A Phase 2, Exploratory Study to Investigate Safety and Efficacy of Doxycycline Monohydrate Hydrogel (NANODOX®) 1% In Atopic Dermatitis

Protocol Approval

As authorized representative of the organizations involved with this study, we hereby give our dated signatures in scientific approval of the obligations and procedures set forth in this investigator initiated protocol as an instrument of proprietary information and instructions for the indicated research project.

Authorization:

Sponsor **Date**
James Talton, PhD
Alchem Laboratories, Inc

Principal Investigator **Date**
Anna De Benedetto, MD
University of Florida College of Medicine
Department of Dermatology

Study Site:
UF Health Department of Dermatology
4037 NW 86th Terrace- 4th Floor
Gainesville Florida 32606

IRB protocol -
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Principal Investigator: Anna De Benedetto, MD – Department of Dermatology, UF

Phone Number: office (352) 294-4427; adebenedetto@ufl.edu

Co-Investigators:

- Gregory Schultz, PhD – Department of Obstetrics & Gynecology, UF

Other research staff:

Sanjuana Rodriquez, PA-C, Department of Dermatology, UF
Kiran Motaparthi, MD – Assistant professor, Department of Dermatology, UF
Jennifer Schoch, MD- Assistant Professor, Department of Dermatology, UF
Kathryn Potter, MD- Resident in Training, Department of Dermatology, UF
Michael Montuno, MD- Resident in Training, Department of Dermatology, UF
Alexandra L. Owens , MD- Resident in Training, Department of Dermatology, UF
Jennifer Nicole Harb, MD- Resident in Training, Department of Dermatology, UF
Bahram Dideban, MD Resident in Training, Department of Family Medicine, UF

Mary Bohannon, CCRA Research Coordinator mebohan@ufl.edu
Lenise Thomas, LPN Research Nurse
Gina Martorana, EPIC specialist, Department of Dermatology, UF
Peter Nadeau, Biological Scientist III in Dr. De Benedetto's lab, Department of dermatology, UF

1. PURPOSE AND BACKGROUND

Atopic Dermatitis (AD) is the most common inflammatory skin disease, affecting about 17% of children and 6% adults in the USA[i, ii]. AD is characterized by skin barrier disruption, an aberrant adaptive immune response (*i.e.,* Th2 polarized) to environmental allergens, susceptibility to cutaneous bacterial infections and intractable itch[iii,iv]. The intense pruritus and cutaneous infections contribute to the morbidity of AD and are major drivers of the reduced quality-of-life associated with this disease[v, vi]. In the World Health Organization 2010 Global Burden of Disease survey, AD has ranked first among common skin diseases[vii]. So far, AD treatments have targeted inflammation with the widespread use of topical and more intermittent use of systemic corticosteroids. In summary, despite its high prevalence, effects on quality-of-life and economic burden – there are few effective treatments for AD.

Doxycycline are tetracycline antibiotics broadly used systemically to treat inflammatory-dermatologic conditions. Several studies in human and animal models have shown doxycycline have anti-inflammatory and pro-healing properties, mainly by blocking tissue proteolytic activity. Doxycycline have been reported to nonselectively inhibit members of the metalloproteinases (MMP) superfamily [reviewed in [viii, ix]]. In addition to this direct inhibitory activity, doxycycline indirectly prevents tryptic kallikreins activation by MMPs[x]. Growing body of evidence suggests that the tetracycline might also directly downregulate Protease Activator receptor (PAR)-2 expression and function, which was also found to play a role in induction of local inflammatory

mediators[xi]. Importantly, the doxycycline antimicrobial activity could lead to reduced Staphylococcus infection/colonization in AD skin, a known trigger of AD flares.

Alchem Laboratories Corp has recently acquired from Nanotherapeutics, Inc the first topical formulation of doxycycline: NanoDOX® Hydrogel, a novel small particle gel suspension of doxycycline 1%. The topical 1% doxycycline monohydrate hydrogel product is available for clinical testing through a FDA-cleared IND obtained in 2008 to treat chronic skin wound and a phase 2 study to evaluate safety and efficacy completed in 2011 at VA hospital in Gainesville ("Safety Study of Topical Doxycycline Gel for Adult Diabetic Lower Extremity Ulcers", Protocol 2008-DOX-NT/003, ClinicalTrials.gov Identifier: NCT00764361). An amendment to the IND 77520 has been filed with the FDA by Alchem Lab for the new IND indication for use in AD.

Safety preclinical studies of NANODOX® were previously evaluated by Nanotherapeutics. Briefly, Franz diffusion cells were used to analyze the amount of doxycycline released out of a NanoDOX® Hydrogel or particle suspension across an exact area with respect to time. These conditions may be used to mimic an exposed wound and the resulting drug release out of the NanoDOX® Hydrogel into the body. Overall, less than 1% w/w of the 15 mg total doxycycline available in the donor chamber in the gel (11.18µg, or 0.08%) as well as the suspension (29.88µg, or 0.21% w/w) diffused across the membrane into the receptor chamber. In a rat wound healing study, NanoDOX® Hydrogel was tested at doxycycline concentrations of 0.0% (placebo hydrogel), 0.3%, 1%, and 3% w/w as well as untreated control. The results of the study demonstrated no significant differences between the histological scoring for all concentrations and the control. All concentrations were judged to be non-irritants versus the control. In humans, NanoDOX® Hydrogel treatment was evaluated in a pilot, randomized, controlled trial of chronic, diabetic, lower-extremity foot ulcers. All four chronic ulcers of diabetic patients treated daily with topical 1% NanoDOX® Hydrogel healed in the 20-week treatment period, whereas only one of three patients treated with vehicle healed (p=0.05). No adverse events were attributed to NanoDOX® Hydrogel treatment[13].

Aim of this protocol is to investigate the safety and clinical efficacy of 1% topical Doxycycline Monohydrate (NanoDOX® Hydrogel) in AD. It is our hypothesis that daily application of NanoDOX® hydrogel blocking cutaneous proteases activity in AD subjects will reduce severity of the disease, by restoring skin barrier function and reducing skin driven inflammation. Importantly, we will also monitor the anti-microbial activity of this product on AD skin, as colonization with Staph aureus is typically associated with disease severity. This work will likely be the first step to developing new therapeutics targeting skin proteases activity.

2. CHARACTERISTICS OF THE RESEARCH POPULATION

Number of subjects We intend to have 15, non-pregnant, non-lactating women and men of any race and ethnicity, ages 18 to 65 yrs, who are generally healthy except for atopic dermatitis diagnosed by the US Consensus criteria complete this study. This may require enrolling up to 30 subjects depending on retention rates and dropouts. Patients can have other allergic conditions such as asthma, allergic rhinitis, food allergies, etc. All AD subjects must have

moderate-severe AD as defined at screening by an Eczema Area and Severity Score (EASI) ≥ 10.

Gender of subjects We will be recruiting both male and female subjects

Age of subjects We will be including subjects between the ages of 18 and 65 years.

Racial and Ethnic Origin We will not have any restrictions based on racial or ethnic origin. We based our target research population racial and ethnic distribution on the data of the 2000 United States Census for the Alachua Area.

Vulnerable subjects We will not be targeting vulnerable subjects, however staff and students of UF and Shands may be enrolled as part of the general population with AD

Inclusion Criteria

- Male or female, 18 through 65 years of age, inclusive who are generally healthy except for active atopic dermatitis diagnosed by the following criteria.
- **Active Atopic Dermatitis**: Subjects *must have* according to medical records, patient account or by medical exam of the investigator:
    - Pruritus
    - Eczema (acute, subacute, chronic)
    - Chronic or relapsing history

  Most subjects *will have* (seen in most cases, adding support to the diagnosis):
    - Early age at onset
    - Atopy
    - Personal and/or family history
    - Xerosis

  Subjects *may have* (these clinical associations help to suggest the diagnosis of AD but are too nonspecific for defining or detecting AD for research or epidemiological studies):
    1. Atypical vascular responses (e.g., facial pallor, white dermographism, delayed blanch response)
    2. Keratosis pilaris/hyperlinear palms/ichthyosis
    3. Ocular/periorbital changes
    4. Other regional findings (e.g., perioral changes/periauricular lesions)
    5. Perifollicular accentuation/lichenification/prurigo lesions
- Moderate to Severe AD: clinical score based on EASI ≥ 10 and/or IGA 1-2.
- If receiving antihistamines, are on a stabilized dose, and expect to maintain this dose throughout the study
- All female subjects of childbearing potential must have a negative pregnancy test at screening visit and must be on an acceptable methods of contraception from the Screening Visit continuously until 30 days after stopping study drug.

Exclusion Criteria:

- As determined by the study doctor, a medical history that may interfere with study objectives (cancer, chronic illness)
- Known allergy to tetracycline
- Hand eczema only (no body involvement).
- Subjects with a systemic infection requiring a course of systemic antibiotics or antivirals within the last 2 weeks
- Unstable AD or any consistent requirement for systemic immune-modulant Rx (e.g. systemic steroids, phototherapy, Cyclosporine)
- History of use of biologic therapy (including intravenous immunoglobulin)
- Recent or anticipated concomitant use of systemic therapies that might alter the course of AD
- Recent or current participation in another research study
- Females who are breastfeeding, pregnant, or anticipate becoming pregnant during the study time frame
- Subjects with a history of keloid formation
- History of lidocaine, epinephrine or Novocain allergy
- History of allergy to tape or other adhesive materials

3. METHODS AND PROCEDURES

Study Design. We will perform a 6 weeks, pilot, investigator-initiated, single-center, open-label clinical study to determine the effect of NANODOX® 1% hydrogel in adult AD patients. The study will be conducted in the UF Health Dermatology Department - Springhill.
Subjects that voluntarily agreed to participate will be consented and enrolled in the study. Qualified subjects will be required to come to UF Dermatology Clinic for at least four visits, approximately 2 weeks apart, with an optional pre-screening (up to 28 days +/- 2 prior to V1) visit if washout is needed and/or to allow subjects to be enrolled in pre-established enrollment dates. Each visit will last approximately 30-60 minutes.

Subjects will be asked to apply NanoDOX® Hydrogel 1% once daily at bedtime for total of four weeks (or until complete clearance) on the chosen target eczematous and adjoining clinically normal (perilesional) area (to cover an area of the size of an index card). NanoDOX® Hydrogel 1% will be provided to the patient in single dose package containing 1.5 cc of hydrogel (equal to 15mg Doxycycline) which is enough to cover a skin area of the size of an index card. Alchem will provide drug for the study at no cost. Of note, for systemic use doxycycline is used at concentration from 100 mg (antimicrobial) twice daily to 40 mg (anti-inflammatory) daily for periods ranging from 14 days to several months. We included non-lesional/perilesional skin to evaluate the safety (e.g. irritant or toxic effects) of the compound on atopic skin, as those subjects have a lower irritancy threshold as is compared to non-atopic subjects[xii]. Also, skin barrier impairment (e.g. increase water loss & increase pH) can be already detected in normal

appearing skin in AD subjects. It is our hypothesis that by normalizing the proteases activities, treatment with NanoDOX® Hydrogel will normalize skin barrier integrity.

Subjects that are on any topical treatment (e.g. steroids, calcineurin antagonist) will be asked to discontinue the use of those medications only in the selected area for one week before starting the study (washout period). During the study, subjects will be allowed to stay on anti-histamine and any topical treatment (except for the treatment area) on a stabilized dose, and expect to maintain this dose throughout the study. In case of acute flare (e.g. intense itch, increase redness, pain or oozing) in the treatment area subjects will be allowed to use rescue medication (e.g. topical steroid or calcioneurin inhibitors that they used before the study) under the PI supervision. The need for rescue topical treatment will be monitored and recorded during the study and it will be used as an indication of the efficacy of the treatment.

Study Design: Evaluation of NanoDOX Hydrogel 1% in AD subjects.

(Vp)	V1	V2	V3	V4
Pre-Screening <28days	Day 0	Day 14 +/-2	Day 28 +/- 2	Day 42 +/- 5
Clinical & itch score, Skin barrier, photographs	X	X	X	X
Swab, Blood & Skin biopsy	X		X	

Screening visit (optional):

1) Consenting and confirmation of inclusion & exclusion criteria
2) Identification of the target area to be treated with NANODOX® and digital picture of the targeted lesion. The picture will not include subjects' identifiers (e.g. facial features, tattoo, ect)
3) possible washout period (topical only in the chosen area – treatment and biopsy sites)
4) scheduling visit 1

Visit 1 (Day 0):

1) Consenting and confirmation of inclusion & exclusion criteria
2) General History and Physical Exam (including review of concomitant medications)
3) Urine Pregnancy test for woman of childbearing potential. Counseling on acceptable birth control measures to use throughout treatment period (28 days) and until 30 days after stopping study drug
4) Questionnaire to clinically characterize AD
5) Clinical score for atopic dermatitis
6) Identification of the target area to be treated with NANODOX® and skin biopsy locations. Arms will be the preferable areas if lesions are present, otherwise trunk or lower extremity
7) Digital pictures of the target area and area used for biopsy
8) Non-invasive skin barrier Measurements: TransEpidermal Water Loss (TEWL). Optional: pH and Stratum Corneum (SC) hydration measurements may also be performed
9) Repeated tape strippings (0, 5, 10, 15 tape-strippings with Cuderm™ tape) of non-lesional and lesional locations from treated area will be collected as part of the TEWL assessment and to assess for SC cohesion/integrity (rate of change in TEWL with repeated strippings).

Serial measurements of TEWL will be performed after tape stripping as previously described[xiii]. This will also allow collecting proteins (eluded from the tape) for proteases activity assessment.

10) Skin swab
11) Optional Skin biopsy (5 mm punch) of a representative skin in the same anatomical area or symmetrical, avoiding the area that will be treated. This biopsy may be performed on D28 at the same time as the post treatment biopsy.
12) Venous blood draw to monitor AD biomarkers
13) Dispense NANODOX® for two following weeks (14 +/- 2 monodose packages)
    - Subjects will be instructed to keep the NANODOX refrigerated at + 4C; subjects will be provided a cooler and ice packs keep the products refrigerated during transport.

13) Provide instructions to complete at home diary.
14) Provide date of Visit 2 and "morning of appointment" instructions

Visit 2 (Day 14 +/-2):

1) Review subject diary and address any questions, concerns, or missed doses
2) Questionnaire to clinically characterize AD, review of medications and possible SE
2) Clinical score for atopic dermatitis
3) Digital pictures of the targeted area
4) Non-invasive skin barrier Measurements: transepidermal water loss. Optional: pH and SC hydration measurements may also be performed.
5) Repeated tape strippings (0, 5, 10, 15 tape-strippings with Cuderm™ tape)
6) Dispense NANODOX® for two following weeks (14 +/-2 monodose packages)
7) Suture removal at pre-treatment biopsy site, if applicable
8) Review Diary instructions
9) Provide date of Visit 3 and "morning of appointment" instructions

In case of complete resolution of the treated area at V2, post treatment biopsy and swab and Venous blood draw will be performed at this visit (instead V3) and patient will only come back for 2 weeks post treatment.

Visit 3 (Day 28 +/-2):

1) Review subject diary and address any questions, concerns, or missed doses
2)Questionnaire to clinically characterize AD, review of medications and possible SE
3) Clinical score for atopic dermatitis
4) Digital pictures of the targeted area
5) Non-invasive skin barrier Measurements: transepidermal water loss. pH and SC hydration measurements may also be performed.
6) Repeated tape strippings (0, 5, 10, 15 tape-strippings with Cuderm™ tape)
7) Skin swab of treatment area
8) Optional skin biopsy (5 mm punch) from treatment site and non-treatment site if not performed at Day 1 visit.
9) Venous blood draw
10) Provide date of Visit 4 and "morning of appointment" instructions

Visit 4 (Day 42 +/-5):

1) Questionnaire to clinically characterize AD, review of medications and possible SE
2) Clinical score for atopic dermatitis
3) Take digital pictures of the targeted area
4) Non-invasive skin barrier Measurements: transepidermal water loss. pH and SC hydration measurements may also be performed.
5) Repeated tape strippings (0, 5, 10,15 tape-strippings with Cuderm™ tape)
6) Suture removal at biopsy site(s)

Study procedures & methods

- **Disease status assessment: 1)** Investigator Global Assessment (IGA), which will provide information of the overall disease (four point scale: clear, mild, moderate, severe) and it is the score requested by FDA for clinical study and **2)** Eczema Area and Severity Index (EASI), a well-accepted and validated scoring system for general disease activity[xiv]. It is the sum of the intensity scores (0 to 3) for four signs: erythema, induration, excoriation, lichenification and it is recorded for targeted treatment areas as well as four regions of the body (i.e., head, trunk, lower and upper extremities). Data will be reported as percent change in EASI score from baseline and each visit. Proportion of participants achieving EASI50 and EASI75 will be determined at each study timepoints. The presence or absence of skin irritation (e.g. macular/papular eruption), redness, atrophy and telangiectasia will be noted at each study visit.

  3) The pruritus intensity will be assessed using the 5-D Pruritus Scale. The pruritus categorical scale is a 4-point scale commonly used in clinical studies of AD and has less of a "middling" effect[xv] The scale is rated as follows: 0: absence of pruritus; 1: mild, pruritus (occasional slight itching/scratching); 2: moderate pruritus (constant or intermittent itching/scratching that does not disturb sleep) and 3: severe pruritus (bothersome itching/scratching that disturbs sleep).
- **Digital pictures:** A digital picture will be taken with a dedicated digital camera. The pictures will be labeled with the subject's study ID and date. Pictures will not include identifying information (e.g. facial features, tattoo, etc). Pictures will be taken at a distance for easy identification of the anatomical area and closer to better capture clinical changes. Digital photographs will be used to record the location and monitor the size of target lesions. Quantification of change in size will be obtained using Image J software.

  At conclusion of the study, two trained physicians/dermatologists will be asked to score clinical pictures (0-4 scale, clear, mild, moderate, severe) in a blind fashion (*i.e.,* pictures will be de-identified and presented randomly/ not in chronologic order). This would allow to objectively confirm the clinical score taken during the study.
- **Skin swab:** Skin swabs will be collected for microbiologic analysis to obtain a semi-quantification of skin bacteria pool, including S. aureus spp. and to determine antibiotic sensitivity. Skin swabs are performed by swiping and rolling a sterile cotton-tipped applicator 4-5 times across the target area. Swabs will be obtained from NanoDOX® Hydrogel sites pre and post treatment and may be repeated as needed to monitor

bacteria growth during study. The microbiology assay will be run by the Clinical microbiology laboratory at the University of Florida (Rocky Point Lab).

- **Optional Skin biopsy:** One skin biopsy sample (5 mm ≈ pencil eraser size) will be obtained if possible within 4 cm from the treatment area or on the contralateral side. Second biopsy will be obtained from the treatment area post-treatment. Biopsy will be performed after injecting a local anesthetic (Lidocaine +/- epinephrine) to minimize pain and bleeding. The biopsy will consist of removing a piece of skin with a sterile 5-mm skin biopsy punch. At the biopsy site one or two sutures will be placed to ensure better healing, minimize blood loss and to provide a more aesthetic scar formation. A pressure bandage will be applied to the biopsy site to minimize the risk of bleeding and infection. We will provide each subject with a wound care instruction sheet.
  Each biopsy will be used for analysis of proinflammatory cytokines (TSLP, IL33, TNFa, IL-1b, and IL-6) and epidermal barrier related protein (e.g. filaggrin, loricrin, claudin1, S100A7) as well as proteases activity (MMPs, elastase) that are typically upregulated in inflammatory skin. This will be done in collaboration with Dr. Gregory Schultz (Professor of Obstetrics/Gynecology & Ophthalmology at the University of Florida, co-PI in the study). It is our hypothesis that NanoDOX® Hydrogel by reducing the proteases activity in the epidermis will enhance the integrity of epidermal barrier structure. Secondarily, we will evaluate changes in inflammatory (and pro-inflammatory) pathways downstream to proteases activity before and after treatment with NanoDOX® Hydrogel. We expect that normalization of molecular pattern parallels clinical improvement (and restore of skin barrier integrity).
- **Tape stripping for proteases activity measurements:** the most superficial layers of the skin will be collected by tape stripping using D-squame (D-squame®, CuDerm, Dallas TX, USA – **Figure 2**). This is a well-established method used in clinical research to gently perturb the skin barrier. The first tape will be discarded to eliminate dirt and remnants of skin products. Each tape will be gently pressed against the skin with standardized pressure for 10 seconds (sec) using a standardized pressurizer (D500–D-squame® pressure instrument, CuDerm, Dallas, TX, USA – **Figure 2**). Non-lesional skin samples will be collected from the volar side of the forearm and lesional skin samples will be collected from an easily accessible area, such as arms or lower legs.
  All subjects will have 20 consecutive tapes collected from lesional skin and 20 consecutive tapes from non-lesional skin. Once the tapes have been collected, as part of this study, we will test different methods to elute the skin proteins from the tapes.
  The corneocytes (cells in the most superficial layer of the skin) or their cellular contents that adhere to the discs must be released from the adhesive coating for subsequent analytical steps. So far several groups were able to use this non-invasive method to analyze for the presence of biomolecules, drugs and other cellular constituents.
- **Skin barrier measurements:** Transepidermal water loss/TEWL, +/- pH and SC hydration measurements will be obtained on treatment area at a non-lesional site and on a site of inflammation. For each of the specific measurements, a probe will be gently applied on the skin surface for few (up to 30) seconds. We will perform all measurements in a climate-controlled examination room with temperatures ranging 20-22°C and consistent humidity (preferably 40%). The patient must be very comfortable in

the room in which they are being tested to ensure that perspiration due to stress or acclimatization does not alter the TEWL. TEWL will be measured at baseline and after tapestripping. SC hydration and surface pH will be assessed on non-lesional and lesional skin using the Corneometer® CM825 and Skin-pH-Meter® PH 905, respectively (Courage and Khazaka Electronic GmbH, Kohn GE – **Figure 1**). TEWL will be assessed using the AquaFlux (Biox, London UK). These measurements should provide data to determine if the treatment has an effect on skin barrier integrity.

- **Venous blood sample:** (4 ml) will be obtained to evaluate biomarkers of disease severity. 4mLs will be collected into a redtop serum blood collection tube and allowed to clot at room temperature for minimum of 30 minutes. Blood will be spanned down and serum pipetted into Eppendorf tubes to be stored in -80°C. Tubes will be labeled with study subject ID, data and Visit number, not subject identifying information Tubes will be stored in Dr. De Benedetto's laboratory for measurement of Th2 biomarkers (markers of disease severity), including serum total IgE and TARC/CCL17.

4. RISK, BENEFITS and PROTECTION AGAINST RISKS

Risks Associated with Digital Picture

There are no known risks for the digital picture. The image will not include identifying features and will be labeled with study subject ID and date. Pictures will be taken with a dedicated camera, stored on a secure server and transferred into RedCap database.

Risks Associated with Barrier Assessments

There are no known risks for the barrier assessments.

Risks Associated with Tape Stripping

The risks associated with tape stripping, theoretically, include the rare possibility of an allergic reaction to the tape and infection. Since the tape is removed immediately after application, the risk of reaction is extremely low. We will exclude study subjects with known allergy to tapes or adhesive material. In previous and ongoing studies involving tape stripping, it has been noted that a very mild erythema may develop immediately after a series of tape strippings on one localized area of skin, presumably due to the mild mechanical disturbance. The erythema is expected to resolve within 12 hours without sequelae. The risk of skin infection is extremely low since only superficial skin layers are removed. Subjects will be provided with wound care information and a department phone number to contact should they have any concerns.

Risks of Phlebotomy

The risks of having blood drawn include some pain when the needle goes in, and a small risk of bruising and/or infection at that site. Some people will get lightheaded, nauseous, or faint. 4mLs is approximately 1 teaspoon of blood and is not likely to cause lightheadedness, however subjects will be observed after blood draw to be sure they are comfortable with deambulation.
Subjects will be encouraged to drink plenty of water prior to the blood draw. Phlebotomy will be performed at the antecubital fossa to minimize pain and bruising. Subjects will be seated during

the blood draw to minimize dizziness and any risk of falling. Pressure will be applied to the site after the draw and a band-aid will be applied to minimize the risk of bleeding and infection.

Risks of Punch Biopsy

The biopsies will be no larger than 5 mm in diameter (about a pencil eraser head). A nonabsorbable suture (1-2 stiches) will be placed in each site for hemostasis and enhanced wound healing. A small scar will remain at the site. The scar will be smaller than the initial biopsy but may also be hypo- or hyperpigmented. Sutures will be removed in 14 (+/- 2) days. The risks of this procedure include adverse reaction to anesthesia (lidocaine), pain, bleeding, infection and hypertrophic scars or keloids. Patients with a history of Novocain or lidocaine allergy or a history of keloid formation will not be allowed to participate in order to minimize the risk of adverse reactions. Biopsy will be performed after injecting a local anesthetic to minimize pain. A pressure bandage will be applied to the biopsy site to minimize the risk of bleeding and infection. Written wound care instruction will be provided to the subjects after the procedure. In case of increased redness, pain or oozing of the sites subjects will be instructed to call the dermatology department if appropriate subjects will be asked to come to clinic in the next 24-48 hours for wound check.

Risks of NANODOXY® 1% hydrogel

Subjects may experience local irritation including redness, pain, stinging or burning sensation in the treatment area. Systemic doxycycline can enhance sensibility to the sun light. It is possible that topical application might temporarily enhance risk of sunburn in the treated site. To minimize risk of sunburn we will instruct the subjects to apply the topical at bedtime and rinse off in the morning. In case of sun exposure subjects should use appropriate sun protection (SPF >30 or clothing). Some patients may experience some irritation from the NANODOX® hydrogel especially in areas of open skin from scratching. Some patients may experience increased dryness from the hydrogel formulation. All patients will be advised to moisturize in the morning and use gentle body wash to minimize these potential side effect.

As for any antibiotic, there might be a risk of inducing antibiotic resistance. To minimize the risk the drug will be used only until the lesion is active/visible. Also we will be monitoring for bacteria growth during the study to determine possible change in bacteria contamination as well as antibiotic resistance.

Preclinical studies have shown that over time (12-24 hours) the color of leftover products in the package might change from a white transparent tone to a more yellow-brown color. Change in color has not been associated with change in efficacy. To minimize risk of skin discoloration we will advise the subjects to rinse off the site in the morning.

Subjects with sensitivity to doxycycline might develop an allergic drug reaction, which could vary from local irritation to more severe systemic reaction. Subjects with sensitivity/allergy to

tetracycline will not be enrolled in the study.

In case of any abnormal reaction we will instruct the subjects to call the dermatology office and stop using NANODOX® until discussing symptoms with study team.

Risks to children or pets are not known and subjects will be instructed to keep the products out of reach from children and pets. Because this is an investigational medication/formulation some side effect may not yet be known. Any adverse reaction will be accounted.

Based on preliminary data, we do not anticipate any complications associated with the use of topical doxycycline.

Common skin reactions with taking oral doxycycline include:
- Redness
- Swelling
- Blistering rash

Common allergic reactions with taking oral doxycycline include:
- Hives
- Shortness of breath
- Swelling of the face, lip, tongue, or throat

Common systemic reactions with taking oral doxycycline include:
- Headache
- Dizziness
- Fever
- Chills
- Rash
- Nausea
- Vomiting
- Diarrhea
- Thrush
- Vaginitis

If a subject experiences an unexpected reaction to the investigational product, it should be recorded as an AE or SAE.

The study agent (NanoDOX™ Hydrogel) consists of doxycycline gel 1% w/w. The 1% w/w doxycycline formulation of NanoDOX™ Hydrogel contains: 1% w/w doxycycline monohydrate in carboxymethylcellulose (CMC), a hydrogel. CMC functions as the vehicle for the doxycycline active ingredient and assists in providing a moist wound environment. The primary function of doxycycline in NanoDOX™ Hydrogel is to promote wound healing by harnessing doxycycline's inhibition of matrix metalloproteinase (MMPs) and TNF-a converting enzyme (TACE). This property of doxycycline is hypothesized as the mechanism of action of NanoDOX™ Hydrogel for enhanced wound-healing.

Alchem will prepare, package and label the investigative material in easy-to-open 1.5 gram disposable packets. Each packet of doxycycline gel will have a two-part tear-off label. The gel should be refrigerated between applications.

Bench Testing

Systemic absorption of the doxycycline component in NanoDOX® Hydrogel through the skin was evaluated by Nanotherapeutics. Briefly, Franz diffusion cells were used to analyze the amount of doxycycline released out of a NanoDOX® Hydrogel or particle suspension across an exact area with respect to time. These conditions may be used to mimic an exposed wound and the resulting drug release out of the NanoDOX® Hydrogel into the body. Overall, less than 1% w/w of the 15 mg total doxycycline available in the donor chamber in the gel (11.18µg, or 0.08%) as well as the suspension (29.88µg, or 0.21% w/w) diffused across the membrane into the receptor chamber.

Animal Testing
In a rat wound healing study, NanoDOX® Hydrogel was tested at doxycycline concentrations of 0.0% (placebo hydrogel), 0.3%, 1.0%, and 3.0% w/w as well as untreated control. The results of the study are:
a) No significant differences between the histological scoring for all concentrations and the control; and,
b) All concentrations were judged to be non-irritants versus the control.

Details relating to additional in vivo and in vitro nonclinical pharmacology and nonclinical toxicology studies of NanoDOX® Hydrogel can be found in the Investigator's Brochure.

Reproductive Risks
The effects of topical doxycycline on an unborn fetus or nursing (breast feeding) infant are unknown. It is possible that use of NANODOX® hydrogel may be associated with unanticipated risks to a pregnancy or fetus. Women of child bearing potential will be counseled to use acceptable birth control methods. Women will not be allowed to participate in this study if they are pregnant, become pregnant, or are nursing an infant.

Property damage risks
Objects in contact with NANODOX® may have discoloration (e.g. bedding, towels, clothes). Subjects are advised to be careful handling the opened NANODOX® packages and to rinse the excess of product from their skin if the observe change in color to minimize the likelihood of stain on towels, rugs or other clothing.

General Risks
When participating in any research study there is a possibility for invasion of privacy or breach of confidentiality.

Adverse Event Reporting
An adverse event (AE) is any observation whether or not considered to be product related, that is unfavorable and unintended and that occurs after any use of an investigational medicinal product.

Any observation of abnormality will be documented on the Adverse event form. The Investigator will document the date of occurrence, severity, resolution, and will assess the relationship of the adverse event to the study drug by assigning a causality assessment. Results from laboratory evaluations which are outside of the laboratory reference range will constitute an adverse event only if the Investigator determines that the result is clinically significant.

Severity will be assessed by the following system:

- Mild: easily tolerated, causing minimal discomfort
- Moderate: sufficiently discomforting to interfere with every day activities
- Severe: prevents normal every day activities

Serious Adverse Events
FDA definition: A serious adverse event results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability / incapacity, results in a congenital anomaly / birth defect. Events fitting these descriptions will be considered adverse events if they occur after treatment with the study drug.

As soon as reasonably possible, subjects will contact the Investigator to report any unfavorable events. The Investigator will assess the case, determine the need for treatment, and consult with Alchem Laboratory, Inc, particularly in the occurrence of:

- A serious adverse event.
- An unusual frequency of non-serious adverse events.
- Death, even if this does not appear to be related to therapy with the study drug.

The Investigator will notify the sponsor (Alchem Laboratory, Inc and CTSI) and the UF Institutional Review Board within approximately 3 working days if a serious adverse event occurs.

Causality assessments

Probable
All of the following apply:
- Reasonable association in time between the administration of the study drug and onset and duration of the reported event.
- Description of the clinical phenomenon should be consistent with, or at least plausible, given the known pharmacology and toxicology of the product.
- There should be no other equally plausible explanation(s) of the case. In particular, concurrent use of other products (and possible interactions) or concurrent disease should be taken into account in the assessment.

Possible
- The causality is one (of other) possible and plausible causes for the described event, but the data do not meet the criteria for inclusion in Category A.

Unknown
- An association with the study drug cannot be discounted, but other factors prevent a conclusion from being drawn.

Unlikely
- Sufficient information exists to establish beyond reasonable doubt that the product was not likely to be the cause of the event.

Classification of adverse events
At the conclusion of the study, Medicinal Dictionary for Drug Regulatory Authorities (MeDDRA) terms will be assigned to all adverse events and will be included in the final study report.

Stopping Criteria
If more than 5 patients report a greater than moderate local side effect (irritation, worsening of rash, etc) or if the clinical dermatological score in the target lesion area as assessed by the clinician has worsened by more than 50% from baseline in more than 5 patients, then all

patients will stop medication until further review and changes made to the protocol. If any unanticipated serious adverse effects are noted all subjects will be notified and changes will be made to future protocols and submitted for approval.

5. DATA ANALYSIS AND DATA MONITORING

Primary Outcomes:
1. Assessment of safety and tolerability of NanoDOX® Hydrogel in AD subjects on lesional and non-lesional (clinically normal appearing) skin, including pain and dermatologic/skin toxicity (incidence, severity, frequency, duration and causality) in lesional skin at target site (NanoDOX® Hydrogel). 2. Improvements rate of IGA and EASI score in lesional skin treated with NanoDOX® Hydrogel before and after treatment. We will quantify clinical improvement as: IGA (0-4) fold improvement from baseline; percent of subjects reaching EASI50 (50% improvement over baseline) or EASI75 (75% improvement over baseline) at each time point post treatment (V2 and V4). 3. Objective Clinical improvement based on digital photography scoring (0-4 scale and size of active lesion), measured as fold improvement over baseline at each time point (V2 and V4).

Secondary Outcomes:
1. Improvements of itch score (0-5 scale) in lesional skin, before and after treatment at each time point. 2. Will evaluate Staphylococcal Aureus rate of colonization (positive or negative), difference in number of growth (0 to 3+++) and antibiotic sensitivity pre and post treatment. Doxycycline could have an effect on AD lesions just by reducing the Staphylococcal Aureus colonization. By integrating these findings with clinical outcomes, drug penetration and molecular changes we will be able to start to address this question. 3. Improvement of barrier function in non-lesional (normal appearing) and lesional skin pre and post treatment (V1 to V3). We will expect that clinical improvement parallels with improvement of barrier function, such as reduced TEWL, increased stratum corneum hydration and reduced pH in active treatment areas. On the other hand, worsening of the measurement will suggest damaging effect of the treatment on the skin, which could be indication of subclinical irritation induced by the treatment and/or vehicle.

The purpose of this study is a proof of concept. We are adopting a similar strategy to Phase II cancer trials that seek a clinically significant response. Using exact binomial methods, we shall obtain point and interval estimates for the fraction of subjects who respond as (a) At least EASI 50% by visit 2 or 4 and (b) At least EASI 75% by visit 2 or 4. We shall also tabulate the IGA score from baseline to visit 4. We would consider 6 or more responses (a) in 15 subjects as promising. The probability of declaring a promising result when the true response rate is 60% or higher is over 96% (power).

Side effects and baseline characteristics will be tabulated qualitatively. The blinded pre-vs post evaluation will be analyzed for IGA scores by the Wilcoxon sign-rank test.

Data Storage and Confidentiality

All data from the clinical trial will be securely stored using the REDCap. We have already obtained funding to work with CTSI to build the REDCap database for the study.

Only the minimal necessary data is being collected. This includes: Name, contact information, age, sex, MRN, history of allergies, dermatologic diagnosis, history of other medical conditions, current medications and clinical scores. This information will be stored in REDcap.
Samples will be assigned unique identifiers and stored separately from the key linking the sample to the identifiers. The electronic key will be stored in REDcap file, accessible only to study personnel. Any paper documents containing identifiers will be kept in a binder in a locked cabinet accessible only to study personnel.

Data Monitoring

The study will be monitored in compliance with the relevant parts of 21 CFR and according to the ICH GCP Guidelines.

A Monitor will visit the Study Center on a pre-arranged schedule to monitor adherence to the protocol and to applicable FDA regulations; and the maintenance of adequate and accurate clinical records. A Case Report Form will be completed for every subject that was enrolled to participate in the Study. The Case Report Form (CRF) will be reviewed in detail, for which the Monitor will have access to subject study records, laboratory data, and other source documentation.

Case Report Forms will be completed as information becomes available or within five days of a Study Visit.

Case Report Forms will be reviewed in detail by the Monitor who will make a decision as to their acceptability. If errors or omissions are found in the course of a data audit, or if clarification of data is required, the Case Report Form(s) in question will be corrected by the Investigator or his/her designee. Data Resolution or Query Forms may be generated on omissions or clarifications, to be completed, signed and dated, and maintained as a part of the CRF.

The Principal Investigator or a Sub-investigator will sign and date the indicated places of the Case Report Form. This signature will indicate that thorough inspection of the data therein has been made and will thereby certify the contents of the form.

The procedures outlined in the protocol and case report forms will be carefully reviewed by the Sponsor, Investigator and staff prior to Study Initiation to ensure appropriate interpretation and implementation. No deviations from the protocol shall be made except in emergency situations where alternative treatment is necessary for the protection, proper care and well being of subjects.

Amendments will be submitted to the IRB for their review and approval prior to implementation. When an amendment to a protocol substantially alters the study design or increases potential risk to the study subject, the Informed Consent form will be revised and if applicable, subject's consent to continue participation will again be obtained.

5. SUBJECTS IDENTIFICATION, RECRUITMENT AND CONSENT

Method of subject identification and recruitment

Dr. Anna De Benedetto will identify potential subjects from the CTSI consent2share cohort, UF dermatology clinics, the IRB-approved Dermatology Research database (IRB201600411) or from advertisements (UF website, fliers and newspaper advertisements) directed at the general population. In the case of clinical subjects, Dr. De Benedetto will provide the subject with a contact phone number for the study coordinator who is approved to enroll and consent subjects. While Dr. De Benedetto will be available for questions, consent might be obtained by other study personnel for subjects she sees in her clinical practice. It will be made clear to the potential subjects that their participation is voluntary and their care at the Department of Dermatology will not be affected by their decision to participate in this study.

Process of consent

Dr. De Benedetto or other team members (e.g. coordinator, nurse, co-investigator) will be meeting directly with the subjects in a private suite. The consent form will be discussed with the potential subject. Time allotted to consenting will be dependent on the subjects' needs, questions, and comprehension of the information in the consent form. Each subject will be encouraged to read the consent form on his/her own and ask any specific questions. At the time of consent, subjects will be offered the option to provide permission to be contacted in the future regarding this and other dermatologic studies. Those who provide permission for future contact will be added to the IRB201600411 approved database.

Subject capacity

We will not be recruiting subjects incapable of giving informed consent**.**

Subject/representative comprehension

The subject's capacity to comprehend the information in the consent form will be assessed by asking questions about the content and encouraging the subject to describe briefly the study procedures.

Documentation of consent

The consent will be documented by signing the consent form by both the study subject and the person obtaining consent. A copy of the consent form will be provided to the subject.

Costs to the subject

Procedures performed during the study will be at no cost to the subject.

Payment for participation

Subjects that agree to participate in the study will receive compensation for their time: $25.00

for the screening visit. Each study visit will be $45.00 and an additional $45.00 for the optional biopsy. The Final visit (Follow up Visit) will also be $25.00. For a total of up to $275.00 including the 2 biopsies or $185.00 without the biopsy if all visits are completed. UF VISA cards will be used for the reimbursement. Payments will be issued to VISA card within 7 business days of completed study visit.

Potential benefits to the subjects

There are no benefits for the individual participant. The investigational medication may temporally improve or worsen the lesion in the target area of application. The nature of this pilot study does not promise benefit for the individual. We hope that this research will lead to a better understanding of atopic dermatitis pathogenesis and pave the way for development of topical medications that aim to repair skin barrier and skin driven inflammation.

Figure 1: **Multi probe System MPA5 –** Courage & Khazaka (Germany) and **Aquaflux** (Biox, London UK).

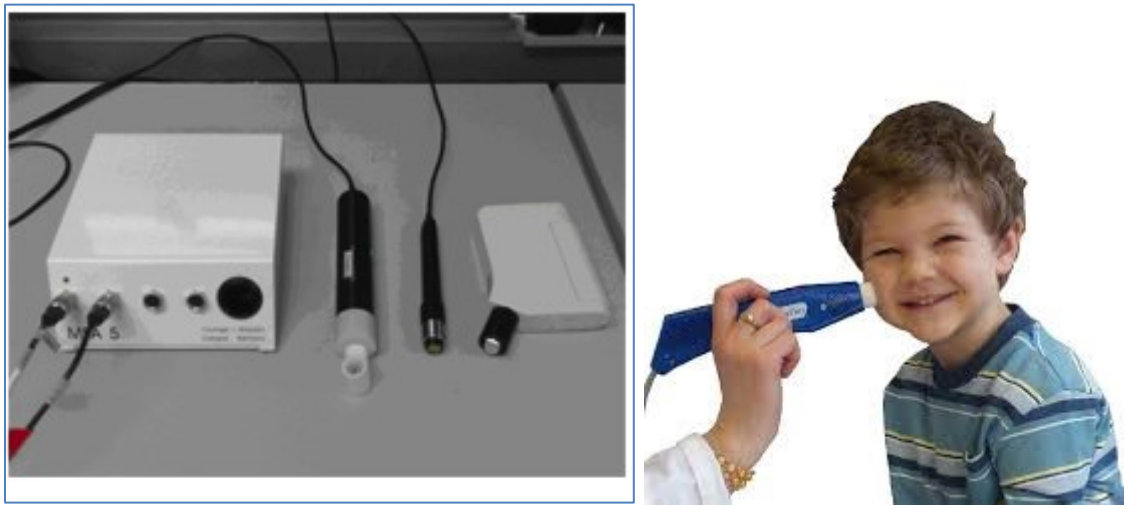
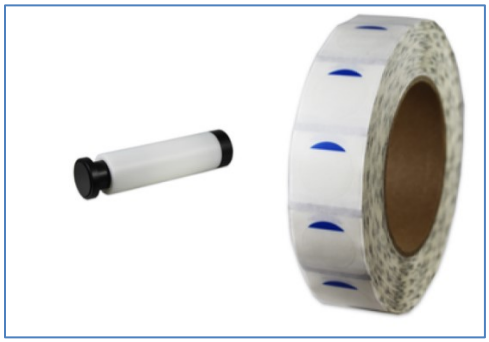


Figure 2**: D500–D-squame® pressure instrument and D-squame®** - CuDerm (Dallas, TX)



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[xi] *J. Periodontology 2015;2:10*
[xii] *Arch Dermatol. 1994 Nov;130(11):1402-7*
[xiii] *Br J Dermatol, 2011. 165(2): p. 329-34*
[xiv] *Br J Dermatol. 2015 Aug;173(2):316-7.*
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