Leiden University
Medical Center

Vulvar Squamous Cell Carcinoma and its Precancerous Lesions

Postgraduate Course Cervix/Vulva

Antwerp 2019

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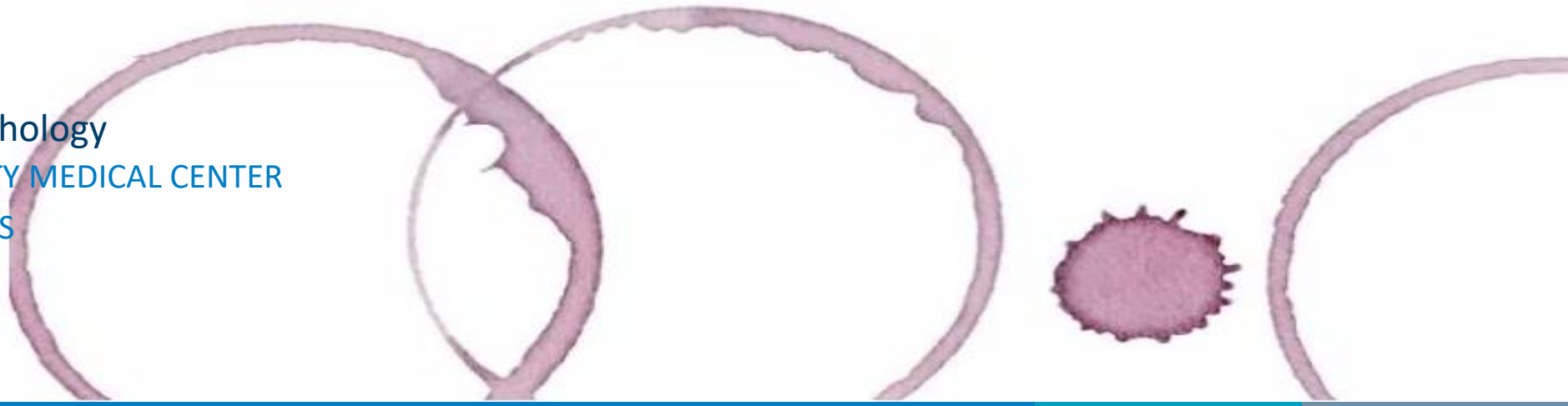
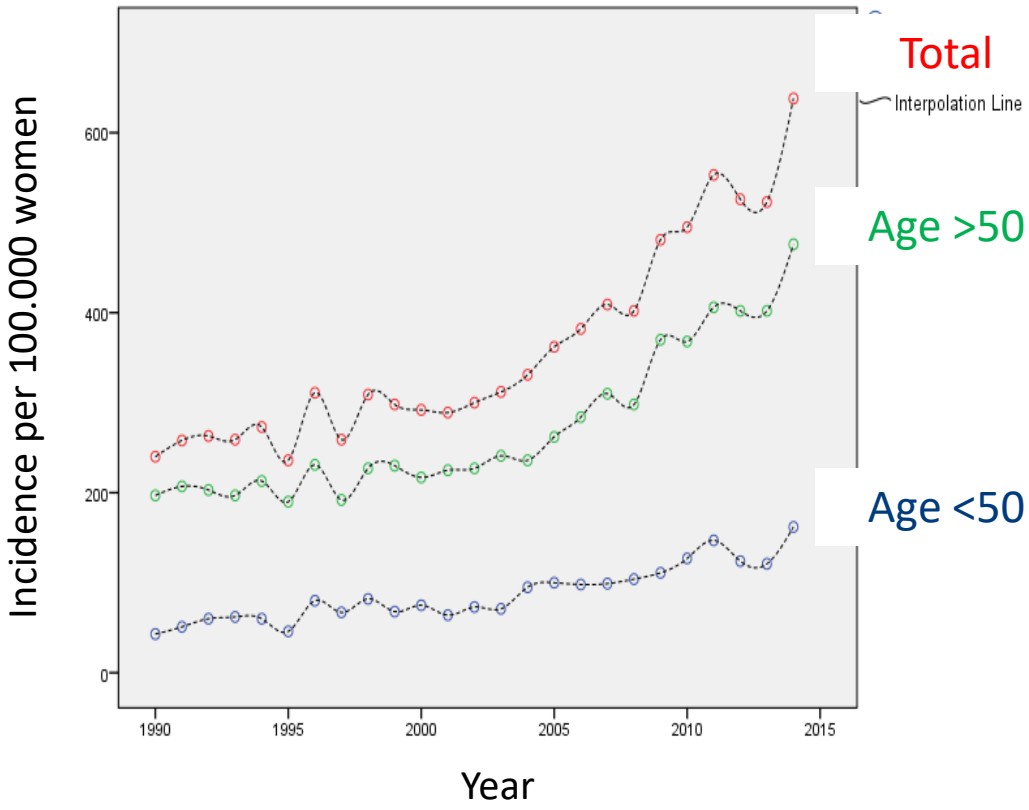
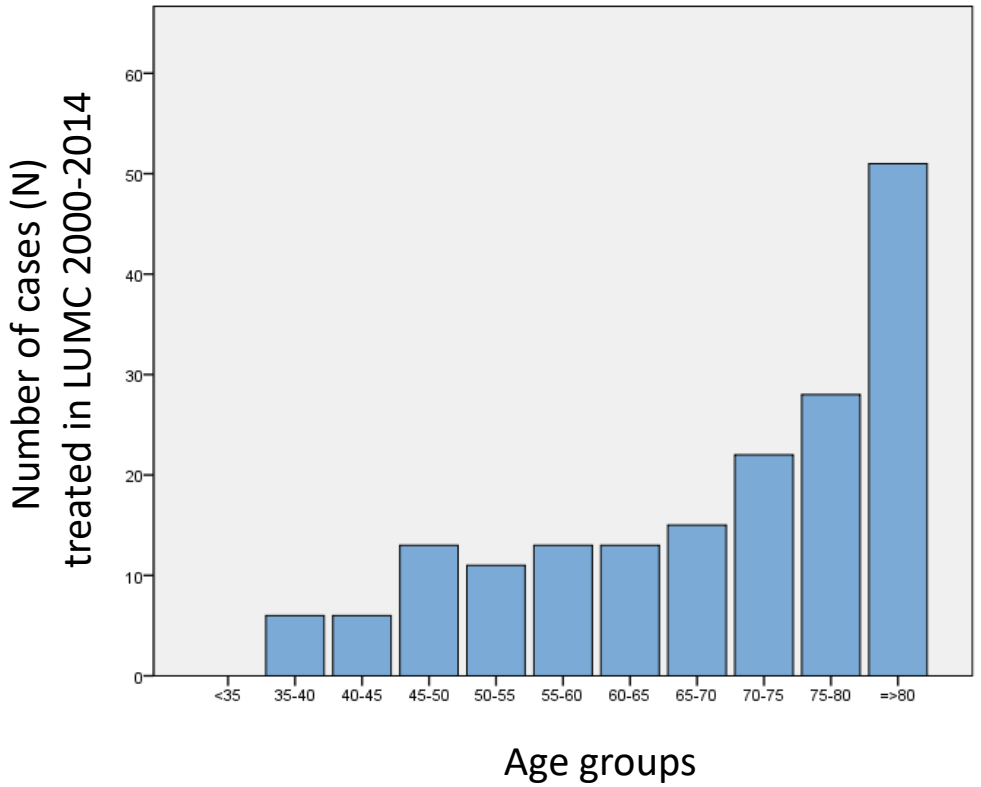
Department Pathology
LEIDEN UNIVERSITY MEDICAL CENTER
THE NETHERLANDS



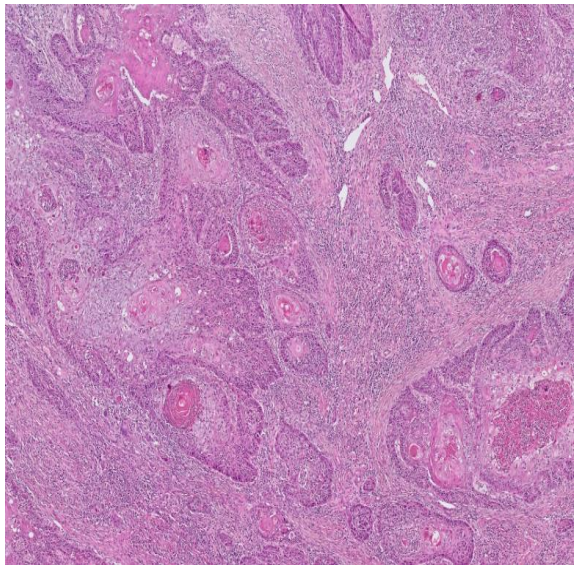
Incidence VSCC in the Netherlands





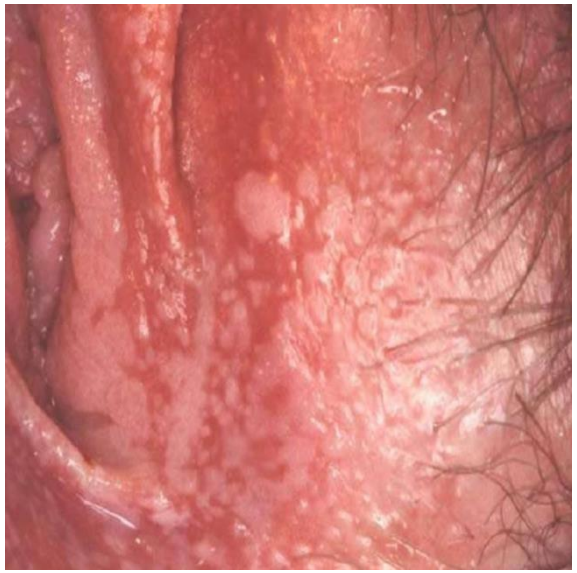
- VSCC is a rare cancer with ~300 new cases each year in NL
- Incidence (in the Netherlands) is rising
- VSCC is a cancer of “the elderly”

VSCC challenging?



Pathologist – pretty straightforward

- Squamous cell carcinoma
- Measuring size, DOI and Margins



Gynaecologist – lots of challenges

- Site specific difficulties – groins!
- Age related co-morbidities
- High recurrence rates
- Need for tailored treatment!

Can we help?

Can we refine our diagnose of VSCC, to provide tools for tailored treatment approaches?

- Tailor extent of surgical treatment
- Tailor adjuvant treatment
- Targeted treatment in recurrent/metastatic setting
- Tailor follow-up schemes

Molecular aspects of VSCC

Molecular genetic understanding of VSCC is in its infancy

Literature search

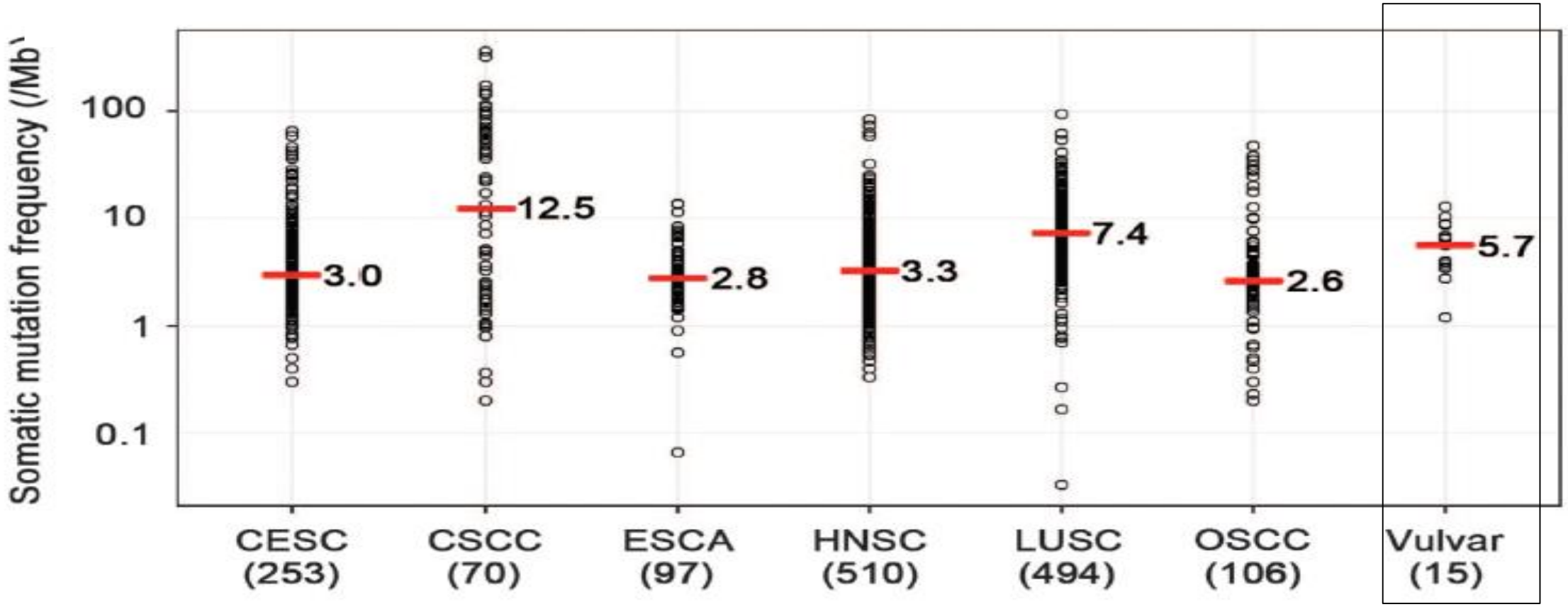
1. Han et al., *Mutational signatures and chromosome alteration profiles of squamous cell carcinomas of the vulva.* Exp Mol Med. 2018 **-> WES and Copy Number Profiling of 15 VSCC**

2. Weberpals et al., *Vulvar squamous cell carcinoma (VSCC) as two diseases: HPV Status identifies distinct mutational profiles including oncogenic fibroblast growth factor receptor 3.* CCR 2017 **-> Targeted NGS 43 VSCC**

3. Nooij et al., *Genomic characterization of vulvar (pre)cancers identifies distinct molecular subtypes with prognostic significance*. CCR 2017 **-> Targeted NGS 36 VSCC + 82 precursor lesions**

4. Watkins et al., *Differentiated exophytic vulvar intraepithelial lesions are genetically distinct from keratinizing squamous cell carcinomas and contain mutations in PIK3CA.* Mod Path 2017 -> **NGS on 12 HPVneg VSCC**

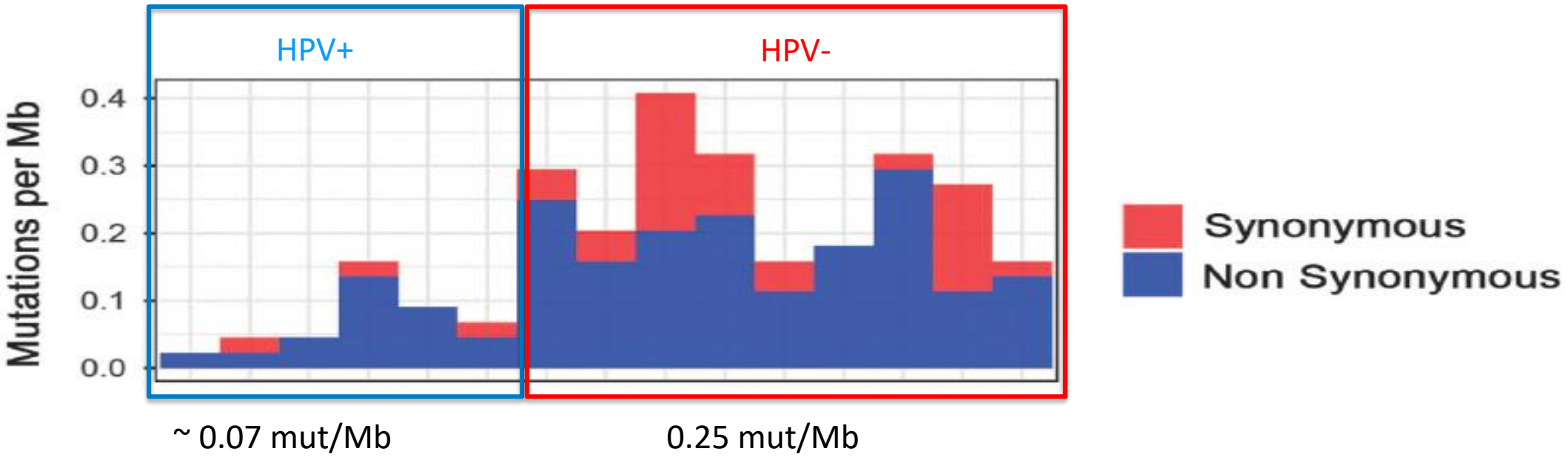
Mutational load in VSCC



Mutational load in VSCC seems relatively low

Han et al., Exp Mol Med. 2018

Cancer driver mutations in HPV- VSCC



Mutational burden indicates HPV status can separate VSCC in two distinct molecular entities

Han et al., Exp Mol Med 2018

Somatic mutations in cancer driver genes by HPV status in VSCC

HPVpos

PIK3CA

..

HPVneg

TP53

CASP8

APC

NOTCH1/2

FAT1

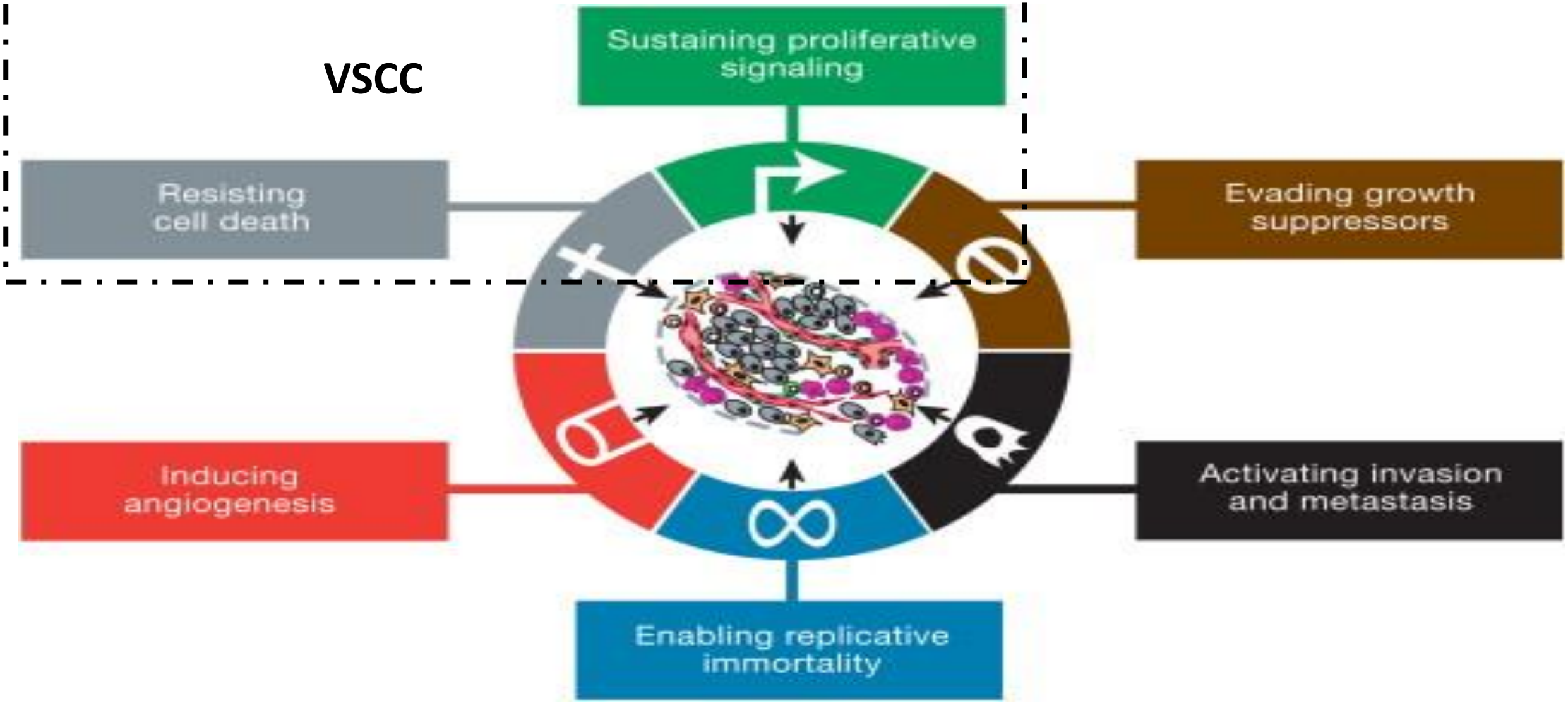
CREBBP

KMT2C

Han et al., Exp Mol Med 2018

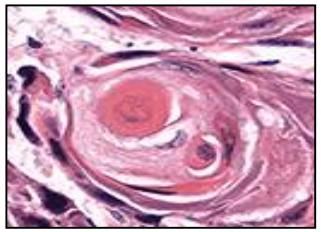
Hallmarks of cancer

VSCC



Hannah and Weinberg Cell 2000

Simplified scheme of pathway alterations in VSCC



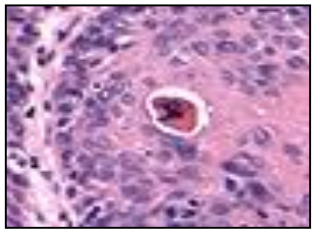
Altered Differentation

Terminal Differentiation

NOTCH1,2,3

TP63

CDKN2A



Resisting cell death

CASP8

Cell Death

TP53

PTEN

PIK3CA

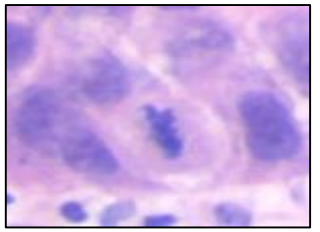
CCND1

RB1

Proliferation

HRAS

Sustained Proliferation



Adapted from Stransky et al., Science 2011

Largest targeted NGS study

Cancer Therapy: Clinical

Clinical Cancer Research

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]

Cohort

82 “Precursors” -> no follow-up

- 22 HSIL/uVIN
- 60 non-HSIL (HSIL, dVIN, LS, VAAD)

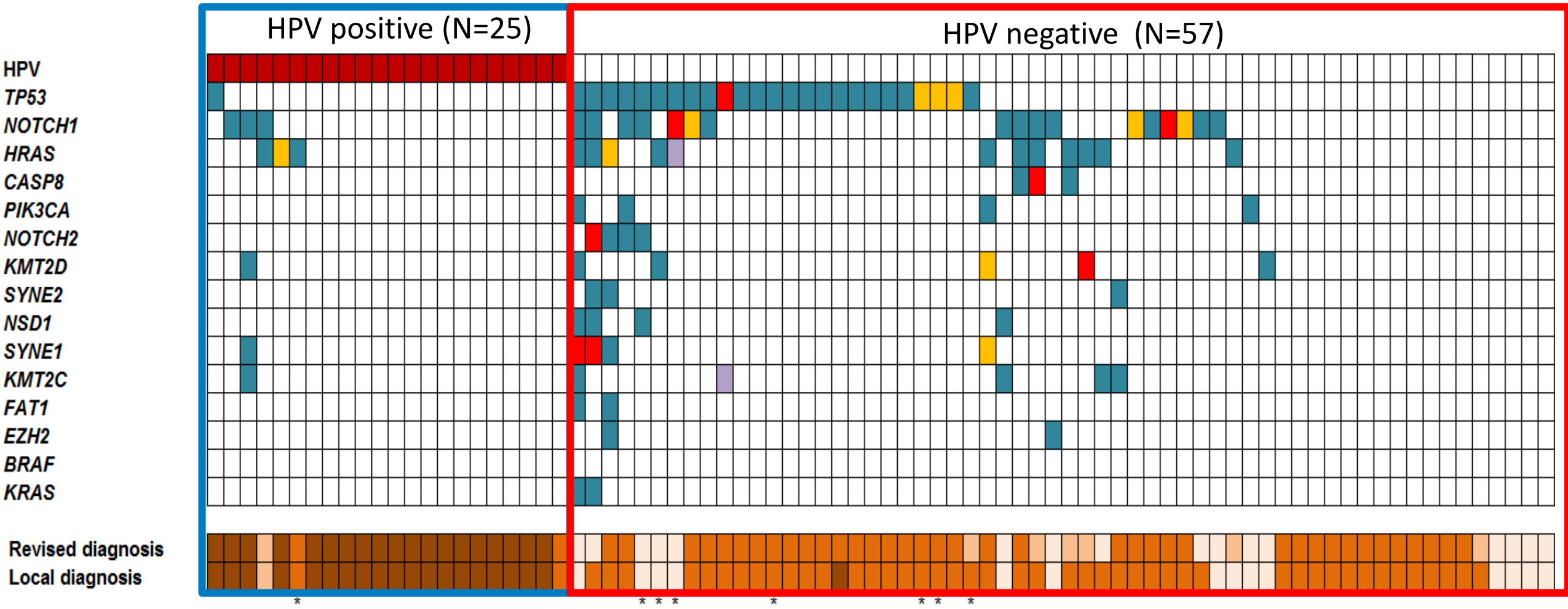
36 unselected VSCC

Methods

HPV typing - Lipa

Targeted NGS - 17 genes involved in HNSCC

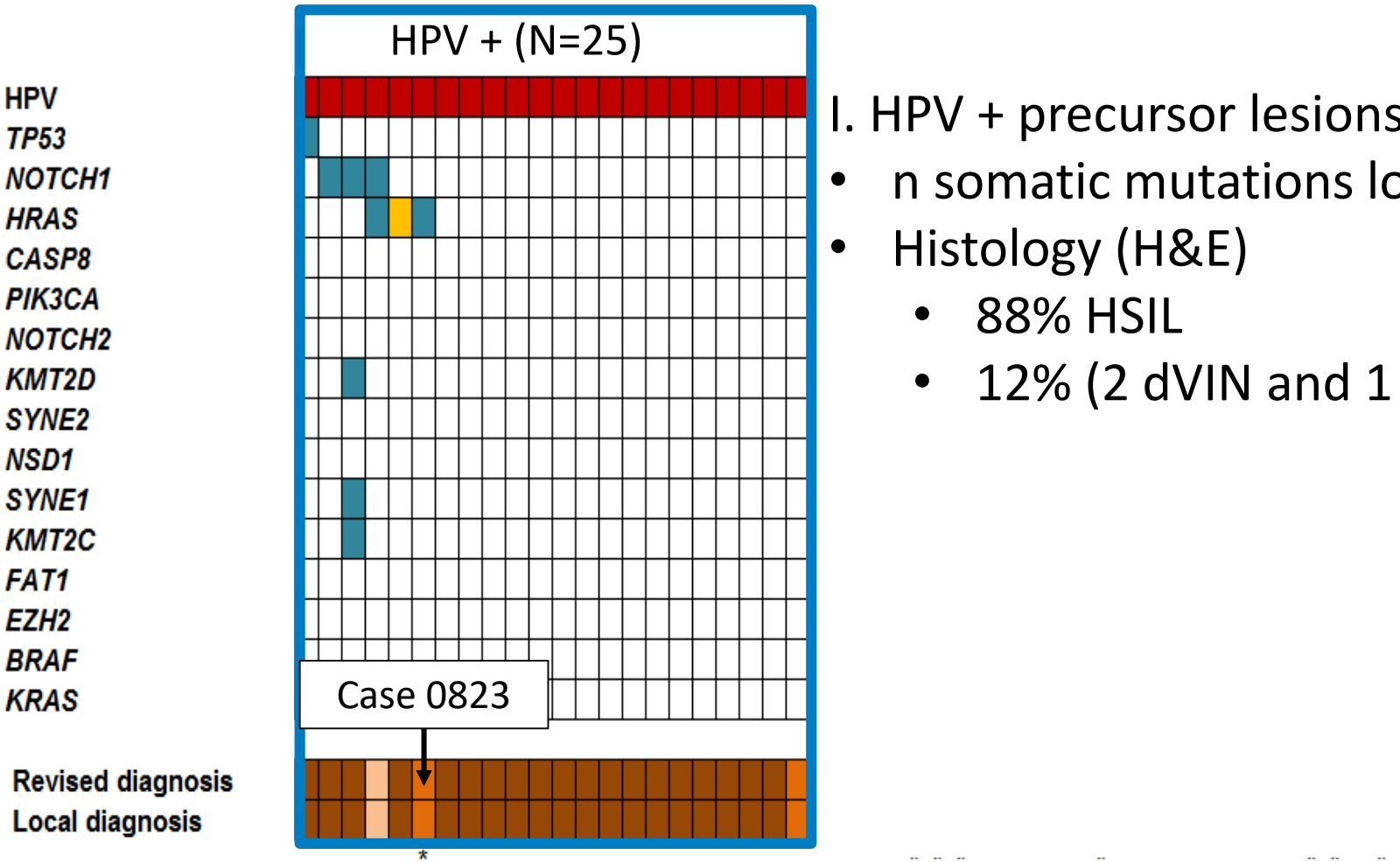
Somatic mutational landscape of "precursor lesions" N=82



* precursor lesion adjacent to vulvar cancer

Nooij et al. CCR 2017

Somatic mutations in HPV positive precursors

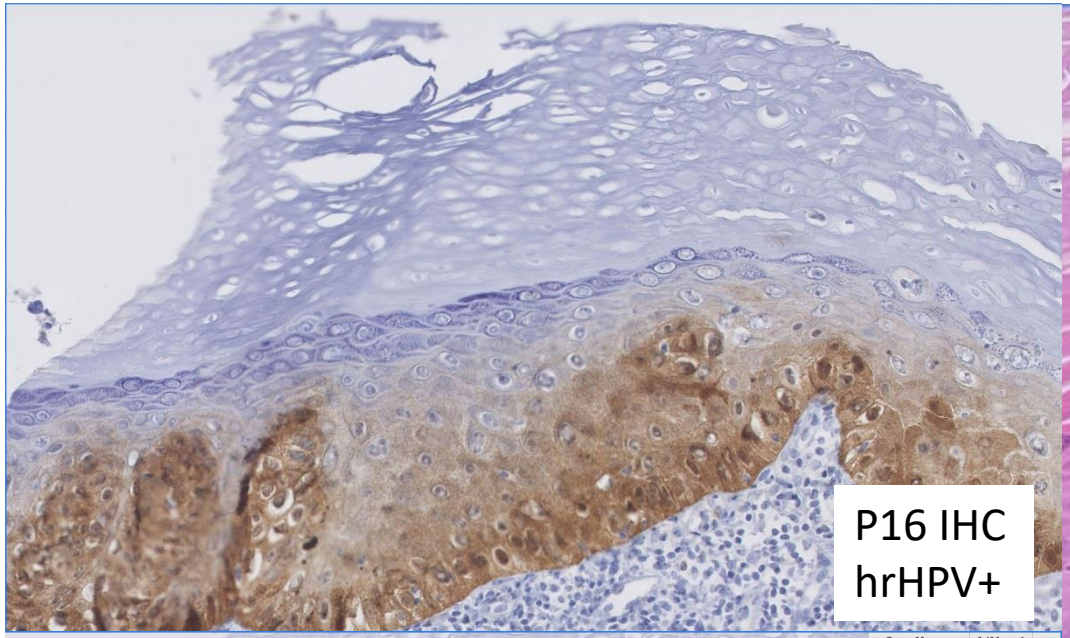


* precursor lesion adjacent to vulvar cancer

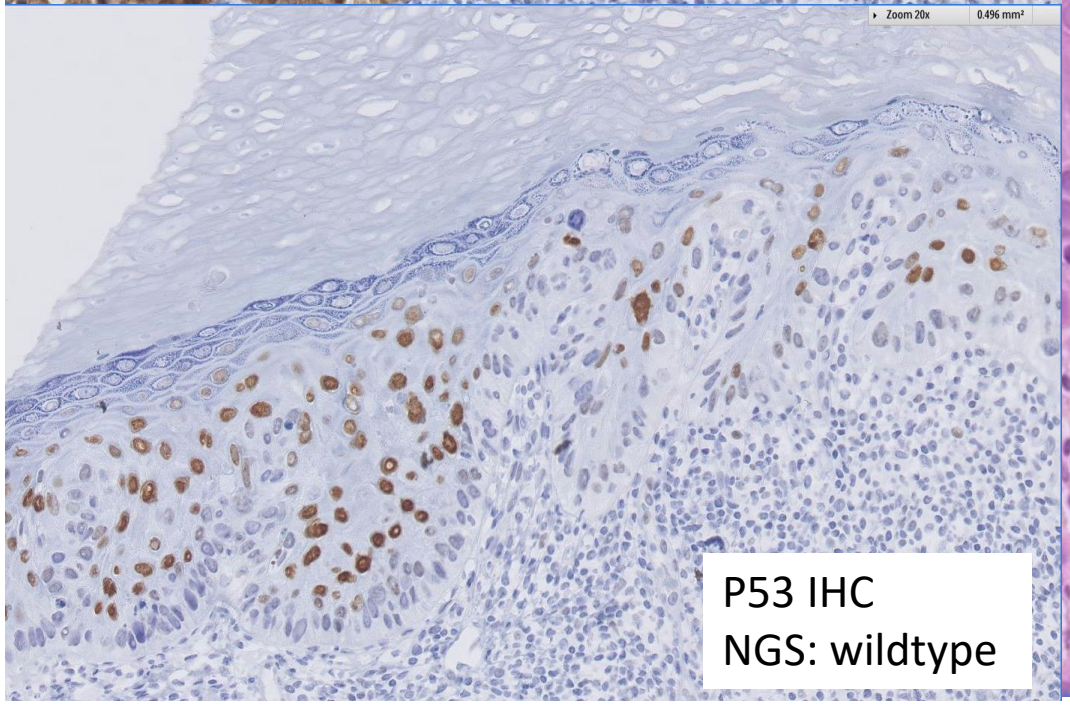
I. HPV + precursor lesions (N=25)

- n somatic mutations low
- Histology (H&E)
  - 88% HSIL
  - 12% (2 dVIN and 1 VAAD/25)

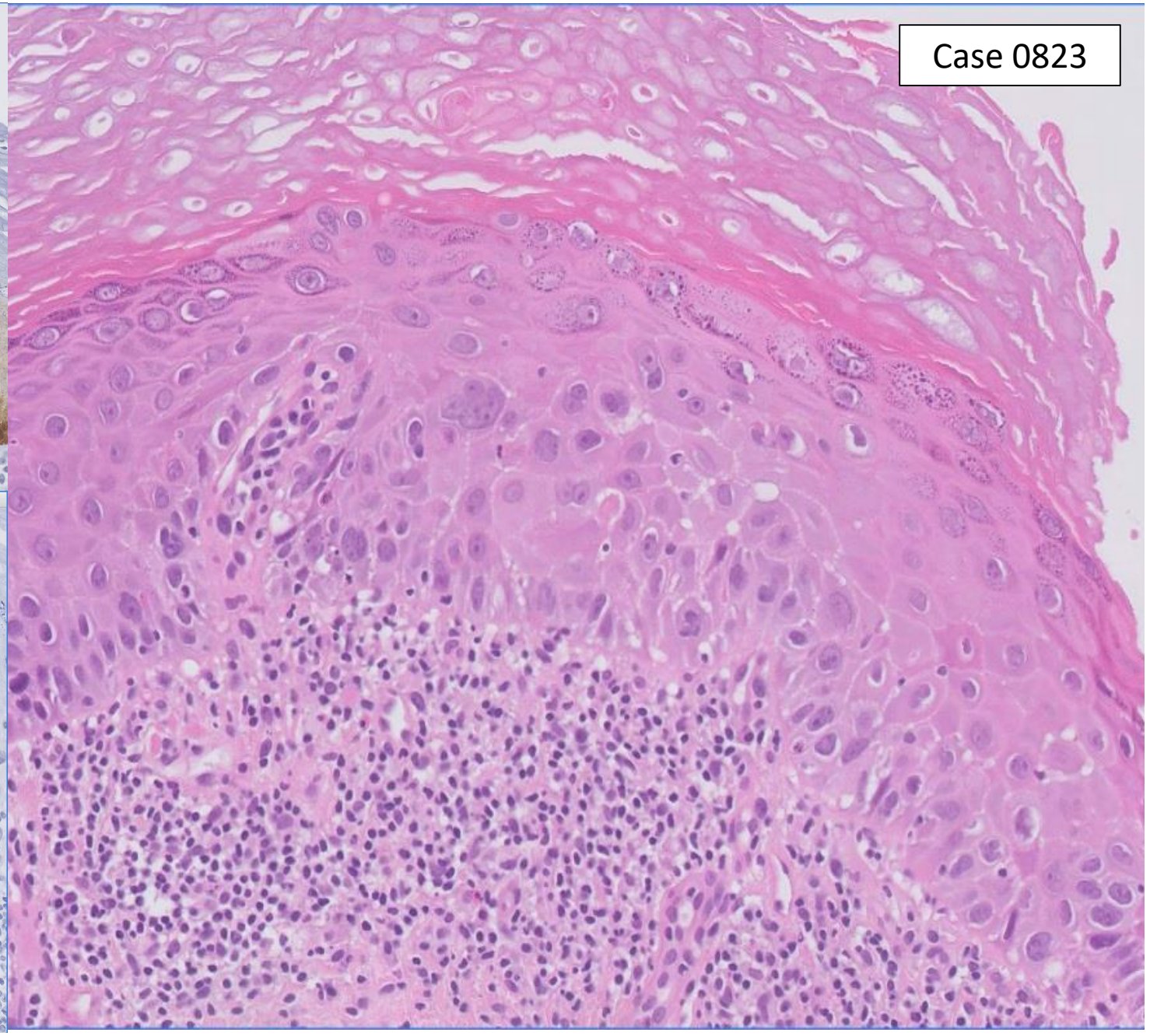
Misdiagnosed as differentiated VIN (dVIN)



P16 IHC
hrHPV+



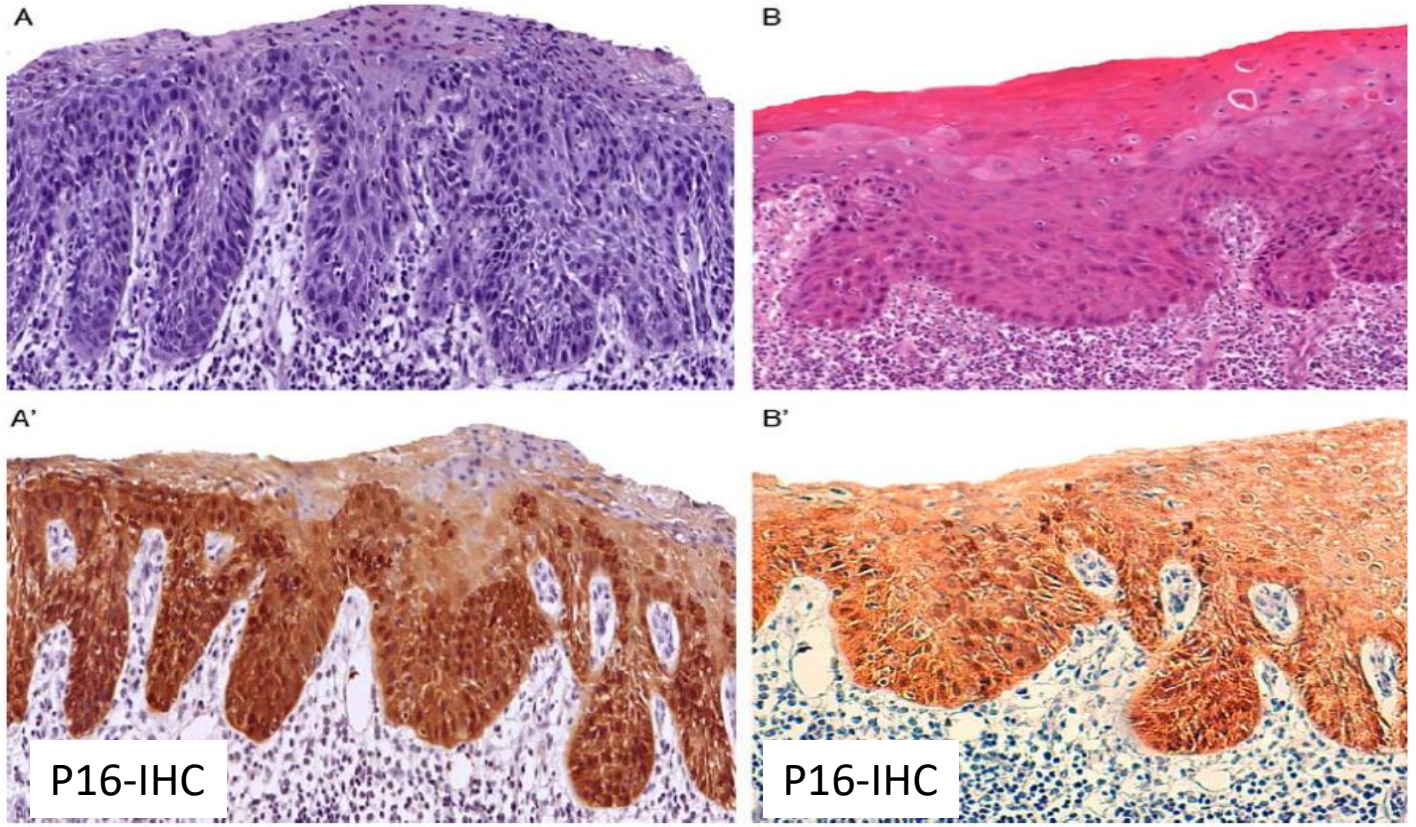
P53 IHC
NGS: wildtype



Case 0823

dVIN mimicking uVIN

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



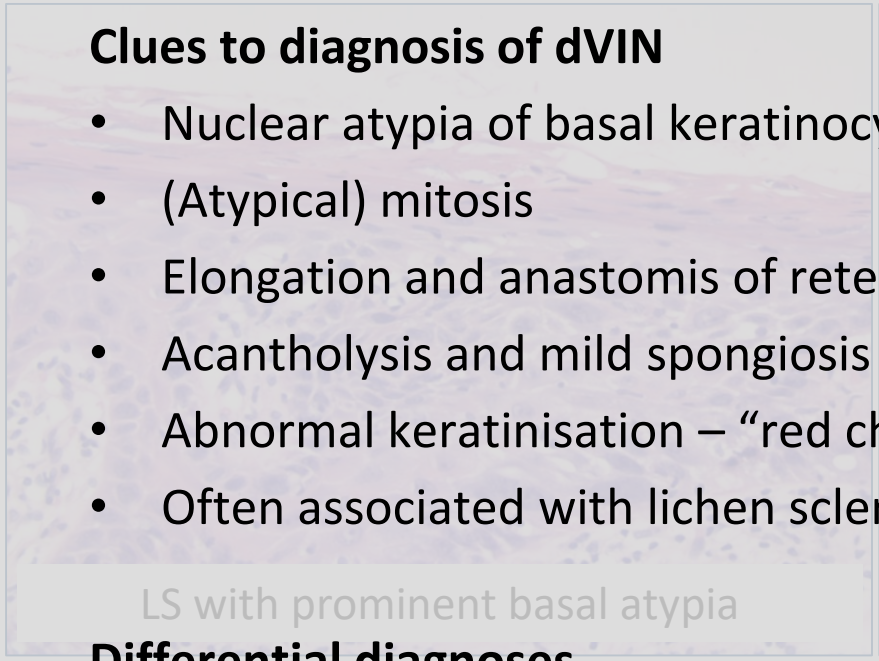
- 326 hrHPV pos lesions
- Diagnosis:
  - 320 HSIL/uVIN
  - 4 dVIN-like
  - 2 LS-like

Rakislova al. Am J Surg Path 2018

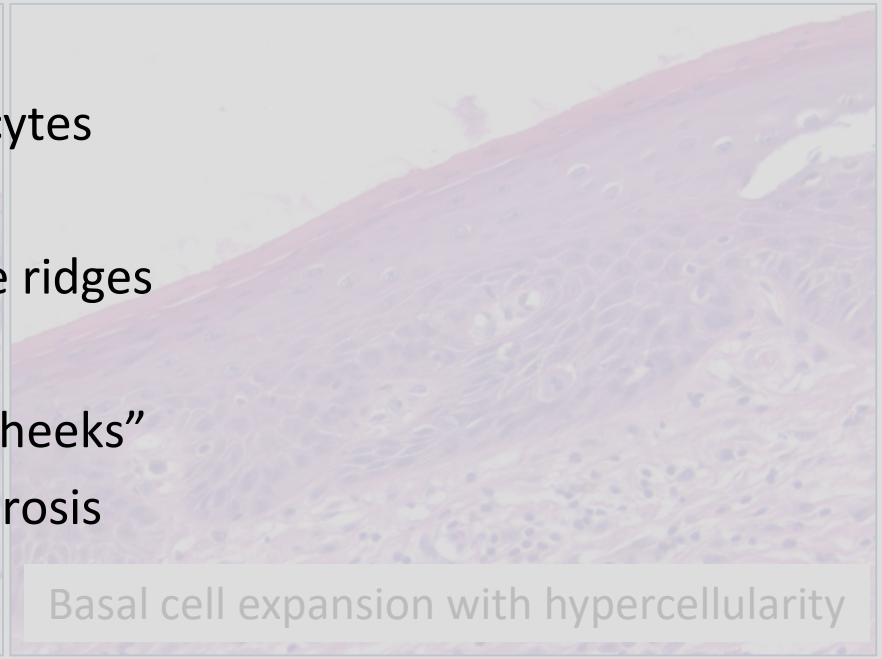
dVIN and its wide morphologic spectrum

Clues to diagnosis of dVIN

- Nuclear atypia of basal keratinocytes
- (Atypical) mitosis
- Elongation and anastomis of rete ridges
- Acantholysis and mild spongiosis
- Abnormal keratinisation – “red cheeks”
- Often associated with lichen sclerosis



LS with prominent basal atypia



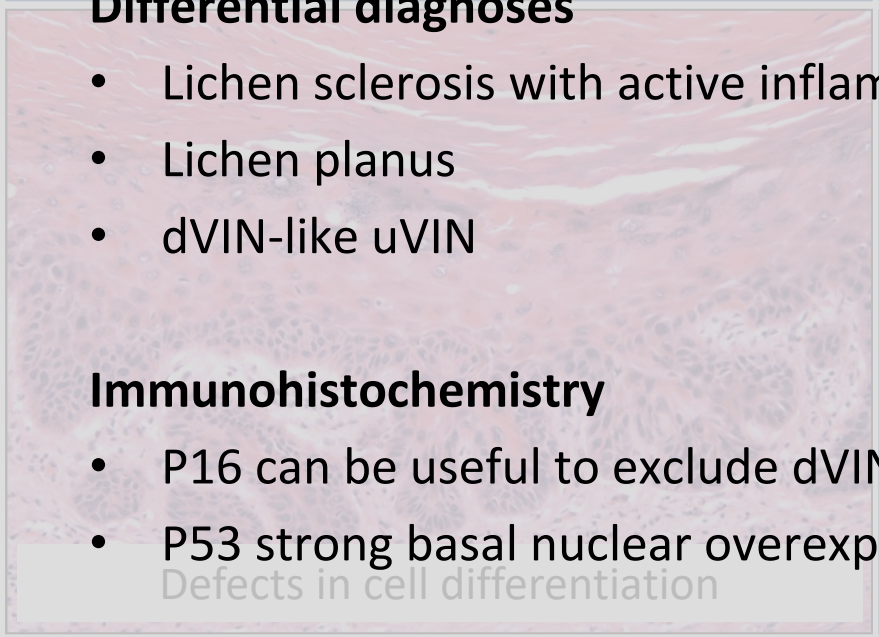
Basal cell expansion with hypercellularity

Differential diagnoses

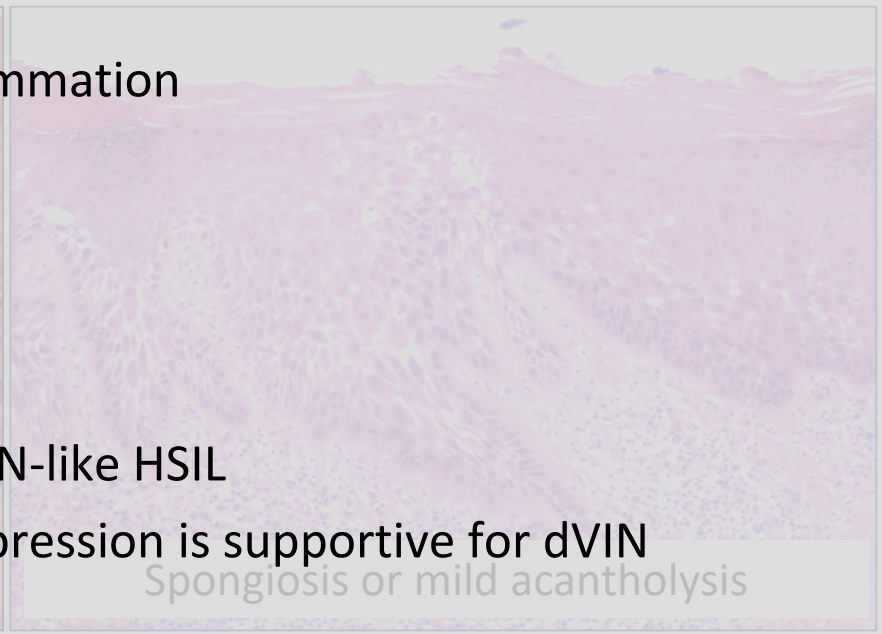
- Lichen sclerosis with active inflammation
- Lichen planus
- dVIN-like uVIN

Immunohistochemistry

- P16 can be useful to exclude dVIN-like HSIL
- P53 strong basal nuclear overexpression is supportive for dVIN

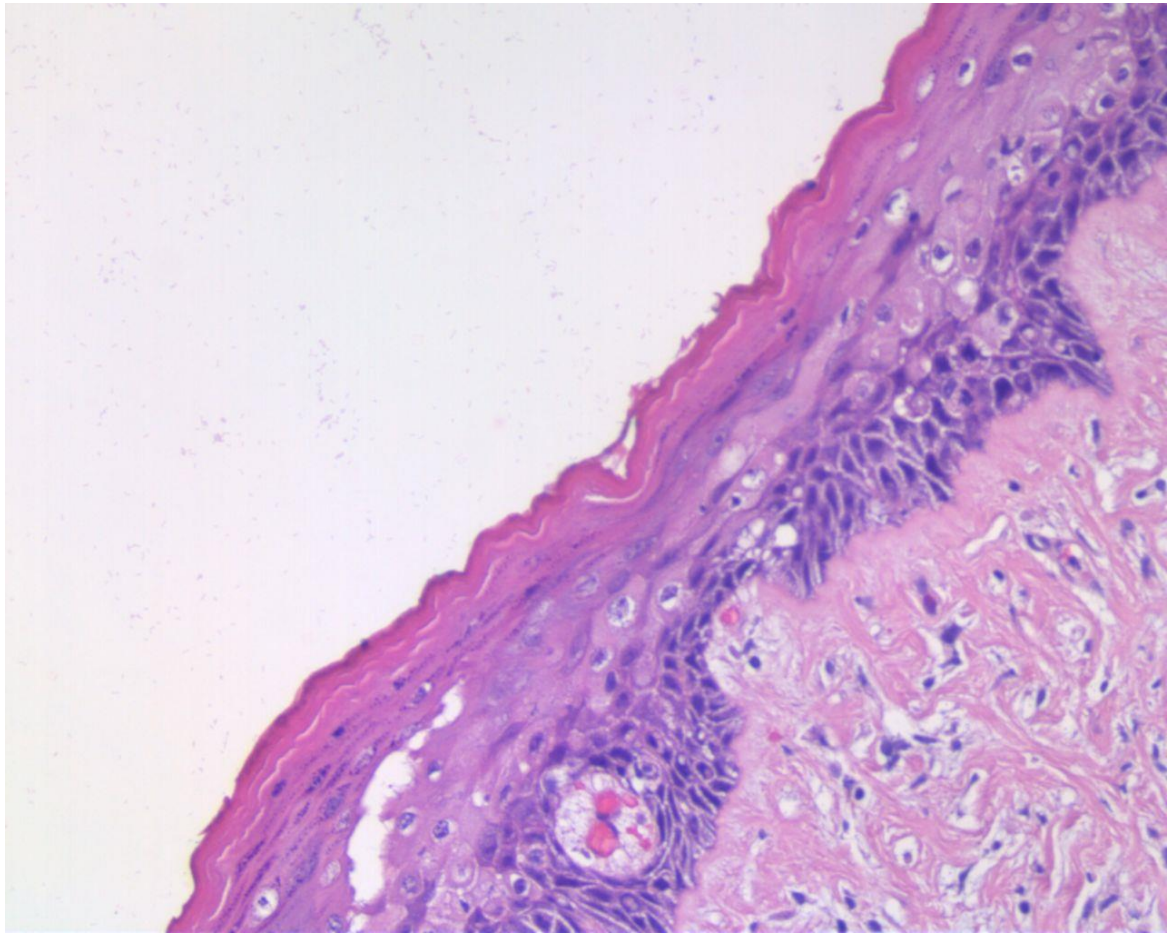


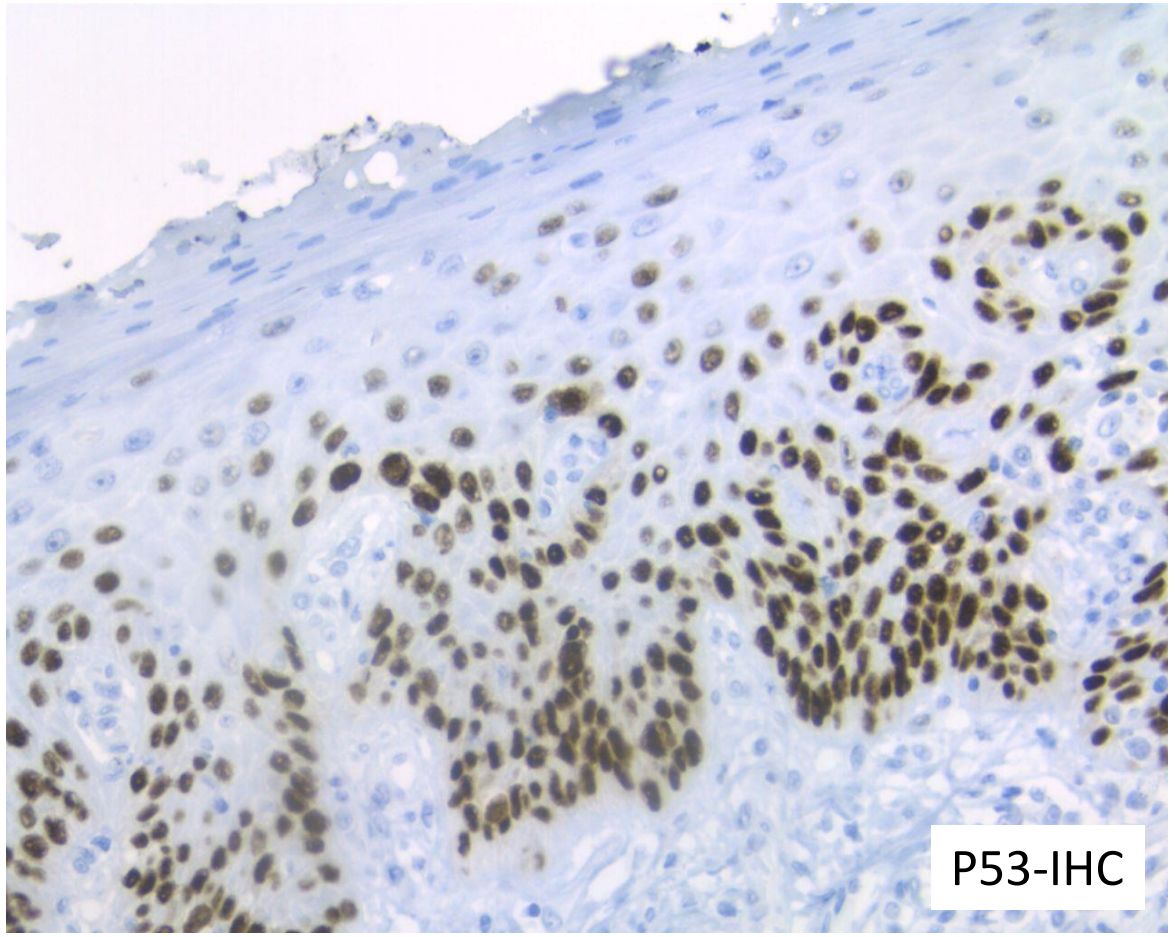
Defects in cell differentiation



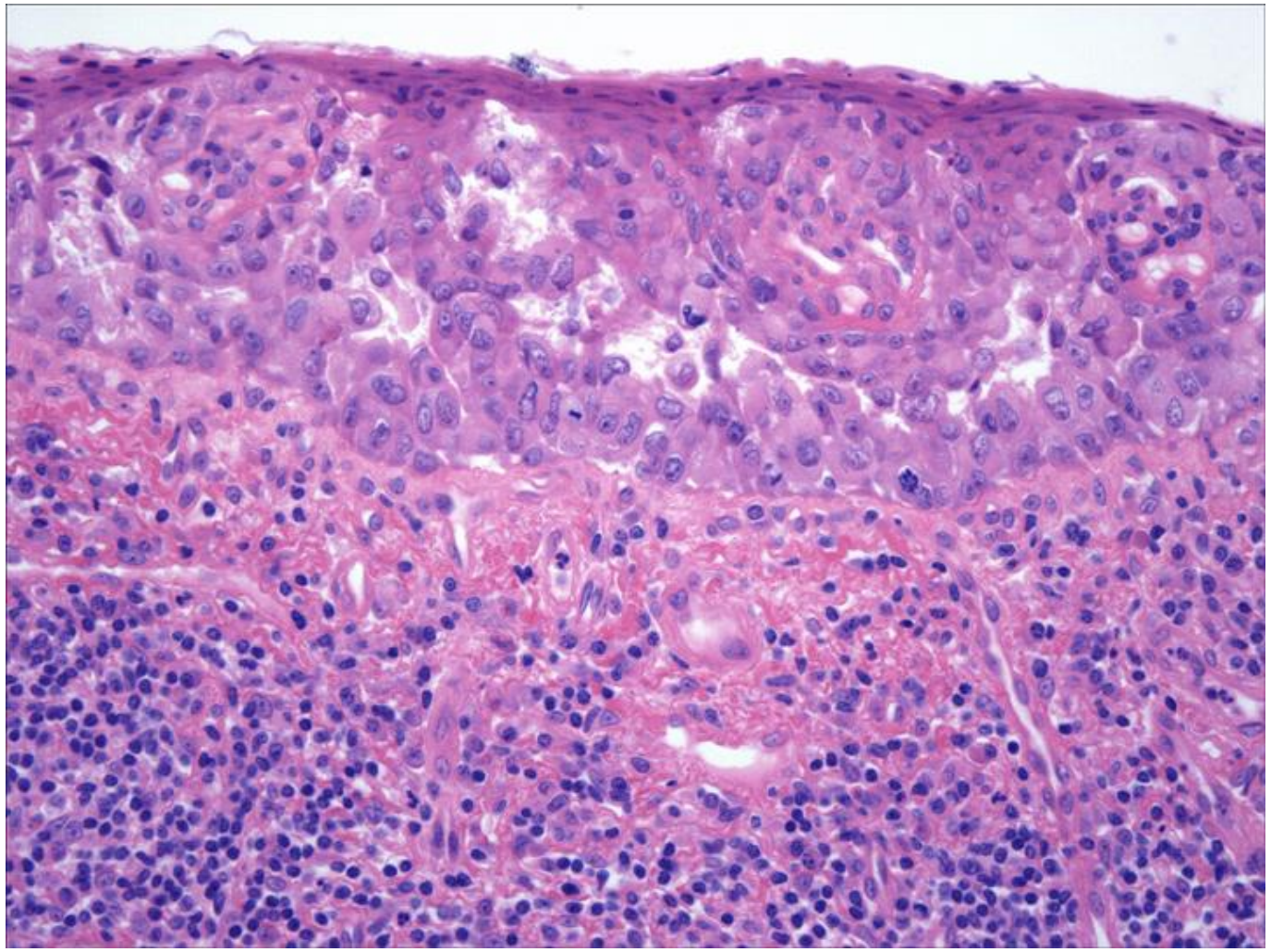
Spongiosis or mild acantholysis

Example of dVIN with abnormal p53-IHC





Stay alert



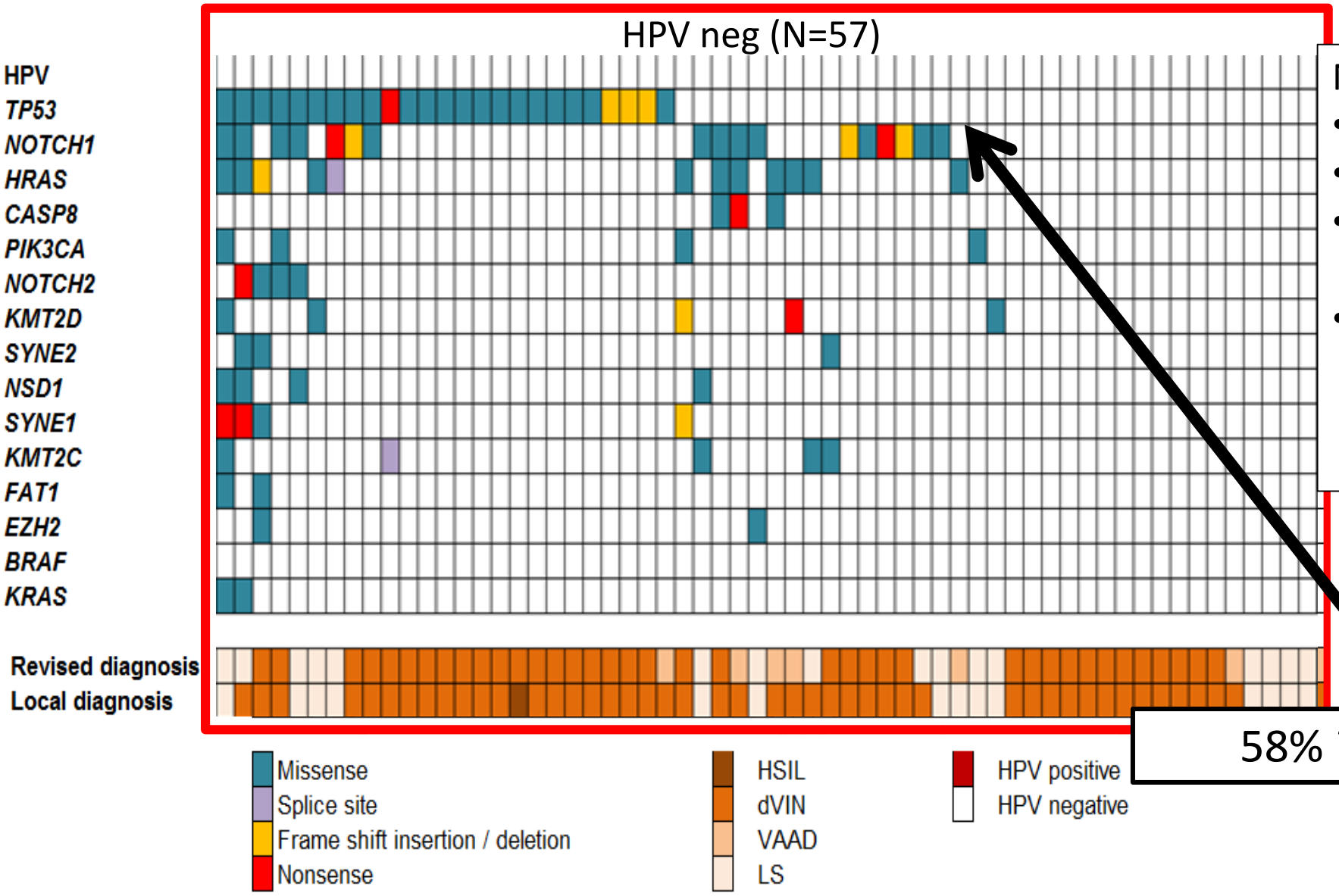
Clues to diagnosis of dVIN

- Nuclear atypia of basal keratinocytes
- (Atypical) mitosis
- Elongation and anastomis of rete ridges √
- Acantholysis and mild spongiosis
- Abnormal keratinisation – “red cheeks”
- Often associated with lichen sclerosis

Diagnosis?

- **Extramammary Paget Disease**

Somatic mutations of HPV negative precursors



N=57

- 42 % *TP53*
- 30 % *NOTCH/FAT1*
- 21 % *HRAS/PIK3CA*

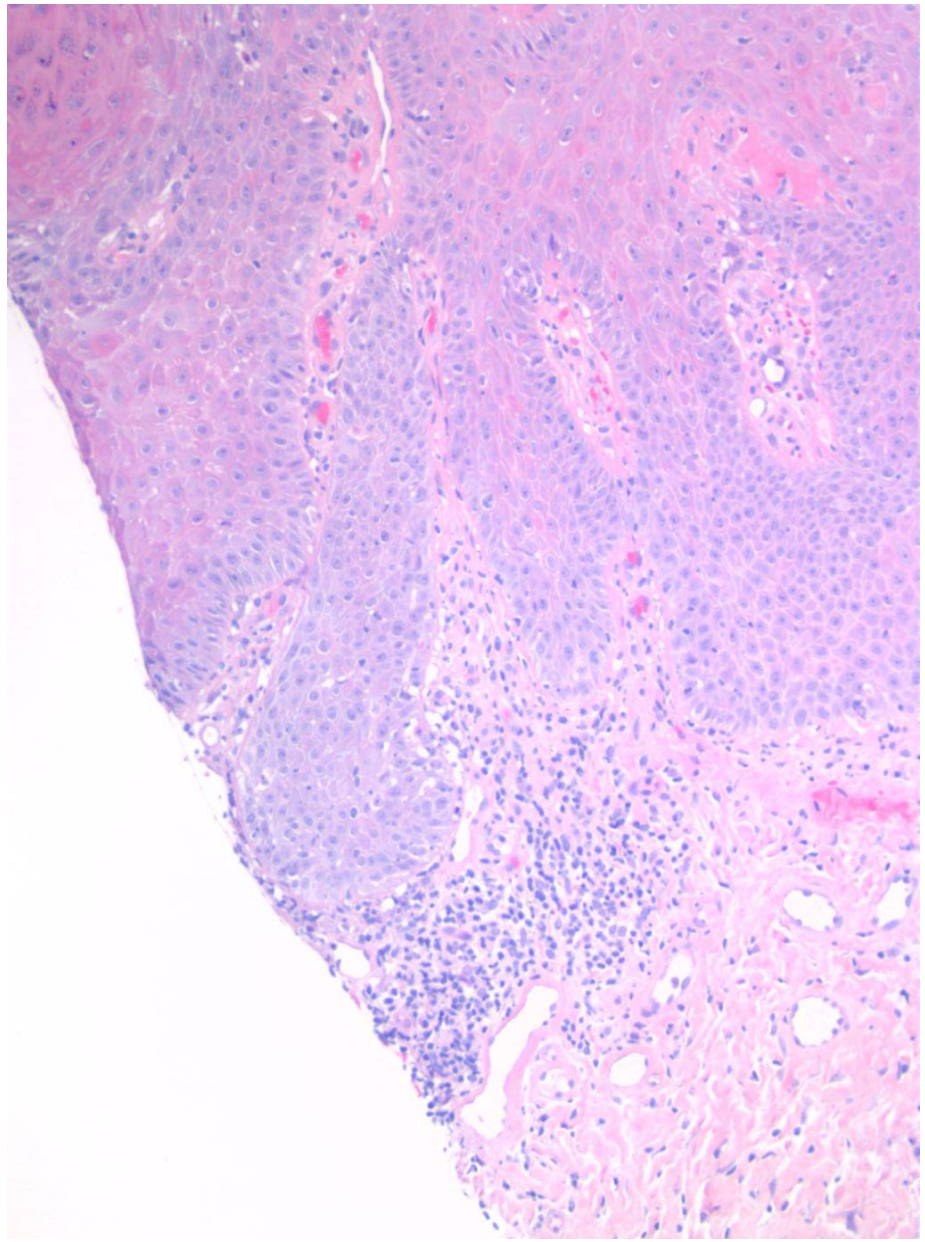
- Histology (H&E)
  - 0% HSIL
  - 61% dVIN
  - 39% LS or VAAD

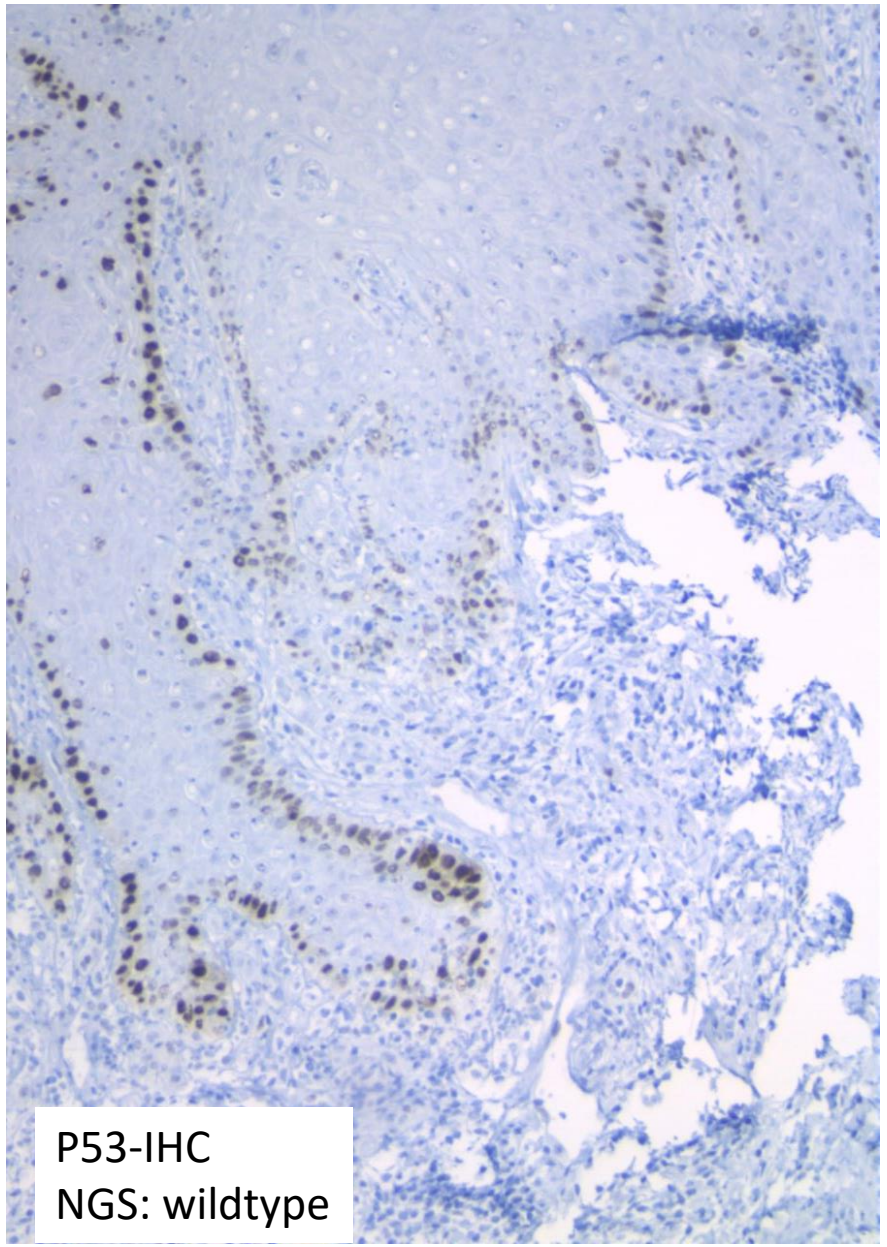
58% *TP53* wildtype!

* precursor lesion adjacent to vulvar cancer

Nooij et al. CCR 2017

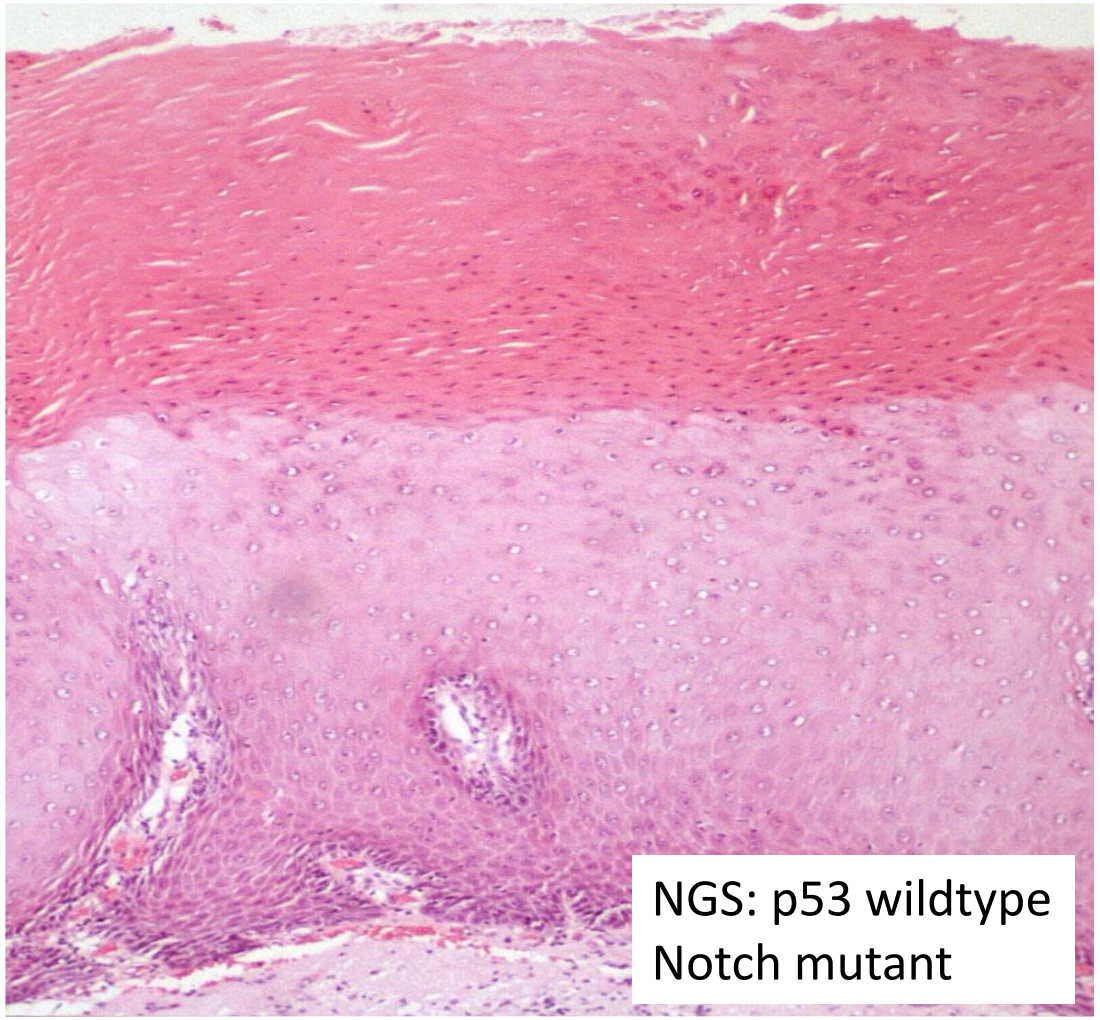
Example of HPV- /*TP53 wildtype* dVIN





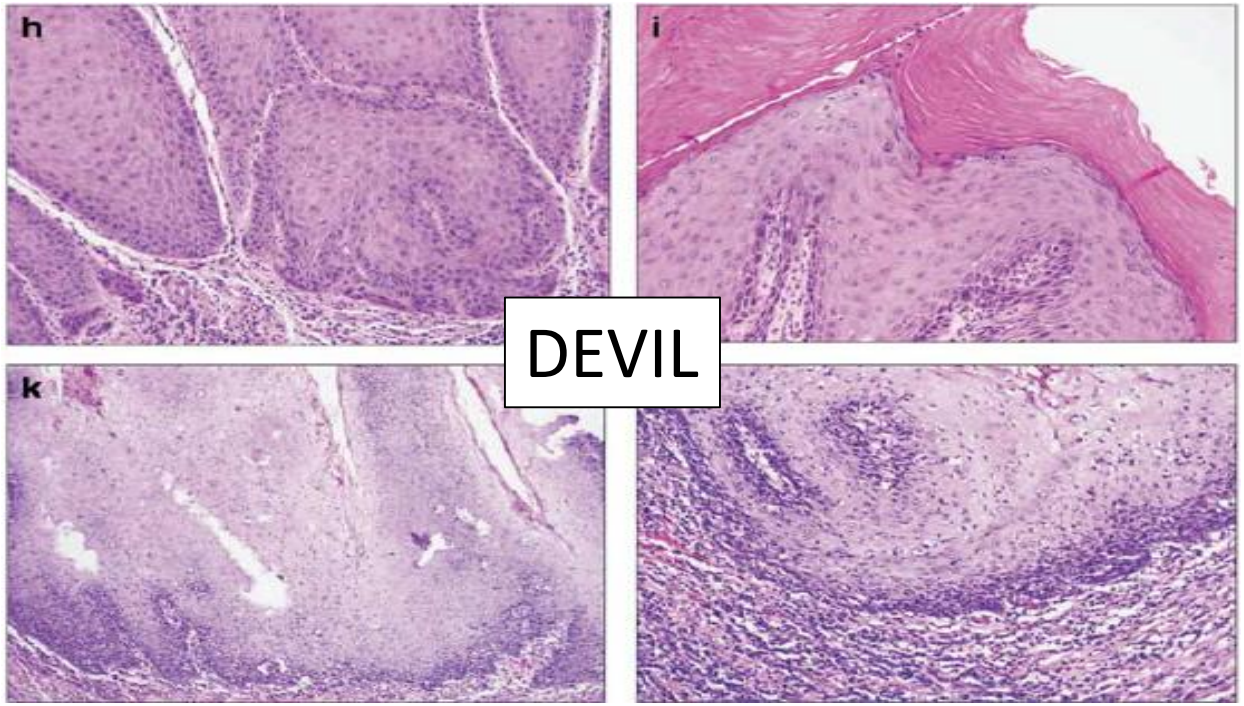
Example of HPV-/*TP53* wildtype VAAD

VAAD



NGS: p53 wildtype
Notch mutant

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

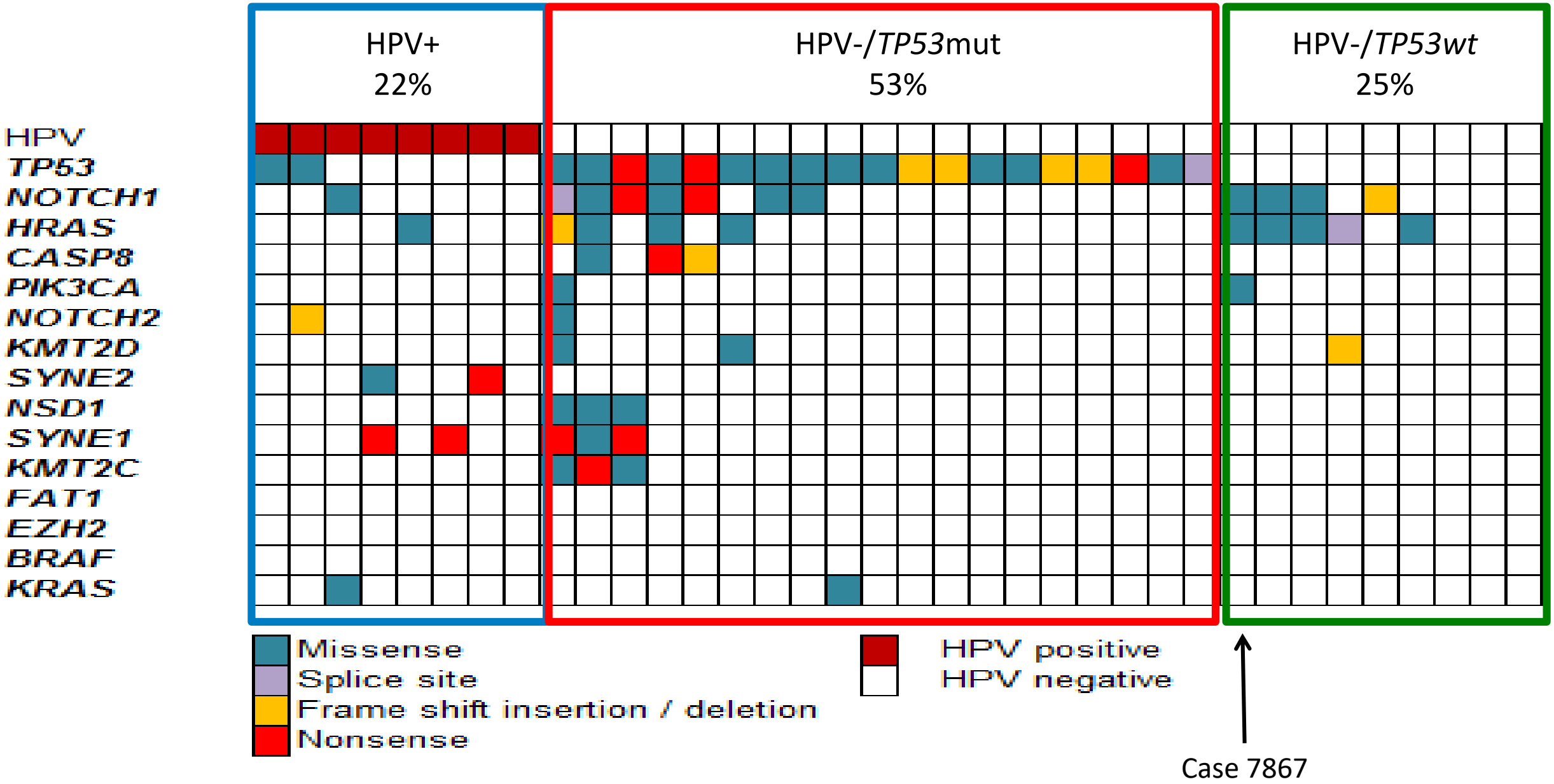


DEVIL

Watkins et al. Mod Path 2017

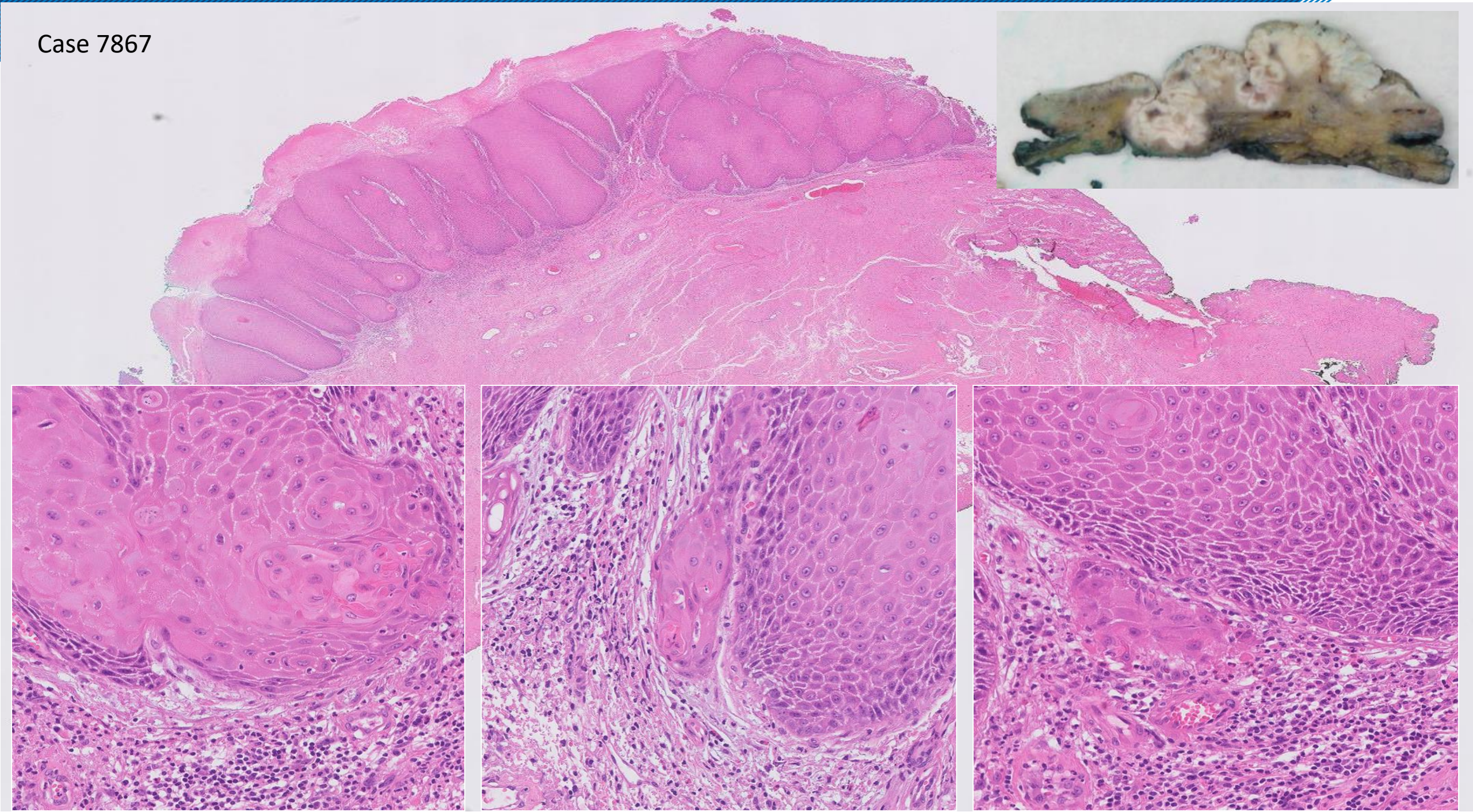
Suggestive of a HPV negative and *TP53* independent pathway – what about cancer?

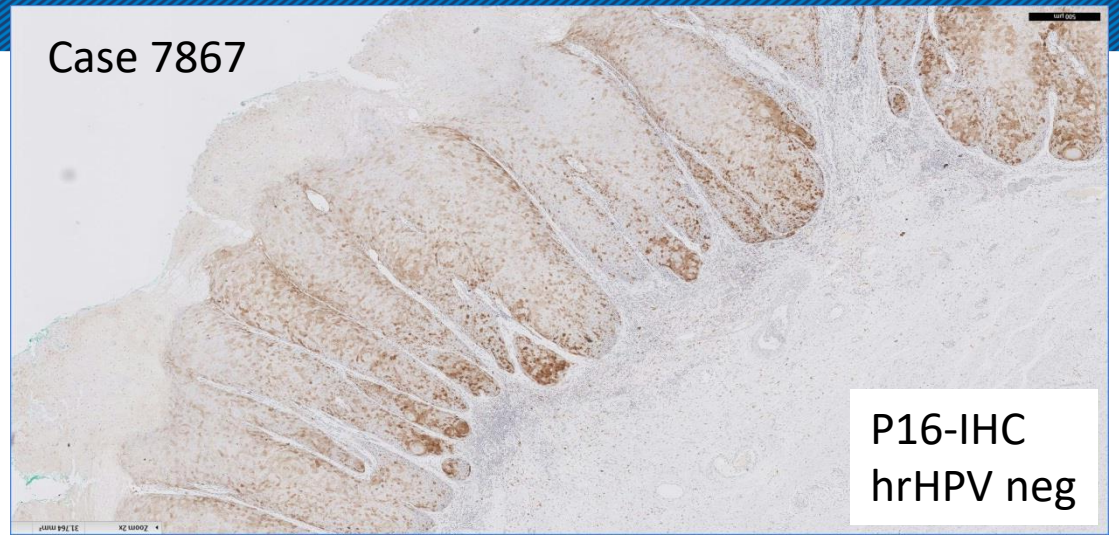
Somatic mutational landscape of VSCC (N=36)

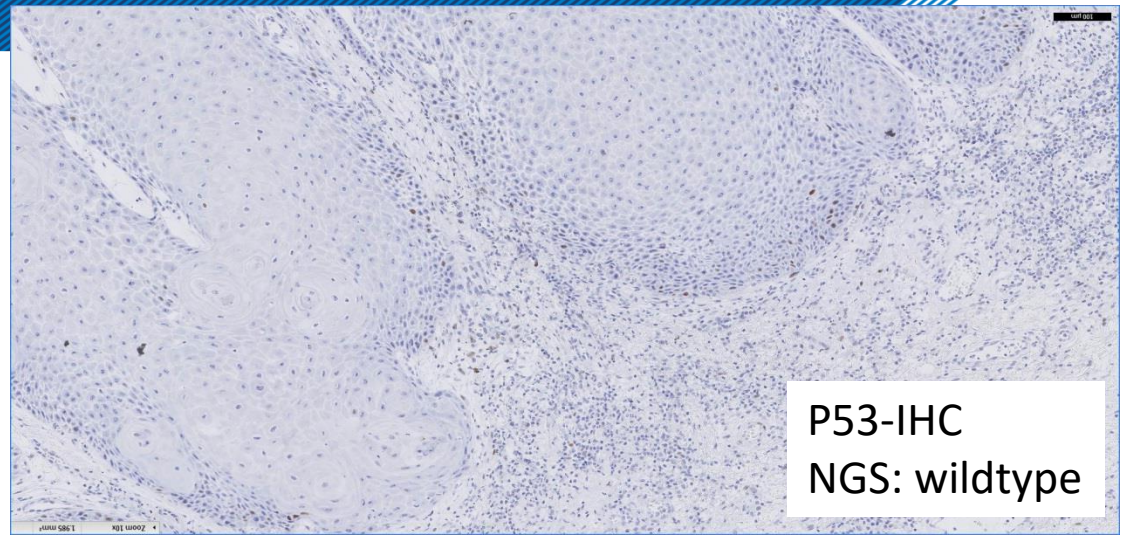


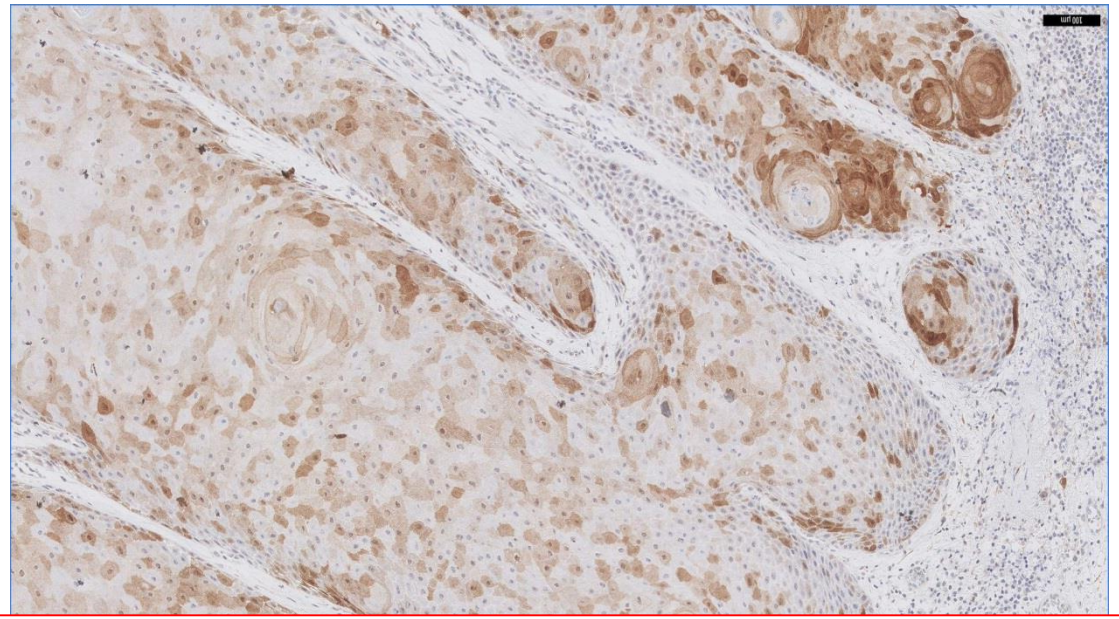
Nooij et al. CCR 2017

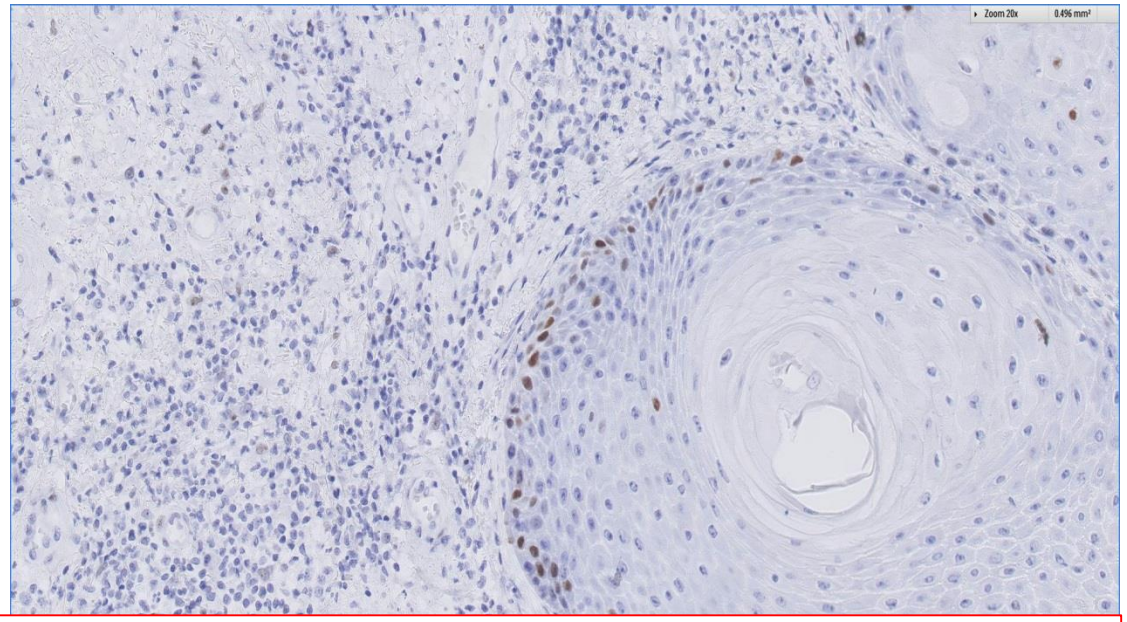
Case 7867





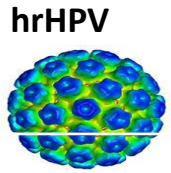






VSCC arising in a background of a differentiated exophytic intraepithelial lesion (DEVIL)
(HPV negative, p53 wildtype)

Simplified model of 3 types of VSCC

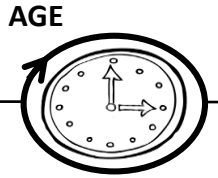


Normal vulvar epithelium

HSIL

PIK3CA

VSCC - Basaloid
VSCC - Keratinizing



NOTCH1/FAT1/HRAS/PIK3CA

VAAD

DEVIL

VSCC - Verrucous

Lichen Sclerosus

TP53 / CDKN2A

dVIN

VSCC - Keratinizing
VSCC - Spindled

Current clinical management

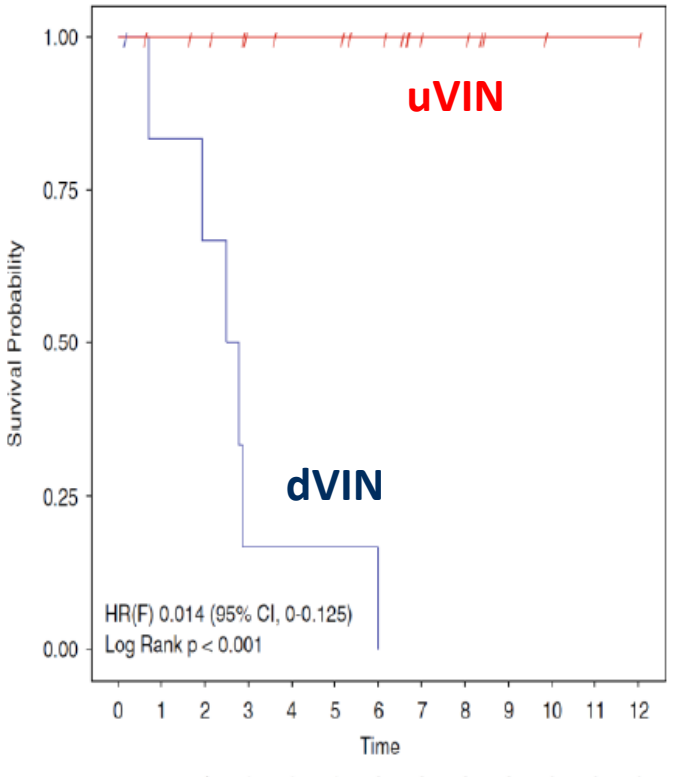
Preinvasive lesions dVIN or HSIL

- Excise with 3 mm margin

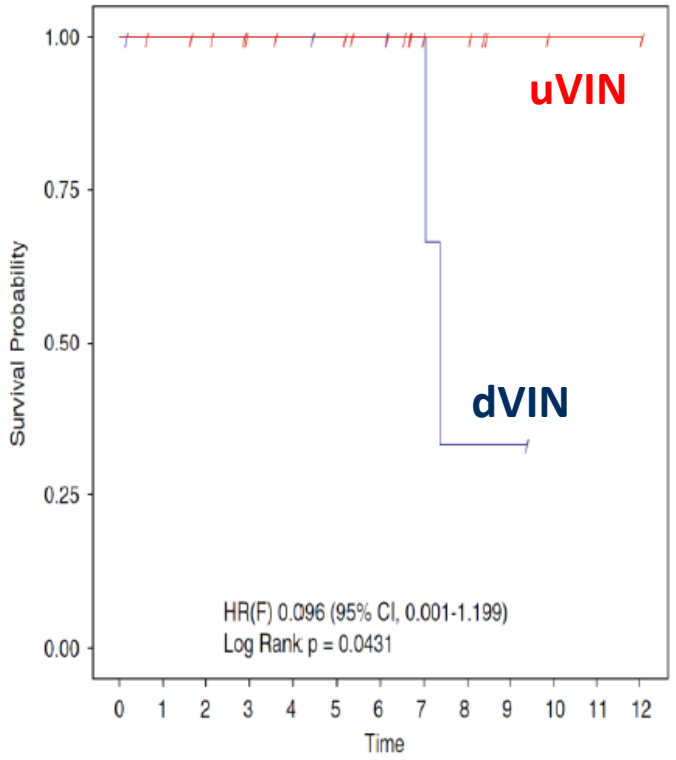
Currently **NO** differences in treatment algorithms based on HPV or p53 Status

Dramatic differences in outcomes uVIN VS dVIN

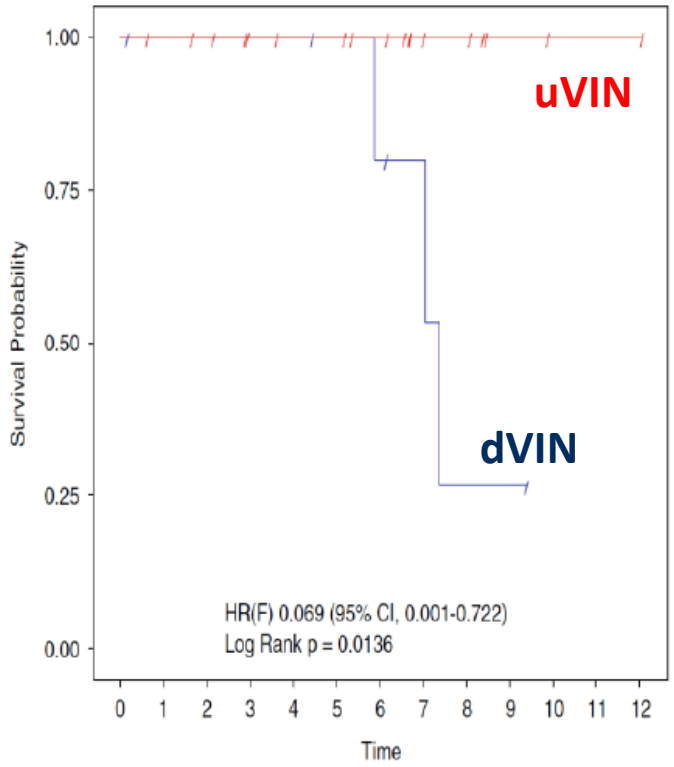
Progression-free Survival



Disease-specific Survival



Overall Survival



- None of the HSIL/uVIN progressed to cancer in median 5 years follow up.
- Recurrence HPV independent SCC 92% (23/25) with median 1.1 years, 22/25 DOD

What about treatment in cancers?

Mc Alpine, Gilks- Int J Gyne Ca, 2017

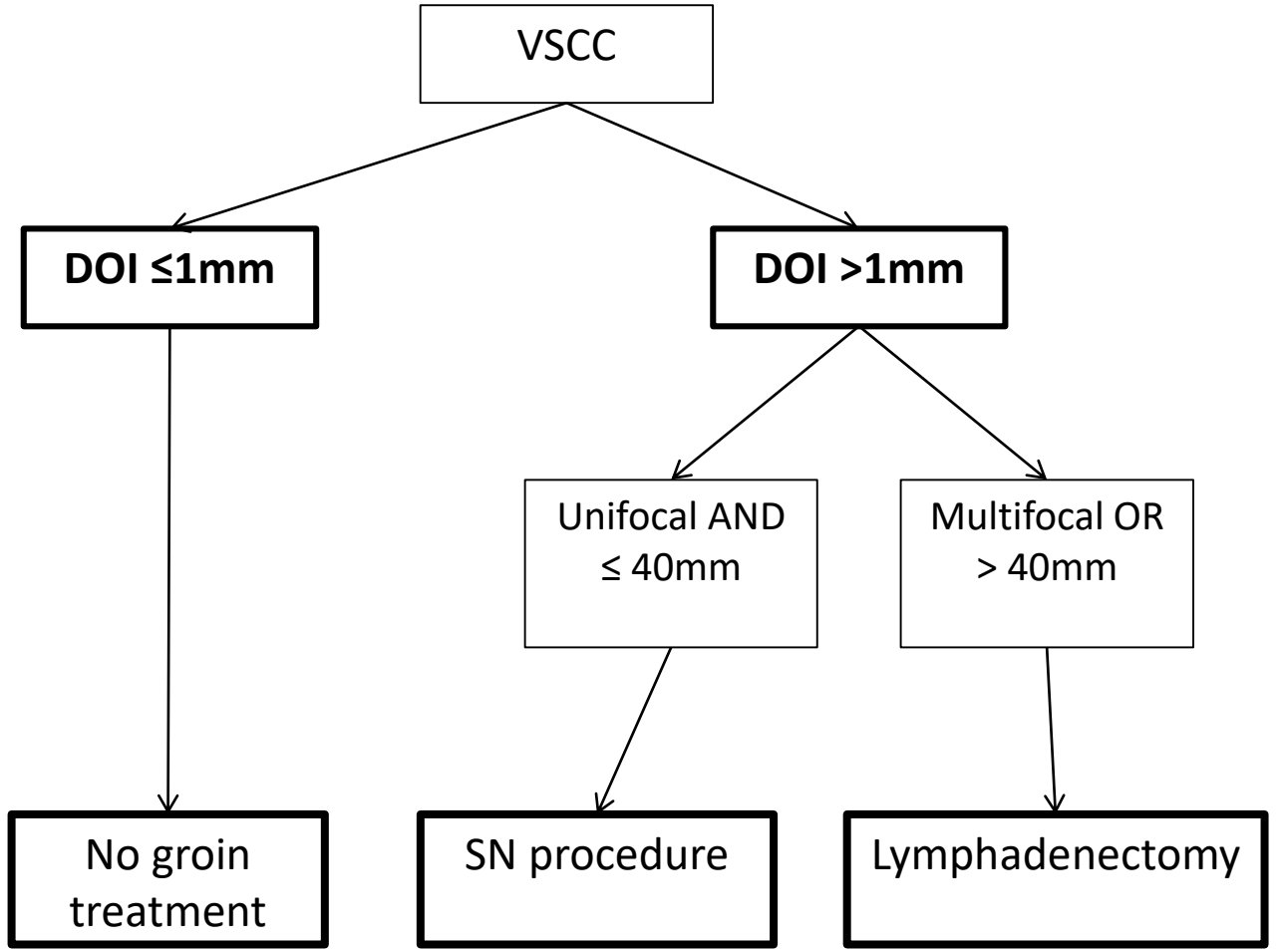
Current clinical management

Preinvasive lesions (dVIN/HSIL):

- Excise with 3 mm margin

Invasive lesion (VSCC):

- “Wide local excision” with 8 mm margin
- Groin treatment dependent on Stage/DOI/Size



DOI and Margin status most critical parameters in (adjuvant) treatment decisions

Do we agree on invasion and 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†§*

TABLE 4. Agreement Among Pathologists for Presence of Invasion, Depth of Invasion, and Tumor Thickness in Vulvar Carcinoma

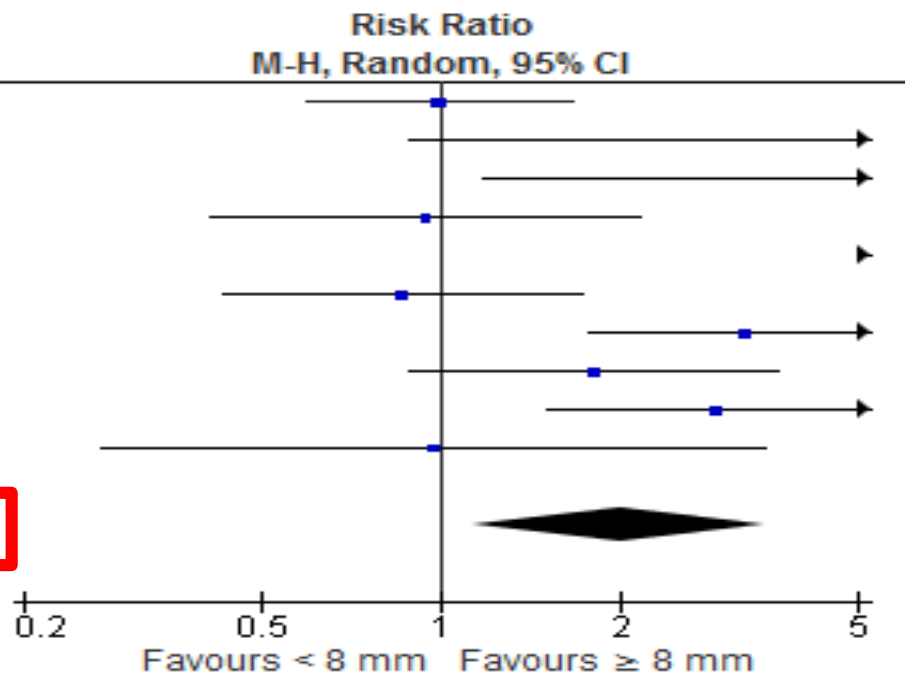
Histologic Parameter	Decision Points	Mean κ (Minimum, Maximum)
Invasion	Present vs. absent	0.24 (0.06, 0.31)
Depth of invasion	≤1 mm vs. >1 mm vs. not measurable	0.50 (0.12, 0.92)
	Noninvasive or invasion ≤1 mm vs. invasion >1 mm*	0.51 (0.22, 0.83)
	All methods of measurement ≤1 mm vs. >1 mm†	0.62 (0.24, 1.00)
	FIGO method ≤1 mm vs. >1 mm†	0.69 (0.23, 1.00)
Thickness	Noninvasive or invasion ≤1 mm vs. invasion >1 mm*	0.49 (0.15, 0.73)
	≤1 mm vs. >1 mm†	0.67 (0.31, 1.00)

The level of reproducibility for assessment of DOI is surprisingly low.
What about margins?

Do margins really matter?

Study or Subgroup	< 8 mm Events	< 8 mm Total	≥ 8 mm Events	≥ 8 mm Total	Weight	Risk Ratio M-H, Random, 95% CI
Baiocchi 2015	18	79	29	126	14.6%	0.99 [0.59, 1.66]
Chan 2007	12	53	0	30	3.3%	14.35 [0.88, 234.13]
De Hullu 2002	9	38	0	39	3.3%	19.49 [1.17, 323.49]
Groenen 2010	11	50	7	30	12.3%	0.94 [0.41, 2.17]
Heaps 1990	17	37	0	91	3.3%	84.74 [5.23, 1373.55]
Iacoponi 2013 (1)	7	22	24	65	13.4%	0.86 [0.43, 1.72]
Rouzier 2002 (2)	15	44	18	171	14.0%	3.24 [1.78, 5.90]
Tantipalakorn 2009	8	24	17	92	13.2%	1.80 [0.89, 3.67]
Viswanathan 2013 (3)	44	116	9	69	13.6%	2.91 [1.51, 5.58]
Woelber 2011	7	72	3	30	9.0%	0.97 [0.27, 3.51]
Total (95% CI)		**535**		**743**	**100.0%**	**1.99 [1.13, 3.51]**
Total events	148		107			

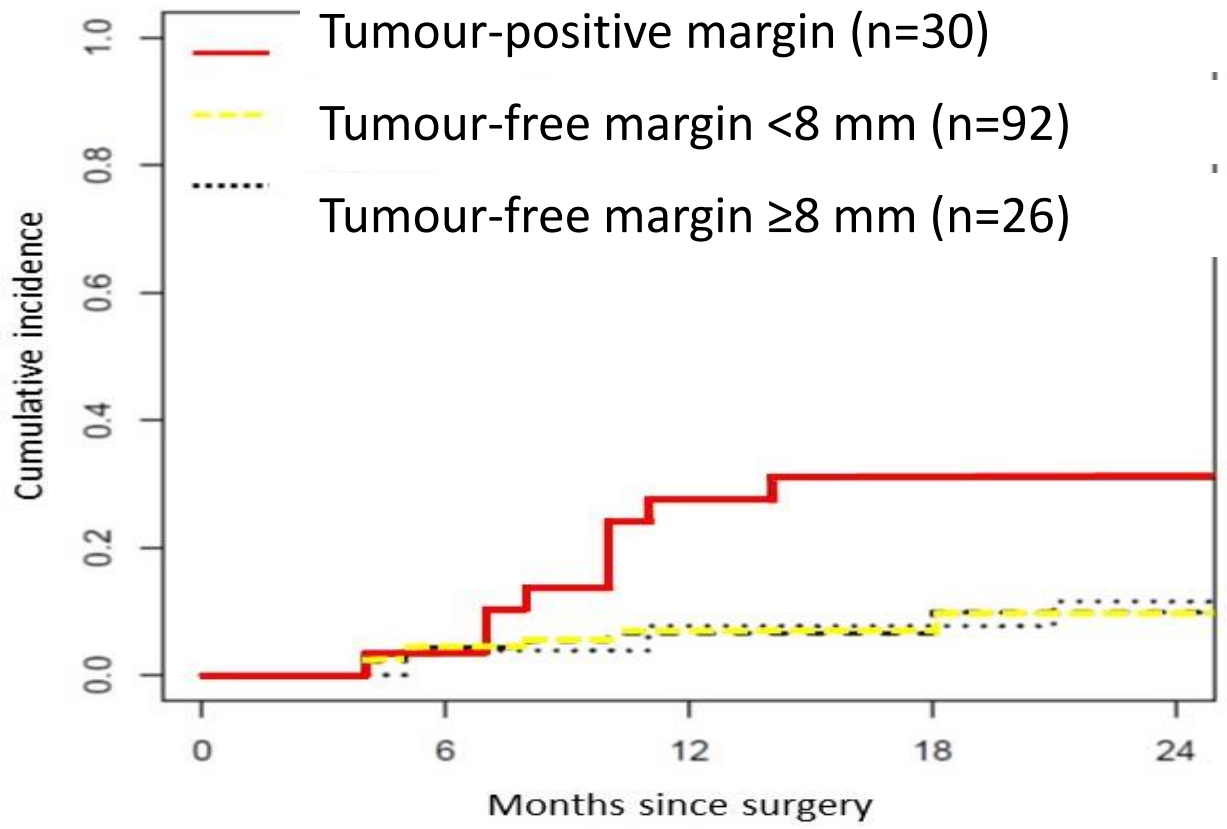
Heterogeneity: Tau² = 0.50; Chi² = 33.86, df = 9 (P < 0.0001); I² = 73%
Test for overall effect: Z = 2.39 (P = 0.02)



Limitations

- No stratification on HPV status
- Studies included positive margins in group of < 8mm
- Late recurrence > 2years included
- Missing data on adjuvant therapy

Do margins really matter, LUMC Cohort study (N=148)



< 8 vs ≥ 8 mm
p=0.808

A tumour-free margin of < 8 mm may NOT be associated with an increased local recurrence risk

Nooij et al., EJC 2016

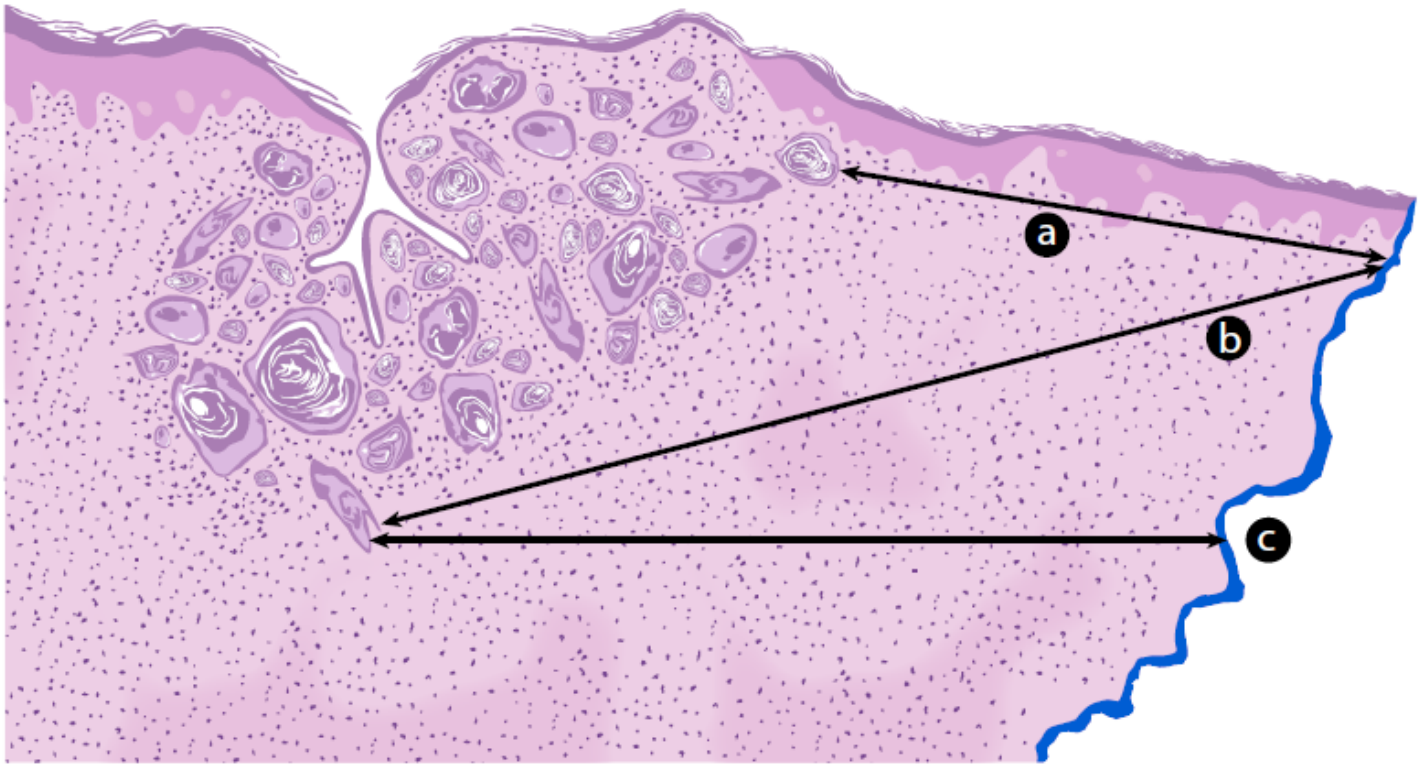
How to measure margins?

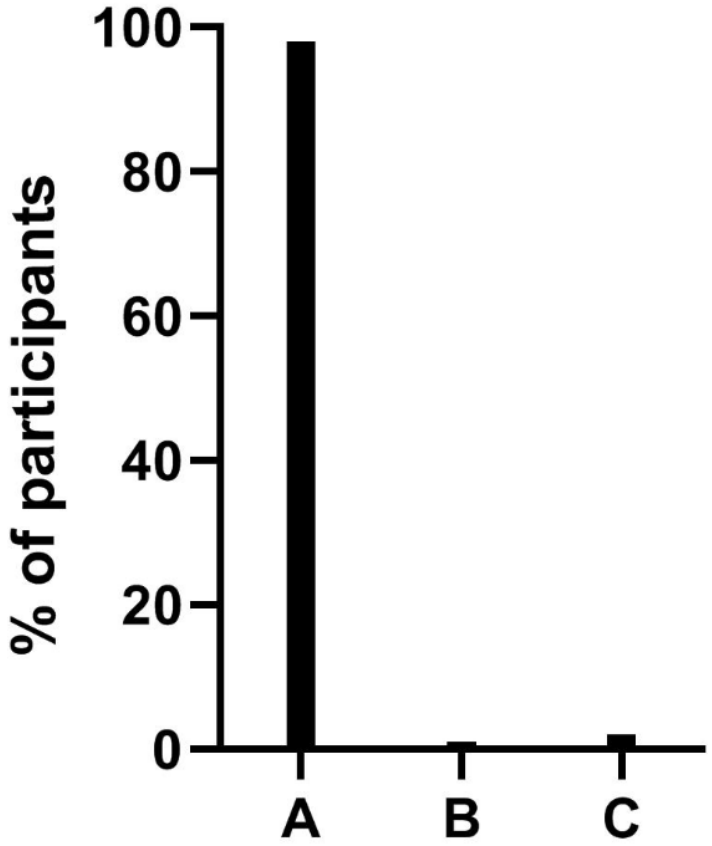
Practical Guidance for Measuring and Reporting Surgical Margins in Vulvar Cancer

Running title: Surgical Margins in Vulvar Cancer

Kim E. Kortekaas[1], Koen van de Vijver[2,], Mariëtte I.E. van Poelgeest[1], Blake Gilks[3], Vincent Smit[4], S. Arif[5], D. Arora[6], A. Faruqi[7], R. Ganesan[8], N.R Griffin[9], R. Hale[10], Y.L. Hock[11], L-C Horn[12], W. Glenn McCluggage[13], P. Mukonoweshuro[14], K.J. Park[15], B. Rous[16], B.Tanchel[8], A.S. Van Rompuy[17], G. van Schalkwyk[18], J. Vella[8], M. Vergine[19], Naveena Singh[7], Tjalling Bosse[4]

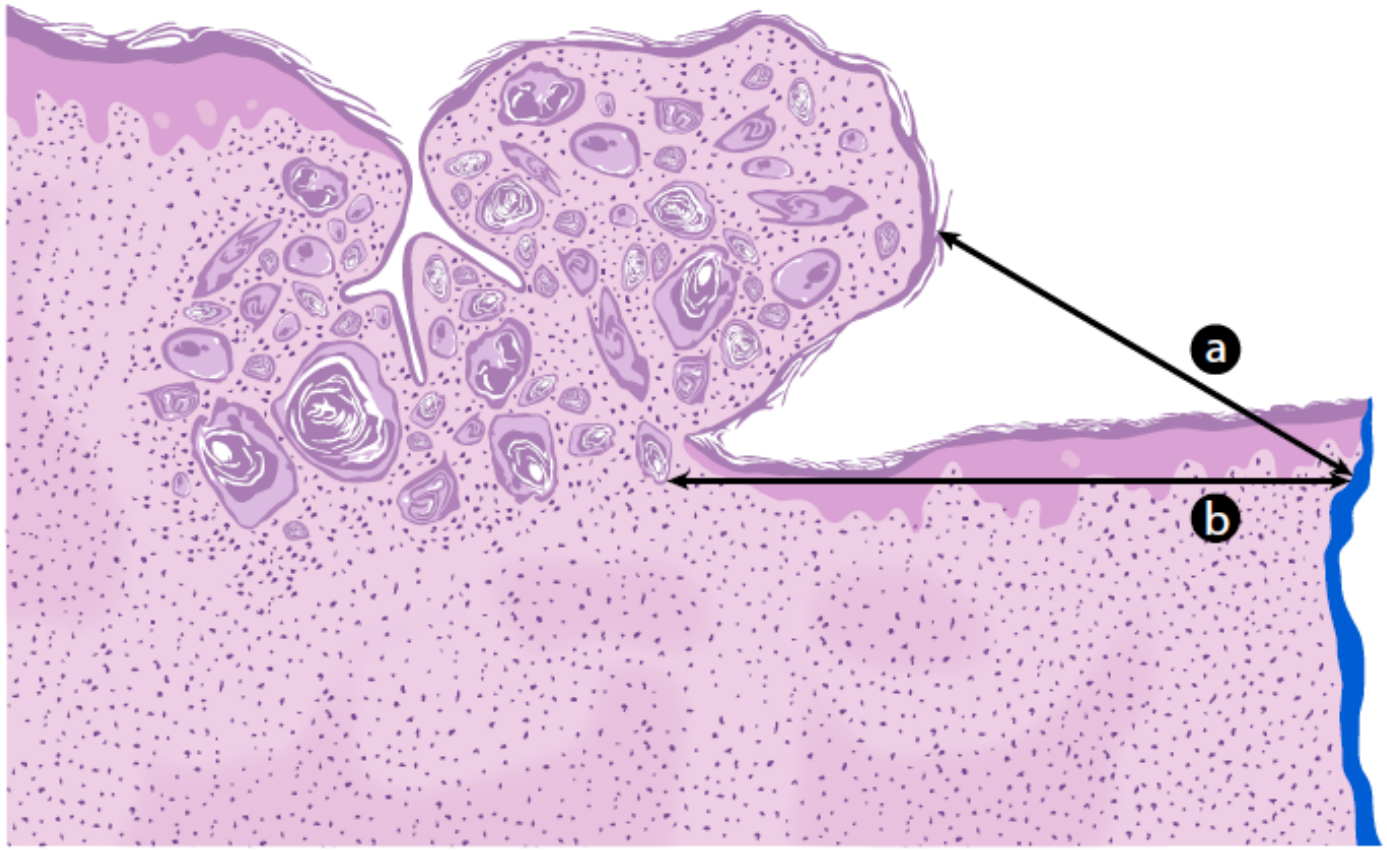
How to measure the Minimal Peripheral Surgical Margin?

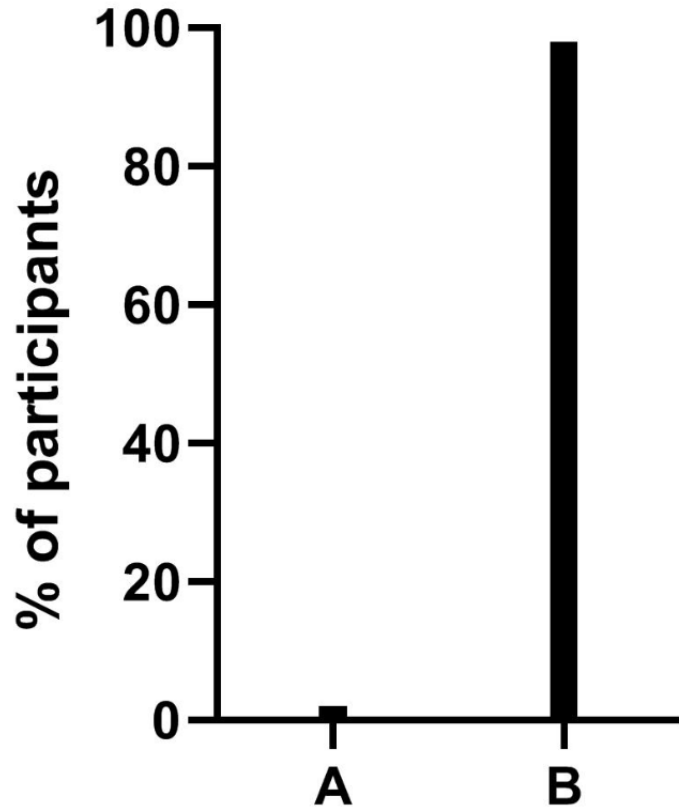




The MPSM is defined as: "*the minimum distance from invasive carcinoma to the inked peripheral surgical margin reported in millimetres*".

How to measure the Minimal Peripheral Surgical Margin?

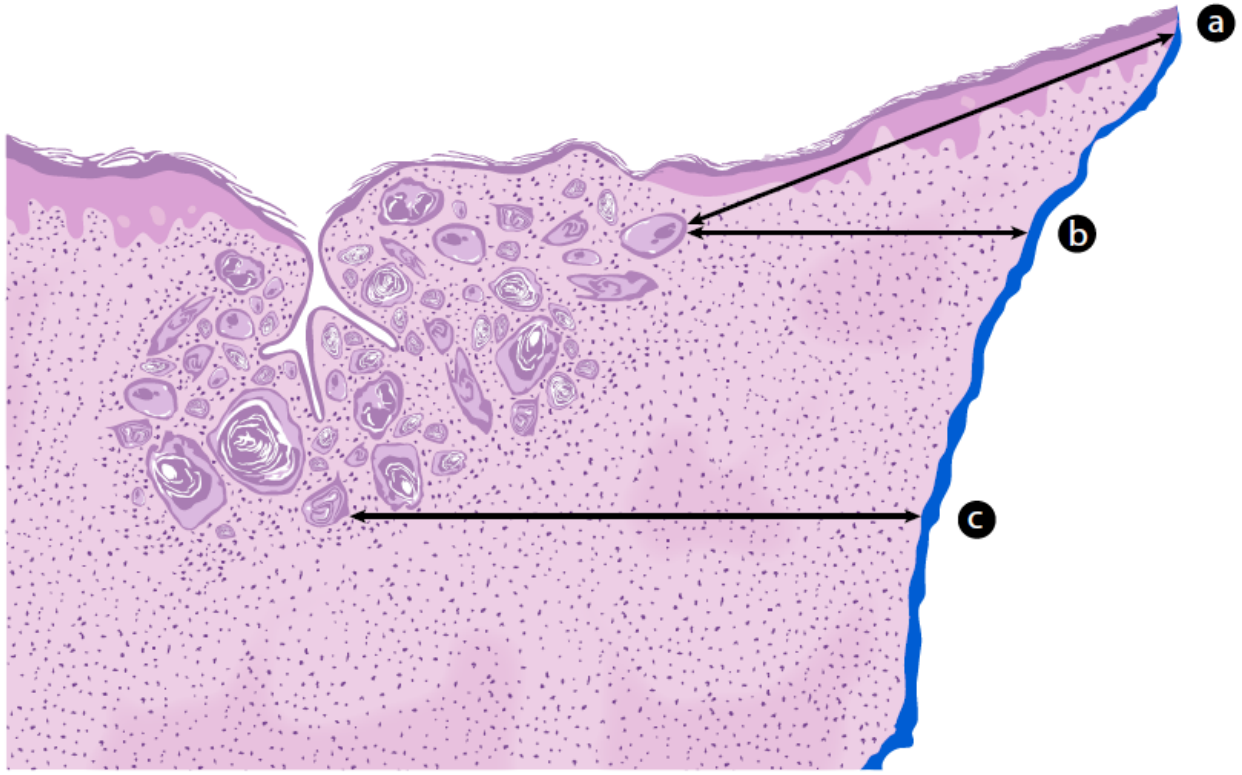


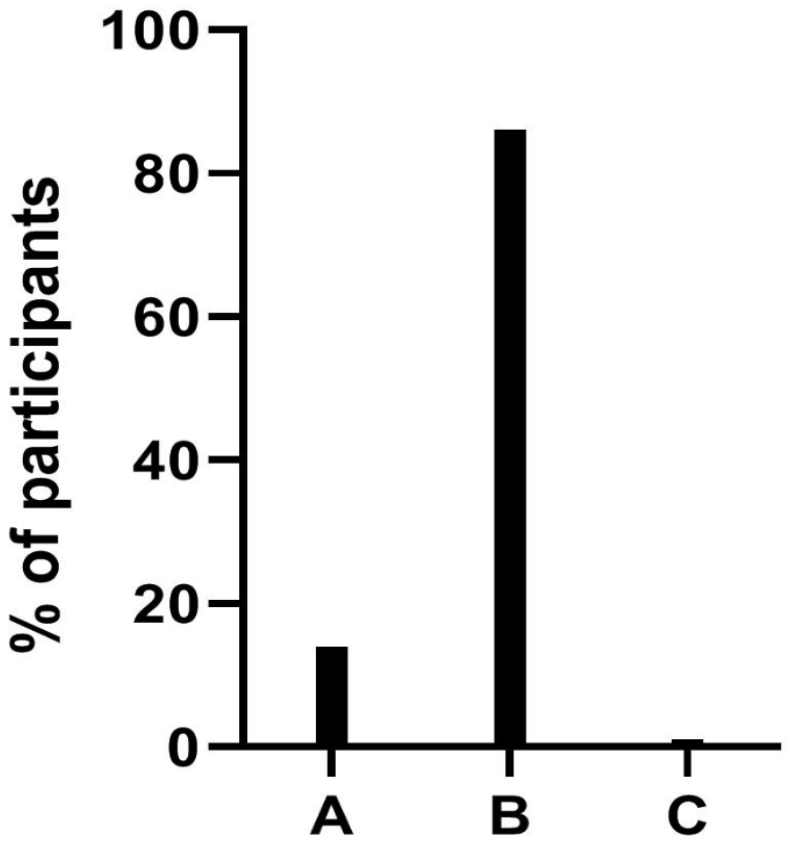


The MPSM is defined as: "*the minimum distance from invasive carcinoma to the inked peripheral surgical margin reported in millimetres*".

-> measurement through tissue

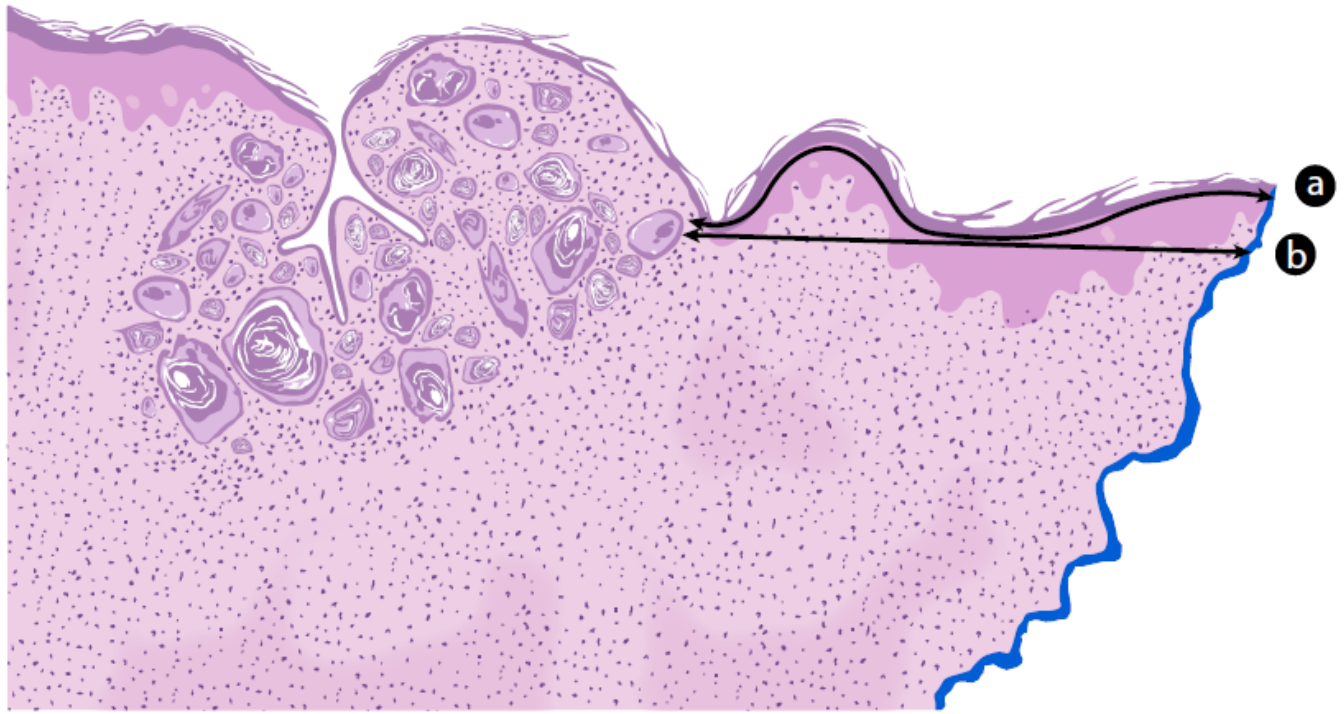
How to measure the Minimal Peripheral Surgical Margin?

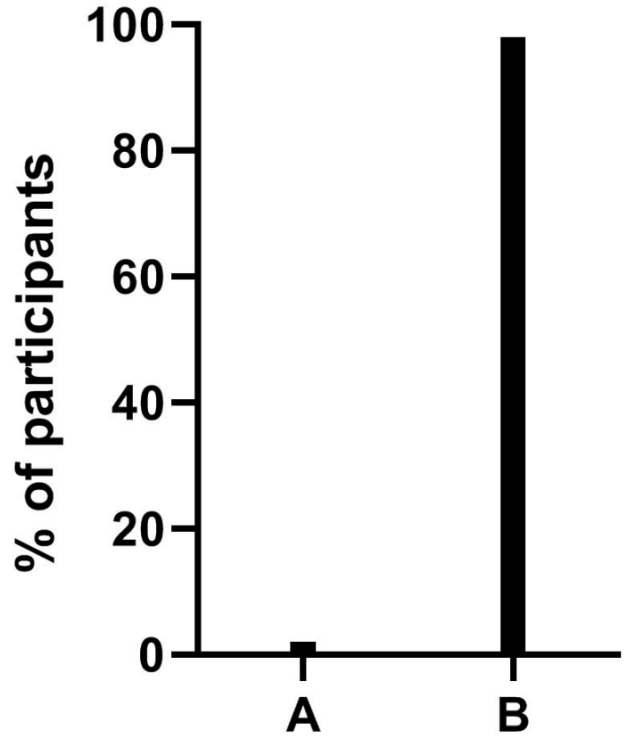




The peripheral margin is roughly perpendicular to the skin surface; this includes the epithelial surface and deeper soft tissue; the MPSM should therefore be measured towards the peripheral stromal edge or surface-epithelial edge, whichever is the shortest.

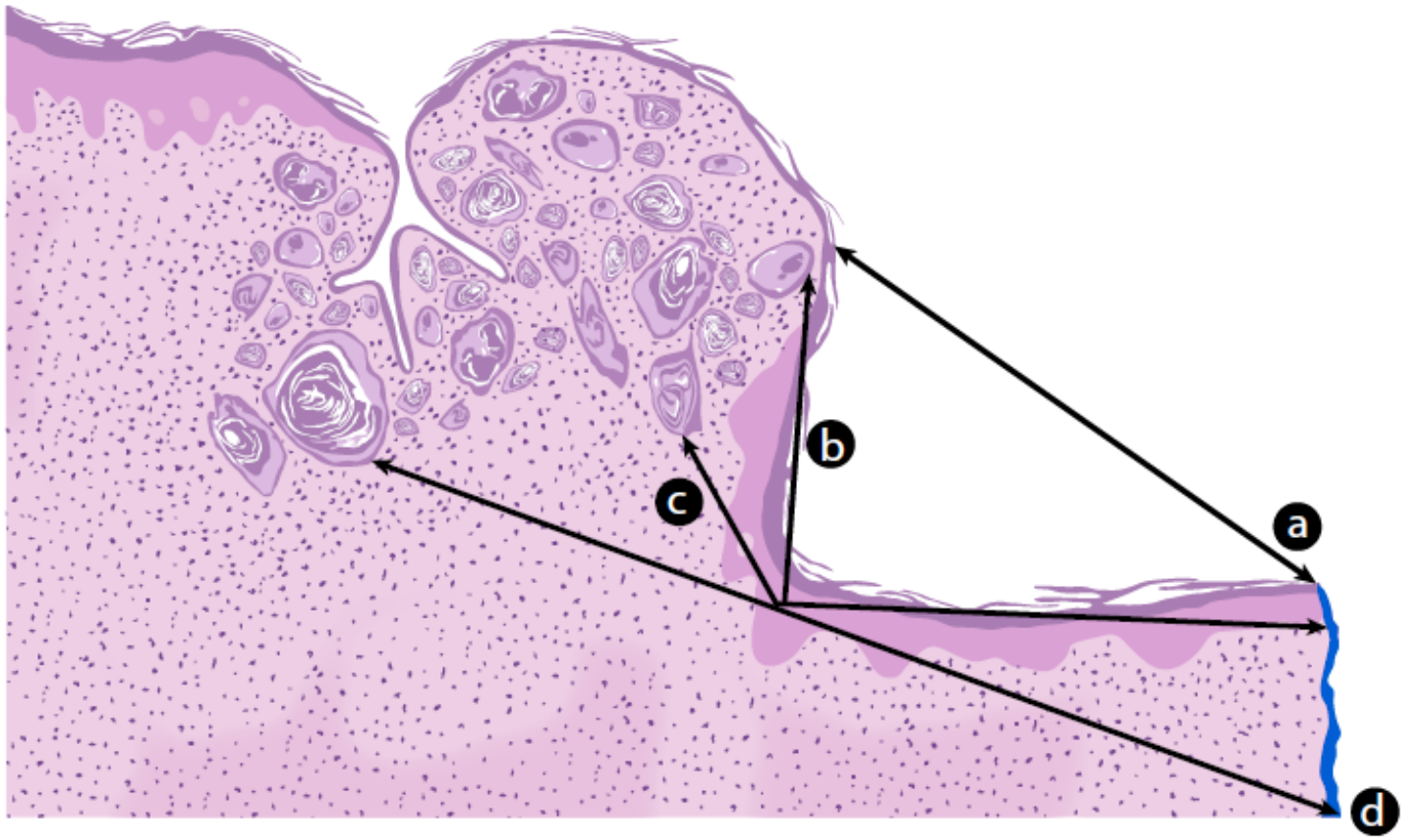
How to measure the Minimal Peripheral Surgical Margin?

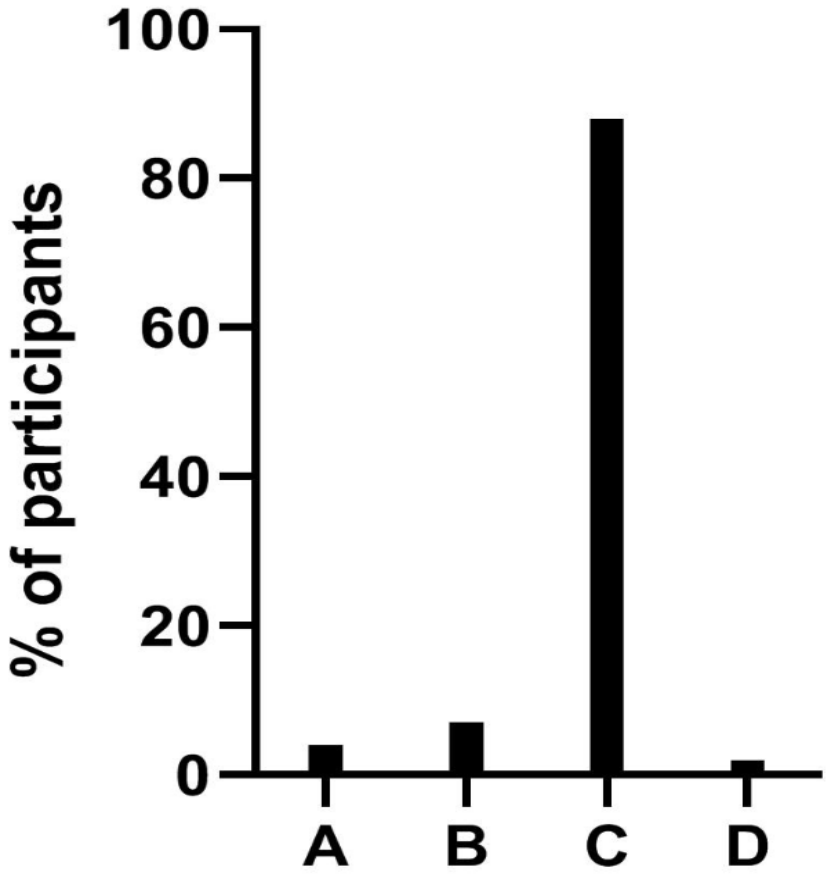




Measuring the MPSM by a curved line is not recommended

How to measure the Minimal Peripheral Surgical Margin?





The MPSM should be measured through tissue and preferably in a straight uninterrupted line, however in some situations a **composite measurement** joined at an angle may be required.

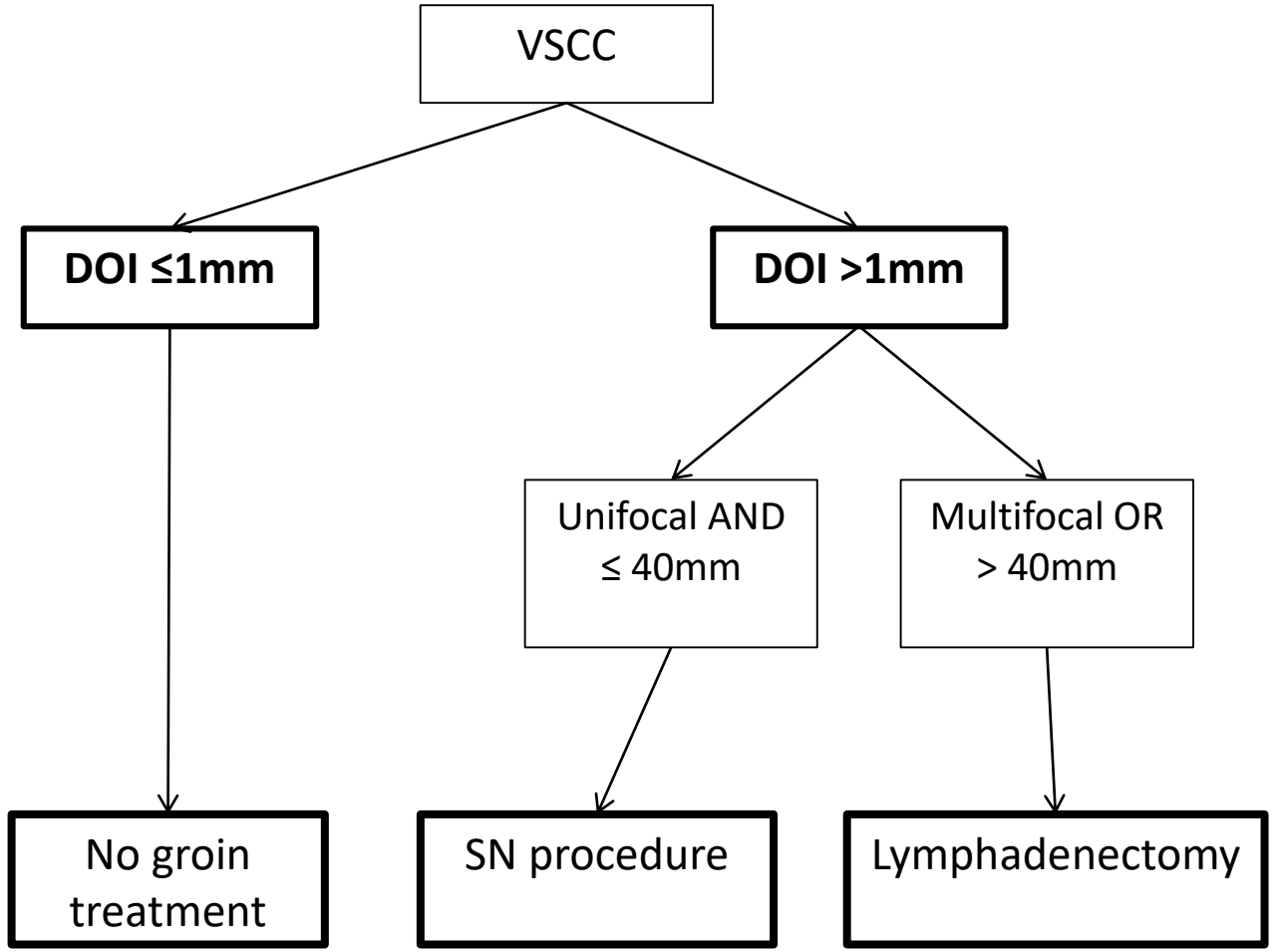
Current clinical management

Preinvasive lesions (dVIN/HSIL):

- Excise with 3 mm margin

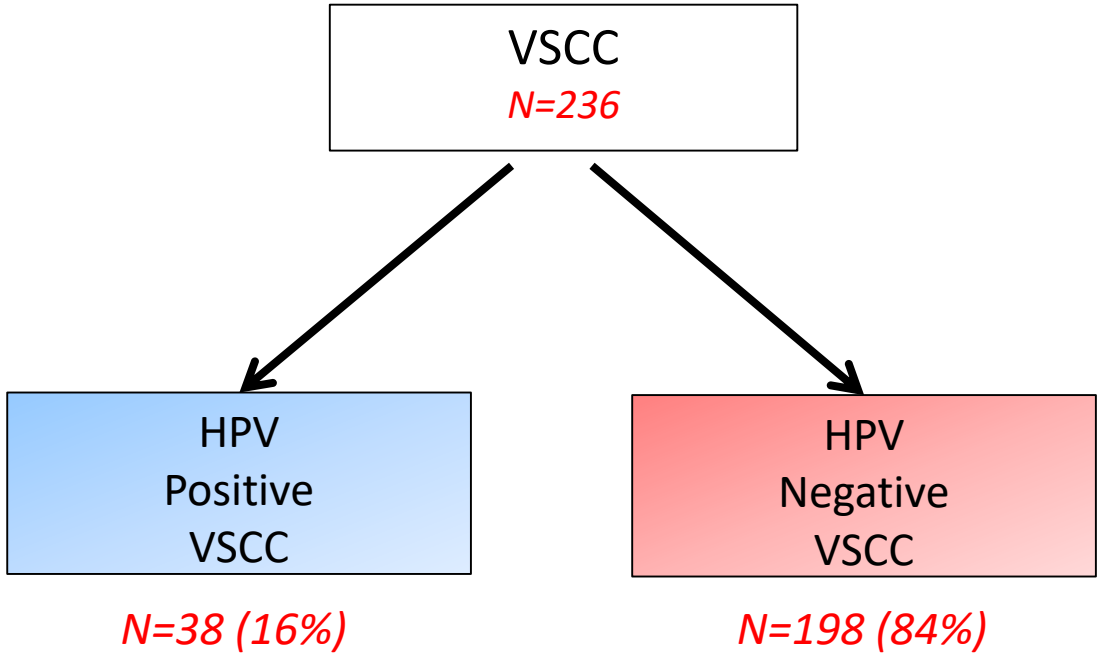
Invasive lesion (VSCC):

- “Wide local excision” with 8 mm margin
- Groin treatment dependent on Stage/DOI/Size



Currently **NO** differences in treatment algorithms based on HPV or p53 Status – Should that be changed ?

Clinical relevance HPV in VSCC



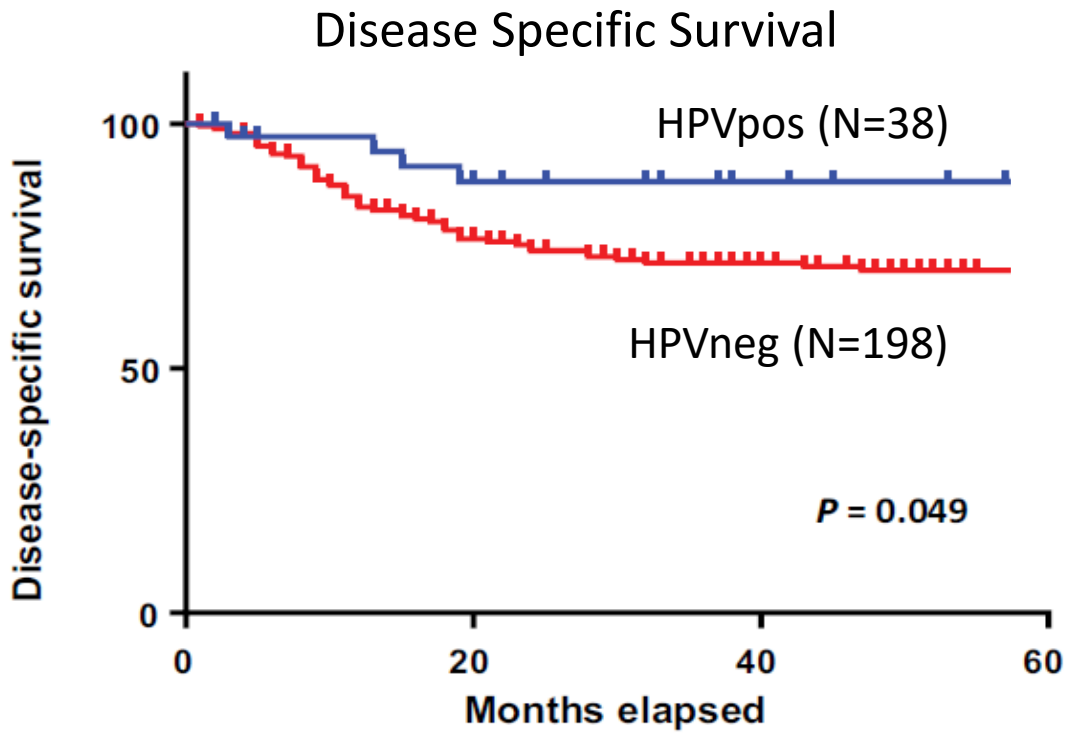


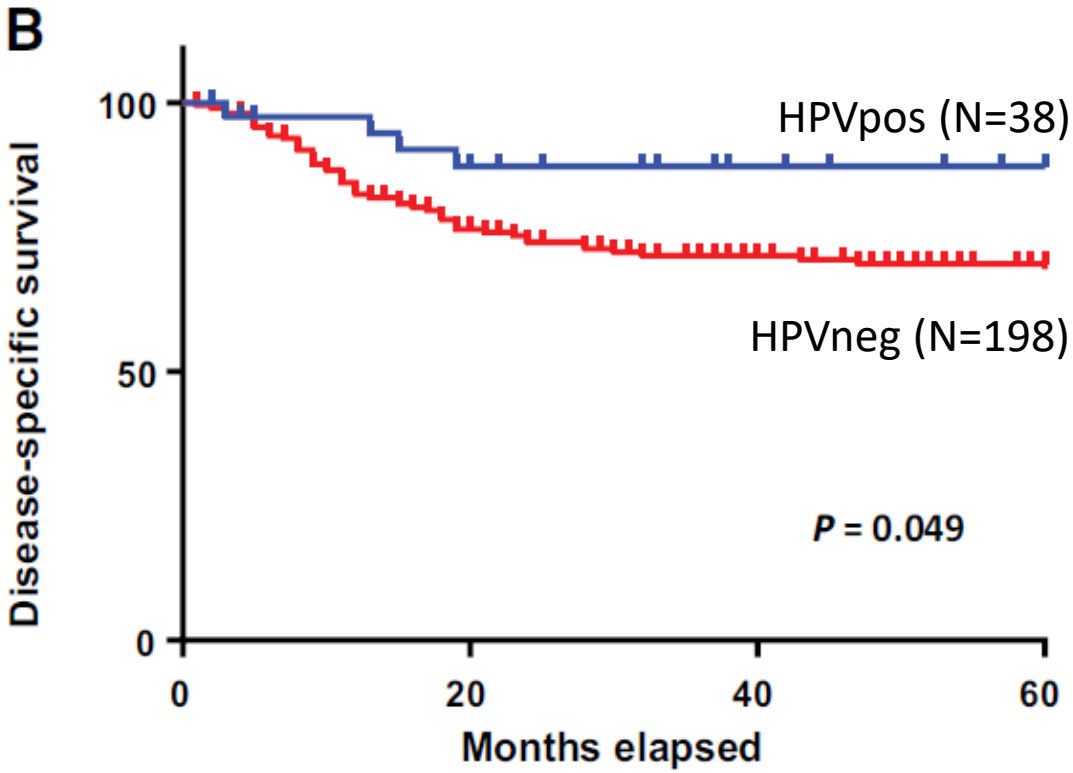
Table 3. Multivariable analysis

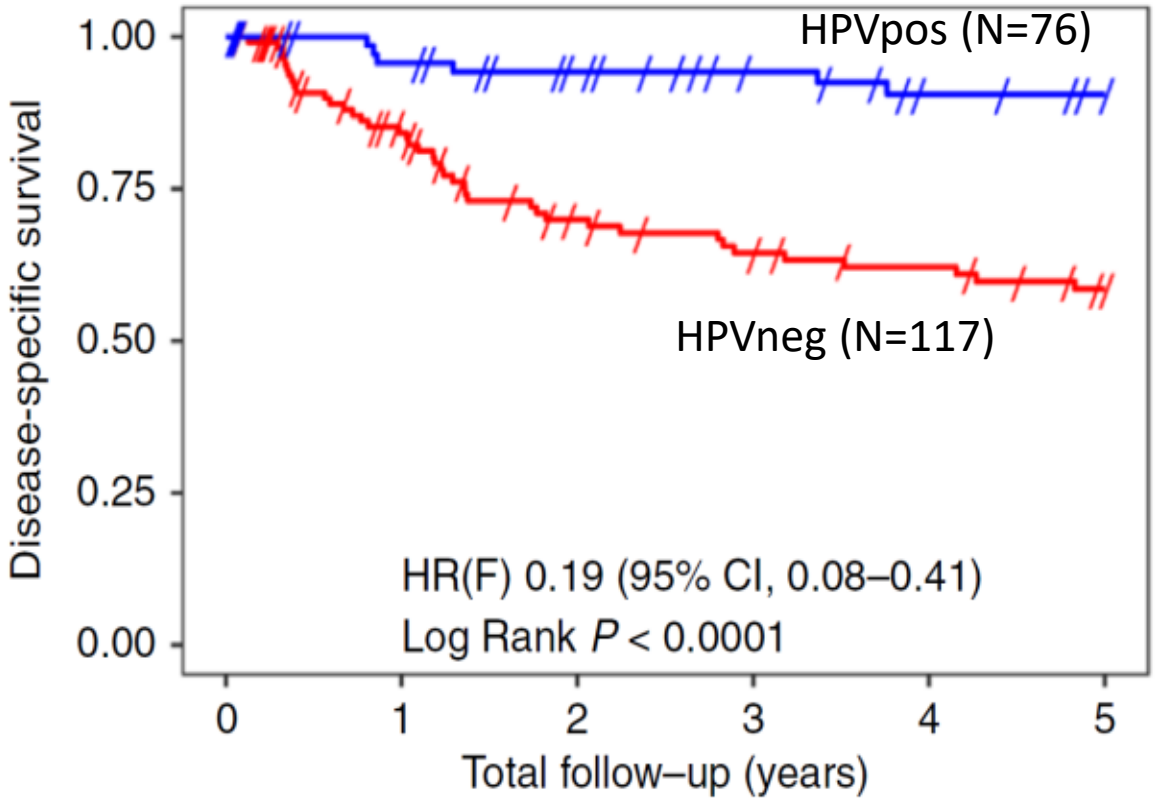
Tumor characteristics	Hazard ratio (95% CI)	*P*
Age (mean in years)	1.024 (1.004–1.045)	**0.021**
Tumor size		
>40 mm vs. ≤40 mm	0.534 (0.291–0.981)	**0.043**
Depth of invasion		
>4 mm vs. ≤4 mm	2.077 (1.174–3.675)	**0.012**
Lymph node status		
Tumor positive yes vs. no	1.119 (0.675–1.856)	0.663
HPV status		
Positive vs. negative	0.287 (0.101–0.819)	**0.020**

Abbreviation: HPV, human papillomavirus.

Nooij et al. CCR 2017

Validated impacts of clinical outcome HPV in VSCC

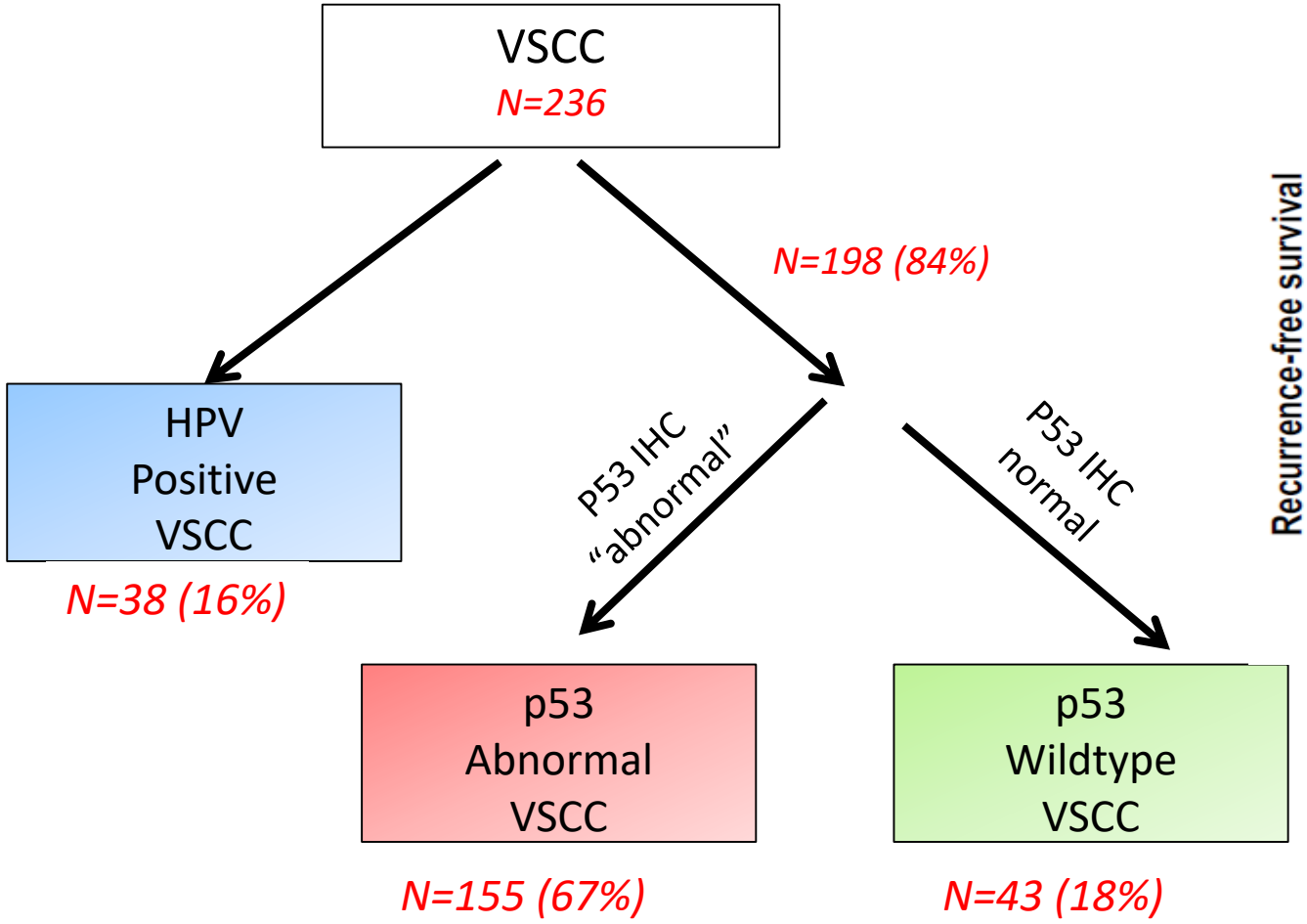


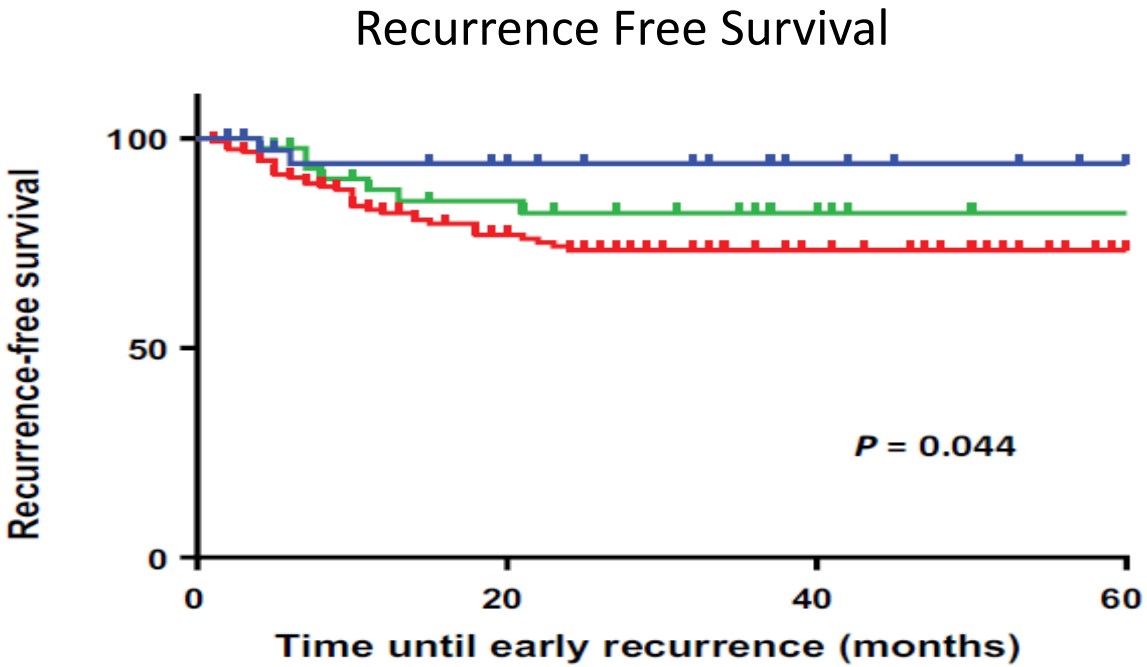


HPV status is a validated independent prognostic factor for disease specific survival in VSCC

McAlpine, Karnezis – Histopathology, 2017

What about p53 stratification?





HPV-/TP53 wildtype subgroup appears to have an intermediate risk of recurrence, but not significant

HPVpos and HPVneg clearly not the same; how to move forward?

- **Stratification by HPV status**
  - In translational research -improve our understanding about the differences
  - In clinical trials -improve our knowledge on treatment effects

- **Opportunities for HPV-dependent treatment algorithms**
  - Primary surgery – HPV status margins/aggressiveness
  - Adjuvant therapy – Who to spare, who to give?
  - Surveillance – Time interval

Role for pathology will become more prominent in treatment decisions, and go beyond measuring size and DOI.

Leids Universitair Medisch Centrum

Acknowledgements

Department of Pathology

Kim Kortekaas

Linda Nooij

Jolijn Trietsch

Dina Ruano

Jan Oosting

Natalja ter Haar

Michelle Osse

Enno Dreef

Vincent Smit

Department of Gynecology

Mariette van Poelgeest

Are we ready for this stratication?

1. How good is our surrogate markers (p16-IHC) for hrHPV status in VSCC?

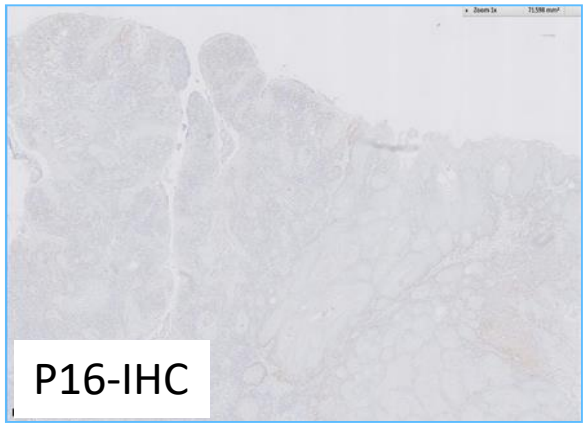
2. How good is our surrogate marker (p53-IHC) for mutational status in VSCC?

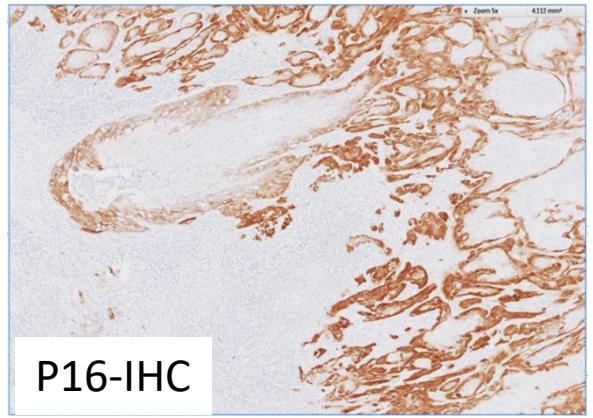
High concordance between P16-IHC and HPV-Lipa in VSCC

Erasmus MC
University Medical Center Rotterdam

N=425
VSCC

N=74	N=2	N=4	N=345
hrHPV+ and P16 pos	hrHPV+ and P16 neg	hrHPV- and P16 pos	hrHPV- and P16 neg





P16-IHC: specificity of 99.4% and sensitivity of 94.9%

Cheng et al, IJGP 2016, Dong et al., AJSP 2015, Riethdorf, Hum Path 2004, de Sanjose EJC 2013, Prigge, IJC 2017

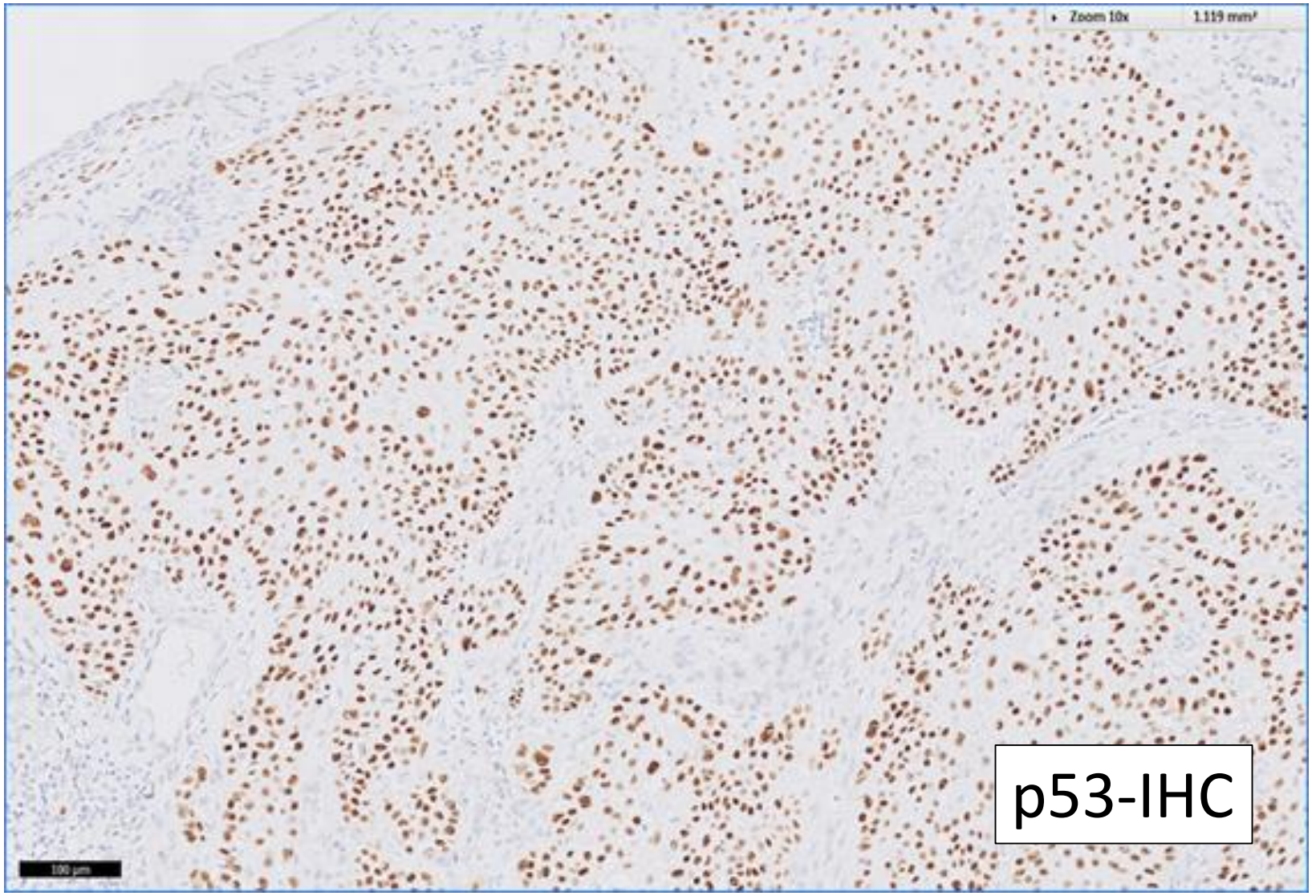
Are we ready for this stratication?

1. How good is our surrogate markers (p16-IHC) for hrHPV status in VSCC?

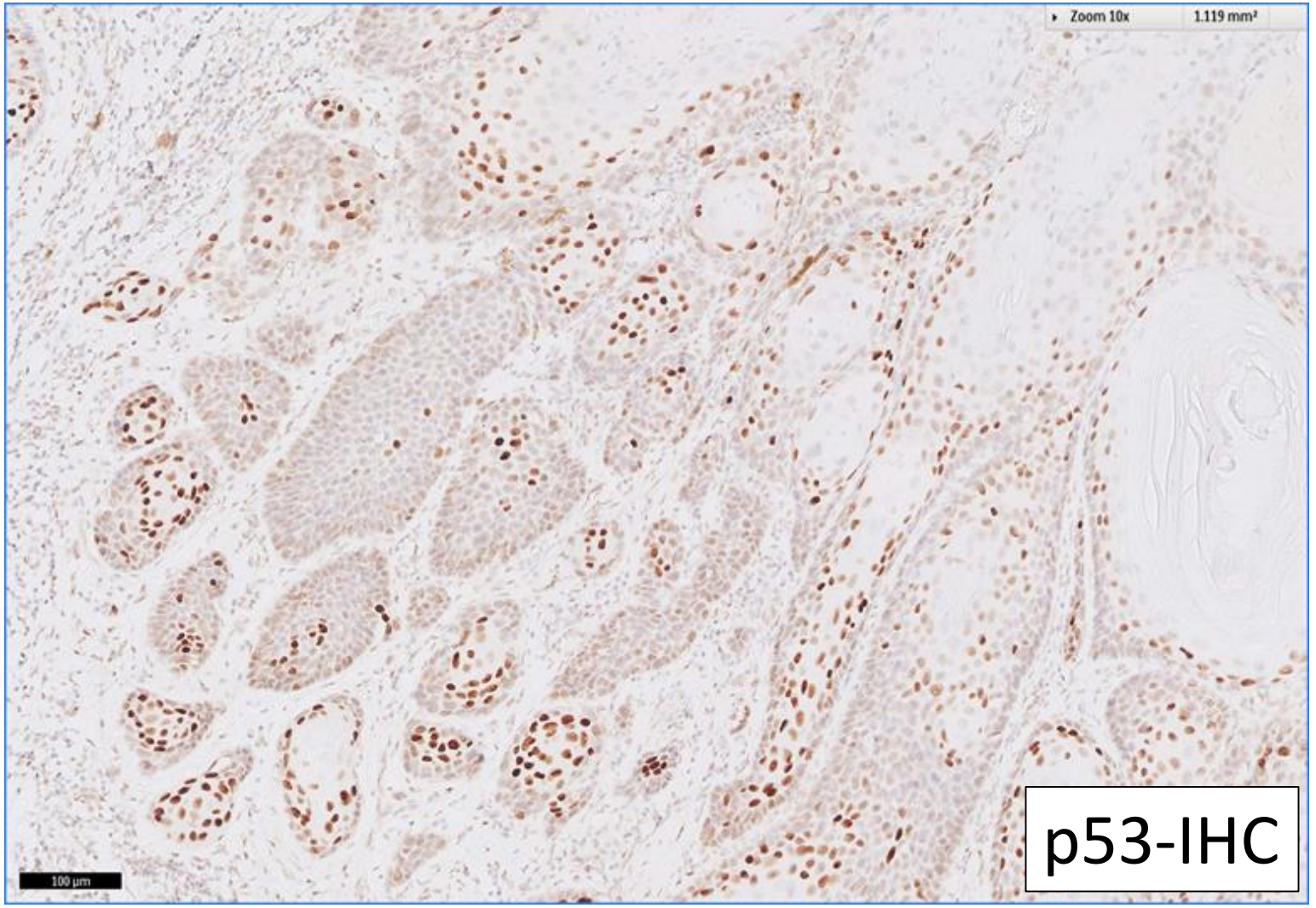
2. How good is our surrogate marker (p53-IHC) for mutational status in VSCC?

- **P53-IHC and NGS on 37 VSCC**

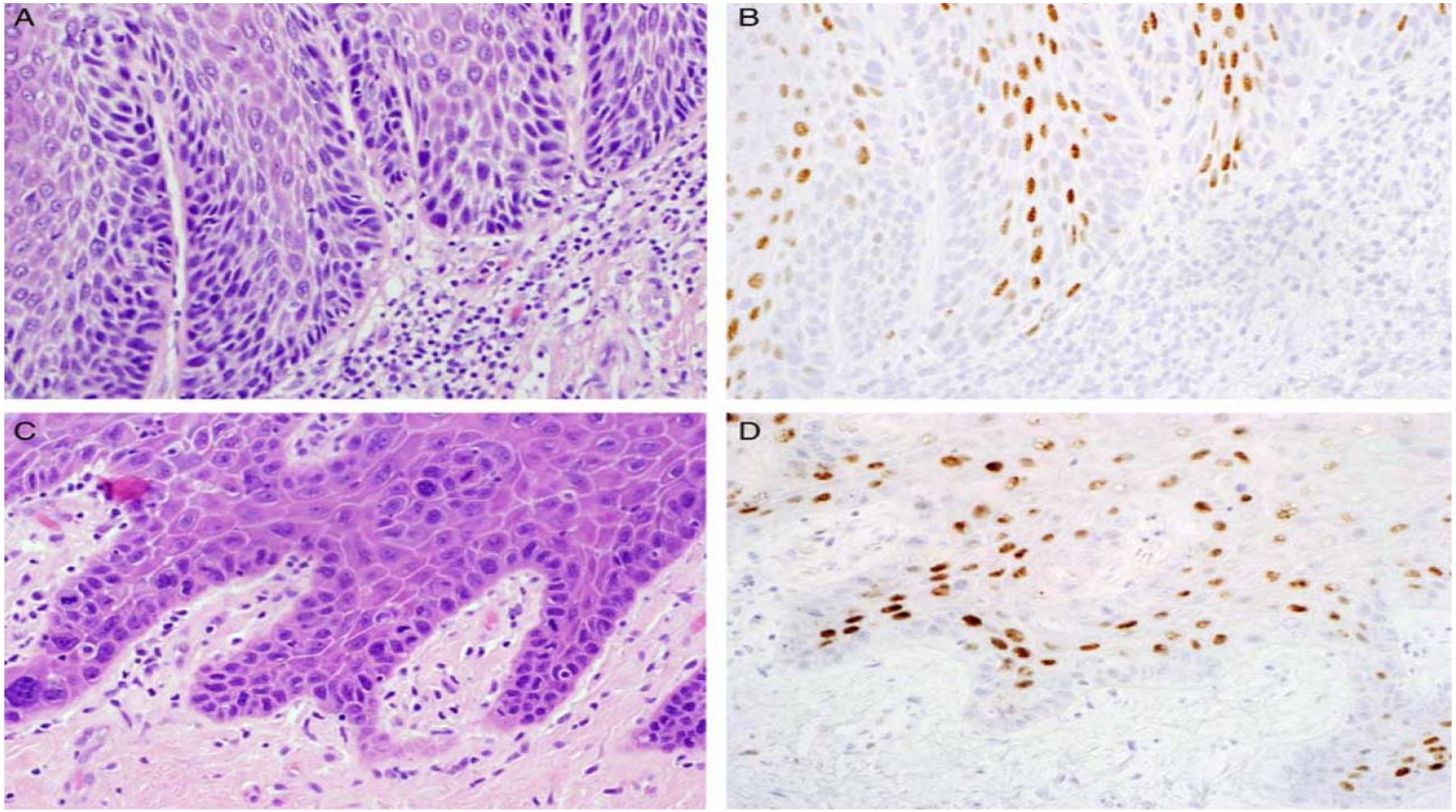
P53-IHC in VSCC: diffuse overexpression



P53-IHC in VSCC: parabasal/mid-epithelial p53 positivity



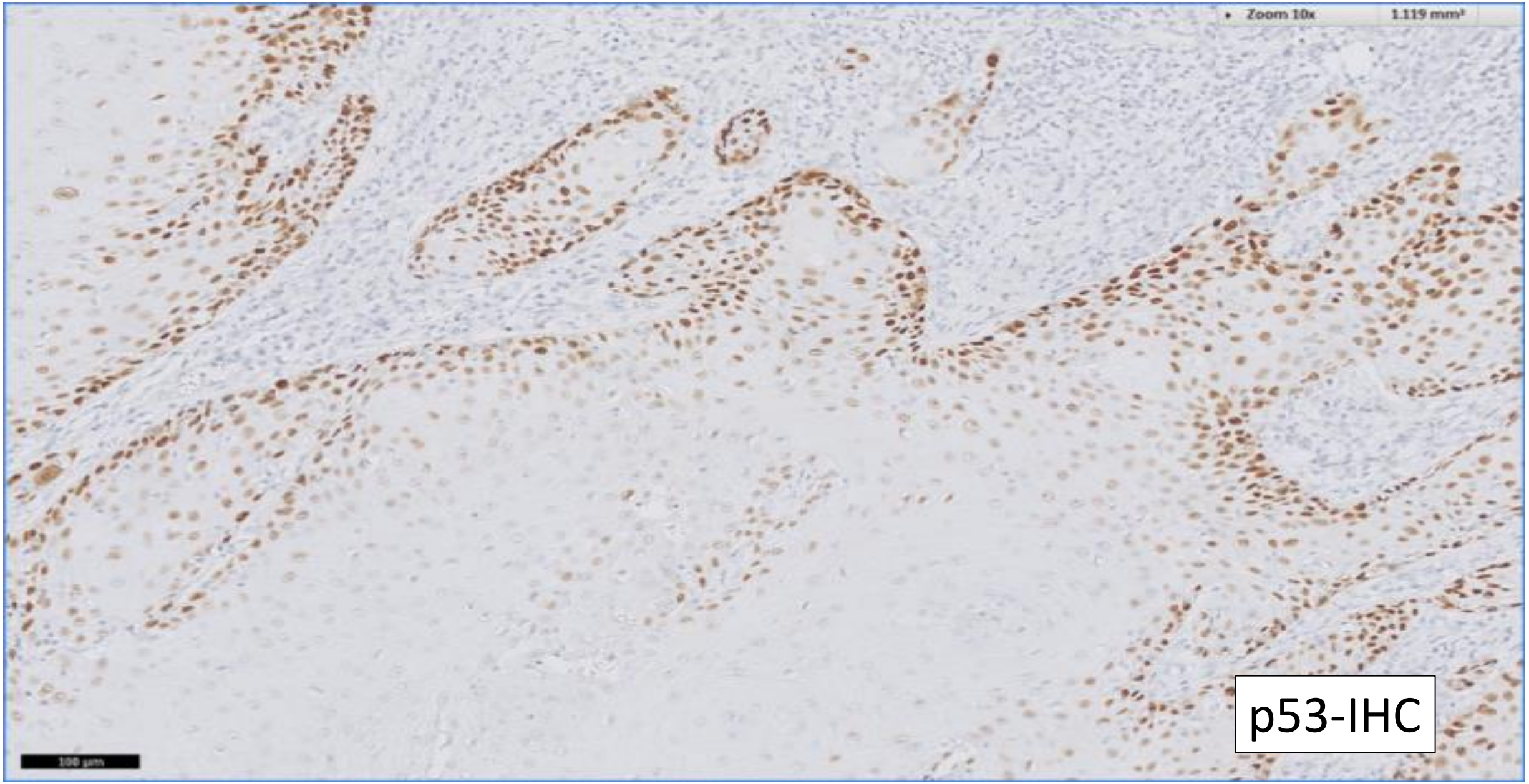
Same patterns also seen in uVIN/HSIL of the vulva



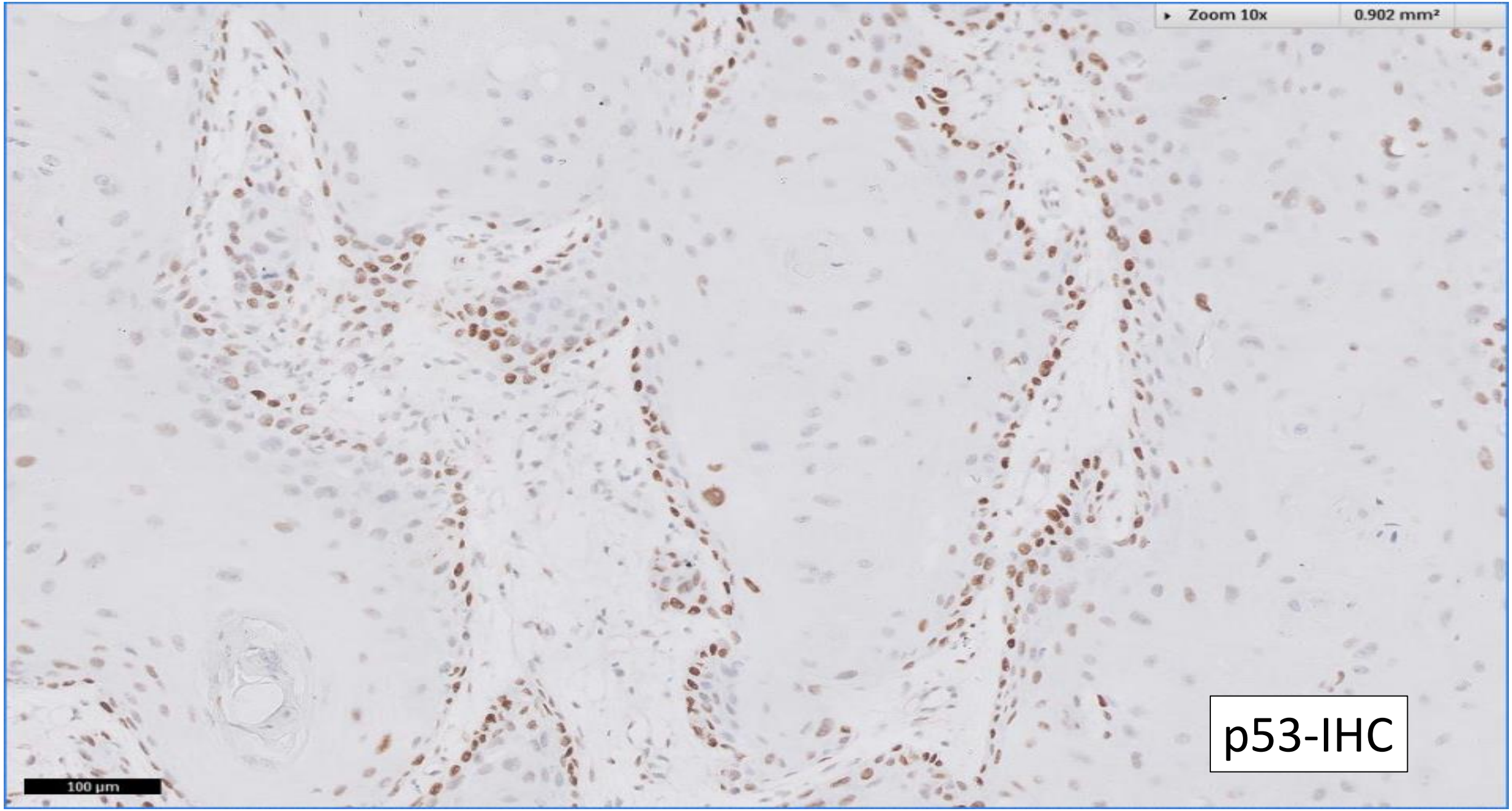
Strong parabasal and mid-epithelial p53 positivity with notable sparing of the basal layer is unique for HPV-associated HSIL and VSCC

Watkins et al., Int J of Gyn Path 2018

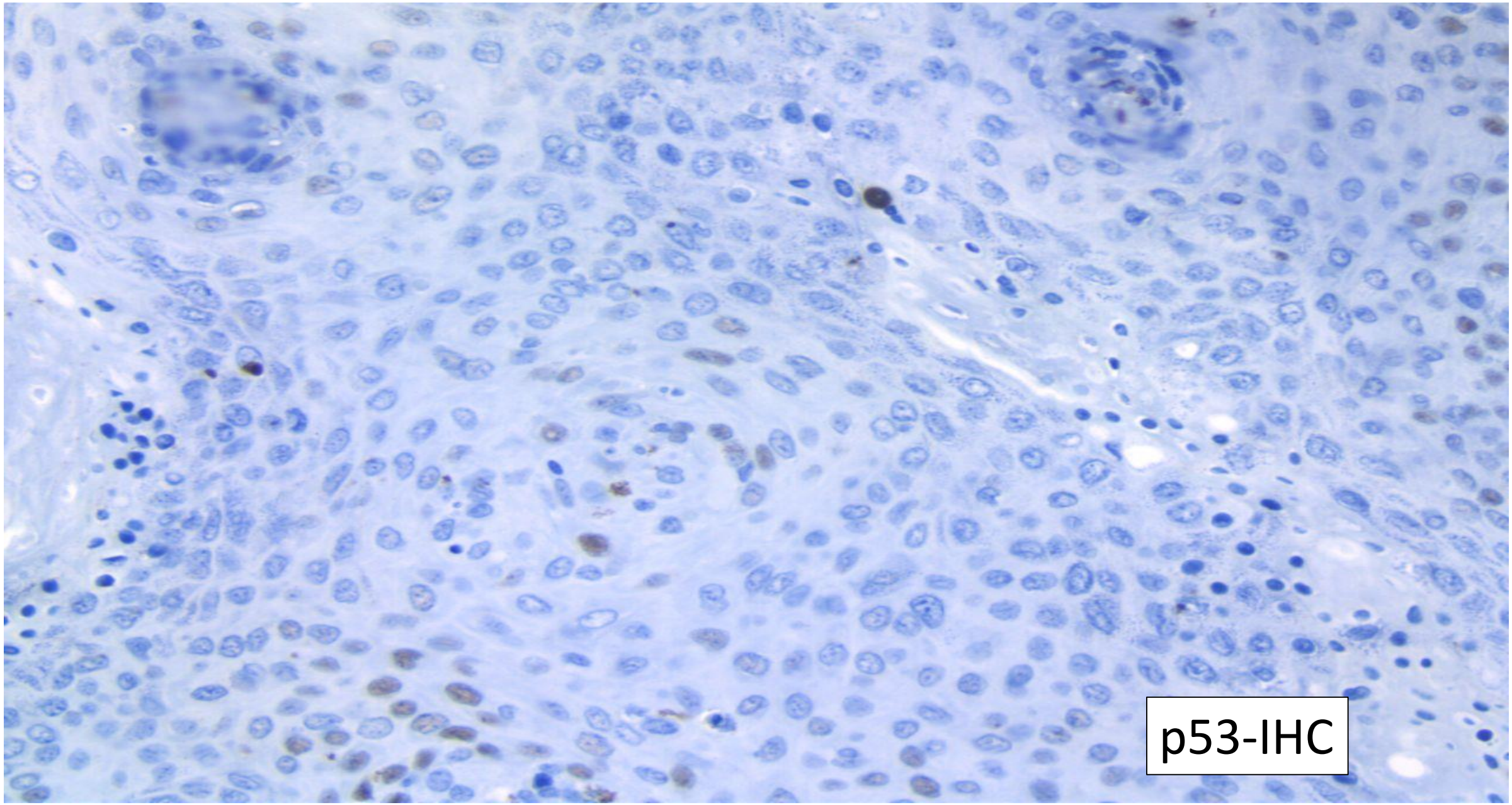
P53-IHC staining pattterns: basal+parabasal expression



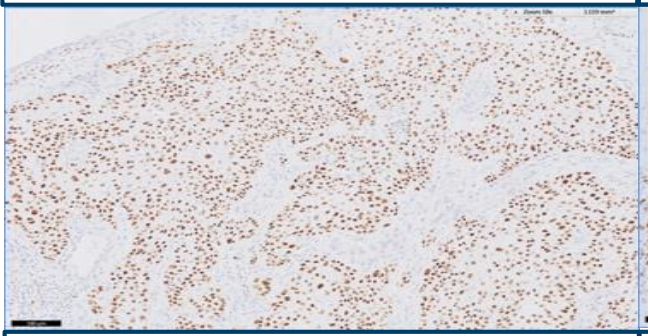
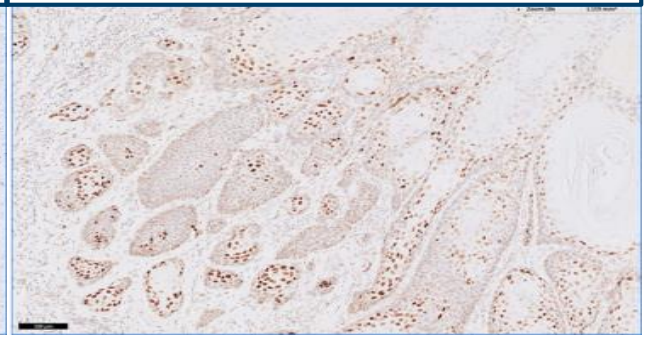
P53-IHC staining patterns: Basal Overexpression

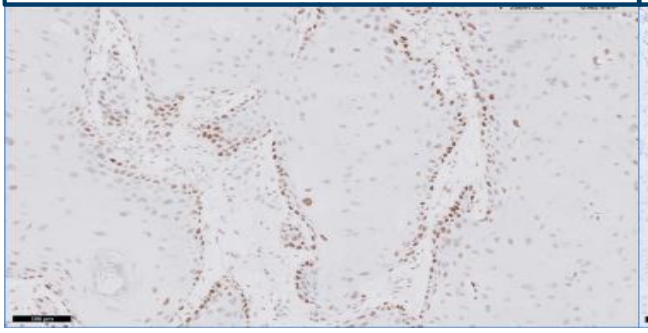


P53-IHC staining patterns: scattered positivity



Summary of p53 staining patterns

I. Diffuse Positivity	II. Parabasal + Basal	V. Scattered Positivity	VI. Mid-epithelial
			

III. Basal Positivity	IV. Complete Absence
	

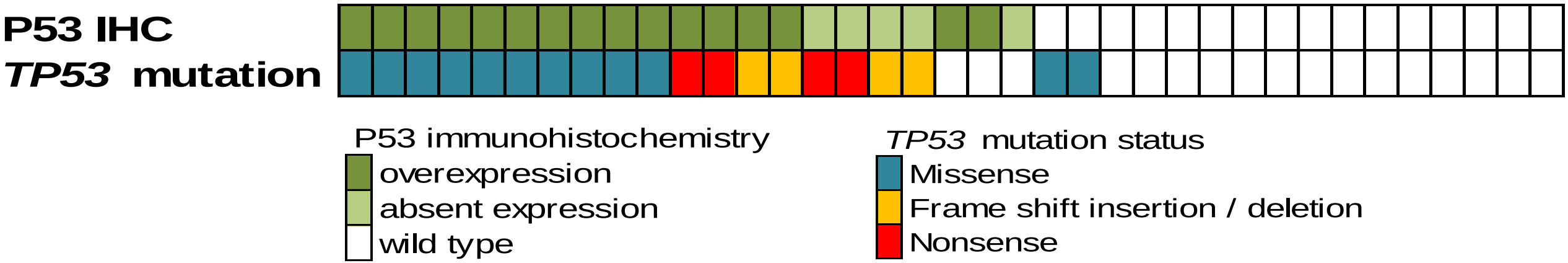
Probably *TP53* mutant	Probably *TP53* wild-type

Future studies required to study reproducible of this pattern-based p53-IHC classification

Kortekaas et al., manuscript in preparation

Concordance p53 IHC - *TP53* mutational status

Kappa = 0.73



Concordance between *TP53* sequencing and P53 IHC in vulvar cancer was surprisingly good, but numbers still too small for definitive conclusions

Take home

Room for optimism regarding tailored treatment options:

- There are at least 2 clinically VSCC precancers and cancers based on HPV status
- P16-IHC is an excellent marker for hrHPV status in VSCC
- There is a potential role for p53, however this needs further investigation

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Acknowledgements

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Natalja ter Haar

Michelle Osse

Enno Dreef

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