# A PHASE 2, MULTI-SITE, RANDOMIZED, DOUBLE-BLIND, VEHICLE-CONTROLLED STUDY OF THE EFFICACY AND SAFETY OF SQUARIC ACID DIBUTYL ESTER IN SUBJECTS WITH RECURRENT HERPES LABIALIS – SINGLE VERSUS TWO-DOSE ARM APPLICATION

**Detailed Protocol**

**Amendment 5**


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## I. Objectives

**Primary Objective:** To compare the efficacy of SADBE versus placebo (DMSO) in the treatment of herpes labialis

**Secondary Objective:** To evaluate the safety and tolerability of SADBE versus placebo (DMSO) in the treatment of herpes labialis

## 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 of warts 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:**

1. No. of days until subject reported first new herpes labialis episode following sensitization dose (Dose A).

**Secondary Outcome Measures:**

1. Incidence of adverse events
2. Number of days until subject reported first new herpes labialis episode following the intensification dose (Dose B).
3. Number of days until subject reported first new herpes labialis episode beginning from 21 days after the intensification dose (Dose B).
4. Number of new herpes labialis episodes during the first 4 months following the sensitization dose (Dose A).
5. Number of new herpes labialis episodes during the first 6 months following the sensitization dose (Dose A).
6. Number of new herpes labialis eposides during the 12-month follow up period following the sensitization dose (Dose A).
7. Number of new herpes labialis episodes during the first 4 months following the intensification dose (Dose B).
8. Number of new herpes labialis episodes during the first 6 months following the intensification dose (Dose B).
9. Number of new herpes labialis eposides during the 12-month follow up period following the intensification dose (Dose B).
10. Number of days with herpes labialis during the first 4 months following the sensitization dose (Dose A).
11. Number of days with herpes labialis during the first 6 months following the sensitization dose (Dose A).
12. Number of days with herpes labialis during the 12-month follow-up period following the sensitization dose (Dose A).
13. For only the subjects who are positive for IgG against HSV-2, number of days until a subject reports his or her first new herpes labialis episode following the sensitization dose (Dose A).
14. For only the subjects who are positive for IgG against HSV-2, number of days until a subject reports his or her first new herpes labialis episode following the intensification dose (Dose B).
15. Average duration of herpes labialis episodes during the 12-month follow up period following the sensitization dose (Dose A).
16. Average duration of herpes labialis episodes during the follow up period following the intensification dose (Dose B).
17. Change in anti-HSV-1 IgG levels between the screening visit and the 2-month visit.
18. Change in lymphocyte counts between the screening visit and the 2-month visit.

Each of the primary and secondary outcome measures will first be made by pooling the two drug treatment groups together and comparing the pooled drug treatment groups versus the placebo.

Secondarily, where appropriate, each of the two drug treatment groups will be separately compared to placebo and compared to each other.

## IV. Subject Selection

### A. Inclusion criteria

1. Age ≥18
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 criteria.
3. Self report having four 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.

### B. Exclusion criteria

1. People that have had treatment with anti viral therapy within 2 weeks before sensitization dose.
2. Pregnant or lactating females.
3. 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 within the past 12 months.
4. Therapy with glucocorticoid or immunosuppressants at time of recruitment or within past 4 weeks, except for inhaled corticosteroids for asthma or topical steroids in sites other than face.
5. History of malignancy (except patients with surgically cured basal cell or squamous cell skin cancers)
6. History of organ transplantation
7. HIV-positive status determined by history at screening or known history of any other immunosuppressive disease.
8. Severe co-morbidities (diabetes mellitus requiring insulin, CHF (EF<50% at baseline will be exclusionary) MI, CVA or TIA within 3 months of screening visit, unstable angina pectoris, oxygen-dependent severe pulmonary disease
9. History of exposure to squaric acid or squaric acid dibutyl ester.
10. Known hypersensitivity to DMSO
11. Any condition judged by the investigator to cause this clinical trial to be detrimental to the patient.
12. Subject is currently enrolled in another investigational device or drug trial(s), or subject has received other investigational agent(s) within 28 days of baseline visit.
13. Previous or current participation in a clinical trial of SADBE to treat herpes labialis.
14. Subject cannot be reliably expected to comprehend or satisfactorily assess a herpetic lesion.
15. Subject has an abnormal skin condition (e.g., acne, eczema, rosacea, psoriasis, albinism, or chronic visiculo-bullous disorder) that occurs in the area ordinarily affected by herpes labialis.
16. Subject has had a vaccine for either HSV-1 or HSV-2.

### C. Randomization

Randomization will be performed by a computer generated randomization table and will be blinded to study investigators and subjects.

Subjects will be randomized to one of three groups on a double blind basis:

1. Only sensitization dose.
2. One treatment dose applied to the patient's upper arm 3 weeks after sensitization dose.
3. Placebo. Will receive DMSO alone (no SADBE) as placebo on a double blind basis.

| Group | Sensitization (Dose A) | 3-weeks after sensitization (Dose B) |
|---|---|---|
| 1 | Arm (2%) | Arm (placebo) |
| 2 | Arm (2%) | Arm (0.5%) |
| 3 (Placebo) | Arm (placebo) | Arm (placebo) |

Subjects in groups 1 and 2 will receive 2% SADBE for sensitization. Subjects in group 2 will receive 0.5% SADBE 3 weeks after the sensitization dose is applied. All subjects will receive their dose at 0 weeks and at 3 weeks whether they have an active cold sore at that time or not. But the presence or absence of active cold sores at the time the doses are applied will be noted.

In order to maintain blinding, the study staff applying study medication will not be involved in any of the subject assessments.

## 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. We plan to randomize and follow for at least 4 months a total of 94 subjects in the study. To achieve this goal, we may have to randomize and enroll 120 subjects and screen up to 200 subjects.

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

### B. Informed Consent

The Investigator or sub-investigator will inform the potential study subject of all aspects of the study and answer all their questions. If the patient 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. No minors will be included in this study.

## VI. Study Procedures

### Screening Visit

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 of inclusion/exclusion criteria
- Medical history and demographics
- Physical examination (targeted skin examination) and vital signs
- 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.
- Photograph mouth and nose area of subject face below the eyes if subject is experiencing an outbreak in that area at the time of the clinic visit.
- Blood collection for
    - CBC with Diff
    - HSV-1 and -2 Ab IgG
- Swab herpetic lesion to collect sample* for
    - HSV-1 and -2 PCR*
- Urine pregnancy test (for female participants of childbearing potential)
- Review of concomitant medications
- Adverse Events collection
- Squaric acid sensitization on the right upper arm (application of Dose A, from a vial labeled xxx-A for Dose A to patient number xxx).

* Viral specimen collection for HSV-1 and HSV-2 PCR testing will be conducted on the first study visit on which the subject has an HSV lesion.

Subjects randomized to one of the squaric acid groups will be given a sensitizing dose of 2.0% SADBE dissolved in DMSO applied to skin on a single spot (approximately 5 mm diameter) in the inner upper arm. The area will be encircled with petroleum jelly and Tegaderm™ dressing will be applied. Subjects assigned to the placebo group will receive DMSO instead of 2.0% SADBE dissolved in DMSO. Subjects will be instructed to remove the dressing and rinse the area three hours after application of the sensitizing dose.

### Week 1

The following procedures will be performed during this visit.

- Physician assessment
- Blood collection
    - CBC with Diff
- Swab herpetic lesion to collect sample* for
    - HSV-1 and -2 PCR*

- Review of concomitant medications
- Adverse events collection
- Collection of diary and photographs
- Photograph mouth and nose area of subject face below the eyes if subject is experiencing an outbreak in that area at the time of the clinic visit.
- Photograph drug application site if subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.

* Viral specimen collection for HSV-1 and HSV-2 PCR testing will be conducted on the first study visit on which the subject has an HSV lesion.

**Intensification Dose (Week 3)**

At 21 days (± 3 days) after the sensitization dose, the following procedures will be performed during this visit:

- Physician assessment (Targeted Skin Examination)
- Urine Pregnancy Test
- Vital signs
- Review of concomitant medications
- Collection of any reported adverse events
- Collection of photographs and diaries
- Photograph mouth and nose area of subject face below the eyes if subject is experiencing an outbreak in that area at the time of the clinic visit.
- Photograph drug application site if subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.
- Squaric acid (or placebo) application on left upper arm (opposite arm from the sensitization dose) (Dose B). This will involve application of Dose B, from a vial labeled xxx-B for Dose B to patient number xxx).
- Swab herpetic lesion to collect sample* for
    - HSV-1 and -2 PCR*

* Viral specimen collection for HSV-1 and HSV-2 PCR testing will be conducted on the first study visit on which the subject has an HSV lesion.

**Month 2**

Two months after the sensitization dose (Dose A), the following procedures will be performed:

- Physician Assessment (Targeted Skin Examination)
- Blood collection
    - CBC with Diff
    - HSV-1 and -2 Ab IgG
- Swab herpetic lesion to collect sample* for
    - HSV-1 and -2 PCR*
- Review of concomitant medications
- Adverse events collection
- Collection of diary and photographs
- Photograph mouth and nose area of subject face below the eyes if subject is experiencing an outbreak in that area at the time of the clinic visit.
- Photograph drug application site if subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.

* Viral specimen collection for HSV-1 and HSV-2 PCR testing will be conducted on the first study visit on which the subject has an HSV lesion.

**Months 3, 4, 5, 6, 7, 8, 9, 10, 11, 12**

The following procedures will be performed during these visits:

- Physician Assessment (Targeted Skin Examination)
- Review of concomitant medications
- Adverse events collection
- Collection of diary and photographs
- Photograph mouth and nose area of subject face below the eyes if subject is experiencing an outbreak in that area at the time of the clinic visit.
- Photograph drug application site if subject is experiencing any redness, rash, or irritation at the drug application site at the time of the clinic visit.
- Swab herpetic lesion to collect sample* for
    - HSV-1 and -2 PCR*

* Viral specimen collection for HSV-1 and HSV-2 PCR testing will be conducted on the first study visit on which the subject has an HSV lesion.

**Photographs**

Digital photographs of the mouth and nose area of each patient will be taken by clinic staff at each clinic visit during which a patient has an outbreak occurring. Eyes will be excluded from the photographs to protect patient privacy.

In addition, patients will be asked to use their own smart phones or digital cameras to photograph herpes labialis lesions on or around the lips or nose once per day during each outbreak the that the patient experiences during the clinical trial. The patients will also be asked to photograph the site of drug application when they experience any redness, rash, or irritation at that site. Those photographs also should be taken once per day on any day they are experiencing rash, redness, or irritation at the drug application site.

**Patient Contacts and Monitoring**

At the initial visit, patients will be instructed to contact the investigators when they have a new outbreak. Patients will be sent text messages weekly asking whether they have had a new outbreak. If they answer yes, they will be contacted by phone to schedule a visit for Provider Assessment and to ask the day the outbreak began. The clinic visit will only be scheduled if it is one of the first three outbreaks for the patient during the study. If they answer no, it will be noted. If they do not reply to 4 consecutive weekly text messages, they will be contacted by phone. If they cannot be reached for 3 consecutive months, they will be dropped from the study and if they had not reported any new herpes labialis outbreaks up to the time of the last response from them, it will be considered that they did not have a new outbreak up to the date of their last response or last clinic visit.

Two to four days after each application of study drug to a subject, the subject will be contacted by telephone to ask about any erythema (redness), itching, or pain at the drug application site or other adverse event. If the subject reports moderate or severe erythema or other adverse event they are concerned about, they will be asked to come in for a clinic visit for evaluation. Patient responses will be recorded as data. They will be asked to rate rash at the drug application site on a scale of 0 to 3, as no rash, mild rash, moderate rash, or severe rash, as in Question 7 of the Patient Assessment diary.

Patients will be asked to come in for a clinic visit during the first three separate herpes labialis outbreaks they have during the study. If they come in for a visit during an outbreak at 7 weeks or more after the sensitization dose, the visit will be considered to be the next monthly visit of their monthly visits at 2-7 months, and the procedures specified for that visit will be performed.

**VII. Risks and discomforts**

Possible side effects of Squaric Acid include:

1. Localized erythema
2. Increasing lesional inflammation.
3. Pruritis
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 participating individuals**

Subjects may or may not benefit from participating in the study. Their skin condition may get better, stay the same, or get worse.

**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. No written or oral communication will be made about any patient with anyone other than the patient, unless the patient so requests. Medical information obtained from the study may become part of the patient's permanent clinic or hospital record, subject to the confidentiality and privacy regulations of the clinic or hospital.

**A. Study Drug Management**

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

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 in a freezer (-20°C ± 5°C, or -13°F to +5°F). Each vial of study medication is labeled with a subject number. Each vial will be transferred to room temperature (23°C ±5°C) to thaw one business day before the clinic visit at which it is scheduled to be used. If the clinic visit for that subject is postponed for some reason, the vial will be stored at room temperature (23°C ±5°C) for up to 3 months after thawing.

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. SADBE is included on the "List of Bulk Drug Substances That Can Be Used To Compound Drug Products in Accordance With Section 503A of the Federal Food, Drug, and Cosmetic Act." SADBE is being proposed for the 503A Bulks List, to appear in § 216.23(a) of Title 21 of the CFR.

A Data and Safety Monitor (DSM) will be appointed, who will be Dr. Thomas Horn, MD, a board-certified dermatologist and Chief Medical Officer of Squarex.. The DSM will advise the IND sponsor regarding both the safety of current and potential subjects, as well as trial validity and merit. The study blind will not be broken unless deemed necessary for the safety and welfare of the study subjects. If the blind needs to be broken, the Principal Investigator will not be informed unless deemed necessary for the safety and welfare of the study subjects.The FDA will be informed of all recommendations made by the DSM to the IND sponsor regarding safety, regardless of whether the definition for serious adverse event is met.

### C. Data and Drug Handling Guidelines

Data will be collected on case report forms and drug accountability logs 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.

### D. 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 (CRF) and the subject's sourcebook.

The original case report form for each subject will be audited to source documents (inpatient/outpatient medical records, clinical assessment profiles) at the study site by the study monitor. In many cases, the case report form may serve as the source document. 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.

### E. 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, dermatological efficacy assessments, photographs of lesions and sites of drug application, all questionnaires, patient diaries, signed informed consent forms, and any other information that Squarex may consider to be necessary to evaluate the efficacy 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.

## F. Adverse event reporting guidelines

### Definitions

Adverse Event (AE) is any untoward medical occurrence associated with the use of the study drug in a subject, whether or not considered drug related.. 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.

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
- The study sponsor decides to suspend or terminate the study.

## X. STATISTICAL METHODS

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; the median, 25th and 75th percentiles, minimum and maximum values for time-to-event endpoints; and the number and percentage of patients in each category for categorical data. 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).

All primary statistical comparisons of 2% SADBE to placebo (i.e., regardless of whether the comparison uses a primary or secondary endpoint) will combine data from patients randomized to treatment group 1 or 2 for testing against data from patients randomized to treatment group 3. Secondary analyses of primary and secondary endpoints will be conducted to assess differences between individual treatment groups.

No interim data analyses are planned. (However, blinded review of safety data may be performed if needed to ensure patient safety.)

### Randomization

Random assignment of patients to three treatment groups will be on a 1:1:1 ratio. Thus the ratio of patients assigned to one of the two 2% SADBE treatment groups will be 2:1 versus placebo.

### Sample Size

The sample size of 94 patients was selected for this study so that a two-sided log-rank at the 5% level of significance would have 90% power under the following assumptions:

- Median times to first new herpes labialis episode of 90 and 40 days for patients treated with 2% SADBE and placebo, respectively
- 2:1 allocation of patients to the two 2% SADBE treatment groups versus placebo
- Patient accrual over a period of 1 year and a total study duration of 18 months

- Patients may be lost to follow-up (and censored according to the rules specified below) according to an exponential distribution with a median of 9 months

The method for controlling the overall two-sided 5% level of significance against the inflationary effects of testing multiple efficacy endpoints is discussed below.

**Primary Endpoint Analysis**

The primary endpoint is the number of days until a patient reports his or her first new herpes labialis episode following the sensitization dose (Dose A). The date of the sensitization dose is defined as Day 1. Therefore, any herpes labialis episodes occurring prior to the sensitization dose, including any episodes between treatment group randomization and the day prior to the sensitization dose, will not be counted when assessing the primary endpoint. Data from patients in treatment groups 1 and 2 will be combined (since patient in both groups receive 2% SADBE for sensitization) and compared against data from patients in treatment group 3 (placebo). The nonparametric log-rank test will be used to test the treatment effect (2% SADBE versus placebo) at the two-sided, 5% level of significance. Patients withdrawn from the study for any reason prior to experiencing a first new episode of herpes labialis will have their primary endpoint censored on the last date of active study participation. Cumulative time-to-endpoint distributions ("survival curves") will be estimated by the Kaplan-Meier method.

**Secondary Endpoint Analyses**

Without taking appropriate measures, testing multiple efficacy parameters inflates the overall significance level since the probability of making a type-1 error in at least one from a set of hypothesis tests is greater than the probability of making a type-1 error in any single test. The hierarchical closed test procedure [Reference #s 5 & 6] will be used to maintain the overall 5% significance level. The order of efficacy endpoints for the hierarchical closed test procedure is:

1. Number of days until a subject reports his or her first new herpes labialis episode following the sensitization dose (Dose A) (primary endpoint)
2. Number of days until subject reported first new herpes labialis episode following the intensification dose (Dose B).
3. Number of days until subject reported first new herpes labialis episode beginning from 21 days after the intensification dose (Dose B).
4. Number of new herpes labialis episodes during the first 4 months following the sensitization dose (Dose A).
5. Number of new herpes labialis episodes during the first 6 months following the sensitization dose (Dose A).
6. Number of new herpes labialis eposides during the 12-month follow up period following the sensitization dose (Dose A).
7. Number of new herpes labialis episodes during the first 4 months following the intensification dose (Dose B).
8. Number of new herpes labialis episodes during the first 6 months following the intensification dose (Dose B).
9. Number of new herpes labialis eposides during the 12-month follow up period following the intensification dose (Dose B).

10. Number of days with herpes labialis during the first 4 months following the sensitization dose (Dose A).
11. Number of days with herpes labialis during the first 6 months following the sensitization dose (Dose A).
12. Number of days with herpes labialis during the 12-month follow-up period following the sensitization dose (Dose A).
13. For only the subjects who are positive for IgG against HSV-2, number of days until a subject reports his or her first new herpes labialis episode following the sensitization dose (Dose A).
14. For only the subjects who are positive for IgG against HSV-2, number of days until a subject reports his or her first new herpes labialis episode following the intensification dose (Dose B).
15. Average duration of herpes labialis episodes during the 12-month follow up period following the sensitization dose (Dose A).
16. Average duration of herpes labialis episodes during the follow up period following the intensification dose (Dose B).

Safety and pharmacodynamic endpoints are not included in the hierarchical closed test procedure.

Each efficacy endpoint will be tested at a two-sided 5% significance level using the statistical procedures described below. Days until a patient reports his or her first new herpes labialis episode following the sensitization dose, the primary efficacy endpoint, will be declared statistically significant if its test p-value is ≤0.05. A secondary efficacy endpoint will be declared statistically significant only if its test p-value is ≤0.05 and all efficacy endpoints preceding it in the hierarchy are statistically significant. In other words, declaration of statistical significance for efficacy endpoints will advance in order down the hierarchy only until a p-value >0.05 is found.

Only the p-value from an endpoint's primary analysis will be used for deciding statistical significance. Supportive (sensitivity) analyses used to demonstrate the robustness of primary results to slight modifications to how endpoints are defined and/or analyzed will not be considered for the purpose of determining primary significance.

The log-rank test (as described above for the primary endpoint) will be used to test secondary time-to-event endpoints. Secondary endpoints that are total or average durations of specific events will be tested using nonparametric Wilcoxon rank sum tests.

**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.

A high-level safety summary will display the numbers of patients within each treatment group and overall who experience one or more AEs in each of the following categories:

- All TEAEs regardless of severity or presumed relationship to study drug
- TEAEs judged related to study drug
- Treatment-emergent serious adverse events (SAEs)
- TEAEs leading to a delay in the application of study drug
- TEAEs leading to discontinuation of study drug
- TEAEs leading to withdrawal from the study

The base summary of TEAEs will show within– and between–treatment-arm incidence rates for each MedDRA primary system organ class and/or preferred term by highest reported severity grade and overall. A separate summary will be produced each of the AE subsets listed above. Additional AE summaries may be produced using safety data from subsets of patients (e.g., patient subsets according to age, sex or race).

## References


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**Appendix A.**

| | | | |
|---|---|---|---|
| Subject #: ☐☐☐ | | *Month* *Day* *Year* | |
| Subject Initials: ☐☐☐ | Visit Date: | ☐☐ ☐☐ ☐☐ | |

## Provider Assessment of Disease Severity

1. Are there HSV lesions present (please circle): Yes No

2. Location of lesions (please choose from the following):

3. Redness of lesions (circle)
   - 0 = no erythema
   - 1 = faint pink,
   - 2 = red
   - 3 = violaceous.

4. Nature of lesion (circle all that apply)
   - a. prodrome (patient reports tingling but no visible lesion)
   - b. erythema
   - c. papule
   - d. vesicle
   - e. erosion or ulcer
   - f. crust
   - g. normal skin

5. 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 #: ☐☐☐ *Month* *Day* *Year*

Subject Initials: ☐☐☐ Date: ☐☐ ☐☐ ☐☐

## Patient Assessment of Disease Severity: please encircle your answers.

1. Do you have any HSV lesions present: Yes No

2. Location of lesions (please label the following):

3. Redness:
   - 0 = no redness
   - 1 = faint pink
   - 2 = red
   - 3 = purple

4. Pain:
   - 0 = no pain
   - 1 = mild pain
   - 2 = moderate pain
   - 3 = severe pain

5. Tingling:
   - 0 = no tingling
   - 1 = mild tingling
   - 2 = moderate tingling
   - 3 = severe tingling

6. Nature of 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

7. Please assess for local irritation from therapy;
   - 0 = no rash,
   - 1 = mild rash
   - 2 = moderate rash
   - 3 = severe rash

Signature: _____

**Appendix C. Short Diary**

**Subject No.** **Month:** **Year:**

| **Date** | **Do you have any cold sores (HSV lesions) present? (If yes, please complete detailed sheet)** | | **Photos Taken** | | **Initials** |
|---|---|---|---|---|---|
| 1 | Yes | No | Yes | No | _____ |
| 2 | Yes | No | Yes | No | _____ |
| 3 | Yes | No | Yes | No | _____ |
| 4 | Yes | No | Yes | No | _____ |
| 5 | Yes | No | Yes | No | _____ |
| 6 | Yes | No | Yes | No | _____ |
| 7 | Yes | No | Yes | No | _____ |
| 8 | Yes | No | Yes | No | _____ |
| 9 | Yes | No | Yes | No | _____ |
| 10 | Yes | No | Yes | No | _____ |
| 11 | Yes | No | Yes | No | _____ |
| 12 | Yes | No | Yes | No | _____ |
| 13 | Yes | No | Yes | No | _____ |
| 14 | Yes | No | Yes | No | _____ |
| 15 | Yes | No | Yes | No | _____ |
| 16 | Yes | No | Yes | No | _____ |
| 17 | Yes | No | Yes | No | _____ |
| 18 | Yes | No | Yes | No | _____ |
| 19 | Yes | No | Yes | No | _____ |
| 20 | Yes | No | Yes | No | _____ |
| 21 | Yes | No | Yes | No | _____ |
| 22 | Yes | No | Yes | No | _____ |
| 23 | Yes | No | Yes | No | _____ |
| 24 | Yes | No | Yes | No | _____ |
| 25 | Yes | No | Yes | No | _____ |
| 26 | Yes | No | Yes | No | _____ |
| 27 | Yes | No | Yes | No | _____ |
| 28 | Yes | No | Yes | No | _____ |
| 29 | Yes | No | Yes | No | _____ |
| 30 | Yes | No | Yes | No | _____ |
| 31 | Yes | No | Yes | No | _____ |

## Appendix D. Study Schema

| | Screening and Sensitization dose (Dose A) application | Week 1 | Intensification Dose (Dose B) (Week 3) | Month 2 | Month 3 | Monthly visits at months 4-12 |
|---|---|---|---|---|---|---|
| | | ± 2 d | +2 W | ±1 W | ± 2 W | ± 2 W |
| Informed Consent | X | | | | | |
| Inclusion/Exclusion | X | | | | | |
| History/Demographics | X | | | | | |
| Physician Assessments (Targeted Skin Examination) | X | X | X | X | X | X |
| Urine Pregnancy Test | X | | X | | | |
| Vital Signs | X | | X | | | |
| Photography | X | X | X | X | X | X |
| Diary distribution and explanation / explanation of taking self photographs. | X | | | | | |
| Photograph Collection and Diary review | | X | X | X | X | X |
| Squaric acid application | X | | X | | | |
| Blood Collection (Processing, storing, shipping, dry ice) | X | X | | X | | |
| CBC with Differential | X | X | | X | | |
| HSV-1 and -2 Ab IgG | X | | | X | | |
| Viral specimen collection* | X | | | | | |
| HSV-1 and -2 PCR* | X | | | | | |
| Concomitant medications/Adverse event collection | X | X | X | X | X | X |

* Viral specimen collection and HSV-1 and -2 PCR in the specimen collection will be conducted on the first study visit on which the patient has an HSV lesion.