Ancillary studies (Core and Non-core)

As discussed (see **HISTOLOGICAL TUMOUR TYPE**), the 2020 World Health Organization (WHO) Classification categorises vulval squamous cell carcinoma (SCC) into two main types, human papillomavirus (HPV)-associated and HPV-independent,[1] with prognostic implications which have already been discussed.[2-6] This new diagnostic approach has consequences since, as discussed, morphology is not always reliable in distinguishing between the two types.[7,8] 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.[1] Similarly, although the HPV-associated and HPV-independent intraepithelial precursors of SCC have distinctive features (see **COEXISTENT PATHOLOGY/PRECURSOR LESIONS**), both HPV-independent premalignant lesions morphologically indistinguishable from high grade squamous intraepithelial lesion (HSIL) and HPV-associated intraepithelial precursors simulating differentiated vulval intraepithelial neoplasia (dVIN) have been described.[9-12] 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, not otherwise specified (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,[13] discrepancies are observed in a small number of cases when the two techniques are applied.[7] 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.[8] 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.[7,8,13] In situ hybridisation for HPV E7 mRNA, one of the oncogenic HPV genes has shown good results in tumours of the uterine cervix,[14] but the experience in vulval lesions is limited.

p16 immunohistochemical staining has shown a good correlation with HPV testing.[3,4,6-8,13] Although isolated cases of HPV-associated tumours with 'negative' p16 staining have been reported in the cervix and vulva,[15] 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.[7] It has also been shown that p16 expression alone is closely associated with prognosis.[2-6] 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.[16] 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.[17,18] 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.[19]

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