

FDA Briefing Document # 2

Preventive Human Papillomavirus (HPV) Vaccines – Background Information

Vaccines and Related Biological Products Advisory Committee

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Karen L. Goldenthal, M.D.
Douglas R. Pratt, M.D., M.P.H.

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Abbreviations used in this document:

- ❑ ADC = Adenocarcinoma. See Appendix B.
- ❑ AIS = Adenocarcinoma *in situ*.
- ❑ ASCUS = Atypical Squamous Cells of Undetermined Significance. A recommendation from The Bethesda 2001 Workshop, May 2001, is to replace the term ASCUS with ASC (Atypical Squamous Cells). See Appendix C.
- ❑ AGUS (or AGCUS) = Atypical Glandular Cells of Undetermined Significance. A recommendation from The Bethesda 2001 Workshop is to replace the term AGUS with AGC (Atypical Glandular Cells). See Appendix C.
- ❑ CIS = carcinoma *in situ*. See Appendix C.
- ❑ CIN = Cervical Intraepithelial Neoplasia (CIN) [histology]. See Appendix C.
- ❑ ECC = Endocervical Curettage.
- ❑ HPV = Human Papillomavirus.
- ❑ HSIL = High Grade Squamous Intraepithelial Lesion. See Appendix C.
- ❑ ICC = Invasive Cervical Cancer. See Appendix A, B and C.
- ❑ LEEP = Loop Electrosurgical Excision Procedure (LEEP).
- ❑ LSIL = Low Grade Squamous Intraepithelial Lesion. See Appendix C.
- ❑ SCC = Squamous Cell Carcinoma. See Appendix B and C.
- ❑ SIL = Squamous Intraepithelial Lesion.
- ❑ TBS = The Bethesda System (cytology).

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Introduction

Human papillomavirus (HPV) has been associated with a number of cancers and clinically important diseases. Most of the preventive HPV vaccine development effort has been directed at cervical cancer and precursor lesions, which will be the major focus of this briefing document. Cervical cancer is a very significant problem for both developed and developing countries, as will be described in detail below.

In addition, HPV has been associated with other diseases that will be described toward the end of this document (See Section entitled Other HPV Associated Diseases). So-called “oncogenic” or “high risk” HPV types, e.g., HPV type 16, have been associated with other cancers, including anal cancer. “Low risk” HPV types, especially 6 and 11, which have been linked with condylomata acuminata (genital warts), are also the targets of preventive vaccine development. Also, HPV has been linked with a relatively uncommon, but life-threatening and often difficult to treat, pediatric disease known as recurrent respiratory papillomatosis.

This document is primarily intended to provide a literature summary of information relevant to cervical pre-neoplastic disease, cervical cancer, and HPV, with some focus on recent information. This document can be viewed as background information for a separate document that addresses options regarding choice of efficacy endpoints¹.

Cervical Cancer: Incidence and Mortality

In the 1930s, cervical cancer was the most common cause of cancer deaths in U.S. women. The introduction of the Papanicolaou (Pap) smear, however, made early detection and treatment of preinvasive disease possible. Incidence and mortality rates for cervical cancer in the U.S. have declined dramatically during the remainder of the 20th century. An estimated 12,900 new cases of cervical cancer and 4,400 deaths due to cervical cancer have been projected for the U.S. for 2001 (Janicek MF & Averette HE, 2001; Greenlee RT et al., 2001). The Surveillance, Epidemiology, and End Results (SEER) data from NCI indicate that a woman's lifetime risk of being diagnosed with cervical cancer in the United States is currently about 0.85 percent, and the risk of dying from the disease is 0.30 percent (Ries LAG et al., 2000). Additional information on the incidence and mortality for cervical cancer in the U.S. can be found in **Appendix A**. (Ries LAG et al., 2000). Although cervical cancer can occur over a broad age range, the mean age at diagnosis is about 52 years (Plaxe SC et al., 1999).

The effects of Pap smear screening have been well documented. It is estimated that cervical cytology screening for women between the ages of 20 and 75 years at least every 3 years decreases the incidence and mortality rate of invasive cervical cancer by about 90% (Eddy, 1990). An estimated 50 million Pap smears are performed per year in the U.S. It is important to remember that a Pap smear is a screening tool that does not render a formal diagnosis of a cervical epithelial abnormality. Histologic diagnosis

¹ That document is entitled: “FDA Regulatory Briefing Document on Endpoints.”

(e.g., from a colposcopically directed biopsy) is required to make a definitive diagnosis.

Although the incidence of cervical cancer has fallen dramatically in the US, it has been noted that the favorable trend has slowed somewhat since the mid-1990s (Janicek MF & Averette HE, 2001; Ries LAG et al., 2000). Of interest, in Finland, between 1991 and 1995, there has been a 60% increase in the incidence of cervical cancer among women <55 years of age. However, the mortality rates in Finland are still decreasing (Dillner J, 2000; Anttila et al., 1999). Also, in Finland, there has been a substantial increase of both moderate dysplasia and severe dysplasia in each successive 5-year-period over the time period 1976–1995 (Anttila et al., 1999).

Worldwide, there are an estimated 400,000-500,000 cases of cervical cancer per year. (CDC, 1999 [review]). Overall, cancer of the cervix is the 3rd most common cancer in women (after breast and colon/rectum). In developing and developed countries, it is the 2nd and 6th most common cancer in women, respectively (Parkin D et al., 1999 [data from 1990]). This geographical disparity between developed and developing countries is thought to be related, in large part, to the absence of effective screening programs in the developing countries. Also, cervical cancer is the leading cause of cancer mortality in women in developing countries, and ranks 7th in developed countries. Cervical cancer causes about 190,000 deaths per year; 78% of this cancer-related mortality occurs in developing countries (Pisani P et al., 1999 [data from 1990]).

Incidence of SCC² versus Adenocarcinoma of the cervix (See Appendix B):

- ✓ Cytology screening appears to have had far less impact on the incidence of adenocarcinoma compared to SCC of the cervix (Smith HO et al., 2000).
- ✓ The relative proportion and absolute incidence of adenocarcinoma and SCC of the cervix have changed in the U.S. and Western Europe, over the past 40 years (Smith HO et al., 2000).
- ✓ In the U.S., adenocarcinomas now represent about 20 to 25% of cervical carcinomas (Smith HO et al., 2000), whereas from 1950 to 1960, they represented only about 5% of cases (Hepler TK et al., 1952; Mikuta JJ et al., 1969).
- ✓ In the U.S., review of the SEER database has shown a clear decrease in the incidence of SCC, and invasive cervical cancer (ICC)³ overall; however, there is an increase in the rate of adenocarcinoma, per **Appendix B** (Smith HO et al., 2000).
- ✓ Also, data from Scandinavia have shown an increase in the proportion of adenocarcinomas relative to SCC, and an absolute decline in the incidence of invasive cervical cancer (ICC) overall and SCC over the recent decades:
 - In Finland, the incidence of adenocarcinoma has increased slightly, while total ICC & SCC declined, from 1955-95, per **Appendix B** (Anttila A et al., 1999).
 - In Sweden, the incidence of adenocarcinoma increased ~ 2-fold between 1958-95, while total ICC & SCC declined, per **Appendix B** (Bergstrom R et al., 1999).
 - In Norway, an increase in adenocarcinoma (1970-84) has been reported, while total ICC & SCC declined (Eide TJ, 1987).

² SCC = Squamous Cell Carcinoma.

³ ICC = Invasive Cervical Cancer.

Overview of Risk Factors Associated with Cervical Cancer

There is a strong association between HPV infection and cervical neoplasia, as will be discussed here and throughout this document. Recently, a systematic review and meta-analysis was published of longitudinal studies on HPV associated relative risk (RR) for and population attributable fraction (PAR%) of HPV16 in cervical neoplasia. (Lehtinen M et al., 2001). The overall findings from the Lehtinen et al., 2001, publication are presented here:

- HPV (“High Risk” types) associated RR for invasive cervical carcinoma (ICC) and carcinoma *in situ* (CIS) of the cervix, **in PCR-based studies, was 17 (95% CI: 8.2-33)**.
- HPV16 associated RRs in **seroepidemiological studies** were 3.3 (95% CI 2.2-4.9) for the unselected population (HPV16 seroprevalence 11.0%), and 12.5 (95% CI 5.5-29) for a population with low HPV16 seroprevalence of 5.3%. Corresponding PAR% estimates of HPV16 based on seroepidemiology studies were 27 and 44%, respectively.

However, the difference between HPV prevalence and the incidence of cervical neoplasia suggests that other cofactors or host characteristics are necessary for the development and progression of the disease.

Cervical cancer has been observed for many decades to have epidemiological features of a sexually transmitted disease. Young age at first intercourse, high number of sexual partners, high parity, cigarette smoking, race, and low socioeconomic status have been reported as significant risk factors for cervical cancer (Janicek MF & Averette HE, 2001).

Studies have been done to examine whether HPV exposure accounts for any, or all, of the link with sexual history. One such study was a population based case-control study performed in Västerbotten County, Sweden (1993-95) to elucidate whether HPV exposure or persistence explains the sexual history-related risk of high-grade cervical intraepithelial neoplasia (CIN 2/3) (Kjellberg L et al., 1999). The subjects included 254 women referred to colposcopy because of an abnormal cervical smear and 320 age-matched women from the general population. The women were interviewed for sexual history, and tested for presence of serum antibodies to HPV types 16, 18 and 33 as well as for the presence of HPV DNA in cervical brush samples.

In this study, for women with 5 or more lifetime partners (N=164), the follow odds ratios (ORs) were obtained:

- The unadjusted OR for CIN 2/3 was 4.4 (95% CI: 1.6 – 15.1).
- The OR for CIN 2/3, adjusted for HPV DNA, was 1.7 (95% CI: 0.4-9.6).
- The OR for CIN 2/3, adjusted for antibodies to HPV types 16, 18 and 33, was 3.1 (95% CI: 1.1-10.6).

Other information on cervical cancer risk in relation to partners is summarized here.

- Subsequent wives of men whose first wife died from cervical cancer are found to be at increased risk for cervical cancer (Keefe KA et al., 2000).
- In Thailand, the risk of invasive squamous cell cervical carcinoma among married women who gave a history of a single sexual partner was assessed. Increased risk was associated with the number of visits to prostitutes per year that the husband had, when he was in his teens and twenties, but not at a later age, and was also associated with the husband using condoms less than 10 percent of the time at those ages (Thomas DB et al. [III.], 2001; Thomas DB et al., 1996).

Immune status appears to represent one important host parameter, with impaired cellular immune function being associated with increased risk in some settings (Lowy DR et al., 2001).

- Cervical squamous intraepithelial lesions (SIL) are more prevalent in HIV-infected women than in women without HIV infection (Duerr A et al., 2001). Recent evidence from linkage studies in the United States and Italy have shown clearly increased rates of cervical cancer in women with HIV (Goedert JJ et al., 1998; Franceschi S et al., 1999).
- Renal transplant recipients are at an increased risk for cervical cancer compared to age matched controls (Porreco R et al., 1975). ICCs also occur at a younger age in these patients compared to the general population (Porreco R et al., 1975; Penn I, 1986).

There are epidemiological data to indicate that *Chlamydia trachomatis* could be an independent risk factor for cervical cancer. To study the possible association of *C. trachomatis* infection as a risk factor for cervical SCC (squamous cell carcinoma), a longitudinal, nested case-control study (3 controls per case) was performed within a cohort of 530,000 women who provided samples to serum banks in Finland, Norway, and Sweden. The data files were linked to respective national cancer registries. In this study, 128 women developed invasive cervical SCC \geq 12 months following serum donation. Of the ten *C. trachomatis* serotypes evaluated by IgG antibodies, serotype G was most strongly associated with SCC [adjusted OR 6.6; (95% CI, 1.6-27.0; adjusted for serum antibodies to HPV 16, 18, 33 & serum cotinine)]. The finding of >1 serotype of *C. trachomatis* also increased the risk for cervical SCC (P<0.001 for trend) (Anttila T et al., 2001).

[Virology of HPV \(See Appendix D\)](#)

Papillomaviruses are epitheliotropic, non-enveloped DNA viruses. Their ~ 8-kb closed, circular, double-stranded DNA genome is surrounded by a 55 nm icosohedral capsid (McLachlin CM et al., 2000). The ORFs (Open Reading Frames) are divided into “early” (E1–E7) and “late” (L1 and L2) regions. The genomic map of HPV 16 is shown in **Appendix D**. The L1 and L2 ORFs encode the major and minor capsid proteins, respectively.

More than 100 HPV types have been detected. Of these, > 80 HPV types have DNA genomes that have been well characterized by sequencing (CDC, 1999 [review]). The more common types of relevance to this document and their disease association are summarized in the Table, below.

Table 1. Genital HPVs. (Modified From Table 20.1, McLachlin CM and Crum CP, 2000)

*Low-risk HPVs	
HPV 6	= Most common HPV type associated with exophytic genital warts (benign); most common in vulvar condylomata, and uncommon in cervical exophytic condylomata.
HPV 11	= Second most commonly associated with exophytic warts. Less common in the cervix.
HPV 42	= Associated with genital warts.
*High risk HPVs	
HPV 16	= Most common cervical HPV, associated strongly with high grade CIN & cervical squamous carcinoma. Associated with some cervical adenocarcinomas.
HPV 18	= Associated principally with cervical adenocarcinomas, adenocarcinomas <i>in situ</i> (AIS), and small cell undifferentiated carcinomas. Also associated with CIN.
HPV 31, 33, 35, 39, 45, 51	= Additional types associated with cervical squamous precursors and invasive cancers, less common than HPV 16. Associated with high grade CIN, but less so than HPV16.
HPV 52, 56, and others	= Occasionally associated with cervical carcinoma.

**Note: This table displays information on both "low risk" and "high risk" types. However, this VRBPAC meeting is focused primarily on "oncogenic" or "high risk" types, especially HPV types 16 and 18.*

The proposed mechanism of action of "high risk" viruses from the Papovaviridae family (e.g., HPV types 16 and 18) is related to their production of oncogenic proteins, E6 and E7, that effectively bind and inactivate tumor suppressor gene products in cervical cells. Specifically, the E6 protein of oncogenic HPVs binds to the p53 tumor suppressor gene product and accelerates its degradation. Also, the E7 protein of oncogenic HPVs binds to the retinoblastoma protein (pRB), another tumor suppressor gene product, and to related proteins, and inhibits their functions. [In this regard, E7 proteins of high risk and low risk types differ in a number of biologic and biochemical properties. For example, E7 protein from HPV types 6 and 11 binds with pRB with ~10-fold less efficiency, compared to E7 protein from HPV types 16 and 18 (Howley et al., 2001).] Also, alteration or abrogation of E2 function through gene disruption or possibly mutation leads to up-regulation of expression of the E6 and E7 genes. Such events may lead to neoplastic transformation (Kubbutat MHG et al., 1996).

Of further interest is that the HPV type 16 E7 protein shares important amino acid sequence likeness with portions of the Ad (adenovirus) E1A proteins and the SV40 large TAg. These conserved regions are essential for the transforming activities of these 3 oncoproteins (Howley PM et al., 2001).

HPV DNA has been found to be integrated, as a partially deleted viral genome, into host chromosomal DNA in many high-grade dysplastic and most invasive cancer cervical (ICC) specimens. When integration of partially deleted viral genome occurs, the HPV E6 and E7 viral oncogenes are preferentially retained and expressed (Lowy DR et al., 2001). HPV DNA integration does not occur in most low-grade cervical lesions. For the latter, the HPV DNA tends to exist in an unintegrated, circular episomal form. Although genomic integration is usually considered to be an important step in malignant transformation, it may not be essential (Keefe KA et al., 2000). The HPV DNA remains episomal in some ICCs associated with HPV type 16 (Lowy DR et al., 2001).

Within the female genital tract, the cervix is the most important target area for infection with high risk HPV types. The squamocolumnar junction of the cervix has been identified as the site at most risk of neoplasia, as will be discussed later. It is thought that HPV infects the cervix or lower female genital tract through defects in the epithelium that expose the basal epithelial cells to virion particles. Specifically, infection most likely occurs via receptors in basal cells known as integrins (McLachlin CM et al., 2000). As the cells with the viral DNA mature and approach the upper layers of the epithelium, the virus replicates and assembles into virions. The mechanism of regulating the switch from HPV plasmid maintenance to vegetative viral DNA replication is unknown (Howley PM et al., 2001) (**See Appendix D**).

There is increasing evidence that intratypic sequence variation (which has been used to study the geographical spread of HPVs) may be important in determining the risk of development of cervical neoplastic disease (Giannoudis A et al. 2001). [The classification of HPV types is based on nucleotide and amino acid sequence data. By definition, the nucleotide sequences of E6, E7 and L1 ORFs of a new type should be no more than 90% homologous to the corresponding sequences of known HPV types. HPVs have been further classified based on sequence similarity to the prototype: (i) “subtypes” (90-98% similarity), and (ii) “variants” (> 98% similarity). (Giannoudis A et al., 2001).]

Because of the difficulty in propagating HPVs *in vitro*, most clinical identifications have relied on methods that identify viral DNA or utilize molecular hybridization, rather than on virus isolation (Lowy et al., 2001).

HPV Antibody

The presence of serum antibodies to the HPV capsid has been proposed as an indicator of lifetime cumulative HPV exposure (Dillner J, 2000). The serum antibody response to the HPV particle is stable over time, and persists after clearance of HPV infection. Thus, it can provide a marker of cumulative HPV exposure that can be used to compare trends over time. Because most HPV infections are cleared spontaneously within weeks to months, many people testing negative for HPV DNA may have had a previous infection. In some subjects, antibody seroconversions can be delayed many months after the detection of viral DNA. The major isotypes of serum antibodies against HPV capsids are IgG1 and IgA. Other Ig G subclasses are only occasionally detected (Wang Z et al., 2000). IgG response may be more stable over time, compared to IgA.

HPV in ASCUS (ASC)⁴, LSIL⁵, HSIL⁶, AGUS (AGC)⁷ and CIN⁸

A number of studies addressing the association of HPV DNA with various cytology and histology findings, as well as longitudinal studies evaluating its association with the development of CIN 2/3 are summarized in this section. In brief, HPV DNA for “high risk” (or oncogenic) types was detected in about 80% and 51% of cervical specimens from women with LSIL and ASCUS, respectively, at baseline, in a recent study (ALTS), described below. Furthermore, in histology specimens, the detection of “high risk” HPV types increases as the CIN grade increases, e.g., 75% of CIN 3 specimens, as described in one study in this section. Also, the finding of DNA for HPV types 16 and 18 is associated with an increased risk for CIN 2/3 in longitudinal studies.

The ASCUS/LSIL Triage Study (ALTS) was a randomized, multicenter clinical trial in the U.S. of the management of women with low-grade and equivocal cervical cytology abnormalities. Within 6 months of an LSIL or ASCUS diagnosis (based on a Pap smear read by a community-based cytopathologist), women underwent a pelvic examination that included collection of cervical specimens for HPV DNA testing by Hybrid Capture II (HCII)[®] assay (detects 13 “oncogenic” HPV types including HPV 16 and 18). Some baseline data from this trial are available.

□ **LSIL** (ALTS Group, 2000):

- ✓ HPV DNA was detected in cervical samples from **79.6% (532 of 668)** by HCII among women referred with LSIL. 642 of 668 women had analyzable test results. Mean age was 25 years.
 - ◆ Cervical specimens from the 1st 210 women with LSIL randomly assigned to the HPV triage arm were also tested by PCR (MY09/MY11/HMB01).
 - ◆ 180/210 (85.7%) were positive by HCII, 180/210 (85.7%) were positive by PCR, and 171 (81.4%) were positive by both methods (total agreement of 91.4%).
 - ◆ In the PCR subset of 210: **24.8% HPV 16 positive; 11% HPV 18 positive.**
 - ◆ Of those with HPV DNA detected by PCR, 106/180 (58.9 %) had > 1 HPV type detected.

□ **ASCUS** (Solomon D et al., 2001):

- ✓ HPV DNA was detected in cervical samples from **50.6% (1766 of 3488)** by HCII among women referred with ASCUS. 3324 of 3488 women had analyzable test results (most common problem = insufficient residual specimen). (No PCR results with individual HPV types were presented).

⁴ ASCUS = Atypical Squamous Cells of Undetermined Significance. A recommendation from The Bethesda 2001 Workshop, May 2001, is to replace the term ASCUS with ASC (Atypical Squamous Cells). See Appendix C.

⁵ LSIL = Low Grade Squamous Intraepithelial Lesion. See Appendix C.

⁶ HSIL = High Grade Squamous Intraepithelial Lesion. See Appendix C.

⁷ AGUS (or AGCUS) = Atypical Glandular Cells of Undetermined Significance. A recommendation from The Bethesda 2001 Workshop is to replace the term AGUS with AGC (Atypical Glandular Cells). See Appendix C.

⁸ CIN = Cervical Intraepithelial Neoplasia (CIN) [histology]. See Appendix C.

Data on HPV testing of AGUS (AGC) are presented here:

- A total of 46,009 nonpregnant female members of the Kaiser Permanente Health Plan, Northern California Region, were studied prospectively in the Borderline Pap Study, described in detail later under the Work-up of ASCUS, etc. Section (Kinney WK et al., 1998). In that study, 137 cases of AGUS were identified. HPV DNA testing, performed with Hybrid Capture II, is provided here (Ronnett BM et al., 1999):
 - ✓ 39/137 (28%) of AGUS cases were HPV positive (no type specific data provided).
 - ◆ 89% (16/18) of those with HSIL or worse at work-up were HPV positive.
- Data on 23 consecutive cases (histology specimens) of adenocarcinoma *in situ* from several US & Polish sites, are presented here (Pirog EC et al, 2000).
 - ✓ All tested positive for ≥ 1 HPV type.
 - ✓ 43.4% (10/23) HPV Type 16 positive. (5/10 also positive for other HPV types.)
 - ✓ 26% (6/23) HPV Type 18 positive.

In France, a study was performed to investigate whether ploidy and oncogenic human papillomavirus types can be correlated with the histological grade of cervical intraepithelial neoplasia (CIN) in the same tissue sections (Monsonog J et al., 1997). At a colposcopy clinic in Paris, 340 new patients were seen between 11/93 and 2/94 for evaluation of "abnormal smears." 258 colposcopically directed biopsies & 82 cone biopsies were obtained. The study evaluated 292 squamous intraepithelial lesions (48 specimens without histological abnormality were excluded). Ploidy assessment (using an image analysis cytometer) and *in situ* hybridization for HPV were performed. Results are shown in the Tables, below.

Table 2^a Histological grade and aneuploidy^c

	N	Aneuploid	%	Chi Sq	P value
Flat condyloma	104	19	18		
CIN 1	51	14	27		
CIN 2	56	31	55		
CIN 3	81	76	94	109 ^b	<0.001
Low grade SIL	155	33	21		
High grade SIL	137	107	78	94.05	<0.001

^aFrom Table 1, Monsonog J et al., 1997 ^bFor Trend

^cPloidy assessment done using an image analysis cytometer

Table 3^a Histological grade and high risk HPV by *in situ* hybridization

	N	HPV types 16, 18, 33*	%	Chi Sq	P value
Flat condyloma	104	15	14		
CIN 1	51	16	31		
CIN 2	56	26	46		
CIN 3	81	61	75	71.4 ^b	<0.001
Low grade SIL	155	31	20		
High grade SIL	137	87	63	98.5	<0.001

^aFrom Table 2, Monsonog J et al., 1997 ^bFor Trend *At least one type present

Table 4^{a,d} Specimens with aneuploidy^c: Histological grade and HPV

	N ^d	HPV types 16, 18, 33*	%	Chi Sq	P value
Flat condyloma	19	3	16		
CIN 1	14	3	21		
CIN 2	31	23	74		
CIN 3	76	58	76	19 ^b	<0.001
Low grade SIL	33	6	18		
High grade SIL	107	81	76	35.5	<0.001

^aFrom Table 3, Monsonego J et al., 1997 ^bFor Trend

^cPloidy assessment done using an image analysis cytometer

^dThis Table only includes specimens with aneuploidy

*At least one type present.

A large natural history study of HPV and cervical neoplasia is described here. Between April 1989 - Nov. 1990, 23,702 women \geq 16 years of age, obtaining a routine Pap smear in the Kaiser Permanente (KP) system in Portland, OR, were recruited for this study (Liaw K et al., 1999). Of these, 17,654 women with a **normal Pap smear at baseline and no history of SIL were followed for the development of incident SIL**. Subjects were followed passively (without intervention to encourage follow-up) by reviewing the computer-accessible records of their voluntary, routine Pap smear examinations from enrollment until the end of Dec. 1994. During that time, KP recommended yearly Pap smears. The mean frequency of Pap smears during follow-up was 0.6 per woman per year, with 22% of the women having none. During follow-up, 380 incident SIL case patients and 1037 matched control subjects were eligible for this nested case-control study. Cervical lavages collected at enrollment and, later, at the time of case diagnosis (or corresponding time for selection of control subjects) were tested for HPV DNA by a PCR-based method.

- 2.5% of controls and 28.6% of HSIL cases were positive for HPV type 16 DNA at baseline (See Table 1 in Liaw K et al., 1999).
- In comparison with initially HPV negative women, those who tested positive for HPV 16 type DNA at enrollment were:
 - ✓ 5.8 times (95% CI, 2.7-12.5) more likely to have LSIL subsequently diagnosed for the first time during follow-up.
 - ✓ **63.9 times more likely (95% CI, 16.4-248.1) to develop HSIL.**
- Incident SIL = 380 cases:
 - ✓ 40.5% (154/380) ASCUS; 47.1% (179/380) LSIL; 12.4% (47/380) HSIL.
- 39% of LSIL cases and 83% of HSIL cases were confirmed by histology.

Another prospective study (1984-86) enrolled 241 women presenting for STD evaluation and with **negative baseline cervical cytology** (Koutsky LA et al., 1992):

- Subjects were followed **every 4 months** (average length of follow-up = 25 months) with cytology, colposcopy, HPV DNA, and testing for other STDs.
- CIN 2 or 3 (biopsy) was diagnosed in 28 women during the study.
- Only 36% of the subjects with CIN 2/3 (10/28) had an LSIL Pap before an HSIL Pap.
- **Those who tested positive for HPV type 16 or 18, had an adjusted RR=11 (95% CI, 4.6-26) for CIN 2 or 3 compared to women w/o HPV.**
- 105 women had negative cytology and were **HPV negative** at their 1st 2 visits.
 - ✓ For this subset, the subsequent 2 year cumulative incidence of CIN 2/3 was 23% for those who became HPV positive (all types) (N=35) **versus** 2% for those who remained HPV negative (N=70).

In a prospective study⁹ in the U.K., 2011 women 15-19 years of age who recently became sexually active were recruited between 1988-92. A publication including the 1075 women who had **normal cytology and HPV negative tests at baseline** is summarized here (Woodman CB et al., 2001), including a Table.

- Subjects were followed **every 6 months** with a Pap smear, and samples were stored for HPV DNA analysis.
- All participants who developed cytology abnormalities were referred for colposcopy and biopsy. However, treatment was postponed until histologic evidence of CIN 2/3.
- Median duration of follow-up = 26 months. Median interval between visits = 7 months. Median number of visits = 4.
- 246 women had abnormal Pap smears during follow-up.
- 28 had a diagnosis of CIN 2 or 3 (14 of each). Median time to diagnosis was 36 months from study entry (range 6.6 to 104 months).
 - ✓ 20 of these CIN 2/3 cases were diagnosed during work-up of the 1st episode of abnormal cytology.
 - ◆ This is 1.9% (20/1075) of the total study population.

Table 5. Risk and duration of HPV infection and risk of CIN 2/3 in 1075 young U.K. women with normal cytology and HPV negative status at baseline. (From Table 2, Woodman CB et al., 2001)

HPV type	6 or 11	16	18	Any
Number positive	45	110	64	407
Cumulative % risk at 3 years (95% CI)	4.3 (2.8–5.7)	10.5 (8.3–12.7)	6.6 (4.8–8.4)	43.8 (40.1–47.5)
Median duration of detection, mos (IQR)	9.4 (6.1–12.9)	10.3 (6.8–17.3)	7.8 (6.0–12.6)	13.7 (8.0–25.4)
Relative risk of high-grade CIN (95% CI)*	3.8 (1.5–9.8)	8.5 (3.7–19.2)	3.3 (1.4–8.1)	7.8 (2.7–22.0)

*Relative hazards ratio of high-grade CIN after type-specific infection, controlling for any other HPV exposure. IQR = Inter-quartile range.

⁹ This study is of special interest, given the number and characteristics of these participants (negative cytology, negative HPV), and the type and frequency of follow-up, as this may be representative of an HPV vaccine efficacy trial.

HPV in Cervical Cancer

As described in this section, HPV DNA has been detected in a high percentage of cervical cancers. HPV type 16 DNA is the most common type detected overall in ICCs, as well as in SCCs separately. On the other hand, HPV type 18 DNA is more common than HPV type 16 in adenocarcinoma of the cervix.

The International Biological Study on Cervical Cancer (IBSCC) was undertaken to determine whether the association between HPV infection and cervical cancer is found worldwide and to investigate geographic distribution of HPV types. For this effort, over 1000 frozen biopsy specimens were collected from 22 countries around the world, from 1989-1992. In each center, collaborators were asked to recruit 50 consecutive cases of invasive cervical cancer, yielding over 1000 frozen biopsy specimens. Slides from all patients were submitted for central histologic review to confirm the diagnosis and to assess histologic characteristics.

In the initial IBSCC, HPV DNA was detected in 93% of the cervical cancer specimens using a PCR assay based on L1 consensus primers (MY09/MY11) (Bosch F et al., 1995). HPV 16 was present in 50% of the specimens, HPV 18 in 14%, HPV 45 in 8%, and HPV 31 in 5%. [Overall: 93% of cases (866/932) HPV-pos; 7% (66/932) HPV-neg; 4% (36/932) double infections]. There was significant geographic variation in the prevalence of some less common HPV types.

Table 6. Most frequent HPV type by histology type of invasive cervical cancer*

<u>Tumor type</u>	<u>HPV type</u>	<u>% of cases</u>
Squamous Cell Carcinoma	HPV 16	51% (451/881)
Adenocarcinoma	HPV 18	56% (14/25)
Adenosquamous	HPV 18	39% (7/18)

*The International Biological Study on Cervical Cancer (IBSCC),
Data from Table 3, Bosch et al., 1995. (Double infections counted twice)

Subsequently, available specimens originally categorized as HPV-negative in the IBSCC study were retested (Walboomers et al., 1999). Retesting of most of the 7% of specimens originally classified as HPV DNA-negative was done by PCR-based assays using more sensitive primers, and with strict control of the amount/quality of malignant tissue in the sections tested. 58 of the 66 HPV-neg samples were available and 55 were deemed adequate for PCR testing. Of these, 40 were HPV-pos and 15 were HPV-neg. Of the 55 retested, only 34 were histologically adequate (i.e., cancer could be identified). 2 of these 34 were HPV negative. Considering the retested cases and excluding histologically inadequate specimens, the HPV prevalence in cervical carcinomas from the IARC specimens was 99.7 % (898/900) (Walboomers et al., 1999). HPV16 was the most common representing 53% of all cases, followed by HPV18 (15%), HPV 45 (9%), HPV31 (6%) and HPV33 (3%). Thus, with the new data, the four

most common types are found in about 80% of all tumors in this study (Muñoz N, 2000).

Table 7. HPV types in cervical carcinoma*

HPV type	1975–85 ^a N=460	1984–95 ^b N=68	1989–92 ^c N=932	1989–92 ^d N=932
HPV16	64%	51%	50%	52%
HPV18	25%	24%	14%	15%
HPV33	3%	0%	3%	3%
HPV any	88%	94%	93%	97% ^e

^a Iwasawa et al., 1996. (Finnish cases)

^b Lehtinen et al. (Unpublished cases, Finland).

^c Bosch et al., 1995. (Global cases)

^d Walboomers et al., 1999. (Global cases, same starting cases as Bosch et al., 1995)

^e 99.7 % with author-defined adequate specimens, Walboomers et al., 1999.

*Modified from Table 3, Paavonen et al., 2000. Includes both SCC and adenocarcinoma.

Adenocarcinoma: This Table summarizes published data on the rate of HPV 18 versus 16, the two most common types, in adenocarcinoma of the cervix. Unlike SCC, HPV 18 is as common or more common in cervical adenocarcinoma compared to HPV 16.

Table 8. Cervical Adenocarcinoma - HPV 18 - Most Common Type

HPV Type	Bosch N=25	Iwasawa N=108	Andersson N=131	Pirog N=67*
HPV 16	28%	17%	24%	36%
HPV 18	56%	56%	37%	36%

Bosch FX et al. *JNCI* 1995; 87:796-802. (Global cases)

Iwasawa A et al. *Cancer* 1996;77:2275–9. (Finnish cases)

Andersson S et al. *Eur J Cancer* 2001;37:246-50. (Swedish cases)

Pirog EC et al. *Am J Pathol* 2000;157:1055–62. (US and Polish cases)

*Excludes 6 HPV neg cases of nonmucinous adenocarcinomas (clear cell, serous, mesonephric).

One study which focused on adenocarcinoma is described here. 131 cervical adenocarcinomas identified through the Swedish Cancer Registry were examined morphologically and then the archival specimens were tested by PCR. The prevalence of HPV negative adenocarcinomas increased with age of the patient in this study. The overall results are presented here:

Table 9. Cervical Adenocarcinomas (Data from Table 2, Andersson S et al., 2001)

Population	HPV 18-pos	HPV16-pos	HPV pos (other types)	HPV neg
≤ 39 yrs (subset)	12/35 (34%)	14/35 (40%)	5/35 (14%)	4/35 (11%)
All	48/131 (37%)	31/131 (24%)	14/131 (11%)	38/131 (29%)

As a final item for this section, in a study in Bangkok, 190 women with invasive cervical cancer were compared with 75 women with *in situ* disease. HPV types 16 and 18, but not types 31/33/35/39, were more common in invasive than intraepithelial tumors, and untyped HPV DNA was found more commonly in the *in situ* lesions, suggesting that *in situ* disease is four times more likely to become invasive if associated with type 16 or 18 than with other types (Thomas DB et al. [II.], 2001).

HPV Incidence and Persistence

Data are summarized in this section from a variety of studies and populations for HPV incidence and persistence. HPV type specific data for types 16 and 18 are presented, when available. Estimates of the median duration of HPV type 16 infection vary from 8 to 12 months (Ho G et al., 1998; Franco EL et al., 1999; Woodman CB, et al., 2001¹⁰).

In one study, college students at a state university in New Brunswick, N.J. were followed at **6 month intervals**. At each visit, a questionnaire on lifestyle was completed, and cervicovaginal lavage was done. Also, a pelvic examination and Pap smear were performed at base line and annually thereafter. The 608 women were seen a total of 2971 times (median, 5 visits each) during an average of 2.2 years of follow-up. Results on persistence for HPV types 16 and 18 are presented in the Table below.

Table 10. Cumulative 24-month incidence & median duration of HPV Types 16 & 18.*

HPV type	Cumulative 24-month incidence† % (95% CI)	Median duration† mo (95% CI)
16	7 (4–9)	11 (7–12)
18	4 (3–6)	12 (6–17)

*From Table 1. From: Ho GYF et al., 1998. Included 608 college women. † Kaplan-Meier method.

In another study, female students 18-20 years old were recruited from the University of Washington, Seattle, from 1990-97 for participation in an ongoing cohort study of the acquisition and natural history of genital HPV infections (Thomas KK et al., 2000). Here, 518 women were followed for an average of 2.9 years. Behavioral information and cervical and vulvovaginal swabs for HPV DNA assay were obtained **at 4-month intervals**. 3939 visits among 518 women were included in the analysis. Among these women, the mean follow-up time was 34.3 months, the mean number of visits per person was 8.3, and the median time between visits was 4.3 months. Acquisition (incidence) of a given HPV type was defined for each subject to be the first positive result for that type after an observed negative result. Incidence rates for the different HPV types during follow-up are included in the table below. (Persistence data not provided.)

Table 11. Incidence of different HPV type-specific infections among the study population.^a

HPV type	Observed	Person-Yrs at risk	Incidence per 100 Person-Yrs	Positive at some visit, ^a N (%)
HPV 16	54	1191	4.7	84 (16.2)
HPV 18	35	1289	2.7	49 (9.5)
HPV 31	26	1322	2.0	33 (6.4)
HPV 45	8	1348	0.6	14 (2.7)
HPV 6	41	1256	3.3	54 (10.4)
HPV 11	4	1369	0.3	9 (1.7)
All typed infections ^b	200	899	22.2	241 (46.5)
All typed infections plus uncharacterized infections ^c	231	832	27.8	281 (54.2)

^aModified From Table 1 from Thomas K et al., 2000; 518 college students included in study.

^bIncludes the following types: 6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, & 58.

^cUncharacterized infections = + with HPV generic probe; not w/specific type or type mix probes.

¹⁰ For Woodman CB et al., 2001 data, see Section entitled HPV in ASCUS (ASC), LSIL, HSIL, etc.

The Ludwig-McGill Cohort Study is a large longitudinal investigation of the natural history of HPV infection and cervical neoplasia in a population of low-income women in São Paulo, Brazil. Incidence and persistence data are available from this study. Subjects were told to return **every 4 months during the first year and then every 6 months thereafter** for cervical specimen collection. The results concerning the dynamics of acquisition and loss of HPV infection during the first four visits for the first 1425 women enrolled in the cohort (Franco EL et al., 1999) are provided in the Table below:

Table 12. Ludwig-McGill Cohort Study: Prevalence & Incidence of HPV Types 16 & 18.

Type	Prevalence at entry (%)	Incidence/100 woman-yrs	Median retention time
HPV 16	2.7%	1.68	8.4 months (95% CI, 6.8-10.0)
HPV 18	0.8%	0.36	*

*Not provided in article. Only 4 incident cases.

One study to investigate HPV presence/persistence on progression of squamous dysplasias enrolled 353 Dutch women (Nobbenhuis MA et al., 1999).

- Mean age 32 years (range 18-55 yrs).
- Referred (1990-1992) to a colposcopy clinic with mild to severe dyskaryosis (dysplasia) on Pap smear.
- No medical history of previous cervical pathology.
- Baseline biopsies were not taken, but colposcopic impression of disease was recorded.
- Subjects were followed every 3 to 4 months with HPV DNA tests, cytology and colposcopy.
- At the last visit, colposcopically directed biopsies were obtained. (Random biopsies were taken if appearance was normal).

Results: Subjects were followed for a mean of 33 months. In this study, 33 women reached *study-defined* clinical progression: an end histology of CIN 3 in ≥ 3 quadrants or a Pap smear suspicious for microinvasion (30 had CIN 3 in ≥ 3 quadrants; 3 had a Pap smear suspicious for microinvasion). No *study-defined* clinical progression occurred in women negative for high risk HPV.

Of those at baseline with mild or moderate dyskaryosis at baseline, 38% (69/182) of those HPV positive versus 3% (3/115) HPV negative progressed to CIN 3 [odds ratio 23 (7-75)]. Using information from Table 1 of that article (baseline characteristics versus clinical progression by end histology), the following calculation was made: Of those at baseline with mild or moderate dyskaryosis and a colposcopic impression of no worse than CIN 2, 38% (68/180) HPV positive versus 3% (3/115) HPV negative individuals progressed to CIN 3 (not considering # of affected quadrants).

HPV Viral Load and Persistence

Data from 3 publications (one on viral persistence; two on viral load) from the **same starting database** are presented here. A group of investigators in Sweden merged data for a cohort of women who had archival cervical cytology smears with data from the National Cancer Registry (NCR) (1. Ylitalo N et al., Cancer Res 2000; 2. Ylitalo N et al., Lancet 2000; 3. Josefsson A et al., Lancet 2000). Critical information:

- Cohort lived in Uppsala County, Sweden.
- Inclusion criteria for study include: Born in Sweden.
- Cases and controls were matched for age & time 1st smear was collected. Controls had to be alive without CIS (carcinoma *in situ*) or worse before date of diagnosis of matched controls.
- First Pap smear had normal cytology, for both cases and controls. (Those without 1st normal Pap smear not included.)
- 732,287 smears from 146,889 women stored at University Hospital.
- Computerized information for 1969-95.
- Mandatory NCR notification for carcinoma *in situ* cases (& invasive cancer).
- 504 incident cases of **squamous cell carcinoma *in situ*** found in NCR database.
- HPV type 16 DNA was quantitated in both baseline cytologically negative smears and later cytology samples. (Not done for histology slides or paraffin block.)
- Other HPV types not analyzed.

Results for recurrent or persistent infection: Using this Swedish Cohort, a **nested case control study** was performed (Ylitalo N et al., Cancer Res 2000):

- 484 women with **squamous cell carcinoma *in situ*** and 619 controls identified who qualified for the **persistence study**. (Smears negative for B-actin, but HPV 16 positive could be included in this cohort, which differs from viral load cohort, below.)
- Among cases, **56% were HPV type 16 + at time of diagnosis**.
- **Median incubation time from infection to CIS was estimated to be 7-12 years**, for HPV type 16 positive women.
- Women whose 1st smear was positive for HPV type 16 DNA: **OR = 4.6 (95% CI 3.1 to 6.8)** for developing squamous cell carcinoma *in situ* compared to those with negative 1st smear (130/483 cases vs. 36/619 controls)
- **Another analysis performed**, using the 2 most recent smears before diagnosis.
- Only women with 2 or more smears, 263 cases and 264 controls, included in this particular analysis (see table below).

Table 13*: Odds ratios (OR) of cervical carcinoma *in situ* in relation to HPV type 16 status.

HPV 16 status in <u>last 2 smears before diagnosis</u> ^b	No. of cases/ controls	OR (95% CI) ^a
Neg- Neg	108/ 235	1 (Reference)
Pos- Neg	26/ 11	4.9 (2.1–11.3)
Neg- Pos	54/ 14	9.7 (4.3–21.8)
Pos- Pos	75/ 4	31.2 (10.6–91.8)
Total	263/ 264	

^aSmears taken the last year before diagnosis have been excluded.

^bOnly analyzed for women with 2 or more smears.

*Modified from Table 2 in Ylitalo N et al., Cancer Res 2000;60:6027-32.

Results for viral load (Ylitalo N et al., [Lancet 2000](#); Josefsson A et al., 2000):

- ✓ 478 women with squamous cell carcinoma *in situ* and 608 controls identified who qualified for nested case control viral load study. (Inclusion: must have \geq one B-actin positive smear.)
- ✓ 60% (288/478) of cases and 16% (99/608) of controls had at least one smear that was positive for HPV type 16 (Per Table 1, Josefsson et al., 2000).
- ✓ The odds ratio (OR) for CIS was highest among women with high viral DNA levels in their cervical smear samples. The increased OR was present over time.
- ✓ At \geq 9 years before CIS diagnosis, women with high HPV type 16 viral loads had an OR of 33.3 (95% CI: 4.7-236.8) for CIS compared to HPV 16-negative women.
- ✓ About 25% of women (95% CI: 0.12–0.32) with a high HPV 16 viral load before age 25 years developed cervical carcinoma *in situ* within 15 years (Ylitalo N et al., 2000).
- ✓ Analysis of the first smear (collected a mean of 7.8 years before CIS diagnosis) showed that women with the 20% highest amount of HPV 16 DNA had an OR of 59 (95% CI: 8-462) for developing cervical carcinoma *in situ* compared to HPV 16 negative women (Josefsson A et al., 2000).
- ✓ However, no single HPV DNA measurement is yet able to predict the risk of cervical cancer. (Josefsson A et al., 2000; Ylitalo N et al., 2000).

Other viral load data:

In another study, data from 217 subjects with ASCUS showed an association between higher levels of viral load of “high risk” HPV types (as assessed by hybrid capture signal strength) and having an underlying CIN 2 or 3 by histology (Cox JT et al., 1995).

In another study described previously (Woodman C et al., 2001), a semi-quantitative method was used to assess viral load. In timed sequences that included both high and low viral load samples, the low viral load readings usually appeared at the beginning or end of the sequence over time.

HLA typing, carcinoma *in situ* (CIS) of the cervix, and persistent infection

A 4th publication from the previously described Swedish CIS database is described here (Beskow AH et al., 2001). The association of variation at the DRB1 and DQB1 loci (HLA class II alleles) with HPV type 16 infection and risk of developing CIS was examined by analysis of 440 cases diagnosed with cervical CIS and 476 age-matched controls in a retrospective case-control study. The infection history was studied by analysis of cervical smears taken at multiple times during a period of up to 27 years (1969-95). After correction for multiple testing, 2 alleles were associated with cervical cancer and only in HPV16-infected patients [DQB1*0602: 102/264 (39%) vs. 130/476 (28%), $p = 0.028$; and DRB1*1501: 104/259 (40%) vs. 132/469 (28%), $p = 0.027$].

Natural History – Cervical Pathology

The histology of the cervix and its relation to neoplasia will be briefly reviewed here. The epithelium of the cervix consists of columnar epithelium located primarily in the endocervical canal and squamous epithelium found predominantly on the ectocervix. The squamocolumnar junction is the site of ongoing squamous metaplasia. This is where the glandular portion is undergoing replacement or transformation by the squamous epithelium, the so-called transformation zone. The transformation zone contains stem cells that may give rise to both squamous and columnar epithelia (Elkas JC, et al., 2000).

Both histologic as well as colposcopic observations indicate that the transformation zone of the cervix is where virtually every preinvasive disease originates. Lesions are thought to arise from the basal cells of the transformation zone (Sheet EE et al., 1999). The transformation zone is the most common site for the development of intraepithelial lesions that may give rise to invasive disease. False negative Pap results may be due to inadequate sampling of the transformation zone, which often regresses into the endocervical canal in postmenopausal women (Cannistra SA et al., 1996).

The histology progression of cervical lesions, i.e., mild dysplasia through carcinoma *in situ*, is illustrated in **Appendix C** and described here (DeMay RM, 1999; Wright TC et al., 1994). The classification (and severity) of the lesion depends on how high the level of undifferentiated cells ascend in the epithelium before the squamous differentiation (if any) begins.

- ❑ Mild dysplasia: The undifferentiated abnormal cells are limited to the lower third of epithelium. Considerable squamous differentiation can occur before the cells are exfoliated at the surface.
- ❑ Moderate dysplasia: Undifferentiated cells occupy at least the lower one third, but no more than two thirds, of the epithelium thickness.
- ❑ Severe dysplasia: Little squamous differentiation occurs, and only in the upper third of the epithelium. At least the bottom two thirds of the epithelium is composed of abnormal, undifferentiated cells.
- ❑ Carcinoma *in situ* (CIS): The full thickness of the epithelium is composed of undifferentiated abnormal cells. The cells at the surface may flatten a little, due to surface tension effects; however, they do not truly differentiate or acquire dense squamous cytoplasm. (Note: CIN 3 includes both severe dysplasia and CIS).

The traditional model of cervical cancer development involves a slow and uncertain progression from normal cervical cells, through pre-cancerous cervical lesions, to increasingly advanced stages of invasive cervical cancer. The pre-cancerous cervical lesions are known as cervical intraepithelial neoplasias, or CIN. CIN 2 and 3, known as high-grade SIL (HSL), are more serious conditions than the early CIN 1 abnormalities. These may develop from CIN 1 lesions.

However, one study (described earlier, follow-up every 4 months with cytology and colposcopy) found that not all women with CIN 2 or 3 had evidence of a transition/progression through CIN 1 first (Koutsky LA et al., 1992; Garnett GP et al., 2000). This study prospectively followed a cohort of 241 women who presented for evaluation of sexually transmitted disease and had negative cervical cytologic tests at baseline.

In another study (also described earlier, with follow-up every 6 months with cytology and referral to colposcopy [with biopsy if needed] for all abnormal cytology), 20/246 (8.1%) of women with abnormal cytology had CIN 2/3 at the 1st work-up. The 1075 women in this cohort had normal cytology and HPV negative tests at baseline (Woodman C et al., 2001).

The average age of patients with low-grade CIN is consistently 5 to 10 years younger than that of patients with CIS, who are in turn of an average age 10 to 15 years younger or so than patients with invasive squamous cell carcinoma (Meanwell, 1988).

The histologic hallmark of transition from carcinoma *in situ* to invasive carcinoma occurs when tumor cells penetrate the epithelial basement membrane and enter the underlying cervical stroma. Once the cervical stroma is invaded, the lymphatics and blood vessels are accessible, and dissemination of the tumor beyond the cervix can occur. The most important prognostic factors for patients with cervical cancers are the lymph node status and size & extent of the primary tumor (Wharton JT & Tortolero-Luna G, 2000, review).

Microinvasion: In the current staging classification scheme for cervical cancer, stage Ia1 is defined as a tumor with stromal invasion no greater than 3 mm in depth beneath the basement membrane, and no wider than 7 mm. Stage Ia2 is defined as a tumor with stromal invasion greater than 3 mm, and no greater than 5 mm in depth and no wider than 7 mm (**Appendix C**).

A meta-analysis which included data on 27,929 patients from 15 studies was performed to evaluate the natural history of SIL (squamous intraepithelial lesions) (Melnikow et al., 1998). In this meta-analysis, the spontaneous regression rate, the risk of progression to a higher grade dysplasia, and the progression rate to invasive cervical cancer of ASCUS, LSIL, and HSIL were examined after 6 and 24 months of observation. The results are presented in the Table below.

Table 14. Natural History of Cervical Dysplasia (From Melnikow J et al., 1998)

Initial Cytology	Progression**		Progression to Invasive Cancer		Regression to Normal*
	6 months	24 months	6 months	24 months	
ASCUS	2%	7.2%	0.06%	0.25%	68.2%
LSIL	6.6%	20.8%	0.04%	0.15%	47.4%
HSIL	6.8%	23.4%	0.15%	1.44%	35.0%

*No relationship was found between the proportion of subjects regressing to normal and the length of follow-up.

**Follow-up smears or biopsies showing a higher grade lesion than the study entry cytology. For HSIL, progression to CIN 3 (from grade 2) or CIS.

A literature review which addresses the history of CIN (since 1950) is described here. Regression, persistence and progression data were obtained by adding the numbers of cases in the respective categories from the reviewed publications, and expressing the results as percentages. (Thus, an article's contribution depended on the # of cases.) Only reports with a "minimal disturbance" of the lesions, i.e., cytology or punch biopsy, at baseline were included. A Table is provided below which summarizes these data.

Table 15. Natural History of CIN (Table 7 from Östör AG, 1993)

	Regress	Persist	Progression to	
			CIN 3	Invasion
CIN 1	57%	32%	11%	1%
CIN 2	43%	35%	22%	5%
CIN 3	32%	<56%		>12%

Limitations of this literature review as noted by Dr. Östör are listed here, e.g.:

- ❑ Biopsy can alter the natural history of CIN.
- ❑ 1950s-1990; Non-uniform diagnostic criteria.
- ❑ Cytology does not always predict histology.
- ❑ "Jigsaw puzzle effect" can occur, e.g., CIN 1 & 3 can exist in same cervix.
 - ✓ A punch biopsy samples 40 mm² from 800 mm² (Takevchi A et al., 1960).
- ❑ Follow-up methods varied.
- ❑ Lack of long-term follow-up.
- ❑ Estimate CIN 3 progression to ICC of ">12%" is likely too low; it may be 20-30% within 5-10 yrs. (Östör AG, 1993; Chang AR, 1990; McIndoe WA et al., 1984)

In conclusion to this Section, it is noted that the pathogenesis of cervical AIS (adenocarcinoma *in situ*) is not as well understood as its squamous counterpart. The existence and progression of glandular dysplasia similar to squamous intraepithelial lesions of the cervix has not been well documented and accepted because of insufficient study. In part because the diagnosis of AIS is less common than squamous CIS, it has been difficult to delineate the natural history of glandular dysplasia (Krivak TC et al., 2001). U.S. data on the mean age at diagnosis of CIS and SCC as well as AIS and adenocarcinoma are provided in this Table.

Table 16. Mean age at diagnosis for Squamous CIS^a, SCC^b, AIS^c and Adenocarcinoma (Cervix)*

Squamous CIS	SCC	△
33.6 yrs of age	51.4 yrs of age	17.9 yrs
AIS	Adenocarcinoma	△
38.8 yrs of age	51.7 yrs of age	13.0 yrs

* Plaxe SC et al., 1999. (Data Source: SEER (Surveillance, Epidemiology, & End Results) database, 1973-95).

^aCIS = Carcinoma *in situ*. ^bSCC = Squamous Cell Carcinoma. ^cAIS = Adenocarcinoma *in situ*.

Findings at Work-up of ASCUS (ASC), AGUS (AGC) and LSIL

Across studies, the proportion of women with CIN2/3 or worse following the work-up for ASCUS (ASC) has ranged from about 6% to 11%. For LSIL, the proportion with CIN 2/3 or worse at work-up has usually ranged from about 16% to 28% (See Table, below). However, several European studies have reported rates as high as 50-70%. For AGUS, the proportion with CIN 2/3 or worse at work-up has usually ranged from about 9-13%, with a special concern about the % of frank cancer, as discussed later in this section.

The Table below summarizes findings at the work-up of LSIL. Some of these articles are also summarized in the text:

Table 17. Frequency of high-grade histology at work-up of those w/LSIL Pap tests varies*

Author	Patient#	% with HSIL** (rare ca.) Number (%)
Lonky (Am J Obstet Gynecol 1999;181:560-6) ^{a,1}	359 (HPV)	39 (10.9%)
	1425 (CIN1)	290 (20.4%)
	1784 (total)	329 (18.4%)
Bolger (Br J Obstet Gynecol 1988;95:1117-9) ^b	91 ^f	36 (40.0%)
Law (J Reprod Med 2001;46:61-4) ^c	534	161 (30.1%)
Spitzer (Obstet Gynecol 1993;82:731-5) ^{a,2}	135	29 (21.5%)
Lee (Int J Gynecol Obstet 1998;60:35-40) ^d	145	45 (31.0%)
Kinney (Obstet Gynecol 1998;91:973-6) ^{a,1}	218 (<40 yo)	36 (16.5%)
	52 (≥40 yo)	5 (9.6%)
Bigrigg (Lancet 1990;336:229-31) ^b	247	124 (50.2%)
Wright (Obstet Gynecol 1995;85:202-10) ^a	217	39 (18.0%)
	106 ^f	9 (8.5%)
Takezawa (J Low Gen Tract Dis 1998,3:136-40) ^{a,2}	972	276 (28.4%)
Andersen (Gynecol Oncol 1995;59:143-7) ^e	103	72 (69.9%)
Total	4498	1161 (25.8%)

*From ASCCP (1), 2001. (modified slightly) (<http://consensus.asccp.org/guidelines.asp>)

**Histology Site: ^aUS, ^bUK, ^cTaiwan, ^dHong Kong, ^eDenmark

¹ HMO ² Specifically noted as high risk

^f Article specifically notes these women have no history of cervical disease

A number of studies that reported findings at work-up of abnormal cytology are presented here.

A total of 46,009 nonpregnant female members of the Kaiser Permanente Health Plan, Northern California Region, were studied prospectively in the Borderline Pap Study. As part of this study, all patients in the cohort who had ASCUS, low-grade SIL, or AGCUS (AGUS) on cervical cytology were recruited to undergo colposcopy with biopsy and/or endocervical curettage. More than 60% (995 with ASCUS, 137 with AGCUS, and 270 with low-grade SIL) of those patients consented to participate. Of the entire 46,009 person cohort, 3.6% and 0.9% and had an initial PAP finding of ASCUS or LSIL, respectively. At work-up, the following was determined:

- 7.3% (73/995) with ASCUS were found to have HSIL or worse histology.
- 15.2% (41/270) with LSIL cytology were found to have HSIL or worse histology.
- 13.1% (18/137) subjects with AGCUS were found to have HSIL or worse histology. (See also Ronnett BM et al., 1999)
- Looking at the Borderline Pap Study from the other direction, 38.8% and 20.1% of the “histologic high-grade SIL+s” were immediately proceeded by a diagnosis of ASCUS or LSIL, respectively (Kinney WK et al., 1998).

In another large prospective study (Southern California Permanente Medical Group, Orange County), the diagnostic utility of TBS-based cervical cytology screening program with colposcopy and biopsy as the criterion standard (Lonky NM, et al., 1999) was evaluated. The population was predominantly employed, middle class and multi-ethnic. In 1996, 93% of Pap smears were read as normal and 5.6% (2195/38,851) as ASCUS (all types). The authors prospectively collected data from all patients who were referred for colposcopy since the start of the policy of routine colposcopic follow-up for any cytologic abnormality on Pap. The authors reviewed the clinical history and the colposcopic and histologic results (if a biopsy or endocervical curettage performed) from 7241 consecutive colposcopy clinic patient visits (both initial referral visits [n = 5585] and return visits [n = 1656]) in which colposcopy (not just repeat Pap smear) was performed. At work-up, the following was determined based on initial colposcopy (See [Appendix E for a complete Table](#)) (Lonky NM et al., 1999):

- 8.9% (278/3118) with ASCUS (all categories) were found to have CIN 2/3 or worse histology.
- 18.4% (329/1784) with LSIL cytology were found to have CIN 2/3 or worse histology.
- 9.4% (6/64) with AGUS were found to have CIN 2/3 or worse histology.
- Only 17% (132 /771) of HSIL cases and 38% (5/13) of invasive cancer cases followed Pap smears suggesting high-grade intraepithelial lesions or cancer, with 77% being discovered after "minor" Pap smear abnormalities.

Other smaller studies where subjects had follow-up with colposcopy, and if indicated, directed biopsies are described here.

- In one study at a university health center, where subjects with a previous history of cervical treatment were excluded, 6.9% (15/217) of women with ASCUS cytology had HSIL (CIN 2/3) (Cox JT et al., 1995).
- In a retrospective chart review series, 6.1% (11/181) of women with ASCUS cytology and 18% (39/217) with LSIL cytology, had confirmed CIN 2 or 3 (Wright TC et al., 1995). In those with no history of treatment for cervical disease, 5.2% (7/136) of women with ASCUS cytology and 8.5% (9/106) with LSIL cytology, had colposcopy with biopsy confirmed CIN 2 or 3 (Wright TC et al., 1995).
- In another report, 9% (11/118) of patients with an initial ASCUS smear were found to have a HSIL (CIN 2/3) on biopsy (Kaufman RH, 1996).

The ASCUS/LSIL Triage Study (ALTS) is a randomized, multicenter clinical trial of the management of women with low-grade and equivocal cervical cytology abnormalities, sponsored by the National Cancer Institute (NCI). Randomization to one of 3 groups was based on the community cytology diagnosis. Women in the ASCUS group who

were randomized to immediate colposcopy had the following results: **11.4%** had biopsy confirmed CIN 2 or worse (**CIN2+**). (Here is the breakdown: **6.3% CIN 2** [72 /1149] + **5.1% CIN 3+** [59/1149]). (Also, 14.5% [167/1149] had CIN 1) (Solomon D et al., 2001).

One area of concern for AGUS (AGC) is the impression that AIS and frank cancer (adenocarcinoma or SCC) may be more common at work-up of AGUS than at work-up for ASCUS or LSIL. Follow-up findings of carcinomas ranged from 3.2% (Goff BA et al., 1992) to 9.4% (Zweizig S et al., 1997). Follow-up findings of adenocarcinoma *in situ* (AIS) ranged from 1.2% (Zweizig S et al, 1997) to 7.9% (Goff BA et al, 1992). (Summarized in Bethesda 2001 AGC forum document.) (Please note that some of these cancers found at work-up of AGC are endometrial cancers, which have not been associated with HPV.)

Also, the findings of carcinoma at work-up of cytology abnormalities, including AGUS (AGC), in a large study are summarized here.

- In 1998, 306 laboratories participated in a Q-Probes study to investigate the follow-up of 16,132 abnormal cytology results (Jones BA et al., 2000). Participants identified abnormal diagnoses from their files that were 1 to 3 years old and searched for follow-up diagnostic material in the lab database. When no lab follow-up was available, lab personnel contacted physician offices for the information. Cytology findings are shown with the % who had carcinoma at work-up, below.
 - ✓ Of those with AGUS, 5.8% (17/293) were diagnosed with carcinoma at work-up (13/293 adenocarcinoma; 4/293 SCC).
 - ✓ Of those w/ASCUS, 0.46% (19/4125) were diagnosed with carcinoma at work-up.
 - ✓ Of those w/LSIL, 0.24% (21/8586) were diagnosed with carcinoma at work-up.
 - ✓ Of those w/HSIL, 2.05% (89/4345) were diagnosed with carcinoma at work-up.

Sources of Error for Screening and Diagnosis

Sources of “error” for cytology including sampling (perhaps the most important), specimen preservation, slide preparation and staining in addition to pathological interpretation (Bishop JW, et al. 1998). It has been estimated that 2/3s of false negatives are due to sampling “error” and 1/3 to detection error (McCrorry DC et al., 1999).

A good example of the impact of sampling is described here. A change of the cervical sampling utensil (from cotton-tipped pins to cervical brushes) in Västerbotten County in Northern Sweden resulted in an immediate 4-fold increase of the incidence of CIN 2 and 3. This was thought to be due to improved detection of prevalent disease (Tord Ångström, personal communication to: Dillner J, 2000).

Clinical Management Overview

Overview articles that cover work-up of an abnormal Pap test and therapeutic options as well as management of invasive cancer are included in the briefing materials (Bristow RE et al., 2000; Janicek MF et al., 2001; Wharton JT et al., 2000). When reading this section, it should be kept in mind that refinements to clinical management recommendations, cytological terminology (see **Appendix C**) and use of adjunctive tests, based on several consensus meetings held in 2001, are expected to be published in the near future.

Colposcopy may be performed upon the first finding of ASCUS or LSIL. Factors such as likely availability for follow-up, immune status, etc., affect decisions in this regard. In general, immediate colposcopy for ASCUS and LSIL is more commonly performed in the U.S. than in Europe. One management option for a finding of ASCUS and LSIL is to repeat the Pap smear in 4-6 months (in the absence of a prior history of abnormal Pap smear and absence of other factors such as HIV disease). In the event of a 2nd abnormal finding, colposcopy and directed biopsy may be indicated to rule out CIN 2/3 or worse.

A single finding of **AGC (AGUS)** by Pap smear should be evaluated by colposcopy with directed biopsy, ECC (endocervical curettage) and depending on the subject's age, endometrial biopsy and possibly other evaluations (Zweizig S et al., 1997; Bristow RE et al., 2000; Jones BA et al., 2000).

Considering the high specificity of Pap smears (McCrory DC et al., 1999), a single Pap test finding of HSIL clearly merits an initial work-up with colposcopy and directed biopsy. In the setting of a cytologic finding of HSIL, the possibility of an underlying invasive carcinoma must be excluded with certainty.

Treatment in general has become less aggressive, and preinvasive disease is seldom treated with hysterectomy (except for AIS, see below). Now, most clinicians favor office procedures such as laser ablation therapy, cryosurgery, or simple loop excision of the transformation zone. Cone biopsy can be performed as the traditional "cold-knife" cone, or loop electrosurgical excision procedure (LEEP). LEEP can be performed in a clinic setting without the need for a major anesthetic or an operating room. On the other hand, cold-knife conization is an operating room procedure that requires general or regional anesthesia. However, thermal artifact may obscure the margins in LEEP pathology specimens.

In general, for biopsy-proven squamous intraepithelial lesions with negative findings on ECC, a satisfactory colposcopy examination (the entire transformation zone can be visualized), and congruent Pap smear and biopsy results, ablative therapies or LEEP may be used.

A CIN 1 (biopsy) found at work-up can be treated, either by ablation (laser ablation or cryotherapy) or excision, or can be followed without therapy (at least over the short-term).

The consensus in the U.S. is that CIN 2 or 3 by biopsy should be treated once diagnosed (Wharton JT et al., 2000, review; Östör AG, 1993). This can be accomplished with an excisional procedure, LEEP or cold-knife conization. Both can provide a satisfactory pathology specimen which is useful, in part, to rule out invasive cancer. Also, for well-visualized lesions, definitive ablative therapy (which will not produce a pathology specimen) may be appropriate (but not if microinvasion is suspected).

There is a preference by some for cold-knife conization instead of LEEP for evaluation and treatment of microinvasive squamous lesions (Jakus S et al, 2000). Cold-knife conization of the cervix (instead of LEEP) is the most appropriate diagnostic procedure for suspected adenocarcinoma *in situ* (Krivak TC et al., 2001).

Adenocarcinoma *in situ* (AIS) has been viewed as a more aggressive disease (than squamous cell carcinoma *in situ*) with a potential for skip lesions (Janicek MF et al., 2001). However, more recently, cold-knife conization has also been proposed as an adequate therapy for adenocarcinoma *in situ* with free margins (Östör AG et al., Gyn Oncol 2000). In addition, based on pathology studies and a very limited clinical experience, cold-knife conization may be adequate therapy for microinvasive adenocarcinoma that meets FIGO stage IA1, provided that the biopsy margins are free (Östör AG, Int J Gyn Path 2000) and the subject receives proper informed consent about the unknown risk of disease recurrence (Schorge JO, 2000) (for subjects with a strong wish to retain fertility). (See last page of **Appendix C**, Staging of Invasive Cervical Cancer.) A multicenter trial in this area would be informative.

With regard to prognosis of microinvasive cervical cancer, in the absence of lymph-vascular invasion, carcinomas invading to ≤ 3 mm (Stage IA1) have $\leq 1\%$ chance of lymph node metastases, thus allowing for conservative surgical resection of the primary tumor by simple extrafascial hysterectomy or conization (Janicek MF et al., 2001; van Nagell JR et al., 1978; DePriest PD et al., 1990).

In a recent series, patients with squamous cell carcinoma invading the cervical stroma to a depth of >3.0-5.0 mm with 7 mm or less in horizontal spread (FIGO Stage IA2) were evaluated. These 94 patients were treated primarily by radical hysterectomy with pelvic lymphadenectomy, and those with lymph node metastases were offered postoperative radiation. Seven of 94 patients (7.4%) had lymph node metastases. The mean duration of follow-up was 6.9 years. The overall 5-year survival rate was 95% in this series. The 5-year survival rate of patients with lymph-vascular space invasion (LVSI) was 89% vs 98% in patients without this finding ($P = 0.058$) (Buckley SL et al., 1996).

Preventive HPV Vaccine Strategies for Cervical Cancer

Preventive HPV vaccines have the potential to be an important public health intervention. The global burden of HPV-related disease is considerable. In addition, there are important limitations with non-vaccine means of primary prevention (CDC, 1999). For example, nonoxynol-9 (N-9) functions largely as a detergent that disrupts lipid envelopes, and thus, has no activity against non-enveloped viruses like papillomaviruses (CDC, 1999). Also, it is unclear whether barrier methods of contraception are helpful in preventing the transmission of HPV infection of the genital tract (Bonnez W et al., 1999).

The major breakthrough in preventive vaccine development was the discovery that L1 (the major capsid protein) can self assemble into virus-like particles (VLPs) when independently expressed at high levels in cultured cells (Schiller JT et al., 2000 [review]). VLPs can assemble when L1 is expressed in mammalian cells, insect cells, yeast, or even bacterial cells. VLPs have been documented to induce high titers of neutralizing antibodies to conformational epitopes when injected into animals.

HPVs do not stably infect or cause disease in experimental animals (review: Schiller JT et al., 2000), which presents important limitations with regard to challenge/ protection animal models to be used for studying human vaccines. However, challenge studies with species-specific PVs and parenteral injection of VLPs have demonstrated a consistently high level of protection (90-100%) against infection in three animal systems; one had a cutaneous route of challenge (cottontail rabbit PV) and two had an oral mucosal route of challenge (bovine PV type 4 and canine oral PV). Although these findings have been viewed as cause for optimism about the potential value of VLPs as preventive HPV vaccines, important issues remain. Specifically, the animal challenge study results, although promising, have not used natural routes of mucosal infection; vaccination strategies which produce greater levels/types of mucosal immunity may ultimately be needed to prevent human infection (CDC, 1999).

Measuring immune responses is an important aspect of vaccine development. The ELISA assay measures both virion neutralizing and non-neutralizing antibodies that bind to the VLP (virus-like particle) preparation. Therefore, it was deemed important to also specifically evaluate the induction of type specific neutralizing antibodies after VLP vaccination. Thus, an HPV type 16 pseudovirion neutralization assay (Schiller JT et al., 2000) has been developed. Neutralizing antibodies may prevent viral infection and spread, e.g., in animal challenge/protection studies, but cell-mediated surveillance of virally infected cells may also be important in the ultimate clearing of infection and disease (Stern PL et al., 2000). Hence, work on CMI assays is also of interest.

It is expected that a vaccine that could potentially prevent more than 50% of cervical cancer would need to be multivalent (Schiller JT et al., 2000 [review]). *In vitro* analyses of antibodies against HPV VLPs strongly suggest that protection from VLP-based vaccines will be largely type specific [e.g., Roden RBS et al., 1996 (both articles)].

Also, natural history studies support the concept that protection is type specific. As described in more detail in the Section on HPV Infection and Persistence, 518 college students were followed for an average of 2.9 years, and cervical and vulvovaginal swabs for HPV DNA assay were obtained at 4-month intervals (Thomas K et al., 2000). In this study, concurrent acquisition of multiple types occurred more often than expected by chance ($p < 0.01$, observed vs. expected, Table 2, Thomas K et al., 2000). However, no 2 types were more or less likely to be acquired concurrently than any other 2 types.

In a study at the Kaiser Permanente system (Portland, OR), subjects were selected from 17,654 women with a normal Pap smear at baseline and no history of SIL who were followed for the development of incident SIL. Follow-up procedures for this group are described previously in this document in the Section on HPV in ASCUS (ASC), etc. Two paired cervical specimens from each of 1124 selected women were tested by PCR.

- Preexisting HPV 16 was generally associated with an increased risk for subsequent acquisition of other types, e.g., the OR of acquiring an HPV Type 18 infection on study, if HPV Type 16 + compared to negative at baseline, was 7.2 (95% CI, 1.9-28).
- Also, “cancer-associated” types (HPV types 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and “low risk” types (HPV types 6, 11, 40, 53, 54, 55, 66, 73, and 83 and PAP155) were evaluated as separate groups, respectively.
- Baseline HPV 16 increased the risk for acquisition of either a “cancer-associated” (OR=2.6; 95% CI, 1.2–5.7) or a low-risk (OR=2.7; 95% CI, 1.1–6.7) HPV type.
- However, the risk for persistence of a “cancer-associated” or a low-risk HPV type was unchanged by initial presence of HPV type 16.

In addition, based on these data, the authors (Liaw KL et al., 2001) suggested that the prevention or removal of HPV 16 is not likely to promote the risk of infection with other HPV types. They further suggested that, particularly on the basis of the persistence data, HPV types tend to act as independent sexually transmitted diseases. Unlike the case for some bacteria, where there can be competition for colonization of specific anatomical sites, it has been suggested that protection against one HPV type should not result in an increase in the prevalence of another (Schiller JT et al., 2000).

One topic that is of interest for vaccine development is distinguishing new HPV infections from a recurrence or reactivation. One author noted an important caveat in studies of the natural history of HPV infection of women past their onset of sexual activity. Specifically, one cannot measure the true incidence of new infections among those negative at enrollment: it is impossible to distinguish a new infection from a recurrence or reactivation of a previously latent infection within the limitations of the molecular sensitivity of the HPV detection method used (Franco EL et al., 1999).

“Latent” HPV infection: HPV latency has been defined as “the presence of viral DNA in the absence of differentiation-dependent virion production” (Stubenrauch F et al., 1999). Data from studies on laryngeal papillomatosis have suggested to some that “latent” HPV may exist. Specifically, despite the removal of infected tissues by therapy, and the “confirmation” that HPV 11 is not present in adjoining tissues, these papillomas tend to rapidly recur. Also, in several recent studies, there has been a 2nd peak of HPV

detection in older women, which has suggested to some that HPV infection might be latent or undetectable for a period of time.

- ✓ In Mexico, a population-based study (1996-9) of HPV prevalence was performed based on an age-stratified random sample of 1,340 women with normal cervical cytology (Lazcano-Ponce E et al., 2001). The 1st peak in HPV prevalence occurred in women ≤ 24 years of age [16% “high risk” (HR) HPV; 0.4% “low risk” (LR) HPV]. A 2nd peak of HR HPV infections was found in women ≥ 65 years of age (17% HR HPV; 6% LR HPV). Among women with HR HPV types, there was a higher intensity of PCR signal in the younger age groups ($p < 0.001$). The authors suggested 2 potential explanations for the 2nd peak: (i) higher HPV exposure of older women when they were young (cohort effect) or (ii) reactivation of latent HPV infections by reduction of immune surveillance or hormonal factors associated with older age.
- ✓ In a recent large population-based survey (Guanacaste, Costa Rica), a 2nd peak was observed among women ≥ 55 years of age. The possibility of reactivation of latent HPV infections was proposed by the authors as one possible explanation (Herrero R et al., 2000). However, in the Guanacaste study, the 2nd peak in women ≥ 55 years of age was mainly “low risk” and noncharacterized HPV types.
- ✓ A study in London among women (N=2988) attending a screening program also showed an increase in HPV detection (high risk types) in women >55 years of age (Cuzick J et al., 1999).

Finally, it is worth noting that capsid proteins are not (in general) of interest as therapeutic vaccines. Since the capsid proteins are not expressed at detectable levels by basal keratinocytes, therapeutic vaccines (not the subject of this VRBPAC) generally have been designed to target other nonstructural viral antigens (Ling M et al., 2000).

Other HPV Associated Diseases

Anogenital Warts (Condylomata acuminata): This non-malignant, but distressing disease is clinically apparent in approximately 1% of the sexually active population in the U.S. (Koutsky L, 1997). Of interest, “latent” infections that can be detected only by the presence of HPV DNA, with neither visible nor histologic abnormality, are probably the most common form of anogenital HPV infection, regardless of HPV type (Handsfield HH, 1997). Visible genital warts usually are caused by HPV types 6 or 11 which are considered “low risk” HPV types (CDC, 1998). About 3/4s of patients with anogenital warts are asymptomatic. Itching and burning, pain, and tenderness are encountered in symptomatic persons (Chuang T-Y, et al., 1984). Depending on the size and anatomic locations, genital warts can be painful, friable, and/or pruritic. HPV types 6 and 11 also can cause warts on the uterine cervix and in the vagina, urethra, and anus; these warts are sometimes symptomatic. Biopsy of genital warts is seldom needed; however, an exception would include cervical warts, in which case a high-grade lesion would need to be excluded prior to therapy (CDC, 1998).

Following sexual transmission, genital warts have an incubation period ranging from 3 weeks to 8 months (average, approximately 3 months) (Oriol JD, 1971; Tuomala RE et al., 1999). This is consistent with a review paper that reports patients with new onset of obvious warts following sexual exposure to a new partner usually develop visible lesions after an average of 2 to 3 months (Handsfield HH, 1997). The transmission rate for genital warts is reported to be 25% to 60% (Oriol JD, 1971); infectivity may be influenced by the number of lesions and by the ages of the lesions. Genital warts of longer duration may be less infectious (Tuomala RE et al., 1999).

The natural history of genital warts, particularly of subclinical HPV infection with types 6 and 11, is not well understood (Handsfield HH, 1997). However, spontaneous remission may occur, as demonstrated by the results of randomized, placebo-controlled therapeutic trials (reported in the 1990s) that indicate a 10 to 20% spontaneous remission rate in untreated lesions over a 3- to 4-month period (Bonnez W et al., 2000).

Recurrent respiratory papillomatosis (RRP): RRP is a disease associated with HPV types 6 and 11 that is manifested by exophytic lesions of the airway. Among pediatric cases, the peak age of diagnosis is 2-4 years of age. RRP has potentially morbid consequences because of its involvement of the airway and the relatively rare risk of malignant conversion (Derkay CS, 2001). Although RRP is likely acquired in most cases during passage through the birth canal, this disease has also occurred in infants delivered by cesarean section. Although HPV could be recovered from the nasopharyngeal secretions of ~30% of infants exposed to HPV in the birth canal, the number of infants expected to manifest evidence of RRP is only a small fraction of this percentage. Specifically, it has been estimated that the risk of a child contracting RRP from a mother who has an active condylomatous lesion and delivers vaginally is about 1 in 400 (Derkay CS, 2001). Currently, the presence of genital warts in a pregnant women is not an indication for cesarean section, to prevent transmission of HPV to the newborn (CDC, 1998; AAP 2000).

Anal Cancer: An estimated 3,500 new cases of anal cancer and 500 deaths due to anal cancer have been projected for the U.S. for 2001 (Greenlee RT et al., 2001). Overall, women get anal cancer slightly more often than men, with a ratio currently of about 4:3. However, in the U.S., there has been an increase in incidence in men under 45 years of age. This has reversed the sex ratio in this younger group (Shank B et al., 2000 [review]). Like CIN, anal intraepithelial neoplasia (AIN) may progress from low to high grade and is found in areas adjacent to frank squamous cell carcinoma of the anus.

Risk factors reported for anal cancer include a history of the following: STDs including anogenital warts, receptive anal intercourse, cervical cancer, and immunosuppression after solid organ transplantation. Despite the evidence that HIV infection increases the likelihood of HPV infection and the subsequent development of high-grade anal intraepithelial neoplasia, it is unclear from actual epidemiological data whether HIV infection itself has a direct effect on the development of anal cancer (Ryan DP et al., 2000 [review]).

HPV data from anal cancer from a population based case control study in Denmark are provided below (Frisch M et al., 1997).

Table 18. HPV in tumors from 388 patients with invasive or *in situ* anal cancer, by PCR. (From Table 5 in Frisch M et al., 1997)

Variable	Women (n=304)	Men (n=84)
	No. of patients (%)	
HPV detected*	282 (93)	58 (69)
High-risk type		
Any†	272 (89)	55 (65)
16	235 (77)	48 (57)
18	18 (6)	4 (5)
31	3 (1)	0
33	20 (7)	3 (4)
Low-risk type		
Any‡	11 (4)	5 (6)
6	3 (1)	2 (2)
11	0	0
HPV not detected	22 (7)	26 (31)

*Some patients had more than one type of HPV.

†The high-risk types included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

‡The low-risk types included 6, 11, 40, 42, 43, and 44.

References

AAP 2000 Red Book: Report of the Committee on Infectious Diseases, 25th ed., Copyright 2000. American Academy of Pediatrics

ALTS Group. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. J Natl Cancer Inst 2000;92:397-402.

Andersson S, Rylander E, Larsson B, Strand A, Silfversvard C, Wilander E. The role of human papillomavirus in cervical adenocarcinoma carcinogenesis. Eur J Cancer 2001;37:246-50.

Andersen ES, Nielsen K, Pedersen B. The reliability of preconization diagnostic evaluation in patients with cervical intraepithelial neoplasia and microinvasive carcinoma. Gynecol Oncol 1995;59:143-7.

Anttila T, Saikku P, Koskela P, Bloigu A, Dillner J, Ikaheimo I, Jellum E, Lehtinen M, Lenner P, Hakulinen T, Narvanen A, Pukkala E, Thoresen S, Youngman L, Paavonen J. Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. JAMA 2001;285:47-51.

Anttila A, Pukkala E, Soderman B, Kallio M, Nieminen P, Hakama M. Effect of organised screening on cervical cancer incidence and mortality in Finland, 1963-1995: recent increase in cervical cancer incidence. Int J Cancer 1999;83:59-65.

ASCCP. (1) Clinical Management of LSIL (draft). Consensus Conference on Cytological Abnormalities and Cervical Cancer Precursors. September 6-9, 2001, Bethesda, MD. <http://consensus.asccp.org/guidelines.asp>

ASCCP. (2) Management of Atypical Squamous Cells (ASC) (draft). Consensus Conference on Cytological Abnormalities and Cervical Cancer Precursors. September 6-9, 2001, Bethesda, MD. <http://consensus.asccp.org/guidelines.asp>

ASCCP. (3) Clinical Management of Atypical Glandular Cells (AGC) (draft). Consensus Conference on Cytological Abnormalities and Cervical Cancer Precursors. September 6-9, 2001, Bethesda, MD. <http://consensus.asccp.org/guidelines.asp>

Benedet JL, Bender H, Jones H, Ngan HY, Pecorelli S. FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology. Int J Gynaecol Obstet 2000;70:209-62.

Bergstrom R, Sparen P, Adami HO. Trends in cancer of the cervix uteri in Sweden following cytological screening. Br J Cancer 1999; 81:159-66.

Beskow AH, Josefsson AM, Gyllensten UB. HLA class II alleles associated with infection by HPV 16 in cervical cancer *in situ*. Int J Cancer Published Online: 16 Jun 2001.

Bigrigg MA, Codling BW, Pearson P, Read MD, Swingler GR. Colposcopic diagnosis and treatment of cervical dysplasia at a single clinic visit. Experience of low-voltage diathermy loop in 1000 patients. Lancet 1990;336(8709):229-31.

Bishop JW, Bigner SH, Colgan TJ, Husain M, Howell LP, McIntosh KM, Taylor DA, Sadeghi MH. Multicenter masked evaluation of AutoCyte PREP thin layers with matched conventional smears. Including initial biopsy results. Acta Cytol 1998;42:189-197.

Boffetta P, Parkin DM. Cancer in developing countries. CA Cancer J Clin 1994;44:81-90.

Bolger BS, Lewis BV. A prospective study of colposcopy in women with mild dyskaryosis or koilocytosis. Br J Obstet Gynaecol 1988;95:1117-9.

Boncz W and Reichman RC. Chapter 133. Papillomaviruses. In: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Eds. Mandell GL, Cheatham OR, Bennett JE, Dolin R. 5th Edition, 1999.

Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995; 87:796-802.

Bristow RE, Montz FJ. Workup of the Abnormal Pap Test. Clinical Cornerstone 3:12-24, 2000.

Buckley SL, Tritz DM, Van Le L, Higgins R, Sevin BU, Ueland FR, DePriest PD, Gallion HH, Bailey CL, Kryscio RJ, Fowler W, Averette H, van Nagell JR Jr. Lymph node metastases and prognosis in patients with stage IA2 cervical cancer. Gynecol Oncol 1996;63:4-9.

Cannistra SA, Niloff JM. Cancer of the Uterine Cervix. N Engl J Med 334: 1030-1037, 1996.

CDC, Division of STD Prevention. Prevention of Genital HPV Infection and Sequelae: Report of an External Consultants' Meeting. Department of Health and Human Services, Atlanta: Centers for Disease Control and Prevention (CDC), December 1999. (Available at: <http://www.cdc.gov/nchstp/dstd/dstdp.html>)

CDC. 1998 Guidelines for the treatment of sexually transmitted diseases. MMWR. 1998;47(RR-1):1–116. <http://www.cdc.gov/mmwr/preview/mmwrhtml/00050909.htm>

Chang AR: Carcinoma *in situ* of the cervix and its malignant potential. A lesson from New Zealand. Cytopathology 1:321-8, 1990.

Chuang T-Y, Perry HO, Kurland LT, et al. Condyloma acuminatum in Rochester, Minn., 1950–1978: I. Epidemiology and clinical features. Arch Dermatol 1984;120:469–475.

Cox JT, Lorincz AT, Schiffman MH, Sherman ME, Cullen A, Kurman RJ. Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 1995;172:946–954.

Cuzick J, Beverley E, Ho L, Terry G, Sapper H, Mielzynska I, Lorincz A, Chan WK, Krausz T, Soutter P. HPV testing in primary screening of older women. Br J Cancer. 1999;81:554-8.

DeMay RM. The Art and Science of Cytopathology. CD-ROM. American Society of Clinical Pathologists (ASCP). 1999.

DePriest PD, van Nagell JR Jr, Powell DE. Microinvasive cervical cancer. Clin Obstet Gynecol 1990;33:846-51

Derkay CS. Recurrent respiratory papillomatosis. Laryngoscope 2001;111:57-69.

Dillner J. Trends over time in the incidence of cervical neoplasia in comparison to trends over time in human papillomavirus infection. Journal of Clinical Virology. 2000;19,7-23.

Duerr A, Kieke B, Warren D, Shah K, Burk R, Peipert JF, Schuman P, Klein RS; HER Study group. Human papillomavirus-associated cervical cytologic abnormalities among women with or at risk of infection with human immunodeficiency virus. Am J Obstet Gynecol 2001;184:584-590.

Eddy DM. Screening for cervical cancer. Ann Intern Med 1990;113:214-226.

Eide TJ. Cancer of the uterine cervix in Norway by histologic type 1970–84. J Natl Cancer Inst 1987;79:199–205.

Elkas JC, Farias-Eisner R, Berek JS. Cervix, Vulva, and Vagina. Chapter 71 in: Clinical Oncology. Editors: Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE. Churchill Livingstone. January 2000.

Franceschi S, Dal Maso L, Arniani S, et al, for the Cancer and AIDS Registry Linkage Study. Risk of cancer other than Kaposi's sarcoma and non-Hodgkin's lymphoma in persons with AIDS in Italy. Br J Cancer 1998;78:966-970.

Freeman J, Hutchison GB. Prevalence, incidence and duration. Am J Epidemiol 1980;112:707-23.

Ferenczy A, Mitao M, Nagai N, Silverstein SJ, Crum CP. Latent papillomavirus and recurring genital warts. N Engl J Med 1985;313:784-8.

Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Desy M, Rohan TE. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999;180:1415-23.

Frisch M, Glimelius B, van den Brule AJC. Sexually transmitted infection as cause of anal cancer. N Engl J Med 1997;337:1350-1358.

Garnett GP, Waddell, HC Public health paradoxes and the epidemiological impact of an HPV vaccine. Journal of Clinical Virology 2000;19:101-111.

Giannoudis A, Herrington CS. Human papillomavirus variants and squamous neoplasia of the cervix. J Pathol 2001;193:295-302.

Goedert JJ, Cote TR, Virgo P, et al, for the AIDS-Cancer Match Study Group. Spectrum of AIDS-associated malignant disorders. Lancet 1998;358:1833-1839.

Goff BA, Atanasoff P, Brown E, Muntz HG, Bell DA, Rice LW. Endocervical glandular atypia in Papanicolaou smears. Obstet Gynecol 1992;79:101-104.

Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. CA Cancer J Clin 2001;51:15-36.

Gulich AE. Cancer Risk in Persons With HIV/AIDS in the Era of Combination Antiretroviral Therapy. The AIDS Reader 10:341-346, 2000.

Handsfield HH. Clinical presentation and natural course of anogenital warts. Am J Med 1997;102:16-20.

Hepler TK, Dockerty MT, Randall LM. Primary adenocarcinoma of the cervix. Am J Obstet Gynecol 1952;63:800-808.

Herrero R, Hildesheim A, Bratti C, Sherman M, Hutchinson M, Morales J, et al. A population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J Natl Cancer Inst 2000;92:464-74.

Hildesheim A, Schiffman MH, Gravitt PE, Glass AG, Greer CE, Zhang T, Scott DR, Rush BB, Lawler P, Sherman ME, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994;169:235-240.

Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural History of Cervicovaginal Papillomavirus Infection in Young Women. N Engl J Med 1998;338:423-8.

Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P, Basu J, Tachezy R, Lewis R, Romney S. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. J Natl Cancer Inst 1995;87:1365-1371.

Howley PM, Lowy DR. Papillomaviruses and their replication. Chapter 65 In: Fields Virology. 4th Edition. Eds: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE. Lippincott Williams & Wilkins. 2001.

Iwasawa A, Nieminen P, Lehtinen M, Paavonen J. Human papillomavirus DNA in uterine cervix squamous cell carcinoma and adenocarcinoma detected by polymerase chain reaction. Cancer 1996;77:2275-9.

Jakus S, Edmonds P, Dunton C, King SA. Margin status and excision of cervical intraepithelial neoplasia: a review. Obstet Gynecol Sur 2000;55:520-7.

Janicek, MF, Averette HE. Cervical Cancer: Prevention, Diagnosis, and Therapeutics. CA Cancer J Clin 2001;51:92-114.

Jones BA, Davey DD. Quality management in gynecologic cytology using interlaboratory comparison. Arch Pathol Lab Med 2000;124:672-81.

Josefsson AM, Magnusson PK, Ylitalo N, Sorensen P, Qwarforth-Tubbin P, Andersen PK, Melbye M, Adami HO, Gyllensten UB. Viral load of human papillomavirus 16 as a determinant for development of cervical carcinoma *in situ*: A nested case-control study. Lancet 2000;355:2189-93.

Kalantari M, Karlsen F, Johansson B, Sigurjonsson T, Warleby B, Hagmar B. Human papillomavirus findings in relation to cervical intraepithelial neoplasia grade: a study on 476 Stockholm women, using PCR for detection and typing of HPV. Hum Pathol 1997;28:899-904.

Kaufman RH. Atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion: diagnostic criteria and management. Am J Obstet Gynecol 1996;175(4 Pt 2):1120-1128.

Keefe KA and Meyskens FL, Jr. Cancer Prevention. Chapter 15 in: Clinical Oncology. Editors: Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE. Churchill Livingstone. January 2000.

Kibur M, Geijerstamm V, Pukkala E, Koskela P, Luostarinen T, Paavonen J, Schiller J, Wang Z, Dillner J, Lehtinen M. Attack rates of human papillomavirus type 16 and cervical neoplasia in primiparous women and field-trial designs for HPV16 vaccination. Sex Transm Inf 2000;76:13–7.

Kinney WK, Manos MM, Hurley LB, Ransley JE. Where's the high-grade cervical neoplasia? The importance of minimally abnormal Papanicolaou diagnoses. Obstet Gynecol 1998;91:973–976.

Kjellberg L, Wang Z, Wiklund F, Edlund K, Angstrom T, Lenner P, Sjoberg I, Hallmans G, Wallin K., Sapp M, Schiller J, Wadell G, Mahlck C, Dillner J. Sexual behaviour and papillomavirus exposure in cervical intraepithelial neoplasia: a population-based case-control study. J Gen Virol 1999;80: 391-398.

Kotloff KL, Wasserman SS, Russ K, Shapiro S, Daniel R, Brown W, Frost A, Tabara SO, Shah K. Detection of genital human papillomavirus and associated cytological abnormalities among college women. Sex Transm Dis 1998;25:243-250.

Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med 1997;102:3-8.

Koutsky LA, Holmes KK, Critchlow CW, Stevens CE, Paavonen J, Beckmann AM, DeRouen TA, Galloway DA, Vernon D, Kiviat NB. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N Engl J Med 1992;327:1272-8.

Krivak TC, Rose GS, McBroom JW, Carlson JW, Winter WE 3rd, Kost ER. Cervical adenocarcinoma in situ: a systematic review of therapeutic options and predictors of persistent or recurrent disease. Obstet Gynecol Surv 2001;56:567-75.

Kubbutat MHG, Vousden KH. Role of E6 and E7 oncoproteins in HPV-induced anogenital malignancies. Semin Virol 1996;7:295–304.

Lazcano-Ponce E, Herrero R, Munoz N, Cruz A, Shah KV, Alonso P, Hernandez P, Salmeron J, Hernandez M. Epidemiology of HPV infection among Mexican women with normal cervical cytology. Int J Cancer 2001;91:412-20

Laimins LA. Human papillomaviruses target differentiating epithelium for virion production and malignant conversion. Semin Virol 1996;7:305-313.

Lee SS, Collins RJ, Pun TC, Cheng DK, Ngan HY. Conservative treatment of low grade squamous intraepithelial lesions (LSIL) of the cervix. Int J Gynaecol Obstet 1998;60:35-40.

Lehtinen M, Kibur M, Luostarinen T, Anttila A, Pukkala E. Prospects for phase III-IV HPV vaccination trials in the Nordic countries and in Estonia. J Clin Virol 2000;19:113-22.

Lehtinen M, Luukkaala T, Wallin KL, Paavonen J, Thoresen S, Dillner J, Hakama M. Human papillomavirus infection, risk for subsequent development of cervical neoplasia and associated population attributable fraction. J Clin Virol 2001;22:117-24.

Liaw KL, Hildesheim A, Burk RD, Gravitt P, Wacholder S, Manos MM, Scott DR, Sherman ME, Kurman RJ, Glass AG, Anderson SM, Schiffman M. A prospective study of human papillomavirus (HPV) type 16 DNA detection by polymerase chain reaction and its association with acquisition and persistence of other HPV types. J Infect Dis 183:8-15, 2001.

Liaw KL, Glass AG, Manos MM, Greer CE, Scott DR, Sherman M, Burk RD, Kurman RJ, Wacholder S, Rush BB, Cadell DM, Lawler P, Tabor D, Schiffman M. Detection of human papillomavirus DNA in cytologically normal women and subsequent cervical squamous intraepithelial lesions. J Natl Cancer Inst 1999;91:954-60.

Ling M, Kanayama M, Roden R, Wu T. Preventive and therapeutic vaccines for human papillomavirus-associated cervical cancers. J Biomed Sci 2000;7:341-356.

Lonky NM, Sadeghi M, Tsadik GW, Petitti D. The clinical significance of the poor correlation of cervical dysplasia and cervical malignancy with referral cytologic results. Am J Obstet Gynecol 1999;181:560-6.

Lowy DR, Howley PM. Papillomaviruses. Chapter 66 In: Fields Virology. 4th Edition Eds: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE. Lippincott Williams & Wilkins. 2001.

McIndoe WA, McLean MR, Jones RW, Mullins PR. The invasive potential of carcinoma *in situ* of the cervix. Obstet Gynecol 1984;64:451-8.

McCrory DC, Matchar DB, Bastian L, Datta S, Hasselblad V, Hickey J, Myers E, Nanda K. Evaluation of cervical cytology. Evidence Report/Technology Assessment No. 5. (Prepared by Duke University under contract no. 290-97- 0014). AHCPH Publication No. 99-E010. Rockville, Maryland: Agency for Health Care Policy and Research, 1999.

McLachlin CM and Crum CP. Papillomaviruses and cervical neoplasia. Chapter 20 in: Holland-Frei Cancer Medicine 5th Edition. Editors: R.C. Bast, Jr., D. W. Kufe, R. E. Pollock, R. R. Weichselbaum, J. F. Holland, E. Frei, III May, 2000 B.C. Decker, Inc.

Melnikow J, Nuovo J, Willan AR, Chan BK, Howell LP. Natural history of cervical squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol 1998;92:727-735.

Mikuta JJ, Celebre JA. Adenocarcinoma of the cervix. Obstet Gynecol 1969;33: 753–756.

Moscicki AB, Shiboski S, Broering J, Powell K, Clayton L, Jay N, Darragh TM, Brescia R, Kanowitz S, Miller SB, Stone J, Hanson E, Palefsky J. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Pediatr 1998;132:277-284.

Monsonogo J, Valensi P, Zerat L, Clavel C, Birembaut P. Simultaneous effects of aneuploidy and oncogenic human papillomavirus on histological grade of cervical intraepithelial neoplasia. Br J Obstet Gynaecol 1997;104:723-7.

Nagai Y, Maehama T, Asato T, Kanazawa K. Persistence of human papillomavirus infection after therapeutic conization for CIN 3: is it an alarm for disease recurrence? Gynecol Oncol 2000;79:294-9.

Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, Risse EK, van der Linden HC, Voorhorst FJ, Kenemans P, Meijer CJ. Relation of human papillomavirus status to cervical lesions and consequences for cervical cancer screening: a prospective study. Lancet 1999;354:20-25.

Muñoz N. Human papillomavirus and cancer: the epidemiological evidence. J Clin Virology 2000;19:1-5.

Oriel JD. Natural history of genital warts. Br J Vener Dis 1971;47:1-13.

Östör AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol 1993;12:186–192.

Östör AG, Duncan A, Quinn M, Rome R. Adenocarcinoma *in situ* of the uterine cervix: an experience with 100 cases. Gynecol Oncol 2000;79:207-10.

Östör AG. Early invasive adenocarcinoma of the uterine cervix. Int J Gynecol Pathol 2000;19:29-38.

Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999;80:827–41.

Pecorelli S, Benedet JL, Creasman WT, Shepherd JH. FIGO staging of gynecologic cancer. 1994-1997 FIGO Committee on Gynecologic Oncology. International Federation of Gynecology and Obstetrics. Int J Gynaecol Obstet 1999;64:5-10.

Penn I. Cancers of the anogenital region in renal transplant recipients: analysis of 65 cases. Cancer 1986;58:611-16.

Pirog EC, Kleter B, Olgac S, Bobkiewicz P, Lindeman J, Quint WG, Richart RM, Isacson C. Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma. Am J Pathol 2000;157:1055-1062.

Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer 1999;83:18-29.

Plaxe SC, Saltzstein SL. Estimation of the duration of the preclinical phase of cervical adenocarcinoma suggests that there is ample opportunity for screening. Gynecol Oncol 1999; 75: 55–61.

Porreco R, Penn I, Droegemueller W, Greer B, Makowski E. Gynecologic malignancies in immunosuppressed organ homograft recipients. Obstet Gynecol 45:359–364, 1975.

Raab SS, Bishop NS, Zaleski MS. Effect of cervical disease history on outcomes of women who have a pap diagnosis of atypical glandular cells of undetermined significance. Gynecol Oncol 1999;74:460-4.

Ries LAG, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Edwards BK (eds). SEER Cancer Statistics Review, 1973-1998. National Cancer Institute, Bethesda, MD, 2000.

Roden RBS, Greenstone HL, Kirnbauer R, Booy FP, Jessie J, Lowy DR, Schiller JT. *In vitro* generation and type-specific neutralization of a human papillomavirus type 16 virion pseudotype. J Virol 1996;70:5875-83.

Roden RBS, Hubbert NL, Kirnbauer R, Christensen ND, Lowy DR, Schiller JT. Assessment of the serological relatedness of genital human papillomaviruses by hemagglutination inhibition. J Virol 1996;70:3298-3301.

Ronnett BM, Manos MM, Ransley JE, Fetterman BJ, Kinney WK, Hurley LB, Ngai JS, Kurman RJ, Sherman ME. Atypical glandular cells of undetermined significance (AGUS): cytopathologic features, histopathologic results, and human papillomavirus DNA detection. Hum Pathol 1999;30:816-25.

Ryan DP, Compton CC, Mayer RJ. Carcinoma of the anal canal. N Engl J Med 2000;342:792-800.

Schiller JT, Hildesheim A. Developing HPV virus-like particle vaccines to prevent cervical cancer: a progress report. Journal of Clinical Virology 2000; 19: 67–74.

Schorge JO, Lee KR, and Sheets EE. Prospective Management of Stage IA1 Cervical Adenocarcinoma by Conization Alone to Preserve Fertility: A Preliminary Report. Gynecologic Oncology 2000;78:217-220.

Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB, Scott DR, Sherman ME, Kurman RJ, and Wacholder S. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia J Natl Cancer Inst 1993 85: 958-964.

Schneider V, Henry MR, Jimenez-Ayala M, Turnbull LS, Wright TC. Cervical cancer screening, screening errors and reporting. Acta Cytol 2001;45:493-8.

Shank B, Enker WE, Flam MS. Neoplasms of the anus. Chapter 104 *in*: Holland-Frei Cancer Medicine 5th Edition. Editors: R.C. Bast, Jr., D. W. Kufe, R. E. Pollock, R. R. Weichselbaum, J. F. Holland, E. Frei, III May, 2000 B.C. Decker, Inc.

Sheets EE. The Cervix. Chapter 6 in: Ryan: Kistner's Gynecology & Women's Health, Seventh Edition, 1999 Mosby, Inc.

Slater D. New Zealand cervical cancer study: could it happen again? BMJ 1988;297:918.

Smith HO, Tiffany MF, Qualls CR, Key CR. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States--a 24-year population-based study. Gynecol Oncol 2000 Aug;78(2):97-105.

Solomon D, Schiffman M, Tarone R for the ALTS Group. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J Natl Cancer Inst 2001;93:293-9.

Spitzer M, Chernys AE, Seltzer VL. The use of large-loop excision of the transformation zone in an inner-city population. Obstet Gynecol 1993;82:731-5.

Stern PL, Brown M, Stacey SN, Kitchener HC, Hampson I, Abdel-Hady ES, Moore JV. Natural HPV immunity and vaccination strategies. Journal of Clinical Virology 2000; 19: 57-66.

Stubenrauch F, Laimins LA. Human papillomavirus life cycle: active and latent phases. Semin Cancer Biol 1999;9:379-86.

Sun XW, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright TC Jr. Human papillomavirus infection in women infected with the human immunodeficiency virus. N Engl J Med 1997;337:1343-1349.

Swan DC, Tucker RA, Tortolero-Luna G, Mitchell MF, Wideroff L, Unger ER, Nisenbaum RA, Reeves WC, Icenogle JP. Human Papillomavirus (HPV) DNA Copy Number Is Dependent on Grade of Cervical Disease and HPV Type. J Clin Microbiol 1999; 37:1030-1034.

Schwartz SM, Weiss NS. Increased incidence of adenocarcinoma of the cervix in young women in the United States. Am J Epidemiol 1986;124:1045-1047.

Takevchi A and McKay DG. The area of cervix involved by carcinoma *in situ* and anaplasia (atypical hyperplasia). Obstet Gynecol 1960;15:134-45.

The 1988 Bethesda System for reporting cervical/vaginal cytological diagnoses. National Cancer Institute Workshop. JAMA 1989;262:931-4.

The Bethesda System for reporting cervical/vaginal cytologic diagnoses: revised after the second National Cancer Institute Workshop, April 29-30, 1991. Acta Cytol 1993;37:115-24.

The Bethesda 2001 Workshop. (1) LSIL / HSIL Forum Draft. Post-meeting recommendations. <http://bethesda2001.cancer.gov/>

The Bethesda 2001 Workshop. (2) ASCUS Forum Draft. Post-meeting recommendations. <http://bethesda2001.cancer.gov/>

The Bethesda 2001 Workshop. (3) Atypical Glandular Cells (AGCs) Forum. Post-meeting recommendations. <http://bethesda2001.cancer.gov/>

Thomas DB, Ray RM, Koetsawang A, Kiviat N, Kuypers J, Qin Q, Ashley RL, Koetsawang S. Human Papillomaviruses and Cervical Cancer in Bangkok. I. Risk Factors for Invasive Cervical Carcinomas with Human Papillomavirus Types 16 and 18 DNA. Am J Epidemiol 2001;153:723–31.

Thomas DB, Qin Q, Kuypers J, Kiviat N, Ashley RL, Koetsawang A, Ray RM, Koetsawang S. Human Papillomaviruses and Cervical Cancer in Bangkok. II. Risk Factors for *In Situ* and Invasive Squamous Cell Cervical Carcinomas Am J Epidemiol 2001;153:732–9.

Thomas DB, Ray RM, Kuypers J, Kiviat N, Koetsawang A, Ashley RL, Qin Q, Koetsawang S. Human Papillomaviruses and Cervical Cancer in Bangkok. III. The Role of Husbands and Commercial Sex Workers. Am J Epidemiol 2001;153:740–8.

Thomas DB, Ray RM, Pardthaisong T, Chutivongse S, Koetsawang S, Silpisornkosol S, Virutamasen P, Christopherson WM, Melnick JL, Meirik O, Farley TM, Rietton G. Prostitution, condom use, and invasive squamous cell cervical cancer in Thailand. Am J Epidemiol 1996;143:779–86.

Thomas KK, Hughes JP, Kuypers JM, Kiviat NB, Lee SK, Adam DE, Koutsky LA. Concurrent and sequential acquisition of different genital human papillomavirus types. Journal of Infectious Diseases 2000;182:1097-1102.

Tuomala RE and Chen KT. Chapter 18- Gynecologic Infections - Part II in: Ryan: Kistner's Gynecology & Women's Health, Seventh Edition, 1999 Mosby, Inc.

Valdini A, Vaccaro C, Pechinsky G, Abernathy V. Incidence and evaluation of an AGUS Papanicolaou smear in primary care. J Am Board Fam Pract 2001;14:172-7.

van Nagell JR, Donaldson ES, Wood, EG, et al. The significance of vascular invasion and lymphatic infiltration in invasive cervical cancer. Cancer 1978;41:228-234. (get ref)

Wharton JT and Tortolero-Luna G. Neoplasms of the cervix. Chapter 112 in: Holland-Frei Cancer Medicine 5th Edition. Editors: R.C. Bast, Jr., D. W. Kufe, R. E. Pollock, R. R. Weichselbaum, J. F. Holland, E. Frei, III May, 2000 B.C. Decker, Inc.

Walboomers, J.M.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J.F., Peto, J., Meijer, C.J.L.M. and Muñoz, N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-19.

Wang Z, Kjellberg L, Abdalla H, Wiklund F, Eklund C, Knekt P, Lehtinen M, Kallings M, Lenner P, Hallmans G, Mahlck CG, Wadell G, Schiller JT, Dillner J. Type specificity and significance of different isotopes of serum antibodies to human papillomavirus capsids. J Infect Dis 2000;181:456–62.

Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, Yates M, Rollason TP, Young LS. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001;357:1831–36.

Wright TC, Sun XW, Koulos J. Comparison of management algorithms for the evaluation of women with low grade cytologic abnormalities. Obstet Gynecol 1995;85:202–10.

Wright TC, Kurman RJ, Ferenczy A: Precancerous Lesions of the Cervix. In Kurman RJ, ed: *Blaustein's Pathology of the Female Genital Tract*. 4th ed. New York: Springer-Verlag NY Inc, 1994.

Xi LF, Demers GW, Koutsky LA, Kiviat NB, Kuypers J, Watts DH, Holmes KK, Galloway DA. Analysis of human papillomavirus type 16 variants indicates establishment of persistent infection. J Infect Dis 1995; 172:747-55.

Yamada T, Manos MM, Peto J, Greer CE, Munos N, Bosch FX, Wheeler CM. Human papillomavirus type 16 sequence variation in cervical cancers: a worldwide perspective. J Virol 1997;71:2463–72.

Ylitalo N, Sorensen P, Josefsson AM, Magnusson PK, Andersen PK, Ponten J, Adami HO, Gyllensten UB, Melbye M. Consistent high viral load of human papilloma virus 16 and risk of cervical carcinoma *in situ*: A nested case-control study. Lancet 2000;355:2194-8.

Ylitalo N, Josefsson A, Melbye M, Sorensen P, Frisch M, Andersen PK, Soren P, Gustafsson M, Magnusson P, Ponten J, Gyllensten U, Adami HO. A prospective study showing long-term infection with human papillomavirus 16 before the development of cervical carcinoma *in situ*. Cancer Res 2000;60:6027-32.

Zweizig S, Noller K, Reale F, Collis S, Resseguie L. Neoplasia Associated with Atypical Glandular Cells of Undetermined Significance on Cervical Cytology. Gynecol Oncol 1997;65:314-318.

Appendix A
Cervix Uteri Cancer (Invasive)
Age-Adjusted SEER^a Incidence AND U.S. Mortality Rates
By Race/Ethnicity (Females only)
(Table V-7 From Ries LAG et al., 2000)

U.S. Incidenceⁱ

	Rate 1992-1998 Rate per 100,000 persons	Trend 1992-1998 EAPC (%) ⁺
Race/Ethnicity		
All Races	8.7	-2.1*
White	8.1	-2.5*
White Hispanic	15.4	-5.5*
White Non-Hispanic	6.9	-2.1*
Black	11.0	-2.1*
Asian/Pacific Islander	10.3	-2.0
Amer Ind/Alask Nat	6.4	-
Hispanic ^b	14.4	-5.5*

U.S. Mortality^c

	Rate 1992-1998 Rate per 100,000 persons	Trend 1992-1998 EAPC (%) ⁺
Race/Ethnicity		
All Races	2.7	-2.3*
White	2.4	-1.8*
White Hispanic	3.6	-3.9
White Non-Hispanic	2.3	-1.5*
Black	5.7	-4.8*
Asian/Pacific Islander	2.7	-0.9
Amer Ind/Alask Nat	2.9	-4.7
Hispanic ^b	3.3	-3.9*

⁺ The EAPC is the Estimated Annual Percent Change over the time interval.

- Statistic not shown. Rate based on less than 25 cases for the time interval. Trend based on less than 10 cases for at least one year within the time interval.

ⁱ Incidence data are from the 11 SEER areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, Atlanta, San Jose-Monterey, and Los Angeles) and Alaska. Unless specified, other tables use data from 9 SEER areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, and Atlanta).

^a Surveillance, Epidemiology, and End Results (SEER) data from NCI

^b Hispanic is not mutually exclusive from whites, blacks, Asian Pacific Islanders, and American Indians/Native Alaskans. For incidence, all 11 SEER areas are included. For mortality, information is included for all states except Connecticut, Oklahoma, Louisiana, and New Hampshire.

^c Mortality data are analyzed from a public-use file provided by the National Center for Health Statistics (NCHS).

* The EAPC is significantly different from zero ($p < .05$).

Appendix B

Squamous Cell Carcinoma and Adenocarcinoma of the cervix in the U.S., Finland and Sweden over time (Figures A-C, below).

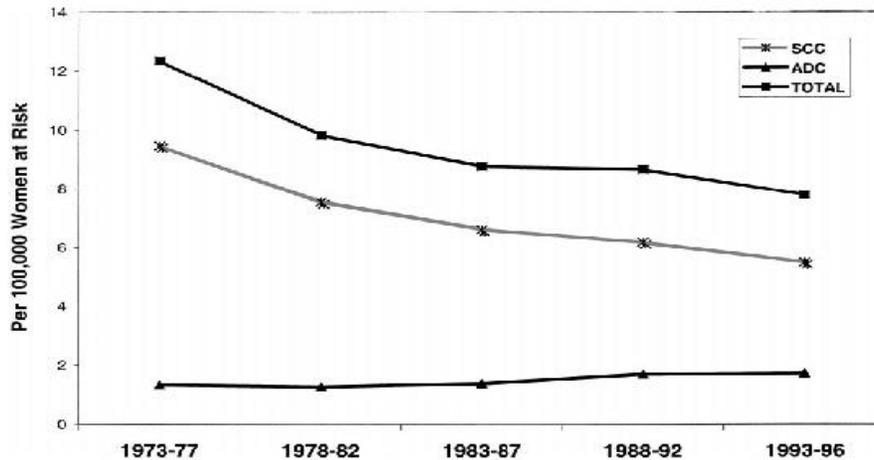


Fig. A. U.S. data. (From Fig. 1, Smith HO, 2000). The age-adjusted incidence rates (per 100,000) for invasive ADC (adenocarcinoma), SCC (squamous cell carcinoma), & total (all invasive cervical cancers inclusive of all histologic types) decreased 37.7% [12.35 (1973–77) vs 7.7 (1993–96)]; decreased 41.9% [9.45 (1973–77) vs 5.49 (1993–1996)], & increased 29.1% [1.34 (1973–1977) vs 1.73 (1993–96)], respectively. The proportion of ADC is increasing relative to total and SCC cases.

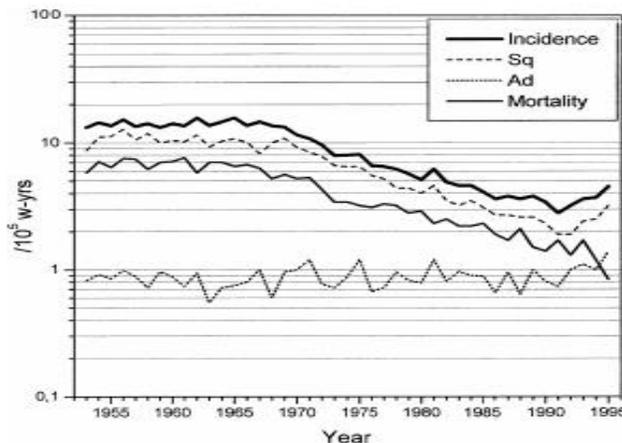


Fig. B. Finnish data. (From Fig. 4, Anttila AA et al, 1999). Cervical cancer incidence and mortality rates in Finland during 1953–1995. (World standardised rates; Sq = incidence of the squamous cell carcinoma of the cervix uteri; Ad = incidence of adenocarcinoma of the cervix uteri.)

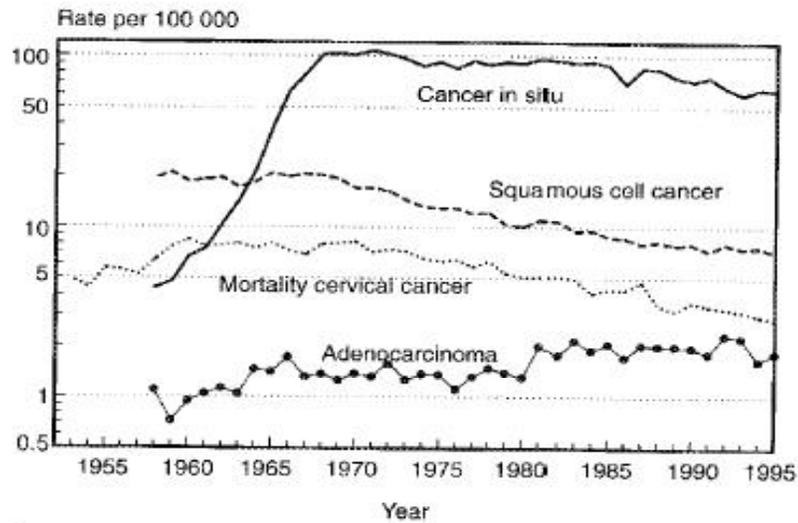
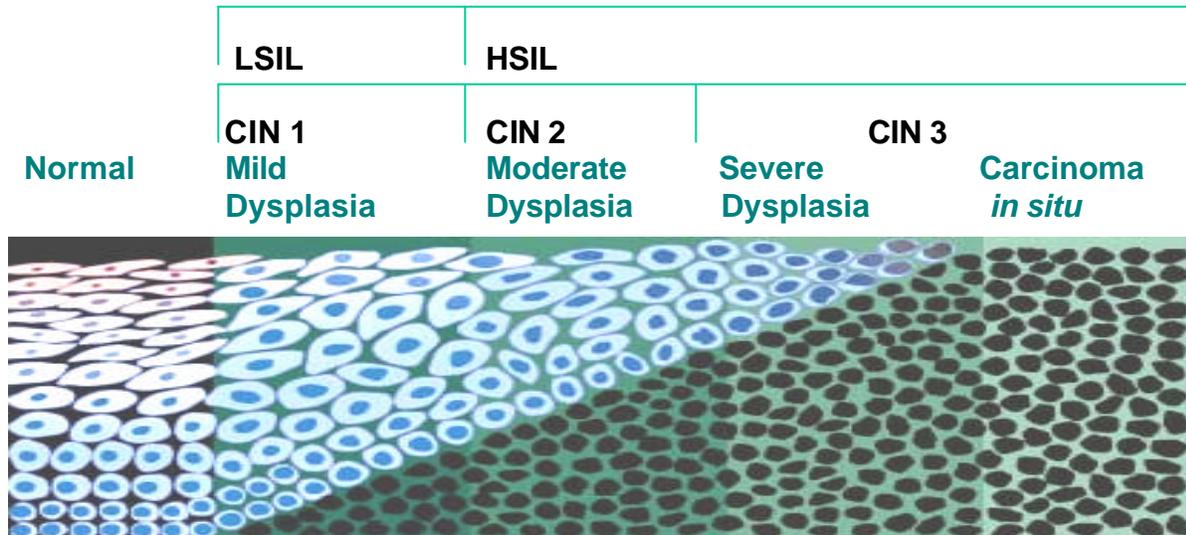
[Appendix B \(continued from previous page\)](#)

Fig. C. Swedish data. (From Fig. 1, Bergstrom R et al., 1999). Trends in age-standardized incidence rates of cancer in situ, squamous cell cancer and adenocarcinoma of the cervix uteri in Sweden, 1958-1995, obtained from the Cancer Registry, and mortality rates from invasive cervical cancer in Sweden, 1953-1992, obtained from the Death Registry.

Appendix C Histology and Cytology (Cervix)

Precursor Lesions of Cervical Carcinoma (Histology)



From Figure 6.13, DeMay RM. *The Art and Science of Cytopathology*. CD-ROM. ASCP. 1999.

Wright TC, Kurman RJ, Ferenczy A: *Precancerous Lesions of the Cervix*. In Kurman RJ, ed: *Blaustein's Pathology of the Female Genital Tract*. 4th ed. New York: Springer-Verlag NY Inc, 1994.

Map of cervical cytology classification schemes. (Figure 2, McCrory DC et al, 1999.)

Classification System	Within Normal Limits	Benign Cellular Changes	Epithelial Abnormalities			
			A	Squamous intraepithelial lesion (SIL)		Invasive carcinoma
The Bethesda System (National Cancer Institute Workshop, 1991)	Normal	Infection reactive repair	ASUS	Low grade (LSIL) High grade (HSIL)		
Richart (1973)			Condyloma	Cervical intraepithelial neoplasia (CIN)		
Reagan (1979) (WHO)		Atypia	Mild dysplasia	Moderate dysplasia	Severe dysplasia	<i>In situ</i> carcinoma
Papanicolaou (Nyrjesy, 1972)	I	II	III		IV	V

ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesions; HSIL = high-grade squamous intraepithelial lesions.

Appendix C (Continued)

Recent recommendations for further modification of cytology terminology.

The Bethesda System (TBS) of classification, designed to further standardize reporting of cervical cytologic findings, was developed after a National Cancer Institute consensus conference in 1988 and was refined in 1991, and again in 2001. A brief summary of some recommendations from **The Bethesda 2001 Workshop**, held April 30 - May 2, 2001, regarding terminology and reporting of cervical **cytology** (not histology) is provided here. (See also <http://bethesda2001.cancer.gov/>)

LSIL/HSIL (The Bethesda 2001 Workshop. (1) LSIL / HSIL Forum Draft)

1. Background: The Proposed Bethesda 2001 System (like the 1991 Bethesda System) sub-divides Squamous Intraepithelial Lesion (SIL) as either
 - **Low-grade squamous intraepithelial lesion (LSIL)** or
 - **High-grade squamous intraepithelial lesion (HSIL)**.Definition: Squamous intraepithelial lesion encompasses a spectrum of noninvasive cervical epithelial abnormalities traditionally classified as flat condyloma, dysplasia/carcinoma in-situ, and CIN. In The Bethesda System, the spectrum is divided into low-grade and high-grade lesions. Low-grade lesions encompass the cellular changes associated with HPV cytopathic effect (so-called koilocytotic atypia) and mild dysplasia/CIN 1. High-grade lesions encompass moderate dysplasia, severe dysplasia, and carcinoma *in situ*/CIN 2,3. *Gynecological cytology cases showing diagnostic HSIL in which there is non-diagnostic cytological evidence of invasion should be diagnosed as HSIL and accompanied by the comment "with features suspicious for invasion".* (The sentence in italics that is directive with regard to reporting possible invasion differs from the 1991 Bethesda System.)
2. As noted above, one recommendation from the Bethesda 2001 Workshop is that the 2-tiered cytology classification of LSIL and HSIL be retained. One reason is that there are data analyzed from a study (ALTS) to indicate that cytologists can distinguish between CIN 1 and CIN 2 (cytology) with a fair to good degree of reproducibility, but that the distinction between CIN 2 and CIN 3 was unreliable (based on a low Kappa value). This supports retaining a two-tiered LSIL / HSIL terminology with the dividing line between CIN 1 (mild dysplasia) and CIN 2 (moderate dysplasia). [The Bethesda 2001 Workshop. (2); M. Schiffman cited].

ASCUS (ASC) (The Bethesda 2001 Workshop. (2) ASCUS Forum Draft)

1. The term ASCUS is being replaced with "**Atypical Squamous Cells (ASC)**," which will have a modified definition and dichotomous qualifiers.
2. A definition for the newly created category of Atypical Squamous Cells was proposed: "Cytologic changes suggestive of a squamous intraepithelial lesion that are quantitatively or qualitatively insufficient for a definitive interpretation."
3. Atypical Squamous Cells (ASC) are qualified as either
 - "Undetermined Significance (ASC-US)" (expected to account for >90% of diagnoses in most labs) or
 - "Cannot Exclude HSIL (ASC-H)."

(Recommendations regarding glandular lesion cytology presented on next page)

Appendix C (Continued)

Glandular lesions (The Bethesda 2001 Workshop. (3) Atypical Glandular Cells [AGCs] Forum)

1. “*Endocervical adenocarcinoma in situ*” should be added as a discrete interpretation when criteria are adequate.
 2. **Atypical Glandular Cells (AGCs)** may be further qualified as:
 - Atypical Glandular/Endocervical/Endometrial Cells (unqualified)
 - Atypical Glandular/Endocervical Cells, Favor Neoplastic
 - specify further in description
 3. Other items:
 - The qualifier “of undetermined significance” is being eliminated. The category is now “Atypical Glandular Cells” (AGC). The term “Atypical Glandular Cells of Unknown Significance” (AGUS or AGCUS) is no longer used.
 - The qualifier “favor reactive” is being eliminated. The concern is that the term does not uniformly elicit an appropriate clinical response & hence some patients harboring unrecognized high grade lesions may not be properly managed.
 - Atypical glandular cells can and should be qualified as to endocervical or endometrial origin whenever possible, i.e., in the majority of cases.
-

International Federation of Gynecologists And Obstetricians (FIGO): Staging Of Invasive Cervical Cancer*

Stage 0	Carcinoma <i>in situ</i>
Stage I	Carcinoma confined to the uterus
Stage IA	Diagnosed only by microscopy (Not macroscopically visible)
Stage IA1	Microscopically measured invasion of stroma ≤ 3 mm depth; ≤ 7 mm horizontal spread; lymphovascular space involvement does not affect classification
Stage IA2	Microscopically measured invasion of stroma > 3 mm to ≤ 5 mm; ≤ 7 mm horizontal spread; lymphovascular space involvement does not affect classification
Stage IB	Clinically visible lesions confined to the cervix and/or microscopic lesions $>$ Stage IA2
Stage IB1	Clinically visible lesions ≤ 4 cm in greatest dimension
Stage IB2	Clinically visible lesions > 4 cm in greatest dimension
Stage II	Carcinoma extends beyond the uterus but has not extended to the pelvic wall or to the lower third of the vagina
Stage IIA	No obvious parametrial involvement
Stage IIB	Obvious parametrial involvement
Stage III	Tumor extends to the pelvic wall and/or involves the lower third of the vagina and/or causes hydronephrosis or nonfunctioning kidney (unless other etiology)
Stage IIIA	Tumor involves the lower third of the vagina with no extension to the pelvic wall
Stage IIIB	Involves pelvic wall and/or causes hydronephrosis or nonfunctioning kidney
Stage IV	Carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum; bullous edema (by itself) is not classified as Stage IV
Stage IVA	Tumor invades mucosa of bladder or rectum and/or extends beyond true pelvis
Stage IVB	Spread to distant organs

* Benedet JL et al., 2000; Pecorelli S et al., 1999.

Appendix D

Figure A. HPV 16 – Genomic Map (From Table 20.3, McLachlin CM and Crum CP, 2000)

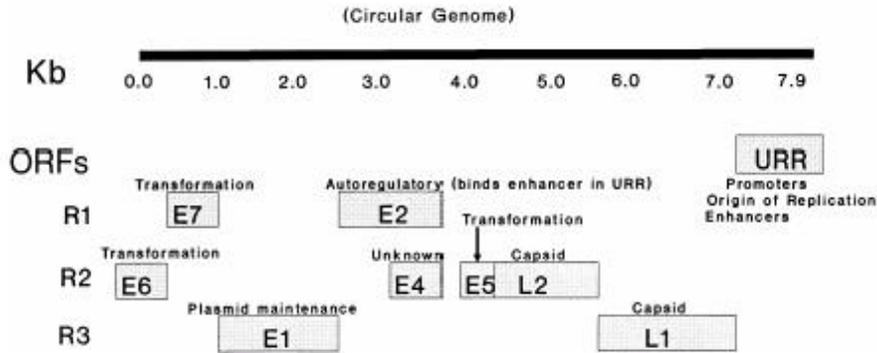
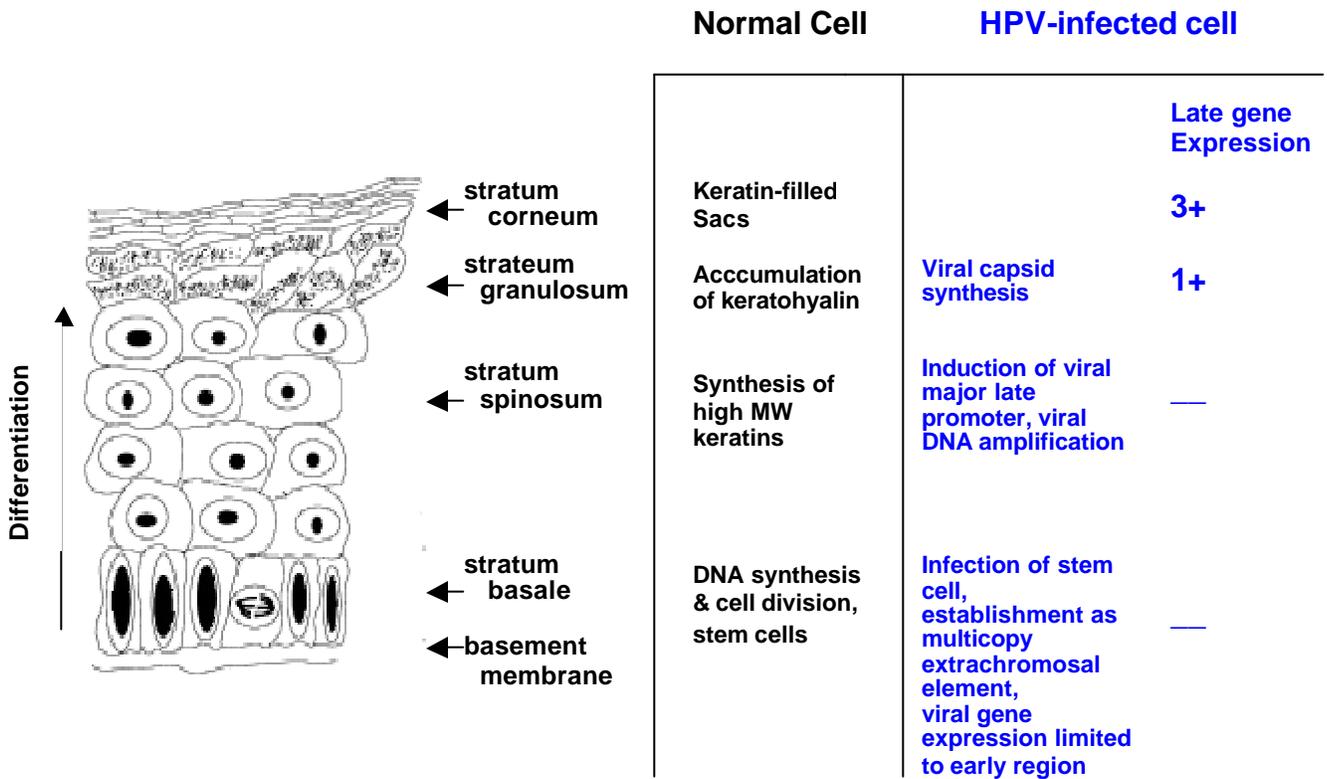


Figure B.



From Figure 1 in Stubenrauch F et al., 1999, & Fig. 1 Laimins LA 1996. The differentiation dependent functions in normal and HPV-infected epithelial cells are depicted here. (Note: There is no granular layer or stratum corneum in non-keratinized squamous epithelia.)

Appendix E**Table. Referral indicators for colposcopy and histologic outcomes****

Referral indicator	Histologic-colposcopic diagnosis								Total
	Negative colposcopy, benign biopsy, Inflammation, or immature metaplasia	Vaginal or vulvar HPV**	Glandular atypia†	Low-grade biopsy of cervix‡	High-grade biopsy of cervix§	ADC in situ*	SCC*	ADC*	
Negative Papanicolaou smear, positive visual	127	5	0	121	33	1	3	0	290
Immature metaplasia	10	1	0	16	2	0	0	0	29
ASCUS undefined	1689	8	11	678	232 (8.9%)	2	1	0	2621
ASCUS reactive process favored	273	0	3	41	14 (4.2%)	0	0	0	331
ASCUS premalignant process favored	91	1	0	45	28 (17%)	0	0	1	166
LGSIL (HPV)	190	17	0	113	37 (10.3%)	1	1	0	359
LGSIL (CIN I)	571	6	9	549	288 (20.2%)	1	0	1	1425
HGSIL	79	1	0	51	123	2	2	2	260
AGUS	40	0	0	18	4 (6.3%)	1	0	1	64
Adenocarcinoma suspected	0	0	1	0	0	0	1	0	2
Squamous carcinoma suspected	0	0	0	1	2	0	0	0	3
Other	24	1	0	10	0	0	0	0	35
SUBTOTAL first visits	3094	40	24	1643	763	8	8	5	5585
Follow-up visits	1591	16	17	26	5	0	0	1	1656
TOTAL all visitsII	4685	56	41	1669	768	8	8	6	7241

All indicators for colposcopy referral were for cytologic findings, except negative Pap smear and positive visual, which indicates a cervical, vaginal, or vulvar lesion was seen but cytologic results were normal.

*SCC = Squamous Cell Carcinoma (cervix); ADC = Adenocarcinoma (cervix)

**Vaginal or vulvar condyloma.

†Atypical endocervical cells.

‡CIN I (mild dysplasia) or condyloma.

§CIN II, CIN III (moderate or severe dysplasia), carcinoma *in situ*.

IIAll patients undergoing colposcopy.

** Table 1 (Modified slightly) from: Lonky NM et al. The clinical significance of the poor correlation of cervical dysplasia and cervical malignancy with referral cytologic results. Am J Obstet Gynecol 1999;181:560-6.