Taking a closer look: non-invasive tools for in-depth characterisation of vulvar diseases

Pagan, L.

CHAPTER 5

Results of a randomized, placebo-controlled, first-in-human trial of topical CY-002 in patients with cutaneous warts

Adapted from Journal of the European Academy of Dermatology and Venereology, 2022. Doi: 10.1111/jdv.18291

Lisa Pagan[1,2], Christina Yfanti[1], Rianne Rijneveld[1], Marina Todd[1], Peter Jongste[1], Joep J. Feijen[1], Erica S. Klaassen[1], Jan Nico Bouwes Bavinck[3], Linda Struijk[4], Maurits N.C. de Koning[4], Lars Prestegarden[5], Tessa Niemeyer-van der Kolk[1], Mariëtte I.E. van Poelgeest[1,2] and Robert Rissmann[1,6,7]

1 Centre for Human Drug Research, Leiden, NL
2 Department of Obstetrics and Gynaecology, LUMC, Leiden, NL
3 Department of Dermatology, LUMC, Leiden, NL
4 DDL Diagnostic Laboratory, Rijswijk, the Netherlands.
5 Cytovation AS, Bergen, Norway
6 Leiden Amsterdam Centre for Drug Research, Leiden University, Leiden, NL
7 Leiden Skin Institute, Leiden, NL

Abstract

BACKGROUND The first-in-class peptide CY-002 was developed to target neoplastic and HPV-infected cells. Topical CY-002 was investigated on patients with cutaneous warts.

OBJECTIVES The primary objective was assessment of safety and tolerability of CY-002 in adults with cutaneous warts. Exploration of efficacy was the secondary objective.

METHODS CY-002 was investigated in a single-centre, randomized, double-blind, placebo-controlled, first-in-human, Phase-I trial including a safety run-in. Sixty-three adult patients with ≥ 1 cutaneous warts (on the hands) received CY-002 (1%) or placebo 1:1. Treatment was topically administered under occlusion for 28 consecutive days. Safety and multi-modal efficacy assessments were performed weekly and at 3-month follow-up.

RESULTS Baseline characteristics were similar except for a higher fraction of treatment-naïve patients among placebo compared to CY-002-treated patients (67.9% versus 33.3%, p=0.009). CY-002 was well tolerated with no differences in adverse events or treatment discontinuations. Exploratory efficacy measures (wart size, clearance, morphology and HPV load) did not differ statistically significant between groups. After CY-002, 2 patients (7.7%) achieved full clearance and 4 patients (15.4%) had clearance of ≥1 warts, compared to respectively 1 (3.8%) and 3 placebo patients (11.5%). Partial clearance was 44% in the CY-002-treated group versus 29% in placebo.

CONCLUSION CY-002 is safe and well tolerated for topical daily use up to 4 weeks. While explorative efficacy outcomes showed no statistically significant outcomes, a trend favouring CY-002 across multiple parameters warrants further studies. The design of this trial allows exploration of efficacy parameters without compromising on primary assessment of novel compound safety and tolerability.

Introduction

Cutaneous warts, or verrucae, are common skin lesions that affect most people at some point in their lifetime. Reported prevalence is 3.6–22% in schoolchildren and 0.84–13% in adults.[1–5] Cutaneous warts are caused by the human papillomavirus (HPV).[6] Some HPV types (e.g. type 16 and 18) have malignant potential in the genitourinary or oropharyngeal tract, whereas others give rise to benign skin lesions. HPV type 1, 2, 7, 27 and 57 most commonly cause cutaneous warts in the general population.[7,8] People with cutaneous warts may report physical or psychological discomfort, including pain or embarrassment.[9] Cutaneous warts can lead to social stigmatisation and lower quality of life.[10,11]

Untreated or unsuccessfully treated warts pose a pool of infection on an individual as well as a community level.[12,13] Current treatment options for cutaneous warts focus on general destruction of the epithelium rather than specifically targeting the HPV-infected keratinocyte.[14–16] The efficacy rate of these treatments varies between 24–40%, with frequently reported side effects including pain, burning sensation and blistering.[17,18] Development of a wart treatment sparing healthy tissue would be of considerable added value. Currently there are no treatments effectively eliminating the HPV infection.[19] Therefore, there is a need for novel treatments that treat cutaneous warts with higher specificity, improved efficacy and reduced side effects.

CY-002 is a synthetic, tumour-targeted membranolytic peptide that aims at an HPV-oriented, immune-targeted cutaneous wart therapy. CY-002 was previously shown to selectively induce cell death in multiple tumour cell lines whilst sparing normal cells *in vitro*.[20] Here, we report the results of a Phase I, first-in-human trial of CY-002 in otherwise healthy subjects with cutaneous warts.

The primary objective of this first-in-human proof-of-concept clinical study was to assess the safety and tolerability of topically applied CY-002 in adults with cutaneous warts. The secondary objective was to explore efficacy of CY-002 on reduction of wart number, size and HPV load after four weeks of daily topical application. Thirdly, new measurement approaches towards a multi-modal follow-up in wart trials were considered.

Methods

STUDY DESIGN

The study was designed as a randomized, placebo-controlled, double-blind, single-centre Phase I, first-in-human trial with a safety run-in. Safety and efficacy of CY-002 was evaluated after 4 weeks of treatment at home, preceded by a separate clinical safety run-in. Patients, study personnel and investigators were blinded throughout study conduct. The study was conducted at the Centre for Human Drug Research, Leiden, The Netherlands from February 2019 to December 2019. The Declaration of Helsinki was the guiding principle for trial execution and the study was approved by the independent medical ethics committee 'Medisch Ethische Toetsingscommissie van de Stichting Beoordeling Ethiek Biomedisch Onderzoek' prior to any procedure. All patients provided written informed consent before enrolment.

STUDY POPULATION

Patients were considered eligible if they were healthy, ≥ 18 years old and had ≥ 1 cutaneous warts on the hand with a diameter ≥ 3 mm. Patients were excluded if they had used wart-removing products within 30–60 days prior to enrolment, depending on the treatment. Effective contraception was required. Warts which were > 6 years old or had been treated with > 5 different treatments were excluded.

STUDY OUTLINE

The safety in-clinic phase was performed in 8 patients. CY-002 or placebo (randomised 1:1) was administered on 5x5 cm healthy skin on the back and on cutaneous warts during 7 consecutive days. Following no safety concerns during the initial phase, 55 ambulatory patients visited the clinical research unit once weekly (baseline, week 1, 2, 3 and 4) during at-home topical treatment of 28 days (*Supplementary Figure 1*). The patients applied one droplet (15–30mg) of CY-002 1% or placebo cream once daily followed by overnight occlusion using Tegaderm™ film (3M healthcare, Maplewood, MN, USA) on max. 3 warts. Patients returned for follow-up after 6 weeks (week 10) and after 12 weeks for end of study (EOS, week 16). Treatment compliance was monitored using a mobile e-diary application.[21] A pre-defined minimum of 21/28 planned applications was considered acceptable to include a patient for efficacy analysis.[22] Patients who failed to apply the study drug were replaced.

SAFETY MEASUREMENTS

The safety assessments comprised of evaluation of adverse events (AE), application site inspection for local tolerability, physical examination, ECG, vital signs (including systolic and diastolic blood pressure, pulse rate and temperature) and clinical laboratory testing. Systematic exposure to CY-002 was assessed in plasma on Day 28 and analysed by Ardena Bioanalytical Laboratory (Assen, The Netherlands) after study completion.

EFFICACY MEASUREMENTS

Efficacy measurements were obtained at baseline and at every following visit to the clinic (*Supplementary Figure 1*).

Wart size and clearance

Dimensions of the target warts (long diameter, short diameter, height and volume) were measured using a digital vernier caliper (0–75 mm) (HBM Machines B.V., Moordrecht, the Netherlands). The wart volume was calculated (volume = π * (diameter/2)^2 * height). Wart clearance was determined by medical study personnel recording total wart count at each visit. Complete clearance was defined as the diameter of the lesion being zero.

HPV typing and quantification

Swab samples were collected by rubbing the surface of the target wart five consecutive times with a sterile, pre-wetted cotton-tipped applicator (Puritan Medical Products, Guilford, Maine, USA), subsequently placed in 1 mL of saline solution and stored at -40 degrees Celsius. HPV type was determined with bead-based XMAP suspension array technology simultaneously identifying 23 wart-associated HPV types from the alpha- (HPV2, 3, 7, 10, 27, 28, 29, 40, 43, 57, 77, 91 and 94), gamma- (4, 65, 95, 48, 50, 60 and 88), mu- (HPV1 and 63) and nu-genus (HPV41) (DDL Diagnostic Laboratory BV, Rijswijk, The Netherlands).[23–25] HPV viral load of HPV types HPV1, HPV2, HPV-4, HPV27 and HPV57 was determined by quantitative PCR in all baseline and follow-up samples if the baseline sample was positive for the respective HPV type.

Morphology

Clinical photography was obtained using conventional 2D and the LifeViz 3D camera.[26] At each study visit, the treated warts were assessed for morphological properties according to the dichotomous nine-point CWARTS

method.[27,28] A 3 mm punch biopsy of the target wart was obtained at the end of study and assessed according to conventional pathological standards at DDL after haematoxylin and eosin (H&E) staining. Quantitative skin morphology analysis was determined by optical coherence tomography (D-OCT VivoSight, Michelson Diagnostics, UK).

Multimodal responder/non-responder analysis

The obtained data from 2D photography in addition to novel techniques (3D photography, OCT imaging and HPV-load analysis) was combined for in-depth study of potential effects. One partial responder after treatment with CY-002 and one non-responder from the placebo group were compared.

STATISTICS

Sample size justification

A group size of 25 patients was considered common for early exploration of safety, tolerability and efficacy of novel topical formulations with a safety run-in group size of 4. This sample size power of 0.8 to detect a difference in means of 24.3 mm^3, assuming that the common standard deviation was 30, using a two-group t-test with a 0.05 two-sided significance level.

Randomization

The patients were randomized 1:1. In the ambulatory part, randomization was performed in blocks of 10. The randomization code was generated in SAS 9.4 by an independent statistician and patient numbers were allocated by chronological enrolment. Patients, study personnel and investigators were blinded. The randomization code was made available for data analysis after study closure and database lock.

Statistical analysis

All safety and statistical programming was conducted with SAS 9.4. Each efficacy parameter was analysed using a mixed model analysis of covariance (ANCOVA) with treatment, time, and treatment by time as fixed factors and patient as random factor and the (average) baseline measurement as covariate. The Kenward-Roger approximation was used to estimate denominator degrees of freedom and model parameters were estimated using the restricted maximum likelihood method.

Results

PATIENTS

Following an acceptable safety profile in the first 8 patients (safety run-in), 55 patients (50 patients and 5 reserves) started treatment in the ambulatory part of the study (Intention to Treat population, ITT). Throughout the study, 52 patients completed treatment (per protocol population, PP) and 49 completed follow-up (*Figure 1* and *Supplementary Figure 2*). Baseline and disease characteristics were comparable for the two treatment groups (*Table 1*). The placebo patients were more treatment-naïve compared to CY-002 patients (67.9% versus 33.3%, p=0.009). Baseline wart morphology according to the CWARTS scoring was similar across groups (*Supplementary Table 1*).[27] Home treatment compliance ranged from 25–28 topical applications with a mean of 27 applications per subject (96.4%). Three patients were replaced due to incompliance failing study drug application for the pre-defined minimum 21 of 28 planned doses. There was no statistically significant difference in treatment adherence between groups.

SAFETY AND TOLERABILITY

Adverse events (AE) were similar between the treatment and placebo groups (*Supplementary Table 2*). No treatment emergent severe AEs or clinically significant changes in vital signs, clinical laboratory results, or ECG occurred in any patients throughout the study. Two patients reported mild application site tolerability issues, with one patient reporting a mild burning sensation lasting 10 seconds after study drug application. All pharmacokinetic samples showed no levels above the lowest level of quantification (LLOQ), concluding that there was no systemic exposure of CY-002.

WART CLEARANCE AND SIZE

Table 2 shows that after treatment with CY-002, 2 patients (7.7%) achieved full clearance and 4 (15.4%) patients had clearance of a minimum of one of the treated warts. In the placebo group, clearance was observed in 1 patient (3.8%) and 3 patients (11.5%), respectively. Three out of 4 responders in the CY-002 group had unsuccessfully used salicylic acid for wart treatment in their medical history. All placebo responders had treatment-naïve warts. Partial clearance (≥50% volume reduction) was observed in 21 treated warts (41%) in the CY-002 group compared to 13 treated warts (25%) following placebo

(p=0.0962) at end of treatment (EOT, week 4). At the end of study (week 16), the partial clearance was 43% (22 warts) in CY-002 group versus 29% (15 warts) in placebo (p=0.0940). There were no statistically significant differences in the clearance rates between groups. Diameter, height and volume of the treated lesions were determined at each visit (*Figure 2*). There were no statistically significant differences in wart dimensions comparing CY-002 and placebo over time. Both the lesions treated with CY-002 and placebo showed reduction in lesion volume within the first week of treatment persisting until the end of study, although the difference between treatment groups was not statistically significant (p=0.0896).

HPV TYPE AND LOAD

The predominant HPV types found >50% of patients were HPV type 27 and 57 (*Table 1*). HPV viral load decreased within the first week after treatment initiation in both the CY-002 and placebo group (*Figure 3*), with high variability found between samples (range 41477–$3{\cdot}10^9$ copies/mL). For CY-002, the HPV viral load remained reduced at the end of treatment (-73.4%,) and up until the end of study (-80.2%). After placebo, a decrease in HPV viral load was observed in the first week, but the results varied during the remainder of the study period (+6.2 at end of treatment, -78.4% at end of study). There were no significant differences between the groups or over time, probably due to high variability observed between samples.

MULTI-MODAL RESPONDER/NON-RESPONDER EVALUATION

Conventional 2D photography, 3D reconstructions and cross-sectional OCT images showed changes in wart characteristics and morphology over time for the CY-002 partial responder (*Figure 4A*), but not for the placebo non-responder (*Figure 4B*). HPV load reduced considerably following the first week of CY-002 treatment (*Figure 4C*), although a spike occurred at follow-up. Morphologically, the responding wart showed extensive roughness and epithelial hyperintensity at baseline in the OCT cross-sectional slides. This signifies a thick epithelium with considerable callous, which can also be seen in the 2D images. The level of roughness and hyperintensity were not present at end of treatment and end of study, although it was observed at follow-up, concurring with HPV-load finding and the 2D images. No distinct morphological changes over time were observed in the placebo subject. HPV load changes in the placebo subject were also less pronounced with no clear pattern.

Discussion

This first-in-human study shows for the first time that topical CY-002 is safe and well tolerated after 4-week treatment. In this study, no statistically significant effect on reduction of wart number, size or HPV load was discerned between treatment and placebo. This poses considerations on mode of action, placebo formulation, drug delivery, and adequate dosing regimen as well as statistical power of the study. Yet, as shown in *Figure 2* and *Figure 3*, the lesions treated with CY-002 showed a potential trend in volume and HPV-load reduction at end of treatment, during follow-up and at end of study. To further substantiate these results, increasing and aligning patient groups with different drug delivery strategies should be considered. Of note, the clearance of ≥1 warts in the placebo group was pronounced (11.5%). Other recent cutaneous warts trial investigating topicals presented 0–11.6% clearance in placebo groups.[25,29] In addition, one recent study with occlusion of a topical reported complete clearance in 1 subject (3.4%) and partial clearance in 1 subject (3.4%) within the placebo group.[30] In this study, the placebo group included more treatment-naïve patients compared to the CY-002 group (imbalance of 67.9% versus 33.3%). It should be emphasized that warts exposed to prior, unsuccessful therapy may be more resistant to other treatments.[31] All placebo patients showing response in this trial were treatment-naïve. In contrast, 75% of responders receiving CY-002 had a previous history of salicylic acid use. Although this small group size does not allow formal comparisons, this observation could partially explain the relatively high placebo response in this study compared to CY-002. This first-in-human study was primarily aimed at assessing the safety and tolerability of CY-002 and was not powered for efficacy.

Early-phase dermatology trials are increasingly introduced to novel techniques for effect exploration guiding drug development.[32] This study presents a combined phenotypical lesion follow-up of a responder and non-responder (*Figure 4*). The addition of 3D photography, HPV load and OCT imaging to conventional wart count allows for detailed observation, suggesting minor changes in HPV load and wart morphology although no effect was observed at group level. Additionally, a trend in partial response was discernible after 16 weeks at the end of study (CY-002 43%, placebo 29%, p=0.0940). Although full lesion clearance is the clinically relevant outcome for wart trials,[19] these positive exploratory observations may substantiate further develop-

ment of CY-002 for the indication of cutaneous warts at different dose levels. The rationale for investigating CY-002 as possible treatment for cutaneous warts comes from its cytolytic activity in malignant neoplasia and its intended mechanism of action directly targeting the HPV-infected cell and subsequently mounting an immune response against HPV.[20] Several potential topical and intralesional compounds targeting immune activation are under investigation, especially for recalcitrant warts and immunocompromised patients.[33,34] Topically, the TLR-7 agonist imiquimod has shown complete wart clearance of 27–89% in immunocompetent and 33–50% in immunocompromised patients in a recent literature review.[35] Intralesional injections of bleomycin have shown 70–95% response rates of treated warts.[36] Administration pain and local blistering limits intralesional application as first-line treatment, prompting development of topical bleomycin spray following several wart abrasion strategies to optimize drug delivery.[37,38] Intralesional *C. albicans* antigen therapy has shown additional efficacy against distant, untreated lesions[39], as have intralesional Bacillus Calmette-Guérin (BCG) vaccine derivatives.[40] Recent trials including an intralesional saline control report wart clearance of 0–40%, showing the marked effect of vehicle or manipulation potentially confounding therapeutic observations.[40–46]

The main strength of this study is its two-tiered design of a first-in-human trial to a novel topical compound in which safety was investigated without compromising on exploring efficacy parameters. Most first-in-human, Phase I trials are carried out clinically for safety surveillance. Here, safety and tolerability could be closely monitored by starting with a run-in population of 8 patients during the in-clinic part with focusing on potential systemic exposure.[47] The subsequent ambulatory phase with weekly follow-up allowed for evaluation of safety with exploration of efficacy. Wart treatment trials have essential challenges and limitations, which add to the low level of evidence for most wart therapy modalities.[19,48] Therefore, this study focused on recognizing and removing potential confounders from its design. Within-subject placebo-control studies ('left-right studies') are flawed as a response of distant warts may occur.[19,49,50] Indeed, recent topical wart trials have reported treatment effects on distant, untreated warts, citing immune activation as a possible explanation.[25,51] A biopsy was only obtained after 16 weeks at the end of this study, as potential effects may also apply to concomitant therapy or interventions on distant lesions.[23,52]

The putative limitation of this study is the lack of confirmation that CY-002 reaches HPV-infected keratinocytes and induces cell death *in vivo*. There was no systemic exposure of CY-002, which is beneficial considering potential systemic effects. However, this may imply lack of dermal penetration. Any interventions to enhance drug delivery may act as a confounder on treatment efficacy, illustrated by the marked placebo effect of intralesional saline injections. Overnight occlusion was included in this study to increase the likelihood of transdermal penetration despite potential confounding. Transdermal drug delivery always poses a key question in dermatology trials.[53,54] This is even more challenging with calloused cutaneous warts. Even salicylic acid, the golden standard in topical treatment, requires erosive intervention.[19] Other experimental wart treatment modalities are often accompanied by drug-delivery enhancing, abrasive methods such as microneedling, tape stripping or lesion shaving, especially in plantar warts.[55–57]

In conclusion, topical CY-002 is considered safe and tolerable when used daily up to 28 consecutive days with no systemic exposure. There was no significant reduction of wart size, number or HPV load comparing active treatment to placebo, although a higher partial response was observed in the warts treated with CY-002. A noteworthy observation in this study is an unprecedented placebo response, which may be the result of overnight occlusion. The design of this data-rich trial with a first-in-its-class topical treatment with a clinical safety run-in followed by ambulatory application allows for in-depth exploration of efficacy parameters without compromising on primary assessment of novel topical compound safety and tolerability.

Table 1 Baseline characteristics.

	Safety run-in		Ambulatory trial	
Characteristic	**CY-002 (n=4)**	**Placebo (n=4)**	**CY-002 (n=27)**	**Placebo (n=28)**
Age (years), mean (SD)	31.0 (9.4)	23.0 (4.7)	28.1 (11.4)	25.1 (9.9)
SEX, N (%)				
Male	3 (75.0)	2 (50.0)	14 (51.9)	12 (42.9)
Female	1 (25.0)	2 (50.0)	13 (48.1)	16 (57.1)
FITZPATRICK SKIN TYPE, N (%)				
I	1 (25.0)	1 (25.0)	2 (7.4)	3 (10.7)
II	3 (75.0)	2 (50.0)	13 (48.1)	12 (42.9)
III	0 (0)	1 (25.0)	7 (25.9)	8 (28.6)
IV	0 (0)	0 (0)	4 (14.8)	3 (10.7)
V	0 (0)	0 (0)	0 (0)	2 (7.1)
VI	0 (0)	0 (0)	1 (3.7)	0 (0)
Height (cm), mean (SD)	174.0 (10.0)	177.9 (16.9)	177.6 (9.5)	174.1 (8.0)
Weight (kg), mean (SD)	69.9 (7.2)	71.5 (11.0)	72.8 (10.0)	72.0 (10.6)
BMI (kg/m^2), mean (SD)	23.2 (1.5)	22.8 (4.4)	23.1 (3.0)	23.8 (3.3)
WART CHARACTERISTICS				
Wart age (years), mean (SD)	1.88 (2.77)	5 (4.69)	2.32 (1.54)	1.92 (1.26)
Total warts per patient (n), mean (SD)	2 (2)	1 (0)	3.56 (3.26)	3.04 (2.3)
Treated warts per patient (n), mean (SD)	1.5 (1)	1 (0)	1.93 (0.96)	2 (0.9)
Long diameter of treated warts (mm), mean (SD)	6.12 (2.5)	4.95 (1.5)	4.61 (1.83)	4.31 (1.7)
Short diameter of treated warts (mm), mean (SD)	4.45 (1.38)	3.95 (1.48)	3.73 (1.36)	3.49 (1.25)
Height of treated warts (mm), mean (SD)	1.32 (0.59)	1.28 (0.34)	0.84 (0.53)	0.83 (0.6)
HPV TYPE OF TARGET WART, N (%)				
HPV2/2var	0 (0)	2 (50)	3 (11.1)	5 (17.8)
HPV4	0 (0)	0 (0)	2 (7.4)	4 (14.3)
HPV27	1 (25.0)	0 (0)	8 (29.6)	8 (28.6)
HPV57	3 (75.0)	1 (25.0)	8 (29.6)	6 (21.4)
HPV65*	0 (0)	0 (0)	1 (3.7)	1 (3.6)
Other**	0 (0)	0 (0)	2 (7.4)	2 (7.1)
Missing	0 (0)	1 (25.0)	2 (7.4)	2 (7.1)

(continuation Table 1)

PREVIOUS WART TREATMENT, N (%)				
Cryotherapy, n (%)	1 (25.0)	2 (50.0)	11 (40.7)	7 (25.0)
Salicylic acid	2 (50.0)	2 (50.0)	10 (37.0)	4 (14.3)
Mono/bi/trichloracetic acid	1 (25.0)	0 (0)	0 (0)	0 (0)
Surgical excision	1 (25.0)	0 (0)	0 (0)	1 (3.6)
Other ***	0 (0)	1 (25.0)	0 (0)	0 (0)
No previous wart treatment	2 (50.0)	1 (25.0)	9 (33.3)	19 (67.9)

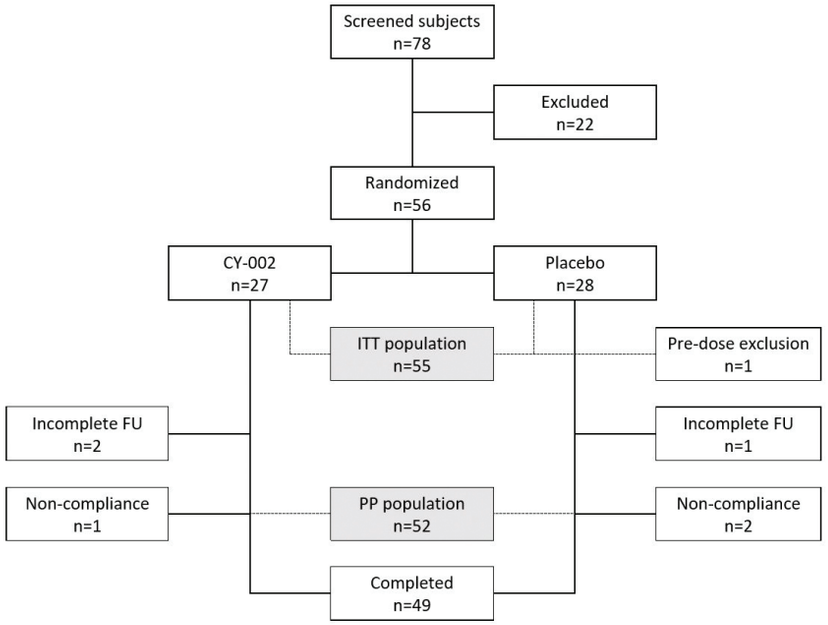
*HPV = Human papillomavirus. * HPV65 was found as a co-infection with HPV4 in 2 patients from the placebo group. ** Other HPV types which were reported once included HPV3, HPV10, HPV88 and HPV95. *** Homeopathic topical formulation. Display of the intention to treat (ITT) population.*

Table 2 Cutaneous wart clearance.

	CY-002 N=26 Treated warts = 51	Placebo N=26 Treated warts = 51	p-value
Subjects with clearance of all treated warts at EOS, N (%)	2 (7.7)	1 (3.8)	0.6092
Subjects with clearance of at least 1 wart at EOS, N (%)	4 (15.4)	3 (11.5)	0.7019
Treated warts completely cleared at EOT (Day 28), N (%)	0 (0)	1 (2)	1.0000
Treated warts completely cleared at EOS (Day 112), N (%)	6 (12)	4 (8)	0.5162
Treated warts with partial clearance at EOT (Day 28), N (%)	21 (41)	13 (25)	0.0962
Treated warts with partial clearance at EOS (Day 112), N (%)	22 (43)	15 (29)	0.0940

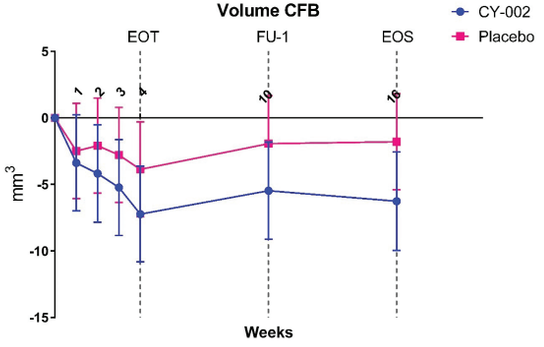
**Partial clearance was defined as >50% reduction in wart volume. Analysis performed on the per protocol (PP) population. EOT = End of Treatment, EOS = End of Study.*

Figure 1 Study flow chart.



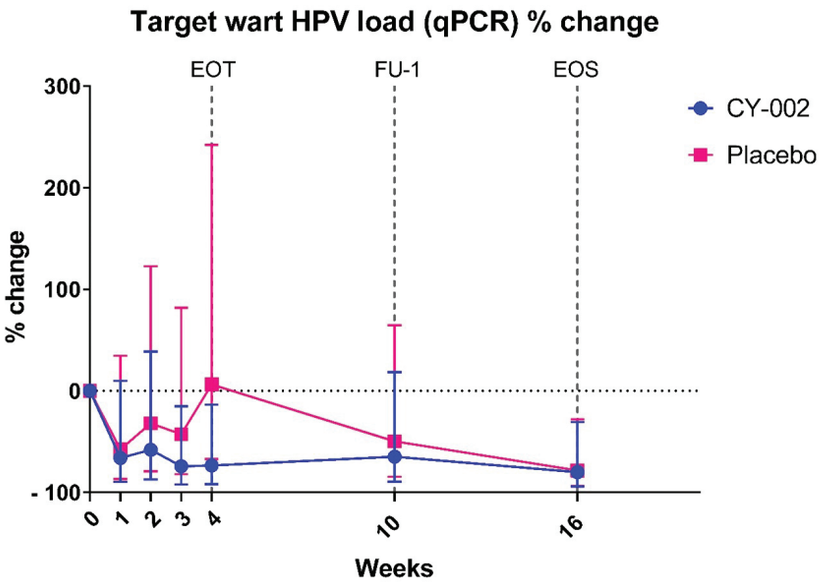
ITT = intention-to-treat population. PP = per-protocol population. FU = follow-up.

Figure 2 Volume change of cutaneous warts over time. In blue, active treatment with CY-002 is shown. In pink, placebo is shown.



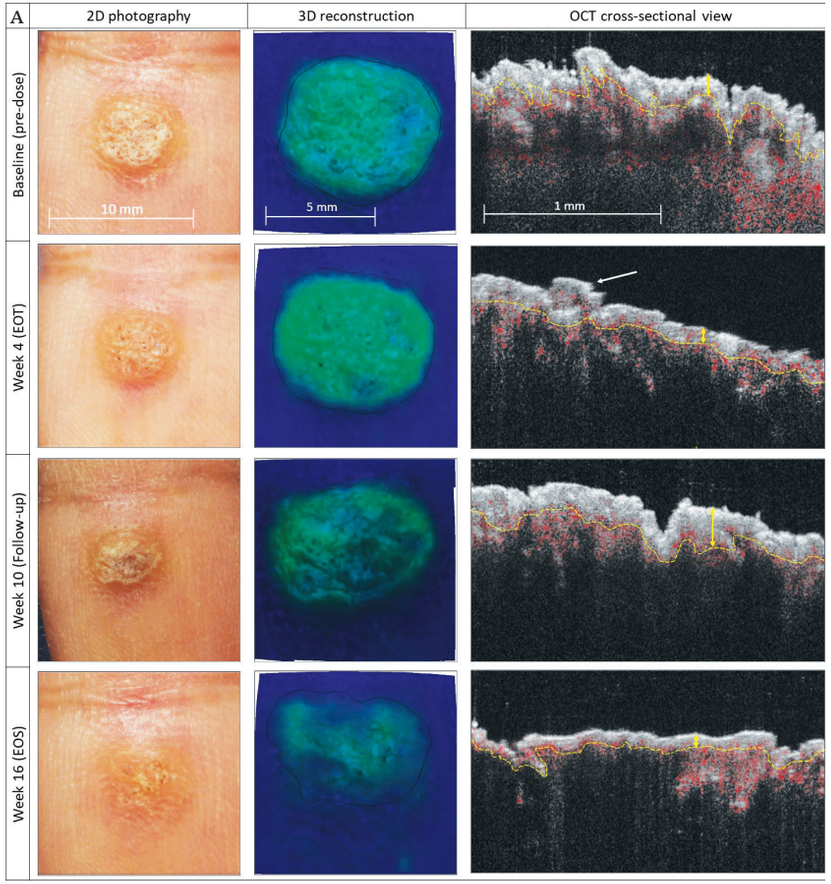
CFB = change from baseline. EOT = end-of-treatment. FU-1 = follow-up visit 1. EOS = end-of-study.

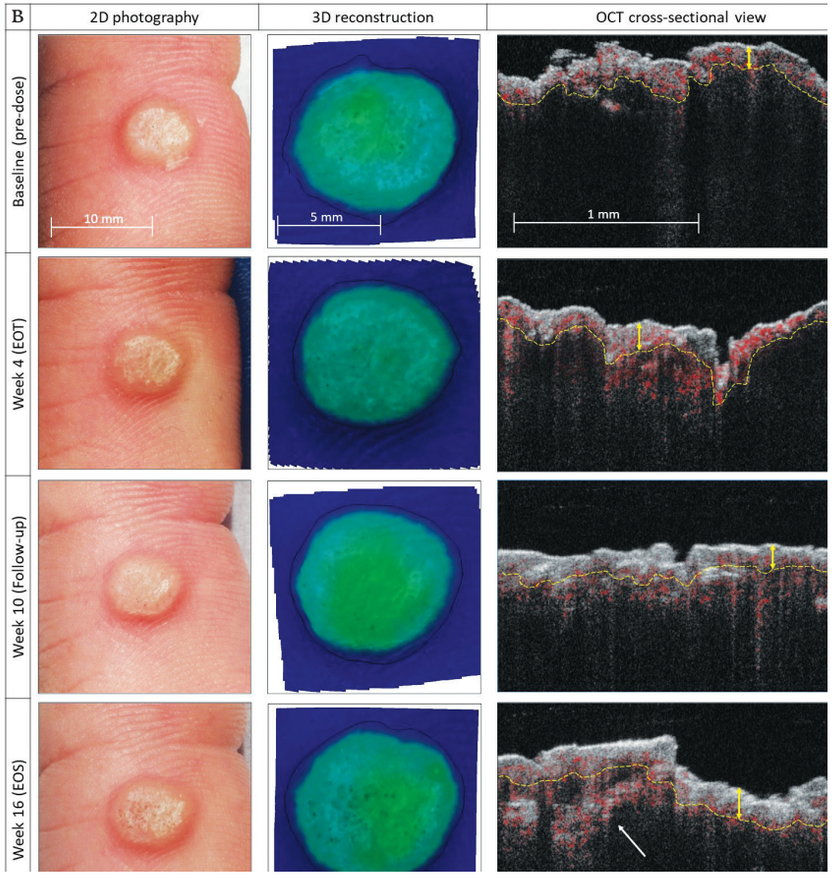
Figure 3 HPV load of target wart, percentage change over time. In blue, active treatment with CY-002 is shown. In pink, placebo is shown.

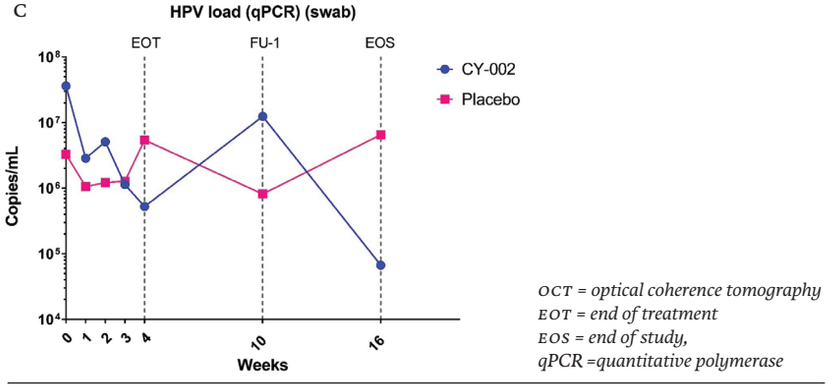


HPV = human papillomavirus. EOT = end-of-treatment. FU-1 = follow-up visit 1. EOS = end-of-study, qPCR: quantitative polymerase chain reaction

Figure 4 Multimodal analysis of responders and non-responders using imaging and sequencing techniques. Imaging techniques shown include conventional photography, stereophotogrammetric three-dimensional photography and optical coherence tomography analysis. A) Individual randomised to active treatment with CY-002. B) Individual randomised to Placebo. C) HPV load as determined by qPCR.







OCT = optical coherence tomography
EOT = end of treatment
EOS = end of study,
qPCR =quantitative polymerase

Supplementary Table 1 Wart morphology of target wart at baseline according to CWARTS during the ambulatory trial.

CWARTS characteristic of TW		CY-002 (n=27)	Placebo (n=28)
Arrangement	Confluent	2 (7.4%)	1 (3.6%)
	Solitary	25 (92.6%)	27 (96.4%)
Level	Elevated	22 (81.4%)	23 (85.2%)
	Skin level	5 (18.5%)	5 (17.9%)
Aspect	Rough/lobed	19 (70.3%)	19 (67.9%)
	Smooth/not lobed	8 (29.6%)	9 (32.1%)
Border	Sharp	23 (85.2%)	23 (82.1%)
	Unsharp	4 (14.8%)	5 (17.9%)
Colour	Skin colour	8 (29.6%)	8 (28.6%)
	Lighter than skin	5 (18.5%)	4 (14.3%)
	White	8 (29.6%)	11 (39.3%)
	Red	5 (18.5%)	3 (10.7%)
	Dark	1 (3.7%)	2 (7.1%)
White skin flakes	Present	16 (59.3%)	21 (75.0%)
	Absent	11 (40.7%)	7 (25.0%)
Capillary thrombosis	Present	13 (48.1%)	13 (46.4%)
	Absent	14 (51.9%)	15 (53.6%)
Border erythema	Present	10 (37.0%)	8 (28.6%)
	Absent	17 (63.0%)	20 (71.4%)
Callus	Present	12 (44.4%)	13 (46.4%)
	Absent	15 (55.6%)	15 (53.6%)

CWARTS = cutaneous WARTS, TW = target wart

Supplementary Table 2 Treatment Emergent Adverse Events – Analysis of the Intention to Treat (ITT) population.

	Safety run-in		At-home trial	
	CY-002 (N=4)	Placebo (n=4)	CY-002 (N=27)	Placebo (n=28)
Total reported adverse events (% subjects)	12 (100%)	8 (75%)	11 (33.3%)	12 (39.3)
Gastrointestinal symptoms	3 (50%)	1 (25%)	1 (3.7%)	0
Application site pain	0	0	1 (3.7%)	0
Skin abrasion*	1 (25%)	1 (25%)	1 (3.7%)	0
Dermatitis**	1 (25%)	0	0	0
Pruritus***	2 (50%)	1 (25%)	0	0
Cystitis	0	0	0	1 (3.6%)
Skin infection****	0	0	0	2 (7.1%)
Upper respiratory tract infection	1 (25%)	1 (25%)	4 (14.8%)	5 (17.8%)
Respiratory tract infection	0	0	0	1 (3.6%)
Influenza like illness	0	0	1 (3.7%)	2 (7.1%)
Headache	3 (75%)	3 (75%)	1 (3.7%)	0
Increased transaminases	1 (25%)	0	0	0
Musculoskeletal pain	0	1 (25%)	1 (3.7%)	1 (3.6%)
Wrist fracture	0	0	1 (3.7%)	0

** Caused by occlusive tape. One patient in Part 2 discontinued treatment on wart #3 / ** Caused by ECG lead adhesive / *** Reported on target area on the back where study drug was applied / **** One subject reported an infection on his foot, 1 patient reported impetigo on the axilla.*

Supplementary Figure 1 Study design of run-in trial.

Day	0	1	2	3	4	5	6	7	14	28
In clinic	X	X	X	X	X	X	X	X		
Outpatient visit									X	X
Treatment application lesions	X	X	X	X	X	X	X	X		
Treatment application healthy skin	X	X	X	X	X	X	X	X		
Safety assessments	X	X	X	X	X	X	X	X	X	X
Efficacy assessments	X	X		X		X		X	X	X
Biopsy										X

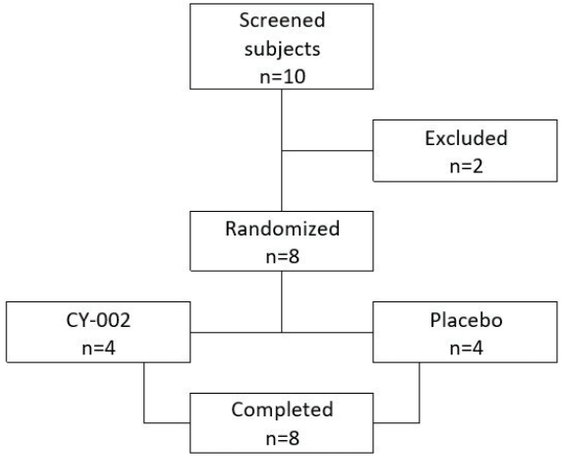
EOT = end of treatment, EOS = end of study

Supplementary Figure 2 Study design of ambulatory trial.

Day	0	7	14	21	28	70	112
Week	0	1	2	3	4	10	16
Outpatient visit	X	X	X	X	X	X	X
Safety assessments	X	X	X	X	X	X	X
Efficacy measurements	X	X	X	X	X	X	X
Biopsy							X

EOT = end of treatment, EOS = end of study

Supplementary Figure 3 Flow-Chart of the run-in trial.



REFERENCES

1 Williams HC, Pottier A, Strachan D. The descriptive epidemiology of warts in British schoolchildren. Br J Dermatol. 1993;128(5):504-511. doi:10.1111/j.1365-2133.1993.tb00226.x

2 van Haalen FM, Bruggink SC, Gussekloo J, Assendelft WJJ, Eekhof JAH. Warts in primary schoolchildren: prevalence and relation with environmental factors. Br J Dermatol. 2009;161(1):148-152. doi:10.1111/j.1365-2133.2009.09160.x

3 Kilkenny M, Merlin K, Young R, Marks R. The prevalence of common skin conditions in Australian school students: 1. Common, plane and plantar viral warts. Br J Dermatol. 1998;138(5):840-845. doi:10.1046/j.1365-2133.1998.02222.x

4 Johnson MT, Roberts J. Skin conditions and related need for medical care among persons 1-74 years. United States, 1971-1974. Vital Health Stat 11. 1978;(212):i-v, 1-72.

5 Beliaeva TL. [The population incidence of warts]. Vestn Dermatol Venerol. 1990;(2):55-58.

6 Bzhalava D, Mühr LSA, Lagheden C, et al. Deep sequencing extends the diversity of human papillomaviruses in human skin. Sci Rep. 2014;4:5807. doi:10.1038/srep05807

7 Bruggink SC, de Koning MNC, Gussekloo J, et al. Cutaneous wart-associated HPV types: prevalence and relation with patient characteristics. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2012;55(3):250-255. doi:10.1016/j.jcv.2012.07.014

8 Hagiwara K, Uezato H, Arakaki H, et al. A genotype distribution of human papillomaviruses detected by polymerase chain reaction and direct sequencing analysis in a large sample of common warts in Japan. J Med Virol. 2005;77(1):107-112. doi:10.1002/jmv.20421

9 Ciconte A, Campbell J, Tabrizi S, Garland S, Marks R. Warts are not merely blemishes on the skin: A study on the morbidity associated with having viral cutaneous warts. Australas J Dermatol. 2003;44(3):169-173. doi:10.1046/j.1440-0960.2003.00672.x

10 Zachariae C, Sand C, Hansen J, et al. Warts in a Cohort of Danish Kidney Transplanted Patients: Impact on Quality of Life. Acta Derm Venereol. 2012;92(6):615-618. doi:10.2340/00015555-1377

11 Akdoğan N, Yıldırım SK, Kültür E, Evans SE. The effect of warts on quality of life in turkish pediatric patients. Turk J Pediatr. 2020;62(6):1028. doi:10.24953/turkjped.2020.06.015

12 Bruggink SC, Eekhof JAH, Egberts PF, van Blijswijk SCE, Assendelft WJJ, Gussekloo J. Warts transmitted in families and schools: a prospective cohort. Pediatrics. 2013;131(5):928-934. doi:10.1542/peds.2012-2946

13 Massing AM, Epstein WL. Natural history of warts. A two-year study. Arch Dermatol. 1963;87:306-310. doi:10.1001/archderm.1963.01590150022004

14 Bruggink SC, Waagmeester SC, Gussekloo J, Assendelft WJJ, Eekhof JAH. Current choices in the treatment of cutaneous warts: a survey among Dutch GP. Fam Pract. 2010;27(5):549-553. doi:10.1093/fampra/cmq047

15 Pezeshkpoor F, Banihashemi M, Yazdanpanah MJ, Yousefzadeh H, Sharghi M, Hoseinzadeh H. Comparative study of topical 80% trichloroacetic acid with 35% trichloroacetic acid in the treatment of the common wart. J Drugs Dermatol. 2012;11(11):e66-9.

16 Kwok CS, Gibbs S, Bennett C, Holland R, Abbott R. Topical treatments for cutaneous warts. Cochrane database Syst Rev. 2012;(9):CD001781. doi:10.1002/14651858.CD001781.pub3

17 Bourke JF, Berth-Jones J, Hutchinson PE. Cryotherapy of common viral warts at intervals of 1, 2 and 3 weeks. Br J Dermatol. 1995;132(3):433-436. doi:10.1111/j.1365-2133.1995.tb08678.x

18 Finley C, Korownyk C, Kolber MR. What works best for nongenital warts? Can Fam Physician. 2016;62(12):997.

19 Sterling JC, Gibbs S, Haque Hussain SS, Mohd Mustapa MF, Handfield-Jones SE. British Association of Dermatologists' guidelines for the management of cutaneous warts 2014. Br J Dermatol. 2014;171(4):696-712. doi:10.1111/bjd.13310

20 Szczepanski C, Tenstad O, Baumann A, et al. Identification of a novel lytic peptide for the treatment of solid tumours. Genes Cancer. 2014;5(5-6):186-200. doi:10.18632/genesandcancer.18

21 Rijsbergen M, Niemeyer-van der Kolk T, Rijneveld R, et al. Mobile e-diary application facilitates the monitoring of patient-reported outcomes and a high treatment adherence for clinical trials in dermatology. J Eur Acad Dermatology Venereol. 2020;34(3):633-639. doi:10.1111/jdv.15872

22 De Geest S, Sabaté E. Adherence to long-term therapies: Evidence for action. Eur J Cardiovasc Nurs. 2003;2(4):323. doi:10.1016/S1474-5151(03)00091-4

23 de Koning MNC, Khoe L V, Eekhof JAH, et al. Lesional HPV types of cutaneous warts can be reliably identified by surface swabs. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2011;52(2):84-87. doi:10.1016/j.jcv.2011.06.016

24 de Koning MNC, ter Schegget J, Eekhof JAH, et al. Evaluation of a novel broad-spectrum PCR-multiplex genotyping assay for identification of cutaneous wart-associated human papillomavirus types. J Clin Microbiol. 2010;48(5):1706-1711. doi:10.1128/JCM.02122-09

25 Rijsbergen M, Niemeyer-van der Kolk T, Hogendoorn G, et al. A randomized controlled proof-of-concept trial of digoxin and furosemide in adults with cutaneous warts. Br J Dermatol. 2019;180(5):1058-1068. doi:10.1111/bjd.17583

26 Rijsbergen M, Pagan L, Niemeyer-van der Kolk T, et al. Stereophotogrammetric three-dimensional photography is an accurate and precise planimetric method for the clinical visualization and quantification of human papilloma virus-induced skin lesions. J Eur Acad Dermatology Venereol. Published online 2019. doi:10.1111/jdv.15474

27 Hogendoorn GK, Bruggink SC, Hermans KE, et al. Developing and validating the Cutaneous WARTS (CWARTS) diagnostic tool: a novel clinical assessment and classification system for cutaneous warts. Br J Dermatol. 2018;178(2):527-534. doi:10.1111/bjd.15999

28 Hogendoorn GK, Bruggink SC, de Koning MNC, et al. Morphological characteristics and human papillomavirus genotype predict the treatment response in cutaneous warts. Br J Dermatol. 2018;178(1):253-260. doi:10.1111/bjd.15758

29 Smith S, Trying S, Grande K, Schlessinger J, Gold M, Shanier S. Hydrogen Peroxide Topical Solution, 45% for Common Warts: Phase 2 Efficacy and Safety Trial Results. J Drugs Dermatology. 2020;19(10):969-976. doi:10.36849/JDD.2020.5054

30 Mahran AM, Twisy HO, Elghazally SA, Badran AY. Evaluation of different concentrations of hydrogen peroxide solution (3% and 6%) as a potential new therapeutic option of nongenital warts: A randomized controlled triple-blinded clinical trial. J Cosmet Dermatol. 2020;19(2):416-422. doi:10.1111/jocd.13021

31 Leerunyakul K, Thammarucha S, Suchonwanit P, Rutnin S. A comprehensive review of treatment options for recalcitrant nongenital cutaneous warts. https://doi.org/101080/09546634202017376 35. Published online 2020. doi:10.1080/09546634.2020.1737635

32 Rissmann R, Moerland M, van Doorn MBA. Blueprint for mechanistic, data-rich early phase clinical pharmacology studies in dermatology. Br J Clin Pharmacol. Published online 2020. doi:10.1111/bcp.14293

33 Thappa DM, Chiramel MJ. Evolving role of immunotherapy in the treatment of refractory warts. Indian Dermatol Online J. 2016;7(5):364. doi:10.4103/2229-5178.190487

34 García-Oreja S, Álvaro-Afonso FJ, García-Álvarez Y, García-Morales E, Sanz-Corbalán I, Lázaro Martínez JL. Topical treatment for plantar warts: A systematic review. Dermatol Ther. 2021;34(1). doi:10.1111/dth.14621

35 Ahn CS, Huang WW. Imiquimod in the Treatment of Cutaneous Warts: An Evidence-Based Review. Am J Clin Dermatol. 2014;15(5):387-399. doi:10.1007/s40257-014-0093-5

36 Soni P, Khandelwal K, Aara N, Ghiya BC, Mehta RD, Bumb RA. Efficacy of Intralesional Bleomycin in Palmoplantar and Periungual Warts. J Cutan Aesthet Surg. 2011;4(3):188. doi:10.4103/0974-2077.91250

37 Al-Naggar MR, Al-Adl AS, Rabie AR, Abdelkhalk MR, Elsaie ML. Intralesional bleomycin injection vs microneedling-assisted topical bleomycin spraying in treatment of plantar warts. Published online 2018. doi:10.1111/jocd.12537

38 Kaul S, Kaur I, Jakhar D, Edigin E, Caldito EG. The diverse methods of bleomycin delivery in cutaneous warts: A literature review. Dermatol Ther. 2021;34(1). doi:10.1111/dth.14401

39 Hodeib AAE, Al-Sharkawy BG, Hegab DS, Talaat RAZ. A comparative study of intralesional injection of Candida albicans antigen, bleomycin and 5-fluorouracil for treatment of plane warts. J Dermatolog Treat. Published online November 12, 2019:1-6. doi:10.1080/09546634.2019.1688236

40 Ebrahim HM, Asaad AM, El desoky F, Morsi HM. Bacillus Calmette-Guerin polysaccharide nucleic acid vs Bacillus Calmette-Guerin vaccine in the treatment of warts: A comparative, double-blind, controlled study. Dermatol Ther. 2021;34(1). doi:10.1111/dth.14549

41 Awal G, Kaur S. Therapeutic Outcome of Intralesional Immunotherapy in Cutaneous Warts Using the Mumps, Measles, and Rubella Vaccine: A Randomized, Placebo-controlled Trial. J Clin Aesthet Dermatol. 2018;11(5):15. Accessed August 3, 2021. /pmc/articles/PMC5955628/

42 Ibrahim AM, Elkot RA, Khashaba SA. Successful Treatment of Multiple Common Warts With Intralesional Ozone. Dermatologic Surg. 2020;46(7):928-933. doi:10.1097/DSS.0000000000002174

43 El Sayed MH, Sayed FS, Afify AA. Intralesional zinc sulfate 2% vs intralesional vitamin D in plantar warts: A clinicodermoscopic study. Dermatol Ther. 2020;33(3). doi:10.1111/dth.13308

44 Nofal A, Yehia E, Khater E, Bessar H. Alternating intralesional purified protein derivative and Candida antigen versus either agent alone in the treatment of multiple common warts. J Am Acad Dermatol. 2020;83(1):208-210. doi:10.1016/j.jaad.2020.01.054

45 Kareem IMA, Ibrahim IM, Mohammed SFF, Ahmed AA. Effectiveness of intralesional vitamin D 3 injection in the treatment of common warts: Single-blinded placebo-controlled study. Dermatol Ther. 2019;32(3). doi:10.1111/dth.12882

46 Abdo HM, Elrewiny EM, Elkholy MS, Ibrahim SM. Efficacy of intralesional methotrexate in the treatment of plantar warts. Dermatol Ther. 2020;33(2). doi:10.1111/dth.13228

47 van der Kolk T, Dillingh MR, Rijneveld R, et al. Topical ionic contra-viral therapy comprised of digoxin and furosemide as a potential novel treatment approach for common warts. J Eur Acad Dermatology Venereol. 2017;31(12):2088-2090. doi:10.1111/jdv.14527

48 Oren-Shabtai M, Snast I, Noyman Y, et al. Topical and systemic retinoids for the treatment of cutaneous viral warts: A systematic review and meta-analysis. Dermatol Ther. 2021;34(1). doi:10.1111/DTH.14637

49 Adalatkhah H, Khalilollahi H, Amini N, Sadeghi-Bazargani H. Compared therapeutic efficacy between intralesional bleomycin and cryotherapy for common warts: A randomized clinical trial. Dermatol Online J. 2007;13(3). doi:10.5070/D362G0G3MJ

50 Bohlooli S, Mohebipoor A, Mohammadi S, Kouhnavard M, Pashapoor S. Comparative study of fig tree efficacy in the treatment of common warts (Verruca vulgaris) vs. cryotherapy. Int J Dermatol. 2007;46(5):524-526. doi:10.1111/j.1365-4632.2007.03159.x

51 Shaheen MA, Salem SAM, Fouad DA, El-Fatah AAA. Intralesional tuberculin (PPD) versus measles, mumps, rubella (MMR) vaccine in treatment of multiple warts: A comparative clinical and immunological study. Dermatol Ther. 2015;28(4):194-200. doi:10.1111/dth.12230

52 Nofal A, Nofal E, Yosef A, Nofal H. Treatment of recalcitrant warts with intralesional measles, mumps, and rubella vaccine: A promising approach. Int J Dermatol. 2015;54(6):667-671. doi:10.1111/ijd.12480

53 Hadgraft J, Lane ME. Skin permeation: The years of enlightenment. Int J Pharm. 2005;305(1-2):2-12. doi:10.1016/J.IJPHARM.2005.07.014

54 Trommer H, Neubert RHH. Overcoming the Stratum Corneum: The Modulation of Skin Penetration. Skin Pharmacol Physiol. 2006;19(2):106-121. doi:10.1159/000091978

55 Huang K, Xie Y, Li M, et al. A Comparative Study: Superficial Shaving Combined With Photodynamic Therapy Versus Cryotherapy in the Treatment of Recalcitrant Plantar Warts. Lasers Surg Med. 2020;52(8):747-752. doi:10.1002/lsm.23212

56 Sumi E, Nomura T, Asada R, et al. Safety and Plasma Concentrations of a Cyclin-dependent Kinase 9 (CDK9) Inhibitor, FIT039, Administered by a Single Adhesive Skin Patch Applied on Normal Skin and Cutaneous Warts. Clin Drug Investig. 2019;39(1):55. doi:10.1007/S40261-018-0712-7

57 Hashmi F, Fairhurst C, Cockayne S, et al. The EVerT2 (Effective Verruca Treatments 2) trial: a randomized controlled trial of needling vs. nonsurgical debridement for the treatment of plantar verrucae. Br J Dermatol. 2017;177(5):1285-1292. doi:10.1111/bjd.15751