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Pilot Study Evaluating the Efficacy of a Topical PDE4 Inhibitor for Morphea

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Confidentiality Statement

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Abbreviations

cAMP	Cyclic adenosine monophosphate
CTCAE	Common Terminology Criteria for Adverse Events
DIET	Dyspigmentation, Induration, Erythema, and Telangiectasias
ERK	Extracellular signal-regulated kinase
hCG	Human chorionic gonadotropin
HRQOL	Health-related quality of life
LoSCAT	Localized Scleroderma Cutaneous Assessment Tool
MAPK	Mitogen-activated protein kinase
PDE-4	Phosphodiesterase-4
SDRC	Skin Disease Research Center (SDRC)
TGFβ	Transforming growth factor beta

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1. Protocol Title:

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2. Purpose of the Study:

To determine the clinical efficacy of crisaborole 2% ointment in the treatment of morphea. Specifically, to:

a. Compare the difference in dermal thickness and fibrosis as measured by histology at baseline and after 12 weeks of crisaborole 2% ointment, applied 2 times a day.
b. Compare changes in physician and patient reported outcomes at baseline, 4, 8 and 12 weeks of treatment.

3. Background & Significance:

Morphea is an autoimmune fibrosing skin disorder of still unknown etiology. It can be associated with significant morbidity, and effective topical and systemic therapies are still lacking. cAMP is known have an anti-fibrotic effect in skin models as well as other organs. It is therefore not surprising that phosphodiesterase-4(PDE4), which degrades cAMP, has been implicated in the pathogenesis in both animal and human studies. Preclinical studies have suggested that PDE4 inhibition may attenuate the oxidative processes that mediate fibrosis[1]. Systemic PDE4 inhibitors demonstrated anti-fibrotic effects in multiple murine skin fibrosis models, including the bleomycin-induced skin fibrosis models, the topoisomerase I mouse model and in murine sclerodermatous chronic graft-versus-host disease[2]. This anti-fibrotic effect was mediated though the blockage of cytokines and other immune mediated mechanisms. Dermal fibroblasts and myofibroblasts in an inflammatory disease such as psoriasis have increased expression of PDE4[3]. Furthermore, PDE4 inhibition reduced TGFβ mediated fibroblast migration and myofibroblast transformation[3].

PDE4 facilitates proinflammatory signals by degrading cAMP in inflammatory cells, smooth muscle cells, and keratinocytes. In mouse models, the anti-fibrotic effect of PDE4 inhibition was mediated though the blockage of cytokines and other immune mediated mechanisms[2]. Keratinocytes play an active part in the modulation of inflammation in a variety of cutaneous inflammatory and immune disorders. Our group has a particular interest in the role of the keratinocyte mediated inflammation and biology[4]. Cross talk exists between cAMP and the RAS/RAF/mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (MEK)/ERK pathway[5]. MEK/ERK has been implicated in the development of fibrosis, and inhibition of this pathway interferes with the profibrotic response to TGFβ[6].

4. Design & Procedures:

A Phase 2, pilot, proof-of-concept, open label, single arm prospective study to assess the safety, tolerability and efficacy of using crisaborole 2% ointment for adult morphea. We will recruit 20 adult (>=18 years of age) patients with morphea.

Patients will be recruited from the Duke University Dermatology Autoimmune Disease Clinic. In addition, patients will also be recruited from the entire Duke System, and from community and academic dermatology practices.

Patients will be identified by their physician, nurse, or other member of their clinical care team. Upon notification by the primary team, the study team will approach patients to discuss the study, treatment protocol, associated procedures and obtain informed consent. Patients who are referred from outside of Duke will have to obtain a Duke Medical Record Number (MRN).

Treatment:

Patients will be asked to apply crisaborole 2% ointment, twice daily, on affected plaques for 12 weeks.

Medication will be provided free of charge by Pfizer, as part of the grant that is funding this study. These will be dispensed by the study group at baseline and during the subsequent clinic visits.

Skin punch biopsy of sentinel plaque:

A sentinel plaque will be selected at baseline. A 4 mm skin punch biopsy will be performed at baseline and at 12 weeks, on the same affected plaque.

If a biopsy has been previously taken of the affected plaque and is available for review, this will be used as the baseline biopsy. Patients who drop out of the study because of progression of their disease or adverse effects will undergo a skin punch biopsy at the time of drop out.

Histologic and genomic assessment of skin biopsy specimen:

Each biopsy specimen will be bisected. Half will be formalin fixed and sent for histologic evaluation. Histologic assessment will be performed by an experienced academic dermatopathologist, who will be blinded to the study design. This will be performed within the Duke Dermatology Skin Disease Research Center (SDRC).

Dermal thickness will be measured and compared before and at the end of treatment.

The other half will undergo processing for Nanostring gene expression analysis. Specimens will be sent to the Nanostring Core Facility at the University of North Carolina (UNC) , de-identified except for study subject number. Gene expression analysis is included for future research purposes only. A Material Transfer Agreement will be completed and executed between Duke and UNC. MTA will be fully executed and uploaded into eIRB prior to any samples being sent outside of Duke.

The Lineberger Cancer Center Translational Genomics Laboratory (TGL) at UNC provides an assortment of analysis services employing the Nanostring™ nCounter platform. These include gene expression, miRNA expression and miRNA gene expression copy number variation analysis, single gene expression, incRNA expression, Chip-String and RNA-Protein profiling. Nanostring™ supplies custom and off-the-shelf assays for various groups of target genes including immunology, inflammation, and pan cancer panels offering pathway, immune and progression profiling. TGL maintains 2 nCounter Prep Stations which can process 12 post-hybridization samples every 3 hours. A generation 2 nCounter digital analyzer can sequentially scan up to 6 cartridges of 12 samples each with a maximum resolution scan requiring approximately 5 hours per

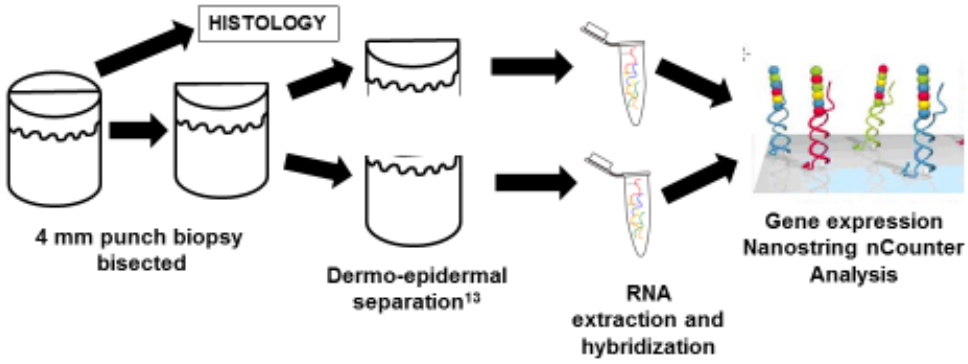
cartridge. Data is provided to the client electronically for analysis using the NSolver v. 3.0 software provided by Nanostring™. LCCC Bioinformatics can also provide Nano Nanostring™ string analysis services. TGL employs 4 trained specialists experienced in assorted types of Nanostring™ assays.

All sample information and metrics are recorded and maintained in the gPATH Lab Information Management System (LIMS) designed and maintained by LCCC Bioinformatics.

To distinguish gene expression profiles in a sample enriched for keratinocytes, the epidermis and dermis will be separated using a previously described protocol that allows for high quality RNA extraction[7]. Total RNA will be isolated from epidermal and dermal portions of each biopsy specimen. RNA will be hybridized to a custom made kit based on the nCounter Human Kinase Gene Expression Code Kit (which includes 519 color-barcoded human kinase genes) with additional genes that screen for innate and adaptive immune response. After washing and cleaning, the sample cartridges will be read in the nCounter SPRINT analyzer which allows automated data collection for up to 24 samples in one day.

Skin sections from the unaffected individuals will be used as control. These are obtained from an IRB exempt protocol that allows us to utilize normal skin from unidentified, healthy donors (Pro00012656, Access to anonymous and de-identified samples).

Genes that show more than 3-fold differences between lesional and non-lesional or normal skin, and between baseline and at 12 weeks, will be analyzed using gene function-based analysis tools such as GeneSpring, and grouped into the categories of cell signaling, structure, cell cycle, survival, and inflammation, as described in our previous studies[4,8].



Retention of data and samples for future research:

With subject agreement, subject samples and data obtained during this research study will be retained for future research under previously established bio and data repositories. This retention of samples will require separate subject consent to Pro00006230 and Pro00006313.

Clinical assessment

Patients will have a clinical assessment at baseline and at weeks 4, 8 and 12. After week 12, patients who need further treatment will undergo standard of care treatment as determined by their primary dermatologist. An optional post treatment follow-up will be performed at week 20.

Physician and patient reported assessment and skin imaging will be performed at baseline, week 4, 8 and 12, and at week 20. Photography will also be performed at baseline and week 12.

Physician assessment will utilize two previously described and validated scoring metrics. The sentinel plaque will be evaluated using Dyspigmentation, Induration, Erythema, and Telangiectasias (DIET) score[9]. Overall disease severity will be evaluated using the Localized Scleroderma Cutaneous Assessment Tool (LoSCAT)[10].

Health-related quality of life (HRQOL) will be measured using Skindex-29[11] which has previously been applied to morphea[12], and has a pruritus component.

Photographs will be taken using a standardized clinic camera.

B-Mode ultrasonographic imaging will be utilized to measure dermal thickness of the sentinel plaque at baseline, week 4, 8 and 12 and post-treatment at week 20.

Summary of Clinical Trial Design

	Baseline	Week 4	Week 8	Week 12	Week 16	Week 20
Crisaborole 2% ointment twice daily	x	x	x	x		
Clinic visit	x	x	x	x		x
LoSCAT scoring	x	x	x	x		x
DIET scoring	x	x	x	x		x
Skin punch biopsy	x			x		
Urine HCG	x	x	x	x		
Skindex 29	x	x	x	x		x
Skin imaging (ultrasound)	x	x	x	x		x
Photography	x			x		x

Primary outcome measure is the change in dermal thickness on skin biopsy at 12 weeks.

Secondary outcome measures are the following:

- Change in DIET score of sentinel plaque at 4, 8 and 12 weeks.

- Change in LoSCAT score at 4, 8 and 12 weeks.
- Skindex-29 at 4, 8 and 12 weeks.
- Change in dermal thickness of sentinel plaque by ultrasonography at 4, 8 and 12 weeks

5. Selection of Subjects:

Inclusion criteria
Subjects eligible for inclusion in this study must fulfill **all** of the following criteria:
1. >= 18 years of age
2. Clinical diagnosis of morphea.
3. <20% Total body surface area involvement.
4. Does not require systemic immunosuppressive therapy for morphea.
5. No changes (additions, subtractions, or dose modifications) in immunosuppressive systemic therapy 2 months prior to starting the study (hydroxychloroquine, methotrexate, mycophenolate mofetil, azathioprine, cyclosporine, cyclophosphamide, prednisone >=10 mg PO daily).
6. No immunomodulating topical therapy (topical steroids or topical calcineurin inhibitor), and no topical vitamin D analogue, 2 weeks prior to starting study.
7. No allergy to crisaborole or vehicle.
8. No known renal disease
9. Able to give informed consent.

Exclusion Criteria
Subjects fulfilling **any** of the following criteria are not eligible for inclusion in this study.
1. Clinical diagnosis of depression or history of suicidal ideation.
2. Pregnant or breastfeeding women, with pregnant women being defined as the state of a female after conception until the termination of gestation, confirmed by a positive urine human chorionic gonadotropin (hCG) laboratory test. Women with a positive urine hCG at any time during the study will be withdrawn from the study.

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6. Subject Recruitment & Compensation:

Patients will be recruited from the Duke Dermatology Outpatient Clinic, including patients referred from Rheumatology for evaluation of morphea. We may also contact dermatologists in the community (within North Carolina), UNC Dermatology, Wake Forest Dermatology and the Durham VA Medical Center, via email and letter, to make them aware of our study so they can refer patients. Prior to any recruiting outside the Duke Derm Autoimmune clinics, we will seek approval for any flyers that are used in recruitment and provide a phone script for those who respond to flyers.

Patients will be given compensation to cover travel expenses as part of their participation in the study, amounting to $50 per visit, or a maximum of $250. A gift card worth $50 will be given at baseline, week 4, week 8 and week 12. A final $50 gift card will be given at the optional week 20 visit, should they choose to participate in it.

7. Consent Process – see Section 14 of the e-IRB submission form.

8. Subject's Capacity to Give Legally Effective Consent:
Subjects must be able to provide consent. Legal representatives will not be used.

9. Study Interventions:
Subjects will be asked to apply crisaborole 2% ointment, twice daily, on affected plaques for 12 weeks.

10. Risk/Benefit Assessment and Safety Considerations:
If a subject agrees to take part in this study, there may not be a direct medical benefit to them. The subject's morphea may improve from using the investigational drug in this study. It is also possible that a subject's condition will not improve on the investigational drug.

The pharmacokinetics of crisaborole as a topical medication have previously been studied[13]. Crisaborole is metabolized to inactive metabolites upon absorption, making it both a safe option for therapy even in relatively extensive disease. There was minimal systemic effect of crisaborole ointment when applied twice daily for 28 days, over up to 35% body surface area, in children 2-17 years or age. Long term studies in adults and children with mild to moderate atopic dermatitis for as long as 48 weeks have also shown a low frequency of side effects[14]. The most frequently reported treatment-related AEs (overall, 10.2%) were worsening of pre-existing atopic dermatitis (3.1%), application-site pain (2.3%), and application-site infection (1.2%). The FDA monograph of crisaborole does not state any requirement for monitoring.

The risks for obtaining skin biopsy samples include a mild amount of pain, bruising, bleeding, or infection at the site of the puncture. Subjects will be instructed in proper wound care to minimize these risks. Subjects may experience scarring at the site of biopsy.

11. Costs to the Subject:
There will be no additional costs to the subjects as a result of being in this study. However, routine medical care for their disease will be charged to them or their insurance company.

12. Data Analysis & Statistical Considerations:
We will aim to recruit 20 patients in the study. We anticipate to see a 30% decrease in dermal thickness at 12 weeks compared to baseline, using histologic evaluation of skin punch biopsy specimens. Using measurements of dermal thickness that we obtained from our archived morphea skin biopsies, we estimated that we need 11 patients to detect 30% change in dermal thickness with a power of 0.80 and an alpha of 0.05. The goal to recruit 20 patients allows for possible drop-outs, while still allowing us to perform statistically significant analysis.

Numeric Results for Wilcoxon Test (Normal Distribution)
Null Hypothesis: Mean of Paired Differences = 0, Alternative Hypothesis: Mean of Paired Differences ≠ 0
Unknown standard deviation.

Power	N	Alpha	Beta	Mean of Paired Differences	Effect S Size
0.81293	4	0.05000	0.18707	1.0	0.3 3.333
0.89861	5	0.05000	0.10139	1.0	0.4 2.500
0.90888	6	0.05000	0.09112	1.0	0.5 2.000
0.89877	7	0.05000	0.10123	1.0	0.6 1.667
0.87994	8	0.05000	0.12006	1.0	0.7 1.429
0.85644	9	0.05000	0.14356	1.0	0.8 1.250
0.83039	10	0.05000	0.16961	1.0	0.9 1.111
0.80310	11	0.05000	0.19690	1.0	1.0 1.000
0.84753	12	0.05000	0.15247	1.0	1.0 1.000
0.80310	11	0.05000	0.19690	0.5	0.5 1.000
0.82792	9	0.05000	0.17208	0.6	0.5 1.200
0.86733	8	0.05000	0.13267	0.7	0.5 1.400
0.87508	7	0.05000	0.12492	0.8	0.5 1.600
0.84741	6	0.05000	0.15259	0.9	0.5 1.800
0.90888	6	0.05000	0.09112	1.0	0.5 2.000

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Machin, D., Campbell, M., Fayers, P., and Pinol, A. 1997. Sample Size Tables for Clinical Studies, 2nd Edition. Blackwell Science. Malden, MA.

Women and minority volunteers will be encouraged to enroll, but no formal subgroup accrual goals are planned.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting.

Months 1-6	Months 7-12	Months 13-18	Months 19-24
Subject recruitment (1-2 patients per month), clinical and objective assessment, collection of biopsies			**Genomic profiling, data interpretation and analysis, and paper writing.**

13. Data & Safety Monitoring:

Subjects will be assigned unique study numbers. The data related to this study will be kept in locked files and an electronic password protected database maintained by the Department of Dermatology research offices. Only unique study numbers but not DUMC history numbers will be supplied as subject IDs for data maintenance and statistical analysis purposes to comply with HIPAA regulations. This study will have routine auditing consistent with Duke Clinical Research Institute and NIH oversights. Subject confidentiality will be maintained.

All samples will be stored and maintained through the conclusion of the study and then destroyed, unless the subjects agree (and separately consent) to the Samples and Data being included in the Duke Autoimmune Serum Tissue Bank (Pro00006313) and Duke Immune Mediated Skin Disease Database (Pro00006230).

This clinical research study will be monitored both internally by the PI and externally by the Duke University Medical Center in accord with their NIH- approved “Institutional Protocol Monitoring Procedures and Guidelines for NIH-Sponsored Research Involving Human Subjects.”

14. **Privacy, Data Storage & Confidentiality** – see Section 12 of the e-IRB submission form.

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