Immunohistochemistry During Mohs Surgery

American Society for Mohs Histotechnology
2017 Annual Meeting

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April 29, 2017

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**I have no conflicts of interest

Objectives:

- Review main indications for use of immunohistochemistry in Mohs surgery
- Discuss tips and pitfalls to increase utility in your Mohs lab and Mohs practice
- Present several illustrative cases to prompt discussion and facilitate troubleshooting

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Main Indications

- Lentigo maligna/melanoma in situ in critical locations where tissue sparing is important, and/or tumors are large, ill-defined, or recurrent
- High-risk squamous cell carcinoma, especially perineural invasion, moderate or poor differentiation, single cells and strands, or lots of background inflammation (CLL)
- Extrammammary Paget's Disease, which is notorious for subclinical extension and skip areas

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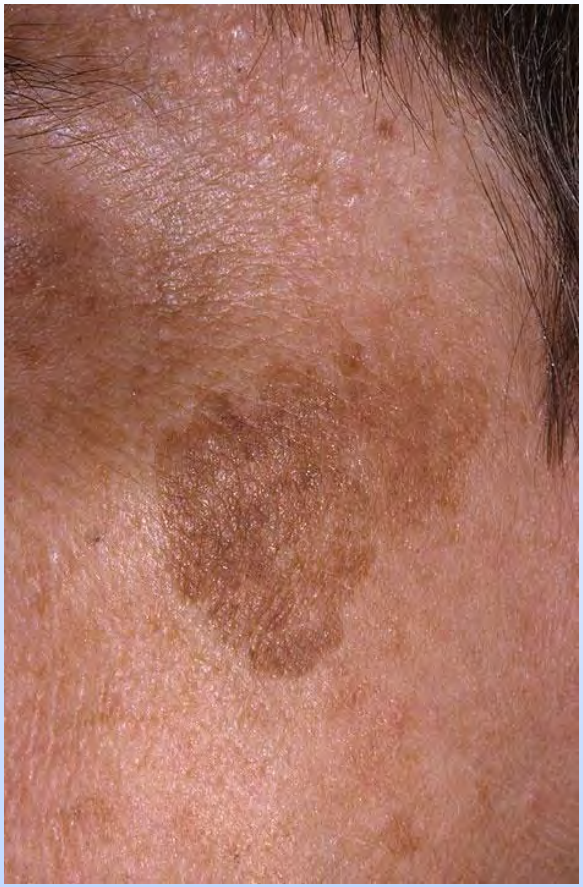
Lentigo Maligna and LMM

- LM and LMM are approximately 10% of all MM, with rising incidence (Swetter JID 2005).
- A.K.A. Hutchinson's melanotic freckle; melanoma-in-situ
- Estimated risk of progression of LM to LMM: 2-5% (Weinstock Br J Dermatol 1987)



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True Confession: I perform Mohs for most facial LM. Why?

- Phone Call: "ENT here; got a dirty margin after excising a facial MM; can you help with the LM at the periphery?"
- Path Report after attempted staged excision of LM with horizontal paraffin sections: ">50% of the epidermis is absent for evaluation, so CPC recommended…" (Proper embedding difficult)
- Patient Says: "Why do I have to come back in 2 days for my next stage? How many times do I have to come back?"

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Immunostains for LM

- MART-1 commonest stain utilized
- Not a panacea, but an adjunct
- Most useful in cases where freeze artifact occurs (pseudokoilocytes), pagetosis, nesting, and if adnexal tracking is occurring.
- Less helpful in my practice for lentiginous involvement
- Ultimately these stains do not predict biologic behavior or study subtle cytologic atypia

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Tips for MART-1

- Cut unstained blocks in advance
- Helps to get a (+) control from your debulking
- Helps to get a (-) control from contralateral area
- Adds about 60 minutes per Mohs stage
- If your H&E shows obvious LM on Stage 1, go on to stage 2…

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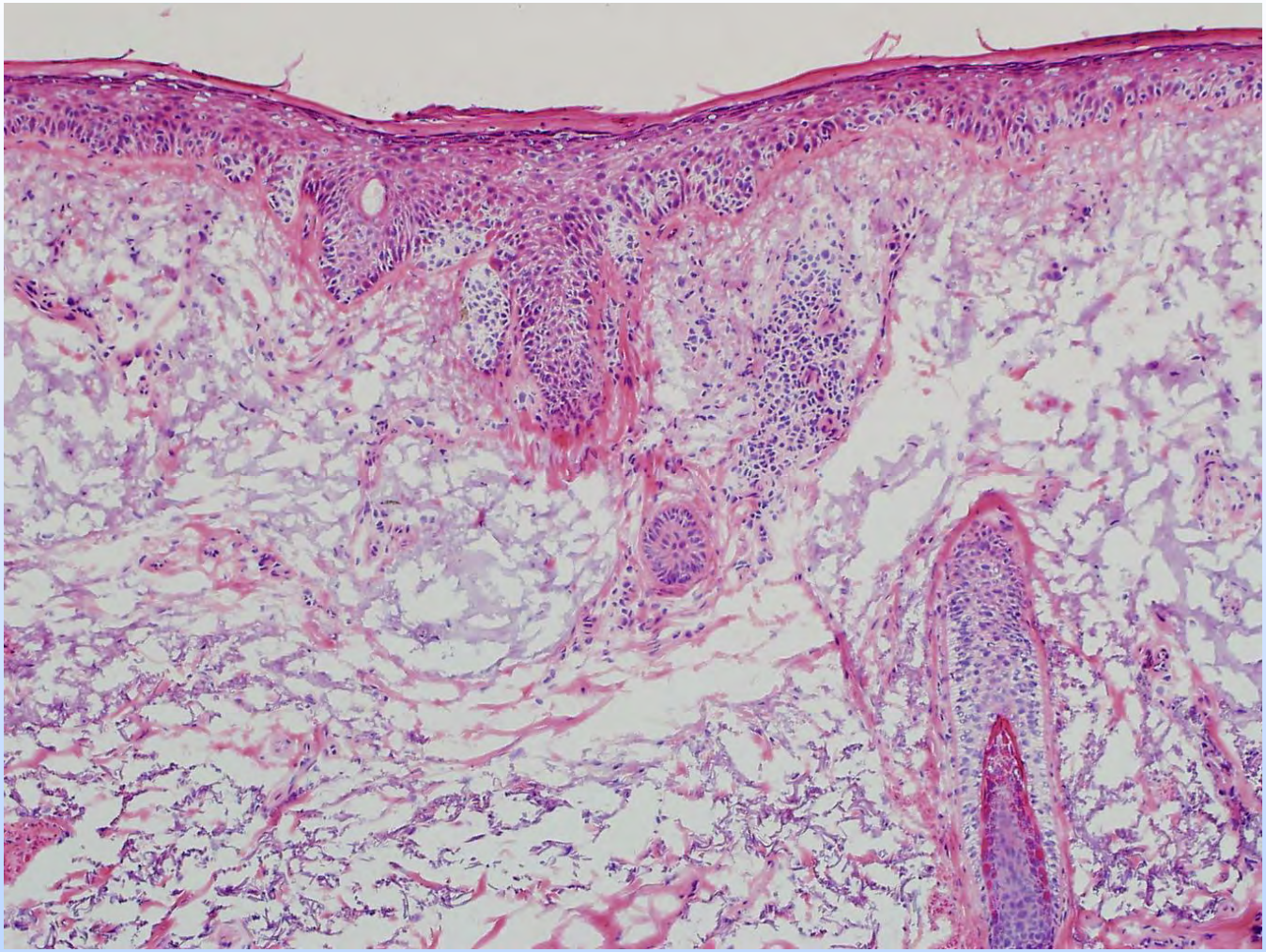
Nested LM + Dermal Inflammation or subtle LMM?



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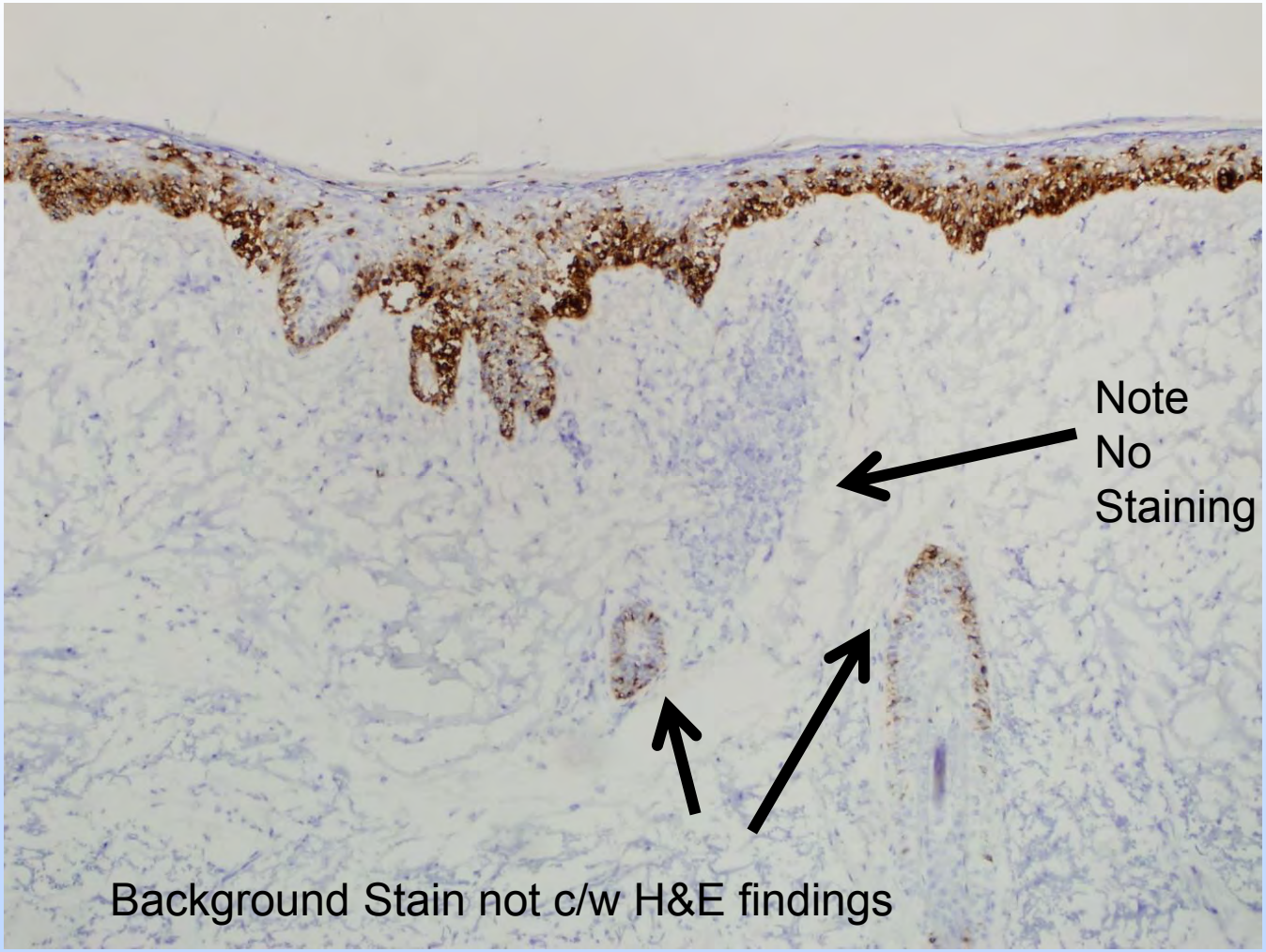
MART-1 Immunostaining



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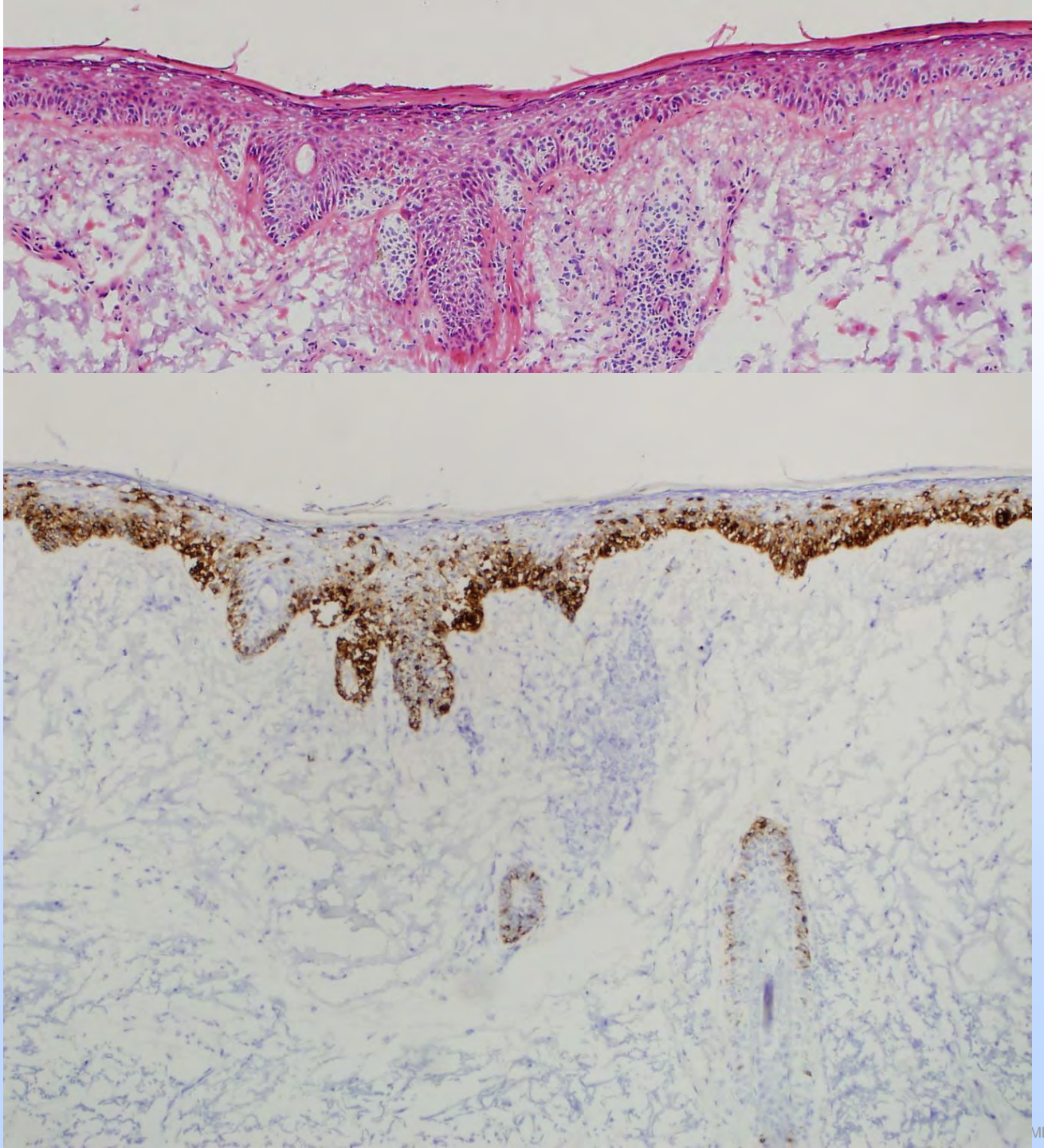
Always helps to compare side-by-side, in parallel



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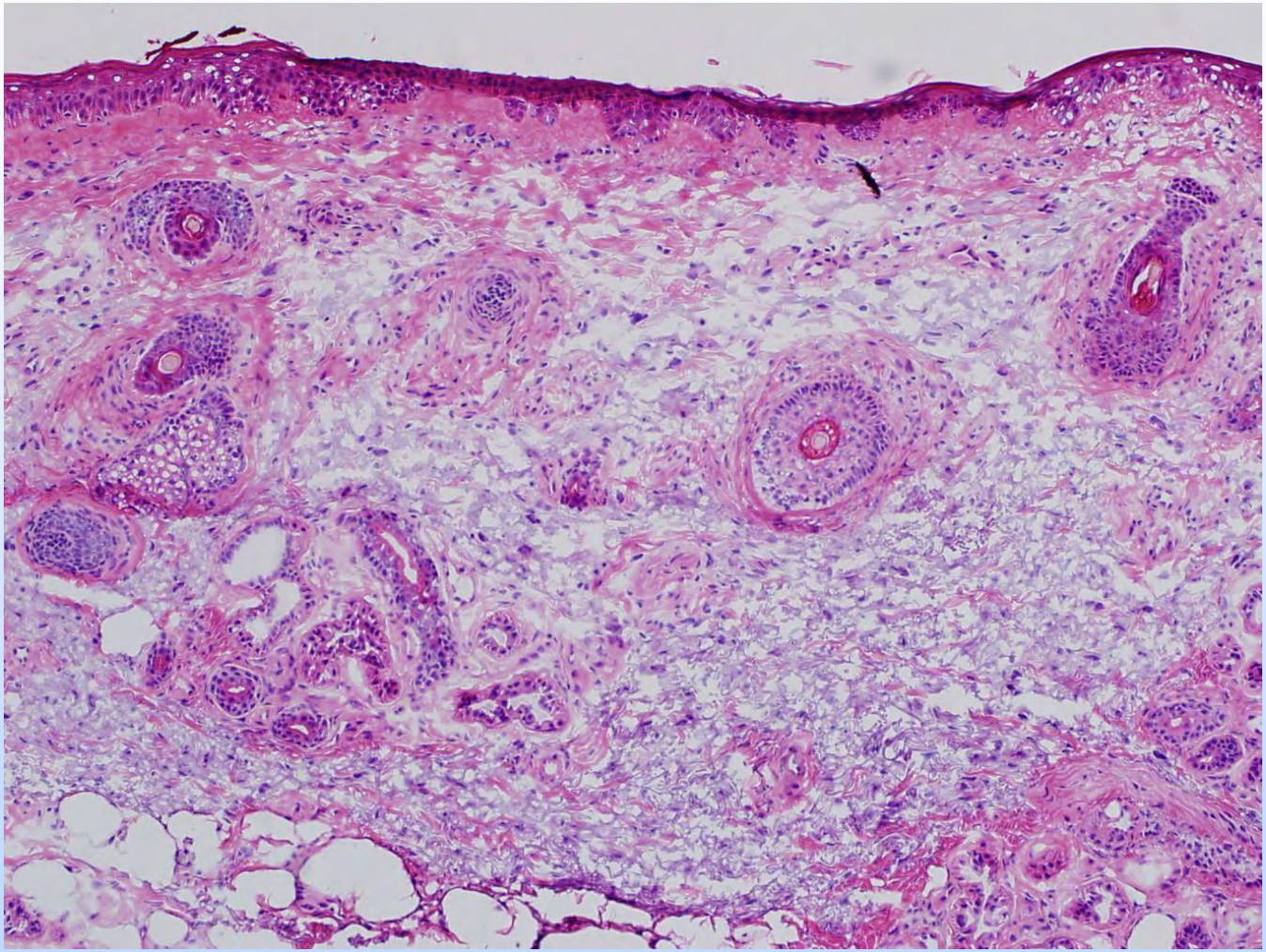
Contralateral Sun-Damaged Skin (“Control”)



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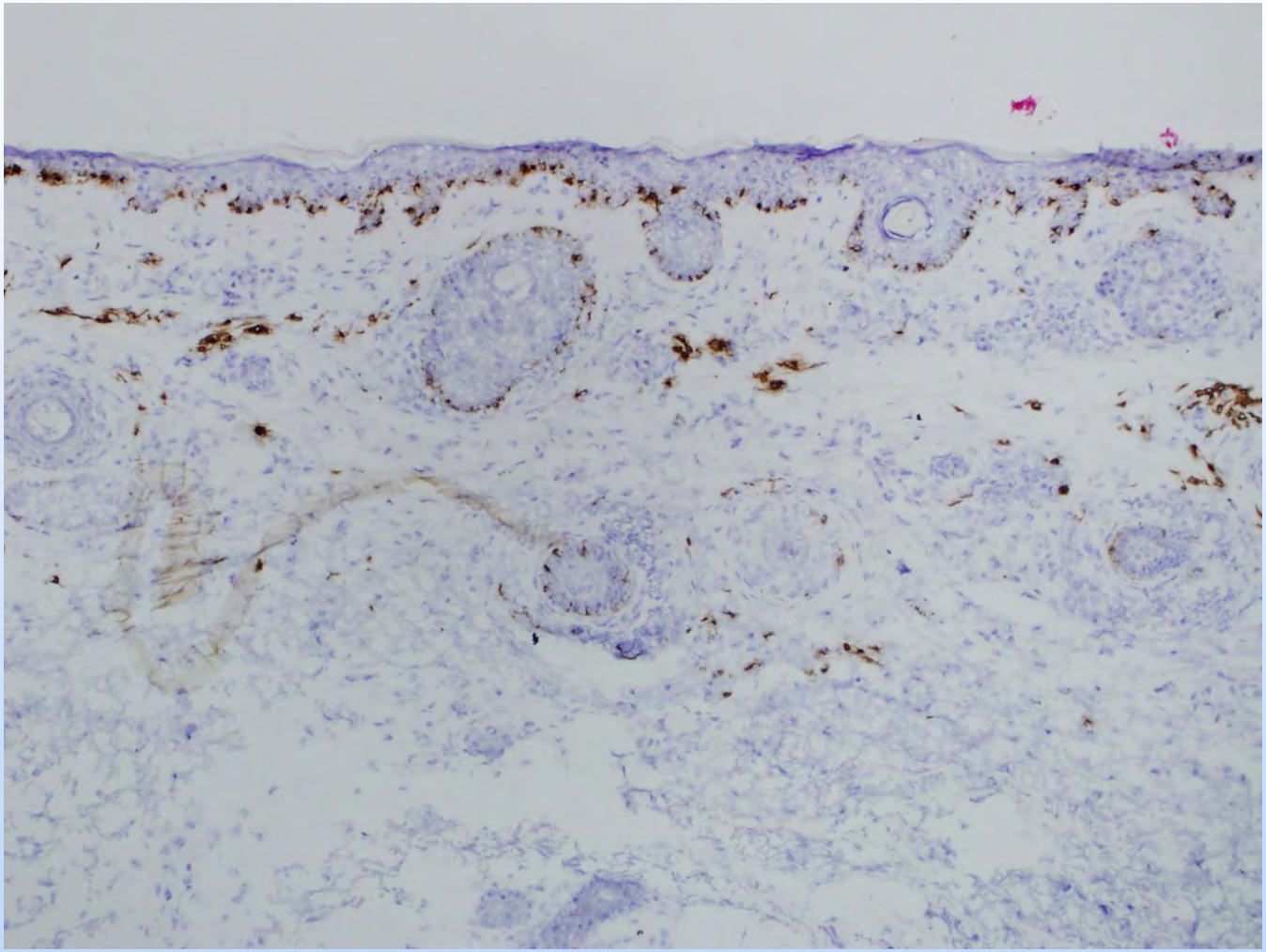
Note Background “Noise” and DEJ increased hypermelanosis.



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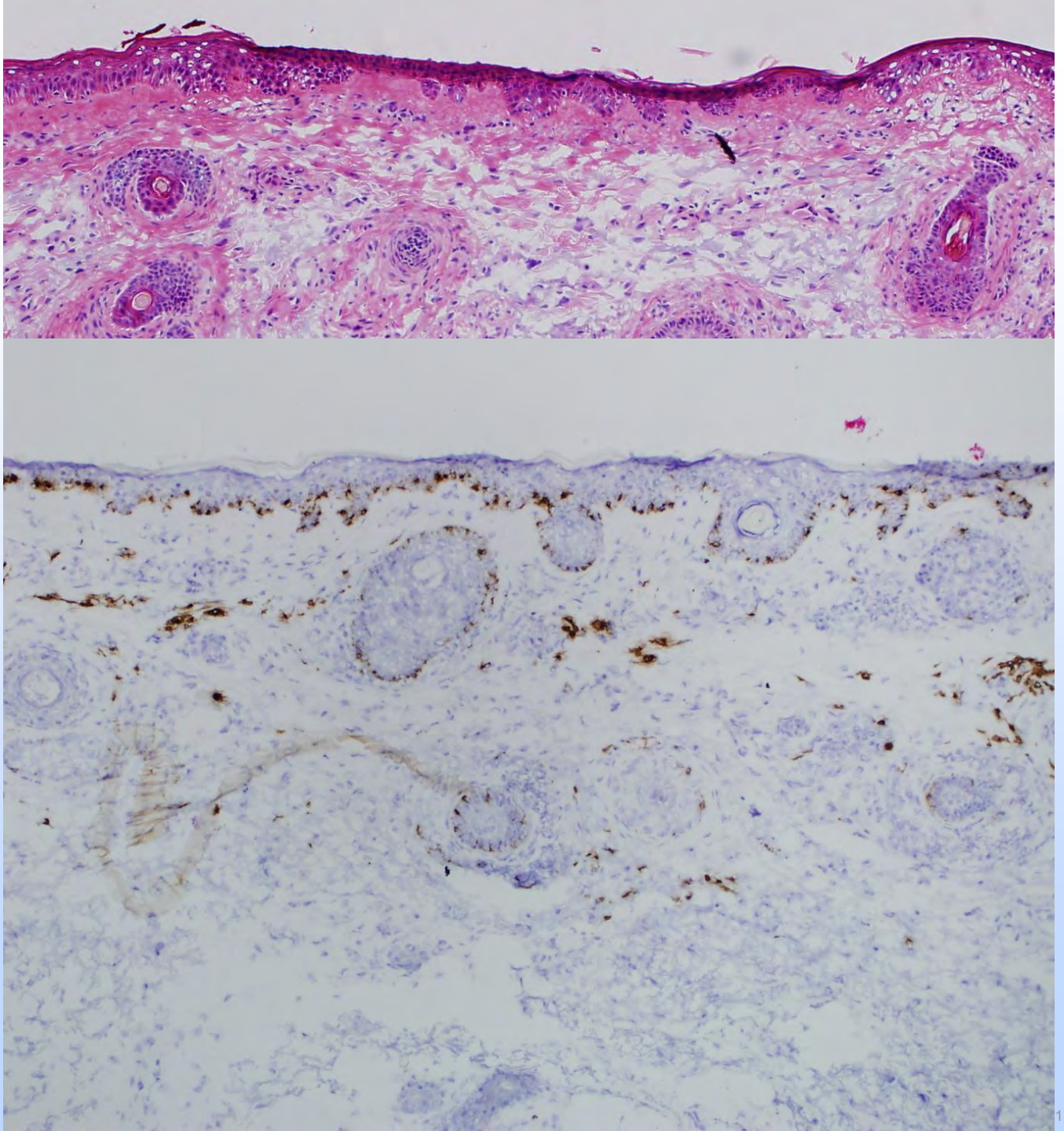
Side-by-side Comparison, H&E, MART-1



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Recent Case Example

- 51 y.o. woman with pigmented lesion since 1998
- Biopsy 2004: MMIS/Lentigo Maligna
- Series of tangential and vertical excisions and partial excisions, possible superficial laser or chemical applications inferolaterally, 2004-2008
- Recurrent pigment 2010, closer to eyelid

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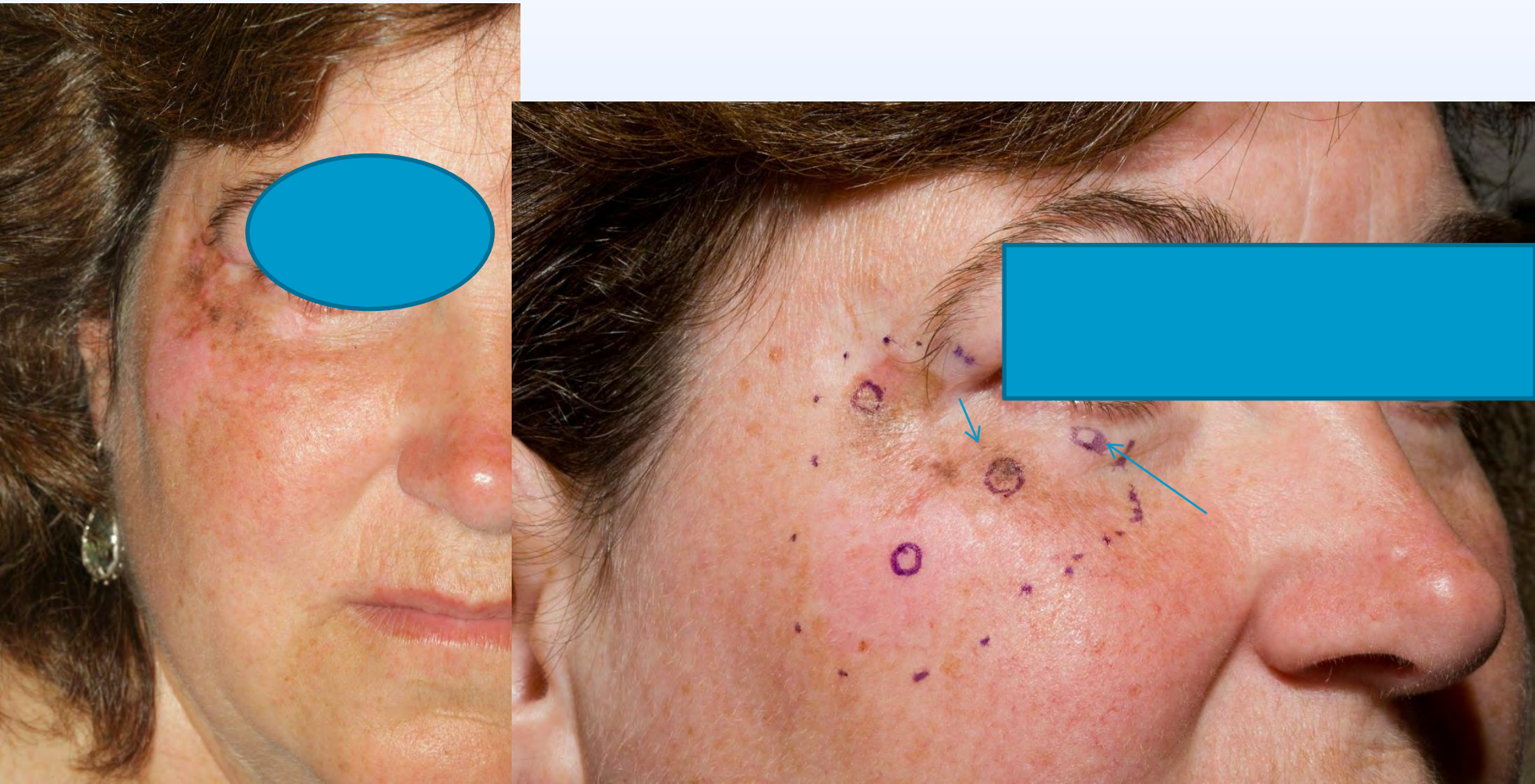
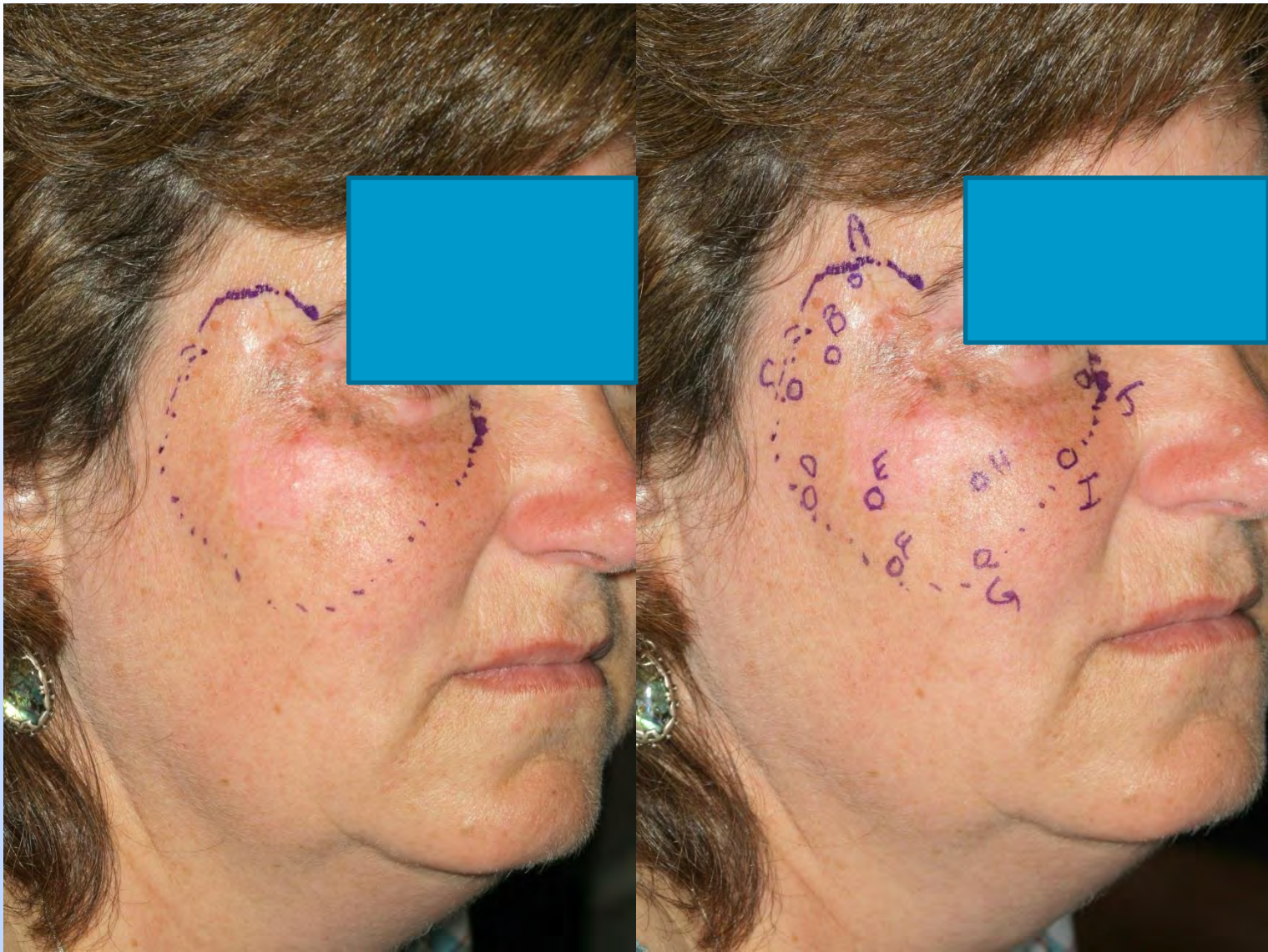
Recurrent LM with Invasive MM; 2 sites MMIS, 2 sites invasive MM; invasive sites where small blue arrows point—note the one near lid margin has no pigment



Additional Scouting Biopsies…All negative except site “H”



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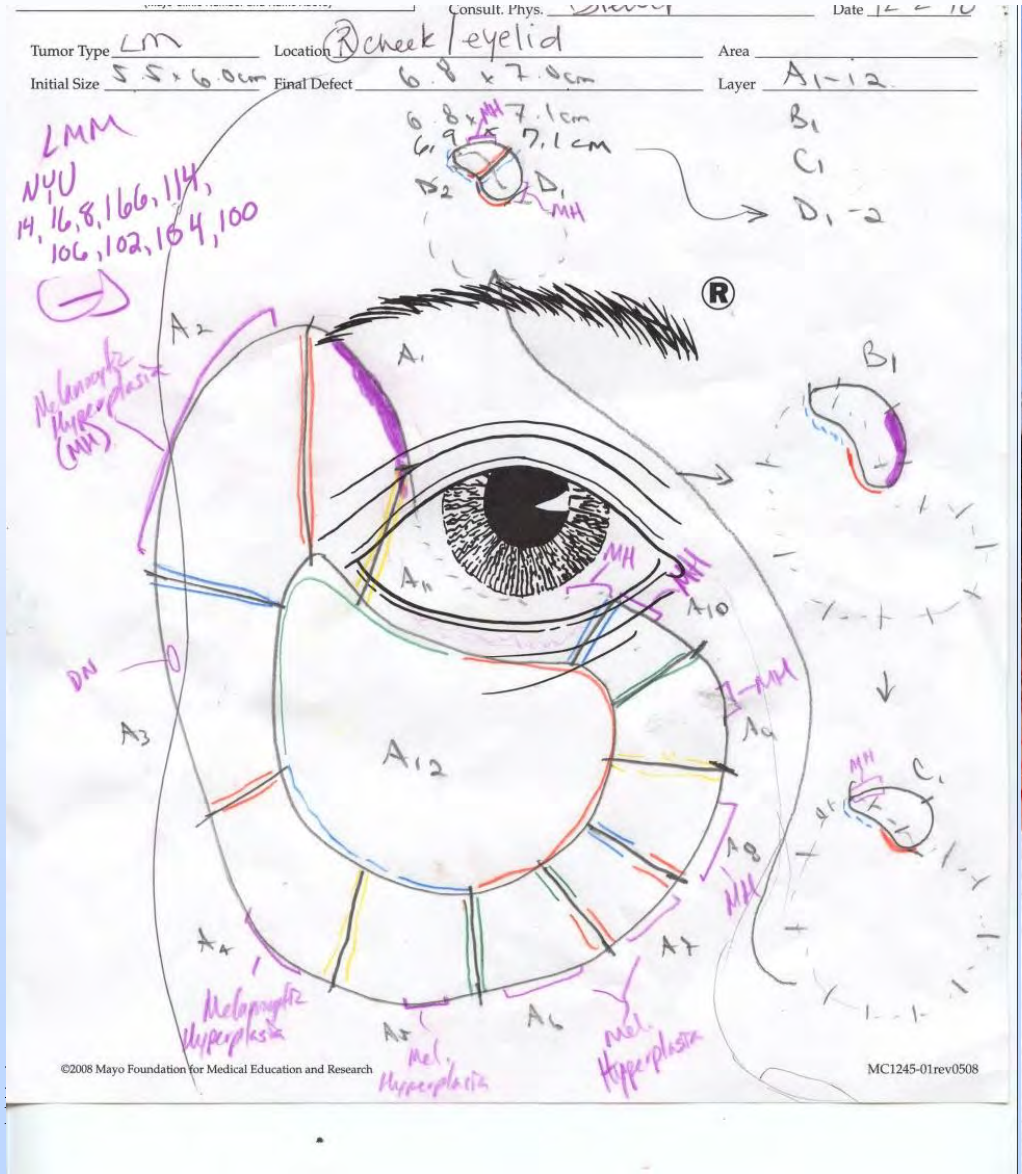
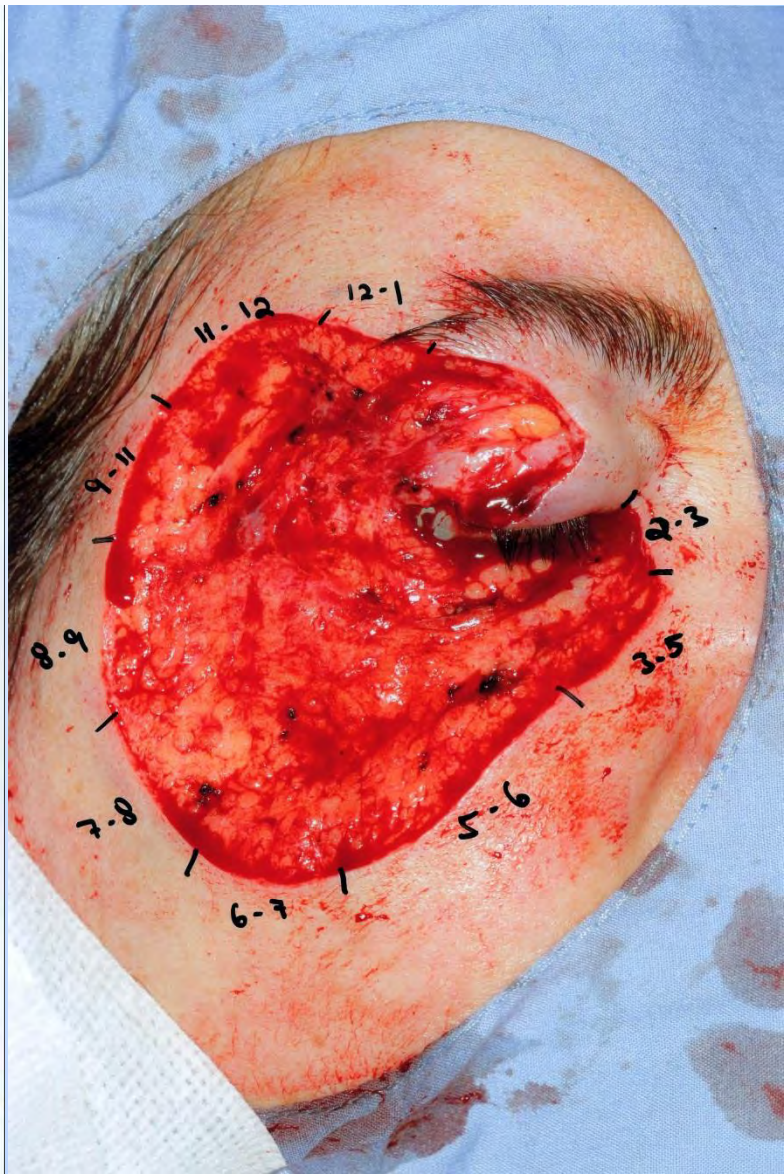
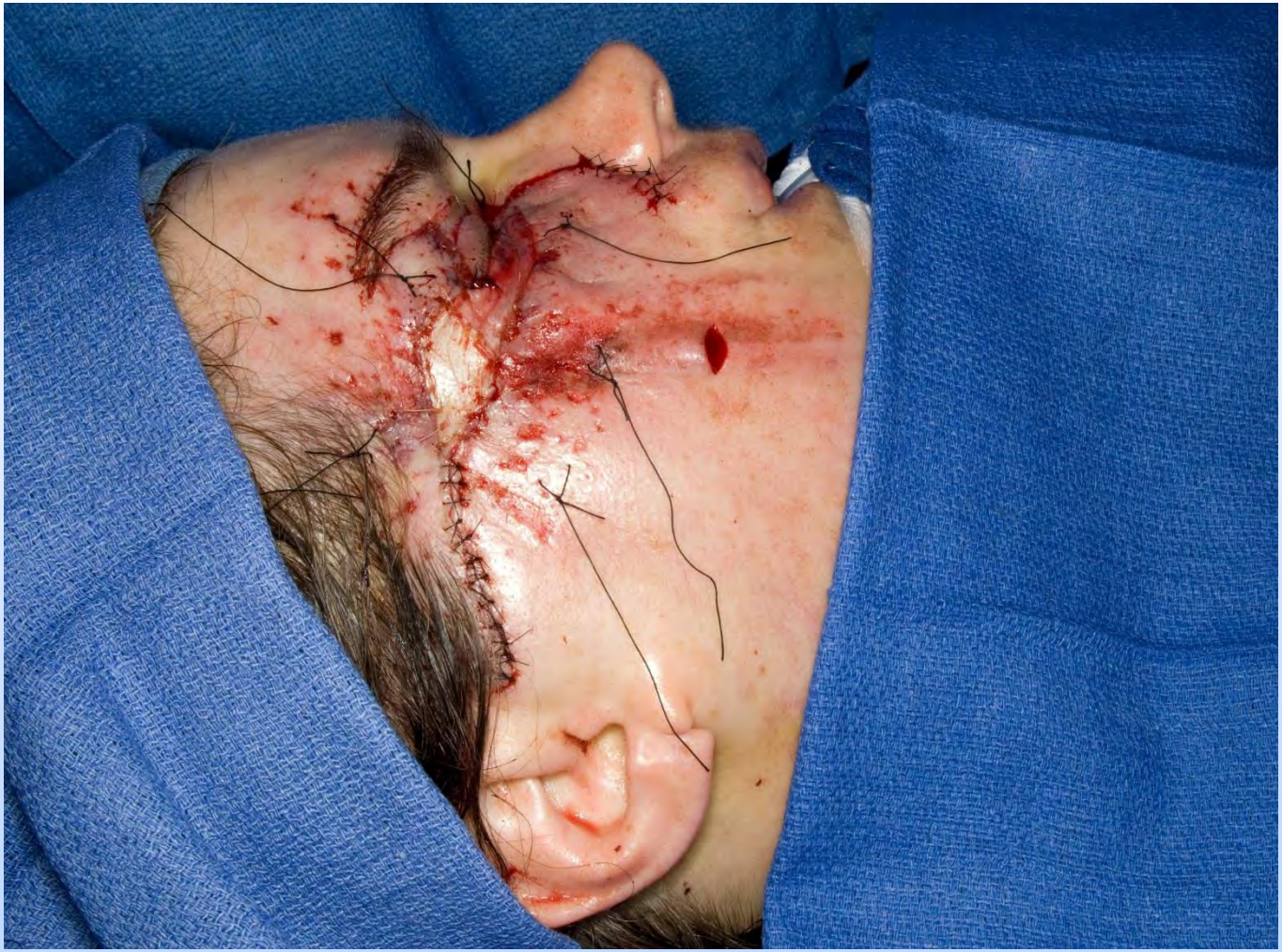
Mohs Map and Mohs Defect





Repair Oculoplastics and Plastic Surgery



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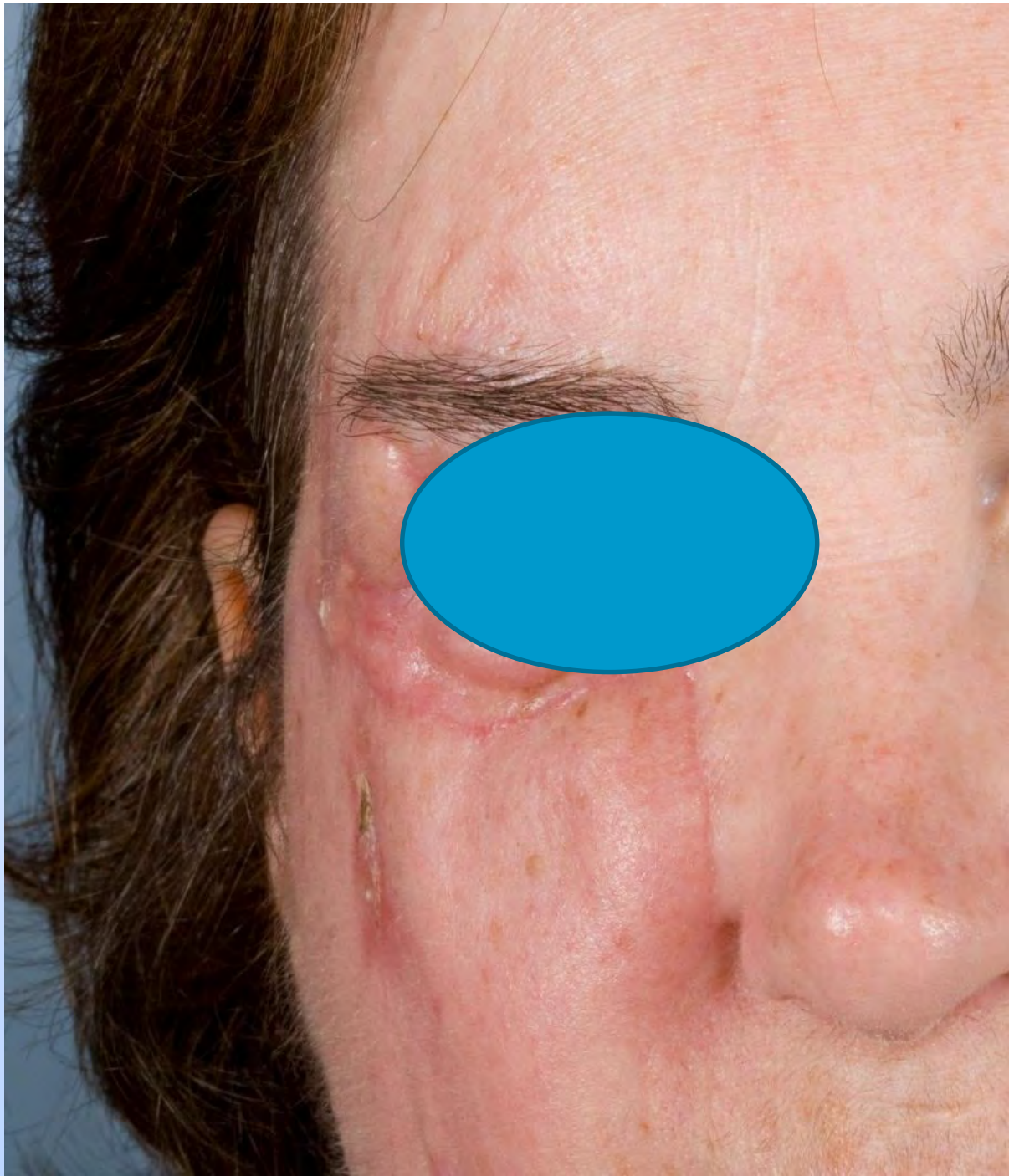
Postop 6 weeks frontal view



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Additional Challenges with LM

- It is often multifocal, so “clear margins” are relative only
- Subtotal initial biopsies may miss invasive MM
- Many LM never progress to invasion, so why treat? If not treated, how does one monitor?
- What constitutes true MM-in-situ?

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What Constitutes LM to me?

- Nested or confluent clearly atypical melanocytes +/- pagetosis. I need to see at least 3 together.
- Mart-1 stains and Melan-A stains may help distinguish melanocytes especially if freeze artifact present, but those stains DO NOT tell the viewer benign vs malignant. They can also stain melanosomes and melanophages…
- Overcalling sun-damaged melanocytes can lead to face-ectomies. "Control" samples may help for comparison.

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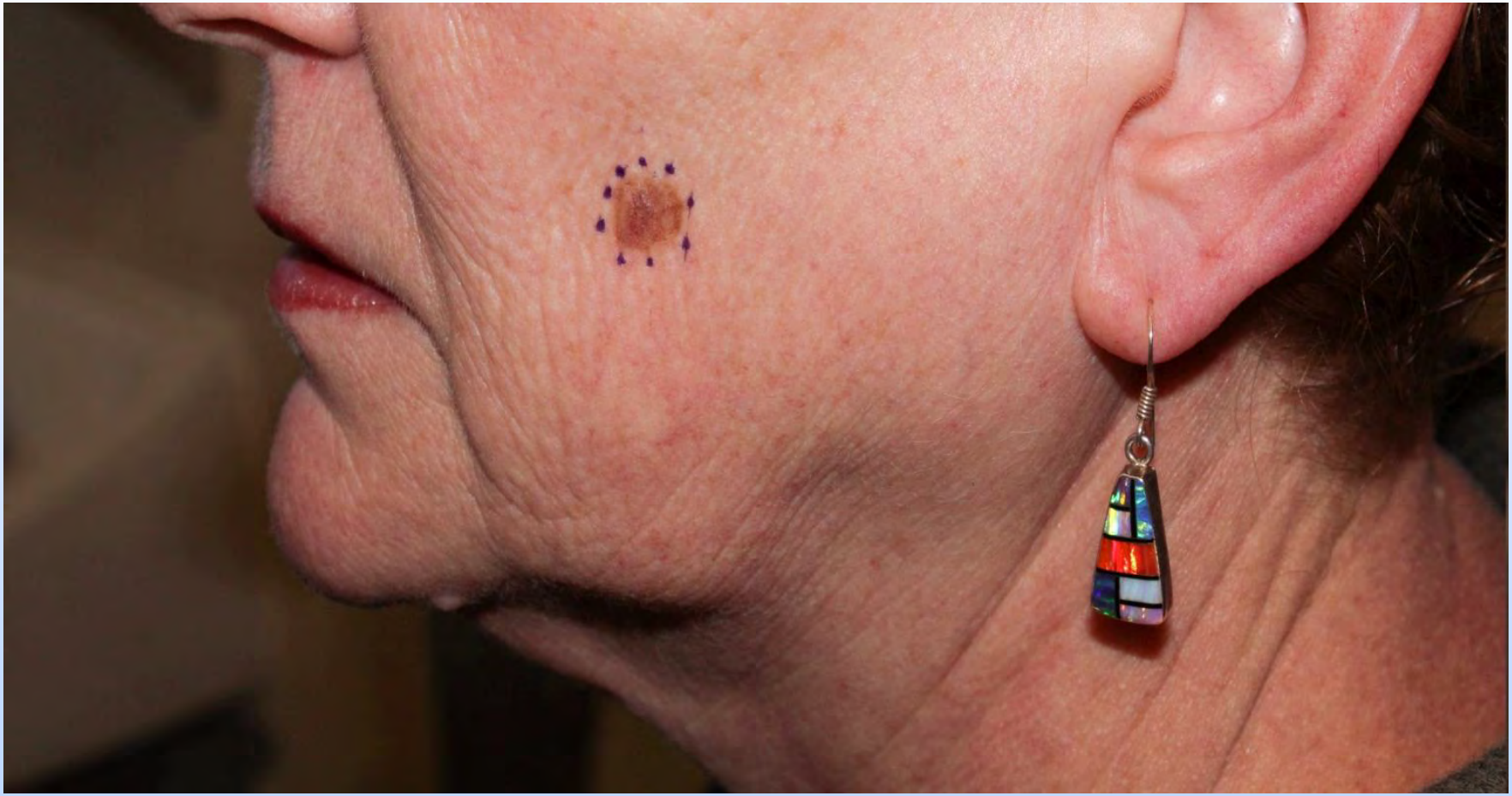
MM Breslow 0.4mm depth: Excision; 1 cm margins



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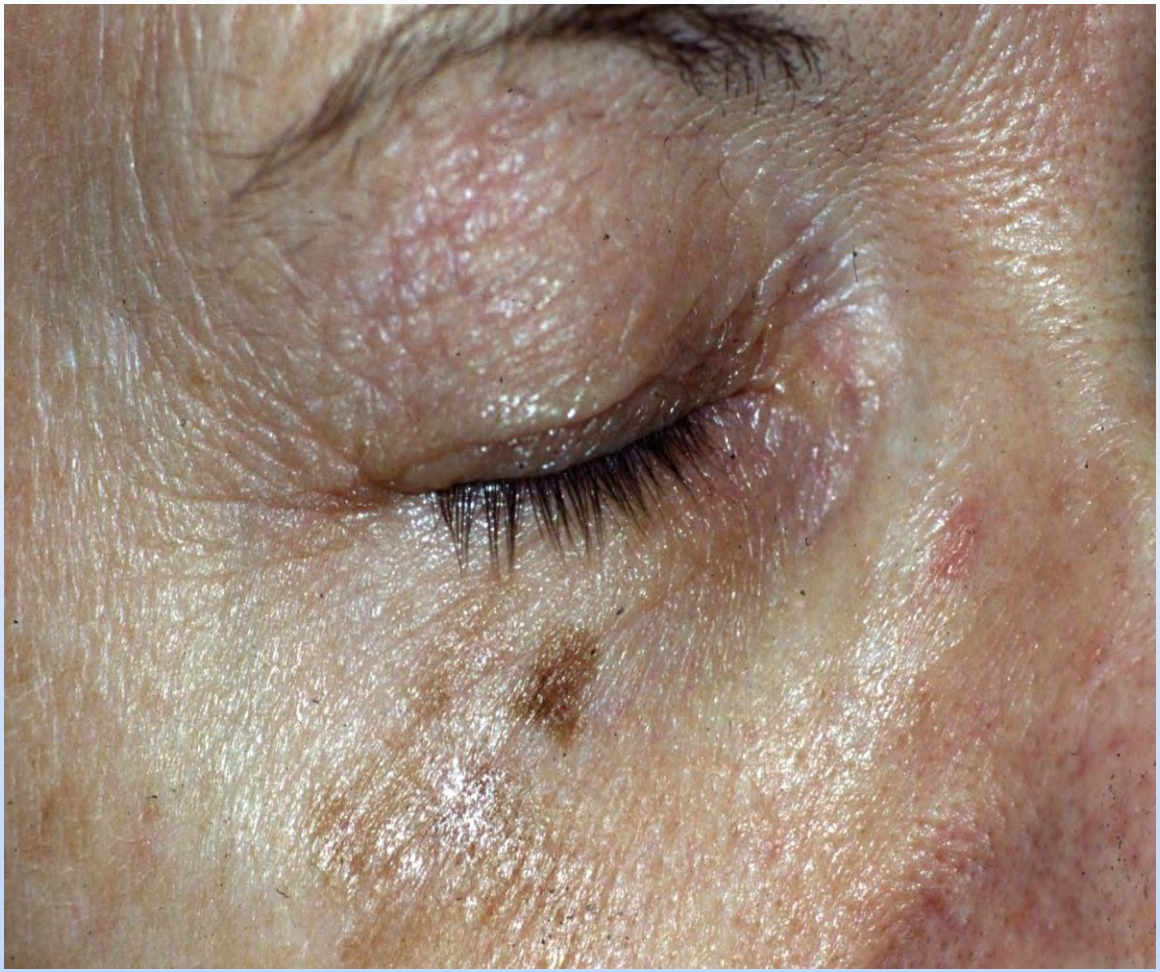
MM in situ: Mohs to conserve skin



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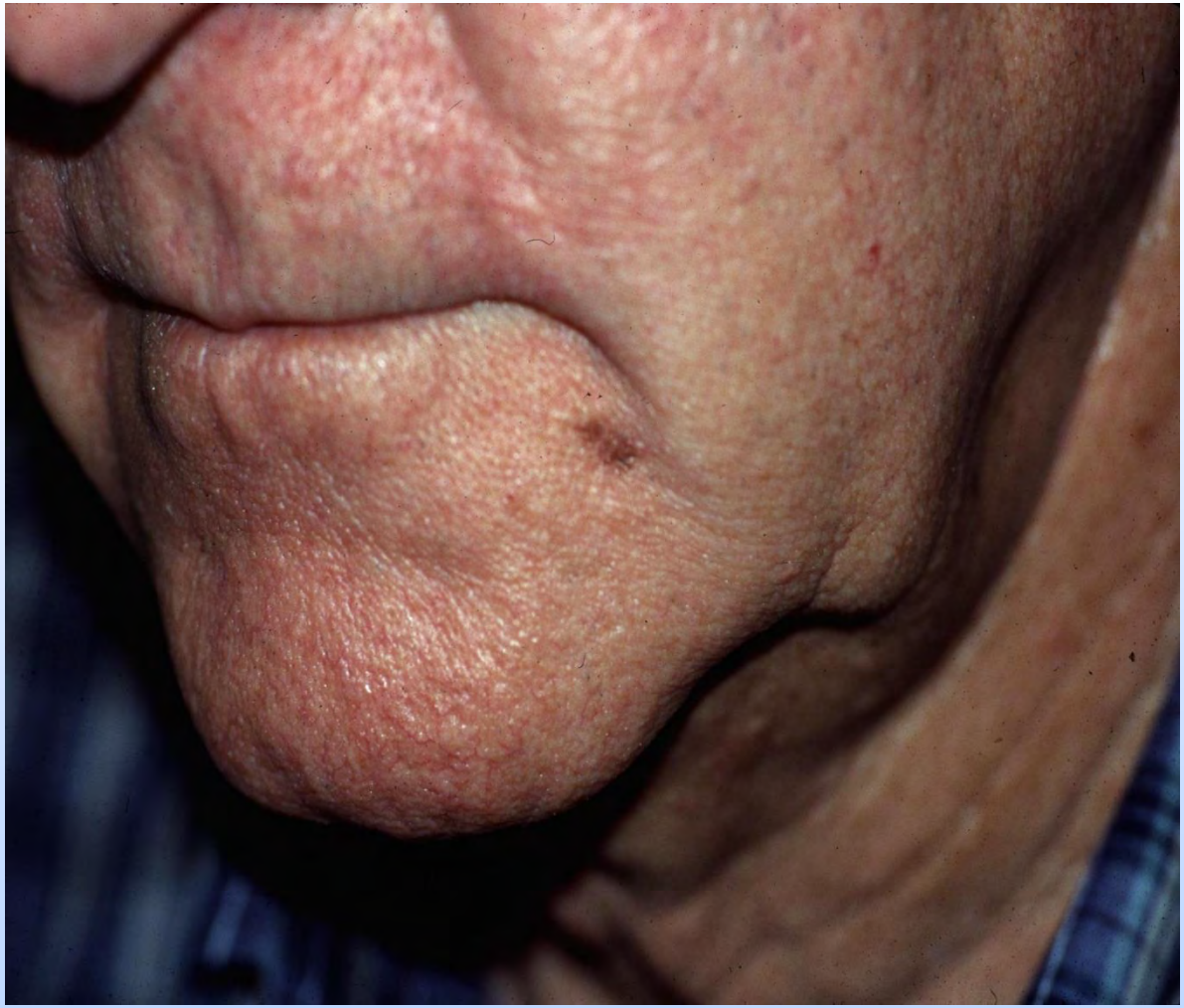
MM in situ: Excision vs Mohs…



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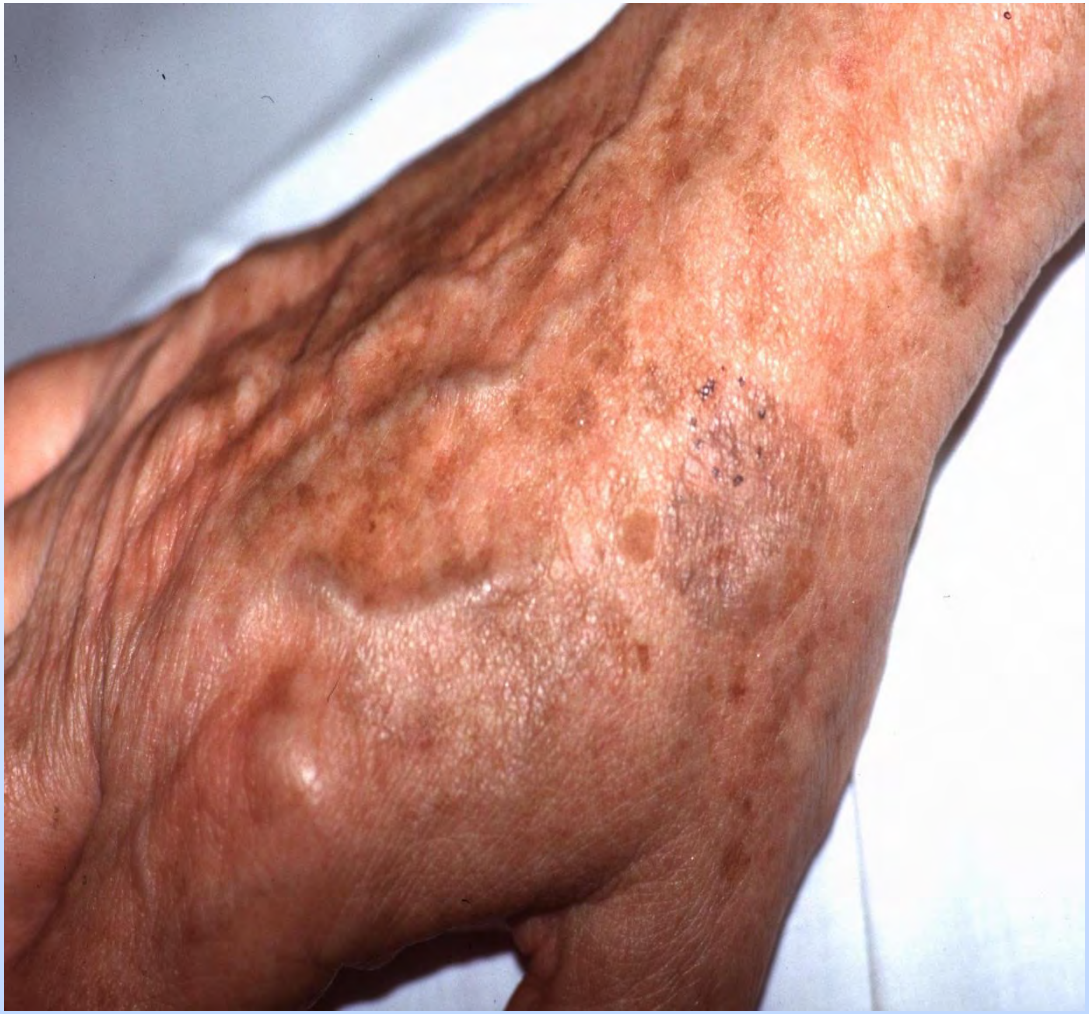
Invasive MM arising in MMIS: Now what?



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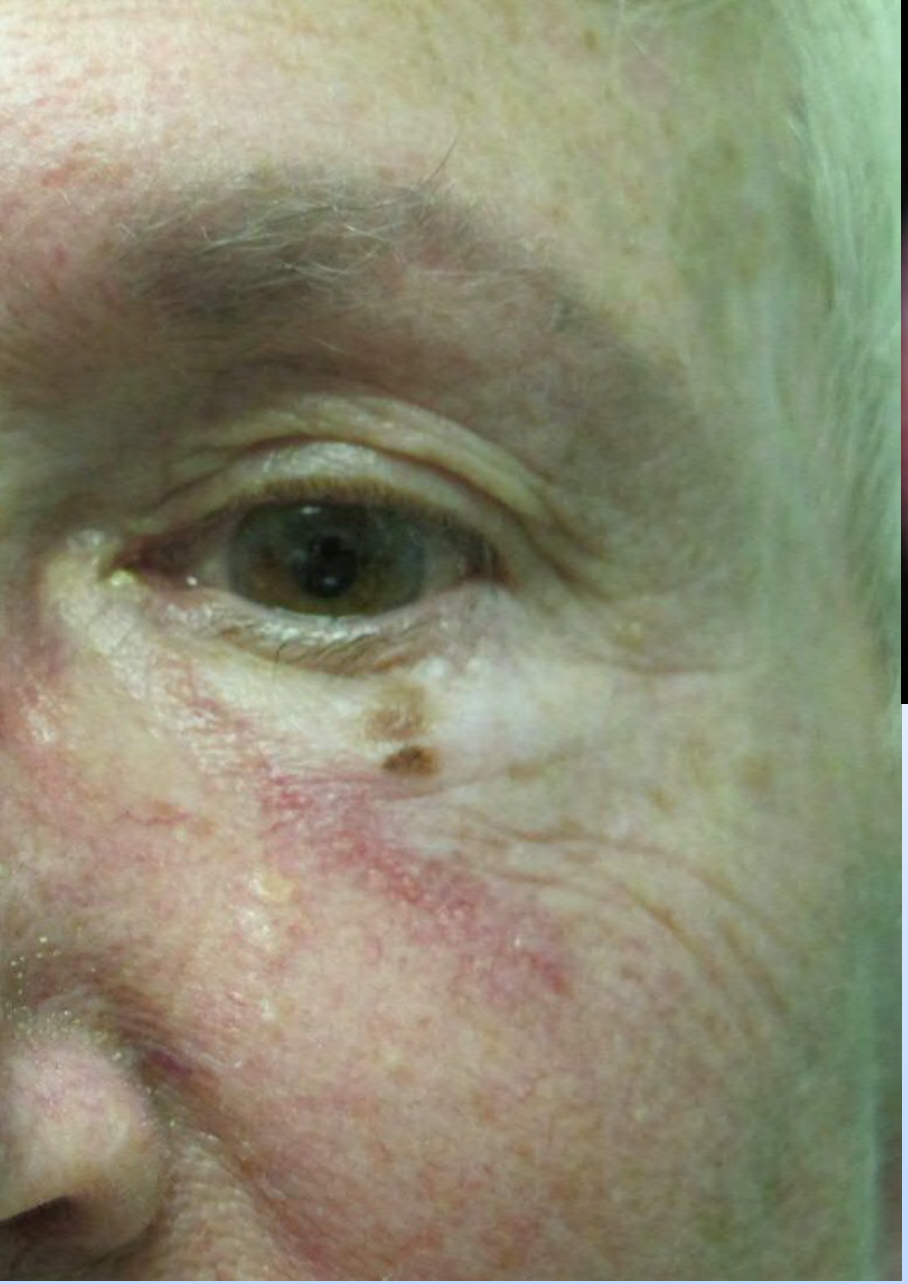

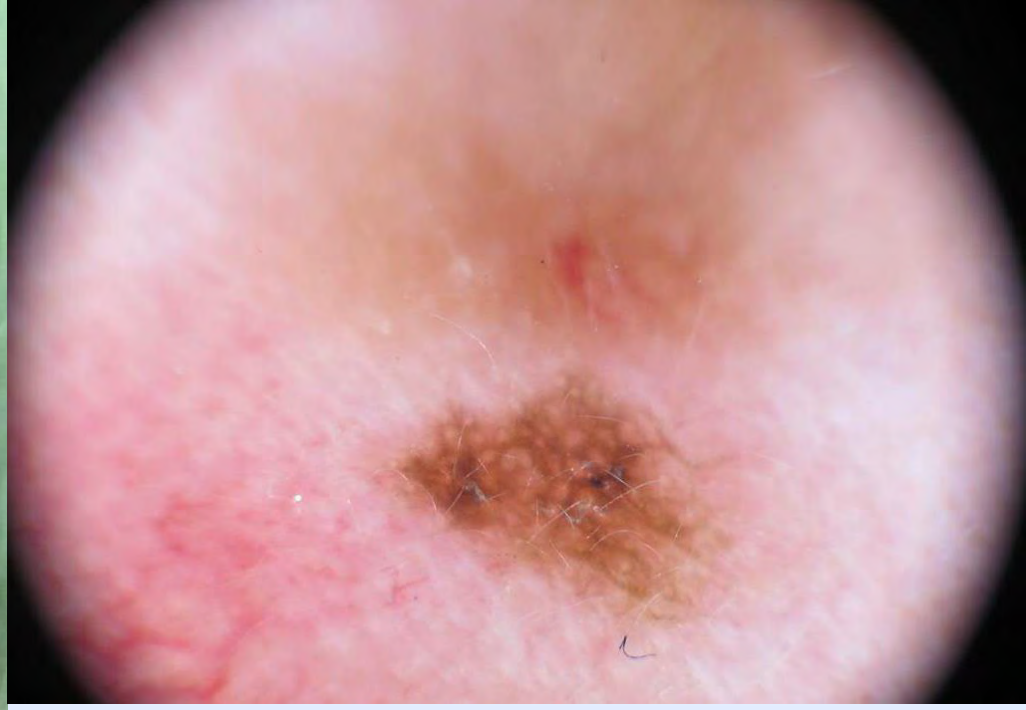

73 y.o. woman with invasive Melanoma 2015, Breslow depth 0.81 mm, 1 mitosis.
ENT excision, small flap, negative SLNB, Residual in situ melanoma at margins; opted for observation.

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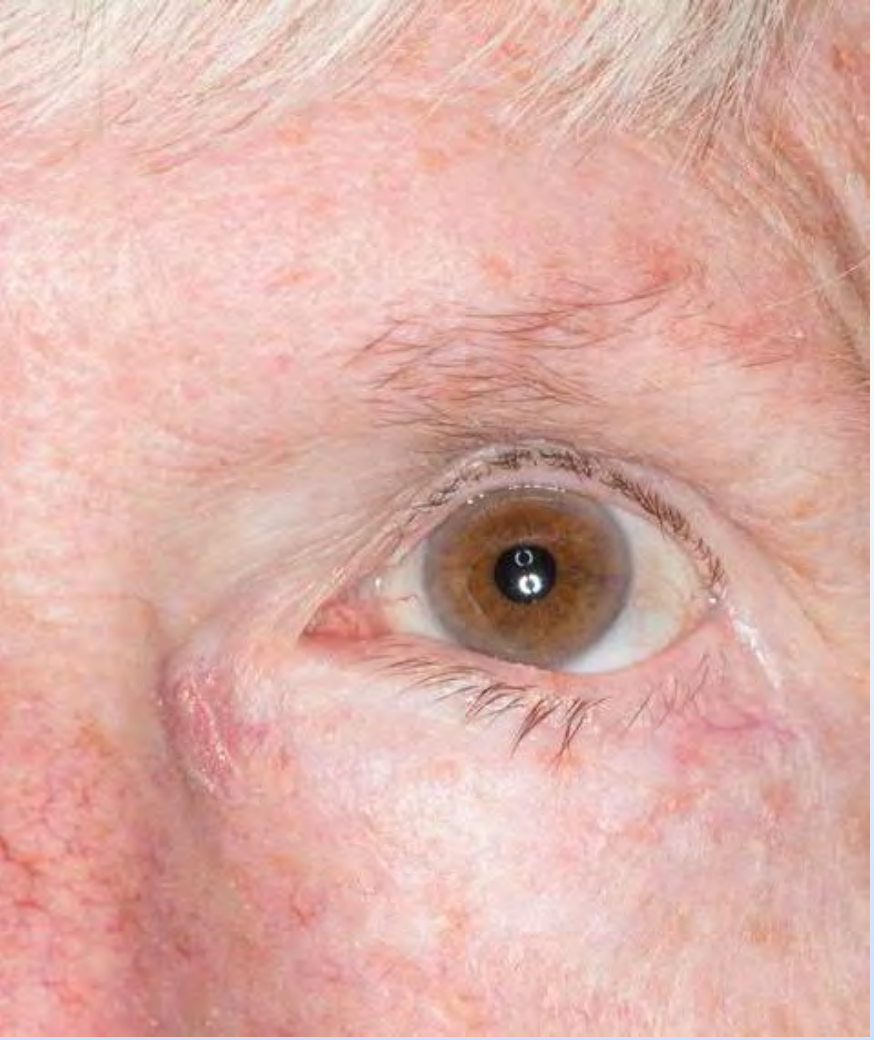

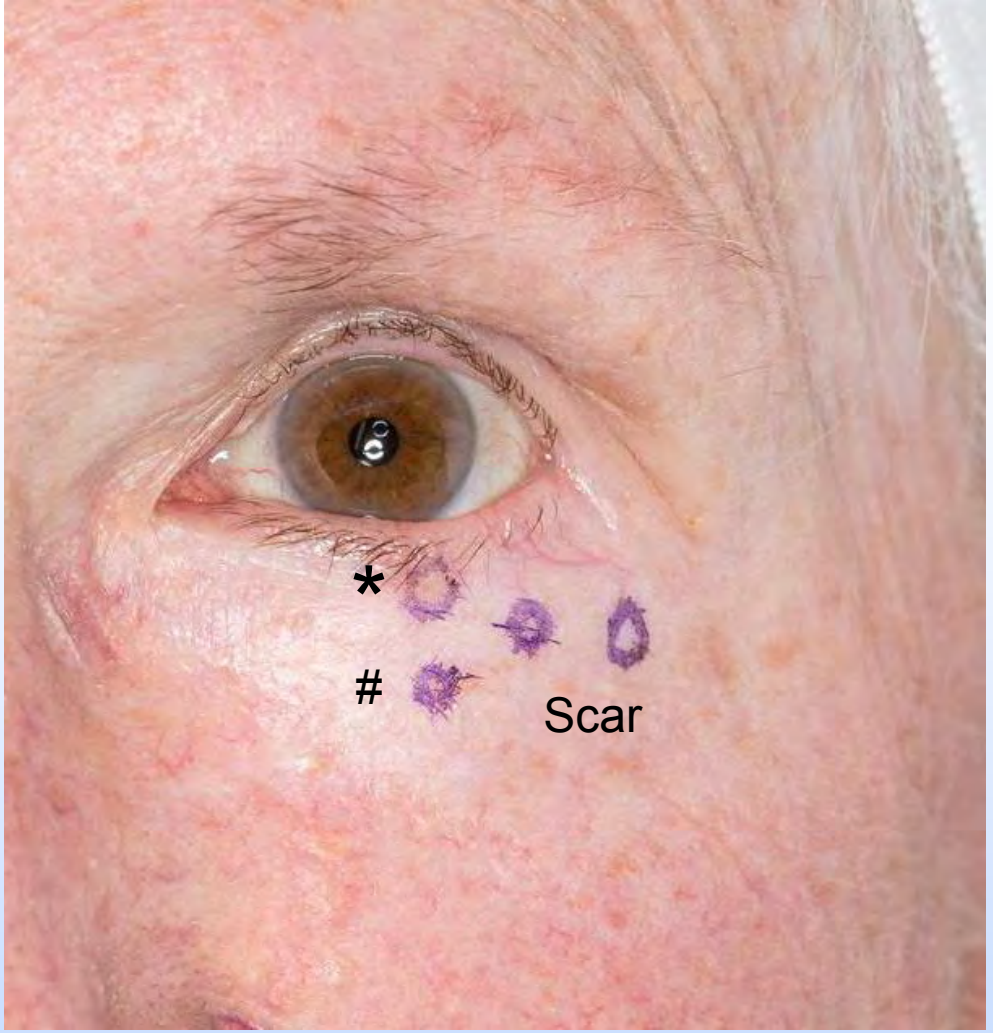

One year later, subtle pink and brown coloration; 2 biopsies showed scar;
1# showed in situ melanoma; 1* showed invasive MM 0.8 mm Breslow depth; Mohs Surgery to assist in tissue conservation around eye (plus minimal clinical signs)

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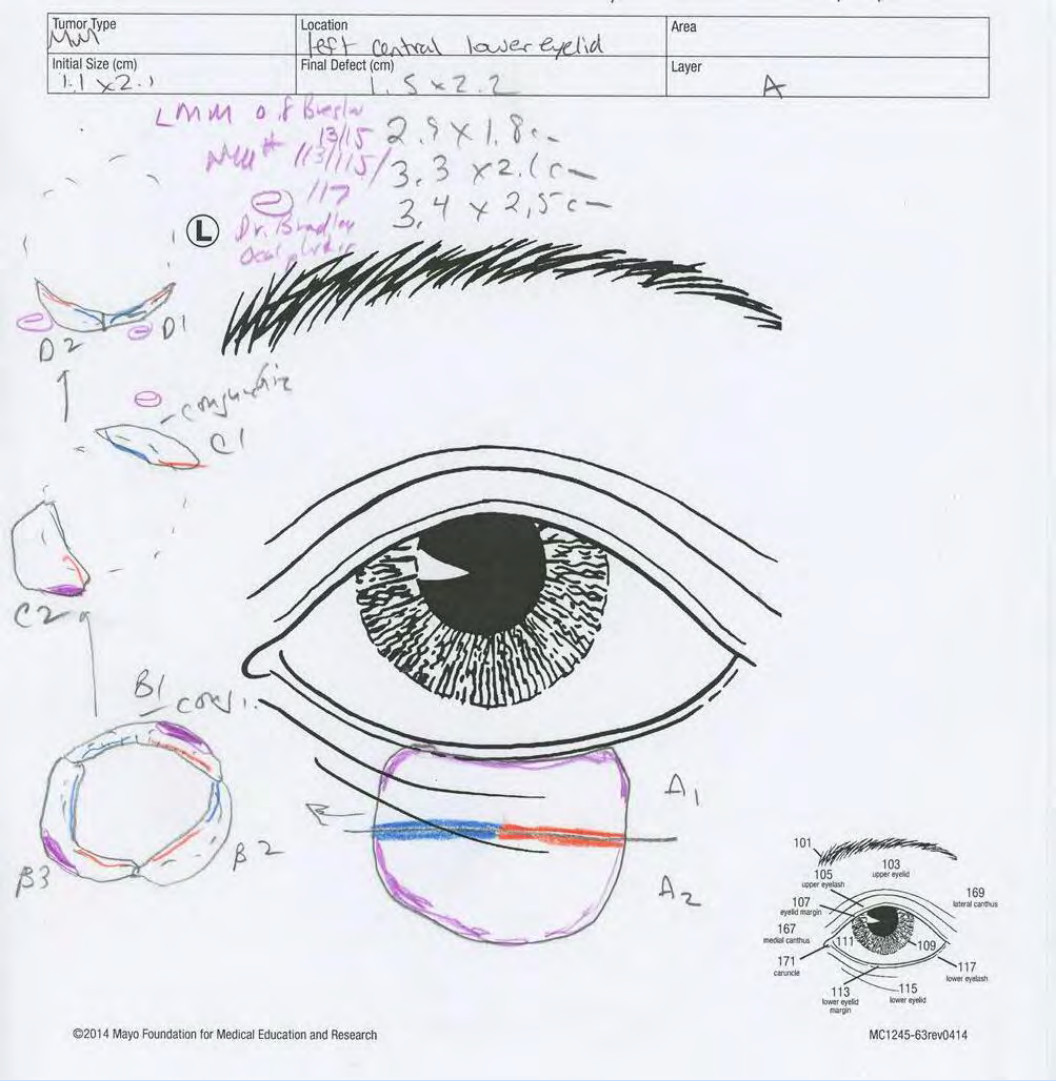

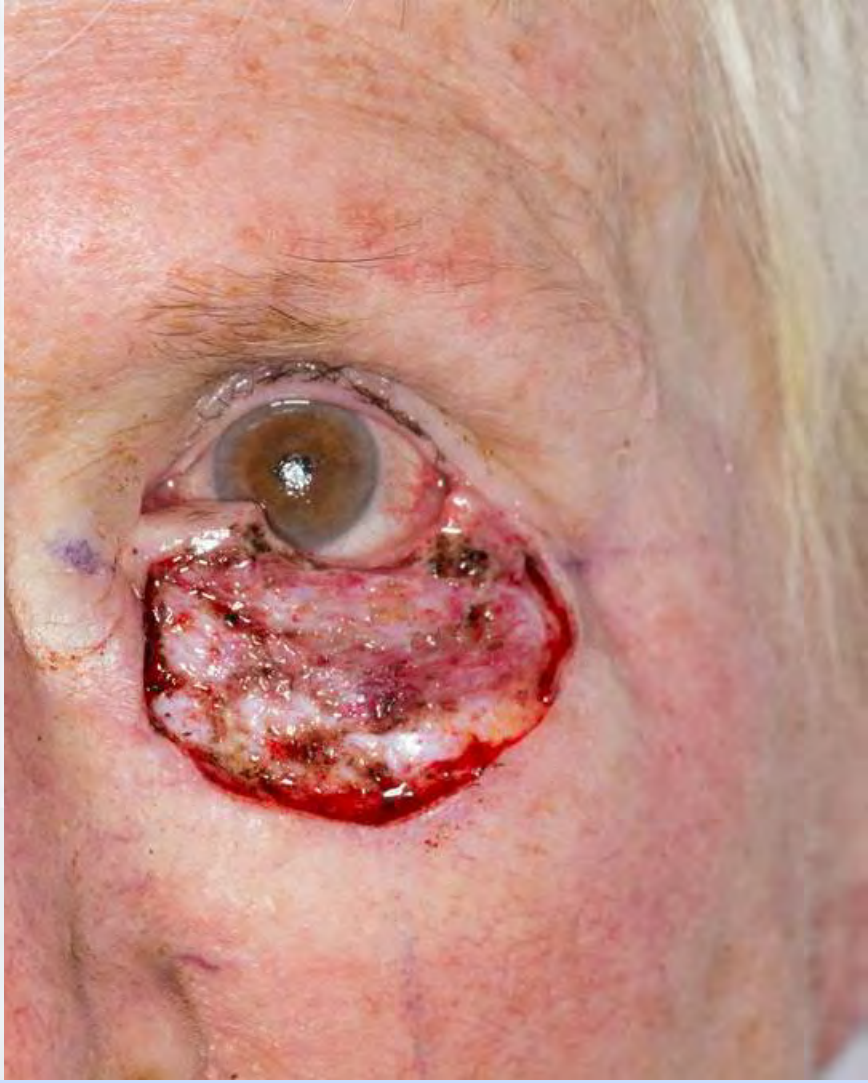

4 stages of Mohs, lots of conjunctival involvement, eyelid sharing procedure for repair

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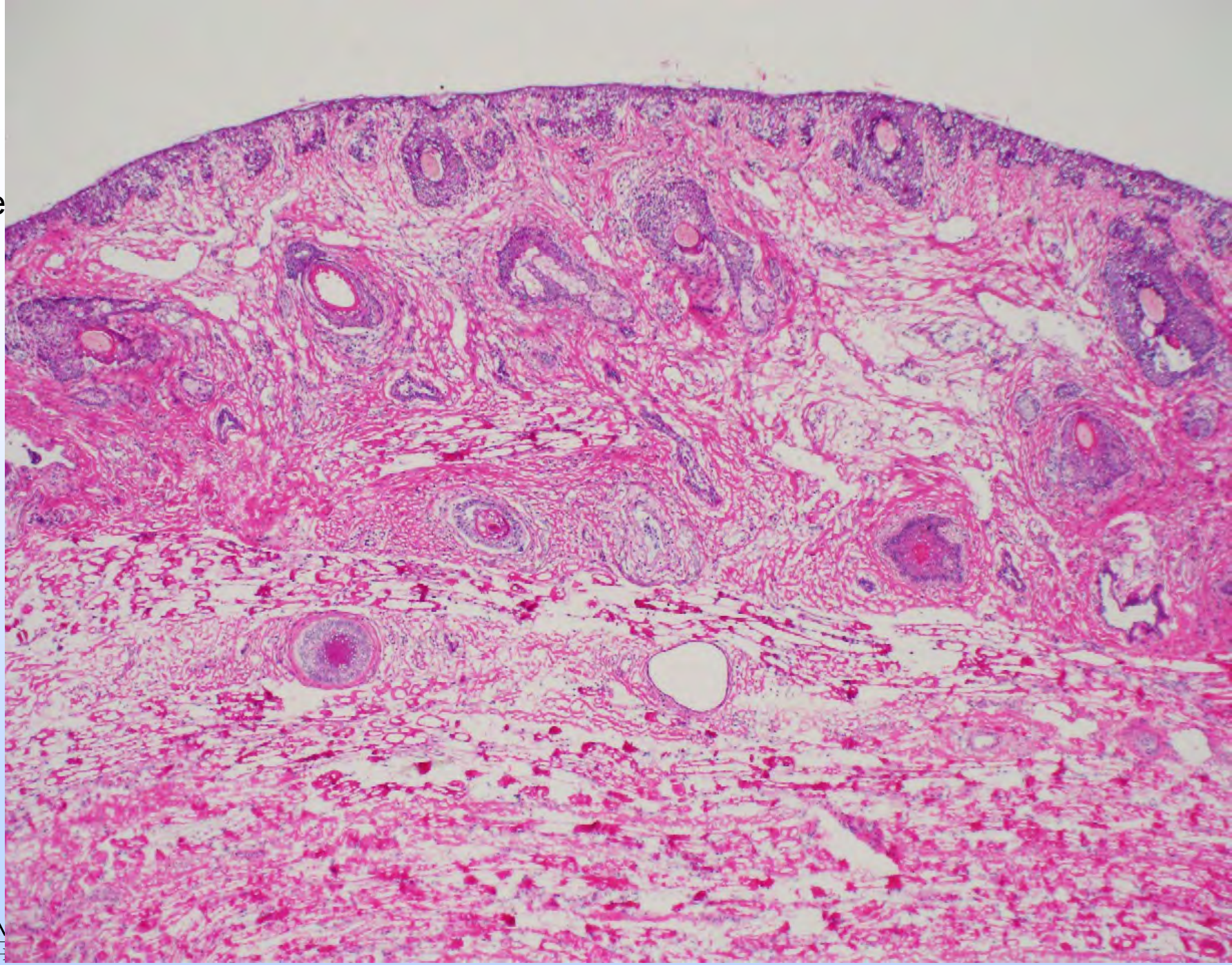
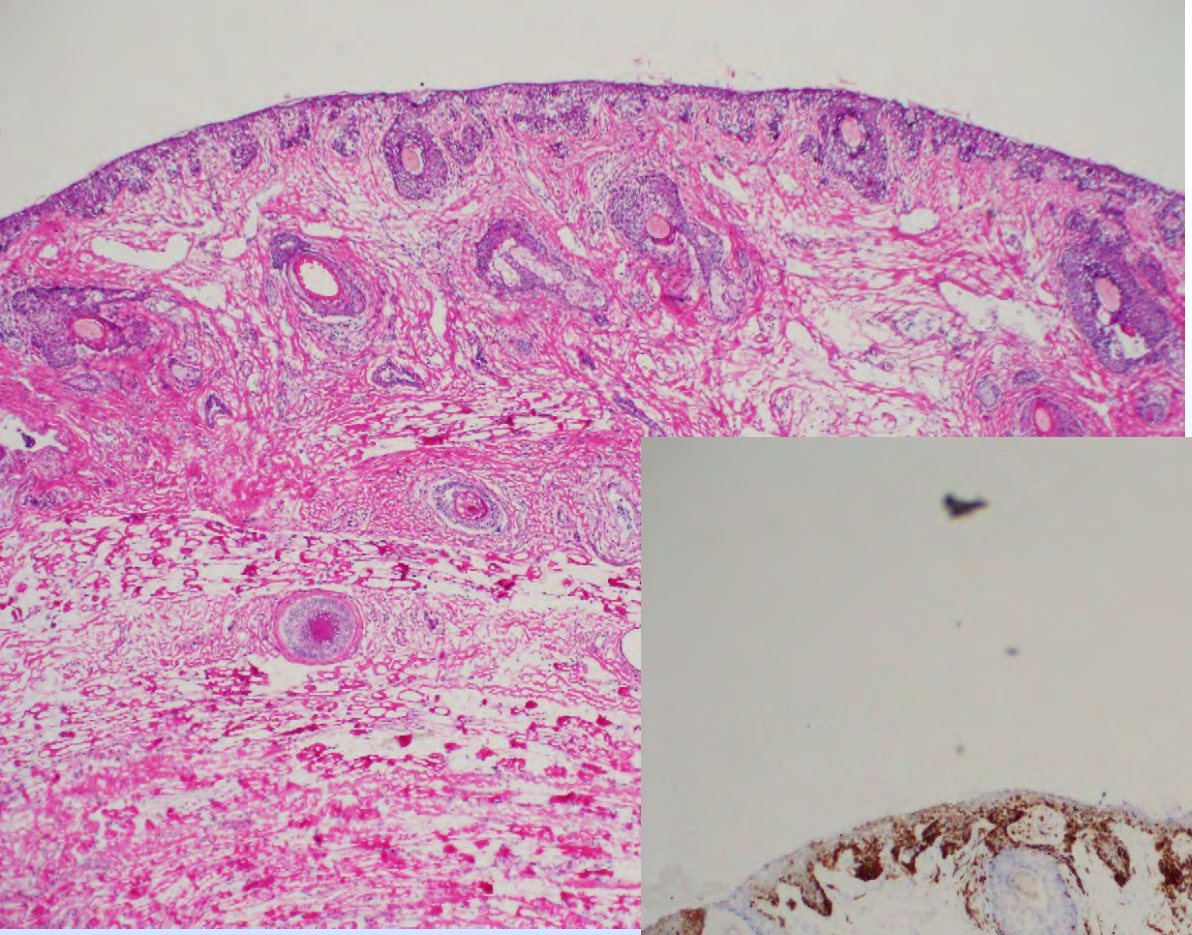
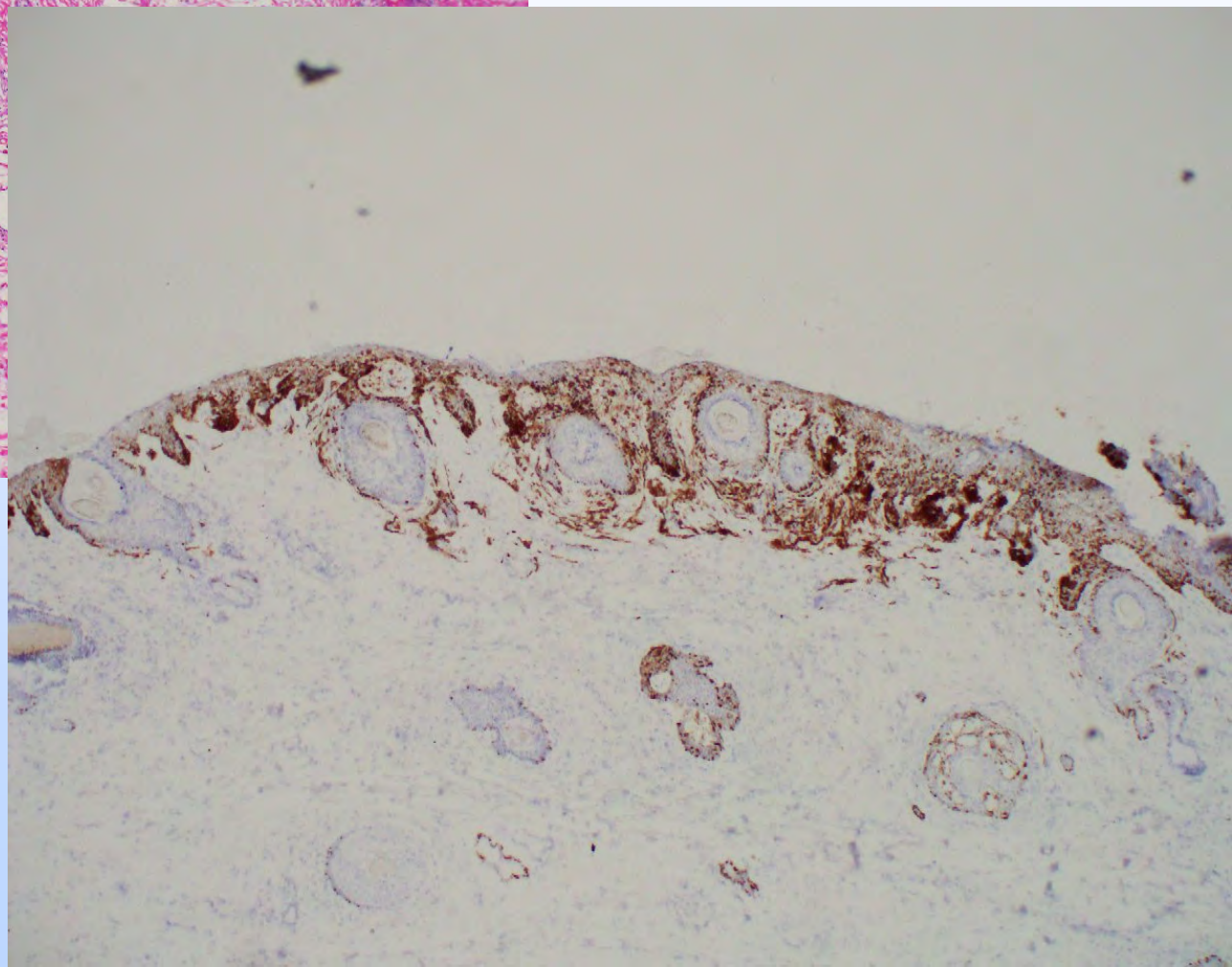
Stage
A1
H&E
4x





Stage
A1
4x
Mart-1



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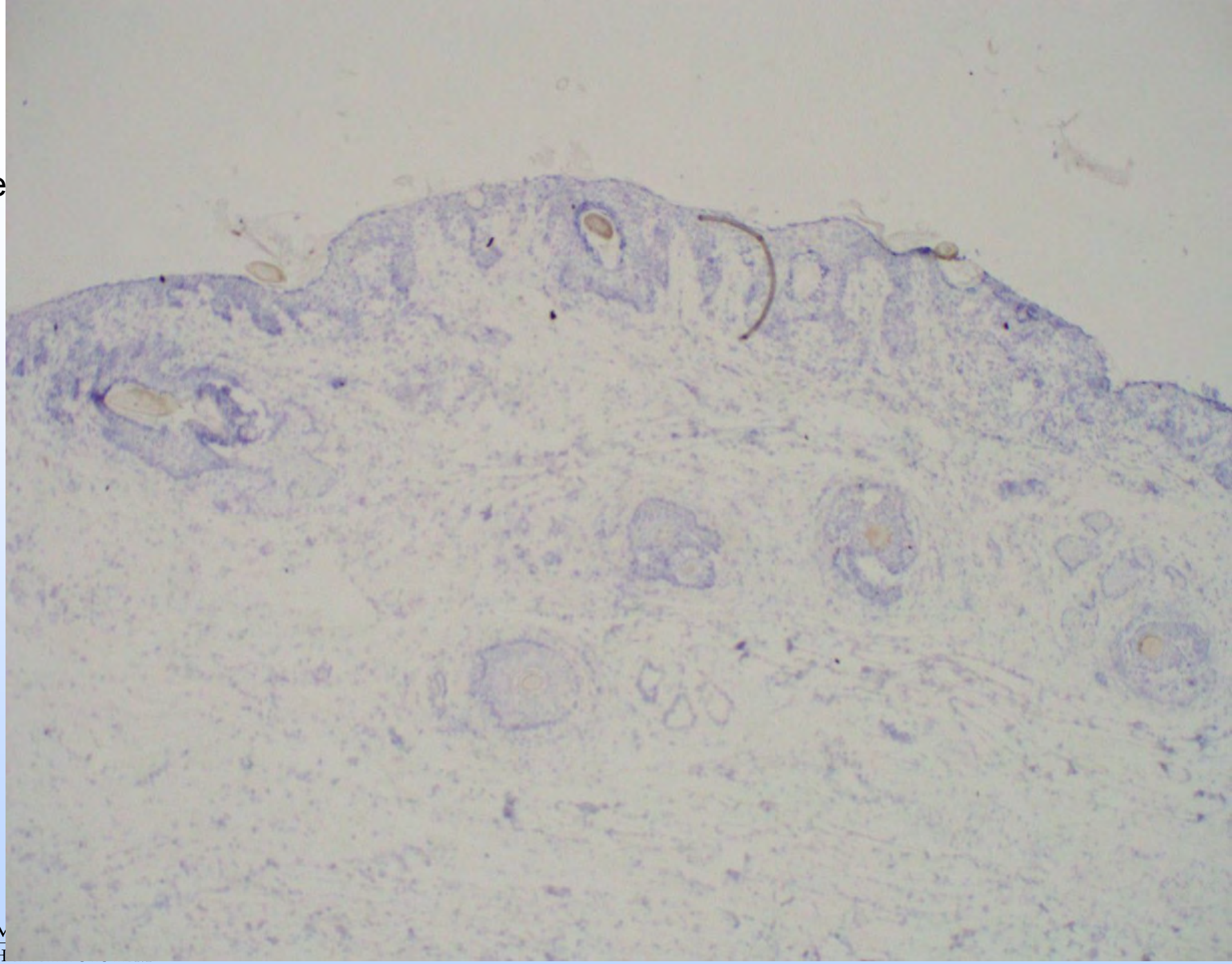
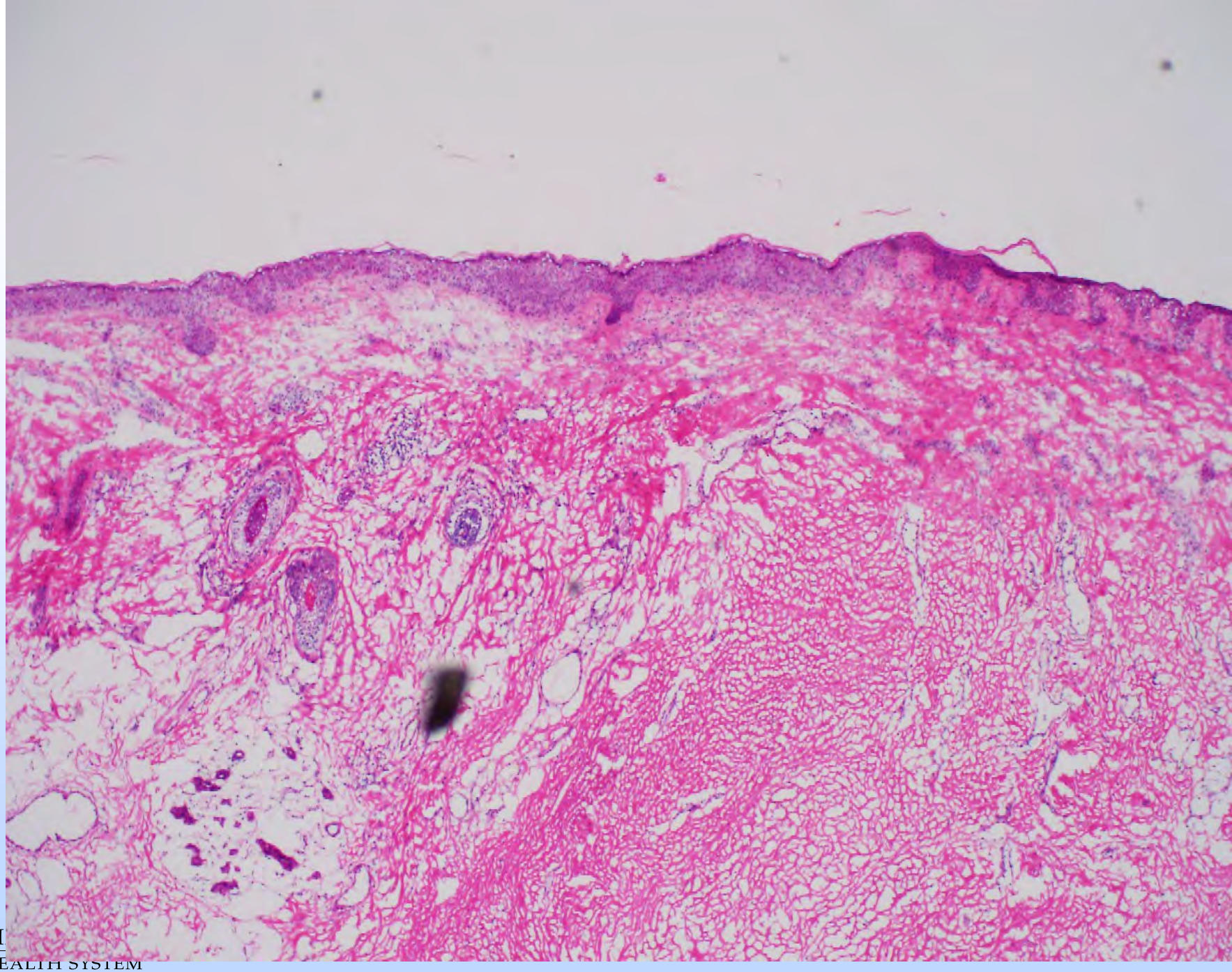
Stage
A1
4x
Neg
Ctrl



Stage
A2
4x



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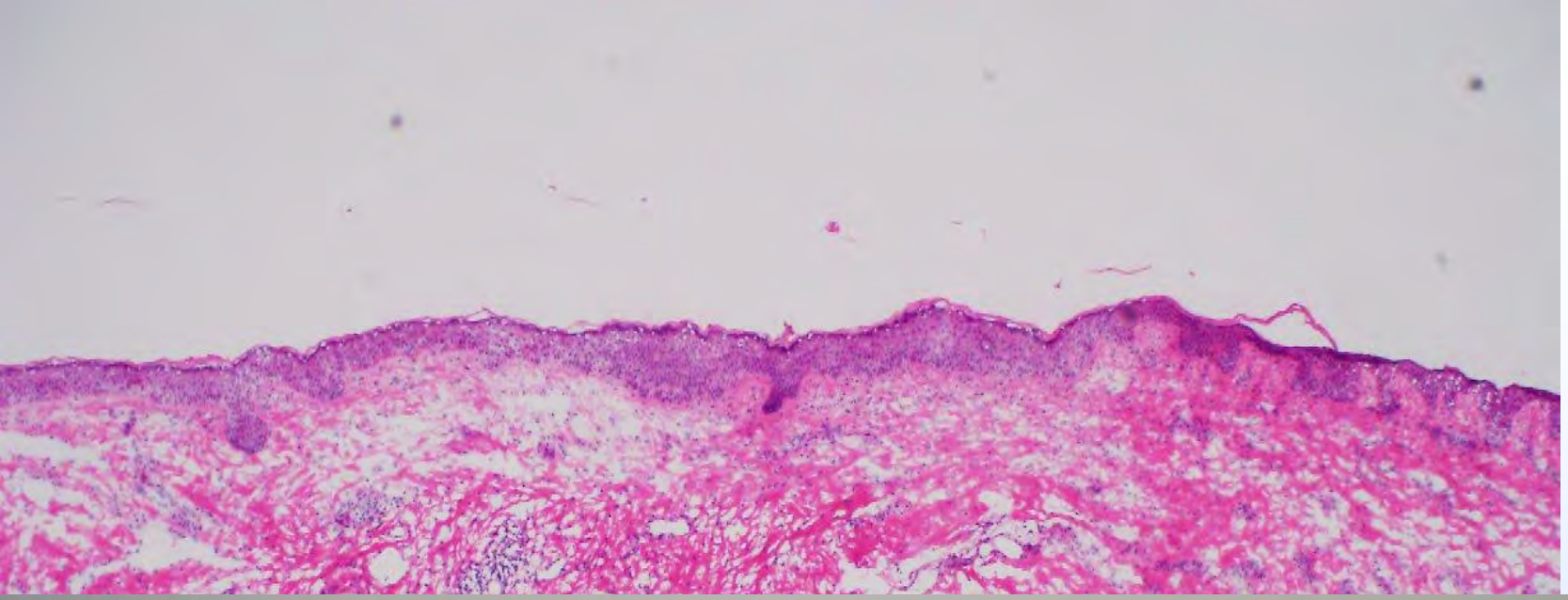
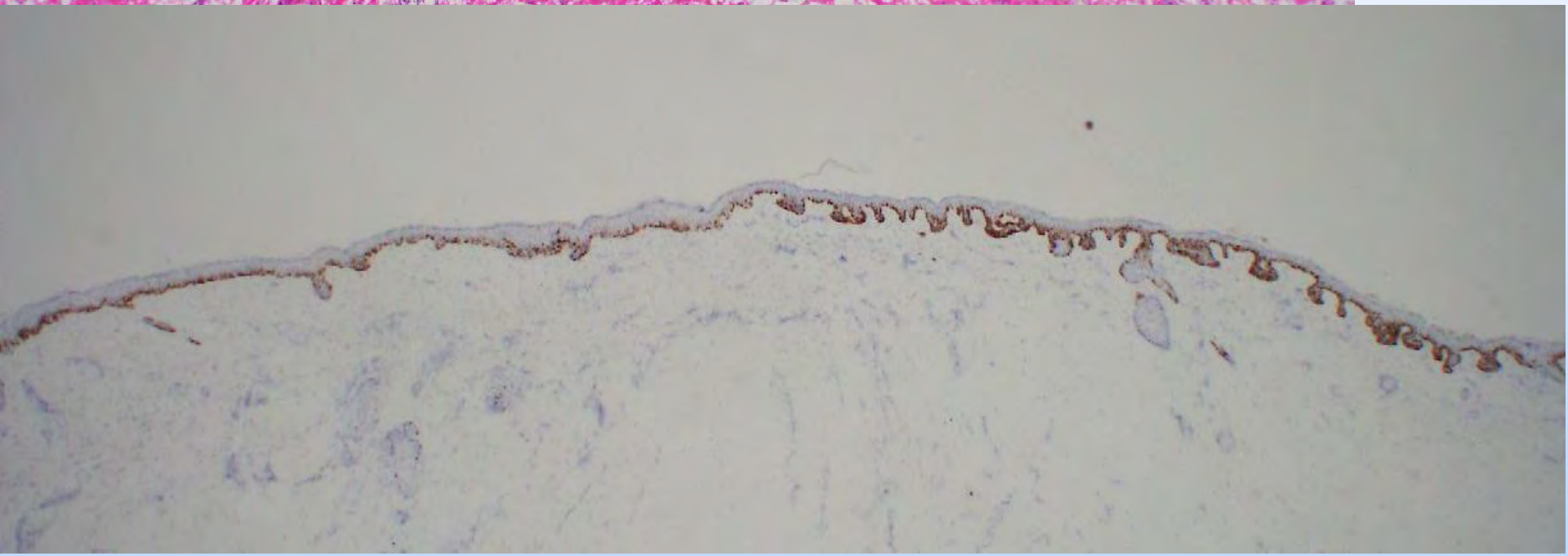


Stage
A2 4x
H&E



Stage
A2 2x
Mart-1

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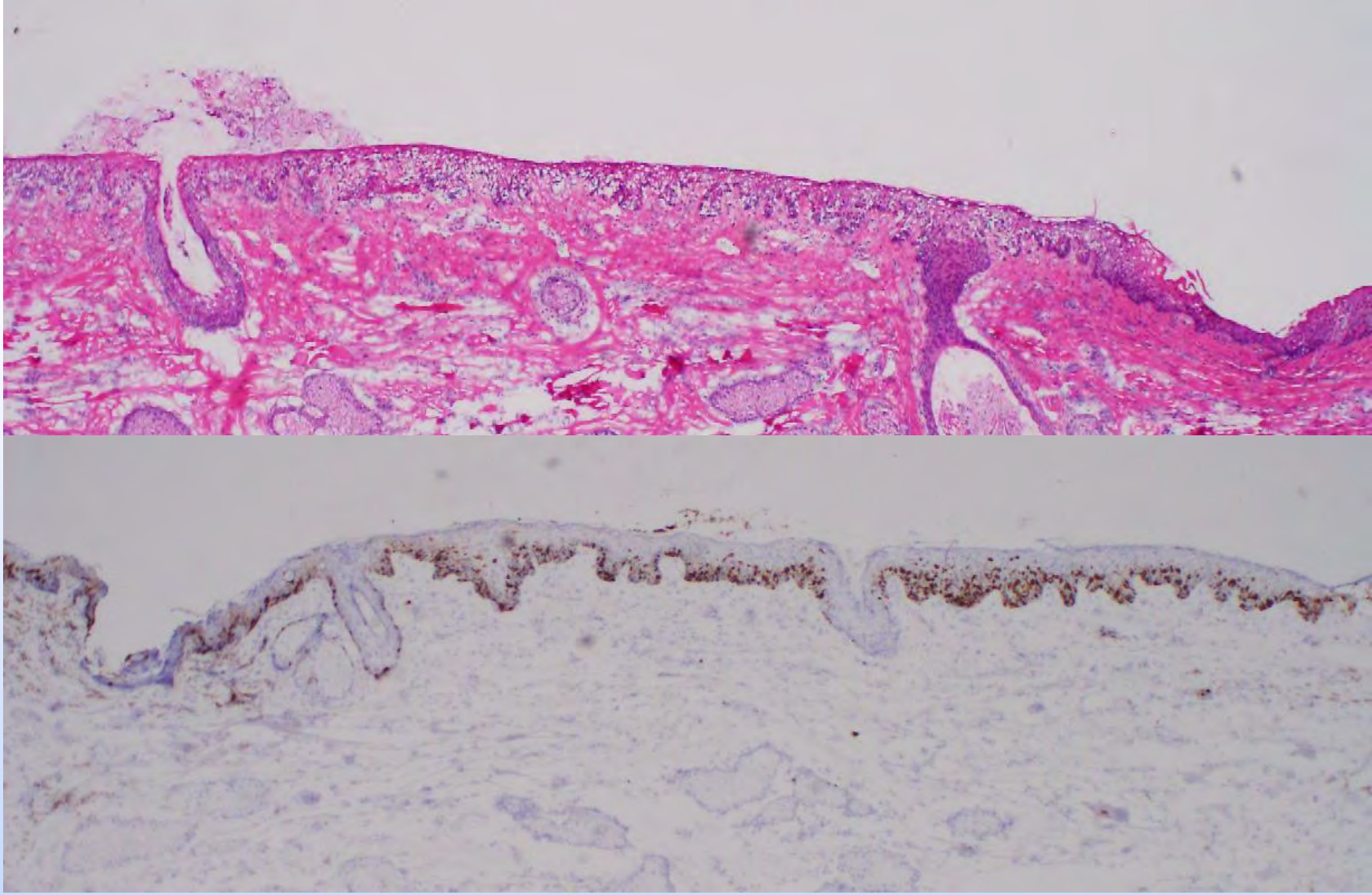
Stage B1 4x H&E Mart-1

Easily Noted MMIS



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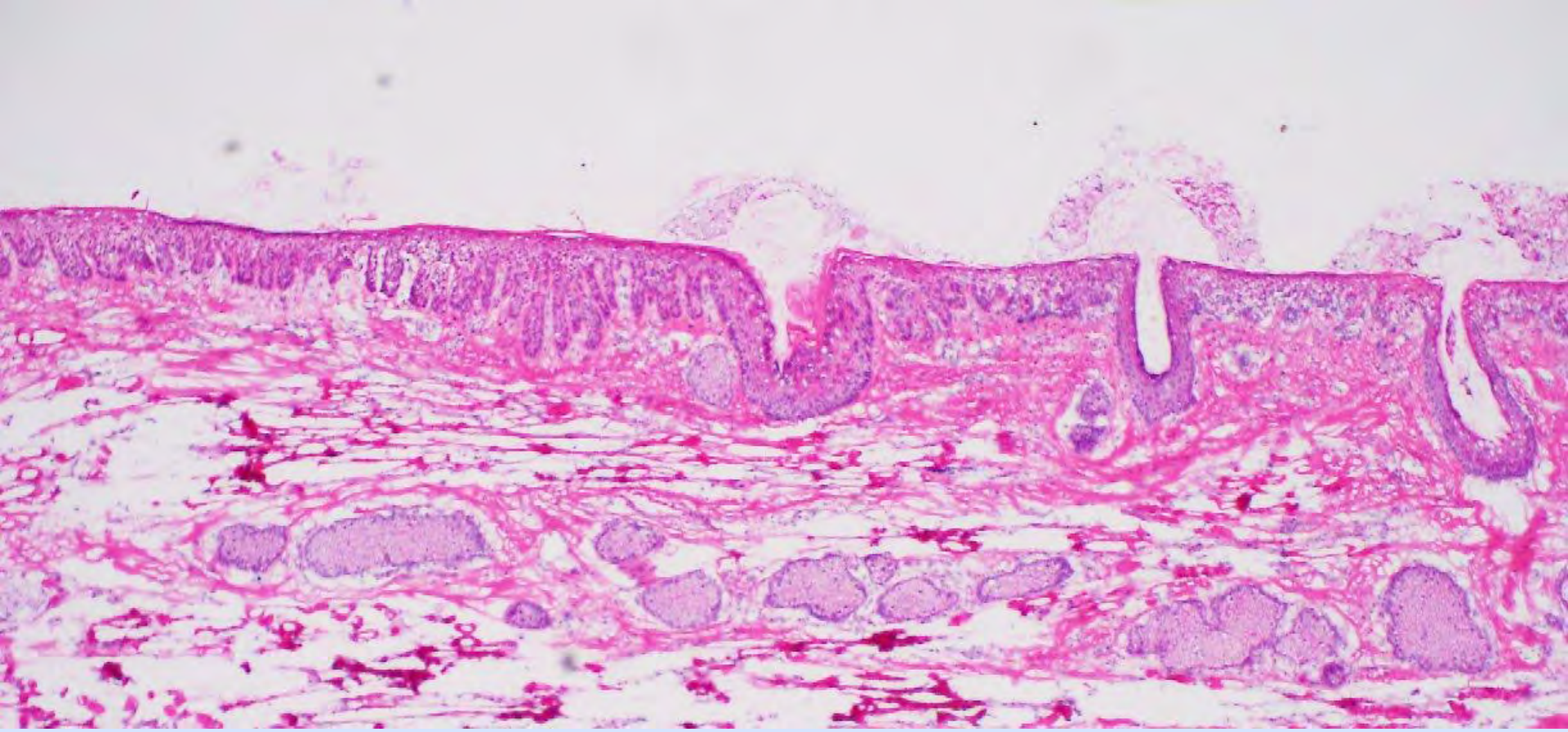

Stage B1 H&E 1x with challenges in viewing nests at lid margin when glandular tissue and freeze changes are mixed nearby

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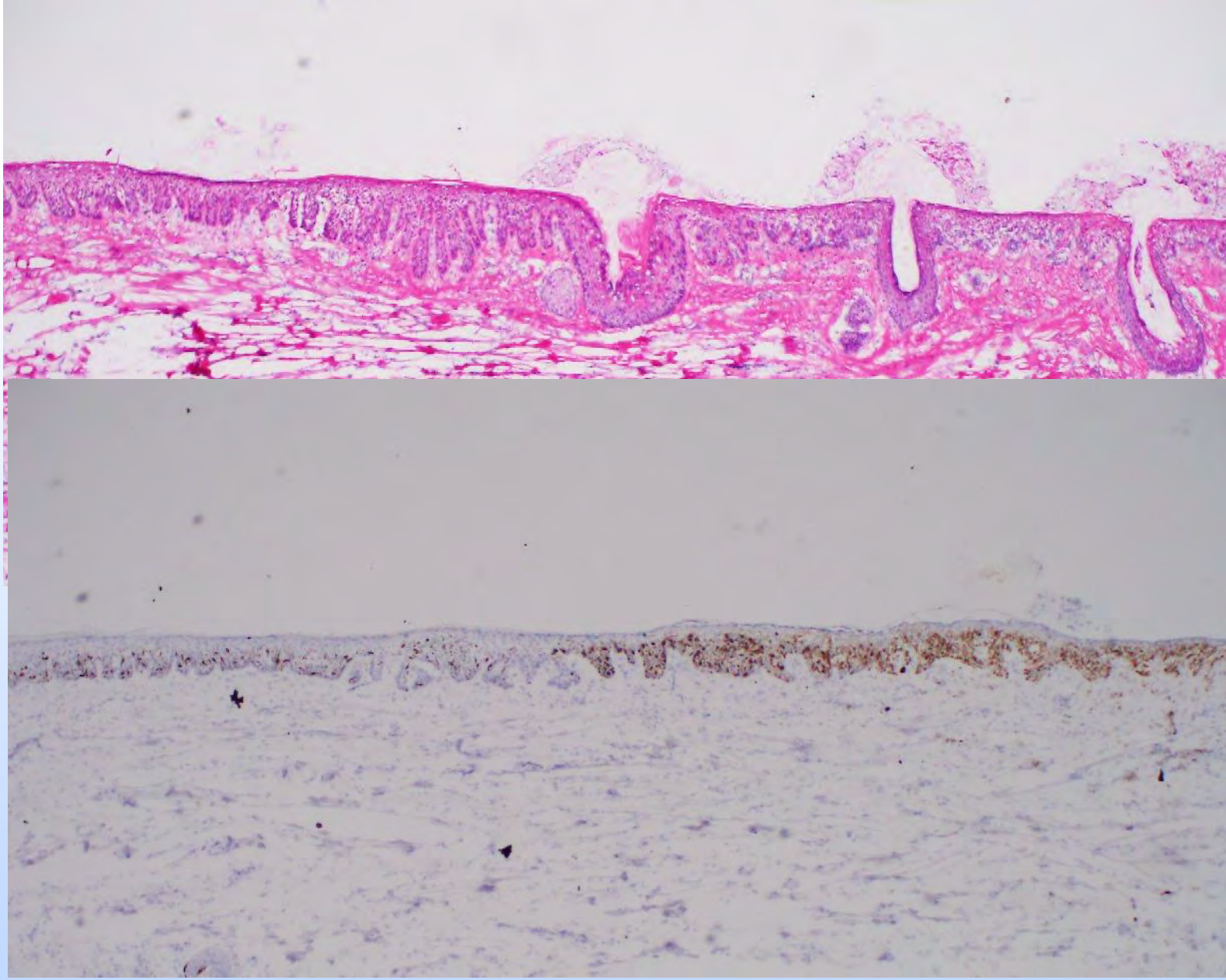
Stage B1 1x H&E and Mart-1

Mart-1 Shows Transition From MMIS to background sun-exposed skin nicely



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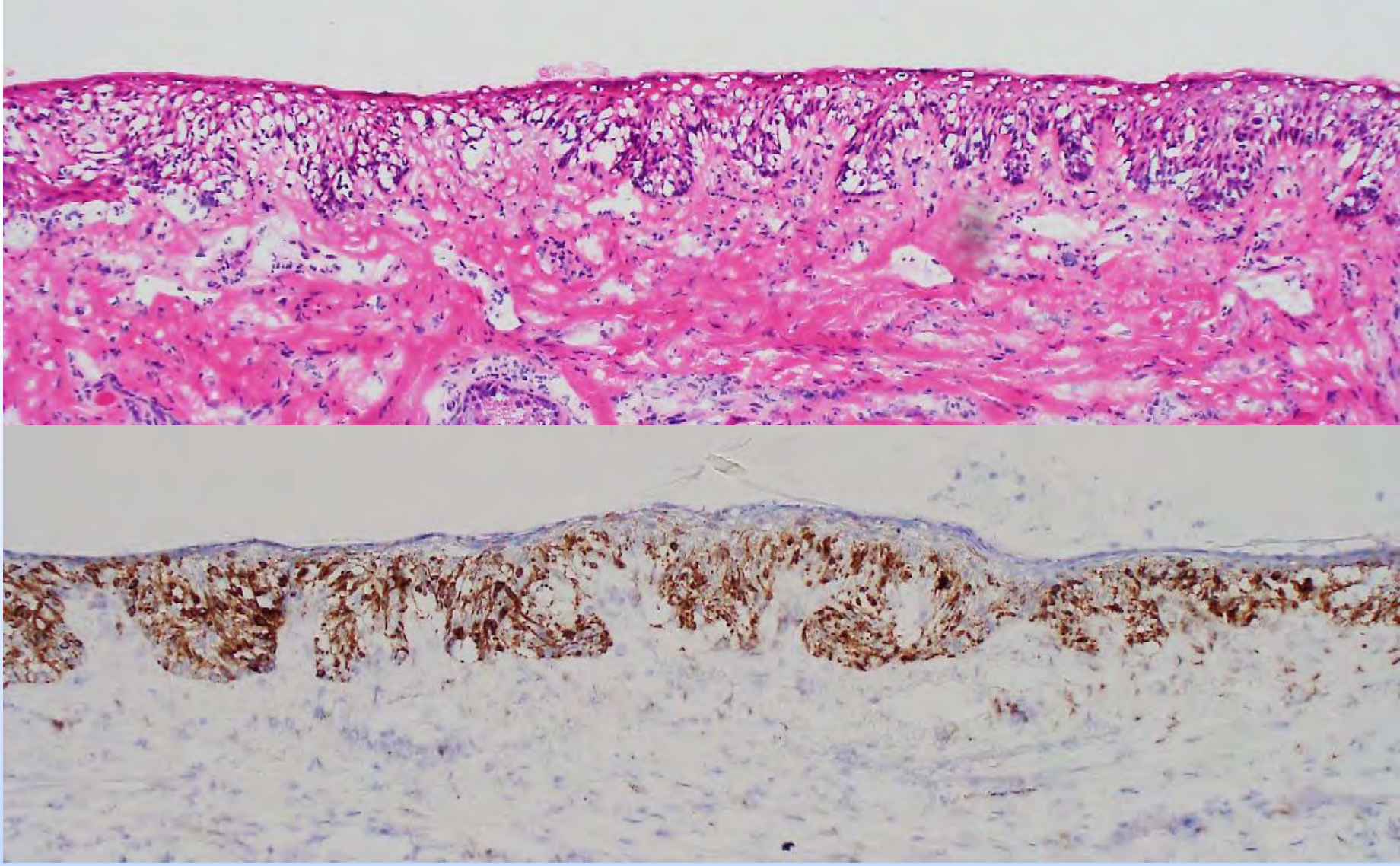

Stage B1 10x H&E with corresponding Mart-1, eyelid margin

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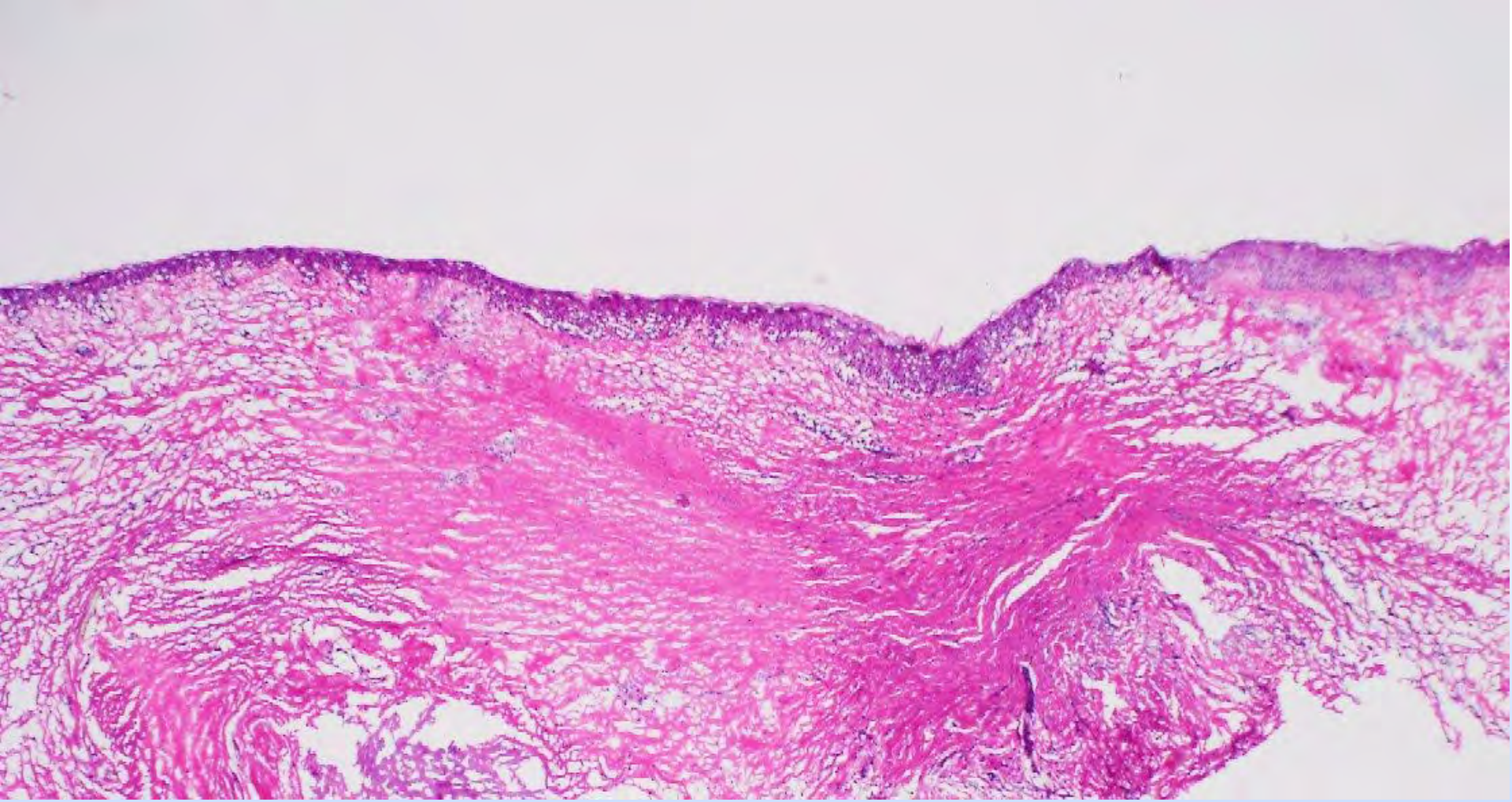

Stage B3 H&E 1x with cells suspicious for MMIS

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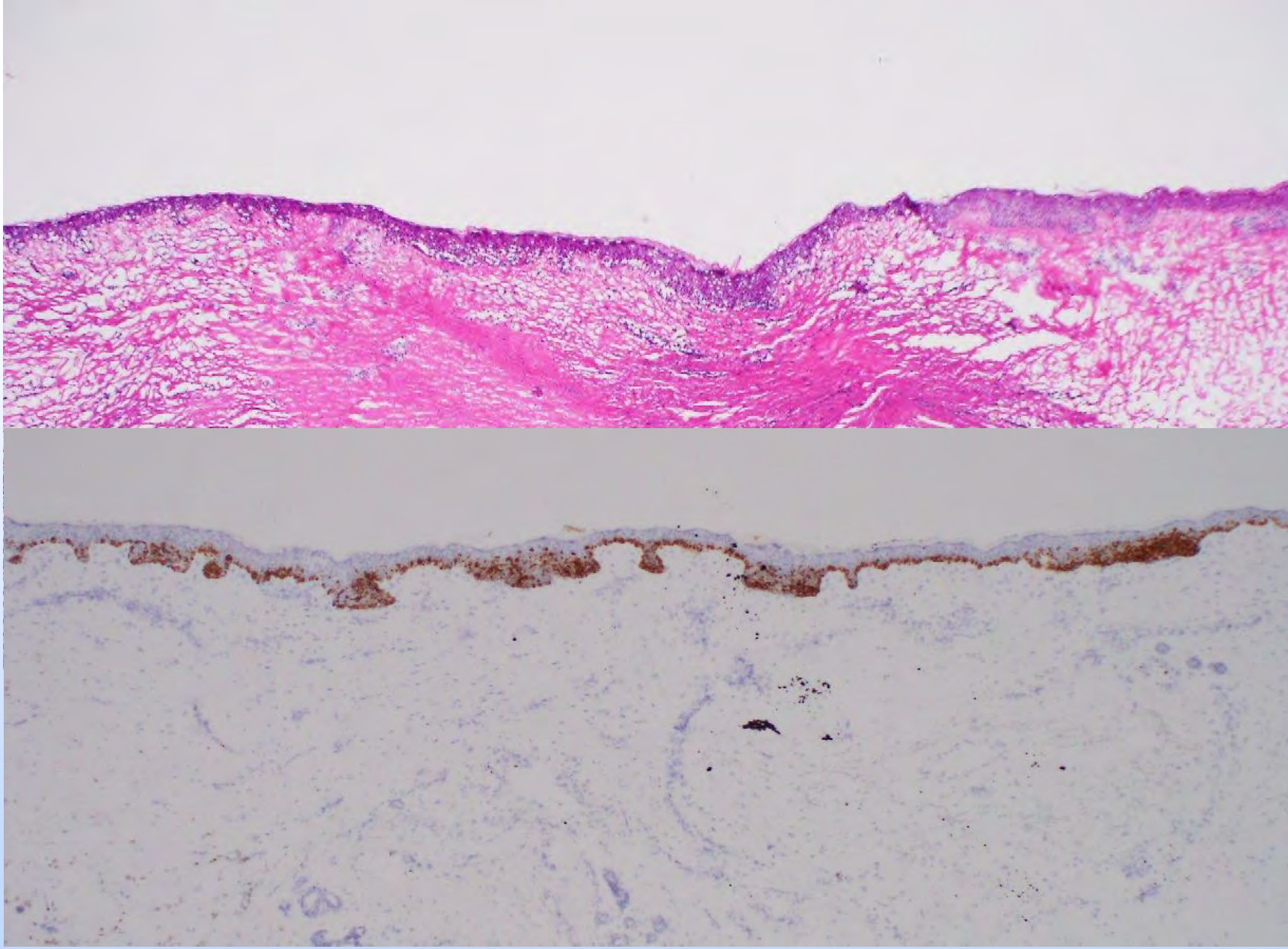
B3 4x
H&E
Mart-1

MMIS
fairly
obvious



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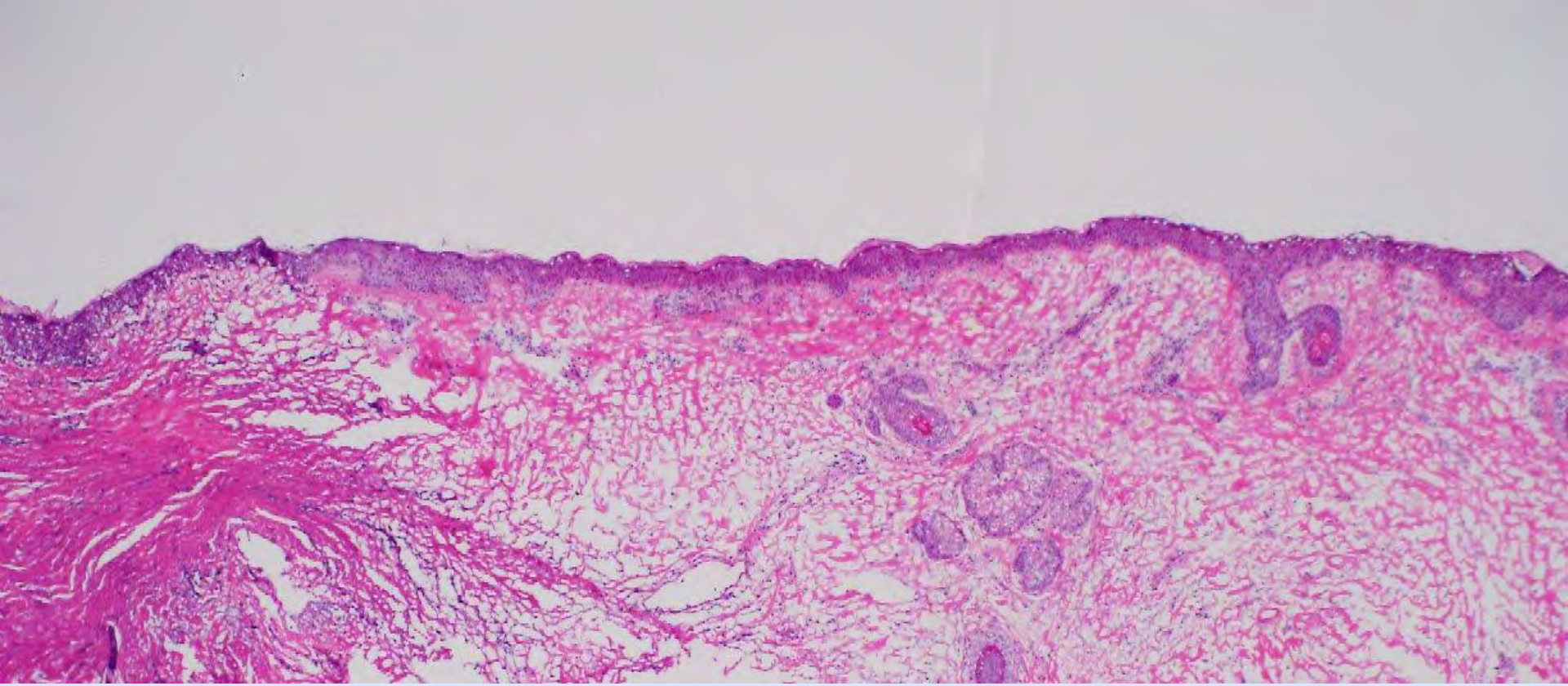

Different area of Stage B3, with suspicious changes to the left, but where does the process end or transition to normal sun-exposed skin?

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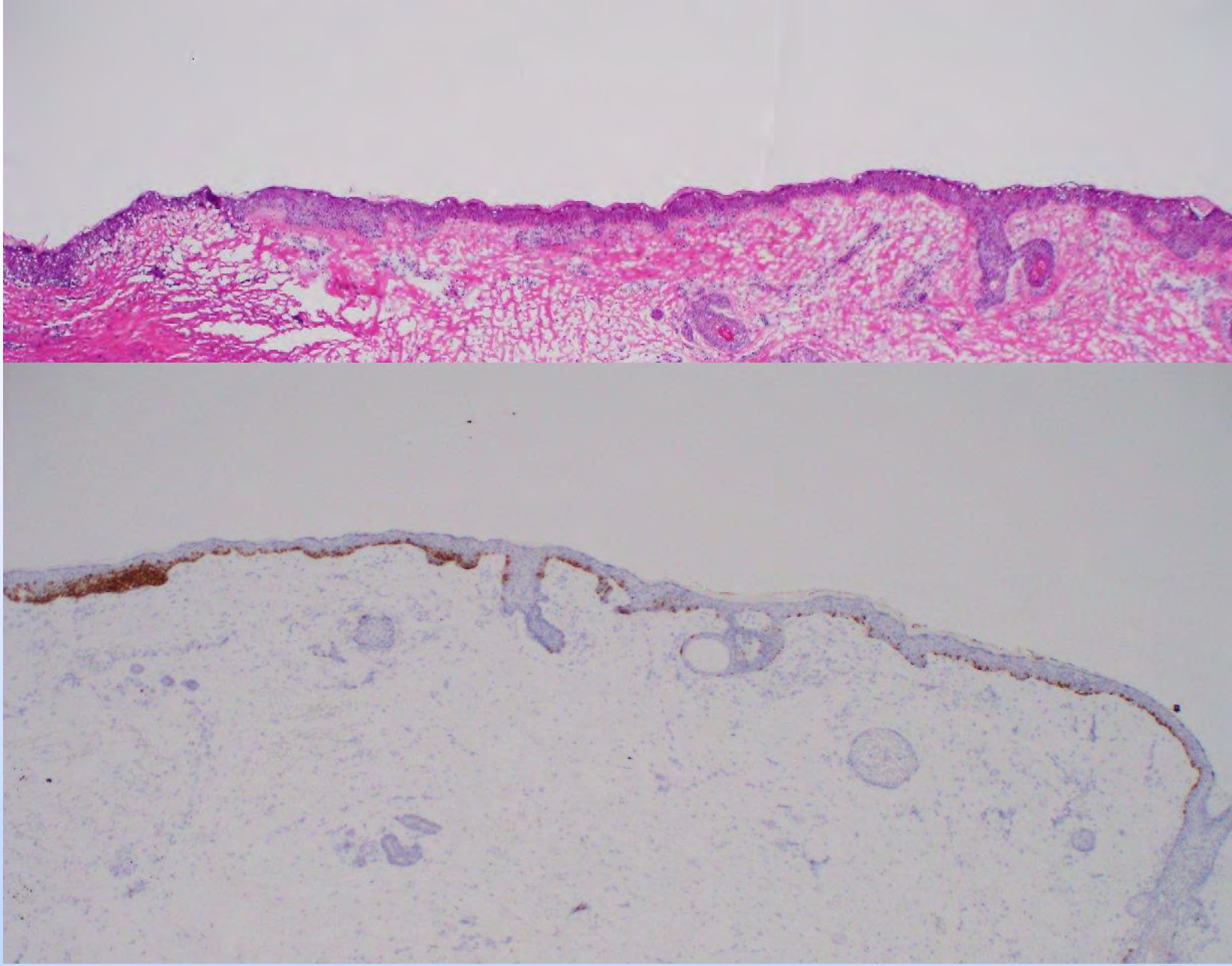
Stage B3
4x
H&E
Mart-1

MMIS and sun-exposed normal challenging but Mart-1 helps greatly



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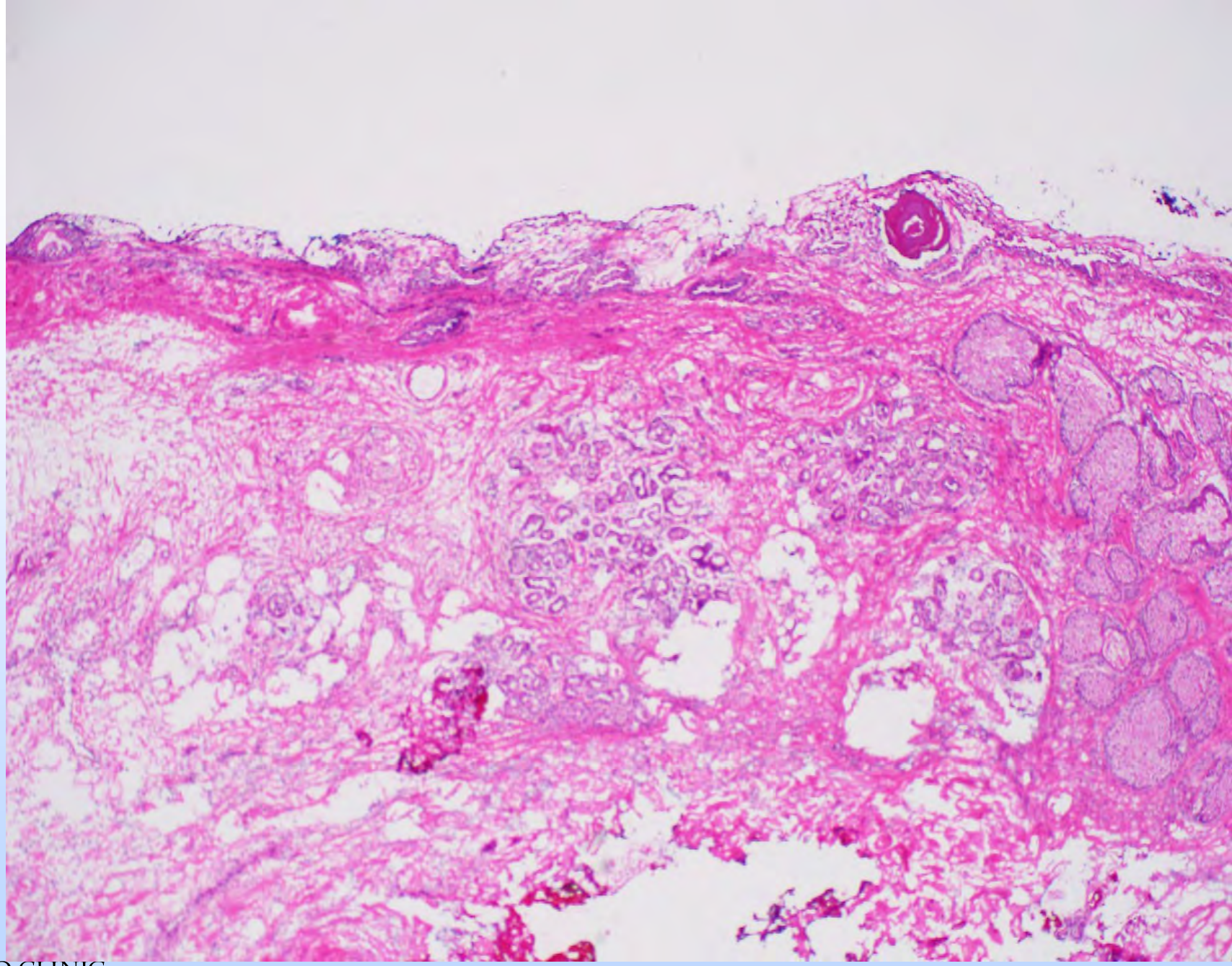
Stage C1 4x H&E

Mucosa conjunc. very difficult



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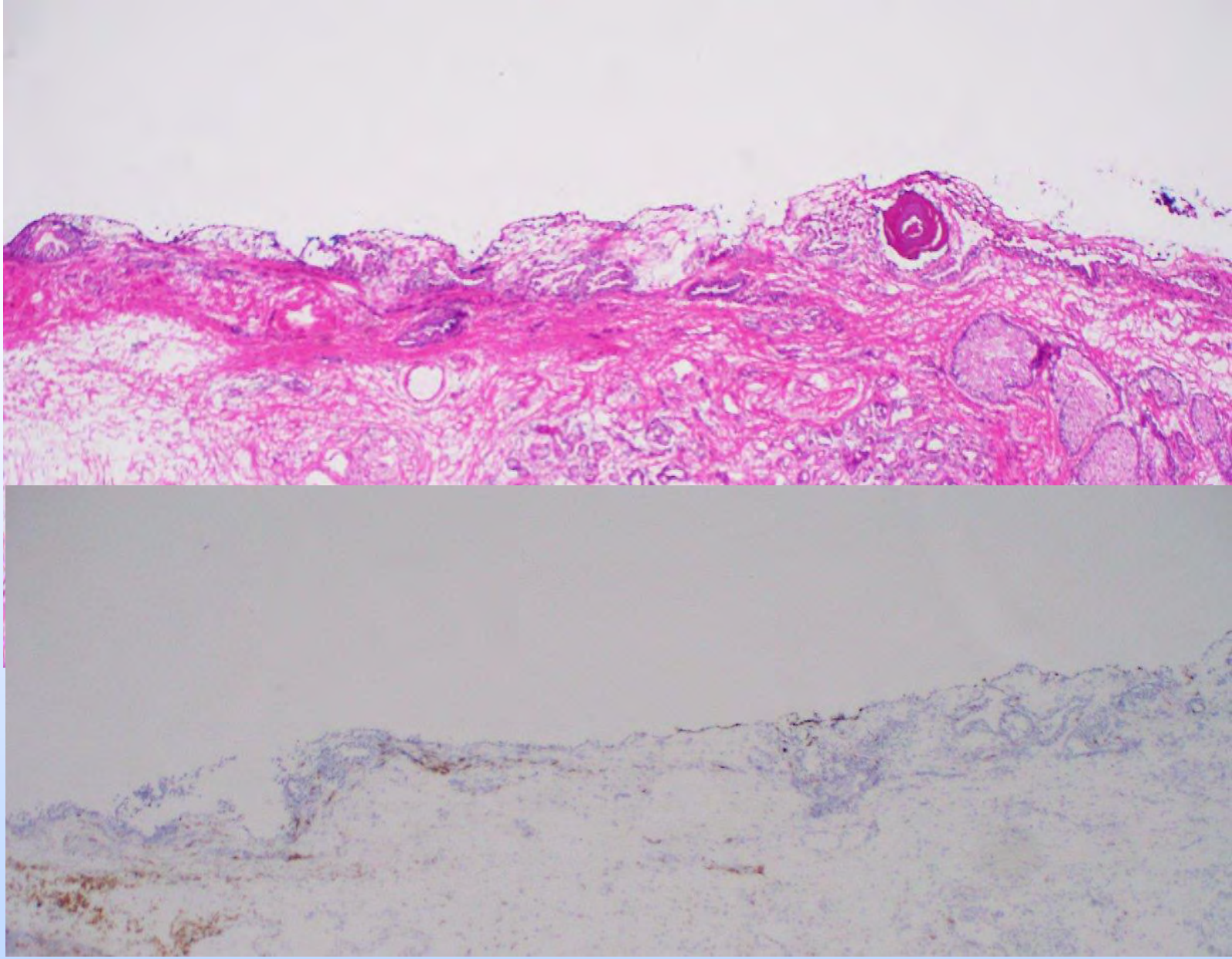
C3 4x
H&E
Mart-1

Immunostain makes it easy to see there is no MMIS

Tumor cleared



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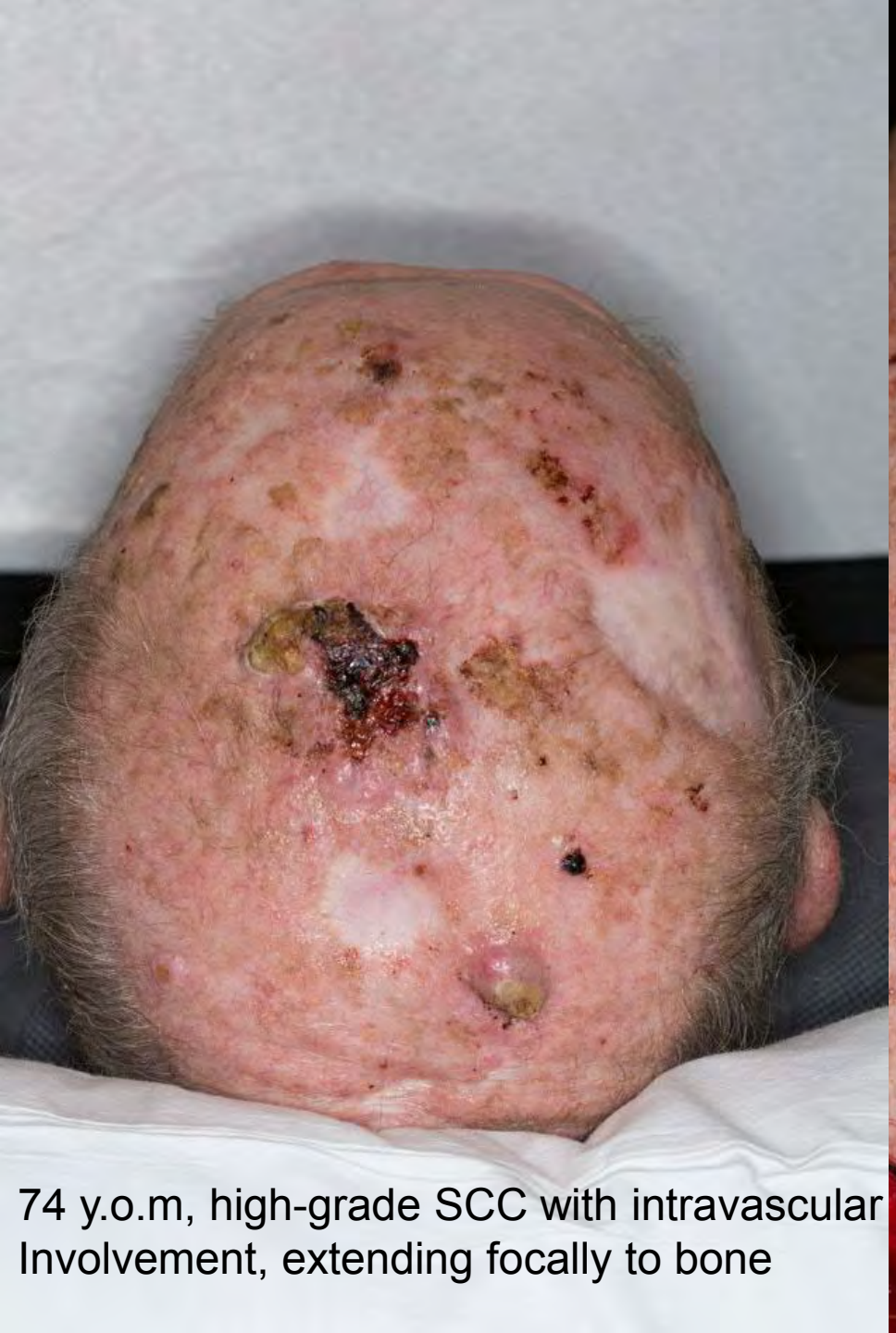
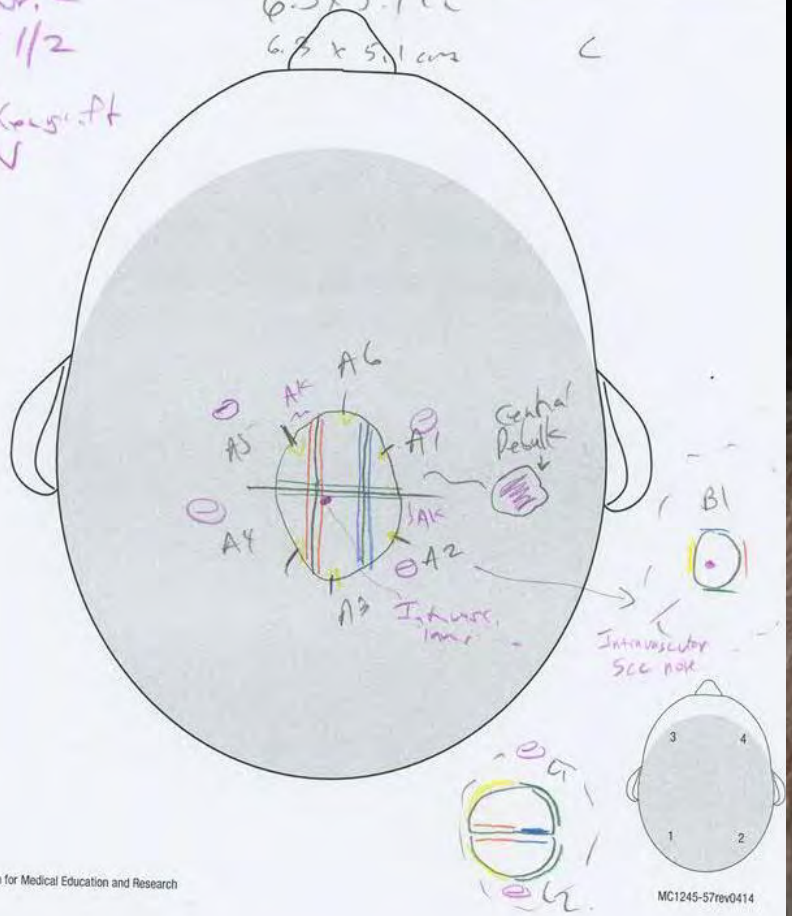


74 y.o.m, high-grade SCC with intravascular
Involvement, extending focally to bone

Tumor Type	Location	Area
SCC	Vertex Scalp	
Initial Size (cm) 2.0x2.6	Final Defect (cm) 6.3x5.1cm	Layer

SCC-Gr.2
Mu# 1/2
Porcine Xenograft
4-0 N

6.3x5.1cm
6.3 x 5.1 cm



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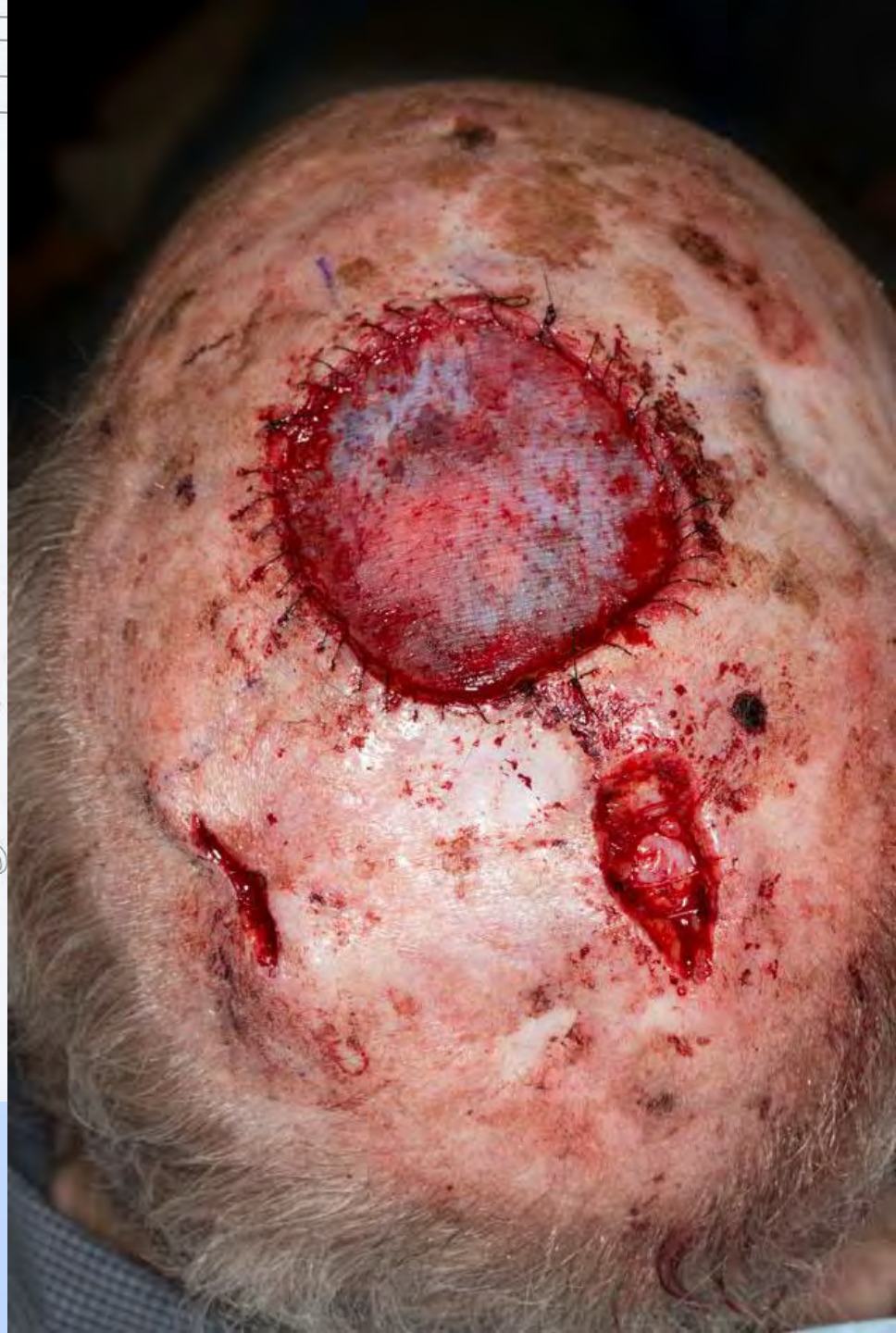

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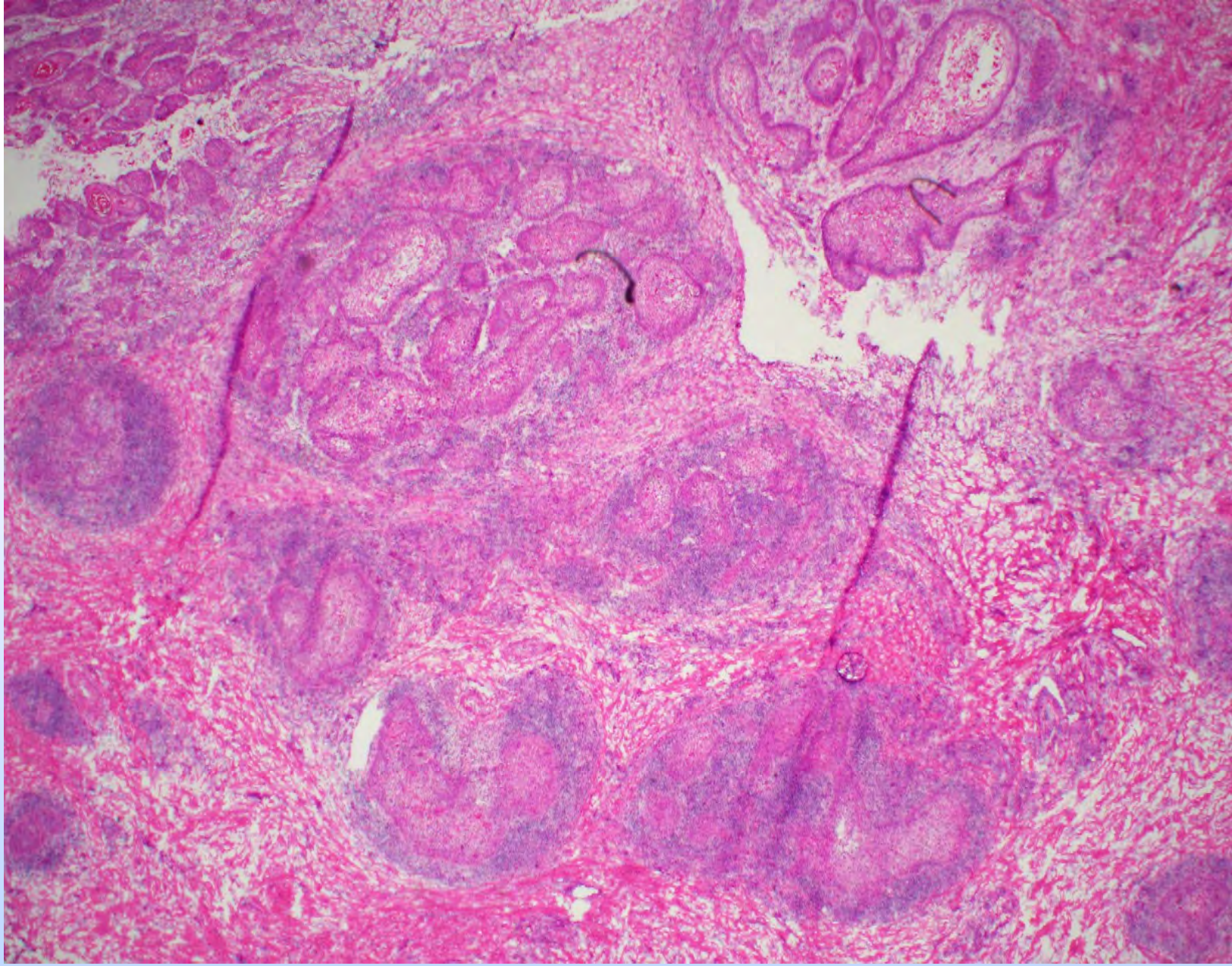
Debulking tissue
2x
H&E

Obvious SCC



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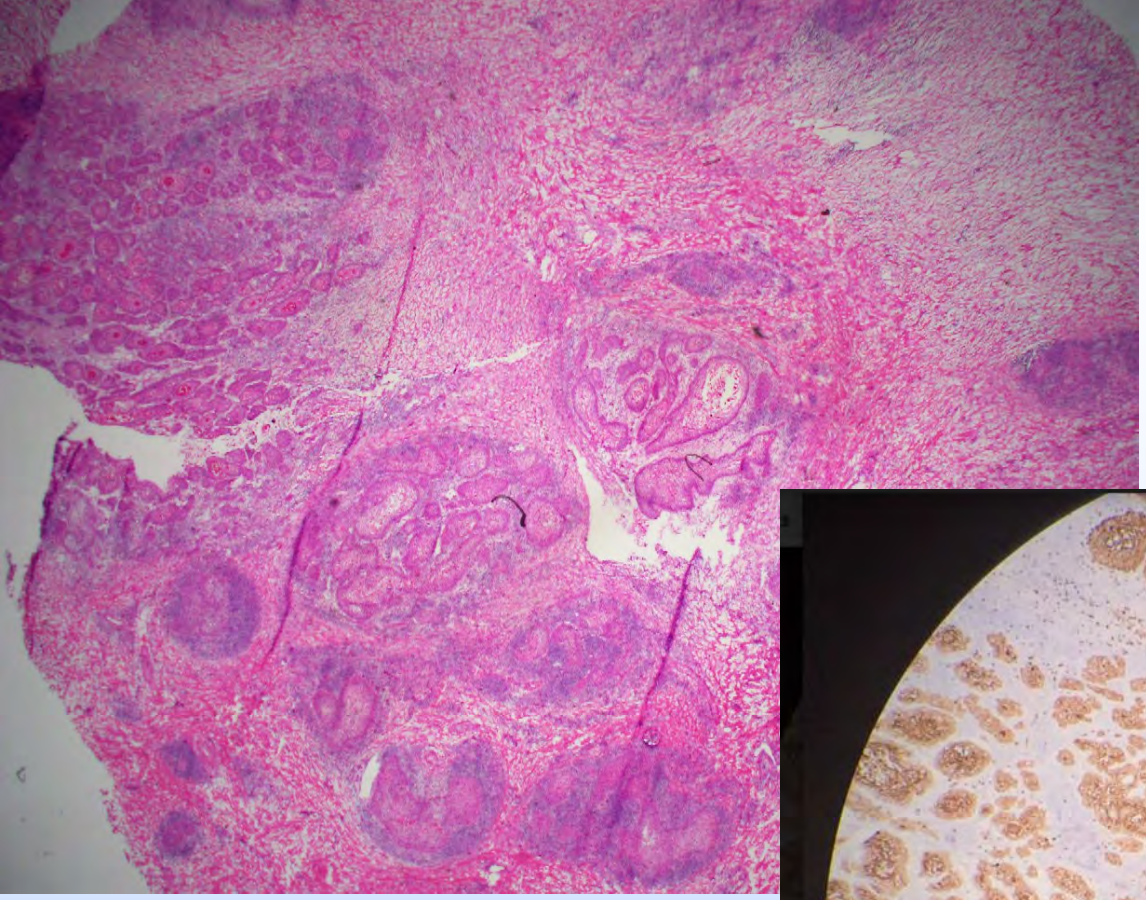
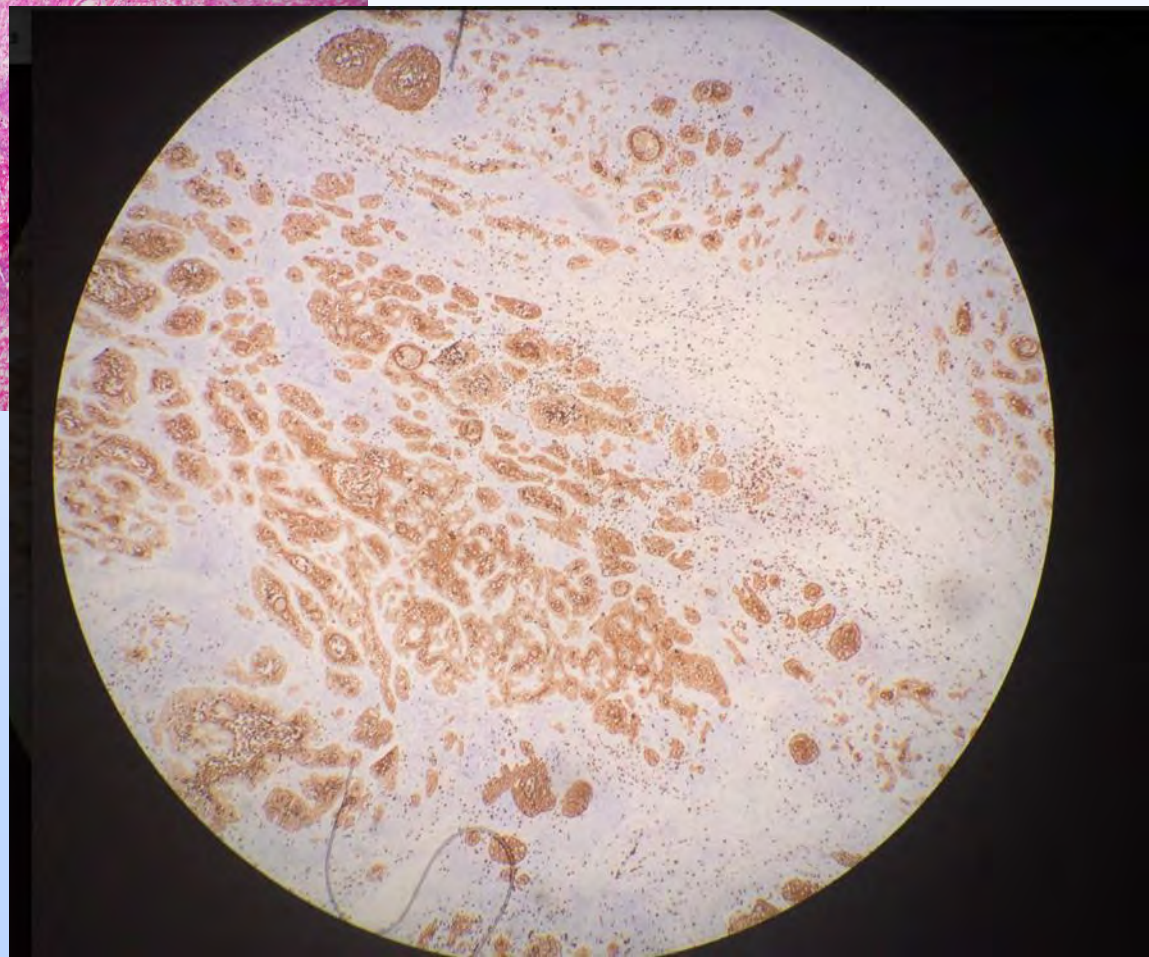


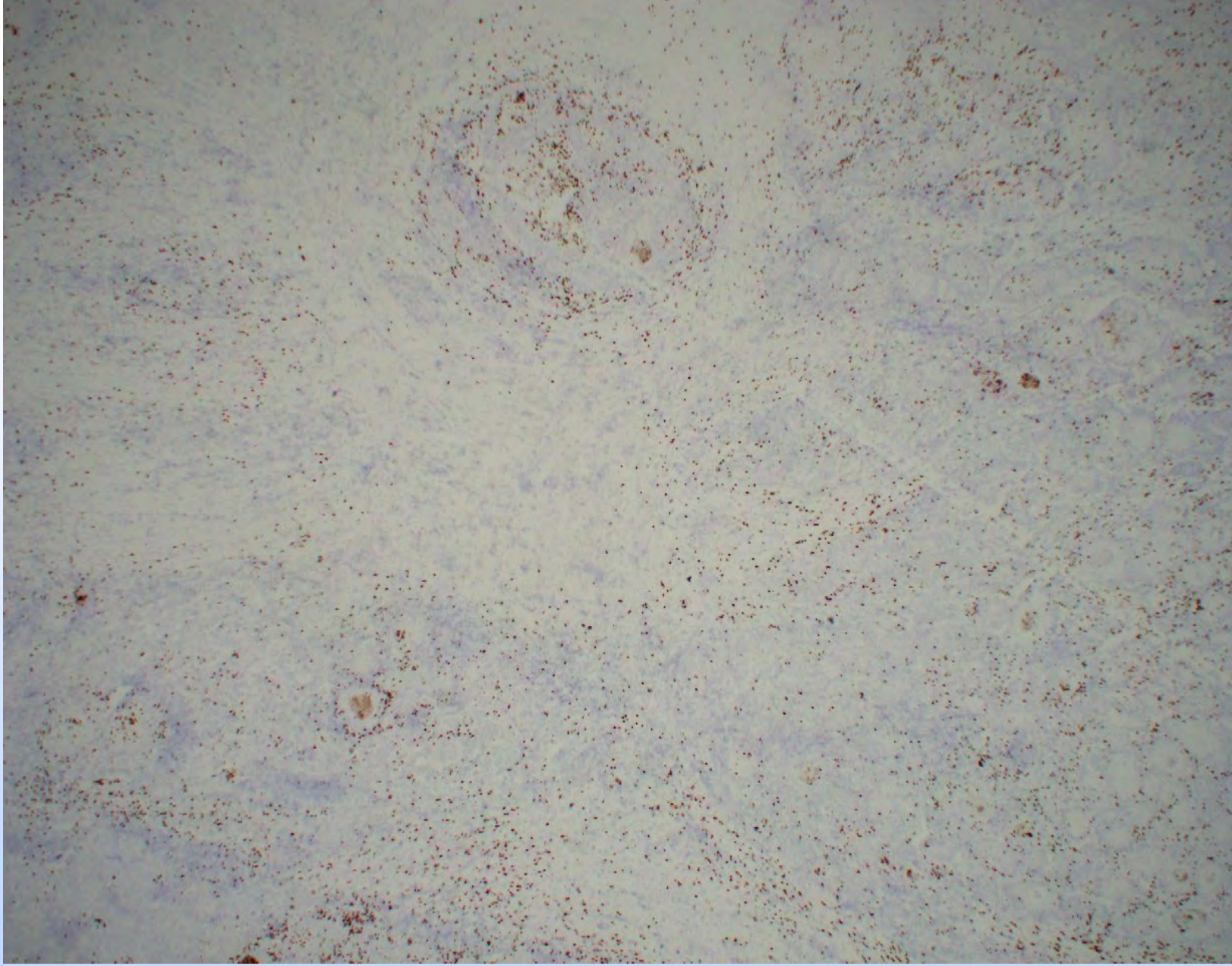
Debulking Tissue 1.25x
H&E
MCK immunostain



MAYO CLINIC
HEALTH SYSTEM

MCK Negative Control:

shows commonly observed background Staining (likely from granulocytes that in some Situations may be addressed with peroxidase)



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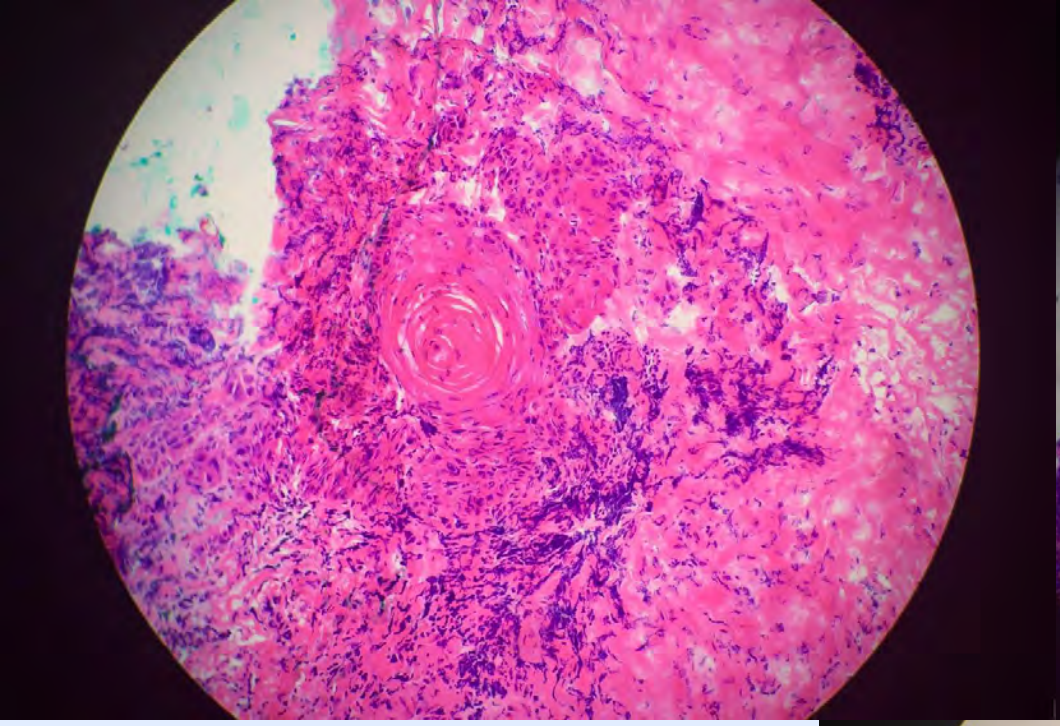
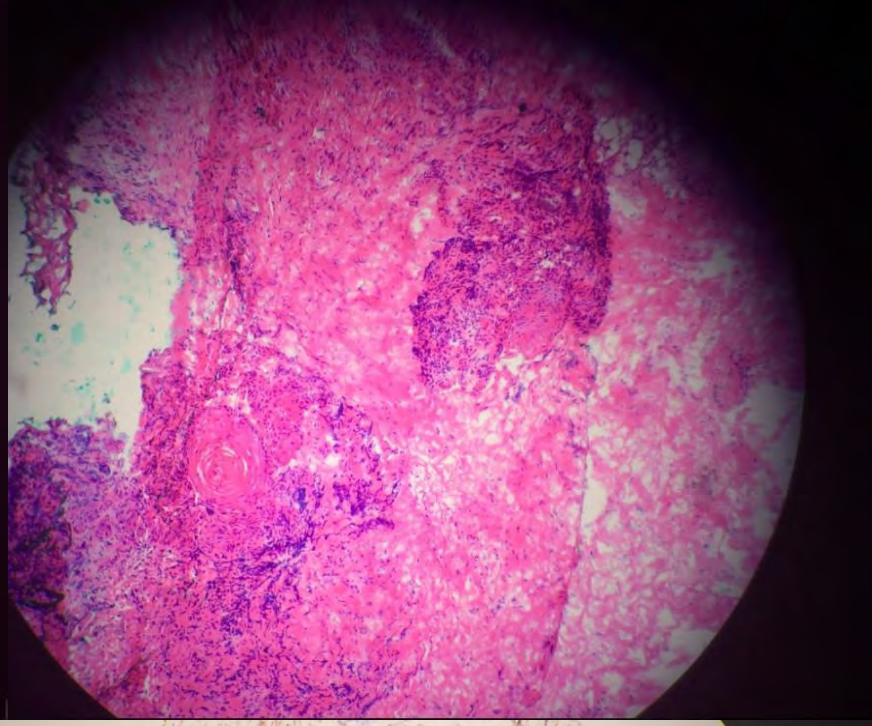
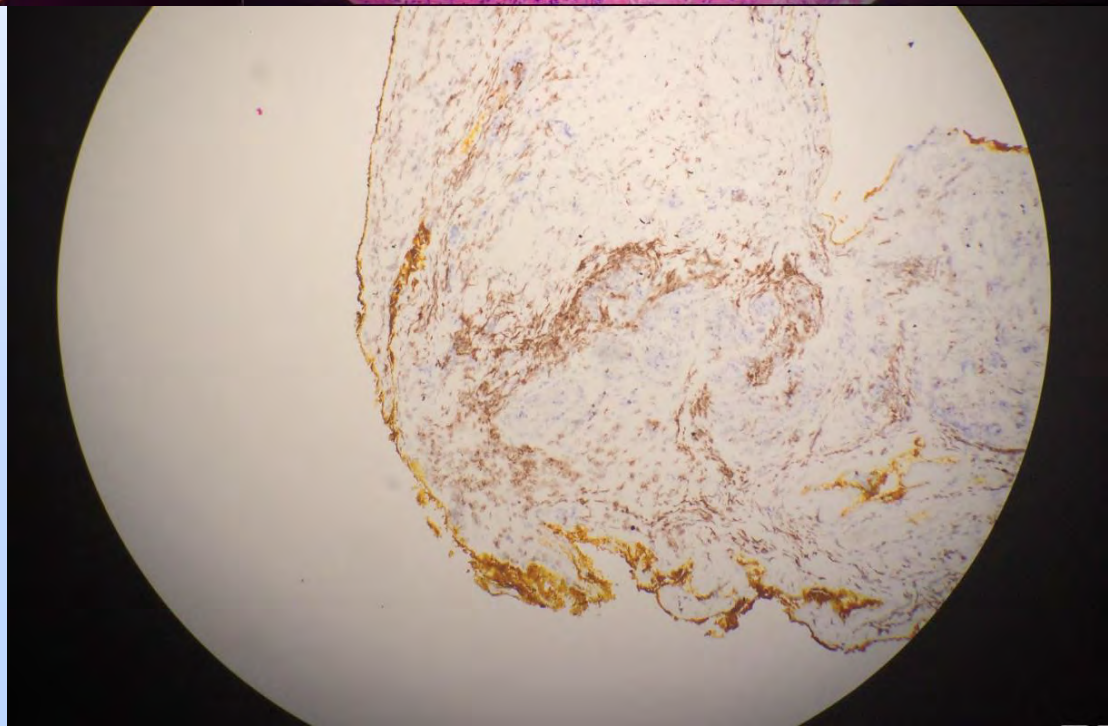




Stage A3 H&E 4x
SCC at deep margin in galea

Once again, NO staining noted with MCK, since unstained slides were not created concurrently when the H&E were cut!

The MCK were cut as an afterthought and SCC was then gone



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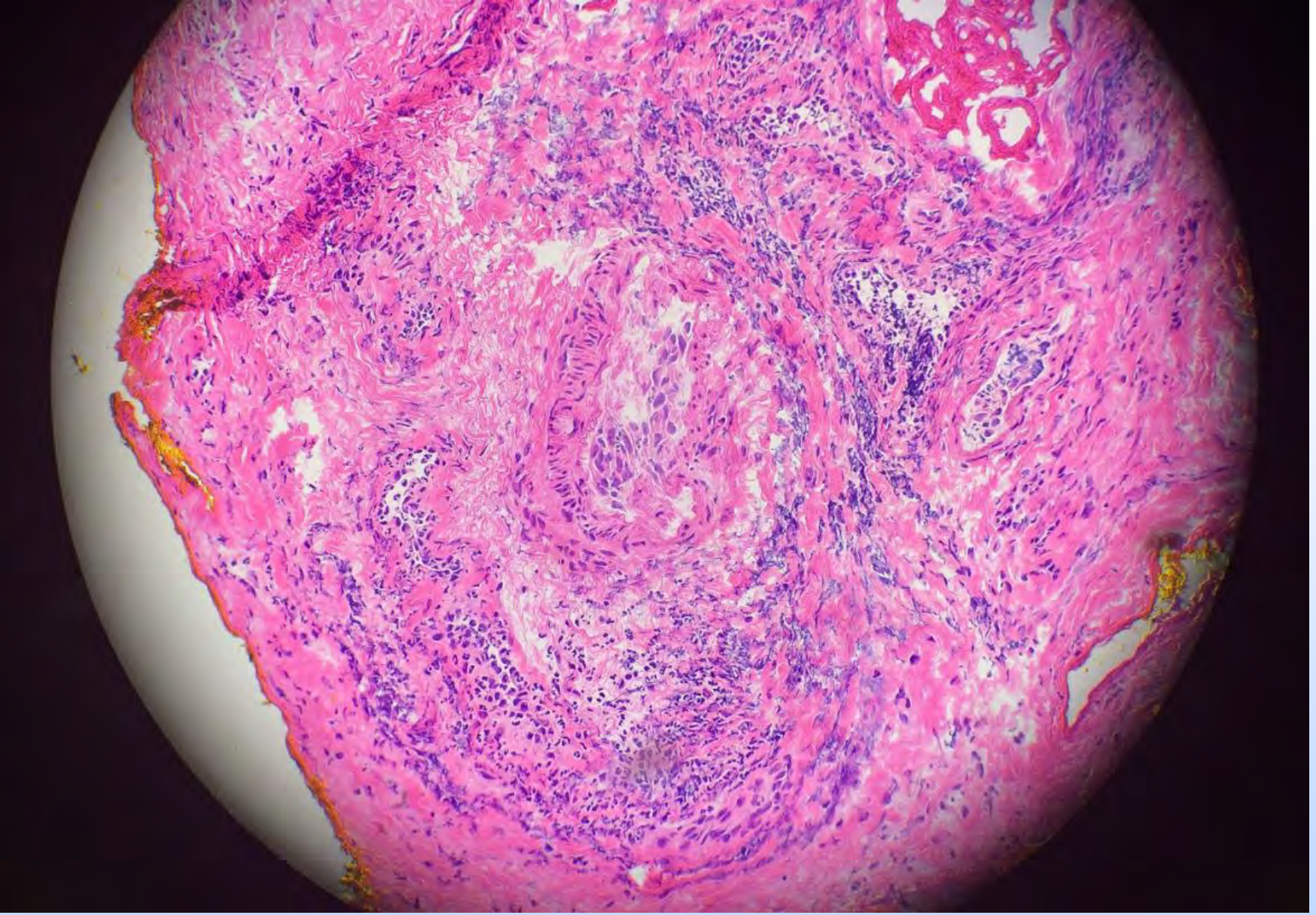
HEALTH SYSTEM



Stage B1 H&E 10x Intravascular SCC
(NOT highlighted with MCK, since MCK stain not concurrent!)

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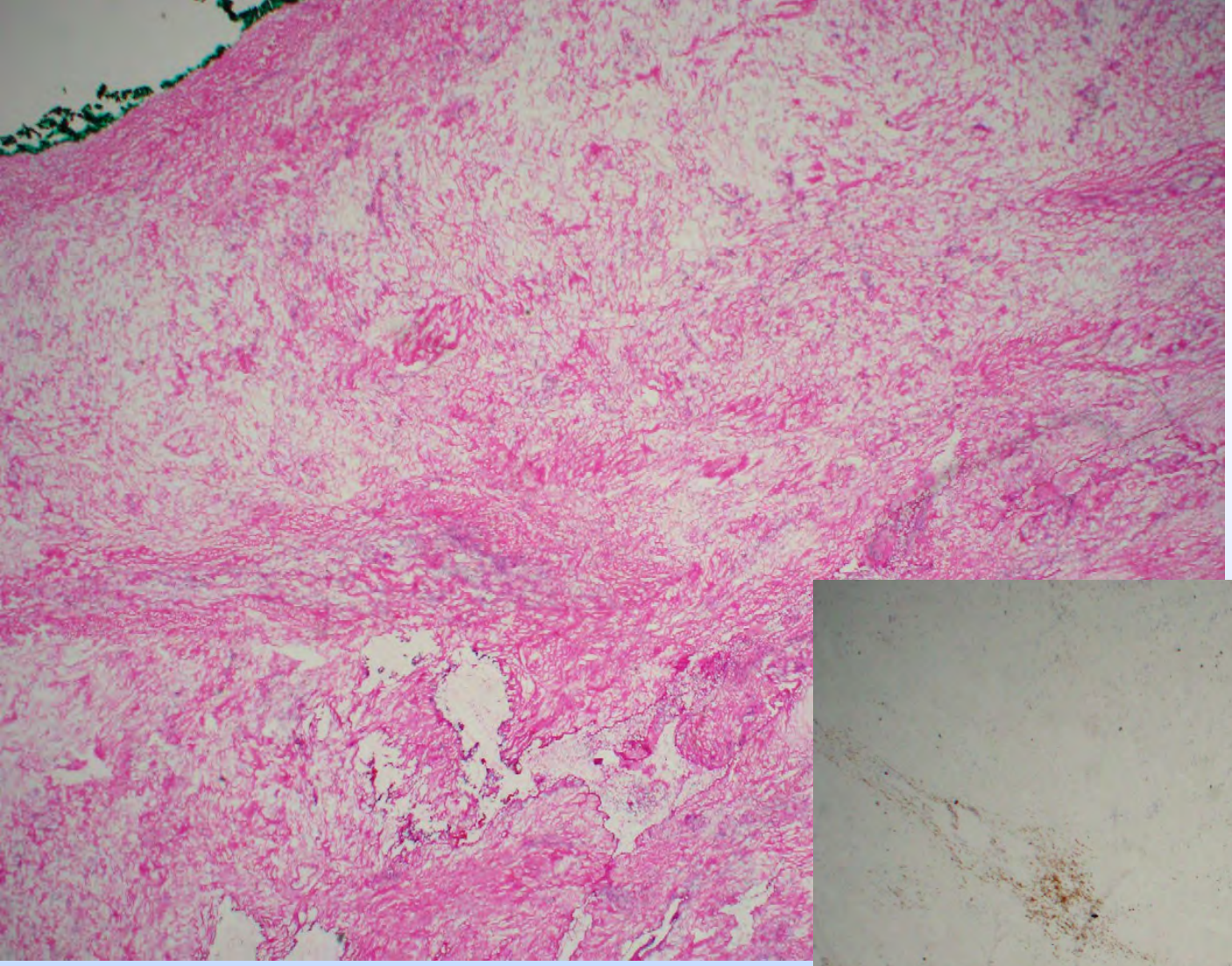
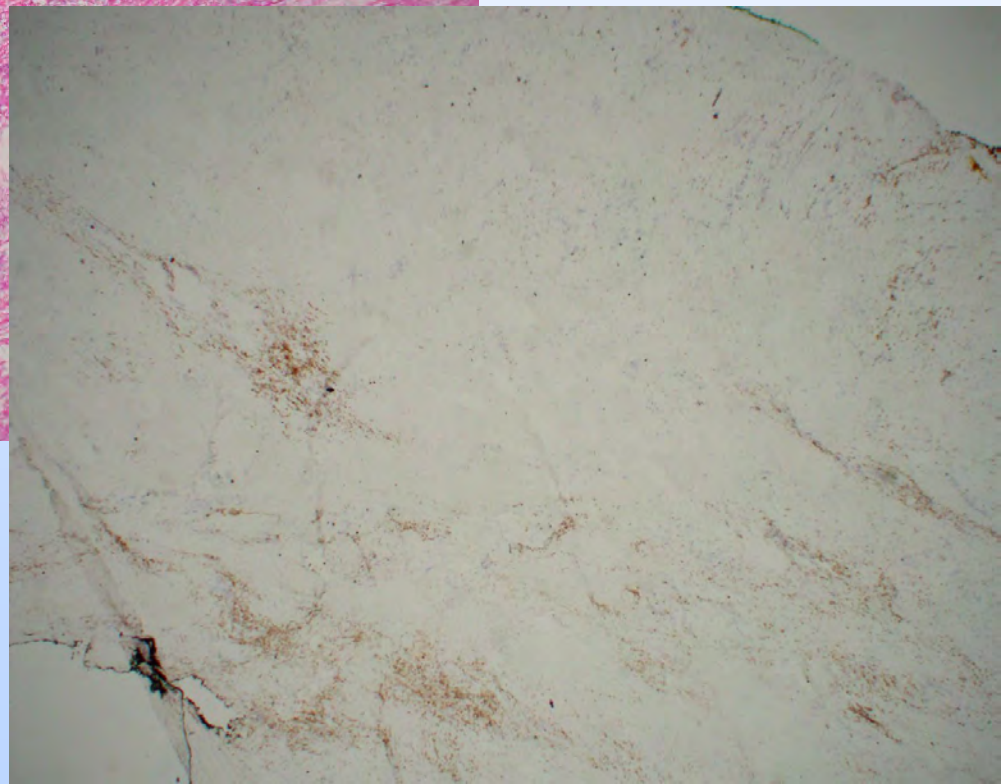


Stage C2 2x
H&E and MCK

MCK can be trusted since 3rd stage was cut concurrently…
No tumor left



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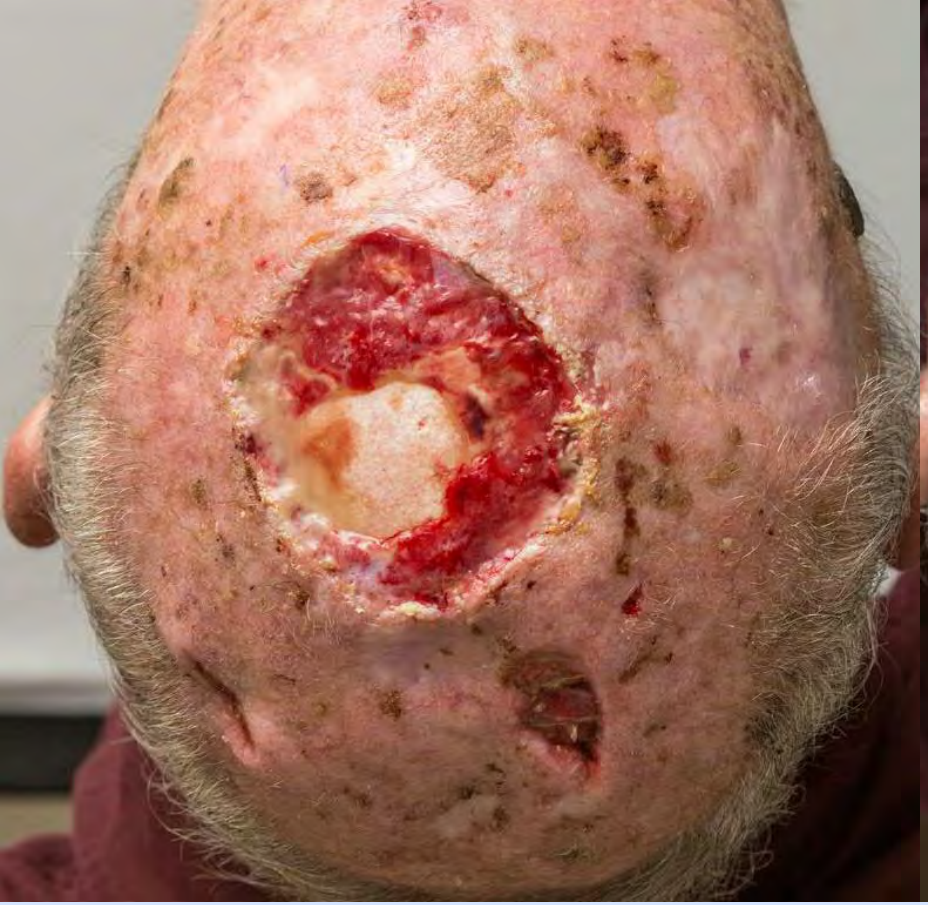
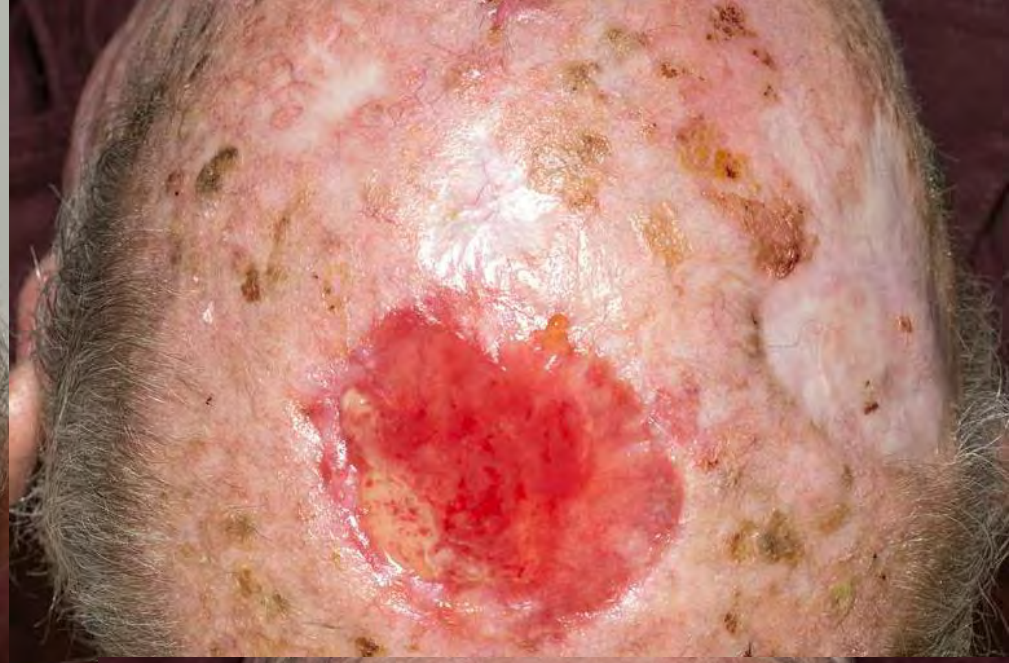
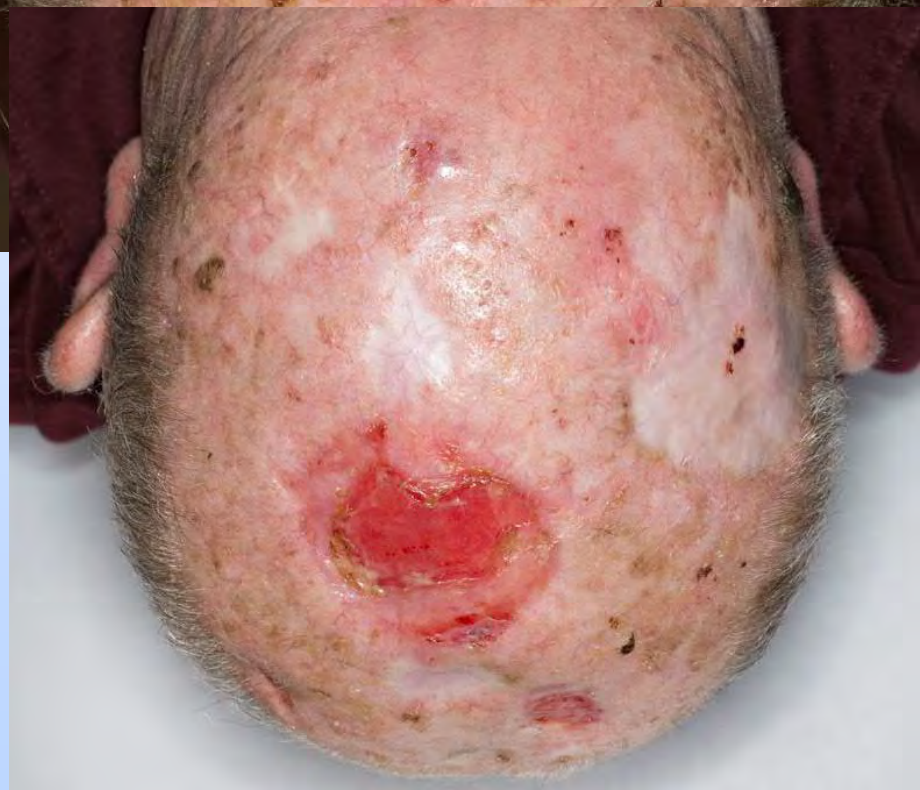
HEALTH SYSTEM







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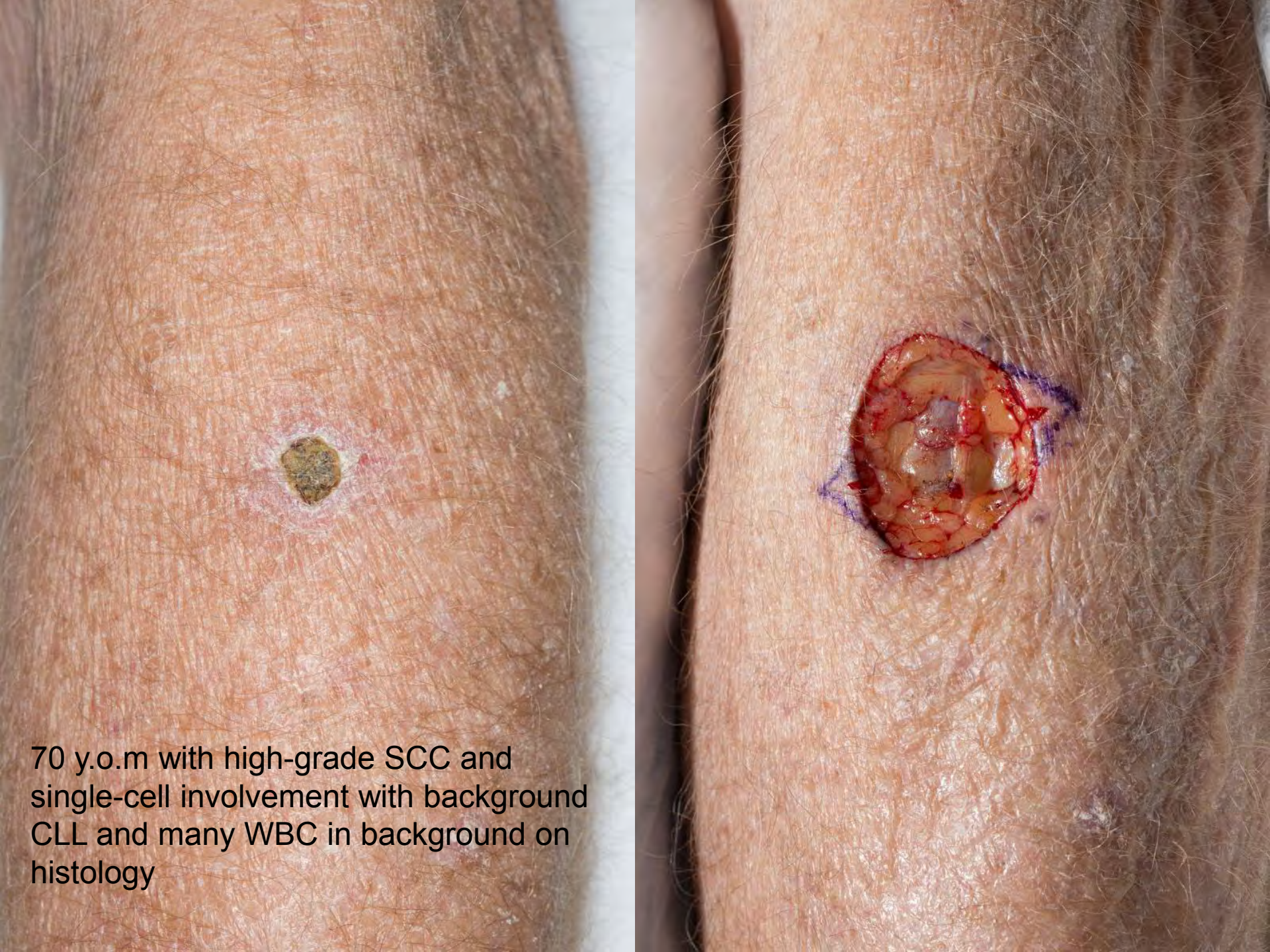
HEALTH SYSTEM

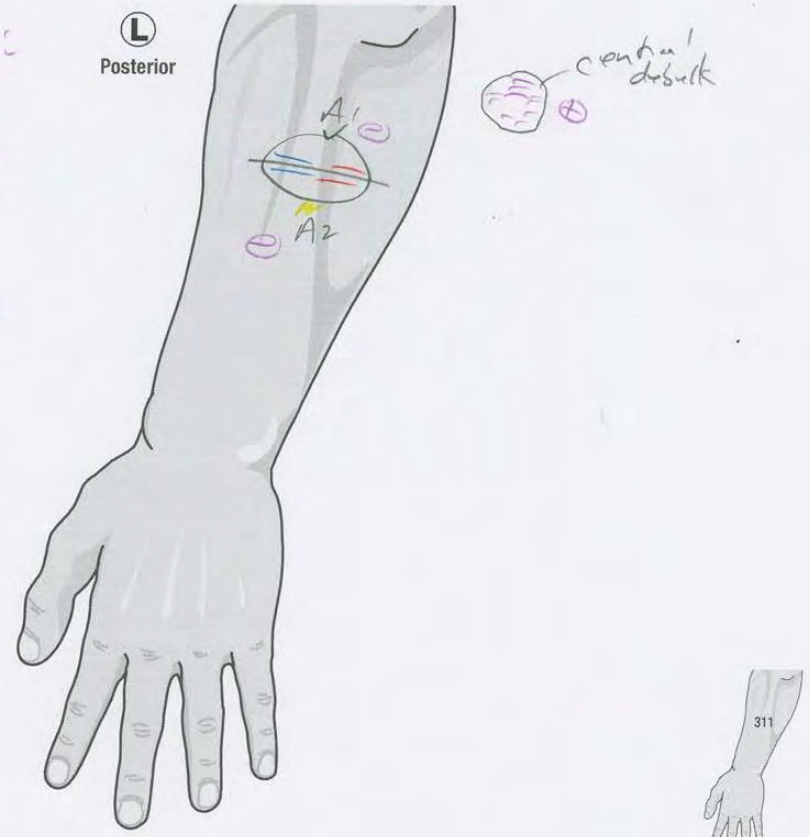


70 y.o.m with high-grade SCC and single-cell involvement with background CLL and many WBC in background on histology

Tumor Type	Location	Area
P-D SCC	(L) forearm	
Initial Size (cm) 1.8 x 1.6	Final Defect (cm) 2.9 x 2.7 c	Layer

SCC - G-3
Mohs 311
⊖
10 repair
3-0 V
4-0 M
5-0 N

CK stain ⊖



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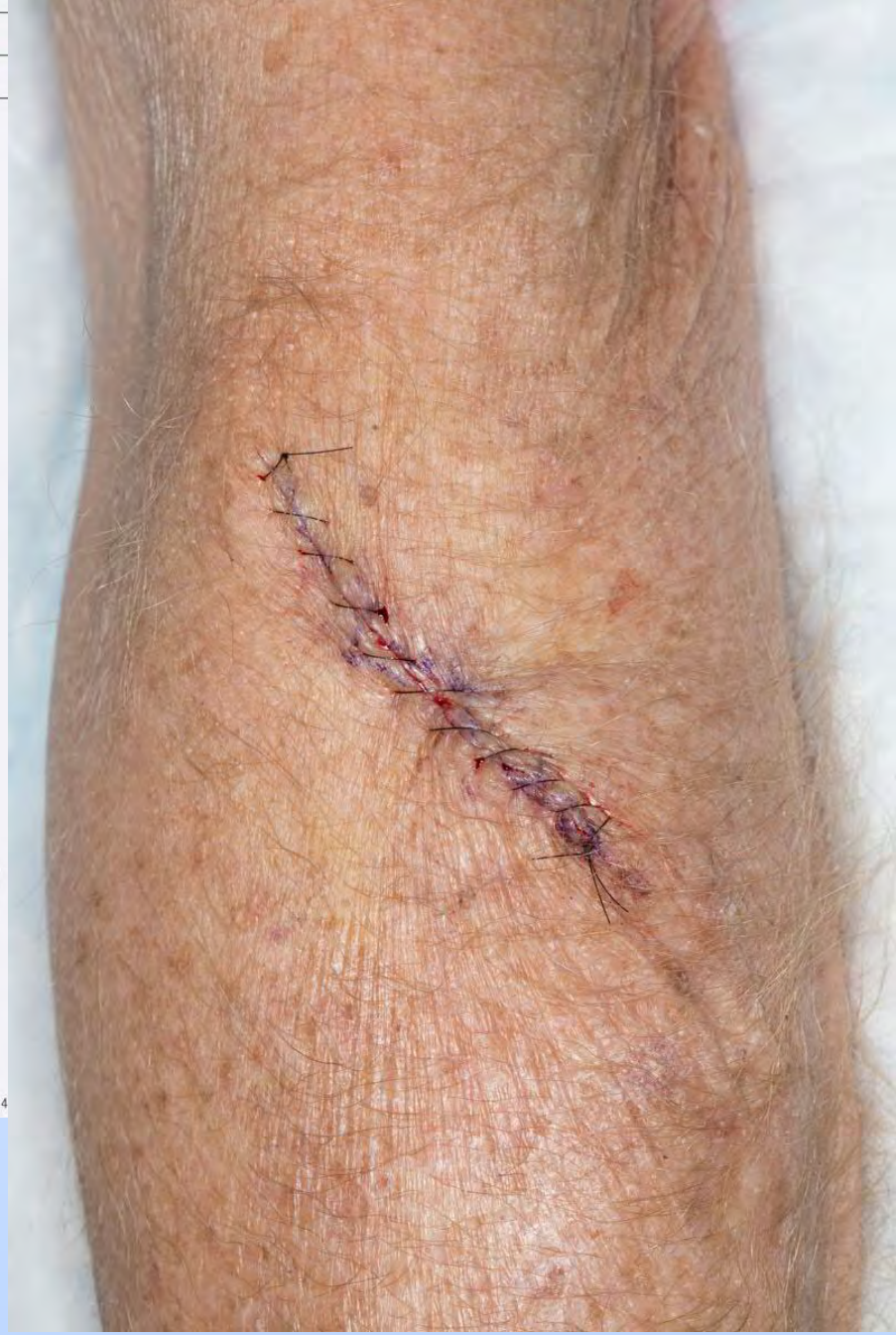
MC1245-67rev0414



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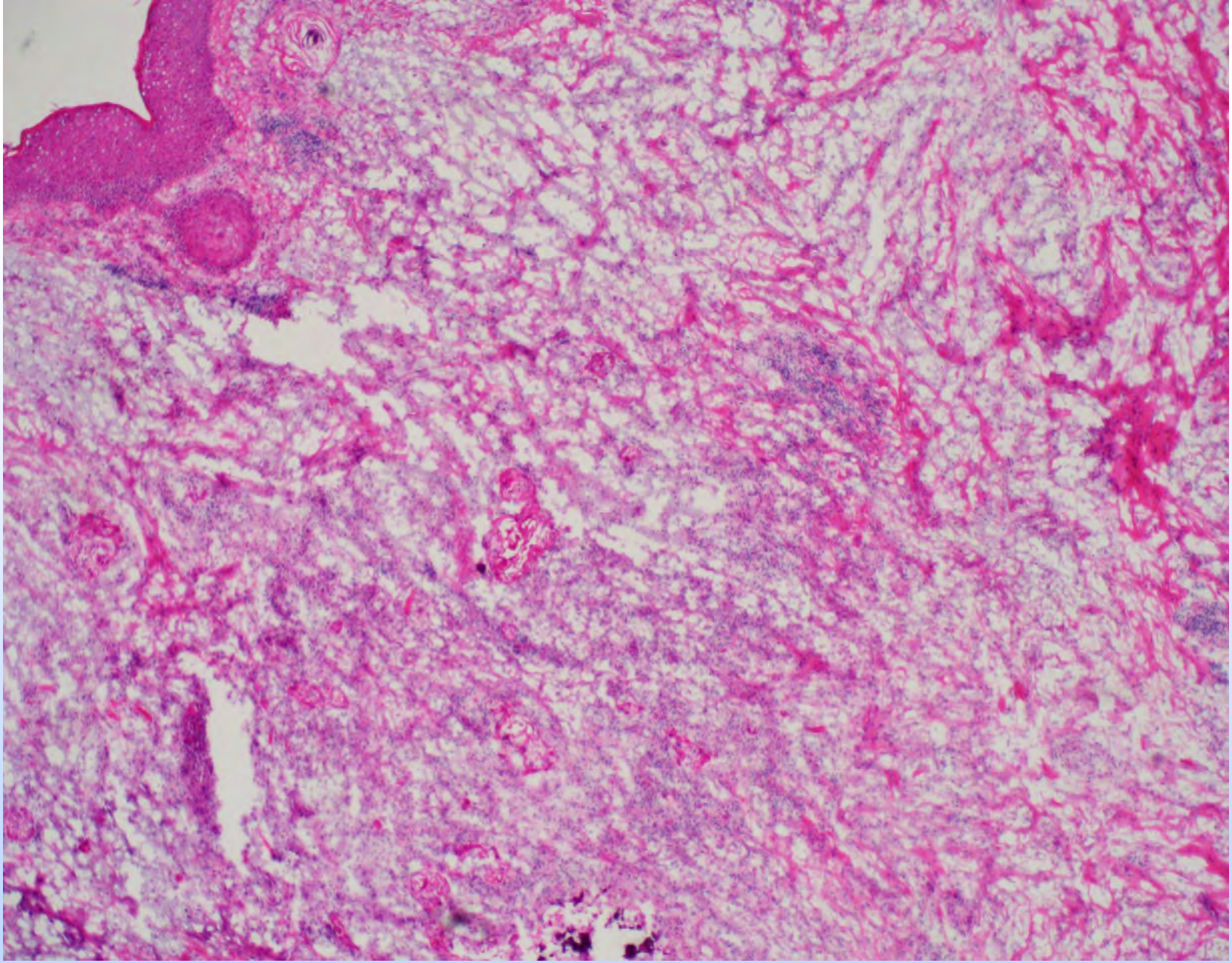
HEALTH SYSTEM



Debulking 4x H&E with patchy SCC mixed with inflammation

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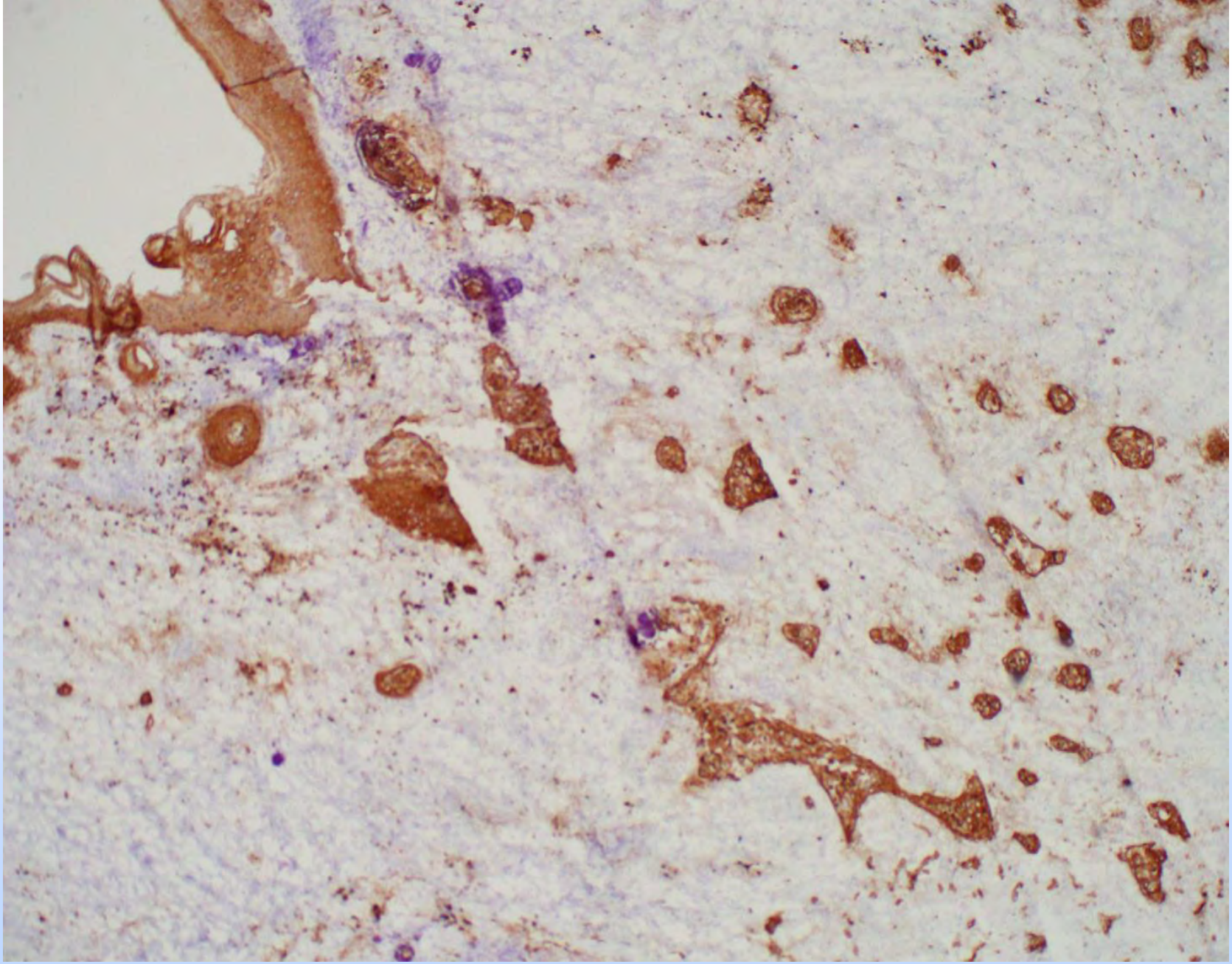

Debulking 4x MCK with patchy SCC easily seen

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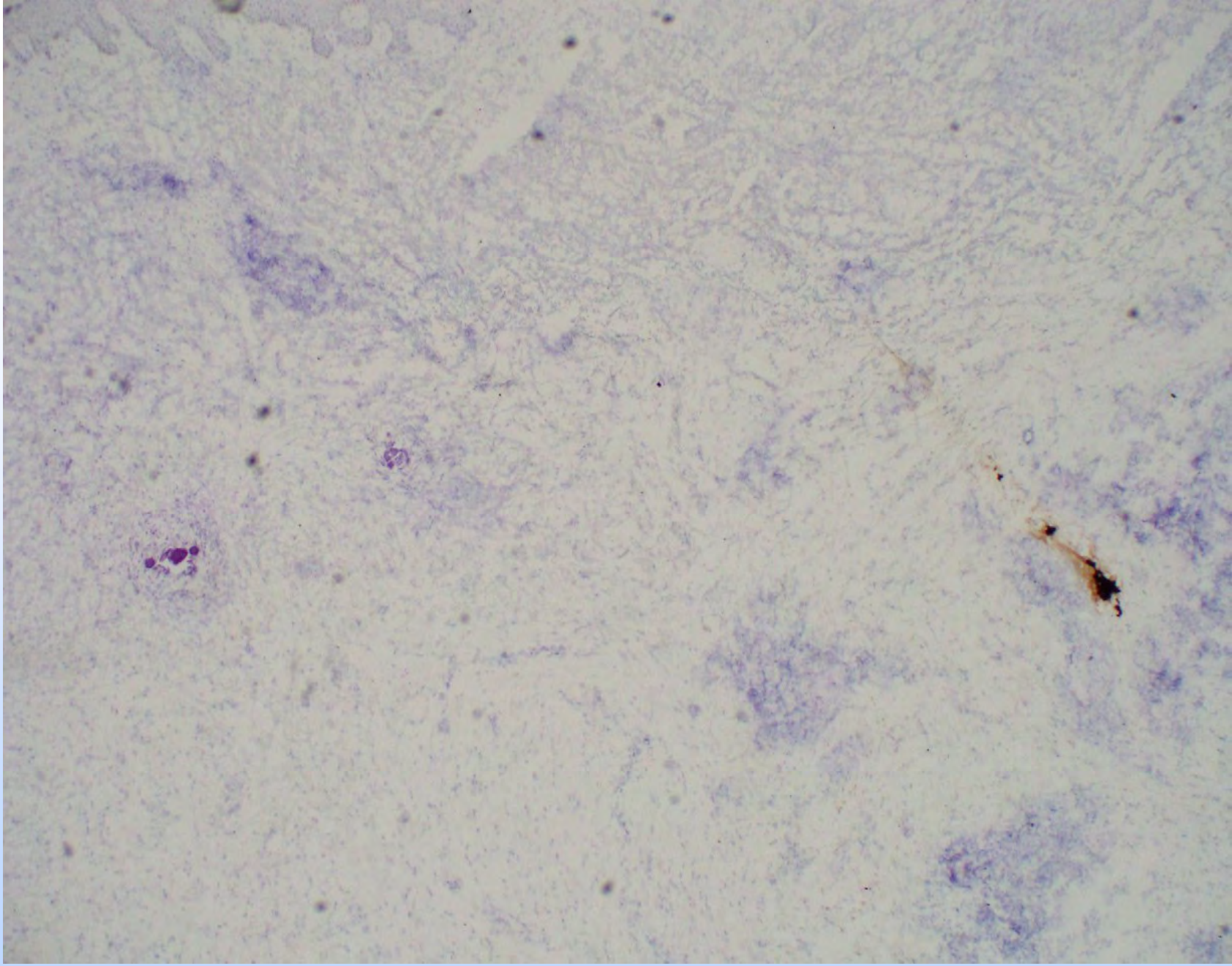
Debulking
MCK
Negative
Control



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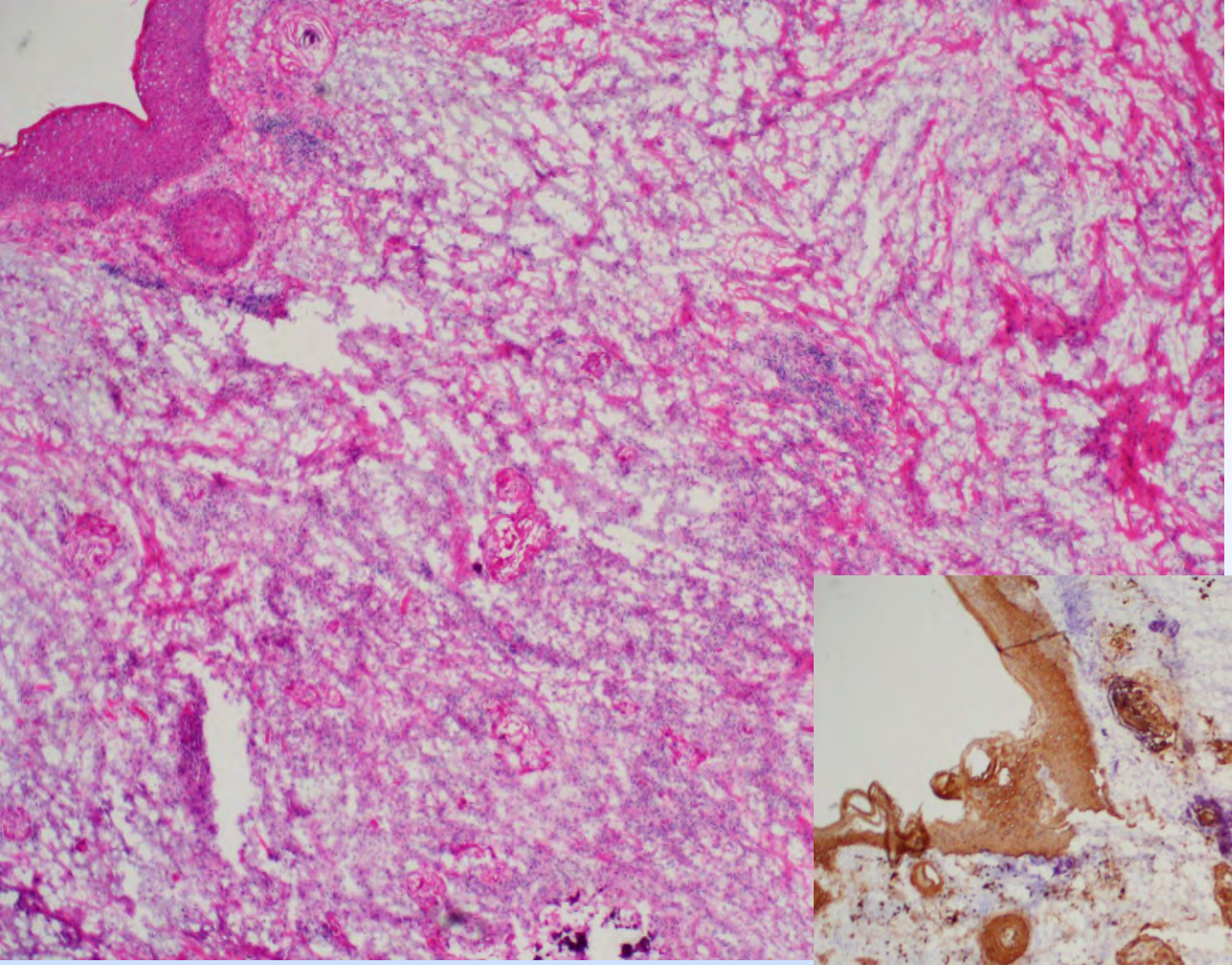
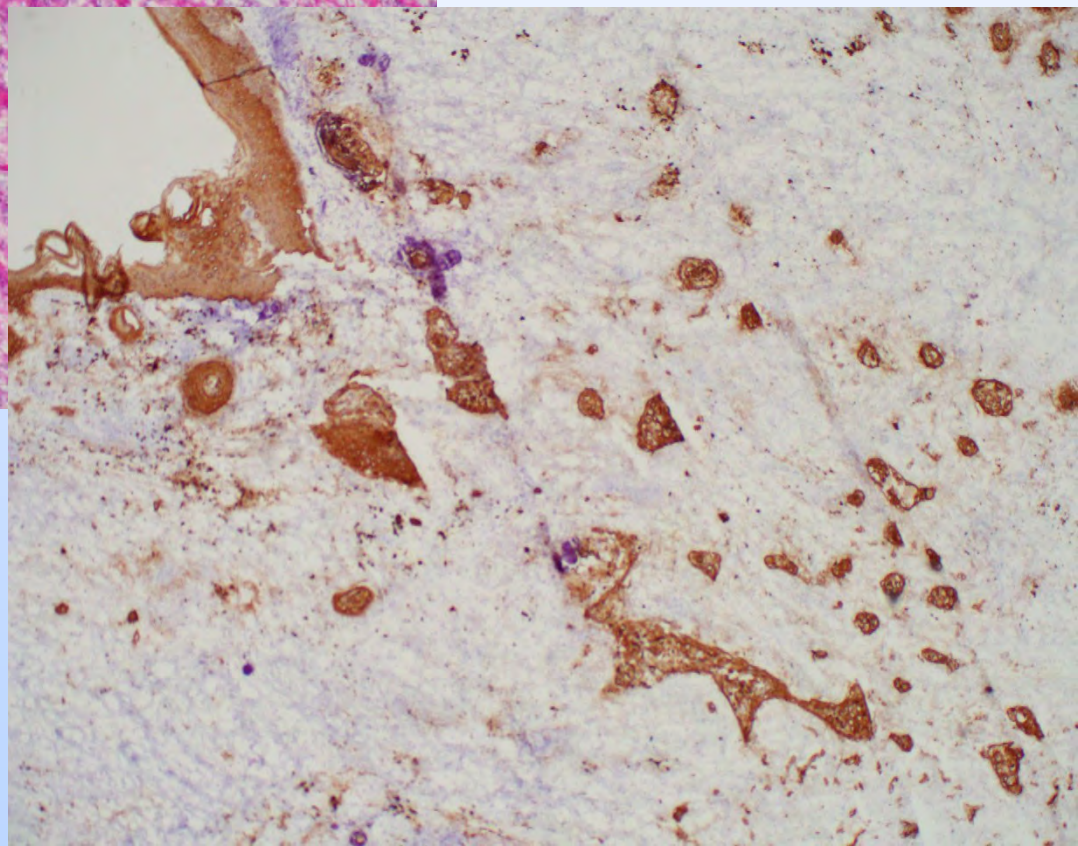
HEALTH SYSTEM



Debulking 4x H&E with MCK highlighting SCC comparison



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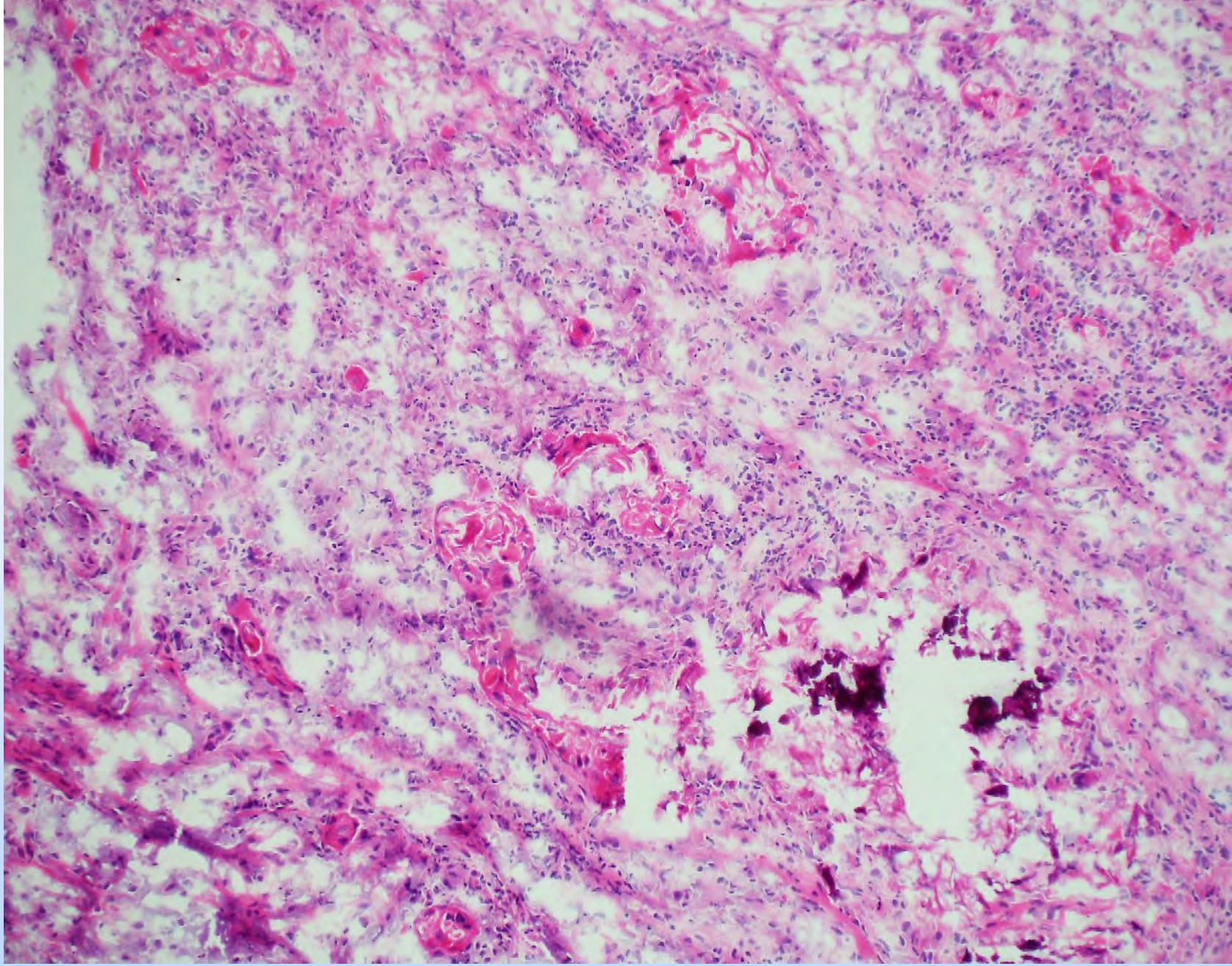
HEALTH SYSTEM

Debulking
10x H&E



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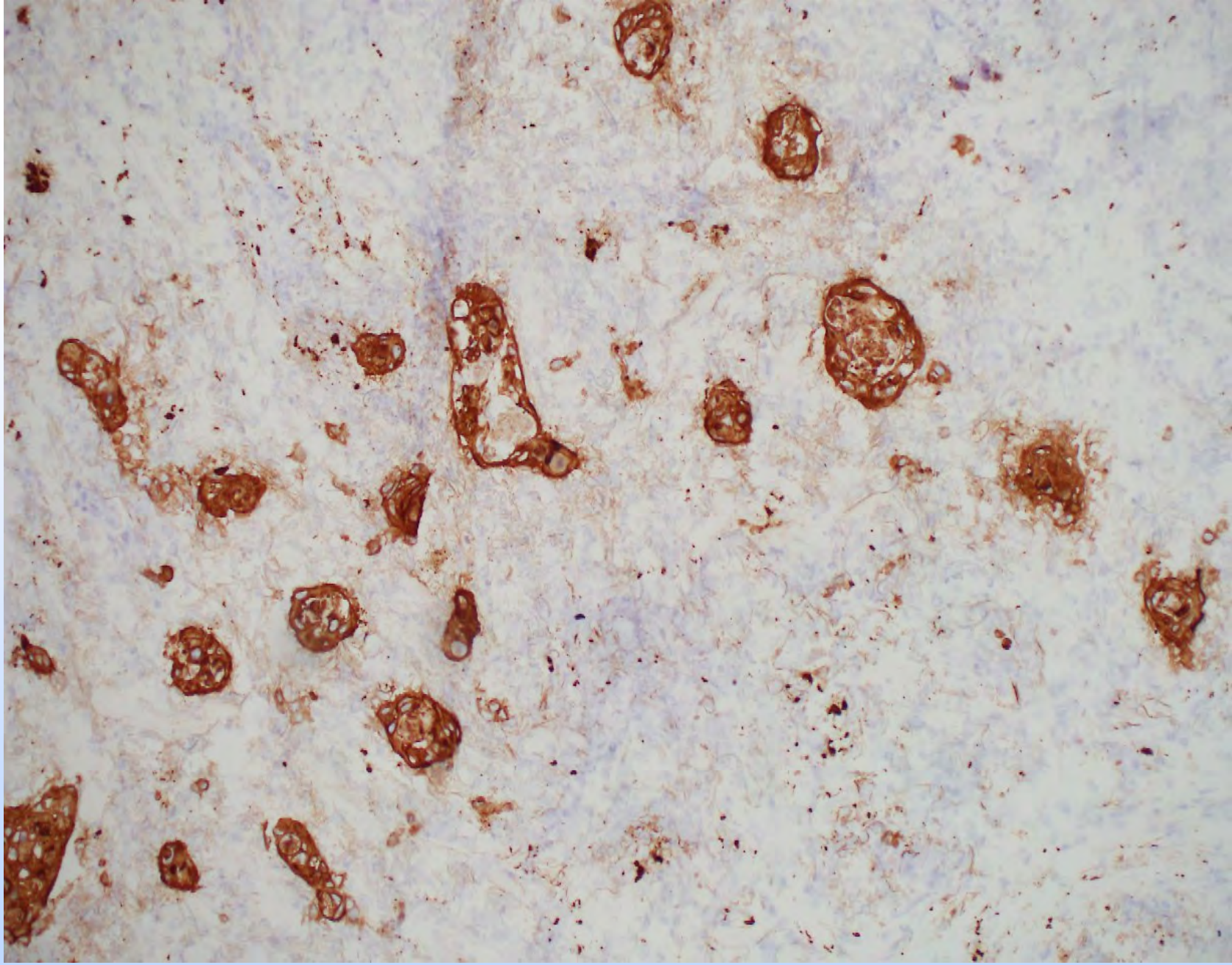
HEALTH SYSTEM

Debulking
10x MCK



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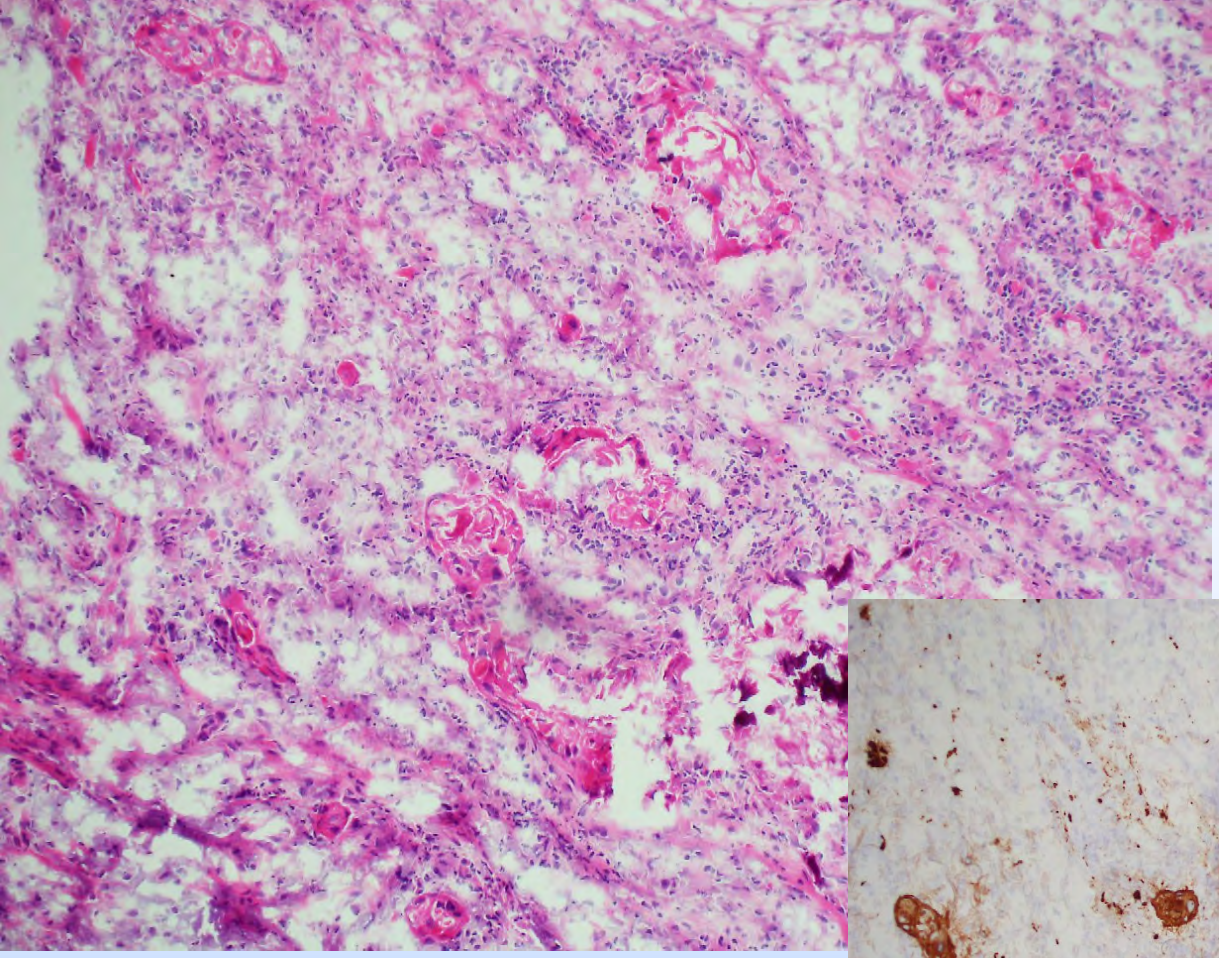
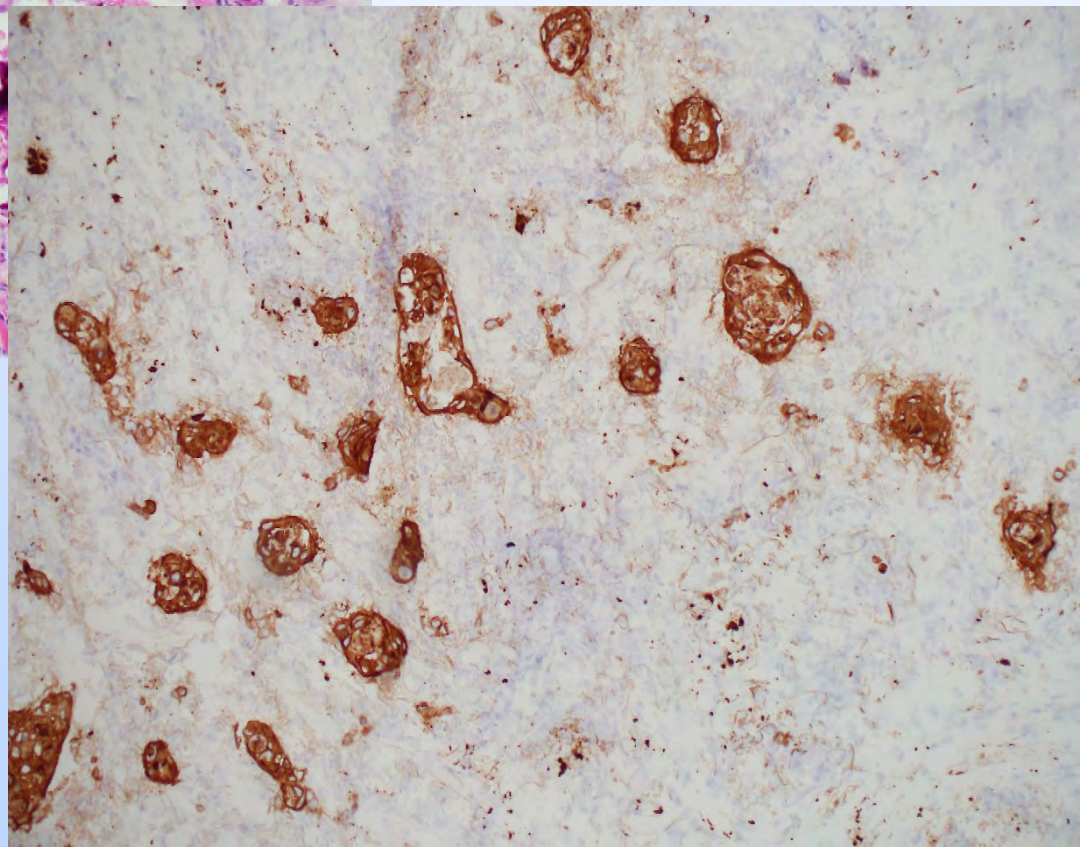
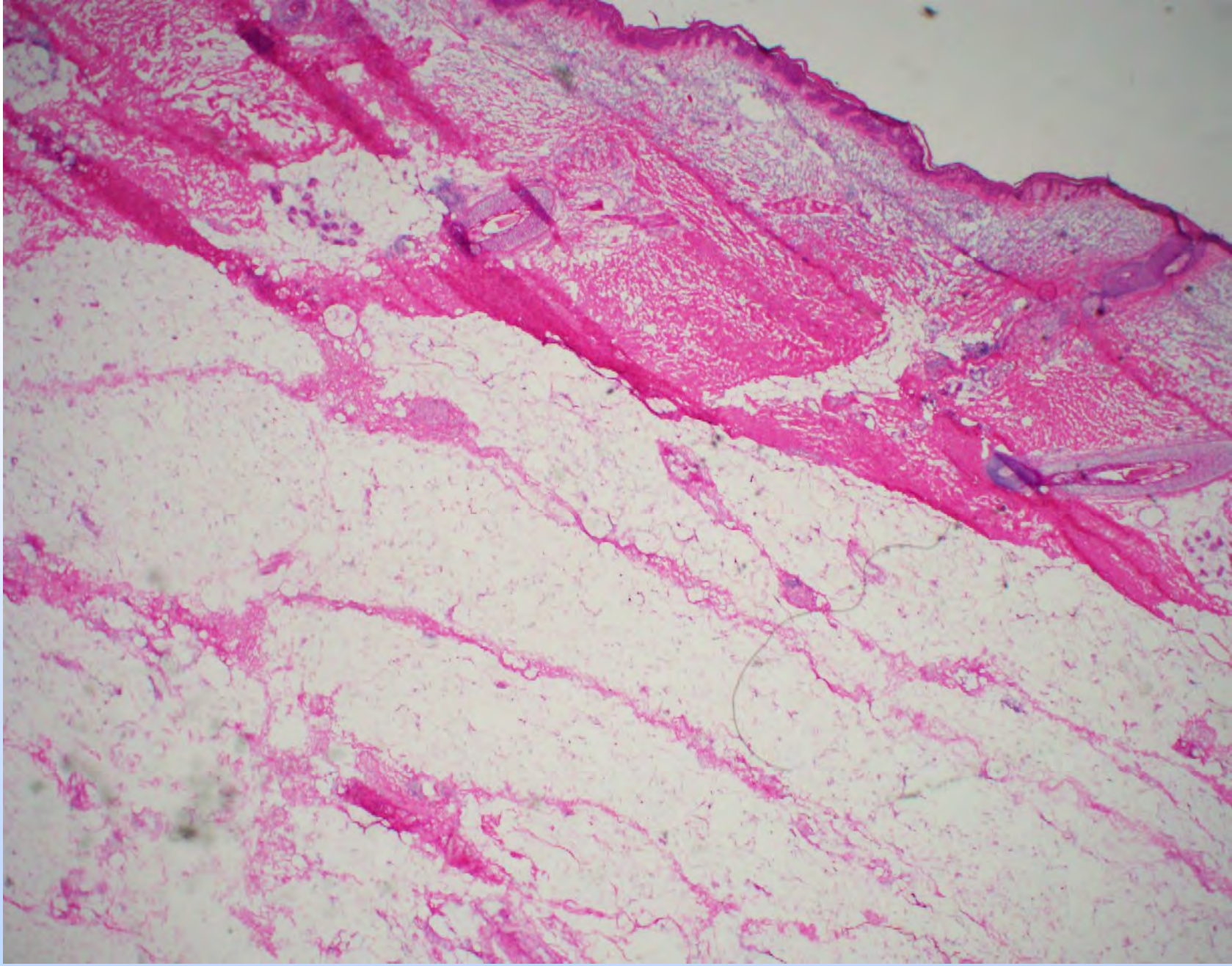


Debulking 10 x H&E with MCK highlighting SCC in background of inflammation and CLL cells comparison





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Stage A1 2x H&E showing no obvious SCC

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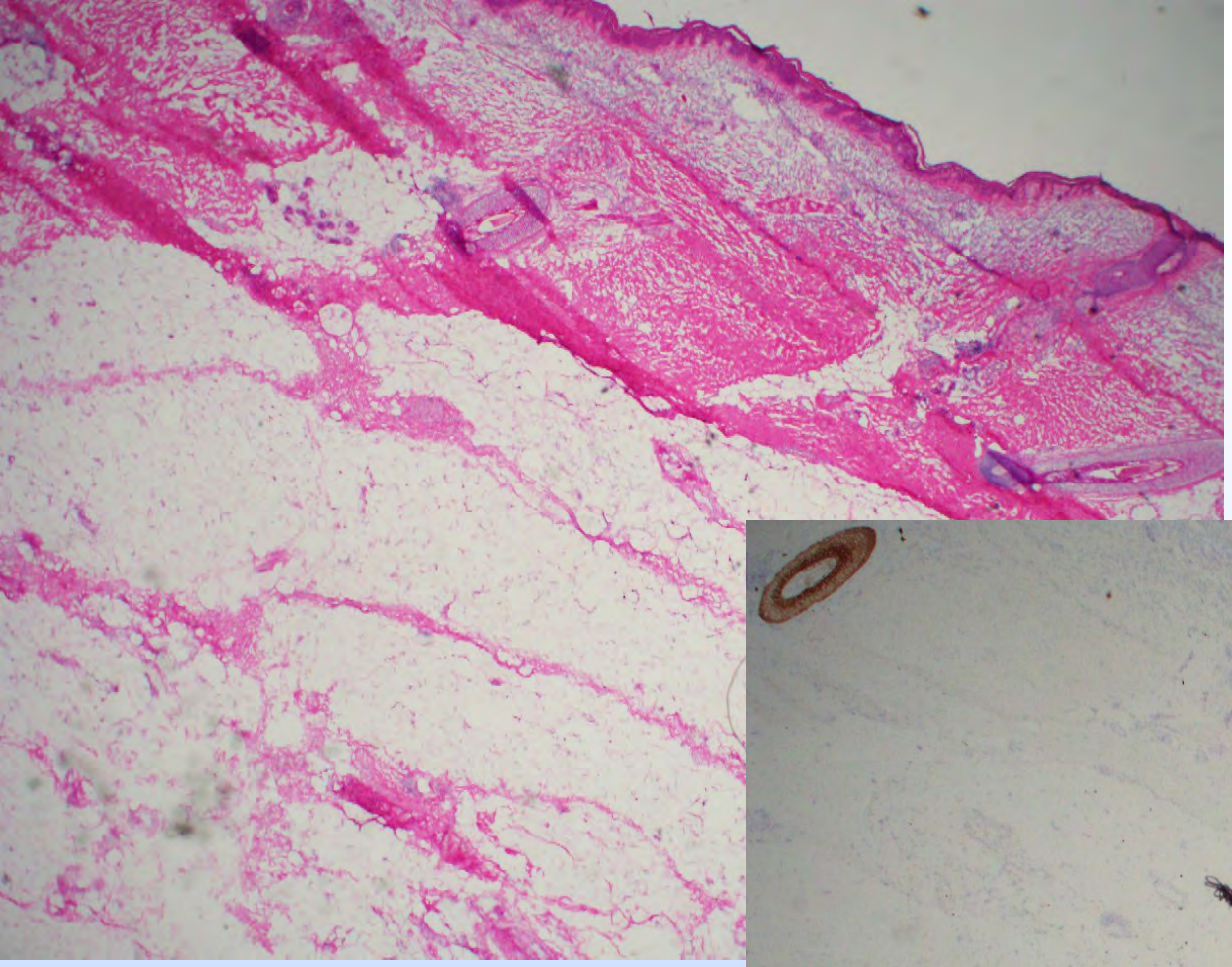
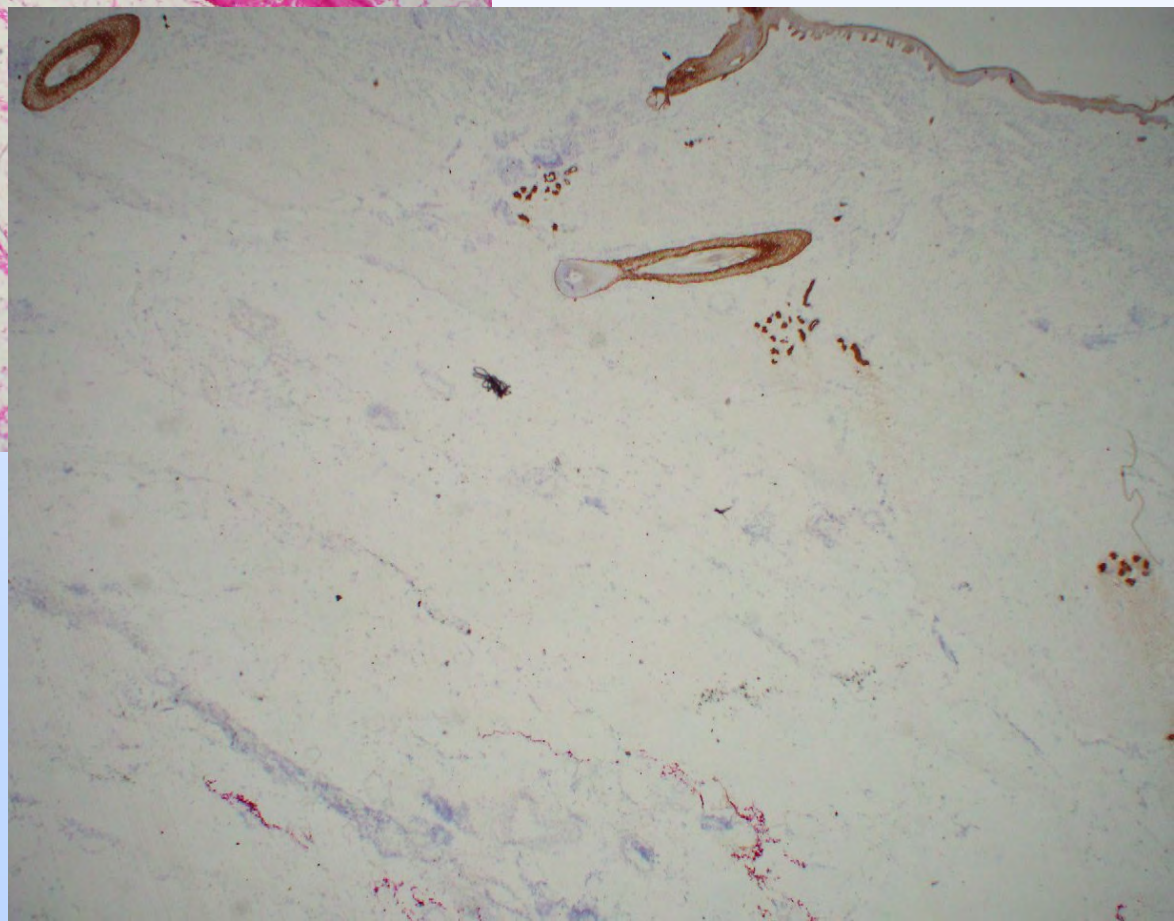


Stage A1 2x MCK stain confirming no SCC. Follicles and eccrine coils highlight



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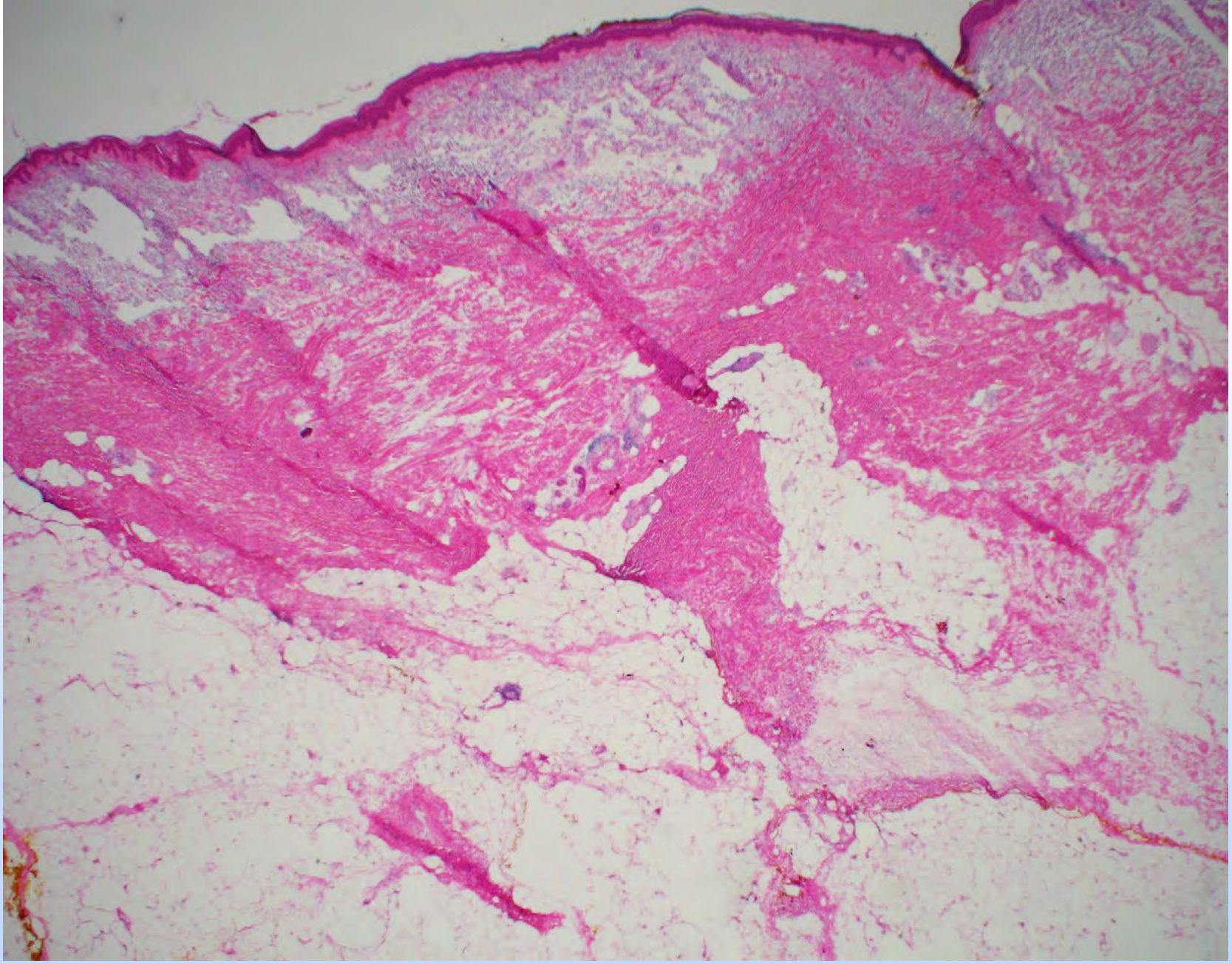
HEALTH SYSTEM



Stage A2 2x H&E showing no obvious SCC

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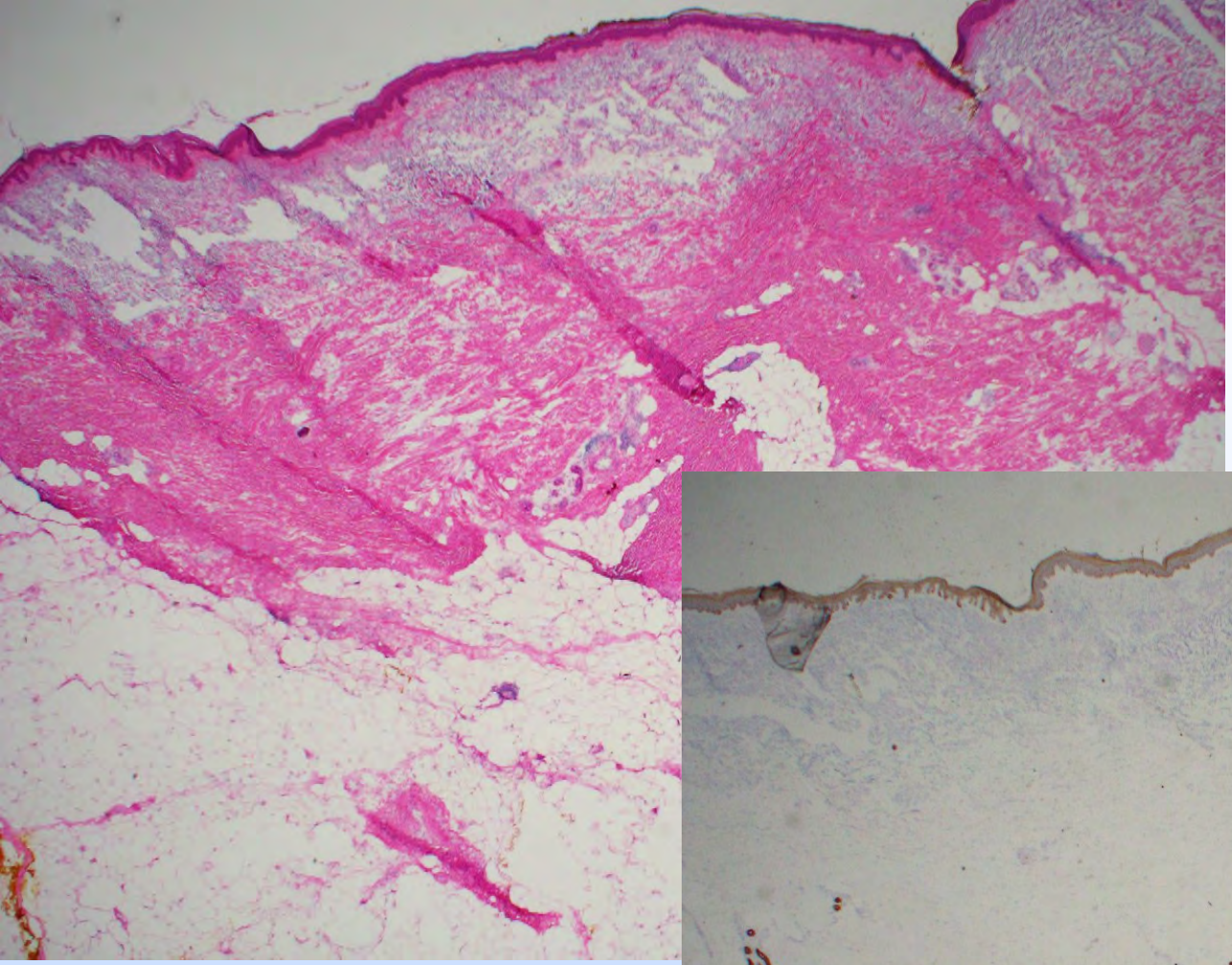
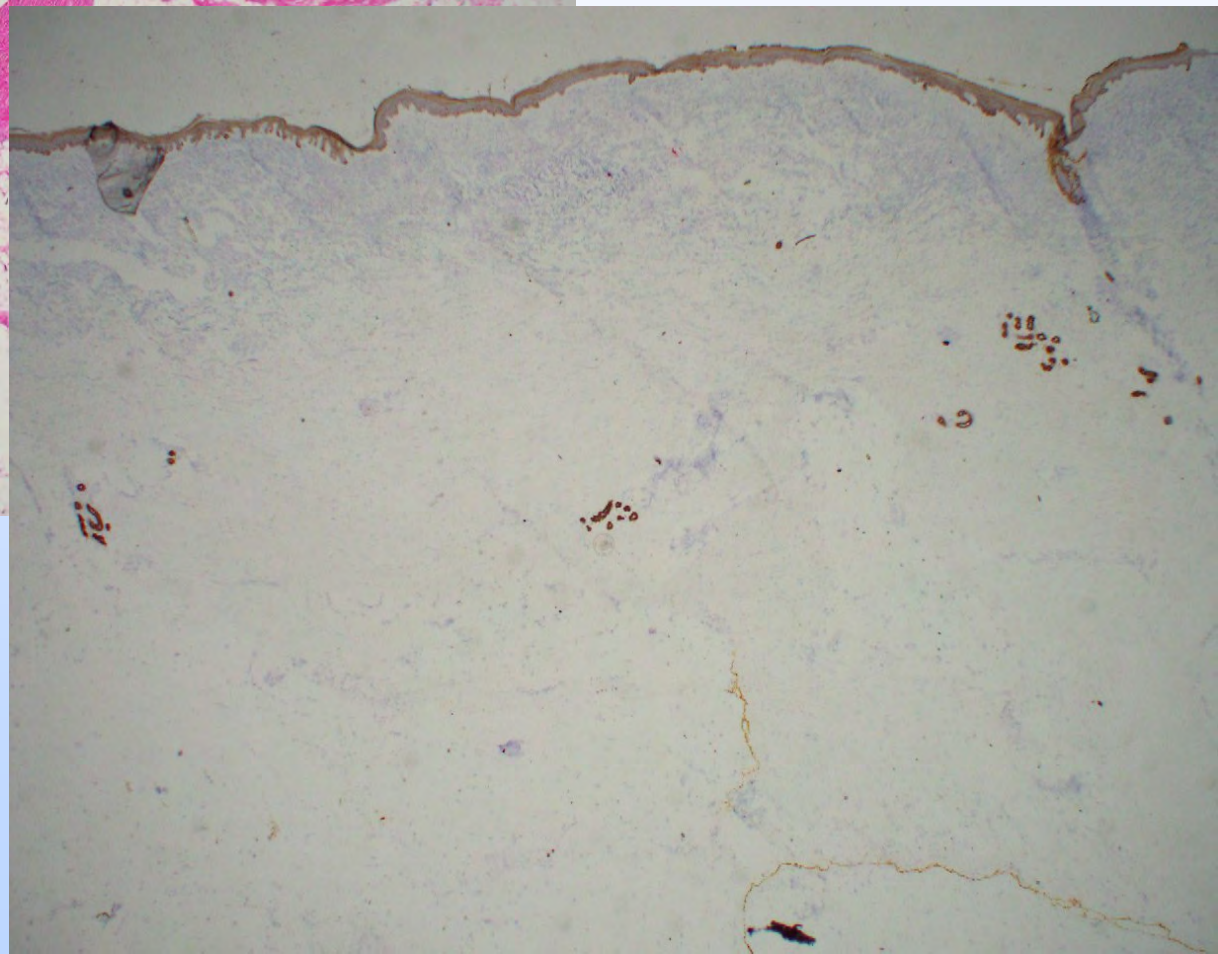


Stage A2 2x MCK stain confirming
no SCC. Follicles and eccrine coils highlight once again



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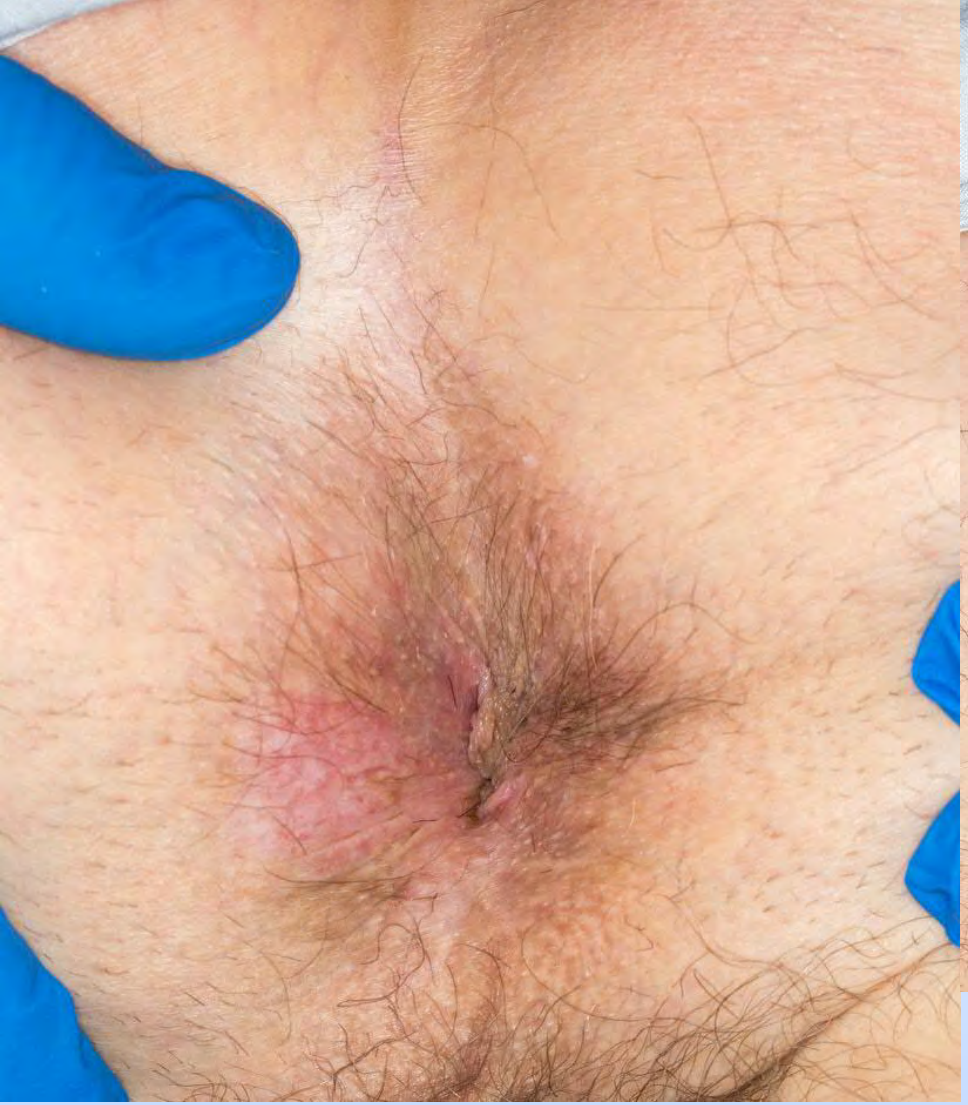
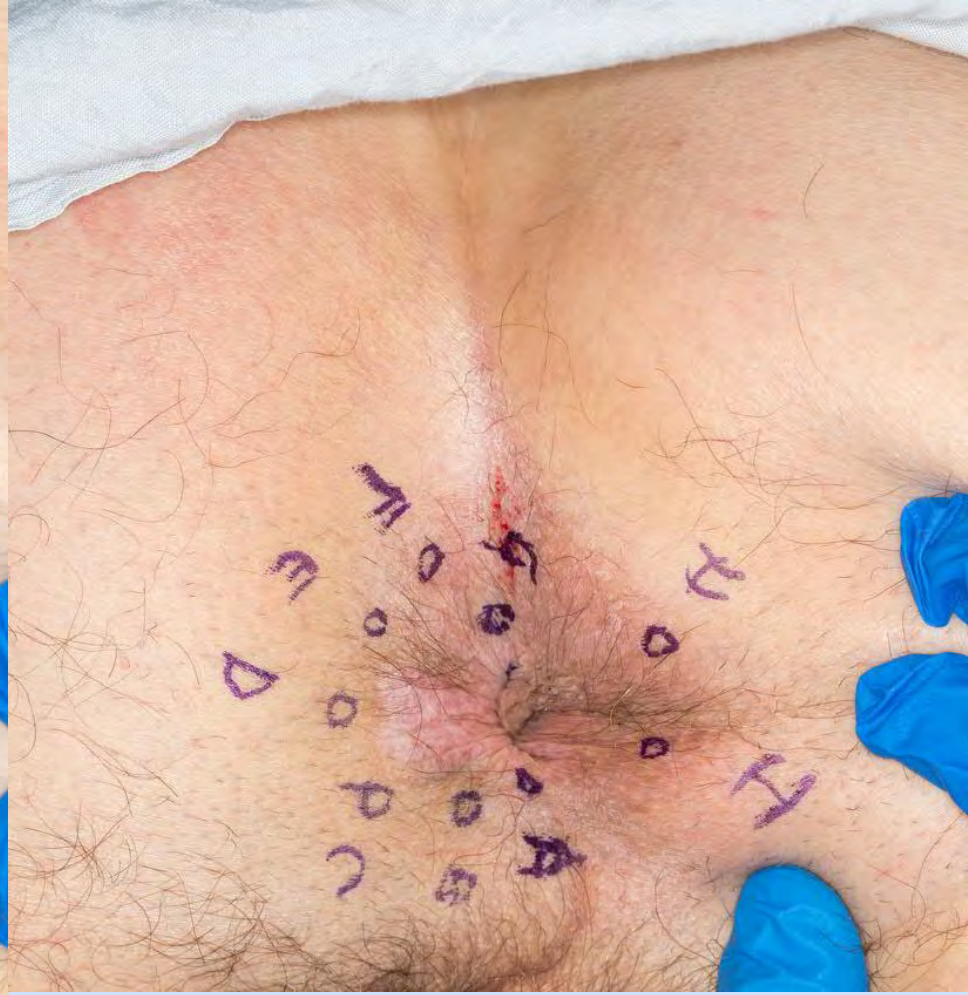
HEALTH SYSTEM





75 y.o. man with ill-defined Extramammary Paget's Disease
No underlying internal malignancy. Scouting biopsies prior to Mohs. Specimens A, G, and anal verge +

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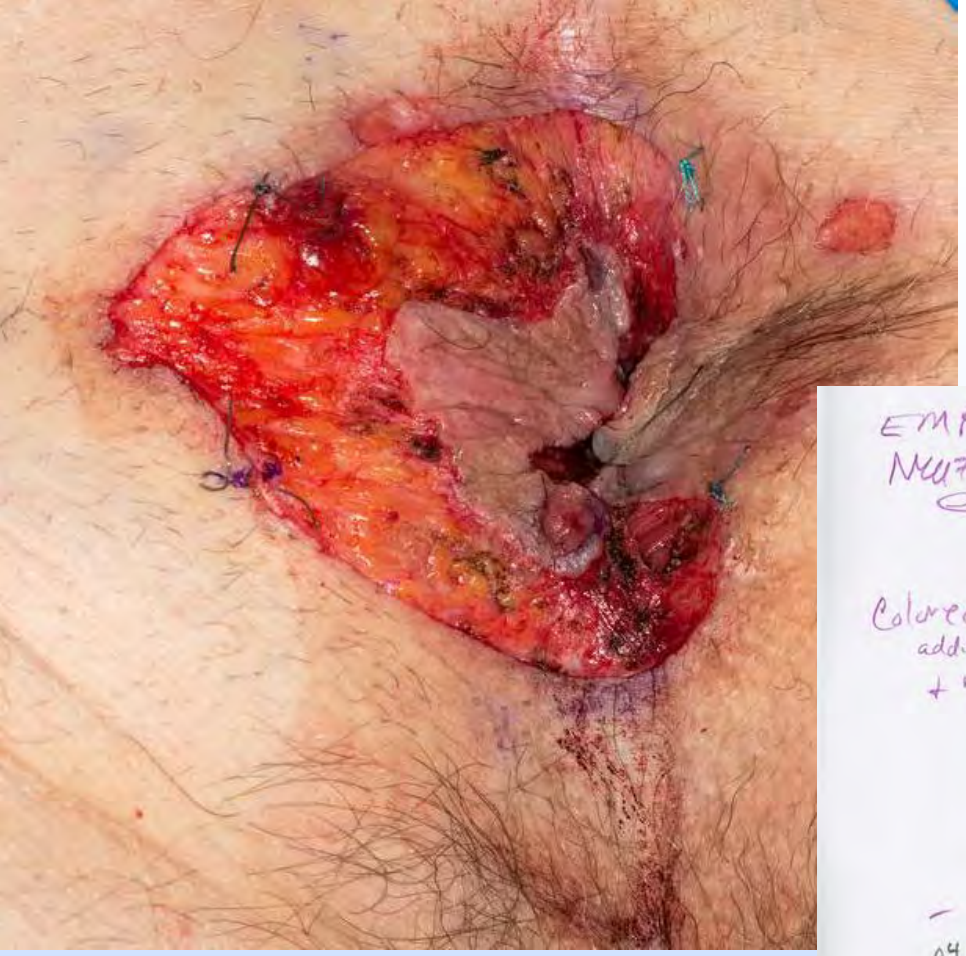
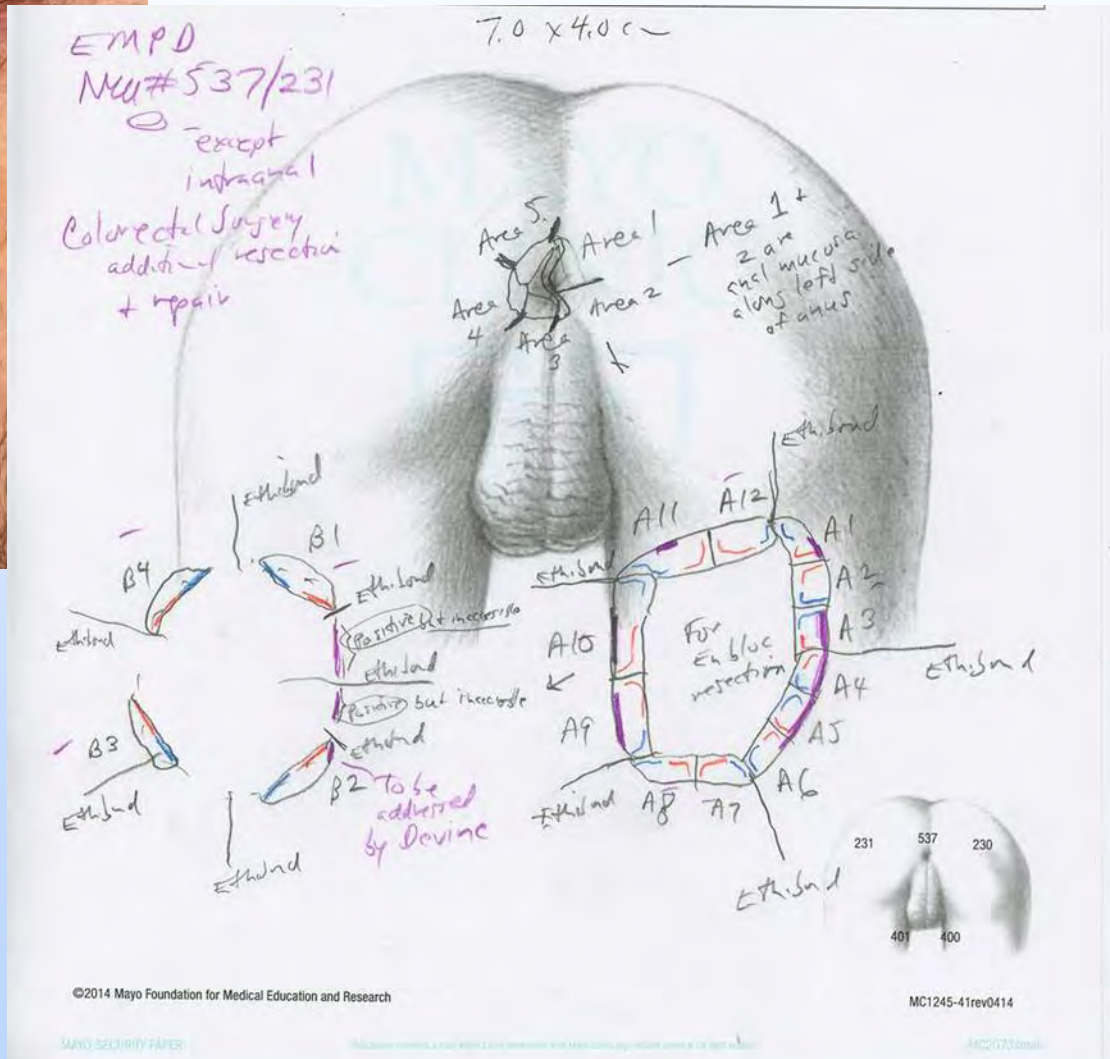
HEALTH SYSTEM





Note sutures used to demarcate corresponding areas on map. Central island of tissue known obvious EMPD to be later resected en bloc. Nearby ulcers from scout bx's

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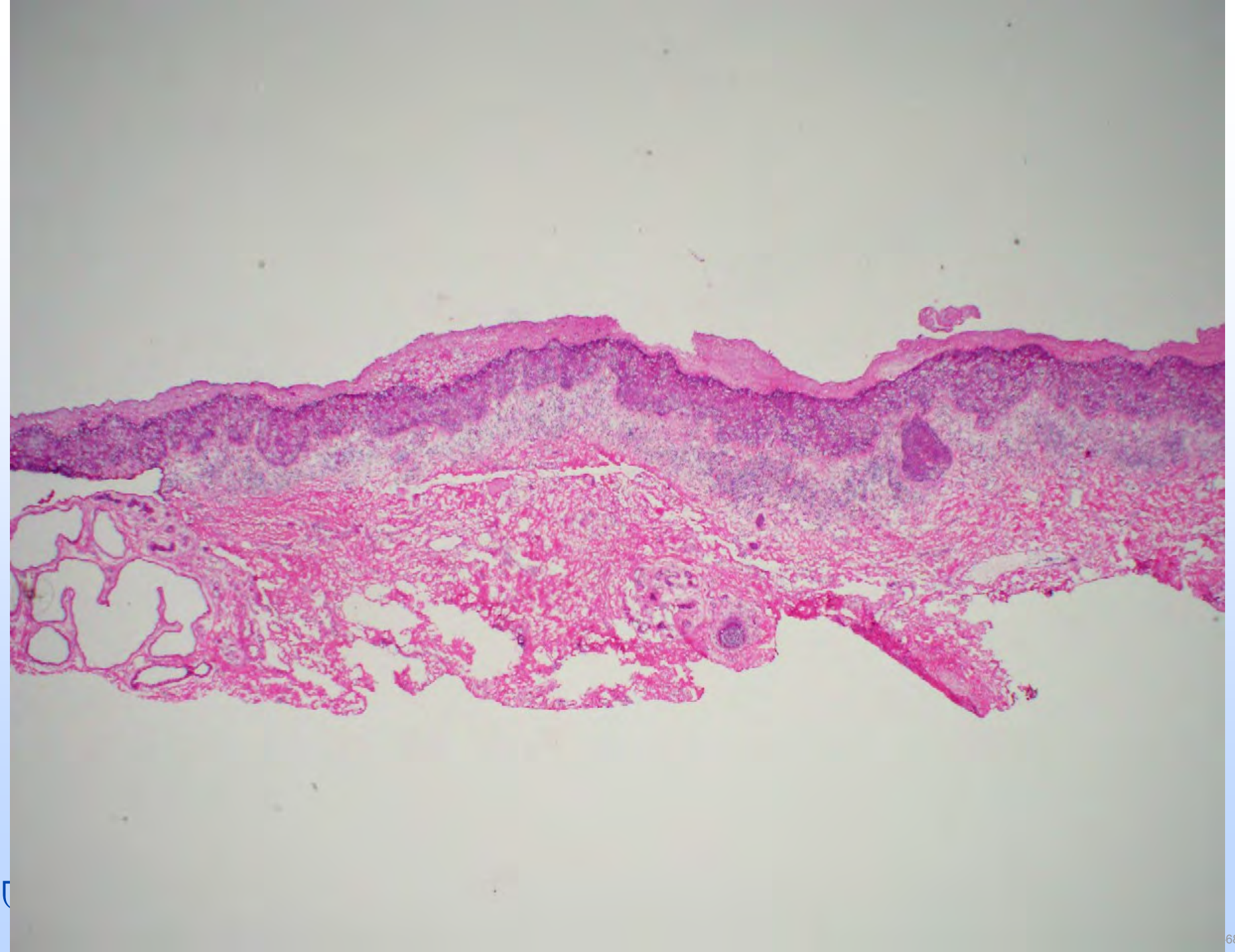
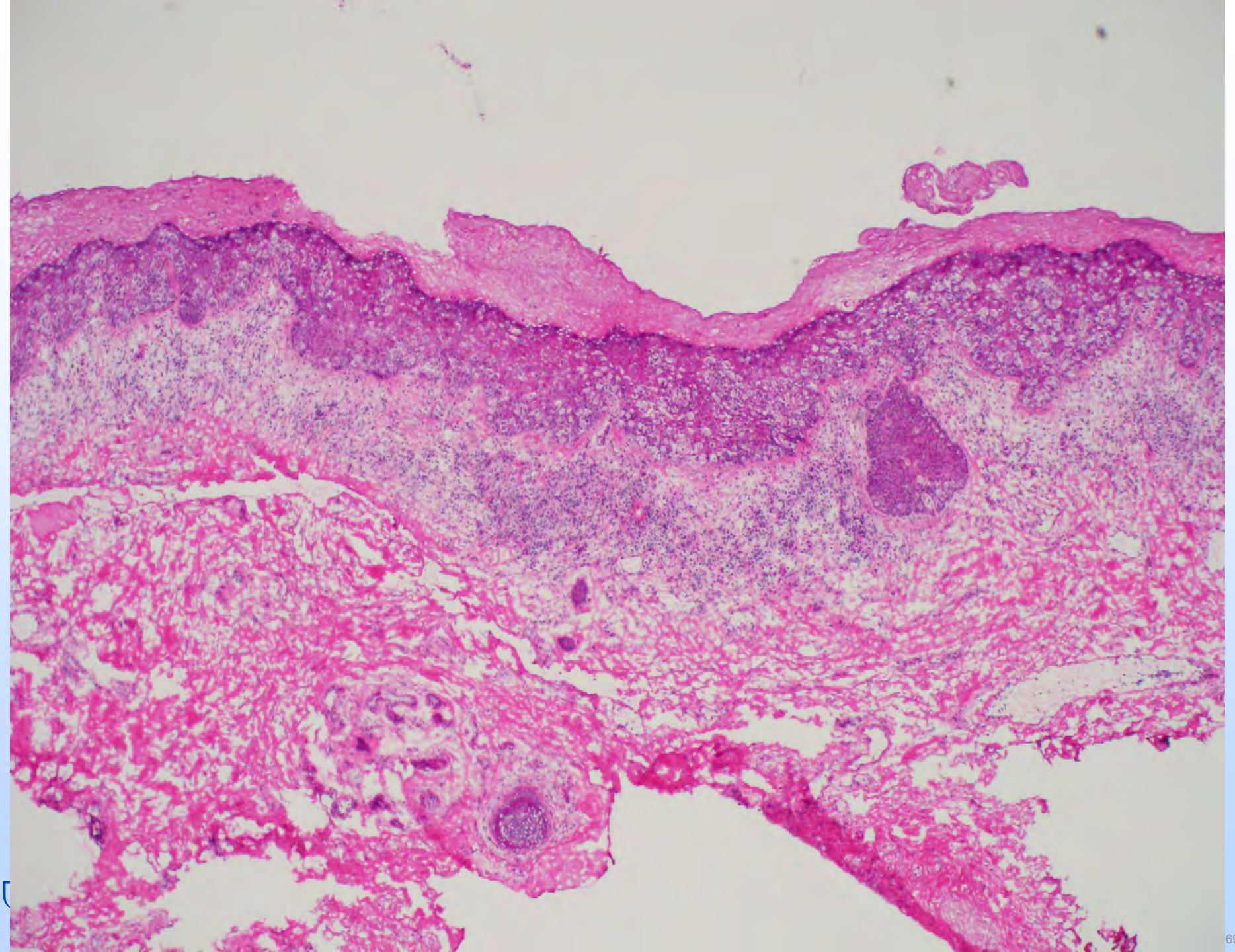
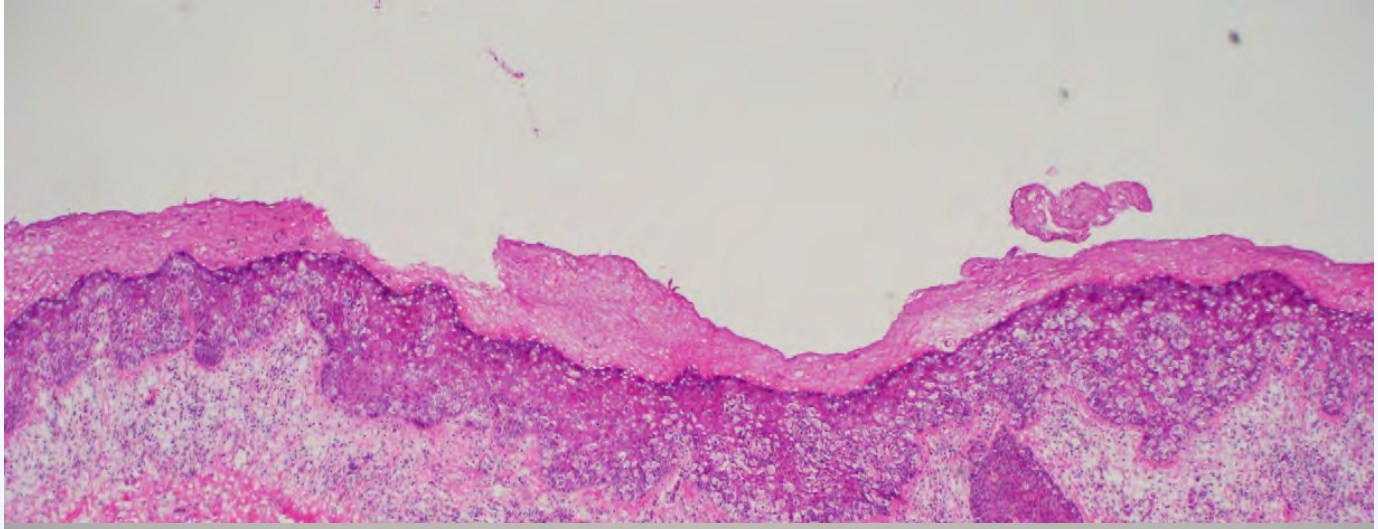
HEALTH SYSTEM

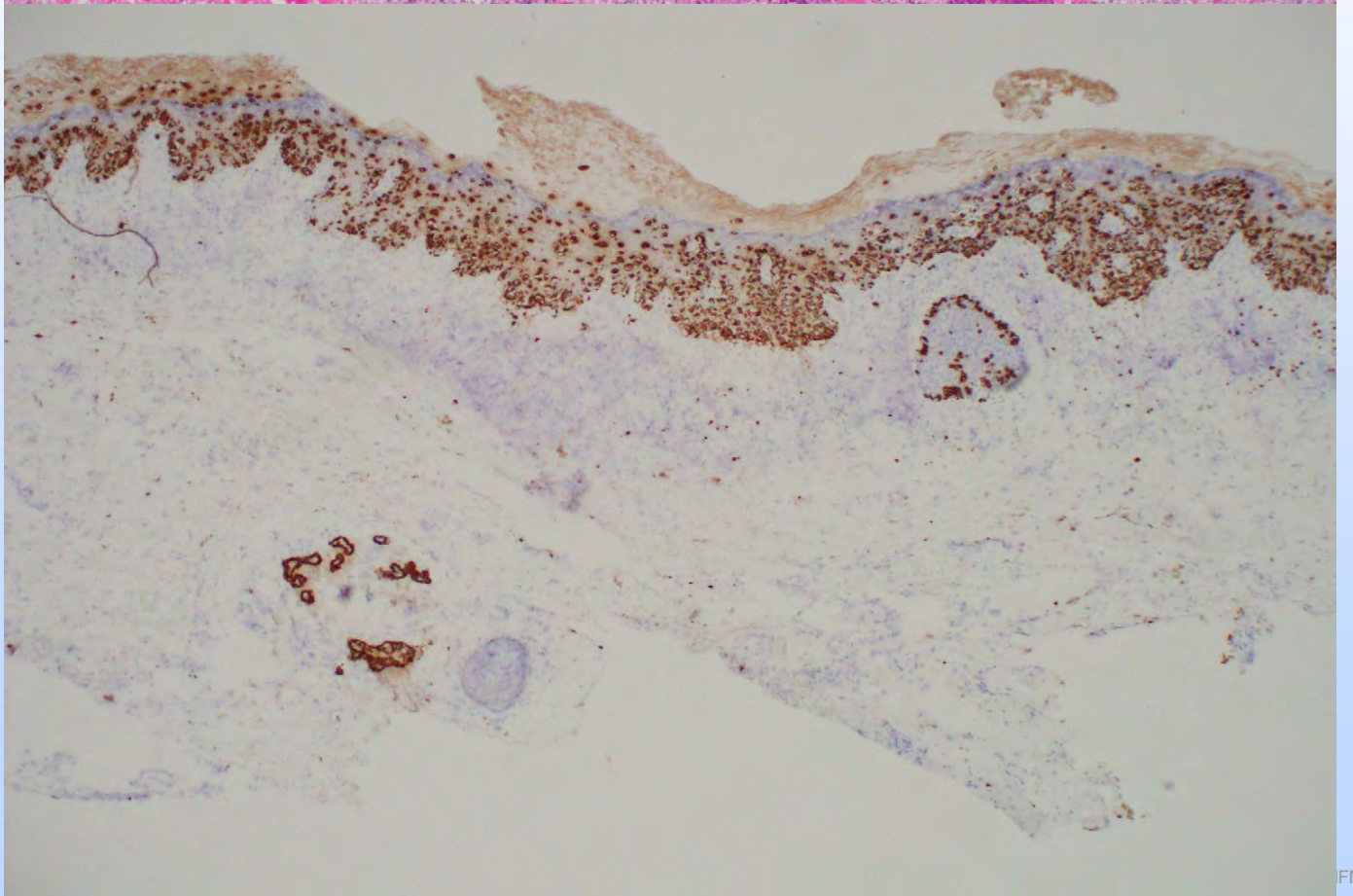




Debulking
EMPD 4x
H&E
CK7

Clearly evident tumor both stains





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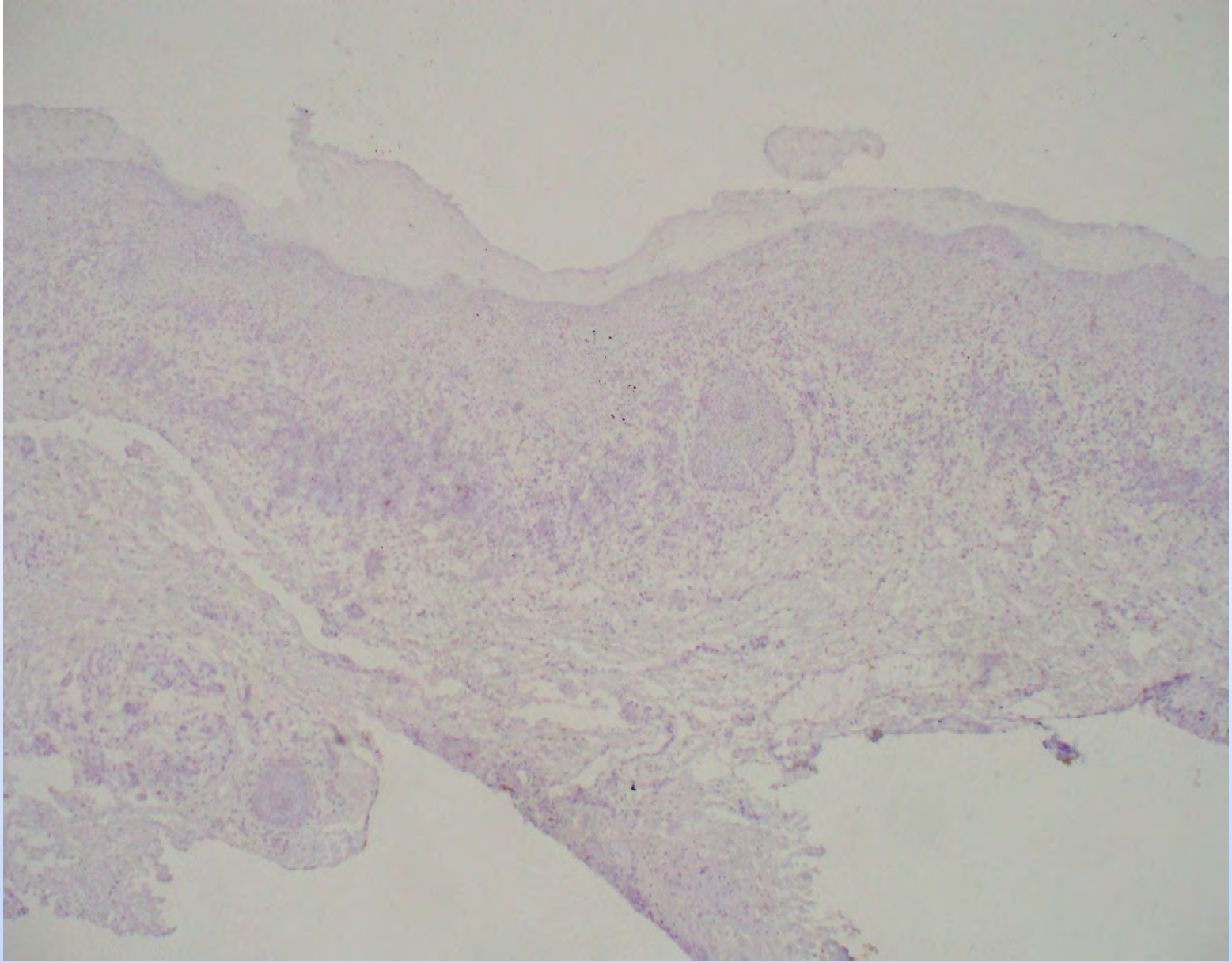
HEALTH SYSTEM

Debulking
CK7

Negative
Control



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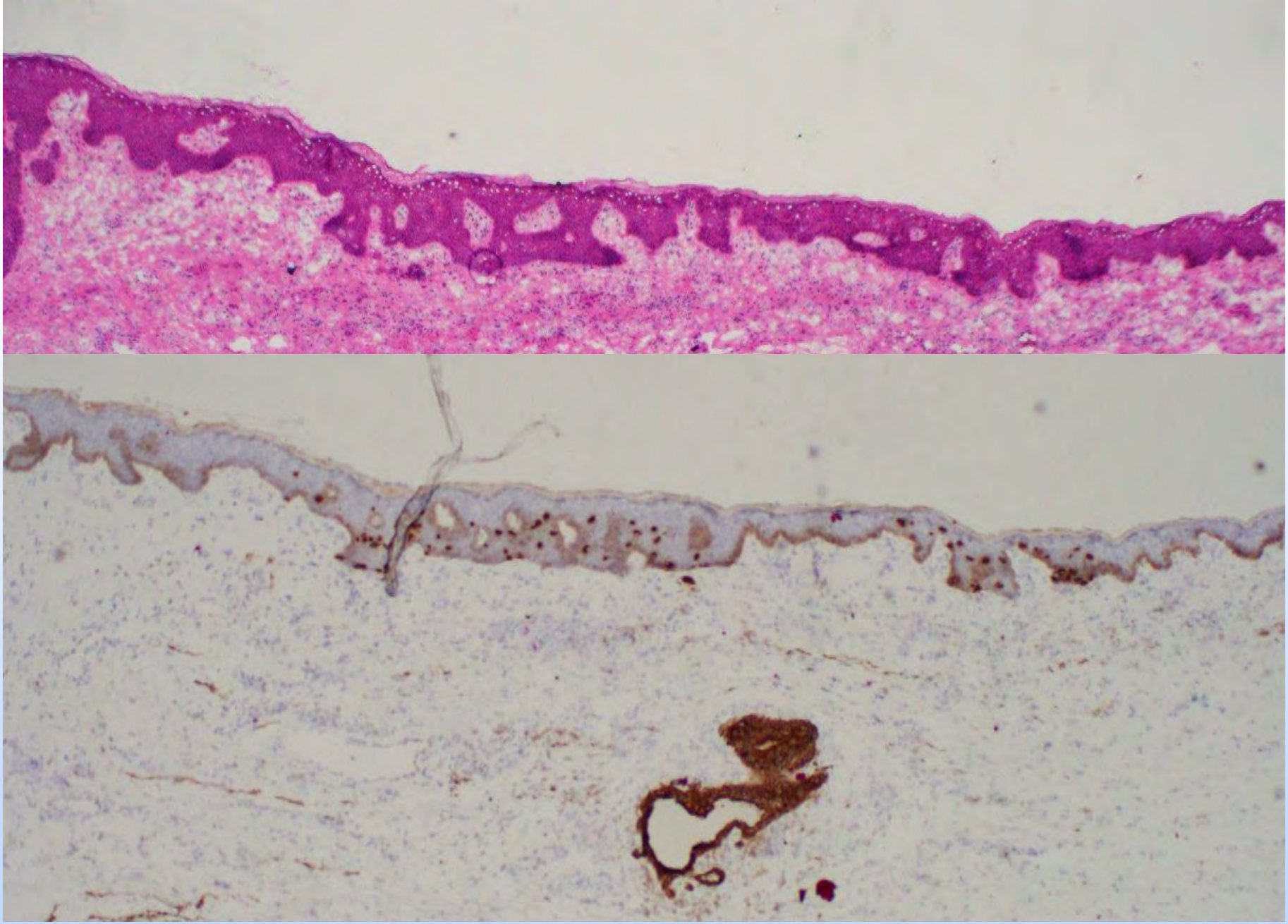
HEALTH SYSTEM



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A1 4x H&E and CK7 Note very subtle H&E but clear immuno findings

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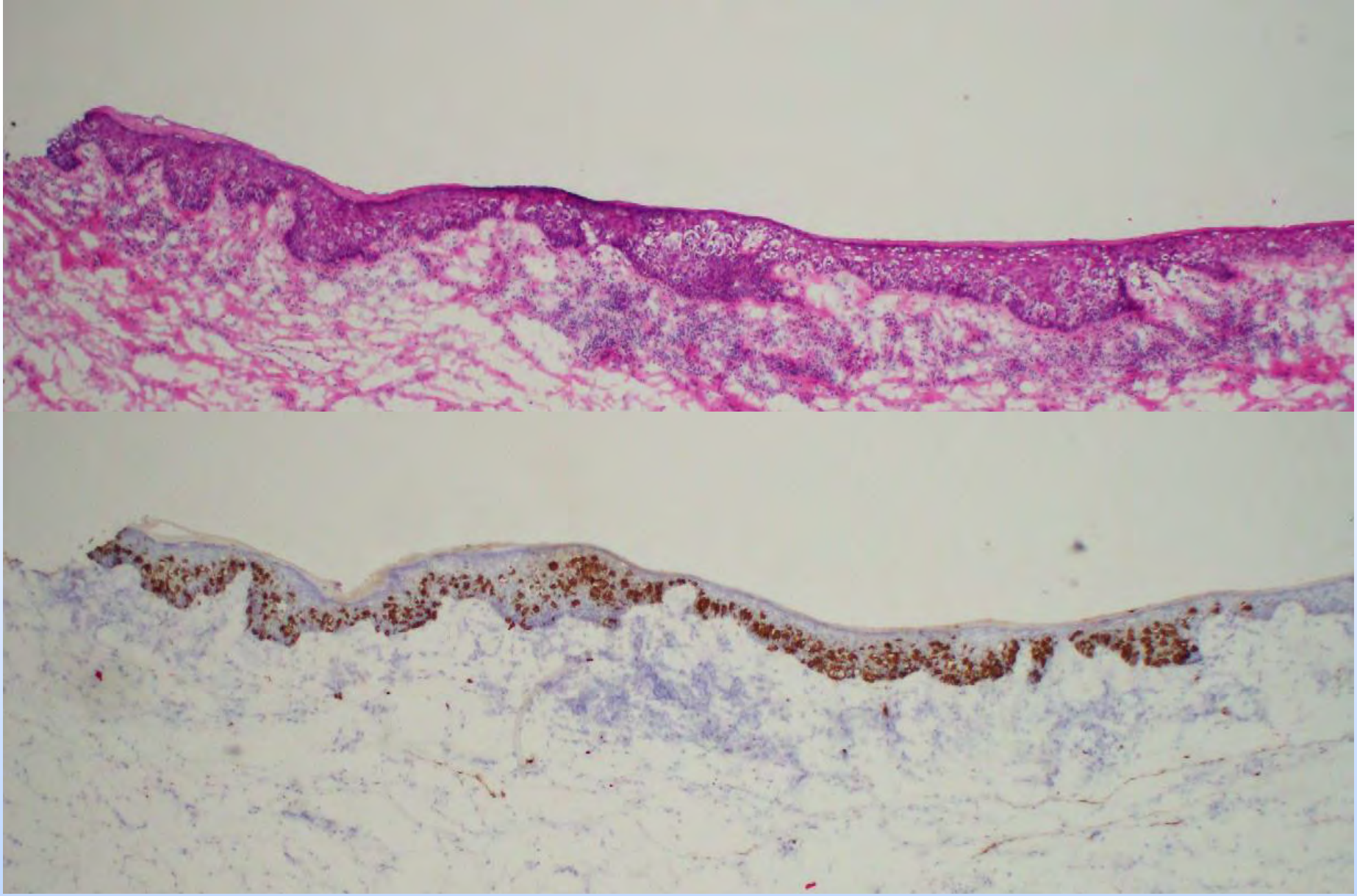

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A4 4x H&E and CK7 Obvious involvement on both stains

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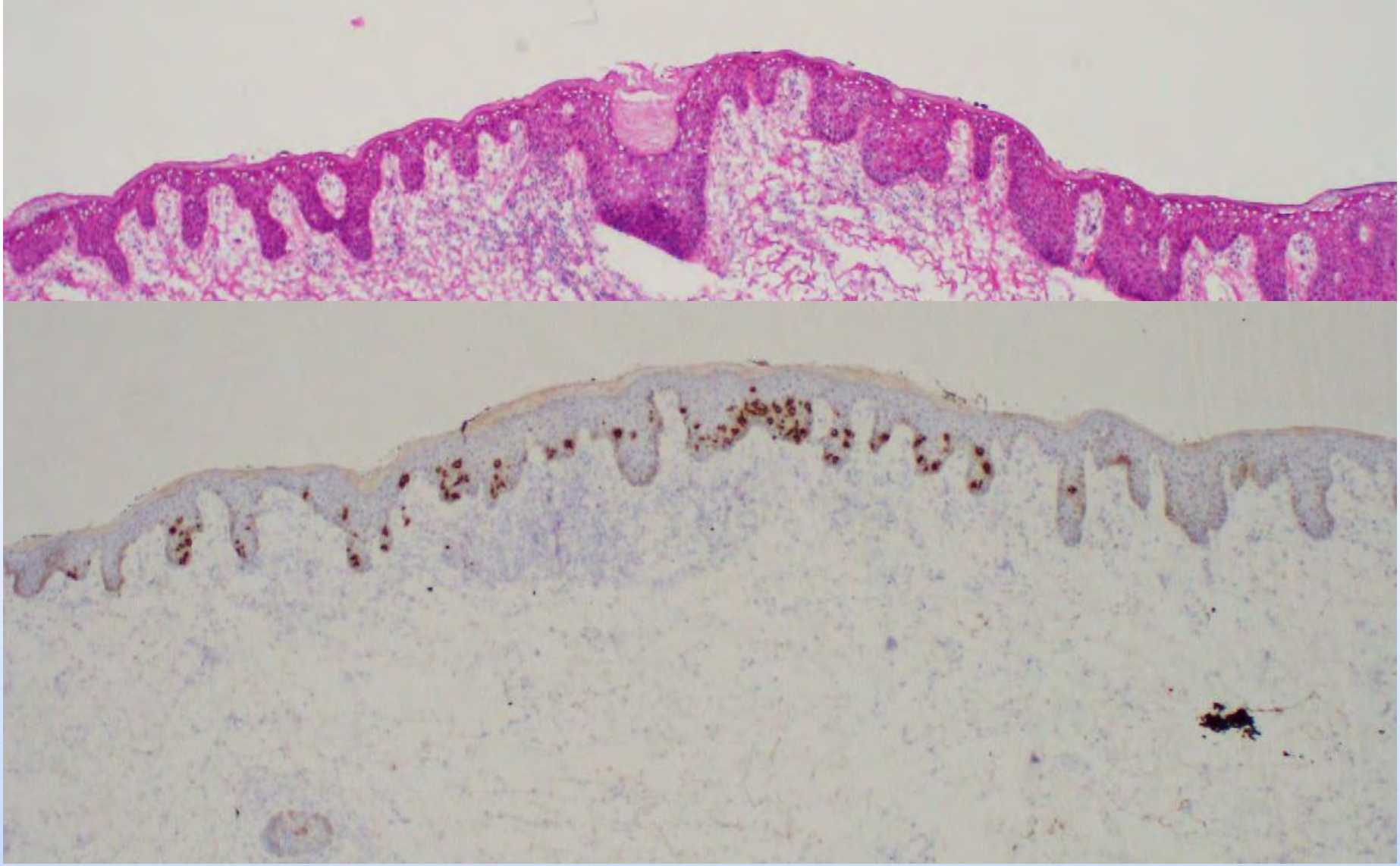

A11 4x H&E and CK7 Much more subtle, CK7 helps greatly

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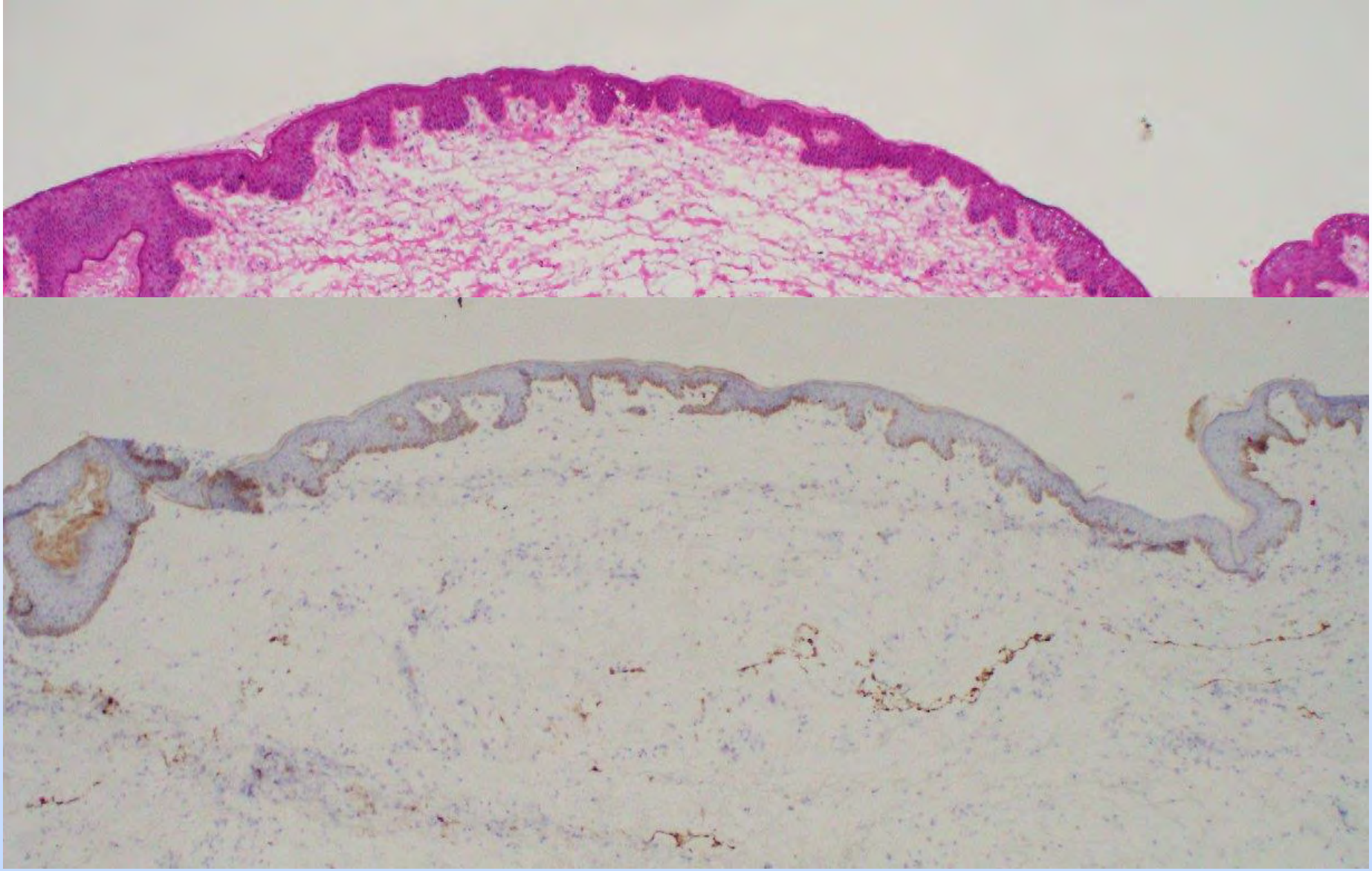

B1 near anal opening 4x H&E and CK7: no tumor. Note background dermal staining

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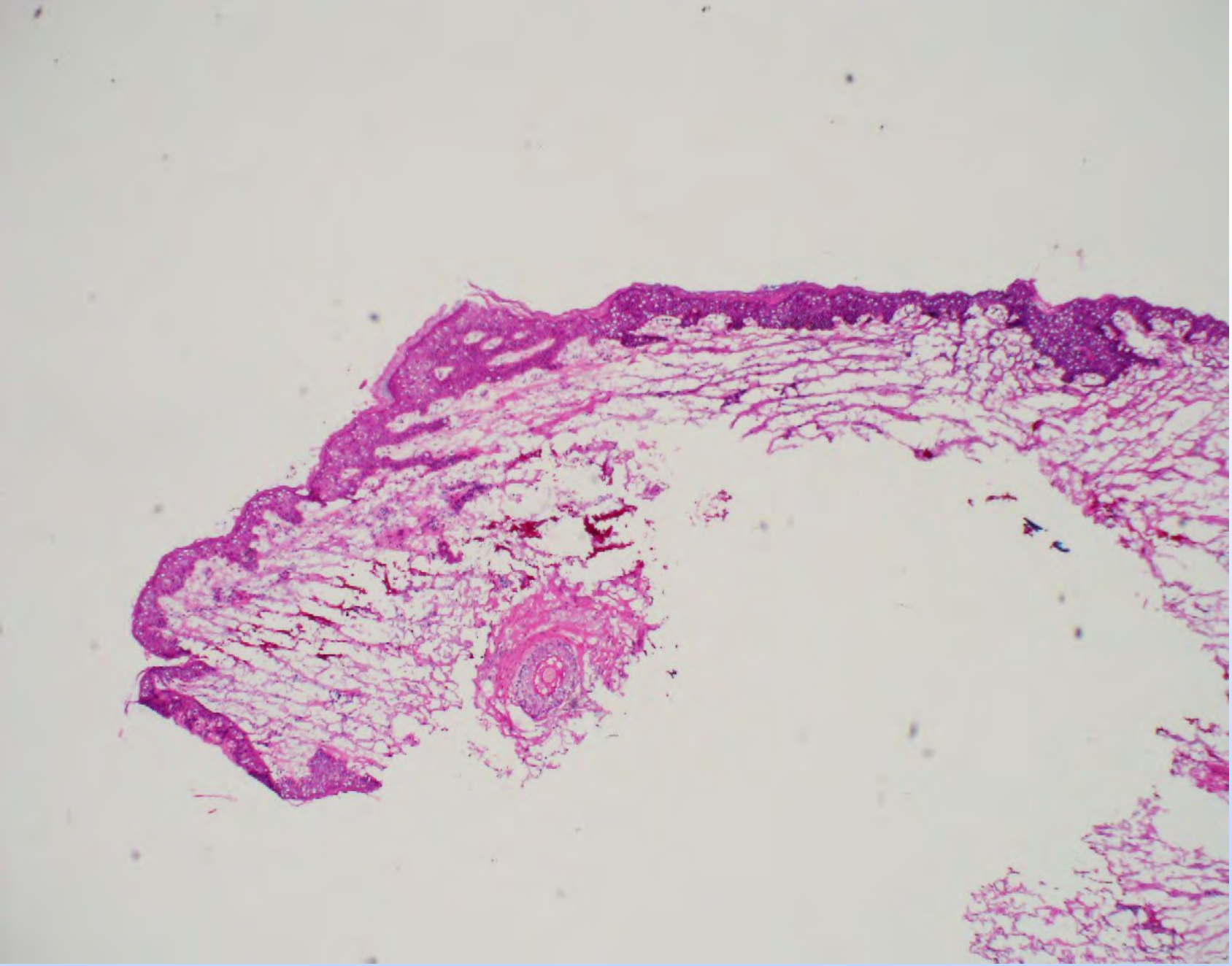

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B2 4x H&E near anus, no obvious tumor

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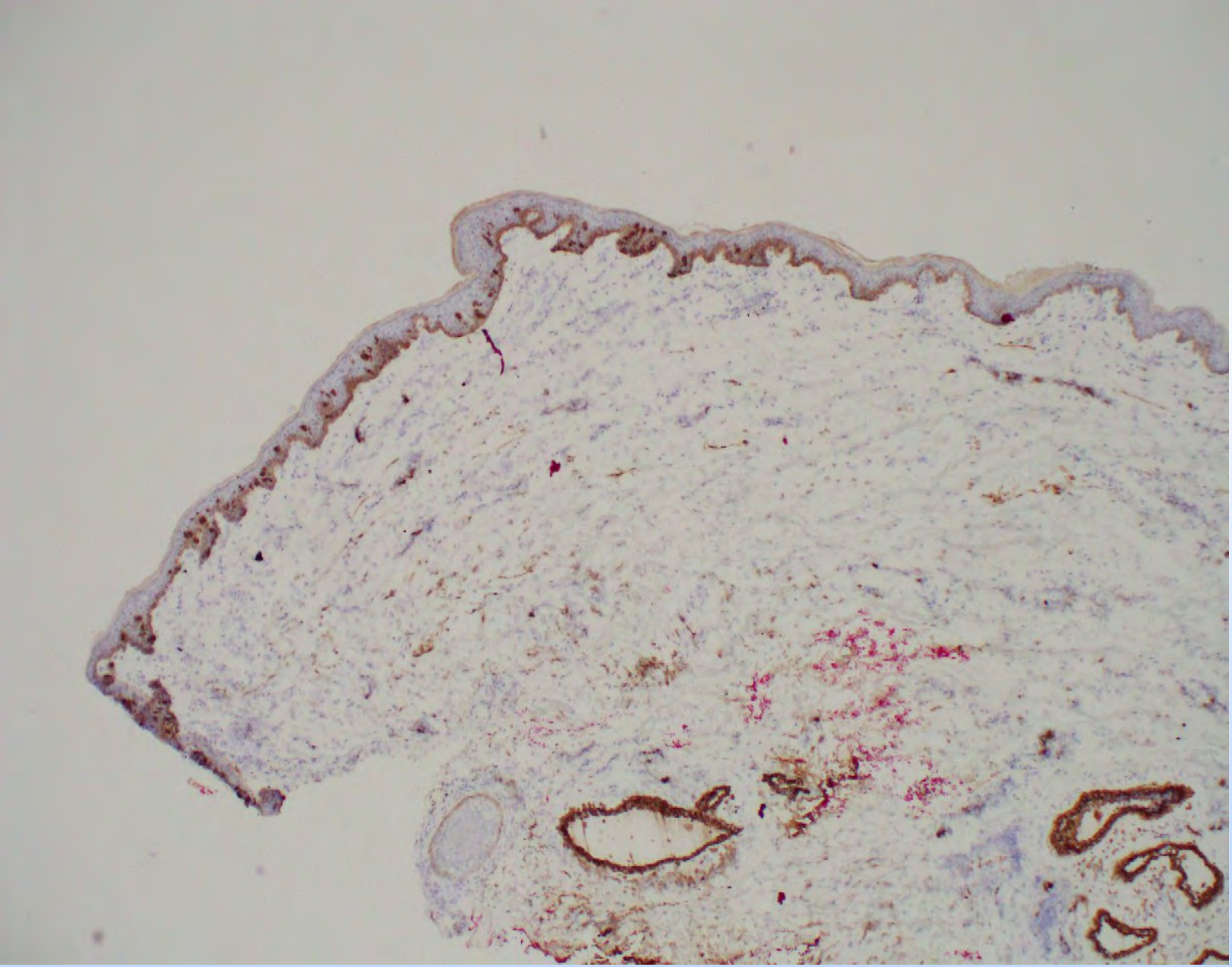

B2 4x CK7 near anus, clearly evident tumor on the left, background and Apocrine glandular staining present

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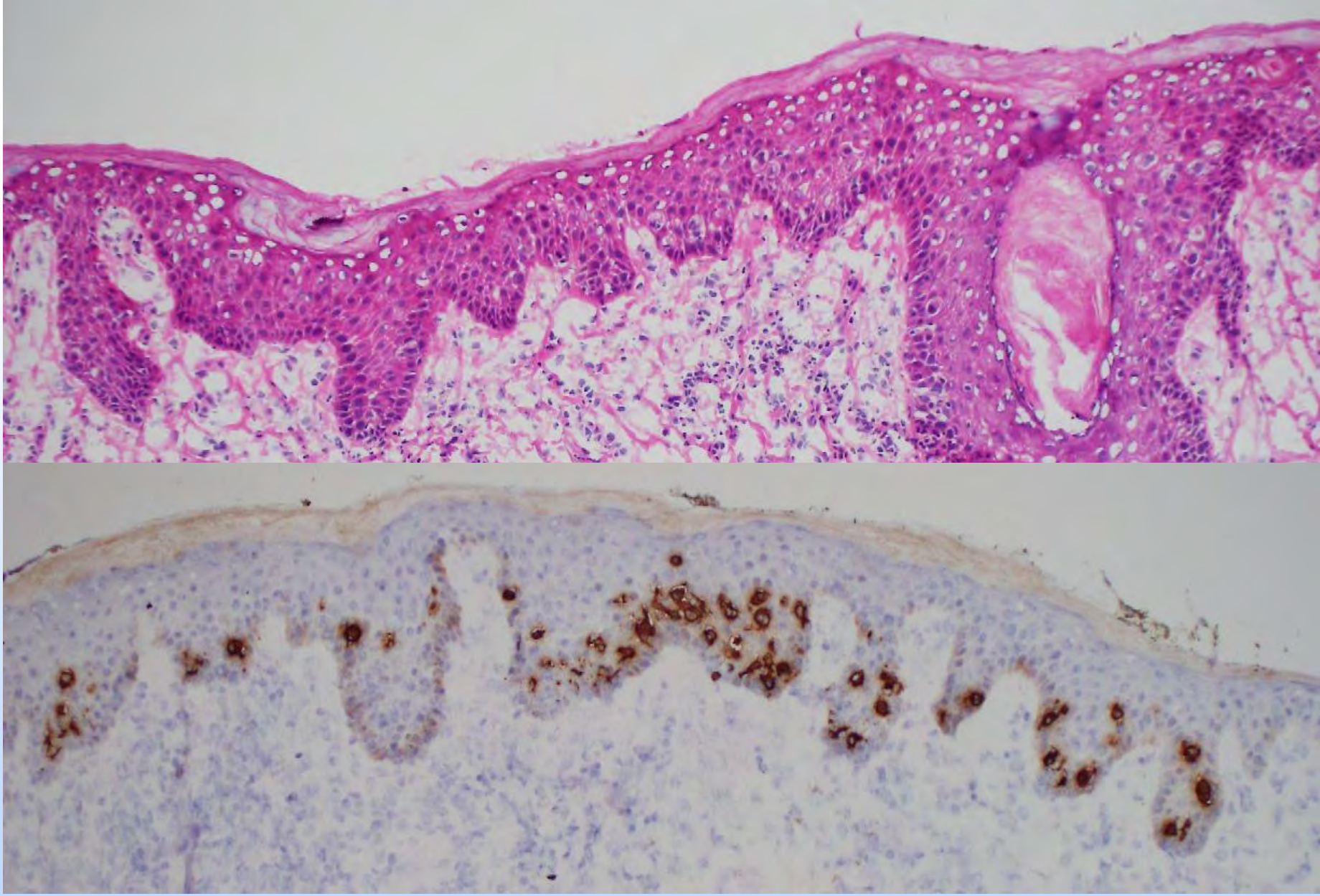

A11 10x H&E with CK7 highlighting Paget cells

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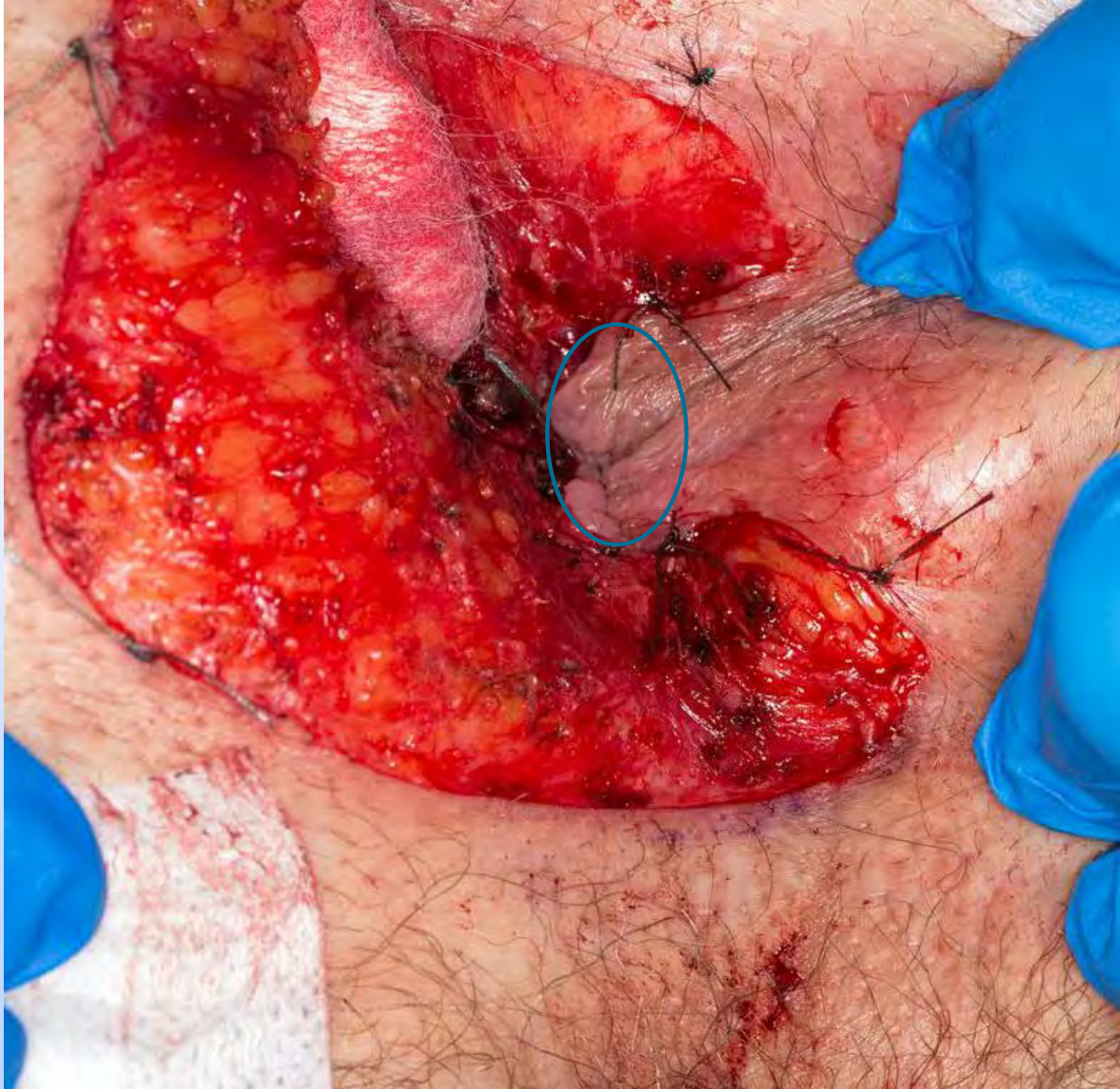
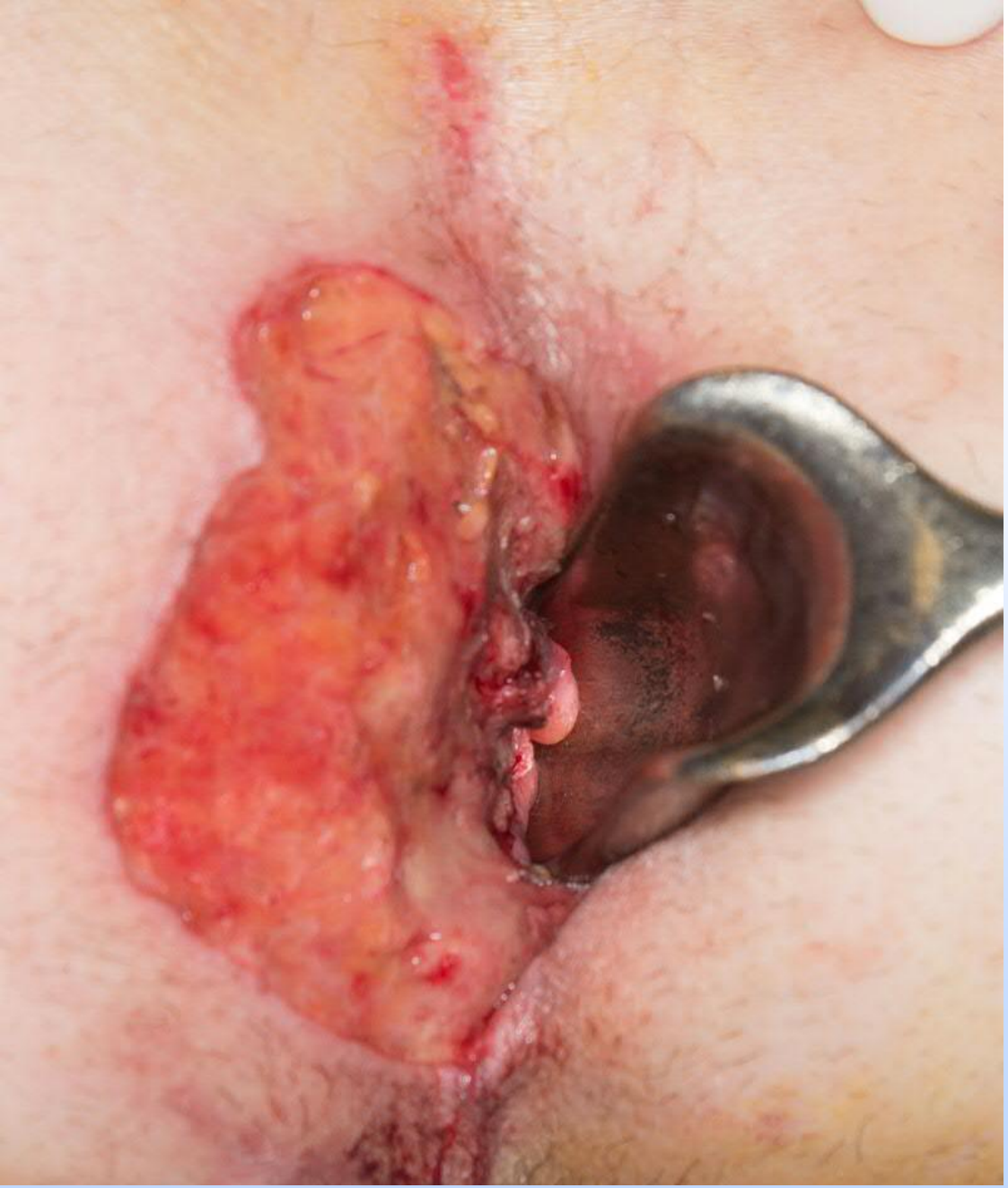
After all margins negative except intraanal areas that were inaccessible by Mohs, and those areas were more widely resected at time of repair next day





Next day, anal speculum helps colorectal surgeon gain internal access.

Wedge resection of additional internal mucosa showed negative margins.

Sufficient tissue spared to allow reconstruction without a colostomy.

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HEALTH SYSTEM

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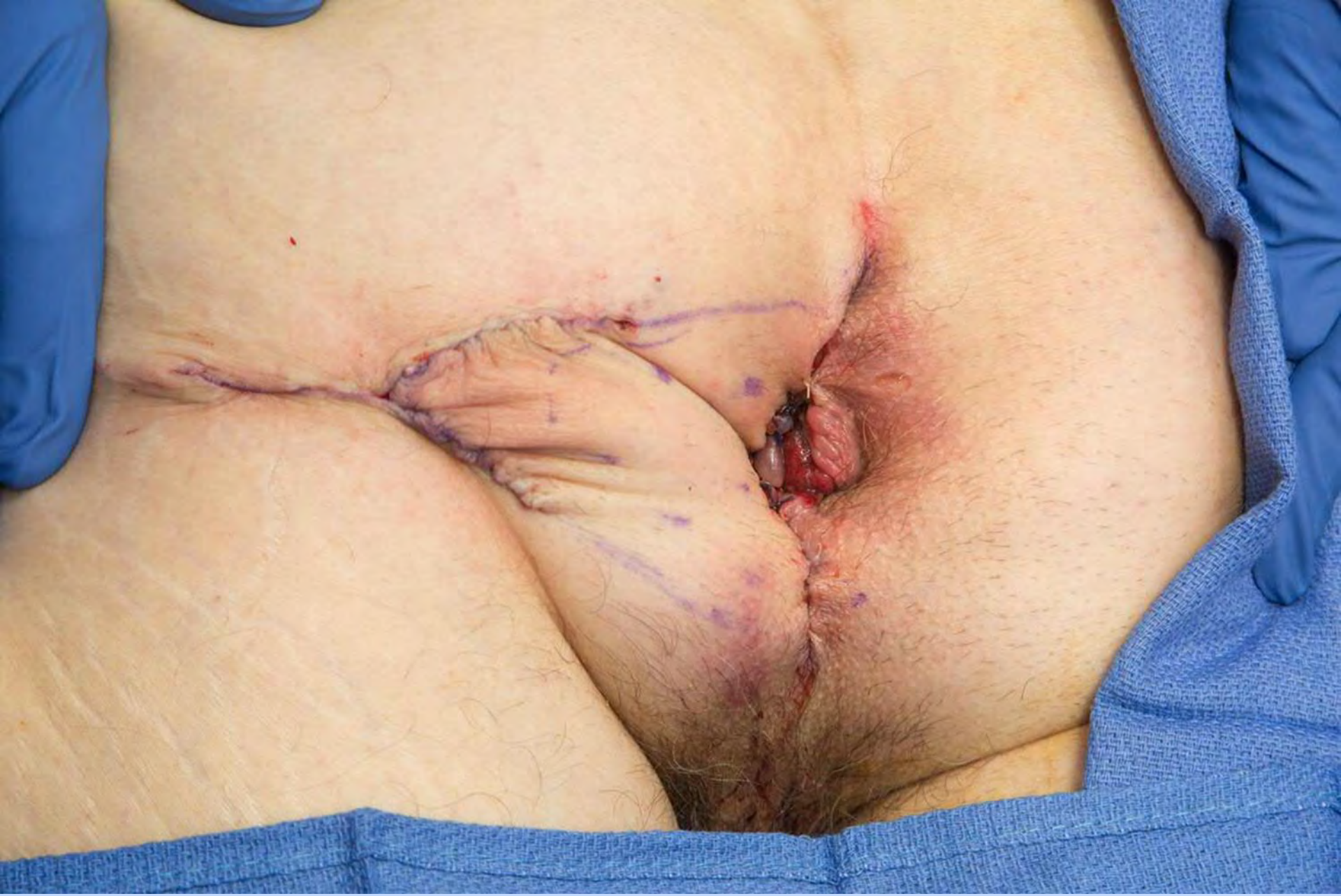

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Large V to Y advancement flap repair

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Key Tips for Immunostains

- Cut at about 5 microns; liquid nitrogen OK
- Make every other, or every third, cut an immuno cut and leave unstained if not sure you will need immunostains
- Remember to get a positive control from the same patient if possible—from the debulking or central clearly involved area, and a sun-exposed normal if MART-1
- Have several good reference controls on hand
- Surgeon should toggle back and forth between H&E and immuno—they are complimentary

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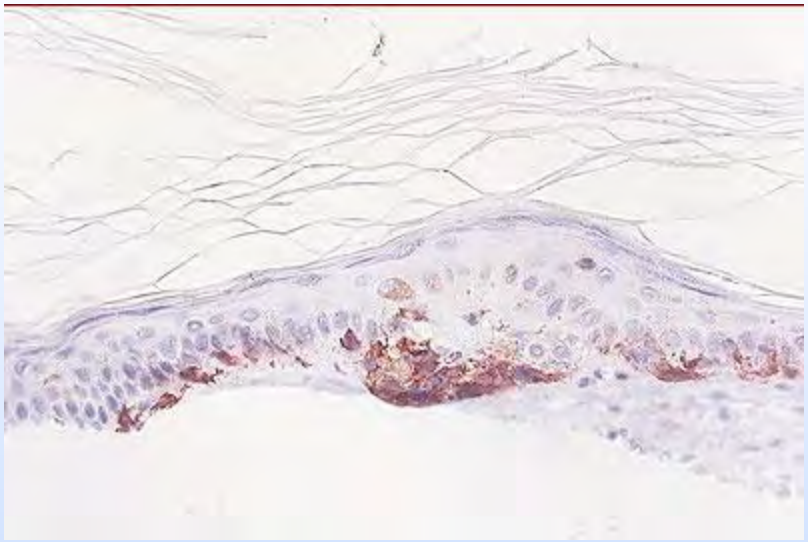
Additional Tips

- Immunostaining and rush paraffin sectioning techniques have been developed in hopes of improving clearance rates, however their use results in increased cost ($30-$100) and procedure time (19-**60**+min) both per slide.
- Mayo uses Leica kits though other brands available. We do a lot of volume so have an automated stainer.



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Mohs for LM Background – Why send debulked central tissue?

- Although melanoma in situ carries nearly a 100% survival rate at 5 years, any level of invasion significantly worsens prognosis
- Rates of an invasion found on debulk specimens initially thought to be LM: 5 to > 50% range in the literature; typical is 5-10% in larger series
- **Some Mohs surgeons will process the entire specimen and not debulk; acceptable, though adds greatly to processing time

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Upstaging of LM to MM at Mayo

- 1994-2012 cases reviewed
- 624 cases of “LM” with subtotal biopsy samples subsequently resected. Largest series to date
- 24 (4%) showed invasion
- Upstaging uncommon but possible, and less than average of pooled prior series

Gardner KH, et al. Upstaging From Melanoma in Situ to Invasive Melanoma on the Head and Neck After Complete Surgical Resection. Dermatol Surg 2015;41:1122-5.

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A Word on MiTF (Microphthlmia transcription factor) Immunostaining…

- A nuclear-staining antibody that may be more specific for atypical melanocytes than MART-1, HMB-45 and others
- How will it perform on frozen sections, and compare regarding cost, reliability, speed??
- Another nuclear stain, SOX-10, may also show utility and should likely be investigated

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MiTF Immunostaining

- Is a nuclear immunostain that may have more specificity than MART-1 for melanocytes, including atypical ones.
- Recent studies performed on fixed tissue
- Black WH, et al. *Am J Dermatopathol* 2011
- Kim J et al. *J Cutan Pathol* 2011
- Christensen KN, et al. Dermatol Surg 2016;42:167-75
  - Showed feasibility and utility of MiTF as an alternative to MART-1 in a pilot study comparing the 2 stains head-to-head in real time

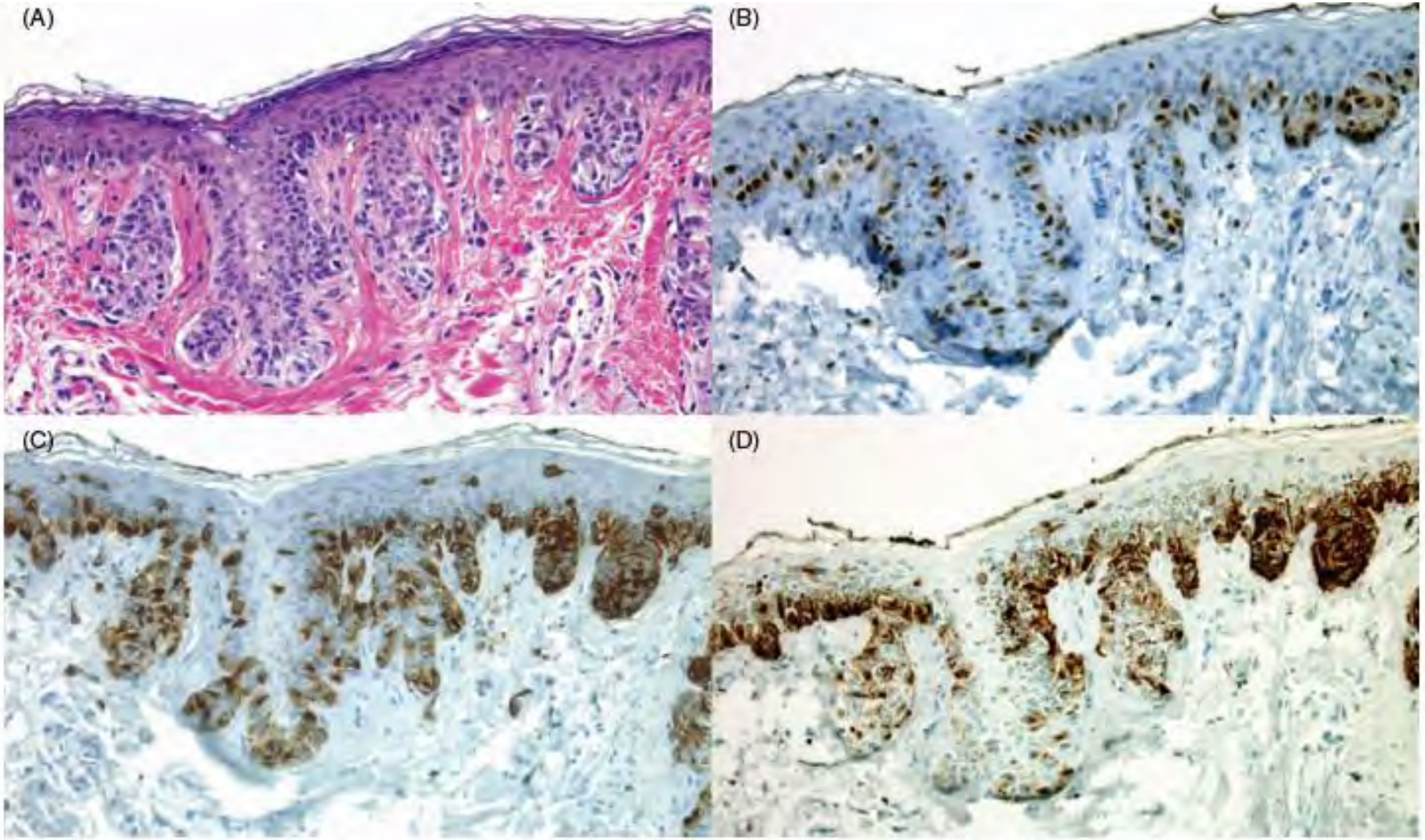

Fig. 3. High-magnification image shows comparison of hematoxylin and eosin (A) and immunohistochemical melanocytic stains in melanoma *in situ*. Nuclear staining by MiTF (B) more clearly identifies intraepidermal melanocytes in melanoma *in situ*. Cytoplasmic Melan-A (C) and HMB-45 (D) highlight increased numbers of melanocytes and basal keratinocytes, even in the presence of Azure blue counterstain.

Kim J et al. *J Cutan Pathol* 2011

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Melanocyte Counts, Mayo Pilot Study, showing improved specificity of MiTF over MART-1 especially for chronic sun-damaged skin used for comparison during Mohs, and for peripheral Mohs margins that were negative for residual MMIS.

Christensen KN, et al. Dermatologic Surg 2016;42:167-75

TABLE 3. Summary of Results

	Control CSDS		*Negative Margin*		*Tumor*	
Stain	*MITF*	*Melan-A*	*MITF*	*Melan-A*	*MITF*	*Melan-A*
Patients	16	16	16	16	12	12
Mean	9.8*	13.7*	8.8*	14.1*	63.5	62.4
SD	3.5	5.9	4.2	5.0	17.7	14.9
MAX	15.2	24.3	17.7	21.7	97.5	86.2
MIN	3.5	5.2	2.7	6.7	41.0	41.8

*Statistical significance ($p < .001$).
MAX, maximum; MIN, minimum.

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Recent Literature Highlights

- Standard vs Mohs approach for over 400 cases of LM, retrospectively, over ~10 years.
- Similar cure rates with slight trend toward fewer recurrences with Mohs and narrower margins, especially in critical anatomic locations.
- Mirzoyev SA et al. J Am Acad Dermatol 2014;70:443-8
- Nosrati A et al. JAMA Dermatol 2017;Feb;epub

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Summary Slide for Treatment Approaches to LM/LMM
Chang KH, et al, Dermatol Surg 2011;37:1069-79.

TABLE 2. Comparison of Modified Surgical Techniques Using Permanent Sections for the Management of Melanoma

	Dhawan, 1990	*Cohen, 1998*	*Clayton, 2000*	*Johnson, 1997*	*Bub, 2004*	*Huilgol, 2004*	*Mahoney, 2005*
Technique	MMS with rush permanent sections	MMS with rush permanent sections	Slow MMS with rush permanent sections	Square technique	Staged excision with radial cuts	Mapped serial excision	Perimeter technique
Section orientation	Horizontal	Horizontal	Horizontal	Vertical	Vertical radial	Vertical	Vertical
Description of technique	45° angled Mohs layers with permanent sections	2- to 3-mm margin, 45° angled Mohs layer with frozen sections read by Mohs surgeon then permanent sections by pathologist	2- to 5-mm margin, 45° angled Mohs layer with permanent sections	5- to 10-mm polygonal margin, double-bladed hair transplant scalpel used to excise peripheral strip of 2–4 mm. Strips sutured or granulated.	Excision of visible tumor with a 2- to 3-mm margin	5-mm margins, sections cut at 1- to 2-mm bread loaf intervals	5-mm margin, 2-mm strips around the surgical margin, strips sutured.
Margin evaluation	Complete	Complete	Complete	Complete	Partial	Partial	Complete
Tumor bulk assessment for invasion	With first stage	With first stage	With first stage	With final stage	With first stage	With first stage	With final stage
Diagram		2- to 3-mm margin	2- to 5-mm margin	5- to 10-mm margin	2- to 3-mm margin	5-mm margin	5-mm margin
View from above							
View from side							

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Conclusions

- There is no single best approach to LM and selected other challenging skin cancers
- Strongest indications when excising LM and EMPD; also recurrent SCC, high-grade SCC, and those close to critical structures or where lots of inflammation or CLL
- Be prepared for subclinical invasion and prepare in advance
- Don’t rely on immunostains as a panacea—they can be a helpful adjunct

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