When chemist Thomas Dougherty went to work at Roswell Park Cancer Institute (RPCI) in 1970, he never could have imagined that he was destined to become the **founding father** of a new treatment modality called photodynamic therapy (PDT), which, in recent decades, has captured the attention of physicians worldwide due to its success in treating diseases and conditions as disparate as cancer, macular degeneration and acne. Dougherty says he went to work at RPCI simply because he had become disillusioned with industrial chemistry, “where the bottom line is what drives things,” and he wanted to try his hand at **biomedical research**, where the top priority is tackling medicine’s most perplexing problems.
ne such problem he immediately encountered in oncology pertained to the fact that tissue that is not fully oxygenated is more resistant to ionizing radiation than tissue that is fully oxygenated. Tumors—because their vasculature tends to be disorganized—are especially prone to being hypoxic and therefore resistant to radiation therapy.

“I spent a lot of time thinking about how to get around this,” says Dougherty, “and I decided that one way to do it would be to make a molecule that produced oxygen when you radiated the tumor, making it more sensitive.”

Using laboratory space he borrowed from the director of RPCI at the time, George Moore, Dougherty set to work on a series of experiments that led to his making a serendipitous discovery that continues to have ramifications today as medical researchers become increasingly intrigued by the potential depth and breadth of PDT.

While using a technique called “vital staining” to test the toxicity of an ionizing sensitizer he had made, Dougherty accidentally discovered that when cancer cells that contained the vital stain (fluorescein diacetate) were exposed to room light, they died.

His interest piqued, Dougherty went to the literature and learned that his experiment demonstrated a well-known phenomenon that was referred to as “the photodynamic effect.”

“Fluorescein, in addition to being highly fluorescent, is also a weak photodynamic agent,” explains Dougherty. “Since these were cancer cells, I wondered if anyone had ever tried to kill cancer this way.”

In the years that followed, Dougherty and his collaborators at RPCI began developing photosensitizer drugs that...
were based on biological molecules called porphyrins, which were known to have two key medicinal properties: they migrate to and accumulate in cancer cells, and they are activated by tissue-penetrating red light to create a cytotoxic event.

It is this cytotoxic event resulting from the interaction of the drug, light and oxygen that defines PDT.

But how does it work?

When the photosensitizer drug is activated in the body by red light from a laser, it produces a molecule called singlet oxygen, an active form of oxygen derived from exogenous oxygen. During its one-millionth-of-a-second life span, this highly reactive molecule oxidizes and kills the targeted cells while doing relatively little damage to surrounding normal tissue.

In 1975, Dougherty and his RPCI colleagues published data in the Journal of the National Cancer Institute in which they described experiments using porphyrin-based photosensitizers and red light to successfully treat mice with mammary tumors.

Over the next few years they treated more than 100 patients at RPCI using these same techniques, and again, outcomes were very promising.

Unfortunately, as many oncology researchers have come to realize over the last few decades, there appears to be no “magic bullet” for the treatment of cancer, and PDT proved no exception.

As research continued and more scientists became interested in experimenting with PDT, drawbacks to the

“Allan Oseroff, MD, PhD, is chair of the Department of Dermatology at Roswell Park Cancer Institute (RPCI) and at the UB School of Medicine and Biomedical Sciences. Since 1992, RPCI has received continuous program project funding from the National Cancer Institute to improve the understanding of photodynamic therapy and its use in the clinic. Oseroff is principal investigator of the grant.

“Fluorescein, in addition to being highly fluorescent, is also a weak photodynamic agent,” explains Dougherty. “Since these were cancer cells, I wondered if anyone had ever tried to kill cancer this way.”
therapy began to percolate to the surface.

The main drawback of the first-generation porphyrin-based photosensitizer is that it accumulates not just in tumors, but also, though to a lesser extent, in some normal tissues, including the skin. Therefore, a major side effect of PDT using the currently FDA-approved agent is that it causes patients to be sensitive to light for four to six weeks after treatment. Also, inflammation at the treatment site is common and can cause problems if not adequately controlled. Selectivity, therefore, is a primary challenge in developing new photosensitizers, with the goal being to design drugs that accumulate only in targeted cells.

Ideally, scientists would like to develop drugs that are activated and highly potent within the red-to-infrared window of the light spectrum because that light penetrates deeply (a few centimeters) into tissue.

Another challenge is that there are so many treatment variables involved in PDT, and each must be exquisitely calibrated with the others if therapy is to be optimally effective. These variables include the amount of drug to be given; the time interval between when the drug is given to the patient and when it is activated by light; how much light is given (called “fluence”); and how fast, or intensely, the light is given (called “fluence rate”). And just in the past few years, scientists have discovered yet another variable to PDT: the immune-system response—but more about that later.

A WORLD OF INTEREST

Despite these challenges, PDT researchers at RPCI and around the world have made enormous strides since the early 1970s, and today PDT is in wide use not only for treatment of solid tumors, but also for a growing number of precancerous and non-cancerous conditions.

A critical milestone to moving the field forward came in 1995, when a porphyrin-based photosensitizer called Photofrin®, which was isolated and developed at RPCI, became the first PDT drug approved by the U.S. Food and Drug Administration.

Currently, Photofrin is approved in the U.S. for general use in esophageal and lung cancers and in high-grade dysplasia associated with Barrett’s esophagus. Outside the U.S., it is approved for use in bladder, stomach, cervical, lung and esophageal cancers.

In addition, researchers at RPCI are currently conducting two phase II clinical trials to evaluate a new topical cream called aminolevulinic acid (ALA) that causes the body to synthesize a natural photosensitizer where the cream is applied. ALA-PDT is being used for the treatment of cutaneous carcinomas, including basal cell, squamous cell, and T and B cell lymphoma. Furthermore, ALA, under the brand name Levulan®, has been approved by the FDA for PDT treatment of actinic keratoses, a precancerous lesion that can lead to squamous cell carcinoma.

OVERVIEW

Photodynamic therapy, at a glance

Photodynamic therapy involves injecting patients with a non-toxic photosensitizing drug that migrates to fast-growing cells. When the drug is exposed to red light, a highly reactive molecule called singlet oxygen is produced, which kills the cells while, for the most part, sparing surrounding healthy tissue.

The concept of photodynamic therapy was first formulated in 1970 by Thomas Dougherty, PhD, a chemist at Roswell Park Cancer Institute and research professor of radiation oncology at the UB School of Medicine and Biomedical Sciences.

Although photodynamic therapy was originally developed as a means to treat cancer, medical researchers are now exploring its potential to treat diseases and conditions as disparate as age-related macular degeneration, atherosclerotic plaques (photoangioplasty), acne, autoimmune diseases and infectious diseases, including AIDS.

To learn more about photodynamic therapy and the interest it is garnering around the world, read “New Light on Medicine” in the January 2003 issue of Scientific American.
ALA’s effectiveness in humans (it failed in mice) was first demonstrated by two Canadian scientists, James Kennedy and Roy Pottier. In 1990, researchers at RPCI began the long and arduous process of discovering how to integrate the drug safely and effectively into PDT regimes. To date, ALA appears to cause less scarring and have better cosmetic outcomes. Also, patients are experiencing light sensitivity for only 24 to 48 hours following treatment and only at the site of application.

Equally promising is a new photosensitizer developed at RPCI called Photochlor®, which is currently being evaluated in clinical trials for treatment of obstructive esophageal cancer, early-stage lung cancer and basal cell carcinoma. Photochlor is considered a second-generation photosensitizer because it is based on chlorin, not porphyrin. Although these two types of drugs have molecular structures that are closely related, they have significantly different photophysical properties; e.g., Photochlor absorbs further into the red portion of the light spectrum, so the activating light penetrates more deeply into tissue.

Studies currently under way show that the new drug doesn’t linger in the system as long as Photofrin; as a result, none of the 60 or more patients who have been treated with Photochlor to date has experienced a photosensitivity reaction.

“THE PERFECT PLACE TO BE”

iven the diverse array of scientific knowledge and instrumentation that researchers must master to develop optimally effective forms of PDT, it’s no surprise that the field is one that thrives on multidisciplinary collaboration and that each clinical advance is intimately tied to basic-science discoveries that help explain how PDT works.

Dougherty is convinced that RPCI has remained the
“Today, we can make the drugs in our labs, evaluate them in terms of toxicology and pharmacokinetics, and then take them all the way through to phase I and II clinical trials. So, literally, we can go from the benchside to the patient.”

worldwide leader in PDT over the past 30 years because the institute has fostered such collaboration by hiring and supporting the physicists, chemists, toxicologists, pharmacologists, immunologists, biologists and clinicians needed to develop the field.

“I have said many times that Roswell Park Cancer Institute has been the perfect place to be,” says Dougherty, who was raised in a neighborhood near the institute. “Today, we can make the drugs in our labs, evaluate them in terms of toxicology and pharmacokinetics, and then take them all the way through to phase I and II clinical trials. So, literally, we can go from the benchside to the patient. And I don’t know too many places where you can do all that in-house.”

This long and proven track record of collaboration between PDT researchers at RPCI is a key reason that the institute has received continuous program project grant funding from the National Cancer Institute (NCI) since 1992 to improve understanding of PDT and its use in the clinic. (In September 2003, the PDT team at RPCI was awarded a second extension to the grant, bringing the total NCI funding for the program project to $18.3 million.)

Principal investigator for the grant is Allan R. Oseroff, MD, PhD, chair of the Department of Dermatology at RPCI and at the University at Buffalo School of Medicine and Biomedical Sciences.

Collaborators on the grant, in addition to Oseroff, include Ravindra Pandey, PhD, who was instrumental in the development of Photochlor and is currently working to design new, third-generation photosensitizers that selectively target brain tumor cells (see page 12); Barbara Henderson, PhD, who is currently investigating the role played by PDT inflammation and changes in the tumor microenvironment; and Sandra Gollnick, PhD, who is seeking to identify how PDT alters the adaptive immune response and how these changes contribute to the efficacy of PDT therapy.
NO OXYGEN, NO PDT

In order for the RPCI investigators to conduct such advanced research, they and other scientists around the world first had to focus their attention on understanding the role of one crucial component in the PDT equation: oxygen.

“In PDT, you have to have oxygen,” says Henderson. “If you don't, then singlet oxygen can’t be produced, which means you can’t kill the cell. So, in PDT, oxygen becomes extremely important; in fact, as important as the photosensitizer drug in the whole PDT equation. And the third component—light—you can play around with in a facile way because you can turn it up or down or off, and you can change its wavelength.”

Oxygen, on the other hand, is a much more difficult component to interact with for a number of reasons, says Henderson. “First of all, oxygenation of tumors is highly variable, and you can never tell how one portion of the tumor is oxygenated compared to another. Second, oxygen delivery to a tumor depends on various physiological and pathological processes, and, finally, things start happening once you do PDT.”

In order to discover how and why “things start happening once you do PDT,” Henderson and her colleagues conducted experiments in the early 1980s in which they used PDT to treat tumors in mice, after which they removed the tumors and studied them in a culture dish.

“Very quickly, we began to see that there were lots of things going on other than just the initial killing of tumor cells by the PDT,” says Henderson. “Whether we took the tumor cells out immediately or took them out one, or two, or four hours later, what we found at the time astonished me.”

What astonished Henderson is this: Despite the fact that

Sandra Gollnick, PhD, is an assistant member of the Photodynamic Therapy Center at Roswell Park Cancer Institute and assistant professor in the Immunology Program, Roswell Park Graduate Division, University at Buffalo.

She is leading studies aimed at identifying how photodynamic therapy (PDT) alters the adaptive immune response and how these changes contribute to the efficacy of PDT therapy. Gollnick played a key role in developing mouse models that convinced researchers there is a basis for PDT immune response in humans.
a large number of the cancer cells survived the initial damage caused by the PDT, most of the tumors eventually died. Clearly, some other cytotoxic mechanism was at work, but what was it?

Henderson says that she and her group “then spent the next ten to 15 years” showing that PDT’s cytotoxic effects involve numerous tissue targets: the tumor cells, which are killed by direct photodynamic action, and the tumor’s vasculature, which can cause tumor-damaging oxygen and nutrient deprivation. Finally, PDT also affects host cells in the tumor, which in turn creates an inflammatory microenvironment that influences host anti-tumor responses.

A clear understanding of these processes became crucial to developing ways to optimally deliver PDT, because, for example, once a tumor’s vasculature is shut down, giving more light becomes a useless exercise since there is no oxygen present to produce the singlet oxygen.

Early in the 1990s, Bruce Tromberg, PhD, at the University of California at Irvine, and Thomas Foster, PhD, at the University of Rochester, further elucidated the oxygen-dependent mechanisms underlying PDT by describing something called photochemical oxygen depletion.

Their description of this phenomenon has helped researchers understand why their intuitive tendency to treat tumors with a high light-dose rate and a large dose of photosensitizer is counterproductive. PDT delivered in this way results in the rapid consumption of available oxygen to produce singlet oxygen, and because the body’s blood vessels cannot resupply oxygen to the treated areas as rapidly as it is being consumed, the tissue—especially in poorly organized tumor blood vessels—becomes hypoxic, which in effect protects the cancer cells from PDT.

Until this process of photochemical oxygen depletion was understood, physicians had assumed that the higher the light-dose rate and the larger the drug dose, the better the cell kill, when in fact just the opposite is true.

Researchers now realize that by adjusting the fluence rate by turning the laser light up or down or by lowering the drug dose, they can control whether photochemical oxygen depletion takes place.

Given this new basis of understanding, scientists at RPCI currently are conducting several large studies aimed at finding ways to calibrate fluence rates and drug dosages to optimize PDT without inducing photochemical oxygen depletion.

### PREDICTING OUTCOMES AT TREATMENT

The oxygen story doesn’t end here, however, as yet another key concept has been discovered at RPCI, which further helps researchers better calibrate how to deliver PDT in an optimally effective way.

When scientists first began developing PDT, it didn’t take them long to realize that dermatology was a natural fit for the new therapy.

“Because PDT involves a drug and light, it’s very easy to apply to the skin,” says Oseroff, who earned a doctorate in applied physics prior to attending medical school.

“Another reason why PDT is being used so extensively in dermatology is, because of the enormous incidence of skin cancer—close to a million cases a year in our country,” he continues, “and since many of these cancers tend not to be life threatening because they are slow growing, the risk of trying a new treatment is quite low. Also, follow-up is straightforward: All you have to do is look; you don’t need to rely on an invasive procedure, such as colonoscopy.”

Although follow-up is straightforward and the success rate for treating skin cancer with PDT exceeds 90 percent, researchers have been frustrated by the fact that when the cancers recur, it’s not until four or five years down the road, a time lapse that has greatly slowed their efforts to formulate adjustments in treatment modalities that will prevent such recurrence.

In an attempt to overcome this problem, RPCI researchers once again were drawn to examining the oxygen component and the crucial role it plays in PDT.

Since the early 1990s, Oseroff and his colleagues have led efforts to understand how a concept called photo-bleaching can be used as a measurement tool to obtain
real-time information about the effectiveness of PDT, thereby providing them with a way to predict outcome.

Essentially, photobleaching describes the following process: When a photosensitizer makes singlet oxygen, a small fraction of the singlet oxygen attacks the photosensitizer itself and destroys it. And, once destroyed, the drug can no longer absorb or fluoresce (so it loses its color) and reaches a “photobleached” state.

In terms of treatment, what this means is that if the rate-of-light delivery is too fast (intense), all the oxygen available in the tissue is consumed by the PDT process, and the treatment stops working.

By measuring the rate of photobleaching using a highly sensitive camera developed at RPCI, Oseroff and his colleagues are predicting that clinicians will be able to determine what the optimum light intensity should be during PDT. “And we’d know it as we were treating the patient. We wouldn’t have to wait five years to find out if what we were doing is right or wrong,” says Oseroff.

Early this year, RPCI received FDA approval to embark on a full-scale trial to investigate the merit of measuring the photobleaching rate in patients undergoing PDT as a means to predict outcome.

**PDT’s Effect on the Immune System**

A ne condition that ALA-PDT (topical-cream PDT) has been especially successful in treating is a genetic disorder called nevoid basal cell carcinoma syndrome (NBCCS), also known as Gorlin’s syndrome. Various organ systems are impacted by NBCCS, including the skin, and it’s not uncommon for individuals with the syndrome to develop tens to hundreds of basal cell carcinomas each year.

Anecdotal evidence growing out of ALA-PDT treatment of these carcinomas, combined with basic-science studies led by Henderson and Gollnick, has spurred the RPCI group to investigate what might yet be the most intriguing aspect of PDT treatment: its effect on the immune system.

Clinicians at RPCI have advanced the treatment of the NBCCS-associated carcinomas by developing a laser-beam splitter system that they use in conjunction with mini-tripod devices that they place on the patient’s skin during PDT treatment. The system accomplishes two things: It splits the light from the laser into as many as eight optical fibers, thereby allowing multiple sites to be treated simultaneously, which significantly shortens treatment time; and, second, it locks the laser beams on each spec of carcinoma, thereby allowing the patient to move during the treatment without shifting the beams off target.

Over the years, the RPCI physicians treating NBCCS patients in this way began to notice a response that caught their attention: When the patients were examined several weeks after the PDT treatment was over, it was evident that some of the cancers were still present; however, when the patients were examined six months later, the cancers were gone.

“And it’s not just that the cells took a really long time to die,” says Oseroff. “Clearly, something else was happening.”

This clinical observation dovetailed with what Henderson and Gollnick were finding in their laboratory studies, leading them and others to question whether PDT triggers an immune-system response.

“In recent years, we have discovered that with PDT you have some very complex immunology issues,” says Henderson. “However, these issues are far from being defined and, like everything in PDT, things are much more complex and paradoxical than you would imagine.”

Henderson and Gollnick’s laboratory investigations, while distinct, are intimately intertwined, as are the two systems they study: Henderson focuses on the innate (natural, or host) immune-system response (i.e., on cells that release inflammatory mediators), while Gollnick focuses on the acquired (adaptive) immune-system response (i.e., the proliferation of antigen-specific B and T cells). Together, they are making intriguing discoveries.

“Most of our patients come from outside of Buffalo,” says Oseroff, who lists points of origin that include South America and Europe, as well as every region in the United States. “The most gratifying part of our work is to provide treatment where there are no other options.”
Hector Nava, MD, is chief of gastrointestinal endoscopy at Roswell Park Cancer Institute and associate professor of surgery at the UB School of Medicine and Biomedical Sciences. Nava was one of the first clinicians in the country to apply photodynamic therapy to the treatment of precancerous lesions of the esophagus (high-grade Barrett’s esophagus). Currently he is principal investigator for a number of clinical trials at RPCI to test Photochlor® in patients with obstructive esophageal cancer.

Due to their thorough understanding of the oxygen component in PDT, the two scientists have been able to devise laboratory experiments in recent years in which they can characterize very clearly what happens in the tumor milieu in terms of oxygen.

In a series of experiments that exploited this ability, Henderson set out to define how oxygen in PDT interacts with the immune system, especially given that oxygen is known to induce transcription factors for inflammatory mediators, which in turn stimulate an adaptive-immune response.

When she looked at the generation of inflammatory mediators in PDT experiments in which the oxygen environment in tumors was carefully controlled, she and her team again encountered a paradox.

"Under certain conditions, you would have thought that the more inflammation you have in PDT, the more you would turn on the immune system, and the better the cure," she says. "Well, think again. Our studies showed that most cures took place when there was a minimum of inflammatory mediators. It was totally unexpected."

Over time, the scientists came to understand the logic behind this riddle: Treatment that is optimal in terms of the dosimetry and the supply of oxygen, kills tumors so effectively that their cells don’t have time to create inflammatory mediators, whereas in regimes that provide subcurative treatment, surviving cells rapidly produce the mediators.

"We are now taking this information and asking, What does it do to the adaptive immune system?" explains Henderson. "We have hypothesized that fewer danger signals clearly prompt less of a response from the adaptive immune response, while more danger signals prompt a stronger response," she says. "And this actually seems to be panning out in our studies."

The studies that are "panning out" are based on mouse models developed by Gollnick that, according to Oseroff, “have worked so convincingly that we said, ‘Yes, there really is a basis for this [immune response] showing up in humans.’”

However, like Henderson, Oseroff is very cautious about extrapolating where the current immunology studies are going, no matter how
“Ten years ago, PDT was hardly ever mentioned at national meetings,” Oseroff says, “and now there are whole sessions on it. And this is all due to the early work by Tom Dougherty, whose contributions have really been extraordinary.”

promising they appear at this juncture.

“We’re a long way from any kind of proof in humans,” he says. “But what we do know is that PDT does all the things that are necessary in patients for inducing an immune response: It kills cells in place, it induces a very strong inflammatory response and it recruits the [adaptive] immune system. Plus, it has available tumor antigen so, in a sense, it’s the perfect situation to vaccinate. But, again, that is the hope, and right now there’s some distance between hope and reality.”

Because PDT also is increasingly being used to treat non-cancerous conditions, it is equally important that clinicians understand the innate, inflammatory immune response to PDT, adds Oseroff.

“For example, with acne or atopic dermatitis—conditions where we know PDT works—it’s the host response that you’re modifying because there’s no tumor. But the host is doing things it’s not supposed to do that may be causing these conditions, and I think it’s going to turn out that we can modulate that.”

**DESTINATION BUFFALO**

Although the challenges to discovering the full potential of PDT may be complex, it is the highly charged atmosphere of research and clinical care at RPCI that makes Buffalo a hub for international PDT research, a fact that is not lost on patients, medical students, residents and fellows who come to RPCI from around the world to be treated and to learn how to provide such treatment.

“Most of our patients come from outside of Buffalo,” says Oseroff, who lists points of origin that include South America and Europe, as well as every region in the United States. “The most gratifying part of our work is to provide treatment when there are no other options.”

Each year, RPCI and the University at Buffalo School of Medicine and Biomedical Sciences sponsor two dermatology fellows. “They do much of their work in PDT,” says Oseroff, “so they really become expert in the therapy and in photomedicine, in general. And once they are done, we actively recruit them to stay in Buffalo and remain a part of the work we are doing here.”

Residents, too, are exposed to as much PDT as possible while training at RPCI, according to Oseroff. “You can’t come through the program without learning about PDT,” he says. “It’s part of our mandate to prepare residents to go out into the community well prepared to provide PDT to patients. We are dedicated to making this a form of treatment they can incorporate into their clinical practice, without it being too complicated. Our goal is to make PDT
Scientists at the institute collaborate closely with researchers at Roswell Park Cancer Institute (RPCI) on photodynamic therapy (PDT), a treatment modality first developed at the cancer institute by chemist Thomas Dougherty, whose contributions have really been extraordinary. He literally was the Johnny Appleseed of the field because in the early days he traveled around the country and, against great odds, got a critical mass of people interested in PDT. And he was able to do this because, although he is trained as a chemist, Tom has always thought like a clinician; he has always wanted PDT to be an effective form of treatment.

Although Dougherty recently assumed emeritus titles at RPCI and UB, he insists that he is not retired.

“Ten years ago, PDT was hardly ever mentioned at national meetings,” Oseroff says, “and now there are whole sessions on it. And this is all due to the early work by Tom Dougherty, whose contributions have really been extraordinary. He literally was the Johnny Appleseed of the field because in the early days he traveled around the country and, against great odds, got a critical mass of people interested in PDT. And he was able to do this because, although he is trained as a chemist, Tom has always thought like a clinician; he has always wanted PDT to be an effective form of treatment.”

Although Dougherty recently assumed emeritus titles at RPCI and UB, he insists that he is not retired.

“We’ve come a long way since the early days, yet we still have so much work to do,” he says from his office at the cancer institute.

“But at least I’m not working here by myself, like it was when I first started.”

In 2000, the University at Buffalo established the Institute for Lasers, Photonics and Biophotonics, which today is considered one of the most advanced and comprehensive facilities for photonics research in the United States.

Scientists at the institute collaborate closely with researchers at Roswell Park Cancer Institute (RPCI) on photodynamic therapy (PDT), a treatment modality first developed at the cancer institute by chemist Thomas Dougherty, PhD (see article on page 8).

The results of one such recent collaboration between the UB and RPCI scientists is the development of a non-release, nanoparticle drug-delivery system for PDT.

Because the ceramic-based nanoparticle never releases photosensitizing drugs into the bloodstream, it has the potential to overcome the primary side effect associated with PDT: the patient’s strong sensitivity to light for four to six weeks after treatment.

Research on this development was published online in June 2003 in the Journal of the American Chemical Society, and a provisional patent has been filed by UB. Lead author of the study was Indrajit Roy, PhD, a former postdoctoral researcher at UB.