Early phase clinical drug development for HPV-induced disorders: novel tools and treatments

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Chapter 1

INTRODUCTION

Human papillomavirus (HPV) infections cause a variety of epithelial lesions ranging from benign common warts to malignant anogenital diseases. The overall disease burden of HPV infections varies correspondingly from minimal cosmetic discomfort to highly debilitating morbidity and even mortality. For the latter category the incidence of HPV-induced diseases still increases, even despite preventive vaccination strategies. As an example, the life-time risk of acquiring a genital HPV-infection is around 80% and almost 10% of these develop into persistent infections related to (pre)malignant diseases.[1,2] Current treatments of HPV-induced lesions consist of medical and surgical therapies that focus on lesion removal instead of eradication of the virus. These treatments are often associated with significant side effects and high recurrence rates. Therefore, there is a strong medical need for new HPV eliminating drugs with an acceptable side effect profile. The scope of this thesis is to elucidate novel pharmacological interventions for HPV-induced diseases by using a question-based developmental approach that includes investigation of the pharmacological effects in an early phase of drug development.[3] The emphasis of the thesis is on the development of new methodological tools to monitor the course of HPV-related diseases in clinical trials, as well as the exploration of successful biomarkers of viral load in HPV infections. This introduction summarizes the biology of HPV and its different types, the pathophysiology of HPV-induced diseases and addresses the relevance of question-based drug development and the development of new methodological tools and biomarkers.

HUMAN PAPILLOMAVIRUS BIOLOGY

Papillomaviruses are small, non-enveloped, circular, double-stranded DNA viruses that belong to the Papillomaviridae family and infect cutaneous and mucosal epithelial cells.[4] The genome of the HPV is distributed into early genes E1-E2, E4-E7 and late genes L1 and L2 (Figure 1).[5,6] The early genes E1, E2, E4 and E5 induce the maintenance, transformation and replication of the viral infection.[6] A persistent infection with a high-risk HPV type can induce the integration of the viral DNA into the human genome, causing overexpression of the E6 and E7 oncoproteins and lead to (pre)malignant progression of the lesion. The late genes encode for viral capsid proteins which are only expressed in highly differentiated epithelial cells and lead to a high number of HPV copies.

Figure 1. Genome organization of the Alpha papillomavirus HPV16. The genome is comprised of a long control region (LCR) and eight genes that are involved in the virus life cycle. This figure is adapted from de Sanjosé 2018 and Doorbar 2015.[9,10] *(see inside front-cover for image in color)*

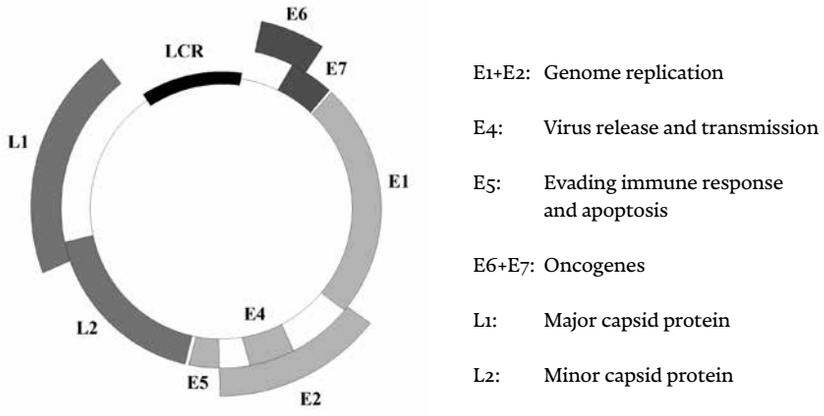
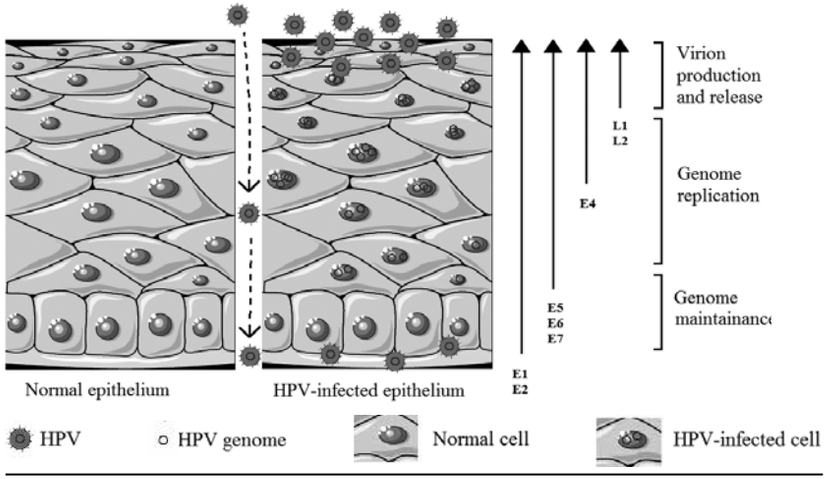


Figure 2. The life cycle of a HPV infection. A diagrammatic representation of the skin is shown after infection with HPV. Often a micro-trauma of the epithelium allows the virus to infect cells in the basal layer of the epithelium (dotted arrow lines). In the basal epithelial cells the virions are internalized and the viral genomes are transferred to the nucleus (genome maintenance). The genome of the virus is replicated in the nucleus and hereafter the virus particles are produced and released. The involvement of the early and late genes is shown with the arrows next to the figure. This figure is adapted from Doorbar 2005.[4] *(see inside front-cover for image in color)*



Infection with HPV requires access of the virus to cells in the basal layer, by micro trauma of the epithelium (Figure 2). The capsid of the virion is cleaved to facilitate the virus internalization.[6,7] Once the virions are internalized they undergo endosomal transport, further uncoating, cellular sorting and subsequent transfer of the viral genome to the nucleus. Infection of the lower epithelial layer is followed by an initial phase of genome amplification in low copy numbers and maintenance of the viral genome.[6] The genome amplification is further facilitated by the upper epithelial layers which results in a high genome copy number. It is hypothesized that the infection of an epithelial stem cell is necessary in the lesion formation process.[6,8]

To date, more than 207 different human papillomavirus (HPV) genotypes have been identified based on the DNA sequence and are divided into five phylogenetic groups: Alpha, Beta, Gamma, Mu and Nu.[5,11] Figure 3 shows the five phylogenetic groups and the subdivision of Alpha papillomaviruses into low-risk (yellow, e.g., cutaneous and anogenital warts) and high-risk (pink, e.g., vulvar high-grade squamous intraepithelial lesions (HSIL)) diseases.

Next to different life-cycle characteristics and disease associations, the HPV types in the different phylogenetic groups can be categorized as either cutaneous or mucosal genotypes based on their tissue preference (Table 1).

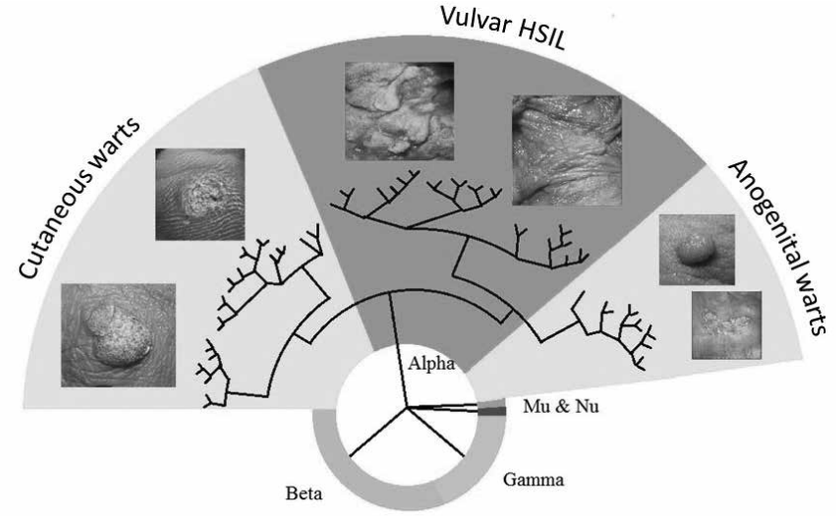
The majority of HPV infections manifest themselves as an asymptomatic infection of the cutaneous or mucosal epithelium, although self-limiting benign growth is also frequently observed. Most HPV types belong to the Alpha papillomaviruses group and these HPV types cause mucosal and cutaneous lesions. This group includes the HPV types that primary cause cutaneous warts and also the genitally transmitted HPV types. The latter mentioned viruses are the most common sexually transmitted pathogens worldwide and some of these are associated with the development of cancer.[4] The Beta papillomaviruses infect the cutaneous epithelia and mostly cause inapparent or latent infections but can also cause non-melanoma skin (pre)malignancies.[5] The Gamma, Mu and Nu papillomavirus mostly causes benign cutaneous lesions.[5]The subdivision of HPV types into low-risk and high-risk depends on their carcinogenicity, i.e. the benign or malignant potential of the virus.[14] Infection by low-risk HPV types is asymptomatic or can cause benign lesions. However, persistent infection with high-risk types can cause (pre)malignant anogenital lesions.[5] The lifetime risk of acquiring a genital HPV infection is about 80% and approximately 40% of female adolescents become infected with a high-risk HPV type at least once.[1,2,15] The prevalence of a high-risk infection decreases with age, being 15% in all women and 9% of all women above 30 years.[2] The majority of these infections are transient and will be cleared by the immune system without causing any lesions.

This thesis describes studies performed in patients with cutaneous warts, anogenital warts and vulvar HSIL, being Alpha papillomaviruses-induced diseases illustrative for typical low-risk (cutaneous warts and anogenital warts) and high-risk (vulvar HSIL) HPV-induced diseases falling within the scope of this thesis.

Table 1. The different human papilloma virus (HPV) groups based on their evolutionary relationship. The HPV types are divided in 5 different groups; Alpha (pink and yellow), Beta (green), Gamma (blue), Mu (purple) and Nu (lilac). The Alpha-papillomaviruses are subdivided as low-risk (yellow) and high-risk (pink) based on the benign or malignant potential of the virus, respectively. Examples of different HPV types, tissue preference and associated diseases are given.[12,13]

Genus + Species	Types	Tissue preference	Diseases
Alpha 1, 13	HPV32, HPV54	Mucosal	Low-risk mucosal lesions
Alpha 8	HPV7	Mucosal	Butcher's wart
Alpha 10	HPV6, HPV11	Mucosal/ cutaneous	Anogenital warts, oral/laryngeal papillomas
Alpha 9	HPV16, HPV31, HPV33	Mucosal	Vulvar and cervical HSIL/carcinoma
Alpha 7	HPV18, HPV45	Mucosal	Cervical HSIL/ carcinoma
Alpha 5, 6, 11	HPV51, HPV56, HPV34	Mucosal	Cervical HSIL/ carcinoma
Alpha 2, 3, 14, 15	HPV3, HPV10	Mucosal	Low-risk mucosal lesions, flat warts
Alpha 4	HPV2, HPV27, HPV57	Cutaneous	Cutaneous warts
Beta 1-5	HPV5, HPV8	Cutaneous	Skin cancer, epidermodysplasia verruciformis
Gamma 1-5	HPV4, HPV60	Cutaneous	Cutaneous warts, epidermoid cyst
Mu 1-2	HPV1, HPV63	Cutaneous	Cutaneous warts
Nu 1	HPV41	Cutaneous	Cutaneous lesions

Figure 3. Phylogenetic tree of human papilloma virus (HPV) demonstrating their evolutionary relationship. HPV types are divided in 5 different groups: Alpha (pink and yellow), Beta (green), Gamma (blue), Mu (purple) and Nu (lilac). The Alpha-papillomaviruses are subdivided as low-risk (yellow) and high-risk (pink) based on the benign or malignant potential of the virus, respectively. Cutaneous warts are caused by low-risk HPV types of the Alpha genus. Typical appearances of a common wart on the hand (left) and a plantar wart (right) are shown. Anogenital warts are also caused by low-risk HPV types of the Alpha genus, but these are phylogenetically different from the HPV types causing cutaneous warts as shown in the tree by the division of the branches. Anogenital warts on the penile shaft (upper) and under the foreskin (lower) are shown. High-risk HPV types of the Alpha genus (pink) cause vulvar high-grade squamous intraepithelial lesions (HSIL) with a high degree of variation in appearance, such as elevated hyperkeratotic white lesions (left) or red lesions (right). *(see inside front-cover for image in color)*



CUTANEOUS WARTS

Cutaneous warts are a common benign skin condition with an estimated prevalence of 3-13% in the general population in the Western world.[16] Most people are affected by cutaneous warts at some time point in their life.[16–19] The vast majority (>80%) of cutaneous warts in the general population is caused by HPV1, 2, 27 and 57.[20–25] Two-third of all warts show spontaneous regression within two years after diagnosis. Although cutaneous warts are benign and usually resolve spontaneously, they cause physical and psychosocial discomfort.[26,27] Cutaneous warts can be subdivided into plantar warts, located on foot soles, and common warts, located on all other skin locations (Figure 1). The diagnosis of cutaneous warts is based on clinical observations. Depending on the HPV-genotype and clinical location, the clinical appearance of common and plantar warts differs but most warts present as hyperkeratotic papules or plaques. Common warts are mostly elevated, scaly, rough and skin-colored papules or nodules, while plantar warts are mostly thick, hyperkeratotic, papules with capillary thrombosis.

The most frequently used treatments for cutaneous warts are cryotherapy, salicylic acid, ablation and surgical excision, aimed at destruction of the infected tissue. The efficacy, recurrence rates and side effects of these therapies are depicted in Table 2. The most commonly used therapy is cryotherapy which consists of the use of liquid nitrogen at a temperature of -196°C to create an area of necrosis below and around the wart.[28,29] Salicylic acid causes chemical ablation of the wart. Ablation with a laser or electrocautery has comparable efficacy rates as surgical excision, however is not often used because of the invasive nature and side effects.[28]

Table 2. Efficacy rates and side effects of the most common treatments for cutaneous warts.

Treatment	Efficacy rate	Side effects
Cryotherapy	29-51%[29–31]	Pain, blistering, scarring, irritation, pigmentation, crust formation[30]
Salicylic acid	16-35%[29–31]	Irritation, pain, blistering, bleeding, pigmentation[30]
Ablation	75-100%[29,31]	Pain, scarring, crust formation, irritation[29]

ANOGENITAL WARTS

Anogenital warts (AGW), caused by low-risk HPV types such as HPV6 and 11, are highly contagious and are the most common sexually transmitted viral disease worldwide with an incidence of 160-289 per 100.000.[1,32–34] Genital warts generally cause minor symptoms such as pruritus and irritation, but most patients report a high psychological burden from the disfiguring nature of the warts and concerns about infecting sexual partners.[35,36] Two prophylactic vaccines are available against HPV, namely 2-valent papillomavirus vaccine (Cervarix®) and 4-valent papillomavirus vaccine (Gardasil®). Cervarix® includes the high-risk HPV types 16 and 18 and Gardasil® includes next to HPV 16 and 18 also the low-risk HPV types 6 and 11. A 9-valent papillomavirus vaccine (Gardasil-9®) recently became available and contains 5 other high-risk

HPV types (31, 33, 45, 52, and 58) next to HPV 16 and 18. Since 2008, the Dutch national immunisation programme included the Cervarix® vaccine for girls aged 12-13. The success rate of immunisation is rather low as the coverage is only 45%.[37] In Australia the Gardasil® vaccine is included in the national immunisation programme and this has resulted in a dramatic reduction of the incidence of genital warts with almost 90%.[38] The diagnosis of AGW is a clinical diagnosis and no additional research, i.e. biopsy, is necessary. AGW can appear as solitary lesion, in clusters or as plaques and can be flat, dome-shaped, keratotic, pedunculated or cauliflower-shaped (Figure 3).

Current therapeutic options for AGW can be divided into topical therapeutical agents (podophyllotoxin, imiquimod) and surgical removal (cryotherapy, excision, electro surgery, laser ablation). Podophyllotoxin is a substance obtained from the rootstock of the may apple plant and causes cell death and destruction of the wart. Imiquimod is an immunomodulator which causes activation of different cytokines, particularly interferon-alpha. The efficacy and recurrence rates and side effects of these therapies are depicted in Table 3.

Table 3. Efficacy and recurrence rates and side effects of the most common treatments for anogenital warts.

Treatment	First/ second line	Efficacy rate	Recurrence rate	Side effects
Podophyllotoxin	First	45-48%[39–42]	4-38%[41,43]	Local inflammation or irritation, erosion, burning, pain, itching[44–46]
Imiquimod 5%	First	27-54%[47–49]	13-19%[48,49]	Erythema, erosion, itching, burning sensation[46,49]
Cryotherapyw	First	27-88%[50–53]	21-40%[51,52]	Pain, ulceration, scarring, irritation, pigmentation[51,52]
Surgical excision	First/second	35-72%[54,55]	19-29%[54,55]	Pain, scarring, crust formation[46,55]
Electro surgery	Second	61-94%[51,56]	22%[51,56]	Pain, scarring, irritation[46,51]
Laser therapy	Second	23-52%[29,46]	60-77%[46]	Pain, scarring, crust formation[46]

VULVAR HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION

Vulvar high-grade squamous intraepithelial lesion (HSIL), previously known as usual vulvar intraepithelial neoplasia (uVIN), is a chronic premalignant vulvar skin disorder caused by a persistent high-risk HPV infection. Vulvar HSIL is in 90% of the cases caused by HPV type 16.[57] The incidence of vulvar HSIL is 2-5 per 100.000 but the incidence is rising and young women in their 30s and 40s are most often affected.[58,59] The majority of patients suffer from long-lasting and severe symptoms like pruritus or pain and the disease is associated with a high psychosocial burden and sexual dysfunction.[60] Vulvar HSIL has a malignant potential of 3-5% when treated, but when left untreated incidence may increase up to 9%.[57,61,62] Only up to 1.5% of vulvar HSIL lesions are reported to regress spontaneously.[61,62]

Vulvar HSIL has a highly variable appearance on clinical examination and may therefore be difficult to diagnose (Figure 3). Diagnosis always has to be confirmed by histopathological analysis. The lesions can be unifocal or multifocal, can present as plaques or papules and may be white, brown or red. The lesional skin can be thickened (hyperkeratosis), split (fissures) or ulcerated. Vulvar HSIL is often a multicentric disease, therefore it is important to examine the cervix, vagina and anus as well.[63]

The aim of vulvar HSIL treatment is symptom relief, restore normal anatomy and prevention of malignant progression. Considering the low malignant potential of the disease, expectative management in combination with close follow-up can be considered if patients have no severe symptoms and there is no suspicion of invasive disease. Treatment options for vulvar HSIL are surgical excision, ablation (laser therapy) and imiquimod (Table 4).

Table 4. Efficacy and recurrence rates and side effects of the treatments for vulvar HSIL.

Treatment	Efficacy rate	Recurrence rate	Side effects
Excision	Not reported	51%[60,64]	Disfiguring, pain[64,73]
Laser therapy	75%[62,74–76]	51%[60,64]	Not reported
Imiquimod	51-58%[64,65,67,77]	11-16%[65,66]	erythema, oedema, pain, erosion/ulceration, fatigue, headache, muscle pain[64–67,69,77]
Cidofovir	46%[69]	6%[70]	Pain, ulcera, fatigue, headache, muscle pain[69]

Further, the treatment is usually associated with psychosocial and sexual burden for the patient. Ablative techniques such as laser therapy offer increased precision and better cosmetic results and are as efficacious as local excision.[64] Surgical interventions aim to remove (excision) or destroy (laser) visible lesions, but do not eliminate the virus, while vulvar HSIL is caused by a persistent HPV infection. Therefore, there are often positive tumour margins

or still presence of HPV infection in the surrounding tissue after surgical intervention, resulting in a high number of recurrence and residual lesions of up to 40%.[64–66] Medical treatments have the advantage that there is minimal disruption of the anatomy compared to surgical interventions. The most commonly used medical treatment is imiquimod which was developed and licensed for anogenital warts but has also shown to be effective in vulvar HSIL.[66–68] Imiquimod is an immunomodulator that destroys abnormal cells by enhancement of the immune response of the body. Other treatments are cidofovir, photodynamic therapy and therapeutic vaccination. Cidofovir is an antiviral therapy and can be used as a local treatment for vulvar HSIL. A recent randomized clinical trial in 180 subjects performed to compare treatment with cidofovir and imiquimod in vulvar HSIL patients showed comparable efficacy and less recurrences in the cidofovir group.[69,70] Photodynamic therapy is based on light-induced oxidation reactions which lead to tissue necrosis and has the advantage that it is well tolerated and that the anatomy of the vulva is preserved.[71] Photodynamic therapy has been evaluated in small samples showing varying results in terms of efficacy.[64] Therapeutic vaccination with a synthetic peptide targeting specific HPV16 has shown to induce clinical responses.[72] Further investigation to evaluate the effect in treating vulvar HSIL is needed. Prophylactic vaccination against HPV has shown to reduce the risk of HPV-related diseases including vulvar HSIL.[64] The option of treatment differs per patient and depends on several factors such as location of the lesions, uni- or multifocality, comorbidity, previous treatment and obviously patient and physician preference.

DEVELOPMENT OF NOVEL THERAPEUTICS WITH NEW METHODS AND BIOMARKERS

Using the information on registered clinical trials as available in the trial registration 'clintrials.gov', 501 clinical trials for HPV-induced diseases were performed in the last decade. Of these studies, 79 were phase 1 trials, 125 phase 2, 83 phase 3, 42 phase 4, and 204 not assigned to a clinical phase. In July 2019, 208 clinical trials in HPV-induced diseases are executed according to the integrity database of Clarivate Analytics.[78] Of these trials, 80 are randomized and 55 are double-blind and placebo-controlled. These high numbers indicate the medical need for the development of new compounds for HPV-induced diseases. A likely explanation is that current treatments are not sufficient given their low efficacy and high recurrence rates.

The clinical development success rate, i.e. the likelihood that an experimental compound investigated in a phase 1 study is approved, was 9,6%, based on data from clinical trials performed in all disciplines from 2006 to 2015.[79] The transition success rate from phase 1 to 2 was 63.2% and from phase 2 to 3 30.7%.[79] Following the traditional four clinical phases of drug development, most early phase studies on new compounds explore safety and tolerability rather than human pharmacology of the specific drug.[80] The low clinical development success rate of approximately 1 out of 10 suggests that the early prediction of the efficacy and safety of a new compound is of crucial importance. Early signaling of limited efficacy could halt the further development of a compound which can save time and resources. However, the pharmacological effects of a new drug are often investigated in later stages of clinical drug development. Question-based development is a new approach integrating the evaluation of the pharmacological effects in an early phase of drug development.[3] By using this approach, specific questions guide the investigation of pharmacodynamics of a compound in an early stage of development. Questions obviously should be tailored to the compound type and indication(s) based on relevancy, but can generally be divided in 5 questions:[3]

1. Does the drug get to the site of action?
2. Does the compound cause its intended pharmacological/ functional effect(s)?
3. Does the compound have beneficial effects on the disease or its pathophysiology?
4. What is the therapeutic window of the drug?
5. How do the sources of variability in drug (e.g. dose, pharmacokinetics, and pharmacodynamics) response in the target population affect the development of the product?

It is essential to utilize the most appropriate methodology to answer these questions. Biomarkers are biological measures of pharmacodynamic drug effects. According to the World Health Organization, a biomarkers is 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease'.[81] The use of biomarkers in an early phase of drug development is of crucial importance to optimize development time and resources. A biomarker refers to a broad subcategory of medical signs of clinically significant patient outcomes that can be accurately and reproducibly measured.[82]

OUTLINE OF THIS THESIS

This thesis describes studies that address different aspects of early clinical phase drug development in three different HPV-related diseases. This thesis is divided into two parts: **section 1** describes the development and use of novel tools in clinical drug development and **section 2** focuses on early phase clinical studies examining safety, tolerability, pharmacodynamic and efficacy parameters of new topical compounds with high pre-clinical potential for the treatment of HPV-induced diseases.

SECTION 1 TOOLS AND BIOMARKERS IN EARLY PHASE CLINICAL TRIALS FOR HPV-INDUCED DISEASES

The clinical trials in this thesis employed new tools and biomarkers for three types of HPV-induced diseases (CW, AGW and vulvar HSIL) in an early phase of drug development with use of the question-based drug development approach. **Section 1** entails a thorough characterization of the implemented tools, including valid information about treatment adherence, (adverse) events and symptoms. **Chapter 2** describes the development, implementation and evaluation of an electronic diary to measure treatment adherence and patient-reported outcomes. **Chapter 3** entails the technical and clinical validation of a stereophotogrammetric 3D camera system.

SECTION 2 NOVEL TOPICAL TREATMENTS FOR HPV-INDUCED DISEASES

Section 2 describes the evaluation of two new topical compounds for HPV-induced diseases: i) omiganan, a small peptide known for its antimicrobial functions which has shown in vitro antiviral and immunomodulatory activity, and ii) ionic contra-viral therapy (ICVT) comprised of digoxin and furosemide which has shown in vitro antiviral activity. **Chapter 4** describes the results of two clinical trials using omiganan in patients with AGW and vulvar HSIL. The purpose of these trials was to assess the safety of omiganan and to explore pharmacodynamics and efficacy of omiganan in these diseases. In **Chapter 5** safety, pharmacodynamics and efficacy of topical ICVT are investigated in patients with cutaneous warts. **Chapter 6** presents the safety and efficacy of topical ICVT in patients with AGW in a randomized, vehicle-controlled trial.

Chapter 7 summarizes and discusses the overarching findings of these studies as well as future perspectives on drug development in HPV-induced diseases. This chapter includes a critical evaluation of the process of drug development and of novel tools and biomarkers in early phase clinical trials in HPV-induced diseases.

REFERENCES

1 Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med. 1997;102(5A):3-8.
2 Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol. 2005;32 Suppl 1:S16-24.
3 Visser Sd. A question based approach to drug development. PhD Thesis, Leiden Univ, Leiden, Neth. 2003.
4 Doorbar J. The papillomavirus life cycle. J Clin Virol. 2005;32 Suppl 1:S7-15.
5 de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology. 2004;324(1):17-27.
6 Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55-70.
7 Frattini MG, Lim HB, Laimins LA. In vitro synthesis of oncogenic human papillomaviruses requires episomal genomes for differentiation-dependent late expression. Proc Natl Acad Sci USA. 1996;93(7):3062-7.
8 Egawa K. Do human papillomaviruses target epidermal stem cells? Dermatology. 2003;207(3):251-4.
9 Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Rev Med Virol. 2015;25 Suppl 1:2-23.
10 de Sanjose S, Brotons M, Pavon MA. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol. 2018;47:2-13.
11 Van Doorslaer K, Li Z, Xirasagar S, et al. The Papillomavirus Episteme: a major update to the papillomavirus sequence database. Nucleic Acids Res. 2017;45(D1):D499-D506.
12 Egawa N, Doorbar J. The low-risk papillomaviruses. Virus Res. 2017;231:119-27.
13 Cubie HA. Diseases associated with human papillomavirus infection. Virology. 2013;445(1-2):21-34.
14 zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2(5):342-50.
15 Brown DR, Shew ML, Qadadri B, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis. 2005;191(2):182-92.
16 Beliaeva TL. The population incidence of warts. Vestn Dermatol Venerol. 1990(2):55-8.
17 van Haalen FM, Bruggink SC, Gussekloo J, Assendelft WJ, Eekhof JA. Warts in primary schoolchildren: prevalence and relation with environmental factors. Br J Dermatol. 2009;161(1):148-52.
18 Kyriakis K, Pagana G, Michailides C, Emmanuelides S, Palamaras I, Terzoudi S. Lifetime prevalence fluctuations of common and plane viral warts. J Eur Acad Dermatol Venereol. 2007;21(2):260-2.
19 Kilkenny M, Merlin K, Young R, Marks R. The prevalence of common skin conditions in Australian school students: 1. Common, plane and plantar viral warts. Br J Dermatol. 1998;138(5):840-5.
20 de Koning MN, Ter SJ, Eekhof JA, et al. Evaluation of a novel broad-spectrum PCR-multiplex genotyping assay for identification of cutaneous wart-associated human papillomavirus types. J Clin Microbiol. 2010;48(5):1706-11.
21 Chan SY, Chew SH, Egawa K, et al. Phylogenetic analysis of the human papillomavirus type 2 (HPV-2), HPV-27, and HPV-57 group, which is associated with common warts. Virology. 1997;239(2):296-302.
22 Hagiwara K, Uezato H, Arakaki H, et al. A genotype distribution of human papillomaviruses detected by polymerase chain reaction and direct sequencing analysis in a large sample of common warts in Japan. J Med Virol. 2005;77(1):107-12.
23 Porro AM, Alchorne MM, Mota GR, Michalany N, Pignatari AC, Souza IE. Detection and typing of human papillomavirus in cutaneous warts of patients infected with human immunodeficiency virus type 1. Br J Dermatol. 2003;149(6):1192-9.
24 Chen SL, Tsao YP, Lee JW, Sheu WC, Liu YT. Characterization and analysis of human papillomaviruses of skin warts. Arch Dermatol Res. 1993;285(8):460-5.
25 Bruggink SC, de Koning MN, Gussekloo J, et al. Cutaneous wart-associated HPV types: prevalence and relation with patient characteristics. J Clin Virol. 2012;55(3):250-5.
26 Massing AM, Epstein WL. Natural history of warts. A two-year study. Arch Dermatol. 1963;87:306-10.
27 Ciconte A, Campbell J, Tabrizi S, Garland S, Marks R. Warts are not merely blemishes on the skin: A study on the morbidity associated with having viral cutaneous warts. Australas J Dermatol. 2003;44(3):169-73.
28 Kwok CS, Holland R, Gibbs S. Efficacy of topical treatments for cutaneous warts: a meta-analysis and pooled analysis of randomized controlled trials. Br J Dermatol. 2011;165(2):233-46.
29 Ockenfels HM. Therapeutic management of cutaneous and genital warts. J Dtsch Dermatol Ges. 2016;14(9):892-9.
30 Bruggink SC, Gussekloo J, Berger MY, et al. Cryotherapy with liquid nitrogen versus topical salicylic acid application for cutaneous warts in primary care: randomized controlled trial. CMAJ. 2010;182(15):1624-30.
31 Dall'oglio F, D'Amico V, Nasca MR, Micali G. Treatment of cutaneous warts: an evidence-based review. Am J Clin Dermatol. 2012;13(2):73-96.
32 Lacey CJ. Therapy for genital human papillomavirus-related disease. J Clin Virol. 2005;32 Suppl 1:S82-90.
33 Bertolotti A, Dupin N, Bouscarat F, Milpied B, Derancourt C. Cryotherapy to treat anogenital warts in nonimmunocompromised adults: Systematic review and meta-analysis. J Am Acad Dermatol. 2017;77(3):518-26.
34 Patel H, Wagner M, Singhal P, Kothari S. Systematic review of the incidence and prevalence of genital warts. BMC Infect Dis. 2013;13:39.
35 Maw RD, Reitano M, Roy M. An international survey of patients with genital warts: perceptions regarding treatment and impact on lifestyle. Int J STD AIDS. 1998;9(10):571-8.
36 Woodhall SC, Jit M, Soldan K, et al. The impact of genital warts: loss of quality of life and cost of treatment in eight sexual health clinics in the UK. Sex Transm Infect. 2011;87(6):458-63.

37 van Lier EA GJ, Oomen PJ, Giesbers H, van Vliet JA, Drijfhout IH, Zonnenberg-Hoff IF, de Melker HE. Vaccinatiegraad en jaarverslag Rijksvaccinatieprogramma Nederland 2017. 2018; RIVM rapport 2018-0008.
38 Garland SM, Kjaer SK, Munoz N, et al. Impact and Effectiveness of the Quadrivalent Human Papillomavirus Vaccine: A Systematic Review of 10 Years of Real-world Experience. Clin Infect Dis. 2016;63(4):519-27.
39 von Krogh G, Szpak E, Andersson M, Bergelin I. Self-treatment using 0.25%-0.50% podophyllotoxin-ethanol solutions against penile condylomata acuminata: a placebo-controlled comparative study. Genitourin Med. 1994;70(2):105-9.
40 Kirby P, Dunne A, King DH, Corey L. Double-blind randomized clinical trial of self-administered podofilox solution versus vehicle in the treatment of genital warts. Am J Med. 1990;88(5):465-9.
41 Edwards A, Atma-Ram A, Thin RN. Podophyllotoxin 0.5% v podophyllin 20% to treat penile warts. Genitourin Med. 1988;64(4):263-5.
42 Syed TA, Khayyami M, Kriz D, et al. Management of genital warts in women with human leukocyte interferon-alpha vs. podophyllotoxin in cream: a placebo-controlled, double-blind, comparative study. J Mol Med (Berl). 1995;73(5):255-8.
43 Syed TA, Lundin S, Ahmad M. Topical 0.3% and 0.5% podophyllotoxin cream for self-treatment of molluscum contagiosum in males. A placebo-controlled, double-blind study. Dermatology. 1994;189(1):65-8.
44 Beutner KR, Wiley DJ, Douglas JM, et al. Genital warts and their treatment. Clin Infect Dis. 1999;28 Suppl 1:S37-56.
45 Beutner KR, Reitano MV, Richwald GA, Wiley DJ. External genital warts: report of the American Medical Association Consensus Conference. AMA Expert Panel on External Genital Warts. Clin Infect Dis. 1998;27(4):796-806.
46 Wiley DJ, Douglas J, Beutner K, et al. External genital warts: diagnosis, treatment, and prevention. Clin Infect Dis. 2002;35(Suppl 2):S210-24.
47 Maitland JE, Maw R. An audit of patients WHO have received imiquimod cream 5% for the treatment of anogenital warts. Int J STD AIDS. 2000;11(4):268-70.
48 Beutner KR, Tyring SK, Trofatter KF, Jr., et al. Imiquimod, a patient-applied immune-response modifier for treatment of external genital warts. Antimicrob Agents Chemother. 1998;42(4):789-94.
49 Edwards L, Ferenczy A, Eron L, et al. Self-administered topical 5% imiquimod cream for external anogenital warts. HPV Study Group. Human PapillomaVirus. Arch Dermatol. 1998;134(1):25-30.
50 Eron LJ, Alder MB, O'Rourke JM, Rittweger K, DePamphilis J, Pizzuti DJ. Recurrence of condylomata acuminata following cryotherapy is not prevented by systemically administered interferon. Genitourin Med. 1993;69(2):91-3.
51 Stone KM, Becker TM, Hadgu A, Kraus SJ. Treatment of external genital warts: a randomised clinical trial comparing podophyllin, cryotherapy, and electrodesiccation. Genitourin Med. 1990;66(1):16-9.
52 Godley MJ, Bradbeer CS, Gellan M, Thin RN. Cryotherapy compared with trichloroacetic acid in treating genital warts. Genitourin Med. 1987;63(6):390-2.
53 Abdullah AN, Walzman M, Wade A. Treatment of external genital warts comparing cryotherapy (liquid nitrogen) and trichloroacetic acid. Sex Transm Dis. 1993;20(6):344-5.
54 Jensen SL. Comparison of podophyllin application with simple surgical excision in clearance and recurrence of perianal condylomata acuminata. Lancet. 1985;2(8465):1146-8.
55 Khawaja HT. Podophyllin versus scissor excision in the treatment of perianal condylomata acuminata: a prospective study. Br J Surg. 1989;76(10):1067-8.
56 Simmons PD, Langlet F, Thin RN. Cryotherapy versus electrocautery in the treatment of genital warts. Br J Vener Dis. 1981;57(4):273-4.
57 van de Nieuwenhof HP, van der Avoort IA, de Hullu JA. Review of squamous premalignant vulvar lesions. Crit Rev Oncol Hematol. 2008;68(2):131-56.
58 Colgan TJ. Vulvar intraepithelial neoplasia: a synopsis of recent developments. J Low Genit Tract Dis. 1998;2(1):31-6.
59 Joura EA. Epidemiology, diagnosis and treatment of vulvar intraepithelial neoplasia. Curr Opin Obstet Gynecol. 2002;14(1):39-43.
60 van Esch EM, Dam MC, Osse ME, et al. Clinical characteristics associated with development of recurrence and progression in usual-type vulvar intraepithelial neoplasia. Int J Gynecol Cancer. 2013;23(8):1476-83.
61 van Seters M, van Beurden M, de Craen AJ. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. Gynecol Oncol. 2005;97(2):645-51.
62 Jones RW, Rowan DM, Stewart AW. Vulvar intraepithelial neoplasia: aspects of the natural history and outcome in 405 women. Obstet Gynecol. 2005;106(6):1319-26.
63 Tatti S, Suzuki V, Fleider L, Maldonado V, Caruso R, Tinnirello Mde L. Anal intraepithelial lesions in women with human papillomavirus-related disease. J Low Genit Tract Dis. 2012;16(4):454-9.
64 Lawrie TA, Nordin A, Chakrabarti M, Bryant A, Kaushik S, Pepas L. Medical and surgical interventions for the treatment of usual-type vulval intraepithelial neoplasia. Cochrane Database Syst Rev. 2016(1):CD011837.
65 Mahto M, Nathan M, O'Mahony C. More than a decade on: review of the use of imiquimod in lower anogenital intraepithelial neoplasia. Int J STD AIDS. 2010;21(1):8-16.
66 Terlou A, van Seters M, Ewing PC, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod: seven years median follow-up of a randomized clinical trial. Gynecol Oncol. 2011;121(1):157-62.
67 van Seters M, van Beurden M, ten Kate FJ, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod. N Engl J Med. 2008;358(14):1465-73.
68 Iavazzo C, Pitsouni E, Athanasiou S, Falagas ME. Imiquimod for treatment of vulvar and vaginal intraepithelial neoplasia. Int J Gynaecol Obstet. 2008;101(1):3-10.
69 Tristram A, Hurt CN, Madden T, et al. Activity, safety, and feasibility of cidofovir and imiquimod for treatment of vulval intraepithelial neoplasia (RT(3)VIN): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol. 2014;15(12):1361-8.
70 Hurt CN, Jones S, Madden TA, et al. Recurrence of vulval intraepithelial neoplasia following treatment with cidofovir or imiquimod: results from a multicentre, randomised, phase II trial (RT3VIN). BJOG. 2018;125(9):1171-7.
71 Fehr MK, Hornung R, Schwarz VA, Simeon R, Haller U, Wyss P. Photodynamic therapy of vulvar intraepithelial neoplasia III using topically applied 5-aminolevulinic acid. Gynecol Oncol. 2001;80(1):62-6.
72 van Poelgeest MI, Welters MJ, Vermeij R, et al. Vaccination against Oncoproteins of HPV16 for Noninvasive Vulvar/Vaginal Lesions: Lesion Clearance Is Related to the Strength of the T-Cell Response. Clin Cancer Res. 2016;22(10):2342-50.
73 Aerts L, Enzlin P, Vergote I, Verhaeghe J, Poppe W, Amant F. Sexual, psychological, and relational fuNCTioning in women after surgical treatment for vulvar malignancy: a literature review. J Sex Med. 2012;9(2):361-71.
74 Hillemanns P, Wang X, Staehle S, Michels W, Dannecker C. Evaluation of different treatment modalities for vulvar intraepithelial neoplasia (VIN): CO(2) laser vaporization, photodynamic therapy, excision and vulvectomy. Gynecol Oncol. 2006;100(2):271-5.
75 Penna C, Fallani MG, Fambrini M, Zipoli E, Marchionni M. CO2 laser surgery for vulvar intraepithelial neoplasia. Excisional, destructive and combined techniques. J Reprod Med. 2002;47(11):913-8.
76 Leufflen L, Baermann P, Jr., Rauch P, et al. Treatment of vulvar intraepithelial neoplasia with CO(2) laser vaporization and excision surgery. J Low Genit Tract Dis. 2013;17(4):446-51.
77 Mathiesen O, Buus SK, Cramers M. Topical imiquimod can reverse vulvar intraepithelial neoplasia: a randomised, double-blinded study. Gynecol Oncol. 2007;107(2):219-22.
78 Integrity database: Clarivate Analytics; [Available from: https://integrity.clarivate.com.
79 Thomas DW JBJ, Audette J, Carroll A, Dow-Hygelund C, Hay M. Clinical Development Success Rates 2006-2015. 2016.
80 Cohen AF, Burggraaf J, van Gerven JM, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. Annu Rev Pharmacol Toxicol. 2015;55:55-74.
81 World Health Organization. Biomarkers in Risk Assessment: Validity and Validation. 2001. Environmental health criteria; 222.
82 Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS. 2010;5(6):463-6.