# IN THE UNTIED STATES DISTRICT COURT FOR THE EASTERN DISTRICT OF MICHIGAN SOUTHERN DIVISION 

DUSA PHARMACEUTICALS, INC., a
New Jersey corporation; and
QUEENS UNIVERSITY AT
KINGSTON, a Canadian academic
Organization
Plaintiffs,
Case: 2:06-cv-10273
Assigned To: Dagan, Patrick J
Referral Judge: Majzoub, Mona K
Assign. Date: 01/20/2006@11:45 a.m.
Description: CMP DUSA
PHARMACEUTICALS, ET AL
v.

AMIRA EL-ALAYLI SOHEIM, M.D., Individually, and d/b/a/
HARPER LASER CLINIC,
Defendants.

## COMPLAINT

Plaintiffs DUSA Pharmaceuticals, Inc. ("DUSA") and Queen's University at Kingston ("Queen's University") (collectively "Plaintiffs"), through their counsel, COX, HODGMAN \& GIARMARCO, P.C., hereby allege the following for their Complaint against Amira El-Alayli Sohcim, M.D. ("Dr. Soheim") and Harper Laser Clinic (collectively "Defendants"):

## JURISDICTION AND PARTIES

1. This is a civil action for patent infringement of United States Patent Nos.

6,710,066 ("the '066 patent") and 5,955,490 ("the '490 patent") under 35 U.S.C. $\$ 271$ (a).
2. This Court has jurisdiction under 28 U.S.C. § 1331 and $\S 1338(a)$.
3. Venue is proper in this Judicial District under 28 U.S.C. $\$ \$ 1391(\mathrm{~b})$ and $\$ 1400$ (b).
4. DUS $\Lambda^{(6)}$ is a corporation organized under the laws of the State of Now Jersey having its principal place of business at 25 Upton Drive, Wilmington, MA 01887.
5. Queen's University is a public university registered in the province of Ontario and located in Kingston, Ontario, Canada.
6. Plaintiffs are informed and believe that Dr. Soheim is a citizen of Michigan, is the owner of, and does business as ("d/b/a") Harper Laser Clinic, located at 20340 Harper Avenuc, Hąper Woods, Michigan, 48225.
7. Plaintiffs are informed and believe that Dr. Soheim practices medicinc in concert with Harper Laser Clinic at 20340 Harper Avenue, Harper Woods, Michigan, 48225.
8. Plaintiffs are informed and believe that Dr. Soheim, in concert with Happer Laser Clinic, commits acts of infringement of the ' 066 patent and the ' 490 patent within this State and Judicial District. A substantial part of the cvents giving rise to the claim occurred in this Judicial District and Division.

## COUNT I <br> INFRINGEMENT <br> OF UNITED STATES PATENT NO. 6,710,066

9. Plaintiffs repeat and re-allcgc each and every allcgation in the foregoing paragraphs as though fully sct forth herein.
10. The '066 patent, entilled "Photochomotherapeutic Mcthod Using 5-

Aminolevulinic Acid and Other Precursors of Endogenous Porphyrins," was duly and lawfully granted on March 23, 2004, by the United States Patent and Trademark Office. The '066 patent is owned by Quccn's University and is cxclusively licensed to DUSA( $\overline{\mathbb{R}}$. See Exhibit A (a true and comect copy of United States Patent No. 6,710,066).
11. DUSA(ß), under its license from Quecn's University, manufacturcs, offers for sale, and sclls phamaceutical compositions containing aminolcvulinic acid under the trademark, LEVULAN ${ }^{\text {(III }}$, for use in the treatment of actinic keratosis, a nonmalignant hyperproliferative skin lesions, as covered by the '066 patent.
12. Upon infonmation and belicf, Dr. Soheim, in concert with Ilarper Laser Clinic, uses aminolevulinic acid containing drug products to treat paticnts for actinic keratosis, and thereby infringes the '066 patent under 35 U.S.C. § 271(a). See Exhibit B (a truc and correct copy of relevant pages of Defendants' wcbsite).
13. Defendants' infringement of the '066 patent is knowing, willful and wanton under 35 U.S.C. § 284 and make this an exceptional case under 35 U.S.C. $\$ 285$.

## COUNT II <br> INFRINGEMENT <br> OF UNITED STATES PATENT NO. 5,955,490

14. Plaintiffs repcat and re-allege each and every allegation in the foregoing paragraphs as though fully sct forth herein.
15. The '490 patent, entitled "Photochemotherapeutic Method Using 5-

Aminolevulinic Acid and Other Precursors of Endogenous Porphyrins," was duly and lawfully granted on September 21, 1999, by the United States Patent and Trademark Office. The ' 490 patent is owned by Qucen's University and is cxclusively licensed to DUSA ${ }^{*}$. See Exhibit C (a true and correct copy of United States Patent No. 5,955,490).
16. DUSA $(\mathbb{R})$, under its license from Queen's University, manufactures, offers for sale, and sells pharmaceutical compositions containing aminolevulinic acid under the trademark, LEVULAN ${ }^{(6)}$, for use in the treatment of acne in human patients, as covered by the ' 490 patent.
17. Upon information and belicl, Dr. Sohcim, in concert with Harper Laser Clinic, uses aminolcvulinic acid containing drug products for the treatment of acne, and uses those aminolevulinic acid containing drug products to treat patients for acne, and thereby infringes the ' 490 patent under 35 U.S.C. § 271 (a). See Exhibit $B$.
18. Defendants' infringement of the ' 490 patent is knowing, willful and wanton under 35 U.S.C. § 284 and make this an exceptional case under 35 U.S.C. $\S 285$.

## COUNT III

FALSE ADVERTISING UNDER § 43(A) OF THE LANHAM ACT
19. Plaintiffs repeat and re-allege each and cvery allcgation in the foregoing paragraphs as though fully set forth herein.
20. DUSA(B), under its license from Qucen's University, manufactures, offers for salc, and sells pharmaceutical compositions containing aminolevulinic acid under the registered trademark, LEVUL $\wedge N^{* *}$, for use in the treatment of actinic keratosis.
21. Neither Dr. Soheim nor Harper Laser Clinic have any rights to use DUSA(Q)'s registered trademark, LEVULAN ${ }^{*}$.
22. Upon information and belief, Dr. Sohcim and Harper Laser Clinic sell aminolevulinic acid for the treatment of actinic keratosis, and advertise aminolevulinic acid for the treatment of actinic keratosis.
23. On their website, Dr. Soheim and Harper Taser Clinic usc DUSA $\Leftrightarrow$ 's registered trademark, LEVULAN ${ }^{(1)}$. See Exhibit B.
24. Dr. Soheim and Harper Laser Clinic have made misleading designations of origins of the aminolcvulinic acid, false and misleading descriptions of lact and false and misleading representations of fact. This is likcly to cause confusion, mistake or deception as to the source of the aminolevulinic acid. See Exhibit B.
25. Dr. Soheim's and Harper Laser Clinic's actions are intentionally designed to likely deceive and confuse consumers in violation of $\$ 1125$ of the Trademark Act ( $\$ 43$ (a) of the Lanham Act).

## COUNTIV <br> TRADEMARK INFRINGEMENT UNDER § 32 OF THE LANHAM ACT

26. Plaintiffs repeat and re-allege cach and every allcgation in the foregoing paragraphs as though fully set forth herein.
27. DUSA ${ }^{*}$, under its license from Queen's University, manufactures, offers for sale, and sclls phamaceutical compositions containing aminolevulinic acid under the registered trademark, LEVULAN ${ }^{(1)}$, for use in the treatment of acne in human patients.
28. Neither Dr. Soheim nor Harper Laser Clinic have any rights to use DUSA ${ }^{\text {tij }}$ s registered trademark, LEVULAN ${ }^{\text {Bi }}$.
29. Dr. Soheim and Harper Lascr Clintic sell aminolevulinic acid, and advertise aminolevulinic acid for the treatment of acne in human patients. See Exhibit C.
30. On their website, Harper Laser Clinic and Dr. Soheim use DUSA ${ }^{(\sqrt{1})}$ s registered tradcmark, LEVULAN ${ }^{(k)}$. See Exhibit B.
31. Upon information and belief, Dr. Sohcim and Harper Laser Clinic use DUSA ${ }^{* 3}$ s registercd trademark, LEVUL $\Lambda N^{\text {* }}$, in connection with the sale, offering for sale, distribution and advertising of the goods in a manner which is likcly to cause confusion, to cause mistake and to dcceive.
32. Dr. Soheim's and Harper Laser Clinic's actions arc intentionally designed to likely deceive and confuse consumers in violation of $\$ 1.114$ of the Trademark Act ( $\$ 32$ of the Lanham Act).

COUNT V

## VIOL ATION OF THE MICHIGAN CONSUMER PROTECTION ACT OF 1976, MCL § 445.901, ET SEQ.

33. Plaintiffs repeat and re-allege cach and every allegation in the foregoing paragraphs as though fully set forth herein.
34. DUSA ${ }^{(1)}$, under its license from Queen's University, manufactures, offers for sale, and sells pharmaceutical compositions containing aminolevulinic acid under the registered trademark, LEVULAN ${ }^{\text {in }}$, for use in the treatment of actinic keratosis and acnc.
35. DUSA ${ }^{10}$, under its license from Qucen's University, uses its federally registered trademark, LEVUL $\Lambda \mathrm{N}^{(\mathrm{k})}$, on its drug, aminolcvulinic acid.
36. Dr. Soheim and Harper Laser Clinic sell aminolovulinic acid, and advertise aminolevulinic acid.
37. Neither Dr. Soheim nor Ilarper Laser Clinic have any rights to use DUSA ${ }^{p}$,s registered trademark, LEVULAN ${ }^{\text {a }}$.
38. On their website, IIarper Laser Clinic and Dr. Soheim use DUSA ${ }^{\text {(t) }}$ s registcred trademark, LEVULAN ${ }^{(1)}$, See Exhibit B.
39. Dr. Soheim and Harper Laser Clinic use unfair, unconscionable or deceptive methods in falsely passing off aminolevulinic acid as DUSA ${ }^{\text {k; }}$ S LEVULAN.
40. Dr. Soheim and Harper Laser Clinic have knowingly misrepresented by advertisement the manufacture or origin or commercial sponsorship of the aminolcvulinic acid drug sold, offered, or exposed for sale.
41. Dr. Sohcim's and Harper Laser Clinic's actions are intentionally or negligently designed to deccive or confuse purchasers in violation of the Michigan Consumer Protection Act of 1976, specifically, but not limited to MCL $\$ \S 445.901(1)(a),(1)(c)$, $(1)(e),(1)(f),(1)(g),(1)(s)$, and $(1)(b b)$, among others.

## COUNT VI MICIIIGAN COMMON LAW TRADEMARK INFRINGEMENT

42. Plaintiffs repeat and re-allege each and cvery allegation in the foregoing paragraphs as though fully set forth herein.
43. DUSA ${ }^{(k)}$, under its license from Queen's University, manufactures, offers for sale, and sclls pharmaceutical compositions containing aminolevulinic acid under the federally registercd, distinctive trademark, LEVULAN ${ }^{\text {*) }}$, for use in the treatment of actinic keratosis and acne.
44. Neither Dr. Sohcim nor Harper Taser Clinic have any rights to use DUSA ${ }^{*}$; trademark, LEVULAN ${ }^{\text {. }}$.
45. Dr. Soheim and Harper Laser Clinic sell aminolevulinic acid and advertise aminolevulinic acid.
46. On their website, Harper Lascr Clinic and Dr. Soheim use DUSA ${ }^{\text {(6)'s }}$ s trademark, LEVULAN ${ }^{n}$. See Exhibit B.
47. Dr. Soheim and Harper Lascr Clinic use DUSA ${ }^{(1)}$ s trademark, LEVULAN ${ }^{(6)}$, in connection with the sale, offering for sale, distribution, and advertising of aminolevulinic acid in a manner which is likely to causc confusion, to cause mistake and to deceive.
48. Dr. Soheim's and Harper Laser Clinic's actions are intentionally designed to likely deccive and confuse consumers in violation of Michigan's common law trademark infringement.

WHEREFORE, Plaintiffs pray that:
a. Defendants be preliminarily and permanently enjoined from infringing United States Patent No. 6,710,066;
b. Defendants be preliminarily and permanently enjoined from infringing United States Patent No. 5,955,490;
c. Defendants be ordered to pay compensatory damages as a result of his infringement of United States Patent No. 6,710,066, including all damages suffercd by Plaintiffs as a result of the infringement, increased by three times for willful behavior;
d. Defendants be ordered to pay compensatory damages as a result of his infringement of United States Patent No. 5,955,490, including all damages suffered by Plaintiffs as a result of the infringement, increased by three times for willful behavior;
e. Defendants be preliminarily and permanently enjoined from promoting and advertising aminolevulinic acid in a confusingly similar manner to DUSA ${ }^{\sqrt{3 i} ;}$ s LEVULAN ${ }^{\text {T }}$,
f. Defendants be ordered to pay compensatory damages as a result of his false and misleading advertising, including all damages suffered by $\operatorname{DUS} \Lambda^{(6)}$ as a result of the false and misleading advertising, increased by three times for willful behavior;
g. Defendants be preliminarily and permanently enjoined from promoling and advertising aminolevulinic acid in a confusingly similar manner to DUSA ${ }^{(k)}$ s LEVULAN ${ }^{\text {® }}$;
h. Defendants be ordered to pay compensatory damages as a result of his trademark infringement of DUS $\Lambda^{(\beta)}$ s rcgistered trademark, IEVULAN ${ }^{(0)}$, including all damages suffercd by DUSA ${ }^{\text {B }}$ as a result of the infingement, increased by three times for willful bchavior;
i. Defendants be ordered to pay actual damages as a result of his unfair and deceptive acts, including all damages suffered by DUSA ${ }^{\text {RN }}$ as a result of the unfair and
deceptive acts, increased by three times for willful behavior pursuant to MCL §§
$445.901(1)(\mathrm{a}),(\mathrm{l})(\mathrm{c}),(\mathrm{l})(\mathrm{c}),(1)(1),(1)(\mathrm{g}),(1)(\mathrm{s})$, and (1)(bb), among others; and
j. Plaintifis be awarded such other further relicf as the Court shall deem appropriate.

Respectfully submitted,


Dated: January 20, 2006

## Exhibit A

## (12)

# United States Patent 

Kennedy et al.
(54) PHOTOCHEMOIIIERAPEUTIC MEITHOD USING 5-AMINOIEVULINIC ACID ANI) OTHER PRECURSORS OF ENDOCTENOUS PORPHYRINS

Inventors: James C. Kennedy, Kingston (CA); Ray H. Pottics, Kingshem (CA)
(73)

Assignee: Quern's University ut KIngsitun, Kiugstor (C.A)
(*) Notice
Subject to any disclaimer, the term of this palent is extcoded or adjusted under 35 U.S.C. 154 (b) by 24 days.

This patent is subject to a terminal disclaimer.
(21) Appl. No.; 09/816,329
(22)
lijed: Маг. 26, 2001
(65)

Irrior Publisution Data
Us 2001/0021370 A1 Scp. 13, 2001

## Related U.S. Application Data

(63) Continuation of application No. ©y2y3,833, tiled on Apr 19, 1999, whis: is is comlimution-in-part of applitation No. $0808 \%$, IB3, filed on Jun. 28, 1993, now Pat. No. 5,422,003, which is a continustion-in-parl of applicention No. 077865, 151, fitcd on Aprr 8, 1992 , now Pat. No. 5,231,946. which is a connimuation-in-purt of eppolication No. (17/783,750), tiled on Oct. 28, 1991, nyw l'al. No $5,211,938$, which is a contimationn of ipplication No. 077386,414, filed on Jul. 28 , 1982, now Pat. No. 5,074,262.
(51) Int. Cl. ${ }^{7}$ $\qquad$ A61K 31/40; A61K 31/195
(52)
U.S. CI. $\qquad$ 514/410; 514/410; 514/561; 514/554; 514/557; 424/9.6; 124/9.61
(58) Field of Seareh $\qquad$ 514/554, 557, 514/561, 410; 424/9.6, 9.61
(10) Patent No.: US 6,710,066 B2
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## (List continued on next page.)

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(74) Attorney, Agent, or Firm-13eth A. Burrons; Folcy \& Linduer


#### Abstract

(57)

ABSTRACT Mcthods of delecting and roatimy rapiclly growing exogchous colls, such as Prolista, or parasites, that preferentially accumulate a photosetivatable porplyrin in which 5 -aminolevalitic acid or precursor thercof is adminisitered to the patient, or comberted tes the exogentus sells, in an amount sudicient to induce syndhesis fluorescence and/or photosensitizing concentrations of a protoporphyrin IX in the exogchous cells, followed by exprosure of the exogenums cells to light of photonctivaliug wavelengths.


15 Claims, 1 Drawing Sheet


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FNGUREJ.


## 1

## PHOTOCHEMOTHERAPEUTIC METHOD USING 5-AMINOLEVULINIC ACID AND OTHER PRECURSORS OF ENIOOGENOUS PORPHYRINS

## CROSS REILRENCE TO RELATLD APPLICATIONS

This application is a continuation of U.S. application So No. 09/293,835, liled Apr. 19, 1999, which in tum is a cimtinuation-in-purt of U.S. application Ser No. 08/082,113, filed Jun. 28, 1993, (now U.S. Pat. No. 5,422,093, issued Iun. 6, 1995), which is a comtinuation-in-part in U.S. application Ser. No. 07/865,151, filed Apr. 8, 1992, (now U.S. Pat. No, $5,234,940$, issued Ang. 10, 199.3), which is a continuation-in-part of U.S. applicalion Ser. No. 07/783, 750), filed (\%ci. 28, 1991 (now U.S. Pat. No. $5,211,938$, issucd May 18,1943 ), which is a continualion of U.S. palent application Ser. Nu. 07/386,414, filed Jul. 28, 1989 (now U.S. Pat. No. $5,079,262$, issuet Jaw. 7, 1992). The disclosurcs of all these applications are incorporated herein by refence.

## FIEI.T) OF INVENTION

This invention clates to the detection and ireatment, by induced fluorescence and photochemotherapy, respectively, of cerrain tissue abnormalitics (both cancerous and nonmalignant of endogenous and exogenous origin), hyperpoliferative cells, and normal cells. The invention atso relates to the deceetion and treatment of abnomalities in body fluids or suspensions of tissucs connaining abnormal cells by induced thuorescence sand photochemotherspy.

## BACKGROUND OF INVENTION

Tissue abowrmalities involving the skin usually ate detected and ussessed by a combination of visual inspection and palpation. In certaim clinical situations the sensitivity of the visual inspection can tre enhaneed by the use of nonwhite light (either ultraviolet or a marrow band in the visible), or by the prior applieation of a connast-entancing agent such as dilute acetic acid or certain stains. Tissues abnormalities that involve surlaces that canmot be palpated (such as the bromehi or the urinary bladder) may le visualized via an appropriate scope. Some specialized seopes eno detect induced fluorescinee. If the abnomality in question is associated with a difticrenece in cither the extent or the patcrn of tissuc vascularization, such a seope may be used w) determine the limits of the area involved by the abnormality, by visualixing an injected bolus of fluorcsccin or other flugreseen malerial as it pusses through the vasculature of hoith the lesion and the adjacent nemmal tissud.

In addition, Iluorescence-delecting scoppes are being used experimentally to identify aress of tis:ue that show strong porphyrin lluorescence followimg the intravenous injection of exogenous porphyrius such as hematophorphyrin IX (IlpIX), hematoporphyrin derivative ( HpL ), or "dihematsporphyrin ether". Such porphyrins tend to accumulate semipreterentially in malignant tissues, but they also aceumulate in tissucs that are regencrating following an injury or in the rapidly growing tissucs of an cmbryo or felus. Normal liver, splecn, and kidney also tend to aceumulate these porphyrims. Using such compouncts and fluoreseence-delecting swopes, areas of malignant tissue tow sirnall to be identified by standard forms of visual inspection have been identified in the bronchi and in the urimary bladder.

Unfortunately, s clinically significant (photosemsitizime) amount of parphyrin may persisi in the skin for at least two
weeks, (cecasionally for more than two months) following the intravenous injection ol $\mathrm{H}_{\mathrm{p}} \mathrm{IX}$, $\mathrm{I}_{\mathrm{p}} \mathrm{D}$, or a semi-puridied preparation of IlpD, such as l'botolin IJ. (Photophrin is a registered trademark of Quadra Logibs, Joe. Vaneonver, British Columbia, Canada.) This means that patients must avoid exposure to sunlight (either direct, or through window glass) for an inconveniontly lang periad of time pustinjection. Understandably, pationt compliance of en is proor, and accidental phototoxic "suubura" is a common occur renee in the wecks following a diagnostie or therapeutic injection of popphyrin. I'ersistent photosensitivity is the major hazard associated with this cechnique, and is the main reation why it is not used mote widely.

The staudad treatments for cancer comprise surgery, adiothecapy and chemotheraply. However, wher lurms of reatment are also known, including pholochemotherapy or pholoclyatuide therapy ( $\mathrm{l}^{\prime}$ 'I' ${ }^{\prime}$ ), based on the disoovery made gever yol years ago that unicellular organims, i.e., eetain rapidly growing cells (such at cells of the Lower Kingom, now relerred to serotista), treated with pettain chemicals will clie when exposed to light. 'Thus, syathetic porphyrins have been shown in vito to protect cells from infections such as parasitcs, eg, tyomastigotes and sphatomustiguter of Tyropanasoma ernzi, J, Parasiol., 75 (i) 1989, p. $970-976$, and gram positive bacteria, mycoplasma and yeasts, Malik et al. J. I'motochemislry and Phowobiology, R. Biology $5281-293$ (1990), $F$ ache is known lo, in vitro, produce intracellular protoporphyin in the presence of exogenous $\Lambda \perp \Lambda$. Kjcidstad, Conference oft Photoserssitivation and Photochemotherapy of Cancer, Det Norske Videnskaps Akademi, Mar. 16-17, 1993, Osko, Norway.
l'DI' is currently being used, on an experimental basis, to treat several different types of eancer as well as certain mon-malignsml lesions such as psoriasis. The paticnt is given a photo-activalable druy that has sume degrec of speciticity for the lisulue being treated. A tisisue volume that includes the target lissue is there exposed to photouctivating light 50 as to destroy the target lissue while causing only mild and reversible damage to the other tissues in the sume treatmont volume.

There are two main types of photochemoherapeutic agents in elinical use at present. The first type, methoxypsoraleas, are given syakemically. Uliraviolet lipht is eswential to activate them. I ocalized exposure of psoralenmontaining lisucs us ultruvislet light induces a localized photochemical reaction that causes the drug to bind covalently to the DNA of living cells, thus destroying their proliferative porential. The sceond type, porphynins and clated photosensitizers, are also given systemically (by intravenous injection), although occasionally they are given cither topically or by intralosional injection. They can be activated by visible (red) light. Jhe localized exposure of porphyrin-containing tissues to such light ordinarily does not induce a chomical rescion between cell components and the porphyrin molecules. Instead, the porphyrins act as calalyst: by trapping the energy of the photoactivating tight. and then passing it on to molecales of oxygen, which in thm are raised to an excited state that is capable of oxidizing adjacent molecules or structures. Cell death is not eaused primarily by damage to the $\mathrm{DN} A$, but by damage to essentia] membrate structures. The goal of phomehumotherapy is sometimes cure (mainly for basal cell carcinomasi), but usually the goal is palliation through koal control when mone of the standard forms of therapy are considered likely to ofter a significano degree of benefil to the patient.

Mothoxypsoralen (ll/VA) therapy is used mainly for the tratment of psotiasis, bat simetimes it is alse used to treat
very superficial cancers that involve the skin (mainly myonsis fungoides). However, there are two serious problems with such treatments. First, the pmoedure has been demonstrated in humans to he carcingenic. Seonnd, the deph at which malignant tissue can be killed is limited to a fow millimeters below the illuminated surface. 'These problems severely limit the usetuluess of the methoxypsoralens for photcothemotherapy.

5-Amino-4-oxopentanoic acid, also known as 5-aminolevulimic acid and ats *-aminolevnimie and (" $\wedge L \Lambda$ ") has been described in the cooss ceforences patents and patent applications first set liords in this specilication tor cotecting and treating rapidly growing colls. Al. A hals als, been reported for use in attenuating the growtla and killing plants and iosects when applied directly to such organisms followed by exposure to light, based on work of Rebeizet al.

Synthelic porphyrins bave alwo heen used as pholochermotherapcutic agents in treating rapidly growing, e.g. rapidly dividing or rapidly melabolizing infeciousis eells, such is infectious pathogens, including proherwal parasites, such ats Plasmodium falciparium (which causes malaria in thumans), varions ohter species of Plasmodia, Ieishmania, and amoebas, pathogenic fungi, and microplasma, including the varions parasitic forms, all such celle and organisms being reffrred to herein as Protista. The term Prolisita ass used here and in the literature refers to the lowest orders of the animas and vegelable kingdoms, single colled or collections of single celled organisms includirg: the eukaryotes, including protuzoa, fungi and algac, and the prokaryotes, which arc bacleria and blue-green algat.
At present, the prophyrins most commonly usel for pholochermotherapy are Iematoporphyrin IX ( HplX ), Hernaloporphyrin derivative ( $\mathrm{I}_{\mathrm{p}} \mathrm{D}$ ) and various semipuritice preparations of HpD such as conomercially aveilable Photofin(iol Il, a semi-puritied form of HpD). When porphyrins are used as photosensitizers, cell death results from damage to cell membrancs. Consequenty, malignan Iransformation is not a surions problem. Moreover, since the visible (red) light that is uised to pholoactivate porphyrins penetrates tissue much more decply than docs the ultraviolet light that must be used to photoactivate methexypsoralens, the depth at which porphyrin-treated tissuc can be killed is substantially greater, Also, since cerain types of prophyrins show a significant tendency to aceumulate preferentially in malignant tissucs, it is sometimes possible to destroy malignant tissue without causing clinically signilicant damage to adjacent normal tissues.
The main problem with the systemic usc of $\mathrm{H}_{\mathrm{p}} \mathrm{IX}, \mathrm{H}_{\mathrm{p}} \mathrm{D}$ ) and Phelofrin II is that photosensitizing concentrations persis. in the skin for several weeks to several months following their adruinistration Consequeatly, severe accidental photoluxic skin reactions may oceur voless the pationt avoidse expesiure to sumlight (cither direct, of filtered through window glasis) until the concentration of the phonscusitizes in the skin has been reduced to a harmess level At prosent, the problem of pholosensitivity fullowidy le administration of prophyrins is tanded by udvisiug the patient to avoid any form of exposure to sumbight (or to very hright arlificial lights) for a pariod of at least twn weeks $\mathrm{p}^{\mathrm{kst}}$-injection, and to initiale subsequent exposure to sumight very cautionsly. Not all patients comply with these instuctions, since it often is quite inconvenient to do 50 , In addition, the use of a sunsereea with a ligh blocking factor is reommender with warning that this will ouly reluce the hazard somewhat, mot eliminate it completely. In a fow cases, pationts whose phatusensilization persisted for more than a month post-
tratment bave heon given large daily desess ol bela-catotenc over a period of several menths in an attempt to provent accidental pholotoxic damage. Finally, attempts have heen made to reduce photoloxicity by applying the photosensitizer topically to a limited area.

However, another type of prollem is cncountered if HpIX or IFT is applied topically in DMSO (dimethylsulfoxide), Azone, or swme other vehicle intended to cohance their diflusion through tissue. The porpliyrins tend to become immobilized wherever they thappened to be when the DMSO or Azome becomes diluted ly nurrial tissuc fluids to such an extent that the porphyrins can no longer diffuse through the tissue (or even remain in solution). Consequently, the opical application of porphyrims uflen is associater with a hoss of specilicity for malignant tissucs, and aormal tissues near the site of application may develon persislent photosensilization from the localized emecentration of porphyrin.

## OBSECT ()F゙ INVFNTION

It is an object of the present inveution to provicle a melbod for the delection of certain types of malignant and nonmalignant cells includity a collection of celis, and lissue abnormalities by induced fluorescence.

It is yet another object of this invention to provide a pholedynamic (photosynthesizing) treatment melhow using an agent which can be administered cither systemically or topically which is not in itself a photosensitizer but which induces the syathesis or aceumulation or both of protoporphyrin IX ( PpIX ) and other cadogenous porphyrins, their precursors and their photoproducts, in rapidly grewing cells, including alonurmal cells in otherwise normal tissues, in vivo or in vitro.

The terms prorphyrin(s) and their precursors acter to compounds produced in vive in the synthesis of heme and other endogeneusily produced photoactivatable compounds including their photoprociucts.

## SUMMARY OF INVENTION

This invention is based on the finding that exogenously administered ALA and other precursors of $\mathrm{P}^{\mathrm{P} P \mathrm{X}}$ are metabolized in patients wo $\mathrm{P}_{\mathrm{P}} \mathrm{IX}$ and that P PLX prefercnanally accumulales in rapidly growing cells, as contrasted with less rapidly growing colls. The rapich growth is corrolated with the metabolic activily, so that the ditterential accumulation is allected by the celative metabolic activity between dillerwint cells.

This invention provides a method for detecting in a patient, a malignam or mon-malignant lesion or aboormality which is sensilive of $\mathrm{P}_{\mathrm{p}} \mathrm{IX}$, namely those which preferentially actumulate $\mathrm{P} p \mathrm{PX}$, comprising administering to said patien an eflective amount of a precursme of P pIX in the biosyntheric parhway for heme so as to induce an aceumulation of $\mathrm{P}_{\mathrm{p}} \mathrm{IX}$ in said lesions, and exposing said lesions to light having a wavelength within the absorption spectrum ol said $\mathrm{P}_{\mathrm{p}} \mathrm{I}$, thereby to induce fluorescence in said lesions.

Another aspoes of this invention is a mecthod for treating malignant and non-malignant lyperpoliferative lesions of the skin, mucosu, endonctrium and urothelium which are sensitive to $\mathrm{P}_{\mathrm{Y} L X}$ in a pationt, comprising aclministering to said parient an effective amount of a precursor of PplX in the binsynthetie pathway for herne so as to induce syathesis or accumulation or both of $\mathrm{P}_{\mathrm{p}} \mathrm{CX}$ or ather endngenous porphyrins, their precursers and their photoprodncts in said lesions, and exposing said lesious to light having a wavelength within the photonctivating action spectrom of said Pple to therehy induce phowactivation in said lesions.
'Inus, the rapidly growing cells involved can be cithor malignant or non-malignant hyperproliferative eells. The hyporpoliferalive cells aan be nomal, rapidy growing cells or abnormal cells in otherwise nomal tissue. The ahnormal cells in an otherwise normat tissue can include abnormal rapidly growing cells endogenons to the patient or abnormat, rapicly growing cells which are exngenous to the patien. These rapiclly growing cells that are cxogenous to the patient shall, for convcnience, be referced to hercby, depending on the degrec of geterality, as rapidly growing exogenous cells, rapiclly growing Protista cells and rapidly growing parasite cells.

One aspect of this invention is induction in vive or in vilos of the hiosyothesis and seleclive acom malation of thoorescing or photosensitizing concentrations of protoporphyrin IX or other endogenons porphyrins such as anproporphyrin I, coproporthyrin III, uroporphytin I, uroparphyrin III, or fluorcscent metalloporphrins such as zinc protoporphyrin IX in Protista and purasites of humans or other animals, hy exposing said Protista and endogenous cells under appropriate conditions in vivn or in vitro to an ellecive concentration of 5 -aminclevulinie acid or other precursior of said porphinin(s) in the hinsynthotic puthway for heme.

Still another aspect of this invention is the detection or cnumeration of Prolista and parasites of humans or other animals, by inclucing in vivo or in vilro (ex vivo) the biosyuthesis and selective ascumulation of Duorescing conecatrations of proloporphycin IX or other endogenous porphyrin in the parasites is dewribed previously, and then usiry such fluorcscence to detect, enumeratc, or otherwise quantify said Protista and parasiles.
Yet another aspect of this invention is the selective killing of Protista and parasites of humans or other animals in vivo or in vitro, by inducing the biosynthesis and selective accumulation of phomsensitizing concentrations of probporphyrin IX or olter condogenous porphyrin in the Protista or condogenous cells as described above, and then exposing the photosensisized parasitus to an effective clase of light of wavelengths lying within the photoactivation spectum of said porphyrin(s) or of photosensilizing photoproducts of suid pophyrin(s) that may be produced during said exposure.
By another aspect of this invention there is provided use of a composition comprising a precursor of proterorphyrin IX in the biosynthetic palhway for heme for the manufacture of a medicament for treating inaliguant and nom-malignant tissue abnormalities and lesions.

In preferred aspects of this inveation the preferred precursor of probsporphyrin IX is 5 -amino-4-()xo-pentancie acid, otherwise known as 5 -aminolcwulinic ucid, and a peterred wavelengh of the photoactivating light is in the range of 625 to 670 חm, more preferably a red light of 625 to 640 mm .

Other objects, features and advantuges of the present invention will become apparent from the following detailed description. It should be understood, howcver, that the dotailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of iflustration ouly, sinee various changes and modifications within the spirit and secope of the invention will become apparent to those skilled in the art from this detailed deseription.

## DETAIIED DESCRIPTION OF TIIE DRAWING

Fig. 1 illustrates the duration of survival of individaral mive follnwing the injection of spleen cells infected with $P$ ?
yoedii. Group ( t ) mice were given spleen oclis that had been exposed in Al $\wedge$ in vivo by then kept in the dark. The average survival of the rcipientus of these cells was 15 days. Group ( 2 ) mice werc given the same number of ect1s from the sumberell suspensinn atter it liad been exposed in photwactivating light. All of thesc mice remained in gond health for 90 days, al which time the experiment was teminated.

## DETAIIET) IHSCRIPTION OF PREFERRET L'MBODIMENT

Protoporphyrin IX (PpIX), a naturally occurring photoscnsitizer, is the immedialc precursor of heme in the heme biosyanhetic pathway. All mucleated cells have at least a minimal capacily to synthesion $\mathrm{I}^{\mathrm{p} P \mathrm{X}}$, since beme is mecessary for the syathesis of various essential hemecontaining enoymes. Certain types of cells and tissucs can synuhesize relatively large quantitios of P PIX. Uucler nurmal conditions, the syrthesis of Pr IX in such cissues is under sucla tight feed-back conirol that the colls produce it at a rate just sufficient to match their need for heme. However, the usual rate-limiting step in the process, the syuthesisi of 5 -sminolevulinic acid, can he hypassed by the provision of cxogenols ALA, porphobidinogen, of other precursor of $\mathrm{P}_{\mathrm{p}} \mathrm{lX}$. Catain tissues and organs will then aceamulate such a large excess of PpIX that they become both fluorescent and photosensitive. At least in the case of the skin, the $\mathrm{P}_{\mathrm{P}} \mathrm{p}$ X appears to be synthesized in situ, $A L \Lambda$, which is commercially available from Sigma (hemical Company and other sources and whith is water suluble, can be administered orally, topically or by injection. The oral and parenera! roules fatal to the induction of elinicaliy uschul concentralions of $\mathrm{p}_{\mathrm{pl}} \mathrm{X}$ in cerlain benign and malignant tissues throughout the budy. () Oly certain types of tissue synthesize and accumulate clinically meciul amounts of $\mathrm{P}_{\mathrm{p}} \mathrm{IX}$ wher provided with an excess of $A \perp$. By the expression "rapidly growing cell" is meant herein any lesion, abnormal cell or aoratal cell that extibits cell growth substantially greater than that of the surrounding tissuss and that preferentially accurnulates protoporphyrin LX from exugenous AI A. Thus, the cells includc rapicly growing cells that sre endogenous to the palient and rapidly growing cxogenous cells such as Protista and parasite cells. The term "rapidly growing cells" is also used here to include living, metabolically active cells as contrasted with motabolically inactive (dead or dormant) cells such as found in the malarial applications of this invention.
At the prosent time, treatment of hasal cell, basosquanous and squamms ocll catcinomas and other lesions of the skin, mioosa (respiatery, digestive, and vaginal), exkmertiom and urothelium is contemplated. Sites, which conld include lesions or cellular abmormalities, generally are those of epithelial or entothelial origin ineluding but not limited to those invalving (i) skin, circulatory system and conjunctiva; (ii) the lining of the mouth, pharynx, cosphagus, sturnach, inteslines and intestinal appondapes, rectum, and anal canal; (iii) the lining of the nasal passages, nasal simmes, nasopharyux, trachea, bronchi, and bronchioles; (iv) the lining of thic ureters, urinary blatder, and urethra; (v) the lining of the valima, uterine cervix, and uterus; (vi) the parictal and visceral pleura; (vii) the lining of the peritumeal and polvic cavitics, ancl the surface of the orgatss contained within thoso cavitics; (viii) the dura mater and meninges; (ix) any tissues or sulupensions of body fluids onntaining abnomal cells, inclucling blood, that can be made accessible to photocativating lighe either in vitro, at time of surgery, in vivo through the sliu via surface irratiation or
via an uptical libre inserted through a needle; ( $x$ ) all exncrinc glands and associaled ducts, including: mammary glands, selbaceous glands, ceruminous glands, sweat glands, and Jacrimial glands; mucus-secreting glands of the digestive, urogenital, atd ruspiratory systemss; salivary glands; liver, bile ducts, and gall bladder; pancreas (excerine component); gastric and intestinal glands; prostat; Cowper's, Barthelin's and similar plands. It is also contemplated that cell alrowrmalitis in the gonads (testes and ovarics), thymus, spleen, lymph notes, bone nartow, lymph and bhood would also be treated according to the invention. Tumors of the nervous system or connective tissues (sarcomas) would also be treated secording to this invention.

Treatment of non-malignant lesions such us genital warts and pesoriasis und of endumetrial tissues for indiculions such ats contriception, waginal bleeding and endometrissis is alsio contemplated.
As used herein the term "skin" includes:
(A) the covering of the external surface of mest of the bekly, eommonly termed the skin.
(B) the covering of the external genitalia:
labia majora, labia minora, clioris, and
glans penis, prepuce, and associates
(C) the covering of the wone of transilino beaween skin and the mucosa of the digestive system: anal verge vermillion border of the lips
(D) the lining of the external auditory mearus, and the covering of the external surface of the rympanic membranc
(E) all cxocrine glands and associated duets that arc located at least partially within an epidermal surface described above, or within the underlying dermis, such as the piloscbaccous units of the skin.
The term "mucosa" includes;
(A) the lining of the whole of the respiratory tract: nasal passages and nasal sinuscs nasal pharynx and associaled structures larynx, vocal cords, and associated structures trachea, bronchi, and bronctioles
(B) the living of the whole of the digestive tiact:
oral cavily and tongue
oral pharynx and laryngeal pharynx
esophagus
stomach
small intestine
large intestinc, caccum, and appendix
sigmend colon and rectum anal canal
(C) the linimg of the whole of the urogenital tact: urothra, bladder, and uretors renal pelvis and renal calyees
vayini, ulerine cervix, ulerus, ind Fallopian
vas delerens, sominal vesicles, cjaculatory
(II) the enojunctiva and the lining of the tear
(F) all exnorine glands and associated ducts that are kecated at least partially within one of the mucosal surfaces desoribed ahove, or within the underlying submucosa.
This invention is especiatly useful for the ireatment of disenses of Protista and parasitic origin, as defined above, particularly acne, malaris and other parasites or lesions resulting from parasites.
The term "parasite" includes parasites of humans and other animals, inclucling parasitic protozoa (both intracellu-
lar and extracellular), parasitic worms (nematodes, trematodes, and cestodes) and parasitic ectoparasites (insects and miles).
The parasitic Protorea include:
malarial parasites of humans or olther animals
malarial parasitos of humans
Plasmodium folciparum
Plasmotium ovale
Plasmoxtiun nataria
Plasmadium vivax
Leishmanial parasites of humans and or other amimals leisthonaial parasites of humans

Leishirrania Irupica
Leishthrania major
Leishunumia dehiopica
Leishmania brasitionsis
Leishmania guyanensis
heistimania panamenis
Leishmania peruviana
l.eishimania mexicana

Leishumania amazonensis
Leishmania pilanoi
I.eishmania gurnhami
heichmania donovani
Letishmanta infantum
Leishmanta chagasi
trypanosomal parasiles of humans and/or othes animals trypanosomial parasices of humans

Trypunosomu cruzi
Trypanosoma brucei gambiense
Trypanosona bructi rhodexiense
amocbic parasites of humans and/or other animals amnehic parasites of hurnans

Entamoeba hivtolyrica
Natglaria species
Acanthamocha species
Dienumoeha fruyilis
miscellaneons protivalan parasites of thumas of other sпimals
miscellaneous proteroan paratsites of humams
Toxoplasma gondii:
Preunocystis carimii
Habesia micmori
Asnapara be:lli
Gryplosporidium
Oydospora spucies Giardia kamblia
Balantidium coli Blastecystis hominis Microsporidia species Samorystis spocios
Some of these miscellaneols protoma cause self-limiting alisease in mormal people, but serivus problems in IIIV 55 palients.
parasitic nematodes in humans and/or other animals
parssilic nematodes in humans
filarial nemalucles
Wuchereria bavcrofti
Brugia malayi
Brugia tinuri
Onchucerca volvulus
I.ona loa

Terrapetalonema perstan:
Tatrapetalomema surepucerca
Mansonella ezzardi
Dirculiaria imbitis

9
Diroflaria tenuis
Dirofilaria repens
intestinal nemastodes
Ascapis Lumbricoides (roundworm)
Necator antericartus (hookworm)
Ancylostoma duodenale (hookworm)
Strongyloides stercoralis (thrcadworm)
Enterobius vermicaluris (pinworm)
Trichuris trichiura (whipworm)
'Irichostrongylus species
Capillaria philippinensis
tissue anmatodos
Trichinella spiralis.
Anasakis species
Pscudoterranova species
Dracunculus medinensis:
parasitic Irematodes in humans andior other animals
parasilic trematosles in humans
Sohastosomu manvoni
Schistosoma haematobium
Schisessoma japonicum
Clonorchis sinensis
Paragonimus species
Opishorchis species
Fasciola hepatica
Melagonimus yokogawai
Heteruphyes heterophyes
Fasciolopis buski
parasitic cestodes in lumans and/or other animals,
parasitic cestodes in humans
Tuenia saginata
Tueria solum
Itymenolepis specios
Diphyllobothrium spueics
Spirtmetra spocics
Echinowocess species
The method of this invention comprises the administration of ALA, other precussors of PplX and other bidngenoms porphyrins, to the patient. The administration can also be in vitro as sppliced to tissucs of the patient, i.e., ex vivo. In ex vivo mothods, tissuc containing the rapidly growing cells are removed from the patient, an effective anomunt of $A T . \Delta$ or endayenous porphyrin is added therew, then the preparation is subjected io photuactivaling: ligh, before beimg reachministered to the patient. The armounts of AI A constituting an effective clase can be delermined by one skillecl in the at by analogy with the doses used for synuthetic porphyrins, based on mililigrams per kilogram body weight for in vive systemic application and the typical concentrations for topical or ex vivo applications. The eompound can be conveniently used orally or intravenously at a dosage of about $10 \mathrm{Lo} 100 \mathrm{mg} / \mathrm{k}$, per single dose, preferedly ats a dosage of $40-50 \mathrm{mg} / \mathrm{kg}$; however split dosiges of $10 \mathrm{mg} / \mathrm{kg}$ fore times jer thay may also be given. The compound can be used topically al a dusie of herween $2 \%$ to $100 \%$, with $100 \%$ being dry puwder. Ex vivo concentrations of the compound are used on coll suspensions in a range of $1-5 \mathrm{mM}$, will a preferred range of $1-2 \mathrm{mM}$; however, if serum is preseal, a hipher dose of about 15 m M should le used. If ex vivo use on whole blood, the coormound is used at alowat 15 mM ; thewe ver, if in ifou kelator, such as Desfermism or des lerroxamine, a lower ennentration may he usecl.
Thus, one application for the rrellos of this invention is the detection and quantitation of parasites by AL -induced lluorescence. The firegoing includes fluonsconce flow cytometry of suspensions of cells or parasiles ex vive, flunrescence micrnecopy of cells, including but not limitel
to 1 iesucs, body fluits, fecal material in vivo or ex vive, and quantative spectrophotolluorimetry of cells, including but not limited to tissucs, fooly fluids, urine, or lecal material in vivo or ex vivo.
Another application for the method of this invention is, the killing of parasites prelerentially photosensitized by cxposure to AT A or an eradogenous porphytin cither in vjvo or cx vivo. The conjunclive, which can be tieatod either topieally or systemically with $A L \Lambda$, followed by, after an appropriate period ol time, exposure of the skio or eonjuctiva to photoactivating light. The parasites can also be present in the peripheral blood, in which case the $\Delta L A$ can be administered systemically, followed by, after an apperpriate rime, which can he easily experimentally delermined, exposirgs the detined area of the skin or the blood passing through a large vein io pholoactivating, light via an uptical guide within a mansparent calheter that has been inserted into the vein. Parasites located within one cm. of the surface of bollow organs that are accessible to liberscopic examination (respiratory tracl, digestive tracl, urogenital tract, abdonimal cavity, pelvic cavily, thoracic cavity) can be diagnowed or treated hy syslemic administration of the AI A. followed by, alter the appropriate period of time, cxposure of the surface of the largel tixsue via an appropriate light euide. Parasites located at sites that are not readily accessible to liturscopic examination can be truatel with the photoactivating light via a light guide that has been surgically iotmaced ints the target area through a needle or following surgery.

Additional applications of the method of this invention are to detect very low levels of metabolically active malariad parasites in peripheral hlood or marrow eell suspensions. Such detcetion can be used in wereen banked blood or as a sercening procedure for patients suspected to have viable malarial parasites. The sereening method using AI A would s be acenmplished by flow eytometry.

Still another applibation for the melhod of this invention would le to distinguish between matabolically active ("viable") and inactive ("non-viable") malarial parasites to evalaste the response to therapy in patients iuteeted with drug-resistant malaria mon quickly than is tow possible. Present methocls for quantitating the lovel of parasitemia do not distinguish between viable and non-viable parasites. Thus, parasitos that have been kitled as a result of recent therapy may not be distioguishable from viable parasiles. If the parasites are in fact resistant oo the specifie drug(s) that are being used for therapy, resistance to these drugs (as shown by failure to reduce the level of parasitemia) may dot become obvious for some time after the initalion of therapy.

In some cascs it might be life-saving in recognize more quickly that a particular ding is not effective. Since AI A inducts lluorscence only in plasmodia that are metabolically active, it is possible w distinguish between "viable" and mophologically similar "mon-viable" malarial parasites in the peripheral bloot, Dings that fail to procluce a decrease in the propotion of the ecythocytes that accurnulate PYIX flomesecnce when exposed to $A I$. A in vilro eould be iden tificd quiekly and replaced by wher drugs that possibly might be more eftective. The lechnology would not necessarily require flow eylomelry, since relalively simple and much less expensive fluommetcrs could be used ill the level of parisitemia is sufficiently high.
lu cuses of partially dog-cesistant mana in which there is a slow response to the drugs, it may be dilticult to know when it is sate to diseontinue therapy. Since $\Delta L A$-induced $\mathrm{p}^{\mathrm{p}} \mathrm{I} \mathrm{X}$ fluorescence can detect viable plasinodia at vecy low levels of parsitemia, the tochuique might he used to verify that the parasitemia has been reduced on undetectable levels
hefore mandemane therapy is dimentinum. Hewover, fow cylomelry would be reguired for such low-level measurements.

The foregoing could also be used in sereen in vitor for susitivity/resistance of the plasmodia 「rom a given patient to selected anti-malarial drugs, since AI A induces fluoresconee only in plasmotia that are metabolically active.

Yet another application of this invention is the selective] photosensitization and killing of malarial parasites in vivo or in vitro by exposing them to photoactivating light. The light would le transinited to the malaria parasites in the circulating blood bither through the skin, via an indwelliag intravenous or intra-atterial catheter or by extracorporeal phatotyoname therapy of blood, ospecially for patients who bave faded to respond to other therapies, paticularly those who might be considered candidates for a therapeutic exchange transtinsjon.

This invention is also particularly applicable to the ticatment of fungal infections. Fungal infectinons arc becoming of inereasing importance in the pasi two decades due to the increasing number of immunocompromised patients, both by chemontherapy and diseases such as AIDS. Immonomppression results in an increased incidence of lungal infections. Fungal infections can be divided into three calegories: cutaneous, subcutaneous, and systemic. Gutameoss infections are by far the most prevalent. Fungel infections predispone their hosts to bacterial superinfections.

The method of the instant invention is cartied out in the same manner as that for synthetic porphyrins previously reported. More speceifeally, the method of this invention is used to decect or treat rapidly growing cells exogenous to the body, including Protista cells and parasitest.

The wayolongel of the photoactivating light is of some importance, ass it has been shown that between 1 and 10 percent of incident red light ( $600.7(0) \mathrm{nm}$ ) can pass theough a slab of human tissue 1 cm thick, whereas enly 0.001 percent or less of blue light (about 400 um) can patis through the same thickness of human tissuc. The photosensitizer wifl, therefore, be pore suceesstill if it absorbs red light. PpIX does silrongly absorb red light. The present approach has several alvanages over the prior art. First endogenous PpIX has a much shorter lialf-life in uomal tissucs (human
 This greatly reduces the danger of accidental phototoxic sikin reactions in the days following treathent. Sucond, the Al A can be applied topically to certain types of lesions. This improves the specificity of the treatment, reduces the danger of aceidental photomxic reacions to a very low level, and greatly reduces the amount of both AI A and PpIX to which the entire body would be exposed if an equally eflective dose of ALA were to be given systemically.

Roth AI $A$ and Ppix aic normal products of motabolism, and are handled quite readily by the binchemieal machinery of the body. However, since very large doses of AT A (like large doses of II I IX or $\mathrm{II}_{\mathrm{P}} \mathrm{D}$ ) are asiocialed with it transient decrase in motor merve conduction velocity, it is dessirable to reduce the dose of ALA to the miniminn that is still
 systemic adninistration. Thitd, P $\mu \mathrm{IX}$ is rapidly inactivated by the pholoactivaling light. Following exposire of tiskues containing PpIX to a therapentic dose of photoactivating light, there is a subsitantial clecreate in photosensitication of the tiskues within the theatment volume. Conscoucotly, if [plX is induced by the topienl application of $A$. $A$ to specific lesions, the paticot can be exposed to sunlight. immerliately posi-treatment withoul danger of serious phototoxicily. Also, the dusimetry of the photoactivating light is great

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simplifist, Fourth, $N, A$ is an effective indueer of $\mathrm{Pp}_{\mathrm{p}} \mathrm{IX}$ when given by mouth, by topical application, or by injection. In conirasi, $\mathrm{H}_{\mathrm{p}} \mathrm{IX}, \mathrm{H}_{2} \mathrm{I}$ ) and Photofrin II are effective in mosit situations only when given by injection. The versatility of AI.A enhances its aceeptability fior routine use by the medical profession, since the oral and topical runtes of administration are much more convenient than the patenteral. Filtli, lie womal and aboomal tissues that ean be photosensitized by the administration of $\angle L A$ are somewhat difterent from those that can be photosensitized by the administration of ITpIX, ItpD or Phowtin II. Consequently, Al. $\Lambda$ would be usefill in elinimal situations in which the other photosensitizers are not.

Thus the present technigue is not merely anolher way to do what can be done already but is, in fact, a significant advance in therapeutic capability.

Without further elaboration, it is helieved that one skilled in the att ean, using the proceding deseription, whilize the present invention to its fallest extent. In carrying out the method of this invention, the quantities of materials utilized are not in themselves critical and can be varied within the seope and spriril of the invention. The following examples are merely illusiralive ol preferred embodiments and not intended to be limitalive of the remainder of the disclesture in any way whatsoever.

## EXAMPLE 1

## Tong Term Pholodynamic Endometrial Ablation

Wals were diviked inlo 2 groups (finnd 7 rats/group) arrd their merine horns were injected with 4 or 8 mg $\Delta L \Lambda$. Fxample 1, of 0.S. application Ser. No. 08/082,113, Ciled Jונ. 2], 1993 (U.S. Pal. No. $5,422,093$ ), wats repealed with the exception that all rats were expesed to light and the time from $A L A$ adminisiralion lo breeding was extended from 10-20 days in $60-70$ days. All wher proceclures were identical os Example 1.

Breeding 60-70 days atter photodynamie irealment with 4 mg Al A resulted in no implanations in the uterine horns treated with Al A ( $n=6$ ) whereas feluses were found in all control urerine horns treated with saline ( $n-6$ ). 'lhese results confirmed the long termendonetrial ablative eftect of PDI: In the groups of rats ( $\mathrm{n}=7$ ) treated with $8 \mathrm{mp} \mu \mathrm{L} / 2$ of 7 became pregrant in $\Lambda \mathrm{L} \wedge$ treated urerine horns compared with 7 of 7 pregrancies in the staline treated horns.

## Hislology

In order to show normal uterine histology of a nonpregnant uterine lorn contralateral to a pregnant uterinc hom on uterinc hotn was ligated at its distal end prior to breeding. At gestation of $10-15$ days monpregnent uterine herris were harvested and histologically proebssed. The uterine muensa was lined with columate opithelium and tbere was hypertrophic infolding of endometrial tissue with torluous glands. In enontrast, prior photodyoanic trealment with AT A cortsistently resuled in an atrophic endomelrium despite the bormonal stimulus ol the eontralateral pregnancey.

## EXAMPIE 2

The precedures of Example 1 (U.S. Pat. No. 5,422,093) were repeated with 1, 2, 3, 4 and 5 homi incubation periods using a lovel of 1 mM of AI.A. No signiffant lluorescence was nbserved in the myometrial samples or in the enclometrial samples incubated for 2 hours. Maximum fluorcscence wath olserved in the endometrial samples incubated for 4 houts.

Endometrial Fhorescence in vivo tollowing Topical Application of Al A in the Non-Human Primate

50 mg of AI A was injecled into the ulerine lumen of an adult, healihy, lemale rhesus, monkey following exposure of the uterus at laparolomy. A hysterectomy was performed 3 hours later and coss sectional slices inconporating codonetrial and myometrial tissue woe taken from the uterime specimen. These stices were subjected to examination hy fluarescence microscopy as in Example 2 and 3 above. Flusrescence was observed throughout the endometrium of all slices. No flmoresence was olberved in the myometrium,

The alove examples clearly illusirate that endometrial ablation in a range o厂 animal species, including humans, hy photodynamic therapy using, AI Acan be achieved with litte or mo damage to the underlying myometrial tisisucs.

## EXAMI'LL 4

## Detection or Treatment of Yeast and Fungi

## A. In Vitro Studies

Clinical joblates of Candida albicans, Cardida glabraia, and Cryphoconcus neoformans and environmenal isolates of Penicillium species, Aspergillus niger, Aspergillus funtgalas, and Allernaria species and Sacchamomyces nerivisiae (brewer's yeasi) oblained from the clinical microbiology laboratories of Kingston General Hospital, Kingsion, Ontario, Canada were used, lhe organisms were plated, and during rapid growih were treated with various concenttations of Al.A varying from 1 mM Io 100 mM by flooding or by using diffusion wells in the agar, while the isolates of I'cnicillinm and Aspergillus were treated with $40 \%$ or $80 \%$ solutions of AI,A in water and the Penicillium species, Alternaria species, Aspergillus riger and Aspergillus fumigalus were treated with $20 \% \mathrm{~L} / \mathrm{L} \Lambda$ in water via diflision wells. Treatment of the various fungi resulted in Пuorescence emission peaks that showed the characteristics of PpIX. Positive PpIX accumulation occurred in both molds and yeasis.

## D. In Vivo Sluchies

The procedure of Giger et al. Infection and Immunity 19 (2) 490-509 (Fobruary 1978) was used with the following modilieations. A elinieal specimen of C , abicans wats replated in blood agar so it was actively growing and left at room temperature for 72 hours. The sample was suspended in TSB to McTarland 0.5 turbidity alter which a 1.0 ml sumple was inoculated into an atrobie culture botle and left shakiog tor 24 hours on a $37^{\circ} \mathrm{C}$. rotor shaker A 10 ml sample was witherawn and emrifuged at 70 , ono rom for 10 minutes to separate the cells from the media, The supernate was discarded and the jeflet resmepended in 10 ml of l s. B . Serial dilusions ( $10^{-9}$ to $10^{-5}$ ) ware mate in and replicated twice on ayar and lelit to incubate for two days at $37^{\circ} \mathrm{C}$. The MeFarland 1.0 smple was entritiged and the pellet resis. pended in 1.0 ma butler bot injection.

On day zero an intradermal injection of the C. albicams suspension (about $7 \times 10^{\circ}$ urganismes $/$ ml saline) was made into the right flank of 5 adult hairless mice. The amount wats just enough to make a small vesiele under the skin, Lesions form by day 2, Latcr, some mice wore given a scond injection on the opposite side.

Three hours prior to their sacrifice, the mice were given $240 \mathrm{mg} / \mathrm{kg} \boldsymbol{A I} . \wedge(10 \mathrm{mg} / \mathrm{ml})$ by iniraperikneal injection,
with the exception of mouse \#3 which was used as a conleol. Flucrescence emission spectra on the live mice were taken every 15 mirnules (mouse $H 1$ readimgsevery 20 minutes) for 3 hours after injection on each lesion, ard al various control ateds of the mice-neck skin llap and lateral side opposite the lesion on mouse 5. Three hoours after the injection of AI $A$ the mice were sactiliced and the lesions were excised. The lesions in mice $1,2,3$, atid 4 were frozen in z-methylbutane cooled to the temperature of liquid nitrogen. The frozen lesions were sectioned and slides were prepared for spectral analysis or fluoresence microseopy, H and E staining lor histokgy, and Grocoll silver stains for fungi identification.

Primary ancl seconclary lesions showed increased PpIX accumalation relative to the control mice.

## EXAMPI_E 5

(1) Selective Induction of the Syuthesis and Aecumulation of $\mathrm{l}^{2}$ rotoporphyrin IX and/or Orher Lndogenous Porphyrins Within Partsites in vivo of in vitro.
In vivo If the parasites in question involve the skin, conjuneliva, oral mucosa, nasal mucosa, anal mucosa, or urothatiom, Al A may be applied directly to the surface of the alfected tissue. If the parasites are located at sites that are not suitable for topical application, an effective amount of AT.A is udministered systernically, either by mouth, by sulyculaneous injection, or by intravenous injection.
[n vitro-' 'he materia] susi]ected ol contaning parasites is ineubated under appropriate eonditions in the presence of an eflective comontration (generally around 5 miM ) of $A L A$.

## TXAMPIE $\mathbb{C}$

## In vivo Studics

The iujection of an ettective dose of 5 -aminolevulinie acid (ALA) into mice intected with $P$ ymehii leads to the accumulation of flumeseing and photomensitiaing concentations of pronoporphyrim within metabolically active parasiles. There is no such accumulation of protoporphyrin within uon-viable patasites, or within nomal erythrocytes or leukocyles. In paratitized arylhrocytes, the potoporplyyin acemmation is localized to the parasite itself.

Metaloolically active (viable) malarial parasites cat bo disinguished readily from parasiles that are inactive (dead), since only parasites that are metaholically active can synthesize proboporphyrin. In addition, metabolically active (vialme) malariad parasites can be killed selectively by exposing iufected blood or cell suspensions to photoactivating wavelengths of light, 'This procedure causes no significant danage to the seeompanying normal erythrocytes and leuknoyles, sinee they do not accumulate enough protopurplyyin do becorne photosensitized.

## EXAMPI.H 7

Demonstration, Quantification, and Analysis of AT AIncured Finoresence Within Firythrocyles Parasilized by $P$. youlii

Notmal mies wore given intraperitancal injections of bloge or spleen bells obtained from mice infocted with $\mu$. yontii. When the malaris was well esmblished, some of the infected mice were given a single intraperituntal injection of 250 mg of A A A per kg of loody weight. Coutrols ineluced infeeted wice that were wot given $\mathrm{NL} \Lambda$, and non-infected mice that were given/not given AI A.

At various intervals thereafter, suspensions of blond and/; or spleen eells were examined by the following lechniques. Fluoresence Microscopy: Red lluorescence developed within parasitized erythrocyles ol mice given $A \mathrm{~L} A$, but mot
within any of the eontrols. This Huorsocnce was localized 1.) the plasmodia.

Flworescence Fkow Cytometry: Iarge umabers of crythrocyles in suspensions of cells lrom the penipheral blood and spleen of heavily paresitized mice given Al A developed red tluorescence. Cells from the contol mine were uniformly ncgative. This technique permilled the rapid delection and enumeration of erythrocyles that contained metalolicallyactive parasiles, and produced relative values for the intensily of AI A-induced flurresence in such crythrocytes.

Spectrophotnfluorometry: Flood and spleen eells from hesvily parasilized mice given AI A were washed and jeldoted by cenirifugation. Proloporphyriu wats the only fluorephere that was identifed by spectrophoto thuorometry. As expected, cell pellets from the control amimals contained only traces of pronoporphyrin.
Demonstration and (quatibalion of AT.A-Ituduced Plobtosedsitization of the Intra-Fryilhmeytic Slage ol $P$ P. yoelii.

Normal mice were given intraperitoncal injoerions of blood or splenen ells obtained from mice infected with $H$ : yoelii. When the malaria was well established, mome of the infected mice were given a single intraperitoneal injection of 250 mg of AI A per kg o「 body weight. Controhs included inferted mise that were not given AI.A, and mon-infected wice that were giventot given $A T A$.

At varbous intervals ithereafter, suspensions ol blood and on spleen cells were exposed to graded doses of photoactivating light. light-incluced lose of viahility of the $I$ ? yoedii was demonstrated by (a) loss of infectivity, or (b) loss of ability to acoumulate the flomescen cleavage product of . calcein-AM.
(A) Infectivity Assay: Mice infected with $P$ yoedii were given a standard dose of AT . A by intraperitoncal injection, Peripheral hlowd and/or spleen eells were collected atter a standard interval, exposed to standatid doses of photonctivating light (including a no-light control) and then injected inks normal mice. If the control (an-light) mice developed malaria and died while the mice given cells that had been exposed to a given dose of light remained free of mataria and lived indelinitely, this was considered to be evidenee that the light trested cell suspensions dicl not contain coough viable plasmodia to cause an infection.

For example, a Ballo/e mousc with advanced mataria ( $P$. yoellh was given an inlaturitonsal injection of 250 mg of AL $\Lambda_{2}$ per kg of body woight. Four homrs later, its spleen cells were suspended in isotonic saline. Half of the spleen cell suspension was placed on ice and ekxposed to photoactivating light. (waveband $600-700 \mathrm{~mm}$, intensity $100 \mathrm{nW} / \mathrm{cm}^{2}$, total dose 540 J (emi'), while the other half was kept on ice in the dark. Balb/e mice were injeeted intraperitnomally with either the light treated or untreated samplo, Survival of the mice was followed for 90 days, FiG. 1 illustrates the suration of survival of individual mice following the injeclion of spleen cells infected with $P$ : yodii.
(B) Photosensitization stuclies (Lx vivo stuclics, direct photeradiation): A group of 4 hairless female mice were used. Two mice were infonterl with $P$ yoctii and 2 other mice wese nou-infected, Mise infocted with malaria were usually in the sth tay following incoulation with plasmodia Mice were divided in twa groupls: one group wat leated with Ar A, the control group was mot treated with $M A$.

Both groupls were then kept in the dark for a poriod of 3 hours. Mice were then sacrificed (nvertoses of chloroform) and inlected blood eells wore obtained from homogenized spleen. Spleens were bomogenized in 3 ee of isoturio saline sollution. From this homogenization 1 es was laken and diluted in 24 es ol butunie saline solution, then from this
dilution 1 ce was taken and placed in tost tubes (a ton.al of 8 lubes). Four lulyes were kept in clark and four lubes were photoirradiated.

The souree of light was a tumpten lamp with a filter for red light ( $600-700 \mathrm{nan}$ ). The beam was 10 cm in diameter and the fluence about $70 \mathrm{nW} / \mathrm{en}^{2}$. 'Ine samples were placed in ice on a turntable ( 3.3 rpm ) to assure a uniform distribution of the light in the targel cells.

To delermine the viability of the plasmodium atter heing irradiated, the contents of each tulbe were itoculated into hairless mico, sand then the mice were libllowed for survival.

Control groups for light alone but not $\Lambda \mathbf{L}$ 人, and Al A hus not fight, were also used to make sule that photsensitization was due to AI A plus lighit.
(C) Spectrnporofluorimetric studiti: A group of 8 hairless 1. female mice were used. Four mice were in the 8 th day posel incoulation with F$/ a$ whodiun youlii with $35 \%$ parasitemia and 4 nomal mice were normal (non-infected). The mice were divided into 4 groups of two mine in cach.
i) 2 infecled mice were given an $11^{2}$ injection of $2.50 \mathrm{mg} / \mathrm{kg}$ of $\mathrm{A} \mathrm{L} A$ in $\mathrm{P}^{2}[3 S$.
ii) 2 nomal (non-infected) mice were also injected II with $250 \mathrm{mg} / \mathrm{kg}$ of $A T$ A in PBS.
iii) 2 refecence controls were included: 2 infected mice wilh malaria and 2 unn-infected mice, none received $\Delta L A$. All 4 groups were kepl al normal room temperature, in the dark, for 4 bous and then sacrificed. Mice were anesthetized with chlorotom and then blood was collected by cardiac puncture (lueparinized sytinge with $20 \mathrm{G} \mathrm{l}^{\prime}$ needle). Approximately 0.4 ce hlood wats colleced and transfered to if 5 ce test tube, kept on ice and in the dark. Tent tubes were Unei centrifiged for 10 minules. Using a spectiophotofino rometer sel for excitation al 410 im ath fluoresence emission at 6.35 m ., fluoresenuce meaturements were taken of the supernant and the pellet.
Hemolysis wili $1 \%$ sapomin was carrica out in samples after the firsi fluorescence weasurements, and the free Plasmolia are cuntrifuged to form a petlet. Then fluoresence measurements were taken from the pelfot and the supernant. Protoperphyrin lluorcseence was detected only in Plathodial pelfet derived from infected mice given $A l$ A.
(D) Fkow eytumeter studibs (Iharmacokinetic studies): A group of 4 hainless female mise were used. Two mice were infecled with $P$. yoelii and 2 other mice were not infected. Mice intected with malaria were usually in the 8 th day past inomation with the infected plamodia. NLA was given directly to the mice ( $250 \mathrm{mp} / \mathrm{kg}$ intraperitneal), then 2 dropes of whole blood were withdrawn at regular intervals of time from the tail of cach monse and placed in 5 ce llow cytometer test tutes containing 0.5 ec of RPMI 1640 and then analyzed by the How cytometer to follow aceurnulation of PpIX. Only infected mice given AI A developed fluocescence in their erythrocyles.
For the in vitro studics, no $\mathrm{AI} A$ was given to the doner mice. Two drops of whole blogit were withdrawn from the tail of each mouse and placed in a 35 mon petri dish contaning 3 be of RPMI I640 withoul phenol red.
i) a petio dish montained infected whole bloud with 5 nm AI, A in RPMI.
ii) a seconsl peiri dish containel iufected whole blood plus RPMT bul not ATA.
iii) a third petri clish contained nomal whole harod cells with 5 mM Al A in RJMI.
iv) a Courth petii dish containct normal whole blood cells with RPMI but aut $A L A$.

All petidishos were incubated at 37 Celsius and rown air enviroument. Samples ( 0.5 ce) were taken at regular inter-
vals from these incubated petri dishos to bo analyzed in tho flow cytometor to follow aceumulation of PplX. Only colls from infected mice developed $\mathrm{P}_{\mathrm{p}} \mathrm{lX}$ flunreseence when incubated with AI.A.
Application of ALA-Indued PrpIX IIIT of the Preatumen of s. Malaria.
Malaria is caused by infection of the host with unicellular prasites known as plasmodia. At one stape in their lite cycle, the plasmodia infect and develop within crythrocytes of the peripheral blood, spleen, and/or marrow. They may infect the liver and certain other organs also.
Of the numerous species of plationclia that have been identified, only a few can infect humans. Plasmodia that cause malaria in mice but not humans provide a sale and convenient model for laboratory studies of malaria.
'Incse cxamples iovolve the murine malarial parasites Phasmodium youlije (lethal strain) and Flasmiodium chathrudi (non-lechal strain) as models for human malaria.

## In vivo Photoscasitization

When mice infected with the murine malarial parasiles $P$ : yoe/ii or $P$ chaboudi were given an adequale dose of S-Aninolevulinic Acid (AT A) hy inlraperibsmeal injection,
what appeared sprectrosespically in bee protergerphyrin ( P pIX) aceumulated in many of ine phasmoctia within erythrocytes of the peripheral blond, spleen, and marrow, However, significan concentrations on $\mu_{\mathrm{p}} \mathrm{X}$ did nol secumulale wilhin the onn-infected erylbrocyles or within the greal majurity of the leakeryless in the infected mice.
a fluorescent material that may have been a complex of protoporphyrin with a light metal (perhapti zinc protoporphyrin) sometimes accumulated in assweciation with the $\mathrm{P}_{\mathrm{P}} \mathrm{IX}$.
following exposurc to an adeguste dose of light of wavelengths within the photoactivation spectrum of $\mathrm{P} p \mathrm{DX}$. the plasmodia that had been exposed to $A L \Delta$ lost their normal ability to atesumulate calecin when exposed to calcein- AM , aud also lost their ability to cause malaris when injected into recipient mice. lowever, the noninfected erychrocytes and the kukocytes in the same coll suspensions showed no morphological evidenee of damage following exposure to the photonetivating lighl.

## In vito Photomensitization

When preripheral blood, spleen, or bone marrow cells; from miee infeeted with the murine malarial parasites $P$. yoelii or $P$, chabaudi were incubated under suilable conditions in the presence of an effective concentration of $\mathrm{AL} A$. what appeared spectroscopically to be protuporphyrits ( P pIX ) accumulated within many of the plasirnodia in erythrucyles of the peripheral blood, splecn, or marrow. llowever, significant concentrations of $\mathrm{l}^{\prime} \mathrm{pIX}$ did not accumulate willin the non-infected crythrocytes or within the great majority of. the leukocytes in the infected mice.
The exposure of metabolically adive $P$ youelii or $P$ : chabaudi to an effective concentration of ALA under suitable conditions in vivo or in vitro leads to the preferential accumulation of חusorescing and photosensitizing conceutrations of PpIX in those plasmodia, but not in non-infected erythrocyles or in the great majority of the leukocytes in peripheral blood, splech, or bone marrow cell suspensions.
Plasmodia-specific AI.A-induced lluorescence can be used to detect and quantitate metabolically active malarial
them in vitro or in vivo to an alequate dose of pholoactivating ligh.

## EXAMPIE K

## Alane

Aene is an inflammatory follicular papular and pustulat enuption involving the skia, Athe freatrment of acme using the method of the instant invention would be considered to be the tratiment of cither (a) condogennus lesions of the sebaceous apparatus of the skin due winalalolicular ly perkerawsis or (b) exogenous bacteria cells present in the ache lesions, particularly Propimibacterium (Coryacbacterina) anne.
Evaluation of PyIX induced llumestence in 8 subjects with rnild to moderale trumeal acene was performed. Bacterial infections ane frejuenty associated with lesions of aene, e.s., $P$. ucres. Followiug cvaluation of baseline acne lesion Hiverconnce, $\Lambda L A$ solution 10 and $20 \%$ was appjed to 10.5 $\mathrm{cm}^{2}$ sites on the chest or back of voluntecrs and evaluated at times $0,3,8$ and 24 hours allen $A L \Lambda$ applicalion. One site of ench eoncentralion wass also oecluled with upapue film for 3 hours and evaluated at similar time points fior comparison with unowluded sites. Flumescence of both anaciform lesions ass well at surrounding normal skin was
 4 -extemely severs: and docamented phongraphically.
In all sulpjects, unoceluded sites had a gradual increase in $\mathrm{I}^{1} \mathrm{pIX}$ fluorescence that was dose ciependent, maximum ut \& hours, specific tor acace lesions and spared normal surrounding skin. These sitcs had woak or no fluoreseence by 24 hours. Little difference in fluorescence intensity was noted by lesion type (corncdoncs vs papules vs pustules) in the same subject, however, time to maximal fluoreseence and traximal fluorusemed intensity was variable from subject in subject. Lesions with surrounding erythema (larger papules and pustulcs) developed fluoreseence extending to the elinical limit of crythema. Vehicle control sites jemained al bascline. In contrast, oceluded siles developed $\mathrm{P}^{\mathrm{p} I X}$ lluoiescence in both acac lesions and nomal surrounding skin that persistod longer than unoceluded sites and remained presemt al 24 hesurs.

## EXAMPIE:

## Cutancous Fungal Infections

Historically, fungal infections have not attacted as much attention as lacterial infections. 'This focus of research has been due to a number of factors, most notally, the high incidence, the degres, anct the oftect of bacterial infections in humans. However, this trend has changed in the past couple of decades. With the increasing mimber of imenumemompromiscet paticnts, both hy ialrogenic (chemotherapy) ausl discase (ADS) canses, the incidence of fungal infections has increased. This has coincicled with an increase in the monbislity and montality ralus due to fungal infections in the last desade.

Fungal inlections calm be divided into thee eategories: cutaneons, subentaneons and systemic. While the systemic inlections (blatomycosis, candibliasis, te) have more serious scquelac, the cotancous infections are much more prevalent lerween 1971 and 1974, fingal infections hacl a
reported tate of sgomo persons in the U.S. with the nouinvasive cutancous infections responsible for $90 \%$ of the cases. ('this is the number of reportent cases. Heennse of the non-life threatening sequelac of cutancous infections, the: actual incident rale is likely much higher.) They were alson cited as the mosit common skin infection.
Gutancous infections can be further divided into thee sub-categories; superficial, dermatophytoses and dermatomycoses. Superficial infections do mot penctate the outer layer of the skin and do mot involve cither the bair or aniss. Tinea niga, black piedra and white pedra are examples of superficial fungal infections. Dermatophytoses ane infeetions of the slim, lair, and nails, and include all hayers of the stratum cornemm. These inlections are cansed by dermatophytes, fung which ramly cane disseminated infertions. 'These organisms elease keratinases, which likely explains their lecalization within the keratinized tissues. These fungi cause litlle mortality, but are a major cause of morbidity worldwide, and in North America a major expenditure of time atal money. These infections predispose their hosis to bacterial superinfections. Dermatomycoses are culaneous infections caused by nou-dermatophytes ad have a greater chame of invasion and dissemination (e.g. superiicial cauclidiasis, mycetoma, sporotrichosis), cspecially in an immunocompromised hosi. However, as staled before, the greater majority of fungal infections are caused by the non-invasive dermatuphyles.

## Dermatophyles

Dermatophytes inclute Trichophyton sppp., Microsponum spp. and Epidermophyton spp. genera. Lewlogically, these fungi are anthrophilic (human to human transmission), woophilic (amimal to human transuission) and goophilic (soil to bundar (ransmission, pussilly via an amimal internediary). Typrically the anthrophilic fungi causc little inflammation (increasing the likelithod of chronic infection) and the zoophilise fungi cause a fimmoular reactinn.

Dermatophyloses ate named "tinca" followed by the body levation (e.g., tine a capitis is an infection of the head). Table 1 lists the dermatophyluses and their cansutive dematuphyte as founc in a survey of cermatologieal visits by U.S. An any personnel. 'This data has been supphrted by data collected from surveys of students, inmales, and bher armed forces personnel in the U.S. The most commen dermatophyte worldwide is $T$ rubrum (survey of major detmatologic conters).

TABLE 1

|  | Incidence ol Dermatophytoses <br>  |
| :---: | :---: |
|  | Inci <br> dence Most common De manophytes (in 1 . to K ) |
| tinna podis | 44\% T. mantagraphyter, T. numum |
| thria mighthen |  |
| tintes cimatis | $15 \%$ T. rubrum, T. mentugrophyex, E. foceoswm |
| tinea cormis. | 13.3, T. Ruhrum |
| cinta harhus: | 4\% 'T. mentagmphytes, T. werrucosum |
| tinea cupilis |  |

## Clinical Presentation

Chese infections are tont life threatening but they can canse a significant amount of dismonfort. 'Typically they cause socaling, fissuring, peeling, itching, burnimg eryhthem, and in some circumsiances, maceration. Tinea capias usu-

## 20

ally causcs reversille lair loss. T. montagrophytes and 2 verrucosum can produce a violent inflammatory reaction. As well, these infections are not pretty and can have serious acsthetic consequences. The outeome of these infections is cither a spontancous cure, a cure by medication, a treatable chronic condition, or a persistent intention despite medication. Thon the presentation and suterme is a function of the dermatophyte virulence and the hosis's delense capabilities. Immenocompromised individuals invariably fare worse than their immonocompetent counterparts.

## Truatment

Dermatophytosos can be theated topically or orally. The advantage of treating topically is that more ageressive (toxic) therapy can be employed, whereas orally, less toxic drags ate reauired. However, topical drags can cause itching, burning, reduess, and sensitization of the infected area. Oral therapy has the advantuge of gaiming access to tissue sites nommally unatlainable to topical therapy (i.e. the nail bers). Lo gain aceess in the site of action, both routes must overcome the body's natural defenses to foreign moolcoules since none of the drogs used are endogenous molcoules. The imidazoles and triazoles are used topically and ketoconazole and griscofulvin orally. Ihowever, keboconazole has a large number of side cfticets, especially if used for a long period of time, and $X$ rubrum and $T$, tonsurans have shown resistance to therapy. Both oral regimens require carctul monitoring and sone patients may not be treated bceause of contraindications.

Antifuagal therapy depends on the thickness of the sitc infeeted. 'Iinua cruris and eorpis require a shorter treatment time than tinca manum and tinca pedis hecause the skin is thinner in the groin and on the body ass compared to the hands and feet. Infections lowalized to the bair follicle roots require 4 to 6 weeks of trealment (root-3-4 man under the skin surface, at 1 rmm/week growth). 'The fingernails require 4-) months of treatment, and the toenails, which grow even skower, require $9-18$ mosnthes of reatroent. Due to wearing shoes, the foet and tocnaik sre also subjected to an ervironment which is conductive to lungal growth (warm, mevist), making it more diflieult to eliminate the infection.
linea minuinm or ouychonyeosis has heen particularly froublesome to treat. Treatment reginems can last as long as 18 months, with eonsiderable time and money invested in the cure. Nail avulsion (removal) is often included in the regimen but may cause considerable postnperative ditenmfort Even so, buly a $75-80 \%$ cure rate can be notained with fingernail infections. The results are more bleak for tuemail infections ( $25 \%$ cure rate). If more than one nail is involved, a permaneme cure is unlikely. It has been estimated that at cast $15-20 \%$ of the U.S. population between the ages of 40 go have onychomyeosis.
Clinical Application of AI.A-Induced Phomsensitization k Chronic Coonsil Infection with Termatoptayte (Trichophyton Species)

An achult mate presented with a chronic slematophytic infection involving the nail of the great toc. The nail itself was badly defonmed ats a result of the infortigu. The surrounding hissues showed widence of chronic low-grate inflammation.
^ $20 \%(\mathrm{w} / \mathrm{w})$ solution of 5 -aminolevulinic acid ( $\mathrm{\Lambda L} \mathrm{~L}$ ) in an sili-in-water emulsion (Glaxal Base) was applied to the tocoail and surmunding tissums, and then covered with a water-resislant phastic dressing (Tegaderri). Four hours later, the Tegaderm and residual crean were removed and the whole ares exposed to photactivating (red) light.

The palient expericuced a typical subjective response while the toe was being exposed to the light-itehing,

## 21

stinging, and a sensation of mild hurning. Upon conpletion of treatment, the toe was erythermatous and somewhat edematous. This gradually decreased over the noxt fow days.

Over the mext fow months, all clinical evidence of the lungal infection vanished. The toenal is now growing withoul deformity.

## EXAMPIE 10

The following arganikrns accumulate fluoroscing and/ar photoseusitizing woncentrations of PpIX when exposed to exogenous Al A:
(1) Protoma
(a) Leishmania-l. donovari
[ AL - A-induced thorescence]
(b) Malaria-Plamuodium yoedii:
[AI.A-induced lluoresernece]
[AI.A-induced photosensitization]

## Plasmudiom chutobadi

[AI A-incluced fluoresenne]
[ALA-incluced phorlusensitization]
(2) Werms
(a) Nematules -Lumbricus terrestris (dewworm)
[A[A-induced Huoreseence]
[ALA-induced photomensilization]
Finterobius vonnicularis (pinworm)
[AI A-induoed fuoresence]
[ $\Lambda L \Lambda$-induced photosensitization]
Ilasmodium yoetii is a malarial parasite that can inlect and grow progressivety to produce a lethal form of malatia in susceptible strains of mice and rats. 'The inventors have found that, when normal mice are injectud with sandard numbers of blood or spleen eells oblained from donors intected with $A$ yoelii, they dic of malaria 10 to 20 days after such injection. This mouse model is applicable to the study of malarial infections in humans, including $P$. vivax, $P$. falaiparam, $P$ malariae, and $P$ ovale.

What is clamed is:

1. A methoul for treating in a Luman patient a ruanmatignant hyperproliterative skin lesion that preterentially accumulates a photoactivatable porphytin, comprising administering to said humen patient in aece thereol an elfective amount of a precursor of protoporphyrin IX thereby accumulating therapeutic levels of said protopor. phyrin IX, and therealler exposing said skin tesion to light cupable of photoactivating said protoporphyrim 1 X .
2. The method of cledim 1 wherein said precursor of protopotphyrin $1 X$ is administered topically.
3. A method for detecting in a humbu pationt a nonmalignant, hyperproliferative skin lesion that proferentially accumulates a photosclivatable porphycin, eomprising
administering to sajo hmman patient in need thered an cticative amount of a ptcentar or protoporphyrin IX therely accumbatiog detectable levels of sajd proteporphyrim IX, and thereafter exposing said skin lesion to light capable of photoactivatiog said protoporphyrin IX.
4. "The methos of clam 3 wherein said precurgor of protoporphyrin IX is administered topically.
5. The method al elam 1 wherein said precussor is 5-aminolevolinic acid.
6. The method of elaim 3 wherein said precussor is 5-aminolevalinic acid.
7. A method of treating a non-malignant, hyperpmliferative skin lesion in a human patient in which protopophyrin IX is produceol fom 5 -aminolevulinit acid, comprising
15 expusing said skin lesion in said human patient to a wavelenght of light within the photoactivating spectrum of pro10porpilyrin (X.
8. A method ol treating a non-malignant, hyperproliferytive skin lesion in a human patient in which protoporphyrin
To 1 X is produced from 5 -aminolovulinic acid, comprising exposing said skin lexion in sad human patient to a wavelength of light withiu the photoactivating spectrum of protoporphyrin I $X$, wherein suid light is gemerated using an antiticial light worce.
25 9. A method according to elaim 7, whereim said light is only within the athorption spectrum of protoporphyrin IX.
9. A muthod accordiog to claim 7 , wherein said wavelonglh of lighe is limited to the group of wivelenglhi ennsistites of 350 to 700 nanomelers.
10. A melhoul aceording to ctaim 7, wherein the photoaclivating light is limited to che cot and blue regions of the spectitm.
11. A method of treating at non-malignant, hyperproliferalive skin lesion, comprisine
(a) aclmimistering to a human pationt a compound that induces accumplation of protoporphyrin IX in said skin lesion and then
(b) exposing siald skin lesion to a wavelength of light within the photwactivating speetrum of protoporphyrin IX.
12. A method according to alam 12, whercin said waveleagth of light is generated usiog an arlificial light sorure.
13. A method according to claim 12, wherein suid wave. length of light is limiled to the group of wavelengths consisting of 350 to 700 nansmeters.
14. A method according to claim 12, wherein the photo. getivating light is linnted to the red and blue regions of the spectrum.

# Exhibit B 



Harper Laser Clinic specializes in Non-Invasive to Minimally Invasive Cosmetic Medicine. We are dedicated to providing our patients with the latest advances in cosmetic procedures, utilizing cutting edge

## Our Specialties include:

Laser Hair Removal

Laser Veīn Removal
BOTOX
Collagen \& Hylaform ${ }^{(8)}$

Laser Skin Rejuvenation
Chemical Peels / Designer
Peels
Sun / Age Spot Removal
BLU-U® Blue Llght Acne
Therapy
Photo Therapy with
Levulan (\$)
Skin Care Products

## Why Choose Harper Laser Clinic...

- We provide Fast, Safe, Permanent hair reduction utilizing the latest laser technology.
- We can safely treat ALL skin types and tanned skin.
- We are not part of a big chaln of hair removal centers. We are a privately owned medical facility. Patients can expect personalized, professional service which is customized to their needs.
- All our procedures are performed only by a physician. (No technicians!) Every detail is centered around safety and quality care for each patient.
- Not all lasers for hair and vein removal are the same. We use a real laser (as opposed to other "laser centers" that use non-laser, light based devises). We offer what we believe is the best laser technology for removal of unwanted hair and unsightly veins. Our laser received many awards including: Most diverse aesthetic laser on the market, Best hair removal laser for dark skin types, Best laser for leg veins.
- Patient satisfaction is our \#1 priority.
- We don't make unrealistic promises. Every procedure is clearly
 explained in detail to our patients prior to any commitments.
- We are open on Saturdays and some evenings to accommodate your schedule.


## Announcements:

We have joined the Magic Family. Listen to Jim Paolucci talk about his procedure on WMGC Detroit Magic 105.1 W. FM, weekdays 3:00 pm-8:00 pm

Jim recently had his first treatment for laser hair removal on his back. Click here to see


Today's Hils \& Yesterdoy's foyerites
"Tune Up Man" from JAMZ 105.9 FM is also a patient of ours. He's getting hair removal for the front of his neck to get rid of those annoying razor bumps! Listen to him talk about his procedure weekdays from 10:00 am-2;00 pm.


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## INTRODUCTION



PDT is a light treatment performed with a topical photosensitizing agent called Levulan (aminolevulinic acid or ALA). When Levulan is applied it is preferentially absorbed by abnormal cells, pores, and oil glands. This makes these areas more susceptible to the light. This technology has allowed us to use it to treat pre-cancerous lesions called actinic keratoses, sun damage, sun freckles or pigmentation from sun damage, and fine lines. It can also be used to reduce oil glands and minimize the appearance of pores. We are also Investigating PDT to treat keratoses pllaris and other skin conditions.


WHAT ARE THE BENEFITS OF PHOTODYNAMIC THERAPY?
Photodynamic therapy is an effective treatment for actinic keratoses, which can eventually turn into skin cancer. Cryotherapy is not effective

when the actinic keratoses are diffuse over a large area. Topical chemotherapy can be used in such cases, but results in months of unsightly crusting. Photodynamic therapy allows effective treatment of actinic keratoses over large areas in one treatment with very little side effects. Photorejuvenation alone has already been effective in the treatment of rosacea, but by adding Levulan we are able to treat it more effectively and usually in fewer treatments. Studies for using photodynamic therapy for acne have yielded good reşults as well.

## HOW MANY TREATMENTS DO I NEED?

Photodynamic therapy is an effective treatment for a wide variety of conditions. It usually can treat these conditions In a series of two or three treatments is performed $2-3$ weeks apart with little or no downtime and very little side effects.

## WHO IS A GOOD CANDIDATE FOR PDT WITH BLUE LIGHT?

Patients with actinic keratoses or sun damaged skin and patient's looking to improve their overall texture and tone and reduce oiliness are good candidates for photodynamlc therapy. Patients with moderate to severe rosacea and/or acne are also good candidates for Photodynamic therapy,

## WHO IS NOT A GOOD CANDIDATE FOR PDT with Blue Light?

If you are pregnant, have been on Accutane within 3 months, or have an active cold sore you should not have this procedure. If you are taking aspirin or blood thinners, you may experience some bruising that can take up to 2 weeks to resolve. If you are tanned or have recently been exposed to the sun in the area you are having treated, you may be more susceptible to potential side effects such as blisters or crusts and/or your treatment may need to be reduced in intensity or postponed untll the tan fades. In addition, patients with pacemakers or internal defibrillators should not have thils procedure. You should advise us of all oral and topical medications that you are currently using prior to treatment as some medications are photosensitizing and PDT should not be performed while you are on them.

## IS THERE ANY DISCOMFORT DURING THE PROCEDURE?

During a PDT with BLU-U ${ }^{T M}$ procedure you will experience little to no discomfort. A slight warmth may be felt in the area being treated.

## WHAT ARE THE POSSIBLE SIDE EFFECTS?

Some patients may experience mild redness and/or peeling after a BLU$U^{\text {TM }}$ with Levulan treatment. This usually lasts for a few days. After Levulan is applied, the area will be sensitive to sunllght or other intense light sources for 48 hours. Therefore, it is important that following a treatment a sunscreen with zinc oxide and SPF\# 30 or higher is used regularly for the 48 hours following. Failure to do this can result in extreme redness and peeling that may last 10 days. Swelling can also occur and although rather uncommon, could last for a few days to a week.

## Advantages of PDT: For Diffuse AK (Actinic Keratosis):

- It is easier for patlents than liquid nitrogen, 5^FU, or Aldara because it is virtually painless, the side effects are minimal, there is a quick recovery time, and few treatments are necessary.

- There is reduced scaring and improved cosmetic outcome compared with other treatment modalities.
- It treats the whole area rather than just spot treating lesions, resulting in clearance of subclinical lesions and prolonged remission,
- It has both medical and cosmetic benefits.


## For Acne:

- It is painless - teenagers love that
- It improves compliance - teenagers often don't use their creams or take their pills
- No oral medications, such as antibiotics or isotretinoin (Accutane(ß), are necessary
- It kills the acne bacteria as well as reducing sebaceous glands - the two things that cause acne.
- It restores the skin integrity to a large degree, resulting in sometimes amazing improvement of not only acne but also acne scarring
- It can cause long-lasting remissions.

For Photorejuvenation:

- It can shorten the number of treatments from 5 to 3 .
- You can take away precancerous cells, something that you cannot do with traditional photorefuvenation.
- You can reduce the oiliness of sebaceous skin, or reduce sebaceous gland hyperplasia.

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Acne - BLU-UN - BOTOXA * Brown Spots - Chemical Peais - Collagen - Hair Removal Hylaformy * Laser Fanial - !evuland - Photodyndmir Therapy * Skin Care Products Skin Rejuverlation * Yein Removal

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Acne is not a just a problem teenagers have. Many young adults, people in their 20's and 30's, have trouble with acne that is hard to control. The BLU-U treatment - using ilght without drugs or antibiotics - may be something for you to try.

## What is acne, and why is it so hard to control?

There are many types of acne. The most common is called acne vulgaris. Almost everyone goes through a period of acne at one time or another. Just beneath the surface of the skin lie hundreds of tiny glands calied sebaceous glands. Their function is to collect and excrete the olly substance called sebum, to keep the skin healthy. However, the tiny pathway for the sebum to escape often becomes clogged, and a bacterium called p. acnes grows inside. In a short time, the area swells up, and forms a pimple on the skin. At certain times of life, this process becomes very active, troublesome and hard to control.


## Is there a new way to control moderate acne?

There are many treatments for moderate acne - creams, washes, medications - mild ones and very strong ones. However, your doctor may decide that certain medications, such as antiblotics, aren't right for you. Your case of moderate acne may not be responding to medications or other conventional treatments, It may be hard for you to keep up with complicated routines of skin care. Maybe it's time to consider something new.
What's the Blue Light Treatment? How does it work? There is now new treatment available that doesn't depend on medication. It's called the BLU-U Blue Light Photodynamic Therapy Illuminator. The BLU-U is a very special blue light that can kill the p.acnes bacteria in your


skin. Treatments are simple - you simply sit with your face close to the light for a short time at a schedule we can set up, usually a 15 -minute session about once or twice per week. The treatments may go on for five weeks or so. It's very safe, it's not hot, it's not painful at all. After some weeks, the blue light can control your acne, or clear it up for a very long period. The BLU-U was cleared by the FDA in 2003 for the treatment of moderate inflammatory acne vulgaris.

What Is Photodynamic Therapy (PDT) with Levulan(9) and BLU-U?
PDT is a light treatment performed with a topical photosensitizing agent called Levulan (aminolevulinic acid or ALA). When Levulan is applied it is preferentially absorbed by abnormal cells, pores, and oll glands. This makes these areas more susceptible to the light. This technology has allowed us to use it to treat pre-cancerous lesions called actinic keratoses, sun damage, sun freckles or pigmentation from sun damage, and fine lines. It can also be used to reduce oil glands and minimize the appearance of pores. We are also investigating PDT to treat keratoses pilaris and other skIn conditions. Patients with moderate to severe rosacea and/or acne are also good candidates for Photodynamic therapy.

## What are the Benefits of PDT with Levulan@?

- It is painless - teenagers love that
- It improves compliance - teenagers often don't use their creams or take their pills
- No oral medications such as antibiotics or isotretinoln (Accutane(ß) are necessary
- It kills the acne bacteria as well as reducing sebaceous glands - the two things that cause acne.
- It restores the skin integrity to a large degree, resulting in sometlmes amazing improvement of not only acne but also acne scarring
- It can cause long-lasting remissions.

Click here for more information about Levulan Treatment

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United States Patent
Kennedy et al.
[11] I'atent Number:
5,955,490
[45] Date of Patent:
[54] PHOTOCHEMOTHERAPEUTIC METHOD USING 5-AMINOLEVULINIC ACIT ANT OTHER PRECURSORS OF ENDOGENOUS PORPHYRINS
[75] huventors: James C. Kennedy; Roy II. I'ottier; Rolyert I., Reid; Arnold Suc-Murales, all of Kingston; Lewls L. 'Tomalty. Invcrary, all of Canada
[7.3] Assignes: Queen's University at Kingston, Kingston, Сапй

121] Appl. No.: 08/465,242
[22] Filed:
Jun, 5, 1995

## Related U.S. Application Data

[63] Cuntimation-in-pant of application No. 08/092,925, Jul. 19, 1093, abandoned, and application No. 08/082,113, Jun. 2 K , 1993, Pat. No. 5,422,093, which is a conlinuation-in-part of application No. 07/865,151, Apr. 8, 1922, Pat. No. 5,234, 140. which is it contimalion in pxart of application No. (177783,730), Oet. 24,1991, Pal. Nos, $5,211,133$, which i:s ; contimuation of application No. 07/386,414, Jul. 28, 1989 , Pat. No. $5,079,262$, said application No. $08 / 092,925$, is a contiouation of application No. 07/865,156, Apr, 8, 1962, abandaned, which is a continuation-in-part of application No. 117/783, 7.30 , Oe:1. 28,1991 , Pat. No. $5,211,998$.
[51] Int. Cl. ${ }^{\text {s }}$ $\qquad$ A6IK $31 / 40 ; \Delta 61 \mathrm{~K} 31 / 195$
U.S. CI. $\qquad$ 514/410; 514/561; 514/814; 514/843; $514 / 263 ; 514 / 895 ; 514 / 899 ; 540 / 145$; 562/567; 424/9.61
[58] Field of Search 514/410, 561 , $514,814,843,899,863,895 ; 540 / 145 ;$ $562 / 5677424 / 9.61$
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## Frequently Asked Questions

## What is acne?

Acne is a common chronle skin condition caused by inflammation of oilproducing sebaceous glands. Acne usually begins between the ages of ten and thirteen and persists for five to ten years. Acne is most common on the face, but can also occur on the back, shoulders, neck, chest, scalp, upper arms and legs. Acne develops when the skin cells don't shed properly - they stick together and plug up the pores. This blockage encourages an oll called sebum and a bacteria called p.acnes to build up in the skin pores, leading to inflammation.

## Can acne be prevented? How is it treated?

Acne is a fact of life for many adolescents. Approximately $90 \%$ of all adolescents and $25 \%$ of all adults experience acne at some point in their lives. In adolescents, acne breakouts are related to the natural release of androgen hormones, which occur during puberty. In adult women, atne is often related to the monthly menstrual cycle. Contrary to popular belief, acne is not caused by eating chocolate, greasy foods or bad hygiene. In the U.S. alone, over one billion dollars is spent on acne medications and treatments each year. In many instances, the money spent yields less than satisfactory results, is cumbersome or causes side effects. Conventional treatments include over-the-counter cleansers and lotions, prescription antibiotics, retinoids and hormonal therapies.

## What is BLU-U $\mathrm{U}^{\text {™ }}$ ? How does it work?

The BLU-U system uses a unique high-Intensity blue-violet light that activates the bacteria-fighting millitia called porphyrins. The porphyrins start a chemical reaction that produces peroxide, which destroys the $P$. acnes bacteria.

## What happens during a treatment?

With BLU-U, a typical treatment session requires approximately 15 minutes. Patients lie comfortably on a bed whlle the therapeutic light is applied; sometimes there is a slight warming sensation. Treatments are described as easy, even relaxing, but most importantly, safe and painless. Patients often listen to music while being treated.

How many treatments are required?
The doctor develops treatment plans based on individual patient needs. Typically, BLU-U treatments are given twice a week for four weeks. It is important to keep treatment appointments recommended by the doctor in order to see maximum results.


## How effective is BLU-U?

The BLU-U system effectively clears $70 \%-90 \%$ of moderate, inflammatory acne in four weeks. Results are especially impressive when compared with conventional treatments, such as topical creams and oral antibiotics.

WIII skin be blemish-free at the end of the BLU-U Treatments?
Though the inflammation will be gone for a majority of patients, some may experience residual redness where the inflamed blemish was tocated. The redness will dissipate over time.

Are the results permanent?
BLU-U treatments usually help controf acne breakouts for four to eight months; after that, occasional touch ups may be necessary.

## Is BLU-U safe? Are there any side effects?

BLU-U is one of the safest acne treatments available. The system's blueviolet light penetrates just deep enough into the tissue to reach the acne target, without adversely affecting surrounding skin. BLU-U has no known side effects.

Who can be treated with BLU-U?
Most people can benefit from treatment. BLU-U has proven to be very effective on mild to moderate, inflammatory acne. Pregnant women and people with photosensltivities should consult with their physician.

Do patients need to prepare their skin before coming in for a BLUU treatment?
Yes. The affected area should be washed with a mild acne cleanser, then rinsed with water and dried. Women should remove makeup before treatment sessions, and should only wear oil-free make-up between appointments. Make-up can be re-applied immediately after a treatment session has been concluded.

What type of skincare is needed between BLU-U treatments?
Patients should consult with their physiclans regarding the daily use of a non-irritating, anti-acne cleanser such as salicylic acid. Patients should avoid touching or picking blemishes at all times. The use of Salicylic acid enhances the effectiveness during and after treatments.

## Are BLU-U treatments FDA-cleared?

Yes. The BLU-U system is one of the first devices to receive FDA clearance to market for the safe and effective treatment of moderate inflammatory acne. After eight, regularly spaced treatments, patients show significant improvement with no side effects noted.

What are the features of BLU-U?
BLU-U Features:

- New paradigm for acne treatment
- High-intensity, narrow spectrum blue-violet light destroys P. acnes bacteria
- Short treatment regimen; 8-10 treatments over 4-5 weeks to achieve impressive results

- No side effects, no pain, no downtime
- Home - Contact us.

Acnc - BLU-U倍 - BOTOXE - Brown Spots - Chemical Peels * Collagen - Hair Removal Hydformg * Laser Faciai - Levulan: P Photodynamic Therapy * Skin Care Produrts Skin Rejuvematon - Vein Removal

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## Exhibit C

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#### Abstract

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Prinary Examiner-Gary E. Hollinden Altorney, Agent, or Firm-Foley \& Lardncs ABSTRACT Methods of detecting, and treating rapidy growing exogconous cells, such as Protista, or parasiles, that preferentially accumulate s photnactivatable porphyrin in which 5 -aminolevulinic acid or precursor thercof is administered to the palient, or contacted to the exugenous cells, in ath amount sufficient to induce synthesis fluorescence and/or photusensitizing concentrations of a prokporphyrin IX in the exogcnous cells, followed by exposure of the exogenous cells is light of photsactivating waveleugths.

12 Claims, 1 Drawing Sheet




13 $\begin{array}{llrr}9 & 10 & 11 & 12 \\ \text { GROUP } 2 \text { (MICE } & 8-13 \text { ) }\end{array}$


## 1

## PHOTOCHEMOTHERAPEUTIC METHOD USING 5-AMINOLEVULINIC ACID AND GTHER PRFCURSORS OF ENDOGENOUS PORPHYRINS

CROSS RLFERENCE TO RFTATED APPLICATIONS

'This application is a continuation-in-part of U.S. application Scr. No. 08/082,113, filed Jun. 28, 1993, (now 1J.S. Pat. No. 5, 122, (093, issued Jun. 6, 1995), which in turn is a contimution-in-part in U.S. application Scer. No. 07/865, 151, filed Apri 8, 1992, (now U.S. Pat. No. 5,234,940, issued Aug. 10, 1493), which is a continuation-in-part of U.S. application Ser: No, 07/783,750, filed Oct. 28, 1991 (ugw U.S. PaI. No. $5,211,9.38$, issued May 18, 1993), which is a continuation of U.S. patent application Ser. No. 07/386,414, filed Jul. 28, 1989 (now U.S. Pat. No. 5,079,262, issucd Jsn. 7, 1992 ). This pateut application is also a CIP ol U.S. Ser. No. (08/092,925, filed Jul. 19, 1993, ABANTOONED which was a continuation of U.S. Scr. No. 07/865,156, filed Apt \& , 1992, ABANDONED which sprplication is a contiduation-in-part of U.S. Ser, No. (17/783, 750, filed Oet. 28, 1991, now U.S. Pat. NO. $05,211,438$, referred to supra. The disclosures of all these applications are incorporated herein by icterence.

## FIEL.T) OF INYENLION

This invention rolates to the detection and treatment, by induced hurescenec and photsehemotherapy, tespectively, of certain tissuc abnorraalitics (both cancerous and monmalignant of endogenous and exogenous origin), hyperproliferative cells, and normal cells. The invention also relates Io the detection aud treatment of abnormalities in body fluids or suspensions of tissucs containing aboormal cells by indued lluorescence and photnchemotherapy.

## BACKGROUND OF INVENTION

'lissue abonmalities involving the skin usually are deteeted and assossed by a combination of visual inspection and palpation. In cortain clinical situations the sensitivity of the visulal inspection can be enbauced by tbe use of nomwhite light (either ultaviolet or a narrow hand in the visible), or by the prior application of a contrat-enhatacing apent such as didute acetic acid or eertain stains, Tissues aboormalities that involve surlices that canoot be palpated (such as the bronchi or the urimary blatider) may to visualized vja an appropriate scope. Some specialized seopes can detect induced fluorescence. If the abmormality in question is associated with a dillerence in cither the extent or the pattern of tissue vascularization, suct a scope may be used to detcrmine the limits of the area involved by the abnomality, hy visualizing au injected bolus of Huoreticein or other fluorescent material ats it passes through the vasculature of both the lesion and the adjacent normal tissue.

In addition, lluoresecnec-retecting seopes are being used experimentally to itentify areas of tissue that show stong porphyrin thoresecnec following the intravenous injection of exagenous porphyrins such ats hematophorphyrin IX ( $\mathrm{H} p \mathrm{SX}$ ), hemaloporphyrin derivative ( HpD ), or "dihematsporphyrin ether". Such porphyrins tend to accumalale semiprelerentially in malignant tissucs, but they also accumulate in tissues that are regencrating follnwing an irijury or in the rapidly growing lisumes of an embryo or fetus. Normal liver, spleco, and kidney also tend to accumulate these porphyrins. Using such compounds and finorseenec-detecling sopes, areas of malignant tisiov too small on be identifled by
slandard forms of visual inspeetion have leen identified in the bronchi and in the urinary bladder.

Unfurtunately, a dinically significant (photosensitizing) amount of porphyrin may persisi in the skin for at Icast two
$s$ wecks, (oceasionally for more than two months) following the iotravenous injection of IIpIX, IppD, ar a semi-puridied preparation of II LD , such as Phololrin IT. (Phorophrin is a registered trademark of Quadra Iogics, Inc. Vancouver, British Cohmbia, Canada.) This means that paticnis musil aveid exposure to sumlight (either dirbet, or through windew glass) for an inconveniently long poriod of lime postinjection, Understamdably, pationt compliance often iss puor, and accidental phototoxic "sunburn" is a common ocenf race in the weeks following a diagoostic or therapeutio injectinn of porphyin. Persistent photosensilivity is the major hazaral assivcialed with this tochnique, and is the main reaskin why it is not used mone widely.

The standard tredunents for cancer comprise surgery, radiontherapy and chemothcrapy. However, ather forms of Ireatrient are aiso known, including photochemotherapy or phonodynamic therapy (IDDT), bascd on the discovery made over 90 years ago that unicellular organisms, i.e., certain rapidly growing cells (such as celle of the I.ower Kingdom, now referred to as Protista), trested with certain chemicals will dic when exposed to light. Thus, synthetic porphyrins have been shown in viro io prolect cells from infections such as parasitcs, e,g., lyromastigotes and sphacromastigotes of Tyropanosoma cruzi, .T. Parasitol., 75(6) 1989, p. 970-976, and gram positive hacteria, mycoplasma and yeasts, Malik of al, J. Phowehemistry and lhotobiokigy, B. Biology 5 281-293 (1990). $I$ acm is known to, in vitro, produce ineracellular protoporphyrin in the presence of cxogenous AI A. Kieldstad, Conferenpe on Photosensitizafion and Photochemotherapy of Cancer, Det Norske Videnskaps-Akadeni, Mar. 16-17, 1993, Osilo, Norway,

P'D' is currently being used, on an experimental basis, to treat several dilferent types of eancer us well as certain non-malignant lesions such as protiasis. I'he putient is given a phow-aclivatable drug that has some degree of specifeity tor the tissue being treated. A tissue volume that includes the tanget tissue is then exposed to photoactivating light wo as w destroy the target tisue while causing only mild and reversible damage to the other tissucs in the same treatinent volume.

There are two main types of pholoclemotherapeutic agents in elinical usc at present. The first type, methoxypsoralens, are given systenically. Ultraviole light is essential to activale them. Localized sxposure of proralencontaining lissues to ultraviolet light inciuces a lucalized photochentical reaction that causes the drug to bind covaleatly to the DNA of living cellb, thus destroying their proliferative potential. The second type, porphyrins and related photosensitizers, are abso given systemically (by intravenoms injection), although occasionally they are given cither topically or by intralesional injoction. They can be activaled by visible (red) light. The lncalized exposure of porphyrin-containing tissues to such light ordimatily toos nos incluce a clemical babtion between cell components and the porphyrin molceules. Insikad, the porphyrins act as catalysts by trapping the energy of the photoactivating light and then passing it on to molecules of oxygen, which in turn are aiscd to an excited state that is onpahle of oxidicing adjacent molecules or struetures, Gell death is not caused primarily by damage in the DNA, but by damage to essential membiane structuces. "The gral of photochemotberapy is sometimes sure (nainly lio basal cell carcinonatis), but usually the gal is palliation though local control when

3
none of the standard forms of therapy are consirlered likely to ofter a significant degree of bencfit on the patient.

Methoxypsoralen (PWVA) therapy is used mainly for the trealment of psofiasis, lyut sometimes it is also nised to treat very supedicial ameers that involve the skio (manly myessis لungoites). However, there are two serious prohlermis with such treatments. Firsh, the procedure hats been (demenstraled in trumans to be carcinegenic. Scoond, the depth at which maligrant tissuc can be killed is limited to a few millimeters beluw the illuminated surlace. These poblems soverely limit the usctulness of the methoxyporalens for phutuchemotherapy.

5-Amino-4-oxopentanoie acid, alse known as 5 -sminolevulinic acid and us $\bar{\delta}$-aminolevulinje acid (" $\wedge L \Lambda$ ") has been alesoribed in the eross referenced patents aud patent applications dirst iet borth in this specilication for detecting and treating rapidly growing cellis. A. A has also betn reported for use in attenuating the growth and killius plents and insects when applied difectly to such organisms Lollowed by exposure to light, based on work of Rebeid et al.

Syuthetic porphyrins have also been used as photsethemotherapeutic agents in treating rapidly growing, c.g, rapidly dividing or rapidly metabolizing infectious cells, such as infoctious pathogens, including protomal parasitics, subh as Plasmodium fakiparium (which causcs malaria in humans), various other species of Plasmodia, Leishmania, and amocbas, pathogenic fungi, and microplasma, including the various parasitic forms, all such cells and organisros being referred to herein as Protista. 'The term Protista as used here and in the literature refers to the lowest orders of the animal and vegetable kingdorns, single eclled or collections of single celled organismis including: the cukaryotes, including proteroa, tung and algae, and the prokaryoles, which are bacteria and bluc-green algac.

At present, the porphyrias mosi cormmonly used for photochemotherapy are Hemamporphyrins 1 X ( $\mathrm{H} p \mathrm{lX}$ ), Hemaloporphytin dcrivative ( $\mathrm{I} p \mathrm{D}$ ) and various semipurified preparations of $\mathrm{H}_{\mathrm{p}} \mathrm{T}$ ) such as commercially available Fhotofrin(0. Il, a semi-purified form of 1 Ip ). When porphy. rius are used as photosemsitizers, cell death resultis trom damage to cell membranes. Conscquently, malignant traisformation is not a scrious prohlem. Morcover, since the visible (red) light that is used to photoactivate porphyrins penetrates tissue much more deeply thar docs the ultraviolet light that musi the used to photoactivate methoxypsoralens, the depth at which porphyrin-treated tissue can be killed is substantially greater. Also, since certain types of porphyrios show a significant tendency to accumulate preficentially in malignant tisuuts, it is sometimes possible to destroy maligasnt tissue without causing clinically significant damage to adjacent normal tissues.

The main problem with the systemic use of $\mathrm{Hp}\left[\mathrm{X}, \mathrm{H}_{\mathrm{p}}{ }^{1}\right.$ ) and Ihnofrin II is that photasensilioing concentrations persist in the skin for several wecks to several months following their adminisiration. Conscopuently, severe accidental phototoxis skin reaclions may oceur umpes the patient avoids exposure to sunlight (either dircet, or filered throngh wimelow glass) until the ennceruration of the photosensition in the skin has heen reduced to a harmiess level. At present, the problom of photosensitivity followiog the administration of porphyrins is handled by advising the patient to avoin any form of exposure to sunlight (or to very bright actificial lights) for a periocl of at least twn weeks post-injection, and in initiate subsequent expmatre of sualight vory cautiously. Not ali patient comply with these instructions, sinec it oflen
is quite inconvemient ly do soi, In addition, the use of a sumsereen with a high blocking fackor is recommended with warning that this will only recluce the hazard smmewhat, not eliminate it completely. In a lew cases, patients whose pholoseusitization persised lor more than a month posttreatment have been given large daily doses of beta-carotene over a period of scveral months in an atempt to provent accidental phototoxic damage. Finally, attempls have been made to reduce phototoxicity by applying the photoscnsitizer topically to a limited area.

Ifowover, another type of problem is encountered if HpIX or $\mathrm{H}_{\mathrm{P}} \mathrm{D}$ ) is applied topically in I)MSO (dinethylsulfoxide), Azonc, or wome other vehicle intendecl of mhance their diflusion through lissus, The pophyrins end to becone immobiliand whecover they happencil to be when the DMSO or Avore becomes clibuted hy normal tissue fluids to such an extent that the prophyrins can no longer clifluse through the tissue (or cenen remain in solution). Coosequently, the topical application of porphyrins ofteo is associated with a lose of specifibity for malignant tissues, anol normal tissucs near the site ol application may develop persistent photosensitization from the localized comentration of pophyrim.

## OMJLCI OF INVENTION

It is an object of the presunt inveution to provide a metbod for the detection of certain typos of malignatt abd toon-
 aboomalities by induced fluorescerice.
It is yet another object ol this invention to provide a phowdynamic (photosynthesizing) treatment method using an agent which can be administered either systemically or tupically which is not in itself a photosenthisizer but which induces the syathesis or aceumulation or both of protoporphyrin IX (PplX) and other endogcnous porphyrins, their precursors and their photoproducti, in rapidly growing cells, including abnomal eells in otherwise nomal lisisues, in vivo $\infty$ in vitro.
The terms porphyrin(s) and their precursors refer to compounds produced in vivo in the synthesis of herne and other endogenously produced photoactivatable compounds jncluding their photoproduck.

## SUMMARY OF INVFNTION

This invention is based on the finding that exogenously adminislered AT A and other precursors of $\mathrm{l}^{\mathrm{l}} \mathrm{plX}$ are metalolized in putients to $\mathrm{P}^{3} \mathrm{pLX}$ and that P PIX preterentially accumulates in rapidly growing cells, as contrasted with less rapidly growing cells. The rapid growth is correlated with the metabolic activity, so that the ditterential accumulation is allected by the relative metabolic activily letween different cells.

Ihis invention provides a method for delecting in a patient, a malignant or non-malignant lesion or abnormality which is sensitive to PpIX , namely those which prefecestially aceumulate $\mathrm{P}_{\mathrm{p}} \mathrm{IX}$, comprising administering to said patient an effective amount of a precursor of $\mathrm{l}^{\mathrm{p} p l X}$ in the biosynthetis pathway for heme so as is induce an aceumulation of PplX in said lesions, and exposing suid losions to light having a wavelength within the ahsopption spectnom of said $\mathrm{l}^{3} \mathrm{pIX}$, thereby t induce fluorescence in said lesions.

Another aspeet of this invention is a method for troating malignant and non-maliymant hyperpoliferative lesions of the skin, moneosa, endometriun and urothelium which are sensitive in $\mathrm{P}_{\mathrm{P}} \mathrm{IX}$ in a patient, comprising administering to saicl patient an eflective anount of a precursor of IPIX in the
biosynthetic pathway for leme so as to induce synthesis or accumulation or both of PpIX or other endogenous porphyrins, their precursors and their photoproducts in said lcsions, and exposing said lesions to light having a wavelength within the photoactivating action spectrum of said $\mathrm{P}^{1} \mathrm{plX}$ to thereby induce phoroactivation in said lesions.

Thus, the rapidly growing eclls involved can be cither malignant or non-malignant lyyperproliferative cells. The hyperproliferative celis can be aurmal, rapidly growing cells or abnormal cells in otherwise normal tissue. The abnormal cells in an otherwise пormal tissuc can include aboormal rapidy growing cells endogenous to the patient or abnorimal, rapiclly growing cells which are exugenous tos the patient. These rapidly growing cells that are exugenows bs the patient shall, for eonvenience, be reforred to herchy, depending on the degree of gencrality, as rapidly growing cxogenous cells, rapidly growing Prolista cells and rapiolly growing parasics cells,

One aspect of this invention is induction in vivo or in vitro of the biosynthesis and selective accumulation of fluorescing or photusensiticing concentralions of proteporphyrin IX or other endogenous porphyrins such as onpmporphyrin 1 , coproporphyrin III, uroporphyrin 1 , uroporphyrin IIl, or fluoresent metallopurphrins such its zinc protoporphyrin IX in Protisia and parasites of humans or other animals, by exposing said Protisha and ondogenous cells under appropriate eonditions in vive or in vitro to an ettective concentration of 5 -aminolevalivic aciel on ofler precursor of said porphryin(s) in the biosynthetic pathway for heme.
Still another aspect of this invention is the deteetion or cnumeration of Protista and parasites of humans or other animals, by inducing in vivo or in vitro (cx vivo) the binsynthesis and selective acoumulation of floorescing concentrations of proteporphyrin IX or other endogenous porphyrin in the parasiles as desuribed previously, and then using such fluorescence to detect, enumerate, or ohterwise quantify said Protista and parasites.
Yel another aspect of this invention is the selective killing of Prolista and parasites of humans or other animats in vivo or in vilro, by inducing the bionsynthesis and selective accumulation of pholosensilizing cincentralions of prosioporphyria IX or other eudhgenous porptyrin in the Protista or endogenous cells as descriled atwoue, and then exposing the pholosensitized puratites to an eflective dose of light of Wavelengths lyimg wilhin the photoactivation spectrum of said purphyrin(s) or of photusensitizing photerproducts of soid porphyrin(s) that may be produced during suid expesure.
By another aspect of this invertion there is provided use of a composition comprising a precursor of protoporphyriu IX in the biosynthetic pathway for herne for the manufacture of a medicament for treating malignant and non-malignant tissue abnomalitics and lesions.
In preferred aspects of this invention the preferred precursor of protoporphysin IX is 5-amino-4-oxo-pentanic acid, otherwise known as 5 -aminolevalinic acid, and a preferred wavelength of the photoactivating light is in the range of 625 to 670 nom, more preterably a red light of 625 to 640 mm .
Other objects, fealures and advantages of the present invention will hecxme apparenl from the following detailed description. It should be understood, however, that the detailed description and apecific examples, white indicating preferred errlogdiments of the invention, are given by way ol illustration only, since various changes and modifications within the spirit and seope of the invention will beenme apparent to these skilled in the art from this detailed deseription.

## DETAIIFD DFAC:RIPTION OF THE DRAWING

FIG. 1 illustrates the duration of survival of individual mice following the injeetion of spleen cells infeeted with $P$. yerelii. Group (1) mice were given spleen wells that had been exposed to ALA in vivo by then kept in the dark. The average survival of the recipients of these cells wis 15 days. Group (2) miee were given the same number of edels from the same cell staspension atier it had been exposed to phowactivaling light. All of these mice remained in good health for 90 days, al which time the experiment was terminited.

## DETAITES TOESCRIPTION OF PREFERRET EMBOTIMENT

Protoporphyrin $I X\left(\mathrm{P}^{\mathrm{p}} \mathrm{X}\right.$ ), a naturally oncuring photosensitizen, is the inuediate precursor of heme in the heme biosynthetic pathway. All molcated cells have at least a minimal capacity to synthesize P plX , since heme is necessary for the synthesis of various essential hemecontaining enzymes. Certain types of celles and tissues can syothesize celatively lace quatities of PplX, Under normal condilions, the synthesis of $\mathrm{P}_{\mathrm{p}}$ IX in such tissues is under such tight foerl-hack enntrat that the cells produce it at a rate just sufficient w, match their need for heme. Itowever, the usual rate-limiling sitep in the process, the synthesis of S-anninolevulinic acid, can be bypassed by the provision of oxogenous $A L \Lambda$, purphobilingen, or other precuser of PPIX. Centain tissues and ongans will then accumulate such a large excess of P pIX that they become both filuorescent and plutusensitive. At least in the case of the skin, the PPIX appoars to be synthesized in situ, $\Lambda L \Lambda$, which is commercially available trom Sigma Chemical Company and other souress and which is water soluble, can bo administerec orally, topicadly or by injection. The nral and parenteral routes load to the induction of clinically uscful concentrations of ppidx in certain benign and malignant tissues throughout the body, Ooly certain types of tissue synthesize and acoumulate elinically useful amounts of PpIX when provided with an execss of AI A. Ry the expression "rapidly growing coll" is meant hercin any lesion, abnormal cell or normal cell that exhihits cell growh substantially greater than that of the surrounding tisenes and that preferentially accumulates protopırphyrim IX from exogenous ALA. Thus, the cells include rapidly growing cells that are endogenous to the pationt and rapicly growing exogenous cells such as Pootista and pansito colls. The tom "rapidly growing cells" is also used here to include living, motabolically active cells as contrasted with motabolically inactive (dead ne dormant) cells such as found in the malarial applications of this inventinn.

At the presenl lime, irestmen of hasal cell, hasomquamous and squamous cell carcinotmas and other lesions of the skin, mucoss (respitalsry, digeslive, and vaginal), endometrium and urothelium is conternplated. Sites, which could include lesions or cellular almornalities, generally are those of epitbelial or endothelial origin inclucling but not limited to those involving (i) skin, ciculatory system and conjunctiva; (ii) the lining of the mouth, pharynx, esophagus, stomach, imlestines and intestinal appendages, rectum, and anal canal; (iii) the lining of the masal passages, nasal sinuses, masopharynx, trachea, boonchi, and bronchioles; (iv) the lining of the ureters, urinary bladder, and urethra; (v) the lining of the vagina, ulerine cervix, and ulerus; (vi) the parictal and visceral ploura; (vii) the lining of the peritoneal and pelvic cavitice, and the surface of the organs contained within those cavitios; (viii) the dura mater and meninges;

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(ix) any tissues or suspensions of body fluids containing abnomal cells, including blood, that con be made acecssible to photoactivating light cither in vitro, at cime of surgery, in vivo througli the skirn via sufface irradiation or via atioptical fibe inserted through a nocde; ( $x$ ) all exoccino glands and associated duets, including: manmary ghands, selhaconis glands, ceruminous glands, sweat glands, and lacrimal glands; mucus-secreting glands of the digestive, umgenital, and respiratory systems; salivary glands; liver, bile duels, and gall hladder; pancreas (exocrine component); gastric and intestinal glands; prostate; Cowper's, Bartholin's and similar glands. It is also contemplated that cell athormalities in the gonads (testes and ovaries), thymus, spleen, lymph nodes, bone matrow, Iymph and blood would also be teated aceording to the invention. Tunners of the nervors system or connetive tissucs (sarcomas) would also be treated accorchjag to this invention.

Treatment of non-malignant lesions such as genilal warts and psoriasis and of erdhmetrial tissues for indications such as conlraceplion, vaginal bleeding and endornelriosis is alson eontemplated.

As used hereit the terme "skint" includes:
(A) the covering of the exterral surface of mosit of the body, commonly termed the skin.
(B) the covering of the extemal gentalia: labia majora, labia minora, clitoris, and associated structures
glans penis, prepuec, and associated structures
(C) the covering of the zone of transition betwecn skin and the mucosa of the digestive system: anal verge vermillion border of the lips
(D) the lining of the excernal audionry meatus, and the covering of the external surface of the tympanic membranc
(E) all exocrine glands and associated ducts that are located at least partially within an epidermat surface deseribed above, or within the underlying dermis, such as the pilosebaccous units of the skin.
The icrm "mucoss" includes:
(A) the lining of the whole of the respiratory tract: naxal passuges and natal sinuses; nasal pharyix and associated structures larynx, vocal cords, and assorciated sitructures tractea, bronchi, and bronchioles
(B) the lining of the whole of the digestive that:
oral cavity and tongue
oral pharynox and laryngeal pharynax
excphagus
stomach
swall intestive
large intestinc, caceum, and appenclix sigmoid colon and reclum
anal canal
(C) the lining of the whole of the urugenital tract: urethra, bladder, and ureters renal pelvis and renal calyces vapinas, uterine cervix, utcrus, and Fallopian tubes vas deferens, seminal vesieles, ejaculatory duch, ampulla of vas, epididymis, and associated structures
(D) the conjunctiva and the liming of the toar duets.
(E) all exocrine glands and associated ducts that are located at least partially within one of the mucosal os surfaces described above, or within the underlying submucossa.

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This invention is cspecially usctul for the treatment of discases of Protista and parasitic origin, as definct above, particularly ucne, malaria and other parasites or lesions ctsulting from paactitcs.

The term "parasite" includes parasiles of humans and other animalo, including parasitic prolozona (holh intracellular and extracellular), parasitic worms (nematodes, trematodes, and cestodes) and parasitic eetopatasites (insectic and miles).
The parasitic Prolonea include:
malarial parasites of humane or other animals
malarial parasiles of humans
Plissmodium falcipar"um
Plasinoditim ovale
Plasmodium malaria
Plasmbdium vivax
leishmanial parasites of humants and or other unimals
Leishumanial parasites of humans
Leishmania tropica
Leishmarnia major
Leishmarnia aethiopica
Leishmunia brusillensis
Leishmania guyanencis
Leishmania panamenis
Leis.shmania peruviana
teeishmania mexicana
Ieissimania amuzonkinsis
Ieishmania pifanoi
Leishmania garnhami
Leishmania donovani
Leiststania infantum
Leishtmania chagasi
trypanosomal parasites of humans and/or other animats
trypanosomal parasites of humsis

## Irypanosoma crazi

Trypanosoma brucei gambiense
Trypurtesoma brucei rhodesionse
amovere parasites of bumans and/or other animals.
amocbic pacasites of humans
Entamoeha histrolyica
Nueglaria species
Acanthamocba spocies
Dientamoeba fragilis
miscellaneows protozoun pariasites of humans on other animals
miscollancous protozana parasites of humans
Toxoplasma gondii
Pnelumocystis carinii
Bubesia microti
Iscospora bechi
Cryptosporicinu.
Syclospara species
Giardia lambla
Balantidium coli
Blastocystis hominis
Microsporislia specios
Sacoocystis spucios
Some of these miscellagenus protozina cause self-limiting discase in normal people, but serious problems in IIIV pationts.
patiaitic nemulodes in humans and/or of ofer animals
parasitic nematoics in humans
filarial uematocles
Wucluceria bancofti
Brugia malayi
Brugial Linuri

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Onchncerca volvilus
Loa loa
Tetrapelalonema perslans
Telrapetalonema sitreplocerca
Mansonella wroardi
Dirofiliaria immilis
Disofilaria tenuis
Dimfilaria repens
intestinal nematoctes
Ascaris lumbricoides (roundwoim)
Necator americanus (hookworm)
Arcylostoma duoslenale (hookworm)
Strengyloides stercoralis (throadworm)
Literobius vermicularis (pinworm)
Trichuris urichiura (whipworm)
Trithostrongylus species
Capillaria philippiumsis
tissue nematodes
Trichinella spipralis
Aпasakis species
Pseudoterranova specics
Dracunculus medinensis
parasitic trematodes in thumans and/or other animals
parasisic tremakedes in humans
Schistosoma mansoni
Schistosuma hatmatohium
Schislosoms japonicum
Clonorchis sinensis
Paragonimus species
Opisthurchis species
lasciola hepalica
Melagonimus yokngawai
lleterophyes helerophyes:
l'asciolopis buski
purasitic cestoces in humans and/or other animals
parasitic costodes in humans
Thenia sabinata
Tannia solium
Hymenolepis species
Diphyllobothrium specics
Spirometra specics
Echinococens species
the method of this invention comprises the adminimat tion of $\Lambda L \Lambda$, other precirsors of Pp XX ad other endegenous porphysins, to the pationt. Jhe adnenistration can alse lee in viton as applicd to tissues of the paticnt, i.e., ex vivo. In ex vivo melhods, tisue containing the rapidly growing colls are removed from the palient, an effective amount of $\Delta \mathrm{L} . \mathrm{A}$ ot endogenous porphyrin is added theretn, then the preparation is subjected to photometivating light, belure being, readmin-
 effective dose can be determined by one skilled in the art by analngy with the doscs used for syathetic porphyrins, basel an milligrams per kilngram body weight for in vivosysteme application and the typical ooneontations for topical or ex vive applications. The compound ban be couveniently used orally or indravenously at a doage of about 10 to $100 \mathrm{mg} / \mathrm{kg}$ fer single tose, preferedly as a dosage of $4050 \mathrm{mp} / \mathrm{k}$; however split dosages of $10 \mathrm{mg} / \mathrm{kg}$ four times por day may also be given. The compromad aso he nese topinally at a dose of between $2 \%$ to $100 \%$, with $100 \%$ being dry powder. Ex vive concentrations of the compound are used on cell suspensions in a range of $1-5 \mathrm{mM}$, with a phetered ramge of 1-2 mM; however, if serum is present, a higher dose of about 15 mM should le ueed. If ex vive usc on whole bloud,

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the compound is used at about 1.5 mM ; however, if an iron kelator, such as Desferol ${ }^{\text {tm }}$ of des ferroxamine, a lower enneentration may be used.
'Thus, one application for the method of this invention is
5 the detection and quantitation of patasites by $\mathrm{Al}_{3} \Lambda$-itduced fluneseence. The forgoing ineludes fluoresenee fow cylometry of suspensions of cells or parasites ex vivo, Husrescence microsenpy of cells, including but not limited to Lisisuet, body fluids, lecal material in vivo or ex vivo, and
10 quantative spectropholohlorimetry of eells, including hus not limiked to tissues, body fluids, urine, or fecal material in vive or ex vivo.

Another application for the method of this invention is the killing of parasiles preferentially photesensitized by expo-
15 sure do AJ.A or an endogenous porphyrin either in vivo or ex vivo. The conjunctiva, which can be treated either topically on systemically with AT A, followed ly, after an appropriate period ol time, exposure of the skin or conjuctiva to phothactivating light. The parasites ean also be present in the ph peripheral blood, in which cate the $A$. $A$ can be administered systemically, follownd by, after an appropriate time, which cao he easily experimentally determined, exposing the defined area of the skin ar the blond pasing through a large vein to photoaclivating light via an optical guide within a
25 Iransparent eatheter that has been inserted into the velin. Parasites located within one cme of the surface of hollow sugans that are aceessible to fibersonnic examination (Iespiratory tract, digestive tracl, urogenital tract, abdeminal cavity, pelvic cavity, horacic esvity) can he diagnosed or
30 treated by systemic administralion of the AT A, followed by, after the appropriate period of lime, exposure of the surface of the target hissue via an appropriate light guide. Parasites bocated at sites that are uot readily acessible to fiberscopic examination can be trealed with the photoactivating light via
35 a light guide that hats been sumpically introduced into the target area thoough a needle or following surgery.

Adelitional applications of the nethod of this invention are to detect very low lovels of metabolinally active malarial parasites in peripheral blood or marow eell suspensions.
40 Such delection can be used to sereen banked blond or as a serecning procodure for pationts suspected to have viable malarial parasites. The screening method using AI A would be aceomplished by flow cytometry.

Still another application for the method of this invention would be to disingeish between metabolically active ("viable") and inaclive ("non-viahle") malarial parasiles lo evaluate the response (o) therapy in pratients infected with drug-resistant malaria more quickly than is now possible. [resent mechools for quantitatiog the leve] of parasitemia do not distinguisll lotwecn vialle and non-viable parasites. Thus, parasites that have been killecl as a result of recent therapy masy not be distinguishable from viable parasites. If the parasites are in fact resistant to the specific druge(s) that ate leing used for herapy, resislance to these drugs (as 55 shown by failure to recluce the level al parasitemia) may not become obvious for some time after the initation of therapy.

In some cases it might be life-saving to recognize more yuickly that a particular drug is not effective. Sirace AI $A$ induces tluosescence only in plasmodia that are metabolically active, it is possible to distinguish between "viable" and morphohosically similar "non-viable" malarial parasites in the peripheral blonk. Drugs that tail to produce a decrease in the proportion of the erylurocytes that accumblate PDidX Пuorescence when exposed to $\mathcal{A I} A$ in vitm could be identified quickly and replaced by other drugs that possibly might be nore ellective. The technology would not necessarily require flow tytometry, sime celatively simple and

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much less expensive fluorometers could be used if the level of parasilemia is suliciently hish.

In cases of partially drus-resistant malaria in whirh there is a slow response to the druys, it may be difticult to know when it is sate to discontinue therapy. Since Al A-indteed PPIX tiucuescence ean detect vialde plananodia at very low levels of parasitentia, the technique might be used to verily that the parasitemia has heen reduced io undelectable levels before maintenance therapy is disecontinued. However, How cytomelry would be required for such low-level measure. ments.

The foregring could alko lee used to scroen in vitro for sensitivity/resistanec of the plasmoxlia from a given patient to setected anti-malarial druses, since ALA imduces tluores. cence only in plasmodia that are melabolically active.

Yet another application of this invention is the selectivel photosensilizalion and killing of malarial parasitos in vivo or in vitro by exposing them to photoretivating light, The light would be transmited to the malaria parasites in the circulating blexd either llorouglı the skin, via an indwelling intabenous or intrataterial watheter or by extracorporeal photodynamic therapy of blood, especially for patients who have failed to resjond on other therapies, particularly those who might he considered candidates for a therapeutic exchange transtusion.

This invention is also paricularly applicable to the treatmeut of fungal intections. Fungal infections are hecoming of incrasing importance in the past two decades due to the incoasing muber of immunocompromised patients, botl, by chemoiherapy and diseases such as AIDS. Immunosupprossion results in an increased incidence of lungal infec. tions. Fungal infections can be divided ink three caleguites: culancous, subcutaneous, and systermic. Cutancous intections are hy far the mosi prevalent. Fungal infections perdispose their hosis to bacterial superinfections.

The method of the instant invention is aspried out in the same manmer as that for syothetic porphyrios previnusiy reported. More specifically, the mothod of this invention is used to detect or treat rapidly growing eells cxigenoms to the borly, including lootista colls and parasites.

The wavelength of the photoactivating light is of some importance, as it has been shown that between 1 and 10 percent of incident red light ( $600-700$ חm $)$ can pass through a slab of buman tissuc 1 rom thick, whereas only 0.001 percent or less of blue light (about 400 nr ) can pass through the same thickness of humsn lissue. The photesensitizer will, therefore, be more sucuessilul if it absorbs rod light. $P_{p} \mathrm{P} X$ does simagly absorb red light. The present appoanh has several advantages over the prioc art. First endngenous PfIX has a much shorter half-life ion nomal tissucs (human
 This greally reduces che danger ot aceidental phototoxic skin reactions in the days following treatment. Seennd, the $A T . A$ can be applied topically in certain types of lesions. This improves the specificity of the treatment, reduces the danger of accidental photoroxic reactions to a very low level, and greatly modiess the amount of both AL A and P PIX to which the entin body would be exposed if an equally ellective dose of $\mathrm{Nl} . \wedge$ were to be given systemically.
 and are handled quite readily by the biochemieal machinery of the body. However, since very large doses of $\Lambda \perp \Lambda$ (like large doses of ITpIX or $I I_{p} D$ ) arc associated with a transient. decrease in motor nerve conduation velocity, it is atcsirable to reduce the dose of $\mathrm{AL} \Lambda$ to the minimmon inal is sall effective. Topical applicarion reguires much less ALA tham systenic aclministration. Third, $\mathrm{P} \mu \mathrm{IX}$ is rapiully inactivated
by the photoactivating light. Followinge exposure of tissucs containing $\mathrm{I}^{2} \mathrm{plX}$ to a therapeutic dose of photoactivating light, there is a substantial decrease in phousensitization of the tissues within the treatment volume. Consequently, if $\mathrm{l}^{\mathrm{p} p \mathrm{X}}$ is induced by the krical application of $\mathrm{L} \Lambda \Lambda$ to specific lesious, the patient can be exposed to sunlight inmediately post-treatment without danger of scrious phototoxicily. Also, the dosimetry of the photoactivating light is greal. simplified. Fourth, ALA is an eftrective inducer oll PpIX when given by mouth, by topiad application, or by injection. In contrast, $\mathrm{HpIX}, \mathrm{HpD}$ and Phololrin II are eifcetive in most situations only when given by injection. The versatility of AT.A enhances its accoptability for rouline use by the medical profession, since the oral and topical routes of administration are much more convenient than the parcoteral. Hitth, the nomal and abnormal tissues that can be photosensitized by the administration of $\mathrm{AT} A$ are somewhat diticrent from those that can be photosensitized by the administration of $\mathrm{Hp} I \mathrm{X}, \mathrm{ItpT}$ ar Photofrim II. Conscquently, $\Delta L \Delta$ would be useful in climical situations in which the other pholosensitizers are not.

Thus the present technique is ant mercly another way to do what can be done already but is, in tact, a signifieant advance in therapeutic capability,

Wihoul further elatoration, it is believed that one skilled in the art can, using the procecling description, utilize the present invention to its lullest extenc. lin carrying out the methed of this invention, the quantities of materials utilized are not in themselves critical and can be varied within the scope and spirit of the invention. The following examples are mercly illustrative of preferred embodiments and not intended to be limitative of the remainder or the disclosure in any way whatsocver.

## EXAMPLE 1

Fiong Term Photodynimic Liodometrial Ablation
Rats were divided into 2 groups ( 6 and 7 rats/group) and their uterine horns were injected with 4 or $8 \mathrm{mg}, ~ \Lambda L \Lambda$. Example 1, of U.S. application Sicr. No. 08/082,113, diled
Jun. 21, 1993 (U.S. Pat. No. 5,422,093), wats repcated with the exception that all rats were exposed to light and the time From AT A administration to breeding was extended from 10-20 days to $60-70$ days. All other procodures were identical to Example 1.

Brecding 60-70 days after photodynamic treatment with $4 \mathrm{mg} \Lambda L A$ resulted in $n<$ implantations in the uterine horns treated with $A L A(n=6)$ whereas feluses were found in al! control uterine horns treated with saline ( $n-6$ ). These results confirmed the long erm endometrial ablative eftect of PDT. In the groups or rats ( nm 7 ) treated with 8 mg AI A 2 of 7 became pregnant in AT.A treated uterine homs conpared with 7 of 7 pregnancies in the salinc treated horns.

## Histology

In order to show nomal uterine hishology ol a nompregnant uterine horn contralateral to a pregrant uterine horn one uterine horn was ligated at its distal und prior to breeding. At gestation of $10-1.5$ days monpregnant uterine horns were harvested and histologically procesised. The uterine mucosa was lined with columnar epihelium and there was hypertrophic infoling of endometrial tissuc with tortuous glands. In contrast, prior photodynamic treatanent with $\Lambda \perp$ ©onsistently resulted in an atrophic endounctrium despite the hotmonal stimutus of the contralateral pregnancy,

## EXAMI'LH. 2

The procedures of Fixample 1 (U.S. Pat. No. 5,122, (1) 4 ) were repeated with $1,2,3,4$ and 5 lout incubation periods

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using a level of 1 mM of Al.A. No signilicant fluorescence was ohserved in the myometrial samples of in the endometrial samples incubated for 2 hours. Maxionum fluorewence was olservod in the endometrial samples incubated for 4 bours,

## EXAMPLE 3

## Endometrial Iluorescence in vivo following Topical

 Application of $\Lambda L \Lambda$ in the Non-human Primate50 mg of $\mathrm{AT} A$ was injected into the uterinc lumen of an adult, bealthy, femate rlesus monkey following, exposure of the utenis at laparotomy. A hysterectomy was performed 3 hours later and erossi sectional slices incorporating endometrial and myometrial tissuc were taken from the ulerime specimen. These slices were sibjected to examination by Iltorescence microscopy as in Example 2 and 3 above. Fhorescence was observed throunhout the enshometrium of all slices. No lluoreseence was ubstived in the myometrium.
'The ahove examplés cloarly illustrate that condometrial iblation in a range of amimal spocics, incluchiug humans, by
 un no damage to the underilying myomerrial tissucs.

## EXAMPLE 4

## Detection or Treatment of Yeast and Pugg

A. In Vitro Studies
(.) Inical isolates of Cantirta albicams, Cardida ghabrata, and C'ryptococcas neoformats and envirommental isolates ol Ponicillium species, Aspergillus miger, Aspergillus fumigalus, and Alternaria species and Sacoharomycer cerivisioe (hrewer's yeast) obstained from the clinical microbielogy laboratories of Kingston General Hospital, Kiugson, Ontarib, Camada were used. The urganisms were plated, and during rapid growth were treated with various concentrations of Al A varying from 1 ma to LO ( m M by flooding or lyy using ulifusion wells in the atgat, while the isolates of Penicillium and Aspergillus were treated with $40 \%$ or $80 \%$ molutions of $\mathrm{LL} \Lambda$ in water and the fenicillium species, Alternaria species, Aspergillus niger and Aspergithus fiumigatus were treated with $20 \% \mathrm{NL} \Lambda$ in water via diftiosion wells. 'lteatment of the varions fungi resulted in fluoresenoee emission peaks that slowed the characteristics of PpIX. Positive PPIX accumulation occurred in both mondes and yeasts.
B. In Vivo Studies;

The procedure of Giger et al. Inlection and Immunity 19 (2) 499-509 (Feb, 1978) was used with the following mondifications. A clinical specimen of C . albicaths was. replated in blood agar on it wats actively growing and left at revon temperature for 72 twors. The smple was suripended in TSB to MeFarland 0.5 turhidity after which a 1.0 m simple was inoculated into an aconbic culture bottle and lefi shaking for 24 hours on a 370 ( 3 rotor thaker. $A 10$ mat sample was withorawn and centrifuged at 70,000 rpu for 10 minutes to separate the colls from the media. The supemate was discarded and the pellet resuspended in 10 ml of 'ISB. Serial dilusions ( $10^{-1}$ to $10^{-5}$ ) were made in and replicated twice on agar and left to inculate for two days at $37^{\circ}$ C.. The Merarland 1.0 sample was centrifuged and the pellet resutipended in 1.0 ml buffer for injection.

On day zero an intradermal injoction of the C. atbicors sunsponsion (about $7 \times 10^{6}$ arganiams/ml salinc) was made into the right tlank of 5 adult hairless mice. The amount wats just ennugh to make a small vesiele uder the skin. Tesions form by clay 2, Latcr, some mice were given a secoud injoction on the opposite side.

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Three hours prior to their sacrifice, the mise were given $240 \mathrm{mg} / \mathrm{kg}$ Al.A ( $10 \mathrm{mg} / \mathrm{ml}$ ) by intraperitoncal injection, with the exceprion of mouse \$3 which was used as a control. Fluorescence emission spectra on the live mice wece taken cvery 1.5 minutes (mouse \#1 readings cvery 20 minules) tor 3 hours after injection on cach lesion, and at varous control ancas of the mice-ueck skin flap and lateral siche opposite the lesion on mouse 5 . Three hours after the injection of AI, A the miec wore sacriliced and the lesions were cxeised. The lesions in miee $1,2,3$, and 4 were frozen in 7-methylbutane cooled to the temperature of liquid nitrogen. The frozen lesions were sectioned and sljdes were prepated for spectral analysis or lluorescence mierascopy, H and E salaing lor histology, and Grocott silver sains for fungi identification.

Primary and secondary lesions showed increased IPplX accumulation relative to the control mice.

## LX MMIगE 5

(1) Selective induction of the synthesis and aceumulation of proknourphyrin IX and/or other encogenous porphyrins within parasites in vivo or in vitro.
In vivo-If the parasites jn question jnvolve the skin, conjunctiva, oral mucosa, nasal mucosa, apal mucosa, of urothalium, $\Lambda L \Delta$ may be applied directly to the surtace of the alfected dissue. If the parasites are located at sites that are now suitable for toprical application, an effective amount of ATA is administered systcmically, either by mouth, by subeutancous injection, or by intravenous injection.

In vitro-'Ibe material suspected of containing parasites is incubated under appropriate conditions in the presence of an ctiective concentration (gencrally around 5 mM ) ol AI.A.

## FXAMPLE 6

In Víva Siludies
The injection of an effeetive duse of 5 -aminolevalinic acid (AI A) into mice infected with $P$ yoelii leads to the acoumulation of llowreseing and photosensitiaing conecaItulions of protoporphyrin within menaholically active parasites. There is no such aceumulation of protopurphyrin within non-viable parasites, or within normal erythrocytes or leukocytes, In parasilized eyphrocyles, the protoporphyrin accumulation is lacalized to the parasile itselt.

Metabolictally active (viable) malarial parasites can be dislinguished resdily from parasites that are inactive (dead), sino only parasites that are metabolinally active cau synthesize protoporphyrin, In addition, metabolically active (viable) malarial parasites can be killed selectively by exposing infected blood or eell suspensions to pholoactivating wavelengets of light, 'Jhis procedure canses no signiticant darmage to the aceompauying normal erythrocytes and leukoybles, since they des not aceumulate enougth protoporphyrin to become photosensitized.

## EXAMPI.F. 7

Demonstration, Quantification, and Aualysis of ALAInduced Flunrewence Within Erydirucytes Parasilized by $P$ ? yonelii

Normal mice were given inlraperitoncal injections of blood or spleen cells obtained From mice infected with $P$. yoedii, When the rualaria was well established, some of the infocted mice were given a single inlraperitoncal injection of 250 mg ol $\mathrm{AL} A \mathrm{~A}$ or kg of budy weight. Controls ineluded irfected mice that were not given $\mathrm{Al}, \mathrm{A}$, and non-infected miee that were given/not given AIA.

At various intervals thereafter, suspensions ar blowel and/ or spleen cells were examined by the following techniculs.
Fluarescence Microserpy: Red fluorescence developed within parasitized crythrncytes of mice given AI A, but not

