A Prospective, Double-Blind, Cross-Over, Pilot Study to Assess Safety and Efficacy of Topical Sirolimus 2% in the Treatment of Plantar Blistering in Patients With Epidermolysis Bullous Simplex (EBS)

Study Protocol and Statistical Analysis Plan

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Clinical Trial Protocol:

A prospective, double-blind, cross-over pilot study to assess safety and efficacy of topical sirolimus 2% for the treatment of plantar blistering in patients with epidermolysis bullosa simplex

Version 6

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1.0 Protocol Summary

The purpose of this study is to investigate a targeted approach for the management of the plantar lesions of epidermolysis bullosa simplex (EBS). The drug to be tested is called sirolimus (Rapamycin), it is a mammalian Target of Rapamycin (mTOR) inhibitor to be used as a topical agent for treatment of the blistering/callus lesions on the soles of feet.

Sirolimus 2% ointment, has been shown to reduce expression of keratin (KRT) 6/16 and improve walking ability in a related condition affecting the feet, pachyonychia congenita, as well as being safe. Since sirolimus may have similar effects on the paired KRT 5/14 which are affected in EBS, we are conducting a prospective, double-blind, randomized, placebo-controlled, crossover study.

Participants will be assigned to treat both feet with either topical sirolimus 2% ointment twice daily or placebo (vehicle-control) for 12 weeks followed by a washout period of four weeks, then retreatment to both feet with the switched intervention followed by a washout period of four weeks, and then a post-study phone call at 8 weeks thereafter.

This study will exploit the naturally occurring transcriptional regulation of keratin sequences, the known gene aberration causing EBS, and assess the potential for mTOR pathway inhibition in treatment of EB patient's plantar lesions.

2.0 Background and Rationale

2.1 Disease background

Epidermolysis bullosa (EB) represents a spectrum of conditions characterized by blistering and mechanical fragility of the skin. Individuals with EB display tremendous clinical diversity, as the dermal-epidermal basement membrane zone contains a number of specialized adhesive structures that link the basal epidermis cytoskeleton to the papillary dermis. The molecular basis of EB has been linked to 18 genes, leading to the classification of 28 clinical (15 EBS, 8 JEB, 14 DEB, 1 Kindler)
EB subtypes and four major EB groups (Fine et al., 2014). With importance to this proposal, the EB simplex (EBS) subtype has been well-characterized genetically, and it is this characterization the authors plan to exploit with the goal of improving current state of the art.

EBS refers to a group of rare inherited disorders caused by blister formation within the epidermis. The clinical features of all forms of EBS manifest with skin

blistering. Palmoplantar-keratoderma, nail dystrophy and shedding, alopecia and oral involvement can be seen. Excruciating pain and severe impairment in quality of life in EBS patients has been reported (Fine at el., Clin Exp Dermatol 2004). Causes of early mortality in EBS patients include infections and protein loss leading to malnutrition, fluid and electrolyte imbalances, and anemia. Patient care has been limited to supportive measures, including wound care, nutritional support, and antibiotics. In 1991, two groups published in Cell and Science independently revealing the first keratin disorder with a genetic basis in humans, EBS. EBS is usually caused by missense mutations in the genes encoding keratin 5 and 14 (KRT5 and KRT14, respectively) leading to a dominant negative effect on the function of normal keratins (Lane et al., J Pathol 2004), resulting in keratin cytoskeleton dysfunction. The prevalence is estimated to be 6 to 30 per 1 million live births.

2.2 Sirolimus

Sirolimus binds to immunophilin FK506 binding protein-12 and creates a complex that inhibits the activation of mTORC1 that leads to decreased translational initiation. Consequently, sirolimus has shown to decrease overall protein synthesis by 20% and cause cell cycle arrest in G1 phase. (Castedo et al., 2001) Other effects of sirolimus include apoptosis, inhibition of T-cell activation and reduced expression of specific proteins involved in angiogenesis and lymphangiogenesis, specifically hypoxia inducible factor-1a (HIF-1a) and vascular endothelial growth factor (VEGF). (Guba et. al 2002)

Systemic use of sirolimus is associated with side effects that include opportunistic infection, mucositis, hypercholesterolemia, decreased renal function, elevation in liver transaminases, thrombocytopenia, neutropenia, and delayed wound healing. Minor adverse cutaneous events of systemic therapy include acne like eruptions, edema, and nail disorders. mTOR inhibitors in topical preparations show promise as an ideal delivery system to minimize systemic adverse effects in the treatment of dermatological disease.

Topical formulations of sirolimus are being used off-label in a variety of dermatological diseases (see Table 1) (Fogel et al, 2015). Formulations are mostly crushed sirolimus from tablet compounded with petrolatum to form an ointment, with a few studies utilizing the commercially available oral sirolimus solution (1mg/ml). Most of the topical formulations were found to have no local adverse effects, with irritation being more common using the aqueous solution. In all cases the systemic level of sirolimus was less than 2ng/ml that is below the standard serum levels required for immunosuppression (5-15 ng/ml). Treatment duration and frequency varied according to condition.

2.3 Rationale

The development of new treatment modalities is critical in improving disease state and the quality of life for EBS patients. Disease classification has changed from descriptive to a detailed pathogenesis over the past several decades, and

now scientists are drawn to ask the question, “How can we approach this disease in a targeted fashion?”

Therapeutic approaches targeting the deleterious effects of abnormal and abundant keratins are emerging. Topical sirolimus 2% is one potential medication yet to be utilized in EBS patients. It has been shown to selectively block 5’oligopyrimidine (TOP) mRNA translation thereby altering mRNAs 5’TOP motifs (Raught et al., 2001). Published sequence data suggest KRT5 mRNA, but not KRT14 mRNA, contain the 5’TOP motif (Hickerson et al., 2009) and thus are viable drug targets of mTOR for the treatment of EBS variants.

3.0 Hypothesis

Our hypothesis is that the mTOR inhibitor sirolimus will down regulate the translation of defective KRT5 protein, therefore ameliorating the severity of EBS in patients affected. Additionally, altering the mTOR pathway may directly affect pain and itch (R Kasper, May 2015, unpublished correspondence). Itch and pain are two of the three top symptoms that matter to patients living with EB (Challenges & Unmet Needs in EB, Jemima Mellerio, EB2015 Conference, Atlanta, GA), both of which are secondary outcome measures in this study.

4.0 Objectives and Scientific aims

Aim 1: To assess the safety of topical sirolimus 2% use in the treatment of plantar lesions in epidermolysis bullosa simplex (EBS).
Aim 2: To assess the efficacy of topical sirolimus 2% to improve the clinical severity of lesional skin, including pain and itch symptoms.
Aim 3: To assess improvement in walking ability.

5.0 Criteria for Subject Eligibility

5.1 Number of Participants

We aim to enroll a total of 8-10 patients in this study at Stanford University.

5.2 Inclusion criteria

- Diagnosis of EBS
- Minimum EBDASI feet activity score of 2/10
- Healthy male or non-pregnant female
- Age –4 years old or older

- Ability to complete 7 study visits, each for approximately 30-60 minutes, including 3 scheduled phone calls within a 40-week period
- Anticipated life expectancy ≥52 weeks
- Males and females of childbearing potential should be using an effective means of contraception.
- Laboratory values within the range of normal unless the PI feels they are not clinically relevant.

5.3 Exclusion criteria

- Allergy to sirolimus or components of the vehicle ointment
- Pregnancy, breast feeding
- Prior history of liver disease
- Serious known concurrent medical illness or infection, which could potentially present a safety risk and/or prevent compliance with the requirements of the treatment program.
- Known immunodeficiency virus or syndrome including those with:
    - Acquired Immunodeficiency Syndrome (AIDS)
    - Human Immunodeficiency Virus (HIV)
    - Hepatitis B
- Prior history of grafting surgeries or other surgeries in the dermatologic treatment area
- History of significant condition in the dermatologic treatment area such as trauma or non-healing chronic wound, which could impair evaluation for the treatment of EBS.
- Use of other investigational drugs within 30 days of the screening visit and/or has not recovered from any side effects of prior investigational drugs or procedure in the affected area (e.g., a biopsy).
- Use of acitretin within the last 1 month
- Use of Roaccutane within last 3 months
- Botox injections to the feet within the last 6 months.
- Participant is planning extra physical activities within the next 3 months.
- Amputated foot

5.4 Withdrawal from the study

Participants must discontinue investigational medicinal product (IMP) and be withdrawn from the study for the following reasons:

- Participant withdraws consent
- Investigator request e.g. non-compliance with visits
- Participant meets any of the above exclusion criteria

6.0 Study Design and Intervention

6.1 Study Design

This study is a prospective, double-blind, randomized, placebo-controlled crossover study at Stanford University School of Medicine, USA. It includes a 6-week screening period, followed by a 4 Phase Pilot Clinical Trial (see Table 2).

Screening: Up to 6 weeks
Phase 1: 12-week treatment period
Phase 2: 4-week wash-out period
Phase 3: 12-week treatment period
Phase 4: 4- week wash out period
Follow-up: 8 weeks after end-of-trial visit.

This research study is expected to take approximately 40 weeks. There will be 2 different parts of the study which the subject may receive for initial treatment period either (a) placebo (no drug) or (b) study drug for 12 weeks, during their enrollment in the 40-week study. After the initial 12-week period, there will be a washout period consisting of 4 weeks, before starting secondary treatment. During the secondary treatment period, a switch will occur and subject will receive either the (a) placebo (no drug) or (b) study drug depending on which treatment was received initially. At some time point during the 40-week study, at least both feet will receive study drug. There will also be a post-treatment visit at week 16 and week 32, with a post study phone call at Week 40. The end of treatment period will occur at Week 12 and Week 28, with the final in-person study visit occurring at week 32. Structured phone calls made by a physician will occur at Week 2, Week 14, Week 18, and Week 40.

The EBDASI feet score needs to be within 20% of baseline to progress to Phase 3. If subject's score does not meet this criteria the Phase 2 wash-out can be extended to 6 weeks.

Treatments are switched after Phase 2.

In-person study visits:

- Week 0 (screening/baseline)
- Week 4
- Week 12
- Week 16
- Week 20
- Week 28
- Week 32

Structured physician phone-call will be scheduled at:

- Week 2
- Week 14
- Week 18
- Week 40

6.2 Recruitment Process

EBS is a rare orphan disease and Stanford is one of several multidisciplinary clinics in the world that focuses on the treatment of EB. With a prevalence of 6-10 per million, there are roughly 3,200 patients in the United States. This study aims to enroll a total of 8-10 patients at Lucile Packard Children's Hospital Stanford. We cooperate with national and international networks of families, researchers, and physicians who care for children and adults with EB and plan to use these groups to recruit subjects for this study. In addition we will use our email listserv (ebcarenetwork@lists.Stanford.edu) to inform our national communities that we are recruiting for this study. Patients have previously signed Consent Forms that have been approved by the Stanford ethics committee to be involved in these databases and contacted. Over 200 people are contained within these databases, 24 of which have EB with known EBS genetic KRT 5/14 mutation. We will access these databases to gain participant names, addresses, phone numbers and email addresses that we will use to invite them to participate in the study. Preference in enrolment will be given to participants in proximity to the study site and those with genetic KRT 5/14 mutations. Genetic testing is not a requirement for EBS diagnoses nor study enrollment. The mechanism of disease in EBS is well known as an autosomal dominant defect in keratin 5 or keratin 14. We will not be genotyping, the cost of genotyping is high at $3-4k per subject. If genotyping is already known, this information will be utilized. Our recruitment methods will not involve material inducements. A telephone recruitment form has been submitted with this IRB request. Study personnel will not receive incentives for recruiting study participants or for any other purpose directly related to the study.

6.3 Compensation for participation

There will be no compensation for participation in this study but travel expenses may be reimbursed e.g. by using IPTAAS (Isolated Patients Travel and Accommodation Assistance Scheme).

6.4 Screening/Baseline Period

Before any study-specific interventions are performed, the appropriate written informed consent must be obtained for the trial and a separate consent for skin biopsy, which is an optional additional procedure.

To assess subject eligibility, all screening assessments as follows should be completed: eligibility criteria, medical history and physical exam, weight and height, demography, vital signs, concomitant medications, Epidermolysis Bullosa Diseases Activity Score (EBDASI), Quality of Life in Epidermolysis Bullosa (QOLEB), collection of blood for: metabolic panel, complete blood cell count with differential, urinalysis, lipid profile.

The participant will also be initiated in how to use the Fitbit and completion of daily diaries and the various different questionnaires to be used in the trial.

6.5 Treatment Period – Phase 1

Participants who meet all eligibility criteria will be randomized 1:1 to receive either topical sirolimus 2% or placebo ointment. The on-site Registered Nurse will teach the participant how to apply the ointment to both feet and will administer the first dose of IMP at week 0. Subsequent twice daily application of IMP to both feet will be performed by the participant, this will continue for 12 weeks.

6.6 Wash-Out Period – Phase 2

After completion of Phase 1 the participant will stop applying the IMP to the feet for a period of 4 weeks at which point their baseline activity will be assessed. If their EBDASI feet score is within 20% of baseline they can proceed to Phase 3.

6.7 Treatment Period – Phase 3

Participants who received topical sirolimus 2% during phase 1 will switch to placebo ointment and vice versa. Twice daily application of IMP will be continued by the participant for a further 12 weeks. The trial will remain double-blinded throughout.

6.8 Wash-Out Period – Phase 4

After completion of Phase 3 participants will enter a further 4-week wash-out period where they will stop applying the IMP to their feet. At the end of Phase 4 they will attend for their final end-of-trial physician visit.

6.9 Follow-up Period

Week 32-40 will be the follow-up period with a structured physician phone consultation at Week 40. Participants who withdraw from treatment will also enter the follow-up period.

7.0 Study Treatment

7.1 Investigational Product

Topical sirolimus 2% and placebo ointment will be made and supplied by PCCA compounding pharmacy. Placebo ointment will consist of vehicle only – i.e. all the same components as the topical sirolimus 2% ointment minus the active

ingredient. Both will have the same appearance, texture and odor and will be in a matching container or tube.

Tubes will contain vehicle or sirolimus 2% ointment in a 60 gram supply, the amount of topical for twice daily application over 2 weeks. We expect 360 grams for each phase of the treatment, 720 grams total with 360 grams intervention and 360 grams vehicle. Vehicle is an ointment plasticized base contained no water that provides at least 6 months sirolimus stability (Chemistry RX, Lars Brichta April 8 communication; PCCA plasticized base information provided in Section 16). 'A kit' describes two components in a plastic bag or cardboard box: (1) vehicle (placebo) or intervention (sirolimus 2%) ointment in 60 gram tubes and (2) our "Study Drug Application Kit Instructions' (provided in Section 16).

Contents of the label will be in accordance with all applicable regulatory requirements.

Study Drug Storage: The IP will be stored at room temperature in a dry place, away from direct sunlight.

At each physician visit the participant will be re-supplied with sufficient IMP until their next visit. The containers will also be weighed to monitor how much IMP is being used.

Patients will be instructed to wear gloves and change gloves between applications.

7.2 Treatment Assignment

Participants who provide informed consent and undergo screening assessments receive a unique participant number. This assignment of participant numbers occurs at the screening and baseline visit and serves to identify data in an anonymous fashion. Participant eligibility will be established at the conclusion of the baseline evaluations (prior to randomization and first dose of IMP at Week 0). Participants who meet all entry criteria will then be randomized in a 1:1 allocation to receive either topical sirolimus 2% or placebo ointment according to a computer-generated randomization list.

The participants and the investigator staff (except the unblinded pharmacist) will be blinded to the treatment assignment. Participants who withdraw from the study following informed consent, but before randomization to study treatment, will be classified as screen failures.

7.4 Blinding

This is a double-blind study. All study staff (which includes the investigator, sub-

investigators, other site staff) and participants will be blinded to the treatment allocation throughout the trial.

The blinded study treatment assignments for individual participants will be maintained by a Central System used by the study pharmacy. Emergency unblinding will be available via the central system in a case where the identity of the IMP must be known in order to treat an emergent adverse experience for an individual patient.

If the treatment blind is broken for a participant in the treatment period, the participant in question will be withdrawn from the treatment period and moved into the follow-up period.

During the study, investigators will not receive central laboratory data that have the potential to unblind a subject's treatment assignment.

7.5 Prohibited concomitant medications

Participants may take other medications for treatment of the symptoms associated with EBS, so long as they do not fall into any of the prohibited medications category (see exclusion criteria).

8.0 Study Assessments

Table 2 shows the schedule of assessments for the study. The investigators (medically qualified) are to conduct all study assessments. The investigator or designee must obtain written informed consent prior to performing any of the study information that will be recorded in source documents.

If a visit cannot be scheduled on the appropriate date, the visit should be re-scheduled as close as possible to the planned date, ideally within 3 days.

The study participants and caregivers will be given specific instructions about gentle skin care and avoid possible irritants on the treated area during the entire study. Patients will also be instructed to inspect the plantar surface daily to ensure no trauma.

8.1 Medical history and examination

A complete medical history will be taken at the Screening Visit. Information from the medical history is important to establish the baseline condition of the participant and will impact the safety monitoring assessments during the study. Any significant medical conditions affecting the participant in the past 5 years should be recorded. The history should include the following:

- A thorough review of systems, including any past or current conditions
- Prior surgical procedures
- Full medication history
- Prior immunosuppressive therapies, including type, number, and duration
- Allergies and significant allergic reactions
- Significant infections, or history of recurrent infection, including urinary and respiratory tract infections
- Smoking history (current or previous smoker, number of packs per day smoked)
- Full physical examination will be performed by an experienced medical practitioner.

8.2 Epidermolysis bullosa simplex history

Details of the participant's EBS history will be collected (e.g. dates of symptom onset, diagnosis) and recorded. These data are critical for establishing subject eligibility, as well as establishing a baseline for subsequent clinical assessments.

8.3 Primary outcome measures

1. Foot function utilizing the validated Foot Health Status Questionnaire (FHSQ) as a change from baseline to the end of each treatment.
2. Safety and tolerability of topical sirolimus 2% ointment (serum trough sirolimus levels, monitoring for adverse events).

8.4 Secondary Outcome Measures

1. Plantar defect size measurements using 3D photography (% change in total defect area) from baseline to the end of each treatment.
2. EB Disease Activity and Scarring Index (EBDASI) feet activity scores as a change from baseline to the end of each treatment
3. Quality of life assessment in EB (QOLEB) as a change from baseline to the end of each treatment.
4. Itch severity measured using 5-D Pruritus Scale as a change from baseline to the end of each treatment.
5. Participants will wear a FitBit® for the duration of the study to assess number of steps walked per day as a change from baseline to the end of each treatment.
6. Changes in foot plantar pressure measurements before and after treatment using the Podotech Elftman Foot Scanner from baseline to the end of each treatment.
7. Molecular biology study of skin biopsies assayed for mTOR pathway inhibition (e.g. determination of phosphoprotein inhibition included ribosomal protein S6, S6 kinase and/or eIF-4E binding protein) as a change from baseline to the end of each treatment.

8.5 Safety Assessments

Safety and tolerability of the medication will be assessed at each visit through recording of adverse events, serious adverse events, evaluation of systemic absorption through measurement of trough sirolimus levels in serum, and additional safety laboratory tests listed below will also be performed.

Investigator assessment of local skin reaction (including increased erythema, edema, weeping/exudates, flaking/scaling/dryness, scabbing/crusting, and erosion/ulceration) will be assessed independently of other adverse effects.

Serum sirolimus levels
Trough serum sirolimus levels are a frequently run outpatient laboratory ELISA assay and will be performed at Stanford University at specific study visits from baseline.

Other Safety Laboratory Assessments
The clinical safety laboratory parameters will be assessed at each study visit. Laboratory tests may be repeated more often if clinically indicated.

All out-of-normal range laboratory values must be evaluated by the Investigator or subinvestigator as to whether they are clinically significant (i.e., require medical intervention or have clinical signs and/or symptoms). Abnormal clinical laboratory parameters that are considered clinically significant by the Investigator will be recorded on the Adverse Event (AE) Case Report Form (CRF) except Baseline visit. Clinically significant laboratory abnormalities noted from the Baseline visit will be recorded as medical history. The AE should be reported as a clinical diagnosis when possible. Laboratory tests will be performed at Stanford Children's Health clinics.

Urine Pregnancy Tests (UPT) will be performed at each scheduled visit on female subjects of childbearing potential starting at Baseline. If a UPT results in a positive test, the subject must be discontinued.

Sample management (collection, storage, shipping, etc…) will be done according to Stanford Clinical Laboratories standards and policies.

Clinical safety laboratories will include:

- Full blood count (CBC, Metabolic Panel, Liver function tests)
- Lipid panel profile
- Urinalysis
- Sirolimus trough (at specific study visits)

These will be performed at each scheduled study visit from screening/baseline visit (7 total), except for sirolimus trough.

Adverse Events (AE) and Serious Adverse Events (SAEs)

1. AE frequency, severity, and relationship to IMP.
2. Frequency of withdrawals due to treatment-related AEs.
3. Frequency of serious adverse events (SAEs)

8.6 Efficacy Assessments

Clinical assessments of the extent and severity of plantar lesions will be performed throughout the study.

Foot Health Status Questionnaire (FHSQ)

The purpose of this questionnaire is primarily to measure foot health related to foot function and quality of life. There are 19 questions regarding foot health and its impact with 4 subscales: foot pain, foot function, footwear and foot health. It has been shown to have excellent validity and ability to detect change in clinical trials and has demonstrated good reliability (Riskowski et al., 2011).

Epidermolysis Bullosa Disease Activity Score (EBDASI)

The EBDASI is a validated scoring system that objectively quantifies the severity of EB affecting the entire body. It has been designed to evaluate the response to new therapies for the treatment of EB. It was developed and validated many time over in the literature (Loh et al., 2014)

EBDASI feet score

Is a subsection of the EBDASI and objectively quantifies activity and damage levels relating to the feet only. Activity is graded according to size and number of lesions (blisters/erosions) in specific anatomical area. Damage is graded according to the presence/absence of the following signs: erythema, pigmentation, poikiloderma, atrophy, hyperkeratosis, scarring and milia.

Method of defect assessment

Tracing of affected wounds will be performed using Visitrak Digital Wound Assessment System. This is a portable device that provides accurate, reproducible data for tracking wound progress (See Figure 2).

Quality of life in Epidermolysis Bullosa (QOLEB)

QOLEB is a Quality of Life Questionnaire specifically designed for people with EB. The QOLEB can be used to identify everyday life occurrences negatively affected by EB. It assesses change in quality of life over time, an important measure when assessing the success of new treatments for EB (Frew et al., 2009).

Plantar Pressure Measurements

The Podotech Elftman foot pressure scanner uses 1600 electronic sensors incorporated into a thin mat to measure pressures at various points on the soles of the feet. This analysis can be performed while standing to get a static analysis or when walking to get a dynamic analysis. Peak pressures can be calculated and can correlate with areas of hyperkeratosis and changes in loading due to pain. A before and after assessment of participants feet using this device will give us insight into how hyperkeratosis has changed as well as

changes in loading and pain. This technology is extensively investigated in rheumatoid arthritis patients (Van der leeden et al 2008).

Fitbit Pedometer Mobility Measurements
Each enrolled study subject will receive a FitBit®/pedometer during their participation in the study to track their activity levels. This purchase will be made using our SPARK grant funding and be provided to subjects at no cost. Will add the information below to the consent and in Section 16.

The FitBit / pedometer will sync stats wirelessly to a deidentified premium account at www.fitbit.com/zip. An Excel form of the data will be downloaded on health protected computers every two weeks. FitBit pedometers have been demonstrated in the literature as reliable and accurate (Storm FA et al. Step Detection and Activity Recognition Accuracy of Seven Physical Activity Monitors. PLoS ONE, 2015).

Erythema score of soles of feet
An erythema score of a specified location on the soles of the feet will be obtained through use of Mexameter® MX 18 to detect any change in erythema at each study visit. This will be an objective measure of local skin irritation at the treatment site. This is an easy, quick and economical tool to measure erythema by reflectance. The instrument is worldwide established and used in many scientific studies.

Skin biopsies assayed for mTOR pathway inhibition
The skin biopsies are optional and will be assayed for transcriptional factors of mTOR pathway inhibition using Western blot / immunohistochemical methods (Transderm laboratory, Santa Cruz USA) e.g. determination of phosphoprotein inhibition included ribosomal protein S6, S6 kinase and/or eIF-4E binding protein. Tissue samples will be transferred to Transderm laboratory deidentified and without identifiable personal health information. A separate consent, and if applicable, assent form(s) will be used.

5-D Pruritus Scale
People with EBS can experience the aggravating symptom of itch. This validated tool will be utilized to assess the effect (if any) topical sirolimus has on this symptom (Elman et al., 2010)

8.7 Adverse Events (AE) and Serious Adverse Events (SAEs)
The investigator is responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE. AEs will be collected from the start of study treatment through the protocol-specified follow-up period.

Definition of an AE: Any untoward medical occurrence in a subject or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
Note: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated)

temporally associated with the use of a medicinal product. This also includes failure to produce expected benefits (i.e. lack of efficacy), abuse, or misuse.

A serious adverse event (SAE) is any untoward medical occurrence that:

a. Results in death
b. Is life-threatening
c. Requires hospitalization or prolongation of existing hospitalization
d. Results in disability/incapacity, or
e. Results in a congenital anomaly/birth defect

8.8 Pregnancy

Women of childbearing potential must have a negative urine pregnancy test within 48 hours prior to starting treatment with study drug. Females of non-childbearing potential are defined as meeting at least one of the following criteria: (1) over the age of 60 years (2) amenorrheic for at least 2 years, (3) have had a hysterectomy and/or bilateral oophorectomy. All other females (including those with tubal ligation) will be considered to be of childbearing potential. At visits 2-12 urine pregnancy testing need only be performed if the subject has missed a menstrual cycle. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child.

9.0 Data and Statistical Analysis

9.1 Hypotheses

The null hypotheses is that there is no difference in efficacy between topical sirolimus 2% and placebo ointment in improving the clinical severity/function/pain scores of the plantar lesions in EBS. The alternative hypothesis is that topical sirolimus 2% is superior to placebo ointment.

9.2 Sample size calculation

Recruitment of **8 subjects** needed to detect an average 30% improvement of foot function score between topical sirolimus 2% and placebo with standard deviation of 40%, with power >0.8 and two-sided significance level of 5% (Stanford Statistician Group, USA).

9.3 Treatment comparisons

The primary comparison of interest is between placebo and topical sirolimus 2% on foot function using the validated Foot Health Status Questionnaire.

Secondary comparisons of interest will be made between placebo and topical sirolimus 2% on the secondary outcome measures.

10.0 Study conduct considerations

10.1 Posting information on Clinical Trials Registers
Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of participants begins. (clinicaltrials.gov)

10.2 Regulatory and ethical considerations
The study will be conducted in accordance with ICH Good Clinical Practice, and the ethical principles that are outlined in the Declaration of Helsinki 2008.

10.3 Records storage
Following closure of the study, the investigator will maintain all site study records in a safe and secure off-site location for duration of 15 years. The records will be easily accessible when needed and will be available for review by regulatory bodies.

All of the records will be maintained in a format other than hard copy (e.g. scanned,electronic).

10.4 Independent data monitoring committee (IDMC)
This will consist of the following:
- [redacted], MD PhD
- [redacted] MB MD FRCP
- [redacted], MD PhD
- [redacted], MD

10.5 Protected Health Information
During screening, patient's initials must be used for tracking purposes. If the subject enrolls in the study, then name and medical number will be necessary as each subject will have a medical chart during the study. Demographic information such as date of birth, phone numbers, and address will be used for communication purposes. Names and medical numbers will be required for lab and other necessary testing, clinic appointments, etc. This includes office visits for the study, medical history and medication history, blood test results, and processing of laboratory samples including tests. However, with exception of initials and subject number, no identifiers are included on survey instruments and questionnaires.

A complete list of health information and identifiers include:
Diagnosis and Procedures by visit
Clinical notes
List of ICD 9 diagnosis
Lab Test Results
Pathology reports
Radiology report
Pharmacy data/Medication list
Gender

Age at Diagnosis
MRN
Names
Contact information (phone number, address, email address)
Date of Birth
Medical History

10.6 Privacy and Confidentiality

Documents pertaining to the study and any other information received will be kept strictly confidential. The data may be reviewed by representatives of regulatory authorities only for the purpose of verifying procedures. Other than this, disclosure of data will only occur if it is required by law.

Physical documents will be kept within folders, which will be locked within the Department of Dermatology, St George Hospital. Electronic data will be kept on locked departmental computers and stored in password-protected files. Only study staff will have access. Records pertaining to the study will be kept for 15 years in a secure location away from the study site. After this amount of time has transpired the records will be disposed of securely.

No identifying information will be included in publications or presentations of the results without prior written consent from the participant.

10.7 Genomic Data Sharing Plan[1]

The data that will be shared:

I will share phenotypic and genotypic data associated with the collected samples by depositing these data at Database of Genotypes and Phenotypes (dbGaP), which is an NIH-funded repository. Additional data documentation and de-identified data will be deposited for sharing along with phenotypic data, which includes demographics, family history of Epidermolysis bullosa simplex disease, and diagnosis, consistent with applicable laws and regulations. I will comply with the NIH GWAS Policy and the funding Institute and Centers existing policies on sharing data on Epidermolysis bullosa simplex disease genetics to include secondary analysis of data resulting from a genome wide association study through the repository. Meta-analysis data and associated phenotypic data, along with data content, format, and organization, will be available at Database of Genotypes and Phenotypes (dbGaP). Submitted data will conform to relevant data and terminology standards.

Who will have access to the data:

I agree that data will be deposited and made available through Database of Genotypes and Phenotypes (dbGaP), which is an NIH-funded repository, and that these data will be shared with investigators working under an institution with a Federal Wide Assurance (FWA) and could be used for secondary study purposes. I agree that the names and Institutions of persons either given or

denied access to the data, and the bases for such decisions will be summarized in the annual progress report. Meta-analysis data and associated phenotypic data, along with data content, format, and organization, will be made available to investigators through Database of Genotypes and Phenotypes (dbGaP).

Where will the data be available:
I agree to deposit and maintain the phenotypic data, and secondary analysis of data (if any) at Database of Genotypes and Phenotypes (dbGaP), which is an NIH-funded repository. The repository has data access policies and procedures consistent with NIH data sharing policies.

When will the data be shared:
I agree to deposit genetic outcome data into Database of Genotypes and Phenotypes (dbGaP) repository as soon as possible but no later than within one year of the completion of the funded project period for the parent award or upon acceptance of the data for publication, or public disclosure of a submitted patent application, whichever is earlier.

How will researchers locate and access the data:
I agree that I will identify where the data will be available and how to access the data in any publications and presentations that I author or co-author about these data, as well as acknowledge the repository and funding source in any publications and presentations. As I will be using Database of Genotypes and Phenotypes (dbGaP), which is an NIH-funded repository, this repository has policies and procedures in place that will provide data access to qualified researchers, fully consistent with NIH data sharing policies and applicable laws and regulations

NOTE: NIH is currently updating their guidance on data sharing plans, which will be available soon. For questions, contact the NIH Office of Extramural Research (OER) at Sharing@nih.gov or your RPM.

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12.0 Tables

Table 1: Review of topical sirolimus use in dermatological disease (Fogel et al., 2015)

Indication	No. of patients	Vehicle	Concentration	Frequency	Duration, wk	Improvement	Irritation*	Serum levels	Study	Year
Chronic erosive oral lichen planus	7	Solution	0.1%	BID	12	Yes	Yes	Undetected/ <1.5 ng/mL	Soria et al[54]	2009
Familial multiple discoid fibromas	1	Solution	0.1%	BID	16	Yes	Yes	Undetected	Wee et al[57]	2013
Pemphigus vulgaris (mucosal lesions only)	3	Mouthwash	0.1%	BID	2-3	No	No	NA	Poot and Jonkman[58]	2012
Tuberous sclerosis facial angiofibromas	1	Ointment	1%	BID	12	Yes	No	Undetected	Haemel et al[59]	2010
	1	Ointment	1%	BID	4	Yes	No	NA	DeKlotz et al[60]	2011
	2	Solution	0.10%	QID/BID	10-23	Yes	Yes	Undetected	Mutizwa et al[61]	2011
	9	Ointment	0.20%	BID	12	Yes	No	Undetected	Wataya-Kaneda et al[62]	2011
	4	Solution/ ointment	0.10%	BID	24	Yes	Yes	NA	Foster et al[5]	2012
	2	Cream ± oral rapamycin	0.10%	QID	12-24	Yes	No	NA	Kaufman McNamara et al[63]	2012
	23	Cream	0.003%-0.015%	QID	24	Yes	2/23 Patients	Undetected	Koenig et al[64]	2012
	10	Ointment	0.40%	3 Times/wk	36	Yes	No	<0.3 ng/mL	Salido et al[65]	2012
	1	Cream	1%	QID	6	Yes	No	<0.3 ng/mL	Truchuelo et al[66]	2012
	2	Ointment	0.20%	BID	12	Yes	No	<0.3 ng/mL	Wataya-Kaneda et al[67]	2012
	11	Ointment/gel	0.20%	BID	12	Yes	No	Undetected	Tanaka et al[68]	2013
	2	Cream	0.10%	QID/BID	52	Yes	No	Undetected	Wheless et al[55]	2013
	19	Ointment	0.1%-1%	BID-3 times/wk	32-120	Yes	No	Undetected (17/19), 5 μg/L (1/19), 8 μg/L (1/19)	Tu et al[69]	2014

BID, Twice daily; *NA*, not applicable; *QD*, once daily; *QID*, four times daily; *Wk*, week.
*Irritation was the only reported side effect in all reports.

Schedule of Events:

Study period	Phase 1[b] Screening/Baseline	Phase 1[b]	Phase 1[b]	Phase 1[b]	Phase 2[c]	Phase 3[d]	Phase 3[d]	Phase 3[d]	Phase 3[d]	Phase 4[e]	Follow-up[f]
Visit Number	1	□ [5]	2	(EOT)[a] 3	□[5] WASH-OUT	4	□ [5]	5	(EOT)[a] 6	7	□ [5]
Study Week	**Week 0**	**Week 2**	**Week 4**	**Week 12**		**Week 16**	**Week 18**	**Week 20**	**Week 28**	**Week 32**	**Week 40**
Informed Consent/Assent (If applicable)	X										
Epidermolysis Bullosa Genotyping (Optional)	X										
Verify eligibility criteria	X										
Vital signs	X		X	X		X		X	X	X	
Medical history	X										
Adverse events	X	X	X	X	X	X	X	X	X	X	X
Serious Adverse events	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X		X	X		X		X	X	X	
Mexameter erythema score	X		X	X		X		X	X	X	
Medical Exam	X			X					X		
Foot Health Status Questionnaire	X		X	X		X		X	X	X	
Defect area measurement using Visitrak	X		X	X		X		X	X	X	
2D photography of feet	X		X	X		X		X	X	X	
EBDASI feet score	X		X	X		X		X	X	X	
EBDASI total score	X			X		X			X		
QOLEB	X			X		X			X		
5-D Pruritus Scale	X			X		X			X	X	
Plantar pressure measurement	X			X		X			X	X	
Study drug collection/weighing/accountability	X		X	X		X		X	X		
Supervise study drug self-administration[l]	X		X			X		X			
Fitbit (daily)	X	X	X	X	X	X		X	X	X	X

Collection of blood for:											
Urine Pregnancy Test[h]	X		X	X		X		X	X	X	
Visit Number	1	□ [5]	2	(EOT)[a] 3	□[5] WASH-OUT	4	□ [5]	5	(EOT)[a] 6	7	□ [5]
Study Week	Week 0	Week 2	Week 4	Week 12		Week 16	Week 18	Week 20	Week 28	Week 32	Week 40
Sirolimus trough level			X	X				X	X	X**	
Urinalysis	X			X		X			X	X	
CBC	X		X	X		X		X	X	X	
Metabolic panel	X		X	X		X		X	X	X	
Lipid profile	X		X	X		X		X	X	X	
Skin biopsy (optional)	X			X		X			X		
Structured phone-call		X			X		X				X

If a washout period is needed prior to baseline, subject has up to 6 weeks before starting treatment.

a. EOT= End of Treatment

b. After screening, subject will be randomized and enter Phase 1, the initial treatment application period. Subject will receive study drug or vehicle during this 12-week treatment period.

c. When Phase 1 is complete, a wash-out period will occur, which will last 4 weeks.

d. Phase 3 is the secondary treatment application period. The subject will receive either study drug or vehicle, switching from treatment initially used in Phase 1 during this secondary 12-week treatment period. Phase 3 may be delayed if subject has not reached 20% baseline EBDASI activity / Visitrak 2cm diameter.

e. Phase 4 will be a 4 week follow-up period after study treatment has concluded.

f. A scheduled phone call will occur at Week 40, after post treatment period at Phase 3.

g. Structured physician phone-call will be scheduled

h. Eligible female subjects of childbearing potential

i. Application occurs at the site on visit days

Table 3: Trial Objectives and Outcome Measures

Primary Objectives	Primary Outcomes
1. To assess for efficacy of topical sirolimus to improve foot function in the majority of participants with EBS at the end of each treatment versus baseline.	Foot function utilizing the validated Foot Health Status Questionnaire (FHSQ) as a change from baseline to the end of each treatment.
	Primary Outcomes
2. Safety and tolerability of topical sirolimus 2% cream	Recording of adverse events, serious adverse events. Evaluation of any systemic absorption of topical sirolimus 2% through measurement of sirolimus trough levels in serum. Additional laboratory tests including full blood count, lipid panel, urea and electrolytes and liver enzymes at baseline through to week 40 will be performed.
Secondary Objectives	**Secondary Outcomes**
3. To assess the efficacy of topical sirolimus to improve clinical severity of lesional skin, including pain and itch symptoms, in the majority of participants with EBS at the end of each treatment versus baseline.	a) Plantar defect size measurements using Visitrak and 2D photography (% change in total defect area) from baseline to the end of each treatment. b) EB Disease Activity and Scarring Index (EBDASI) feet activity scores as a change from baseline to the end of each treatment c) Quality of life assessment in EB (QOLEB) as a change from baseline to the end of each treatment. d) Itch severity measured using 5-D Pruritus Scale as a change from baseline to the end of each treatment. e) Participants will wear a FitBit® for the duration of the study to assess number of steps walked per day as a change from baseline to the end of each treatment. f) Changes in peak foot pressures and loading, before and after treatment using the Podotech Elftman foot pressure scanner as a change from baseline to the end of each treatment. g) An erythema score of the soles of the feet will be obtained through use of Mexameter® MX 18 to detect any change in erythema at each study visit. This will be an objective measure of local skin irritation at the treatment site. h) Molecular biology study of skin biopsies assayed for mTOR pathway inhibition (e.g. determination of phosphoprotein inhibition included ribosomal protein S6, S6 kinase and/or eIF-4E binding protein) as a change from baseline to the end of each treatment.

Figure 1. Schematic of the proposed study

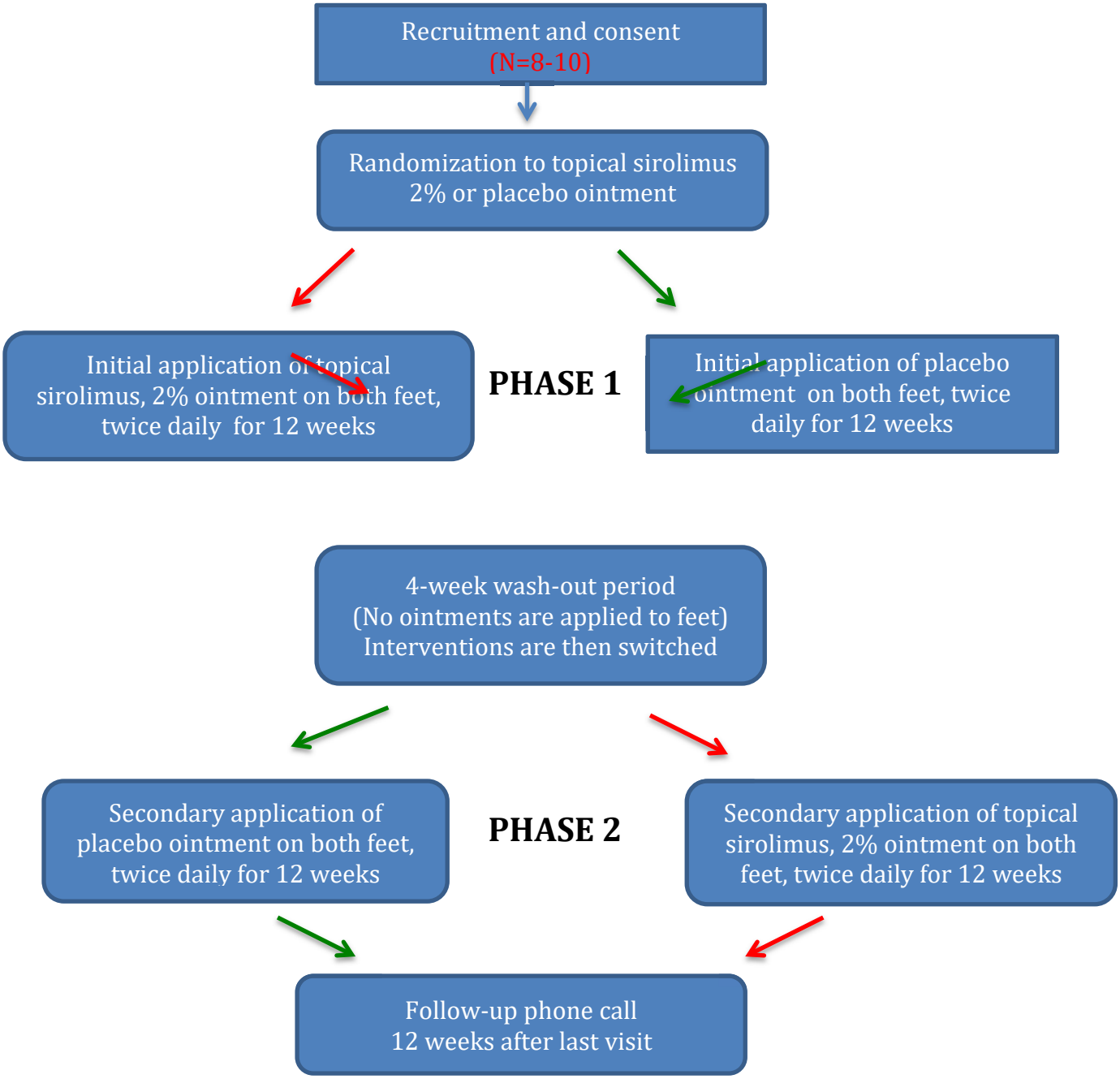
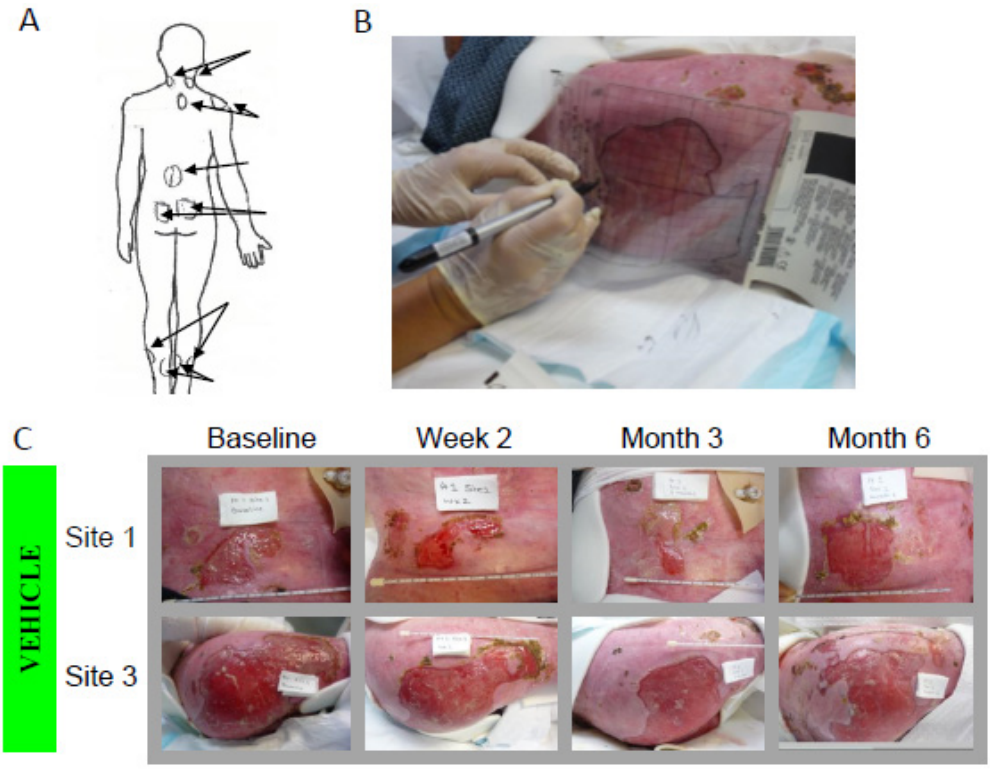


Figure 2. Method of defect measurement using Visitrak Digital Wound Assessment System

Tracing of affected areas (hyperkeratosis or wounds)



A. Selection of paired chronic wounds in a patient with RDEB.
B. Wound size measurements made by 2-D tracing method.
C. RDEB wound ulcers improved after vehicle injections at baseline, with a maximum response at 3 months (Venugopal et al., JAAD 2013). The same visitrak method will be used to trace affected areas in EBS.