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ORIGINAL ARTICLE

Results of phase 2 trials exploring the safety and efficacy of omiganan in patients with human papillomavirus-induced genital lesions

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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 AGW 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 analysing 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. Human papillomavirus load significantly reduced after 12 weeks of treatment with omiganan compared to placebo (−96.6%; 95% confidence interval −99.9 to −7.4%; P = .045) in AGW patients only.

Conclusion: Topical omiganan appears to be safe in patients with AGW and vulvar HSIL and reduced human papillomavirus load after 12 weeks of treatment in AGW patients.

KEYWORDS

dermatology, medication safety, pharmacokinetics, pharmacodynamics, virology

PI statement: The authors confirm that the Principal Investigator for this paper is Jacobus Burggraaf, MD, PhD and that he had direct clinical responsibility for patients.

Trial registration ClinicalTrials.gov: AGW trial: NCT02849262, registration 29 July 2016, enrolment 26 July 2016, https://clinicaltrials.gov/ct2/show/NCT02849262 Vulvar HSIL trial: NCT02596074, registration 4 November 2015, enrolment 1 December 2015, https://clinicaltrials.gov/ct2/show/NCT02596074

1 | INTRODUCTION

Anogenital warts (AGW) and vulvar high-grade squamous intraepithelial lesion (HSIL), formerly referred to as usual type vulvar intraepithelial neoplasia, 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 defence 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 immunomodulatory 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 antiviral activities. Omiganan also stimulates the maturation of dendritic cells, reduces the M2-type (protumour) macrophage profile and enhances the M1-type (antitumour) macrophage profile.[25] Furthermore, omiganan is widely studied in several clinical trials in nonviral, 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 2 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.

What is already known about this subject

- Current therapies often fail as treatment for human papillomavirus-induced genital lesions.
- Current therapies have many side effects and lesions have high recurrence rates.
- There is a strong medical need for new therapies eliminating the virus with minimal side effects.

What this study adds

- This study is the first to demonstrate antiviral activity of topical omiganan in adults with anogenital warts.

2 | METHODS

2.1 | 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, 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 20 mm in diameter or 120 mm^2 surface. Exclusion criteria were active treatment of the lesions within 28 days prior to enrolment, 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 enrolment. 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 sex 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.

2.2 | 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 (AEs), physical examination, echocardiography, and laboratory blood and urine tests. AEs were recorded according to the Medical Dictionary for Regulatory Activities (MedDRA) version 19. Physical examination and echocardiography 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 (predose and 10, 20, 30, 60, 120 and 180 min after dosing) and tested by validated high-performance liquid chromatography–tandem mass spectrometry with a limit of quantitation of 1.0 ng/mL of omiganan.

Clinical assessments were performed at each study visit by the same 2 investigators and gynaecologist. In the AGW trial, 3 warts were selected for size measurements based on size and reproducibility of repeated measurements; target wart and biopsy warts 1 and 2. Wart size reduction was assessed in longest and shortest diameter and height (mm) was assessed using a digital Vernier calliper (0–150 mm, Conrad Electronic Benelux B.V., Oldenzaal, the Netherlands) and 3D photography.[29] Wart clearance was defined as complete disappearance of the wart. In vulvar HSIL patients, clinical response was determined based on the RECIST guidelines.[30] Complete response was defined as the complete disappearance of target lesions. A reduction >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 prewetted cotton-tipped stick. The cotton stick was placed in 1 mL of saline solution and stored at −40°C. Swabs for polymerase chain reaction (PCR) analyses were taken before omiganan was applied. All swabs were analysed 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 350 μL of swab, 100 μL of 0.9% NaCl and an added volume of 50 μL of 10 times concentrated phosphate buffered saline. Nucleic acids were eluted in a volume of 50 μL 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 10× and retested to confirm that PCR inhibition was no longer present. The 10× diluted sample was used in the HPV quantitative PCR (qPCR) and viral load was corrected for the 10× dilution. The baseline 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 3 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 5 μL 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 analysed 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 3-mm biopsy was obtained at screening (biopsy wart 1), week 12 (biopsy wart 2) and week 24 (target wart) in case of AGW and 2 3-mm 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 2 equal pieces and were further evaluated by the Leiden University Medical Centre department of pathology. One piece/biopsy was assessed according to conventional pathological standards by the same gynaecological pathologist after haematoxylin and eosin staining.[31,32] 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. The expression of HPV was corrected using the expression of the housekeeping genes ACTB and RPL11.

Patient-reported outcomes were determined by a study-specific smartphone application (e-diary) and a paper questionnaire.[33] In the e-diary application, the symptoms pain and itch were assessed by a numeric rating scale 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).[34] 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.

2.3 | 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 predefined intention-to-treat population, which comprised of all enrolled patients who received at least 1 dose of study treatment. The pharmacodynamics and efficacy analyses were conducted in the per protocol population, which consisted of the intention-to-treat population with at least 1 postbaseline assessment and no major protocol deviation. All

efficacy and pharmacodynamic endpoints were analysed 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 2-sided Fisher exact and a 2-sided Wilcoxon exact rank test were used to analyse wart clearance. Graphs were made using GraphPad Prism (version 6.05 for Windows, GraphPad Software, La Jolla, California, USA). All statistical tests were 2-tailed with α-level of .05.

3 | RESULTS

3.1 | 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 1 AGW patient was excluded due to a major protocol deviation (study procedure incompliance) and 1 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.

3.2 | Safety, tolerability and pharmacokinetics.

There were no serious AEs and no study discontinuations or withdrawals due to AEs. 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 AEs. 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 2 weeks after having consulted the research team, due to AEs (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. Omiganan was undetectable in all blood samples.

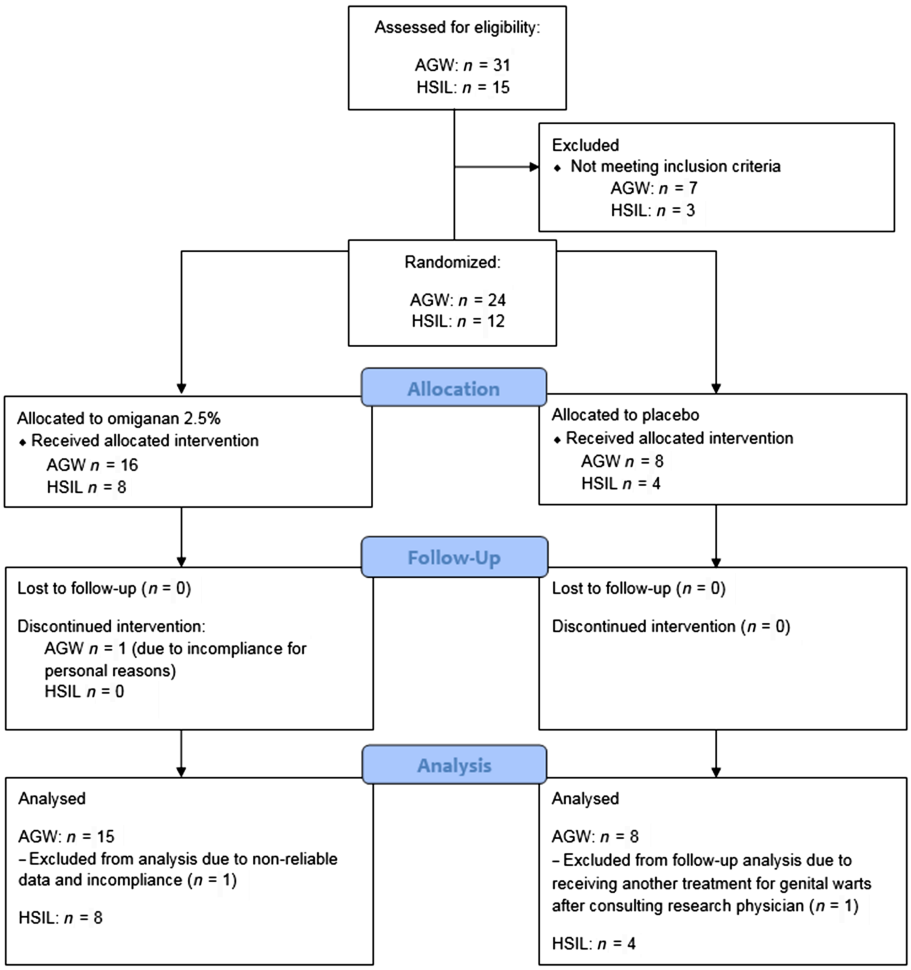

FIGURE 1 Integrated flowchart of the anogenital warts (AGW) and vulvar high-grade squamous intraepithelial lesions (HSIL) trial. In the AGW trials, 31 subjects were screened and 24 (77%) enrolled. Of the 24 remaining subjects, 16 were randomly assigned to treatment with omiganan and 8 to placebo. One 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

	Anogenital warts trial			Vulvar high-grade squamous intraepithelial lesions trial		
Characteristics	**Omiganan**	**Placebo**	**Total**	**Omiganan**	**Placebo**	**Total**
Sex, *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 (y), 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 (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 (y), 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)
Human papillomavirus (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

(Continues)

TABLE 1 (Continued)

Characteristics	Anogenital warts trial			Vulvar high-grade squamous intraepithelial lesions trial		
	Omiganan	Placebo	Total	Omiganan	Placebo	Total
- 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 coinfection (HPV6 and HPV51).
[c]Two patients had a coinfection (HPV16 and 18, HPV16 and 33).

3.3 | 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% confidence interval [CI] −51.8 to −0.7%; P = .054). Biopsies showed no difference in histology between the 2 groups of both trials, although in AGW patients treated with omiganan 3 biopsies were not taken because of clearance of the lesion while the in the AGW placebo group 1 biopsy was missing due to clearance of the lesion.

3.4 | HPV clearance

The median viral load in LN copies/μL 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 statistical significant decrease during the study period compared with the placebo group (−96.6%; 95% CI −99.9 to −7.4%; P = .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 h after treatment with omiganan a decrease was seen compared to the swabs predose (−73.1%; 95% CI −94.3 to 25.9%; P = .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).

3.5 | 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 = .81 and P = .66, in pain and itch respectively). For AGW, most patients did not experience any pain and there was a minimal interpatient variability for both symptoms. There was no difference in total QoL score between the treatment groups in both vulvar HSIL and AGW patients (P = .16 and P = .94 respectively), see supplemental figures. The treatment adherence was 99% in both trials.

4 | DISCUSSION

This study demonstrates that despite the lack of clinical efficacy, omiganan reduced the viral load of AGW and demonstrated a favourable 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

TABLE 2 Treatment-emergent adverse events

	Anogenital warts				Vulvar high-grade squamous intraepithelial lesions			
	Omiganan (*n* = 16)		Placebo (*n* = 8)		Omiganan (*n* = 8)		Placebo (*n* = 4)	
	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects
System organ class/preferred term	*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)	-	-	-	-	-	-

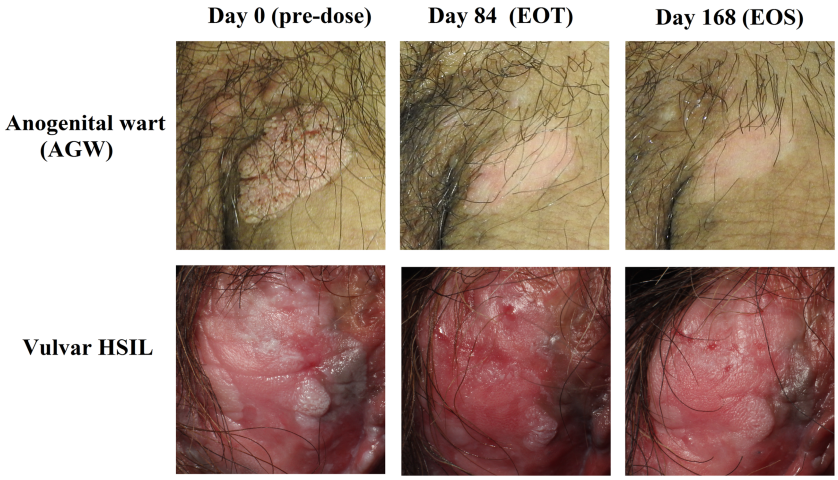

FIGURE 2 Photography assessments of lesions over time. Photography of patients with anogenital warts and vulvar HSIL, both patients were treated with omiganan. Predose (day 0) the lesions are clearly visible. Upon treatment, the genital warts clearly resolve and the vulvar HSIL remained the same. The patient in the first picture had total clearance of the genital warts at EOS, but a postinflammatory hypopigmentation had 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)

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 noninvasive 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,[25] the reduction of the viral load in AGW patients support these in vitro results. In the vulvar HSIL trial, we tested the viral load 4 h 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) *vs* 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 2 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 4 variant lineages,

TABLE 3 Clinical efficacy after treatment with omiganan or placebo

		Anogenital warts (AGW)				Vulvar high-grade squamous intraepithelial lesions (HSIL)			
Assessment		**Predose**	**EOT**	**EOS**	***P*-value**	**Predose**	**EOT**	**EOS**	***P*-value**
Lesion count – Mean (SD)	Omiganan	14.9 (14.5)	13.9 (17.2)	12.1 (15.8)	.17	2.0 (0.9)	2.0 (0.9)	1.9 (1.0)[a]	.58
	Placebo	21.2 (12.0)	23.5 (14.2)	18.1 (13.7)		3.8 (1.5)	3.5 (1.7)	3.3 (2.1)[a]	
Long diameter in mm – Mean (SD)	Omiganan	4.4 (1.6)	4.5 (1.8)	3.8 (2.7)	.98	-	-	-	-
	Placebo	5.0 (1.1)	6.0 (3.9)	4.8 (2.5)		-	-	-	
Short diameter in mm – Mean (SD)	Omiganan	2.9 (1.0)	2.7 (1.2)	2.6 (2.0)	.23	-	-	-	-
	Placebo	3.7 (0.9)	4.5 (2.5)	3.7 (2.0)		-	-	-	
Height in mm – Mean (SD)	Omiganan	1.4 (0.8)	1.5 (1.0)	1.2 (1.1)	.05	-	-	-	-
	Placebo	1.2 (0.6)	2.3 (2.0)	1.3 (0.7)		-	-	-	
Sum longest diameter in mm – Mean (SD)	Omiganan	-	-	-	-	35.0 (17.7)	31.3 (12.0)	32.5 (11.6)	.79
	Placebo	-	-	-		47.8 (20.7)	41.8 (22.9)	44.5 (27.6)	
Viral load swab in LN copies/μL – Mean (SD)	Omiganan	5.1 (4.2)	2.3 (4.8)	1.1 (6.2)	.045	4.8 (2.6)	5.7 (2.4)	4.8 (2.2)	.96
	Placebo	3.6 (5.3)	4.9 (5.2)	2.5 (7.0)		7.3 (2.2)	7.4 (3.2)	7.4 (2.3)	
Relative HPV expression biopsy – Mean (SD)	Omiganan	0.5 (0.8)	0.2 (0.4)	0.3 (0.3)	.11	2.2 (1.4)	4.3 (2.8)	2.1 (1.9)	.67
	Placebo	0.2 (0.2)	0.2 (0.2)	0.6 (0.5)		1.3 (1.1)	1.1 (1.1)	0.6 (0.6)	
Histology	Omiganan	AGW 16/16	AGW 11/15 Other 2/15Normal 2/15	AGW 9/15 Other 3/15[b] No biopsy 3/15[c]	N.A.	HSIL 8/8	HSIL 7/8 No dysplasia 1/8	HSIL 6/8 No dysplasia 2/8	N.A.
	Placebo	AGW 8/8	AGW 6/8 Other 0/8Normal 2/8	AGW 3/7 Other 3/7[b] No biopsy 1/7[c]		HSIL 4/4	HSIL 3/3[3]	HSIL 2/3[d]no dysplasia 1/3[d]	

Lesion count, dimensions and human papillomavirus (HPV) expression are shown as mean (SD). The viral load swab is described as mean (SD) of the LN copies/μL. EOT = end of treatment, EOS = end of study; SD = standard deviation.

[a]At the EOT, visit a biopsy of wart 2 was performed.

[b]Seborrhoeic 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.

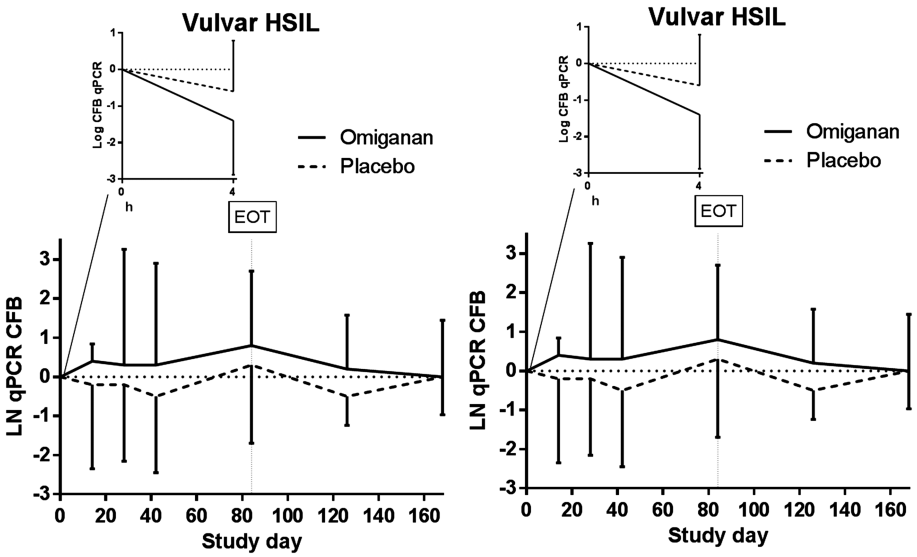

FIGURE 3 Viral load shown as change from baseline (CFB). From all swabs nucleic acid was isolated to determine the viral load of human papillomavirus (HPV) by use of quantitative polymerase chain reaction (qPCR) analysis of viral load in swabs was performed using a mixed model with treatment, time and treatment by time as fixed factors and subject as random factor. All statistical tests were 2-tailed with an α-level of 0.05. The viral load of anogenital warts patients decreases during the study period in the active treatment group, while the placebo group first shows an increase of viral load. A statistically significant difference was found when comparing the HPV load of anogenital warts patients treated with omiganan compared to placebo (−96.6%; 95% confidence interval −99.9 to − 7.4%; $P = .045$). The viral load of vulvar high-grade squamous intraepithelial lesions (HSIL) patients remains stable during the study period and no statistical difference was found when comparing the HPV load of vulvar HSIL patients treated with omiganan compared to placebo. EOT, end of treatment

compared with 2 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 nonadherence reasons; however, nonadherence was related to personal reasons, and not to treatment-related reasons or AEs. 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 included 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-naive 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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COMPETING INTERESTS

G.F. was employee of the sponsor. All other authors have no competing interests to declare.

CONTRIBUTORS

Melanie Rijsbergen wrote the manuscript, designed research, performed research, analysed data; Rianne Rijneveld wrote the manuscript, designed research, performed research, analysed data; Marina

Todd and Gary L. Feiss wrote the manuscript, designed research, analysed data; Stijn T.P. Kouwenhoven wrote the manuscript, performed research; Koen D. Quint wrote the manuscript, designed research, performed research, analysed data; Dirk C.J.G. van Alewijk wrote the manuscript, designed research, performed research, analysed data; Maurits N.C. de Koning wrote the manuscript, designed research, performed research, analysed data; Erica S. Klaassen wrote manuscript, designed research, analysed data; Jacobus Burggraaf wrote the manuscript, designed research, performed research, analysed data; Robert Rissmann wrote the manuscript, designed research, performed research, analysed data; Mariette van Poelgeest wrote the manuscript, designed research, performed research, analysed data.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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