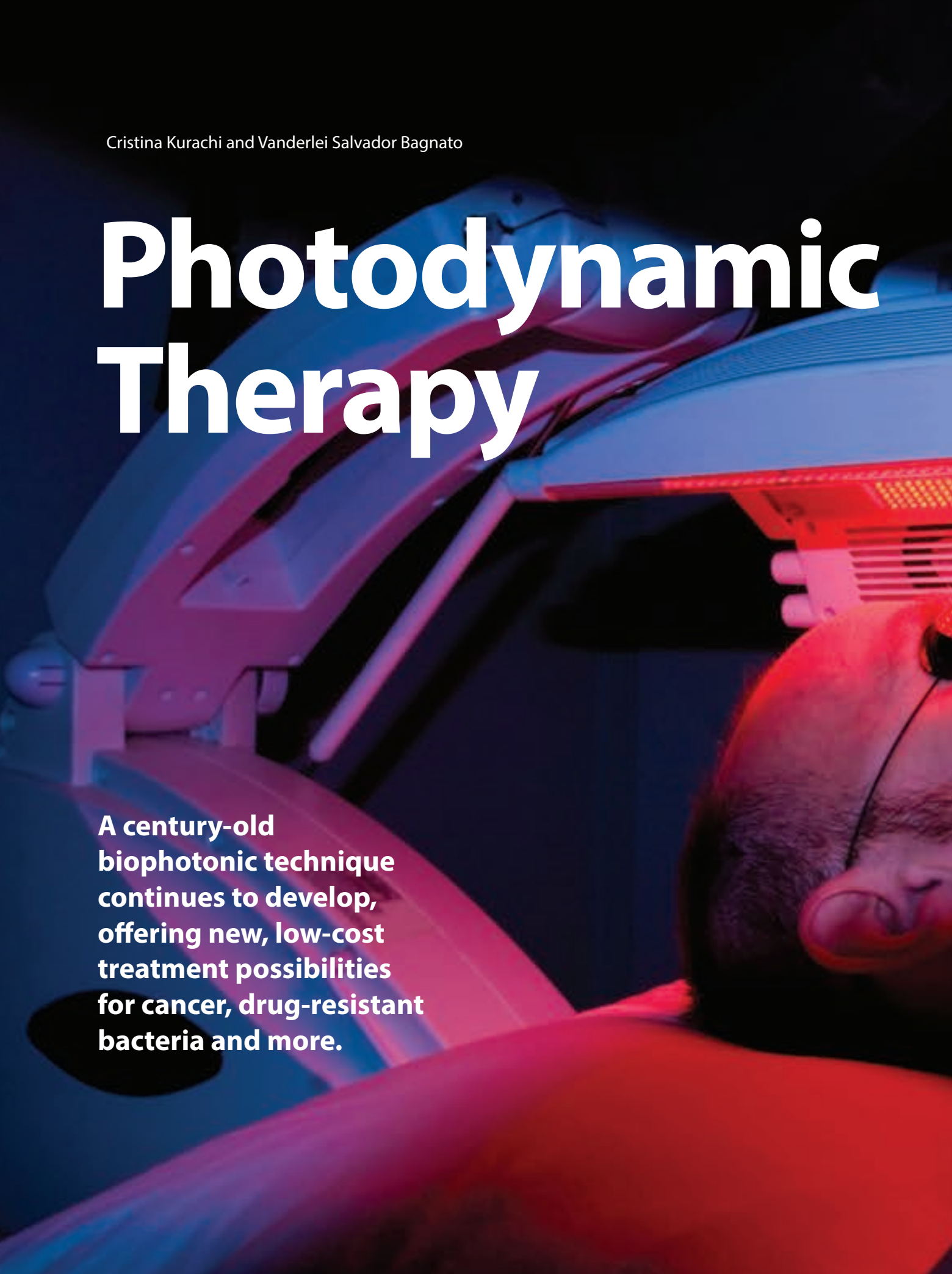
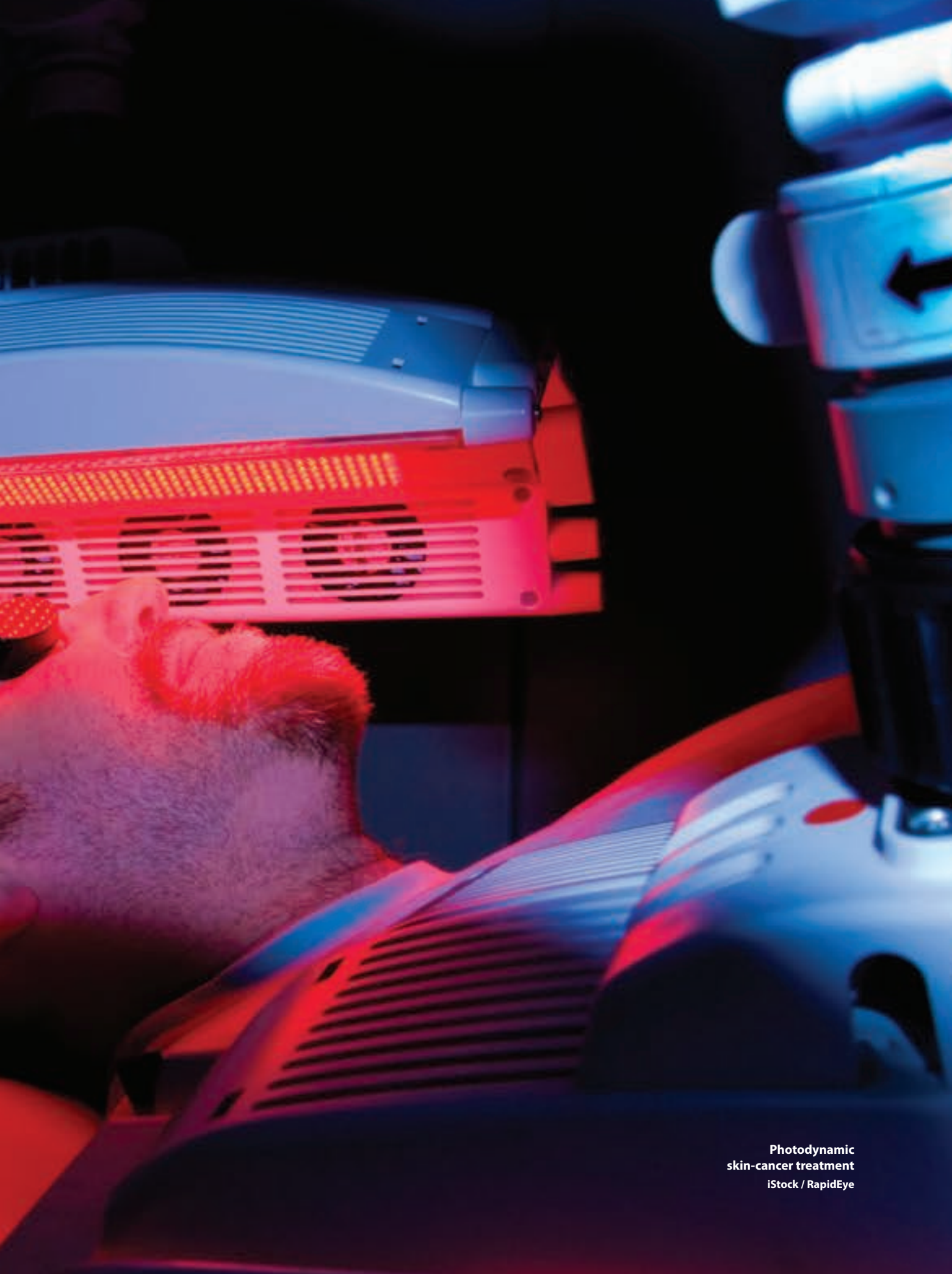
Cristina Kurachi and Vanderlei Salvador Bagnato

Photodynamic Therapy



A century-old biophotonic technique continues to develop, offering new, low-cost treatment possibilities for cancer, drug-resistant bacteria and more.



Photodynamic skin-cancer treatment
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Since ancient times, humans have been fascinated by the therapeutic possibilities of sunlight. More recently, the desire to use light as a tool in the life sciences has given rise to biophotonics, which combines great advances in our understanding of medicine, and of light-tissue interactions, with the recent technological development of light sources, to create light-based techniques for diagnosing and treating specific diseases.

One such biophotonic technique is photodynamic therapy (PDT). This technique offers a targeted, localized treatment approach to cancers and infectious lesions, by using light and specialized photosensitive compounds to produce disease-killing reactive oxygen species. While PDT was first proposed more than a century ago and has developed steadily ever since, recent years have seen substantial success in trials in some new therapeutic areas, with some significant advantages in some cases relative to conventional surgical techniques. And, while a number of challenges remain toward more widespread adoption, the approach—which requires little specialized equipment and can be applied in a range of treatment settings—could prove particularly promising in low-resource areas in the developing world.

Photodynamic therapy offers a targeted, localized treatment approach to cancers and infectious lesions.

How PDT works

The first significant reference on the systemic use of a photosensitizer, and its potential therapeutic value, came in 1900, from J. Prime, a French neurologist. Prime orally treated patients with eosin, a fluorescent acid, as a treatment for epilepsy, and observed that after treatment, those patients showed dermatitis in sun-exposed skin areas.

Influenced by Prime's example, in 1903, H. von Tappeiner and H. Jesionek decided to investigate whether this skin damage could be used to induce tumor necrosis. They began topically applying eosin to specific areas, and subsequently illuminating those areas, to treat skin cancer—the first reported clinical application of PDT on tumors. In 1907, von Tappeiner and another partner, A. Jodlbauer, demonstrated the probable mechanism of treatment—the oxygen dependence of the photosensitizer reaction—and introduced the term photodynamic action to describe these interactions.

More than a hundred years later, while the tools have changed, the basic approach of PDT remains the same. A specific compound, or photosensitizer, is applied at a specific concentration onto diseased tissue. The photosensitizer is then illuminated with a light source, which triggers a chemical reaction that causes the creation of reactive species, especially singlet oxygen (oxygen in one of several highly reactive, excited electronic states). The light source's detailed characteristics, such as wavelength, irradiance and fluence, must be tuned to the optical characteristics of the photosensitizer, mainly its absorbance and the wavelength that offers the maximum quantum efficiency to produce singlet oxygen.

The singlet oxygen—which, being highly reactive, operates over a short active distance, and only during actual tissue illumination—reacts with biological molecules, such as proteins and membrane components, in the cell microenvironment to return to the less reactive (triplet) state. This process results in damage to cellular components, mainly in the cell membrane and mitochondria, the organelles responsible for the energy production, and thus essential for the cell metabolism. The final response is cell death.

Moreover, because PDT has fewer side-effects than chemotherapy, radiation therapy and surgery, it also can function as a palliative treatment, aiming at reducing or controlling the size of the tumor rather than eliminating it entirely, and resulting in a better overall condition and experience for the patient. The main PDT side-effect is skin and eye photosensitivity that can persist for up to six weeks, a condition observed only if systemic sensitization is used (see below). In this case, the patient must avoid sunlight exposure during the photosensitivity period, or skin burns and eye damage can occur.

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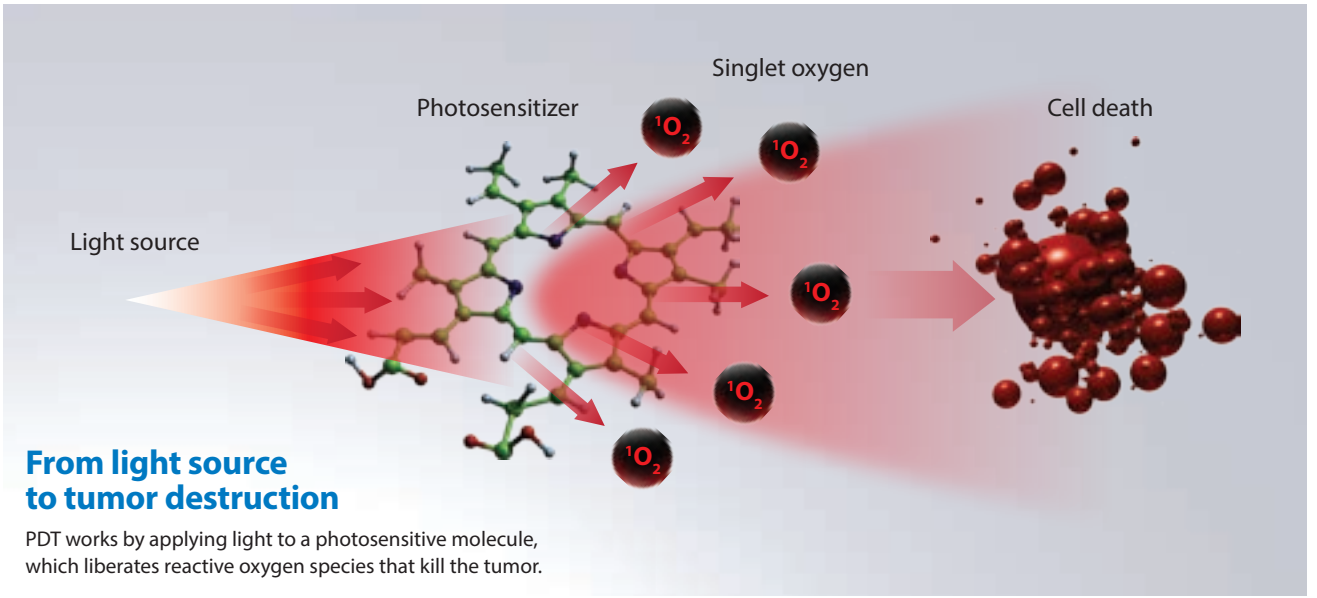


Phil Saunders / Adapted from animation by P.M. Lacerra (2002), courtesy of C. Kurachi

Setting the stage: Systemic and local sensitization

In principle, PDT can be used both for the treatment of skin cancers and other external conditions, with light delivered using simple external sources, and for some internal cancers, with the light delivered via endoscopes or fiber optic catheters. Different tumors and tumor locations, however, will call for two very different approaches to actually administering the photosensitizer:

Systemic photosensitization.

If the tumor is internal or not easily reached, the photosensitizer is usually delivered orally or through intravenous injection, and distributed across the entire body, reaching all organs. This broad, systemic approach can work because tumor cells tend to absorb more of the photosensitizer than noncancerous cells do—the result of the tumor cells' higher metabolism and differences in pH and vascularization (blood-vessel development) relative to noncancerous cells, as well as of an increased concentration of low-density lipoproteins at the tumor cell membrane. Similarly, the photosensitizer remains in the cancer cells longer than in normal cells.

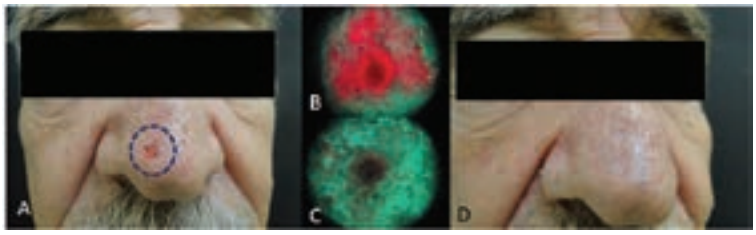
As a result, after a period that varies from hours to days depending on the photosensitizer molecule, the tumor tissue reaches a threshold concentration significantly higher than that of surrounding cells, at which point PDT can proceed. At this stage, the tumor is illuminated with the specific activating wavelength, and the photodynamic reaction begins to take place at the cancer cells or tumor blood vessels.

Nowadays, two systemic photosensitizers are approved for clinical applications: porphyrin, which is excited at wavelengths of 630 nm; and chlorin, active at wavelengths of 660 nm. An obvious limitation of systemic PDT for cancer treatment at these visible wavelengths is that the light can penetrate only a limited distance into biological tissues, which reduces the potential volume that can be treated. With the approved PDT sensitizers, it is possible to treat tumors up to 1 cm in thickness in the best scenario.

To overcome the depth limitation, PDT can be used in combination with surgery or radiation therapy, performed in multiple sessions, or done using multiple fiber optic irradiation sites, an approach known as interstitial PDT. (In this last approach, multiple optical fibers are inserted into the tumor at different, precisely predetermined sites to apply the light dose and kick off local production of the tumor-killing reactive oxygen species.) And scientists are exploring still other strategies to beat the penetration limit, including photosensitizers that absorb at near-infrared wavelengths, pulsed illumination and other ways of improving optical coupling into tissues.

Local photosensitization.

For cutaneous lesions such as skin cancers, the photosensitizer delivery is local: a cream that delivers a "pro-drug"—consisting of a molecule that will, through metabolic processes, induce production of an endogenous photosensitizer in the tissue upon application—is topically applied directly to the lesion. Pro-drugs currently used for topical photosensitization include aminolevulinic acid (ALA)



A PDT result

In this patient, PDT treatment of superficial basal cell carcinoma at the nose (left) showed a good cosmetic and clinical result after two MAL PDT sessions over six months (right). No malignant cells were present in a biopsy taken at 30 days after the second PDT session.

Courtesy of C. Kurachi

and methyl aminolevulinate (MAL); both of these induce production of an endogenous porphyrin, called protoporphyrin IX (PpIX), that acts as the photosensitizer.

An obvious advantage of local photosensitizers such as MAL and ALA is that, in contrast to systemic photosensitizers, they focus only on the lesion area, and thus avoid the broad skin and eye photosensitization experienced with systemic chlorin and porphyrin, which can take from one to six weeks to wear off. The treatments are also quite effective, with average complete-response rates (the percentage of the treated lesions that show complete elimination of cancer cells) reported in the literature at around 85 to 90 percent.

Another, very different potential use for PDT is rapidly developing as well: therapy to inactivate microbial pathogens that are resistant to conventional antibiotics.

In addition to those highly satisfactory response rates, PDT with local photosensitization has better cosmetic outcomes relative to surgery—and, because the treatment in most cases does not require anesthesia medication or advanced treatment facilities, it can easily adapt to low-resource settings. The key disadvantage, once again, is depth of penetration: for treatment of the superficial basal cell carcinoma, topical PDT is limited to tumors of up to only 2 mm thickness.

PDT versus the "superbugs"

Given PDT's success in treating certain cancers, especially skin cancers, the community is actively investigating potential protocols for other kinds of cancers and potentially malignant lesions, including other kinds of skin lesions such as actinic and cheilitis keratoses, cervical and vaginal conditions such as intraneoplasia and HPV-condiloma lesions, and esophageal cancers and colon polyps. But another, very different potential use for PDT is rapidly developing as well: therapy to inactivate microbial pathogens that are resistant to conventional antibiotics.

One of the main motivations for this application is the emergence of so-called superbugs: multi-drug-resistant microorganism species. These species have evolved in response to the widespread success and use of antibiotics, and represent a significant public-health issue for the future. Such microbes, however, are still vulnerable to the damage that can be caused by singlet oxygen.

The key problem to be resolved is how to select for the drug-resistant bacteria, while protecting the other tissues. Highly reactive singlet oxygen will induce molecular damage to important biomolecules wherever it is produced. It is thus necessary to find a photosensitzer that will be taken up more efficiently by the target microbes than by the surrounding cells. Fortunately, most microorganisms are highly responsive to photodynamic action, so a lower photosentizer concentration and light dosage can be used than for tumor treatment, limiting damage to the host tissue.

Photosensitization for antimicrobial PDT involves topical application of a photosensitizer solution, gel or cream to the infected tissue, followed by an incubation time, before tissue illumination, of a few minutes to an hour, depending on the photosensitizer and lesion type. The most common photosensitizers used are blue dyes, like methylene and toluene blue, but the porphyrins, chlorins, hypericin and, more recently, curcumin have been also investigated for antimicrobial PDT.

Several PDT protocols have been tested, both *in vitro* and *in vivo*, for infected lesions mainly caused by bacteria and fungi. Examples of lesions for which PDT has efficiently fought microbial

action include infected cutaneous lesions, vaginal and oral candidiasis, tooth canal infection, periodontal disease, onychomycosis and pythiosis. Antimicrobial PDT has also been tested for blood disinfection, oral-cavity decontamination and overall skin decontamination.

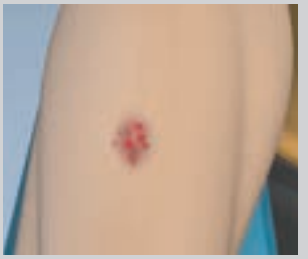
A particular advantage of PDT in these kinds of local antimicrobial applications relative to broader-spectrum antibiotic treatment, of course, is that PDT is a local treatment, with limited side effects—particularly as the photosensitizer is light activated and has a low "dark" toxicity (that is, a low toxicity in the absence of stimulation at the target wavelength). For example, a conventional (and not completely successful) treatment for onychomycosis, a fingernail and toenail fungal infection, involves prolonged, systemic use of a liver- and kidney-toxic drug. PDT, using the naturally occurring plant chemical curcumin as the photosensitizer, has shown curative results after five to seven sessions.

Antimicrobial PDT also faces a challenge: how to handle biofilms—complex, well-organized multispecies accumulations of microorganisms that can form on healthy and diseased tissue. These structures resist the delivery of photosensitizers and can form a protective microenvironment against photodynamic action. Strategies under investigation to overcome this obstacle include the use of nanostructures for photosensitizer delivery, mixtures of photosensitizers tuned to penetrate biofilms, and combined use with other disaggregation mechanisms, such as the use of ultrasonics that can help to mechanically disrupt the biofilm structure.

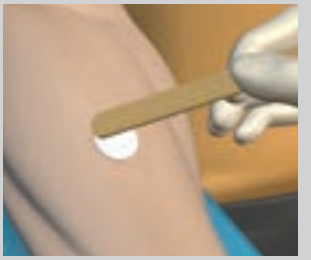
Challenges and opportunities

Beyond cancer and drug-resistant infections, PDT is finding continual expansion to meet other medical needs. One recent example has been the successful application of PDT for treating diabetic foot ulcers. In Brazil, for example, diabetes afflicts nearly 19 percent of the elderly population, and foot complications from diabetes constitute the most common cause of traumatic foot amputations. The use of PDT for these foot ulcers has been shown to prevent foot amputation in more than 80 percent of treated cases.

A SAMPLE PDT PROTOCOL

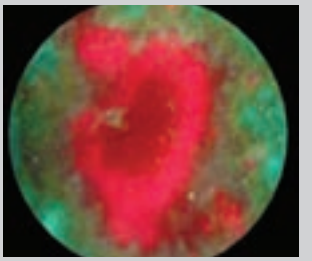


1 Lesion, such as superficial basal cell carcinoma (sBCC), is scraped and cleaned to remove dead cells and ensure photosensitizer penetration.



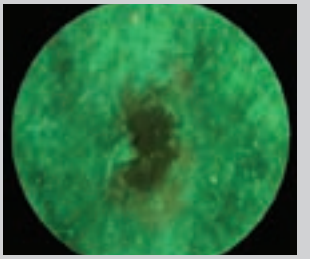
2 Pro-drug cream, such as ALA or MAL, is applied to the lesion.

3 The cream is "incubated" for a minimum time (three hours), to allow adequate production of photosensitizer (PpIX) within the tumor.



4 Wide-field fluorescence monitoring shows PpIX production in tumor and normal surrounding skin (red zones indicate central area of the sBCC lesion and green zones show autofluorescence).

5 After incubation, the lesion is illuminated with 630-nm (red) light at 150 J/cm^2, spurring production of singlet oxygen from the PpIX.



6 Fluorescence imaging after illumination shows the superficial PpIX photobleaching.

Adapted from animation by P.M. Lacerra (2002), courtesy of C. Kurachi

The case for PDT as a treatment alternative has never been stronger, owing to its possible use in low-resource settings in the developing world.

PDT for superbugs: *In vitro* experiment using curcumin as the photosensitizer and 450-nm LED illumination for antimicrobial PDT. Courtesy of C. Kurachi

PDT also faces some challenges blocking more widespread adoption—particularly for its longest-standing application, the treatment of tumors. While we tend to speak of it colloquially as a single disease, cancer is actually many diseases, and individual cancer lesions have a complex and diverse biology. That diverse tumor biology leads to distinct differences in tissue optical characteristics, oxygenation and vascularization, all of which can greatly affect PDT response.

Ideally, therefore, PDT planning must consider these inherent tumor characteristics for an improved photosensitizer delivery and tissue illumination. That, in turn, implies a customized PDT dosimetry—that is, carefully defining the illumination parameters (wavelength, irradiance, fluence and energy) depending on tumor optical properties, mainly absorbance and scattering at excitation wavelength, tumor oxygenation and photosensitizer concentration. Any medical treatment that needs such customized treatment planning can find difficulty getting broadly approved and applied, and in many cases surgical resection may seem like a "simpler" option.

Yet, from another perspective, the case for PDT as a treatment alternative has never been stronger, owing to its possible use in low-resource settings in the developing world. The incidence of cancer is increasing in developing countries as a result of both population aging and lifestyle changes; meanwhile, global health agencies have documented substantially poorer cancer survival rates in developing countries, owing partly to insufficient treatment. PDT protocols could form a highly attractive solution for part of this dilemma particularly in low-resource settings, owing to PDT's comparatively low cost, low side effects, and lack of requirement for a specialized health facility.

Indeed, researchers have recently focused on making the cost even lower—for example, by leveraging handheld, battery-operated LED devices (for photoactivation) and smartphone imaging (for measuring fluorescence) to extend PDT to areas with little electricity or medical infrastructure. Such dedicated instrumentation for illumination, coupled with better local knowledge of the treatment and diagnostic auxiliary tools for customized treatment planning, could provide the key to greater global use of this effective, low-cost biophotonic application. OPN

Cristina Kurachi (ckurachi@gmail.com) and Vanderlei Salvador Bagnato are with the São Carlos Institute of Physics, University of São Paulo, Brazil.

References and Resources

- *Biomedical Photonics Handbook*, T. Vo-Dinh, ed., CRC Press (2003).
- *Advances in Photodynamic Therapy: Basic, Translational, and Clinical*, M.R. Hamblin and P. Mróz, eds., Artech House (2008).
- A. Jemal et al. "Global cancer statistics," CA: A Cancer Journal for Clinicians **61**, 69 (2011).
- *Highlights in Skin Cancer*, Pierre Vereecken, ed., Intech (2013).
- S.P. Songca and O.S. Oluwafemi. "Photodynamic therapy: A new light for the developing world," African J. Biotech. **12**, 3590 (2013).
- L. Pires et al. "Photodynamic therapy in *Pythium insidiosum*—An *in vitro* study of the correlation of sensitizer localization and cell death," PLoS One, e85431 (2014).
- A. Spaeth et al. "Improving photodynamic inactivation of bacteria in dentistry: Highly effective and fast killing of oral key pathogens with novel tooth-colored type-II photosensitizers," J. Med. Chem.**12**, 5157 (2014).
- H.H. Buzzá et al. "Photodynamic therapy: Progress toward a scientific and clinical network in Latin America," Photodiag. Photod. Ther. **13**, 261 (2015).
- J. Hempstead et al. "Low-cost photodynamic therapy devices for global health settings," Sci. Rep. **5**, 10093 (2015).
- J.P. Tardivo et al. "Development of the Tardivo algorithm to predict amputation risk of diabetic foot," PLoS One, e0135707 (2015).
- A.P. Silva et al. "Development and comparison of two devices for treatment of onycomycosis by photodynamic therapy," J. Biomed. Opt. **20**, 061109 (2015).