

**A PHASE 2, MUTI-CENTER STUDY OF REPEAT DOSING OF SQUARIC ACID  
DIBUTYL ESTER IN SUBJECTS WITH HERPES LABIALIS**

**Detailed Protocol**

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## I. Objectives

**Primary Objective:** To assess local and generalized adverse events with repeat topical application of 2% and 0.5% squaric acid dibutyl ester (SADBE) in subjects with frequent herpes labialis (4 or more episodes in the previous 12 months).

**Secondary Objective:** To assess efficacy of repeat topical application of 2% and 0.5% SADBE in the prevention of herpes labialis episodes.

## II. Background

Primary oral infection with the herpes simplex virus (HSV) typically occurs at a young age, is asymptomatic, and is not associated with significant morbidity. After primary oral infection, HSV may persist in a latent state in the trigeminal ganglion and later reactivate as the more common herpes labialis, or “cold sores.” Common triggers for reactivation are well known and include ultraviolet light, trauma, fatigue, stress, fever, inflammation, and menstruation. These lesions affect up to 45 percent of the U.S. population. They classically manifest as a well-localized cluster of small vesicles along the vermilion border of the lip or adjacent skin. The vesicles subsequently rupture, ulcerate, and crust within 24 to 48 hours. Spontaneous healing occurs over seven to 10 days.

In immunocompetent patients, herpes labialis usually is mild and self-limited. However, pain, swelling, and cosmetic concerns may prompt physician consultation. Orally administered antiviral agents, such as acyclovir (Zovirax) or valacyclovir (Valtrex), have a modest clinical benefit if initiated during the prodrome. Topical treatment with 1% penciclovir cream (Denavir) may reduce healing time and pain slightly, even if initiated after the prodrome. However, reduction in healing time with systemic or topical agents is modest.

Squaric acid dibutyl ester (SADBE) is a topical immunotherapeutic agent used in the treatment of verruca vulgaris and alopecia areata. During a recent FDA Compounding Advisory Committee Meeting, it was recommended that squaric acid dibutylester be included on the list of bulk drug substances allowed for use in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act. And SADBE has now been so listed under section 503A.

A study completed by Lee et al of 29 patients with recalcitrant warts demonstrated complete clearance in 69% of patients with application every 2-4 weeks. Silverberg et al showed a complete clearance in 58% of patients (n=61) when SADBE was applied 3 times weekly.

SADBE has also been used with some success in the treatment of alopecia areata. In a review of the literature, Rokhsar et al noted a 50% to 60% success rate of SADBE in use for hair re-growth in this population.

SADBE has been reported to cause eczema, lymphadenopathy, blistering, allergic contact dermatitis, skin hypopigmentation, a burning sensation after application, and systemic reactions including fever and arthralgias. A study completed by Oglio et al of eight patients treated with SADBE for warts noted only mild and well tolerated side effects of erythema, desquamation, cutaneous edema, pruritus, burning, and pain.

SADBE induces a delayed-type hypersensitivity response which in warts, is believed to induce the killing of virally infected cells by cytotoxic lymphocytes. This influx of lymphocytes into lesional tissue may also enhance the recognition and processing of viral antigens, leading to clonal expansion of effector cells. It is hoped that SADBE will offer subjects a safe and effective therapeutic option to decrease the frequency and severity of future herpes labialis outbreaks through these mechanisms.

A placebo-controlled clinical study completed at Massachusetts General Hospital showed that squaric acid prevented recurrence of herpetic lesions. The effect of SADBE of delaying new herpes labialis outbreaks was highly significant ( $p < 0.01$ ) as compared to placebo. (Throughout this document, where the term "squaric acid" is used, it refers to squaric acid dibutyl ester (SADBE).)

### III. Outcomes

#### Primary Outcome Measure:

##### Localized adverse events:

Particularly, localized erythema, itching, and pain at the drug application site.

##### General adverse events:

Particularly, generalized allergic reaction, and any eczematous dermatitis anatomically distant from the drug application site and consistent with autosensitization, other than dermatitis of a herpes labialis lesion, that is assessed as possibly related to the study medication.

#### Secondary Outcome measures:

- Number of days until subject reported first new herpes labialis episode following the first dose on day 0.
- Number of days until subject reported first new herpes labialis episode following the dose applied at the 9-week visit for groups A and B.

### IV. Subject Selection

#### A. Inclusion criteria

1. Age  $\geq 18$  and  $\leq 65$
2. Clinical diagnosis of herpes labialis, which may be made at the screening visit based on the patient's self-reported history of symptoms. An active herpes labialis outbreak at the time of entry into the clinical trial will neither be required nor will be an exclusion criterion.
3. Self report having four (4) or more episodes of herpes labialis in the past 12 months. Subjects will NOT be told that four-or-more episodes in the previous 12 months is the entry criterion. Subjects will be asked "How many separate episodes of cold sores have you had in the previous 12 months?" They will be included if they give an answer of four or more and excluded if they give an answer of three or fewer.
4. At least half of the subject's episodes of the previous 12 months should be vesicular in nature and at least half preceded by prodromal symptoms. Prodromal symptoms may include tingling, itching, burning or pain before the development of a herpetic lesion.

#### B. Exclusion criteria

1. Pregnant or lactating females.
2. Current or recurrent non-herpetic infection or any underlying condition that may predispose to infection or anyone who has been admitted to the hospital due to bacteremia, pneumonia or any other serious infection in the last 12 months.
3. Therapy with glucocorticoid or immunosuppressants at time of recruitment or within past 4 weeks prior to the screening visit, or at any time during the study (including inhaled corticosteroids for asthma), except for topical steroids in sites other than face.
4. History of malignancy (except patients with surgically cured basal cell or squamous cell skin cancers).
5. History of organ transplantation.

6. HIV-positive status determined by history at screening or known history of any other immunosuppressive disease.
7. Severe co-morbidities (CHF [NYHA class II or worse], MI, CVA or TIA) within 3 months of screening visit, current unstable angina pectoris or oxygen-dependent severe pulmonary disease.
8. Known hypersensitivity to Dimethyl sulfoxide (DMSO).
9. Any condition judged by the investigator to cause this clinical trial to be detrimental to the patient.
10. Subject is currently enrolled in another investigational device or drug trial(s), or subject has received other investigational agent(s) within 28 days of the screening visit.
11. Previous exposure to SADBE (squaric acid or squaric acid dibutyl ester).
12. Subject has an abnormal skin condition (e.g., acne, eczema, rosacea, psoriasis, albinism, or chronic vesiculo-bullous disorder) that occurs in the area ordinarily affected by herpes labialis
13. Subject has an abnormal skin condition (e.g., eczema, rosacea, psoriasis, albinism, or chronic vesiculo-bullous disorder) that occurs in the inner aspect of either upper arm (the area where drug will be applied).
14. Subject has had a vaccine for either HSV-1 or HSV-2.
15. Subject has had treatment with anti-viral therapy (including ABREVA) within 2 weeks before first dose of SADBE or at any time during the study.

### **C. Treatment Groups**

Subjects will receive on a double blind basis one of two treatment regimens:

- Group A: 15 subjects, treated with 2% SADBE on day 0 and with 2% SADBE on the visits at week 3, week 6, week 9, and month 8.
- Group B: 15 subjects, treated with 2% SADBE on day 0 and with 0.5% SADBE on the visits at week 3, week 6, week 9, and month 8.

And subjects will be recruited on an open label basis to two other treatment regimens:

- Group C: 15 subjects, treated with 2% SADBE on day 0, month 3, and month 6.
- Group D: 15 subjects, treated with 2% SADBE on day 0 and month 6.

The blind only applies between groups A and B. A subject and the site personnel will know which subjects are in group C and group D, and will know that a subject is in either group A or B, but not whether the subject is in group A or is in group B.

### **V. Subject Enrollment**

Preliminary eligibility will be determined based on study staff interviews of interested subjects over the phone. Eligible subjects will then be scheduled for a screening visit. Sixty (60) subjects overall will be enrolled.

#### **A. Methods of Enrollment**

All subjects who sign an informed consent form (ICF) and are screened will be documented on the enrollment log. A note will be made in the source documentation verifying that the subject has willingly signed the ICF prior to participation in any study procedures. All enrolled subjects will receive a subject number to ensure their protected health information (PHI) and subject anonymity.

Adult men and women of any race or and ethnic group may participate in this study.

Women of childbearing potential must agree to use adequate birth control while in the study and for a period of one month after use of the study medication. Examples of acceptable contraceptive methods include, abstinence, oral contraceptives, implantable hormonal contraceptives, or double barrier methods.

No minors (i.e., <18 years of age) will be included in this study.

## **B. Informed Consent**

The Investigator or designee will inform the potential study subject of all aspects of the study and answer all their questions. If the subject agrees to be a study subject, they will document their consent in writing by signing an ICF. If a subject needs more time to think about study participation, they will be given a copy of the consent and sign it upon return if they elect to participate. The investigator is responsible for using a consent form that has been approved by the IRB and is the most current version. If a new version of the consent form is approved by the IRB while a subject is in the treatment portion of the study, then the investigator or designee will inform the subject of the changes and, if the subject agrees to continue treatment, both the investigator and subject should sign the updated form.

## **VI. Study Procedures**

### ***Screening Visit (Day 0) (Groups A, B, C, and D)***

During the screening visit, the investigator will discuss with each subject the nature of the study, its requirements and its restrictions.

The following will be performed:

- Review and sign ICF
- Medical history and demographics
- Physical examination (targeted skin examination) and vital signs (oral temp, blood pressure, heart rate, respiration rate after resting supine for 5 minutes)
- Urine pregnancy test (for female participants of childbearing potential)
- Review of concomitant medications
- Review of inclusion/exclusion criteria
- Diary distribution and explanation and explanation of the need for subjects to complete paper or electronic diaries during each day of the study during which they are experiencing a herpes labialis outbreak or redness, irritation, or rash at the drug application site.
- Explanation of the need for subjects to take digital photographs with their own smart phones or digital cameras of affected areas of their face on each day of the study that they are experiencing a herpes labialis outbreak and to take photographs of the drug application site on each day of the study during which they are experiencing redness, rash, or irritation at the drug application site.
- Blood collection
  - 3 ml EDTA whole blood collected for CBC with differential
  - 10 ml serum collected for anti-HSV-1 and anti-HSV-2 IgG assays.
- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak
- Targeted skin exam (at drug application site). Exclude the subject from the study if the subject is experiencing any redness, rash, or irritation at the drug application site before drug application.
- Adverse events collection.
- When all Inclusion and Exclusion Criteria has been reviewed and the subject meets eligibility requirements, the subject will then be permitted to receive study medication.
- SADBE application (2% SADBE solution for all subjects) on the inner aspect of the left upper arm (application of Dose 1, from a vial labeled xxx-1 for Dose 1 to patient number xxx). The drug solution (supplied by Squarex) is topically applied to the inner aspect of the upper **left** arm of the subject and covered with Tegaderm. The drug application

area should be approximately 1 cm in diameter. (Subsequent doses will be applied on alternate arms to maximize time between applications to the same spot.) Subject is advised to remove the Tegaderm and rinse and wipe off the application spot with a wet paper towel 6-12 hours after drug application and then dry the application spot. When applying the drug solution, the cotton swab should be rested in the drug solution vial and the vial and swab weighed together on a scale with precision to 0.0001 grams immediately before dosing. Immediately after dosing, the swab is placed in the same vial again on the scale and the weight measured again. The weights before and after drug application are recorded. The difference in weight is the weight of drug solution applied. Investigators should attempt to apply 20-30 mg of drug solution.

### **Day 3 (3 days after the Day 0 visit) (Groups A, B, C, and D)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site) (Score for erythema at drug application site on a 0-3 scale.)
- Photograph the drug application site.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection. (Query subject to score pain at drug application site, itching at drug application site, and redness at drug application site, each on a 0-3 scale, as in the subject diary.)

### **3 weeks (Day 21 ±3) (Groups A and B only)**

The following procedures will be performed during this visit:

- Urine pregnancy test (for female participants of childbearing potential)
- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site)
- Photograph the drug application site if the subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection.
- SADBE application (2% or 0.5% on double blind basis) on the inner aspect of the right upper arm (application of Dose 2, from a vial labeled xxx-2 for Dose 2 to patient number xxx). The drug solution (Supplied by Squarex) is topically applied to the inner aspect of the upper **right** arm of the subject and covered with Tegaderm. Subject is advised to remove the Tegaderm and rinse and wipe off the application spot with a wet paper towel 6-12 hours after drug application and then dry the application spot. When applying the drug solution, the cotton swab should be rested in the drug solution vial and the vial and swab weighed together on a scale with precision to 0.0001 grams immediately before dosing. Immediately after dosing, the swab is placed in the same vial again on the scale and the weight measured again. The weights before and after drug application are recorded. The difference in weight is the weight of drug solution applied.

**3 weeks plus 3 days (3 days after the 3-week visit) (Groups A and B only)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site) (Score for erythema at drug application site on a 0-3 scale.)
- Photograph the drug application site.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection. (Query subject to score pain at drug application site, itching at drug application site, and redness at drug application site, each on a 0-3 scale, as in the subject diary (see Appendix B).

**6 weeks (Day 42 ±3) (Groups A and B only)**

The following procedures will be performed during this visit:

- Urine pregnancy test (for female participants of childbearing potential)
- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site)
- Photograph the drug application site, if the subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection.
- SADBE application (2% or 0.5% on double blind basis) on the inner aspect of the left upper arm (application of Dose 3, from a vial labeled xxx-3 for Dose 3 to patient number xxx). The drug solution (Supplied by Squarex) is topically applied to the inner aspect of the upper **left** arm of the subject and covered with Tegaderm. Subject is advised to remove the Tegaderm and rinse and wipe off the application spot with a wet paper towel 6-12 hours after drug application and then dry the application spot. When applying the drug solution, the cotton swab should be rested in the drug solution vial and the vial and swab weighed together on a scale with precision to 0.0001 grams immediately before dosing. Immediately after dosing, the swab is placed in the same vial again on the scale and the weight measured again. The weights before and after drug application are recorded. The difference in weight is the weight of drug solution applied.

**6 weeks plus 3 days (3 days after the 6-week visit) (Groups A and B only)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site) (Score for erythema at drug application site on a 0-3 scale.)
- Photograph the drug application.

- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection. (Query subject to score pain at drug application site, itching at drug application site, and redness at drug application site, each on a 0-3 scale, as in the subject diary.)

**9 weeks (Day 63 ±3) (Groups A and B only)**

The following procedures will be performed during this visit:

- Urine pregnancy test (for female participants of childbearing potential)
- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak, or has since the previous clinic visit.
- Targeted skin exam (at drug application site)
- Photograph the drug application site if the subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection.
- SADBE application (2% or 0.5% on double blind basis) on the inner aspect of the right upper arm (application of Dose 4, from a vial labeled xxx-4 for Dose 4 to patient number xxx). The drug solution (Supplied by Squarex) is topically applied to the inner aspect of the upper **right** arm of the subject and covered with Tegaderm. Subject is advised to remove the Tegaderm and rinse and wipe off the application spot with a wet paper towel 6-12 hours after drug application and then dry the application spot. When applying the drug solution, the cotton swab should be rested in the drug solution vial and the vial and swab weighed together on a scale with precision to 0.0001 grams immediately before dosing. Immediately after dosing, the swab is placed in the same vial again on the scale and the weight measured again. The weights before and after drug application are recorded. The difference in weight is the weight of drug solution applied.

**9 weeks plus 3 days (3 days after the 9-week visit) (Groups A and B only)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak, or has since the previous clinic visit.
- Targeted skin exam (at drug application site) (Score for erythema at drug application site on a 0-3 scale.)
- Photograph the drug application site.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection. (Query subject to score pain at drug application site, itching at drug application site, and redness at drug application site, each on a 0-3 scale, as in the subject diary.)
- Blood collection
  - 3 ml EDTA whole blood collected for CBC with differential.

**3 months (3 months after day 0 ±7 days) (Group C only)**

The following procedures will be performed during this visit:

- Urine pregnancy test (for female participants of childbearing potential)
- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak, or has since the previous clinic visit.
- Targeted skin exam (at drug application site)
- Photograph the drug application site if the subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection.
- SADBE application (2% SADBE) on the inner aspect of the right upper arm. The drug solution (Supplied by Squarex) is topically applied to the inner aspect of the upper **right** arm of the subject and covered with Tegaderm. Subject is advised to remove the Tegaderm and rinse and wipe off the application spot with a wet paper towel 6-12 hours after drug application and then dry the application spot. When applying the drug solution, the cotton swab should be rested in the drug solution vial and the vial and swab weighed together on a scale with precision to 0.0001 grams immediately before dosing. Immediately after dosing, the swab is placed in the same vial again on the scale and the weight measured again. The weights before and after drug application are recorded. The difference in weight is the weight of drug solution applied.

**3 months plus 3 days (3 days after the 3-month visit) (Group C only)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak, or has since the previous clinic visit.
- Targeted skin exam (at drug application site) (Score for erythema at drug application site on a 0-3 scale.)
- Photograph the drug application site.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection. (Query subject to score pain at drug application site, itching at drug application site, and redness at drug application site, each on a 0-3 scale, as in the subject diary.)

**6 months (6 months after day 0 ±7 days) (Groups C and D only)**

The following procedures will be performed during this visit:

- Urine pregnancy test (for female participants of childbearing potential)
- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site)
- Photograph the drug application site if the subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications

- Adverse events collection.
- SADBE application (2% SADBE) on the inner aspect of the upper arm. The drug solution (Supplied by Squarex) is topically applied to the inner aspect of the subject's (upper **left** arm for Group C, or the upper **right** arm for Group D) and is then covered with Tegaderm. Subject is advised to remove the Tegaderm and rinse and wipe off the application spot with a wet paper towel 6-12 hours after drug application and then dry the application spot. When applying the drug solution, the cotton swab should be rested in the drug solution vial and the vial and swab weighed together on a scale with precision to 0.0001 grams immediately before dosing. Immediately after dosing, the swab is placed in the same vial again on the scale and the weight measured again. The weights before and after drug application are recorded. The difference in weight is the weight of drug solution applied.

**6 months plus 3 days (3 days after the 6-month visit) (Groups C and D only)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site) (Score for erythema at drug application site.)
- Photograph the drug application site.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection. (Query subject to score pain at drug application site, itching at drug application site, and redness at drug application site, each on a 0-3 scale, as in the subject diary.)
- Blood collection
  - 3 ml EDTA whole blood collected for CBC with differential.

**7 months (30 days  $\pm$ 7 days after the 6-month visit) (Groups C and D only) (End of Study visit for Groups C and D)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site)
- Photograph the drug application site.
- Collection of photographs and diaries from subject.
- Vital Signs
- Adverse events collection.
- Blood collection
  - 3 ml EDTA whole blood collected for CBC with differential.

**8 months (8 months after day 0  $\pm$ 14 days) (Groups A and B only)**

The following procedures will be performed during this visit:

- Urine pregnancy test (for female participants of childbearing potential)
- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site)

- Photograph the drug application site if the subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection.
- SADBE application (2% or 0.5% on double blind basis) on the inner aspect of the upper arm (application of Dose E, from a vial labeled xxx-E for Dose E to patient number xxx). The drug solution (Supplied by Squarex) is topically applied to the inner aspect of the upper **left** arm of the subject and covered with Tegaderm. Subject is advised to remove the Tegaderm and rinse and wipe off the application spot with a wet paper towel 6-12 hours after drug application and then dry the application spot. When applying the drug solution, the cotton swab should be rested in the drug solution vial and the vial and swab weighed together on a scale with precision to 0.0001 grams immediately before dosing. Immediately after dosing, the swab is placed in the same vial again on the scale and the weight measured again. The weights before and after drug application are recorded. The difference in weight is the weight of drug solution applied.

**8 months plus 3 days (3 days after the 8-month visit) (Groups A and B only)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site) (Score for erythema at drug application site.)
- Photograph the drug application site.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection. (Query subject to score pain at drug application site, itching at drug application site, and redness at drug application site, each on a 0-3 scale, as in the subject diary.)

**9 months (30 days  $\pm$ 7 days after the 8-month visit) (Groups A and B only) (End of Study visit for Groups A and B)**

The following procedures will be performed during this visit:

- Query subject if the subject is currently having a herpes labialis (cold sore) outbreak or has since the previous clinic visit.
- Targeted skin exam (at drug application site)
- Photograph the drug application site.
- Collection of photographs and diaries from subject.
- Vital Signs
- Review of concomitant medications
- Adverse events collection.
- Blood collection
  - 3 ml EDTA whole blood collected for CBC with differential.

## **Photographs**

Digital photographs of the drug application site of each patient will be taken by clinic staff at each clinic visit during which a patient has any erythema or other localized adverse event at the study drug application site. Photographs of the drug application site will also be taken at each clinic visit 3 days after a drug application, regardless of whether the subject is then experiencing any erythema or other localized adverse event at the study drug application site.

In addition, patients will be asked to use their own smart phones or digital cameras to photograph the site of drug application when they experience any redness, rash, or irritation at that site. Those photographs should be taken once per day on any day they are experiencing rash, redness, or irritation at the drug application site and on the first day after rash, redness, or irritation have resolved.

## **Patient Contacts and Monitoring and Patient Diaries**

At the initial visit, patients will be given paper journals or instructed on electronic journals, which will be available through a website. Patients will be contacted weekly by text message, E-mail message or phone call. Each patient will be asked if he or she experienced a new outbreak and if he or she experienced any adverse events. Each patient will be reminded to fill out his or her diary on any day that he or she experienced an outbreak or an adverse event.

All patients will be instructed to fill out their diaries at least weekly, even if they have not had an outbreak or adverse event to document the lack of outbreaks and adverse events.

## **VII. Risks and discomforts**

Possible side effects of squaric acid dibutyl ester include:

1. Localized erythema
2. Increasing lesional inflammation.
3. Pruritus
4. Contact dermatitis
5. Lymphadenopathy
6. Vitiligo or leukoderma
7. Generalized allergic reaction
8. Blistering
9. Burning sensation with application
10. Fever
11. Arthralgias

## **VIII. Potential Benefits**

### **A. Potential benefits to subjects**

Subjects may or may not benefit from participating in the study.

### **B. Potential benefits to society**

Information gathered from this study may help other people in the future with herpes labialis.

## **IX. Monitoring and Quality Assurance**

Written informed consent will be obtained from each subject at the initiation of the screening visit. In all cases, the consent will be witnessed by an appropriate health care professional. A copy of the signed consent form will be given to the subject to keep. All efforts will be made to insure the privacy rights of the study subject.

### **A. Study Drug Management**

Study medication will be provided by Squarex and shipped directly to the clinical site.

All study medications will be retained in secure and restricted access storage by the Investigator's designee for the duration of the study. All study medication will be stored frozen (-15 to -25°F or -26 to -32°C). Each dose of study medication should be removed from the freezer and placed at room temperature one business day before the appointment of the patient for that dose of study medication. If the patient cancels the appointment, the thawed dose of study medication should continue to be stored at room temperature for up to 3 months. Controlled access to study medications will be maintained until Squarex, Inc. has completed final drug accountability and provided instructions for drug return and destruction.

### **B. Data and Safety Monitoring Plan**

This study is considered to be moderate risk to human subjects as the study drug is not FDA approved, but is commonly used for treatment of warts and alopecia areata.

### **C. Data and Drug Handling Guidelines**

Data will be transcribed to an Electronic Case Report Form (ECRF) and will be complete and accurate based on available source documentation. Corrections of data will be made in a manner that allows Squarex to track changes according to FDA regulations. The investigator will respond to inquiries regarding data errors, inconsistencies, and missing data in a timely fashion.

The study site will keep all study records, including source docs, CRFs, signed ICFs, regulatory papers, patient logs, drug accountability logs, etc. until Squarex determines that they can be returned or destroyed. The investigator will follow the procedures outlined in the protocol and discuss any deviations with Squarex.

#### **Site Monitoring of Source Data**

The principal investigator and members of the study staff, not directly involved in clinical assessments or clinical study procedures, will monitor the study. All data relevant to the assessments outlined in this protocol will be recorded in the case report form (ECRF) and the subject's sourcebook.

The original case report form for each subject will be audited to source documents at the study site by the study monitor. The study staff monitor will review the progress of the study to ensure proper study conduct and accurate data collections through ongoing reviews of Case Report Forms, clinical records, and administrative documents. Reviews will be made at least once a month.

## **D. Sponsor Monitoring of Study Data**

Representatives of Squarex will also monitor this study's data regularly via scheduled monitoring visits. Monitoring procedures include pre-study preparations, site initiation visit, interim monitoring visits, and study close-out preparations and visits. During sponsor's visits, the Investigator will provide Squarex's monitors with access to all protocol regulatory documents, all medical data collected on the participating subjects, screening logs, enrollment logs, drug accountability logs and the drug supply, case report forms, signed informed consent forms, and any other information that Squarex may consider to be necessary to evaluate the safety of the investigational product and patient safety. Both the monitors and study staff will review the accuracy and completeness of case report form entries, all log entries, source documents, and informed consent documents.

## **E. Adverse event reporting guidelines**

### **Definitions**

Adverse Event (AE) is any untoward medical occurrence in a subject that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

Serious Adverse Event (SAE) is any untoward medical occurrence that:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability or incapacity,
- Is a congenital anomaly/birth defect, or
- Is another medically important condition

### **Reporting and Documenting Adverse Events**

All untoward medical occurrences that occur after the subject signs a consent form should be documented as an AE. The Investigator should ensure that all events that occur during the study period are recorded. All AEs should be followed until resolution or until, in the Investigator's judgment, they are chronic and stable. If an emergency situation should occur, appropriate medical measures should be taken to stabilize the subject.

General adverse events will be elicited by the physician and his or her staff through examination and general interview. Adverse events specific to evaluating the primary outcome of the study (e.g., localized erythema, itching, and pain at the drug application site) will be captured through examination and direct questioning. For example, a subject will be asked, "Have you had any redness around the area where the medication was applied?" Subjects will be asked to complete a weekly entry into an electronic or paper diary where information on the occurrence or nonoccurrence of specific application-site adverse events will be recorded.

Documentation of AEs includes: date and time of onset and resolution of AE, intensity, frequency, seriousness, related interventions and outcome. The Investigator must also evaluate the probability of a causal relationship of the AE to the study treatment as being: "definite, probable, possible, unlikely, or unrelated." Intensity of adverse events will be graded as mild, moderate, or severe according to the following criteria:

Mild: symptoms that are easily tolerated and transient in nature with minimal or no impairment of normal activity

Moderate: symptoms that are poorly tolerated, are sustained, and interfere with normal activity

Severe: symptoms that are incapacitating and render the subject unable to work or participate in many or all usual activities

All SAEs will be reported to the IRB according to the IRB's requirements. They will also be reported to the study sponsor and FDA according to regulatory guidelines.

### **Study Discontinuation**

At any time after enrollment, a subject may be discontinued. Reasons for discontinuation of a subject from the study will include, but may not be limited to, the following:

- Subject is found to be intolerant to a required study procedure at any time point
- Subject experiences a serious adverse experience at any time point.
- Subject develops an inter-current illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree.
- Subject begins a medication that, in the judgment of the investigator, may affect assessments of clinical status to a significant degree.
- Subject becomes pregnant while participating in the study
- Subject enrolls in another investigational study
- Subject requests to withdraw from the study
- Subject who is non-compliant with study specific visits
- The study sponsor decides to suspend or terminate the study.

Subjects will be discontinued from the study if they have a moderate or severe rash at a drug application site (score of 2 or 3 on Patient Assessment for pain or redness, but not itching, or score of 2 or 3 for erythema on Provider Assessment on a scale of 0-3) at any time, if they have any SAE, and if they have any generalized allergic reaction assessed as possibly related to the study medication or any eczematous dermatitis anatomically distant from the drug application site and consistent with autosensitization, other than dermatitis of a herpes labialis lesion, that is assessed as possibly related to the study medication. Discontinued subjects will be followed until the adverse event resolves.

## **X. STATISTICAL METHODS**

A brief overview of the statistical analysis plan is presented below. Complete details of the planned analysis will be documented in a full Statistical Analysis Plan, which will be finalized before locking the study database.

Statistical analyses will be performed using SAS software. Default estimation methods in version 9.4 of SAS are always used unless an alternative is specified below. In general, data summaries will include the mean, standard deviation, median, minimum and maximum values for continuous data (e.g., changes in CBC measurements); the median, 25th and 75th percentiles, minimum and maximum values for time-to-event endpoints (e.g., number of days until subject reported first new herpes labialis episode following the first dose on day 0); and the number and percentage of patients in each category for categorical data (e.g., number of subjects experiencing specific adverse events). Pointwise 95% confidence intervals (CI) will also be estimated for the mean (continuous data), median (time-to-event endpoints) or percentage of patients (categorical data). A series of power transformations (including the log transformation) may be applied to observed values of continuous outcome measures if the distribution of raw (i.e., untransformed) data do not appear to follow a normal distribution.

There is no plan for case-control matching that would permit pairing of subjects between the four treatment arms. Therefore, unpaired data analyses will be performed on screening data across the four groups. Analysis of variance (ANOVA) models will be fit to numeric outcome measures such as serum cytokine levels. Any categorical outcome measures will be summarized using within-group frequency tables.

Longitudinal data from patients (screening to the end of study) will be analyzed using appropriate statistical techniques appropriate for paired observations such as mixed-model for repeated measures to estimate 95% confidence intervals for the difference between screening and follow-up values.

The Sponsor plans to perform an interim data analysis after the week 9 visits. The blind will be maintained for the site personnel and patients until the end of the study. (The blind only applies between groups A and B. A subject and the site personnel will know which subjects are in group C and group D, and will know that a subject is in either group A or B, but not whether the subject is in group A or in group B.)

## **Sample Size**

The primary and secondary objectives of this study will be addressed with descriptive statistics and subjective comparisons of outcome measures between the four groups and within each group from screening to the end of follow-up for each subject. Although statistical methods will be employed to estimate means, variances and confidence intervals, no formal statistical hypotheses will be tested. Therefore, the sample size of 15 subjects per group (60 subjects total) was selected purely based on clinical judgement.

## **Safety Analyses**

Reported adverse event (AE) terms will be mapped to MedDRA preferred terminology. All reported events will appear in AE listings, however only treatment-emergent adverse events will be summarized. A treatment-emergent adverse event (TEAE) is an AE that starts or increases in severity any time after the first application of any study drug up to 30 days following the last application of any study drug.

Reported adverse event (AE) terms will be mapped to MedDRA preferred terminology. All reported events will appear in AE listings, however only treatment-emergent adverse events will be summarized. A treatment-emergent adverse event (TEAE) is an AE that starts or increases in severity any time after the first application of any study drug up to the time the patient withdraws from the study and, if the patient withdraws from the study within 30 days after any application of study drug, then for a minimum of 30 days following the last application of any study drug.

Incidence rates of treatment-emergent adverse events will be summarized within treatment group at the MedDRA preferred term and primary system organ class levels. Similar summaries will be made for subsets of AEs such as (1) those judged by the Investigator to be related to study treatment, and (2) serious adverse events (SAEs). Other routine safety assessments (e.g., clinical laboratory parameters and vital signs) will be summarized by standard deviation, median, minimum and maximum observed values and changes from baseline values. NCI-CTCAE severity grades for laboratory parameters and other endpoints measured on an ordinal categorical scale may be summarized using shift tables (a two-way frequency table pairing baseline value with the most extreme post-baseline result).

## **References**

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6. Koch GG, Gansky SA. Statistical Considerations for Multiplicity in Confirmatory Protocols. *Drug Information J*. 1996;30:523-533. Available at: <http://ferran.torres.name/download/shared/general/Multiplicity%20dj302612.pdf>. Accessed February 5, 2015.

**Appendix A.**

Subject #:	<input type="text"/>	<input type="text"/>	<input type="text"/>		<i>Month</i>	<i>Day</i>	<i>Year</i>
Subject Initials:	<input type="text"/>	<input type="text"/>	<input type="text"/>	Visit Date:	<input type="text"/>	<input type="text"/>	<input type="text"/>

**Provider Assessment of Local Irritation from Therapy**

1. Please assess for local irritation from therapy. Please circle the choice that most accurately describes the treatment response:

- 0 = no rash,
- 1 = mild rash
- 2 = moderate rash
- 3 = severe rash in need of treatment with topical hydrocortisone

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

## Appendix B

Subject #:	<input type="text"/>	<input type="text"/>	<input type="text"/>		<i>Month</i>	<i>Day</i>	<i>Year</i>
Subject Initials:	<input type="text"/>	<input type="text"/>	<input type="text"/>	Date:	<input type="text"/>	<input type="text"/>	<input type="text"/>

### **Patient Assessment of Disease Severity and Local Irritation from Therapy: please encircle your answers.**

1. Please assess for local irritation from therapy at the drug application site on your arm;  
0 = no rash (redness)  
1 = mild rash (redness)  
2 = moderate rash (redness)  
3 = severe rash (redness)
2. Please assess for local irritation from therapy at the drug application site on your arm;  
0 = no pain  
1 = mild pain  
2 = moderate pain  
3 = severe pain
3. Please assess for local irritation from therapy at the drug application site on your arm;  
0 = no itching  
1 = mild itching  
2 = moderate itching  
3 = severe itching
4. Do you have any HSV lesions present:    Yes            No
5. Location of lesions (please label the following):



6. Redness at HSV lesion:  
0 = no redness  
1 = faint pink  
2 = red  
3 = purple
7. Pain at HSV lesion:  
0 = no pain  
1 = mild pain  
2 = moderate pain  
3 = severe pain
8. Tingling at HSV lesion:  
0 = no tingling  
1 = mild tingling  
2 = moderate tingling  
3 = severe tingling

9. Nature of HSV lesion (check all that apply):
- a. prodrome (tingling, no visible rash)
  - b. erythema (redness)
  - c. papule (raised skin)
  - d. vesicle (fluid-filled raised skin)
  - e. erosion or ulcer (open sore)
  - f. crust (dry sore)
  - g. normal skin

Signature: \_\_\_\_\_

### Appendix C: SQX P2c Study Schema for all Groups (A, B, C, D)

Groups	A, B, C, D	A, B, C, D	A, B	A, B	A, B	A, B	A, B	A, B	C	C	C, D	C, D	End Study C, D	A, B	A, B	End Study A, B
Schedule of Procedures	Screening Day 0	Week 0 Follow-up 3 Days after Wk 0	Week 3	Week 3 Follow-up: 3 days after Wk 3	Week 6	Week 6 Follow-up: 3 days after Wk 6	Week 9	Week 9 Follow-up: 3 days after Wk 9	Month 3	Month 3 Follow-up: 3 days after Month 9	Month 6	Month 6 Follow-up: 3 days after Month 6	Month 7	Month 8	Month 8 Follow-up: 3 days after Month 8	Month 9
Informed Consent	X															
Inclusion/ Exclusion	X															
History/Demographics	X															
Physician Assessments (Targeted Skin Exam)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Pregnancy Test	X		X		X		X		X		X			X		
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Photography	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Diary Distribution/ Instruction	X															
Instruction for taking Self-photographs	X															
Collect Photographs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Collect Diary		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Collection (Processing, storing, shipping, dry ice)	X							X				X	X			X
CBC with Differential	X							X				X	X			X
Anti-HSV ½ IgG testing	X															
Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Collect Adverse Events	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Squaric Acid Application	X		X		X		X		X		X			X		

### Appendix D: SQX P2c Study Schema for Subject Groups A&B

Groups	A, B,	A, B,	A, B	A, B	A,B	A, B	A, B	A, B	A, B	A, B	End of Study (A, B)
<b>Schedule of Procedures</b>	Screening Day 0	Week 0 Follow-up 3 Days after Wk 0	Week 3	Week 3 Follow-up: 3 days after Wk 3	Week 6	Week 6 Follow-up: 3 days after Wk 6	Week 9	Week 9 Follow-up: 3 days after Wk 9	Month 8	Month 8 Follow-up: 3 days after Month 8	Month 9
Informed Consent	X										
Inclusion/ Exclusion	X										
History/Demographics	X										
Physician Assessments (Targeted Skin Exam)	X	X	X	X	X	X	X	X	X	X	X
Urine Pregnancy Test	X		X		X		X		X		
Vital Signs	X	X	X	X	X	X	X	X	X	X	X
Photography	X	X	X	X	X	X	X	X	X	X	
Diary Distribution/ Instruction	X										
Instruction for taking Self-photographs	X										
Collect Photographs		X	X	X	X	X	X	X	X	X	X
Collect Diary		X	X	X	X	X	X	X	X	X	X
Blood Collection (Processing, storing, shipping, dry ice)	X							X			X
CBC with Differential	X							X			X
Anti-HSV ½ IgG testing	X										
Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X
Collect Adverse Events	X	X	X	X	X	X	X	X	X	X	X
Squaric Acid Application	X		X		X		X		X		

### Appendix E: SQX P2c Study Schema for Subject Group C

Group	C,	C,	C	C	C	C	End of Study Group C
<b>Schedule of Procedures</b>	<b>Screening Day 0</b>	<b>Week 0 Follow-up 3 Days after Wk 0</b>	<b>Month 3</b>	<b>Month 3 Follow-up: 3 days after Month 9</b>	<b>Month 6</b>	<b>Month 6 Follow-up: 3 days after Month 6</b>	<b>Month 7</b>
Informed Consent	X						
Inclusion/ Exclusion	X						
History/Demographics	X						
Physician Assessments (Targeted Skin Exam)	X	X	X	X	X	X	X
Urine Pregnancy Test	X		X		X		
Vital Signs	X	X	X	X	X	X	X
Photography	X	X	X	X	X	X	X
Diary Distribution/ Instruction	X						
Instruction for taking Self-photographs	X						
Collect Photographs		X	X	X	X	X	X
Collect Diary		X	X	X	X	X	X
Blood Collection (Processing, storing, shipping, dry ice)	X					X	X
CBC with Differential	X					X	X
Anti-HSV ½ IgG testing	X						
Concomitant Medication	X	X	X	X	X		X
Collect Adverse Events	X	X	X	X	X		X
Squaric Acid Application	X		X		X		

## Appendix F: Study Schema for Subject Group D

Group	D	D	D	D	End of Study Group D
Schedule of Procedures	Screening Day 0	Week 0 Follow-up 3 Days after Wk 0	Month 6	Month 6 Follow-up: 3 days after Month 6	Month 7
Informed Consent	X				
Inclusion/ Exclusion	X				
History/Demographics	X				
Physician Assessments (Targeted Skin Exam)	X	X	X	X	X
Urine Pregnancy Test	X		X		
Vital Signs	X	X	X	X	X
Photography	X	X	X	X	X
Diary Distribution/ Instruction	X				
Instruction for taking Self-photographs	X				
Collect Photographs		X	X	X	X
Collect Diary		X	X	X	X
Blood Collection (Processing, storing, shipping, dry ice)	X			X	X
CBC with Differential	X			X	X
Anti-HSV ½ IgG testing	X				
Concomitant Medication	X	X	X		X
Collect Adverse Events	X	X	X		X
Squaric Acid Application	X		X		