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”

### Incidence VSCC in the Netherlands

<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>Age &gt;50</th>
<th>Age &lt;50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td></td>
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<td>1995</td>
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<td>2000</td>
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<td>2010</td>
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<tr>
<td>2015</td>
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</tbody>
</table>

Number of cases (N) treated in LUMC 2000-2014

- Total
- Age >50
- Age <50
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 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 genetic understanding of VSCC is in its infancy

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 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

Sustaining proliferative signaling

Resisting cell death

Evading growth suppressors

Inducing angiogenesis

Activating invasion and metastasis

Enabling replicative immortality

Hannah and Weinberg Cell 2000
Simplified scheme of pathway alterations in VSCC

Altered Differentiation

Terminal Differentiation

NOTCH1,2,3

TP63

CDKN2A

Resisting cell death

CASP8

Cell Death

TP53

PTEN

PIK3CA

Sustained Proliferation

HRAS

Adapted from Stransky et al., Science 2011
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

HPV positive (N=25) HPV negative (N=57)

Revised diagnosis
Local diagnosis

* precursor lesion adjacent to vulvar cancer

Nooij et al. CCR 2017
Somatic mutations in HPV positive precursors

I. HPV + precursor lesions (N=25)
   - Few somatic mutations low
   - Histology (H&E)
     - 88% HSIL
     - 12% (2 dVIN and 1 VAAD/25)

* precursor lesion adjacent to vulvar cancer
Misdiagnosed as differentiated VIN (dVIN)

P16 IHC
hrHPV+

P53 IHC
NGS: wildtype

Case 0823
Differentiated Vulvar Intraepithelial Neoplasia-like and Lichen Sclerosis-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
dVIN and its wide morphologic spectrum

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

**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
Example of dVIN with abnormal p53-IHC
Clues to diagnosis of dVIN

• Nuclear atypia of basal keratinocytes
• (Atypical) mitosis
• Elongation and anastomosis 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

P53-IHC
NGS: wildtype
Example of HPV-/TP53 wildtype VAAD

Differentiated exophytic vulvar intraepithelial lesions are genetically distinct from keratinizing squamous cell carcinomas and contain mutations in \textit{PIK3CA}

Watkins et al. \textit{Mod Path} 2017

NGS: p53 wildtype
Notch mutant

Suggestive of a HPV negative and \textit{TP53} independent pathway – what about cancer?
Somatic mutational landscape of VSCC (N=36)

HPV+ 22%

HPV-/TP53mut 53%

HPV-/TP53wt 25%

Nooij et al. CCR 2017
Example of a HPV/p53 wildtype VSCC 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
- hrHPV
- hsIL
- PIK3CA
- NOTCH1/FAT1/HRAS/PIK3CA
- AGE
- VaAD
- DEVIL
- TP53 / CDKN2A
- dvIN

VSCC - Basaloid
VSCC - Keratinizing
VSCC - Verrucous
VSCC - Keratinizing
VSCC - Spindled

Lichen Sclerosis
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

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

- No stratification on HPV status
- Studies included positive margins in group of < 8mm
- Late recurrence > 2 years included
- Missing data on adjuvant therapy
A tumour-free margin of < 8 mm may NOT be associated with an increased local recurrence risk

Nooij et al., EJC 2016
Practical Guidance for Measuring and Reporting Surgical Margins in Vulvar Cancer

Running title: Surgical Margins in Vulvar Cancer

Kim E. Kortekaas¹, Koen van de Vijver², Mariëtte I.E. van Poelgeest¹, Blake Gilks³, Vincent Smit⁴, S. Arif⁵, D. Arora⁶, A. Faruqi⁷, R. Ganesan⁸, N.R Griffin⁹, R. Hale¹⁰, Y.L. Hock¹¹, L-C Horn¹², W. Glenn McCluggage¹³, P. Mukonoweshuro¹⁴, K.J. Park¹⁵, B. Rous¹⁶, B. Tanchel¹⁸, A.S. Van Rompuy¹⁷, G. van Schalkwyk¹⁸, J. Vella⁸, M. Vergine¹⁹, Naveena Singh⁷, Tjalling Bosse⁴
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”.

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-> measurement through tissue
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 \textit{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

VSCC  
N=236

HPV Positive VSCC  
N=38 (16%)

HPV Negative VSCC  
N=198 (84%)

Disease Specific Survival

HPVpos (N=38)  
P = 0.049

HPVneg (N=198)

Table 3. Multivariable analysis

<table>
<thead>
<tr>
<th>Tumor characteristics</th>
<th>Hazard ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean in years)</td>
<td>1.024 (1.004-1.045)</td>
<td>0.021</td>
</tr>
<tr>
<td>Tumor size</td>
<td>0.534 (0.291-0.981)</td>
<td>0.043</td>
</tr>
<tr>
<td>Depth of invasion</td>
<td>2.077 (1.174-3.675)</td>
<td>0.012</td>
</tr>
<tr>
<td>Lymph node status</td>
<td>1.119 (0.675-1.856)</td>
<td>0.663</td>
</tr>
<tr>
<td>HPV status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive vs. negative</td>
<td>0.287 (0.101-0.819)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Abbreviation: HPV, human papillomavirus.

Nooij et al. CCR 2017
HPV status is a validated independent prognostic factor for disease specific survival in VSCC.
What about p53 stratification?

HPV Positive VSCC

N=38 (16%)

HPV Negative VSCC

N=155 (67%)

N=43 (18%)

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.
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Vincent Smit

Department of Gynecology
Mariette van Poelgeest
Are we ready for this stratification?

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

N=425
VSCC

N=74
hrHPV+ and P16 pos

N=2
hrHPV+ and P16 neg

N=4
hrHPV- and P16 pos

N=345
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
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 patterns: basal+parabasal expression
P53-IHC staining patterns: Basal Overexpression
P53-IHC staining patterns: scattered positivity
Summary of p53 staining patterns

<table>
<thead>
<tr>
<th>I. Diffuse Positivity</th>
<th>II. Parabasal + Basal</th>
<th>V. Scattered Positivity</th>
<th>VI. Mid-epithelial</th>
</tr>
</thead>
<tbody>
<tr>
<td>III. Basal Positivity</td>
<td>IV. Complete Absence</td>
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</tr>
</tbody>
</table>

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