



CASE  
COMPREHENSIVE  
CANCER CENTER



A Cancer Center Designated by the  
National Cancer Institute

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Protocol Date: 4/13/2022

STUDY TITLE: **Vitamin D supplementation as a neoadjuvant for photodynamic therapy of actinic keratoses**

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SPONSOR: Investigator-initiated (Dr. Maytin)

SUPPORT/FUNDING: National Cancer Institute (NCI)  
National Institutes of Health (NIH), Bethesda MD

SUPPLIED AGENTS: Levulan Kerastick (FDA-approved drug)  
Cholecalciferol (over-the-counter vitamin)

IND #: The study uses nutritional Vitamin D and has been determined  
by the U.S. FDA to be IND-exempt (IND Letter#138378)

**Title:**

**Principal Investigator:** Edward V. Maytin, M.D. Ph.D.

PRINCIPAL INVESTIGATOR SIGNATURE:

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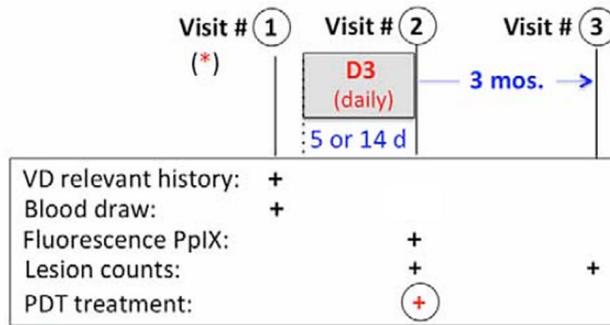
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## SUMMARY OF CHANGES

Protocol Date	Section	Change
		Initial IRB approval

## STUDY SCHEMA

Explanatory text for this timeline can be found in the legend to Figure 5.



(\*) Visit 1 will take place at least 20 days before Visit 2, to allow time to process the serum 25OH-D3 test results

## PROTOCOL SUMMARY

Protocol Number/Title	<b>Vitamin D supplementation as a neoadjuvant for photodynamic therapy of actinic keratoses</b>
Study Phase	Phase II
Brief Background/Rationale	This is a prospective clinical trial in which 30 patients will receive a brief (5-day or 14-day) supplementation with 10,000 IU of Vitamin D, prior to receiving aminolevulinic acid (ALA)- blue light PDT.
Primary Objective	Primary Endpoint(s) Determine whether acute supplementation (neoadjuvant Vitamin D3), adjusted according to baseline Vitamin D status, can improve the clinical PDT response relative to patients receiving PDT alone.
Secondary Objective(s)	Secondary Endpoint(s) Determine whether gene polymorphisms in VDR and CYP27B1 are predictive for the degree of responsiveness to Vitamin D as a neoadjuvant for PDT.
Exploratory Objective(s)	Exploratory Endpoints (s) Does prior supplementation with 10,000 IU of Vitamin D increase the accumulation of protoporphyrin IX (PpIX) within actinic keratoses after application of ALA solution prior to illumination with blue light PDT? Does prior supplementation with 10,000 IU of Vitamin D enhance the clinical clearance of actinic keratoses after PDT?
Correlative Objective(s)	Correlative Endpoint(s) Is there a correlation between Vitamin D receptor (VDR) polymorphisms and PDT treatment outcomes for actinic keratoses with and without prior Vitamin D supplementation?
Sample Size	30 participants (to be compared against already obtained control group)
Disease sites/Conditions	Actinic keratosis
Interventions	Patients receive Vitamin D3 10,000 IU daily for 5 or 14 days depending on VitD baseline level. They then undergo PDT for treatment of actinic keratosis.

## ABBREVIATIONS

CCCC	Case Comprehensive Cancer Center
CRF	Case Report Form
CRU	Clinical Research Unit
DSTC	Data Safety and Toxicity Committee
FDA	Food and Drug Administration
ICF	Informed Consent Form
IRB	Institutional Review Board
PRMC	Protocol Review and Monitoring Committee
SOC	Standard of Care
CCF	Cleveland Clinic Foundation
UH	University Hospitals
AK	Actinic Keratosis

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## 1.0 Introduction

Evidence accumulated over the past few years has led to an interesting new hypothesis, namely, that certain pharmacological and hormonal factors that promote terminal differentiation within skin cancer and precancer cells can also improve the responsiveness of these lesions to PDT. One such regulatory factor is vitamin D (VitD). The other is 5-fluorouracil (5FU), an FDA-approved topical agent that is used routinely to treat squamous precancers of the skin. This is a proposal to evaluate the use of vitamin D supplementation as a neoadjuvant for Photodynamic Therapy (PDT) in the treatment of adults with actinic keratoses, as well as measure biomarkers that are relevant to VitD and 5FU metabolism that might be predictive of PDT outcome. We will test a hypothesis (based upon our extensive preclinical studies in mice) that induction of a transient spike in serum Vitamin D levels through administration of dietary Vitamin D (cholecalciferol; D3) can significantly increase PDT efficacy and improve the clearance of actinic keratoses.

The biomarkers to be examined include serum VitD levels at the time of PDT, and the presence/absence of gene alleles that correlate with expression of several proteins involved in VitD and 5FU metabolism. The latter are allelic variants of the VitD receptor (VDR); 1-alpha-hydroxylase (CYP27B1); and thymidylate synthase. The presence of these biomarkers will be correlated to the improvement in AK lesion counts at the patient's routine follow-up visit 3-6 months after PDT treatment. Potential benefits of the research could be the development of a combination VitD-PDT regimen with improved efficacy, as well as the development of a simple test that predicts which patients will respond well vs. respond poorly to PDT, and who might benefit from nutritional correction with VitD supplements or from pre-administration of topical 5FU prior to PDT. The blood collection and assays will be paid for by NIH grant R01 CA204158 (Dr. Maytin, PI).

### 1.1 Background of Actinic Keratosis

Skin cancer is more common than any other human cancer. Cutaneous squamous cell carcinoma (SCC), the second most common form of skin cancer, has an overall metastasis rate of 2% to 3%, but can have a metastasis rate as high as 30% to 40% depending on the location, size, depth, invasion, and immunosuppression<sup>1</sup>. Squamous cell carcinoma primarily develops from precursor lesions called *actinic keratoses* (AKs) that typically affect fair-skinned individuals and are associated with excessive sun exposure<sup>2</sup>. Although surgical removal with cryosurgery (liquid nitrogen) is an effective, standard-of-care treatment for AKs, this treatment can leave unsightly scars and can be difficult for large numbers of AKs. Photodynamic therapy (PDT) is an effective new alternative for AKs, especially when treating large areas of field cancerization<sup>3</sup>.

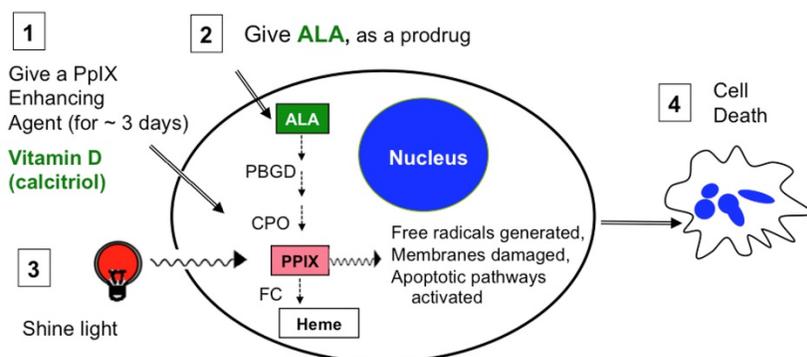
### 1.2 Photodynamic therapy (PDT)

PDT is a technique that combines a photosensitizing drug and an intense light source to kill tumor cells (reviewed in [1-4]). In the most commonly-used type of PDT, **5-aminolevulinic acid (5-ALA; ALA;** a precursor of porphyrin synthesis within cells) is administered to cause selective enhancement of protoporphyrin IX (PpIX) synthesis within tumor cells. PpIX is a porphyrin intermediate that strongly absorbs visible light; in other words, PpIX is the actual photosensitizer, whereas ALA is a pro-drug. The methyl ester of ALA, methyl-aminolevulinate

(MAL), can also be used as a pro-drug; MAL is cleaved to ALA within the cells. ALA is converted into PpIX through a number of enzymatic steps, whereupon PpIX can absorb photons of visible light, creating highly reactive singlet oxygen species that damage membranes and kill cells [2]. Killing is tumor-specific because ALA is selectively taken up and converted to PpIX at a higher rate in neoplastic cells than in normal tissues. To activate the PpIX, visible light (between 400 nm and 700 nm wavelengths) is employed because the PpIX molecule contains several absorption peaks in this range that can be used to advantage. In the United States, there are only two FDA-approved light sources currently available. By far the most common is a *blue light* source, Blue-U™, 400 nm) which targets the main PpIX absorption peak called the "Soret band", also shared with hemoglobin. A *red light* source (RhodoLED™, 635 nm) was also recently approved.

The **main advantage** of PDT with either light source is the fact that PDT, unlike surgical excision or ionizing radiation, can eliminate skin cancer and precancers without scarring. In fact, PDT has been shown to exert anti-fibrotic and anti-scarring effects [5-7].

Over the past ten years, the Maytin laboratory has shown that when squamous cancer cells in animal models are driven to a more terminally differentiated state by exposing them to certain differentiation-promoting agents, including 5-fluorouracil and Vitamin D, the cancer cells become much more sensitive to PDT [4, 8, 9]. The current proposal aims to test this hypothesis using oral Vitamin D as a neoadjuvant with PDT of actinic keratoses in humans.



**Figure 2.** Principle of differentiation-enhanced PDT of epithelial cancer.

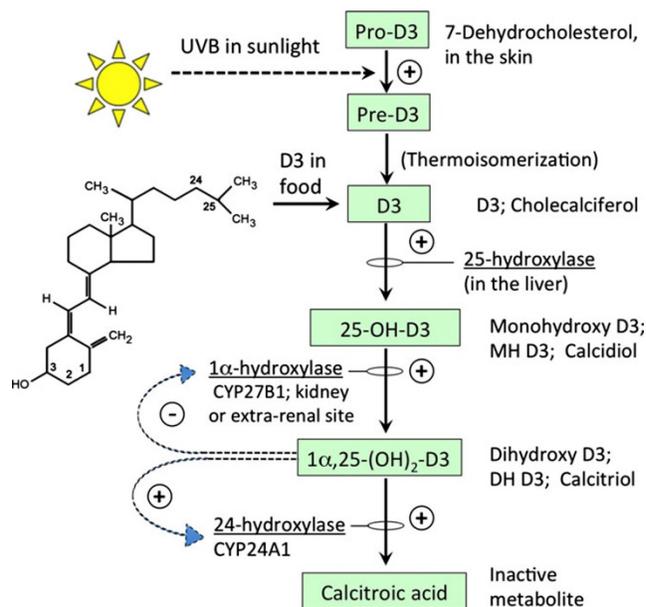
### 1.3 Combining differentiation-enhancing agents with PDT to improve outcomes.

Starting over a decade ago, our group initiated preclinical efforts to enhance ALA-PDT efficacy, not by trying to produce better light sources to improve ALA delivery (already well-optimized), but instead by considering the biology of the NMSC tumor cell. Our fundamental observation was the discovery that production of the PS (PpIX) in epithelial cancer cells can be affected by agents that enhance epithelial differentiation and change the activity of the heme-synthetic pathway (**Fig. 2**). Preconditioning regimens using methotrexate (MTX), 5-fluorouracil (5FU), or vitamin D (VD) improve selective accumulation of PpIX within squamous tumors, rendering the cancer cells more susceptible to PDT-mediated killing while sparing normal tissues; reviewed in [4]. To translate these findings for the first time in the clinic, in 2018 we completed a clinical study of one combination regimen (neoadjuvant 5FU and MAL-PDT) for actinic keratoses (AK) of the face and scalp (ClinicalTrials.gov NCT01525329). When one half of the face was pretreated with 5FU, and the other half was left untreated prior to MAL application and illumination with red light, the results showed nearly a 2-fold clinical benefit in terms of lesion clearance [10]. Although the 5FU combination approach was advantageous for many patients

with actinic keratoses, some 5FU non-responders were seen in our trial. Therefore, not everyone benefits from this particular combination. In fact, therapeutic responses to any combination PDT regimen will likely be patient-specific, and therefore it is very important to have several different treatment options available.

#### 1.4. Vitamin D (VD) as a promising option to enhance PDT.

Vitamin D is a very interesting alternative to other PDT-enhancing agents. 5FU and MTX both act by targeting DNA synthesis, whereas VD acts by regulating multiple physiological targets. As shown in Fig. 3, vitamin D3 (*D3*; cholecalciferol) is a natural pro-hormone produced in the skin via a reaction catalyzed by sunlight. It can also be obtained from the diet. *D3* undergoes sequential addition of two hydroxyl groups, first in the liver and second in the kidney. The final active form, calcitriol (1,25diOH-*D3*) binds to the vitamin D receptor (VDR), and the liganded receptor binds to DNA in the nucleus to regulate the expression of multiple target genes, including those that regulate calcium metabolism, growth, and differentiation [11]. Our preclinical data show that calcitriol can also stimulate changes in gene expression that result in increased ALA-mediated PpIX production in skin tumors.



**Figure 3.** Regulation of enzymes that lead to production of calcitriol. The natural agent, *D3*, is made in the skin during sun exposure, or obtained from the diet. *D3* is hydroxylated once in the liver, and again in the kidney. Activity of 1α -hydroxylase (CYP27B1) is highly regulated in the kidney but not in extrarenal tissues or in tumors. Calcitriol downregulates its own production by inhibiting CYP27B1, and upregulates its degradation by increasing the enzyme CYP24A1. [12]

In our early preclinical work in mice, we used the active form of VD (calcitriol) to elicit a beneficial enhancement of PDT for skin cancer. However, a problem with calcitriol is its inherent risk of inducing *hypercalcemia*. If one could use natural *D3* instead, we reasoned, then the risk of driving up serum calcium levels would be obviated. Also, approval by the FDA would not be required since *D3* is not a drug. However, we first had to show that dietary *D3* can work, which is not at all obvious when one considers the many steps in VD metabolism that are highly regulated (**Fig. 3**). Normal serum levels of *calciol* (the form of VD measured in patients) are typically 1,000-fold higher than serum levels of *calcitriol*, underscoring just how tightly the production of calcitriol is regulated [4]. One cannot simply assume that giving oral *D3* supplements will raise calcitriol levels. However, an important notion suggested by the Feldman group at Stanford is that many tumors make their own 1α -hydroxylase, allowing them to convert calciol to calcitriol directly within tumor tissues [13, 14]. To test whether dietary *D3* can

circumvent normal VD regulation and drive enhanced ALA-mediated PpIX production in skin tumors, we performed experiments using oral D3 supplementation in SCC-bearing mice. The results were highly positive and showed significant PpIX induction within tumors [12]. Therefore, we believe it is now time to move into the clinic to perform clinical trials that can establish oral D3/PDT as a new combination therapy for skin cancer. VD represents a potentially excellent neoadjuvant for clinical PDT for two reasons. First, our preclinical data in cell and animal models suggest a large beneficial effect. Second, VD is by far the safest of the three agents we have identified as PDT-enhancers. Methotrexate is a systemic drug with potential liver toxicity. 5FU is a systemic drug used for colon cancer and as a topical agent for early SCC, but may not penetrate thick tumors. Oral D3, by comparison, is very safe and should provide deep delivery to nodular BCC or moderately thick SCC *in situ*. Preliminary data to support these assertions are offered in the next section.

In this clinical trial, we propose to administer natural vitamin D to AK patients prior to PDT, in order to potentially improve their therapeutic response to PDT. After informed consent, we will collect a sample of blood from the patients to do two types of measurements, as follows. (1) serum levels of 25-hydroxy-vitamin D3 (25OH-D3; calcidiol), the vitamin D intermediate routinely measured in clinical assays. Calcidiol is a surrogate for the final active form of vitamin D (1, 25-diOH-D3; calcitriol); calcitriol is the hormonal form that binds to the VDR and mediates most of vitamin D's physiological actions, including upregulation of PpIX synthesis within tumor cells [8]. (2) DNA from the patients' leukocytes (white blood cells) to test biomarkers that have been reported to predict the activity of two proteins that regulate Vitamin D synthesis and hormonal activity, namely, 1-alpha-hydroxylase (CYP27B1) and Vitamin D Receptor (VDR), respectively. Details about the PCR-based assays for these biomarkers are discussed in Section 10 (Correlative/Special Studies).

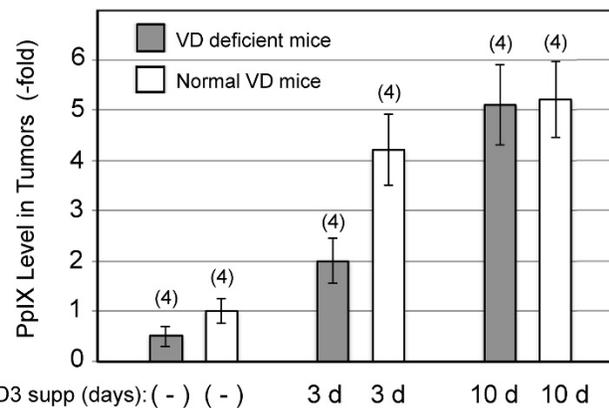
### **1.5 Preliminary data in mice, supporting the idea that VD status affects PDT outcomes and that neoadjuvant VD effectively boosts PpIX levels in precancerous and cancerous lesions**

Our clinical studies are based upon the Maytin laboratory's preclinical work over the last decade [4], showing that epithelial tumors are more responsive to ALA-based PDT when "primed" by pre-exposure to VitD. Cancer cells in culture[15] and NMSC tumors in mice[8] were shown to produce more PpIX and more photoactivation (tumor cell killing) if first exposed for ~3 d to calcitriol. Our enthusiasm for this approach was temporarily dampened because calcitriol, if overdosed, can induce hypercalcemia. However, we have now shown that D3 (the natural dietary form of VitD, which carries little to no hypercalcemic risk) can selectively elevate PpIX levels in murine NMSC tumors as efficiently as systemic calcitriol [12]. Based upon these preliminary data, clinical studies in the current proposal are designed to test the hypothesis that oral D3 supplements, administered over a relatively short time, can boost the effectiveness of ALA-PDT for NMSC in human patients. In mice, we have now shown that D3 (the natural dietary form of VD, which carries little to no hypercalcemic risk) can selectively elevate PpIX levels in murine skin tumors as efficiently as systemic calcitriol [12]. Based upon these preliminary data, clinical studies in the current proposal are designed to test the hypothesis that oral D3 supplements, administered over a relatively short time, can boost the effectiveness of ALA-PDT for NMSC in human patients. In addition, because the majority of people in the U.S. are VD deficient, we will

assess whether or not a patient's VD status (deficient, normal, or replete) can predict therapeutic responsiveness to ALA-PDT.

It has long been thought that VD may be beneficial for suppressing carcinogenesis, an important factor in the development of precancerous and cancerous lesions. For example, in an oral carcinogenesis protocol, VD receptor (VDR) knockout mice developed more BCC than their wild type littermates [16]. BCNS patients tend to be VD deficient, which may hasten BCC development [17]. BCC tumor cells harbor the VDR [18, 19]. The latter fact may be useful if using VD to stimulate PpIX production requires the VDR (which we hope to partially validate by collecting patients' leukocyte DNA and examining VDR gene sequences directly).

Other preliminary data using **oral D3 supplementation** in mice with skin tumors (**Fig. 4**) indicate that VD-deficient individuals may be significantly less responsive to ALA-PDT than subjects with normal VD levels. In tumor-bearing mice that were rendered VD deficient on a D3 free diet (as verified by 25OH-D3 serum assay), the administration of ALA to induce PpIX synthesis in tumors produced only 50% of the usual amount of PpIX (Fig. 4; 1<sup>st</sup> and 2<sup>nd</sup> bars). In the VD deficient mice, a short course of neoadjuvant D3 (10,000 IU/d of a human equivalent dose) for 3 d failed to boost intratumoral PpIX production to the same extent as in VD replete mice (Fig. 4; 3<sup>rd</sup> and 4<sup>th</sup> bars). However, if the course of neoadjuvant D3 was lengthened to 10 d, then both the VD-deficient and VD-normal mice experienced the same benefit, in terms of similar increases in PpIX levels (Fig. 4; 5<sup>th</sup> and 6<sup>th</sup> bars). These data inform our selection of conditions for D3 supplementation in the clinical trials. We will give a neoadjuvant D3 boost to patients with AKs, prior to ALA-PDT, that is tailored to their initial baseline VD levels. Thus, patients with normal VD levels will take D3 pills for 5 days, whereas patients with low VD levels will take D3 for 14 d.



**Figure 4.** Effect of VD deficiency upon production of PpIX photosensitizer in skin tumors. *Nude* mice were made VD deficient by feeding for 4-weeks with a no-D3 diet (gray bars); controls ate a normal diet (white bars). Serum levels of 25OH-D3 in the VD-def mice dropped to  $3 \pm 2$  ng/mL, compared to  $25 \pm 4$  ng/mL in normal mice (data not shown). SCC cells (A431) were implanted s.c. and allowed to grow for 10 d. The neoadjuvant D3 regimen (oral D3, using a 10x high D3 diet) was given for 3 d or for 10 d, immediately prior to giving ALA by i.p. injection. Four hours later, tumors were harvested for PpIX analysis by confocal fluorescence microscopy of frozen tumor sections.

### 1.6 Preliminary data in humans suggest that PDT can be enhanced by supplementing with Vitamin D.

There is good reason to think that endogenous VD levels are important for growth and development of NMSC. Literature data show that low 25OH-D3 levels correlate with higher cancer risk in human populations, with evidence being fairly convincing for BCC [17, 20, 21], but still understudied for SCC [22]. Vitamin D may have a protective effect against UVB-induced damage by preventing formation of cyclobutane dimers in DNA [23-25] and by inducing DNA repair enzymes [26]. Many of these effects are mediated through the VD receptor (VDR),

since VDR knockout mice are more susceptible to developing skin cancers, both SCC [27] and BCC [16]. Questions remain about whether all VDR-mediated effects are dependent upon calcitriol or other VD metabolites [27, 28]. Nevertheless, the VDR appears important as a suppressor of skin cancer development. We postulate that skin cancers in patients with low VD levels may be harder to cure with PDT than in patients with normal VD levels, due to lower activity of the heme synthesis pathway, or if patients have a variant of VDR which is less transcriptionally active.

Recently, an association was found between VDR polymorphisms (allelic variants of the VDR gene) and cancer risk. Epidemiologic approaches have shown a strong correlation between VDR polymorphisms and the risk for melanoma and nonmelanoma skin cancer development [29, 30]. In a paper from 2001 by Whitfield et al., the variants of the VDR were studied. The FokI polymorphism is frequently associated with nonmelanoma skin cancer and is known to alter the function and expression levels of the VDR[31]. The most common FokI polymorphism, which we define as the F allele, is a protein of 424 residues encoded from DNA on exon II. A single nucleotide substitution in FokI, three residues upstream from the F allele start site, creates a new translation start site that encodes a protein of 427 amino acids. We define this as the f allele. Individuals with the ff, Ff, and FF have the least, intermediate, and most active vitamin D receptor transcriptional activity, respectively. The most active VDR's had 7-fold greater activity in comparison to the low activity receptors. The FokI polymorphisms interact with another polymorphic site (L/S), which also alters the transcriptional activity of the VDR. This other polymorphic site has adenosine (A) repeats in the 3'-UTR of the VDR gene[32]. This stretch of DNA varies widely, but can be divided into long (L) and short (S) groups. The long (L) group contains 17-24 adenosine repeats. The short (S) group contains 10-15 adenosine repeats. VDR variants with the LL genotype had around 8-fold higher transcriptional activity than the SS variants. When VDR variants with the FF\*LL genotype were compared to the variants with the ff\*SS genotype, a 10-15 fold increase in transcriptional activity was found[31].

### **1.7. Rationale for the experimental design of our proposed trial**

This is a phase 2 clinical trial of patients with actinic keratosis (AK) that will examine a combination regimen of Vitamin D plus PDT, and compare its efficacy to controls who underwent PDT alone in a prior study from the Maytin group (IRB 16-1615). Patients will be enrolled at the Cleveland Clinic. Each patient will be matched to patients from the prior study based upon baseline calcidiol levels.

In order to assess the accumulation of protoporphyrin IX in the skin, a noninvasive fluorescence dosimeter will be used to measure PpIX accumulation in areas of both actinic damage and normal skin[33]. Serum levels of calcidiol, a routine clinical marker of vitamin D status, will be measured in each patient in order to match each patient with a matched control patient. The overall outcome of the Vitamin D/PDT regimen will be evaluated against the control group by finding the percent reduction of AK lesions at the 3-month follow-up visit. Patient blood samples will be taken to determine assess the relationship between VDR polymorphisms and treatment response in patients undergoing photodynamic therapy for AK.

## 2.0 OBJECTIVES

The main objective of this proposal is to determine whether oral vitamin D3 supplements, used as a neoadjuvant for PDT, can be used to enhance PDT treatment of AKs. Our primary hypothesis is that prior supplementation (5 or 14 days) with 10,000 IU of Vitamin D3 enhances the efficacy of photodynamic therapy (PDT) in the treatment of actinic keratoses (AK) by increasing the accumulation of protoporphyrin IX (PpIX) inside the precancerous cells, leading to increased photoactivation during PDT. A sub-hypothesis is that it may be possible to predict which patients will respond to the neoadjuvant VitD, based upon the allelic status of their VDR. Our long-term goal is to develop a novel and practical way to make PDT more effective in the treatment of AKs and perhaps other nonmelanoma skin cancers as well.

### 2.1 Primary Objective

- **Determine whether acute supplementation (neoadjuvant Vitamin D3), adjusted according to baseline Vitamin D status, can improve the clinical PDT response relative to patients receiving PDT alone.**

#### **Aim 1: Does prior Vitamin D supplementation increase the effectiveness of PDT for the treatment of actinic keratoses (squamous cell carcinoma precursor lesions)?**

Patients who come to the dermatology clinic for PDT of facial AK will be invited to enroll in a prospective clinical trial in which they will receive a brief (5-day or 14-day) supplementation with 10,000 IU of Vitamin D, prior to receiving aminolevulinic acid (ALA)- blue light PDT. A baseline vitamin D (calcidiol) level will be taken for each patient. Patients will be matched to other patients from prior studies involving PDT only, based on their baseline vitamin D level.

**Subaim 1a:** Does prior supplementation with 10,000 IU of Vitamin D increase the accumulation of protoporphyrin IX (PpIX) within actinic keratoses after application of ALA solution prior to illumination with blue light PDT?

Thirty minutes after applying the photosensitizing agent, ALA, a fluorescence dosimeter will be used to measure PpIX accumulation in areas of both actinic damage and normal skin<sup>17</sup>. The level of calcidiol, a clinically accepted marker of vitamin D status, will be measured in each patient.

**Subaim 1b:** Does prior supplementation with 10,000 IU of Vitamin D enhance the clinical clearance of actinic keratoses after PDT?

Percent reduction of actinic keratoses at a 3-month follow-up visit will be used to evaluate the overall outcome of PDT with and without prior Vitamin D supplementation.

### 2.2 Secondary Objective(s)

- **Determine whether gene polymorphisms in VDR and CYP27B1 are predictive for the degree of responsiveness to Vitamin D as a neoadjuvant for PDT.**

#### **Aim 1: Is there a correlation between Vitamin D receptor (VDR) polymorphisms and PDT treatment outcomes for actinic keratoses with and without prior Vitamin D supplementation?**

Leukocyte DNA will be collected to assess the relationship between VDR polymorphisms and treatment response in patients undergoing photodynamic therapy for actinic keratoses. Patients

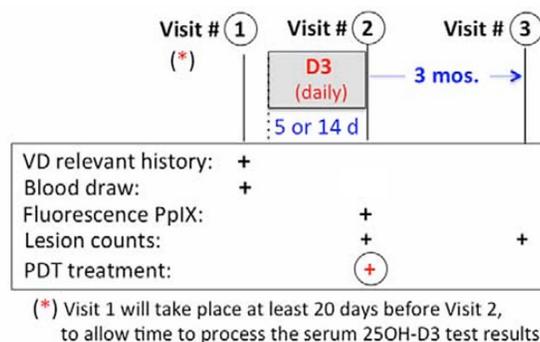
will be genotyped to determine the presence of F/f and L/S alleles. These findings will be correlated with the therapeutic responsiveness to ALA-PDT, with or without vitamin D enhancement.

These studies propose to test and demonstrate that vitamin D3 pretreatment can be used to enhance porphyrin accumulation and PDT efficacy in the treatment of AKs. PDT is approved by the FDA for the field treatment of widespread precancerous lesions of the skin. In Europe, PDT is also approved for the treatment of squamous cell carcinoma and basal cell carcinoma of the skin. We expect this clinical trial to support the foundation for a new treatment modality, vitamin D3-enhanced PDT, for the treatment of AKs and other nonmelanoma skin cancers. This data would benefit public health by demonstrating this approach to be a simple, safe, and effective treatment for AKs, as well as supporting its use as a nonscarring alternative to surgery for early squamous cell carcinomas and basal cell carcinomas.

### 3.0 STUDY DESIGN

#### 3.1 Study Design Protocol

This is a non-randomized interventional trial, in which the study group will be compared to a baseline cohort of patients from a previous study (IRB 16-1615) who received the same regimen of PDT, but without any Vit D. Our protocol is shown in Fig. 5. The dose of D3 chosen for this study reflects the best currently available evidence regarding efficacy, safety, and practicality of oral D3 dosing. In our mouse studies, the human equivalent of 10,000 IU given daily for 10 d, provided better PpIX enhancement than 5,000 IU/d x 10 d, with no evident toxicity [12]. In normal mice, 3 d of daily 10,000 IU were just as good as 10 d for boosting PpIX levels in tumors; however, the 3-day regimen worked only about half as well in VD-deficient mice (prelim data; Fig. 4). Importantly however, 10 d were sufficient to restore the PpIX elevating effect in VD deficient animals (Fig. 4). Therefore, we will tailor the neoadjuvant D3 dose in this clinical trial, according to initial serum 25OH-D3 levels, to maximize convenience and safety, while testing for equivalence of therapeutic benefit. Specifically, study patients with normal VD levels will take a daily 10,000 IU D3 pill for 3-5 d, while patients with suboptimal VD levels will take the D3 pill for 10-14 d. (Ranges are given to compensate for patients possibly missing a day or two). Regarding safety, the doses of D3 that physicians use to treat VD deficiency can be remarkably high, including 5,000 IU (oral, daily, continuous), 50,000 IU (oral, twice weekly for 5 weeks), and 500,000 IU (i.m., single dose); all are reported safe and effective[34]. Our dosing regimen is well within these safe ranges.



**Figure 5. Study Design.**

At **Visit 1**, patients will be given oral D3 to take for either 5 days or 14 days (according to their normal or deficient 25OH-D3 level, respectively) prior to the PDT treatment at **Visit 2**. Mandatory F/U visit at 3 months (**Visit 3**). After that, patients will be followed clinically every 6-12 months.

Study patients will have three mandatory visits. At Visit #1, patients will sign the ICF and have their blood drawn. A vitamin D-relevant history will be taken, and CIS lesions counted by a clinician. The patient will be given enough D3 pills (10,000 IU each) to be taken daily at home, beginning at either day -5 or day -14, as per their assignment (see above). At Visit #2, to assess how much PpIX is generated in the skin, ten lesions will be measured using our fluorescence probe dosimeter (before and after ALA application). PDT will then be performed. At Visit #3 (which is 3-6 months after PDT), the patient's lesions will be counted to assess the treatment response).

#### 3.2 Number of Subjects

A total of 30 patients with at least 10 AKs will be needed to complete the study.

### 3.3 **Replacement of Subjects**

If a study subject withdraws for any reason, up to 6 months after the start of this study, then a new subject can be recruited in his/her place.

### 3.4 **Expected Duration of Subject Participation**

#### 3.4.1 *Duration of Therapy*

The study consists of a single blood draw, to be collected by routine phlebotomy on the day of PDT treatment or within 1 week of the treatment.

The principal investigator reserves the right to temporarily suspend or prematurely discontinue this study. The date and reason for discontinuation will be documented. Every effort will be made to complete the appropriate assessments.

#### 3.4.2 *Duration of Follow Up*

Patients will be followed for 3 months, as specified in the study Calendar; see **section 11.0**. The treatment (PDT) will occur at Visit 2, followed at 3 months by a follow-up evaluation (within a window extending from 3-6 months after Visit 2) .

#### 4.0 PATIENT SELECTION

This section shows a checklist of criteria (sections 4.1 to 4.3) that must be met in order for a patient to be considered eligible for this study. A checklist with this information will be used to confirm a patient's eligibility. It will be completed for each patient, then signed and dated by the treating physician, and a nurse or physician unaffiliated with the principal investigator, as shown below.

PATIENT'S NAME \_\_\_\_\_

MEDICAL RECORD # \_\_\_\_\_

A) SIGNATURE OF PERSON DETERMINING ELIGIBILITY \_\_\_\_\_ DATE: \_\_\_\_/\_\_\_\_/\_\_\_\_  
mm dd yyyy

B) SIGNATURE OF PERSON CONFIRMING ELIGIBILITY: \_\_\_\_\_ DATE: \_\_\_\_/\_\_\_\_/\_\_\_\_  
mm dd yyyy

C) INVESTIGATOR SIGNATURE: \_\_\_\_\_ DATE: \_\_\_\_/\_\_\_\_/\_\_\_\_  
mm dd yyyy

#### 4.1 Inclusion Criteria

4.1.1 Patients can be males or females of at least 18 years of age. Patients need to have actinic keratoses in sufficient numbers (>10) to warrant PDT therapy in our clinic.

#### 4.1.2 *Female subjects must not become pregnant during the study*

The effects of 5-aminolevulinic acid (Levulan™) on the human fetus are unknown. For this reason, women of child-bearing potential must agree to use contraception (double barrier method of birth control or abstinence) prior to study entry, and throughout study participation. Should a woman become pregnant or suspect that she is pregnant while she is participating in this study, she should inform the treating physician immediately.

4.1.3 Subjects must be **able** to understand and **willing to sign a written informed consent document**.

#### 4.2 Exclusion Criteria

4.2.1 Pregnant or nursing.

4.2.2 At risk for hypercalcemia (renal disease, sarcoidosis, etc.)

4.2.3 Using topical retinoids, since these can exacerbate the post-PDT erythema reaction.

4.2.4 Using any topical treatment on their AKs; must stop at least one month prior.

4.2.5 Currently undergoing treatment for other cancers with medical or radiation therapy.

4.2.6 Patients with a known hypersensitivity to 5-aminolevulinic acid or any component of the study material.

4.2.7 Patients with history of a photosensitivity disease, such as porphyria cutanea tarda.

4.2.8 Currently participating in another clinical trial.

#### 4.3 Inclusion of Women, Children, and Minorities

Men and women at least 18 years of age of any ethnic group are eligible for this trial, as long as they fulfill the eligibility criteria.

## **5.0 REGISTRATION**

### **5.1 Recruitment**

Patients who have been scheduled to receive PDT in our dermatology clinics will be given written information prior to their visit, including a copy of the Informed Consent (IC) that describes the purpose of the study. If the patient indicates that he/she is interested, the physician or study nurse will review the IC with the patient on the day of PDT and answer all questions. After the patient signs the IC, the patient will be directed to the phlebotomy lab in the Crile building (Crile A1) to have blood drawn.

### **5.2 Consent:**

When a patient is referred for the study, he/she will be given information about the study and all its components. An Informed Consent will be mailed to the patient at least 72 h prior to the first visit. If the patient continues to be interested in the study, he/she will be scheduled for the first visit in Dermatology. Once the patient arrives in clinic, the study will be explained once again. If the patient indicates understanding and wishes to enroll in the study, he will be asked to sign the consent form with the understanding that he can change his mind at any time during the study. The informed consent must be signed by the patient, and by a parent or legal guardian if the patient is a minor, and witnessed. The participant will be given contact phone numbers to call if questions arise as the study proceeds.

### **5.3 Randomization:**

There will be no randomization procedures.

## **6.0 TREATMENT PLAN**

**6.1. VISIT 1:** As part of the Informed Consent process, the patient will be seen in the clinic for a full screening visit (**Visit 1**), including a review of medical records and a physical exam. Informed Consent will be obtained on-site. AK lesions will be carefully evaluated to determine if the patient meets eligibility criteria (> 10 AK lesions). A study nurse from the CRU will draw two tubes of blood, one for serum D3 assay, the other for leukocyte collection (buffy coat) and preparation of DNA. The patient will be sent home after scheduling the date of the next visit, in approximately 1 month.

**Evaluation procedures at Visit 1:** Blood will be collected in two tubes: one with an anticoagulant (e.g. heparin) and the other without. The nonheparinized tube will be sent to CCF Clinical Laboratory Medicine for routine measurement of vitamin D (calcidiol) serum levels. The heparinized tube will be transported to Dr. Maytin's laboratory for preparation of leukocyte DNA (see Procedure B).

**VISIT 2: Treatment procedure:** The patient will return on the treatment day, and all AK lesions will be carefully marked with a water-soluble pen, photographed and mapped. PDT will then be conducted as per our routine procedure, as follows. The prodrug (5-aminolevulinic acid, 5-ALA; Levulan® Kerastick™, 20% Topical Solution) is applied to the entire involved area of skin, allowed to dry, and left on the skin surface for a period of time between 15 to 30 min (for AK of the face and scalp). During that time, the 5-ALA prodrug is selectively absorbed by preneoplastic cells and is converted within the cells' mitochondria into protoporphyrin IX (the molecule that constitutes the actual phototarget). The patient is positioned under the light source (Blu-U, 400 nm wavelength), wearing protective yellow goggles, and is illuminated for 30 min. Upon completion of PDT, aftercare steps are explained to the patient, and then the patient is sent home with emollient samples and written aftercare instructions. A questionnaire of side effects during the 6 days post-PDT will be filled out at home.

**Visit 3: Final evaluation visit (3 months post-PDT):** AK lesions will be counted and photographed.

### **6.2 Duration of Therapy**

The target duration of therapy **3 months**. In the absence of treatment delays due to adverse events, treatments may administered unless one of the following criteria applies:

- (i) Intercurrent illness prevents administration of treatment.
- (ii) The investigator considers it, for safety reasons, to be in the best interest of the patient.
- (iii) Unacceptable adverse event(s), including: general or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator; patient decision to withdraw from treatment (partial consent) or from the study (full consent); pregnancy during the course of the study for a child-bearing participant; death. The Principal Investigator reserves the right to temporarily suspend or prematurely discontinue this study.

### **6.3 Duration of Follow Up**

Patients will be followed for 3 months, as specified in the study Calendar; **section 11.0**. The single treatment will occur at Visit 2, at 3 months prior to the last visit. No formal study follow-up is planned after completion of 3 months, but every effort will be made to encourage patients to return at 6 month intervals for 2 years for routine examination, at which time will document AK status (clearance/recurrence) in our PDT database registry, and provide additional standard-of-care as needed.

## **7.0 DOSING DELAYS / DOSE MODIFICATIONS**

In photodynamic therapy, two types of dosing must be considered: (1) the dose of prodrug; (2) the dose of light. The prodrug, Levulan™, is a liquid preparation with a fixed concentration of 20% aminolevulinic acid (ALA) that is delivered topically as a uniform film that dries upon application to the skin. The delivered dose of ALA cannot be changed, but the effective concentration of the photosensitizer (PpIX) in the skin can be controlled by shortening or lengthening the incubation time. Once ALA has been converted into PpIX within the tumor cells, PpIX is activated by shining the light (either blue or red wavelengths). Photons are delivered from an FDA-approved light source (Blu-U™ for blue light at 400 nm; Aktelite™ for red light at 635 nm) at a fixed dose rate, measured in Joules/m<sup>2</sup>/sec. The product of the dose rate and the illumination time gives the final light dose. The light dose that is safe and effective for treatment of actinic keratosis using a continuous blue light illumination protocol is 20 J/cm<sup>2</sup> (Kaw, Maytin et al, J Am Acad Dermatol, 2019, in press). In the proposed study, all AKs will be treated with blue light at 20 J/cm<sup>2</sup>, following a standardized ALA incubation time of 30 minutes prior to illumination.

## **8.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS**

The following is a list of AEs (Section 8.1) and the reporting requirements associated with observed AEs (Sections 8.3 and 8.4).

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

### **8.1 Adverse Events and Potential Risks**

#### **8.1.1 *Levulan<sup>TM</sup>***

ALA is a natural component found in all cells in the human body. The application of ALA mixed in an alcohol-based vehicle (Kerastick) is not associated with any risks or side effects.

#### **8.1.2 *Photodynamic Therapy***

When ALA is converted into PpIX in tumor cells, and illuminated with light, the following signs and symptoms are known to occur as part of the therapeutic response:

- (i) Stinging and/or burning sensation: >50%
- (ii) Erythema (localized redness): 35%
- (iii) Edema, localized: >35%
- (iv) Peeling, transient: <50%
- (v) Blister formation: rare

#### **8.1.3 *Photodynamic Therapy response (with local cutaneous effects):***

The constellation of transient local symptoms of stinging and/or burning, itching, erythema and edema as a result of LEVULAN KERASTICK Topical Solution plus BLU-U treatment was observed in all clinical studies of LEVULAN KERASTICK for Topical Solution Photodynamic Therapy for actinic keratoses treatment. Stinging and/or burning subsided between 1 minute and 24 hours after the BLU-U Blue Light Photodynamic Therapy Illuminator was turned off, and appeared qualitatively similar to that perceived by patients with erythropoietic protoporphyria upon exposure to sunlight. There was no clear drug dose or light dose dependent change in the incidence or severity of stinging and/or burning.

#### **8.1.4 *Extra-cutaneous adverse experiences reported:***

In Phase 3 studies, no non-cutaneous adverse events were found to be consistently associated with LEVULAN KERASTICK Topical Solution application followed by blue light exposure. No clinically significant patterns of clinical laboratory changes were observed for standard serum chemical or hematologic parameters in any of the controlled clinical trials.

#### **8.1.5 *Safety in pregnancy:***

No carcinogenicity testing has been carried out using ALA. No evidence of mutagenic effects was seen in four studies conducted with ALA to evaluate this potential. No assessment of effects of ALA HCl on fertility has been performed in laboratory animals. It is unknown what effects

systemic exposure to ALA HCl might have on fertility or reproductive function. Therefore, Levulan is considered as Pregnancy Category C.

## 8.2 Definitions

### 8.2.1 *Adverse Events*

An **adverse event** (AE) is any unfavorable or unintended event, physical or psychological, associated with a research study, which causes harm or injury to a research participant as a result of the participant's involvement in a research study. The event can include abnormal laboratory findings, symptoms, or disease associated with the research study. The event does not necessarily have to have a causal relationship with the research, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of the interventions and interactions used in the research; the collection of identifiable private information in the research; an underlying disease, disorder, or condition of the subject; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject. In general, adverse events that are at least partially the result of (a) or (b) would be considered related to the research, whereas adverse events solely related to (c) or (d) would be considered unrelated to the research.

**External adverse events** are adverse events experienced by subjects enrolled in multicenter clinical trials at sites other than the site(s) over which the Institutional Review Board has jurisdiction.

**Internal adverse events** are adverse events experienced by subjects enrolled at the site(s) under the IRB's jurisdiction for either multicenter or single-center research projects.

8.2.2 **The significance of an adverse event** is used to describe the patient/event outcome or action criteria associated with events that pose a threat to a patient's life or functioning (i.e., moderate, severe or life threatening). Based on the National Cancer Institute Guidelines for the Cancer Therapy Evaluation Program, severity can be defined by the following grades of events:

**Grades 1** are mild adverse events. (e.g., minor event requiring no specific medical intervention; asymptomatic laboratory findings only; marginal clinical relevance)

**Grades 2** are moderate adverse events (e.g., minimal intervention; local intervention; non-invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

**Grades 3** are severe and undesirable adverse events (e.g., significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

**Grades 4** are life threatening or disabling adverse events (e.g., complicated by acute, life-threatening metabolic or cardiovascular complications such as circulatory failure, hemorrhage, sepsis; life-threatening physiologic consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure, therapeutic endoscopy or operation).

**Grades 5** are fatal adverse events resulting in death.

### 8.2.3 *Serious Adverse Events*

A *serious adverse event* (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:

- Results in *death*.
- Is a *life-threatening* adverse experience. The term life-threatening in the definition of serious refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- Requires *inpatient hospitalization or prolongation of existing hospitalization*. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
  - The admission results in a hospital stay of less than 12 hours OR
  - The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study) OR
  - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care).

However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of “medically important” and as such may be reportable as a serious adverse event dependant on clinical judgment. In addition where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.

- Results in *persistent or significant disability/incapacity*. The definition of disability is a substantial disruption of a person’s ability to conduct normal life’s functions.
- Is a *congenital anomaly/birth defect*.
- Is an *important medical event*. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

### 8.2.4 *Expectedness*

Adverse Events can be Expected or Unexpected.

*An expected adverse event* is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event is usually listed in the Investigator Brochure, consent form or research protocol.

*An unexpected adverse event* is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

### 8.2.5 *Attribution*

Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study drug.
- Probable – The AE is likely related to the study drug.
- Possible – The AE may be related to the study drug.
- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

### **8.3 Reporting Procedures for All Adverse Events**

*This section is not yet determined, pending the outcome of our application to the FDA.*

All participating investigators will assess the occurrence of AEs throughout the subject's participation in the study. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's case report form, subject's medical records, and/or any other institutional requirement. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study), requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event.

The investigator will provide the following for all adverse events:

- Description of the event
- Date of onset and resolution
- Grade of toxicity
- Attribution of relatedness to the investigational agent
- Action taken as a result of the event
- Outcome of event

In this study, descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 available at <http://ctep.cancer.gov> will be utilized for AE reporting.

Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

### **8.4 Serious Adverse Event Reporting Procedures**

*This section may need revision if Sponsor or Funding Source of study has specific reporting requirements for trial, and/or if study is conducted under an IND.*

Serious adverse events that occur beginning with the signing of the informed consent, during treatment, or within 30 days of the last dose of treatment must be reported to the CCF Principal Investigator.

Investigative sites will report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting serious adverse events.

**8.4.1 Pharmaceutical Sponsor Reporting: N/A**

**8.4.2 FDA Reporting: N/A**

**8.5 Data Safety Toxicity Committee**

It is the Case Comprehensive Cancer Center's Principal Investigator's responsibility to ensure that ALL serious adverse events are reported to the Case Comprehensive Cancer Center's Data Safety Toxicity Committee. This submission is simultaneous with their submission to the Sponsor or other Regulatory body.

## 9.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the commercial agents administered in this study can be found in Section 8.0.

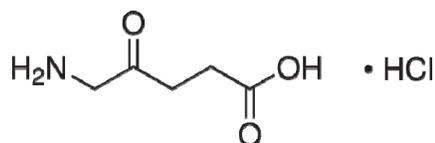
9.1. Levulan<sup>TM</sup> The following information about Levulan<sup>TM</sup> is cited from the package insert:

**Chemical Name:** 5-aminolevulinic acid (ALA)

**Other Names:** *delta*-aminolevulinic acid

**Classification:** Topical agent

**Molecular Formula:** The chemical name for ALA HCl is 5-amino-4-oxopentanoic acid hydrochloride (MW = 167.59). The structural formula is represented below:



**Mode of Action:** Photosensitization following application of LEVULAN KERASTICK Topical Solution occurs through the metabolic conversion of aminolevulinic acid to protoporphyrin IX (PpIX), a photosensitizer, which accumulates in the skin to which LEVULAN KERASTICK Topical Solution has been applied. The absorption of light results in an excited state of porphyrin molecules, and subsequent spin transfer from photoreactive porphyrins to molecular oxygen generates singlet oxygen, which can further react to form superoxide and hydroxyl radicals.

**Metabolism:** ALA is converted to PpIX within mitochondria. PpIX is then destroyed upon illumination with the incident light (see above).

**Product description:** LEVULAN® KERASTICK® (aminolevulinic acid HCl) for Topical Solution, 20%, a porphyrin precursor, contains the hydrochloride salt of aminolevulinic acid (ALA), an endogenous 5-carbon aminoketone. The LEVULAN KERASTICK for Topical Solution applicator is a two component system consisting of a plastic tube containing two sealed glass ampules and an applicator tip. One ampule contains 1.5 mL of solution vehicle comprising alcohol USP (ethanol content = 48% v/v), water, laureth-4, isopropyl alcohol, and polyethylene glycol. The other ampule contains 354 mg of ALA HCl as a dry solid. The applicator tube is enclosed in a protective cardboard sleeve and cap.

**Solution preparation:** The 20% topical solution is prepared just prior to the time of use by breaking the ampules and mixing the contents by shaking the LEVULAN KERASTICK applicator. The term “ALA HCl” refers to unformulated active ingredient, “LEVULAN KERASTICK for Topical Solution” refers to the drug product in its unmixed state, “LEVULAN

KERASTICK Topical Solution” refers to mixed drug product (in the applicator tube or after application), and “LEVULAN KERASTICK” refers to the applicator only.

**Storage requirements:** Levulan should be stored at room temperature, 20°-25 °C (68°-77 °F).

**Stability:** The ALA medication in a LEVULAN KERASTICK is stable for over 2 years, if unmixed. After mixing, the LEVULAN KERASTICK Topical Solution should be used immediately following preparation (dissolution). Solution application must be completed within 2 hours of preparation. An applicator that has been prepared must be discarded 2 hours after mixing (dissolving).

**Route of administration:** Levulan™ Kerastick will be applied to the skin, as directed by the manufacturer. Specifically, the glass ampules within the Kerastick will be crushed; the Kerastick shaken for 2 min to allow the powder to adequately dissolve in the vehicle; the liquid applied to the selected areas of skin using foam-tip applicator at the end of the Kerastick, and allowed to air dry for at least 3 min. The study subject will be advised to avoid sunlight until he/she will return to the clinic for the illumination phase of the treatment.

**Drug Procurement:** Levulan™ Kerastick will be purchased from Sun Pharmaceuticals.

**Drug Accountability:** The investigator or designated study personnel are responsible for maintaining accurate dispensing records of the study drug. All study drugs must be accounted for, including study drug accidentally or deliberately destroyed. Under no circumstances will the investigator allow the investigational drug to be used other than as directed by the protocol. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

**Drug Destruction:** Levulan Kerastick™ can be used only once after mixing. Immediately after application to the patient's skin, the remaining Kerastick will be discarded.

### 9.2. **Cholecalciferol (Vitamin D3 supplements)**

While D3 is not a drug, we describe it here to clarify its source and handling. A large batch of D3 tablets, 10,000 IU per tablet, will be purchased from a commercial source (e.g., NatureMade from Costco, Inc.) and several tables will be sent for testing at a commercial analysis facility to verify the cholecalciferol content (Heartland Assays, Ames, Iowa). These tablets will be stored in the CCF Research Pharmacy, under temperature-controlled conditions.

[NOTE: The CCF Research Pharmacy, under guidance of John Petrich RPh, will also create inert pills made of inert methylcellulose for use as placebo in this randomized study].

### 9.3. **Light Sources for PDT**

#### **Blue light source: (Blu-U):**

This lamp is FDA-approved, and used routinely in our dermatology clinics.

#### **Noninvasive Fluorescent Measurement Device (NIR):**

This custom-made fluorescent detector, consisting of a safe, low-energy HeNe laser (equivalent to a classroom pointer) and an optical detector to collect weak red fluorescence, was IRB-approved and routinely used in our CCF IRB protocol, 09-1050.

## 10.0 CORRELATIVE / SPECIAL STUDIES

### 10.1 *Processing of blood for serum 25-hydroxy-vitamin D3 measurements.*

#### **Rationale:**

Here we will address the question of whether a patient's overall vitamin D status (as measured by 25OH-D3 serum levels) can affect PpIX production within skin tumors, and the subsequent clinical response to PDT. A large proportion of people in northern climates are VD deficient, especially in winter, as diagnosed by low serum 25OH-D3 levels [35, 36]. There is high variability in the results of different studies, due to multiple factors including sunlight/season, age, ethnicity, and laboratory technique, and to the endpoints being used (bone loss; PTH levels, etc.). Thus, controversy exists about how to define low VitD levels using terms such as "deficiency", "insufficiency", etc. However, most authorities agree that a 25OH-D3 level above 20 ng/mL is required for normal health, with the range of 20 – 60 ng/mL being acceptable to avoid VitD toxicity [35, 36]. In our study, we will use 20 ng/mL as our threshold to define normal Vit D levels. Patients with a suboptimal level (below 20 ng/mL) will be classified into two groups, either moderately deficient (10-19 ng/mL), or severely deficient (<10 ng/mL) for analytical purposes. Thus, the patients' 25OH-D3 levels will be grouped in tertiles (severely deficient, moderately deficient, or optimal) when interpreting the relationship between VitD level and PDT treatment response.

#### **Method:**

The specimen required is 1 mL of blood, collected in a serum separator (gold) tube. The assay is a radioimmunoassay (RIA) performed in the Cleveland Clinic Institute of Pathology and Laboratory Medicine. Results are expected back in 3-6 days. Details are at CCF Guide to Laboratory Services, available on the CCF Intranet at <http://intranet.ccf.org/pathology>.

### 10.2 *Processing of blood for preparation of DNA from leukocytes.*

#### **Rationale:**

DNA sequence differences in the VDR may be useful as a biomarker to predict responsiveness to combination VitD/PDT in individual patients. In the past few years, excitement has been growing about an association between polymorphisms (allelic variants) in the VDR gene and an elevated cancer risk (including breast, prostate, colon, and skin cancer) [20, 21]. Over 300 studies on this topic are now listed in PubMed. Epidemiologic approaches show a strong correlation between VDR polymorphic alleles (named for DNA restriction enzymes that define the altered nucleotide sites) and the risk of melanoma [22] and nonmelanoma [23, 24] skin cancer development. For NMSC, studies have primarily implicated FokI, TaqI, BsmI, and ApaI as potentially important predictors. Overall, the FokI polymorphism is the most interesting, not only because it is very frequently associated with NMSC [24, 25], but also because it is one of the few polymorphisms known to alter VDR levels and function [26]. In their elegant 2001 paper, Whitfield et al. showed that Fok 1 interacts with a second polymorphic site (L/S) which together define a wide range of transcriptional activity of the VDR [31]. FokI detects a single nucleotide substitution (the f allele) in exon II that creates a new translation start site (encoding a protein of 427 amino acids) located three residues upstream from the usual start site (defined by the F allele, and yielding a protein of 424 residues). When 20 different human fibroblast lines were transfected with a VDR-responsive reporter gene ± calcitriol, and each cell line's DNA analyzed to correlate the genotype with VDR transcriptional activity, it was found that ff was the least active genotype, Ff was intermediate, and FF was the most active, over a 7-fold range of

transcriptional activity. The second element of this story involves adenosine (A) repeats in the 3'-UTR of the VDR gene, which vary widely in size between individuals but segregate into a "long" group (L) with 17-24 A's in the repeat, and a "short" group (S) with 10-15 A's in the repeat [31]. The VDR variants with an LL genotype were ~8-fold more active than VDR with a SS genotype. When F/f and L/S alleles were evaluated together, the FF•LL genotype was 10-15 fold more active than the f/f•SS genotype. We plan to perform genotyping of our patients' DNA to determine the status of the F/f and L/S alleles, and to correlate these findings with therapeutic responsiveness to ALA-PDT, ± D3 supplementation.

As an alternative (or additional) assay, we plan to look at single nucleotide polymorphisms (SNPs) in CYP27B1, the 1 $\alpha$ -hydroxylase enzyme that regulates 1,25diOH-D3 production. According to a study from the group at Univ. Arizona, four SNP variants of CYP27B1 (R107H, V166L, S356N, and V374A) show markedly reduced 1 $\alpha$ -hydroxylase activity, while one variant (V166L) shows increased activity. The presence of these allelic variants will be established by qPCR, to see whether they correlate with responsiveness to PDT.

#### **Method:**

The complete procedure for extracting blood for leukocyte DNA preparation is given in Appendix 1.

Briefly, blood will be collected in a 10 mL lavender top Vacutainer tube with EDTA. The phlebotomist in the outpatient Crile A61 will call Dr. Maytin's technician/designated staff to inform him/her that a specimen is waiting; the technician will then pick up the sample and transport it to Dr. Maytin's lab. The blood should stay at room temperature, and NOT be put on ice (which would activate platelets). A simple and reliable kit from Qiagen will be used to prepare the DNA. The first step involves adding a solution to lyse the cells and precipitated the proteins; after this stage, the tube can safely remain at room temperature for weeks prior to DNA extraction (see Appendix 1).

The analysis of DNA sequences using polymerase chain reaction (PCR) is a standard procedure in Dr. Maytin's laboratory. We will employ published DNA primer sequences, described in the publications listed in the Rationale for (B) given above.

#### **Safe storage of the patient samples**

- [Describe where samples will be stored, maintained and for how long.](#)

DNA samples will be stored in a locked minus 80 degree ultrafreezer, in Dr. Maytin's laboratory (room ND4-25A in the Lerner Research Institute). The samples will be maintained for up to 10 years.

- [Will samples contain subject identifiers or are names replaced with codes and a separate key?](#)

Blood sample tubes and data sheets in the laboratory will be labeled with a code consisting of the first 5 digits of the patient's 8-digit MRN along with the date that the phlebotomy was performed (i.e. the date of the blood draw). This should ensure anonymity of the data while preventing mistakes when linking the laboratory results to the correct patient. Study personnel with password-protected access to the database registry will use the code to unequivocally

identify the subject's file within the Oracle database and thereby enter the subject's laboratory results into the proper data field.

### **Procedure (C): Correlation of biomarker results to clinical outcomes (Database Registry)**

Clinical parameters will be extracted from the EPIC electronic medical record, for each study patient, at the treatment visit and the 3 month follow-up. The data fields to be used in this study are identical to items already IRB-approved in our established PDT database (IRB 09-953), with the exception that we have added 4 new fields to capture results of the Vitamin D tests and biomarker results. This strategy will allow us to eventually add clinical parameter data from this study into the larger comprehensive database, but the biomarker data will continue to reside separately in the study-specific database.

**Privacy and confidentiality:** Medical information obtained in this study will remain in the Oracle-based database registry within the Department of Dermatology. Information contained in this database is confidential and can only be viewed by investigators and study staff who have password access. Data from the assays conducted in Dr. Maytin's laboratory will be de-identified on all tubes, vials, and notebooks through use of the coding system described in the previous paragraph. Again, investigators with password-protected access to the database will be the only people able to break the code when the time comes to interpret the results (to correlate lab results with clinical outcomes for individual patients).

- [Describe how subjects may withdraw the use of their samples.](#)

The Informed Consent document informs the subject that he/she can withdraw permission for use of their samples at any time, and explains how to do this by contacting the Principal Investigator (PI) in writing.

- [Will any samples or research data be given to external sponsors or other 3rd parties?](#)

No.

- [Will any genetic testing be undertaken \(i\), and shared with subjects \(ii\)?](#)

(i) Yes; (ii) No.

The genetic sequence of very specific loci in genes that encode the Vitamin D receptor (VDR), and in the genes for 1-alpha-hydroxylase and thymidine synthase (enzymes which metabolize Vitamin D and 5-fluorouracil respectively), will be examined for the specific purpose of asking whether the presence of a particular variant statistically correlates with PDT treatment outcomes. There are no known diseases that are associated with these vitamin D enzymes nor the TS promoter variants. Therefore, because no known information with the potential to cause harm exists within the limited DNA sequences to be examined, we have no plans to share any DNA sequence information with the subjects. However, the Informed Consent will instruct subjects that they are free to withdraw permission for use of their DNA data at any time by contacting the PI in writing.

## **11.0 STUDY PARAMETERS AND DETAILED STUDY CALENDAR**

Each step of the study has been described in detail under Section 6.0, “Treatment Plan”. Each study visit, and events occurring between visits, are laid out in the Table in section 11.6 (“Calendar”).

**Allowances for variations in scheduling:** A visit window of **3 months** is allowed when scheduling the final follow-up assessment visit.

In brief, the study consists of the following activities:

### **11.1 Initial evaluation and screening (Visit 1)**

Initial exam, and review of previous records; blood draw for 25-OH-D3 levels (and leukocyte DNA, for VDR receptor analysis). Vitamin D study pills will be given to the patient.

11.2 **At home:** Study nurse will call patient to instruct patient on when to start Vitamin D supplementation.

### **11.3 PDT treatment (Visit 2)**

All AK lesions are measured clinically and photographed. Noninvasive fluorescence dosimetry may be performed on up to 6 lesions. *Levulan Kerastick<sup>TM</sup>* will be applied to each lesion and left to incubate for 30 minutes. **Blue light** (Blu-U device, 20 J/cm<sup>2</sup>, 33 minutes) will be administered.

11.4 **At home:** Study nurse will call patient to check up on the patient and record side effects at 24-48 h.

### **11.5 Final observation (Visit 3)**

At month 3, the subject will return for final exam and photography of lesions.

11.6 **Calendar.** (Please see next page for **STUDY CALENDAR**)

**CALENDAR. Timeline of procedures for patients enrolled in the study.**

**Yellow:** Clinic visits. **Gray:** events taking place at home. **Green:** steps that are critical to decision-making.

	VISIT 1 No PDT	Start VD	VISIT 2 PDT 1	Phone Call	VISIT 3 No PDT
<b>Scheduled visit: →</b> <b>Procedure: ↓</b>		5 d or 14 d before PDT	Day 0		Month 3 *
Review previous clinical records	X				
Blood draw	X				
Dispense Vitamin D3	X				
Call, and tell the patient to start taking pills		X			
Pill Count (verify patient took pills)			X		X
Skin exam and lesion counts	X		X		X
Photograph lesions			X		
Topical ALA application			X		
Measure PpIX fluorescence on selected lesions at 30 minutes			X		
Blue light exposure (20 J/cm <sup>2</sup> )			X		
Phone call to patient, to record side effects at 24-48 hr post PDT				X	

\*Time window for scheduling Visit 3 will be 3-6 months after Visit 2, to match the protocol of the control group (IRB 16-1615).

## **12.0 MEASUREMENT OF EFFECT**

### **12.1.1 *To establish a diagnosis of AK.***

AK will be diagnosed through a combination of touch and visual inspection.

## **12.2 Methods for Evaluation of AK Response**

### **12.2.1 *Number of AK Lesions Measured from Clinic Visits and Photographic Images***

(Primary Objective)

Percent reduction of actinic keratoses at a 3-month follow-up visit will be used to evaluate the overall outcome of PDT with prior Vitamin D supplementation. This will be achieved by counting AK lesions at visit 2 and visit 3.

### **12.2.2 *Noninvasive fluorescence measurements (NIF) of lesions***

(Secondary Objective)

The *maximal levels of PpIX*, measured 30 minutes after Levulan application at each treatment session, will indicate the degree of accumulation of porphyrins in the areas of skin with actinic damage.

### **12.3 *Tolerability and patient satisfaction***

(Secondary Objective)

We will collate the results from the patient symptom score sheets filled out by each patient in the week following each PDT treatment. We will also record erythema (on a scale of 1 to 4+), from clinical exam and from the photographs, in the treated areas just prior to the second treatment in each PDT cycle. The absence/presence of pain will be recorded on a 0-to-10 visual/analog scale.

### **12.4 *Correlate tumor response to Vitamin D3 serum levels, and VDR status*** (Secondary

Objective)

See Section 10.0 for description of how the blood samples will be handled and analyzed.

## **13.0 RECORDS TO BE KEPT / REGULATORY CONSIDERATIONS**

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

### **13.1 Data Reporting**

The OnCore Database will be utilized, as required by the Case Comprehensive Cancer Center, to provide data collection for both accrual entry and trial data management. OnCore is a Clinical Trials Management System housed on secure servers maintained at Case Western Reserve University. OnCore properly used is compliant with Title 21 CFR Part 11. Access to data through OnCore is restricted by user accounts and assigned roles. Once logged into the OnCore system with a user ID and password, OnCore defines roles for each user which limits access to appropriate data. User information and password can be obtained by contacting the OnCore Administrator at [oncore-registration@case.edu](mailto:oncore-registration@case.edu).

OnCore is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. This study will utilize electronic Case Report Form completion in the OnCore database. A calendar of events and required forms are available in OnCore.

### **13.2 Regulatory Considerations**

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

#### ***13.2.1 Written Informed consent***

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the subject.

#### ***13.2.2 Subject Data Protection***

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

#### ***13.2.3 Accessing Electronic Medical Records for University Hospitals Health System:***

*Not applicable.*

#### **13.2.4 Retention of records**

The Principal Investigator of The Case Comprehensive Cancer Center supervises the retention of all documentation of adverse events, case report forms, source documents, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with national and international regulations and the institution in which the study will be conducted, or for the period specified by the sponsor, whichever is longer. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

#### **13.2.5 Audits and inspections**

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the Center to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

#### **13.2.6 Data Safety and Monitoring Plan**

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI regulations.

## **14.0 STATISTICAL CONSIDERATIONS**

### **Primary objective:**

#### **Statistical Analysis**

Patients receiving neoadjuvant D3/PDT will be first matched to control patients who received PDT alone (in our previous study, IRB 16-1615) in a 1:1 ratio based upon the baseline 25OH-D3 values. The 25OH-D3 values will have three strata: normal blood levels, intermediate, and low. The matching will also be stratified, i.e., normal level patients who received D3/PDT will be matched to normal level patients who received PDT, and so on. Within each stratum, the matching will be done with the optimal algorithm by considering 25OH-D3 as a continuous variable. The outcome,  $\Delta C$ , (% decrease in lesion number), will then be compared between the two matched groups using the paired t-test. Statistical significance will be established at a two-sided p-value  $< 0.05$ . As a sensitivity analysis, a linear mixed-effect model will be used to adjust the comparison for baseline 25OH-D3 values. A random two-way ANOVA model will also be used to include therapy, D3 strata and their interactions in the model to examine whether the treatment effect differs by strata.

#### **Sample size estimates:**

We estimated the variance within our actinic keratosis (AK) study population by using data from our prior pilot studies that examined PDT for AK of the face and scalp [10]. The mean clearance of the AK lesion counts was 70%, with a SD of 24% (n=30) three months after PDT. We hypothesize that the clearance rate with neoadjuvant D3 will be 90%. For this effect size, we would need 30 patients to achieve a power of 90% (assuming a common SD of 24%, two-sided alpha=0.05, 0.1 correlation). As an enrollment estimate, we aim to recruit 35 patients.

#### **Secondary objectives:**

- To assess the level of PpIX accumulation in AK lesions at treatment visits, in the absence or presence of neoadjuvant D3. (fluorescence dosimetry measurements)
- To assess tolerability of the technique. (Pain scale measurements)
- To assess patient satisfaction with the technique. (Cosmetic result, and questionnaire)
- To assess D3 serum levels (in serum) and VDR status (in leukocyte DNA), and to correlate these results to clinical outcomes.

Characteristics of the above parameters will be summarized as means and standard deviations (medians and quartiles) or percentages and frequencies. We will also compare the characteristics of screened and randomized patients using appropriate two-sample comparison procedures (t-test, Chi-squared test, etc.).

Analyses of study endpoints will follow the intention-to-treat (ITT) principle. For each relevant

endpoint, we will summarize its trajectories over study visits by randomization assignment for each patient. Linear mixed-effects models will be the primary analytic tools to analyze continuous endpoints by accounting for within-patient randomization, multiple lesions from the same patient and repeated measurements over time. The models will include fixed effects of treatment and visit and multiple random effects that represent the hierarchical data structure. Similarly, generalized linear mixed-effects models will be applied to categorical endpoints (binomial data, e.g., number of lesions with CR). The analysis for time to clearance will be based on a random-frailty survival model, where lesions without CR will be considered to have clearance times censored at the end of study. Further exploratory analyses will adjust these models by controlling for important initial parameters (e.g., initial tumor size).

All analyses will be conducted using SAS 9.3 (Cary, NC). Results will not be adjusted for multiple comparisons, and statistical significance will be established with a two-sided p-value < 0.05.

**Estimate of the accrual rate:** Patients who have been scheduled to receive PDT in our noninvasive cutaneous oncology clinics will be given written information prior to their visit, including a copy of the Informed Consent (IC) that describes the purpose of the study. If the patient indicates that he/she is interested, the physician or study nurse will review the IC with the patient on the day of PDT and answer all questions. We estimate an enrollment of 1 patient each week.

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## APPENDIX I

### APPENDIX 1. DNA EXTRACTION FROM WHOLE BLOOD

Ed Maytin 5-Nov-2011, from Nancy Rebert (technologist in Dr. JP Achkar's lab in Pathobiology)  
Notes on blood sample handling for R21 Efudex/PDT study

#### OVERVIEW:

1. Collect blood from patient in lavender top 10 ml Vacutainer tube, with EDTA.
2. The blood should stay at room temperature; do NOT put on ice (which activates platelets)
3. The blood must undergo the first processing step (RBC lysis and centrifuge spin) within 24 hr after collection. After that, the WBC pellet can stay at room temp in the cell lysis buffer for weeks to months before processing and collection of the DNA.

#### REAGENTS TO PURCHASE:

You'll need 3 Genra Puregene kit components from Qiagen, plus standard alcohols:

• RBC Lysis solution (Qiagen Cat# 158904)
• Cell Lysis buffer (Qiagen Cat# 158908)
• Protein Precipitation solution (Qiagen Cat# 158912)
100% isopropanol (2-propanol)
70% ethanol
Tris-EDTA, pH 8 (warmed to 60 °C)

#### OTHER SUPPLIES NEEDED:

50 ml conical tubes	Large table-top centrifuge
15 ml tubes, and tube rack	Ice bucket
5 ml and 10 ml plastic pipettes	Timer
Pipet-Aid	Alcohol resistant pen (VWR Lab Marker)
1000 µl Pipetman and tips	Labels
	Gloves, disposable (wear at every step)

#### STEPS PRIOR TO DNA EXTRACTION: (Wear mask, or work behind glass in hood)

1. Label a 50 ml conical tube with blood specimen ID, and fill the tube with the following volume of RBC lysis solution:  
For 9 ml of blood, use 27 ml of lysis solution  
If < 6 ml of blood, use 18 ml  
If > 9 ml of blood, use 30 ml
2. Invert the blood specimen to mix; then transfer the blood to 50 mL tube with the lysis solution.
3. Incubate tubes for 5 min at room temp; gently invert the tubes 3x's during the 5 min period
4. Centrifuge at 2,000 x g (RCF) for 5 min.

5. Carefully pipet off the supernatant with a 25 mL pipet, into a spare 50 ml conical tube, retaining the white cell (WBC) pellet. Leave ~ 200µl on the pellet. (Be sure to cap the waste tube tightly dispose in Biohazard)
6. Resuspend the pellet by Vortexing 10 sec.
7. Add 12 ml Cell Lysis Buffer to the pellet. Vortex for 20 sec. This lyses the cells and precipitates the proteins. [The tube can be stored at room temp for months, up to 2 years.]

-----  
DNA EXTRACTION:

8. Add 4 ml Protein Precipitation Solution to the 12 ml of lysate. Shake vigorously for 20 sec.
9. Centrifuge at 2,000 x g for 5 min. After centrifugation, the precipitated proteins should form a tight, dark brown pellet. If the supernatant is not clear, or if pellet is not tight, vortex again and incubate 5 min on ice. Then repeat centrifugation. (*Nancy not too strict about the clarity aspect; still gets good result even if slightly brown*).
10. Add 12 ml of 100% Isopropanol to a labeled 50 ml conical tube.
11. Pour supernatant from Step 9 into the Isopropanol tube. Mix the sample by gently inverting 50 times. Centrifuge at 2,000 x g for 3 min.
12. Pour off supernatant into waste, watching the pellet carefully. Briefly drain the tube on absorbent paper.
13. Add 12 ml of 70% ethanol to the 50 ml tube. Shake the tube to wash the pellet. Centrifuge at 2,000 x g for 1 min.
14. Pour off supernatant into waste, watching the pellet carefully. If pellet slides, roll tube as you drain. Briefly drain the tube on absorbent paper. Let air dry for 5-15 min after carefully wiping tube with a fresh, clean Kimwipe. (*Do not overdry, or cannot re-dissolve the DNA.*)
15. Using the 1000 µl pipetter and disposable tip, add 400 µl Tris-EDTA (warmed to 60 °C) to the 50 ml tube. Run the tube along a microtube rack to mix.
16. Incubate in a 60 °C waterbath for 1 hr. Transfer the DNA into a labeled 1.5 ml tube with cryo label. Samples may be stored at 4 °C for short period, and at -20 °C for long-term storage.