A Prize for Cancer Prevention

Harold Varmus[1,*]
[1]Meyer Cancer Center, Weill Cornell Medicine, New York, NY 10065, USA
*Correspondence: varmus@med.cornell.edu
http://dx.doi.org/10.1016/j.cell.2017.08.020

This year's Lasker-DeBakey Prize for Clinical Research to Douglas Lowy and John Schiller celebrates the science behind one of the greatest advances in the history of cancer research: the development of vaccines that prevent infection and thus prevent tumor induction by pathogenic strains of human papilloma virus (HPV).

Before describing the science that produced HPV vaccines, it is useful to contemplate the sometimes-paradoxical relationship between cancer and prevention.

Prevention: The Backseat Driver of Improved Cancer Statistics

Cancer-related journalism is dominated today by advances in cancer treatment, especially immunotherapies and drug therapies based on mutant cancer genes. Still, prevention strategies are responsible for a major portion of the recent steady decline, about 1.5% per year, in the overall death rate for cancers in the US (Jemal et al., 2017). Reduced use of tobacco accounts for much of that decline; the incidence rates for lung cancers, the major cause of cancer mortality here and globally, have been falling for males and now females for several years, with a delayed relationship to smoking practices. Avoidance of occupational and environmental exposures to asbestos, UV and X-irradiation, and other recognized carcinogens have also contributed to the declines. And detection of certain kinds of pre-cancerous lesions and early cancers—especially cervical (by Pap smears), colorectal (by fecal blood tests and endoscopies), melanoma (by skin exams), and breast (by mammography)—have prevented deaths from those cancers, too.

Cancer prevention is nevertheless underappreciated; we will always hear more pleas for cures than for prevention. Preventing cancer does not produce survivors who know the bullet they've dodged, so we lack grateful patients whose possible tumors have been prevented. The protected individuals and their families are not making donations to cancer research or marching for better prevention. Furthermore, for many kinds of cancers, no clear strategy for prevention can yet be envisioned, in part because we have learned that cancers commonly arise from our inherently mutation-prone machinery for DNA replication, DNA repair, and cell division.

In contrast, prevention has always dominated thinking about the control of infectious diseases, especially acute viral infections for which vaccines are the first port of call. But even in those not-so-rare situations in which infectious agents— viruses, bacteria, parasites—contribute to carcinogenesis, establishing a causal connection between cancers and microbes, especially those as prevalent as HPV, Epstein-Barr virus, *Helicobacter,* or schistosomes, can be difficult; only a subset of infected individuals may develop a cancer, and the latency is likely to be many years in duration.

But when the connection to an infectious cause is made, the result can be powerful, especially if there is a route to an effective vaccine. This was first demonstrated by the now-universally used vaccine against hepatitis B virus (HBV)—cheap, effective, and non-toxic— that has already reduced the incidence of HBV-associated hepatoma, formerly one of the most common lethal cancers worldwide. And now, we have HPV vaccines with the potential to reduce the still-high incidence of cervical and several other potentially lethal types of cancer (see Figure 1A) associated with infection by certain strains of HPV.

The Strange History and Properties of Papilloma Viruses

The remarkable potency and effectiveness of the HPV vaccines being celebrated by this year's clinical award seem all the more extraordinary in view of some of the unusual features of papillomaviruses: the many genetically and antigenically distinct strains of HPVs, now numbering more than 100, with varying carcinogenic potency; transmission through mucosal surfaces, a route of infection potentially refractory to immune protection; and the failure to propagate HPVs in conventional cell cultures, usually a requirement for making viral vaccines.

This virus class was first studied long ago by three giants in tumor virology: Peyton Rous (famed for his discovery of the iconic Rous sarcoma virus), Richard Shope, and J.W. Beard. A filterable factor in extracts from benign papillomas (warts) found in cottontail rabbits induced papillomas upon injection into naive rabbits; the warts sometimes turned into squamous carcinomas, especially when exposed to chemical carcinogens (Rogers and Rous 1951). Particles found in human, bovine, rabbit, and other papillomas appeared to be essentially indistinguishable symmetrical particles, about 50–60 nm in diameter and composed of 72 pentamers of the major capsid protein (Baker et al., 1991). These papilloma viruses, including the most commonly studied bovine and human versions (BPV and HPV), contain circular, double-stranded DNA genomes of about 8,000 base pairs. We now know that the papillomavirus genomes are organized in a stereotypic manner, encoding a few "early" proteins required for DNA replication and at least two "late" proteins, L1 and L2, that assemble to form the coat of the virus particles.

Epidemiological observations had long hinted that carcinoma of the uterine cervix might be a sexually transmitted disease. For those of us old enough to remember
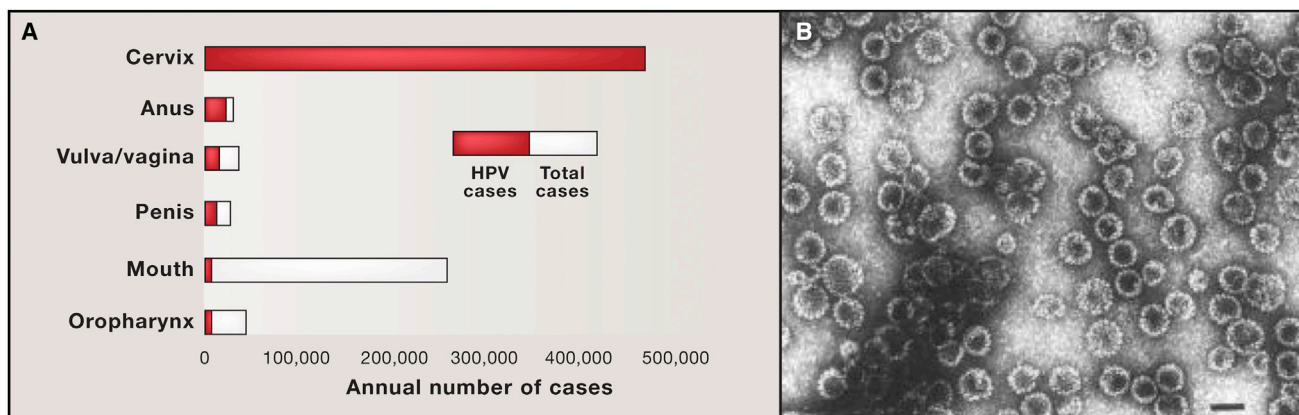
Figure 1. Preventing Cancer Associated with Human Papilloma Virus Infection
(A) The global incidences of human cancers associated with human papilloma virus (HPV) (horizontal bars); the red coloring denotes the number of cases directly attributable to infection with oncogenic strains of HPV. Adapted from Parkin, 2006.
(B) Electron microscopic view of virus-like particles composed of L1 protein from HPV-16. Micrograph from Kirnbauer et al., 1993.

the uncertainties about its possible infectious cause (for many years, a herpes virus was viewed as the leading contender), Harold zur Hausen's demonstration that many cervical cancers contain DNA belonging to one of the many types of HPV was a bombshell (Dürst et al., 1983). The likely conclusion—that more than half of the tumors were caused by a single strain of HPV, type 16—was reached despite features of HPV that have continued to complicate the study of these viruses.

HPVs have never been efficiently propagated in culture, so there has been no ready means to classify them with traditional serological methods. But because their relatively small DNA genomes differ substantially, they could be grouped, well before DNA sequencing became routine, by DNA hybridization. Thus, zur Hausen's finding, that one type of HPV was commonly found in cervical carcinomas, was a powerful indicator of a requirement for infection by select strains to produce cervical cancer. As discussed later, variations in the regional prevalence and carcinogenic potency of the many types of HPV continue to influence epidemiological and prevention strategies.

Despite zur Hausen's compelling evidence for a causative role of HPV in cervical cancer, it was not apparent how to put that information to use for patient benefit. In an era in which large armies of investigators were trying to understand oncogenes found in RNA and DNA tumor viruses—especially the retroviruses, poly-omaviruses, and adenoviruses—it was natural for papilloma virologists to seek and study the oncogenic loci in HPV and other papillomavirus genomes. Using cell-based assays for oncogenesis after DNA transfer, viral genes—mainly the "early" genes E6 and E7—were implicated in transformation. Strikingly, these two genes were found to do what other DNA tumor virus oncogenes do: interfere with the actions of the now-well-known mammalian tumor suppressor genes, *P53* (Scheffner et al., 1990) and *Retino-blastoma-1* (Dyson et al., 1989). These findings were fascinating, but they did not provide obvious avenues to prevent or treat cervical cancer.

Envisioning a Vaccine Based on Virus-like Particles
John Schiller and Doug Lowy, working on papilloma viruses (mainly BPV, but also HPV) in the National Cancer Institute's intramural program, had also devoted most of their efforts to papilloma virus oncogenes (Schiller and Lowy, 2011). But because BPV could be studied in cell culture (by transforming rodent cell lines with infectious virus, not just by DNA transfection), Lowy and Schiller were positioned to perform quantitative assays, measure neutralizing antisera, and think about the structural attributes of papillomavirus proteins or particles that induce a protective immune response (Schiller and Lowy 2011).

In turning their attention to prevention of virus infection, they recognized that a traditional viral vaccine—live, attenuated, or killed—would be impractical or unethical (Schiller and Lowy 2011); HPV could not be grown in culture to make a conventional vaccine, and a virus particle containing oncogenes was not, in any case, likely be medically acceptable. So, they considered the option of making virus-like particles (VLPs) composed solely of papillomavirus protein, lacking viral nucleic acid. There were important precedents: Robert Garcea's group had made such particles by self-assembly of coat proteins from the distantly related polyomaviruses (Salunke et al., 1986), and an effective, widely used vaccine against HBV was composed of VLPs containing viral surface antigen produced in yeast (Valenzuela et al., 1982). There were also reasons to believe that properly assembled capsid proteins would be more antigenic and more likely to induce neutralizing antibodies than would individual viral proteins in solution.

These initial ideas about a papilloma-virus vaccine were confirmed, using BPV as an effective model, in a remarkable paper published by the NCI group in 1992 (Kirnbauer et al., 1992). Expression of only the major BPV virion protein, L1, made in insect cells infected by a baculo-virus vector, produced abundant, uniform, correctly sized particles; after injection into rabbits, those VLPs induced high titers of neutralizing antibodies that protected cultured cells from infectious BPV.

Of course, as beautiful as this was, the results did not ensure that oncogenic

strains of HPV, especially type 16, would behave in the same way, nor did they predict whether the immune response would protect against infection of mucosal surfaces or whether this approach would be amenable to scaled-up production or commercial viability.

Indeed, the first issue proved initially problematic: efforts to reproduce the BPV-based findings with the commonly used HPV-16 L1 DNA clone from a cervical cancer produced few and inappropriately sized virus-like particles, similar to earlier reports from an Australian team that had also included the L2 protein (Zhou et al., 1991). Lowy and Schiller were skeptical of this discrepancy, in part because a member of their laboratory group was able to make abundant VLPs by expressing an L1 gene from a rhesus monkey papilloma virus closely related to HPV-16 (Schiller and Lowy, 2011). When the original HPV-16 L1 clone was sequenced and compared with the L1 gene from other HPV-16 genomes obtained from non-malignant lesions, a single difference (Asp202His) was noted in the predicted L1 protein sequence. Use of the putative wild-type clones of HPV-16 L1 reassuringly restored production of VLPs to levels comparable to those observed with BPV (Kirnbauer et al., 1993).

Reduction to Practice: Making, Testing, and Improving HPV Vaccines

Despite these promising findings and the magnitude of the problem that cervical cancer poses to human health, it was not initially easy to find commercial partners to take on the scientifically difficult and commercially risky task of making a viable vaccine against a sexually transmitted pathogen. Schiller and Lowy (2011) have recounted a fateful meeting with the vaccine pioneer at Merck, Maurice Hilleman, who immediately embraced the concept and convinced the company to proceed with HPV vaccine development. Shortly thereafter, other companies, notably MedImmune and GlaxoSmithKline (GSK), were also able to take up the challenge, thanks to non-exclusive licensing practices at the NIH.

Many choices are required for the development of any vaccine. An HPV vaccine that was dependent on VLPs composed only of L1 protein, however

simple in concept, was no exception. In this case, the choices included the following: the expression system for production of L1 and VLPs (Merck shifted to bakers yeast, *S. cerevisiae* [Mach et al., 2006]); the HPV strains to be included in the vaccine (another highly oncogenic strain, type 18, was used by both Merck and GSK, and Merck included two strains commonly found in genital warts, types 6 and 11); the recipe for presentation of the VLPs (Merck scientists reassembled VLPs in vitro from L1 pentamers [Mach et al., 2006]); the inoculation schedule (initially three doses, months apart); and the design of efficient clinical trials.

Safety trials quickly revealed that HPV VLPs were well tolerated and induced high titers of neutralizing antibodies in human subjects (Harro et al., 2001), but the choice of appropriate endpoints for large-scale, controlled efficacy trials was particularly vexing, involving many sectors: scientific, regulatory, and commercial. Since the major purpose of the vaccines was protection against cancer, looking simply for reduction in the frequency of infection by relevant types of HPV might not have been a reliable indicator of success (only a subset of infected women develop cervical cancer, and many infections regress naturally). But waiting to observe a reduction in cancer incidence would not only be slow, it would be ethically unacceptable; standard care dictates ablation of any pre-malignant lesions detected by regular Pap smears. As a compromise, it was generally agreed to follow two metrics: presence of viral DNA by HPV strain-specific polymerase chain reaction (PCR) assays and, more importantly, the appearance of intermediate or high-grade cervical intraepithelial neoplasias (CIN2 and CIN3).

The trials conducted with the two major HPV vaccines (Merck's quadrivalent and GSK's bivalent vaccine) in various patient populations have been uniformly, indeed dramatically, successful (reviewed by Schiller and Lowy, 2011, and Schiller et al., 2012) and have led to widespread licensure and use.

But the story does not end here: HPV vaccination has yet to fulfill its potential. Some of the remaining problems are inherent in the scientific plan; others have social origins and became quickly apparent.

Incomplete Protection

Even successfully vaccinated women remain at risk of disease caused by strains less commonly implicated in the causation of cervical cancer; HPV16 and HPV18 are together responsible for only about 70% of cervical cancers in the U.S. and for lower percentages in some other places. Therefore, it remains necessary to advise vaccinees, both here and abroad, to continue surveillance for early lesions. A nonavalent Merck vaccine that includes VLPs from seven oncogenic strains offers 90% protection and was recently approved (Petrosky et al., 2015), but this does not eliminate the dilemma. Designing a truly universal HPV vaccine (or at least one that protects against infection by all known disease-producing strains) continues to be a valuable quest.

Neglecting Males

Since HPV vaccines were developed principally to prevent cervical cancer, by far the most prevalent of the HPV-associated cancers (see Figure 1), public health campaigns and financial supporters have emphasized the vaccination of girls. But immunization of boys would restrict the frequency of virus transmission and enhance herd immunity. Furthermore, it would protect them from lethal cancers, including the HPV16-initiated oropharyngeal cancers that are rising in frequency in some places, including in the U.S. (Pytynia et al., 2014).

Inadequate Use

Rates of vaccination of the primary target population, adolescent females, have been disappointing, even in many advanced economies, including the U.S., and the vaccines are generally unused or rarely used in many developing countries. The failure to make universal use of a method that could prevent lethal cancers in hundreds of thousands of people worldwide each year may seem surprising, but many factors are at work: the cost (initially about $360 for a full three-injection course in the U.S.); the recommended multi-dose vaccination protocol, initially requiring three health care visits, independent of other vaccinations; inadequate education of parents and health care workers about the benefits of the vaccine; social resistance to a vaccine predicated on the assumption that 9- to 11-year-old

recipients will soon have sexual relationships; and more general opposition to immunization, based on fears of undocumented toxicities.

Efforts have been made to surmount these barriers with financial support for purchase of vaccines (e.g., through the Global Alliance for Vaccines and Immunizations [http://www.gavi.org/support/nvs/human-papillomavirus/]), shortening of the immunization schedule from three doses to two (https://www.cdc.gov/media/releases/2016/p1020-hpv-shots.html), and reports from authoritative groups, like the President's Cancer Panel (https://deainfo.nci.nih.gov/advisory/pcp/annualreports/hpv/index.htm), but opportunities to save lives are lost every day.

A Final Word about Immunization and Cancer Prevention

This Lasker Prize is more than just a reward to two individuals for good science and for the design of a vaccine that prevents cancers and saves lives. It is also a prestigious endorsement of a global community of scientists and health care professionals attempting to harness the immune system to prevent infection and disease. This validation is sorely needed at a time when a grass-roots anti-vaccine movement and a powerful handful of national leaders are undermining confidence in immunization, placing the public at risk of contracting dangerous but fundamentally preventable infections.

It is impossible to identify the individuals who are the beneficiaries of any prevention strategy, including vaccination. But it is easy to recognize those who suffer the consequences of failing to use the vaccines that human ingenuity has produced. If this year's prize prompts greater attention to the value of vaccines, the Lasker Foundation will have done much more than reward two ingenious scientists.

REFERENCES

Baker, T.S., Newcomb, W.W., Olson, N.H., Cowsert, L.M., Olson, C., and Brown, J.C. (1991). Biophys. J. 60, 1445–1456.

Dürst, M., Gissmann, L., Ikenberg, H., and zur Hausen, H. (1983). Proc. Natl. Acad. Sci. USA 80, 3812–3815.

Dyson, N., Howley, P.M., Münger, K., and Harlow, E. (1989). Science 243, 934–937.

Harro, C.D., Pang, Y.Y., Roden, R.B., Hildesheim, A., Wang, Z., Reynolds, M.J., Mast, T.C., Robinson, R., Murphy, B.R., Karron, R.A., et al. (2001). J. Natl. Cancer Inst. 93, 284–292.

Jemal, A., Ward, E.M., Johnson, C.J., Cronin, K.A., Ma, J., Ryerson, A.B., Mariotto, A., Lake, A.J., Wilson, R., et al. (2017). J. Nat. Cancer Inst. 109 http://dx.doi.org/10.1093/jnci/djx030.

Kirnbauer, R., Booy, F., Cheng, N., Lowy, D.R., and Schiller, J.T. (1992). Proc. Natl. Acad. Sci. USA 89, 12180–12184.

Kirnbauer, R., Taub, J., Greenstone, H., Roden, R., Dürst, M., Gissmann, L., Lowy, D.R., and Schiller, J.T. (1993). J. Virol. 67, 6929–6936.

Mach, H., Volkin, D.B., Troutman, R.D., Wang, B., Luo, Z., Jansen, K.U., and Shi, L. (2006). J. Pharm. Sci. 95, 2195–2206.

Parkin, D.M. (2006). Int. J. Cancer 118, 3030–3044.

Petrosky, E., Bocchini, J.A., Jr., Hariri, S., Chesson, H., Curtis, C.R., Saraiya, M., Unger, E.R., and Markowitz, L.E.; Centers for Disease Control and Prevention (CDC) (2015). MMWR Morb. Mortal. Wkly. Rep. 64, 300–304.

Pytynia, K.B., Dahlstrom, K.R., and Sturgis, E.M. (2014). Oral Oncol. 50, 380–386.

Rogers, S., and Rous, P. (1951). J. Exp. Med. 93, 459–488.

Salunke, D.M., Caspar, D.L., and Garcea, R.L. (1986). Cell 46, 895–904.

Scheffner, M., Werness, B.A., Huibregtse, J.M., Levine, A.J., and Howley, P.M. (1990). Cell 63, 1129–1136.

Schiller, J.T., and Lowy, D.R. (2011). History of Vaccine Development, S.A. Plotkin, ed. (Springer), pp. 265–284.

Schiller, J.T., Castellsagué, X., and Garland, S.M. (2012). Vaccine 30 (Suppl 5), F123–F138.

Valenzuela, P., Medina, A., Rutter, W.J., Ammerer, G., and Hall, B.D. (1982). Nature 298, 347–350.

Zhou, J., Sun, X.Y., Stenzel, D.J., and Frazer, I.H. (1991). Virology 185, 251–257.