Diploma Thesis

Primary Imiquimod Therapy versus Surgery for the Treatment of Vulvar Intraepithelial Neoplasia (VIN) – Analysis and Measurement of the Photo Documentation from the PITVIN Study

submitted by

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Graz, 10.02.2022

Statutory Declaration

I hereby declare on my honor, that I have written this thesis independently and without external help. I have not used sources other than those specified, and I have explicitly marked all material which has been quoted either directly or indirectly from the sources used.

Graz, 10.02.2022 *Dina Misut eh.*

Acknowledgments

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To my husband Elvin, I want to thank you for all your patience and strength in keeping me moving towards the fulfilment of my dreams.

Finally, I wish to thank the rest of my family: my dad, my grandmothers, my sisters, my son and my parents in law for their infinite love and encouragement.

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Glossary and Abbreviations

ACOG – The American College of Obstetricians and Gynecologists

AGO - Arbeitsgemeinschaft für Gynäkologische Onkologie

CR – complete response

CRF – case report form

dVIN – differentiated vulvar intraepithelial neoplasia

FDA – U.S. Food and Drug Administration

HPV – human papilloma virus

hrHPV – high-risk human papilloma virus

ISSVD - International Society for the Study of Vulvovaginal Disease

ITT – intention-to-treat

NR – no response

NSAR – non-steroidal antirheumatic

PP – per-protocol

PR – partial response

RCT – randomized controlled trial

SCC – squamose cell carcinoma

uVIN – usual vulvar intraepithelial neoplasia

vH-SIL – vulvar high-grade squamous intraepithelial lesion

VIN – vulvar intraepithelial neoplasia

vL-SIL – vulvar low-grade squamous intraepithelial lesion

wPR – weak partial response

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Zusammenfassung

Hintergrund

Die vulväre intraepitheliale Neoplasie (VIN) ist eine prämaligne Dysplasie atypischer Keratinozyten im Vulvarepithel (1). Das bösartige Potenzial der VIN wird auf 8 bis 86% geschätzt (2). Die Inzidenz des HPV-bedingten Subtyps, der vulvären hochgradigen squamösen intraepithelialen Läsion (vH-SIL), nimmt weltweit zu, insbesondere bei Frauen vor der Menopause. Die chirurgische Entfernung sichtbarer VIN-Läsionen ist der Goldstandard in der Behandlung von VIN/vH-SIL, doch ist sie weder nebenwirkungsfrei, noch bietet sie eine langfristige Sicherheit vor einem Wiederauftreten der Krankheit. Imiquimod 5% Creme wurde als Off-Label-Behandlung bei Patientinnen mit vH-SIL seit mehreren Jahren eingesetzt. Die Creme kann die lokale Immunantwort verändern und die Clearance von hrHPV bewirken.

Ziel dieser Diplomarbeit ist es, einen Beitrag zu den zunehmenden Erkenntnissen über die Wirksamkeit, Sicherheit und Nichtunterlegenheit der topischen Imiquimod-Behandlung im Vergleich zur chirurgischen Exzision bei vH-SIL (VIN2-3) Patientinnen zu leisten.

Methoden

Diese Diplomarbeit wird im Rahmen der Analyse und Interpretation von Fotodokumentation aus der PITVIN-Studie durchgeführt. Es handelt sich um eine multizentrische, randomisierte, kontrollierte Studie (RCT), an der 110 Frauen im Alter von 19–82 Jahren mit histologisch gesichertem vH-SIL teilgenommen haben.

In dieser Diplomarbeit wurde die Veränderung der Anzahl der Läsionen und der Gesamtfläche der Läsionen (Summe der einzelnen Läsionenflächen, die bei einer Kontrolle vorhanden waren) unter der gegebenen Behandlung (Imiquimod oder Operation) im Verlauf der klinischen Kontrollen gemessen und analysiert. Ferner wurde der Einfluss der Ausgangsläsionsfarbe auf die Wirksamkeit der Behandlung untersucht und bewertet. In der Diplomarbeit wurden insgesamt 68 Patientinnen einbezogen (ohne die restlichen 39 Patientinnen aus der PITVIN Studie aufgrund unvollständiger Daten), wobei die primäre Imiquimod-Gruppe 36 Patientinnen und die primäre chirurgische Gruppe 32 Patientinnen umfasste.

Ergebnisse

Bei der Intention-to-treat-Analyse wurde sechs Monate nach der Baseline (bei U6) bei 30/36 (83,3 %) Frauen, die mit topischem Imiquimod behandelt wurden, und bei 28/32 (87,5 %) Frauen nach einem chirurgischen Eingriff ein vollständiges klinisches Ansprechen festgestellt. Die Haltbarkeit nach einem Jahr bei Patientinnen, die als vollständige Responder eingestuft wurden, wurde bei U12 ermittelt, wobei drei weitere Patientinnen in der Imiquimod-Gruppe ein vollständiges Ansprechen entwickelten (33/36) - 91,7 %, und zwei weitere Patientinnen aus der chirurgischen Gruppe (30/32) - 93,8 %.

Bei den mit topischem Imiquimod behandelten Patientinnen ging die Zahl der Läsionen nach sechs Monaten ($P<0,001$) und nach 12 Monaten ($P<0,001$) im Vergleich zum Ausgangswert statistisch signifikant zurück.

Zu Beginn der Behandlung war der Anteil der Patientinnen mit leukoplakischen Läsionen (75 %) und mit pigmentierten Läsionen (25 %) in der Imiquimod-Gruppe und der chirurgischen Behandlungsgruppe gleich. Die Korrelationsanalyse zwischen der Farbe der Läsionen zu Beginn der Behandlung und der Wirksamkeit der Behandlung, die bei der U6 und der U12 in beiden Behandlungsgruppen ermittelt wurde, ist als nicht signifikant ($p>0,05$) zu werten.

Schlussfolgerung

Die primäre topische Behandlung mit 5%igem Imiquimod ist eine wirksame, sichere und nicht unterlegene Alternative zur Operation bei der Behandlung von VIN (vHSIL). Die Ausgangsfarbe der Läsion sollte kein relevantes Kriterium für die Wahl zwischen Imiquimod oder einer Operation als optimale primäre Behandlungsmethode darstellen und ist ein unbedeutender Aspekt für die Wirksamkeit der Behandlung bei vH-SIL-Patientinnen.

Abstract

Introduction/Background

Vulvar Intraepithelial Neoplasia (VIN) is a preinvasive dysplasia of atypical keratinocytes within the vulvar epithelium (1). The malignant potential of VIN is yet to be determined; however, it is estimated to range between 8% up to 86% (2). The incidence of its HPV related subtype, a vulvar high grade squamous intraepithelial lesion (vH-SIL), marks a worldwide increase particularly among premenopausal women. Currently, surgery is still the most common treatment choice for VIN/vH-SIL, however, it is neither side-effects free nor does it offer a long-lasting absence of disease recurrence. Imiquimod 5% cream has been introduced as an off-label treatment for vH-SIL, capable of altering the local immune response and eliciting the clearance of hrHPV.

This thesis aims to contribute to growing evidence regarding the effectiveness, safety, and non-inferiority of topical imiquimod treatment relative to surgical excision in vH-SIL (VIN2-3) patients.

Methods

The thesis is conducted as a part of the analysis and interpretation of photographical documentation from the PITVIN study. It is designed as a multicenter randomized controlled trial (RCT) enrolling 110 women aged between 19-82 years with histologically confirmed vH-SIL (110 patients enrolled, 3 lost to follow-up).

This thesis measured and analyzed the change in lesion number and total lesion area (Σ of individual lesion areas present at the control) under the given treatment (imiquimod/surgery) throughout clinical controls. Furthermore, the influence of the baseline lesion color on the effectiveness of the treatment was assessed and evaluated. The thesis incorporates a total of 68 patients (it excludes the residual 39 patients enrolled in the PITVIN study due to incomplete data), resulting in a primary imiquimod group containing 36 patients and primary surgery group of 32 patients, respectively.

Results

By intention-to-treat analysis, complete clinical response six months after the baseline (at U6) was seen in 30/36 women treated with topical imiquimod (83.3%) and 28/32 (87.5%) women after surgical intervention. One year durability in patients listed as complete responders was determined at U12, with three additional patients in the imiquimod group developing complete response (33/36) - 91.7% and two additional patients in the surgery group (30/32) - 93.8%.

Patients treated with topical imiquimod witnessed a statistically significant decline in the number of lesions at six months ($P<0.001$) and at 12 months ($P<0.001$), relative to the baseline lesion number.

At the baseline, an equal portion of patients presented with leukoplakic lesions (75%) and pigmented lesions (25%) between imiquimod and surgical treatment groups, respectively**.** Correlation analysis assessing the influence of the baseline lesion color for the treatment effectiveness determined at the U6 and the U12 in both treatment groups is insignificant ($p>0.05$).

Conclusion

Primary topical 5% imiquimod treatment is an effective, safe, and non-inferior alternative to surgery in the treatment of VIN (vHSIL). Pretreatment (baseline) lesion color should not represent a relevant criterium favoring imiquimod or surgery as an optimal primary treatment modality and is an insignificant aspect for the treatment effectiveness in vH-SIL patients.

1 Introduction

Vulvar Intraepithelial Neoplasia (VIN) is a treatable premalignant lesion of the vulvar skin (3). Cellular changes described through VIN are confined within the vulvar epithelium and do not pass the basement membrane. Though the condition is rare, it carries a risk of invasive progression. On average, "9% of untreated VIN patients progress to invasive vulvar cancer within 1–8 years and 3.3% progress after the treatment" (4).

The International Society for the Study of Vulvovaginal Disease (ISSVD) classifies VIN as a "low-grade squamous intraepithelial lesion of the vulva (vL-SIL), high-grade squamous intraepithelial lesion of the vulva (vH-SIL) and differentiated VIN (dVIN)" (5).

Patients with VIN may be asymptomatic or report diverse symptoms such as chronic and severe pruritus, pain, discomfort and burning sensations (6). Macroscopic changes of the vulvar skin affected with the VIN lesion range from color changes (red, brown, white or grey patches), skin thickening, skin thinning up to skin lacerations.

Complete spontaneous remission is uncommon, as even patients receiving the treatment develop recurrences. Currently, surgical removal of all visible lesions is still a state-of-the-art treatment for VIN (7). Surgery is neither side effects free nor does it offer a reliable absence of VIN recurrence. The surgery itself does not permanently eliminate the underlying cause of VIN – a persistent human papillomavirus (HPV) infection and may leave significant impairments in terms of psychosexual dysfunction and aesthetic changes. Imiquimod 5% cream has been introduced as an alternative, non-invasive, off-label treatment for vH-SIL. Being a topical immune response modulator, imiquimod is capable of completely eradicating hrHPV in complete responders (1), as reported by Mathiesen et al. (2007) with complete histologic response in 81% (8), and van Seters et al. (2008) with partial and complete clinical response in 46% and 35% (9) of patients treated with imiquimod at six months, respectively. To date, the comparison of topical imiquimod versus surgery as a primary treatment entity for VIN is not reported in a single RCT study (10). The PITVIN study aims to evaluate the extent of clinical response comparing two treatment options (primary topical imiquimod treatment vs primary surgical intervention) in 110 patients with histologically confirmed vHSIL (VIN 2,3). The primary endpoint of the study is defined as a complete clinical response (i.e., 100% reduction in a primary lesion size) at six months from the therapy onset. The study also aims to evaluate on the degree of reduction in the lesion size, histologic remission, local HPV clearance, amount and extent of surgical procedures, immune cells in

the epidermis and health-related quality of life, including sexuality, based on the treatment patent received (11).

Within the framework of this diploma thesis, the complete photographic evidence of the patient's vulvar lesions (histologically confirmed vH-SIL) collected throughout the PITVIN study over 9 clinical visits (at the baseline, first-month control k1, second-month control k2, third-month control k3, fourth-month control k4, fifth-month control k5, sixth-month control U6, ninth-month control U9 and 12th-month control U12) is calibrated and chronologically measured. Numerical data provided from these measurements is used in the PITVIN study for further statistical analyses.

Change in the lesion number and in the total lesion area (Σ individual lesion areas present at one control) in correlation to the randomized therapy group at the baseline, sixth-month control (U6) and 12th-month control (U12) is further statistically analyzed and evaluated in this thesis. Firstly, the decline in the lesion number based on the different treatment received is noted. Secondly, the extent of decline in a primary lesion area at patients' controls at sixth- (U6) and 12th (U12)-month from the baseline is assessed. Finally, the relationship between the pretreatment lesion color at the baseline (determined from the patients' baseline lesion photograph) and the clinical response at the sixth-month control (U6) and the 12th-month control (U12) is determined.

1.1 Objective of this diploma thesis

The main goal of this diploma thesis is to contribute to growing evidence regarding the effectiveness, safety and non-inferiority of primary topical imiquimod treatment relative to surgical excision in vH-SIL (VIN2-3) patients, as a part of the analysis and interpretation of photographic documentation from the PITVIN study. It is led by the hypothesis that the primary imiquimod treatment is non-inferior to primary surgery in terms of visible lesion clearance with additional benefits related to its use (in terms of cosmetical vulvar outcome, ease of treatment application etc.).

In the scope of writing this diploma thesis, photographic data from total of 107 patients (110 patients enrolled, 3 lost to follow-up) was used to determine and compute the lesion number and lesion area (in mm^2) over nine defined clinical controls within 12 months (RCT and short clinical follow-up phase of the PITVIN study). At each clinical control, a picture of

patients' vulvar region was taken, uploaded to the image measuring software, calibrated and the lesion number and total lesion area were calculated and exported in the Excel table. Results obtained through lesion number and area measurements within this thesis are used for the intention-to-treat (ITT) and per-protocol (PP) analyses of the PITVIN study. Thus, this thesis also aims to provide the additional information about VIN in general, contributing to its better understanding and optimal management.

This diploma thesis analyses and compares two treatment entities by comparing visible lesion presence (no visible lesion = complete remission) and total lesion area in patients' diagnostic follow-ups (at six-months and 12 months after the baseline). Objective measurements of the lesion area were conducted in the 'IMagic IMS' software. For each patient's clinical visit, the vulvar region including the lesion(s) and a ruler were photo documented. Additionally, for precise documentation purposes, the evaluating physician marked the approximate lesion size, location and dispersion pattern in patient's case report form (CRF) and in the 'CLIN case' program.

The software 'IMagic IMS' provides a quantitative assessment (numerical and dimensional) of the available photo data, which was used to accurately compute the area(s) of the vulvar lesion(s) throughout the consecutive patient follow-ups. If a patient presented with multiple VIN lesions, the total lesion area was calculated from the sum of individual lesion areas. This feature demonstrated a particular importance in the imiquimod group, since in the most cases, the complete remission (absence of macroscopic, visible lesion) occurred as a gradual process over the course of the treatment, opposite to surgery, where the lesion clearance occurred immediately after the intervention.

It is important to note that the results from this diploma thesis are based on patient assessments/lesion measurements at the baseline, sixth-month control (U6) and 12th-month control (U12), and do not further track patient follow-ups (i.e. the long term follow-up of the PITVIN study), at which time clearance, persistence, recurrence or emergence of new lesions might have been observed.

1.2 Theoretical Background

1.2.1 Definition and classification of VIN

The term vulvar intraepithelial neoplasia (VIN) defines a premalignant proliferation of atypical keratinocytes within the squamous epithelium of the vulva (1). VIN lesion is considered a precursor of invasive squamous cell carcinoma (SCC) (12).

Previously, numerous terms have been used to classify malignant precursor lesions of the vulva, including VIN grades 1, 2, 3 and Bowen's disease (13). In 2004, the International Society for the Study of Vulvovaginal Disease (ISSVD) reclassified VIN (14) into three subgroups, according to respective histopathological and clinical differences. It distinguished between "HPV-associated, high-grade squamous intraepithelial lesion/usual VIN (HSIL/uVIN), and the non-HPV-associated, differentiated VIN (dVIN)" (15), associated with the underlying inflammatory dermatosis. The third subtype refers to an unclassified type or VIN not otherwise specified. The acronym VIN is used by the ISSVD in order to describe high-grade lesions exclusive of grading specification (6).

In 2015, the ISSVD issued an update on the Lower Anogenital Squamous Terminology (LAST), with the aim to concordantly unite the classification of HPV-associated lesions. According to this update, terminology for vulvar squamous intraepithelial lesions distinguishes between:

- Low-grade vulvar lesions termed as vulvar low-grade squamous intraepithelial lesions (vL-SIL). These encompass benign lesions (human papillomavirus effect), are not cancerous and are linked with low-risk HPV infection.
- High-grade vulvar lesions, which account for the majority of VIN cases and are further classified into:

  → Vulvar high-grade squamous intraepithelial lesions (vH-SIL), formerly known as VIN usual type, are associated with high-risk HPV infection, carrying a risk for malignant transformation.

  → Differentiated VIN lesions (dVIN) linked to chronic inflammatory dermatosis (lichen) with a greater potential to progress to invasive vulvar cancer (16).

In line with the latest 2015 ISSVD terminology, 'vulvar HSIL' replaced the term 'usual type VIN (uVIN)' from the 2004 ISSVD terminology. Low-grade SIL of the vulva or vulvar LSIL

encompass flat lesions (flat condyloma or HPV effect) with basal atypia and koilocytic changes (17). The classification of the differentiated type (dVIN) remained unchanged between the ISSVD 2004 and ISSVD 2015 terminology. Changes in the ISSVD terminology for classifying VIN over the past three decades can be seen in **Table 1.**

2015 ISSVD Terminology	2004 ISSVD Terminology	1986 ISSVD Terminology
Vulvar low-grade squamous intraepithelial lesions (vL-SIL)	Flat condyloma, HPV effect	VIN 1
Vulvar high-grade squamous intraepithelial lesions (vH-SIL)	Usual-type VIN (uVIN); subdivided: → VIN, warty/bowenoid type → VIN, basaloid type VIN, mixed type (warty or basaloid)	VIN 2 VIN 3
Differentiated type (dVIN)	Differentiated type (dVIN)	Differentiated type (dVIN)

Table 1 *ISSVD Terminology from the years 1986, 2004 and 2015 (17)*

1.2.2 Epidemiology

Even though VIN is a rare condition, worldwide cancer registries report a steadily increasing incidence over the past three decades, particularly in premenopausal women. In developed countries, VIN is identified as one of 12 neoplasia with increasing incidence (18). This increase is predominantly witnessed amongst white females compared to women of black, Asian or Hispanic origin (19).

The Surveillance, Epidemiology and End Results program revealed an increase in the age-adjusted incidence rate of VIN from 1.1 to 2.1 per 100,000 women-years during the observational period between the 1970s and 1980s (20). The University Hospital of Vienna, Austria documented a substantial rise in the incidence of VIN, with "an average of 7 new cases per year presented during the period from 1985 to 1988, and around 25 new cases per year during the period from 1994 to 1997" (21). Similar observations were reported from the United States, where the incidence of VIN increased by 411% against 20% for invasive

vulvar cancers (18). This worldwide reported increase is most likely linked to an increase in the incidence of HPV infection rates; however, it is also important to note that not all vulvar intraepithelial lesions are related to HPV infection.

At the same time of increasing incidence of VIN, the mean age of diagnosis decreased, with women under 55 accounting for almost 75% of cases. The age at which VIN is diagnosed seems to follow a bimodal peak distribution, with a major peak observed in women aged 40-49 and followed by a minor peak in women older than 55 years (21).

Vulvar low-grade squamous intraepithelial lesions (vL-SIL) do not possess the potential of becoming invasive tumors and manifest clinically either as anogenital wards (condyloma accuminatum) or as Buschke-Lowenstein tumors (16). Anogenital warts occur in 90% of cases in premenopausal patients, who carry a persistent infection with low-risk HPV-type 6 or HPV-type 11 (22). Younger patients tend to develop multifocal lesions, whereas postmenopausal women mainly develop single, solitary herds. Relatively high rates of vL-SIL are reported in France, with "229 cases per 100,000 adolescents and women aged 15–65 years diagnosed by gynecologists" (23) and "107 cases per 100,000 women seen by general physicians" (24).

Vulvar high-grade squamous intraepithelial lesions (vH-SIL) are considered an HPV associated malignancy, as according to the most recent multicenter study on HPV distribution in VIN and vulvar cancer, "HPV DNA was found in 86.7% of the 587 VIN examinations. In the same study, 91.6% of patients carried a single HPV infection, with HPV 16 being the most commonly isolated type (77.3%), followed by HPV 33 (10.6%) and HPV 18 (2.5%)" (25). The younger the patients were, the more likely it was to detect a HPV infection – women under 37 years had it in 93.0%, whereas women over 62 years had it in 76.7% of cases (25).

Differentiated VIN (dVIN) develops due to inflammatory skin conditions (such as lichen sclerosus), without association to an underlying HPV infection. In the past 15 years, "the incidence rates of dVIN witnessed a nine-fold increase from 0.013/100,000 to 0.121/100,000 patients"(26). dVIN affects mainly postmenopausal women between their 60s to 80s. Studies have shown that only a small portion of high-grade VIN patients had dVIN, whereas the majority were diagnosed with vH-SIL (27).

The introduction of a worldwide HPV vaccination program is expected to cause a significant reduction in HPV-associated malignancies, VIN included (28). However, the increasing age

of the population and higher awareness of dVIN are likely to lead to higher relative and absolute incidence rates of dVIN in the near future (29).

1.2.3 Pathogenesis

dVIN and vH-SIL are two primary precursor lesions to invasive vulvar squamous cell carcinoma, equivalent to their HPV association. Histologically, "vH-SIL lesions (previously uVIN) progress to basaloid or warty SCC, whereas dVIN progresses to keratinizing SCC" (30). With the majority of SCC developing from dVIN (non HPV-related), the majority of reported VIN develops in its absence, with dVIN accounting for only 2-10% of reported cases (6). Based on the correspondent HPV infection status, different pathogenic pathways between dVIN and vH-SIL are proposed.

HPV is a small, circular, double-stranded DNA virus (31), that spreads when skin-to-skin contact with an infected tissue occurs. Genital HPV infection is regarded as sexually transmitted. To date, over 100 different HPV types have been identified (32) and subdivided according to their oncogenic potential into high-risk and low-risk types. Low-risk HPV types, associated with condyloma of vL-SIL, are HPV-6 and HPV-11. High-risk types include HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45, with HPV-16 being the single most prevalent type isolated in vH-SIL lesions (25). In the pathogenesis of vH-SIL, the body's immune response fails to produce adequate protection against the high-risk HPV types. The insufficient immune response is linked to diverse host factors and virus persistence (33). Host factors include age, patient medical history, immunity, smoking, sexual behavior and lifestyle. The majority of anogenital HPV infections are self-clearing – 90 % of cases subside on their own within two years (34). HPV-induced cancers typically occur several years after the primary infection (35) due to the potential of local reinfection and latency periods. In such cases, where "the viral infection persists, the oncoproteins E6 and E7 can longer interfere with cellular control mechanisms" (36), causing cellular transformation and apoptosis inhibition. HPV oncoprotein E6 leads to downregulation of tumor suppressor gene p53 (37), whereas E7 causes inactivation of retinoblastoma tumor suppressor gene (pRb), leading to overexpression of $p16^{ink4a}$ and $p14^{arf}$ and eventually to hyperproliferation of affected cells (38). "Most v-HSIL lesions have positive immunohistochemistry to p16, and negative to p53" (39).

Non-HPV related squamous lesions (dVINs) are associated with lichen sclerosus and lichen simplex chronicus (40). Oncogenic potential of those precursors to vulvar SCC is more potent than of the HPV-related lesions. However, the pathophysiology of dVIN leading to vulvar SCC is more complex to understand, nevertheless it appears to be related to chronic oxidative genetic damages (6). At a molecular level, dVIN is associated with p53, NOTCH1 and HRAS mutations. According to the target sequencing study, "NOTCH1 was present in 20% of dVINs, and HRAS was present in 10% of dVINs. The same study also reported that in HPV-negative vulvar carcinoma cases, 33.3% have NOTCH1 mutations, and 27.8% carry HRAS mutations" (41). Vulvar carcinogenesis in the early stage of transformation from dVIN demonstrates upregulated regions of microsatellite instability and genetic hypermethylation (6). In contrast to vH-SIL, the majority of dVIN has positive immunohistochemistry to p53 and negative to p16 (39). **Figure 1** shows different pathogenic pathways of VIN development and subsequent vulvar oncogenesis.

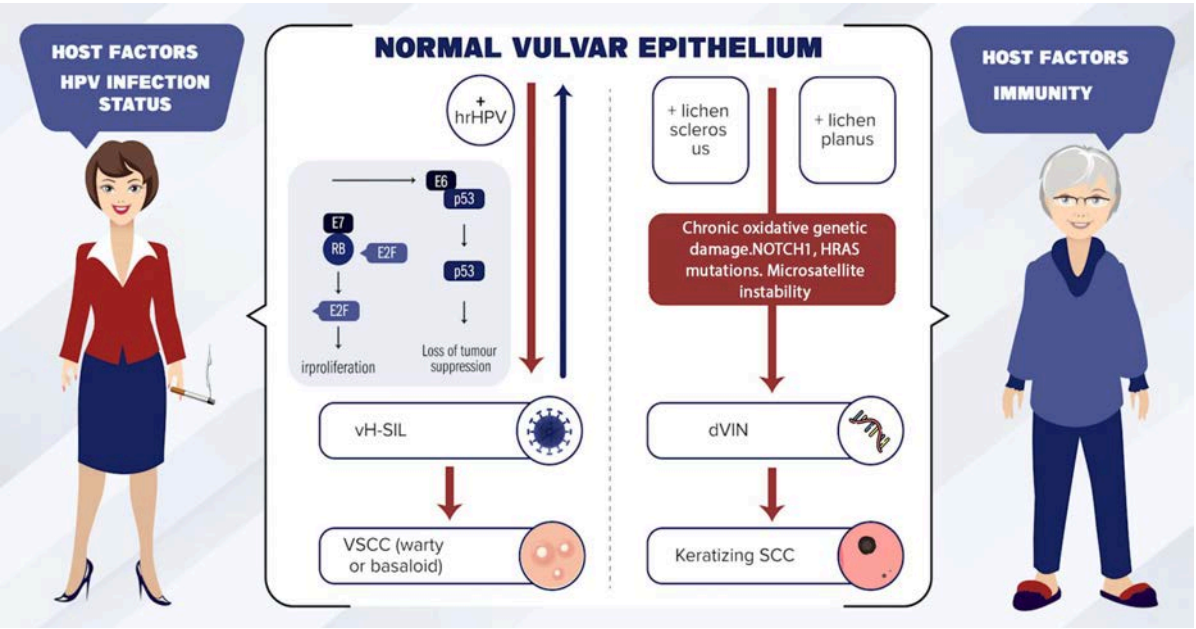


Figure 1 *Schematic representation of pathophysiological pathways of vH-SIL and dVIN*

1.2.4 Diagnosis

VIN patients can either be symptomatic or present without apparent symptoms of VIN. 60% of patients show non-specific warning signs (42) such as chronic and severe pruritus, pain (mostly dyspareunia), discomfort, provoked bleeding or burning sensations. The extent of symptom intensity appears to correlate with lesion severity, as 30% of patients with low-grade lesions are asymptomatic (16). Macroscopic visual assessment of the vulvar region during gynecological examination is a first step towards the diagnosis, though VIN has no pathognomonic macroscopic characteristics (43). Even though the clinical examination with several morphological features of VIN represents the essential aspect of the diagnostic workup, lesion biopsy with histology is the gold standard for diagnosing suspicious lesions (44).

1.2.4.1 Clinical Examination and Characteristics

Clinical examination of the vulvar region should be done under complete exposure and suitable lighting (6), systematically and thoroughly, not omitting the examination of perineum, perianal areas, vagina and cervix. Visual examination is done with the naked eye, although the use of magnifying glass or colposcope can be beneficial. Vulvoscopy and acetowhitening (acetic acid 5% solution) may aid a more in-depth examination, especially in identifying sites for guiding biopsy (45). During the examination, attention should be given to the areas of the vulva that differ from the rest of the region, especially those with erythema, atrophy, pigmentation changes, lichenification, fissures, ulcerations, erosions and scars. **Figures 2 and 3** show the diversity of possible clinical lesion presentation in vH-SIL patients.

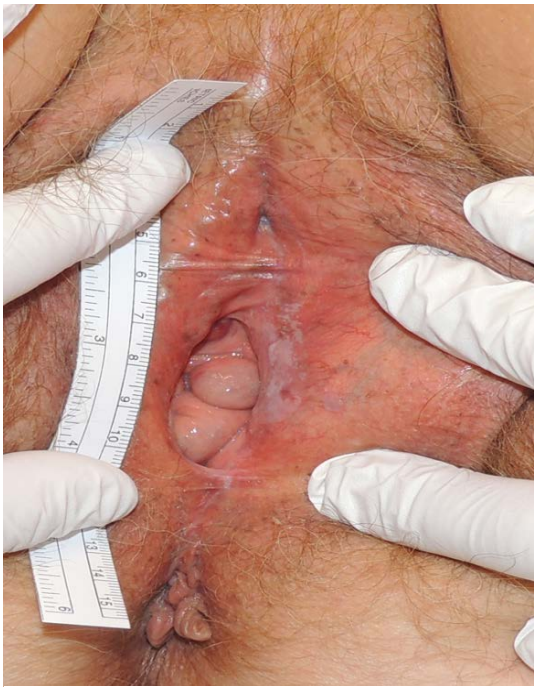


Figure 2 *58-year-old patient with multicentric vH-SIL presenting as white flat patches with erosions and lacerations in the left labia minora*

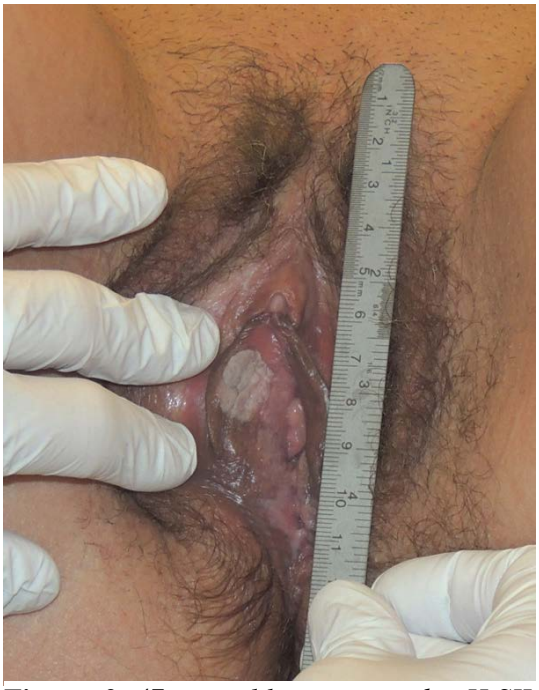


Figure 3 *47-year-old patient with vH-SIL presenting with unifocal white-grayish plaque in the right labia minora*

vH-SIL (uVIN) and dVIN differ not only based on their epidemiologic characteristics but also based on their clinical appearance (17). In vH-SIL, lesions tend to invade the hair-free mucous areas, in a multifocal pattern, mainly around the introitus. Those lesions are usually polymorphic (elevated or papillomatous) and sharply defined, however, they may also appear as irregular plaques with varying degrees of coalescence (16). The presence of intermittent vulvar symptomatology, such as dyspareunia and itching, may indicate vH-SIL. A common finding in vH-SIL is a multicentric lesion dispersion pattern (affecting vulva, cervix, vagina and anus) emphasizing the underlying 'HPV field effect' (involving squamous epithelium from the cervix to perianal area) (46).

The clinical manifestation of dVIN has a less specific lesion pattern than in vH-SIL. Such lesions appear "unicentric and unifocal, as erythematous-leukoplakic discolorations (hyperkeratotic), predominantly as treatment-resistant plaques or elevated nodules, affecting mainly hairy areas of the vulva" (46). History of prolonged vulvar pruritus and discomfort in women with lichen sclerosus or lichen planus is often present (6). An accurate distinction between dVIN and the associated underlying dermatosis remains one of the clinical

challenges of this condition (6). Schematic representation of the differences in the clinical manifestation between the two entities of VIN – vH-SIL and dVIN can be seen in **Figure 4.**

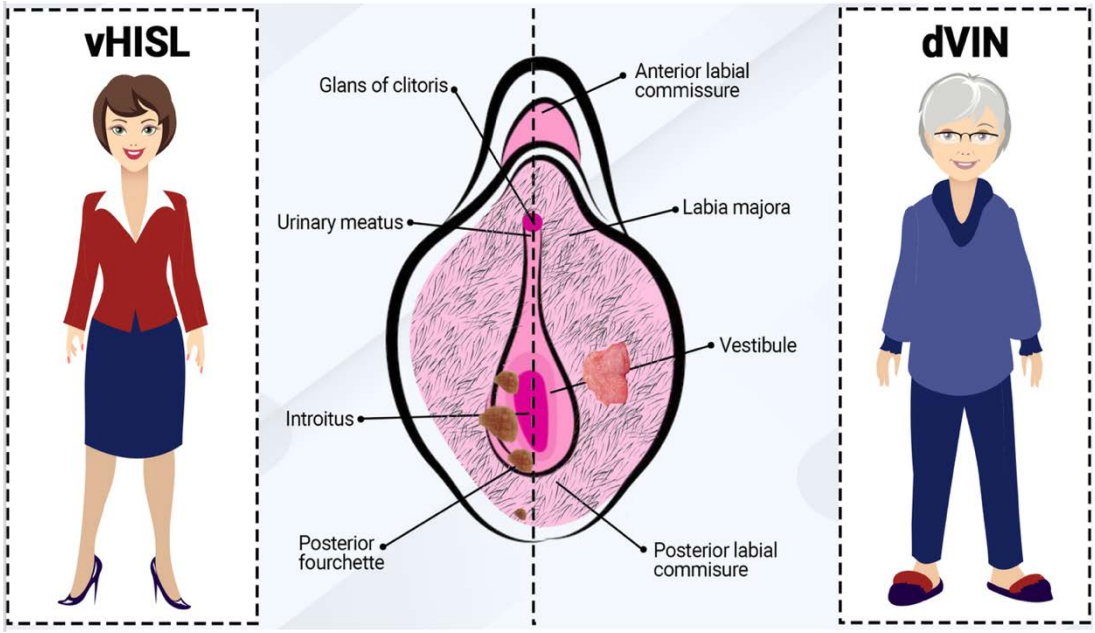


Figure 4 *Schematic representation of clinical manifestation in vH-SIL and dVIN*

1.2.4.2 Biopsy

Small incision biopsy or a punch biopsy (**Figure 6**) from the edge of the lesion, including a sample of normal tissue, is the gold standard in diagnosing VIN (26). Nevertheless, even experienced gynecological pathologists tend to misclassify HPV-related from HPV-independent intraepithelial lesions when relying on the morphology alone in at least 20% of cases. Thus, the correct categorization incorporates the usage of histopathology with immunohistochemistry (IHC) for p16 and p53 (47). Block positive p16, and negative p53 phenotype supports transforming HPV infection. Pathological diagnosis of dVIN is not as conclusive as in vH-SIL, as distinguishment between lichen sclerosus and dVIN is not a step-by-step defined process but more of a biological continuum with the gradual transformation towards SCC (48). In such cases, "IHC for p53 shows basal cell positivity of the same pattern in dVIN, lichen sclerosus and lichen simplex, respectively" (49).

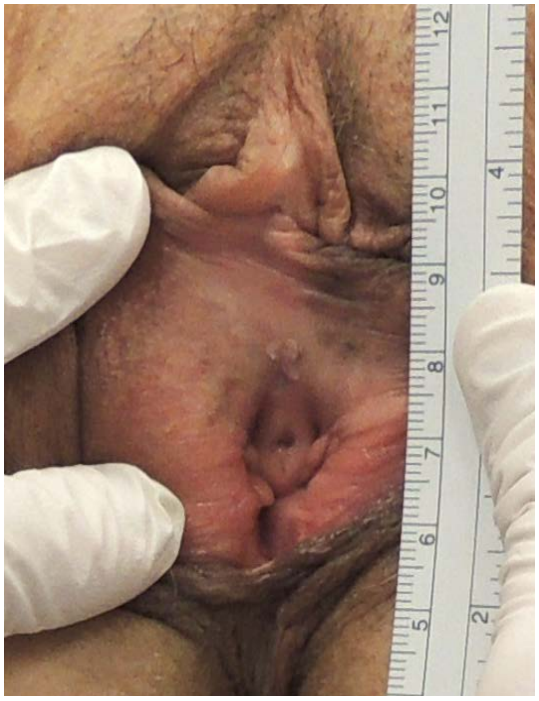


Figure 5 *Patient with a solitary 2mmx2mm vH-SIL lesion at 12 o'clock around the introitus*

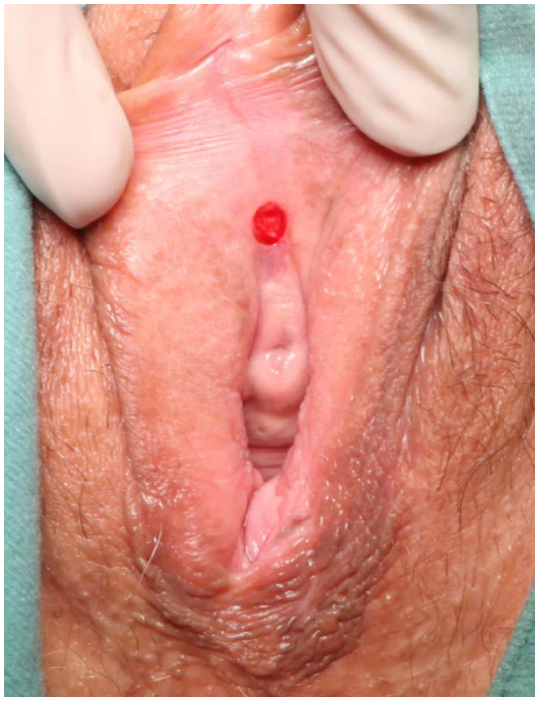


Figure 6 *The same patient following a punch biopsy of the lesion at 12 o'clock around the introitus*

1.2.5 Management

Optimal treatment for VIN is directed by lesion characteristics and host factors, with an ultimate goal of symptom, recurrence and progression freeness. In that sense, therapeutic modalities for VIN include surgical procedures and medical therapy. Complete spontaneous remission is uncommon, as even patients receiving the treatment develop recurrences. Several studies noted recurrency rates for treated vH-SIL patients (uVIN) to range between 25-51% (50)(51). In the most extensive single-institution study on VIN (by Johnes RW et al.), the risk of progression in treated patients was reported to be 4.2% (51). Currently, surgery is still the most common treatment choice for VIN, however high morbidity and recurrences increase the necessity for less invasive and more effective, individualized treatment options. In younger women, who tend to develop multifocal lesions, the initial treatment of choice should aim towards minimal psychosexual impairment (i.e. usage of topical agents) (52). Following the recommendations from the 2016 Cochrane Review of medical and surgical interventions for the treatment of VIN, patients who develop low- or no-response to medical treatment should receive a second-line surgical excision (10).

1.2.5.1 Surgical interventions

Surgical procedures can be performed using cold-knife excision, loop electrosurgery or laser CO_2, among which similar effectiveness rates have been reported (51). "Surgical excision of all visible lesions with large safety margins is the standard treatment for VIN" (53), particularly in patients with unifocal, unicentric herds. On the one hand, the advantage of an invasive approach lies in the opportunity for histological analysis with the exclusion of invasion and the assessment of resection margins. Patients with unaffected resection margins show slightly lower – though still high – risk of recurrence when compared to patients with involved margins (2). On the other hand, the radical treatment leaves mutilating changes in vulvar tissue, including local scarring, without offering reliable and long-lasting lesion absence. **Figures 7 and 8** show the development and formation of local changes affecting the excised vulvar area. **Figure 7** represents the effect of local scarring observed three months post-surgery, resulting in a long-lasting change in the vulvar tissue. In the **Figure 8**, surgery results after one year can be seen. An additional limitation of the surgical approach is that persistent hrHPV infection is not eradicated at the source (10); consequently, the visible is only being removed. However, if the invasion is suspected or diagnosed via biopsy, and in cases of dVIN, surgery remains the desired treatment (52).

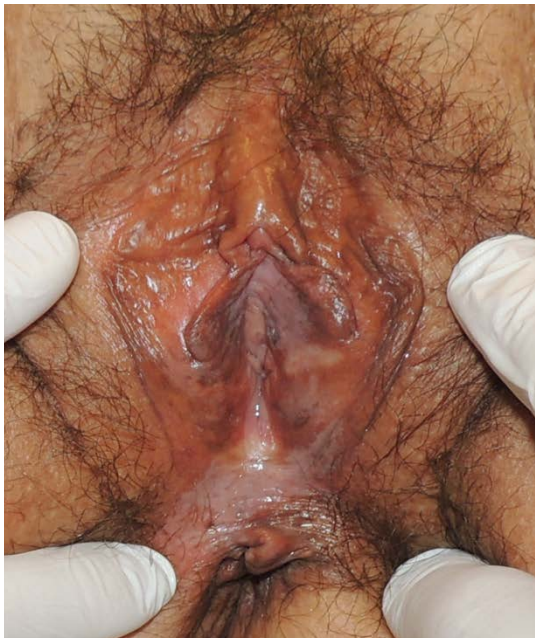


Figure 7 *Patient three months post-surgery, visible formation of the local scar tissue at the perineum*

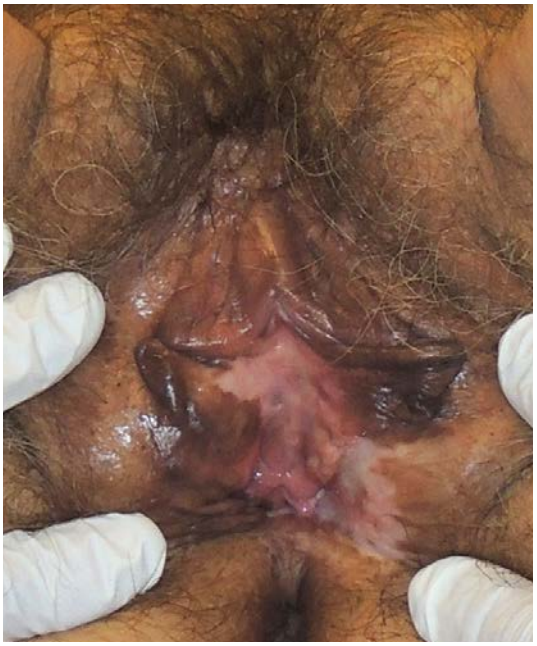


Figure 8 *Patient's control one year after the surgery with apparent scar tissue*

1.2.5.2 Medical interventions

As an alternative to surgery, topical treatments are emerging, hoping to surpass the invasive approach while improving the success rates in primary therapy goals (in the first line recurrence and invasion freeness, psychosexual satisfaction). The effectiveness of such agents has been increasingly studied, however according to the 2016 Cochrane Systematic Review on medical and surgical interventions for the treatment of VIN, it is only reported in isolated cases or short study series. Historically, several medical agents, including topical 5-fluoruracil, trinitrochlorobenzene, bleomycin, and others, have been disregarded due to their inefficacy or aggressive side effects (10).

Topical immune response modulator, imiquimod, is proving a promising treatment for vH-SIL, having been accepted and recommended by a number of scientific societies and guidelines worldwide (54)(52)(55). However, FDA still does not approve any topical treatment for VIN. "Imiquimod is authorized for the treatment of external anogenital warts" (56). The mechanism of action in imiquimod is related to enhancing the body's immune response, though imiquimod itself does not possess direct antiviral properties (16). "It clears HPV through an agonistic effect on Toll-like receptor 7, causing the activation of dendritic cells and cytokine secretion (interferons, tumor necrosis factor α and interleukins)" (33). Recommended dosing regimen by the ACOG includes three times weekly application of 5% imiquimod cream on the lesion area for 12-20 weeks, with vulvoscopic assessment in 4–6-week intervals (56). Local side effects are related to inflammatory reactions and include burning sensation following application, redness, itching, irritation and pain, while systemic side effects include flu-like symptoms with headache, myalgia and apathy (9). Van Seters et al. (2008) led a study on 52 patients with VIN2-3 receiving either imiquimod or placebo, with the conclusion that imiquimod proved as an effective treatment. In particular, the lesion size was reduced by more than 25% at 20 weeks in 81% of women treated with imiquimod and HPV cleared from the lesion in 58% of patients in the imiquimod group, when compared with 8% in the placebo group (9). The associated 2016 Cochrane systematic review of medical interventions for VIN by Lawrie et al. (10) noted that small lesions relate to good response to imiquimod, while multifocal lesions pose a risk factor for recurrence. In cases of residual lesions or recurrences, surgical second-line treatment is needed.

2 Material and methods

2.1 PITVIN Study design

The PITVIN study is designed as a multicenter randomized controlled trial (RCT) enrolling 110 patients with histologically confirmed vH-SIL (formerly classified as VIN 2-3) (11). Study sites include the division of gynecology at the Medical University of Graz (the main study site), the department of gynecology at the Medical University Vienna and dep. of gynecology and obstetrics at the MedUni Innsbruck, dep. of gyn. and obst. at the Landeskrankenhaus Salzburg, dep. of gyn. Krankenhaus Barmherzige Brüder Graz and the dep. of obst. and gyn. at Klinikum Klagenfurt. The study is registered as an AGO trial. The PITVIN study is designed in three phases. The first phase encompasses RCT (Randomised Controlled Trial), with a duration of six months, followed by a short-term clinical control (lasting six months) and proceeded by a long-term follow-up (5 years) (11). **Figure 9**

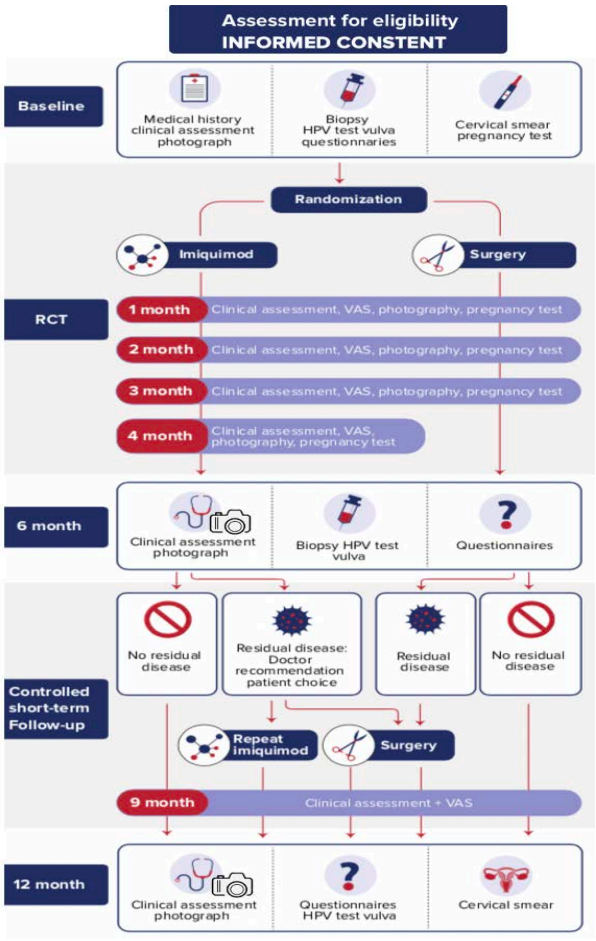


Figure 9 *Schematic representation of the PITVIN study design (RCT and controlled short-term follow-up)*

2.2 Diploma thesis design

This diploma thesis is conducted as an integral part of the PITVIN study in terms of data analysis, focusing on its first two phases - RCT and short-term clinical controls. Precisely, it measures and analyzes photographically documented vulvar lesions from 107 patients (110 patients enrolled, three patients lost to follow-up) to determine the lesion number and total lesion area (in mm^2) at the baseline, one month after baseline (k1), two months after baseline (k2), three months after baseline (k3), four months after baseline (k4), five months after baseline (k5), at the sixth-month control (U6), at the ninth-month control (U9) and at the 12^{th} -month control (U12). The generated measurements are provided for the PITVIN study.

Further statistical analysis, elaboration and evaluation of gathered data in this thesis incorporate a total of 68 patients (107 patients completely measured, 39 patients with incomplete data from U6, U12 or both excluded). The change in the lesion number and total lesion area under the given treatment (imiquimod/surgery) from the baseline to the U6 and U12 was investigated. Additionally, the influence of the baseline lesion color on the treatment effectiveness at U6 and U12 was also examined.

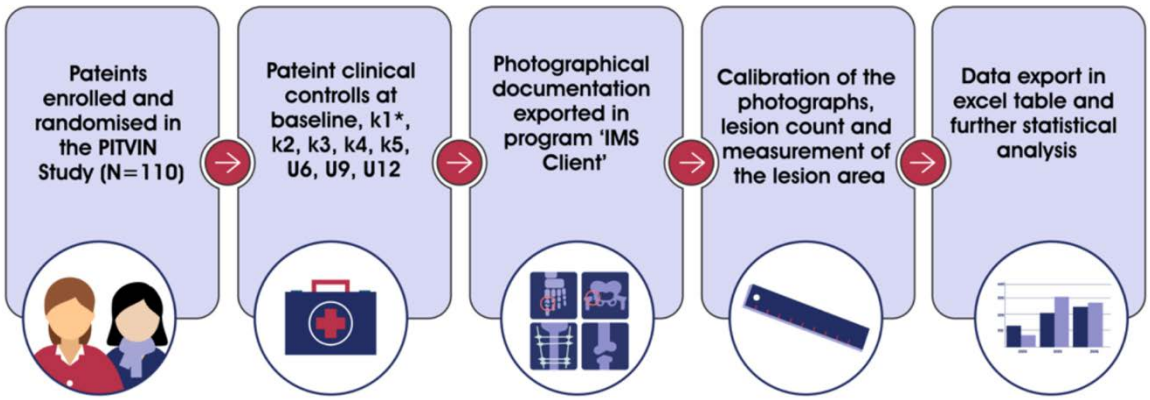


Figure 10 *Schematic representation of the design of this diploma thesis*
**k and U indicate clinical control and number signifies the number of months after the baseline*

For each enrolled and randomized patient, photographic documentation was uploaded in the software 'IMagic IMS' and respectively calibrated. In correlation to patients' case report form (CRF), the total lesion number and corresponding lesion size have been marked and computed. **Figure 10** shows the flowchart of this diploma thesis design. Additionally, the

lesion color at the baseline was also noted, as seen on the corresponding vulvar photograph. In connection with the methodical aspect of this thesis, all anonymously randomized patients were listed in the main Excel table (see Table app. 1), according to their assigned number (i.e. G01).

2.3 Patient recruitments

Patient recruitments for the PITVIN study took place at the above listed study sites with a total of 110 recruited patients in the time period between 2013 and 2020.

Inclusion criteria for the PITVIN study included:

- → Histologically confirmed VIN (only usual type, formerly VIN 2-3)
- → Macroscopically visible, measurable lesion(s)
- → Female patient, age 18 - 90 years
- → Contraception (for premenopausal women)
- → Informed consent

Exclusion criteria:

- → Evidence of invasion
- → History of cancer or severe inflammatory dermatosis of the vulva
- → Pregnancy, lactation
- → Immunodeficiency
- → Any treatment for VIN within the previous three months
- → Known hypersensitivity to imiquimod (11)

2.4 Randomization

Patients who met the inclusion criteria for the PITVIN study were randomized electronically in the program www.randomizer.at for N=110 patients. “They were allocated either to the primary imiquimod treatment or primary surgery and stratified by unifocal or multifocal disease” (11).

From 110 enrolled and randomized patients, 56 were allocated to the imiquimod and 54 to the surgery group in the PITVIN study. In the imiquimod group, one patient was lost to follow up and one developed lung cancer, four stopped the treatment and four changed to surgery. The surgery group lost one patient who declined the consent and one patient changed to imiquimod (11).

In the scope of writing this diploma thesis, photographic data from 107 patients through a total of 9 clinical controls (baseline, k1, k2, k3, k4, k5, U6, U9, U12) was chronologically measured. Obtained results (lesion number, lesion area and lesion baseline color) are subsequently directly used for the statistical analysis and results of the PITVIN study. Corresponding to conducted measurements, the PITVIN study assessed its primary endpoint (clinical response to imiquimod at 6 months) also in a total of 107 patients (11).

2.5 Treatment groups

2.5.1 Primary imiquimod treatment

Treatment with imiquimod 5% cream is patient self-administered for a period of 4 months, with a possible extension for up to 6 months (11).

As part of the PITVIN study protocol, enrolled patients received detailed instruction and written information regarding the use of imiquimod cream, including ways of monitoring and reducing the side effects related to its use. A thin layer of imiquimod cream is applied to the lesion and left to remain overnight without being covered (11). Application frequency:

- → once a week for two weeks
- → twice a week the following two weeks
- → if tolerated, three times a week for the last weeks.

In the case of systemic side effects, the use of NSAR was allowed. Upon occurrence of severe side effects, the application frequency was reduced; however, a treatment gaps of no longer than 7 days were allowed.

During the controlled short-time follow-up, patients with no response, weak partial response or recurrent disease had the option of continuing treatment with imiquimod or switching to

surgical therapy. The decision to discontinue the treatment was based on the doctor's recommendation and the patient's choice, as directed by the PITVIN study protocol.

2.5.2 Primary surgical treatment

Patients randomized to receive primary surgical management were to receive either ablation or excision, based on the clinical findings and surgeon's preference (11). Patients were informed beforehand of the chosen surgical procedure. Following the excision, the histological analysis with the exclusion of invasion and the assessment of resection margins took place.

2.6 Schedule

The table below (**Table 2**), shows the schedule for the first two phases of the PITVIN study. Orange marked areas represent time periods statistically analyzed in this diploma thesis – the measurement of lesion number and sizes according to the photographic evidence recorded at patients' visits at defined controls.

	Clinical assessment	Photo documentation	Biopsy	HPV test	Questionaries	Study diary (VAS)
Baseline	x	x	x	x	x	
K1*	x	x				x
K2	x	x				x
K3	x	x				x
K4	x	x				x
U6	x	x	x	x	x	
U9	x	x				
U12	x	x		x	x	

Table 2 *Overview of the time schedule and desired clinical procedure at defined patients' controls*

**number indicates the number of months after the baseline assessment; orange shaded boxes represent areas involving this diploma thesis*

2.7 Primary endpoint(s) of the PITVIN study vs this diploma thesis

"The primary endpoint of the PITVIN study is defined as a complete clinical response, i.e., the clinical absence of vulvar lesion in 100% in 6 months, after randomly allocated primary treatment with either imiquimod or surgery" (11).

This thesis also concentrates on the same primary endpoint and evaluates it based on the photographic evidence, whether the lesion presence at U6 (6^{th} month control) and U12 is seen or absent in terms of complete clinical remission (complete remission = lesion number 0, total lesion area 0 mm^2).

The leading hypothesis of this thesis is that the primary imiquimod treatment is a safe, effective and non-inferior treatment option compared to primary surgery in terms of visible lesion clearance at U6 and U12 in the vH-SIL patients.

2.8 Secondary endpoints of the PITVIN study vs this diploma thesis

In addition to the main study outcome evaluating the complete clinical remission, "the secondary endpoints of the PITVIN study consider evaluation of clinical response (defined as % decline in lesion size), histologic remission, HPV clearance, the extent of surgical intervention, immunohistochemical changes, aesthetic outcome, quality of life, and treatment satisfaction between two treatment groups" (11).

This thesis also concentrates on the clinical response, defined as a % reduction in the lesion area. Previously photographed images of the vulvar region containing all visible lesions were uploaded in the program 'IMagic IMS'. This program has a feature to calibrate and compute the total lesion area in mm^2. Lesion areas were assessed at the baseline, 1 month after baseline (k1), two months after baseline (k2), three months after baseline (k3), four months after baseline (k4), five months after baseline (k5), at the sixth-month control (U6), at the ninth-month control (U9) and at 12^{th}-month control (U12). In this diploma thesis, the baseline, U6 and U12 are furtherly addressed and the degree of reduction in the lesion size is interpreted as follows:

- No response (NR): reduction in lesion size ≤ 25%
- Weak partial response (wPR): reduction in lesion size between 26-75%
- Strong partial response (stPR): reduction in lesion size between 76-99%

- Complete response (CR): 100% reduction in lesion size

Figure 11 offers a schematic list of endpoints and aims of this thesis and PITVIN study, with intersecting region demonstrating areas of common interest: photo documentation and resulting clinical response.

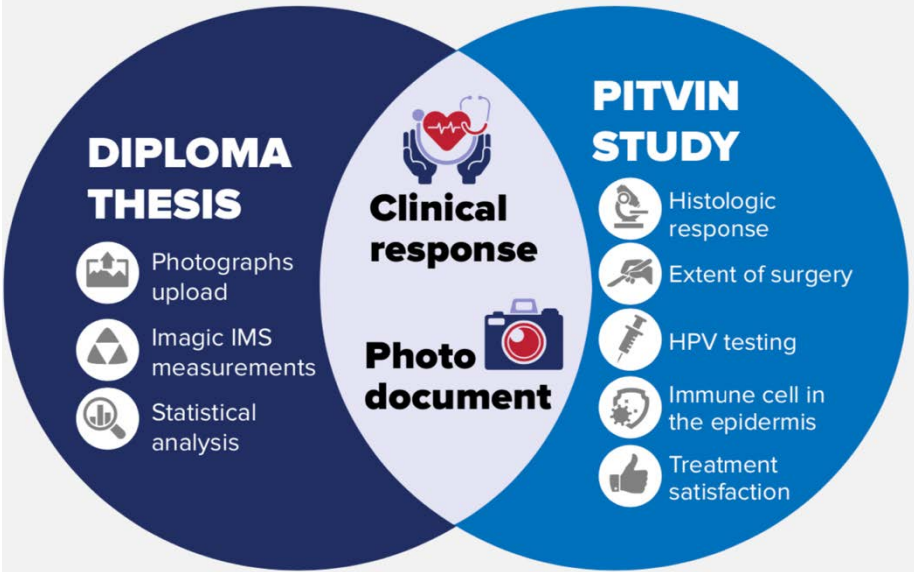


Figure 11 *Venn's Diagram showing primary and secondary endpoints of this thesis and the PITVIN study, with - clinical response and photo documentation – representing the areas of intersection/interest between the two*

2.9 Data analysis

2.9.1 The 'IMagic IMS' Software

Raw data analysis was done in the software 'IMagic IMS', where the complete photographic evidence from the PITVIN study was imported. Image calibration, processing, measurement and annotation occurred in the same program. Firstly, each imported image was calibrated based on the ruler included in the original clinical photograph. Automated measurements with dot-by-dot image measuring tool generated lesion area in mm^2. The program has an Excel built-in feature to automatically export lesion numbers and corresponding areas in a table format.

An example of a measurement from the baseline picture of the patient assigned to the primary imiquimod group is seen in **Figures 12 and 13**.

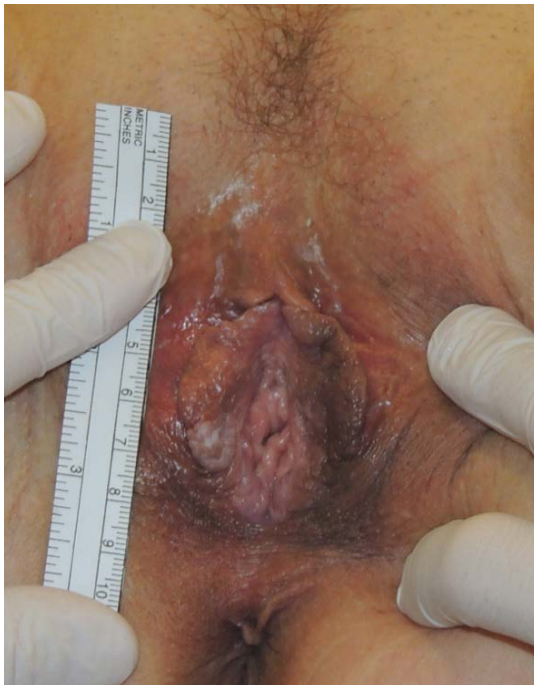


Figure 12 *Raw photographical data uploaded in the 'IMagic IMS' software*

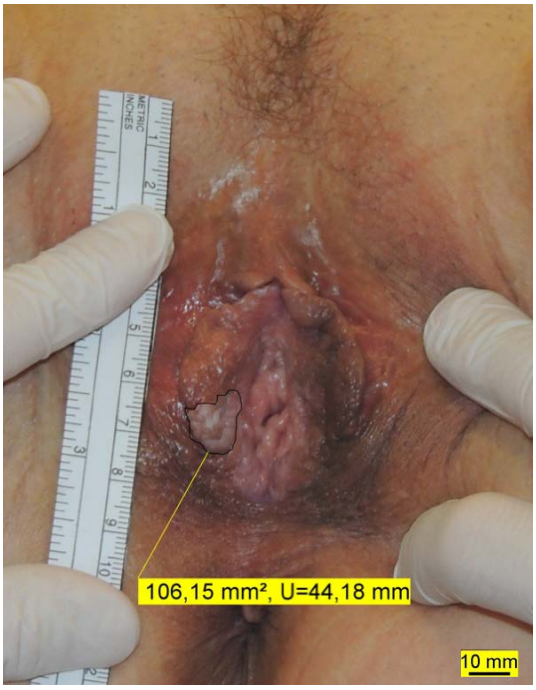


Figure 13 *Calibrated and measured lesion area in mm²*

2.9.2 Statistical analysis

A total of 110 patients were enrolled and randomized in the PITVIN study, with three patients lost to follow-up, resulting in 107 patients. Form the 107 patients, 54 patients allocated to the imiquimod group were included in the intention-to-treat analysis (ITT), and 46 were included in per-protocol analyses (PP) in the PITVIN study. The surgery group had 52 patients included in the per-protocol analyses and 53 in the intention-to-treat analysis. The primary PITVIN study outcome, complete clinical response after six months, was assessed in a total of 107 patients (11).

In order to address the main objective of this diploma thesis, an intention-to-treat (ITT) analysis is conducted. This diploma thesis incorporates 107 patents with corresponding photographic lesion data. It measures the lesion number and total lesion area (Σ of areas of all lesions present at the control) throughout several defined clinical controls (baseline, k1, k2, k3, k4, k5, U6, U9 and U12). However, the objective and statistical analysis of this thesis

refer to the measurements obtained from the baseline, sixth-month control (U6) and twelfth-month control (U12).

As photographic data from U6, U12 or both clinical controls from several patients allocated to both treatment groups was incomplete; those patients have been omitted for the ease and precision of statistical analyses in this diploma thesis. Thus, the final raw data used for the statistical analysis in this thesis incorporates measurements of the total of 68 patients - surgery group including 32 and imiquimod group 36 patients. **Figure 14** schematically represents population numbers and the final ITT population included in this diploma thesis.

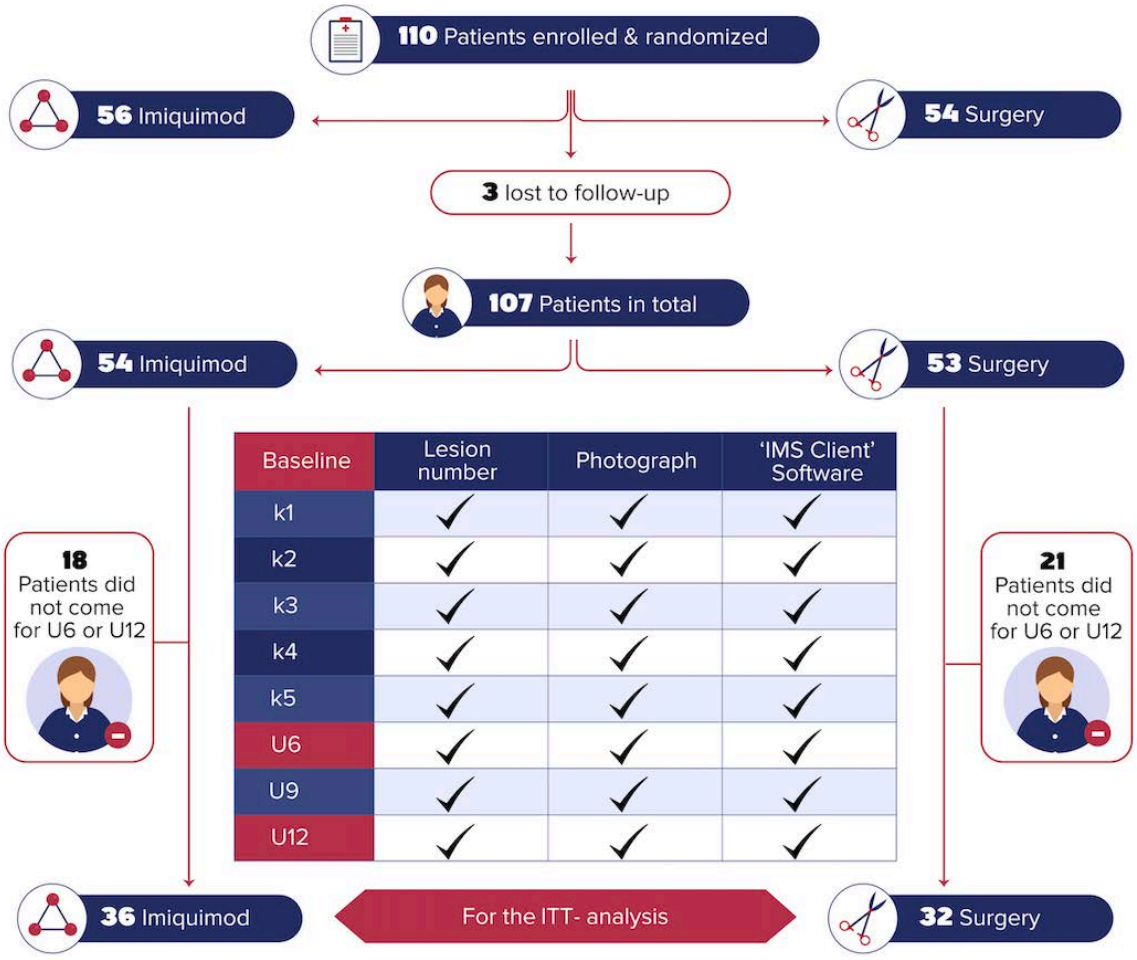


Figure 14 *Diploma thesis ITT population*

Measurement of lesion sizes at respective clinical assessments based on photographic documentation is conducted using 'IMagic IMS' and marked in an Excel™ table. The data was coded based on the treatment (1- primary imiquimod, 2- primary surgery) and statistically analyzed using IBM SPSS statistics program.

Data analysis in this thesis was done as an intention-to-treat analysis (ITT), meaning that the patient was always analyzed, and the clinical response evaluated based on the group allocated by randomization, not taking into account possible treatment changes thereafter (i.e. patient randomized to become treatment with imiquimod, but changed to invasive treatment after six months).

Statistical analysis was conducted in the SPSS software (version 25), with statistical significance being established when $P<0.05$. Non-normally distributed data were reported using median and range, and its related groups were compared using the Kruskal-Wallis test. Mean and 95% confidence interval (CI) were computed for continuous variables. Categorical variables were expressed in the form of the number and percentage, and their particular groups were compared using Pearson's chi-square test with Fisher's exact test. Correlation analysis was conducted using Spearman's rank correlation coefficient. For the graphical presentation of the data, charts and tables were used, which were created using Microsoft Excel™ and GraphPad Prism software (version 8). Figures in the thesis were drawn and configured using Adobe Illustrator, Adobe Photoshop and Microsoft Paint programs for Windows.

3 Results

The main raw data table is represented in the Appendix under **Table app. 1**. In total, data has been gathered and measured for 107 patients. Furthermore, 39 patients, where the data from the U6, U12 or both was incomplete or missing, have been omitted in all of the statistical analysis in this diploma thesis, to include the total of 68 patients for the statistical analysis (see **Figure 14**). As seen in the **Figure 15**, 36 patients in the imiquimod group and 32 patients in the surgery group, respectively, have been further statistically analyzed. The table with omitted patients in this diploma thesis is to be found in the Appedix material under **Table app. 2**. The table has been coded based on the treatment group the patient was randomized to (1 – imiquimod, 2 – surgery).

Throughout the timeline of the PITVIN study, the total of 10 patients from the imiquimod group switched to receive additional surgery and 2 patients from the surgery group switched to the imiquimod treatment. As the majority of those patients who switched groups throughout the course of the PITVIN study did not show up for the U6, U12 or both clinical controls, those patients have not been included in the statistical analysis in this diploma thesis. However, from the set of patients who have been included in the statistical analysis in this thesis (N=68), two patients from the imiquimod group changed to surgery, and none of the patients form the surgery group changed to imiquimod.[1] The **Figure 15** shows a flowchart of the patient numbers in each treatment group (N) and the final ITT-population numbers.

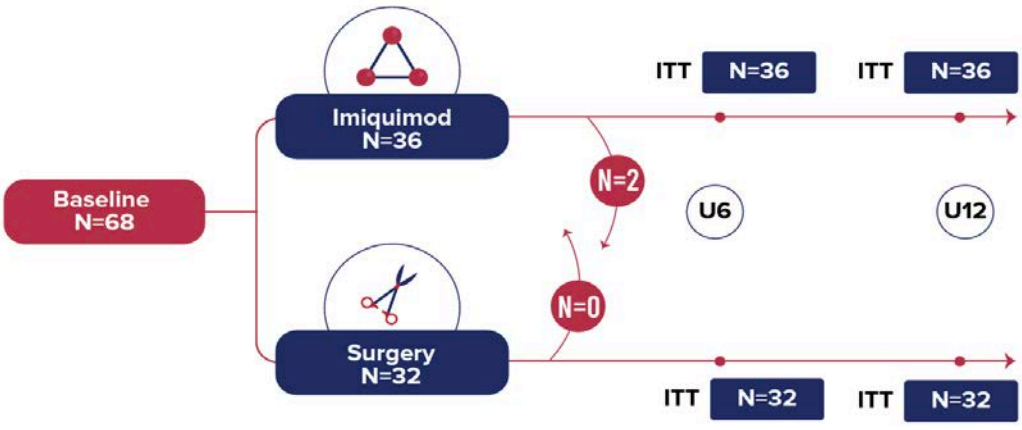


Figure 15 *Flowchart of the diploma thesis ITT population*

[1] Patietns G-02 and G-28 changed from the imiquimod to surgery within the course of the PITVIN study

As previously mentioned, the study population in this diploma thesis will be addressed as an ITT (intention-to-treat) population, meaning that:

Imiquimod group - all patients who received at least one imiquimod application as a first treatment after randomization are considered as an ITT-population (N=36)

Surgery group – all patients who received at least one surgical intervention as a first treatment choice after randomization are addressed as an ITT-population (N=32)

3.1 Baseline demographic characteristics

Statistical analysis in this diploma thesis includes 68 patients with histologically diagnosed vulvar HSIL. Out of them, 36 (53%) patients received topical imiquimod treatment, while 32 (47%) were treated with primary surgical therapy.

At the baseline, patients who received imiquimod treatment had a significantly higher number of lesions, with a median ranging from 1 to 11 lesions (median of 1), in contrast to a range of 1-7 lesions (median 1) among the surgical treatment group (P=0.039). There was no statistically significant difference between both groups regarding the total area of lesions at the baseline (P=0.618). **Table 3**

Table 3 Baseline demographic characteristics

	Imiquimod (N=36)		**Surgical (N=32)**		**P-Value**
	Median(range)	**Mean (95CI%)**	**Median(range)**	**Mean (95CI%)**	
Number of Lesions	1 (1-11)	1.5 (0.9-2.2)	1 (1-7)	1.8 (1.2-2.3)	0.039
Area of lesions (mm^2)	139 (24-708)	193.4(134.95-251.76)	143 (17-1181)	247.2 (145-349.4)	0.618
Abbreviations; CI=Confidence interval					

3.2 Outcome regarding decline in the lesion number

At each patients' clinical control, the lesion number was noted. Median and mean for the total number of lesions at sixth-month control (U6) and 12th-month control (U12) in two different treatment groups were calculated.

Six months after the baseline (U6) there was no statistically significant difference (P=0.765) between imiquimod and surgical treatment regarding the number of lesions, with a median number of 0 (0-2) lesions.

Twelve months after the baseline (U12), the median lesion number was 0 (0-2) and 0 (0-4) between imiquimod treatment and surgical treatment groups, respectively. There was no statistically significant difference regarding the number of lesions seen at U12 between both treatment groups (P=0.731). **Table 4**

Table 4 Number of lesions at 6- and 12-months post-treatment

	Imiquimod (N=36)		**Surgery (N=32)**		**P-Value**
	Median(range)	**Mean (95CI%)**	**Median(range)**	**Mean (95CI%)**	
At 6 months (U6)	0 (0-2)	0.2 (0.0-0.4)	0 (0-2)	0.2 (0.0-0.3)	0.765
At 12 months (U12)	0 (0-2)	0.1 (0.0-0.3)	0 (0-4)	0.2 (-0.1-0.417)	0.731

Abbreviations; CI=Confidence interval

Among patients who received imiquimod treatment, there was a statistically significant decline in the number of the lesions at six months (P<0.001) and at 12 months (P<0.001) relative to the baseline lesion number. A graphical representation of a decline in the lesion number between the baseline and U6 can be seen in the **Figure 16** as a downward sloping trend-line. The y-axis shows median number of lesions in the imiquimod group, while the x-axis represents the time period after the treatment begin (corresponding to the patients' visit). No significant difference was observed between the number of lesions at 6 and 12 months (P=0.453) after the treatment begin.

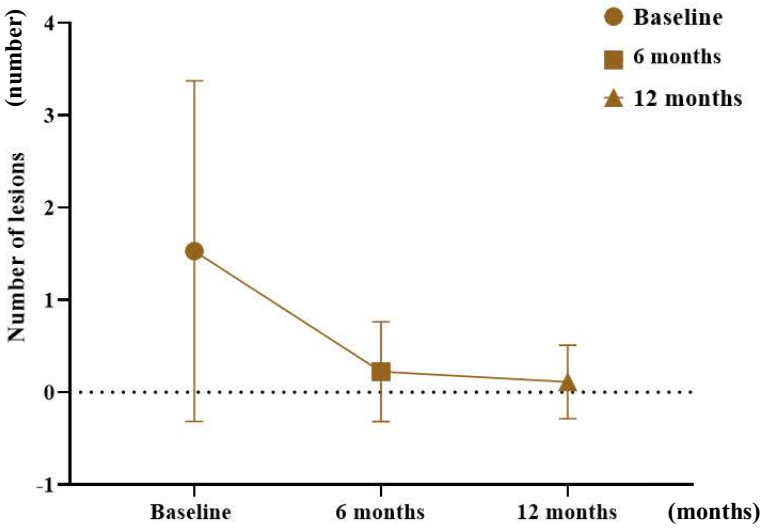


Figure 16 *Line graph of mean and 95%CI with error bars showing the number of lesions at baseline, at sixth month control (U6) and at 12th month control (U12) of imiquimod treatment*

As for the surgical treatment, the mean baseline lesion number declined notably straight after the procedure and remained low for the remaining period until U6. Furthermore, there was no statistically significant difference observed at 6 and 12 months after the baseline in terms of lesion number in the surgery group (P=0.289). This means that the reduction in the lesion number observed at the U6 was maintained for the minimum of additional 6 months, until the U12. This decline in the lesion number from baseline to U6 and up to U12 under the surgical treatment is graphically represented in the **Figure 17.**

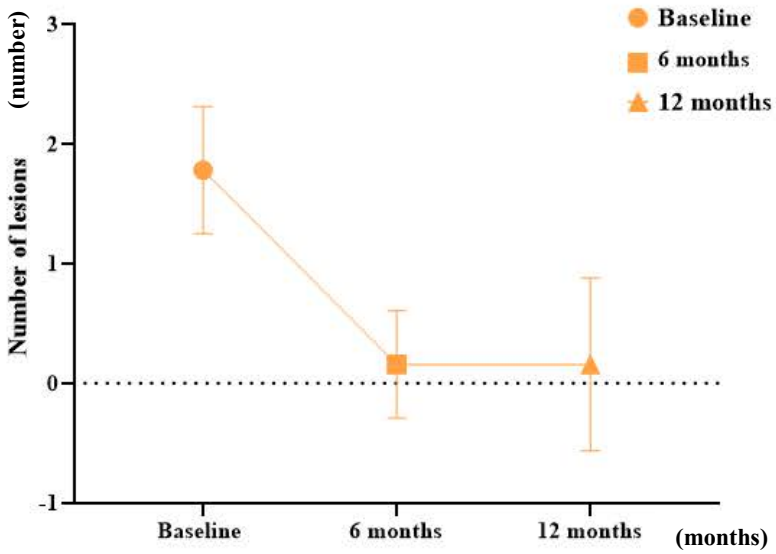


Figure 17 *Line graph of mean and 95%CI with error bars showing the number of lesions at baseline and after 6 and 12 months of surgical treatment*

3.3 Outcome regarding the decline in total lesion area (mm^2)

Total lesion area reduction over the course of treatment (from the baseline to U6 and U12) was investigated. It was assumed that surgery will show cut-off values, with complete absence of the lesion several months after the surgery, with questionable recurrence rates at later controls. It was noteworthy to investigate how fast the decline in the lesion area could be assumed under the topical imiquimod therapy.

When comparing the two treatments, six months after treatment begin (U6), there was no statistically significant difference (P=0.618) between the imiquimod and surgical groups regarding the total area of lesions, with median of 0 (0-208) and 0 (0-70) mm^2, respectively. One-year (U 12) after the baseline, the median lesion area remained 0 (0-41) and 0 (0-114) mm^2 among imiquimod and surgical groups, without a significant difference between the two groups (P=0.807). **Table 5**

Table 5 Area of lesions at 6- and 12-months post-treatment

	Imiquimod (N=36)		**Surgery (N=32)**		**P-Value**
	Median(range)	**Mean (95CI%)**	**Median(range)**	**Mean (95CI%)**	
At 6 months (U6)	0 (0-208)	12.1 (-1.9-26.0)	0 (0-70)	5.2 (-0.6-11.0)	0.618
At 12 months (U12)	0(0-41)	2.5 (-0.6-5.6)	0 (0-114)	5.6 (-2.7-13.9)	0.807
Abbreviations; CI=Confidence interval					

In the imiquimod group, the mean lesion area at U6 and U12 was determined. Among patients who received imiquimod treatment, a decline in the lesion area to the median of 0 (complete absence) was already observed at the sixth month control (U6) in 83%. This reduction in the lesion area was statistically significant at six months (P<0.001) and at 12 months (P<0.001), in contrast to the baseline size. This significant difference was absent when comparing the lesions' area at 6 and 12 months (P=1). **Figure 18**

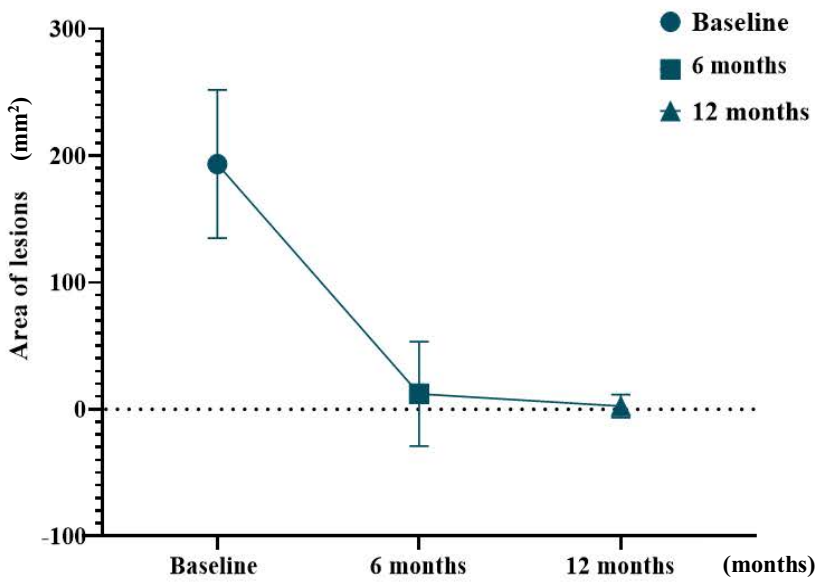


Figure 18 *Line graph of mean and 95%CI with error bars showing the area of lesions at baseline, at sixth month control (U6) and at 12th month control (U12) of imiquimod treatment*

Regarding the surgical treatment, the lesion area was significantly reduced at sixth month (P<0.001) and at 12th month control (P<0.001), relative to pretreatment (baseline) size. There was no statistically significant difference (P=0.625) regarding the lesion area at 6 and 12 months post-surgical therapy. **Figure 19**

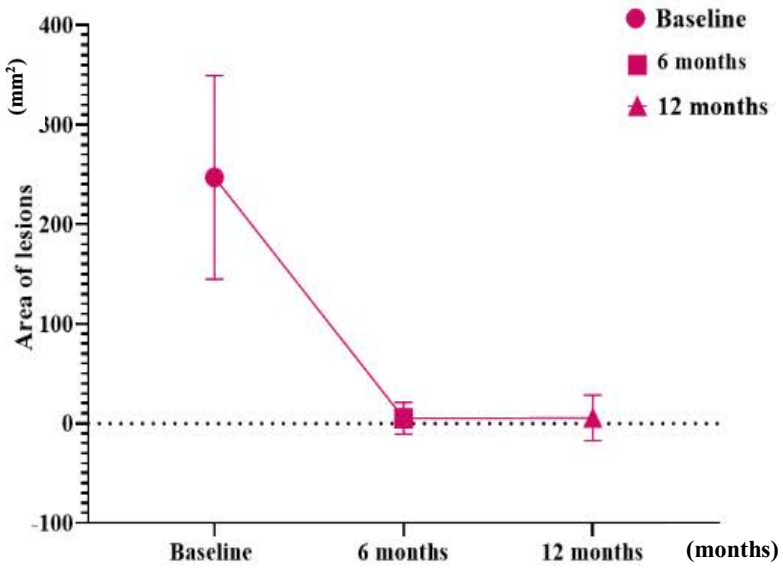


Figure 19 *Line graph of mean and 95%CI with error bars showing the total lesion area at the baseline, at the sixth month control (U6) and at 12th month control (U12) of surgical treatment*

3.4 Baseline lesion color and the effectiveness of the treatment

The relationship between the lesion color at the baseline and the clinical response between the two treatment groups was analyzed. The color of the lesion was determined as seen on the photograph at the baseline. In the imiquimod group, 9/36 patients had brown (pigmented) lesions (25%), while 27/36 patients presented with white (leukoplakic) lesions (75%). In the surgery group, 8/32 had pigmented and 24/32 presented with leukoplakic lesions. There was an equal proportion of patients presenting with white lesions (75%) and brown pigmented lesions (25%) among the imiquimod and surgical treatment groups, respectively**.**

Table 6

Table 6 Lesion color at the baseline			
	Imiquimod (N=36)	**Surgery (N=32)**	**Total (N=68)**
Pigmented	9 (25%)	8 (25%)	17 (25%)
Leukoplakic	27 (75%)	24 (75%)	51 (75%)

The lesion color distribution at the baseline among the ITT population can be seen in the Pie chart (**Figure 20)** below. Three quarters of the patients from both treatment groups presented with white lesions at the baseline.

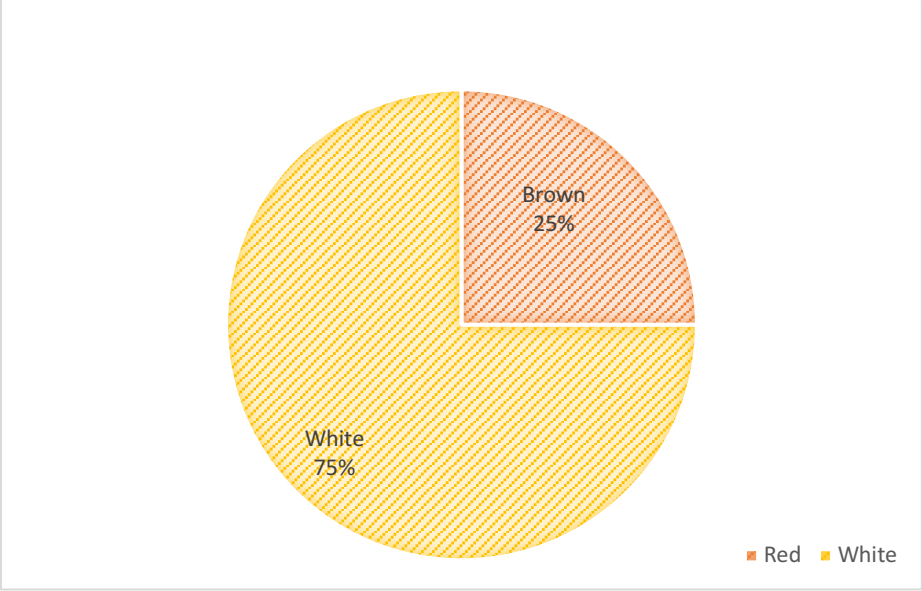


Figure 20 *Pie chart of lesion color distribution at the baseline*

The **Table 7** shows Pearson chi-square test performed on each treatment group for U6 and U12. The chi-square test statistics of all the groups is insignificant, which concludes that no correlation between the baseline lesion color (pretreatment lesion color) and the effectiveness of the treatment in terms of clinical response could be established.

Table 7 Analysis of the relationship between the baseline lesion color and the treatment outcome

	U6		**U12**	
	Imiquimod treatment	**Surgical treatment**	**Imiquimod treatment**	**Surgical treatment**
Pearson Chi-Square Value	3.674	0.396[b]	0.632	0.705
df	2	1	1	2
Asymp. Sig. (2-sided)	0.159	0.529	0.426	0.703

In this concern, **Table 8** also demonstrates, that no statistically significant correlation between the lesion color at the baseline and the response at 12 months (r=-0.059, P=0.628) could be established. Furthermore, there was no statistically significant correlation between the baseline lesion color and the lesion number and lesion area at U6 and U12.

Table 8 The correlation between the baseline lesion color and the outcomes at U6 and U12

Variables	Correlation coefficient	P-Value
Number of lesions at 6 months	0.148	0.229
Number of lesions at 12 months	0.036	0.769
Area of lesions at 6 months	0.150	0.221
Area of lesions at 12 months	0.040	0.745
Complete response at 12 months	-0.059	0.628

Abbreviations; P-Value= Probability Value

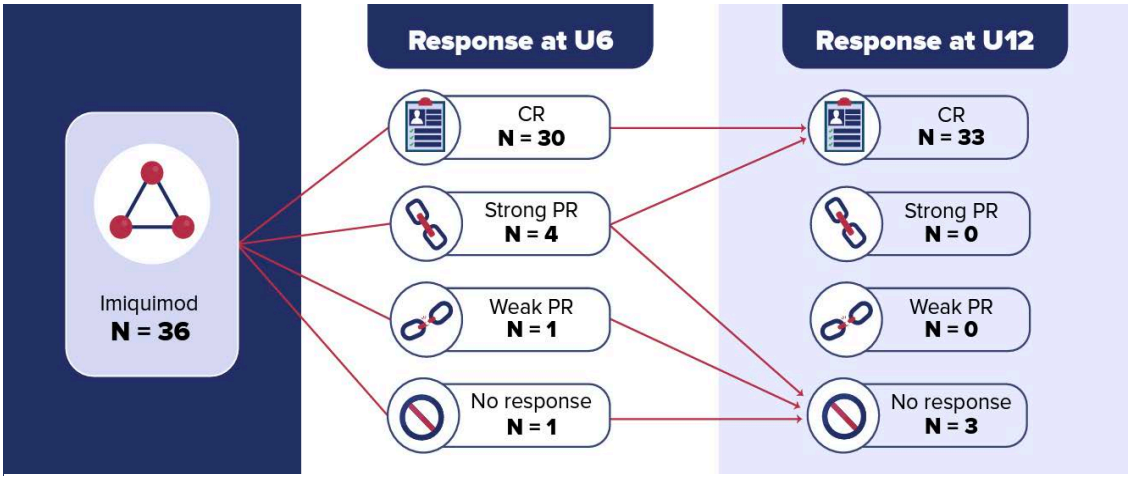
3.5 Response to treatment

In order to show the non-inferiority of imiquimod treatment to standard surgical treatment, comparison of the effectiveness rates (reduction in total lesion size) at 6- (U6) and 12-months (U12) after the baseline was conducted.

The degree of reduction in the lesion size is classified as:

- No response (NR): reduction in lesion size ≤ 25%
- Weak partial response (wPR): reduction in lesion size between 26-75%
- Strong partial response (stPR): reduction in lesion size between 76-99%
- Complete response (CR): 100% reduction in lesion size

Six months after the baseline (at U6), complete clinical response was seen in 30 patients treated with topical imiquimod (83.3%) and 28 (87.5%) patients after surgical intervention (P=0.793). For the same time period, a strong partial response to treatment was revealed among 4 (11.1%) patients within the imiquimod treatment group and 3 (9.4%) among the surgical treatment group. One year durability in patients listed as complete responders was further evaluated. At the U12, 3 additional patients in the imiquimod group developed complete response. At U6 those 3 patients were listed as strong responders, two of them received secondary surgery and one patient received an additional imiquimod treatment. Both additional interventions in those patients resulted in the complete lesion clearance at U12 (**Figure 21)**. The total of 3 patients were listed as non-responders at U12. Two out of three patients in NR imiquimod group at U12 received secondary surgical treatment and one patient refused additional interventions.



***F*igure 21** *Clinical response at 6 months (U6) and 12 months (U12) after the start of imiquimod treatment. Clinical response at U6 and U12 was classified as no response, weak partial response, strong partial response or complete response (CR)*

In the surgical group, the difference among the complete clinical response rates at six and twelve months after the baseline was observed, with an additional two patients with complete response at U12. Non responders (2 patients) received additional surgery after the U12. **Figure 22**

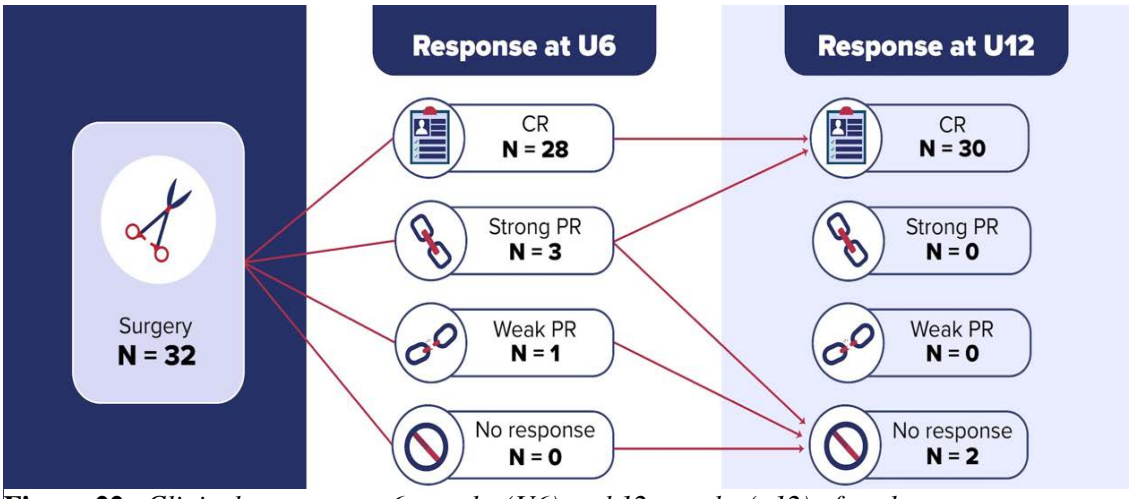


Figure 22 *Clinical response at 6 months (U6) and 12 months (u12) after the surgery*

The above represented **Figures 21** and **22** correlate with the **Table 9**:

Table 9 Response to imiquimod and surgical treatment at U6 and U12			
	Imiquimod treatment	**Surgical treatment**	**P-Value**
	Number (%)	**Number (%)**	
At 6 months (U6)			
No response	1 (2.8%)	0 (0%)	_____
Weak partial response	1 (2.8%)	1 (3.1%)	_____
Strong partial response	4 (11.1%)	3 (9.4%)	_____
Complete response	30 (83.3%)	28 (87.5%)	0.793
At 12 Months (U12)			
No response	3 (8.3%)	2 (6.3%)	0.743
Complete response	33 (91.7%)	30 (93.8%)	

Bar charts for the U6 and U12 with coded clinical response classification (CR, wPR, sPR and NR) are shown below. In the bar chart for the U6, blue bars are representing patients' outcome under the imiquimod treatment, while red bars represent surgically treated patient (**Figure 23).**

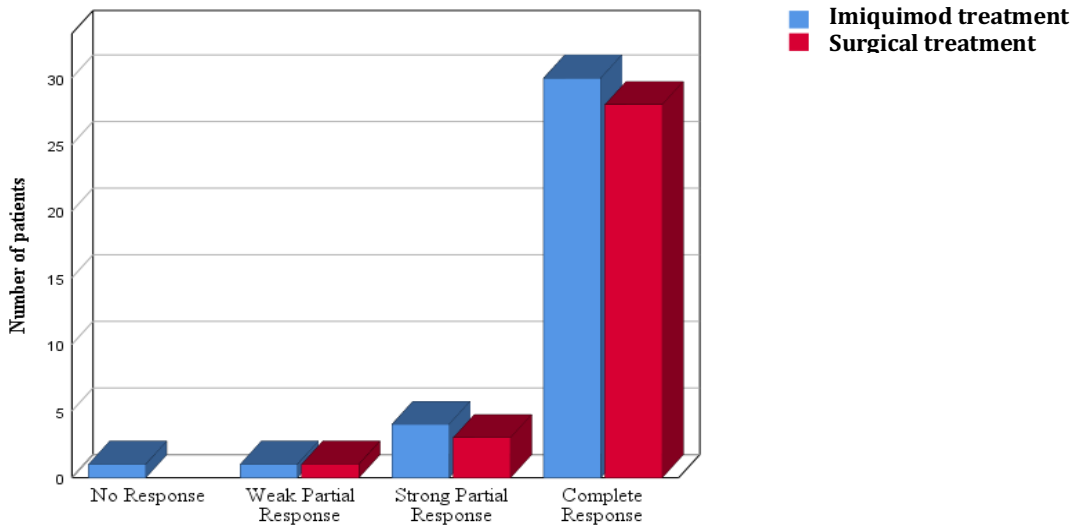


Figure 23 Bar chart representing clinical response to imiquimod and surgical treatment at sixth -month control (U6)

Figure 23 shows that the complete clinical response (100% lesion clearance) can be observed slightly more often under the imiquimod treatment than following surgery in the period of 6 months after the treatment initiation (after the baseline). Thus, it can be stated that a similar clinical response rate between the two treatments is to be assumed in the time period of six months after the start of the treatment. Surgery group did not have a patient with no response at U6, as all visible lesions were completely excised.

Furthermore, the clinical effectiveness was determined for the period of U12 and is graphically represented in the **Figure 24**. Both treatments demonstrate an absence of weak partial response (wPR) and strong partial response (sPR) in the 12th month control. This means that the lesions with no response and partial responses from the U6 either completely cleared (the treatment needed longer time after the U6 to demonstrate the effectiveness or the secondary treatment was initiated) or that they did not respond to treatment (NR).

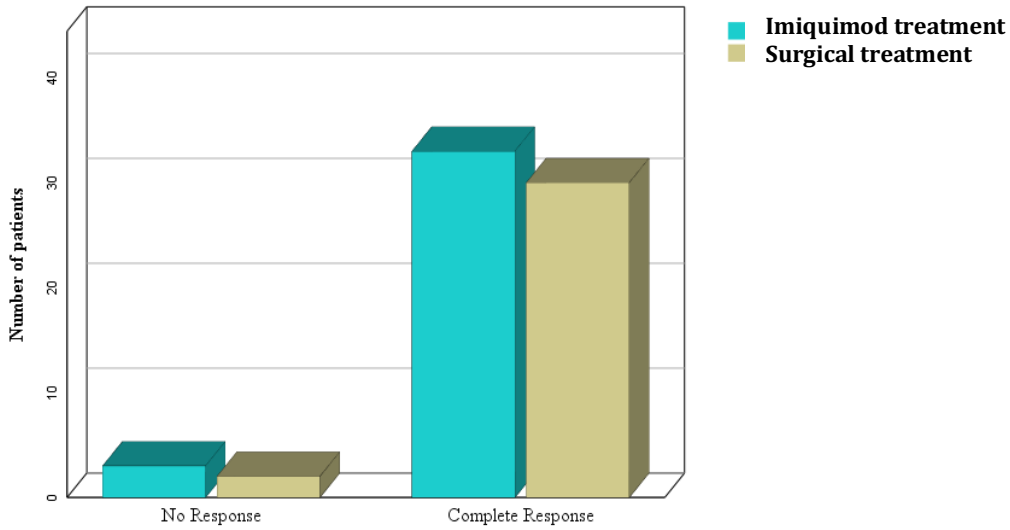


Figure 24 Bar chart representing clinical response to imiquimod and surgical treatment at 12th month control (U12)

3.6 Example of a patient with good clinical response to imiquimod

The presented case is a 39-year-old white female patient presenting with anamnestic recurring vulvar pruritus, discomfort and dyspareunia. She is a mother of one child, born via c-section. The patient has no history of other illnesses or regular medicament use. She is a non-smoker. Total number of sexual partners was no more than 2. Clinical and histological diagnosis confirmed vHSIL without evidence of invasion.

The patient was randomized to receive topical imiquimod therapy, which was applied according to the PITVIN study protocol. The patient was adherent to the study medication protocol and tolerated the given medication without occurrence of any major side-effects.

Clinically, the patient presented with a single leukoplakic lesion in the lower middle portion of the right labia minora. At the baseline, right before the initiation of the imiquimod treatment, the lesion area was measured at 106mm^2 (**Figure 25**). At the first month control (k1), the lesion area decreased to 100mm^2. However, as seen in the **Figure 26**, it can be assumed that the lesion area declined even more, as the integrity of the lesion was not as solid as seen at the baseline. Erosions throughout the lesion with reactive changes across the midline are noticeable, leading to rather uneven, opposed to a concentric decline in the lesion size.

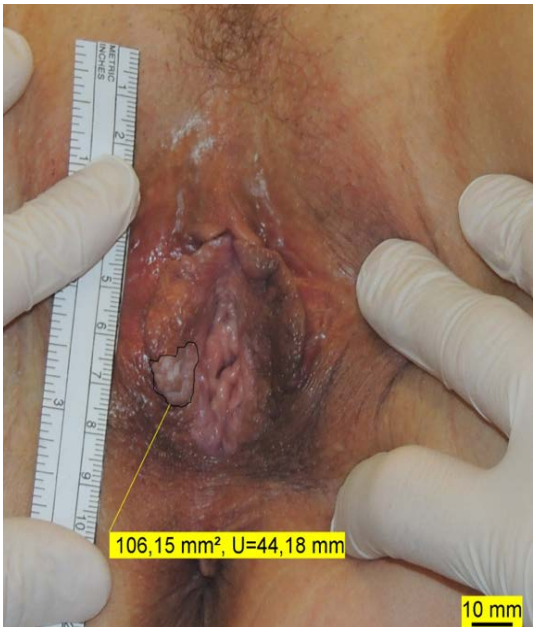


Figure 25 *Baseline lesion area before the imiquimod treatment*

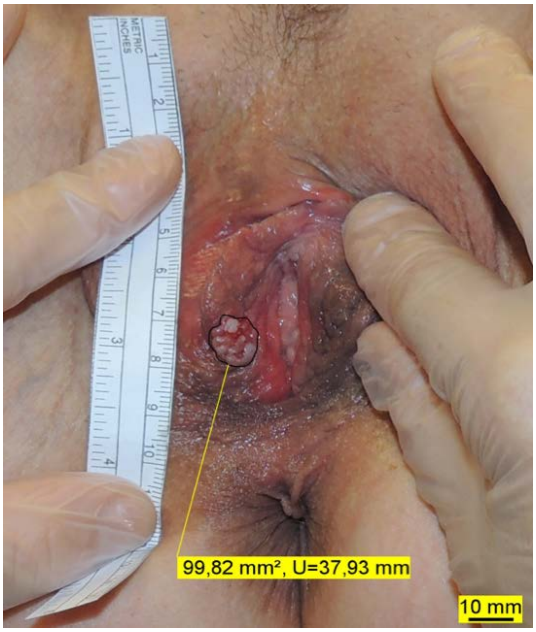


Figure 26 *The same patient with lesion measurement at the first month control (k1)*

Following the previously arranged clinical control at the third month after the baseline (k3), the lesion reduced in size to roughly 14mm^2. Erosive changes, as seen in the **Figure 26** are absent and the epithelial layer of the lesion is becoming increasingly similar to the surrounding healthy tissue, as seen in **Figure 27**. One month after the k3, at k4 the lesion was completely eradicated and the vulvar architecture fully maintained, as seen in the **Figure 28**.

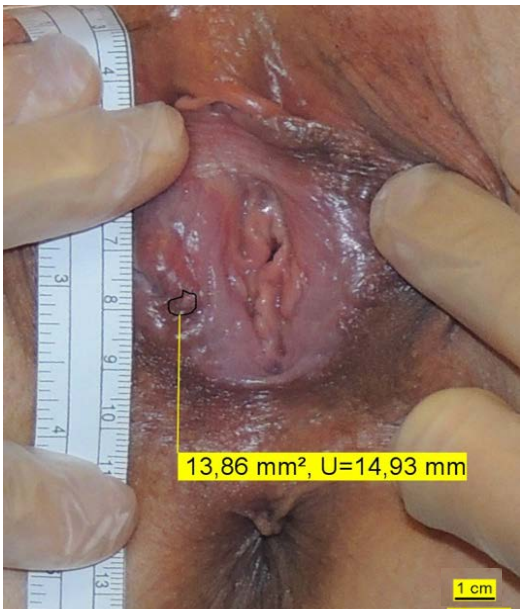


Figure 27 *Lesion area third month post baseline (k3)*

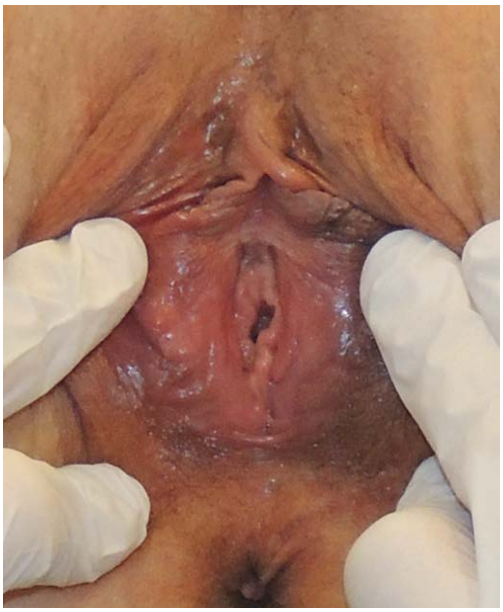


Figure 28 *Lesion clearance at the fourth month control (k4)*

During the patient follow-up, there were no lesion recurrences in the time period between k4 to U6 and U12, as seen in the figures below (**Figure 29** and **Figure 30**).

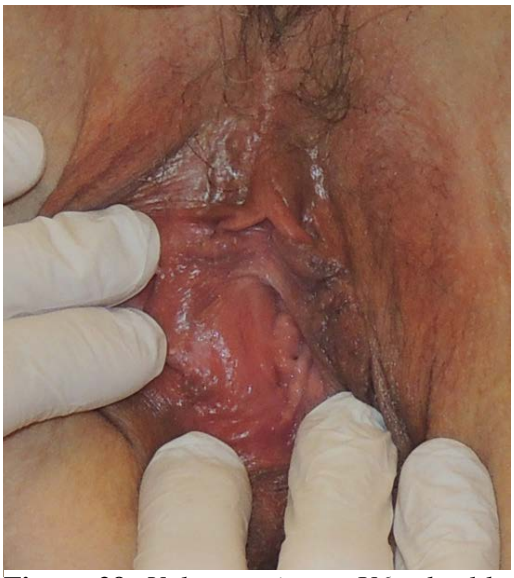


Figure 29 *Vulvar region at U6 – healthy vulvar area*

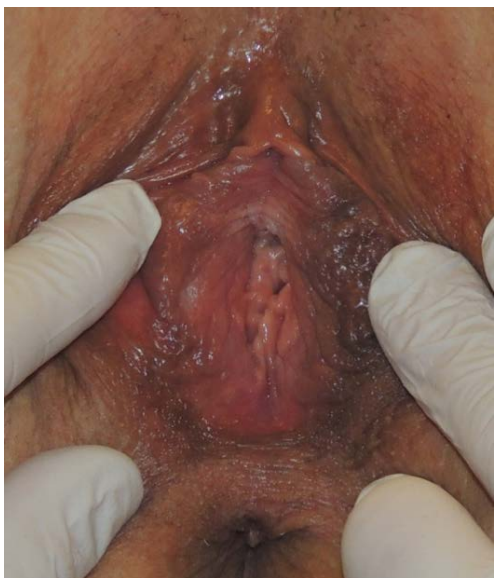


Figure 30 *Vulvar region at U12 without macroscopic lesion presence*

3.7 Example of patient's clinical response post-surgery

A presented example shows the diagnostic and therapeutic follow-up for a patient in her fifties presenting initially at gynecological examination after noticing a small white lump in the vulvar region, which started to be increasingly uncomfortable and itchy. Her last period was at the age of 51 years and since then the patient has experienced recurrent vulvar dryness and dyspareunia. Past medical history does not reveal any gynecological interventions, apart from two spontaneous deliveries of her two children. Patient is a non-smoker and does not regularly use medication. She is sexually active. Clinical diagnosis and biopsy revealed vHSIL. The patient was randomized to receive a primary surgical lesion removal.

At the baseline, the lesion represented as a white plaque with papulose (elevated) region at the transition between right labia minora to perineum, with the total area measuring 56mm^2 (**Figure 31**). The lesion was entirely removed through surgery, leading to an instant post-surgical lesion clearance (**Figure 32**). However, as seen in the **Figure 32**, the surgery led to local destruction and irritation of the vulvar tissue, that had previously been affected by a VIN lesion. Also noticeable is that the surgeon preformed excision with wide safety margins, leading to a larger increased vulvar area involved in the wound healing process.

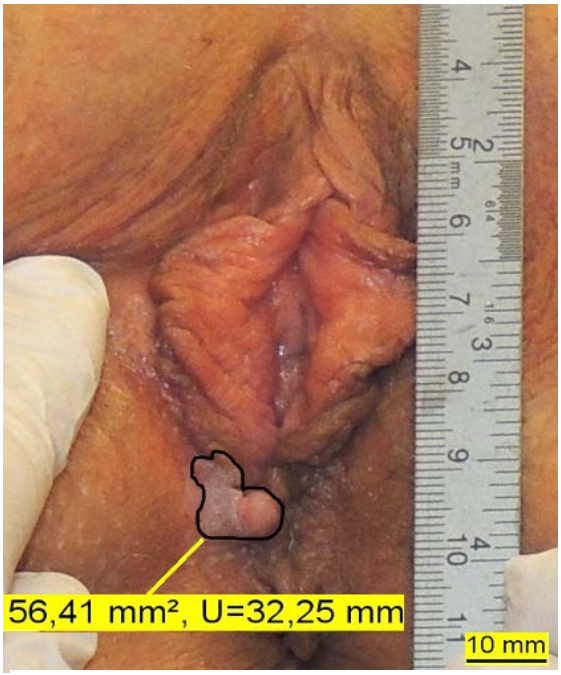


Figure 31 *Baseline lesion area of 56mm^2 before surgical removal*

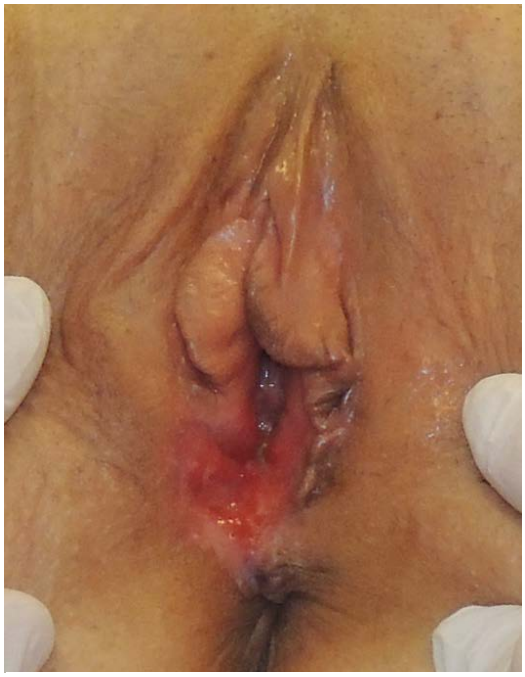


Figure 32 *Vulvar region photographed one month after surgery at k1*

Further clinical controls revealed the process of post-surgical wound healing, with noticeable formation and consecutive maturation of the scar tissue. The change in the operated vulvar area from U6 to U12 is seen in the **Figures 33 and 34**. **Figure 34**, taken of the patient at U12 shows the remodeling phase of scar formation with permanent changes to the vulvar skin. Lesion was absent at all clinical controls after the baseline.

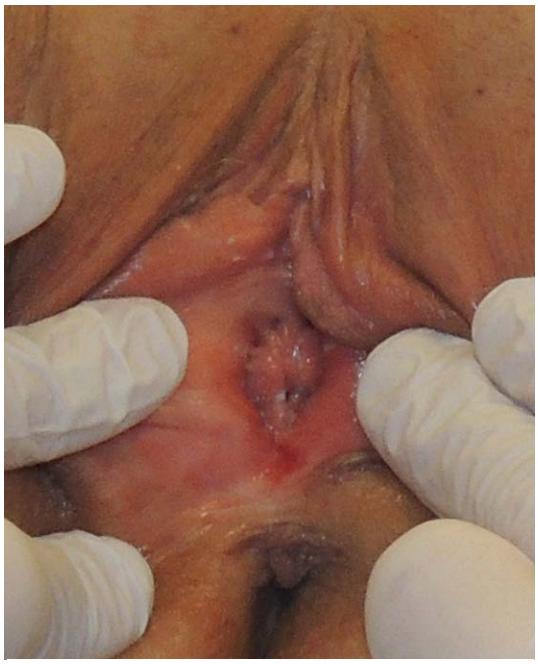


Figure 33 *Vulvar region at U6 with erosive changes 6 months after surgery*

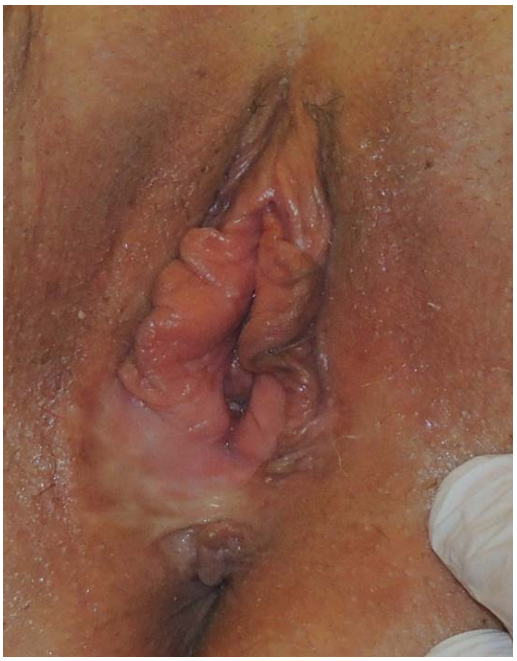


Figure 34 *Patient at U12 without lesion presence, but prominent scar tissue*

4 Discussion

The primary objective of this diploma thesis – to demonstrate the non-inferiority of topical imiquimod to standardized surgical treatment of VIN (vHSIL) - was assessed in 68 patients. The imiquimod treatment for patients with VIN represented a safe and effective treatment option. The treatment was associated with a reduced number of VIN lesions and a decline in the lesion area six- and 12-months after its initiation. Furthermore, the imiquimod was associated with a similar pattern of complete response as surgical intervention.

Statistical analysis included an ITT population of 36 patients in the imiquimod group and 32 patients in the surgical group, respectively. By intention-to-treat analysis, complete response six months after the baseline (at U6) was seen in 30/36 patients treated with topical imiquimod (83.3%) and 28/32 (87.5%) patients who had surgical intervention. One year durability in patients listed as complete responders was further evaluated. At the U12, three additional patients in the imiquimod group developed complete response, and three patients were listed as non-responders (NR). Two out of three patients in the NR imiquimod group at U12 received secondary surgical treatment later in the follow-up, and one patient refused additional interventions. In the surgery group, two additional patients developed a complete response at U12 from the U6, and two patients showed no response for the entire period of 12 months after the baseline.

A number of studies and systematic reviews, including Mathissen et al. (2007), Le at al. (2007), Van Sets and at. (2008) and Tristam et al. (2014) report on the success of imiquimod in the treatment of VIN. Complete clinical response for imiquimod treatment in the Mathissen et al. was noted in 81% of cases (8), which is in accordance with the 83% complete clinical response at U6 reported in this diploma thesis. In the above-mentioned studies, no-response to the imiquimod treatment ranges from 9 to 30% (1), whereas no response rate to imiquimod in this thesis lies at 2.8% at U6 and 8.3% at U12. Terlou et al. (2011) investigated a long-term response to imiquimod for at least five years as a follow-up of complete response rates of the Van Sets et al. study. It demonstrates the long-term effectiveness of imiquimod treatment with the addition that such patients are expected to have “a significantly better global quality of life than patients who recurred after the imiquimod treatment” (3).

Over the course of photographic data analysis conducted as a part of this diploma thesis, classification of lesion presence vs lesion absence was made. However, it is essential to note that this distinction was based on clinical macroscopic assessment, meaning the lesion might still be there. In most cases, there was histological diagnostic confirmation at U6, though it is open to discussion as to whether patients who were listed as complete responders without histologic confirmation indeed are lesion-free. This distinction was especially questionable in cases of multifocal disease since, in order for a patient to be regarded as a complete responder, all lesion sites had to be clear. However, it is highly probable to assume that the clinical distinction is reliable, as majority of imiquimod patients listed in the complete responder group at U6 remained lesion free for the following six months (till U12). Two patients from the imiquimod group with clinically persistent lesions at U6 showed at the same control histologically complete resolution and consequently complete clinical response in the period after U6. It is to assume that those two patients were already lesion free at the U6, only that the clinical appearance seemed to falsely indicate the lesion presence. The long-term follow-up of the PITVIN study will lead to further evidence of disease recurrence rates in these patients.

As this diploma thesis concentrates on the percentage reduction in the lesion area, it is also important to note that patients treated with imiquimod experienced different grades of lesion reduction over different clinical time periods. Most often, in the period of medicament application or shortly after, the lesion area appeared as an active, pigmented and inflamed area, which made the distinction between lesion vs skin inflammation difficult. This limitation was observed mainly in the cases of imiquimod patients listed as weak- and strong partial responders at early clinical controls, as the topical treatment could lead to reactions and inflammatory changes in the area of normal skin, making the measurement of the lesion area mistakenly larger than it is in reality. From the imiquimod group, 6 patients had either partial- (strong/weak) or no response to the treatment at U6. However, in 50 % of those patients complete response was achieved throughout the next 6 months, until the U12 clinical control – either those patients were already histologically lesion free at U6 or the effect took place after the additional treatment interventions from the U6 to the U12.

The data calculation in this diploma thesis was done as an ITT analysis, which offers a reliable reflection of patients' behavior in the real world and is therefore recognized as a standardized and favorable statistical assessment procedure in clinical studies. From the population of patients investigated in this thesis (N=68), two patients from the imiquimod

group changed to secondary surgery and are listed as complete responders at U12, as no visible lesion was present at that time. This implies that the rate of complete responders from the imiquimod group in per-protocol analysis would be lower. The surgical group did not have patients who switched to the imiquimod treatment from the subset of patients included in the statistical analysis in this diploma thesis (considering the period of baseline-U6-U12).

The quality of photographic documentation was an important factor affecting the lesion area measurements. As the PITVIN study is a multicenter study that lasted for several years (2013-2020 patient enrollment), a standardized system of lesion photographing was developed. For a photograph to be included in the study documentation, it needed to incorporate at least one vulvar region picture (including well visible VIN lesion(s)) per patient per clinical control, with an obligatory presence of a ruler for the later photograph calibration purposes. Despite all precautions and instructions regarding the photographing procedure, not all photographs met the quality criteria, as certain centers failed to incorporate the ruler in the picture. In such cases, the lesion area measurement was still performed by calibrating based on the other objects present in the picture. For example, an examiner's finger was often calibrated to 1,5 cm - 2 cm to allow for the lesion area measurement. This was identified as one of the main outliers of the data analysis process, which led to possible inconsistencies for several follow-up lesion area measurements – i.e., lesion area measurement at k5 being smaller than the lesion area at U6, followed by complete lesion absence at the following clinical control. Additional inaccuracy encountered in the lesion area measurement regards the angle at which the photograph was taken. For example, if the photograph of the lesion was not taken at an angle perpendicular (at 90^{o}) to the lesion, the computed lesion area measurement could appear either smaller or larger than it was in reality. This error was more significant in cases of smaller lesions, as a percentage error in measurements of such lesions is greater than for the bigger lesions.

The surgical group had zero patients with no response at U6. However, some patients showed early recurrences and had repeat surgical interventions within a few months after primary surgery, resulting in complete response at U6. Two patients had recurrent lesions at U12. The long-term follow-up of the PITVIN study will provide valuable information on long-term recurrence rates after surgery.

The imiquimod treatment showed long-term efficacy during the 12 months study period. This long-term effect of imiquimod was described in several studies, including Terlou et al.

(2011), and justified by the fact that imiquimod alters hosts immune response and leads to clearance of hrHPV in complete responders (3). In patients where the clearance of the hrHPV is achieved, no further recurrence or progression shall be expected in the long run.

In cases of patients presenting with multifocal lesion dispersion, small lesion areas or non-well demarcated lesions, topical imiquimod treatment represents a favorable approach. Large lesions tend to shrink under the imiquimod treatment, not as fast and not as effectively as in cases of smaller lesions. Therefore, relatively large vulvar lesions shall be evaluated for the need of surgical removal. Terlou et. al (2011) advise on imiquimod treatment prolongation until the regression has stopped and consider a surgical approach afterwards (3). In the cases of non-well-demarcated lesions, surgical removal may result in the excision of more-than needed healthy tissue to ensure safe resection margins. This may lead to larger than needed wound healing areas, with consecutively possible more extensive scarring. The alternative imiquimod approach offers a less invasive, though still effective treatment option.

The findings from this diploma thesis are based on patient assessments/lesion measurements at the baseline, sixth-month control (U6) and 12th-month control (U12). The long-term patient follow-up of the PITVIN study is still ongoing and will provide information on long-term recurrence rates.

In summary, statistical results of this diploma thesis conclude that a primary imiquimod 5% cream is an effective, safe and non-inferior alternative to surgery in the treatment of VIN (vHSIL). The sooner and greater the reduction in the lesion area under topical imiquimod is observed, the more likely it is that the lesion will completely clear. In other words, if no effect or a weak reduction in the lesion area in the first six months from the treatment start occurs, the lesion will likely show weak-to-no response to the topical imiquimod further in the course of the treatment. Therefore, it may be reasonable for patients with limited clinical response (i.e. weak reduction in primary lesion size) to change to the other treatment option (i.e. surgery) earlier in the course of clinical follow-ups. The results demonstrate that both treatment options (imiquimod and surgery) lead to effective lesion clearance at U6 and U12, with a minor difference in the effectiveness rates between the two treatment methods (for the periods of U6 and U12). Furthermore, this thesis also concludes that a baseline lesion color should not represent a predictor characteristic influencing the effectiveness of the treatment (imiquimod vs surgery) and that it should not be a relevant criterium favoring imiquimod or surgery as an optimal primary treatment modality.

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6 Appendix

6.1 Table app. 1

The main raw data table with lesion number and lesion area measurements from 110 patients (3 lost to follow-up[2]), resulting in 107 patients throughout 9 clinical controls. Color interpteration: w – leukoplakic, r – pigmented.

Pat ID	Group	Number of Lesions								Area (mm2)								Color
		Baseline	K1	K2	K3	K4	K5	U6	U9	U12	Baseline	K1	K2	K3	K4	K5	U6	U9	U12	
GB-01	Imiquimod	1	1	1	1	x	x	x	x	x	124	108	103	384	x	x	x	x	x	r
GB-02	Imiquimod	1	1	x	1	0	x	0	0	0	133	187	x	94	0	x	0	0	0	r
GB-03	Operation	1	1	x	x	0	x	0	0	0	263	3	3	x	0	x	0	0	0	w
GB-04	Operation	1	0	0	0	x	x	0	0	0	240	0	0	0	x	x	0	0	0	w
GB-05	Imiquimod	2	0	0	x	0	0	0	0	x	31	0	0	x	0	0	0	0	x	r
I-01	Imiquimod	10	9	9	x	x	x	x	x	x	620	538	484	x	x	x	x	x	x	r
I-02	Operation	5	0	0	0	0	0	0	0	x	217	0	0	0	0	0	0	0	x	w
I-04	Operation	1	1	x	0	0	0	0	0	2	69	32	x	0	0	0	0	0	228	w
I-05	Operation	2	0	0	0	0	0	0	0	0	638	0	0	0	0	0	0	0	0	r
I-06	Imiquimod	1	1	1	1	0	x	0	0	0	198	90	86	40	0	x	0	0	0	w
I-07	Imiquimod	1	x	0	0	0	0	0	0	0	211	x	0	0	0	0	0	0	0	w
S-01	Operation	1	x	x	x	x	x	x	x	x	166	x	x	x	x	x	x	x	x	w
S-02	Imiquimod	1	1	1	1	1	x	1	x	x	45	38	45	32	12	x	19	x	x	w
S-03	Operation	7	0	x	x	x	x	1	1	0	317	0	x	x	x	x	54	52	0	w
S-04	Operation	1				x	x	x		0	33				x	x			0	w
G-01	Operation	1	0	0	0	x	x	x	x	0	206	0	0	0	x	x	x	x	0	w
G-02	Imiquimod	1	2	2	0	1	2	2	1	0	649	560	820	0	114	303	136	63	0	r
G-03	Operation	2	0	x	x	x	x	0	x	0	421	0	x	x	x	x	0	x	0	r
G-04	Imiquimod	1	1	1	1	0	x	0	0	0	226	68	37	7	0	x	0	0	0	r
G-05	Operation	1	0	0	0	x	x	0	0	0	58	0	0	0	x	x	0	0	0	w
G-06	Operation	1	1	1	1	0	x	0	0	0	72	40	27	27	0	x	0	0	0	w
G-07	Imiquimod	1	1	1	1	1	0	0	0	0	102	93	44	83	90	0	0	0	0	w
G-08	Operation	1	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	w
G-09	Imiquimod	1	1	1	1	1	1	1	1	0	419	143	63	53	58	56	32	8	0	w
G-10	Imiquimod	1	1	1	1	0	0	0	0	0	142	179	164	80	0	0	0	0	0	w
G-11	Operation	5	4	4	1	0	x	0	0	0	131	159	230	19	0	x	0	0	0	w
G-12	Imiquimod	1	1	1	1	1	x	0	x	0	129	108	95	99	29	x	0	x	0	r
G-13	Operation	1	0	0	0	x	x	0	0	0	96	0	0	0	x	x	0	0	0	r
G-14	Operation	1	0	0	0	x	x	0	0	0	81	0	0	0	x	x	0	0	0	r
G-15	Operation	1	0	0	0	0	x	0	x	0	208	0	0	0	0	x	0	x	0	w
G-16	Imiquimod	3	3	2	x	x	x	x	x	x	383	376	203	x	x	x	x	x	x	r
G-17	Imiquimod	1	1	1	x	x	0	0	1	1	167	54	30	x	x	0	0	17	41	r
G-18	Imiquimod	1	x	x	1	1	1	0	x	0	436	x	x	282	228	19	0	x	0	r
G-19	Imiquimod	6	4	x	0	0	0	0	0	x	93	55	x	0	0	0	0	0	x	r
G-20	Operation	1	0	0	x	0	x	0	0	0	54	0	0	x	0	x	0	0	0	w
G-21	Imiquimod	5	3	0	0	0	x	0	0	0	83	72	0	0	0	x	0	0	0	r
G-22	Imiquimod	1	0	0	0	x	0	0	0	0	31	0	0	0	x	0	0	0	0	w
G-23	Operation	1	0	0	0	0	x	0	0	0	125	0	0	0	0	x	0	0	0	w
G-24	Operation	1	0	x	x	0	x	0	x	x	179	0	x	x	0	x	0	x	x	w
G-25	Operation	1	0	x	x	x	x	0	0	0	1181	0	x	x	x	x	0	0	0	w
G-26	Operation	1	0	0	0	0	x	0	0	0	200	0	0	0	0	x	0	0	0	w
G-27	Operation	1	0	x	x	x	x	0	x	0	287	0	x	x	x	x	0	x	0	w
G-28	Imiquimod	1	x	x	x	x	x	2	x	0	209	x	x	x	x	x	208	x	0	r
G-29	Imiquimod	1	1	1	1	1	1	x	0	0	137	135	123	122	100	41	x	0	0	w
G-30	Imiquimod	1	1	1	1	1	x	x	x	x	1070	1598	383	370	137	x	x	x	x	w
G-31	Imiquimod	1	0	0	x	0	x	0	0	0	27	0	0	x	0	x	0	0	0	w
G-32	Imiquimod	1	1	x	0	x	0	0	0	0	26	5	x	0	x	0	0	0	0	r
G-33	Operation	1	0	0	x	x	x	x	x	x	205	0	0	x	x	x	x	x	x	w
G-34	Imiquimod	1	1	1	1	0	x	0	0	0	106	100	61	14	0	x	0	0	0	w
G-35	Operation	1	0	x	0	x	x	0	x	0	903	0	x	0	x	x	0	x	0	w
G-36	Operation	3	0	0	x	0	x	0	0	0	108	0	0	x	0	x	0	0	0	w
G-37	Operation	1	0	x	0	x	x	0	0	0	47	0	x	0	x	x	0	0	0	w
G-38	Imiquimod	3	2	1	1	1	x	1	x	x	662	423	310	161	155	x	26	x	x	w
G-39	Operation	1	0	0	0	0	x	0	0	0	117	0	0	0	0	x	0	0	0	w
G-40	Operation	1	0	0	x	0	x	0	0	0	35	0	0	x	0	x	0	0	0	w

[2] G-19, G-63, and G-54 had no follow-up

G-41	Operation	1	1	x	x	x	x	x	x	x	126	96	x	x	x	x	x	x	x	w
G-42	Imiquimod	1	1	0	0	x	x	0	x	x	81	5	0	0	x	x	0	x	x	w
G-43	Imiquimod	1	1	1	1	1	x	1	0	0	78	38	27	16	12	x	10	0	0	w
G-44	Imiquimod	1	1	0	x	0	x	0	0	0	83	15	0	x	0	x	0	0	0	w
G-45	Operation	5	0	0	x	1	x	2	x	0	982	0	0	x	37	x	28	x	0	r
G-46	Imiquimod	1	1	1	1	0	x	0	0	x	153	93	25	14	0	x	0	0	x	w
G-47	Operation	1	0	x	0	0	x	0	0	0	141	0	x	0	0	x	0	0	0	w
G-48	Operation	1	0	1	1	1	0	1	0	x	50	0	x	56	14	0	25	0	x	w
G-49	Imiquimod	1	x	x	x	x	x	x	x	x	68	x	x	x	x	x	x	x	x	w
G-50	Imiquimod	7	7	7	6	0	x	x	x	1	563	183	148	60	0	x	x	x	18	w
G-51	Operation	1	x	0	x	x	x	0	x	x	38	x	0	x	x	x	0	x	x	w
G-52	Imiquimod	1	1	1	1	1	1	1	1	x	57	56	55	3	9	5	3	17	x	w
G-53	Operation	1	x	x	0	x	0	0	0	0	28	x	x	0	x	0	0	0	x	w
G-54	Operation	1	0	0	x	0	x	0	x	x	205	0	0	x	0	x	0	x	x	w
G-55	Imiquimod	1	x	x	x	x	x	x	x	x	435	x	x	x	x	x	x	x	x	
G-57	Operation	4	0	0	0	0	x	0	0	0	189	0	0	0	0	x	0	0	0	w
G-58	Imiquimod	1	1	1	1	0	x	1	0	0	291	267	56	31	0	x	35	0	0	w
G-59	Imiquimod	1	1	1	1	0	x	0	0	0	126	85	65	23	0	x	0	0	0	w
G-60	Operation	2	0	x	0	x	x	0	0	0	145	0	x	0	x	x	0	0	0	w
G-61	Operation	2	0	x	0	x	x	0	0	0	169	0	x	0	x	x	0	0	0	r
G-62	Imiquimod	3	3	1	0	0	x	0	0	0	108	87	64	0	0	x	0	0	0	w
G-63	Imiquimod	1	1	1	1	x	1	1	x	x	1421	1363	592	374	x	254	140	x	x	w
G-64	Imiquimod	1	1	0	x	0	x	0	0	0	252	236	0	x	0	x	0	0	0	w
G-65	Imiquimod	1	1	1	1	0	x	1	1	1	24	21	21	12	0	x	15	12	13	w
G-66	Operation	1	0	x	0	x	0	0	x	0	97	0	x	0	x	0	0	x	0	w
G-67	Operation	3	0	x	0	x	x	0	0	x	444	0	x	0	x	x	0	0	x	r
G-68	Imiquimod	1	1	x	x	1	x	0	x	0	203	59	x	x	24	x	0	x	0	w
G-69	Operation	1	x	0	0	x	x	0	0	x	334	x	0	0	x	x	0	0	x	w
G-70	Operation	1	0	0	x	x	x	0	0	x	146	0	0	x	x	x	0	0	x	w
G-71	Operation	1	0	x	x	x	x	0	x	x	496	0	x	x	x	x	0	x	x	w
G-72	Operation	1	x	x	x	x	x	x	x	x	66	x	x	x	x	x	x	x	x	w
G-73	Imiquimod	5	5	5	5	x	x	0	0	x	147	118	108	64	x	x	0	0	x	w
G-74	Imiquimod	4	4	5	4	x	x	0	0	2	158	151	121	59	x	x	0	0	36	w
G-75	Imiquimod	1	1	x	1	0	x	0	x	x	127	89	x	42	0	x	0	x	x	w
G-76	Operation	4	x	3	x	4	x	0		x	198	x	105	x	236	x	0	75	x	w
G-77	Imiquimod	11	3	2	1	x	1	0	0	0	238	196	63	25	x	12	0	0	0	r
G-78	Imiquimod	1	1	0	x	0	x	0	x	0	65	21	0	x	0	x	0	x	0	w
G-79	Imiquimod	1	1	1	0	x	x	0	0	0	708	465	139	0	x	x	0	0	0	w
G-80	Operation	1	x	x	x	x	x	0	x	0	160	x	x	x	x	x	0	x	0	w
G-81	Operation	1	0	0	x	x	x	0	x	x	42	0	0	x	x	x	0	x	x	w
G-82	Imiquimod	1	0	x	0	x	x	0	x	0	26	0	x	0	x	x	0	x	0	w
G-83	Imiquimod	1	1	x	1	x	x	x	x	x	29	17	x	4	x	x	x	x	x	w
G-84	Operation	1	0	0	x	x	x	x	x	x	104	0	0	x	x	x	x	x	x	r
G-85	Operation	1	0	0	x	x	x	0	0	x	341	0	0	x	x	x	0	0	x	r
G-86	Imiquimod	1	1	1	x	x	1	0	x	0	216	184	27	x	x	5	0	x	0	r
G-87	Imiquimod	1	1	x	x	1	x	0	x	0	623	312	x	x	103	x	0	x	0	w
K-01	Chirurgie	2	x	0	x	0	0	0	0	0	203	x	0	x	0	0	0	0	0	w
K-02	Imiquimod	1	1	x	1	x	1	0	0	0	77	77	x	68	x	40	0	0	0	w
K-03	chirurgie	1	x	x	x	x	x	1	x	1	119	x	x	x	x	x	70	x	66	w
W-01	Chirurgie	2	0	0	0	1	1	1	2	4	106	0	0	0	86	26	14	69	114	w
W-02	Imiquimod	1	1	1	x	1	0	0	0	0	136	75	57	x	56	0	0	0	0	w
W-03	Imiquimod	1	1	x	1	0	0	0	0	0	114	41	x	28	0	0	0	0	0	w
W-04	Imiquimod	1	1	x	x	x	x	0	0	0	141	137	x	x	x	x	0	0	0	w

6.2 Table app. 2

The table with omitted patients in this diploma thesis. The table has been coded based on the treatment group the patient received (1,00 – imiquimod, 2,00 – surgery). Also, the baseline color of the lesion has been coded, with 1,00 - brown and 2,00 - white lesions.

Group	Grouping	Number_Basline	Number_U6	Number_U12	Area_Basline	Area_U6	Response U6	Coded_U6	Area_U12	Response U12	Coded_U12	Color
Imiquimod	1,00	1,00	,00	,00	133,00	,00	CR	3,00	,00	CR	3,00	1,00
Imiquimod	1,00	1,00	,00	,00	198,00	,00	CR	3,00	,00	CR	3,00	1,00
Imiquimod	1,00	1,00	,00	,00	211,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	2,00	,00	649,00	136,00	stPR	2,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	226,00	,00	CR	3,00	,00	CR	3,00	1,00
Imiquimod	1,00	1,00	,00	,00	102,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	1,00	,00	419,00	32,00	stPR	2,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	142,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	129,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	1,00	167,00	,00	CR	3,00	41,00	NR	,00	2,00
Imiquimod	1,00	1,00	,00	,00	436,00	,00	CR	3,00	,00	CR	3,00	1,00
Imiquimod	1,00	5,00	,00	,00	83,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	31,00	,00	CR	3,00	,00	CR	3,00	1,00
Imiquimod	1,00	1,00	2,00	,00	209,00	208,00	NR	,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	27,00	,00	CR	3,00	,00	CR	3,00	1,00
Imiquimod	1,00	1,00	,00	,00	26,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	106,00	,00	CR	3,00	,00	CR	3,00	1,00
Imiquimod	1,00	1,00	1,00	,00	78,00	10,00	stPR	2,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	83,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	1,00	,00	291,00	35,00	stPR	2,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	126,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	3,00	,00	,00	108,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	252,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	1,00	1,00	24,00	15,00	wPR	1,00	13,00	NR	,00	1,00
Imiquimod	1,00	1,00	,00	,00	203,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	4,00	,00	2,00	158,00	,00	CR	3,00	36,00	NR	,00	2,00
Imiquimod	1,00	11,00	,00	,00	238,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	65,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	708,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	26,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	216,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	623,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	77,00	,00	CR	3,00	,00	CR	3,00	1,00
Imiquimod	1,00	1,00	,00	,00	136,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	114,00	,00	CR	3,00	,00	CR	3,00	2,00
Imiquimod	1,00	1,00	,00	,00	141,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	263,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	240,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	2,00	,00	,00	638,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	7,00	1,00	,00	317,00	54,00	stPR	2,00	,00	CR	3,00	2,00
Surgery	2,00	2,00	,00	,00	421,00	,00	CR	3,00	,00	CR	3,00	1,00
Surgery	2,00	1,00	,00	,00	58,00	,00	CR	3,00	,00	CR	3,00	1,00
Surgery	2,00	1,00	,00	,00	72,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	17,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	5,00	,00	,00	131,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	96,00	,00	CR	3,00	,00	CR	3,00	1,00

Surgery	2,00	1,00	,00	,00	81,00	,00	CR	3,00	,00	CR	3,00	1,00
Surgery	2,00	1,00	,00	,00	208,00	,00	CR	3,00	,00	CR	3,00	1,00
Surgery	2,00	1,00	,00	,00	54,00	,00	CR	3,00	,00	CR	3,00	1,00
Surgery	2,00	1,00	,00	,00	125,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	1181,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	200,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	287,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	903,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	3,00	,00	,00	108,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	47,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	117,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	35,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	5,00	2,00	,00	982,00	28,00	stPR	2,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	141,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	4,00	,00	,00	189,00	,00	CR	3,00	,00	CR	3,00	1,00
Surgery	2,00	2,00	,00	,00	145,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	2,00	,00	,00	169,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	97,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	,00	,00	160,00	,00	CR	3,00	,00	CR	3,00	1,00
Surgery	2,00	2,00	,00	,00	203,00	,00	CR	3,00	,00	CR	3,00	2,00
Surgery	2,00	1,00	1,00	1,00	119,00	70,00	wPR	1,00	66,00	NR	,00	2,00
Surgery	2,00	2,00	1,00	4,00	106,00	14,00	stPR	2,00	114,00	NR	,00	2,00