2. Summary

Title
A phase 2, randomized, double blind, vehicle controlled, parallel group study to explore the efficacy, pharmacodynamics and safety of topical ionic contra-viral therapy (ICVT), comprised of digoxin and furosemide in actinic keratosis (AK)

Short Title
ICVT in AK.

Principal investigator & Trial Site
R. Rissmann, PhD, Centre for Human Drug Research, Zernikedreef 8, 2333 CL Leiden, The Netherlands. Medical responsibility J. Burggraaf, MD PhD, Centre for Human Drug Research, Zernikedreef 8, 2333 CL Leiden, The Netherlands.

Background & Rationale
Actinic keratoses (AK) are common skin lesions which appear clinically as erythematous, scaly plaques on sun-exposed skin. As UV radiation has been recognized the main risk factor, they are typically located on the face, scalp, neck and extremities. The prevalence of AK lesions in adults increase with age: less than 10 percent for 20- to 29-year-olds, approximately 80 percent for 60- to 69-year-olds, and more than 80 percent for 70-year-olds and older. On histology examination AK is a proliferation of neoplastic keratinocytes in the epidermis, characterized by architectural disorder, with features of abnormal shape and size of keratinocytes, nuclear atypia and hyperkeratosis. AK may enter spontaneous remission or remain stable. However, importantly, AKs are also known as precursor lesions of squamous cell carcinoma (SCC). Furthermore, the annual burden for the treatment of AK has been estimated at around 900 million dollars in the USA alone. (Dodds A et al, 2014, Berlin JM, 2010).

Investigations have suggested a role for human papilloma virus (HPV) and the development of AK into SCC. In 2005, Weissenborn and colleagues determined viral DNA loads of six frequent HPV types (5, 8, 15, 20, 24 and 36) by qPCR in AK, NMSC and perilesional tissue. HPV viral load was highest in AK compared with non-melanoma skin cancer (NMSC) and perilesional tissue. It was suggested that active HPV replication and presumably enhanced gene expression may stimulate keratinocyte proliferation and contribute to carcinogenesis in early stages of NMSC development. (Weissenborn et. al. 2005).

In 2006 the association between HPV infection and SCC development was further explored. Presence of HPVL1 and E6 seroreactivity and viral DNA were determined for HPV types 5, 8, 15, 16, 20, 24, and 38 in three study groups: SCC patients, AK patients and controls without any history of skin tumors. After recruitment, the response rate was between 75% and 85% in all groups. Eyebrow hair was collected from 57 controls, 126 AK, and 63 SCC cases, and blood from 53 controls, 118 AK, and 55 SCC cases. HPV DNA positivity was most prevalent in the AK cases (54%) compared with the SCC (44%) or tumor-free controls (40%). (Struijk et. al 2006). An additional study from 2009 published similar results for a long-term persistence of betapapillomavirus (betaHPV). Eyebrow hairs were collected from 171 participants and tested for 25 different betaHPV types in 1996 and 2003. Of the total betaHPV infections detected, 30% were found to persist. After accounting for AK at baseline, persistence of betaHPV DNA resulted in a 1.4 fold increase in risk of having AK on the face in 2007 (Plasmeijer E et. al. 2009).

Maruho (sponsor) is investigating ICVT (CLS003), comprised of digoxin (0.125%) and furosemide (0.125%) as a potential treatment for HPV-mediated and associated diseases including cutaneous warts, anogentical warts (AGWs), and high-grade squamous intraepithelial neoplasion (HSIL), the later formerly referred to as usual type vulvar intraepithelial neoplasia (uVIN). This study was the

first study of ICVT in AK. The ionic properties of digoxin and furosemide interact with the cell membrane ion cotransporters Na+/K+- ATPase and Na+-K+-2Cl- co-transporter-1 and thereby inhibit the K+ influx on which DNA viruses rely for replication. In an in vitro study, published in 2006, digoxin and furosemide inhibited replication in DNA viruses, herpes simplex virus, varicella zoster virus, human cytomegalovirus and adenovirus. Both drugs prompted antiviral effects by extracellular K+; these effects were most potent when digoxin and furosemide were used in combination (Hartley C, 2006). In two exploratory clinical studies of CLS003 in patients with cutaneous warts tolerability and short-term safety was established as well as efficacy in terms of viral load reduction and dimensional changes of the lesions.

Based on the scientific literature on the potential association of HPV with AK, the study results with ICVT supported the concept of an investigation of ICVT in the therapeutic management of AK. This study intended to assess the clinical efficacy and pharmacodynamics of ICVT as a potential new treatment for AK. Clinical efficacy by means of clinical outcomes (i.e. clearance of the lesions, AK-FAS) and sub-clinical parameters / biomarkers on the skin and systemic ones were assessed.

Objective(s)

Primary Objective

- To explore the pharmacodynamics of ICVT comprised of digoxin and furosemide (dual agent), digoxin (single agent), furosemide (single agent) in patients with AK.
- To evaluate clinical efficacy of ICVT comprised of digoxin and furosemide (dual agent), digoxin (single agent), furosemide (single agent), and vehicle gel.

Secondary Objectives

- To evaluate the safety and tolerability of ICVT comprised of digoxin and furosemide (dual agent), digoxin (single agent), furosemide (single agent).

Methodology

This study was a phase 2, double-blind, vehicle-controlled, parallel group, efficacy and pharmacodynamic study of ICVT in AK. Thirty-two (32) subjects with Actinic Keratosis participated in the study and were dosed. Per patient two AK fields were selected; one AK field was treated with the topical formulation and the other AK field was used as untreated comparator.

The total duration of the study for each subject was up to 126 days divided as follows:

- Screening: Up to 35 days before dosing;
- Treatment and study assessments: Days 0 to 42 (EOT)
- In clinic visits: Days 0, 21, 42
- Follow-up visit: Days 84 and 126 (EOS)

Main parameters

Tolerability/safety

Plasma digoxin levels were determined by therapeutic drug monitoring (TDM) on Day 21 and Day 42. Adverse events (AE) were collected throughout the study, at every study visit. Vital signs were collected at baseline and at every study visit. Physical examination, laboratory safety tests and 12-lead ECGs were performed at screening and Day 126 (EOS).

Pharmacodynamic/Efficacy

Pharmacodynamic and efficacy effects of CLS003 were assessed on Day 0, 21, 42 (EOT), 84, 126 (EOS) by clinical AK scores (CCC, AK-FAS and IGS) and morphology assessment by 2D and 3D photography, dermoscopy, skin surface biomarkers and local biopsy biomarkers.

Investigational drug
ICVT topical formulation containing digoxin (0.125%) and furosemide (0.125%), topical formulation containing digoxin only (0.125%), and topical formulation containing furosemide only (0.125%).

Comparative drug
Vehicle gel with identical appearance served as placebo.

Participation and demographics
67 subjects were screened of which 32 subjects were enrolled into the trial. Thirty-two (32) subjects were randomized 1:1:1:1 in blocks of 4. Demographics and baseline characteristics were comparable across the 4 treatment groups.

Statistical Methods
Treatment effects were analysed by the repeatedly measured PD parameters, each parameter was analysed with a mixed model analysis of covariance (ANCOVA) with treatment, time, and treatment by time as fixed factors, subject as random factor and the (average) baseline measurement as covariate.

Endpoints
- Difference in IGS from (baseline) placebo
- Difference in count of lesions per visible per field
- Total clearance present/absent at EOT and/or EOS
- Difference in field morphology scores based on dermoscopic photos of AK lesions as scored by 3 independent dermatologist of the LUMC from (baseline) placebo
- Change in Haemoglobin Average levels as assessed by quantification of 3D Antera photos
- Difference in IHC expression in biopsies (Ki-67, p53) at EOS
- Difference in genotype or viral load of expressed betaHPV types in biopsies/swabs of target lesions

The AE coding dictionary for this study was Medical Dictionary for Regulatory Activities (MedDRA). It was used to summarize AEs by primary system organ class (SOC) and preferred term (PT). All AEs were displayed in listings. The listing contained subject number, treatment, visit, dosing date, SOC, PT, start date and time of AE, duration of AE, severity, SAE, relationship and chronicity.

Results and Discussion
Safety and Tolerability
This clinical phase 2 study in patients with facial AK showed that topical treatment with ICVT (dual agent), digoxin (single agent) and furosemide (single agent) was safe and in general well tolerated. No serious adverse events (SAEs), discontinuations due to AEs or deaths occurred during the study. The AE profile was comparable for all treatments. All Treatment Emergent Adverse Events (TEAEs) were of mild (n=36) severity. Six of these TEAES were classified as ‘possibly or probably’ treatment related, contributing of administration site irritation (n=4) and by skin exfoliation (n=2). Within the ICVT treatment group, one probably related TEAE was observed (non-recurring administration site irritation). All plasma digoxin levels were below the lower limit of quantification for all subjects at all time points.

Efficacy/pharmacodynamics
Compared to the placebo group and the untreated fields, the treated fields with ICVT did not show a statistically significant difference in complete clinical clearance, lesion count per field or the Investigator Global Score. For the Investigators Global Score, a statistically significant difference was observed between the digoxin group and the placebo (p=0.0016), ICVT (p=0.0014), furosemide (p=0.0029) and the untreated group p=<0.001). This result is based on a decrease of the IGS score for the digoxin treated group over time (compared to baseline).

ICVT did show a statistically significant lower 'field morphology % pigmentation score' up to EOT compared to placebo (p=0.0077), digoxin (p=0.0082), furosemide (p=0.0002) and the untreated field (p=0.0007), based on a decrease of this score for the ICVT group over time (compared to baseline). In addition, ICVT showed a statistically significant lower 'total morphology score' at EOT compared to placebo (p=0.0014). However, the placebo group also showed to be significantly higher at EOS compared to digoxin (p=0.0209), furosemide (p=0.0090) and to the untreated field (p=0.0043). It was therefore concluded that the 'total morphology score' of the placebo group significantly increased during treatment period compared to active treatment groups.

The viral load of HPV in swabs and biopsies showed a high degree of variability and no clear trend over time or between different treatment groups. Overall, HPV93 was the most observed HPV subtype within AK (at baseline and EOS).

Adherence/exposure
24 subjects were randomized to one of the three active treatment groups, i.e. ICVT, digoxin and furosemide respectively and 8 subjects were randomized to vehicle, i.e. placebo. Administrations were performed on consecutive days and only sporadically subjects did not comply to the daily treatment regimen. All dose administrations at home were recorded via an electronic mobile application. The mean weight of applied study medication per day across the treatment groups was 178 mg/day for ICVT, 177 mg/day for digoxin, 204 mg/day for furosemide and 197 mg/day for placebo.

Summary - conclusions
Pharmacodynamic results
In all clinical evaluation scores of AK, no statistically significant differences were found between the ICVT and the placebo group. In fact, the digoxin treatment showed the strongest improvement of AK over time, based on the clinical score IGS. However, for the non-clinical pharmacodynamic endpoints, ICVT did show a statistically significant lower 'field morphology % pigmentation score' compared to placebo. This observation was not assisted by other trends or significant differences between ICVT and placebo in the non-clinical pharmacodynamic analysis.

Safety results
The results from the current study show that ICVT, furosemide or digoxin treatment show to be safe and in general well tolerated in patients with AK of the face. The overall incidence of TEAEs was similar among subjects of different treatment groups.

Overall conclusion
The results from the current clinical phase 2 study showed that ICVT (dual agent), digoxin (single agent) and furosemide (single agent) are safe to administer to patients with facial AK's. No consistent trends in pharmacodynamic and efficacy endpoints were observed between the ICVT and the placebo or untreated group. Overall, the results of this phase 2 study indicate that application of topical ionic contra-viral therapy applied with an average dose of 177mg/day on facial AKs (histological grade 2), does not seems to improve clinical scores or pharmacodynamic features of this disease as measured with the in this study applied techniques.