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

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SECTION II

**NOVEL TOPICAL
TREATMENTS FOR
HPV-INDUCED
DISEASES**

Chapter 4

RESULTS OF PHASE 2 TRIALS EXPLORING THE SAFETY AND EFFICACY OF OMIGANAN IN PATIENTS WITH HUMAN PAPILLOMAVIRUS-INDUCED GENITAL LESIONS

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Abstract

AIMS To assess safety and tolerability and explore pharmacodynamics and efficacy of omiganan in external anogenital warts (AGW) and vulvar high-grade squamous intraepithelial lesions (HSIL).

METHODS Two randomized controlled trials in patients with external anogenital warts and vulvar HSIL were conducted. Patients received topical omiganan 2.5% or placebo gel once daily for 12 weeks with a follow-up of 12 weeks. Safety and tolerability were monitored and pharmacodynamics and clinical efficacy of omiganan were assessed by analyzing lesion count, size and viral load. Self-reported pain, itch and quality of life were assessed by an electronic diary and questionnaire.

RESULTS Twenty-four AGW and 12 vulvar HSIL patients were enrolled. All patients had a high treatment adherence (99%). No serious adverse events occurred and all adverse events (N=27) were mild, transient and self-limiting. The treatment groups were not different in terms of safety and tolerability, lesion count and size and patient-reported outcomes pain, itch and quality of life. HPV load significantly reduced after 12 weeks of treatment with omiganan compared to placebo (-96.6%; 95% CI -99.9 to -7.4%; p=0.045) in AGW patients only.

CONCLUSIONS Topical omiganan appears to be safe in patients with AGW and vulvar HSIL and reduced HPV load after 12 weeks of treatment in AGW patients.

Introduction

Anogenital warts (AGW) and vulvar high-grade squamous intraepithelial lesion (HSIL), formerly referred to as usual type vulvar intraepithelial neoplasia (uVIN), are human papillomavirus (HPV)-induced genital lesions.¹ AGW is the most common sexually transmitted viral disease with a worldwide incidence of 160-289 per 100,000 and is mostly caused by the low-risk HPV type 6.²⁻⁵ Vulvar HSIL is caused by high-risk HPV types, mostly type 16, and has an incidence of 1-2 per 100,000.⁶⁻⁸ Vulvar HSIL has a malignant potential of 3% when treated, but up to 9% if left untreated.^{6,9,10} AGW and vulvar HSIL generally cause similar symptoms such as pruritus and irritation and most patients suffer from a high psychological and sexual burden.¹¹⁻¹³

The current treatment of AGW and vulvar HSIL consists of ablative/surgical and medical treatments with efficacy rates of 50-90% and high recurrence rates of 15-70%.^{3,14-20} Surgical treatments are often mutilating and painful and medical treatments have notable side effects such as erythema, irritation, ulceration and pain.^{12,19,20} Therefore, novel and more effective treatment options with an acceptable side effect profile are needed.

Omiganan pentahydrochloride, a synthetic analogue of indolicidin, is a small antimicrobial peptide from the cathelicidin family that has an important role in the first line immune defense of the skin and has antimicrobial properties against bacteria and fungi.²¹⁻²⁴ Omiganan has demonstrated to block HPV infection in vitro and has shown immuno-modulatory effects in primary human immune cell cultures.²⁵ The peptide modifies responses driven by cell surface-expressed toll like receptors, in particular type I and type III interferon responses which are important for efficient anti-viral activities. Omiganan also stimulates the maturation of dendritic cells, reduces the M2-type (pro-tumor) macrophage profile and enhances the M1-type (anti-tumor) macrophage profile.²⁵ Furthermore, omiganan is widely studied in several clinical trials in non-viral, dermatological conditions.²⁶⁻²⁸ The combination of its antiviral and immunomodulatory properties makes topical omiganan a promising compound for the treatment of HPV-induced diseases. The main objective was to investigate the effects of topical omiganan in patients with genital dysplasia. The dose rationale was based on preclinical data where anti-HPV effects were observed at dose strength of 25 mg/g.

In this article, we report the results of two randomized controlled trials to assess safety and tolerability and to explore pharmacodynamics and clinical

efficacy of topically applied omiganan once daily in patients with AGW and vulvar HSIL. These studies were the first to test an antimicrobial peptide for its antiviral properties in HPV-induced diseases. The results of both studies are combined to establish a profile incorporating different HPV-induced diseases.

Materials and methods

STUDY DESIGN, PATIENTS AND RANDOMIZATION

Two randomized, double-blind, placebo-controlled, parallel-group, phase 2 trials in patients with AGW and vulvar HSIL were performed at the Centre for Human Drug Research (CHDR), Leiden, The Netherlands. Patients of ≥18 years with biopsy-proven HPV positive AGW or histologically proven vulvar HSIL were included. Eligible patients had at least 3 external AGW or a vulvar HSIL of 20mm in diameter or 120mm² surface. Exclusion criteria were active treatment of the lesions within 28 days prior to enrollment, immunosuppression, pregnancy, breast-feeding or inability to use effective contraception during the treatment period and at least 90 days afterwards. All participants gave written informed consent before randomization. The studies were approved prior to any clinical activities by the independent Dutch Medical Ethics Committee (Stichting BEBO, Assen, The Netherlands).

Patients were randomly assigned to topical omiganan or placebo gel in a 2:1 ratio by a computer-generated list prepared by an independent statistician. In the vulvar HSIL trial subject numbers were sequentially allocated by chronological enrollment. In the AGW trial male patients were numbered sequentially from 1 upwards and female patients from 24 downwards in order of inclusion and were randomized in blocks of 3, to enable the inclusion of at least 3 patients of each gender per treatment. Patients, study personnel and investigators were blinded for allocated treatment throughout the study. Patients of the vulvar HSIL trial could be enrolled in an open label study part B with an additional 12 weeks treatment and 12 weeks follow-up, if there were no safety or tolerability issues in the double-blind study part and no suspected progression.

STUDY PROCEDURES

The participants were instructed by the study physician or nurse practitioner to apply the study drug once daily at approximately the same clock time

for 84/85 consecutive days in the AGW and vulvar HSIL trial respectively. The amount of study drug depended on the number and size of lesions with a maximum of 37.5 mg omiganan per day. Patients returned for a visit at the study site after 2, 4, 6 (HSIL only), 8 (AGW only) and 12 weeks and 2 visits were planned during the 12-week follow-up period.

Safety and tolerability assessments were performed by monitoring of adverse events (AE), physical examination, ECG and laboratory blood and urine tests. AEs were recorded according to the Medical Dictionary for Regulatory Activities (MEDDRA) version 19. Physical examination and ECG were performed at screening and end of study visit. Blood and urine samples were collected at screening, end of treatment and end of study visit. Pharmacokinetic blood samples were taken at the last treatment day of the vulvar HSIL trial (pre-dose and 10, 20, 30, 60, 120 and 180 minutes after dosing) and tested by validated HPLC/MS/MS with a LoQ of 1.0 ng/mL of omiganan.

Clinical assessments were performed at each study visit by the same two investigators and gynecologist. In the AGW trial, three warts were selected for size measurements based on size and reproducibility of repeated measurements; target wart and biopsy wart 1 and 2. Wart size reduction was assessed in longest and shortest diameter and height (mm) was assessed using a digital Vernier caliper (0-150 mm, Conrad Electronic Benelux B.V., Oldenzaal, the Netherlands). Wart clearance was defined as complete disappearance of the wart. In vulvar HSIL patients, clinical response was determined based on the RECIST guidelines.²⁹ Complete response was defined as the complete disappearance of target lesions. A reduction more than 30% of the sum of the longest diameter of the target lesions was classified as partial response. Disease progression was defined as the increase of the target lesion and was expressed as the sum of longest diameters of >20%, or the development of new lesions.

Swabs were collected at each study visit by rubbing the surface of the target lesion for 5 consecutive times with a pre-wetted cotton-tipped stick. The cotton stick was placed in 1 mL of saline solution and stored at -40 degrees Celsius. Swabs for PCR analyses were taken before omiganan was applied. All swabs were analyzed batch-wise at the end of study to limit variability. Nucleic acid isolation was performed on the MagNA Pure 96 system, using the DNA and Viral NA Large Volume Kit. The input for the nucleic extraction procedure was 350µL of swab, 100µL of 0.9% NaCl and an added volume of 50µL of 10 times concentrated phosphate buffered saline. Nucleic acids were

eluted in a volume of 50µL of elution buffer. All DNA isolates were tested for PCR inhibition by a real-time PCR detecting a plasmid spiked in the PCR mix. An increase in cycle threshold value of the plasmid spike in clinical samples would indicate PCR inhibition. When PCR inhibition was found the DNA from the swab was diluted 10x and retested to confirm that PCR inhibition was no longer present. The 10x diluted sample was used in the HPV qPCR and viral load was corrected for the 10x dilution. The base line swab DNA isolates were tested on HPV genotype using the SPF10-LIPA25 version 1 (Labo Bio-medical Products B.V., Rijswijk, The Netherlands). The viral load of HPV 6 and HPV 11 in case of AGW and HPV16 in case of vulvar HSIL was determined by three separate qPCRs in swabs of lesions that were positive for the respective HPV type as determined on the baseline sample. The target of the quantitative PCRs was the E6 open reading frame and an input volume of 5µL of isolated nucleic acid was used per qPCR. Determining viral loads was done by comparing the cycle threshold value of the clinical samples with the cycle threshold values of a standard curve specific for each HPV type and analyzed in each qPCR run. Quantitative PCRs were validated and limit of detection and lower limit of quantification values for HPV6, HPV11 and HPV16 were 14/100, 12/100 and 8/32 copies/PCR respectively.

One 3mm biopsy was obtained at screening (biopsy wart 1), week 12 (biopsy wart 2) and week 24 (target wart) in case of AGW and two 3mm biopsies were obtained at screening, week 6, week 12 and week 24 of the same lesion in case of vulvar HSIL. The AGW biopsies were directly cut into two equal pieces and were further evaluated by the LUMC department of pathology. One piece/biopsy was assessed according to conventional pathological standards by the same gynecologic pathologist after hematoxylin and eosin (H&E) staining.^{30,31} HPV-typing was performed in the screening biopsy using INNO-LIPA HPV genotyping Extra II (INNO-LIPA; Fujirebio Europe, Ghent, Belgium). From the other piece/biopsy RNA was isolated by means of the Nucleospin Kit RNA XS (Macherey-Nagel, Düren, Germany), cDNA was synthesized and thereafter the expression of HPV6 (AGW) or HPV16 (vulvar HSIL) was determined using real-time PCR with a primer targeted for E6.

Patient-reported outcomes were determined by a study-specific smartphone application (e-diary) and a paper questionnaire.³² In the e-diary application, the symptoms pain and itch were assessed by a numeric rating scale (NRS) once daily during the treatment period on a scale from 0 to 100 (0: no pain/pruritus and 100: worst pain/pruritus). The paper questionnaire was

filled out during the visit days and consisted of questions from a validated vulvar HSIL questionnaire (supplemental data).³³ All answers were scored and higher scores indicated a higher level of burden of disease. Scores were added up, providing mean total quality of life (QOL) scores. Higher QOL scores indicated a lower QOL. Treatment adherence, the actual administrations divided by the expected administrations, was monitored by the e-diary to register daily dose administration and to remind patients; in case patients did not fill in the e-diary, they were contacted and asked whether they applied the drug. The expression of HPV was corrected using the expression of the housekeeping genes ACTB and RPL11.

STATISTICAL ANALYSIS

Due to the exploratory nature of the trials and the first-in-indication setting, sample size was determined empirically. Safety analyses were conducted in the pre-defined intention-to-treat (ITT) population, which comprised of all enrolled patients who received at least one dose of study treatment. The pharmacodynamics and efficacy analyses were conducted in the per protocol population, which consisted of the ITT population with at least one post-baseline assessment and no major protocol deviation. All efficacy and pharmacodynamic endpoints were analyzed using a mixed model analysis of covariance (ANCOVA) using treatment, time and treatment by time as fixed factors, patient as random factor and the baseline value as covariate with SAS 9.4 for Windows (SAS institute Inc., Cary, NC, USA). A two-sided Fisher's exact and a two-sided Wilcoxon exact rank test were used to analyze wart clearance. Graphs were made using GraphPad Prism (version 6.05 for Windows, GraphPad Software, La Jolla, California, USA). All statistical tests were two-tailed with α -level of 0.05.

Results

PATIENT CHARACTERISTICS

Between November 2015 and July 2017, eligibility for participation in the AGW trial and vulvar HSIL trial was assessed in 31 and 15 individuals respectively (Figure 1). Twenty-four patients with AGW and 12 patients with HSIL were randomized. Data of one AGW patient was excluded due to a major protocol deviation (study procedure incompliance) and one AGW patient was excluded from analysis of the follow-up period due to use of concomitant treatment

for AGW (after consultancy with research physician). Seven subjects were enrolled in part B of the vulvar HSIL trial. Baseline characteristics of all patients are described in Table 1. The evaluation of HPV type showed that HPV6 (83.3%) and HPV16 (83.3%) were the most frequently occurring types in AGW and vulvar HSIL patients respectively.

Figure 1. Integrated flowchart of the AGW and vulvar HSIL trial. In the AGW trials 31 subjects were screened of whom 24 (77%) were enrolled. Of the 24 remaining subjects, 16 were randomly assigned to treatment with omiganan and 8 to placebo. One subject in the omiganan group discontinued the intervention due to personal reasons, the end of study visit was performed prematurely. One subject in the placebo group was excluded from analysis in the follow-up period because of use of concomitant medication for AGW. In the vulvar HSIL trials 15 subjects were screened of whom 12 (80%) were enrolled. Of the 12 remaining subjects 8 were randomly assigned to treatment with omiganan and 4 to placebo. All subjects completed the study.

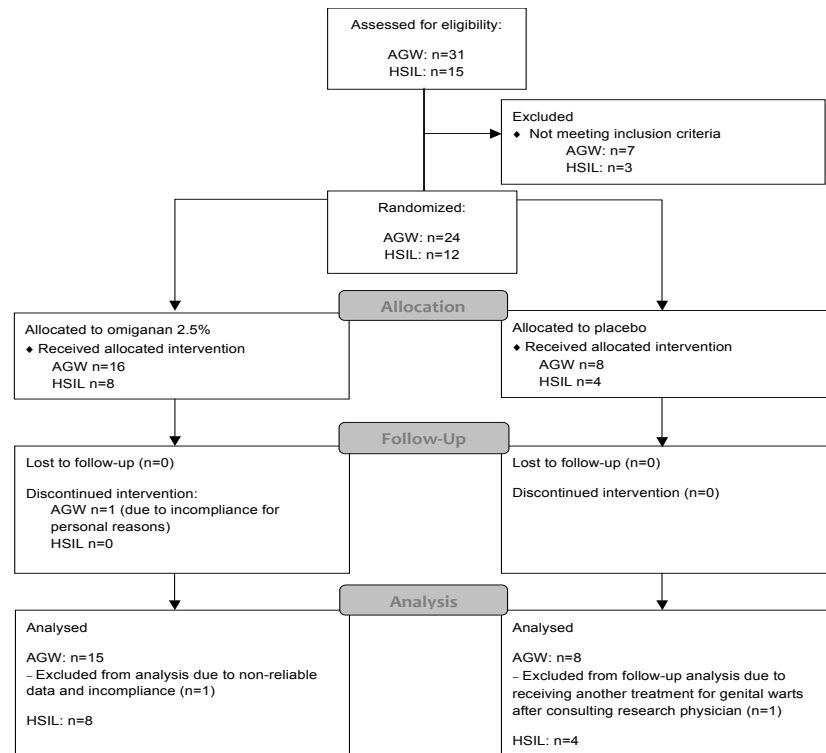


Table 1. Baseline characteristics of patients.

Characteristics	AGW trial			Vulvar HSIL trial		
	Omiganan	Placebo	Total	Omiganan	Placebo	Total
GENDER - N (%)						
Female	6 (37.5)	3 (37.5)	9 (37.5)	8 (100)	4 (100)	12 (100)
Male	10 (62.5)	5 (62.5)	15 (62.5)	-	-	-
AGE IN YEARS - median (range)	30 (20-46)	40 (23-64)	33 (20-64)	55 (35-71)	46 (29-51)	52 (29-71)
NUMBER OF LESIONS - median (range)	8 (4-62)	18 (10-43)	11 (4-62)	2 (1-3)	4.5 (2-6)	2.5 (1-6)
LESION SIZE IN MM - median (range)						
Long diameter	4.3 (2.4-7.8)	4.7 (4.2-7.3)	4.4 (2.4-7.8)	16 (10-40)	10 (5-30)	16 (5-40)
Short diameter	3.0 (1.4-4.8)	3.8 (2.1-5.2)	3.3 (1.4-5.2)	10 (5-20)	7 (4-30)	10 (4-30)
Height	1.3 (0.2-3.0)	1.3 (0.5-2.1)	1.3 (0.2-3.0)	-	-	-
DISEASE DURATION In years - median (range)	1.7 (0.3-10.5)	1.5 (0.2-5)	1.6 (0.2-10.5)	5.0 (0.1-25.8)	5.5 (0.9-8.5)	5.3 (0.1-25.8)
HPV TYPE BIOPSY - N						
HPV6	13 ^b	7	20 ^b	-	-	-
HPV11	-	1	1	-	-	-
HPV16	-	-	-	6 ^c	4	10 ^c
HPV18	-	-	-	1 ^c	-	1 ^c
HPV33	-	-	-	2 ^c	-	2 ^c
HPV44	1	-	1	-	-	-
HPV51	1 ^b	-	1 ^b	-	-	-
HPVX ^a	2	-	2	1	-	1
Previous treatment - N (%)	11 (69)	8 (100)	19 (79)	7 (88)	4 (100)	11 (92)
Cryotherapy	5	2	7	-	-	-
Surgical excision	3	1	4	3	1	4
Podofyllotoxine	4	7	11	-	-	-
Imiquimod	4	3	7	6	3	9
Sinecatechins	1	0	1	-	-	-
Trichloroacetic acid	1	0	1	-	-	-
Laser	-	-	-	2	1	3
Vaccination trial	-	-	-	1	0	1
SMOKING						
No	10	4	14	2	0	2
1-15/day	5	3	8	4	3	7
15+/day	1	1	2	2	1	3
SYMPTOMS						
no	13	4	17	5	3	8
itch	2	4	6	0	0	0
pain	1	0	1	0	0	0
itch and pain	0	0	0	3	1	4

a: HPVX= unidentifiable HPV type. / b: One patient had a co-infection (HPV6 and HPV51) / c: Two patients had a co-infection (HPV16 and 18, HPV16 and 33)

SAFETY, TOLERABILITY AND PHARMACOKINETICS

There were no serious adverse events and no study discontinuations or withdrawals due to adverse events. An overview of all treatment-emergent AEs is provided in Table 2. All AEs (N=34) were mild, transient and self-limiting. There were no systemic treatment-emergent adverse events. Almost all administration site symptoms (11/12) were in the omiganan group and probably related to the treatment. One AGW patient did not apply the investigational product for two weeks after having consulted the research team, due to adverse events (pruritus, erythema and irritation at application site). Hereafter, the investigational product was re-introduced and was applied every other day until the end of treatment without re-emergence of the side-effects.

Table 2. Treatment-emergent adverse events.

System organ class / preferred term	AGW				Vulvar HSIL			
	Omiganan (N=16)		Placebo (N=8)		Omiganan (N=8)		Placebo (N=4)	
	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects
	N	N (%)	N	N (%)	N	N (%)	N	N (%)
Any event	8	7 (43.8)	1	1 (12.5)	3	3 (37.5)	0	0 (0)
ADMINISTRATION SITE CONDITIONS								
Burning sensation	1	1(6.3)	1	1(12.5)	2	2 (25)	-	-
Erythema	1	1(6.3)	-	-	-	-	-	-
Induration	1	1(6.3)	-	-	-	-	-	-
Pruritus	4	3(18.8)	-	-	1	1 (12.5)	-	-
Skin hypopigmentation	1	1(6.3)	-	-	-	-	-	-

CLINICAL EFFICACY

Clinical impressions of patients with genital warts and vulvar HSIL treated with omiganan are shown in Figure 2. The clinical efficacy of omiganan in genital warts and HSIL is summarized in Table 3. There was no difference in clearance of both AGW and vulvar HSIL between omiganan and placebo and no differences were found in study part B of the vulvar HSIL trial. A trend towards a decrease in the mean height of AGW in the omiganan

group was detected compared to the placebo group (-30.3%; 95% CI -51.8 to -0.7%; p=0.054). Biopsies showed no difference in histology between the two groups of both trials, although in AGW patients treated with omiganan three biopsies were not taken because of clearance of the lesion while the in the AGW placebo group one biopsy was missing due to clearance of the lesion.

Figure 2. Photography assessments of lesions over time. Photography of a patient with anogenital warts (A) and vulvar HSIL (B), both patients were treated with omiganan. Pre-dose (day 0) the lesions are clearly visible. Upon treatment, the genital warts clearly resolve (A) and the vulvar HSIL remained the same (C). The patient of picture 2a had total clearance of the genital warts at EOS, but a post-inflammatory hypopigmentation has occurred at the lesion site. Day 0 is before start of treatment, day 84 is at the end of treatment (EOT) and day 168 is at the end of study (EOS). (see back-inside cover for image in color)

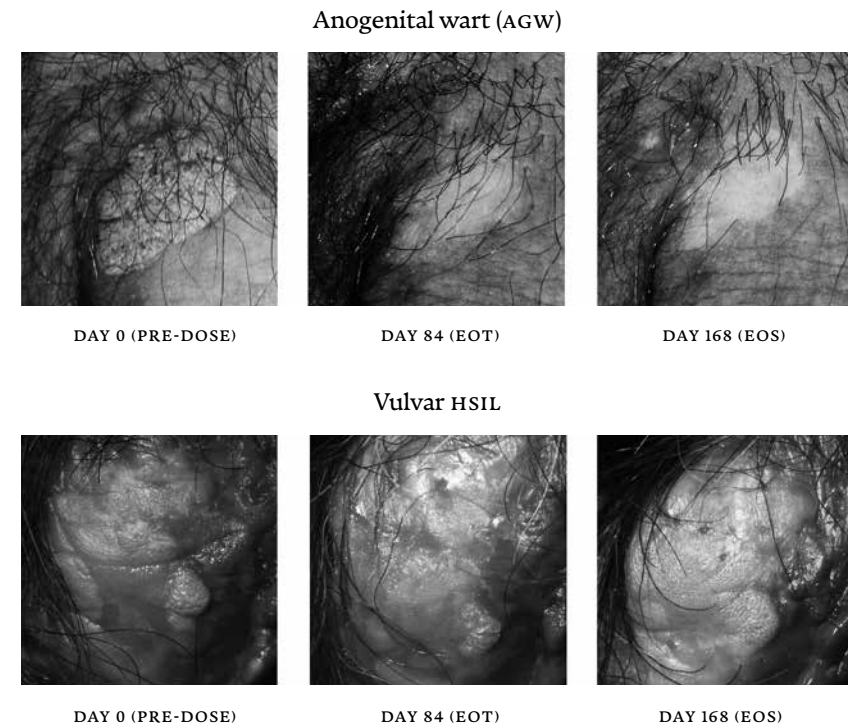


Table 3. Clinical efficacy after treatment with omiganan or placebo. Lesion count, dimensions and HPV expression are shown as mean (SD). The viral load swab is described as mean (SD) of the LN copies/microliter.

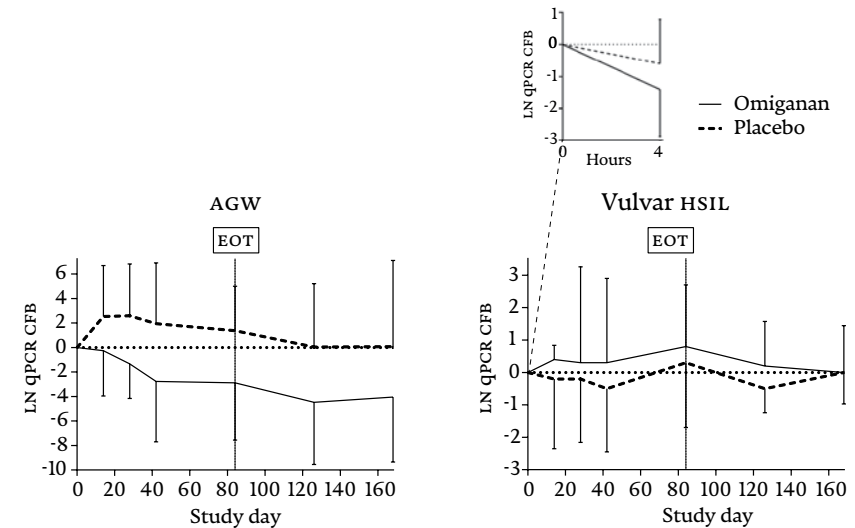
Assessment	AGW		Vulvar HSIL				P-value	EOS	P-value
	Pre-dose	EOT	EOT	EOS	Pre-dose	EOT			
Lesion count - mean (SD)	Omiganan 14.9 (14.5) Placebo 21.2 (12.0)	13.9 (17.2) 23.5 (14.2)	12.1 (15.8) 18.1 (13.7)	1.9 (1.0) ^a 3.3 (2.1) ^a	2.0 (0.9) 3.8 (1.5)	2.0 (0.9) 3.5 (1.7)	0.17	1.9 (1.0) ^a 3.3 (2.1) ^a	0.58
Long diameter in mm - mean (SD)	Omiganan 4.4 (1.6) Placebo 5.0 (1.1)	4.5 (1.8) 6.0 (3.9)	3.8 (2.7) 4.8 (2.5)	- -	- -	- -	0.98	- -	-
Short diameter in mm - mean (SD)	Omiganan 2.9 (1.0) Placebo 3.7 (0.9)	2.7 (1.2) 4.5 (2.5)	2.6 (2.0) 3.7 (2.0)	- -	- -	- -	0.23	- -	-
Height in mm - mean (SD)	Omiganan 1.4 (0.8) Placebo 1.2 (0.6)	1.5 (1.0) 2.3 (2.0)	1.2 (1.1) 1.3 (0.7)	- -	- -	- -	0.05	- -	-
Sum longest diameter in mm - mean (SD)	Omiganan - Placebo -	- -	- -	32.5 (11.6) 44.5 (27.6)	35.0 (17.7) 47.8 (20.7)	31.3 (12.0) 41.8 (22.9)	-	32.5 (11.6) 44.5 (27.6)	0.79
Viral load swab in LN copies/ μ L - mean (SD)	Omiganan 5.1 (4.2) Placebo 3.6 (5.3)	2.3 (4.8) 4.9 (5.2)	1.1 (6.2) 2.5 (7.0)	4.8 (2.2) 7.4 (2.3)	4.8 (2.6) 7.3 (2.2)	5.7 (2.4) 7.4 (3.2)	0.045	4.8 (2.2) 7.4 (2.3)	0.96
Relative HPV expression biopsy - mean (SD)	Omiganan 0.5 (0.8) Placebo 0.2 (0.2)	0.2 (0.4) 0.2 (0.2)	0.3 (0.3) 0.6 (0.5)	2.1 (1.9) 0.6 (0.6)	2.2 (1.4) 1.3 (1.1)	4.3 (2.8) 1.1 (1.1)	0.11	2.1 (1.9) 0.6 (0.6)	0.67
Histology	Omiganan AGW 16/16 Placebo AGW 8/8	AGW 11/15 Other 2/15 Normal 2/15	AGW 9/15 Other 3/15 ^b No biopsy 3/15 ^c	HSIL 6/8 No dysplasia 2/8	HSIL 8/8 No dysplasia 1/8	HSIL 7/8 No dysplasia 1/8	N.A.	HSIL 6/8 No dysplasia 2/8	N.A.
	Omiganan AGW 8/8 Placebo AGW 3/7 Other 3/7 ^b Normal 2/8	AGW 6/8 Other 0/8 Normal 2/8	AGW 3/7 No biopsy 1/7 ^c	HSIL 4/4 No dysplasia 1/3 ^d	HSIL 3/3 ³	HSIL 2/3 ^d No dysplasia 1/3 ^d			

EOT= end of treatment, EOS= end of study. / a At the EOT visit a biopsy of BW2 was performed / b Seborrheic verruca, (hyperkeratotic) papilloma, fibro epithelial polyp / c Due to clearance of the wart no biopsy was performed / d One patient refused the biopsies at the EOT and EOS visit

HPV CLEARANCE

The median viral load in LN copies/ μ L as measured with swabs at baseline was 4.6 (range -6.9 – 12.1) for AGW and 5.7 (range 1.9 – 9.5) for vulvar HSIL. In the AGW trial, the amount of viral DNA in the omiganan group showed a statistically significant decrease during the study period compared with the placebo group (-96.6%; 95% CI -99.9 to -7.4%; p=0.045), Figure 3a. There were no differences in viral load in vulvar HSIL swabs between omiganan and placebo (Figure 3b). Remarkably, in the swabs 4 hours after treatment with omiganan a decrease was seen compared to the swabs pre-dose (-73.1%; 95% CI -94.3 – 25.9%; p=0.08). The relative expression of HPV measured in the biopsies showed no differences between omiganan and placebo in patients with AGW and HSIL (supplemental figures).

Figure 3. Viral load shown as change from baseline (CFB). A qPCR was performed to determine the HPV load in the swabs of both trials. Analysis of viral load in swabs was performed using a mixed model with treatment, time and treatment by time as fixed factors and subject as random factor. All statistical tests were two-tailed with an α -level of 0.05. A statistical significant difference was found when comparing the HPV load of AGW patients treated with omiganan compared to placebo (-96.6%; 95% CI -99.9 to -7.4%; p=0.045) (A). No statistical difference was found when comparing the HPV load of vulvar HSIL patients treated with omiganan compared to placebo (B).



PATIENT-REPORTED OUTCOMES

For vulvar HSIL, both pain and itch showed a high inter-patient variability and no statistical significant differences were seen between the treatment groups ($p=0.81$ and $p=0.66$, in pain and itch respectively). For AGW, most patients did not experience any pain and there was a minimal inter-patient variability for both symptoms. There was no difference in total QOL score between the treatment groups in both vulvar HSIL and AGW patients ($p=0.16$ and $p=0.94$ respectively), see supplemental figures. The treatment adherence was 99% in both trials.

Discussion

This study demonstrates that despite the lack of clinical efficacy, omiganan reduced the viral load of AGW and demonstrated a favorable safety profile in AGW and HSIL patients. The distribution of the low and high-risk HPV types in AGW and vulvar HSIL patients was similar to the distribution found in literature.^{5,6} This is the first study to show the use of skin swabs as a biomarker in the genital area related to antiviral treatment monitoring. We previously showed that skin swabs can be used for antiviral treatment monitoring for cutaneous warts.^{35,36} These studies showed a good correlation of the HPV load between swabs and gold standard biopsies. Performing swabs instead of multiple biopsies has major advantages: a lower patient burden because of its non-invasive nature, the ability to assess the viral load of a single lesion over time and its lack of curative effect, i.e. by removing the lesion or by inducing an immune response. Previous research has shown that the viral load as assessed in cervical swabs is inversely related to HPV16 clearance and may therefore predict whether HPV infections become persistent or not.³⁷ A significant reduction in HPV load was found in swabs of treated AGW patients, which could suggest a suppression of the viral infection by omiganan. In the *in vitro* experiments we found that omiganan blocks HPV infection and has immune-modulatory effects, the reduction of the viral load in AGW patients support these *in vitro* results.²⁵ In the vulvar HSIL trial we tested the viral load 4 hours after administration of the drug and we observed a decrease of the viral load in the omiganan group. It is tempting to hypothesize that omiganan reduced the viral load shortly after application and that extended

exposure (twice daily dosing) would have been more efficacious. Skin swabs from viral lesions do not represent a homogeneous sample matrix like blood. However, HPV viral load determination has previously been shown to be a reliable marker in prior studies.³⁶ To mitigate the risk of high variability in viral load measurements in the skin swabs a standard protocol was used by trained clinicians dedicated to the project.

We showed remarkable differences in omiganan treatment on viral load in patients with low-risk HPV type (AGW) versus patients with high-risk HPV type induced lesions (vulvar HSIL). In AGW patients, we noted a significant decrease in low-risk HPV load after omiganan treatment, opposed to no difference in high-risk HPV load in vulvar HSIL patients. While HPV6 and HPV16 are all in the Alpha genus of the phylogenetic tree of Papillomaviridae which is based on the DNA sequence of the L1 open reading frame, both HPV types exhibit different biological properties. HPV6 is mostly implicated in benign lesions whereas HPV16 is the main cause of HPV-induced cancers. In this study we investigated the effect of omiganan on two distinct lesion types. AGW is a benign lesion, whereas vulvar HSIL is a premalignant lesion, which can progress to vulvar cancer. Also, analyses of complete genome sequences have found that HPV16 is more diverse with four variant lineages, compared with two variant lineages for HPV6.^{38,39} These differences along with their oncogenic properties may lead to the hypothesis that omiganan can interfere with the low-risk HPV type 6 and 11, but may not be effective for high-risk HPV type 16. A possible explanation might be that high-risk HPV types cause integration of the viral DNA into the human genome and overexpression of the E6 and E7 oncoproteins, which are not amenable to omiganan treatment.

Limitations of our study include the small sample sizes in the AGW and HSIL trials, the lack of dose ranging in both trials and the absence of measurements of omiganan in the lesions, which makes it hard to draw conclusions about the efficacy of the drug. One patient was excluded from the efficacy analyses because of non-adherence reasons, however non-adherence was related to personal reasons, and not to treatment-related reasons or adverse events. Biopsies in AGW patients were taken from different lesions and no biopsies were taken if a lesion was cleared. It should therefore be noted that the AGW trial comprised of subjects with treatment-resistant AGW that had been present for a longer time. It cannot be excluded that omiganan might have shown higher efficacy rates in subjects with recently developed, treatment-naïve AGW. Strengths of our study are the well-controlled study

designs of the AGW and HSIL trials, the use of biomarkers and innovative methodology such as the e-diary and the combination of face-to-face visits at the clinic and remote assessments by the smartphone application.

Because the reduction in viral load did not translate in clinically meaningful effects, we suggest to further develop omiganan as a potential new therapy for HPV-induced genital lesions using a higher dose (twice daily treatment) or in combination with other compounds such as imiquimod. In a human skin inflammation model study we found that omiganan can enhance the effect of imiquimod.⁴⁰ Therefore, we hypothesize that the combination therapy of omiganan and imiquimod may show synergistic effects and lower recurrence rates in patients with AGW and a follow-up study with combination treatment of omiganan and imiquimod is currently under development.

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