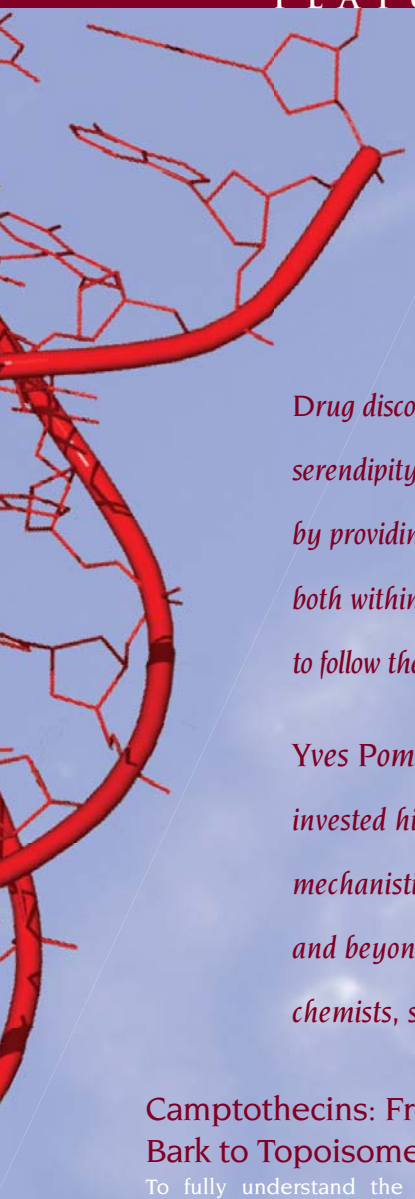


The DNA of Drug Discovery

Drug discovery, like research in general, relies on a fine balance between directed exploration and serendipity. With the goal of translating basic scientific insights into cures, CCR fosters this balance by providing the infrastructure to make new connections among seemingly disparate research efforts—both within the NCI and extramurally—and by providing new tools and opportunities for investigators to follow the therapeutic directions generated by their science.

Yves Pommier, M.D., Ph.D., Chief of CCR's Laboratory of Molecular Pharmacology, has invested his career in studying DNA processing mechanisms, with an eye towards turning his mechanistic insights into new generations of drugs. And thanks to innovative collaborations within and beyond NCI that have bridged his knowledge of molecular biology with the expertise of chemists, such drugs may be closer to hand.

Camptothecins: From Tree Bark to Topoisomerase

To fully understand the story behind Pommier's quest, one must look back 40 years. In the 1960s, while working on a contract with NCI, Monroe Wall, Ph.D., whose credits already included the purification of the anti-cancer wonder drug paclitaxel (Taxol®) from the bark of the Pacific yew tree, identified a second cancer-fighting compound—camptothecin—from the bark of a tupelo tree found only in China and Tibet. Wall studied camptothecin and synthesized derivatives, but without a known mechanism of action, the compounds languished at NCI's Natural Products Branch. Some 20 years later, in 1985, an NCI-supported academic/commercial collaboration of researchers at Johns Hopkins University, University of Florida, and SmithKline (now GlaxoSmithKline, or GSK) provided the first evidence that a DNA processing enzyme called topoisomerase I (topo I)—which makes cuts in DNA double helices, permitting them to relax for transcription or replication—was the camptothecins' molecular target.

At the time the camptothecins-topo I link was announced, Pommier's group was studying topoisomerase II (topo II), a related enzyme and known target of chemotherapeutic agents like doxorubicin. Thus, he was well positioned to study the cellular mechanisms of action of this new class of compounds. He and others confirmed that topo I was indeed the camptothecins' anti-cancer target and that the drugs turned normal topo I into a deadly enzyme by jamming it irreversibly onto the cell's DNA. Pommier's group also showed that human cancer cell lines could evolve resistance to camptothecins, invariably due to a mutation in the topo I gene. Within ten years of the confirmation of topo I's role, two camptothecin drugs had been FDA approved—topotecan (Hycamtin®) and irinotecan (Camptosar®).

> ...If there is one family of topo I inhibitors, might not there be another that would prove more powerful still?

Limited by their chemical stability and toxicity, camptothecins were not suitable for widespread drug development efforts. However, if there is one family of topo I inhibitors, might not there be another that would prove more powerful still? This was the question that Pommier and his colleagues decided to attack. But to do so, they needed help.

Panning for New Topo I Inhibitors

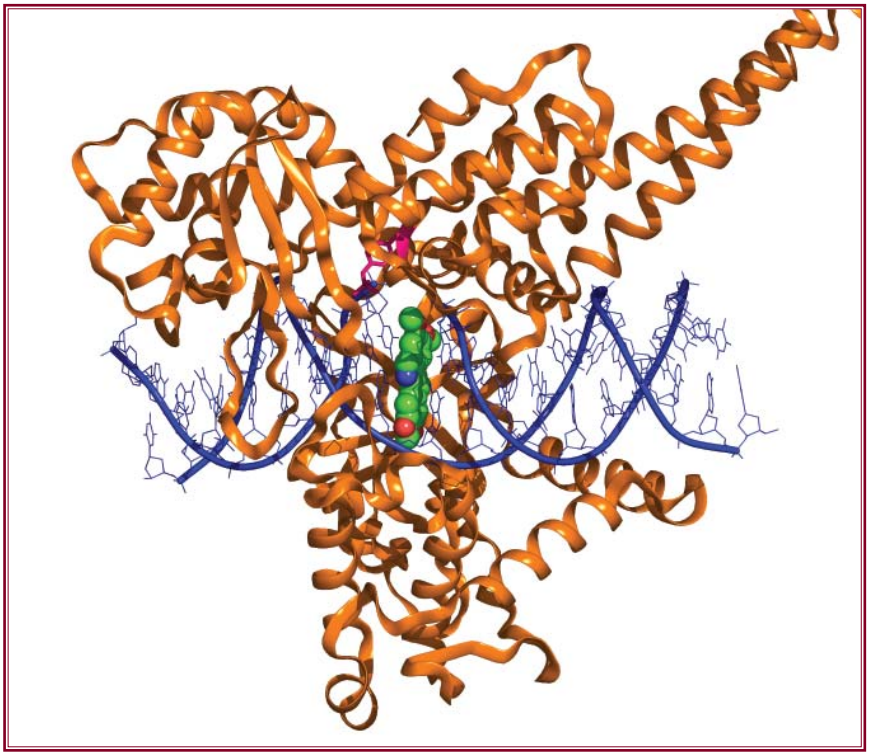
Prior to his death a few years ago, Ken Paull, Ph.D., worked with NCI's Developmental Therapeutics Program (DTP) to screen compounds for anti-cancer activities. NCI has 60 distinct standardized cancer cell lines, the so-called NCI-60, that its scientists use to screen compounds at five different concentrations for their ability to inhibit growth. Since no two cell lines are identical, compounds with different mechanisms of action affect the proliferation of individual cancer cell lines differently. Paull and his colleagues realized that by comparing dose-response profiles across all 60 cell lines, they could classify compounds with related mechanisms of action; drugs that affect all of the cell lines in a similar way are likely to operate via a similar mechanism. Paull formalized this logic in a computer algorithm called COMPARE.

Familiar with Paull's work, Pommier decided to see if COMPARE could pick out compounds that work like camptothecin. When they struck gold, the compound they identified, an indenoisoquinoline, turned out to be the byproduct of another serendipitous event captured by the NCI. The compound, synthesized by chemist Mark Cushman, Ph.D., at Purdue University, was the result of an "unexpected, undesired reaction," as he put it, that occurred as he attempted to synthesize the anti-leukemia agent nitidine chloride. Instead of discarding it, Cushman placed the indenoisoquinoline compound in the NCI-60 database, where it sat untouched for 18 years, until he received a phone call from Paull.

Cushman immediately set to work making indenoisoquinoline analogs—400–500 of them—which he sent to NCI for Pommier's group to test against purified topo I and in cell culture for structure-activity relationships. The data led Pommier and Cushman to focus on the two most promising candidates, which are now on the verge of entering the clinic for the first time. "At this point, we've done preclinical and toxicology work, and the clinical protocols have been written," said Pommier.

But advancing these compounds from the chemistry lab to the clinic would not have been possible without the DTP, which as a mission takes lead compounds that show promise in cell culture and puts them through the many hurdles of animal experiments and formulations that must be cleared before first-in-human trials. For example, the academic synthesis protocols that Cushman develops may bear scant relation to the synthesis processes necessary for the high-volume commercial manufacturing steps that a pharmaceutical company must employ. Similarly, the drugs that Pommier tests *in vitro* are all dissolved in DMSO, which is toxic to human beings, and so must be assessed for solubility in non-toxic solvents as well. The DTP has even enabled the development of a biomarker for topo I inhibition that research clinicians will use in their clinical trials, the phosphorylation of histone γ-H2AX. This biomarker was first associated with DNA damage by another CCR investigator, William Bonner, Ph.D. Another NCI colleague, James Doroshow, M.D., Director of NCI's Division of Cancer Treatment and Diagnosis, collaborates with CCR's Laboratory of Molecular Pharmacology and has been a key player in the development team leading to the clinical evaluation of the indenoisoquinolines at the NIH clinical center.

Pommier and Postdoctoral Fellow Thomas Dexheimer, Ph.D., continue to collaborate with Cushman to test new potential topo I inhibitors. "When you have one target, you want to have more than one type of drug," said Pommier. "Even drugs in the same family, such as irinotecan and topotecan, have different clinical profiles. We're making the assumption, but I think it is likely to be the case, that the indenoisoquinolines are going to have a different clinical profile from any of the camptothecins. And we have many arguments to say why they have advantages, but the proof will become apparent when we give these compounds to patients."



(*Image: Y. Pommier, CCR*)

Indenoisoquinolines *(green)* glue complexes of topoisomerase I *(brown)* and DNA *(blue)* together.

Nature Plus Nurture: The Consortium Approach

Citing the examples of paclitaxel and camptothecin, Pommier is convinced that Nature has many more hidden treasures that could benefit mankind's health: "Nature has taken a long time to optimize for us," he said. "Although we now have powerful methods for visualizing and predicting compounds' structural features and binding activities, rational drug design is not the only way forward." Rather, screening and rational drug design are complementary parts of an overall drug discovery strategy that Pommier and his colleagues are using to go after another cancer target, the DNA repair enzyme Tyr-DNA-PDE, or TDP. TDP repairs the stalled DNA replication caused by topo I inhibitors, so cells that are missing TDP are hypersensitive to topo I inhibitors.

> Pommier is convinced that Nature has many more hidden treasures that could benefit mankind's health.

Because TDP had no known inhibitors, Christophe Marchand, Ph.D., a Staff Scientist in Pommier's group, spearheaded high-throughput screening against TDP in collaboration with the NIH Chemical Genomics Center (NCGC) up the road in Gaithersburg, Md. Although Marchand had already developed an assay for the Pommier laboratory's in-house screening system when they began their collaboration, he needed to reoptimize it for the NCGC, more or less on his own.

"We got lucky," he said of the success of his early optimization attempts. After less than a year, NCGC was convinced that it could screen its entire compound library of over 300,000 compounds against Marchand's TDP inhibitor assay, a screen that was completed in the first week of June 2008.

The TDP project now includes more than just Pommier's group and the NCGC. NCI's Chemical Biology Consortium has since taken an interest in the work and set up an entire team of investigators, supported by dedicated project managers, to promote the development of TDP inhibitors "from bench to bedside." Across NCI, more than 20 investigators meet regularly to share data and plan new experiments, including using synthetic chemistry to design better inhibitors based on the structural analysis of lead compounds (see "SCSORS Takes the Lead").

Marchand counts the success of this project to date among his proudest achievements and is excited about the collaboration and the opportunities afforded by a large consortium in overcoming practical obstacles. "For the first time, I have the feeling that we are surfing on big waves."

"The resources are amazing, although they aren't always connected up as well as we'd like," Pommier noted when describing the path he took to establish a collaboration with the NCGC. "The NCI is a powerful place for this kind of work."

Learn more about Yves Pommier's research at http://ccr.cancer.gov/staff/staff.asp?profileid=5812 and http://discover.nci.nih.gov/pommier.

To learn more about camptothecin and other natural products, see "The Natural Products Repository: A National Drug Development Resource," page 9.

SCSORS Takes the Lead

To tap into the vast reservoir of possible synthetic organic chemistry, the NIH has developed a new Semi-Custom Synthesis On-line Request System (SCSORS) in conjunction with the company ChemNavigator, Inc. SCSORS has been funded mostly by NCI with additional financial support from the NIH Chemical Genomics Center (NCGC).

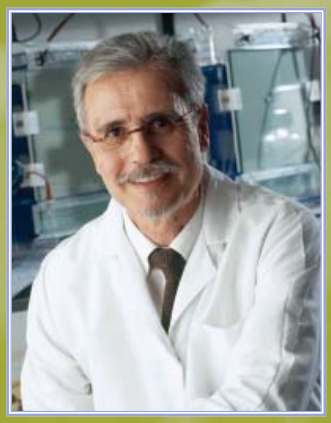
The new SCSORS project will provide the NIH (and the NIH Roadmap-associated screening centers) access to the world's supply of synthetic chemistry available for drug discovery. It will also help NIH scientists to access specific chemical samples, in amounts ranging from milligrams to kilograms, from thousands of synthetic chemists at suppliers registered in the system.

NIH researchers will be able to use SCSORS in three ways:

1) By proposing specific structures for which they request a SCSORS quotation
2) By submitting a structure—typically a lead generated from an assay—to ChemNavigator's affiliated chemistry procurement service, which will do a "medicinal chemistry expansion" of this structure and present a series of analogs for selection and approval before submitting them to suppliers
3) By requesting that a structure (or structures) be presented to suppliers as is, with the requests, "What can you do with this molecule? Which analogs do you think you can synthesize, and at what cost?"

The hope is that using the SCSORS strategy will allow the NIH to acquire chemical samples at less than 10 percent of the internal cost of synthesis while accessing global chemical expertise and diversity.

In the long-term, SCSORS will become an archive of commercially accessible custom chemistry products for pharmaceutical research. The project's leaders expect that its database will grow to over 250 million substances in the coming two years.



(Photo: R. Baer)

Yves Pommier, M.D., Ph.D.

Yves Pommier, M.D., Ph.D.

Pommier heads the Laboratory of Molecular Pharmacology at NCI where his research has centered on DNA processing mechanisms and on two enzyme classes in particular—cellular DNA topoisomerases and HIV integrase.

In addition to his focus on the role of DNA topoisomerases in cancer, Pommier began studying HIV integrase in 1993 in response to the widespread call to arms in the research community for the development of AIDS therapies. Pommier's group reported the first HIV integrase inhibitor and proceeded to develop several more.

Pommier joined the NIH in 1981 after receiving his degrees from the University of Paris, France. Although he does not do clinical work himself, he is glad that he was encouraged to receive both an M.D. and a Ph.D. He was quickly frustrated as a hematology/oncology resident by the paucity of treatments available for the cancers that ravaged his patients; he attributes the direction of his career in molecular pharmacology and translational research to these clinical experiences.

"You think differently," he says, explaining that he does not lose sight of the goal of turning research into cures. "I've had great fun [studying DNA processing]. But it would be so pleasing to discover one drug and make a difference."

(Photo: R. Baer)

Christophe Marchand, Ph.D.

Christophe Marchand, Ph.D.

Marchand's career epitomizes personal initiative. As an undergraduate in Reims, France, Marchand wrote to the organizer of an international meeting in Paris on DNA-drug targeting and convinced him to waive the attendance fees. At the meeting, he met his first mentor in the field, Cambridge University's Michael Waring, Ph.D., who took the fledgling scientist under his wing. For the next five years, Marchand spent every summer at Cambridge, returning in the fall with another publication under his belt.

Waring introduced Marchand to Claude Hélène, Ph.D., Head of an INSERM unit in the Laboratory of Biophysics at the French Natural History Museum, who supervised Marchand's doctoral work on DNA triple helices—molecules composed of three rather than two spiraling strands of nucleic acids. Marchand's passion for these intriguing molecules is still evident in his voice. "I had a revelation when I heard Hélène talking about DNA triple helices—there was a spark in my head—the applications seemed almost endless." The drug he developed for his thesis, which specifically identifies DNA triple helices, is now in the Sigma catalog.

Marchand, currently a Staff Scientist, has been in Pommier's group for ten years. His primary expertise is the study of HIV integrase, but he has broadened his focus to include the development of high-throughput screening assays. Although he does not rule out returning to France some day, he is pleased with his current position, which affords no shortage of opportunities for anyone with initiative.

(Photo: R. Baer)

Thomas Dexheimer, Ph.D.

Thomas Dexheimer, Ph.D.

Dexheimer came to the NIH two years ago, motivated to pursue postdoctoral work with Pommier after hearing him give a seminar at the University of Arizona (UA) where Dexheimer was completing his Ph.D. His doctoral work also focused on DNA with an eye towards drug discovery, so the transition was a natural one. In the lab of UA's Laurence Hurley, Ph.D., Dexheimer studied DNA secondary structures—G-quadruplexes, so called because of their four-stranded guanine-enriched composition. Most G-rich regions are in promoters, and Dexheimer had hoped to design drugs to stabilize G-quadruplexes in cells as a means of targeting proto-oncogene promoters.

Dexheimer is currently involved in both the TDP and topo I inhibitor projects. Although he arrived after the two lead indenoisoquinoline inhibitors of topo I were discovered, he continues to look for new compounds which may have different yet advantageous clinical profiles. He also hopes that since TDP's and topo I's mechanisms of action are linked, the two projects may intersect in combination therapies.

Dexheimer knows that the odds of turning a lead compound into a successful drug are very low. But that does not temper his excitement in the search for new classes of topo I inhibitors. "My father won the Wisconsin state lottery when I was in high school," he noted, a windfall that helped pay for Dexheimer's college undergraduate chemistry degree (and a few other things, like a hot tub). "I'm an optimist."