I. 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, 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 verrucae vulgaris and alopecia areata. 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.

II. Hypothesis and Endpoints

Hypothesis: Squaric acid dibutyl ester will significantly decrease the frequency and severity of herpes labialis outbreaks compared to placebo.

A. Primary Endpoints:

1. Determine the effect of squaric acid on frequency and severity of herpes labialis outbreaks compared to placebo. Including:
   a. Days with lesions in the time period from 2-8 month after the last treatment.
   b. Days until first subject-reported recurrence (distinct new outbreak) after the last treatment.
2. Determine the safety and tolerability of squaric acid for the treatment of herpes labialis. Cumulative pain as determined by the sum of pain levels each day of the treated outbreaks, will also be determined

B. Secondary Endpoints:

1. Change in anti-HSV-1 IgG titer before and after treatment.
2. Proportion of patients positive for PBMC anti-HSV-1 proliferative response in the final blood draw (4 months after the last treatment), and the change in the proportion after treatment as compared to before treatment.
3. Average numerical measured PBMC anti-HSV-1 proliferative response at 4 months after the last treatment, and the ratio of the numerical PBMC anti-HSV-1 proliferative response after treatment versus before treatment.
4. Proportion of patients positive for a PBMC cytotoxic response against HSV-1-infected LCL at 4 months after the last treatment, and the change in the proportion after treatment as compared to before treatment.
5. Average numerical PBMC cytotoxic response against HSV-1-infected LCL at 4 months after the last treatment, and the ratio of the numerical PBMC cytotoxic response after treatment versus before treatment.

II. Subject Selection

Subjects will be recruited from advertisements in local newspapers, posters and Partners wide e-mail seeking interested subjects. Interested subjects will call and undergo a preliminary eligibility screen followed by a formal screening appointment at the Clinical Unit for Research Trials in Skin (CURTIS) at the Massachusetts General Hospital, at which time the protocol will be explained in detail and informed consent, will be obtained. Subjects may also be apprised of
the study by information given to primary care physicians and dermatologists at MGH so that interested subjects can contact the investigators.

**Inclusion criteria**

1. Age ≥18.
2. Subject willing and able to give informed consent.
3. Patients who have a clinical diagnosis of herpes labialis.
4. Patients who self report having six or more episodes of herpes labialis in the previous 12 months.

**Exclusion criteria**

1. Subject has been treated with anti-viral therapy within 2 weeks prior to the screening visit.
2. Pregnant or lactating females.
3. Current or recurrent 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.
4. Therapy with glucocorticoid or immunosuppressant at time of recruitment or within past 4 weeks, except for inhaled corticosteroids for asthma.
5. History of malignancy (except patients with surgically cured basal cell or squamous cell skin cancers who will be eligible).
7. Positive-HIV status determined by history at screening or known history of any other immuno-suppressing disease.
8. Severe comorbidities (diabetes mellitus requiring insulin, CHF (EF<50% at baseline will be exclusionary) of any severity, MI, CVA or TIA within 3 months of screening visit, unstable angina pectoris, oxygen-dependent severe pulmonary disease.
9. 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.
10. Subjects who have known hypersensitivity to Squaric acid or any of its components.
11. History of recent alcohol or substance abuse (< 1 year).
12. Any condition judged by the investigator to cause this clinical trial to be detrimental to the patient.
13. History of psychiatric disease that would interfere with the patient’s ability to comply with the study protocol.
14. History of non-compliance with other therapies.

**III. 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.
Informed Consent

Written informed consent will be signed prior to screening evaluation and testing by a licensed physician investigator. Subjects will be informed that they may withdraw from participation in the study at any point.

Randomization

Subjects will be randomized to squaric acid 0.2% versus squaric acid 0.5% vs. placebo at the baseline visit in a 1:1:1 ratio. Randomization will be performed by a computer generated randomization table and will be blinded to study investigators and subjects.

60 subjects in total will be randomized to the three treatment groups.

V. Study procedures

All doses will be administered by topically saturating a cotton swab with the appropriate solution of drug (or no drug for placebo) in dimethylsulfoxide (DMSO), and then rolling the swab over the largest lesion then present to thoroughly wet the lesion without dripping over other areas of the skin. The solutions will be in 20 ml amber bottles. To quantify the amount of drug solution applied, the cotton swab will be weighed before and after soaking it in the study medication and after application of the drug. The difference in weight will be recorded as the mass of drug solution applied.

Screening visit 1:

Patients with herpes labialis will be screened for the study at the Massachusetts General Hospital. Those who are eligible and willing to participate will be enrolled. Our goal is 60 subjects with evaluable data. To achieve this goal, we may screen up to 100 subjects.

During the screening visit, after informed consent is obtained, subjects will undergo a medical history and physical exam, which will include vital signs and lab assessments. Patients randomized to squaric acid 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 Vaseline and a bandage will be applied. Patients in placebo control group 1 will be given DMSO on a single spot on the upper inner arm. The purpose of the sensitizing dose is to induce sensitivity to SADBE, so that after a 2 week initiation period, the subjects will likely be sensitive to the immunogen SADBE and the next time they are exposed to SADBE they will develop a DTH response.

Each patient will also be given a diary containing the Patient Assessment of Disease Severity worksheet which is to be completed daily while participating in this study.
Patients will also be given a camera and will be instructed to photograph their lips daily from the time they experience any symptoms such as tingling pain, burning or itching until three days after the symptoms disappear. Patients may also use their cell phone to take daily pictures as desired.

Subjects woman who is sexually active, you must use adequate birth control while in this study and for one month after using the study medication. Adequate forms of birth control include:

- Hormonal contraceptives for at least 3 months
- Implants
- Injections
- Intruterine Device (IUD)
- Condom and spermicide
- Diaphragm and spermicide

Some methods of birth control will not work when you are taking certain drugs or using certain products. You can still become pregnant even if you use an acceptable birth control method. The study requires all females to complete pregnancy tests throughout the study.

Visit 1 summary:
- Informed Consent
- Eligibility criteria
- Medical History
- Targeted physical exam
- Vital Signs
- Laboratory assessments including:
  1. Qualitative and quantitative measurement of anti-HSV-type-1 IgG antibodies and quantitative measurement of anti-HSV IgM antibodies.
  2. White blood cell counts and differential.
  3. Peripheral blood mononuclear cells (PBMC) proliferative response to a uv-inactivated laboratory HSV type 1 strain.
  4. PBMC cytotoxicity response against HSV-1-infected lymphoblastoid cell line (LCL).
  5. Pregnancy test
- Randomization
- Application of sensitizing dosage versus placebo
- Diary and camera distribution

Visit 2:

This visit will occur upon the first appearance of a herpes labialis lesion and after waiting at least 14 days after the sensitizing dose. At this visit, the lesion will be cultured for HSV detection and for polymerase chain reaction (PCR). The viral type (e.g., HSV-1 or HSV-2) will be determined.
A positive result for HSV culture or HSV presence by PCR will not be required for inclusion or for continuation in the study. A pregnancy test will also be conducted.

The provider will complete a targeted physical examination and the Provider Assessment of Disease Severity worksheet.

After completion of the culture and assessment, the patient will be treated by applying the appropriate solution topically to the largest lesion then present on the lip or vermillion border of the lip. Drug will only be administered once to the patients at this time and patients will only be treated within 72 hours of the onset of symptoms.

If the first lesion appears 14 days or more after the sensitizing dose, the patient will be given the first treatment dose on the same visit after swabbing the lesion for detection of virus. If lesion appears less than 14 days after the sensitizing dose, the patient will only receive cultures, and must return again for initial treatment during a subsequent outbreak.

Visit 2 summary:
- Eligibility criteria
- Targeted physical exam
- Provider Assessment of Disease Severity worksheet.
- Vital Signs
- Laboratory assessments including:
  1. Pregnancy test
  2. HSV cultures
- Photography
- Application of squaric acid 0.2% versus squaric acid 0.5% versus placebo

Visit 3, 5:
Safety visits. These will occur 2-4 days after the first and second treatment doses. The provider will complete a targeted physical examination and the Provider Assessment of Disease Severity worksheet. Photography will also be performed.

Visit 4:
After a period of at least 21 days after the first treatment dose and up to at most 120 days after the first treatment, at a time when the patient is experiencing one or more herpes labialis lesions, they will receive a repeat dosage of the treatment to which they have been previously randomized. Treatment will be administered to the largest lesion then present on the patient’s lip or vermillion border of the lip. Prior to treatment at this visit, the provider will complete a targeted physical examination, pregnancy test when indicated, and the Provider Assessment of Disease Severity worksheet.

Patients will only be treated within 72 hours of the onset of symptoms.
Patients will only receive a maximum of two treatments in the study. If after 120 days past the first treatment the patient has not again experienced a herpes labialis lesion, the patient will not receive the second treatment dose.

Visit 4 summary:
- Targeted physical exam
- Provider Assessment of Disease Severity worksheet.
- Vital Signs
- Laboratory assessments including:
  1. Pregnancy test
- Photography
- Application of squaric acid 0.2% versus squaric acid 0.5% versus placebo

Subsequent visits

The patients will be monitored every 4-6 weeks for 8 months after their last treatment.

The provider will complete a targeted physical examination and the Provider Assessment of Disease Severity worksheet at each visit.

Four months after the second treatment (or after the first treatment if the patient has not yet received a second treatment), the patient will be asked to repeat the following labs:
  1. Qualitative and quantitative measurement of anti-HSV-type-1 IgG antibodies and quantitative measurement of anti-HSV IgM antibodies.
  2. White blood cell counts and differential.
  3. Peripheral blood mononuclear cells (PBMC) proliferative response to a uv-inactivated laboratory HSV type 1 strain.
  4. PMBC cytotoxicity response against HSV-1-infected lymphoblastoid cell line (LCL).

At the patient’s final visit (8 months after last treatment), they will be asked to turn in their camera and daily diaries.

**Quantitative measurement of anti-HSV-type-1 IgG antibodies.**

Because there is no commercially available quantitative testing for HSV IgG antibodies, serum will be collected and banked at MGH and later processed.

**Early termination**

Subjects who meet any of the criteria will not receive study drug during the 2nd outbreak:

- Pain of skin – severe pain, limiting self care ADL (CTCAE Grade 3)
• Skin ulceration – combined area of ulcers >2 cm, full-thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to the fascia (CTCAE Grade 3)

• Intense or widespread constant pruritus, limiting self-care ADL or sleep, requiring oral corticosteroid or immunosuppressive therapy (CTCAE Grade 2)

• Development of urticarial lesions requiring oral intervention (CTCAE Grade 2)

Subjects who do not receive the second treatment will continue to come for monthly follow-up visits to monitor the progress of their HSV.

VI. Biostatistical Analysis:

Power Analysis:

The primary efficacy endpoints will be time to recurrence after treatment and the number of days with herpes labialis lesions during the period from two to eight months after the last treatment. We believe the treatment may or may not reduce the duration or severity of the outbreaks that are treated, but will reduce the frequency and severity of subsequent outbreaks. We are allowing two months after the last treatment for the treated outbreak to resolve and the immune response caused by the treatment to fully develop.

If the treatment doubles the time to recurrence or halves the number of days with lesions, and the standard deviation of these measurements in each group is 70% of the mean values, then a group size of 11 will be required in order for P to be < 0.05. A group size of 20 allows for some attrition of patients enrolled in the study and the possibility of detecting a smaller than 2-fold difference in outcomes between treated and control groups.

VII. Potential Benefits

Subjects may or may not receive any benefit from being in the study. Their skin condition may get better, stay the same, or get worse. Information gathered from this study may help other people in the future with herpes labialis.

VIII. Risks and discomforts

Risks of Squaric Acid may include:

1. Localized erythema
2. Increasing lesional inflammation.
3. Pruritus
4. Contact dermatitis
5. Lymphadenopathy
6. Vitiligo or leukoderma
7. Risk of bruising and pain with blood draw.
8. Allergic reaction
9. Blistering
10. Burning sensation with application
11. Fever
12. Arthralgias

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 hospital record, subject to the confidentiality and privacy regulations of the Massachusetts General Hospital.

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. The safety contact for this study is Dr. Alexa Kimball.

The study will be monitored by the principal investigator and members of the study staff. All data relevant to the assessments outlined in this protocol will be recorded in the case report form (CRF) and the subject’s sourcebook.

Adverse event reporting guidelines

a. 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
b. 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.

X. 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:

1. Subject is found to be intolerant to a required study procedure at any time point
2. Subject is noncompliant with protocol restrictions and requirements such as:
   a. Inability to use study product
   b. Failure to return for 2 study visits
3. Subject experiences a serious adverse experience at any time point.
4. Subject develops an inter-current illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree.
5. Subject begins a medication that, in the judgment of the investigator, may affect assessments of clinical status to a significant degree.
6. Subject becomes pregnant while participating in the study
7. Subject enrolls in another investigational study
8. Subject requests to withdraw from the study
9. The study sponsor decides to suspend or terminate the study.

References

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 each lesion (circle)
   a. dry and crusted
   b. ulcerated and exudative.

5. Please assess for local irritation from therapy. Please circle the choice that most accurately describes the treatment response:
   a. 0 = no rash,
   b. 1 = mild
   c. 2 = moderate
   d. 3 = strong rash in need of treatment with topical hydrocortisone

________________________________________________  ______________
Signature         Date
Patient Assessment of Disease Severity: please encircle your answers.

1. Do you have any cold sore (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 each lesion
   dry and crusted
   open sore and draining

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

Kimball Squarex 11-26-13
### Visit Schema

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^a: Lab studies will be repeated only at the 4 month (from first treatment) follow up visit