Contemporary Clinical Trials Communications 17 (2020) 100534

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: http://www.elsevier.com/locate/conctc

Research paper

Efficacy of AV2-Salicylic acid combination therapy for cutaneous warts: Study protocol for a single-center randomized controlled trial

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ARTICLE INFO

Keywords:
Cutaneous warts
Antiviral drug
Study protocol
Clinical research
AV2-Salicylic acid combination therapy
HPV genotyping
Randomized controlled trial
Omnivirol

ABSTRACT

Cutaneous warts comprise an extremely common condition caused by infection with the human papillomavirus (HPV). Although most verrucae will disappear spontaneously, many patients do seek treatment. Current wart treatments do not target the cause of the lesion directly, resulting in variable treatment efficacies and high wart recurrence rates. AV2 is a broad-spectrum antiviral drug, that is capable of deactivating HPV. It is however not able to destruct the already infected cells, which raises the need for an additional ablative treatment i.e. salicylic acid (SA). Implementation of AV2-Salicylic acid (AV2-SA) combination therapy would ensure permanent lesion clearance by on the one hand inactivation of HPV by AV2, and on the other hand elimination of the lesion by SA treatment.

The primary aim of this study is to assess the efficacy of AV2-SA treatment versus standard SA treatment, by comparing cure and recurrence rates of cutaneous warts between the two treatment groups (at 12 weeks and six months after randomization). The second aim is to assess the safety and tolerability of AV2-SA therapy. The third aim is to identify subgroups of cutaneous warts that have favorable response to treatment, by comparing cure rates in an HPV genotype-specific manner.

This randomized controlled trial will enroll 260 participants with cutaneous warts who will either receive the AV2-SA combination therapy or SA control treatment. Real time monitoring will be possible by daily photographs sent via WhatsApp™ (a messaging application) as well as online follow-up questionnaires administered on several occasions. HPV genotyping will be performed on swab self-samples.

1. Introduction

Cutaneous warts or verrucae are very common with a varying worldwide prevalence of 0.84–12.9% [1]. The prevalence rate in children and young adults is even higher and reported to reach 30% [2]. Verrucae are caused by infection with the human papillomavirus (HPV) and although most verrucae disappear spontaneously, many patients do seek treatment because their lesion is painful, persistent or interfering with normal function. An armamentarium of wart treatments is currently at disposal, starting from folk remedies to over-the-counter medications and more aggressive clinic-based treatments. Unfortunately, none of these treatments seem to produce consistent results and reported efficacies often vary widely depending on several factors (e.g. age, compliance, immunocompetence). A systematic review conducted by the Cochrane Skin Group assessed the effects of different wart treatments [3]. A modest efficacy of salicylic acid (SA) topical treatment was observed in pooled data of five placebo-controlled trials with average clearance rates of 73% (0–84%) vs. 48% (10–65%) respectively. As to cryotherapy there was inconclusive evidence concerning the efficacy when compared with placebo and other simpler and safer treatments. In total, 21 trials with placebo groups were evaluated and an average clearance rate of 27% (0–73%) in the placebo groups after an average period of 15 weeks (4–24 weeks) [3] was found. These data highlighted the lack of an optimal treatment for verrucae and resulted in some practitioners recommending that warts should not be treated at all [4]. Unfortunately, there is currently no reliable mean of predicting which warts will clear spontaneously and which will remain for years. Although in theory a policy of not treating warts is recommended, in practice many people do consult healthcare professionals and are

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https://doi.org/10.1016/j.conctc.2020.100534
Received 20 August 2019; Received in revised form 8 January 2020; Accepted 19 January 2020
Available online 21 January 2020

List of abbreviations

Abbreviation	*Explanation*
AML	Algemeen Medisch Laboratorium
AV2	Antiviral 2
AV2-SA	Antiviral 2 – Salicylic Acid
CRF	Case Report Form
GRAS	Generally Recognized As Safe
HPV	Human Papillomavirus
OV	Omnivirol
qPCR	Real-Time Polymerase Chain Reaction
REDCap	Research Electronic Data Capture
SA	Salicylic Acid
UA	University of Antwerp
v/v	Volume/Volume percent
w/v	Weight/volume percent

treated due to the social stigma and morbidity associated with visible warts [2,3]. Furthermore, treatment is also necessary in order to prevent spread of infection in the general population [5].

Contemporary wart treatments intend to simply destroy the infected cells (i.e. physically or chemically ablate warts), and do not have any specific antiviral mode of action. Hence the high wart recurrence rate of 12.5–70% amongst these treatments, due to residual HPV particles [2]. Currently, there are no specific molecular inhibitors that directly target HPV. By eliminating the source instead of only affecting the lesion, these drugs would make wart recurrence unlikely [6]. Unfortunately, most of these strategies are still in the very early stages of investigation, and since the appearance of vaccines, research into direct antiviral agents against HPV has been suspended [6].

Recently, Cesa Alliance has developed a broad-spectrum antiviral drug, called AV2 (Omnivirol™), that has proven to be highly effective in treatment of cervical lesions caused by HPV [7]. AV2 is a combination of FDA GRAS-label approved organic compounds (natural essential oils: carvone, eugenol, geraniol, and nerolidol) that is postulated to be able to prevent viral entry and proliferation by deactivating the infectious virions before they enter the cell [7].

Although AV2 is capable of deactivating the source of the lesion, it is however not able to destroy the already infected cells. In order to ensure that AV2 is able to reach the epidermal basal layer, which contains the infectious reservoir, and deactivate the viral particles thus preventing future re-infection and recurrence of the lesion, an additional ablative treatment is necessary. Salicylic acid (SA) formulations are the most commonly used preparations in the treatment of warts [2]. SA is an organic acid that destroys epidermal cells and softens hyperkeratotic epidermis. Implementation of AV2-SA combination therapy would ensure permanent lesion clearance by on the one hand inactivation of HPV by AV2, and on the other hand elimination of the lesion by SA treatment.

Furthermore, a recent study by Bruggink et al. revealed that the HPV genotype influences the natural course and response to treatment for plantar warts, hereby suggesting that HPV genotyping could potentially be used to optimize wart treatment schemes [8]. An additional study concluded that from all patient- and wart-specific characteristics analyzed, HPV genotype most strongly predicted treatment response in warts [9]. The authors advised that for development of new wart therapies it is essential to take HPV DNA testing into account in order to determine the most optimal treatment. Therefore, this project not only intends to evaluate the efficacy of standard SA treatment versus AV2-SA combination therapy against cutaneous warts, but also to investigate the predictive value of HPV genotyping regarding treatment response.

2. Objectives

The primary objective of this study is to assess the efficacy of AV2-SA treatment versus standard SA treatment by comparing: (1) cure rates of the index warts between the two treatment groups at 12 weeks after enrolment; and (2) recurrence rates of index warts between the two treatment groups at six months after enrolment.

The secondary objectives are: (1) to assess the safety and tolerability of AV2-SA therapy and identify the maximum tolerable dosage; (2) to identify HPV type-specific subgroups of cutaneous warts that have a favorable response to treatment; (3) to compare time to clearance and change in size of index wart between the two treatment groups; (4) to compare clearance of all verrucae, the number of verrucae remaining and potential re-infection at six months after enrolment between the two treatment arms; (5) to determine the genotype-specific distribution of wart-associated HPV types in a Belgian population (according to the age, wart location, postal code, etc.); and (6) to investigate the prevalence of mucosal HPV types in cutaneous warts.

3. Method and analysis

3.1. Trial design and setting

This study comprises two trials i.e. Phase I and Phase II. The Phase I trial is designed to optimize the AV2 treatment dose. The Phase II trial is a double-blind, single-center, two-armed, randomized controlled trial with equal randomization. Participant progress through both trials is shown in Figs. 1 and 2. Specimens will be processed and analyzed at medical laboratory AML, Antwerp, BE. Data management and statistical analysis will take place at the University of Antwerp (UA), Antwerp, BE.

3.2. Recruitment and eligibility

Advertising material will be distributed in local community areas frequently accessed by the general public including movie theatres, shopping centers, pharmacies, local dermatology and general practices, hospitals, high schools, and public swimming pools. Individuals responding to an advert will be screened for eligibility by phone. Table 1 provides a list of all the inclusion and exclusion criteria. At the baseline appointment, the study coordinator will again ensure that the patient is eligible, take informed consent, and check that all baseline data have been completed. Subsequently the study coordinator will firstly photograph the lesion and instruct the patient on follow-up procedures, secondly take a sample for HPV genotyping, and thirdly administer the allocated treatment. The recruitment will continue until 260 patients have been enrolled and at least 50% of the study population comprises persistent warts. Persistent warts are defined as warts resistant to previous treatment and/or warts older than six months.

3.3. Sample size estimation

The previously mentioned systematic review conducted by the Cochrane Skin Group found six studies comparing SA treatment with placebo, with an average SA cure rate of 70% [3]. This trial is powered to show a 15% difference in effectiveness between standard SA treatment and AV2-SA combination therapy. In order to achieve 80% power, 5% two-sided significance, difference in cure rates of 70% versus 85% at 12 weeks, and allowing for 10% loss to follow-up, a sample size of 130 patients in each treatment group will be required (i.e. 260 patients in total).

3.4. Randomization

The study drugs will be randomized at the site of the manufacturer based on a computerized randomization list [11]. The study center will receive sequentially numbered vials, with no knowledge of the

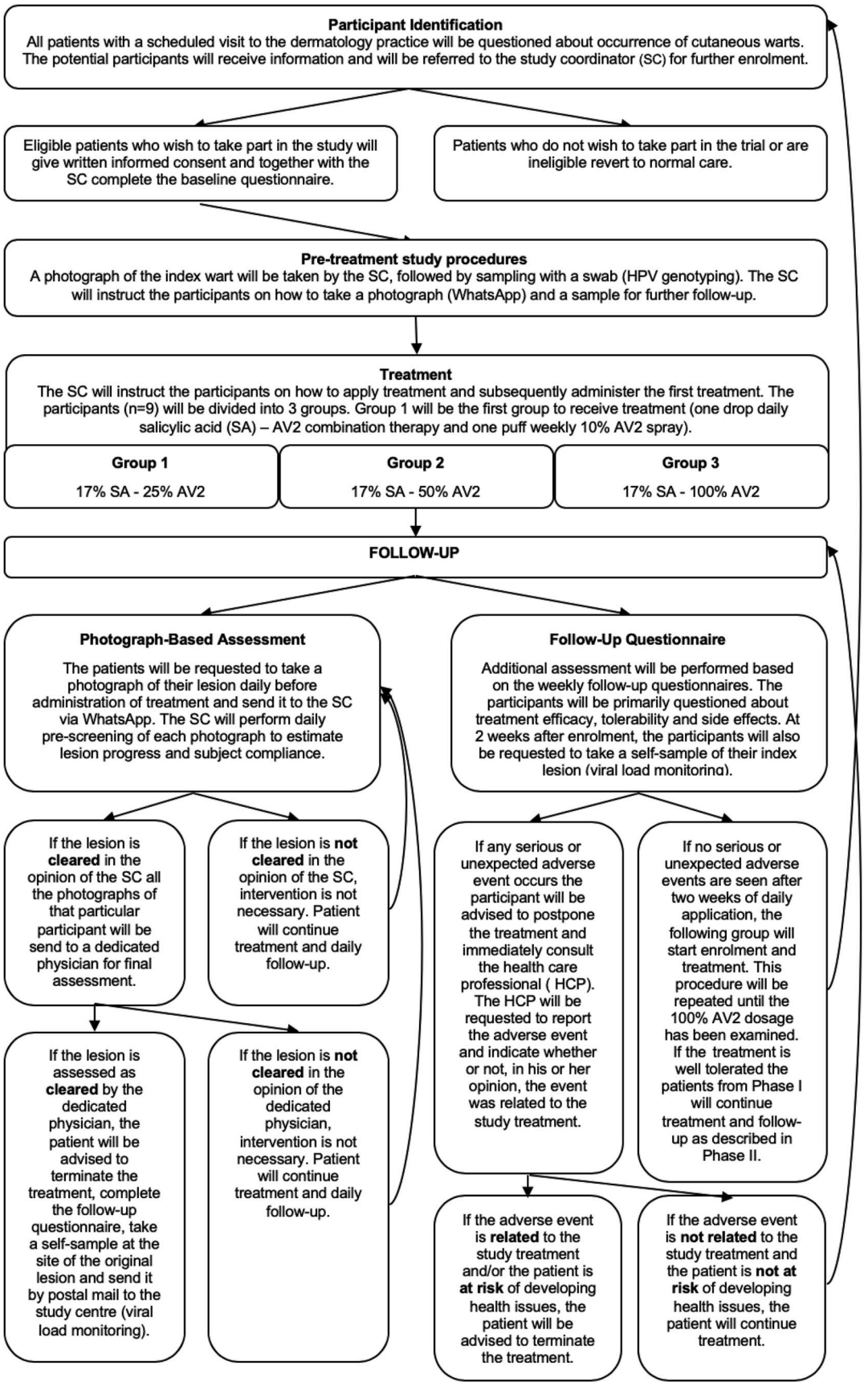

Fig. 1. Flow chart Phase I trial.

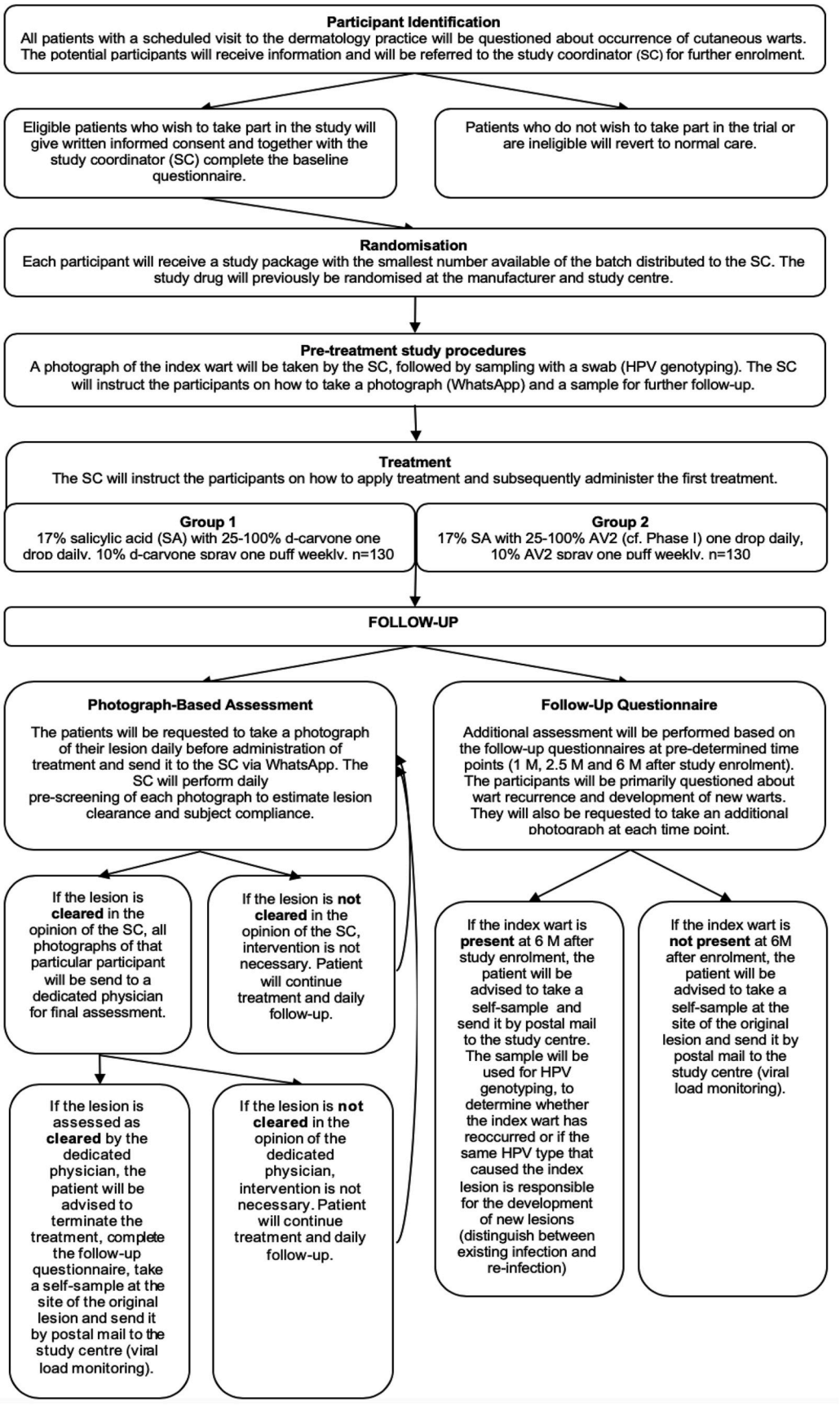

Fig. 2. Flow chart Phase II trial.

Table 1
Details of outcome measures and data collection forms. The majority of the outcomes are responses to questions that require a 'Yes' or 'No' answer or open comments, except for pain which will be measured by a 0–4 numeric pain rating scale.

Measure	Source: Content
Eligibility	**Inclusion Criteria**: patient exhibiting one or more cutaneous warts; aged 12 years or older; agrees to refrain from using prescription or supplemental antiviral medications without first obtaining permission of the coordinating trial dermatologist; able to read Dutch; signed informed consent; able to self-assess and use WhatsApp for follow-up (all Y/N). **Exclusion Criteria**: patient only exhibiting facial and/or seborrheic warts; not suitable for salicylic acid (SA) treatment due to a medical history of severe diseases (e.g. hepatitis, renal or liver dysfunction, cardiovascular, or gastrointestinal disorders, etc.), impaired healing or neuropathy (e.g. due to diabetes, peripheral vascular disease or any other condition); known or suspected allergic or adverse response to SA, AV2 or its components; immunocompromised patient; patient had already participated in another clinical trial concerning treatment for cutaneous warts within six months before enrolment in this study or is currently in a trial evaluating other treatments for his/her warts (all Y/N).
Demographic Details	**Baseline Questionnaire**: date of birth; sex (M/F); postal code.
Wart anamnesis	**Baseline Questionnaire**: General History: number of warts; wart type (verruca vulgaris, verruca plantaris mosaic or simple, verruca plana, verruca filiformis); average size (mm); location; persistence (<6months, >6months); previous treatment (Y/N, if yes specify). Index Wart History: wart type; average size (mm); location; persistence (<6months, >6months).
Efficacy Of Treatment	**Follow-Up Questionnaire:** Index wart: clearance (Y/N) → if cleared date of clearance; if not cleared potential recurrence Y/N. Other warts: still present (Y/N) → if not present date of clearance; if present number of warts and location (inside a radius of 0.5 cm around the original position/another position = 'new warts' → if new warts, inside a radius of 3 cm around the index lesion (Y/N; if yes number of warts).
Side Effects Of Treatment	**Follow-Up Questionnaire:** pain scores (numeric pain rating scale 0 = no pain at all – 4 = extreme level of pain); another side effects (Y/N, if yes specify).
Treatment Compliance	**Follow-Up Questionnaire:** use of additional treatments (Y/N; if yes specify); comments about treatment (open text).
Photograph-Based Outcome Assessment	**Photograph-Based Assessment Form:** Study Coordinator: date at which the photograph is taken; treatment day; index wart cleared (Y/N); size of index wart (mm). Dedicated Physician: photograph interpretable (Y/N); index wart cleared (Y/N); remarks (open text).
HPV Genotyping	**Laboratory Form:** date at which the sample is taken; treatment day; HPV (pos/neg; if pos specify HPV type(s)).

allocation table, and randomly assign new study numbers to each vial (this to ensure blinding of the study drug manufacturer). The participants will be sequentially assigned a unique subject number, corresponding to the new vial number, at the time of enrolment.

4. Interventions

4.1. Phase I

A dose-finding trial will be conducted on nine patients in total, divided in three groups. The first group will daily apply one drop of 25% AV2 (v/v) – 17% SA (w/v) formulation directly on the lesion, in addition to one puff of 10% AV2 (v/v) spray weekly. The additional weekly spray application is intended to prevent re-infection and formation of new warts in the direct environment of existing lesions. Substance tolerance and adverse events will be assessed via weekly follow-up questionnaires (Table 1). The patients will daily take a photograph of their index lesion (the largest and thickest wart) and send it via WhatsApp™ (a freely available messaging application) to the study coordinator, enabling real-time monitoring of treatment progress (see Fig. 3 for further details). The photographs will also be used as proof of daily treatment and daily reminders will be send in order to improve patient compliance. If no serious or unexpected adverse events are seen after two weeks of application, the second group will start with 50% AV2 (v/v) – 17% SA (w/v) treatment. This procedure will be repeated to a maximum dose of 100% AV2 (group 3) if no issues occur. If the treatment is well tolerated, the patients from Phase I will continue treatment and follow-up as described in Phase II.

4.2. Phase II

Participants will be randomized to receive either: (1) the optimal AV2 concentration as determined in Phase I, in combination with 17% SA (w/v) one drop daily and 10% AV2 (v/v) spray one puff weekly; (2) equivalent d-carvone concentration as AV2 concentration in group 1, in combination with 17% SA (w/v) one drop daily and 10% d-carvone (v/v) spray one puff weekly. D-carvone is a component of AV2 that has no anti-viral properties on its own and will be used to provide a fragrance to the SA control treatment in order to ensure blinding. The participants will give the same treatment to all their warts, but only the index lesion will be used for primary outcome assessment. The daily application will be continued for 12 weeks or until the wart is completely cleared. The weekly spray administration, however, will be continued for entire 12 weeks, even after the lesion has cleared (in order to prevent reinfection).

5. Outcome measures

Baseline data will be collected using a baseline questionnaire at time of enrolment. The main measures will be demographic details and previous wart anamnesis (Table 1).

The primary outcome measures (i.e. presence or absence of index wart) will be assessed via follow-up questionnaires (Table 1) and photographs. A wart is considered cured if it is no longer visible (skin color and skin lines are re-established) and cannot be palpated anymore by hand. Patients will complete the follow-up questionnaire on three to four occasions (at time of wart clearance, one month, 12 weeks and six months after enrolment) and take a daily photograph of the index lesion for a maximum period of 12 weeks or until the index wart is cleared, and at six months. The participants will take all photographs according to a standardized protocol (Fig. 3). The final blinded outcome assessment

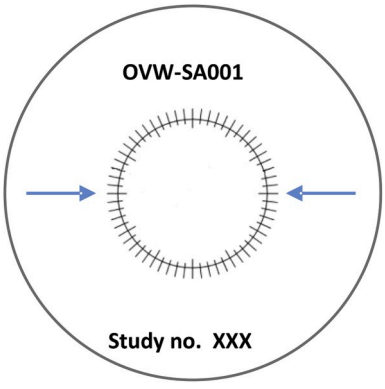

Fig. 3. Example of a follow-up sticker that will be used for photograph-based outcome assessment. The circular stickers have an opening at the center and are provided with a measuring scale and a subject number. The photograph will always be taken from the same distance and angle, with the index wart at the center of the sticker. The subject number and the wart measurements will always be clearly legible.

based on photographs will be performed by two dedicated physicians who are unaware of the treatment group to which the patient is allocated. After confirmation of wart clearance by both physicians, the patient will terminate the treatment, complete a follow-up questionnaire, and take a self-sample of the skin surface at the site of the original lesion.

The secondary outcomes (i.e. general treatment efficacy, side effects and compliance) will also be assessed via online questionnaires (Table 1). HPV genotyping will be performed on several occasions (at time of enrolment and/or wart clearance, and at 12 weeks and six months after enrolment). Samples will be collected and stored according to the previously described optimized sampling protocol [13]. The DNA extraction will involve overnight Proteinase K and EDTA digestion, followed by automated extraction on the NucliSENS easyMAG system (bioMérieux) [13]. The samples will be analyzed with two separate in-house PCR assays capable of detecting the most prevalent cutaneous HPV types as well as the most relevant mucosal types, i.e. respectively the newly developed wart-associated HPV qPCR assay and the HPV Riatol genotyping assay [13,14]. In addition, a cellularity control will be performed on every sample by amplification of the beta-globin gene [14].

5.1. Adverse events

The patients are instructed to consult the participating healthcare professional immediately at onset of any adverse events. Any adverse events will be reported using the adverse event form. When appropriate an assessment of severity, causality, regularity and intensity will also be performed. The clinical course of each event will be followed until resolution or until it has been determined that the study treatment is not the cause. The possible treatment-related adverse events due to salicylic acid treatment are pain, blistering, irritation to the skin, burning sensation, and allergic contact reaction [2]. AV2 has no reported side effects.

6. Data management

All data will be treated with the strictest confidentiality. The study coordinator will keep a locked subject identification log with the names and allocated subject number of the enrolled patients. The case report forms and samples will only be identified by the subject number. All collected data will be incorporated in the trial master file using the REDCap (Research Electronic Data Capture) data management system [15]. Data quality checks will be undertaken to ensure the accuracy of the data. Paper study documents will be retained in a restricted access archive at the study center. Electronic records will be stored on a secure, password protected server within the UA indefinitely.

7. Statistical analysis

Baseline data and treatment details will be analyzed using descriptive statistics (i.e. standard deviation, means, percentages). Proportions of patients with complete wart clearance will be compared using chi-square test. To identify subgroups of cutaneous warts that have favorable response to treatment, cure rates of different treatments will be compared within specific HPV types using 95%CIs, relative risks and risk differences. A logistic regression model will be used to adjust the primary analysis for important prognostic variables (e.g. age, previous treatment, type of wart, persistence). A Cox proportional hazards model will be used to compare the time to clearance of cutaneous warts between the two treatment groups and between different HPV types adjusting for the same covariates as for the primary outcome. Participants will be right censored if they are lost to follow-up or if their verrucae have not cleared. The incidence of all suspected adverse treatment reactions will be summarized by treatment group.

7.1. Missing data

The amount of missing baseline data is expected to be minimal as data monitoring will be performed at regular time intervals. If any issues arise subjects will be contacted in order to resolve them. An 'intention to treat' analysis will be used i.e. all patients will be included in their initially randomized groups whether or not they received their allocated treatment. If the status of a patient cannot be verified the patient will be treated as not having a cleared index wart in the primary analysis.

7.2. Trial completion

Participants will have the option, at any time, to withdraw from the study. Participants may withdraw for the following reasons: development of safety issues; failure of the participant to adhere to protocol requirements; or the participant wished to exit the trial. In case of a withdrawal the change of circumstances form must be completed to ensure appropriate follow-up.

8. Ethics

This study has been approved by the Ethics committee of Antwerp University Hospital (B300201734040). The written information that the participants will receive, clearly describes the potential risks and benefits of participation, the voluntary nature of participation, and how confidentiality will be maintained. All participants will give written informed consent prior to entry into the study. The participant will be informed if new information comes to light that may affect the participant's willingness to participate in the trial. The trial will be conducted in full compliance with local regulations governing the conduct of clinical studies.

9. Dissemination

The results of this study will be published in a peer reviewed medical journal as well as presented at international scientific conferences. Furthermore, participants will be offered the opportunity to obtain a summary of the findings on completion of the study.

10. Conclusion

This will be the first randomized controlled trial to evaluate the efficacy of an HPV-targeted treatment for cutaneous warts. The AV2-SA combination therapy is expected to significantly enhance treatment efficacy and substantially decrease wart recurrence, which is the main cause of patient frustration. The results of this study will make further research into efficacy of AV2-treatment against other HPV-related diseases certainly interesting. In addition, if we are able to confirm that HPV genotype indeed influences the natural course and treatment efficacy in cutaneous warts and make additional statements about the response to treatment of warts infected with other HPV types, we could provide physicians with crucial information necessary to determine the optimal treatment for individuals with cutaneous warts (personalized medicine). In other words, this study could prevent patients from receiving unnecessary therapy, sparing them from painful adverse effects and costs of treatment.

Trial status

Recruitment to the study began in March 2018 and has been completed in January 2019. The follow-up is still in progress and will be completed in July 2019. The analysis will be conducted in September 2019.

Bulleted statements

Cutaneous warts are very common infectious disorders caused by human papillomavirus (HPV). Current wart treatments exhibit variable treatment efficacies and high wart recurrence rates. This randomized controlled trial intends to examine the efficacy of a new antiviral drug (AV2) that targets the source of the infection, making wart recurrence unlikely. The unique study design allows for real-time monitoring of treatment progression and patient compliance (via daily photographs). If treatment response to AV2 is proven to be HPV type-dependent, future triage of patients according to HPV type is recommended in order to ensure treatment efficacy and minimalize side effects and treatment costs.

Funding sources

This study is funded by the AML-Riatol chair (General Medical Laboratory, Antwerp, Belgium) awarded to the University of Antwerp. The University of Antwerp is the study sponsor and is legally responsible for the initiation and management of the study. The sponsor representative is Prof. Dr. Johannes Bogers. The study drug was donated by Cesa Alliance (Strassen, Luxembourg), represented by Reiner van Tilborg.

Declaration of competing interest

The authors have no conflict of interest to declare.

IRB approval status: Reviewed and approved by Ethics committee of Antwerp University Hospital (B300201734040).

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