Efficacy of Intralesional Vitamin D Injection for Treatment of Common Warts: A Randomized Controlled Trial

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EFFICACY OF INTRALESIONAL VITAMIN D INJECTION FOR TREATMENT OF COMMON WARTS: A RANDOMIZED CONTROLLED TRIAL

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Study Product: *Cholecalciferol (Vitamin D3)*

Protocol Number: (IRBe) *19-010219*

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
Co-I	Co-Investigator
CRF	Case Report Form
ECH	Employee and Community Health
ED	Emergency Department
HER	Electronic Health Record
EMR	Electronic Medical Record
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IND	Investigational New Drug Application
IRB	Institutional Review Board
IU	International Unit
Non-UPIRTSO	Non-Unanticipated Problem Involving Risk to Subjects or Others
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
UPIRTSO	Unanticipated Problem Involving Risk to Subjects or Others

Study Summary

Title	Efficacy of Intralesional Vitamin D injection for Treatment of Common Warts: A Randomized Controlled Trial
Running Title	Vitamin D injection for Treatment of Common Warts
Protocol Number	19-010219
Phase	Clinical study phase II
Methodology	Double blind; Randomized, placebo – controlled design
Overall Study Duration	The study will run until the n is reached with target based on sample size calculation.
Subject Participation Duration	Maximum of 168 days
Single or Multi-Site	Single
Objectives	In this study, we aim to explore the efficacy of intralesional vitamin D injection into cutaneous warts by comparing the rates of wart improvement and resolution between a population randomly selected to receive either blinded Vitamin D wart injections or placebo.
Number of Subjects	80 (40 intervention, 40 control)
Diagnosis and Main Inclusion Criteria	Adult ECH patients seen at the Mayo Clinic Rochester practices suffering from one or more cutaneous warts.. Both recalcitrant and non-recalcitrant warts will be included.
Study Product, Dose, Route, Regimen	Cholecalciferol (Vitamin D3), injection of 0.3 ml 40,000IU, intralesional (specifically at the base of the wart with needle centered under the wart) at baseline visit and weeks 4 and 8 as needed.
Duration of Administration	56 days
Reference therapy	Placebo
Statistical Methodology	Primary Endpoint: Complete regression of index wart. Secondary Endpoints: Partial regression of index wart as measured in percent surface area lost. Complete or partial regression of up to 7 other warts followed.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Warts are very common benign proliferations of epidermal cells. Warts are typically treated by traditional destructive modalities, such as cryotherapy, topical salicylic acids, candida antigen immunotherapy, and even laser surgery. All of these treatment modalities can be painful and expensive, and disappointingly, many warts are refractory to treatment and require multiple treatment sessions[1,3].

Topical Vitamin D3 has been used for wart treatment with success[14-17]. Although the pathophysiology of Vitamin D on warts is not completely clear, it is speculated that Vitamin D may regular cell proliferation and can modulate cytokine production affecting innate immunity and has an important role as immunomodulator[13]. Recent case reports and case series of intralesional Vitamin D3 have shown promise as well, but these studies are limited[18-23].

Our study will seek to determine whether an injectable low-dose form of vitamin D is efficacious in treatment of cutaneous warts. Our randomized, double-blind, placebo-controlled methodology will provide level A evidence of efficacy. If efficacious, this would represent a translational science opportunity for production of an injectable vitamin D3 product for intradermal injection for cutaneous warts.

The population to be studied will include adults ≥ 18 years old seen at the Mayo Clinic Rochester practices suffering from one or more cutaneous warts as diagnosed by the examining physician at baseline visit based on typical diagnostic characteristics and able to provide consent. Both recalcitrant and non-recalcitrant warts will be included.

1.2 Investigational Agent

Vitamin D is a fat-soluble vitamin that is produced endogenously when ultraviolet rays from sun exposure trigger vitamin D synthesis. Vitamin D is also naturally present in foods and is widely available as a dietary supplement.

An article in Science in 2006 discussed the effects of activated vitamin D receptors as immunomodulatory and inhibiting cell replication. Activation of toll-like receptors on macrophages induce an antimicrobial peptide through an increase in vitamin D receptors and vitamin D-hydroxylase genes, properties of vitamin D that may explain its potential mechanism of action as an immune modulator, which may represent an efficacious and low cost treatment for the common wart.[13]

Vitamin D3 will be supplied as cholecalciferol 40,000 IU/mL by Mayo Production Lab. 40,000 IU/ml is the formulation that Mayo Clinic has been mixing for administration as treatment for hypovitaminosis D. This drug product will be dispensed through the research pharmacy in 1.5 mL vials containing 0.3 mL of the drug product prepared in sesame oil.

1.3 Clinical Data to Date

Cutaneous warts are a common dermatologic affliction caused by human papillomaviruses (HPVs) which affect the epithelium of skin. Cutaneous warts include common, plantar and flat warts. Diagnosis of a wart is usually straightforward, based on typical epidermal thickening and keratinization that developed over weeks to months and visible capillaries in the base.[1] Spontaneous remission occurs in the majority of cases of cutaneous warts in children within 1-2 years but may take several years in adults particularly among those without intact cellular immunity. A 2006 Cochrane review of 21 randomized clinical trials of topical treatments for warts found an average wart regression rate for placebo of 27% over an average of 15 weeks.[2] Up to a third of cases do not resolve but become recalcitrant to destructive therapies.[1,3]

Standard therapy for warts begins with over-the-counter products that produce physical destruction of the lesion including topical salicylic acid (SA) and low-potency cryotherapy. Studies done have been mostly of poor methodologic quality, and widely heterogeneous (varying strengths and formulations of SA, paring down of wart prior to treatment or not, varying locations of warts, widely varying cryotherapy durations of a few seconds to 30 seconds of one or more freeze cycles). SA appears to be about 1.6 times more likely to clear warts than placebo (meta-analysis of 5 studies of 333 patients) and has demonstrated mean cure rates of 49% vs 23% for placebo (pooled analysis of 16 studies with 813 patients with variable durations up to 18 months).[4] In one 13 week randomized trial of SA 40% ointment (for comparison, Compound W in the US is 17% liquid) vs cryotherapy by cotton-tipped applicator dipped in liquid nitrogen vs placebo, cure rates for all sites were 15% vs 46% vs 7% with lower efficacy of cryotherapy for plantar warts (30% vs 33% with SA ointment). A 23% rate of spontaneous resolution of warts (placebo arm) was noted in that 13 week trial.[5,6] Treatment guidelines from both specialty (British Association of Dermatologists) and generalist physician groups (American Academy of Family Physicians) recommend SA and/or cryotherapy as first line treatment.[1,7]

Patients presenting to their primary care provider for treatment of warts usually receive in-office cryosurgery with or without paring or curettage. In cases where warts are recalcitrant to first or second-line therapies, immunotherapy (e.g. Candida antigen at Mayo Clinic and elsewhere also MMR, BCG or PPD), and topical or intralesional 5-fluorouracil or bleomycin, electrocoagulation (e.g., Hyfrecator), surgical excision, or laser ablation may be used. All of these office-based therapies can be painful, cause blistering, scarring and hypo or hyperpigmentation, distort nails in the case of periungual warts, risk recurrence, and cost substantial time and money.[1,6,8,9]

At Mayo Clinic Rochester's ECH Procedure Clinic as in the Dermatology wart clinic practice, intralesional immunotherapy with Candida antigen repeated every 3 weeks up to 5 sessions is the third-line therapy for refractory warts. Yet, only 39% of patients in a retrospective review of patients receiving Candida antigen in the Mayo Clinic Dermatology practice achieved complete wart clearance after up to 6 injections though 41% had a partial response.[10] Other studies have reported a higher efficacy of Candida antigen. In the original report of use of Candida antigen in

a retrospective review by Philips, Ruhl and Pfenninger in 2000 of 149 patients in a Family Medicine residency in Michigan, a 72% complete efficacy was reported.[11] A prospective study of 34 pediatric and adult patients with recurrent or recalcitrant warts who were given 3 injections of Candida antigen demonstrated 56% clearance of all warts, and 38% non-response rate. All of the patients in both of these studies had been skin-tested for Candida sensitivity prior to treatment. A retrospective review of 55 pediatric Dermatology clinic patients without skin-testing mentioned in the paper showed complete resolution in 87%.[12] The lower efficacy of the Mayo Clinic Candida antigen experience compared to other published data was explained in part by the lack of pre-treatment skin test to assure Candida sensitivity, the use of the Candida only for recalcitrant warts, and potential misclassification bias as patients were not seen back for a final visit to assure clearance but anecdotally (mentioned in the paper) sometimes simply cancelled that visit because their warts had cleared.[10]

In addition to a suboptimal efficacy, patient experiences of injection pain, blistering, swelling, rare flu-like symptoms, wheezing, and the low risk of anaphylaxis with Candida (as with any antigen or allergen therapy)[10], low efficacy, and costs in time, money, lost work productivity while returning for repeated treatments warrant a search for alternative approaches .

Vitamin D intralesional injection may be a good alternative. An article in Science in 2006 discussed the effects of activated vitamin D receptors as immunomodulatory and inhibiting cell replication. Activation of toll-like receptors on macrophages induce an antimicrobial peptide through an increase in vitamin D receptors and vitamin D-hydroxylase genes, properties of vitamin D that may explain its potential mechanism of action as an immune modulator, which may represent an efficacious and low cost treatment for the common wart.[13]

A number of small case reports and case series have suggested efficacy of topical vitamin D in regression of cutaneous warts.[14-17] Four studies have examined intralesional or sub-lesional vitamin D in treatment of recalcitrant warts. Abdel Kareem et al reported the results of a single blinded placebo controlled trial using vitamin D3 in a dose of 60,000 IU in 0.2 ml in 2 injections given 1 month apart vs a saline placebo in a small population in Turkey of 50 patients ages 12 – 50 years, most of whom were suffering cutaneous warts of the hands. The methodology of recruitment, randomization, and assessment of confounding variables and objective response were not described. They noted side effects of itching and pain in 60% of vitamin D recipients (n=30) vs 20% of controls (n= 20). Vitamin D3 resulted in complete wart clearance in 40% of those treated vs. 5% in placebo saline control subjects. They noted a better response in patients with fewer and shorter duration of warts and noted regression of distant non-treated warts with complete resolution in 2 of the 30 cases as well[18]. This study added evidence to two prior studies using similar doses of vitamin D3 given as an intralesional injection. A retrospective analysis case series in Turkey found a 70% clearance after 1 month of recalcitrant plantar warts after one treatment session. Treatment involved injections of up to 5 warts per patient each with 0.2 ml of vitamin D3 (7.5 mg/ml; 300,000 IU/ml) into the base of the wart using a 21 g needle after pre-injecting the wart with 0.1 ml prilocaine. The mean patient age was 28.6 years and mean duration of wart was 4.3 years. They observed an 80% complete clearance of plantar warts after a second intralesional injection was given to the remaining 3 of 14 patients who had a partial response defined as < 50% decrease in lesion size while 3 of 14 patients who failed to respond at all did not consent to a second treatment. These above resolution rates were based on 6 month

follow up. No patient experienced any adverse effects other than pain during prilocaine injection. No hypervitaminosis D or systemic side effects were observed. This study was neither blinded nor controlled[19]. In an open label case series of 64 patients in India, 0.2 to 0.5 ml of vitamin D3 (15 mg/ml; 600,000 IU/ml) was injected by 26 g needle into the base of up to 5 warts refractory to other treatments every 3 weeks for 4 sessions. Complete clearance was seen in 90% of patients. Roughly half of the warts were palmoplantar and half common warts elsewhere. The mean age of these patients was 24 years but ranged 8 – 66 years and mean duration of warts were 14 months and had not responded to treatment with at least 2 treatment modalities. All patients had transient pain at the time of injection, and a few developed erythema or edema at the injection site that resolved within a week. No signs of hypervitaminosis D or systemic side effects were observed. They followed patients for 6 months; 2 of the 64 had recurrence[20]. Fathy et al recently reported a three arm trial described as a comparative interventional case control study of 60 patients in Egypt with ≥3 recalcitrant or recurrent plantar warts. Subjects received intralesional injection of vitamin D3, Candida antigen, or saline by 30 g needle using a pre-injection application of an ice pack for 2 minutes every 3 weeks up to 3 injections in the largest wart. The vitamin D3 dose was 60,000 IU in 0.6 ml injected into the base of the largest plantar wart. The Candida antigen dose was 0.1 ml and was only used on patients who exhibited a 5mm or greater wheal immune response to a 0.1 ml skin test. They found significantly better reduction of warts with vitamin D (70% excellent resolution defined as 75-100% reduction of warts) and 30% non-response compared with both Candida (25% excellent response and 5% very good (50-75% wart reduction) and 15% good (25-50% reduction)and placebo (no response in any patients). In 4 vitamin D subjects who were excellent responders, 35% of warts elsewhere (7 of 20) showed complete clearance. In 4 Candida subjects who were complete responders, 20% of warts elsewhere (4 of coincidentally also 20 total) completely cleared. The only side effect reported for vitamin D was pain during injection.[21] Kavya et al performed an open label study in India of intralesional vitamin D3 in 42 patients with both palmoplantar warts (23) and verruca vulgaris (18) and saw a 79% complete response and 14% moderate response. They also used 0.2 ml of vitamin D3 15 mg/ml (600,000 IU/ml) injected with a 27 g needle into the base of each wart pretreated with 0.2 ml of lidocaine 20 mg/ml.[22] Another study compared vitamin D3 and PPD and used 0.5 ml of vitamin D3 (600,000 IU/ml; 15 mg/ml) injected into the base of each wart up to 4 warts (maximum vitamin D3 dose of 1.2 million IU) every 2 weeks for up to 4 treatments. 80% of patients treated with vitamin D3 injection had complete clearance of warts and another 15% showed at least moderate clearance.[23]

None of these studies had sufficiently robust methodology to demonstrate with confidence that vitamin D treatment is efficacious. All were done in countries outside of the US. All 3 of the injection trials used a fairly high dose of vitamin D that may potentially in vitamin D-replete individuals result in harm though none was noted.

1.4 Dose Rationale

In their 2014 guidelines for wart management, the British Association of Dermatologists authors made some clear recommendations for future studies on cutaneous wart therapies.[1] They recommended single treatment arms rather than combined therapies, sufficient sample size based on formal sample size calculations, true randomization of patients to treatment, reporting of complete cure of all warts, blinding of outcome evaluation, and follow up both 12 weeks after treatment and 6-12 months post-treatment.

In previous studies of intralesional vitamin D3 in wart treatments, high dose Vitamin D3 was used.[18-23] This study proposes to use cholecalciferol (Vitamin D3), injection of 0.3 ml 40,000 IU, intralesional (specifically at the base of the wart with needle centered under the wart) at baseline visit and weeks 4 and 8 as needed. This regimen is similar to the standard of practice used at Mayo Clinic Rochester's ECH Procedure Clinic and in the Dermatology wart clinic practice with Candida injections, where standard practice includes intralesional immunotherapy with Candida antigen is repeated every 3 weeks up to 5 sessions and is a third-line therapy for refractory warts. In addition, our proposed injection regimen is similar to the previous case reports and case series which used high dose intralesional injections in 4 week intervals.

1.5 Risks and Benefits

Risks of being in the study include the possibility of some pain or discomfort and bleeding at the injection site. There is a low risk of infection, scarring, redness or discoloration of the skin, swelling or itching at the injection site, as well as the risk of no improvement of the wart. The post injection survey will assess if any of these signs or symptoms occur.

This study may not make the subject's health better, but anything we learn may benefit others in the future. There is a chance the subject may see some improvement in their wart(s).

2 Study Objectives

We aim to explore the efficacy of intralesional vitamin D injection into cutaneous warts by comparing the rates of wart improvement and resolution between a population randomly selected to receive either blinded Vitamin D wart injections or placebo.

3 Study Design

This study is a randomized, double blind, placebo-controlled phase 2 trial of the safety and efficacy of Vitamin D3, in the treatment of cutaneous warts. Subjects will be recruited through the Mayo Classifieds, an internet website posting, and/or via flyers and will contact the research study coordinators to express interest in the study. Subject will be asked baseline inclusion questions and may take a photo of their index wart to determine eligibility for the study. Once eligibility is determined the study coordinators will consent (either digitally or via paper), enroll and randomize the subject. Following enrollment the subject will be seen for examination to include measurement of wart(s),photo-documentation, to receive injection of either Vitamin D or placebo into the index wart, and to collect baseline data. Subject visits for intralesional injection and follow up will be conducted in exam rooms on Baldwin 4 during regular hours 8 am to 5 pm, Monday through Friday. Baseline data will be collected in the form of a questionnaire to include age, sex, presence/absence of immunosuppressive comorbidity or medication use, location(s) of warts, approximate time each wart has been present, prior treatments used for these warts and month/year those treatments were utilized, size of each wart, and photo-documentation of the wart(s) using the Mayo PhotoExam app with paper ruler placed near the wart with images saved to the EHR. Size (surface area) of each wart will be calculated based on one-half the mean of the measurement of two diameters of the wart at right angles (the mean wart radius) squared and

multiplied by pi. Photos of the warts and size measurements of 2 wart diameters at 90 degree angles from each other will be collected at baseline and each subsequent visit and the measurements entered into an Excel spreadsheet to calculate surface area of each wart at each visit. Subjects with more than one wart will be given the choice of which wart they would like injected. Only one wart will be treated but the others up to 8 measured, photodocumented and observed for evidence of regression during the study. Confirmation of wart measurements by use of the PhotoExam images by study Co-I's blind to treatment and surface area calculations in Excel will be compared to those resulting from measurement at the time of the subject visits to ensure data integrity. The principle investigator will be responsible for ensuring data integrity by comparing measurements recorded at subject visits and measurements based on PhotoExam observations by the Co-I's for the first 3 subjects and then every 10^{th} patient. Any discrepancy will result in an increase in monitoring and discussions with both observers to ensure consistency. Patients will return at a scheduled time to the ECH Procedure Clinic for injection and post-injection care and education as per the study schedule below.

Subjects will be contacted by email to complete a REDCap survey regarding side effects of treatment one week after each visit including pain, pain score, redness, pruritus, malaise, myalgia, and fever. These surveys will be reviewed weekly by the PI to ensure subject safety. In addition, subjects will be contacted by email to complete a REDCap survey regarding pain and pain score each week after the initial survey, until they report for 2 weeks without pain. This survey will also be sent to those subjects who do not receive an injection. They will be seen back 4 weeks and 8 weeks (+/- 1 week) after the first injection to receive an injection in the index wart if it is still present (subject may send a photo of their index wart to study coordinator to determine if the injection is needed so that medication can be ordered ahead of time), and to measure and photo-document all warts. Subjects will also be seen 12 weeks and 24 weeks (+/- 1 week) after the first injection to measure and photo-document all warts. Subjects will be questioned at each visit to ensure that they have not been using any other wart treatments.

3.1 *General Description*

Randomized double-blind placebo controlled trial

3.2 Number of Subjects

80 (40 intervention, 40 control)

3.3 Duration of Participation

Maximum of 168 days.

3.4 Primary Study Endpoints

Complete regression of index wart.

3.5 Secondary Study Endpoints

Partial regression of index wart as measured in percent surface area lost.

Complete or partial regression of up to 7 other warts followed.

3.6 Identification of Source Data

The following source data will be directly recorded on the Case Report Form (CRF):

- Location of wart, time wart present, prior treatments, prior treatment dates
- Visit, wart appearance, if injection was given, any liquid escape during injection, pain score immediately after injection, pain score 1 minute after injection, and pain score 5 minutes after injection.
- Concomitant Medications
- Patient Reported Side effects

The following source data will not be directly collected in the Case Report Form (CRF), but will be captured in supportive documentation (study source documents, EMR):

- PhotoExam wart image

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- Adult ECH patients seen at the Mayo Clinic Rochester practices
- Patients suffering from one or more cutaneous warts as diagnosed by the examining physician at baseline visit on typical diagnostic characteristics
- Able to provide consent
- Both recalcitrant and non-recalcitrant warts will be included

4.2 Exclusion Criteria

- Patients with prior use of home or office-based destructive treatments for this wart(s) in the last 1 month with SA or cryotherapy
- Immunoadjuvant therapy for warts in the last 4 months (e.g Candida)
- History of vitamin D injection of warts ever
- High-dose vitamin D supplementation (>4,000 IU daily or equivalent) in the preceding 3 months
- Pregnancy or lactation
- Facial or genital warts
- Lesions not felt by the examining clinician to be a wart (e.g., corns or calluses)
- Immunosuppression (to include immunosuppressive medications or conditions as judged by the physician evaluating the patient at the baseline visit).
- Allergy to sesame oil

4.3 Subject Recruitment, Enrollment and Screening

- From the Principal Investigator or Co-Investigator clinical practices
- Information that is to be given to potential subjects (flyers)
- Advertisement on Mayo Classifieds and/or internet website posting
- Screening requirements/Evaluation and documentation of inclusion/exclusion criteria
    - If subject is an adult and has a wart on their skin
    - Wart is not located on face or genitals
    - Subject has not been treated with destructive treatment like Compound w or similar products or freezing spray in the last month
    - Subject has not had injection therapy into the wart in the last 4 months
    - Subjects has not been using high does vitamin D (more than 4,000 IU daily) in the last 3 months
    - Subject is not pregnant or breast-feeding
    - Subject does not have any immune suppressing disease or is on immune suppressing medications
    - Subject is not allergic to sesame oil

4.4 Early Withdrawal of Subjects

The subject may decide to withdraw at any time. Subjects should tell the Principal Investigator if they decide to withdraw early and they will be advised whether any additional tests need to be done for their safety.

4.4.1 When and How to Withdraw Subjects

Study subjects will be withdrawn for any subject who is intolerant of intralesional injections or has an adverse reaction to include infection, persisting pain, substantial pruritus, malaise, myalgias, fever or any other systemic symptoms suspected of being related to the injections. The risk of these is very low.

Following each visit, there will be a one week follow up survey of post-injection symptoms including pain, pain score, redness, swelling, itching and a one month follow up visit will be requested to examine and photodocument the injected wart. An additional survey will also be sent each week after to assess pain and pain score until the subject reports for 2 weeks without pain

The risk of hypervitaminosis D in subjects not taking high dose vitamin D with the low doses of 12,000 IU vitamin D3 that we are using in this study is negligible and will not be monitored. In the event of concern for adverse events in study participants (i.e., participant presents to ED with symptoms that may be consistent with Vitamin D toxicity such as confusion, polyuria, polydipsia, anorexia, vomiting, and muscle weakness or local inflammation at the site of injection) Ivana Croghan, PhD will have 24/7 access to REDCap to break the blind and supply study drug administration information.

The study will be stopped for any unanticipated problems involving risks to subjects or others until these are understood and explained sufficiently to resume the study and for unexplained adverse outcomes or life threatening adverse events.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

For all subjects, one week post-injection, follow up surveys (administered by study coordinators and completed/answered by subjects) will be reviewed weekly by the principle investigator. Additional surveys will be sent each week after until subject reports for 2 weeks without pain.

5 Study Drug

5.1 Description

Most pharmacologic data relates to oral dosing of vitamin D3. Studies have found that healthy adults reach 25(OH)D3 plasma concentrations of about 20 ng/mL with oral dosing of 600 to 800 IU vitamin D per day.[24,25] Other studies have found that a high single dose of 150,000 IU orally raises serum 25(OH)D peaking to 139.0 nmol/l at day 7 then slowly declines, while subjects taking 5,000 IU daily peaks to high of 130.5 nmol/l, with no clinical significant elevations in calcium or phosphorus in daily oir single high dose regimens.[26]

5.2 Treatment Regimen

Cholecalciferol (Vitamin D3), injection of 0.3 ml 40,000IU, intralesional (specifically at the base of the wart with needle centered under the wart) at baseline visit and weeks 4 and 8 as needed.

5.3 Method for Assigning Subjects to Treatment Groups

The research pharmacy will perform the randomization and assign a number associated with a specific treatment drug.

5.4 Preparation and Administration of Study Drug

Subjects will be randomized to receive an intralesional (specifically at the base of the wart with needle centered under the wart) injection of 0.3 ml of cholecalciferol (vitamin D3) in sesame oil 40,000 IU/mL prepared from a fresh 1.5 ml vial and the unused portion of the vial discarded. These injections will be provided in an exam room near the ECH Procedure Clinic on Baldwin 4. Controls will receive 0.3 ml of a similarly clear and equally viscous solution of sesame oil packaged in the same appearing vials. The wart to be injected will be sprayed for a few seconds until it turns white with ethyl chloride to reduce discomfort of the injection. Injections of 0.3 ml will be performed using a 3/4” 30 g needle on a Luer-lock 1 ml syringe under the center of the wart being treated avoiding entering the skin from more than one entry point, repositioning the needle tip if liquid runs out from under the wart or from the wart as it is being injected. Any escape of injectate during the injection will be recorded. A Band-Aid will be applied and participants instructed to remove it after 1 hour unless there is continued bleeding. Vials will be ordered from the pharmacy as required for investigational medications for each individual patient and picked up by study coordinators from the pharmacy. These vials will either contain active drug (vitamin D3 in sesame oil) or placebo (sesame oil)), randomization performed by the pharmacy. A spreadsheet will be completed for each study subject who arrives at the procedure clinic to record patient study number, Mayo Clinic number, vial A/B (or however pharmacy

chooses to identify study drug vs placebo) used, any escape during injection of injectate, and pain score immediately, 1 minute and 5 minutes after injection.

5.5 Subject Compliance Monitoring

Patients will be present for face-to-face visits injection of the study medication, which will ensure the medication is appropriately administered.

5.6 Prior and Concomitant Therapy

Patients with prior use of home or office-based destructive treatments for this wart(s) in the last 1 month with SA or cryotherapy, immunoadjuvant therapy for warts in the last 4 months (e.g. Candida) history of vitamin D injection of warts ever, high-dose vitamin D supplementation (>4000 IU daily or equivalent) in the preceding 3 months, or immunosuppressive medications or conditions as judged by the physician evaluating the patient at the baseline visit.

5.7 Packaging

Cholecalciferol (vitamin D3) in sesame oil 40,000 IU/mL injections will be prepared from a fresh 1.5 ml vial and the unused portion of the vial discarded. Controls will receive a similar clear and equally viscous solution of sesame oil packaged in the same appearing vials. Study drug and placebo are provided by Mayo Production Lab and dispensed by Research Pharmacy. 90 study drug vials and 90 placebo vials will be provided by Research Pharmacy.

5.8 Masking/Blinding of Study

The pharmacy will prepare a stock of vials of vitamin D3 and placebo at intervals when requested. At their subsequent visit, participants will receive the same vial containing the same unknown as they received at their prior visit.

5.9 Receiving, Storage, Dispensing and Return

Subjects presenting for enrollment will be randomized by the pharmacy to receive either placebo or vitamin D3 for their injection, procured from the pharmacy at the time of enrollment by prescription and prior to each future visit, and picked up by Department of Family Medicine research coordinators.

5.9.1 Receipt of Drug Supplies

Mayo Production Lab will ship the vitamin D3 and placebo to the Research Pharmacy and the Department of Family Medicine research coordinators will pick up prior to each study visit.

Upon receipt of the vitamin D3 and/or placebo, an inventory will be performed and a drug receipt log will be completed as appropriate for the Research Pharmacy.

5.9.2 Storage

Mayo Research Pharmacy will store the vitamin D3 and placebo.

5.9.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the Research Pharmacy.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Screening Visit: At this visit once it has been determined if the subject qualifies for this study and they agree to participate, they will be randomly assigned by chance (like a coin toss) to receive either Vitamin D or placebo injections into the wart of their choice. Neither the subject nor the study team can choose which group they'll be assigned to; we will find out at the end of the study.

6.2 Baseline Visit 1: The wart will be examined, photographs taken for measurements, medications will be reviewed and the subject will complete an initial study questionnaire. The wart will be sprayed for a few seconds with ethyl chloride to reduce discomfort just prior to receiving the injection. Within a week following the visit, the subject will receive a brief survey via email to complete which asks about their symptoms and pain. Each week after, the subject will receive an additional survey asking about pain until they report for 2 weeks without pain.

6.3 Visit 2 and 3 (Week 4 and Week 8) Visits: The subject will receive an injection in the index wart if it is still present (subject may send photograph ahead of time to study coordinator to determine),. At the visit, photographs will be taken of all warts for measurements, medications reviewed and they will be asked to assess their pain. A week following the visit a survey will be sent via email which asks about their symptoms and pain. Each week after, the subject will receive an additional survey asking about pain until they report for 2 weeks without pain.

6.4 Visit 4 (Week 12): The subject will return to have the wart examined. Photographs will be taken of all warts for measurements and medications reviewed. If the subject still reports of pain, a survey will be sent to assess until they report for 2 weeks without pain.

6.5 Study Completion (Week 24): The subject will return to have the wart examined. Photographs will be taken of all warts for measurements and medications reviewed. Payment for their participation will be processed at this time.

Only one wart will receive injections, however up to 8 others may be photographed, measured, and observed for any changes during the study.

Photographs of the subjects wart will be added to their electronic medical record.

The subject will be informed which group they were in after the study is completed and analyzed. If they continue to have persistent warts, they will be referred back to their primary care provider for treatment and provided with education for treatments available over the counter.

Schedule of Events:

Time point	Screening Visit [1]	Baseline/ Visit 1 (+ 7 Days) [1]	Visit 2 (+/- 7 Days)	Visit 3 (+/- 7 Days)	Visit 4 (+/- 7 Days)	Study Completion (+/- 7 Days)
Week	**0**	**0**	**4**	**8**	**12**	**24**
Day	**0**	**1**	**28**	**56**	**84**	**168**
Informed consent	**X**					
Randomization	**X**					
Vit. D Study Questionnaire		**X**				
Inject study medication		**X**	**X**[5]	**X**[5]		
Vitamin D Study Visit Form		**X**	**X**	**X**		
Take photo of wart		**X**[6]	**X**[6]	**X**[6]	**X**	**X**
Concomitant Meds.[2]		**X**	**X**	**X**	**X**	**X**
REDCap Side effects survey[3]		**X**	**X**	**X**		
REDCap Pain survey[4]		**X**	**X**	**X**	**X**	
Subject remuneration						**X**

1. Screening and Baseline visit could take place on same day if patient prefers
2. To ensure subject has not been using any other wart treatments
3. REDCap side effects survey will be emailed one week after each visit
4. REDCap pain survey will be emailed one week after the REDCap side effects survey for every week until subject reports for 2 weeks without pain
5. If the index wart is still present
6. Subject may take photo of index wart ahead of time to determine if injection is needed

7 Statistical Plan

7.1 Sample Size Determination

We can reasonably estimate an efficacy rate of up to 3 intralesional injections of vitamin D into recalcitrant warts as high as 90% for and as low as 40%. Given the study design of a dichotomous outcome as dependent variable of complete resolution of the wart(s) in the two groups to be compared – intervention and control - and an anticipated incidence of 5% spontaneous resolution of recalcitrant warts over the course of the 24 weeks of the study and an anticipated effect size of the intervention of 35% additional complete resolution based on the Abdel Kareem study with a Type I/II Error Rate alpha of 0.05 and power of 80%, we would need a sample size of 54 composed of 27 subjects receiving the intervention and 27 controls. We will increase enrollment to 30 in each group to account for drop outs. Since Abdel Kareem enrolled pediatric patients age 12 and older who tend to have a higher spontaneous resolution of warts than adults, we can reasonably base our sample size calculation on his study. If a high percentage of the patients we enroll have non-recalcitrant and non-plantar warts, we can assume a higher

spontaneous resolution rate but also anticipate a higher response to the intervention which will balance (e.g. sample size calculation for a 25% spontaneous resolution rate in previously untreated non-recalcitrant warts but a higher 60% response rate to vitamin D would give a same sample size calculation of 60 with 30 subjects in each arm)

Endpoints
Primary:
Complete regression of index wart.

Secondary:
Partial regression of index wart as measured in percent surface area lost.
Complete or partial regression of up to 7 other warts followed.

7.2 Statistical Methods

Descriptive Statistics

There should be no significant temporal bias as season does not appear to affect wart refractoriness or resolution (though perhaps the increased vitamin D generated in skin in the summer would suggest a higher rate of spontaneous resolution if our hypothesis is correct). Secondary outcomes of partial resolution of warts based on visually and palpable size and photo-documented appearance will be recorded and reported as well. Fisher's exact test will be used to compare effects of placebo vs vitamin D3 injection on wart resolution.

Handling of Missing Data

Adherence to the protocol will be assessed by the study investigators through examination of data ensuring randomization and blinding, and self-reports of the study participants. Data from participants who for whatever reason deviate from the protocol will be handled by an intention to treat method in the final statistical analysis.

Primary Hypothesis: Vitamin D intralesional injection into cutaneous warts results in higher rates of complete resolution than saline placebo injection.

Fisher's exact test will be used to compare effects of placebo vs vitamin D3 injection on wart resolution.

Secondary Hypothesis

Partial regression of index wart as measured in percent surface area lost.

Multivariable linear regression will be used to assess the effect of vitamin D injection on final wart surface area, adjusting for initial surface area in the model.

Complete or partial regression of up to 7 other warts followed.

Fisher's exact test will be used to compare effects of placebo vs vitamin D3 injection on complete resolution of all additional warts. Multivariable linear regression will be used to assess the effect of vitamin D injection on final wart surface area of all additional warts, adjusting for initial surface area of the additional warts in the model.

Interim Analysis

The study dataset will be routinely monitored by the principle investigator and research coordinators to screen for any duplicate data entry and ensure sequential data input. Any adverse events or safety concerns will be immediately reported to the principle investigator and documented.

7.3 Subject Population(s) for Analysis

- All-treated population: Any subject randomized into the study that received at least one dose of study drug

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event

Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- persistent or significant disability or incapacity
- substantial disruption of the ability to conduct normal life functions
- birth defect/congenital anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as 112 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values

There will be no routine clinical laboratory monitoring for the purpose of this study. However, if subjects present with signs or symptoms of vitamin D toxicity to a medical care provider, values for standard laboratory workup including 25(OH) vitamin D concentration, calcium, phosphorus, creatinine would be monitored as well as any follow-up labs within the study's follow-up period.

A clinical laboratory abnormality will be documented as an adverse event if any of these values are found to be above normal ranges.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization for diagnostic or elective surgical procedures for a preexisting condition.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the adverse event worksheet. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

The sponsor-investigator must also notify the FDA (and sponsors must notify all participating investigators) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting under § 312.32(c)(1)(i)-(iv).

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 Unmasking/Unblinding Procedures

In the event of concern for adverse events in study participants (i.e., participant presents to emergency department with symptoms that may be consistent with vitamin D toxicity such as confusion, polyuria, polydipsia, anorexia, vomiting, and muscle weakness or local inflammation at the site of injection) Ivana Croghan, PhD will have 24/7 access to REDCap to break the blind and supply study drug administration information. Documentation of this will be kept in the subject's source document.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction.

Data Management

Subject inclusion criteria will be ascertained by study coordinators at enrollment during the screening visit. A questionnaire completed at this time will be reviewed to ensure both verbal and written affirmation of inclusion criteria and absence of exclusion criteria. In addition to completion of the questionnaire by the study subjects, all warts will be photo-documented at baseline and at each follow up visit with ruler placed near the wart and those images used for confirmation of wart measurements and surface area calculations by the study PI or Co-Investigators blinded to treatment to ensure accurate surface area calculations. Measurements of 2 diameters of each wart will be entered into REDCap for standardized surface area calculations.

Data Security and Confidentiality

Data will be stored in a password protected encrypted file on a study laptop or desktop computer kept in the Department of Family Medicine Research Office in Baldwin and automatically backed up several times daily. Data will be kept for 3 years after completion of the study. Access to the computer will be limited to the PI, Co-I's, and study coordinators. Images of the warts will be stored in a secure electronic form in Epic for each patient's PhotoExam wart image.

Data Quality Assurance

Department of Family Medicine research study coordinators will monitor the data closely throughout the study, to ensure the data in the database accurately reflects data on the CRFs.

Data Clarification Process

Adherence to the protocol will be assessed by the study investigators through examination of data ensuring randomization and blinding, and self-reports of the study participants. Data from participants who for whatever reason deviate from the protocol will be handled by an intention to treat method in the final statistical analysis. The primary investigator will evaluate any data queries and discuss discrepancies with any investigators involved with that particular data acquisition.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

The subjects information will be coded by unique numbers, research materials will be stored in locked cabinets, and data stored electronically on password-protected computers.

The sponsor-investigator will retain the specified records and reports for whichever is longer:

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study is financed through the Parker D. Sanders & Isabella G. Sanders Professorship funds and departmental small grant funds.

12.2 Subject Stipends or Payments

Each participant will receive $25 per study visit completed and will be paid at study completion.

13 Publication Plan

Stephen Merry, MD, MPH, holds the primary responsibility for the publication of the results for the study.

The study will be registered on ClinicalTrials.gov prior to subject recruitment and enrollment. The results will be posted to ClinicalTrials.gov within 12 months of final data collection for the primary outcome.

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