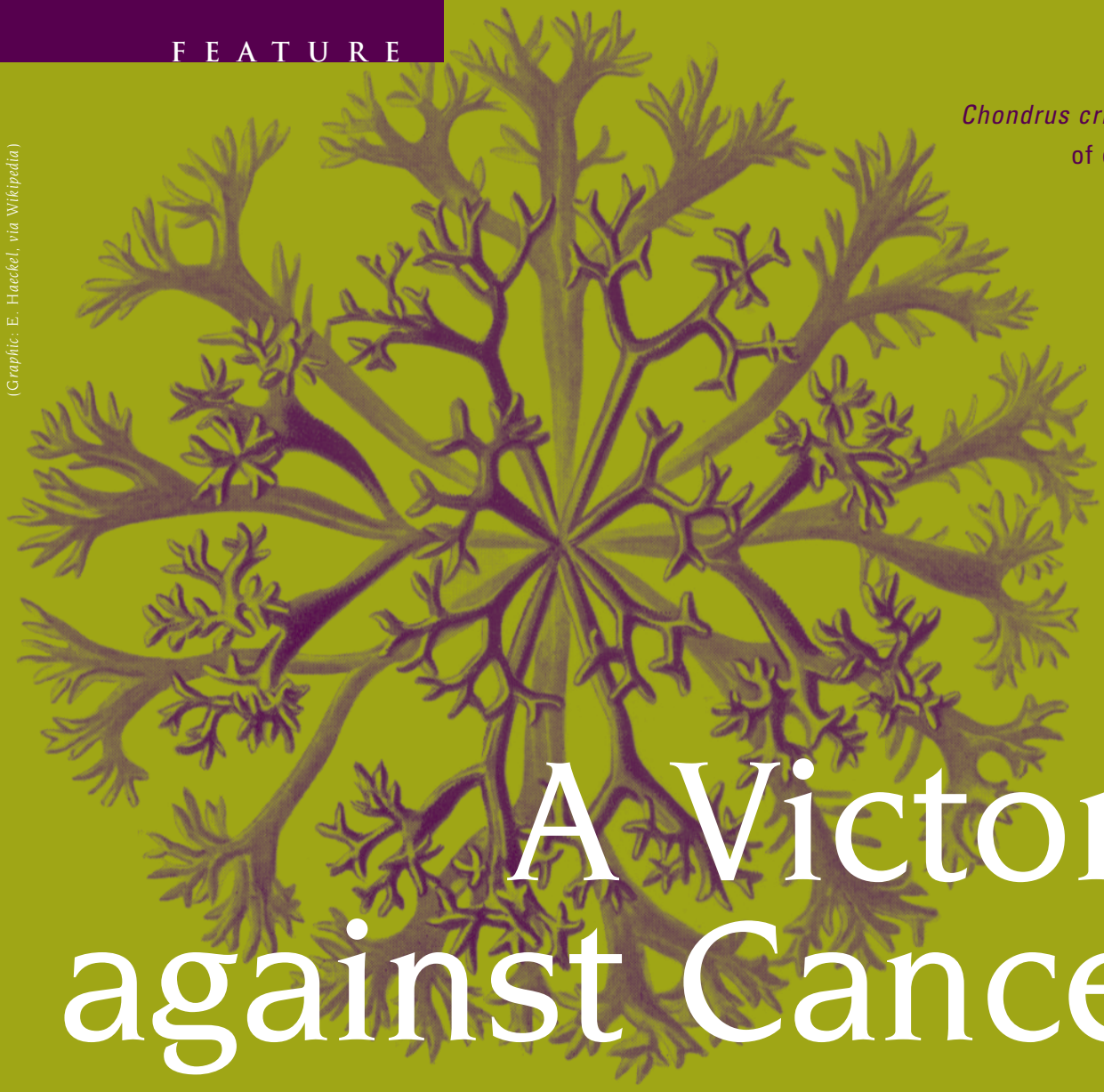

Chondrus crispus, source of carrageenan.

(*Graphic*: E. Haeckel, *via Wikipedia*)

A Victory against Cancer

25 YEARS IN THE MAKING

(*Graphic*: C. Buck, B. Trus, CCR)

In the early 1980s, CCR scientists heard the news that researchers had established the link between human papillomavirus (HPV) and cervical cancer, including those in NCI's Division of Cancer Epidemiology and Genetics (DCEG). This announcement set off a new quest: to craft a vaccine against a form of cancer that, at the time, claimed the lives of more than 5,000 American women each year.

Nearly 25 years later, success: in 2006, the U.S. Food and Drug Administration (FDA) approved the first HPV-blocking vaccine to protect against cervical cancer. Approvals in Canada and Europe soon followed.

Apart from its significance as a landmark event in women's health, the FDA's approval also marked a victory for CCR scientists Douglas Lowy, M.D., and John Schiller, Ph.D., who laid the biological foundation for the HPV vaccine. Rather than rest on their laurels, these two lead scientists from CCR's Laboratory of Cellular Oncology and their colleagues are already looking toward alternate ways of fighting or preventing cervical cancer, including the next generation of HPV vaccines and topical microbicides that might address some of the significant challenges of delivering a vaccine in the developing countries where it is most needed.

(*Photo: R. Baer*)

Two decades of work by Douglas Lowy, M.D. (left), John Schiller, Ph.D. (right), and their collaborators and students could help protect millions of women around the world from HPV's cancerous consequences.

A Vaccine with Impact

HPV vaccines promise to make a big difference in women's health. The American Cancer Society estimates that 11,150 American women will be diagnosed with invasive cervical cancer this year; nearly 3,700 will succumb to it.

A unique feature of HPV is the ability of one of its component proteins, L1, to assemble itself into empty shells called virus-like particles (VLPs). Because they contain no viral genomic material, these particles cannot cause infection on their own. What they can do, though, is mimic the presence of a viable virus and trick the immune system into mounting an anti-HPV immune response. The pair found that immunization of animals and even human volunteers with these L1-based particles could stimulate production of large numbers of antibodies; serum taken from vaccinees protected cultured cells from HPV infection in the laboratory.

NCI licensed the VLP technology to two pharmaceutical companies, Merck and GlaxoSmithKline (GSK), both of which subsequently developed HPV vaccines for clinical use. Both vaccines protect against HPV types 16 and 18, which cause up to 70 percent of all cervical cancer cases worldwide. Merck's vaccine, marketed under the name Gardasil®, also protects against HPV types 6 and 11, which cause 90 percent of genital warts. Both vaccines were remarkably successful in Phase III clinical trials, showing themselves to be 100 percent effective at preventing the premalignant cellular changes caused by the relevant virus types. Thus far, protection has remained solid after four years of follow-up. However, it appears that the vaccine cannot clear HPV infections that have already become established.

The FDA approved Gardasil in June 2006 for women and girls ages nine to 26 years. The vaccine, which is given in a series of three injections, was evaluated and approved in six months under the FDA's priority review process, which is used for products with potential to provide significant health benefits. GSK is expected to apply for approval of its vaccine, Cervarix™, in 2007.

Taking it Further

The considerable public health impact of this work has introduced NCI researchers to issues that most basic scientists do not face. How will the vaccine be delivered? Will the people who need it be able to get access to it? Those issues have spurred them on ever since.

> HPV vaccines promise to make a big difference in women's health.

"The current vaccine has implementation limitations that will make it difficult for poor women to get it—and they're the women who need it most because they have no Pap screening," Schiller said. "It's expensive to make and deliver this vaccine. We're trying to make better approaches that are very simple to deliver."

A new approach can be highly complex when developed in the laboratory, but has to be simple to produce and distribute. Lowy and Schiller are developing new therapeutic technologies capable of meeting those goals while still providing effective protection against HPV (see "A Topical Option").

While the current Merck and GSK vaccines are based on L1, the virus's L2 protein appears able to confer broad protection against more types of HPV. However, it is less effective than L1 VLPs at triggering virus-neutralizing antibody responses. Lowy and Schiller are working with Richard Roden, Ph.D., at the Johns Hopkins University—a former fellow in the Lowy lab—to improve the protein's ability to spur on these responses.

Fighting two diseases with one vaccine is an efficient way of keeping costs down. Schiller is working with Denise Nardelli-Haefligher, Ph.D., at the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland, to see if L1 can be incorporated into the typhoid vaccine, which is still used in developing countries. It may also be possible to formulate the dual-action vaccine as a nasal spray, easing the logistics of transport and administration. Such a preparation elicited strong immune responses in preclinical tests in mice.

"We can easily grow liters and liters of this. It doesn't require high-tech production methods," Schiller said. "Plus, a small oral dose for every villager would be much easier than giving three intramuscular injections." They are moving forward with companies in India to conduct clinical trials of both vaccines.

Helping This Generation

While preventing future HPV infections remains a central goal, Lowy does not want to abandon the millions of women who are already infected. The Centers for Disease Control and Prevention reported in February, 2007, that about one in four females ages 14 to 59—the equivalent of 25 million American girls and women—are infected with HPV.

"We need to recognize that the vaccine is not going to do anything for the millions of women who are already infected with HPV and who remain at increased risk for cervical cancer," Lowy warned. "The vaccine is for the next generations of women. But let's not lose sight of the current generation and the need to help them reduce their incidence of cancer."

He is hoping to see inexpensive DNA-based HPV tests made available to women globally, along with appropriate follow-up

"The vaccine is for the next generations of women. But let's not lose sight of the current generation and the need to help them reduce their incidence of cancer."

A Topical Option

In their continued search for alternate and easier ways to block human papillomavirus (HPV) infection, John Schiller, Ph.D., and his colleagues designed a high-throughput screening assay to test a wide range of compounds in hopes of finding ones that can prevent HPV infection. So far, a widely-used thickening agent extract from seaweed, called carrageenan—which can be applied as a topical gel or cream—has shown the most promise in laboratory tests.

"The screen let us test lots of drugs and other compounds very easily to see if they can act as a topical microbicide," Schiller said. "Carrageenan popped out as something that worked amazing well, and it's already [used] in some topical products."

The fact that carrageenan is inexpensive and "generally recognized as safe" by the U.S. Food and Drug Administration (FDA) for food and topical, including vaginal, applications increases its appeal to the scientists who are eager to find products that are safe and effective for humans. However, further studies are needed to determine whether products containing carrageenan can inhibit HPV infection and prevent sexual transmission of cancer-associated HPV in humans.

In preclinical animal studies, Schiller's group tested carrageenan-based over-the-counter sexual lubricants in a mouse cervicovaginal infection model developed in his laboratory. Even at a one million-fold dilution, these lubricants could prevent genital HPV infection in these mice. As for human studies, the Schiller lab has teamed with Terri Cornelison, M.D., Ph.D., and colleagues in the Division of Cancer Prevention to clinically test carrageenan's ability to prevent sexually transmitted HPV infections.

Although a topical microbicide would be a significant addition to the anti-HPV arsenal, it does have some drawbacks. For example, it would have to be used prior to every occasion of sexual contact, as opposed to a vaccine, which requires just a few injections. "I don't see it as a replacement for the vaccine," Schiller said, "but it may be a complement to the vaccine to give coverage against more types of HPV or where the vaccine is not available."

Carrageenan may have other uses as well. HPV can also be passed from mother to child during birth and, in rare cases, lead to serious diseases, including juvenile onset recurrent respiratory papillomatosis. The relative safety of carrageenan-containing infant formulas suggests that a cervical gel containing carrageenan may be a viable option for preventing HPV transmission during childbirth.

treatment. (To put the need in perspective, more than half a million women worldwide are currently stricken with invasive cervical cancer each year, with a quarter million deaths.) Thanks to work done by DCEG's Mark Schiffman, M.D., M.P.H., and Diane Solomon, M.D., HPV-DNA tests are known to be cost effective when used in women with inconclusive Pap test results. DNA-based HPV testing is currently approved for screening of women over age 30, with some insurers now providing coverage. Digene Corporation, which has partnered with the Bill & Melinda Gates Foundation, is field-testing a new HPV-DNA test for use in developing countries. If proven effective, it will be available at a cost much lower than current DNA-based tests, according to Lowy.

Beyond Cancer

To capitalize on the fact that VLPs are "really good at inducing antibody responses," Schiller is exploring their ability to fool the immune system into making antibodies that target "self" proteins—which the body normally tolerates—that play roles in chronic diseases, such as Alzheimer's disease or HIV infection. For example, in the context of Alzheimer's, Schiller can imagine sopping up the beta-amyloid protein that makes up Alzheimer's-causing plaques with antibodies before it can coalesce in the brain.

In HIV, he is aiming at the CCR5 receptor. People lacking this receptor do not develop AIDS after infection with HIV, indicating that this receptor is necessary for the virus to take hold. If the body could be tricked into making autoantibodies that compete for the receptor, perhaps HIV infection could be prevented or controlled.

VLPs may even work for contraceptive vaccines. With researchers in India, Schiller is testing a vaccine that targets a protein required for a fertilized egg to implant in the uterine wall, preventing pregnancy without affecting women's hormone levels or menstruation.

Right Place at the Right Time

Schiller, who began as a Postdoctoral Fellow in Lowy's lab, credits CCR's research environment for a lot of the progress they have made. "I stayed because CCR is a very good place to do research," explained Schiller. "We were studying the basic biology of a virus, but we had no experience in vaccines or immunology. However, no one said we couldn't try to develop this vaccine." He is convinced that if he and Lowy had been in the extramural program at the beginning of their search, they would have had to write a grant, which likely would have been rejected because they did not have the relevant track record in immunology or virology. Within CCR, however, they had the freedom to operate with confidence that they could justify their work in the long-term.

Recognition

The 2006 approval of the first human papillomavirus (HPV) vaccine ushered in a host of awards for the NCI scientists who made possible development of the HPV vaccine.

For their work on HPV, Douglas Lowy, M.D., and John Schiller, Ph.D., of the Laboratory of Cellular Oncology received a 2006 DHHS *Secretary's Award for Distinguished Service*. The award was shared with other NCI investigators who made significant contributions to HPV research including: Allan Hildesheim, Ph.D., and Mark Schiffman, M.D., M.P.H., in the Division of Cancer Epidemiology and Genetics, and Diane Solomon, M.D., in the Division of Cancer Prevention.

Lowy and Schiller's efforts also earned them the *3rd Annual David Workman Award* from the Samuel Waxman Cancer Research Foundation, the 2007 *Dorothy P. Landon-AACR Prize for Translational Cancer Research*, the 2007 *Award for Excellence in Technology Transfer* from the Federal Laboratory Consortium for Technology Transfer, and the 2007 *American Medical Association Nathan Davis Award for Outstanding Government Service*.

"We are simply symbols of the many people who have made critical contributions to understanding the relationship between papillomavirus infection and cervical cancer," Lowy said. Both Lowy and Schiller are quick to point out that the recognition has been nice, but that they remain humbled by the insightful research done by so many of their colleagues.

Leveraging CCR Progress through His Students

John Schiller, Ph.D., encourages his trainees to follow their own paths. "We try to set the postdocs up with broad, open-ended projects, where we can build an enabling technology in an area that's not fully explored," he said. He keeps his lab small enough to enable his postdocs to direct their projects and take them with them when they continue their careers at other institutions. He generally has three postdocs, two technicians, and a staff scientist. A great deal of responsibility falls to the postdocs.

He can list several success stories. One fellow, Bryce Chackerian, Ph.D., took work on virus-like particles (VLPs) and autoantibodies with him to the University of New Mexico. Richard Roden, Ph.D., who worked on the L2 vaccine in Schiller's lab, took that technology with him to the Johns Hopkins University.

"The nice thing is we're not competing for grants with my former postdocs," Schiller explained. "If we were in the extramural community, we'd be applying for the same research funds." But being in the intramural program, Schiller can let each fellow get a good head start and expand on a technology that they launched in Schiller/Lowy lab.