

# From Discovery to Treatment

Pitt has the edge in developing drugs.

Research attacks widespread killers like TB, as well as rare diseases.



Before network news programs, newspapers, and magazines praise its potential with powerful adjectives like “revolutionary” and “breakthrough,” a new drug has to wend its way up long, steep hills littered with speed bumps, potholes, and dead ends; and, with little warning, it has to overcome considerable, sometimes overwhelming, odds on its long journey into the human body.

Pharmaceutical company executives, having ushered the drug’s critical and costly final steps to market, usually win the right to announce their newest creation to the world. But many researchers—chemists, biologists, and pharmacologists among them—who helped rear the drug from birth through adulthood go unnoticed by the public. They are a drug’s behind-the-scenes architects and builders who first asked, “What if...?”

At the University of Pittsburgh, these investigators take center stage each day as they search for agents powerful enough to bring down life-threatening diseases like cancer. Although the process is long, deliberate, and marked with unforeseen setbacks, the thought of creating a drug with the potential to save thousands, perhaps even millions, of lives fuels drug discovery research at Pitt.

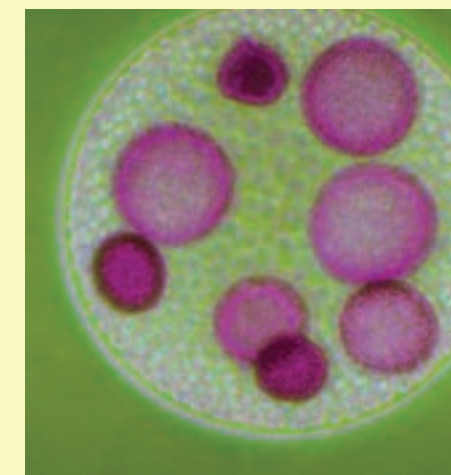
“We’ve known the cause of cancer for 25 years,” says Allegheny Foundation Professor John S. Lazo, director of the Fiske Drug Discovery Laboratory, University of Pittsburgh; Codirector

of the Molecular Therapeutics/Drug Discovery Program, University of Pittsburgh Cancer Institute; and chair of Pitt’s Department of Pharmacology.

“We’ve known what the fundamental problem is with cystic fibrosis for 25 years. The gene was known. We know it’s mutated. We know a lot about its biology. But translating that into something that is a therapy is a huge intellectual activity.

“And that’s what we are trying to deal with,” Lazo continues. “How you take this very basic information that’s being generated by all of our colleagues at the University of Pittsburgh and throughout the world and convert it into something that you can put into a little pill, swallow, and feel better is the process that gets our hearts beating faster.”

Pitt bolstered its odds considerably and solidified its place among the elite drug discovery universities in 2002, when the National Institutes of Health’s (NIH) National Institute of General Medical Sciences awarded Pitt a \$9.6 million grant to create the



Center of Excellence in Chemical Methodologies and Library Development, making it one of the first two such centers in the country. In the future, researchers will be able to “check out” some 50,000 compounds from a chemical library to screen against drug targets.

To head the new program, the University selected Peter Wipf, professor of chemistry and 2002 American Association for the Advancement of Science

Fellow. Wipf and his colleagues at the center, among them Kay Brummond, associate professor of chemistry and vice director of the center, now have access to the latest high-tech equipment, which allows them to build and purify large numbers of chemical compounds that could be used to discover new drug candidates.

The University landed the NIH funding for the Center for Excellence, in part, based on the strength of the Department of Chemistry's Center for Combinatorial Chemistry (CCC), also led by Wipf. The University created the CCC in 1998 to strengthen its drug discovery program. The University created the University of Pittsburgh Cancer Institute (UPCI) in the same year.

In September 2002, just a month prior to winning the grant to create the new chemical library, the University of Pittsburgh Cancer Institute (UPCI) was one of 15 centers in the country to receive a five-year, \$2.7 million grant from the National Cancer Institute (NCI) to develop anticancer agents for testing in Phase I clinical trials.

As part of this NCI grant, investigators are examining, among other things, angiogenesis inhibitors, which prevent blood vessels from growing from surrounding tissue onto solid tumors; "apoptosis-inducing" compounds, which play a role in a cell's death; and gene therapies.

### Constructing Chemical Libraries

Organic compounds often act like smart molecular missiles. Researchers fire them at such targets as proteins or DNA in hopes of scoring a "hit," measured by the increase or decrease in activity in the target. It's extremely important for scientists to engineer and construct these molecules properly; otherwise, the number of compounds tested, if flawed, might do little to

increase the likelihood of hitting the target.

To create these compound libraries, Wipf and his team use combinatorial strategies, constructing diverse molecular scaffolds and adding chemical groups to them to create a variety of shapes, which can be screened for useful properties. To build these agents accurately, researchers must understand issues like solubility, toxicity, and molecular structure and reactivity. They also need to have considerable knowledge of the specific targets.

When a missile hits the bull's-eye, the potential new drug then moves on to the "lead stage" for further testing in a more complex system, like a mouse. This requires Wipf and his colleagues to make larger amounts, or "scale up synthesis," for researchers.

"If I find something that looks really interesting," explains Lazo, who partners with Wipf on several projects, "I go over to Peter's lab, and I say, 'Here's a chemical structure. Can you

make me a personalized library around the structure that we can then put back in our system and refine it?'...Five years ago, I was lucky if I could get my hands on 500 compounds, [but with advances in technology] I now have 25,000."

In building libraries, Wipf and his research team often look to Mother Nature for help. One such project that he and his colleagues are investigating involves calyculin A, a molecular structure that comes from a marine bug called cyanobacterium, or blue-green algae. Calyculin A's target is a family of enzymes called phosphatases that Wipf says acts like the "off switch" (its better known relative, kinases, serves as the "on" switch) in cell cycles. By inhibiting phosphatases from functioning continuously in malignant cells, the scientists hope to discover possible anticancer drug leads.

"We have to learn how to make bonds and break bonds," says Wipf. "We basically synthesize these molecules from scratch. And in the process of doing so, we learn how to manipulate



every single atom in the particular framework that makes up this molecular or chemical scaffold."

One of Wipf's colleagues and a close drug discovery collaborator, Dennis Curran, Distinguished Service Professor and Bayer Professor of Chemistry, also is building new libraries of compounds with natural products. One of his research groups synthesized approximately 100 "known and new camptothecin analogs," or anticancer agents, that he believes are "excellent preclinical candidates" for treating stubborn solid tumors.

Curran also champions fluororous chemistry as a means to build libraries of drug candidates quickly and efficiently. In 2000, he helped found Fluorous Technologies, a Pittsburgh company that uses the patented technology developed by Curran to synthesize and separate molecules that can be used for any variety of drug targets. Using a technique called fluororous mixture synthesis, researchers tag molecules with fluorocarbons, which allows them to identify and separate compounds rapidly.

### Preparing for Clinical Trials

Many promising drug leads eventually end up in the lab of Merrill Egorin, professor of medicine and pharmacology and codirector (with Lazo) of UPCI's Molecular Medicine and Drug Discovery Program.

"[The compounds we receive] all have some kind of wart or pimple. Our job is to find and figure out how you put a patch over it and make it go away," says Egorin. "We're always saying, 'OK, so we now have a drug that we can formulate, that we can deliver, and it's stable enough, and it gets into cells. Does it work?'"

"At each of these steps, at each of these hurdles, you get dropouts.

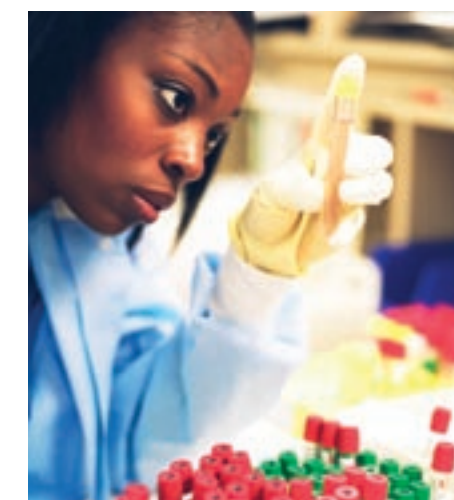
At each of these steps, sort of the fun of playing the game is looking at what happens and talking to people at early stages of discovery and development and saying, 'Here are my data. Here's what I think they mean. Can you make something better?'"

As principal investigator on the NCI-funded contract to evaluate anticancer drugs being considered for clinical trials (mentioned previously), it is Egorin's job to "ask the tough questions," as Lazo puts it. Egorin and his team examine how the drug reacts in living systems (starting with lab animals) over an extended period of time, including how the drug is absorbed, distributed, metabolized, excreted, and localized in tissues.

"We're sort of like the people in the trenches," Egorin says with a laugh. "We're the GIs. And I think Peter [Wipf] and his colleagues are the ones doing all the satellite-based guidance—fancy stuff. We're down in the mud, and they're up in the airplane."

No matter what their positions on the drug discovery continuum, the Pitt researchers' ultimate goal is to create drugs that will find their ways into clinical trials so that real people can benefit from their healing power. Lazo says that he and Wipf have "personal pacts" to get three compounds into preclinical trials each year and to get at least one compound on the market in the next 10 years.

Lazo understands the Herculean effort it takes to usher a new drug to market. In 1996, he founded a Pittsburgh-based pharmaceutical company called ProIX with Garth Powis, professor of pathology and pharmacology at the University of Arizona, and D. Lynn Kirkpatrick, a former professor of chemistry and biochemistry at the University of Regina. They identified a redox (relating to oxidation and reduction) human oncogene found in many human cancers. Their research involves developing



drugs that will restrain the "signaling pathways [responsible for the abnormal cell growth] activated by this gene." Its lead candidate, a drug called PX-12 that targets the redox signaling protein thioredoxin, is currently in Phase I clinical trials.

### Moving from Laboratory to Living Organism

For a drug to move out of a flask and into a living system—like a mouse—it needs an exceptionally sound mode of transportation, one strong enough to hold up against a full-fledged assault by an immune system and fast and accurate enough to speed through the blood directly to the target.

"It's one thing to get a molecule that works really well in a test tube," explains Lazo. "It's a huge jump to then say, 'Can I put it into a cell? When I swallow it, does it disappear in two seconds or does it stay around long enough to do what it's supposed to do? And where does it go?'"

Drug *delivery* is as influential on a compound's progression as any other factor. Many drugs with lots of potential will no doubt wither on the vine without a solid delivery system. One area of research where this is readily apparent is in gene

therapy, where investigators search for ways to replace or repair damaged genes with normal, healthy genes.

One team of Pitt researchers—led by Leaf Huang, professor of molecular genetics and biochemistry, pharmacology, and bioengineering and director of Pitt's Center for Pharmacogenetics—has pioneered a new drug delivery method using nonviral vectors. (Investigators use vectors to transport DNA to a specific cell.) Although more attention has been paid to viral vectors for drug delivery because of their high efficiency, Huang and his team have focused on their less toxic counterparts, nonviral vectors, to deliver plasmid DNA to diseased cells.

Surrounded by layers of oily organic compounds called lipids, DNA molecules are transported through the body to their target cells, past the enzyme barriers, and into the cell's membrane to begin their repair work. Some of the Huang team's nonviral vectors, named DC-chol liposomes, have so far been tested in eight different clinical trials on patients with cancer and cystic fibrosis.

Drugs that make it to Phase I clinical trials, although still miles from market, in many ways have already achieved success. The Pharmaceutical Research and Manufacturers of America estimates that only five out of every 5,000 potential new drugs tested on animals will make it to clinical trials. And only one out of those five will win FDA approval. The Food and Drug Administration, which is responsible for approving new drugs in the United States, estimates that the average journey is eight-and-a-half years for the fortunate few drugs that make it to market.

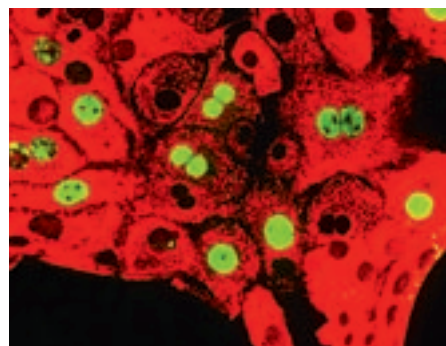
"If you're an industrial chemist, you'd like to see your compounds going into clinical trials," says Wipf. "A lot of compounds don't make it through Phase I or Phase II—a lot of compounds. But from a chemist's point of view, it's

a great satisfaction to see a compound being used in humans, even if it doesn't make it onto the market, because the barriers are high and unpredictable and we don't understand a fraction of what's going on in the complex human organism. And [chemists] don't have the tools to make good predictions, because all of the evaluations are simplified animal and cell studies."

### Pitt Advantage: Discovery to Development

Drug discovery has mushroomed into big business, requiring cavernous pockets. With costs in the millions (and potential payoffs in the billions), heavily financed pharmaceutical companies try to secure the rights to promising new drugs as they move through costly clinical trials and closer to market.

In 2001, the Tufts Center for the Study of Drug Development estimated that the cost of drug discovery—bringing a drug from an idea to market—is \$802 million. This was up from an estimated \$231 million in 1992 and \$54 million in the late 1970s. And,



*Drug discovery and development have been and continue to be a central focus of research at the University of Pittsburgh Cancer Institute. In the drug discovery and development laboratories of the Research Pavilion, numerous advanced technologies are applied to rapidly develop and screen drugs and test their effects on different cells within the body.*

according to IMS Health, a pharmaceutical market research and consulting firm, in 2001, drug companies invested \$30.3 billion on research and development marketing campaigns to reach consumers and doctors.

According to Egorin, with the ability to nurse a drug from infancy all the way through Phase II clinical trials, the University of Pittsburgh has a decided advantage over most universities.

"The neat thing here is that we really do have a vertically integrated drug discovery/drug development program," says Egorin. "We're federally funded on every level. We're one of only 15 NCI-funded facilities that do Phase I studies. The UPCI plays a major role in the Eastern Cooperative Oncology Group [one of the largest clinical cancer research organizations in the country]. My lab turns out to be a reference lab for Cancer and Leukemia Group B, which is one of the other big cooperative groups. We can do our in-house Phase I studies, and we can then take them to multi-institution Phase II trials. We also have a subcontract with the California Cancer Consortium to do Phase II studies."

Because Pitt is an institution of higher learning and research and not a business, it also is allowed to tackle lesser-known "orphan diseases." According to NIH, an orphan disease affects fewer than 200,000 Americans. There are currently 6,000 of these known rare diseases, and although the incidence rate of each is small, combined they afflict 25 million Americans. They include ailments like sickle cell anemia, Tay-Sachs disease, hemophilia, Fanconi anemia, Tourette syndrome, and Lou Gehrig's disease.

"If you're a drug company, you want to make a billion dollars a year," says Lazo. "If your disease only affects 30,000 people, it is not on the company's radar screen. Period. The market's too small.

"However, it turns out that one out of six people is affected by orphan diseases. So there's a huge opportunity for us to fight diseases that may not be something that affects lots of people but affects a number of individuals. And if we can make it more attractive for a drug company to pick them up and develop them, then we'd be really happy. But we're not driven by profit. It would be wonderful if [the drug we develop] would help lots of people, but Peter and I are excited about going after these rare diseases, also," says Lazo.

Orphan diseases have been rising in public awareness in the United States since 1983, when Congress passed the Orphan Drug Act, which provides research grants for investigators searching for cures. In 1993, the NIH created the Office of Rare Diseases to further advance these findings, and in 2002, President Bush signed into law two bills—the Rare Diseases Act and the Rare Diseases Orphan Product Development Act—which nearly doubled funding for orphan disease research.

### New Approaches to Drug Discovery

One disease that's anything but rare, *Mycobacterium tuberculosis* (TB), has the attention of Graham Hatfull, Eberly Family Professor in Pitt's Department of Biological Sciences, and his research team.

In the early 20th century, TB ravaged the nation, killing slowly and painfully. But in 1944, with the discovery of streptomycin, TB virtually vanished from public view—at least in developed countries like the United States. But TB is hardly a disease of the past.

The World Health Organization estimates that TB infects one third of the world's population, killing three million people each year. If treated consistently and aggressively with multiple antibiotics, TB usually is not a problem. However, in many resource-

starved countries where people are unable to follow these procedures, new drug-resistant strains are making it more difficult to stop, thus more deadly. "If you don't comply with the [antibiotic] regimen, you provide a seeding ground for the development of drug-resistant strains," says Hatfull. In many instances, "the mere usage of antibiotics is actually fostering the development of strains that are untreatable by the [regular] methods, so there's clearly a need for new approaches to drug discovery."

To learn more about a harmful organism, Hatfull's lab focuses on bacteriophages—viruses found in bacteria. According to Hatfull, phages are like genetic tool boxes that allow researchers to determine what role specific genes in mycobacteria play in the growth process. By understanding the molecular biology and genetic makeup of these organisms, researchers can then hopefully begin to create new vaccines and more powerful anti-TB drugs.

"If the focus is on understanding the underlying biology, you actually generate a reservoir of incredibly important information that enables you to develop new types of strategies in dealing with particular diseases," says Hatfull.

Biology and chemistry have long been close bedfellows in drug discovery. And now, with the Human Genome Project unveiling some of the mysteries of human DNA, a vast new world of research possibilities exists, perhaps more than ever, for researchers from a variety of disciplines.

"It used to be the paradigm that drug discovery was chemistry-driven," says Wipf. "It's not the only paradigm any more, but many alternatives are still in their infancy. [Chemistry and biology] are almost like DNA and proteins. You still argue which one is the more fundamental starting point, but without proteins you can't translate the information into action. [In other



*University of Pittsburgh School of Pharmacy graduate students develop skills and techniques useful in drug discovery and research in the school's well-equipped laboratories.*

words,] what's the use of a book if you can't read the language?"

Whoever is driving the discovery machine, Pitt researchers don't seem to mind, because they are all working toward the same goal: to create drugs that will someday help somebody. And based on the ongoing research across campus—of which only a molecule-size portion is represented here—the University seems well positioned to reach its drug discovery goals.

"If you were to ask around the United States, 'Where does drug discovery occur?' I think the University of Pittsburgh would be in the top five," says Lazo. "And I would like to think that five years from now we'll be *the* center for work in drug discovery. We certainly have the fire power in terms of the faculty and research activity to be there." ■