THE ANNUAL GLOBAL MEETING OF THE INTERNATIONAL GYNECOLOGIC CANCER SOCIETY - 2019

Emerging Developments and New Concepts in Gynaecological Pathology (Sponsored by the ISGyP)

"ADVANCES IN THE PATHOLOGY OF VULVAR CARCINOMA AND ITS PRECURSORS."

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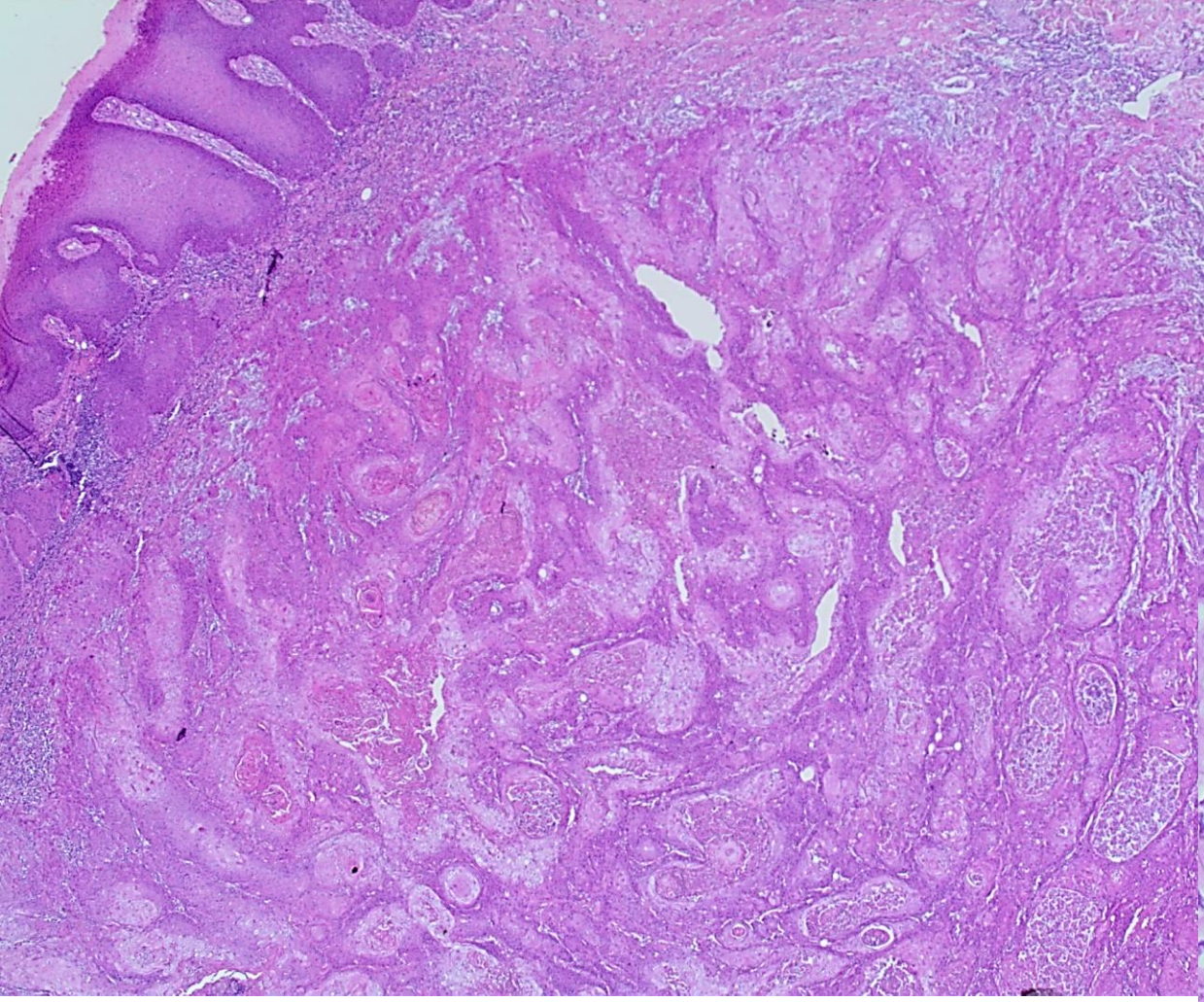
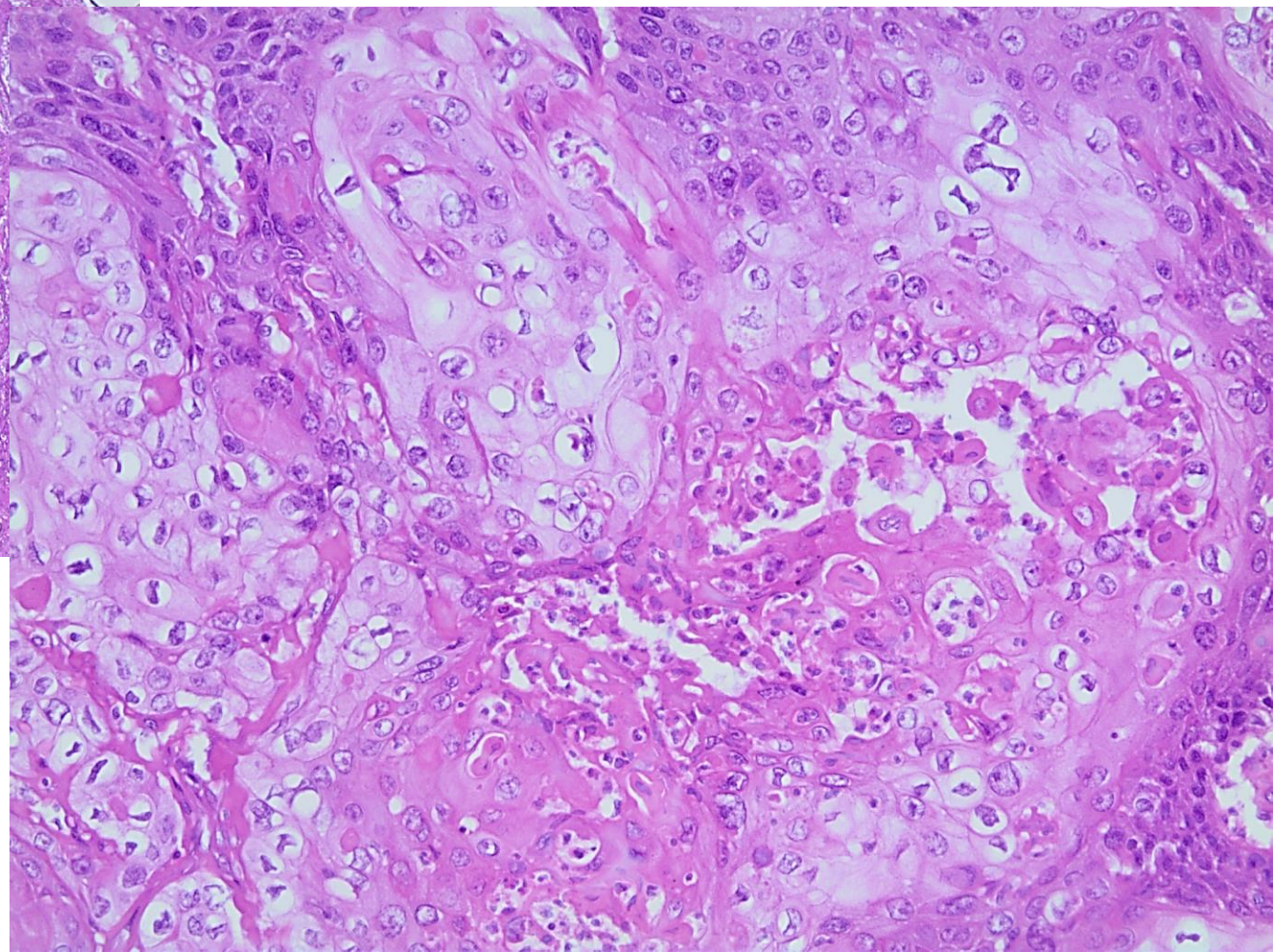
Faculty Disclosure

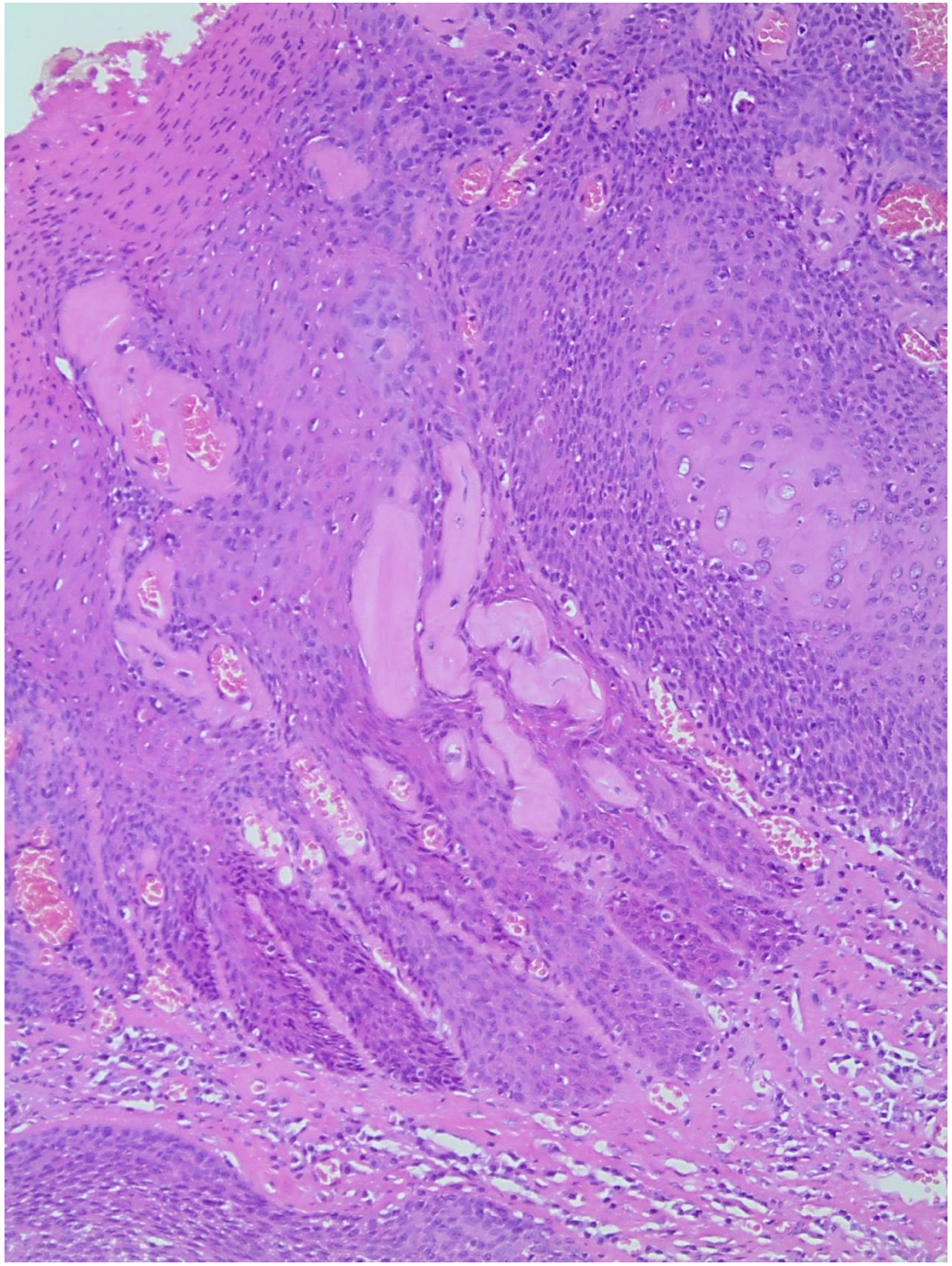
	No, nothing to disclose
X	Yes, please specify:

Company Name	***Expenses***	*Consulting/ Advisory Board*	*Funded Research*	*Royalties/ Patent*	*Stock Options*	*Ownership/ Equity Position*	*Employee*	*Other (please specify)*
ISGyP	**x**							

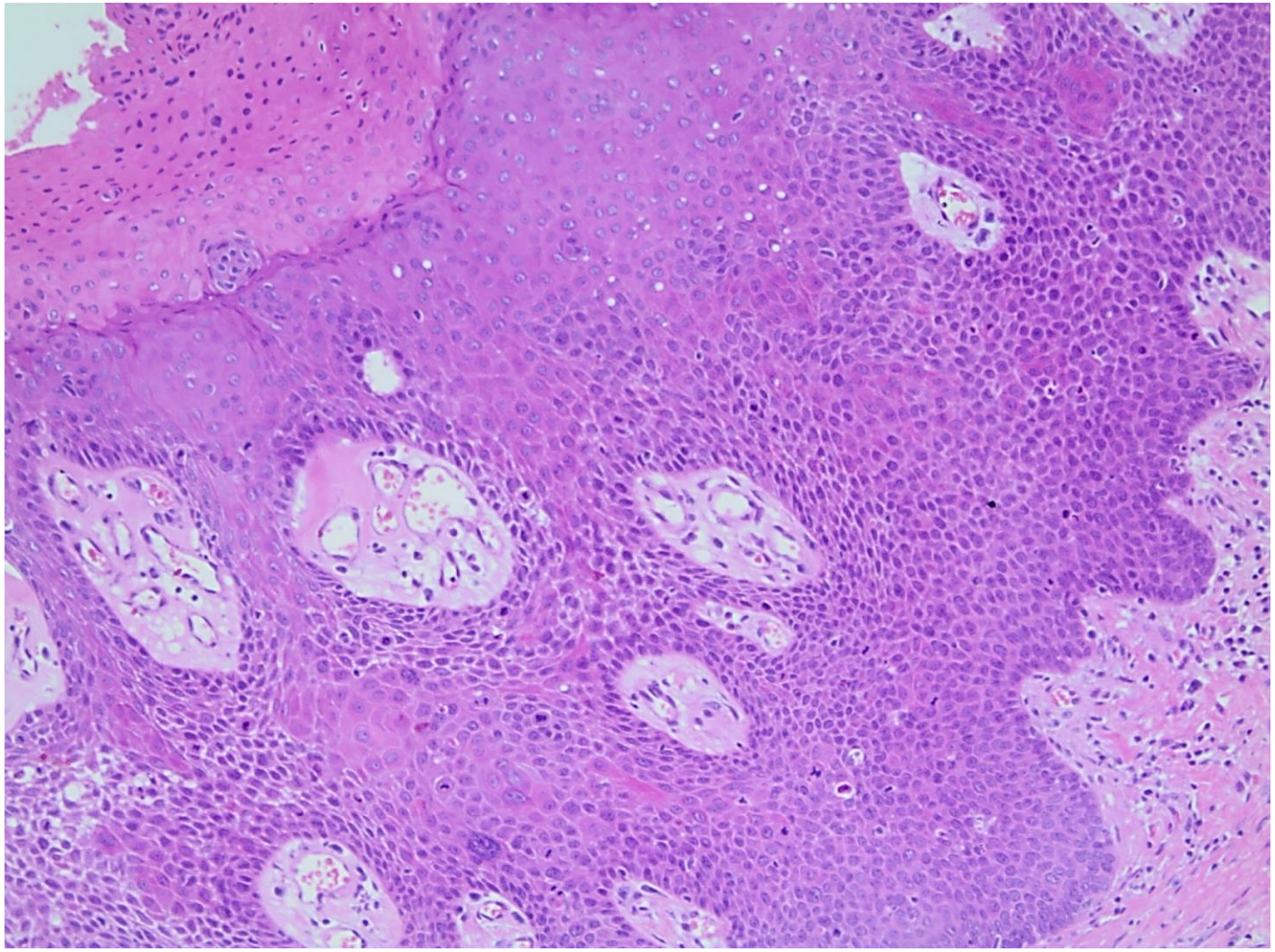
Vulvar mass, 63 y.o.





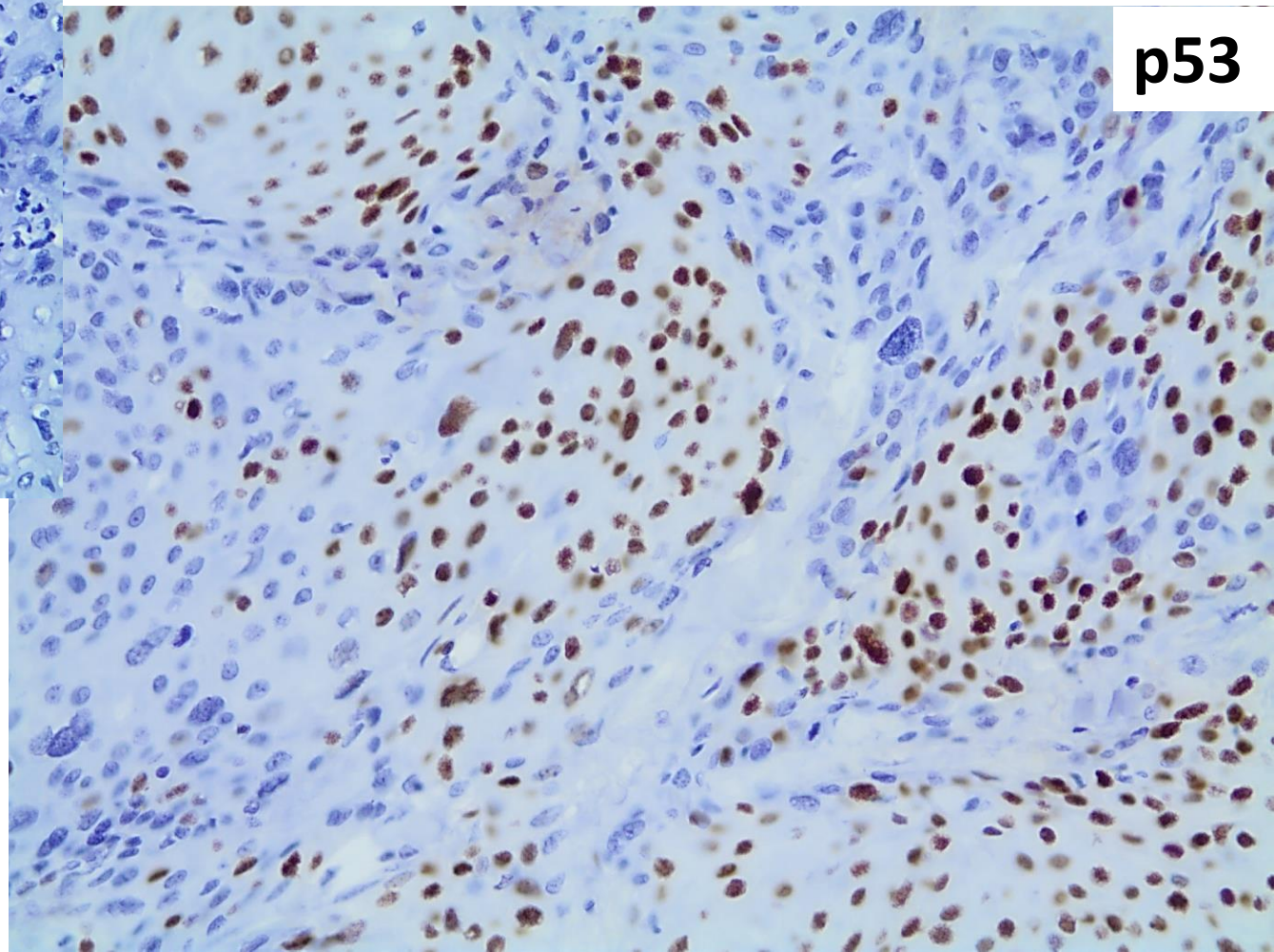


Periphery of the mass



p16

Vulvar mass

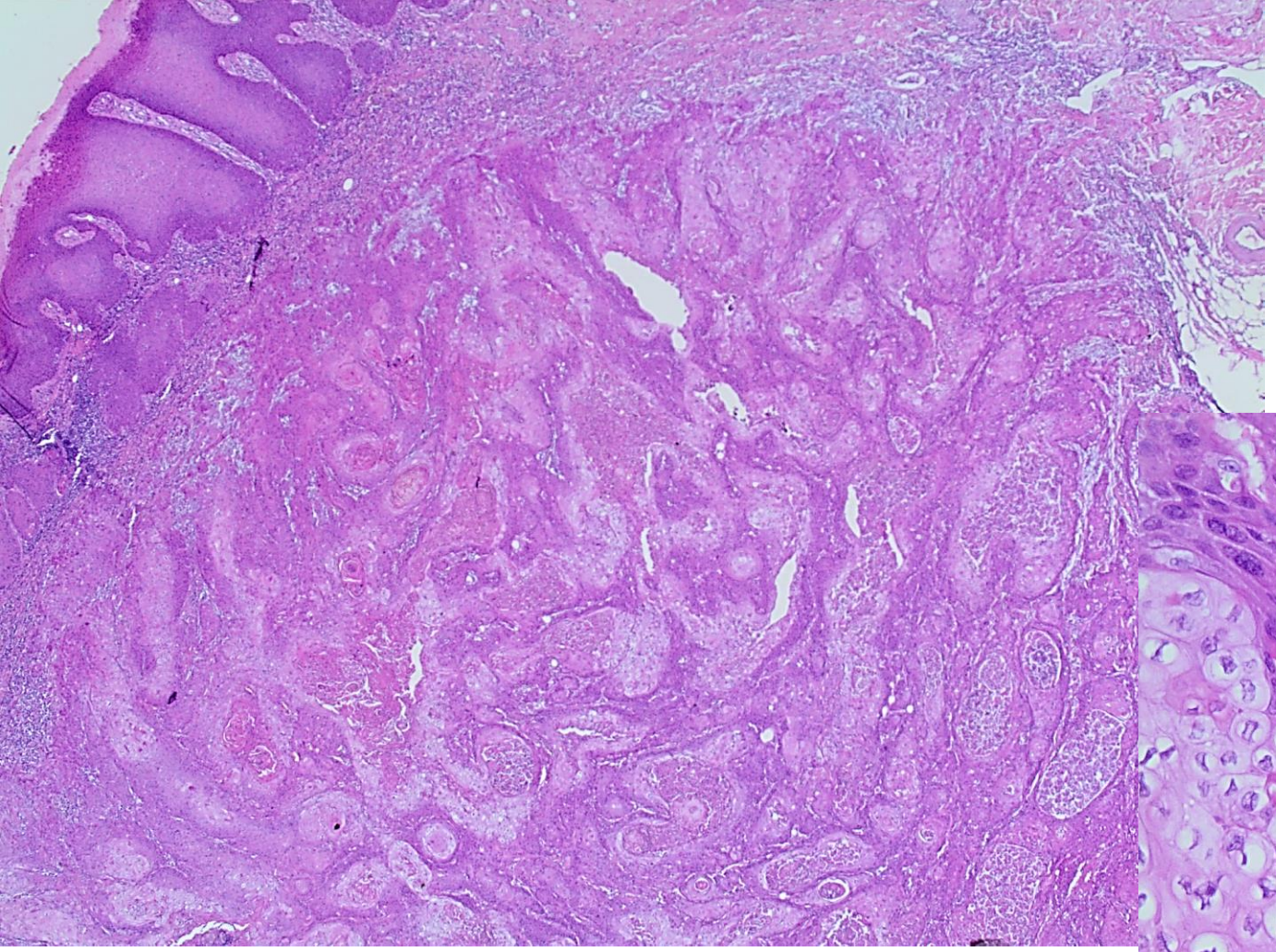


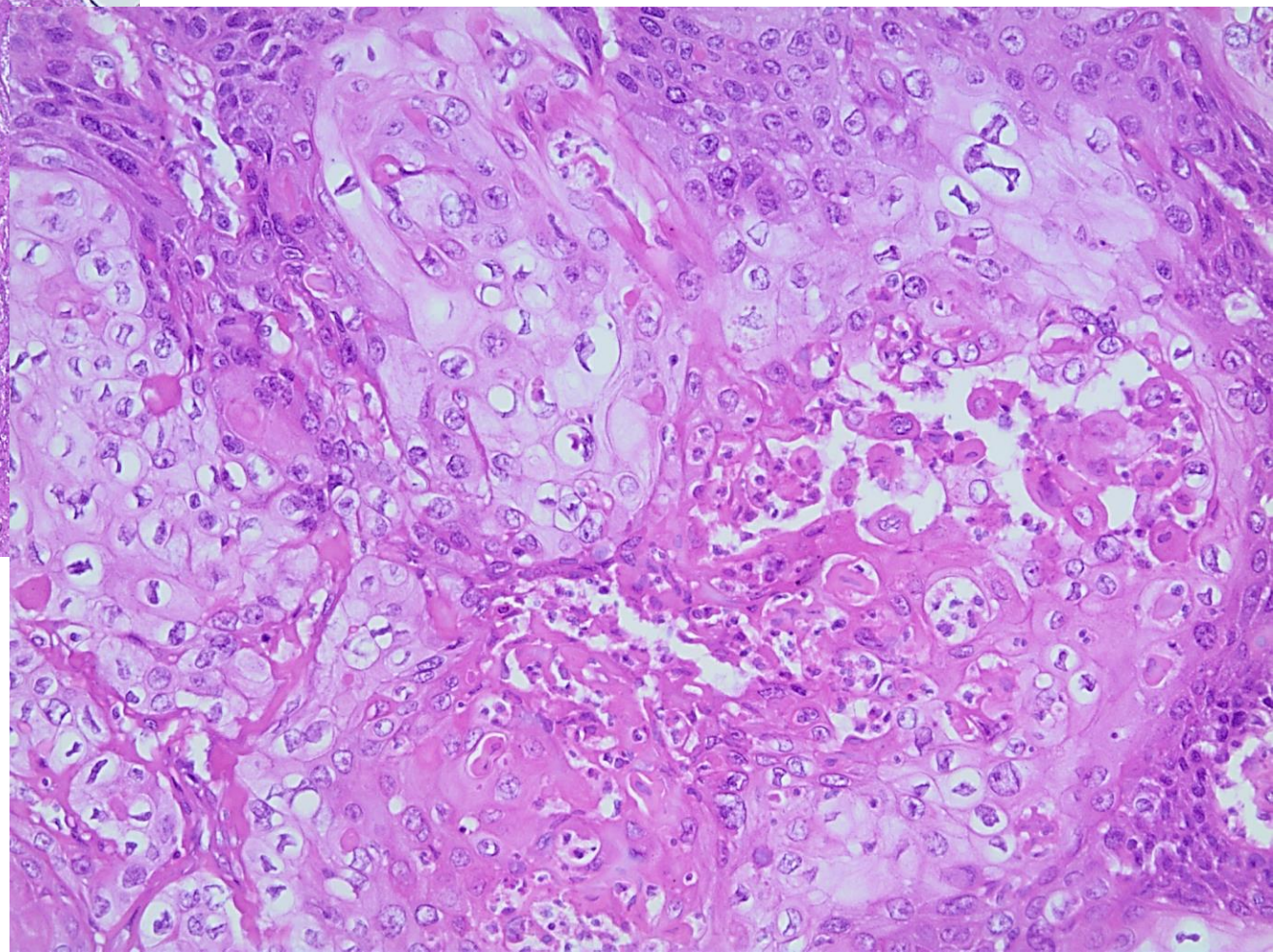
Non-HPV associated keratinizing SCC (?)

p16-negative and p53-positive (?)

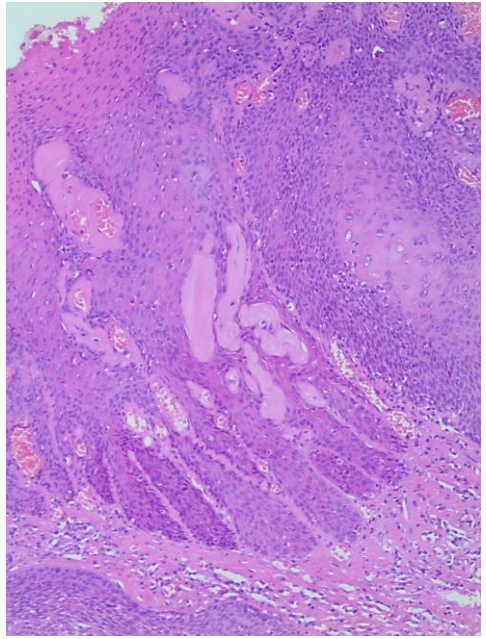
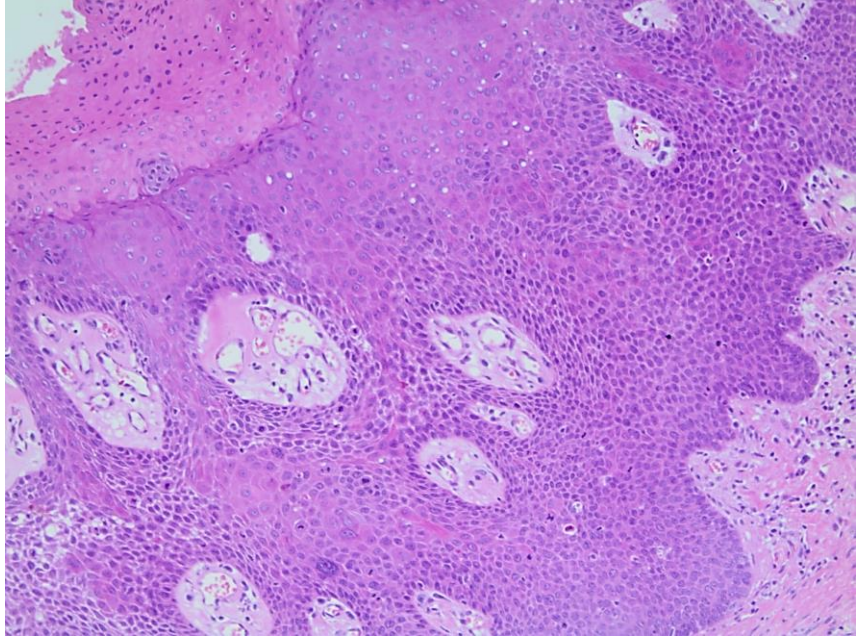
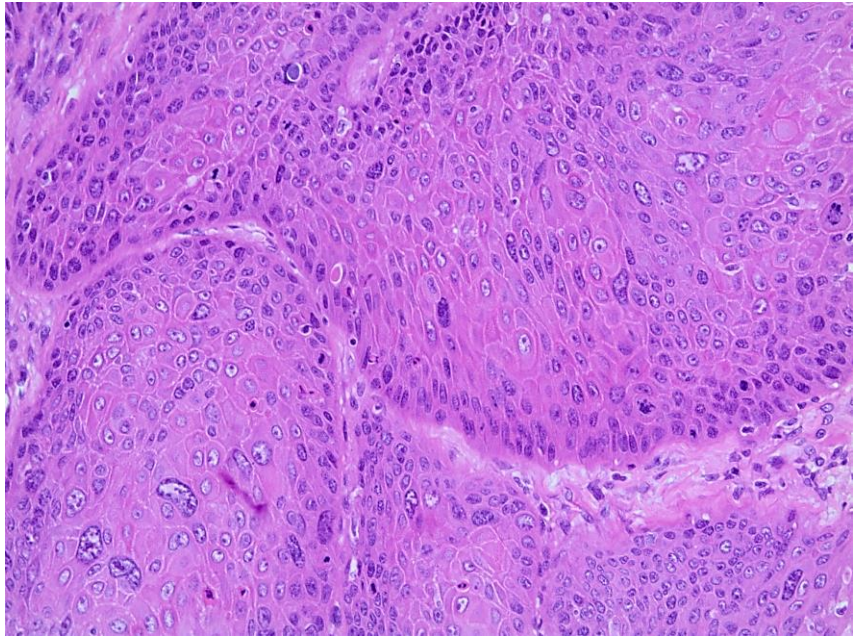
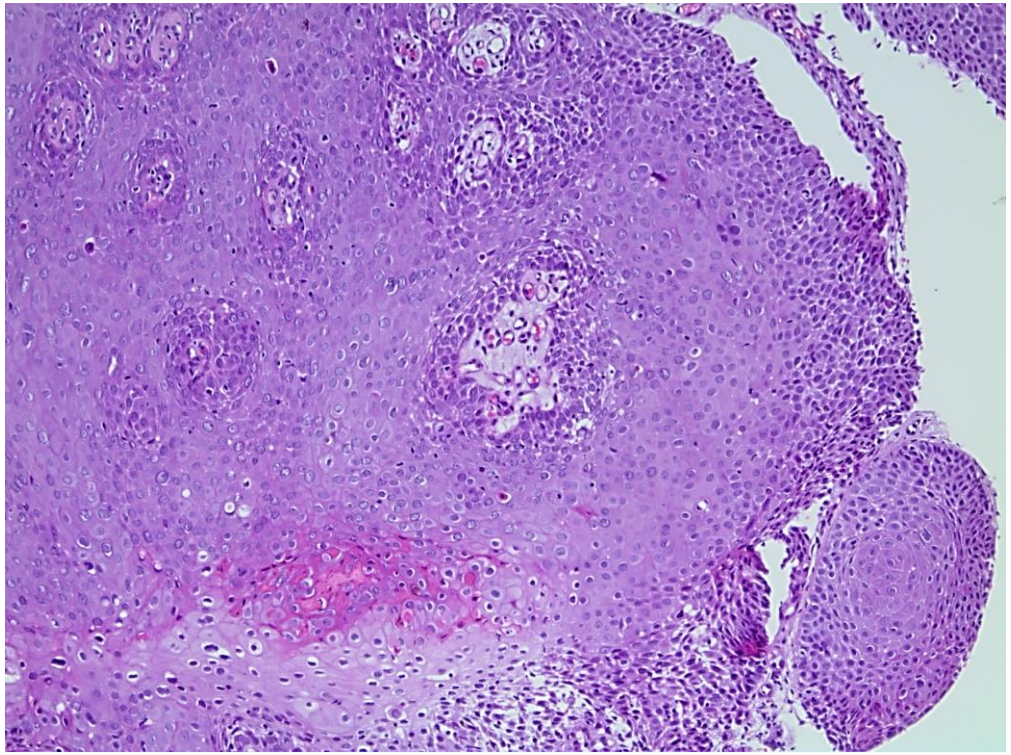
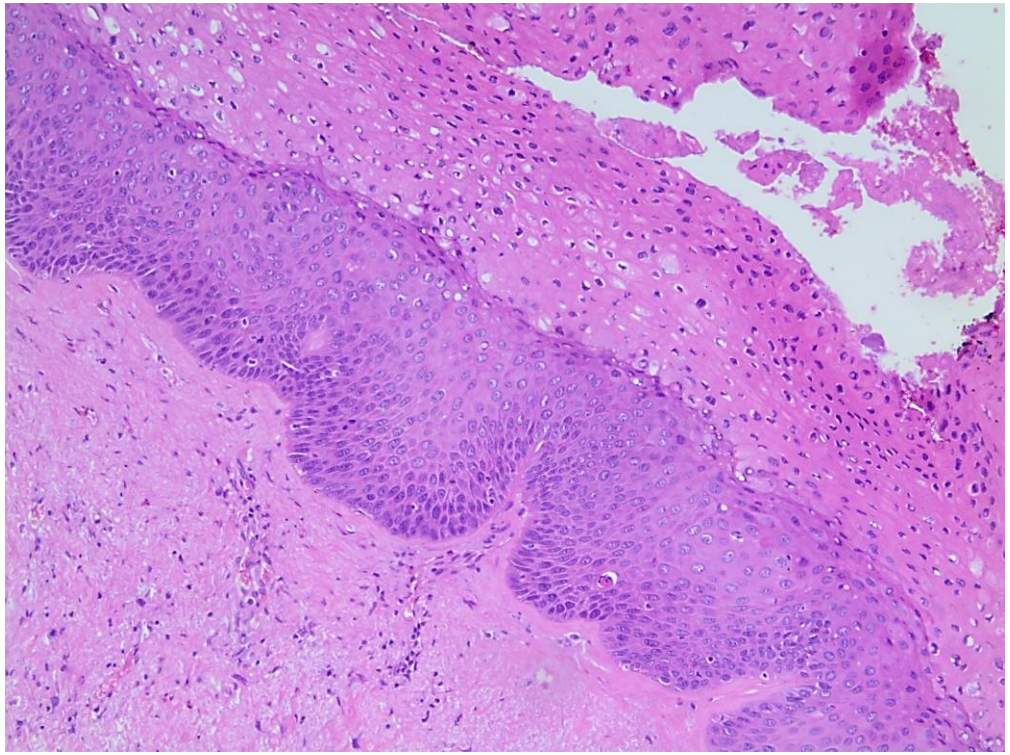
lichen sclerosus and dVIN (?)

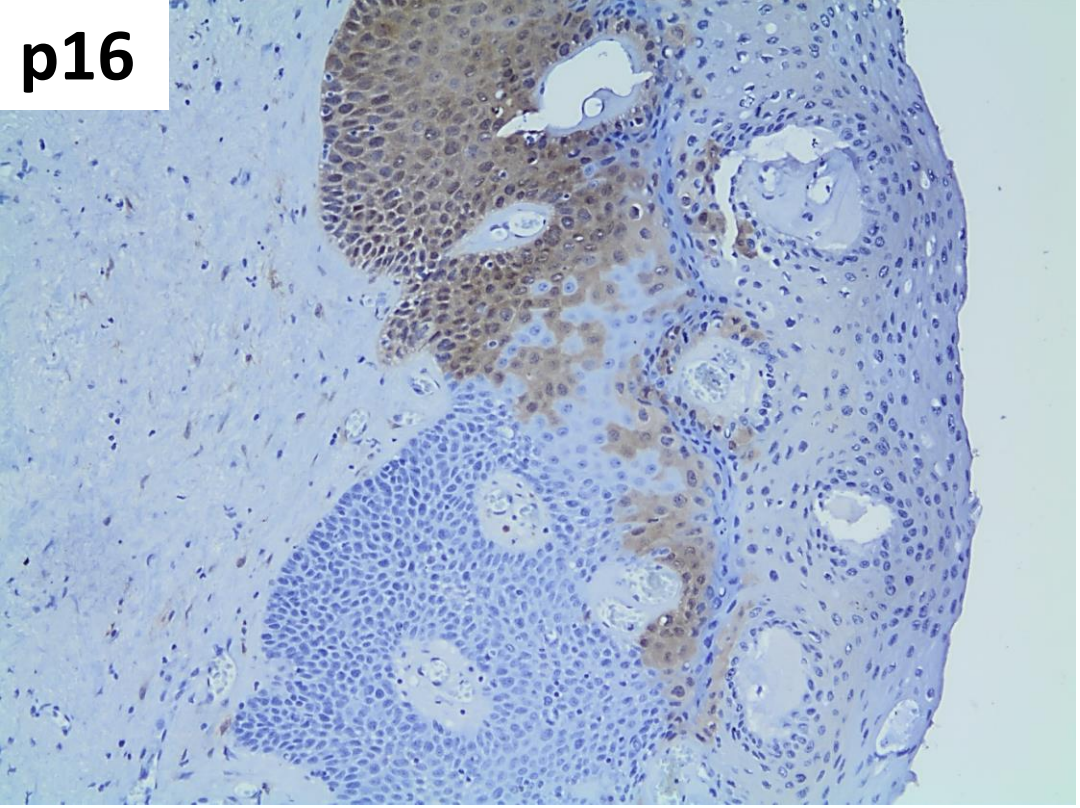
Vulvar mass, 63 y.o. (2012)





- Previous diagnosis of LS (6 y. before)
- Previous diagnosis of LSIL (5 y. before)
- Previous diagnosis of uVIN (3 y. before)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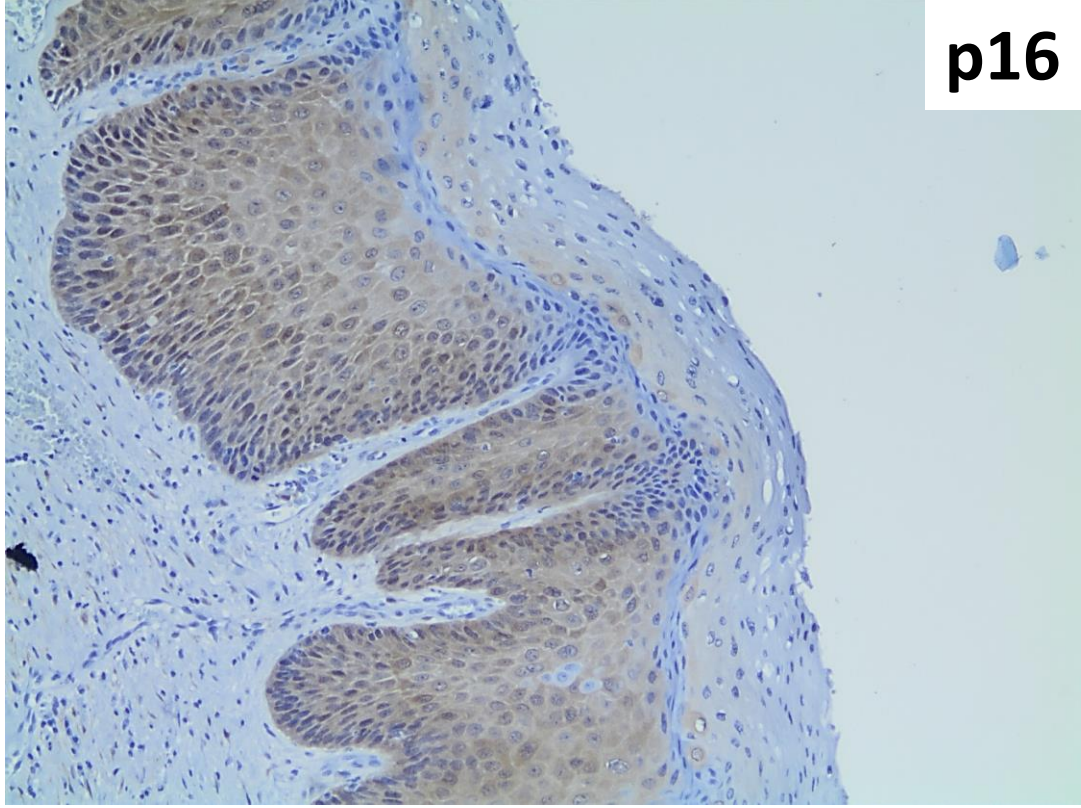
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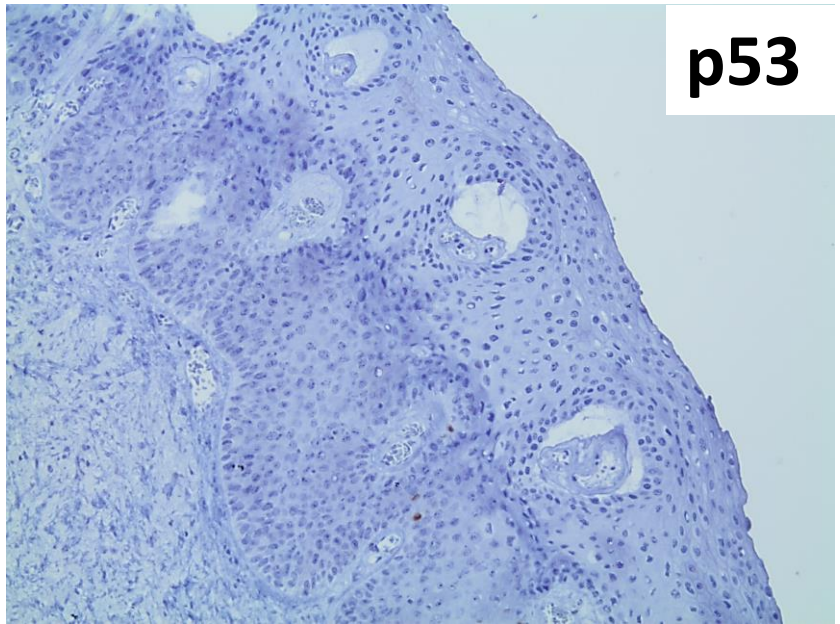
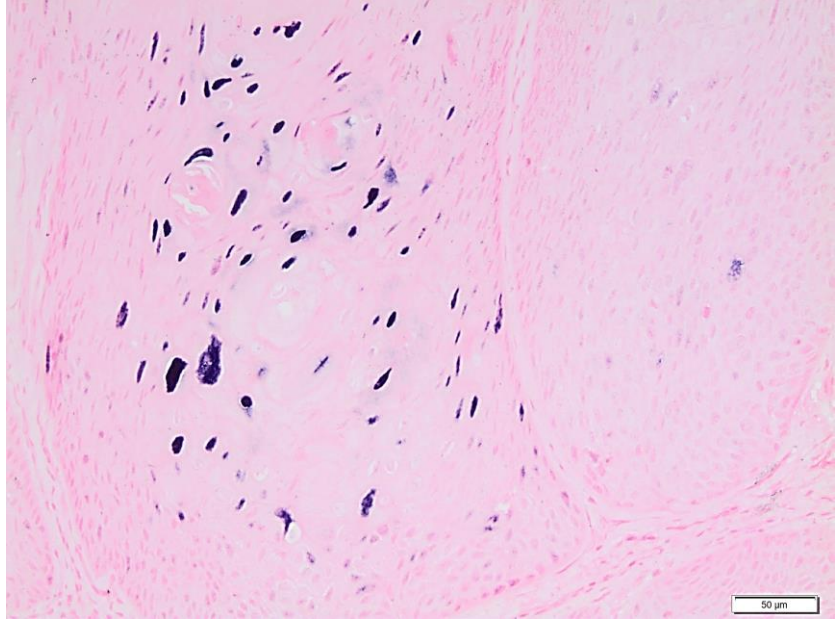
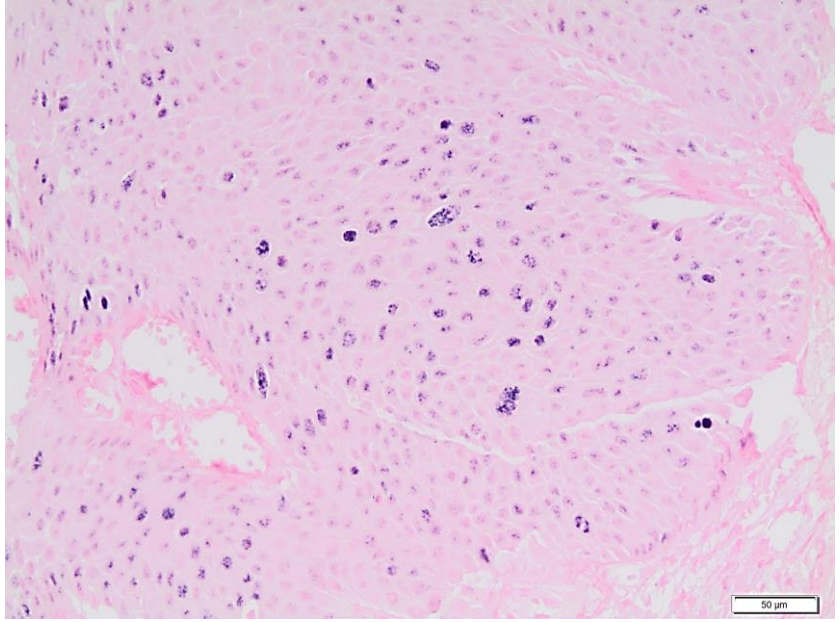
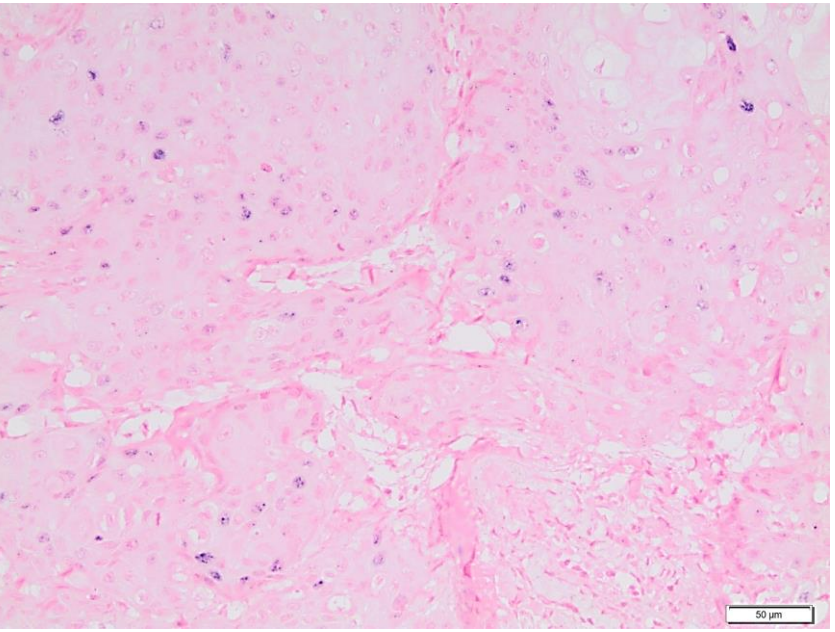
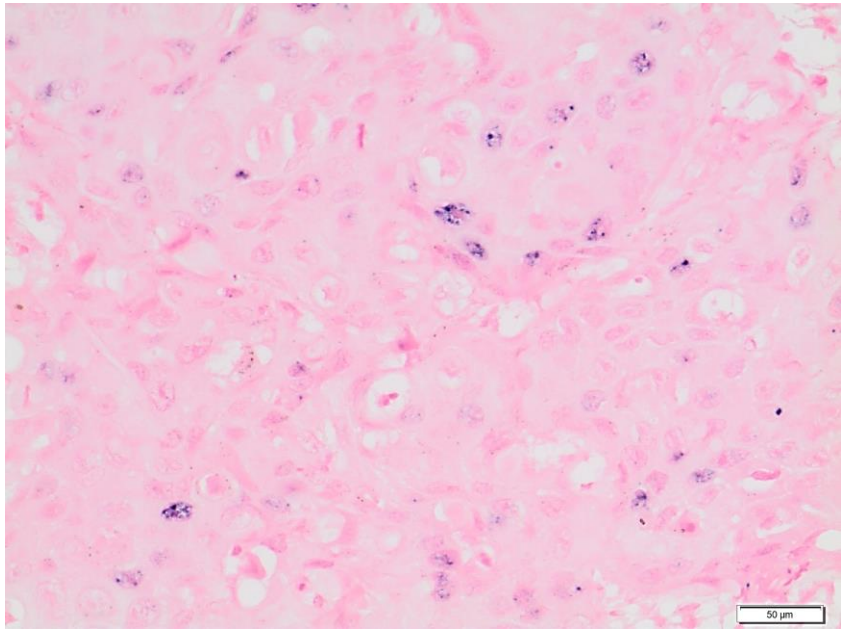

p53

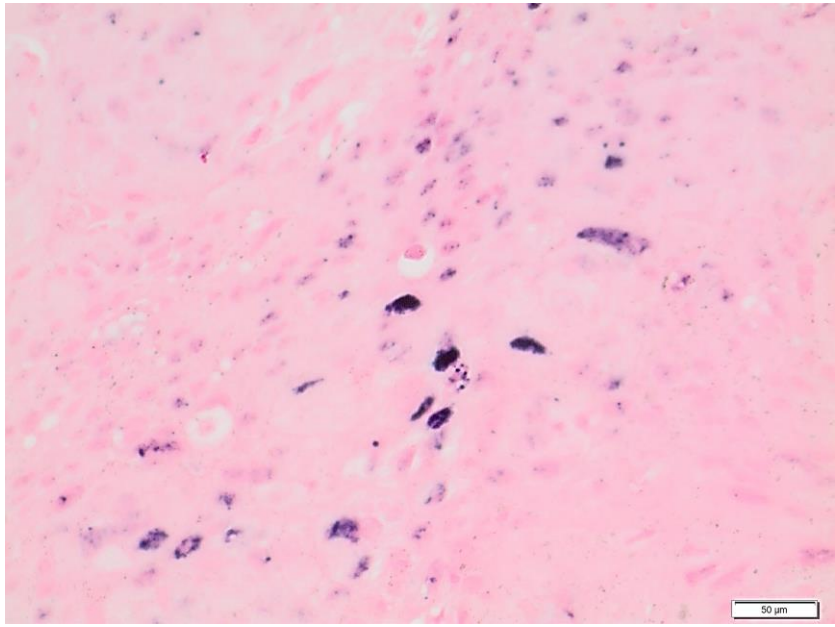




HR-HPV DNA-ISH



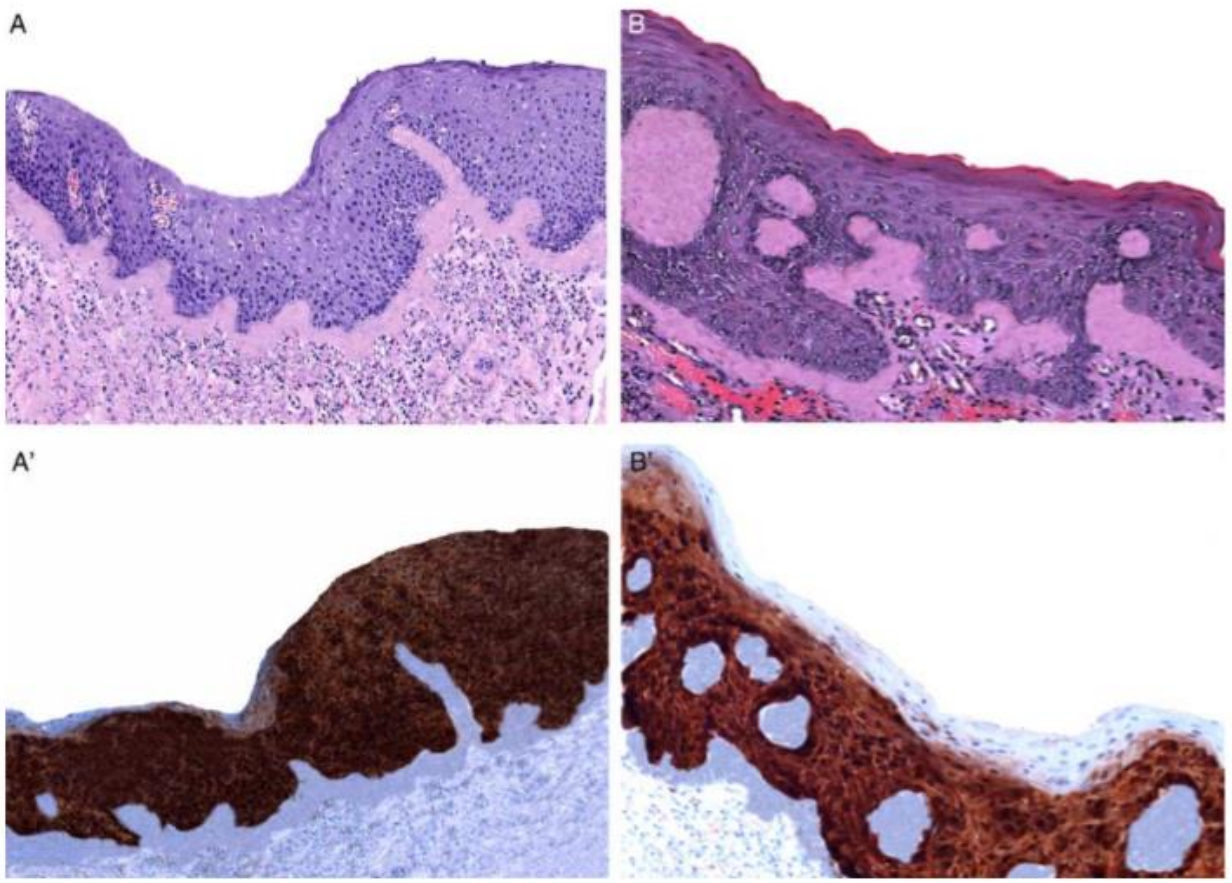


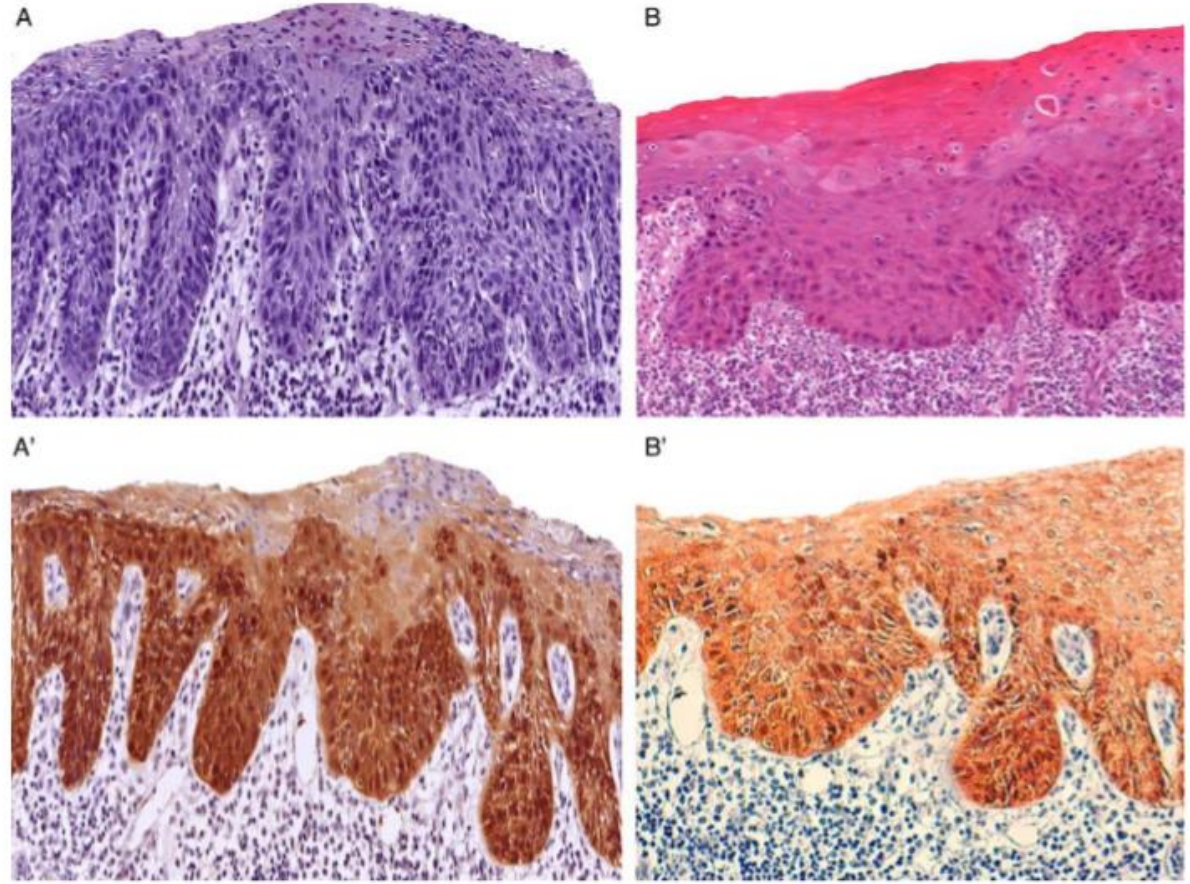


Am J Surg Pathol • Volume 42, Number 6, June 2018

Differentiated Vulvar Intraepithelial Neoplasia-like and Lichen Sclerosus-like Lesions in HPV-associated Squamous Cell Carcinomas of the Vulva

Natalia Rakislova, MD, Laia Alemany, MD, PhD,†‡ Omar Clavero, MD,†‡ Marta del Pino, MD, PhD,§ Adela Saco, MD, PhD,* Beatriz Quirós, BSc,†‡ Belen Lloveras, MD, PhD,‖ Maria Alejo, MD, PhD,¶ Gordana Halec, MD, PhD,# Wim Quint, MD, PhD,** Silvia de Sanjosé, MD, PhD,†‡ Jaume Ordi, MD, PhD,* and on behalf of VVAP study group*





dVIN-LIKE AND LS-LIKE LESIONS IN HPV-ASSOC. VSCC

- 326 DNA-HPV (+) tumors; HPV typing, HPV E6*I mRNA, P16.
- Conclusive association with HPV based on: p16 (+) and/or mRNA (+) (in addition to DNA-HPV positivity).
- **14 (4,3%) cases with unusual intraepithelial lesions (7 dVIN-like features, 5 adjacent LS-like lesions, 2 dVIN-like/LS-like lesions).**
- **HPV 16 (+), P16 (+) and mRNA (+) in 3/7 dVIN-like lesions, 2/5 LS-like lesions, 1/2 dVIN-like/LS-like lesions.**
- **P16 positive in all dVIN-like and LS-like lesions in tumors conclusively associated with HPV.**

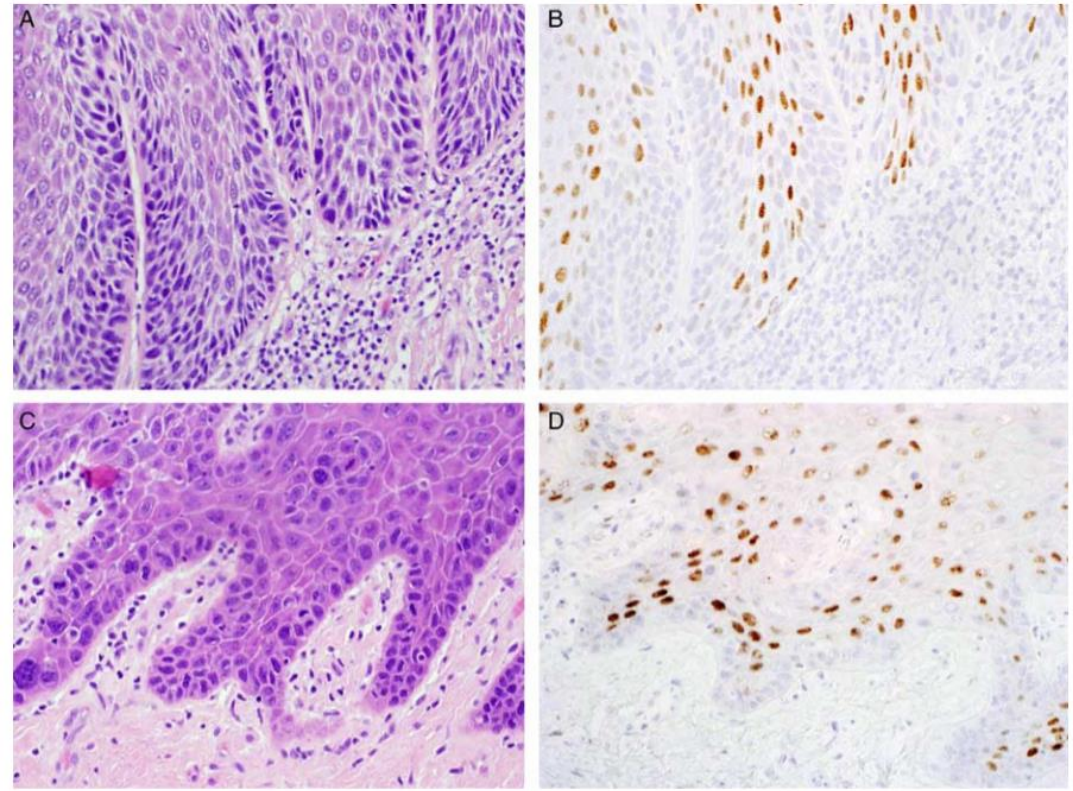
Rakislova N, Alemany A, Clavero O et al. - AJSP, 2018, 42, 828-35.

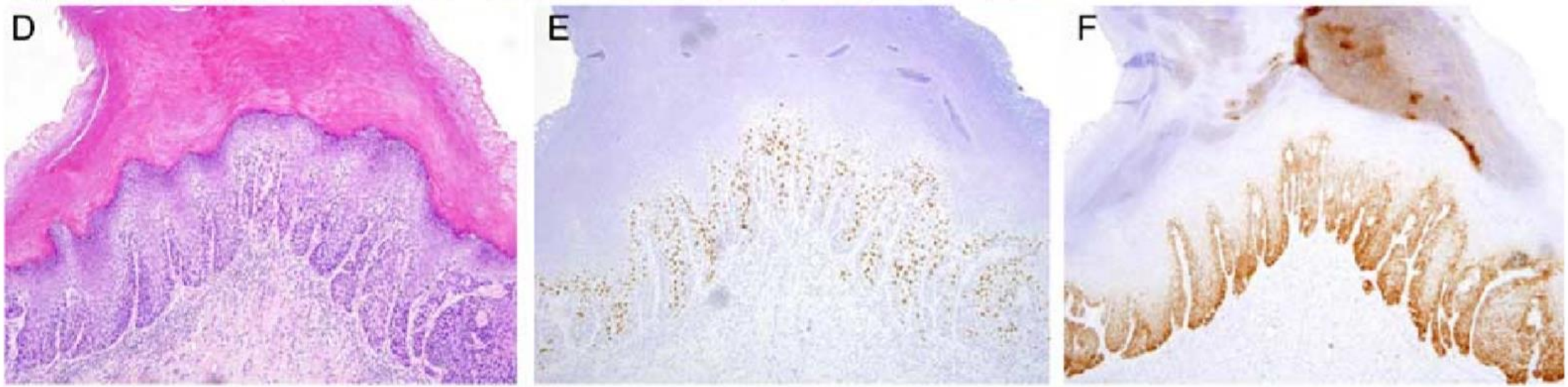
Original Article

Classic Vulvar Intraepithelial Neoplasia With Superimposed Lichen Simplex Chronicus: A Unique Variant Mimicking Differentiated Vulvar Intraepithelial Neoplasia

Jaclyn C. Watkins, M.D., M.S., Eric Yang, M.D., Ph.D., Christopher P. Crum, M.D., Michael Herfs, Ph.D., Tarik Gheit, Ph.D., Massimo Tommasino, Ph.D., and Marisa R. Nucci, M.D.

Int J Gynecol Pathol Vol. 38, No. 2, March 2019

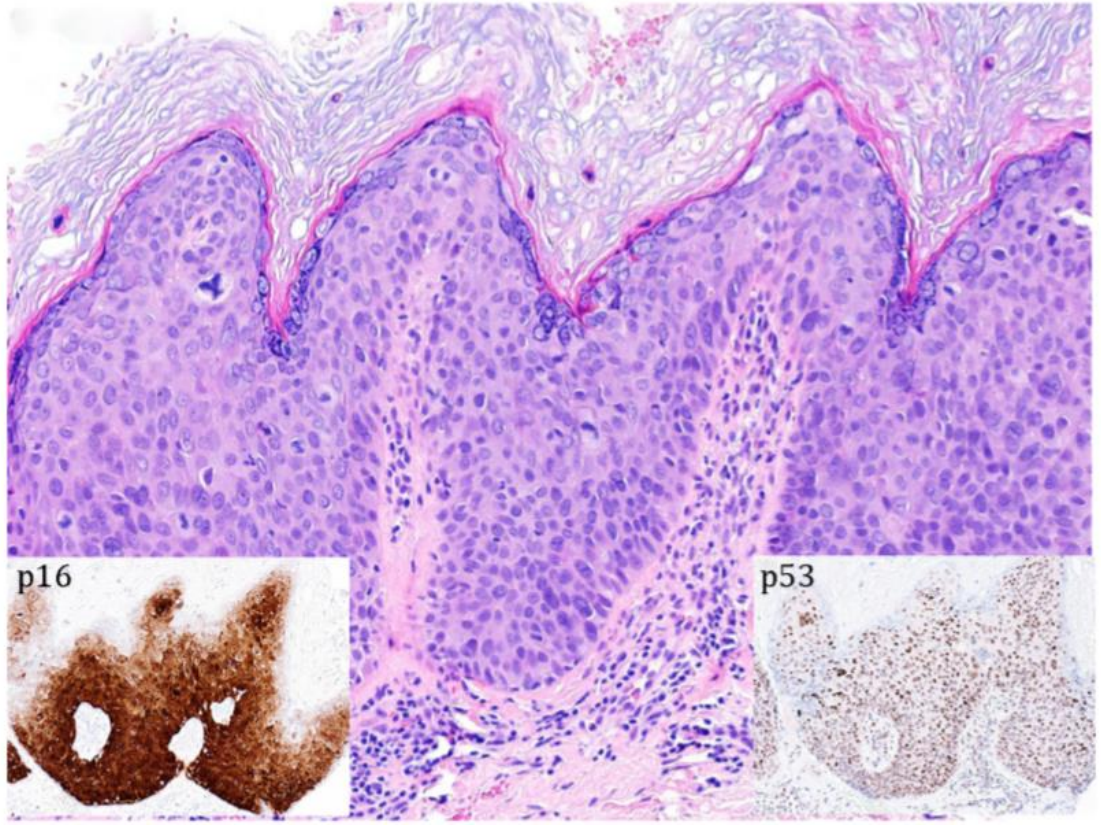


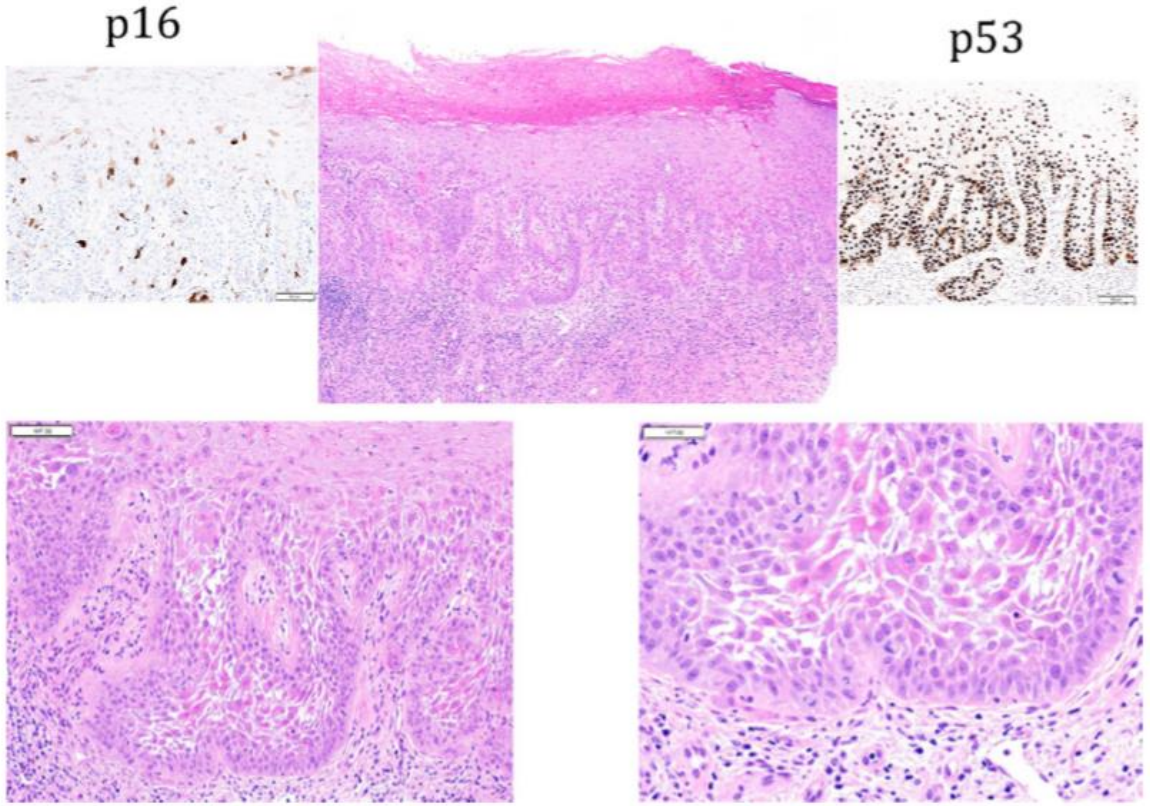


uVIN WITH SUPERIMPOSED LSC

- 12 cases of dVIN; 9 cases of LSC; 9 cases of uVIN + LSC.
- Morphology; p16, p53, ki-67; HPV genotyping.
- **dVIN: abnormal maturation, basal atypia; p16(-); p53 (+) of moderate to strong intensity in basal and parabasal layers.**
- **cVIN + LSC: hyperchromasia in basal 3 to 4 layers, basal to full-thickness atypia, apoptosis; P16: positivity reduction or loss in maturing keratinocytes; P53: parabasal and mid-epithelial weak to moderate positivity with sparing of the basal layer.**

Watkins JC, Yang E, Crum CP et al. - IJGyP, 2018, 38, 175-82.

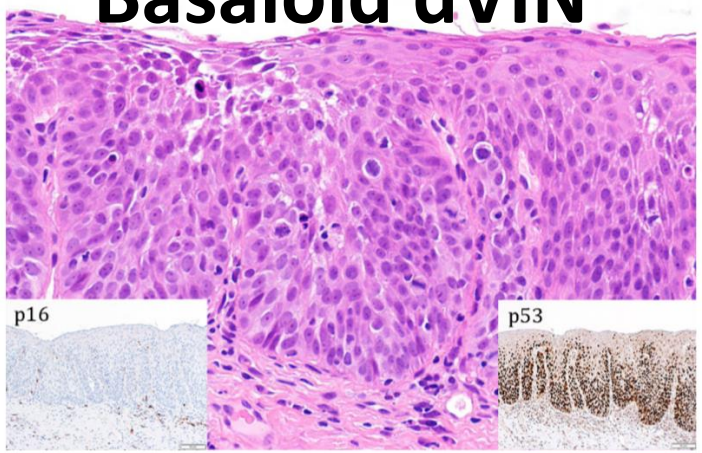




uVIN

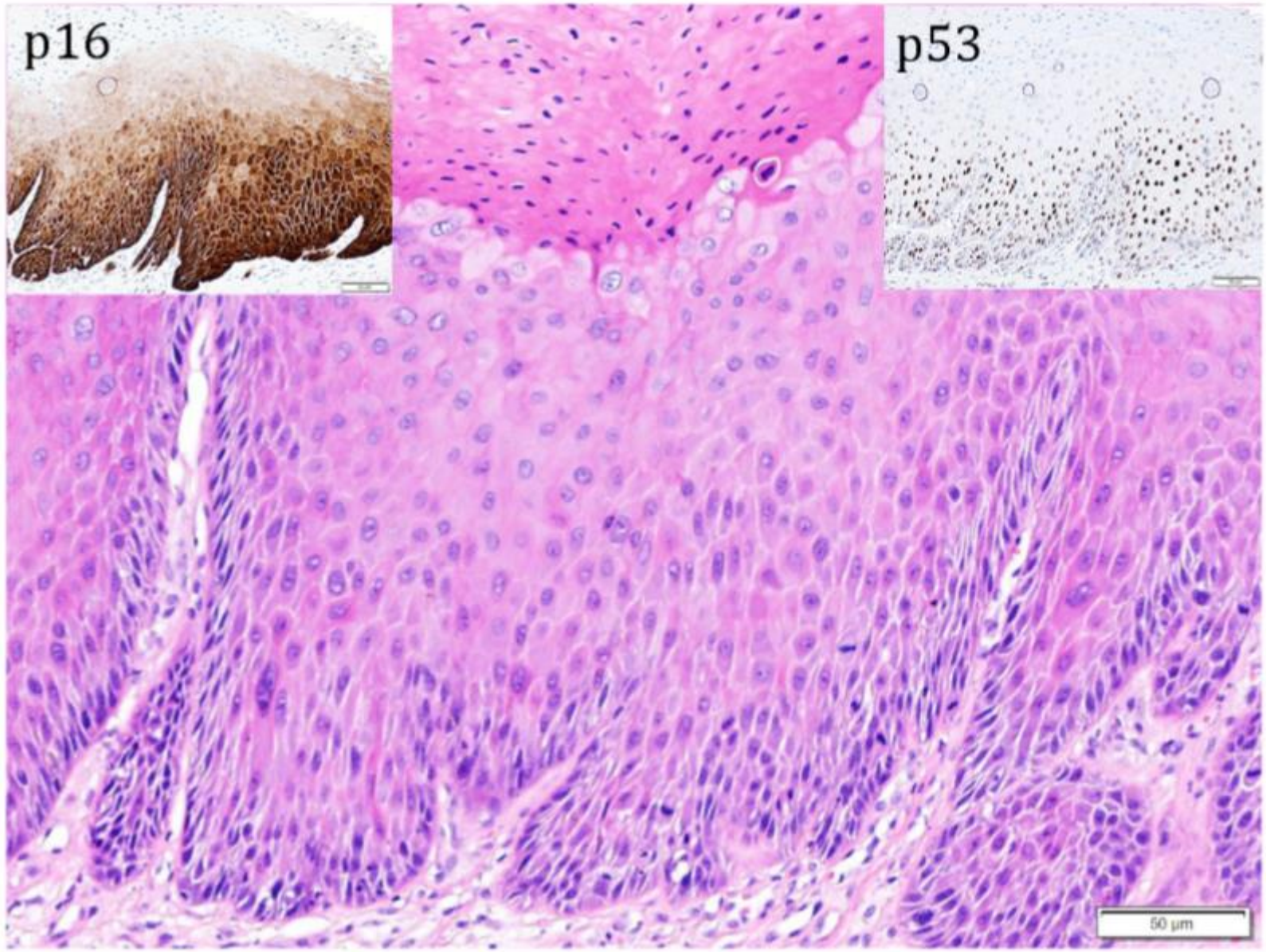
Basaloid dVIN

dVIN



Cohen PA, *et al. Int J Gynecol Cancer* 2019;**29**:821–828.

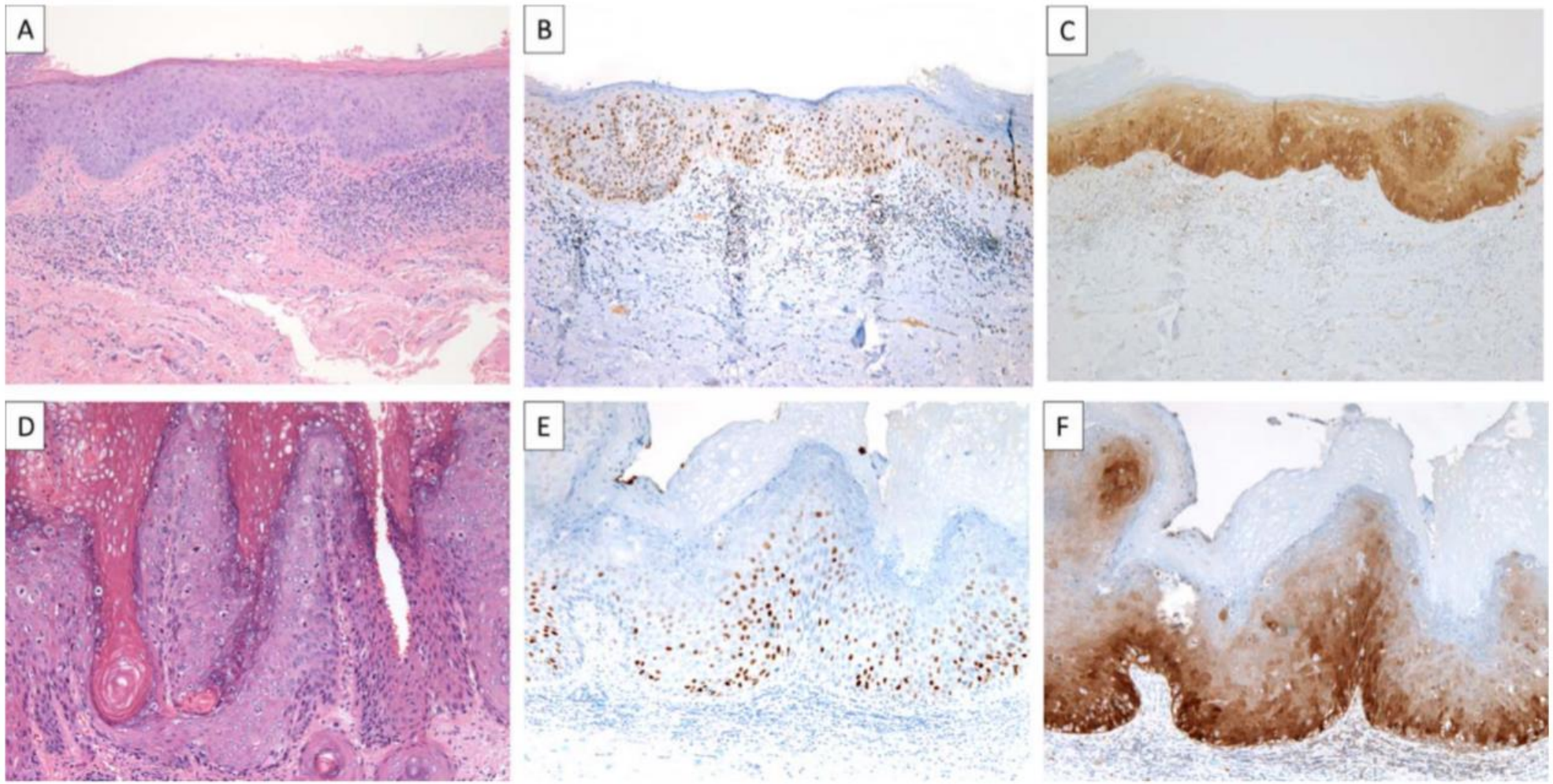
“Keratinizing (“dVIN-like”) uVIN (HSIL)”



Cohen PA, *et al. Int J Gynecol Cancer* 2019;**29**:821–828.

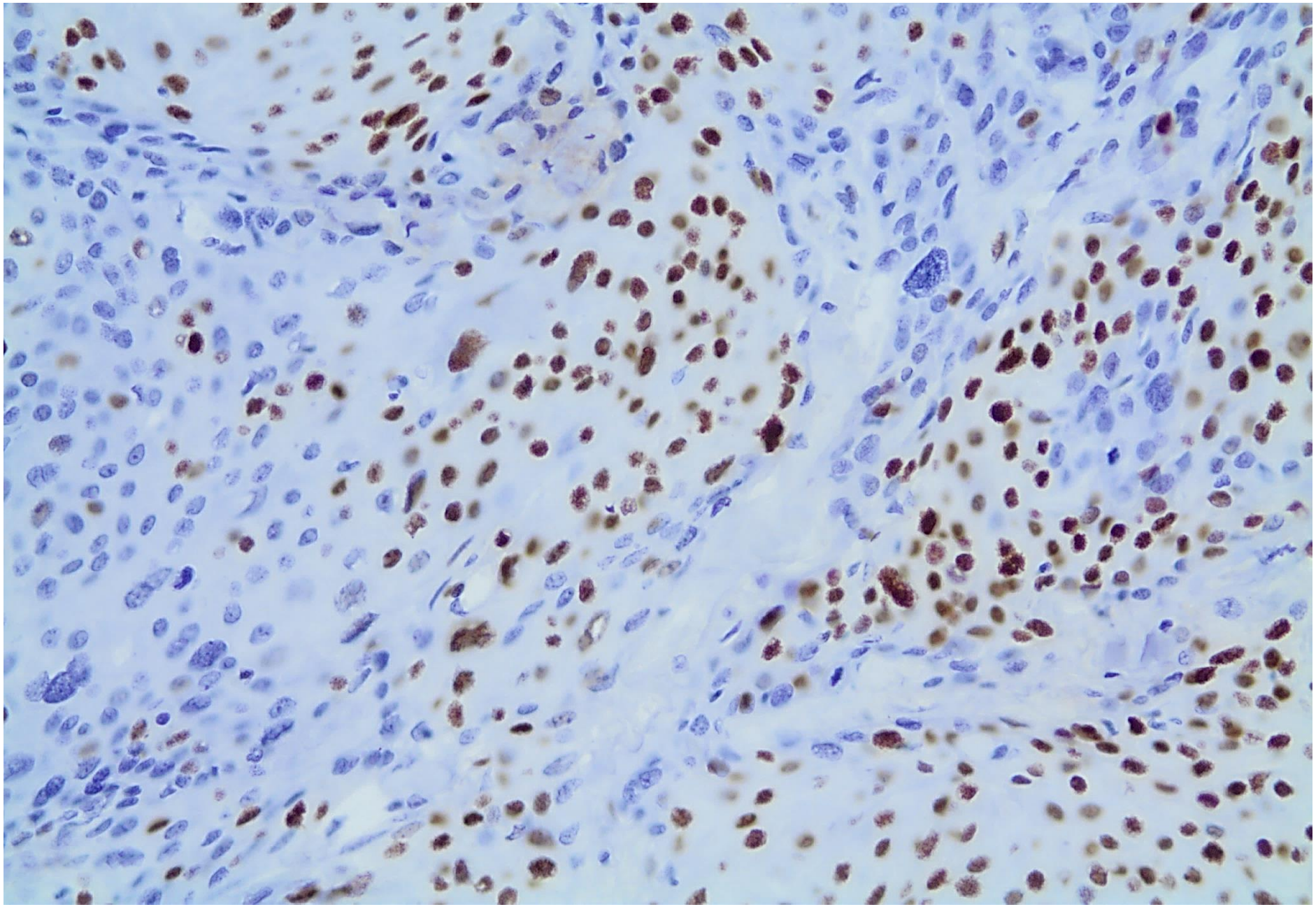
Accentuated p53 staining in usual type vulvar dysplasia—A potential diagnostic pitfall

Matthew Jeffreys[a], Susanne K. Jeffus[a], Michael Herfs[b], Charles Matthew Quick[a,*]

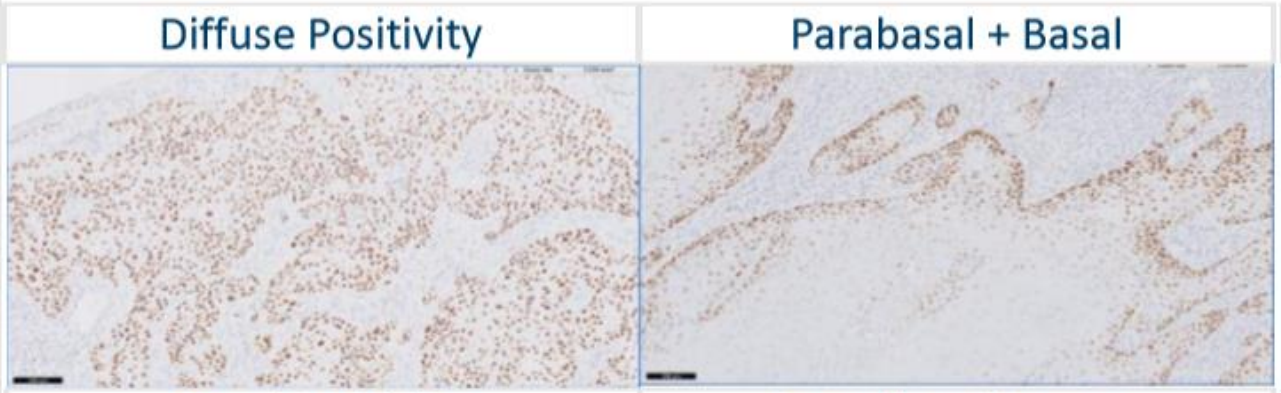


Pathology - Research and Practice 214 (2018) 76–79

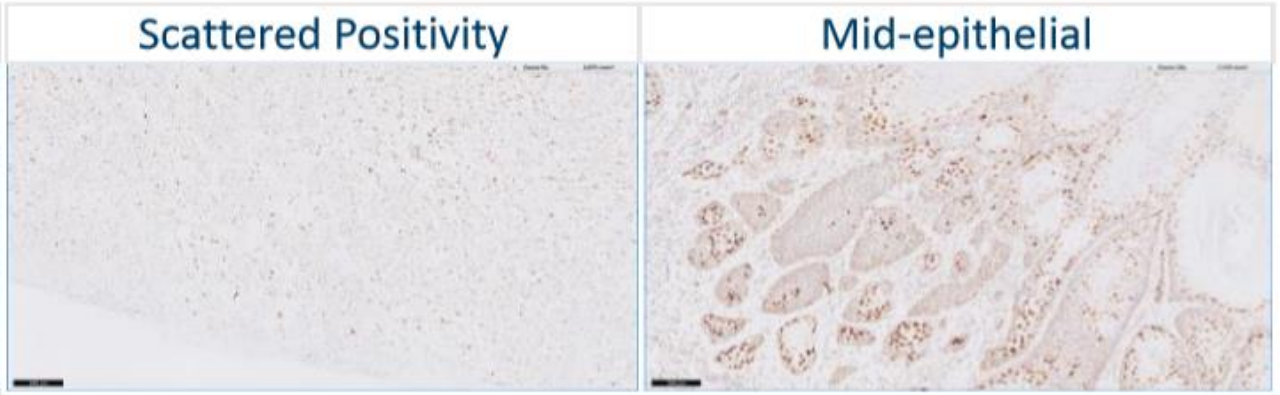
p53: possibly “accentuated” (i.e., high expression) wild type



p53 staining patterns: Summary



Probably *TP53* mutant



Probably *TP53* wild-type

Kortekaas et al., manuscript in preparation (Courtesy of T. Bosse)

(HOWITT BE – USCAP 2019)

Genomic Characterization of Vulvar (Pre)cancers Identifies Distinct Molecular Subtypes with Prognostic Significance

Linda S. Nooij[1,2], Natalja T. ter Haar[1], Dina Ruano[1], Natalia Rakislova[3], Tom van Wezel[1], Vincent T.H.B.M. Smit[1], Baptist J.B.M.Z. Trimbos[2], Jaume Ordi[3], Mariette I.E. van Poelgeest[2], and Tjalling Bosse[1]

- 36 VSCC and 82 precursors; NGS, p53 IHC, HPV testing.
- Three molecular subtypes:

- HPV(+) p53wt; HPV(-) p53abn.; HPV(-) p53wt (frequent NOTCH1 mutations).

- Local Recurrence Rates:

- HPV(+) p53wt: 5,3%; HPV(-) p53abn.: 22,6%; HPV(-) p53wt: 16,3%,

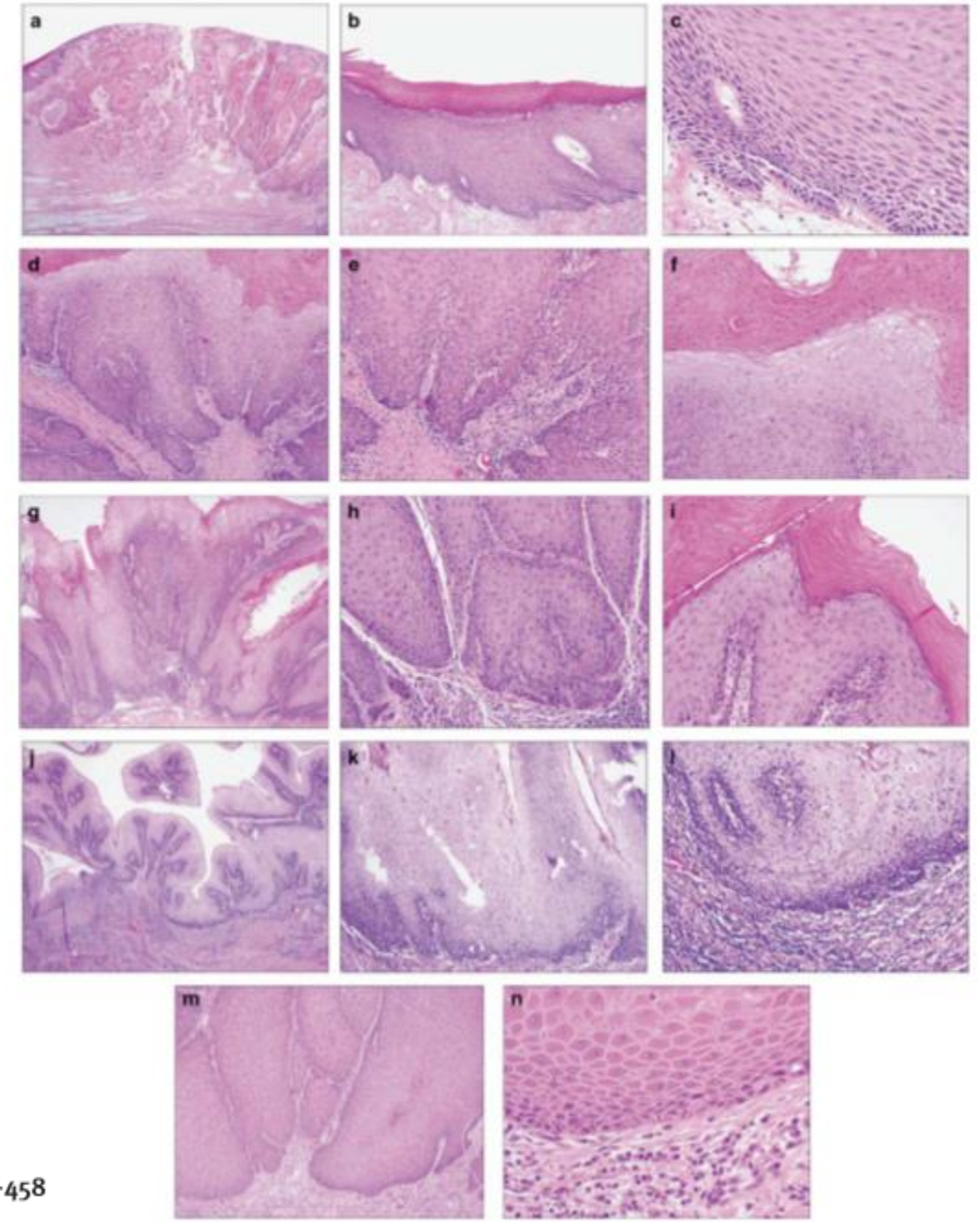
- **HPV positivity: independent prognostic factor for favourable outcome in multivariable analysis.**

Clin Cancer Res, 2017, Nov 15; 23(22):6781-9.

Differentiated exophytic vulvar intraepithelial lesions are genetically distinct from keratinizing squamous cell carcinomas and contain mutations in *PIK3CA*

Jaclyn C Watkins[1], Brooke E Howitt[1], Neil S Horowitz[2], Lauren L Ritterhouse[1], Fei Dong[1], Laura E MacConaill[3], Elizabeth Garcia[1], Neal I Lindeman[1], Larissa J Lee[4], Ross S Berkowitz[2], Marisa R Nucci[1] and Christopher P Crum[1]

- 11 atypical verruciform lesions.
- **"atypical verruciform hyperplasia"*;** Vulvar Acanthosis with Altered Differentiation (VAAD); verruciform LSC.
- Compared to 14 HPV-negative VSCC.
- *** PIK3CA (73%) and ARID2 (55%) mutations.**
- *** No p53 mutations.**
- *** One case progressed to a p53-mutated VSCC.**
- **Direct precursor or risk factor ("Differentiated Exophytic Vulvar Intraepithelial lesion: DE-VIL"): verruciform architecture, abnormal diff., no invasion, absence of HPV changes, no significant basal atypia, p53 IHC: wt.**



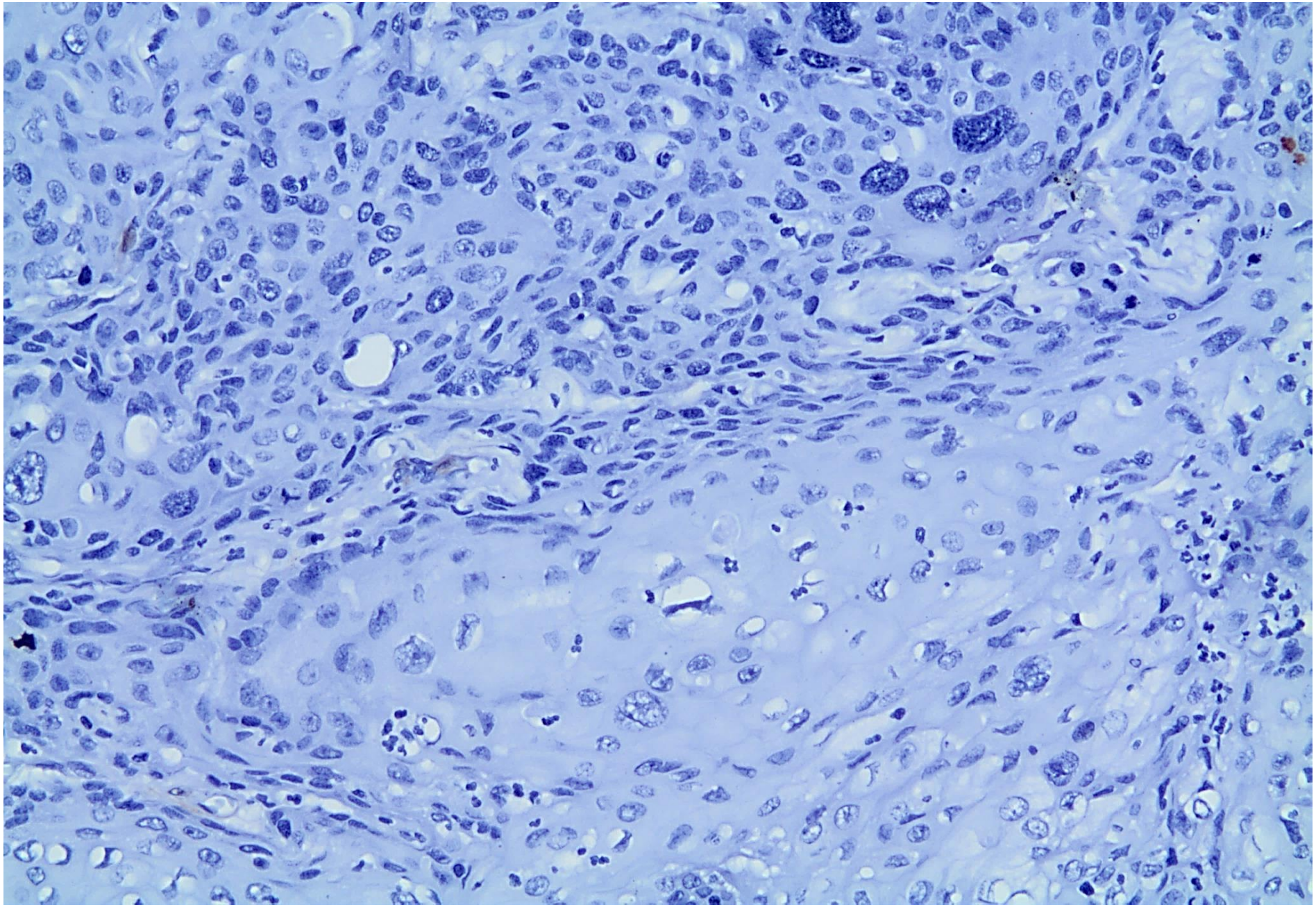
MODERN PATHOLOGY (2017) 30, 448–458

Table 1 Studies of somatic mutations in vulval squamous cell carcinoma that used next generation sequencing

Study	Year	Number of patients	Diagnosis and HPV status	Sequencing method	Gene	Mutation frequency HPV+	Mutation frequency HPV–
Weberpals et al[40]	2017	43	VSCC	NGS	TP53	9%	62%
			22 HPV+		PIK3CA	27%	19%
			21 HPV–		CDKN2A	9%	14%
					HRAS 1	4.6%	24%
					PTEN 2	9%	0%
					FGFR3	14%	4.8%
					KIT	18%	9.5%
Han et al[41]	2018	15	VSCC	WES	TP53	0%	56%
			9 HPV+		CDKN2A	0%	11%
			6 HPV–		HRAS	0%	11%
					FAT1	0%	44%
					PIK3CA	33%	0%
					BRCA2	17%	11%
					FBXW7	17%	11%
Zieba et al[42]	2018	81	VSCC	NGS	TP53	46%	41%
			52 HPV+		CDKN2A	25%	21%
			29 HPV–		PIK3CA	7%	10%
					HRAS	7%	3%
					FBXW7	3%	10%

Cohen PA, *et al. Int J Gynecol Cancer* 2019;**29**:821–828.

P16 negative in the invasive component: inactivating CDKN2A mutation?



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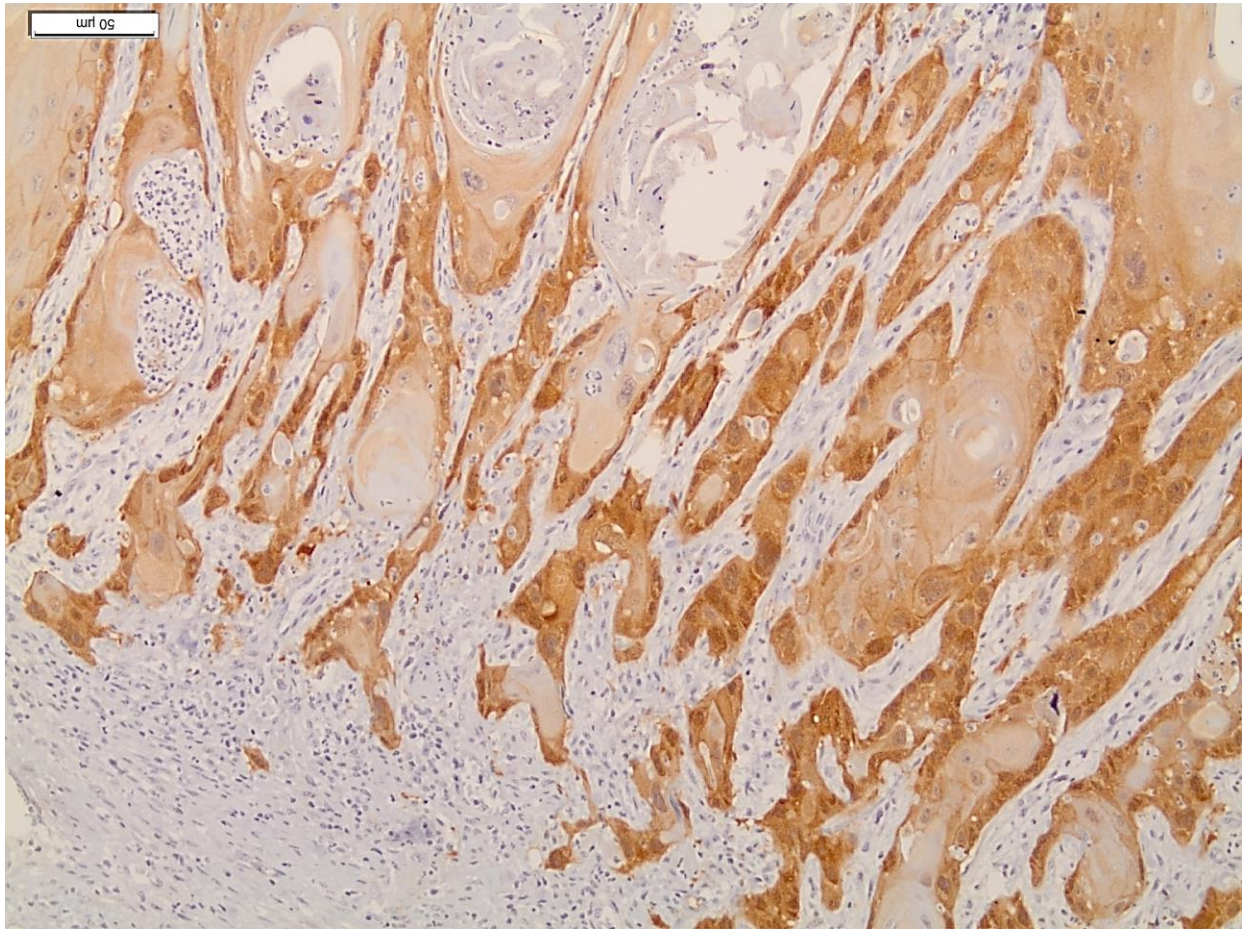
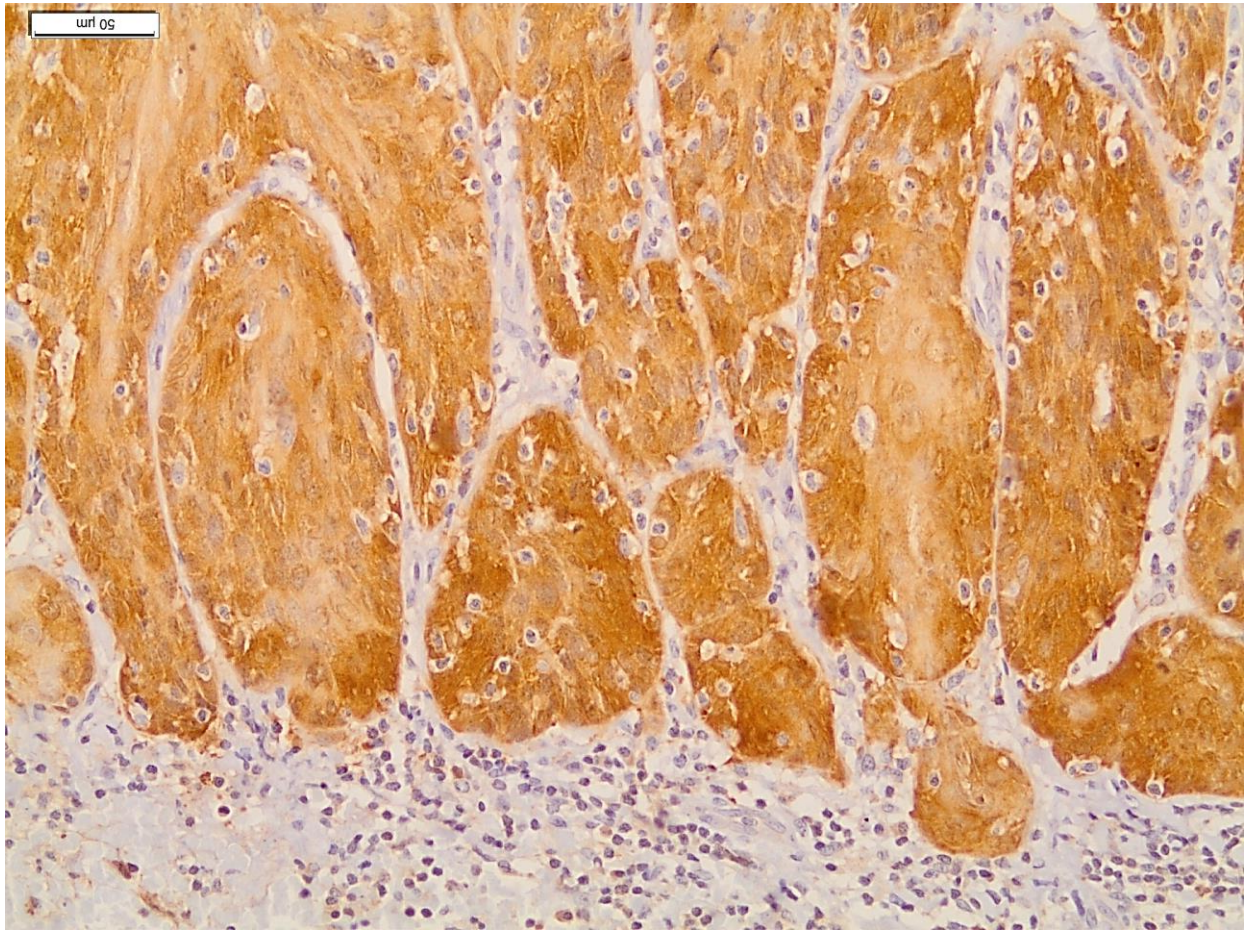
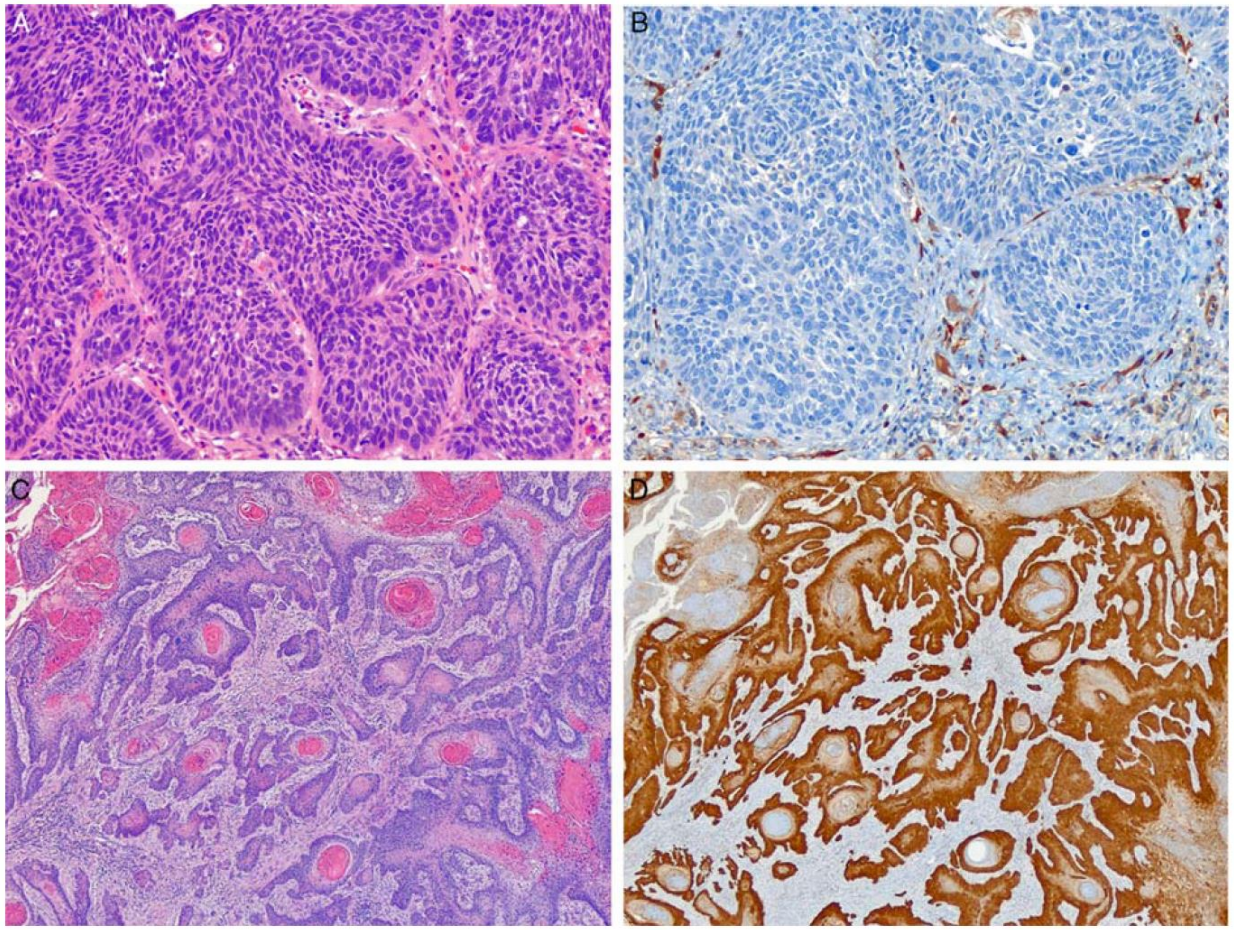
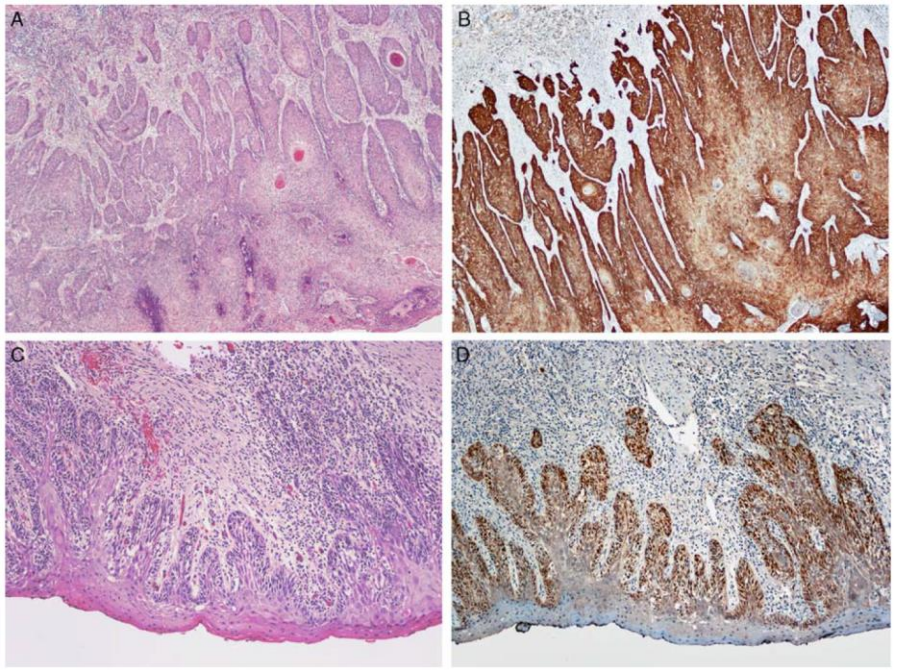


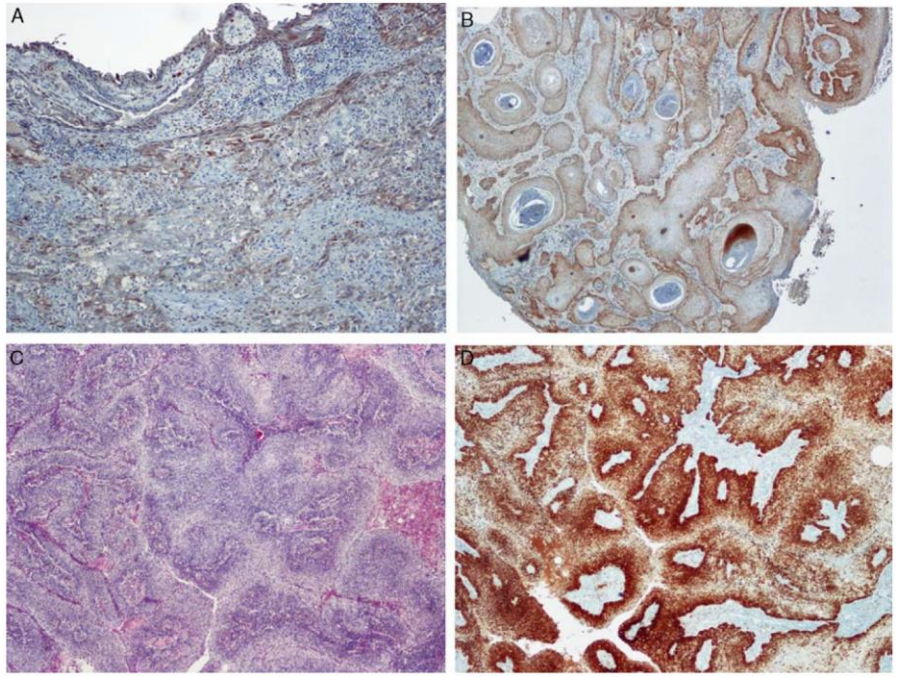


p16 Immunostaining Allows for Accurate Subclassification of Vulvar Squamous Cell Carcinoma Into HPV-Associated and HPV-Independent Cases

Angela S. Cheng, B.Sc., Anthony N. Karnezis, M.D., Ph.D., Suzanne Jordan, M.Sc., F.I.M.L.S., Naveena Singh, F.R.C.Path., Jessica N. McAlpine, M.D., and C. Blake Gilks, M.D.







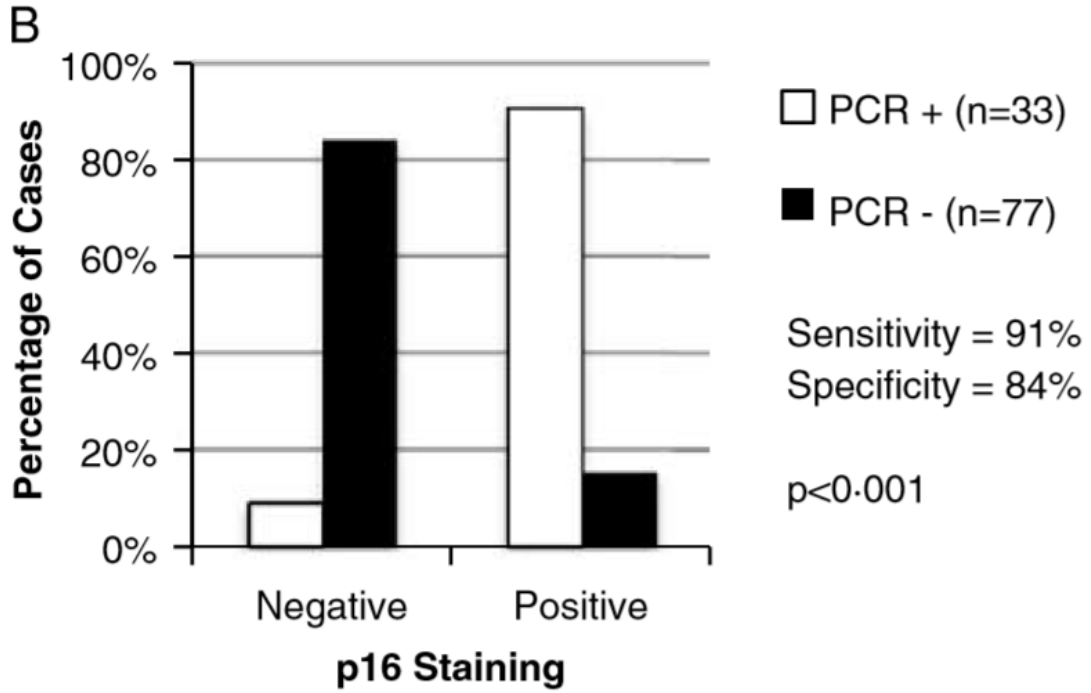
IHC FOR P16 AND HPV STATUS

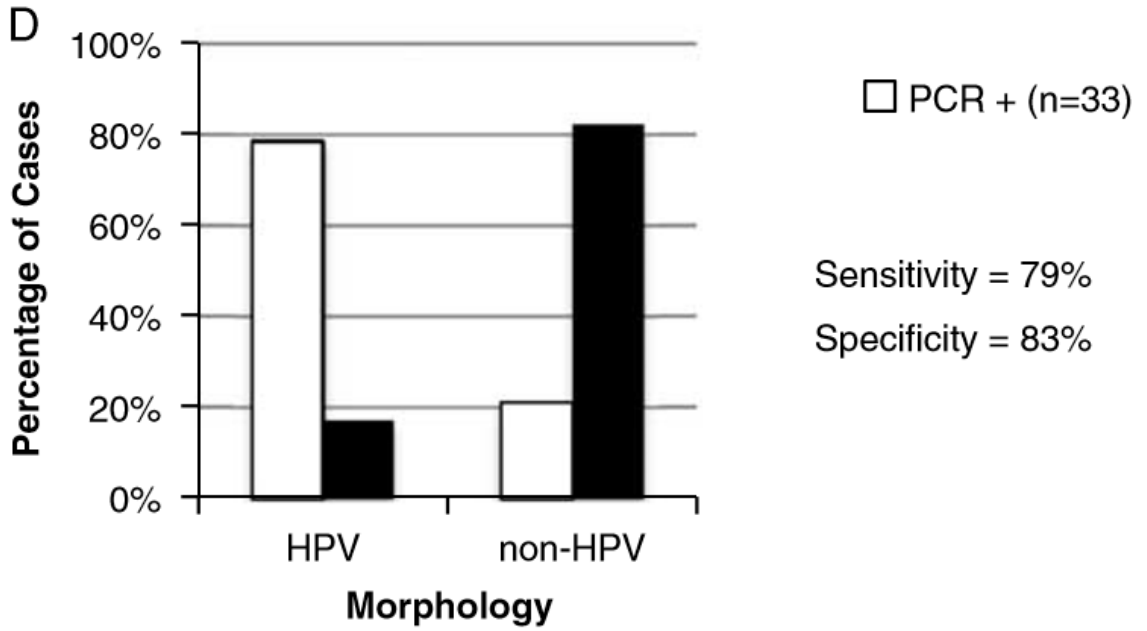
- 201 tumors.
- morphology was correlated with P16 IHC; discrepancies analysed by PCR for HPV DNA.
- **83 % concordance between morphology and p16 IHC (165/196).**
- **most discrepant cases were well differentiated keratinizing tumors with p16 positivity (94% of these were HPV-positive by PCR).**
- **P16 sensitivity and specificity for classification of vulvar SCC as HPV-associated or HPV-independent: 100% and 98,4%.**

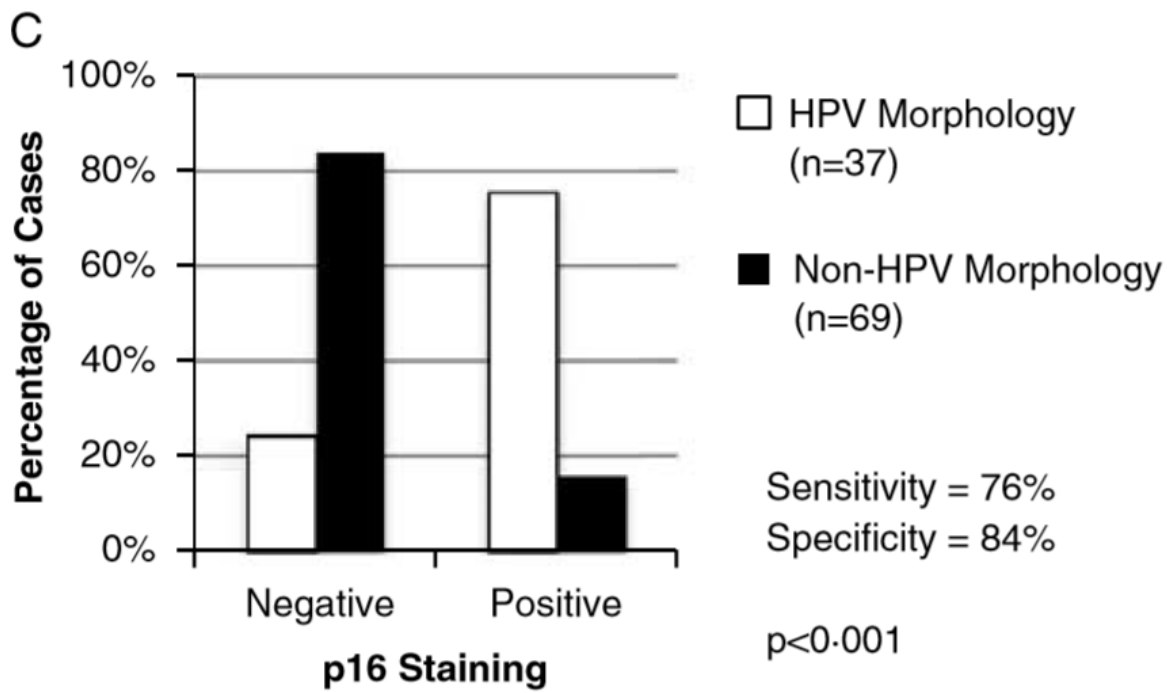
Cheng ES, Karnezis AN, Jordan S et al. - IJGYP, 2016, 35(4), 384-93.

HPV-independent Vulvar Squamous Cell Carcinoma is Associated With Significantly Worse Prognosis Compared With HPV-associated Tumors

Ghassan Allo, M.D., Mei Ling Yap, M.D., Julie Cuartero, M.D., Michael Milosevic, M.D., Sarah Ferguson, M.D., Helen Mackay, M.D., Suzanne Kamel-Reid, Ph.D., Ilan Weinreb, M.D., Danny Ghazarian, M.D., Ph.D., Melania Pintilie, Ph.D., and Blaise A. Clarke, M.D.







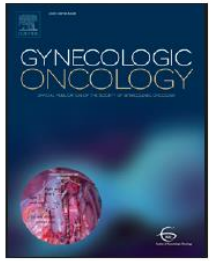
IJGYP, 2019, Jul 3 (Epub ahead of print).

HPV ASSOCIATION AND PROGNOSIS

- 114 tumors.
- Morphologic and multimodal HPV analysis (PCR, DNA-ISH, RNA-ISH, P16 IHC).
- **HPV morphology (36,7 %); PCR(+) (31,9%); DNA-ISH(+) (14%); RNA-ISH(+) (27,3%); p16(+) (37,8%).**
- **univariate analysis: HPV morphology, p16(+), DNA-ISH(+) and RNA-ISH(+) associated with better 5-yr PFS.; DNA-ISH(+) associated with better 5-yr OS.**
- **multivariate analysis: HPV morphology, p16(+) and RNA-ISH(+) associated with better 5-yr PFS.**
- **Routine reporting of HPV status is recommended.**

Allo G, Yap ML, Cuartero J et al. - IJGYP, 2019, Jul 3 (Epub ahead of print).

Prognostic importance of human papillomavirus (HPV) and p16 positivity in squamous cell carcinoma of the vulva treated with radiotherapy



Larissa J. Lee [a,f,*], Brooke Howitt [b,f], Paul Catalano [d,f], Cynthia Tanaka [a], Rita Murphy [a], Nicole Cimbak [a], Rebecca DeMaria [a], Paula Bu [a], Christopher Crum [b,f], Neil Horowitz [c,f], Ursula Matulonis [e,f], Akila N. Viswanathan [a,f]

Gynecol Oncol. 2016 Aug;142(2):293-8.

HIGHLIGHTS

- We evaluated HPV genotype, p16 status and outcome for vulvar SCC treated with RT.
- HPV or p16 positivity was associated with better PFS and fewer in-field relapses.
- HPV status is prognostic for women with vulvar SCC treated with radiotherapy.

Table 2
Concordance of p16 immunostaining and HPV status.

		p16-Positive	p16-Negative
All patients	56	20	36
HPV-positive	15	14 (93%)	1 (7%)
HPV-negative	41	6 (15%)	35 (85%)

Table 4 Contemporary studies evaluating HPV/p16 status and their relationships with outcomes

Study	Patients	Radiation regimen	HPV detection	p16 Detection	Prognostic value
Yap et al[4]	40	Preop, postop, definitive	PCR	IHC	LRR: yes DFS: yes OS: no
Lee et al[5]	57	Preop, postop, definitive	PCR	IHC	IFR: yes PFS: yes OS: possible
Kim et al[17]	56	"Curative RT"	Hybrid capture 2	-	DFS: no OS: no
Alonso et al[13]	98 (9 RT)	"Radio/chemo" and adjuvant RT	PCR	IHC	DFS: no OS: no
Lindell et al[12]	75 (24 RT)	Adjuvant RT	PCR	-	RFS: yes DSS: yes OS: yes
Larsson et al[18]	130	Unknown	PCR	Yes (unknown method)	PFS: no OS: yes
McAlpine et al[19]	201 (61 RT)	Unspecified	-	IHC	PFS: yes DSS: yes
Wakeham et al[20]	62 (12 RT)	Surgery/CRT, CRT or RT alone	PCR	IHC	PFS: yes OS: possible
Weberpals et al[21]	43 (21 RT)	CRT, surgery/RT	PCR	IHC	PFS: no OS: no
Rasmussen et al[22]	Meta-analysis	-	-	-	DFS: yes OS: yes
Current study	39	Adjuvant RT	-	IHC	IFR: possible OS: no

Abbreviations: CRT = chemoradiation therapy; DFS = disease-free survival; DSS = disease-specific survival; HPV = human papillomavirus; IFR = in-field relapse; IHC = immunohistochemistry; LRR = locoregional relapse; OS = overall survival; PCR = polymerase chain reaction; PFS = progression-free survival; RFS = recurrence-free survival; RT = radiation therapy.

Dohopolski MJ, Horne ZD, Pradhan D et al. - Int J Radiation Oncol Biol Phys, 2019, 103: 152-60.

IHC FOR P16 AND p53; HPV STATUS

- 92 cases; stage I.
- p16 (+): “diffuse, strong, band-like involving at least two-thirds of the tumor thickness”.
- P53 (+): “+2 and +3 score; dark intense nuclear staining”.
- **P16 (+), HPV (+) patients: less likely to recur, no tumor-related deaths.**
- **P53 (+) patients: 3X more likely to recur and almost 7X more likely to die from vulvar cancer.**
- ***Tumor size > 4 cm.: 4X increase in disease-specific mortality.***

Hay CM, Lachance JA, Lucas FL et al. - J Lower Gen Tract Dis, 2016, 20(3): 252-6.

Review Article

The prognostic value of p16 and p53 expression for survival after vulvar cancer: A systematic review and meta-analysis

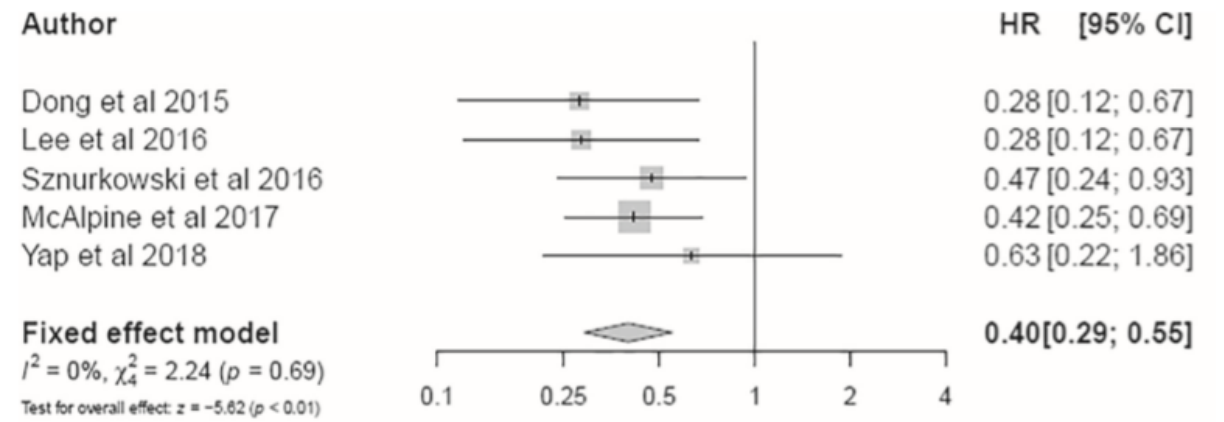
Freja Lærke Sand [a,1], Ditte Maria Bjerno Nielsen [a,1], Marie Hoffmann Frederiksen [b], Christina Louise Rasmussen [a], Susanne K. Kjaer [a,c,*]

Gynecol Oncol. 2019, 152: 208-17.

HIGHLIGHTS

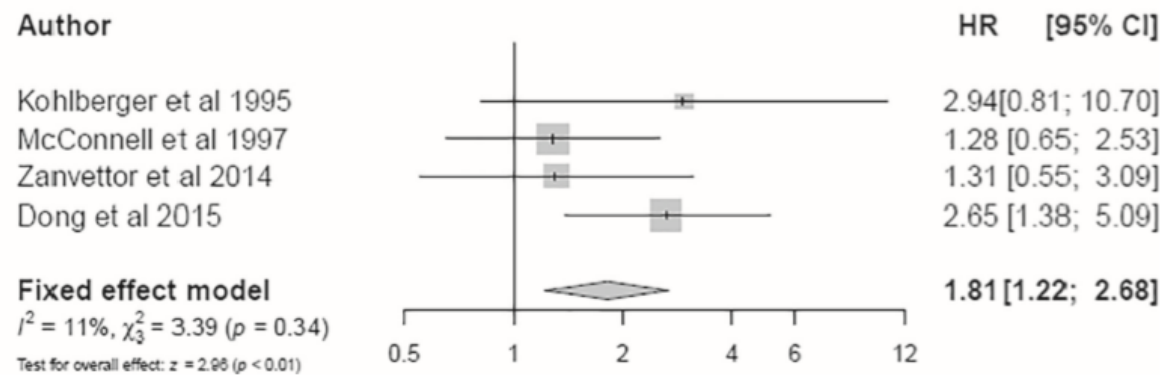
- Women with p16 positive vulvar cancers had better survival compared to p16 negative.
- p53 positive vulvar cancers had a less favorable survival compared to p53 negative.
- p16 and p53 may be clinically useful prognostic markers for vulvar cancer patients.

A



HR, hazard ratio; CI, confidence interval; VSCC, vulvar squamous cell carcinoma

B



HR, hazard ratio; CI, confidence interval; VSCC, vulvar squamous cell carcinoma

PD-L1 expression on immune cells is a favorable prognostic factor for vulvar squamous cell carcinoma patients

Jacek J. Sznurkowski[1], Anton Żawrocki[2], Katarzyna Sznurkowska[3], Rafał Pęksa[2] and Wojciech Biernat[2]

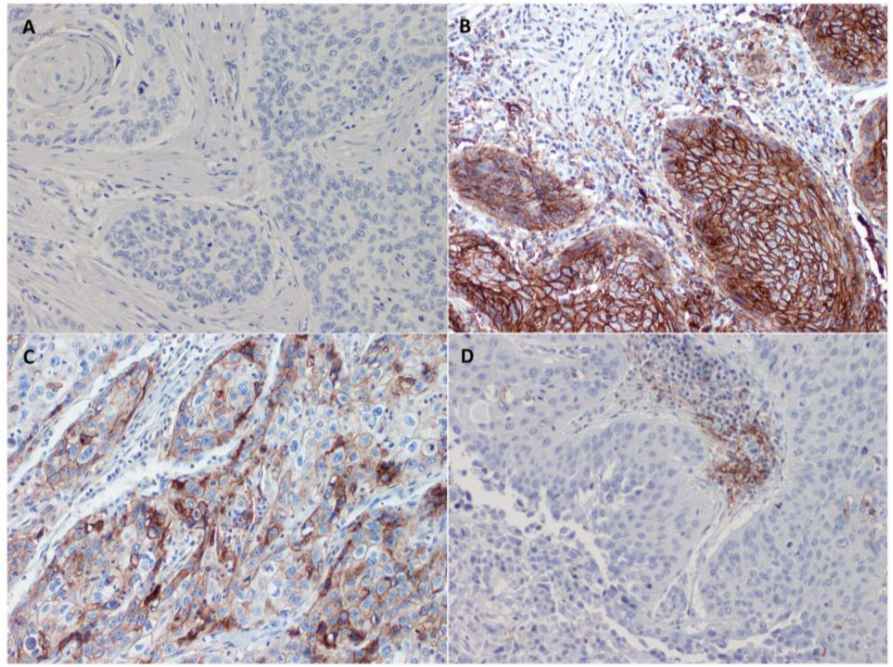
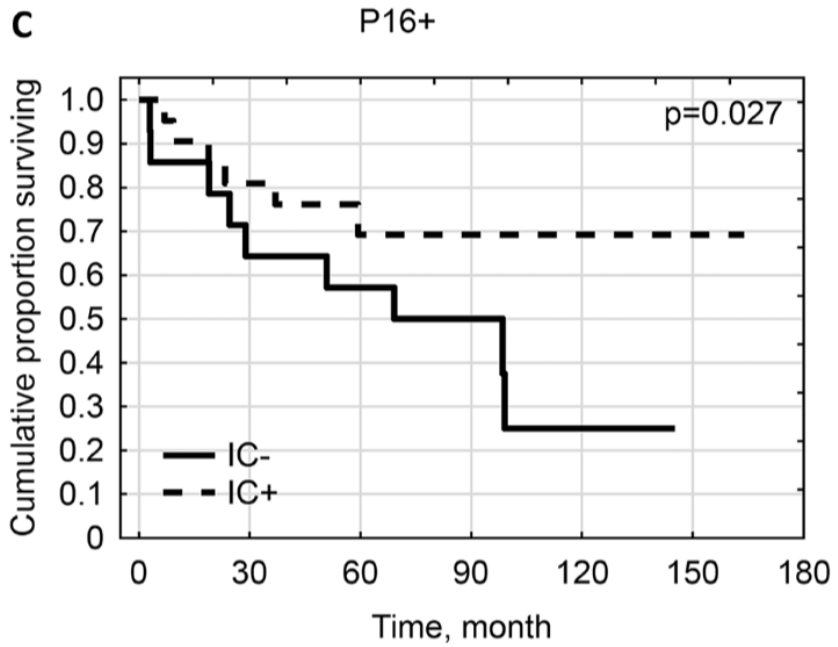


Table 4: Multivariate analyses of survival in vulvar cancer patients

Variables	Categories	Overall survival		p
		HR	95% CI	
Nodal status	Negative for metastases	1	1.50-5.02	0.019
	Positive for metastases	2.74		
Histologic Grade	Low (G1)	1	1.33-5.90	0.007
	High (G2+G3)	2.80		
p16 status	Positive	1	1.13-3.95	0.001
	Negative	2.11		
IC-PD-L1	negative	1	0.25-0.83	0.010
	positive	0.45		



Oncotarget, 2017, (8) 52: 89903-12

PD-L1 EXPRESSION ON IMMUNE CELLS

- 84 tumors; p16 and DNA-HPV; CD8, CD4, FOXP3, CD56, CD68, GZB.
- PD-L1 (22C3) positivity defined as $\geq$ 5%.
- Positivity on cancer cells (32,1%): correlated with higher infiltration of CD4+, CD8+, FOXP3+ and CD68+ cells.
- **Positivity on peritumoral imune cells** (60,7%): correlated with higher infiltration of intraepithelial FOXP3+ cells; **independent favorable prognostic factor for OS.**
- Positivity of cancer cells but not imune cells: more frequent in p16(-) tumors; HR-HPV: no correlation with PD-L1 status.

Sznurkowski JJ, Zawrocki A, Sznurkowska K et al. – Oncotarget, 2017, (8) 52: 89903-12

PD-L1 receptor expression in vulvar carcinomas is HPV-independent

M. Choschzick[1] · A. Gut[1] · D. Fink[2]

Table 1 Relationships between PD-L1 expression and clinicopathological features in vulvar carcinomas

		All (*n*)	PD-L1 IHC				*p* value
			Negative (*n*)	Weak (*n*)	Moderate (*n*)	Strong (*n*)	
		55	15	25	11	4	
Age (years)	Median	69	69	66	77	77	n.s.
Histologic tumor type	Keratinizing	26	6	16	4	0	n.s.
	Non-keratinizing	23	9	5	5	4	
	Basaloid	6	0	4	2	0	
Tumor stage	pT1a	4	0	1	3	0	0.04
	pT1b	31	7	15	6	3	
	pT2	10	4	5	1	0	
	pT3	4	3	1	0	0	
	pT4	3	1	1	1	0	
Nodal stage	pN0	17	5	8	3	1	n.s.
	pN1a,b	5	0	3	2	0	
	pN2a-c	10	3	3	1	2	
Grading	G1	6	3	2	5	0	n.s.
	G2	29	7	16	5	1	
	G3	16	4	5	3	2	
HPV OOoO	Negative	25	6	13	4	2	n.s.
	Positive	21	5	9	5	2	
Overall survival	PD-L1 negative/weak vs. moderate/strong	48	Hazard ratio 1.16; 95% confidence interval 0.36–3.78				0.8

n.s. not significant

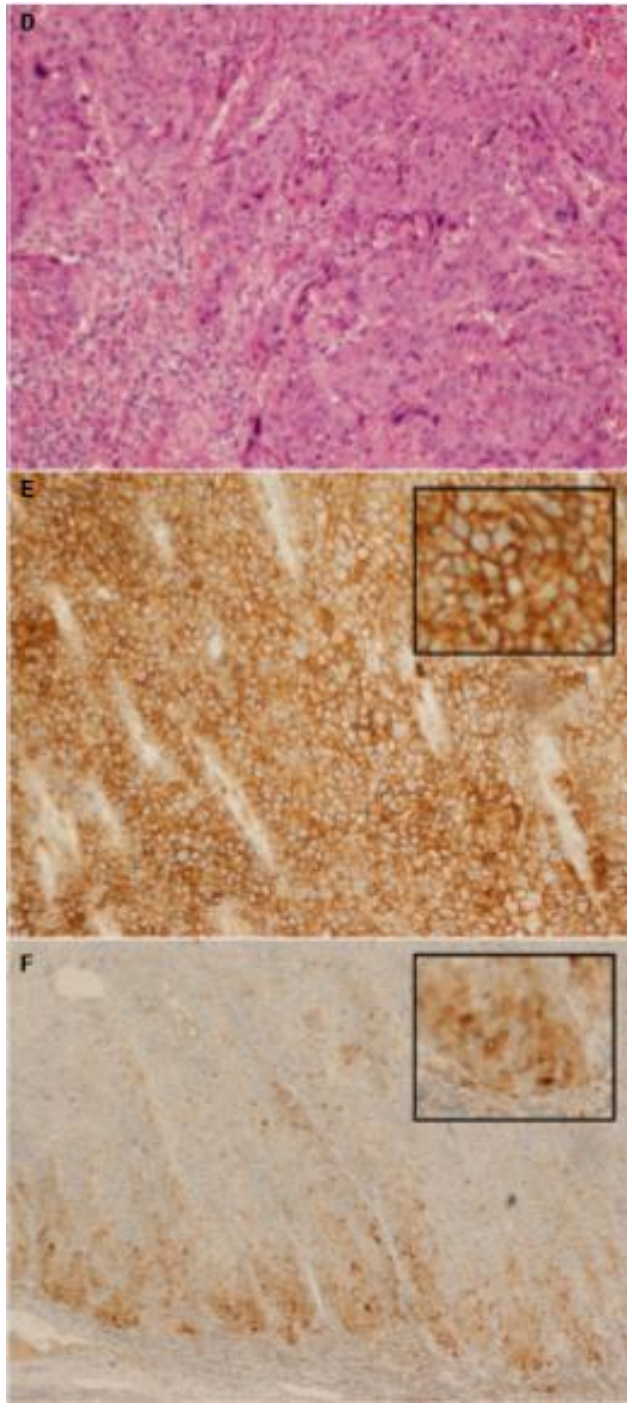
Virchows Archiv, 2018, 473: 513-6.

Histopathology 2019, 74, 256–268. DOI: 10.1111/his.13723

PD-L1 and IDO expression in cervical and vulvar invasive and intraepithelial squamous neoplasias: implications for combination immunotherapy

Zachary Chinn, Mark H Stoler & Anne M Mills

	+PD-L1 Tumor	+PD-L1 Immune	PD-L1 CPS ≥ 1	+IDO Tumor	+IDO Immune	IDO CPS ≥ 1
dVIN	0% (0/2)	50% (1/2)	50% (1/2)	0% (0/2)	50% (1/2)	50% (1/2)
	1–5%: 0/2	1–10%: 0/2		1–5%: 0/2	1–10%: 1/2	
	6–10%: 0/2	11–25%: 0/2		6–10%: 0/2	11–25%: 0/2	
	11–25%: 0/2	26–50%: 1/2		11–25%: 0/2	26–50%: 0/2	
	26–50%: 0/2	>50%: 0/2		26–50%: 0/2	>50%: 0/2	
	>50%: 0/2			>50%: 0/2		
Vulvar SCC, HPV-associated	63% (10/16)	44% (7/16)	81% (13/16)	13% (2/16)	19% (3/16)	25% (4/16)
	1–5%: 5/16	1–10%: 4/16		1–5%: 0/16	1–10%: 3/16	
	6–10%: 3/16	11–25%: 3/16		6–10%: 2/16	11–25%: 0/16	
	11–25%: 1/16	26–50%: 0/16		11–25%: 0/16	26–50%: 0/16	
	26–50%: 0/16	>50%: 0/16		26–50%: 0/16	>50%: 0/16	
	>50%: 1/16			>50%: 0/16		
Vulvar SCC, dVIN-associated	75% (3/4)	75% (3/4)	75% (3/4)	100% (4/4)	75% (3/4)	100% (4/4)
	1–5%: 0/4	1–10%: 1/4		1–5%: 4/4	6–10%: 2/4	
	6–10%: 2/4	11–25%: 2/4		6–10%: 0/4	11–25%: 1/4	
	11–25%: 0/4	26–50%: 0/4		11–25%: 0/4	26–50%: 0/4	
	26–50%: 1/4	>50%: 0/4		26–50%: 0/4	>50%: 0/4	
	>50%: 0/4			>50%: 0/4		



PD-L1 AND INDOLEAMINE DIOXYGENASE 2,3 (IDO) EXPRESSION

- 13 uVIN3, 2 dVIN, 16 HPV-associated * SCC and 4 dVIN-associated SCC; (P16 diffuse expression in > 70% of tumor cells).*
- Positivity defined as staining in ≥ 1% of tumor cells (membranous for PD-L1 (22C3) and cytoplasmic for IDO); Cut-points: 1-5%; 6-10%; 11-25%; 26-50%; > 50%.; CPS.
- PD-L1 (+) in 63% HPV-assoc. and 75% of dVIN-assoc. tumor cells.
- IDO (+) in 13% of HPV-assoc. and 100% of dVIN-assoc. tumor cells, mostly focal; CPS ≥ 1 more frequent in dVIN-assoc. SCC, but focal (≤ 10%).
- Majority of intraepithelial lesions were negative.

Chinn Z, Stoler MH, Mills AM – Histopathology, 2019, 74, 256-68.

High numbers of activated helper T cells are associated with better clinical outcome in early stage vulvar cancer, irrespective of HPV or p53 status

Kim E. Kortekaas[1,3], Saskia J. Santegoets[3], Ziena Abdulrahman[3], Vanessa J. van Ham[3], Marij van der Tol[2], Ilina Ehsan[3], Helena C. van Doorn[4], Tjalling Bosse[5], Mariëtte I. E. van Poelgeest[2†] and Sjoerd H. van der Burg[3*†]

Abstract

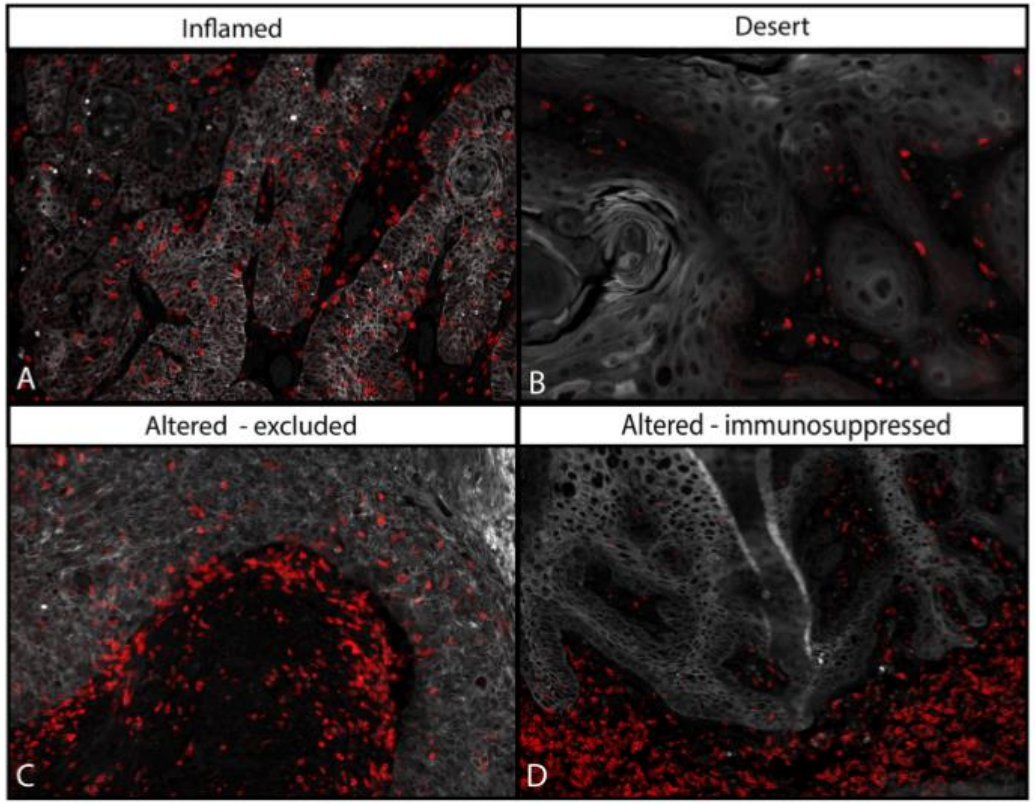
Background: Vulvar squamous cell carcinoma (VSCC) has been suggested to consist of three subtypes; HPV-positive, HPV-negative mutated *TP53* or HPV-negative *TP53* wildtype, with different clinical courses. To analyze the immune infiltrate in these molecular subtypes and its impact on clinical outcome, an in-depth study of the tumor immune microenvironment was performed.

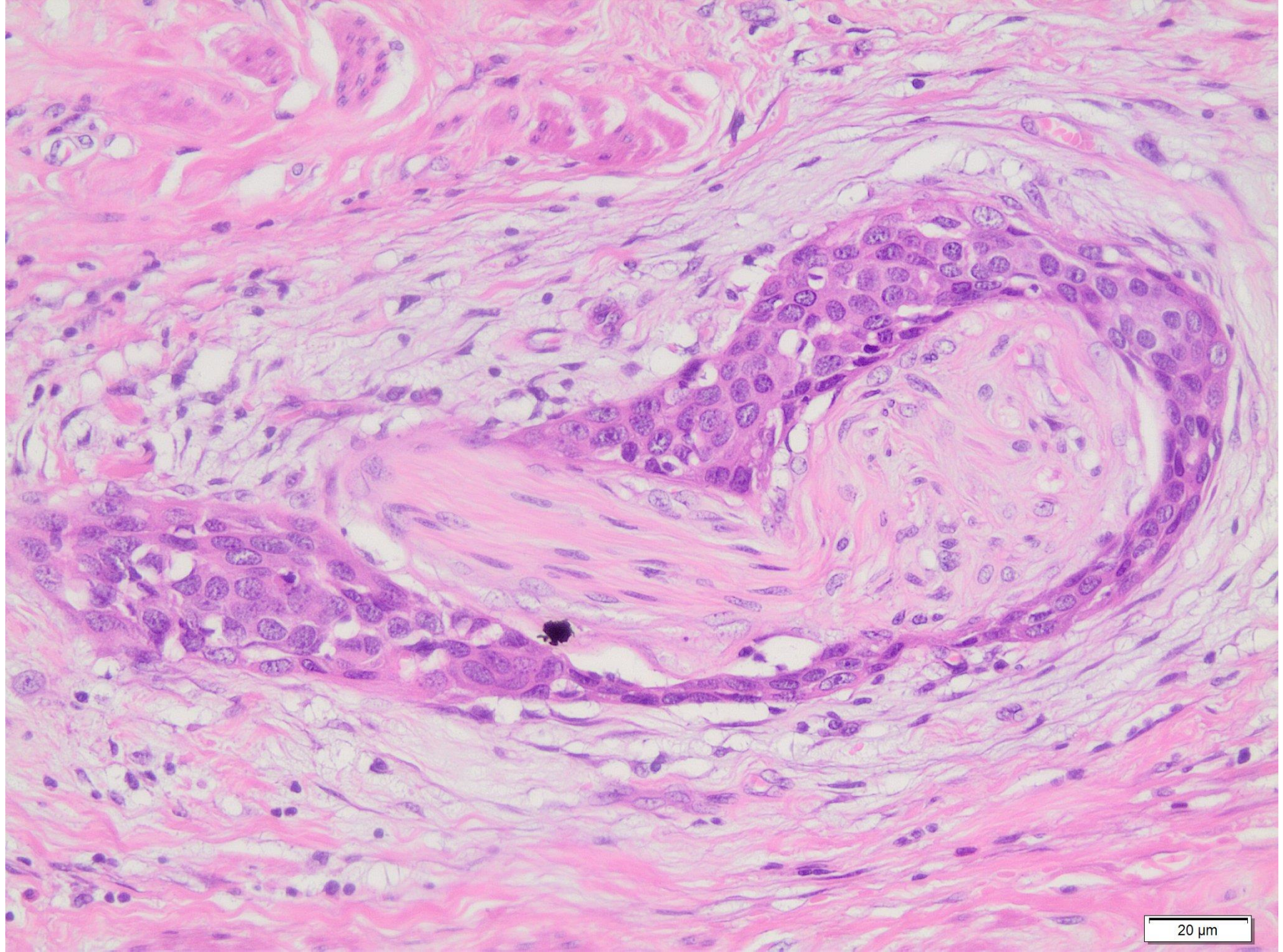
Methods: Sixty-five patients with invasive VSCC matched for age, FIGO stage and treatment modality, were grouped according to the presence of HPV and p53 protein expression status. Archived tissues were analyzed for intraepithelial and stromal expression of CD3, CD8, Foxp3, PD-1, and pan-keratin in randomly selected areas using immunofluorescence. Additional phenotyping of T cells was performed ex-vivo on VSCC ($n = 14$) and blood samples by flow cytometry. Healthy vulvar samples and blood served as controls.

Results: Based on T-cell infiltration patterns about half of the VSCC were classified as inflamed or altered-excluded while one-third was immune-deserted. High intraepithelial helper T cell infiltration was observed in 78% of the HPV-induced VSCC, 60% of the HPVnegVSCC/p53wildtype and 40% of the HPVnegVSCC with abnormal p53 expression. A high intraepithelial infiltration with activated ($CD3^+PD\text{-}1^+$), specifically helper T cells ($CD3^+CD8^-Foxp3^-$), was associated with a longer recurrence-free period and overall survival, irrespective of HPV and p53 status. Flow cytometry confirmed the tumor-specific presence of activated ($CD4^+PD\text{-}1^{++}CD161^-CD38^+HLA\text{-}DR^+$ and $CD8^+CD103^+CD161^-NKG2A^{+/-}PD1^{++}CD38^{++}HLA\text{-}DR^+$) effector memory T cells.

Conclusion: This is the first study demonstrating an association between intraepithelial T cells and clinical outcome in VSCC. Our data suggest that abnormal p53 expressing VSCCs mostly are cold tumors whereas HPV-driven VSCCs are strongly T-cell infiltrated.

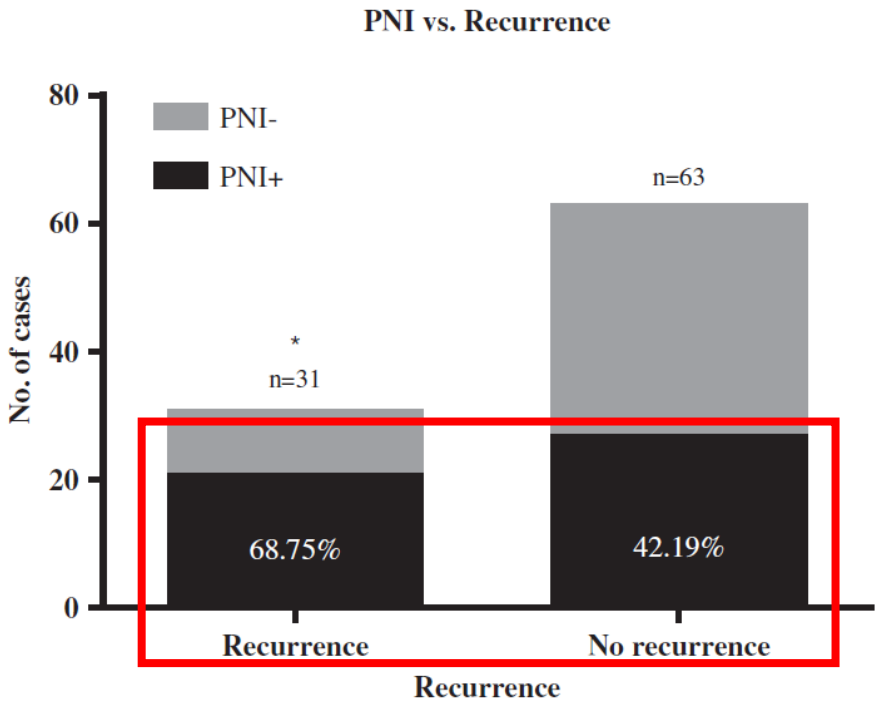
Journal for ImmunoTherapy of Cancer (2019) 7:236

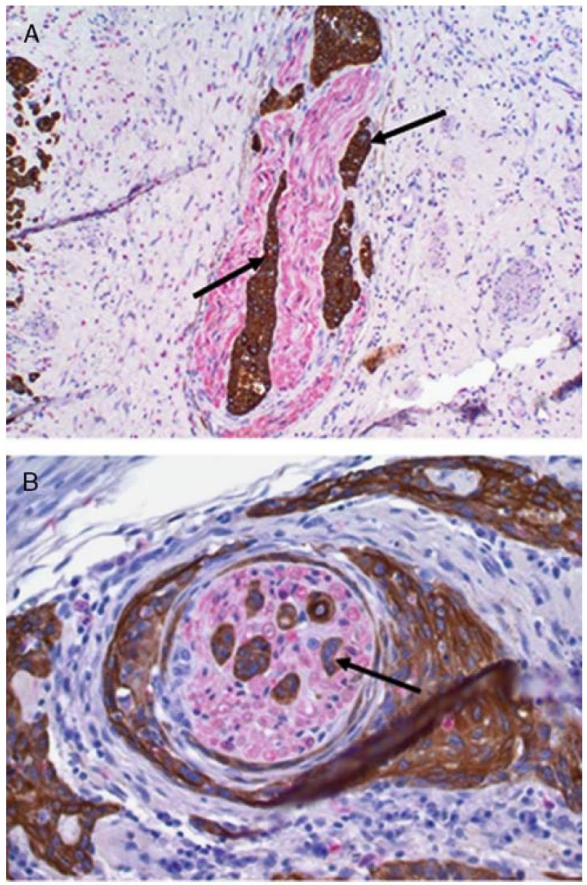


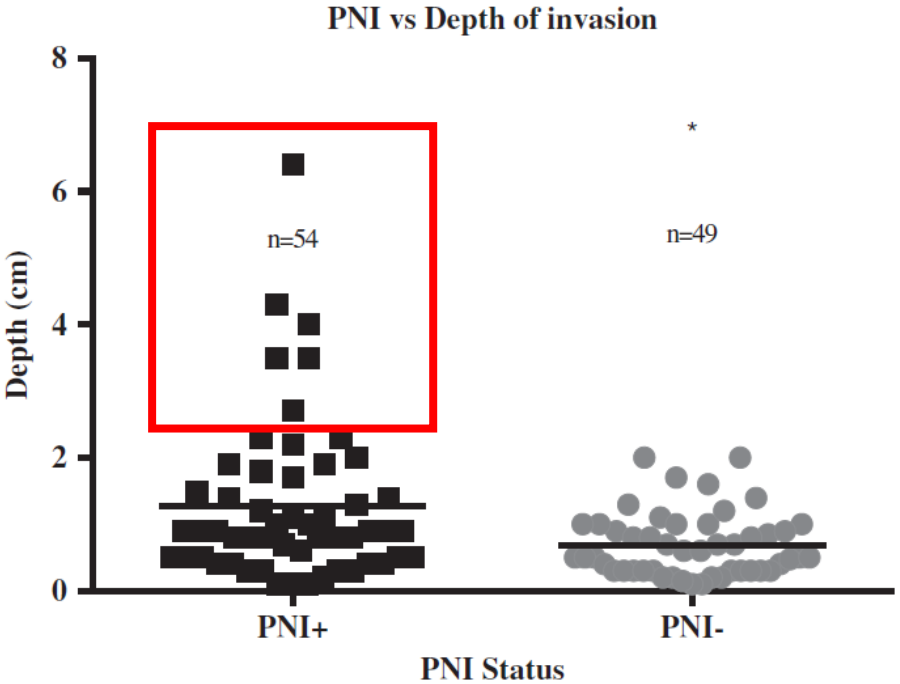

20 µm

Perineural Invasion Is an Independent Pathologic Indicator of Recurrence in Vulvar Squamous Cell Carcinoma

Emily R. Holthoff, BS, Susanne K. Jeffus, MD,* Ashita Gehlot, MD,† Rebecca Stone, MD,†*
Stephen W. Erickson, PhD,‡ Thomas Kelly, PhD, Charles M. Quick, MD,**
*and Steven R. Post, PhD**







(*Am J Surg Pathol* 2015;39:1070–1074)

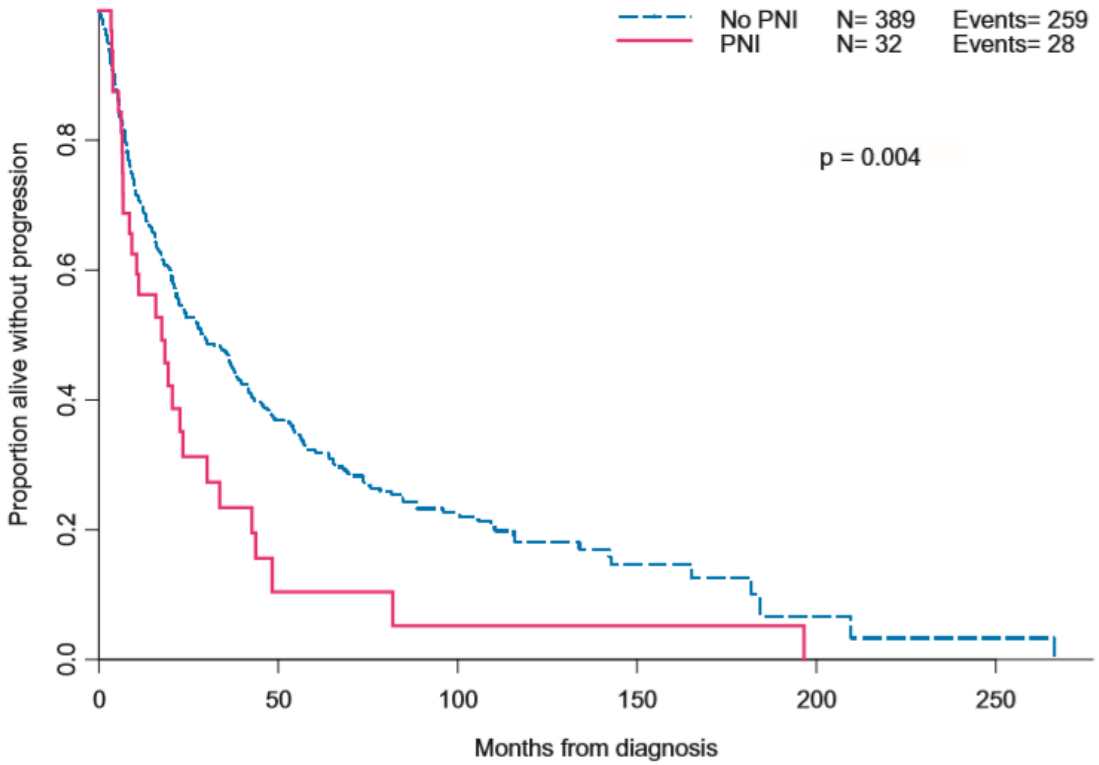
Perineural invasion (PNI) in vulvar carcinoma: A review of 421 cases☆

Mila Pontremoli Salcedo [a,b], Anil K. Sood [b], Ricardo dos Reis [c], Preetha Ramalingam [d], Chunling Chen [e], Michael Frumovitz [b], Anuja Jhingran [f], Brandelyn Pitcher [g], Pedro T. Ramirez [b], Kathleen M. Schmeler [b,*]

Gynecol Oncol. 2019, 152: 101-5.

HIGHLIGHTS

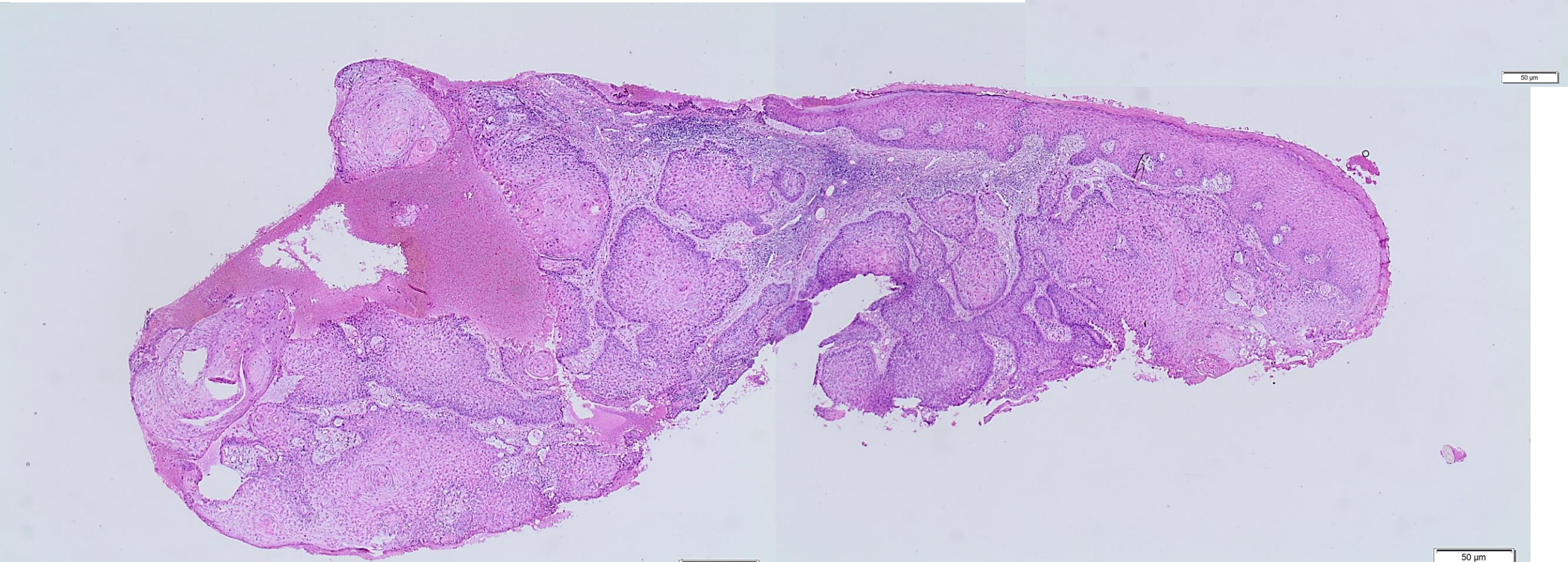
- Perineural invasion should be considered a poor prognostic factor in vulvar carcinoma.
- Perineural invasion was associated with higher stage disease.
- Perineural invasion was associated with poorer overall survival.



Overall survival			
Univariate model			
Variable	Description	HR (95% CI)	p-Value
PNI	PNI vs. No PNI	2.73 (1.83–4.07)	<0.001
Multivariable model			
Variable	Description	HR (95% CI)	p-Value
PNI	PNI vs. No PNI	2.71 (1.78–4.13)	<0.001
Stage	III/IV vs. I/II	1.70 (1.32–2.20)	<0.001
Recurrence free survival			
Univariate model			
Variable	Description	HR (95% CI)	p-Value
PNI	PNI vs. No PNI	1.65 (1.11–2.44)	0.004
Multivariable model			
Variable	Description	HR (95% CI)	p-Value
PNI	PNI vs. No PNI	1.64 (1.08–2.48)	0.020
Stage	III/IV vs. I/II	1.58 (0.24–2.02)	<0.001

POORLY ORIENTED AND/OR FRAGMENTED SPECIMENS...

How to measure the depth of invasion (DOI)?



Interobserver Agreement for Assessing Invasion in Stage 1A Vulvar Squamous Cell Carcinoma

Amal Abdel-Mesih, MD, Dean Daya, MD,† Kazu Onuma, MD,* Monalisa Sur, MD,† Shangguo Tang, MD,† Noori Akhtar-Danesh, PhD,‡§ Odette Boutross-Tadross, MD,‖ Kathy M. Ceballos, MD,¶ William Chapman, MD,# Terence Colgan, MD,** Pratima Deb, MD,* Marisa R. Nucci, MD,†† Esther Oliva, MD,‡‡ and Alice Lytwyn, MD†§*

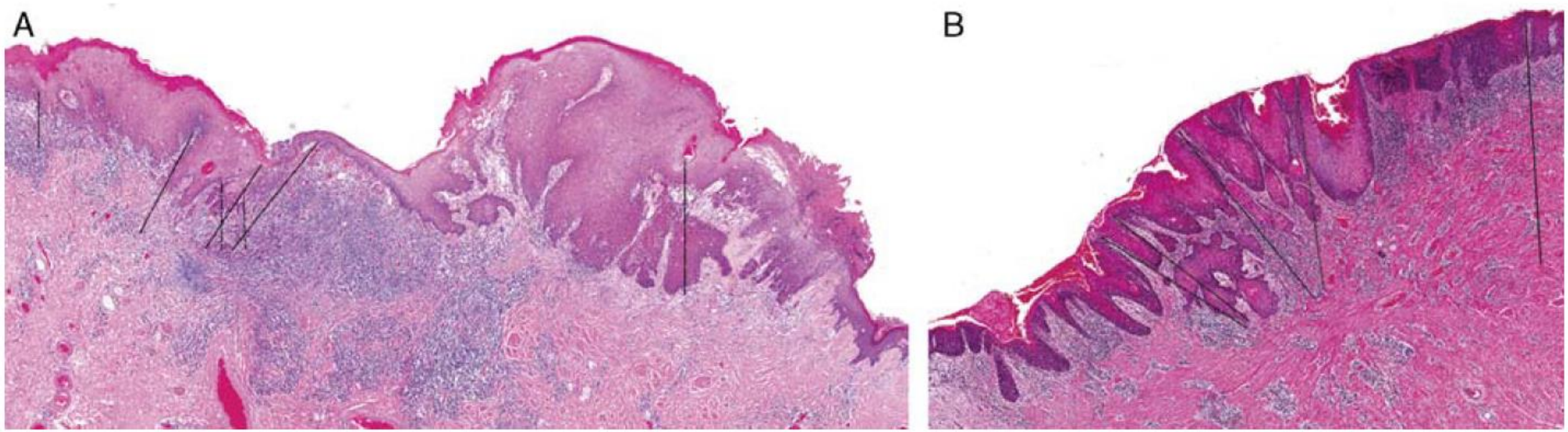


FIGURE 4. A and B, Examples of 2 cases in which pathologists varied in their ways of measuring depth of stromal invasion using the FIGO method. Each line shows the location where at least 1 pathologist made the measurement for depth of invasion (hematoxylin and eosin stain).

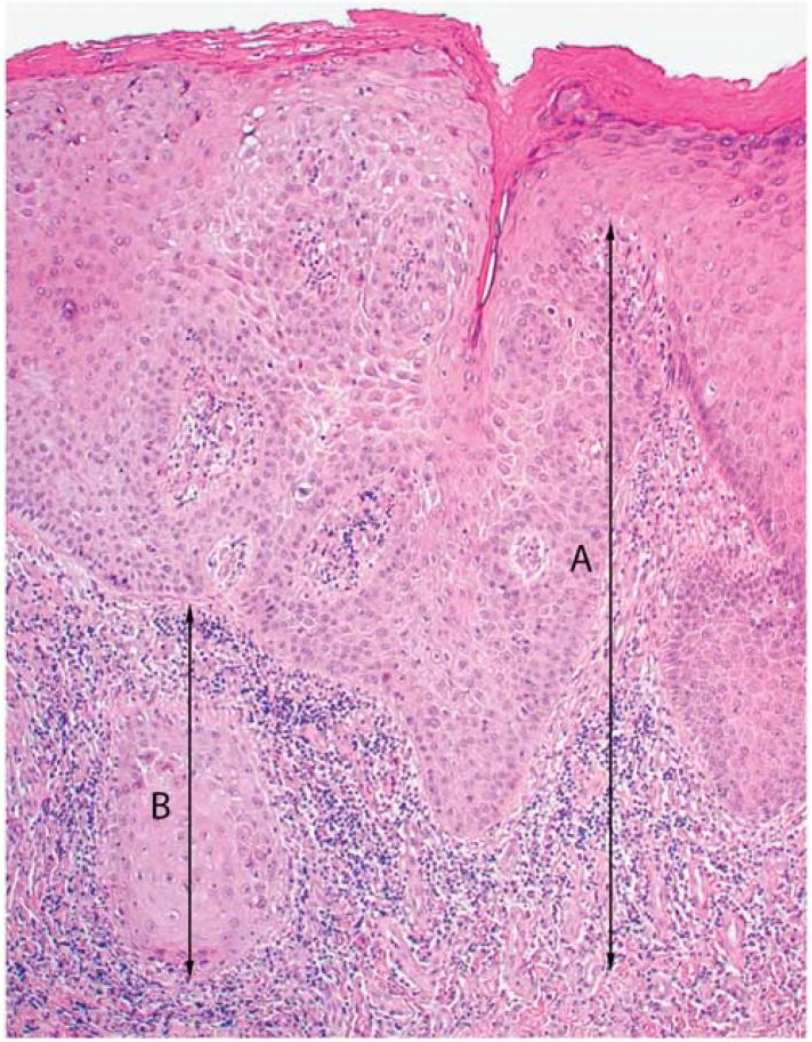
(*Am J Surg Pathol* 2013;37:1336–1341)

INTEROBSERVER AGREEMENT FOR ASSESSING INVASION IN STAGE IA VULVAR SCC

- 45 cases with depth of invasion (DOI) originally reported as ≤ 5mm.
- 11 gynecological pathologists.
- **agreement for diagnosing invasion was only fair...**
- **agreement for measuring depth of invasion and tumor thickness was moderate...**
- **interpretation of the location of the "adjacent most superficial dermal papila" varied among observers...**

Abdel-Mesih A, Daya D, Onuma K et al. - AJSP, 2013, 37, 1336-41.

ALTERNATIVE METHOD TO ASSESS THE DOI



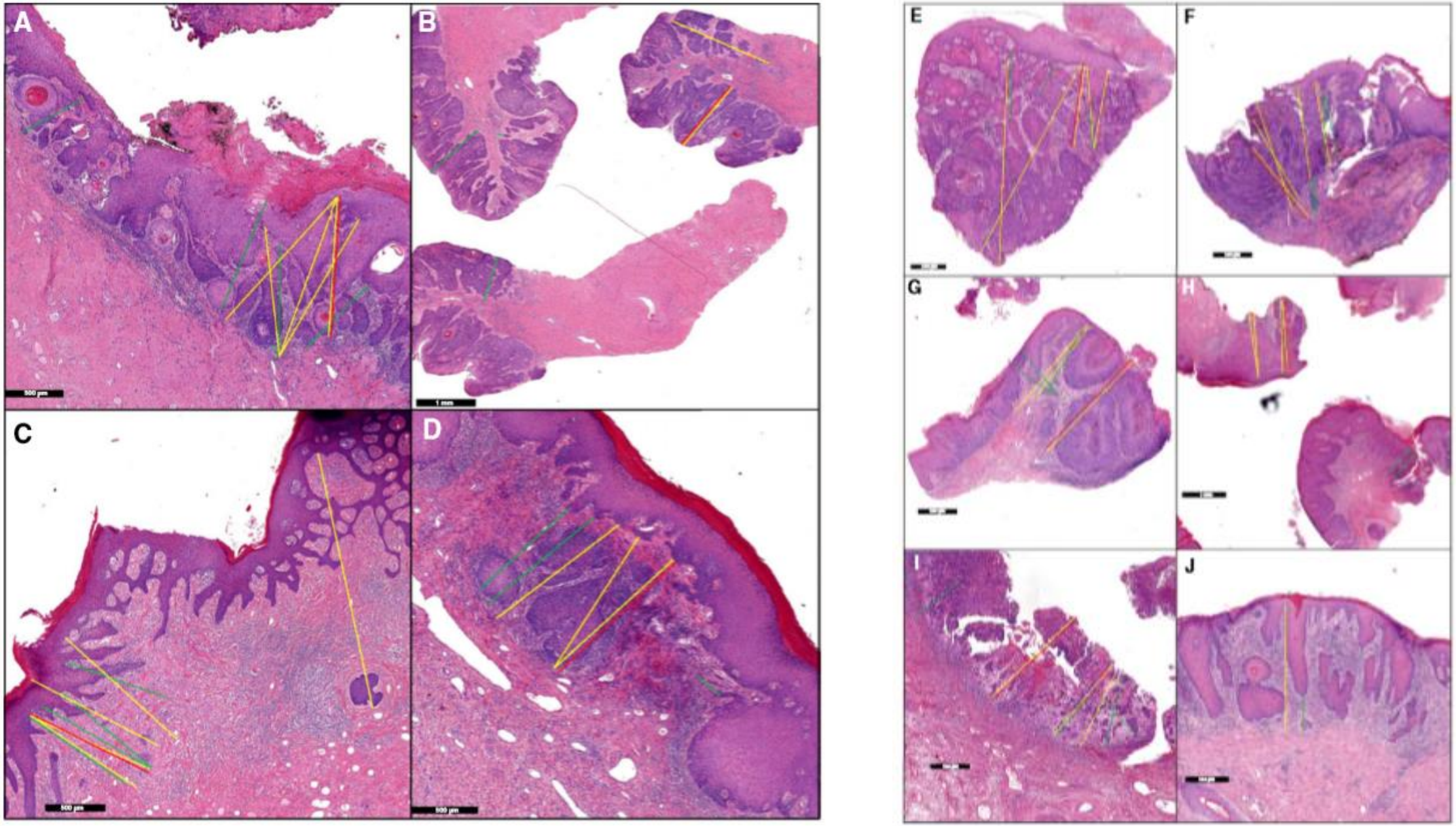
- 148 patients with known lymph node data.
- FIGO stage IB or higher.
- **median DOI: 5,5 mm (traditional) x 3,6 mm (alternative).**
- **69 stage IB patients: 13 downstaged to stage IA.**
- **downstaged patients developed less recurrences (15% x 39%) and had higher disease-specific survival (100% x 84%) than patients who remained stage IB.**

van den Heiden LCG, Massuger LFAG, Jonkman JK et al. - Mod Pathol, 2015, 28, 295-302.

DOI: INTEROBSERVER AGREEMENT AND PITFALLS

- 50 digitally scanned slides of VSCC with a DOI of approximately 1 mm.
- independently assessed by 10 specialized and 4 in training pathologists; DOI measured with the conventional and alternative methods.
- **Conventional method: moderate agreement; 85% vs. 89,4% (conventional vs. alternative: no notable difference).**
- **Pitfalls: which invasive nest is deepest; presence of invasive growth and where it starts; curved surface; carcinoma located on the edge of the tissue block; ulceration; different measurement methods.**

Pouwer AW, Bult P, Otte I et al. - Histopathology, 2019, 75, 413-20.



Pouwer AW, Bult P, Otte I et al. - Histopathology, 2019, 75, 413-20.