5. Summary of Data Reported and Evaluation

5.1 Human papillomavirus (HPV) infection

Papillomaviruses are a family of DNA viruses that have a double-stranded, closed, circular genome of 7000–8000 base pairs and a non-enveloped T=7 icosahedral capsid. Approximately 100 human papillomaviruses (HPVs) have been molecularly cloned and sequenced, and other putative types have been identified based on polymerase chain reaction products that represent partial genomes. HPVs have a strict species-specific tropism and infect only mucosal or cutaneous epithelia. Analysis of HPV genomes, and those of a number of animal papillomaviruses, has led to the development of robust phylogenetic trees that form a stable framework for the placement of additional HPV types as they arise. A newly proposed taxonomy and nomenclature follows generally accepted criteria. Higher order phylogenetic assemblages are considered to be a genus; for example, the genital HPVs belong to the genus alpha-papillomavirus and the cutaneous epidermodysplasia verruciformis-associated HPVs belong to the genus beta-papillomavirus. Clusters of lower order

are known as species, which are closely related phylogenetically; while members of the species have distinct genomes, they have identical or very similar biological or pathological properties. For example, the species alpha-9 includes HPV types 16, 31, 33, 35, 52 and 58. Among genital HPVs, the nomenclatures 'high-risk' and 'low-risk' are widely used and refer to types that are frequently found in cervical cancers versus types that are rarely or never found in cervical cancers. Sequencing of a hypervariable region from many isolates of HPV 16 has provided evidence that HPVs are ancient viruses that have co-evolved with their hosts. A continuing area of investigation is the determination of how variation within a type affects the pathogenic potential of that type.

The structure of the viral genome can be divided into three parts: a non-coding region that contains the origin of viral replication and multiple elements that regulate transcription of the viral genes; an early region that encodes non-structural viral proteins; and a late region that encodes two capsid proteins, L1 and L2. HPVs are thought to infect cells in the basal layer of the epithelium and establish maintenance of the viral genome as a low-copy number nuclear plasmid. The E6 and E7 gene products promote cellular replication of at least some suprabasal cells that harbour the HPV genome. As infected cells move further up in the epithelium, epithelial differentiation promotes replication of productive viral DNA, expression of the late genes, assembly of the capsid and encapsidation of a chromatinized viral genome.

The repetitive structure of 72 capsomers that forms the papillomavirus capsid is highly immunogenic. Combined use of monoclonal antibodies, mutagenesis and the recently derived crystallographic structure of a capsomer has localized neutralization epitopes to type-specific surface-exposed loops. Virus-like particles that originate from the expression and self-assembly of L1 provide an antigen target to measure naturally occurring antibodies. In response to HPV infection, most, but not all, women develop immunoglobulin G antibodies directed towards type-specific conformational epitopes on L1. These antibodies have a low titre and are slow to develop, but generally persist for many years. Serum and cervical immunoglobulin A antibodies to L1 also develop, but are lost quickly. While antibodies to other HPV proteins may be elicited, no reproducible assays have been developed for their measurement, except for antibodies to HPV 16 and 18 E6 and E7, which are seen quite frequently in individuals who have invasive cancer.

During the past decade, many techniques have been applied for the detection of HPV infection and disease. Visual inspection of the cervix or genital tract, colposcopy, cytology and histology are all used to detect clinical manifestations of HPV-associated disease. The most reliable confirmation of current HPV infection is by assays that detect HPV nucleic acids. Hybrid Capture 2™, which employs cocktails of probes, is widely used clinically. Most studies use polymerase chain reaction methods that permit determination of HPV types, and many protocols use primers that amplify a conserved region of the L1 gene that can then be hybridized to (or interrogated with) type-specific probes. Primer pairs for both genital and cutaneous HPV types have been developed. Real-time polymerase chain reaction assays facilitate the quantitation of viral load in a specimen, and sequencing allows the identification of viral variations. Serological assays that use virus-like particles

can provide a measure of past HPV infection. Taken together, these methods have contributed greatly to understanding the natural history of genital HPV infections.

Transmission of genital HPVs occurs primarily through sexual intercourse. Annual rates of incident infection in young women are approximately 5–15%, and infections by high-risk types, particularly HPV 16, are the most frequent. Overall HPV positivity in cytologically normal women has been reported at levels of between 1.5% and 39%. The incidence and prevalence of HPV infections peak in young adults in most study populations, and the prevalence of specific HPV types has been reported in numerous studies. Although the age of the women who were tested and the type of polymerase chain reaction methods used could introduce some variability, clear geographical differences in prevalence exist, and, as anticipated, the prevalence of HPV infection was also higher in populations of commercial sex workers or women and men who were infected with human immunodeficiency virus. However, there is a paucity of studies on the natural history of HPV infection in men and on HPV infection at non-genital sites.

Viral DNA persists for a median of approximately 1 year, and high-risk types persist somewhat longer than low-risk types. As HPVs induce cellular proliferation, HPV infection may result in a range of morphological manifestations. While the development of more severe lesions such as stage 3 cervical intraepithelial neoplasia may be due to accumulated events, some infections may cause the rapid appearance of this lesion. When HPV DNA is no longer detectable by polymerase chain reaction amplification, it is uncertain how often the viral infection has been cleared rather than remaining either latent or at extremely low levels of persistence. In addition, viral clearance is associated with regression of the lesions.

The classification of the cytological and histological changes that precede invasive cervical cancer has changed over time as understanding of the link between HPV infection and the appearance of lesions has improved. In 2001, the Bethesda cytological classification was revised. The dichotomous division of squamous intraepithelial lesions into low-grade and high-grade is based on virological, molecular and clinical observations. The term low-grade more frequently reflects a transient HPV infection, whereas the term high-grade implies a lesion in which high-risk HPVs are present. The terms cervical intraepithelial neoplasia 1, 2 and 3 are used for cytological classification in some European countries, but are generally used for histological classification. Grade 3 cervical intraepithelial neoplasia, which subsumes the diagnoses of severe dysplasia and carcinoma *in situ*, has been proposed as the proximal precursor to invasive cervical cancer. In addition to cervical intraepithelial neoplasia, HPVs cause similar lesions in glandular cervical epithelium, as well as in the epithelia of the vulva, vagina, anus, penis and some other non-genital sites. Benign lesions are also found in a variety of genital and non-genital sites.

The treatment of benign genital lesions may be accomplished using cytotoxic treatments, e.g. with trichloroacetic acid, podophyllin, podofilox, 5-fluorouracil; immunomodulation with agents such as imiquimod or interferons; or physical ablation, e.g. with a laser, through excision by surgery or with laser electrocautery excision. Numerous efforts are under way to develop therapeutic vaccines that could be used as adjuvant therapies. These approaches attempt to stimulate powerful cell-mediated immune responses and most

frequently target the viral E6 and/or E7 proteins. While some preliminary studies provide evidence of immunological and, in some cases, clinical responses, no strategy has yet been tested in large, blinded trials. In contrast, the realisation of a prophylactic vaccination to prevent HPV infection has been accomplished. Three trials have provided proof of the principle that vaccination with virus-like particles can elicit strong antibody responses that seemingly protect against genital infection and clinical disease in a type-specific manner. The next decade should determine whether durable protection against HPV infection can be achieved, and large randomized controlled trials should provide evidence of a reduction in HPV-induced neoplasia.

5.2 Human carcinogenicity data

Cancer of the cervix

The evidence evaluated for determination of the carcinogenic potential of individual HPV types derived primarily from three lines of epidemiological data that include results predominantly from HPV type-specific case–control studies, as well as prospective cohort studies and case series from five continents. Traditionally, prospective cohort studies are considered to provide the highest level of evidence; however, in the case of HPV, they have limitations because of the small number of cancer end-points and the need to focus on the surrogate end-point of grade 3 cervical intraepithelial neoplasia. For this reason, results from case–control studies are emphasized because of the far larger number of invasive cancers that have been evaluated. Virtually all cervical cancers contained HPV DNA. The large and comprehensive case series permitted consideration of the relative frequency of different HPV types across cervical lesions of increasing severity. Finally, the classification of risk for HPV types derived from epidemiological evidence was considered in the light of phylogenetic classifications.

Strong epidemiological evidence confirmed the previous evaluation that HPV types 16 and 18 are carcinogenic to humans. The evidence for carcinogenicity was strongest for HPV 16. In addition, a convincing association, mainly from case–control studies, was found for HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66. HPV types 26, 68, 73 and 82 were associated with cervical cancer in some case–control studies but were rarely found in case series and were not associated with an increase in risk in prospective studies; overall, the epidemiological data for these types were not considered to show a consistent association. Despite a large amount of HPV type-specific data that failed to demonstrate an association, HPV 6 and 11 have been reported in very rare cases, but they did not contribute meaningfully to the burden of cervical cancer. Fewer data were available for other HPV types.

Anogenital cancers other than those of the cervix

Cancer of the vulva

Vulvar cancers include basaloid, warty and squamous-cell cancers. Case series showed that the prevalence of HPV DNA in vulvar cancers was higher in basaloid and warty tumours (> 50% in most studies) and lower in keratinizing squamous-cell carcinomas (approximately 5% in most studies). Seroepidemiological studies were consistent with HPV DNA studies and supported a causal role for HPV 16 in the pathogenesis of these subsets of vulvar cancers. The evidence for HPV 18 was less strong, but was suggestive. Studies of the carcinogenicity of HPV 6, 11, 31 or 33 in basaloid and warty tumours of the vulva were limited by the small number of tumours reported. Most verrucous carcinomas were not associated with HPV. However, HPV 6 or 11 have consistently been detected in the rare verrucous carcinoma that arises from Buschke-Löwenstein tumours.

Cancer of the vagina

HPV DNA was detected in a high proportion of vaginal cancers (> 50% in most studies), particularly that of HPV 16. Combined with the seroepidemiological data, the studies of vaginal cancer consistently showed a strong association with HPV 16 and a less consistent association with HPV 18.

Cancer of the penis

Similarly to vulvar cancers, penile cancers include basaloid, warty and keratinizing squamous-cell cancers. Case series showed that the prevalence of HPV DNA in penile cancers was higher in basaloid and warty tumours (> 50% in most studies) and lower in keratinizing squamous-cell carcinomas. Studies of the carcinogenicity of HPV 6, 11, 31 or 33 in penile cancer were limited by the small number of tumours reported. However, HPV 6 or 11 have consistently been detected in the rare verrucous carcinoma that arises from Buschke-Löwenstein tumours.

Cancer of the anus

As in vaginal and cervical cancers, HPV DNA was detected in a high proportion of anal cancers using polymerase chain reaction (> 50% in most studies), particularly that of HPV 16. The data on HPV DNA prevalence combined with the seroepidemiological data on anal cancer indicated that there was a consistent association with HPV 16 and a less consistent association with HPV 18. Studies of the carcinogenicity of HPV 6, 11, 31 or 33 in anal cancer were limited by the small number of tumours reported. However, HPV 6 or 11 have consistently been detected in the rare verrucous carcinoma that arises from Buschke-Löwenstein tumours.

Cancers of the aerodigestive tract

Cancer of the oral cavity

HPV DNA was detected in a limited number of cancers of the oral cavity (tongue, floor of the mouth, gum, palate and other sites of the mouth). The range of detection was wide with an estimated average of approximately 25%. HPV 16 was detected in about 70% of HPV-positive cases and HPV 18 in a smaller fraction. Several studies that compared tumours with normal tissue detected HPV DNA more frequently in the tumours. Several seroepidemiological case–control studies and one prospective study indicated an increase in risk for oral cavity cancer associated with HPV 16.

Cancer of the oropharynx

HPV DNA was consistently detected in a substantial fraction of cancers of the oropharynx with an estimated average prevalence of 35%. HPV 16 DNA was detected in approximately 80% of HPV-positive cases. Several studies that compared tumours with normal tissue revealed large differences in HPV DNA detection, particularly that of HPV 16 in cancer of the tonsil. Seroepidemiological case–control studies and one prospective study showed marked increases in risk associated with serological markers of expression of HPV. These associations were much stronger than those observed for cancer of the oral cavity.

Cancer of the oesophagus

Some studies detected HPV DNA in cancers of the oesophagus, but others reported negative results. Seroepidemiological studies also gave contradictory results, although some prospective studies showed a positive association.

Cancer of the larynx

HPV DNA was detected in a variable fraction of cancers of the larynx. Limited and contradictory data resulted from comparisons of tumours and normal tissue. Some cross-sectional and prospective seroepidemiological data suggested a modest association with HPV 16 and 18. In patients with recurrent papillomatosis, some well documented reports pointed to an involvement of HPV 6 and 11.

Cancer of the skin and conjunctiva

The prevalence of HPV DNA was high in squamous-cell skin carcinomas, especially among immunosuppressed patients. HPV DNA was, however, frequently detected at very low copy number in normal skin specimens from the general population and immunosuppressed patients. Case–control studies consistently showed an association between HPV types of genus beta, also known as epidermodysplasia verruciformis-associated HPV, and squamous-cell carcinoma of the skin. Some HPV types from the genus beta were considered to be carcinogenic in patients who have epidermodysplasia verruciformis.

HPV 16 DNA was highly prevalent in rare squamous-cell carcinomas of periungual skin, which provides evidence for a carcinogenic role of HPV in these tumours.

The presence of HPV in some conjunctival squamous-cell carcinomas and the results of a small case–control study from Uganda suggested an association between HPV and these carcinomas.

The Working Group recognized that assays and sampling methods that can detect epidermodysplasia verruciformis-related and cutaneous HPV types are still in an early phase of development; this prevented more detailed conclusions on the role of individual HPV types in non-melanoma skin cancers.

Cancer at other sites

High- and low-risk types of HPV were reported, mainly in a number of case reports, in cancers of other organs including the colon, ovary, breast, prostate, urinary bladder and nasal and sinonasal cavity. The significance of these findings is questionable, since other studies that analysed cancers of the same sites failed to confirm these data. Data on the relationship of HPV with lung cancer were also equivocal, although it was noted that, in the setting of recurrent papillomatosis, a small number of case series consistently reported detection of HPV 11 DNA. At present, there is a lack of evidence from case–control studies to support a possible involvement of HPV infections in tumours of these organs.

Co-factors

Tobacco smoking

Regardless of differences in the prevalence of current and past use of tobacco across the many studies and populations evaluated, tobacco smoking was consistently associated with risk for stage 3 cervical intraepithelial neoplasia and invasive squamous-cell carcinoma of the cervix. The risk estimates for this association were consistently in the range of 2.0 regardless of the study design (retrospective versus prospective), the restriction criteria employed (any HPV type-positive versus high-risk HPV-positive) and the covariates included in the model. Concordant with the vast amount of evidence that indicates that tobacco constituents are carcinogens and that smoking reduces immunological function, these data demonstrated that smoking is a co-factor with HPV in the development of invasive cervical cancer.

Hormonal contraceptives

The association between the use of oral contraceptives and squamous-cell cancer of the cervix was complicated by the different formulations used across geographical regions and changes in the formulations that have occurred within populations over time. In addition, it may not be possible to account adequately for the effect of Papanicolaou test smear screening and the consequent bias from the detection of lesions on the estimates of the association between the use of oral contraceptives and cervical cancer.

It was not possible to assess the strength of the association between the use of other hormonal contraceptive formulations such as injectable contraceptives and Norplant and the risk for cervical intraepithelial neoplasia and cancer. More research is needed to evaluate

adequately the risk associated with these commonly used formulations in developed and developing countries.

Parity

The data that support an association between parity (three or more children) and increased risk for cervical cancer were consistent.

Nutrients

Although numerous studies to assess the association between nutrient status and cervical disease have been carried out in the past few decades, very few restricted their analyses to HPV-positive women and most had small sample sizes. Therefore, the evidence in support of an association is too limited to draw conclusions for any one nutrient in the circulation or in the diet.

Infectious co-factors

Other than the human immunodeficiency virus (see below), *Chlamydia trachomatis* was the co-infection most consistently reported to have an epidemiological association with cervical neoplasia and invasive cervical cancer, after statistical control for the effects of HPV infection. The actual point(s) in the multistage process of tumorigenesis that might be affected by *C. trachomatis* remains uncertain. As residual confounding by HPV (due to shared sexual risk factors) is a potential source of bias, the possible association between *C. trachomatis* and HPV-associated cervical tumorigenesis must be viewed as an intriguing but unproven relationship. There was less consistent epidemiological evidence supporting a role of other sexually transmitted infections in cervical cancer, including herpes simplex virus 2. In contrast, cervical inflammation in general might play a role in HPV infection and cervical cancer, a possibility that could explain in part some of the conflicting findings reported by epidemiological studies conducted on an agent-specific basis and requires further investigation.

Human immunodeficiency virus (HIV)

A large number of studies consistently demonstrated an association between cervical HPV infection and HIV infection and between cervical intraepithelial neoplasia and HIV infection. A high proportion of HIV-positive women were infected with multiple HPV types and cervical HPV infection was more likely to persist in HIV-positive women than in HIV-negative women. Similarly, there was a strong association between anal HPV infection and HIV infection and between anal intraepithelial neoplasia and HIV infection in both men and women. The prevalence of anal HPV infection was very high in HIV-positive men who had sex with men and approached 100% in some studies. The prevalence of anal HPV infection exceeded that of cervical HPV infection in studies of HIV-positive women and HIV-negative women at high risk for HIV infection.

Studies of the effect of highly active antiretroviral therapy on the natural history of cervical HPV infection and cervical intraepithelial neoplasia used a wide variety of

measures of outcome and methodologies, which rendered definitive conclusions difficult. Some studies showed no effect of this therapy on the natural history of cervical intraepithelial neoplasia while others showed a modest reduction in its incidence and progression and an increase in its regression. This therapy appeared to have little or no effect on the natural history of anal HPV infection and anal intraepithelial neoplasia.

Data from cancer and acquired immune deficiency syndrome registry matches from developed countries consistently showed an increase in the incidence of cervical and anal cancer as well as that of vulvar, vaginal, penile and non-melanoma skin cancers in HIV-positive individuals compared with the general population. The degree to which the incidence of cervical cancer is increased among HIV-positive women in developing countries (i.e. Africa) was not as clear. No data were reported on the prevalence of different HPV types in invasive cancers in HIV-positive individuals. It is therefore not known whether this differs from those in HIV-negative individuals. Similar to the limited beneficial effect of highly active antiretroviral therapy on cervical and anal intraepithelial neoplasia, emerging data suggest that this therapy has not led to a reduction in the incidence of HPV-associated cervical and anogenital cancers.

5.3 Animal carcinogenicity data

Several animal papillomaviruses are carcinogenic in their natural hosts. Malignancies include cancer of the genital tract in monkeys, cancer of the upper gastrointestinal tract and urinary bladder in cattle, skin cancer in rabbits, dogs and rodents, cancer of the oral cavity in dogs, cancer of the vulva and perianal region in sheep and sarcoids in equids.

The rhesus monkey papillomavirus 1 is the most relevant to high-risk alpha-HPV: it infects the genital mucosa, is sexually transmitted and induces lesions that can progress to squamous-cell carcinomas.

Among the various bovine papillomaviruses (BPV), BPV-2 and BPV-4 are associated with cancer. Co-factors in pasture bracken fern that have been identified as chemical mutagens and immunosuppressants are prerequisite for the occurrence of cancer. BPV-2 has consistently been associated with cancer of the urinary bladder, and the E5 protein, which has strong transforming activity, is expressed in cancer cells.

BPV-4 causes papillomas of the upper gastrointestinal tract that can develop into carcinomas. Viral DNA is gradually lost during carcinogenesis and alterations in cellular proto-oncogenes (*ras* and *EGF-R*) and tumour-suppressor genes (*p53*) accumulate during progression.

Infection of equids as the heterologous host by BPV-1, and occasionally BPV-2, causes aggressive and persistent sarcoids. Viral DNA is present in the tumour and the E5 oncoprotein is consistently expressed. BPV infection of equids is the only documented case of natural cross-species infection by a papillomavirus.

Cross-species transmission also takes place between cottontail rabbits (*Sylvilagus floridanus*) infected with cottontail rabbit papillomavirus (CRPV) and domestic rabbits (*Oryctalagus cunniculus*), but only when the two species are housed together.

CRPV-induced papillomas progress to carcinomas at a much higher frequency in domestic rabbits than in cottontail rabbits, which implicates the genetic background of the host in the neoplastic process. Increased malignant progression of persistent warts is linked to alleles within the hypervariable region of class II DQ alpha genes.

The transforming proteins SE6, LE6 and E7 of CRPV are consistently expressed in all cancers. Experiments on overexpression in rodent cells also determined a transforming role for E8, which may represent an orthologue of the HPV E5 protein. Most genes, except for *E4* and *E5*, are necessary for the induction of papillomas in domestic rabbits. Single amino acid mutations in the context of the full genome within the trans-activation domain of E2, that maintain the replication function of E2, caused a dramatic loss in the efficiency of the induction of papillomas and carcinomas.

BPV-associated carcinogenesis has highlighted the interaction between the virus and environmental co-factors, and the functional analysis of BPV-transforming proteins has helped elucidate the proteins of HPVs. CRPV-associated carcinogenesis has features that may be relevant to HPV-associated non-melanoma skin cancer in humans.

In addition, animal papillomaviruses are highly valuable for the study of the interaction between the virus and the host immune system. BPV, CRPV and canine oral papillomavirus have been used extensively in vaccination experiments that first proved that prophylactic vaccination with capsid proteins or virus-like particles prevents infection. The minor capsid protein L2 of BPV and CRPV presents a virus neutralization epitope which is common to the L2 proteins of many papillomavirus types. This raises the possibility of developing polyvalent vaccines against HPV.

The observation in different animal papillomavirus models that therapeutic vaccination with viral early proteins can induce tumour regression is of major importance for the development of therapies against HPV-induced disease.

5.4 Other relevant data

Studies have been designed to determine how HPVs cause cancer. One hallmark of HPV-associated cancers is the frequent integration of the viral genome into random sites within the human genome, which leads to a clonal selection of cells in which HPV oncogenes E6 and E7 are up-regulated. HPVs play an active role in cervical cancer, because the expression of E6 and E7 is required for the continued growth and tumorigenicity of cervical cancer-derived cell lines. Another hallmark of cervical cancers is the accumulation of specific genetic alterations, most notably on chromosomes 1, 3 and 5, that are predicted to contribute to carcinogenesis. Their accumulation is probably the direct consequence of the expression of HPV oncoproteins that induce genomic instability through the dysregulation of cell-cycle regulatory machinery during G2/M and/or induction of centrasome abnormalities.

HPVs do not infect animals. Therefore, other experimental systems, including biochemical and tissue culture assays and transgenic animal-based models, have been used to determine the biological properties of HPVs that relate to their carcinogenic potential.

Relevant biochemical properties of the HPV-encoded oncoproteins E5, E6 and E7 include the inactivation of tumour suppressors, the modulation of cell-cycle regulatory, DNA-repair and apoptotic processes, the de-regulation of gene expression and the activation of signal transduction pathways. Properties bestowed on cells in tissue culture by HPVs that relate to their carcinogenicity include immortalization and genomic instability, as well as alterations in cell proliferation, differentiation, responses to DNA damage and apoptosis. In transgenic mice, HPV oncogenes, either individually or together, can induce cancers of the skin and cervix, two sites at which HPVs are implicated in human cancer. Good correlations have been found between the biochemical activities of the individual viral gene products that contribute to the formation of cancer in these animal model systems and those that contribute to their tumorigenic potential in tissue culture. Of particular importance are the interaction of E7 with the tumour-suppressor proteins pRb, p21 and p27, the interaction of E6 with p53, Bak and PDZ domain proteins, the ability of E6 to activate telomerase and the ability of E5 to stimulate the activity of the growth factor receptor.

Many of the experimental studies to date have focused on the analysis of high-risk HPVs, particularly HPV 16. There is a strong correlation between the tumorigenic activity of these high-risk HPVs in experimental systems and their carcinogenic activity in humans. Experimental studies carried out on other HPVs provide valuable insight into their carcinogenic potential. Of particular importance are studies of epidermodysplasia verruciformis-associated cutaneous HPVs, such as HPV 8 and HPV 38, that suggest that they play a role in human cancer.

The immunobiology of HPV infections, the role of the humoral and cellular components of the immune system in the control of these infections and HPV-associated cancers and the modulating effect of HPVs on the immune system were also assessed. Studies in these areas provide evidence that the immune system can respond to and may provide control over HPV infections and perhaps also HPV-associated cancer, and that HPVs in turn have the potential capacity to modulate the immune system at multiple levels.

A role of the human immune system in HPV infections is demonstrated by the fact that inflammation is commonly observed in regressing lesions, that immunosuppressed patients are at an increased risk for HPV infections and associated neoplasia and that HPV lesions respond positively to non-specific immune modulation.

With regard to specific cellular immune responses to HPVs, $CD4^+$ T-helper and $CD8^+$ cytotoxic T-lymphocyte immune responses against viral proteins can be induced in animal models. Moreover, in women infected with high-risk HPVs, cytotoxic T-lymphocyte responses to E7 can be detected in tumour infiltrates. Responses of these lymphocytes to HPV 16 E6 also are detected in women who have HPV 16-positive premalignant lesions. In contrast, a lack of cytotoxic T-lymphocyte responses to HPV 16 E6 was noted in women who had persistent HPV 16 infections. The specific role of cytotoxic T-lymphocytes in mediating the regression of HPV-associated lesions remains unclear.

With regard to immunomodulation by HPVs, tissue culture-based studies suggest that E5 and E7 oncoproteins of high-risk HPVs can modulate the cell-surface levels of major histocompatibility complex class I and class II molecules and inhibit the function of transporters associated with antigen presentation, respectively. In human cervical cancers, major histocompatibility complex class I is down-regulated whereas class II is up-regulated. In addition, the E6 proteins of both high- and low-risk HPVs and the E7 protein of high-risk HPVs can modulate the activity of several factors that regulate interferon-responsive pathways, which mediate the innate immune response and modulate antigen-specific responses. Furthermore, the risk for cervical cancer could be affected by genetic polymorphisms in the major histocompatibility complex class I and II genes.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of HPV 16 in the cervix, vulva (basaloid and warty tumours), vagina, penis (basaloid and warty tumours), anus, oral cavity and oropharynx.

There is *sufficient evidence* in humans for the carcinogenicity of HPV 18 in the cervix.

There is *sufficient evidence* in humans for the carcinogenicity of HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 in the cervix.

There is *limited evidence* in humans for the carcinogenicity of HPV 16 in the larynx and periungual skin (squamous-cell carcinoma).

There is *limited evidence* in humans for the carcinogenicity of HPV 18 in the vulva (basaloid and warty tumours), vagina, penis (basaloid and warty tumours), anus, oral cavity and larynx.

There is *limited evidence* in humans for the carcinogenicity of HPV 6 and HPV 11 in the larynx (squamous-cell carcinoma) and in the vulva, penis and anus (verrucous carcinomas of the latter three sites).

There is *limited evidence* in humans for the carcinogenicity of HPV genus-beta types in the skin (squamous-cell carcinoma). In the rare case of patients with epidermodysplasia verruciformis, there is compelling evidence for the carcinogenicity of HPV genus-beta types 5 and 8 in the skin (squamous-cell carcinoma).

There is *limited evidence* in humans for the carcinogenicity of HPV in the conjunctiva (squamous-cell carcinoma).

There is *inadequate evidence* in humans for the carcinogenicity of HPV in the oesophagus, lung, colon, ovary, breast, prostate, urinary bladder and nasal and sinonasal cavities.

Overall evaluation

HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 are *carcinogenic to humans (Group 1)*.

HPV 6 and HPV 11 are *possibly carcinogenic to humans (Group 2B)*.

Some types of HPV genus beta are *possibly carcinogenic to humans (Group 2B)*, with the notable exception that HPV 5 and HPV 8 are carcinogenic to patients with epidermodysplasia verruciformis.

Evaluations in the *IARC Monographs* provide a qualitative assessment of carcinogenicity. The HPV types that have been classified as *carcinogenic to humans* can differ by an order of magnitude in risk for cervical cancer. The Working Group cautions that the design of HPV screening tests must also consider other factors that are discussed in the General Remarks.