4. Synopsis

Name of Sponsor/Company:
Cutanea Life Sciences

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Wayne, PA 19087, The United States

Protocol number: CHDR1607 **Sponsor protocol number:** CLS003-CO-PR-003

Name of Finished Product:	**Name of Active Ingredients:** Digoxin (0.125% w/w) and furosemide (0.125%).

Title of Study: A phase 2, randomized, vehicle-controlled, double-blind study to explore the efficacy, pharmacodynamics and safety of topical ionic contra-viral therapy (ICVT) comprised of digoxin and furosemide in HPV-induced genital lesions of immunocompromised and immunocompetent patients

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Studied Period:	**Phase of Development:**
Oct 2017 – Oct 2018	2

Background and Rationale
Human papillomavirus (HPV) infection is the most common sexually transmitted disease worldwide and can result in benign, premalignant and malignant lesions of the genital skin and genital mucosal surfaces. Among immunocompromised patients, the prevalence of low as well as high risk HPV-infections is higher than in the immunocompetent population, and therefore HPV-induced genital lesions are more common in this group of patients[1;2].
Current surgical therapies for HPV-induced genital lesions are not directed at clearance of the HPV infection, but at physical removal of the lesions and stimulating the host immune system. Recurrence rate is high and often the currently available treatments cause a high physical and psychosocial burden [2;3].
Cutanea Life Sciences (CLS) is investigating various formulations with digoxin and furosemide as a potential treatment for HPV infections of skin and other similar tissue. In a published *in vitro* study in 2006 [4], the cardiac glycoside digoxin and loop diuretic furosemide inhibited replication in DNA viruses, herpes simplex virus, varicella zoster virus, human cytomegalovirus and adenovirus. The effects were most potent when digoxin and furosemide were used in combination as the topical formulation CLS003. This new approach, described as Ionic Contra-Viral Therapy (ICVT), is suggested to be most effective via topical application.
One potential viral target of ICVT is human papillomavirus (HPV) in associated cutaneous and mucosal lesions. Specifically, the ionic properties of digoxin and furosemide were noted to inhibit the K+ influx on which DNA viruses rely for replication. These drugs interact with the cell membrane ion co-transporters Na^+/K^+-ATPase and Na^+-K^+-$2Cl^-$ co-transporter. A previous study was conducted with a group of 80 patients with cutaneous warts, which demonstrated ICVT to be effective, safe and well tolerated.
This study is intended to explore clinical efficacy and safety/tolerability of ICVT as a potential treatment for benign and premalignant HPV-induced genital lesions in immunocompetent and immunocompromised patients. This includes 3 different patient populations: i) immunocompetent patients with anogenital warts (AGWs), ii) immunocompromised patients with anogenital warts and iii) immunocompromised patients with

vulvar high grade squamous intraepithelial neoplasia (HSIL), formerly referred to as usual type vulvar intraepithelial neoplasia (uVIN). Since digoxin / furosemide ICVT' mode of action is in part independent of the immune system and directly targeted to eradicate the causative HPV, we hypothesize this therapy to be of value in this specific group of individuals.

Objectives:

Primary objectives

- To explore the pharmacodynamics of the ionic contra-viral therapy CLS003 in immunocompromised and immunocompetent patients with benign and premalignant HPV-induced genital lesions.
- To evaluate clinical efficacy of CLS003 compared to vehicle in immunocompromised and immunocompetent patients with benign and premalignant HPV-induced genital lesions

Secondary objectives

- To evaluate the safety and tolerability of CLS003 in immunocompromised and immunocompetent patients with benign and premalignant HPV-induced genital lesions

Methodology:

The study consisted of 2 parts. Part 1 is a randomized, double-blind, vehicle-controlled design to assess the pharmacodynamics, safety, tolerability and efficacy of topical CLS003 in patients with benign and premalignant HPV-induced genital lesions. The total study period for part 1 will be approximately 23 weeks: 5 weeks for screening, 6 weeks of double-blind treatment and 12 weeks of follow-up. During the part 1 treatment period subjects will visit CHDR every three weeks. During the part 1 follow-up period subjects will visit CHDR at 6 week intervals. Patients who completed part 1 without safety or tolerability symptoms/patterns could be enrolled in part 2 of the trial, i.e. an open label, extended use of topical CLS003. In part 2 consist of 8 weeks treatment and 12 weeks of follow-up. During the treatment period of part 2 subjects will visit CHDR every four weeks. During the follow-up period of part 2, subjects will visit CHDR at week 12.

Pharmacodynamic / efficacy

Pharmacodynamic and efficacy effects of CLS003 were assessed on Day 0, 21, 42 (EOT), 84 and Day 126 (EOS) by clinical assessment of lesion on-site with lesion size, standardized clinical photography and 3D photography, swab and biopsies of the lesions.

Tolerability / safety

Adverse events (AE) were collected throughout the study, at every study visit. Vital signs were collected at baseline and at every study visit. Laboratory safety tests and 12-lead ECGs were performed at screening and day 126 (EOS). Plasma digoxin levels were determined by therapeutic drug monitoring (TDM) at the end week 3 (day 21) and 6 (day 42).

Number of patients:

Planned

Group 1: 24 immunocompetent AGWs patients
Group 2: 16 immunocompromised AGW patients
Group 3: 8 immunocompromised vulvar HSIL patients

Included Part 1

Group 1: 24 immunocompetent AGWs patients
Group 2: 1 immunocompromised AGW patients
Group 3: 3 immunocompromised vulvar HSIL patients

Included Part 2

Group 1: 12 immunocompetent AGWs patients
Group 2: 0 immunocompromised AGW patients
Group 3: 3 immunocompromised vulvar HSIL patients

Inclusion criteria

For enrollment of subjects the following criteria must be met:

1. Vulvar HSIL or AGW patients, ≥ 18 years of age, in general, stable good health (with the exception of the immunocompromised disorder) as per judgment of the investigator based upon the results of a medical history, physical examination, ECG, chemistry, hematology.

2. In case of the immunocompromised patient group(s): having an immunosuppressive disease or receiving immunosuppressive therapy for any reason including but not limited to; patients with auto-immune disease, HIV patients, transplantation patients
3. In case of the genital warts patient group(s): have at least 3 genital warts (only applicable for study part 1).
4. In case of vulvar HSIL patient group: at least one lesion that can be accurately measured (using RECIST criteria) in at least one dimension with longest diameter ≥20 mm OR in 2 perpendicular dimensions that when multiplied together give a surface area ≥120 mm² (only applicable for study part 1).
5. If female of childbearing potential, have a negative urine pregnancy test at Screening and Day 0, and is willing to use effective contraception during the study and 3 months afterwards (i.e. oral, implanted, injectable, IUD, diaphragm, condom, tubal ligation, abstinence, or are in a monogamous relationship with a partner who has had a vasectomy)
6. Able to participate and willing to give written informed consent and to comply with the study restrictions
7. Ability to communicate well with the investigator in the Dutch language
8. Willing to refrain from using other topical products in the treatment area, or prohibited medications for the duration of the study

Exclusion criteria
Eligible subjects must meet *none* of the following exclusion criteria:

1. Significant, uncontrolled or unstable disease in any organ system as per judgment of the investigator (regardless of association with the immunosuppressing disorder/therapy), including but not limited to: psychiatric, neurologic, cardiovascular, pulmonary, gastrointestinal, hepatic, renal, endocrine, hematologic or respiratory disease
2. Have used or received any topical genital wart treatment, cryotherapy, electrocoagulation, surgery in the treatment area within 28 days prior to enrolment
3. Have used or received any topical vulvar HSIL treatment, laser therapy or surgery in the treatment area within 28 days prior to enrolment
4. Have any current relevant skin infections in the treatment area other than genital warts or vulvar HSIL (inclusively, but not limited to atopic dermatitis, lichen sclerosis, lichen planus or psoriasis)
5. Have a known sensitivity to any of the investigational product ingredients, including digoxin and furosemide
6. Participation in an investigational drug or device study within 3 months prior to screening or more than 4 times in the past year
7. Loss or donation of blood over 500 mL within three months prior to screening.

Test Product, Dose and Mode of Administration:
Investigational drug: CLS003; topical formulation (gel) containing digoxin (0.125% w/w) and furosemide (0.125%); Lot number B170013

Duration of Treatment: 42 days, QD

Reference Therapy, Dose and Mode of Administration:
Comparative treatment Formulation

- Vehicle formulation (placebo); Lot number B170012 with identical appearance

Statistical Methods:
Analysis Populations
-The safety population will be defined as all subjects who were validated (randomized) and received at least one dose of study treatment.
-The analysis population for pharmacodynamics/efficacy is defined as all subjects who were validated (randomized), received at least one dose of study treatment, and have at least one post-baseline assessment of the parameter being analyzed.

Efficacy/ Pharmacodynamic Analyses
Following metrics were determined:

For both cohorts:

- Lesion (vulvar HSIL or wart) size reduction as absolute and percent reduction in lesion diameter as measured by caliper and 3D photography
- Change in patient-reported outcomes (QoL and patient-reported clearance)
- HPV viral load assessment (quantitative PCR including HPV genotyping in swabs and biopsies)
- Change in the HPV viral load (nominal, natural log transformed, and natural log of viral load per DNA copies) as determined by qPCR in swabs and biopsies
- Mean HPV viral load (nominal, natural log transformed, and natural log of viral load per DNA copies) in swabs and biopsies
- Histology (regression of vulvar HSIL or AGWs, HPV genotyping)
- Local immunity status (Histological changes in immune cells in the mucosa/submucosa)

For vulvar HSIL cohort

- Vulvar HSIL, size and reduction in lesion size (clinical assessment of lesions by RECIST, absolute reduction in lesion size, lesion size reduction (percentage) as measured by calliper and 3D photography
- Percentage clearance of vulvar HSIL lesions
- Proportion of patients with all vulvar HSIL lesions cleared
- Histology (regression of high grade dysplasia to no dysplasia)
- Histological recurrence (progression of no dysplasia to high grade dysplasia) in the Part 1 follow-up period

For genital wart cohort:

- Wart size and reduction in wart size of the target wart (absolute reduction in lesion size, lesion size reduction (percentage)) as measured by caliper and 3D photography
- Percentage clearance of genital warts
- Proportion of patients with all genital warts cleared
- Clinical recurrence in the Part 1 follow-up period

Treatment effects were analyzed by repeated measures analysis of variance (mixed model) using treatment, time and treatment by time as fixed factors and subject as random factor. To determine the differences among the treatments, contrasts on the EOT visit (Day 42), the EOS visit (Day 126) and on all post-treatment assessments were calculated.

Safety Analyses
Data listings and descriptive statistics were performed of adverse events, vital signs, physical examinations and all laboratory tests.

Results and Discussion

Group 1, part 1
Participation and demographics
32 subjects were screened of which 24 subjects were enrolled into the trial. Sixteen (16) subjects were randomly assigned to CLS003 treatment and 8 subjects were assigned to placebo. Demographics and baseline characteristics were comparable across the 2 treatment groups. All subjects completed the study.

The analysis populations consisted of 24 subjects.

Efficacy/pharmacodynamics:
Warts were counted during every visit. In the CLS003 group, there was a change in mean lesion count observed from 15.0 (SD 12.7) pre-dose to 15.0 (SD 12.2) at EOT further to 9.9 (SD 8.1) at EOS. In the placebo group, there was a change in mean lesion count observed from 12.8 (SD 5.9) pre-dose to 13.3 (SD 7.4) at EOT further to 6.6 (SD 4.5) at EOS. No statistically significant difference in wart count was observed between the CLS003 and placebo (p=0.89).

Wart clearance was derived from the count of all warts present at baseline and showed no statistically significant differences between the two groups (P=0.53). At EOT, in the CLS003 group there was a decrease

in the mean wart count of 24.1% compared to 0.6% in the vehicle group (p=0.58). At EOS, in the CLS003 group there was an increase in the mean wart count of 8.8% compared to 36.1% in the vehicle group (p =0.49).

Wart size was measured during every visit. In the CLS003 group, there was a change in mean of longest diameter of target warts observed from 5.1 mm (SD 2.7) pre-dose to 5.5 mm (SD 3.0) at EOT further to 4.1 mm (SD 3.5) at EOS. There was no statistical significant difference of the longest diameter (p=0.49), shortest (p=0.84) diameter and height (p=0.43) of the target wart between the CLS003 and vehicle group. No statistical significant difference was observed in longest (p=0.92) and shortest diameter (p=0.92) and the height (p=0.48) of the 3D measurements of the target warts between both treatment groups.

At baseline, the HPV subtype was assessed in the biopsies were we found that 22 out of 24 patients (92%) were positive for HPV6. No difference was found in the HPV6 viral load between the two groups in the swabs (p=0.68) and in the HPV6 expression in the biopsies (p=0.27).

The pruritus and pain score were collected daily during the treatment period (day 0 - day 42) by using an e-diary app and during the follow-up visits by a questionnaire. We found a statistical significant difference in pruritus score between the CLS003 and the placebo group (p=0.03), where the placebo group had a reduction of the pruritus. In the CLS003 group we observed a change in mean of the pruritus score from 9.4 (SD 19.0) pre-dose to 3.4 (SD 7.0) at EOT to 8.6 (SD 22.7) at EOS, and in the placebo group we observed a change in mean of the pruritus score from 7.5 (SD 9.9) pre-dose to 2.0 (SD 4.5) at EOT to 3.0 (SD 6.7) at EOS. There was no statistical significant difference in pain score between the CLS003 and the placebo group (p=0.31).

Different questions regarding the Quality of Life (QoL) and sexual function of the patients were assessed during each study visit. Of all these questions, a total score of quality of life was measured. There was no statistical significant difference (p=0.62) in the total score of QoL between the CLS003 and the placebo group.

Safety and Tolerability
CLS003 was well tolerated by the subjects and did not result in any clinically significant changes in any safety laboratory parameters, vital sign measures and ECG recordings. No treatment related study discontinuation or SAE occurred. The AE profile was comparable for all subjects across treatment groups. The most frequent occurring treatment-emergent AEs was application site discomfort (n=6; 33%) and resolved without intervention. Application site discomfort was mild in all cases and considered probably related to the study drug. Investigation of blood samples did not show any presence of digoxin, indicating that no systemic effects were detectable upon this low dose.

Adherence / exposure
In total 16 subjects received approximately 200 mg CLS003 once daily on all external anogenital warts for 42 consecutive days. With an electronic diary we measured a mean treatment adherence of 96%.

Group 1, part 2
Twelve (12) of 24 subjects chose to enroll in Part 2 of the trial. There were no differences in wart count, wart size measurements, HPV load in swabs and patient reported outcomes between start and end of the study observed. Treatment with CLS003 was well tolerated by the patients and did not result in any clinically significant changes in safety laboratory parameters, vital signs and ECG recordings. No SAE occurred and there were no discontinuations due to AEs.

Group 2&3, part 1&2
Because the lack of efficacy in group 1 it was decided in consultation with the Independent Ethics Committee to discontinue the inclusion for part 2 and 3.
One (1) subject was included in group 2 and 3 subjects in group 3. There was no difference in wart count, wart size measurements and patient reported outcomes between start and end of the study observed. Treatment with CLS003 was well tolerated by the patient and did not result in any clinically significant changes in safety laboratory parameters, vital signs and ECG recordings. No SAE occurred and there were no discontinuations due to AEs.

Summary - conclusions

Pharmacodynamic/efficacy *results:*
In the clinical evaluation of the treatment of cohort 1 part 1, no differences were found lesion count and lesion size (long and short diameter, height) between CLS003 and placebo. The viral load of HPV in swabs and biopsies showed no difference between the CLS003 and the placebo group. In the patient reported outcomes a reduction of itch was found in the placebo group compared to CLS003 (P=0.03).

Safety results:
This study found that once daily administration of CLS003 containing digoxin+furosemide for the treatment of genital warts and vulvar HSIL for 42 consecutive days was well tolerated by the subjects and did not result in any clinically significant changes in any safety laboratory parameters, vital signs measures and ECG recordings. No serious adverse events (SAEs), discontinuations due to adverse events or deaths occurred during the study.

Overall conclusion:
The results from the current study show that CLS003 is safe to be administered to patients with genital warts and vulvar HSIL. In all pharmacodynamic and efficacy endpoints no differences were found between the CLS003 and the vehicle group.
Because the lack of efficacy in group 1 it was decided in consultation with the BEBO to stop the inclusion for this trial.

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