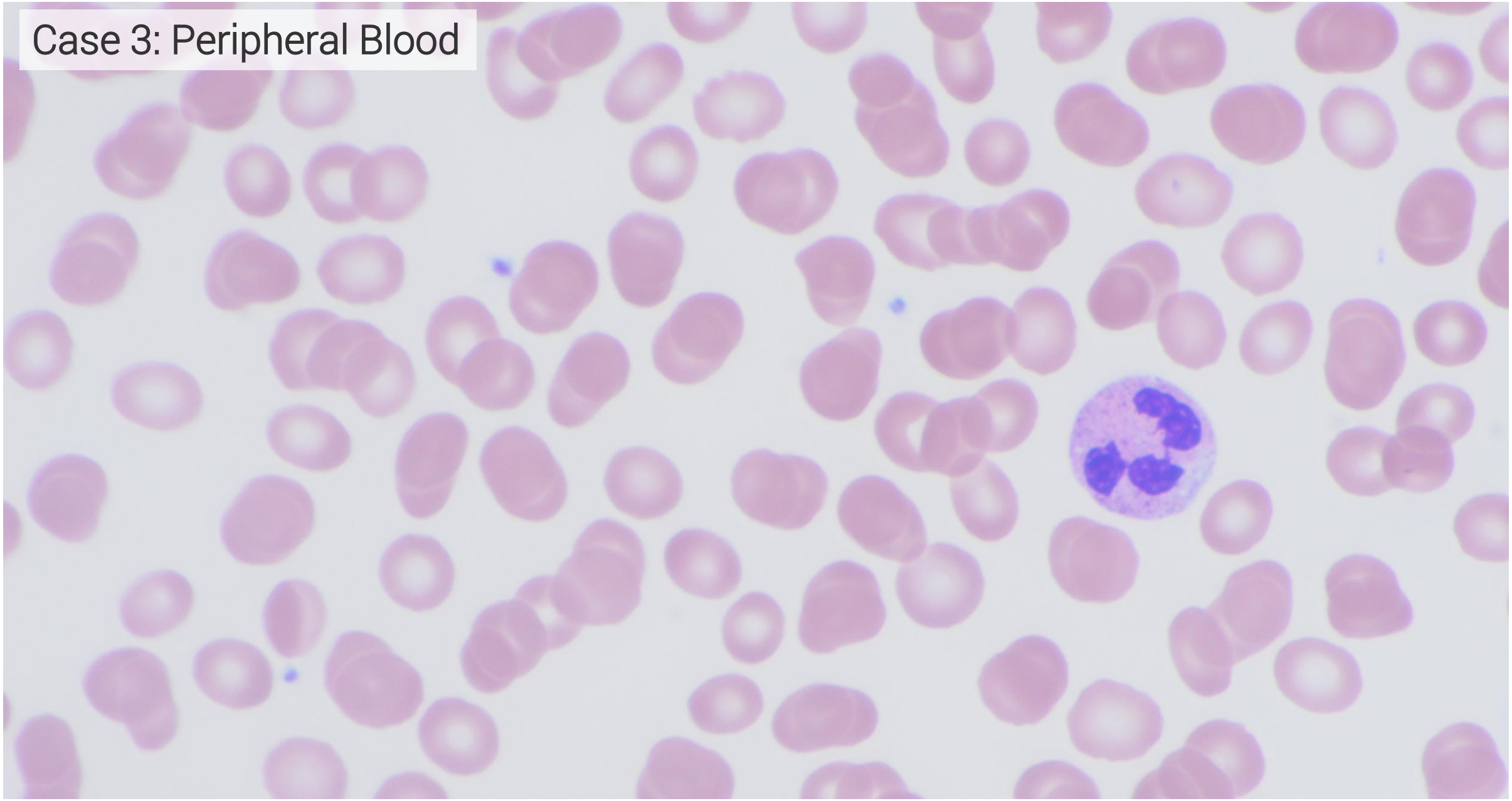
Case 3

Case 3:

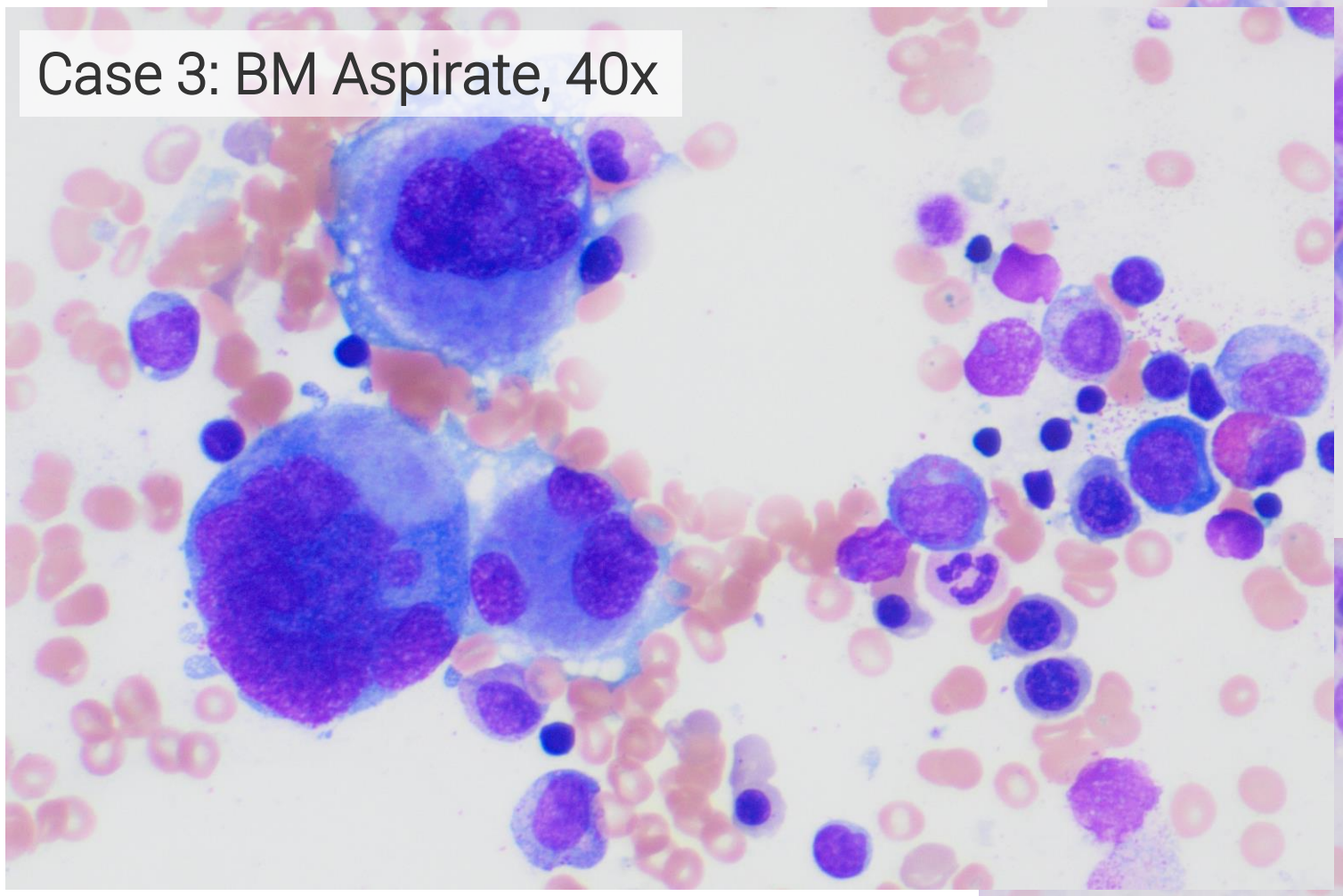
- 25-year-old female with a history of adrenal cortical carcinoma, previously treated with chemotherapy, who presents for bone marrow evaluation of persistent thrombocytopenia

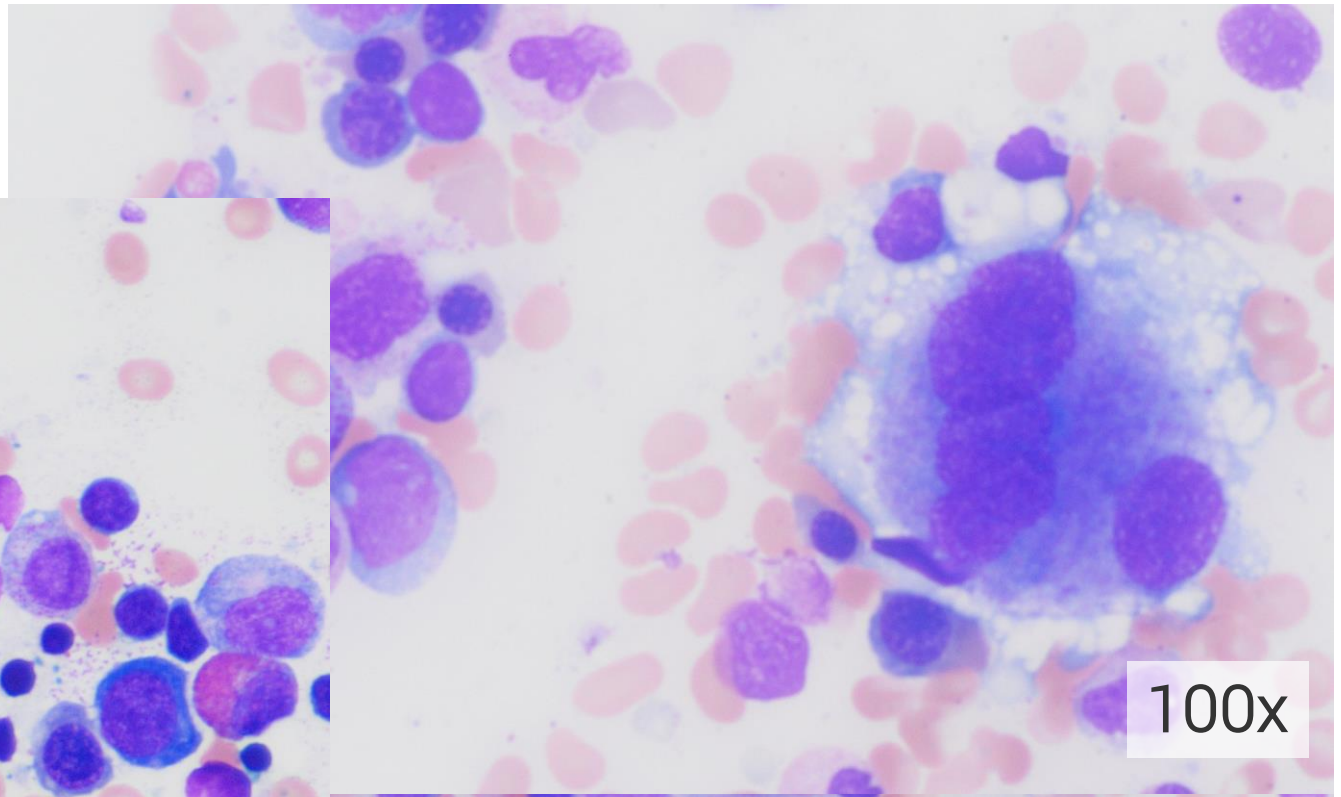
CBC Data	
Hemoglobin (g/dL)	13.4
Hematocrit (%)	38.7
MCV (fL)	93.7
MCHC (g/dL)	34.6
WBC (k/uL)	6.28
ANC (k/uL)	3.7
Platelet Count (k/uL)	82 (↓)
MPV (fL)	11.8

Case 3: Peripheral Blood

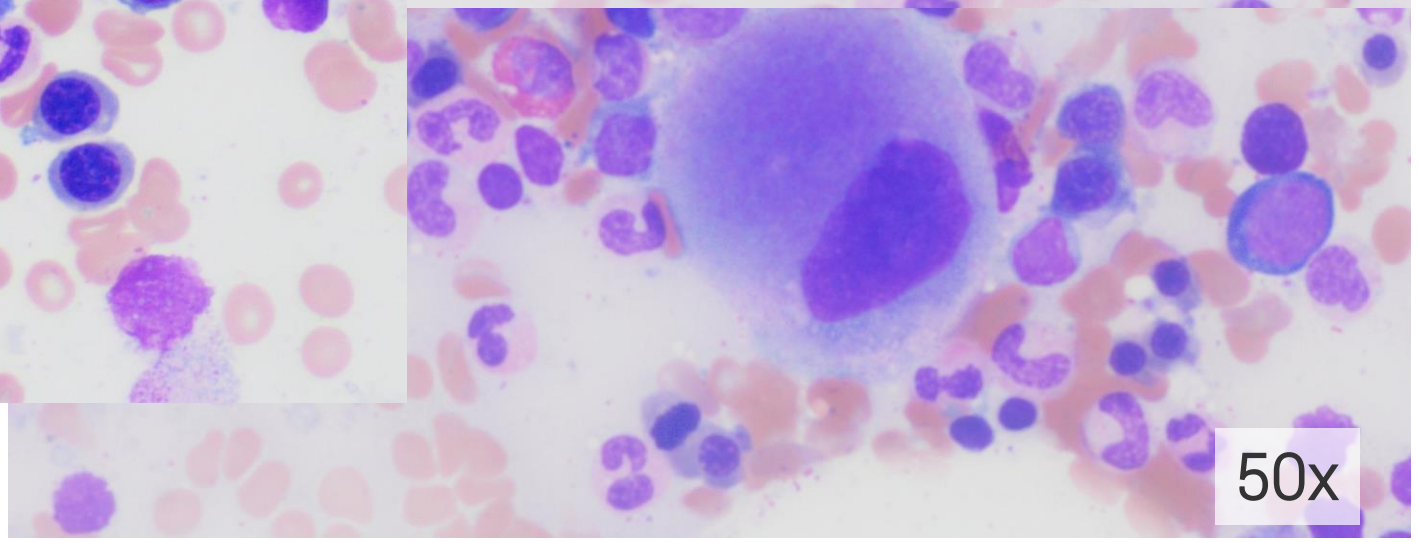


Case 3: BM Aspirate, 40x



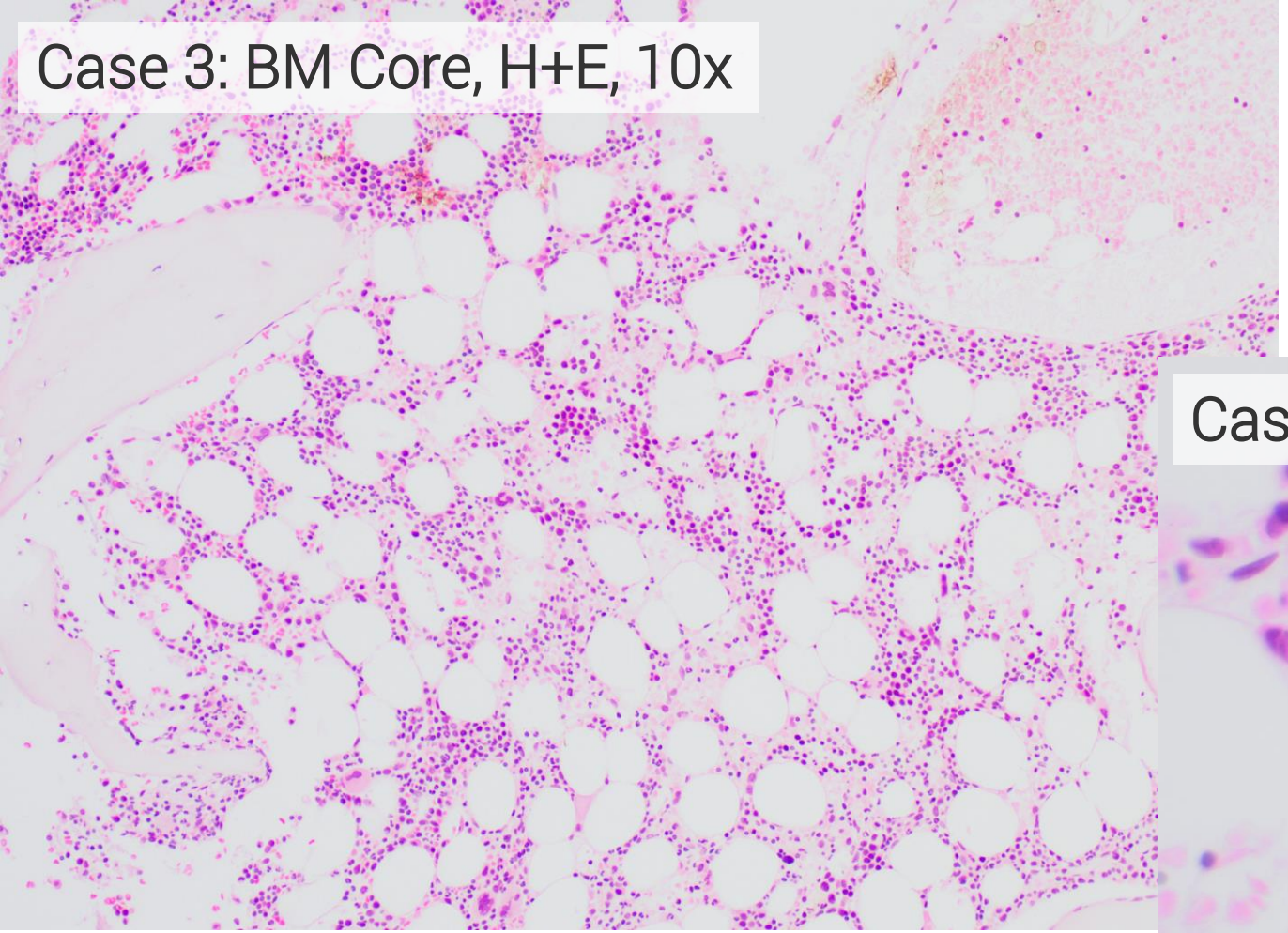


100x

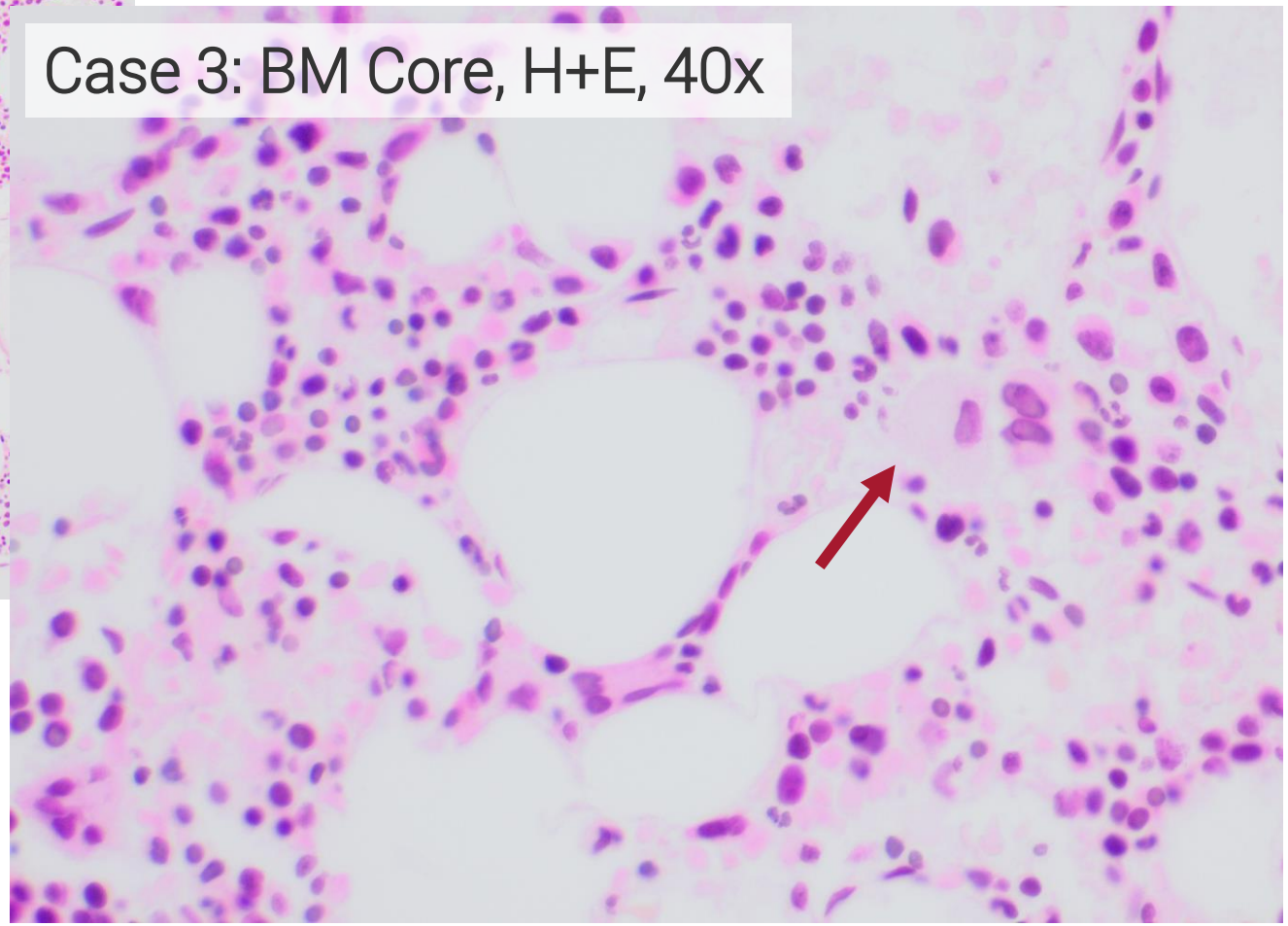


50x

Case 3: BM Core, H+E, 10x

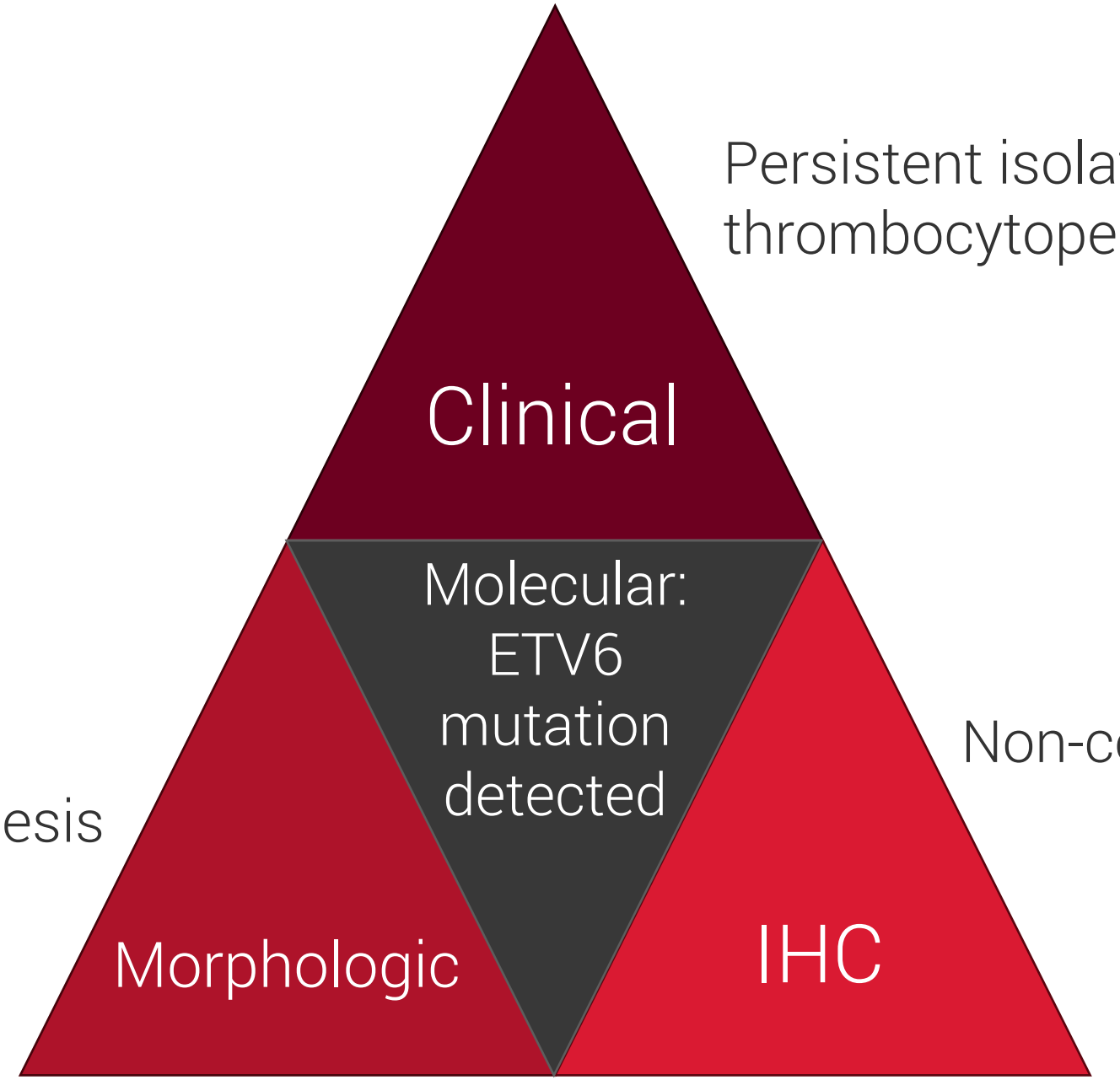


Case 3: BM Core, H+E, 40x



Case 3:

- Flow Cytometry:
  - » No abnormal population identified.
- Cytogenetics:
  - » Normal Female Karyotype: 46,XX[20]
- Myeloid Mutation Panel by NGS:
  - » One tier 1 mutation detected in *ETV6,* c.1195C>T, p.Arg399Cys



Persistent isolated thrombocytopenia

Mild dysmegakaryopoiesis

Non-contributory

Molecular:
ETV6 mutation detected

Young patient with a solid tumor and persistent isolated thrombocytopenia

Mild dysmegakaryopoiesis

Non-contributory

When does a clonal finding + persistent cytopenia + dysplasia not equal a myeloid malignancy?

Rule out everything else…

- Clonal hematopoiesis of indeterminate potential + nutritional deficiency, autoimmune disease, infection, drugs, other systemic illnesses

Rule out everything else…

- Clonal hematopoiesis of indeterminate potential + nutritional deficiency, autoimmune disease, infection, drugs, other systemic illnesses
- Germline syndromes

Germline Predisposition to Myeloid Neoplasms

Germline predisposition with platelet disorder	Germline predisposition with other organ systems affected	Germline predisposition without platelet disorder or other organ
• RUNX1 • ANKRD • ETV6		

How I diagnose myeloid neoplasms with germline predisposition

Nisha Patel, DO,[1] and Katherine R. Calvo, MD, PhD[1,2]

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WHO

ICCS

syndrome-like disorders
- Neurofibromatosis
- CBL syndrome**

Germline Predisposition to Myeloid Neoplasms

Germline predisposition with platelet disorder	Germline predisposition with other organ systems affected	Germline predisposition without platelet disorder or other organ systems affected

• RUNX1
• ANKRD26
• ETV6
	• GATA2
	• SAMD9/SAMD9L
	• Bone Marrow Failure syndromes:
	  • Fanconi Anemia
	  • Shwachman-Diamond Syndrome
	  • Severe Congenital Neutropenia
	  • Diamond-Blackfan Anemia*
	  • Telomere Biology Disorders
	• Down Syndrome
	• Rasopathies:
	  • Noonan Syndrome, Noonan syndrome-like disorders
	  • Neurofibromatosis
	  • CBL syndrome**
		• CEBPA
		• DDX41
		• TP53

* Included in ICCS but not WHO

** Included in WHO but not ICCS

Germline Predisposition to Myeloid Neoplasms with Platelet Disorders

Predisposition Syndrome	Preceding Findings All have mild to moderate thrombocytopenia	Other Characteristics All autosomal dominant inheritance
RUNX1	• Mild to moderate bleeding tendency	• 40% lifetime risk of hematologic neoplasm
ANKRD26	• Normal to mild bleeding tendency	• Most common • ~10% prevalence myeloid neoplasm
ETV6	• Mild to moderate bleeding symptoms	• 50% risk of hematologic malignancy, 20% of those are B-ALL • Risk of other non-heme malignancies: colon, duodenal, breast, renal cell carcinomas, meningiomas, skin cancers

Germline Predisposition to Myeloid Neoplasms with Platelet Disorders

- Category of germline disorders can show thrombocytopenia and baseline dysmegakaryopoiesis
  - » not considered indicative of a myeloid neoplasm especially when there are no other cytopenias, dysplasias, and normal karyotype
- These patients are important to identify:
  - » as NOT having MDS, or other platelet disorder (ITP)
  - » surveillance for risk of development of a myeloid neoplasm, identification of other family members, and for transplant implications

Case 3: Final Diagnosis

- *ETV6* c.1195C>T, p.Arg399Cys, 43.6% variant allele fraction
  - » Had been reported previously both as a probable somatic and as a germline finding

- *ETV6* confirmed as germline mutation by skin biopsy
- No evidence of myeloid malignancy
- Undergoing surveillance for myeloid malignancy and additional affected family members identified

Agenda

Challenge Cases

Can we develop an approach to unexpected ancillary findings?

Approach

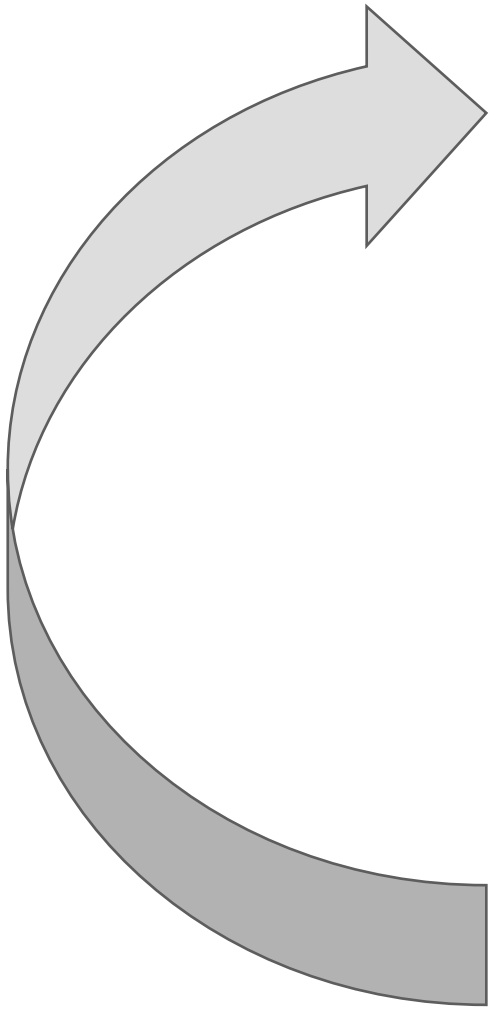
- The process of trouble shooting an unexpected result starts before you order the test.
  - » Case 1: t(11;14)

Approach

When ordering ancillary testing, consider:

- » Pre-analytic factors
- » Analytic factors
- » Post-analytic factors

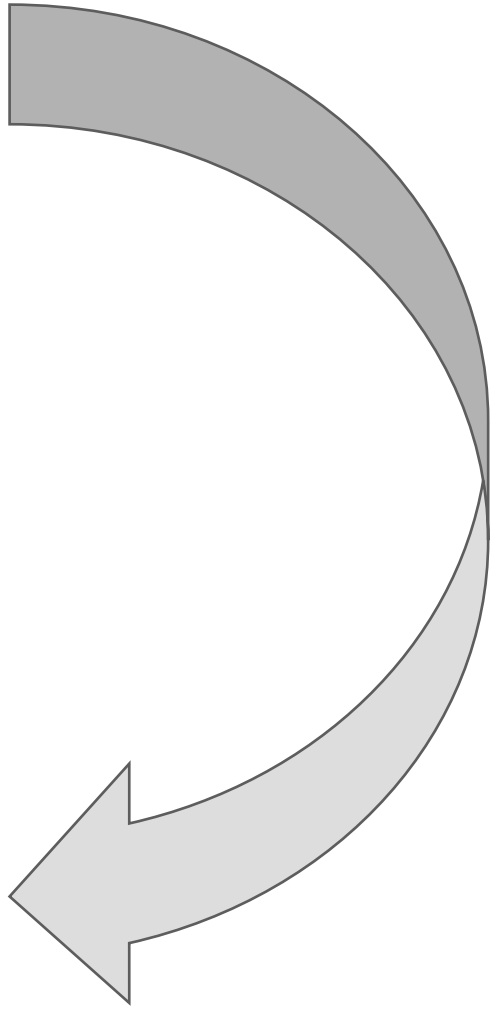
Approach



When ordering ancillary testing, consider:

- » Pre-analytic factors
- » Analytic factors
- » Post-analytic factors

When you get an unexpected result, reconsider:



Approach

- Pre-analytic factors:
  - » Case factors
    - Why am I ordering this?
  - » Specimen factors
    - Section submitted:
      - › Is there enough tissue or tumor represented?
        - ○ False negative results
        - ○ Pseudoclonality
      - › Undergone processing that can affect testing
        - ○ Appropriate fixation, decalcification
      - › Is the specimen tumor or germline or both?

Approach

- Analytic factors:
  - » Is there an issue with the result?
    - Lab may need to investigate
  - » What are the limitations of that particular test?
    - Are there other options?

Approach

- Post-analytic factors:
  - » Is the result concordant with the clinical and histologic features?
  - » If not, process begins!

Take Home Points

- Notice when your cytogenetic or molecular testing doesn't fit with your impression
- Each method has limitations that can help to explain a discrepant result, and may be able to point to a next step
- The lab can help trouble-shoot a discordant result!

Thanks!

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