



Diagnosis of Vulval Cancers Under the Microscope

Supporting Images are found in the document

Type of vulval cancer	Microscopic features	Immunohistochemistry markers
Vulvar intraepithelial neoplasia/ differentiated vulvar intraepithelial neoplasia. (HPV independent)	Basal cell atypia Abnormal keratinocyte differentiation	Standard – p16 – cyclin dependent kinase inhibitor for all VINs more specifically CDK4 and CDK6. Cyclin Dependent Kinase regulates the cell cycle and binds with cyclin proteins to elicit its effects in the different phases. This prevents phosphorylation of Rb. Later, phosphorylated Rb becomes bound with E2F transcription factors in the nucleus preventing gene transcription. The normal role for p16 as a tumour suppressor gene is to prevent progression of S phase. It regulates Growth 1 (G1)/S phase (DNA replication). P16 is involved in responding to DNA damage, cellular stress and RAS oncogene/MAPK signalling, oxidative stress and ageing. It accumulates when transforming HPV (it is mainly applied for prognosis and needs to be combined with other immunohistochemical markers to increase accuracy) Loss of p16 is an indicator of dysregulation of retinoblastoma signalling pathway and increased progression. Some cases of non-HPV, protein is silenced or lost by mutation, deletion or methylation of the promoter CpG islands. CpG is a section in the DNA that consists of cytosine followed by guanine). Block positive staining – It helps distinguish HPV independent and dependent. P53 staining of basal layer – not recommended in small biopsies due to difficulty visualising normal epithelium. If there is a presence of



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		non-block staining for p16 then p53 can be used. CK17 stronger diffuse expression in DVIN over uVIN and lichen simplex chronicus. Lichen simplex chronicus is a thick eczematous skin that develops in the area with constant rubbing, scratching and irritation. It is associated with stress. It causes a thickened, leathery, hyperpigmented skin due to chronic inflammation
Squamous cell carcinoma	No grading. Lymphovascular and/or perineural invasion (it had infiltrated or affect the lymph and nerves respectively).	P16 positive staining P16 is a cyclin dependent kinase inhibitor for VIN. It accumulates/increases when transforming HPV. The viral E7 oncoprotein inactivates Rb independent of CDK4 AND CDK6 activity. Loss of functional Rb affects the function of p16. This leads to overexpression of p16 immunostaining. This contributes to tumour development and proliferation independent of HPV tumourigenesis.
Vulval Paget's disease (VPD)	Rare intraepithelial adenocarcinoma. The stem cells present on the skin (epidermis) or in the sweat-oil glands (apocrine-sebaceous unit) is premature and has the ability to differentiate into different types of cells (pluripotency). There are boundaries (demarcated) and painful eczema-like	The tumour cells express the following: cytokeratin 7 – keratin proteins found in the cytoplasm of the cells. carcinoembryonic antigen (CEA) – protein produced in the foetus and also some cancers especially the colon. It is a tumour marker. Apocrine cell marker GCDFP15 – sweat gland that occurs in the hairy parts like the armpit and the groin. This helps to distinguish VPD from other intra-epidermal neoplasms such as malignant melanoma in situ and VIN. The borders of the lesions seen clinically correlate poorly with the histological extent of



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	lesion if 20% invasion has grown underneath the stroma (connective tissue). This commonly appears in the labia majora. Large round cells with pale cytoplasm. Red eczematous scaly plaques with white islands Nucleus and nucleolus are distributed throughout the lining epithelial as single cells.	the disease, which may account for the high rate of recurrence after primary surgery.
Vulvo-vaginal melanoma	It is distinctive from mucosal and cutaneous sites. The predominant location is different between cutaneous and mucosal melanoma.	PD-L1 (56 %) PD1 (75 %) Programmed cell death protein 1 (PD-1) receptor of T lymphocytes. It can associate with PD-L1 ligand on T cells and inactivate the T cells. This lowers the immune response. P16 commonly absent or less nuclear expression. Its presence does not rule it out. P16 applied with other microscopical findings to distinguish difference between benign melanocytic lesions and melanoma. Loss of p16 found in cutaneous melanoma and sinonasal melanoma and correlates with disease progression and adverse prognosis. Indicators for adverse prognosis are: increased tumour thickness, ulcer, recurrence, metastasis and less survival.



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Adenoid Cystic Carcinoma	Rounded islands of uniform malignant epithelial cells with a cribriform pattern and scant cytoplasm. Nucleus (dense staining/hyperchromatic) with basaloid appearance. They can be cord-like or massive sheets and cribriform cavities. Pure adenoid cystic carcinomas of the vulva are independent from HPV infection. Slight less eosinophils Hyalin structure Basophilic mucin.	Epithelial membrane antigen (EMA/MUC1) – It is a type of protein joined with a sugar (glycoprotein) and it aims to provide barrier function. It is overexpressed in many malignancies. It is expressed in glandular, luminal epithelial and haematopoietic cells. However, they are not found in the lining of the skin (epithelium), red blood cells, immune cells (natural killer cells) and mesenchymal cells. The mesenchyme are undifferentiated cells derived from the mesoderm and found in the skeletal, connective, blood and muscle cells. It prevents the formation of E-cadherin/beta catenin complex. EMA/MUC1 is commonly expressed along cytokeratin stains. Smooth Muscle Actin (SMA) – This is a form of actin that helps in the motility or movement of cells (alpha) and helps maintain the surrounding of the cell membrane called the cytoskeleton (beta and gamma). It can be found in the pericytes, smooth muscle cells, myofibroblasts and myoepithelial cells. Pericytes are star-shaped cells that are found around the capillaries and small blood vessels. It is found in normal, reactive or neoplastic tissue. carcinoembryonic antigen (CEA) – protein produced in the foetus and also some cancers especially the colon. It is a tumour marker. CD117/ C-KIT– It is a receptor tyrosine kinase for a growth factor receptor protein called kit protein. It helps develop and survive mast cells, haematopoietic stem cells, melanocytes, germ cells and cells of Cajal. Interstitial cells of Cajal (ICC) are found in the gastrointestinal tract from the oesophagus to the anus. It regulates smooth muscle cell function and motility in collaboration with the enteric nervous system (myenteric plexus, Auerbach's plexus, circular and longitudinal
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		muscles). Mutation in C-Kit can cause the interstitial cells of Cajal to grow into hyperplastic sheets and malignant gastrointestinal stromal tumours (GIST). Stem cell factor (SCF) is the ligand that binds to C-KIT. This is produced by the smooth muscle and enteric nerves, mediates the effect of insulin and Insulin growth factor (IGF-1) on ICC. When there are smooth muscle atrophies (waste of normal developed organ or tissue due to degeneration of cells), the resulting decrease in the SCF results in a loss of ICC. Vulva atrophy can also become thinner and less elastic due to less oestrogen in postmenopausal women. Vimentin - It is an intermediate filament protein made by mesenchymal cells. It helps identify mesenchymal origin of some tumours. This includes endothelial, vascular smooth muscle cells and endometrial stroma cells. It also helps to identify blood and immune cells, melanocytes, endometrial glands and male reproductive cells (Leydig and Sertoli cells). Leydig cells make the testosterone and Sertoli makes the sperm. They nourish germ cells (spermatids). Absence of vimentin confirms fat-like or other spaces that lack cellular lining. It also helps to distinguish between endocervical adenocarcinoma (vimentin negative) and endometrial carcinoma (vimentin positive). Negative staining of Carcinoma is an indication of Kruckenberg tumour. This is a gynaecological condition where there is malignant in one or both ovaries. The cause is a trans coelomic spread of primary growth in the stomach or intestines. Other examples of negative staining are urological where it affects the kidneys (chromophobe renal cell carcinoma, renal oncocytoma), soft tissue tumours (sarcoma) and immune (thymic carcinoma).
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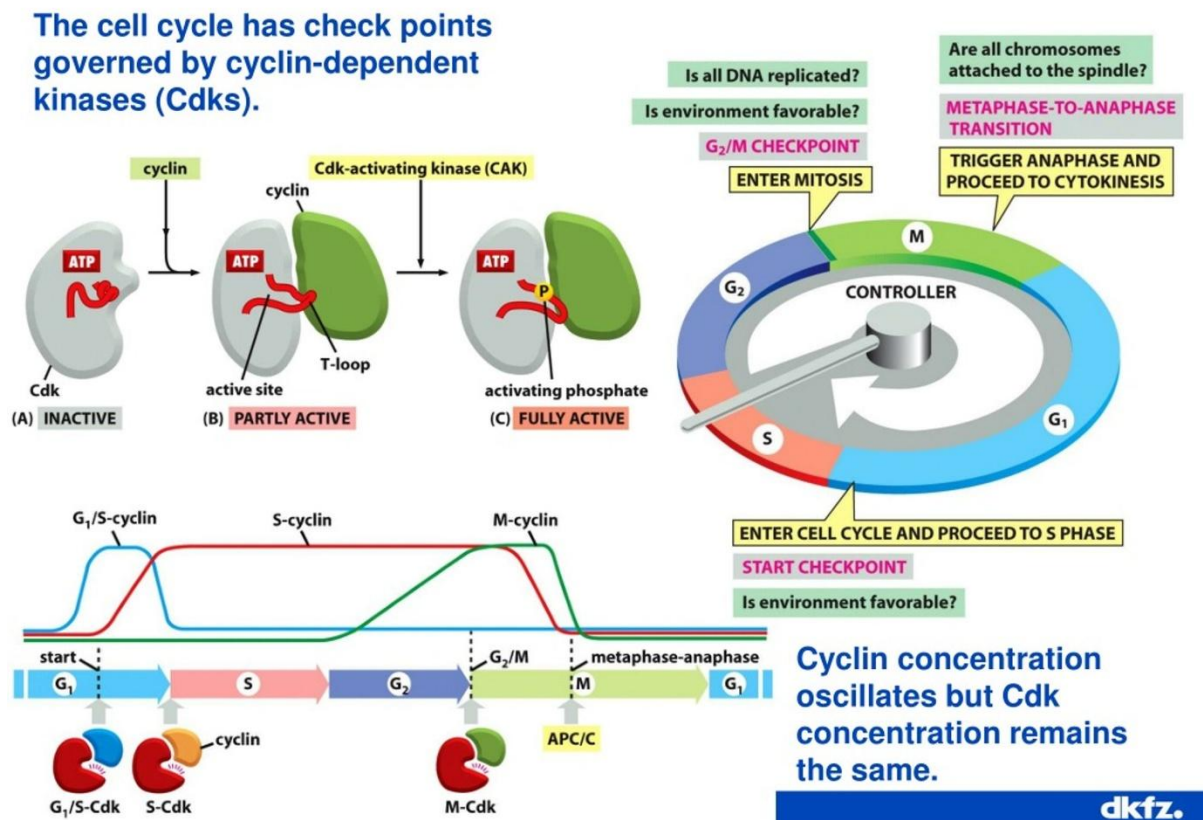
		High Ki-67 index – Ki-67 is a protein and indicates of how cancer cells are dividing. Higher percentage or scores with that the cells are growing faster. However, it cannot be used alone to determine the stage of the cancer.
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Supporting Images for histological images and Immunohistochemistry markers of Vulval Cancers

Figure 1: The cell cycle



The cell cycle is a series of events that living organisms undergo in cell division. It consists of the G₀ (quiescent) phase, Gap 1 (G₁) phase, S phase, Gap 2 (G₂) phase, and Mitosis (M phase). In G₀, cells leave the cell cycle and become dormant. They postpone making a decision to divide or not divide again. The G₁ phase is the longest but most important. It is approximately 11 hours for the cell to grow and accumulate changes. It then passes the restriction point and enters the S phase. The S phase is where DNA replication takes place. This is where the chromosomes, thread-like structures containing DNA material, are duplicated. This phase takes approximately 8 hours. During the G₂ phase, additional growth takes place and proteins are made to help with cell division. The G₁, G₂, and S phases are collectively called interphase. Once interphase is complete, mitosis begins, where one cell divides into two, and each daughter cell receives the same number of chromosomes as the parent cell. This is part of growth and repair. All of the phases in the cell cycle require regulation; this is achieved through checkpoints and proteins called cyclins and cyclin-dependent kinases. The checkpoints evaluate the environmental conditions and integrity of the genome, and control where the



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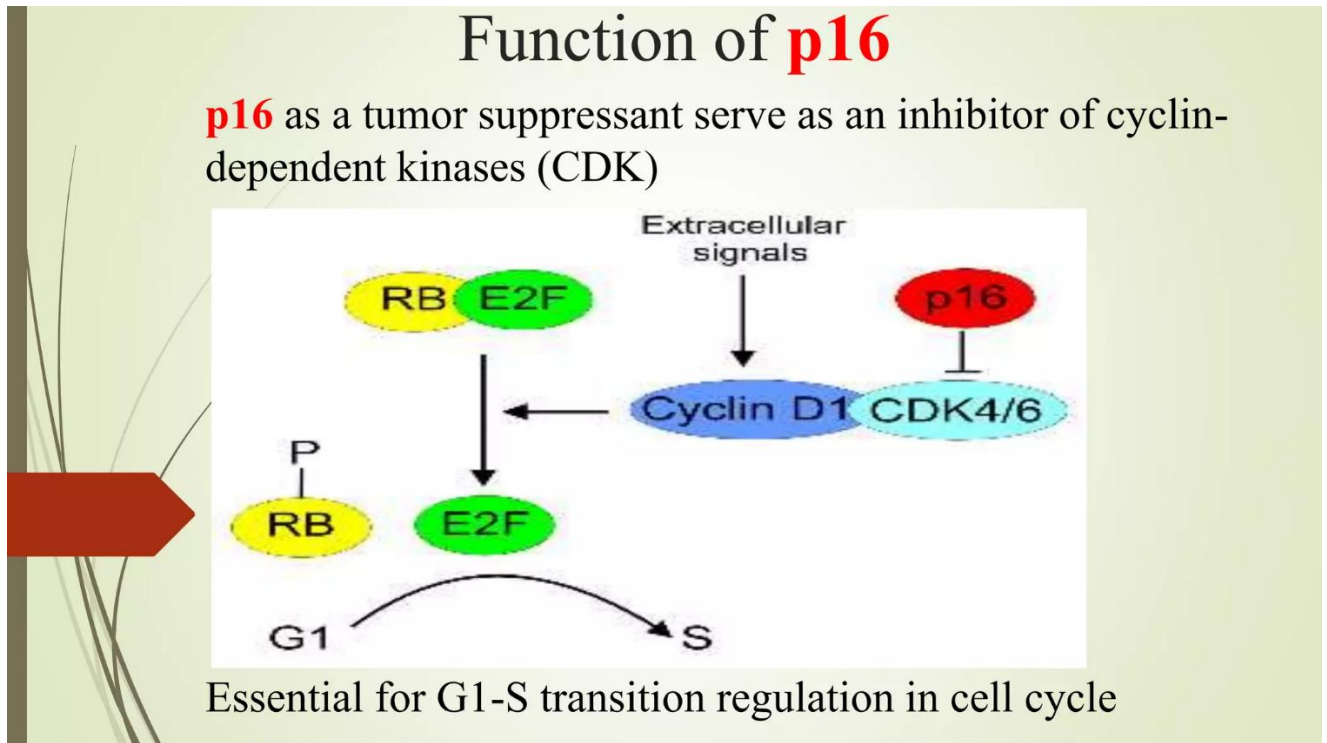
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cell progresses into the cell cycle. If there are any errors, they are repaired before progressing. There are four checkpoints: the restriction point, G1, G2, and the metaphase checkpoint. Cyclins, as their name suggests, fluctuate in level throughout the cell cycle. They become complementary and bind to cyclin-dependent kinase (CDK) and activate it. For instance, G1 cyclin binds to CDK to form a cyclin-CDK complex. The cyclin-CDK complex then phosphorylates proteins needed for the cell to progress to the next stage of the cell cycle; in this case, the proteins and enzymes needed for DNA synthesis that takes place in S phase.

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Figure 2: The Function of p16

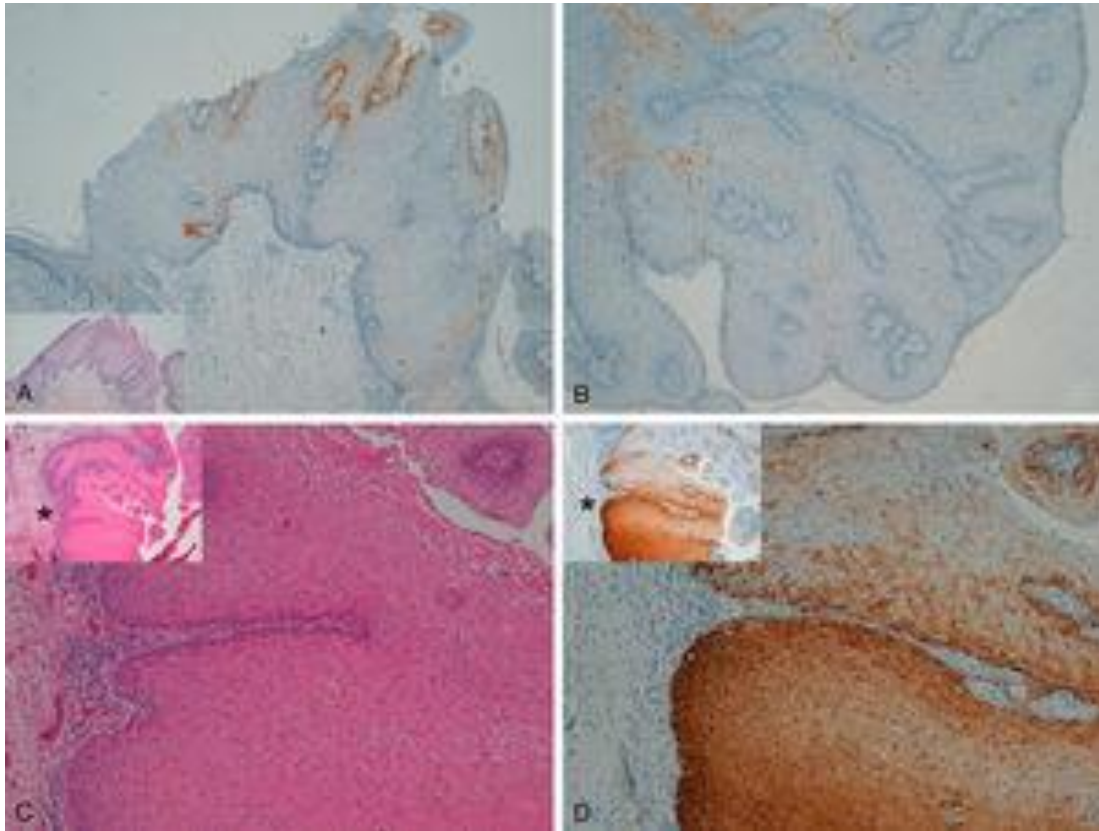


P16^{INK4A} is a tumour suppressor gene that inhibits cyclin-dependent kinase alongside p21 (Cip/Kip). The INK proteins associate with the cyclin-dependent kinase (cdk4/6) that commonly binds with Cyclin D1. Loss or dysregulation of p16 leads to cancer. Retinoblastoma (Rb) is a tumour suppressor protein and facilitates the G1-S phase transition where DNA replication takes place. This is achieved by RB regulating the activity of E2F transcription factors and interfering by binding to it via a section in E2F called the transactivation domain to block E2F function. The E2F transcription factors are vital in the expression of genes that encode proteins needed for DNA replication to take place in the S phase. The RB also interacts with HDAC (histone deacetylase) to prevent E2F target genes from being transcribed; HDAC deacetylates histones and increases chromatin compaction. Chromatin is a substance that contains DNA and proteins and is where chromosomes are made. They can be seen microscopically as euchromatin and heterochromatin. Histones are proteins that stabilise the DNA structure by binding to the nucleic acid and forming nucleoprotein.

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Figure 3: P16 positive and negative staining



Staining of the tumour suppressor protein p16 has been used to differentiate between lesions (Corbalán-Vélez et al., 2011). Image A presents p16 staining in genital warts with haematoxylin and eosin at X16 magnification. The magnification of the same sample rose to X40 for better observation of differences. In both cases, there is negative staining for p16. However, in isolated cells of genital warts, there is positive staining for p16.

Image C presents a genital wart of the vulva without carcinoma, which shows positive staining for p16. The magnification is X100.

Image D presents p16 staining at x100 magnification.

There are insets present in both Image C and D where there is a x40 magnification.

It is important to note that the positive immunostaining for p16 is strongly associated with mucosal or alpha subtypes of human papillomavirus (HPV) in cervical and genital squamous cell carcinoma (SCC). SCCs of the cervix originate via a single oncogenic pathway. This is particularly notable in subtypes 16, 18, 33, and 35, where 16 and 18 are oncogenic for cervical cancer.



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However, if it is squamous cell carcinoma present in the genital area (vulva, penis), it is p16 negative.

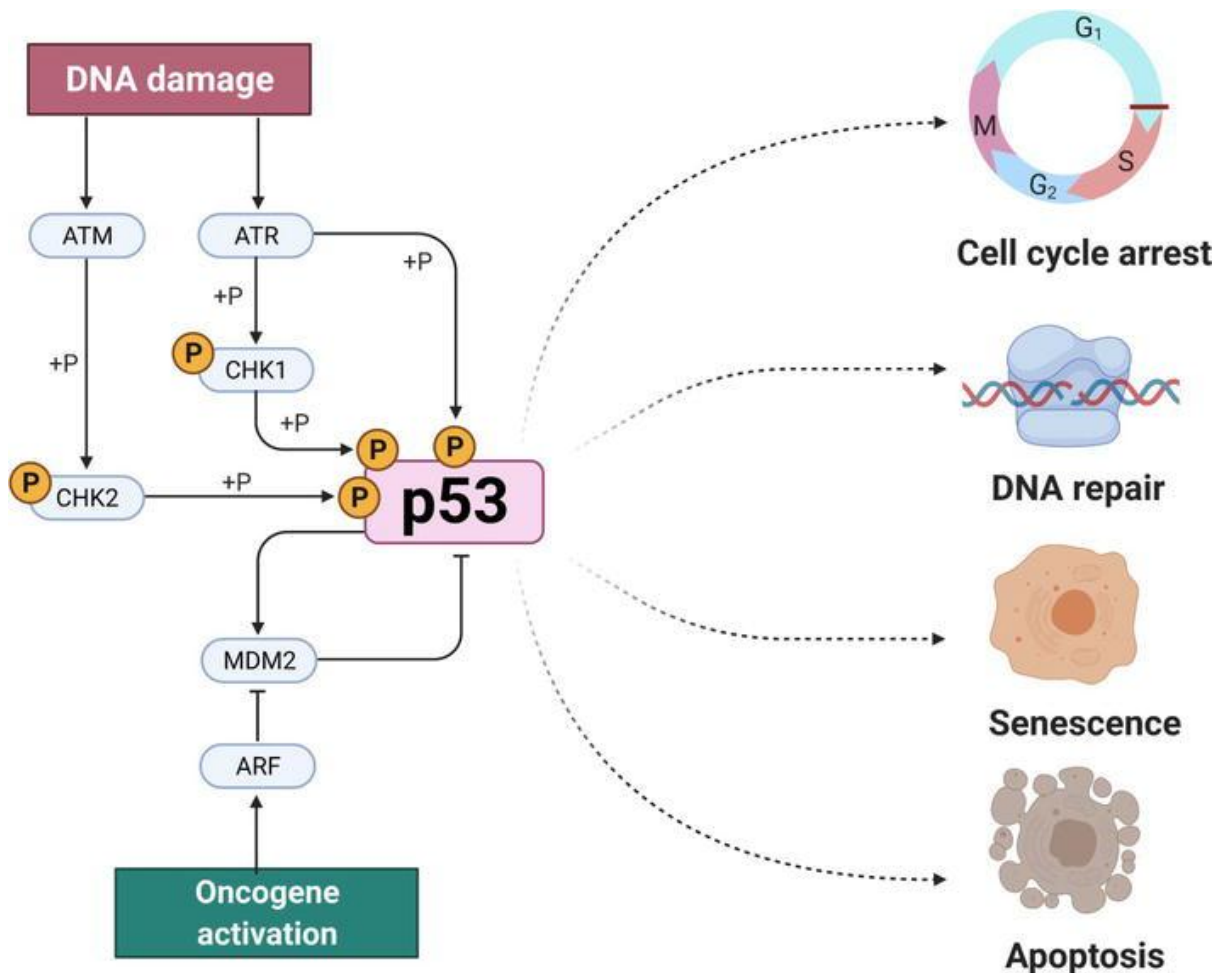
If it is present in intraepidermal and invasive SCC, they are p16-positive, whether genital or extragenital. This helped to locate additional structures, for instance, dermal microinfiltration or the influx of normal skin appendages.

On the other hand, extragenital invasive SCC is negative for p16 staining in areas that are invaded by the carcinoma but are focally positive in the epidermis that is adjacent to the SCC - the damaged areas by the sun where there is severe dysplasia and actinic keratosis lesions. The change in p16 protein presence is associated with progression to the invasive phase. Dysplasia is the abnormal development of the epithelium, whereas Actinic keratosis is the overgrowth of the skin that is characterised by red spots with a scaly surface in patients who are overexposed to the sun and may become malignant.

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Figure 4: P53 regulation

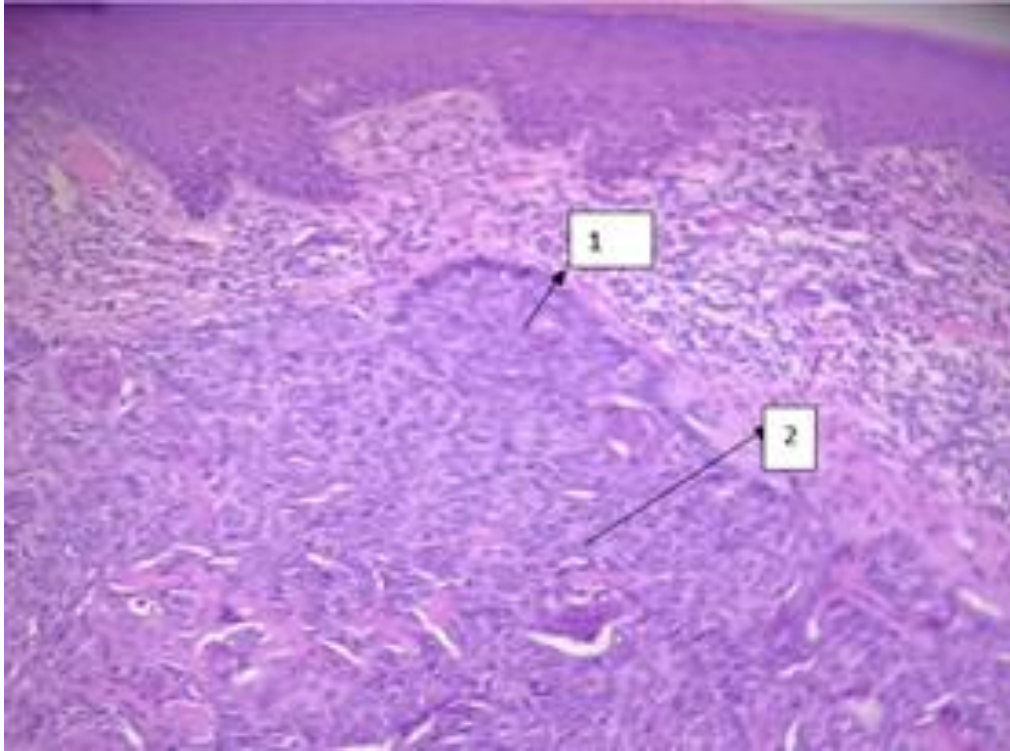


P53 under normal conditions (wild-type) is the guardian of the genome whose purpose is to suppress tumours, maintain the integrity of DNA, and induce cellular events like apoptosis (cell death), senescence (ageing), DNA repair, and to stop the cell cycle in response to DNA damage, stress, or hypoxia (low oxygen). P53 is activated by the process of phosphorylation (the addition of phosphate groups). Under mutant-type p53, hot-spot mutations provide a growth advantage for tumours and are mainly associated with squamous cell carcinoma and basal cell carcinoma rather than melanoma. It is regulated by a protein/enzyme (ubiquitin ligase) called MDM2. MDM2 is a transcriptional target for p53; however, MDM2 also negatively regulates p53, causing its degradation by binding to p53's transactivation domain and transporting it to the cytoplasm to be targeted by proteasomes, structures where degradation takes place.

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Figure 5: Basal cell carcinoma

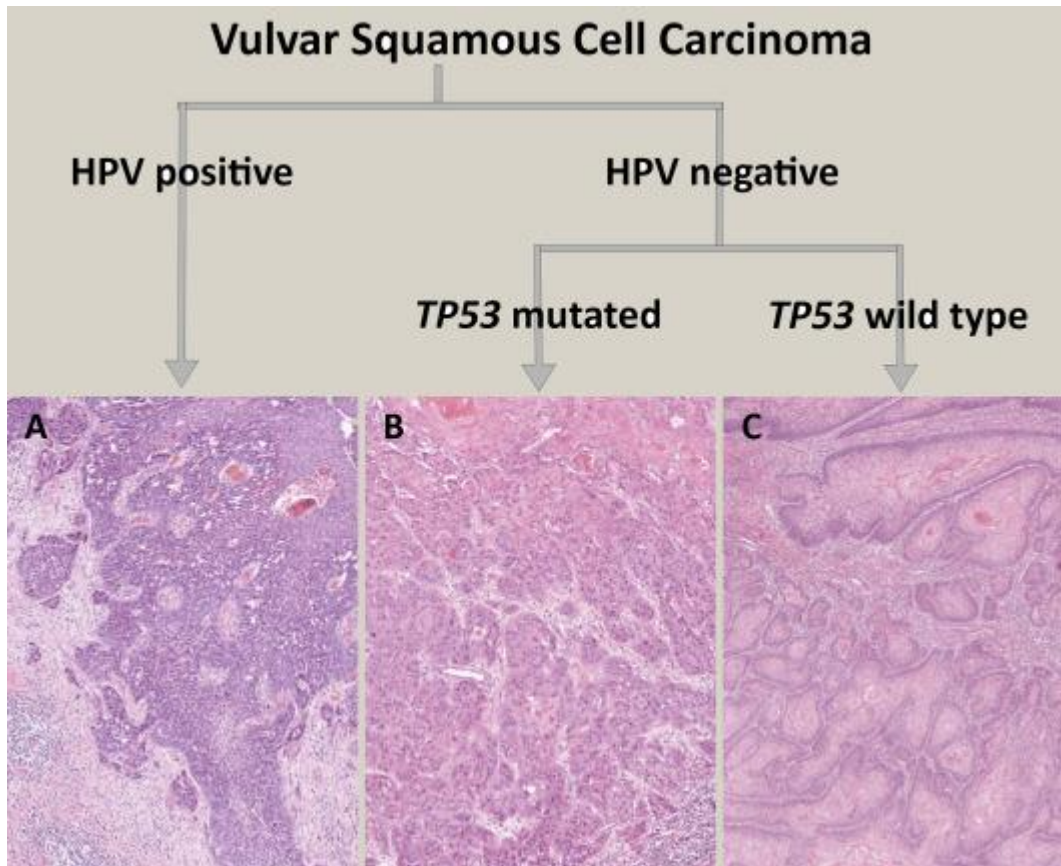


Basal cell carcinoma of the vulva. It is characterised by the basaloid cells, basophil nucleus, little cytoplasm and individual mitosis/cell division. Basophils are a type of immune cells that has a lobed nucleus and coarse granules stained purple-black with Romanowsky stains. They normally ingest foreign particles. There are nests made in a fence-like manner (palized). There are some stroma cells that form spaces (Aguilar *et al.*, 2024)

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Figure 6: Vulvar Squamous cell carcinoma with and without HPV and TP53 mutations



There is a distinctive presentation of histological features exhibited by all three molecular subgroups: Image A presents HPV-positive vulvar squamous cell carcinoma. This is the most common form, and it presents basaloid or warty features. It is positive for p53, HPV, and p16. However, the staining patterns are dim in the mid-epithelium.

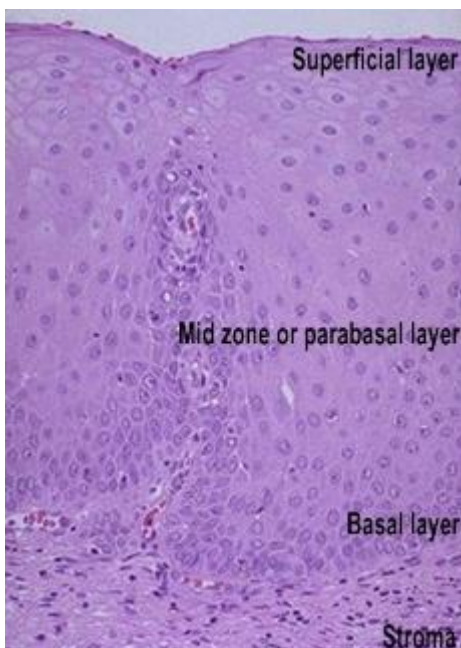
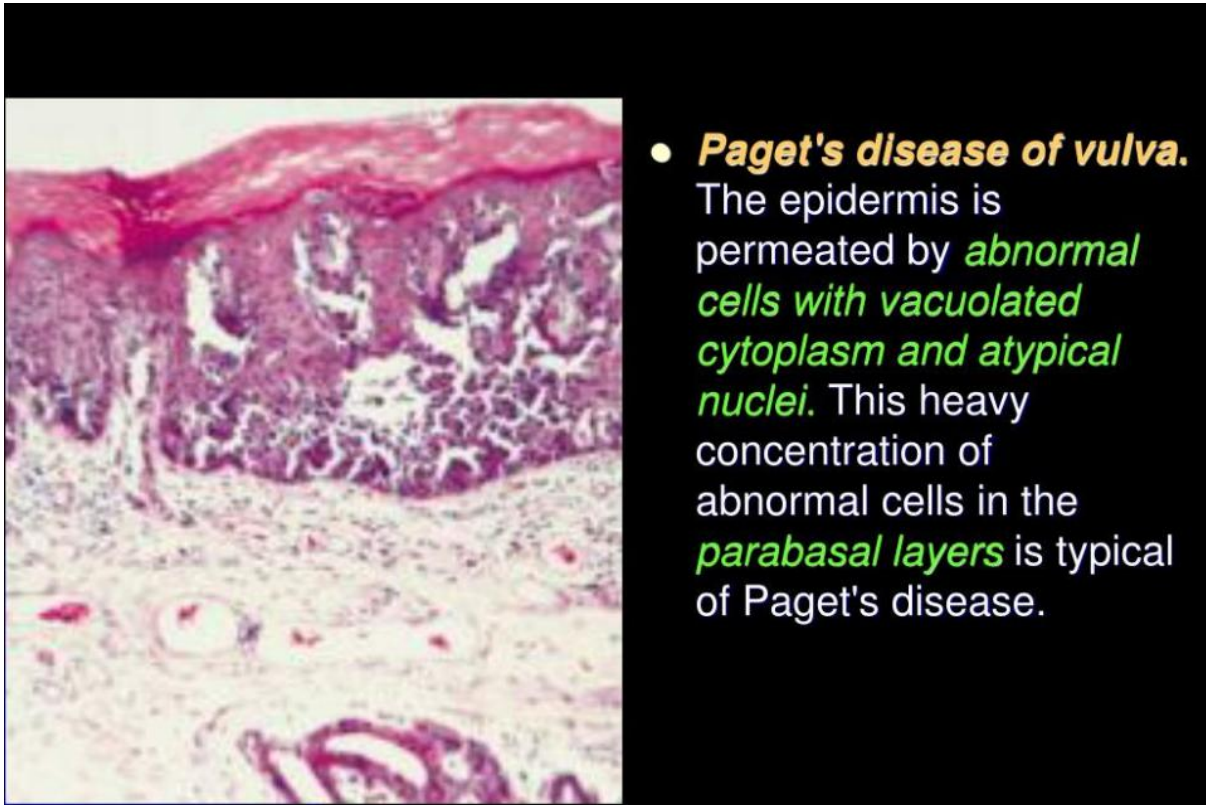
Image B presents HPV negative and TP53-mutated (positive for TP53 mutation) vulvar squamous cell carcinoma. Amongst the histological features are the presence of keratin and nuclear atypia. There is overexpression in the basal/parabasal layers. Other features may be absent in staining procedures. However, the cytoplasm has staining patterns.

Thirdly, the HPV negative and TP53 wild-type vulvar squamous cell carcinoma has a wider invasive pattern. It is well differentiated, with p16 immunohistochemistry and HPV in situ hybridization negative; however, scattered expression is present here.

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Figure 7: Vulvar Paget's disease

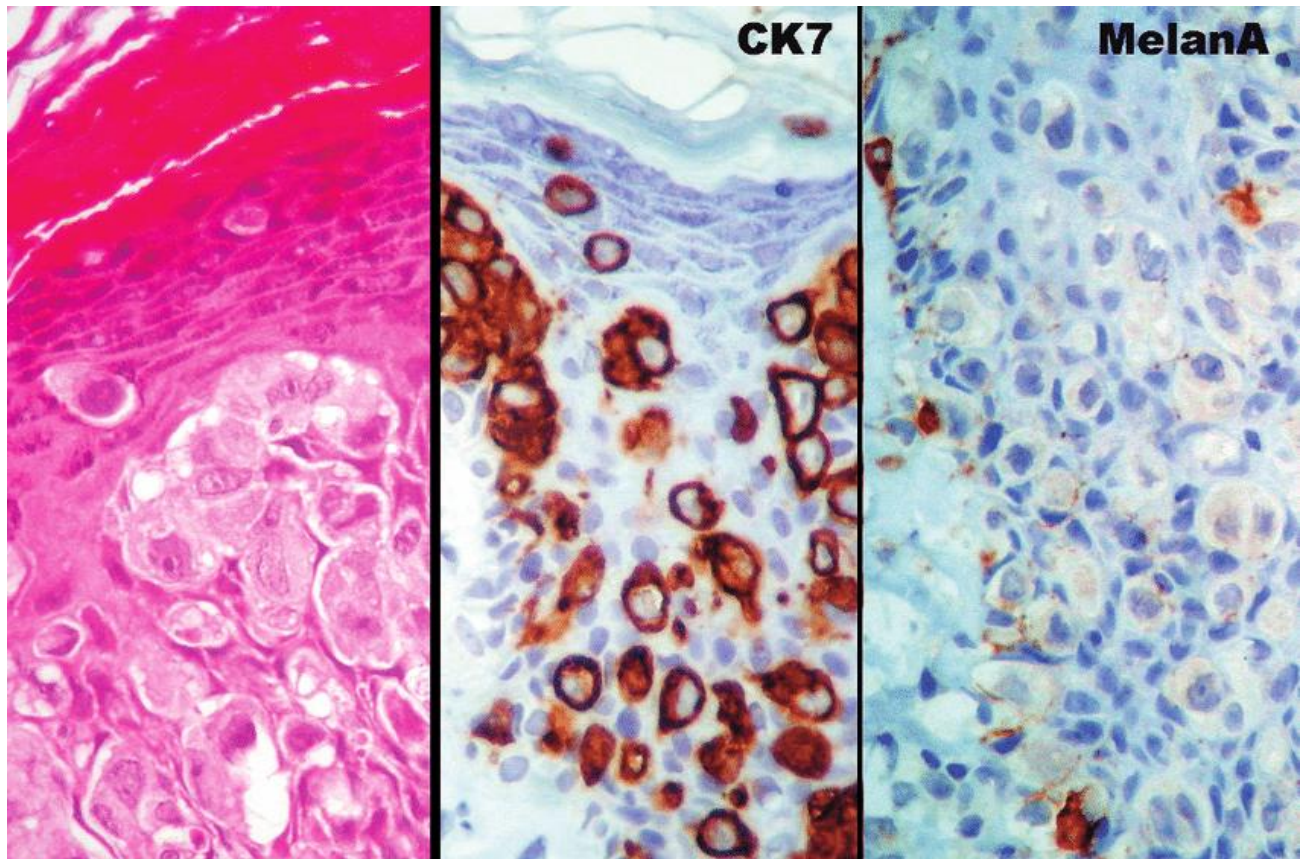


The histological structure of the skin to help illustrate where the layers of superficial, parabasal and basal are located.

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Figure 8: Vulvar Paget's disease – H and E, CK7 and MelanA

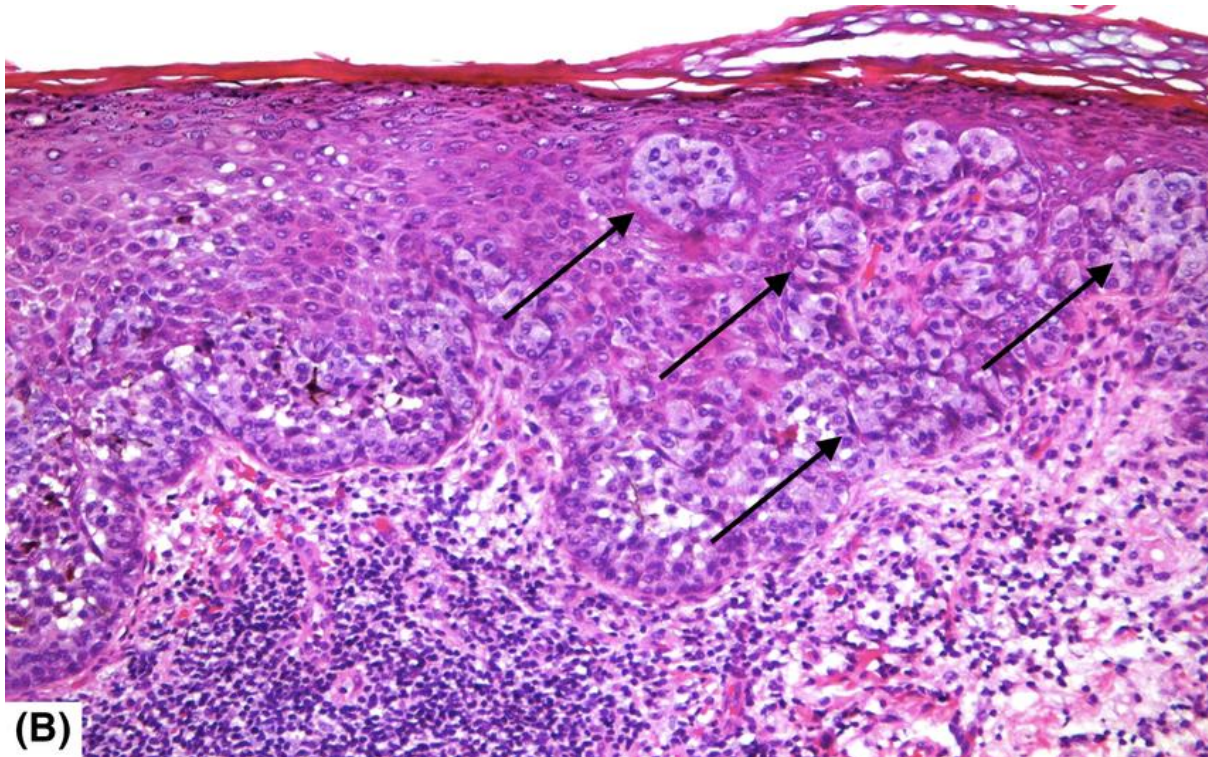


The main histological features of vulval skin are large cells in different forms (pleomorphic), whether alone or in groups. The keratinocytes have clear chromatin, a nucleolus, and gray-blue cytoplasm that may be vacuolated with haematoxylin and eosin staining. Chromatin contains DNA and protein. The nucleolus is found within the nucleus and contains RNA and helps to make proteins via ribosomes. There are also normal keratinocytes (left). However, there is positive staining for CK7 and negative staining for Melan-A. (Anton *et al.* 2011)

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Figure 9: Vulvar Paget's disease – H and E

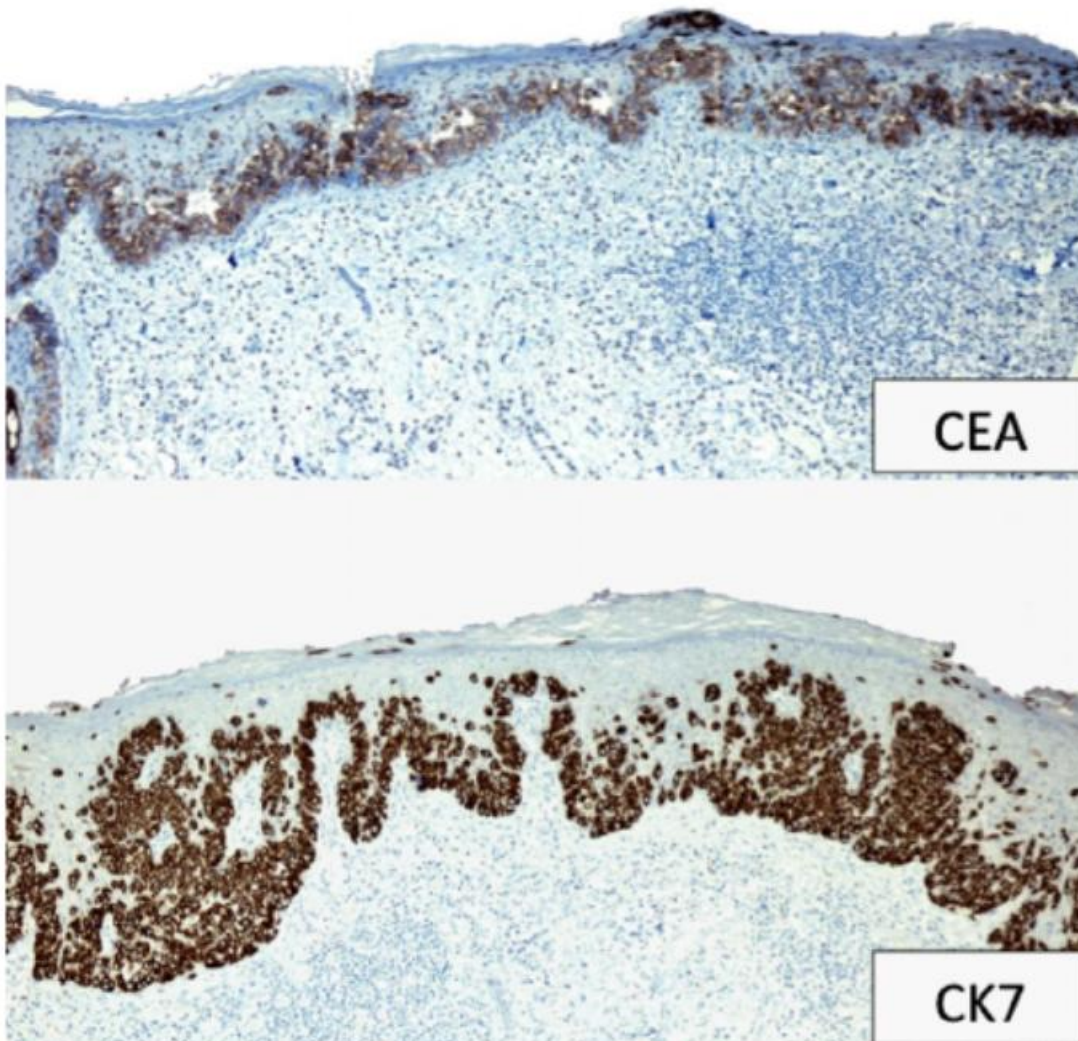


A closer presentation of atypical large Paget cells that have clear cytoplasm and lots of nuclei using Haematoxylin and Eosin staining. The magnification will be increased to x400 (Vera-Kellet, C. and Hasbun, C.,2020)

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Figure 10: Vulvar Paget's disease – CEA and CK7

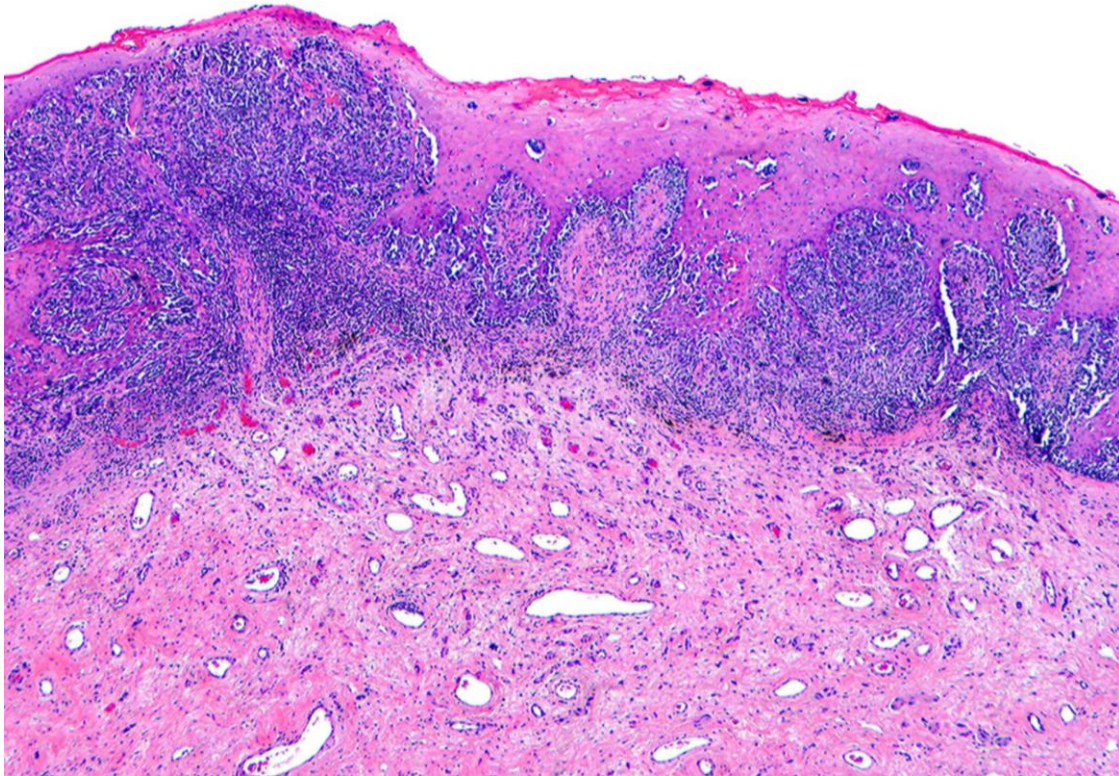


Positive Immunohistochemistry staining for CEA and CK7 with extramammary Paget's disease (EMPD). (Vera-Kellet, C. and Hasbun, C.,2020)

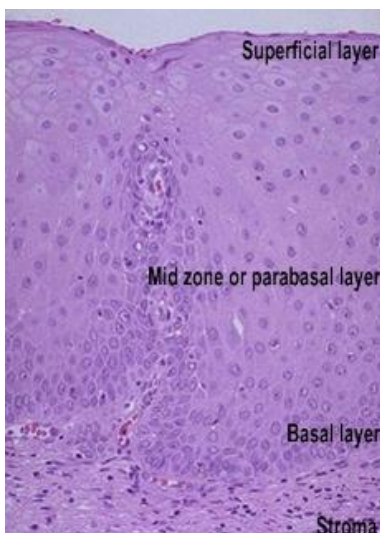
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Figure 11: Vulvar Vagina Melanoma



This is a histological presentation of several architectural patterns from Clinical Tree (2024) that alter the normal structure of the stratified squamous epithelium. There is lentiginous pattern across the basal layer and are separated by a few nests. Lentiginous refers to a continuous proliferation of atypical singular melanin-producing cells (melanocytes). This is not always the case as sometimes there is a nodular appearance where deep growth is absent into the intraepithelial region. Haematoxylin and Eosin staining was performed.

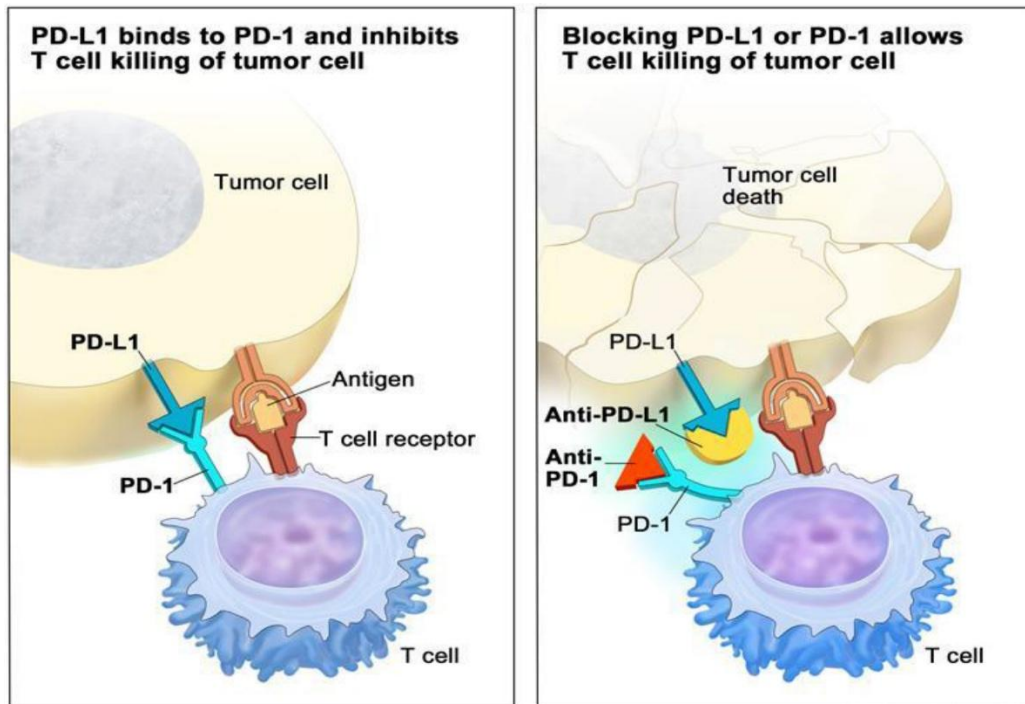


The histological structure of the skin to help illustrate where the layers of superficial, parabasal and basal are located.

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Figure 12: The interaction between PDL and PD-L1

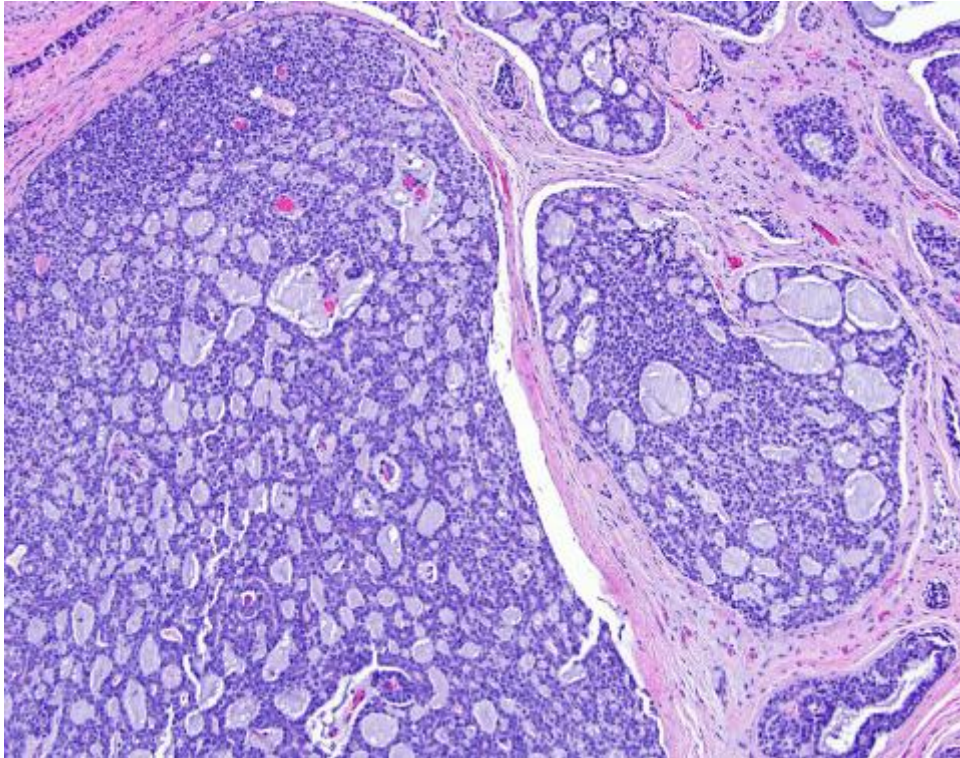


Programmed cell death protein 1 (PD-1) is a receptor on T lymphocytes. It can associate with the PD-L1 ligand on T cells and inactivate the T cells. This lowers the immune response and allows tumours to grow. Cancer Immunotherapies like Pembrolizumab, Atezolizumab, Cemiplimab and Nivolumab are checkpoint inhibitors that counteract this by preventing the tumour cells from deactivating the T-cells. The malignant cells commonly escape as they mimic the signals released by healthy cells, causing unregulated proliferation. PD-1 and CTLA-4 checkpoints are found on T-cells but not exclusively; PD-1 can also be found on other types of T cells, for instance, B cells (produce antibodies), macrophages (engulf disease-causing substances non-specifically), regulatory T-cells, and natural killer cells that toxically kill cancer cells. Alternatively, PD-L1 is found on cancer cells. There is a poor prognosis when there is overexpression of PD-1 ligands on tumours (Rasmy *et al.*, 2019)

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Figure 13: Adenoid Cystic carcinoma



This image from Adenoid cystic carcinoma (ACC) is from Mahjabin et al. (2023). ACC rarely occurs in the uterine cervix and vulva and accounts for less than 1% of all lower female genital tract malignancies. Bartholin's glands are even rarer. It mainly arises in the salivary glands in the head and neck cancer region and the breast. Here, in the image above, we can see ACC of the vulva with a tissue sample stained with Haematoxylin and Eosin. This image has a magnification of x100. ACC has cribriform growth of small, uniform tumour cells that vary in size, with hyperchromatic nuclei in the epithelium. There are pseudocystic spaces that are filled with mucus and basophils. Basophils are a type of immune cell that boosts the immune response by ingesting foreign particles, and they contain heparin and histamine.

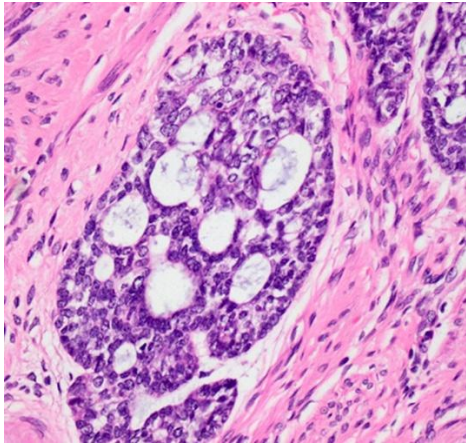
Other adenoid cystic adenocarcinomas have tubular and solid growth patterns. It is sometimes confused with benign conditions, for instance, Bartholin gland cysts and abscesses. In some cases (not this image), there is perineural invasion (invading the nerves) and lymphatic infiltration (build-up of lymphocytes/immune cells). Most adenoid cystic carcinomas have MYB and MYBL1 gene rearrangements (Mahjabin et al., 2023). 66.7% of vulvar ACCs harboured NFIB rearrangement (Xing and Lu, 2018).

It is important to note that adenoid cystic carcinoma is different from adenoid basal carcinoma. The latter does not have cribriform architecture, intraluminal secretions, or a

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basement membrane. It has less nuclear atypia, minimal stromal invasion, and no stromal reaction. Pure ACC is unlinked to high-risk HPV in the vulva and cervix. However, mixed differentiation of adenoid cystic carcinoma in the cervix is associated with high-risk HPV.



Cribriform patterns with secretions. Image is from Mohamed, Means and Chen, 2026)



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