Title: Photodynamic Therapy-Induced Immune Modulation: Part III

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Photodynamic Therapy-Induced Immune Modulation: PART III

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1.0 Background

This study is Part III of the Photodynamic Therapy-Induced Immune Modulation research. Part III is designed as a double-blinded proof of concept of feasibility study to define if the immunosuppression associated with photodynamic therapy (PDT) can be blocked by treatment with cyclo-oxygenase-2 (COX-2) inhibitor celecoxib in comparison to placebo. PDT consists of application of the photosensitizer 5-aminolevulenic acid followed by treatment with a blue light. PDT is used to treat pre-cancerous actinic keratosis on large areas of skin [1]. These studies are a continuation of ongoing studies that indicate that the lipid mediator platelet-activating factor (PAF) is generated in skin following PDT, and that PDT suppresses the immune system. **Our hypothesis is that PDT-generated PAF results in the immunosuppression associated with PDT**. Therefore, we propose that a treatment to block that immunosuppression could protect the patient undergoing PDT. Unfortunately, blockers of the PAF system are not currently commercially available. However our research studies using mice indicate that PAF- and PDT-induced immunosuppression is blocked by treatment with COX-2 inhibitors (2,3). We would like to conduct this research study **as a proof of concept**.

This double-blinded placebo-controlled study will test if a seven day treatment with COX-2 inhibitor celecoxib (200mg BID) following PDT will 1) block PDT-induced immunosuppression, and 2) enhance the effectiveness of PDT as measured by decreased numbers of actinic keratosis at 6 months and 12 months following PDT in comparison to placebo treated. This protocol will recruit subjects undergoing PDT or control subjects (not undergoing PDT) at Department of Dermatology at Boonshoft School of Medicine at Wright State University where the PI is Chair of Pharmacology & Toxicology, with joint appointment in the WSU Department of Dermatology. These studies are temporarily funded by WSU Department of Pharmacology and Toxicology.

Please note that this study will recruit both subjects who are planning to undergo PDT as a part of their management of their actinic keratosis and normal control subjects. This study will compare the effects of celecoxib vs placebo on the immune effects and effectiveness of PDT. This study also assesses if celecoxib alone modulates immune responses in control subjects. Thus, there will be 4 groups: PDT + Celecoxib, PDT + placebo, Control + Celecoxib and Control + placebo. We plan to randomize the subjects and one of the study personnel (coordinators who will not have any contact with subjects except for making up the medicines will be unblinded). These studies take advantage of the large numbers of patients undergoing PDT at WSU.
These studies include:

- Research subjects (those who receive PDT therapy)
    - Skin testing with intradermal Candida and Trichophyton antigens on two separate occasions.
    - Reporting a pain scale of 0-10
    - Urine collection (7 samples)
    - laboratory serum collection (2 venipunctures)
    - PDT-conducted for clinical reasons
    - 7 days of Celecoxib 200mg 2x per day or placebo
    - Follow up visits months 6 and 12 to assess actinic keratosis in treated areas.

- Control subjects

- Skin testing with intradermal Candida and Trichophyton antigens on two separate occasions.
- Urine collection (7 samples)
- Laboratory serum collection (2 venipunctures)
- 7 days of celecoxib 200mg 2 x per day or placebo.

2.0 Rationale and Specific Aims PART III

This protocol is designed to address multiple questions that have arisen since we have discovered that 1) PDT in mice generates PAF and related oxidized glycerophosphocholines (ox-GPCs); and that 2) PDT suppressed systemic immunity against the allergen DNFB (dinitrofluorobenzene) in wild-type but not PAF-R-knockout mice [3]; and 3) the literature indicates that PDT induces localized immunosuppression [4]; and 4) our ongoing studies that PDT of human subjects results in PAF agonists in their skin; and finally, 5) our ongoing studies that PDT of human subjects results in immunosuppression.

To test immunosuppression in humans, we plan two strategies. The first strategy will test skin testing against Candida and Trichophyton antigens, before and after PDT treatment.

We can also measure immune responses by taking peripheral blood mononuclear cells (PBMCs) from blood of subjects before/after PDT and incubate them with candida and trichophyton as well as other common antigens, to see if can measure an immune response (by measuring gamma-interferon by EIA). We can also measure levels of immunosuppressive T cells (Tregulatory cells) in the blood as well as the Treg-associated cytokine interleukin-10 (IL-10). Previously, we have demonstrated that when PAF is made, increased Tregs and IL-10 can be found in the mice. Again, our studies have demonstrated in mice that COX-2 inhibitors can block immunosuppression associated with PDT, and PAF agonists [2,3,4].

This study will also collect urine specimens from the PDT-treated subjects (before and after PDT) to test them for COX-2 metabolites and reactive-oxygen-associated markers (isoprostanes). We will also test urines in control subjects treated with celecoxib vs placebo.

We hypothesize that these studies will demonstrate the following:
1. PDT will result in immunosuppression (as measured by increased IL-10 in serum, and lymphocyte assays, as well as diminished candida/trichophyton skin testing).
2. PDT will result in increased COX metabolites and isoprostanes in urine.
3. Short-term Celecoxib treatment at commonly used dosages following PDT will inhibit the PDT-induced immunosuppression in comparison to placebo treatment.
4. Short-term Celecoxib treatment following PDT will block the increased COX metabolites in urine, but will have no effect on the isoprostanes.
5. Subjects undergoing PDT treated with celecoxib will exhibit decreased levels of erythema and decreased pain/tenderness in comparison to placebo control.
6. PDT will be more effective (as measured by less actinic keratosis in the PDT-treated areas at 6 and 12 mo following PDT) in those subjects who underwent Celecoxib treatment compared to placebo treatment.
7. Control (non-PDT) subjects will not experience any differences in immune responses in those treated with celecoxib versus placebo treatment.

This proof of concept study is valuable as it may form the basis of future studies to test if cyclooxygenase inhibitors can block PDT-mediated systemic immunosuppression, and potentially improve the long-term outcomes of PDT.

3.0 Inclusion/Exclusion Criteria

Inclusion Criteria for control subjects

- Adult age 45 or older
- Caucasion (Fair skin, Fitzpatrick types I and II)
- Ability to understand and consent to the instructions of the study
- Have access to stable transportation

Inclusion Criteria for study subjects

- WSU dermatologist has prescribed PDT for the treatment of actinic damage (Presence of precancerous actinic keratoses whose treatment necessitates PDT with the BLU-U).
- Undergoing PDT on greater than 5% body surface area: face and scalp, face and dorsal surface of arms, face and chest, face and back, or dorsal surface of arms alone, chest alone, or back alone.
- Caucasian (Fair skin, Fitzpatrick types I and II)
- Adult-age 45 or older
- Ability to understand the informed consent and comply with instructions and have stable transportation.

Exclusion Criteria for all subjects

- PDT on less than 5% body surface area (eg, forehead)
- Present treatment with corticosteroids or Non-steroidal anti-inflammatory drugs (e.g., cyclooxygenase inhibitors) within past 2 months (except low-dose 81 mg aspirin).
- On antioxidant supplements (e.g., vitamin C) for past 2 months
- Tanning bed use within last 3 months
- PDT treatments within last 3 months
- Significant health issues that could affect your immune system (e.g., uncontrolled Diabetes Mellitus, Rheumatoid arthritis, skin rashes, psoriasis) that could interfere with testing
- Pregnant or nursing
- No immunosuppression, and on no immunosuppressive medications or NSAIDS within past 30 days (except low-dose [81 mg daily] aspirin).
- No signficant underlying diseases that could potentially interfere with the immune assays or cardiac or renal or liver problems.
- History of blood clot or hypercoagulable state or GI bleed/ulceration.

All subjects will be asked the screening questions below as part of the inclusion/exclusion criteria:

- Are you 45 years old or older?
- Do you regularly use tanning beds?

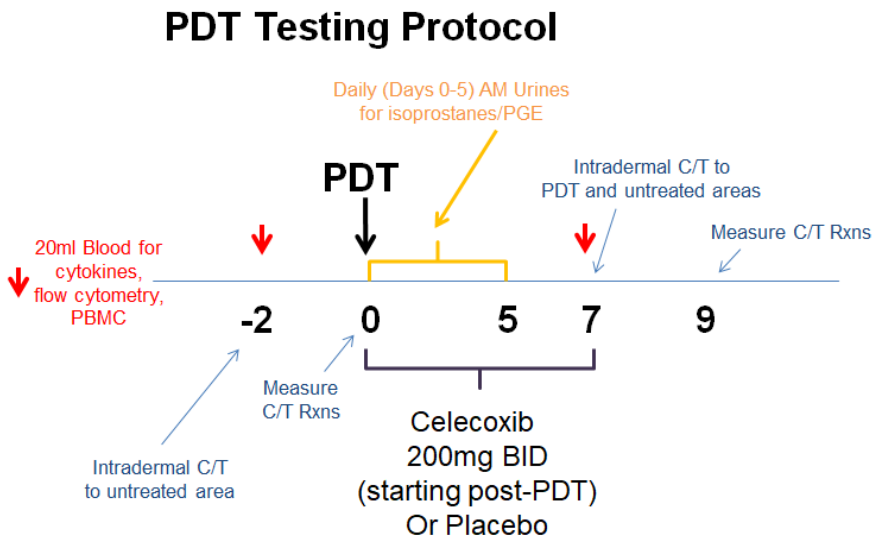
- Are you being treated for anything that requires UV-treatments?
- Have you had any diseases that got worse when you went in the sun?
- Are you using topical or oral anti-inflammatory doses of NSAIDS or steroid?
- Have you ever had any abnormal reactions or allergies to NSAIDS?
- Are you taking any medications that warn you to stay out of the sun?
- Have you ever had a reaction to medications that were applied to your skin?
- Do you have severe sugar diabetes, heart problems or liver problems or other health problems that affect your immune system?
- Have you had significant sun exposure or sunburn within the last 2 weeks? Tanning bed exposure in the past 3 months?
- Have you had PDT in past 3 months?
- Have you ever had a blood clot or other bleeding issues?
- Have you ever had a stomach ulcer or bleeding from GI tract?
- Are you pregnant or nursing?

4.0 Enrollment

Subjects who will be undergoing PDT for actinic keratoses and controls will be recruited from the Dermatology clinics at WSU. Control subjects may also be recruited from other sites or other practices. The potential subjects will be told by their physician about the study. They will be reminded that this study will in no way benefit them directly and that their participation is only voluntary, and if they choose not to participate it will not impact their care or the interest of their providers in their case. In addition, advertising flyers placed in the Wright State Department of Dermatology clinics will be used to seek PDT subjects and control subjects. The flyers will require subjects who are interested in participating in the study to contact the PI, or the research office. The PI, or study team member, will meet with the potential subjects. The PI will determine enrollment status based on the inclusion and exclusion criteria. Each subject will be given all of the procedures and risks in writing and a signed informed consent statement will be obtained from each subject prior to enrollment in the study. The subjects will also be informed of the need to avoid sun exposure or tanning bed use for 3 months prior to PDT or during the study.

5.0 Study Procedures

Outlines of testing for PDT-treated subjects. Please note that the Controls (see picture on page 9) will undergo all procedures EXCEPT the PDT. Controls also will not have actinic keratosis counted/mapped and will not be following up to have actinic keratosis mapped at 6 and 12 months.



DAY -2. Consent, Skin testing, Venipuncture

- Informed consent. Explanation of the study, listing of all the required dates to return to the clinic for the study, and a signed informed consent will be obtained
- Intended PDT treatment site will be noted, photographed and recorded
- Actinic keratosis mapping. Numbers of AKs will be counted and mapped on a diagram to allow us a base recording for future comparisons
- Venipuncture. 20ml of blood will be drawn by routine phlebotomy.
- Intradermal skin testing application. An area on inner (volar) arm where planned PDT will not be taking place will be photographed, and intradermal injections of 0.1 ml standardized solutions of commercially available Candida and Trichophyton protein antigens, or saline will be intradermally injected into skin to form small wheals. The area will be photographed.

DAY 0. Read skin testing results, Urine specimen, PDT, Start celecoxib or placebo

- Intradermal skin testing reading. The skin reactions to the Candida and Trichophyton antigens will be visualized, photographed, and the size of the reactions measured using calipers.
- Urine specimens. Urine Sample will be obtained, and 6 sterile pre-labelled urine cups will be provided to the subject for home urine collection along with an orange opaque biohazard bag to store the samples in the freezer until they are returned to the research site.
- Photodynamic Therapy (PDT). PDT is a standard treatment at the WSU Department of Dermatology clinics and approximately 75-80 procedures are performed annually. PDT is being conducted for clinical reasons and will therefore be submitted to the patient/patient's insurance as routine care. **Patients sign a separate informed consent for the PDT therapy** and the PDT will follow standard clinical guidelines. Control subjects will not undergo the PDT treatment.
- Celecoxib. The subjects in the study arms which receive Celecoxib will be provided #14 capsules of 200mg celecoxib or 14 placebo capsules and instructed to take 1 capsule in the morning and 1 capsule in the evening. They will be instructed to start the medication the same day (2 doses) and take as prescribed for a total of seven days. The subjects will be provided a 7 day pill dispenser with AM/PM components for their use. Funds from the study budget

will pay for the prescription so that there will not be a cost to the subjects. The dose of celecoxib was similar to that used long-term in previously reported studies where subjects were treated with celecoxib 200mg BID for ~ 1 year. This is a dose and time frame that based upon our murine studies, should inhibit PAF-induced immunosuppression.

DAY 1-5 Urine Home Collection

At night (~8 h post-PDT) on the day of PDT treatment, and days 1-5 First morning urine samples will be obtained in the sterile cups provided to the subject, each placed in the biohazard bag and stored in the freezer. The subject will bring the urine samples back to the research clinic at day 7 study visit. Subjects will be asked to fill out a Urine Collection Record form. Also, they will rate the pain associated with the PDT treatment on the Skin Pain Visual Analogue Scale (VAS). The scale will be 0 (no skin pain) to 10 (severe skin pain).

DAY 7. Return of urine samples, Repeat skin testing to control and PDT-treated sites, Venipuncture.

- Urine samples returned to the research site from research subjects
- Subjects will bring empty pill dispensers.
- Intradermal skin testing application. An area on opposite inner (volar) arm where PDT did not take place, as well as a PDT-treated site (extensor arms, scalp, etc) will be photographed, and intradermal injections of 0.1 ml standardized solutions of commercially available Candida or Trichophyton protein antigens, or saline will be intradermally injected into skin to form small wheals. The areas will be photographed.
- Reporting amount of pain. The 0-10 pain scale will be used again to query the subject as to amount of discomfort in the PDT-treated sites.
- Venipuncture. 20ml of blood will be drawn by routine phlebotomy.

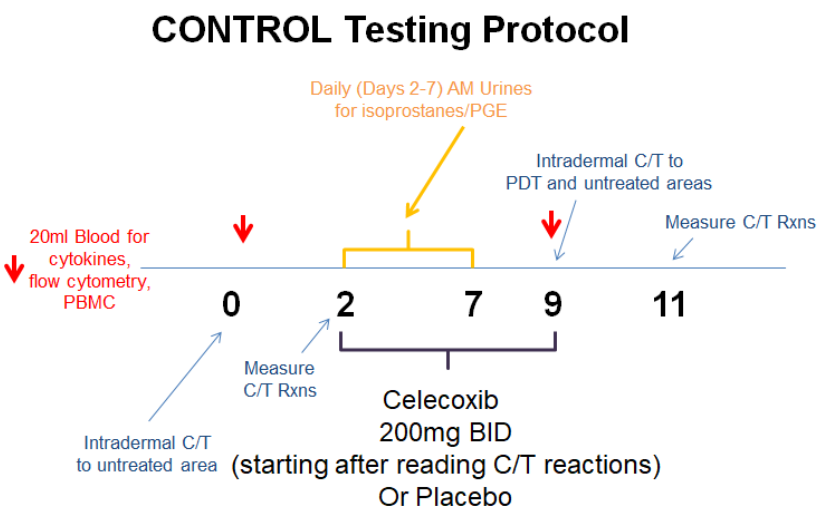
DAY 9. Read skin testing results

- Intradermal skin testing reading. The skin reactions to the Candida and Trichophyton antigens will be visualized, photographed, and the size of the reactions measured using calipers.

6 months and 12 months.

The subjects who were treated with PDT will be seen when they return to the dermatology clinic for their routine follow up care. We will use a body map to record the current numbers and sites where they have the precancerous AK lesions. Their current numbers and sites will be compared to the numbers and locations of their AKs pre-treatment with PDT obtained at visit Day 0. This comparison will help to define effectiveness of the therapy. This information will become part of their medical record.

CONTROL SUBJECTS



DAY 0. Consent, Skin testing, Venipuncture, Urine specimen

- Informed consent. Explanation of the study, listing of all the required dates to return to the clinic for the study, and a signed informed consent will be obtained
- Venipuncture. 20ml of blood will be drawn by routine phlebotomy.
- Intradermal skin testing application. An area on inner (volar) arm will be photographed, and intradermal injections of 0.1 ml standardized solutions of commercially available Candida or Trichophyton protein antigens, or saline will be intradermally injected into skin to form small wheals. The area will be photographed.
- Urine specimens. Urine Sample will be obtained, and 6 sterile pre-labelled urine cups will be provided to the subject for home urine collection along with an orange opaque biohazard bag to store the samples in the freezer until they are returned to the research site.

DAY 2. Read skin testing results, Start celecoxib or placebo

- Intradermal skin testing reading. The skin reactions to the Candida and Trichophyton antigens will be visualized, photographed, and the size of the reactions measured using calipers.
- Celecoxib. The subjects in the study arms which receive Celecoxib will be provided #14 capsules of 200mg celecoxib or 14 placebo capsules and instructed to take 1 capsule in the morning and 1 capsule in the evening. They will be instructed to start the medication the same day (2 doses) and take as prescribed for a total of seven days. The subjects will be provided a 7 day pill dispenser with AM/PM components for their use. Funds from the study budget will pay for the prescription so that there will not be a cost to the subjects. The dose of celecoxib was similar to that used long-term in previously reported studies where subjects were treated with celecoxib 200mg BID for ~ 1 year. This is a dose and time frame that based upon our murine studies, should inhibit PAF-induced immunosuppression.

DAY 9. Repeat skin testing, Venipuncture.

- Subjects will bring empty pill dispensers.

- Intradermal skin testing application. An area on opposite inner (volar) arm where previous skin testing did not take place will be photographed, and intradermal injections of 0.1 ml standardized solutions of commercially available Candida or Trichophyton protein antigens, or saline will be intradermally injected into skin to form small wheals. The areas will be photographed.
- Venipuncture. 20ml of blood will be drawn by routine phlebotomy.

DAY 11. Read skin testing results

- Intradermal skin testing reading. The skin reactions to the Candida and Trichophyton antigens will be visualized, photographed, and the size of the reactions measured using calipers.

Compensation.

The subjects will be compensated for involvement in the study.
Research subjects require more visits and procedures than control subjects.

Breakdown for each event is as follows:

Consent	$20.00	PDT ($20), Control ($20)
Venipuncture	$10.00	PDT ($20), Control ($20)
Urine collection	$5.00	PDT ($35), Control ($35)
Skin testing APPLICATION	$15.00	PDT ($45), Control ($45)
Skin testing READING	$20.00	PDT ($40), Control ($40)
Bonus for completing study	$65.00	PDT ($65), Control ($65)
Follow up visits at 6 and 12 months	$25.00	PDT ($50)

Hence the research (PDT) subjects may receive up to **$ 275.00** and the control subjects up to **$225.00**.

There is no cost for parking at the Wright State Physician's Building. Compensation is based upon completed study procedures and overall study completion. Since completion of study procedures are critical to the study findings if a subject cannot come to their scheduled visit (and cannot be quickly rescheduled) they will be removed from the study. Another subject will be enrolled in their place. Our goal is to enroll 48 subjects who complete the study.

6.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Risks of the study are as follows:

1. Photodynamic therapy. The risks to PDT are explained to the patient at the time of therapy decision and obtaining the informed consent for that standard of care procedure. PLEASE NOTE THAT PDT TREATMENT IS ALREADY RECOMMENDED FOR THE TREATMENT OF THE SUBJECT. THEY WILL BE SIGNING A SEPARATE INFORMED CONSENT FOR THE TREAMTENT AND THEY/THEIR INSURANCE AS APPROPRIATE WILL BE BILLED FOR THE TREATMENT.
2. Photography - the risk of photography is the possible loss of confidentiality. Pictures will not be of recognizable body parts or markings. Photos will be

labeled with the study number and a code that does not identify the subject. Photos will not include any identifying information.

3. Urine samples/non-invasive skin measurement with calipers. There are no risks to these.
4. Intradermal skin testing. These procedures are used in dermatology and allergy to define the ability of a host to respond to the allergen of interest. The majority of individuals will test positive to Candida protein antigens, and approximately 50% of individuals test positive for antigens from the common dermatophyte fungus Trichophyton (which causes skin infections like “athlete’s feet”). The side effects are localized skin inflammation and rash and occasionally can be painful. Very rarely an individual can have a systemic reaction. If we do note that the subject’s first skin testing results in exaggerated reactions, then the subject will not undergo further testing with this antigen.
5. Celecoxib Celecoxib is a commonly used NSAID, which is COX-2 specific. It was designed to have anti-inflammatory effects but have minimal side effects such as gastric distress, etc. There have been multiple studies examining the long-term effects of celecoxib 200mg BID. In particular, there was a published multicenter trial that indicated that long-term celecoxib was effective for diminishing the incidence of non-melanoma skin cancers [7]. It should be noted that in these studies the incidence of side effects of the celecoxib was similar to placebo. Long-term use of COX-2-selective inhibitors have been associated with increased cardiovascular events due to a pro-coagulant effect [6]. However, this is not associated with short-term use. There is a slight risk of GI bleeding. These effects will be minimized as we will recruit subjects with no history of GI ulceration/bleeding/blood clots.
6. There is also the potential risk of loss of confidentiality, but this will be minimized as photos of the PDT- and treated sites will not allow the person to be identified, and will not expose buttocks/genitals. Samples will be labeled with numeric numbers --III-001, III-002, III-003 etc. and the study number (XXXX). Example III-001-XXXX.

Risks of exaggerated PDT-related sunburn will be minimalized as subjects will be queried about abnormal reactions to sun or artificial sources of light, as well as if they are taking any medicine which is potentially photosensitizing.

Adverse Event and Serious Adverse Event Collection and Reporting

Information related to any adverse event will be collected at every study visit. The NCI-CTCAE (Common Terminology Criteria for Adverse Events) volume 4.03 published June 14, 2010 will be utilized for definition, grading scale and ‘study relatedness’ criteria of the event. Dr. Travers will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

An adverse event (AE) means any untoward medical occurrence in a subject associated with the use of an intervention that does not necessarily have a causal relationship with the intervention. It can be any unfavorable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the investigation, whether or not considered intervention-related.

An unanticipated problem can be any unexpected, related or possibly related to the intervention, and increases risk to participants or others more than was previously known. All three must be true to have an unexpected problem. Each unanticipated problem is assessed as to the impact on subject safety.

A serious adverse event (SAE) is any AE that results in one of the following outcomes:

- Death
- Life threatening
- Requires hospitalization or prolonged hospitalization
- A congenital anomaly/birth defect
- Persistent or significant disability/incapacity
- Requires intervention to prevent permanent impairment or damage

All reports will simultaneously be submitted to NIAMS, through KAI. This will include:

- Safety reports and meeting deliberations, including findings of adverse events and any comments, minutes, or recommendations resulting from the review of these periodic reports or meetings.
- Expedited reporting, including SAEs and unanticipated problems, along with the independent assessment of such events, as appropriate, should be reported in an expedited manner.

Each AE will be accompanied with the severity grade. The grades are as follows:

- Grade 1 Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*.
- Grade 3 Hospitalization: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
- Grade 4 Life-threatening consequences: urgent intervention indicated.
- Grade 5 Death

*Activities of Daily Living (ADL) refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, etc.

Grade 1 Mild and Grade 2 Moderate represent AEs that will be recorded at the time of occurrence and reported to DSMB at the next semi-annual meeting. DSMB meeting documents will be forwarded to the local IRB, as well as to the NIAMS, through KAI.

Grade 3 – Grade 5 are considered SAEs. Serious adverse events include death, are life threatening, requires hospitalization or prolonged hospitalization, a congenital anomaly/birth defect, results in persistent or significant disability/incapacity, or require intervention to prevent permanent impairment or damage. All SAEs will be reported to the local IRB, the DSMB, and to NIAMS through KAI within 48 hours.

In addition to severity grade, each AE will have the relationship to the study intervention assessed by Dr. Travers. The degree of certainty about causality will be graded using the categories below.

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE.

- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event.

Data Monitoring Plan
Dr. Travers will be the individual responsible for monitoring the safety environment of the participants. He will establish a Data and Safety Monitoring Board (DSMB) to assist in the safety oversight of this trial. Dermatology chair and board-certified dermatologists, Julian Trevino, MD, and Max Rubin, MD, will also serve on the DSMB. Also, Dr. R. Michael Johnson, MD, FACS, a board-certified plastic surgeon will serve on the DSMB. The DSMB will review the results of this study on a semi-annual basis and provide the Institutional Review Board (IRB) with a copy of that review report. Dr. Travers is not part of the DSMB as he is the PI for the trial.

In the event of adverse outcome (e.g., exaggerated skin reaction to skin testing antigen), this will be treated appropriately (with no physician cost from Dr Travers, though a pharmacy fee could be incurred if any prescription agents needed) and will be noted and reported to the DSMB for their review. Moreover, if any SAE or unanticipated events occur, these will also be reported to NIAMS within 48 hours. Non-serious AEs will be reported to the DSMB semi-annually versus immediately.

Dr. Travers is an experienced (~20 years) board-certified dermatologist who would be experienced in dealing with any potential adverse events should one arise. More importantly, Dr. Travers' role as a PI is to monitor the following: 1) ensuring that only subjects who meet the eligibility criteria are enrolled; 2) the informed consent process will be conducted appropriately and all subjects consented before proceeding with any study procedures; 3) Data will be collected and analyzed as outlined in the protocol; 4) Privacy and confidentiality of the subjects will be maintained; 5) Documentation of dropouts; 6) Documentation and reporting of all adverse events.
He will be assisted by study coordinator Elizabeth Cates, who will assist with non-invasive components of the study. Another study coordinator (Amy Williams) will be in charge of dispensing the celecoxib vs placebo and will serve as unblinded personnel.

7.0 Study Withdrawal/Discontinuation

Participants will be instructed that taking part in this study is voluntary and they may choose not to take part or may leave the study at any time. Emphasis will be stressed to the participants that leaving the study will not result in any penalty or loss of benefits that they are entitled.

8.0 Statistical Considerations

Statistical support will be provided by Dr. Genxin Li from WSU who will be collaborating with the PI. There are numerous outcomes in this study. They include: 1) levels of isoprostanes and PGE2 in urine before/after PDT; 2) Skin immune reactions; 3) T cell reactions; 4) numbers of actinic keratosis in the PDT-treated areas at 6 and 12 months. Moreover, these will be compared to control subjects control sites/pre-PDT as well as treatment with celecoxib/no celecoxib. Outcomes will be analyzed using McNemar's Test comparing the PDT-treated vs non-PDT treated area and celecoxib vs no celecoxib exposure. Secondary outcomes will be analyzed similarly using McNemar's tests for binary outcomes and paired t-tests for numeric outcomes. Exact and/or non-parametric methods will be considered if standard test assumptions are not met.

The sample size of 12 per group for PDT + celecoxib, PDT placebo, control + celecoxib and control placebo. Use of 12 allows reasonable discrimination for pilot studies (Pharmaceut. Stat. 4:287-291, 2005 [7]). Once we have obtained preliminary data from these subjects and controls, we will have Dr. Li perform power analysis to define if more subjects are needed, and submit a new/amended study if appropriate.

9.0 Privacy/Confidentiality Issues

There is also the potential risk of loss of confidentiality, but this will be minimized as photos of the will not allow the person to be identified, and will only expose a small areas of buttocks and will not show any breasts/genitals. All conversations and procedures will take place in Wright State Physician's building in the dermatology clinic in a private examining room. The data will be kept in the locked office of the PI. Samples and pictures from subjects will be coded by numbers as outlined above. Photos will not be of recognizable body parts or markings.

10.0 Quality Control

Quality control (QC) procedures will be done on all regulatory documents, research subject records and original source documentation. Any missing data will be communicated to the PI and coordinators for resolution.

Following written Standard Operating Procedures (SOPs), the Clinical Research Operations Manager will verify that the trial is conducted, documented, and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements.

The investigational site will keep records of all original source data. If requested, the site will provide access to original source documents to applicable regulatory agencies.

11.0 Follow-up and Record Retention

The duration of the study is expected to be 4 years from the first enrolled subject, and the duration of record retention will be 10 years. After this time, records will be archived. **We do not plan to biobank any samples**.

12.0 COVID-19 Safety Plan

All subjects are phone screened for COVID-19 symptoms the day before their appointment. When they arrive for their appointment they are instructed to call the research office prior to entering the building. Subjects are permitted to enter the building once research staff are ready to bring them back to the patient area. Everyone is required to wear masks when entering the building and have their temperature taken. Masks are provided to subjects who do not have one.

The research staff are required to wear masks and check for symptoms prior to reporting to work.

Patient room and equipment are cleaned and disinfected according to health authority guidance. During the research visit, subjects and research staff need to maintain the appropriate social distancing requirement when procedures are not being performed.

In regards to contact tracing, the research office keeps track of all appointments on a calendar to remember who has been in the office. These appointments have the subject's contact information such as full names, phone numbers, email addresses and approximate appointment length. Should subjects need to contact the research office, a copy of the informed consent with the office's contact information is listed on the first page.

13.0 References

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14.0 Abbreviations.

AE; Adverse Event
ALA; aminolevulinic acid
BSA; Body surface area
DNCB; dinitrochlorobenzene
EIA; enzyme-linked immunoabsorbant assay
NSAIDs; non-steroidal anti-inflammatory drugs
Ox-GPCs; oxidized glycerophosphocholines
PDT; photodynamic therapy
PAF; platelet-activating factor
PGE2; prostaglandin E2
QC; Quality control

SAE; Serious Adverse Event
WSP; Wright state physician's building
WSU; Wright state university