## Carcinoma of the Vulva

### Histopathology Reporting Guide

<table>
<thead>
<tr>
<th>Field</th>
<th>Example</th>
</tr>
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<tbody>
<tr>
<td>Family/Last name</td>
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<tr>
<td>Given name(s)</td>
<td></td>
</tr>
<tr>
<td>Date of birth</td>
<td>DD – MM – YYYY</td>
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<tr>
<td>Patient identifiers</td>
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<tr>
<td>Date of request</td>
<td>DD – MM – YYYY</td>
</tr>
<tr>
<td>Accession/Laboratory number</td>
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</tbody>
</table>

Elements in **black text** are **CORE**. Elements in **grey text** are **NON-CORE**.

### CLINICAL INFORMATION (select all that apply) (Note 1)

- Information not provided
- History of previous cancer, specify
  - ...
- Prior neoadjuvant therapy, specify
  - ...
- Other, specify
  - ...

### OPERATIVE PROCEDURE (select all that apply) (Note 2)

- Not specified
- Wide local excision
- Partial radical vulvectomy
- Total radical vulvectomy
- Lymph nodes, specify site(s)
  - ...
- Other, specify
  - ...

### SPECIMEN DIMENSIONS (Note 3)

<table>
<thead>
<tr>
<th>Width</th>
<th>Height</th>
<th>Depth</th>
</tr>
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<tbody>
<tr>
<td>mm</td>
<td>mm</td>
<td>mm</td>
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</tbody>
</table>

- Cannot be assessed, specify
  - ...

### TUMOUR SITE (select all that apply) (Note 4)

- Left vulva
  - Not specified
  - Labium majus
  - Labium minus
  - Bartholin gland

- Right vulva
  - Not specified
  - Labium majus
  - Labium minus
  - Bartholin gland

### TUMOUR DIMENSIONS (Note 5)

- Maximum horizontal tumour dimension
  - mm

- Depth of invasion
  - mm

- Cannot be assessed, specify
  - ...

### BLOCK IDENTIFICATION KEY (Note 6)

(List overleaf or separately with an indication of the nature and origin of all tissue blocks)

### HISTOLOGICAL TUMOUR TYPE (Note 7)

(Value list based on the World Health Organization Classification of Female Genital Tumours (2020))

- Squamous cell carcinoma, HPV-associated
- Squamous cell carcinoma, HPV-independent
- Squamous cell carcinoma, NOS
- Basal cell carcinoma
- Bartholin gland carcinoma, specify type
- Neuroendocrine carcinoma, specify type
LYMPHOVASCULAR INVASION (Note 8)
- Indeterminate
- Not identified
- Present

PERINEURAL INVASION (Note 9)
- Not identified
- Present

MARGIN STATUS (Note 10)

Invasive tumour
- Cannot be assessed
- Not involved
  - Distance of tumour from closest skin or mucosal margin [ ] mm
    Specify closest margin, if possible
  - Distance of tumour from deep margin [ ] mm
    Specify closest margin, if possible
- Involved
  - Specify margin, if possible

Precursor lesions
- Not applicable
- Cannot be assessed
- Not involved
  - Distance of high grade precursor lesion from closest margin [ ] mm
    Specify closest margin, if possible
- Involved
  - Specify margin, if possible

LYMPH NODE STATUS (Note 11)

Sentinel lymph nodes (inguinofemoral)
- Cannot be assessed
- No nodes submitted or found

Site 1
- Number of nodes examined [ ]
- Number of positive nodes [ ]
- Size of maximum tumour deposit [ ] mm
- Extracapsular spread
  - Not identified
  - Present

Site 2
- Number of nodes examined [ ]
- Number of positive nodes [ ]
- Size of maximum tumour deposit [ ] mm
- Extracapsular spread
  - Not identified
  - Present

Classification of sentinel nodal metastasis
- Isolated tumour cells (<0.2 mm)
- Micrometastasis (0.2-2 mm)
- Macrometastasis (>2 mm)

If sentinel node positive
- Identified with ultrastaging including immunohistochemistry
- Identified with ultrastaging without immunohistochemistry
- Identified without ultrastaging

Regional non-sentinel lymph nodes (inguinofemoral)
- Cannot be assessed
- No nodes submitted or found

Site 1
- Number of nodes examined [ ]
- Number of positive nodes [ ]
- Size of maximum tumour deposit [ ] mm
- Extracapsular spread
  - Not identified
  - Present

Site 2
- Number of nodes examined [ ]
- Number of positive nodes [ ]
- Size of maximum tumour deposit [ ] mm
- Extracapsular spread
  - Not identified
  - Present

Classification of nodal metastasis
- Isolated tumour cells (<0.2 mm)
- Micrometastasis (0.2-2 mm)
- Macrometastasis (>2 mm)

Clinically fixed or ulcerated lymph nodes
- Not known
- Present
Carcinoma of the Vulva

Non-regional lymph nodes (other than inguinofemoral; includes pelvic or other sites)

- Cannot be assessed
- No nodes submitted or found

**Site 1**

- Number of nodes examined
- Number of positive nodes
- Size of maximum tumour deposit (mm)

**Extracapsular spread**

- Not identified
- Present

Classification of sentinel nodal metastasis

- Isolated tumour cells (<0.2 mm)
- Micrometastasis (0.2-2 mm)
- Macrometastasis (>2 mm)

**COEXISTENT PATHOLOGY/PRECURSOR LESIONS (Note 12)**

- None identified
- Present (select all that apply)

- Low grade squamous intraepithelial lesion (LSIL), HPV-associated
- High grade squamous intraepithelial lesion (HSIL), HPV-associated
- Vulval intraepithelial neoplasia (VIN), HPV-independent
- Lichen sclerosis
- Other, specify

**ANCILLARY STUDIES (Note 13)**

- Not performed
- Performed (select all that apply)
  - p16 immunohistochemistry*
  - HPV testing*
  - p53 immunohistochemistry
  - Other, specify test(s) and result(s)

Representative blocks for ancillary studies, specify those blocks best representing tumour and/or normal tissue for further study

* Core for squamous cell carcinomas.

**PATHOLOGICALLY CONFIRMED DISTANT METASTASIS (Note 14)**

- Not identified
- Present, specify site(s)

**PROVISIONAL PATHOLOGICAL STAGING (Note 15)**

**FIGO (2009 edition)**

- I Tumour confined to the vulva
  - IA Lesions ≤2 cm in size, confined to the vulva or perineum and with stromal invasion ≤1.0 mm; no nodal metastasis
  - IB Lesions >2 cm in size or with stromal invasion >1.0 mm, confined to the vulva or perineum, with negative nodes
- II Tumour of any size with extension to adjacent perineal structures (1/3 lower urethra, 1/3 lower vagina, anus) with negative nodes
- III Tumour of any size with or without extension to adjacent perineal structures (1/3 lower urethra, 1/3 lower vagina, anus) with positive inguinofemoral lymph nodes
  - IIIA (i) With 1 lymph node metastasis (≥5 mm), or (ii) 1-2 lymph node metastasis(es) (<5 mm)
  - IIIB (i) With 2 or more lymph node metastases (≥5 mm), or (ii) 3 or more lymph node metastases (<5 mm)
  - IIIC With positive nodes with extracapsular spread
- IV Tumour invades other regional (2/3 upper urethra, 2/3 upper vagina), or distant structures
  - IVA Tumour invades any of the following: (i) upper urethral and/or vaginal mucosa, bladder mucosa, rectal mucosa, or fixed to pelvic bone, or (ii) fixed or ulcerated inguinofemoral lymph nodes
  - IVB Any distant metastasis including pelvic lymph nodes

* Reprinted from Int J Gynaecol Obstet., Volume 105(2), Hacker NF, Revised FIGO staging for carcinoma of the vulva, pages 105-6, 2009, with permission from Wiley.

**The depth of invasion is defined as the measurement of the tumour from the epithelial-stromal junction of the adjacent most superficial dermal papilla to the deepest point of invasion.**
**TNM Staging (UICC TNM 8th edition 2016)**

**TNM Descriptors** (only if applicable) (select all that apply)

- m  - multiple primary tumours
- r  - recurrent
- y  - post-therapy

### Primary tumour (pT)

- TX  Primary tumour cannot be assessed
- T0  No evidence of primary tumour
- Tis  Carcinoma in situ (preinvasive carcinoma), intraepithelial neoplasia grade III (VIN III)
- T1  Tumour confirmed to vulva or vulva and perineum
  - T1a Tumour 2 cm or less in greatest dimension and with stromal invasion no greater than 1.0 mm
d
  - T1b Tumour greater than 2 cm and with stromal invasion greater than 1 mm
d
- T2  Tumour invades any of the following structures: lower third urethra, lower third vagina, anus
d
- T3\d  Tumour invades any of the following perineal structures: upper 2/3 urethra, upper 2/3 vagina, bladder mucosa, rectal mucosa; or fixed to pelvic bone
d

### Regional lymph nodes (pN)

- NX  Regional lymph nodes cannot be assessed
- N0  No regional lymph node metastasis
- N1  Regional lymph node metastasis with the following features:
  - N1a One or two lymph node metastases each less than 5 mm
  - N1b One lymph node metastasis 5 mm or greater
- N2  Regional lymph node metastasis with the following features:
  - N2a Three or more lymph node metastases each less than 5 mm
  - N2b Two or more lymph node metastases 5 mm or greater
  - N2c Lymph node metastasis with extracapsular spread
- N3  Fixed or ulcerated regional lymph node metastasis


d The depth of invasion is defined as the measurement of the tumour from the epithelial-stromal junction of the adjacent most superficial dermal papilla to the deepest point of invasion.

\d T3 is not used by FIGO.
Definitions

CORE elements

CORE elements are those which are essential for the clinical management, staging or prognosis of the cancer. These elements will either have evidentiary support at Level III-2 or above (based on prognostic factors in the National Health and Medical Research Council (NHMRC) levels of evidence\(^1\)). In rare circumstances, where level III-2 evidence is not available an element may be made a CORE element where there is unanimous agreement in the expert committee. An appropriate staging system e.g., Pathological TNM staging would normally be included as a CORE element.

The summation of all CORE elements is considered to be the minimum reporting standard for a specific cancer.

NON-CORE elements

NON-CORE elements are those which are unanimously agreed should be included in the dataset but are not supported by level III-2 evidence. These elements may be clinically important and recommended as good practice but are not yet validated or regularly used in patient management.

Key information other than that which is essential for clinical management, staging or prognosis of the cancer such as macroscopic observations and interpretation, which are fundamental to the histological diagnosis and conclusion e.g., macroscopic tumour details, may be included as either CORE or NON-CORE elements by consensus of the Dataset Authoring Committee.

Scope

The dataset has been developed for the pathological reporting of resection specimens of primary carcinomas of the vulva. In some patients with a prior diagnosis of vulval carcinoma (especially squamous), it is not clear whether a ‘new’ lesion is a recurrence or an independent neoplasm and the dataset can also be used for such tumours, especially when these ‘arise’ from the surface squamous epithelium. Molecular studies have shown that some of these ‘recurrent’ neoplasms exhibit similar mutations and are clonally related to the original tumour and are likely to represent true recurrences while others are clonally unrelated with different mutations and are likely to represent new neoplasms.\(^2\) In those rare cases where more than one primary tumour is present, separate datasets should be completed for each neoplasm. These should include all the elements in this dataset, except for lymph node status which does not need to be documented separately for each tumour.

Haematopoietic neoplasms, mesenchymal neoplasms, mixed epithelial and mesenchymal neoplasms, malignant melanomas, other non-epithelial malignancies and metastatic tumours are excluded from this dataset.

The authors of this dataset can be accessed here.

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Note 1 – Clinical information (Core)

In most International Collaboration on Cancer Reporting (ICCR) datasets, clinical information is a non-core element but the Carcinoma of the Vulva Dataset Authoring Committee felt that clinical information is vital in reporting vulval carcinomas and thus this is included as a core element. In reporting a vulval carcinoma, knowledge of a history of any prior vulval tumour (including the site), precursor lesion or treatment is important. While in many cases, this information can be identified from the laboratory information system/electronic care record, this is not always the case and this information should be provided by the clinician on the specimen request form. This is especially important with vulval squamous carcinomas since tumour recurrence is common. In some patients with a prior vulval squamous carcinoma, it is not clear from a pathological perspective whether a ‘new’ lesion is a recurrence or an independent neoplasm and the dataset can also be used for such tumours if an ‘origin’ can be seen from the overlying squamous epithelium. Knowledge of a history of a prior precursor lesion or inflammatory dermatosis is also important. Information regarding a history of a prior malignancy is important in reporting those rare primary vulval adenocarcinomas since a metastasis should always be excluded before rendering such a diagnosis. Knowledge of a history of any prior neoadjuvant therapy (chemotherapy, radiotherapy, chemoradiation) is important since this can have a marked effect on the pathological appearances of the neoplasm (gross and morphological).

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Note 2 – Operative procedure (Core)

Wide local excision refers to removal of the full thickness of vulval skin or mucosa with preservation of subcutaneous fat and other deep tissues (older terminologies include partial vulvectomy, superficial vulvectomy, skinning vulvectomy).3-5 Wide local excision is usually performed for pre-invasive or non-malignant lesions or for diagnostic purposes where cancer has not been ruled out.

Radical vulvectomy (partial or total) is usually performed for biopsy confirmed invasive carcinoma and involves removing the vulval tissue down to the deep fascia. Radical vulvectomy may include removal of the clitoris with prepuce, the labia majora, labia minora, a portion of vagina, urethra, and/or anus.3-5 It is desirable that orientation of the specimen is provided by the surgeon to enable evaluation of margin status; this may be achieved by the placing of sutures or by provision of a diagram or photograph.

Wide local excision and radical vulvectomy procedures will be tailored depending on the tumour size, pathological diagnosis, patient wishes/expectations, likely impact on psychosexual function and tumour location with respect to proximity to other vital structures.

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Note 3 – Specimen dimensions (Core)

Although not necessary for staging, clinical management or prognosis, it is recommended that the specimen dimensions be recorded on the pathology report.6-9 This gives clinicians dealing with the patient an indication as to how radical a resection has been undertaken.

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**Note 4 – Tumour site** (Core and Non-core)

Detailing the anatomical site of a vulval carcinoma is important for the following reasons: tumours located close to or in the midline can be associated with bilateral or contralateral lymph node involvement because the lymphatic vessels anastomose across the midline, particularly in the clitoral and the anterior labium minus regions and midline/ clitoral involvement is associated with a worse prognosis which is possibly related to unfavourable histopathological characteristics of the tumours (more likely to be human papillomavirus (HPV)-independent).10–12

The tumour site should be provided by the surgeon and, as discussed above, the placing of sutures or the provision of a diagram or photograph may be helpful. If determination of the tumour site is not possible, it may be necessary to liaise with the surgeon.

The tumour laterality (right vulva, left vulva, midline, involvement of other structures) is regarded as a core item, while involvement of the labium majus, labium minus and Bartholin gland is regarded as non-core.

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**Note 5 – Tumour dimensions** (Core)

Accurate measurement of tumour dimensions in vulval carcinomas is important for staging, patient management and prognostication. Tumour dimensions should be measured in millimetres (mm). The maximum horizontal dimension is the greatest tumour dimension measured parallel to the skin surface. This measurement is typically made based on macroscopic assessment for larger tumours but for very small tumours this may be best measured or can only be measured on the histological section. A second horizontal dimension taken perpendicular to the first and also parallel to the skin surface is often included in the pathology report but this is not necessary for staging, management or prognostication. The depth of invasion must also be reported and this is discussed in more detail below.

Note that the final pathology report should only contain one set of measurements; in other words, there should not be separate gross and microscopic measurements in the report. The single set of measurements provided should be based on a correlation of the gross and microscopic features, with gross examination being more important for some tumour measurements and microscopic examination for others.

In providing the final tumour dimensions, the measurements in a prior specimen, for example an excisional biopsy, may need to be taken into account. Although it may overestimate the maximum horizontal extent, it is recommended to add together the maximum horizontal measurement in different specimens when calculating the final horizontal extent. The depth of invasion can be taken as the maximum (largest) depth of invasion in the two different specimens.

If the tumour involves a margin (skin, mucosal or deep), a comment should be made regarding the possibility of underestimation of the horizontal dimension or depth of invasion.
Measurement of depth of invasion

As discussed, the maximum depth of tumour invasion must be measured in all cases since invasion >1 mm signifies greater than Stage IA and typically results in inguinofemoral lymphadenectomy being undertaken. This measurement is taken from the most superficial dermal papilla adjacent to the tumour to the deepest point of invasion (conventional measurement) (refer to Figure 1). An alternative method of measuring the depth of invasion has been proposed whereby the depth of invasion is measured from the basement membrane of the deepest adjacent dysplastic (tumour free) rete ridge to the deepest point of invasion.\textsuperscript{13,14} This method of invasion results in ‘downstaging’ of some Stage IB tumours to IA. In one study, the downstaged patients developed less recurrences and had a higher disease-specific survival compared with the patients who remained Stage IB.\textsuperscript{13} Using the alternative method for measuring depth of invasion would have resulted in 19\% of patients with vulval squamous cell carcinoma not undergoing lymphadenectomy with less treatment-related morbidity. In another study, all tumours which were downstaged using this method of measuring depth of invasion had no nodal metastasis, lymphovascular or perineural invasion.\textsuperscript{14} Although the results of these studies are promising, more prospective data on a higher number of patients is necessary before this alternative method of measuring depth of invasion can be recommended and currently the conventional method is recommended.

There is significant interobserver variability in assessment of superficial invasion, including disagreements about to whether or not there is invasion and whether the invasion is ≤1 mm or >1 mm (Stage IA versus Stage IB).\textsuperscript{15,16}
Figure 1: Schematic diagram showing measurement of depth of invasion in vulval carcinomas. A shows the traditional (recommended) method of measurement from the adjacent most superficial dermal papilla to the deepest point of invasion while B shows an alternative (not currently recommended) method from the basement membrane of the deepest adjacent dysplastic (tumour free) rete ridge to the deepest point of invasion. Permission courtesy of Mr Norm Cyr.

Note 6 – Block identification key (Non-core)

The origin/designation of all tissue blocks should be recorded. This information should ideally be documented in the final pathology report and is particularly important should the need for internal or external review arise. The reviewer needs to be clear about the origin of each block in order to provide an informed specialist opinion. If this information is not included in the final pathology report, it should be available on the laboratory computer system and relayed to the reviewing pathologist. It
may be useful to have a digital image of the specimen and record of the origin of the tumour blocks in some cases.

Recording the origin/designation of tissue blocks also facilitates retrieval of blocks for further immunohistochemical or molecular analysis, research studies or clinical trials.

**Note 7 – Histological tumour type (Core)**

All tumours should be typed according to most recent edition of the World Health Organization (WHO) Classification of Tumours of Female Genital Tumours, 5th edition, 2020. The ICCR dataset includes 5th edition Corrigenda, June 2021. Squamous cell carcinoma (SCC) is by far the most common carcinoma to arise on the vulva. Traditional histologic subtyping of SCC, using terms such as keratinizing, non-keratinizing, basaloid and warty, has been superseded by human papillomavirus (HPV) status as the major determinant of classification. Vulval SCC is divided into HPV-associated and HPV-independent types. HPV-independent SCC have a worse prognosis with significantly worse recurrence free and overall survival compared to HPV-associated SCC. There is also growing evidence that HPV-independent SCC are less responsive to radiotherapy. HPV-associated SCC are secondary to persistent infection by oncogenic high-risk HPV (most commonly types 16 and 18) and are associated with smoking, immunosuppression and often multifocal disease including HPV-associated lesions in other areas of the lower female genital tract (vagina, cervix) and anal/perianal regions. HPV-independent SCC often arise in the setting of lichen sclerosus and chronic inflammation. Verrucous carcinoma falls under the umbrella of HPV-independent SCC. The majority of HPV-associated SCC exhibit basaloid or warty morphology, while HPV-independent SCC tend to be keratinizing; however, a significant percentage of cases (15-20%) will show overlapping morphologic features. The nature of any adjacent precursor lesion may be useful in helping to determine the HPV status. However, in practice, ancillary testing is necessary to determine the HPV status given the overlap in morphology in some cases (see Note 13 ANCILLARY STUDIES). When HPV status cannot be confidently determined or resources are not available to undertake ancillary testing, a morphological diagnosis of SCC, not otherwise specified (NOS) is acceptable, although this is not recommended.

Most, but not all, HPV-independent vulval SCC are associated with TP53 mutations. However, a proportion are TP53 wild-type and there is growing evidence that these may have an intermediate prognosis between HPV-associated SCC and HPV-independent TP53 mutated neoplasms.

Grading of vulval SCC is not recommended and is not included in this dataset. Grading has not been shown to consistently correlate with clinical outcome. In fact, there is a paradox in that HPV-independent SCC, which tend to be keratinising and often well-differentiated have a worse prognosis than HPV-associated SCC which are typically non-keratinising, basaloid and poorly differentiated. In addition, no validated grading system exists for vulval SCC.

Basal cell carcinomas are histologically identical to their counterparts occurring in other cutaneous locations. A variety of adenocarcinomas rarely arise in the vulva and these should be diagnosed using the 2020 WHO Classification. These may be of mammary gland type (various types as in the breast), of sweat gland origin (various types), intestinal type or arise from Paget disease (invasive Paget). Before diagnosing a primary vulval adenocarcinoma, a metastasis from elsewhere should always be considered, and correlation of the clinical picture (including the past history) with pathological features, including immunohistochemical studies, may assist.
A variety of carcinomas (squamous, glandular, ‘salivary-type’ and other) can arise from the Bartholin gland. To be considered a Bartholin gland primary, the tumour should involve the anatomic region of the Bartholin gland and be histologically compatible with an origin in Bartholin gland with no alternative primary site identified elsewhere; preferably normal Bartholin gland tissue should be present in the vicinity of the neoplasm.

Neuroendocrine neoplasia is classified according to the 2020 WHO Classification (neuroendocrine tumour, small cell neuroendocrine carcinoma, large cell neuroendocrine carcinoma, mixed neuroendocrine-non-neuroendocrine carcinoma, Merkel cell carcinoma). Some vulval neuroendocrine carcinomas are driven by HPV-infection, while some Merkel cell carcinomas are driven by polyomavirus.

Table 1: World Health Organization classification of malignant epithelial tumours of the vulva.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>ICD-O codesa</th>
</tr>
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<tbody>
<tr>
<td>Squamous cell carcinoma, HPV-associated</td>
<td>8085/3</td>
</tr>
<tr>
<td>Squamous cell carcinoma, HPV-independent</td>
<td>8086/3</td>
</tr>
<tr>
<td>Squamous cell carcinoma NOS</td>
<td>8070/3</td>
</tr>
<tr>
<td>Basal cell carcinoma NOS</td>
<td>8090/3</td>
</tr>
<tr>
<td>Adenocarcinoma of anogenital mammary-like glands</td>
<td>8500/3</td>
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<tr>
<td>Bartholin gland lesions</td>
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<tr>
<td>Squamous cell carcinoma NOS</td>
<td>8070/3</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>8200/3</td>
</tr>
<tr>
<td>Carcinoma, poorly differentiated, NOS</td>
<td>8020/3</td>
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<tr>
<td>Adenosquamous carcinoma</td>
<td>8560/3</td>
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<tr>
<td>Neuroendocrine tumour NOS</td>
<td>8240/3</td>
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<tr>
<td>Myoepithelial carcinoma</td>
<td>8982/3</td>
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<td>Epithelial–myoepithelial carcinoma</td>
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<td>Paget disease, extramammary</td>
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<td>Porocarcinoma NOS</td>
<td>8409/3</td>
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<td>Adenoid cystic carcinoma</td>
<td>8200/3</td>
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<tr>
<td>Adenocarcinoma, intestinal type</td>
<td>8144/3</td>
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</tbody>
</table>

a These morphology codes are from the International Classification of Diseases for Oncology, third edition, second revision (ICD-O-3.2). Behaviour is coded /0 for benign tumours; /1 for unspecified, borderline, or uncertain behaviour; /2 for carcinoma in situ and grade III intraepithelial neoplasia; /3 for malignant tumours, primary site; and /6 for malignant tumours, metastatic site. Subtype labels are indented. Incorporates all relevant changes from the 5th Edition Corrigenda June 2021. © World Health Organization/International Agency for Research on Cancer. Reproduced with permission.
Note 8 – Lymphovascular invasion (Core)

Lymphovascular invasion is an adverse prognostic factor associated with increased risk of local recurrence, lymph node metastasis and poorer survival in vulval SCC.\textsuperscript{36-39} Two recent systematic reviews have highlighted some conflicting data on the prognostic significance of lymphovascular invasion from different studies,\textsuperscript{29,40} but it should be noted that the criteria for lymphovascular invasion were often not specified and there might be substantial variability in terms of diagnostic thresholds. The published studies also did not distinguish between focal or extensive lymphovascular invasion.\textsuperscript{29,40}

Caution is needed when distinguishing genuine lymphovascular invasion from mimickers, such as ‘carry-over’ of tumour cells into lymphovascular spaces or retraction artefacts. In one study of vulval carcinomas, the use of D2-40 immunohistochemistry as a marker of lymphatic vessels demonstrated improved detection of lymphovascular invasion as compared to morphology alone.\textsuperscript{41}

While usually straightforward, the assessment of lymphovascular invasion may be difficult in a minority of cases, for which the reasons may include (but are not limited to) suboptimal fixation or cauterisation artefacts. In such cases, examination of multiple levels and/or immunostaining for endothelial or lymphatic markers (such as CD31, CD34, D2-40) may be employed to assist with the decision-making. Cases that are still equivocal after taking additional steps may be reported as ‘indeterminate’ for lymphovascular invasion, but this designation should only be sparingly used and it is useful to provide the reason in a comment in the report.

Note 9 – Perineural invasion (Non-core)

Perineural invasion has been specifically evaluated by several retrospective studies, which demonstrated an association with significantly shorter overall survival and disease-free survival in patients with vulval SCC.\textsuperscript{42-44} Perineural invasion is also an independent predictor of local recurrence based on multivariate analysis in two studies.\textsuperscript{44,45}

Immunohistochemistry was used as an adjunct to identify perineural invasion in several studies which showed its prognostic value,\textsuperscript{43-45} either by S100 alone or dual immunohistochemistry with S100 and AE1/3. Immunohistochemistry may be useful to assist with cases that are morphologically inconclusive or suspicious for perineural invasion.

Note 10 – Margin status (Core and Non-core)

Appropriate sections need to be taken to include the nearest peripheral epithelial/ mucosal margin and the deep margin.

Vulval cancer patients with positive or ‘close’ surgical margins are at high risk of local recurrence. A clearance of at least 8 mm from the tumour has been suggested as the distance required to significantly reduce this risk of local recurrence. Recent studies, however, show no difference in recurrence between <8 mm and ≥8 mm tumour free surgical margin.\textsuperscript{46,47} It is also likely that the risk of recurrence with regard to tumour distance to the nearest margin differs between HPV-associated and HPV-independent SCC.\textsuperscript{19} It is clear that there are multiple problems in measuring the distance to
surgical margins with no clear guidelines as to how measurements should be undertaken. Separate gross and microscopic distances to margins should not be provided on the pathology report, but rather a single set of measurements. To ensure a standardised approach regarding margin measurements for vulval carcinomas, it is recommended that surgical margins should be inked and the following recommendations adhered to:48

- Involvement of a peripheral (skin, mucosal) surgical margin by tumour should be recorded and the margin specified if possible.
- The minimum distance from invasive carcinoma to the peripheral margin should be reported and the margin specified if possible.
- This peripheral surgical margin should be roughly perpendicular to the skin/mucosal surface; this includes the epithelial surface and deeper soft tissue.
- The peripheral margin should be measured toward the peripheral stromal edge or surface-epithelial edge, whichever is shorter.
- The minimum peripheral margin should be measured through tissue and preferably in a straight uninterrupted line; however, in some situations (collarette), a composite measurement including separate linear measurements joined at an angle may be required.
- Measuring the distance to the margin by a curved line in the context of an irregular surface, which is now possible due to the increased use of digital pathology, is not recommended, unless this is felt to represent a truer measurement, for example, when a length of uninvolved skin is embedded curved/folded in order to fit into a paraffin block.
- Involvement of a peripheral margin by a high grade precursor lesion (HPV-associated high grade squamous intraepithelial lesion (HSIL) or HPV-independent vulval intraepithelial neoplasia (VIN)) should be recorded and the margin specified if possible; p53 immunohistochemical staining may be of value in assessing margin involvement by HPV-independent VIN (see Note 13 ANCILLARY STUDIES). Margin involvement by a low grade precursor lesion (low grade squamous intraepithelial lesion (LSIL)) does not need to be recorded.
- Although there is no clear evidence to support the value of recording the distance of high grade precursor lesions from the nearest peripheral margin and thus this cannot be considered a core element, it is recommended that this measurement be included in the report and collection of this data prospectively may facilitate future studies which will determine the importance of this. This measurement is made along the epithelial surface. The distance from the margin of a LSIL does not need to be recorded.
- The minimum distance of invasive tumour to the deep soft tissue margin should also be recorded. In general, this should be measured from the deepest infiltrating tumour nest to the deep soft tissue margin. However, if the deep margin is irregular, the closest deep margin may not necessarily be at the point of deepest invasion; in such cases, this should be taken into account when providing this measurement.

Note 11 – Lymph node status (Core)

Lymph node involvement in vulval cancer is one of the most important adverse prognostic parameters,29 and the appropriate management and pathological assessment of regional (inguino-femoral) lymph nodes is considered the most important factor in reducing mortality from early vulval cancer.49 Regional nodal assessment is therefore typically indicated in all carcinomas (with the exception of basal cell carcinomas) that are greater than International Federation of Gynecology and Obstetrics (FIGO) Stage IA (pT1A) on clinicopathological assessment, i.e., those that exceed 20 mm
in maximum size, those with greater than 1 mm depth of invasion and those of any size that involve adjacent structures (lower third of urethra, lower third of vagina or anus).50,51 Clinically suspicious/palpable inguinal nodes should be biopsied. Tumours that are <40 mm in size and ≥20 mm from the midline are usually managed by an ipsilateral inguinofemoral lymphadenectomy. Bilateral inguinofemoral lymphadenectomy is typically undertaken in tumours larger than 40 mm, those that cross or are located within 20 mm of the midline, or those that clinically or radiologically are felt to have positive ipsilateral lymph nodes.52 Significant changes in surgical practice in the last decades, both in terms of vulvar excision and nodal assessment have led to publication of algorithms to help direct surgical procedure.

When lymphadenectomy is performed, one or more sections of all identified nodes should be submitted for histological examination, including sections containing perinodal fat to confirm the presence or absence of extracapsular extension, especially if grossly suspected. For nodes which are grossly involved by tumour, representative sampling is acceptable whereas nodes which are not suspicious should be submitted in their entirety after sectioning at 2 mm intervals perpendicular to the long axis of the node. Ultrastaging does not need to be performed for lymphadenectomies (see discussion on sentinel lymph node biopsy (SLNB) below)).

Lymph node status is a powerful indicator of local recurrence and survival. The site, size and nature of nodal metastasis all influence prognosis and are integral to tumour stage. Involvement of regional lymph nodes represents Stage III, and this is further subdivided according to the number of involved nodes, the maximum size of the deposit and the presence or absence of extracapsular spread. It has been shown in multivariate analysis that extracapsular lymph node spread is an independent prognostic factor for earlier recurrence and overall survival.44 The presence of fixed or ulcerated inguinofemoral lymph nodes as determined by clinical examination, or of involvement of non-regional, including pelvic, lymph nodes, upstages the carcinoma to Stage IVA or IVB respectively. The anatomic location and number of lymph nodes dissected, the number containing tumour and the size of the largest tumour deposit should be accurately documented in the pathology report.

In recent years, owing to the high morbidity of groin dissection, SLNB has become the standard of care in some vulval cancers.53-55 SLNB can be performed for unifocal lesions which are confined to the vulva and less than 40 mm in size, with no prior vulval or groin surgery or radiation, and in the absence of clinically palpable or radiologically suspicious nodes. The evaluation of sentinel lymph nodes should follow an established locally agreed protocol. It should be documented whether or not an ultrastaging procedure has been carried out and whether nodal metastases have been detected on routine histological examination (without ultrastaging) or by ultrastaging, including cytokeratin immunohistochemistry. Sentinel (and non-sentinel) nodal involvement should be recorded as presence of isolated tumour cells (ITC), micrometastases (MIC) or macrometastases (MAC). An ideal ultrastaging protocol used should detect almost all MIC (0.2-2 mm). The anatomic location and number of lymph nodes dissected, the number containing tumour, the size of the largest tumour deposit and the presence or absence of extracapsular spread should be accurately documented in the pathology report. According to TNM8,56 nodal involvement should be recorded as the presence ITC (<0.2 mm), MIC (0.2-2 mm) or MAC (>2 mm). MAC are regarded as pN1, MIC as pN1 (mi) and ITCs are pN0 (i+); ITCs do not upstage a carcinoma. The possibility of performing radiologically-guided fine needle aspiration cytology (FNAC) of suspicious lymph nodes should be considered. A positive result enables the surgeon to immediately perform a bilateral inguinofemoral lymphadenectomy, thus avoiding an unnecessary SLNB.
**Note 12 – Coexistent pathology/precursor lesions (Core)**

Recording the presence of precursor lesions and coexistent pathology is important for vulval SCC since this gives insight into the pathogenesis of the tumour, specifically whether it is HPV-associated or HPV independent. Margin involvement by a high grade precursor lesion is also important.

A variety of non-invasive lesions may be present in association with SCC. Some are considered to be precursor lesions while others, such as lichen sclerosus, are not considered to be a precursor lesion but rather a chronic inflammatory condition that increases the risk of HPV-independent SCC and cancer recurrence when present at surgical margins.

The presence of the following should be noted in the setting of vulval SCC: HPV-associated squamous intraepithelial lesion (LSIL or HSIL), HPV-independent VIN and lichen sclerosus.

Vulval squamous precursor lesions are classified into HPV-associated and HPV-independent. The HPV-associated lesions predominantly comprise HSIL (VIN 2/3). LSIL in the vulva is uncommon aside from condylomatous lesions. HPV-associated precursor lesions are associated with smoking, immunosuppression and often multifocal disease including HPV-associated lesions in other areas of the lower female genital tract (vagina, cervix) and anal/perianal regions. HPV-independent precursor lesions, collectively termed ‘VIN, HPV-independent’, include differentiated VIN (dVIN) and two uncommon lesions termed vulvar acanthosis with altered differentiation (VAAD) and differentiated exophytic vulvar intraepithelial lesion (DEVIL). The latter two lesions show significant morphological overlap and are likely part of a spectrum of HPV-independent precursor lesions. dVIN is typically associated with TP53 mutations while VAAD and DEVIL usually do not contain mutations.

Biomarkers may be useful for appropriate classification of precursor lesions given that both HPV-independent premalignant lesions morphologically indistinguishable from HSIL and HPV-associated intraepithelial precursors simulating dVIN have been described (see **Note 13 ANCILLARY STUDIES**).

**Note 13 – Ancillary studies (Core and Non-core)**

As discussed (see **Note 7 HISTOLOGICAL TUMOUR TYPE**), the 2020 WHO Classification categorises vulval SCC into two main types, HPV-associated and HPV-independent, with prognostic implications which have already been discussed. This new diagnostic approach has consequences since, as discussed, morphology is not always reliable in distinguishing between the two types. It implies that the use of ancillary techniques, namely p16 immunohistochemistry and/or HPV molecular testing, are considered as essential to correctly classify vulval SCC. Similarly, although the HPV-associated and HPV-independent intraepithelial precursors of SCC have distinctive features (see **Note 12 COEXISTENT PATHOLOGY/PRECURSOR LESIONS**), both HPV-independent premalignant lesions morphologically indistinguishable from HSIL and HPV-associated intraepithelial precursors simulating dVIN have been described. Therefore, p16 staining and/or molecular testing (see below) are also highly desirable in classifying precursor lesions. p16 immunohistochemistry and/or HPV testing is considered a core element in cases of vulval SCC. In practice, almost all laboratories will perform p16 immunohistochemistry rather than HPV testing. As discussed earlier, when HPV status cannot be confidently determined or resources are not available to undertake ancillary testing, a morphological diagnosis of SCC, NOS is acceptable, although this is not recommended. This is especially likely in laboratories in developing countries and including these ancillary techniques as a core element may enable laboratories to introduce these tests. If p16 immunohistochemistry and/or HPV testing has been performed on a diagnostic biopsy, it does not need to be repeated on the resection specimen.
although it is useful to record the results on the report of the resection specimen. Similarly, these tests do not need to be repeated on a tumour recurrence.

As discussed, the two accepted tools for confirming an HPV-association are the direct identification of HPV products (DNA or mRNA) and block-type staining for p16, a cell protein typically overexpressed in transforming HPV infections. Although the results of both methods are usually in agreement and it has been proposed that a positive result with both techniques is the gold standard for classifying a tumour as HPV-associated, discrepancies are observed in a small number of cases when the two techniques are applied. Moreover, most laboratories are not likely to have access to HPV testing and, as discussed, p16 immunohistochemistry is likely to be the method of choice in most laboratories.

One of the main challenges of HPV molecular testing methods in vulval samples is that HPV identification is usually performed on formalin-fixed, paraffin-embedded tissues, which may result in limitations due to fragmentation of DNA and RNA, associated with the tissue processing. Thus, highly sensitive methods, such as SPF10 polymerase chain reaction (PCR) testing are the most used tests, but large series have reported both false positive and false negative results with this test. In situ hybridisation for HPV E7 mRNA, one of the oncogenic HPV genes has shown good results in tumours of the uterine cervix, but the experience in vulval lesions is limited.

p16 immunohistochemical staining has shown a good correlation with HPV testing. Although isolated cases of HPV-associated tumours with ‘negative’ p16 staining have been reported in the cervix and vulva, there is evidence indicating that the accuracy to classify a tumour as HPV-associated or HPV-independent is probably higher for p16 than for most of the available HPV tests. It has also been shown that p16 expression alone is closely associated with prognosis. In addition to its high accuracy, p16 immunohistochemistry is available in most pathology laboratories. It is important to stress that only so-called ‘block-type’ p16 staining in a squamous lesion (in situ or malignant) is supportive of an association with oncogenic high-risk HPV. Block-type staining in an in-situ lesion is defined as strong and continuous typically nuclear and cytoplasmic (less frequently only nuclear) immunoreactivity in all epithelial cells in the basal and parabasal layers with upward extension. Upward extension must involve at least the lower one-third of the epithelial thickness and expression must extend for at least 6 cells across. It is acknowledged that the criteria defining the horizontal and upward extent are arbitrary but these serve to improve specificity. In HPV-associated SCC, there is typically diffuse positive staining involving almost every tumour cell but keratinous areas may be negative. It also needs to be stressed that p16 staining should not be reported simply as positive since HPV-independent premalignant and malignant lesions and non-neoplastic tissues may exhibit focal (so-called mosaic) staining. Instead terms such as ‘block-type’, ‘abnormal’ or ‘aberrant’ should be used in the pathology report, or alternatively when the term positive is used this should be qualified as diffuse or ‘block-type’.

Other ancillary studies are regarded as non-core and when undertaken the results should be documented on the pathology report. One of the most useful markers is p53 and many HPV-independent vulval SCC contain TP53 mutations. Almost all HPV-associated vulval SCC and high grade precursor lesions exhibit a ‘wild-type’ pattern of p53 immunoreactivity while many, but importantly not all, HPV-independent SCC and precursor lesions exhibit ‘mutation-type’ immunoreactivity. Classification of p53 staining in such lesions as ‘wild-type’ or ‘mutation-type’ is not always straightforward with different patterns of both types of staining being described. In addition, there is emerging evidence that not all HPV-independent SCC and precursor lesions are associated with TP53 mutations and that TP53 wild-type tumours may have a better prognosis than those harbouring TP53 mutations. p53 staining may be helpful in assessing margin involvement by HPV-independent dVIN; this may be subtle histologically and mutation-type p53 staining at a margin may be useful in confirming margin involvement.
Additional biomarkers, such as PD-L1, may become useful in the future as the role of immune checkpoint inhibitor therapy in vulval squamous carcinomas becomes established through ongoing clinical trials.\textsuperscript{76}

Note 14 – Pathologically confirmed distant metastasis (Core)

Documentation of known metastatic disease is an important part of the pathology report. Such information, if available, should be recorded with as much detail as is available including the site, whether the specimen is a histopathology or cytopathology specimen and with reference to any relevant prior surgical pathology or cytopathology specimens.

Note 15 – Provisional pathological staging (Core)

The pathological staging must be provided on the pathology report and is therefore a core element. The term ‘provisional pathological staging’ is used in this dataset to indicate that the stage that is provided may not represent the final tumour stage which should be determined at the multidisciplinary tumour board meeting where all the pathological, clinical and radiological features are available.\textsuperscript{49,56,77,78}

The latest version of either FIGO or TNM staging, or both, can be used depending on local preferences.\textsuperscript{49,56,77,78} The FIGO Staging System is in widespread use internationally and is the system used in most clinical trials and research studies. However, Union for International Cancer Control (UICC) or American Joint Committee on Cancer (AJCC) versions of TNM are used or mandated in many parts of the world.\textsuperscript{56,78} With regards to updating of staging systems, there is collaboration between FIGO and those agencies responsible for TNM with an agreement to adopt changes to FIGO staging. Following the introduction of a new FIGO Staging System, the amendments are usually incorporated into TNM (both UICC and AJCC) versions at a later date. Apart from minor discrepancies in terminology, the UICC and AJCC systems are broadly concurrent.

A tumour should be staged following diagnosis using various appropriate modalities (clinical, radiological, pathological). While the original tumour stage should not be altered following treatment, TNM systems allow staging to be performed on a resection specimen following non-surgical treatment (for example chemotherapy, radiotherapy); in such cases, if a stage is being provided on the pathology report (this is optional), it should be prefixed by ‘y’ to indicate that this is a post-therapy stage.

In cases where more than one primary tumour is present, a separate pathological stage should be provided for each tumour and, as stated in the scope, separate datasets should be completed for each neoplasm.

The reference document TNM Supplement: A commentary on uniform use, 5th Edition (C Wittekind et al. editors) may be of assistance when staging.\textsuperscript{79}
References

1 Merlin T, Weston A and Tooher R (2009). Extending an evidence hierarchy to include topics other than treatment: revising the Australian 'levels of evidence'. *BMC Med Res Methodol* 9:34.


