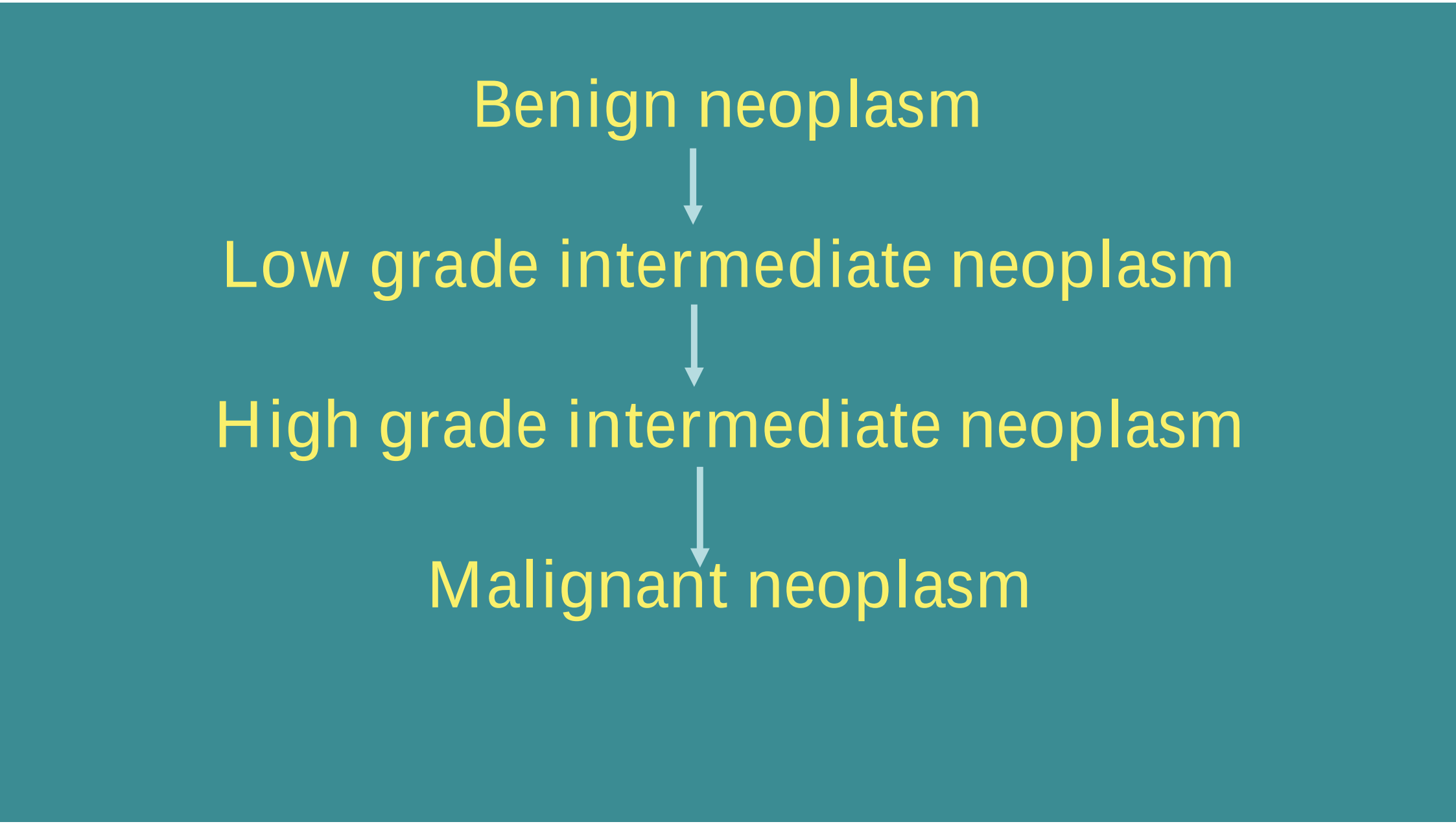
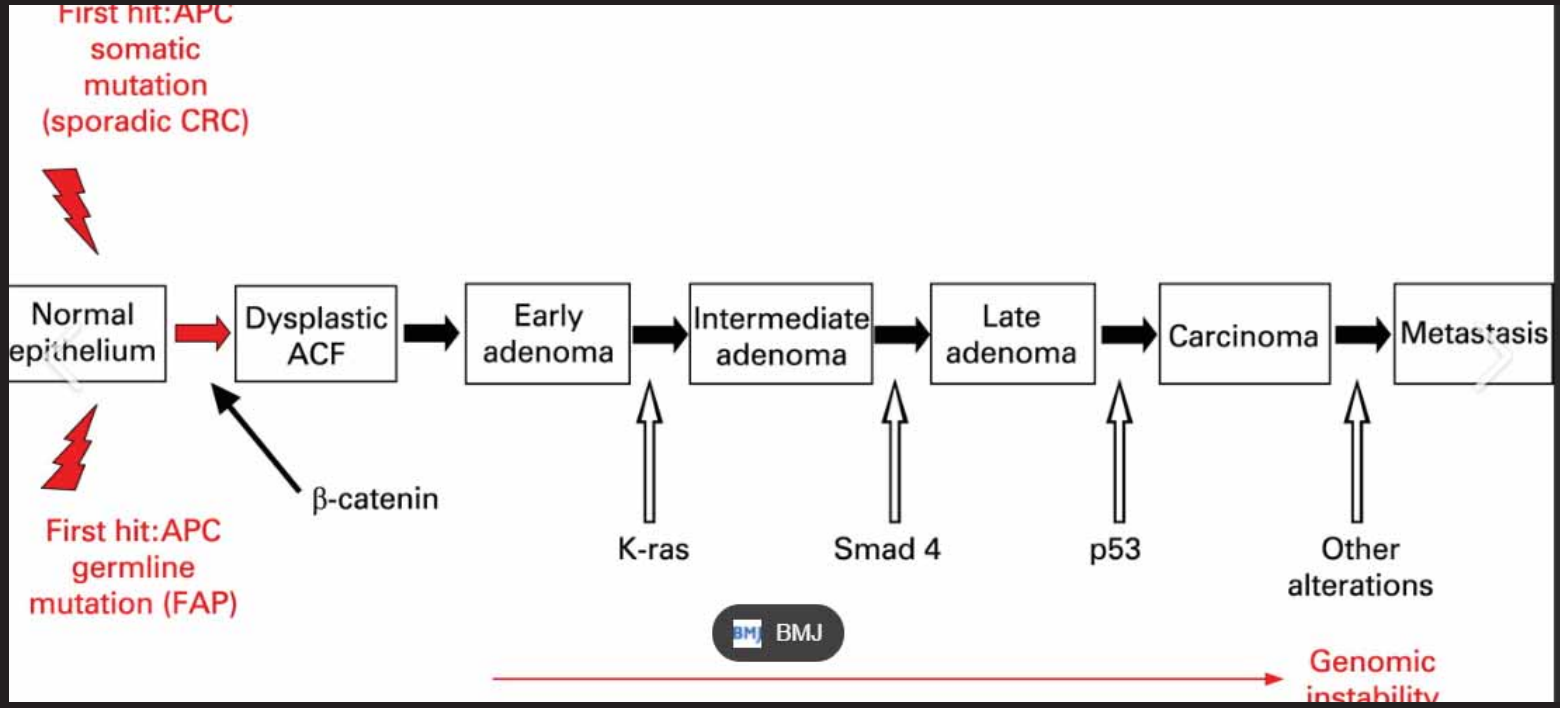
# Clinical, morphologic and genomic correlations of melanocytic neoplasm: When do multiple populations mean tumor progression?

Philip E. LeBoit, M.D.

Depts. of Pathology and Dermatology

University of California, San Francisco


Benign neoplasm
Low grade intermediate neoplasm
High grade intermediate neoplasm
Malignant neoplasm


First hit:APC somatic mutation (sporadic CRC)
Normal epithelium
Dysplastic ACF
Early adenoma
Intermediate adenoma
Late adenoma
Carcinoma
Metastasis
β-catenin
K-ras
Smad 4
p53
Other alterations
First hit:APC germline mutation (FAP)
BMJ
Genomic instability

Journal of Pathology
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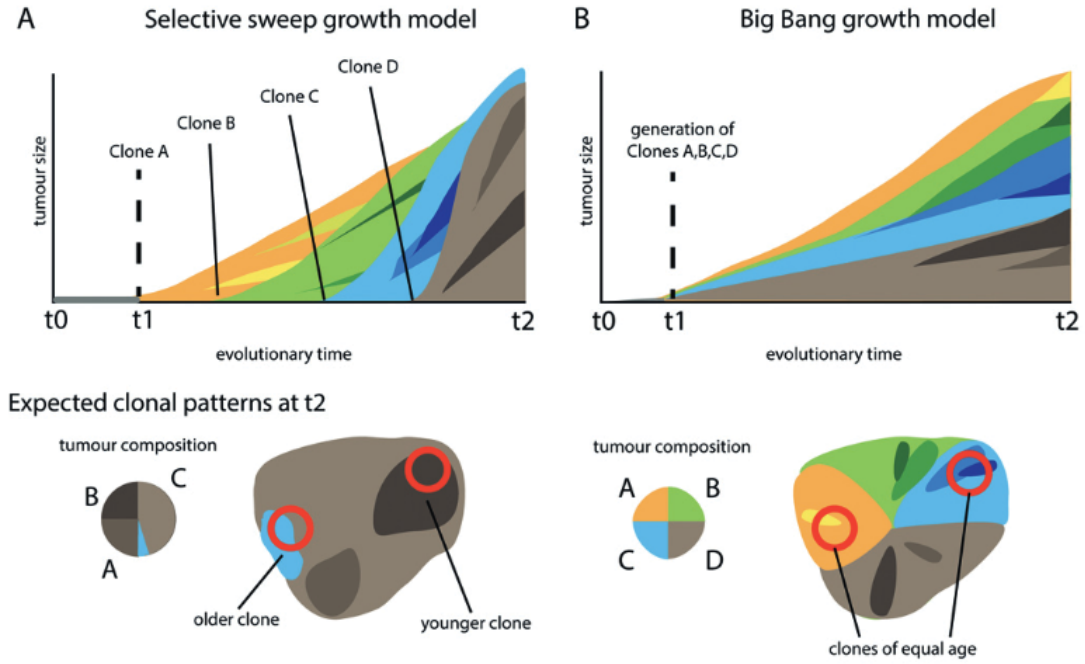
INVITED REVIEW

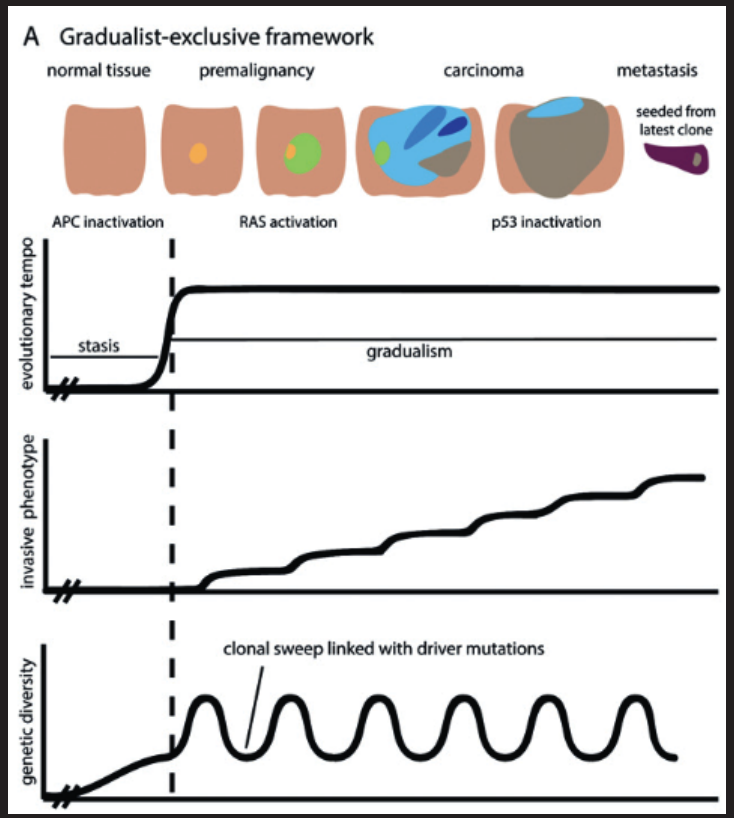
# New paradigms in clonal evolution: punctuated equilibrium in cancer

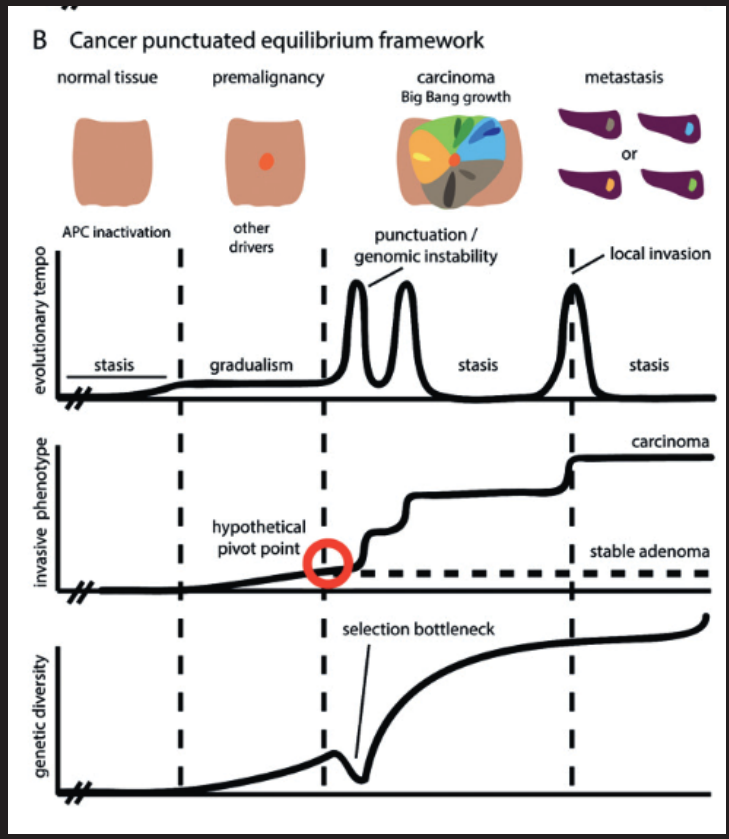
William CH Cross,* Trevor A Graham and Nicholas A Wright

*Centre for Tumour Biology, Barts and the London School of Medicine and Dentistry, London, EC1 2AD, UK*

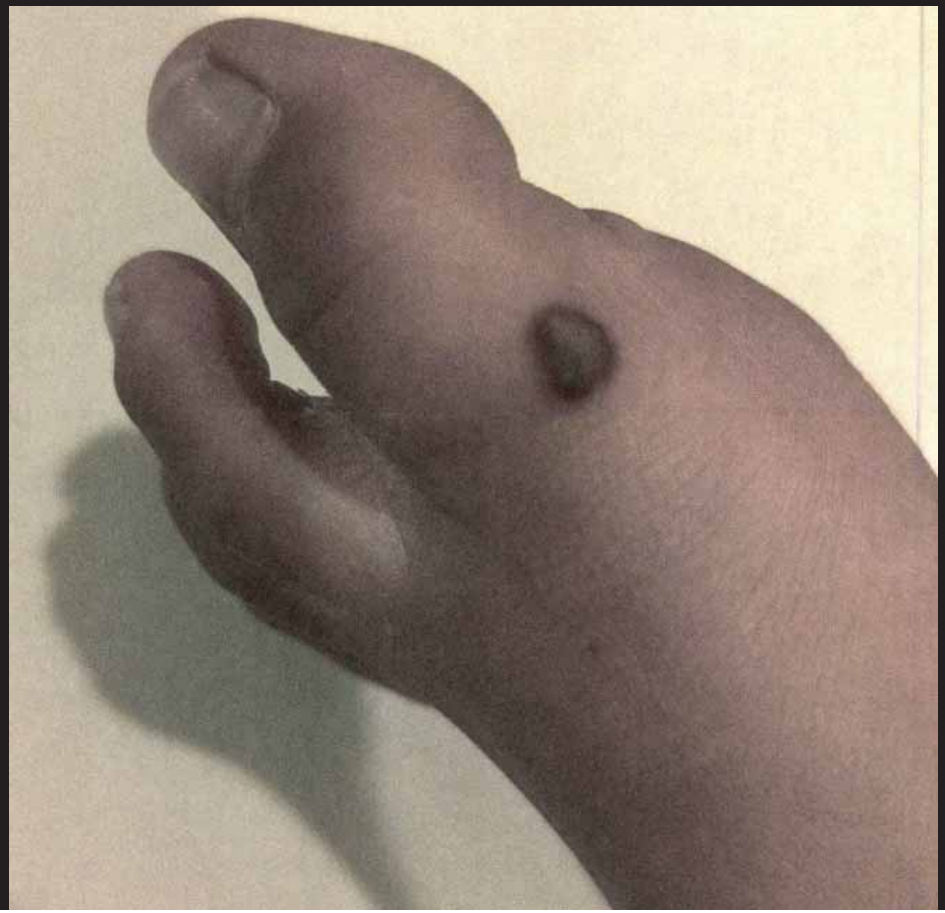
**New paradigms in clonal evolution: punctuated equilibrium in cancer**

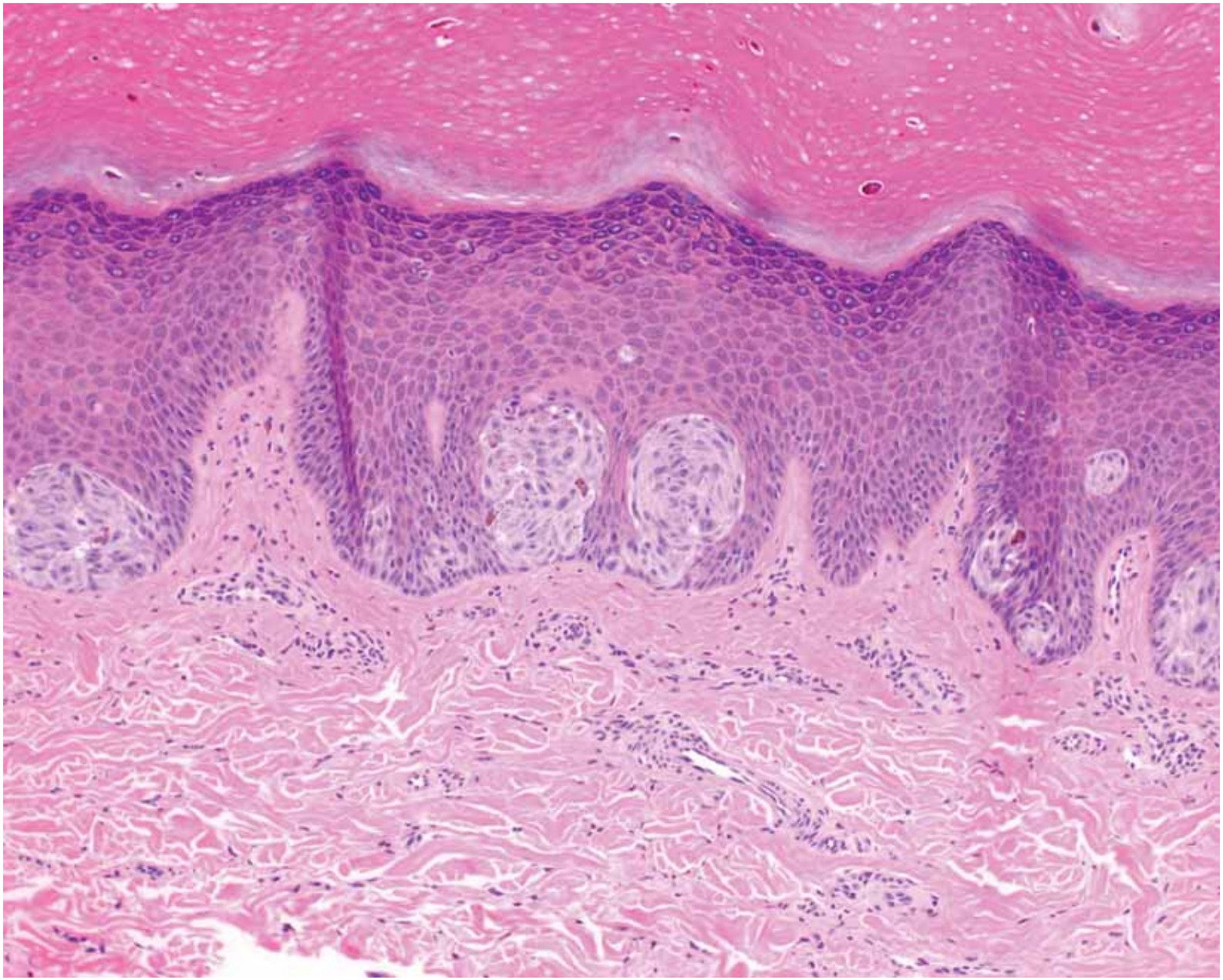


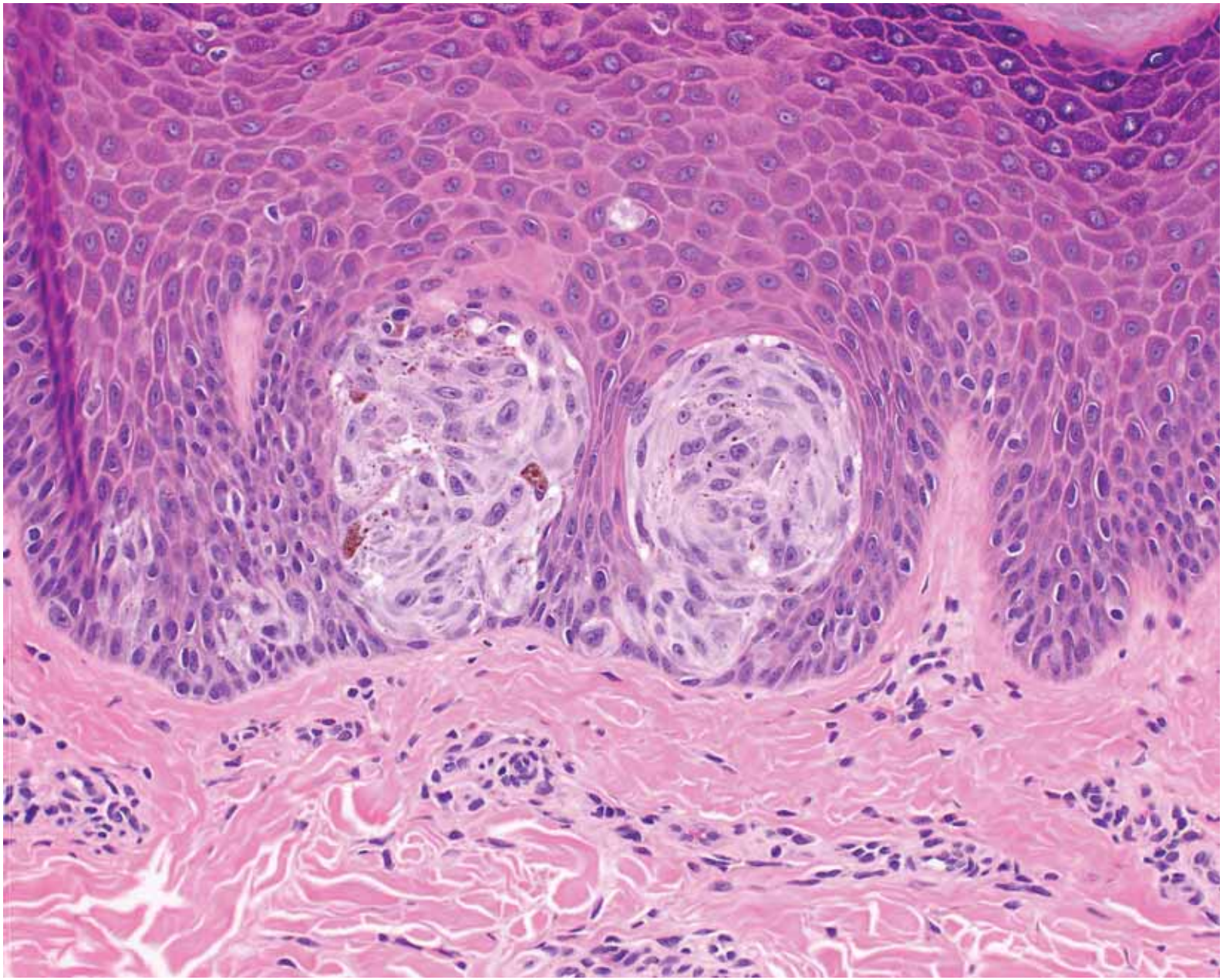

A Gradualist-exclusive framework
normal tissue
premalignancy
carcinoma
metastasis
seeded from latest clone
APC inactivation
RAS activation
p53 inactivation
evolutionary tempo
stasis
gradualism
invasive phenotype
genetic diversity
clonal sweep linked with driver mutations

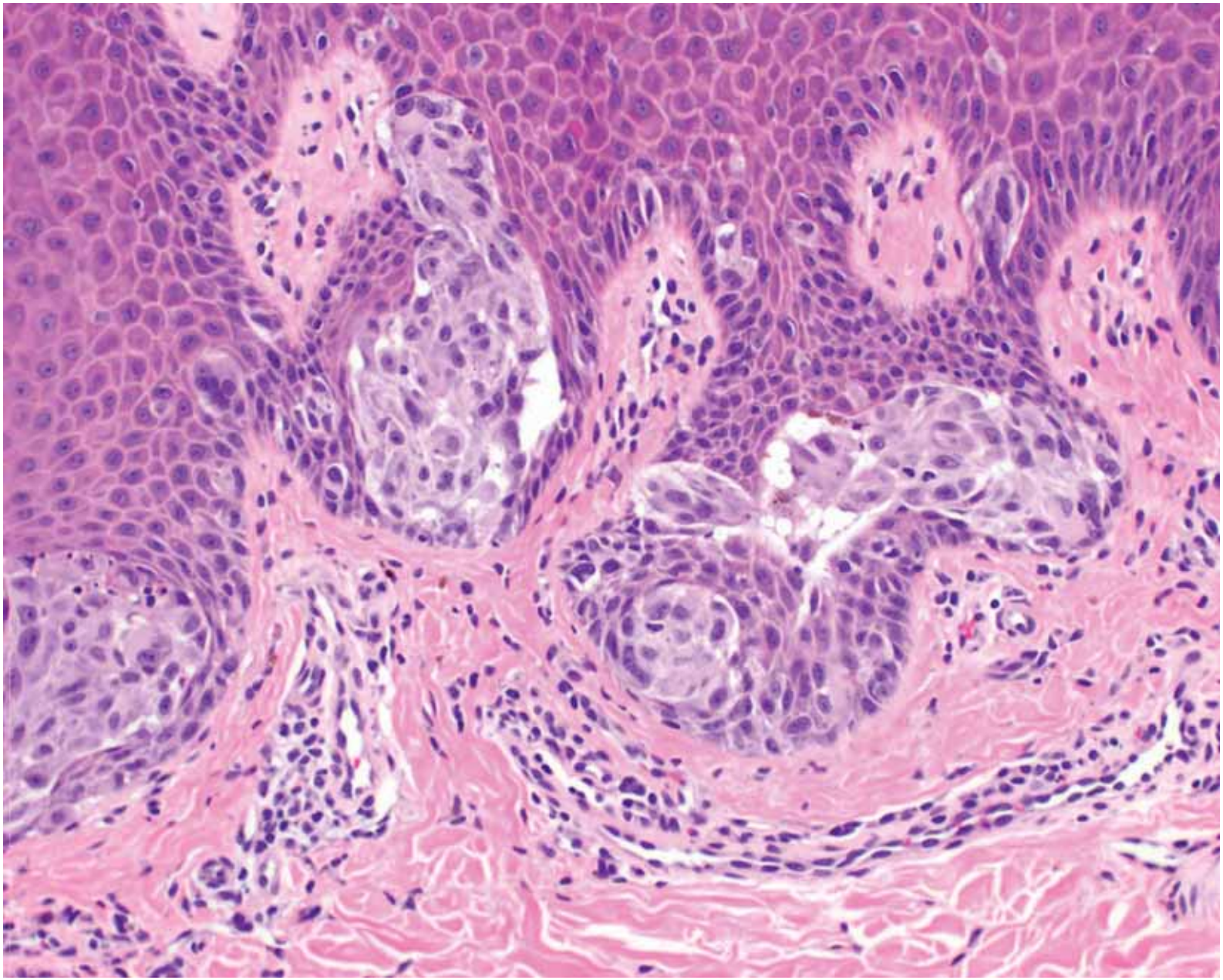

B Cancer punctuated equilibrium framework
normal tissue
premalignancy
carcinoma
Big Bang growth
metastasis
or
APC inactivation
other drivers
punctuation / genomic instability
local invasion
evolutionary tempo
stasis
gradualism
stasis
stasis
carcinoma
invasive phenotype
hypothetical pivot point
stable adenoma
selection bottleneck
genetic diversity

# 13 year old girl, right base of first toe









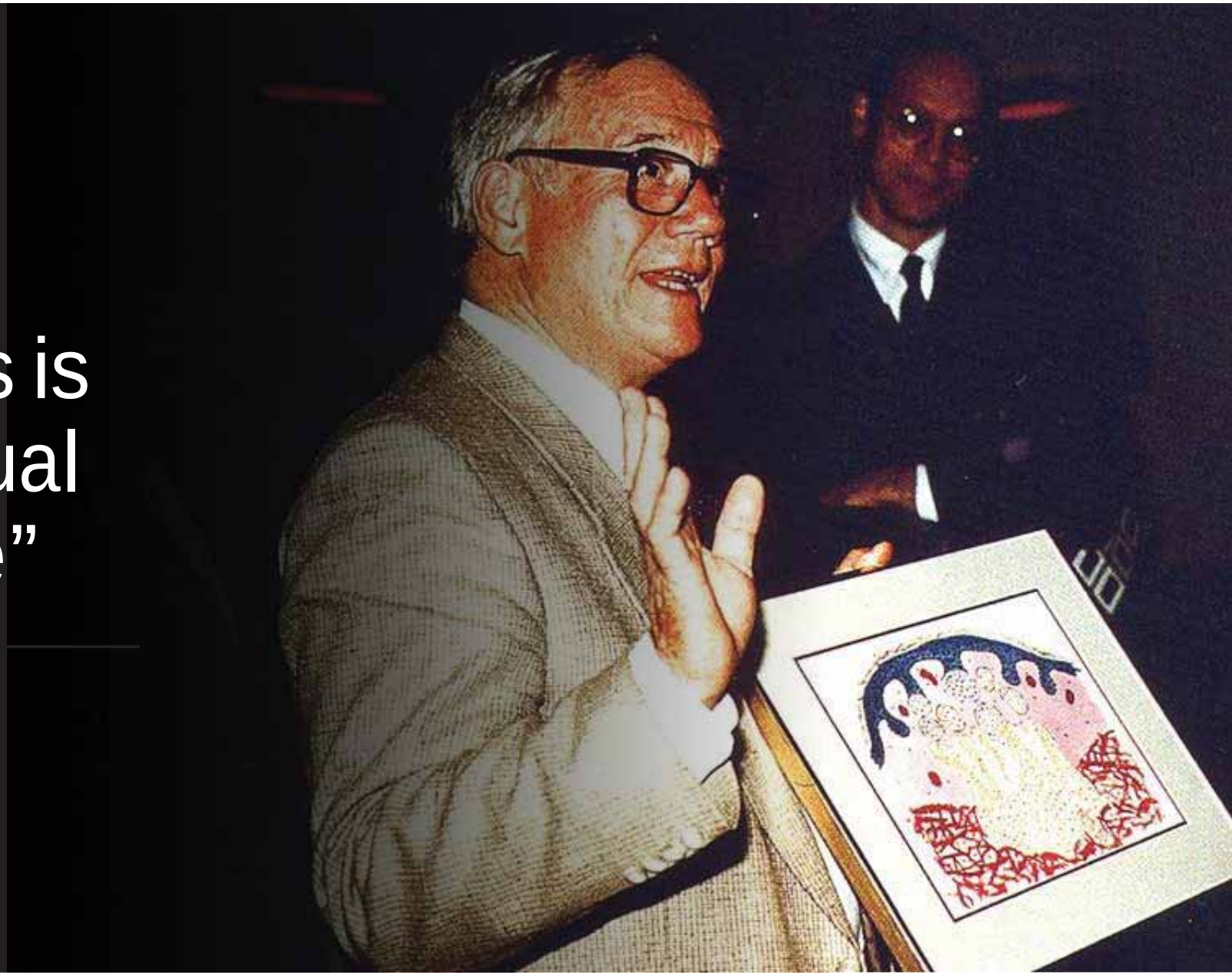
p16

# What is the diagnosis?

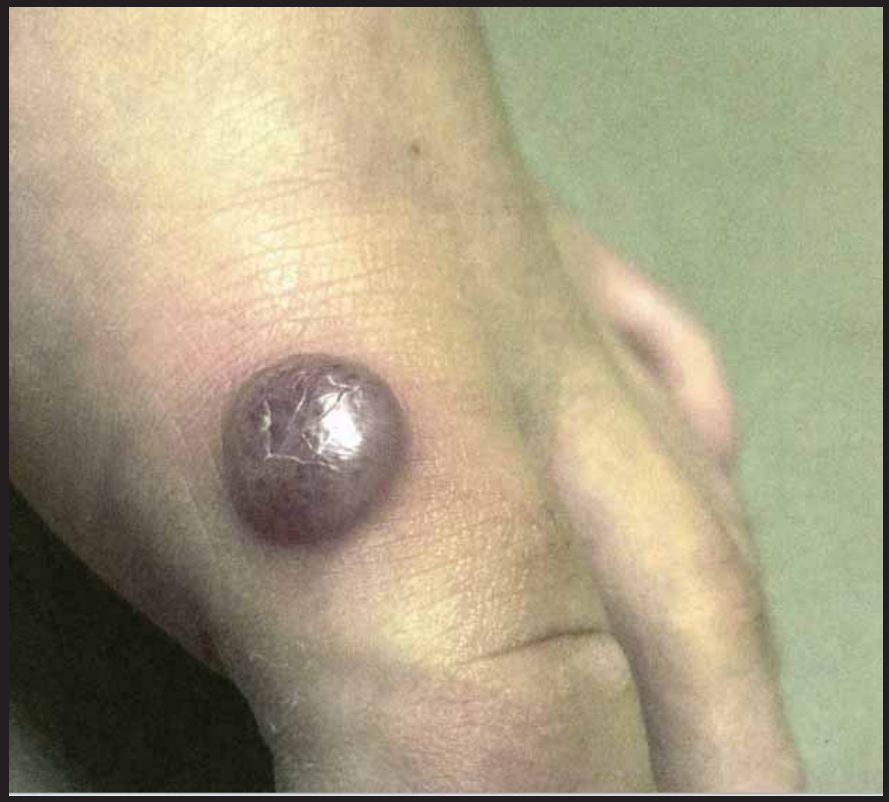
- Spitz nevus
- Junctional atypical Spitz tumor
- Melanoma in situ
- There must be a trick!

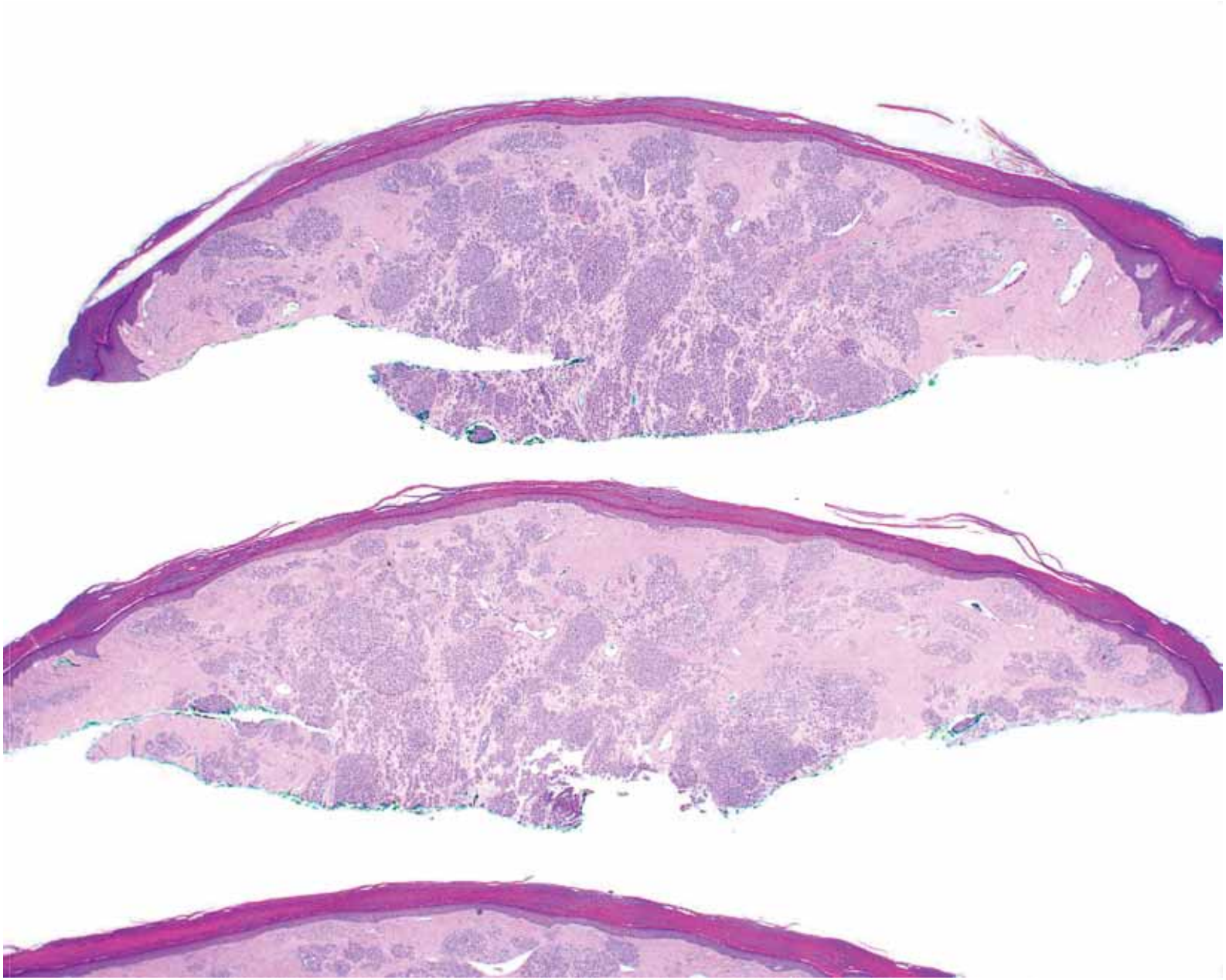
“A diagnosis is an intellectual catastrophe”

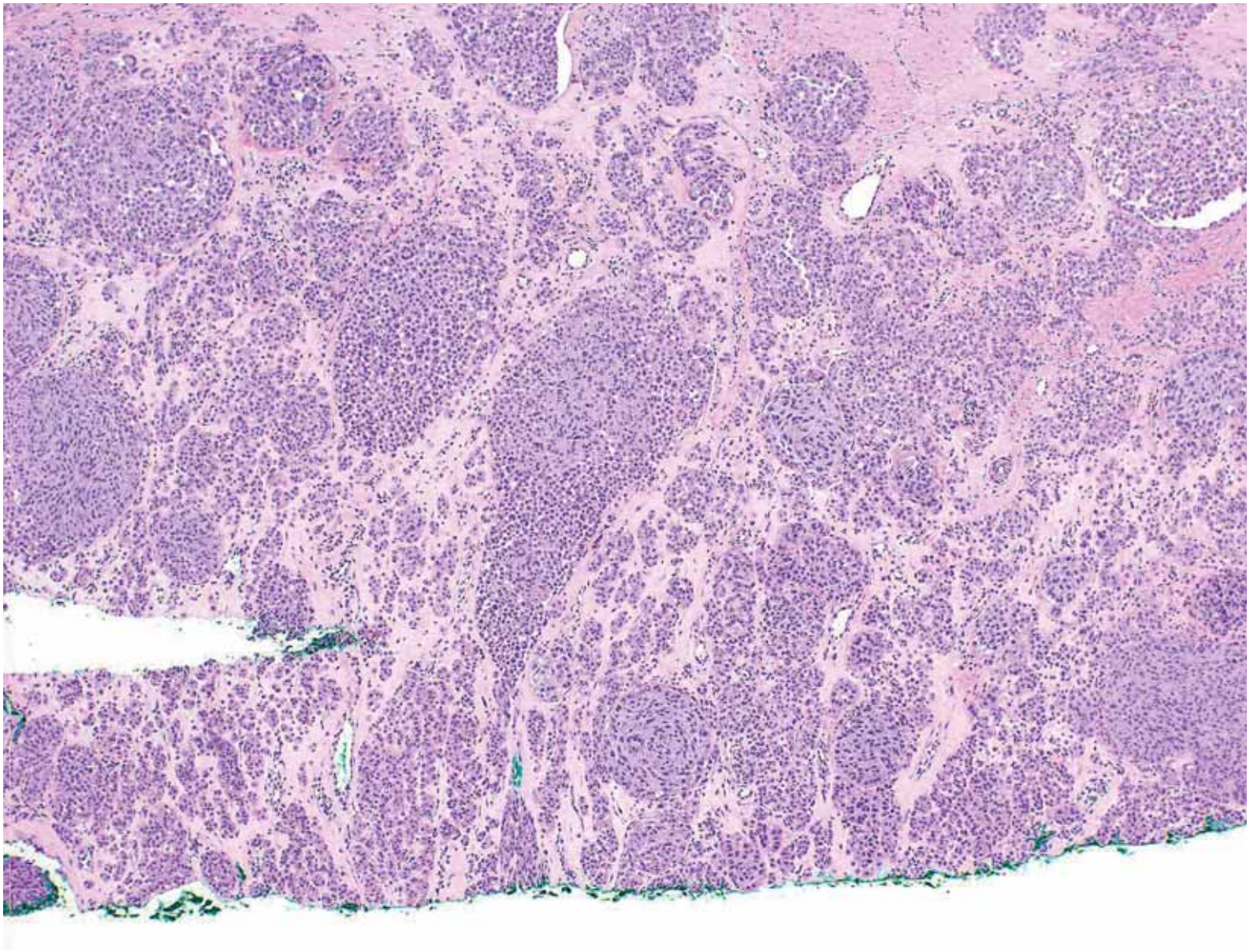
*-Wallace H Clark, Jr*

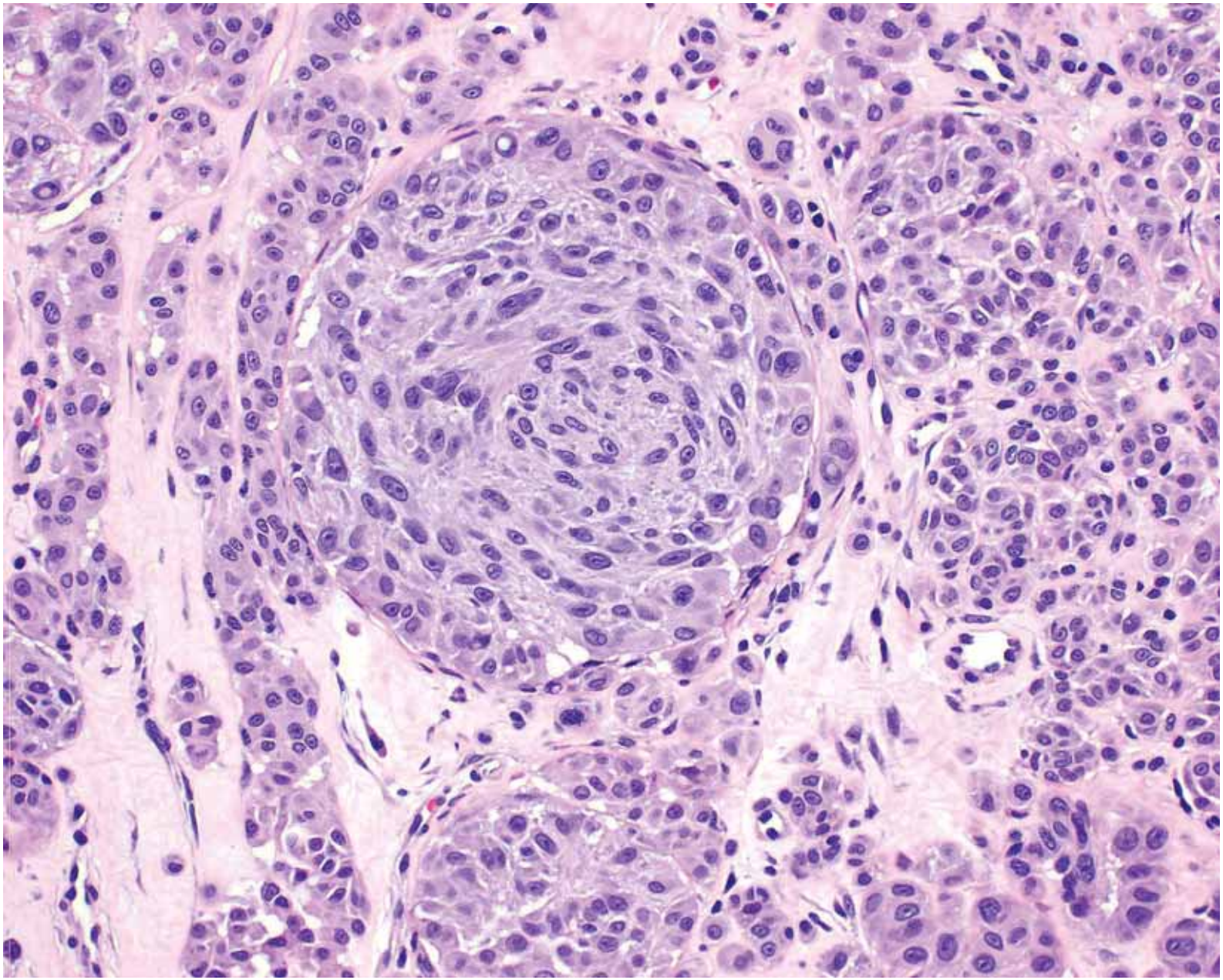


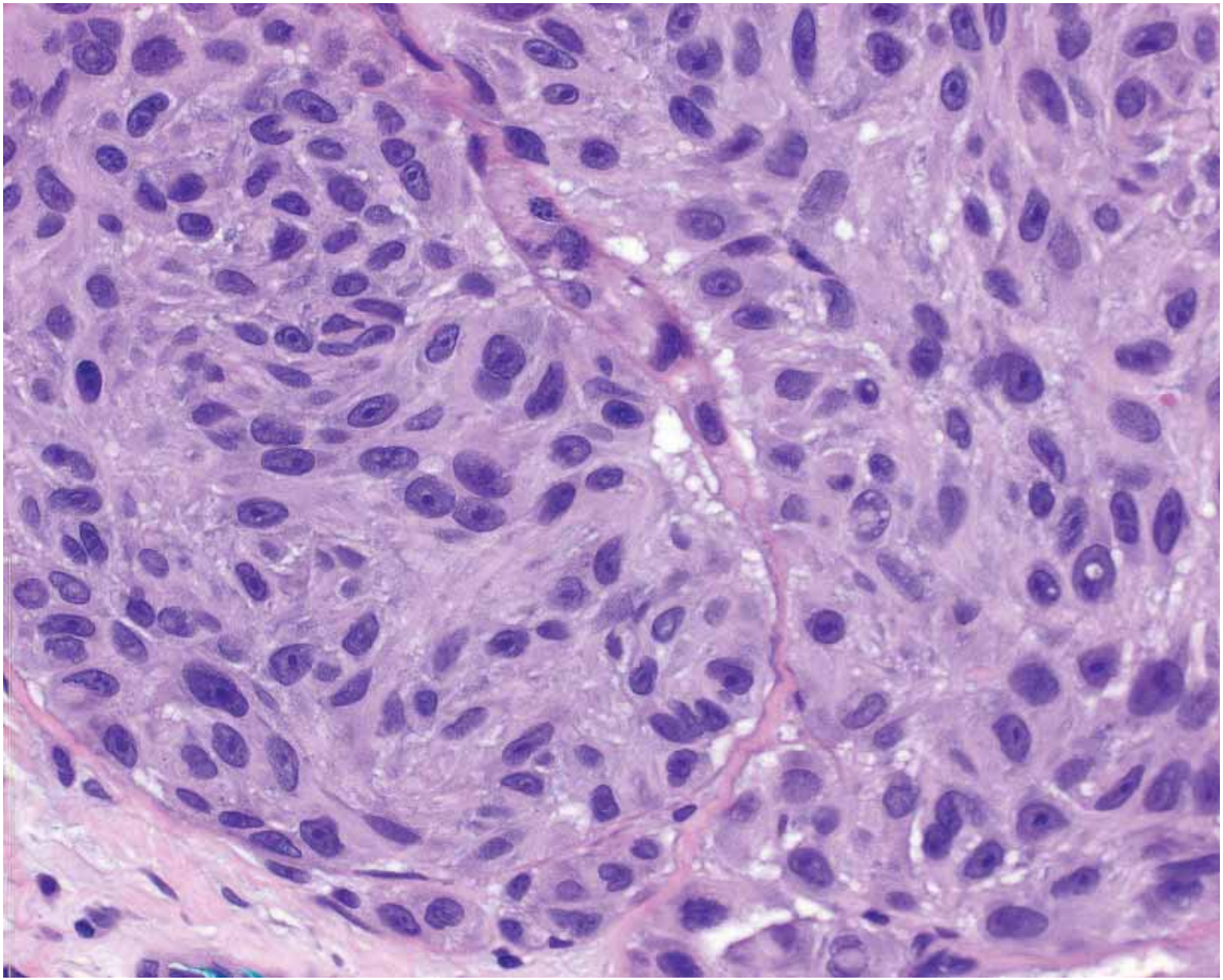
# Several months later, left first toe

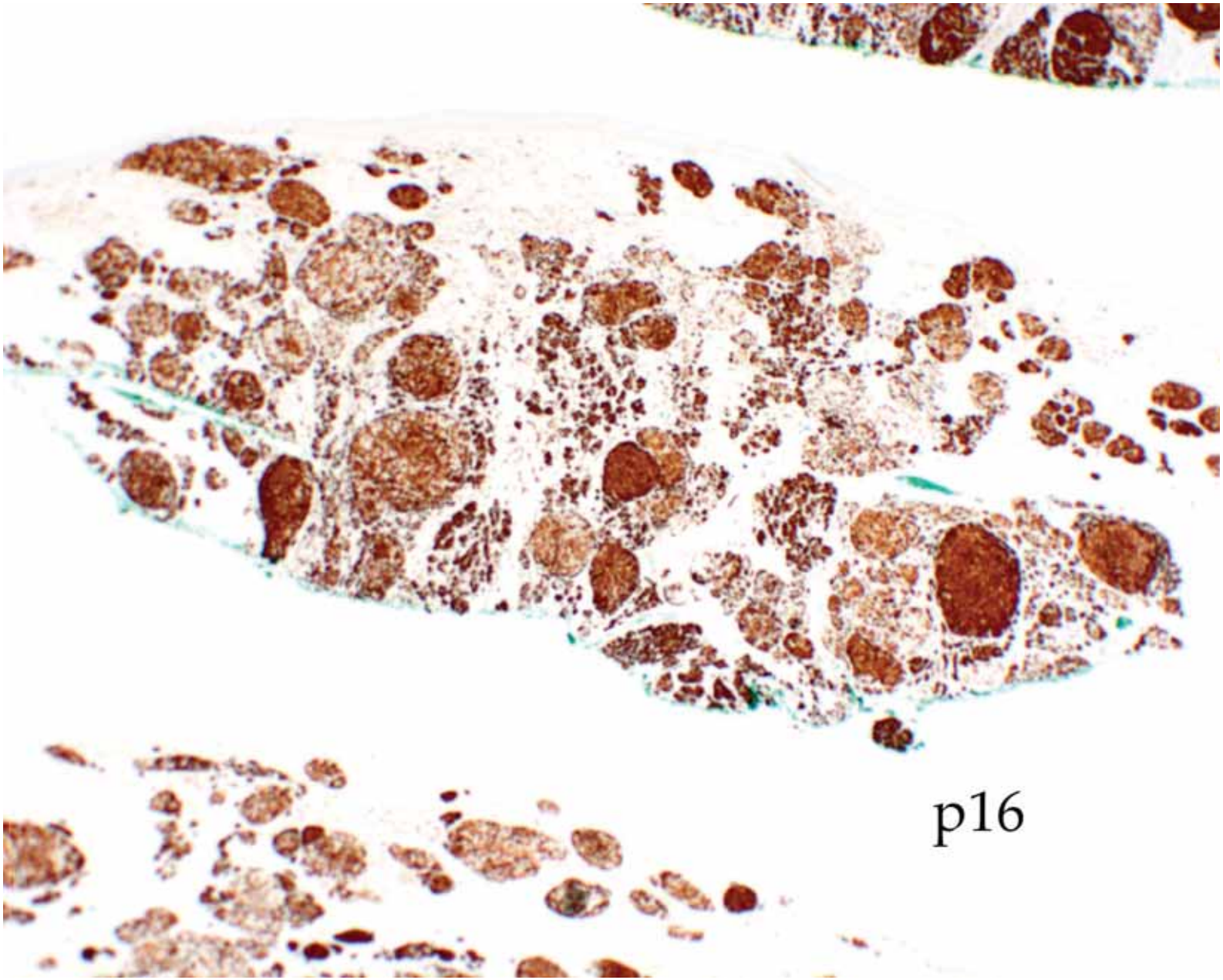








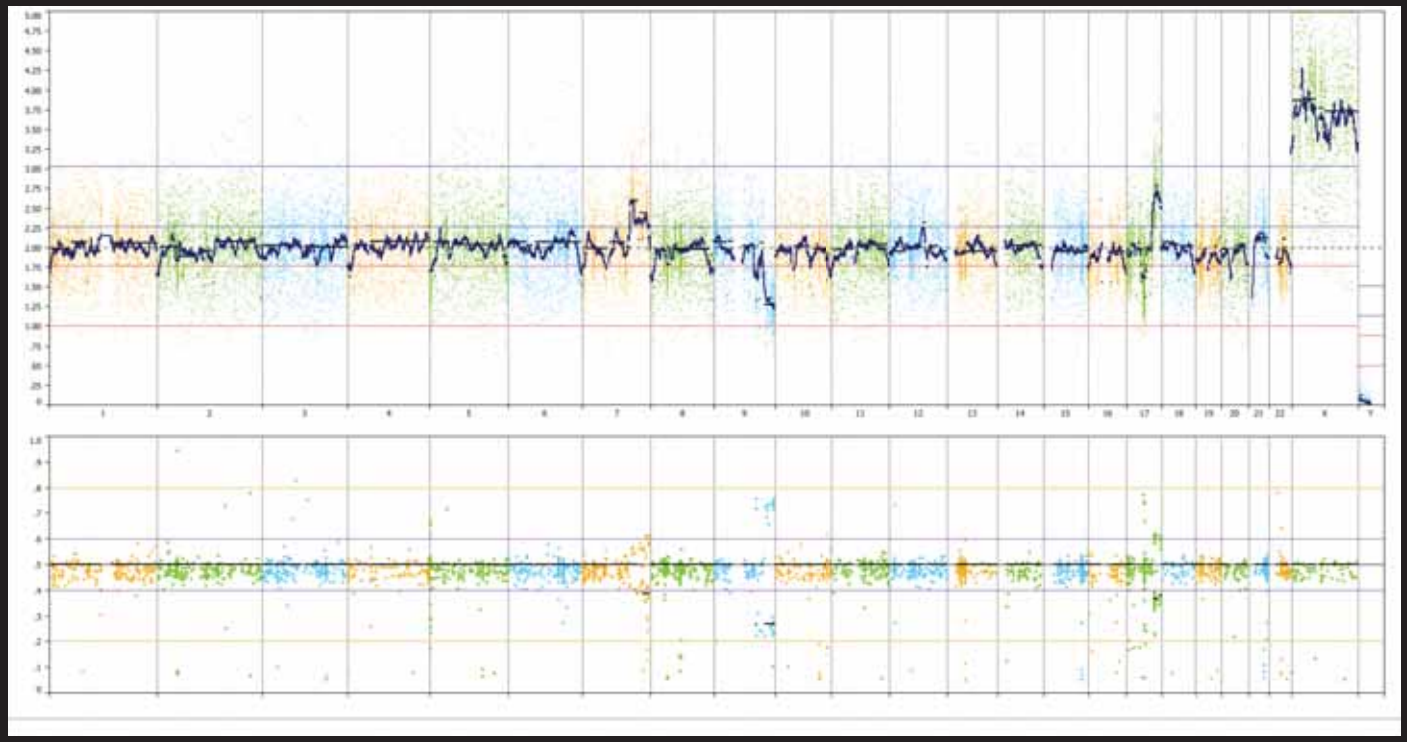



p16

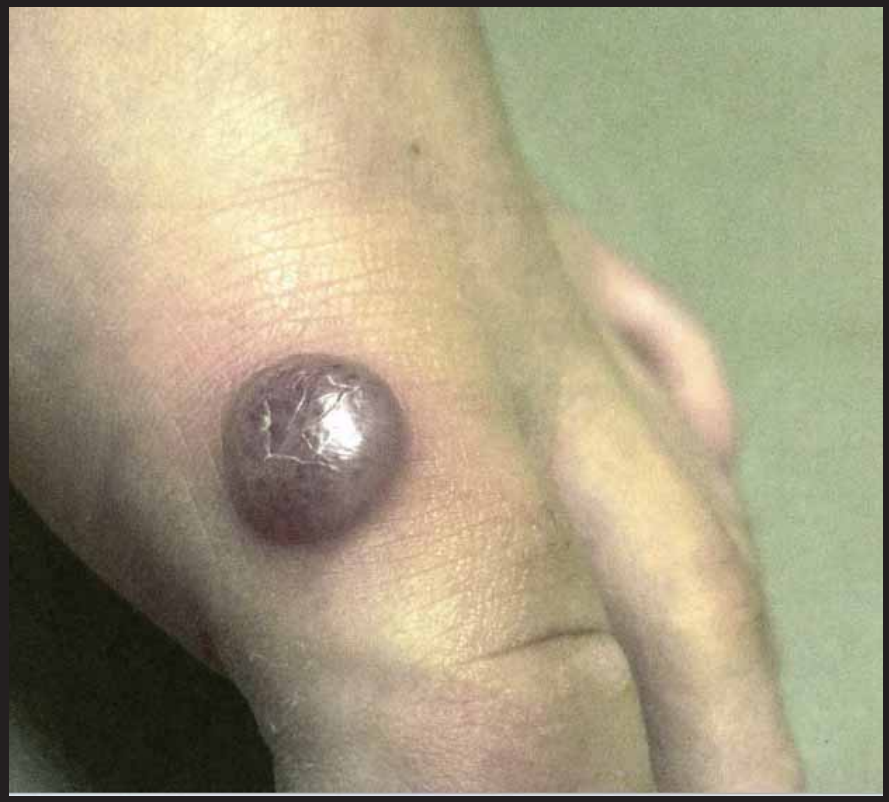
## PATHOGENIC AND LIKELY PATHOGENIC ALTERATIONS

| VARIANT | TRANSCRIPT ID | CLASSIFICATION | READS | MUTANT ALLELE FREQUENCY |
|---|---|---|---|---|
| CAPRIN1::ROS1 fusion | NM_005898/NM_002944 | Pathogenic | 169 | N/A |

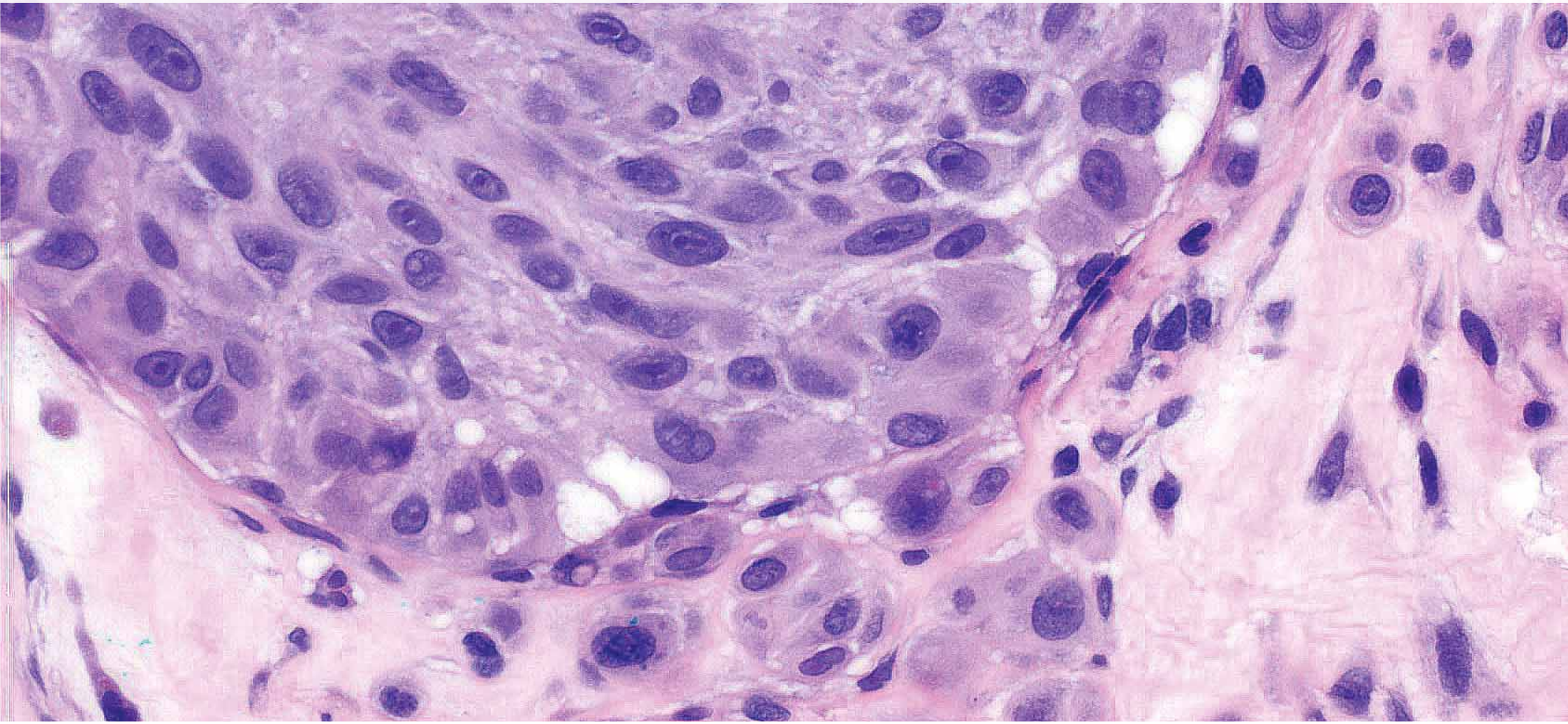
'Reads' indicates the number of unique DNA molecules sequenced. 'Mutant Allele Frequency' indicates the percentage of the reads with the respective 'Variant' and is affected by the degree of normal cell contamination of the sample and whether the variant is fully clonal or subclonal. 'Pathogenic' and 'Likely Pathogenic' classifications are based on CCGL molecular pathologist/geneticist interpretation of data from somatic and germline databases and published literature. Variants classified as 'Possibly Pathogenic' have unknown significance but occur in genes or molecular pathways known to be recurrently altered in the tumor type.

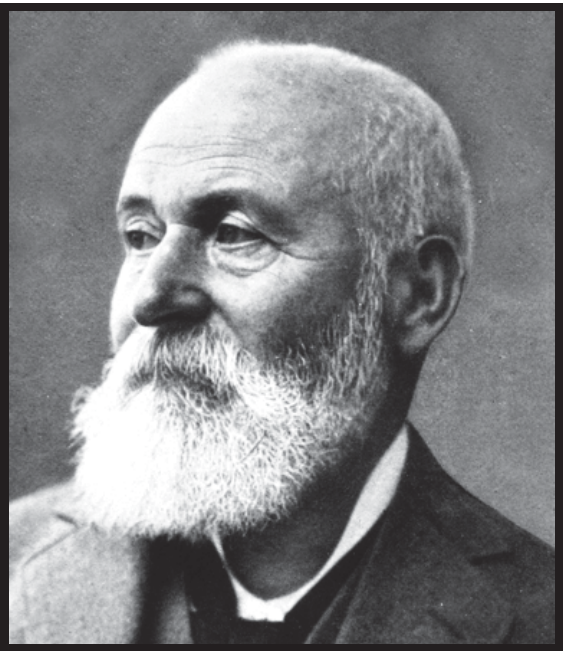


# So what happened?



# Clonal selection/tumor progression?

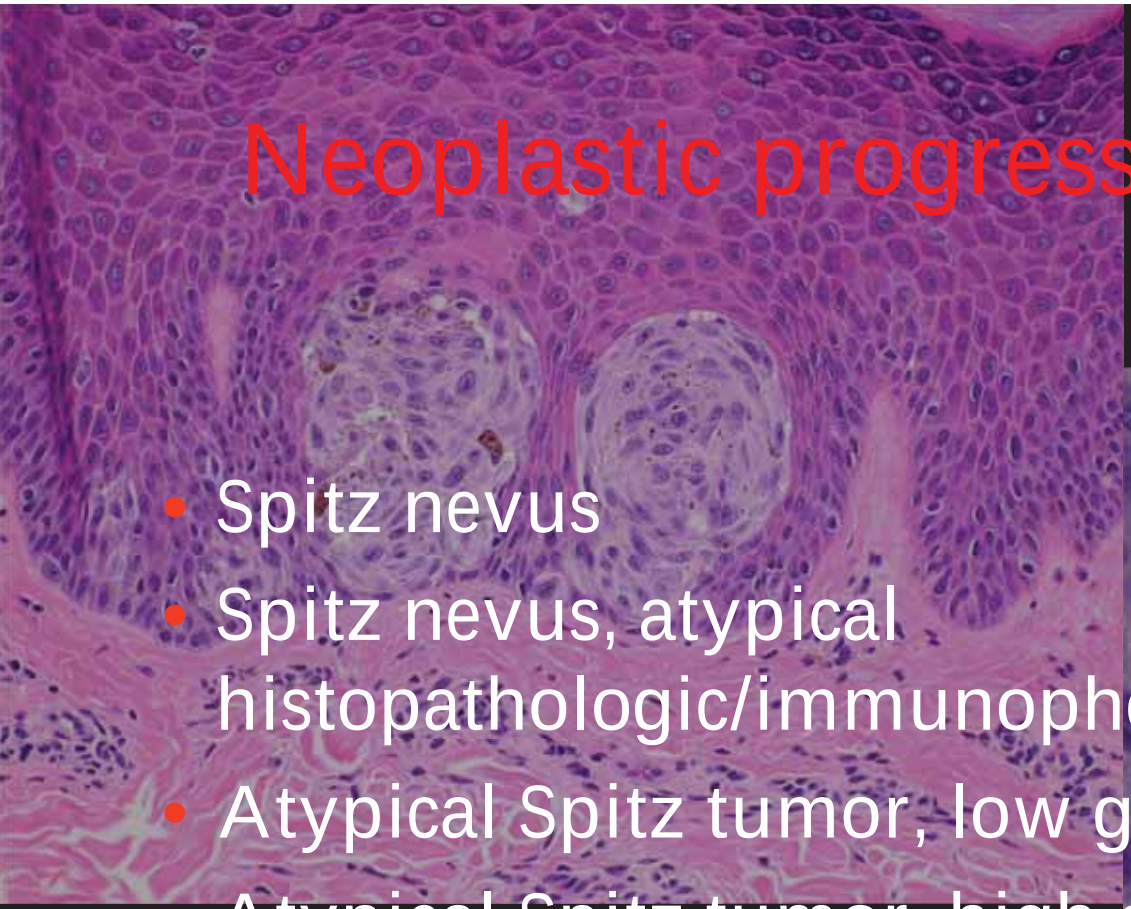




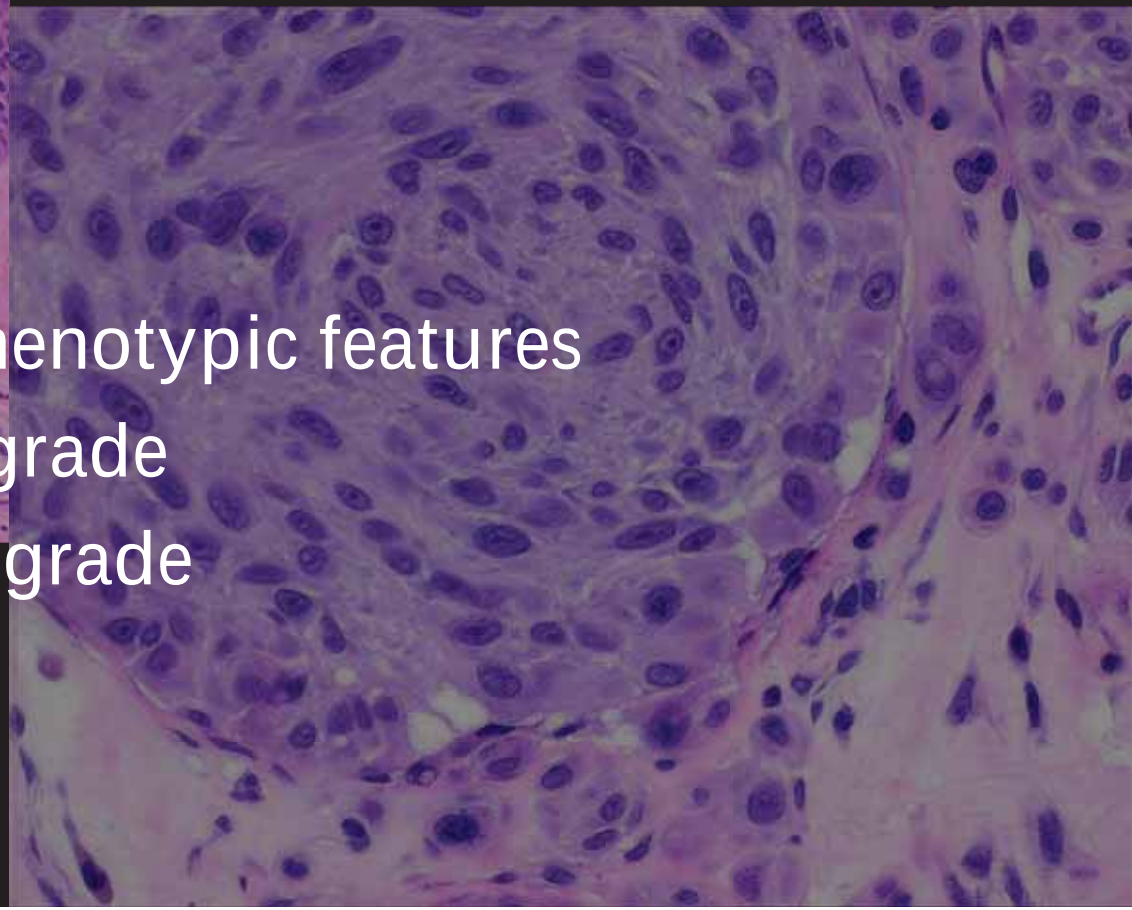
# Nevogenesis

- Descending nevi (abropfung theory of Unna)
- Ascending nevi (hochsteigerung theory of Cramer)
- Mixed ascending and descending

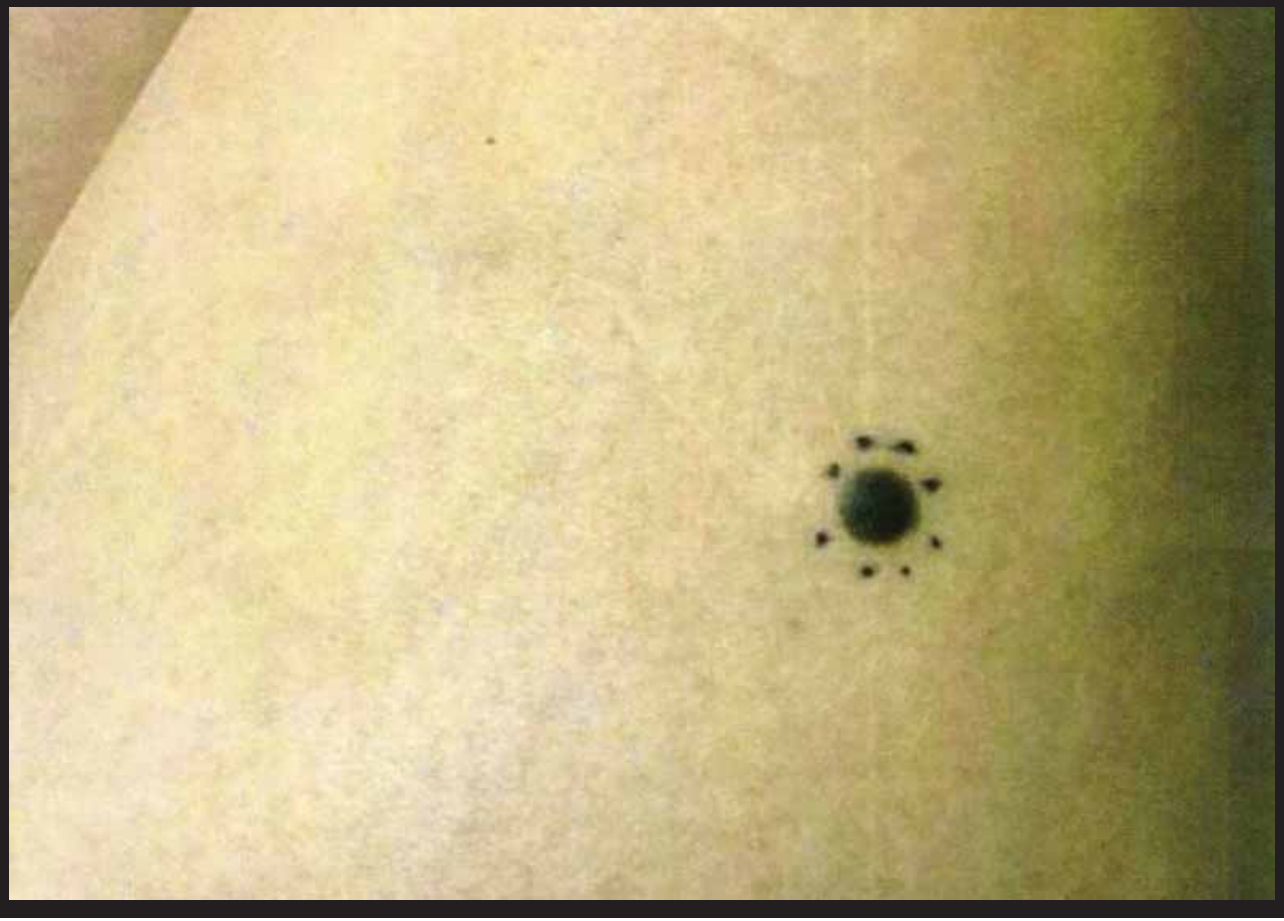
# Neoplastic progression in Spitz tumors

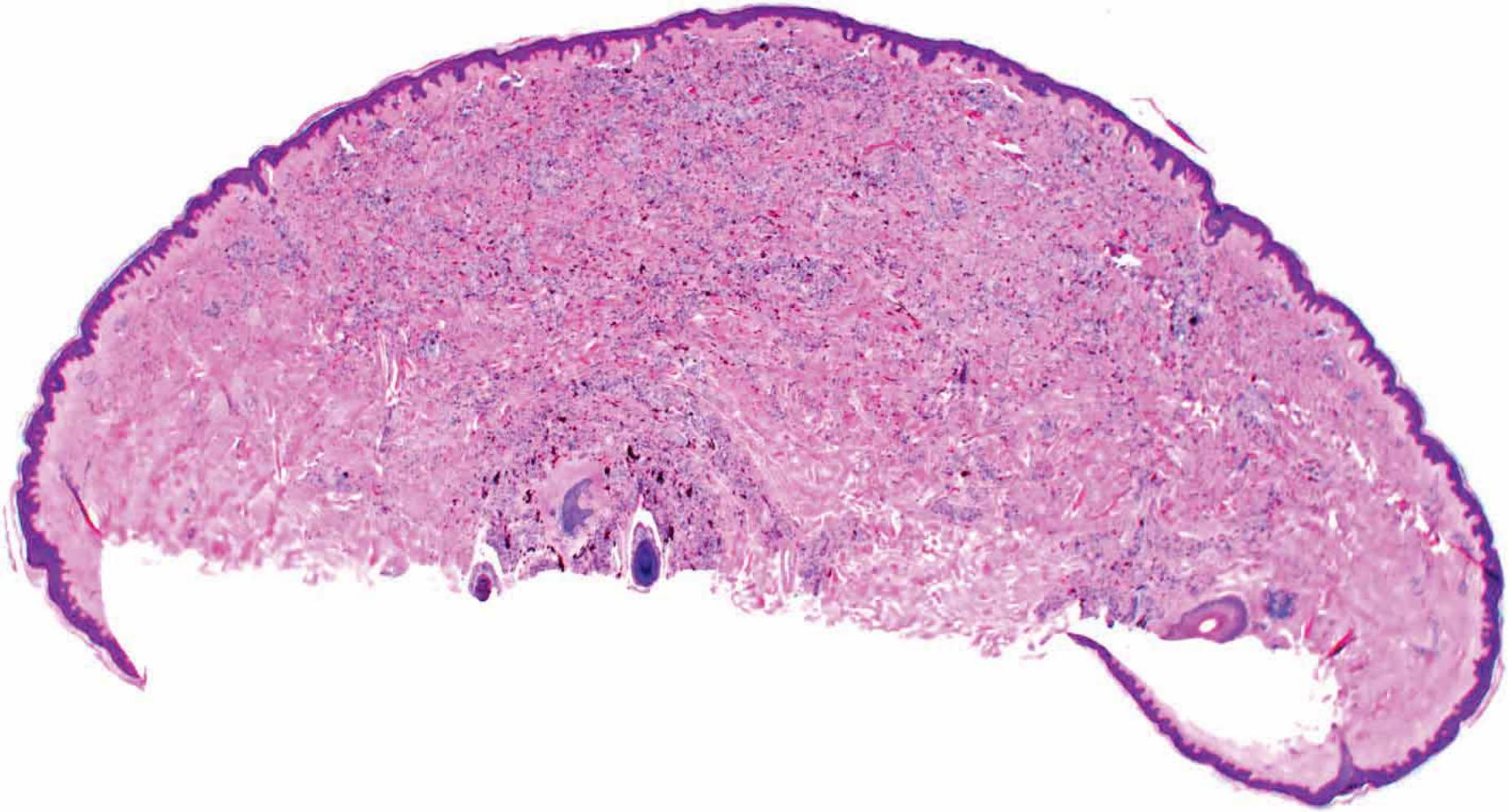


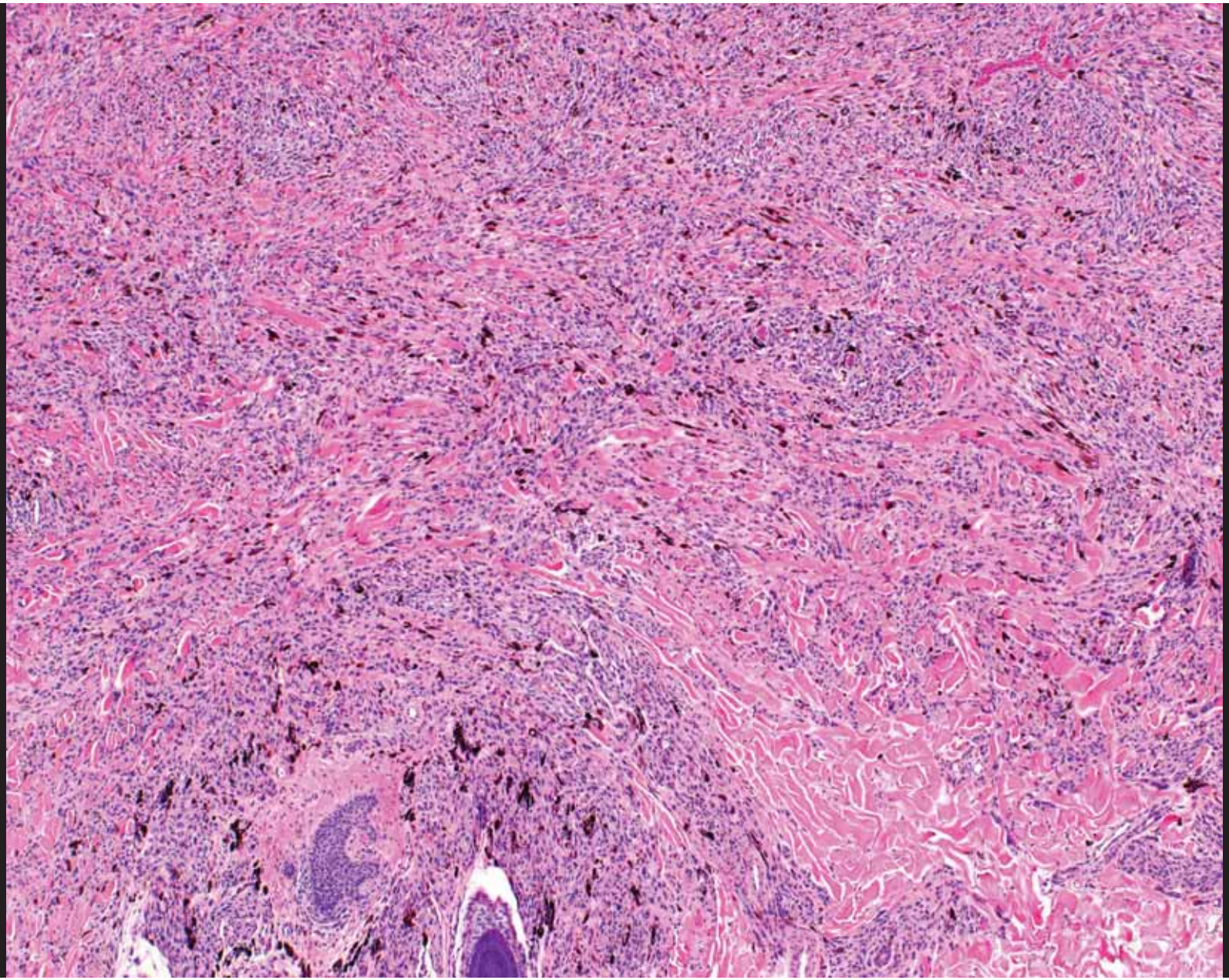
- Spitz nevus
- Spitz nevus, atypical histopathologic/immunophenotypic features
- Atypical Spitz tumor, low grade
- Atypical Spitz tumor, high grade
- Spitz melanoma

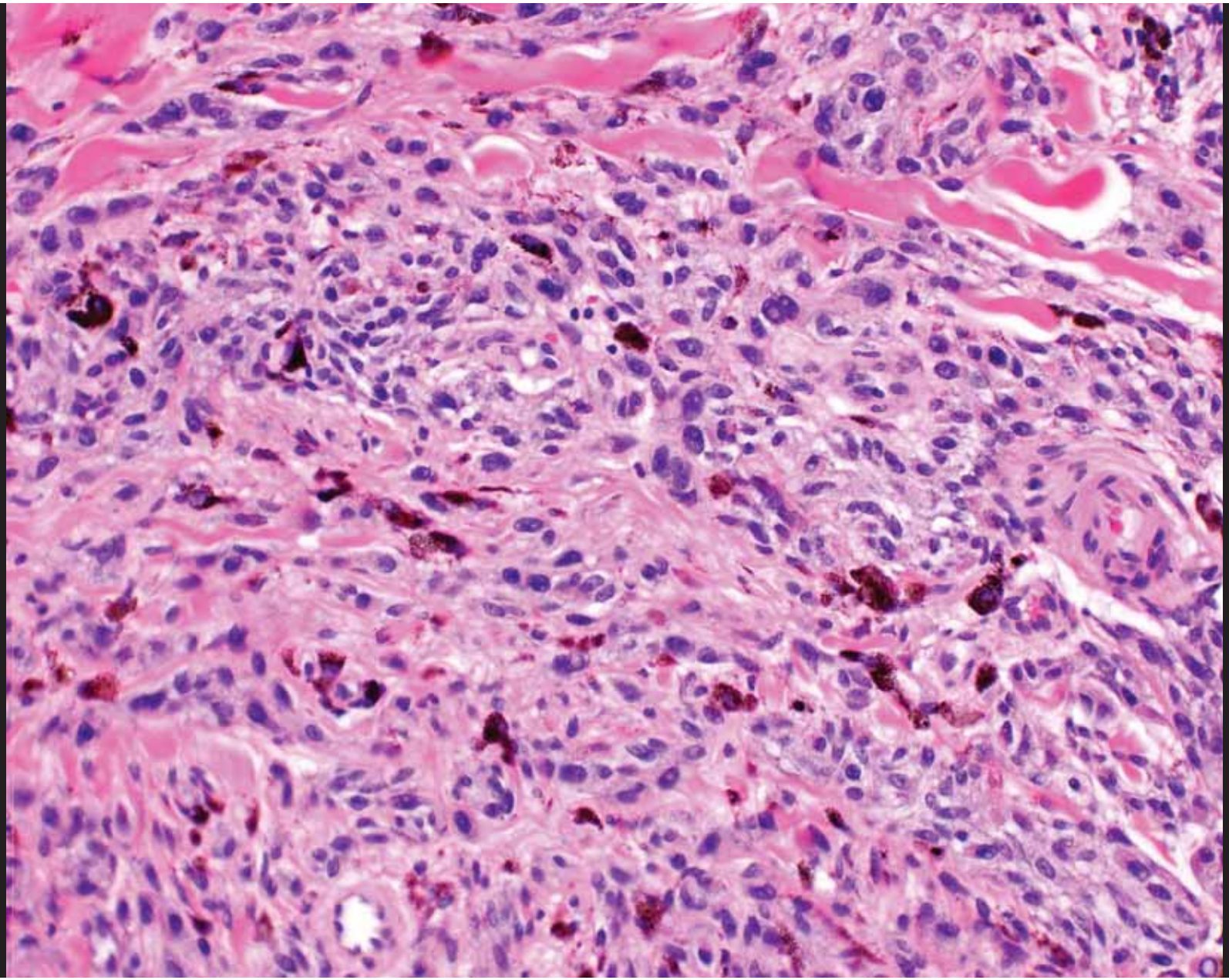


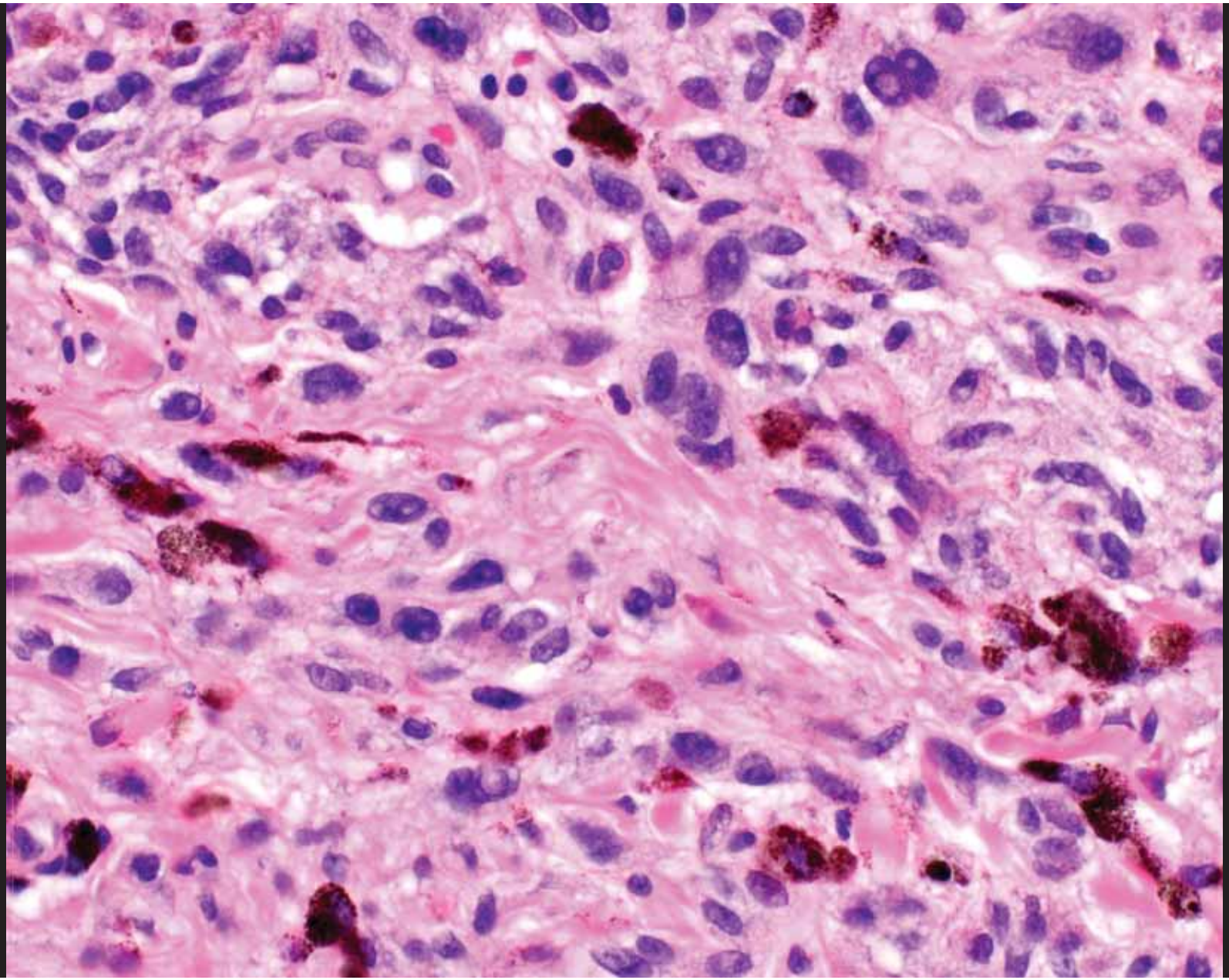
# 25 year old woman, right thigh

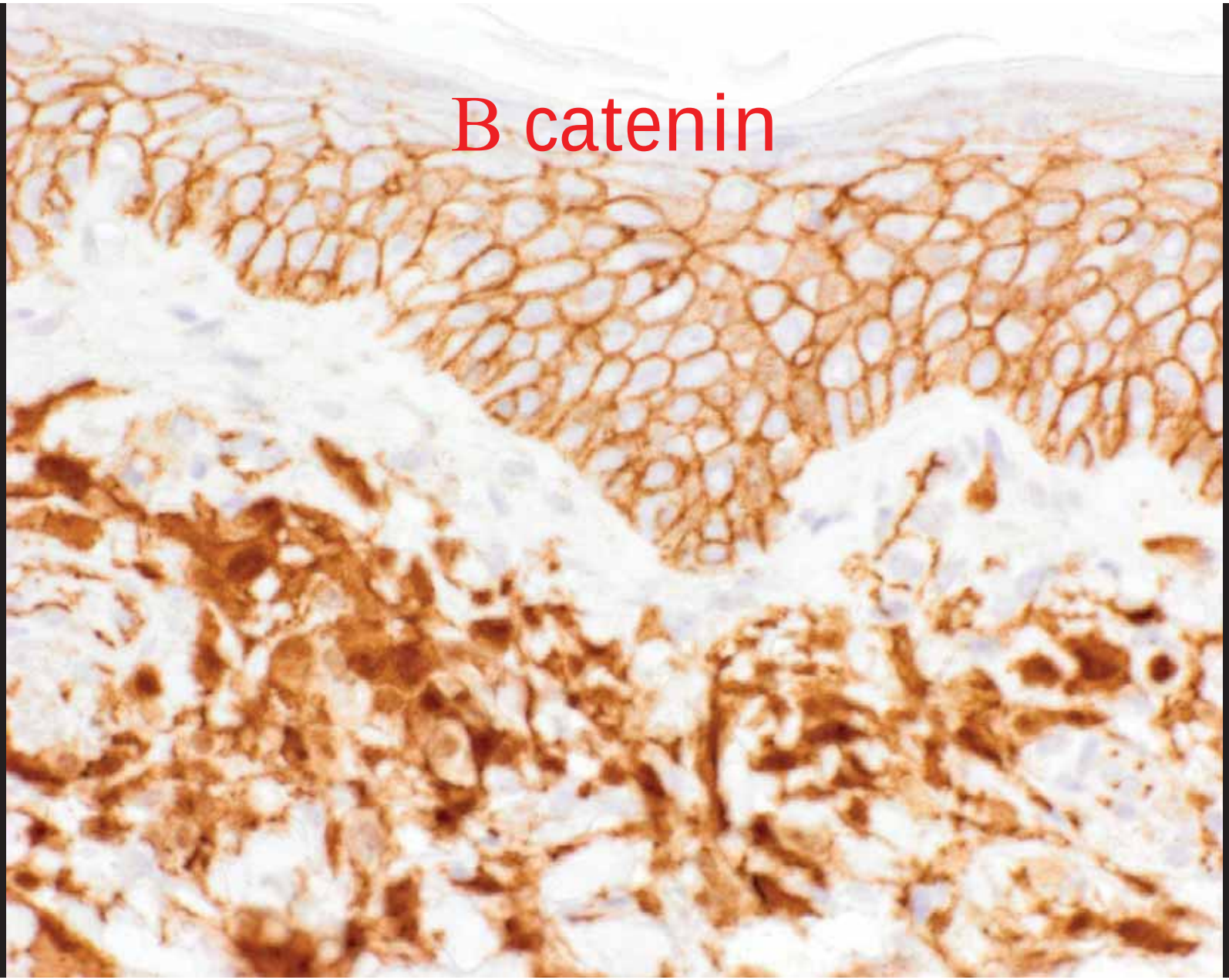


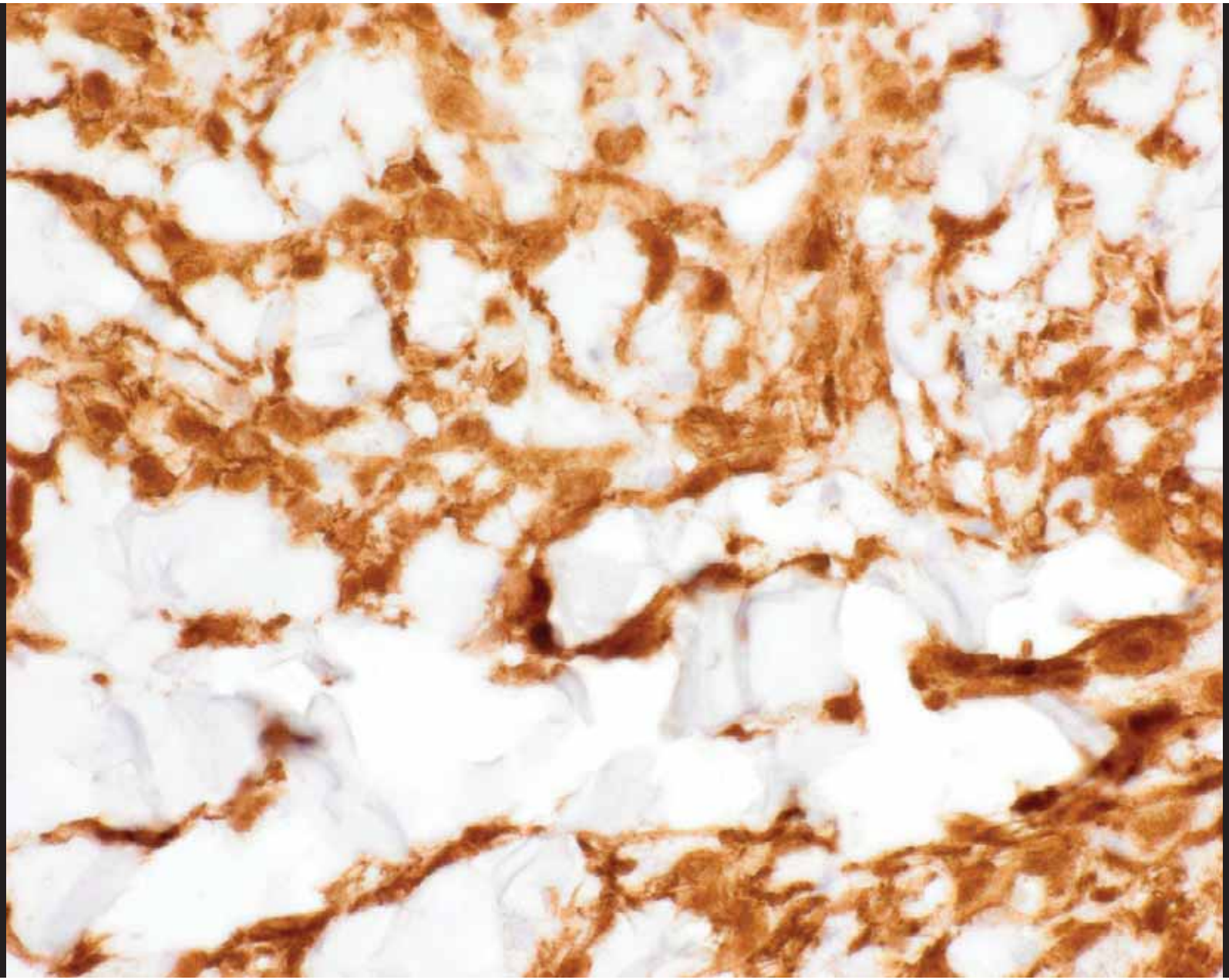


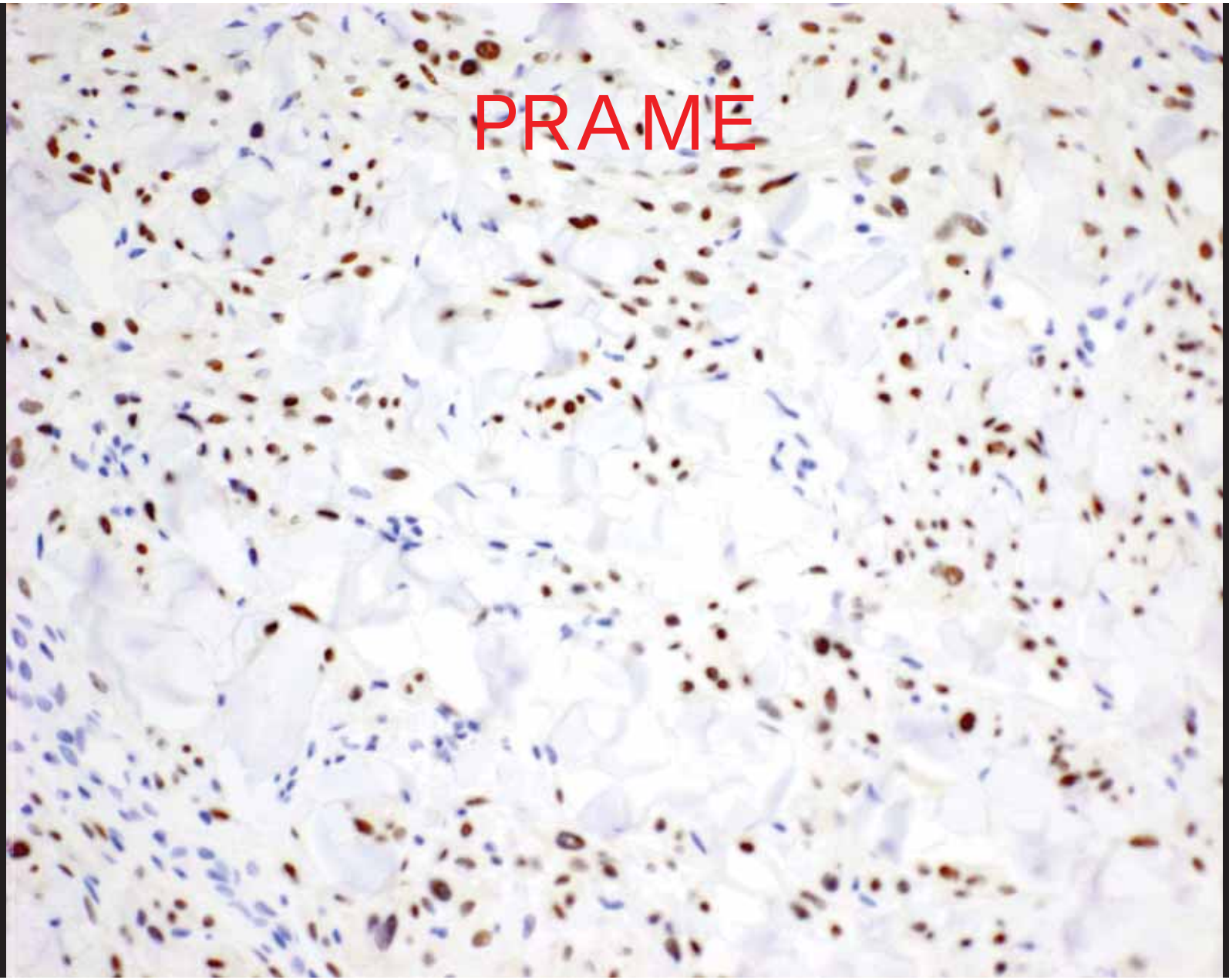







B catenin




PRAME

The American Journal of Surgical Pathology *13(1): 39–44, 1989*



# Deep Penetrating Nevus

James A. Seab, Jr., COL., MC, USA, James H. Graham, M.D., and Elson B. Helwig, M.D.

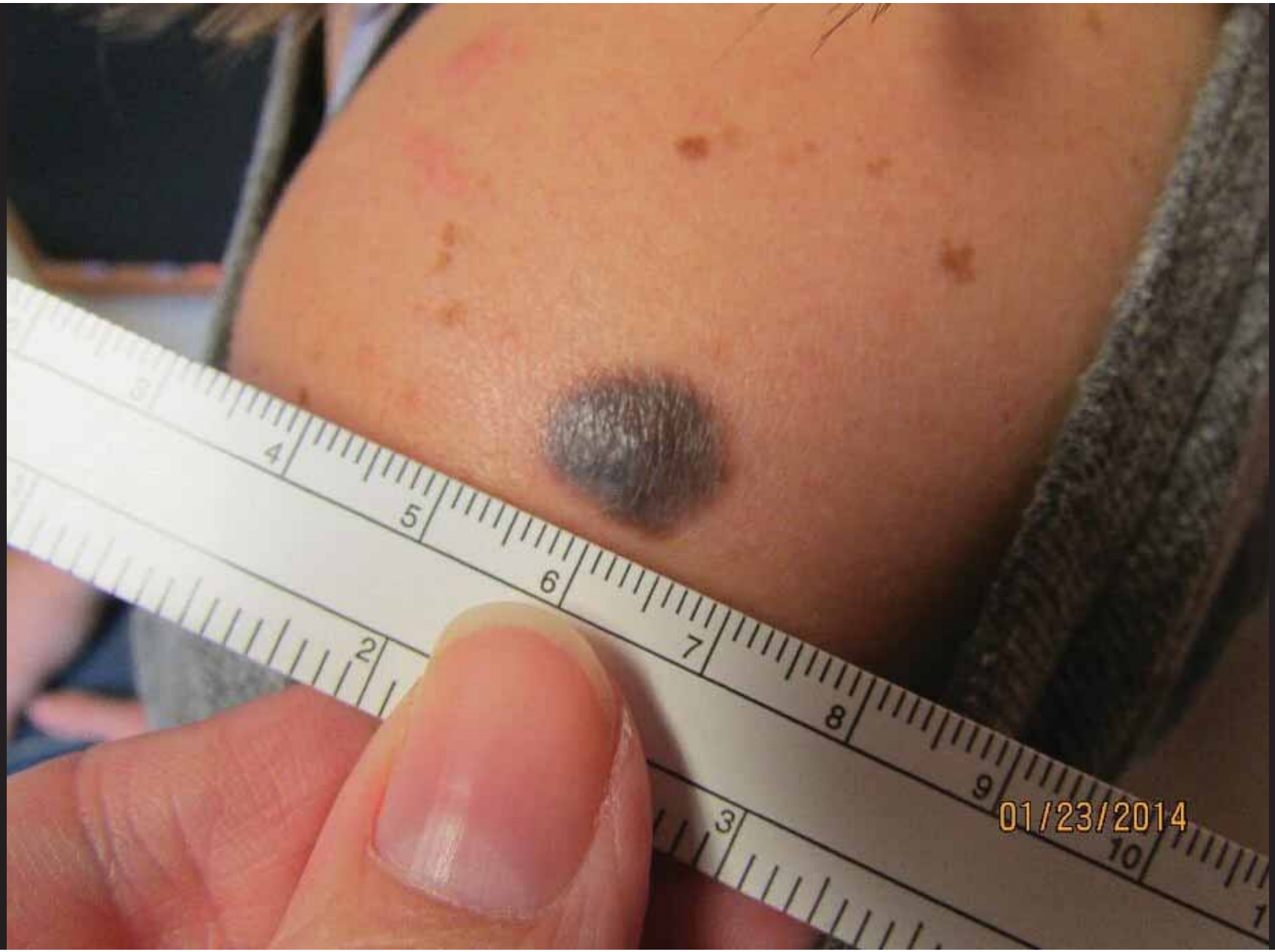
We report a clinical and histologic study of 70 patients, each with a single melanocytic lesion termed "deep penetrating nevus" (DPN). The lesions are most commonly found on the face, upper trunk, or proximal extremities of patients between the ages of 10 and 30 years. Typically they are darkly pigmented. Histologically they are characterized by loosely organized nests of pleomorphic pigmented cells that penetrate deep into the reticular dermis and often to the subcutaneous fat. Follow-up was obtained from 48 patients. It ranged from 1 to 23 years (mean, 7 years). Despite an initial histologic diagnosis of malignant melanoma in 29% of the cases, there were no local recurrences and no distant metastases. It is important to differentiate DPN from malignant melanoma. The characteristic histologic features of DPN also allow its differentiation from spindle cell and epithelioid cell nevi and blue nevi.

**Key Words:** Deep penetrating nevus—Nevus—Malignant melanoma.

*Am J Surg Pathol 13(1): 39–44, 1989.*

"Deep penetrating nevus" (DPN) is a term used at the Armed Forces Institute of Pathology (AFIP) for a distinctive cutaneous pigmented tumor that heretofore has not been defined as an entity. Some lesions described as combined nevus are probably examples of DPN (2). DPN shares some clinical and histologic features with those of blue nevus (BN), cellular BN, and spindle cell and epithelioid cell nevus (SEN). However, when a large number of cases of DPN are examined, they are remarkably uniform histologically and have features that clearly distinguish them, in most cases, from other types of nevi.

Because of their characteristic cellular pleomorphism and deep infiltration of the reticular dermis, lesions of DPN are often misinterpreted by pathologists as malignant melanoma (MM). However, our experience at the AFIP indicates that they are benign. We studied 70 patients with DPN and analyzed the available follow-up information to define and document the diagnostic histologic features and clinical behavior of this cutaneous pigmented lesion.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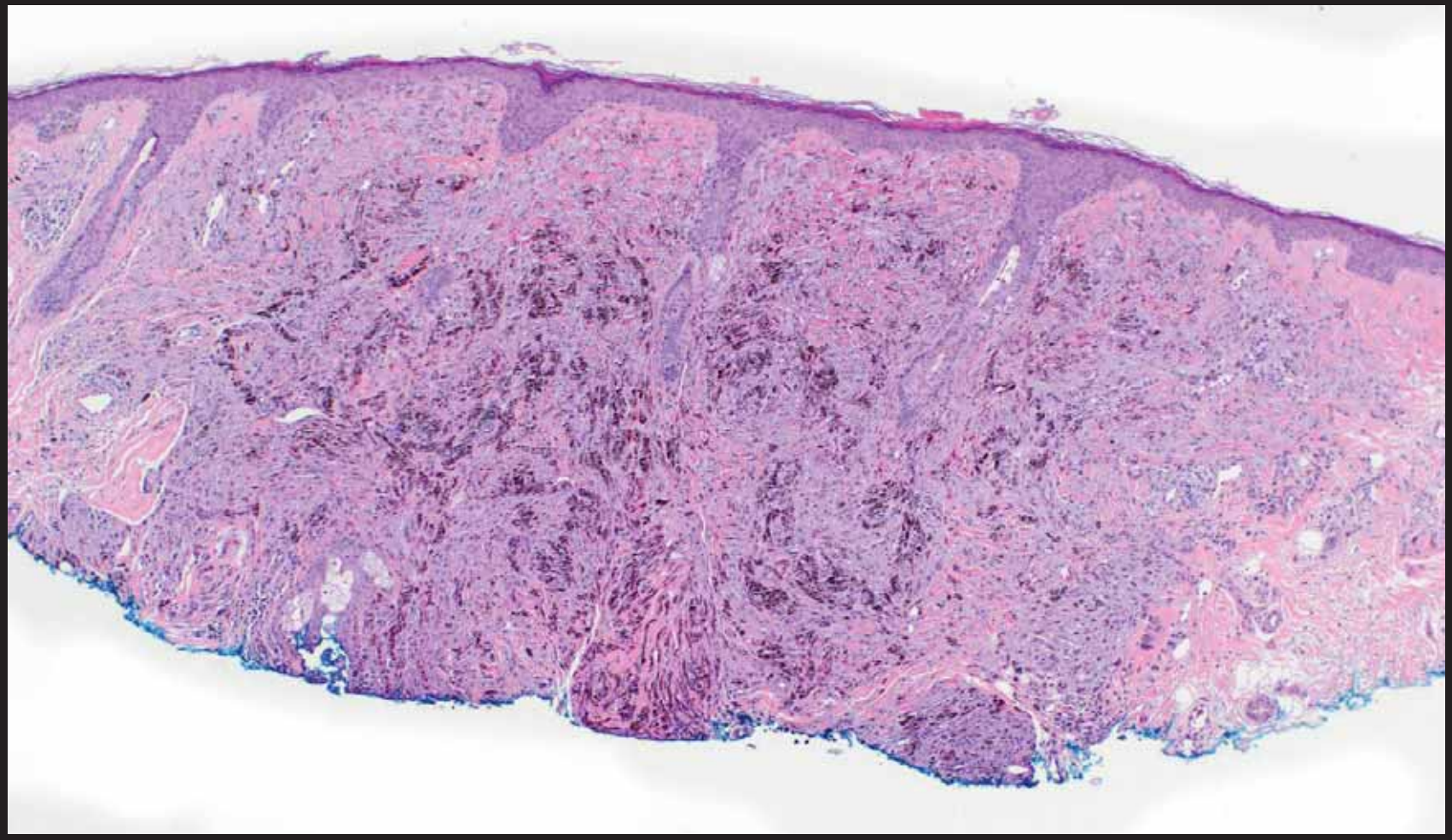
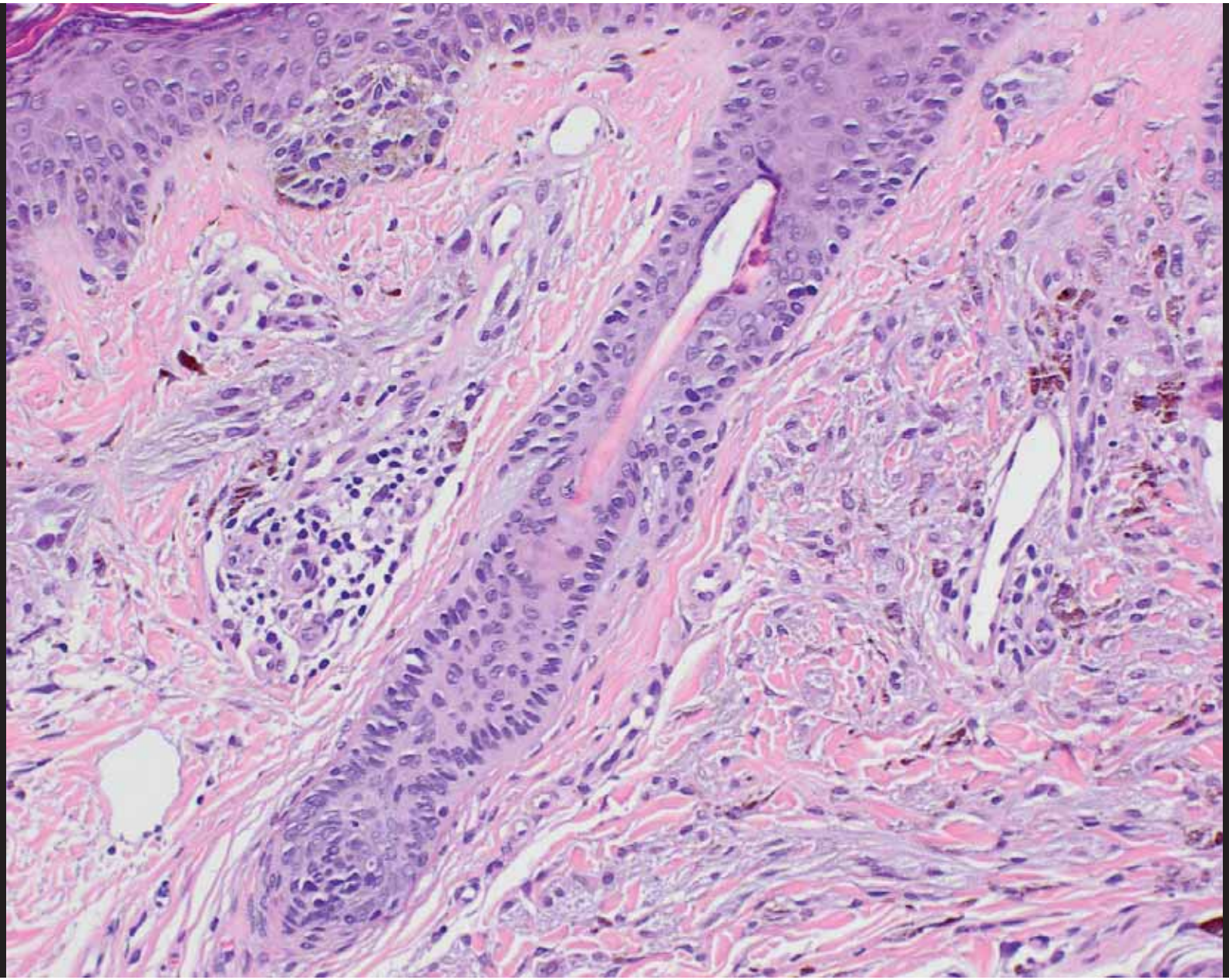
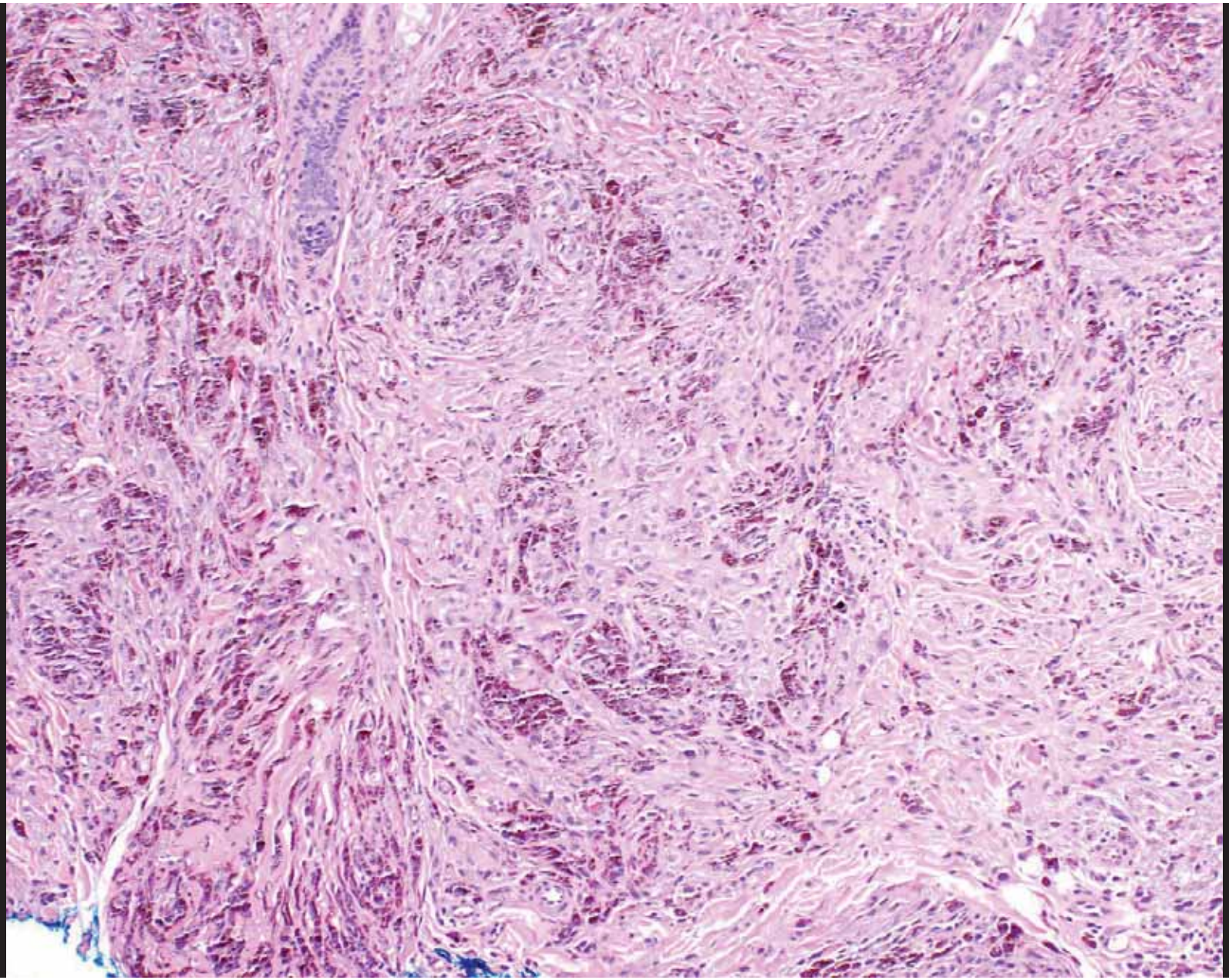
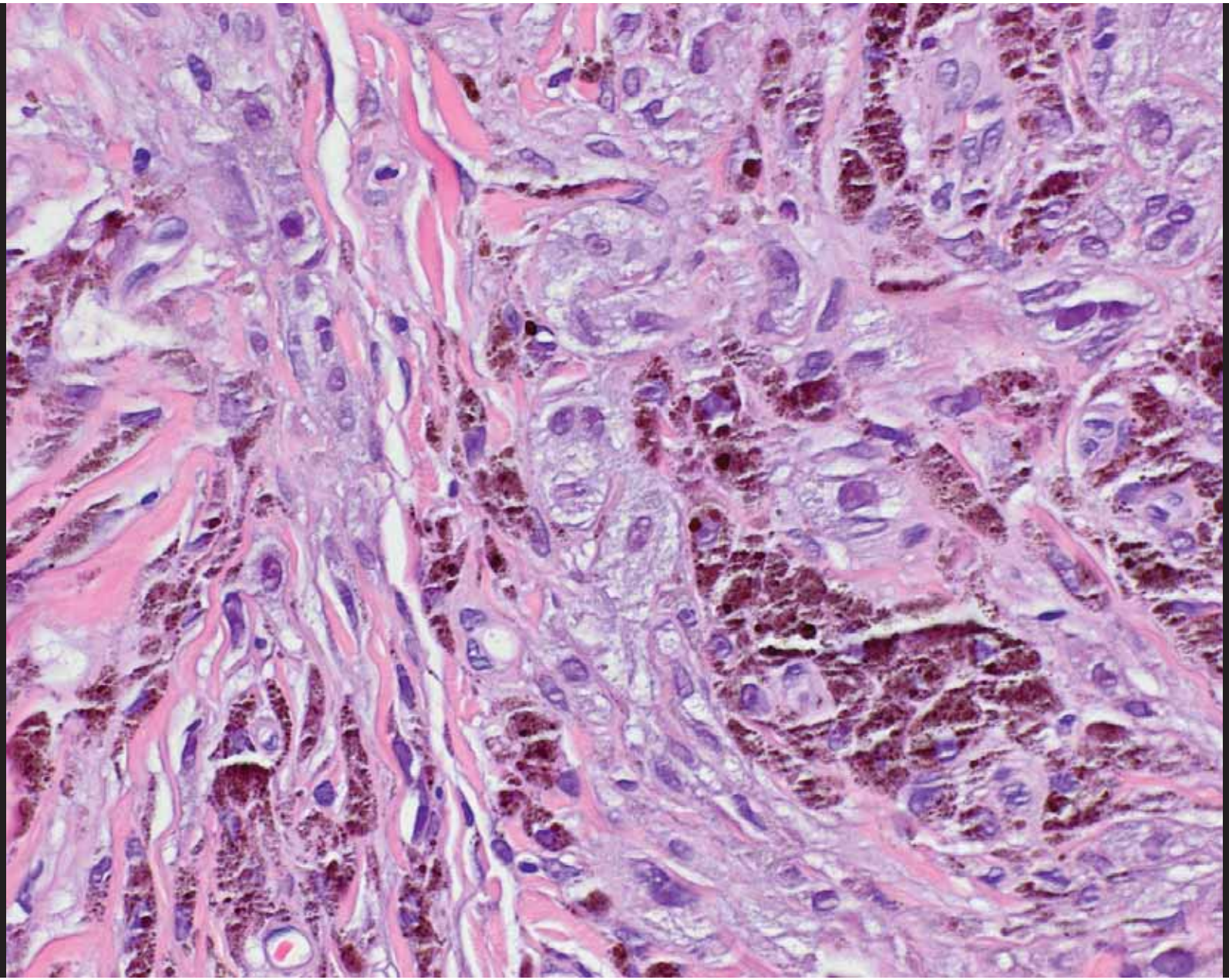








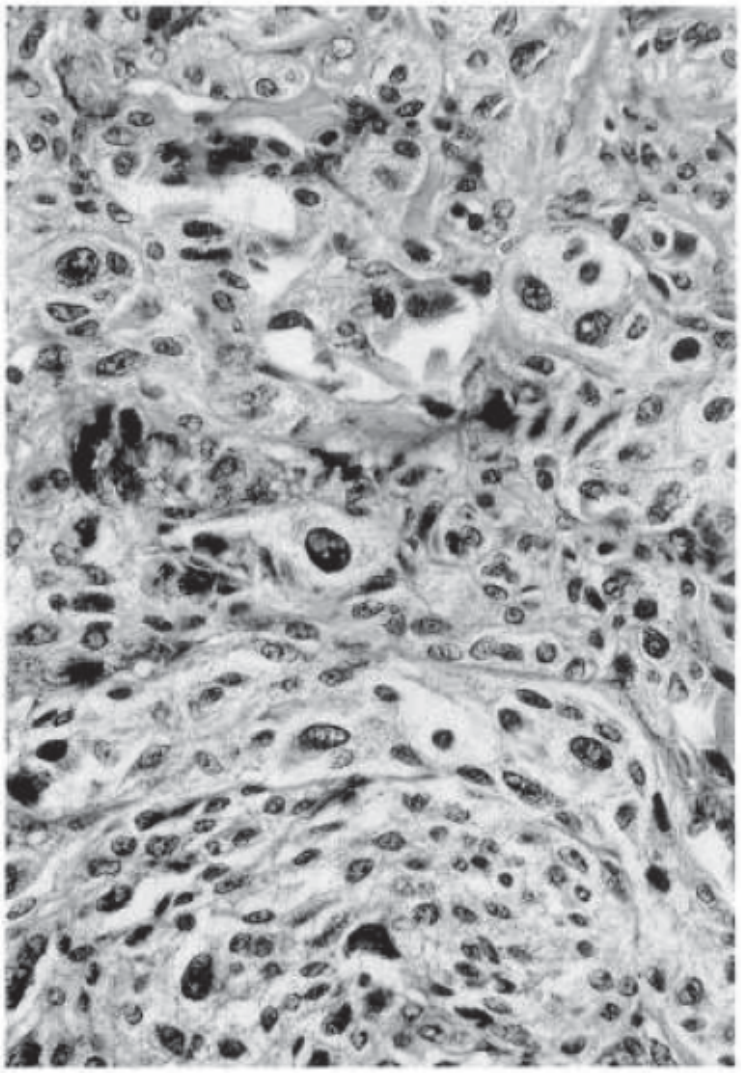


**FIG. 7.** High magnification of a typical lesion of DPN showing pleomorphic nuclei with vacuoles and pseudoinclusions. Note the absence of mitoses.

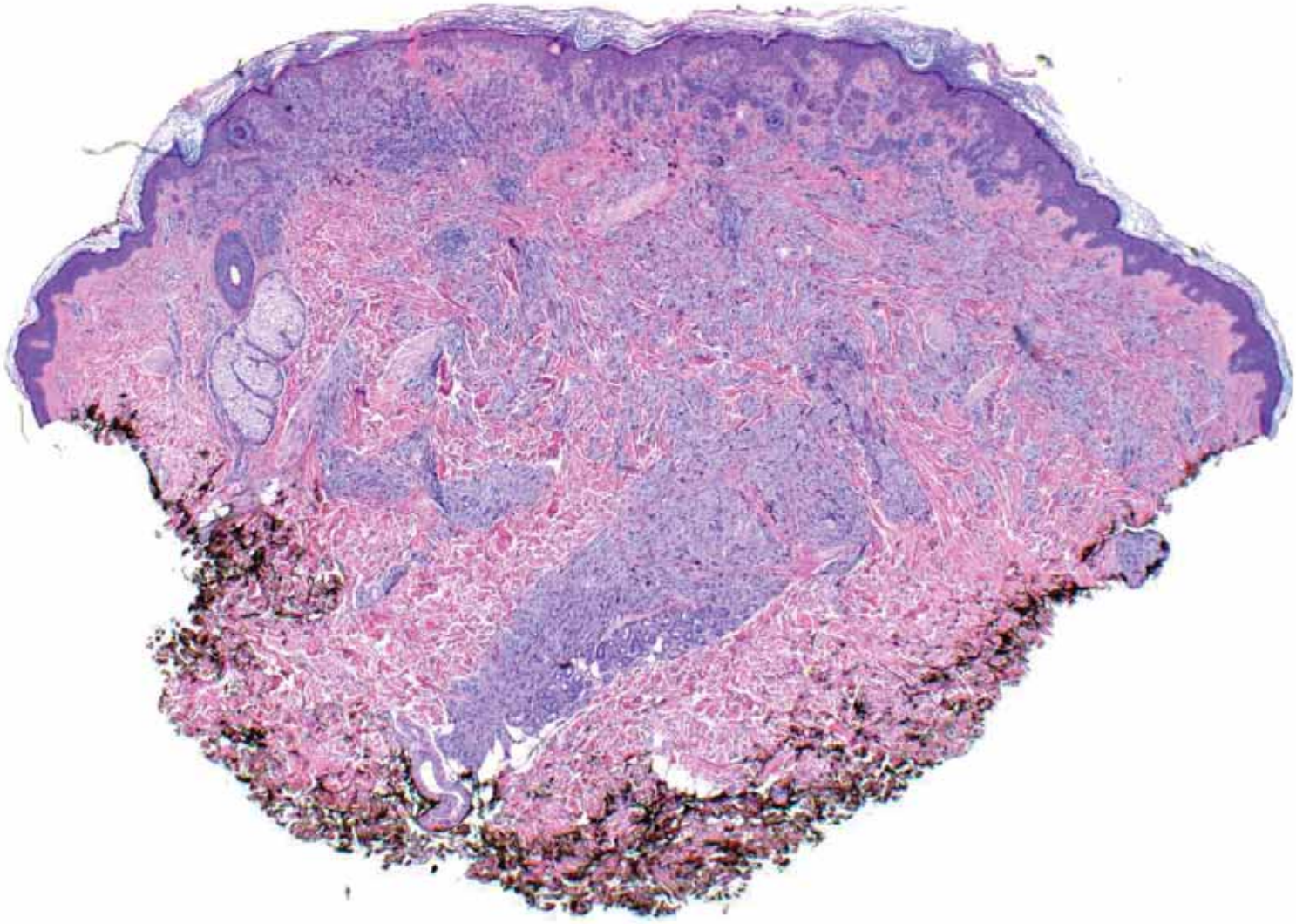
# Deep penetrating (plexiform spindle cell) nevus

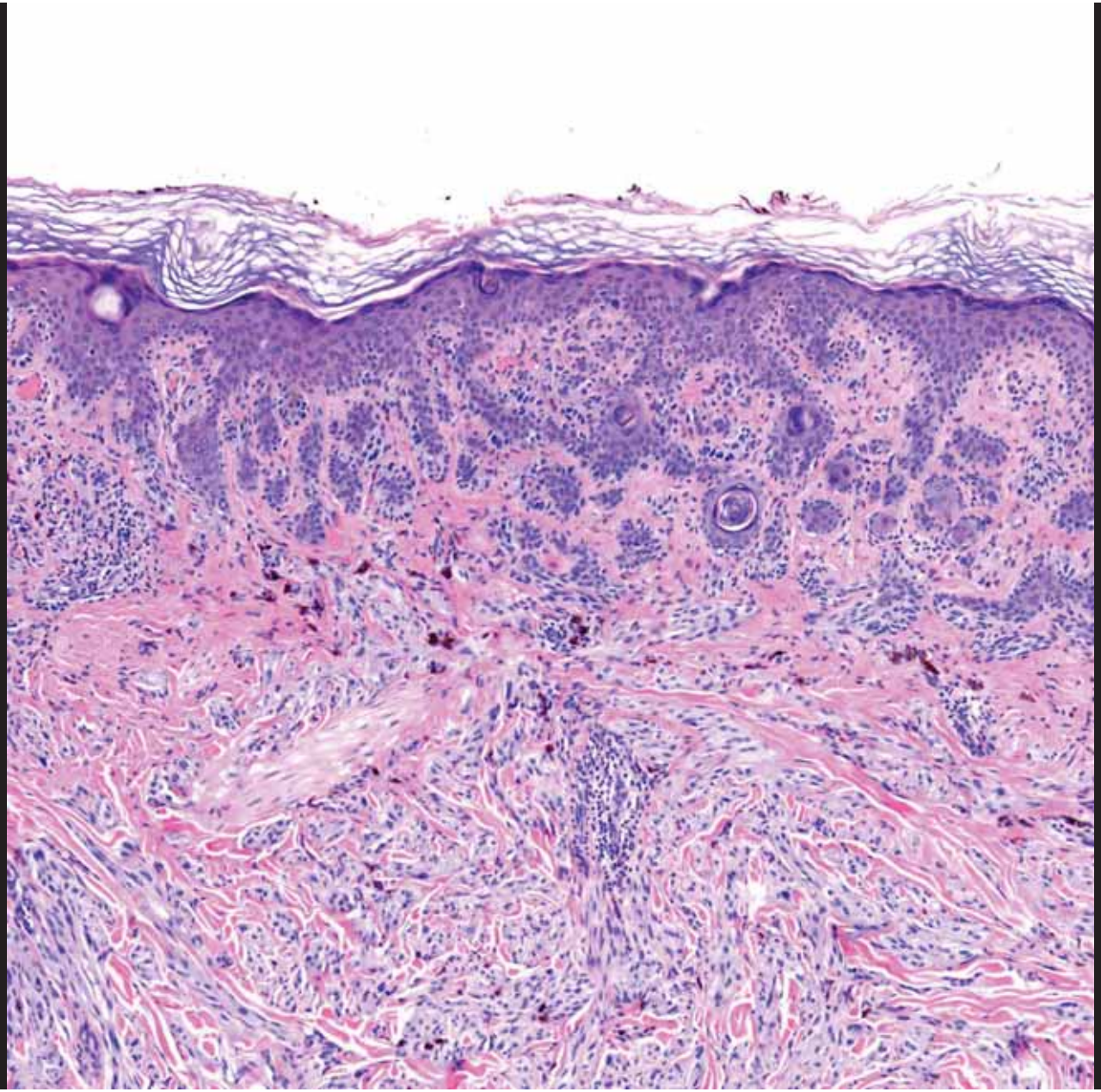
*A frequent participant in combined nevus*

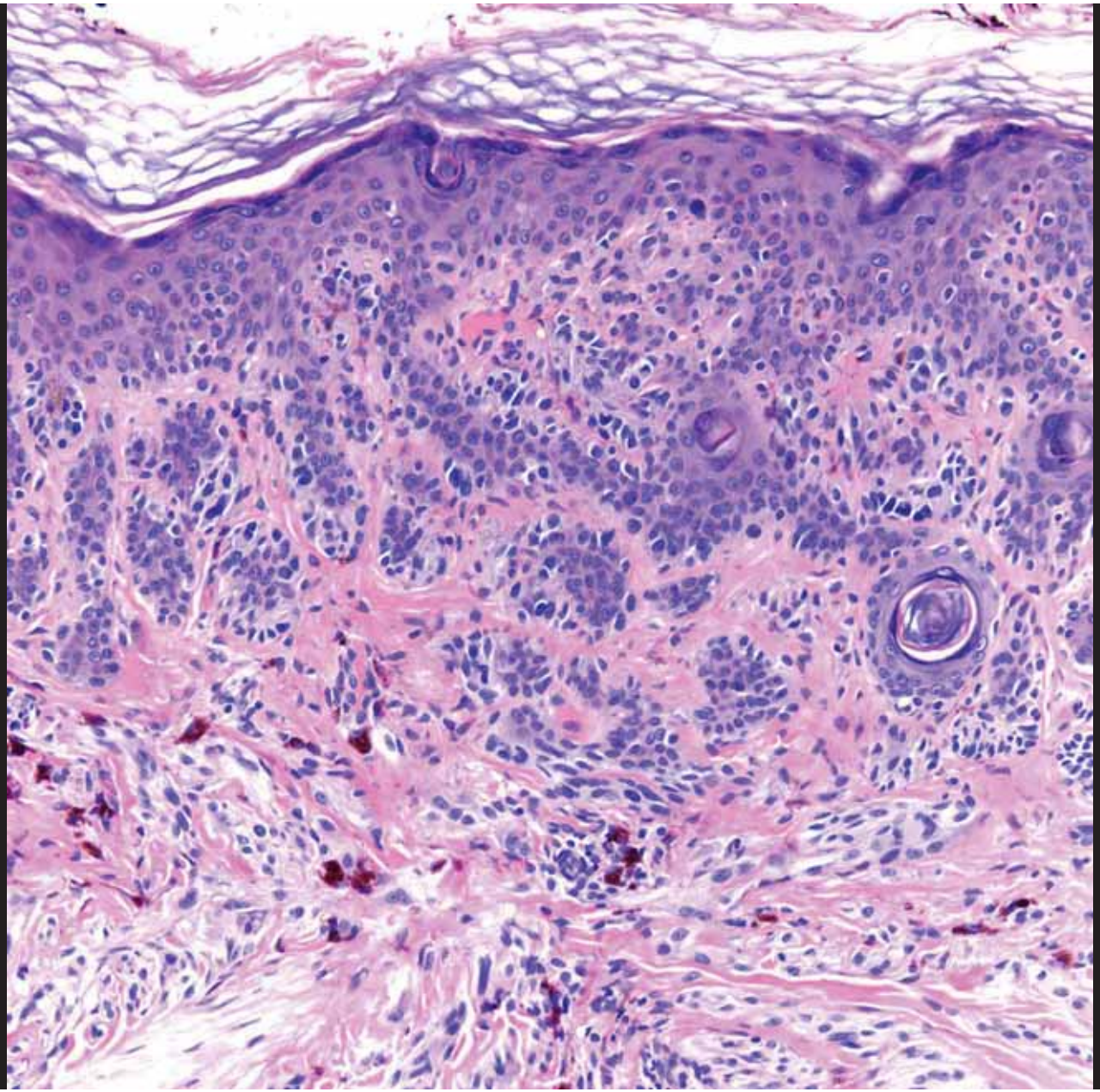
This report describes 41 patients with lesions similar to those previously termed "deep penetrating" or "plexiform spindle cell" nevus (DPN). DPN occurs primarily during the first four decades, is somewhat more common in females, and has a predilection for the face, trunk, and proximal extremities. It is usually

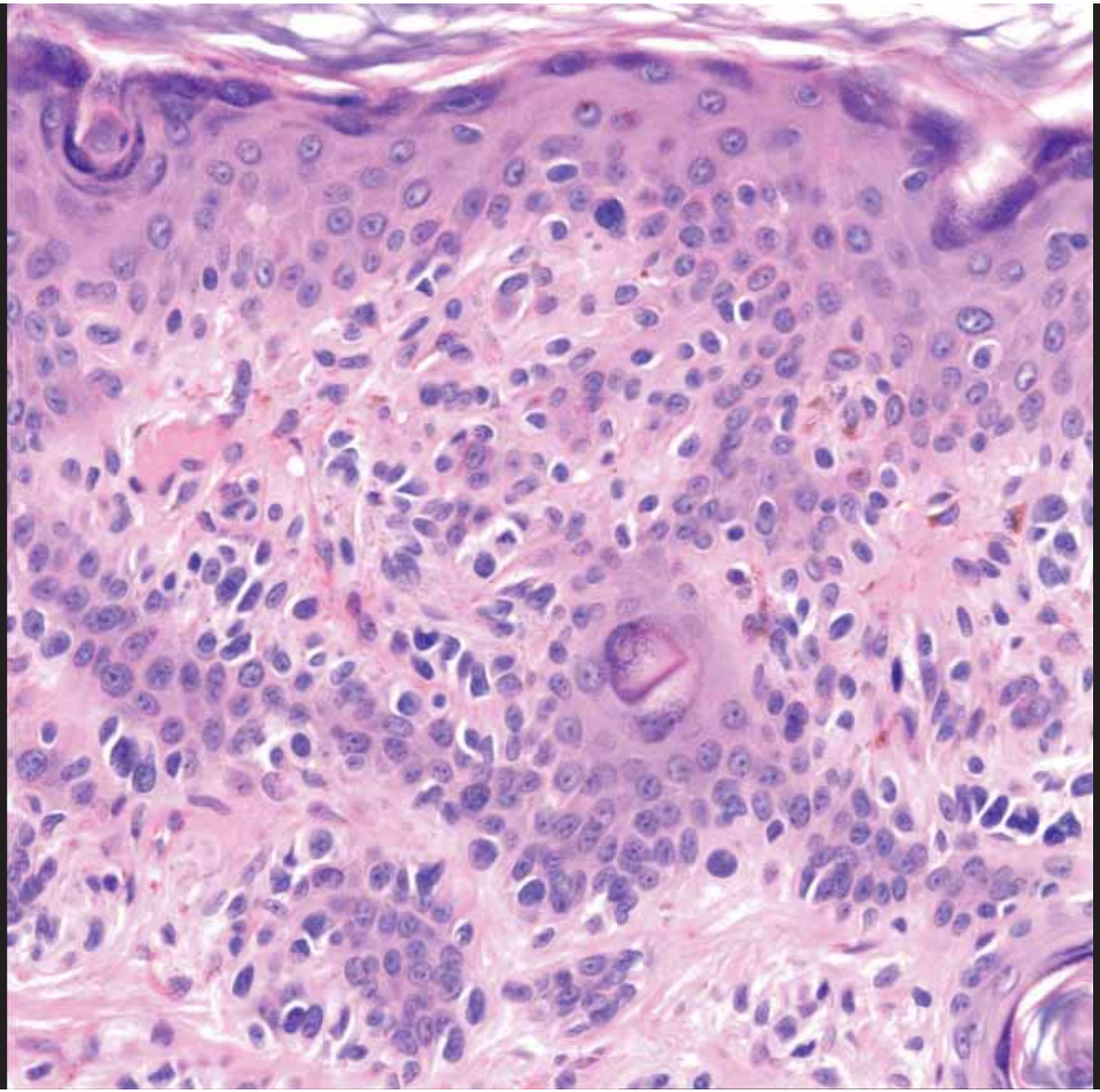
**Philip H. Cooper**
Departments of Pathology and Dermatology, University of Virginia Health Sciences Center, Charlottesvilla, USA

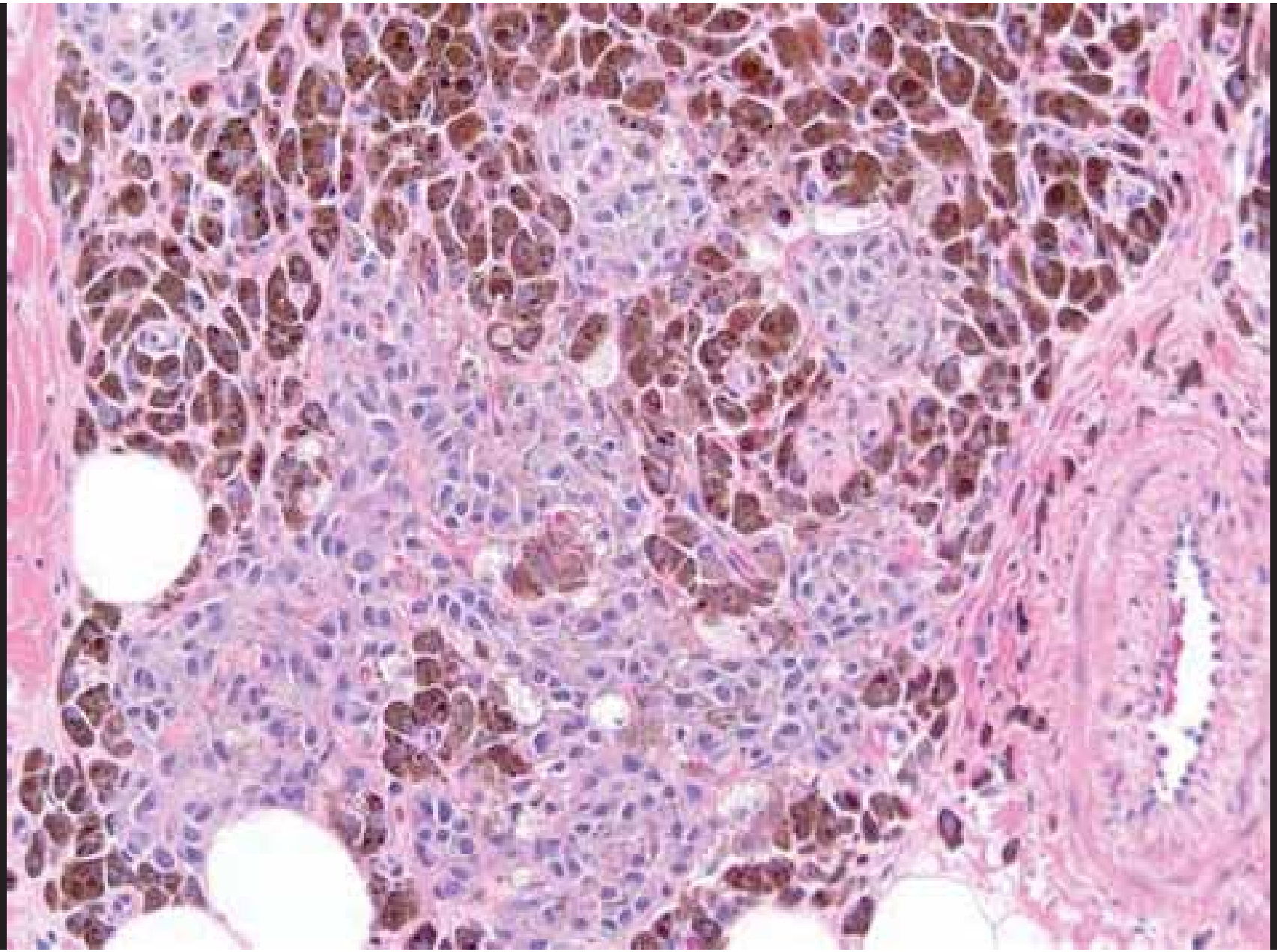
Cooper PH. Deep penetrating (plexiform spindle cell) nevus. A frequent participant in combined nevus. J Cutan Pathol 1992: 19: 172–180.











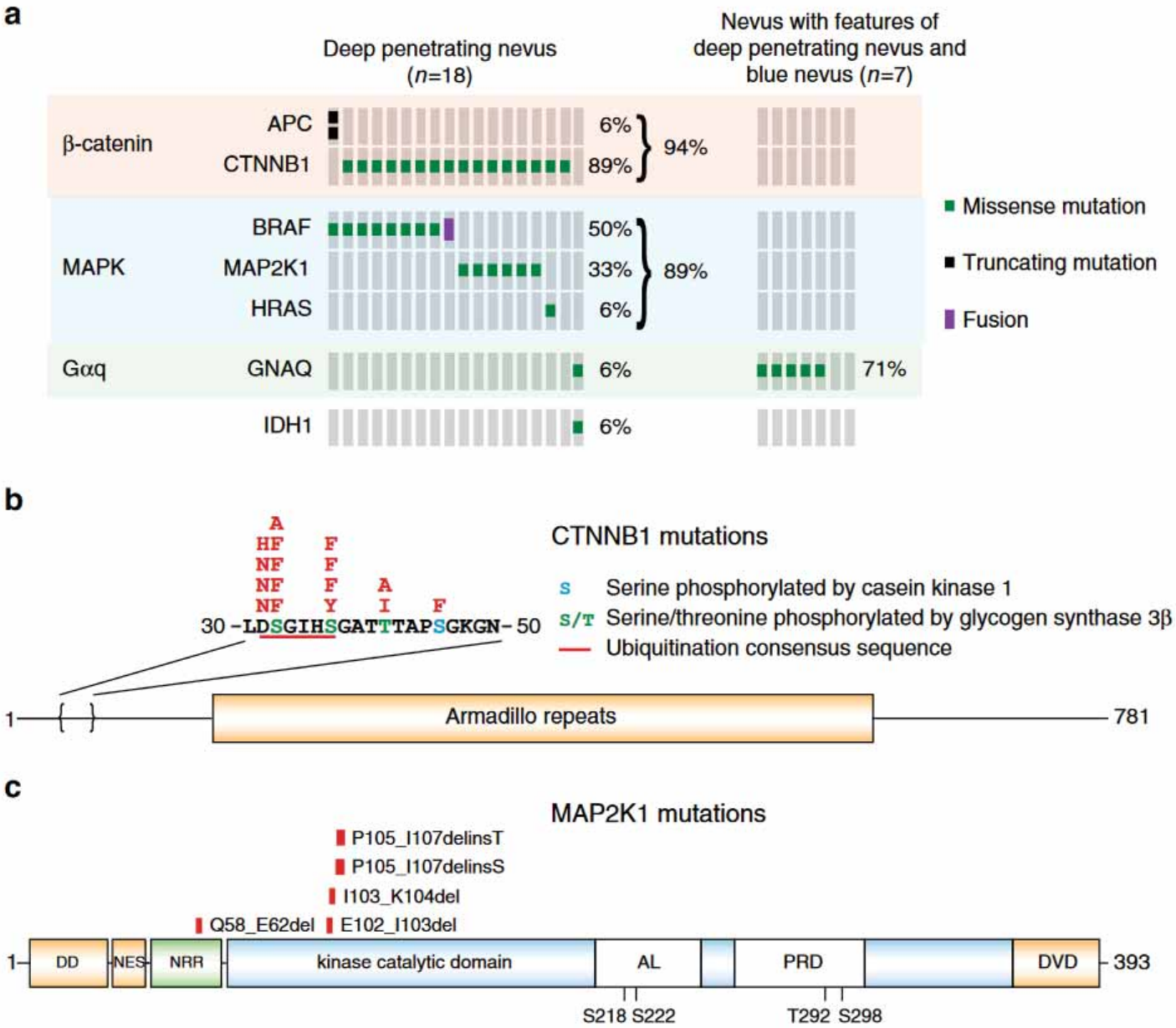
nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-017-00758-3 OPEN

# Combined activation of MAP kinase pathway and β-catenin signaling cause deep penetrating nevi

Iwei Yeh[1,2], Ursula E. Lang[2], Emeline Durieux[3], Meng Kian Tee[1], Aparna Jorapur[1], A. Hunter Shain[1], Veronique Haddad[4], Daniel Pissaloux[4], Xu Chen[1], Lorenzo Cerroni[5], Robert L. Judson[1], Philip E. LeBoit[1,2], Timothy H. McCalmont[1,2], Boris C. Bastian[1,2] & Arnaud de la Fouchardière[4]


a
Deep penetrating nevus (n=18)
Nevus with features of deep penetrating nevus and blue nevus (n=7)
β-catenin
APC
CTNNB1
6%
89%
94%
MAPK
BRAF
MAP2K1
HRAS
50%
33%
6%
89%
Gαq
GNAQ
6%
71%
IDH1
6%
Missense mutation
Truncating mutation
Fusion
b
CTNNB1 mutations
S Serine phosphorylated by casein kinase 1
S/T Serine/threonine phosphorylated by glycogen synthase 3β
Ubiquitination consensus sequence
30 -LDSGIHSGATTTAPSGKGN- 50
1
Armadillo repeats
781
c
MAP2K1 mutations
P105_I107delinsT
P105_I107delinsS
I103_K104del
Q58_E62del
E102_I103del
1
DD
NES
NRR
kinase catalytic domain
AL
PRD
DVD
393
S218 S222
T292 S298

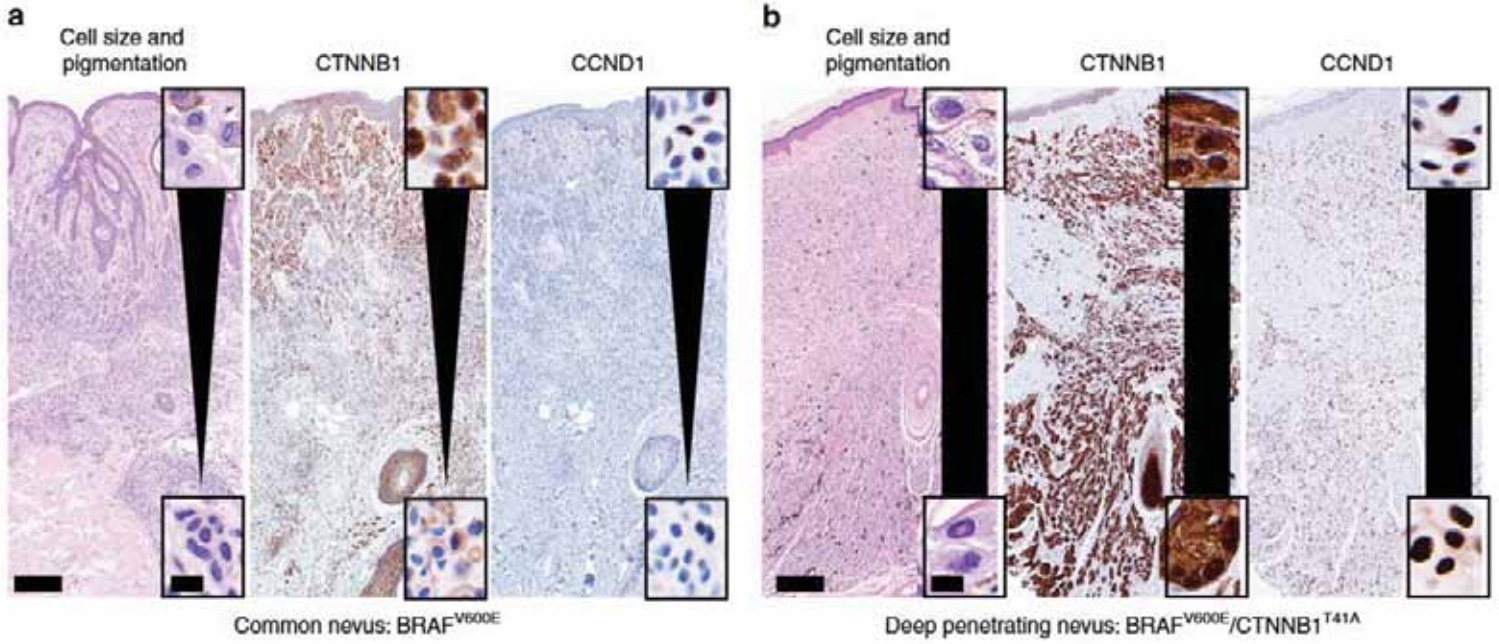
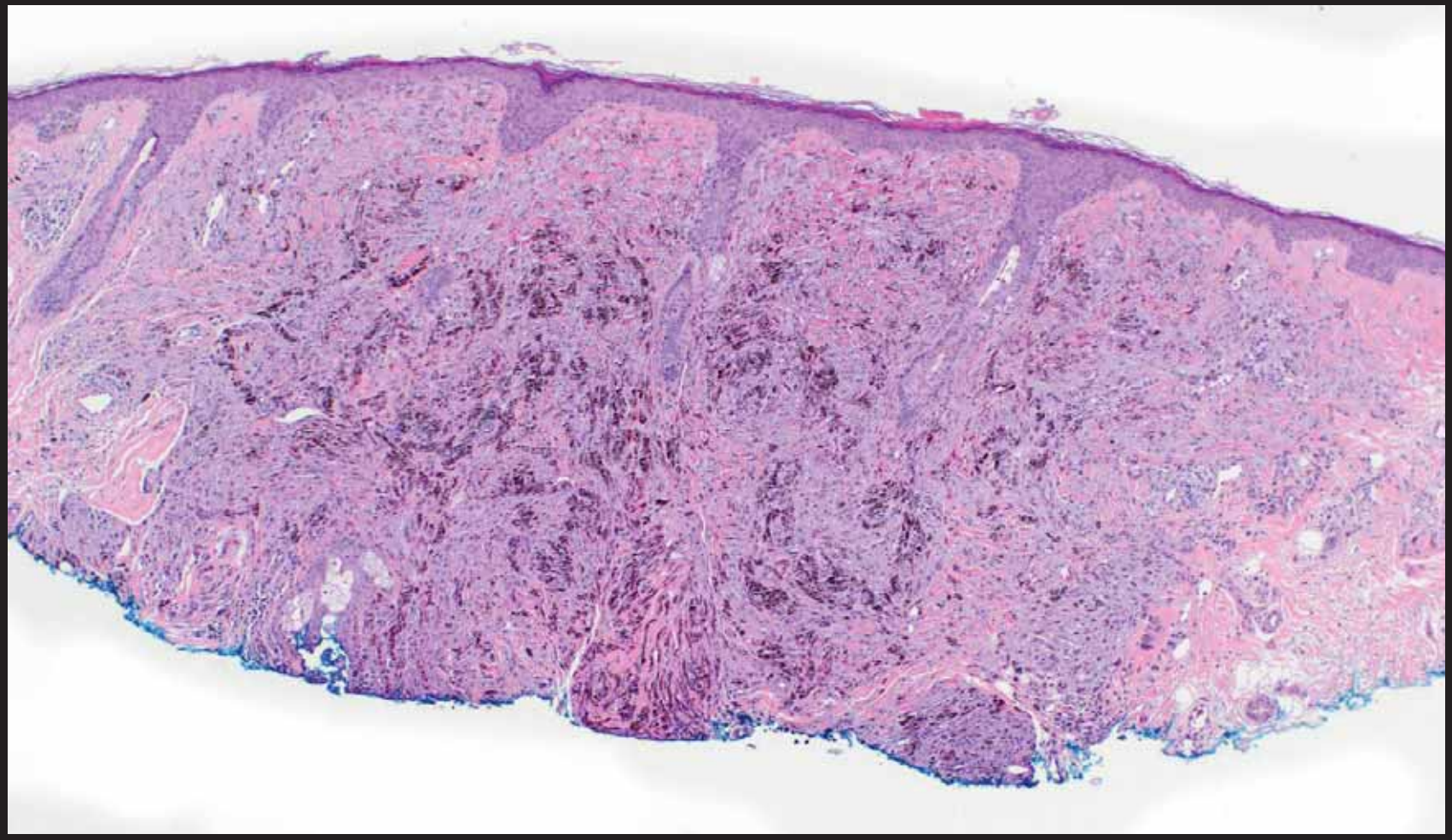
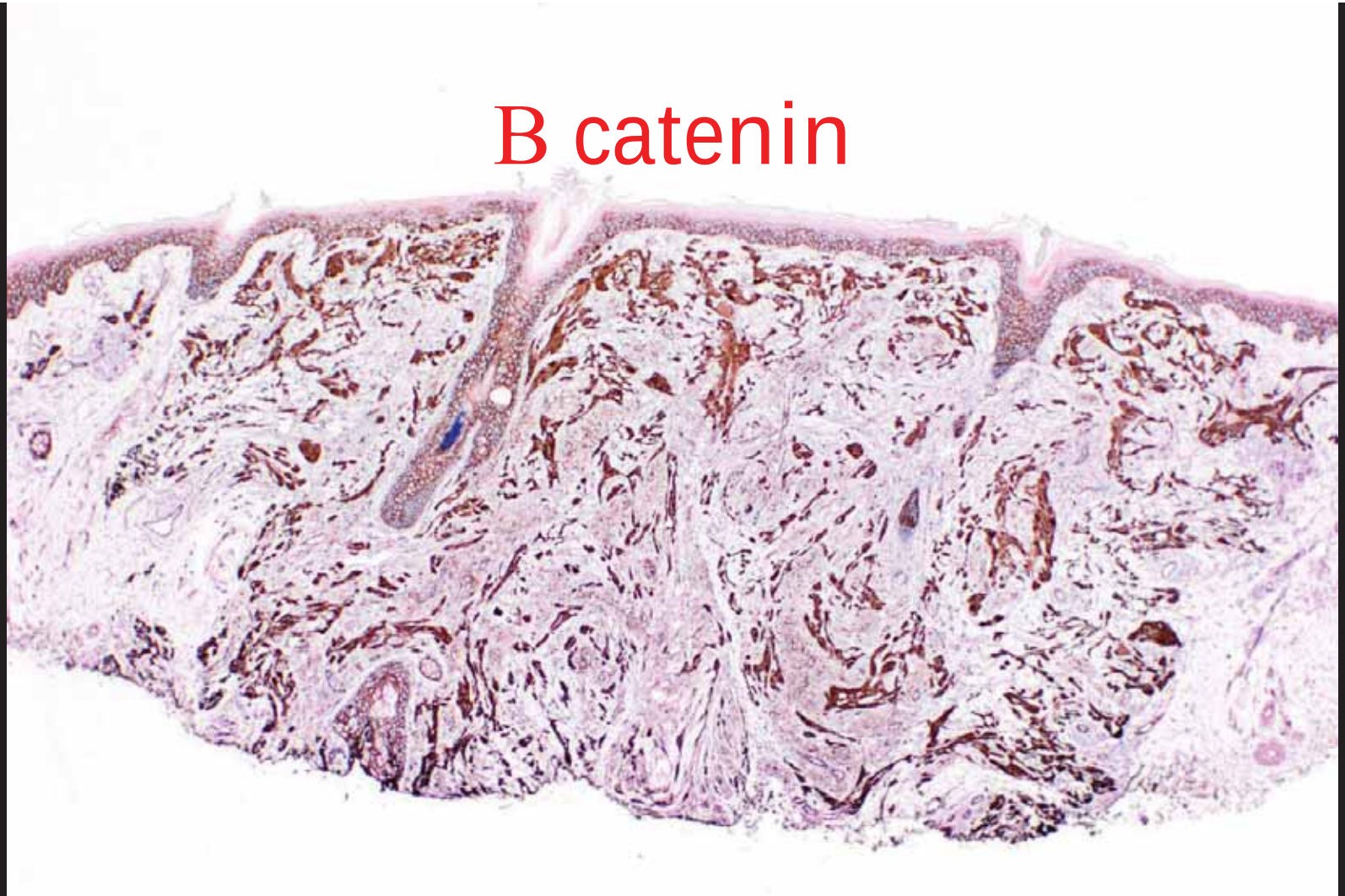
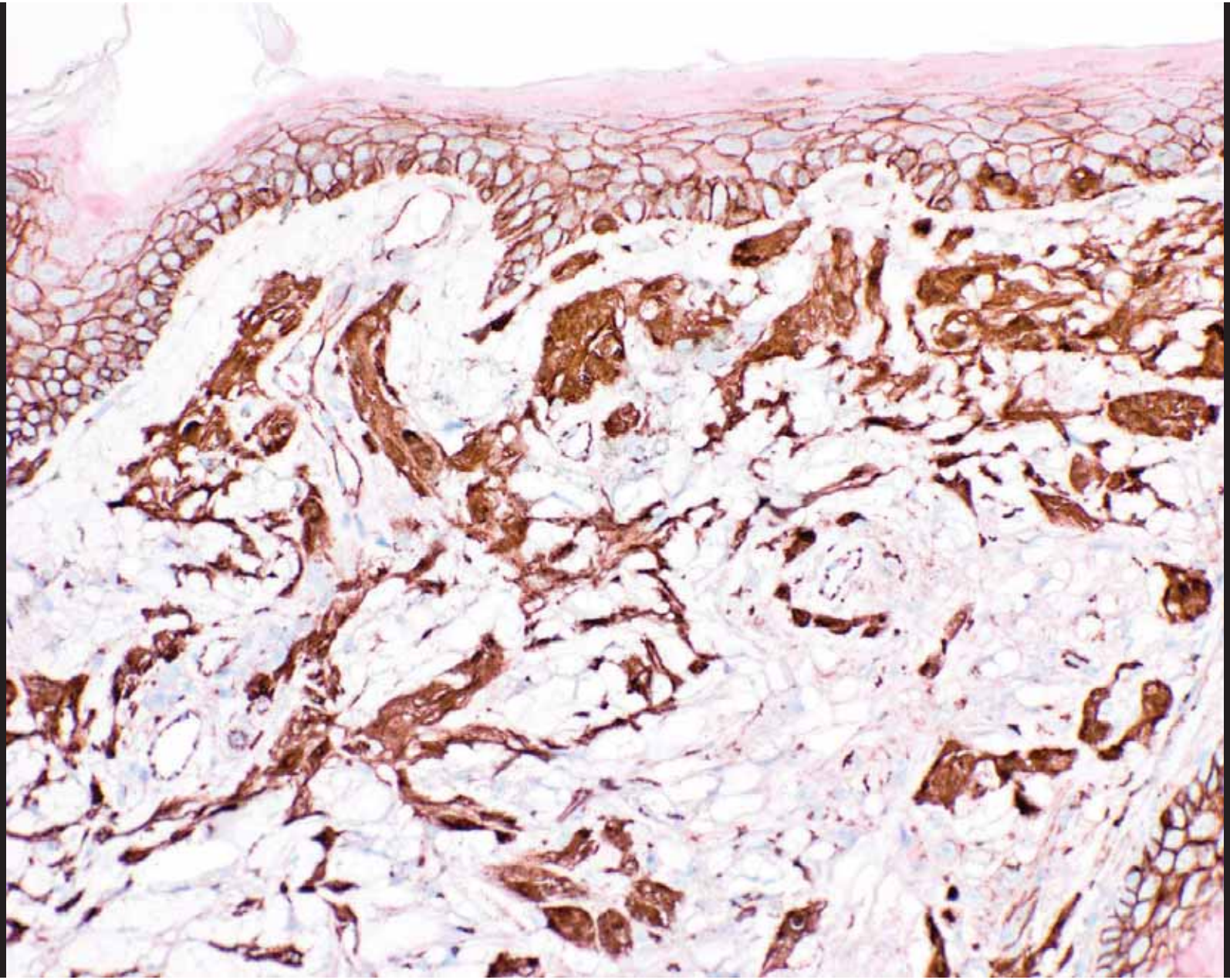


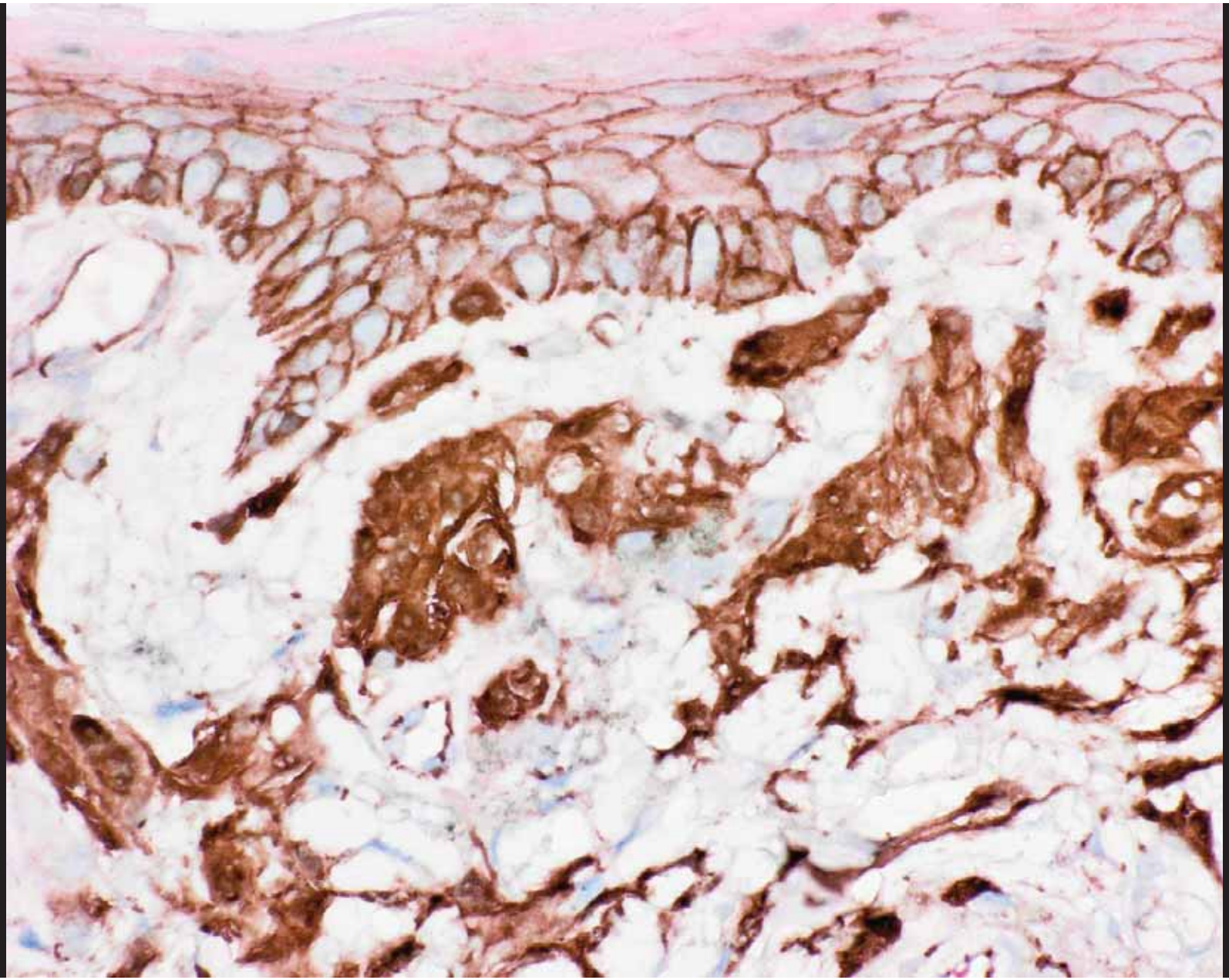

**Fig. 2** Cell size and pigmentation decrease with β-catenin and cyclin D1 levels in common nevi in contrast with deep penetrating nevi. **a** BRAF$^{V600E}$ mutant common nevus. *Scale bars*: 300 microns, inset 12.5 microns. **b** BRAF$^{V600E}$/CTNNB1$^{T41A}$ mutant DPN. *Scale bars*: 250 microns, inset 12.5 microns. Hematoxylin and eosin staining *left* and immunohistochemistry for β-catenin *center* and cyclin D1 *right*. *Insets* show high power views of melanocytes close to the epidermis *top* and in the deep dermis, away from the epidermis *bottom*. Melanocyte size and pigmentation diminish and β-catenin and cyclin D1 expression levels decrease with distance from epithelium in common nevus **a** but not in DPN **b**



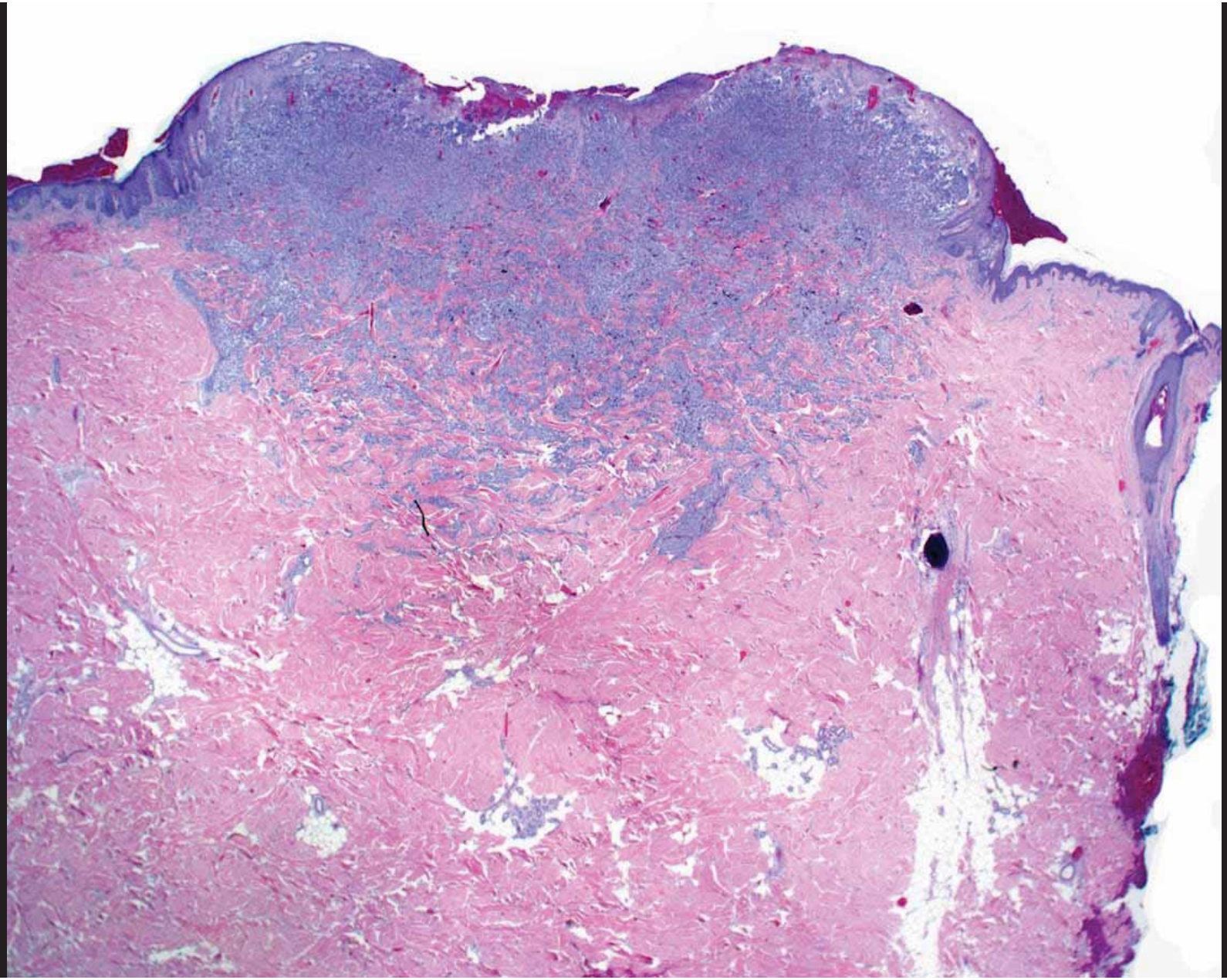
# B catenin

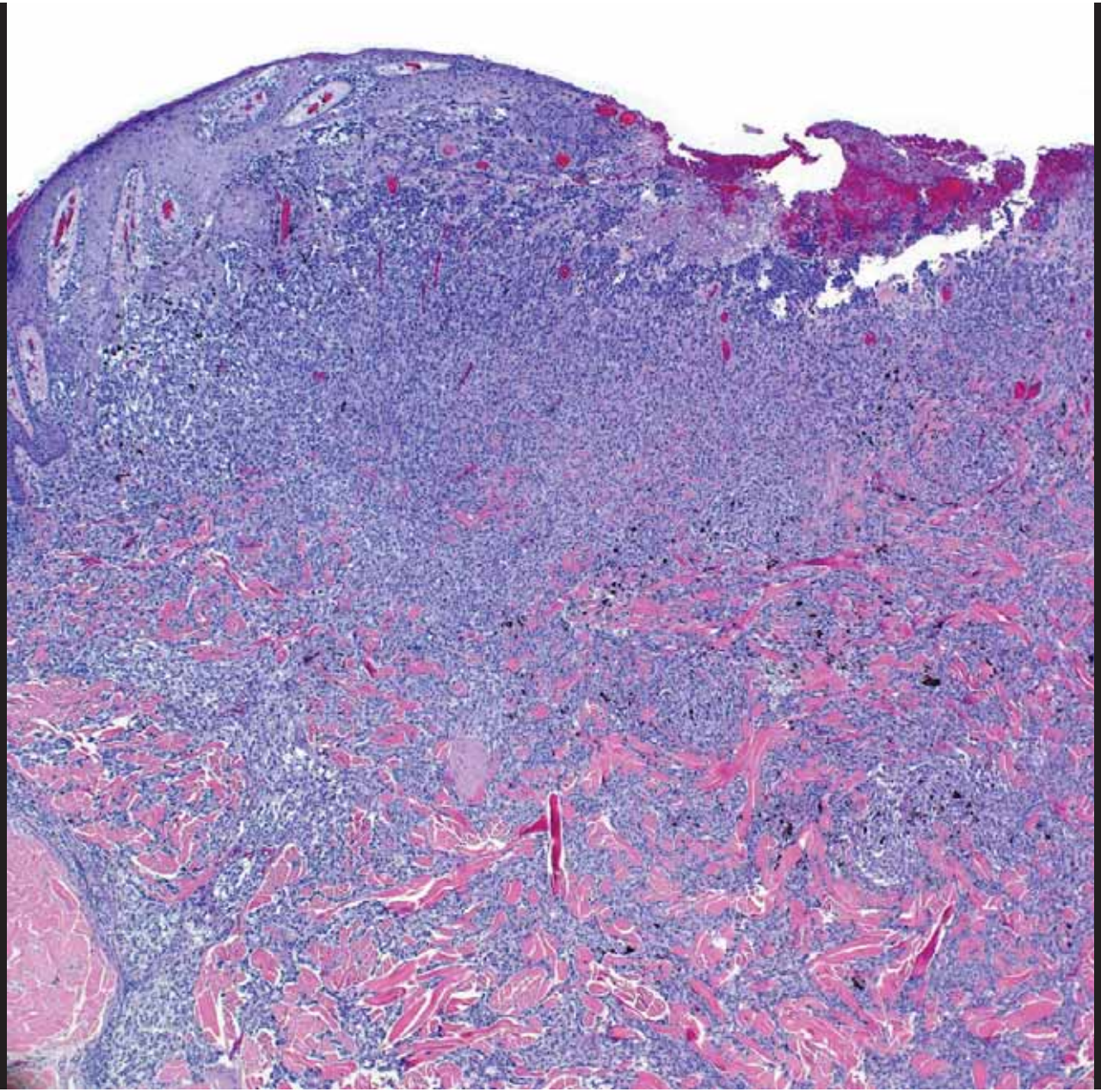


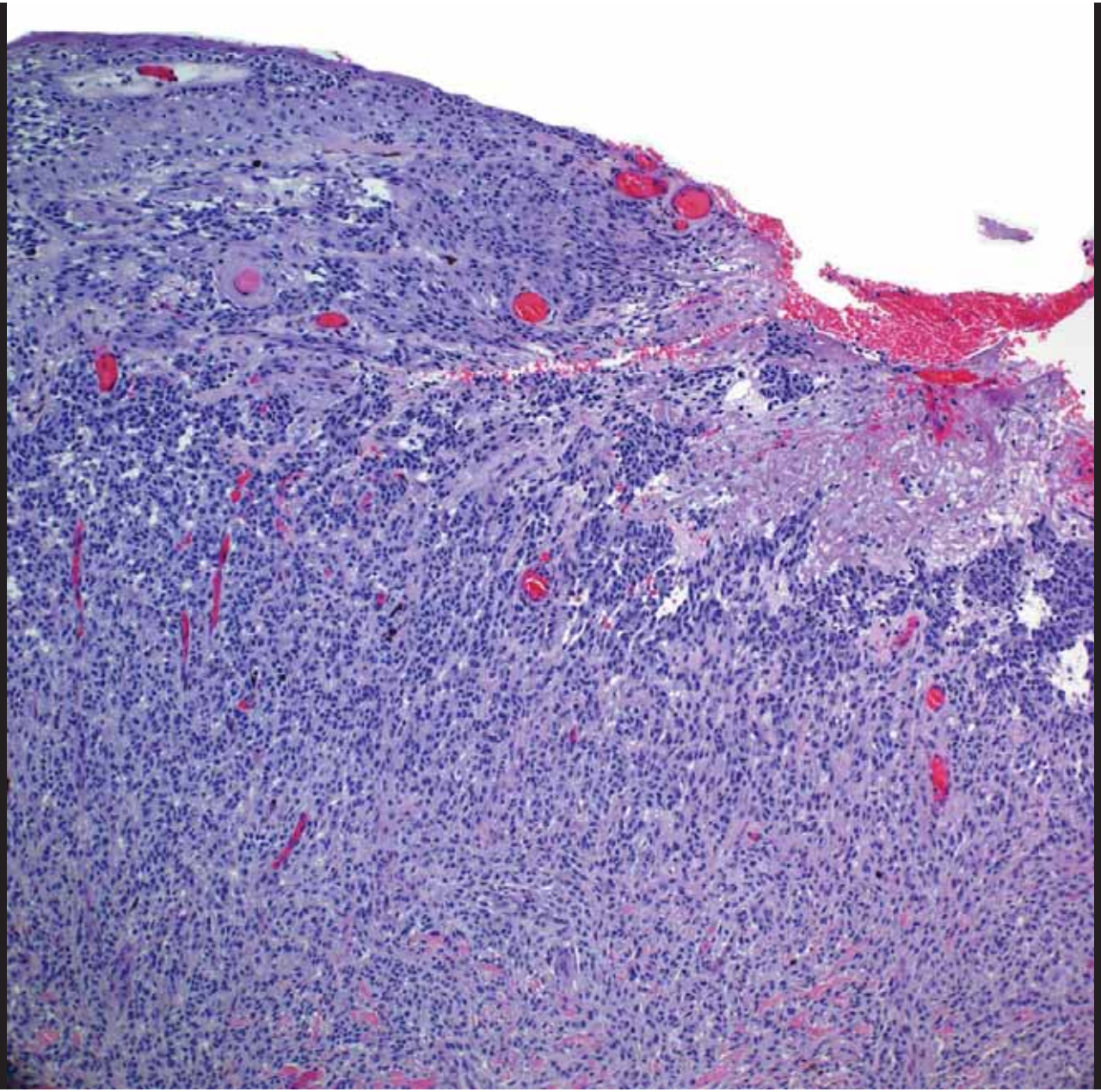


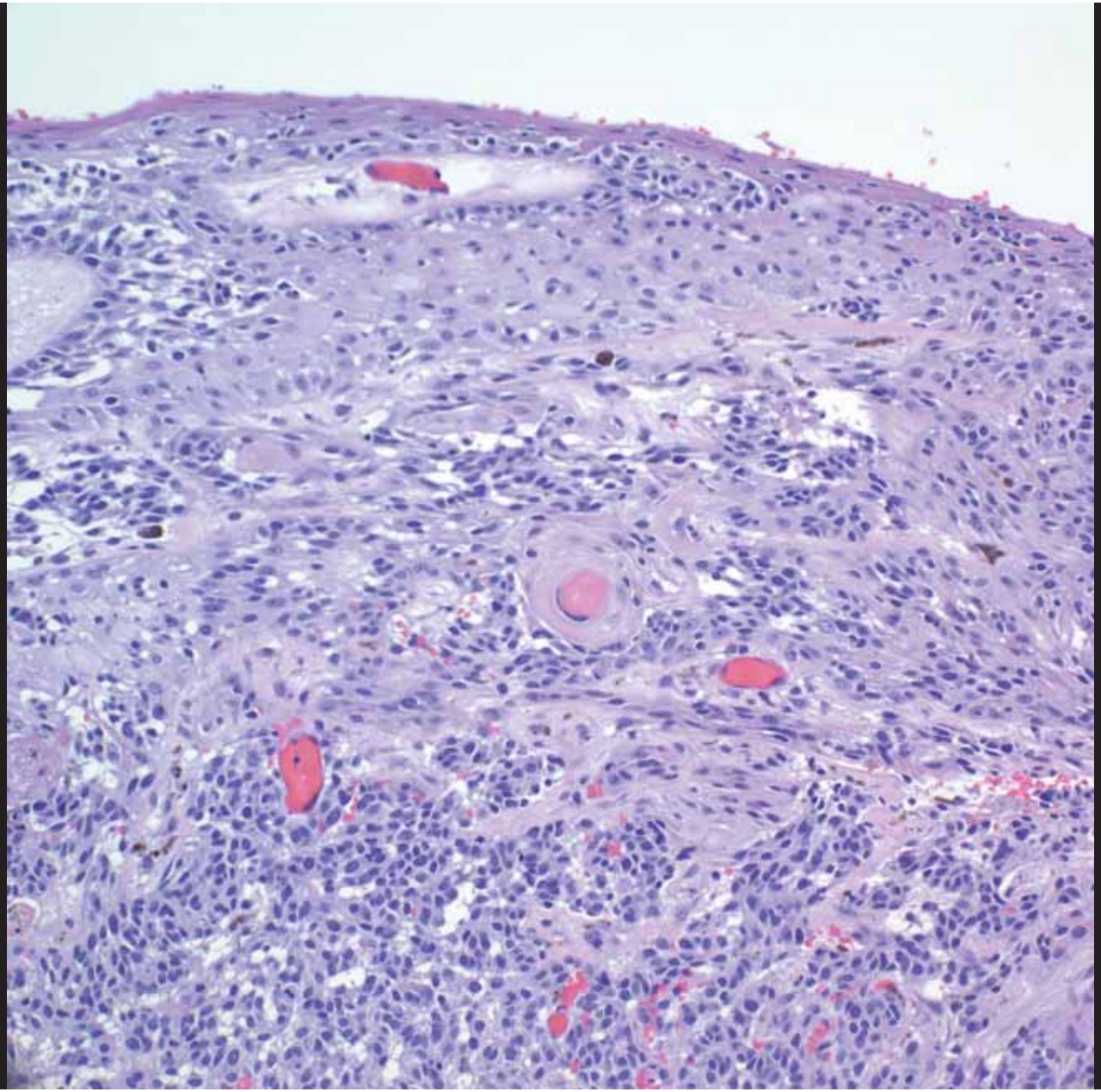


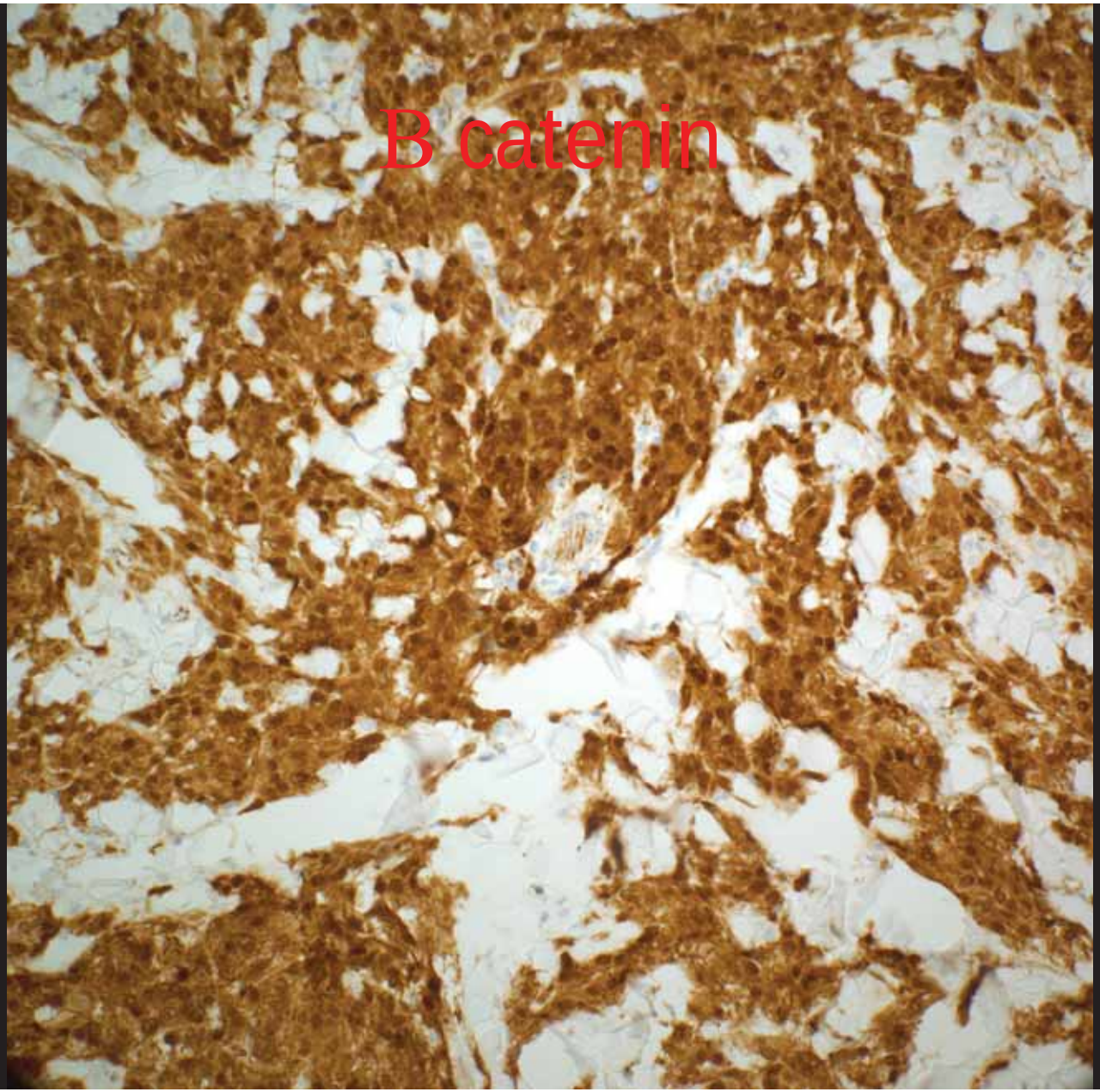
# Deep penetrating nevus-like melanoma / WNT activated melanoma

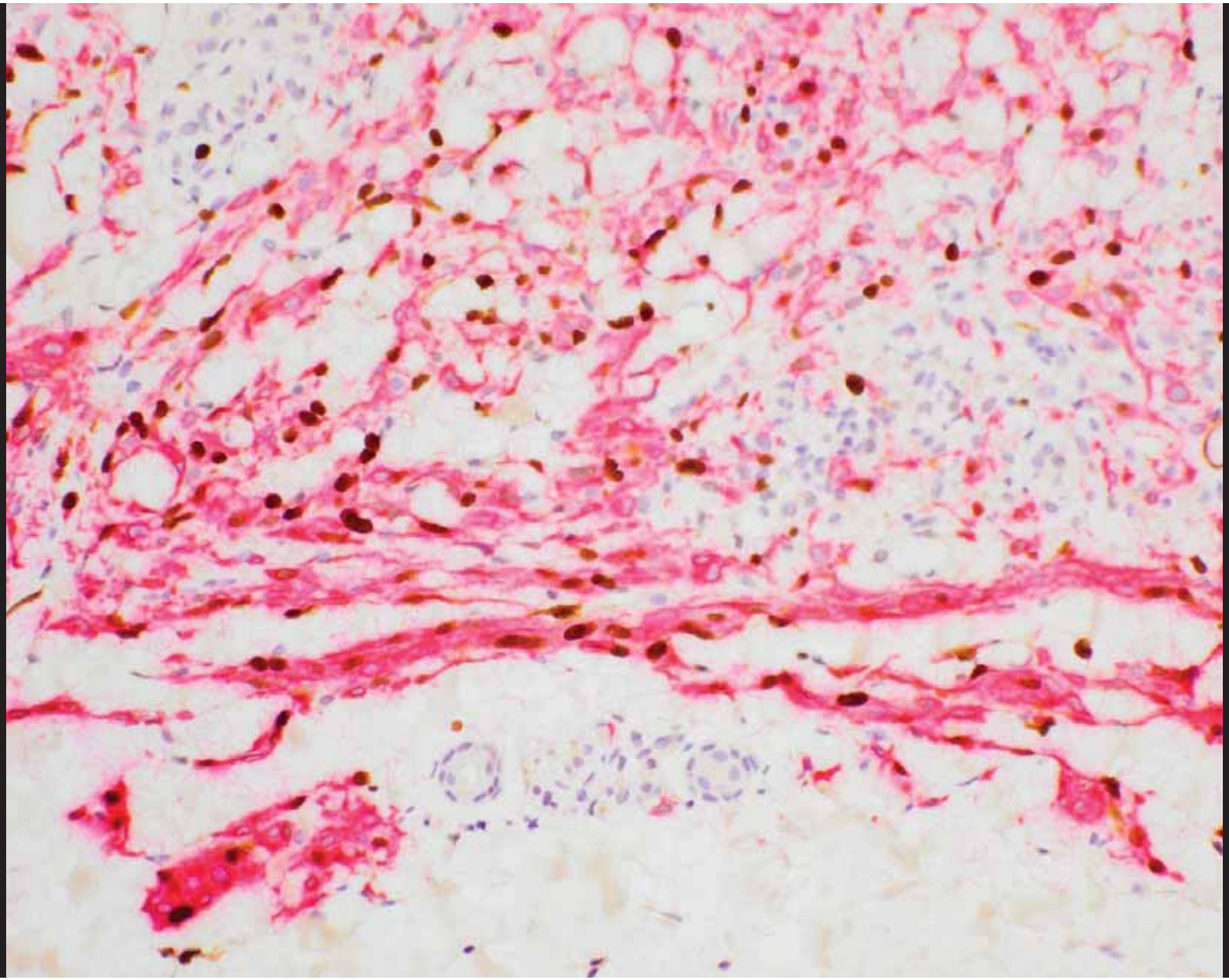


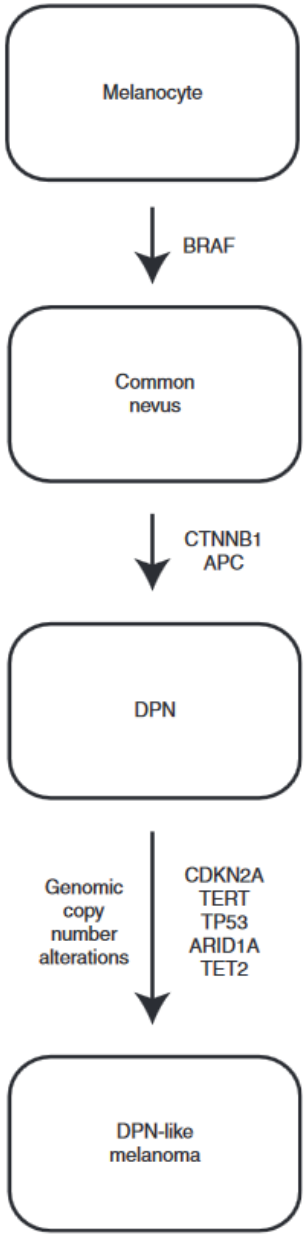


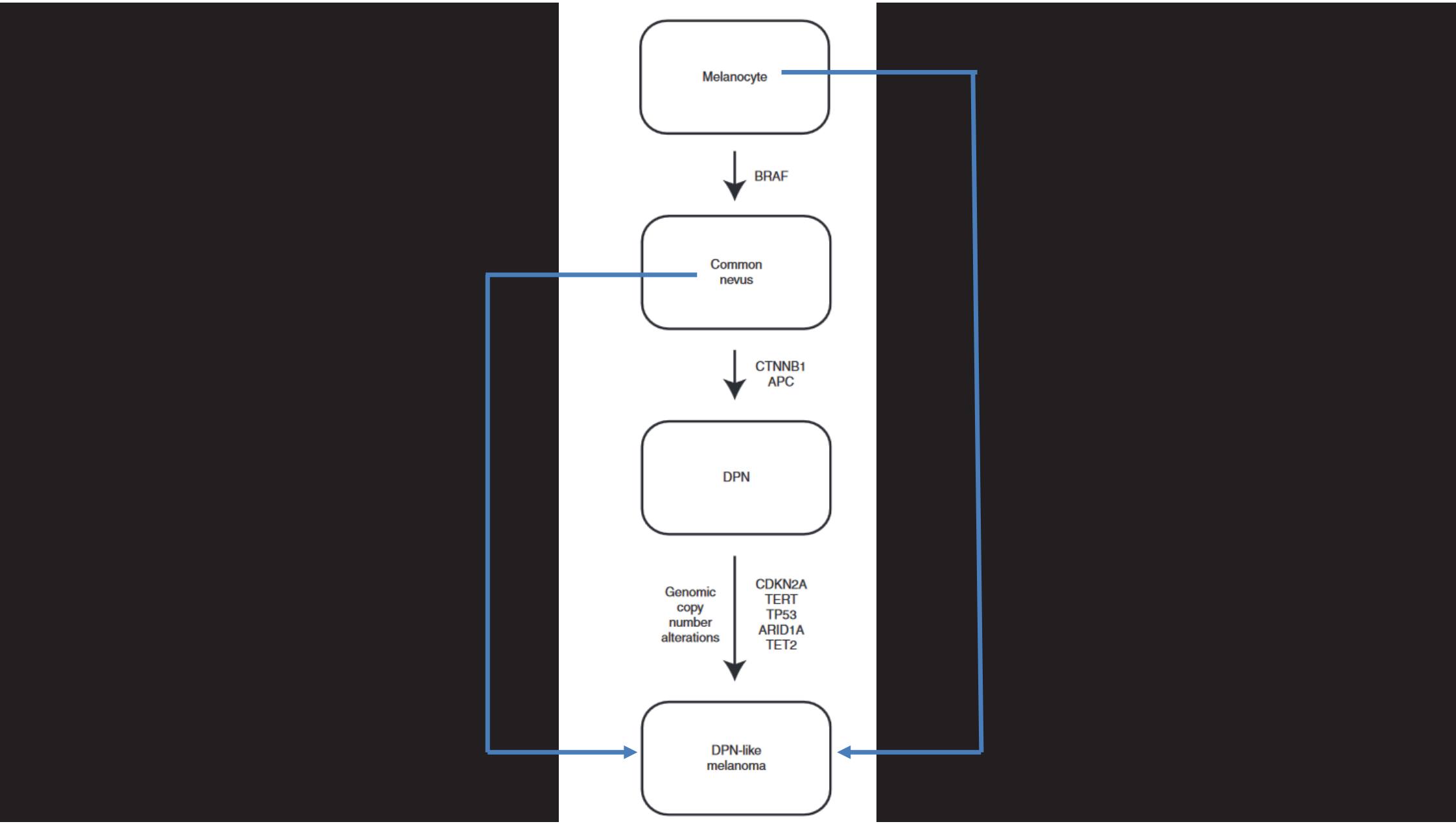




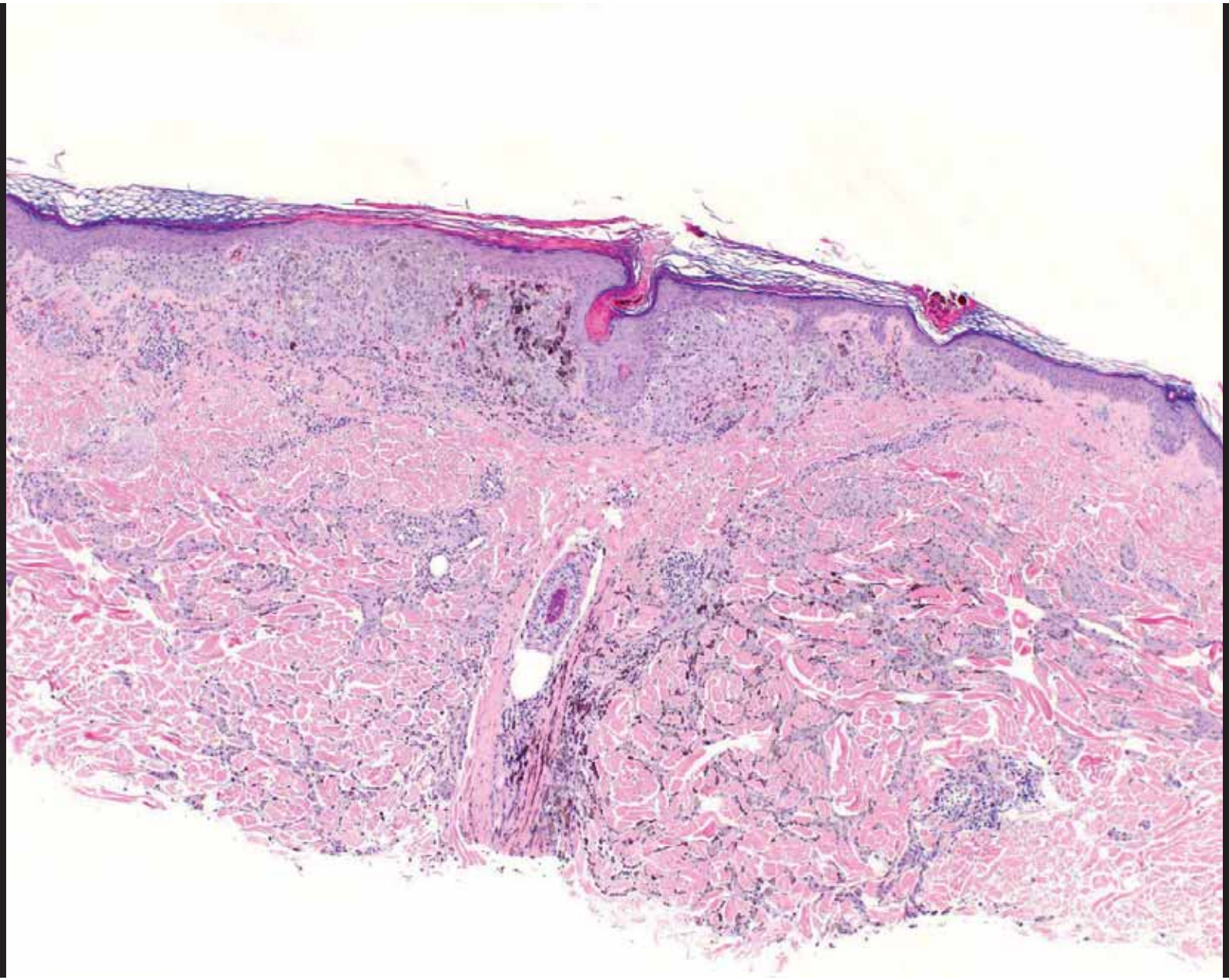

B catenin

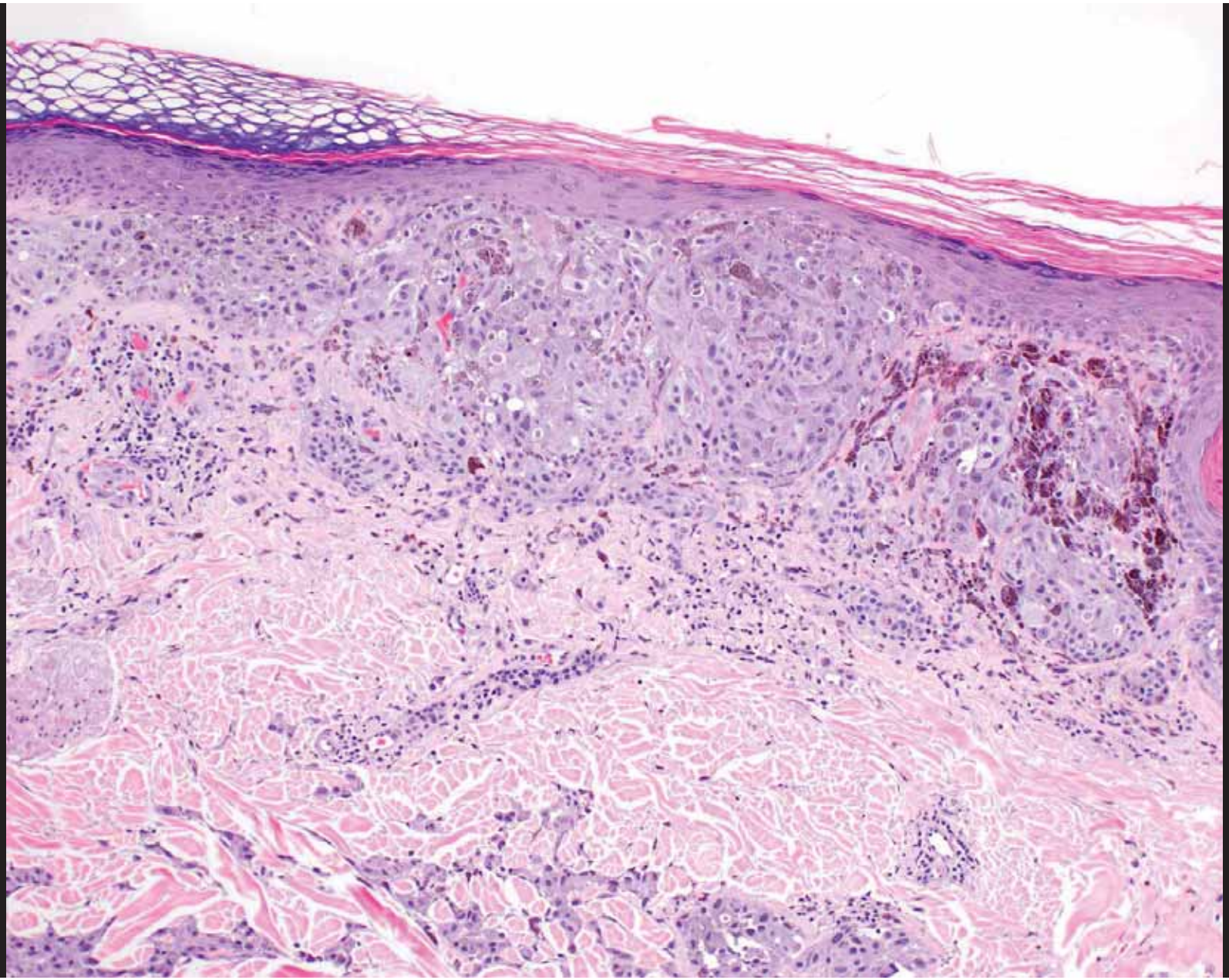


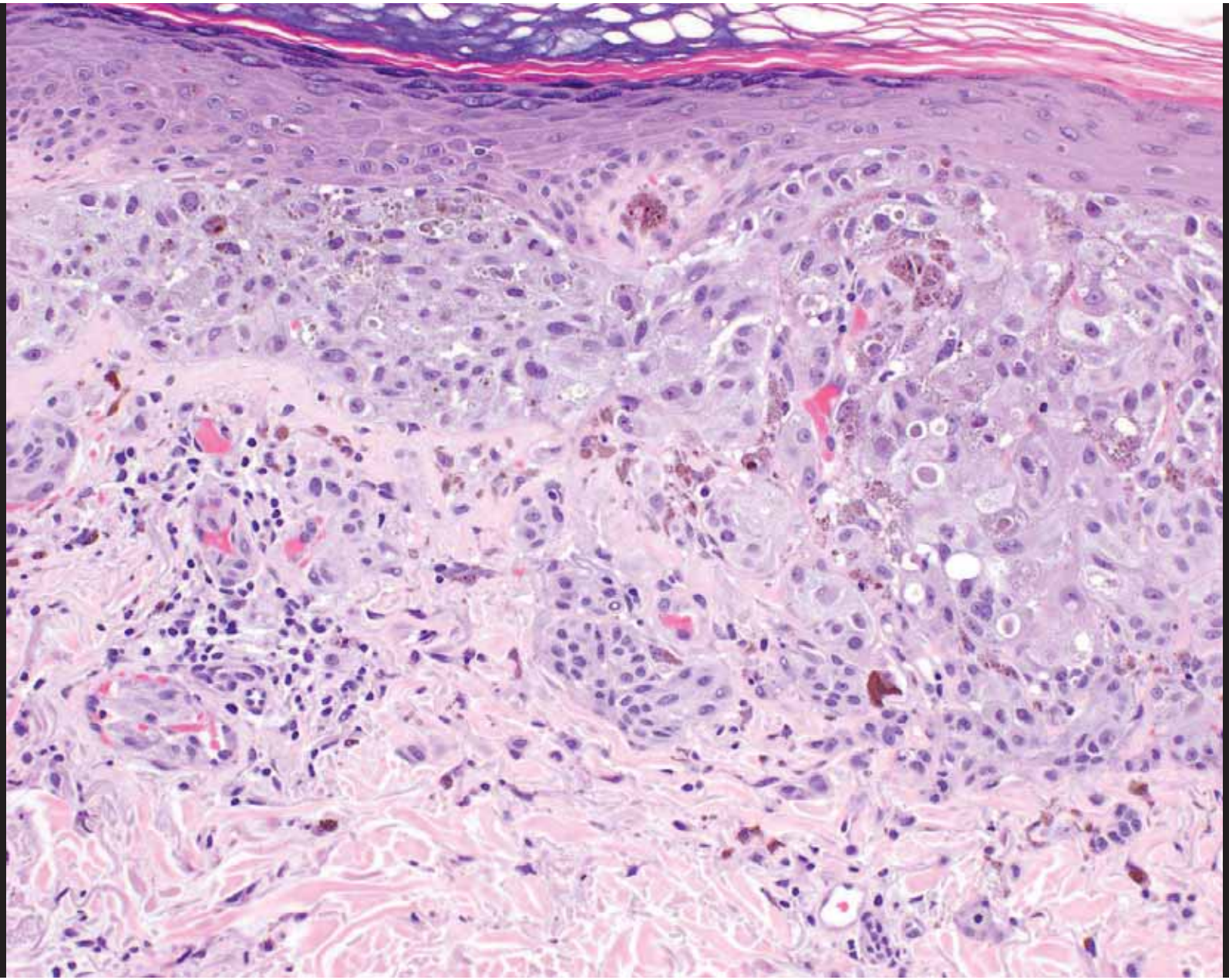

Melanocyte
BRAF
Common nevus
CTNNB1
APC
DPN
Genomic copy number alterations
CDKN2A
TERT
TP53
ARID1A
TET2
DPN-like melanoma

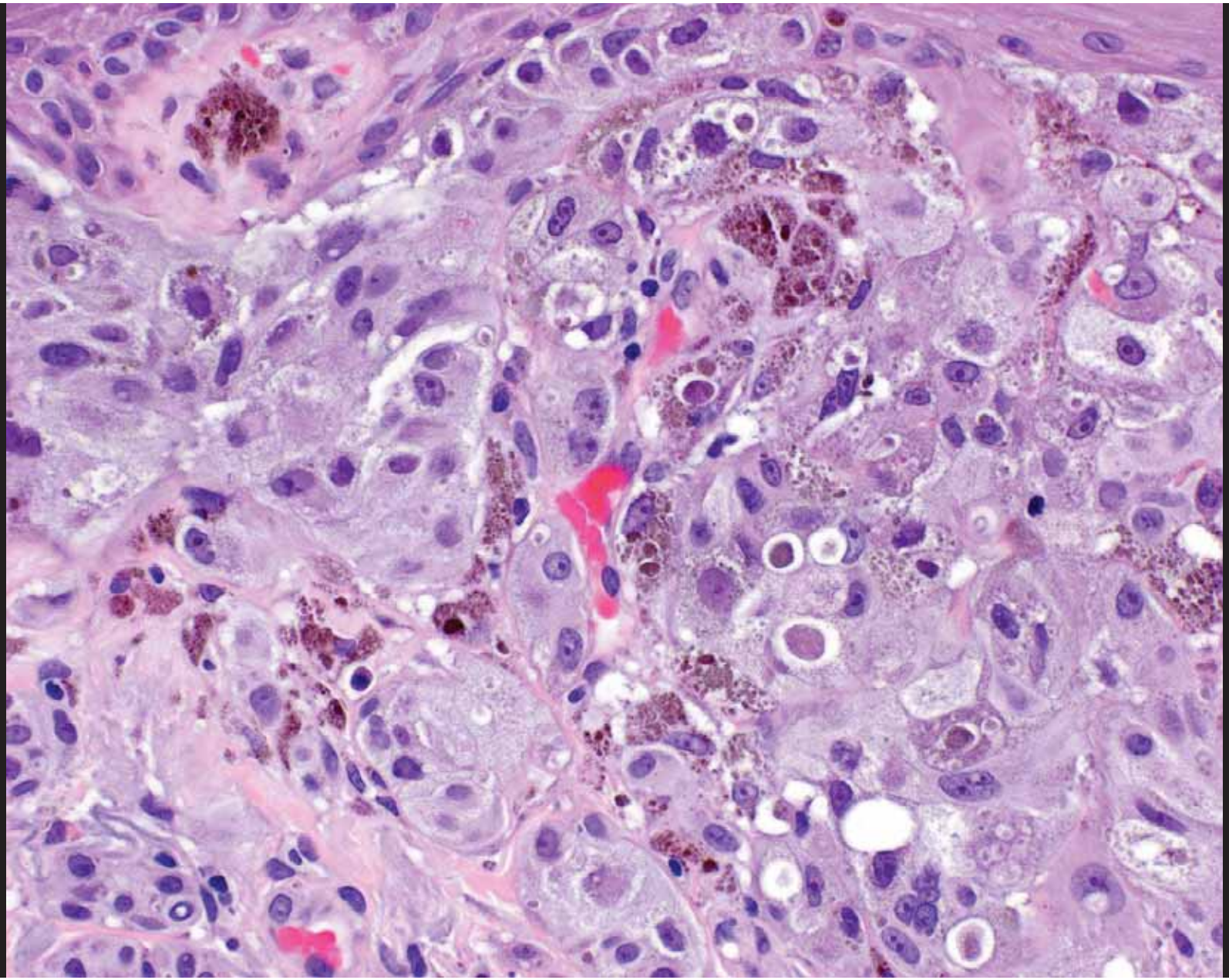

Melanocyte
BRAF
Common nevus
CTNNB1
APC
DPN
Genomic copy number alterations
CDKN2A
TERT
TP53
ARID1A
TET2
DPN-like melanoma

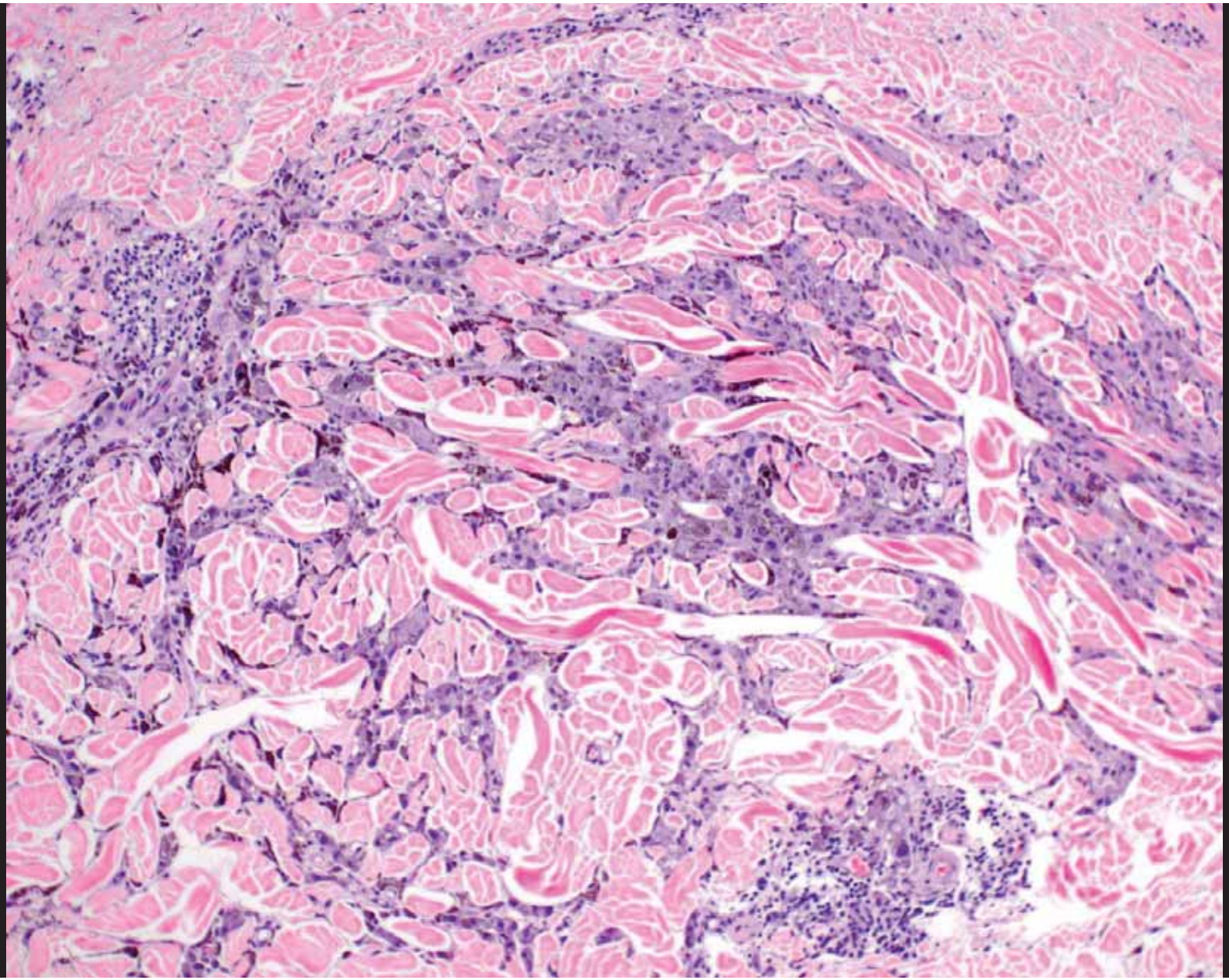
# Tumor progression from conventional to DPN-like melanoma

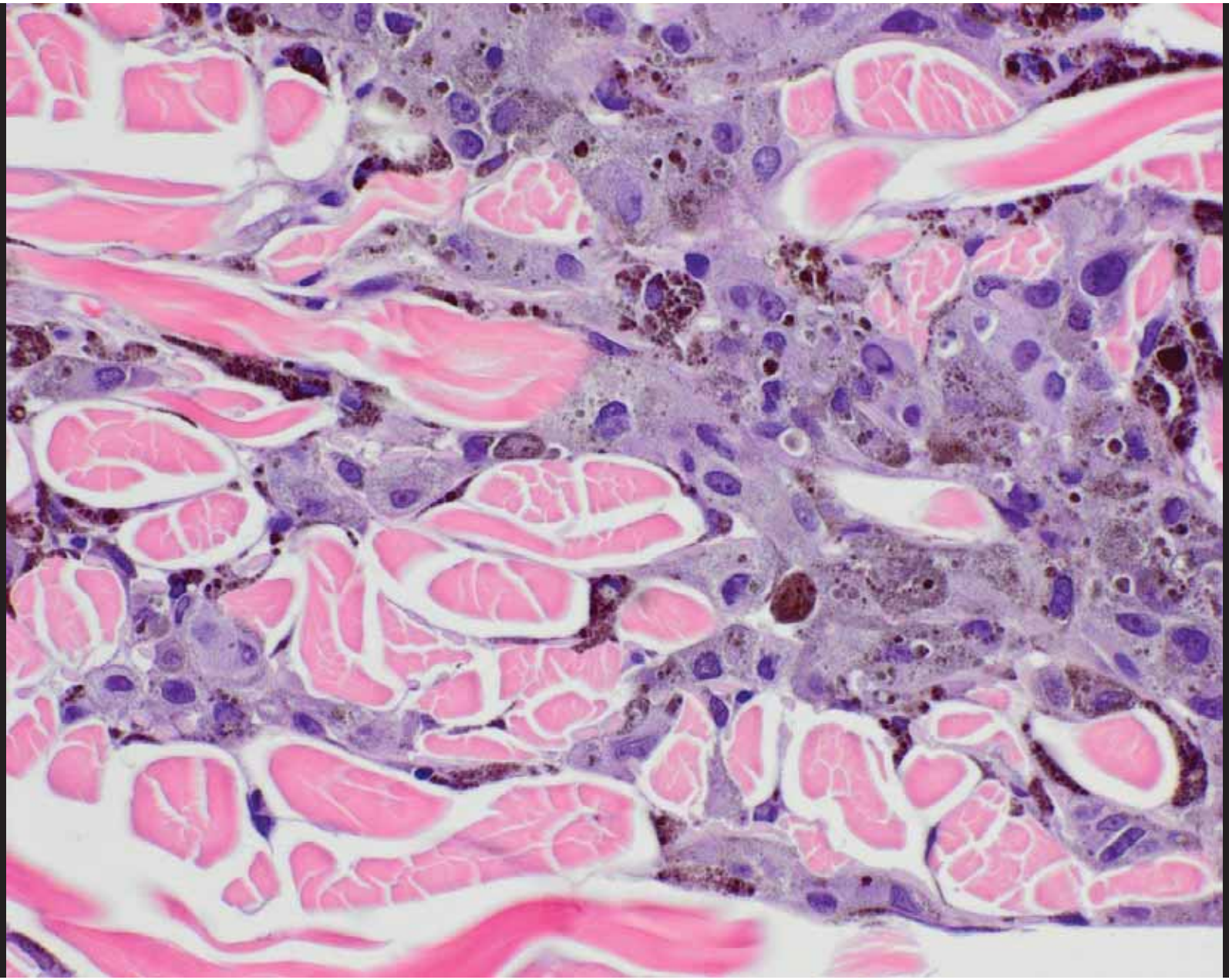




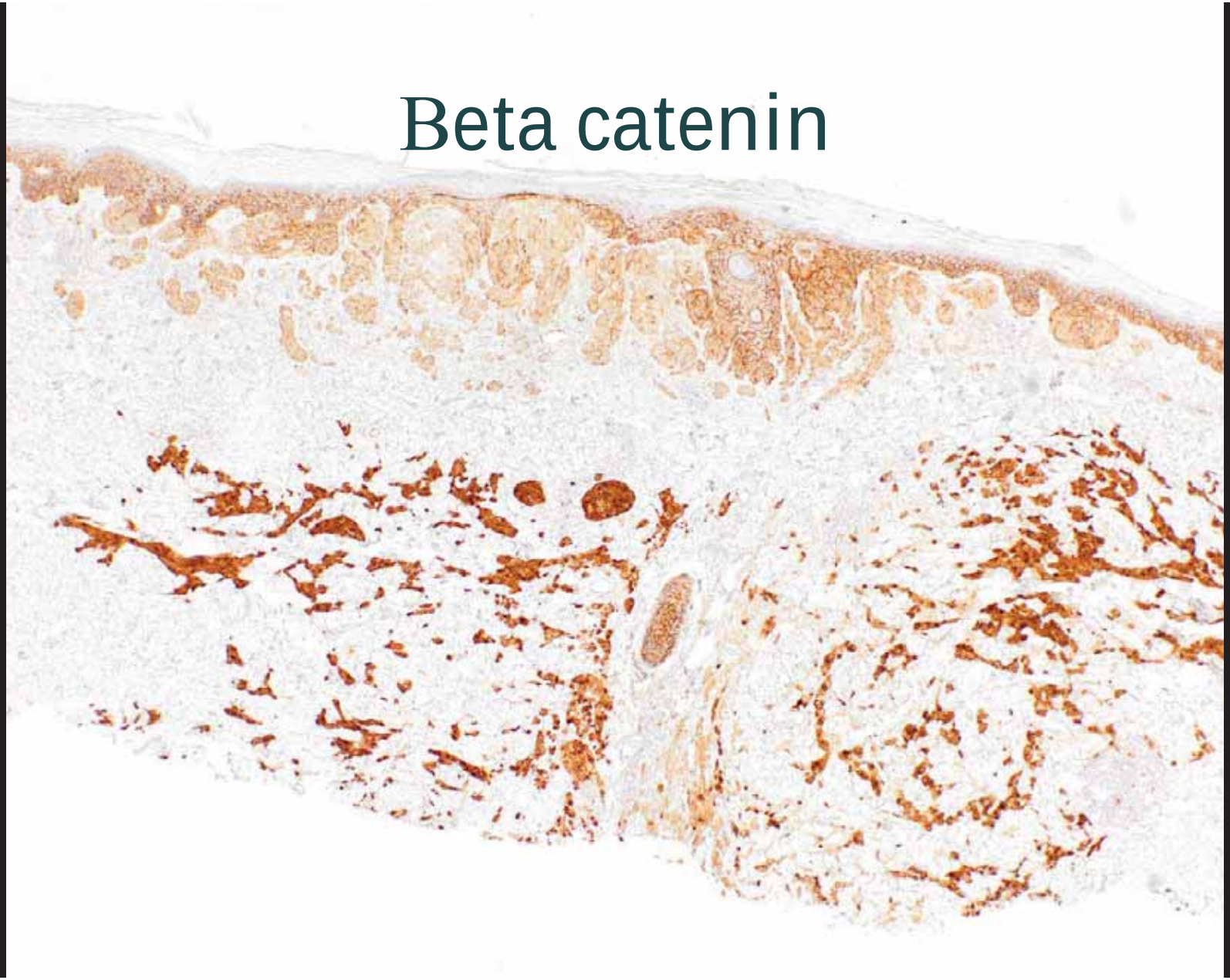


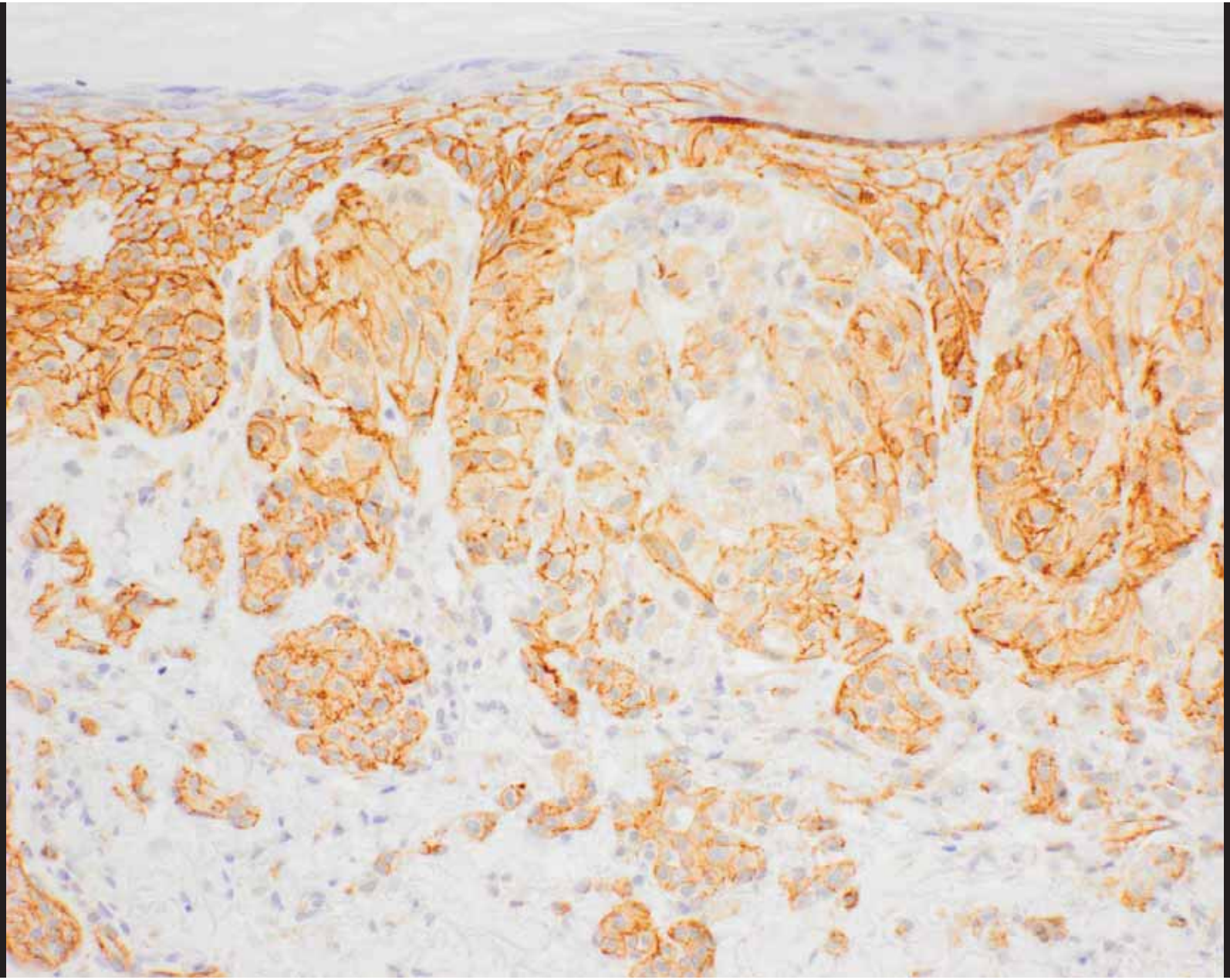


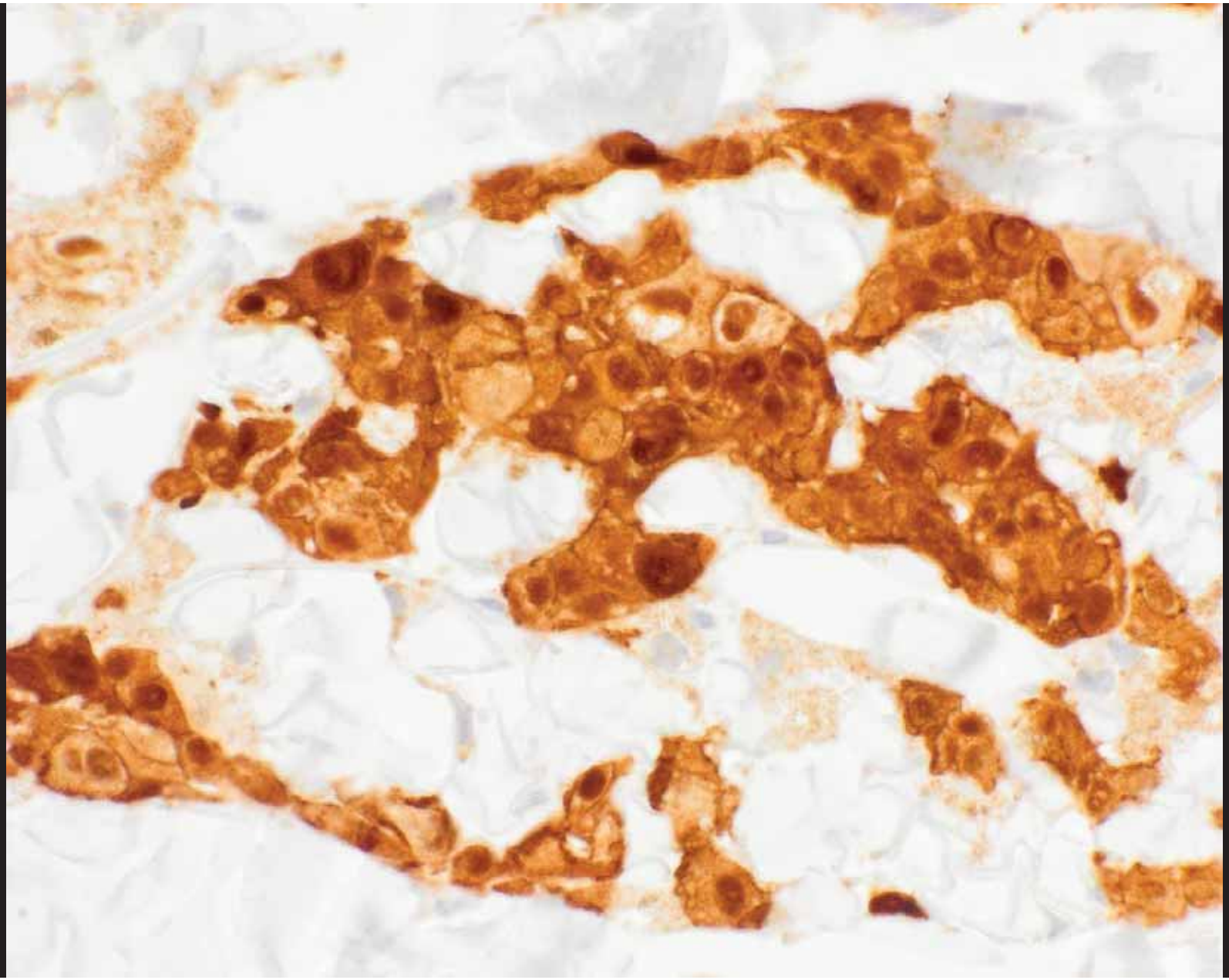




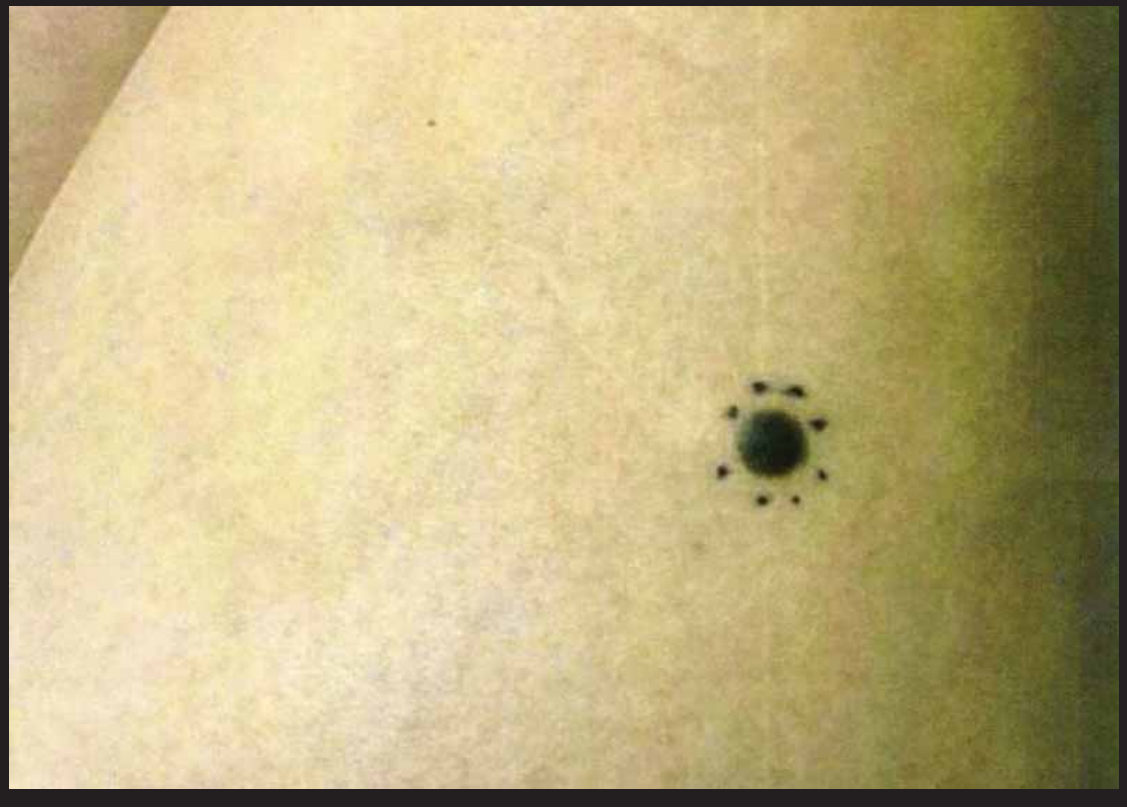
# Beta catenin

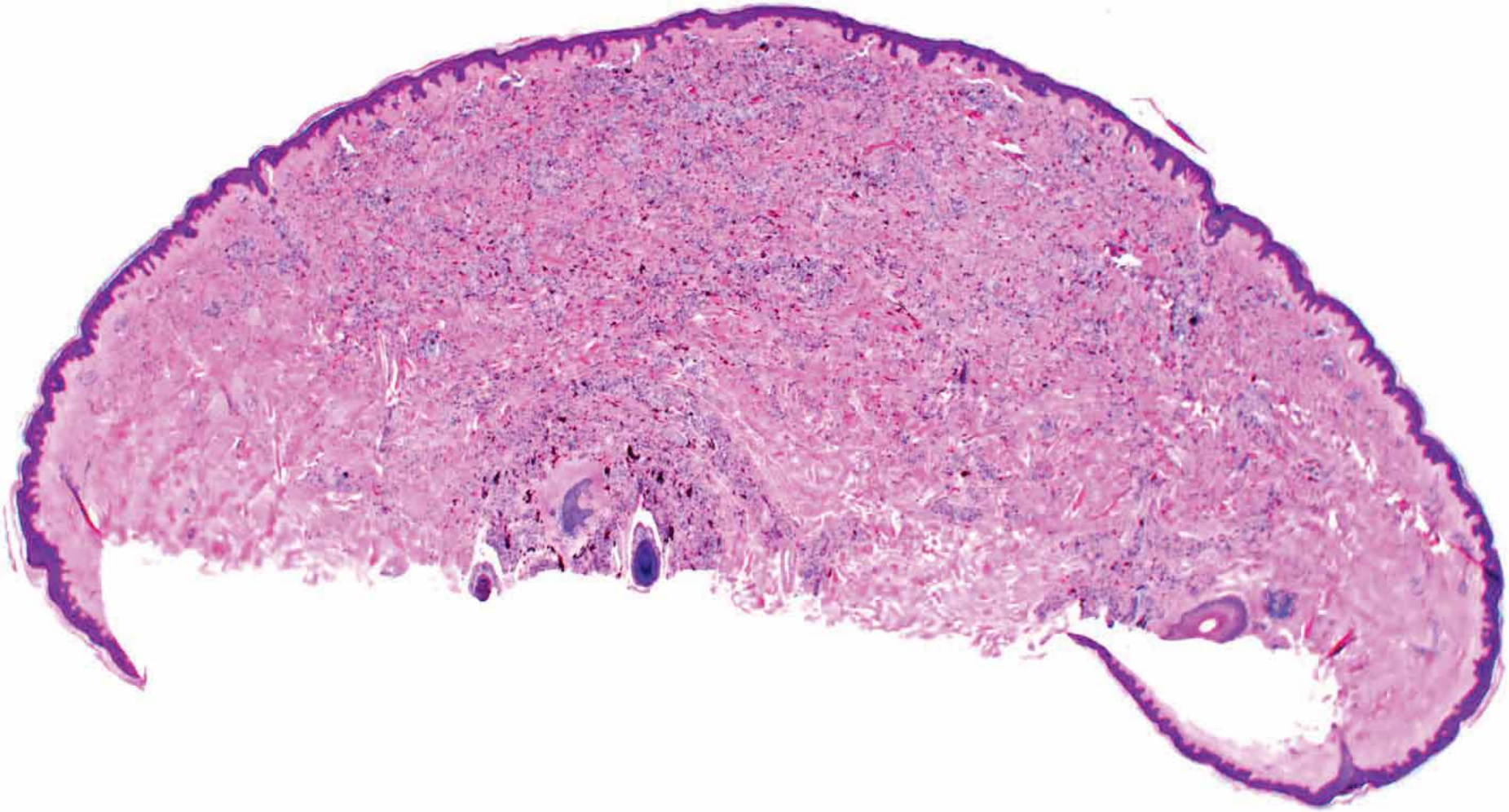


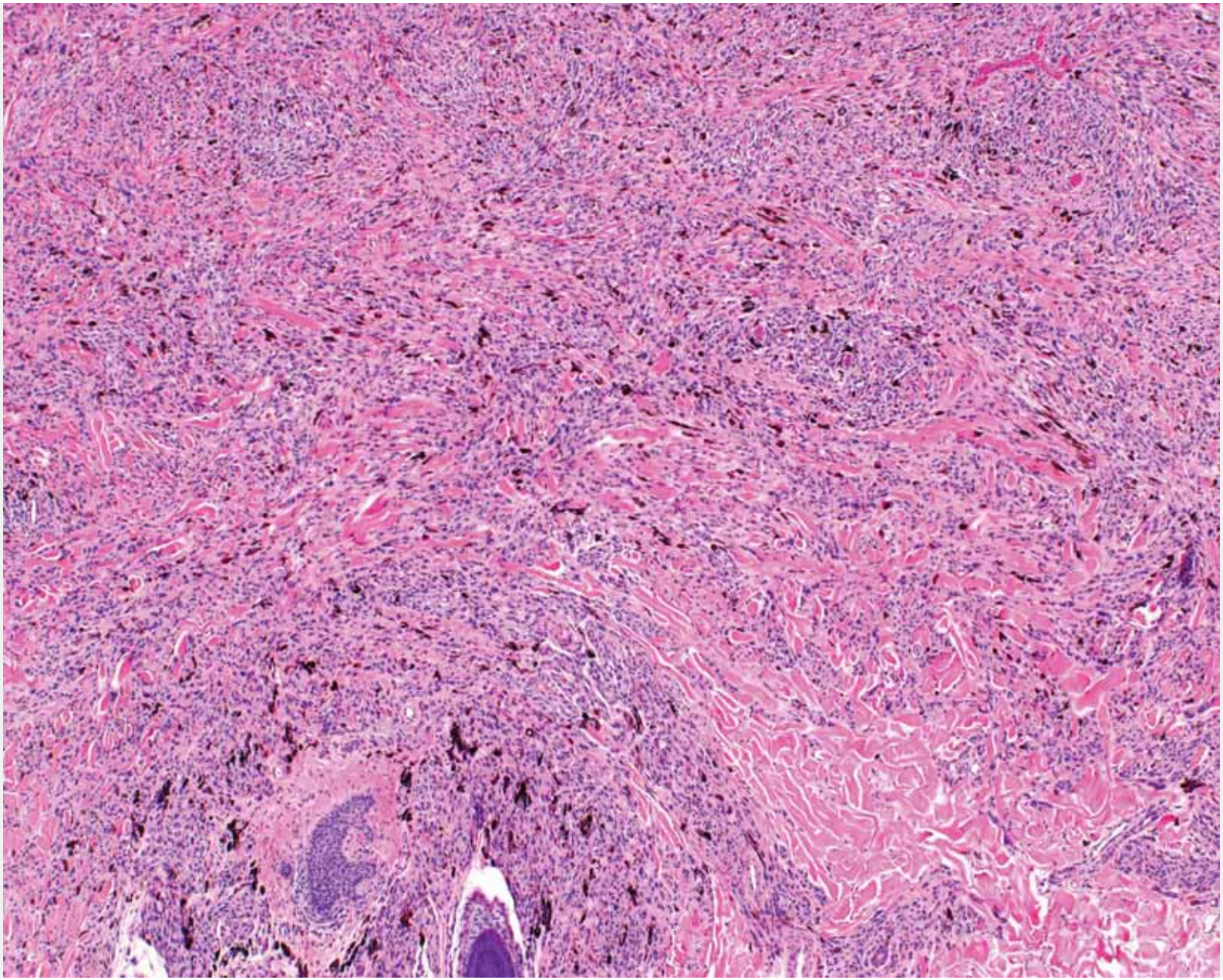


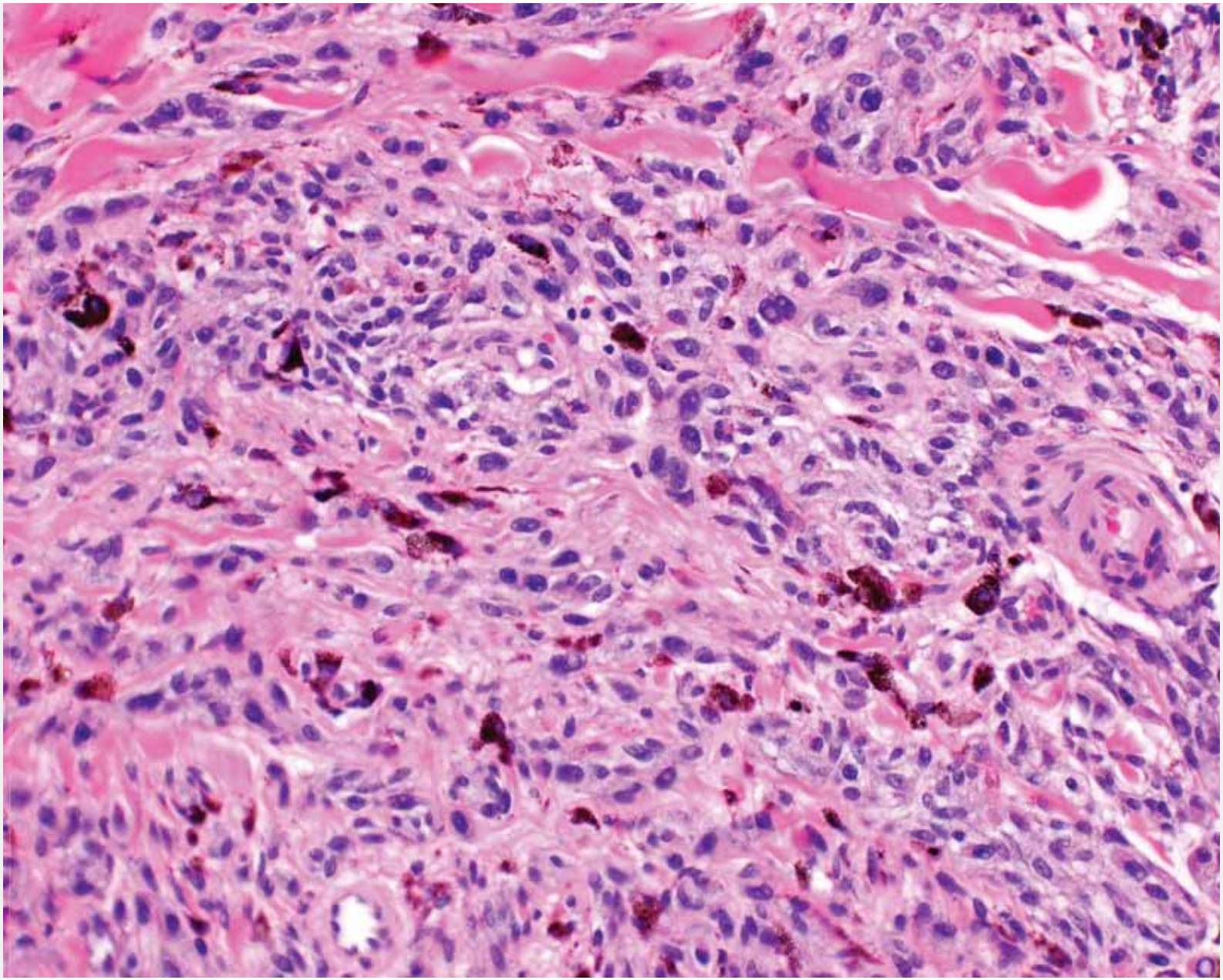


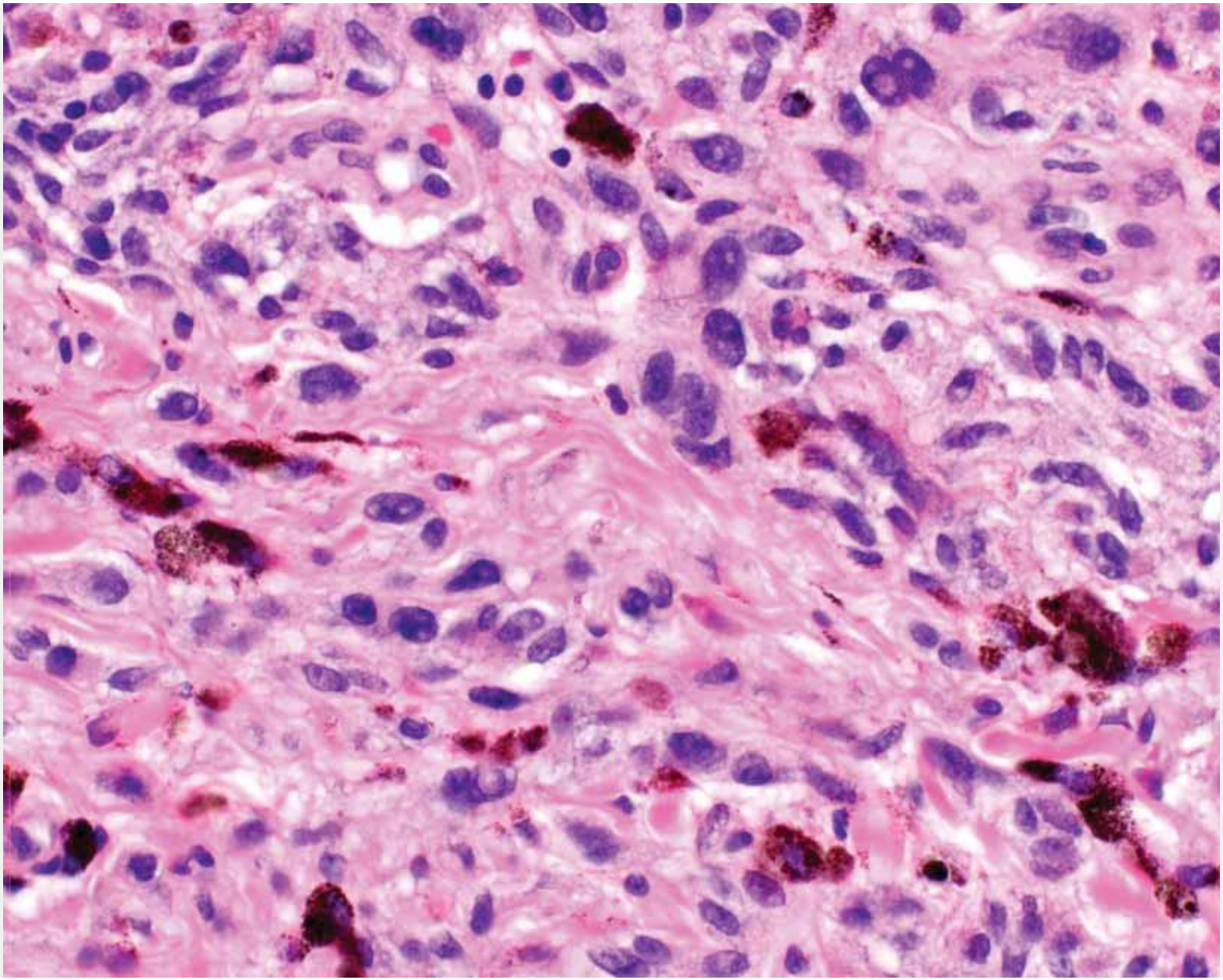
# 25 year old woman, right thigh

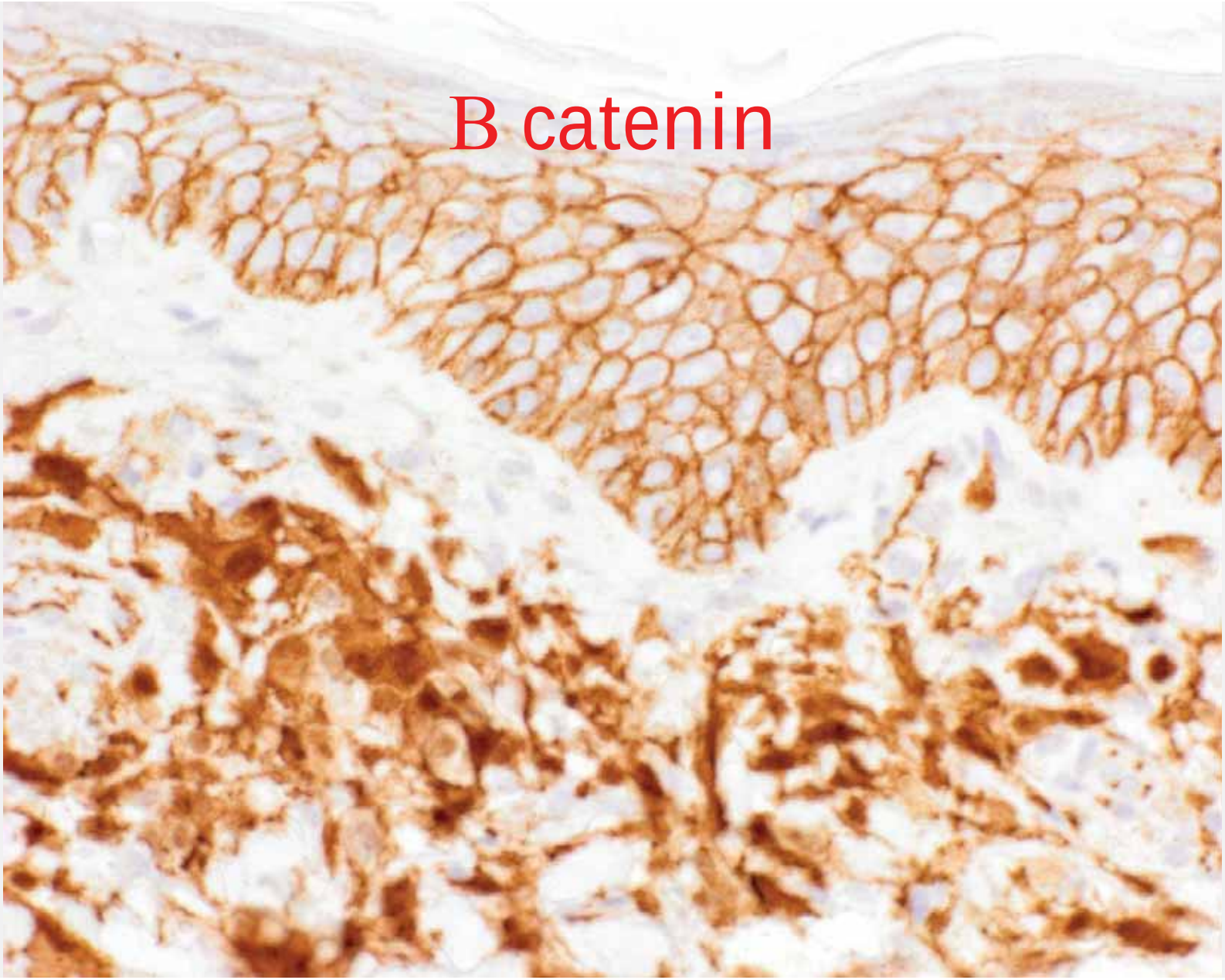


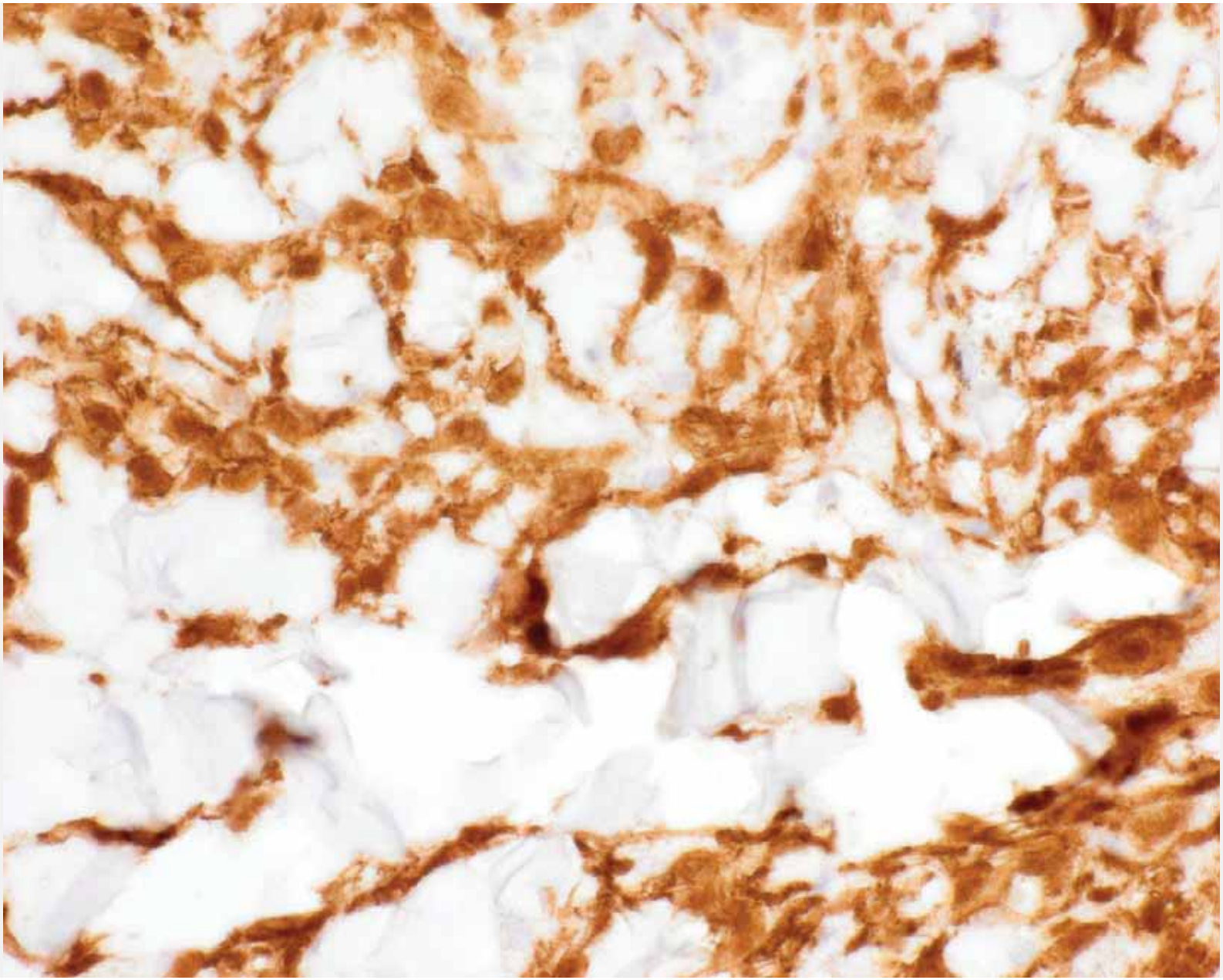


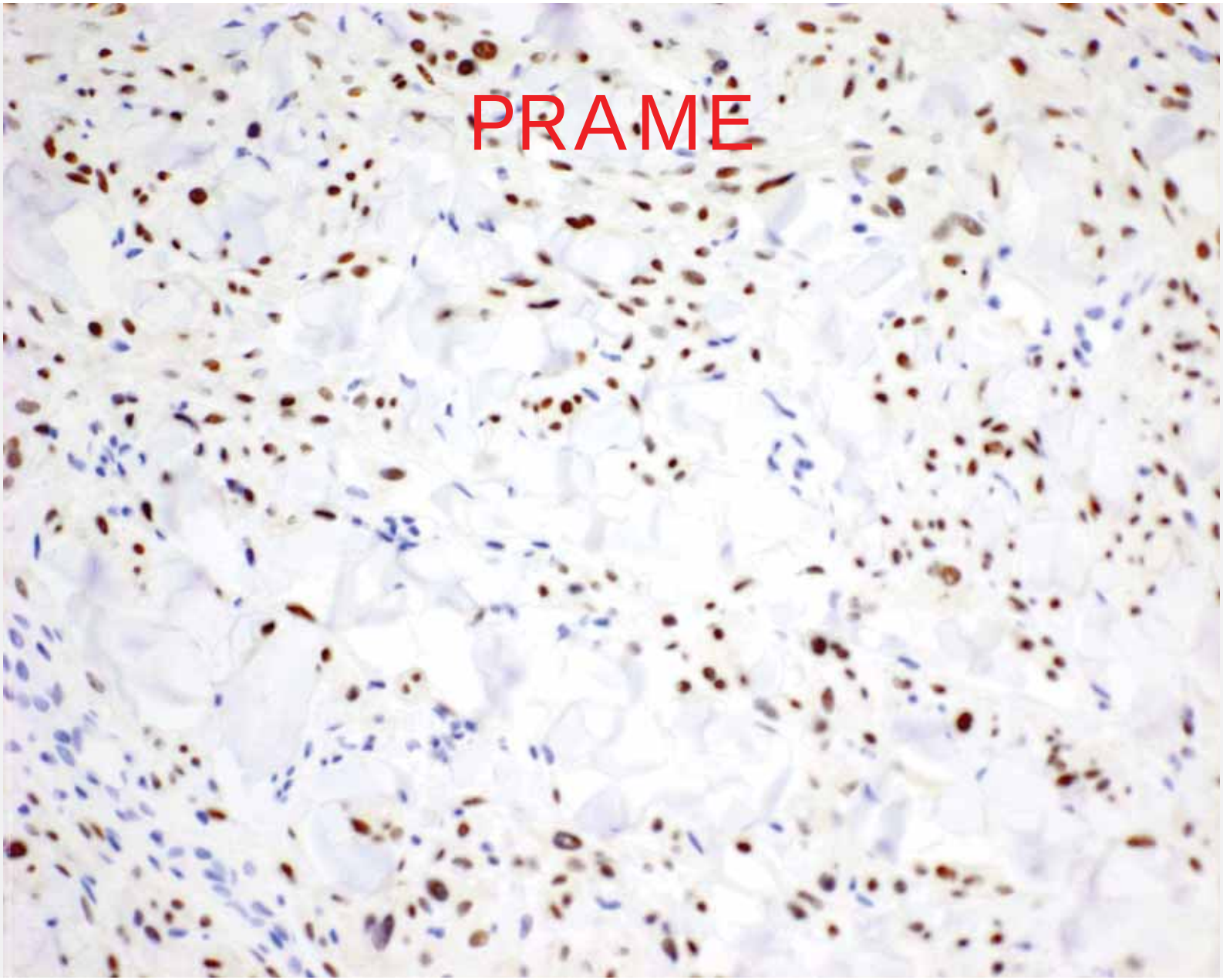




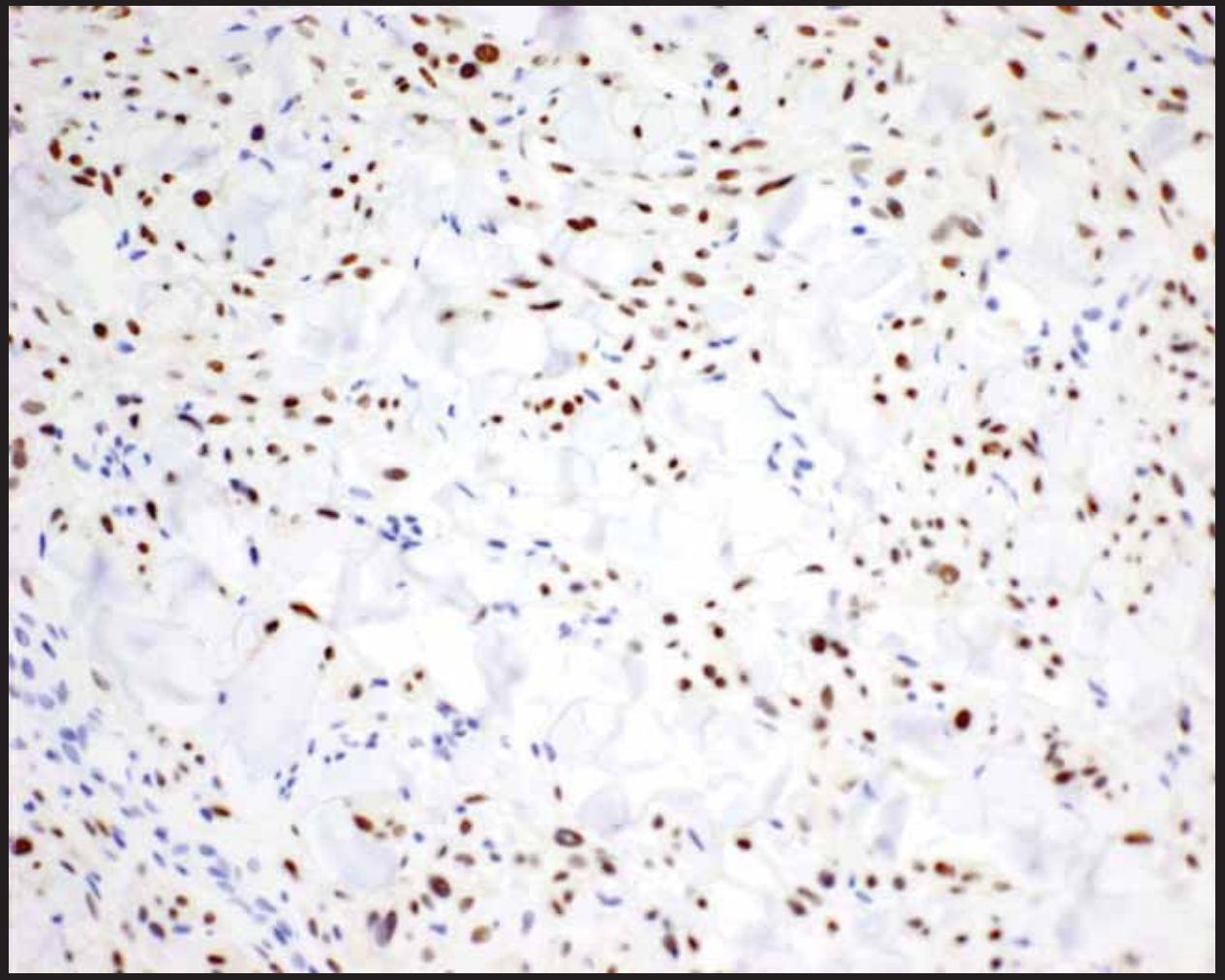



B catenin




PRAME

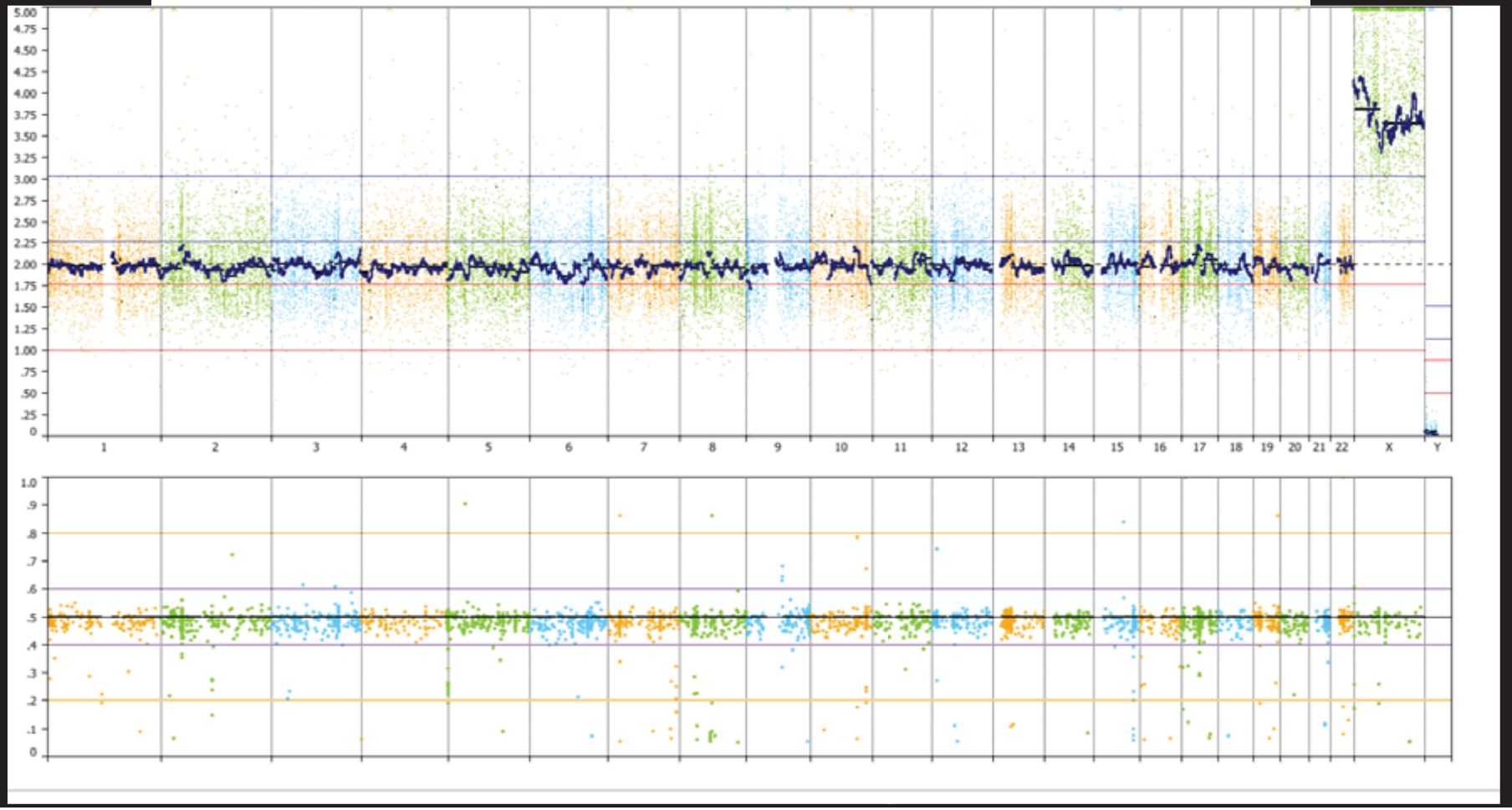
Possibly
Right
Auften
Makes
Errors



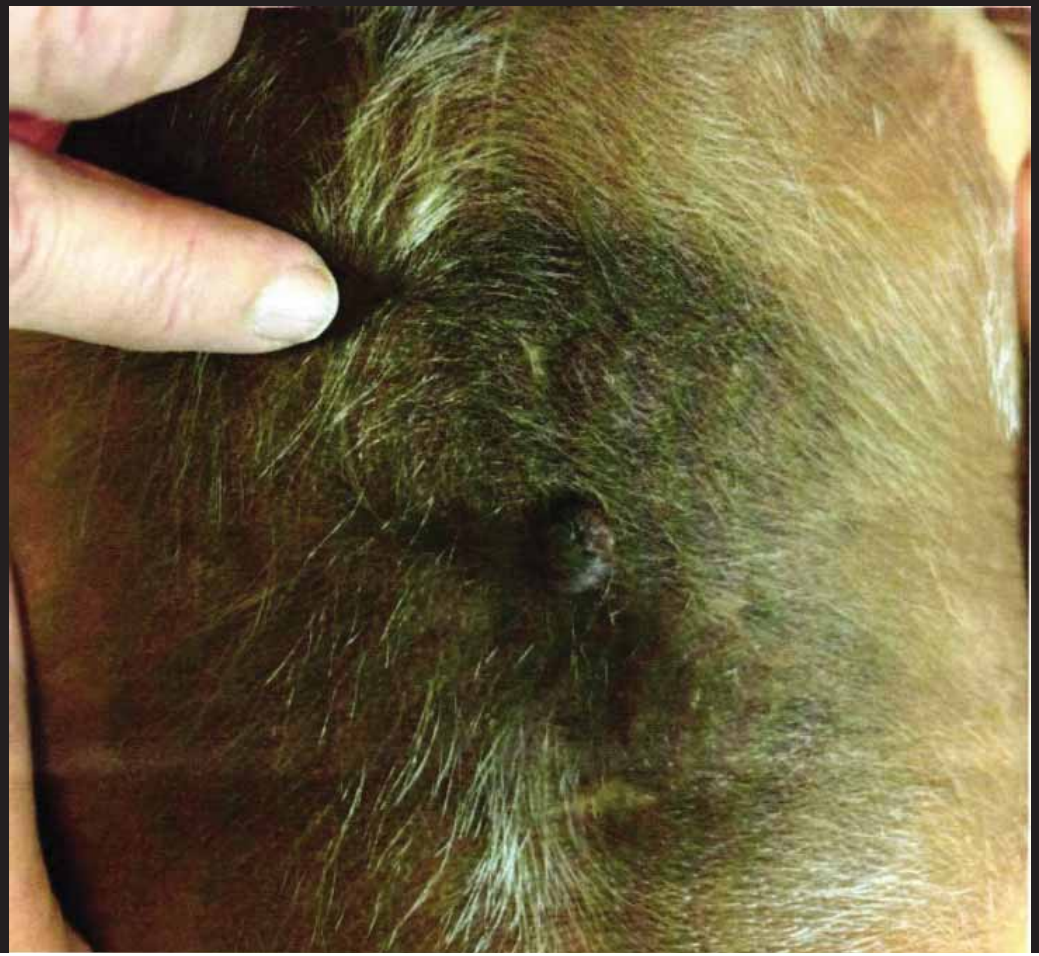
## PATHOGENIC AND LIKELY PATHOGENIC ALTERATIONS

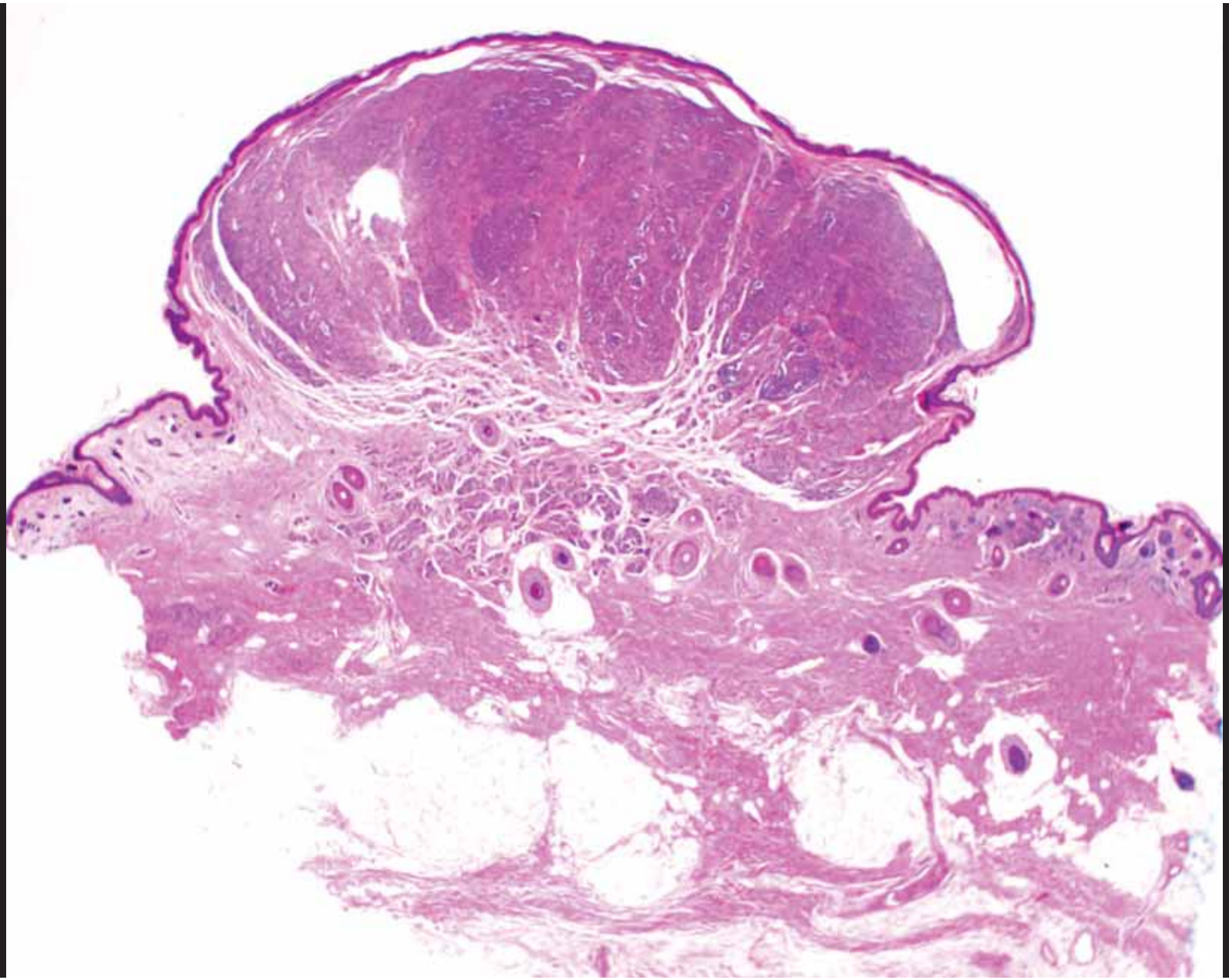
| VARIANT | TRANSCRIPT ID | CLASSIFICATION | READS | MUTANT ALLELE FREQUENCY |
|---|---|---|---|---|
| BRAF p.V600E | NM_004333.4 | Pathogenic | 1013 | 27% |
| CTNNB1 p.S33F | NM_001904.3 | Pathogenic | 1017 | 23% |

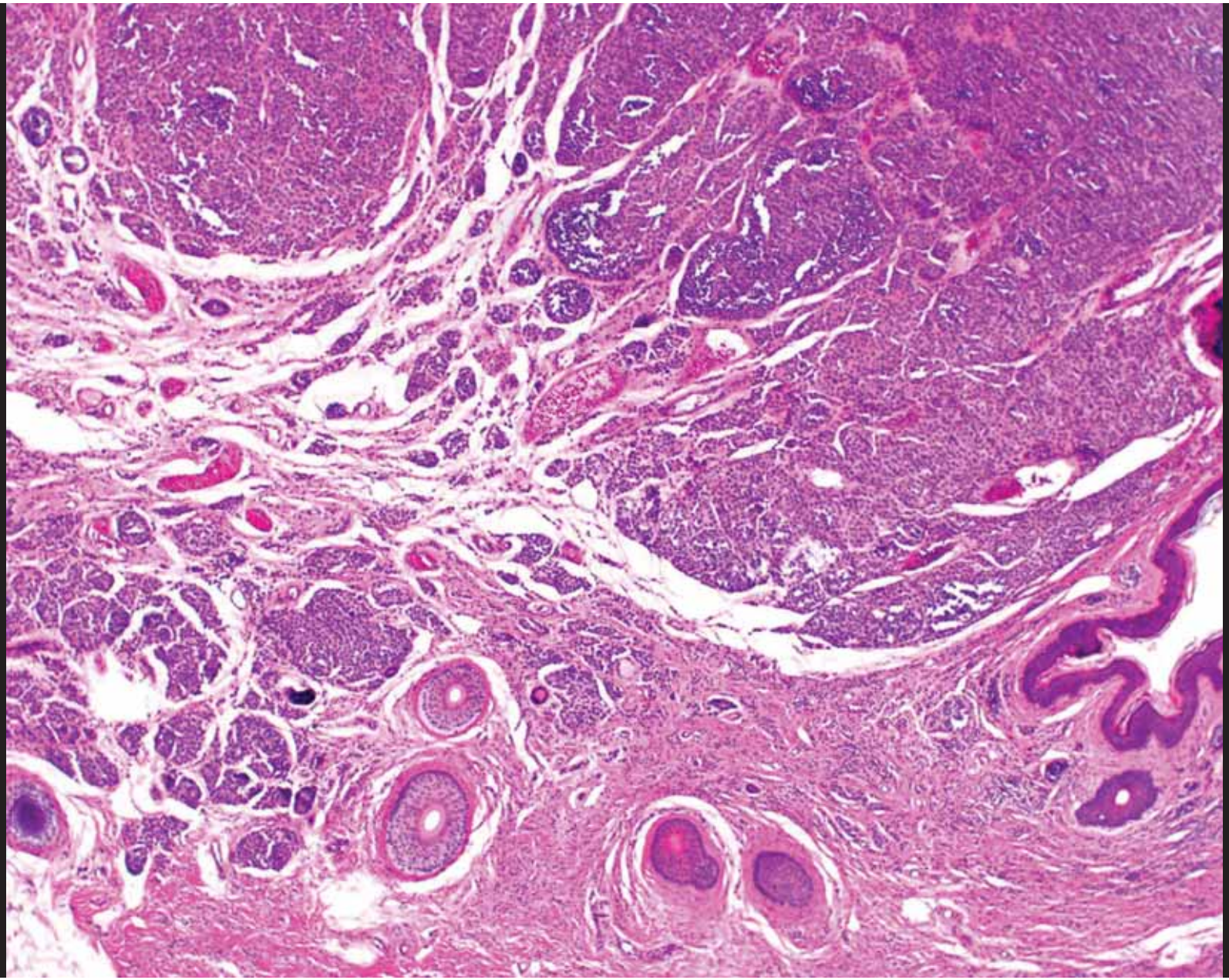
'Reads' indicates the number of unique DNA molecules sequenced. 'Mutant Allele Frequency' indicates the percentage of the reads with the respective 'Variant' and is affected by the degree of normal cell contamination of the sample and whether the variant is fully clonal or subclonal. 'Pathogenic' and 'Likely Pathogenic' classifications are based on CCGL molecular pathologist/geneticist interpretation of data from somatic and germline databases and published literature. Variants classified as 'Possibly Pathogenic' have unknown significance but occur in genes or molecular pathways known to be recurrently altered in the tumor type. Official gene symbols may change over time. See Gene List in Appendix for prior gene symbol aliases.

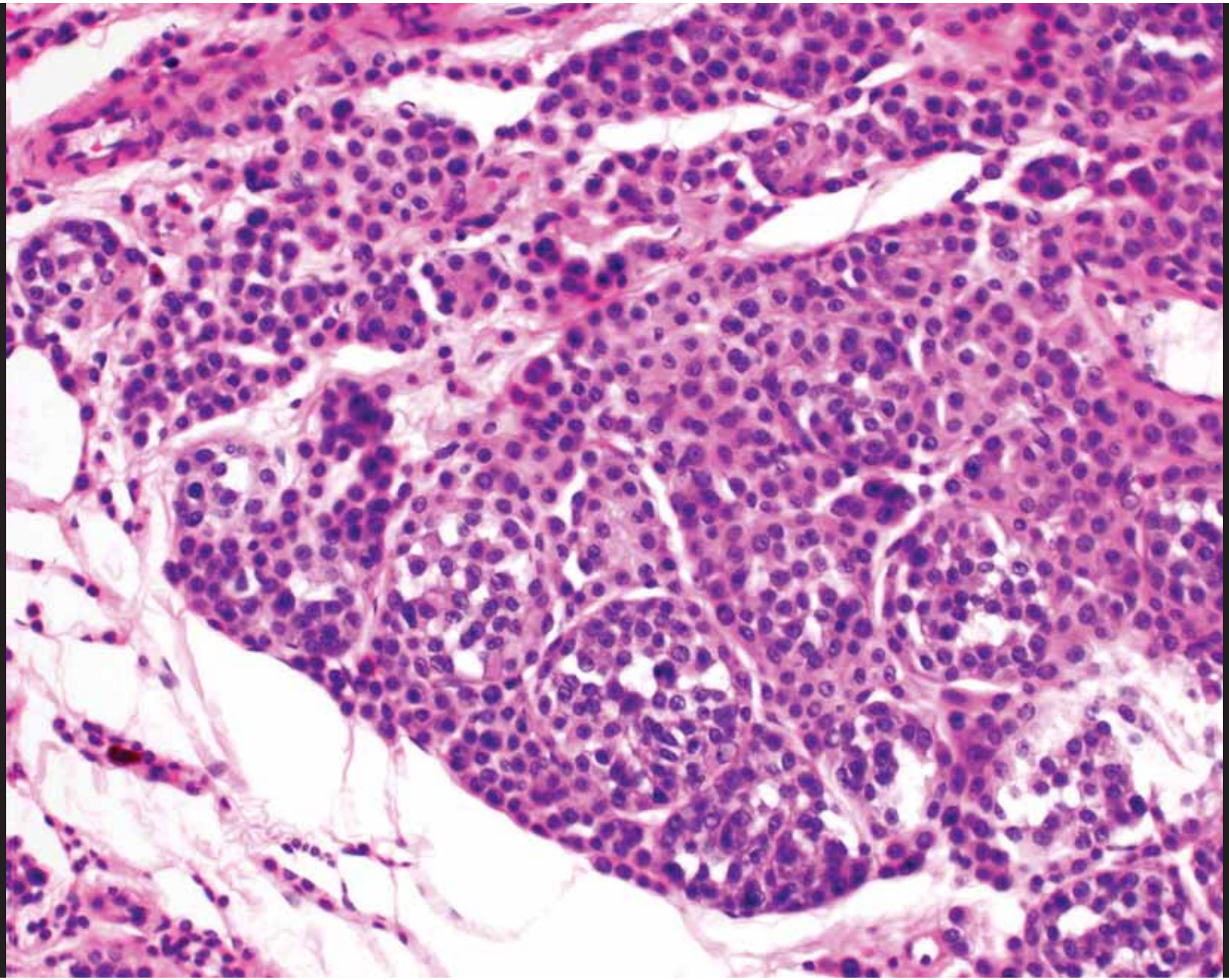


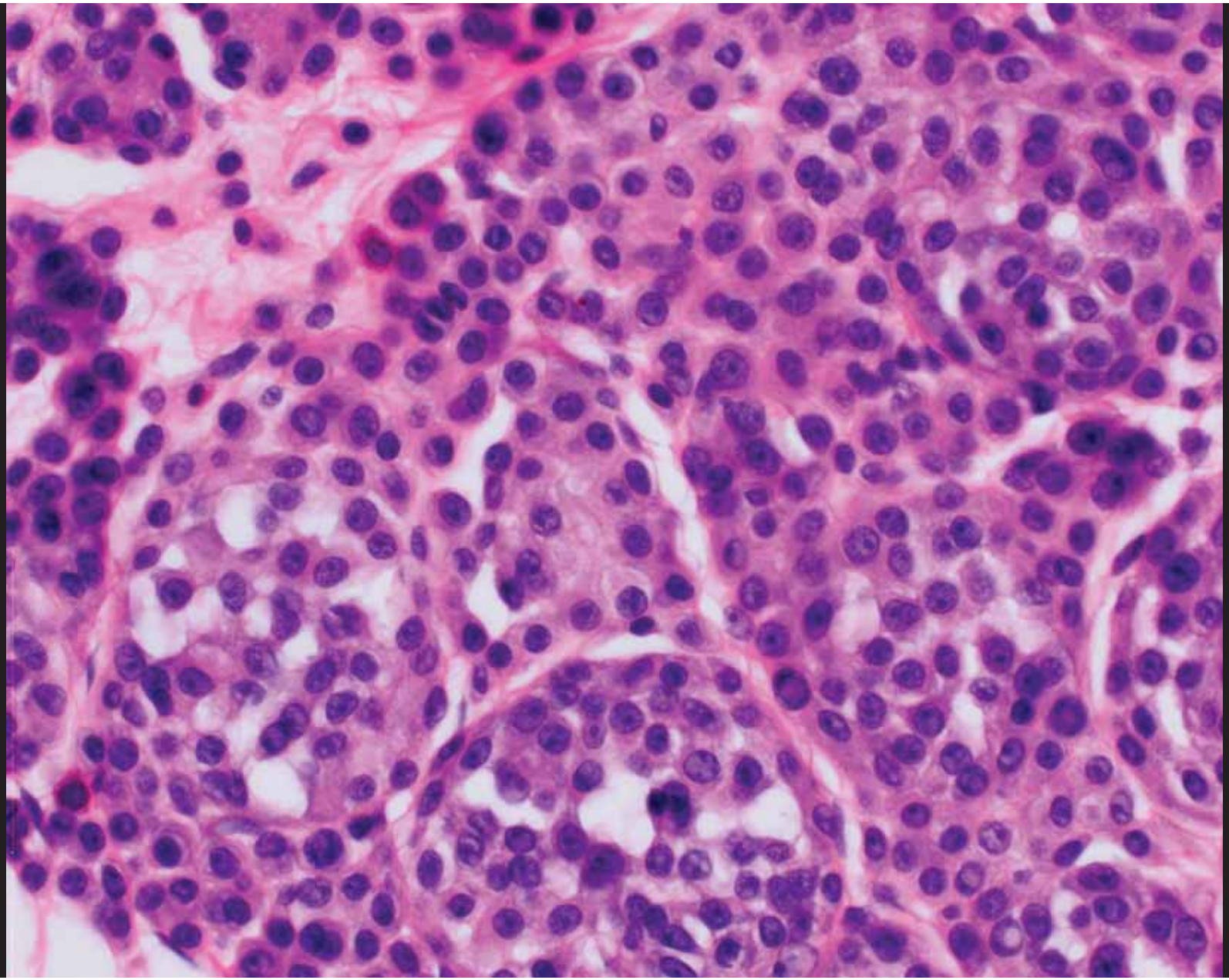
2 year old girl, 22 X 18 cm nevus on back a few weeks after birth, recently developed two soft nodules

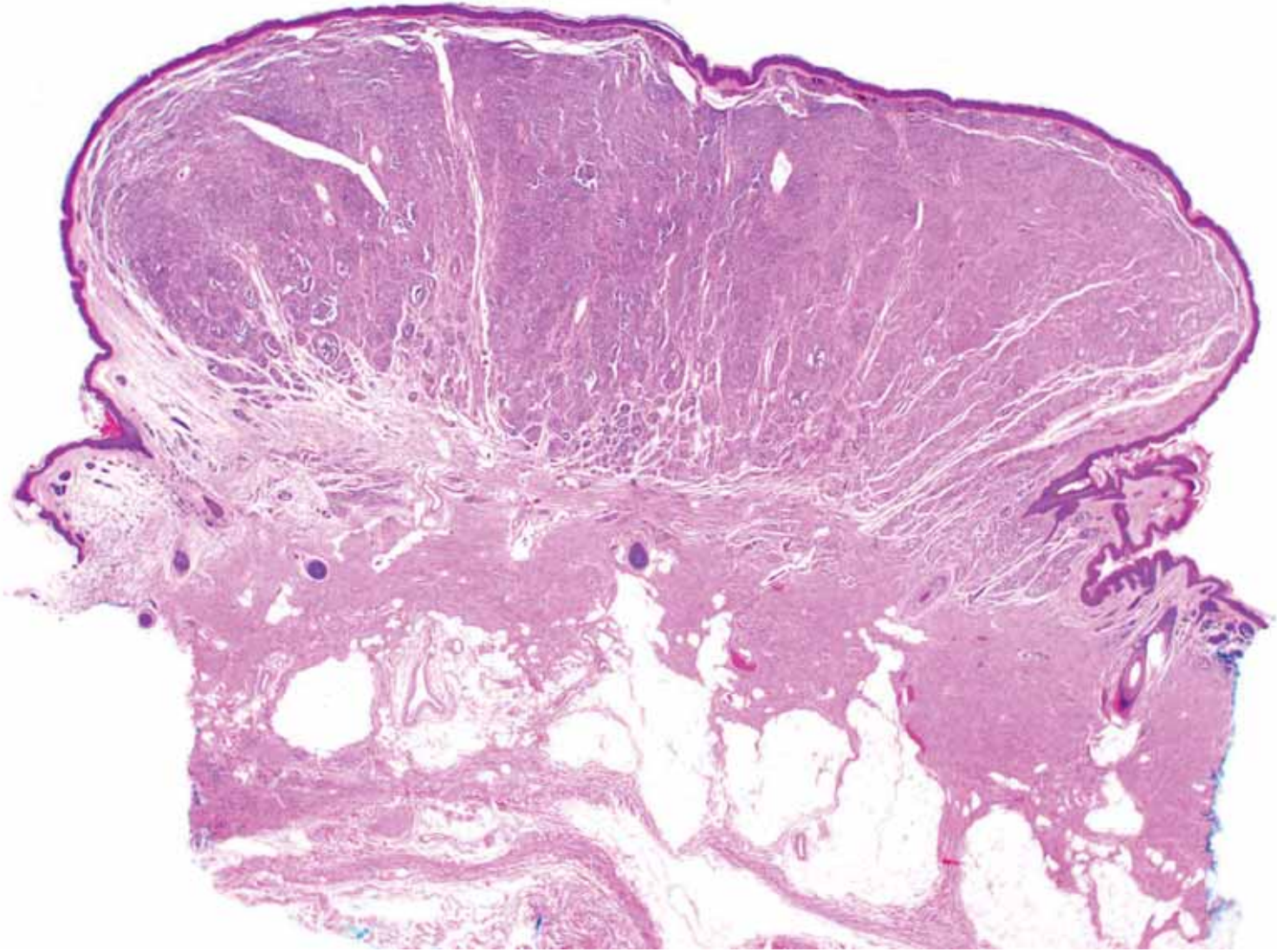


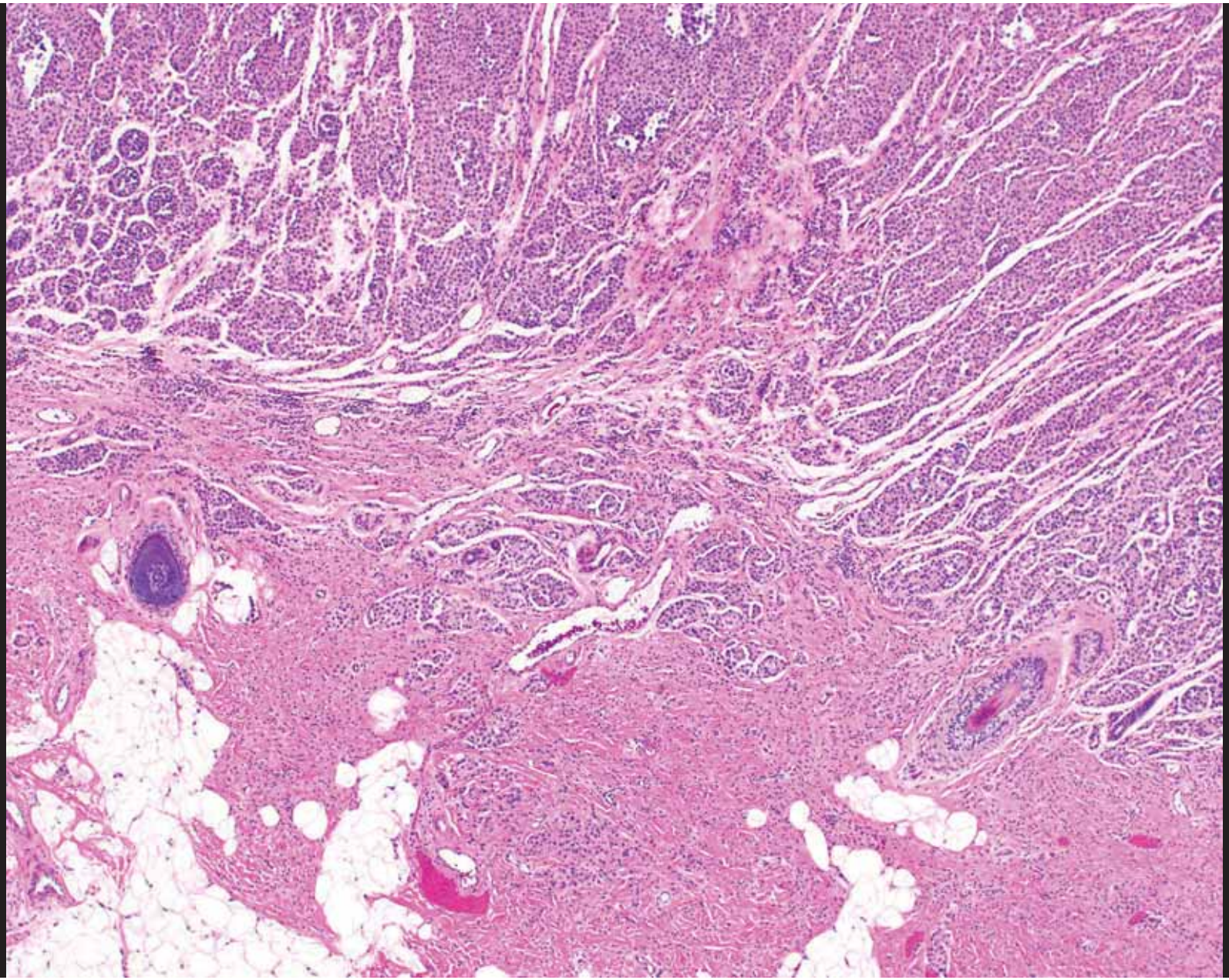


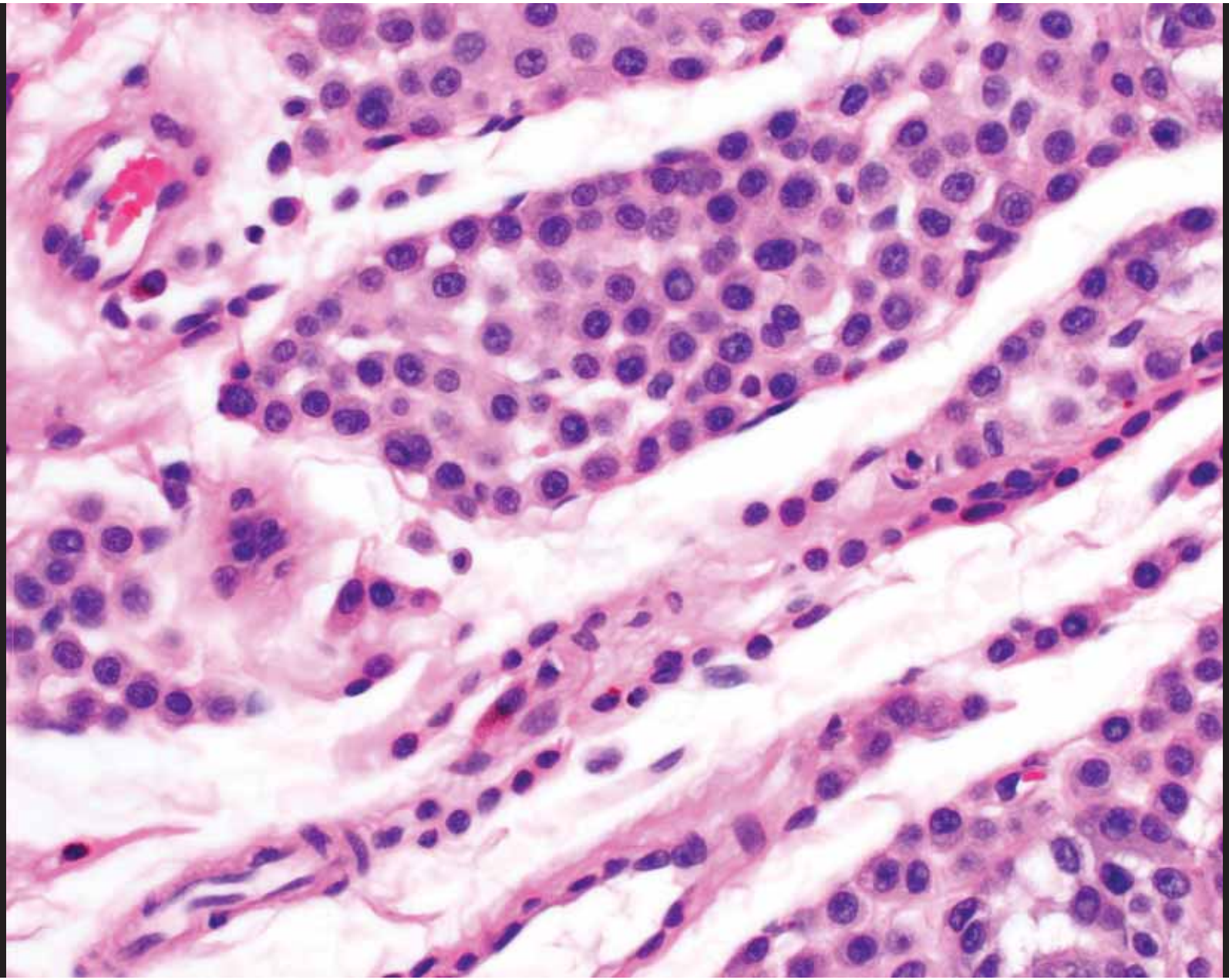


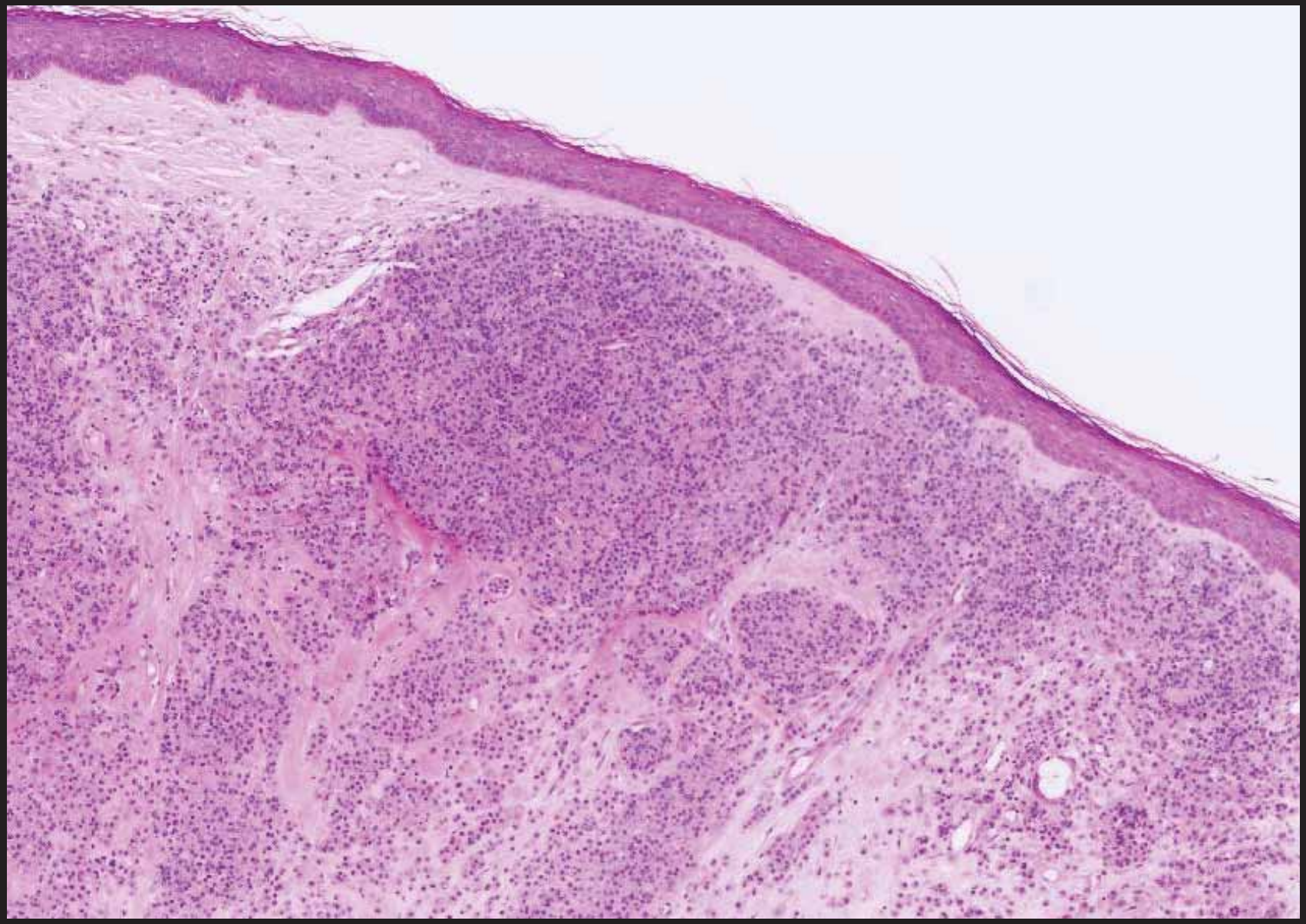


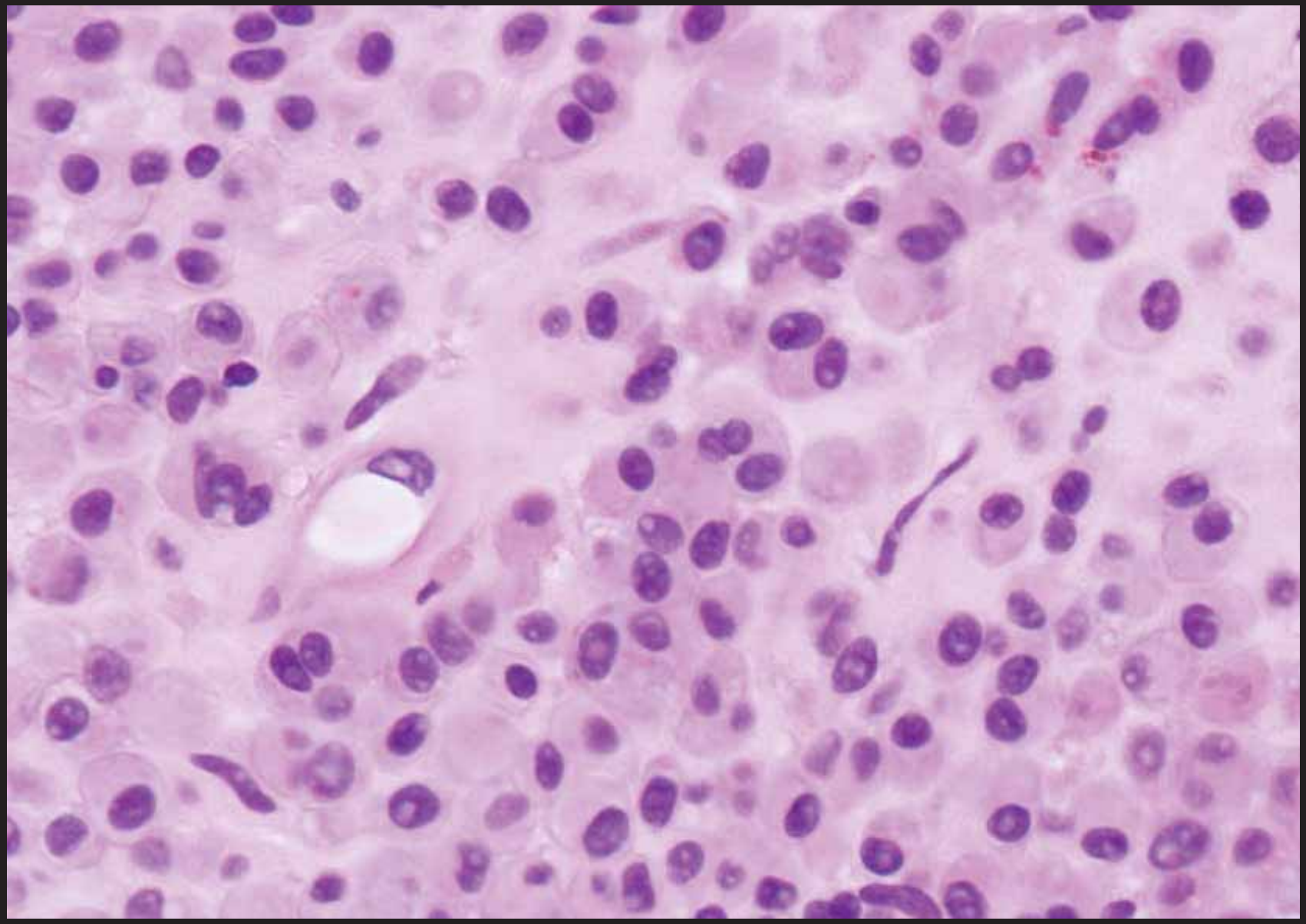


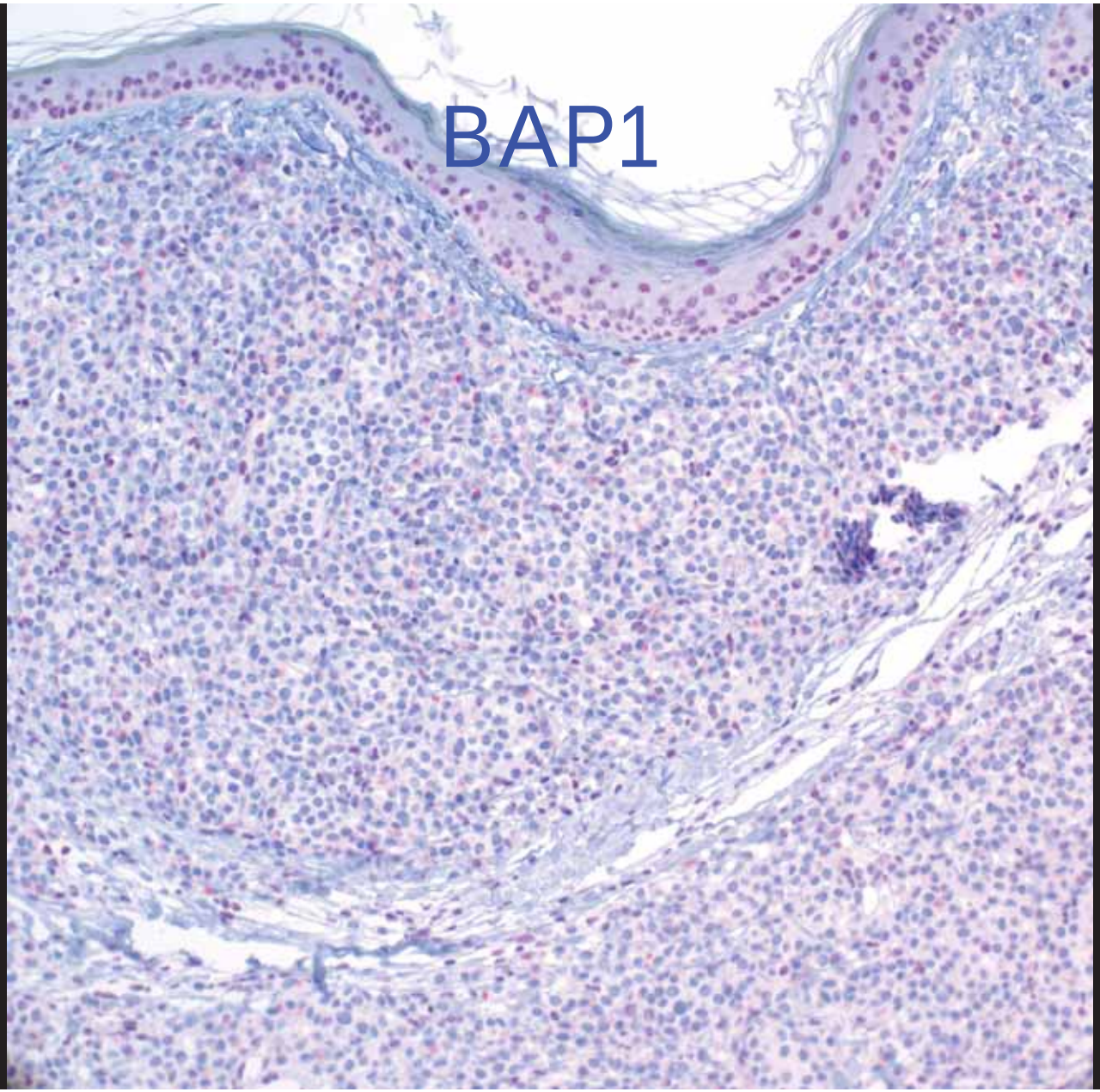


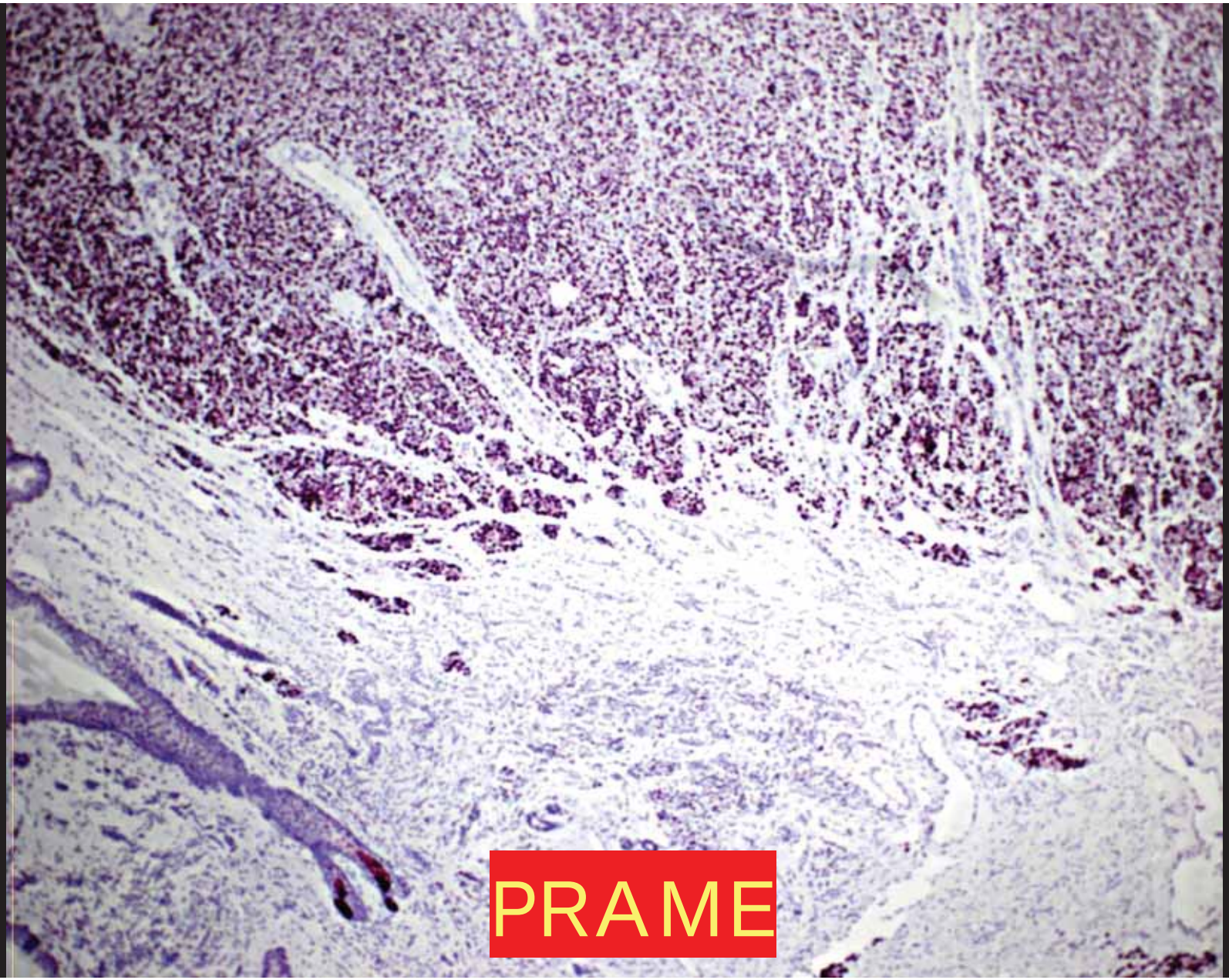


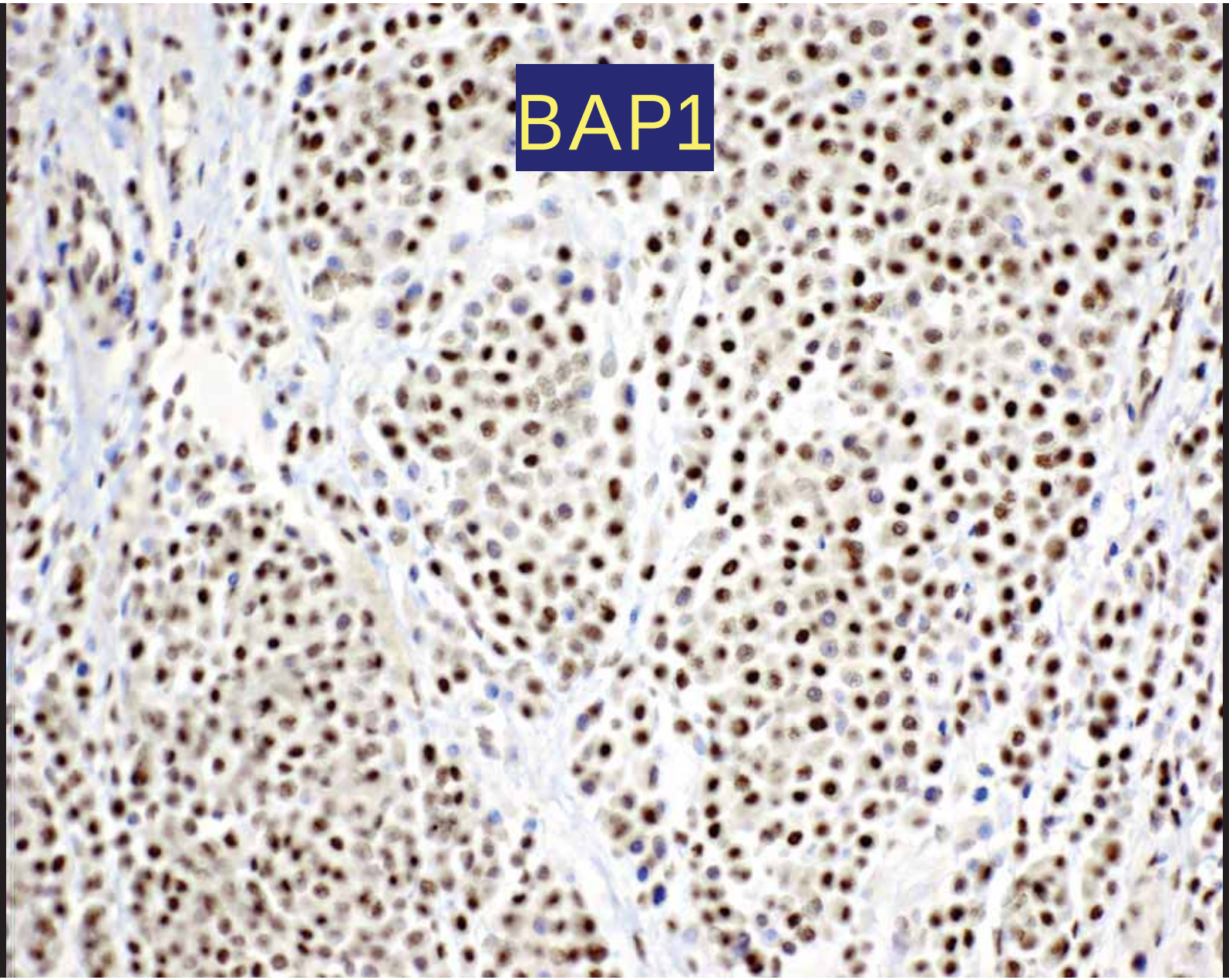







BAP1


PRAME


BAP1

## VARIANTS OF UNKNOWN SIGNIFICANCE

| Gene | Mutation effect | Variant allele fraction |
|---|---|---|
| PPP2R2A | c.761A>G p.Y254C Missense variant NM_001177591 | 52.7% |
| DPYD | c.938T>C p.V313A Missense variant NM_000110 | 43.7% |
| BAP1 | c.1721C>T p.A574V Missense variant NM_004656 | 41.6% |
| ERCC2 | c.2047-13_2048del p.R683fs Frameshift NM_000400 | 41.0% |
| WNK1 | c.2176_2219delins(46) p.I726fs Frameshift NM_213655 | 34.8% |

## GENOMIC VARIANTS

**Potentially Actionable** — **Variant Allele Fraction**

**NRAS** p.Q61K Missense variant (exon 3) - GOF 36.3%

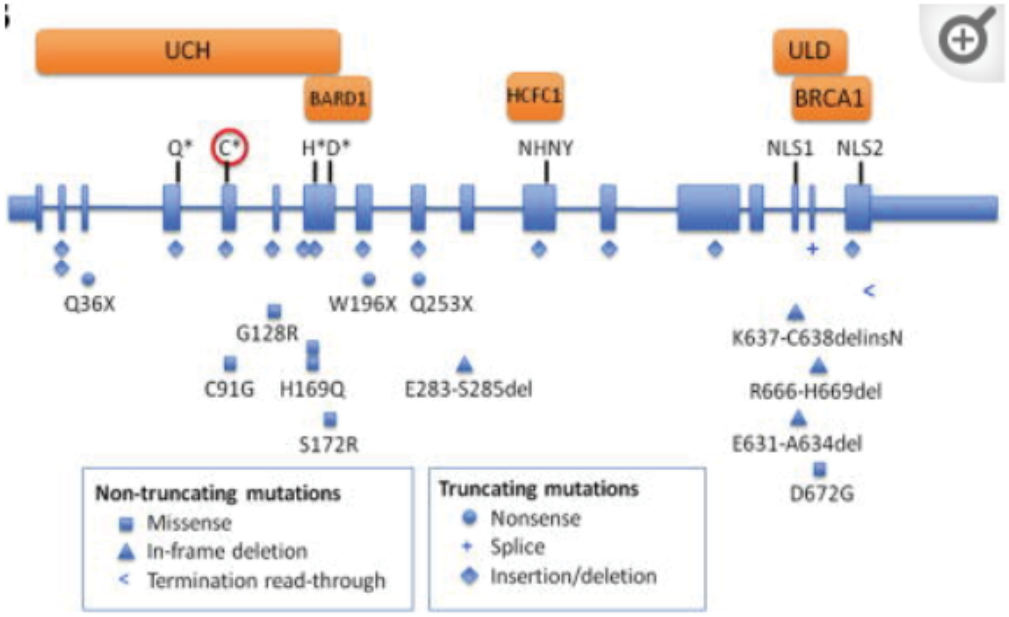
**Tumor / Normal Matched Analysis (Potential Germline)**

Phil,

As we discussed earlier, it seems that the C91 is the primary catalytic residue in BAP1, located in the UCH domain at the N-terminus of BAP1. Truncating or deletion mutations in this region would inactivate BAP1, as would missense of Cysteine 91 to anything else (cysteine is the only amino acid with a sulfhydryl group). Other mutations in the UCH domain could impair BAP1 activity, especially those in the "critical" residues of Q85, C91, H169 and D194 within the active site. These are indicated by asterisks in the figure below from this paper, which also has more information if you are interested. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3087380/.

Cheers,

-Rony



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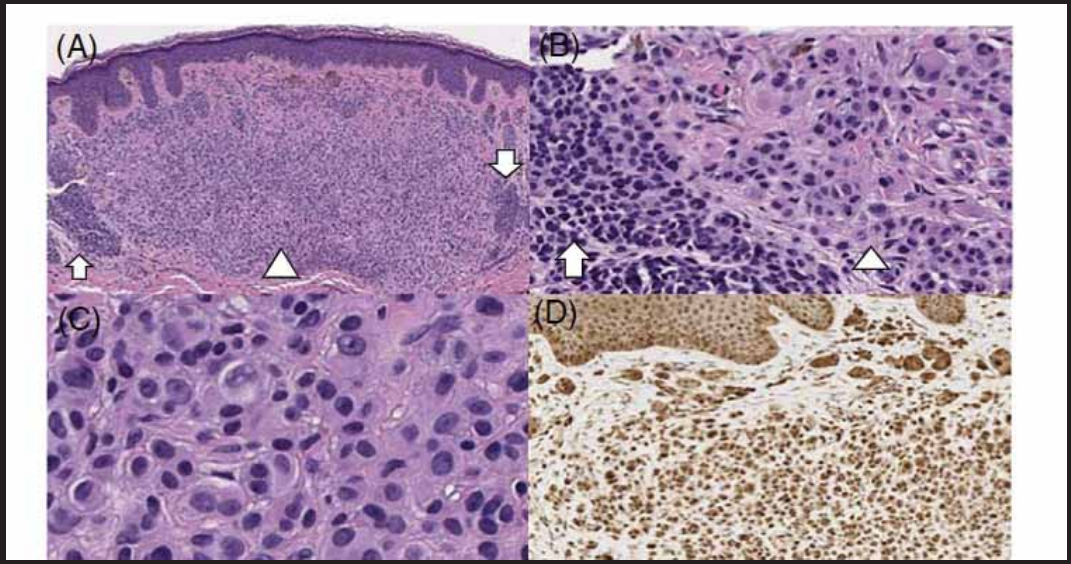
CASE REPORT

JCP JOURNAL OF CUTANEOUS PATHOLOGY WILEY

# A case of molecularly confirmed *BAP1* inactivated melanocytic tumor with retention of immunohistochemical expression: A confounding factor

Konstantinos Linos MD | Aaron E. Atkinson PhD | Shaofeng Yan MD, PhD | Gregory J. Tsongalis PhD | Joel A. Lefferts PhD



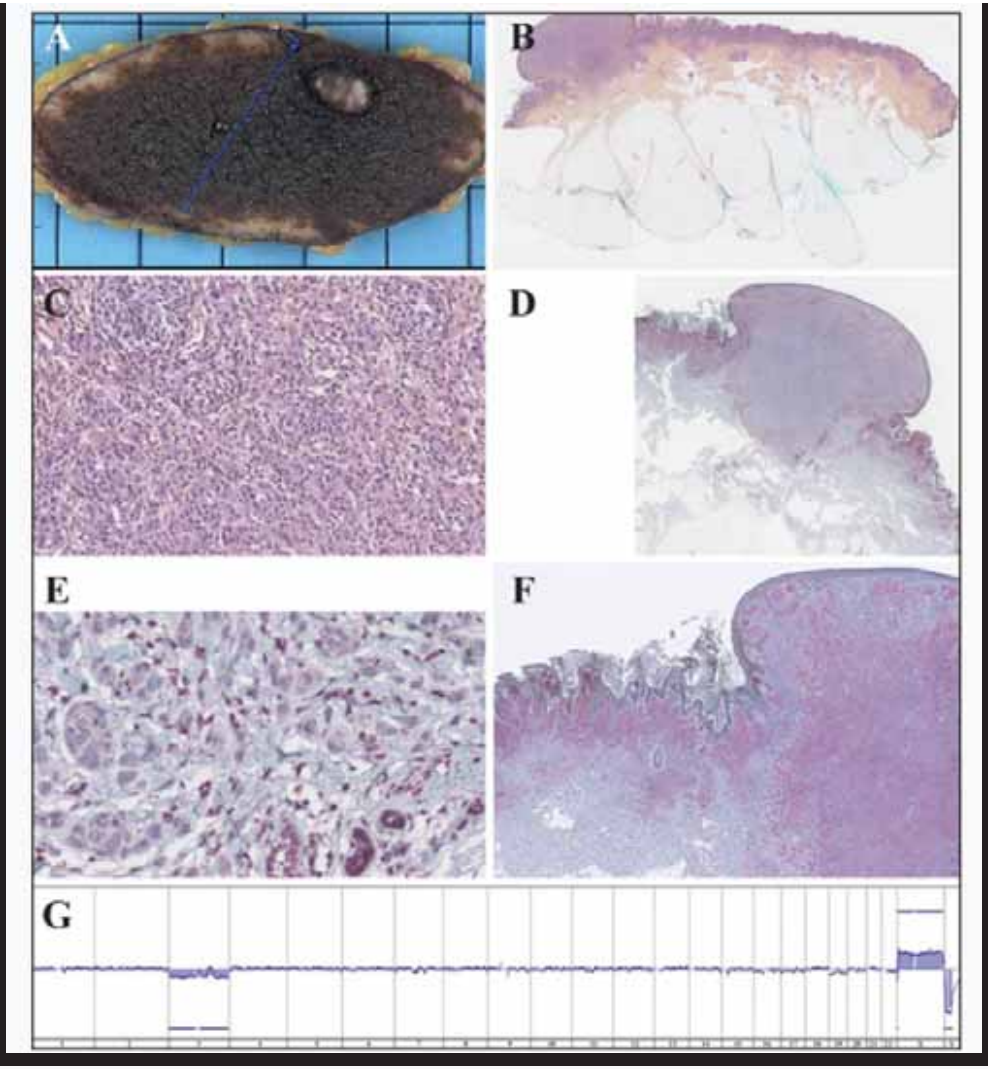


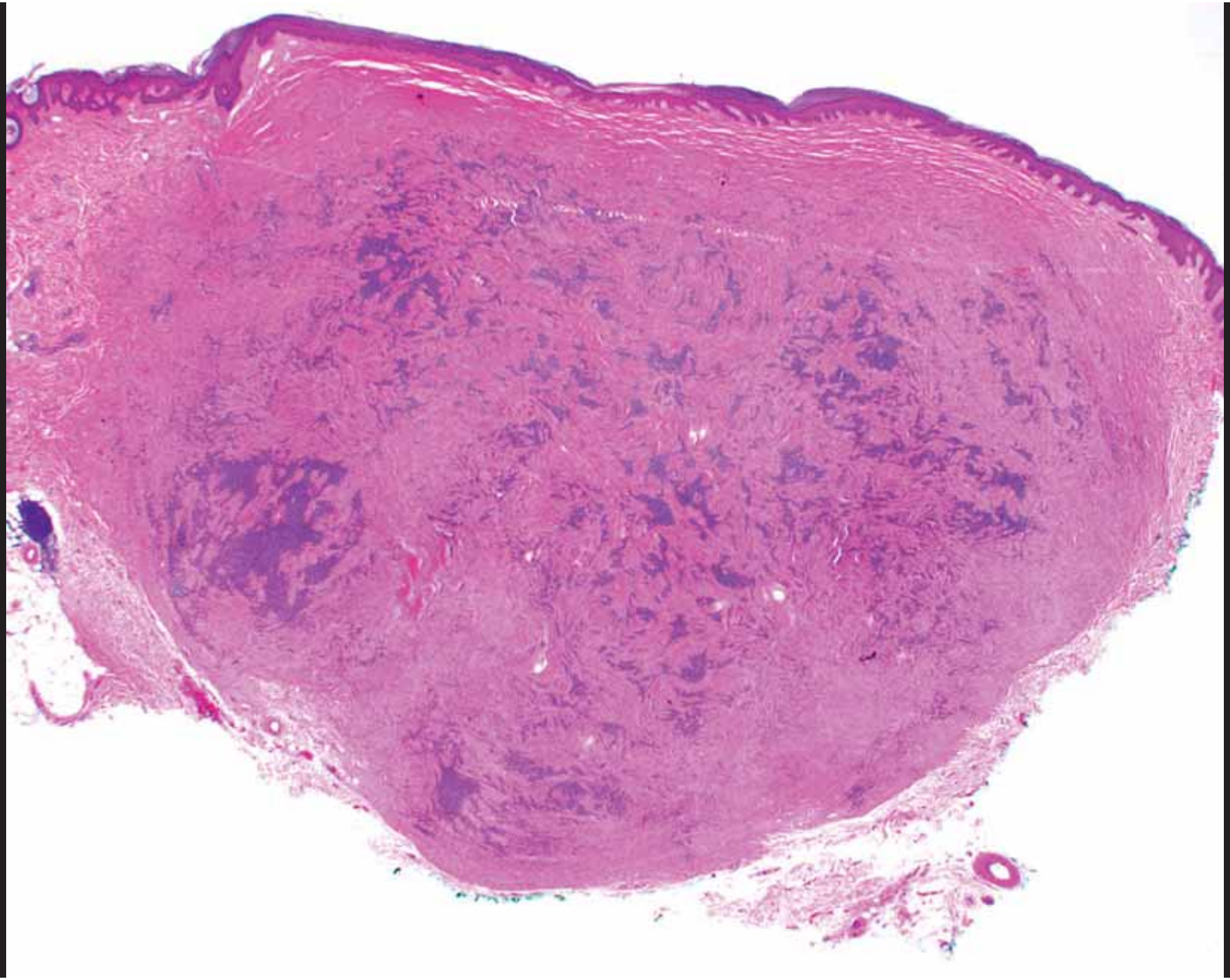

**Figure 1**

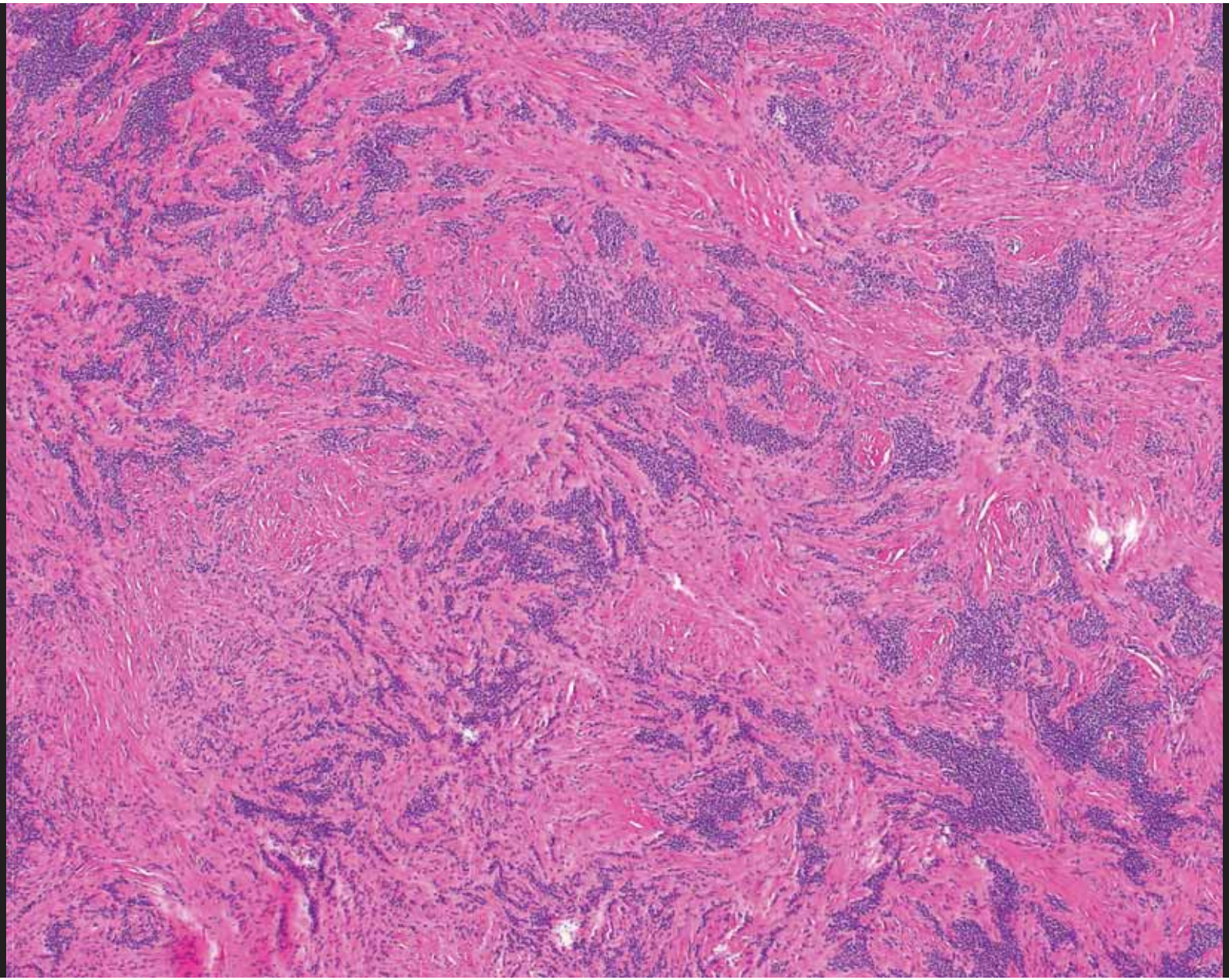
**A) Macroscopy: medium-sized congenital nevus with partially depigmented centimetric nodule B) Low power microscopy: dense dermal nodule disrupting the architecture of the congenital nevus. C) High power microscopy: large epithelioid melanocytes with images of kissing lymphocytes. D) BAP1 IHC: loss of nuclear expression in melanocytes restricted to the nodule. E) BAP1 IHC: close up view of loss of expression. Notice positive staining in sweat gland. F) BRAF: diffuse cytoplasmic expression. G) aCGH: loss restricted to chromosome 3**

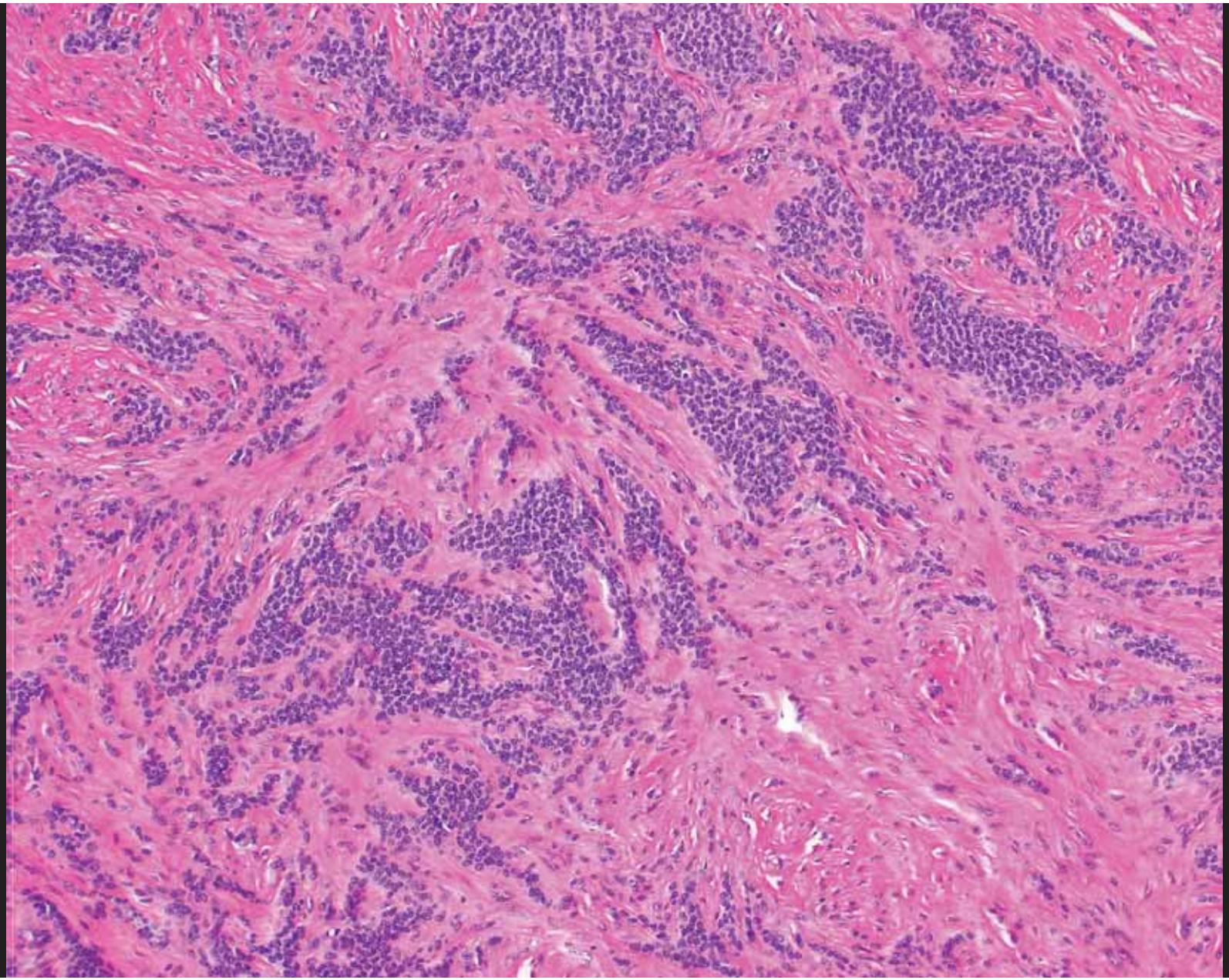
# OR MOSIAICISM WITH ONE AND TWO HITS?

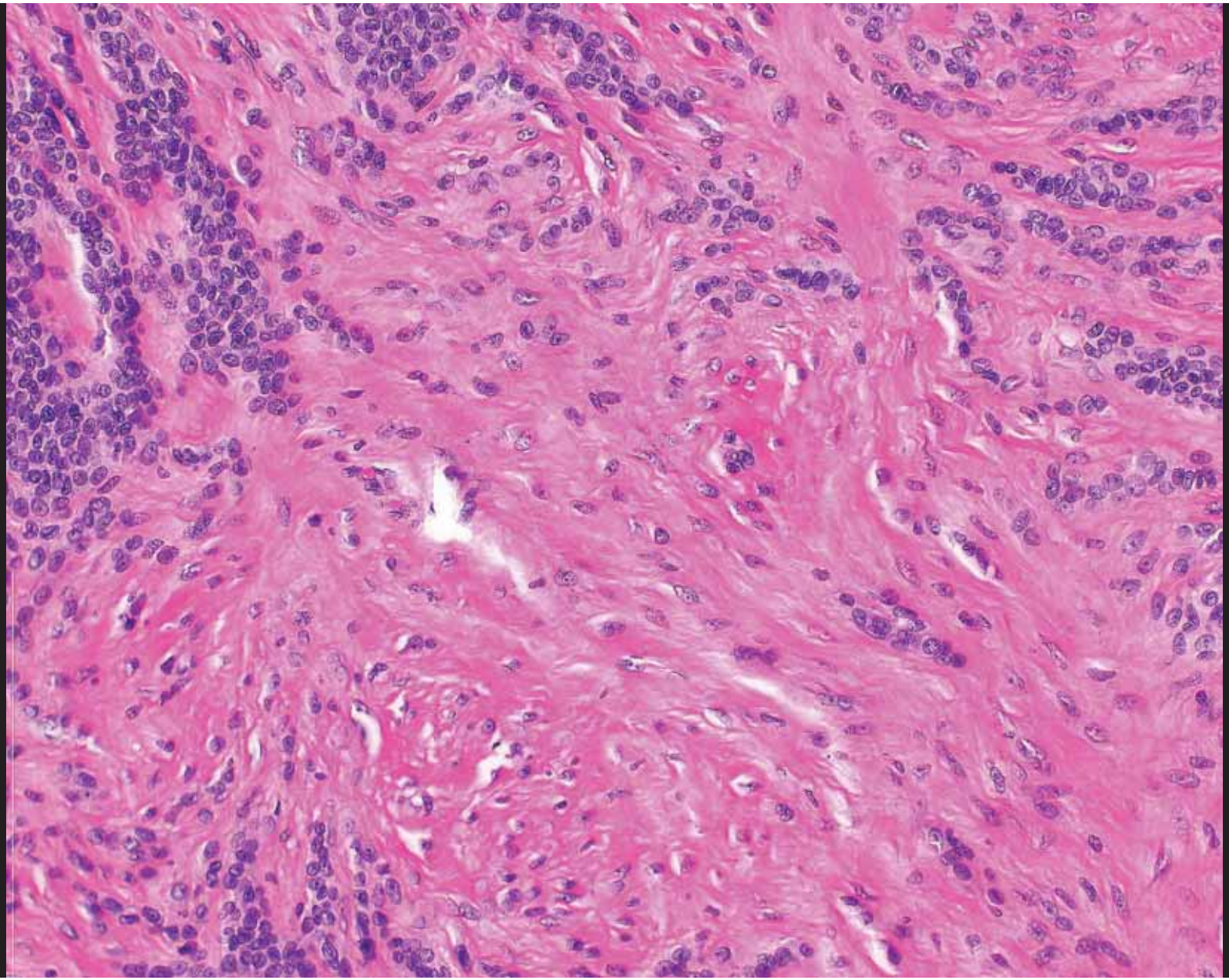
Is this tumor progression?

20 year old woman, gluteal cleft, r/o malignancy









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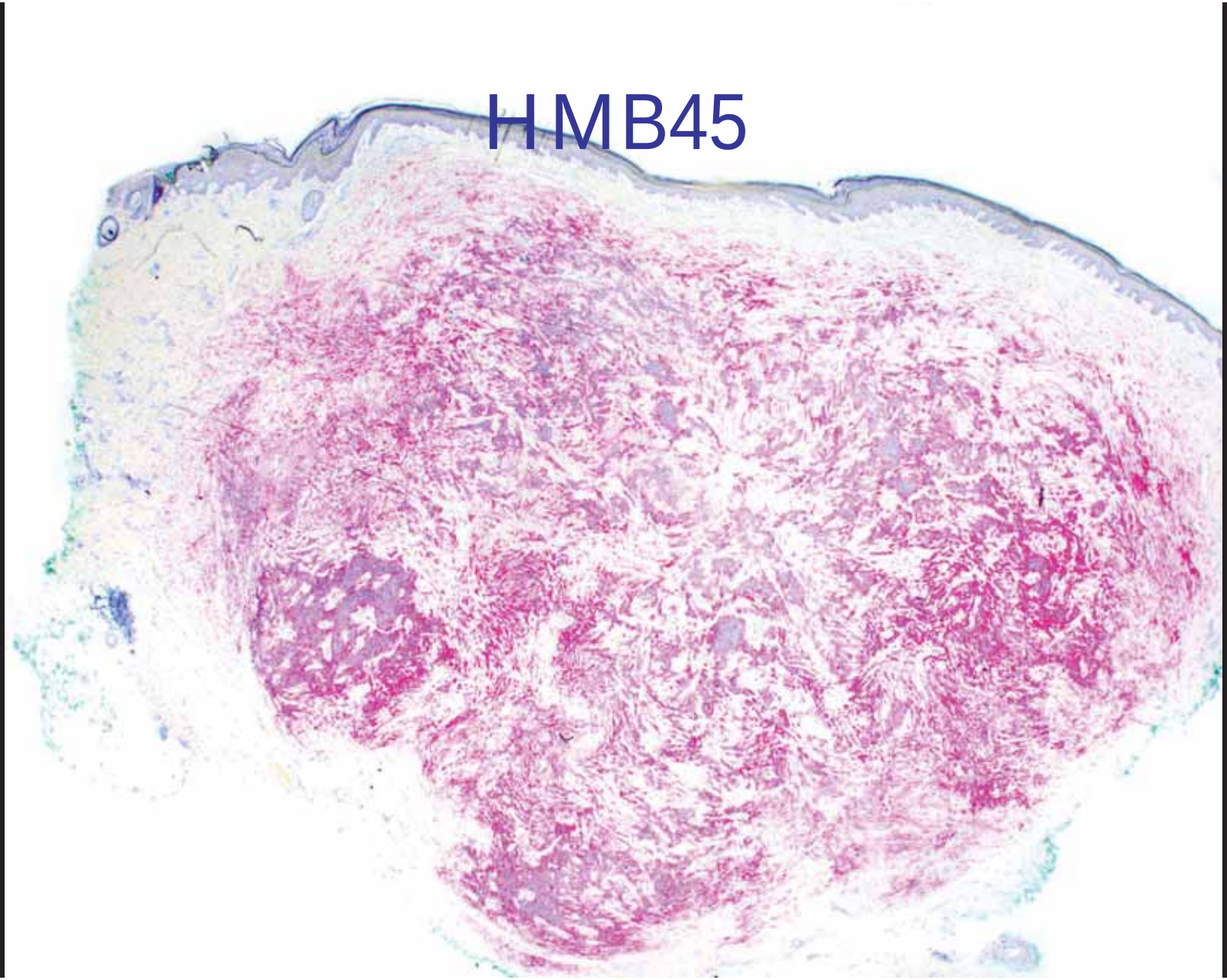
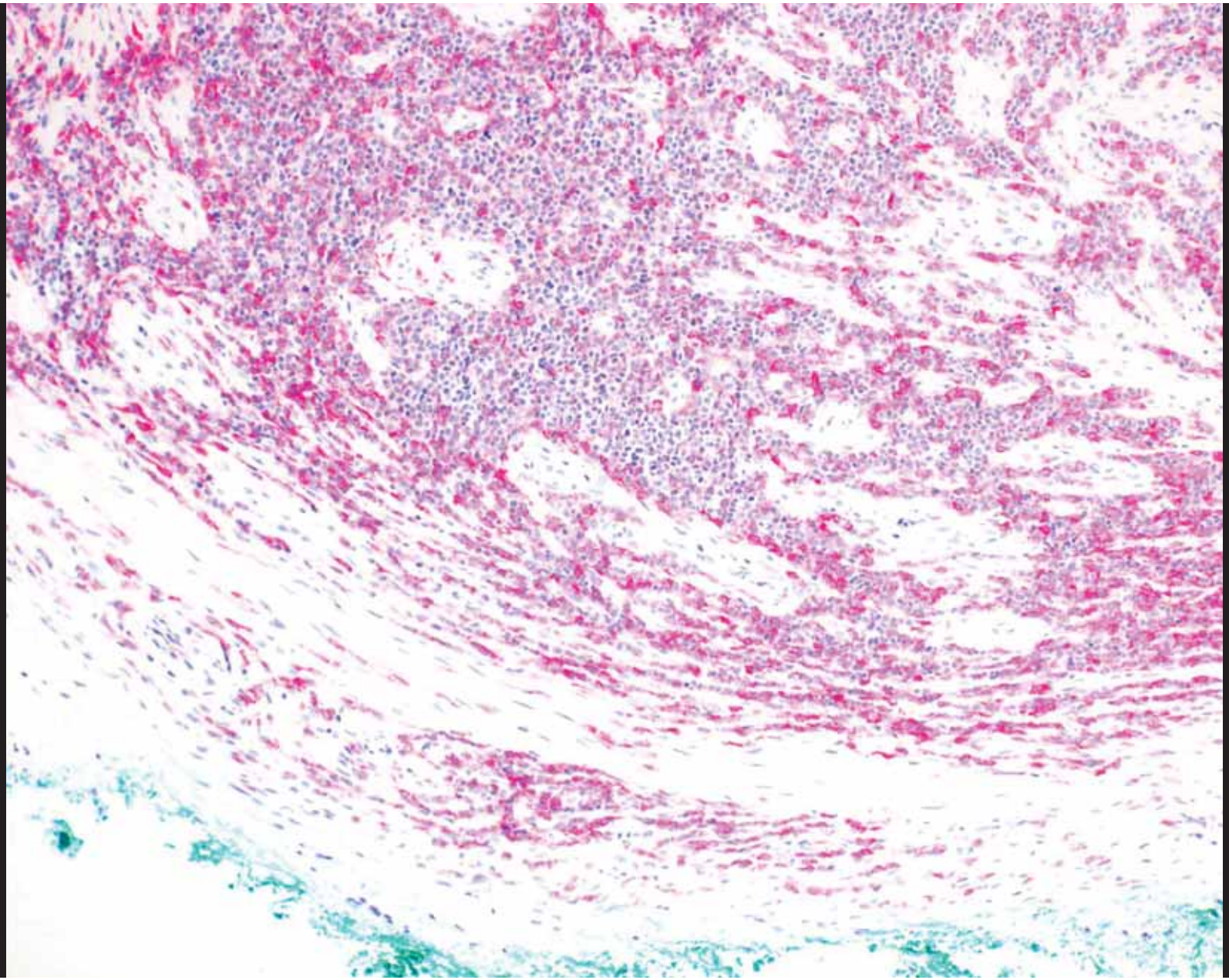
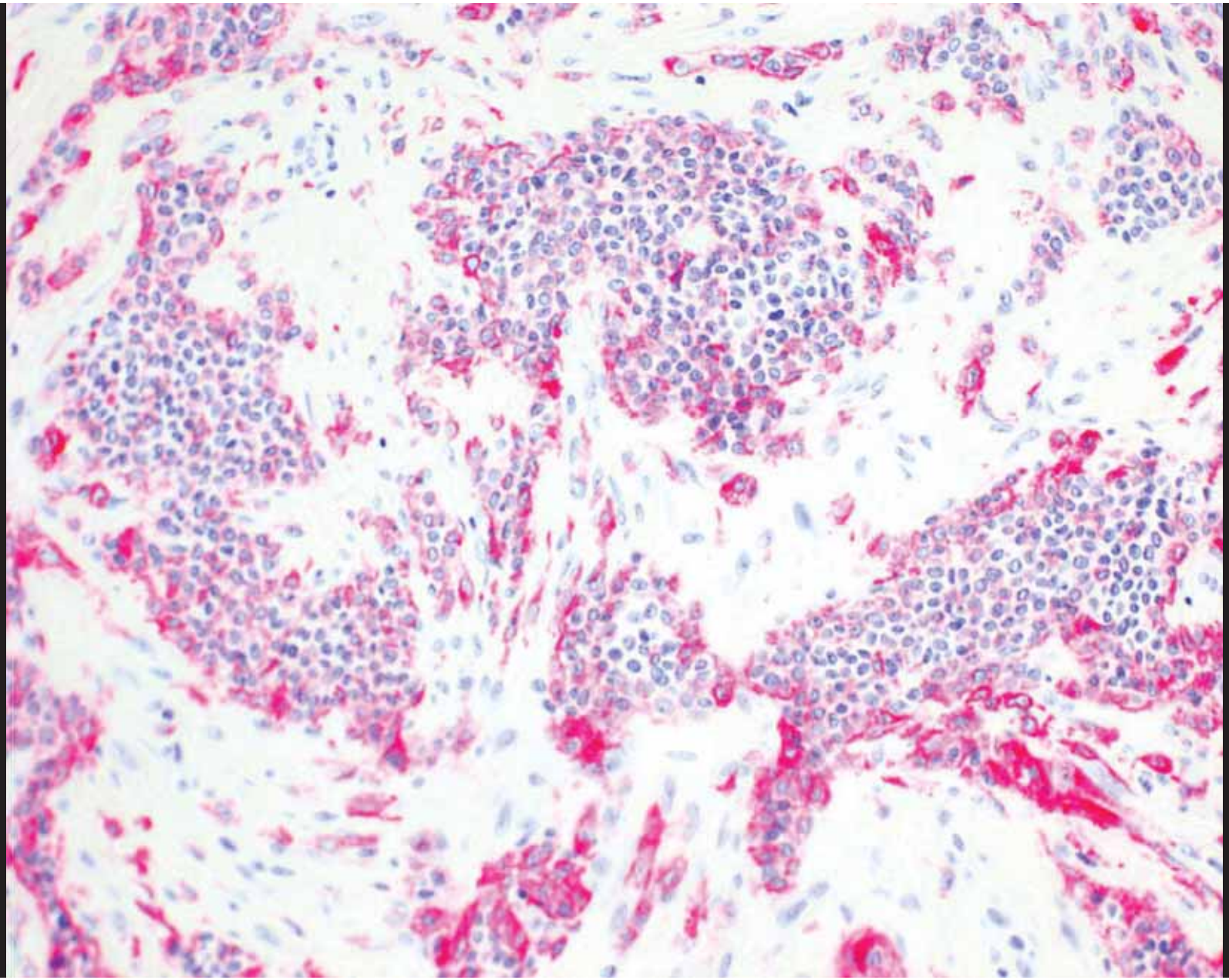






Protein kinase C fusion

(RNAseq courtesy of Dr. Arnaud de la Fouchardiere)

One fusion

## TWO POPULATIONS OF MELANOCYTES

Mod Pathol 36 (2023) 100286

MODERN PATHOLOGY

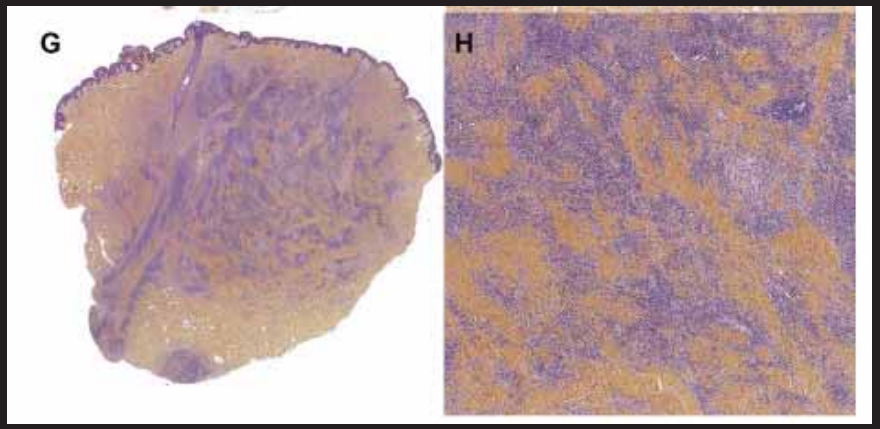
Journal homepage: https://modernpathology.org/

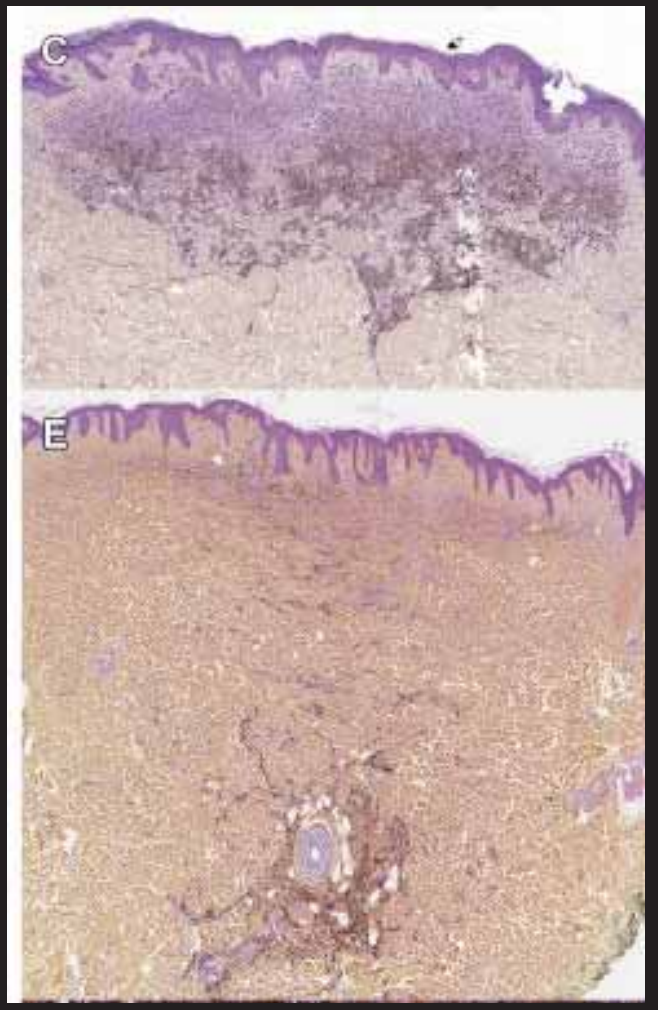
Research Article

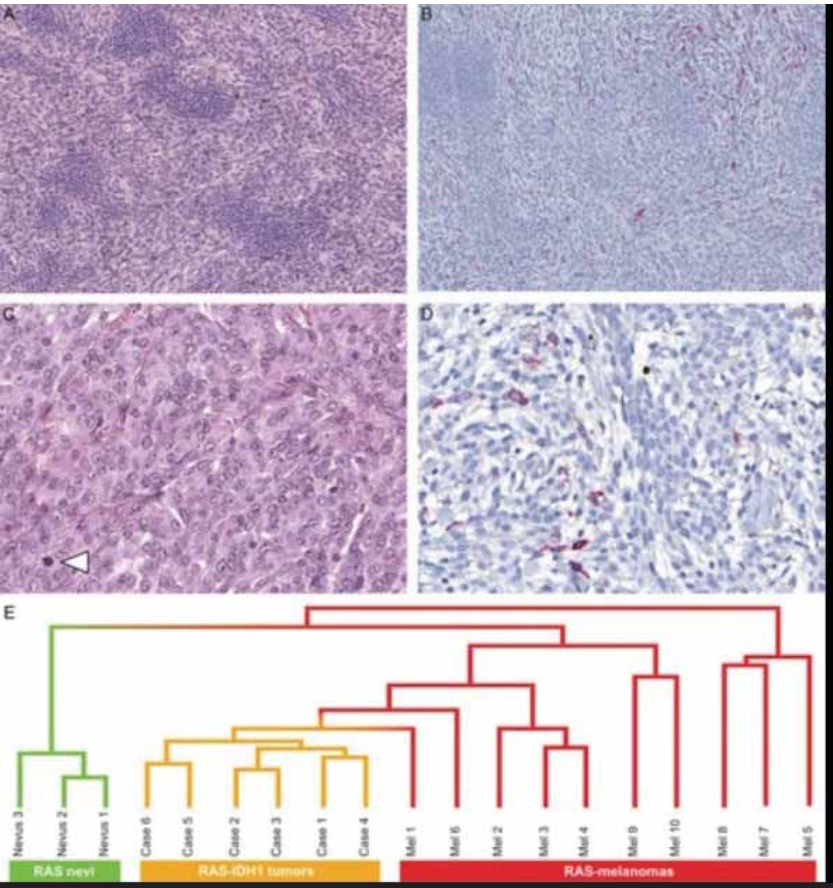
# Histologic and Genetic Features of 51 Melanocytic Neoplasms With Protein Kinase C Fusion Genes

Arnaud de la Fouchardière[a,b,*], Daniel Pissaloux[a,b], Aurélie Houlier[a], Sandrine Paindavoine[a], Franck Tirode[b], Philip E. LeBoit[c,d], Boris C. Bastian[c,d], Iwei Yeh[c,d]

[a] Department of Biopathology, Centre Léon Bérard, Lyon, France; [b] Department of Research, University of Lyon, Université Claude Bernard Lyon 1, Cancer Research Centre of Lyon, Lyon, France; [c] Department of Dermatology, Helen Diller Family Cancer Center, University of California, San Francisco, San Francisco, California; [d] Department of Pathology, Helen Diller Family Cancer Center, University of California, San Francisco, San Francisco, California



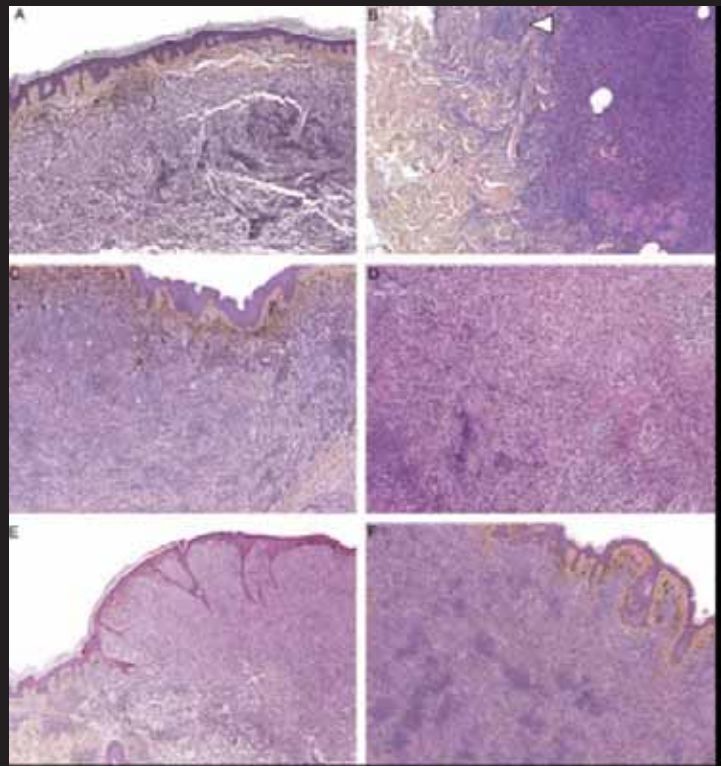


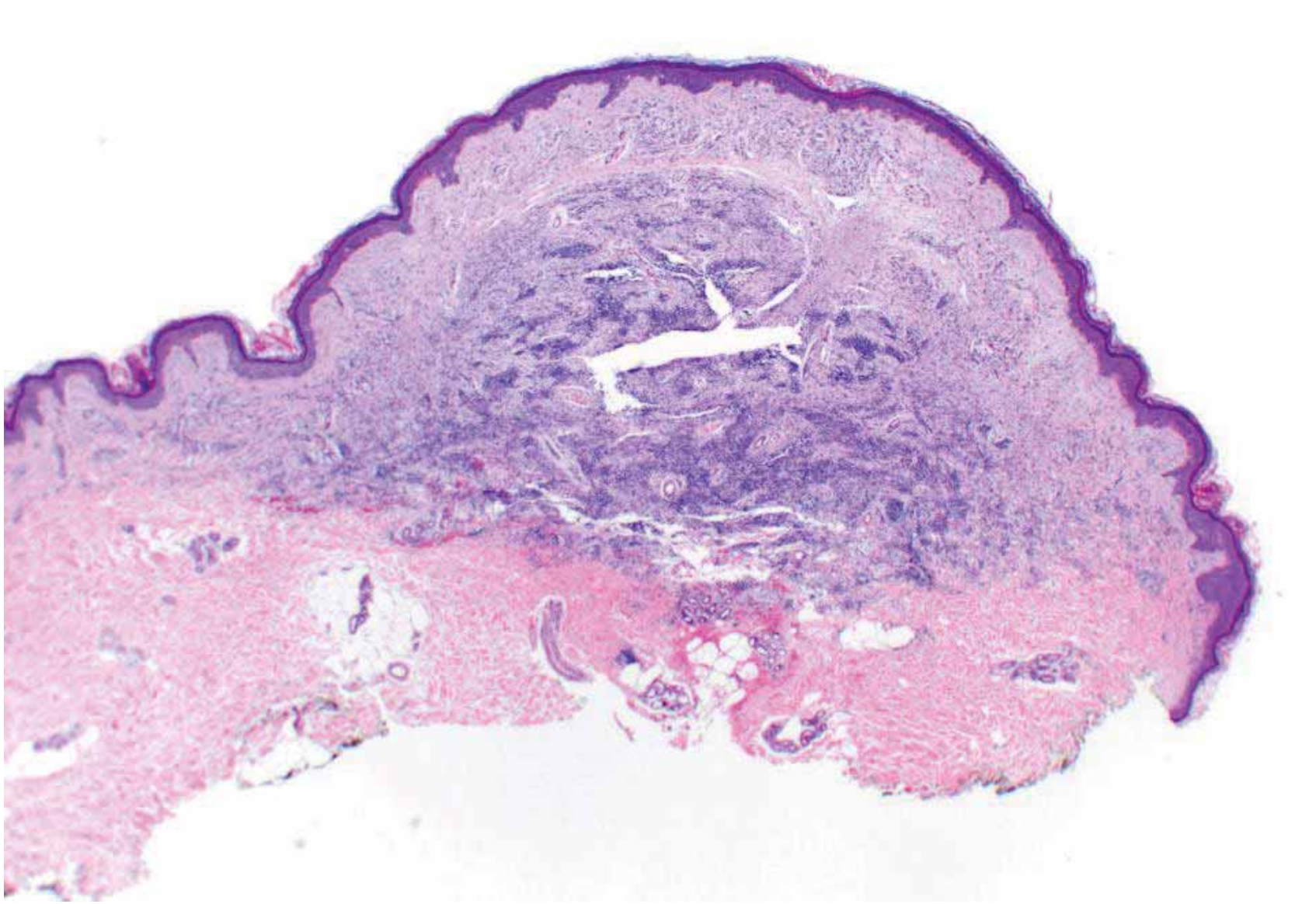

ORIGINAL ARTICLE

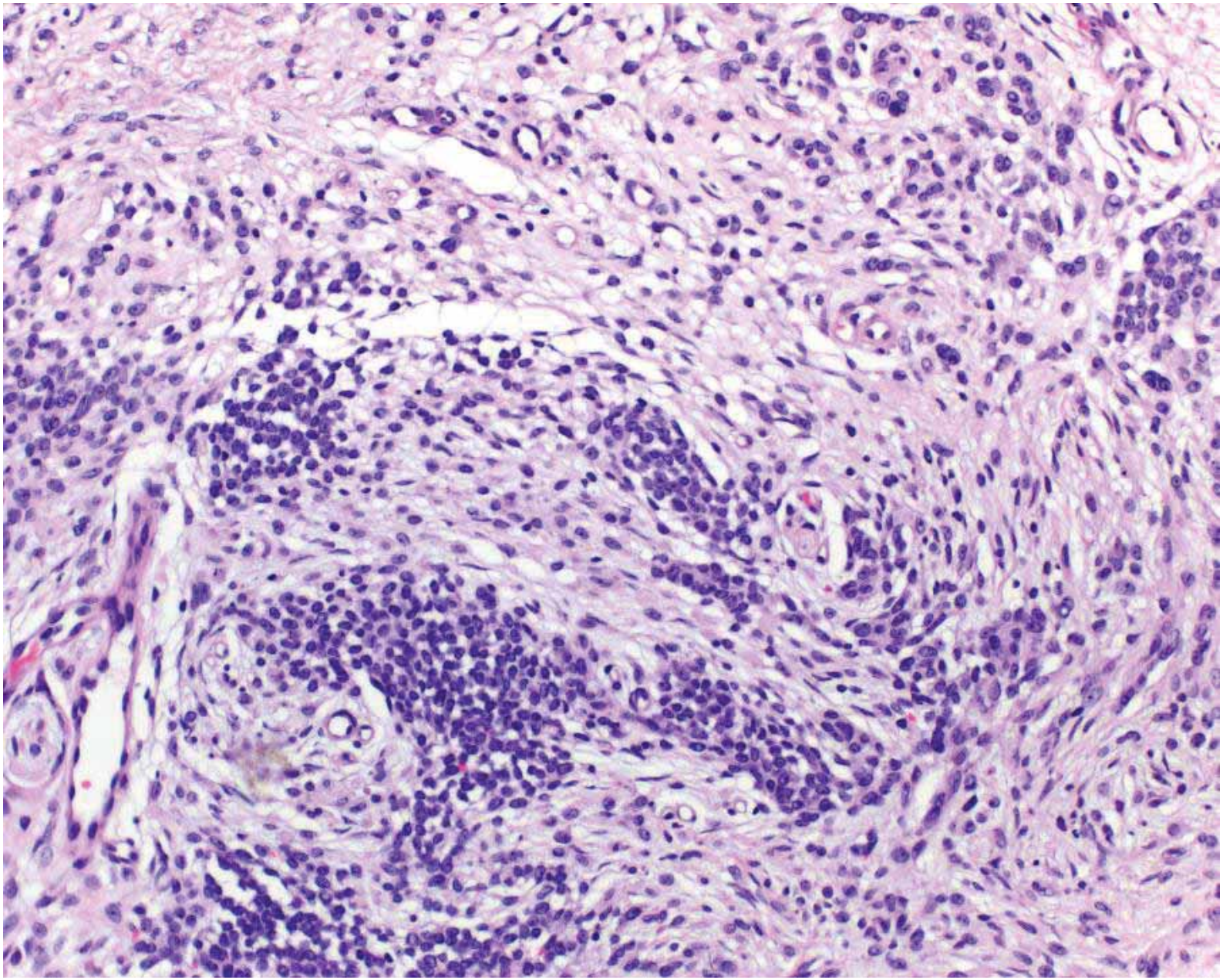
# Cutaneous Melanocytic Tumors With Concomitant *NRAS*$^{Q61R}$ and *IDH1*$^{R132C}$ Mutations

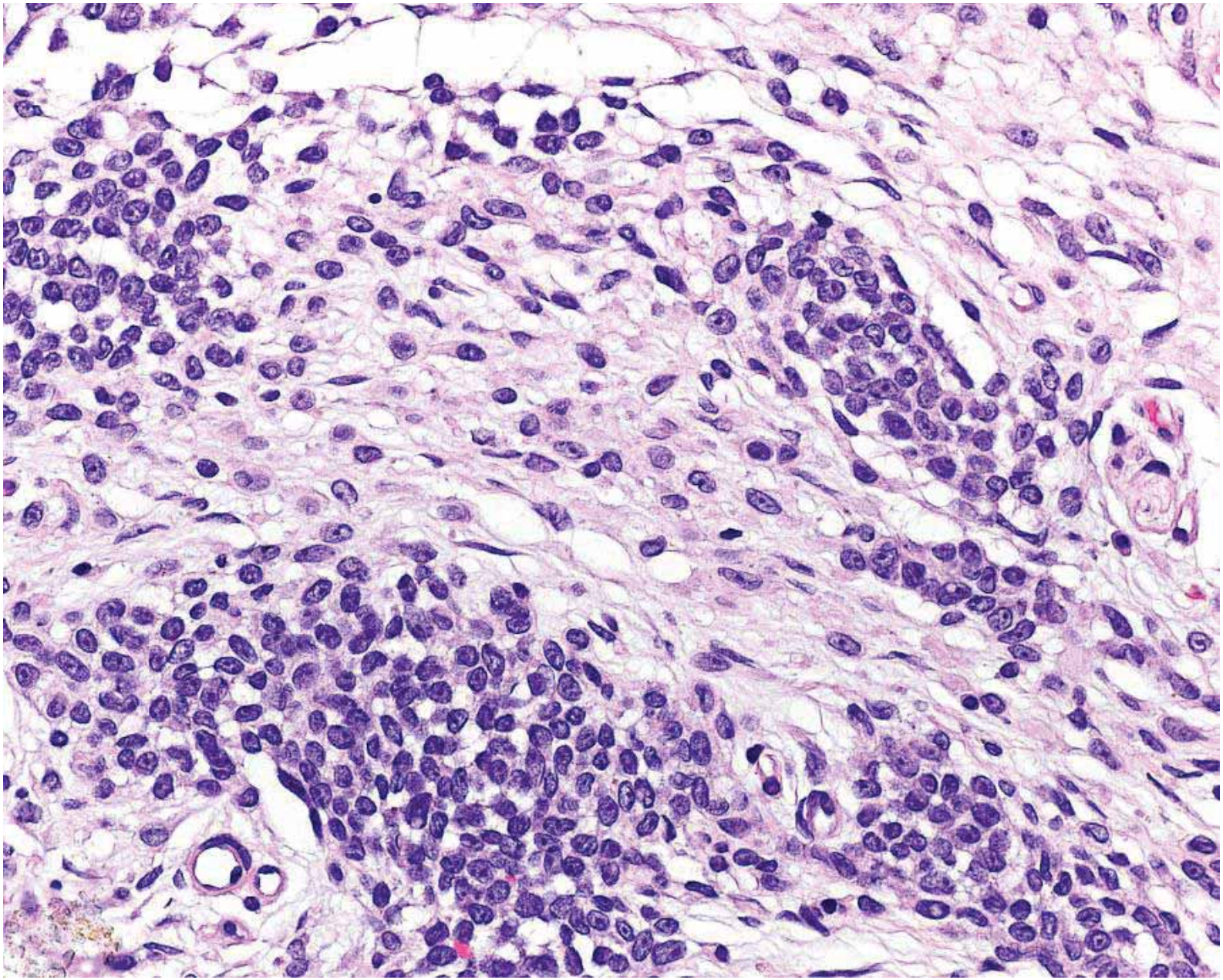
## *A Report of 6 Cases*

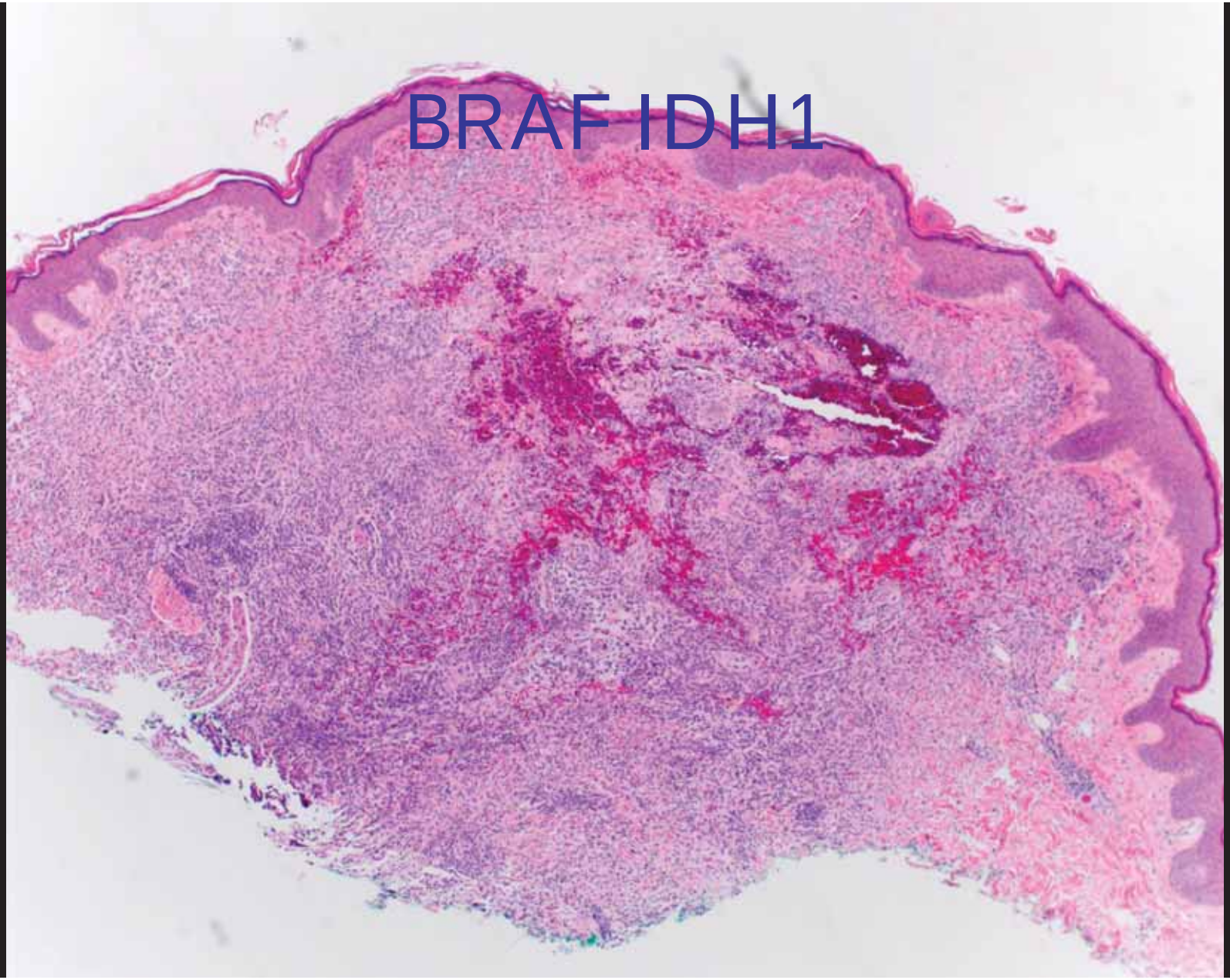
*Nicolas Macagno, MD, PhD,*† Daniel Pissaloux, PhD,‡§ Heather Etchevers, PhD,† Véronique Haddad, PhD,‡ Beatrice Vergier, MD, PhD,‖ Sandrine Sierra-Fortuny, MD,¶ Franck Tirode, PhD,‡§ and Arnaud de la Fouchardière, MD, PhD‡§*

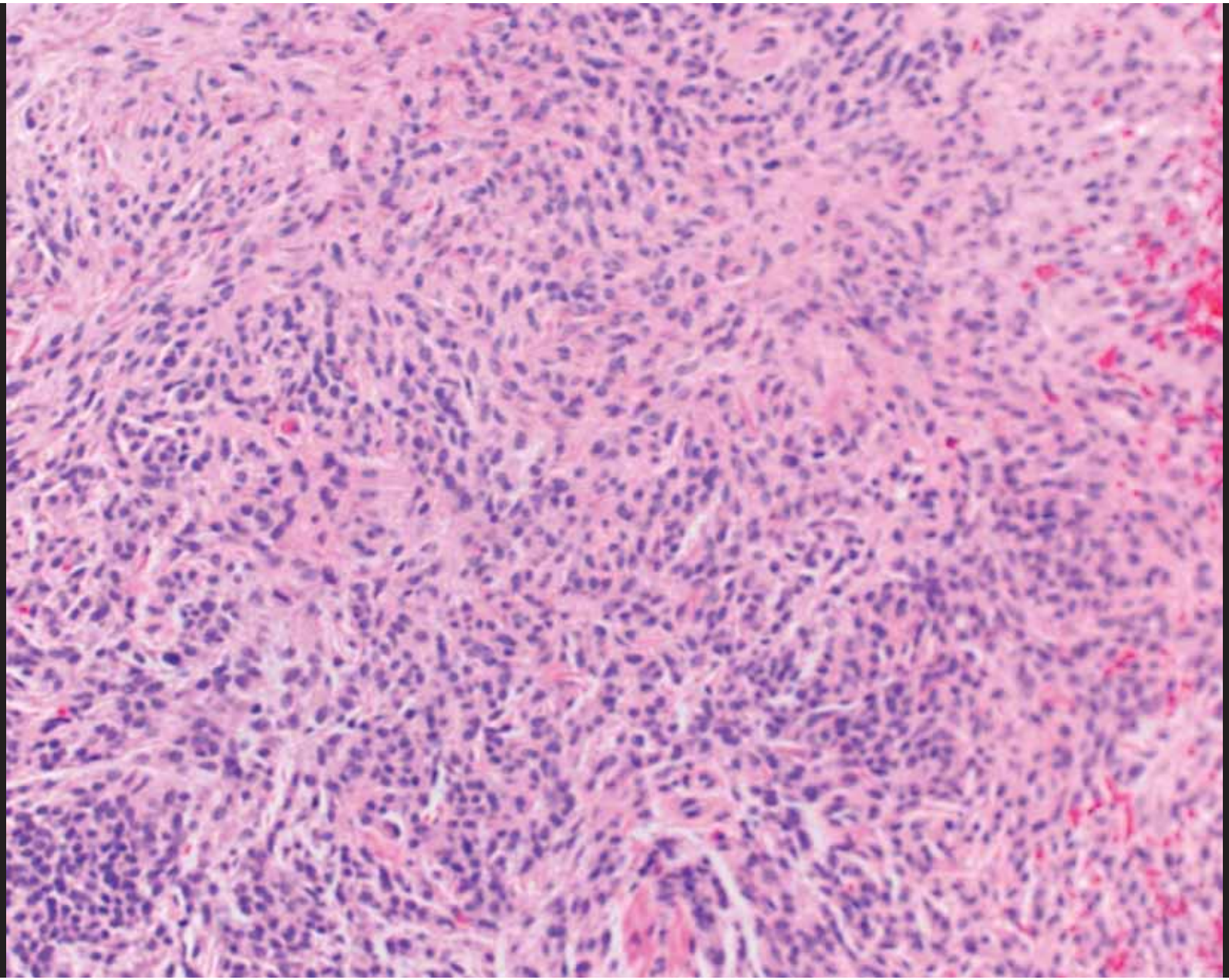


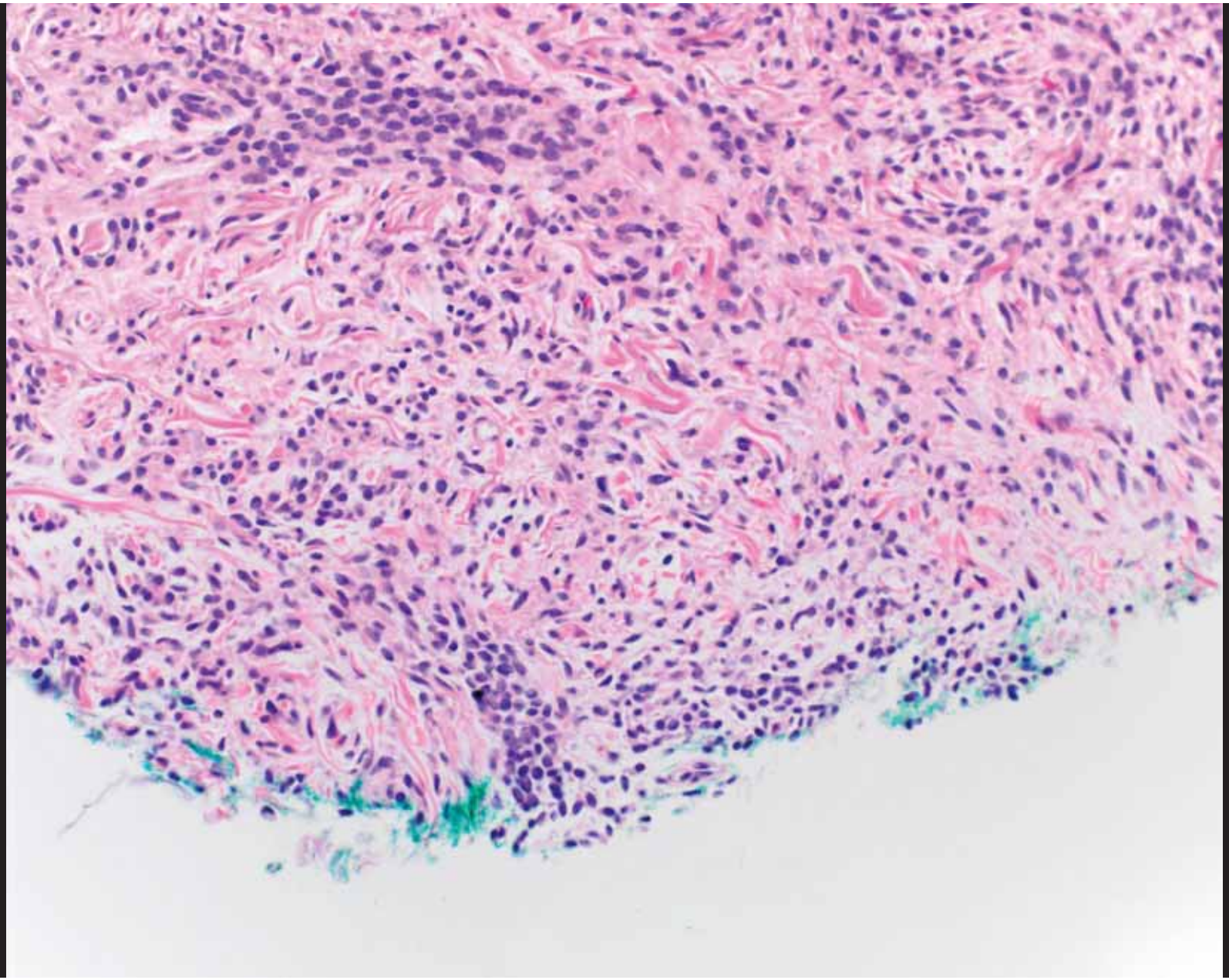







BRAF IDH1





ORIGINAL ARTICLE

# Clinical and Pathologic Findings of Spitz Nevi and Atypical Spitz Tumors With *ALK* Fusions

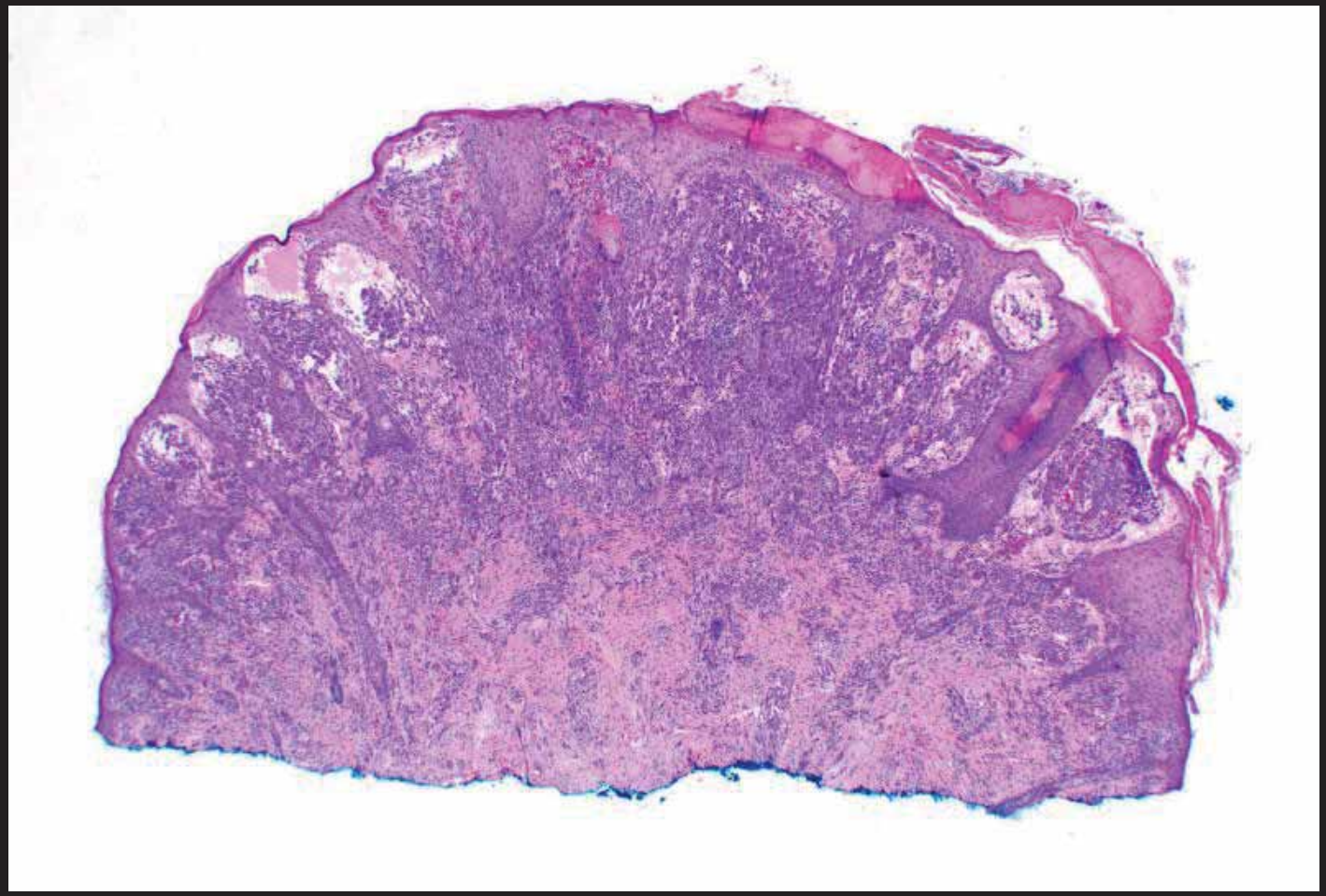
*Klaus J. Busam, MD,* Heinz Kutzner, MD,† Lorenzo Cerroni, MD,‡ and Thomas Wiesner, MD‡§*

**Abstract:** Spitz tumors represent a group of melanocytic neoplasms that typically affect young individuals. Microscopically, the lesions are composed of cytologically distinct spindle and epithelioid melanocytes, with a range in the architectural display or the cells, their nuclear features, and secondary epidermal or stromal changes. Recently, kinase fusions have been documented

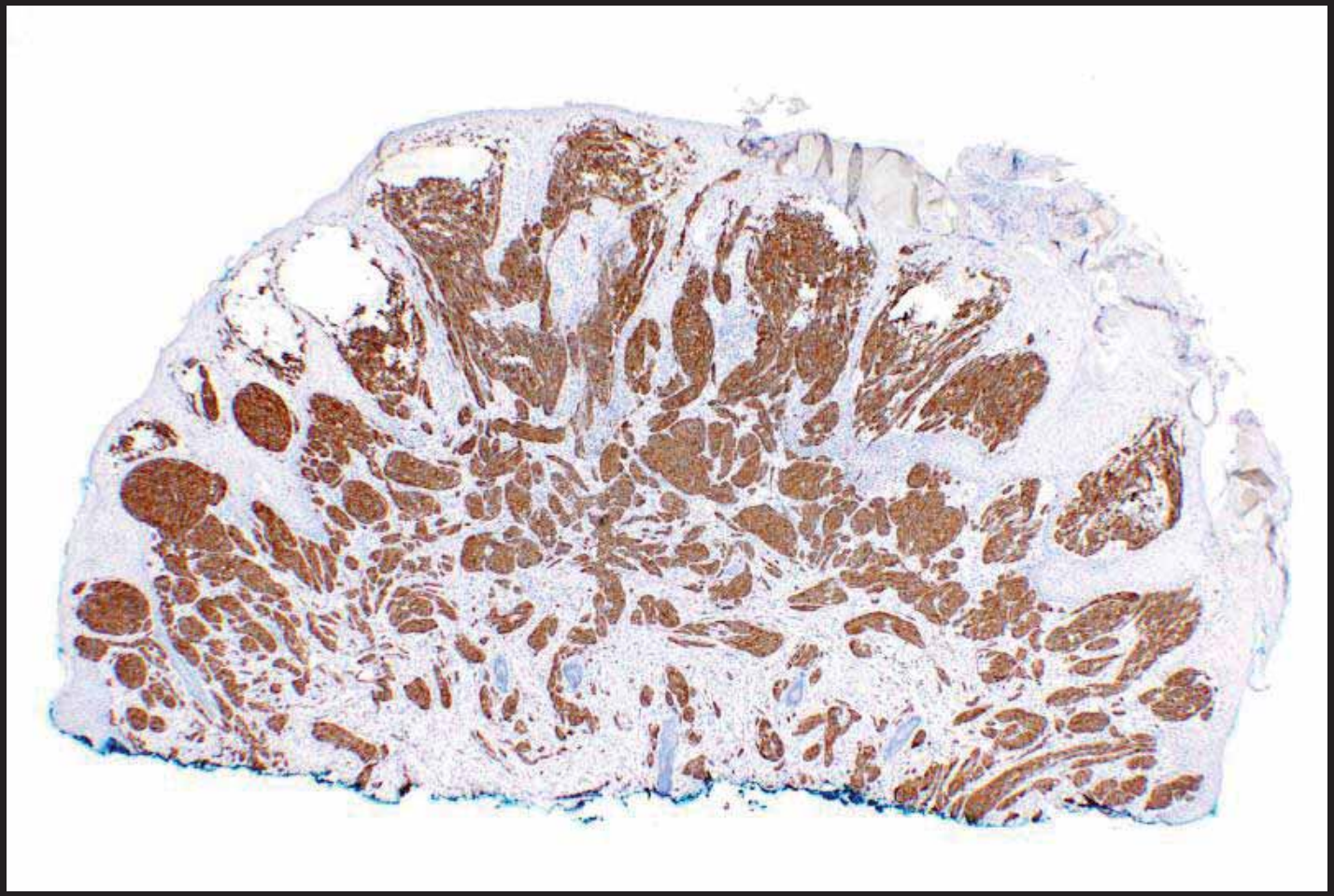
munohistochemistry and FISH enable the accurate identification of this morphologic and genetic distinct subset of spitzoid neoplasms.

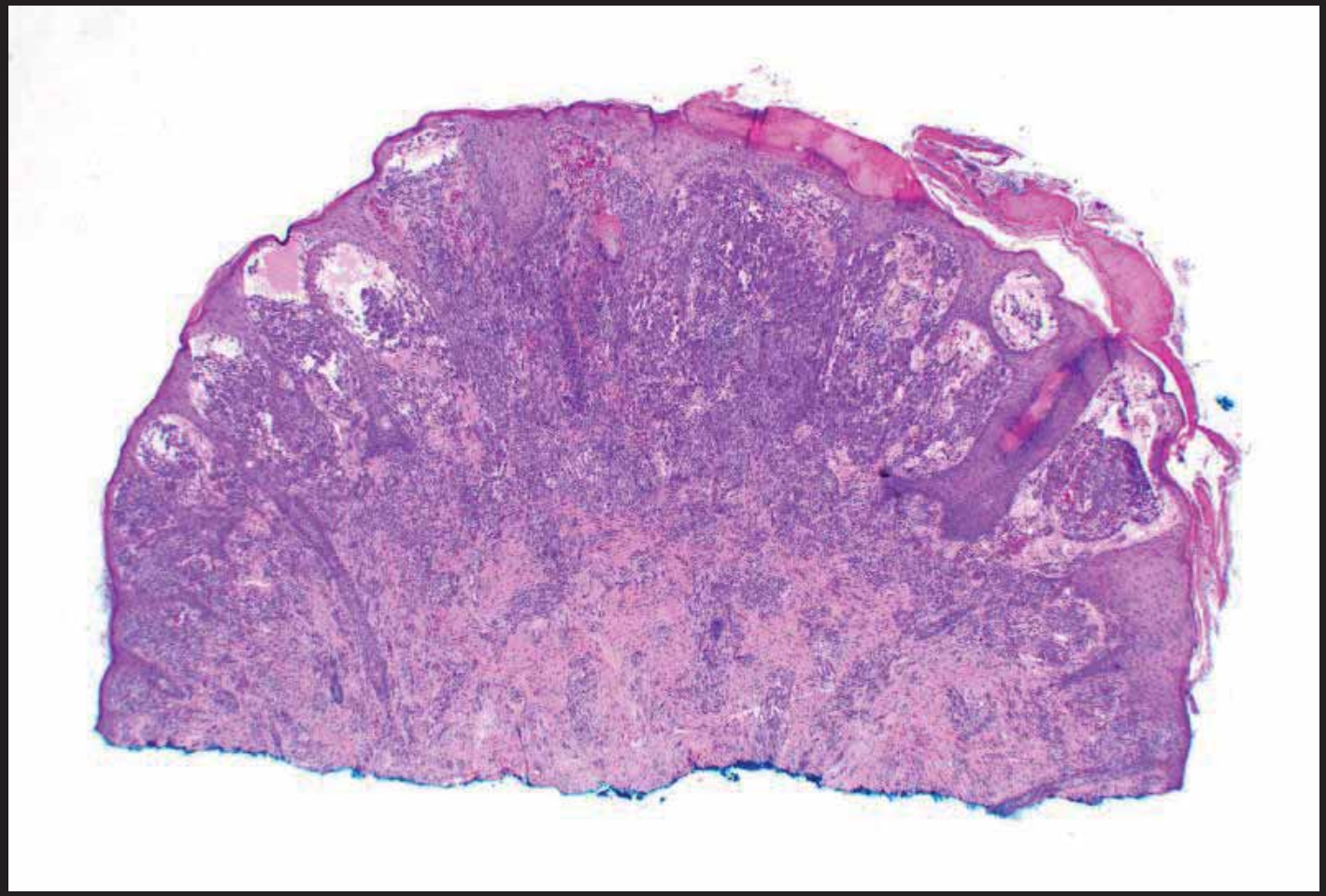
**Key Words:** kinase fusion, melanocytic nevus, Spitz nevus, ALK

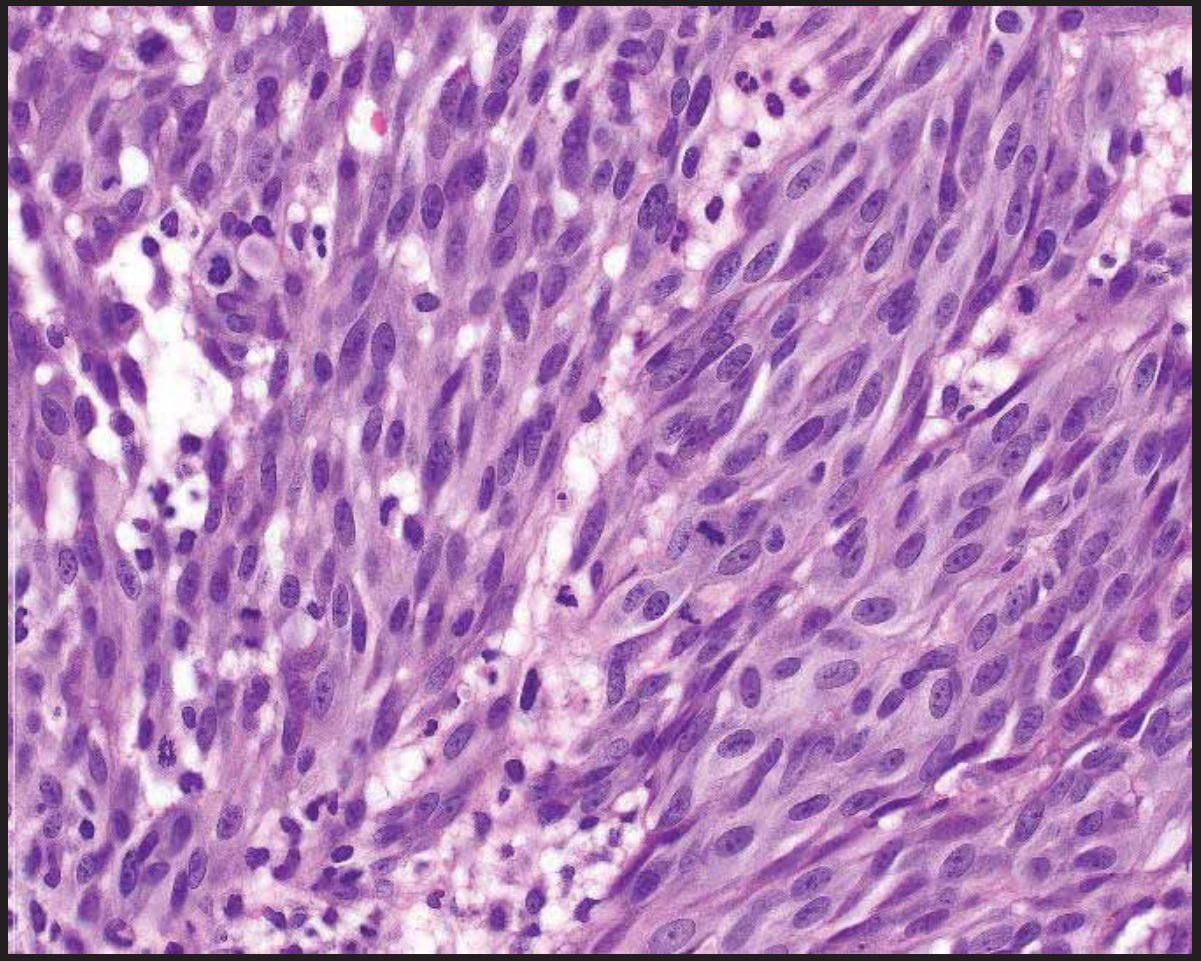
(*Am J Surg Pathol* 2014;38:925–933)



# ALK immunostain

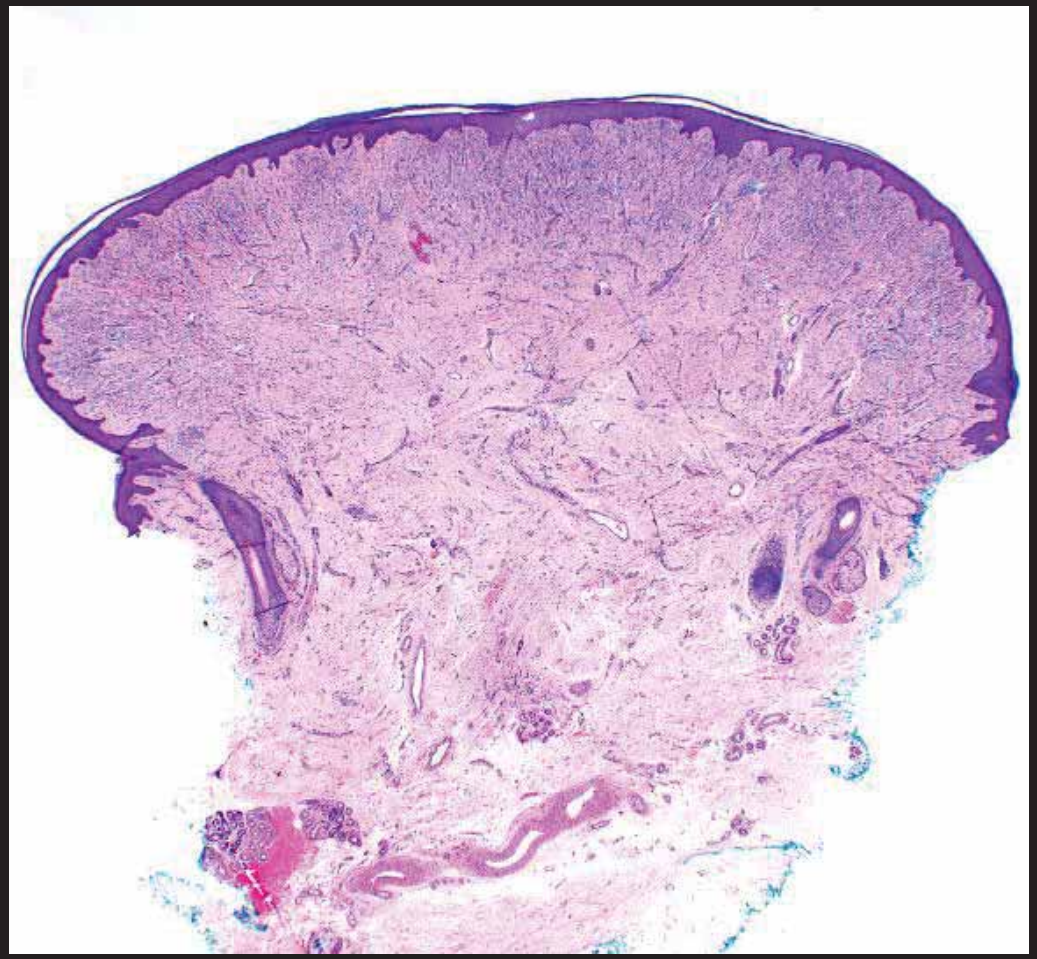


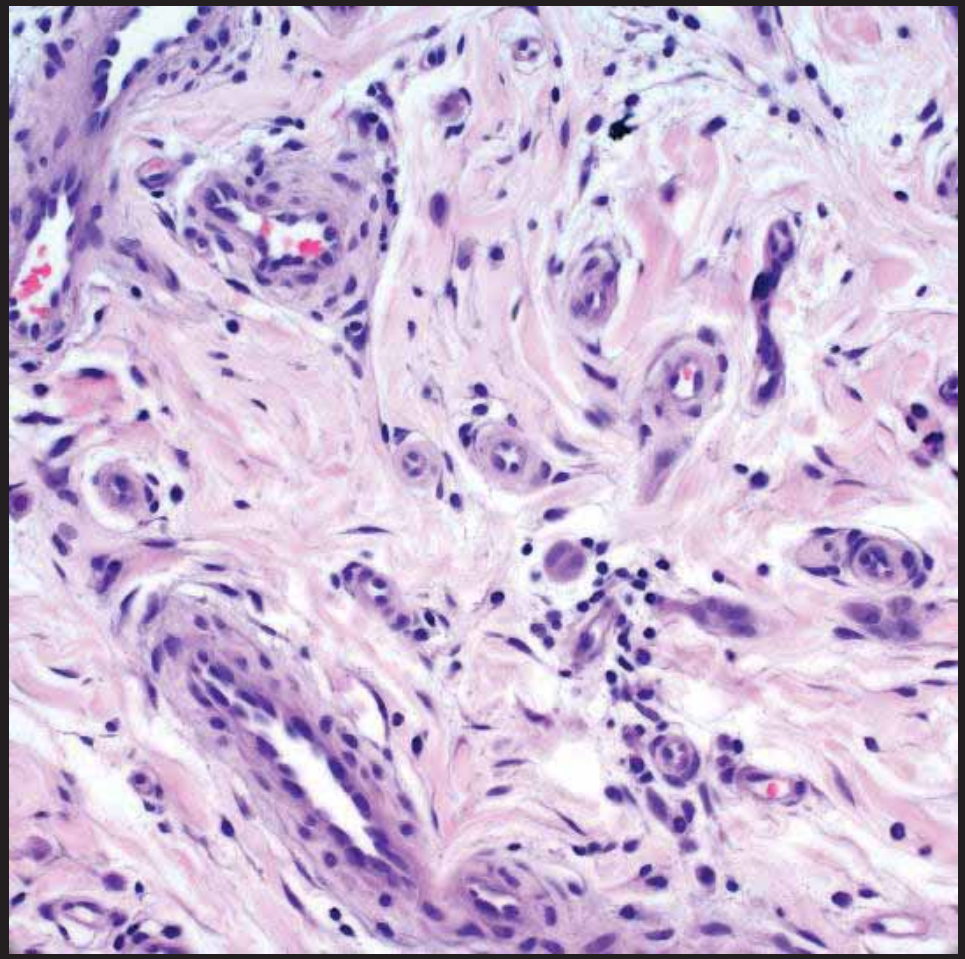




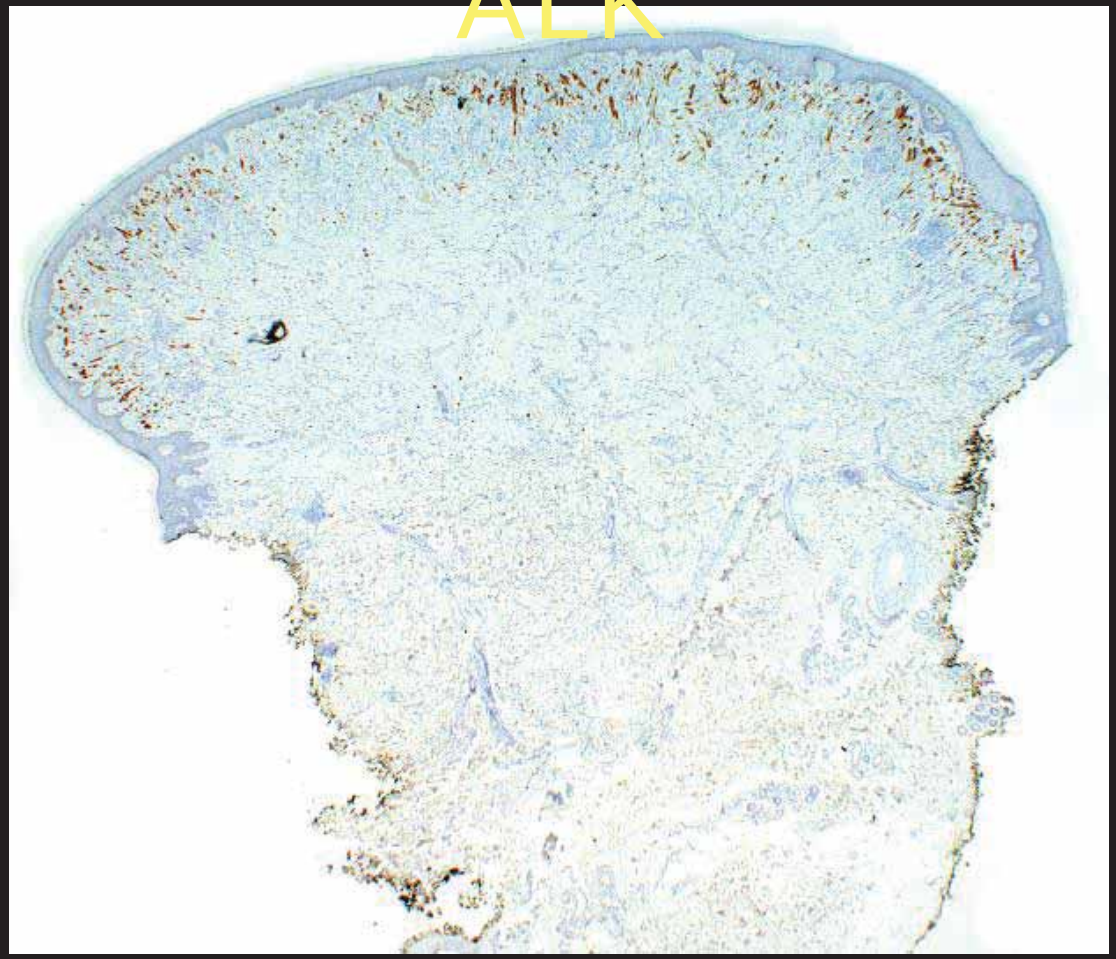
1. Very large nests with a high mitotic rate are just fine in the upper part of an ALK fused Spitz nevus

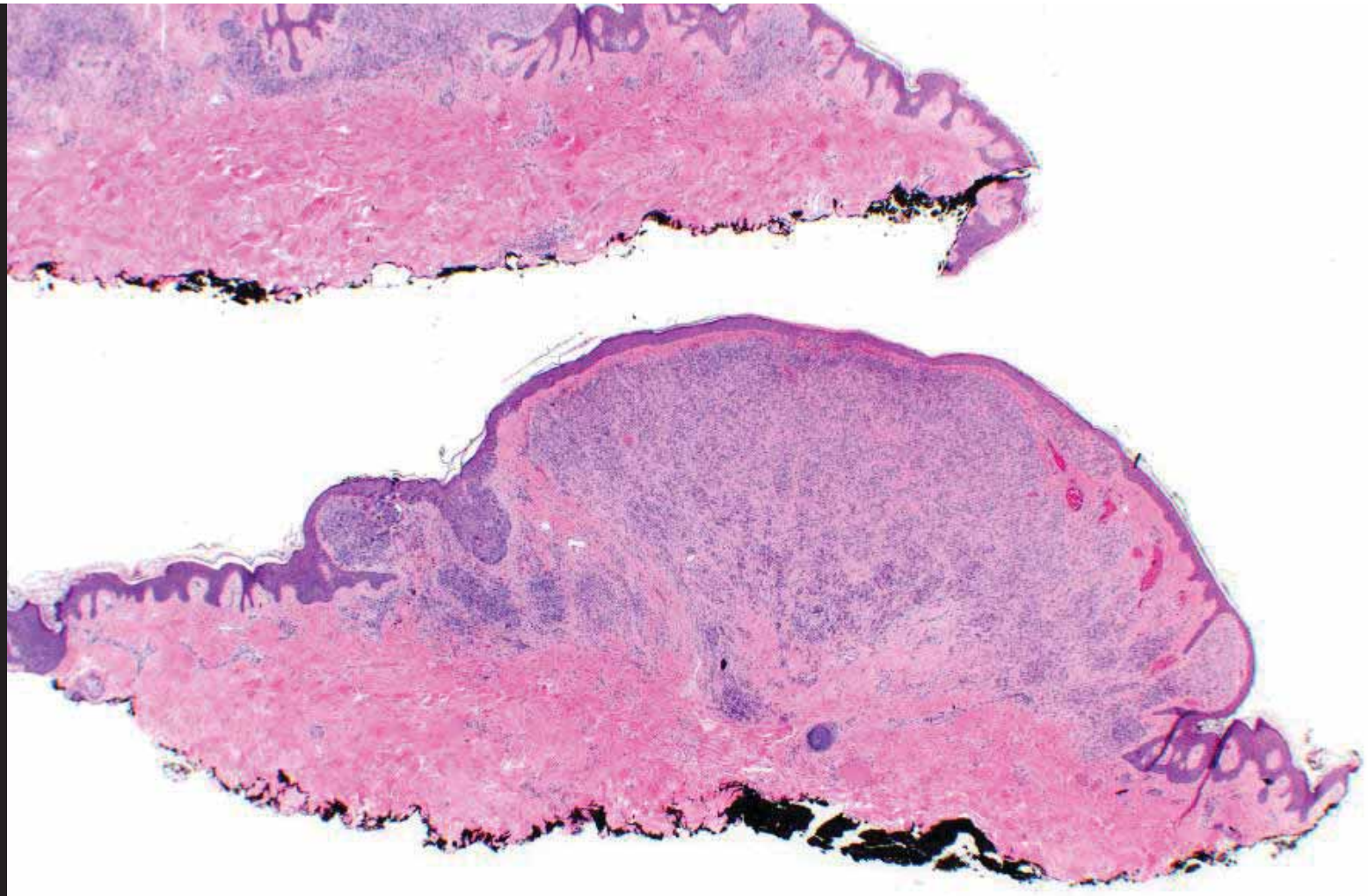
2. Superficial biopsies of ALK fused Spitz nevus may show ONLY these nests



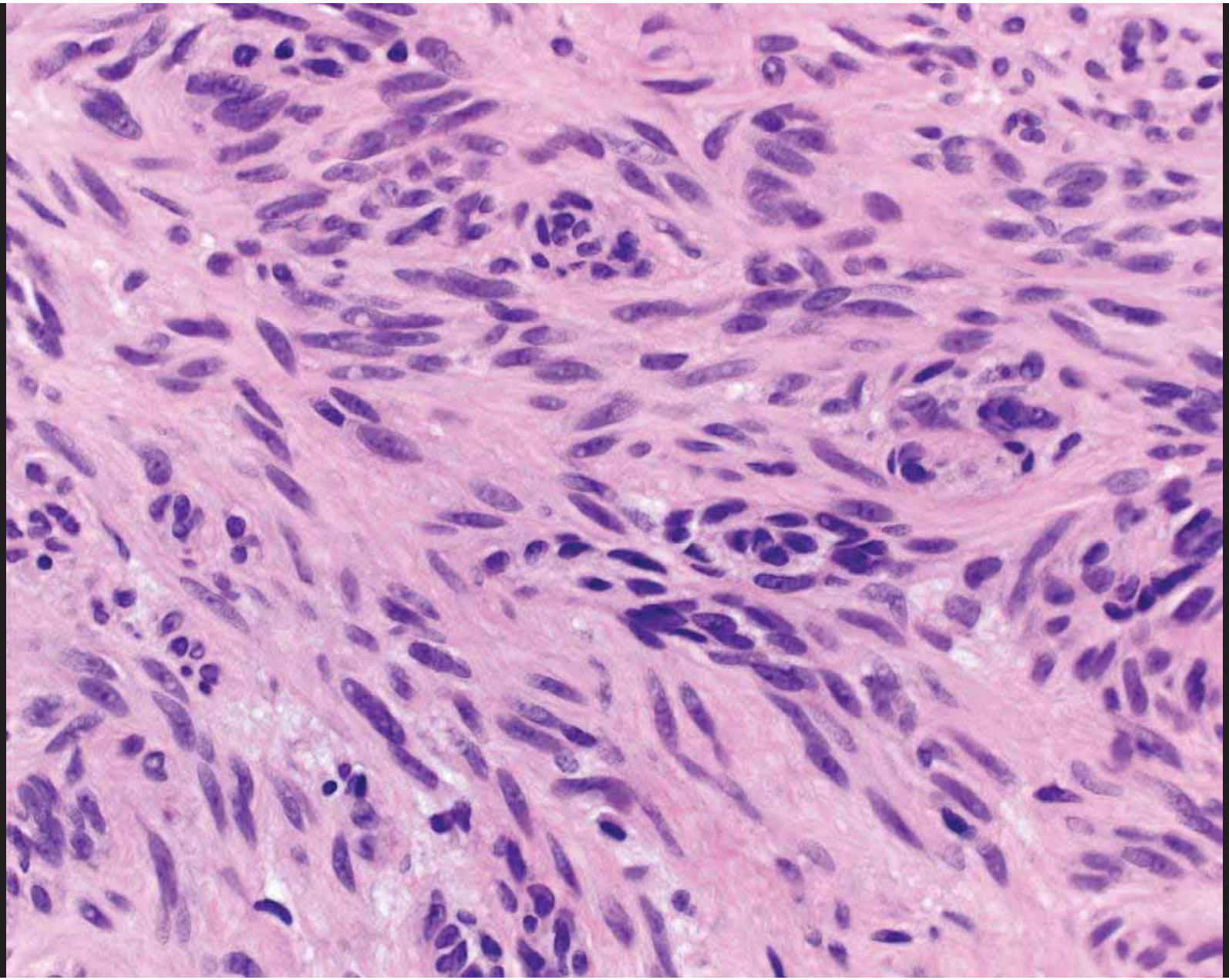


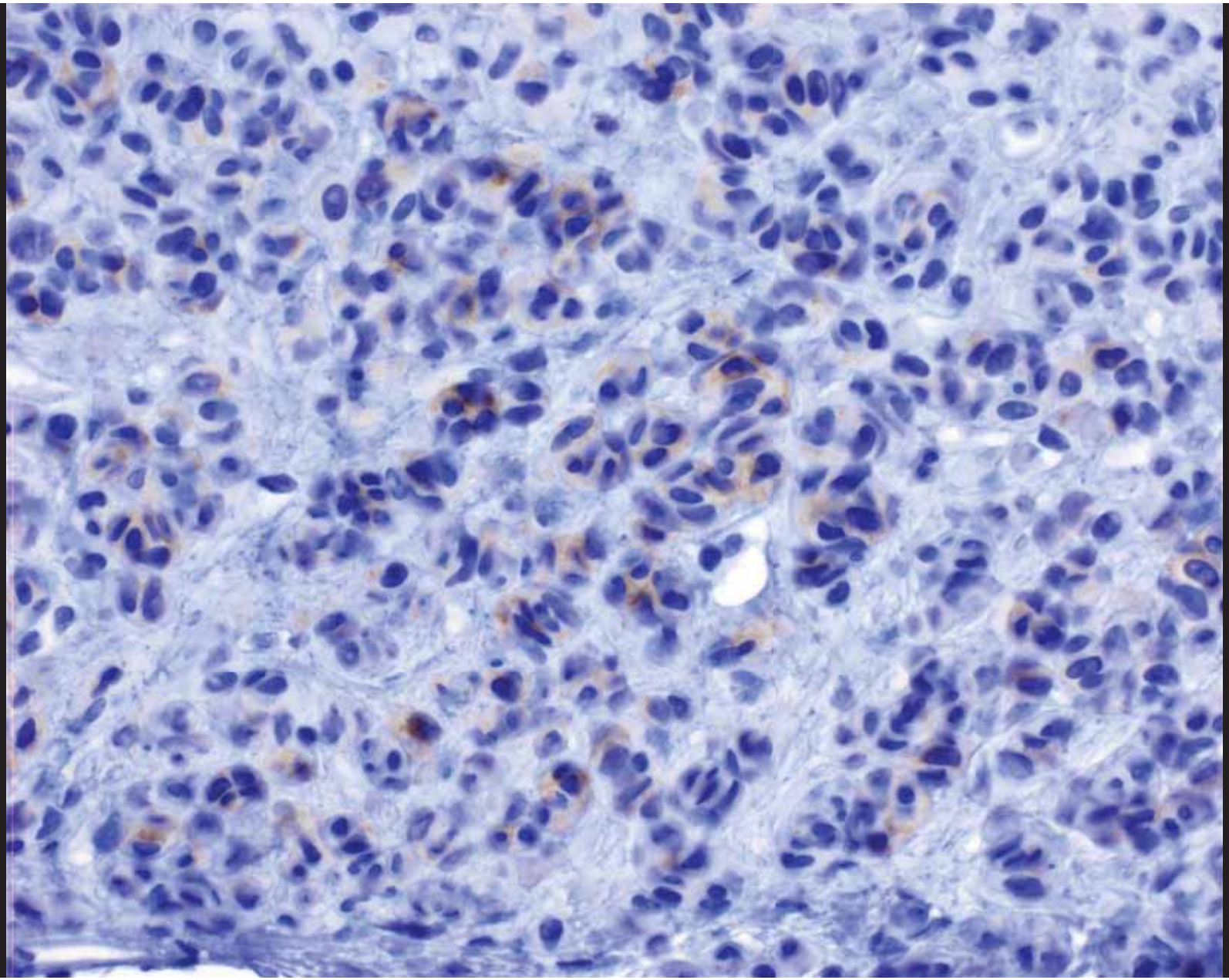
# ALK





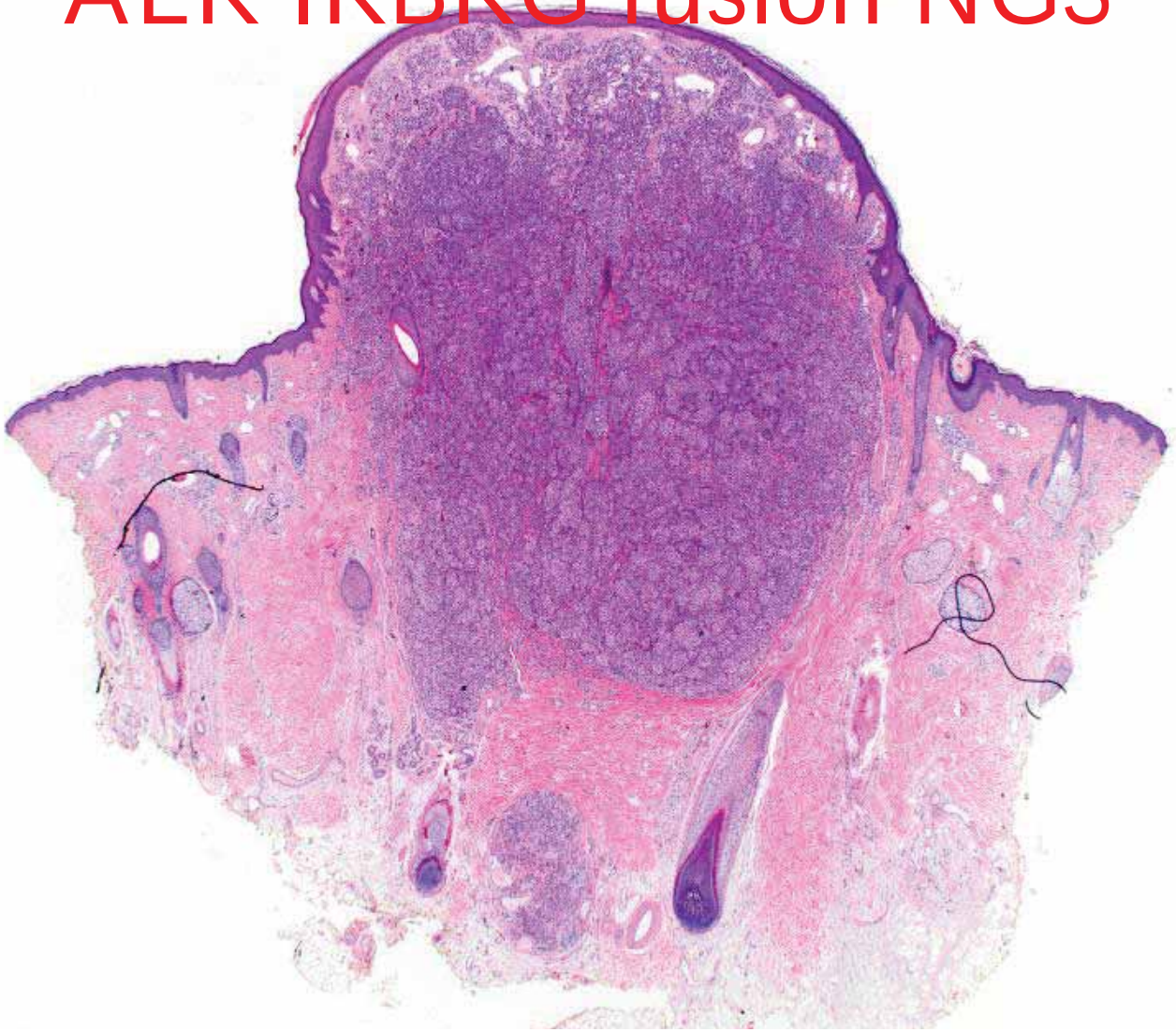
MYO5A-ALK

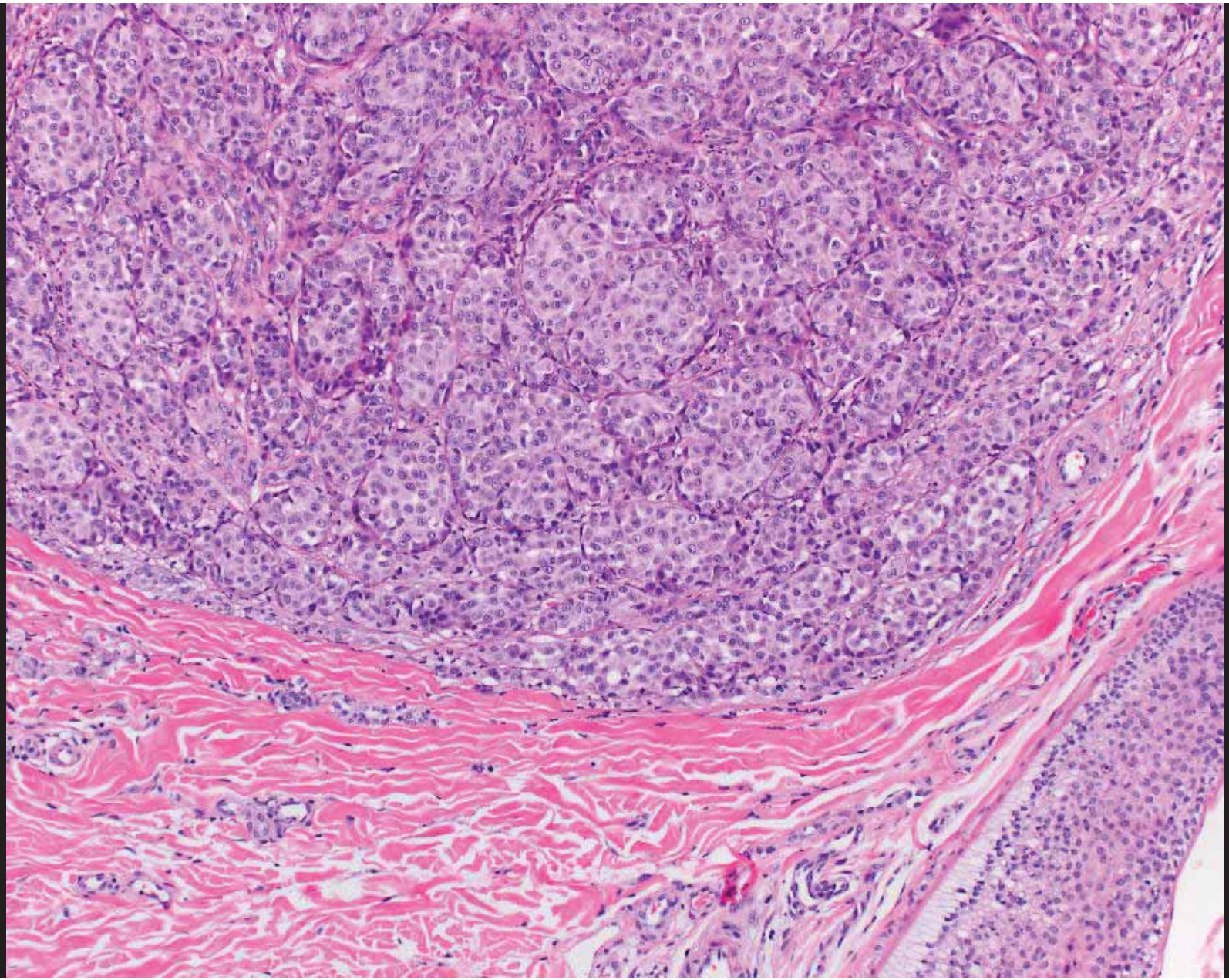


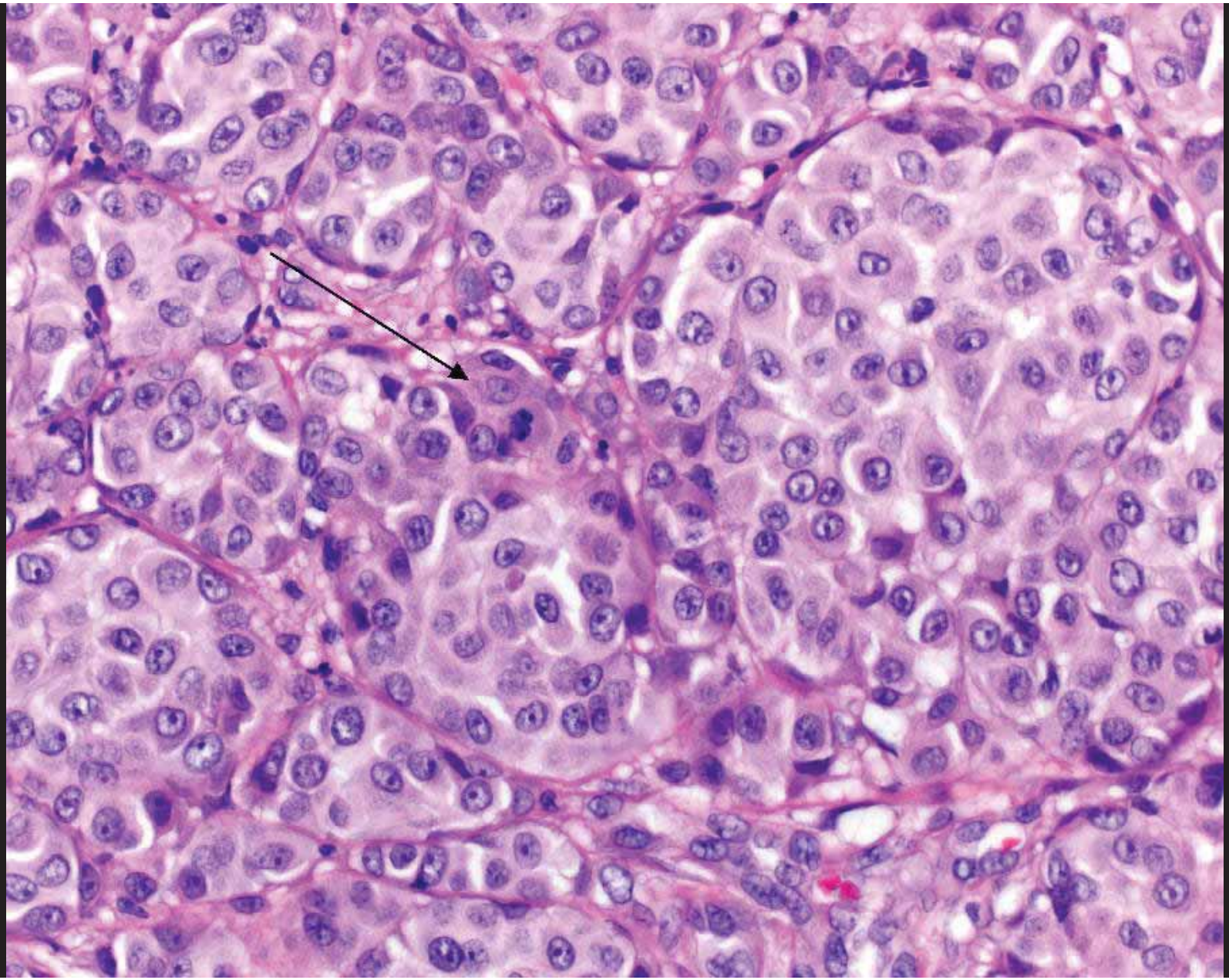


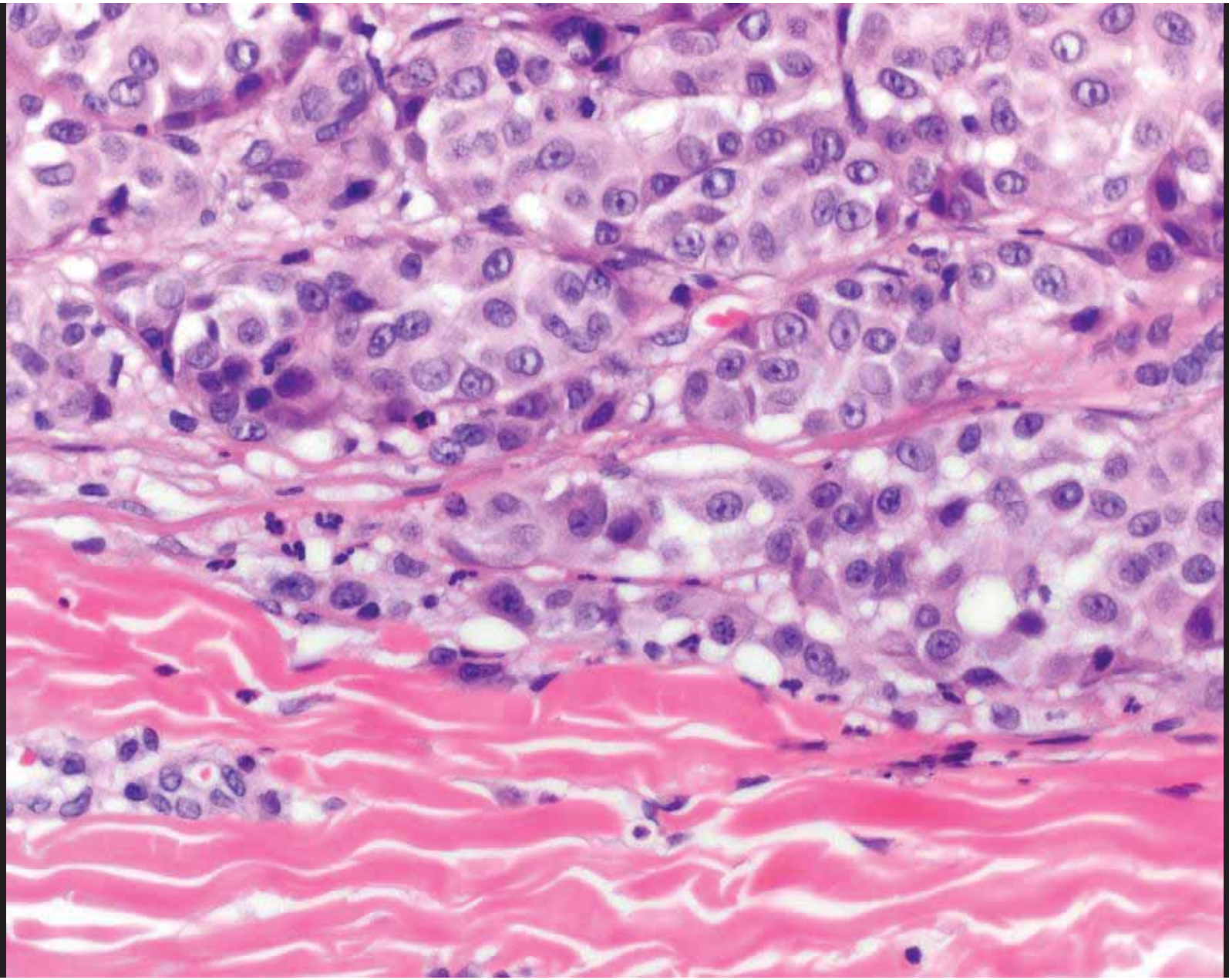
Intermediate grade: atypical
Spitz tumor, ALK fusion

# ALK IKBKG fusion NGS

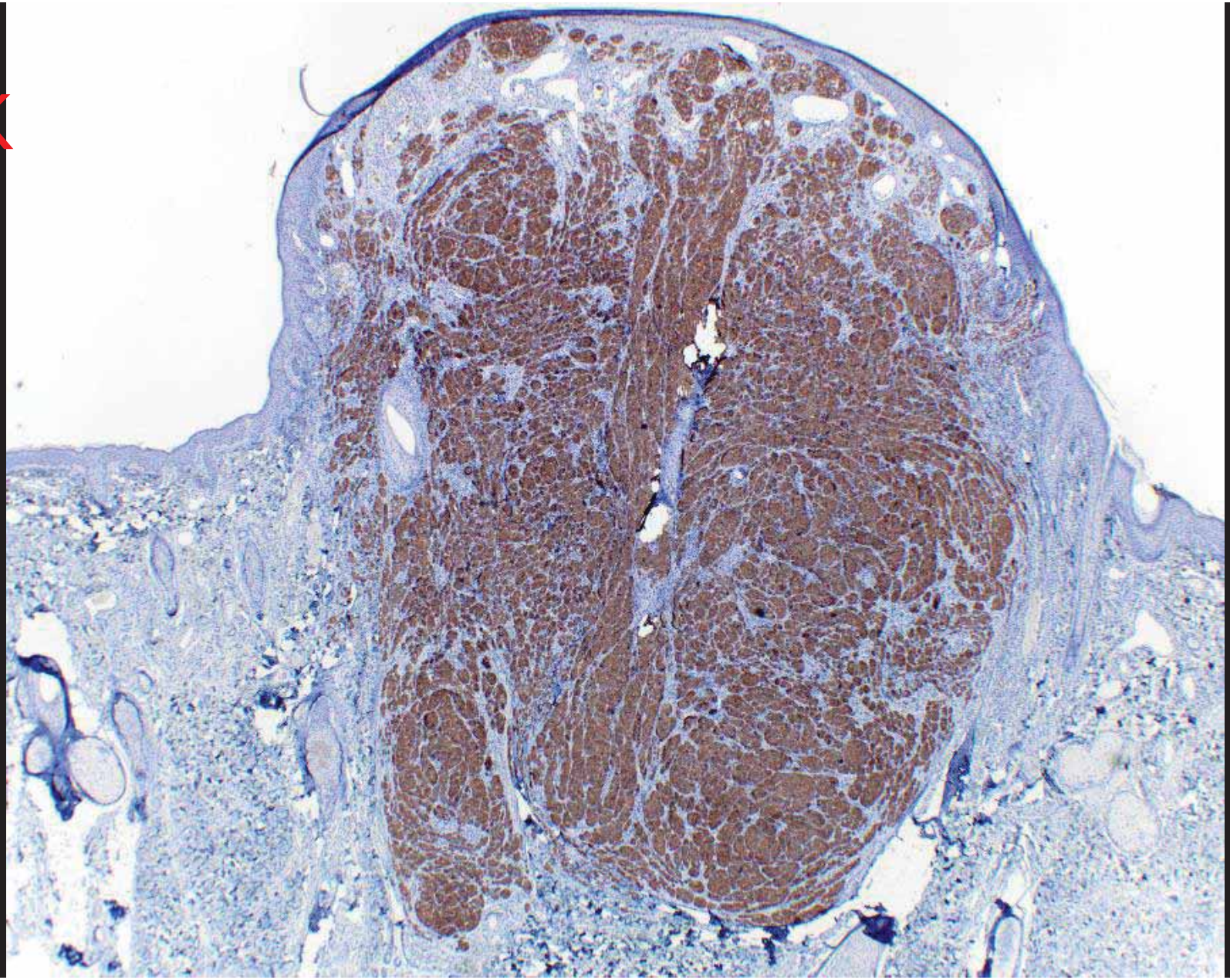






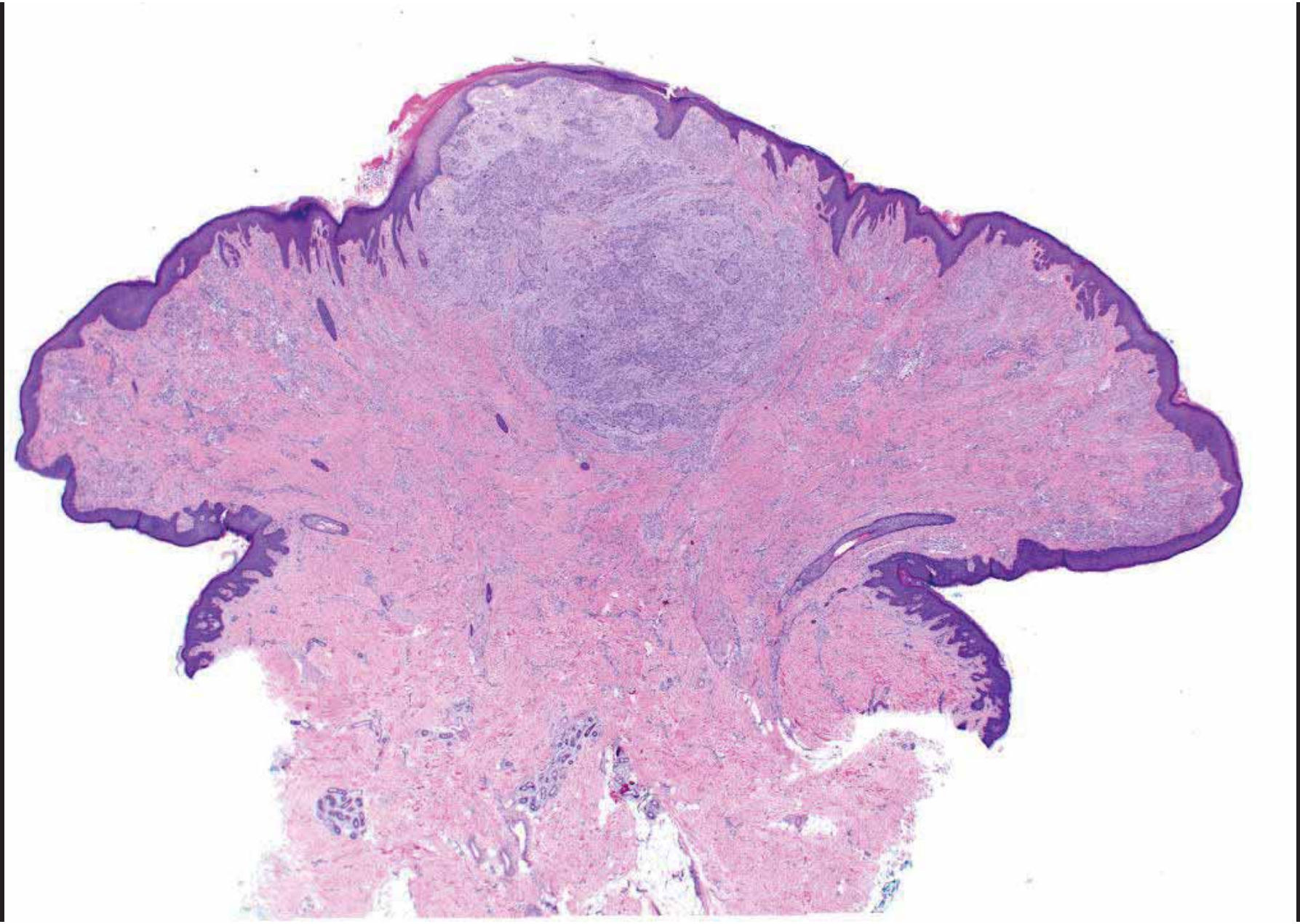


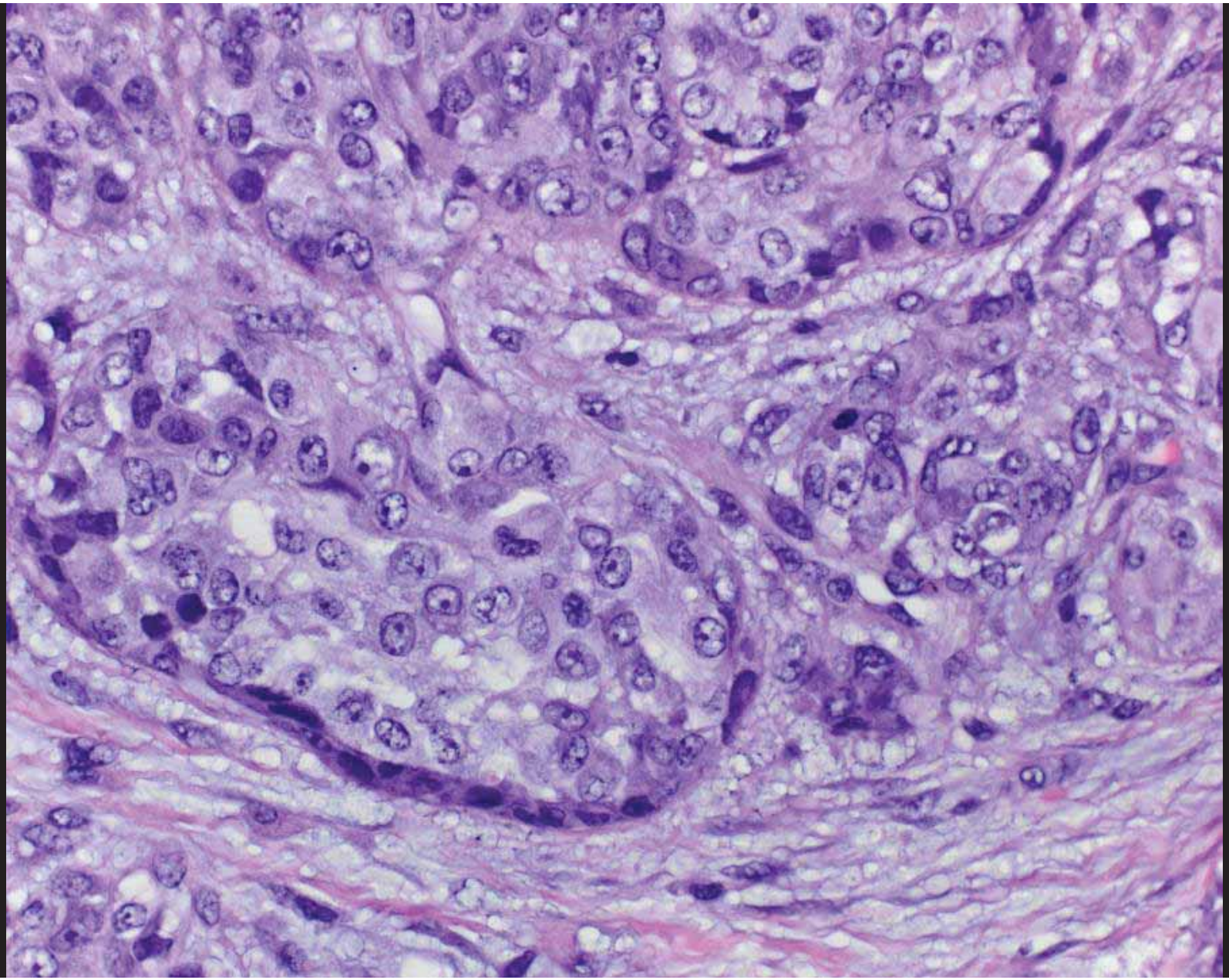
ALK

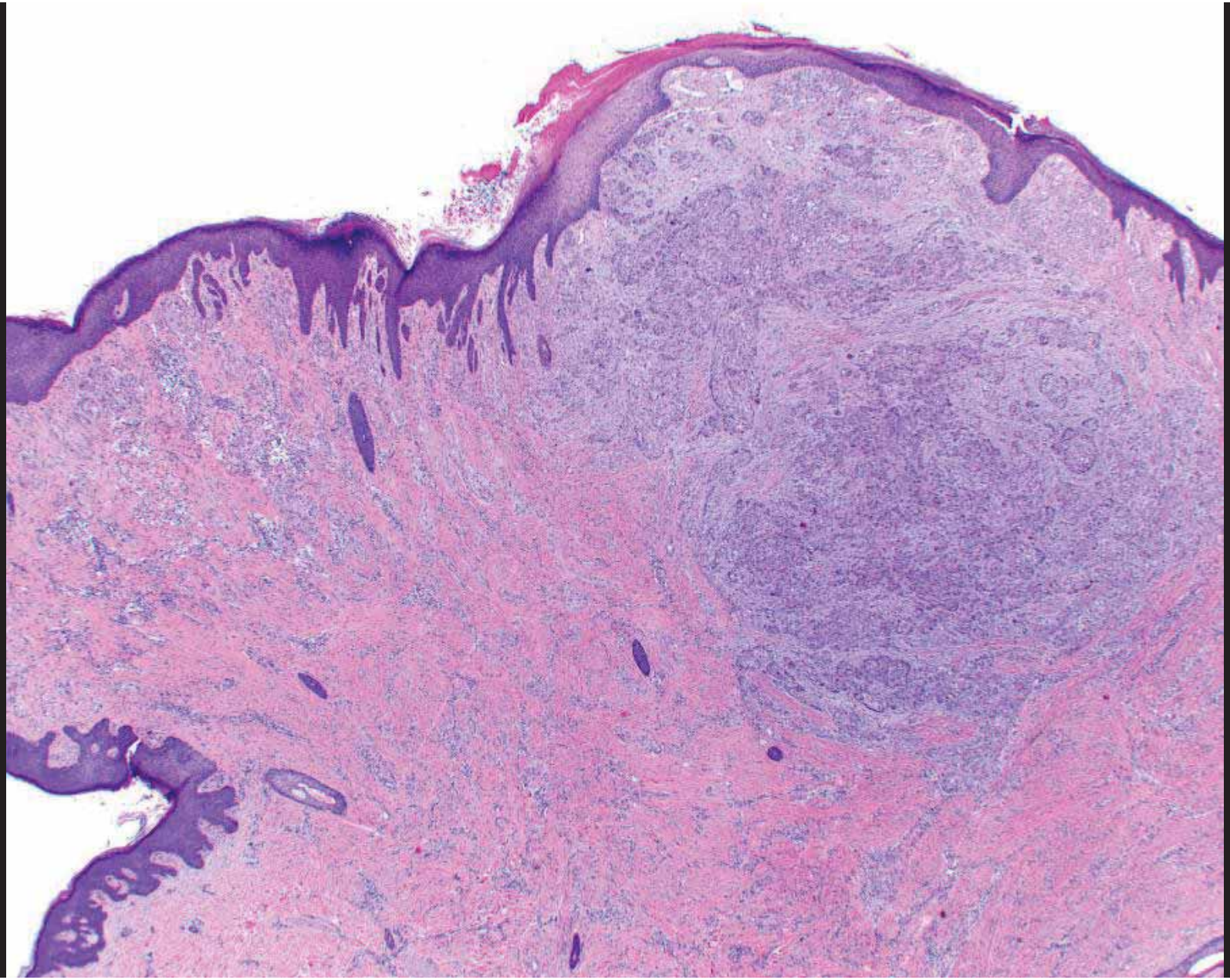


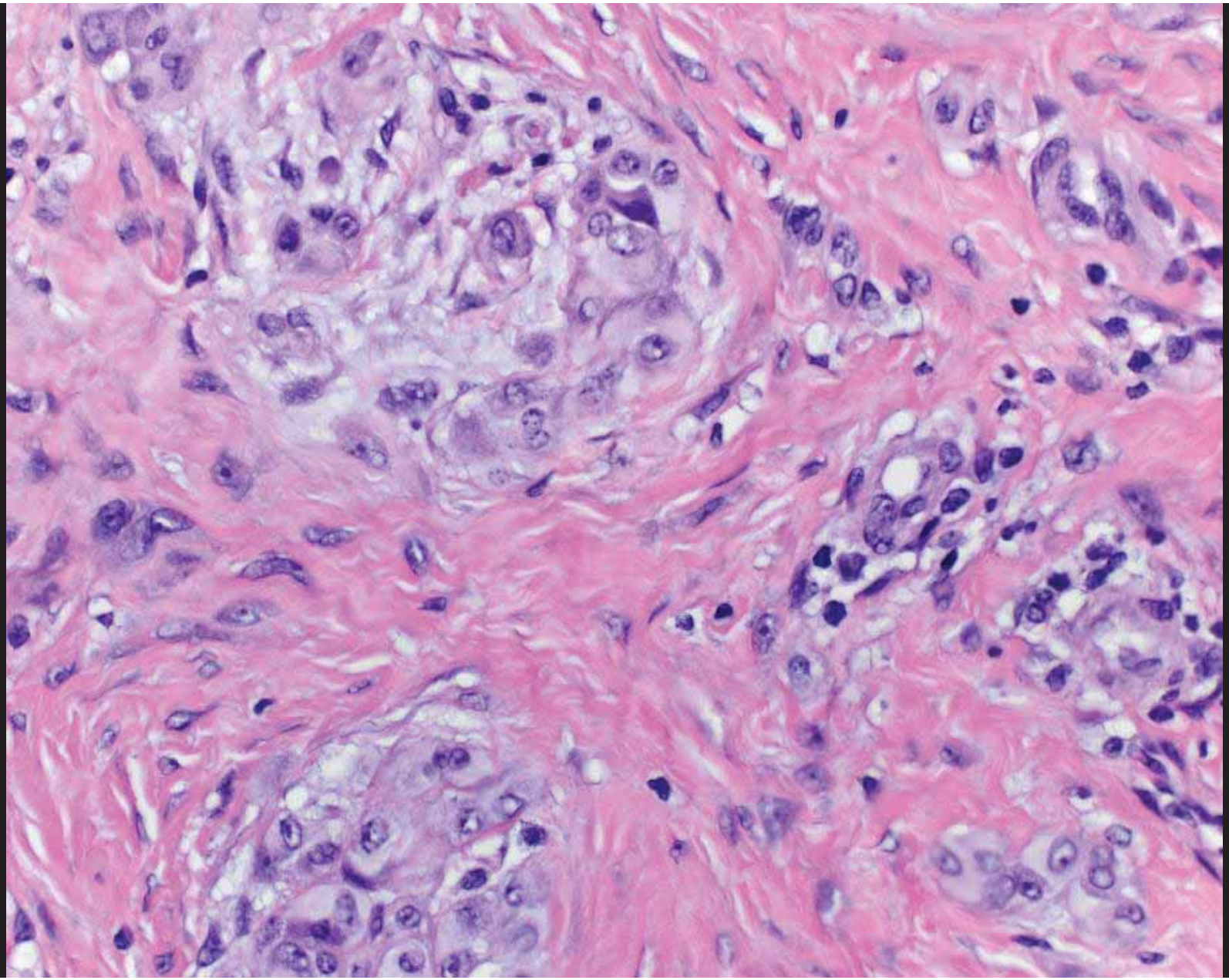
ALK fused

# HIGH GRADE ATYPICAL SPITZ TUMOR

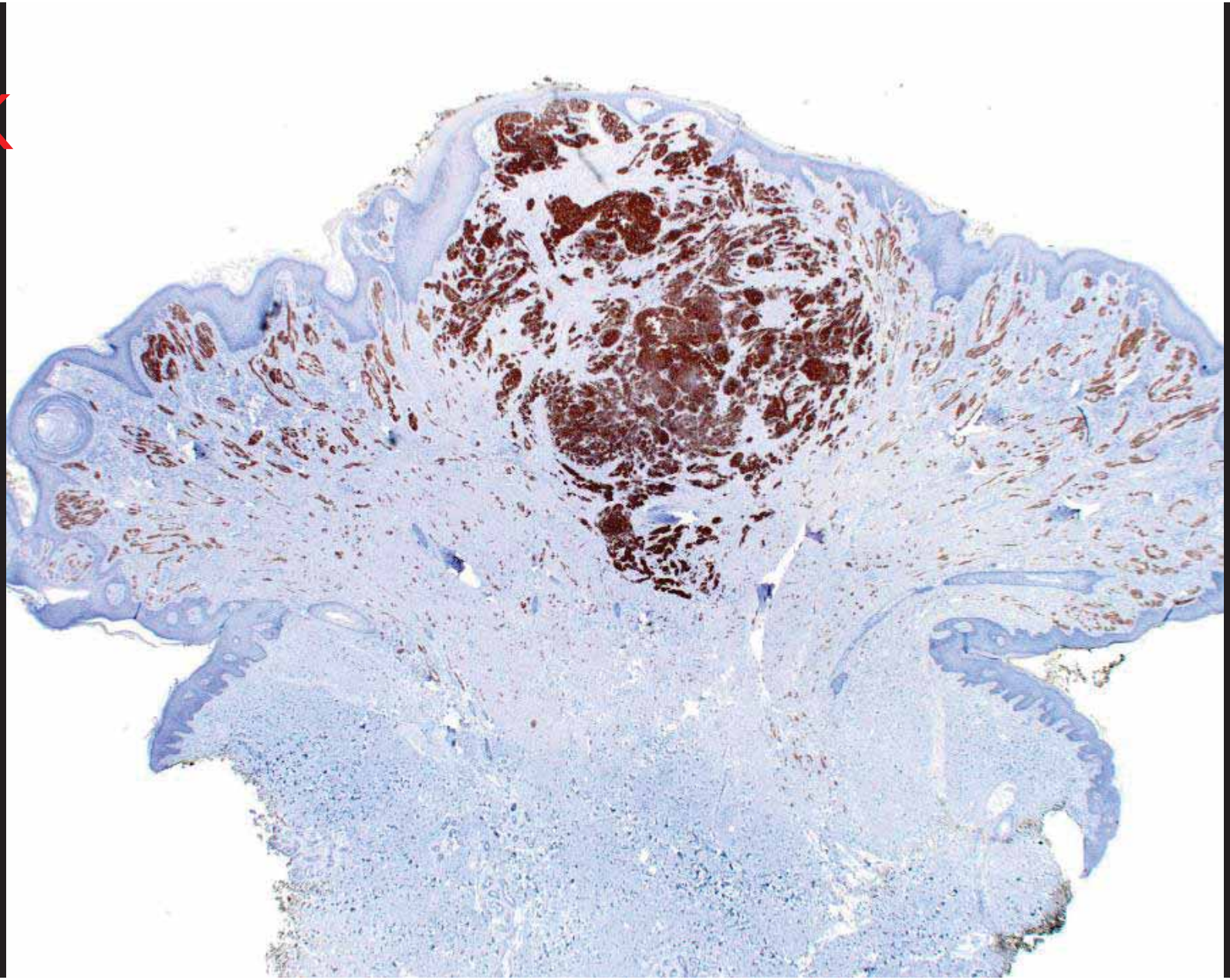




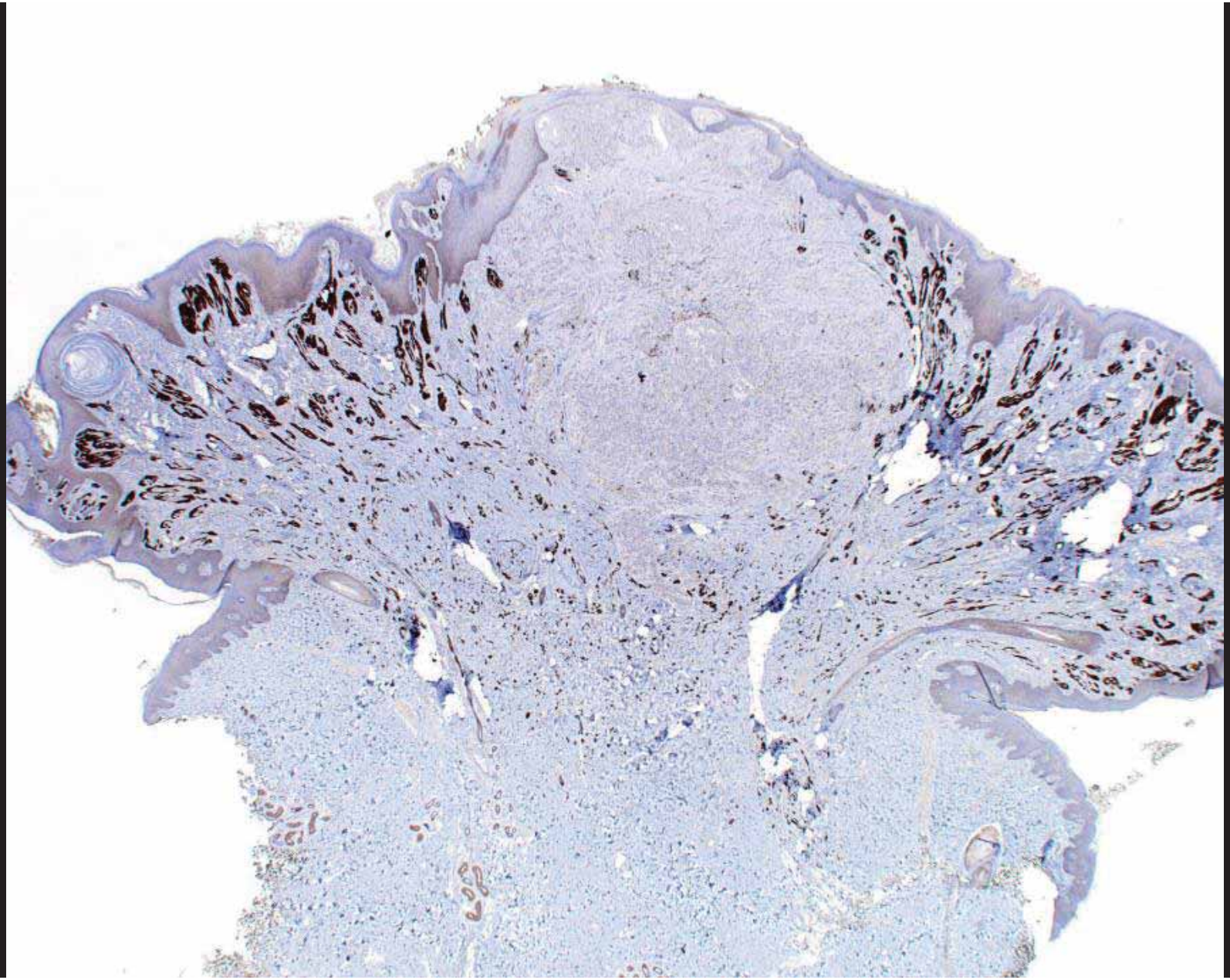




ALK



P16



# Two NGS studies- 1) background

- TPM3-ALK rearrangement
- PTPRD mutation, 43%

Flat profile

# More atypical portion

- TPM3-ALK rearrangement
- PTPRD mutation, 43%
- Deep deletion, cdkn2a
- Losses 1p (partial), 2p, 3p, 9p, and 15qter

# Spitz melanoma

- Spitz lineage initiating event
- Additional progression events (homozygous loss of CDKN2A, mutations in hTERT, CDK4, p53, etc.)
- Spread beyond local lymph nodes, but prognosis seems better than classic melanoma

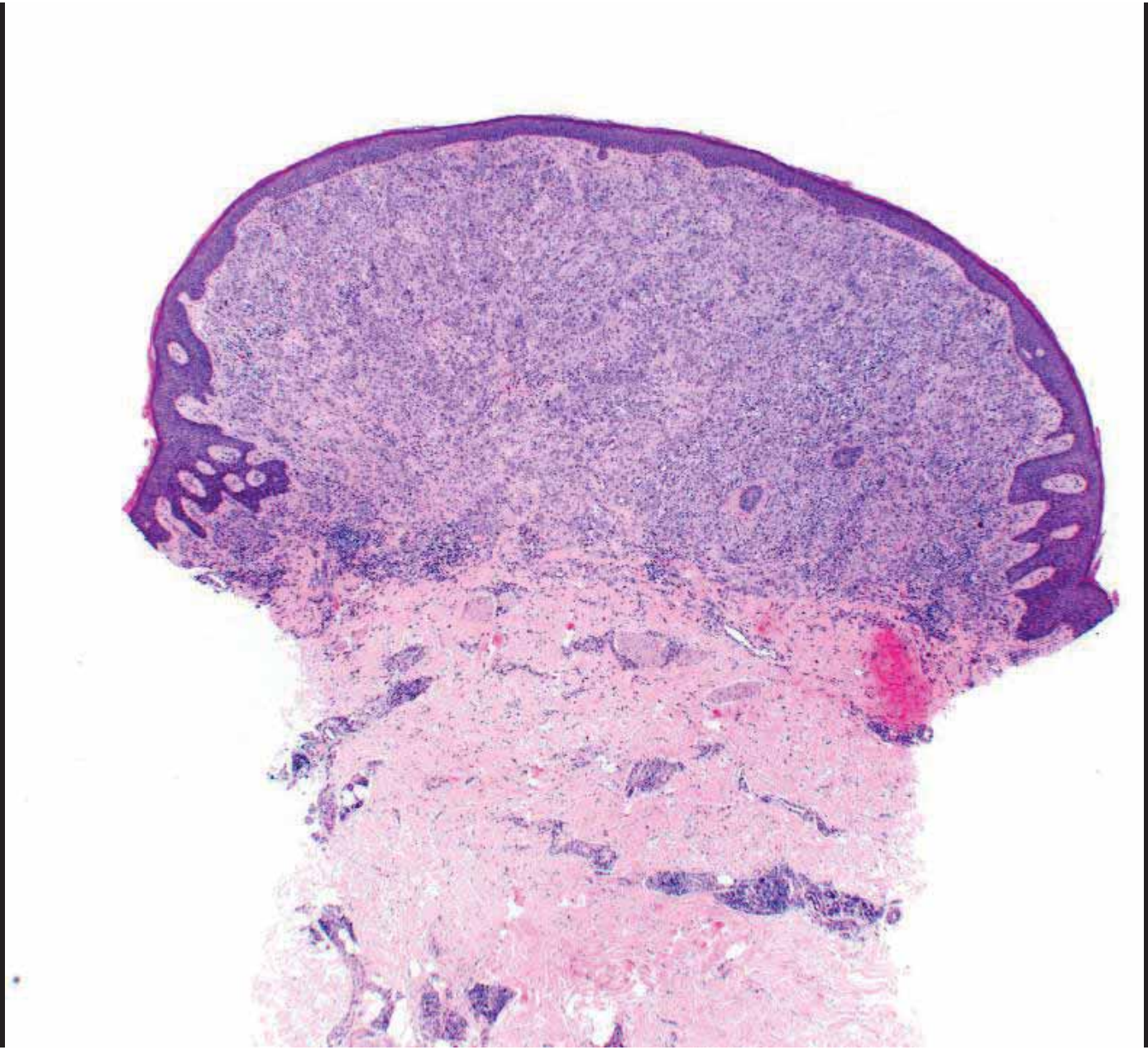
nature medicine

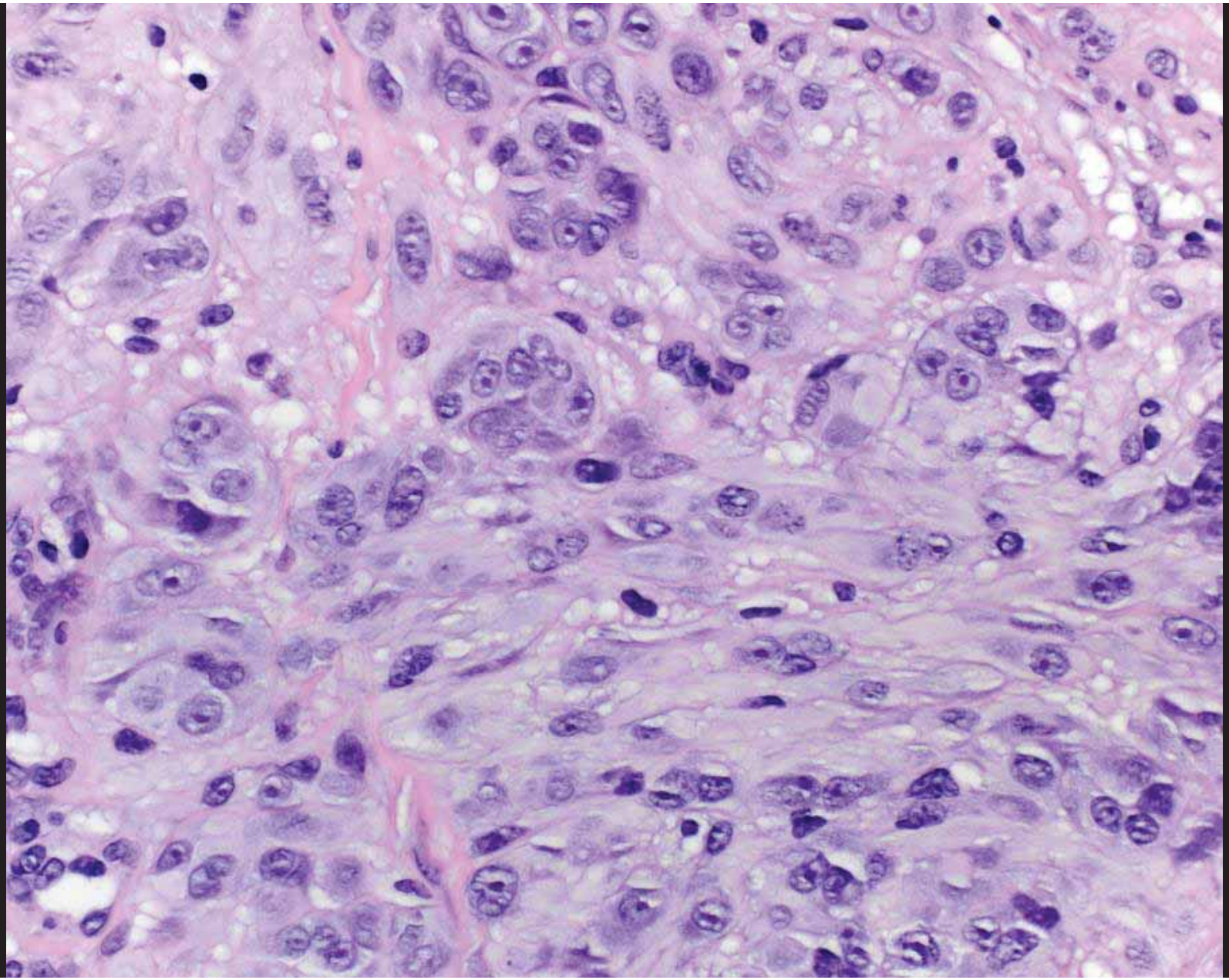
LETTERS

https://doi.org/10.1038/s41591-019-0373-y

# Clinical genome sequencing uncovers potentially targetable truncations and fusions of *MAP3K8* in spitzoid and other melanomas

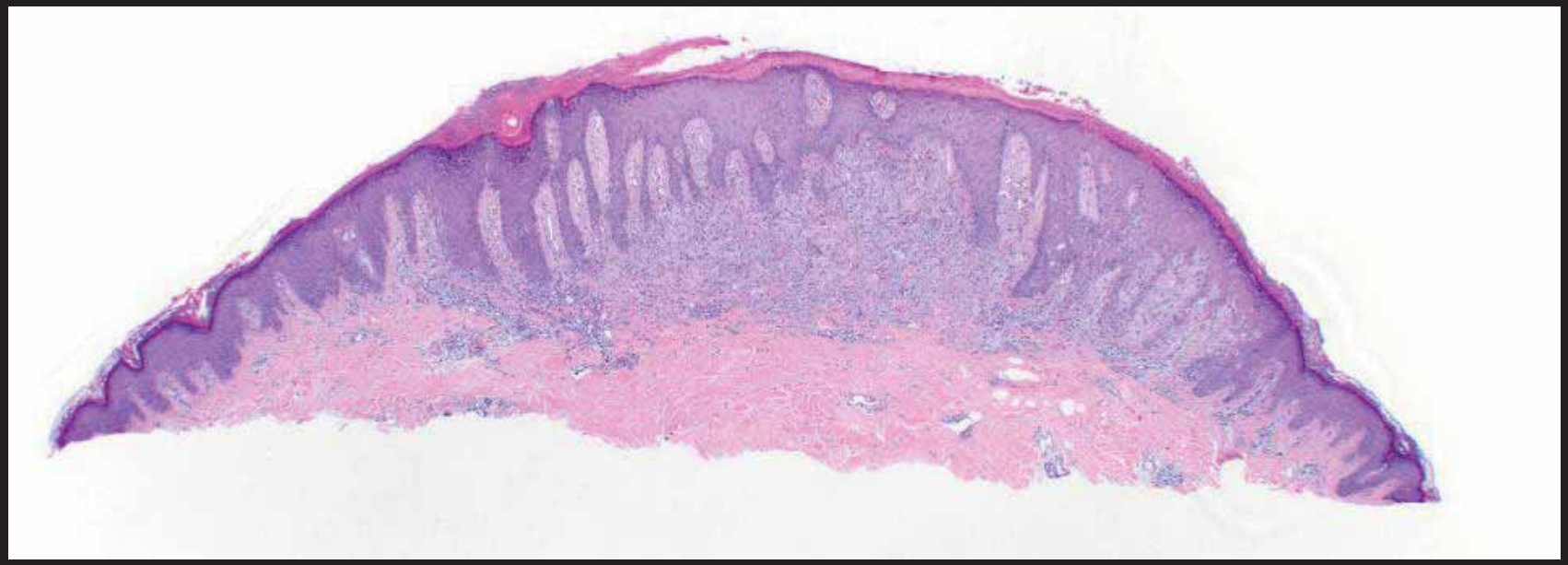
Scott Newman[1*], Liying Fan[2], Allison Pribnow[3,4], Antonina Silkov[1], Stephen V. Rice[1], Seungjae Lee[5], Ying Shao[1], Bridget Shaner[1], Heather Mulder[1], Joy Nakitandwe[5], Sheila Shurtleff[5], Elizabeth M. Azzato[5], Gang Wu[1], Xin Zhou[1], Raymond Barnhill[6], John Easton[1], Kim E. Nichols[3], David W. Ellison[5], James R. Downing[5], Alberto Pappo[3], Philip M. Potter[2], Jinghui Zhang[1*] and Armita Bahrami[3,5*]

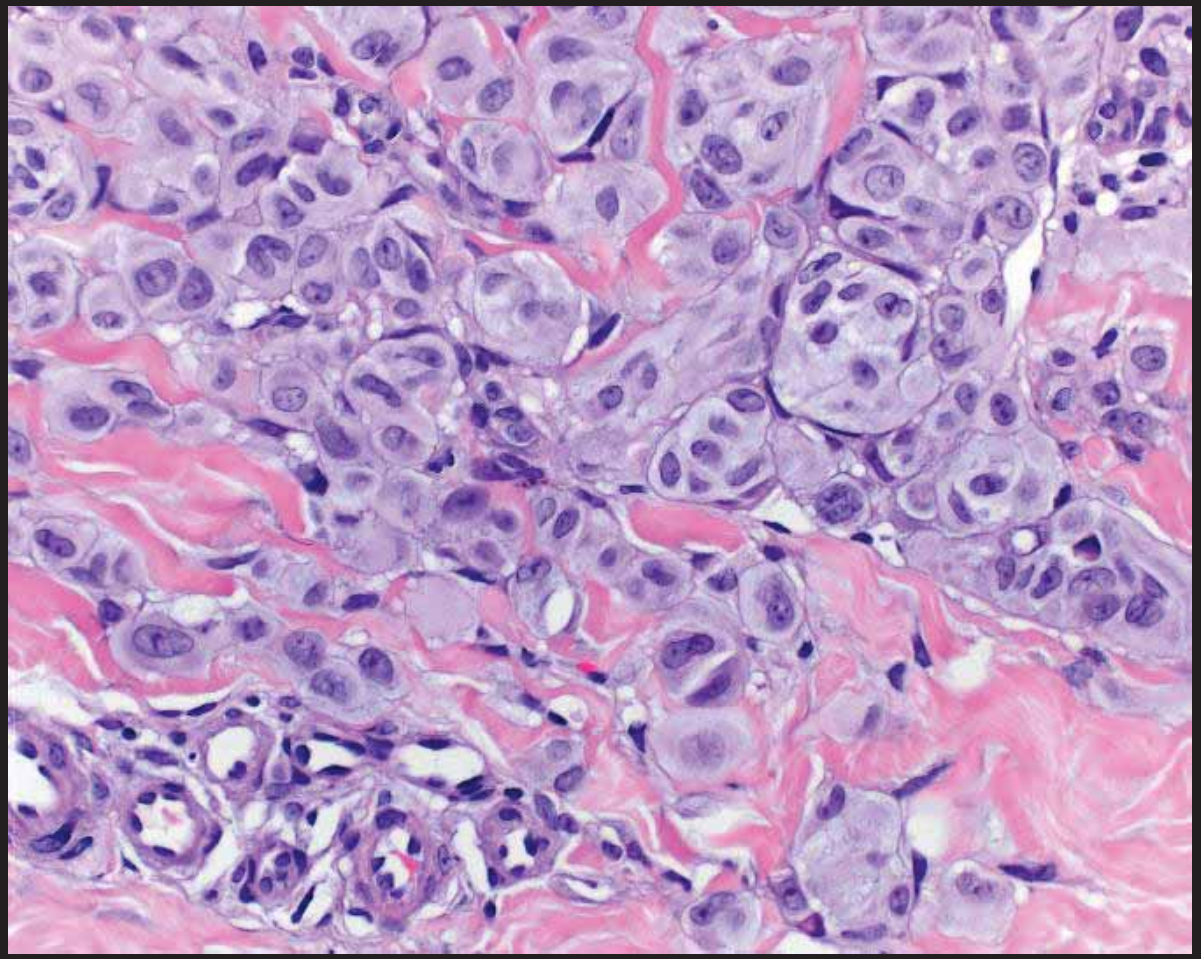




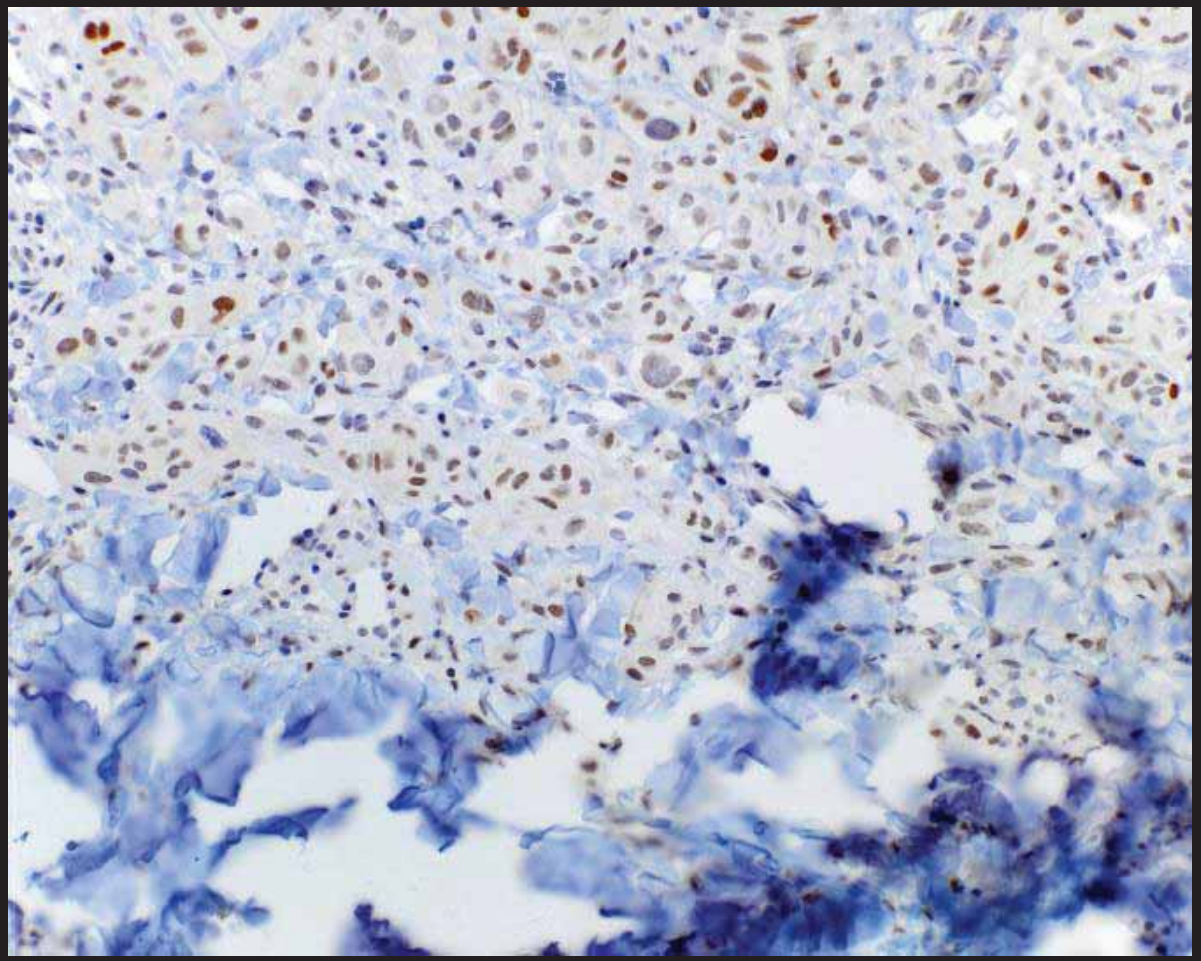
# 18 year old male, left knee

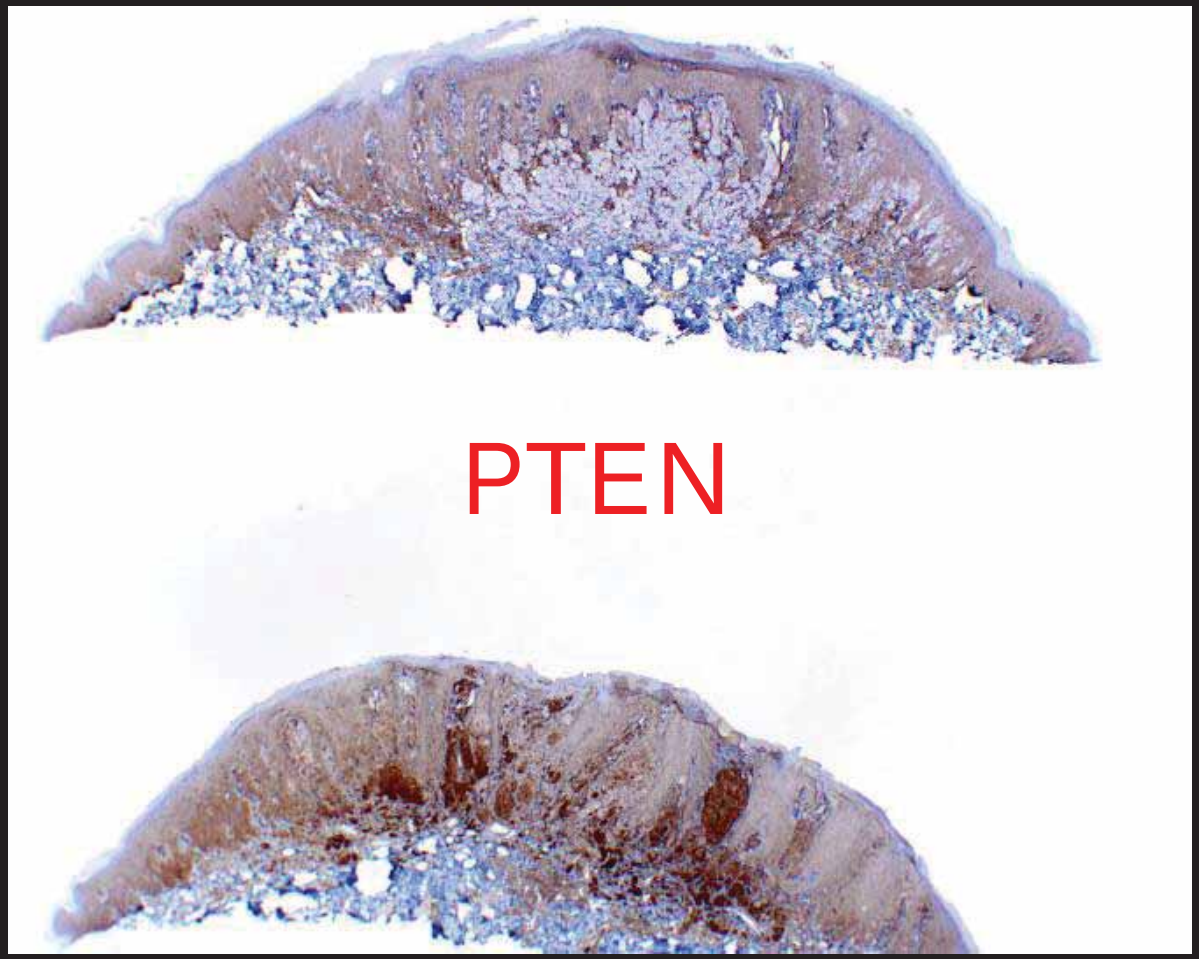
- MAP3K8 fusion
- missense V126D mutation of CDKN2A
- loss of the wild-type allele on chr. 9p, loss involving chr. 10
- inactivating mutation in PTEN

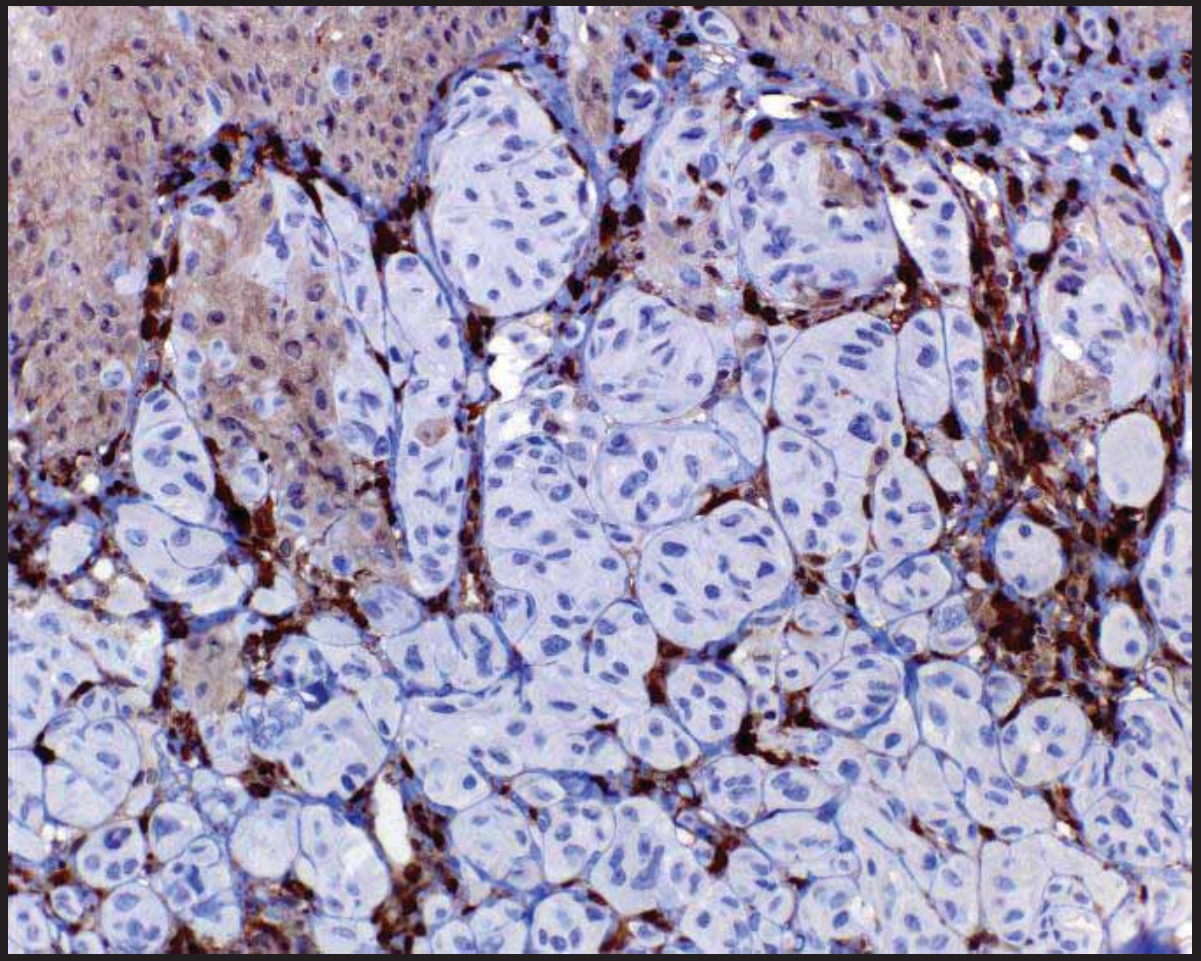


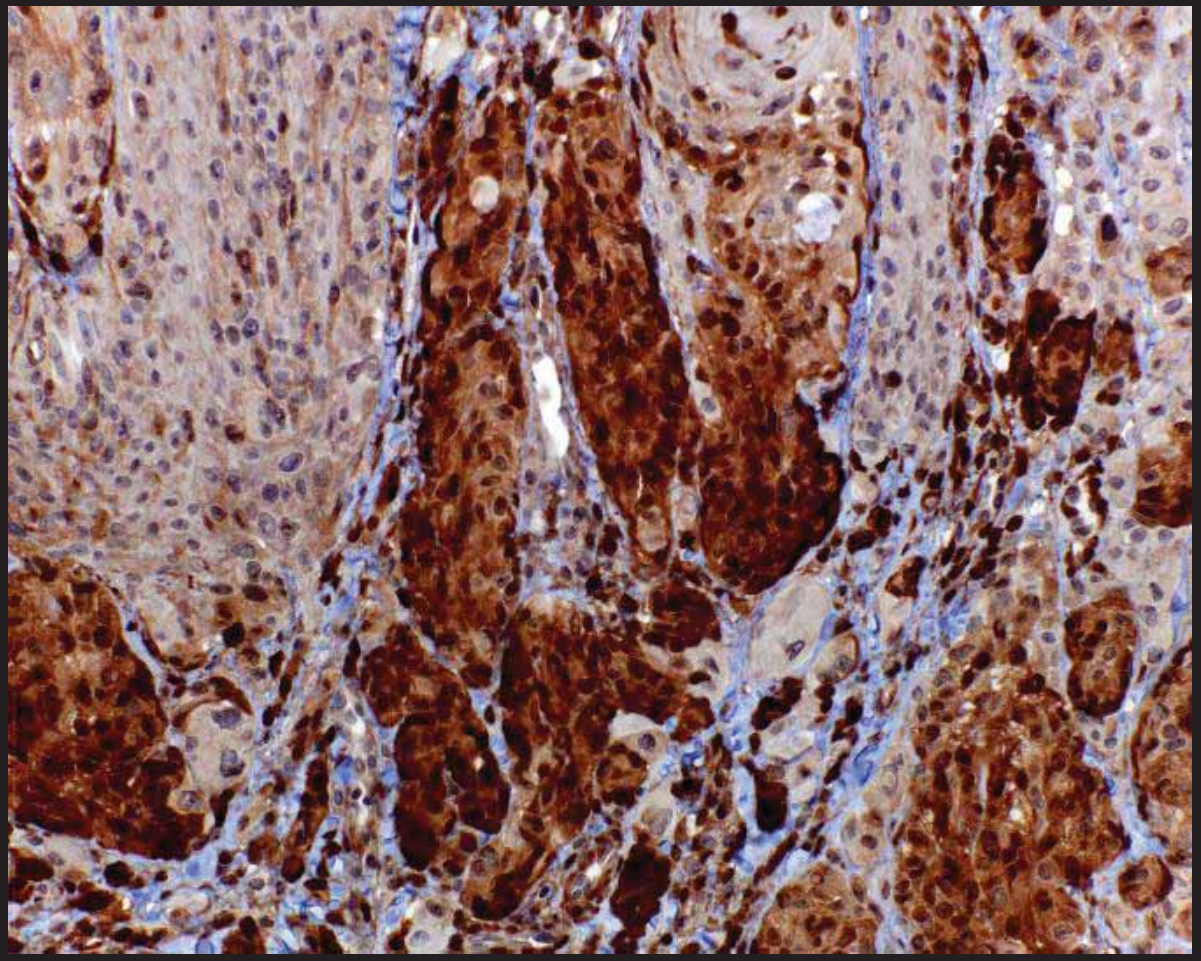


# PRAME

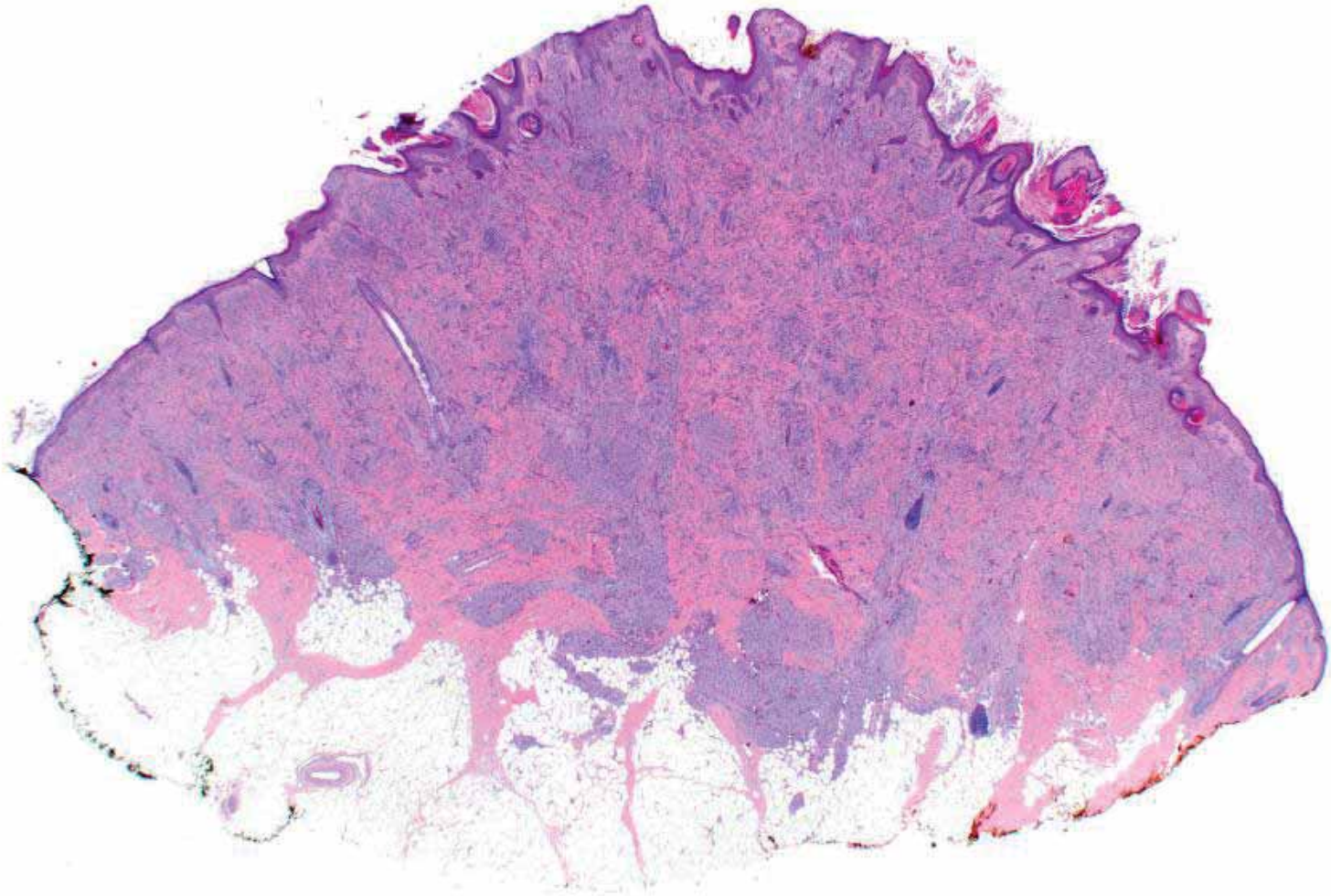


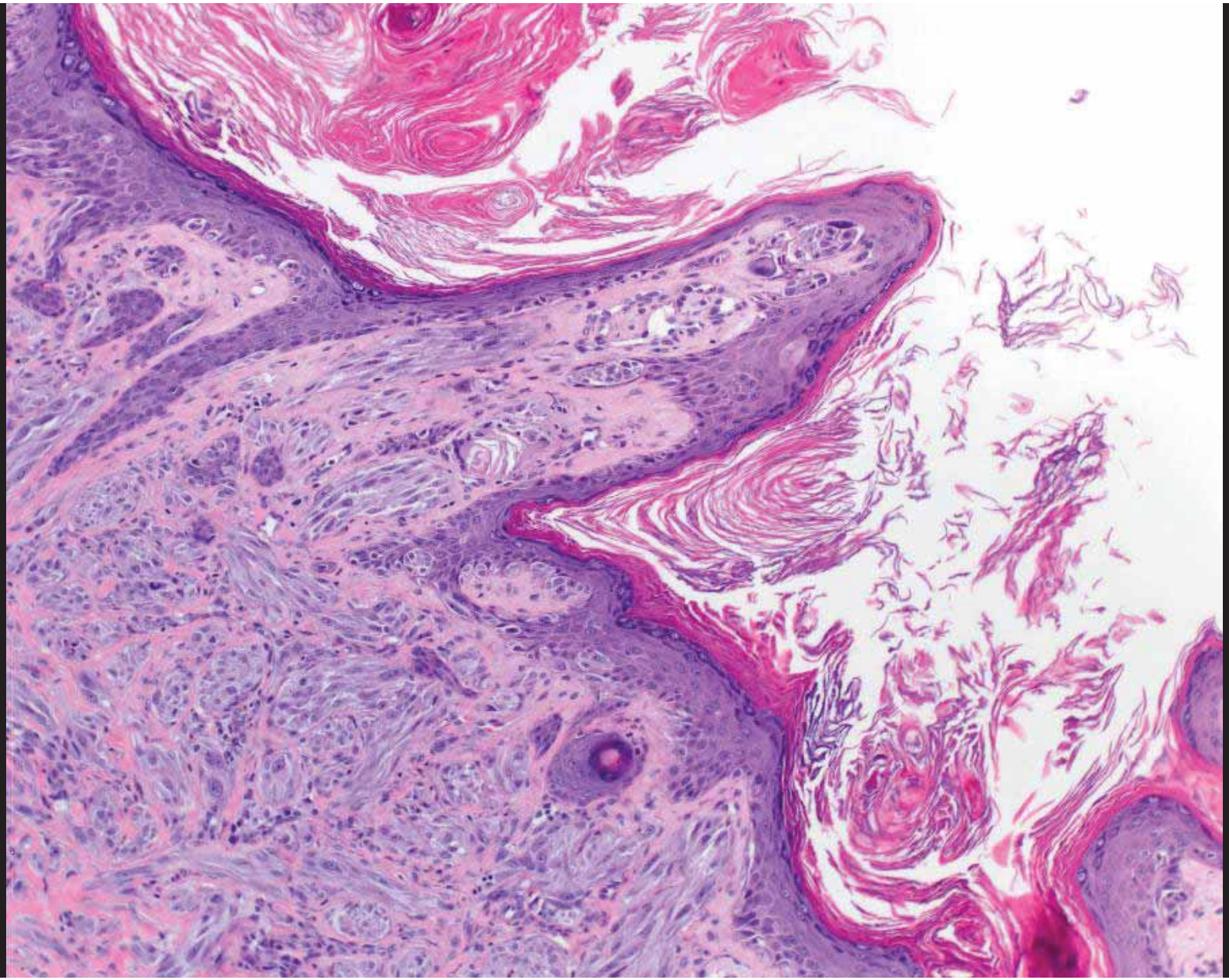

PTEN

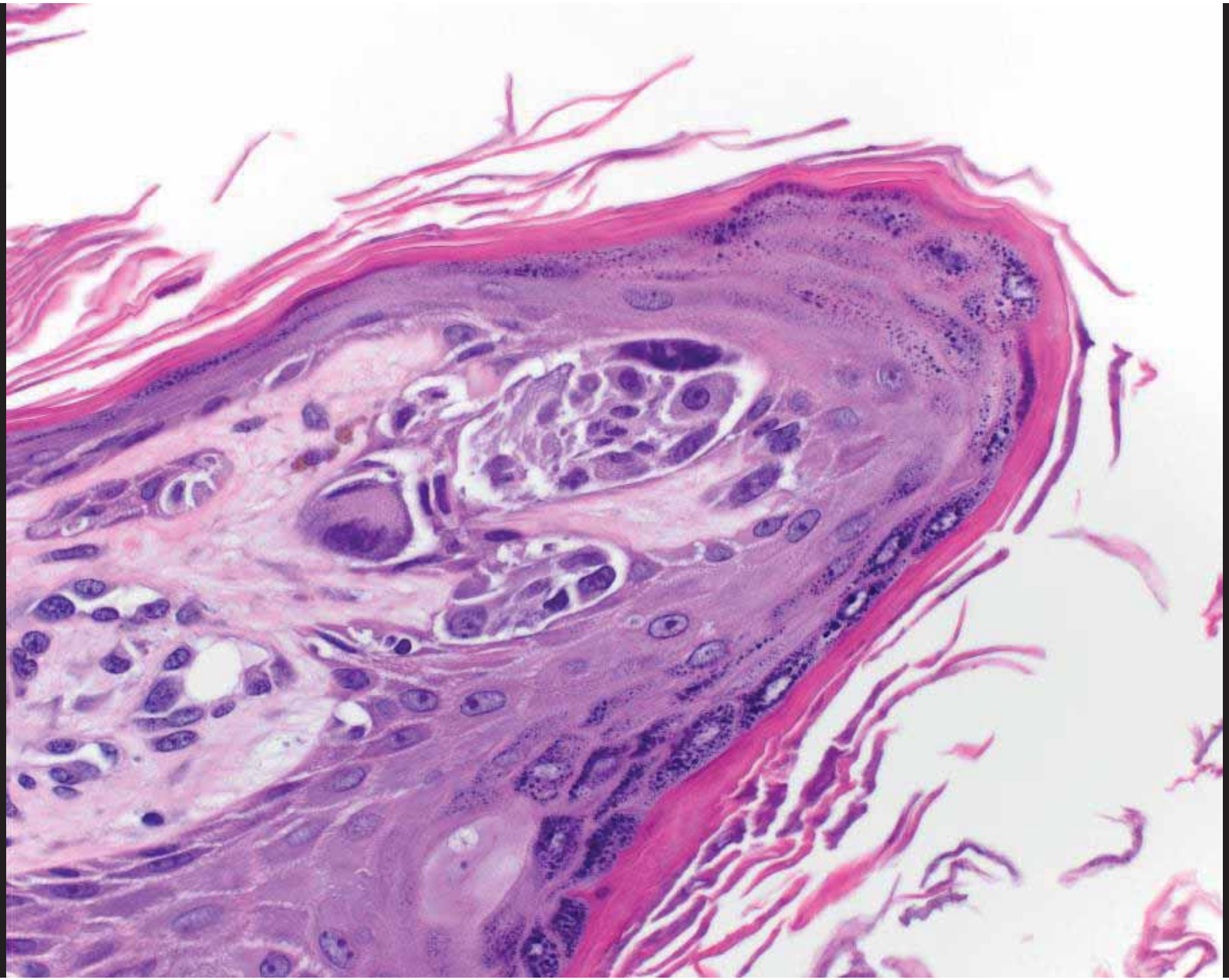


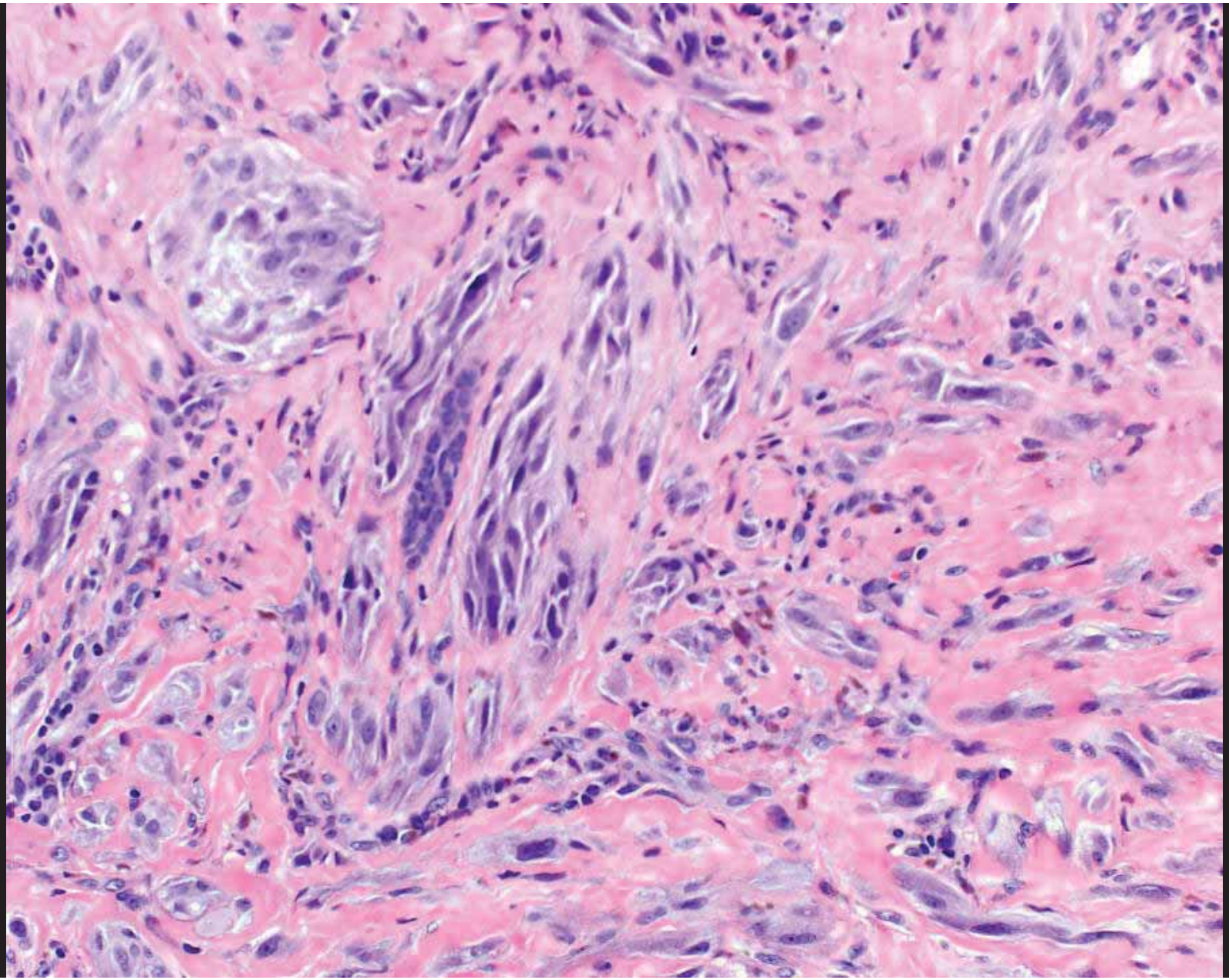


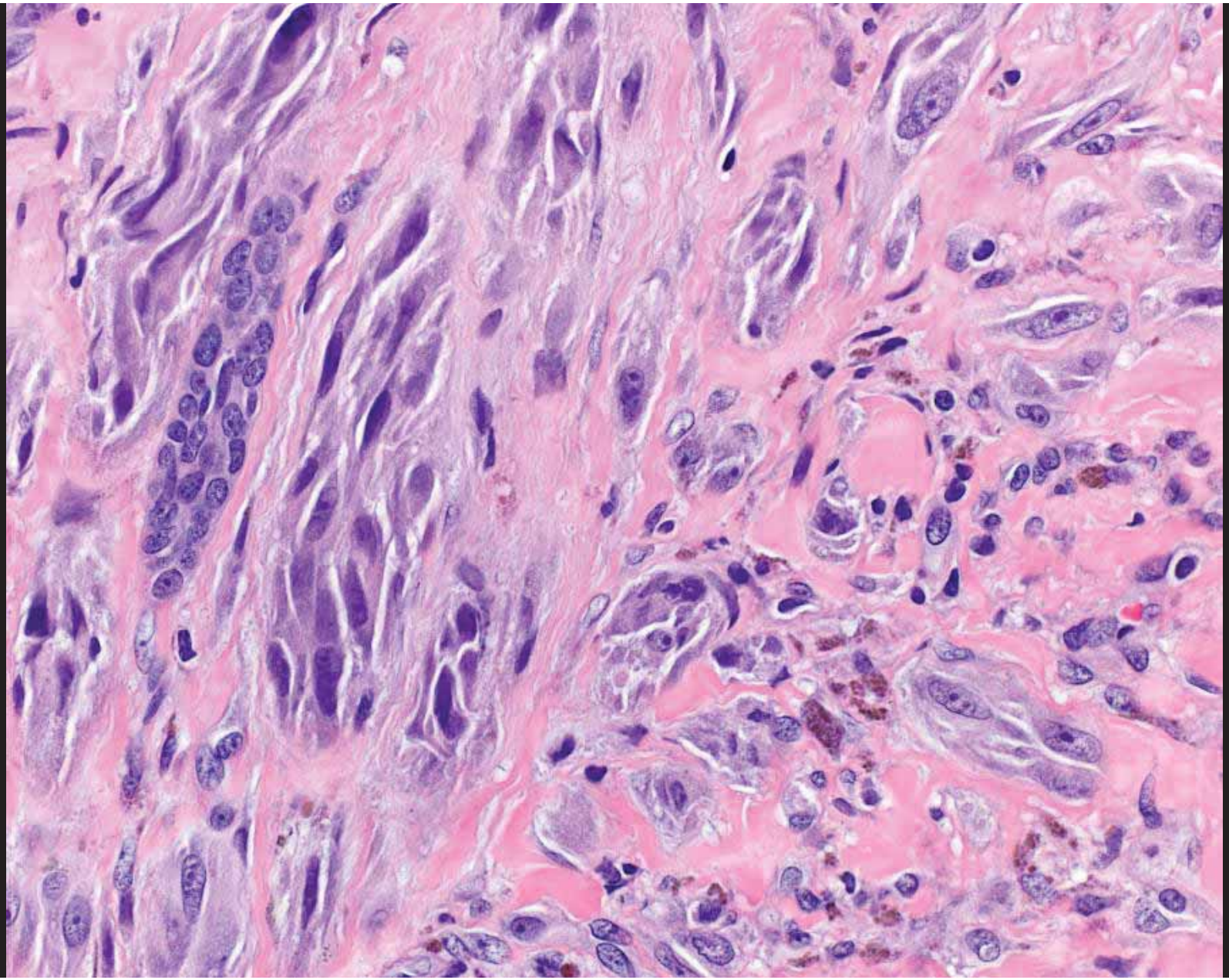
# Morphology/genomics final exam

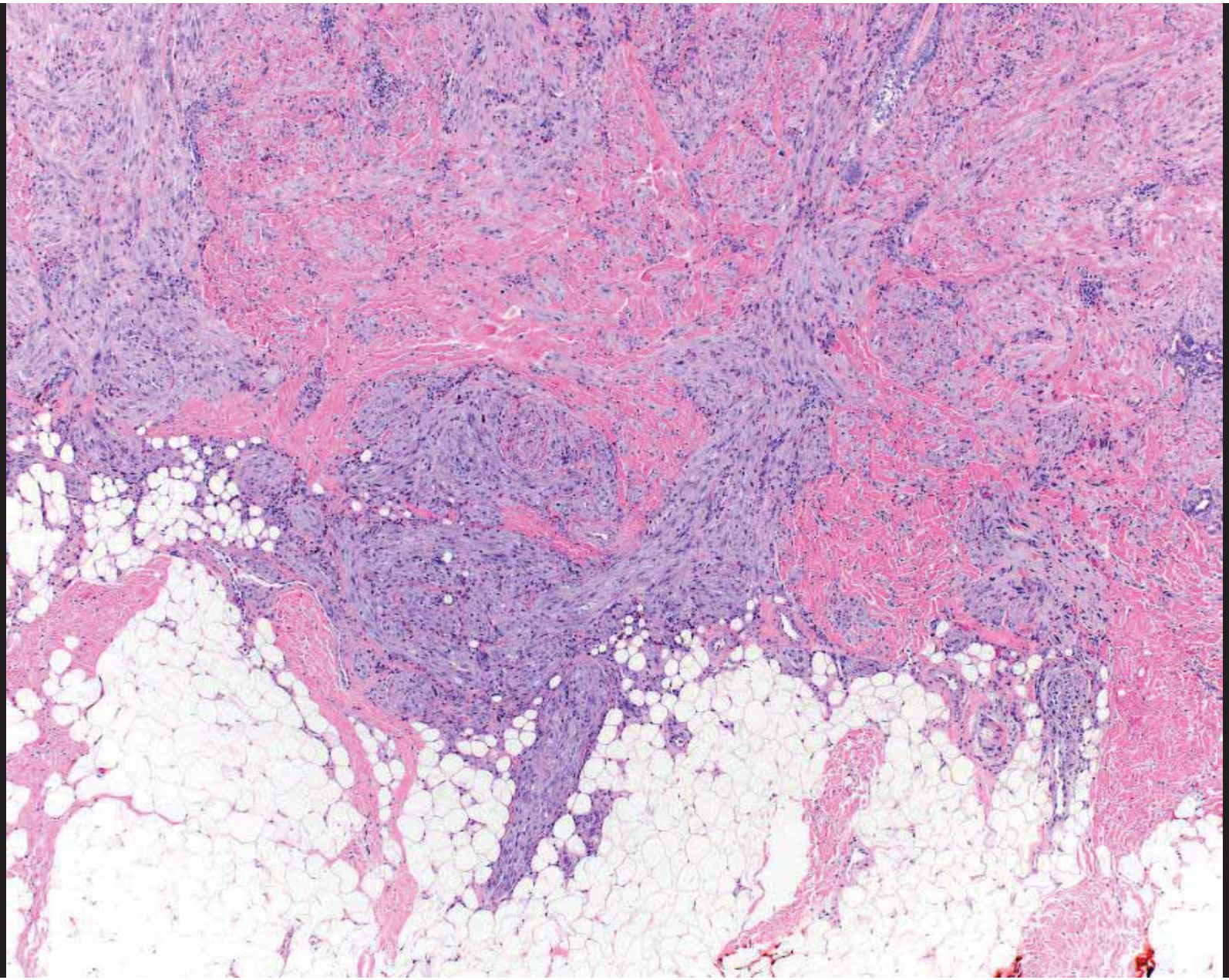


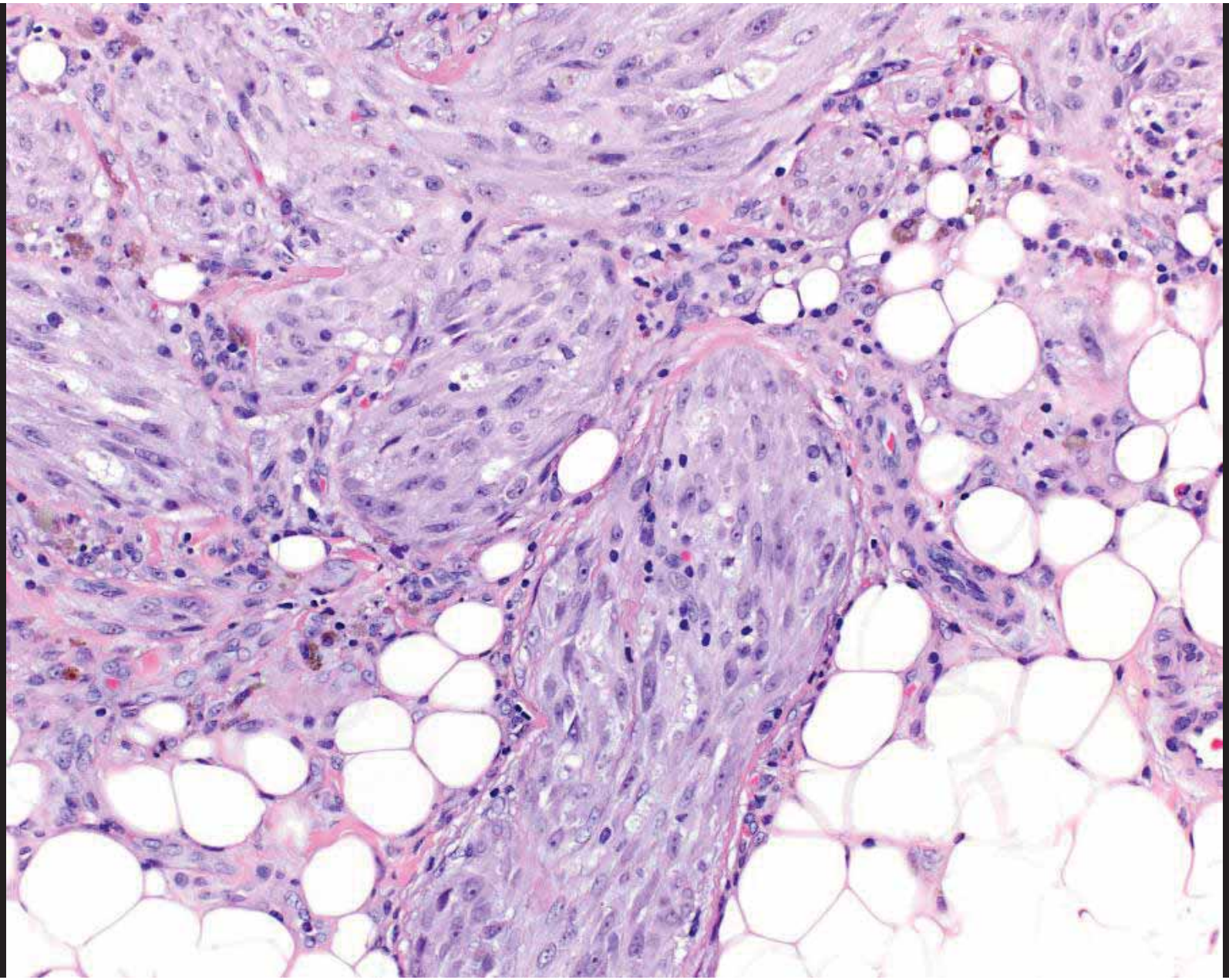


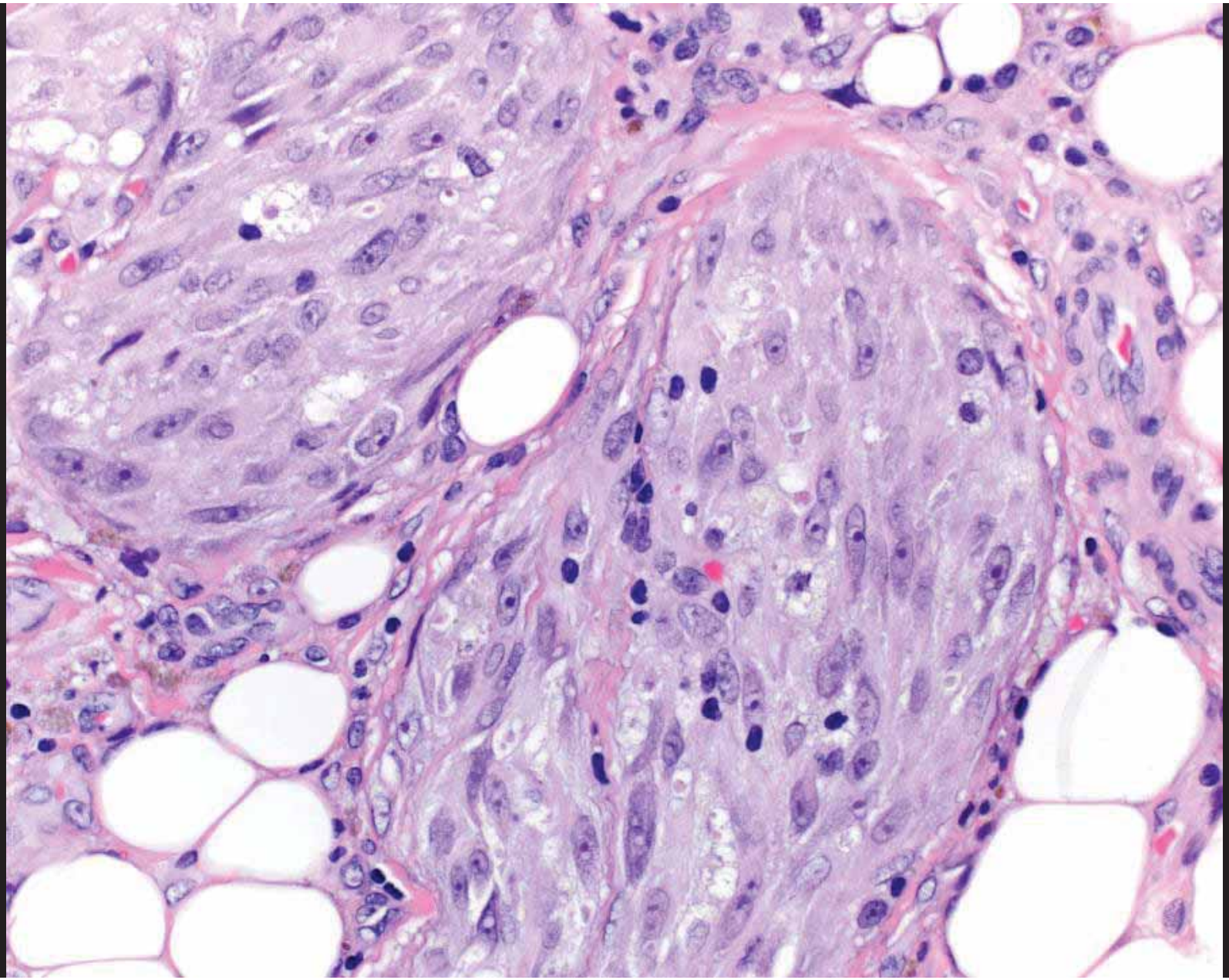




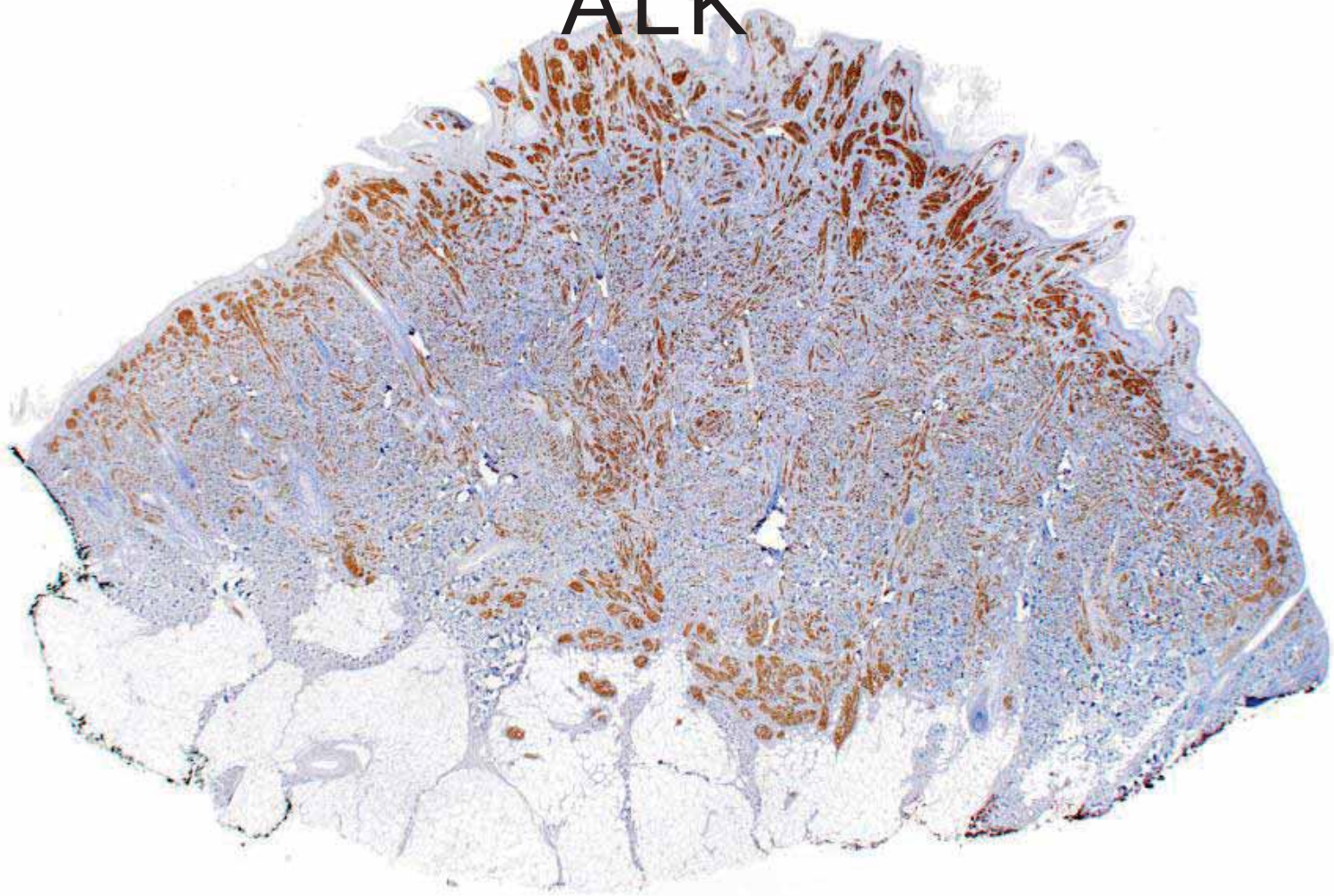


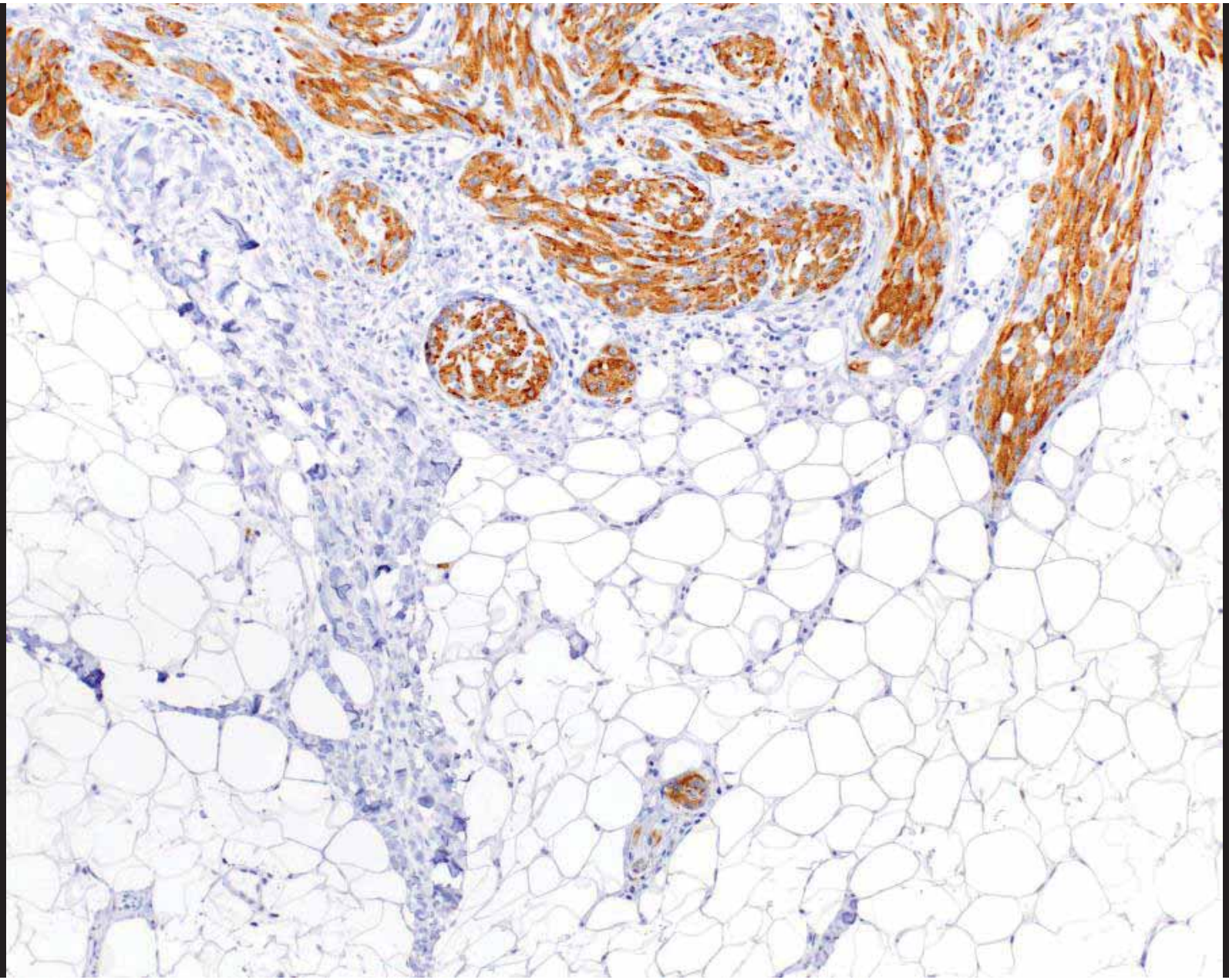


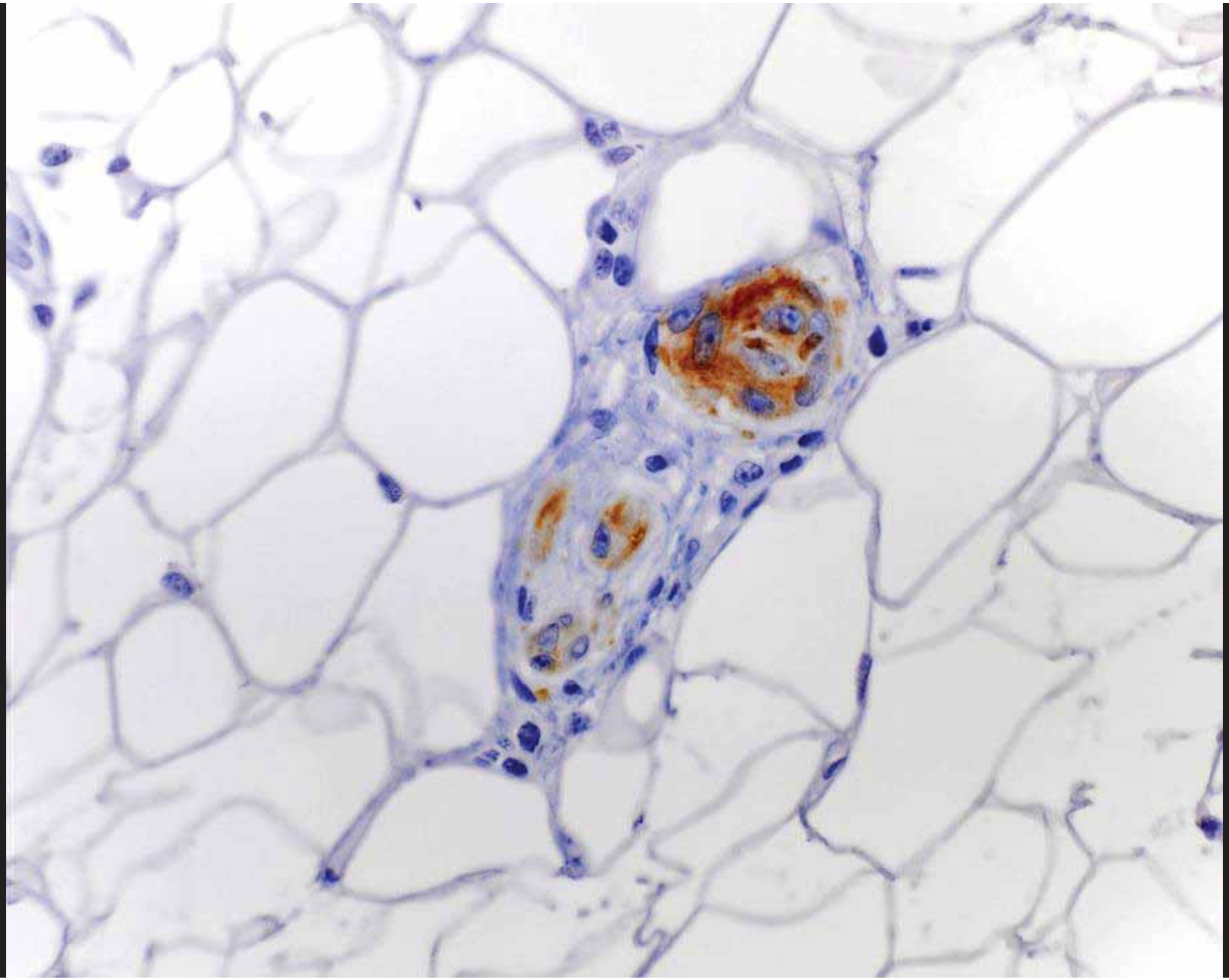




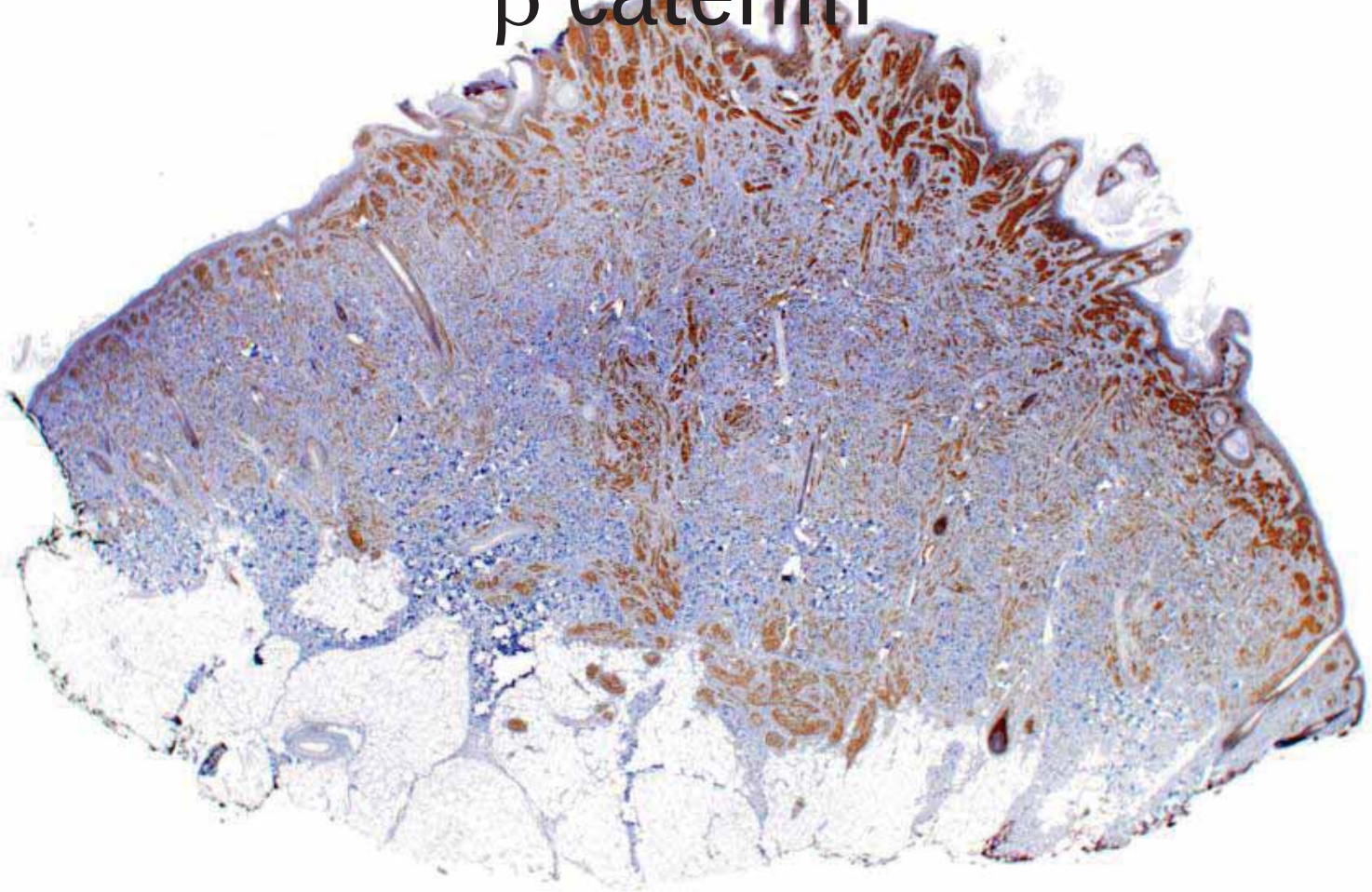
ALK

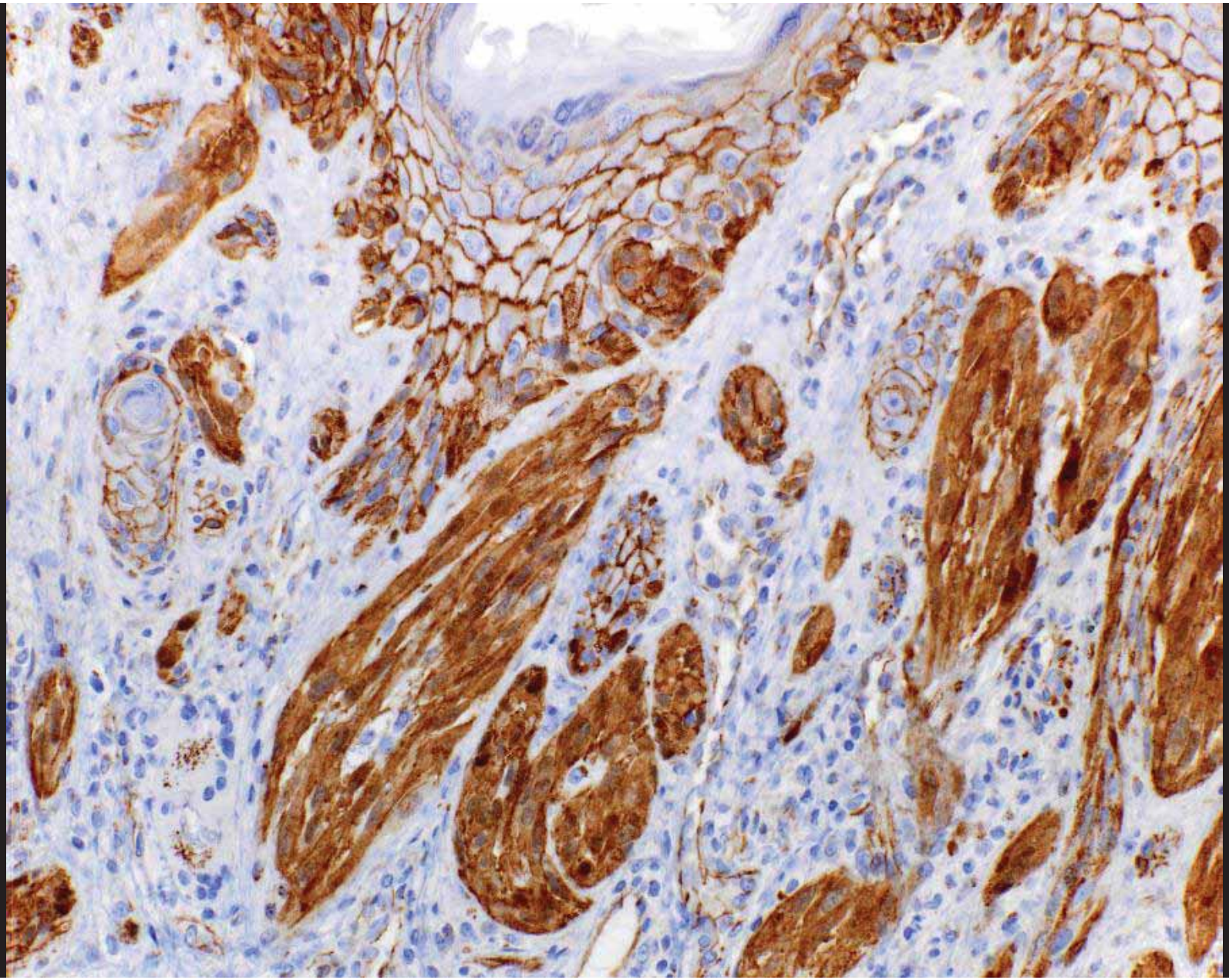


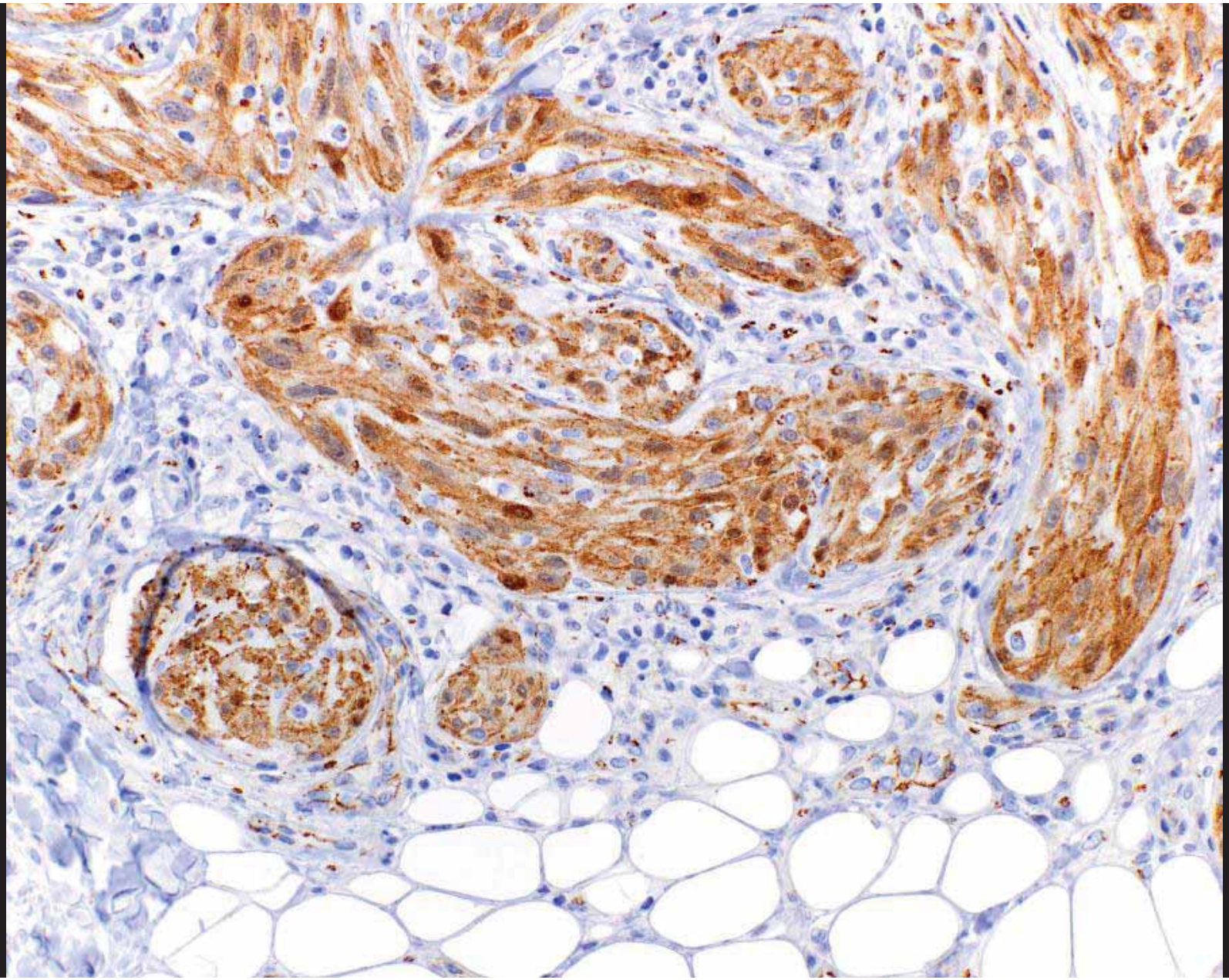




# β catenin







# Conclusions

- Multiple populations of melanocytes can mean tumor progression
- Or not…
- Tumor progression can be from any grade to the same or a higher grade
- We will know a lot more about tumor progression when we can include transcriptomics/proteomics