

*Chapter 1*

**Introduction**

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## Abstract

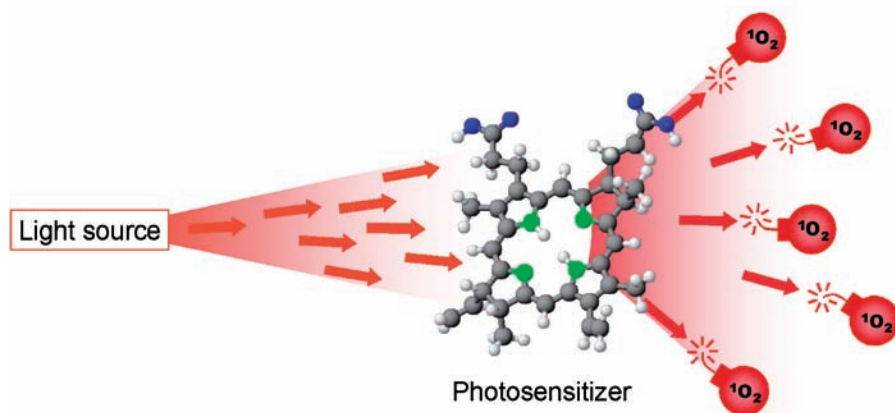
This book is intended to be a very practical handbook for physicians who would like to add photodynamic therapy (PDT) to their clinical practice. It is concerned primarily with the specific type of PDT that involves administration of the porphyrin precursor 5-aminolevulinic acid (ALA), and which therefore is commonly referred to as ALA-PDT.

Chapter 1 provides brief descriptions of some basic physicochemical and biological mechanisms that are involved in ALA-PDT, and discusses some of their clinical implications. More detailed discussions are provided in Chapter 2, which is an in-depth coverage of the scientific principles involved in photosensitization. Subsequent chapters discuss the application of ALA-PDT to a variety of anatomical sites and clinical situations. Chapter 2 includes supplementary information of the physics of light delivery, and the Appendix a listing of suppliers of instrumentation used in ALA-PDT.

### 1.1. Outline of the Theory and Technique of ALA-PDT

Light is a form of energy. Molecules of certain chemical compounds (photosensitizers) have the ability to absorb a photon of visible light and then transfer most of their absorbed energy to a molecule of oxygen. This causes a transient increase in the chemical reactivity of the oxygen molecule, and converts it into a relatively strong oxidizing agent known as singlet oxygen (Figure 1). PDT makes use of light-induced singlet oxygen to kill cells by causing lethal oxidative damage to biologically important structures.

The excited states of both the photosensitizer and oxygen have very short half-lives. Consequently, in order to be effective, molecules of both must be in very close proximity to biologically important cellular structures. Moreover, the



**Figure 1.** A photosensitizer molecule absorbs light of appropriate wavelength and can excite multiple oxygen molecules to a biologically reactive state (singlet-oxygen, <sup>1</sup>O<sub>2</sub>).

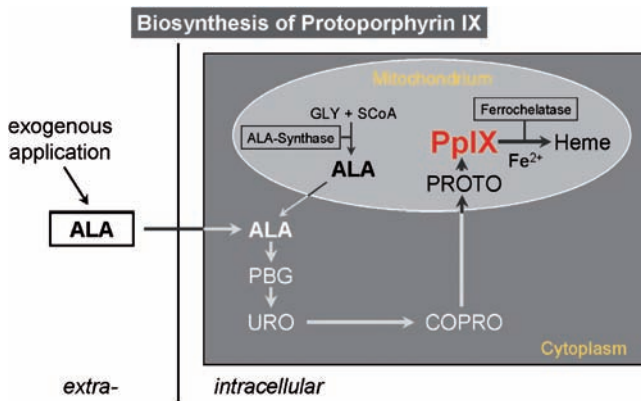
primary damage to cells occurs only while they are actually being exposed to the photoactivating light, although lethal cascades that were initiated during such an exposure may continue long after the treatment light has been turned off.

In order to become activated, the photosensitizer must absorb the light. The light therefore must be of wavelengths that lie within the absorption spectrum of the photosensitizer. However, since tissue contains pigments and particulate material that can absorb or scatter light in a wavelength-dependent manner, the particular photoactivating wavelengths that are selected should be ones that are neither absorbed nor scattered strongly by the tissue through which it passes. The choice of a wavelength for PDT usually is a compromise between strong absorption by the photosensitizer and good transmission by the tissue. For very superficial lesions, the major peak in the absorption spectrum works well, but for deeper lesions it is necessary to use light whose wavelength is more toward the red.

Ideally, the phototoxic damage will be restricted to the target tissue, although in practice we often accept a reasonable differential effect. The target tissue therefore must accumulate substantially more of the photosensitizer than does adjacent or underlying or overlying non-target tissue.

### 1.1.1. Tissue Specificity

The photosensitizer used in ALA-PDT is protoporphyrin IX (PpIX), which is synthesized *in situ* from exogenous ALA rather than given to the patient as a preformed molecule (Figure 2). The administration of exogenous ALA bypasses the rate-limiting step in the biosynthesis of heme, and thus forces each step in the pathway to produce its product at the maximum rate possible for that particular step. Since PpIX is the immediate precursor of heme, cells in which the rate of synthesis of PpIX is greater than the rate at which it can be converted into heme, excreted, or otherwise lost to the cell will accumulate PpIX.



**Figure 2.** Protoporphyrin IX is synthesized within the intracellular biosynthesis of heme. Upon exogenous delivery of ALA, PpIX is accumulated.

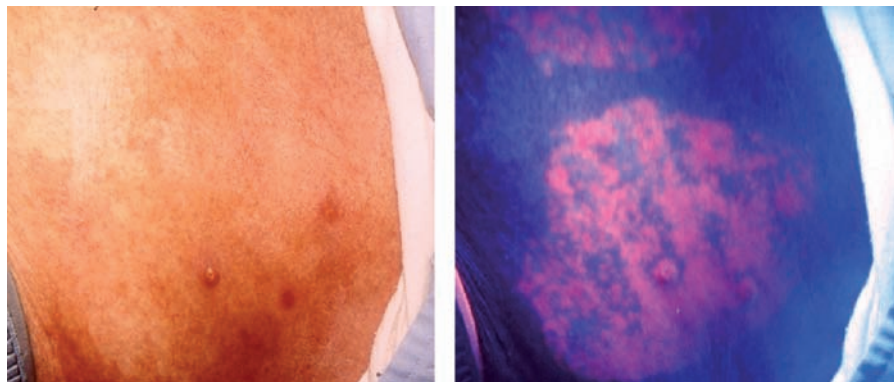
The amount of PpIX that accumulates in malignant, premalignant, and certain other abnormal tissues usually is significantly greater than the amount that accumulates in normal tissues of similar origin. When exposed to exogenous ALA, malignant tissues show a strong tendency to become much more photosensitive than the normal tissues from which they were derived. This is the primary reason for the tissue specificity found with ALA-PDT. Secondary reasons for tissue specificity may involve differences in the capacity of the cells to repair oxidative damage, or differences in the concentration or location of some compound that can function as an anti-oxidant and thus render singlet oxygen harmless.

### 1.1.2. Intracellular Targets

Photosensitizers other than ALA are administered intravenously as preformed molecules. They enter the blood stream, and then enter cells through their plasma membranes. The selective distribution of such photosensitizers depends upon physicochemical differences between the different types of cells, and the phototoxicity that results is a function of the intracellular location and concentration of the photosensitizer. In contrast, PpIX is synthesized by the mitochondria, the primary source of energy for the cell. Oxidative damage to such structures interferes with energy metabolism and can lead to cell death. Once the extra PpIX is produced in the mitochondria, it cannot all be converted into heme. Thus a significant amount will diffuse into the cytoplasm and may eventually find its way into other organelles with the exception of the nucleus. The fact that PpIX was not introduced as a preformed molecule, but rather synthesized *in situ*, means that the final destination of excess ALA-induced PpIX may be quite different from the site of localization of preformed photosensitizers.

### 1.1.3. Fluorescence

As well as being a good photosensitizer, PpIX is strongly fluorescent. It is possible to use ALA-induced fluorescence to locate tiny patches of abnormal tissue. It is possible also to measure the effectiveness of a course of chemotherapy by quantifying changes in the intensity, area, or volume of that fluorescence. For example, fluorescence induced *in vivo* may be used to locate patches of T-cell lymphoma (Figure 3) in the skin and to follow their response to either radiation therapy or chemotherapy. A decrease in fluorescence indicates a decrease in overall metabolic activity (the biosynthesis of PpIX from ALA requires energy), and may correspond to cell death. Again, flow cytometry can be used in conjunction with PpIX fluorescence (induced *in vitro*) to follow the response of leukemic cells to a course of chemotherapy. Failure to observe a decrease in fluorescence intensity of individual leukemic cells might indicate that the chemotherapeutic agent in question is ineffective, or alternatively that only drug-resistant cells are being measured. For such a study, it is very important to use fluorescence intensity standards, and to



**Figure 3.** Cutaneous T cell lymphoma (mycosis fungoides) (left). ALA-induced PpIX fluorescence induced in cutaneous T cell lymphoma (right).

distinguish between a decrease in total cell number and a generalized decrease in their overall metabolic activity.

#### *1.1.4. ALA Administration and Approval*

Unlike other photosensitizers, ALA can be administered both topically and systemically. Dissolved in a suitable vehicle, it can pass through intact layers of keratin. Dissolved in water or in saline, it rapidly passes into the lining of the digestive, respiratory, and urogenital tracts. Injections (intra-dermal, intratumoral, intravenous, or into the pleural, peritoneal, or pelvic cavities) are effective also. In clinical studies, systemic application is usually realized by oral delivery of ALA dissolved in water or juice. Altogether, it is a very versatile drug.

Topical administration has several advantages. The photosensitized area is restricted to the site of application, and there are no systemic effects. However, the maximum depth at which a significant phototoxic effect can be produced is perhaps not much about 1 mm. Intradermal injection may be helpful at the periphery of large nodular tumors, and direct injection of tumor nodules may be effective if the tumor contains areas of devitalized tissue that can function as slow-release reservoirs for the ALA. A solution of ALA held in the mouth for 15 or 20 min will photosensitize malignant and premalignant tissues of the oral cavity, and ALA solutions may be injected into the urinary bladder, the uterus, and the vagina. In all such situations, the effect of ALA is localized primarily to the site of application, although in some cases there may be a small amount of leakage systemically.

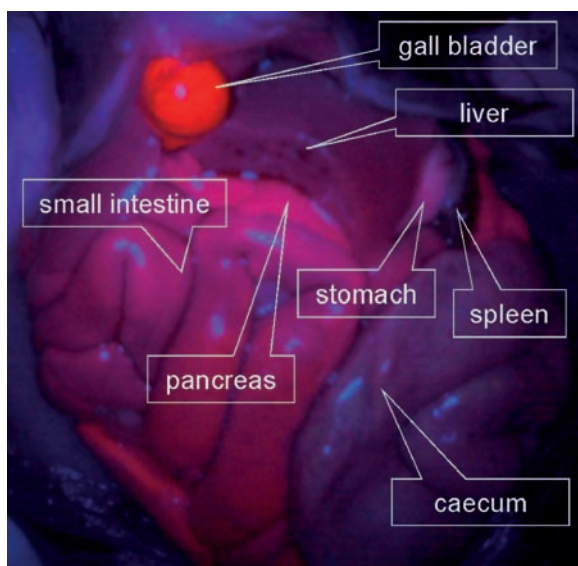
Both the oral administration of ALA and its intravenous injection may lead to undesirable systemic effects. These include occasionally reported nausea and vomiting, tachycardia and hypotension, as well as a general skin photosensitivity for up to 48 h. It is possible to avoid most of these problems by reducing the dose of ALA. Liver function enzymes may remain elevated for several

weeks, but this elevation is rarely as severe as that produced routinely by chemotherapy.

ALA can be administered as ALA hydrochloride or as ester derivatives. The ester derivatives have different polarities and some derivatives can be useful to treat deeper lesions, since some esters can penetrate deeper into the lesion. The methyl-ester derivative is approved in Europe and Australia for treatment of skin disorders (Actinic keratosis and basal cell carcinoma, Metvix by Photocure, Norway and Galderma, France; see also Chapter 3, 3.1.1.), the hexyl-ester derivative is approved in Europe for endoscopic fluorescence diagnosis of bladder cancer (Hexvix, Photocure). ALA has FDA-approval (USA) also for treatment of actinic keratosis with a blue light source (Levulan Kerastick together with Blu-U light source, DUSA, USA). All other clinical indications are investigational procedures performed in clinical studies.

#### 1.1.5. ALA/PpIX Clearance

When ALA is administered by the oral or intravenous route, much of it is cleared from the body as it passes through the liver. The liver has a very large capacity to synthesize PpIX, and most of the ALA-induced PpIX that is synthesized by the liver is excreted via the bile. The kidneys excrete another large fraction. The residual ALA can be converted into PpIX by every cell in the body except mature erythrocytes (which lack the necessary mitochondria). Some normal tissues accumulate relatively large amounts of PpIX, but others accumulate almost none (Figure 4). In the absence of liver or kidney disease,



**Figure 4.** Red PpIX-fluorescence 3 h after intraperitoneal injection of 100 mg/kg ALA into a mouse immediately postmortem. Half of the liver had been removed. The bile in the gall bladder shows the strongest fluorescence.

intracellular PpIX returns to normal levels 36–48 h following the systemic administration of the ALA. Until such clearance occurs, the whole body remains at least somewhat photosensitized.

When ALA is administered by topical application, the situation is somewhat different. The stratum corneum, stratum granulosum, and stratum spinosum of the epidermis function as a slow-release reservoir for the ALA, so that instead of being exposed to a relatively short pulse of ALA, the cells of the skin are exposed to ALA over a period of time that may last as long as 12–16 h following its initial application. PpIX continues to be synthesized during this whole time. Consequently, the clearance of PpIX that is synthesized in response to the topical application of ALA is slower than that which follows the systemic administration of ALA, the exact time varying with the thickness of the reservoir tissues. Toenails and the skin on the soles of the feet are relatively slow to lose their PpIX, while the thin skin of the inner arm clears rather quickly. However, in most cases the PpIX concentrations are close to normal 3–4 days after its application. This delayed clearance is usually not a problem, since photobleaching during treatment destroys much of the PpIX.

## **1.2. Factors Affecting Photodynamic Therapy**

Many different types of cells are capable of repairing minor oxidative damage. However, if a large number of oxidative events take place within a relatively short period of time, the capacity of a cell to repair itself can be overwhelmed. For effective PDT, oxidative damage must be produced faster than the damaged cells can repair it, until eventually the damage becomes too great for cell survival. The rate at which such damage is produced depends upon a number of interacting factors, some of which are described below.

### *1.2.1. Oxygen*

The concentration of oxygen molecules within cells at the target site immediately prior to therapy is important. This concentration decreases during the course of therapy as oxygen molecules are used up in the oxidative reactions. The oxygen can be replenished to some extent so long as there is adequate circulation and diffusion within the target tissue, but the progressive onset of edema during therapy may cause a progressive decrease in this rate of replenishment. It is therefore quite possible for the concentration of oxygen at the target site to limit the effectiveness of PDT.

The total dose of light is the product of the intensity times the duration of exposure. Using lower intensities of photoactivating light over a longer period of time will use up the oxygen more slowly and may permit replenishment during the course of treatment. Dose fractionation (multiple doses of light separated by intervals of dark time) may be helpful also. However, the onset of tissue edema often interferes with the diffusion of oxygen, and it may be

necessary to wait as long as 48 h between fractions for the target tissue to become re-oxygenated. It should be noted that at very low light intensities, phototoxic damage may occur so slowly that cellular repair processes negate most of the damage.

### *1.2.2. Photosensitizer*

Within limits, the concentration of a photosensitizer in areas immediately adjacent to vital intracellular structures largely determines the effectiveness of the therapy. The half-life of a molecule of photosensitizer that has absorbed the energy of a photon of light is very short, as is the half-life of the resulting singlet oxygen. Light-activated photosensitizer loses its excess energy, and singlet oxygen loses its chemical reactivity without having any therapeutic effect if it is generated too far distant from the intended target.

With other photosensitizers, the usual way to solve this problem is to flood the cell with the photosensitizer. However, ALA-induced protoporphyrin is unique in that it is synthesized within the mitochondria, and therefore is located right at the heart of a cell's primary energy-producing chain of biochemical reactions. A little damage at such a site can have lethal consequences.

Normal tissues may differ in their response to ALA. For example, the normal skin of a child often accumulates significantly more PpIX than does the normal skin of an older person. It is therefore sometimes necessary to adjust the dose of ALA downward in order to obtain a good differential effect. This is particularly important when using ALA-induced PpIX to locate tiny areas of abnormality in the skin, or to follow the response to treatment of subcutaneous nodules that are too small to detect by palpation. It is possible to adjust the ALA concentration to increase the contrast so that the normal skin shows little or no PpIX fluorescence while the abnormal tissues, although definitely less bright than before, are much more visible against the non-fluorescing background.

Protoporphyrin is photobleached very rapidly during the treatment process. As will be explained below, this greatly simplifies the light dosimetry for ALA-PDT.

### *1.2.3. Light*

Three factors are important – the wavelength(s) of the photoactivating light, (b) the total dose of light at the target site, and (c) the intensity (dose rate) of the light.

The wavelength(s) must be capable of being absorbed by the photosensitizer molecules. One might predict that the major peak in the photosensitizer absorption spectrum would be the ideal photoactivating wavelength. However, it often happens that these particular wavelengths are strongly absorbed by blood or other body pigments, or are scattered too strongly by cells and other particulate material to permit deep penetration of the tissues. In general, if deep penetration is required, the most effective wavelengths are those at the red end

of the photosensitizer absorption spectrum (since blood transmits red wavelengths). The blue end of the spectrum can be effective for treating more superficial lesions.

The total dose of light is important, but so also is the dose rate (the intensity). Too low a dose rate, and the target tissue may repair much of the phototoxic damage. Too high a rate, and the light is wasted because the concentration of either oxygen or the photosensitizer becomes rate-limiting. Heating of the tissue can become a problem also if the dose rate is too high, especially if red wavelengths are not used, since most of the energy of the non-red wavelengths is absorbed in the superficial layers of the target tissue. In general, light intensity should be reduced if the site is anatomically a poor heat sink (*i.e.*, the ears and the tips of the fingers), or if edema is interfering with removal of excess heat by the blood.

#### *1.2.4. Light Dosimetry*

Unlike other photosensitizers, protoporphyrin is photobleached very rapidly during treatment. This greatly simplifies dosimetry of the light, since the phototoxic damage can be determined primarily by both the absolute and relative concentrations of photosensitizer that are present in the normal and malignant tissues prior to treatment. Once the photosensitizer in the normal tissue has been photobleached, the addition of a huge excess of light can cause no additional phototoxic damage.

Example – the target lies deep within some normal tissue that we do not wish to destroy. The target tissue contains much more photosensitizer than does the normal, but since the light intensity decreases as we go deeper, giving a lethal dose of light to the target may require us to give a much higher dose to the normal tissue that overlies the target.

If a photosensitizer does not photobleach very rapidly, even a relatively low concentration of that photosensitizer in the superficial tissues could result in serious phototoxic damage if we attempt to give a lethal dose to the target tissue that lies beneath. However, protoporphyrin IX is destroyed by light, and once it has been destroyed in the overlying normal tissues, those tissues may be given any amount of additional light without untoward consequences. It is therefore possible to expose the deeper target tissues to a lethal dose of light while causing only mild and easily repairable damage to more superficial normal tissues, merely by increasing the duration of exposure to the photoactivating light.

Example – the target is a lesion on a complex curved surface (for example, the nose and adjacent cheeks). It is almost impossible to expose every part of such a surface to equal intensities of photoactivating light. If we try to give every part of that surface at least the minimum effective dose, then some parts will be given an overdose. Conversely, if we make sure that no part receives an overdose, then some parts will be underdosed.

This would present a difficult problem in light dosimetry if we were using a photosensitizer that did not photobleach readily. However, if we are using

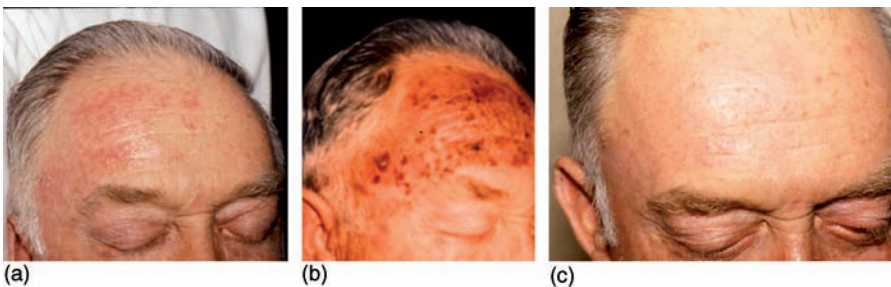
PpIX, then we do not worry about giving too much light, but instead deliberately overdose. The PpIX in the normal tissues will be photobleached long before those tissues experience more than mild damage that is easily repaired.

In summary, when using ALA-PDT we are concerned primarily with giving a curative dose of light to the target. We do not worry about overdosing the normal tissues within the treatment field, since photobleaching occurs and the phototoxic effect is determined primarily by the concentration of photosensitizer that was originally present in the tissues. This assumes that there is a good PpIX concentration differential between the normal and the malignant tissues. If there is not, then reducing the amount of ALA administered may increase that differential, although it also may decrease the degree of photosensitization of the target tissue. Longer exposure times may then be necessary to destroy the target.

### 1.3. Clinical Applications of ALA-PDT

Some of these applications are well-established clinical procedures, others are widely used but still in the process of being accepted, and still others are considered highly experimental. Some are for the treatment of obvious lesions, others for identification of the boundaries of indistinct lesions, and still others for prevention of the further development of occult lesions. A few of the applications are for cosmetic purposes. Examples are given below.

- (i) Premalignant lesions – of the skin (Figure 5), oral mucosa, esophagus, bladder mucosa, vulva, vagina, and cervix.
- (ii) Malignant lesions (all types) – for cure, local control, palliation, prevention, detection, and identification of the boundaries of lesions (malignant glioma).
- (iii) Bacterial infections – Propionibacterium acne.
- (iv) Fungal infections (superficial) – onychomycosis.
- (v) Viral infections – verucca vulgaris and other warts.



**Figure 5.** (a) Actinic keratosis prior to treatment. (b) Actinic keratosis 3 days after ALA-PDT treatment with red light. (c) Actinic keratosis 30 days after ALA-PDT. Note remaining nodule.

- (vi) Parasitic infections – cutaneous and mucocutaneous leishmaniasis.
- (vii) Rapid quantification of response to treatment – leukemia, T-cell lymphoma of skin, recurrent carcinoma of breast, malaria
- (viii) Cosmetic procedures – rejuvenation of skin, removal of excess hair

## **1.4. Interaction between ALA-PDT and the Immune System**

By itself, ALA-PDT is able to kill malignant cells by either necrosis or apoptosis. However, there is strong suggestive evidence that, in at least some clinical situations, the immune system acts in cooperation with ALA-PDT to destroy those malignant cells.

### *1.4.1. Squamous Cell Carcinoma of the Skin*

In the presence of a normal immune system, squamous cell carcinoma of the skin usually can be destroyed quite readily by ALA-PDT. However, if the immune system is suppressed (either by uncontrolled AIDS or by the strong immunosuppressive therapy that is given routinely to patients with kidney or heart transplants), the destruction of squamous cell carcinomas of the skin by ALA-PDT becomes very much more difficult, and may in fact be impossible. In contrast, patients whose AIDS is under control and transplant patients whose maintenance dose of immunosuppressive agents has been significantly reduced respond to ALA-PDT like patients who have a normal immune system.

### *1.4.2. Kaposi's Sarcoma*

Patients whose immune system has been suppressed by uncontrolled AIDS often develop a form of Kaposi's sarcoma that fails to show any clinically significant response to ALA-PDT. In contrast, patients who develop the classical slowly progressive age-onset type of Kaposi's sarcoma respond to ALA-PDT very well. In some cases, the treatment of lesions at one anatomical site results in the apparent spontaneous eradication of previously untreated lesions at distant sites.

The mechanism responsible for the synergy between ALA-PDT and the immune system has not yet been identified, but in at least some cases it appears to be more than a simple "mopping up" of a few residual malignant cells that happened to survive the PDT. It may be that the oxidation of biological material generates strong new antigens that can cross-react with the malignant cells. Another possibility is that the inflammatory response induced by ALA-PDT in close association with the targeted malignant cells stimulates non-specific immune and phagocytic cells, which then attack and destroy any residual malignant cells. Alternatively, products of the inflammatory response may stimulate a tumor-specific cellular immune reaction that has the capacity to destroy malignant cells throughout the body.

## **1.5. Conclusions**

ALA-PDT can be very helpful in clinical situations in which standard forms of therapy are not appropriate, such as patients with basal cell nevus syndrome who cannot be treated with ionizing radiation and yet have far too many lesions to treat by surgery. It can be used also to salvage certain lesions that have failed maximum safe dose radiation therapy, and it is being used routinely as the primary form of treatment for superficial basal cell carcinoma, Bowen's disease, and actinic keratosis. The following chapters discuss certain other indications.

