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Low-cost enabling technology for image-guided photodynamic therapy (PDT) of oral cancer

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DETAILED PROTOCOL

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BACKGROUND AND SIGNIFICANCE: Oral cancer is defined as the cancer of the lip, mouth, tongue, and buccal cheek. Based on these criteria, oral cavity cancer is the 8th most frequent cancer in the world among males and 14th among females, the main risk factors being tobacco and alcohol use. 'Oral cancer' OR 'mouth cancer' OR 'tongue cancer'; the use of these terms generated a list of numerous MeSH entry terms, which included subheadings of these main terms to include mouth neoplasms, oral neoplasms, cancer of the mouth, and head and neck cancers. Oral cancer is a heterogeneous group of cancers arising from different parts of the oral cavity, with different predisposing factors, prevalence, and treatment outcomes. It is the sixth most common cancer reported globally with an annual incidence of over 300,000 cases, of which 62% arise in developing countries. There is a significant difference in the incidence of oral cancer in different regions of the world, with the age-adjusted rates varying from over 20 per 100,000 population in India, to 10 per 100,000 in the USA, and less than 2 per 100,000 in the Middle East. In comparison with the U.S. population, where oral cavity cancer represents only about 3% of malignancies, it accounts for over 30% of all cancers in India (1). More than 80,000 new cases are reported every year and it is the leading cause of cancer death among Indian men (2, 3). Oral cancer in India affects mostly those from the lower socioeconomic groups, that is, people from the lower socioeconomic strata of society due to a higher exposure to risk factors such as the use of tobacco, zarda, khaini, chewing gutka, mawa, and kharra, which are all dry mixtures of flavorings, areca nut flakes and powdered tobacco.

Earlier detection of oral cancer offers the best chance for long-term survival and has the potential to improve treatment outcomes and make healthcare affordable. Even though clinical diagnosis occurs via examination of the oral cavity and tongue, which is accessible by current diagnostic tools, the majority of cases present to a healthcare facility at later stages of cancer subtypes, thereby reducing chances of survival due to delays in diagnosis. Current treatment options, primarily surgery and/or radiation, can be curative if cancer or dysplasia is caught at a sufficiently early stage. Though even in these cases treatment may be disfiguring with significant impact upon quality of life, and many patients do not seek medical attention until the disease has progressed to a point where radical operation is required (Stage III/IV), often entailing block dissection and removal of the entire lymphatic drainage of the neck. Despite the radical operation, disease still recurs, leading to an overall survival rate of less than 70% of these cases (4). Even the less drastic excisional surgeries for early stage disease often cause cosmetic issues and persistent chewing, swallowing, speech problems. Radiotherapy, when feasible, typically preserves more tissue, but side effects in the oral cavity, including xerostomia, mucositis, ulceration, alterations in taste, and fibrosis, can also present major quality of life issues.

Furthermore, it is crucial to note that even given the limitations of these approaches, both surgery and radiotherapy require significant medical infrastructure and highly trained clinicians that are often not available in the resource-limited settings where these malignancies are disproportionately prevalent. In fact it is estimated that approximately 100 million people globally do not have access to radiotherapy machines, which cost about $6,000,000 and last for 8–10 years if maintained properly, which they are often not. As a result of these limitations, patients who do not have immediate access to high quality health care will far too often not receive timely intervention. All of these factors point to the urgent need for the pursuit of therapeutic strategies that are not only effective, but can feasibly be adapted for implementation with low-cost components in rural areas with little or no access to electricity, let alone the medical resources of a major clinical center.

With these considerations in mind, the present proposal makes the case that photodynamic

therapy (PDT)-based treatment of oral cancer, already shown to be effective in the traditional clinical setting (5–10), is inherently conducive to adaptation into a low-cost battery-powered platform that addresses the requirements for EMERGING ECONOMY implementation.

PDT Background: PDT is a photochemistry-based modality for the treatment of cancer and non-cancer diseases that works by preferential light-mediated cytotoxicity to target tissues (11). PDT is based on the observation that certain photosensitizers (PS) accumulate preferentially in malignant tissues. PDT is clinically approved for the treatment of a number of carcinomas (12, 13) and shown to be effective with remarkable healing of the mucosa in oral cancer and pre-cancer applications while surgical procedures frequently result in scar formation (chen et al 2012).

PDT involves delivering visible light of the appropriate wavelength to excite the PS to an excited state, leading to intersystem crossing to the long-lived triplet state which can produce singlet oxygen or other cytotoxic species, in addition to fluorescence emission for tumor imaging (14). It is also well established that PDT is inherently a theranostic modality whereby the photosensitizing compounds function simultaneously as imaging and cytotoxic agents. The modest preferential accumulation and activation of photosensitizers in neoplastic tissues provides both site-specific cytotoxicity, and selective visualization of tumors using fluorescence contrast to demarcate the boundaries of cancerous and healthy tissues, which have been studied by our group and others (14), i.e., PDT is a localized therapy and has minimal side effects unlike systemically delivery chemotherapy.

Early work led by Dr. Stephen Bown and Dr. Colin Hopper in the UK (clinical consultants on this proposal) showed that PDT for treatment of oral cancer and dysplasia produced consistent epithelial necrosis and excellent healing after treatment (5, 6). In their initial study of 18 patients presenting with squamous cell carcinoma (SCC) or premalignant lesions of the buccal mucosa, floor of the mouth, tongue or alveolus, the maximum depth of necrosis was 1.3mm and complete epithelial necrosis was achieved. Yet no necrosis in muscle was reported for all cases.6 Patients received analgesia during therapy but importantly there was excellent healing in all cases, with even the largest lesions completely healed in 3-5 weeks and none of the patients had perceived changes in sensation within the oral cavity after healing.

Similar results from PDT therapy on oral lesions have been reproduced by other groups and at other centers in Europe and Asia (7-9, 15, 16). In a study by Tsai et al (provide reference here), patients with oral lesions had minimal to no scarring with excellent cosmetic outcome. At 6 month followup, post therapy, in the five hyperplasia subjects, complete response was observed in four subjects while partial response in the remaining one subject. This study and others (5) showed that LED light-mediated topical ALA-PDT is as effective as the laser light-mediated topical ALA-PDT for treatment of oral lesions.

A review of the 5-Aminolevulinic acid-mediated photodynamic therapy for oral cancers and precancers has been provided in Chen et al 2012 (5). This clinical literature collectively concludes that PDT is a safe and effective approach, with remarkable healing and is especially effective for early stage cancerous and precancerous lesions of the oral cavity. It is for this reason that the clinical validation of the present proposal focuses on treatment of patients with T1N0M0 disease with no lymph node involvement or distant metastasis. That being said, this is a highly active and rapidly evolving field, in which new photosensitizers and light delivery methods indicate promise in achieving greater depths of penetration.

To the best of our knowledge, most of the previous ALA-PDT studies used laser light to treat a variety of premalignant and malignant human lesions; Tsai et al and Yu et al first tested the efficacy of an LED light-mediated ALA-PDT on oral premalignant lesions, however these were not battery operated. Laser machines can provide light with specific mono-wavelength; the laser system is well designed, more stable, and power adjustable. However, it is heavier, more bulky, and more expensive. An LED light device can provide light with a range of wavelengths and has the advantages of being a simpler, smaller, lighter, less expensive, and more portable light source than the laser machine. The present project however focuses on developing a technology for the situations in which PDT is already satisfactorily shown to be safe and effective. Furthermore, by coordinating the clinical validation with efforts at broader dissemination, it is hoped that the ability to treat relatively early stage disease in a manner that is actually available, accessible and attractive (not too expensive or painful or requiring good infrastructure or costly travel to a major

medical center and possible disfigurement) will in fact reduce the number of patients presenting at late stages whose treatment require highly demanding surgical procedures.

Motivated by all these factors and excellent studies by several research groups around the world, here is a compelling case for PDT in rural India, not only because it has excellent results for early stage disease relative to the other options, but also for patients in rural areas who might otherwise get no treatment at all due to the cost, logistics of travel to a clinic, etc.

Photosensitization by 5-aminolevulinic acid (ALA)-induced accumulation of protoporphyrin IX (PpIX): In this proposal, we adopt the well-established technique of photosensitization by administration of ALA 5-aminolevulinic acid (ALA) (17, 18). This approach has been widely used in dermatology PDT applications (19–23) and in all the clinical oral cancer PDT studies cited above. In fact, the first clinical report of photosensitization by oral, systemic administration of ALA was by Drs. Bown and Hopper and their colleagues, and it was for oral cancer patients. Because administration of ALA only temporarily overloads the natural heme synthesis pathway, photosensitization lasts no more than several hours. This is a crucial consideration in a setting where sunlight exposure is difficult to avoid.

LED-based light source for PDT therapy: The light source to be used for PDT treatment is a fiber-coupled, 635nm (peak wavelength) LED housed inside a small aluminum enclosure, which contains the LED itself and all associated electronics. The device is battery powered and provides a stable output power of 42mW continuously for approximately 8 hours before battery change is required. A simple on/off switch controls it and also has a power selector dial that can reduce the internal current through the LED to regulate power to 4 preset settings between approximately 10 and 42mW. The fexible fiber bundle (approx. 1cm diameter) extends approximately one meter from the enclosure. The distal end of the fiber, which delivers light to the oral cavity, is fitted with an interchangeable light delivery applicator, which attaches to the end of the fiber. The applicators have variable size light output apertures from 0.5,1.0,1.5 and 2cm that can be selected based on lesion size. These are plastic [poly(methyl methacrylate) (PMMA)] or other similar material pieces that contain an internal reflector to direct light at a 90-degree angle from the fiber bundle with external dimensions of 1cm by 2cm. During treatment the distal end of the fiber, including the applicator, will be covered to approximately 10 cm down the fiber bundle from the end by a transparent hygienic plastic sheath. This device is adapted from an earlier prototype battery- operated LED PDT light source that has been characterized and shown to produce tumoricidal efficacy comparable to a turn-key laser system in two recent peer-reviewed publications (24,25).

The device described here has been built and tested in the US and is to be imported to India for the proposed study. As an investigational device, the process for obtaining a Certificate of Exportability (COE) from the United States Food and Drug Administration (FDA) is described under section 801(e)(1) of the FD&C Act Chapter VIII: Imports and Exports. Pending ICMR review of this protocol, a letter from the Secretary of Commerce at the Indian Embassy in Washington, D.C. may be provided to the FDA to provide assurance that the device is not in conflict with the laws of India, the Indian government has full knowledge of the status of the device in the U.S.; and import is permitted or not objected to. Once this assurance has been provided, the investigators will provide additional technical documentation to the US FDA who may then provide a COE for this device. Once the required documentation, including a letter from the Secretary of Commerce, is provided to the FDA, it is anticipated that a COE can be granted within 20 days.

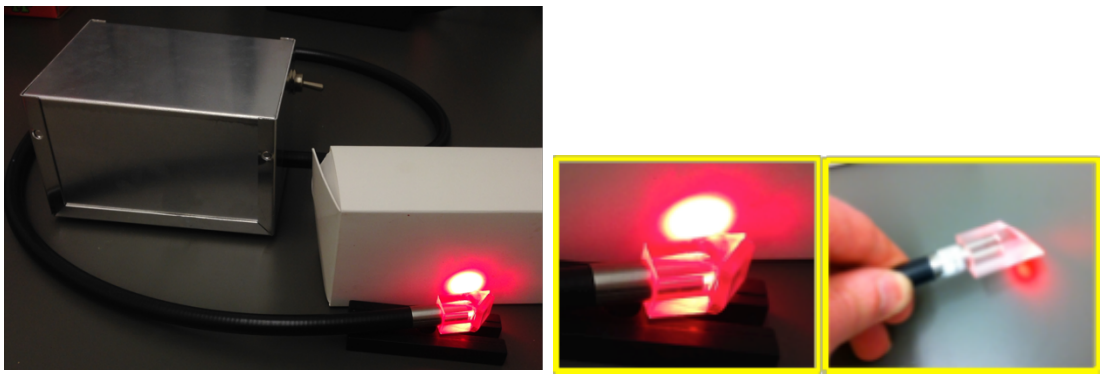

Figure 1: Battery operated light source for oral cavity PDT treatment. The fiber coupled battery-powered device is shown with two different prototype light applicators (left panel).

Smart-phone based fluorescence imaging: An inherent advantage of this PDT approach is that the therapeutic agent (PpIX, via ALA administration) also doubles as a fluorescence contrast agent. After administration of ALA and allowing a 2-3h period of PpIX conversion and accumulation in the malignant tissue, imaging can be performed to gain a more sensitive/specific view of tumor margins that will primarily be used in the current application to guide the precise placement of the fiber-coupled applicator for PDT treatment. A secondary priority, will be a forward looking validation study of high contrast baseline and follow-up fluorescence images of the treatment site that will be compared with histopathological evaluation of corresponding biopsies. This study will evaluate the feasibility of using PpIX fluorescence images to monitor treatment response in the clinic and, as the technology is considered for dissemination into, neighboring rural villages. In the long-term and with the proper resources and development, this approach may also serve as a rapid source of documentation for potential telemedicine interaction between a clinician and a field healthcare worker to provide additional guidance if needed.

This proposal develops an approach in which photosensitizer (PpIX)-based fluorescence images can be obtained via smartphone or using an adaptation of a standard commercial intraoral camera. So, following a single administration of a photosensitizing agent, the oral cavity can be illuminated with blue light to more clearly identify margins on the smartphone screen before proceeding with the PDT treatment without need for application of any additional exogenous agents. The latter follows using the compact, hand-held, red-light system in which a sheathed fiber optic with a light diffusing applicator is placed in the patient's mouth for the duration of therapy (approximately 30-45 minutes total exposure broken into 10 minute exposure segments with a 2-3 minute breaks after each segment). The smartphone-mounted LED array to be used for PpIX fluorescence imaging in this study is characterized in a recent publication (24). The smartphone-mounted illuminator or intraoral camera, to enable non-invasive fluorescence imaging of the oral cavity can be exported by the same FDA export request mechanism discussed in the preceding paragraph.

In fact it is reasonable to envision a more ambitious implementation of PpIX imaging as a global health technology for oral cancer screening. For the sake of focus, the emphasis on treatment rather than diagnosis here is a decision based on the realistic scope of the proposal rather than capability of the technology, and we will apply for additional funding to develop the diagnostic potential of this approach for emerging economy settings.

SPECIFIC AIMS/OBJECTIVES:

Aim 1: Adaptation and calibration of existing PDT treatment and imaging technologies for oral cancers to a low-cost, battery powered format. In dialog with design criteria from emerging economy site clinical collaborators, we will develop a low-cost light delivery device by adapting high-output 635 nm LED(s) coupled to a multimode fiber with an oral insert applicator to illuminate a sufficient area (for typical lesion sizes) with the necessary power density (~50mw/cm^2) for clinically feasible PDT treatments. The required power density over these areas will be verified by a series of calibration experiments using established optical tissue phantoms. Leveraging the well-established tumor selectivity and

multifunctional capability of ALA-PpIX to serve also as a contrast agent for optical tumor imaging, a simple approach using low cost filters and the imaging capability of a smartphone to ascertain disease margins (also on follow- up) and guide fiber applicator placement will also be developed in Aim 1 building on prelim data. All engineering design will be conducted in close dialog with PSI.

Aim 2: Optimization of LED-based ALA-PpIX PDT and Imaging. In dialog with Aim 1, we will **(A)** conduct preclinical PDT studies in an established mouse model to 1) validate tumor depth of necrosis in an implanted tumor model by histology **(B)** We will conduct pilot sessions in healthy subjects at AMU in India, to assess uniformity of light delivery in the human oral cavity.

Aim 3: To validate clinical performance of PDT treatment of oral cancers and dysplasia at emerging economy sites. Initially we will conduct clinical studies on 30 eligible patients with T1N0M0 oral lesions on location at AMU, which treats approximately 500 oral cancer patients per year. All patient studies will be conducted according to local institutional guidelines that are also compliant with the NIH requirements. Ultrasound and/or biopsy will also see patients at the hospital for follow-up examination and to assess therapeutic response.

Aim 4: To establish a long-term business, dissemination and healthcare training plans for sustainable implementation. This is facilitated by our industry partners, DUSA pharmaceuticals (subsidiary of India-based SunPharma) and PSI. Our team includes expertise in international oncology care and global health delivery and will forge partnerships with smaller care providers in surrounding villages to "train the trainers" in implementing our technology, establish relationships with local organizations and develop a more detailed business plan for distribution of both the PDT agent (ALA) and the low-cost device for PDT treatment. If successful, the PDT-based cancer treatment technology established here could have broader impact in neighboring countries in South Asia (Pakistan, Sri-Lanka) where oral cancer is also disproportionately prevalent. It would also have impact on other cancers such as cancers of the uterine cervix, which is still a significant problem in rural India.

Study Recruitment and Procedures: Study participants will be recruited from patients referred to JN Medical College with histologically confirmed T1N0M0 oral lesions. The sites of these lesions, which are typically squamous cell carcinoma, are in order of occurrence: the gingivobuccal sulcus, cheek, floor of mouth and tongue. All of these lesion sites in the oral cavity should be accessible for light delivery with the proposed fiber-coupled applicator system.

1. **Pre-study procedures/Screening**: Patients come to ENT/Oral and Maxillofacial/Plastic and Reconstructive surgery outpatient facilities. Typically, an ENT specialist handles early cases (biopsy proven malignancies), while ENT and plastic surgeons jointly deal with advanced cases. Patient screening will take place in outpatient departments. The investigator/s will interview each subject to develop a brief, relevant medical history and determine that all selection criteria are met. The subject will receive an explanation of the study objectives, possible risks and benefits of the study. After history evaluation of the patients, Blood work will be done to check liver functions Test. After the blood report and inclusion and exclusion criteria, the consultant will discuss and educate the patients about photosensitization and PDT therapy. If patients are willing to take part in the study, co-investigator will obtain a signed consent form. After obtaining a signed consent form, the consultant will schedule an appointment for PDT therapy.

Questions asked of participants <u>before treatment</u> will seek to understand:

1. Why is the patient seeking treatment?
2. What are the bothersome symptoms: pain, difficulty eating, swallowing, speaking, etc.?
3. What medications, treatments, ayurvedic and herbal supplements are currently being taken?

4. What is the patient's previous health history, including liver problems, tuberculosis, yellowing of skin or eyes (often undiagnosed hepatitis A or B), etc.?
5. What is the patient's current use of NSAIDs?
6. Practical considerations such as the availability of transportation, cost, home environment and availability of caregivers.
7. What is the patient's current use of tobacco, including willingness to quit and what is their access to tobacco and nicotine treatments and treatment programs?

Subject enrollment:

1. If a potential subject is eligible and still interested, identifiable healthcare information may be collected including: last name, address, and birthdate, contact information, etc. This method ensures that identifiable healthcare information will be collected only from those persons who likely meet eligibility criteria. Those people who do not meet entry criteria will have only given non-identifiable health information.
2. For those subjects who have given identifiable healthcare information and have signed a consent form, but are not subsequently enrolled, a failure log will be maintained.
3. Logs for pre-screening, enrollment and failure to enroll will be used to track relevant information.
4. The subjects must verbally acknowledge understanding of the informed consent, and sign the consent form accordingly.

Study Facility: The study is to be conducted at one location within the JNMC hospital. All procedures will be performed at the Department of Radio-diagnosis. The following sections describe the procedures to be followed for this clinical study. The study does not necessitate the use of any invasive interventions at the test sites. All data collection and procedure techniques are standard medical practice and are known to be safe.

Inclusion Criteria:

1. Age above 21 years, males or females
2. Subject has read and signed a written informed consent form
3. Subject is willing to have ALA administered and wait for 2-3 hours
4. Subject is willing to receive red light irradiation in the mouth via the fiber probe/applicator and is willing to have the fiber probe/applicator in the mouth for a maximum time of 1 hour
5. Subject is willing to allow investigators to take measurements using smart phone imaging before, during and after light treatment
6. After the procedure, the subject is willing to avoid spicy, hot, or oily foods for at least 6 hours
7. Subject willing to wear full sleeve shirt and full-length garments for couple of hours.

Exclusion Criteria:

1. Pregnancy or nursing (ALA is a drug that belongs to FDA pregnancy category C).
2. History of photosensitivity diseases (e.g., lupus erythematosus, porphyrias).
3. Therapy with any photosensitizing medication, e.g., thiazides (for the treatment of high blood pressure), fluoroquinolones, griseofulvin, or sulfonamides (for the treatment of infections), sulfonylureas (for the treatment of diabetes), phenothiazines (for the treatment of emotional problems), and other medications reported to cause photosensitivity within the last 6 months.
4. Subject is unable or unwilling to comply with the study requirements.
5. Subject has any conditions or scars within the location of the test sites that may interfere with the treatment or evaluation.
6. Allergy to porphyrins or ALA.
7. Subject has received laser treatment within 6 months in the area of the treatment.
8. Subject is participating in other potentially confounding research, e.g., currently enrolled in a clinical study of any other unapproved investigational drug or device.
9. Any other condition or laboratory value that would, in the professional opinion of the

investigator, potentially affect response or participation in this clinical study.
10. Subject has inadequate organ function.
11. Subject has co-morbid systemic illnesses or severe concurrent disease.
12. Subject is being treated for vascular disease.
13. Subject is an employee of the participating sites directly supervised by the investigator.
14. Subject with invasive deep carcinoma evaluated by biopsy.
15. Subject is currently being treated for other cancers with medical or radiation therapy.
16. Subject has AIDS or other infectious diseases, including tuberculosis, hepatitis or herpetic lesions (oral herpes).
17. Subject has oral submucous fibrosis (OSF) resulting in patient inability to comfortably hold the light applicator in mouth.
18. Subject has HPV positive tumor.
19. Subject has the tumor at Oropharynx area.
20. We will exludes the patients of the all other oral lesions at sites except: the gingivobuccal sulcus, cheek, floor of the mouth and tongue.

Procedure after enrollment: We plan to enroll 30 patients with oral cancer in this study. The study will take place at JNMC, Aligarh. A timeline of key steps in the clinical protocol is shown in **Figure 2**. This timeline of procedures considers that many patients may need to travel from remote rural areas with poor access to transportation, so it cannot be assumed that patients have convenient access to return to the clinic for multiple repeated biopsies.

***Day One**:*
Baseline imaging; Fluorescence measurement: PpIX fluorescence will be recorded using 405nm excitation light delivered from an LED source powered by a smartphone. This is a non-invasive system. The PpIX fluorescence will be photographed using the camera embedded in the smartphone. The advantage of this step is that in the long term it will obviate the need for biopsies to establish PpIX concentrations and could allow for online dosimetry customized to individual patients. Three photographs will be taken in each subject: first photograph will be taken prior to given ALA to the patients, second photograph after 2-3 hours of ALA incubation, and third photograph will be taken immediately after PDT treatment.

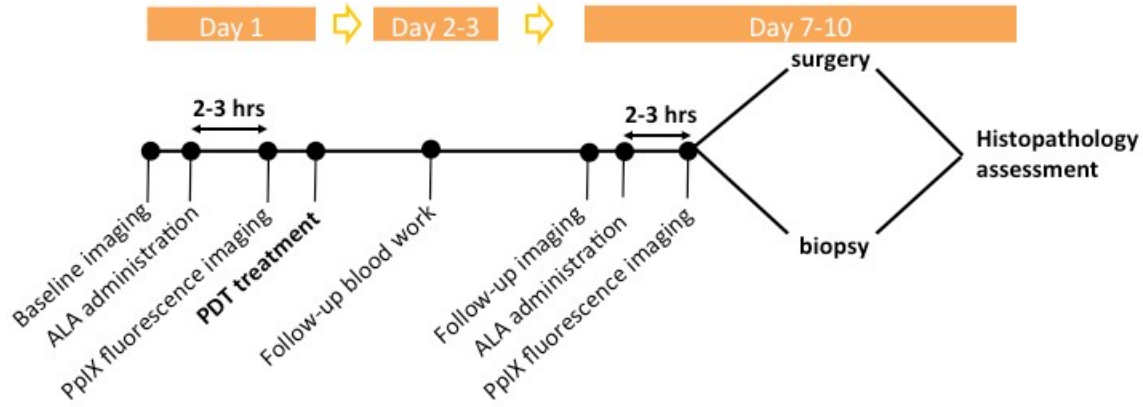

Figure 2: Timeline of procedures for clinical PDT protocol

ALA preparation and administration: The FDA-approved ALA from DUSA Pharmaceuticals, Inc., Wilmington, MA will be used in powder form. Patients will initially receive a baseline ultrasound of the oral cavity prior to ALA administration. One hour prior to ALA administration, analgesic (Aceclofenac, HIFENAC-SR (200mg);Intas Pharmaceuticals, India) and antiemetic (Domperidone, Domperon, 10mg; Cadila Pharmaceuticals, Inc., India) will be given to patients orally to avoid nausea and pain. ALA, totaling 60 mg/kg, will be administered orally via three repeated doses of 20 mg/kg of ALA dissolved in either orange juice or a soft drink, which lowers the pH of the solution, at 0, 1 and 2 hours.

Imaging Procedure before treatment: After allowing 2–3 hours for PpIX conversion and accumulation in the malignant tissue, a baseline image of the tumor will be obtained using the smartphone PpIX imaging approach. In this clinical validation study, these PpIX fluorescence images will be used to make treatment decisions beyond visual guidance for applicator placement on the lesion.

PDT Procedure: Immediately after the brief imaging session (approximately 5 minutes), the PDT treatment will proceed. Depending on the exact fractionation schedule determined, a total fluence of 100J/cm^2 at the lesion surface will be delivered with an area of applicators or compressed applicator in for approximately 30-45 minutes. Placement will be such that the full extent of the lesion falls within a 1mm margin of the applicator area. Every 10 minutes, a 2-3 minute break is provided to the patient and the study personal will check for the correct placement of the probe. The clinician will conduct applicator placement with the assistance of a nurse as needed so that it fits tightly in contact with the active surface facing the lesion. Care will be required when pressing an applicator tipped light delivery system hard against tissue. If one presses too hard and the tissue is rendered temporarily hypoxic, there will be no PDT effect. During therapy patients will leave their mouth closed around the optical fiber. Depending on the exact orientation of the lesion within the oral cavity a mouth guard may be used to aid applicator positioning, or if this improves patient comfort. Also, to aid in stabilization of the fiber and improve patient comfort, an ergonomic external positioning mount may be employed to help maintain the optical fiber in position. Provided that care is taken that no incidental body movements during irradiation lead to significant shifting of movement of the applicator, patients will be able to read a magazine, watch TV (if available) or other similar activities from a seated position. Small lesions may receive a complete treatment in this time interval, while (as noted above), larger lesions may require one or more additional applications. Patients will receive analgesic Aceclofenac, HIFINAC-SR (200 mg); Intas Pharmaceuticals, India to avoid pain. Appropriate Laser safety glasses will also be provided to the patients during irradiation.

Post-treatment imaging: At the conclusion of the PDT treatment, the light delivery fiber will be removed and a follow-up smartphone fluorescence image will be obtained for later analysis of PpIX photo bleaching. Patients will receive guidance on resumption of normal activities. After the procedure, patient will be advised to avoid foods that are spicy, hot or oily for at least 6 hours and advised to take analgesic as needed Aceclofenac, HIFENAC-SR (200 mg); Intas Pharmaceuticals, India. to avoid pain. Patients will also be advised to stop smoking, and chewing tobacco product.

Questions for participants on survey to be administered after treatment, and at follow-up will seek to understand:

1. The tolerability of the treatment and, in particular, any side effects such as nausea, vomiting, pain after treatment, ability to eat and drink after treatment, and photosensitivity.
2. What are current symptoms and their severity, in order to capture trajectory and worsening/no change/resolution of previously identified bothersome symptoms?
3. Pragmatic issues associated with cost, transportation for follow-up (including non-PDT) treatments, lab work to monitor LFTs (bilirubin, biliverdin, AST), and follow-up visits.
4. Adherence to recommended plan of treatment (literacy, cultural variation).
5. Current tobacco use, access to tobacco and nicotine treatment programs/treatments. (Information about quitting tobacco chewing will also be made available to patients as needed).

***Day Two to Three**:* Routine complete blood investigations including a liver function test (LFT) will be performed at 1 to 2 days following ingestion of ALA. If abnormalities are noted, follow-up blood work will be conducted that includes a renal function test (blood urea, serum creatinine, uric acid) and blood electrolytes measurement.

***Day Seven to Ten**:* At a follow-up examination, 7 days after treatment, ultrasound of the oral cavity will be conducted to assess treatment response by lesion area (cm^2) and depth (mm). As part of this study, ALA will also be re-administered (unless a particular patient had an adverse reaction to ALA in the initial treatment) so that follow-up fluorescence imaging can also be conducted. Again, at this stage, fluorescence imaging is included for validation of the procedure itself, not to decide the next steps in treatment for this patient cohort. However, as shown in Figure 2, the follow-up ultrasound will be a decision point to determine whether a given patient needs to undergo surgical excision of residual tumor (possibly with additional radio- and/or chemotherapy as determined by the clinician team), or if no residual disease is present, a biopsy will be conducted at the original disease site for histological confirmation of treatment response, and/or disease progression (development of invasive disease). If the radiologist reports residual tumor after the ultrasound procedure, an ENT surgeon will perform biopsy and the tissue will be preserved in formalin and sent for histopathology assessment. The depth of necrosis evaluated from the histopathology will serve as end dpoint for evaluating the technology. This histopathology data will also be used for validation of imaging to establish fluorescence contrast agreement with histologically confirmed malignant tissue.

Follow-up Model:
Patients who have good response to PDT will not need to undergo surgery, which would compromise the excellent healing of the mucosa that has been reported with this modality (7,9). At the same time, patients whose disease has partial or no response to PDT will still receive the same standard of care (surgical excision, chemo/radiation) they would have if they had not enrolled in the study. It is also possible that the PDT treatment, even if response was not complete, could reduce the scope of excision required in these subjets. Therefore at the end of the study we will have established the utility of this technology without interrupting the flow of treatment/management of the patients whose disease has partial or no response to PDT. Regardless of the flow of this study, additional follow-up on all patients will be carried out at 3 months, 6 months, 9 months and 1 year after treatment. All patient data obtained will be appropriately de-identified as per local institutional guidelines before off-site analysis, which will continue throughout the study.

The clinical validation study will also serve as an opportunity to gather patient data that will be invaluable in two capacities 1) to obtain important information immediately relevant to individual patient care and 2) with a mind toward sustainability we will seek to understand cultural barriers to acceptance or compliance with the proposed PDT treatment that will be accounted for in outreach and training plans. The present validation study is not powered to make comparison between different interventions, but rather to provide a validation that the safety and efficacy of the low cost approach is equivalent to previous reports of PDT treatment for oral cancer in traditional medical settings at JNMC.

Role of Clinicians
Role in recruitment and prescreening: Oral malignancy cases reporting in our OPD will be examined, evaluated and screened for enrolling under the study. 30 patients with T1N0M0 and fulfilling other inclusion criteria will be considered for PDT. Each patient will undergo thorough clinical examination and investigation as per protocol. All the patients will be explained the objectives, benefits and possible risks of the study. The physician will also discuss and educate the patients on PDT therapy and its procedure. Once they agree co-investigator along with a person with no conflict of interest will take consent under video graphic recording.

Role in conducting the study and followup: Dr. Hasan, in conjunction with Dr. Siddiqui, will be responsible for recruiting patients and defining the selection criteria for patients. Dr. Hasan will be one of the physicians responsible for performing photodynamic therapy of oral cancer in the clinical trial proposed and also prescribing the required analgesic and antiemetic drugs. He will also contribute to the coordination of education programs focused on training physicians and healthcare workers to deliver the treatment protocols. Dr. Hashmi is the co-Investigator of the project and collaborating on the surgeries and treatment performed for the clinical studies during trial. After first PDT regimen we will follow-up the study for the out come. In the follow up study If the radiologist reports residual tumor after the ultrasound procedure, an ENT surgeon will

perform biopsy and the tissue will be preserved in formalin and sent for histopathology assessment. Based on the histopathalogical analysis, the clinicians will determine the patients who have good response to PDT will not need to undergo surgery, as it would compromise the excellent healing of the mucosa that has been reported with this modality. At the same time, patients whose disease has partial or no response to PDT will still receive the surgical excision, chemo/radiation by the clinicians. It is also possible that the PDT treatment even if response was not complete could reduce the scope of excision required in these subjects. Regardless of the flow and output of this study, the clinicians will followup on all patients at 3 months, 6 months , 9 months and 1 year after treatment.

Objective drop criteria for the removal of a subject from the study include the following*:*

1. Subject verbalizes desire to no longer participate in the study, which will be monitored by utilizing "ongoing consent" during the study.
2. PIs reserves the right to terminate any subject based on his expert judgment of potential increased risk and/or non-compliance with the study protocol

Bio-statistical analysis:

1. Number of patients required for the study: The estimated study size of 30 patients is based on an estimated effect size of 1.2 (p-value, two-tailed students t-test) to measure significance of change between pre and post treatment mean lesion surface area in cm^2 from published estimates of variance in PDT response in oral lesions (reference to Fan et al paper). This estimate assumes a statistical power of 0.80 and alpha level of 0.05 and accounts for an attrition rate of 10–15% of patients not completing the study."

2. Analysis on follow up response data: " We emphasize that the intent of the study is to test the technology for future clinical trials and each patients' pre-treatment biopsy will serve as control to his/her post-treatment biopsy data. Statistical comparisons between the pre and post-treatment histopathological images will be performed via two-tailed paired student's t-test. The biostatistician assigned to this project will be consulted to perform comprehensive statistical analysis on the data.

Risks and possible discomforts:

1. Potential effects experienced during the irradiation: stinging or burning sensations. We do not expect major discomfort or severe pain during the irradiation. If a study subject feels major discomfort or pain during the treatment, we will provide analgesic ceclofenac, HIFENAC-SR (200 mg); Intas Pharmaceuticals, India. If major discomfort is constant despite the changes, we will stop the treatment immediately.
2. Potential effects experienced after the irradiation: Swelling and reddening. The reddening and swelling is a response to the light irradiation, which is the intent of the treatment. This response will usually fade within 48–72 hours but may persist longer.
3. Cutaneous photosensitivity: ALA-induced PpIX causes cutaneous photosensitivity. So avoidance of inadvertent exposure to sunlight or artificial light is recommended for 24–48 hours. Patients will be provided with the appropriate clothing, including a full sleeve gown, to cover skin totally. In addition, patients will also get the sunglasses to wear and will be advised to stay indoors.
4. There is a risk of adverse reactions to ALA. Cutaneous photosensitivity after the administrations of ALA last approximately 24–48 hours. High doses of ALA have been associated with transient elevations of serum transaminase, nausea and vomiting. To reduce the side effect of nausea for patients taking oral ALA dose, we will provide an anti-emetic drug. On the day of procedure, patients will be pre-medicated with Domperidone (Domperon, 10mg; Cadila Pharmaceuticals, Inc., India), one hour prior to oral administrations of ALA.
5. Psychosocial (non-medical) risks: psychological risks are not anticipated during this

study. However if any participants experience emotional distress during the study, the study staff will assist them to resolve this stress with appropriate referrals. Steps to prevent this outcome will include detailed explanation of the study, what to expect from the study treatments and careful screening of subjects who may be at higher risk of experiencing distress from the study procedures as described.

6. Patients who participate may experience discomfort from venipuncture to obtain 10ml of blood, which is required pre- and post-study procedure to perform a liver function test. A trained phlebotomist will perform the procedure here to reduce the associated discomfort.
7. Cardiovascular effects: though these are rare events, patients might experience hypotension and bradycardia.

Details of Funding agency/ Sponsors and fund allocation:
National Institute of Health, USA,

Subcontract was awarded by Massachusetts General Hospital, Boston, USA
Fund Allocation:

Budget in Indian currency:	**Total Budget**	**Budget for Indian Investigator**
i) Staff	**Rs.41,610,240**	**9,321,984**
ii) Equipment		
iii) Contingency (including Chemicals, reagents etc.)	**Rs.2,028,600**	**1,955,016**
iv) Travel (including Manpower training)	**Rs.2,520,000**	**1,323,000**
v) Miscellaneous		
Total	**Rs.46,158,840**	**Rs.12,600,000**

Methods for ensuring safety of subjects

1. Small sample size (n = 30).
2. Exclusion of patients who are prone to higher risk of side effects associated with photosensitization - as defined by inclusion/exclusion criteria (i.e. lupus erythematous, porphyries).
3. Baseline study tasks: history and abbreviated physical exam.
4. All subjects will be informed that their participation is strictly voluntary.
5. Ongoing “Process of consent” to ensure continued voluntary participation throughout the study.
6. Extensive explanation of procedures and expected results prior to consent and throughout the study.
7. Close follow-up of all study patients during study participation, including monitoring of vital signs during light exposures. 8. 24-hour availability of co-investigators and study nurse during the study.
9. Anonymity and confidentiality will be kept strictly confidential; only authorized research staff will have access to study data (including all photographs taken of their test sites).
10. All subjects will be asked to sign an informed consent form detailing the risks, benefits, the nature of their participation and the estimated time involved in the study.
11. Subjects will be informed that adverse events are unlikely and unexpected. However, subjects will be instructed to report any adverse effects immediately.
12. Periodic monitoring visits by study sponsor, weekly research meetings of study staff, continuing review of data by the principal investigator (PI).
13. We will utilize previously defined safe parameters as determined in previous human study (IRB 2004-9-002217) which was done by our Massachusetts General Hospital collaborators.

Consent Procedures: 30 patients with T1N0M0 and fulfilling other inclusion criteria will be considered for PDT. Each patient will undergo thorough clinical examination and

investigation as per protocol .The subjects will meet with one of the licensed physician investigators to discuss the study and the entire research in detail. All the patients will be explained the objectives, benefits and possible risks of the study. The informed consent document will be reviewed with the subject orally and the potential subject will be allowed to read the entire document. The physician will also discuss and educate the patients on PDT therapy and its procedure. Subjects will have as much time as necessary to consult with family members or their physician before enrolling in the study. If the subject understands and accepts the document, they will sign it or, when appropriate, they will make their thumb print upon it. The estimated time for the review and the explanation of the consent form is about 45 minutes. Once they agree co-investigator along with a person with no conflict of interest will take consent under videographic recording. After the subject is enrolled, the baseline evaluation and treatments may commence after signing the consent form.

Publication of the Results: The results of this study may be published in a medical publication, journal, or may be used for teaching purposes. Additionally, this study and its results may be submitted for inclusion in all appropriate health authority study registries, as well as publication on health authority study registry websites, as required by local health authority regulations. Selection of first authorship will be based on several considerations, including, but not limited to study participation, contribution to the protocol development, and analysis and input into the manuscript, related abstracts, and presentations in a study. However study participants' names or other identifying information will not be used for these purposes without their permission.

Maintenance of the data collected during trial: All study subjects will be assigned a study number which will be the only subject identifier used throughout the study. A log of subject names and study numbers will be kept in a locked file in a locked office accessible only to study staff. As data is collected, it will be entered into case report forms by the study nurse and stored in a password protected computer database, identifiable only by subject study number. All photographs taken of the treatment site will be identified by subject study numbers and will be kept in patient specific study records in the same locked file as other study logs. Photographs will be taken of the test site and will not include subject faces. We will cover the face or region if any identifiable marks are in the photograph. Photos may be used for publications at the end of the study, but names will not be included.

Proposed compensation and reimbursement of incidental expenses and management of research related and unrelated injury/ illness during and after research period: We will offer you the care needed to treat any injury that directly results from taking part in this research study. We reserve the right to bill your insurance company or other third parties, if appropriate, for the care you get for the injury. We will try to have these costs paid for, but you may be responsible for some of them. For example, if the care is billed to your insurer, you will be responsible for payment of any deductibles and co-payments required by your insurer. Injuries sometimes happen in research even when no one is at fault. There are no plans to pay you or give you other compensation for an injury, should one occur. However, you are not giving up any of your legal rights by signing this form. If you think you have been injured or have experienced a medical problem as a result of taking part in this research study, tell the person in charge of this study as soon as possible. The researcher's name and phone number are listed in the consent form.

Monitoring and Quality Assurance:

a. **Independent monitoring of source data**: A study coordinator at MGH shall monitor all source data and ensure that the data are accurately documented on the study case report forms. All source data shall be organized and filed into individual subject binders. Any pertinent notes to file regarding the subject shall be documented by the study coordinator and kept in the applicable subject binder. The PI shall review data on an ongoing and consistent basis throughout the duration of the study. All subjects must be assigned a study

identification number that is the only item that appears on the subject binder and photographs.

The study shall be performed in accordance with all applicable, Ministry of Health and Family Welfare, Government of India act (Drugs & Cosmetics Act 1945) which was amended in June 16th, 2015, the requirements of the Food, Drug and Cosmetics Act (the "FDCA") and similar or successor legislations, any policed issued by the U.S. Food and Drug Administration (the FDA), the ICH Guidelines for Good Clinical Practice (the "GCP Guidelines") and the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") and any regulation and official promulgated here under.

b. **Safety monitoring**:

Data to be used for safety monitoring will include the following:

- Review of any adverse events.
- Review of enrollment, dropout rates, any protocol deviations and protocol adherence.
- Review of medical history to ensure patient safety: medical history, and ongoing clinical evaluations (including participant's objective and subjective response to cooling exposure).
- Review of any subjective report of discomfort experienced by participants.

The Data Safety Monitoring Board will consist of 7 members namely:

1. Prof. Anjum Pervez, Department of Medicine, JNMC (*Physician*)
2. Prof. Moied Ahmad, Department of Anesthesia, JNMC (*Intensivist*)
3. Prof. Asif Ali, Department of Biochemistry, JNMC (*Scientist*)
4. Mr. Salman Khalil, Asst. Prof., Department of Community Medicine (*Statistician*)
5. Dr. Ruhi Khan, Department of Medicine, JNMC (*Medical Oncologist*)
6. Dr. G.S. Hashmi, Assoc. Prof, Dept. Of Oral & Maxillofacial Surgery, ZADC, AMU (*Surgical Oncologist*)
7. Dr. Mohd. Akram, Assoc. Prof., Department of Radiotherapy, JNMC (*Radiation Oncologist*)

c. Outcome monitoring: The validity and integrity of the data will be ensured by close adherence to the protocol and meticulous documentation of the data as it is collected. Case report forms will be completed within hours to days after the subject's visits. Separate source documents will be utilized during the actual data collection and carefully reviewed after completion of the study visit and prior to entry into the final case report forms. Completeness and accuracy of study documents will be supervised by the study nurse and reviewed by the principal investigator on a weekly basis. Each subject will have two study books. One will serve as the source document, the other as the final study record. Additionally, study staff will participate in a weekly study meeting, which will serve to provide a collaborative review and analysis of subject recruitment, selection, enrollment, retention, participant's response to treatment, safety profile and data collection and documentation. The study sponsor (NIH) will source of integrity and validation of data and protocol adherence.

d. Adverse event reporting guidelines: Serious, expected/unexpected, adverse events will be reported to the IRB within 24 hours of the event followed by a full written report within 10 working days/14 calendar days. Mild/moderate, expected/unexpected adverse events will be reported to the IRB in writing within 20 working days/30 calendar days. Adverse events will also be summarized in a progress report at the time of continuing review. The Principal Investigator acknowledges the following PHRC & FDA definitions of adverse events which will be utilized to guide recognition and management of such events in the trial. Serious adverse events: are events that result in any of the following outcomes: death; a life threatening experience; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability/incapacity; or a congenital anomaly/birth defect. In addition, events that may not result in death, but may be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Mild adverse events: Awareness of signs or symptoms, but easily tolerated; are of minor irritant type; causing no loss of time from normal activities; symptoms would not require

medication or a medical evaluation; signs and symptoms are transient.

Moderate adverse events: Discomfort severe enough to cause interference with usual activities; persistent or requiring treatment.

Unexpected adverse events are defined as any event, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigative plan or elsewhere in the current application, as amended. "Unexpected", as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

Expected adverse events are defined as any event, the specificity or severity of which is consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is consistent with the risk information described in the general investigative plan or elsewhere in the current application, as amended.

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