Vulvar squamous cell carcinoma : genetics, morphology and clinical behaviour

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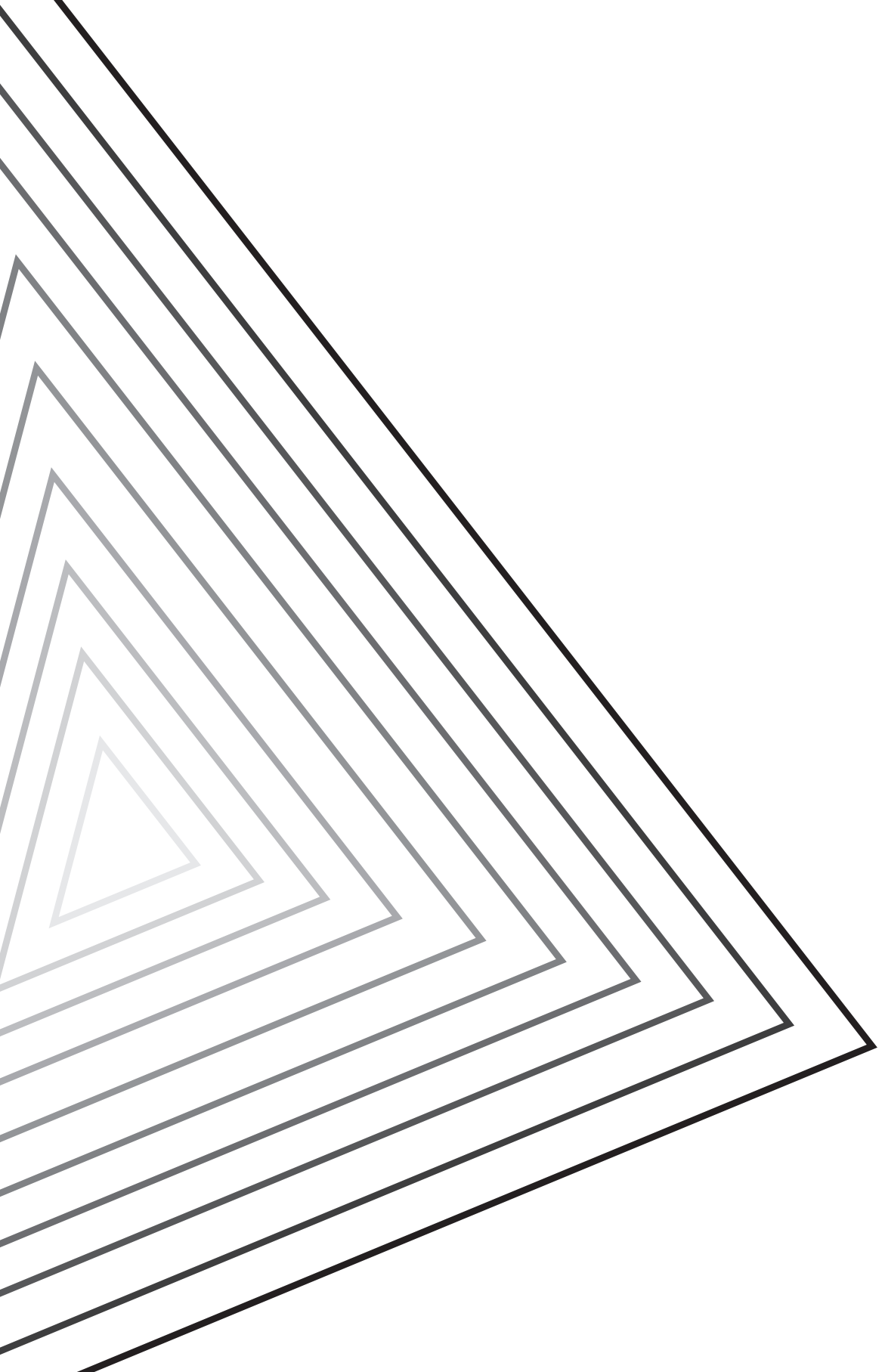

Chapter 1

General introduction

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General introduction

Although vulvar cancer is a rare gynaecologic malignancy with 2 new cases per 100,000 women each year, its impact on the often older aged patients can be devastating (1-7). When detected at an early stage, vulvar cancer can be treated curatively by surgical excision (8). Some patients however, suffer from rapid recurrences and progression (9). Vulvar cancer that has spread to the urethra, anus or lymph nodes requires larger or wider surgery, or a combination of surgery and radio- and chemotherapy, which in turn results in higher morbidity rates. This sometimes mutilating treatment has a major impact on the quality of life, because patients suffer from wound healing problems, lymph oedema and nerve damage that can result in sexual dysfunction and incontinence (10-12).

Currently, the aetiology of vulvar cancer has been far from unravelled. The majority of vulvar cancers are squamous cell carcinomas. It is well accepted that approximately 40% of all new cases of vulvar squamous cell carcinomas (VSCCs) are associated with a persistent infection by a high risk variant of the human papilloma virus (HPV) and that the other 60% are HPV negative and associated with mutations in the *TP53* gene (3;13;14). The HPV positive vulvar cancers are preceded by usual vulvar intraepithelial neoplasia (uVIN), a condition that has a malignant potential of 9-16% in untreated women (1;15) (figure met HE coupe). This type of vulvar cancer affects younger women and is associated with smoking and a higher number of sexual partners (1;3;16).

Differentiated vulvar intraepithelial neoplasia (dVIN) is the precursor lesion of the non HPV related vulvar squamous cell carcinoma (figure 1). This type of VIN is very hard to recognise and is mainly seen adjacent to vulvar cancer. Whether this means that it is a precursor lesion that rapidly progresses to cancer before it can be detected as a solitary lesion is still being debated (17;18).

Markers that can predict patient outcome other than lymph node metastases have not been established yet (19;20). This thesis contains work to further understand and predict why vulvar cancer can behave as aggressively as it sometimes does.

Treatment of vulvar cancer

The International Federation of Gynaecology and Obstetrics (FIGO) staging system uses clinical and histological parameters to subdivide patients into risk categories (table 1 FIGO staging) (21). Early stage vulvar cancer has a fairly good prognosis, but the prognosis rapidly declines with increasing stage (table 2 FIGO stage and survival) (22).

Surgical excision is the primary treatment for low stage vulvar cancer (12). Depending on the size and spread of the tumour, other treatment modalities come to play. In the Netherlands, patients with unifocal VSCC with a diameter <4 cm

Table 1 *FIGO staging in vulvar cancer:*

Stage	TNM Classification	Description
0	Tis N0 M0	Carcinoma in situ, intraepithelial carcinoma
I	T1 N0 M0	Confined to the vulva or perineum; no nodal metastasis
A	T1a N0 M0	Lesions =< 2 cm with stromal invasion, =< 1 mm
B	T1b N0 M0	Lesions > 2 cm in size or stromal invasion, > 1 mm
II	T2 N0 M0	Adjacent spread to the lower urethra, the vagina, or the anus, no nodal metastasis
III	T1,2 N1a,b N2a,b,c M0	Tumour confined to vulva or adjacent spread to the lower urethra, the vagina, or the anus and positive inguinofemoral lymph nodes
A	T1,2 N1a,b M0	One lymph node metastasis >= 5mm or 1-2 lymph node metastases < 5 mm
B	T1,2 N2a,b M0	Three or more lymph nodes < 5mm or 2 or more lymph nodes >= 5mm
C	T1,2 N2c M0	Lymph nodes with extracapsular spread
IV A	T1,2 N3 M0	Tumour with fixed or ulcerated lymph nodes
	T3 anyN M0	Tumour with spread into upper urethra/vagina, bladder, rectal mucosa, bone or fixed to pelvic bone
IV B	Any T Any N M1	Any distant metastasis, including pelvic lymph nodes

(FIGO 2009 and UICC 7th ed.)

Table 2 Five year survival according to FIGO Annual Report 2006, using the 1995 FIGO staging system:

Stage I: 79%
Stage II: 59%
Stage III: 43%
Stage IV: 13%

without suspicious groin nodes are generally treated with radical local excision and a sentinel node procedure (23). Tumours with a diameter exceeding 4 cm or multifocal tumours are generally treated with radical local excision and unilateral or bilateral inguinofemoral lymphadenectomy. In patients with tumours in FIGO stage III or higher but with contraindications for extensive surgery, such as high age and comorbidity, radical local excision is performed without inguinofemoral lymphadenectomy, followed by (chemo)radiation.

All of these treatments can be mutilating and have high morbidity rates (10;11). Wound healing problems, lymph oedema, and sexual dysfunction regularly occur. These negative effects of treatment can have a major impact on the patient's quality of life (10).

Aetiology of vulvar squamous cell carcinoma: two different pathways

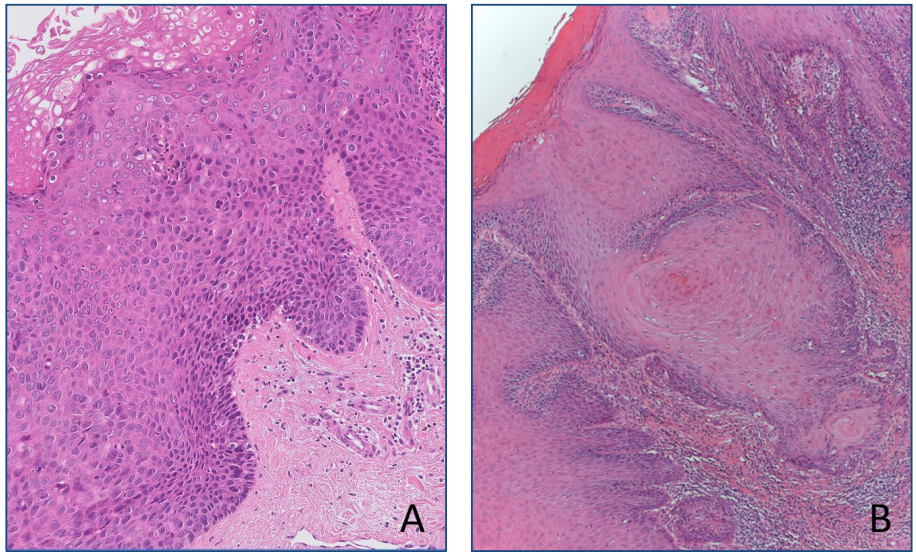
Cancer is the uncontrolled growth of abnormal cells, which can occur when the DNA in our cells is damaged and fails to be repaired. It can arise in virtually any cell type within the body. Skin cancer is one of the most prevalent types of cancer worldwide and is most often caused by DNA damage through ultraviolet irradiation

from sunlight. Well known skin cancer types are, amongst others, melanomas, basal cell carcinomas and squamous cell carcinomas.

The skin of the outer parts of the female genital tract, the vulva, can also be affected by cancer, but usually through a completely different etiologic pathway than in skin cancer on other locations of the body. As said before, most vulvar cancers are squamous cell carcinomas, a type of cancer arising from the squamous epithelium of the vulvar skin.

The pathogenesis of vulvar cancer can be divided into two different pathways: an HPV dependent, and an HPV independent route. Approximately 40% of all vulvar cancers are caused by a persistent infection with high risk HPV (3;13;14). HPV's are common and contagious viruses that can cause abnormal cell growth which can result in warts, and in some cases, cancer. More than 100 types of HPV have been described, of which 15 are 'high risk' types that can cause malignancies. These high risk HPV's are the main cause of cervical cancer and its precursor lesion cervical intra-epithelial neoplasia (CIN) (24). They can also cause the much less prevalent vulvar cancer and usual vulvar intraepithelial neoplasia (uVIN) (1;2;15;25). CIN usually goes unnoticed by its host, but VIN itself can be an unpleasant condition, resulting in itching and pain. Of VIN patients not receiving treatment, 9-16% will progress towards vulvar cancer (1;15). uVIN patients that do receive treatment have a risk of developing cancer from their VIN lesion of approximately 3% (figure 1a).

Figure 1 *Usual VIN and differentiated VIN*



HE slides of a usual VIN (A) and differentiated VIN (B). Note the uniform cells with hyperchromatic nuclei and little maturation in uVIN and the mature cells with eosinophilic cytoplasm in dVIN.

It is estimated that over 80% of women will have an HPV infection at least once in her life, whereas only a very small number of these women will suffer from cancer caused by HPV infection. In most cases, the HPV infection is transient, and the body is able to clear the virus by itself within 1 to 2 years (24). Some women, however, are unable to rid the virus, which finds a way to integrate itself in the human DNA and causes cell growth and, in some cases, eventually cancer. Smoking and a compromised immune system (for example by HIV/AIDS) are factors that have been proven to result in a higher risk of persistent HPV infection and therefore a higher risk of HPV associated cancer (24). Other than that, little is known about why some women are unable to clear the HPV infection.

The other 60% of vulvar cancers are not associated with HPV infections, but with lichen sclerosis, a chronic, autoimmune inflammatory disease of the skin. Lichen sclerosis progresses towards cancer in 3-5% of patients (1;15). This pathway has a worse prognosis than the HPV positive pathway, and affects older patients than the HPV positive pathway, with an average age at diagnosis of 70 versus 40 years (1-3;15;25). Approximately 3-5% of women with lichen sclerosis will develop vulvar squamous cell carcinoma. Differentiated VIN (dVIN) is thought to be the precursor lesion of HPV negative vulvar cancers, but it is hardly ever diagnosed before invasive cancer has developed. This is probably due to its subtle clinical and histological appearance, which makes it hard to recognise by clinicians and pathologists, but it could also mean that dVINs progress very rapidly into vulvar cancer (3;15) (figure 1b).

Somatic mutations in the HPV negative pathway

The HPV negative pathway is associated with lichen sclerosis, but also with somatic mutations. The most frequently mutated and well-studied gene in vulvar cancer, and in fact in any type of cancer, is the *TP53* (tumour protein 53) gene. This gene is the guardian of the cell cycle: it stops cells from dividing when aberrations in the DNA are detected. *TP53* malfunctioning because of a somatic mutation leads to an uncontrolled cell cycle and chromosomal instability, which can lead to the formation of tumours (26). Studies on somatic mutations in vulvar cancer other than in *TP53* are limited in number and size. Holway and Growden have found that quite a large percentage of the vulvar cancers and carcinomas in situ they studied carry mutations in *PTEN (*Phosphatase and tensin homolog), but the study sizes are rather small (27;28). O'Nions, Soufir and Gasco have reported mutations in *CDKN2A* (cyclin-dependent kinase inhibitor 2A) (29-31). Both *PTEN* and *CDKN2A* are also tumour suppressor genes that are involved in signalling pathways that control the cell cycle and stop cells from dividing or even cause cells to undergo apoptosis (32;33). Studies of melanoma, lung, colorectal, and breast carcinomas have shown that the somatic mutation status can be used to predict prognosis and guide tumour-spe-

cific treatment strategies (34-37). Clinical trials have shown promising outcomes of targeted therapies, such as in targeting the PI3K/AKT/mTOR pathway in colorectal cancer (35).

Epigenetic alterations

In addition to genetic mutations, in which the DNA sequence is changed, VSCC might also develop under influence of epigenetic changes. Epigenetic changes, such as hypermethylation, are heritable changes in gene expression without changes in the DNA sequence. Hypermethylation functions as a switch that can turn genes on or off, thus inactivating tumour suppressor genes (38-43). Hypermethylation of the promoters of *RASSF2A*, *MGMT,* and *TSP1* has been described in vulvar cancer (40).

Predictive morphological factors in vulvar cancer

As mentioned in the first part of the general introduction, clinical and histological parameters that constitute the FIGO staging system have been studied carefully and have proven their prognostic and clinical value in large cohorts of patients. Identifying more risk factors for poor survival is important to further reduce the mortality and morbidity of vulvar cancer patients, but few studies have found new factors of clinical importance (19). To date, lymph node involvement is the only accurate prognostic factor for survival and recurrence (20).

Recent publications have focussed on the potential prognostic value of morphological characteristics in vulvar squamous cell carcinoma (44). Vulvar squamous cell carcinomas with a spray patterned or spindle cell morphology are thought to represent a subgroup of vulvar cancers with a worse prognosis than 'conventional', solid vulvar cancers. Spindle cells are thin, slender, elongated cancer cells that infiltrate stromal tissue and that occur either as single cells or as cords of cells rather than as groups or islands. Spindle cell carcinoma is seen in many different types of cancer and is associated with a worse prognosis in cancers of the oral cavity, oesophagus and lung (45-49). Spindle-shaped epithelial cancer cells have lost their typical epithelial characteristics and seem to gain the ability to infiltrate the underlying stromal and lymphvascular tissue and bud from the primary tumour (50).

Epithelial to Mesenchymal Transition

Research by the Brazilian group of Rocha/AC Camargo Cancer Center (44) has pointed out that this invasive growth pattern in vulvar cancer might be caused by a process called Epithelial to Mesenchymal Transition (EMT). EMT is a process

that naturally occurs during embryonic development, but can also be seen during cancer progression (51). Cells undergoing EMT lose their epithelial characteristics, such as their cobble stone shape, cell-cell-adhesion and basal cell polarity, and gain mesenchymal traits that provide them with the capacity to migrate as single cells through the extracellular matrix (51). This way, EMT supports the process of solid tumours converting to invasive separate cells. Several signalling pathways control EMT, amongst others Wnt and NOTCH signalling. One of the major inducers of EMT is Transforming Growth Factor-β (TGF-β). In healthy cells, TGF-β has a tumour suppressor role, but in malignant cells, its role changes and TGF-β will induce cell motility and angiogenesis (52;53). Another cell motility promoter that is thought to be involved in EMT is L1-cell adhesion molecule (L1CAM), which is an emerging prognostic factor for metastasis in many cancer subtypes. L1CAM is a membrane glycoprotein involved in neural development where it has two roles: a cell adhesion function, and a cell motility promoting function. It is expressed by normal nerve tissue, but it is sometimes detected on tumour cell surface (54). The role and relevance of L1CAM has been studied in gynaecological cancers such as endometrial and ovarian cancer, but not yet in vulvar cancer.

Thesis outline

the etiology of HPV positive vulvar cancer is quite well understood, but the HPV independent axis remains to be unravelled. This thesis aims to gain knowledge on the origin of this type of vulvar cancer through the study of two mechanisms: genetic and morphological alterations in vulvar cancer.

In order to study somatic mutations in vulvar cancer, suitable techniques and methods had to be selected, that match the small numbers of patients, the relatively low quality and quantity of available DNA and the lack of prior knowledge we have from the available literature on somatic mutations in vulvar cancer other than *TP53*. **Chapter 2** describes the design of a somatic mutations profiling panel using mass spectrometry that is created especially for gynaecological cancers, focussing on the mutations that are most relevant in cervical, endometrial, ovarian and vulvar cancer. **Chapter 3** contains the results from applying this panel to a cohort of 108 vulvar cancer patients that were treated for primary VSCC in the Leiden University Medical Center between 2000 and 2009. In a review of the literature (**chapter 4**), these data are compared to the current knowledge on genetic and epigenetic changes in vulvar cancer and its precursors.

The majority of vulvar cancers are squamous cell carcinomas, but within this cancer type, morphological sub classifications can be made that can help us understand and predict the progression of vulvar cancers. **Chapter 5** describes a vulvar squamous cell carcinoma type called squamous cell carcinoma with spindle

cell morphology. This type of cancer progression is in some cases thought to develop through the process of EMT, which is reflected by the expression of EMT inducing molecule L1CAM. **Chapter 6** reports the prevalence and prognostic value of L1CAM expression in separate groups of vulvar cancer patients from the Leiden and the Groningen University Medical Centers. In **Chapter 7**, the relation between mutational and morphological data is studied by testing solid and spindle cell carcinomas and their recurrences and metastases for somatic mutations. **Chapter 8** gives an overview of the findings of this thesis and sheds some light on possible future research.

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