

An Inside Attack on Cancer

*Riding aboard
new molecules
synthesized at
Livermore,
radioisotopes can
detect, diagnose,
and treat cancer
with unparalleled
precision.*

CANCER is always a dreaded diagnosis. Even with improvements in treatment results over the last few decades, cancer is still the second leading cause of death in this country.

Treatments for cancer include cutting, burning, and poisoning—surgery, radiation therapy, and chemotherapy—any combination of which is often highly successful in eradicating cancer cells. However, cancer that metastasizes, spreading to multiple sites in the body, has proved to be difficult to treat. Therapy with beams of radiation is only successful for localized cancers. At the same time, the 5-year survival rate for patients with detectable metastatic cancer who receive chemotherapy is less than 20 percent for many cancers.

Given that almost three-quarters of all cancer deaths involve cancers that have metastasized, finding an effective treatment method is a top national health priority. Livermore is facing this challenge head-on with a far-reaching set

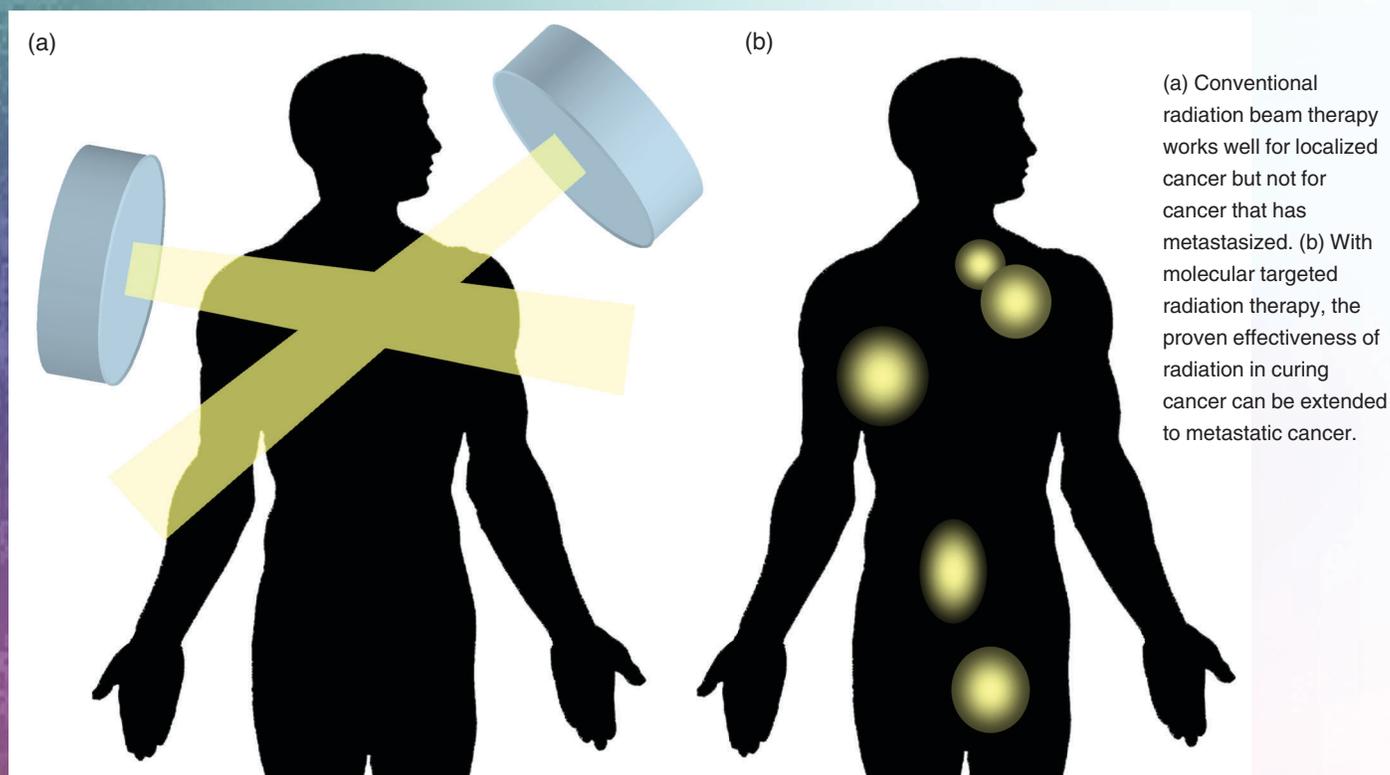
of projects overseen by medical physicist Christine Hartmann-Siantar, director of Livermore's Glenn T. Seaborg Institute. In pursuing this work, biochemists, computational biologists, material scientists, chemists, and physicists in two Livermore directorates—Chemistry and Materials Science and Biology and Biotechnology Research Program—are collaborating with scientists at the University of California (UC) at Davis Cancer Center.

Hartmann-Siantar had been principal investigator for development of PEREGRINE, a treatment planning program for radiation beam therapy that couples Livermore's storehouse of radiation transport data with powerful simulation tools and desktop computers. (See *S&TR*, May 1997, pp. 4–11; April 2001, pp. 15–17.) PEREGRINE, named for the patron saint of cancer patients, has since been commercialized and is now available to hospitals as a tool for accurately targeting cancer tumors with radiation beams.

“A couple of years ago, PEREGRINE was in the technology transfer phase, and the team was asking what we could do to save the next 100,000 cancer patients using radiation,” Hartmann-Siantar says. “Beam therapy cannot treat cancer that has metastasized. We wanted to know how we could address widespread cancer.”

At about the same time, Livermore and the UC Davis Cancer Center formed a research collaboration to fight cancer. In part as a result of that joint venture, the UC Davis Cancer Center was named a designated cancer center by the National Cancer Institute, one of the National Institutes of Health. Together, Livermore and UC Davis are seeking better ways to prevent, diagnose, and treat cancer.

This cancer-fighting initiative brought Hartmann-Siantar and others at Livermore together with many experts at the UC Davis Cancer Center, including physicians Sally and Gerald DeNardo, leaders of the Section of Radiodiagnosis and Therapy in the Molecular Cancer Institute at the UC



Davis Medical School. The DeNardos are pioneers in the treatment of cancers with radiation administered internally. They, together with Hartmann-Siantar and others at Livermore and UC Davis, envisioned much of the work that goes on today in four projects that are described here.

One team is perfecting a new way to get radiation inside the body and directed only at cancer cells. New molecules being synthesized in the laboratory will lock on to specific proteins, in a process known as molecular targeting. When the specially designed molecules are tagged with a radioactive isotope, deadly radiation can be delivered straight to cancer cells.

A problem with chemotherapy is that the toxicity it delivers to tumors is only slightly higher than what it delivers to healthy tissue. The beauty of targeted radionuclide therapy is that diseased cells receive a much higher fraction of drug. The radioactive material is busily destroying cancerous tissue while normal, healthy tissue stays healthy. This translates to no more nausea or hair loss for the patient.

Another team is developing a new imaging system that uses molecular

targeted radionuclides to reveal and diagnose breast cancer tumors. A third project is combining such images with computer software similar to PEREGRINE to make the planning of molecular targeted radiation therapy as specific to each patient as possible. Finally, a fourth project is using subcellular imaging to take snapshots that show how radioisotopes interact with cells to kill tumors.

Unfortunately, none of the systems currently under development will be available for patients in the immediate future. Says Hartmann-Siantar, "Even if we are wildly successful, it will be at least 10 and more likely 20 years before our advances mean widespread cures for metastatic cancer. After research and development are done, the phases 1, 2, and 3 trials take several years. That's just the way it works."

Delivery System for the Cure

Because radiation is a proven killer of cancer cells, researchers have been searching for years for the best way to get radiation inside the body and directed specifically at tumors.

In 1985, a team led by the DeNardos was the first to use monoclonal antibodies

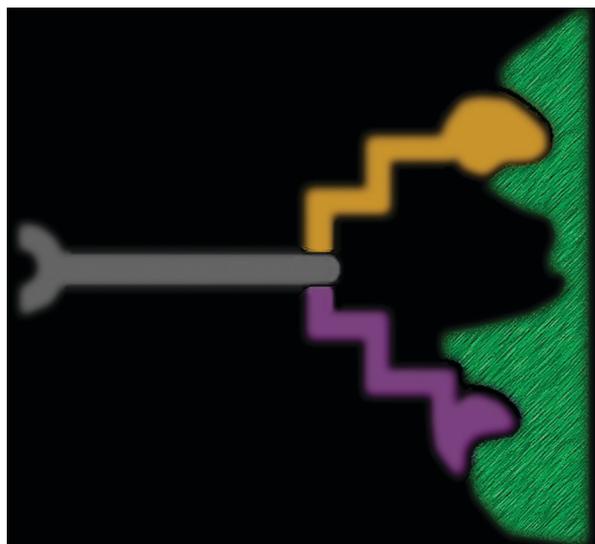
tagged with a radioisotope to treat cancer patients. Monoclonal antibodies are laboratory-produced substances that can locate and bind to cells wherever they are in the body. Many monoclonal antibodies are used in cancer detection or therapy; each one recognizes a different protein on certain cancer cells. Monoclonal antibodies can be used alone to stimulate the immune system, or they can be used as a system to directly deliver drugs, toxins, or radioactive material to a tumor. The first monoclonal antibodies were produced entirely from the cells of mice, which meant that rejection by the human body was common. In recent years, methods for "humanizing" monoclonal antibodies have greatly reduced the rejection rate.

Over the years, the DeNardos have treated more than 200 patients with radionuclide-tagged monoclonal antibodies for non-Hodgkin's lymphoma, prostate, and metastatic breast cancers. Lymphoma is a cancer of the lymphatic system, a network of thin vessels and nodes in the body whose function is to fight infection. Lymphoma is a particularly difficult cancer to treat because its tumors tend to be small and widespread. Cancers of the prostate and breast are carcinomas. Accounting for at least 80 percent of all cancers, carcinomas begin in the lining layer—epithelial cells—of organs.

The DeNardos' patients were typically at the end of the line, looking only to gain a few more months of life after not responding to conventional chemotherapy and radiation. Despite their grim prognosis, 60 percent of the patients responded to radionuclide-tagged antibody treatment, and 30 percent of that number have celebrated with complete remissions.

As internal radiation therapy for cancer was gaining ground in research hospitals, a project to develop synthetic antibodylike molecules began at

This diagram shows how a linker molecule will connect molecules that bind to two sites on a protein. When two molecules are connected with a linker, they bind with up to a million times higher affinity than does each molecule alone. The goal is to develop synthetic high-affinity ligands (SHALs) that bind to cancer cells. When tagged with a radioactive substance, the SHAL serves as a delivery system for cancer-killing treatment.



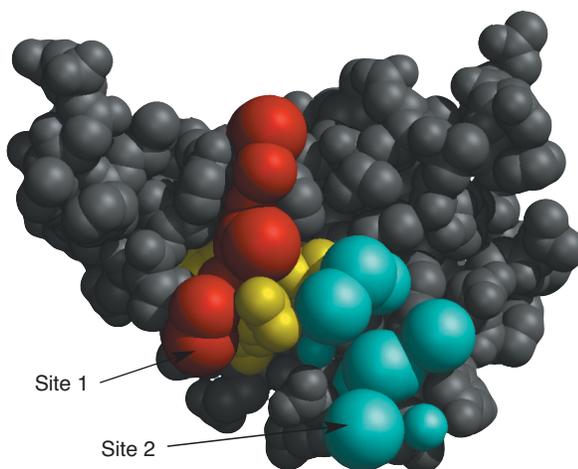
Livermore about three years ago. (See *S&TR*, June 2002, pp. 4–11.) The original goal for this work was to design molecules to bind to and capture proteins of biowarfare agents for fast, efficient detection. It was Gerald DeNardo who suggested to Livermore researchers that synthetic molecules could easily be tagged with radionuclides and used for cancer treatment.

Biochemist Rod Balhorn heads the team of biologists and chemists at Livermore who are producing the synthetic high-affinity ligands, or SHALs. The synthesis of a SHAL in the laboratory is the culmination of a process that integrates computations and experimental selection. A SHAL has two ends, each of which is a small molecule selected for its affinity to bind to a part of a particular protein (as determined through computational modeling by Felice Lightstone and other members of Mike Colvin's biomolecular modeling team). The two ends are combined by a linker molecule to create an entirely new molecule that will bind to the target protein thousands or even millions of times more strongly than either one of the original small molecules would have.

Livermore's first cancer-fighting SHAL, synthesized by Julie Perkins, binds to a receptor protein known as HLA-DR10 that is found on the surface of almost all non-Hodgkin's lymphoma cells. This SHAL, which will carry the radioactive isotope yttrium-90, is designed to rapidly pass through the liver and kidney to minimize the systemic damage that can occur when antibodies carry radionuclides.

"The new high-affinity ligands will have the selectivity of monoclonal antibodies without the 'baggage' that comes with antibodies," says DeNardo.

Initial laboratory testing of the non-Hodgkin's lymphoma SHAL at UC Davis will be to verify that it is selective for cancer. Researchers are examining



The first synthetic high-affinity ligand (SHAL) for cancer is designed to bind to HLA-DR10, a receptor protein found on the surface of almost all non-Hodgkin's lymphoma cells. The two sites on the HLA-DR10 molecule that the SHAL binds to were identified by Felice Lightstone.

the response of many kinds of tissue to the SHAL, using tissue arrays that have various types of healthy tissue—heart, liver, kidney, breast, and so on—as well as some cancerous tissue. The first SHAL tested has been shown to bind selectively to human lymphoma cells, and it doesn't bind to normal cells lacking the HLA-DR10 receptor. Future tests will use mice implanted with a human cancer to determine if the SHAL selectively localizes in the tumor, a feature critical for effective tumor targeting. The team will also be designing SHALs for prostate cancer and metastatic breast cancer in the next few years.

Imaging to Detect

Radionuclide-tagged SHALs or monoclonal antibodies that bind tightly to cells can also serve as a diagnostic tool for cancer. The gamma rays they emit can be detected to reveal precisely where cancer cells are located. A team led by Livermore physicist Kai Vetter is developing a high-resolution gamma-ray imager designed to improve the odds of detecting breast cancer.

When a mammogram indicates the presence of a lesion in the breast, a biopsy must be performed. Yet 80 percent of such biopsies reveal a benign rather than a malignant lesion. Patients and doctors alike want to reduce the problem of false-

positive mammograms, reduce the need for invasive biopsies, make mammograms more sensitive, and generally improve breast cancer detection.

Today's gamma-ray detection systems can only detect lesions greater than about 10 millimeters across, which is too large to improve detection and treatment of breast cancer. Livermore's new technology is applying radiation detection systems developed for national security to the detection of breast cancer lesions just 1 to 2 millimeters in size.

Other isotope detection systems require that radiation emanating from the tumor source be aligned, or collimated. The new Livermore detector eliminates this need for collimation. Instead, it relies on recent developments in segmented semiconductor detectors and digital signal processing to measure the spatial distribution of the outgoing, tumor-based gamma rays. Because some gamma-ray energy is lost in the collimation process, eliminating collimation makes Livermore's new device just that much more efficient. Thus, technology advances have made it possible to realize the full potential of the gamma-ray imaging concept in the new detector.

An initial demonstration of Livermore's gamma-ray imager prototype will use small radioactive test lesions embedded in material designed to mimic the tissue of a woman's breast.

In developing the prototype, Vetter is working closely with UC Davis physicians and technical staff to optimize the imager's usefulness in a clinical setting.

Imaging to Plan the Attack

The ability of targeted molecular radionuclides to locate tumors is being put to another use as well. By taking images of patients after they have received a small diagnostic radionuclide dose, physicians can determine exactly where the drug is distributed in the body. No other cancer treatment can provide that kind of dose information.

A team of researchers at Livermore, Montana State University, Idaho National Engineering and Environmental Laboratory (INEEL), and UC Davis is putting that dose data to work in new

treatment planning system known as Modality-Inclusive Environment for Radiotherapeutic Variable Analysis, or MINERVA. While the initial emphasis in the development of MINERVA is on targeted radionuclide therapy, the system can be used for any kind of external or internal radiotherapy or combination thereof.

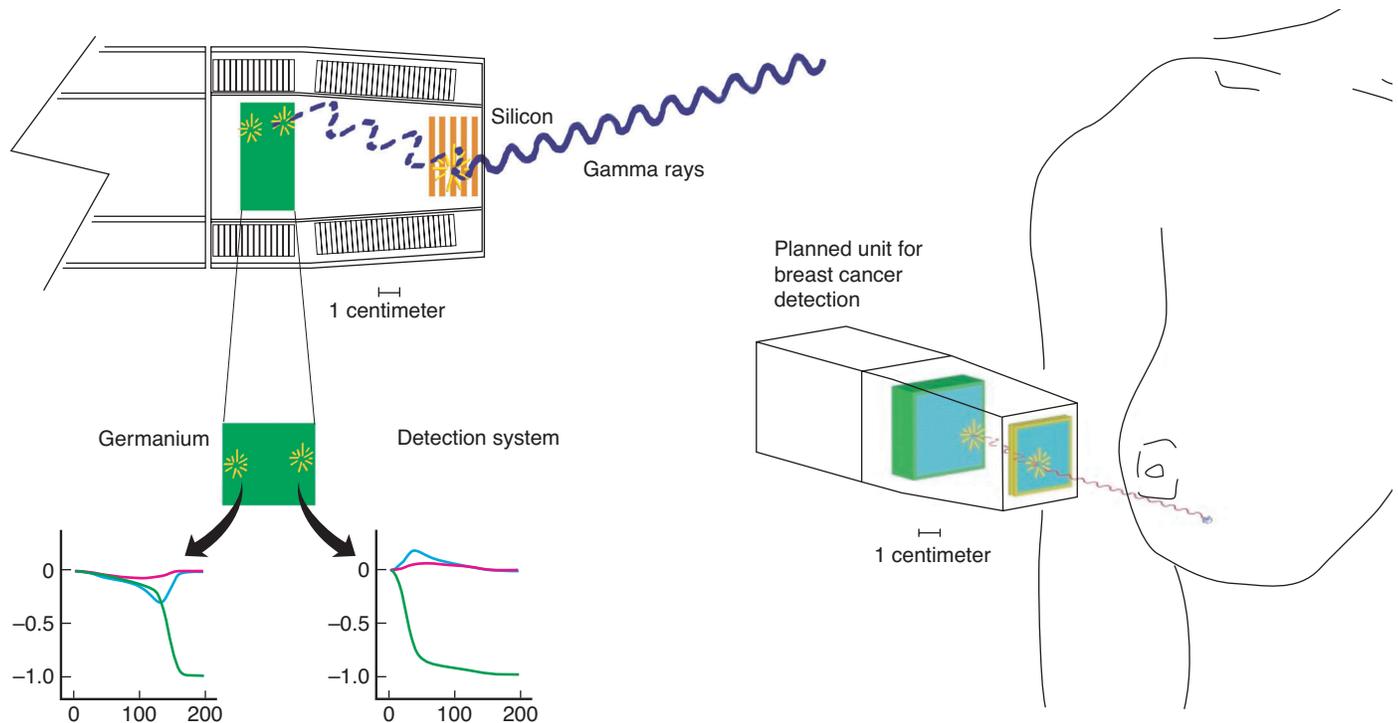
The team is making use of radiation-response data that have been accumulated over decades of conventional radiation beam therapy. They anticipate that this valuable data can be used to refine the estimates of what it will take to make targeted radiation therapy cure metastatic cancer while avoiding injury to healthy organs.

In MINERVA, INEEL's computational dosimetry system for neutron radiotherapy is being merged

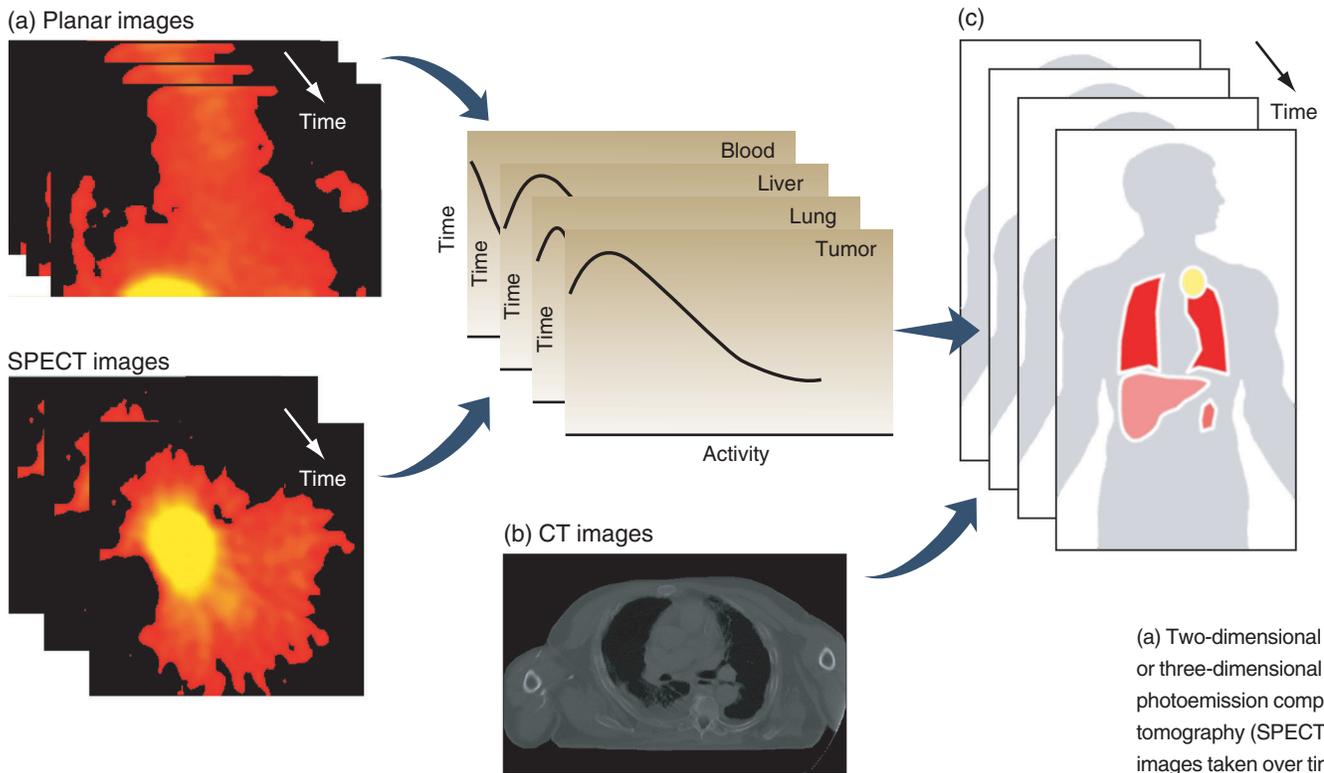
with Livermore's fast, three-dimensional Monte Carlo PEREGRINE simulations for photon-electron therapy. Montana State is writing most of the user interface, and UC Davis is providing its expertise in targeted radiotherapy.

Says radiation physicist Joerg Lehmann, who directs Livermore's part of the effort, "Targeted radiotherapy has been in trials for many years and there are other planning programs around. But their dosimetry data are less accurate than MINERVA's will be."

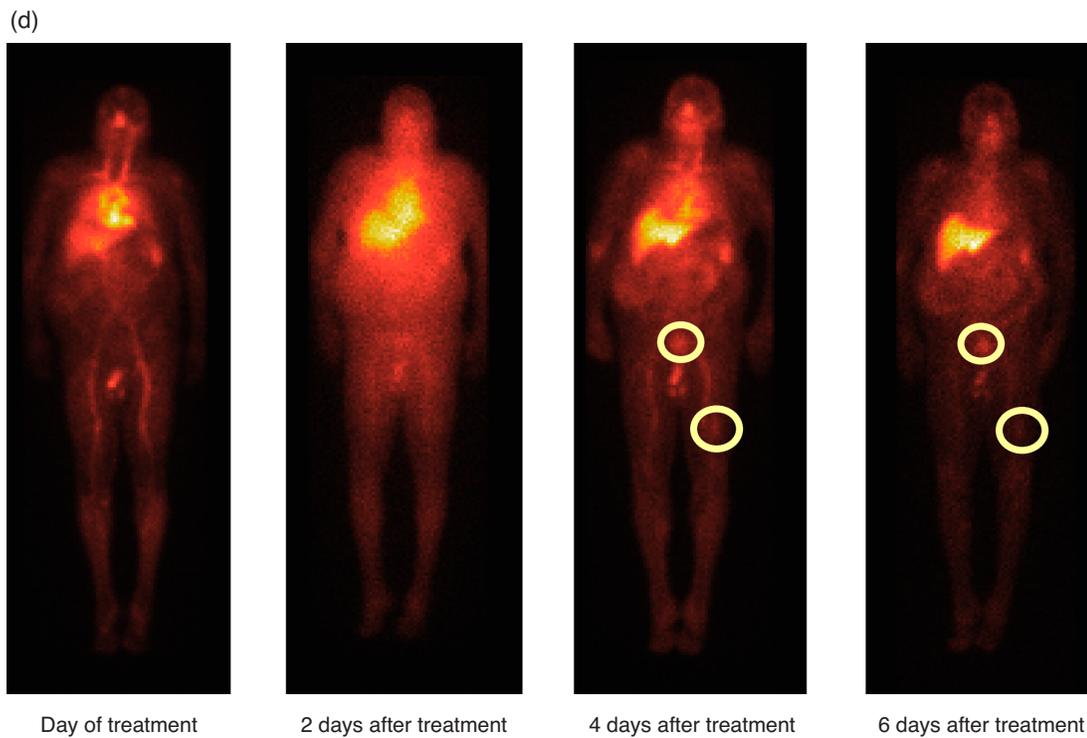
Currently, dosimetry is based either on the patient's body surface alone or on risk assessment approaches used in diagnostic nuclear medicine. At the same time, treatment planning is not based on the patient's particular anatomy. The end result is that most patients are undertreated as doctors strive to avoid damage to normal



Livermore's new system for detecting breast cancer tumors relies on recent developments in segmented semiconductor detectors and digital signal processing. Unlike other gamma-ray detectors, this system does not require collimation (alignment) of the radiation emanating from the tumor source. Because some gamma-ray energy is lost in the collimation process, not requiring collimation increases system efficiency.



(a) Two-dimensional planar or three-dimensional single photoemission computed tomography (SPECT) images taken over time after a patient ingests a small diagnostic dose of a synthetic high-affinity ligand tagged with a radionuclide. Those images and (b) computed tomography (CT) images of the patient's anatomy are the basis for MINERVA's Monte Carlo radiation simulations. (c) MINERVA's simulations result in verifiable quantitative data on the amount of radiation that the patient has absorbed and where the radiation dose is distributed in tumors and critical normal organs. (d) The physiology of an individual patient determines how much radiation dose is delivered.



organs. No one wants the cure to kill the patient.

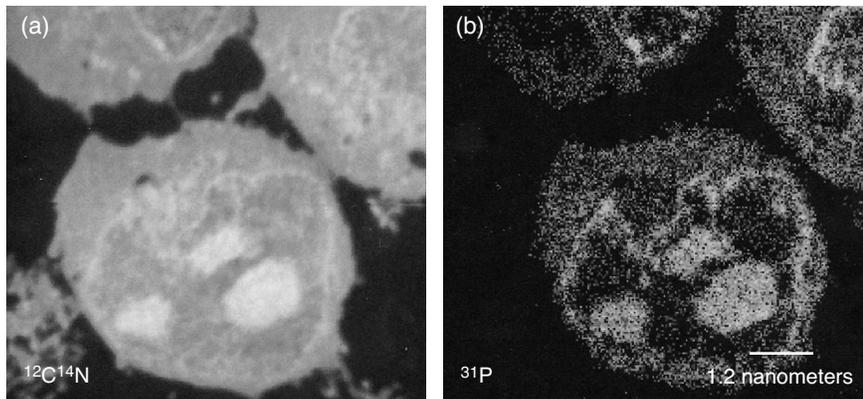
In contrast, MINERVA is designed to produce a customized treatment plan for each patient. When the system is up and running, it will reveal the time-dependent activity of radiation in the body. First, an initial test dose of

radiation in a SHAL or monoclonal antibody is administered to the patient. Then a series of images is taken of the radioisotope in the body over time using either two-dimensional planar images or three-dimensional single photoemission computed tomography (SPECT) images. The distribution of radiation activity in

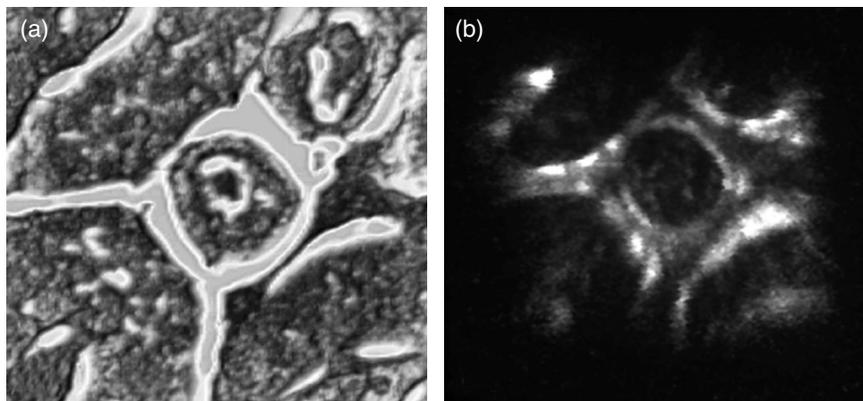
the body is based on these images and a set of computed tomography (CT) scans of the patient's body that show the location of organs. Monte Carlo radiation simulations will provide verifiable quantitative data on the amount of radiation that the patient has absorbed and where the radiation dose is distributed in tumors and critical normal organs.

Then comes decision time for the oncologist: Will this patient benefit from this kind of therapy? If so, how much dose should be administered to the particular patient?

At present, the resolution in available isotope imaging technologies is poorer than that of a CT scan. Isotope images also tend to be time-consuming to obtain. But as Livermore's collimatorless technology becomes available, the image and resolution will improve markedly, leading to even better data on the activity of the radiopharmaceutical.



Secondary ion images of Raji cells showing the distribution of (a) nitrogen (imaged as the $^{12}\text{C}^{14}\text{N}$ ion) and (b) phosphorus (^{31}P). These images were obtained with Livermore's new NanoSIMS and have a spatial resolution of approximately 50 nanometers. The ovoid-shaped bright regions in the center of the cell indicate nitrogen and phosphorus are concentrated in nucleoli. The membrane separating the cell nucleus is also clearly visible in the phosphorus image. The cell diameter is approximately 8 micrometers.



Two views of tubules in the cortex of an yttrium-treated mouse kidney. (a) Transmitted light photomicrograph. The tubule diameter is approximately 30 micrometers. (b) Secondary ion image showing the distribution of yttrium-89 in mouse kidney. Yttrium is not found in tubules but is concentrated in the spaces between tubules. The concentration of yttrium is highly variable from one tubule to another. Understanding how targeted radiopharmaceuticals—represented here by yttrium-89—are distributed in cells can lead to more effective cancer treatment.

A Look Inside Cancer Cells

Once a tagged monoclonal antibody or SHAL attaches itself to a cancer cell, how does the radionuclide attack the cell and work its deadly magic? Researchers elsewhere have attached three different radioactive nuclides (iodine-131, copper-67, and yttrium-90) to Lym-1, a monoclonal antibody used to treat non-Hodgkin's lymphoma, with varying degrees of clinical effectiveness. The reasons for the differences in effectiveness are currently unknown.

To examine the specific effects radionuclides have on cells, a Livermore team recently began using a novel form of mass spectrometry with unprecedented spatial resolution to study the distribution of isotopes within individual cells. A goal is to determine the toxicities of various radioisotope-tagged molecules in both cancer and normal tissues and whether or not the localization of the drug can be correlated to its effectiveness.

In secondary-ion mass spectrometry (SIMS), ions with a few kiloelectronvolts

of energy bombard a solid sample in a vacuum chamber. In this process, called sputtering, surface atoms are ejected from the sample, ionized, and sent into a mass spectrometer for analysis. The secondary ions provide a direct measure of the elemental, isotopic, and molecular composition of the uppermost atomic layers of the sample's surface. SIMS has been used at Livermore for more than 17 years for high-precision analyses of many kinds of samples—weapons materials, radioactive waste to be stored at Yucca Mountain, meteorites, and even counterfeit money.

“About two years ago, we wanted to be able to characterize biological materials with SIMS,” says physicist Ian Hutcheon, who has been working with SIMS for 25 years. “But cells are very small, from 1 to 10 micrometers in size, and the spatial resolution with conventional SIMS wasn't good enough.”

So, Livermore purchased the NanoSIMS, a new instrument designed specifically for quantitative imaging of biological materials. Livermore's NanoSIMS, only the eighth instrument of its kind in the world, provides a spatial resolution of better than 50 nanometers, roughly 100 times better than that of conventional SIMS. The unit arrived in December 2002, and installation began in January. Although the NanoSIMS is not yet fully operational, the first studies have already provided a glimpse of cellular microstructure with remarkable clarity.

Well before the NanoSIMS was delivered, Hutcheon and his team began to develop procedures and standards for using SIMS on biological samples. Traditionally, SIMS has been used primarily on inorganic substances. Unlike inorganic materials, biological samples are largely water and behave badly in the high vacuum of a SIMS instrument. But Hutcheon's team overcame this problem by developing a biological sample-preparation technique

that removes the water while preserving the biochemistry and composition of the sample as well as its microstructure and morphology.

Using isotopically labeled monoclonal antibodies in both normal and cancer cells, the team began studying the distribution of yttrium-89 and iodine-127—stable surrogates for the radioisotopes often used in cancer therapy—in kidney, liver, and tumor samples of a mouse infected with lymphoma. The NanoSIMS images reveal for the first time just how yttrium-89 delivered by the molecule accumulates in a mouse's kidney. The yttrium is not found in the kidney tubules but rather is concentrated in spaces in between tubules. The images also show that the amount of yttrium is quite variable from one tubule to another. Data such as these offer great promise in understanding the toxic effect to the kidneys of different radionuclides. This examination of the cellular distribution of various molecular targeting radionuclides will help to determine the effectiveness of various radiopharmaceuticals in treating tumor cells as well as what, if any, effect that the pharmaceuticals have on normal organs.

“Using SIMS to examine biological samples is very new,” adds Hutcheon. “Our NanoSIMS is only the second one in the country with full-blown capabilities.”

One Patient at a Time

Hartmann-Siantar expects that by the end of five years, phase 1 clinical trials of

Livermore's customized cancer-fighting SHALs will be under way while the collimatorless gamma-ray imaging device, MINERVA, and NanoSIMS will be in use in research settings.

The high-resolution images from the collimatorless imager will begin to diagnose not only breast cancer but other cancers and diseases whose presence can be revealed with radioisotopes. MINERVA will be available to assist physicians in providing individualized treatment decisions, while the images from NanoSIMS will be offering cellular-level explanations for drug behavior in both mice and patients.

Because each of us is unique, one-size-fits-most medical treatments should not be the norm. In a more perfect world, we would be offered treatment options tailored to our own physiology. These new technologies are helping to bring that world a bit closer.

—Katie Walter

Key Words: gamma-ray detection, Modality-Inclusive Environment for Radiotherapeutic Variable Analysis (MINERVA), non-Hodgkin's lymphoma, PEREGRINE, radiation treatment, radiopharmaceuticals, secondary-ion mass spectrometry (SIMS), synthetic high-affinity ligand (SHAL).

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