

Protocol Title: A STUDY IN ATOPIC DERMATITIS TO DETERMINE SERUM AND SKIN BIOPSY BIOMARKERS IN PATIENTS RECEIVING TOPICAL CORTICOSTEROIDS (TCS) AND FOLLOWING TCS WITHDRAWAL

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Short Title: Biosample Collections in Atopic Dermatitis

This study will be conducted according to the principles of Good Clinical Practice (GCP), including the archiving of essential documents.

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INTRODUCTION

Atopic Dermatitis (AD) is a chronic relapsing and remitting inflammatory skin disorder affecting all age groups. Clinically AD is characterized by xerosis, erythematous crusting rash, lichenification, an impaired skin barrier and intense pruritus (Bieber 2008).

AD is one of the most common dermatologic diseases and the prevalence has increased over the past two to three decades; 15% to 30% of children and 2% to 10% of adults are affected (Williams et al. 2006). Overall, the disease burden is high and quality of life (QoL) in AD patients is severely impacted, even when compared to other skin diseases such as psoriasis (Keibert et al. 2002).

The therapeutic approach to AD primarily consists of trigger avoidance, skin hydration with bathing and use of emollients and anti-inflammatory therapies consisting predominantly of topical corticosteroids (TCS). For patients with moderate to severe disease, a step up in therapy is required, including an increase in the potency and frequency of TCS. However, long term (months) use of TCS, particularly high potency TCS, is not recommended due to the increased incidence of both local and systemic side effects (Simpson EL 2010). In cases where high doses (potency and frequency of use) of TCS are unable to control disease, topical calcineurin inhibitors (TCIs) may be used to control disease and/or allow a reduction in TCS potency particularly at high-risk sites. Recent guidelines provide a number of options for patients not responding to TCS and/or TCI depending on the clinical assessment of disease activity (Ring et al. 2012; Schneider et al. 2013), that include, phototherapy and immunosuppressive agents such as oral corticosteroids, cyclosporine, azathioprine, methotrexate and mycophenolate. Although these medications may be effective, significant toxicities limit their long term use. The continued use of these agents despite the harmful side effects and limitations speaks to the high unmet medical need for safer and more effective therapies.

Therapy targeted at central pathologic mediators has promise as a means to maximize efficacy and limit toxicity. The high expression of IL-13 in AD skin (Gittler 2012, Choy 2012) indicates that lebrikizumab, an anti-IL-13 monoclonal antibody, may represent such a targeted therapy. Genentech plans to perform a Phase II study to assess the efficacy and safety of lebrikizumab in patients who have persistent moderate to severe AD that is inadequately controlled by TCS. The design of this study includes a run-in periods on daily prescribed TCS for 2 weeks, followed by randomization to placebo and treatment arms remaining either on or off background TCS, respectively. Assessment of pre-determined biomarker candidates have been incorporated into the planned Phase II study design to characterize the mechanism of action (MOA) and treatment effect of lebrikizumab on biomarkers of AD disease. However, the pre-determined biomarkers were identified from studies of AD patients who had been withdrawn from TCS weeks prior to providing samples, and it remains to be determined how well these markers are representative of AD disease on a background of TCS. Therefore, the purpose of this proposed, interventional study will be to collect serum and lesional and non-lesional skin from moderate to severe AD patients both on, and after withdrawal from a stable TCS regimen in order to provide samples that can answer this outstanding question.

Study Objective

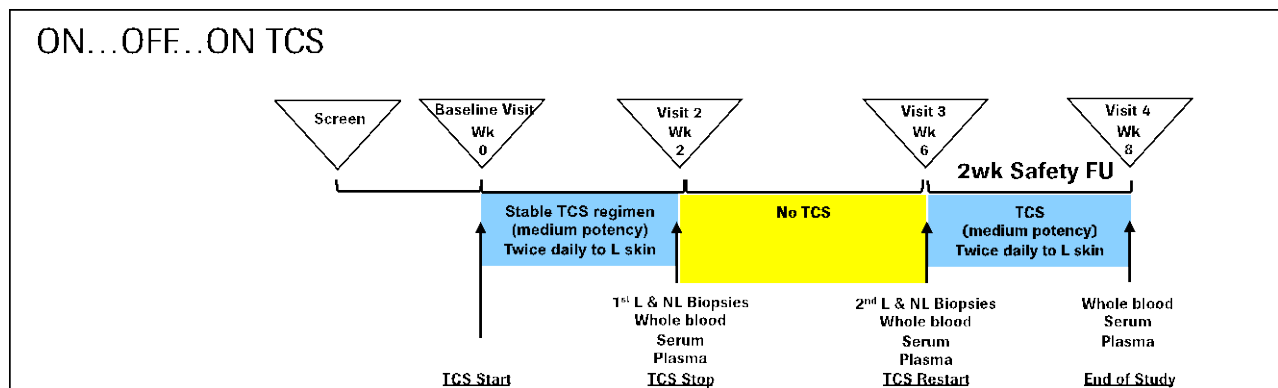
To assess the differential expression of AD biomarkers in serum, plasma, and skin biopsies from both lesional and non-lesional skin in moderate to severe AD patients in the presence of TCS and after withdrawal from TCS.

INVESTIGATIONAL PLAN

Study Design

This exploratory study consists of four visits to the investigator site post screening: at Weeks 0 (baseline), 2, 6 and 8 (Figure 1). At the first visit (baseline), patients who have met the screening eligibility criteria will be started on a stable TCS regimen for a total of two weeks. At the second visit (Week 2) following two weeks of therapy on stable TCS, patients will stop TCS and blood, serum, plasma and two 6mm punch biopsies (one lesional (L) and one non-lesional (NL) skin) will be obtained. At the third visit (Week 6), after four weeks receiving no TCS, the same sample collections will be repeated and the patients will then enter a two week safety follow up. At the end of the safety follow up (last visit, Week 8) and after obtaining written informed consent by the patient, blood, serum and plasma samples may be collected.

Figure 1. Study Design



RATIONALE FOR BIOMARKER ASSESSMENTS

The sensitivity of IL-13/atopic dermatitis–related biomarkers to topical corticosteroid is unknown. This study is intended to increase this understanding and will ultimately inform selection of steroid-resistant biomarkers that can be evaluated in subsequent interventional trials where Lebrikizumab is provided as an adjunctive therapy to topical corticosteroid.

To that end, blood and skin biomarkers, including but not limited to eosinophils, IgE, IL-13, CCL-13 and CCL-17, will be evaluated for differential gene expression and protein levels in samples obtained from atopic dermatitis patients on and off topical corticosteroid treatment. The skin evaluations will include Hematoxylin & Eosin (H&E) staining to evaluate the pathologic skin phenotype, and immunohistochemistry (IHC) for biomarkers of dermatopathologies including but not limited to epidermal hyperplasia (eg. KI16, Ki67), epidermal differentiation (eg. Filaggrin), and inflammatory cell infiltration (eg. eosinophils).

Additionally, differential gene expression analysis of lesional and non-lesional skin collected from patients on and off topical corticosteroid will be evaluated to measure steroid responsive genes, including but not limited to FKBP51.

Whole blood for genomic analyses of predisposing factors, including, but not limited to, the two common filaggrin mutations, R501X and 2282de14 will also be determined.

SAMPLE SIZE

Assuming the proportion of non-differentially expressed genes is above 0.997 and that the gene expression variances are following an Inverse Gamma(3, 0.3) distribution (as observed in Gittler et al, 2012 for non-differentially expressed genes between acute and chronic AD), approximately 10 patients would be needed to detect a two-fold change in the gene expression levels (corresponding to a difference in log₂ expressions of one) between the presence of TCS and after withdrawal of TCS, while controlling the false discovery rate (FDR) at 5%.

Figure 1 shows the average power versus sample size for different proportions of non-differentially expressed genes

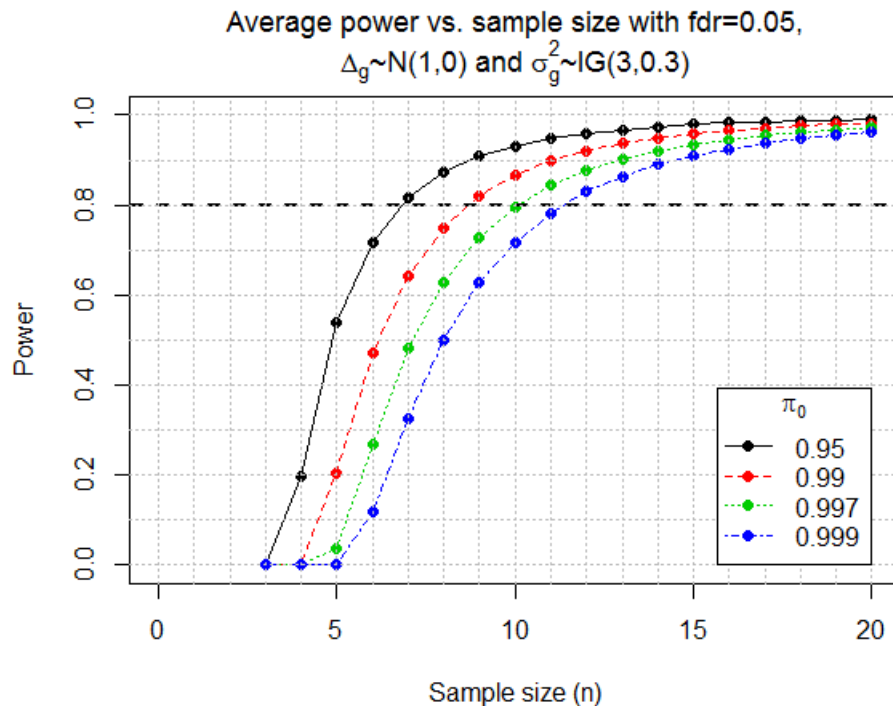


Figure 1: Sample size vs. power for one-sample two-sided t-test with effect size of one and various proportions of non-differentially expressed genes with FDR controlled at 5%. Variances are assumed to follow an Inverse Gamma(3,0.3) distribution.

Furthermore, the sample size of 10 patients provides over 80% power to detect differences between the two regimens (TCS and no TCS) for epidermal thickness and epidermal hyperplasia as measured by Ki67. Differences are either 70 microns in epidermal thickness assuming a standard deviation of 50 microns and a two-sided type I error rate of 0.05, or 40 Ki67 cells/mm assuming a standard deviation of 30 Ki67 cells/mm and a two-sided type I error rate of 0.05, respectively.

STUDY VISITS

See schedule of assessments for details

Visit 1 (Baseline – Week 0)

- Written informed consent will be obtained for those patients meeting eligibility criteria.
- Medical history and concomitant medications will be recorded.
- Skin severity scores (including EASI and IGA) will be assessed.

- Protocol prescribed TCS treatment (Triamcinolone 0.1%, twice daily to lesional skin) and daily emollient use will be initiated.

Visit 2 (Week 2): Patients receiving TCS

- Skin severity scores (including EASI and IGA) will be assessed.
- Total TCS use will be recorded at each visit by measuring tube weights (g) and questioning of the patients to primarily assess treatment compliance.
- Concomitant medications will be recorded.
- Blood, serum and plasma samples will be obtained (Table A).
- UCSF labs will perform CBC and differential.
- Skin biopsies will be collected from one lesional and one non-lesional site, as determined by the site investigator (Table A).
- Photographs will be taken to record the lesional and non-lesional biopsy sites.
- Protocol prescribed TCS treatment will be stopped for a subsequent period of 4 weeks. Patients will continue use of daily emollient

Visit 3 (Week 6): Patients receiving no TCS

- Skin severity scores (including EASI and IGA) will be assessed.
- Use of TCS over the prior 4 weeks of the study and other concomitant medications will be recorded
- Blood, serum and plasma samples will be obtained (Table A).
- UCSF labs will perform CBC and differential.
- One lesional and one non-lesional skin biopsy will be obtained adjacent to those previously collected on Visit 2, as determined by the site investigator (Table A).
- Photographs will be taken to document the biopsy sites.
- Patients will enter a two week period of safety follow up during which time protocol prescribed TCS treatment will be re-initiated. Patients will use daily emollient.

Visit 4 (Week 8): End of Study

- Skin severity scores (including EASI and IGA) will be assessed.
- Use of TCS and other concomitant medications will be recorded
- Optional blood, serum and plasma samples will be obtained from those patients who provide additional consent for these sample collections (Table A).

Table A – Time and Events Table

Mandatory Samples	Visit 2 (week 2)				
	Prepare as	Aliquots	Labelled as	Final Volume/tube	Tube Type
10 ml Whole blood	EDTA	1	CBC	3.5ml	Lavendar top
	EDTA	1	DNA Collection	6ml	Lavendar top
10 ml Blood (1 collection tube) => 5 ml Serum	Serum	1	Periostin – Split 1	0.5ml	Sarstedt FBT
	Serum	4	Serum PD – Splits 2-5	1ml	Polypropylene Tube
6 ml Blood (1 collection tube) => >2ml Plasma	Plasma-EDTA	2	EDTA Plasma PD – Splits 1 through 2	1ml	Polypropylene Tube
4 ml Blood (1 collection tube) => >2ml Plasma	Plasma-Li Heparin	1	Li Heparin Plasma PD –Split-1	1ml	Polypropylene Tube
6mm punch biopsy of lesional skin	Bisect	1	Skin for RNA	3mm	RNA Later
		1	Skin for dermatopath	3mm	Formalin fixed
6mm punch biopsy of non-lesional skin	Bisect	1	Skin for RNA	3mm	RNA Later
		1	Skin for dermatopath	3mm	Formalin fixed

Mandatory Samples	Visit 3 (week 6)				
	Prepare as	Aliquots	Labelled as	Final Volume/tube	Tube Type
4 ml Whole blood	EDTA	1	CBC	3.5ml	Lavendar top
10 ml Blood (1 collection tube) => 5 ml Serum	Serum	1	Periostin – Split 1	0.5ml	Sarstedt FBT
	Serum	4	Serum PD – Splits 2-5	1ml	Polypropylene Tube
6 ml Blood (1 collection tube) => >2ml Plasma	Plasma-EDTA	2	EDTA Plasma PD – Splits 1 through 2	1ml	Polypropylene Tube
4 ml Blood (1 collection tube) => >2ml Plasma	Plasma-Li Heparin	1	Li Heparin Plasma PD –Split-1	1ml	Polypropylene Tube
6mm punch biopsy of lesional skin	Bisect	1	Skin for RNA	3mm	RNA later
		1	Skin for dermatopath	3mm	Formalin fixed
6mm punch biopsy of non-lesional skin	Bisect	1	Skin for RNA	3mm	RNA later
		1	Skin for dermatopath	3mm	Formalin fixed

Optional Samples	Visit 4 (week 8)				
	Prepare as	Aliquots	Labelled as	Final Volume/tube	Tube Type
4 ml Whole blood	EDTA	1	CBC	3.5ml	Lavendar top
10 ml Blood (1 collection tube) => 5 ml Serum	Serum	2	Periostin – Splits 1 and 2	0.5ml	Sarstedt FBT
	Serum	4	Serum PD – Splits 3-6	1ml	Polypropylene Tube
6 ml Blood (1 collection tube) => >2ml Plasma	Plasma-EDTA	2	EDTA Plasma PD- Splits 1 through 2	1ml	Polypropylene Tube
4 ml Blood (1 collection tube) => >2ml Plasma	Plasma-Li Heparin	1	Li Heparin Plasma PD -Split-1	1ml	Polypropylene Tube

Blood volumes collected per patient per visit:

Visit 2 (week 2) ~30ml

Visit 3 (week 6) ~24ml

Visit 4 (week 8) ~24ml (optional)

A patient may donate ~80ml blood during the trial.

Study Population

15 subjects with moderate to severe AD are planned to be enrolled in this study which, based on a predicted 30% drop-out rate, is considered to be adequate so that at least 10 patients complete the study.

Study Eligibility

Subjects who meet all of the inclusion criteria and none of the exclusion criteria at the time of screening will be eligible for enrollment in the study.

Inclusion Criteria :

The following inclusion criteria must be met for study entry:

1. Male or female patients 18 – 75 years of age
2. AD diagnosed by the Rajka/Hanifin criteria at the time of screening and that has been present for at least 1 year
3. At least one area of moderate to severe active AD amenable to study biopsy and TCS treatment.
4. A washout period prior to screening for those patients who have previously received the following medications:
 - a) Cyclosporine/Oral Steroids/Imuran/Mycophenolate Mofetil/Other systemic immunosuppressants: 4 weeks
 - b) Phototherapy: 4 weeks
 - c) Biologics: 5 half lives of the drug

Exclusion Criteria :

Patients who meet any of the following criteria will be excluded from study entry:

1. Evidence of other concomitant skin conditions (e.g., psoriasis or contact dermatitis)
2. Use of topical calcineurin inhibitors within 4 weeks of screening
3. Hypersensitivity to TCS or to any other ingredients contained by the emollient or TCS product used during the study
4. Evidence of active skin infection at screening or baseline visit
5. Evidence or history of active or latent infections such as tuberculosis or hepatitis C
6. Patient clinical condition is not appropriate for treatment with protocol prescribed TCS
7. Use of an investigational agent within 4 weeks prior to screening or within 5 half-lives of the investigational agent, whichever is longer
8. Use of a tanning booth/parlor within 4 weeks before the baseline visit
9. Use of any anti-histamine medication within 4 weeks before the baseline visit.

10. History of any condition (e.g. bleeding diathesis) that may predispose the patient to complications associated with the planned skin biopsy procedures
11. Known current malignancy or current evaluation for a potential malignancy, including basal or squamous cell carcinoma of the skin or carcinoma in situ
12. Other clinically significant medical disease that is uncontrolled despite treatment that is likely, in the opinion of the investigator, to impact the patient's ability to participate in the study or to impact the study pharmacodynamic (PD), or safety assessments
13. Unwillingness or inability to comply with the study protocol for any other reason.

Rules for withdrawal from the Study in Individual Participants:

- The investigator feels study withdrawal is in the patient's best interest
- Withdrawal of consent by patient (or representative)
- The need for prohibited therapies, including:
 - Phototherapy
 - Systemic agents including oral corticosteroids, cyclosporine or other systemic immunosuppressants
 - TCS with higher potency than protocol-prescribed, for more than one day in any given week of the study (baseline to Week4)
- Skin infection
- Any other serious infections
- Inadequate compliance to the study protocol

Patients withdrawn from the study will no longer receive study drug (Triamcinolone 0.1%) but will continue in the study for safety assessments until completion.

STUDY PROCEDURES

Patient Treatment Regimen

1) Baseline (Week 0) until 2nd visit (Week 2): All patients will be treated with a stable topical treatment regimen. Patients must demonstrate compliance with this baseline therapy, as determined by the investigator's interview of the patient and measuring TCS tube weights at Visit 2 (Week 2). The regimen will consist of:

i) Emollients:

- Applied to all xerotic skin daily
- Only basic bland emollient which may include
 - o Vaseline (Unilever, London, UK); moisturizers such as
 - o Aquaphor (Beiersdorf AG, Hamburg, Germany),
 - o Plastibase (E.R. Squibb & Sons Inc., New Brunswick, NJ, USA)
 - o Cetaphil cream (Galderma Laboratories, SA, Lausanne, Switzerland)
 - o CeraVe cream (Coria Laboratories Ltd, Fort Worth, TX, USA)

ii) Triamcinolone 0.1%:

- To be applied twice daily to all lesional skin; for 2 weeks (Week 0 to Week 2)

2) Visit 2 (Week 2): Triamcinolone will be stopped and for the following 4 weeks patients will only apply emollient to xerotic skin daily.

3) Visit 3 (Week 6): Patients will resume the protocol prescribed TCS (Triamcinolone 0.1%, applied twice daily to lesional skin) treatment regimen for the duration of the two week safety follow up i.e. end of the study.

4) Visit 4 (End of Study, Week 8): Final safety assessments will be performed and optional blood, serum and plasma samples will be obtained from those patients who provide additional consent for these sample collections.

Unscheduled Visits

Patients who experience significant worsening of AD between visits 2 and 3 will return to the study site for evaluation of their clinical condition. At this time the site Investigator will determine if it is safe and in the patient's best interests to continue off TCS. If not, it is at the Investigator's discretion to perform Visit 3 assessments at the unscheduled visit and restart TCS. In this case the End of Study visit 4 would occur two weeks after the unscheduled visit.

Safety

Safety will be assessed in all patients who are entered into the study and until study completion (Week 8) or early termination.

Safety assessment will be performed at each study visit (Baseline, Wk2, Wk6 & Wk8) and patients will be provided a study site telephone number to contact the Investigator at any time to report any adverse events or to discuss any questions or concerns.

Safety will be assessed by non-directive questioning and symptom directed physical examination and all identified adverse events (AEs) will be recorded by the study site. Other evaluations, including clinical laboratory tests may be performed at the discretion of the study site investigator.

After informed consent and initiation of TCS, all AEs will be reported until study completion (Week 8) or early termination. Verbatim descriptions of adverse events will be coded using appropriate MedDRA terms. If collected, clinical laboratory data, which may include serum chemistry, hematology evaluations, or skin cultures will be summarized and analyzed by descriptive statistics. If collected, descriptive summaries of ECG findings will be summarized.

During the study the following specific safety monitoring for skin infection will be performed:

- If, in the opinion of the site investigator, a skin infection is present, a culture of the affected area(s) should be performed to identify the microbe and exclude fungi or viruses, including eczema herpeticum. Treatment of infections with antimicrobial medications will be at the Investigator's discretion and as per local Institutional practice. Therapy will be modified as appropriate based on the results of microbial culture results.
- If the patient's infection does not demonstrate improvement following antibiotic therapy, the patient will stop study drug but continue to participate in the study until completion of the study.

The safety outcome measures for this study are frequency and severity of treatment-emergent adverse events

Whole Blood Collection

Blood collection timepoints will be at week 2 and week 6 with an additional blood collection at week 8 from patients who give specific consent to participate in this optional collection.

At visits where blood will be collected, venipuncture will be performed using aseptic techniques and universal precautions, and drawn into vacutainers.

Whole blood will be collected at baseline for genotyping that includes but is not restricted to filaggrin status as described in Table A.

Whole blood will be collected for complete blood counts (CBC) as described in Table A.

Whole blood will be collected for serum as described in Table A.

Additionally, plasma will be prepared from a final blood collection as follows.

- 6mL of whole blood will be collected for EDTA plasma.
- 4mL of whole blood will be collected for Li plasma as described in Table A.

Topical analgesia will be offered for all venous puncture procedures, according to local guidelines and practices.

Skin Biopsy

The skin biopsies will be performed as described in Appendix 1.

Each 6mm punch biopsy tissue will be divided in two with one half placed into RNALater for PCR (as described in Table A) and the other half formalin fixed and paraffin embedded for subsequent dermatopathology evaluation.

Responsibilities for collected samples:

UCSF Responsibilities:

1. Dr. Arron will be responsible for:
 - Conducting the study according to the final study protocol as summarized in Figure 1 and according to the principles of Good Clinical Practice (GCP).
 - Providing the Research Ethics Board (REB) with all appropriate material, including the informed consent document.
 - Obtaining REB approval for the proposed studies.
 - Providing patient clinical data associated with these samples and a current sample inventory log within the clinical manifest.
 - Providing the Biologic Material, defined as follows: blood, serum, plasma and skin samples from study participants as outlined in Table A.
 - Perform CBC and differential through UCSF clinical labs (Table A).
 - Storing Biological Material in alarm-monitored $\leq -70^{\circ}\text{C}$ freezer within 1 hour of collection and until shipment to sponsor.
 - Shipping Biological Material with each patient visit according to Sponsor's specifications.
2. Unless specifically outlined in the study-specific guidelines and plans, Dr. Arron will perform all tasks and activities in accordance with UCSF standard operating procedures (SOPs).
 - Dr. Arron will provide Genentech with copies of all UCSF SOPs utilized in the execution of this project.

Genentech Responsibilities:

1. Provide labels, collection tubes/containers and materials described in Table A and Appendix 1.
2. Receive and process all shipped biosamples in accordance with all relevant Genentech SOPs.
3. Genentech will receive requested biosamples for evaluation of prospective disease biomarkers intended for future interventional trials in atopic dermatitis patients on a background of TCS.
4. The samples may be evaluated by a third party selected by Genentech and may be used for further exploratory analyses as deemed necessary by Genentech.
5. Any leftover blood samples may be used by Genentech and its representatives, affiliates, and collaborators for additional biomarker research and/or the development of diagnostic tests that help with the detection or understanding of medical conditions.

Length of Study

This study will be for a total of 8 weeks, composed of 3 time periods: TCS treatment (2 weeks), no TCS treatment (4 weeks), and safety follow-up with protocol prescribed TCS treatment (2 weeks).

End of Study

The end of this study is defined as the date when the last patient, last visit (LPLV) occurs or safety follow-up is received from the last patient, whichever occurs later.

ETHICS

Research Ethics Board

The Principal Investigator (PI) will provide the Research Ethics Board (REB) with all appropriate material, including the informed consent document. The study will not be started until appropriate REB approval of the protocol, the informed consent (IC) document, and all recruiting materials is obtained in writing by the investigator and Sponsor. Appropriate reports on the progress of the study will be made to the REB and Sponsor by the Principal Investigator in accordance with applicable governmental regulations and in agreement with the policy established by Sponsor.

Ethical Conduct of the Study

This study will be conducted in accordance with the protocol, Good Clinical Practice (GCP) according to the International Conference on Harmonization (ICH) guidelines, and regulatory requirements for the participating institution. The study will be conducted under a protocol reviewed by an REB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the patients will be respected; the physicians conducting the study do not find the hazards outweigh the potential benefits; and each patient will give his or her informed consent without duress.

Patient Information and Consent

A properly executed, written, informed consent form, in compliance with GCP according to ICH guidelines will be submitted by the investigator to the REB for review and approval prior to the start of the study. Before entering patients into the study, a copy of the REB-approved IC will be reviewed with the potential participant, and signed and dated. The investigator will provide a copy of the signed informed consent form to each patient and will maintain a copy in the patient's study file.

INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The study will be conducted by Dr. Sarah Tuttleton Arron, MD, PhD. The medical procedures will be conducted or supervised by Dr Sarah Tuttleton Arron, MD, PhD.

REFERENCES

1. Bieber 2008
2. Williams et al. 2006
3. Simpson EL 2010
4. Ring et al. 2012
5. Schneider et al. 2013
6. Gittler 2012
7. Choy 2012

APPENDIX 1

Skin Biopsy Procedures

At intervals stated in the protocol 6 mm skin punch biopsies will be obtained from lesional and non-lesional skin. It is important to carefully follow the steps outlined below.

A. **MATERIALS** - Provided in bulk by Genentech

Methanol	Scalpel	Suture kit
6 mm punch	Labels	-
50 ml conical tube	Formalin filled collection jars	-
2x2 Gauze Pads	RNAlater filled collection tubes	-

B. **BIOPSY SITE SELECTION**

Biopsies should be performed by the Investigator as per the site's standard practices and following the instructions described below.

1. **First biopsies – week 4 (visit 2):**

a. Lesional skin

- i. The 6 mm punch biopsy should be taken from the most involved chronic active erythematous (scaly) lesions.
- ii. Biopsies from the folds of the upper extremities, followed by lower extremities are preferred, followed by the trunk, in that order (although investigators should have flexibility).
- iii. Each biopsy should be divided in two using a scalpel, with one half placed into labeled collection jars containing formalin, and the other half prepared in RNAlater as described below.

b. Non-lesional skin

- i. The 6 mm non-lesional sample should be collected from the most normal appearing skin in a relative proximity to the lesional skin biopsy site, at least 5 cm away from the lesion (at least 1 cm away, if 5 cm is not possible).
- ii. Each biopsy should be divided in two using a scalpel, with one half placed into labeled collection jars containing formalin, and the other half prepared in RNAlater as described below.

c. Photograph the site from which the biopsies will be collected to document the site of biopsy, identify biopsy sites at the time of subsequent visits and as reference to assess any biopsy related complications.

2. Second biopsies - week 6 (visit 3):

a. *Lesional skin*

- i. The week 8 "lesional" biopsy should be taken from the same area location as the week 4 lesional sample. If the lesion has cleared, collect the sample close to the previous biopsy scar (>5mm but not further than 1cm from the biopsy scar). Do not biopsy the previous biopsy scar.
- ii. Each biopsy should be divided in two using a scalpel, with one half placed into labeled collection jars containing formalin, and the other half prepared in RNAlater as described below.

b. *Non-lesional skin*

- i. The week 8 non-lesional sample should be taken close to the area of the previous biopsy scar of the healthy appearing nonlesional skin biopsy (>5mm but not further than 1cm from the biopsy scar). Do not biopsy the previous biopsy scar.
- ii. Each biopsy should be divided in two using a scalpel, with one half placed into labeled collection jars containing formalin, and the other half prepared in RNAlater as described below.

c. Photograph the site from which the biopsies will be collected to document the site of biopsy, identify biopsy sites at the time of subsequent visits and as reference to assess any biopsy related complications.

C. FREEZING THE BIOPSY SPECIMEN IN RNALATER

1) DO NOT leave the tissue at room temperature or on wet ice for an extended period of time.

2) Place the remaining half of the biopsy specimen in the tube of RNAlater solution at 4°C.

It is important that the sample be completely immersed in the solution.

3) Store overnight at 4°C, then move samples to -70°/-80°C until shipment. Samples must be frozen before shipment.

D. SHIPMENT OF BIOPSY SPECIMENS

Ship samples according to sponsor's specifications based on sample type.