

Opportunities and challenges for human papillomavirus vaccination in cancer

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Abstract | The discovery of genotype 16 as the prototype oncogenic human papillomavirus (HPV) initiated a quarter century of laboratory and epidemiological studies that demonstrated their necessary, but not sufficient, aetiological role in cervical and several other anogenital and oropharyngeal cancers. Early virus-induced immune deviation can lead to persistent subclinical infection that brings the risk of progression to cancer. Effective secondary prevention of cervical cancer through cytological and/or HPV screening depends on regular and widespread use in the general population, but coverage is inadequate in low-resource settings. The discovery that the major capsid antigen L1 could self-assemble into empty virus-like particles (VLPs) that are both highly immunogenic and protective led to the licensure of several prophylactic VLP-based HPV vaccines for the prevention of cervical cancer. The implementation of vaccination programmes in adolescent females is underway in many countries, but their impact critically depends on the population coverage and is improved by herd immunity. This Review considers how our expanding knowledge of the virology and immunology of HPV infection can be exploited to improve vaccine technologies and delivery of such preventive strategies to maximize reductions in HPV-associated disease, including incorporation of an HPV vaccine covering oncogenic types within a standard multitarget paediatric vaccine.

High-risk human papillomavirus (hrHPV) is an established carcinogen in the cervix, penis, vulva, vagina, anus and oropharynx¹. Approximately 5% of all cancers can be attributed to HPV, but the fraction linked to HPV varies markedly by geographical region and level of economic development. Worldwide, almost all 530,000 cases per annum of cervical cancer are HPV-driven. HPV-associated cancer of five other sites accounts for a further 113,400 cases^{2,3}. Persistence of hrHPV infection is the main factor driving dysplasia and increased risk of cancer development⁴. In the absence of intervention, the risk of cervical cancer rises with increasing dysplasia to around 50% in women with high-grade cervical intra-epithelial neoplasia (CIN3)⁵. The worldwide prevalence of HPV infection in women without cervical lesions is 11.7%, with higher rates in Latin America, Eastern Europe and sub-Saharan Africa². Successful prophylactic vaccination against hrHPV types can prevent dysplasia and thus cervical cancer⁴. Prevention of hrHPV infection is also important for men as it also causes anogenital and oropharyngeal cancer in both sexes, as well as penile cancer^{6,7}. Immunosuppressed individuals are also at higher risk of HPV-associated cancers^{8,9}.

Extensive virological and epidemiological studies provide an understanding of the natural history of HPV and carcinogenesis and reveal opportunities to harness the host immune response to prevent and/or treat HPV disease. This Review focuses on the development and impact of current HPV prophylactic vaccines and the prospects for continued improvements for maximal cancer control, although recent clinical data provide early signs that therapeutic approaches can increase clearance rates of existing infection and disease, suggesting additional opportunities to more quickly reduce HPV-associated cancer rates^{10,11}.

Oncogenic HPV genotypes

Papilloma viruses have an ~8 kb, histone-bound, double-stranded DNA genome encoding six early genes (*E1*, *E2*, *E4*, *E5*, *E6* and *E7*) and two late genes (*L1* and *L2*) that produce its non-enveloped T = 7d capsid¹². HPVs are characterized by genotype, defined as >10% *L1* DNA sequence divergence from other known genotypes (generally termed 'types'), and numbered in order of discovery¹³. More than 200 HPV types have been identified thus far¹⁴. The HPV types within the β -species and γ -species

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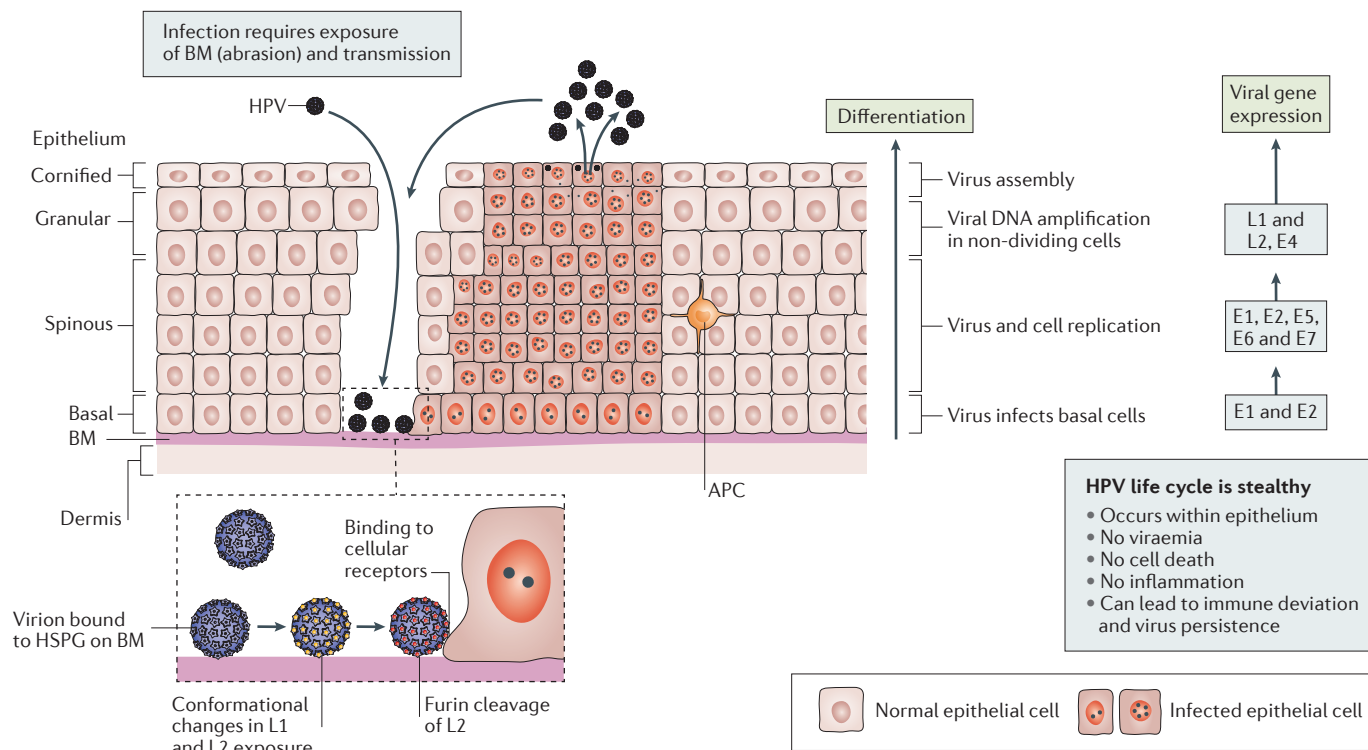


Figure 1 | The life cycle of HPV. Abrasion, which leads to denudation of the basement membrane (BM) from epithelial cells, provides access to the basal keratinocytes. During the course of human papillomavirus (HPV) infection, the virus binds to heparin sulfate proteoglycans (HSPGs) and/or laminin 5 on the BM through the major capsid protein L1 (REFS 95, 149–151). This triggers conformational changes in the capsid that further expose the minor capsid protein L2, including a conserved site on the L2 amino terminus that is susceptible to cleavage by extracellular furin^{152,153}. Furin cleavage of L2 reveals several conserved protective epitopes of L2, including residues 17–36, on the capsid surface¹⁵⁴ and is critical to infection. This is followed by virus uptake into the target basal keratinocyte¹⁵⁵. Several uptake pathways have been implicated, none of which are necessarily mutually exclusive¹⁵⁶. In the infected basal cells (which might include stem cells), the viral genome replicates and establishes ~50 HPV episome copies, which then segregate between the daughter progeny as the cells undergo cell division. The early viral proteins E6 and E7 are key to stimulating the continued proliferation and milieu for E1 and E2-driven vegetative viral genome replication to a very high copy number. Terminal differentiation of infected cells in the upper epithelial layers activates the expression of E4 and then L1 and L2 to package the very high copy numbers of the viral genome. The virions are released as E4 disintegrates the cytoke­ratin filaments, and the keratinocyte remnants are sloughed off the epithelial surface. Thus, the viral life cycle is completed without directly causing cell death and without systemic viraemia or apparent inflammation to avoid alerting the local immune responses²⁰. APC, antigen-presenting cell.

are typically benign and infect skin^{15,16}. Infection may be clinically unapparent or produce cutaneous warts that generally resolve within a year but can be recalcitrant, especially in immunosuppressed patients⁹. The α -species includes ~40 HPV types that are trophic for genital mucosa and spread by sexual intercourse. Many α HPV types produce benign disease, for example, genital warts caused by HPV-6 and HPV-11. However, there are 13 hrHPV types, HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59 and HPV-68, defined as carcinogenic or probably carcinogenic, plus a group of possibly carcinogenic types (HPV-26, HPV-30, HPV-34, HPV-53, HPV-66, HPV-67, HPV-69, HPV-70, HPV-73, HPV-82 and HPV-85)^{1,17}. Ninety-nine per cent of cervical cancers contain HPV¹⁸, with HPV-16 accounting for 50–60% and HPV-18 accounting for ~20%, and the remaining fraction is caused by the other oncogenic types in the

α 7, α 9 and, to a lesser extent, the α 5, α 6 and α 11 species¹⁷. Therefore, all licensed HPV vaccines directly target HPV-16 and HPV-18, but the most recently approved vaccine also targets the next five most common types in cervical cancer (HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58) and the two low-risk types most prevalent in genital warts (HPV-6 and HPV-11).

HPV infection in the cervix

HPV infection typically occurs at a site of epithelial abrasion, and the squamocolumnar junction of the cervix is particularly susceptible to transformation by hrHPV¹⁹. Infection is localized to a few cells surrounding the wound and is not lytic. Rather, infection is stealthy and durable. The life cycle of the virus is completely dependent on the differentiation of the epithelium and does not cause cell death, and there is no systemic viraemia²⁰ (FIG. 1).

Early extracellular events. HPV infects the epithelium (FIG. 1, magnified area) by binding of L1 to heparan sulfate proteoglycans (HSPGs) on the basement membrane that are exposed by an abrasion. Binding induces a conformational change in the capsid, revealing the amino terminus of L2, which is then available for cleavage by extracellular furin. L2 cleavage by furin is a prerequisite to virus uptake and internalization over several hours by the target cell²¹.

Virus uptake and delivery of its genome to the nucleus. Uptake of the bound virus occurs via endosomal vesicles and is L1-mediated. Once the virus has entered the cell, L2 is essential for infection as the endosomal virus engages the retromer and travels towards the nucleus. While L1 is being degraded, L2 mediates its escape in complex with the viral genome from the endosome into the *trans*-Golgi network^{22,23}. The entry of the L2-genome complex into the nucleus is dependent on cell cycle progression and occurs upon transient nuclear membrane breakdown during mitosis²⁴. Once inside the nucleus, the L2-genome complex associates with promyelocytic leukaemia nuclear bodies (termed ND-10), and early viral transcription is initiated^{25,26}.

Viral transcription programmes and completing the viral life cycle. Early viral proteins E1 and E2 co-opt the host DNA replication machinery to establish 50–100 episomal copies per cell²⁵. Use of the host replication machinery ensures a low error rate and thus the rate of viral evolution is very slow, meaning that immune escape through mutation is not an important issue for HPV vaccination because variants are of the same serotype²⁷. The productive phase of the unique life cycle of HPV exhibits exquisite spatial and temporal regulation following keratinocyte differentiation in the squamous epithelium (FIG. 1). Triggered by epithelial differentiation of daughter basal cells leaving the basement membrane, the switch to high copy number (>10³ per cell) HPV genome replication and the expression of the late viral (capsid) genes for virion assembly occur only in the upper, terminally differentiated layers of the epithelium, and virus-laden squames are shed. Although vaccination with the capsid antigens can trigger cellular immune responses, they are not therapeutic because the basal epithelial cells harbouring HPV express only the early genes^{10,11}. Consequently, most therapeutic vaccines target E6 and/or E7, as the other early viral proteins are not typically expressed in cancer or not obligatory for tumour cell viability^{10,11}.

To access host replication factors, the virus drives the host keratinocyte into S phase¹². This occurs through expression of the three viral oncoproteins E5, E6 and E7. E5 increases growth factor signalling, in particular epidermal growth factor receptor (EGFR), to promote exit from G0–G1. E6 binds and triggers proteasomal degradation of p53 and other pro-apoptotic factors to promote cell survival and PDZ proteins to promote de-differentiation of cells. E6 also activates MYC expression and telomerase to promote cell survival. E7 binds to multiple targets, notably RB, to overcome

the restriction point (FIG. 2). The viral oncoproteins encoded by β HPV types share some targets but act somewhat differently¹⁶.

Ectopic expression of E6 and E7 of hrHPV, but not low-risk HPV (lrHPV), is sufficient to immortalize primary keratinocytes and induce genomic instability¹². Carcinogenesis is not beneficial to the virus but is an unfortunate consequence of viral oncogene expression and the resultant genomic instability and dysregulated proliferation¹² (FIG. 2). Oncogenicity varies among hrHPV, but HPV-16 and HPV-18 are associated with the most rapid carcinogenic progression⁴. Additional genetic hits are necessary for carcinogenesis; smoking, oral contraceptives and multiparity are cofactors for cervical cancer²⁸ and ultraviolet light is a cofactor for non-melanoma skin cancers¹⁶.

Self-limited versus persistent infection

Fortunately, the majority of hrHPV infections do not progress to cancer but instead resolve spontaneously. Thus, the tension between persistence and clearance is central to the carcinogenic potential of hrHPVs. It remains controversial whether clearance is control to below the level of detection or complete elimination of the virus^{29–32}. There has been much interest in whether this dichotomy reflects infection of a stem cell (which would remain in the basal layer, producing HPV⁺ daughter cells) or infection of a more differentiated keratinocyte (for example, an HPV⁺ transit-amplifying cell, which rises through the epithelium and is lost). Likewise, the site of infection may also be important, with recent evidence suggesting that the CK7⁺ and p63⁻ cells of the squamocolumnar junction are residual embryonic cells that provide a niche for cervical carcinogenesis³³.

There is an increased burden of HPV-associated cancers in patients with a compromised immune system, whether iatrogenically in organ transplant recipients⁹, via co-infection with HIV⁸ or via certain genetic syndromes (for example, epidermodysplasia verruciformis or warts, hypogammaglobulinaemia, immunodeficiency and myelokathexis (WHIM) syndrome)³⁴. These observations, as well as studies in animal models¹², support a central role for cellular immunity in the natural control of HPV infection.

Neutralizing antibodies (nAbs) can be protective, but these are triggered only after infection and cannot eliminate virus-infected cells. However, the relevance of capsid-binding antibodies is indicated because numerous genotypes exist with great variation in the immunodominant L1 surface loops that form neutralizing epitopes that are type-restricted³⁵. However, the roles of macrophages and natural killer (NK) cells in the control of infection, as well as defence mechanisms within the infected keratinocyte, are less well understood. Langerhans cells are the primary antigen-presenting cell (APC) in the epithelium and are likely critical to the recognition of infection and the induction of HPV-specific cellular immunity³⁶.

Most times, a combination of innate and adaptive immunity eliminates infection³⁶ (FIG. 3). Effector T cells targeting early viral proteins (notably E2, E6 and E7)

Retromer

A complex of proteins that is important for recycling of transmembrane receptors from endosomes to the *trans*-Golgi network.

Type-restricted

Reactive with the same (homologous) type but not most other types, although weakly cross-reactive with some very closely related types. This contrasts with 'type-specific', which denotes reactivity only to the homologous type.

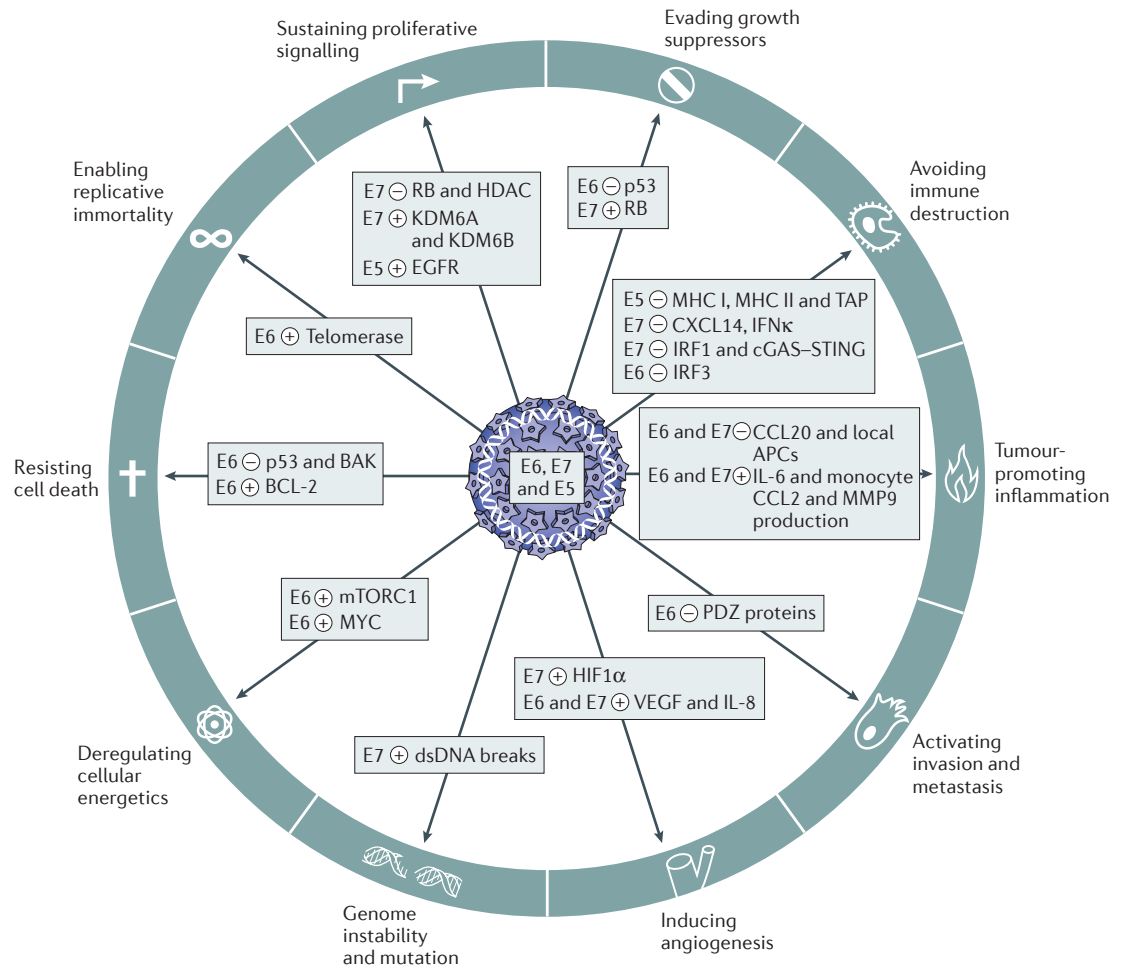


Figure 2 | Hallmarks of cancer affected by high-risk HPV. While high-risk human papillomavirus (hrHPV) infection alone is not sufficient to cause cancer, it regulates pathways that promote the hallmarks of cancer¹⁵⁷, via E6, E7 and E5 (REF. 1 2). This includes sustaining proliferative signalling via E7 and E5; enabling replicative immortality via E6; activating invasion and metastasis (that is, subverting keratinocyte differentiation via E6); deregulating cellular energetics via E6; and inducing local angiogenesis via E6 and E7, through the dysregulation of pathways as indicated in the figure. While this dysregulation triggers growth suppressors that promote cell death, they are restrained by E6 (via degradation of p53 and BCL-2 homologous antagonist/killer (BAK) and upregulation of BCL-2). The derailing of cell cycle control results in double-stranded (ds)DNA breaks, genomic instability and mutations that can lead to additional ‘hits’ that drive towards lethal cancer¹². Numerous mechanisms to avoid destruction by promoting immune deviation have been evolved by hrHPV, as reviewed in REFS 158, 159. E6 and/or E7 expression interferes with several aspects of innate immune activation, including detection of viral DNA by cyclic GMP–AMP synthase–stimulator of interferon genes protein (cGAS–STING) and Toll-like receptor 9 (TLR9); production of interferon- κ (IFN- κ) and CXCL14, which are important for Langerhans cell chemotaxis; suppression of pro-inflammatory responses — including through type 1 interferon signalling (interferon regulatory factor 1 (IRF1)) — and production of CC-chemokine ligand 20 (CCL20), to prevent the recruitment of antigen-presenting cells (APCs) to the site of infection. E5 interferes with T cell recognition through disruption of antigen presentation via modulation of major histocompatibility complex (MHC) I and MHC II cell surface expression and downregulation of transporter associated with antigen processing (TAP), while neutralizing antibody (nAb) production is hampered by release of virions outside the body rather than into systemic circulation. Furthermore, E6 and E7 expression induces production of interleukin-6 (IL-6), which recruits monocytes that generate tumour-promoting inflammation via local expression of CCL2 and matrix metalloproteinase 9 (MMP9). Such virus-mediated immune interference combines to promote the persistence of the infection and thereby promote the risk of cancer. EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; HIF1 α , hypoxia-inducible factor 1 α ; KDM6A, lysine-specific demethylase 6A; VEGF, vascular endothelial growth factor. Figure adapted with permission from REF. 157, Elsevier.

Immune deviation

Mechanisms that subvert the development of an effective anti-viral immune response, thereby facilitating the establishment of a persistent infection.

can attack the virus-infected cells. Helper T cells that recognize L1 facilitate the induction of nAbs that can prevent virus transmission and reinfection of the host³⁷. However, with HPV persistence, lesion progression can be driven by de-repressed expression of

E6 and E7, which can result from methylation of the E2 promoter but is most commonly attributed to viral integration¹². This leads to immune deviation by compromising the innate immune system via a series of interacting and self-reinforcing events mediated through

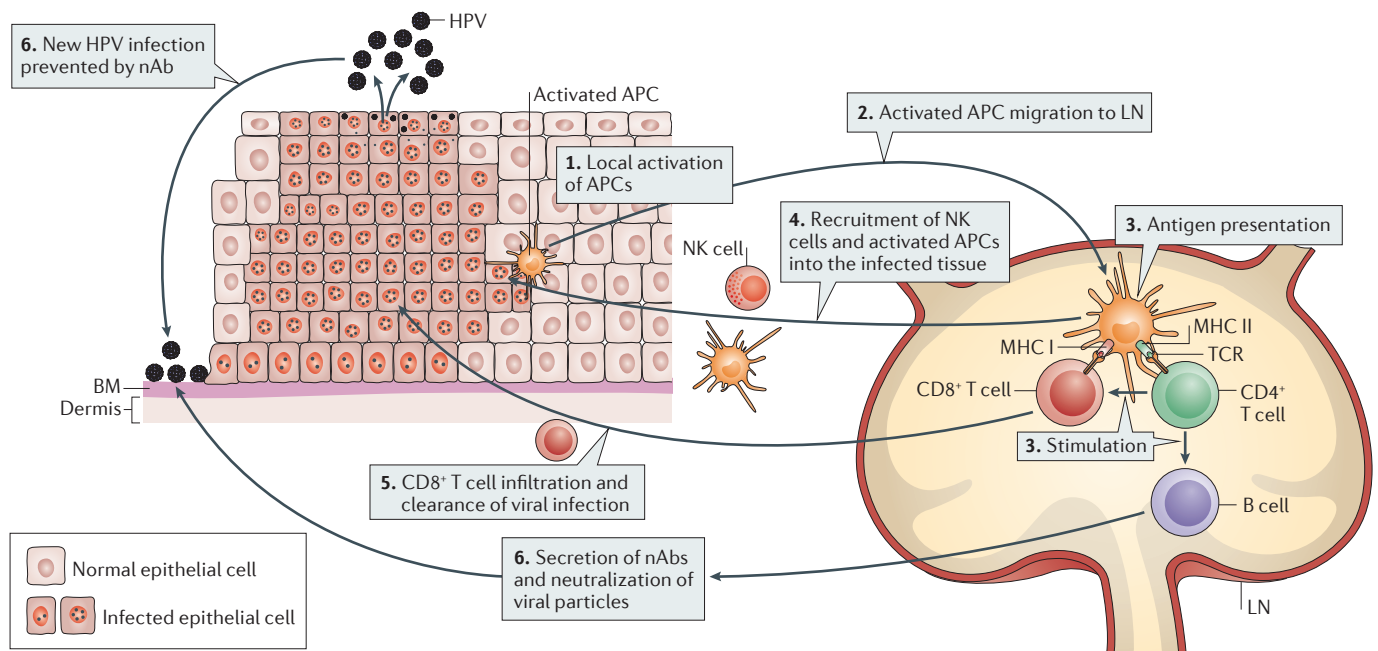


Figure 3 | Natural immune control of HPV infection. The immune system controls most human papillomavirus (HPV) infections before cancer can develop. The process of virus uptake into epithelial cells occurs over several hours and thus offers a time window for the action of vaccine or naturally induced neutralizing antibodies (nAbs). The first step is the detection of damage by the innate immune response arm via local antigen-presenting cells (APCs) and their activation (step 1). The secretion of pro-inflammatory cytokines and chemokines supports the viral antigen processing and migration to loco-regional lymph nodes (LNs) (step 2). Here, activated APCs stimulate various viral-antigen-specific CD4⁺ T cells that can either help activation of CD8⁺ T cells (for example, in targeting early viral antigens) or help B cells to produce nAbs that are, for example, directed against capsid proteins (step 3). The local activation of the innate immune response results in the attraction of nonspecific effectors (such as natural killer (NK) cells), the secretion of interferons (which can directly affect the HPV infection) and the attraction of more APCs to further drive activation of adaptive immunity (step 4). This inflammatory state provides the signals to attract the effector CD8⁺ T cells, which can target the virus-infected cells in the basal layers of the epithelium and are critical to clearance of the virus infection (step 5). Long-lived plasma cells secrete nAbs that can access the infection site either by transudation from the blood to the mucosal secretions or by serous exudation. Only the viral particles, and not the HPV-infected cells, can be targeted by nAbs, which are thus unable to cure infection but can stop further infections (step 6). Such antibody responses in women occur many months after HPV infection, and the levels detected are not necessarily sufficient to prevent a subsequent infection by the same virus type. It is likely that long-term natural protection against a specific HPV-type infection is the result of cell-mediated immunity, with nAbs contributing to a much lesser extent. BM, basement membrane; MHC, major histocompatibility complex; TCR, T cell receptor.

modulation of different immune activation pathways. For example, E6 and/or E7 interfere with recognition by intrinsic immune receptors cyclic GMP–AMP synthase–stimulator of interferon genes protein (cGAS–STING) and Toll-like receptor 9 (TLR9), including through interferon regulatory factor 1 (IRF1) or IRF3 inhibition, and thereby block innate signalling of the viral genome presence while suppression of interferon- κ (IFN κ), CXC-chemokine ligand 14 (CXCL14) and CC-chemokine ligand 20 (CCL20) production prevents the recruitment of APCs to the site of infection. Such events compromise and/or delay the induction of an adaptive immune response (FIG. 2), facilitating viral persistence and increased risk of cancer. E6 and E7 overexpression also compromises cellular DNA repair and induces extra centrosomes (leading to genomic instability^{38–40}), and it can further increase immune escape^{41–44}. This and additional cofactors provide genetic changes driving malignant transformation over 1–2 decades after initial HPV infection¹².

Vaccine requirements

Induction of nAbs is the major basis of vaccine-induced protection but requires immunization with the killed or attenuated natural pathogen or a subunit vaccine. An ideal vaccine should provide protection against all hrHPV types, and also the possibly carcinogenic types, to eliminate the need for cervical screening¹⁷. Sterilizing immunity may be required, as the cervical location of infection lacks secondary lymphoid tissue wherein substantial numbers of memory B cells could reside, ready to produce antibody at sufficient levels and, in time, to neutralize the virus before uptake⁴⁵. Unfortunately, the levels of type-specific antibody produced in natural infection are often insufficient to protect against subsequent reinfection. Therefore, a vaccine should deliver an improved response compared with natural serological responses.

To ensure protective immune responses throughout sexually active life, high and sustained levels of serum antibody are desired following HPV vaccination. The ability of natural HPV exposure to improve B cell

Sterilizing immunity
A humoral response that completely neutralizes the viral inoculum such that no infection occurs upon natural exposure.

Vaccination coverage

The per cent of vaccinated individuals in a predefined susceptible population.

Raft-derived viruses

The complete papillomavirus life cycle can be accomplished in vitro using organotypic raft culture, including the production of native virions.

Opsonization

A process, such as antibody binding, by which a pathogen is marked for ingestion and eliminated by phagocytes.

memory is not known. Therefore, maximal longevity of antibody levels directly induced by vaccination is needed. The current model suggests that plasma cells are imprinted with a predetermined lifespan. This model is based on the magnitude of B cell signalling that occurs during induction of an antigen-specific humoral immune response⁴⁶. Importantly, the magnitude and longevity of antibody responses are increased by adjuvants, for example, alum and MF59, which, among other effects, promote slow-release antigen depots to prolong antigen exposure, and danger signals such as monophosphoryl lipid A, which promote B cell activation via TLRs^{47,48}.

The ultimate goal is the best use of resources to have the maximum impact in preventing HPV-associated cancer. The key elements of a suitable vaccination programme will ideally be the selection of an optimal immunization age (and possible catch-up cohorts), the decision on sex-independent vaccination or whether female-only vaccination provides sufficient vaccination coverage, and how to integrate vaccination with programmes for

surveillance and treatment of HPV disease. Currently, the main problem is the lack of access to HPV vaccination in low-income and middle-income countries (LMICs), where cervical screening is inadequate if at all present and wherein >86% of cervical cancer cases and 88% of related deaths occur^{2,3}. With a predominance of sexual transmission, the coverage requirements for HPV vaccination will likely be <95% because of herd immunity⁴⁹.

Virus-like particle vaccines

In the early 1990s, it was discovered that recombinant expression of L1 in a range of systems yielded virus-like particles (VLPs) that were devoid of the oncogenic viral genome and immunologically similar to native virions — a eureka moment for vaccine development⁵⁰. Studies in diverse animal models have consistently shown that vaccination with L1 VLP, even without adjuvant, elicited high and durable titres of nAbs that protected against experimental viral challenge, as demonstrated by passive-transfer studies^{51,52}. However, it was also shown that the protection elicited by L1 VLP was type-restricted, indicating that to achieve broad protection, an HPV vaccine would likely need to contain L1 VLP of several key types. As 70–80% of cervical cancer is caused by HPV-16 and HPV-18¹², these two types were the focus of the first vaccines, with the intent of achieving broader protection through cross reactivity or expansion of the range of VLP types.

Assessing vaccine immunogenicity and efficacy. Vaccine development starts with the task of assessing safety and immunogenicity. The serological assays used to assess immunogenicity are outlined in BOX 1. These approaches differ considerably in the spectrum and potential relevance of the detected antibodies (BOX 1; FIG. 4). Efficacy depends on the integrated effects of antibody specificity, avidity and local in vivo concentration and potentially effector cells, such as phagocytes or local memory B cells. These qualities will also influence the potential for cross-protection and longevity.

The two pioneering vaccines (TABLE 1) — a bivalent (2vHPV) vaccine (L1 VLPs of HPV-16 and HPV-18 with AS04, an adjuvant comprising aluminium hydroxide and monophosphoryl lipid A) marketed as Cervarix and a quadrivalent (4vHPV) vaccine (L1 VLPs of HPV-6, HPV-11, HPV-16 and HPV-18 with an aluminium hydroxyphosphate sulfate (AHSS) adjuvant) marketed as Gardasil — were shown to induce antibody titres 2–3 orders of magnitude higher than those seen in natural infection, as assessed by various different serological assays⁵³. As it takes 1–2 decades from hrHPV infection to the development of cervical cancer and because the ablation of CIN2/3 precursor lesions protects against cervical cancer, the presence of such precursor lesions is used as the efficacy end point of clinical trials. In registration trials and subsequent population surveys, vaccines based on L1 VLP proved remarkably safe as well as effective for protection against new infections and disease associated with the types targeted by each vaccine. Thus, they were approved for protection against anogenital infection and disease associated with the types used to

Box 1 | Common assays to measure humoral immunity induced by vaccination**Competitive Luminex immunoassay (CLIA)¹³⁸**

- Advantages: robust and high throughput
- Disadvantages: measures antibodies that bind to a single neutralization epitope, yet there are multiple surface loops that contain protective epitopes. This assay does not detect crystallizable fragment (Fc)-dependent antibody effects and has not yet been adapted for L2-specific responses.

Virus-like particle enzyme-linked immunosorbent assay (VLP ELISA)¹³⁹

- Advantages: sensitive, robust and high throughput
- Disadvantages: measures both neutralizing (those that block virion binding and those that neutralize post-binding) and non-neutralizing antibodies (non-nAbs) (FIG. 4). It typically uses L1 VLP but can substitute with pseudovirions (PsVs) as an antigen to permit detection of L2 antibodies. This does not detect Fc-dependent antibody effects.

In vitro neutralization test (IVNT)¹⁴⁰

- Advantages: measures functional presence of L1-neutralizing and L2-neutralizing antibodies with moderate throughput
- Disadvantages: measures both neutralizing but not non-nAbs (FIG. 4). This assay does not detect Fc-dependent antibody effects. PsVs of different types have very different particle:infectivity ratios¹⁴¹, and this assay is complex to perform under Good Laboratory Practice (GLP) as compared with CLIA or VLP ELISA.

There is some evidence that PsVs are not structurally identical to the native virus^{142–144}. Raft-derived viruses and detection of early mRNA can be substituted, but this is challenging to scale. The standard assay has poor sensitivity to L2-specific nAbs because the in vitro assay does not fully recapitulate the extracellular matrix-bound phase of infection. However, modifications to the assay have been designed to better mimic this process and increase sensitivity to L2-specific neutralization^{78,145,146}.

Passive transfer and in vivo challenge^{77,147}

- Advantages: sensitive and follows actual events of human papillomavirus (HPV) infections, potential for opsonization or complement effects and measuring disease outcomes
- Disadvantages: this assay is technically challenging, labourious and expensive if studying large cohorts and does not address cellular contribution to protection when relevant. Although it measures Fc-dependent effects, care must be taken when crossing species, for example, human antibodies into mice or rabbits. It has been used in dogs with canine oral papillomavirus challenge^{51,52}. To measure HPV-specific immunity, PsVs carrying luciferase are used for vaginal challenge of mice or for a disease end point, and PsVs carrying cottontail rabbit papillomavirus genome (termed quasivirion) are used for skin challenge in rabbits. In the latter model, multiple HPV quasivirion challenges can be performed in the same animal¹⁴⁸.

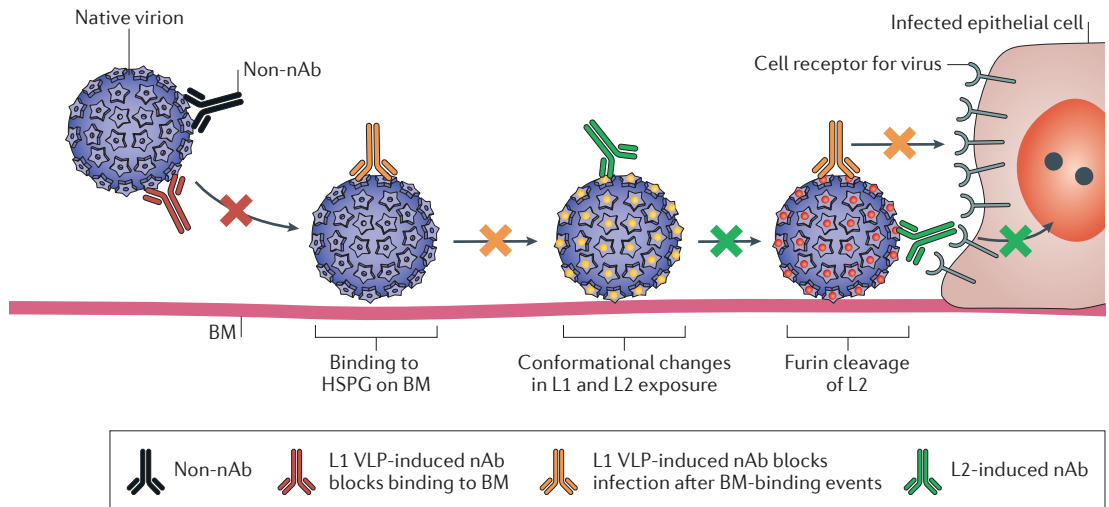


Figure 4 | Antibody-mediated protection. Vaccination with human papillomavirus (HPV) capsid antigens can induce different types of type-specific antibodies, most of which can bind to the native virion, but not all will necessarily neutralize the virus by preventing uptake by the target cell. Antibodies depicted in black represent non-neutralizing antibodies (non-nAbs), which are not able to directly influence the infectivity of the virus. The available data suggest that an initial step that can be blocked by some L1- virus-like-particle (VLP)-induced nAbs is the binding to heparin sulfate proteoglycans (HSPGs) on the basement membrane (BM) (indicated by red cross)^{160,161}. Orange nAbs represent those that can potentially influence infectivity after HSPG-binding events that occur before and after changes to L2 (indicated by orange cross). These antibodies are detected with different assay types (BOX 1). Immunization with L1 VLPs cannot reflect all the potential structures through which antibodies may be able to block the infection process (FIG. 1), most notably L2, which is poorly immunogenic in natural infection. Nevertheless, L2 is a potentially effective target for prophylaxis. L2 vaccination induces nAbs (depicted in green) that neutralize the virion after binding and only after a conformational change in the capsid and cleavage of L2 by extracellular furin to render L2 protective epitopes, such as residues 17–36, accessible to antibody binding (indicated by green cross)¹⁴⁶. L2-specific antibodies have a much lower titre and avidity than L1 VLP-specific antibodies. The L2 epitope spacing will probably not allow bivalent binding of this antibody. The different types of antibodies may include recognition of different epitopes of L1 or L2 molecules. Late events associated with virus uptake and processing by the cell may also be interfered with by nAbs. There are clearly still gaps in our knowledge of the infection process and the nature of antibodies that can influence the process, including the contribution of phagocytes that recognize the crystallizable fragment (Fc) of capsid-reactive antibodies.

Quasivirion

An infectious virion comprising the papillomavirus capsid that has packaged a heterologous papillomavirus genome, typically produced in 293T cells expressing a human papillomavirus (HPV) capsid delivering a cottontail rabbit papillomavirus genome.

Herd immunity

The indirect protection for individuals who are not immunized that occurs when a large percentage of their surrounding population has become immune through vaccination such that the likelihood of exposure of a vaccine-naive member of the general population to the infectious disease is substantially reduced.

CIN2/3

Moderate and/or high-grade cervical intraepithelial neoplasia, the precursor lesion of cervical cancer and the disease detected by cervical screening with Papanicolaou smear and conformational biopsy.

make the VLP^{54–57}. Of note, there is no approval of these vaccines yet for the prevention of HPV-associated oropharyngeal squamous cell carcinoma (OPSCC), and this reflects the lack of a validated precursor lesion in the oral cavity for use as a disease end point in efficacy trials⁵⁸. HPV tests for cervical cancer screening have been licensed in some countries. Therefore, the option of using persistent HPV infection over 6 or 12 months as an end point for the approval of vaccines, especially of next-generation vaccines and of vaccines for the prevention of HPV-associated OPSCC, is being considered^{59,60}.

Cross-protection, dosing schedules and longevity.

Vaccine-mediated, type-specific protection has been shown to be durable for at least a decade. Importantly, this protection was not absolutely type-specific because significant cross-protection (particularly by vaccination with the 2vHPV vaccine) was observed against very closely related types from within the same species, for example, HPV-16 with HPV-31 and HPV-33, or HPV-18 with HPV-45. Indeed, while only 52% of CIN3 is associated with HPV-16 and HPV-18, vaccination with the 2vHPV vaccine showed an efficacy of 93% in protecting against CIN3, irrespective of the HPV type^{55,57}. Although the substantially lower titres of cross-neutralizing

antibody compared with those of type-specific antibodies raised concern about the durability of cross-protection⁶¹, there has been little evidence of the waning of cross-protection in patients who have been vaccinated three times as per recommendation⁶². Indeed, recent data from Scotland confirm cross-protection through a decrease in the prevalence of HPV-31, HPV-33 and HPV-45 during the 5-year follow-up after vaccination with the 2vHPV vaccine^{63,64}.

Both the 4vHPV vaccine and the 2vHPV vaccine induce sustained levels of antibodies against HPV-16 and HPV-18, but the responses are not identical. Five years after vaccination, females aged 18–45 years who received the 2vHPV vaccine showed higher serum nAb responses than those given the 4vHPV vaccine, where seropositivity rates against HPV-18 decreased across all age groups⁵³. In addition, vaccination with the 2vHPV vaccine produces a T_H1 (T_H1) cell-biased response that favours immunoglobulin G1 (IgG1) and IgG3 serum responses^{65,66}, while vaccination with the 4vHPV vaccine produces a T_H2-biased response that favours IgG1, IgG4 and IgA serum responses⁶⁷. These vaccines use different adjuvants and L1 expression systems to generate VLP — differences that potentially affect relative immunogenicity (titre), longevity and breadth of immunity.

Geometric mean titres (GMTs). The simple arithmetic mean of the logarithms of the last positive dilution of each serum that binds to and neutralizes a fixed viral inoculum.

An important factor in vaccine immunogenicity is also the age of the recipients. For example, in females aged 10–17 years who received the 2vHPV vaccine, the geometric mean titres (GMTs) of IgG are at least twice as high as those of females aged 18–25 years⁶⁸. Subsequent immune-bridging studies demonstrated that 9–14-year-old females who received two doses of the 2vHPV vaccine with an interval of 0 and 6 months showed non-inferior immune responses compared with those who received three doses of the 4vHPV vaccine on a schedule of 0, 2 and 6 months, and the efficacy against disease was established in these groups⁶⁹. On the basis of such studies, the World Health Organization (WHO) has now recommended two doses of the 4vHPV vaccine or the 2vHPV vaccine for 9–15-year-old females. Remarkably, recent evidence suggests that durable type-specific immunity

can be achieved with a single dose of either the 2vHPV vaccine⁷⁰ or the 4vHPV vaccine⁷¹. Analysis of the impact of bivalent HPV vaccination in a population of 14–18-year-old females in the UK also shows evidence of type-specific protection but not cross-protection following a single dose of vaccine⁷². At this point, both the 2vHPV vaccine-vaccinated and the 4vHPV vaccine-vaccinated populations appear to be potently and durably protected against the HPV types they were designed to prevent. A major randomized trial (ESCUDDO) to test the efficacy of single-dose vaccination is underway, and it will also provide some independent comparisons between HPV vaccines⁷³.

A 9-valent (9vHPV) vaccine, Gardasil 9, that contains VLPs from HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58 has now been

Table 1 | HPV vaccines targeting capsid antigens that are licensed, in or advancing towards clinical trial

Name	Antigen	System	Adjuvant	Status	Party
Cervarix (2vHPV vaccine)	L1 VLP of HPV-16 and HPV-18	BEVS	Aluminium hydroxide and MPL	Licensed	GSK
Gardasil (4vHPV vaccine)	L1 VLP of HPV-6, HPV-11, HPV-16 and HPV-18	Yeast	AHSS	Licensed	Merck
Gardasil 9 (9vHPV vaccine)	L1 VLP of HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58	Yeast	AHSS	Licensed	Merck
Cecolin	L1 VLP of HPV-16 and HPV-18	<i>E. coli</i>	Aluminium hydroxide	Phase III ¹¹⁹	Xiamen Innovax
Gecolin	L1 VLP of HPV-6 and HPV-11	<i>E. coli</i>	Aluminium hydroxide	Phase II ¹²⁰	Xiamen Innovax
L1 capsomers	L1 capsomers of HPV-16	<i>E. coli</i>	unknown	cGMP production	R. Garcea, University of Colorado–Boulder ^{113,114}
RG1-VLP	HPV-16 L1-L2 (17–36) VLP	BEVS	Aluminium hydroxide	cGMP production	R. Kirnbauer, NCI ¹³⁰ , Pathovax LLC
L2-AAV	L2 peptides of HPV-16 and HPV-31 displayed on AAV VLP	BEVS or 293T cells	unknown	cGMP production	2A Pharma ¹³¹
L2 multimer	Fusion protein of L2 ~11–88 of HPV-6, HPV-16, HPV-18, HPV-31 and HPV-39	<i>E. coli</i>	Alum	cGMP production	Sanofi, BravoVax ^{76,129}
L2-thioredoxin	L2 peptide displayed on thioredoxin	<i>E. coli</i>	unknown	cGMP production	M. Muller, DKFZ ¹³⁴
AX03	L2 peptide displayed on bacteriophage	<i>E. coli</i>	unknown	cGMP production	Agilvax, NIAID ¹³⁴
L1-E7 VLP	HPV-16 L1-E7 VLP	BEVS	None	Phase I	Medigene AG ¹³⁵
TA-CIN	HPV-16 L2E7E6 fusion protein	<i>E. coli</i>	None	Phase II	Cantab Pharmaceuticals, Xenova ^{136,137}
TA-GW	HPV-6 L2E7 fusion protein	<i>E. coli</i>	Aluminium hydroxide or AS03	Phase II	Cantab Pharmaceuticals, GSK ¹⁶²

The last three vaccines are being tested in a therapeutic context because they also include early antigens. The remainder are intended for prophylactic use only. Several other companies are developing human papillomavirus (HPV) vaccines based on L1 in China and India, including some that are in advanced clinical trials; Walvax (HPV-16 and HPV-18), China National Biotech Group (HPV-16, HPV-18, HPV-52 and HPV-58) and Health Guard (HPV-16, HPV-18 and HPV-58), Serum Institute of India (HPV-6, HPV-11, HPV-16 and HPV-18). They are not listed as we could not access the complete details. 2vHPV vaccine, bivalent HPV vaccine; AAV, adeno-associated virus; AHSS, aluminium hydroxyphosphate sulfate; BEVS, baculovirus expression vector system; cGMP, cyclic GMP; CIN, cervical intraepithelial neoplasia; DKFZ, German Cancer Research Center; *E. coli*, *Escherichia coli*; GSK, GlaxoSmithKline; GW, genital warts; MPL, monophosphoryl lipid A; NCI, US National Cancer Institute; NIAID, US National Institute of Allergy and Infectious Diseases; TA, tissue antigen; VLP, virus-like particle.

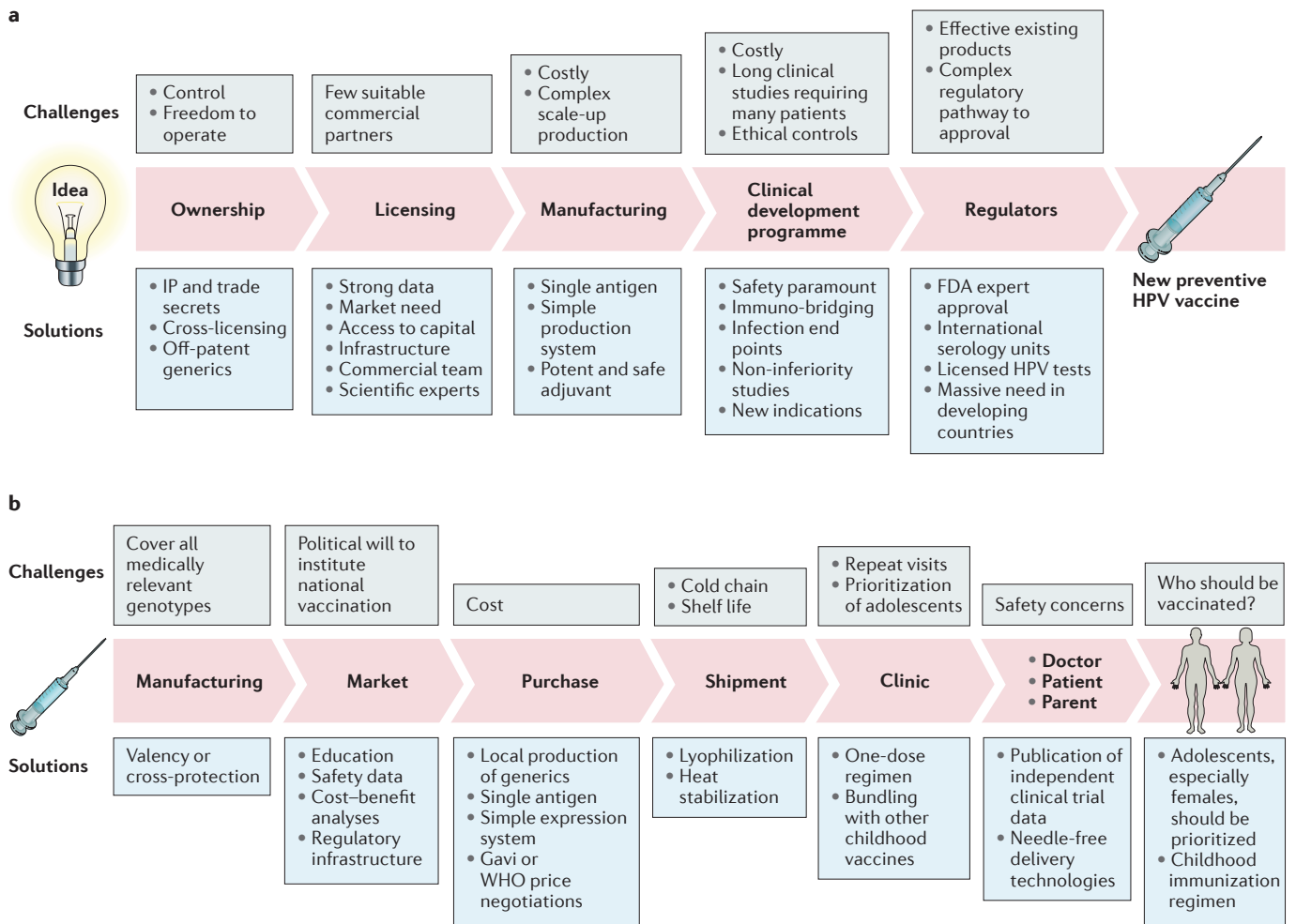


Figure 5 | Development and implementation of HPV vaccines. a | The challenges and possible solutions to bringing a novel preventive human papillomavirus (HPV) vaccine from its initial invention through licensure. **b** | Challenges and possible solutions to achieving global use of licensed HPV vaccines and herd immunity and thus recognizing the full potential of cancer prevention. FDA, US Food and Drug Administration; Gavi, Global Alliance for Vaccines and Immunization; IP, intellectual property; WHO, World Health Organization.

Biosimilar

A type of biological product that is licensed (approved) because it is highly similar to an already approved biological product, known as the biological reference product, and has been shown to have no clinically meaningful differences from the reference product.

Pseudovirion

(PsV). A single round infectious virion comprising the papillomavirus capsid that has packaged a reporter plasmid, typically produced in 293TT cells, and delivering either luciferase or green fluorescent protein (GFP).

Anamnestic response

A more rapid and profound immune response upon re-exposure to an antigen that occurs after initial exposure or vaccination and is driven by immunological memory.

tested in comparison with the 4vHPV vaccine from the same manufacturer. The 9vHPV vaccine prevented infection and disease related to HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58 in a susceptible population of 16–26-year-old females and generated an antibody response to HPV-6, HPV-11, HPV-16 and HPV-18 that was non-inferior to that generated by the 4vHPV vaccine. The 9vHPV vaccine did not demonstrably prevent infection and disease related to HPV types beyond the nine types covered by the vaccine, although the study was not powered to do so⁷⁴.

Effectors of protection

An understanding of the mechanism of protection is important to identify the relevant immune correlates of protection and thus facilitate the development of biosimilar and second-generation prophylactic vaccines (FIG. 5a). Passive transfer into naive animals of L1 VLP-specific immune serum, IgG or purified monoclonal antibodies that show *in vitro* neutralizing activity protects against experimental animal papillomavirus or HPV pseudovirion

(PsV) challenge^{51,52,75,76} (BOX 1). Although L1 VLP vaccines induce very high titres of serum neutralizing IgG, protection can be achieved with much lower titres⁷⁷, as seen with cross-protection and, as discussed below, with L2-based vaccines^{21,78}. FIGURE 4 summarizes the different phases of early infection and potential specificities of antibodies that may be relevant to neutralization.

The role of nAbs as the primary effector of protection is widely accepted; however, this concept does not consider the contribution of cellular immunity and a potential therapeutic benefit. Vaccination with L1 VLP does not confer a therapeutic benefit in most disease models or clinical studies⁷⁹, although there have been hints of activity⁸⁰. L1 VLP immunization does induce a robust L1-specific CD8⁺ T cell response, but basal keratinocytes harbouring HPV do not detectably express L1 and thus presumably escape this response. The activity of T helper cells is important to achieve a robust response. L1 VLP challenge studies demonstrate the induction of a rapid anamnestic response^{81,82}, which has been suggested to prolong protection if titres decline.

Immunogenicity of capsid proteins

The data emerging from the UK showing that HPV-16 and HPV-18 L1 VLP immunization reduces the occurrence of HPV-6-associated and HPV-11-associated genital warts⁸³, as well as the reports of the high efficacy of single-dose L1 VLP immunization on HPV prevalence and disease^{70–72}, merit further discussion. One possibility to explain the unexpected cross-protection is that the adjuvant AS04 in the bivalent vaccine induces a particularly effective T cell response against L1, which will cross-react to HPV-6 and HPV-11 L1 proteins and act either directly, by enhancing local innate control, or by providing help for subsequent specific, therapeutic adaptive immunity against other viral targets. AS04 contains the TLR4 agonist monophosphoryl lipid A and aluminium salts and is particularly effective in activating APCs, inducing cytokines that enhance the adaptive immune response and inducing a T_H1-type response, thereby enhancing humoral and cellular responses⁴⁸. T_H1 immune cell-derived IFN γ induces anti-viral protein effector functions, leading to inhibition of viral transcription or translation and infection⁸⁴. Such events are likely to contribute to local control of HPV infections.

A key feature of L1 VLPs is their high immunogenicity, which is present even without adjuvant and also in immunocompromised patients with HIV^{85,86}. As mentioned before, even a single dose of the 2vHPV or 4vHPV vaccines is sufficient to induce a robust, durable IgG response indicative of antibody class switching, somatic hypermutation, affinity maturation and memory B cell development that does not require subsequent re-exposure or boosting^{70,71}. This immunogenicity likely reflects the highly ordered and closely packed 3D structure of L1 VLP⁸⁷. By comparison, denatured L1 protein is not an effective immunogen⁸⁸, while capsomers, comprising L1 pentamers, are strongly immunogenic but do not achieve the titres of L1 VLP (which are formed from 72 capsomers) without the use of adjuvants^{89,90}. The close spacing of the epitopes is also important, likely reflecting their ability to perform bivalent immunoglobulin binding and crosslinking of B cell receptors⁸⁷. The remarkable immunogenicity of L1 VLPs may also derive from a direct activation of immature dendritic cells and induction of key chemokines, cytokines and co-stimulatory molecules central to effective antigen presentation^{91,92}.

By contrast, the L2 protein does not form VLP on its own and is weakly immunogenic when given without an adjuvant⁹³. There are only 12–72 copies of L2 per virion compared with 360 L1 in the capsid, and thus L2 is spaced further apart^{94,95}, which potentially contributes to the poor immunogenicity of L2 in the context of the capsid compared with that of the immunodominant L1 (REF. 93). Nevertheless, vaccination with L2 is protective, although the antibody response elicited by L2 is characterized by a lower titre and avidity than that elicited by L1 VLP⁹⁶. We speculate that these differences may reflect, in part, the inability of antibodies directed against L2 to bind bivalently to the capsid because of the greater separation of L2 epitopes compared with L1 epitopes^{87,97}. Despite these differences, vaccination

with L2 using an adjuvant such as alum provides durable immunity in animal models, and passive-transfer studies show that low titres of nAbs with moderate avidity suffice for protection^{76,97,98}. Interestingly, the subdominant protective epitopes of L2 are well conserved between types and broadly cross-protective in animal models^{97–99}.

Antibody-mediated protection

Vaginal fluids of vaccinated patients contain neutralizing IgG at levels consistent across the menstrual cycle but much lower than the levels in serum⁴⁵. Notably, studies in animals show that protection also occurs at extragenital sites, and passive intraperitoneal transfer of nAbs even just before experimental HPV challenge provides robust protection. This suggests that abrasion of the epithelium at the site of infection is sufficient to support exudation of serum IgG and neutralize the inoculum⁷⁵. It is unclear whether the viral inoculum can reactivate putative local memory B cells to produce sufficient neutralizing IgG locally in time to prevent infection, although such an anamnestic response might be important if antibody titres have declined substantially many years after vaccination^{81,100,101}.

An important question is which serum level of antibody is correlated with immunity. To date, there has not been enough breakthrough infection for type-specific immunity in vaccinated patients to determine the minimum titre required for protection. However, it may be possible to approach this question by analysing the level of cross-protection that is often insufficient in patients and associated with much lower titres of cross-neutralizing antibodies¹⁰². International serologic units have been established for HPV-16 and HPV-18, and ongoing efforts to further this standardization are critical¹⁰³. Immune-bridging studies have been used to obtain licensure for vaccination in female and male adolescents and young adult females¹⁰⁴ for a two-dose vaccination schedule¹⁰⁵ and for using the 9vHPV vaccine for vaccination against HPV-16 and HPV-18⁷⁴. These efforts underline the growing importance of serological standardization and the need to define a minimum titre that correlates with protection, especially for the development of low-cost biosimilar and L2 vaccines⁶⁰.

Application of preventive HPV vaccines

The primary factor limiting the impact of vaccination is inadequate population coverage¹⁰⁶ (FIG. 5b). In a few developed countries and some LMICs using school-based vaccination programmes, high and sustained levels of vaccination coverage (70–90%) have been achieved when targeting adolescent females^{64,107–109}. This level of vaccination coverage is sufficient to achieve a significant reduction in hrHPV prevalence and associated cervical lesion rates, as well as herd immunity in males within the general population¹¹⁰. Unfortunately, even in most highly developed countries, such a level of vaccination coverage has not been consistently achieved. Critically, global estimates of HPV vaccination delivery by region and income level show virtually no significant delivery to many poorer populations of

Breakthrough infection
An infection that occurs despite prior vaccination.

women worldwide¹⁰⁷. The relatively high cost of the current vaccines is one contributing factor. Although efforts such as the [Gavi Vaccine Alliance](#) (see related links) have reduced the price of the current vaccines for developing countries with the greatest burden of cervical cancer, the commitment of national governments to support the logistics for establishing and sustaining efficient vaccination programmes is central to any long-term success¹¹¹. Unfortunately, vaccination programmes compete with screening-based approaches for funding, and vaccination has little or no benefit for those already infected with HPV⁷⁹. The HPV-FASTER protocol, which includes extending routine vaccination programmes to females up to 30 years of age in conjunction with at least one HPV screening test for females at 30 years of age or older may, based on modelling studies, accelerate the reduction in cervical cancer incidence. Indeed, modelling suggests that temporary introduction of catch-up vaccination for older females, although less effective, would advance the date to achieve cervical cancer reduction by several years. Refining this protocol for a particular population will require further investigation, including cost-effectiveness modelling to determine the age ranges for HPV vaccination and screening intervals in public health programmes, and to estimate possible impact based on the resources available¹¹².

Targeting adolescent females before sexual debut was considered the best strategy to deliver cervical cancer protection and has shown remarkable success⁶⁴. However, given the durable responses to HPV vaccination seen thus far, it is likely feasible to implement a childhood regimen and even to piggyback HPV vaccines in the same vial with other childhood vaccines administered within existing regimens to reduce infrastructure demands. This would need to be phased in to replace the current vaccination schedule for adolescents so that no child reaches sexual debut without HPV vaccination.

The need for refrigeration of the vaccine is another hurdle in underdeveloped nations. This may be addressed with lyophilized formulations or with heat-stable capsomer preparations^{113,114}. Thus, a combination of technical improvements, additional low-cost producers and the continued commitment of public health professionals to demonstrate the safety and benefits of HPV vaccination is critical for global implementation (FIG. 5b).

In areas of limited resources, there has been debate about whether to prioritize vaccination of adolescent females or to promote sex-independent vaccination. It is clear that females suffer the greatest burden of cancer caused by HPV, but to achieve herd immunity most effectively¹¹⁵, to address other HPV-related cancer types and to reach men who have sex with men, it is recommended to vaccinate adolescent males as well. Indeed, immunization of adolescent males also improves the resilience of programmes, accelerates the reduction in HPV prevalence¹¹⁶, increases cross-protection and provides a health benefit to unvaccinated females¹¹⁵. Co-infection with HIV is associated with increased risk of HPV-associated cancers. Patients with HIV respond to HPV vaccination with titres suggestive of protection, although this remains unproven^{86,117,118}.

Promising avenues for HPV vaccination

TABLE 1 summarizes HPV vaccines either licensed or in clinical development. Emergence of local biosimilar vaccine production is likely to advance sustainable implementation of HPV vaccination worldwide by reducing costs and promoting access. Indeed, a major effort is underway to develop two additional bivalent HPV vaccines using L1 VLP purified from *Escherichia coli*. These vaccines are Cecolin, which targets HPV-16 and HPV-18 and is in a phase III clinical trial¹¹⁹, and Gecolin, which targets HPV-6 and HPV-11 (REF. 120). Likewise, local production of the currently licensed vaccines could reduce pricing substantially¹¹¹.

There remains considerable interest in developing preventive HPV vaccines with broader coverage of HPV types. Currently, cervical screening, which is of considerable cost, is continued even in vaccinated patients because of the potential for disease caused by hrHPVs other than those targeted by the licensed vaccines. Notably, the predictive value, benefit:risk ratio and cost-effectiveness of cytologic screening are remarkably reduced in vaccinated women because of the dramatic reduction in the prevalence of CIN2/3 (REFS 110, 121, 122). The lower incidence of disease in vaccinated women alters the key performance indicators of cervical cytology, colposcopy referral criteria, colposcopy practice and histology reporting¹²³. This impact might be mitigated to some degree by increasing intervals between screening and/or switching to HPV testing (which could include self-testing) as the primary screening modality¹²⁴. Although a dual approach of screening and vaccination is being promoted¹¹², the logistics and price of this approach will still be very challenging in many developing countries. However, childhood vaccination in this context has proved feasible for many pathogens¹⁰⁶, and future developments in HPV vaccination that piggyback other paediatric immunization regimens are a good option for improving vaccination coverage and delivering substantial population protection with limited resources.

The cutaneous β HPV and γ HPV species also have significant clinical impact^{15,125}. Prevention of common foot and hand warts associated with γ HPV types would reduce health-care costs¹²⁵. In addition, β HPV types are associated with non-melanoma skin cancer in patients with immunity compromised through treatment (for example, organ transplant recipients), HIV co-infection or hereditary disease (epidermodysplasia verruciformis or WHIM syndrome)^{15,16}. While vaccination would likely need to start in childhood¹²⁶, a recent animal study has suggested that VLP vaccination prevents the development of non-melanoma skin cancer in infected immunocompromised hosts^{126,127}.

A major challenge for the L1 VLP technology is the complexity of manufacturing a sufficiently multivalent formulation to comprehensively target the plethora of HPV types associated with disease. The 9vHPV vaccine targets the seven most common types detected in cervical cancer (TABLE 1), but including more L1 VLPs to cover the remaining high-risk types (each causing a very small fraction of cancers) is likely to make the vaccine prohibitively costly. The alternative approach is to find a single

broadly protective antigen. The amino terminus of L2 harbours several well-conserved protective epitopes⁹³, such as residues 17–36, recognized by monoclonal antibody RG1^{75,128}.

Neutralizing epitopes of L1 VLP are conformational, but they are linear for L2 and thus can be readily linked to further broaden immunity and fused with an adjuvant, for example, a TLR agonist^{76,129}, to boost immunogenicity and extend protection. The immunogenicity of L2 protective epitopes can potentially be improved by displaying these epitopes on the immunodominant surface loops of L1 VLP¹³⁰; the VLP of other viruses (such as adeno-associated virus (AAV))¹³¹ or bacteriophages^{132,133}; or on a thioredoxin scaffold¹³⁴ (TABLE 1). Several of these products are being prepared for early phase trials. The success of the L1 VLP vaccines demonstrates the potential of this approach but also represents a commercial hurdle unless L2 vaccines can also be useful in other indications (FIG. 5a).

There is still a massive global burden of HPV disease, and, unfortunately, vaccination with capsid antigens alone is not therapeutic for pre-existing infection. In an effort to combine both prophylaxis and therapeutic activity, early viral antigens E7 and/or E6 have been incorporated into L1 VLP or fused with L2 (REFS 135,136) (TABLE 1). Both approaches have been explored in early phase therapeutic studies and found to be both immunogenic and well tolerated. In these small trials, there was limited evidence of therapeutic activity, and prophylactic efficacy was not examined^{135,137}. Given the delayed and uneven implementation of prophylactic HPV vaccination programmes, there remains a need to screen for and treat HPV-associated cervical disease. FIGURE 5 summarizes the challenges and some potential solutions for delivering substantial improvement in effective prophylactic HPV vaccination worldwide.

Conclusions

As the aetiology of hrHPV in anogenital and oropharyngeal cancers is well established, there are continuous efforts to fully implement vaccination programmes

globally and to broaden coverage to all medically significant genotypes. In parallel, population-based HPV testing and/or cytological screening programmes can identify females for ablative therapy to prevent cervical cancer. The burden of HPV-related cancer falls primarily on the disadvantaged and medically underserved populations of the world, and, therefore, a focus on addressing issues constraining the delivery of these remarkably effective and safe interventions (as well as standard-of-care surgery and chemoradiation for patients with cancer) remains paramount. The prevention of HPV-related head and neck cancers would be a very beneficial, although unproven, outcome of vaccination. Preventive HPV vaccines to target a broader range of HPV types are being developed. While some may even protect against cutaneous warts and non-melanoma skin cancer associated with βHPV, the primary aim would be to reduce and eventually eliminate the need for continued cervical cancer screening. Indeed, there are important questions of how appropriate are current cervical cancer screening paradigms in properly vaccinated females, given their reduced predictive value and increased cost:benefit ratio, and what alternative screening technologies (for example, self-testing and point-of-care testing) or screening and vaccination combinations (for example, HPV-FASTER) might be substituted. In the long term, and provided there is sustained immunity, incorporating an HPV vaccine against oncogenic types within a multitarget paediatric vaccine regimen offers a viable strategy to improve vaccination coverage without added visits and ensures that everyone receives vaccination before sexual debut; however, this approach would need to be phased in. For current generations exposed to a high prevalence of HPV infection, the use of scarce resources for vaccination and screening must be carefully optimized through mathematical modelling to maximize benefits, as exemplified by HPV-FASTER¹¹². Finally, the success of HPV vaccines in preventing cancer ultimately revolves around the effective communication of their safety and benefits to policymakers, physicians, patients and their families.

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Author contributions

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Competing interests

[The authors declare competing interests: see Web version for details.](#)

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