Molecular alterations in Spitizoid tumours and significance to the practising pathologist

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Liverpool Dermatopathology symposium

February 11, 2020

Icahn School of Medicine at Mount Sinai

I do not have any relevant conflicts of interest

- Consultant Sanofi pharmaceuticals

I. BAPomas

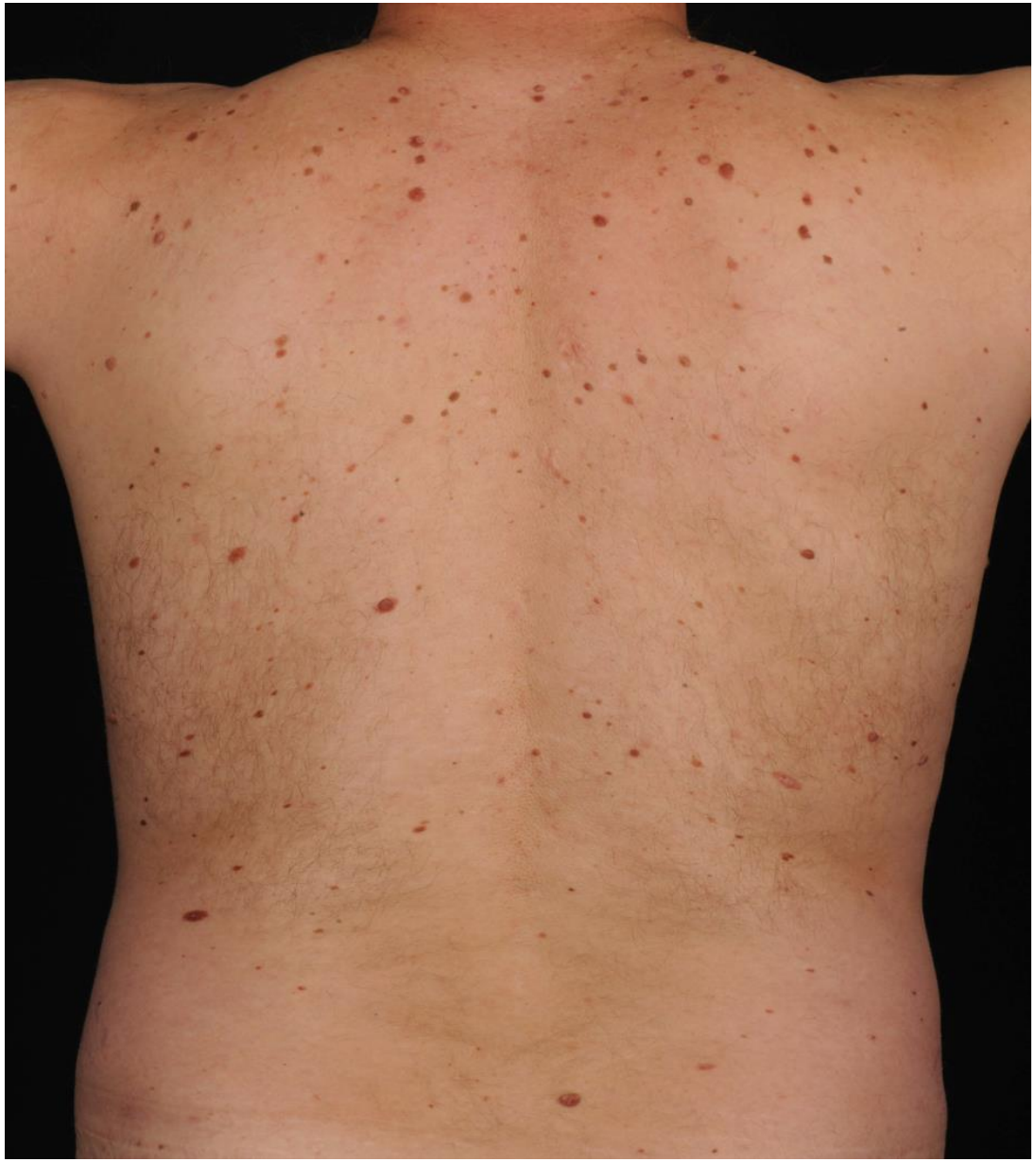
AKA: atypical Spitz tumor, melanocytic BAP1-associated intradermal tumor, nevoid melanoma-like proliferation, or BAP1-inactivated melanocytic nevus/melanocytoma (WHO)
or BAPomas

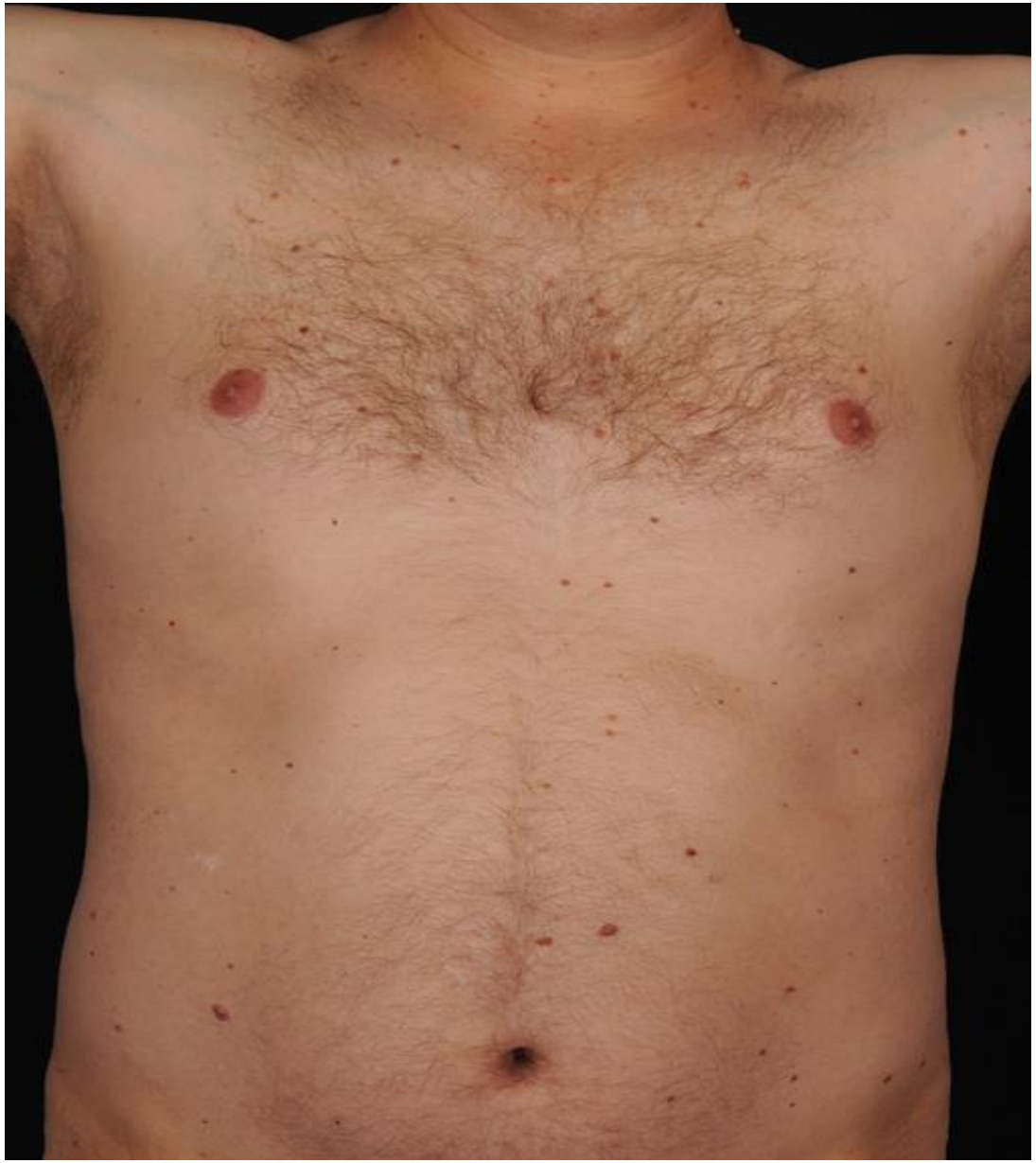
BAP1

- BRCA1 associated protein-1
- Functions as a tumor suppressor, regulating cell growth and division and cell death
- Involved with ubiquitin metabolism (prolongs survival and growth)

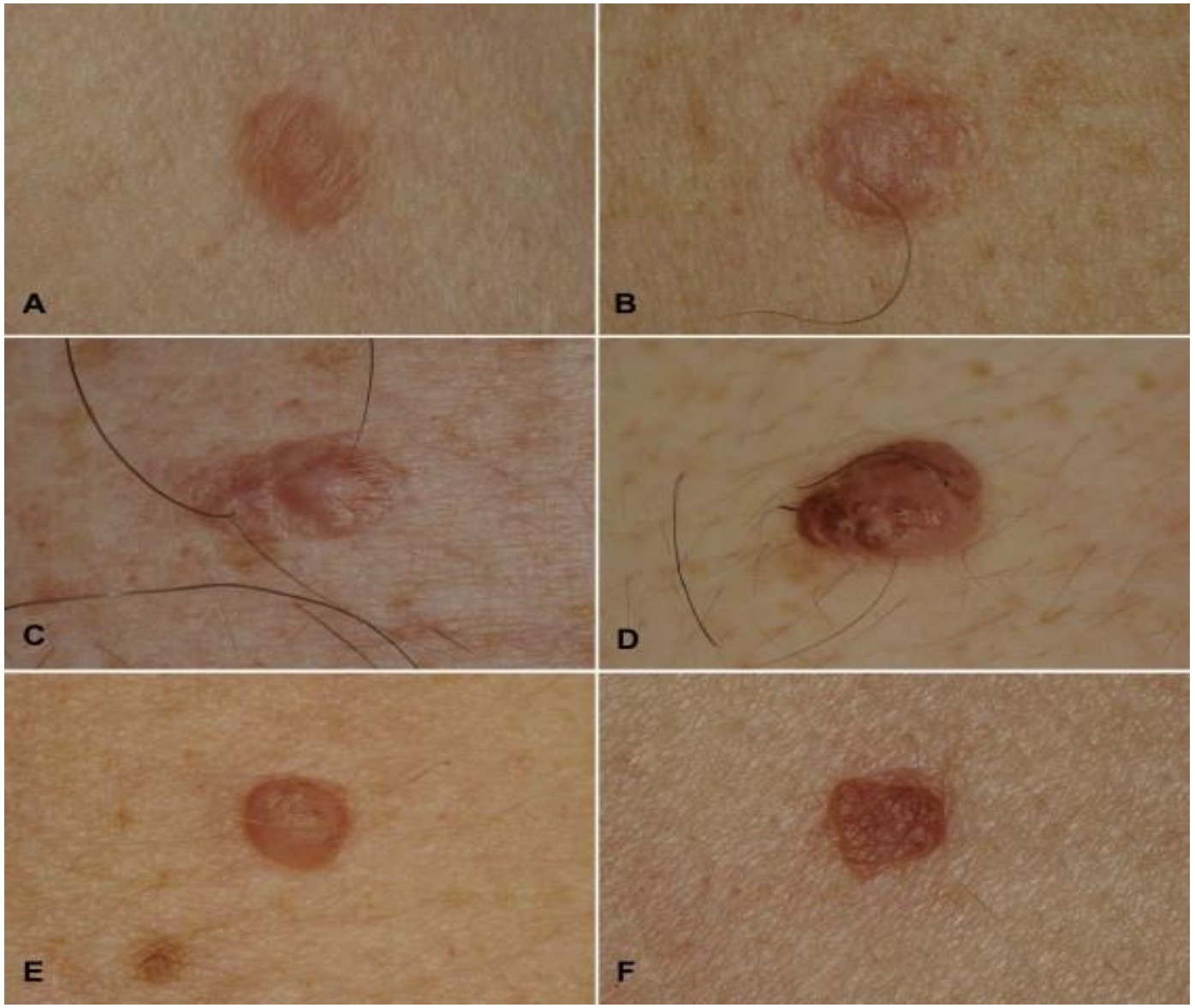
BAP1 mutations (germline)

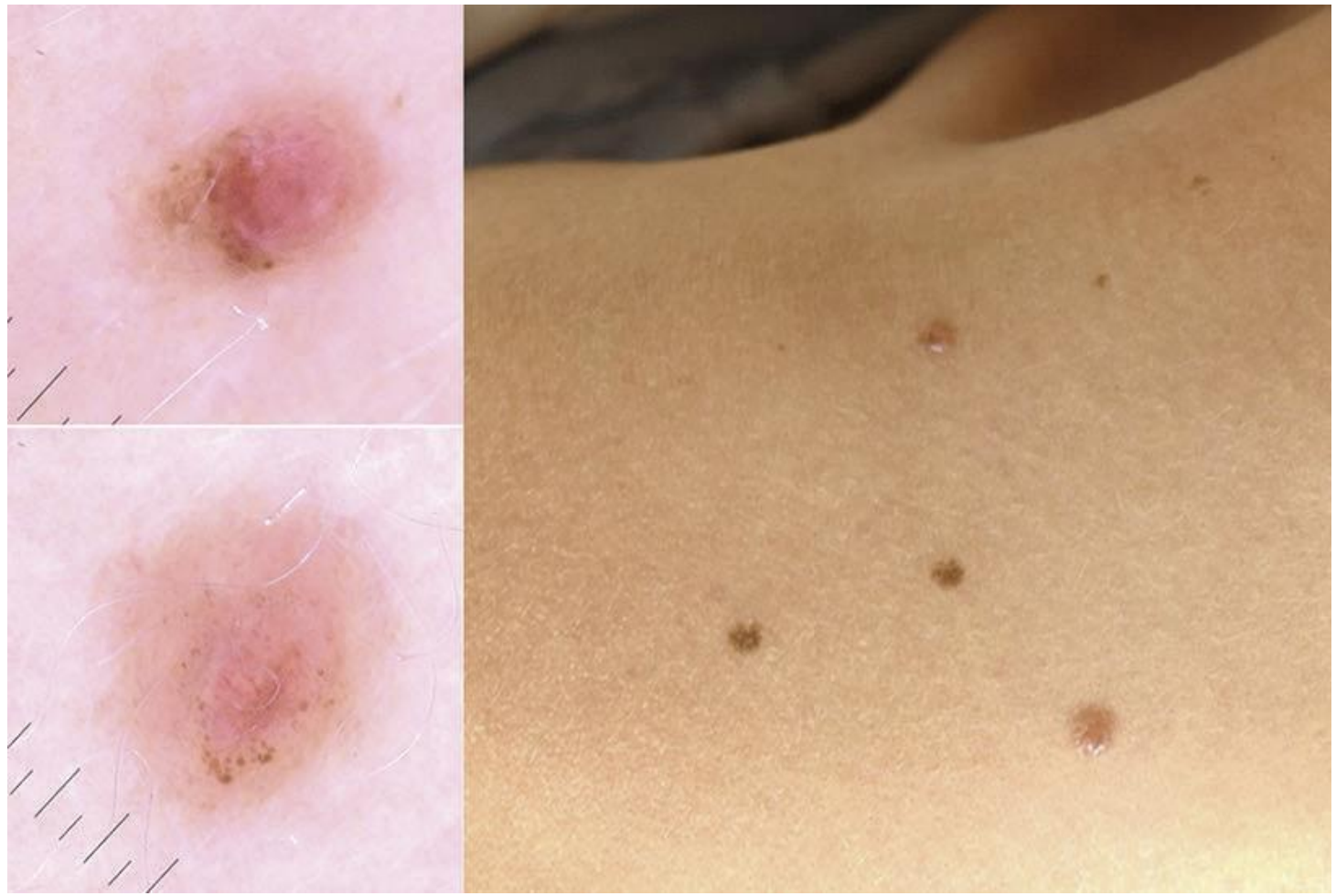
- Cancer susceptibility syndrome, called BAP1 tumor predisposition syndrome (BAP TPDS)
- Uveal melanomas, renal cell carcinomas, mesotheliomas and multiple other tumors still to be diagnosed and increasing list
- Patients develop multiple nevi and MM
- Sporadic mutations are possible (3p21.1)

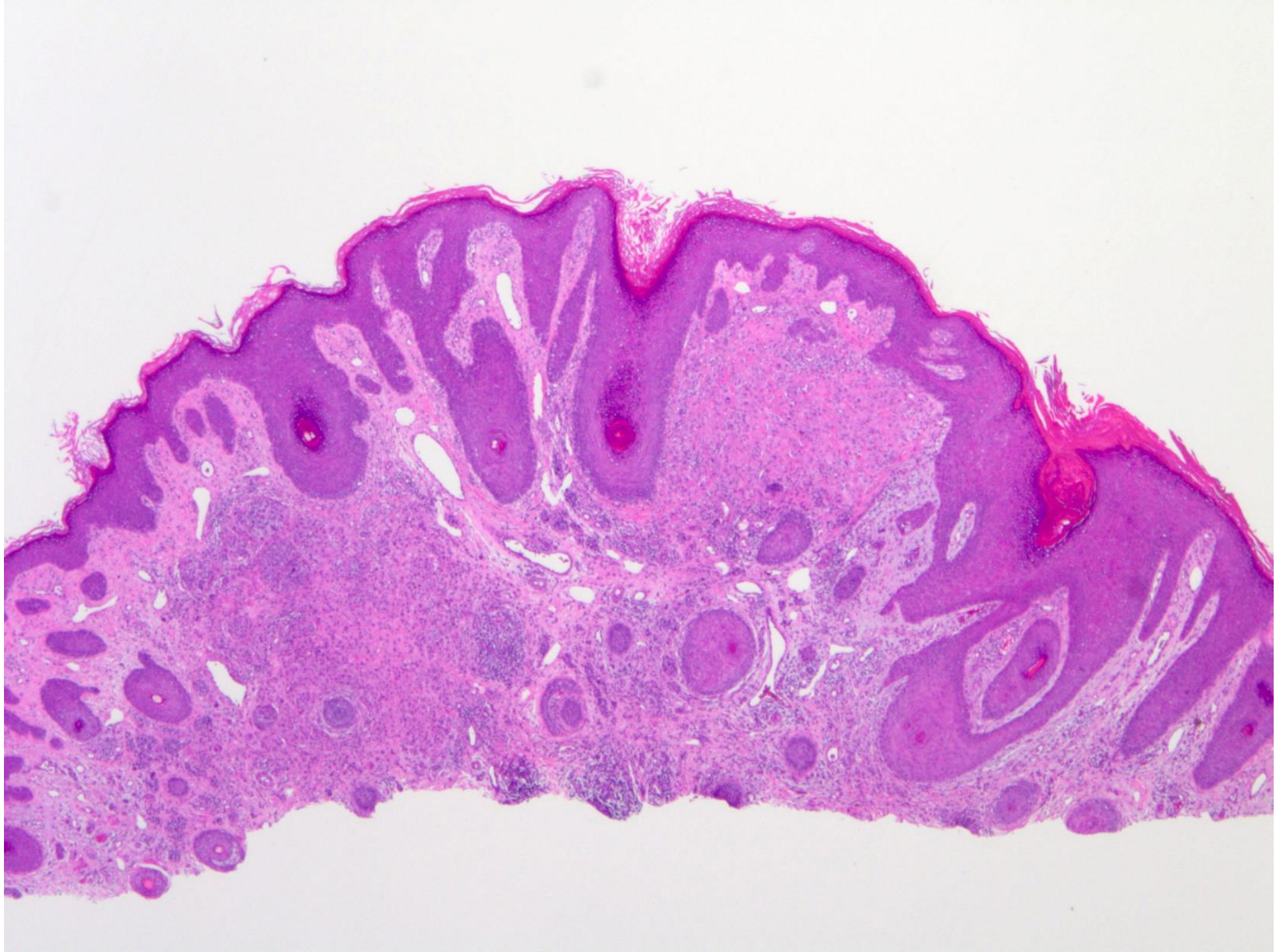


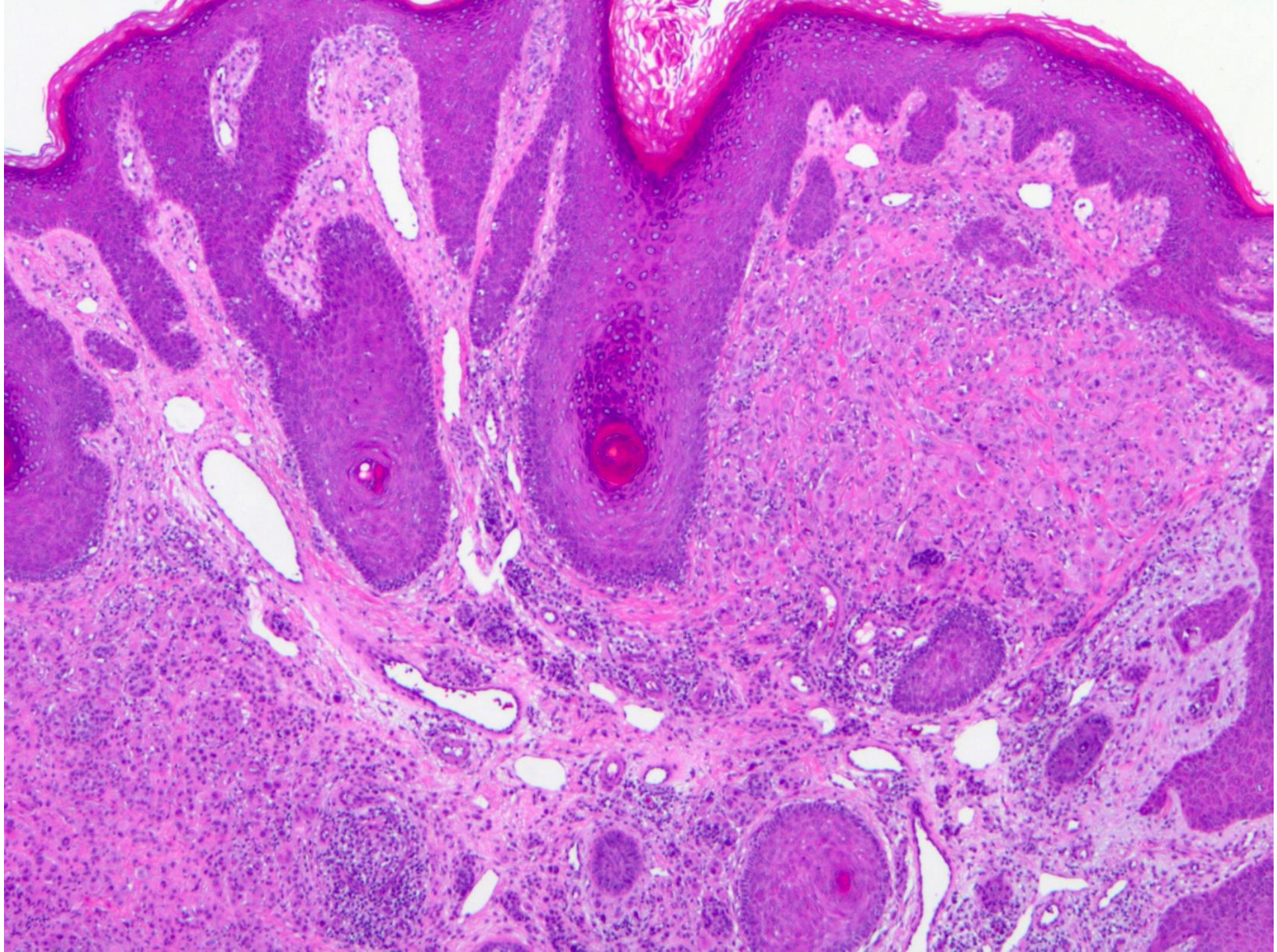


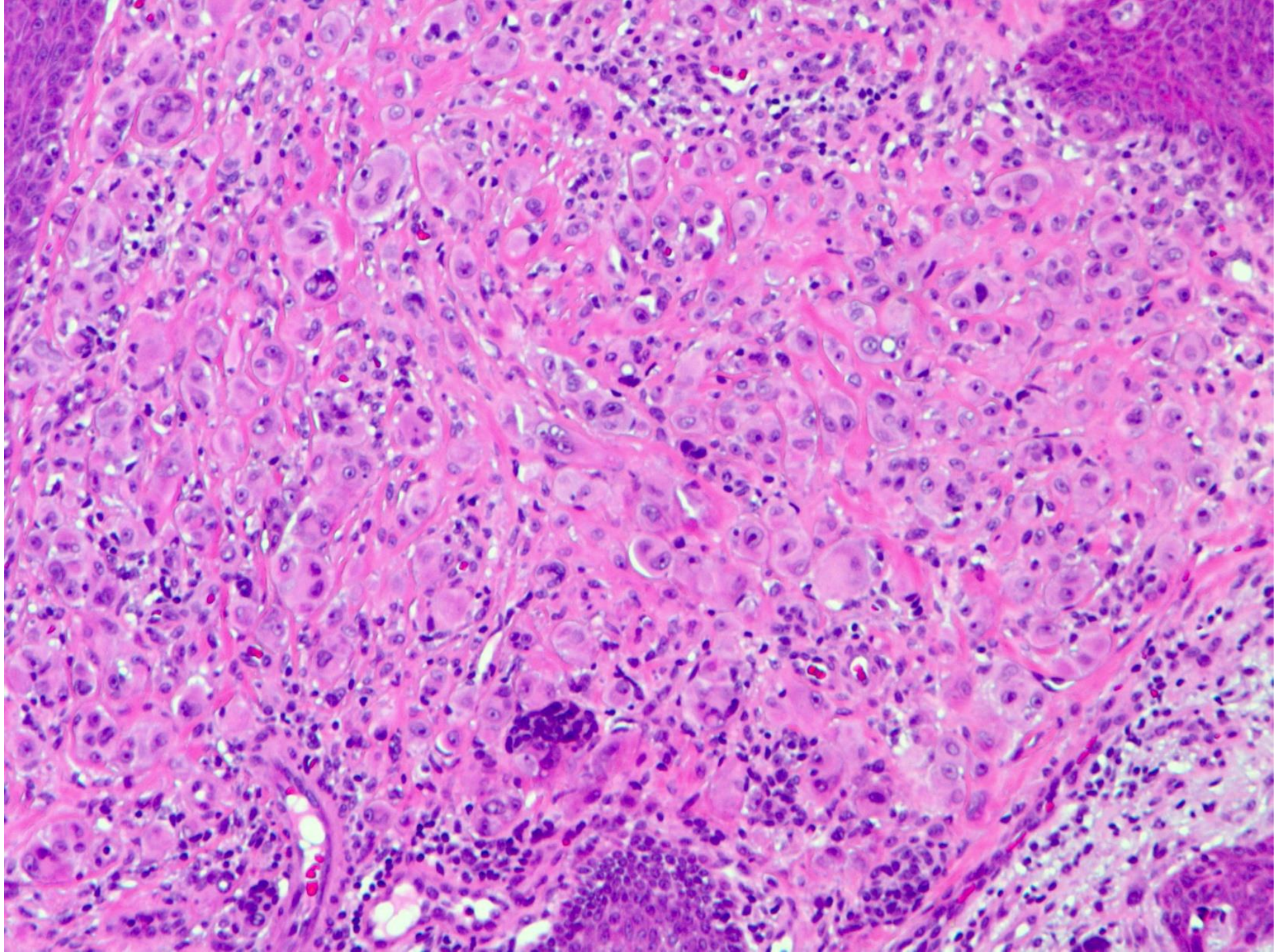
Wiesner nevus

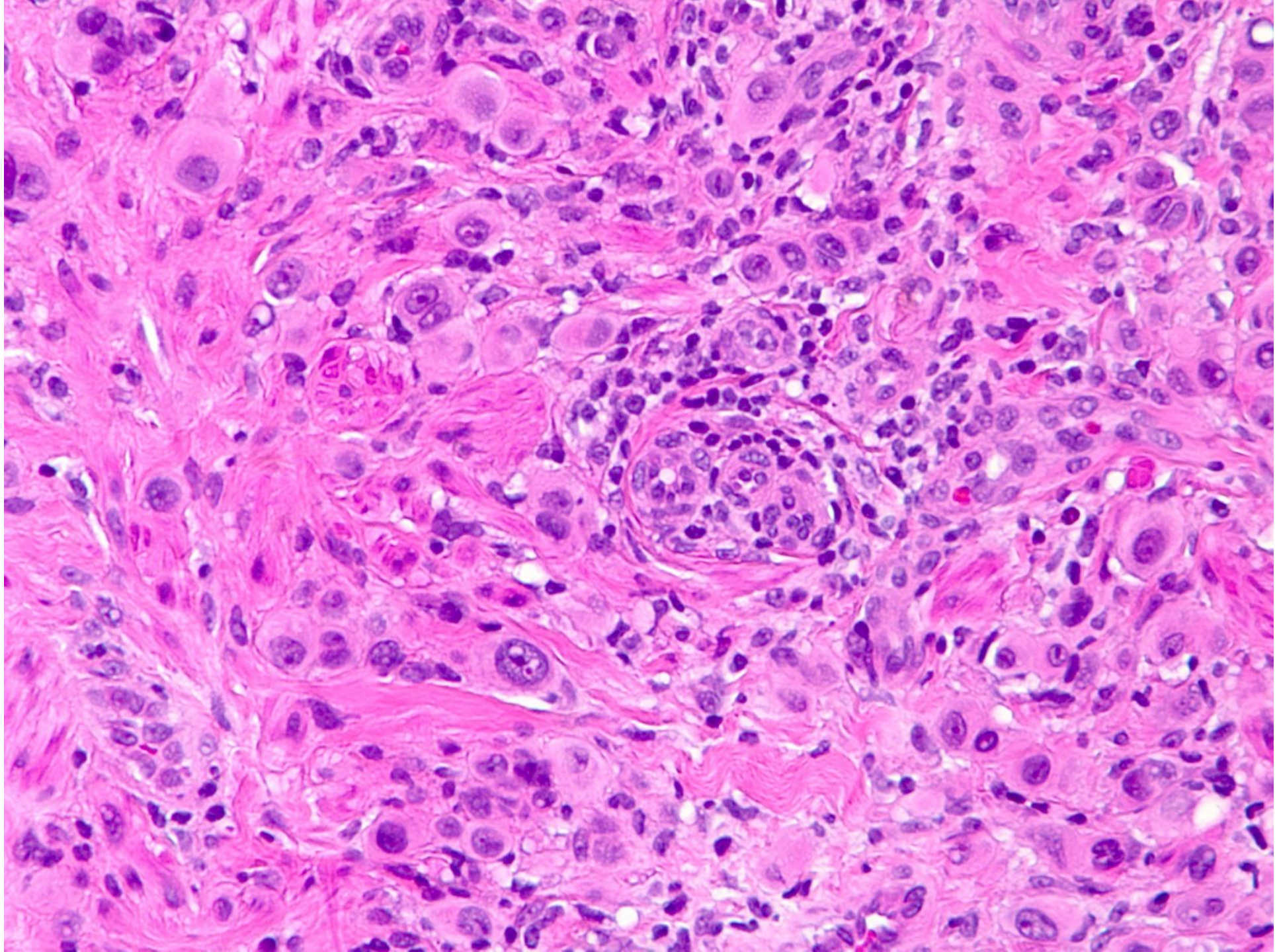


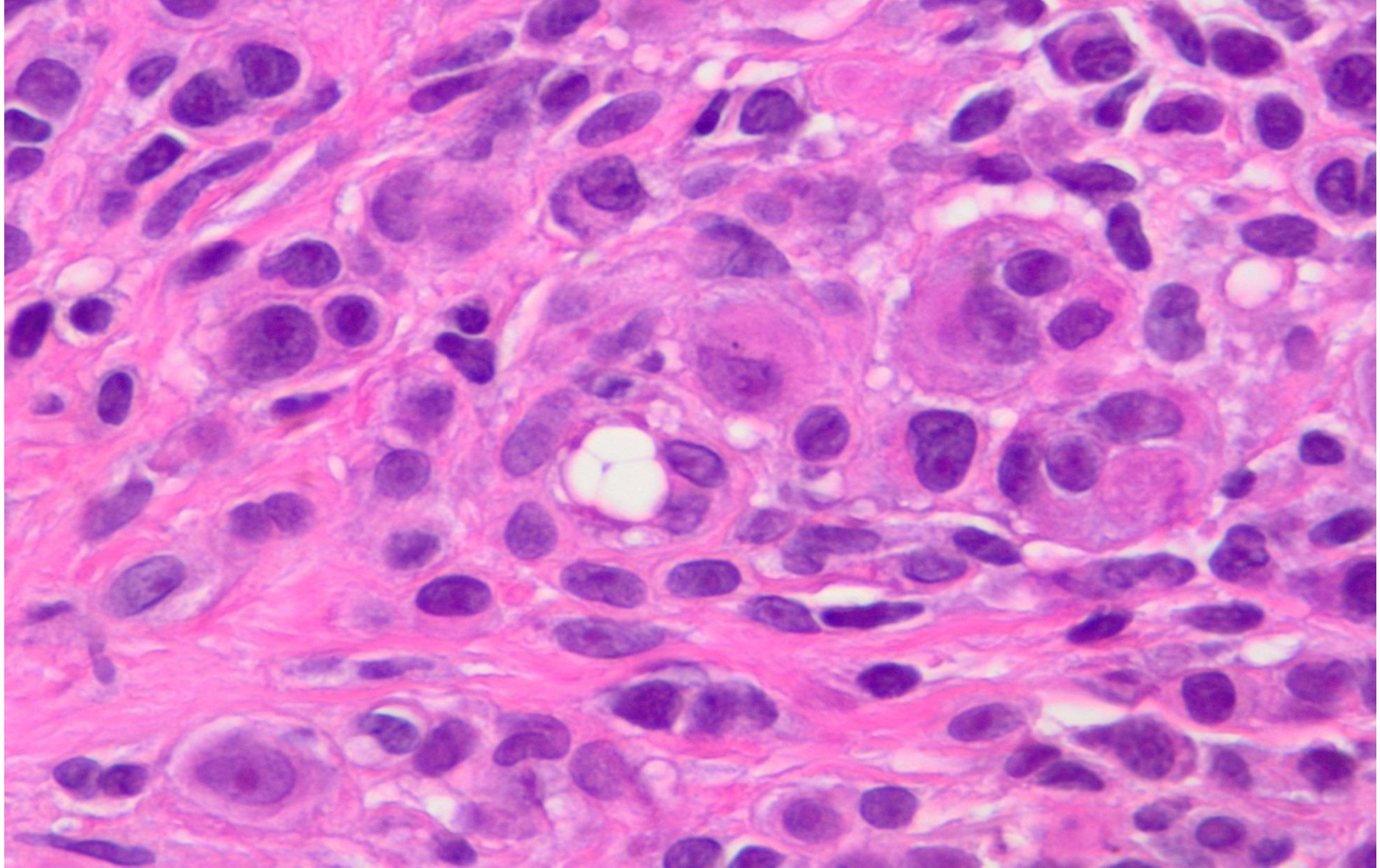


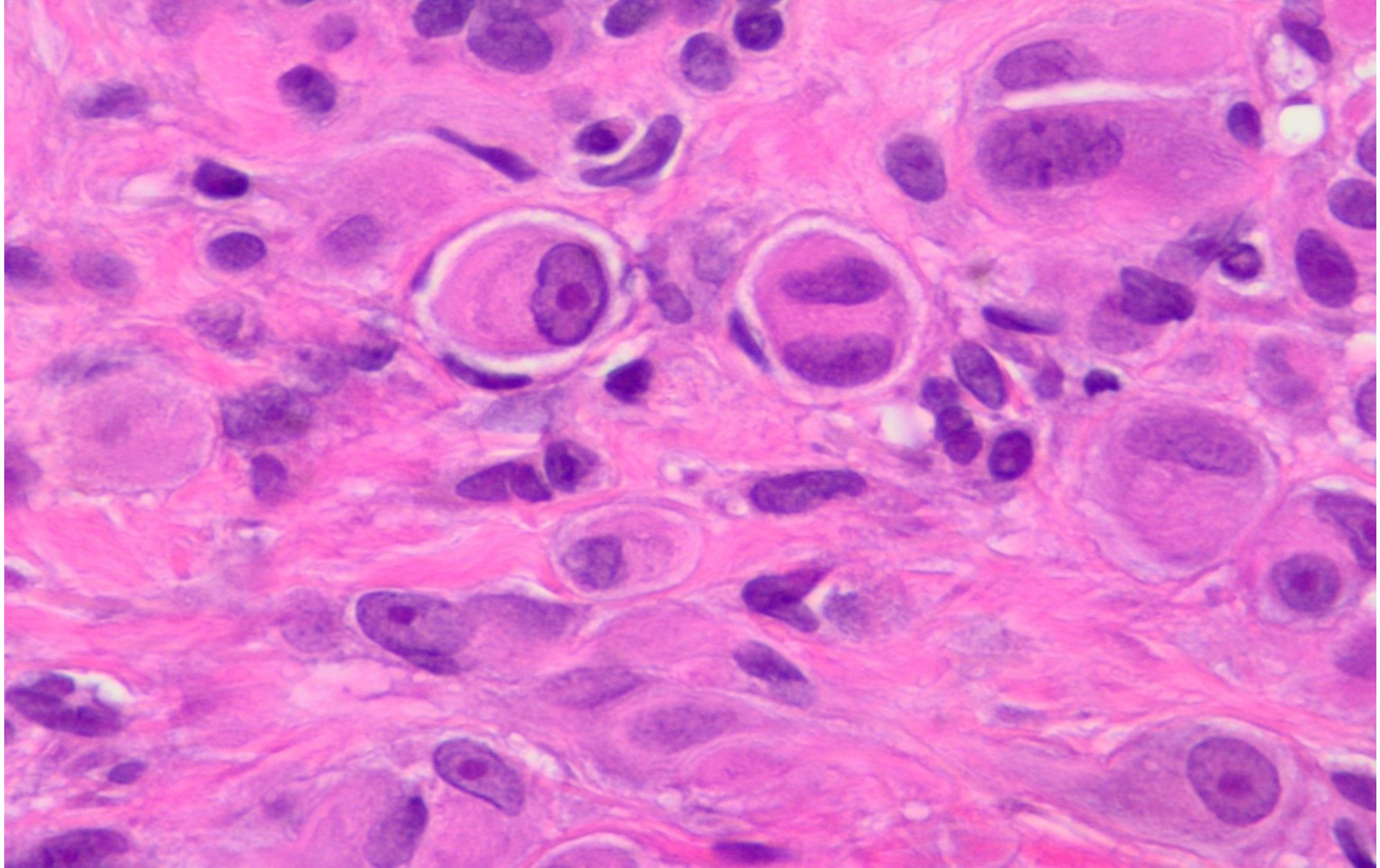


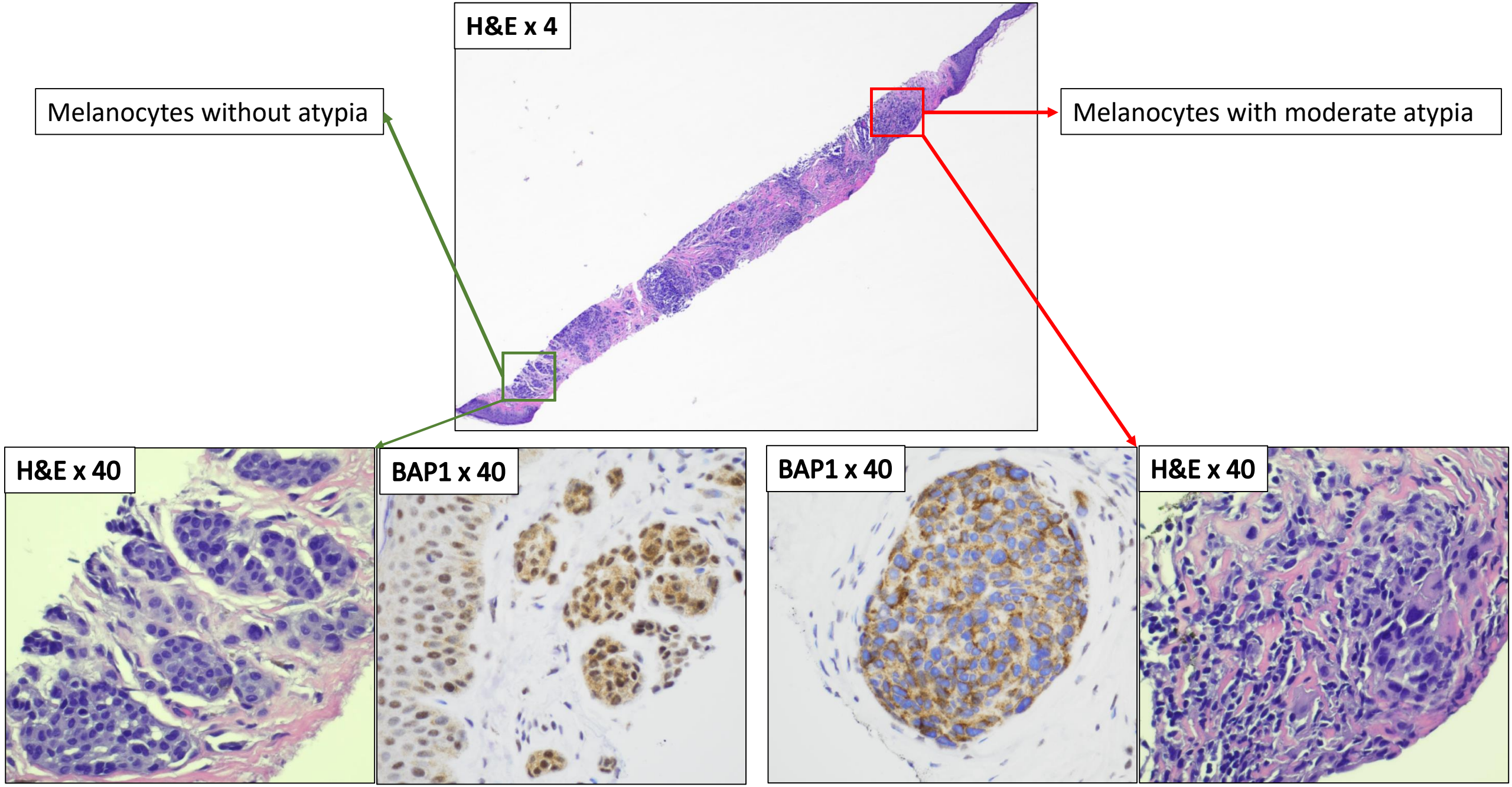


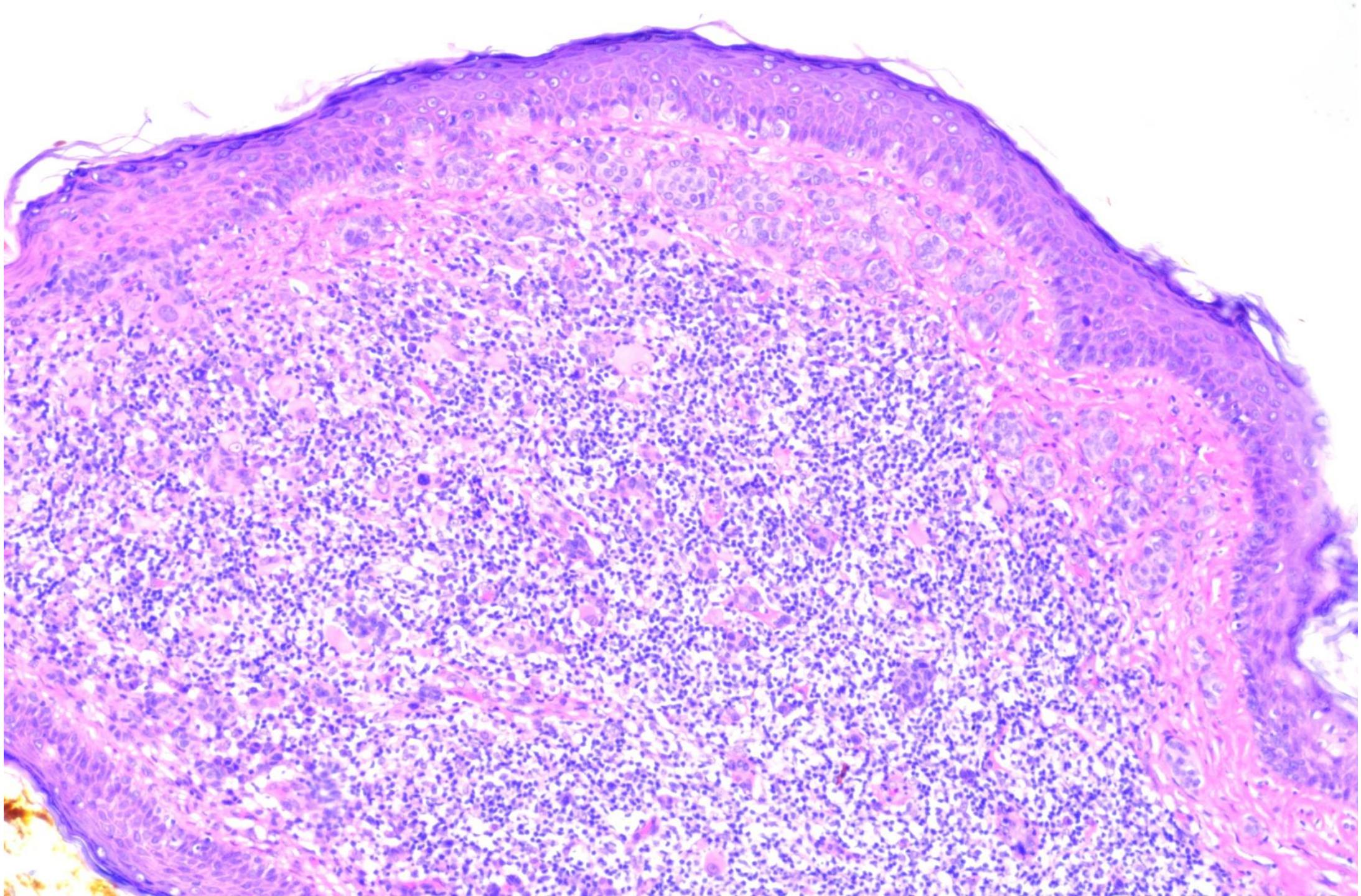


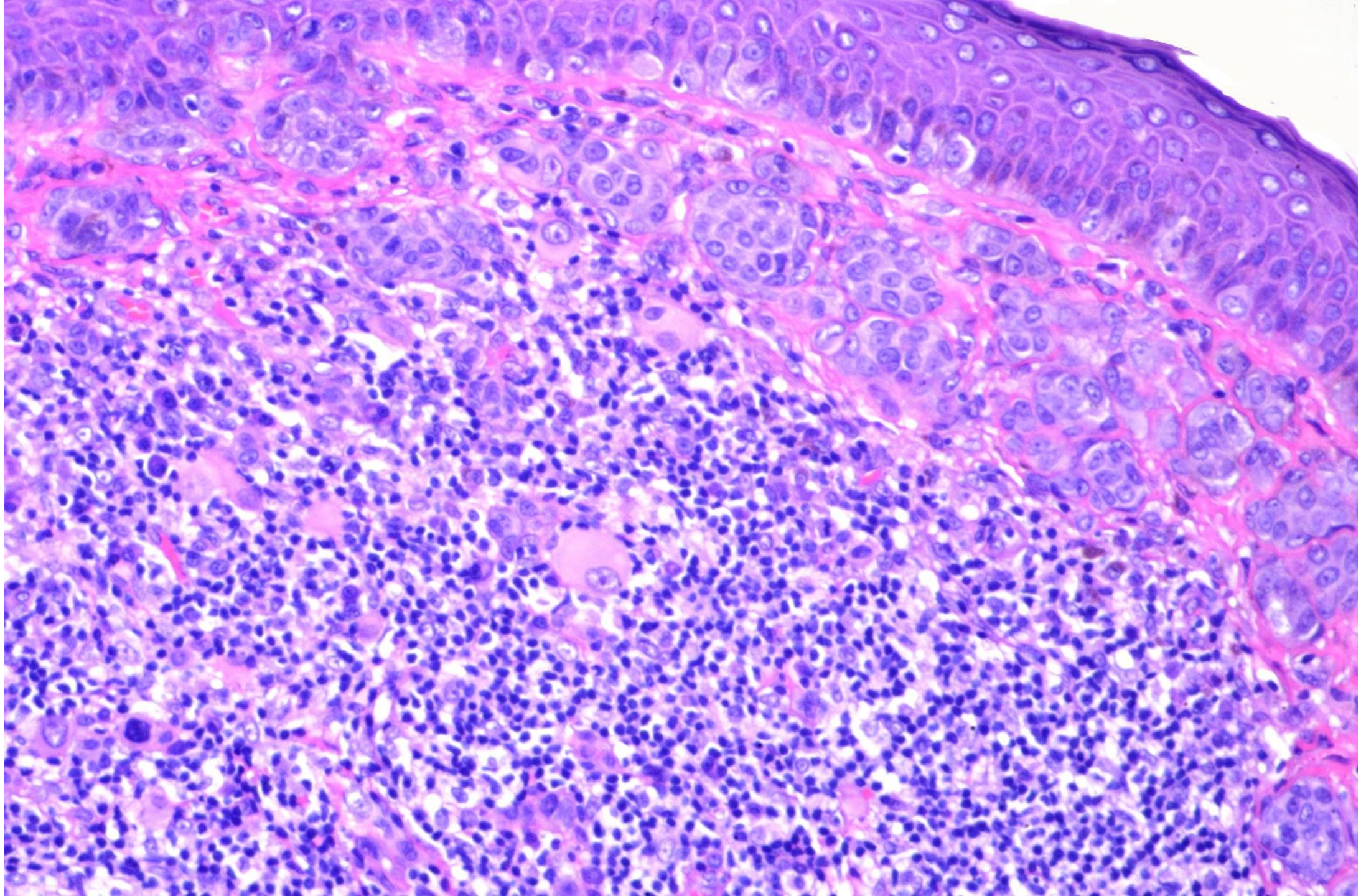


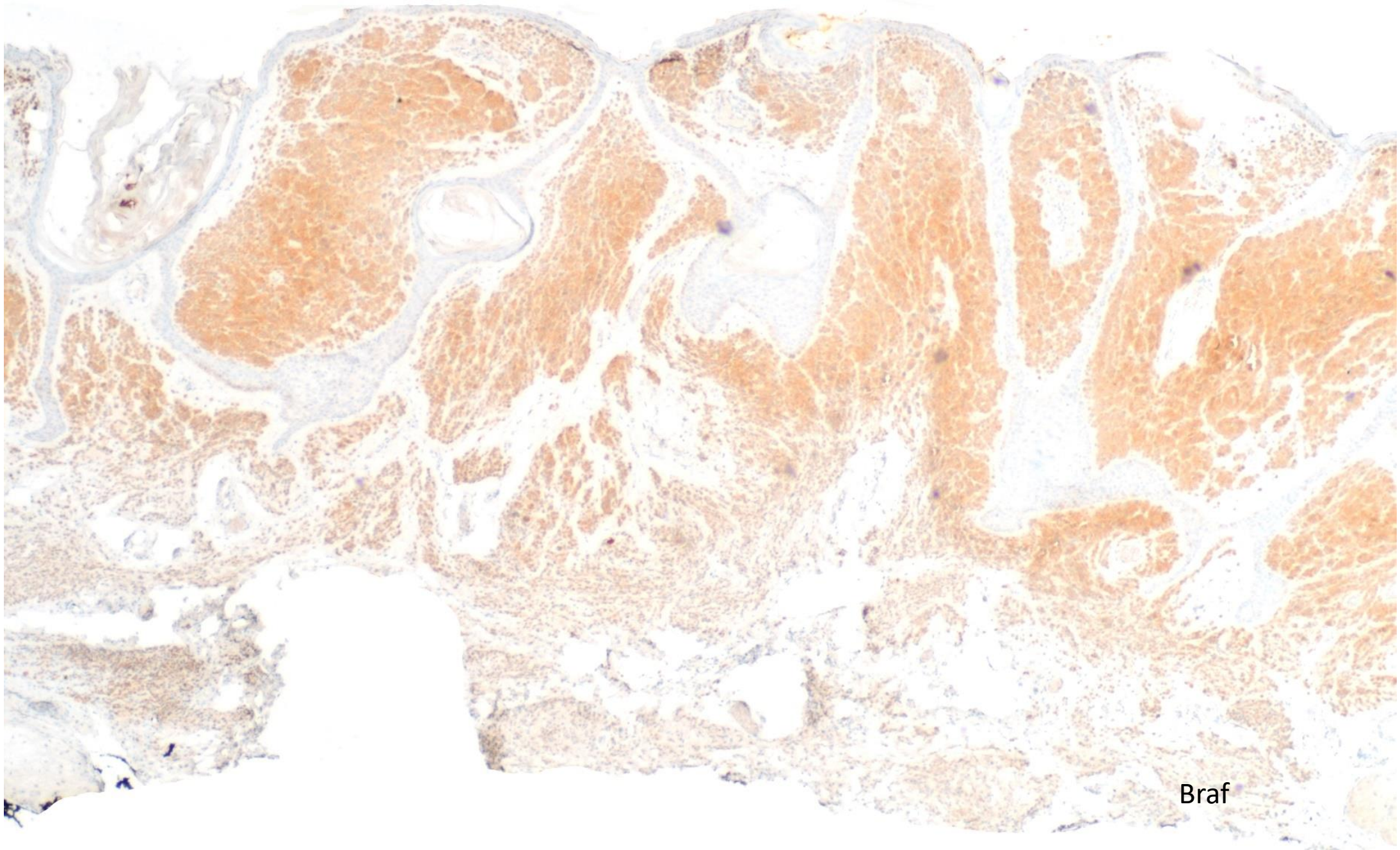


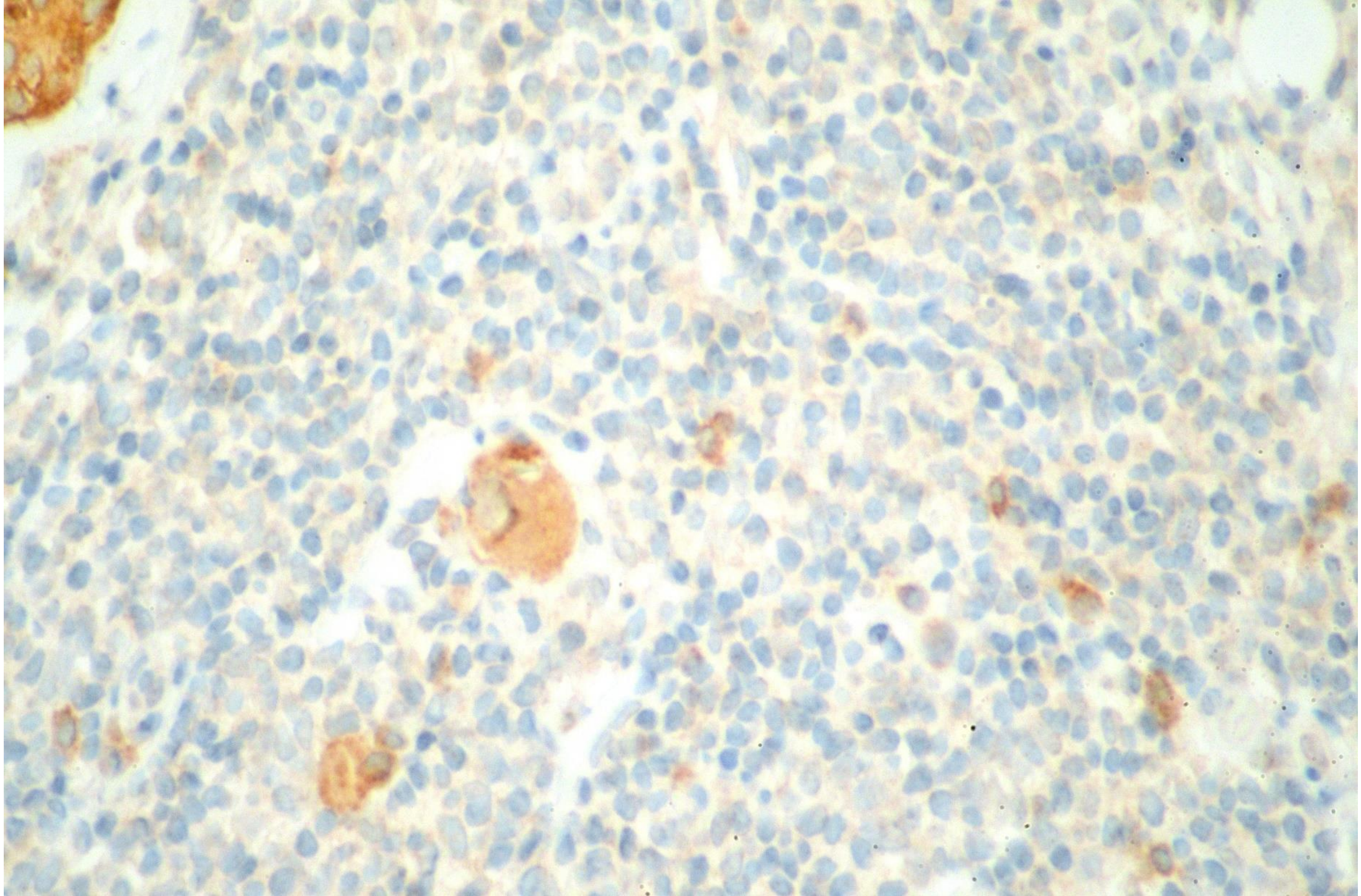


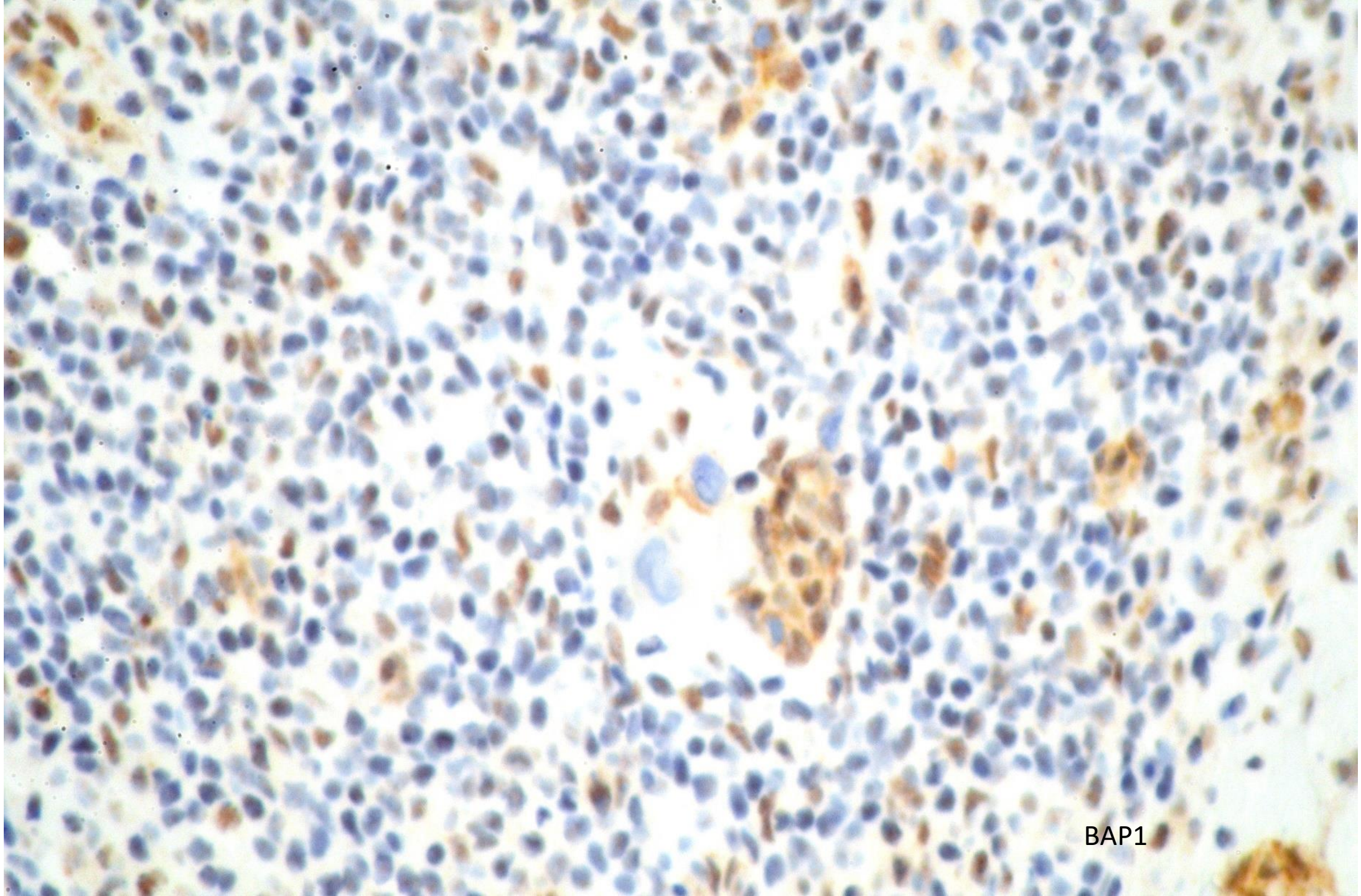

H&E x 4
Melanocytes without atypia
Melanocytes with moderate atypia
H&E x 40
BAP1 x 40
BAP1 x 40
H&E x 40






Braf




BAP1

Definition by molecular genetics and IHC

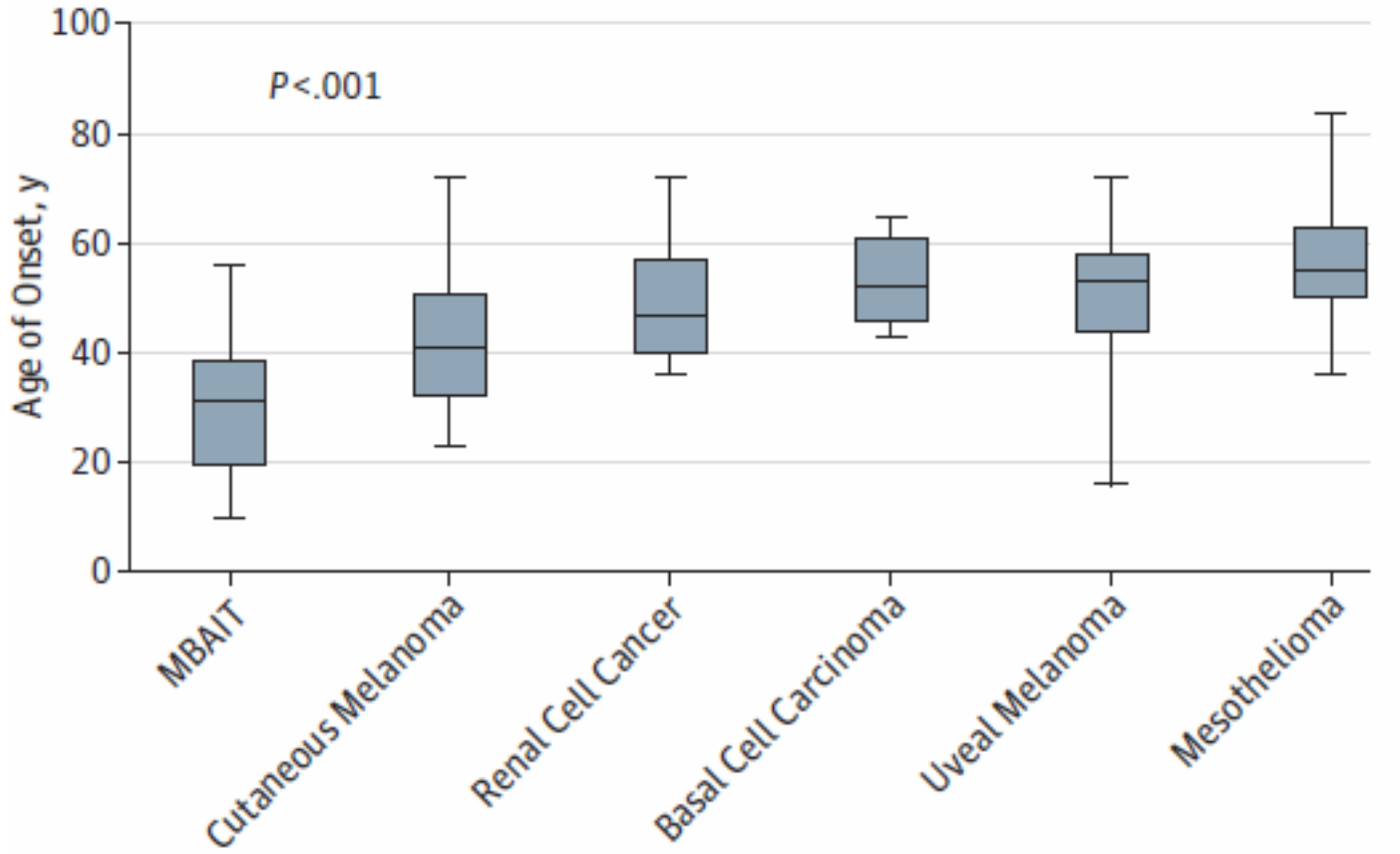
- The usual presence of a standard V600 E Braf mutation in all melanocytes and the absence of BAP1 reactivity in the larger melanocytes (a mutation of both copies of the gene). Distinction from Spitz.
- Germline versus sporadic (3p21.1)

What role can dermatologist and dermathologists play?

Skin examination: other studies

- If a patient has a germline mutation the chance of finding a BAPoma with a total skin exam is 75.5%
- If a patient has a BAPoma found incidentally, chance of the syndrome (germline vs sporadic) 49%

Figure 3. Median Ages of Onset of Tumors Associated With the BAP1 Syndrome



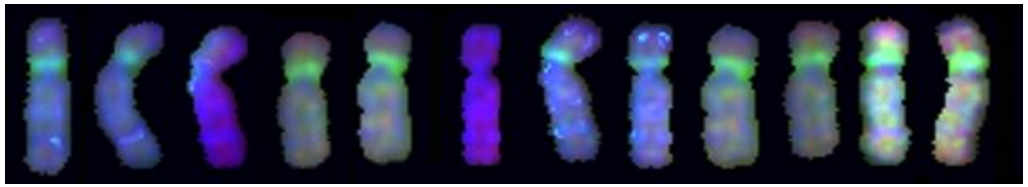
melanocytic BAP1-mutated atypical intradermal tumors

New Study

- JNCI: 181 families, large worldwide study
- Four core tumors UM, RCC, meso, CM all at high levels averaging around 20%
- Earlier age than background
- New tumors were added: BCC, cholangiocarcinoma, meningioma much greater than background
- Histologic types may be important: clear cell renal cell, rhabdoid meningioma
- Chance of carrier developing one tumor is 84.9%
- Number of cases diagnosed is most likely greatly underreported; carriers not always identified and only families are followed

II. Chromosomal changes in nevi

Comparative Genomic Hybridization



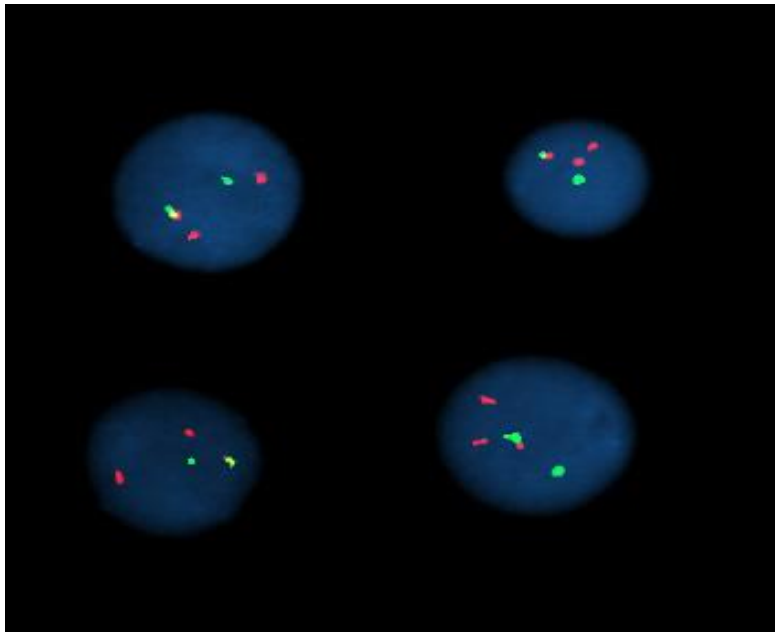
Can detect chromosomal aberrations in the entire genome of a tumor (compared to a control)-traditional and array methods

Melanomas have a variety of portions of chromosomal copy number changes (increases and changes with progression):

- Gains of 1q, 6p, 7, 8q 17q, 20q
- Loss of 6q, 8p, 9p, 10q
- Nevi usually do not; exception 11p gain Spitz nevus

Disadvantages: Need abundant tumor tissue with 30% of tissue containing gene alteration

Fluorescence in situ Hybridization: FISH



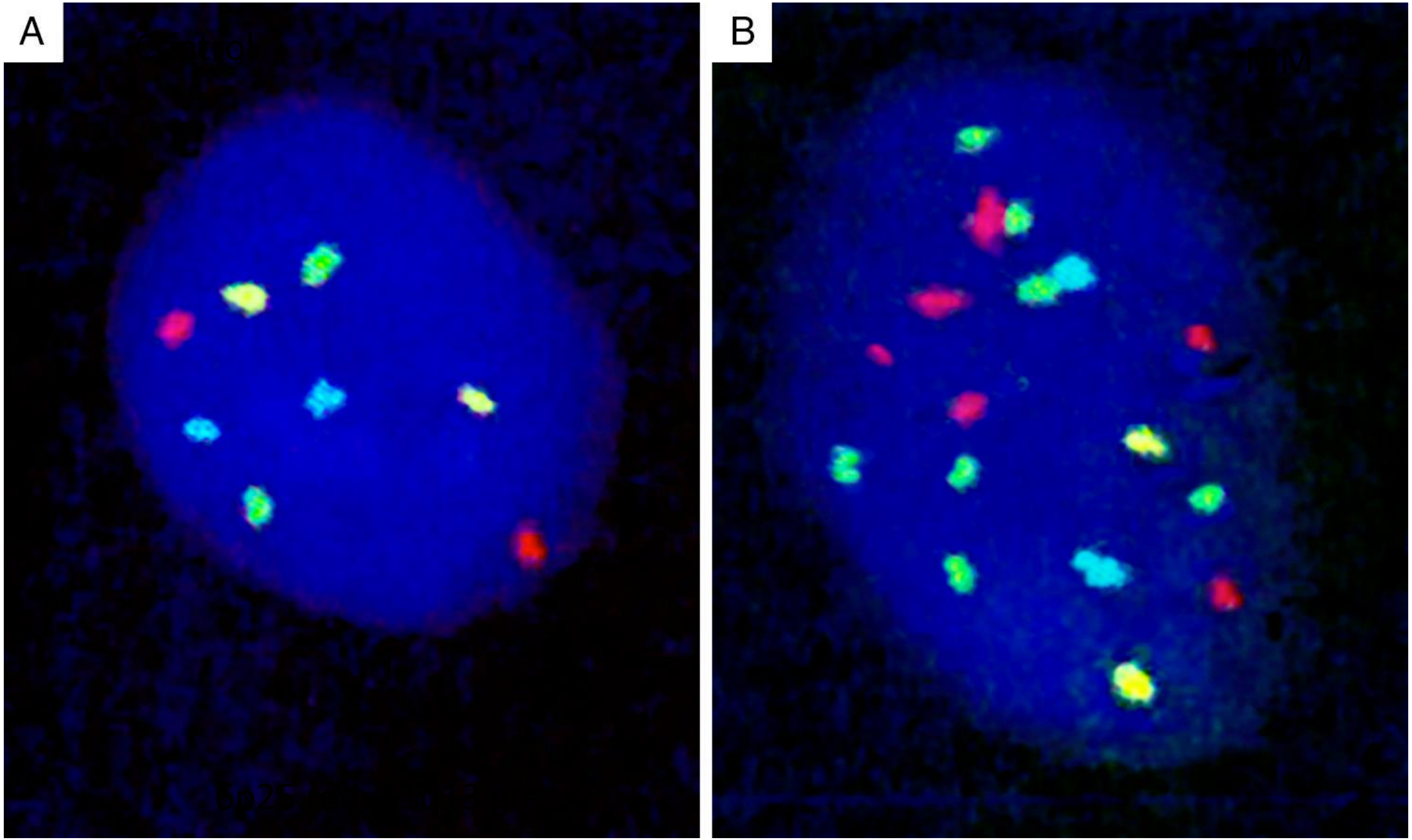
Can be used in FFPE tissue (easy to do) with less tissue; must identify tumor

Short probes of defined DNA sequences and attached tags fluorescent tags are added to cells in a mitotically active cell preparation

Different colors of probes can be used to differentiate signals and determine if chromosomal anomalies are present in specific chromosomal regions (altered copy number)

Genes in probes (hotspots)

- RREB1-Ras responsive element binding protein, 6p25, red
- MYB-myeloblastosis proto-oncogene, 6q23, gold
- CCND1-cyclin D1, 11q13, green
- CMYC-regulator gene transcription factor, 8q24 (not used, not for Spitz nevi), aqua
- CDKN2A-P16, melanoma gene, 9p21, gold
- CEP6 (aqua), CEP9 (green) (controls)

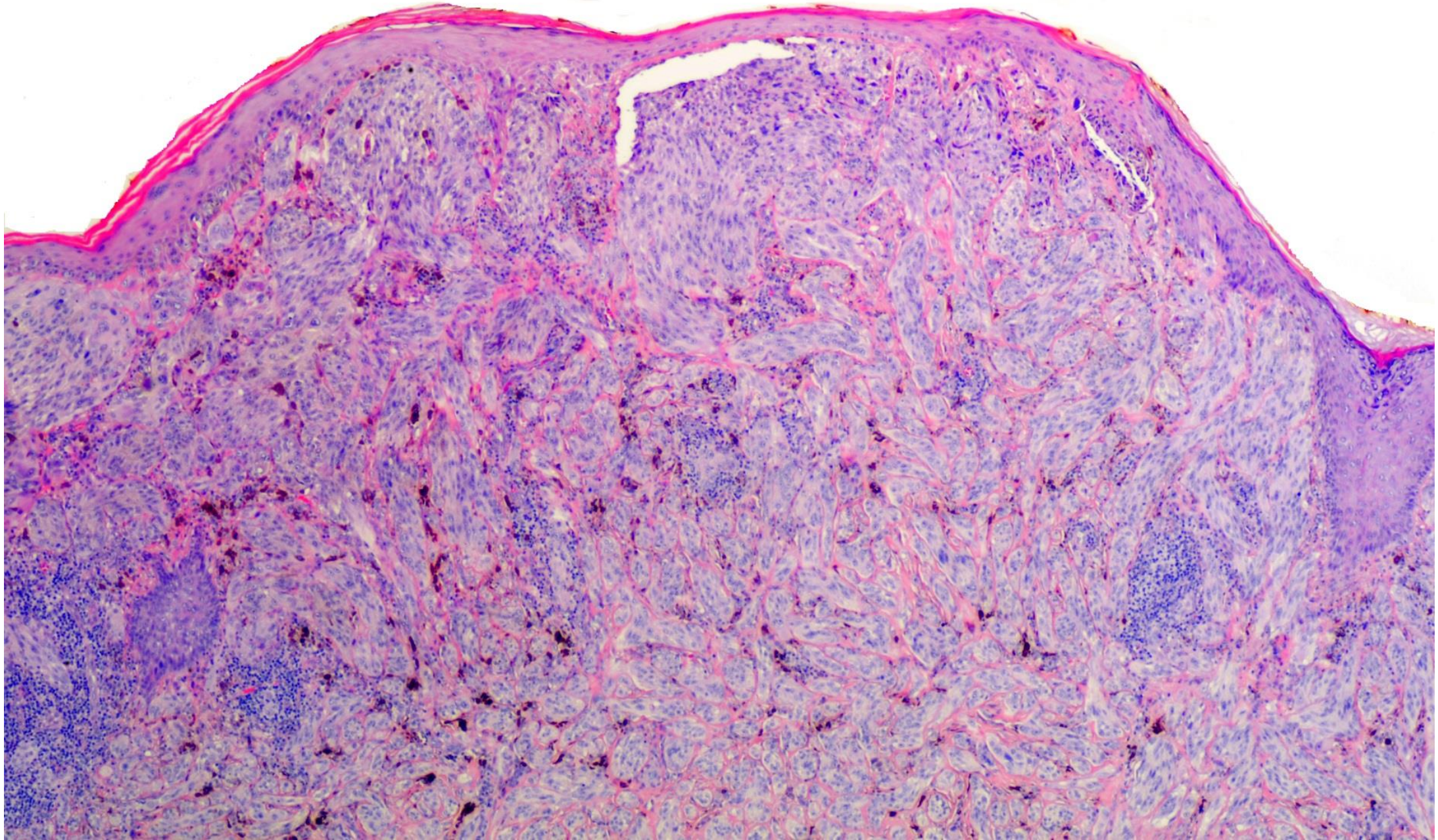

A
B

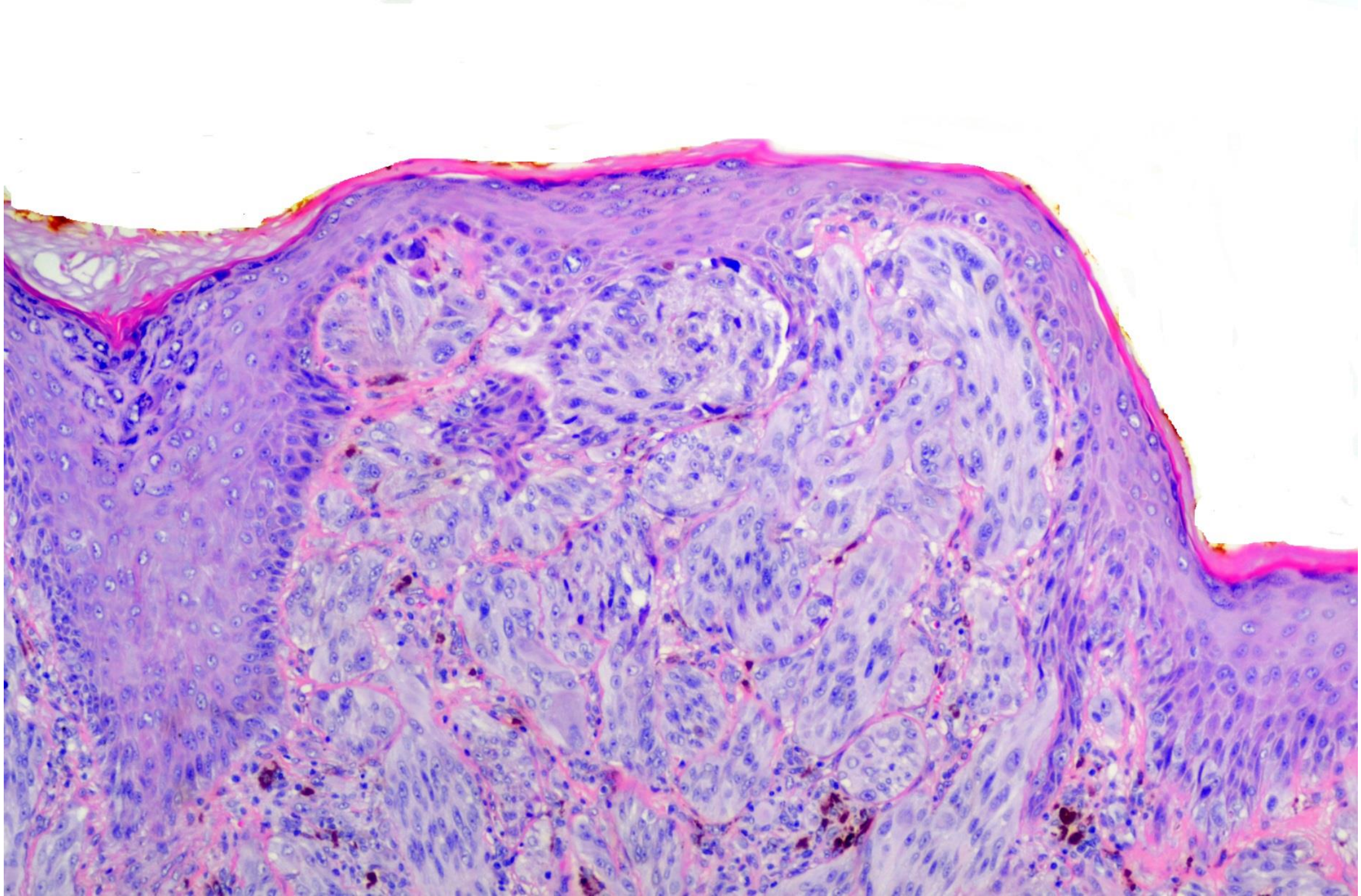
Histologic features noted (criteria for atypical Spitz)

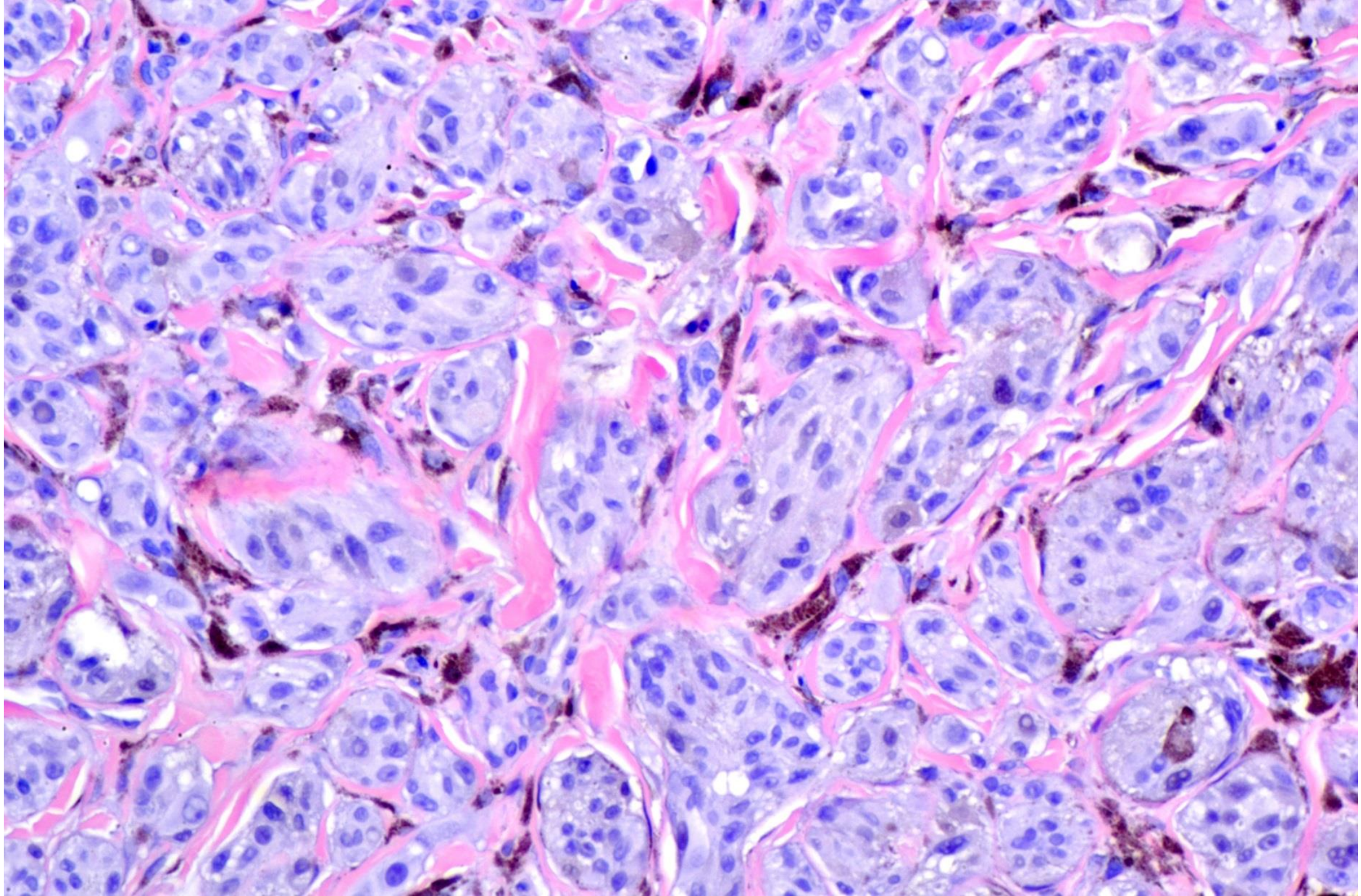
- Expansile dermal nodule, deep extension, deep mitoses, abundant melanin at depth, marked nuclear pleomorphism, asymmetry, necrosis, epidermal atrophy, cells within lymphatic vessels presence of ulceration, large dermal nests, dermal sheets of cells, lack of maturation, inflammation, sclerosing pattern, overall mitoses, pagetoid spread, compound versus intradermal; spindled, epithelioid, or combination ; HMB 45 staining pattern (lack of zonation)

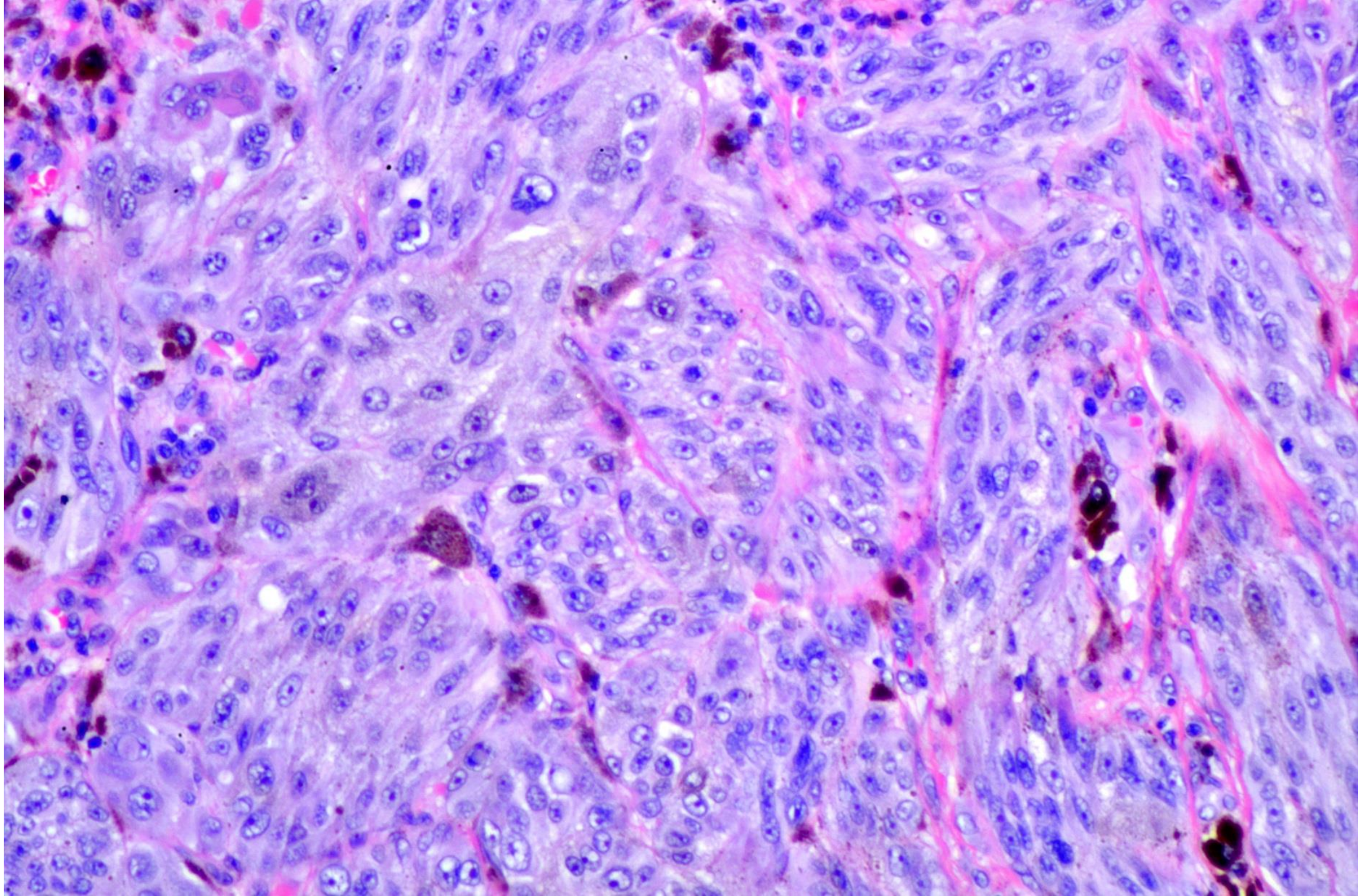
Case 1

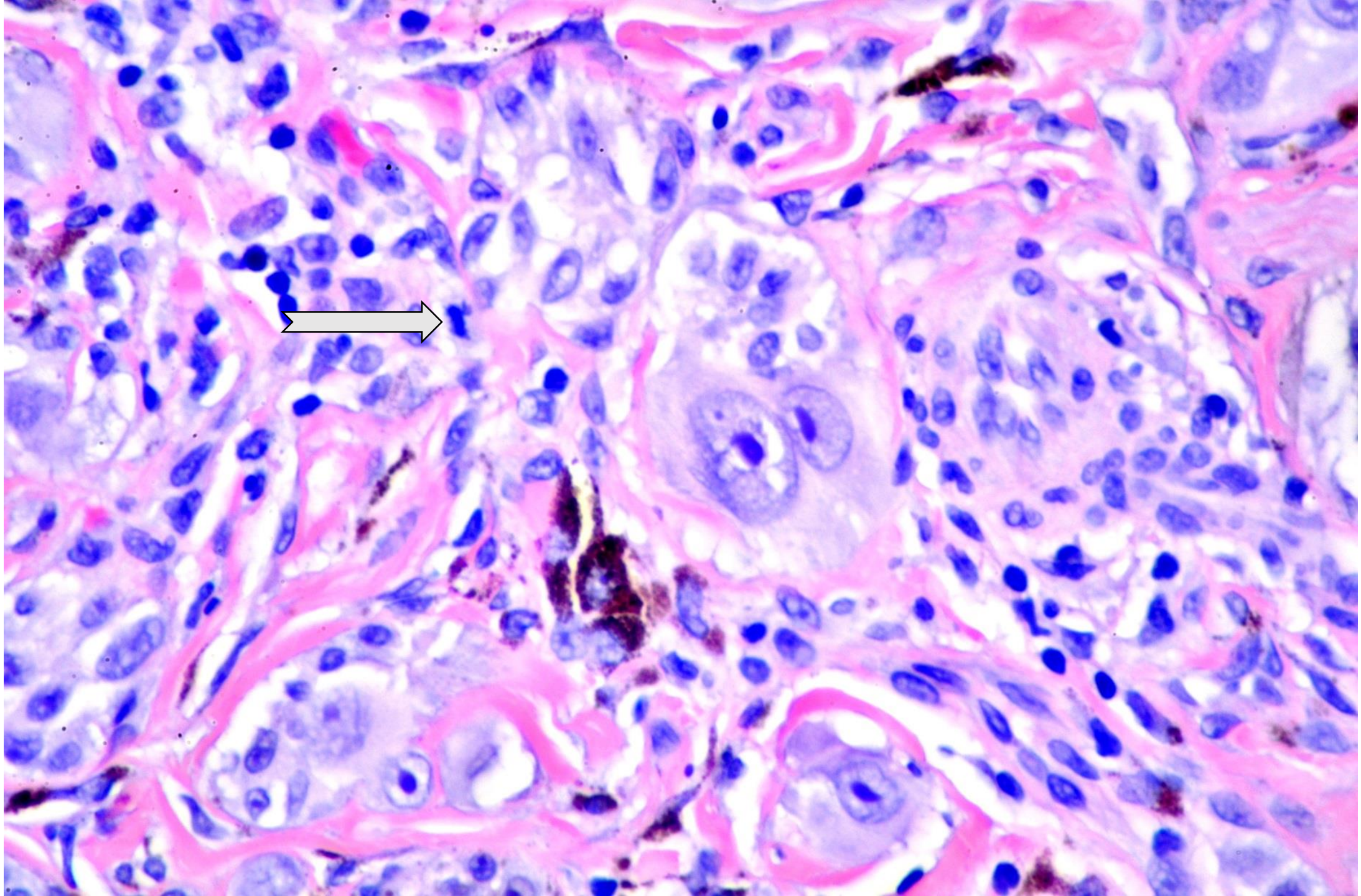
- 14 year old male
- Lesion right helix
- Clinical diagnosis: DN









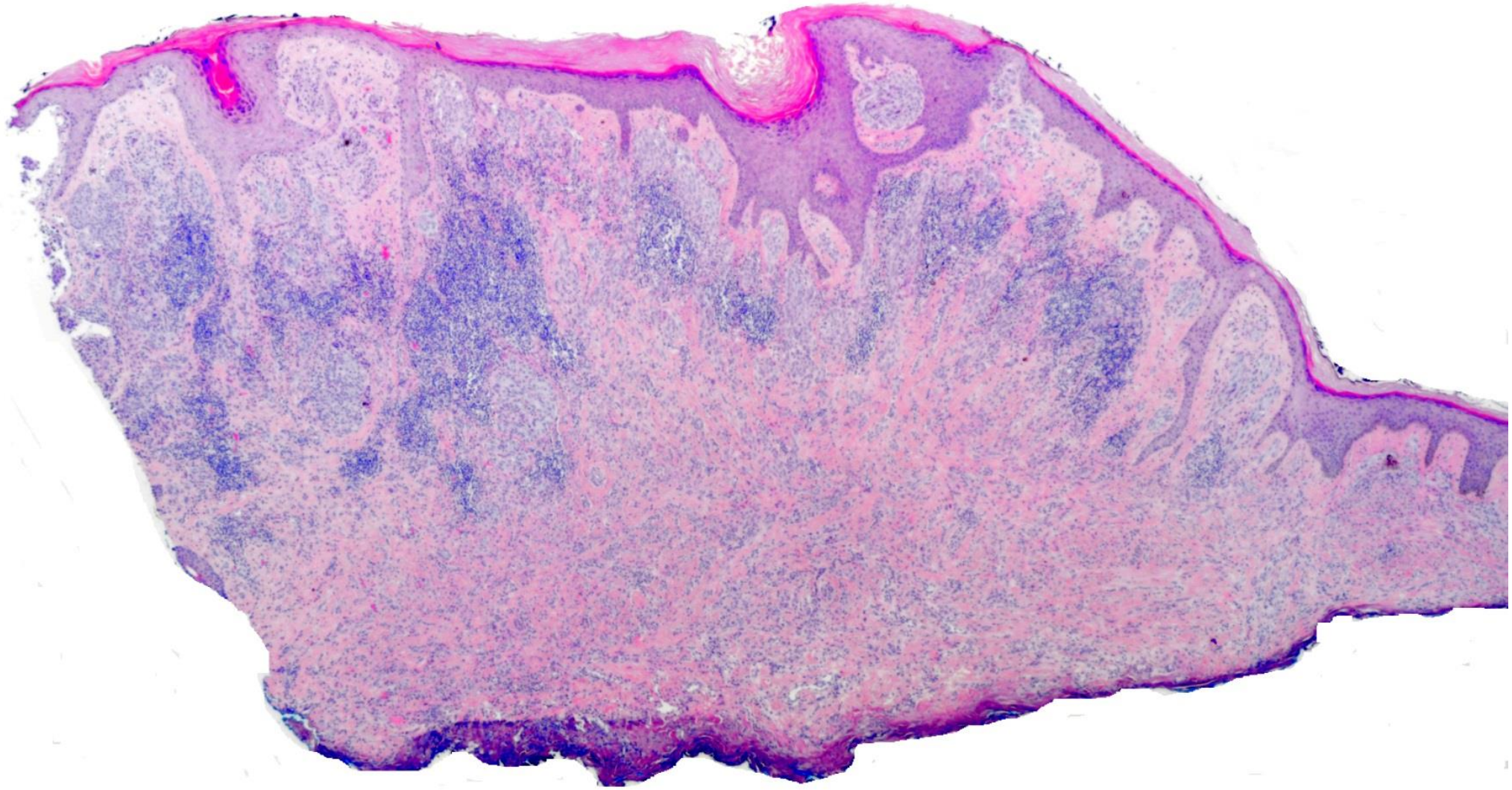


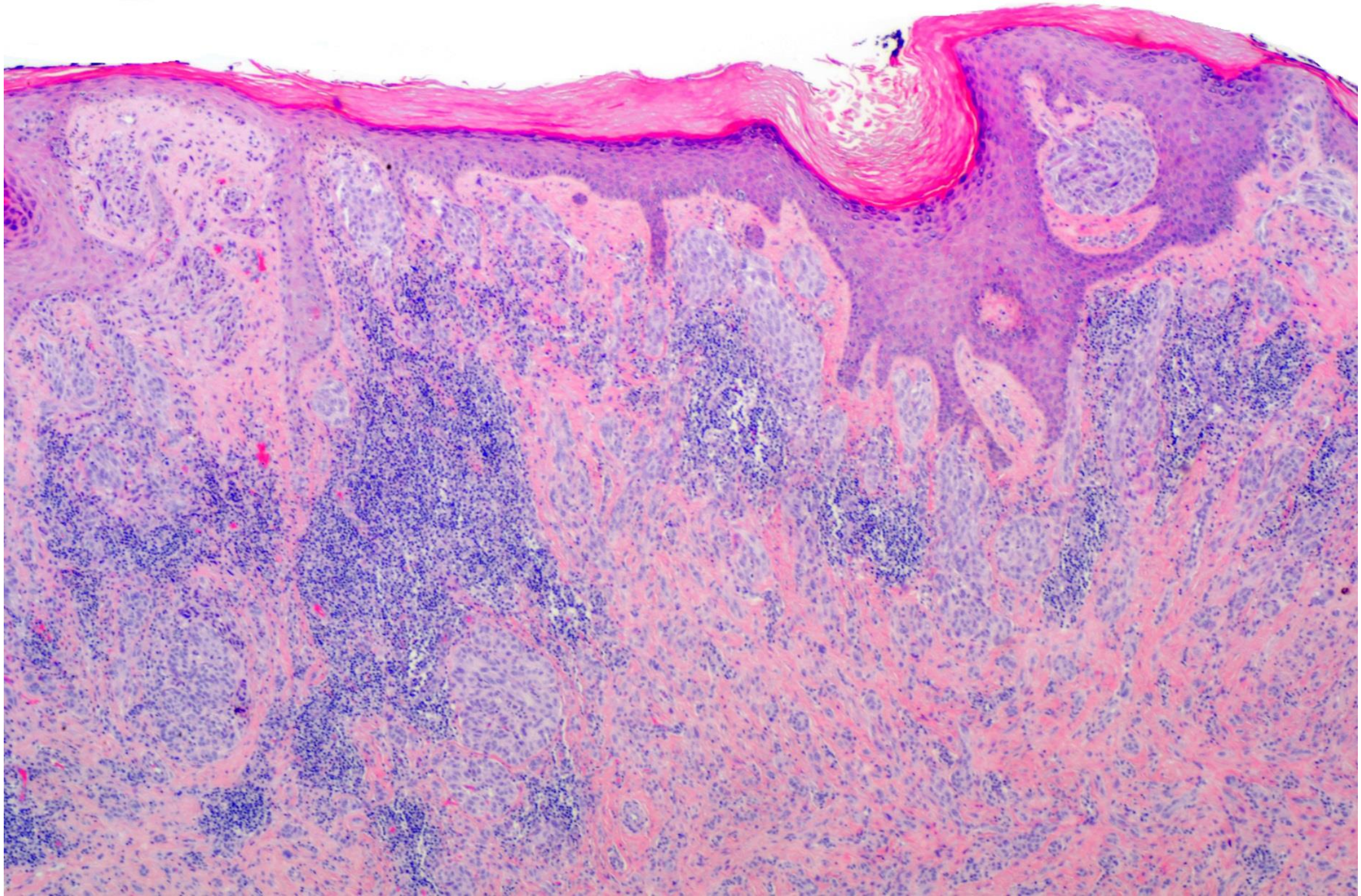
Results

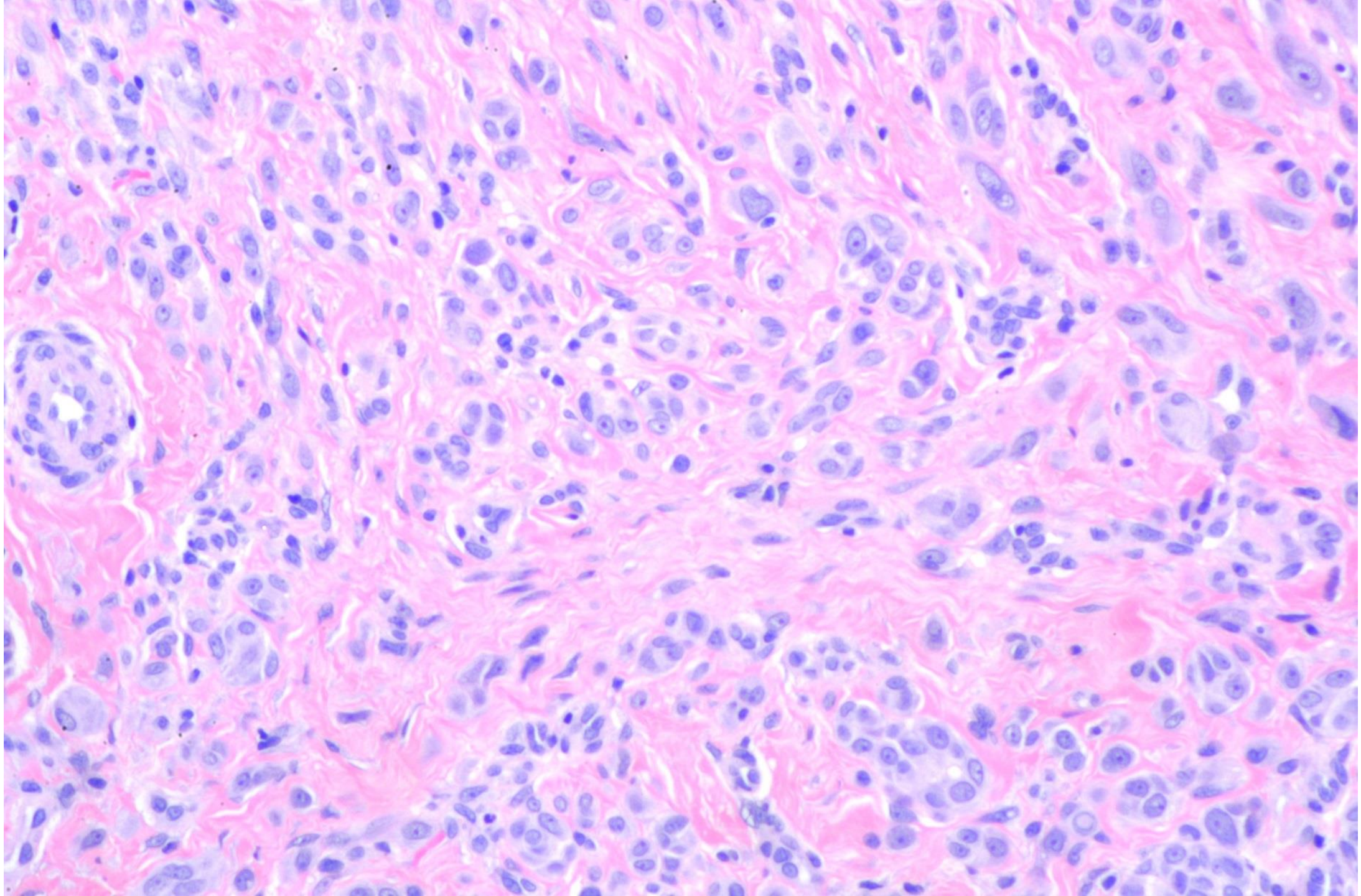
- FISH: Increased 11q13 (CCND1); and 6p25 (RREB); intermediate risk

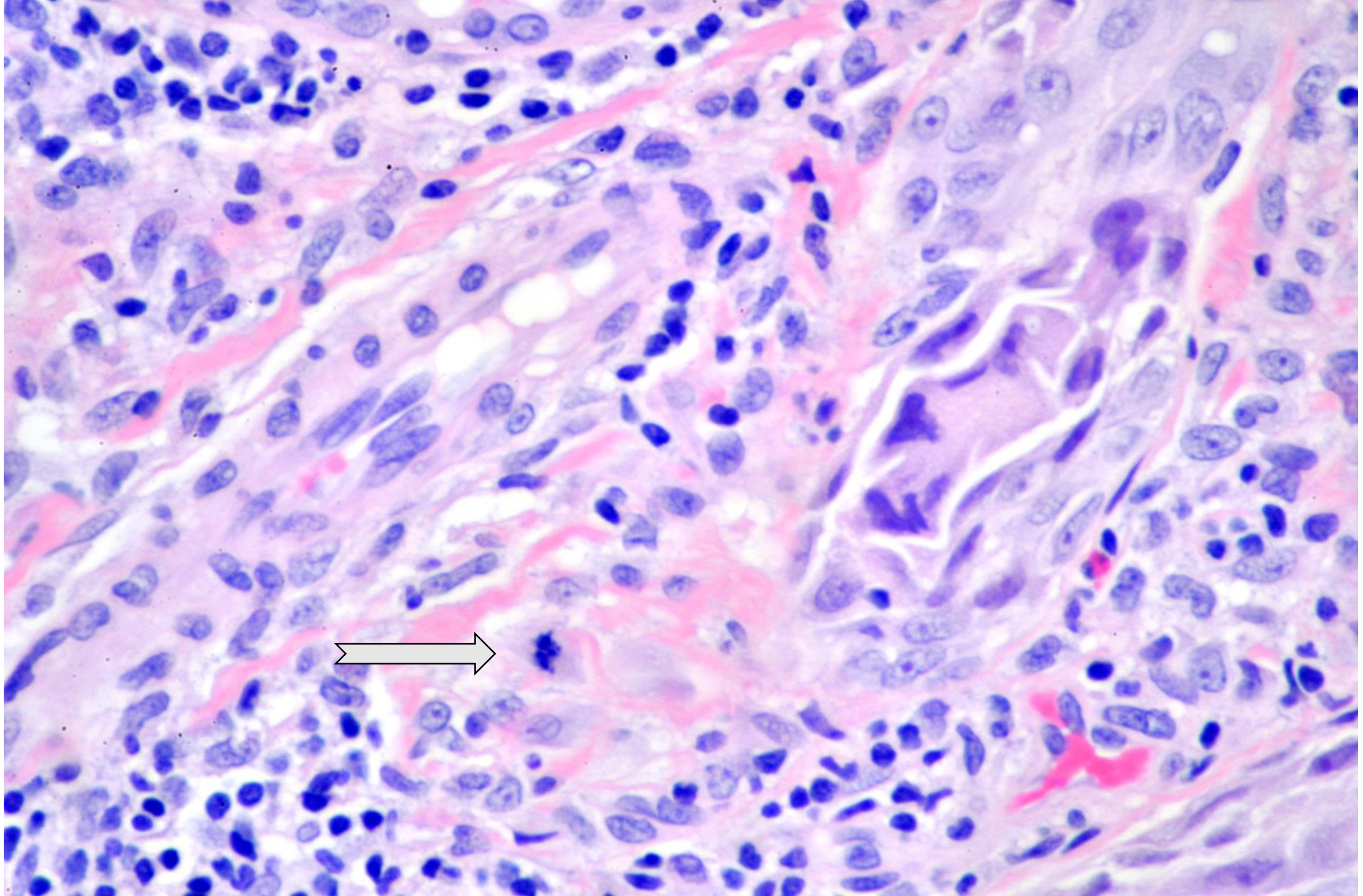
Case 2

- 41 year old female
- Lesion left forearm
- Clinical diagnosis BCC vs poroma









Results *

- FISH: increased 6p25 RREB, intermediate risk
- *

Histologic features predictive of + FISH (Mount Sinai experience)

- Mitoses: 67% vs 12%, P=.01
- Deep mitoses 67% vs 8% p=.005
- Sheets of melanocytes 67% vs 19% p=.04

III. Gene fusions in melanocytic neoplasms

Next generation sequencing in melanocytic neoplasms

- Kinase fusions are the most common
- Translocation is one of many mechanisms for its development
- Fusion transcript results in a chimeric protein
- Results in loss of regulatory domain
- Constitutive activation of kinase
- Activation of downstream pathways
- Usually mutually exclusive of one another and other mutations

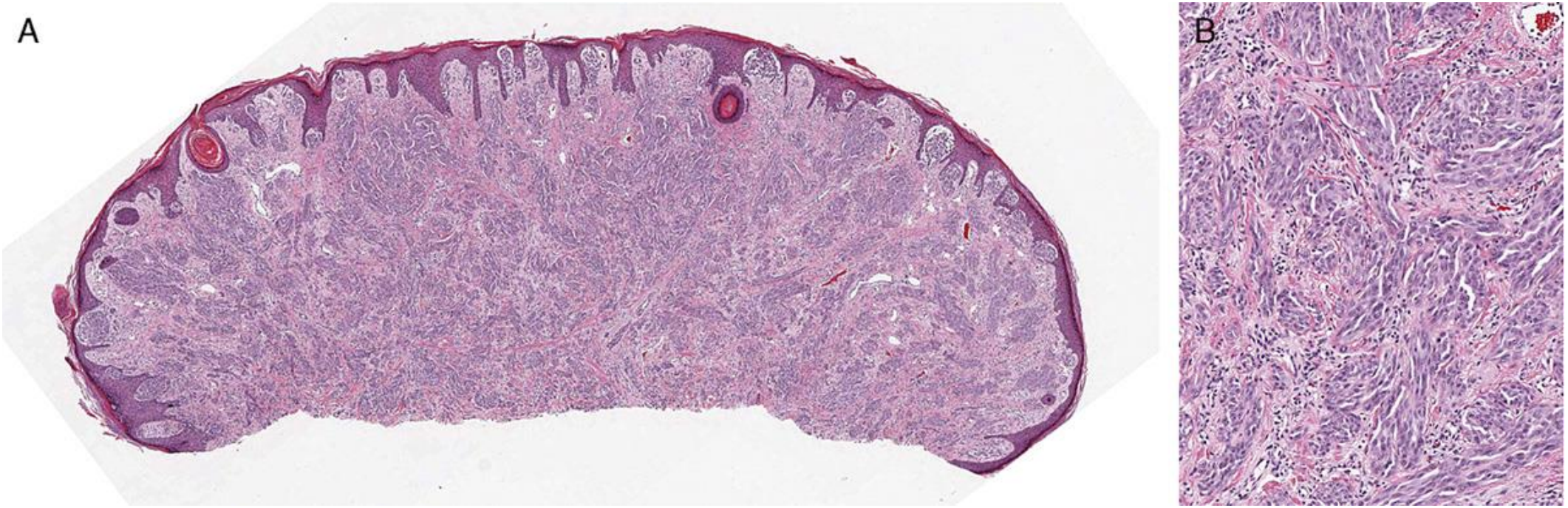
Types of fusions

- ROS1, ALK, RET, BRAF, NTRK1, MET, NTRK3

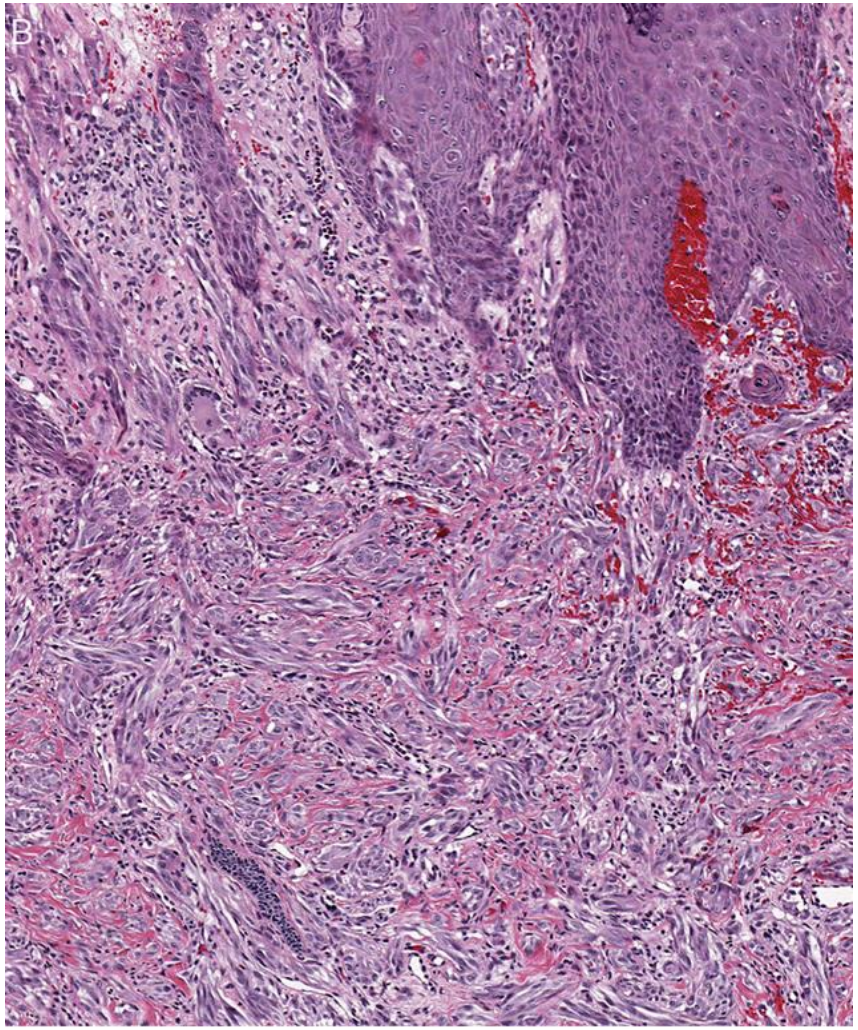
ALK fusion

- Average age 13
- Large size: Average 7.15 mm
- Exophytic verrucous
- Pathology: Large spindle cell, wedge shaped pattern, plexiform

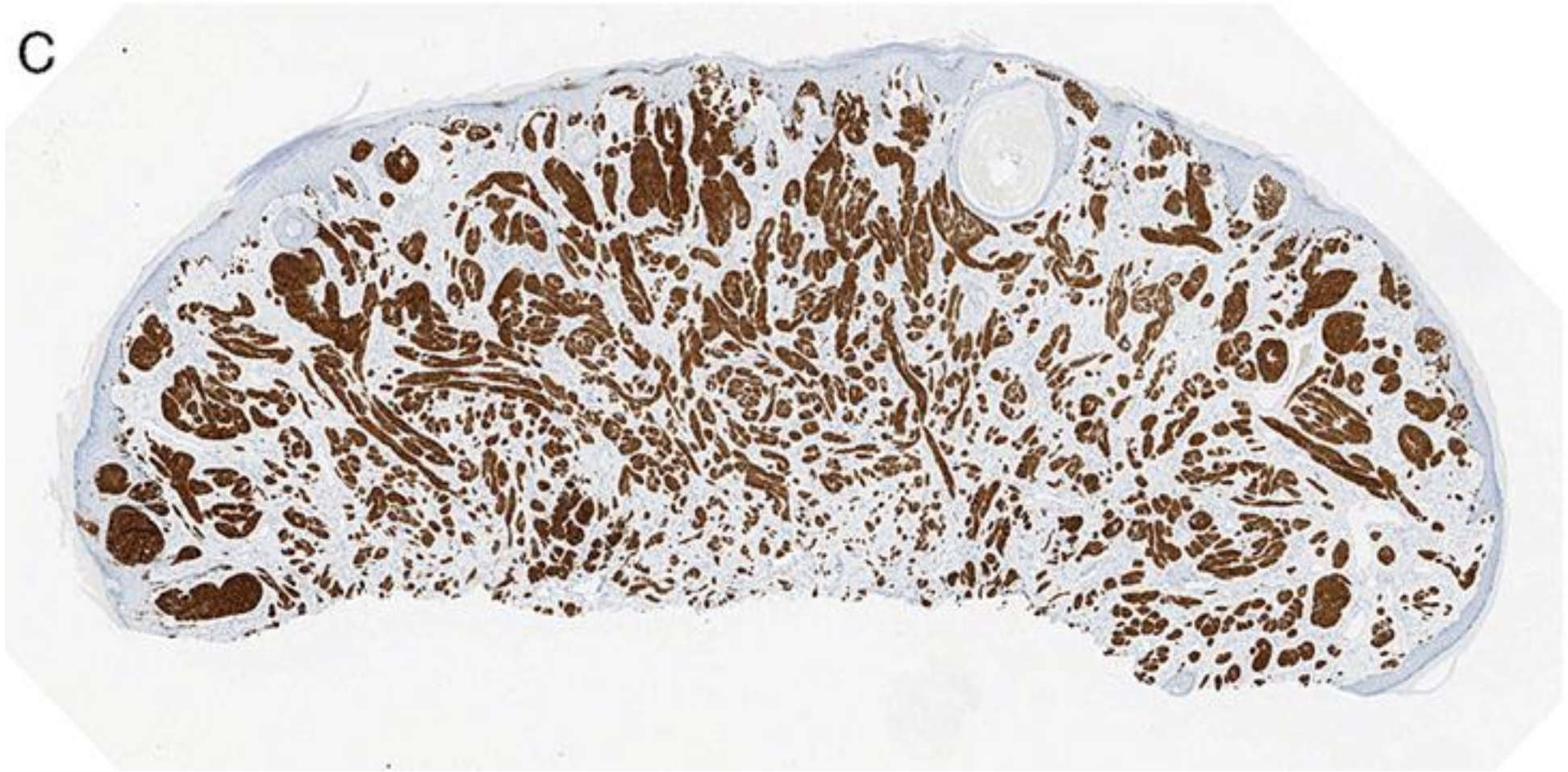
Pathology



Pathology



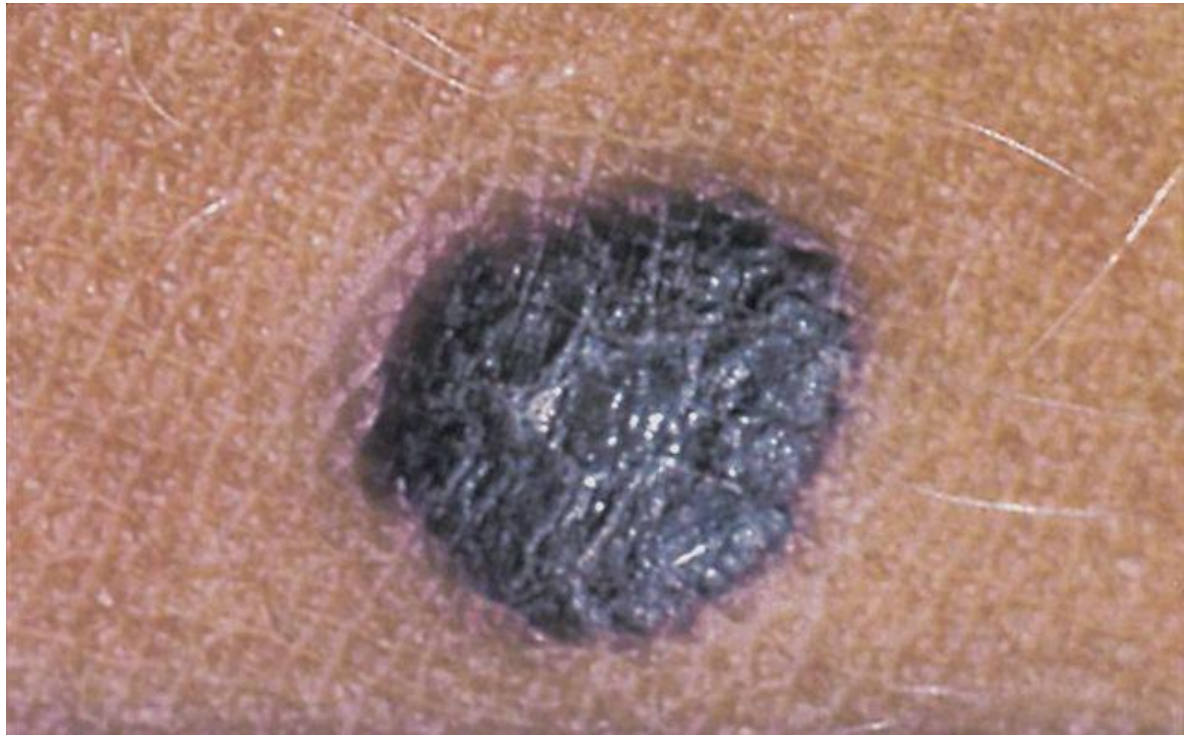
Immunopathology

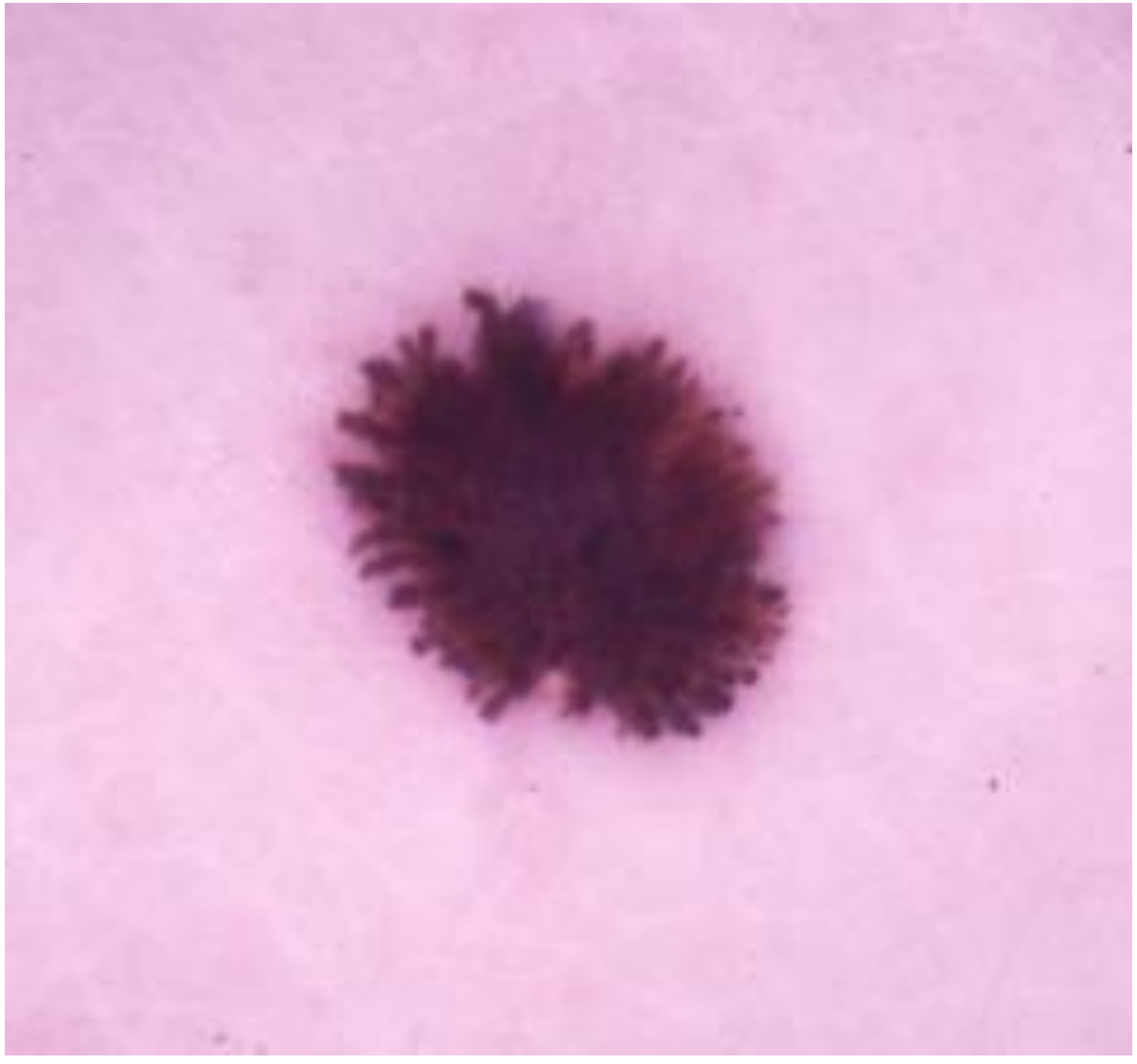


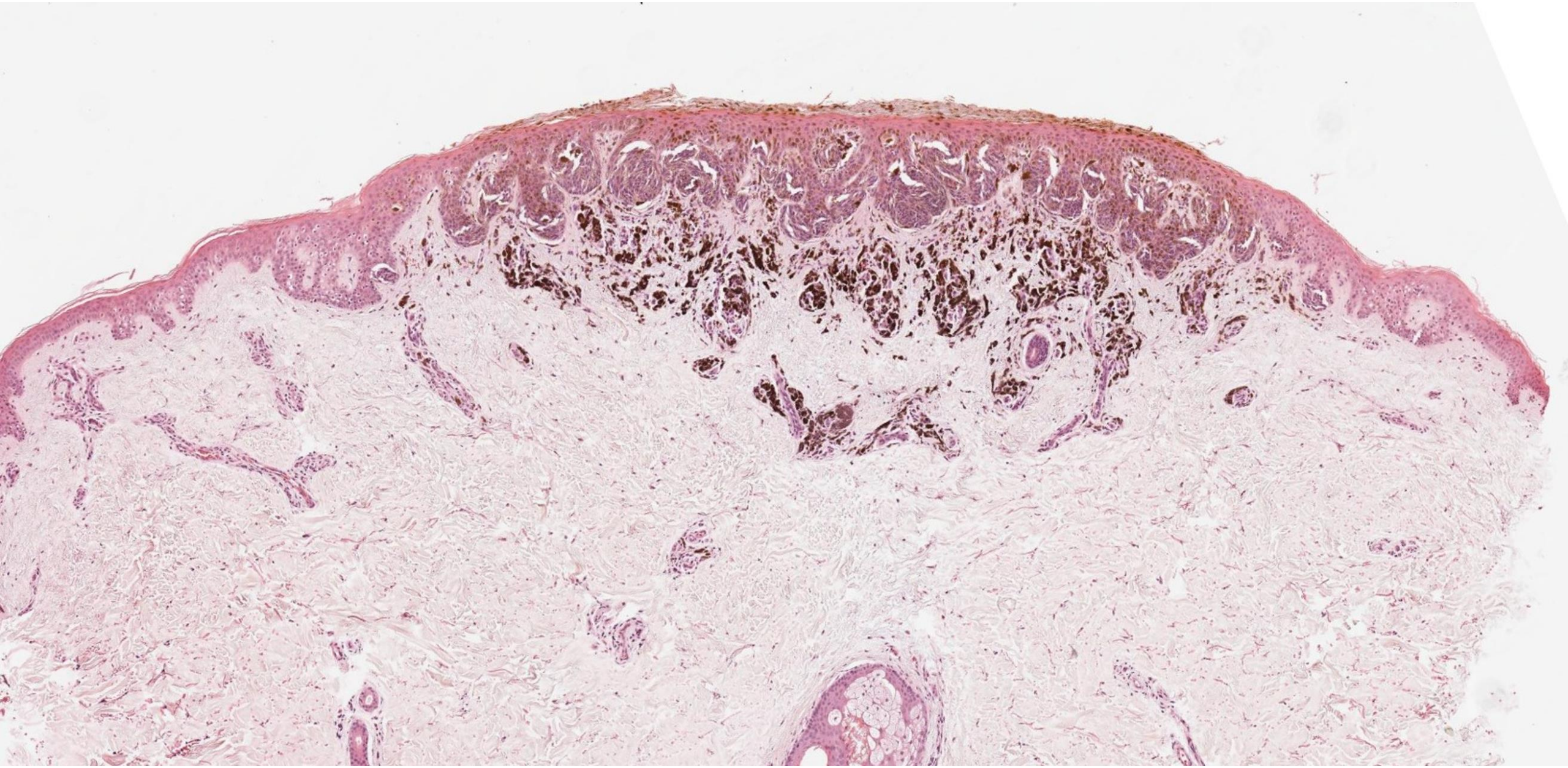
Molecular Genetics

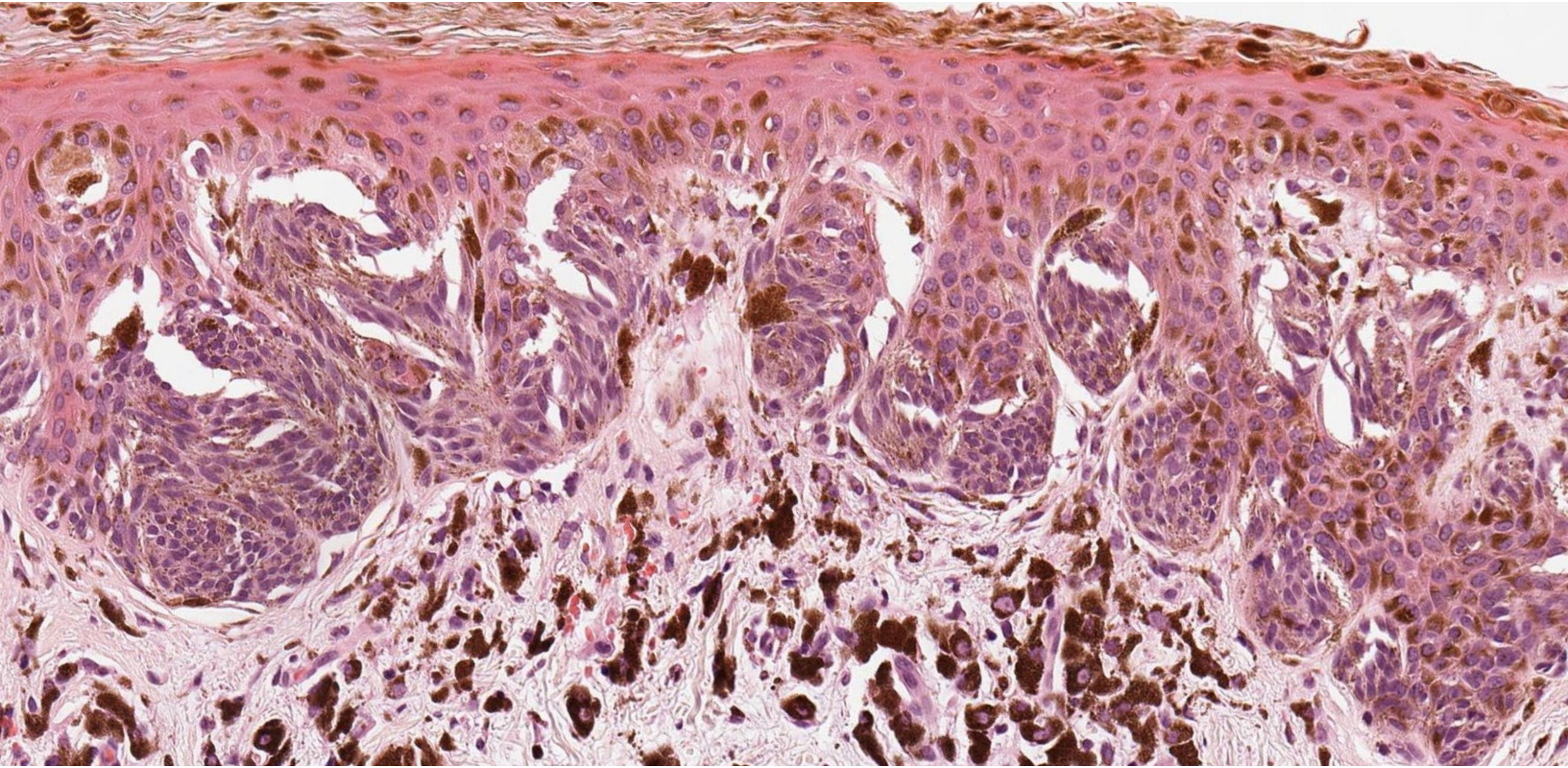
- ALK-TPM3
- ALK-DCTN1
- CGH : no chromosomal changes

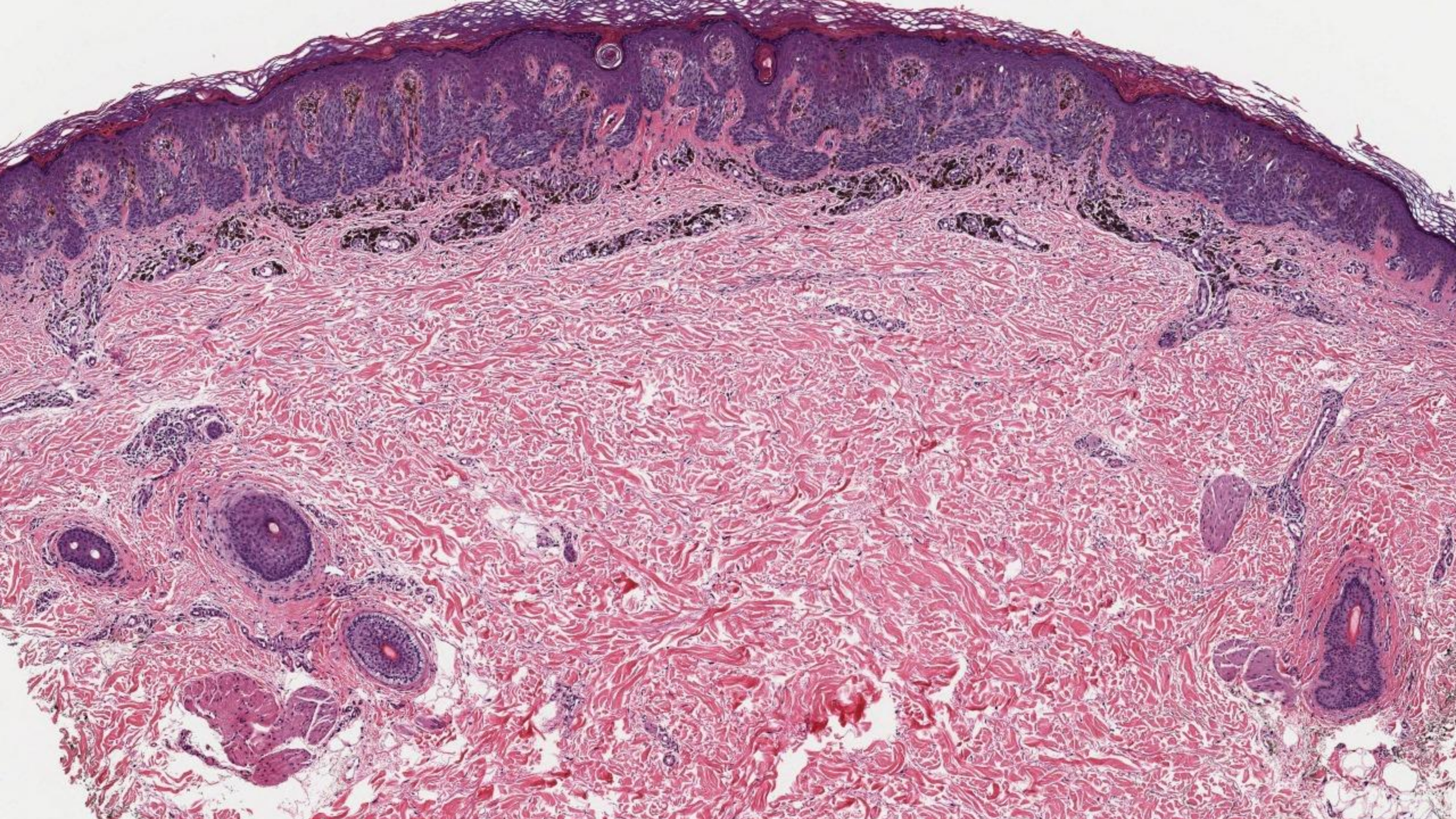
Pigmented spindle cell nevus of Reed: fusion genes











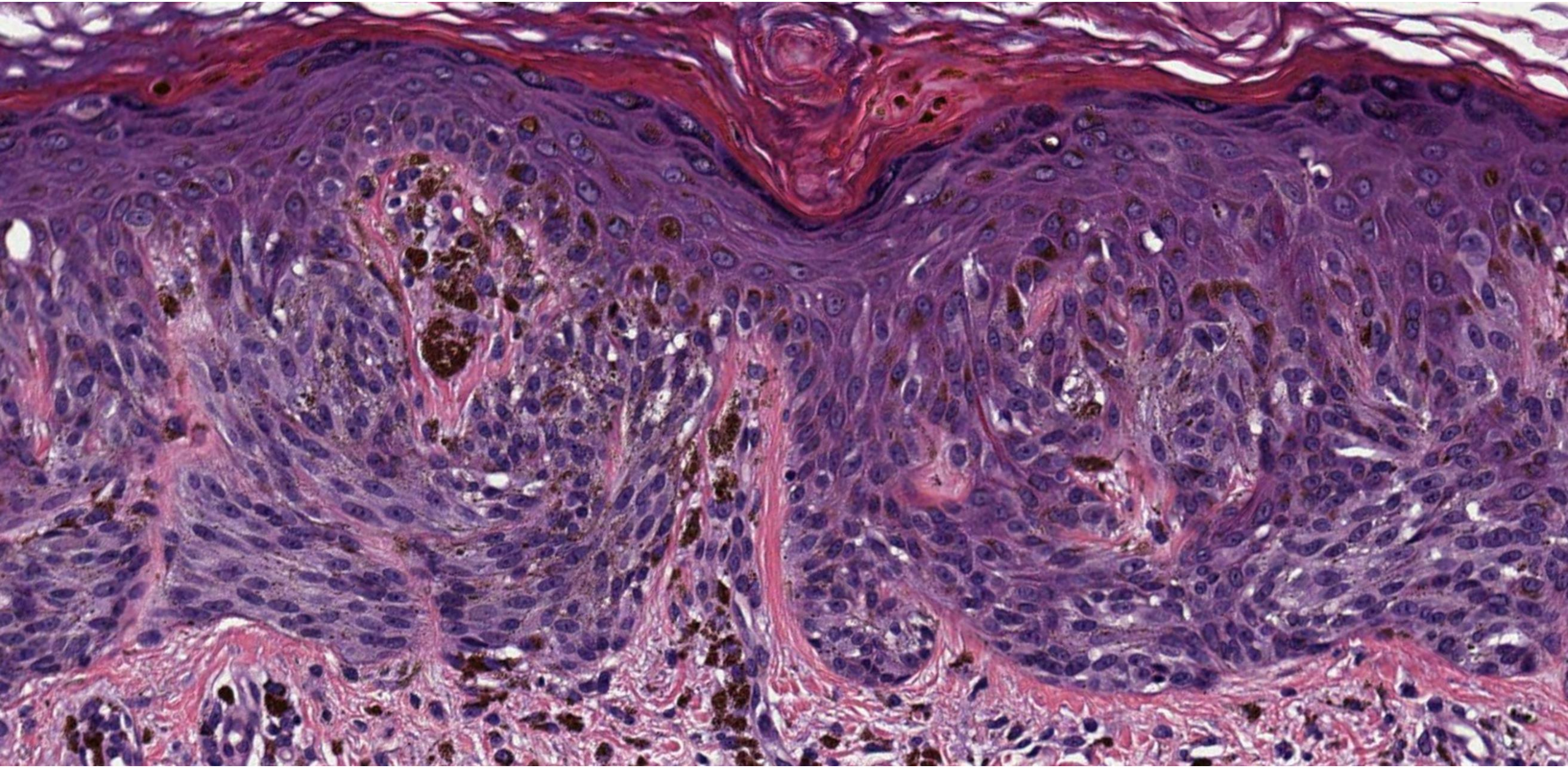
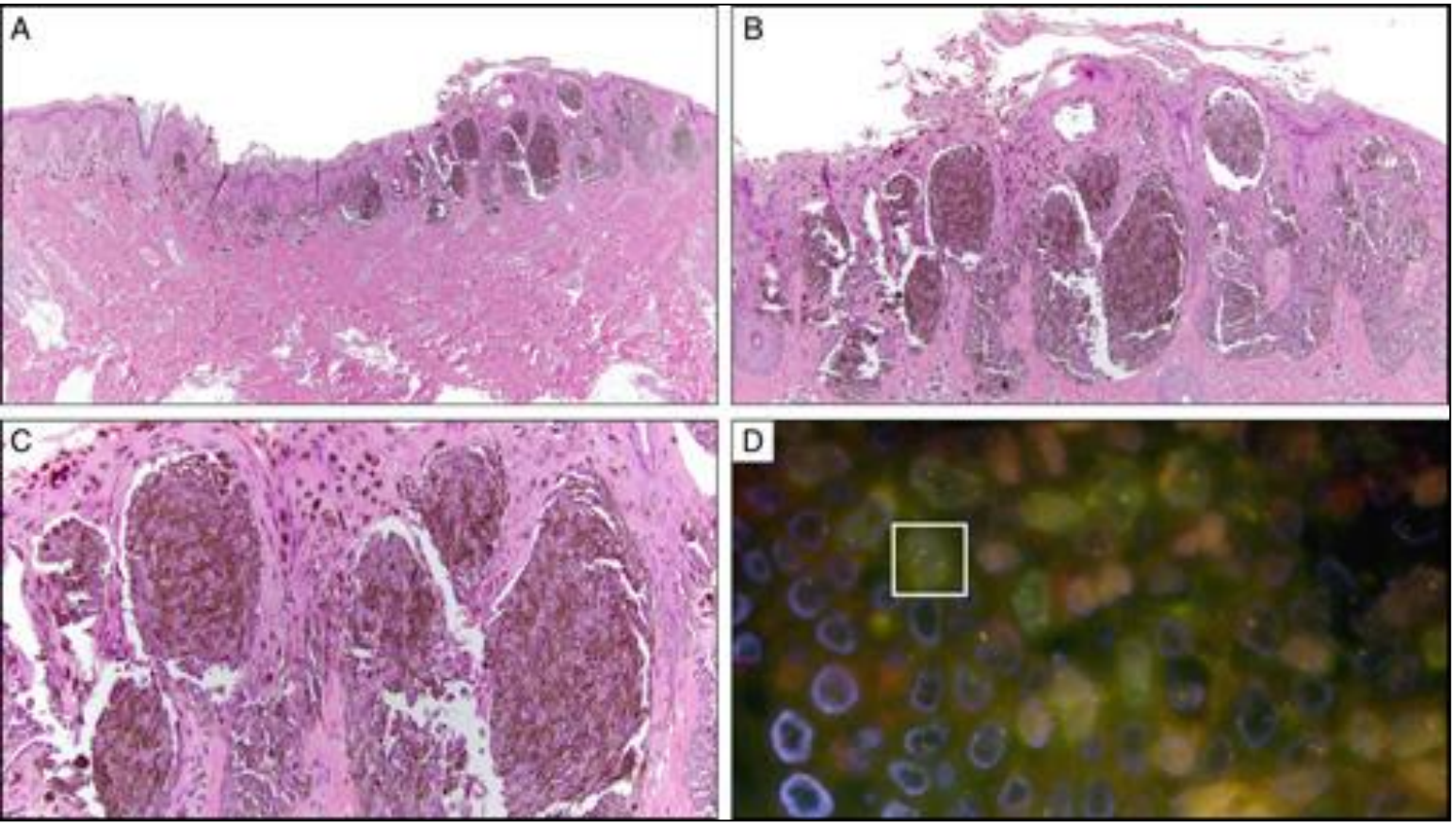


FIGURE 3



Genomic Fusions in Pigmented Spindle Cell Nevus of Reed.

VandenBoom, Timothy; Quan, Victor; Zhang, Bin; Garfield, Erin; Kong, Betty; MD, PhD; Isales, Maria; MD, MPH; Panah, Elnaz; Igartua, Catherine; Taxter, Timothy; Beaubier, Nike; White, Kevin; Gerami, Pedram

American Journal of Surgical Pathology. 42(8):1042-1051, August 2018.
DOI: 10.1097/PAS.0000000000001074

FIGURE 3 . NTRK3 fusion PSCN of Reed (hematoxylin and eosin). A, Low-power view showing a well-circumscribed melanocytic lesion encased by epidermal hyperplasia. B, Expansile, heavily pigmented nests with prominent clefting from the adjacent epidermis. C, Highly spindled, heavily pigmented melanocytes without significant nuclear atypia. D, NTRK3 FISH break-apart probe showing separation of green and red signals, confirming NTRK3 fusion status. The white box highlights a representative cell showing separation of 5' and 3' ends of NTRK3.

TABLE 1

Case	Diagnosis	Age (y)	Sex	Location	Fusion	Silhouette	Cell Size	Cytologic Atypia
1	Tumor of Reed	2	M	Canthus, left lateral	MYO5A-NTRK3	Dome-shaped	Large; epithelioid component	Moderate
2	PSCN of Reed	30	F	Arm, right upper	MYO5A-NTRK3	Plaque-like	Small	Moderate
3	PSCN of Reed	35	M	Thigh, left posterior	MYO5A-NTRK3	Plaque-like	Small	Mild
4	PSCN of Reed	8	M	Leg, right lower	MYO5A-NTRK3	Plaque-like	Large	Moderate
5	PSCN of Reed	27	F	Thigh, right	MYO5A-NTRK3	Plaque-like	Large	Moderate
6	PSCN of Reed	39	F	Thigh, right	MYO5A-NTRK3	Plaque-like	Large	Moderate
7	PSCN of Reed	9	F	Knee, left	MYO5A-NTRK3	Plaque-like	Large	Moderate
8	PSCN of Reed	6	F	Thigh, left	MYO5A-NTRK3	Plaque-like	Large	Moderate
9	PSCN of Reed	17	F	Trunk, left	MYO5A-NTRK3	Plaque-like	Small	Moderate
10	PSCN of Reed	26	M	Shoulder, right anterior	MYO5A-NTRK3	Plaque-like	Large	Moderate
11	PSCN of Reed	2	M	Helix, right	MYO5A-NTRK3	Plaque-like	Large	Moderate
12	PSCN of Reed	13	F	Neck, right	ETV6-NTRK3	Plaque-like	Large	Moderate
13	PSCN of Reed	28	M	Back, mid	ETV6-NTRK3	Plaque-like	Small	Mild
14	PSCN of Reed	34	M	Back, left lower	MYO5A-MERTK	Plaque-like	Small	Moderate
15	PSCN of Reed	29	F	Arm, right upper	MYO5A-MERTK	Plaque-like	Large	Moderate
16	PSCN of Reed	30	F	Arm, left	MYO5A-ROS1	Plaque-like	Large	Moderate
17	PSCN of Reed	62	F	Thigh, right lateral	MYO5A-RET	Plaque-like	Small	Mild
18	PSCN of Reed	27	F	Elbow, left	ETV6-PITX3	Plaque-like	Large	Moderate
19	PSCN of Reed	39	F	Arm, left upper lateral	—	Plaque-like	Large	Moderate
20	PSCN of Reed	33	F	Arm, left upper	—	Plaque-like	Large	Moderate
21	PSCN of Reed	23	F	Wrist, right	—	Plaque-like	Large	Moderate
22	PSCN of Reed	33	F	Forearm, left	—	Plaque-like	Large	Severe
23	PSCN of Reed	26	F	Hip, right	—	Plaque-like	Large	Moderate
24	Spitz tumor	18	M	Mandible, right	MYO5A-NTRK3	Plaque-like	Small	Moderate
25	Spitz nevus	41	F	Thigh, left	MYO5A-NTRK3	Dome-shaped	Small	Mild

F indicates female; M, male.

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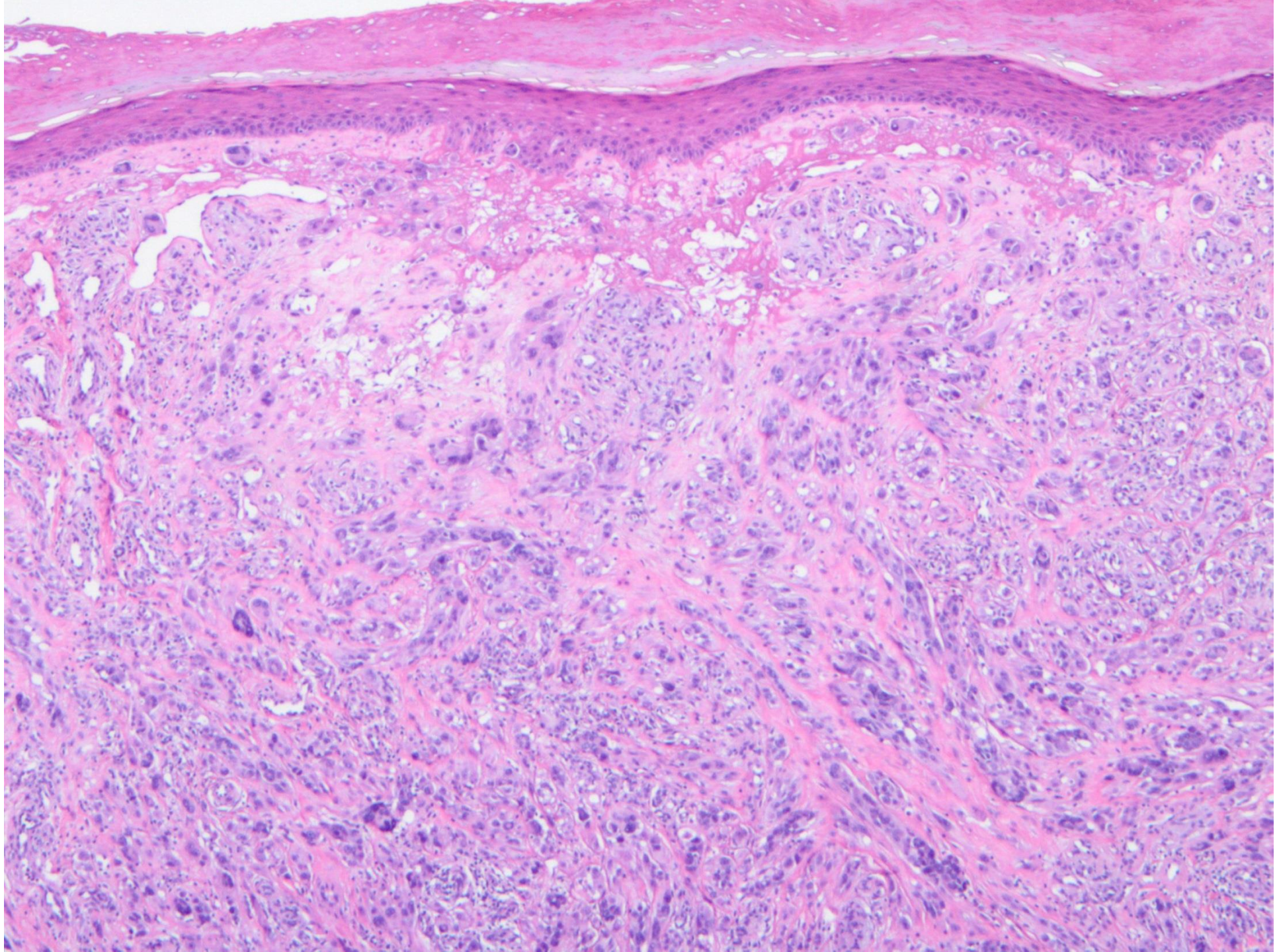
TABLE 1 Clinical and Histopathologic Characteristics of PSCN of Reed and NTRK3-positive Melanocytic Neoplasms

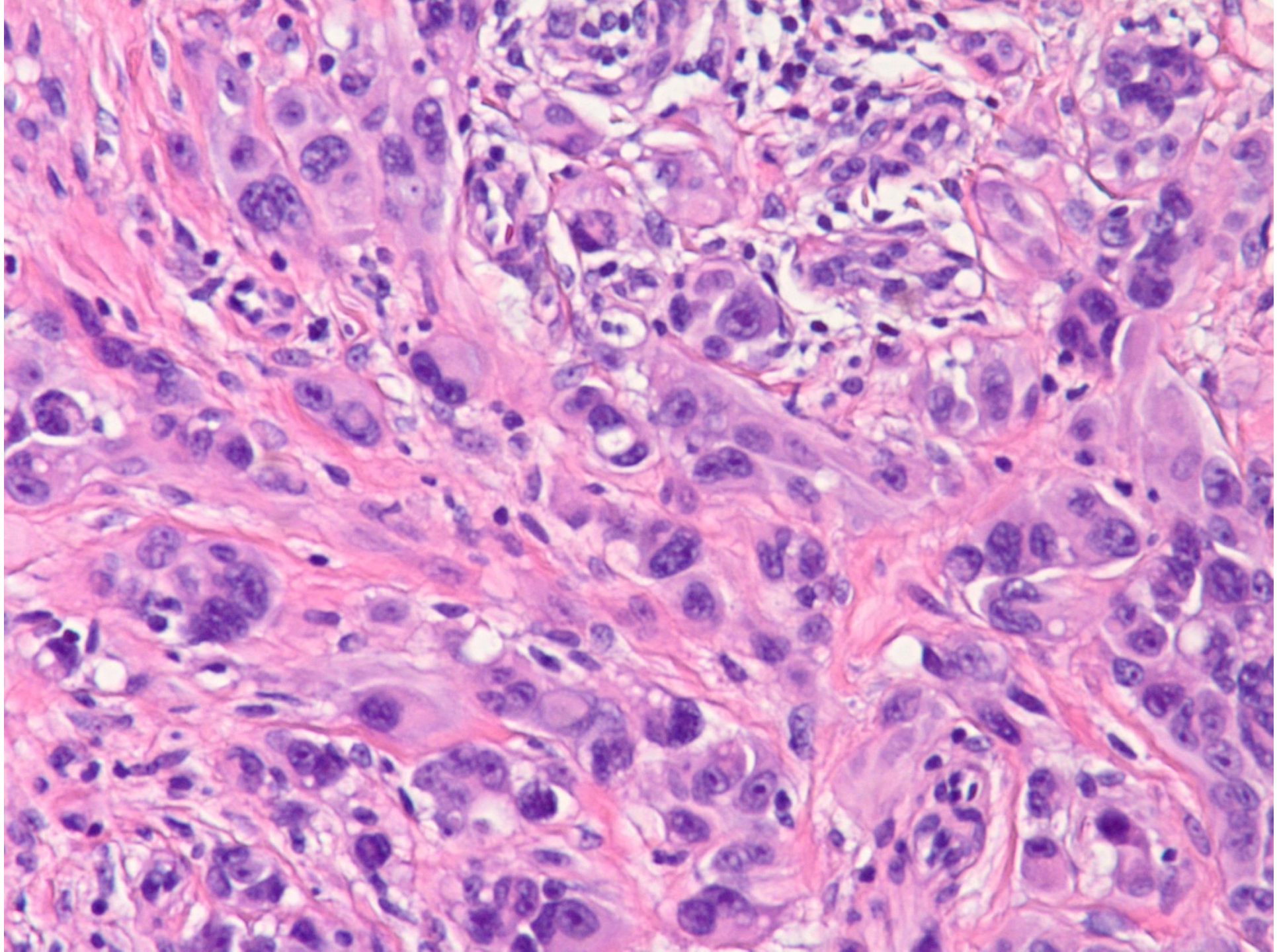
Mechanism

- NTRK3 (neurotrophic tyrosine receptor kinase) promotes proliferation and migration of melanocytes
- Downstream activation of other genes AKT, MAPK

MAPK genes

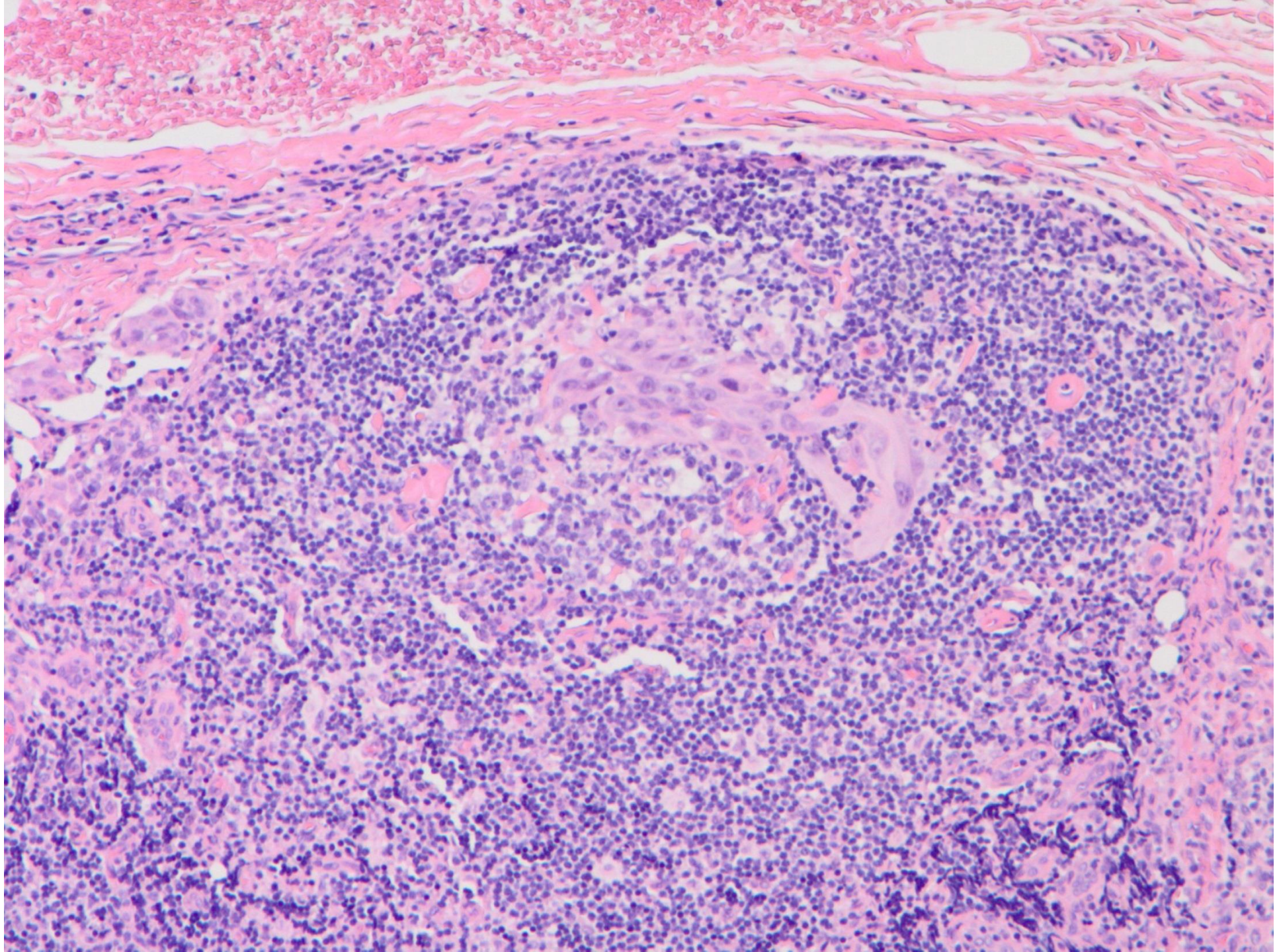
- In 9-33% of Spitz tumors; MAP3K3, MAP3K8, andMAP2K1
- Single most important driver of Spitz melanomas
- Up to fifty % of this fusion type are Spitz melanomas or AST
- In younger patients (median 18)

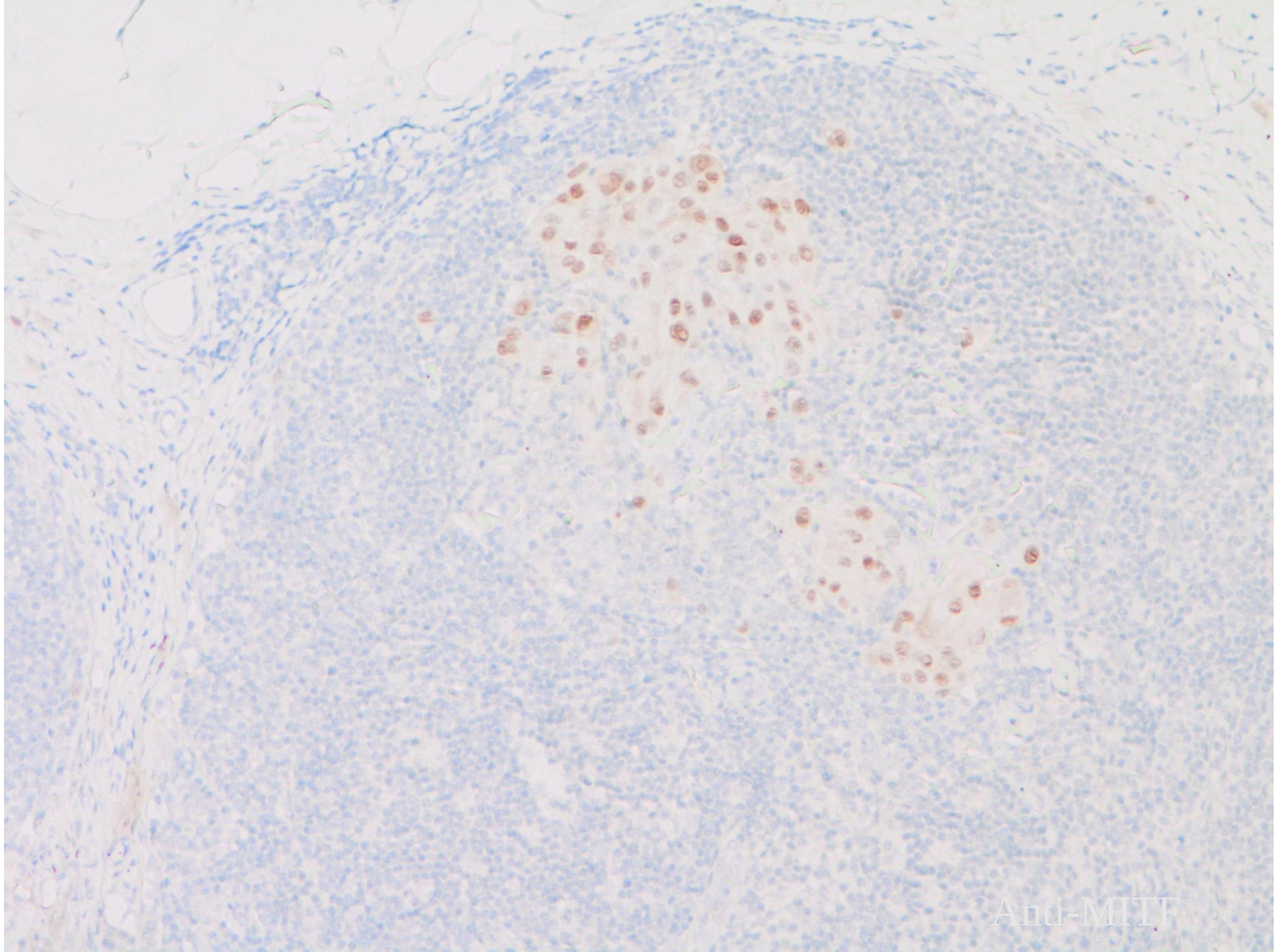




Histology

- Epithelioid
- High grade nuclear atypia
- Poor maturation
- Mitotic figures
- Syncytial type cells




Anti-MITF

Prognosis

- Cases diagnosed as melanoma had involvement of sentinel lymph nodes
- Complete lymph node dissection
- Follow up at nine months no evidence of disease
- One with recurrence at 10 months

Fusion genes/proteins

- Many others: *ROS1*, *NTRK1,* MET, and *RET* or the serine-threonine kinase *BRAF.* Likely others
- Expression is mutually exclusive
- Active site is maintained with constitutive activation by second part of fusion; loss of regulatory element
- Some types are much more commonly associated with malignancy (BRAF).

IV. Gene panel

Gene expression

- 182 archived melanocytic lesions four institutions
- Cases selected by pre-defined clinical outcome
  - Malignant: Distant mets beyond sentinel lymph node, originally stage I-III
  - Benign lesions long term no adverse outcome
- Sensitivity and specificity and sensitivity 93.8 and 96.2 %

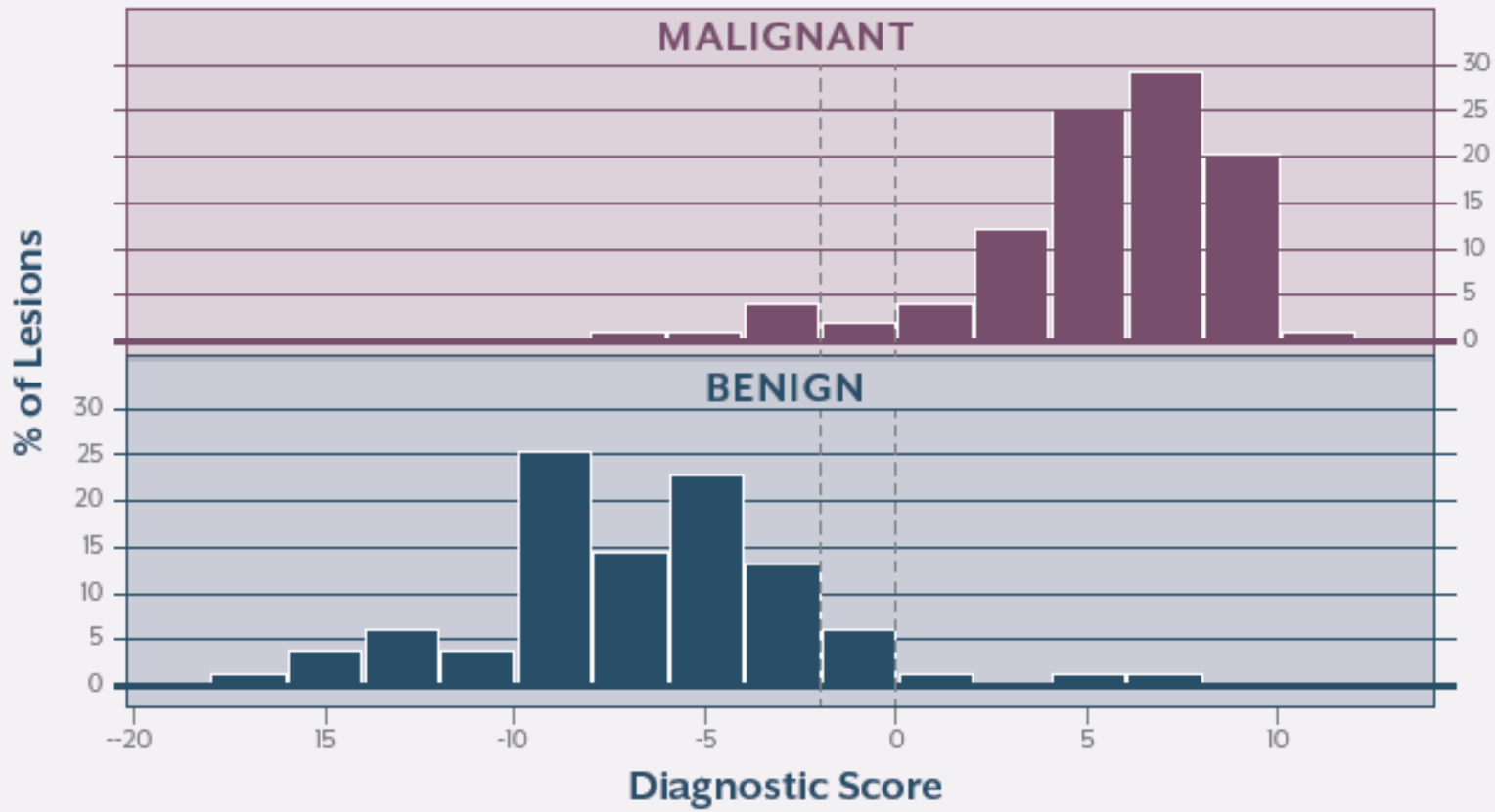
RNA in FFPE, qRT-PCR

- 23 genes (from website)
  - PRAME a single gene involved in cell differentiation
  - S100A9 S100A7, S100A8, S100A12, P13a group of genes involved in multiple cell signaling pathways
  - CCL5, CD38, CXCL10, CXCL9, IRF1, LCP2, PTPRC and SELL involved in tumor immune response signaling
  - 9 Housekeeping genes measured to normalize RNA expression for analysis

Scoring system

- Weighted algorithm (from prior validation studies)
- Scores given
  - -16.7 to 2.1 benign
  - -2.0 to 0.1 indeterminate
  - 0.0 to 11.1 malignant

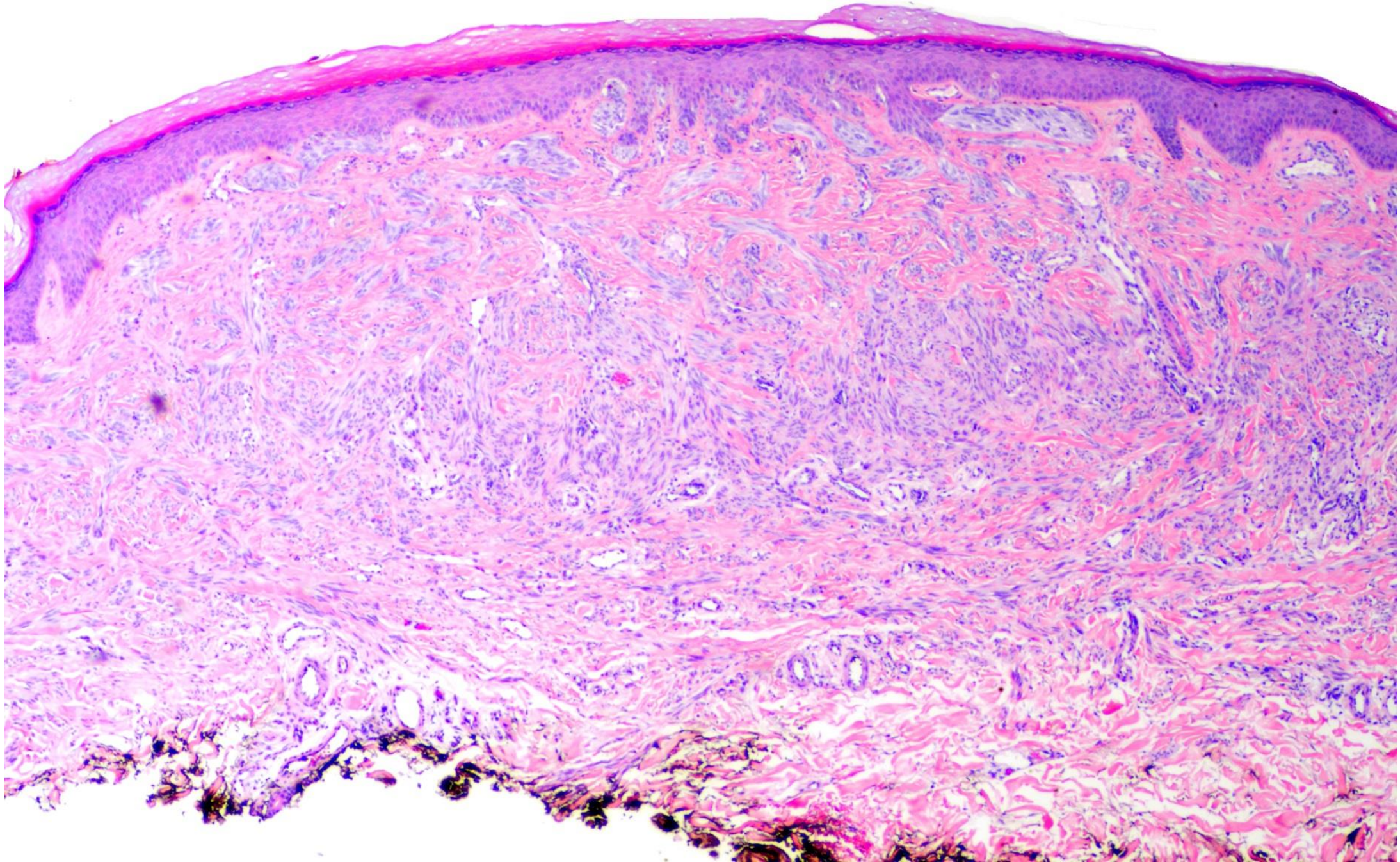
Figure 1. Score distribution

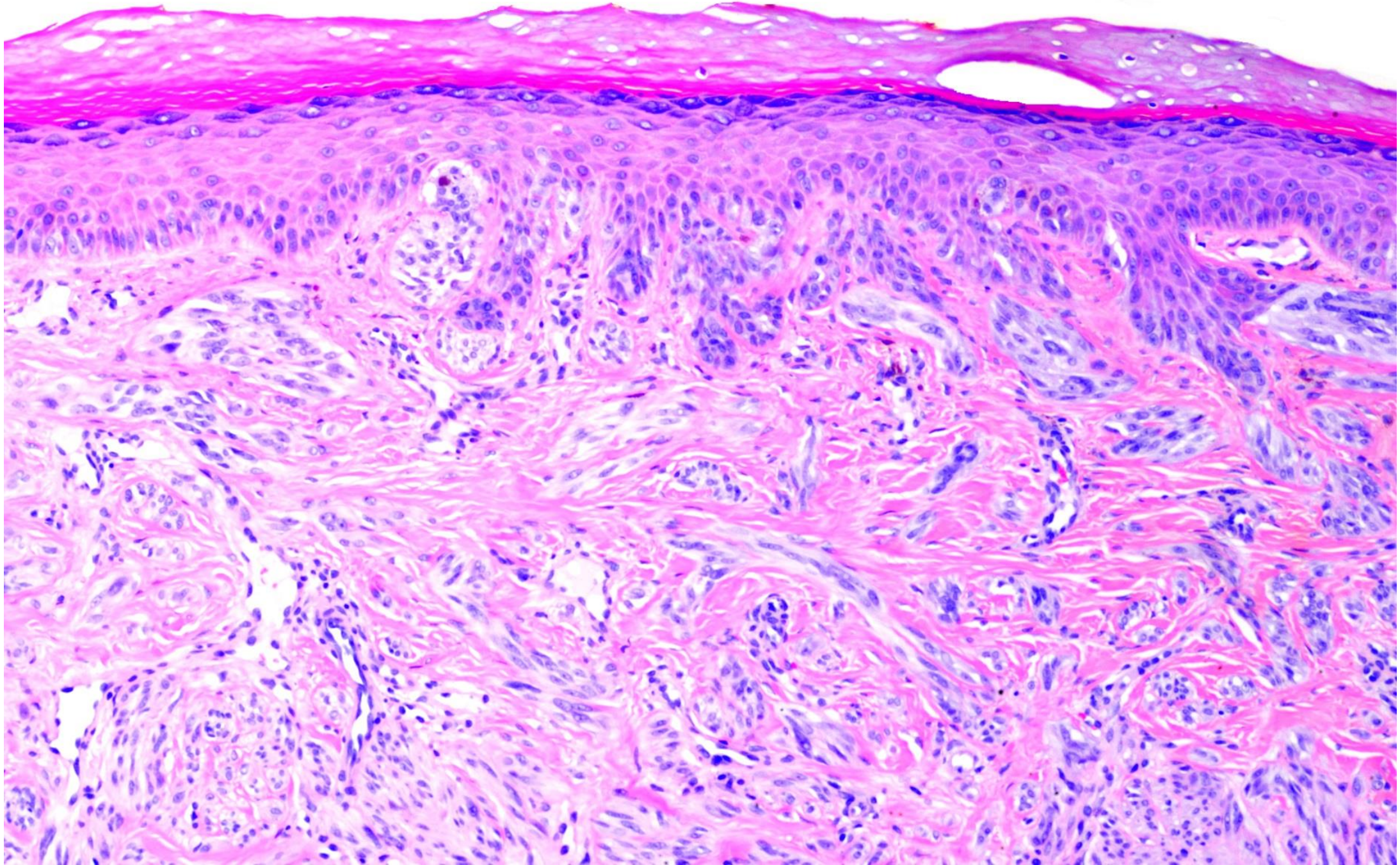


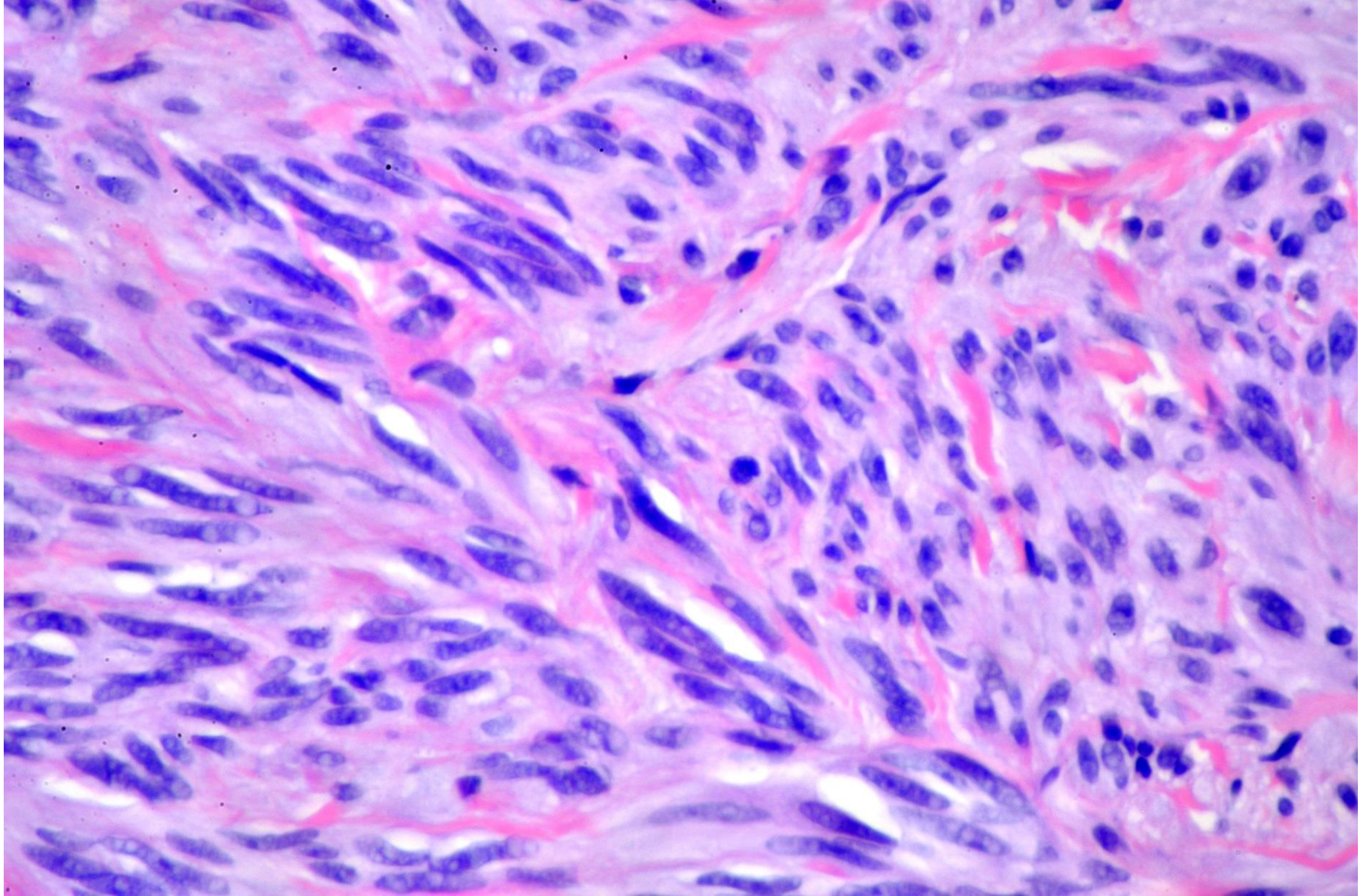
Myriad My Path: Mount Sinai

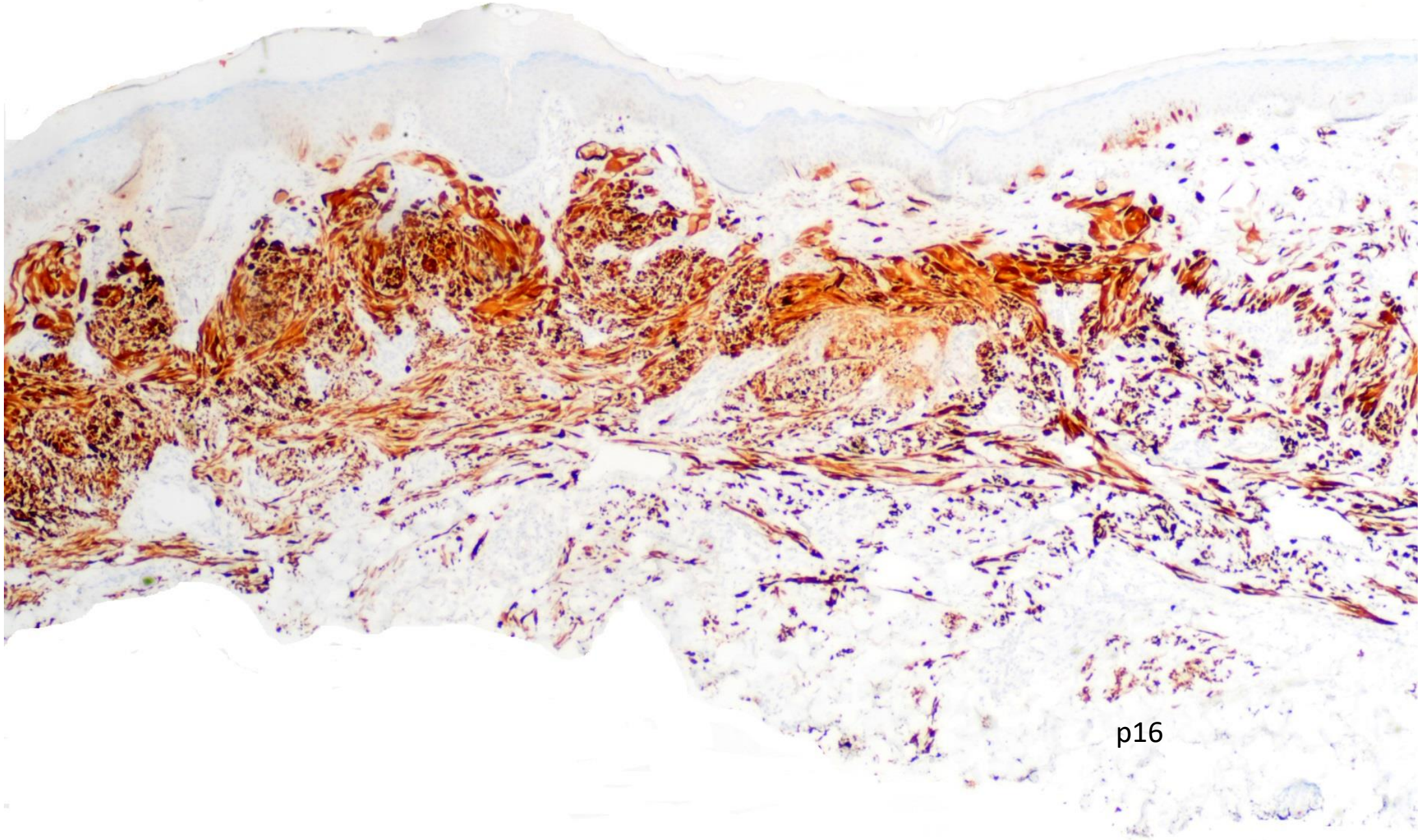
- Concordance with histologic diagnosis in almost all cases
- Concordance with FISH in almost all cases

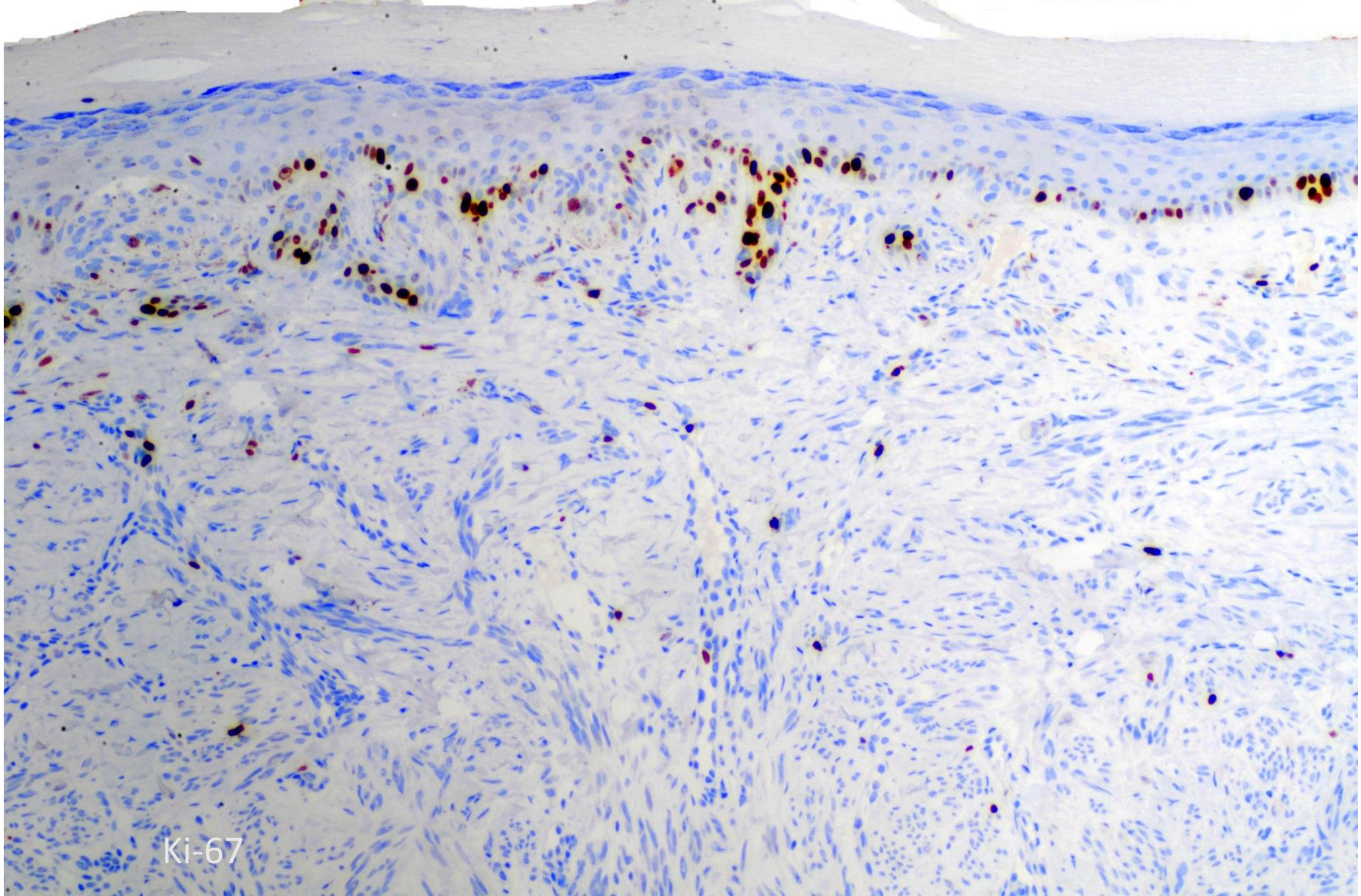
26 year old lesion left wrist

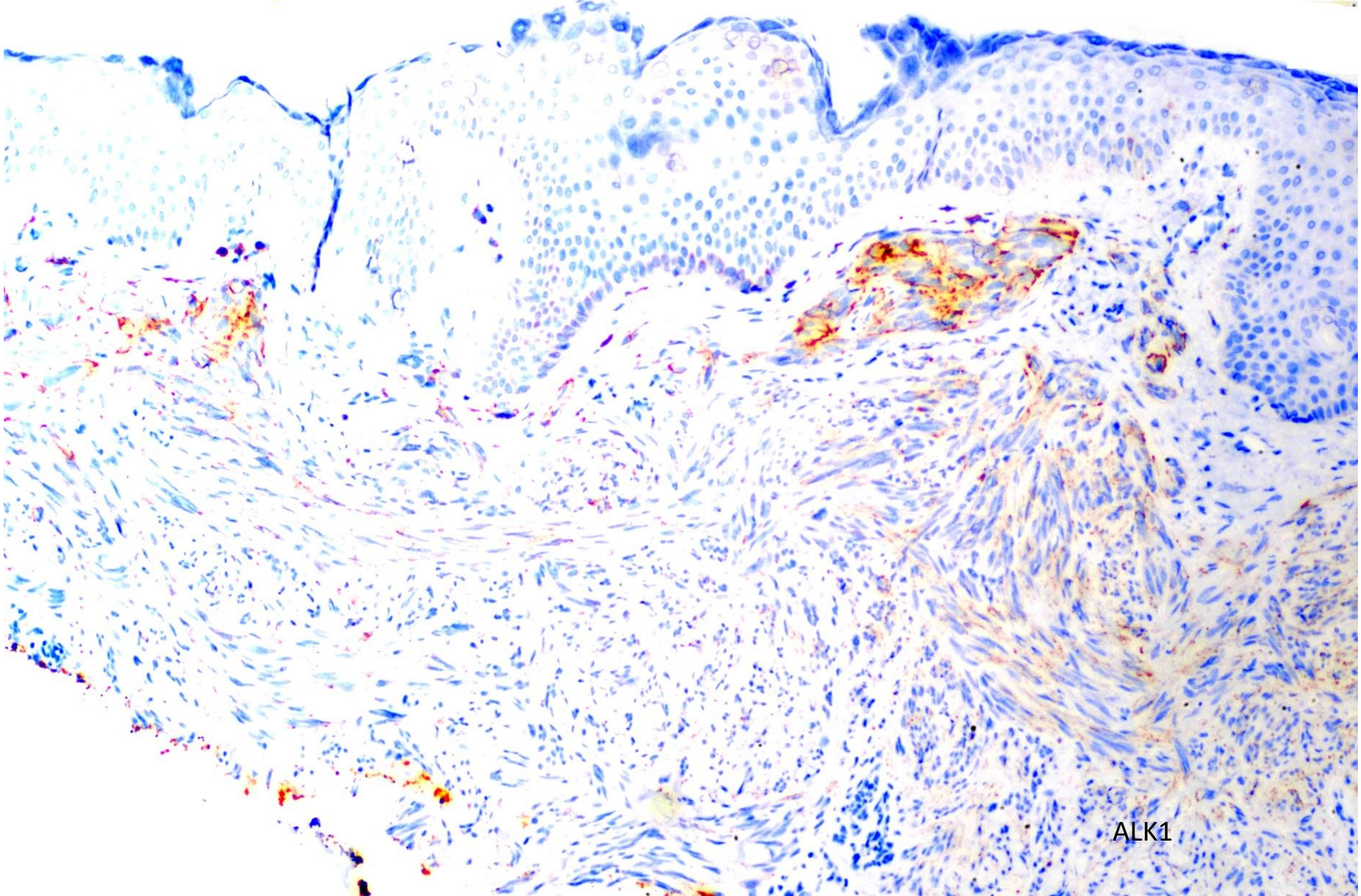






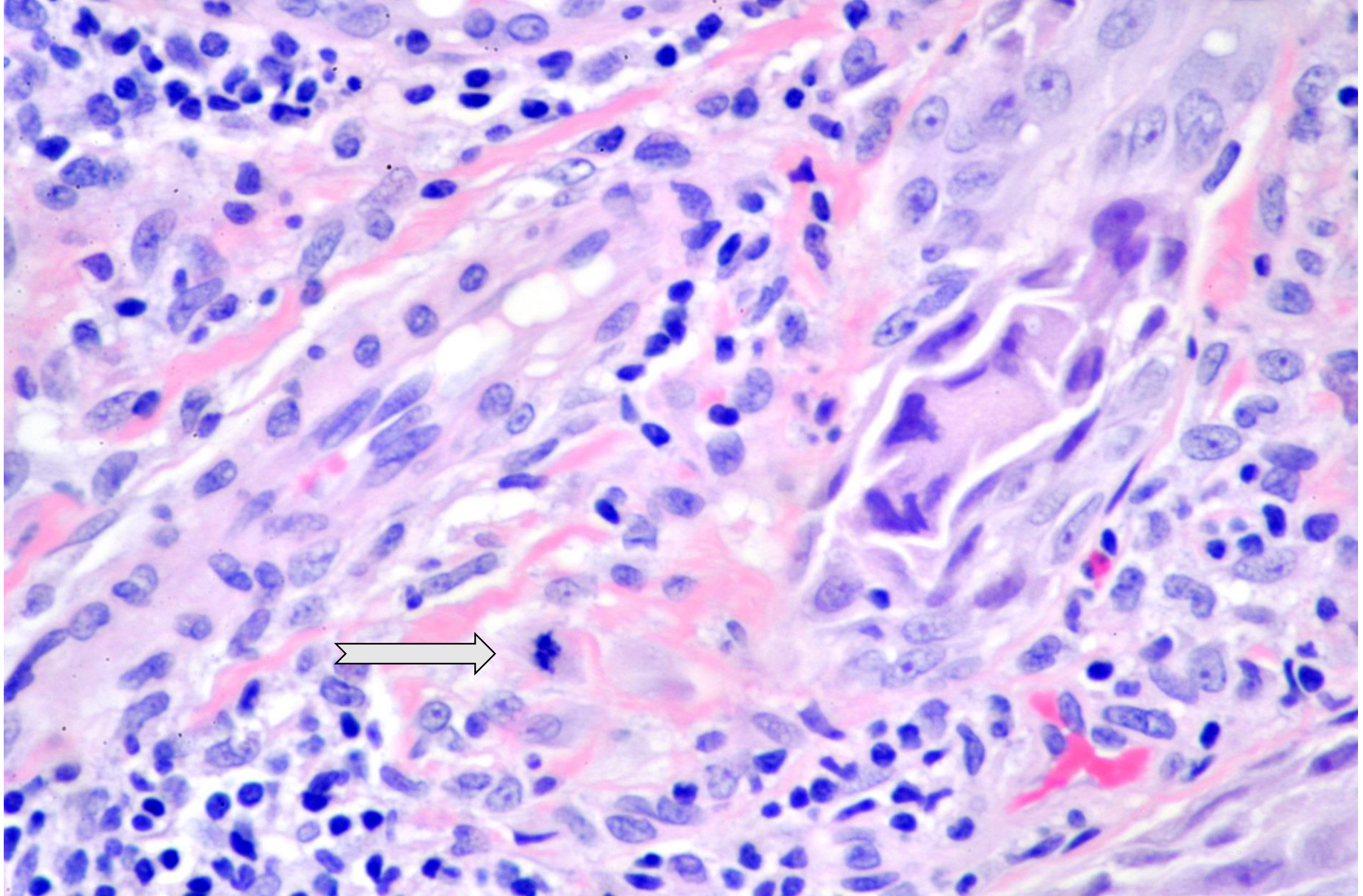

p16


Ki-67


ALK1

Results

- IHC: P16 (+), ALK1 (+) –favors benign/atypical
- Cytogenetics: 6q23 loss (MYB), low risk lesion , 50% sentinel node only, infrequent in other areas non sentinel, in transit or distant mets
- Myriad: -10.5 (Benign)



Results Case 2*

- FISH: increased 6p25 RREB, intermediate risk
- Gene profile: -3.8 (benign)*

Is there one single test that can help discriminate between a melanoma and nevus?

V. PRAME

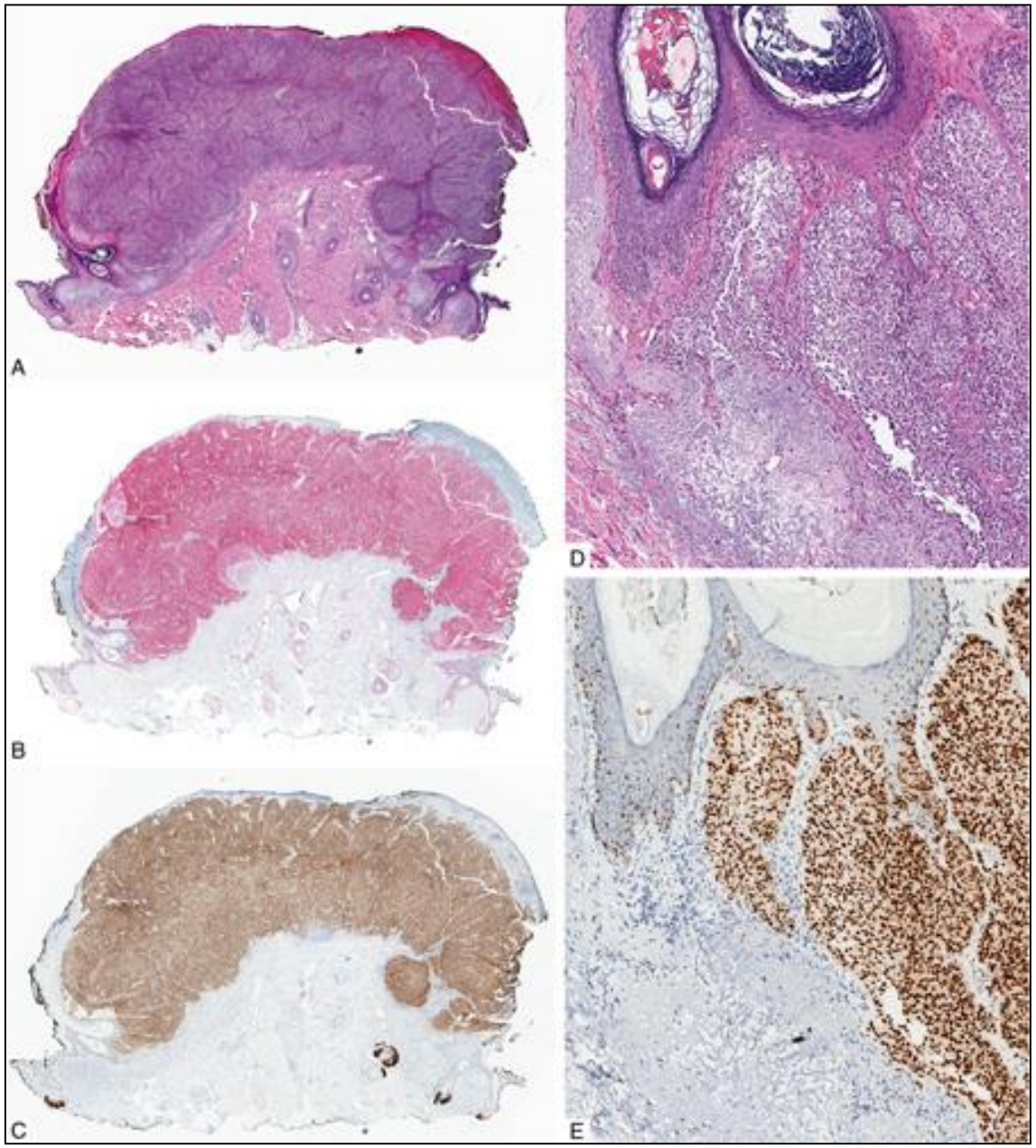
PRAME

- PReferentially expressed Antigen in MElanoma
- Antigen first recognized in melanoma cell lines by cytolytic T cells (1997)
- Present in many other types of tumors
- Normally expressed in testis but not significantly in other tissues
- Hence, used in gene expression assays (myPath Melanoma, DecisionDx ®-PRAME, DermTech) and now by immunohistochemistry

Materials and Methods (first study)

- Commercially available rabbit monoclonal antibody
- Abcam Anti-PRAME antibody [EPR20330] (ab219650)
- Tested on nevi (145) and (255) melanoma of all types, primary and metastatic (one study)
- Positive only if 4+ staining nuclear

FIGURE 2



PRAME Expression in Melanocytic Tumors.
Lezcano, Cecilia; Jungbluth, Achim; Nehal, Kishwer; Hollmann, Travis; MD, PhD; Busam, Klaus

American Journal of Surgical Pathology. 42(11):1456-1465, November 2018.
DOI: 10.1097/PAS.0000000000001134

FIGURE 2 . Primary melanoma. A, Ulcerated polypoid tumor from the neck of a 73-year-old man. B, IHC for Sox10: the tumor cells are homogenously immunoreactive for Sox10. C, IHC for PRAME: the tumor cells diffusely express PRAME. D, Melanoma is present in both the epidermis and dermis (H&E-stained section). E, IHC for PRAME highlights both in situ and invasive tumor cells.

FIGURE 4

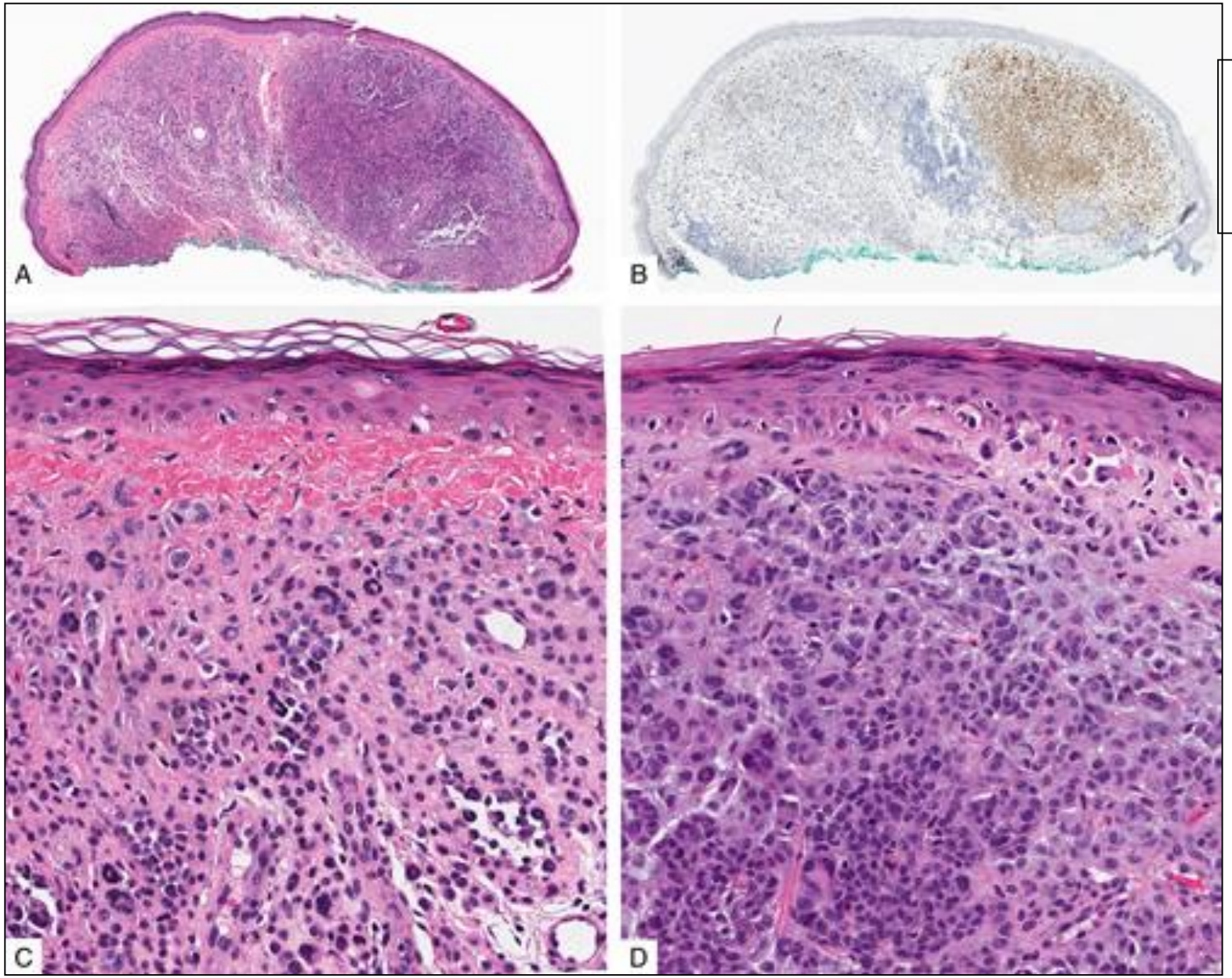


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FIGURE 4 . Melanoma associated with a melanocytic nevus in the ear of a 63-year-old man. A, Nodular silhouette of the lesion with a more densely cellular tumor cell population on the right side of the lesion. B, IHC for PRAME stains only the densely cellular nodule. C, The less cellular area shows cytologic features of a melanocytic nevus. D, The PRAME-positive tumor cells are cytologically atypical. Cytogenetic analysis of the tumor cells revealed a number of chromosomal aberrations, including loss of 9p and gain of 8q (not shown).

FIGURE 6

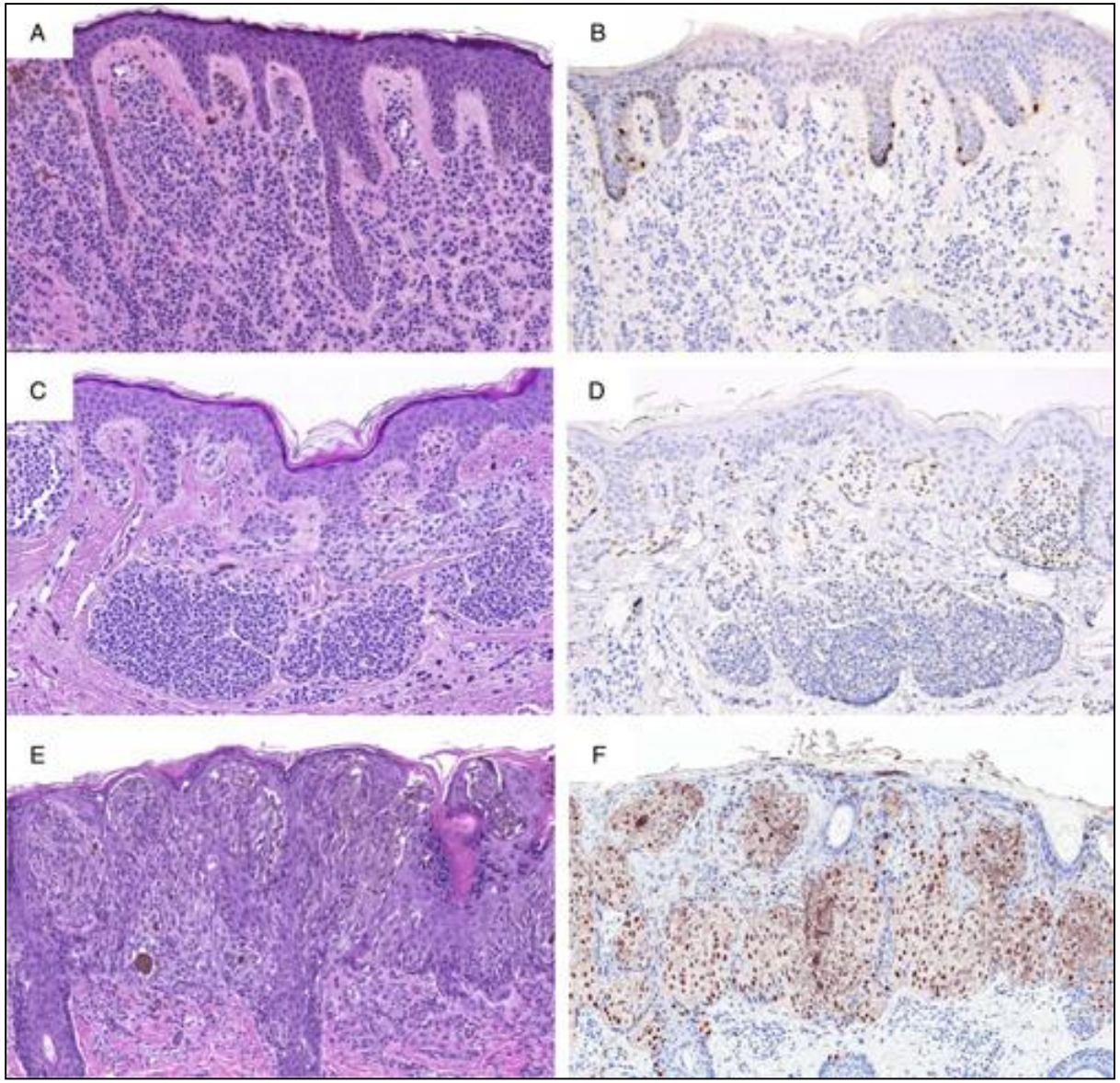

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FIGURE 6 . PRAME immunoreactivity in nevi. A, Ordinary melanocytic nevus (H&E-stain). B, A few junctional melanocytes express PRAME (1+). C, Compound dysplastic nevus (H&E-stain). D, The center of the lesion contains a number of PRAME-positive melanocytes at the dermoepidermal junction and in the superficial dermis (2+). E, Predominantly junctional Spitz nevus on the cheek of a child (H&E-stain). F, The intraepidermal lesional melanocytes diffusely label for PRAME (4+).

TABLE 1

Melanoma Type	In Situ Only	Invasive	Total
Superficial spreading	12/12	37/41	49/53
Lentigo maligna	24/27	15/17	39/44
Acral	7/7	10/11	17/18
Nodular	NA	9/10	9/10
Other*	2/2	6/8	8/10
Subtotal†	45/48	77/87	122/135
Desmoplastic‡	NA	7/20	7/20
Total	45/48	84/107	129/155

*This category includes (proportion of cases with 4+ PRAME): lentiginous vulvar in situ melanomas (2/2), nevoid melanoma (2/2), malignant melanoma ex-blue nevus (0/1), cutaneous paramucosal (3/3), and unclassified invasive melanomas (1/2).

†Subtotal = all melanomas except for desmoplastic melanomas.

‡This category comprises (proportion of cases with 4+ PRAME): spindle cell melanomas with variable desmoplasia, including pure (0/4) and mixed (6/14) desmoplastic melanomas, and spindle cell neurotropic (1/2) melanomas.

NA indicates not available.

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TABLE 1 Primary Cutaneous Melanomas With Diffuse (4+) PRAME IHC Expression

TABLE 3

Type of Melanocytic Nevus	Diffuse (4+) IHC PRAME Expression	Focal (1 or 2+) IHC PRAME Expression
Common acquired nevus	0/40	4/40 (1+)
Dysplastic (Clark's) nevus	0/60	10/60 (1+)
		1/60 (2+)
Blue nevus	0/10	0/10
Spitz nevus	1/10	1/10 (1+)
Deep penetrating nevus	0/3	0/3
Traumatized/ recurrent nevus	0/15	1/15 (2+)
		1/15 (1+)
Congenital nevus	0/2	0/2
Nodal nevus	0/5	0/5
Total	1/145	18/145

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TABLE 3 PRAME IHC Expression in Melanocytic Nevi

Margins

FIGURE 5

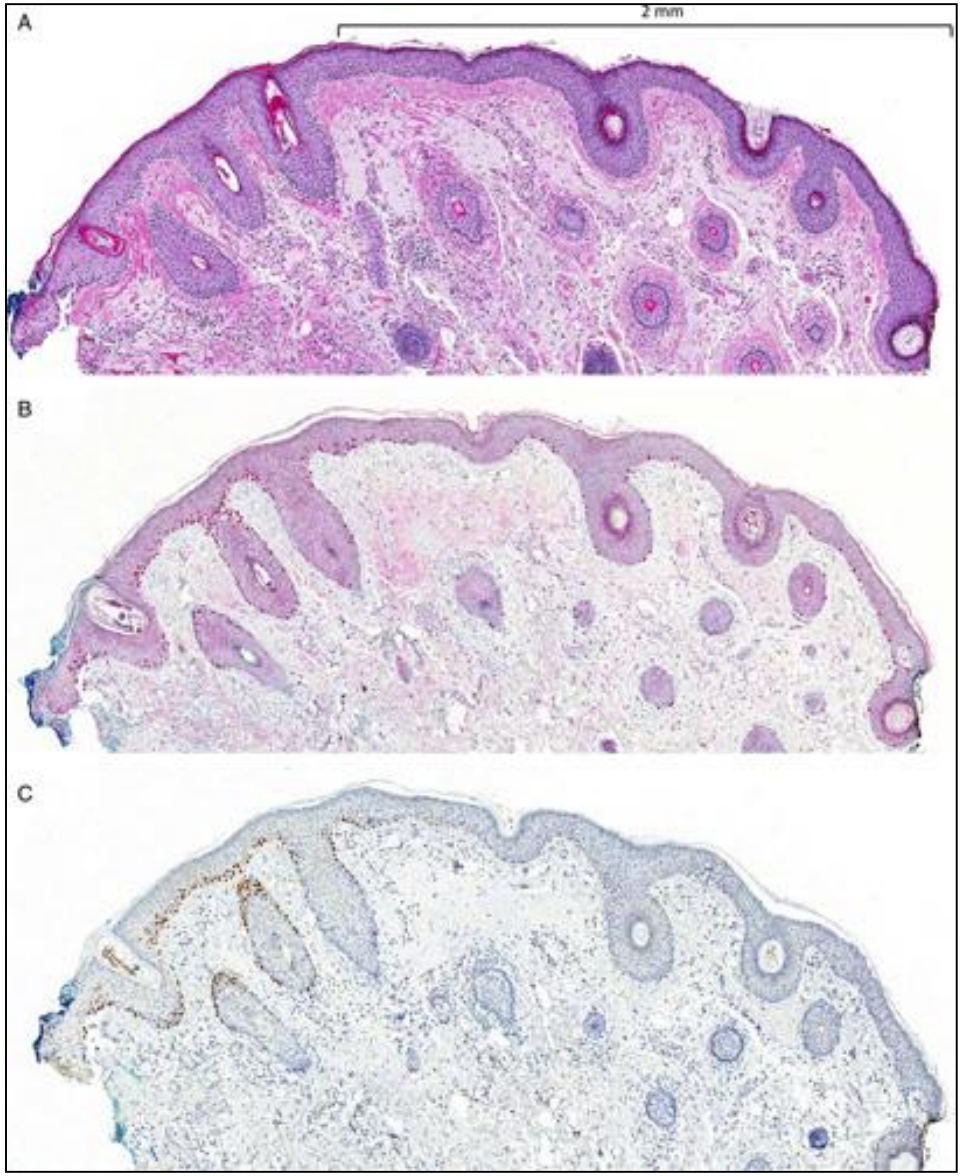

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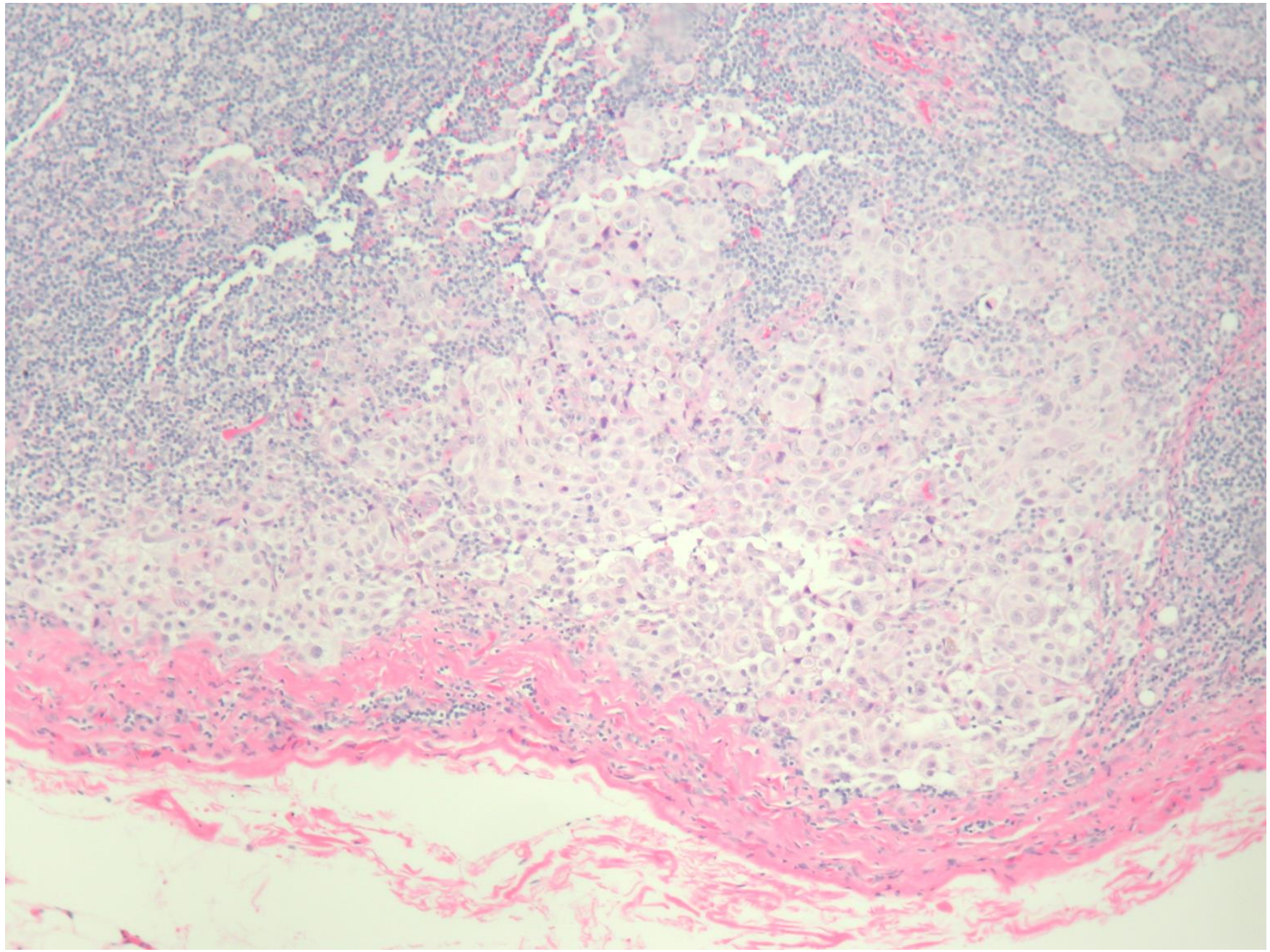
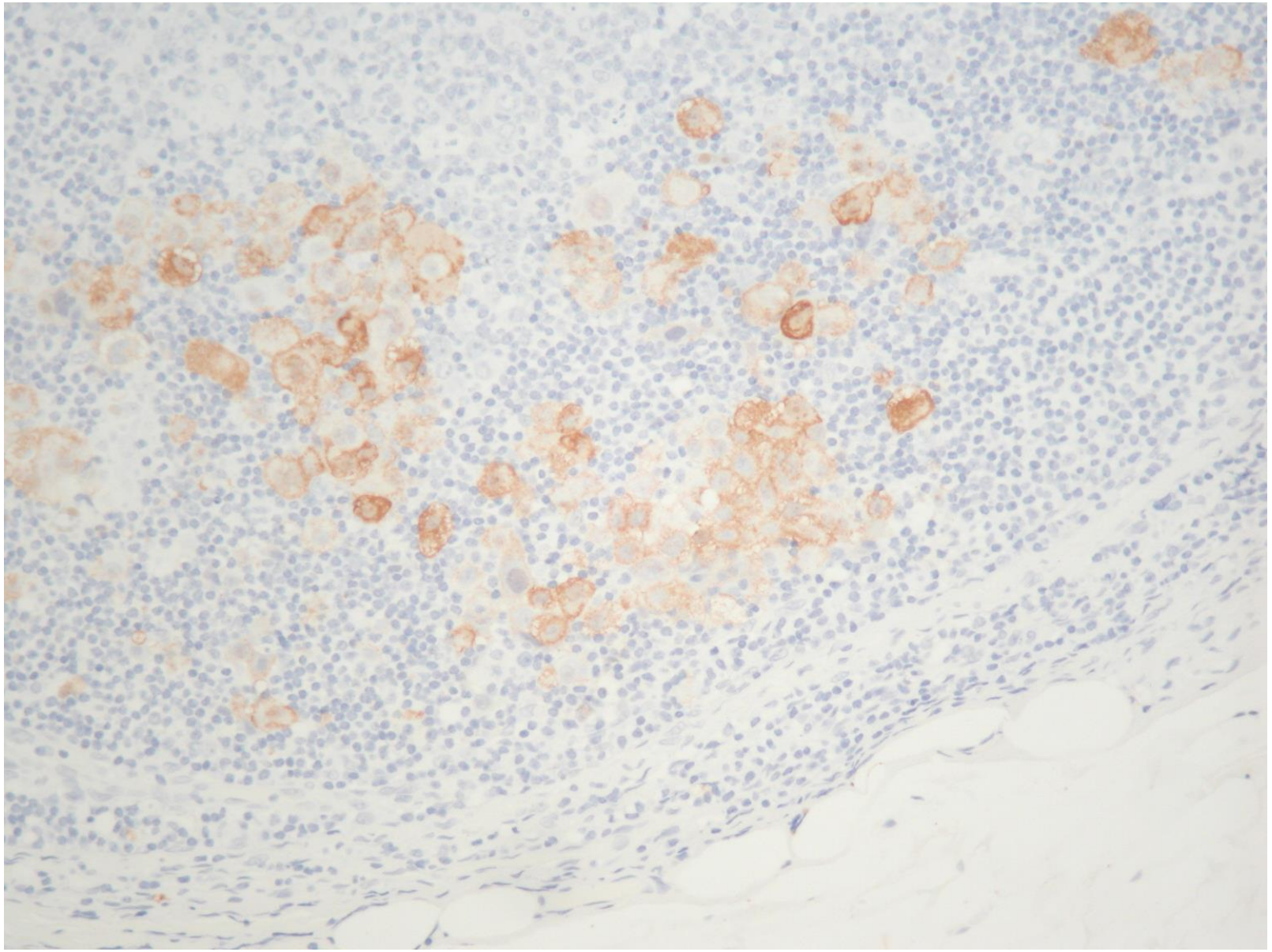
FIGURE 5 . Section from a staged excision of a lentigo maligna melanoma in situ with a rim of normal skin. A, H&E-stained section. The left blue-inked section edge faced the tumor debulk. The right side represents the outer rim-margin-of the excision. B, IHC for Sox10. C, IHC for PRAME. While the melanoma in situ strongly labels for PRAME, the melanocytes of the adjacent benign skin are negative.

Nodal nevi

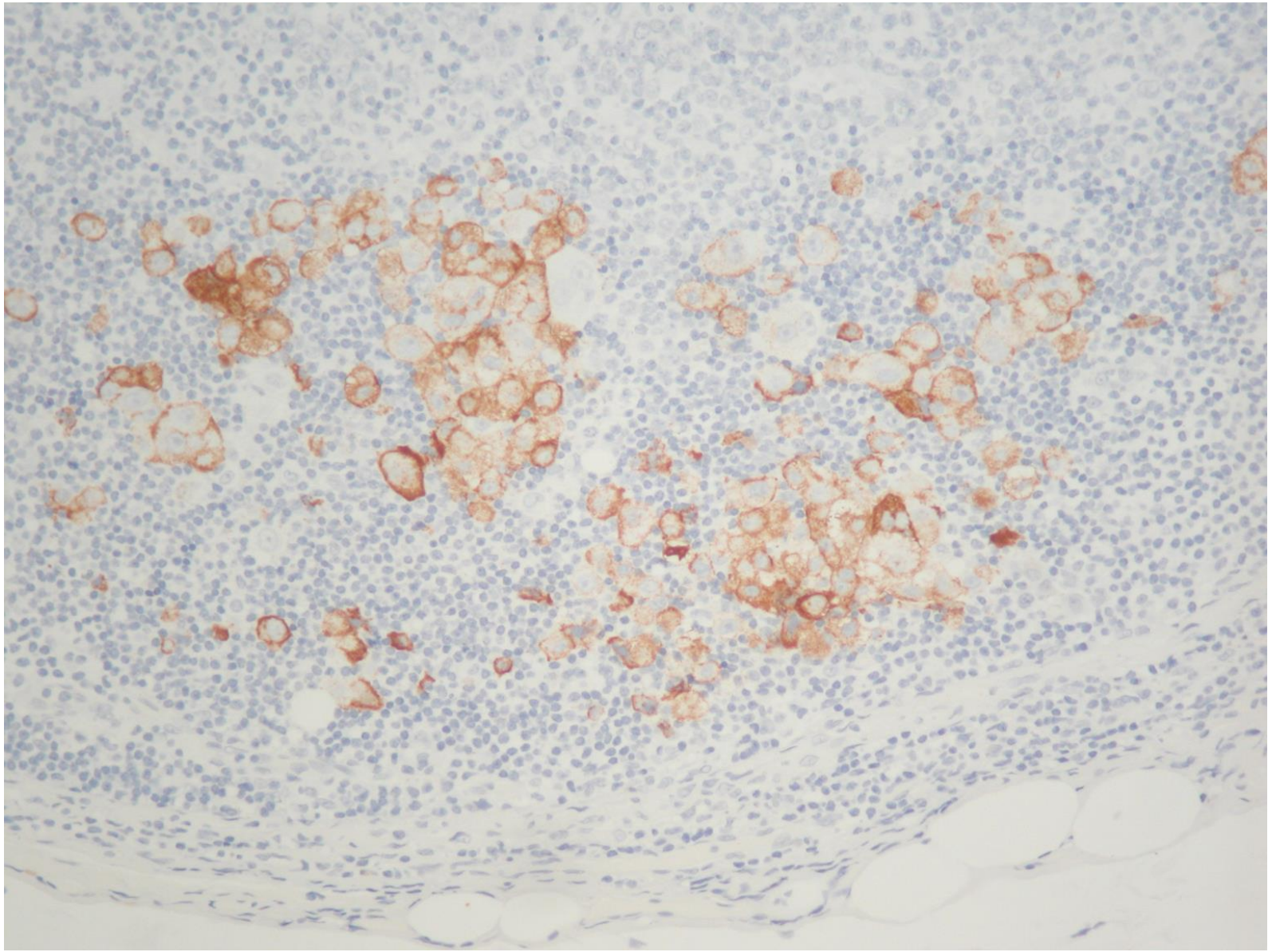
- Occurs in % of lymph node excisions
- More frequent in lymph node excisions for melanoma
- Commonly occurs in the capsule of the node but can occur elsewhere
- Any histologic type of nevus can occur, most commonly blue and acquired
- Immunophenotype of acquired: HMB45 (-), Ki-67 (-)
- Makes staging problematic
- N of TNM:

Sentinel lymph nodes are still used for staging
Use of melanoma (melanocyte) specific and nonspecific markers

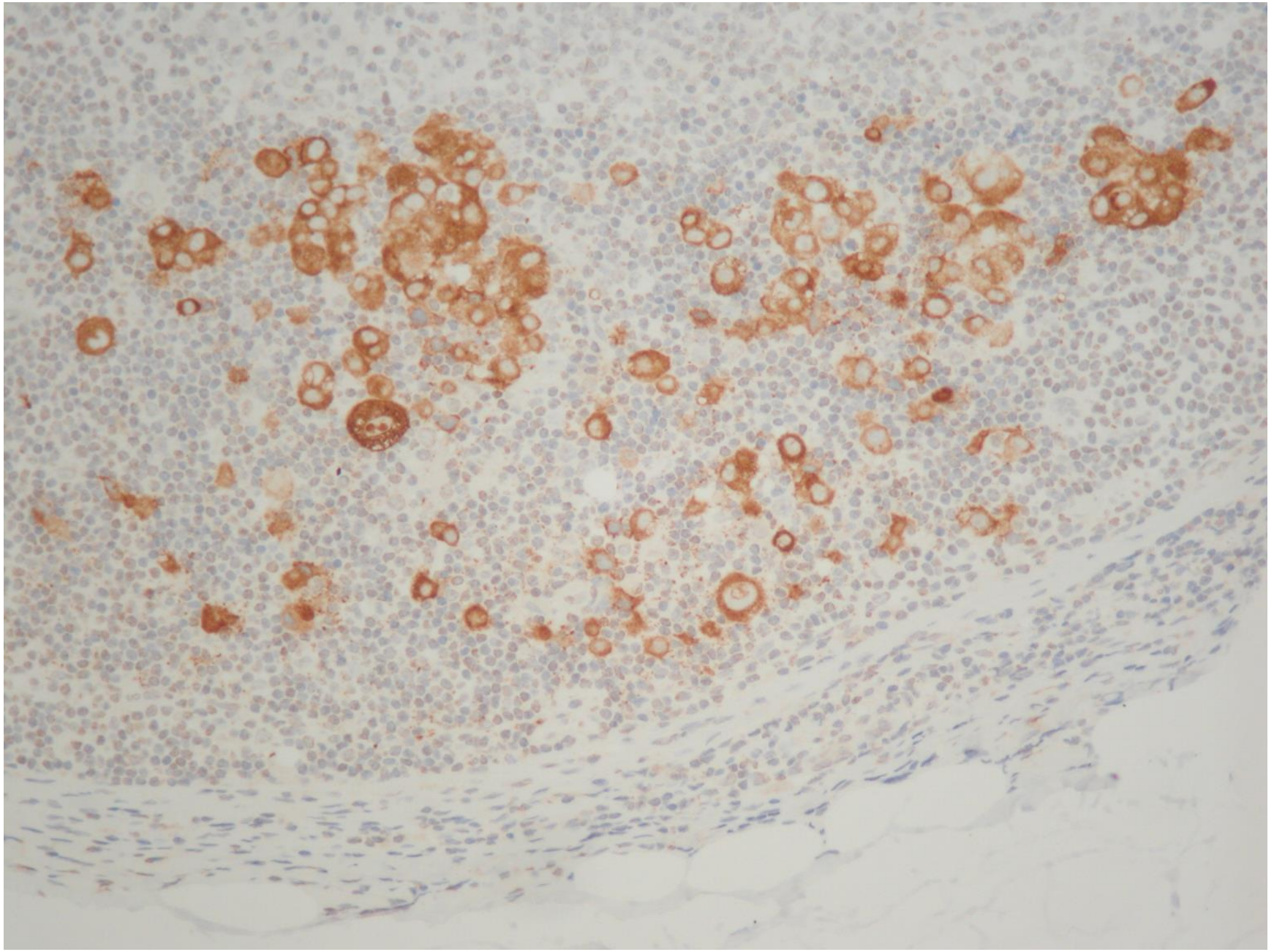




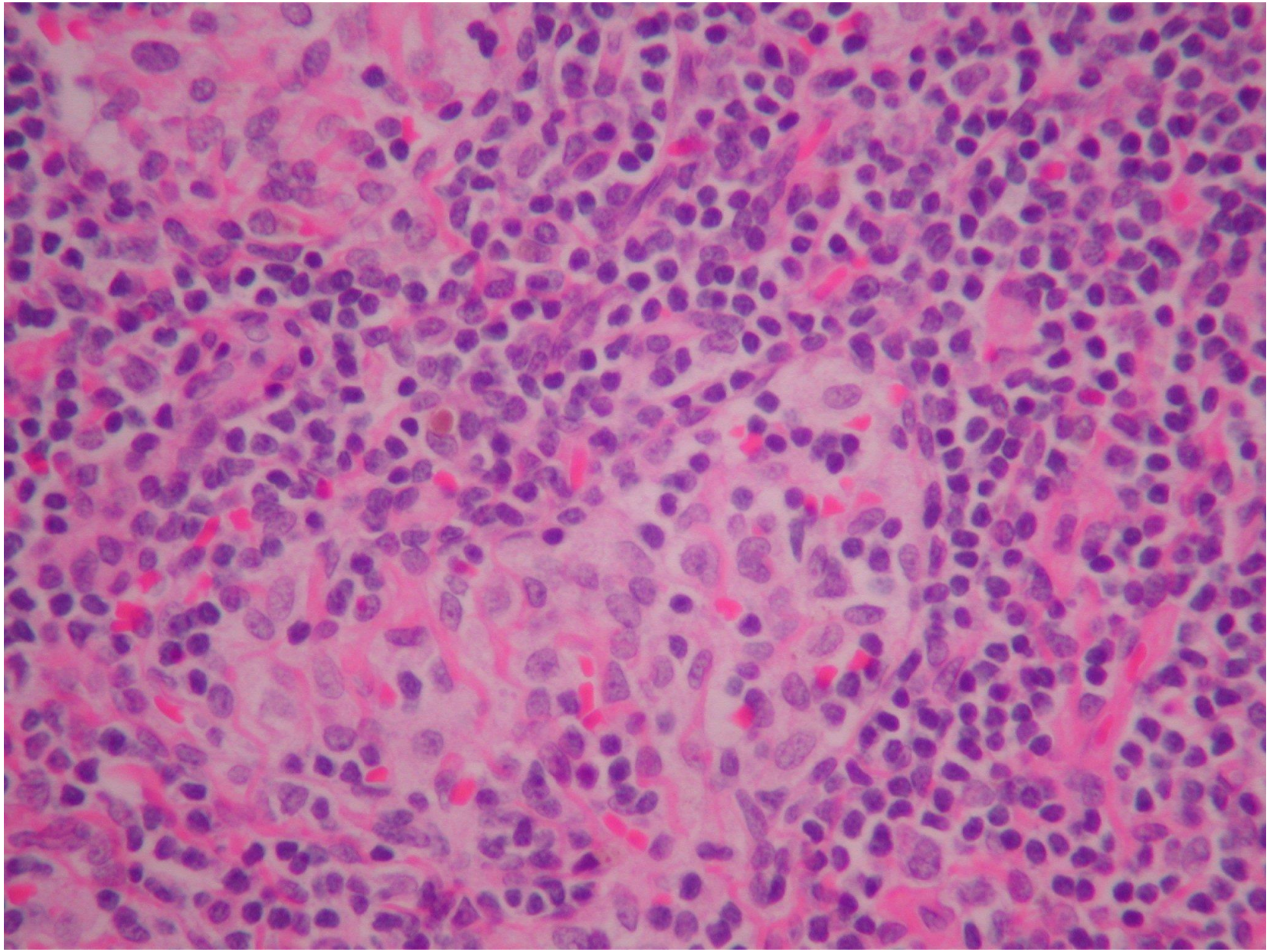
S-100

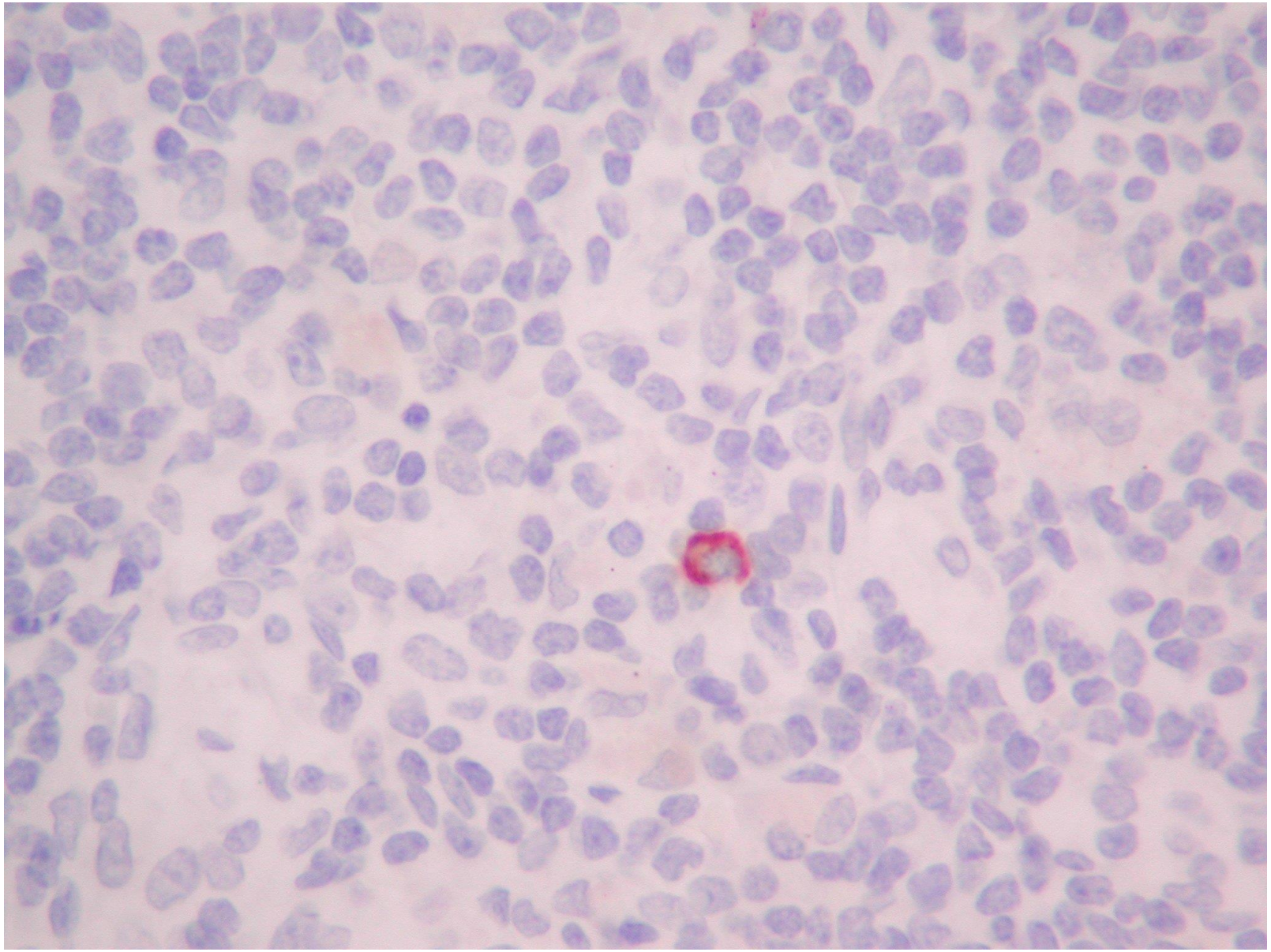


HMB45

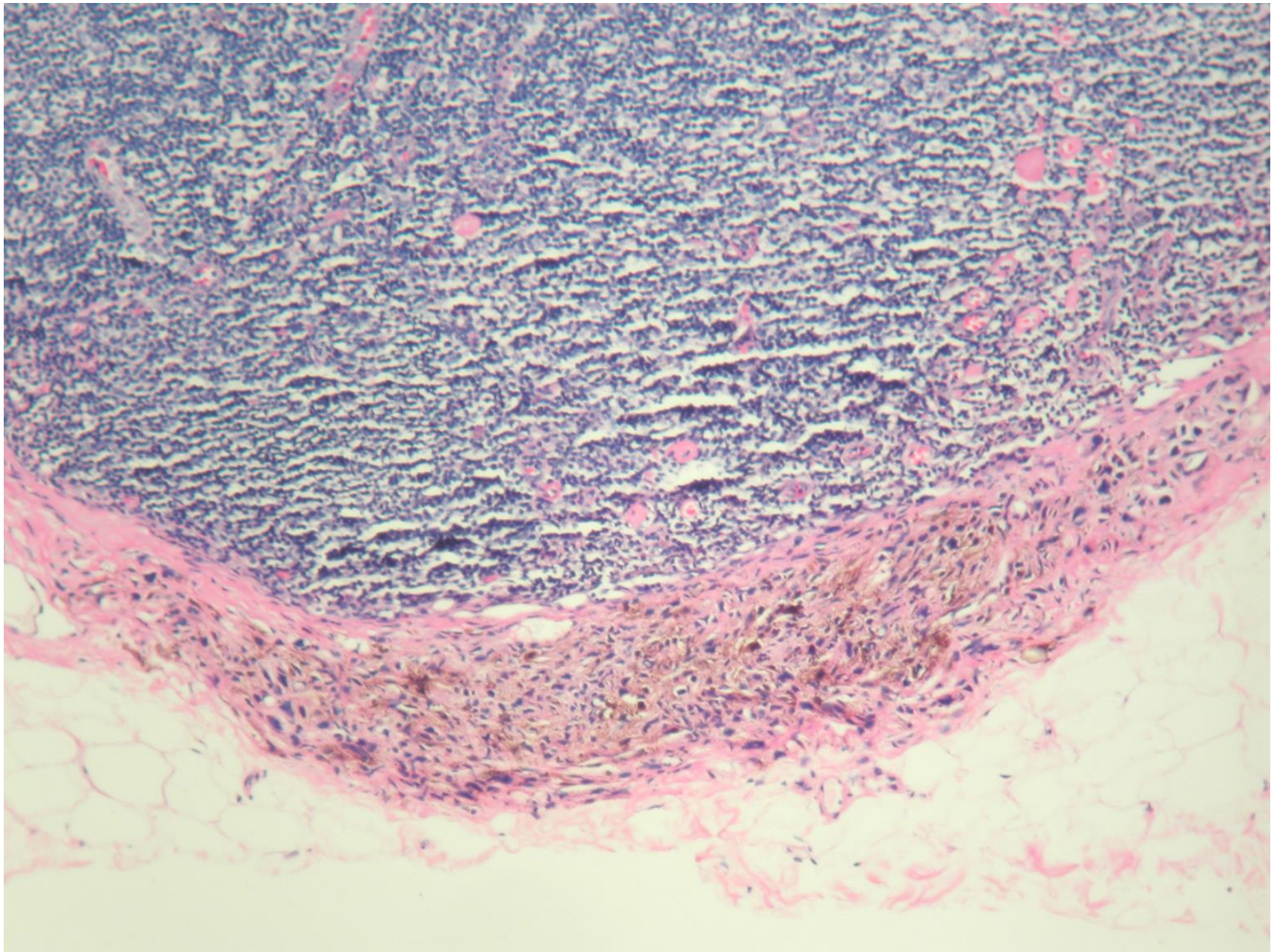


Melan-A





Is this a positive node?



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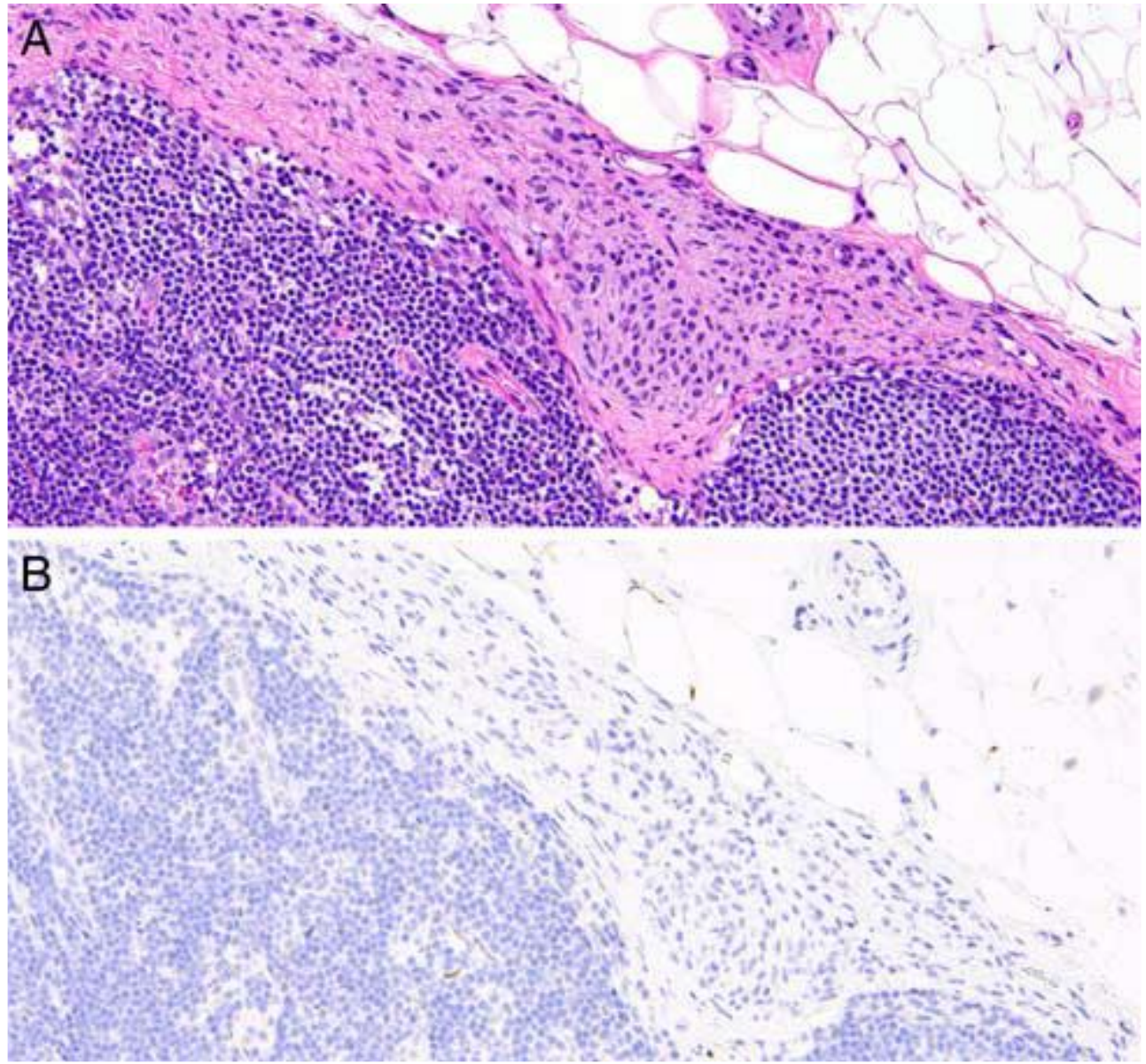
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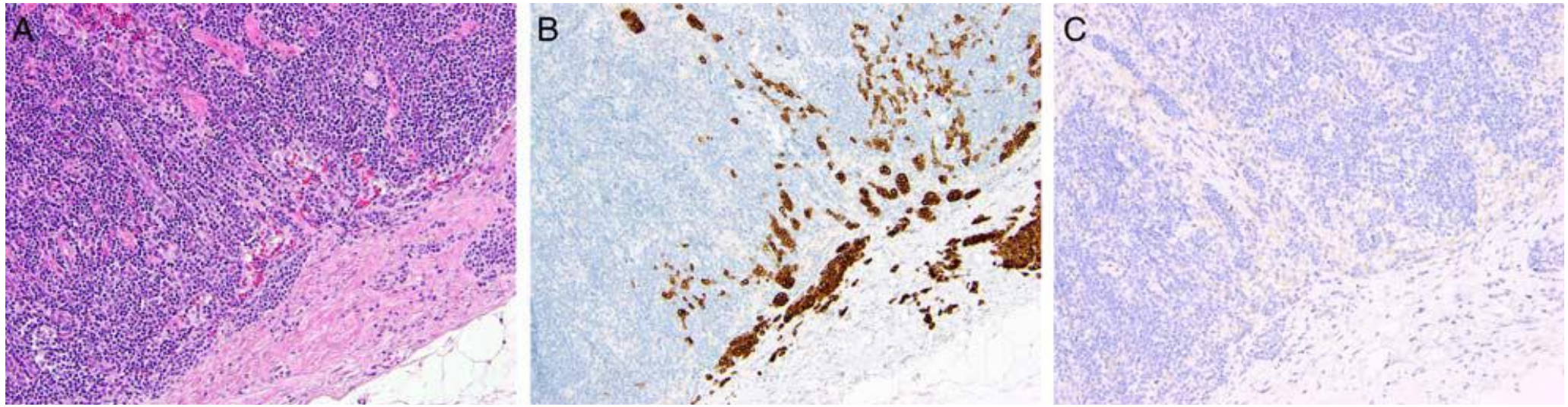
B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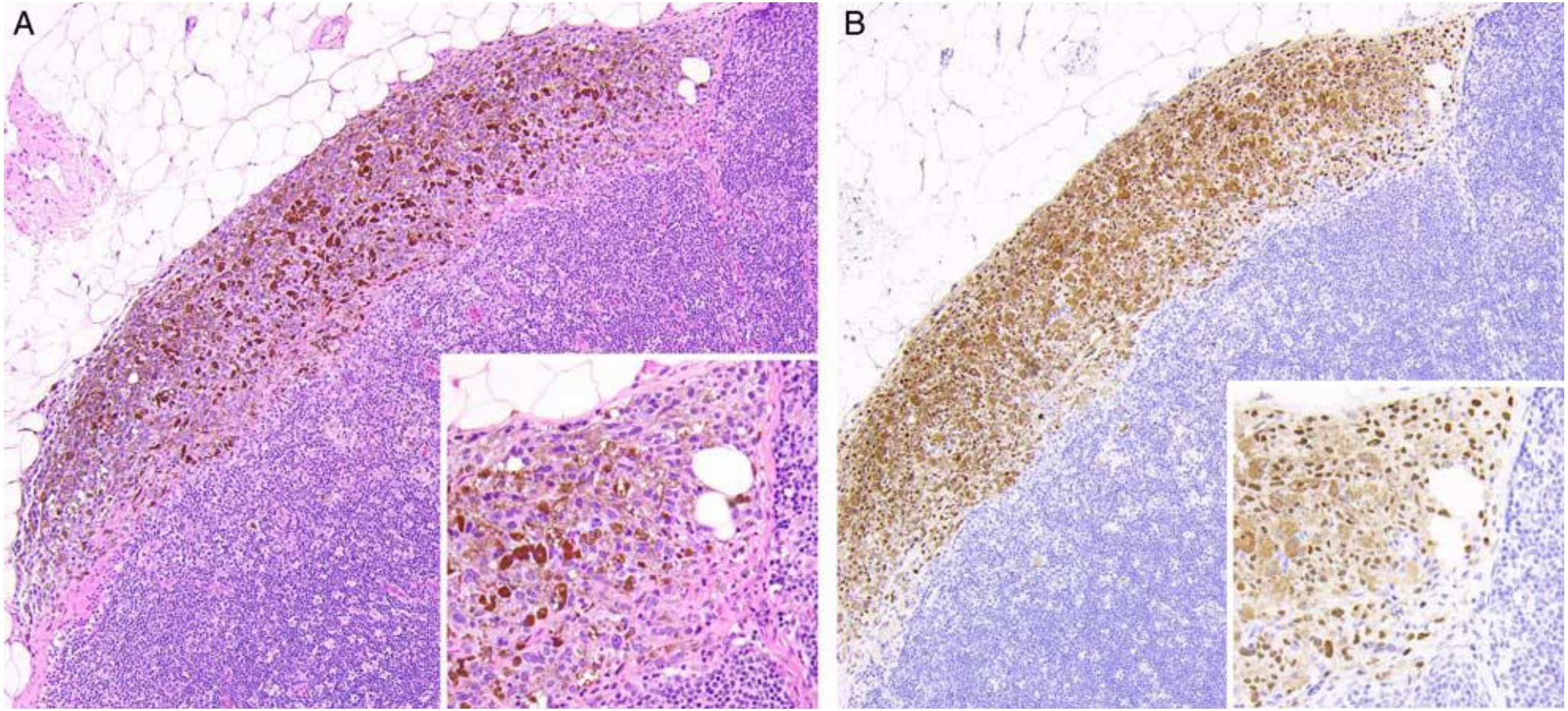
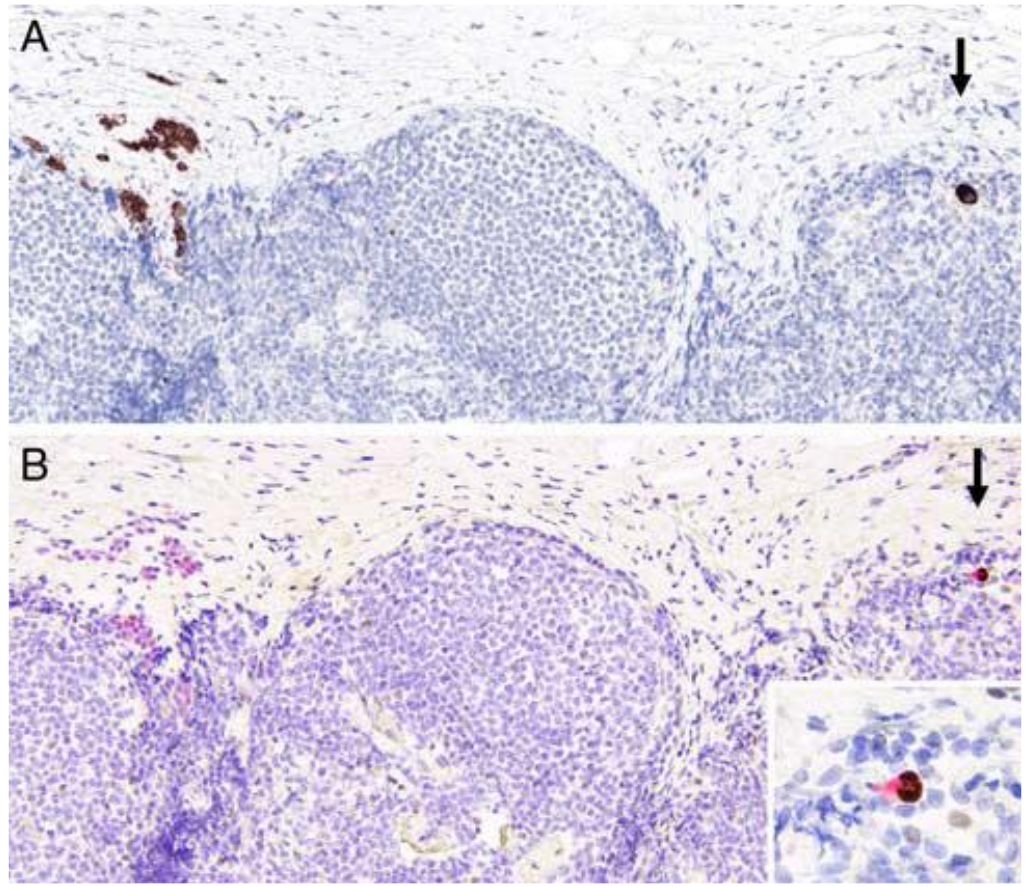
B
C


A
B


A
B