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Preface

Preventing mortality and morbidity from cervical cancer

Neal M. Lonky, MD, MPH
Guest Editor

Over 50 years ago, Papanicolaou and Traut's discovery changed the way we view cancer screening. The link between cytological sampling and histopathology was made, and the natural history of cervical carcinoma was explored. We now face a multi-billion dollar "cervical cancer prevention" practice model and industry which has traditionally advocated screening with the conventional Papanicolaou smear, followed by diagnostic colposcopy, biopsy, and potential treatment of precursors for the "at-risk" patient. Despite all of our efforts, the initial significant reduction in cervical cancer incidence and mortality has reached a plateau in the last decade. The prevalence of high grade precursors and high risk behavior is on the rise.

This issue refocuses on the desired endpoint, as our goal all along has been to the end the suffering and mortality associated with cervical carcinoma. To achieve that goal we must re-examine the etiology, natural history, the existing, and new strategies used in caring for women prone to develop cervical neoplasia and carcinoma. We must measure the value of interventions on several levels which include the impact on the quality of care (outcomes), the quality of services rendered (accessible, culturally sensitive care), and cost effectiveness or benefit.

The intent of the authors of this issue is to provide a guide for women's health care givers that is hinged upon finding effective interventions in the context of three levels of prevention. Our introductory articles set the context with background information, and provides a financial "primer" on standardizing and measuring the value of health care interventions. We then "begin with the end in

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mind'' by addressing saving the lives of women with invasive cervical carcinoma (tertiary prevention). The subsequent section deals with the identification of cervical intraepithelial neoplasia (CIN) and the prevention of progression of these precursors toward malignancy (secondary prevention). The concluding section focuses on primary prevention through the identification and management of the ''at-risk'' patient for cervical carcinoma, and the emerging vaccination strategies which may potentially prevent the development of CIN precursors or alter the progression of those with established precursors.

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Neal M. Lonky, MD, MPH
Member, Board of Directors
Southern California Permanente Medical Group
Director of Medical Education and Research Chairman
Kaiser Permanente
Dept. of Obstetrics and Gynecology
1188 N. Euclid Street
Anaheim, CA 92801, USA
Clinical Professor, Dept. of Obstetrics and Gynecology
University of California, Irvine, CA, USA

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Paying for prevention
Standardizing the measurement of the value of health care interventions

Steven A. Vasilev, MD, MBA, FACOG, FACS

Department of Gynecologic Oncology, Kaiser Permanente, 4900 Sunset Boulevard, Building M, Los Angeles, CA 90027, USA

In the context of all the clinical aspects of primary, secondary, and tertiary prevention of cervical cancer, how does one measure and stratify the added value of screening strategies? Upon adoption of widespread Papanicolaou (Pap) smear screening in the United States, invasive cervical cancer incidence decreased by 36% from 1973 to 1991, accompanied by a 42% reduction in the age-specific mortality rate [1,2]. Although this success is credited to cytologic screening, no prospective randomized trial has ever demonstrated that the mortality reduction is attributable to screening. Nevertheless, this success story is widely accepted because of overwhelming epidemiologic evidence. When designing screening programs for the future, epidemiological evidence is a pivotal issue; determining quality and effectiveness of new programs and new technologies should be done by modeling, using high-level evidence, and continuing critical analysis of population-specific outcomes rather than by retrospective trial and error. Demonstrable and reproducible clinical effectiveness is an absolute requirement for a believable cost effectiveness analysis.

Despite the widespread availability of screening, women continue to develop cervical cancer [3]. Because nearly 50% of cervical cancers in the US occur in patients who have never been screened and 60% of cases develop in patients who have not been screened in the past 5 years [4], an argument has been made that widespread periodic screening of all women would further reduce the overall incidence of cervical cancer, eventually eliminating it. Barriers to access of Pap smear surveillance have been identified and have been the subject of numerous reviews [5–9].

It is unfortunate that even when Pap screening is readily available and utilized, it might not alter outcomes. Screening had been performed in 63% of women under age 45 who died of cervical cancer in Scotland between 1982 and 1991

E-mail address: Steven.A.Vasilev@kp.org (S.A. Vasilev).

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[10]. Gay and others [11] reported that 20% of women with carcinoma in situ (CIS) or invasive cervical cancer had a normal Pap smear within the preceding year. Thus, it appears that episodic screening might not be sufficient to prevent development of cervical cancer using standard screening strategies. Many patients present with symptoms, and half have been recently screened with at least standard cytologic testing.

Despite the major contribution of the Pap smear to cervical cancer prevention, the conventional Pap smear has significant limitations. Using histologic confirmation as the gold reference standard, the Pap smear's sensitivity might be as low as 20% to 30% [12]. Understanding the clinical limitations of the Pap smear is essential in counseling patients and in designing cost effective screening strategies. Although Pap smears per se are relatively inexpensive, they can lead to further diagnostic workups (eg, colposcopy, biopsy) and patient anxiety. Thus, the financial and emotional costs of Pap smears go well beyond the test itself. These costs by no means diminish the many positive aspects of Pap smear screening, but they include variables that can be difficult to factor into any cost effectiveness equation. It is critical to consider that false-positive or atypical results lead to excessive additional testing and psychological stress for the patient.

From a resource consumption standpoint, screening for cervical cancer and its precursors represents a rapidly expanding, technology-driven, $4 billion per year industry, yet despite "advances," only 50% of at-risk patients are identified. Despite this increasing resource consumption, knowledge about the sensitivity, specificity, and predictive values of new adjunctive testing technologies is meager. The lack of an adequate agreed-upon reference standard case is the overwhelming reason that Pap smear characteristics are not properly assessed or compared in published studies. Thus, even though screening expenditures are on the rise because of variable—and sometimes haphazard—incorporation of new tests, many cervical cancers and precursor lesions are still not detected with Pap smears or adjunctive technologies.

To develop optimal, cost effective screening strategies, a myriad of important questions need to be addressed, including the following. How is cost effectiveness best defined? Is cost effectiveness the best economic analysis tool for screening outcomes? How does one measure the value of a screening intervention, and by what health outcome is this best determined? At what age should screening begin, and how does this impact cost versus benefit? How should abnormal Pap smears be interpreted, and can lesions be stratified according to risk, thus allowing the diversion of follow-up resources to patients who are most likely to benefit from them? What are the limitations of standard cytologic screening in this regard? Can advances in technology help increase the positive and negative predictive value of current screening strategies, optimizing intervention and limiting unnecessary diagnostic evaluation? How can an agreed-upon reference standard case to compare new screening technologies be formed? Finally, incorporating all of the above questions, how does one include and consider all these variables in making complex decisions in the face of uncertainty? These issues and others are the subject of this article.

Decision analysis primer

The lack of a formal approach to multifactorial risk decisions is akin to preparing an annual income tax statement in your head. Some clinical decisions might be (or seem to be) straightforward. When multiple variables and outcomes are entertained, however, a free-form decision, especially in the context of a program planning committee, will predictably fail to take all issues into weighted consideration [13].

Decision analysis rests upon modeling and the concept of *expected value*. Using computer-assisted modeling programs such as DATA (TreeAge Software, Williamstown, MA) [14], anticipated uncertainties are put into perspective and analyzed using Markov (recursive) processes [15]. An influence diagram is usually initially constructed that defines the factors that affect the decision and how they are related. From this diagram a decision tree is created, which follows these basic principles: (1) time flows from left to right, with all events in proper sequence; (2) all clinically important final outcomes must be represented; (3) nodes represent a decision, an uncertainty, or an outcome; (4) branches emanating from a decision node represent all known available options; (5) branches emanating from a chance node represent all possible clinically important outcomes; and (6) probabilities of events are assigned at each chance node and payoffs are assigned at each terminal outcome node.

Fig. 1 represents a rudimentary decision tree for a much simpler screening process, colo-rectal cancer screening tests that are ordered in several possible combinations. Probabilities based on the best available published and local outcome evidence are assigned to each chance node. The tree can then be "rolled back" by the computer program to determine which path is the most likely to provide the best desired outcome, and at what expected value (ie, the outcome value that can be expected on average). The utility outcome or payoff measure can be defined as optimal detection, minimal morbidity, optimal cost structure, quality of life, and so forth. For any decision or chance node branch, a sensitivity analysis should be performed to determine which decision and chance points have the greatest relative uncertainty effects on the final outcome.

Validity of decision analysis must be iteratively reviewed during model construction and at the time of analysis. The following key questions should be kept in mind: (1) Was the appropriate decision model used? (2) Were all appropriate strategies included in a clinically appropriate sequence? (3) Were all clinically relevant outcomes considered? (4) Was an explicit and appropriate process used to collect and transform the available evidence into the probabilities used within the tree? (5) Were appropriate utilities assigned to the possible outcomes? (6) Were the appropriate sensitivity analyses conducted? The last item is particularly important because the quality of input equals the quality of output. If the entire result is overly dependent on a chance or decision data point that is not precise or not well substantiated, the definitiveness of the result questionable.

Serious limitations of decision analysis include: (1) availability and quality of data in formulation of chance node probabilities, (2) potential oversimplification

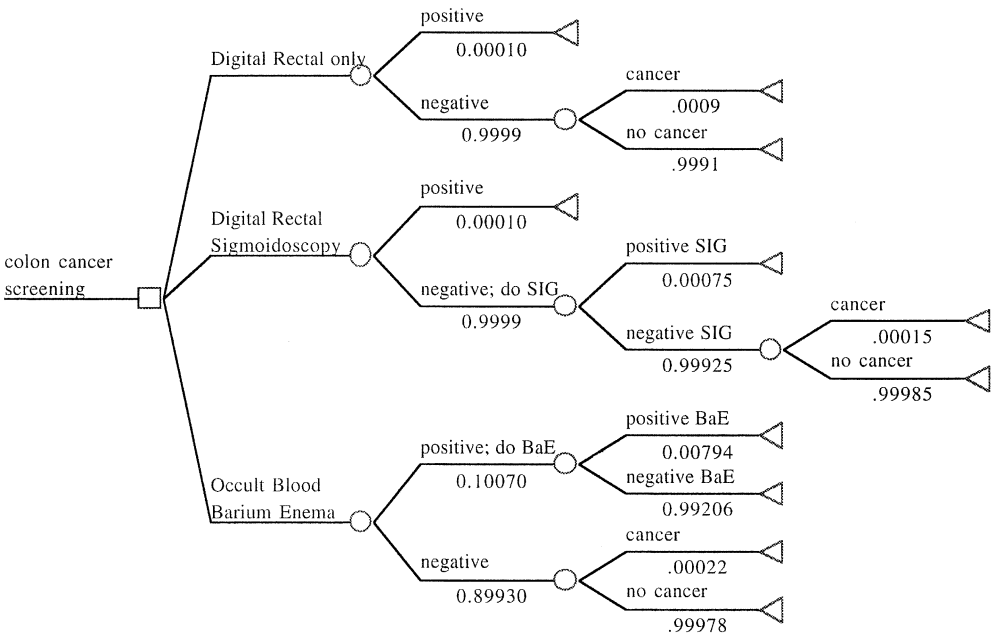

Fig. 1 A sample decision tree for colon cancer screening, a simpler process.

of complex medical decisions, and (3) possibility of subjective assignment of utilities to outcomes.

Although decision analysis is a complex subject, information technology and user-friendly, inexpensive computer programs such as DATA are leveling the playing field [14]. Several useful references can provide enough background to the reader such that basic decision analysis can be readily incorporated into relatively complex clinical decision making [13,16–20]. It would seem desirable to routinely enlist the help of such tools in cervical cancer screening program development rather than to employ free-form, less robust methods to evaluate new technologies and potentially complex combined strategies.

Economic analysis primer

An economic analysis concentrates on choices and tradeoffs in resource allocation, whether they are established diagnostic testing, treatment intervention, or application of emerging technologies. The ultimate economic outcome of interest is usually "cost" in relation to some utilization or outcome parameter. Costs are exceedingly difficult to correctly define and assign [21,22]. Furthermore, the perspective (eg, payer versus provider versus patient versus societal) of the analysis determines the types of costs considered. Table 1 lists types of costs generally considered in various analyses and their definitions. The list is by no

Table 1
Costs

Cost	A sacrifice of resources, regardless of whether or not it is accounted for as an asset or expense (NOT = expense per se)
Expense	A cost charged against a revenue in a given accounting period (NOT = cost per se)
Direct medical cost	Costs of medical services provided
Direct nonmedical cost	Costs of additional related services such as transportation, transfer of materials
Indirect cost	Costs indirectly impacting patient care such as administration, housekeeping, engineering
Direct variable cost	Costs that change in direct proportion with changes in volume of service provided
Direct fixed cost	Costs that do not change as volume changes within a relevant range of activity
Semi-fixed/step cost	Costs that increase in steps with volume or outcome, such as academic salary adjustments
Total cost	Variable costs + fixed costs
Average cost	Total cost divided by the total quantity of output
Marginal cost	Addition to total cost that results from one additional unit of output or benefit
Opportunity cost	A forgone benefit that could have been realized from the best forgone alternative use of a resource: time, money, health benefit, etc
Intangible cost	Costs of pain and suffering
Morbidity cost	Costs of economic loss because of work missed
Mortality cost	Costs of economic productivity loss because of death

means exhaustive. For example, if a study on cost effectiveness is conducted over a long period of time, issues such as short-term versus long-term costs arise. These time frames have different intrinsic distributions of fixed and variable direct costs.

The economic cost accrued today is not the same as that over a period of time because of inflation and cost of money provided (ie, interest or hurdle rates), at which point discounting methods must be employed [23,24]. If the study period exceeds 1 year, a discount rate to adjust for the present value should be applied. This rate is usually 3%, but reach up to 7% [25].

Because of difficulties in measuring the subjective costs of pain, suffering, morbidity/mortality, and some opportunity costs, most analyses concentrate on the direct costs of providing a medical service, but this can skew the true clinical meaning and validity of a given study when interpreting results across different subpopulations.

Many economic analyses have been based upon cost estimates from Medicare data. These analyses are based on diagnostically related groups (DRGs). Many associated costs incurred by the patient, provider, or health care delivery system might not be considered in an analysis that is based on Medicare DRG cost data even though these costs will clearly affect the cost effectiveness ratio. Furthermore, proxy-based measures of cost such as cost-to-charge ratios can be misleading because of charge fluctuations and an inconsistent relationship between true costs for rendering a service and charge for doing so [26]. For this reason, economic analysis validity is enhanced greatly when a specific measurement of cost such as activity-based costing/management is used [27]. Specific ''micro-costing'' and reference-based ''gross-costing'' are highly dependent on the quality and applicability of source data. Neither is necessarily crossapplicable between different patient subpopulations or care delivery systems [28].

Even the seemingly simple task of accurately defining and assigning a cost structure is foreboding. When that is performed, however, the next equally challenging step is assigning a value to a given health benefit from an intervention or test. If there is an objective medical measurement such as blood pressure readings, this must be accurately defined and used. In the absence of (or as a supplement to) objective measurements, quality adjusted life years (QALYs) provide a common metric for differentiating between interventions that require a patient's subjective preferences regarding outcomes [29–31]. An alternative is the health years equivalent (HYE) metric, which incorporates the likelihood of deterioration or improvement in the patient's condition over time [32]. These metrics have been determined by patient utility preferences and community-defined preferences, with no consensus as to which is the better metric [29,33]. Unfortunately, there is no good way to assess QALYs and HYEs per intervention when a patient has multiple comorbidities. Both methods have their critics, but a better alternative is elusive.

Economic analysis studies are often mislabeled. Several types exist and are dependent upon the goal of the study. First and foremost, prior to any economic

Table 2
Economic Analyses

Question	Outcome units	Study design
Which of several similar interventions that yield *similar* outcomes should be chosen?	Equal medical outcomes	Cost minimization analysis
Which of several interventions that yield clinically *different* outcomes should be chosen?	Medical units (eg, mmHg pressure)	Cost effectiveness analysis
Which of several similar interventions that affect quality of life or patient preferences should be chosen?	QALY or HYE	Cost utility analysis
Which of several different interventions with differing outcomes, also expressed in terms of cost, should be chosen?	Monetary	Cost benefit analysis

Cost units for all study designs are in monetary terms (eg, dollar).
Abbreviations: HYE, health years equivalent; QALY, quality adjusted life years.

analysis, well-documented, evidence-based data regarding pure clinical effectiveness should be sought. When clinical effectiveness has been established, the goal of economic assessment is defined and questions formulated, yielding the appropriate study design, as summarized in Table 2.

Cost minimization analysis

Cost minimization analysis, the simplest analysis, determines which is the least costly of clinically equivalent interventions. An example might be comparison of equivalent same-generation, same side effect profile, same coverage spectrum antibiotics from different vendors. The output is simply the least costly alternative among clinically equivalent agents.

Cost effectiveness analysis

When comparing several interventions with different clinical outcomes, the effectiveness of the intervention can be compared using clinical effect on the same medical units (eg, medication A and B effect on mmHg reduction in blood pressure). This type of study is called a cost effectiveness analysis (CEA). If the outcome units differ, some common denominator must be sought, such as survival. When the costs and clinical effects for study intervention are determined, a cost effectiveness ratio (C/E) is reported [31,34–36]. Comparisons can then be objectively drawn between interventions.

Although most often an *average* total cost is used in reporting cost effectiveness, the optimal assessment should be based on *marginal* cost versus marginal benefit (ie, how much more cost is associated with one more unit of benefit or with the next most effective option) [13,37].

An additional requirement of appropriate reporting of CEA studies is presentation of alternative, scenario-based sensitivity analyses, which indicates the stability or definitiveness of the reported findings [35].

Cost utility analysis

When utility or preference is the outcome, reported as QALY or HYE, the analysis becomes a cost utility analysis (CUA), a specific type of cost effectiveness assessment. CUA determines the clinical outcome benefits gained in terms of a *time tradeoff* of preference for raw life years gained versus the quality of life in those years. The alternative is a *standard gamble* technique, which asks the patient to rate the utility of a sure outcome (eg, chronic pain) versus a gamble on a possible alternative outcome with an intervention (eg, motor nerve damage with surgical intervention).

Cost benefit analysis

A cost benefit analysis (CBA) is used less frequently in health care because of the difficulty of assigning a monetary amount to an outcome such as a QALY gained or medical complication avoided. The intervention cost and benefit are *both* expressed in monetary terms, such that the interventions can be compared for best value for dollar spent in health care delivery. From a societal perspective, however, this ''weakness'' is also a strength because the scope of application is broader than CEA and CUA. Neither CEA nor CUA can be used to assess how much one should spend on housing, food, environmental concerns, or education in relation to health care. CBA can do this in principle because all comparisons are in monetary terms.

Ideally, a decision analysis tree using cost and utility outputs should be structured rather than a pure spreadsheet cost model. This arrangement offers greater versatility by visually representing the decision and chance issues (nodes), and it can calculate effectiveness, cost effectiveness, dollars per QALY for a given intervention, and so forth.

Economic issues specific to cervical cancer screening

While physicians are held increasingly accountable for functioning within health care budgets (ie, profit/loss-driven restrictions), a well-designed cost effectiveness analysis is still rarely used to make decisions regarding health service policy. Few studies have specifically addressed the cost effectiveness of cervical cancer screening and management [38–41]. In fact, a recent review of studies published over the past 35 years failed to identify *any* reports that adequately addressed health outcomes and cost effectiveness tools in designing cervical cancer screening programs [42].

Most publications compare results of screening using new technology with an expert panel review of cytologic specimens. In this prevalent scenario, the tests are

not independent measures and do nothing to relate the screening test findings to the true histopathologic status of the cervix, making determination of false-negatives—thus sensitivity, specificity, and negative predictive value—impossible [42]. It is difficult, if not impossible, to arrive at cost effectiveness ratios when the clinical effectiveness of various screening modalities are not clearly defined.

In addition, most studies have applied standard reimbursement rates to estimate cost savings for particular management models without examining real applications. Costs, charges, and reimbursement are too often used interchangeably, making study comparison and meta-analysis difficult.

In an attempt to address these issues, there have been several expert panel consensus reports describing how cost effectiveness analysis should be performed and interpreted in the health care setting [43]. A recent, specific report describes the recommendations of a panel of cost effectiveness studies convened as part of the International Consensus Conference on the Fight Against Cervical Cancer. Recommendations for cost effectiveness studies included: (1) the use of clearly defined reference case methods to support comparisons across studies, (2) the use of a consistent standard of evidence on the clinical effectiveness of different screening strategies, (3) further research into the costs and effectiveness of different screening and treatment strategies for cervical cancer, (4) further research into screening and treatment strategies in a wide range of countries, (5) easily accessible and detailed descriptions of the methods and supplementary analyses underlying published studies, (6) greater use of newly developed models of cervical cancer, and (7) greater revelation of potential conflict of interest by researchers [44].

As previously mentioned, the challenge of correctly capturing all costs is foreboding. The most obvious costs attributable to Pap smear screening and the management of abnormal smears are those related to patient contact with the health care system (eg, office visits) and to the incremental costs of cytologic evaluation, interpretation of specimens, adjunctive testing, and the treatment of affected individuals. Common direct costs of these interventions, as estimated by a Medicare allowable proxy, are summarized in Tables 3 and 4.

Direct costs of a particular screening and management strategy must be assessed in the context of opportunity costs. Opportunity costs can be defined as the amount of alternative services that must be sacrificed to screen more patients or offer more effective (with incrementally increasing direct costs) screening services. Because a relationship is presumed to exist between Pap smear screening program expenditures and their impact on cervical cancer mortality, as additional resources are allocated, the expected final effect is an incrementally decreasing death rate. At some point, however, additional input into a screening program will produce negligible effects in terms of further reducing cervical cancer. As noted in the primer above, economists define the change in total costs that occurs with a one-unit change in output as the marginal cost. Health care planners must consider how much it costs to prevent each additional case of cervical cancer when determining how to allocate scarce resources. Performing multiple, expensive tests on patients within the health care system will eventually be at the cost of

Table 3
Estimated unit costs of medical services

Type of resource	CPT code/ DRG	Average Medicare Reimbursement in 1997, $
Cervical screening (excludes physician time)		
Papanicolaou smear	88156	25.00
Speculoscopy		18.50
Physician visits		
Physician visit, new patient level 2 (initial screen visit)	99202	490.00
Initial consultation	99205	129.00
Physician visit, established patient, level 2 (follow-up screen visits)	99212	27.00
Stage IA: age 40 y or older	99213	39.00
Physician visit, established patient, level 5 (histological workups)	99215	93.00
Physician visit, inpatient, day 1, level 2	99222	111.00
Physician visit, inpatient, days 2+, level 2	99232	52.00
Treatment service for treating squamous intraepithelial lesions		
Cryosurgery		118.00
Cone biopsy		1097.00
LEEP		305.00

Costs are derived from American Medical Association CPT '96, Professional Edition [84] and St. Anthony's DRG Optimizer. CPT indicates Current Procedural Terminology; DRG, diagnosis related group; and LEEP, loop electrosurgical excision procedure.

recruiting new patients into screening systems and providing even the most basic of tests. In a world with finite resources, there will always be tradeoffs, and any strategy design must factor this in.

It is illuminating for purposes of comparing screening strategies to look at the number needed to screen (NNS) to prevent one death from cancer. The NNS is simply the inverse of the absolute risk reduction of death realized by screening. An approximate NNS value for cervical cancer screening using compiled data is 1100, meaning that 1100 women need to be screened regularly for 10 years to prevent one death from cervical cancer. This number could be calculated for comparison of various screening strategies, intervals, and age ranges. Established screening tests for cancer, including yearly mammography for women over age 50, have an NNS of 500 to 1100. In contrast, the more controversial position of screening women 40 to 49 years of age with mammography results in an NNS of 3125; this represents a screening strategy that is one-fifth as effective and five times more expensive.

Identifying how often to screen

Screening has the potential for generating excessive spending when patients who will never develop cancer are repeatedly screened or overtreated for early

precursors. Thus, identifying subsets of patients who require less frequent screening might lead to a better utilization of resources. It follows that supplemental tests to the Pap smear in primary screening that triage patients into risk groups might ultimately allow for more cost effective care. New technologies have the potential to increase total cost by increasing the identification and treatment of clinically insignificant lesions. In contradistinction, if a patient group that is at high risk for cervical cancer is not screened, savings accrued by withholding services might be offset by the relatively high costs of managing advanced cancers [39]. Based on incidence and weighted average reimbursement, the direct annual costs of cervical cancer treatment in the US were reported by the National Cancer Institute to be $1.7 billion in 1996. This does not include the loss of work force productivity and costs related to individuals and society.

Fortunately, cervical intraepithelial neoplasia (CIN) meets many of the criteria required to fit into an idealized screening model. CIN is a common problem, with nearly 2.5 million cases of low-grade dysplasia diagnosed from 50 million Pap smears performed yearly in the US [45]. The time of progression from CIN to CIS, with few exceptions, is estimated to be 10 to 15 years, creating a lengthy lead time during which the disease can be identified and effectively eradicated [46]. The Pap smear is an easy-to-perform, well tolerated, relatively inexpensive procedure, but the cost effectiveness of any screening program must be measured not in terms of its ease of implementation but in terms of clear endpoints and measures of effect. Screening must translate into objective, reproducible outcomes such as decreased cancer incidence, decreased death rate, or decreased overall spending while minimizing morbidity. Ideally, all of these factors are considered in the equation. The text box below summarizes common measures of

Table 4
Estimated costs of treating cervical cancer[a]

Treatment service	Average cost per patient, $
Stage IA	
Conization	1097
Simple hysterectomy	7423
Stage IB-IIA	
Radical hysterectomy/pelvic lymphadenectomy	10504
Radiation	9798
Stage IIB	
Radiation	9798
Stage III	
Radiation	6436
Stage IV	
Radiation	6436
Palliative care	1820

[a] Excludes 5-year follow-up. Costs are derived from American Medical Association CPT '96, Professional Edition [84] and St. Anthony's DRG Optimizer [85].

screening effectiveness. The reference case outcomes should be agreed upon for study or model comparison.

Measures of screening effectiveness

Relative and absolute risk reduction
Gain in life expectancy
Cost per case detected
Cost per life saved
Gain in quality adjusted life years (QALYs)
Number needed to screen (NNS)

Although mathematical models suggest that annual Pap smear screening reduces the rate of invasive cancer by 93%—versus 91% at 3 years and 84% at 5 years [47]—there might be subsets of patients who benefit the most from less frequent screening.

There is no question that increasing Pap smear frequency is an expensive way to save lives. The widely cited Markov model designed by Eddy over a decade ago evaluated the cost effectiveness of alternative conventional Pap screening regimens [48]. Among women screened from 20 to 75 years of age, the marginal cost per life year gained ranged from $10,000 (screening every 4 years versus no screening), to $262,800 (screening every 2 years versus every 3 years), to greater that $1 million (screening every year versus every 2 years).

Identification of lower-risk patient subsets for less frequent screening might translate not only into direct cost savings but also into fewer unnecessary diagnostic tests and less associated patient anxiety. While models have been published, no prospective data exist that adequately clarify optimal screening intervals. Furthermore, the epidemiologic evidence for high-risk categorization is still rather rough. Thus, one key unanswered clinical question that carries a major economic impact remains clarifying optimal screening intervals. Another is at what age women should begin and end screening.

What age range should be screened?

The peak age-specific rate for CIN occurs in the late 20s; for CIS it is approximately age 35, and for invasive cancer it is 55 to 60 years [49]. This pattern suggests that screening might be more effective in older women than in younger women when defined by detection of more advanced precursor lesions or early cancer. Some young women clearly develop cervical cancer, thus would also benefit from early detection. Whether or not it is cost effective to initiate screening at age 18 opposed to beginning with age groups that are more likely to harbor high-grade lesions (30- to 35-year-old women) is unclear.

From a societal and broad economic impact perspective using Markov modeling and marginal analysis, the resource consumption tradeoff implications are clearly defined. Nonetheless, current recommendations for individual patient care err on the side of caution. The American College of Obstetricians and Gynecologists (ACOG) has recommended annual Pap smear surveillance for all women who are or who have been sexually active or who have reached the age of 18 [50]. After three or more normal Pap smears, less frequent screening can be offered to "low-risk" patients. ACOG also notes that certain patients might be at low risk for dysplasia and cancer and can therefore be screened less frequently [50].

Several lines of evidence support initiating screening earlier rather than later. Using a Markov model, Eddy [48] found that a 20-year-old woman with average risk reduced her lifetime risk of squamous cell cancer of the cervix from 0.7% to 2.5% by screening. On the other hand, when viewed from a marginal analysis standpoint, the same model suggests that varying the age for beginning screening from 17 to 29 years, or ending screening at age 65 for women who had been regularly screened up to that age, made little difference to overall health outcomes. In another mathematical model, the reduction in cumulative rate of cervical cancer was estimated to improve by 7% by initiating screening at age 20 rather than age 35 [47]. From an epidemiologic review it is known that following a government decision not to pay for Pap smears in women younger than age 35 unless they had three children, cervical cancer deaths doubled in England during the 1960s [51]. Finally, some researchers support initiation of screening at age 18 based on concerns related to a younger age peak incidence of adenocarcinoma, although this testing would come at a high marginal cost because of the relative rarity of cervical adenocarcinoma.

Sexual norms in the US lend support to earlier screening because a large number of young women are sexually active and therefore have a higher risk of human papillomavirus (HPV) transmission, which is recognized as a causative agent in the development of cervical dysplasia and carcinoma. The benefits associated with contraceptive counseling and sexually transmitted disease screening performed concurrently with Pap screening are difficult to calculate, but they should measure into a global health benefit analysis.

If all women are screened at a younger age, more rather than less screening will be performed, thus potentially increasing total costs and workup-related anxiety. Data by Lynge and Poll [52] and Arneson and Kao [53] suggest that even if periodic screening is begun at a young age, it need not necessarily be performed yearly. In Lynge and Poll's [52] study of Danish women, the 5-year risk of developing an invasive cervical cancer was 48% lower in women after one negative smear and 69% lower after two to four negative smears than in women who were not screened. If a screened woman developed cervical cancer, she was more likely to present with an earlier stage of the disease [53]. According to the investigators, "women with one previous negative smear have a zero risk of developing cervical cancer during the first year following the negative smear. The incidence among these women increases with length of time since the negative smear and reaches the level of unscreened women during the

fifth year of follow-up. Women with two to four previous negative smears also have a negligible risk of developing cervical cancer during the first 2 years following the last negative smear. The incidence among these women increases less over time than the incidence for women with one previous negative smear. No cases of cervical cancer were observed among 7716 women with five or more previous negative smears.''

Current controversies regarding screening age are not limited to younger women. One report noted that 25% of cervical cancers and 41% of all cervical cancer deaths occurred in women older than 65 years of age [54]. In another study, 65% of a group of cervical cancer patients 65 years of age and older had not had a Pap smear until diagnosis, but 88% had seen a physician in the preceding 3 years [55]. Thus, opportunities do exist to screen women in this age group without necessarily increasing the cost of physician contact. Surprisingly, not all states allow uniform coverage of Pap smears as a screening tool for women who are eligible for Medicaid [56].

Continued screening results of patients older than 65 years of age might translate into decreased mortality, so some authorities recommend continued screening throughout a patient's lifetime. Data have suggested, however, that low-risk groups of elderly patients can be identified as candidates for either no screening or screening at 3-year intervals based on previous Pap smear histories [57,58]. If this contention is supported in large, well-designed trials, greater cost savings might be realized.

Using a multiple state Markov model, Fahs et al [59] evaluated the cost effectiveness of screening elderly women. Compared with no screening, the cost per life year gained ranged from $1666 to $33,693: once at age 65 ($1666), every three years ($5956) or annually ($33,693). Similarly, for conventional Pap screening of low-income elderly women the cost savings was $5907, and 3.72 years of life were gained per 100 Pap tests performed [60].

Costs of precursor lesion triage

It has been suggested that introduction of The Bethesda System terminology—in particular, the epithelial cell abnormality, atypical squamous cells of undetermined significance (ASCUS)—might be responsible for at least a doubling of the number of ''abnormal'' Pap smears, thereby increasing interventions and costs [61]. The problem is compounded because 20% to 60% of patients with smears that indicate ASCUS will ultimately be found to have dysplasia on follow-up evaluation with colposcopically directed biopsies [62–66]. Most of these lesions are low-grade, but high-grade lesions are not uncommon, representing 30%. Given the shorter timeline to possible invasion, this creates a clinical, economic, and medico–legal nightmare because there are 2 to 3 million women who are found to have ASCUS yearly in the US [67].

The frequency of ASCUS diagnoses should not exceed 5% of Pap test findings, or two to three times the frequency of smears that indicate dysplasia/

squamous intraepithelial lesion (SIL) [68]. Medico–legal concerns related to the inherent false-negative rate of Pap smears are perceived to be largely responsible for an excessive use of this category [69]. True increases in the incidence of HPV infection might also play a role. Subtle abnormalities are increasingly diagnosed as "possibly dysplastic," because pathologists concerned about claims of missed diagnoses err on the side of caution [69,70]. It is not clear whether the increased recognition of these abnormalities translates into diminished cancer deaths or merely results in increased allocation of resources to diagnosis and treatment of clinically insignificant lesions.

It is important to remember that even when cytologic evidence points to the presence of a precursor lesion, not all women with SILs develop cancer. The incidence of low-grade changes is greater than that of high-grade changes and far greater than that of invasive cancer. From a *retrospective* point of view, dysplasia has a relatively long lead time for progression; the peak age is 15 years earlier than that for patients with invasive cancer [71]. The problem is that no one has *prospectively* been able to accurately predict the progression and regression rates of low-grade lesions in any individual patient.

Low-grade lesions

Approximately 2.5 million women in the US are cytologically "diagnosed" each year with low-grade lesions [18]. Many investigators have attempted to define better management strategies to triage this group of patients. The three studies described in Table 5 are representative. Nash and colleagues [72] addressed a group of 45 patients with HPV infection defined by strict criteria as the only abnormality on colposcopically directed biopsies. These patients were prospectively followed at 3- to 6-month intervals with Pap smear, colposcopy, biopsy, and endo cervical curettage (ECC). While 40% had spontaneous regression of their lesions at an average time of 13.7 months, 33% progressed to CIN. Whether or not this reflects true biologic behavior in terms of spontaneous regression of dysplastic lesions or other factors is unclear. This study and others suggested that biopsy of lesions can either remove small lesions entirely or generate a local inflammatory effect that leads to the eradication of the adjacent dysplastic lesion [46].

Montz et al [73] reported on a cohort of 492 patients with either ASCUS or low-grade SIL on a referral Pap smear. They performed colposcopy at presentation and biopsied the cervix only if colposcopy findings were suggestive of high-grade lesions. Moderate dysplasia or worse was encountered at initial colposcopy

Table 5
Spontaneous regression and progression of low-grade lesions in three studies

Study	N	Spontaneous regression	Progression
Nash et al [72]	45	40%	33%
Montz et al [73]	294	71%	3%
Nasiell et al [74]	555	62%	16%

in 19% of patients. The study subgroup of 294 patients with initial Pap smear and colposcopic diagnosis of low-grade SIL was followed with Pap smears and colposcopic exams, but without biopsy, at 3-month intervals for 9 months. Nearly 71% of patients had spontaneous regression to normal, and only 3% progressed to a more significant lesion. This study and others are limited by no histologic confirmation of negative colposcopic findings. Although this confirmation would be ideal, ethical considerations and sampling error, even if a biopsy were performed, preclude such a gold standard.

In Sweden, patients are not routinely treated for dysplasia unless they have high-grade changes. Nasiell et al [74] reviewed 555 patients with a first abnormal Pap smear showing mild dysplasia (low-grade lesion formerly classified as CIN I). All patients had an initial colposcopy and were followed with Pap smears at 3- to 12-month intervals. Progression was defined as cytology consistent with CIN III (high-grade SIL) or invasive cancer. A 62% regression rate with a mean follow-up period of 39 months was noted. Persistent abnormalities were seen in 22% of patients (10% mild, 12% moderate dysplasia), and progression occurred in 16%. Two cases of invasive cancer occurred in patients who were lost to follow-up for 2 to 6 years before cancer was eventually diagnosed. This underscores the critical importance of reliable, routine surveillance when managing patients expectantly. Although lesions can regress, recurrence and progression is a clear risk.

Despite estimated progression rates of CIN of 15% to 33%, the observed rates of cervical cancer are far lower than those predicted by mathematical models. While data support the use of follow-up Pap smears without treatment in select patients with low-grade changes, this approach carries significant risk. Most series have shown that as many as 20% of patients with low-grade abnormalities on Pap smear will actually have a higher-grade lesion when visually examined by colposcopy and biopsied [73]. Visual evaluation by way of colposcopy is therefore essential in initial management. If no lesion is noted or a well-trained colposcopist finds that the lesion is consistent with low-grade SIL, then serial Pap smears at 3- to 4-month intervals might be reasonable [75]. For worsening cytology, repeat colposcopy and biopsy are generally performed.

The duration of follow-up before treatment has not been defined. In Sweden, expectant management is the rule unless progression to CIN III (high-grade SIL) is seen. In the US, differences in access to care, compliance problems, and medico–legal constraints might force a provider to give earlier treatment.

Few studies have addressed conservative follow-up patterns in terms of cost. As noted above, there are a number of clinical behavior uncertainties that preclude complete cost analysis. These uncertainties introduce potential for wide variance in outcomes when modeled by Markov processes. While it would seem reasonable to expect that less treatment would translate into less cost, in some practice settings, serial Pap smears with or without colposcopy might be more costly than initial colposcopy, biopsy, and treatment. Therefore, see-and-treat strategies have been purported to be cost effective in low-resource and poor compliance settings, but excessive treatment morbidity for low-grade lesions is a

tradeoff. Well-designed prospective studies are needed to define the most cost effective management. The initial results of one such study were recently published, the National Institutes of Health-sponsored prospective ASCUS/low-grade squamous intraepithelial lesion (LSIL) Triage Study (ALTS) on this subject. Three thousand four-hundred and eighty-eight patients with ASCUS and 1572 with LSIL were randomized to determine whether or not the ''wait and see'' approach is appropriate in patients with low-grade abnormalities [76]. Patients with ASCUS and LSIL were randomized to serial liquid-based cytology Pap smear follow-up at 6-month intervals, immediate colposcopy and biopsy, or HPV DNA testing to stratify patients by risk into expectant management or immediate colposcopy groups. Analysis of ALTS data is ongoing. When long-term data from the study become available, the ALTS investigators plan to analyze the cost effectiveness of the three options under study.

High-grade lesions

The management of high-grade lesions is less controversial. While their overall incidence is low, their risk of progression to cancer is substantial. Studies detailing the natural history of high-grade lesions show that the risk of progression to invasive cancer is approximately 6% by 3 years and as much as 71% by 12 years [46]. A variety of low-cost procedures for treatment with acceptable morbidity exist. For selected patients with high-grade lesions, some have advocated a see-and-treat approach [77–79].

Costs of treating cancer and precursor lesions

Costs associated with treating precursor or invasive lesions are procedure-, resource-, and site/provider-specific. Extensive microcosting would have to be done to arrive at the exact direct costs, both fixed and variable, of providing a therapeutic service. In the end, because of the differences between sites/providers, this extensive, resource-intensive assessment would still not be crossapplicable to all other sites/providers. It is therefore usually easier, although not as pure, to use Medicare data based on DRGs as previously described to capture the most obvious direct costs (Tables 3, 4). An average 3% annual discount rate is often used for the reference case analysis [25].

Indirect costs for the subpopulation under study are then considered, which relate to lost earnings resulting from time lost, disability, and mortality attributable to cervical cancer screening, dysplasia, and cervical cancer. Models usually introduce key assumptions regarding time lost to screening, workup, and treatment. According to one review, individuals who were histologically diagnosed and treated for LSIL and high-grade squamous intraepithelial lesion (HSIL) lost 1.6 days per year [80]; patients with cervical cancer reported 35.4 lost days per year. Indirect costs associated with cervical cancer screening, dysplasia, and

cancer are then calculated by multiplying hours lost by a blended median hourly earning rate, such as that derived from Bureau of Labor Statistics data [81].

Mortality costs reflect productivity losses after cervical cancer deaths, taking into consideration life expectancy at age of death [82] and changing patterns of earnings at successive ages. In the case of retired individuals, no good proxies for opportunity costs of time or loss of life have been established.

The costs associated with each treatment scenario must be evaluated for relevance and completeness. Resource use that is germane to the analysis and nontrivial in magnitude should ideally be included in the reference case analysis and sensitivity analysis. Unfortunately, data regarding standard cost estimates is lacking, precluding accurate comparisons between studies and models. Furthermore, most often it is not clear what proportion of total costs in any given study represents variable versus fixed costs. Ideally, variable costs, which reflect the value of the service that changes because of the intervention being considered, should be included. Fixed costs, which remain constant over the long-run regardless of the level of production, should be excluded. Finally, costs should reflect marginal or incremental resource consumption to achieve a given health state rather than average costs. The foregoing overview merely scratches the surface of cost issues related to CEA studies that consider screening, diagnostic, and treatment scenarios.

As an example, a recently described cost effectiveness Markov decision model described cost considerations of preventing cervical cancers using three different treatment strategies for preinvasive disease. A hypothetical cohort of 100,000 women with CIN II and III was followed and analysis performed for marginal costs. In the analysis of CIN II, cryotherapy was the least expensive ($41 million) and least effective (95% cure and 1454 cancers prevented). Vaginal hysterectomy was the most expensive ($1.2 billion) and most effective (99% cure rate and 1475 cancers prevented). The loop electrosurgical excision procedure (LEEP) was more effective than cryotherapy with a 96% cure rate and an additional 19 cancers prevented. Relative to cryotherapy, however, LEEP carried a marginal cost of $31,394 per additional cure and $1.8 million per additional cancer prevented. For CIN III, cryotherapy was also the least expensive ($46 million) and least effective (91% cure and 2154 cancers prevented). Vaginal hysterectomy was the most expensive ($1.2 billion) and most effective (99% cure and 2206 cancers prevented). LEEP was more effective than cryotherapy, with a 94% cure rate and an additional 44 cancers prevented. Relative to cryotherapy, LEEP carried a marginal cost of $17,564 per additional cure and $1 million per additional cancer prevented [83–85].

Limitations of the Pap smear: setting the stage for adjunct testing

The public's assumption that the Pap smear is a precise tool for cancer detection has led many to believe that cancer after a normal Pap smear must imply malpractice [86]. To design and implement believable CEA-based cervical

cancer screening programs, what the Pap smear can and cannot do must be clearly defined at the outset.

The Pap smear can often, but not always, show the presence or absence of abnormal cells consistent with the histologic diagnosis of dysplasia or cancer. It cannot, by itself, distinguish which patients with dysplasia will have a course marked by spontaneous regression from those who will ultimately develop a cancer if left untreated. For cervical cancer screening to be effective, repetitive screenings at some, not yet agreed upon, regular intervals are required. Even a programmatic approach cannot defeat all factors involved in low Pap screening sensitivity, however.

It has always been assumed that if an early cervical cancer or precursor lesion were present, the Pap smear would eventually detect it. This assumption has led to studies in which the Pap smear's sensitivity was determined either by: (1) re-evaluating negative Pap smears and calculating sensitivity values based on the view that misread slides represent the only potentially missed cases of cancer or precancer, or (2) retrospectively reviewing the Pap smear history of women with confirmed diagnoses of cervical cancer. Thus, accurate sensitivity and specificity estimates for the Pap test that require histologic confirmation of both positive and negative results remain incompletely defined because of practical and ethical considerations. Construction of 2×2 contingency tables is impossible without knowledge of true prevalence or incidence. The best available estimates come from meta-analyses by Fahey et al and the Agency for Healthcare Policy and Research (AHCPR), which used a histologic diagnosis as the gold reference standard [12,87]. From these analyses, which were based on the sensitivity and specificity and receiver operating characteristic (ROC) modeling, the screening Pap is better suited to rule in—but not rule out—disease.

Inadequate sampling of the transformation zone, poor collection and fixation of the specimen, and inclusion of excessive blood, inflammatory material, or necrotic material can obscure or preclude a correct cytopathologic diagnosis. Clinicians can improve the quality of specimens submitted by using appropriate collection devices and following established techniques [75]. Studies have shown that in 12% to 25% of patients diagnosed with CIS or cancer who had previous ''negative'' Pap smear results, the smears were actually unsatisfactory for interpretation on re-review [86,88]. Improvement in technique is certainly a cost-free proposition.

It has recently been reported that some precancerous lesions might fail to shed cells in sufficient quantities for cytologic detection. Because of abnormal expression of intercellular adhesion molecules, dysplastic cells are simply bound tightly at the surface and are not available for transfer to the slide or liquid medium for detection [89].

A particular problem related to screening is the case of adenocarcinoma. Pap smears are somewhat limited in the ability to sample the endocervical canal completely [58]. No supplemental test to date offers unique advantages in terms of reducing the risk of adenocarcinoma [90].

The 1987 Wall Street Journal article regarding ''lax laboratory practices'' in cytology screening was a rude awakening for the lay public [91,92]. Inevitably,

the first response focused on accusations and human interpretation errors. Hence, the Clinical and Laboratory Improvement Ammendments of 1988 (CLIA) were hastily passed. Ten percent of Pap smears are required to be rescreened for quality control. This approach has many critics, with good reason. In a case–control study by Lynge et al [93] evaluating smear misclassification, 106 Pap smears from 53 cases of invasive cervical cancer were matched against 530 controls without a diagnosis of CIN or cancer and were re-reviewed. The investigators found that with improved cytopathology that eliminated misclassification of positive smears, the proportion of prevented cancers could increase from between 62% and 72% to between 83% and 86%. This could be achieved with a 2% increase in the workload, but to identify all cases from all smears correctly, including those read as unsatisfactory, a 31% increase in cost would be required. Other studies have raised similar concerns.

In the end, CLIA legislation did not result in eradication of the false-negative Pap smear rate. As part of a market opportunity business response to a public health problem, venture capital-backed, technology-driven efforts began to create a "better" Pap test.

Advances in specimen collection and interpretation

Specimen collection

ThinPrep (Cytyc, Marlborough, MA) and Autocyte (Roche Corp, Burlington, NC) methods were introduced to improve the quality of specimen collection. The sample liquid suspension is used to create a series of ThinPrep slides, optimizing cell preservation and reducing artifacts that hinder interpretation. Subsequent clinical trials purported improved sensitivity despite study design flaws [94,95]. The test added approximately $5 to $10 to the cost of each Pap smear.

Cytology automation

A different approach to lowering the false-negative rate of Pap smears addresses the large number of cells on each slide that must be evaluated. Since 50,000 to 300,000 cells are on each slide, rare but abnormal events might be missed by human eyes. Psychological habituation and fatigue can easily become factors when a cytotechnologist's caseload of 60 slides per day means reviewing 3 to 18 million cells per day. Thus, based on the premise that abnormalities are often present but not recognized, computerized neural network screening systems like PAPNET (Neuromedical Sciences Inc, Suffern, New York) and NeoPath's Autopap300 (Tripath Imaging, Burlington, NC) were introduced [96]. On the strength of computerization, these systems represented an improvement over a rudimentary 1950s cytoanalyzer scanning device. Although they both received FDA approval for both rescreening and primary screening strategies, cost effectiveness was not proven [97–101]. Several corporate mergers and technology acquisitions led to

the formation of TriPath Imaging, whose Autopap300 is now FDA approved for primary screening in low-risk populations as a "safe and effective" method despite a persistent false-negative rate. Other technologies are entering the arena, each promoting incremental technologic advances and touting improved sensitivity and specificity over conventional cytologic screening. Industry-driven research has produced a myriad of supporting published clinical trials of variable quality on automated screening. The added cost per patient screened has averaged $40.

Assessment of liquid-based and automated cytology

The New Zealand Health Technology Assessment (NZHTA) systematically reviewed the international evidence base for clinical and cost effectiveness of replacing conventional screening with automated, semiautomated, and liquid-based technologies [90]. The assessment team employed an exhaustive computerized search. More than 700 articles were identified, of which all but 26 were disqualified because of design limitations, mainly relating to lack of verification by histology or at least an adjudicated panel cytology review. Their findings included the following conclusions.

- Estimates of test sensitivity and test specificity for the new devices could not be reliably determined. The research reviewed provides no evidence for improved detection of high-grade abnormalities by new devices for cervical screening.
- Estimates of test sensitivity and specificity were the main source of uncertainty in the economic models investigating the cost effectiveness of new devices. In economic models in which improved detection from the introduction of new devices was assumed, the impact of new devices on days of life saved was extremely small for women screened at 3-year intervals.
- Any increases in sensitivity resulting from the introduction of new devices might come at the cost of decreased specificity. This would lead to increases in false-positive results with extensive direct and indirect costs and negative impact on quality of life.
- Higher-quality research is required to generate valid estimates of test sensitivity and specificity. Methodological limitations that must be addressed include the application of appropriate reference standards for verification of cytological diagnoses, including test negatives. Economic modeling studies will be more meaningful with more valid estimates of test characteristics.

It is important that promotional information for new devices is balanced by material for health professionals and for patients based on key findings of independent evidence such as found in the NZHTA report. Additionally, legal

avenues should be investigated to restrict advertising of unsubstantiated claims for new devices.

A US study population model examined a 20- to 65-year-old screening age range. New technologies increased life expectancy by 5 hours to 1.6 days, varying with the technology and the frequency of screening. The cost per year of life saved rose from $7777 with quadrennial screening to $166,000 with annual screening. PAPNET produced more life-years at a higher cost per year of life saved. When used with triennial screening, however, each of them produced more life-years at lower cost than conventional Pap testing every 2 years. The C/E of each technology improved with increases in the prevalence of disease, decreases in the sensitivity of conventional Pap testing, and increases in the improvement in sensitivity produced by the technology. From this model, it appears that technologies that increase the sensitivity of Pap testing will be more cost effective when incorporated into infrequent screening. True documented increases in sensitivity and decreases in cost might eventually make each technology more cost effective [98].

Another US study population model combined the use of the ThinPrep with increased screening compliance, which suggested that it would be more cost effective in decreasing cervical cancer incidence than simply increasing the screening rate with conventional Pap smears to Healthy People 2010 goals. Using Markov processes, the model followed a theoretic cohort of 100,000 women aged 20 through 80 years. Assumptions included three compliance rates inclusive of racial differences (self-reported, Healthy People 2000 and Healthy People 2010 compliance) and different Pap test sensitivity for conventional Pap versus liquid-based cytology. Given these assumptions in the context of foregoing discussion regarding limitations of true sensitivity values, benefit was seen from both increased compliance and the use of liquid-based cytology. Increasing screening compliance to Healthy People 2010 goals resulted in 22% and 17% reductions in cervical cancer incidence for Caucasian women and African American women, respectively. Substituting liquid-based cytology with no change in compliance resulted in cervical cancer incidence reduction by one third. Cost per life year saved for African American women was $10,335 versus $17,967 for Caucasian women [102].

Whether or not automation and liquid-based media truly improve sensitivity for significant lesions or add an economic burden is not clear. Certainly, the New Zealand summary stresses lack of CEA, or even clinical proof when screening continues on a yearly basis across all age ranges without regard for risk factors. If widely implemented, added technologies of this type would contribute to a direct incremental screening cost of $30 to $257 per contact. Even without addressing the significant added cost of unnecessary workup and treatment, the gross added yearly cost to the system would range from $1.5 billion to a staggering $30 billion based on an estimated 50 million Pap smears performed yearly in the US.

What is clearly needed to create a more rational and cost effective approach to screening is a triage system that separates patients whose lesions are at risk to progress from those whose lesions are not. A number of adjuvant tests have been

introduced that might prove useful in this regard. These tests include HPV detection and new techniques for visualizing acetic acid-stained cervical specimens. In the future, molecular markers will certainly play a role, but they are too far from widespread clinical application and are therefore not discussed here.

In vitro HPV typing

The association of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 with high-grade dysplasia and cancer has led to HPV panel tests [103]. With improvements in technology, HPV testing has become widely available by way of Digene's Hybrid Capture II assay (HCII; Digene Corporation, Gaithersburg, MD). It has been proposed both in primary screening and in triaging patients into risk groups [104]. In one study, 1985 patients were evaluated by routine Pap screening and HPV testing [105]. Colposcopy with biopsy identified 81 cases of high-grade lesions in 231 patients with screening abnormalities on either Pap smear or a positive HPV test. In 45 of 81 patients the Pap smear was abnormal, in 61 of 81 cases the HPV test was positive, and in only 25 of 81 patients were both the Pap smear abnormal and the HPV test positive. It is unknown whether lesions from patients who were HPV-positive but cytologically normal would have spontaneously regressed or would have been detected by subsequent Pap screening prior to the onset of invasive cancer. In a separate, prospective series, patients with negative cytology but a positive HPV test had an 11-fold increase in the risk of developing a high-grade lesion within a 2-year follow-up period [106]. In young, sexually active women, HPV infection patterns might change over time, weakening the significance of a single test. The best utility for HPV testing remains unclear, but the ALTS trial has stated a case for using HCII as an effective ASCUS triage tool, identifying those at high risk for significant dysplasia [76]. Unfortunately, the preliminary results regarding HCII for LSIL triage are not as encouraging because 85% of these patients are HPV-positive, limiting triage utility [107]. The CEA results of that prospective study remain to be released as longer-term results become available. The average cost of the HCII assay is $60.

In vivo adjuvant tests

Visual methods to enhance the Pap smear have been described, one of which is PapSure (Watson Diagnostics, Morristown, NJ). PapSure combines Pap testing and a visual component. The visual component, speculoscopy, uses 4 × to 6 × magnification of an acetic acid-stained cervix with a low-intensity blue light of specific wavelength to visualize potentially abnormal areas on the cervix. Dysplasia appears clearly as white areas that stand out from the unaffected, blue tissue background. The study is interpreted as positive or negative based on the dichotomous presence or absence of white lesions; no grade is assigned. When

used in combination with the Pap smear, less than a 3% false-negative rate has been reported [108–110]. A negative PapSure thus carries a 99% negative predictive value. Using well-defined and tested algorithms, patients can be triaged on the basis of visual and cytologic positive tests, potentially reducing costs of immediate colposcopic evaluation and treatment. Massad and associates [38] reported that speculoscopy was a cost effective alternative to routine colposcopy in a mathematical model for managing patients with atypia. In addition, using a Markov model and well-defined reference case screening and treatment scenario CEA, Taylor et al showed that biennial screening with PapSure provides cost savings for screened 18- to 65-year-old women compared with annual Pap screening alone [111]. The cost of the visual speculoscopy portion of PapSure is $20.

Cervicography has been compared with the follow-up Pap smear in identifying patients with atypia who require further colposcopic evaluation. In a series of 97 patients with ASCUS, 42% of colposcopically detected lesions would have been missed on repeat Pap smear versus 11% with cervicography [112], but cervicography was associated with a significantly higher false-positive rate. Overall, investigators concluded that the cost-per-case using cervicography for triage was equal to using follow-up Pap smears, but it was one-third higher than that of offering colposcopy to all patients at presentation. In another cohort series of 967 women who had a normal Pap smear within 1 year, 38 (3.9%) were identified as having CIN II/III (high-grade SIL) on biopsy. Colposcopy and biopsy were performed based on either positive cytology or cervicography. Sensitivity for cervicography alone was 45%, and the positive predictive value was 17% for the 38 cases of high-grade lesions (CIN II/III). Adding HPV testing or HPV testing with cytology increased sensitivity to 60% and 68%, respectively. Using costs based on Medicare's allowable reimbursement rates, a cost-per-case of CIN II/III was $1687 with Pap and HPV versus $1816 with cervicography and a cytologic examination [113].

Nonetheless, cervicography and digital colposcopic imaging were used as the visually-based gold standard safety net in the ALTS trial, making an implicit case for some type of visual reference standard in cervical cancer screening.

Summary

From what perspective the cost analysis is viewed (the patient, provider, payer, society, or others) is important. Simple measures by the clinician to collect and produce an adequate specimen do not increase cost and aid in the screening and interpretation of smears. Likewise, following established management protocols for the evaluation of abnormal Pap smears, including biopsy of all grossly abnormal cervical lesions, should reduce delays in diagnosis and optimize care. It is not clear if spending more on tests that enhance the accuracy of Pap smears would lead to a greater reduction in cancer incidence than if the money were spent to include a greater proportion of women in primary screening. Because

overtreatment accounts for an excessive portion of expenses, perhaps information gained from adjuvant testing should be directed at decreasing surveillance in patients with clearly low risk.

Although clinical promise is evident, the cost effectiveness of tests beyond the Pap smear have not been clearly demonstrated. Even clinical effectiveness is not well defined because of practical and ethical limitations in determining true sensitivity and specificity by histologic confirmation and other issues discussed herein. Because of modeling assumptions, study flaws, and lack of reference case standards, directly comparing the published studies is almost impossible.

When viewed from the perspective of caring for individual patients, the need for reducing the rate of false-negative Pap smears is clear. Optimally, new tests that augment the Pap smear should demonstrate cost effectiveness. Adjuvant tests must mainly be measured in terms of the clinical impact they can produce for any individual patient. There is little use for tests that lead to an increased diagnosis of clinically insignificant lesions. The adjuvant test within a specified program should ultimately lead to a gain in life expectancy from the detection and treatment of CIN and early-stage cervical cancers. Adjuvant tests should also be able to stratify patients based on risk so that less time-intensive surveillance and follow-up care is offered to patients who have low risk while treatments are reserved for patients who can be expected to benefit most from them.

For health care planning of large segments of the population, cost effectiveness must be considered by the payer and provider. The natural history of dysplasia and invasive cancer make the likelihood of eventually detecting a dysplastic lesion in a patient who is regularly screened high, thus reducing the clinical impact of many, but certainly not all, negative smears in the presence of clinical lesions. Nevertheless, limitations of Pap smear screening must be recognized and patients must be adequately counseled. Each adjuvant test adds cost, and the cost effectiveness of new technology must be addressed in well-designed trials, at the center of which must be agreed-upon reference standard cases. Finally, there is the question of whether cervical cancer incidence can be decreased more by improving the tests for patients who are already screened or by improving access for the unscreened population.

From a societal perspective, health care can ill afford the costs associated with mass rescreening or adding duplicative adjuvant testing to all Pap smears unless societal and legislative initiatives funnel money from other segments (eg, environment, food, housing) based on strict monetary CBA policy making. Even reaching the lofty goal of screening every woman in the US with conventional Pap smears was estimated by Eddy to range from $2 billion to $6 billion (in 1990 $), depending on screening frequency [114]. Medico–legal concerns no doubt raise the costs of medical care by favoring overdiagnosis and overtreatment. Legal reforms might remedy some of these problems and possibly reduce costs.

In a perfect world resource constraints are not an issue, the disease process is completely defined, medical objectives are well formulated, legal issues are immaterial, a test would does what it is designed to do. In such a world a sound

strategy would be easier to design and implement. In the real world, however, the foregoing factors are constraining and any recommendation will by definition have its flaws, so one global recommendation does not currently exist. Cervical cancer screening represents only one of many public health issues competing for resources. The optimal screening strategy will ultimately depend on the cost effectiveness threshold of a given setting. This socially accepted threshold, which touches on the "worth" of a human life, remains to be defined [115].

The objective truth is that, from a resource consumption and value-added perspective, many public health programs with a low cost-per-life saved are relatively underfunded while many environmental regulations with a high cost-per-life saved are issued each year. To illustrate, a program to detect and treat breast cancer among women over the age of 50 has been estimated to cost less than $15,000 per life-year saved, whereas the cost-per-life-year saved of a regulation to reduce airborne exposure to benzene is approximately $5 million [116]. The future of this disparity will rest in the presence or absence of CBA-based legislative policy initiatives [117].

Given that there are choices to be made, the optimal yardstick against which *all* resource-competing programs are measured should be marginal benefit versus marginal cost. In the case of cervical cancer screening, the benefit is the marginal cost per life year gained until the quality of life adjusted life-year gained can be accurately determined. If society cannot bear the costs of all services, then it will be up to individual patients to best assess where they wish to concentrate their resources. From an individual patient's perspective, higher costs might appear to be worth the perceived additional security that some tests offer. Some manufacturers of augmenting tests are counting on this and are directly marketing services to the consumer. If patients decide to add personal resources for a clinically effective intervention that adds to personal health benefits, then that should be their prerogative.

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Traditional management of invasive cervical cancer

David O. Holtz, MD[a], Charles Dunton, MD[b,*]

[a]*Department of Obstetrics and Gynecology, Jefferson Medical College, 111 South 11th Street, Philadelphia, PA 19107, USA*
[b]*Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Albert Einstein Medical Center, 5501 Old York Road, Philadelphia, PA 19141, USA*

Cervical cancer continues to be one of the most frequent and deadly cancers affecting women worldwide. In developed countries that have implemented screening programs, the incidence of cervical cancer has markedly decreased, whereas the incidence of cervical intraepithelial neoplasia (CIN) has risen. Effective treatment of CIN decreases the incidence of and the mortality from cervical cancer. In the United States in 2001, it was estimated that there were approximately 15,000 cases and 4000 deaths from invasive cervical cancer. This article reviews the state-of-the-art of treatment in the US for cervical cancer.

FIGO staging

Because of the worldwide scope of cervical cancer, it remains a clinically staged disease. In 1994 the International Federation of Gynecology and Obstetrics (FIGO) revised their staging system (Table 1). Stage I was revised to better reflect understanding of the biology of this disease. The newly developed Stage IA1 is a minimally invasive tumor that can be treated more conservatively. The addition of Stage IB2 recognizes the influence of the size of the lesion on prognosis and treatment.

Current clinical and radiographic modalities used to stage cervical cancer include examination under anesthesia, cystoscopy, proctoscopy, intravenous urogram, and chest radiograph. In the US, however, most clinicians use CT scanning information to determine disease status prior to treatment. CT scanning, while not changing the stage, might change the proposed treatment modalities. The information from other pretreatment testing is not included in staging because this

* Corresponding author.
E-mail address: duntonc@einstein.edu (C. Dunton).

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Table 1
FIGO staging for cervical carcinoma, 1994

Stage 0	Carcinoma in situ, CIN III
Stage I	Carcinoma strictly confined to the cervix
	Stage IA: Preclinical carcinomas, diagnosed only by microscopy
	Stage IA1: Stromal invasion <3 mm in depth, <7 mm in width
	Stage IA2: Stromal invasion 3–5 mm in depth, <7 mm in width
	Stage IB: Clinical lesions, all grossly visible lesions, or preclinical lesions greater than IA
	Stage IB1: <4 cm
	Stage IB2: ≥ 4 cm
Stage II	Carcinoma extends beyond the cervix but does not extend to the pelvic side-wall; involves the vaginal, but not the lower third.
	Stage IIA: No obvious parametrial involvement
	Stage IIB: Obvious parametrial involvement
Stage III	Carcinoma extends to the pelvic wall; on rectal examination, there is no cancer-free space between the tumor and the pelvic wall; involves the lower third of the vagina; all cases of hydronephrosis or nonfunctioning kidney.
	Stage IIIA: No extension to pelvic wall
	Stage IIIB: Extension to pelvic wall or hydronephrosis or nonfunctioning kidney
Stage IV	Carcinoma has extended beyond the true pelvis or has clinically involved the mucosa of the bladder or rectum; bullous edema does not permit a case to be allotted to stage IV
	Stage IVA: Spread of the growth to adjacent organs
	Stage IVB: Spread to distant organs

testing is not necessarily available on a widespread basis worldwide. Clinical staging has an error rate of between 24% to 39% because of failure to palpate parametrial involvement or discover nodal metastases [1]. CT scanning has been used in attempts to determine whether or not there is parametrial involvement present or involvement of lymph nodes. CT scanning has a low accuracy rate (30–60%) in assessing parametrial tumor invasion, however [1]. CT scanning might also underestimate the extent of disease caused by microscopic tumor involvement in lymph nodes or other areas. Currently, the Radiation Therapy Oncology Group (RTOG) and the Gynecologic Oncology Group (GOG) have an ongoing study to determine the usefulness of CT scan and MRI on operability in Stage I lesions. As these data become available, they might change current management of early-stage lesions based on diagnostic imaging techniques.

Prognostic factors

Tumor depth

There is a correlation between the incidence of pelvic nodal metastases in the depth of stromal invasion in Stage IB, IIA, and IIB carcinoma of the cervix [2]. Patients with less than 5 mm of stromal invasion had less than a 1.0% chance of nodal metastases, whereas greater than 5 mm to 9.9 mm of invasion conferred a

12.4% chance of nodal metastases and greater than 30 mm conferred a 61.5% rate of nodal metastases [1].

Parametrial involvement

In a study of 32 patients with high-risk Stage I cervical cancer who underwent a radical hysterectomy, multivariate analysis showed that parametrial involvement was an independent poor prognostic indicator for the progression free interval ($P = 0.043$) regardless of nodal status. In addition, patients with positive lymph nodes had worse disease-free intervals and survival if the parametria were also involved [3].

Lymph node involvement

Status of nodal involvement is associated with recurrence and survival. In a study of 545 patients with negative pelvic lymph nodes, 3-year disease-free survival was 85.6% compared with 74.4% in patients with positive nodes [4]. Five-year survival rates also significantly decreased with the number of involved pelvic nodes (62% for one node, 36% for two nodes, 20% for three or four nodes, and no survivors for five nodes or greater) [5]. Lymphovascular space involvement (LVSI) on conization specimens has also shown to be an independent risk factor poor prognosis in Stage IB and IIA disease [6]. A multivariate analysis of 301 patients showed that patients with less than 6 mm depth of invasion, no LVSI, or no involved lymph nodes have a 5-year disease-free survival rate of 91.0%, whereas the survival rate falls to 43% in patients with greater than 2 cm depth of invasion with LVSI and positive lymph nodes [7].

Race

In the US, the relative risk of mortality for African American women versus Caucasian women was 1.30 (95%CI 1.14–1.48) in a multivariate model including demographics, FIGO stage, tumor characteristics, and treatment according to data obtained from the surveillance, epidemiology, and end results (SEER Program). Also in that study it was noted that African American women were more likely to present with advanced disease. Treatment also varied by race, with African Americans receiving surgery less often and radiotherapy more often than Caucasian women [8].

HIV status

Patients who are HIV positive have been observed to have more aggressive disease and poorer prognosis. This phenomenon is felt to be caused by the immunocompromised state of the patient, which prevents development of HPV immunity and normal responses to dysplastic cells. A case–control study of

16 HIV-positive patients and 68 controls found worse stage, poorer response to therapy, and higher recurrence and death rates in the HIV-infected patients [9]. This finding has not been confirmed by other studies, however [10].

Current treatment by stage

Carcinoma in situ and CIN III

Current methods for treatment of CIN include cryotherapy, laser vaporization, laser excision, loop electrosurgical excision procedures (LEEP), and cold knife conization. Hysterectomy, either abdominal or vaginal, remains part of the continuum of treatment of preinvasive disease, but it is not currently accepted as a primary modality. Treatment of preinvasive disease should be based on the size of the lesion, the need to determine if an invasive cancer is present, and the availability of treatment methods. In general, cryotherapy is less effective in large lesions and large preinvasive lesions might harbor an occult invasive lesion. Recent studies have found an almost 1% incidence of microinvasion or invasion on LEEP specimens performed for preinvasive disease [11]. Over the past several years, LEEP has become the preferred modality of treatment in the US. It needs to be emphasized, however, that ablative methods are as effective and can be utilized. Recurrence after the excisional or ablative techniques is on the order of 5% to 10%. Most patients can be cleared of their disease with a repeat excisional procedure. In patients who have recurrence after ablative procedures, it is often more prudent to excise these lesions because the possibility of an underlining invasive disease is present [12].

In certain incidences, the use of thermal excision techniques is not preferred. In cases of suspected early invasion in squamous lesions, that thermal artifact might preclude an accurate diagnosis and determination of margin status. These factors can be critical in deciding whether or not conization alone is sufficient treatment or if the patient needs a radical hysterectomy. Additionally, in the management of glandular lesions, while controversy exists, many experts feel that cold knife conization is the preferred diagnostic method of choice. Again, thermal artifact can interfere with interpretation of depth of invasion and margin status in glandular lesions [13].

Microinvasive stage IA1

Stage IA1 lesions are defined as minimally invasive lesions. This definition must be based on conization specimens and not colposcopically directed biopsies. With uninvolved margins, less than 3 mm invasion, and no lymphovascular space involvement, conservative treatment might be possible. In women who have a desire for future childbearing, conization with negative margins can be considered as a safe alternative to hysterectomy [13]. There are, however, occasional case reports of patients who have metastases with microinvasive disease [14]. In

patients not desiring future fertility, the standard of care includes a simple hysterectomy, which can be performed abdominally or vaginally. Stage IA1 tumors have an excellent prognosis with the outlined management scheme [15].

Treatment for stages IB and IIA

Patients have traditionally been offered definitive radiation or radical surgery for Stage IB and IIA carcinoma of the cervix with similar 5-year disease-free survival rates (74–83%) [16]. Radical hysterectomy had been reserved for patients with fewer comorbid medical conditions, younger patients with a desire to preserve sexual functioning, and for smaller lesions. Morbidities associated with radical hysterectomy include risk of anesthesia, blood loss, and risk of injury to the urinary tract. Ureteral injury and fistula has been reported to follow 5% to 10% of radical hysterectomies [17]; however, more recent data have shown a reduction in that rate to 1% to 2% [1]. Most complications from radical surgery are short-term with the exception of bladder atony.

Complications of radiation therapy include both short-term and long-term complications including radiation cystitis or proctitis and bowel and urinary fistula that rarely close spontaneously and frequently require diversions. Radiation therapy can also induce vaginal stenosis and results in menopause. Even in patients with ovarian transposition receiving postoperative pelvic irradiation, only 4 in 24 retained ovarian function [18].

The use of radiation therapy after radical hysterectomy has been proposed in certain cases. It has been recognized that patients with high risk factors such as bulky lesions, deep stromal invasion, involved nodes or surgical margins, and LVSI have high rates of recurrence. The results have been mixed, however, with some studies showing an increase in 5-year survival rate and some studies showing no improvement in overall survival [19,20]. The GOG sponsored a randomized trial of radiation therapy versus no further therapy in 273 patients with Stage IB cervical cancer after radical hysterectomy. Radiation therapy significantly reduced the risk of local reoccurrence (from 6% to 25%) but increased the rate of moderate to severe adverse events [21]. The authors currently use radiation treatment postoperatively for high-risk Stage IB to IIA tumors in patients whose life expectancy justifies the additional risk of adverse events.

Stages IIB, III, and IVA

In the US, patients with advanced cervical carcinoma have traditionally been treated with radiation therapy alone. The goal of radiation therapy is to achieve cytotoxic doses to the cervix, parametrium, and pelvic lymph nodes. External beam radiation can treat these regions, but the dose is limited by normal pelvic structures (bladder, rectum, and small bowel). Additional radiation dosing can be applied directly to the cervix with intrauterine and intravaginal applicators loaded

with radioactive sources (eg, Fletcher-Suit). Bulky or irregularly shaped cancers can also be treated with interstitial needles placed within the tumor. Both methods achieve a "boost" of radiation to the internal cervical os (point A) and pelvic sidewall (point B) without affecting the small bowel [22].

The 5-year survival rates for Stages IB and IIA are 87% to 90% and 62% to 83%, respectively [23]. The survival rates fall for Stages IIB, III, and IV carcinoma of the cervix to 62% to 68%, 33% to 48%, and 14%, respectively [24–26].

Chemoirradiation

Recent randomized, controlled trials have examined concurrent chemotherapy with radiation therapy for advanced cervical cancer. Theoretically, the two therapies work synergistically. Chemotherapeutic agents act as a radiosensitizers, inhibiting the repair of sublethal damage and providing systemic therapy not otherwise treated by local radiation therapy. Four randomized studies investigating the use of cisplatin-based chemotherapy in conjunction with radiation therapy have shown improvements in patient survival [27–30]. Three of these studies looked at Stage IIB to IVA and one study looked specifically at bulky Stage IB cervical carcinoma. All four studies showed a significant risk reduction and progression-free survival for patients receiving cisplatin-based regimens in addition to radiation therapy (RR 0.48–0.57, $P < 0.001$ in all studies) and for death (risk ratio [RR] 0.54–0.61, $P < 0.001$ in all studies). Keyes et al found a significant decrease in recurrence rate (21% cisplatin/XRTversus 37% Radiation Therapy (XRT) only, $P < 0.001$) and a significant increase in three year survival (83% versus 74%, $P < 0.001$). Morris et al found a similar benefit in the 5-year survival rate (73% versus 58%, $P < 0.001$). There were no deaths related to chemotherapy in any of the four studies, though patients receiving cisplatin-based chemotherapy did experience an increase in adverse side effects. These studies led to the announcement of a new standard of care for treatment of cervical cancer by the National Cancer Institute. Current GOG and RTOG studies include radiation in combination with paclitaxel with cisplatin, cisplatin with hyperthermia, oral capecitabine, and cisplatin combined with celecoxib and 5-fluorouracil.

Recurrent cervical cancer

Locally recurrent

Cervical cancer can recur locally after either radiation therapy or radical surgery. If a patient has recurrence after radical surgery, radiation therapy is instituted, and it is often successful [31]. While there are no current studies on use of chemotherapy in conjunction with radiation in this setting, clinicians can

extrapolate for the studies on advanced disease and elect to treat with combined modalities. In the setting of central local recurrence after radiation, pelvic exenteration is considered.

Exenteration was first described in 1948 [32]. Patient selection is critical to the success of exenterative procedures and to avoid associated morbidities and mortalities. Elderly patients or patients with multiple medical conditions might not be appropriate for exenterative surgery. Candidates for this procedure should have resectable local recurrence without distant or nodal disease, so studies such as liver function tests, CT scans of the abdomen and pelvis, and intravenous pyelogram are critical prior to exenterative surgery [33]. Even with meticulous preoperative patient selection, 30% of operations are aborted intraoperatively because of disseminated disease, involved lymph nodes, or parametrial fixation [34].

Results of exenterative surgery were reported on 143 pelvic exenterations performed between 1969 and 1986 in Birmingham. The overall mortality was 6.3%, the 5-year survival rate overall was 50% (63% in patients with anterior exenterations and 42% with total exenterations). The 5-year survival rate (82%) was observed in patients who had mobile masses less than 3 cm in diameter greater than 1 year from the end of their radiation therapy [35]. Gastrointestinal complications were low. Small bowel obstruction and fistulas were seen in 2.2% of patients, and rectovaginal fistula was seen in 5.3% of patients. Urinary complications in patients with continent conduit with irradiated bowel include hydronephrosis and pyelonephritis (7%), ureteral reflux (5%), ureteral stricture (3%), anastomotic leakage (8%), and stone formation (4%). Metabolical acidosis is also a concern in patients after continent conduit surgery [36].

Distant metastatic disease

Results of chemotherapy in the management of recurrent and disseminated cervical cancer have been disappointing. Squamous cell tumors are (in general) chemoresistant. Cervical cancers also tend to reoccur locally in previously irradiated, poorly perfused tissue, which limits chemotherapy. Single-agent chemotherapy (eg, adriamycin, bleomycin, methotrexate, paclitaxel) obtains response rates of 10% to 38% [31]. Cisplatin as a single agent achieves an 18% response rate with minimal adverse effects [37]. Recently, a Phase II trial of cisplatin and paclitaxel showed a 46% response rate [38].

Neoadjuvant chemotherapy prior to radical surgery has been proposed. Retrospective data suggest an improvement in 5-year survival rates [39]. Investigators have completed Phase II studies of neoadjuvant cisplatin/gemcitabine and cisplatin/vincristine with 95% and 82% response rates, respectively [40,41].

Multiple studies are in progress examining novel chemotherapies. The current GOG Phase III trial compares cisplatin alone and in combination with topotecan. Antimetabolites gemcitabin and capecitabine, as well as the alkylating agent ifosfamide, are being studied as single agents or in combina-

tion with cisplatin. Immunotherapy using HPV 16 E6 and E7 peptides is also being studied.

Special considerations

Glandular lesions of the cervix

Adenocarcinoma and adenosquamous carcinoma account for about 15% of invasive cervical carcinomas [42]. There seems to be a rising incidence of adenocarcinoma in the general population, but this might be a result of a decrease in squamous cell disease because of mass screening [43]. Of concern, the incidence of adenocarcinoma of the cervix appears to be increasing in younger women in the US [44].

Controversy exists regarding the prognosis for adenocarcinoma versus squamous cell carcinoma of the cervix [7,45,46]. Because these lesions arise higher in the cervical canal, early detection is difficult and cytology is less effective. This might lead to differences in outcome when compared with squamous cell lesions.

The treatment of glandular carcinoma of the cervix has traditionally been similar to treatment of squamous cell carcinoma of the cervix. There are no randomized, controlled studies specifically looking at treatment of adenocarcinoma of the cervix. There does appear to be some advantage to primary surgery in early-stage disease (RR 1.7–2.9) [47].

For advanced-stage cervical adenocarcinoma, radiation therapy continues to be the mainstay. Studies of radiation therapy versus chemotherapy and radiation actually include patients with glandular carcinoma of the cervix; however, few studies have looked specifically at the effectiveness histological type on the outcomes of chemotherapy and radiation.

Cervical carcinoma and pregnancy

Cervical cancer during pregnancy complicates management. The clinician must take into account both the desires of the patient to maintain the pregnancy and adequate treatment of the cancer. Approximately 30% of cervical cancers are diagnosed during childbearing years with 3% of cervical cancers diagnosed during pregnancy and only 0.05% of all pregnancies complicated by cervical cancer. The reported incidence ranges from 1.1 to 10.6 cases per 10,000 pregnancies, depending on whether or not in situ carcinomas and postpartum patients are included [48]. Factors that are important to consider are the stage of disease at the time of diagnosis, the gestational age of the fetus, and the patient's desires.

Diagnosis of cervical cancer during pregnancy can be delayed because symptoms of early cervical cancer are similar to those of uncomplicated pregnancy. Vaginal bleeding can complicate both cervical cancer and a normal pregnancy. Additionally, cervical lesions can be mistaken for an ectropion or a decidual relation of the cervix. The diagnosis of cervical cancer should be

considered along with abnormal vaginal bleeding and any lesion of the cervix should be evaluated by biopsy.

Colposcopy and cervical biopsy are safe during pregnancy despite the increased propensity for bleeding following biopsy. The number of unsatisfactory colposcopies should be decreased because of the eversion of the transformation zone during pregnancy. Cervical colposcopy during pregnancy can be more difficult, however, because of the size of the cervix and vaginal relaxation. Either vaginal wall retractors or a condom placed over the speculum might be necessary to fully visualize the cervix. It has been the author's practice to perform biopsies for suspected invasive carcinoma or significant high-grade lesions, whereas others have recommended biopsies for suspicions of all high grade lesions. Experienced colposcopists can defer biopsy and closely follow patients with low-grade lesions during pregnancy. Because of the reported incidence of patients discovered with cervical cancer postpartum, however, a biopsy of any suspicious lesion or referral of the patient for a second opinion should be considered.

The indication for a cervical conization during pregnancy is for the evaluation of an invasive lesion. Should the diagnosis of invasion be made on a colposcopically directed biopsy, conization is not necessary. If there is a biopsy of a microinvasive carcinoma and definitive therapy is planned, cervical conization should be performed.

Significant complications have been reported with cervical conization and pregnancy, including hemorrhage, spontaneous abortion, premature delivery, and infection. To decrease the amount of bleeding, a cervical cerclage technique has been proposed [49]. Other authors have proposed a shallower ''coin-shaped'' specimen or a ''wedge'' excision to decrease morbidity. While LEEP procedures have been proposed during pregnancy, cautery artifact can obscure the diagnosis and complicate the decision for radiation or radical surgery. Treatment of carcinoma in situ (CIS) during pregnancy by loop excision has been published. In most series, treatment of CIS during pregnancy shows persistence of the disease in the postpartum period. Some authors have proposed loop excision during the first trimester of pregnancy for biopsy-proven CIN III. Persistent dysplasia was found in two of nine patients in this series and no invasive cancers were found [50]. The consensus among experts is that CIS does not need treatment and can be followed safely during pregnancy.

Management of Stage I cervical cancer patients during pregnancy is similar to nonpregnant patients. Again, recommendations for treatment must be based on gestational age, the patient's desire for continuation of the pregnancy, and stage. Patients with diagnosed Stage 1A1 cervical cancer diagnosed by cervical conization who wish to maintain the pregnancy can delay treatment until fetal maturity. Some authors suggest close follow-up with colposcopy every 4 weeks. Postpartum patients who have completed childbearing might consider a simple hysterectomy.

Patients with Stage 1B disease who do not desire continuation of pregnancy can undergo radical surgery or radiation therapy as definitive treatment. Patients who are greater than 20 weeks gestation can be observed and await fetal maturity.

The literature available suggests that a delay of treatment with Stage I disease has no survival advantage compared with undergoing immediate treatment. The number of patients in the literature is small, however.

In early-stage cervical carcinoma, while data do not exist that the route of delivery effects outcome of patients in patients with Stage 1B disease, the risk of hemorrhage might argue for cesarean section followed by delayed radical hysterectomy or cesarean radical hysterectomy.

In more advanced Stage II to Stage IV cancers, radiation therapy is generally the preferred treatment modality. Again, the age of gestation must be taken into consideration. The effects of delay of therapy in more advanced cases are unknown. Certainly, in advanced cases, if fetal maturity is present, cesarean delivery should be affected followed by radiation therapy.

Current data suggest that the risk of mortality is not adversely affected by pregnancy, nor does cervical cancer adversely affect pregnancy outcomes. Consideration of referral to a professional who is familiar with the treatment of cervical cancer during pregnancy and referral to a high-risk obstetrician should be considered [48].

Summary

- Cervical carcinoma is staged clinically by examination and simple radiological procedures. CT and MRI can, however, be used to guide management.
- Prognosis is best made by tumor size, depth of invasion, parametrial involvement, nodal status, LVSI, and histology.
- CIN III and CIS can be treated by ablative or excisional procedures. Hysterectomy should not be the primary treatment.
- Microinvasive (<3 mm) Stage IA cervical carcinoma can be treated conservatively with conization in patients who desire fertility, but the standard of care remains simple hysterectomy.
- Stages IB and IIA can be treated with either radical hysterectomy or radiation therapy dependent upon the patient's health and preference. Risk factors after radical hysterectomy (eg, bulky tumors, deep invasion, involved nodes or margins, LVSI) might warrant adjuvant radiation therapy.
- Chemoirradiation is the current standard of care for treatment for Stages IIB, III, and IVA. Some clinicians also use this modality in patients with Stage IB disease who are undergoing radiation as the primary treatment.
- Locally recurrent disease can be treated with either radiation (after radical hysterectomy) or pelvic exeteration (after primary radiation therapy).
- Exenteration in appropriately selected patients yields 5-year survival rates up to 82% with low complication rates. Many exenterations are aborted intraoperatively because of distant or unresectable disease, however.
- The incidence of glandular carcinoma of the cervix is rising, particularly in younger women.

- Cervical carcinoma detected during pregnancy requires the combined efforts of the gynecologic oncologist and the maternal–fetal medical specialist to determine the timing and method of treatment.

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New developments in the treatment of invasive cervical cancer

Samuel S. Im, MD, Bradley J. Monk, MD*

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of California, Irvine Medical Center, Chao Family Comprehensive Cancer Center, 101 The City Drive, Building 23, Room 107, Orange, CA 92868, USA

Worldwide, cervical cancer is one of the most common cancers in women [1]. About 450,000 women are diagnosed with cervical cancer each year around the world, and another 200,000 women succumb to the disease annually [2,3], especially in developing countries where Papanicolaou smear screening has been insufficiently implemented. In the United States, where the cervical cancer rate has steadily declined since the mid-1940s because of screening practices, there are still approximately 13,000 new cases of invasive cervical cancer and 4100 cancer deaths each year [4].

Through surgery and radiation treatments, early stage cervical cancer has an excellent prognosis. With more advanced disease, however, the hope for prolonged survival is poor. For many decades there has been little change in the overall survival rates of women with locally advanced cancer. Fortunately, concurrent cisplatin-based chemotherapy with pelvic irradiation has been shown to improve survival, and it represents one of the most significant advances in the treatment of cervical cancer over the past three decades [5–9].

Chemotherapy has also received considerable attention in recent years for the treatment of metastatic or recurrent carcinomas. In addition to newer chemotherapy agents, combinations of cytotoxic compounds and other novel biologic therapies are under investigation. Rapid advances in molecular biology and human cancer genetics appear ready to pave the way for other novel and potentially active cancer treatments. For example, the study of human papillomavirus (HPV)-induced carcinogenesis (which is now believed to be an important factor in the cause of cervical cancer) and its two oncogenes might have a major impact in the near future on how patients are treated. HPV vaccines are on the horizon. Moreover, as cervical cancer biology is studied in greater detail,

* Corresponding author.
E-mail address: bjmonk@uci.edu (B.J. Monk).

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clinicians are developing a much better understanding of the impact that angiogenesis and hypoxia play in the overall treatment of cervical cancer. Hopefully, these future advances will continue to improve outcomes and translate into significant survival benefits.

Current treatments

Surgery

With early-stage invasive cervical cancer (Stage IA1 or microinvasive cancer) a simple hysterectomy is generally recommended. With such minimal invasion, the chance of spread is considered to be minimal, thus less radical surgery is performed. Östör noted in his series of 1324 patients from Scandinavia with 1 to 3 mm of stromal invasion that only about 1% of these patients were found to have nodal metastasis [10]. In agreement with Östör's findings, in their series of 1704 patients who met the Federation of International Gynecologists and Obstetricians (FIGO) Stage IA1 definition, Creasman et al found that only 17 of the patients recurred; three died from their disease after surgical therapy [11]. With increased experience and substantial supportive data, conservative management of early invasive cervical cancer is now generally accepted. In cases in which future fertility is desired, cervical conization might also be acceptable provided that the surgical margins are free of any invasive or preinvasive disease.

The conservative management of Stage IA1 (or microinvasive disease) has been limited to squamous cervical cancers. The treatment of early-stage adenocarcinomas of the cervix has traditionally been radical hysterectomy with pelvic lymphadenectomy. The argument for this more aggressive treatment for nonsquamous tumors was based on the assumption that the architectural complexity of endocervical glands, with deep invaginations, branching, and tunnel formation, made the measurement of depth of stromal invasion difficult. The term microinvasive adenocarcinoma of the cervix now appears more frequently in the literature, however. In 1997, FIGO included both squamous and glandular disease in the definition for Stage IA1 and microinvasive cervical cancer [12].

Data on the conservative management of adenocarcinoma of the cervix are limited. Most of the literature has been devoted to evaluating the use of cervical conization for carcinoma in situ disease and subsequent rates of recurrence. More recently, patients who strongly desired a fertility-sparing procedure for their microinvasive disease have been treated with cervical conization if the diagnosis is Stage IA1 adenocarcinoma and the margins of resection are uninvolved. Schorge et al reported on their experience with five patients with Stage IA1 adenocarcinoma cervical cancer [13]. Although their follow-up was limited to less than 2 years, none of the participants treated with a cervical cone had evidence of recurrence at the end of the study period. McHale et al also reported on four other patients with Stage IA1 adenocarcinoma who opted for cervical conization as definitive treatment of their disease [14]. Three of the four women

subsequently delivered viable infants. Although cervical conization cannot be advocated as standard treatment for early microinvasive glandular lesions, it could be an option for a well-informed patient who is willing to accept the risks involved with conservative management in the hope of maintaining fertility.

The conventional treatment of women with Stage IA2, IB, and IIA cervical carcinoma consists of either radical hysterectomy and bilateral pelvic lymph node dissection or radiation therapy combining whole pelvic teletherapy with local brachytherapy. These treatment modalities are recognized as equally efficacious with respect to local control and survival. Surgery is often preferred to radiotherapy in younger women because ovarian function is eliminated and sexual function often is compromised following radiation. In addition, late complications of radiation are avoided when patients are treated with surgery alone.

For more than a decade, the sentinel lymph node procedure has been investigated in the treatment of melanoma, breast, and vulvar cancers [15–17]. The primary goal of the procedure is to identify the first (sentinel) draining lymph node from the anatomical region during the surgery. If it is free of disease, an assumption is made that other nonsentinel nodes are also negative, and the rest of the lymph node dissection is avoided. Recently, Lantzsch et al reported their experience with the sentinel lymph node procedure in 14 Stage IB1 cervical cancer patients [18]. Sentinel lymph nodes were identified in 13 patients (eight unilaterally and five bilaterally) out of 14 using a radiolabelled marker. Only one patient demonstrated positive sentinel lymph nodes, and the two sentinel nodes were the only histopathologically positive nodes from the lymphadenectomy, perhaps accurately depicting the real nodal status. The sentinel lymph node procedure is still under investigation in the treatment of cervical cancer and will need further clinical evaluation. If it proves to be accurate, however, the morbidity of complete pelvic lymphadenectomy might be avoided.

Radiation

The main treatment modality for advanced-stage cervical cancer is radiotherapy with concurrent chemotherapy. Even in earlier-stage disease, this treatment has emerged as a notable alternative to radical surgery. Radiotherapy is also used after radical hysterectomy to minimize future recurrences. Gynecologic Oncology Group (GOG) Protocol 92 was designed to determine whether or not postoperative pelvic radiotherapy reduces the rate of cervical cancer recurrence and decreases the mortality rate in Stage IB cancer patients with risk factors such as large tumor diameter, deep stromal invasion, and presence of lymphovascular space invasion [19]. The objective of the study was to improve the 30% local recurrence rate among patients with intermediate risk factors predictive of recurrence. The failure rate has remained unchanged for decades. As summarized by Sedlis et al, 277 women who met the criteria for the study were recruited and randomized to either the adjuvant radiotherapy group or the no further treatment group after radical hysterectomy. For the adjuvant radiotherapy group, there was a 47% reduction in risk of recurrence when compared with the group that did

not undergo further treatment. There was a three-fold higher incidence of grade 3/4 adverse events in the radiotherapy group, although adverse events were still relatively infrequent and self-limiting.

In attempt to better define radiotherapeutic practices and outcomes in the management of cervical cancer, Eifel et al assessed the patterns of radiotherapy care for patients with cervical cancer from 62 randomly selected radiation therapy facilities throughout the United States [20]. These findings suggested that many of the nonacademic radiation facilities treated small numbers of cervical cancer patients. In 83% of the nonacademic facilities, each center treated fewer than three patients per year. In addition, Eifel et al concluded that only 32% of the centers completed the treatment in 8 weeks or less, which is the time frame that is maximally efficacious and represents an acceptable treatment duration. Another notable finding was an increase in utilization of high dose-rate (HDR) brachytherapy as an alternative to traditional low dose-rate (LDR) intracavitary irradiation. HDR intracavitary radiation therapy appears to be gaining wider acceptance with its advantages of shorter treatment period for the patient and less radiation exposure for the medical personnel. Although there are insufficient data comparing HDR with LDR brachytherapy, it appears that even small facilities that have relatively little experience with cervical cancer are beginning to use the HDR intracavitary treatment modality.

Radiotherapy has been used with great success in earlier-stage cervical cancer. A combination of external beam irradiation and intracavitary brachytherapy has achieved excellent locoregional disease control and survival for patients. Eifel et al reported on their series of 701 Stage IB1 cervical cancer patients who had been treated with radiation alone [21]. The 5-year disease-specific survival rate for this group was 90%. Perez et al and Lowrey et al also reported an excellent prognosis with radiotherapy in this cancer stage group [22,23]. Similarly, Stage IIA disease treated with radiotherapy has a high survival rate, ranging between 70% to 85% [22–24]. Unlike earlier-stage cervical cancer, in which radical surgery is a valid alternate treatment option, more locoregionally advanced disease requires radiotherapy as a primary treatment. For Stage IIB disease, the 5-year survival rate ranges between 65% to 75%; for Stage IIIB it is 35% to 50%, and for Stage IV it is between 15% to 20% [22–25]. Radiation treatment for these stages requires a careful balance between whole pelvic external-beam irradiation with local brachytherapy. Patients receive between 4000 to 6000 cGy of external pelvic irradiation during the course of the treatment. For traditional LDR brachytherapy, the dose rate at point A is maintained at 40 to 50 cGy/hour. HDR intracavitary therapy delivers greater than 100 cGy/minute using a high-activity cobalt or iridium source.

Chemotherapy

For years, chemotherapy was considered to be ineffective in the treatment of invasive cervical cancer. Its role in cervical cancer was limited to treating patients with advanced metastatic disease or patients with recurrences who could not be

otherwise salvaged with pelvic exenteration. Then, in 1999, five landmark papers reported significant improvement in survival for advanced cervical cancer patients through the concurrent administration of cisplatin-based chemotherapy with radiation therapy in women with locally-advanced disease [5–9]. These five studies were all mutli-institutional, randomized, controlled trials that demonstrated improved survival with combination therapy (Table 1). The trials differed in their inclusion criteria, chemotherapy regimen, and radiotherapy schedule, but all showed statistically significant in progression-free survival and overall survival for the investigational group. All five of the trials included cisplatin in their chemotherapy regimen, and the National Cancer Institute issued a strong recommendation for inclusion of cisplatin-based chemotherapy for women who require radiotherapy in the treatment of cervical cancer [26]. Weekly single-agent cisplatin at 40 mg/m^2 or cisplatin plus 5-flurouracil are the current regimens of choice to be given concurrently with radiotherapy.

Previously, Sedlis et al had shown that postoperative radiotherapy results in improved overall local control for Stage IB cervical cancer patients [19]. GOG Protocol 109 included concurrent chemotherapy with postoperative radiotherapy [7]. The patients included Stages IA2 to IIA cervical cancer patients with the highest risk factors for recurrences (positive nodes, positive margins, parametrial extension). The patients were randomized to either radiotherapy alone or radiotherapy plus chemotherapy. The concurrent chemotherapy group showed significantly better progression survival and overall survival when compared with the radiotherapy alone group.

Table 1
Five multi-institutional randomized, controlled trials comparing concurrent chemoradiotherapy with radiotherapy alone

Trial	FIGO stage	Patient No.	Progression-free survival (%)	(P-value)	Survival rate (%)	(P-value)
Keys et al, 1999	IB2					
RT versus		186	63		74	
RT + CDDP		183	79	< 0.001	83	0.008
Morris et al, 1999	IB2–IVA					
RT versus		193	40		58	
RT + CDDP + 5-FU		195	67	< 0.001	73	0.004
Rose et al, 1999	IIB–IVA					
RT versus		177	47		50	
RT + CDDP or		176	67	< 0.001	66	0.004
RT + CDDP + 5-FU + HU		173	64	< 0.001	67	0.002
Whitney et al, 1999	IIB–IVA					
RT + HU versus		191	47		43	
RT + CDDP + 5-FU		177	57	0.033	55	0.018
Peters et al, 2000	IA2–IIA					
RT versus		116	63		71	
RT + CDDP + 5-FU		127	80	0.003	81	0.007

Abbreviations: 5-FU, 5-flurouracil; CDDP, cisplatin; HU, hydroxyurea; RT, radiotherapy.

Recurrent disease

Management of persistent or recurrent cervical cancer has not improved significantly, even with current advancements in chemotherapy. Cisplatin has a response rate of approximately 20% [27], and other agents such as paclitaxel and ifosfamide have been added successfully to single-agent cisplatin with increased response rates [28,29]. Increasing the cisplatin dose is also associated with an increased response rate [30]. All three of these approaches have been studied in prospective randomized trials without any increase in survival (Table 2). Because the addition of paclitaxel is associated with almost twice the response rate compared with single-agent cisplatin without additional significant morbidity, this combination is considered to be the standard regimen in the palliative management of cervical cancer. Cisplatin alone is an acceptable alternative because of its convenience and the absence of alopecia. Disappointingly, however, patient survival rates have not changed.

Other chemotherapeutic agents appear to be active as single agents and might show some promise in the treatment of recurrent cervical cancer. Vinorelbine is a semisynthetic vinca alkaloid that has a cytotoxic effect on cancerous cells through disruption of microtubule assembly by binding to tubulin dimers. Microtubules are an essential element during cell division, and the antimicrotubule action of vinorelbine results in dissolution of the mitotic spindle apparatus, leading to metaphase arrest. The European Organization for Research and Treatment of Cancer recently reported on vinorelbine as a single agent in cervical cancer patients with metastatic or recurrent disease [31]. They observed 17% partial response and 20% stable disease, and there were minimal side effects from the drug. In another Phase II trial studying vinorelbine, Pignata et al reported on their experience with the combination of cisplatin and vinorelbine chemotherapy in the treatment of recurrent cervical cancer [32]. They observed an overall response rate of 46.7%. Irinotecan is another chemotherapeutic agent that shows promise against recurrent cervical cancer. It is a topoisomerase-1 inhibitor with a bio-

Table 2
Prospective randomized GOG trials involving cisplatin in dose escalation study and in combination study with ifosfamide and paclitaxel for advanced, recurrent, or persistent cervical cancer patients

Trial	Overall response rate (%)	Progression-free survival (mo)	Median survival time (mo)
Bonomi et al, 1985			
50 mg/m^2 q 3 wk	20.7	3.7	7.1
100 mg/m^2 q 3 wk	31.4 ($P = 0.015$)	4.6 (no P value)	7.0 (NS)
Omura et al, 1997			
Cisplatin	17.8	3.2	8.0
Cisplatin + ifosfamide	31.1 ($P = 0.004$)	4.6 ($P = 0.003$)	8.3 (NS)
Moore et al, 2001			
Cisplatin	19.4	2.8	8.8
Cisplatin + paclitaxel	36.2 ($P = 0.002$)	4.8 ($P < 0.001$)	9.7 (NS)

Abbreviations: NS, not statistically significant.

logically active metabolite, SN-38. SN-38 is approximately 1000 times more potent than irinotecan as an inhibitor of DNA topoisomerase-1. In Phase II trials irinotecan has shown response rates of 21% to 24% in recurrent cervical cancer patients. With promising Phase II results for these two drugs, the GOG is currently developing a study to compare cisplastin plus paclitaxel versus cisplatin plus vinorelbine versus cisplatin plus irinotecan. Importantly, vinorelbine's mechanism of action is distinct from paclitaxel, another microtubule active drug, as is the mechanism of irinotecan is distinct from topotecan, another topoisomerase inhibitor. Idealy, these differences in action will translate into clinically significant differences in response. To evaluate the addition of a topoisomerase-1 inhibitor with platinum-based therapy, GOG Protocol 179 is currently evaluating the combination of cisplatin and topotecan in a Phase III trial in advanced-stage, recurrent, or persistent cervical cancer patients.

New and novel treatments for the future

HPV and human therapeutic vaccine trials

A sexually transmitted agent has long been implicated in the cause of cervical cancer. In the mid-1970s, HPV emerged as a potential carcinogenic cofactor involved in cervical carcinogenesis. Subsequent studies showed that cervical cancer is highly associated with HPV infection. HPV is detected in more than 99% of cervical cancers and is now considered to be one of the main factors in cervical cancer development [33].

Over 100 HPV genotypes have been cloned and sequenced [34]. Among them about 35 HPV types infect the female genital tract [35]. HPV types 6 and 11 are usually associated with common genital condyloma, thus pose a low risk for invasive cervical carcinoma. HPV types 16, 18, 31, 33, 45, 52, and 58 carry a higher risk for cervical cancer. HPV 16 is detected in about half of all squamous cell cervical cancers and is the main focus for much cervical cancer and HPV research [36].

The HPV genome can be grouped into early and late genes. The early genes E2, E6, and E7 might play an important role in carcinogenesis. In particular, E6 and E7 are known oncogenes. The E6 encodes for a 16 to 19 kDa protein, and it binds to the tumor suppressor protein p53 and causes its degradation by an ubiquitin proteolysis pathway [37,38]. E6 has also been linked to the telomerase activation and immortalization of cells. E7 encodes for a 10 to 14 kDa protein, which has transforming activity and regulatory functions [37]. It binds pRB (retinoblastoma-susceptibility protein) and modulates cell cycle control. The late proteins consist mainly of L1 and L2. L1 is the major capsid protein for HPV and the L2 is the minor capsid protein. The capsid proteins form the icosahedric capsids; 72 capsomeres to enclose the HPV genome.

With HPV emerging as a major factor in cervical cancer pathogenesis, there is great interest in targeting HPV to prevent viral infection and to treat precancerous

and cancerous lesions. Development of an HPV vaccine has been a major area of interest in achieving this goal. The late proteins of HPV, consisting mainly of L1 and L2, have been used exclusively in the development of prophylactic vaccines. The L1 and L2 proteins are the most antigenic of the HPV-encoded proteins [37]. Vaccines containing these proteins should elicit a strong humoral immune response (antibodies) from the host against these capsid antigens and theoretically protect the vaccinated individual from HPV infection.

For invasive cervical cancer patients, a therapeutic vaccine against HPV early proteins is required. The ubiquitous presence of E6 and E7 in the cellular cytoplasm during all stages of cervical cancer makes them ideal targets for the vaccine. The therapeutic vaccine should elicit a strong cellular immune response and cytotoxic T lymphocytes (CTL) should eradicate the tumor tissue.

Earlier human therapeutic vaccine trials have been limited to advanced cervical cancer patients with the hope of eliciting some immunologic or therapeutic response. Borysiewics et al from United Kingdom reported administering a recombinant vaccinia virus that expressed E6 and E7 proteins of HPV 16 and 18 to patients with advanced-stage and recurrent cervical cancer [39]. Eight patients were recruited for the study. None of them had any side effects from the vaccination, but only one patient showed any specific CTL response to the vaccine.

As in the above clinical trial, the use of viral vectors has been a popular way to deliver E6 and E7 genes to targeted cells. Viral vectors, which had been developed for gene therapy, became useful tools in assisting with vaccine development. The underlying concept has been that the delivery of E6 and E7 genes with subsequent expression of these proteins inside cancerous cells would lead to major histocompatibility complex (MHC) I-dependent antigen presentation and destruction of the targeted cells expressing the antigen.

Peptide vaccines using E6 and E7 proteins have also been used in clinical trials. Steller et al tested the human leukocyte antigen (HLA)-restricted HPV 16 E7 peptide vaccine in a Phase I trial involving patients with refractory vaginal and cervical cancers [40]. Twelve patients were recruited for the study. Each patient was given four inoculations of the vaccine at 3-week intervals. Like the viral vector vaccine, there were no side effects from the vaccination, but no clinical response was elicited from the vaccine. Although there was no clinical improvement, this study showed that even in advanced-stage cervical cancer patients, specific cellular responses could be generated and augmented by peptide vaccination. In a similar clinical trial, van Driel et al vaccinated 19 advanced cervical cancer patients with HPV 16 E7 peptide vaccine [41]. Although no specific CTL response against the E7 peptide was elicited, this study further bolstered the notion that even in advanced-stage cervical cancer patients, vaccines can be safely administered and are feasible.

DNA vaccination is another alternative in therapeutic vaccine development for cervical cancer. DNA vaccines allow for large-scale production and high purity, making them a more attractive option than peptide vaccines. Shi et al reported their experience in using an HPV 16 E7 mutant with markedly decreased

transforming activity as a DNA vaccine [42]. They tested their DNA vaccine in a murine model, which showed a highly immunogenic effect with marked elevation in the E7-specific CTL response. Furthermore, when the vaccinated mice were challenged with tumor cells, they were 100% protected. These results showed that DNA vaccination is a viable option in human cervical cancer vaccine development.

In contrast to attenuated viral vaccines, which induce immunity through low-grade infection with nonvirulent yet intact virus, DNA vaccines do not have the ability to propagate, thus limiting their effectiveness in immune induction. To enhance the immunity of DNA-based vaccines, genes that encode for proteins that have the capacity to enhance immunogenicity are fused with HPV genes of interest. Heat shock proteins have been used in this capacity along with endosomal/lysosomal targeting signal proteins [43–46]. The resulting fused DNA vaccines markedly enhanced the potency of the vaccine by boosting the immune induction.

Another approach to increasing the potency of DNA vaccines has been the use of dendritic cells (DCs), which are highly efficient antigen presenting cells. Wang et al transfected the DCs with the E7 gene to construct an E7-containing DC vaccine [47]. The vaccine was then administered to the murine model, which showed high humoral and cellular immune responses. The potency of the vaccine was highest when delivered through the intramuscular route. Similarly, de Bruijn et al pulsed DCs with E7 proteins and elicited an E7-specific CTL response from the vaccinated mice [48]. When the vaccinated mice were challenged with tumor cells, they showed marked antitumor activity.

Anti-angiogenesis compounds

Angiogenesis is an important element in the progression of solid tumors because synthesis of new blood vessels is an essential process in tumor invasion and metastasis. There is growing evidence that angiogenesis plays an important role in cervical cancer pathogenesis. In cervical cancer, there is upregulation of the angiogenic promoter vascular endothelial growth factor (VEGF) and down-regulation of angiogenic inhibitor thrombospondin-1 (TSP-1). These two factors are usually downregulated and upregulated, respectively, by the tumor suppressor protein p53 in the angiogenic signaling pathway. In cervical cancer, however, p53 is rapidly degraded by the activity of the HPV oncogene E6, possibly leading to the promotion of angiogenesis.

The level of angiogenesis in tissue is usually determined by the measurement of the expression of angiogenic factors such as VEGF and TSP-1. Analysis of tumor microvessel density (MVD), which is determined through immunohistochemical staining of vascular endothelial cell markers, is also used to evaluate angiogenesis [49]. Dobbs et al recently demonstrated a direct association of MVD and VEGF expression with increasing levels of cervical neoplasia in cone biopsy and hysterectomy specimens [50]. The same relationship was also reported by Dellas et al [51]. Using similar angiogenesis measurement meth-

odology, Tokumo et al showed that the expression of VEGF was highly correlated with MVD in primary invasive cervical cancer from 73 patients treated with radical hysterectomy [52].

The association between angiogenic activity and clinical outcome has yet to be determined, but recent studies seem to suggest an inverse relationship between angiogenic activity and a favorable prognosis. Obermair et al in Vienna reported that in their series of 166 women with Stage IB disease who had been treated with radical hysterectomy, the estimated 5-year survival rate for patients whose tumors had MVD of less than or equal to 20 per high-power field to be 90% compared to 63% for patients with tumors that had an MVD of greater than 20 [53]. More importantly, multivariate analysis showed this association to be an independent prognostic factor. Kodama et al in Japan showed that TSP-1 expression was also an important prognostic factor in cervical cancer [54]. Cervical cancer patients demonstrating TSP-1 mRNA expression showed significantly better prognosis than patients lacking TSP-1 expression.

Given the association between angiogenesis and tumor progression, inhibition of tumor-induced angiogenic activity stands out as an attractive therapeutic target for a novel cervical cancer treatment. It seems implicit that pharmacological inhibitors of angiogenesis could arrest tumor progression. Many novel angiogenesis inhibitors are currently being tested in Phase I and II clinical trials to test for activity in advanced and recurrent cervical cancer. In a recent Phase I study of the novel angiogenesis inhibitor TNP-470, complete remission was demonstrated. GOG is currently evaluating SU5416, a VEGF inhibitor, and bevacizumab, a recombinant anti-VEGF monoclonal antibody, in Phase II trials [55].

Anemia in cancer

Anemia is a common finding in cervical cancer patients. It results from chronic illness and myelosuppressive cancer treatments, and it can lead to intratumor hypoxia, which has been associated with development of resistance to ionizing radiation and some forms of chemotherapy. Recent clinical studies seem to suggest that persistent tumor hypoxia can lead to enhanced malignant progression and aggressiveness of the tumor through clonal selection and genomic changes [56]. It appears that hypoxia might play a role in further loss of capacity to differentiate and to undergo apoptosis for the tumor cells along with upregulation of angiogenesis, which would increase tumor progression and metastasis, potentially impacting long-term survival.

A retrospective study from Canada recently evaluated the effect of anemia and subsequent transfusion on 605 cervical cancer patients treated at seven radiotherapy centers [57]. The study showed a significant increase in local control of the disease, progression-free survival, and overall survival when the hemoglobin level was consistently maintained above 12 g/dL throughout the radiation treatment. Survival was also significantly worse for patients who began the therapy with a hemoglobin level below 12 g/dL or patients whose level subsequently fell below 12 g/dL during the treatment period. When the 5-year

survival rate was analyzed, there was a 24% difference between the groups whose hemoglobin level was above and below 12 g/dL.

Transfusion is an effective method of raising the hemoglobin level in anemic patients, but there are inherent, well-defined risks to the procedure. There is also a limited supply of blood products, which is heavily dependent upon blood donations. Thus, recombinant human erythropoietin (r-HuEPO) has been evaluated as an alternative to transfusion in raising the level of hemoglobin in patients with cervical cancer [58–60]. R-HuEPO stimulates the proliferation of committed erythroid progenitor cells and effects their differentiation into normoblasts, thereby increasing the hematocrit.

Although there is compelling evidence that raising the hemoglobin level can improve poor prognosis, which is associated with a low-presenting hemoglobin level, there are currently no prospective randomized studies addressing this issue. GOG is currently investigating the role of hemoglobin level in advanced cervical cancer patients undergoing concurrent chemoradiation therapy. GOG Protocol 191 is a Phase III trial with two arms of concurrent cisplatin and radiotherapy patients. The patients are randomized to a control group (who will be transfused only when their hemoglobin level falls below 10 g/dL) and investigation group (who will receive r-HuEPO throughout the treatment and will be transfused if hemoglobin falls below 12 g/dL).

Summary

Cervical cancer is a preventable disease that is curable when it is detected early. For advanced-stage cancer, the prognosis is worse. Over the years, much progress has been made in radiation therapy and in chemotherapy, but it took three decades for the arrival of concurrent chemoradiation therapy, which significantly improved the survival among women with advanced cervical cancer. This fact underscores the need and the importance for continuing efforts in clinical research. While current standards of therapy are being fine-tuned as more information is being gathered, great strides are being made in the areas of molecular and cancer biology. Novel treatments for cervical cancer appear to be imminent in the near future.

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In vitro conventional cytology
Historical strengths and current limitations

Mark Spitzer, MD

New York University School of Medicine and North Shore University Hospital, 4-Levitt, 300 Community Drive, Manhasset, NY 11030, USA

Papanicolaou and Traut first reported the use of exfoliative cervical cytology for the diagnosis of cervical cancer and precancer [1]. They obtained cellular material from the vaginal pool. Ayre reported the use of a wooden spatula to scrape cellular material directly from the cervical transformation zone [2]. When used in a regular screening program, conventional cervical cytology has been a hugely successful screening tool. Cervical cancer was once the most common gynecologic malignancy in the United States, and it remains so in countries that lack regular cytological screening programs. Eighty percent of the almost half-million cases of cervical cancer worldwide occur in developing countries, where only 5% of the female population have had a Papanicolaou (Pap) smear in the past 5 years [3]. In these countries, cervical cancer is a leading cause of cancer death. In contrast, in countries with a regular screening program, cervical cancer is only the tenth leading cause of cancer death. In 1986 about 50% of women in developed countries had been screened for cervical cancer compared with 5% of women in developing countries [4]. Perhaps the best observational studies demonstrating the benefit of cervical cytological screening are those reporting cervical cancer death rates in the Scandinavian countries before and after the institution of regular screening programs [5–9]. After the introduction of regular screening, cervical cancer death rates dropped between 8% and 73% [5]. The greatest drop occurred in Iceland and corresponded to the highest rate of participation [5,6], while in Norway, the country with the lowest participation rates, the mortality rate was almost unchanged [8,10].

In the US, where conventional cytology has been used to screen women for more than 50 years, cervical cancer death rates have decreased dramatically. Current estimates are that there will be 12,800 cases of invasive cervical cancer that will result in 4600 deaths annually in the US [11]. A woman's lifetime risk of being diagnosed with cervical cancer in the US is currently 0.83%, and the risk of

E-mail address: mspitzer@nshs.edu

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dying from the disease is 0.27% [12]. This represents a 79% reduction in the incidence of cervical cancer and a 75% reduction in the mortality rate since 1950 [12]. The dramatic drop in incidence and mortality rates are largely attributable to the success of Pap smear screening [13–15]. The decrease has been so dramatic that it is one of the few interventions to receive an "A" recommendation from the US Preventive Services Task Force even though there are no randomized trials demonstrating its effectiveness [15].

Nevertheless, despite seemingly universal availability of cytological screening in the US, cervical cancer rates have not decreased significantly in the past 15 years, and have definitely not dropped to zero. This fact can be attributed to a combination of a failure to screen selected populations of women at risk and the failure of Pap smears to detect disease in some women. Over the past several years there has been a tremendous amount of attention focused on the latter issue, but both matters deserve intense scrutiny.

Failure to screen

Schwartz et al reviewed women who developed cervical cancer in Connecticut between 1985 and 1990 [16]. They separated these women into four categories: (1) those who developed cervical cancer in the absence of any prior screening, (2) those who had false-negative Pap smear reports, (3) those who were inadequately treated, and (4) those who had rapidly progressive cancers.

They found that the largest group (28.5%) was comprised of women who had never been screened. Compared with the rest of the groups, these women tended to be older (average age 64 years). The screening interval in an additional 23.5% of women who developed cervical cancer was more than 5 years, meaning that they had been underscreened. The population of patients who were never screened was also more likely to have advanced disease than all of the other groups taken together. Schwartz et al [16] estimated that if the Connecticut data were applied nationally, almost half of the cases of cervical cancer would be linked to inadequate or absent Pap smear screening. Furthermore, it can be assumed that because the never-screened population had more advanced disease that this group is responsible for more than half of the mortality from cervical cancer. In reviewing the European experience, Kenter et al [17] reported that 72.9% of patients with a diagnosis of cervical cancer in the Netherlands between 1980 and 1989 had never been screened. Kreuger and Beerman [18] reported that 57.0% of women diagnosed with cervical cancer in the Rotterdam area were not screened because they were either too old or too young to meet screening criteria. Stuart et al [19] reported that 30.1% of women with cervical cancer in the Alberta, Canada area had never been screened, and an additional 15.4% had not been screened within 3 years. Summarizing the American experience, the National Institutes of Health consensus statement in 1996 stated that "one half of the women with newly diagnosed invasive cervical carcinoma had never had a Pap smear and another 10% have not had a smear in the past five years" [20].

Estimates from the 1992 National Health Interview Survey supplement on cancer found that 90% of women had at least one Pap smear in their lifetime and that 67% had one within the past 3 years [21]. In a 1997 Gallup Organization telephone survey of 1000 women aged 18 years and older, 98% reported having had prior Pap tests [22].

Certain groups are more likely to be underscreened. Older women, uninsured and poor women, ethnic minorities—especially Hispanics, elderly Blacks, and women in rural areas—are at greater risk for being underscreened. Although the mortality rate from cervical cancer has dropped more than 70% in Caucasian women, the incidence of cervical cancer among African American women is still 65% higher and mortality rates remain more than double that for non-Hispanic Caucasians [23]. In some populations, the higher incidence and mortality from cervical cancer can be attributed to a failure to screen. The lack of screening might explain many of the differences in cervical cancer rates among ethnic groups [24], which has been attributed to lack of access to health care or the individual's refusal to take advantage of such access. In other populations, however, women are screened yet fail to follow up when a cytological abnormality is identified. Studies have shown that up to 15% to 42% of women fail to obtain evaluation and treatment following an abnormal Pap smear [25–28].

A number of factors have been associated with nonadherence to screening recommendations. Unfortunately, although some reasons for nonadherence are common to all groups, other factors are unique to specific populations. Southeast Asian women are more likely to be nonadherent, as are single, younger, and less educated women [24]. Another study noted that young women who attended an adolescent clinic were more likely to follow up [29]. Sanders et al used a structured interview to elicit reasons for noncompliance. The reasons cited were lack of understanding, inaccessibility of information, and staff attitudes [30]. Lerman et al also cited lack of understanding but noted fear of cancer and forgetting the appointment were also barriers to follow-up care [31]. In a survey of Mexican women, reluctance to be examined by a male health care provider, inconvenient clinic schedule, and poor communication were cited as barriers [32]. Still other studies have noted that 10% to 18% of women with abnormal Pap smears had never even been notified of their abnormality [33,34].

Although the vast majority of American women are screened regularly for cervical cancer, a small percentage ($< 10\%$) have never been screened or are underscreened. The importance of screening these women cannot be underestimated because more than half of all the women in the US with cervical cancer and an even greater percentage of women who die of cervical cancer are underscreened. Education, support services, economic incentives, and the use of intensive tracking systems have all proven to be successful in increasing the rate at which women adhere to screening and follow-up recommendations. Unfortunately, no single intervention or group of interventions can reach all women. Interventions must be tailored to meet the needs of each population of women. Despite the barriers, the implications of failure are such that all available resources must be marshaled in this attempt.

Problems with conventional cytology

Although the majority of all cervical cancers occur in unscreened and underscreened women, the majority of recent efforts to reduce the incidence of cervical cancer have focused on improving the quality of cervical cancer screening tests and reducing the false-negative results of cervical cytology. Much technology has been developed to detect cervical cancer and precancer. Although colposcopy is generally used in the US as a diagnostic tool to direct cervical biopsies, studies have shown that colposcopy is also an excellent screening tool [35–37]. Unfortunately, the cost of screening colposcopy and a shortage of trained colposcopists make it an impractical tool for cervical cancer screening in the US. Because of its etiologic role in cervical cancer, testing for the human papillomavirus (HPV) has been proposed as a cervical cancer screening tool. Almost 100 genotypes of HPV are known, and approximately 20 of them infect the cervix. Low-risk HPV types are unlikely to be associated with cervical cancer, so they are not thought to have a role in cervical cancer screening [38,39]. Even high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58) whose role in cervical carcinogenesis is well established might have only a limited role in cervical cancer screening because of the high prevalence of the virus in young, sexually active women [40]. Cervicography is a form of photographic colposcopy that was proposed for use in cervical cancer screening [41,42]. Initial reports showed that the false-positive rate of this technique was too high, and modifications of the reporting system to resolve this problem led to a false-negative rate approaching 50% [40]. Direct visual inspection (DVI) is proposed primarily for use in underdeveloped countries that lack the resources for a cervical cytology screening program, and speculoscopy, a form of enhanced DVI that employs chemiluminescent illumination, is currently FDA approved in the US only as a screening adjunct to cervical cytology [40].

Historically, the false-negative rate of cervical cytology has been reported to be between 6% and 55%; it is most commonly reported in the neighborhood of 20% [43–47]. This figure formed the basis of the American Cancer Society's recommendation that, after three consecutive negative smears, Pap smears can be performed at 3-year intervals [48]. Using such an analysis 80% of the lesions would be discovered, in the first year; in the second year 80% of the remaining 20% would be discovered (16%), and in the third year 80% of the final 4% would be discovered, calculating to a sensitivity of 99.2% after 3 years. The Agency for Health Care Policy Research (AHCPR) analyzed 85 manuscripts addressing the sensitivity of conventional cervical cytology and concluded that the sensitivity of a single smear is 51% [49]. Using this same rationale as outlined above, after three consecutive yearly Pap smears, the sensitivity would be only 88.2%.

An additional limitation on the accuracy of studies reporting the sensitivity of cervical cytology is the choice of a "gold standard." Many studies estimated their false-negative rate by rescreening the same slides [49]. This approach has it limitations because rescreened smears are subject to the same errors that caused them to be falsely negative in the first place. A further limitation is the failure to

evaluate women with negative cytology to assess whether or not they actually have disease (so-called verification bias). Failure to evaluate these women can lead to an underestimation of the false-negative rate. Only three studies have identified patients undergoing initial Pap smear screening and verified at least some of the negative Pap tests by screening colposcopy. The largest of these studies was by Baldauf et al [50]. In this study, a 10% random sample of 1539 women with negative Pap smears underwent colposcopy and biopsy to verify disease status. Davison and Marty [51] studied 200 women, and Hockstad [52] tested 73 women; all of the test-negative women in these two studies had their disease status verified with the reference standard test. The studies estimated the sensitivity of conventional cervical cytology at 56%, 53%, and 29%, respectively, and their specificity at 98%, 100%, and 97%, respectively [50–52]. The AHCPR meta-analysis [49] and a similar analysis by Fahey et al [53] were based on a histologic ''gold standard'' and found that the sensitivity of cytology was considerably lower than had previously been predicted.

These studies [50–52] and the meta-analyses [49,53] assume that all false-negative cytology is caused by screening and interpretive errors. Specifically, they assume that in each case the abnormal cells were on the slide and were missed by the screener or were misinterpreted by the pathologist. They further assume that the cellular findings on true positives and false-negatives are the same except that they were identified in the true positive smear and missed on the false-negative smear. Finally, they assume that if the false-negative Pap smear was caused by a collection error, that this was a random error rather than a predilection for a particular cervical lesion to not shed its abnormal cells.

Each of these assumptions is flawed. Evidence has shown that errors in sampling and preparation are the underlying cause of two-thirds of false-negative conventional Pap smears [49,54,55]. Reasons for false-negative results can include failure of the abnormal cells to exfoliate and adhere to the collection device, failure of the abnormal cells to be transferred from the collection device to the slide, or difficulty in preparation of the slide relating to air drying and other artifacts [56].

Hutchinson et al showed that the most commonly used devices for obtaining cervical cytology transferred fewer than 20% of their collected cells on to the glass slide [54]. Furthermore, because this transfer was heterogeneous, one can never be certain if the abnormal cells made it off the device and on to the glass slide. Variability and errors in technique further limit the predictability and reliability of conventional cytology. Pap smears might tend to be clumped, air-dried, or cover only a small portion of the glass slide. These issues weaken the sensitivity of the technique.

When a spatula and an endocervical brush are used to perform conventional cytology, the quality of the smear can be improved by using the spatula before the endocervical brush [57], which will minimize contamination by blood. The spatula is first rotated around the cervical os maintaining contact with the cervix throughout. The endocervical brush is then inserted into the os then rotated 180°. Rotating the brush more than 180° increases the risk of bleeding. Both samples are then smeared on to a glass slide, covering as much of the slide as possible.

Both sides of the spatula are smeared on to the slide and the endocervical brush is rotated on to the slide with slight pressure. Smears should be obtained before any digital examination. Intercourse, lubricants, douches, and intravaginal medications should be avoided for 24 hours prior to the examination. Pap smears should be postponed if the patient is bleeding or has marked vaginitis.

One factor critical to the ability of a cytotechnologist to detect abnormal cells on a Pap smear is their degree of alertness. The *Wall Street Journal* article by Walter Bogdonich exposed the high-volume, low-quality methods of some commercial laboratories [58] and heralded the Clinical Laboratory Improvement Act, which limited the number of smears a cytotechnologist is permitted to interpret on a daily basis and instituted several quality control regulations [59]. Nevertheless, Bosch et al demonstrated that when cytotechnologists were alerted to the potential for false-negative results they were able to identify the false-negative smears, but at the cost of tripling the number of smears identified as abnormal and doubling their screening times [60].

The assumption that screening and interpretative errors are random events is also untrue. Abnormal smears that are falsely reported as negative are both qualitatively and quantitatively different than smears that are correctly recognized as abnormal on initial screening [60,61]. False-negative smear results are associated with small, hypochromatic nuclei with little anisokaryosis [60]. False-negative results are associated with fewer cells [62], smaller cells [62,63], and single cells rather than cell clusters [62]. Slides containing fewer than 50 abnormal cells are 24 times more likely to have a false negative report than slides with more than 200 abnormal cells [64]. Because conventional cytology smears are heterogeneous, abnormal cells might be found on one smear while being absent on another simultaneously obtained smear.

In a review of patients with false-negative smears who were later found to have cervical cancer, DeMay found that errors were often related to (1) the presence of few, bland-appearing, or small abnormal cells, (2) cytologists offering an interpretation of a smear that on rescreening should have been read as unsatisfactory or less than satisfactory, or (3) tissue fragments rather than single cells [65].

Careful adherence to the criteria set by the Bethesda classification system, specifically with respect to the definition of an unsatisfactory smear is essential. Despite its success, however, the rate of false-negative cervical cytology cannot be reduced beyond a certain level. Quality assurance studies indicate that skilled screening cytologists have an irreducible false-negative fraction of at least 5% [66]. Cells from necrotic tumors might not be recognizable, and certain small or bland-appearing cells can be overlooked. Sampling might still miss lesions if they were small or eccentric on the cervix or high in the endocervical canal. Given these sources of error, the greatest sensitivity that can be expected is in the range of 90% [67].

Despite the demonstrated capability of conventional cervical cytology to reduce cervical cancer mortality, the Pap smear is not a very good test. Pap smear screening is more accurate when a higher cytological threshold (eg, high

grade squamous intraepithelial lesion (HSIL)) is used to detect a high-grade lesion. It does not discriminate well between normal variants and low-grade dysplasia. The reported accuracy of Pap smear screening is extremely dependent on the prevalence of disease and issues like ''workup'' bias and imperfect reference standards for defining who really has disease. When using only the best studies to draw a conclusion, the specificity of Pap smear screening is between 97% and 100% and its sensitivity is between 29% and 56%. These findings are much lower than previous reports and the commonly believed estimates.

Cost effectiveness of conventional cytology

When evaluating any screening test, several costs must be factored into the analysis. With respect to conventional cervical cytology, in addition to the cost of interpreting the smear, the costs of obtaining the smear, processing the smear, and rescreening negative smears must also be considered. Furthermore, if the insensitivity of the Pap smear necessitates more frequent sampling to confidently exclude disease, then this must also be factored into the cost of the test. Finally, the cost of managing women with abnormal Pap smears must be considered.

A variety of studies have analyzed the cost effectiveness of conventional cytology [68–70]. Eddy's [68] target population for initial screening was asymptomatic 20-year-old women of average risk, whereas Fahs et al [69] assessed women over the age of 65. Both analyses were conducted from the payer's perspective. The models arrived at different conclusions based on their inherent assumptions. For example, Eddy [68] used 85% sensitivity in his base case analysis, whereas Fahs et al [69] used 75% sensitivity in their model. This different strategy resulted in different cost estimates. When considering screening in women who had prior regular screening, Eddy [68] and Fahs et al [69] estimated different marginal cost effectiveness ratios: $52,241 per year of life saved versus $33,572 per year of life saved, respectively. Increasing the screening interval affects the cost effectiveness of cervical cytology in a number of ways. Although having less frequent Pap smears increases the risk of missing significant disease and increases cost caused by undiagnosed cancers, it allows minor-grade lesions to regress without being identified and worked up. This saves money because these lesions do not have a significant premalignant potential.

Increasing the screening interval or increasing the sensitivity of the Pap smear increase its cost effectiveness, but the most significant impact on improving cost effectiveness can be made by increasing participation in existent screening programs [71]. In addition to evaluating diagnostic accuracy of conventional cytology, the AHCPR addressed the cost of Pap testing to determine cost effectiveness of the procedure [49]. For women aged 20 to 64 years, the total cost (including office visit and processing costs) in 1997 dollars was $38.68. The estimate was higher for women aged 65 years and older ($47.73) unless Medicare reimbursement was considered, which reduced the amount to $35.01.

Summary

Despite the fact that cervical cytology screening programs have dramatically reduced the prevalence of cervical cancer in the US, women continue to develop and die from the disease. The most important observation contributing to this failing is that 60% of women with invasive cancer have not had a Pap smear in the previous 5 years (or have never had one). The most clinically effective and cost effective approach to reducing the incidence of cervical cancer is to screen the unscreened population. Recent evidence has also noted that the sensitivity of conventional cytology is also much lower than was previously believed. Much recent investigation has been directed at identifying the reasons for this low sensitivity and identifying ways to improve it. Only by improving the sensitivity of cervical cancer screening and participation in screening programs can the prevalence, morbidity, and mortality from cervical cancer be further reduced.

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In vitro adjuncts to the Pap smear

Juan C. Felix, MD[a,b,*], Charles Amezcua, MD[a]

[a]*Department of Pathology, Keck School of Medicine, University of Southern California, 1240 North Mission Road, Room 1L23, Los Angeles, CA 90003, USA*
[b]*Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, 1240 North Mission Road, Los Angeles, CA 90003, USA*

Background

The Pap smear has been recognized widely as the most effective cancer screening test in the history of medicine. Introduced by Dr. George Papanicolaou into clinical practice circa 1940 [1], it is widely believed that the use of this test has been has been responsible for the drastic reduction in the incidence and mortality of cervical cancer in the United States, Canada, and much of Western Europe in the past 50 years [2–5].

The ingenious technique of collecting exfoliated cells from the cervix, placing them on a glass slide, and examining under them under a microscope remained largely unchanged for more than 50 years. Only when a series of scandals in various laboratories in the eastern United States became public did the efficacy of the Pap smear enter into question and efforts to improve it were considered.

The first documented incident of deficiencies in gynecologic cytology laboratories was reported by the United States Air Force. Allegations that claimed inaccuracies in Pap smear diagnosis performed by a contract laboratory between 1972 and 1977 resulted in an investigation by governmental agencies into the matter. These investigations led to the discovery of large numbers of underdiagnoses of test results on Air Force personnel and their dependants that were largely attributed to poor regulation of laboratory personnel and large workloads [6]. In 1987, a highly publicized investigative report published in the *Wall Street Journal* denounced the egregious practices of a few cytology laboratories in the Eastern United States. The report exposed the policies of several high-volume, low-cost laboratories that encouraged excessive productivity of their screening cytotechnologists at the cost of accuracy. Similar problems

* Corresponding author. Dept. of Pathology, Keck School of Medicine, University of Southern California, 1240 North Mission Road, Room IL23, Los Angeles, CA 90003, USA.
E-mail address: felix@hsc.usc.edu (J.C. Felix).

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had been documented in other laboratories [7] yet had not reached such widespread attention. Greatly spurred on by public outcry, further government investigations into these and other allegations brought forth by these articles led to the recommendation of guidelines in the practice of cytology that culminated in an amendment of the Clinical Laboratories Improvement Act (CLIA) in 1988 [8]. CLIA '88 established workload limits on cytotechnologists who screened slides and instituted performance standards for laboratories and laboratory professionals. The regulations passed as part of this act limited to 100 the number of cytology slides that a cytotechnologist could screen in a 24-hour period, established a minimum of 10% rescreen of slides initially deemed as normal by a cytotechnologist, and mandated remediation of cytotechnologists for clinically significant underdiagnoses.

In subsequent years, results of numerous studies that evaluated the sensitivity of the Pap smear were published in peer-reviewed medical literature. Published figures on the sensitivity of the Pap smear ranged widely from 31% to 89%, largely depending on the design, population, and endpoint of the study [9–17]. In the three series in which the cause of false-negative results was investigated, screening errors were less common than errors of sampling, in which the slides were rescreened carefully and no abnormal cells were found [10,11,18]. These data strongly indicated that the limitations of the conventional Pap smear were caused by more than just poor laboratory practice or human error on the part of cytotechnologists. The systematic evaluation of the conventional Pap smear culminated with the publication of two metaanalyses of the world literature [19,20]. Both of these studies shocked the medical community in strongly establishing that the sensitivity of the Pap smear for the detection of cervical cancer precursors was less than 50%.

On the laboratory front, the regulations imposed by CLIA presented laboratory directors with a seemingly unending list of challenges. Increasing demands for cytotechnologists that resulted from the workload limitations and increasing use of the Pap smear by the baby boomer generation were met with escalating costs, lower reimbursements, and a diminishing workforce of cytotechnologists. Temporary relief was found in decreasing the number of slides submitted per patient by combining the cervical, endocervical, and vaginal samples onto a single slide instead of submitting two or three separate slides despite a paucity of data that proved equal efficacy of this method [21]. Despite these measures, many laboratory workers in the field remained alarmed at the prospect of not being able to cope with the demands for screening cervical cytology in the future. Advances in image analysis and increased speed of computer processors allowed for efforts to develop computerized instruments that could assist or even replace human cytotechnologists in the tedious chore of screening Pap smears. Although several efforts were undertaken to design devices that would evaluate the conventional Pap smear, other groups believed that limitations inherent to a conventionally prepared slide posed insurmountable impediments to computer analysis. The limitations identified could be divided into sampling limitations and preparation limitations.

Limitations of the conventional Pap smear

The efficacy of the conventional Pap smear is predicated on the presumption that if an abnormality exists in the cervix, abnormal cells will be collected by a Pap smear device and transferred onto the glass slide. This theory presupposes that cells representative of the abnormality either exfoliate or can be avulsed by the collection device at the time of the Pap collection. It also assumes that if collected, all the cells are deposited on the glass slide or that the population transferred onto the slide contains an adequate representation of the abnormal cells. The first of these premises was recently put into question. In a study of women with cervical cancer precursor lesions and a false-negative Pap test result, the expression of E-cadherin, an adhesion molecule, showed aberrant patterns of expression when compared to women whose lesions were detected by the Pap smear. The authors suggested that these lesions had a biologic reason for resisting collection as the barrier for the false-negative Pap smear result [22]. Although the true incidence of this phenomenon has not been established firmly, the persistence of false-negative Pap test results despite the use of improved collection and preparation devices suggests that it may be considerable. The second premise was proved incorrect by Hutchinson et al, who showed that commonly used devices for the performance of the Pap smear collected between 600,000 and 1.2 million cervical epithelial cells but that fewer than 20% of these collected cells were transferred onto the glass slide [23]. The knowledge that most of the epithelial cell sample was never transferred to the slide provided a viable explanation for the high prevalence of true-false negative rate reported in these studies. Particularly disturbing was the realization that the transfer of cells to the glass slide is a random event and is statistically prone to error if the population of abnormal cells is not homogeneously distributed throughout the specimen.

Little effort has been devoted to addressing the problem of failure to capture abnormal cells from the cervix. The difficulty in addressing these issues stems from the fact that the collection is performed in a poorly controlled clinical setting. Little is known about the group of patients whose lesions resist collection. Much more effort has focused on the way in which the Pap tests are prepared once the specimen has been collected from the cervix.

Preparation of the conventional Pap smear by the clinician is a highly variable and poorly controlled technique. Optimal application of cells onto a glass slide should be performed in a systematic fashion to spread evenly the epithelial cells across the entire surface of the slide and maximize the transfer of cells while minimizing clumping. The transfer of cells onto the slide must be done rapidly to fix the specimen promptly and avoid air drying or degeneration. In addition to these technical challenges, uncontrollable variables exist that affect the optimization of the conventional Pap smear. The presence of inflammatory cells and blood competes for available area on the glass slide. In severe cases, the inflammatory cells or blood could replace or obscure the epithelial cells and create an impediment to visual analysis. Finally, inflamed epithelial cells and normal epithelial

cells in the late luteal phase form thick, three-dimensional aggregates that again pose an obstruction to the clear visualization of the sample. Studies that evaluated the adequacy of the Pap smear reported that more than 15% of all Pap smears are limited because of the presence of obscuring blood, inflammation, or thick areas of overlapping epithelial cells [24,25].

The stated limitations of the conventional Pap smear were believed by many investigators to pose insurmountable obstacles to the successful development of a computer-assisted screening device. Inherent limitations of the conventional Pap smear had to be addressed and overcome to create a computer-assisted technology that was superior to the conventional Pap smear. Liquid-based, thin-layer technology was conceived out of the necessity to improve the physical state of the Pap smear to allow for the accurate evaluation by a computerized device.

Principles of efficacy of liquid-based cytology

Liquid-based, thin-layer technology was developed to overcome the technical limitations of the conventional Pap smear. The technology was developed to address specifically the five major limitations posed by the conventional Pap smear: (1) failure to capture the entire specimen obtained from the patient, (2) inadequate fixation of the sample, (3) random distribution of abnormal cells in the sample, (4) obscuring elements, and (5) technical variability in the quality of the smear. The collection of cells directly into a liquid fixative addresses the first two limitations. By immersing the cervical collection device into the liquid fixative, the cells are fixed instantly, which avoids the potentially damaging contact with the dry slide and minimizes postcollection degeneration and air drying. If proper technique is observed, most of the cells retrieved by the sampling devices are rinsed into the liquid media, which captures virtually the entire sample obtained from the patient into the vial.

Mechanical mixing of the cells follows collection of cells into liquid fixative. Although the different products that use this technology use different methods of mixing, the principle is the same: mixing the cells creates a homogenous sample in which abnormal cells, if present, are evenly distributed throughout the sample. Specimen homogeneity directly addresses the potential flaw related to the false-negative rate of the conventional Pap smear, possibly caused by a failure to include nonrandomly distributed abnormal cells onto the glass slide. Sample homogeneity is critical because no slide captures the entire sample collected from the patient but rather contains only a relatively small aliquot of the collection. Hutchinson et al, who produced multiple slides from abnormal samples and identified abnormal cells in virtually all of the slides, demonstrated the efficacy of this process [26]. The effect of liquid collection and sample mixing seems to afford a beneficial effect to the consistent identification of lesions regardless of the method used. Khalbuss et al used a modified electric toothbrush to mix liquid-fixed residual cells obtained after a conventional Pap smear. Slides were produced by simple cytocentrifugation onto glass slides. Despite the simplicity

of the procedure, diagnostic equivalency to the conventionally prepared Pap smear was demonstrated [27].

The final two limitations of the conventional Pap smear—obscuring elements and thick sample—are addressed in different fashions by the products currently available. The ThinPrep (Cytyc Corp., Boxborough, MA) uses a polycarbonate cylinder that holds a membrane with an 8-μm pore size at the end to mix and subsequently suction the medium. As the collection fluid of the sample passes through this semi-permeable barrier, the membrane detains epithelial cells and infectious organisms, but much of the debris and some inflammatory cells are allowed to pass. When sufficient epithelial cells accumulate on the membrane, as determined by a pressure sensor, the suction is discontinued and the membrane is placed against the glass slide to transfer the cells.

The Autocyte Prep (TriPath Imaging, Inc., Burlington, NC) uses a liquid gradient onto which the sample is layered after vigorous vortexing. The sample and gradient are then centrifuged. The gradient preferentially concentrates epithelial cells and partially depletes the final sample of extraneous material, blood, and inflammatory cells. An aliquot of this filtrate is then transferred by robotic pipette onto a chamber and the sample is allowed to settle onto the slide by gravity. Both techniques result in consistent, thin-layer preparations of epithelial cells that are depleted of extraneous elements. Both products produce slides that contain 50,000 to 75,000 cells per slide in circular areas.

Efficacy of liquid-based, thin-layer technology

The efficacy of liquid-based, thin-layer cytology has been assessed by numerous clinical trials. Two types of study designs account for most of the published studies: the split-sample design and the intended use, direct-to-vial design. The split-sample studies accrue patients in whom a single sample collection is performed. The sample is used to prepare a conventional Pap smear. The residual material that remains on the collection device is then rinsed in the collection media and sent for thin-layer preparation. This study design suffers from a beneficial bias in favor of the conventional Pap smear because it is prepared first and may deplete the remaining sample of abnormal cells for thin-layer preparation. The second type of study is the intended use, direct-to-vial design in which women have their cervical sample directly deposited to the liquid collection medium. Comparison of the technology to its conventional counterpart is performed by obtaining matched populations of historical controls. This type of study also suffers from numerous biases, including differences in the population studied, selection bias on the basis of ability to afford a more expensive technology known to the cytology readers (possible Hawthorne Effect), and high-risk patient selection to a test perceived to have superior sensitivity. To date, no published data that evaluated prospective, randomized trials comparing these technologies to the conventional Pap smear exist.

Also of great importance is the fact that despite the large body of literature that reflects studies conducted with the liquid-based, thin-layer technology, none has been subjected to the rigor of the current "gold standard" of cervical cancer precursors (ie, a comparison to colposcopy). Randomized clinical trials with a colposcopy arm as measure of "truth" have been recommended for in vivo visual tests, and optical devices have not been performed with the in vitro modalities. Despite the limitations of the current data, the number of patients studied is currently more than 500,000 subjects, with a preponderance of data indicating a significant benefit of liquid-based, thin-layer technology over the conventional Pap smear in the detection of cervical cancer precursor lesions and in the improvement of specimen adequacy.

Early clinical studies that compared ThinPrep and the SurePap (formerly known as Autocyte Prep and Cyto-Rich) were performed on early versions of the devices that later underwent significant modifications. These studies are not reviewed in detail because the devices tested were replaced with newer versions that are the only ones clinically available. The importance of these early trials was in the demonstration of diagnostic equivalence to the conventional Pap smear despite the adverse bias introduced by the split sample study design [18,21,28–38].

Most of the more recent studies use versions of the automated devices approved by the Food and Drug Administration (FDA) (Tables 1–4). The studies are listed by design and divided into studies conducted in a split-sample fashion and studies conducted in the intended-use, direct-to-vial design. Examination of the data summarized reveals that liquid-based cytology outperformed the conventional Pap smear in the detection of cervical cancer precursors. Only one study published failed to find more squamous intraepithelial lesion (SIL) in the liquid-based slides than in the conventional smear, which shows a nonsignificant 3% decrease in the detection of SIL [38]. The equivalent or superior performance of liquid-based, thin-layer slides is particularly impressive in the split-sample studies, in which cases that involve the conventional smear showed that no lesions were found to represent SIL on the leftover cells. The range of improvement afforded by liquid-based cytology in these split-sample studies

Table 1
Performance of the ThinPrep in split-sample studies

		Conv.	TP		ASCUS		Unsatisfactory		SBLB	
Reference	No. of cases	SIL (%)	SIL (%)	% Increase	Conv. (%)	TP (%)	Conv. (%)	TP (%)	Conv. (%)	TP (%)
Lee [41]	6747	8.0	9.4	18.4	7.7	7.4	1.6	1.9	27.8	19.8
Roberts [42]	35,560	2.0	2.3	11.7	N/A	N/A	3.5	0.7	8.3	20.0
Corkill, 1997	1583	2.7	5.6	109.5	3.7	5.1	N/A	N/A	2.2	0.3
Shield [43]	300	7.0	8.3	19.1	N/A	N/A	17.3	6.3	N/A	15.3
Wang [44]	972	4.4	6.0	34.9	N/A	N/A	N/A	N/A	N/A	N/A
Hutchinson [40]	8636	4.9	5.2	6.0	1.8	7.5	N/A	N/A	N/A	N/A

SBLB, satisfactory but limited by; Conv., conventional Pap; TP, thin prep pap.
Data from references [23,41,43,44,72,73].

Table 2
Performance of the ThinPrep in direct-to-vial studies

	No. of cases	Conv.	TP		ASCUS		Unsatisfactory		SBLB	
Reference	Conv. / TP	SIL (%)	SIL (%)	Increase (%)	Conv. (%)	TP (%)	Conv. (%)	TP (%)	Conv. (%)	TP (%)
Weintraub [75]	13,067/18,247	1.0	2.9	190	1.6	2.7	0.7	0.3	30.9	10.9
Papillo [53]	18,569/8541	1.6	2.5	56	9.0	6.6	0.2	0.4	4.8	4.4
Bolick [25]	39,408/10,694	1.1	2.9	164	2.3	2.9	1.0	0.3	17.8	11.6
Dupree [51]	22,323/19,351	1.2	1.7	40	4.9	4.6	2.0	3.8	N/A	N/A
Guidos [52]	5423/9583	1.3	4.7	262	2.0	3.4	1.2	0.5	21.4	0.7
Carpenter [49]	5000/2727	7.7	10.5	36	12.5	6.9	0.6	0.3	19.4	10.5
Diaz-Rosario, 2000	74,756/56,339	1.8	3.2	79	4.8	4.5	0.2	0.7	22.0	18.7
Weintraub [55]	129,619/39,455	0.6	2.3	141	1.5	2.4	0.3	0.2	27.8	8.1

SBLB, satisfactory but limited by; Conv., conventional Pap; TP, thin prep pap.
Data from references [49–51,53,55,61,74,75].

ranged from a low of 6% to a high of 110% improvement with the ThinPrep technology and from a low of -3% to a high of 137% with the SurePap technology (Tables 1, 3). On average, the improvement seen with the ThinPrep device summarized from these studies was 15% [39–44], with a similar 18% improvement in the series of samples summarized from the SurePap studies [24,38,45–48]. It is important to note that some series selected laboratories at which the rate of SIL was lower than the national average, wherein the liquid-based cytology resulted in more accepted rates and the improvement may have been influenced by participation in a research audit.

Direct-to-vial studies using ThinPrep technology revealed such marked improvements in the detection of SIL that much of the medical community began to suspect that the increased diagnoses of SIL may have been the result of "overcalls" on the part of overzealous cytopathologists in these studies rather than true detection of abnormalities. Summary of the direct-to-vial studies for the ThinPrep device shows a 140% improvement in the detection of SIL over the historical conventional Pap smear controls (Table 2) [25,49–55]. These clinical

Table 3
Performance of Auto-Cyte Prep in split-sample studies

		Conv.	Prep		ASCUS		Unsatisfactory		SBLB	
Reference	No. of Cases	SIL (%)	SIL (%)	Increase (%)	Conv. (%)	Prep (%)	Conv. (%)	Prep (%)	Conv. (%)	Prep (%)
Vassilakos [47]	560	3.8	4.6	24	12.9	7.7	5.4	3.8	28.3	8.4
Takahashi [38]	2000	3.5	3.4	−3	1.1	4.6	N/A	N/A	N/A	N/A
Wilbur [48]	286	4.2	9.1	117	13.6	13.3	3.5	1.1	30	16
Bishop [24]	8983	5.2	5.9	13	6.2	6.0	1.0	0.6	28.1	15.8
Kunz [45]	554	1.4	3.4	137	9.6	3.3	19	12	N/A	N/A
Minge [46]	14,539	4.4	5.8	32	6.9	5.9	0.9	0.6	N/A	N/A

SBLB, satisfactory but limited by; Conv., conventional Pap; TP, thin prep pap.
Data from references [24,38,45–48].

Table 4
Performance of the Auto-Cyte Prep in direct-to-vial studies

	No. of cases	Conv.	Prep		ASCUS		Unsatisfactory		SBLB	
Reference	Conv. / Prep	SIL (%)	SIL (%)	Increase (%)	Conv. (%)	Prep (%)	Conv. (%)	Prep (%)	Conv. (%)	Prep (%)
Vassilakos [57]	15,402/32,655	1.1	3.6	224	3.7	1.6	1.9	0.4	13.4	2.7
Vassilakos [58]	88,569/111,358	2.0	3.2	63	3.0	1.2	1.5	0.2	4.6	1.2
Vassilakos [54]	19,923/81,120	1.2	3.4	283	3.5	1.9	N/A	N/A	N/A	N/A
Tench [56]	10,367/2231	1.0	1.7	67	3.8	5.5	2.9	0.4	31	16

SBLB, satisfactory but limited by; Conv., conventional Pap; TP, thin prep pap.
Data from references [56–59].

data strongly support the FDA labeling, which states that ThinPrep is superior to the conventional Pap smear for the detection of cervical cancer precursor lesions.

Summary of the direct-to-vial studies for the Autocyte Prep device is similarly impressive, with more than 200% increase in the detection of SIL over historical conventional controls (Table 4) [56–59]. It is necessary to mention that three of the four direct-to-vial Autocyte Prep studies did not use the FDA-approved instrument to produce the slides but rather used manual pipetting [57,58]. The one direct-to-vial study for the Autocyte Prep that did use the FDA-approved instrument and procedure, however, showed an increase in the detection of SIL of 67% [56].

Confirmation that this increase in SIL is true detection of dysplasia rather than ''overcalls'' by cytopathologists can be found in several studies in which subsets of patients with biopsy follow-up are available. Papillo et al found a statistically significant increase in specificity of a diagnosis of SIL of the ThinPrep (81%) over the conventional Pap smear (72%) [53]. Diaz-Rosario et al found equivalent specificity as determined by biopsy-proven dysplasia between the ThinPrep (74%) and the conventional Pap (79%) smear [50]. Finally, Hutchinson et al also reported biopsy correlation data from a population-based study in Costa Rica [40]. In their study, the specificity of the ThinPrep diagnosis of 85.4% when compared to biopsy results had a slightly better correlation to biopsy of the conventional Pap smear at 88.8%. In all three studies the superior sensitivity combined with the specificity reported led to a significant increase in the detection of biopsy-proven dysplasia. Although fewer data exist for the Autocyte Prep, two reports comment on biopsy correlation. Vassilakos et al reported a statistically significant improvement in correlation between the ThinPrep diagnosis and biopsy result when compared with the conventional Pap smear [59]. The improvement in correlation was particularly notable among cases diagnosed by the ThinPrep slide as high-grade squamous intraepithelial lesion (HSIL), in which 90% of biopsies confirmed the diagnosis. Finally, Tench et al reported preliminary biopsy correlation in 30 cases with available data. In this small subset, biopsy confirmed abnormal (SIL) ThinPrep diagnoses in 26 of the 30 cases, which suggested adequate specificity.

Another concern voiced regarding liquid-based, thin-layer technology was directed at the rise in the diagnosis of atypical squamous cells of undetermined significance (ASCUS) in a few of the series. Although many series report an

absolute decrease in the frequency of a diagnosis of ASCUS, all of the series report a decrease in the ASCUS-to-SIL ratio. This parameter is considered a more representative measure of performance because the detection of more disease is accompanied by the detection of all abnormalities, including nondiagnostic abnormalities such as ASCUS. The improvement in nondiagnostic abnormalities seen in the ASCUS category is also seen for the diagnosis of atypical glandular cells of undetermined significance (AGUS). Ashfaq et al reported a significant improvement in the detection of adenocarcinoma of the cervix, with a 65% decrease in the false-negative rate in the diagnoses of adenocarcinomas by the ThinPrep method over the conventional Pap smear and a 64% increase in the specificity rate of a diagnosis of AGUS or adenocarcinoma [60]. Similar findings were reported by Guidos and Selvaggi, who also noted an improvement in the diagnosis of glandular lesions using liquid-based cytology [61].

Computer-assisted screening

Computer-assisted screening devices have been shown to reduce the incidence of false-negative Pap test results when used in a quality control mode to rescreen cases with a diagnosis of within normal limits [62,63]. One instrument (the Autopap 300, TriPath Imaging, Inc., Burlington, NC) is approved by the FDA for primary screening with the capability of rendering machine-only diagnoses of "within normal limits" for the 25% of the lowest-risk tests in a non–high-risk screening population. Studies that evaluated the efficacy of the Autopap 300 in this primary screening modality have shown an increased sensitivity in the detection of squamous intraepithelial lesions [64,65]. Usage issues regarding the definition of the high-risk patient and unacceptability of many conventional Pap smear slides to be read by the device combined with the increase in cost and low reimbursement rates by insurance carriers have delayed the widespread acceptance of this technology.

Computer-assisted devices designed to screen liquid-based, thin-layer slides circumvent many of the technical problems faced in screening the conventional Pap smear. The Autopap 300 has been approved recently by the FDA to screen Autocyte Prep slides, grant 25% of smears with lowest risk of harboring an abnormality an "automated diagnosis" of within normal limits, and assign the remaining tests a rank for risk of having an abnormality. Studies that evaluated the efficacy of the Autopap 300 demonstrated that the device can assign correctly a high-risk score to cases of high-grade cervical intraepithelial neoplasia (CIN). In the study by Vassilakos et al, 100% of HSIL and cancers were assigned correctly a high-risk ranking [66]. Clinical trials that evaluated other devices are already at an advanced phase and will be considered for approval in the near future.

In a series of 583 patients, Takahashi et al found that an interactive computer analysis system, the Autocyte Screen (TriPath Imaging Inc., Burlington, NC), yielded a false-negative rate of only 1.8% (detecting 55 of 56 SIL) and triaged only 21% of cases for pathologist review [67]. In a series of 1676 thin-layer preparations, Bishop et al reported that the Autocyte Screen detected an improved sen-

sitivity in the detection of SIL, with the computer-assisted screening yielding a 98% sensitivity rate compared to a sensitivity rate of 89% by manual screening alone [68]. Although larger clinical trials are needed, these early results offer great promise for an improvement in screening sensitivity while reducing human effort and time usage. Thin-layer technology largely has reduced obstacles for the use of computer imaging, which allows optical analysis of single cells rather than clusters. When used in conjunction with thin-layer slides, computer-assisted screening devices offer tremendous promise for the future, particularly at a time in which the number of human cytotechnologists is decreasing while demand for screeners is increasing.

It is important to mention that despite abundant data regarding "in vitro" technologies, to date no study has subjected these technologies to the rigor of the current gold standard of colposcopy. Such studies are needed to substantiate the claimed false negative rates of these technologies.

Molecular testing of residual material in the vial

An unexpected benefit of liquid-based, thin-layer technology was discovered upon the realization that abundant cellular material remained in the vial after the production of the slide. It is estimated that on the average one tenth or less of the cellular material is used to make the test slide. The remainder of the cellular material is destined to be discarded. With the advancement of molecular testing, however, biologists began using the residual material to test for the presence of infectious organisms. To date, successful out-of-vial testing has been shown for human papillomavirus (HPV), *Chlamydia trachomatis*, gonorrhea, and herpes simplex virus. The ability to detect infections in the residual volume of the collection fluid offers numerous opportunities, including the simplification of collection devices and minimization of routing errors, to which multiple samples from one patient fall prey. Most exemplary of the benefits of this adjunct technology is the case of HPV testing for women with a cytologic diagnosis of ASCUS.

Recently, results of two large clinical trials showed that detection of high oncogenic risk HPV types using the Hybrid Capture II assay effectively separates patients with a cytologic diagnosis of ASCUS into a group with a high likelihood of having CIN 2 or CIN 3 and a group that is at no increased risk of harboring high-grade CIN. Patients with a diagnosis of ASCUS who are high oncogenic risk HPV positive have a significant risk of harboring high-grade CIN. In contrast, women with a diagnosis of ASCUS who are high oncogenic risk HPV negative are at no increased risk of having a HSIL over women with a normal Pap smear result [69,70]. The HPV test using the Hybrid Capture II test can be performed by separately collecting a sample of cells into a transport medium or by using the residual cell sample from the liquid-based cytology sample. The latter option offers the clinically desirable option of automatically testing the sample of patients with a cytologic diagnosis of ASCUS without the necessity of an additional patient visit or patient clinician interaction. If a patient is found to be HPV positive, she is

referred for colposcopy for further diagnostic evaluation. If the patient is high-risk HPV negative, however, the risk of a HSIL is low enough to recommend screening at the routine interval. This triage strategy has been shown to reduce unnecessary colposcopic examinations in 45% to 60% of women with ASCUS and reduce the morbidity, anxiety, and cost associated with that procedure [69,70].

Cost analyses of triage strategies have shown a cost savings in the management of patients using HPV testing over previous cytology-based or colposcopy-based strategies [69]. Results from the National Cancer Institute–sponsored ASCUS-Low Grade SIL Triage Study (ALTS) showed a statistically significant improvement in the detection of CIN 2 and CIN 3 when patients were triaged to either immediate colposcopy or using Hybrid Capture 2 HPV detection as compared to patients followed with Pap smears [70]. These data have encouraged us to advocate the use of high oncogenic risk HPV testing in patients with a cytologic diagnosis of ASCUS to define the likelihood of dysplasia in the patient and the need for a colposcopic evaluation.

Screening with combined modalities

The improvements seen with the various individual in vitro adjuncts to the Pap test in the detection of cervical cancer and its precursor lesions have made many investigators consider the use of multiple simultaneous modalities to create a superior detection test. The high negative predictive value for cervical cancer and its precursors of a negative high-risk HPV test in a population of patients with a Pap test result of ASCUS [69,70] has led to the assumption that HPV negativity in all patients may connote absence of a cervical cancer precursor lesion and a lowered risk of acquiring one over an extended period of time. This latter fact would allow safely the prolongation of screening intervals for cervical cancer prevention from the currently recommended 1- to 3-year intervals to 3- to 5-year intervals. Recently, Vassilakos et al found that a combination of high-risk HPV detection and automated screening of liquid-based, thin-layer Pap smears could separate women with cervical lesions that were ASCUS or greater into a human review group from a group with negative cytologic findings for an automated review only. The authors predict that 51% of patients would be classified as negative and avoiding the costly review while maintaining 99.6% negative predictive value [71]. Similar combination modality is actively being pursued in an effort to achieve more sensitive yet cost-effective cervical cancer screening programs and probably represents the future of cervical cancer screening.

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Use of visual screening methods for cervical cancer screening

Thomas C. Wright, Jr, MD[a,*],
Lynette Denny, MMed (O&G), PhD, FCOG (SA)[b],
Louise Kuhn, PhD[c], Sue Goldie, MD, MPH[d]

[a]*Department of Pathology, Columbia University, 630 West 168th Street, New York, NY 10032, USA*
[b]*Department of Obstetrics and Gynecology, Groote Schuur Hospital, Main Road, Observatory 7925, University of Cape Town, Cape Town, South Africa*
[c]*Gertrude H. Sergievsky Center, Columbia University, 630 W. 168th St., New York, NY 10032, USA*
[d]*Harvard Center for Risk Analysis, Harvard School of Public Health, 718 Huntington Ave. Boston, MA 02155, USA*

Over the past decade there has been a resurgence of interest in using non-cytologic methods to screen for cervical disease in low resource settings, in which cytology is simply not available, and in developed countries, where it is hoped that combining cytologic screening methods with visual screening methods might reduce the error rate inherent in cytologic screening methods. There are two general types of visualization methods. The first type includes visualization methods that use broad-band light (ie, the entire spectrum of light) to illuminate the cervix. The simplest of the methods that use broad-band light is visual inspection of the cervix with the naked eye. This method is usually performed after the application of a 3% to 5% acetic acid solution and is often referred to as direct visual inspection (DVI). Other visual screening techniques that use broad-band light include colposcopy and cervicography, in which one obtains a 35-mm photograph of the cervix after applying a 3% to 5% acetic acid solution and allows experts to review the photograph to determine whether a significant lesion is present.

Simple visual methods for identifying cervical cancer precursor lesions predate cervical cytology as a method to screen for cervical cancer and its precursor

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* Corresponding author.
E-mail address: tcw1@columbia.edu (T.C. Wright,).

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lesions. Before the introduction of cytologic screening programs, clinicians in the United States and Western Europe used visual screening methods. Screening using visual methods was discontinued once cervical cytology became available, however, because of the perceived superiority of cytology with respect to test performance characteristics, including sensitivity and specificity. The recent resurgence of interest in visual screening methods is a result of several factors. Many public health officials have realized that cytologic screening is not practical, or even possible, in many developing countries, where cervical cancer rates are the highest in the world. In contrast to cervical cytology, which requires a well-maintained laboratory infrastructure and highly trained cytotechnicians, many visual screening methods are relatively simple and do not require a laboratory, are inexpensive and, perhaps most importantly, provide an immediate result, which means that patients can be screened and treated at a single visit. This is a major advantage in areas of the world in which communications and transportation are limited. Another factor that stimulated interest in visual screening methods is the increasing recognition by clinicians and patients of the limitations inherent in cervical cytologic screening. Recent meta-analyses have suggested that a single conventional Pap test misses 40% to 50% of cases of biopsy-confirmed high-grade squamous intraepithelial lesions (SIL) and cervical cancers [1,2]. This failure rate is leading to the development of adjunctive screening methods designed specifically to augment the sensitivity of cytology-based cervical cancer screening programs for countries in which cytologic screening is already readily available and widely practiced.

The second general category of visualization methods includes electro-optical devices capable of detecting cervical disease. These devices are capable of measuring various parameters, including the uptake of fluorescent compounds by the cervix, the endogenous fluorescence emitted from the cervix when exposed to various wavelengths of light, and the response that tissues produce to specific wavelengths of light or electrical impulses. Based on these biophysical measurements, which are analyzed by mathematical algorithms, the devices then predict the underlying tissue histology.

There is currently significantly more information available regarding the performance of the visual methods, such as DVI, than there is for the devices that incorporate electro-optical sensors. Large-scale clinical and epidemiologic studies of DVI have been completed in several studies and demonstrate clearly that in selected low-resource settings DVI is attractive as a low-cost screening method. Currently, large-scale trials are being conducted that are designed to evaluate how best to incorporate DVI into cervical cancer screening programs. In this article the authors review recent studies that have investigated the performance of visual screening methods as primary screening methods and as adjuncts to cytologic screening. Particular emphasis is placed on the potential role of the simple visual screening methods in low resource settings and on novel strategies that are being developed to allow cervical cancer screening to be implemented in areas of the world that lack the infrastructure necessary for screening programs based on cervical cytology.

Simple visual screening methods

Terminology

The simple visual screening methods use broad-band light (ie, the entire spectrum of light) to illuminate the cervix, and the examiner makes a diagnosis or interpretation based on the appearance of the cervix. The simple visual screening methods can be subdivided based on two general properties. The first is whether magnification is used. The second is whether a chemical stain, such as an iodine or 5% acetic acid solution, is used to enhance differences between normal and abnormal tissues. Different visual screening methods include DVI, which is the inspection of the cervix after the application of a dilute solution of acetic acid (also known as visual inspection, the acetic acid test, cervicoscopy, and visual inspection with acetic acid); the Schiller's iodine test, in which the cervix is inspected with the naked eye after the application of Lugol's iodine; speculoscopy, which is the inspection of the cervix after the application of a dilute solution of acetic acid using a low (4–6 ×) magnification device and a special chemiluminescent light; and cervicography, which requires that a photograph be obtained of the cervix after the application of a dilute solution of acetic acid and the photograph be interpreted by a specially trained expert. Comparing the results obtained in different studies is difficult because not only have the different studies used various methods but also they are referred to by different names, even when the same method is used. A listing of some of the different names used to refer to the visual screening methods is provided in Table 1.

Table 1
Terminology used for visual screening methods

Term used for method	Magnification	Enhancement
Schiller test Lugol's iodine test Visual inspection with Lugol's iodine	No	Iodine staining
Downstaging	No	No
Direct visual inspection acetic acid washes acetic acid visualization acetic acid screening test visual inspection with acetic acid cervicoscopy acetic acid test	No	3%–5% acetic acid solution
Aided visual inspection gynoscopy avioscopy DVI with magnification visual inspection with acetic acid and magnification	2.5–4×	3%–5% acetic acid solution
Speculoscopy	4–6× magniification	3%–5% acetic acid solution
Cervicography	35-mm photograph	3%–5% acetic acid solution

Another issue that makes the terminology surrounding visual screening methods confusing is that the criteria used to define what constitutes a "positive" screening result vary considerably among different studies. These variations potentially could result in differences in test performance and may explain why different results have been observed in different studies. The criteria used to define a "positive" test in the different studies are discussed in the specific subsections that discuss the individual tests.

Issues regarding determination of test performance

In most visual screening studies, the gold standard of colposcopy and cervical biopsy has not been applied uniformly, because women with negative visual screening examinations and negative cervical cytology results usually have not undergone colposcopy. This limitation in study design may produce a significant verification bias in determining the true prevalence of disease, and sensitivity and specificity cannot be evaluated directly in most visual screening studies. There are several ways to correct partially for this bias statistically, but sensitivity and specificity usually can only be estimated. To reduce this problem, some studies have compared the performance of visual screening tests to that of cytology using parameters other than sensitivity and specificity. These parameters include comparing the detection rates of high-grade SIL (cervical intraepithelial neoplasia (CIN) 2,3) using the different tests, comparing the ratio of sensitivity of the two tests, and simply reporting the approximated specificity of each screening test and the positive predictive value.

It is also important to realize that even studies that have applied the reference or gold standard, such as colposcopy or cervical biopsies, to all women in a study may not produce results that are free of bias. Colposcopy and the pathologic interpretation of cervical biopsies are highly subjective, and the skill and experience of the colposcopist or the pathologist clearly have a profound impact on performance. With respect to colposcopy, most practicing colposcopists readily admit that colposcopy is a difficult technique to master. Although a recent analysis of the sensitivity and specificity of colposcopy in the published literature reported overall rates of sensitivity and specificity of 96% and 48%, respectively, this estimate is most likely overly optimistic [3]. This is because most women evaluated in these studies were referred for colposcopy on the basis of having had an abnormal Pap smear and the colposcopy was performed in a large academic center.

The sensitivity and specificity rates of colposcopy when performed in women with two or more negative screening tests would be expected to be much lower because most of these women do not have significant disease and many of them have metaplastic regions that can be colposcopically confused with SIL. In a recent analysis of the performance of colposcopy when performed as part of a South African screening study, the authors observed poor performance, even-though all of the colposcopic examinations were performed by highly experienced gynecologic oncologists [4]. Out of 96 patients with biopsy-confirmed high-grade SIL (CIN 2,3) who underwent colposcopy, only 2 (2%) had no col-

poscopic lesion identified; however, 26 (27%) had what was considered to be a "trivial" colposcopic lesion. Similarly, 41% of the women who had what was considered a "significant" colposcopic lesion had no SIL or cancer identified on cervical biopsy and 27% had only a low-grade SIL (CIN 1) [5]. The authors attributed the poor performance of colposcopy in this study to the fact that almost half of all women who were enrolled underwent colposcopy based on the results of a positive visual screening test, HPV DNA testing, or cervical cytology.

The histopathologic interpretation of cervical biopsies is also prone to error. Multiple studies have documented high rates of interobserver and intraobserver variation in the diagnosis of SIL [6,7]. Pathologists have a particularly difficult time reproducibly distinguishing between inflamed immature squamous metaplasia and CIN 2. Because there is a high rate of cervicitis and cervicovaginal infections among women in many low-resource settings, biopsies obtained from visual screening studies from these areas might be especially prone to misclassification. Because of these considerations, it is clear that even in studies in which all women screened also have undergone colposcopy, there may be a significant misclassification as to disease status.

Specific visual screening methods

Visual methods used in current clinical practice

Schiller test

The concept of visual screening for cervical cancer began with Walter Schiller, who developed the Schiller test in 1929 [8,9]. The Schiller (iodine) test, which is also referred to as the Lugol's iodine test, consists of applying an iodine solution to the cervix and viewing the cervix with the naked eye. Glycogenated epithelium takes up the iodine and stains a dark brown, whereas nonglycogenated epithelium, including most SIL and invasive cancers, do not stain and appear a yellowish red. At the time it was introduced, the Schiller test was initially well received by gynecologists, who had no other method for screening women for cervical cancer precursors [9]. As the Schiller test began to be more widely used, however, it was recognized that the test was nonspecific. Areas of immature squamous metaplasia within the transformation zone and columnar epithelium in areas of cervical ectopy do not contain glycogen and do not stain with iodine. As a result, most women with a positive Schiller test result do not have significant cervical disease. Because colposcopy was not available and biopsy instruments at that time were designed to take relatively large tissue samples, the Schiller test became unpopular and was largely discontinued after the introduction of cytologic screening methods, which were considerably more specific.

Recently, investigators from the International Agency for Research on Cancer (IARC) who work in India and Africa have begun evaluating the Schiller test in large-scale screening studies. Although this method has been known widely for more than 50 years by the terms "Schiller's test" or "Lugol's iodine test," these

investigators have begun using the term "visual inspection with Lugol's iodine (VILI)" to refer to this method. The rationale for evaluating the performance of the Schiller's test is the hope that the use of two visual screening tests, when combined with other visual screening methods such as DVI, will result in increases in sensitivity and specificity. Although the results of these studies have not been published formally, they have been presented at several meetings and are considered by the investigators to be promising.

Downstaging

Screening for cervical cancer by visual inspection was widely advocated by the World Health Organization (WHO) in the 1980s as a way to provide screening services in low-resource settings in which cytology was not available [10,11]. The goal of the WHO approach was to detect early-stage cervical cancers in asymptomatic women who could be cured by a simple hysterectomy. The detection was to be done by visualizing the cervix using a vaginal speculum and using the naked eye to inspect it without the aid of magnification or chemical enhancing agents such as iodine or acetic acid. This approach sometimes has been referred to as unaided visual inspection but was more commonly referred to as downstaging because it was designed to detect early, asymptomatic cancers visually. Downstaging was most extensively evaluated in India with the early studies enrolling symptomatic women who attended outpatient Indian gynecologic clinics.

The first large-scale study of downstaging began in 1976 and included 11,760 women who underwent downstaging and had a Pap smear [12]. Of the 215 cancers (including carcinoma in situ) that were detected at the first visit, 88 (41%) were classified as suspicious for cancer on visual inspection. Women with cytologic evidence of dysplasia but no cancer were followed with 3 monthly examinations that included a Pap smear, a visual examination, and a colposcopic examination. 63 incident cases of cases of cancer were identified in this group, and in 33 (52%) visual inspection revealed "suspicious" findings at the time the cancer was detected. Cytology correctly identified 71% of the cases of early cancer and colposcopy identified 87%. Two other Indian studies that included predominately symptomatic women enrolled from a hospital setting reported a better sensitivity of visual screening for high-grade disease but a much lower specificity [13].

Subsequently, the IARC sponsored several large screening programs in India based on visual inspection. One was a population-based study conducted in rural areas of the state of Maharashtra [14]. In this study, two trained paramedic workers performed visual inspection of the cervix without magnification or chemical enhancing agents and classified the cervix into three categories: normal, low abnormal, or high abnormal (Table 2). A Pap smear was then obtained. Of the 3748 women invited to participate, 2135 (57%) agreed to be examined. 1120 (57%) of the women had an abnormal cervix using the low threshold and 118 (6%) using the high threshold. In this study seven invasive cervical cancers and five CIN 3 lesions were identified by cytology and histology. Of the

Table 2
Criteria for defining an abnormal cervix in IARC Maharashtra study

	Abnormal appearing	
Normal appearing	Low threshold criteria	High threshold criteria
No obvious lesions	Reddish looking cervix, Erosion Unhealthy cervix Erosion bleeds on touch Unhealthy cervix bleeding on touch Polyp Hypertrophied elongated edematous cervix Suspected growth Growth	Erosion bleeding on touch Unhealthy cervix bleeding on touch Suspected growth Growth

12 women with CIN 3 or invasive cervical cancers, 10 (83%) were identified by visual screening using the low threshold and 6 (50%) were identified using the high threshold.

Another IARC-sponsored study was conducted in Kerala state [15]. In this study, married women aged 30 years or older, particularly women with symptoms suggestive of cervical cancer, were screened visually by a cytotechnician trained in visual inspection and had a Pap smear taken. The visual appearance of the cervix was classified into one of the categories indicated in Table 3. A total of 2843 women underwent screening; of those women, 1564 (55%) had a normal appearing cervix, 1100 (39%) had a cervix with an abnormal appearance using the lower threshold, and 179 (6%) had an abnormal appearing cervix using the higher threshold criteria. By cytology, 10 (0.6%) of the women had CIN 2 and 27 (1%) had CIN 3 or cancer. Downstaging using the higher threshold correctly identified only 29% of the CIN 2 lesions, 31% of the CIN 3 lesions, and 55% of the invasive cervical cancers.

A low specificity of visual inspection when performed without the aid of magnification or chemical enhancement (ie, downstaging) was observed in most of the Indian studies and most have reported a low sensitivity for high-grade SIL

Table 3
Classification of visual appearance of the cervix in Kerala study

	Abnormal appearing	
Normal appearing	Low probability of disease	High probability of disease
No obvious lesions	Unhealthy cervix Cervicitis Hypertrophied cervix Polyp Congestion Discharge Prolapse	Bleeding on touch Suspicious growth, ulcer Hard, indurated, irregular cervix

Table 4
Results of studies using downstaging as a screening test

Author	No. of women	Threshold	Detection of HSIL and cancer	
			Sensitivity (%)	Specificity (%)
Singh [13]	44,970		62[a]	89[a]
Bhargava	3608		92[b]	37[b]
Sujathan	3602		93	38
Nene [14]	2135	Low	83	43
		High	50	95
Wesley [15]	2843	Low	66	55
		High	50	95

[a] For invasive cervical cancer only.
[b] For all grades of dysplasia and invasive cancer.

as opposed to invasive cervical cancer (Table 4). Because of the poor performance of downstaging in these large studies, most authorities have concluded that downstaging offers little merit as a cancer screening test [16]. However, clinicians should view the cervix carefully every time a vaginal speculum examination is performed to ensure that an obvious cancer is not present.

Direct visual inspection

Description of method. To increase the sensitivity of visual screening methods, many investigators wash the cervix with a 3% to 5% solution of acetic acid before inspecting it. A 3% to 5% acetic acid solution is routinely used during colposcopy to enhance the detection of CIN and early invasive cancers. Tissues composed of cells with a high nuclear:cytoplasmic ratio typically turn white after the application of acetic acid, a process referred to as acetowhitening. Although the exact mechanism of action that is responsible for acetowhitening of CIN lesions after the application of a dilute acetic acid wash is unknown, the use of an acetic acid wash allows cervical cancer precursors—as opposed to only early invasive cervical cancers—to be identified visually. Evaluating the cervix with the naked eye after the application of acetic acid has been referred to by various terms, including acetic acid washes, acetic acid visualization, acetic acid screening test, visual screening with acetic acid, visual inspection, cervicoscopy, and DVI (see Table 1) [16–21]. The authors believe that DVI and the acetic acid test are the most appropriate terms for this technique because they distinguish this method clearly from other visual inspection methods that use acetic acid, such as speculoscopy, cervicography, and even colposcopy. In this article the authors use the term "DVI" because they have used it in their other published studies.

In some studies, DVI has been augmented by using low-power, hand-held magnifying devices, including a 2.5× magnifying device (Gynoscope) and a 4.0× magnifying device with a built-in light source (Aviscope PATH, Seattle, WA). The magnified view of the cervix observed with these devices is between that observed with the naked eye and with a colposcope. This approach has been variously referred to as DVI with magnification, visual inspection with acetic

acid and magnification, avioscopy, and gynoscopy. In this article the authors use the term "DVI with magnification" when magnification is used.

Large clinical studies. Several studies have been published on DVI of the cervix using the naked eye after applying a 3% to 5% acetic acid solution (ie, DVI) as either a primary screening method or an adjunctive screening used in combination with a Pap smear (Table 5). These studies have enrolled women from diverse clinical settings, including China, Italy, India, Zimbabwe, South Africa, Europe, and the United States. The results obtained with DVI by the different investigators have been remarkably consistent given the diverse clinical settings. In the first large clinical study of DVI that was reported from Italy, 2105 women had a Pap smear obtained, a 3% to 5% acetic acid solution was applied to the cervix, and the cervix was inspected with the naked eye. A 35-mm photograph of the cervix (eg, cervigram) also was obtained. Women with an abnormality on any of the three tests were referred for colposcopy. DVI examinations were classified as positive in 25% of the women, 15% had a positive cervigram, and 4% had an abnormal Pap smear. DVI identified seven (88%) of the eight cases of biopsy-confirmed SIL, whereas cytology and cervicography each identified five (63%). The Italian study concluded that DVI is more sensitive than cytology but less specific [20].

In another clinical study of DVI, 2426 South African women were screened using a combination of Pap smears that were processed on site in a mobile van and DVI [17]. The age of women ranged from 20 to 83 years, with a median age of 31 years. Women with an SIL on Pap smear or a positive DVI examination were referred for immediate colposcopy, biopsy, and, if indicated, loop excision. Of the 76 women with positive DVI examinations, cytologic evidence of SIL was identified in 61 (80%). Of the 31 women found to have biopsy-confirmed high-grade SIL, DVI correctly identified 20 (64%). Of the 253 women with biopsy-confirmed low-grade SIL identified in this study, however, DVI only detected 35 cases (13.8%). In this study, a relatively high specificity of DVI was observ-

Table 5
Results of studies using direct visual inspection as a screening test

			Detection of HSIL and cancer	
Author	Country	Number	Sensitivity (%)[a]	Specificity (%)[a]
Cecchini [20]	Italy	2105	88	75
Ottaviano [21]	Italy	2400	94[a]	90[a]
Megevand [17]	South Africa	2426	66	98
Sankaranarayanan [22]	India	2135	90	92
Sankaranarayanan [16]	India	1351	96	65
Chirenge [23]	Zimbabwe	2148	77	64
Denny et al [24]	South Africa	2,944	65	84
Belinson et al [25]	China	1997	71	74
Denny et al [4]	South Africa	2754	73	79

[a] estimated from numbers provided in manuscript and may not reflect adjustment for verification bias

** For all grades of dysplasia and invasive cancer

ed. Only 3.1% of all women screened were classified as having a positive DVI examination.

More recently, six large, well-controlled studies of DVI have been published from India, Zimbabwe, China, and South Africa. In the first study from India, Sankaranarayanan et al used cytology and DVI to evaluate 3000 women [22]. DVI was performed by two cytotechnicians who took a 1-month training course in DVI and were taught to identify acetowhite lesions, nabothian cysts, cervicitis, cervical cancers, and various other cervical conditions. The training also included observing colposcopic examinations in women with and without cervical lesions. DVI examinations were classified as positive in 298 (10%) of the 3000 women. Pap smears were classified as abnormal (atypia or SIL of any grade) in 307 (10%) of the women. An additional 215 (7%) of the women had an abnormal appearing cervix but without an acetowhite lesion. The estimated sensitivities of DVI and cytology were similar in this study (ratio of estimated sensitivities 1.05; $P = 0.25$). Of 51 cases of biopsy-confirmed high-grade SIL or cancer, 46 (90%) were DVI positive, and the Pap smear was classified as positive in 44 (86%) of the cases. The estimated specificity of cytology and DVI was 0.92.

Another study from India by the same investigators found a higher sensitivity but much lower specificity [16]. In the second study from India, 1351 women aged 22 to 70 years who presented for routine cervical cancer screening had a speculum examination by a trained nurse who performed a naked-eye examination of the cervix, took a Pap smear, and then performed a DVI examination. Women who had a grossly abnormal appearing cervix, an acetowhite lesion detected by DVI, or any degree of cytologic atypia were referred for colposcopy. 509 (38%) of all women screened had a positive DVI examination, 205 (15%) had an abnormal Pap smear result, and 107 (8%) had a grossly abnormal appearing cervix but a negative Pap smear result and DVI examination. At colposcopy, 62 (0.5%) of the women were found to have biopsy-confirmed high-grade SIL (CIN 2,3), and nine invasive cancers were identified. DVI detected 68 (96%) of the biopsy-confirmed high-grade SIL (CIN 2,3) and invasive cervical cancers, whereas cytology alone detected only 44 (62%). The detection rate of high-grade SIL (CIN 2,3) and cancer was 53.6 cases per 1000 women screened compared to 34.7 per 1000 women screened for cytology. This rate yields a sensitivity ratio of 1.54 ($P < 0.001$). The estimated specificity of DVI was significantly lower than that of cytology, however, with 441 women without disease being classified as having a positive DVI examination compared to 161 by cytology. The positive predictive value for high-grade SIL (CIN 2,3) and cervical cancer was 14% for DVI compared to 22% for Pap smears in this study.

A lower sensitivity but equivalent specificity was reported in another recent study from Zimbabwe by Chirenje et al that included a total of 10,934 women between the ages of 25 and 55 years [23]. Screening was performed by six trained nurse midwives who used a wooden Ayres spatula to take a Pap smear and then performed a naked-eye visual inspection of the cervix after the application of 4% acetic acid. The first phase of the Zimbabwean study included

8731 women. In the first phase, the DVI examination was classified as abnormal in approximately 20% of the women screened, and the positive predictive value of DVI for high-grade SIL or cancer was 26%. DVI had a detection rate for biopsy-confirmed high-grade SIL that was similar to that of cytology. The second phase of the study included a total of 2203 women. In the second phase, all women underwent colposcopy, including women with negative colposcopic examinations. This fact is important because it allows verification bias to be eliminated, and the sensitivities and specificities of cytology and DVI can be measured accurately. In the second phase, the DVI examination was classified as positive in almost 40% of all women screened, and DVI had a sensitivity of 77% and specificity of 64% for biopsy-confirmed high-grade SIL or cancer. For comparison, Pap smears obtained using only a wooden Ayres spatula had a sensitivity of only 44% but a specificity of 91%.

Over the past 6 years the authors have been conducting a study in Cape Town, South Africa, in which previously unscreened black women between the ages of 35 and 65 years have been enrolled. All women undergo an extensive examination performed by a trained nurse that includes a Pap smear, HPV DNA testing for high-risk HPV types using the Hybrid Capture test, a DVI examination, and a cervigram. Women with an abnormality on any of the four screening tests are referred for colposcopy. In the first phase of the study, which included 2944 women, to be classified as HPV DNA positive, women had to have relatively high levels of high-risk types of HPV DNA detected. The results the authors obtained using DVI in this setting were similar to those obtained by the studies from India and Zimbabwe [24]. Biopsy-confirmed high-grade SIL (CIN 2,3) or cancer was identified in 86 (3%) of the women screened. The DVI examination was classified as abnormal in 18% of the women, Pap smears were classified as low grade squamous intraepithelial lesion (LSIL) or higher in 8%, high-risk HPV DNA was detected in 16%, and the cervigram was atypical or more in 13%. DVI correctly identified 67% of the cases of high-grade SIL (CIN 2,3) or cancer, Pap smears identified 78%, and HPV DNA testing using the Hybrid Capture test identified 73%.

In the second phase of the study, 2754 women were screened using the four different tests, but all women found to be high-risk HPV DNA positive by Hybrid Capture II, which detects approximately 1 pg of HPV DNA, were referred for colposcopy. In the second phase almost half of all women screened underwent a colposcopic examination, which reduced the potential for verification bias. The nurse who performed the DVI examinations in the second phase of the study had no previous experience in performing DVI and had not performed any gynecologic or obstetric procedures for more than 10 years. Despite these differences between the first and second phases of the study, the performance of DVI observed in the second phase of the study was similar to that observed in the first phase (Table 5). Almost identical results recently have been reported in a study from China in which DVI was performed by gynecologic oncology fellows and younger gynecologic oncologists (Table 5) [25].

Comparison of the results obtained with DVI with results obtained using cervical cytology indicates that, in general, the sensitivity of the two tests is

comparable, but in most studies the specificity of DVI is considerably lower than that of cervical cytology. For example, in the first phase of the authors' South African study we found that the estimated sensitivity and specificity for the identification of biopsy-confirmed high-grade SIL (CIN 2,3) and cancer of conventional cervical cytology evaluated in Cape Town were 76% and 93%, respectively, when cases diagnosed as atypical squamous cells of undetermined significance were classified as negative [24]. For comparison, the sensitivity and specificity of DVI were 67% and 83%, respectively. In the second phase of the authors' Cape Town study, the sensitivity and specificity of conventional cytology for the identification of high-grade SIL (CIN 2,3) and cancer declined to 0.62, but specificity remained high at 96%. In the second phase of the study the sensitivity of DVI for high-grade SIL (CIN 2,3) and cancer was 73% and the specificity was 79%. When considered in terms of a screening test in a population in which the prevalence of high-grade cervical disease is only several cases per hundred women screened, the low specificity results in a low positive predictive value for DVI. In the South African study the positive predictive value of DVI was 13%, which means that only approximately one out of eight women who test positive have a high-grade cervical lesion.

Key remaining issues

Several important unresolved issues regarding the performance of DVI remain. One of the most important unresolved issues is the best way to define a "positive" DVI screening test. DVI test results are usually reported as being negative, positive, or suspicious for invasive cancer, although some investigators also have used a "borderline" or "indeterminant" category. Positive tests are frequently defined as dense, well-defined acetowhite lesions that are adjacent to the squamocolumnar junction. Cervices with faint, ill-defined areas of acetowhitening and small dot-like areas of acetowhite epithelium are frequently classified as negative [26]. Recently the authors evaluated two different definitions of what constitutes a "positive" DVI result among 2754 women examined as part of the second phase of their South African study [4]. In this study the clinician who performed the DVI examination was specifically trained to classify DVI findings into five categories (Table 6). "Definite lesions" were defined as acetowhite lesions with a well-circumscribed border, "ill-

Table 6
Different categories used to classify results of direct visual inspection

Category	Results
Suspicious	Cervical ulcer or exophytic growth suspicious for carcinoma
Definite lesion	Acetowhite lesion with well-circumscribed border
Nonconfluent scattered lesions	Focal small, punctuated areas of acetowhitening usually involving the transformation zone
Ill-defined lesions	Poorly circumscribed and faintly acetowhite
No lesion	No acetowhite lesion visible

defined lesions'' included lesions that were poorly circumscribed and faintly acetowhite, and ''nonconfluent scattered lesions'' were focal, small, punctuated areas of acetowhitening that usually involved the transformation zone. Women with any cervical lesion identified during the DVI examination were referred for colposcopy.

When only ''definite lesions'' were classified as a positive result, the sensitivity of DVI for high-grade SIL (CIN 2,3) was 58%. The sensitivity significantly increased to 70% when ''any lesion'' was classified as a positive result. The increase in sensitivity observed with expanding the definition of what constitutes a positive test result was accompanied by a significant decrease in test specificity from 84% to 79%. For comparison, the sensitivity of conventional cervical cytology using a cut-off of LSIL to define a positive test result produced a sensitivity of 0.57 and a specificity of 0.96. These findings represented the only comprehensive evaluation of the performance of DVI using different definitions of what constitutes a ''positive'' test result. The results of this study suggested that the approach advocated by some investigators of restricting the definition of a positive test result to only well-defined, dense lesions to increase the specificity of DVI-based screening will result in a significantly poorer sensitivity than would be obtained using a broader definition of what constitutes a ''positive'' result.

Another issue that requires resolution is the potential role of magnification in DVI and whether magnification significantly improves the performance of DVI. Although magnification has been advocated as a way to improve the performance of DVI, minimal data are available to support the use of magnification over simple examination using the naked eye. In the first phase of our South African study, we used a hand-held 2.5× magnifying device during DVI. The study design prevented a determination of whether the use of the magnifying device actually improved test performance. The second phase of the screening study that enrolled almost 3000 women was designed specifically to evaluate the impact of using a 4× hand-held magnifying device that uses green liquid crystal diodes (LCD) crystals to illuminate the cervix with a green light (Aviscope, PATH, Seattle, WA). The screening examination was designed to force a break between the inspection of the cervix with and without magnification and findings from the first examination without magnification that were recorded before, and independent of, the findings of the examination that was performed with magnification. Using this approach the authors were able to determine the impact of magnification on the performance of DVI. Magnification resulted in a slight but nonsignificant increase in sensitivity for high-grade SIL (CIN 2,3) from 70% to 74% when the definition of ''positive result'' was the presence of any acetowhite lesion. The nonsignificant increase in sensitivity was accompanied by a slight but significant drop in specificity from 79% to 77%, however. In the authors' opinion these results indicate that magnification does not dramatically improve the performance of DVI, and because magnifying devices are somewhat expensive and require periodic maintenance they do not recommend their routine use for DVI.

In this same study the authors also were able to evaluate the importance of other factors, such as patients' age, parity, presence of sexually transmitted

infections, and HIV status. No significant differences in the sensitivity and specificity of DVI were associated with the presence or absence of *Neisseria gonorrhea, Chlamydia trachomatis, or Trichomonas vaginalis* or by age, parity, contraceptive use, or across the time frame of the study. DVI had a significantly lower specificity among HIV-infected women compared to uninfected women, and there was a nonsignificant trend toward greater specificity in women over the age of 50 years, which became significant when women were classified as postmenopausal or not. These results suggest that demographic differences among studies (at least when restricted to women over the age of 35 years) and the prevalence of coexistent sexually transmitted infections do not explain the differences that have been observed in the performance of DVI in different studies. Based on this conclusion it is most likely that the differences in performance of DVI observed in the various studies listed in Table 5 are caused by differences in the definitions used to define what constitutes a DVI-positive result, errors in the determination of the prevalence of cervical disease in women in the studies, and the skill and training of the clinical examiners.

Should DVI become widely adopted for use in low-resource settings, it will be necessary to train large numbers of mid-level clinicians, such as nurses, in how to perform DVI examinations. Several international health groups have developed comprehensive training programs and instruction manuals oriented specifically toward mid-level clinicians in low-resource settings. Training programs typically include didactic and practical hands-on components. Although it is logical to expect that the experience and training of the clinical examiner will have a significant effect on the performance of a highly subjective skill such as DVI, few studies actually have examined directly the impact of these variables. In the authors' South African studies they have looked carefully for evidence of changes in the performance of individual examiners over time but have found no consistent trends in clinical performance. This observation may be caused by the fact that they have had a relatively small number of nurses perform the DVI examinations and the nurses have been closely supervised.

Investigators from International Agency for Research on Cancer (IARC) have presented data at international meetings that suggest that with increasing experience, DVI examiners classify fewer women as having a ''positive'' DVI examination, although it is unclear whether this results in decreased sensitivity. Recently, Sellors et al had three clinicians who were experienced in visual screening techniques score 114 cervical photographs taken after the application of a 5% acetic acid [26]. The degree of intraobserver agreement among the three observers was found to be only moderate (pair-wise unweighted kappa statistic of 0.54–0.60). The performance of DVI when conducted using cervical photographs varied considerably among the three observers. Among the three observers the sensitivity of DVI for identifying high-grade SIL (CIN 2,3) and cancer varied from 87% to 97%. Specificity also varied considerably, ranging from 58% to 39%.

One of the interesting findings of the study was that although the three observers agreed before the study on the definition of what constituted a positive

DVI result, the observer with the greatest amount of colposcopic experience had the highest sensitivity and the lowest specificity. This result is not surprising, because although colposcopy and DVI evaluate the cervix after the application of a 3% to 5% solution of acetic acid, conceptually the methods are different. Colposcopy was developed as a method to evaluate women with abnormal screening test results. High-grade lesions are relatively common in this population, and the primary goal of colposcopy is to ensure that high-grade cervical disease is not missed. Current colposcopic practices emphasize identification of all areas of acetowhitening and the liberal use of colposcopically directed biopsy, even of minor cervical abnormalities. In contrast, DVI was designed as a screening test, and high-grade lesions are relatively uncommon in this population. False-positive test results are of considerable concern in the screening setting because these women, most of whom do not have a significant cervical lesion, require further evaluation or treatment.

The previous discussion highlights the need for effective quality control measures should screening programs based on DVI become widely adopted. Cytology-based screening programs are relatively easy to control for quality because a proportion of slides that are reviewed by one technician can be reviewed easily by another. The performance of individual screeners can be monitored and retraining instituted as required. With DVI no slide or radiographic film can be reviewed at a later date; therefore quality control measures for DVI must be more indirect than they are for cervical cytology. Possible ways to provide quality control for DVI-based screening programs are to have examinations observed periodically by supervisors, obtain photographs periodically of the cervix at the same time as DVI is performed and have the photographs reviewed by experts, monitor the performance of individual examiners over time, and require periodic retraining and retesting of examiners. Critics of visual screening have argued that because DVI is inherently more difficult to control for quality than cervical cytology, it should not be considered for primary screening. The authors do not find this argument to be convincing, however. Screening programs based on DVI are inherently no more difficult to control for quality than are other screening programs, such as blood pressure monitoring and tuberculin testing, which are based on simple physical examinations but are considered highly effective [27]. The authors fully recognize that providing quality assurance programs for any type of screening program is logistically difficult in low-resource settings.

Cervicography

Description of method

Cervicography is a visual screening method introduced in the 1970s that uses a specially designed 35-mm camera to take photographs of the cervix after the application of a 3% to 5% solution of acetic acid. The film is then sent to a central facility, where it is processed under strict quality controlled conditions and evaluated by a specially trained expert skilled in colposcopy. The theory

behind cervicography is that because experts evaluate the cervical photographs they are better able to identify cervical lesions and discriminate between high-grade SIL (CIN 2,3) and more trivial lesions than the mid-level clinicians who perform DVI.

Results of studies. When cervicography was first introduced, several relatively small studies were conducted that suggested that it was superior to cervical cytology for the detection of high-grade SIL (CIN 2,3) and cervical cancer. In general, although the results of these early studies were promising, the studies were relatively small and most had significant methodologic flaws. More recently, two large, well-designed screening studies critically evaluated the performance of cervicography and compared it with HPV DNA testing and cervical cytology as a screening test. One of these studies was the National Cancer Institute's Costa Rican study; the other was the authors' South African study. The Costa Rican project is a large, population-based study of the natural history of cervical neoplasia that is being conducted in a region of Costa Rica with consistently high age-adjusted rates of cervical cancer. At the enrollment examination, women were screened using a cervigram, two types of Pap tests (liquid-based and conventional), and a HPV DNA test. Participants were referred for colposcopy if (1) physical examination was suspicious for cervical cancer, (2) the Pap test was abnormal (atypical squamous cells or more), (3) there was a "positive" cervigram. As a quality control mechanism, 2% of all women with normal screening tests were referred for colposcopy.

The sensitivity of cervicography when performed under routine conditions was found to be poor. Cervicography correctly identified only 52% of all the biopsy-confirmed high-grade SIL (CIN 2,3) and invasive cervical cancers (Table 7) [28]. The specificity of cervicography was reasonable, however; only 5% of women were referred for colposcopy on the basis of an abnormal cervigram. For comparison, conventional cytologic screening identified 77% of the cases of high-grade SIL (CIN 2,3) or cervical cancer and had a specificity of 94%. The authors obtained similar results in their Cape Town study. In the Cape Town study, women between the ages of 35 and 65 years underwent a screening examination that included HPV DNA testing, conventional cervical cytology,

Table 7
Results of studies using cervicography as a screening test

		Detection of high-grade SIL and cancer			
		Cervicography		Conventional cytology	
Country	No. of women	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
South Africa	2944	58	91	76	94
Costa Rica					
routine read	8460	52	95	77	94
optimized read	3645	64	94	77	94

DVI, and cervicography. Women with high levels of high-risk types of HPV DNA, LSIL or greater cervical cytology, DVI positivity, or a positive cervigram were referred for colposcopy. In the authors' study, 11% of enrollees did not have cervigrams available for review either because of problems with the camera at the time of the examination or because the cervigrams were classified as technically defective. Of women who had readable cervigrams, 58% with biopsy-confirmed high-grade SIL or cancer had positive cervigrams and the specificity of cervicography was 91% [24].

To determine the best possible performance that can be obtained with cervicography, Schneider et al retrospectively reevaluated the performance of cervicography in the National Cancer Institute's Costa Rican project. A stratified sample of 3645 women selected from the original group of 8460 women was reevaluated by a second reviewer, who was blinded to all clinical data [28]. When the two evaluators disagreed, a third evaluator reviewed the slides. As part of the reevaluation process, all of the histology specimens originally diagnosed as low-grade SIL (CIN 1) or more and a subset of specimens classified as normal also were reevaluated by a second pathologist. Agreement between the initial and second reviewer when classifying cervigrams as either "negative" or "positive" was found to be only moderate when compared to the results expected by chance alone (kappa statistic of 0.5). This result indicates that there is considerable variability in the readings obtained when different evaluators read a cervigram. The sensitivity of the "arbitrated" or "optimized" cervigram readings was considerably better than the sensitivity of the "routine" cervigram readings. The optimized readings correctly identified an additional 12% of the women with high-grade SIL (CIN 2,3) or cancer compared to the sensitivity obtained with a single reviewer (Table 7).

When the "arbitrated" cervigram reading and the "arbitrated" pathology diagnosis was used, the sensitivity of cervicography for identifying high-grade SIL or cervical cancer increased to 64%. Under these conditions cervicography would have referred a total of 7% of all women screened for colposcopy. In this study, the impact of various characteristics, such as patient age, degree of acetic acid effect, presence of metaplasia or a congenital transformation zone, and ability to see the squamocolumnar junction on the performance of cervicography, also were assessed. Sensitivity was considerably higher in younger, as opposed to older, women. In women younger than age 50, sensitivity was 66%, whereas in women aged 50 years or older it was 37% [28]. Of the other characteristics that were evaluated, only an increasing quality of the acetic acid effect was associated with a higher sensitivity. Several characteristics were associated with small but statistically significant reductions in specificity, including premenopausal status, entirety of the lesion not visible, and presence of a congenital transformation zone. The importance of this study is that it demonstrates the optimal results that can be obtained using cervicography. Even when performed under optimal conditions, however, cervicography identified significantly fewer cases of high-grade SIL or cervical cancer than did conventional cytologic screening (Table 7).

Summary of cervicography results

Based on the results obtained from the large screening studies, cervicography does not seem to have an adequate sensitivity, even when the performance of the test is highly optimized, to be used as a stand-alone screening test. In at least one low-resource setting, high rates of technically deficient cervigrams were obtained when the test was performed by trained nurses. Although it is possible that cervicography may be useful as an adjunctive screening method in conjunction with cytology in settings in which cytologic screening is routinely conducted, no large, well-designed studies demonstrate clinical use of cervicography as an adjunctive screening method in populations at relatively low risk for having cervical disease.

Speculoscopy

Description of method

Speculoscopy is essentially DVI that has been modified to use a special disposable low-intensity blue-white chemiluminescent light source and 4 to 6× magnification obtained using a hand-held magnifying device or a magnifying loupe. The light source is referred to as Speculite and the test is marketed in the United States under the trade name PapSure (Watson Laboratories, Corona, CA) when performed in tandem with a Pap smear. It is important to recognize that in contrast to DVI, speculoscopy is not considered to be a replacement for cervical cytology for primary screening but is considered to be an adjunctive method that should be used in combination with cervical cytology for primary screening.

Results of studies. Over the past several years three relatively large studies evaluated the clinical use of combining speculoscopy with cervical cytology for primary cervical cancer screening. In contrast to the aforementioned visual screening procedures, no speculoscopy studies were performed exclusively in low-resource settings. The first study was that of Westlake et al, in which primary care providers from 276 medical practices were given a 2-hour training course in speculoscopy and cervical cytology collection techniques [29]. The providers then used a combination of cervical cytology and speculoscopy to examine women who presented for routine screening. Although this study was relatively large (n = 5692 women), it had several limitations. The first limitation was that only women with "abnormal" screening test results were referred for colposcopic evaluation. This limitation is common in many studies of screening tests and offers a significant potential for verification bias because there is no way of knowing the prevalence of cervical lesions in women who had negative results on the screening tests. Because considerably more women were classified as being "positive" using speculoscopy as compared to cervical cytology and were referred for colposcopy, this could result in speculoscopy seeming to be more sensitive than cytology simply because more women are referred for colposcopy.

A second limitation of the study was that approximately half of the women with a positive screening test result who were referred for colposcopy did not

actually undergo a colposcopic examination. This failure could result in an underestimation of the prevalence of cervical disease in the population by approximately 50% and lead to underestimates of the sensitivity of both screening methods. The final methodologic shortcoming of this study was that cervical cytology tests reported as having atypical squamous cells (ASC) were classified as "negative" and those women were not referred for colposcopic evaluation. It is well recognized that 40% to 50% of all cases of biopsy-confirmed high-grade SIL are in women with ASC cytology results, and classifying these results as "negative" produces an erroneously low estimate of the sensitivity of cervical cytology [30,31]. In the recent National Cancer Institute-sponsored ASC of undetermined significance LSIL Triage Study (ALTS) clinical trial, the sensitivity rate of a repeat cervical cytology for identifying cases of biopsy-confirmed high-grade SIL or cancer was 0.85 when repeat cervical cytology diagnosed as ASC or more was classified as an abnormal result. This rate dropped to 0.59 when ASC was classified as "negative" and only women with a result of LSIL were referred for colposcopy [32]. Despite these methodologic limitations, the Wertlake et al study provided a good estimate of the specificity of speculoscopy and cervical cytology.

Among the 5692 women screened, a total of 32 cases (0.56%) of biopsy-confirmed high-grade SIL were identified. The number is similar to the prevalence of high grade squamous intraepithelial lesion (HSIL) cytology results reported by the College of American Pathologists survey of cytology laboratories in the United States in 1996 but most likely represents only half of the cases of high-grade SIL that would have been identified if all of the women with a positive screening result had actually undergone colposcopy [33]. Adding speculoscopy to screening cervical cytology in the Wertlake et al study increased the detection of biopsy-confirmed high-grade SIL (CIN 2,3) by 52%. Using a cut-off of "LSIL or greater" to define a "positive" cervical cytology, only 21 (66%) of the 32 cases of biopsy-confirmed high-grade SIL that were identified using both methods combined were identified using cytology alone. In that study, 151 (2.6%) of all women screened had a cervical cytology result of LSIL or HSIL. In contrast, 692 (12%) of all women screened were classified as having a "positive" speculoscopy examination, and 799 (14%) of the women were classified as being "positive" with either test (Table 8). The specificity rates of these tests when used for the detection of biopsy-confirmed high-grade SIL were 97% for cervical cytology, 88% for speculoscopy, and 86% for the two tests combined.

The study by Edwards et al of the performance of speculoscopy has a similar design as the study of Wertlake et al but is considerably smaller ($n = 689$) [34]. The study was conducted at four Kaiser Permanente Medical Centers in Southern California, where speculoscopy examinations were performed by nurse practitioners and midwives who underwent a 2-hour training course. ASC cervical cytology results were classified as "positive," and women with the ASC results were referred for colposcopy. A total of nine cases of biopsy-confirmed high-grade SIL were identified among the 689 women screened (ie, 1.2% of all women screened). Cervical cytology was classified as ASC or

Table 8
Performance of speculoscopy for detection of high-grade squamous intraepithelial lesions or cancer

	Cytology (alone)			Speculoscopy and cytology		
Study	Sensitivity (%)	Specificity (%)	Referral (%)	Sensitivity (%)	Specificity (%)	Referral (%)
Wertlake et al [29][a]	66	97	2.6	100[b]	86	14
Edwards et al [34]	67	95	4.9	100[b]	86	15
Loiudice et al [35][a]	76	94	7.4	100	< 80	> 23

[a] Cytologic results of atypical squamous cells were classified as "negative."
[b] By definition because women who were negative on both tests did not undergo colposcopy.

more in 34 women (4.9%), and speculoscopy was classified as "positive" in 79 women (11%). Either test was positive in 102 (15%) of the 689 women screened. The specificities of the tests when used for the detection of biopsy-confirmed high-grade SIL (CIN 2,3) were 95% for cervical cytology, 88% for speculoscopy, and 86% for the combination of the two tests (Table 8). Cervical cytology alone using ASC as a cut-off identified six (67%) of the nine cases of high-grade SIL.

The recently published large cooperative Italian study on speculoscopy addressed the study design issues of the two earlier studies [35]. The study was conducted at 32 hospitals and university gynecology departments and enrolled 3300 women between 18 and 45 years of age (median age, 33 years) who presented for routine screening. All women underwent a standard pelvic examination, cervical cytology, speculoscopy, and colposcopy in succession. Biopsies of colposcopically identified lesions were obtained and 10% of women who had negative results on cytology, speculoscopy, and colposcopy had cervical biopsies obtained for quality control purposes. Of the 3300 women who were examined, 316 (9.6%) had an abnormal cervical cytology, including 72 (2.1%) cases of ASC, 224 (6.8%) cases of LSIL, and 20 (0.6%) cases of HSIL. Speculoscopy identified 733 (22%) of the women screened as having "abnormal" results, which is 2.3 times the abnormal cervical cytology rate. The total number of women who had either positive cytology or speculoscopy results cannot be determined exactly from the manuscript, but it seems to have been at least 761 (23% of all women screened). A total of 25 cases of biopsy-confirmed high-grade SIL (CIN 2,3) was identified among the 3300 women screened (0.76% of all women screened). For the purposes of determining test performance, the Italian cooperative study defined a "positive" cervical cytology as LSIL or HSIL. Using this definition, the sensitivity of cervical cytology for biopsy-confirmed high-grade SIL was 76% (19 of 25 cases). Importantly, every case of biopsy-confirmed high-grade SIL (CIN 2,3) identified by colposcopy was identified using the combination of cervical cytology and speculoscopy (Table 8). These results corroborated the high sensitivity of the combination of speculoscopy and cervical cytology observed in the earlier studies.

Summary of speculoscopy results

The Papsure procedure thus far has been targeted for the industrialized world, and its use in addressing the low-resource setting awaits further studies. Based on the three studies described earlier, it is clear that a screening program that combines cervical cytology and speculoscopy would identify significantly more cases of biopsy-confirmed high-grade SIL (CIN 2,3) than would screening using cytology alone (Table 8). It is also evident from these three studies, however, that the combined screening approach would classify increased numbers of patients as being screening-test positive, compared to cervical cytology alone. These numbers range from 14% to 23% of all women screened. Addressing how best to evaluate or follow up with women who are speculoscopy-positive but cytology-negative is a major challenge to the introduction to speculoscopy into routine cervical cancer screening programs because it is unlikely that clinicians and payers will be willing to perform colposcopy on such a large segment of the screening population. There is a high level of uncertainty with respect to the histologic diagnosis of SIL (CIN) on cervical biopsies, and excessive use of colposcopy can result in the overdiagnosis of SIL (CIN) and overtreatment of women [36]. One potential approach to managing women who have positive results on speculoscopy but who have negative cervical cytology results is to defer colposcopy for 6 months and to perform colposcopy only if the cervical cytology becomes abnormal or the speculoscopy examination is persistently abnormal [37]. In one study that evaluated this approach, after 6 months 29% of the initially positive speculoscopy examinations among women without cytologic abnormalities had reverted to normal [37]. In the women who had persistently positive results by speculoscopy but negative results by cytology, 81% were found at colposcopy to have biopsy-confirmed low-grade SIL (CIN 1) and 8% had high-grade SIL (CIN 2,3). Overall, SIL was identified in 91% of the women whose speculoscopy examination result was positive at the second visit.

Another issue that requires further study is whether speculoscopy—when used as an adjunct to cytology—provides sufficient clinical benefit to warrant its expense and extra effort, as compared to simply performing DVI at the time of cervical cytology. The chemiluminescent light (ie, Speculite) required for speculoscopy costs several dollars and requires that the examining room lights be dimmed, which may be awkward for patients and providers. In two published series in which DVI, DVI with low power magnification accompanied by blue light filtration of the projected light (to simulate the coloration of the blue-white chemiluminescent light used during speculoscopy), and speculoscopy were performed in succession, the sensitivity of speculoscopy was proven superior to projected illumination for the detection of SIL (CIN) and the rate of false-positive examinations was lower than that of visualization with incandescent projected illumination [38,39]. Controlled clinical trials of the two methods in which patients are randomized upon enrollment into a speculoscopy or DVI group, in combination with cytologic screening, are still needed.

Potential clinical impact of visual screening methods

Primary cervical cancer screening

Need for alternative methods

Cervical cancer is the second most common cancer of women, accounting for approximately 200,000 deaths yearly worldwide [40]. The IARC estimated in 1990 that there were approximately 371,600 new cases of cervical cancer [41]. Cervical cancer comprises 10% of all cancers diagnosed in women. For every woman who dies from invasive cervical cancer, approximately 17 potential years of life before age 70 are lost. This rate results in a worldwide loss of approximately 3.4 million women-years of life before age 70 from cervical cancer each year [42].

The impact of cervical cancer is greatest in low-resource settings. Cervical cancer is the most common female cancer in many areas of Africa, Central and South America, and Asia, where it constitutes 20% to 30% of all cancers in women. In contrast, in high-resource settings, such as in North America, Northern and Western Europe, and Australia, cervical cancer accounts for only 4% to 6% of female cancers. Age standardized incidence rates for cervical cancer in 1985 varied from 7.6 in Western Asia to 46.8 in Southern Africa [43].

Much of the difference in age standardized incidence rates for cervical cancer between low- and high-resource settings can be attributed to differences in screening for cervical cancer and its precursors. In areas of the world in which cytologic screening is routinely performed, a threefold to fivefold reduction in the incidence of cervical cancer has been observed after screening was introduced. The impact of screening programs is clearly demonstrated by the Scandinavian experience [44]. In Finland, a national cytologic screening program was begun in the 1950s, and cervical cancer rates in Finland are currently among the lowest in the world—5.5 cases per 100,000 women. In contrast, Norway did not develop a nationwide screening program, and a much smaller reduction in cervical cancer rates occurred. The rate of invasive cervical cancer in Norway continues to be three times higher than that of Finland—15.6 cases per 100,000.

Unfortunately, there continue to be significant barriers to implementing comprehensive cytologic screening programs in many regions of the world. Perhaps the most important of these barriers is competing health needs. Seventy percent of female deaths in poor countries are caused by either communicable diseases, such as tuberculosis, malaria and HIV, or from maternal and perinatal causes. The average maternal mortality rate in sub-Saharan Africa is 650 per 100,000 live births compared to 10 per 100,000 live births in the United States. UNAIDS estimates that as of 2001 there were 28.5 million people living with HIV/AIDS worldwide [45]. Despite its importance as the most common cause of cancer-related deaths, other health issues are frequently given a higher priority. Additional barriers to implementing cervical cancer screening include war and civil unrest, which have been endemic in many countries for decades, and widespread poverty. For example, 25% of all medical personnel are be-

lieved to have been killed during the recent genocide in Rwanda. Poverty may be the greatest barrier to screening. Only 26% of families in sub-Saharan Africa have running water or proper sanitation facilities. In such areas, health care services are often poorly developed and tend to focus on curative, rather than preventive, health.

Another barrier to implementing comprehensive cytologic screening programs is the nature of the screening test itself. If cytologic screening programs are to be effective, several requirements must be met. The first requirement is for a high-quality cytology laboratory. Developing and maintaining a high-quality cytology service is not an easy undertaking. Interpreting cervical cytology smears is considered by many pathologists to be one of the most difficult tasks in pathology, and obtaining a high level of proficiency typically requires several years of training in conjunction with motivation and good pattern recognition skills [46]. Maintaining skills, once they have been obtained, is also not simple. Maintaining skills requires an ongoing continuing medical education program and access to cervical biopsies from women diagnosed as having an abnormal Pap smear. Cytology laboratories also require close supervision by trained laboratory managers and established quality assurance programs. This quality assurance includes careful monitoring of how Pap smears are processed and interpreted, rescreening of a given percentage of smears diagnosed as being within normal limits, work load limits to ensure that smears are not screened too quickly, and laboratory-wide correlation between the cytologic results and the findings observed at colposcopy. Guidelines for setting up and maintaining a cytology service have been discussed in depth in several publications [47].

The fact that Pap smears are usually not interpreted at the point of clinical care also produces a barrier to screening. An infrastructure must be in place that allows smears to be transported from the clinic site to the cytology laboratory, laboratory results to be transmitted back to the clinical site, and patients with abnormal results to be tracked down and notified that they require further evaluation and treatment. In low-resource settings, each of these steps offers an opportunity for the screening program to break down, and smears, results, or patients can be lost.

Visual screening strategies

Visual screening tests lack many of the disadvantages of cytologic screening for low-resource settings. Visual screening seems to be considerably easier to learn than cervical cytology. Although the minimal training requirements for performing visual screening are not known, complete training in either DVI or speculoscopy usually takes less than a week, rather than the months to years required for a cytotechnician to become proficient in cervical cytology. More importantly, the results of visual screening are instantly available, which greatly reduces the infrastructure requirements of the screening program. The availability also allows screening and treatment to be incorporated into a single clinic visit, which eliminates the need to track patients with abnormal screening results and reduces the risk that patients will be lost to follow-up.

The large screening studies that have compared visual screening modalities with cervical cytology have found uniformly that these technologies have a sensitivity equivalent to that of cytology for detecting high-grade SIL (CIN 2,3) or cancer but that in many clinical scenarios the specificity is considerably lower (see Table 5). Screening programs based on DVI or speculoscopy as a primary screen might be expected to be cheaper to initiate and maintain than a traditional cytologic screening program but are just as effective. DVI and speculoscopy have low positive predictive values, however, which means that primary screening with either technology alone would classify considerable numbers of women without cervical disease as being screen positive. In some of the studies described in Table 5 this would be more than one third of all women screened. Using conventional triage algorithms, these women would be referred for colposcopic evaluation and biopsy. In many resource-poor settings, colposcopic services are either not available or are limited. Expanding colposcopic services sufficiently to allow all women classified as screening test positive by these visual technologies to undergo colposcopy actually might be more costly than providing high-quality cytologic screening. Alternative strategies for handling women who have positive visual screening test results must be developed.

One approach to the low positive predictive value of either DVI or speculoscopy would be to treat all women with a positive visual examination result using inexpensive methods such as cryosurgery. This approach is frequently referred to as ''screen and treat'' and has been advocated by some researchers for particularly low resource settings in which no other screening options are available. ''Screen and treat'' offers several advantages in that mid-level providers, such as nurse midwives, could be used to provide the service and, importantly, screening and treatment could be performed at a single visit. This service would eliminate the need to track patients with abnormal test results and reduce the risk that patients are lost to follow-up. Several unknowns about this approach must be evaluated before it can be recommended for widespread implementation, including the complication rate of cryosurgery when performed by mid-level providers in a resource-poor setting, the success rate of cryosurgery when used to treat high-grade SIL (CIN 2,3) in the absence of colposcopic guidance, and the acceptability to patients of a screening program in which up to 40% of all women screened receive cryosurgery.

Cryosurgery has been used widely for almost 40 years to treat SIL and has been shown to be an effective treatment for cervical cancer precursors. Most of the published series are from large teaching institutions, in which the procedure has been performed under optimal conditions after colposcopic evaluation. Under these conditions, published rates of treatment success are 80% to 90%. Treatment failures increase with larger lesions and lesions that extend into the endocervical canal. There is only one publication on the success rates obtained with cryosurgery when performed in a setting in which colposcopy is not available. This study was conducted in Ibadan, Nigeria [48]. Women with persistent abnormal Pap smears were first evaluated using blinded, four-quadrant

punch biopsies. If biopsy-confirmed SIL was identified, the women underwent treatment. Of 22 women with high-grade SIL (CIN 2,3) treated using either electrocautery (n = 10) or cryosurgery (n = 12), persistent disease was identified in 12 (55%). In contrast, none of the 20 women with high-grade SIL (CIN 2,3) treated using excisional methods (including 12 by cold-knife conization and 9 by hysterectomy) had persistent disease identified.

Another problem that might be encountered with cryosurgery in resource-poor settings is the poor treatment response observed with large, high-grade lesions. In some series, large, high-grade SIL (CIN 2,3) that involve four quadrants of the cervix have had a 30% or more failure rate after cryosurgery [49,50]. Large, high-grade lesions would be expected to be more common in a poorly screened or previously unscreened population. Because of unknowns with respect to the safety and effectiveness of "screen and treat" programs, the authors believe that it is premature to advocate the adoption of these programs for low-resource settings. Currently studies are underway in South Africa that are designed to evaluate "screen and treat" programs directly, and in the near future it should be known whether such programs are truly safe and effective.

An alternative approach to cervical cancer screening that retains many advantages of the "screen and treat" approach in which all women found to have an abnormal screen undergo immediate cryosurgical treatment would be to sequentially screen women using two tests. This approach, to which the authors refer as "two-stage" cervical cancer screening, would greatly increase the positive predictive value of a "positive" screening test and would reduce the number of women who undergo unnecessary treatment [51]. In the "two-stage" approach to cervical cancer screening, the authors would use a low-cost but relatively nonspecific test, such as a test using visual inspection, to screen all women for the presence of cervical abnormalities. Women with an abnormality identified with the first test would then be rescreened using a second test, such as an HPV test or a Pap smear. Only women classified as abnormal using both tests would be identified for treatment, either with or without colposcopic evaluation. Although widely used in clinical medicine, a "two-stage" screening approach has not been used in cervical cancer screening.

Introducing a "two-stage" screening approach that incorporates sequential screening tests (the second performed only if the first is positive) would markedly reduce the number of women being referred for colposcopy or undergoing unnecessary treatment, while retaining many of the benefits available from "low technology" screening approaches that incorporate screening methods, such as DVI, with treatment for abnormal results in the absence of colposcopic triage. A "two-stage" approach that combines an initial screen of all women using a visual technology with secondary HPV DNA testing or cytology for women with abnormalities detected by the visual test would reduce by approximately 80% the number of "high technology" secondary tests required. This has important implications for low-resource settings in which access to tests, such as cervical cytologic, is severely limited. Of equal importance is the fact that

because of its relatively poor specificity, the "low technology" approach of combining a single, relative, nonspecific, initial screening test with immediate treatment of all women with a positive initial test would result in considerable unnecessary treatment. Because treatment is relatively expensive and is associated with various adverse outcomes, including cervical stenosis, hemorrhage, infection, increased shedding of HIV from the cervix, and potentially increased susceptibility to infection with HIV, treatment of women who lack cervical disease should be minimized whenever possible, especially in resource-poor settings. A "two-stage" screening approach achieves this by greatly increasing the specificity of the screen. For example, when DVI is used as an initial screen and HPV DNA testing is used as the secondary screen, only 4% of the population screened are classified as abnormal and approximately one in four have high-grade SIL (CIN 2,3) or cervical cancer. The impact that different "two-stage" screening strategies would have in South Africa is shown in Fig. 1.

It should be noted, however, that "two-stage" screening also has certain limitations. The reduction in unnecessary treatment achieved using a "two-stage" screening approach is accompanied by a reduction in sensitivity because of the introduction of a second screening test. The magnitude of the reduction in

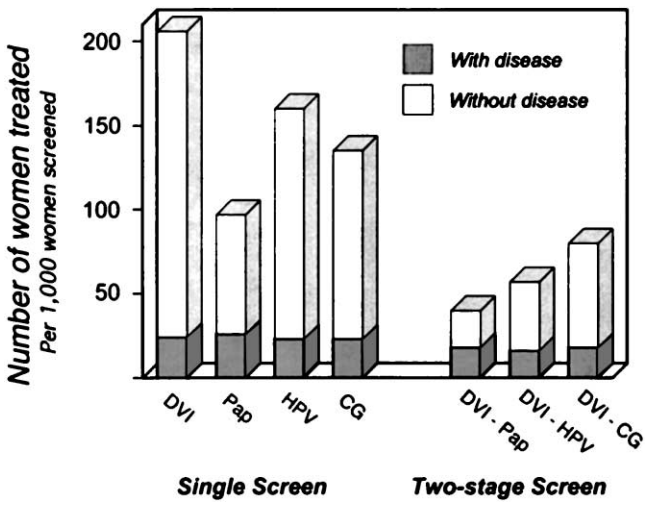

Fig. 1. Projected outcomes using a "two-stage" screening approach in Cape Town, South Africa. These results were obtained in black South African women aged 35 to 65 years who had never participated in cervical cancer screening. HPV DNA testing was performed using the first generation Hybrid Capture HPV DNA assay, which is less sensitive than the currently available HPV DNA assays. Results are presented as the number of women per 1000 women screened who would be classified as screen positive (total bar) and the number of women who are screen test positive with biopsy-confirmed high-grade SIL (CIN 2,3) or cancer. (*From* Denny L, Kuhn L, Risi L, et al. Two-stage cervical cancer screening: an alternative for resource-poor settings. Am J Obstet Gynecol 2000;183: 383–8; with permission.)

sensitivity is directly proportional to the sensitivity of the second screening test. Using data from a study conducted in South Africa, "two-stage" screening using DVI as the first test followed by a conventional cervical cytology of women who are DVI positive would have had a sensitivity of only 58% for the detection of high-grade SIL (CIN 2,3) or cancer. If DVI were followed by HPV DNA testing of DVI-positive women and only women with high levels of high-risk types of HPV DNA referred for treatment, the sensitivity of the screening program for high-grade SIL and cancer would have been 51% and the specificity 98%. Similar calculations recently have been performed using data from a Zimbabwean screening study. In the Zimbabwean study, DVI alone had a sensitivity for biopsy-confirmed high-grade SIL (CIN 2,3) of 77% and a specificity of 64%. If a program of DVI followed by HPV DNA testing of DVI positive women were adopted, sensitivity would decrease to 64% and specificity would increase to 0.82 [52]. If a "two-stage" screening program of DVI followed by conventional cytology of DVI positive women were adopted, sensitivity would be reduced to 38% and specificity would increase to 94%. Another limitation of "two-stage" screening approaches is that because only the visual technology provides an immediate result, introducing a second screening test requires that tracing and follow-up protocols be incorporated into the screening process.

Evaluation of alternative screening programs

Comparing the overall effectiveness of screening programs that incorporate different screening tests and strategies is difficult. To determine the impact that a program would have on cervical cancer incidence it would be necessary first to implement the screening program and then follow the population over several decades. Developing an optimal cervical cancer screening policy for any particular target population requires that a wide range of variables be taken into account. These variables include the age at which to start screening, the age at which to stop screening, how frequently to perform screening, how to manage screen-positive women, and the screening method. No single clinical trial or single longitudinal cohort study will ever be able to consider all of these different variables; therefore, health policy experts frequently use mathematical decision models to help inform policy. Mathematical models can be a useful way of evaluating alternative screening strategies because they can take knowledge derived from empirical studies and extrapolate it to other screening settings and different time horizons. The mathematical models that are used to evaluate different screening strategies combine information about the natural history of cervical disease and the performance of different screening tests obtained from various clinical settings. The models are capable of extrapolating costs and health effects of the screening intervention beyond the time horizon that can be observed in clinical studies. In addition to incorporating key biologic and clinical information, mathematical models can provide quantitative insight into the different components of the screening process and investigate how cost-effectiveness ratios will change as key parameters are changed.

Recently a policy analysis of different cervical cancer screening strategies for low-resource settings using data from a South African study to inform the model was conducted [53]. The model was designed to compare the clinical benefits and cost effectiveness of the different strategies should they be implemented in South Africa. It is a state transition computer-based mathematical model that simulates the natural history of HPV-induced neoplasia and cervical cancer screening, diagnosis, and treatment in a cohort of previously unscreened 30-year-old black South African women. Life expectancy and life-time costs were outputs of the model. In this model a societal perspective was taken (ie, all benefits and costs are included, regardless of who benefits or who pays) and comparative performance was measured by the incremental cost-effectiveness ratio, which is the additional cost of performing a given screening strategy compared with the next least expensive strategy. The key finding of this policy analysis was that single lifetime screenings using either DVI or HPV DNA testing coupled with treatment of all women who are screen positive (ie, ''screen and treat'') are incredibly attractive.

A single lifetime screening performed at age 35 using DVI with immediate treatment of all DVI-positive women using cryotherapy would reduce lifetime incidence of cervical cancer by 26% and actually would be less expensive than not screening because the cost of the screening is completely offset by the savings that are obtained from preventing 26% of all cervical cancers [53]. In South Africa the mean lifetime cost of cervical cancer care in the absence of screening is high ($40 per woman) because cervical cancer is common and surgical and radiation therapy is available in the public sector. This is an exciting finding because it means it actually costs more not to screen than to screen. A single lifetime screening using HPV DNA testing coupled with treatment of all HPV DNA-positive women using cryotherapy would be somewhat more effective and reduce cervical cancer by 32%, but it would cost $1.13 to $1.61 per woman more than the costs associated with not screening. Conventional cytologic screening coupled with colposcopy of women who are screening test positive and treatment of only women with biopsy-confirmed SIL is less effective and more expensive than the ''screen and treat'' strategies that incorporate DVI or HPV DNA testing. A once-in-a-lifetime screen with cytology at age 35 would reduce a woman's risk of cervical cancer by only 17% and would cost $6.44 per woman compared with no screening. In accordance with other studies, the authors' modeling indicated that targeting a single lifetime screen to women at age 35 provides the best balance between costs and clinical benefits.

Although cost-effectiveness studies can be helpful for illustrating the tradeoffs between different policy alternatives, they represent only one of many inputs in policy decision making. Other factors, including qualitative considerations such as the willingness of a society to accept overtreatment of screen-positive women who lack cervical disease, the acceptability of ''screen and treat,'' the availability of sufficient numbers of mid-level clinicians to conduct a country-wide ''screen and treat'' program, and preexisting cytology capacity, have an impact on the most appropriate screening strategy for a given setting.

Visual methods in development

Devices that use electro-optical sensors

Overview

The entire cervical cancer screening process is subjective and depends on the interpretations of three different medical disciplines, including the cytologist's interpretation of the Pap smear, the colposcopist's interpretation of the appearance of the cervix done with a colposcope, and the pathologist's interpretation of a cervical biopsy. All three steps of the screening process are well recognized to have an inherent high rate of error, which adds enormous costs to cervical cancer prevention. What is needed is a less subjective and more cost-effective method for detecting precancerous cervical lesions. Over the last decade several devices have been developed that use electro-optical sensors to detect cervical cancer and cervical cancer precursors. These devices use sensitive electronic detection devices to measure differences in the biochemical and physical properties of normal and neoplastic tissues. The ultimate goal of the newer devices is to provide an instant and objective assessment of the cervical epithelium that can be achieved without histologic or cytologic sampling of the tissue. Possible clinical applications of these newer visual screening devices that incorporate electro-optical sensors include (1) the triage of women with minor cytologic abnormalities, (2) as an adjunct or aid to colposcopy and possibly as an actual replacement for the cervical biopsy, (3) as an adjunct to other screening methods, such as cytology or DVI for routine screening, (4) as a primary stand-alone screening method.

(TruScan, Polartechniques, Ltd, Sydney Australia) Polarprobe

The TruScan device is a small, portable device that uses low-level electrical impulses and light pulses at various frequencies to determine whether cervical tissue is normal or neoplastic. The device compares measurements that are obtained from a given cervix using a small diameter, pencil-type device and compares the measurements with those produced by known cervical tissue and whose characteristics or tissue signature are stored within a databank. Using the TruScan, tissue is classified into one of three categories: normal, low-grade SIL (CIN 1), and high-grade SIL (CIN 2,3). The handpiece or probe is approximately 17 cm long and has a 5-mm diameter tip. The handpiece contains the electro-optical detection system and is connected by a flexible cable to a computer console. Cervical tissue is evaluated by passing the 5-mm tip of the device across the cervix over a 1- to 2-minute period. Initial studies, using an earlier prototype, suggest that false-positive and false-negative rates in the order of 10% are achievable [54,55]. Clinical trials that compare the performance of TruScan to other screening modalities using colposcopy and biopsy as the standard for comparison are lacking in the peer reviewed literature.

In vivo spectroscopy

Several other devices under commercial development use principles of in vivo spectroscopy to evaluate cervical tissue. In vivo spectroscopy is based on the well-established principle that epithelial tissues that are abnormal have different optical properties than normal tissues and that these optical differences can be used to determine whether a tissue is normal or abnormal. Devices that are currently under development for diagnostic purposes use various approaches, including fluorescence spectroscopy, white light elastic backscatter spectroscopy, infrared spectroscopy, Raman spectroscopy, image analysis of visible images, or combinations of the different methods. The key principle of fluorescence spectroscopy is that when a tissue is illuminated with low-power light at specific wavelengths it produces autofluorescence (ie, it emits light of a different wavelength), which can be captured and analyzed using electro-optical sensors. The spectral content of this autofluorescence is determined by the concentrations of chromophores, such as collagen, elastin, FAD, and NADH, in the tissue and by the concentration of molecules, such as hemoglobin, absorb the autofluorescence produced by the tissue. Although our understanding of the biophysical mechanisms responsible for alterations in the autofluorescence spectra that occur with neoplastic transformation is incomplete, changes in spectral characteristics have been used to diagnose preinvasive and invasive lesions at various body sites [56–58].

Several studies have investigated the use of in vivo spectroscopy to diagnose cervical SIL. One study described a noncontacting device that measured autofluorescence and spectral backscatter from the cervix of women referred for the colposcopic evaluation of an abnormal cervical cytology. In this setting the device produced a specificity for detecting SIL (of any grade) of 89% to 93% and a specificity of 93% to 94% [59]. Another fluorescence spectroscopic device has been described that uses a probe that is placed in direct contact with the cervix and has a sensitivity for the detection of SIL (of any grade) of 82% and a specificity of 68% [60]. Rather than simply measure endogenous autofluorescence, another approach that has been used at body sites such as the bladder is to topically apply photosensitizing agents such as 5-aminolevulinic acid and measure fluorescence induced by the compound rather than autofluorescence. 5-aminolevulinic acid is a chemical compound that causes the intracellular accumulation of protoporphyrin IX in neoplastic epithelium. Protoporphyrin IX is a strongly fluorescent molecule that can be detected using simple electro-optical sensors [61]. Several groups are currently evaluating this approach for the detection of cervical neoplasia [62].

Based on the preliminary data that are available from the noncommercial prototype devices, it seems that the approach of using in vivo spectroscopy to identify cervical disease is promising. A recent review compared the performance of in vivo spectroscopy with other diagnostic techniques, such as cervical cytology, cervicography, and colposcopy, using colposcopic biopsy as the reference standard [63]. Fluorescence spectroscopy was found to perform better than colposcopy and other techniques in the diagnosis of SIL, and this review

suggested that fluorescence spectroscopy has the potential to be a useful diagnostic test. The size of some devices, scope of expertise needed to use the equipment, and potential cost are challenges for its widespread incorporation into settings in which primary cervical cancer screening is performed.

Summary

This article has considered recent advances in visual screening methods. Devices that use electro-optical sensors offer great potential in various clinical roles, but considerable additional work is required to develop these devices and it is unlikely that they will come into widespread clinical use in the next 5 years. In contrast numerous studies, demonstrate that simple visual screening methods, such as DVI, have a sensitivity for the detection of women with biopsy-confirmed high-grade SIL (CIN 2,3) and cancer that is equivalent to that of conventional cervical cytology. The primary disadvantage of the simple visual screening methods is poor specificity. These methods classify up to 30% of all women screened as being test positive and as a result new strategies toward managing DVI positive women must be developed before simple visual screening methods can be adopted for routine screening. Enhanced visual methods that use cervicography and speculoscopy may be more specific and improve detection of biopsy-confirmed SIL, but the added time and expense to perform either of these methodologies must be considered and justified. Currently numerous studies are evaluating the best strategies for incorporating visual screening methods into cervical cancer screening programs. In the near future we should be able to determine whether these approaches should be incorporated into routine clinical care.

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Efficient triage of the "screen-positive" at-risk patient

Ramon M. Cestero, MD, FACOG[a,b,*],
W. Benson Harer Jr, MD, FACOG[b,c,d]

[a]*Department of Women's Health, Arrowhead Regional Medical Center, 400 North Pepper Avenue, Sixth Floor South, Colton, CA 92324, USA*
[b]*Western University of Health Sciences, 309 E. Second Street/College Plaza, Pomona, CA 91766-1854, USA*
[c]*Department of Obstetrics and Gynecology, Riverside County Regional Medical Center, 26520 Cactus Avenue, Moreno Valley, CA 92555, USA*
[d]*The American College of Obstetricians and Gynecologists, 409 12th Street SW, Washington, DC 20024-2188, USA*

Cervical cancer-related deaths have decreased markedly following the introduction of exfoliative cytology and mass population-based screening for the detection of cervical cancer and its precursors. That is the good news. The bad news is that the number of new cases of cervical cancer and the annual death rates have not shown any significant further decrease over the past two decades. In the United States alone, it was estimated that in 2001, 12,900 new cases of cervical cancer were diagnosed and 4400 women died from a disease that the vast majority of clinicians believe to be mostly preventable [1]. On a worldwide scale, approximately 500,000 cases of cervical cancer are diagnosed annually, representing 12% of all female cancers, and about half of these women are expected to die from the disease [2]. Although at least half of the cases of cervical cancer occur among unscreened women, a significant number of cases develop in women who have had some degree of screening, albeit not necessarily on a regular basis. This has prompted a review of current screening and management strategies in an attempt to optimize their application and improve results.

Many new and exciting technologies have expanded the number of adjuncts available to the clinician during the past few decades. These include, but are not limited to, the advent of fluid-based monolayer cytology, the development of reliable tests for human papillomavirus (HPV) DNA, cervicography, speculo-

* Corresponding author. Department of Women's Health, Arrowhead Regional Medical Center, 400 North Pepper Avenue, Sixth Floor South, Colton, CA 92324 USA
E-mail address: rcesteromd@earthlink.net (R.M. Cestero).

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scopy, contact biophysical screening techniques, and computerized automated cytology screening, among others. Other exciting technologies such as non-invasive systems designed to detect the optical signature of cervical cancer precursor lesions in vivo are in active clinical trial stages. Despite this apparent flurry of technological progress, colposcopy remains the gold standard in modern gynecology as the final diagnostic technique for cervical cancer.

To discuss the options available to the clinician once a woman is found to be "at-risk," it is necessary to define how at-risk status is determined. Unless the patient presents with visible pathology or exhibits risk factors such as a history of high-risk sexual behavior, previous sexually transmitted diseases (especially infection with *Chlamydia trachomatis* [3]), immunosuppression, and so forth, cytology is considered by a majority of clinicians to be the most cost-effective means of identifying the asymptomatic patient at risk for harboring cervical cancer or a precursor lesion. This concept is firmly entrenched despite recent data indicating that the "real world" sensitivity of the conventional Papanicolaou (Pap) smear is much lower than was previously believed.

In 1998, the American College of Obstetricians and Gynecologists commissioned the Agency for Health Care Policy and Research to conduct an evidence-based meta-analysis of the efficacy of cervical cytology. The study, which was conducted at Duke University and published in 1999, showed that the sensitivity of conventional cervical cytology is no greater than about 51%, validating similar data published by Fahey in 1995 [4,5]. Although the specificity of the Pap smear has been shown to be about 98%, the findings of this study indicate that 49% of women who were reassured in good faith by their healthcare providers after a negative conventional Pap smear might actually harbor a cervical cancer precursor lesion or even invasive cervical cancer. In response to previous concerns confirmed by these disturbing findings, a number of corrective measures have been instituted or considered. These include the placement of limitations upon the workload assigned to individual cytotechnologists per specific time period, the replacement of conventional glass slide cytologic screening by fluid-based monolayer cytology, and the utilization of adjuncts to cytologic screening, all of which are intended to improve the sensitivity and efficacy of primary screening modalities. Some adjunct technologies have been available for some time, and others are being actively developed at this writing. This article addresses the clinician's current and forthcoming options.

Recent developments

Two major developments regarding cervical cancer occurred in 2001. In May 2001, a workshop was convened by the National Institutes of Health (NIH) to revise The Bethesda System (TBS) [6] of cytologic classification and to address areas of confusion that still persisted after more than a decade since its design and implementation. TBS was initially developed in 1988 under the auspices of the NIH at a similar workshop in Bethesda, Maryland as a replacement for the

previous five-tier Papanicolaou classification system that had been in use since its initial adoption more than 50 years ago. TBS, among other desirable features, established general categories, introduced new and uniform descriptive terminology and diagnoses, and attempted to establish uniform standards for specimen adequacy, all of which were intended to aid the clinician in determining the most appropriate management of cytologic abnormalities among the screened population [6]. Shortly thereafter, cytopathology laboratories in the United States were required to file federal reports using TBS. Unfortunately, more than a decade since its implementation, and even following its interim revision in 1991 at a second workshop, TBS remains controversial, and its application and interpretation is still at times confusing to clinicians and other health care providers. These issues were addressed and theoretically resolved at the Bethesda 2001 conference.

The second major event was the Consensus Conference for the Management of Cytologic Abnormalities and Cervical Cancer Precursors, which was sponsored by the American Society of Colposcopy and Cervical Pathology (ASCCP) and held in Bethesda, Maryland in September 2001 under the auspices of the NIH. In the same spirit of the Bethesda System 2001 workshop, it was convened in an attempt to clarify and standardize the various management options available to clinicians in response to either cytologic abnormalities identified under TBS or to histopathologic diagnoses of cervical cancer precursor lesions. The approximately 150 attendees at the conference included gynecologists, gynecologic oncologists, family physicians, pathologists, cytopathologists, cytotechnologists, and epidemiologists. They represented 29 organizations and Federal agencies, which shared their expertise to arrive at a consensus. The final recommendations and consensus guidelines established during these two landmark conferences should be available early in 2002, subsequent to the submission of this article for publication.

Approach to the patient with abnormal cytology

Despite its shortcomings, there is no doubt that the Pap smear has served as an invaluable tool whose use has led to the dramatic decrease in cervical cancer witnessed in the past half-century in developed countries with established screening programs. Fortunately, its relative lack of sensitivity is offset by the fact that it is used to screen for the presence of a disease that in most cases is characterized by a prolonged intraepithelial preinvasive stage, which allows for timely intervention if there is adequate access to screening programs. Sensitivity is also augmented if the test is repeated regularly at appropriate screening intervals. Unfortunately, half of the cases of invasive cervical cancer in the US are diagnosed in women who have not had a recent Pap smear. Kinney and colleagues have shown that even among patients with access to a prepaid health plan, 60% of cervical cancer cases occur in patients who have had interaction with the health care system within the 3 years immediately preceding diagnosis but they did not get screened [7]. The success of the entire cytologic screening process depends

upon optimal linkage of its various interrelated components (ie, initial sampling utilizing proper technique, sample transport, preservation, preparation, interpretation, reporting back to the healthcare provider, and identification of the patient as a candidate for further diagnostic procedures).

In managing the patient who exhibits abnormal cytology, the clinician must keep in mind that in spite of its proven value, cytology is not a diagnostic tool, but rather a screening test designed to identify women who are at risk and require additional diagnostic procedures. Conceptually, repeating an abnormal cytology to determine the need for further diagnostic studies assigns diagnostic capabilities to a screening test known for its less than optimal sensitivity. This further introduces concerns about its scientific appropriateness and raises potential liability issues if significant disease is missed. The combined published data considered at the TBS 2001 workshop showed that the sensitivity of repeat cytology is no more than 67% to 85% and might therefore unacceptably delay timely intervention or result in the loss to follow-up of patients who have been identified as being "at-risk" by an abnormal cytologic screen [8].

Based upon their morphological, viral, and biological similarities, TBS groups together into the low grade squamous intraepithelial lesion (LSIL) category cytologic smears that show HPV-related cytopathic changes (koilocy-

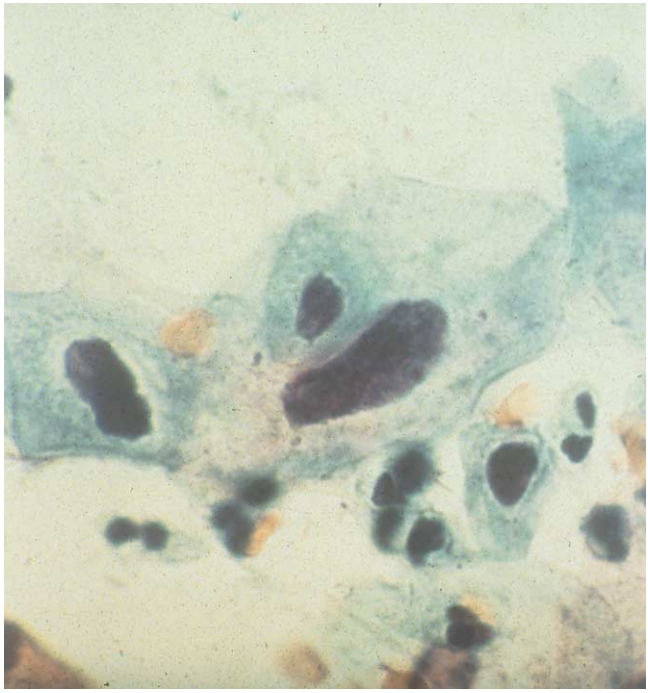

Fig. 1. Cytology of ASCUS. This equivocal (ASCUS) cytologic smear shows rare abnormal intermediate cells that contain deeply hyperchromatic nuclei, suggesting possible HSIL (ASC-H under TBS 2001). (*Courtesy of* A. Ferenczy).

tosis) and smears that show cervical intraepithelial neoplasia (CIN) 1 (mild dysplasia) [6]. Lonky et al have raised the concern that this grouping might result in the delayed diagnosis of high-grade lesions because the prevalence of significant disease has been shown to be higher among patients showing LSIL cytology suggestive of CIN 1 when compared with patients showing only koilocytotic atypia [9]. It has also been shown that minimally abnormal cytology can herald the presence of significant cervical cancer precursor lesions or even invasive cancer (Figs. 1–3). In the study published by Kinney and Manos, histologically-proven high-grade dysplasia was found to be preceded by minimally abnormal atypical squamous cells of undetermined significance (ASC-US) cytology in 39% of patients, by atypical glandular cells of undetermined significance (AGUS) cytology in 10%, and by LSIL in 20%. In only 31% of their cases did high grade squamous intra epithelial lesion (HSIL) cytology correctly predict a high-grade precursor lesion [10].

Today it is widely accepted that 5% to 15% of patients who exhibit an abnormal cytologic screen classified as ASC-US (Bethesda 2001 classification), and about 15% to 18% of women with cytology suggestive of LSIL will harbor high-grade

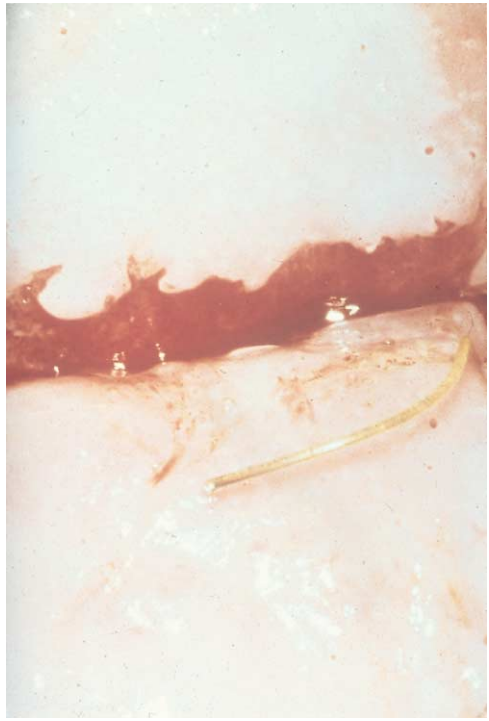

Fig. 2. Colposcopic examination of the same patient shown in Fig. 1. After application of 5% acetic acid, note sharply demarcated, raised, acetowhite epithelium on anterior cervical lip consistent with high-grade SIL (CIN 3). An IUD string is visible on the posterior lip near the external os. (*Courtesy of* A. Ferenczy).

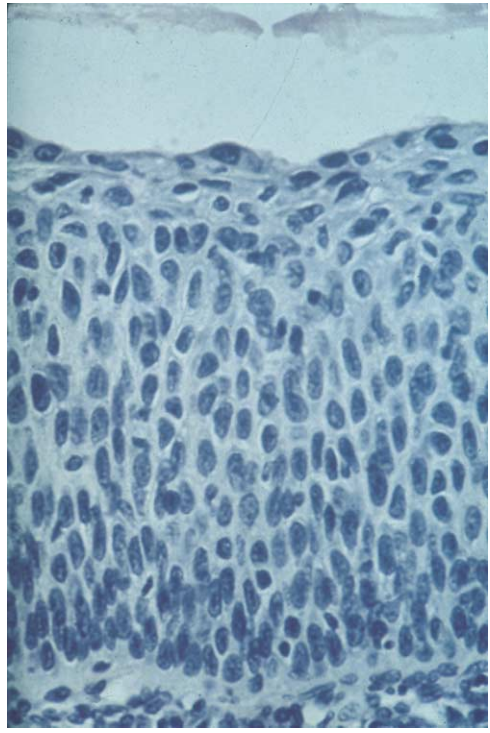

Fig. 3. Histology of the lesion corresponding to the images seen in Figs. 1 and 2. Note the full-thickness involvement of epithelium by neoplastic basal–parabasal cells. Histologic diagnosis is CIN 3. (*Courtesy of* A. Ferenczy).

precursor lesions or even frank carcinoma [8,11]. It should therefore not be assumed that patients with minor or equivocal cytological atypia harbor nothing more than lesions of equivalent or correspondingly minor histologic severity (Figs. 4–6). Although repeat cytology might still be a valid option in some clinical situations, it is generally anticipated that the 2001 consensus conferences will recommend either immediate colposcopy or triage utilizing HPV DNA testing as the preferred response to equivocal cytology. Immediate colposcopy is definitely warranted whenever screening cytology reveals a high-grade lesion or the presence of glandular cytologic atypia [8,12,13].

The role of colposcopy and directed biopsy

The vast majority of physicians would also agree that colposcopy is the optimal and most appropriate initial response in the evaluation of the ''at-risk,'' screen-positive patient. It allows an experienced examiner to determine the size and distribution of any lesions present and arrive at a clinical impression as to their significance while assessing the risk of stromal invasion. This determination is of

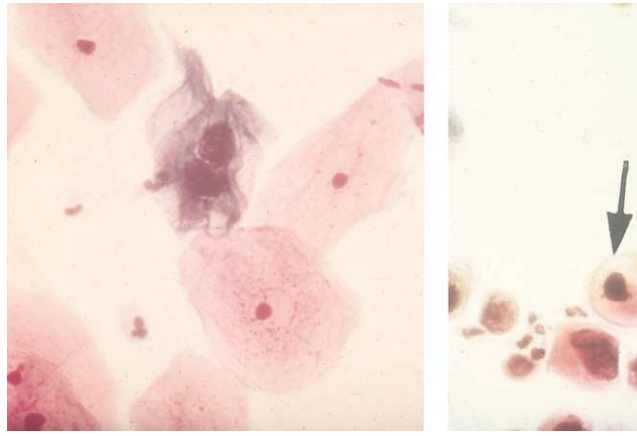

Fig. 4. Cytology of LSIL. Note on the left two overlapping intermediate cells with large, hyperchromatic nuclei and abundant cytoplasm, consistent with the cytologic diagnosis of LSIL. On the right there is a single suspicious parakeratotic cell (*arrow*) associated with several neoplastic-appearing parabasal cells. Because of their scarcity on the slide, a definitive cytologic diagnosis of HSIL cannot be made. Colposcopy was nevertheless recommended. (*Courtesy of* A. Ferenczy).

critical importance in the selection of any subsequent diagnostic or treatment modality. Colposcopy permits the identification and histologic sampling of the most clinically significant areas of an identified lesion by allowing directed rather than random biopsy, thus enhancing the accuracy of the triage of the patient at risk by providing an objective histopathologic diagnosis. The disadvantages of offering colposcopy to all women with an abnormal screening are (1) its relative cost when compared with some other modalities, (2) patient discomfort, (3) the anxiety understandably generated by referral for the procedure, and (4) the concern that even in experienced hands colposcopy might lead to overdiagnosis and overtreatment of nonsignificant disease with low malignant potential. Despite these potential disadvantages, cost analysis models have shown that immediate colposcopy is more accurate and cost effective than a program of repeat cytology with colposcopy, which is reserved only for patients with persistent cytologic abnormalities [14].

The success of colposcopic triage is entirely dependent upon the skill and experience of the colposcopist. Even in the presence of adequate colposcopic expertise, it is often impossible to correctly identify and detect areas of early stromal invasion [15]. Furthermore, despite the opinion of some respected colposcopists [16], the majority of expert colposcopists feel uncomfortable predicting the absence of disease in the columnar epithelium of the cervical transformation zone without histologic confirmation. Occult adenocarcinoma might not be visualized, or it might be present yet evade colposcopic diagnosis because adenocarcinomas do not always exhibit the characteristic colposcopic features that serve as markers to identify squamous lesions. For these reasons, many experts recommend the liberal use of directed biopsy rather than only one or two ‘‘representative’’ biopsies.

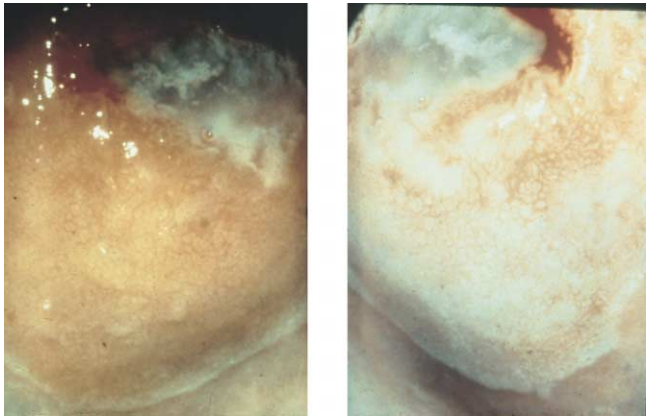

Fig. 5. Colposcopic examination of the patient shown in Fig. 4. The cervix is seen on the left prior to the application of 5% acetic acid solution. On the right, an abnormal transformation zone is visible, showing a dull acetowhite lesion with sharp margins and a highly irregular, coarse, mosaic vascular pattern. This appearance is consistent with the colposcopic diagnosis of CIN 3. (*Courtesy of* A. Ferenczy).

Other clinicians advise endocervical curettage in all nonpregnant women at the time of colposcopic examination, although this is controversial [17]. Despite its unquestioned value, colposcopy is not a diagnostic technique, but a valuable part of triage of the patient identified as being at risk. Histology is still the gold standard and the definitive diagnostic test, although the spectrum of progression from normal histology to cancer requires arbitrary subdivisions of changes upon which pathologists cannot always reproducibly agree.

The role of HPV DNA testing

At present, the association between high-risk oncogenic types of HPV and the development of cervical cancer and its precursor lesions is well established [18,19]. Consequently, the development of sensitive, highly reliable molecular hybridization technology for the detection of HPV DNA has been well received by the medical community. The only commercially available FDA-approved HPV DNA test (Hybrid Capture II, Digene Corporation, Gaithersburg, MD) is based upon microplate technology. With built-in amplification, it provides RNA probes for the most commonly found low- and high-risk HPV types (6, 11, 42, 43, 44 and 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, respectively). It is ten times as sensitive as its tube-based predecessor, and it can detect HPV DNA levels as low as 1.0 pg/mL. Receiver operating characteristic analysis of the Hybrid Capture II test at this threshold has shown an adequate balance between sensitivity and specificity for the detection of high-grade disease and cancer [20].

The initial enthusiasm generated by the ready availability of reliable testing for HPV DNA has been tempered by data documenting that both low- and high-risk HPV types are prevalent in the general population among women with normal or

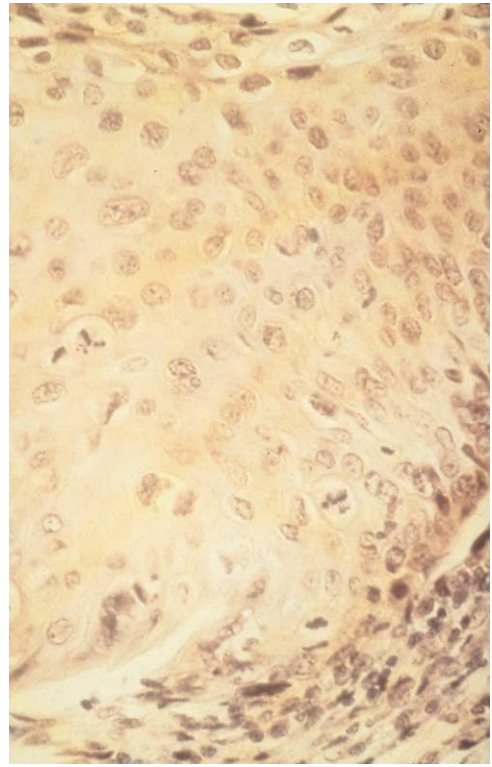

Fig. 6. Histology of the lesion corresponding to the images seen in Figs. 4 and 5. Note that the entire epithelium is involved by neoplastic basal/parabasal cells with irregular atypical nuclei exhibiting abnormal mitotic figures *(middle left and lower right of photograph*). The histologic diagnosis is CIN 3. (*Courtesy of* A. Ferenczy).

abnormal cytology [21]. A positive HPV DNA test result is especially prevalent among young women, reaching a peak around the ages of 20 to 24. Prevalence gradually declines with age until age 40 to 45, while a secondary peak has been observed around age 55 or older [22]. The vast majority of HPV infections are transient, with a median clearance time of 8 months; even infections with high-risk HPV types are cleared in about 12 to 13 months [23]. Only about 10% to 20% of HPV infections persist and have the potential to produce true cancer precursor lesions [24]. Because of its high prevalence, mass screening for HPV DNA among the general population appears to have little or no value, especially in women under the age of 30, although perhaps it could be useful in patients older than age 30. The test appears to be of value in the triage of women who exhibit equivocal or minimal squamous cytologic abnormalities, however (ASC-US).

Preliminary data from the ongoing ALTS trial (ASCUS/LSIL Triage study, National Cancer Institute) suggests that HPV DNA testing can efficiently identify women who harbor underlying high-grade cervical cancer precursor lesions (CIN

2+) among patients with equivocal or minimally abnormal cytology [25]. Kinney and Manos have shown that the largest proportion of high-grade cancer precursor lesions is found among patients who exhibit minimally abnormal cytology (ASC-US) [10]. To provide maximum patient protection and minimize the possibility of missing a significant lesion, routine, immediate colposcopy of all women with ASC-US cytology has been proposed and is actually practiced in many institutions and screening centers. The increasing frequency with which clinicians encounter minimally abnormal or equivocal cytology and the lack of universal access to expert colposcopic resources make the colposcopic examination of such patients either economically or otherwise impractical in many clinical settings, however.

Several investigators have studied the potential value of self-collected vaginal samples for the detection of HPV DNA [26,27]. In preliminary studies, the sensitivity of patient-obtained samples for the detection of high-grade precursor lesions (CIN 2–3) and invasive cancers compared favorably with samples obtained by healthcare practitioners, although self-testing appears to be somewhat less specific. If validated by further studies, this technique could be utilized among at-risk populations in underdeveloped countries or in other clinical settings with limited access to trained health care providers. Testing for high-risk HPV DNA types appears to be a more sensitive indicator than cytology for the detection of high-grade cervical disease. Because of its low specificity, however, especially in young women, it cannot be recommended as an effective primary screening tool. Nevertheless, this test might yet prove to be a valuable adjunct as a prognosticator of future risk, as a means of safely increasing cytologic screening intervals, and perhaps in the evaluation of postmenopausal women with equivocal cytology.

Liquid-based cytology and reflex HPV DNA testing

Fluid-based monolayer cytology has become available during the past decade and offers several advantages over the conventional glass slide technique [28]. Several studies have consistently demonstrated that fluid-based cytology improves overall specimen adequacy, results in fewer equivocal or unsatisfactory smears, and increases the detection of both low- and high-grade cervical disease [29–34]. The improved specimen quality decreases the need to recall patients for retesting, and the technique offers an opportunity to test residual sampling material for HPV DNA and other pathogens such as *C. trachomatis*. Despite these apparent advantages over conventional cytology, the managed care establishment has been reluctant to adopt liquid-based cytology because of cost considerations and concerns that increased detection of nonsignificant disease with low potential for malignancy might lead to unnecessary treatment.

The need for patient recall to obtain a repeat cytologic sample reduces cost efficiency and raises the possibility of less than optimal patient compliance with potential loss to follow-up. The use of liquid-based monolayer cytology allows HPV DNA testing of residual material for up to 3 weeks after collection if the sample is classified as equivocal (ASC-US). By using liquid-based monolayer

cytology, Manos and Kinney have proposed an algorithm based upon "reflex" HPV DNA testing of the residual material of equivocal ASC-US samples with immediate referral to colposcopy of HPV-positive cases and repeat cytology/HPV testing in 6 months for HPV-negative patients [35]. The authors calculate that this algorithm offers an overall sensitivity of 96.9% for the detection of significant disease (CIN 2+) among this population and it will result in an equal number of patients from either group (39%) referred to colposcopy. Polymerase chain reaction is the investigational laboratory gold standard for HPV detection, but it is currently not widely applicable in clinical settings. Further studies, with a reduction in the incidence of cervical cancer as an endpoint, are needed to establish how HPV DNA testing can be optimally integrated into screening methodologies [24].

Response to abnormal glandular cytology

TBS replaced the previous Papanicolaou category of "glandular cell atypia" with "atypical glandular cells of undetermined significance" (AGUS). AGUS includes cellular changes that are more severe than those suggestive of a reactive process, but they are still not sufficient for the diagnosis of frank adenocarcinoma. AGUS is an uncommon diagnosis, and from its inception the response to this cytologic category has been anything but uniform. While the term seems to indicate minimal risk of significant disease, past experience has demonstrated that about 39.6% of such cases harbor squamous interepithelial lesion (SIL), most commonly high-grade [36], 5.8% show adenocarcinoma in situ (AIS), and 5.8% are found to represent frank carcinoma on follow-up [37]. The data discussed in the ASCCP Forum preceding the 2001 ASCCP Consensus Conference showed that SILs are present in 9.1% to 54.3% of women with cytologic diagnoses of AGUS. AIS was found in 0% to 7.9% of cases, and from less than 1.0% to as many as 9.4% showed invasive squamous carcinomas or adenocarcinomas [8]. Based upon this and additional data [12,13], it is recommended that patients with atypical glandular cervical cytology undergo colposcopy and endocervical sampling with endometrial sampling when appropriate rather than repeat cytology. Postmenopausal patients and patients with other additional cytologic risk indicators (such as atypical cells suggestive of an extrauterine origin) should undergo further appropriate diagnostic procedures to identify the source.

Other adjuncts

Cervicography (National Testing Laboratories, 3460 Whitby Lane, High Ridge, MO) consists of high-resolution photography of the cervix under magnification using a specially designed camera (cerviscope) following application of acetic acid. The resulting cervigrams are sent to a processing center and reviewed by an expert consultant who makes recommendations for further diagnostic steps if needed. This technique has shown better sensitivity than cytology for the detection of CIN, but it has not been widely accepted because of its low specificity,

increased cost, and unnecessary colposcopy referrals for patients without significant disease [38]. The inherent delay in management and the patient anxiety caused by the need to send the cervigrams to a distant site for review are unavoidable.

A direct tissue contact technique that utilizes optical and dielectric biophysical tissue parameters and a computer algorithm to determine the "signature" of the cervical epithelium and predict the likelihood of a cancer precursor lesion is currently undergoing clinical trials [39]. Initial experience with the device has shown concordance with histology in 85% of low-grade dysplasias, 90% of high-grade dysplasias, and 99% of invasive cancers [40]. This technology offers promise as an adjunct to the Pap smear or, potentially, as an alternative to cytologic screening in specific settings.

Speculoscopy is another FDA-approved adjunct performed in conjunction with Pap smear screening that combines the effect of low-power magnification and chemiluminescent light in a darkened room to detect characteristic changes in the cervix following the application of acetic acid. This technique is easily learned and has demonstrated increased sensitivity over cytology alone (82%) and offers a negative predictive value of more than 99% when cytology and speculoscopy show no evidence of abnormal lesions. Abnormal speculoscopy correlates well with subsequent abnormal colposcopic findings, with a positive predictive value of 97% [41]. It might have potential cost benefits when applied in combination with conventional cytology by allowing increased screening intervals in patients enrolled in cancer detection programs. Direct visual inspection (DVI) of the cervix following application of acetic acid is not truly an adjunct to cytologic screening, but rather a simple, relatively crude, moderately sensitive screening tool that is hindered by its low specificity. DVI seems to have proven value in settings with limited health care resources, however [42,43].

Automation in cytologic screening is increasingly gaining acceptance [28]. Sophisticated algorithms now allow automated primary and secondary screening of both conventional and fluid-based cytology with reproducible results, although it is anticipated that this technology will be applied only to liquid-based samples in the future. Automated cytology removes all subjectivity from its interpretation and is not limited by human workload guidelines, reducing the need for training large numbers of cytotechnologists. It can reduce the rate of false-positive cytology results, but some of its critics believe that its cost could be greater than that of establishing laboratory quality control programs based on review of samples by a second cytotechnologist. While this concern might prove to be false, there is additional concern that automation could eventually limit cytology to a small number of large, national laboratories with the financial resources to establish automation, yet its overall long-term effect of reducing the incidence and mortality from cervical cancer remains to be demonstrated. Neither liquid-based nor automated improvements address inadequate sampling, which is thought to be the largest component of inaccurate results.

Finally, laser-induced fluorescence and broadband reflectance spectroscopy are promising new technologies in which directed incident light induces measurable tissue signatures that are specifically associated with the biochemical and mor-

phological epithelial changes of cervical cancer precursor lesions [44–46]. A similar fluorescence-based imaging system has received FDA approval as an adjunct to white light bronchoscopy for the detection of lung cancer. In future clinical applications, optical detection systems could allow immediate, quantitative, noninvasive assessments for the detection of premalignant or malignant lesions of the cervix.

The future: molecular, genetic, and biochemical markers

The current understanding of the complex molecular, genetic, and biochemical alterations necessary to allow the transformation of normal cervical epithelium into invasive cancer increases exponentially on an almost daily basis. Previously seldom-utilized terms such as loss of heterozygosity (LOH), genomic instability, cellular proteins p53, pRb, and p16, gene deletions, chromosomal alterations, nuclear matrix proteins, and other molecular and biochemical markers, might become part of the daily vocabulary of the next generation of health care practitioners involved in screening for cervical cancer and its precursor lesions.

Determination of DNA instability by immunohistochemical staining with DNA antibody is being studied as a marker for progression toward malignancy among cervical cancer precursor lesions [47,48]. Although the genetic basis for the transformation of cancer precursors into invasive malignancy is not clearly understood, LOH manifested by specific allelic gene losses and microsatellite instability has been observed in a significant number of these cases [49]. These genetic markers might in the future help define lesions that are at high risk for progression into invasive cancer.

A total of eleven human mucin genes (MUC) have been identified so far. They are numbered in the chronological order of their initial description. MUC4 is a human mucin gene sequence expressed by the endocervical epithelium that is believed to play a protective role and perhaps be involved in intracellular signaling. It is strongly detected in intraepithelial neoplastic cervical epithelia, suggesting that it is activated during dysplastic transformation, and it might become a useful marker for this process in the future [50]. Other markers for cellular proliferative activity such as proliferating cell nuclear antigen and mitotic index have shown correlation with the transformation of low-grade lesions into CIN 3 and cervical carcinoma, and they could help predict the behavior of these precursor lesions [51,52].

The potential value of HPV E6/E7 mRNA as a marker in the screening and early detection of cervical neoplasia is also currently under study (R.M. Richart, personal communication, 2002). In addition, the cyclin-dependent kinase cellular inhibitor protein p16, which is not found in normal squamous cervical epithelium, is overexpressed as a consequence of the binding of pRB by HPV E7 oncoproteins during the process of dysplastic transformation. The utilization of monoclonal antibody assays against p16 to identify dysplastic cells in cervical smears and histologic samples could perhaps lead to the development of an entirely new class

of sensitive, specific, and cost efficient tests for the early detection of cervical cancer and its precursors [53–55].

Summary

At this time little, if any, of the vast knowledge base being generated by current research is available for practical application within screening programs, although the path is opening for the development of future methodologies that might someday lead to the successful eradication of cervical cancer. Current efforts to develop a vaccine against HPV are rapidly progressing and are promising. In the meantime, a better understanding and utilization of the technologies at hand will result in a successful, organized approach to the detection of early cervical cancer and its precursors [56]. The adoption of any new screening technologies must result in a documented reduction in the incidence of cervical cancer. Until then, colposcopy and HPV DNA testing when indicated appears to remain as the undisputed gold standard in the evaluation of the patient at risk.

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Management of precursor lesions of cervical carcinoma: history, host defense, and a survey of modalities

J. Thomas Cox, MD

Gynecology Clinic, Health Services, University of California, Santa Barbara, CA 93106, USA

The ability to identify the precursor lesion to invasive cervical cancer by evaluation of cervical cell changes on the Papanicolaou (Pap) smear provided the opportunity to alter the natural history of a lesion, that for some, would lead to unalterable consequences. The first description of the existence of a precursor lesion to cervical cancer occurred in Sir John Williams's presentation in 1886 on the identification of a case of very early ''cervical cancer''. This was later referred to as carcinoma in situ (CIS), and even later, as cervical intraepithelial neoplasia grade 3 [1]. Before the instigation of widespread, cervical cytologic screening, invasive cervical cancer was the first or second most common cancer among women, often striking at the age of greatest contribution to family and society. To this day, the majority of women live in areas that are not privileged to have the resources for preventative cervical screening, and, therefore, suffer the consequence [2]. Although cervical cancer incidence and mortality had begun to fall in the United States before the onset of cervical screening, few question that early detection and treatment of significant cervical cancer precursors has been primarily responsible for the decrease in cervical cancer incidence from second to ninth among cancers in women in this country [3,4]. An analysis of five, long-term studies in the follow-up of conservative treatment of cervical intraepithelial neoplasia (CIN), also known as squamous intraepithelial lesion, demonstrated a reduction in the risk of invasive cervical cancer by 95% for at least 8 years [5]. Estimation of the exact contribution of screening and treatment to these reductions has been hampered by lack of comprehensive, accurate statistics during the era before the introduction of screening [6]. Despite this problem, the best evidence indicated that the number of cervical cancers that occurred in the United States during the year (1943) that Papanicolaou and Traut published their monograph on the microscopic examination of cells exfoliated from the cervix, would be equivalent to 50,000 cases if it were based upon 1996 U.S. population statistics

E-mail address: cox-t@ucsb.edu

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[7]. In 1950, 8000 women died of cervical cancer [8], which would be equivalent to 16,000 to 20,000 cases today [6]. Certainly the dramatic 75% reduction in incidence and 74% decrease in mortality could not have occurred to this extent in the absence of screening and treatment of precancerous cervical lesions [6,9].

During the first 3 decades following the introduction of cervical screening, treatment approaches were limited by lack of knowledge of the natural history of cervical precursor lesions, and by the absence of a relatively nontraumatic procedure for evaluation of women with abnormal cervical cytology. Although the first description of the koilocyte was by Koss and Durfee in 1956 [10], it was not until 1976 that the etiology of these "balloon" cells was clearly established by Meisels and Fortein [11] to be human papillomavirus [11]. HPV was not proclaimed to be the primary, and perhaps necessary, agent in the etiology of cervical cancer until 1995 [12]. The introduction of colposcopy and new surgical treatment options beginning in the 1960s dramatically altered the incidence of cervical cancer by interceding with the potential for progression of true cervical cancer precursors. The first of the new treatment options was cryotherapy, which was followed in the 1970s by carbon dioxide laser, and in the late 1980s by loop electrosurgical excision procedure (LEEP). Increased understanding of the high prevalence of HPV and associated low-grade cervical lesions, and the relatively low risk for progression to cervical intraepithelial neoplasia, grade 3 and cancer (CIN 3+) has made treatment guidelines for low-grade disease less clear.

Although the primary approach to any documented cervical cancer precursor has traditionally been surgical, an increasing trend to follow women with low-grade cervical intraepithelial neoplasia, grade 1 (CIN 1) and to treat only high-grade cervical intraepithelial neoplasia, grade 2 and 3 (CIN 2, CIN 3, CIS) has taken hold over the last decade in the absence of clear guidelines for management. This changed in September 2001 when guidelines for the management of abnormal cervical cytology and cervical cancer precursors were developed at a conference in Bethesda, Maryland that was sponsored by the American Society for Colposcopy and Cervical Pathology (ASCCP). The conference was attended by representatives from 29 participating professional organizations, federal agencies, and national and international health organizations [13]. These guidelines will serve as a basis for discussion in this article of the management of women with abnormal cervical cytology, with and without a documented cervical cancer precursor lesion. The guidelines were consensus and evidence-based to the limit of the available literature. Guidelines are always in evolution, however, and the horizon is bright with potential for moving away from primary surgical treatments to more rational approaches that focus on the interruption of the viral life-cycle or on enhancement of the host immune response, or both.

Management of cervical cancer precursors before colposcopy

Before the introduction of cervical screening with the Pap smear, cervical neoplasia was not diagnosed until the woman was symptomatic with bleeding

or pain that was secondary to invasion. The inability to diagnose precancerous lesions precluded any possibility of interrupting the natural history of progressive precursor lesions before invasion. The road to identification and treatment of cervical cancer precursors began in 1926 simultaneously in two countries; a Romanian named Dr. A. Babes, and a Greek, George Papanicolaou, introduced the concept of vaginal cytologic sampling as a means for detecting cervical cancer. Despite the publication of the work of Babes in the French literature in 1928 [14] and the presentation in the same year by Papanicolaou of his data on vaginal smears [15], it was not until 1943 that publication of Papanicolaou and Traut's "Diagnosis of Uterine Cancer by the Vaginal Smear" [16] introduced the concept of screening for cervical neoplasia by cervical cytology to the medical community in the United States. During the 1930s and 1940s the development of two other methods of diagnosis, colposcopy and Schiller's staining, had profound influence over treatment options. These methods were developed simultaneously with cervical cytology and initially were used widely only in Europe and in some areas of South America.

Hans Hinselmann developed colposcopy in the early 1900s in Germany; as with the early years of cervical cytologic screening, the procedure was initially intended only to detect invasive cervical cancer in its earliest stage [17]. Hinselmann soon realized that the colposcopic characteristics of intraepithelial neoplasia were different from those of invasive cancer [18]. Unfortunately, colposcopy was not introduced to the United States until the 1960s [19]. This delay created a vacuum in precancer diagnostics that was filled less than adequately by the use of Schiller's test.

Schiller documented that the absence of glycogen in squamous carcinomas was in contrast to the glycogen that was found in normal, estrogenized cervical and vaginal mucosa [19]. With the use of an iodine stain, later termed Schiller's solution, he discovered that normal cells stained dark brown, whereas columnar epithelium did not stain, and neoplastic epithelium took on a mustard-yellow color (Fig. 1). Schiller immigrated to the United States in 1932. The Schiller's iodine test was too nonspecific for primary screening of the cervix because many nonneoplastic conditions did not stain brown. With the advent of cervical cytology screening in the United States in the late 1940s, however, Schiller's testing was often used to delineate an area of the cervix to be excised following high-grade abnormal cytology.

Electrocauterization, or "hot cautery" of postdelivery cervical eversions became common practice during the 1930s and 1940s in the belief that eradicating postpartum cervical "erosions" would prevent cervical cancer from developing. Subsequent follow-up over the ensuing decades documented that women treated by this method had a significantly reduced risk of cervical cancer, which is likely to have occurred because of the destruction of the "area-at-risk", the transformation zone [20,21]. The procedure fell out of favor, however, as women complained of considerable discomfort and the foul, odorous discharge that often lasted for several weeks [21]. Some clinicians used this procedure in the United States until the early 1970s for the treatment of CIN [22]; "hot

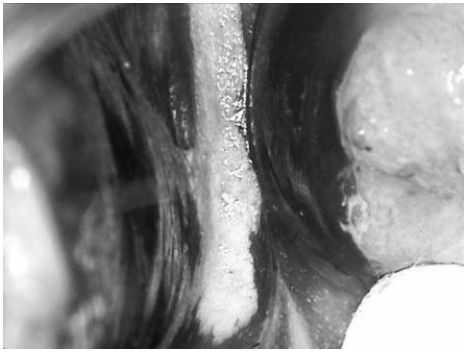

Fig. 1. This 25-year-old referred for the colposcopic evaluation of an LSIL Pap smear had a normal cervix on colposcopy. Staining the vagina with Schiller's solution located the source of the abnormal cells in a sharply circumscribed, nonstaining area in the right vaginal fornix. Many noncorrelating Pap smears, particularly LSIL, will have lesions in the vagina that confirm that the source of the abnormal cells are not cervical in origin. The most important use for Schiller's staining is in the location of vaginal HPV-induced lesions. The cervix can be seen on the right and stains entirely normally right up to the squamocolumnar junction. The elongated nonstaining area to the left of the cervix was biopsied and the histology was interpreted as a low-grade lesion.

cautery'' continues to be used in Europe and many other areas of the world for this purpose [23].

During the early decades that followed the recognition of an intraepithelial phase for cervical cancer, the absence of a clear understanding of the differences in risk between the intraepithelial nature of the precursor process and frank invasion led to similar treatment for both. Hence, treatment of intraepithelial neoplasia consisted primarily of radical hysterectomy and was reserved for very high-grade lesions [21]. In 1949, Galvin and Telinde [24] were widely criticized for suggesting that this radical procedure be modified for young women by only removing 2 centimeters on each side of the parametrium, preserving one ovary, and eliminating lymphadenectomy. Three years later, Graham and Meigs [25] reported that a simple hysterectomy was adequate for treatment of intraepithelial neoplasia.

Beginning in the early 1950s, cold knife conization gradually replaced hysterectomy for the treatment of severe dysplasia, but not for carcinoma in situ [26]. Cold knife ''cone'' was first performed by Lisfranc in 1816 [27] long before CIN was described; during the first half of the twentieth century the procedure was primarily reserved for the treatment of cervicitis and ectropion [21]. Even when cold knife cone became established for treatment of severe dysplasia in the 1950s, it was often considered a temporary option to be followed by hysterectomy when child-bearing was over. The real breakthrough in diminishment of this radical approach to treatment of precursor lesions did not occur until the advent of colposcopy in the United States in the 1960s.

The advent of cervical cytology screening in the United States: impact on diagnosis and treatment

From the late 1940s until the introduction of new cytologic terminology by the 1988 Bethesda System, cytologic interpretations were reported in the classification system that was developed by Papanicolaou and others. The abnormal interpretations in the Papanicolauo Classification were designated Class II, for cellular changes considered to be reflective of inflammation, repair, nonspecific atypia, or specific infection (including human papillomavirus), Class III for cellular changes that were considered to be mildly or moderately abnormal, Class IV for severe dysplasia or "carcinoma in situ", and Class V if cancer was suspected [28]. Until the late 1960s, women with cytology that was read as Class II or III were managed by repeating the Pap smear in 6 to 12 months; the only other options before the introduction of colposcopy was to either randomly biopsy Schiller's nonstaining areas or to completely excise these areas by conization [28]. Because "random" biopsies were not particularly accurate, and conization was too drastic for "minor" cytologic changes, cytologic follow-up was the only reasonable alternative. Conization was reserved for diagnosis and treatment of high-grade Pap smears. The result was underevaluation and undertreatment for the 10% to 30% of women with Class II and III smears who had high-grade disease [28,29]. An occasional invasive cervical cancer developed or was not detected because of the loss to follow-up over the "standard-of-care" two normal follow-up Pap smears [30].

Additionally, some women with misclassified Class IV or V smears were overtreated by cervical conization [28]. Others suffered the misfortune of a lack of understanding of the similar risk presented by the diagnosis of severe dysplasia and carcinoma in situ. Women with any grade of dysplasia detected in the cone specimen were considered "cured" and were returned to routine screening, whereas women with CIS were considered to be at continued risk for invasive cervical cancer and hysterectomy was recommended even if the woman had not completed childbearing. Therefore, the absence of an effective, minimally invasive method to more completely evaluate the cervix following high-grade cytology led to either conization or hysterectomy. Evaluation and treatment were often synonymous and women were at-risk for significant complications, including bleeding, infection, cervical stenosis, cervical incompetence, and interruption of the potential for childbearing.

Treatment of precursor lesions following the introduction of colposcopy

Although colposcopy was introduced to the US in the early 1960s, widespread training in colposcopy did not bring it into common use until the mid to late 1970s. Soon thereafter, the dichotomy of treating women with severe dysplasia by conization and women with CIS by hysterectomy fell into disfavor. The recognition of the essentially identical natural history of these artificial "subdivisions",

and of the imprecision in the histologic differentiation, clarified the impropriety of the approach [31]. Colposcopy provided the means for detecting whether a lesion was present and, if so, its location and degree of severity. It also led to an increased understanding of the natural history of precursor lesions. Now that these lesions could be identified, it was no longer necessary to remove a large portion of the cervix to identify the source and validity of a Pap smear interpretation.

Increased diagnostic capability promoted in-office treatment methods, such as cryotherapy, laser ablation, and loop electrosurgical excision. Cold knife conization, the mainstay of diagnosis and treatment for more than 30 years, was largely supplanted by these procedures [32–35]. By the late 1980s an evolving understanding of the association of human papillomavirus with cervical precancer and cancer, the increased availability of colposcopy and of conservative outpatient treatment methods, and the advent of a new cytology classification system dramatically changed the management of cervical preinvasive disease. With the arrival of the new century has come evaluations of new treatment modalities that use improved understanding of the host immune response to HPV. To understand optimal treatment of patients with CIN, it is most important to understand the host immune response to HPV.

Treatment of cervical precursor lesions

The role of the host immune response

Understanding of the immune response to HPV is critical in determining the optimal treatment strategies for CIN. Successful treatment of cervical precursor lesions ultimately depends upon the success of the host immune response in preventing recurrence; individuals with compromised immunity have a very high rate of recurrence following any treatment modality [36]. The primary immune response to an established HPV-infection is cellular but humoral immunity is likely to play a significant role in the prevention of infection. In the normal cervix, the lymphocyte population is mostly B-lymphocytes that are capable of mounting a humoral immune response as the first line of defense against initial infection (Fig. 2) [37]. In contrast, B-lymphocytes become a minor part of the population of immune responsive cells in the presence of CIN 3 where the primary cells of cellular immunity, natural killer cells (NKCs) and cytotoxic T lymphocytes, predominate [37].

The host immune response to most viral infections is usually quite rapid. Quick identification of a viral invader will typically activate antigen-presenting cells and release local cytokines within 24 to 48 hours of the detection of virus. Within 3 to 5 days of viral infection, activated T cells migrate to regional lymph nodes where antigen-specific cytotoxic T-lymphocytes are created (Fig. 3) [38]. Antigen-specific CD4 cytotoxic T-lymphocytes produce antibodies, whereas antigen-specific CD8 cytotoxic T-lymphocytes return to the site of the infection within 5 to 7 days postinfection to mount a cellular immune response. This

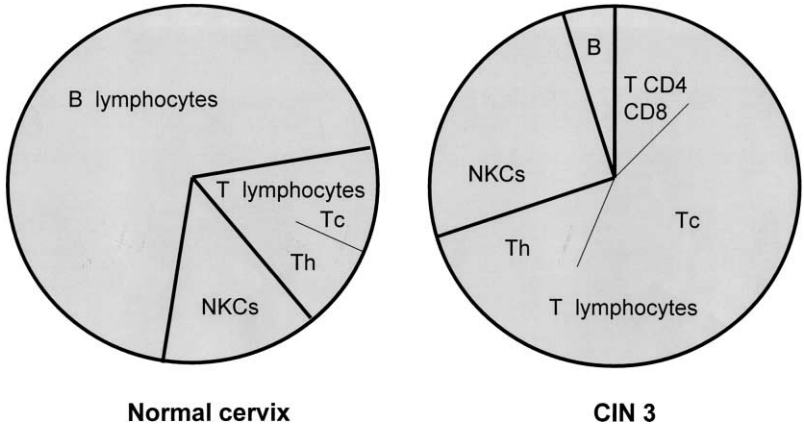

Fig. 2. The lymphocytic population of the cervix changes dramatically in the presence of cervical intraepithelial neoplasia. B cells predominate in the normal cervix which supports the theory that an antibody response is the first line of defense to infection. As an indication of the relative lack of importance of local cellular immunity in the absence of disease, T cells and natural killer cells comprise only slightly more than 25% of the lymphocytic population in the normal cervix. In contrast, in women with CIN-3, T cells comprise almost three quarters of the immune-responsive cells in the cervix, natural killer cells comprise almost one quarter, and B cells comprise only a very small percentage. This illustrates the importance of local cellular immunity in fighting disease once established, and of antibody-producing B cells in providing defense when no disease is present. (Courtesy of P.A. Crowley-Nowick, MD, Boston Massachusetts)

immune response clears infection and provides immunity to reinfection by way of retention of T-helper and cytotoxic T-lymphocyte memory of the viral antigen. Unfortunately, detection of HPV is typically much slower and, hence, the immune response to HPV is usually delayed considerably in comparison with other viral infections.

There are several reasons why HPV is so elusive. Because cervical precursor lesions do not penetrate below the basement membrane, the primary exposure of HPV in the absence of invasion is to the epithelial host-defense mechanisms. Although the epithelium is a good primary barrier to infection, any breach in the epithelium facilitates HPV infection of the basal epithelium where there is little viral replication and no cell lysis [39]. Therefore there is little antigen available to be detected by the immune surveillance of the host. After a period of latency, when a lesion occurs, accelerated viral DNA replication in differentiating cells begins. These differentiating squamous epithelial cells, or keratinocytes, have little antigen-presenting capability despite viral replication that results initially in 25 to 50 viral genomes per cell [39]. As the keratinocytes mature in the upper layers of the epithelium, viral assembly of a protein capsule surrounds the DNA core; this creates an infective unit that is shed from the surface in the dead or dying cells [40]. During this entire process the HPV-infected cells remain intact, as HPV does not kill or lyse the cells. Therefore, the entire HPV life cycle occurs

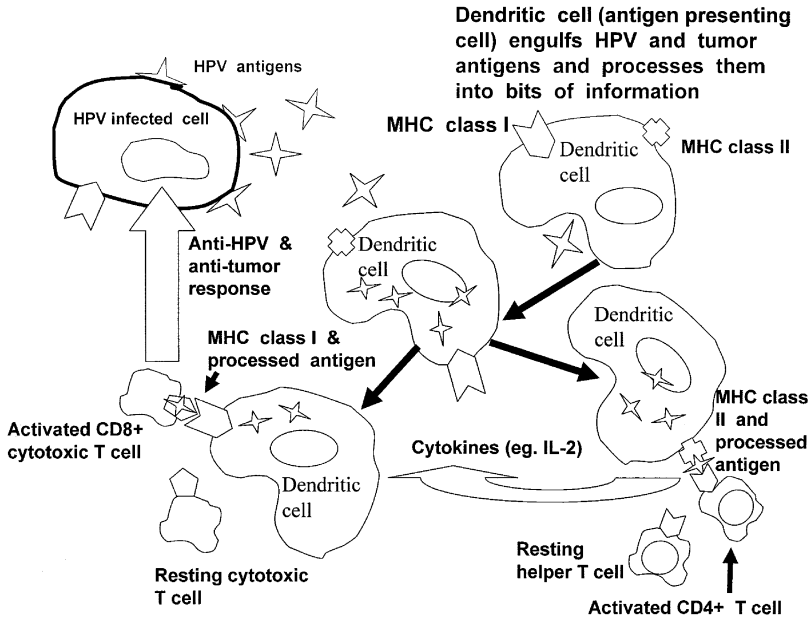

Fig. 3. Induction of cytokines help in the recognition of the presence of HPV and results in an immunologic cascade. Although keratinocytes are not good antigen-presenting cells, when HPV or tumor antigens can be detected on an HPV-infected cell, dendritic cells will engulf these antigens and process them into bits of information that can be used in initiating an immune response. Dendritic cells may present MHC Class I and MHC Class II processed-antigens on their surface. The MHC is a region of the human chromosome 6 that is responsible for producing glycoproteins that are expressed on the surfaces of most cells. These glycoproteins "present" foreign antigen-derived peptides on the cell surface for T-cell recognition. MHC Class I processed-antigens are presented in the regional lymph nodes to resting cytotoxic T cells, which become activated CD8+ cells. MHC Class II processed-antigens are presented to resting helper T cells, which become activated CD4+ cells. Activated CD4+ cells produce cytokines, such as IL-2, interferon, and tumor necrosis factor, that further promote recognition of HPV antigens and recruitment of macrophages, monocytes, and dendritic cells at the site of infection. Activated CD8+ cells also migrate back to the site of infection to mount an anti-HPV, antitumor response.

without the virus ever being released outside of the protection of the infected keratinocytes. Hence, the immune system has little opportunity, in the absence of treatment-induced cell lysis, to be exposed to the HPV genome. Also, HPV may be ignored by the immune system, because the virus does not cause inflammation and HPV genes are expressed at very low levels [41]. Additionally, the HPV genome has mechanisms to evade the host immune response [42]. For instance, it was demonstrated that HPV E6 and E7 interfere with MHC class I presentation of antigens and E7 can suppress the interferon signal and the pRb tumor suppressor block [43,44]. The latter results in liberation of E2F transcription factors, which play key roles in promoting host-cell and viral DNA synthesis. The E6 protein

suppresses the p53 anti-oncogene pathways that are important in preventing the accumulation of genetic damage that may lead to cancer [45].

Any treatment modality that lyses HPV-infected keratinocytes will expose HPV-antigens to the macrophages and mononuclear cells that initiate immune recognition. Therefore, ablative methods that leave a large load of killed HPV behind should theoretically provide the greatest immune response and long-term immune memory to HPV [46]. However, significant differences in posttreatment HPV-detection and in clearance of precursor lesions were not demonstrated for women who were treated with ablative methods in comparison with those who were treated by excision of the lesion [4]. It is possible that any treatment modality leaves behind enough lysed HPV-infected cells to initiate immune recognition (Fig. 4).

The effectiveness of the local cellular immune response can be measured by several parameters, including viral load, detection of dendritic and other immune-response cells, and detection of cytokines. Rising and falling viral levels probably

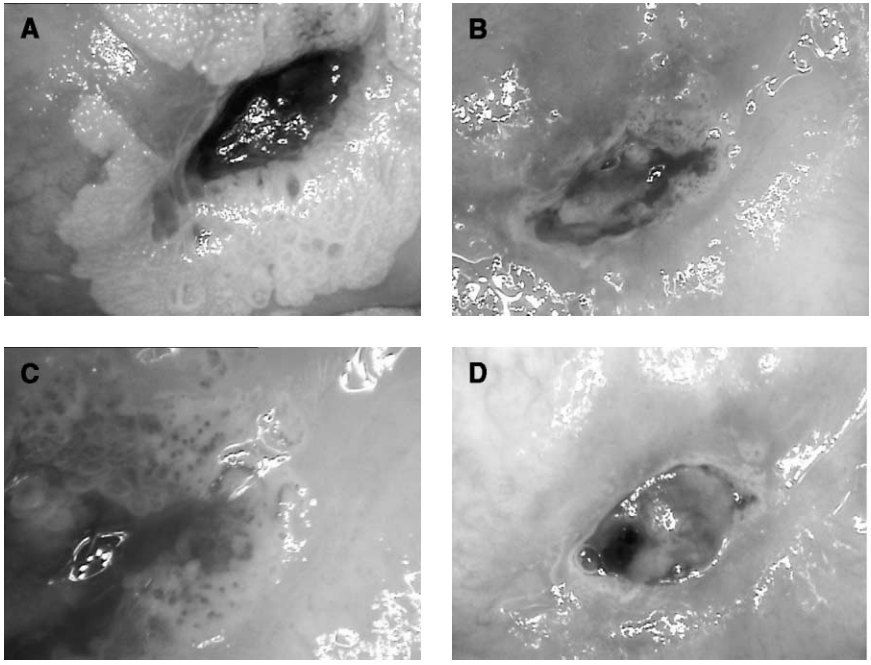

Fig 4. (A) This patient presented 4 months post-cryotherapy for CIN 1 with this colposcopic appearance that is consistent with extensive recurrence of low-grade cervical HPV changes. No further treatment was elected at that time in the expectation that a significant immune response could still occur. The patient was seen again 4 months later with only a tiny area of mild acetowhite and linear punctation, (B) often seen post-cryosurgery, visible in the magnified view (C). (D) By the third follow-up 3 months after the second examination her cervix had completely returned to normal. The spontaneous resolution might have occurred without the previous ablative therapy, or it is possible that the release of a large load of killed HPV from lysed cells may have initiated an immune response that was responsible for the subsequent resolution.

mirror the effectiveness of the host cellular immune response [47]. When CIN persists, the inability of the immune system to clear the lesion is reflected in decreased detection of dendritic cells; there is evidence that HPV-type influences the threshold for dendritic suppression [48]. For instance, women with CIN 3 or cancer have a decreased ability to mount a T-helper cell type 1 (TH1) immune response to HPV E6 E7 when compared with women with CIN 1, or to HPV-infected women who do not have lesions [49,50]. The inability to mount such a response, either genetically or acquired, may predispose the patient to persistent HPV infection and the sequelae of CIN 3 and cancer [51]. Decreased production of interferon gamma and interleukin-2 by NKCs was detected in patients with persistent condyloma; an inverse association between the grade of cervical neoplasia and interleukin 2-production by mononuclear cells in response to HPV-16 E6 and E7 peptides was demonstrated in vitro [49,50].

HPV-induced oncogenesis requires long-term viral persistence [52,53]. Hence, the subset that is at risk for neoplastic progression is the 10% to 20% of patients who continue to express the disease, or who "recur" after a lesion-free interval; in either case, they have not been able to suppress the infecting HPV. In these individuals, immunity to the HPV infection may not be triggered until after HPV integration and other cellular events that contribute to malignant transformation have occurred [54]. Presumably, most people in this subset have a reduced immunocompetence to HPV of unknown etiology. An understanding of the immune response to HPV provides insight into where this paradigm might be influenced to possibly overcome the advantage that HPV has in evading host defenses (Fig. 5).

Treatment modalities

Treatment modalities that are presently in use, or have been used previously but have fallen out of favor, can be divided into chemically destructive, surgical ablative, and surgical excisional methods.

Chemical destructive treatments

Trichloracetic acid (TCA) and 5-fluorouracil (5-FU) are two chemically destructive methods that have been proposed for treating CIN [21]. Neither has been studied adequately as a treatment for CIN and neither has achieved widespread use for this indication. TCA is a mainstay in the treatment of external genital warts and 5-FU continues to be the most commonly used modality in treating actinic keratoses and early skin cancers.

Trichloracetic acid

Trichloracetic acid was suggested as a possible treatment for the cervix as its cytodestructive properties, safety profile, and low cost all seem to be favorable. TCA has generally been proposed only as a treatment for CIN 1, either as a primary treatment or for small areas of recurrence posttreatment by other

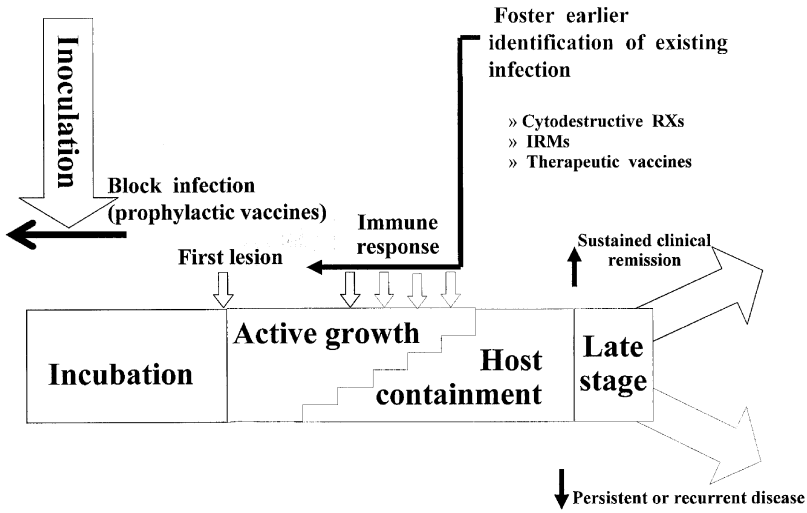

Fig. 5. Prevention of infection by a successful, prophylactic HPV vaccine at the time of transmission would block the occurrence and risk of epithelial lesions and HPV-induced lower genital tract cancers. Although results in various studies are encouraging. commercially available HPV vaccines remain at least a few years away and even when available, the restricted number of HPV types in HPV vaccines will ensure that some lesions will continue to develop and require treatment. After HPV-induced lesions develop, host containment and clearance will follow spontaneous immune recognition but this response may be slow or may never occur. Therefore, fostering earlier identification of existing infection is the next opportunity to change the equation. Doing so moves the immune response to an earlier time in the course of the infection. Because any cytodestructive treatment may result in earlier immune recognition by releasing killed viral antigens from lysed cells, all of the treatment options for cervical precursor lesions may foster earlier immune recognition. Immune response modifiers (IRM) may also promote earlier immune recognition by initiating cytokine release without the prerequisite of initial identification of HPV. Therapeutic HPV vaccines, if successful, would stimulate an immune response to existing infection, but to date, no therapeutic vaccine of any type is in commercial use. Therapeutic vaccine trials that target HPV E2, E6, or E7 are in progress and results are pending. The goal of all of these modalities is to promote earlier immune recognition that results in either prevention or clearance of infection, with an increase in sustained clinical remission that decreases persistent or recurrent disease.

modalities. The use of TCA for the treatment of CIN has never been widely accepted because of the concern that treatment of only surface cells by a topically applied solution might leave CIN buried in gland ducts that are not accessible to the cytodestructive effects of the TCA [21].

5-Fluorouracil

Topical 5-fluorouracil is a chemodestructive agent with a primary mechanism of action of interference with DNA and RNA synthesis. 5-FU is commonly used to treat skin cancer precursor lesions; during the 1980s it became widely used for the treatment of vaginal condyloma and vaginal intraepithelial neoplasia [54]. In the process of treating vaginal HPV lesions, many investigators also presumed that it had therapeutic efficacy in treating low-grade cervical disease; an oc-

casional therapeutic trial confirmed some efficacy [55]. Initial enthusiasm for the use of 5-FU in the vagina was soon replaced by concern over reports of intractable vaginal and introital ulcers, development of vaginal adenosis in denuded areas, significant, acute introital pain, and occasional chronic vulvar vestibulitis syndrome. Additionally a report of finding clear cell adenocarcinoma in areas of 5-FU-induced vaginal adenosis [56] eliminated any expectation that this medication will be of use in the routine treatment of CIN unless a delivery system can be developed that does not expose the entire vagina to the medication. Several studies evaluated the application of a bilaminar, bioadhesive polymeric film that contains 5-FU directly to the cervix to control the dose, site, and duration of application of the cytotoxic drug [57,58]. Side-effects were reported as being minimal but efficacy was not established [57].

One potential, clinical use for 5-FU is in the prevention of recurrence of CIN post-treatment in HIV-positive women. Maiman et al [59] reported on an unmasked, randomized control trial of 101 HIV-positive women who were treated for CIN by standard excisional or ablative procedures that received either 6 months of biweekly treatment with vaginal 5-FU cream (2 g) or underwent 6 months of observation. Treatment with 5-FU was significantly associated with prolonged time to CIN development, decreased risk of recurrence (28% recurrence in the 5-FU therapy group and 47% in the observation group), and decreased grade of recurrences that occurred (8% CIN 2/3 in the 5-FU treated group and 31% in the observational group). These very favorable results in the prophylaxis of individuals who are at very high-risk of recurrence post-treatment support the use of 5-FU in this setting.

Cervical ablative procedures

Electrodiathermy

Cauterizing the cervix with an electric ball and needle is a common method for treating cervical cancer precursor lesions in Australia and Europe, but is rarely used in the United States. One large study of 1864 cases (two thirds were CIN 3) reported cure in 97.5% following treatment with electrodiathermy [60]. Additionally, 44 of the 62 patients with recurrent disease were cleared by either directed biopsy or by repeat diathermy. Other investigators reported clearance rates of 85% to 94% [61,62]. As with other ablative procedures, the intent of electrodiathermy is to destroy the entire transformation zone and some of the proximal endocervical canal. The procedure requires a standard diathermy unit capable of generating 40 to 45 Watts, and a needle and ball electrode [63]. After administration of general anesthesia or quite comprehensive local anesthesia, a radial cut that is 5 to 7 mm deep and which extends 2 to 3 mm beyond the iodine-negative areas, sets the margins of the treatment. The portion of the cervix that is within this radial incision is ablated with a ball electrode, leaving a crater with a depth of at least 7 mm. Postoperative instructions and complications are similar to those for laser treatment. Although there has been concern that cervical stenosis would occur at an increased rate with this procedure, it was shown to be of no

greater degree than with other ablative procedures. Increased scarring may occasionally occur and was shown to prolong labor for some women [63].

Cryosurgery

Cryosurgery became the primary procedure used to treat CIN in the 1970s; it continues to be widely used for treatment of low-grade CIN and for some high-grade lesions. Cryosurgery is performed under stringent patient selection guidelines that triage less suitable lesions to other treatment procedures and results in cure rates quite comparable to either laser treatment or to LEEP [4]. Success rates for all grades of CIN range from 86% to 91.6% [64,65]. The procedure destroys tissue down to a level of 4 to 5 mm. Beyond that, because of the warmth of the underlying vessels, the temperature of the tissue does not fall to the level that is required for cell death. For the majority of preinvasive lesions, this depth of penetration is adequate; it was shown that even with gland duct involvement the clearance rate for cryosurgery is not statistically different from excisional techniques [4]. Success of cryosurgery, as with other treatment methods, is related to the size of the lesion rather than to its grade [4,66] [Table 1]. The one other aspect that diminishes the effectiveness of cryosurgery is the extension of the lesion more than 4 to 5 mm into the canal [4,67].

To eliminate the risk of inadequately treating an early invasive lesion, several prerequisites must to be met before any outpatient ablative surgery is performed for the treatment of CIN (Box 1). Additionally, cryosurgery requires somewhat more restrictive criteria than either laser treatment or electrodiathermy in that the lesion should not extend more than 5 mm into the canal and the cryoprobe should cover the entire lesion to minimize the risk of persistence. One advantage of cryosurgery is that it is relatively painless and can be done without anesthesia or analgesia. The procedure should be done with nitrous oxide; an appropriate cryosurgery probe tip should be chosen that best conforms to the cervical portio

Table 1
Freeze failure rate of CIN

Severity	Lesion size	Failure percentage
CIN 1	1[a]	9%
	2[b]	12%
	3[c]	50%
CIN 2	1	8%
	2	10%
	3	26%
CIN 3	1	5%
	2	19%
	3	56%

From Burke L. Evolution of therapeutic approaches to cervical intraepithelial neoplasia. J Lower Genital Tract Dis 1997;1:267–73; with permission.

[a] lesion involves 1cm or less of the transformation zone

[b] lesion size between a and c

[c] lesion involves the entire transformation zone

Box 1. For any ablative cervical treatment for cervical intraepithelial neoplasia, the following criteria must be met:

Full colposcopic evaluation by an experienced colposcopist.
Complete visualization of the entire transformation zone and lesion.
No suspicion of invasion on cytology or colposcopy that has not been satisfactorily eliminated as a possibility.
Endocervical sampling has excluded the presence of endocervical disease.
There is no significant inconsistency between cytology, colposcopy, and histology.
The patient is reliable and can be expected to follow-up as directed.

In addition, cryotherapy requires somewhat more restrictive criteria in that:

No more than the proximal 5 mm of canal be involved.
The lesion should, ideally, be covered by the cryoprobe.

and covers the entire extent of the lesion. If the lesion is more than 1.5 times greater than the surface area of the probe tip, more than one probe application will be required [66]. The probe temperature is from $-65^{\circ}C$ to $-85^{\circ}C$, far below the $-20^{\circ}C$ that is required for cell destruction. One cannot see the depth of the freeze, but it can be estimated as the depth approaches the lateral spread of the ice-ball by 1:1.3. The areas at 3 and 9 o'clock, however, are less prone to freeze as deeply because the presence of the uterine vascular tree in these areas impedes heat loss. As a result, recurrences postcryosurgery are frequently in these lateral areas. The most common causes of treatment failure are an inadequate freeze because of low refrigerant pressure, poor probe application, insufficient freeze time, large three or four quadrant lesions, or extension of disease into the endocervical canal [66–72].

CO_2 laser

Laser vaporization of cervical precursor lesions was first introduced in the late 1970s; it was used extensively in the treatment of all grades of CIN up through the early 1990s. The high cost of the equipment and of regular maintenance, and the training required to expertly perform CO_2 laser procedures eventually decreased its availability and use in favor of procedures that were shown to be equally efficacious but without these negatives. The introduction of LEEP in the late 1980s began to reduce the use of laser vaporization because it required far less training and was relatively inexpensive to purchase and to maintain. Additionally, the trend towards the use of generalists to evaluate and manage

women with abnormal Pap smears favored procedures that did not require high technical expertise. Although cryotherapy and LEEP are now the preeminent procedures that are used to treat CIN, the CO_2 laser continues to serve an important role in the treatment of very large cervical lesions. Laser vaporization can be performed in an outpatient setting, and success rates of 90% to 96% reported [73–76]. Benedet et al documented that nearly 96% of women with CIN who were treated by laser vaporization were cleared of their disease [76]; this similar to the 5.5% recurrence rate that was reported in another study of 2130 women who were treated by laser vaporization [77]. In the latter study, two women were subsequently found to have microinvasion and one had frankly invasive cancer. Invasive cancer was reported to occur in a very small percentage of women who were treated by any of the ablative methods and emphasizes the need to follow strict management guidelines as outlined in Box 1 [78].

Laser vaporization of the cervix should always be done under colposcopic control. Selection of patients for laser ablation should follow rules that are similar to selection for cryotherapy [66] except that laser can successfully treat lesions that extend somewhat further into the endocervical canal or peripherally beyond the cervicovaginal junction. Several studies reported that laser vaporization is more effective for larger lesions, for high-grade lesions, for lesions that extend into the cervical canal, and for lesions with gland duct involvement [72,79]. As discussed earlier, however, more recent studies indicated that it is only the size of the lesion and not the grade that determines the differences in clearance rates between cryosurgery, laser vaporization, and LEEP [4]. Laser vaporization of CIN should destroy the tissue to a depth of 5 to 7 mm and should be performed under local anesthesia. Major postoperative side effects of laser vaporization include heavy bleeding in 1%; an occasional infection was reported, as was cervical stenosis. Complications are minimal when adequately trained clinicians perform the procedure.

Cervical excisional procedures

Excisional biopsy by cervical biopsy forceps

In essence, any conization is an excisional biopsy, as is excision of the lesion by one or more cervical "punch" biopsies. The first description of the use of punch biopsies for diagnosis and treatment was made in 1949 [21,80]. The intention was to excise much of the transformation zone and squamocolumnar junction by biopsy in women with abnormal cervical cytology. Now that colposcopy provides information on the size and location of the lesion, it is possible to excise small lesions successfully by simple biopsy. Not proceeding to further treatment modalities that fully treat the transformation zone is usually reserved for low-grade precursor lesions or small post-treatment recurrences. Cure rates for treatment by excisional biopsy were reported to be 82% for CIN 1, 68% for CIN 2, and 46% for CIN 3; this indicates that stand-alone treatment by biopsy must be reserved for low-grade lesions [21,80,81].

Loop electrosurgical excision procedure

Loop electrode excision procedure and large loop excision of transformation zone (LLETZ) are different terms for essentially the same procedure [63], although LEEP encompasses treatment by electrosurgical loop excision anywhere throughout the lower genital tract; LLETZ applies only to cervical treatment. The use of circular or other shaped wire electrodes to excise lesional tissue is not new; so-called "hot-coned" biopsies of the cervix were done 50 years ago. This early excisional procedure fell out of favor because of the inability to control deep burns which had resulted in cervical stenosis and infertility. A revival of interest in this procedure occurred with the advent of electrosurgical generators that could convert 60 Hz low-frequency, alternating current into a high-frequency AC of 350,000 to 700,000 Hz (350 to 700 kHz) [63,82]. The advantage of this frequency is that it cuts by vaporizing tissue rather than by burning it and does not cause muscle twitching or spasm. This significant improvement in technology increased patient comfort and limited thermal damage. Development of the modern LEEP procedure occurred when these electrical advantages were coupled initially with Cartier's [83] innovations in small loop biopsy of the cervix and the subsequent development by Prendiville et al [35] of the larger loops that are now used.

LEEP has been used to excise lesions throughout the lower genital tract, however concerns over possible deep thermal injury to the vagina and possible scarring of the vulva and penis prompted many clinicians to continue to perform laser vaporization or conservative modalities to treat these areas. In a few years LEEP virtually replaced laser vaporization in most protocols for the treatment of CIN. LEEP has several advantages over other procedures that includes: (1) preservation of tissue for histologic diagnosis, (2) relatively inexpensive equipment (compared with laser vaporization), and (3) speed and simplicity of the procedure [84]. Because LEEP produces a specimen, it is ideal for high-grade lesions where there may be concern for areas of microinvasion or for low-grade lesions in which biopsy did not detect a high-grade lesion that correlates with a referral high-grade Pap smear. Cure rates have been reported to be between 73% and 95% [85–88] and are consistent with the rates reported for other modalities. This procedure was widely overused in the early to mid-1990s, as evidenced by reports in 20% to 65% of either negative LEEP specimens or specimens that showed only CIN 1 [89–91]. These high numbers reflect both high-grade lesions that were completely removed by biopsy before the LEEP and some women with misclassified high-grade (HSIL) Pap smears that do not represent a significant precursor lesion [92].

CO_2 laser conization

Laser can also be used for conization, and for clinicians with appropriate training the procedure can produce a better specimen with fewer potential complications than cold-knife cone [93,94]. In contrast to laser ablation, which requires a defocused spot size and lower power density to vaporize large areas and coagulate blood vessels up to 2 mm size, the spot size used for laser as an excisional tool is small (0.25 to 0.8 millimeters) with a power density of greater

than 1,000 watts/cm [63]. This requires greater skill because the beam is more difficult to control at this power density. Most of the characteristics of laser and cold-knife cone are similar and will be discussed together below.

Cold-knife conization

Cervical cold-knife cone was the standard excisional procedure of the cervix for nearly 50 years following the first description of "cone biopsy" for treatment of cervical neoplasia by Martzloff in 1938 [95]. Although it is a minor procedure, it is one of the most difficult to do well. It has become more problematic in the last few years because of the replacement of cold-knife cone by LEEP, which has reduced skills for many. Additionally, many residency programs no longer train in expert use of the cold-knife cone. Hemorrhage and secondary stenosis are the most common complications, followed by dysmenorrhea and decreased adequacy of cytologic and colposcopic evaluation. All of these adverse effects are proportional to the size of the cone, which can be varied in its shape depending upon the location of the disease [63,93]. When the disease is confined to the ectocervix, only the ectocervical disease needs to be removed and the canal may be spared. Conversely, if there is disease on the ectocervix and in the canal, a large, shallow ectocervical cone may be followed by a narrow, cylindrical endocervical cone, sparing some stroma that would have otherwise been removed in one large cone [93]. Disease entirely in the canal can be successfully evaluated and treated by a long, cylindrical cone.

Cold-knife cone is commonly done under general anesthesia in an outpatient setting. When possible, an effort is made to excise only 4 to 5 mm laterally around the canal. This incorporates gland ducts, while reducing the potential for perforating the cervix or transecting the cervical branch of the uterine artery. When it is important to evaluate the majority of the canal, such as in the management of cytology suggestive of adenocarcinoma in situ (AIS) or cancer, or a high-grade lesion that extends into the canal beyond visualization, the cone should be 2 to 2.5 cm in length. The excised cone bed can be sutured with several approaches, but a running, interlocking stitch of absorbable material seems to decrease the potential for postoperative stenosis [66].

Hysterectomy

Hysterectomy is rarely done for CIN alone because of the morbidity associated with this procedure in comparison with any of the cervical excision procedures and the comparable clearance rates with the latter. Most hysterectomies for CIN are done in the presence of another indication for this procedure, such as endometriosis, significant symptomatic fibroids, or intractable dysfunctional uterine bleeding. Hysterectomy was often used when high-grade CIN extended to the endocervical margin of the lesion. However, reports of the low-risk of subsequent detection of cervical cancer in patients with positive cone margins, but without further treatment, reduced enthusiasm for this approach unless the patient has completed her family and understands the pros and cons of follow-up versus hysterectomy [96].

Alternative methods of treatment

Several alternative methods for treating CIN were evaluated over the past decade; some are presently available for clinical use and others were entirely in the realm of research. All of the presently available treatment modalities for cervical cancer precursor lesions are directed at surgical removal of the lesion by either ablation or excision; there is no clinically available treatment for CIN that is either directly antiviral or stimulates an immune response. Therefore, recurrences are most commonly secondary to inadequate control by local cellular immunity of adjacent infected, but pretreatment morphologically normal appearing epithelium. Several primary and adjunctive treatment modalities are presently under investigation that are designed to either boost the host immune response, or to disable the viral mechanisms that promote HPV replication, transcription, and transformation.

Modalities that promote the immune response to HPV

Dietary measures

Beta-carotene

Several case-control and cohort studies identified an association of dietary antioxidant micronutrients with a reduced risk of certain human malignancies [97–99]. The micronutrients most associated with reduced cancer risk are carotenoids, tocopherols, and vitamin C. Beta-carotene, a carotenoid metabolized to retinol, has been studied extensively. Palan et al [99] demonstrated that the mean plasma levels of carotenoids (beta-carotene, lycopene, and canthaxanthin), and alpha-tocopherol, were significantly lower in women with CIN and cervical cancer [99]. Peng and colleagues [97] evaluated 10 micronutrients in plasma and in tissue of women with CIN or cervical cancer; only the concentrations of beta-carotene and cis–beta-carotene were lower in both than in controls [97]. The other eight micronutrients were decreased in the plasma but increased in histological sections of CIN and cancer from the same patients. This suggested that not all of the micronutrient concentrations in plasma reflect the micronutrient concentrations in cervical tissue and that maintaining an adequate plasma and tissue concentration of beta-carotene may be necessary for the prevention of cervical cancer and precancer. Other investigators concluded that these findings indicate a potential role for antioxidant deficiency in the pathogenesis of CIN and carcinoma of the cervix [100]. In contrast, a nested, case-control study in Finland and Sweden found that levels of retinol or unoxidized alpha-tocopherol in the blood were not indicators of risk for cervical cancer [101]. Analysis of the joint-effect of retinol and high-risk HPV seropositivity revealed that low levels of retinol were associated with a statistically significant synergistic interaction with HPV-16, 18, and 33. The investigators concluded that retinol might act as an effect modifier of the HPV-associated risk for cervical cancer.

Proving the therapeutic efficacy of the administration of beta-carotene, has, however, been elusive to date. Comerci et al [100] tested the therapeutic efficacy of beta-carotene in cervical intraepithelial neoplasia and described a possible mechanism of action. Transforming growth factor (TGF) beta 1 is a potent growth inhibitor of epithelial cells; loss of TGF-beta 1 or loss of responsiveness to it may be important in the progression of cervical intraepithelial neoplasia to invasive cervical cancer. Retinoids may promote the induction of TGF-beta [100]. A significant increase in intracellular TGF-beta 1 was noted in cervical epithelial cells in patients with cervical intraepithelial neoplasia after treatment with beta-carotene; this demonstrated regulation of a TGF-beta isoform in response to administration of beta-carotene [102]. There are no definitive data that show that supplementation of the diet with beta-carotene or other micronutrients increases the rate of regression of established cervical precursor lesions. The only randomized placebo control trial did not show a difference in regression of CIN or HPV persistence over a period of 9 months of administration of daily 30-mg oral dose of beta-carotene [102]. Despite the apparent lack of efficacy of short-term administration of beta-carotene, the strong epidemiological data and verification of a possible mechanism of action that may decrease the rate of progression to cancer support the recommendation for a diet that is rich in these micronutrients. Longer-term studies may be necessary to prove that supplementation with vitamin C and beta-carotene is helpful in reducing the risk of progression.

Folic acid

It was suggested that folate, vitamin B_{12}, vitamin B_6, and methionine may function to prevent cervical cancer by interfering with HPV transcriptional activity through their role in DNA methylation and one carbon metabolism. However, generally supportive results in epidemiological case control studies [103–109] have not been validated in interventional trials. As with investigation of carotenoids, this may be secondary to the short duration of all interventional studies to date [103]. Additionally, folate has a role in the synthesis and repair of DNA. Vitamin B_{12}, which shares a pathway with folate metabolism, and homocysteine, which is a marker of low folate and B vitamin concentrations, were suggested as modulators of risk for cervical cancer [108]. Elevated serum homocysteine levels (tHey) enhanced the normal increased risk for CIN for women who smoke, have increased parity, or have documented HPV 16 infection; this suggested that elevated plasma tHcy is a risk factor for cervical dysplasia and that it enhances the effects of other risk factors [109]. It is unknown whether tHcy is serving as a marker of folate deficiency or is acting through other mechanisms. A recent large, case-control study found that serum homocysteine levels were strongly and significantly predictive of invasive cervical cancer risk [105]. In contrast, Potischman et al [106] were not able to demonstrate a significant difference in the mean plasma folate levels between women with Stage I or Stage II cervical cancer and controls.

Butterworth et al [103] demonstrated that the adjusted odds ratio for HPV-16 for cervical dysplasia was only 1.1 among women with folate levels higher than

660 nmol/L but was 5.1 among women with lower levels; this indicated that folate deficiency may be an important cofactor to HPV 16 in lesion development or progression. The same group was not able to demonstrate a significant difference in the rate of regression of disease or in the rate of papillomavirus type 16 infection in a 6-month placebo control trial of folic acid administration to women with CIN 1 or 2 [103]. Other investigators noted that although plasma vitamin B_{12} levels are inversely associated with HPV persistence, no significant association can be observed between HPV persistence and the level of dietary intake of folate, vitamin B_{12}, vitamin B_6, or methionine from food or food and supplements combined [107]. Although the epidemiological and interventional studies do not seem to coincide, recommending a diet that is rich in these micronutrients cannot be argued against and a case can be made for recommending dietary supplementation with folic acid and the B vitamins.

Other interventions

Smoking cessation

A retrospective analysis of the literature from 1966 through 1998 showed that although several recent large studies demonstrated that smoking is associated with a greater incidence of cancer of the cervix, vulva, penis, and anus, as well as several head and neck cancers in a dose-dependent fashion, other studies have not shown any correlation between smoking and cervical dysplasia after multivariate adjustment [110]. However, the association of smoking with progression of cervical dysplasia seems to be strong enough to conclude that smokers are at increased risk for cervical cancer and for failure to respond to treatment for CIN [111]. The mechanism of action for the increased risk of cervical neoplasia for smokers continues to be somewhat hypothetical, but local immunosuppression and direct carcinogenic effects of cotinine, nicotine, and nitrosamines may be contributory [112,113]. The absence of cervical cancer in celibate women indicates that the presence of these carcinogenic substances alone does not promote cervical cancer [114] . Cigarette smoking was shown to significantly decrease the density of Langerhans cells and their function in normal cervical epithelium and in CIN [115].

Smokers also have a higher risk of treatment for CIN being unsuccessful. One case control study evaluated the history of smoking among 958 women who were treated for CIN, 77 (8%) of whom experienced treatment failure [111]. Current smokers had a threefold increased risk of treatment failure for CIN compared with nonsmokers; it was noted that cigarette smoking functioned independently of HPV detection. The investigators concluded that women who smoked and those who continued to be HPV positive post-treatment would benefit by longer, more intensive follow-up. One large interventional study demonstrated a clear relationship between reduction in smoking and changes in cervical immune cell counts [116]. Reduction in smoking by 20 to 40 cigarettes per day was significantly associated with an increase of between 6% and 16% in counts of Langerhans cells, CD8, and total lymphocytes. Heavy smoking was significantly

associated with an increase in persistent HPV infection. The data are sufficiently strong to advise all women that smoking increases their risk for cervical cancer and decreases the success rate of treatment for cervical cancer precursor lesions. Therefore, promotion of smoking cessation should be included in any treatment program for women with cervical cancer precursor lesions who smoke.

Use of oral contraceptives

Oral contraceptive use was reported as a risk factor for cervical cancer in numerous studies [117–121]. This raises the question of whether women with cervical precursor lesions should consider coming off hormonal contraception. Until recently it was not clear whether OCs increased the risk of cervical cancer independent of the risk attributable to HPV, whether they act as cofactors to HPV in cervical oncogenesis, or whether the increased risk merely reflects secondary associations that are attributable to confounding by HPV [119]. Although one recent analysis did not show a significant increased risk for CIN 2 or 3 among women with a history of prolonged oral contraceptive use once HPV was taken into account [119], a large, multicenter, case control study by the International Agency for Research on Cancer (IARC) found increased risk for CIS and cancer among OC users that was independent of HPV [121]. The IARC study group pooled data from eight case-control studies of patients with histologically-confirmed invasive cervical cancer and from two studies of patients with carcinoma in situ to evaluate the effect of oral contraceptives and HPV detection on the risk of these lesions. Compared with persons who had never used OCs, patients who had used oral contraceptives for less than 5 years did not have an increased risk of cervical cancer. The odds ratio for persons who had used oral contraceptives for 5 to 9 years was 2.82 (95% CI 1.46–5.42) and the odds ratio for persons who had used oral contraceptives for 10 years or longer was 4.03 (95% CI 2.09–8.02). These risks did not vary by time since first or last use. The investigators concluded that long-term use of oral contraceptives could be a cofactor that increases the risk of cervical carcinoma by up to fourfold in women who are positive for high-risk HPV DNA. These findings may not be in conflict if the cofactor effect of sex steroid hormones is at the level of persistence and progression of HPV-induced oncogenesis rather than in progression of low- to high-grade CIN.

Oral contraceptives have also been implicated in an increased risk for adenocarcinoma in situ [120]. Among women who were born after 1945, the relative risk for AIS increased with the duration of OC use; the highest risk (five times the risk of nonusers) was noted for those women who had taken oral contraceptives for 12 or more years. The possible mechanism of action of oral contraceptives on cervical cancer risk has been discussed in numerous studies. Eversion of the cervical columnar epithelium with activation of the immature metaplastic process may increase the epithelia at-risk [114]. Oral contraceptives were shown to decrease serum folate levels which causes megaloblastic changes in cervical epithelial cells [122]; this suggested a possible explanation for the increased incidence of CIN and cervical cancer that was noted in women who

have low levels of folate [103]. More recent studies focused on the immunomodulating effects of sex steroid hormones and their promoting effects on the expression of viral oncogenes [123,124]. Estrogen stimulates antibody- and cell-mediated immune responses which increases cytokine production in the mucosa that may reduce susceptibility to primary HPV infection [124]. Estrogen or progesterone are also associated with progression to cervical cancer in persistent HPV-infected individuals. Beta estradiol was shown to increase by eightfold the transcription of the open reading frames of E6 and E7 [125]; the levels of HPV-18 E6 and E7 mRNA are significantly increased by estradiol [126]. The increased expression of HPV E6/E7 by estrogen is likely to be the cause of the growth stimulation of HPV-positive cervical cancer cells by estradiol noted in vitro [126]. Progesterone and progestins also seem to modulate expression of HPV genomes. The antiprogesterone RU 486 blocks the demonstrated progesterone-induction of HPV gene expression in cervical keratinocytes; this confirmed the role of progestins in modulating the natural history of HPV in cervical precursor lesions [123].

What does this mean for women with known cervical cancer precursor lesions who are on oral or other forms of hormonal contraception? Should clinicians recommend that these women consider other forms of contraception? Such questions must be answered with full understanding of the natural history of cervical precursor lesions and accounting for the potential risks and benefits of hormonal contraceptives within that context. First, it seems that the potential cofactor-effect of oral contraceptives is most likely to be late in the natural history of cervical precursor lesions; this gives most HPV-infected women on hormonal contraceptives in the United States ample time for detection and treatment because of the requirement of participation in Pap screening to obtain these contraceptives. Additionally, the increased risk of pregnancy in women who stop oral contraceptive use after CIN has developed would complicate the management and follow-up of CIN [114]. The evidence at this time does not seem to be sufficient to recommend that women with CIN stop using oral contraceptives. However, many women in resource-poor countries have access to long-term oral contraceptive use but not to routine cervical cancer screening. These data suggest that extra effort should be made to include these women in cervical screening programs [121].

What lies ahead?

Markers for progression

Management of women referred for the evaluation of low grade squamous intraepithelial lesion (LSIL) and HPV positive atypical squamous cells of undetermined significance (ASC-US) cytology and found to have only low-grade CIN or less on colposcopy is presently limited by the inability to predict which women are at risk of progression (see elsewhere in this issue). The result is

intensive follow-up for all women with $\leq$ CIN 1 managed expectantly, most of whom may not be at great risk. Successful prediction of risk by markers that either identify genetic variants of high-risk types more likely to induce transformation, or increased activity of HPV genomes and proteins known to be involved in increasing abnormality would greatly facilitate rational management. Some of the current candidates being studied besides HPV variants include the expression of a tumor suppressor gene called FHIT, HPV E6 and E7 messenger RNA, expression of p16, loss of heterozygosity at specific chromosomal loci indicating the accumulation of mutations, and DNA ploidy [127–131]. Additional markers may enable the identification of a permissive immunity that is more likely to be susceptible to HPV persistence. Although there is expectation that one or more markers may be eventually documented to predict those at highest risk of progression, there is currently no marker identified that is close to clinical usefulness.

New treatment modalities

Antisense gene therapy

High-risk HPV genes E6 and E7 are expressed in cervical cancer cells, thus making them suitable targets for gene therapy [132]. Down-regulation of oncogene expression by antisense-based gene therapy has been extensively studied, and in some cases, therapeutic effects were demonstrated [133]. Antisense gene therapy involves the cloning of the full-length HPV 16 E6 or E7 cDNA in reverse orientation. Choo et al [132] demonstrated that the delivery of the antisense gene construct of HPV 16 E7 resulted in the reduction of HPV-16 E7 protein expression and in cell proliferation in CaSki cells 239. These changes were accompanied by cell cycle arrest, up-regulation of the retinoblastoma gene pRB, and down-regulation of HPV 16 E2 regulatory proteins. The result was inhibition or retardation of the tumorigenicity of CaSki cells in vivo.

The E7 antisense apoptosis and antitumor immune response seems to be enhanced by codelivery with the interleukin-12 cytokine gene [134]. The combination of antisense and cytokine gene therapy resulted in complete regression of 26 of 28 (93%) HPV 16 DNA-positive cervical tumors in mice. Complete regression was also demonstrated in tumors located 1 cm from the treated tumors; this confirmed the induction of a systemic antitumor effect. Antisense gene therapy may hold promise as an adjunct for women with invasive cervical cancer who are treated with conventional approaches; its incorporation in treatment guidelines will await therapeutic trials and its usefulness for high-grade precursor lesions has not been evaluated.

Immunotherapies

Human leukocyte ultrafiltrate

Several immunotherapies are presently being investigated for the treatment of cervical cancer precursor lesions. One report on the administration of systemic

leukocyte ultrafiltrate to 97 HPV-positive women who had no evidence of CIN found a very high rate of suppression or clearance of HPV [134]. Following the administration of human leukocyte ultrafiltrate, 86 women were HPV negative at week six; the remaining 11 women tested negative after completion of a second course of therapy. Although these results are encouraging, in the absence of a randomized control trial the data can only be considered preliminary.

Interferon

Interferons are cytokines that are released initially as a front-line defense of innate immunity to foreign antigens and have antiproliferative and antiviral activity [135,136]. Interferon has been shown to boost the host immune response to HPV, but virtually all of the data on safety and effectiveness of interferon is on the treatment of external genital warts (EGWs) rather than CIN. Intralesional and systemic injection of interferon were studied extensively in the 1980s for the treatment of EGWs [135–138] but variable efficacy, high cost, and the necessity of multiple office visits for injection have limited its use primarily as an adjunct after surgical ablation of EGWs and vulvar intraepithelial neoplasia (VIN).

Indirect evidence for the importance of interferon in modulating the immune response to CIN exists. For example, reduced epithelial and subepithelial IFN-gamma, as well as increased subepithelial interleukin-10 synthesis was demonstrated in all grades of HPV 16–positive CIN when compared with normal cervical epithelium; this suggested that the cytokine aberrations may play a role in the development and progression of HPV 16–associated cervical precancer [139]. Data on the use of interferons in treating CIN have only recently become available. In vitro and in vivo studies of the systemic administration of alpha-IFN 2a or interferon beta and 13 cis-retinoic acid (13cRA) strongly supported the enhanced effectiveness of the two agents when used in combination to treat neoplasia. One study of the topical treatment of low-grade cervical HPV lesions indicated greater clearance with interferon-beta in combination with 13cRA and tamoxifen compared with interferon-beta alone [138]. Toma et al [140] administered 13cRA orally and alpha-IFN 2a intramuscularly for 8 weeks to 14 women with CIN 2 and seven women with CIN III. They noted that 13 (62%) of the women had histologically-verified objective responses (six complete and seven partial). Systemic administration of interferon beta was evaluated in a randomized control trial of 121 women with recurrent CIN [141]. Women who were given interferon were significantly more likely to be free of CIN at 6- and 12-month evaluations (79%) than women in the placebo group (54%). These results indicated that various interferons administered systemically with or without 13cRA may increase the rate of resolution of cervical HPV infection and of CIN. However, the high cost of interferon and the systemic side effects will likely limit the use of this approach.

Imiquimod

Imiquimod is the first of a family of hundreds of molecules called immune response modifiers to achieve approval for the treatment of EGWs. Because a number of other molecules in this family are being evaluated for the treatment of

CIN, a discussion of the probable mechanism of action is appropriate. Although the majority of immunomodulatory agents that are available or in development inhibit pathways that are involved in immune activation, imiquimod is unique because it activates immune function [142]. In vitro studies showed that imiquimod has no direct antiviral effects, but it does exhibit antiviral and antitumor effects in vivo through induction of cytokines and enhancement of cell-mediated cytolytic antiviral activity [142]. Members of the imidazoquinoline family, such as imiquimod and resiquimod, act by inducing cytokine secretion from monocytes or macrophages (interferon-alpha, interleukin-12, tumor-necrosis factor-alpha) [143]. The immune response that is initiated seems to quite similar to that which would occur naturally in spontaneous immune recognition of HPV, but occurs after application of an IRM without the requirement of first identifying the presence of HPV. The immune response that is generated leads to a TH1-dominance and cell-mediated immunity that has been used clinically to treat several viral infections besides HPV, including herpes, and the pox virus that is responsible for molluscum contagiosum. Although the primary function of the imidazoquinoline family is the enhancement of antigen-presentation by dendritic cells, they also act on B cells, leading to the synthesis of antibodies, such as IgG2 [143].

Application of imiquimod to genital HPV lesions was shown to stimulate significant increases in mRNA for IFN-alpha, IFN-gamma, and 2′,5′ oligoadenylate synthetase (2′,5′-AS) as well as a tendency toward increases in TNF-alpha and interleukin-12 p40 [144]. Significant increases in mRNA for CD4 and a trend toward increases in CD8 were observed in patients who were treated with imiquimod; this suggested activation of a cell-mediated immune response that correlated with decreased viral load as measured by HPV DNA and L1 mRNA [144]. These effects on HPV markers were accompanied by an apparent decrease in mRNA expression for markers of cell proliferation, and an increase in mRNA for markers of normal keratinocyte differentiation and for tumor suppressors. Arrese et al [145] compared the densities and distributions of inflammatory cells in external HPV-induced condyloma and high-grade lesions that did not respond to imiquimod with similar, untreated lesions. All inflammatory cells except factor XIIIa-positive dendrocytes were similar in density and distribution between both groups; this demonstrated that lesions that did not respond to imiquimod have reduced density of dermal dendritic cells that may be responsible for diminished cytokine production and failure of imiquimod treatment.

Studies are underway in the treatment of all grades of CIN by IRMs, but in the absence of completed studies, the only available data are anecdotal from off-label use of these medications. It is expected that the immune response stimulated by an IRM to HPV-induced CIN would be similar to that stimulated by HPV-induced EGWs, but verification of this assumption awaits further study.

Therapeutic vaccines

Therapeutic vaccines are extensively reviewed elsewhere in this issue so they are not covered in this article. Their potential importance in the management of

women with cervical cancer precursor lesions cannot be understated [146]. It is possible that vaccines for all HPV types may never be made, or are in the very distant future. The requirement for continued cervical screening and treatment will likely continue for the foreseeable future.

Chemopreventive agents

New approaches that are being evaluated for the prevention and treatment of cervical cancer precursors include indole-3-carbinol and Cidofovir. These natural and synthetic compounds fall in the realm of chemopreventive agents that may intervene in the early precursor stages of carcinogenesis and prevent the development of invasive disease.

Indole-3-carbinol

Dietary indole-3-carbinol (I-3-C) was shown to produce clinical benefits for cervical cancer and laryngeal papillomatosis, and to cause apoptosis of breast and cervical cancer cells, but not normal keratinocytes, in vitro and in vivo [147]. I-3-C and the estrogen metabolite 2-hydroxyestrone was shown to abrogate estrogen-increased expression of HPV oncogenes by competing with estradiol for estrogen-receptor binding. Mice that expressed transgenes for HPV-16 developed cervical cancer when they were given 17 beta-estradiol chronically, but only 2 of 24 transgenic mice who were given I-3-C administered at physiological doses developed cervical cancer in contrast to 19 of 25 in the control group who were fed the control diet. Data from these animal trials prompted the investigation of I-3-C in a randomized, placebo control trial in humans, which documented no regression of CIN2-3 in the placebo group, but approximately 50% of the I-3-C treated group had complete regression [148]. There was no statistical difference in the detection of HPV between the placebo and treated groups; this was consistent with the known apoptotic effect of I-3-C on dysplastic cells and also with its lack of immunostimulatory or direct antiviral effects. Indole-3-carbinol is available as a supplement. These studies suggest that it may be appropriate to recommend their use for women with known CIN. At least, as discussed earlier, encouraging a diet that is rich in indole-3-carbinols may be helpful.

Cidofovir

Cidofovir is an acyclic, nucleoside phosphonate derivative with broad-spectrum anti-DNA virus activity that has been evaluated for the treatment of CIN 3. The mechanism of action of cidofovir may be the result at least in part, of the induction of apoptosis and is associated with accumulation of the tumor suppressor proteins p53 and pRb [149]. Snoeck noted that 7 of the 15 patients with CIN 3 who were treated topically with Cidofovir 1% gel had complete return to normal histology, five patients had a partial response that was characterized by the persistence of CIN II–III lesions, and one patient regressed to CIN 1 [150]. Only two patients did not show any difference in the histology. Four of the seven

patients with complete histologic regression became HPV-negative by PCR. The effect was specific for dysplastic epithelium as no normal tissue was affected by the treatment. The investigators have demonstrated complete regression of laryngeal papillomatosis and other severe HPV-induced proliferative lesions. Cidofovir may hold great promise if further studies demonstrate such dramatic results with similarly recalcitrant lesions.

Photodynamic therapy

Photodynamic therapy (PDT) is a novel treatment modality that produces local tissue necrosis of dysplastic epithelium with laser light after prior administration of a photosensitizing agent. PDT does not affect normal surrounding tissue [151,152]. Animal model studies suggested that successful treatment with PDT that resulted in long-term resolution of the disease process involved an cytotoxic T-lymphocyte (CTL)-driven immune reaction [153]. Topical 5-aminolevulinic acid-based (5-ALA) photodynamic therapy produced complete response rates of more than 90% for nonmelanoma skin cancers, which are mostly HPV-negative [153]. It was shown to induce higher porphyrin fluorescence in CIN compared with the surrounding epithelium [154]; the increased fluorescence corresponding generally to an increased grade of abnormality (normal tissue [1.0], CIN 1 [1.3], CIN 2 [1.21], and CIN 3 [2.35]) [151]. Therapeutic trials of 5-ALA followed by PDT in the treatment of cervical and vulvar cancer precursors were disappointing [152,153]. Hillemanns and colleagues [152] did not find any improvement in high-grade CIN lesions treated by PDT with an argon-ion-pumped dye laser at 635 nm following topically-applied 5-ALA. Similar, disappointing results were recently reported for the treatment of high-grade vulvar intraepithelial neoplasia (VIN 2–3); a short-term response was noted in only one third (10 of 32) [153]. Unifocal lesions were more responsive than multifocal and pigmented lesions. VIN nonresponders were more likely to show HLA class I loss compared with responders; HLA class I down-regulation was significantly greater in the carcinomas (82% total loss) than VIN (28% total or partial loss). None of the cases with class I down-regulation responded to PDT, and 50% of cases that showed total class I loss subsequently developed superficial invasion. The results indicated that poor cellular immune response is an important determinant in the high failure rate.

Better results were achieved in a trial of 24 cases of CIN 1–3 that were treated by PDT following topically-applied dihematoporphyrin ether [155]. Sixty-eight percent of the patients were disease free at 12 months; four of the seven failures or recurrences occurred at lower laser energy densities. Side effects reported in all studies were minimal; no patients experienced local necrosis, sloughing, scarring, or systemic symptoms, and only mild vaginal discharge was noted by some patients [155]. The majority of the data on the treatment of cervical and vulvar HPV-induced high-grade lesions has not been consistently encouraging and does not support recommendation of the use of this treatment approach at this time.

Summary

Before the initiation of screening and treatment for cervical cancer precursors, approximately 3% to 4% of women were destined to eventually develop cervical cancer. During the last 50 years the rate of cervical cancer incidence and mortality has decreased by more than 75% primarily because of the widespread availability of cervical cytologic screening and of treatment for documented cervical precancer. Successful screening of the entire population and appropriate treatment of lesions could theoretically reduce this risk to one tenth of the risk of an unscreened population [7,28]. The relatively recent understanding of the etiology of cervical cancer precursor lesions and of the immune response to them has given new direction to management options that incorporate healthy habits and dietary measures as part of traditional ablative or excisional treatment options.

As we look to the future we can expect that new markers that more specifically identify individuals at-risk for cervical precancer and cancer will be developed and take precedence in cervical screening. At the same time, treating the cause of these lesions, rather than the result, should provide less traumatic and more successful therapies. To this end, harnessing the immune system through immune response modifiers and HPV vaccines seems to be on the horizon, as do new chemopreventative approaches. Of all human cancers, only cervical cancer, once the second most common cancer among women, stands on the threshold of being virtually eliminated.

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Management of women with cervical cancer precursor lesions

J. Thomas Cox, MD

Gynecology Clinic, Health Services, University of California, Santa Barbara, CA 93110, USA

There are a number of guiding principles that can be applied to the management of all women with cervical intraepithelial neoplasia (CIN), as well as for women not found to have CIN but who are likely to be at some continued risk due to the probability that the inability to document disease at colposcopy most likely reflects cellular changes secondary to human papillomavirus (HPV) that may regress, persist, or progress. Although the management guidelines discussed in this article are either evidence-based or are based on expert opinion where the published literature is scant or inconclusive, some of the ''guiding principles'' are not based on definitive scientific validation; however, based on discussion in ''Management of precursor lesions of cervical carcinoma: history, host defense, and a survey of modalities'' in this issue, they may be helpful. These include proactive dietary measures aimed to improve the immune response and educational measures designed to reduce anxiety and empower the patient with a sense of control.

Encouraging healthful habits

It is clear in ''Management of precursor lesions of cervical carcinoma: history, host defense, and a survey of modalities'' in this issue that there is enough evidence to recommend a diet rich in vitamin C, folic acid, vitamin B_6, vitamin B_{12}, beta-carotene, and indole-3-carbinols [1–6]. Although there may not yet be compelling data that diets rich in these substances increase the rate of regression and decrease the rate of persistence and progression of established CIN [7], recommendation for a balanced diet of fruits and vegetables cannot be argued against and may provide benefits. Dietary supplementation of 800 to 1200 mg/d of folic acid and 1000 to 2000 per day of vitamin C and the B vitamins may also be of some benefit, particularly if the patient does not feel that she can eat a diet

E-mail address: cox-t@sa.ucsb.edu

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rich in citrus, dark green vegetables, carrots, and yellow squashcruciferous vegetables such as broccoli, cauliflower, cabbage and Brussels sprouts . It may also be helpful to note that excesses can also interfere with the ability of the immune system to respond. Therefore, drug use, excessive alcohol intake, and inadequate sleep should all be avoided as much as possible. Most importantly, women who smoke should be advised that smoking has an adverse effect on the ability of their immune system to clear HPV and may increase their risk for cervical cancer [8,9]. Many patients given clear recommendations for healthy living will find that they have been given an opportunity to be active in their own management, giving them a sense of empowerment over their disease process. This psychological advantage may potentially outweigh the potential benefits of many of these measures.

Promoting a positive attitude

The psychological stresses that many women feel secondary to an abnormal Pap result and subsequent management can be a threat to both the emotional and the physical well-being of the patient and should be dealt with in a constructive manner. Education is the key to reducing stress related to all aspects of management of abnormal cytology [10,11]; therefore, it is important to provide information about the nature and cause of an abnormal Pap, the natural history of HPV-induced lesions, and options for management and treatment. Factors that affect the impact of the information include the timing of the information, whether it is given orally or in written material [12], the communication skills of both the clinician and the patient, the readability (and reading level) of the materials, and individual coping styles [13]. Education can be in the form of written material or frank verbal discussion. Perhaps providing both is optimal; although verbal communication establishes a relationship of trust, anxiety often blurs retention of the information, which can be supplanted by written materials perused at the patient's leisure [12]. Messages should be delivered that help the patient understand the commonness of HPV and the relative lack of risk for most who contract this virus; however, it is also important to reconfirm the importance of following through with management recommendations. Anxiety can be greatly decreased by emphasizing at the start that the Pap is a test for cells that may lead to cancer if not detected and treated and that only rarely does an abnormal Pap reflect the presence of cancer [14]. Counseling may be beneficial for women not relieved by these measures, because it has been proven to be beneficial for women with more life-threatening diseases such as cancer or AIDS [15].

Deciding on treatment or expectant management

For most women with CIN 2, CIN 3, or adenocarcinoma in situ (AIS), the only option is the choice of procedure, for only pregnant women having a

documented high-grade lesion would be followed expectantly. The one exception to this statement is the potential for following compliant adolescents with CIN 2, as many will spontaneously resolve [16]; however, the high rate of regression of low-grade squamous lesions provides the patient with the opportunity to be followed expectantly without initial treatment, provided that she is reliable for follow-up and does not find the "wait and see" approach to be more anxiety-producing than the prospect of being treated. Ideally, decisions regarding treatment versus observation are best made in consultation with the patient and after full discussion of the pros and cons of each approach. Each patient is unique and may have very different coping mechanisms that promote one approach over another. If observational follow-up is elected, it is important to ensure that the patient will continue to be in the area, or if not, that access to care will be available. If treatment is chosen, then a procedure that best fits the clinician's skills as well as the patient's needs must be chosen.

Choosing the procedure for women requiring treatment

Despite the promise of several investigative treatment approaches that either harness the immune system or target viral promoting regions, available therapies will continue for the immediate time to be limited to either ablative or excisional methods, aided by support of optimal host immunity through nutritional enhancement and removal of known immune depletors such as smoking. Once the need for treatment has been determined, the available options include cryotherapy, diathermy, laser ablation or excision, loop electrosurgical excision (LEEP), and cold knife cone (see "Management of precursor lesions of cervical carcinoma: history, host defense, and a survey of modalities" in this issue). Cryotherapy has been touted for its low complication rate, ease of use, reliability, low cost, and the possibility that there may be an advantage in leaving a large killed HPV load within disrupted cells that may enhance immune recognition [17,18]. Proponents of laser vaporization point out that this procedure is much more easily tailored to lesion location and size than cryotherapy and even holds some advantage over LEEP when lesions are very large. However, as discussed previously, laser use for the treatment of CIN has significantly diminished from its peak in the early 1990s secondary to the high expense of the equipment and its maintenance, the requirement for extensive training, and the potential for more serious injuries such as inadvertent burns. Proponents of excisional modalities such as laser cone and LEEP have stressed that occult adenocarcinoma in situ or microinvasive carcinoma have been reported to occur in 2% to 3% of specimens excised by LEEP, questioning the safety of ablative procedures [19,20].

Three nonrandomized trials and five randomized trials comparing cryotherapy and laser use have not shown statistically significant differences in clearance rates; however, there is a striking variability among these rates [20–28]. For example, recurrence or persistence has ranged from 30% for laser compared with 14% for cryotherapy in the study by Kwikkel et al [22] to 14% for cryotherapy

Table 1
Failure rates for cryotherapy, laser user, and LEEP from four nonrandomized and six randomized trials

Study	Year	Total patients	Failure rate (%)[a]		
			Cryotherapy	Laser	LEEP
Nonrandomized					
Wright [29]	1981	334	14%	3%	NA
Townsend [26]	1983	200	7%	11%	NA
Ferenczy [23]	1985	294	9%	4%	NA
Gunasekera [29]	1990	199	NA	8%	5%
Randomized					
Kirwan [25]	1985	98	17%	11%	NA
Kwikkel [22]	1985	101	14%	30%	NA
Berget [24]	1987	204	9%	10%	NA
Berget [27]	1991	187	4%	8%	NA
Alvarez [28]	1994	375	NA	4%	7%
Mitchell [21]	1998	390	24%	17%	16%

Although failure rates differ substantially from study to study, differences between each modality are not significant.
From Cox JT. Management of cervical intraepithelial neoplasia. Lancet 1999;353:857–9; with permission.
[a] Persistence and recurrence combined.

and only 3% for laser in the study by Wright [29] (Table 1). One randomized and one nonrandomized trial comparing large-loop excision of the transformation zone (LLETZ) with laser use have shown no difference [28,30]. These trials have found an association of persistent or recurrent disease with three prognostic variables: large lesion size, high lesion grade, and endocervical gland involvement; however, the randomized trial conducted by Mitchell et al [21] assessed the effectiveness of cryotherapy, laser use, and LEEP in the management of CIN stratified by prognostic variables that may have accounted for the differences in failure rates noted in these previous studies. The data provided irrefutable evidence of the comparable efficacy of each of these methods, as did a recent review of 23 randomized and quasirandomized trials of seven different surgical treatments in women with CIN [30]. In the Mitchell et al study, the complication rate varied slightly (2% for cryotherapy, 4% for laser use, and 8% for LEEP) but was not statistically significant [21]. All groups had less than 1% infection and a less than 1.5% incidence of cervical stenosis. The only major difference in risk was an increased rate in postoperative bleeding for LEEP of 4.6%, compared with 2.3% for laser use and 0% for cryotherapy. Persistent and recurrent disease combined was reported to be slightly more common for women treated with cryotherapy (24%) compared with laser use (17%) and LEEP (16%), but the difference was not statistically significant. After controlling for endocervical gland involvement, lesion size, grade and location, HPV status, age, and smoking history, only lesion size was statistically associated with persistence. Lesions involving more than two-thirds of the surface of the cervix were more than 19 times more likely to be persistent post-treatment than smaller lesions, regardless of the treatment modality chosen. In contrast, recurrence—defined as new disease

detected following a previous negative post-treatment visit—was more than twice as likely for women with any of the following characteristics: over 30 years of age, HPV 16 or 18 positive, or having a history of previous treatment for CIN [31]. An increased recurrence rate in women previously treated for CIN and in older women is consistent with the premise that women in these groups have some decreased ability to clear HPV.

Reports of an increased rate of cervical cancer following the switch from excisional to ablative procedures have prompted some investigators to conclude that direct punch biopsy is an inadequate endpoint technique for judging the severity of an epithelial lesion, and therefore may not always be a reliable endpoint for determining treatment [32]. Buxton found that 47% of the 132 women who had discordant biopsy and loop specimens had a more severe lesion, including three cases of AIS and one microinvasive carcinoma [32]. Mac Indoe also questioned the accuracy of colposcopy-guided biopsy when 2 of 196 patients with CIN considered to be suitable for ablative treatment were found on laser cone to have microinvasive carcinoma; another patient had AIS [33]. Sze et al [34] reviewed 15 studies involving 1975 patients comparing the accuracy of colposcopic biopsy with later excisional treatment. Although they documented that only 1% to 10% of the patients in each of these studies had lesions more severe on excision than they had on pretreatment biopsy, 16 invasive cancers were missed by pretreatment colposcopically directed biopsy. Protection from ablation treatment of a microinvasive or early invasive cancer is afforded in part by treatment guidelines that restrict ablative procedures to lesions not extending more than 4 to 5 mm into the canal, because large high-grade lesions that are at greatest risk for harboring an occult invasive cancer are more likely to extend the farthest into the canal [35].

One major concern regarding treatment of cervical cancer precursor lesions has always been the potential for long-term effects on fertility. Theoretically, treatment of CIN could impair fertility by causing cervical stenosis, decreasing cervical mucus, decreasing cervical competence, or by tubal scarring secondary to post-treatment infection [18,36]; however, an extensive review of the entire literature on the impact of cryotherapy, laser use, LEEP, cold knife cone, and electrocoagulation diathermy on fertility found no significant alteration of either fertility or pregnancy for any procedure other than cold cone biopsy, which did have increased rates of second trimester abortions, preterm labor, and low birth weight infants related to the volume and cephalocaudal length of tissue removed [37]. In contrast, single laser conization and LEEP procedures generally produce smaller excised specimens and have not been found to be associated with problems with pregnancy.

Treatment guidelines have generally advocated excisional procedures for lesions with gland duct involvement, for high-grade lesions irrespective of lesion size, or any large lesion extending beyond two quadrants; however, the randomized trial reported by Mitchell does not appear to support this approach, because it provides the clearest evidence that, other than cost and concern over the small, but not zero, risk of missing AIS or microinvasive cancer almost all of the other pros

and cons of cryotherapy, laser use, and LEEP in the management of CIN would appear to have little consequence in the choice of procedure. It is clear that clinician and patient preference and cost considerations, not concern over potential differences in efficacy, complications, or fertility, should dictate choice of treatment. The one caveat to this generalization is the detection of one unsuspected microinvasive cancer in the Mitchell study in the LEEP group (0.77%) [21]. This continues to give credence to treatment protocols that designate excision of large high-grade lesions, which are at greatest risk for microinvasive loci.

Management guidelines for treating women with CIN

Recently published guidelines on the management of women with abnormal cervical cytology and the treatment of women with cervical cancer precursor lesions will likely guide clinical care in this area for some time [16,38]. Therefore, the evidence-based Guidelines developed at the September 2001 American Society for Colposcopy and Cervical Pathology (ASCCP) consensus workshop will provide the basis for the discussion of both observational and active treatment management scenarios for these lesions [16]. The Guidelines are considered "consensus" guidelines because the meeting was attended by voting representatives from 29 participating professional organizations, federal agencies, and national and international health organizations. All guidelines were eventually approved by more than two thirds of the representatives, and the majority was approved by 70% to 90%.

Management of women with $\leq$CIN 1

Follow-up without treatment (observation or expectant management)

For nearly 30 years, the theory that CIN represented a disease continuum with progressive potential from CIN 1 to CIN 3 promoted the treatment of all grades of precursor lesions [39]; however, the recognition that women with normal immunity suppress HPV-induced low-grade lesions in at least 70% of cases, and that the CIN spectrum does not reflect progression from CIN 1 to CIN 2 to CIN 3 [40–43], has dramatically changed this approach. In addition, interobserver variability studies have introduced uncertainty as to the accuracy of a histopathological diagnosis of CIN [44]. All these issues have coupled with the present inability to predict the biological potential of a CIN 1 lesion to complicate the management of women with low-grade lesions. Expectant management of women with CIN 1 has been shown to have a risk of detection of CIN 2, 3 during follow-up that is similar to the risk of CIN 2, 3 in atypical squamous cells of undetermined significance (ASC-US) cytology [45]. Even women referred for evaluation of low-grade squamous intraepithelial lesion (LSIL) or HPV-positive ASC cytology and not found to have CIN 1 at colposcopy are at continued increased risk for CIN 2,3

until proven by conscientious follow-up to be persistently clear, either on repeat Pap or by HPV testing [45]. Therefore, management algorithms will be similar for women with documented CIN 1 and for women referred for Pap interpretations highly associated with high-risk HPV types but not found on initial colposcopy to have a lesion that correlates with the Pap. In other words, these findings suggest that women with $\leq$CIN 1 in the follow-up of HPV positive ASC-US and any LSIL should be managed similarly; therefore, the move over the last 10 years toward expectant management of women with documented CIN 1 and the traditional expectant management of women referred for the evaluation of LSIL- and HPV-positive ASC Paps but not found to have CIN at colposcopy come together.

For some, the downside of long-term observation is continued anxiety over the uncertainty, while for others the opportunity to avoid treatment is a positive. Observational management protocols can increase the workload of clinic staff in following large numbers of women with minor cervical abnormalities with as great a diligence as women with more significant lesions. In addition, prolonged follow-up increases the number of office visits, patient notifications, and treatment for the 20% to 30% that have lesions that persist or progress. Jones et al [46] determined that accelerated repeat cytology with or without colposcopy is effective in detecting the development of major grade lesions; however, data from the Ascus/LSIL triage study (ALTS) trial differed with this finding unless the cytology threshold for referral back to colposcopy was set at $\geq$ASC, and at this threshold, so many women require repeat colposcopy that this approach may not be cost-effective. Some follow-up protocols recommend periodic colposcopic examinations in addition to repeat cytology to decrease the risk that CIN 2, 3 could be missed; however, this approach is costly, and colposcopy is known to increase anxiety related to the exam [47]. Most $\leq$CIN 1 cases that spontaneously resolve do so before the end of 2 years of follow-up; however, longer follow-up is not considered unsafe for compliant women even though progression to CIN 2, 3 is more likely the longer high-risk HPV persists [48].

Using ALTS longitudinal follow-up data, Guido et al [45] compared the sensitivity for detection of CIN 3 or cancer (CIN 3+) and the percentage of re-referral to colposcopy of various strategies for the management of women referred for the evaluation of LSIL or HPV-positive ASC and found initially to have only CIN 1 or less (Table 2). The cumulative CIN 3+ diagnosed over the 2 years of follow-up in this group was 7%. All management strategies with high sensitivity to detect CIN 3 were found to result in re-referral to colposcopy of over half of the women. HPV testing at 12 months had approximately 95% sensitivity for CIN 3 with re-referral of 55% of women, whereas repeat cytology at 6 and 12 months cumulatively detected approximately 85% of the CIN 3 with rereferral of 60% of women to colposcopy and an extra office visit for all. This data, combined with evidence that only persistent HPV progresses to CIN 3 [49,50] and that testing for high-risk HPV detects most CIN 3 [51] has established a single repeat HPV test as an alternative to two repeat Paps in the follow-up of women with $\leq$CIN 1 [16].

Table 2
Follow up of women referred for the evaluation of LSIL- and HPV-positive ASC and found to have ≤ CIN 1 at initial colposcopy

Test	Interval	Sensitivity for CIN 3+[a]	% referred to colposcopy
HPV test	12 mo	95%	55%
Repeat Pap ≥ ASCUS	6 and 12 mo	85%	60%

HPV, human papillomavirus; ASCUS, atypical squamous cells of undetermined significance.
Women referred for the evaluation of LSIL and HPV positive ASC found to not have high-grade (CIN 2,3) disease at initial colposcopy (women with ≤ CIN 1) continued to be at increased risk of CIN 3 over the 2-year ALTS follow-up. Both management strategies re-referred over half the women to at least one more colposcopy. A single HPV test at 12 months detected 95% of all the CIN 3 that would be found over the 2-year folow-up, whereas two repeat liquid-Paps detected 85%.
Data from Guido R, Solomon D, Schiffman M, Burke L. Comparison of management strategies for women diagnosed as CIN 1 or less post-colposcopic evaluation: *Data from* the ASCUS and LSIL Triage Study (ALTS), a multicenter randomized trial [abstract]. J Low Gen Tract Dis 2002;6:176; with permission.

[a] % of all CIN 3+ detected over the 2-year follow-up of women with ≤ CIN 1 referred originally for the evaluation of LSIL- or HPV-positive ASCUS.

Several issues related specifically to expectant management should be taken into account when deciding whether to use follow-up rather than treatment in any particular scenario. These issues include: interobserver variation in the reading of cytology and histology and in colposcopic interpretations; the rate of resolution or persistence of CIN 1 and of subsequent detection of high-grade CIN and cancer; the perceptions and preferences of the patient; the risk of loss to follow-up; the cost of treatment options in comparison with expectant management; and whether the colposcopy was satisfactory or unsatisfactory.

Interobserver variability

There is significant observer variation noted in all modalities (cytology, colposcopy, and histology) used to determine whether a woman should be offered treatment for CIN, and if so, by what method. Significant differences in interpretation of CIN 1 have been reported from numerous studies [44,52,53], accounting for overtreatment of some women with no disease and undertreatment of others with high-grade CIN either missed on colposcopic biopsy or misclassified by under-grading of the histology. Stoler and Schiffman [44] reported only 43% agreement between the clinical center pathology diagnosis of histologic CIN 1 and the expert pathology review committee (Table 3). Disagreement was greatest between CIN 1 and normal, with 41% downgraded to normal by the review panel and 13% upgraded to CIN 2,3. In contrast, Roteli-Martin et al [54] reported that interobserver agreement between two pathologists in the classification of histology as no HPV lesion, CIN 1, or CIN 2-3 was substantial (Kappa 0.638) when based upon the combined presence of binucleation, multinucleation, abnormal mitosis, koilocytosis, and dyskaratosis. The authors concluded that the

Table 3
Interobserver variability, ALTS: original versus quality control group diagnosis divided into "disease" versus "nondisease" at different binary cutpoints

Disease cutpoint	Specimen type	Kappa
WNL versus ≥ ASCUS	Enrollment monolayer	0.56 (0.54–0.58)
	Colposcopic biopsy	0.47 (0.44–0.50)
	LEEP histology	0.46 (0.36–0.55)
≤ ASCUS versus ≥ LSIL	Enrollment monolayer	0.64 (0.62–0.67)
	Colposcopic biopsy	0.55 (0.52–0.58)
	LEEP histology	0.52 (0.44–0.60)
≤ LSIL versus ≥ HSIL	Enrollment monolayer	0.51 (0.46–0.55)
	Colposcopic biopsy	0.68 (0.64–0.71)
	LEEP histology	0.69 (0.63–0.75)

Interobserver variability is similar for cytology, colposcopic biopsy, and LEEP histology as demonstrated by only moderate agreement between the clinical center and quality control pathology group diagnosis for each of these modalities. Moderate agreement is reflected by a kappa of 0.41–0.60, with substantial agreement between 0.61–0.80. The worst agreement is between normal and atypia, but agreement between atypia and low-grade lesions and between low-grade and high-grade lesions is only marginally better.
Adapted from Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA 2001;285:1500–5; with permission.

histologic interpretation of CIN 1 was reproducible and did not promote unnecessary treatment of minor abnormalities; however, others would disagree with this assessment and contend that the individuals reading the cytology and histology slides and the colposcopist both heavily bias the diagnosis and subsequent management decisions [55].

For women referred to colposcopy for the evaluation of definitive HPV findings, such as HPV-positive ASC and young women with LSIL, the line between normal and low-grade is often very fine. Heatley [56] noted that the outcome of follow-up of 43 women with minor histologic changes that were suggestive but not diagnostic of HPV and of 30 women with histologic CIN 1 was similar in the two groups in terms of regression to normal, persistence of low-grade disease, or subsequent detection of high-grade CIN. Heatley concluded that CIN 1 and minor histologic alterations not definitive but likely due to HPV should be managed similarly. Long-term ALTS follow-up data appear to confirm this conclusion [45].

Evaluation of interobserver variability in real-time colposcopic impressions would also likely be significant, but for the obvious reason of respect for patient privacy, colposcopic interobserver variability studies using multiple observers have been limited to the evaluation of colpophotographs or video or digital images. Several studies have reported that considerable interobserver variability and variation in diagnostic accuracy in scoring cervical images, particularly at the lower end of the spectrum of abnormality, has the potential to lead to overtreatment [57,58]. This risk is increased by the tendency of colposcopists to rely considerably on the referral Pap interpretation in formulating the colposcopic

impression. Interobserver variability in colposcopic interpretation may be even a bigger clinical problem than similar variability in the reading of cytology [57,58]. Inter- and intraobserver variability in interpreting colposcopic images and selecting the site for biopsy was reported by Hopman et al [59] to be in the same moderate range as observer variation in other subjective diagnostic tests such as cytology and histopathology. Sideri [60] reported on the observer variability in the colposcopic evaluation of women with LSIL cytology, noting that the detection of CIN 2 and 3 is influenced by the subjectivity of the colposcopic examination and should be considered when planning optimal management for patients referred for the evaluation of low-grade cytologic abnormalities. One evaluation of 813 women with a median age of 29.0 years (range: 15–71 years) referred to colposcopy with their first abnormal Pap determined that the sensitivity for detecting CIN 2 or 3 by cytology was 41%, whereas that for colposcopy was 67% and the combination of both missed 25% of the high-grade lesions [61]. Colposcopy underestimated more small lesions and more CIN 2 than CIN 3. The finding of significant underdiagnosis of high-grade lesions in this and in other studies [62] and of only moderate interobserver agreement in the colposcopic impression [44] raises the issue of the reliability of initial diagnosis of low-grade CIN and of the follow-up methods for women not treated for CIN 1.

Sensitivity and specificity of follow-up tests

Evaluations of the sensitivity of cytology in the follow-up of Paps already detecting abnormal cells have been severely biased by many limiting factors. Several recent meta-analyses have attempted to limit these factors by eliminating all but the least biased studies [63–65]. One meta-analysis determined that repeat cytology had a mean sensitivity of 66%, somewhat better than the mean sensitivity of 58% for primary screening Paps [64]. Another meta-analysis, commissioned by the Agency for Health Care Policy Research, evaluated 12 of the least biased articles in the literature on the accuracy of the Pap in both primary screening and in following women with prior abnormal Paps [65]. As would be expected, the threshold for referral to colposcopy determined the sensitivity and specificity of repeat cytology, with a threshold of $\geq$ ASCUS resulting in the highest sensitivity but the lowest specificity. Sensitivity fell at the threshold of referral of $\geq$ LSIL, and considerably more so with $\geq$ high-grade intraepithelial neoplasia (HSIL), with specificity improving at each elevated threshold. The overall sensitivity ranged from 30% to 87% and specificity from 86% to 100%.

These meta-analyses do provide information on the number of repeat Paps required to provide adequate reassurance that no disease is missed. Most Pap follow-up protocols have recommended three or four normal repeat Paps at 4- to 6-month intervals before returning the patient with $\leq$ CIN 1 to routine screening; however, the ALTS trial demonstrated that two repeat liquid-based Paps (ThinPrep Pap, Boxborough, MA) detected 85% of the CIN 3+ that was found during the 2-year follow-up of women with $\leq$ CIN1 [45]. The ASCCP Consensus

Conference used this higher sensitivity (85%) as a basis for recommending that these women can return to routine screening after two repeat normal cytologies; however, some clinicians may be concerned that 85% is still too low and that even with liquid-based cytology the Pap needs to be repeated three times to minimize the risk of missing significant disease. It is this false-negative rate and the rate of "loss to follow-up" that largely determines the success of any postcolposcopy repeat Pap management option, while the rate of abnormals on repeat largely determines the cost-effectiveness.

The data by Guido et al [45] provide evidence that 95% of the CIN3+ detected in women with $\leq$ CIN 1 postcolposcopy can be detected by a single HPV test at 12 months. This would appear to be more cost-effective than management by repeat cytology because of the decrease in the number of office visits and decreased referral to colposcopy required to obtain equivalent sensitivity. A repeat Pap threshold of $\geq$ ASC-US will refer a large percentage of women with continued repeat abnormality at 6 months, and even at 12 months many women ultimately destined to clear CIN 1 will continue to have cellular change that will be reflected in their Pap. This dilemma could be resolved if increasing the threshold of referral back to colposcopy of $\geq$ LSIL was safe. Indeed, this would clearly be the case for most women with $\leq$ CIN 1 being followed by cytology. However, one retrospective study in the UK of cytological surveillance of 1781 women with the UK equivalent of LSIL (mild dyskaryosis) revealed that only 3 of the 10 carcinomas that occurred during follow-up were in the 434 women who were lost to follow-up. The remaining seven were not detected, despite reasonable Pap follow-up using higher thresholds for referral to colposcopy [66].

Rate of resolution, persistence, and subsequent detection of high-grade CIN and cancer

The study of the natural history of CIN has always been hampered by interobserver variability in colposcopic impressions and in cytologic and histologic interpretations, and by the potential alteration of the course of the disease by biopsy and treatment. Also, many studies have followed the natural history of lesions by cytology only, taking histologic samples only with colposcopic or cytologic interpretation of progression and adding the variability in sensitivity and specificity of cytology and of colposcopy to the mix. In addition, following the natural history of progression of CIN to invasion can happen only by misfortune, relegating this important step to in vitro experiments. Therefore, statistics on rates of progression, regression and persistence depend on imperfect data. Several retrospective studies following the natural history of CIN long-term by cytology alone documented regression of low-grade Pap abnormalities in approximately 50% of patients [46,66,67]. A prospective follow-up of 89 women with histologic CIN 1 by cytology and colposcopy performed every 3 months in a low-risk private practice population documented spontaneous resolution in 75% within 1 year [68]. The median time to resolution was 9 months (n = 38), and only one CIN 2 appeared to represent progression (1.1%); one patient was lost to

follow-up. The author concluded that expectant management of CIN 1 is safe and cost-effective in a reliable patient population.

The most widely quoted statistics on the natural history of CIN are those compiled by Ostor [43] in a 1993 review of all papers on this subject published between 1952 and 1992, including data on 4504 patients with CIN 1. The data varied widely depending on study size, length of follow-up, and whether the diagnosis was established by biopsy alone, cytology alone, or a combination of the two. When stratified into various grades of severity, 57% of CIN 1 regressed to normal, 32% persisted, and 11% either progressed or were subsequently found to have CIN 3 not detected by initial evaluation. The author noted that in the majority of these studies the diagnosis was established by biopsy, which may have altered the natural history of the lesion by increasing the rate of regression. Despite the concerns over the imperfect measures in many of the studies, 2-year follow-up in the ALTS trial of untreated CIN 1 appears to confirm the "progression" rate noted in the Ostor study, and a recent meta-analysis of the natural history of CIN 1 derived similar statistics [69].

A prospective evaluation of women with $\leq$CIN 1 evaluated a cohort of 220 women whose disease was neither biopsied nor treated at the initial examination [70]. All had HPV DNA testing by PCR and were followed with interval colposcopic examinations and repeat Pap tests for a limited time period. Biopsy confirmed progression to CIN 2,3 occurred in 41 (18.6%), persistence of CIN I/condyloma in 41 (18.6%), and regression to $<$CIN I/condyloma in 138 (62.7%). HPV DNA positivity and current oral contraceptive use were the only independent predictors of progression when age at diagnosis, the number of follow-up visits, and time to progression were controlled. The study highlighted the clinical role that HPV testing can play in the observational management of CIN 1 lesions.

Follow-up of women with low-grade CIN must take into account the potential for a high-grade lesion to develop during follow-up or that a high-grade lesion may already exist that was not correctly diagnosed by the referral cytology, the colposcopy or biopsy placement, or histologic evaluation. Several studies have shown that women with low-grade CIN followed cytologically are at higher risk than women with normal cytology for high-grade CIN and cervical cancer over the long term [66,71,72]. Therefore, expectant management of CIN 1 is not totally without some risk. Most of these have occurred in women lost to follow-up, highlighting the importance of evaluating the patient to be followed for the likelihood of compliance and ensuring that the follow-up system is tight and reliable.

Loss to follow-up

Even with the most intensive follow-up reminder system, compliance can be a problem; furthermore, the longer the follow-up, the more likely that compliance will not occur. The requirement that women having observation for CIN 1 be followed at 6-month intervals for a minimum of 2 years ensures that a significant proportion will not have adequate surveillance because of lack of compliance.

This factor must always be taken into account when deciding whether to treat or to follow low-grade cervical disease. One study evaluated compliance for 219 low-income women with biopsy-proven CIN 1 who were given the choice of cryotherapy or cytology surveillance, followed by cytologic testing every 4 months until three consecutive results were normal [73]. Only 37% of the total group successfully completed follow-up at the clinic, 30.1% transferred or were referred, and 32.9% were lost to follow-up, indicating that successful completion of a commonly recommended protocol for serial cytology follow-up is very low.

A number of other factors have been identified that affect adherence to follow-up among women with abnormal Paps. Some of these factors involve individual characteristics, such as demographics, social support, lack of understanding, and fear [74]. Compliance may also be related to factors that pertain to the health care system, such as clinic hours, sensitivity of staff and providers, cost, and the reliability of the follow-up notification system. In the cases of cervical cancer that developed during follow-up of mild dyskaryosis, Robertson et al [66] identified several reasons for loss to follow-up, including poorly organized call and recall systems, transient populations, and a host of psychological and societal barriers. Intuitively, it would be expected that the more return visits required for adequate reassurance, the more likely that loss to follow-up would occur. A number of strategies have been successful in improving follow-up, including telephone counseling, educational programs, and economic incentives [74].

The management recommendations for women with CIN 1 made by both the 2001 ASCCP Consensus Conference and by the 1992 British National Health Service recommend treatment if the patient is considered at risk for noncompliance [16,75]. To achieve the maximum compliance, patients must be given a comprehensive explanation of the need for regular follow-up and the risks of not doing so. In addition, a sound follow-up tracking and notification system must be in place, and the patient should not be from a population known to be transient.

Patient perceptions and preferences

Cervical screening can take women who have no clinical signs or symptoms of disease and by virtue of a positive Pap result make patients out of people who otherwise feel well [13]. The literature is replete with evidence that notice of an abnormal Pap result creates significant anxiety [10,47,76,77]. Adverse reactions include feelings of vulnerablity, helplessness, and anger, as well as fear about fertility, cancer, and mortality [13]. Additionally, partners often worry more about the abnormal result than the woman receiving it [78], particularly now that an abnormal result is known to often be secondary to a sexually transmitted virus. The result can be threatening to sexuality and relationships as well as to the recipient's feeling of well-being. Bjork and Hagstrom [10] noted that the level of anxiety generated does not vary significantly with the level of cytologic abnormality in that the number of women who reacted with medium to strong anxiety when given a cytologic result of CIN 1 (15/22) did not differ from those given the result of CIN 2/3 (15/21); however, appropriate counseling did decrease

anxiety for 64% of the women with a low-grade Pap result compared with only 38% of the women given a high-grade result. Despite the low-risk of a low-grade Pap result, 45% of these women worried that they had cervical cancer.

Numerous studies have shown that most women have very little information on HPV and even less on the association of this virus with abnormal Paps, cervical precancer, and cancer [79]. Some studies have shown that women given counseling about HPV did not suffer psychosocial consequences when given a positive result on HPV testing [80]; however, most women feel that they had inadequate counseling about HPV that resulted in adverse emotional and sexual repercussions [81]. In a study by Ramirez et al [79] of college-age women given the hypothetical circumstance of testing positive for HPV, emotions selected by more than 50% of the group included feelings of fear, anger, guilt, anxiety, confusion, filthiness, regret, and panic. Women with the highest degree of anxiety were most likely to refuse to be tested for HPV. Perception of risk amongst those electing to be tested did not correlate with actual HPV results, because approximately 35% of the women tested positive whether or not they perceived themselves to be at risk.

Preparation by appropriate counseling has been shown to reduce anxiety for women in the cervical cytology screening program [47,76]. One clinic noted that an explanatory video before colposcopy or treatment significantly reduces anxiety [47], and others have shown similar favorable results by providing educational brochures [82]. Women receiving educational brochures when notified of their abnormal Pap report were significantly less distressed on the Brief Symptom Inventory and were less anxious about the abnormal Pap smear, the fear of cancer, and their future health than women who did not receive the brochure; furthermore, these women performed significantly better when answering questions about dysplasia, colposcopy, and recommended follow-up.

Once a cervical abnormality has been documented, both treatment and nontreatment management options may generate considerable anxiety. Patient preference for any particular management option is often driven by the degree of anxiety experienced, with the most anxious women more likely to choose the most active management strategy [83]. For some women, concern over potential pain and misperceptions about the real risk of complications and threats to infertility may result in the option of treatment generating the most anxiety, while others may find the ''wait and see'' approach to be the most daunting. Most women want to participate in decisions about their care but find the information confusing and often difficult to obtain from their clinician [84]. The inherent power structure of medical practice combined with time pressures often make it difficult for doctors to provide the detailed information and reassurance patients need when a diagnosis is distressing and treatment is daunting [84]; however, preparatory information given either verbally or in writing before treatment of a documented lesion has been shown to significantly reduce anxiety and to promote improved recovery [12]. Given that both conservative treatment and close surveillance are reasonable options in the management of compliant women with CIN 1, with no conclusive evidence to support one strategy over another, the informed preference of women affected by these decisions should be of primary importance.

Cost of treatment in comparison with observation

Analysis of the cost of various management options is always problematic because of the variability in local factors. Hamm et al [85] performed a clinical decision analysis comparing treatment of women with CIN 1 with expectant management. The authors used several baseline assumptions to conclude that expectant management led to a better outcome for the 57% of patients who have spontaneous resolution of their disease requiring no treatment, but that the delay in treatment for some having expectant management required more costly surgical procedures (loop electrosurgical excisional procedure, conization, or, rarely, hysterectomy) than did those treated with immediate cryotherapy. A number of unknowns, however, prohibited a valid cost comparison. Part of the difficulty in determining the cost of one management strategy compared with another has been the lack of a universal approach to expectant management. Most expectant management includes a repeat cytology and office visit every 6 months, but there is significant variability in what threshold cytologically initiates repeat colposcopy, and whether colposcopy is routinely built into the follow-up of these women, and if so, at what interval. The option of using HPV testing may also significantly alter the cost–benefit analysis.

Satisfactory versus unsatisfactory colposcopy

The risk of missed disease in the cervical canal is virtually nonexistent when the colposcopy is satisfactory; however, when the colposcopy is unsatisfactory and either negative for a lesion, or the lesion is only CIN 1 and extends into the canal beyond colposcopic visualization, expectant management may leave an undiagnosed high-grade lesion, or even cancer, in the canal. Although there is limited data regarding the risk of occult CIN 2+ in the canal when evaluation of LSIL and ASC-HPV positive cytology is unsatisfactory and no high-grade lesion is identified on the portio, the consequence of missing an occult invasive cancer has prompted some to call for a diagnostic excisional procedure in this setting, particularly for women who are referred for LSIL [86]. One study of women having a cervical excisional procedure for CIN 1 reported that 12% of women who had unsatisfactory examinations accompanied by CIN 1 in the endocervical curetting had CIN 2,3 detected in the cone specimen, and even 7% of those with a negative ECC had high-grade CIN detected in the cone specimen [87].

Expectant management of women with ≤CIN 1: management algorithm

All of the above considerations promote giving women with CIN 1 the option of expectant management if the patient is most comfortable with this approach and is deemed compliant, and if the facility responsible for follow-up is equipped to manage the patient long-term with a tight call and recall system. Because women who are referred for the evaluation of HPV-positive ASC-US and LSIL but who are not found to have CIN at colposcopy are at similar risk for sub-

sequent detection of high-grade CIN as women with biopsy-proven CIN 1, these women may be managed expectantly with similar protocols. Therefore, based on the Guido et al [45] data, when these prerequisites are all confirmed, management of $\leq$ CIN 1 can be by either repeat cytology every 6 months or a single HPV test in 12 months, with return to repeat Pap in 12 months for all women who test negative on two consecutive repeat cytologies or one HPV test negative for high-risk types. Any repeat abnormal Pap at a threshold of $\geq$ ASC-US, or a positive HPV test, would be best referred to colposcopy.

Active management of women with CIN 1: treatment algorithm

Treatment of the cervix in women with CIN 1 continues to be an option. Some will argue that limited sampling of the cervix by cervical biopsy leaves too much of a risk of missed occult CIN 2,3 to not proceed with treatment, even when the colposcopy is satisfactory. For example, one large interlaboratory comparison of cytology, biopsy, and cone specimens found that women having a cervical excision procedure for CIN 1 had high-grade CIN in the loop, laser, or cold cone specimen in 23% to 55% [88]. CIN 1 can be treated by procedures that either ablate or excise the abnormal tissue and the entire transformation zone. Treatment choice for women not previously treated can be based on clinician and patient preference and whether or not the colposcopy is satisfactory and the entire limits of the lesion can be seen. Treatment procedures have been discussed previously and include the ablative modalities of cryotherapy, electrofulguration, laser ablation, and the excisional modalities of LEEP, laser and cold-knife conization. However, women previously treated may have "skip" lesions within the canal; therefore, retreatment of women for recurrent or new disease is best done using an excisional method.

Controversy continues as to whether an endocervical sampling procedure should be performed before any ablative procedure when the colposcopy is satisfactory. The controversy is generated partly because the sensitivity of endocervical sampling is poor and partly because a positive endocervical sampling in the setting of a satisfactory colposcopy with a normal appearing endocervical canal is exceedingly rare; however, a number of studies have shown that failure to perform an endocervical sampling procedure before cervical ablation has been associated with a higher risk of subsequent detection of high-grade disease and cervical cancer [88,90]. Others have found little value of endocervical sampling in this setting, with the likelihood of cases being reported in the absence of an endocervical sample being secondary to inability to accurately identify the squamocolumnar junction and the limits of the lesion. Nevertheless, new management guidelines being published by the ASCCP recommend endocervical sampling before any cervical ablative procedure [16].

Controversy also exists regarding the necessity for performing a diagnostic excisional procedure on most women with CIN 1 and an unsatisfactory colposcopic examination, but the ASCCP guidelines call for the management of these women by LEEP, laser, or cold cone, unless the patient is pregnant or is an

adolescent [16]; however, some physicians may feel that the low risk of missed invasive cancer in this setting warrants close follow-up rather than an excisional procedure. One noncontroversial recommendation is that ablative procedures are not appropriate for women being treated for CIN 1 when the colposcopy is unsatisfactory, even if the endocervical sampling is normal [16]. Pretreatment endocervical sampling may be helpful, but it is not mandatory when an excisional procedure is expected because the canal will be evaluated within the excised portion and an endocervical sample beyond the excisional margin may be obtained at the time of the procedure. When performed at this time, an endocervical sample has been shown to correlate with margin status and to be predictive of residual disease at a subsequent procedure [89,90].

It is important, however, to not treat or perform a diagnostic excisional procedure on women who are pregnant unless invasive cancer is present or of significant concern. In addition, it is not helpful to treat women with CIN 1 who are immunosuppressed (see section below on immunosuppression), and it is appropriate to consider following an adolescent with CIN 1 and an unsatisfactory colposcopy, because the risk of occult invasive disease at this age is virtually nonexistent, and spontaneous regression is common.

Management of CIN 2 and 3

In many other countries, CIN 2 is managed expectantly and CIN 3 is treated; however, in the United States, both are managed similarly because it is clear that the ability of pathologists to reliably differentiate between these lesions has only been moderate, and the risk of progression of definitive CIN 2 is higher than that for CIN 1, although not as high as for CIN 3 [44,66,91]. As with the treatment of women with CIN 1, women with CIN 2, 3 and a satisfactory colposcopy can be treated equally successfully by either ablative or excisional methods [21,91]; however, the risk of a missed occult cancer is much greater when the lesion is high-grade, and this risk increases with large CIN 3 lesions [20,92,93]. Therefore, large, high-grade lesions may be best treated by excisional methods that allow histologic evaluation, even though numerous studies have failed to demonstrate a significant difference in clearance rates for high-grade CIN treated by either ablative or excisional methods. Despite these statistics, many physicians prefer to have a histologic specimen when any high-grade lesion is treated, and patients with recurrent CIN 2,3 are best treated with a cervical excision procedure. As with CIN 1 lesions, the entire transformation zone should be included within the treatment area [94].

Ablative procedures are contraindicated in the treatment of women with CIN 2,3 and an unsatisfactory colposcopy, except when used only peripheral to a central cervical excision procedure to eradicate disease outside the area not excised. The requirement for an excisional procedure is due to both the need to be sure that all high-grade disease is adequately treated, and to eliminate the risk of an occult invasive carcinoma within the endocervical canal, which is up to 7% in the setting of CIN 2,3 and an unsatisfactory colposcopy [89,95]. Either cold-

conization, laser cone, or LEEP are acceptable treatment options, because they all produce comparable success rates, though cervical distortion may be more common with cold-knife conization [95,96].

Expectant management of CIN 2, 3 with repeat cytology and colposcopy is not acceptable for most women with a high-grade lesion. The only exceptions to this general rule are women who are pregnant, and some very young women (adolescents) who are considered reliable for follow-up (see discussion below). Unless there are other compelling reasons for performing a hysterectomy, this procedure is considered unacceptable as primary therapy for CIN 2, 3 [16].

CIN 2, 3 in Pregnancy

Unless invasive cancer cannot be ruled out, high-grade disease detected during pregnancy is generally followed until postpartum because of the low risk of progression to invasion and the potential to regress following delivery [97,98]. In addition, cervical excision procedures performed during pregnancy increase the risk of premature delivery and are often complicated by excessive bleeding [98,99]. Follow-up is generally by cytology and colposcopy, but timing of management protocols varies. Even though these lesions are high-grade, the relative increase in immune response postpartum and the decrease in hormonal influences that promote progression result in regression in up to 69% [100].

CIN 2 in adolescents

Adolescents with CIN 2 often have transient disease, even though CIN 2 is considered to be high-grade. In addition, cervical cancer is virtually nonexistent during the adolescent years. Therefore, the risk of a missed opportunity to treat preinvasive disease is very low for women below age 20 who are found to have CIN 2, and the possibility of spontaneous resolution is reasonably high. These issues have prompted the ASCCP Guidelines to note that observation with colposcopy and cytology at 4- to 6-month intervals for 1 year is acceptable for adolescents with biopsy-confirmed CIN 2, provided colposcopy is satisfactory, endocervical sampling is negative, and the patient accepts the risk of occult disease. In addition, the patient should be considered to be highly reliable for follow-up. In contrast, it is never considered appropriate to manage CIN 3 expectantly except when the woman is pregnant, as previously discussed.

Treatment of the immunosuppressed

Immunosuppression results in a higher prevalence of single and multiple HPV types, more rapid progression to CIH 2,3, increased rates of both CIN and cervical cancer, and high recurrence rates following treatment of CIN [101–107]. In addition, cytology does not appear to perform as well in immunosuppressed

Table 4
Sensitivity of cytology in immunosuppressed women

Author	Total CIN	Pap		Sens
		Abnl	WNL	
Maiman	32	1	12	0.08
Wright	398	65[a]	15	0.81
Tweddel	21	10	3	0.77
Korn	52	22	13	0.63
Fink	51	15[a]	8	0.65

The sensitivity of the Pap for high-grade disease in women with human immunodeficiency virus varies considerably from a low of 0.08% in the study by Maiman to a high of 81% in the study by Wright where the threshold of referral to colposcopy was set at ASCUS. In general, the sensitivity is somewhat lower than that reported by the majority of studies in immunocompetent women.

[a] $\geq$ ASCUS.

women (Table 4). Therefore, management protocols for preinvasive lesions are quite different for immunocompromised women. The New York Cervical Disease Study (NYCDS) followed immunosuppressed women with low-grade CIN for 1 year who were either treated by cryotherapy or observed without treatment. Although 56% in the treatment group remained disease-free at the end of 1 year in comparison with 24% in the observation group, treatment did not decrease the risk of progression, which was noted to be the same in both groups (T.C. Wright, personal communication, 2001) Additionally, these clearance rates are significantly less than for immunocompetent women who are generally reported to clear CIN in 85% to 92% of cryotherapy-treated cases and in and 50% to 70% of cases observed without treatment [4,43]. The same NYCDS group also found no difference in clearance rates for HIV-positive women treated with either cryosurgery or LEEP. Disappointing success rates and lack of data confirming reduced risk of progression following treatment of low-grade lesions supports the observational management of HIV-positive women with these lesions.

Response rates reported in the treatment of immunosuppressed women with CIN 2 and 3 are also much lower than for immunocompetent women and vary depending on the CD4 cell count and margin status. The risk of recurrence with a negative margin is 48.9% compared with 68.4% with a positive margin [104]. The very high rate of recurrence reported for women with negative margins is particularly at variance with the low rate reported for immunocompetent women with negative margins. Tate et al [107] reported even higher recurrence rates. Recurrence post-cold cone was 90%, and all women having either LEEP or cryosurgery recurred. Even with hysterectomy, 60% had recurrence of HPV-induced lesions at the vaginal cuff. Therefore, it appears that no procedure is more effective than another. Despite high recurrence rates for CIN, treatment appears to be effective in preventing progression to invasive cervical cancer [104].

The inadequacy of treating CIN in the immunosuppressed has promoted the evaluation of other nonsurgical modalities, either as an adjunct to surgical treatment or as primary treatment to either increase the immune response or to

decrease cellular proliferation. As discussed in the preceding article, Maiman et al reported that women treated for CIN 2 or 3 by surgical excision followed by vaginal application of 5-FU had approximately half the recurrence rate over 18 months of follow-up (28%) when compared with women randomized to the non-5FU treated observation-only arm (47%) [108]. The remarkable success of this adjunctive treatment has prompted its addition to many protocols following the treatment of high-grade CIN in the immunosuppressed.

Most clinics also administer highly active antiretroviral therapy (HAART) to increase the immune response. At Columbia University, treatment of high-grade CIN is restricted to patients who are HAART compliant due to the high (75%) treatment failure rate for noncompliant women (T.C. Wright, personal communication, 2001). Women remaining HAART compliant are treated a second time when there is recurrence, but further recurrences are followed on a long-term basis every 4 months without retreatment with colposcopy to ensure that invasion has not occurred. Hysterectomy is the final option for women if invasion is detected or appears eminent.

Posttreatment follow-up of women with CIN 2, 3

Treatment of CIN is generally successful, yet the risk for subsequent development of invasive cervical cancer remains higher than for women never having documented CIN [109–115]. One large study of 2116 women assessed the rate and duration of the risk of developing invasive cervical cancer over a period of 8 years of follow-up postablative or postexcisional treatment [111]. Thirty-three women developed invasive cancer during this follow-up period, which amounts to 5.8 per 1000 treated women and an incidence of 85 per 100,000 years. The authors concluded that even with a reduction in the risk of invasive cervical cancer by 95% following conservative outpatient therapy of CIN, invasive cancer does occur even with careful, long-term follow-up. This amounted to a risk for cervical cancer that was five times greater than that among the general population of women in the same locale in England at that time, emphasizing the need for careful follow-up of women for at least 10 years after conservative treatment of CIN. Others have suggested more intensive follow-up for women treated for CIN who are over the age of 40 or 50 because of the higher risk of subsequent detection of invasive cancer for older women reported in several studies [115,116].

In multiple studies, rates of recurrence or persistence of CIN range from 1% to 21% [20,109,117,118], with large lesions and lesions with involved margins having the highest treatment failure rates [20,110,119,120]. Risk of persistent or recurrent disease has been traditionally considered to be significantly related to whether or not the margins of the excised specimen were clear of disease [117,121,122]. Although there does appear to be a higher risk for recurrence in women with positive cone margins, studies that have used multivariate analysis to control for contributing factors have not confirmed margin status to be a

uniformly independent predictor of success of treatment. Reich et al [110] followed 390 women with positive margins after cold knife conization for CIN 3 with colposcopy, cytology, histology, and pelvic examination for a mean of 19 (range: 6–30) years. Over three quarters (78%) remained free of CIN 3, but 22% had persisting or recurrent CIN 3 and 6 developed invasive carcinoma. Five carcinomas were microinvasive, developing between 3 and 23 years, and the sixth was a stage 2B carcinoma detected at 8 years. Fifty-three patients with persistent CIN 3 were diagnosed within 1 year of conization; 25 developed recurrent CIN 3 after a median of 3 (range: 2–28) years. Persisting or recurrent disease was more common in patients in whom both the endocervical and the ectocervical cone margins were involved than in those in whom only the ectocervical or the endocervical margin was positive (52% versus 17% and 21%, respectively); however, margins of LEEP specimens are often difficult to interpret, and up to 40% are considered positive [123]. Because of this high frequency of positive margins and the fact that the majority of women with involved margins remain disease-free during follow-up, most studies have concluded that expectant management is reasonable for patients with CIN 3 and positive margins found following any surgical excisional procedure, with the requirement that these women have careful follow-up, particularly during the first year [110,123,124].

Age over 40 years, glandular involvement, and satellite lesions were determined in one study to be related to the reappearance of CIN after loop excision with clear margins [125]. Combining LEEP with laser vaporization of the excised crater base has been shown to reduce the risk of recurrence post-treatment when compared with treatment of women by LLETZ alone regardless of margin status [126]. In this study of 289 women with CIN 2, 3 treated by LLETZ and laser vaporization (cases) and 137 similar in disease, margin status, and other characteristics treated by LLETZ alone (controls), recurrence occurred in 21.4% of controls and in none of the cases. Although these results are very compelling, addition of laser vaporization following a surgical excision procedure to the standard protocol for treating CIN 2, 3 would be burdensome because of added expensive required in purchasing and in maintaining the laser equipment.

The majority of follow-up protocols in the United States recommend follow-up by Pap alone, combinations of Pap and colposcopy, or more recently, HPV DNA testing. Repeat cytology has been shown to be generally successful in detecting up to 90% of recurrent persistent high-grade lesions identified following excisional therapy [117,125,127] but this success depends upon the reliability of the patient to return for the multiple repeat Paps required to provide adequate reassurance. Typical repeat cytology protocols call for repeat Pap every 4 to 6 months for the first year and every 6 months for the second year, but the threshold for referral back of colposcopy has been quite variable. Although many perform colposcopy with repeat cytology during the first year after treatment, there has not been benefit proven in overall outcome when compared with repeat Pap-only protocols [127]. The long-term risk of invasive cervical cancer following treatment of CIN has prompted the recommendation of continuing ac-

celerated surveillance of these women for many years after treatment [114,122]. For example, Zaitoun et al [122] recommended a national policy in England of returning women with treated CIN of any grade to a normal 3-year recall after 5 years of accelerated follow-up, except for cases of CIN 3 with positive margins, for which they recommended follow-up with annual Paps for 10 years.

Numerous studies have documented clearance of HPV DNA in the majority of women successfully treated for CIN and increased risk for women remaining HPV positive, suggesting that testing for HPV DNA may be very effective in post-treatment follow-up (Fig. 1) [128–133]. One study tested 141 women for HPV with Hybrid Capture 2 who were scheduled to be treated for CIN by surgical excision and then retested these women for HPV DNA at 3, 6, and 12 months postsurgery [132]. At the 12-month follow-up visit, 94% of patients who tested positive for HPV pretreatment no longer had detectable high-risk HPV DNA. Jain et al [130] evaluated 32 women with negative postcone margins following treatment for CIN 2, 3, documenting that 100% of the 25 women testing HPV-negative posttreatment were completely clear of CIN. This high negative predictive value was documented in another study of women with positive margins after conization who underwent subsequent hysterectomy. All 23 women who were HPV DNA–negative had no residual disease in the hysterectomy specimen [131]. In addition, very high sensitivity for residual CIN was confirmed, as all 27 women having residual disease were HPV DNA–positive. The predictive value of persistently detected same-type HPV post-treatment was doc-

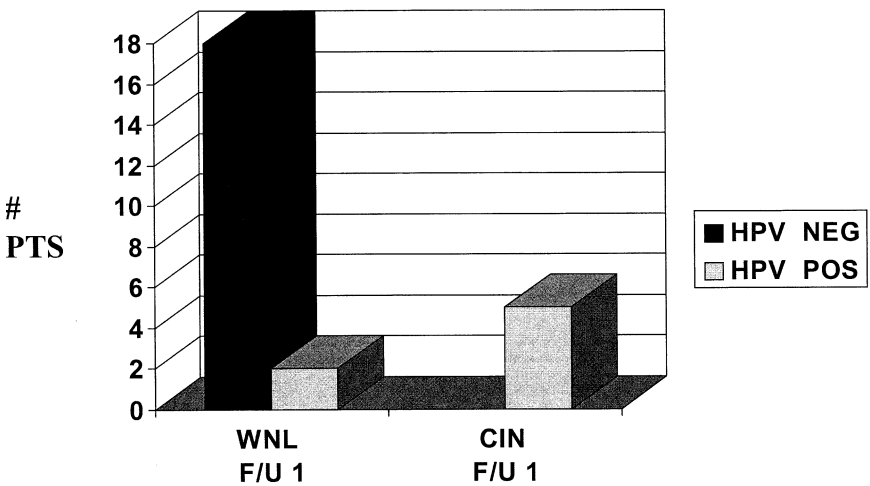

Fig. 1. Predicting reoccurrence postcryotherapy by HPV testing. Consistent with average postcryotherapy "cure" rates, 85% (20/24) of women treated with cryotherapy were HPV-negative at the 4- to 6-month follow-up colposcopy. Only one of these 20 HPV-negative women had biopsy-proven disease (CIN 1). In contrast, all four HPV-positive women had colposcopic or biopsy-proven CIN. (*From* Cox JT. Clinical role of HPV testing. In: Lorincz AT, editor. Obstet Gynecol Clin North Am 1996;23:811–51; with permission.)

umented in another study that for 5 years followed women who were initially positive for HPV 16 or 18 and treated for CIN 3 [134]. The unadjusted odds ratio for women positive for HPV 16 or 18 at both the pretreatment and at the 6-month post-treatment visit compared with women HPV-negative at both was 8.0 (95% CI 2.13–30.37). Clearly, the high sensitivity, the high negative predictive value, and the predictive value of a persistently positive HPV test all indicate that testing for persistent HPV post-treatment is likely to become the standard for post-treatment evaluation.

The ASCCP Guidelines provide two options for follow-up of women post-treatment for CIN 2, 3 [16]. The standard guideline is to follow these women by either repeat Pap or by a combination of cervical cytology and colposcopy at 4- to 6-month intervals until at least three cytological results are ''negative for squamous intraepithelial lesion or malignancy.'' If all these results are negative, then ongoing annual cytological follow is recommended, whereas any repeat abnormal Pap of ASC or greater requires repeat colposcopy if not already done. The guidelines also offer HPV testing as an alternative follow-up option for these women. The HPV test should be done no sooner than 6 months after treatment, and if positive for high-risk types of HPV, colposcopy is recommended, whereas the high negative predictive value of a negative HPV test and a normal repeat Pap allows the patient to safely return to annual Paps. It must be understood, however, that a positive HPV test without documentation of persistent disease is not a reason to repeat conization or hysterectomy.

Women having a surgical excision procedure with either a positive margin or a postprocedure positive endocervical sampling containing CIN are best followed by adding endocervical sampling to the 4- to 6-month colposcopic exam [16]. Detection of CIN 2, 3 in posttreatment follow-up is best managed by either repeating the cervical excisional procedure, or if the patient has completed childbearing, by performing a hysterectomy.

Summary

An understanding of the natural history of HPV-induced precancer and cancer, and of the immune response to HPV and to these lesions, has significantly changed the management of lower genital tract neoplasia. New management guidelines incorporate this understanding, providing a more rational approach to diagnosis and treatment. Understanding that low-grade HPV-induced lesions are not true cervical cancer precursor has fostered expectant management of women with these lesions; however, management approaches are still hampered by the inability to better predict who is at risk for high-grade intraepithelial neoplasia and cancer and who is not; this is particularly problematic in the expectant management of CIN 1. In addition, the decision whether or not to treat these low-grade lesions may depend on a number of complex factors that take into account the woman's preferences and reliability for follow-up, as well as a host of issues related to costs and the reliability of the original diagnosis and the tests used for follow-up. Management options for high-grade cervical cancer precursor lesions

are much more definitive, because the option of expectant management is not given except in pregnancy and for adolescents with CIN 2. New markers that better predict which women with high-risk HPV are at highest risk for subsequent development of a true cervical cancer precursor lesion appear to be on the horizon and may make the management of low-grade lesions as clear as present guidelines for their high-grade cousins. Until that time, understanding all the issues involved in expectant and in active management of cervical HPV-induced lesions will help provide women with the best care possible.

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Risk factors related to the development and mortality from invasive cervical cancer Clinical utility and impact on prevention

Neal M. Lonky, MD, MPH[a,b,*]

[a]*Department of Obstetrics and Gynecology, Kaiser Permanente, 1188 North Euclid Street, Anaheim, CA 92801, USA*
[b]*Department of Obstetrics and Gynecology, University of California Irvine School of Medicine, Irvine, CA, USA*

The link between evidence, outcomes, and risk of invasive cervical cancer

Although prevention of cervical cancer hinges upon the identification and intervention of a cohort of patients at higher risk for developing and dying from cervical malignancy, the definition of the ''at-risk'' group remains controversial. The use of epidemiological, clinical, and biomolecular evidence in defining low- and high-risk cohorts in the most cost effective manner possible is the foundation of the current strategy of prevention of death and suffering related to cervical cancer. Based on far-ranging retrospective studies, it is commonly believed that since its introduction, the employment of the conventional Papanicolaou (Pap) smear and deployment of an organized Pap smear screening program has contributed to a lower morbidity, mortality, and incidence of cervical cancer by 70% to 80% in the United States. This was accomplished through segregating a population based on cytologic evidence and managing patients who have abnormal results with colposcopy, biopsy, and treatment [1–4]. Following a dramatic initial decline, the incidence and death rates from cervical cancer have remained between 12,000 to 16,000 and 3500 to 5000, respectively, in the US. The benefit of the conventional Pap smear approach has been debated and the value of other preventative interventions examined [5].

The use of cytologic evidence in defining a woman who is at risk of developing and dying from cancer of the uterine cervix has limitations. Cytological screening can be augmented with newer technologies, epidemiological and medical informa-

* Department of Obstetrics and Gynecology, Kaiser Permanente, 1188 North Euclid Street, Anaheim, CA 92801, USA.
E-mail address: neal@lonky.com

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tion, concomitant clinical findings, history regarding health-seeking behavior, and long-term monitoring based upon the immune competence of the patient. The goal of secondary prevention of cervical cancer is to remove or reduce risk factors related to the development or harboring of precursor lesions and reduce or eliminate the probability of progression of such lesions toward malignancy. When the discovery point is malignancy, clinicians strive to find the disease in a treatable stage in which a long-term cure is achievable. The cost, both financial and human, must be weighed in the accomplishment of that goal. The epidemiological and medical correlates to the discovery of the ''at-risk'' lesion help define the ''at-risk'' patient, who becomes the focus of more aggressive interventions. The focus of this chapter is to explore the various risk factors related to cervical cancer and the practical context in which they can be applied. The ability to link clinical outcomes (disease presence, persistence, progression, and recurrence) with antecedent risk factors is strengthened by a new understanding of the molecular mechanisms that are responsible for malignant transformation.

Clinical aims of risk factor analysis in cervical cancer prevention

Prior to examining the factors that contribute to the risk of developing and dying from cervical cancer, one must keep in mind the settings or context in which they will be used as we decide when the application of new technology, health services, or treatments are indicated, based on good evidence.

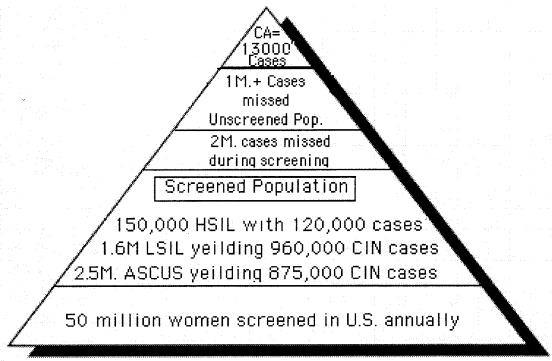
How and with what tools do we practice cervical cancer screening?

During the screening encounter, the clinician must ask if it is warranted to expend precious health care dollars on overcoming the significant error rate (false negative rate is approximately 50%) of the conventional Pap smear and pelvic examination [6]. Are extra expenditures related to adjunctive technologies or services appropriate for the entire population, or can a subset of patients be selected in which the investment is more effective? Cervical cancer, premalignant lesions, productive viral infections, and benign proliferations can share features related to the important outcome variable that one seeks to discover during screening. The goal is to identify epithelial changes consistent with neoplastic transformation (increased cell density or nuclear atypia) causing leukoplakia or whitening following acetic acid application (acetowhitening), endophytic or exophytic growths, abnormal or atypical vasculature, ulceration, or tissue degeneration from necrosis. Although the at-risk patient is identified through in vitro techniques (cytology-based) or in vivo techniques (direct visualization or other bedside techniques), the signs related to disease are ultimately confirmed by histologic sampling. A woman's risk of developing true precursor lesions is of concern, especially when accompanied by evidence of oncogenic HPV viral infection. The discovery of oncogenic HPV DNA from the lower genital tract of patients without neoplastic lower genital tract disease is problematic because true

risk has yet to be defined based on solid prospective evidence. Finding biopsy-proven premalignant lesions and early cancer and managing patients appropriately is the linchpin of any secondary cervical cancer prevention program. Any tool that finds the patient at risk of developing and dying from the disease is most valued, but women with neoplasia must first be diagnosed then their prognosis estimated based on multiple coincident risk factors. Prevention of neoplasia is the focus primary prevention, and it would ultimately obviate the need for secondary or tertiary prevention.

Who gets referred for diagnostic colposcopic evaluation when there is a high prevalence of abnormal screening tests with poor specificity in targeting patients destined to die from progression of cervical cancer precursors?

The management of low-grade cytologic abnormalities including atypical squamous cells of uncertain significance (ASCUS), suspicious visualized lesions, or evidence of oncogenic HPV DNA in the patient with normal cervical cytologic findings poses a challenge to the health care provider. These challenges might burden to the health care system. Fig. 1 shows the prevalence of precursors in the US from which invasive cancer will emerge [7]. It is impossible to predict who will develop lesions or suffer from progression to fatal invasive cancer during a future observation period. The lesions must be found and managed, even if the clinician



Assumptions : 50 million women screened annually, Pap smear sensitivity near 50%, 10% prevalence rate of CIN in screening population, 80% of HSIL colposcoped yields CIN or worse on biopsy, 60% of LSIL colposcoped yields CIN or worse on biopsy, 35% of ASCUS colposcoped yields CIN or worse on biopsy, AGUS and other reasons for referral not shown.

Fig. 1. Estimate of pool CIN precursors leading to annual incident invasive uterine cervical cancer cases. Assumptions: Fifty million women screened annually; Pap smear sensitivity near 50%; 10% prevalence rate of CIN in screening population; 80% of HSIL colposcoped yields CIN or worse on biopsy; 60% of LSIL colposcoped yields CIN or worse on biopsy; 35% of ASCUS colposcoped yields CIN or worse on biopsy; AGUS and other reasons for referral not shown. (*From* Lonky NM. Overview of cervical cancer screening: the present standard of care. In: The future of diagnostics. Atlanta, GA. p. 5–9.)

elects to observe for regression, persistence, or progression. The most direct method of finding and evaluating the "at-risk" lesion to define the "at-risk" patient is to add colposcopy during screening and sample any suspicious lesion with directed biopsy. In some cases excision might be warranted, and viral, genetic, or biochemical testing for markers linked to malignant potential can aid in treatment decisions. Several studies have documented the superior sensitivity of a single colposcopy over a conventional Pap test in screening for cervical premalignant and malignant lesions [8,9]. Because there are lesions hidden near or within the endocervix or lesions too small or subtle to detect with a single colposcopic examination, some clinicians have advocated the combination of visual inspection with colposcopy and conventional Pap smear or testing for HPV in primary screening [10,11]. Enthusiasm for the utility of colposcopy in screening for finding true precursor lesions is tempered by its low positive predictive value, high overcall rate, significant cost, and a requirement for specialized training, which is not available to all clinicians already performing screening examinations. Screening colposcopy and the colposcopic biopsy can cause discomfort, bleeding, and loss of income from missed work. This is especially true of colposcopic screening in young women because of the high prevalence of "look-alike" benign lesions and the lower prevalence of true pathology, leading to a low specificity [12]. Low specificity is also a function of the investigator's choice of the defining "gold standard" target precursor lesion (all dysplasia versus high-grade dysplasia) and the thoroughness of the evaluation method (Loop Electrosurgical Excision Procedure (LEEP) specimen or directed biopsy) used to define a "disease-free" state [13]. Established or emerging technologies—most of which aid in the identification of lesions—seek to offer the ability to detect true precursors with comparable sensitivity and improved specificity over colposcopy in an affordable manner (either alone or in combination with Pap smear screening) [14–17]. Their utility will hinge on the cost associated with any improvement in sensitivity, their widespread acceptance, and ultimate reduction in disease incidence resulting from true cancer precursor eradication.

Which preinvasive lesions are significant and require treatment? When should patients be treated and with what technique?

The goal of clinicians is to find all patients with invasive lesions, clinically manage lesions of uncertain malignant potential, and adequately treat lesions with a high malignant potential. The correlation of risk factors on a case-by-case basis is crucial in every treatment decision. Specific treatment strategies related to the evidence are covered in another section of this book. Under certain circumstances, conservative management might be a valid option, but it requires the ability to monitor patients over time. Adequate follow-up care might be related to factors that might or might not be under the patient's control. In addition, there might be a medical–legal risk associated with observation, waiting for host immune recognition, and clearance of a premalignant lesion, especially if the patient is lost during follow-up.

How and when are primary prevention interventions employed?

Should clinicians only counsel and intervene in high-risk patients or is the prevalence of disease so pervasive and the ability to detect lesions so compromised that these interventions should be applied to all patients? At what point in the life cycle are the interventions most effective? Young women are especially at risk when the probability of sexual transmission of HPV in the cervix undergoing metaplastic transformation is increased [18]. Does the benefit of intervention also prevent onset, morbidity, and mortality from other cancers or other diseases? One obvious example would be the effect of smoking cessation on the reduction of cervical cancer and other malignancies and the prevention of cardiovascular disease. Another would be the effect of safe sex practices on the reduction of HPV infection, lower and upper genital tract infection, prevention of infertility, and a reduced risk of AIDS.

What should be the long-term follow-up care of patients who are treated for or are observed to have intraepithelial lesions?

Which risk factors best predict when additional resources are not needed to track and diagnose the development of recurrent or persistent disease? As an example, new evidence suggests that typing for oncogenic HPV DNA in specimens during subsequent visits can help guide clinicians on the length and intensity of screening and colposcopic services [19].

Risk factor identification and utility

Cervical neoplasia occurs in patients who might possess some or many of the risk factors described in the sections below. The analysis of the contribution of any single risk factor can be confounded by other coincident risk factors present. In measuring the strength of the association of the variable with the untoward outcome (relative risk, odds ratio), the author prefers studies that recognize this and attempt to control for bias in the statistical analysis of data or the study design.

Risk related to oncogenic transformation from HPV infection

HPV, a member of the Papovavirus family, contains a double-stranded DNA genome of approximately 8000 base pairs in length, a nonenveloped virion, and an icosohedral capsule. The antigenic portion of the virus is the capsid protein, which is shared by all HPV viral types. The types of HPV virus therefore cannot be distinguished serologically. Identification of HPV subtypes requires DNA hybridization or sequence analysis in cases in which the L1, E6, and E7 gene sequences are unique [20]. HPV infects the host by way of penetration and insinuation into the skin and mucous membranes, causing epithelial proliferation. More than 40 types of HPV have been shown to specifically target and infect the epithelium of the anogenital tract. The most common subtypes have been stratified into three

Table 1
Oncogenic-risk grouping of anogenital human papillomavirus

Low oncogenic risk	6, 11, 42, 43, 44, 53
High oncogenic risk	16, 18, 45, 56, 58
Other high risk types	31, 33, 35, 39, 51, 52, 59, 68

From Wright TC, et al. Precancerous lesions of the cervix. In: Kurman RJ, editor. Blaustein's pathology of the female genital tract; 5th edition. New York, NY: Springer-Verlag; 2001. p. 253–324; with permission.

risk groups based on their oncogenic potential (Table 1) [21]. The definition of oncogenicity is predominantly based on retrospective evidence, whereas non-oncogenic types are almost never found in invasive cancers and high-risk types are commonly found in high-grade dysplasia and cancers. Types 6, 11, 42, 43, and 44 are found in benign laryngeal papillomas and lower genital tract condyloma and are thought to have little or no oncogenic potential. High-risk HPV types (16, 18, 45, 56, and 58) comprise the majority of types found in invasive squamous cell carcinomas of the lower genital tract [22,23]. Types 31, 33, 35, 39, 51, 52, 59, and 68 were originally placed into an "intermediate" risk group, but more sensitive tests for their presence have suggested that these types are also considered to be high-risk oncogenic viruses. Types 66 and 69 are even less common oncogenic viral subtypes [24]. The epidemiological link between the presence of HPV infection and cervical cancer has been recognized for many years [25,26]. A recent international study showed that 93% of invasive cancer specimens had DNA evidence of HPV infection as revealed by a polymerase chain reaction (PCR)-based test. This study confirmed that infection with HPV increases the risk of cancer independent of other epidemiological risk factors associated with sexual activity or infectious disease exposure [27]. Walboomers and associates further evaluated the majority of specimens, which were initially deemed HPV-negative. The specimens were retested using HPV serum antibodies and HPV DNA analysis targeting different open reading frames of the virus. In all but 6% of the original HPV negative cases that were adequate for analysis, the presence of HPV types 16, 18, 31, 33, 39, 45, 52, or 58 were confirmed [28]. It is widely agreed that oncogenic transformation is either induced by the aforementioned types of the HPV virus or HPV serves as a cofactor for facilitating transformation in the lower genital tract of women who are otherwise prone to malignancy because of other risk factors (either the cause or a co-factor).

The epidemiological association of HPV infection was strengthened significantly when it was discovered that it is possible for two oncogenes specifically coded for by HPV DNA, E6 and E7, to block or degrade the tumor suppressor genes p52 and pRb, especially in patients who were infected with subtypes 16 or 18 [26]. Oncogenic potential is related to the ability of the oncogenes to integrate into the human genome and undergo transcription and expression. Oncogenic potential differs with each viral type. Retrospective evidence of oncogenic HPV infection has been documented in almost all cases of cervical cancer. Up to 40% of asymptomatic populations tested (predominantly young women) without pre-

invasive or invasive disease also show evidence of such infection limiting enthusiasm of HPV testing in primary screening [29–32]. In some cases, the presence of common oncogenic strains of HPV without a current lesion might precede the emergence of an intraepithelial neoplasm by months or years [33]. A prior study showed that patients infected with HPV 16—but not 18, 31, or 45—the viral load as measured by viral DNA copies using a fluorescent PCR assay was correlated with a higher risk of cervical intraepithelial neoplasia (CIN) 2 or 3 [34].

The majority of women with evidence of prior HPV infection will not develop or die from cervical cancer. It is now known that the virus can be recognized immunologically and cleared by the host in the majority of individuals who are infected. The persistence of the high-risk virus types in testing over time seems to be more predictive of oncogenic transformation because it suggests that immune recognition, viral clearance, and subsequent disease regression has not occurred. This is especially true in older women who test positive and have not had recent sexual exposure to HPV.

Without widespread testing of asymptomatic patients, most women are unaware of their HPV status. The patient might provide anecdotal recollections of being treated for condyloma of the lower genital tract in the past. Less commonly, the patient might recall having an abnormal Pap smear and undergoing a test that was positive for HPV. Confirmation of oncogenic types portends higher risk, especially when there is confirmation by means of direct tissue studies from a cervical lesion after HPV testing. A positive Hybrid Capture II test (Digene Corporation, Gaithersburg, MD) performed on cytologic samples that have been exfoliated and collected serves as marker of prior infection (not necessarily active infection) with integration of HPV DNA in affected lower genital tract epithelium. Women who have immunologically cleared the virus or an associated intraepithelial neoplasm might still test positive, making routine testing for HPV as an indicator of a patient's risk of harboring disease problematic and inefficient. Recent intercourse with an HPV-infected partner (with or without obvious condyloma) might also confound evaluation if semen is retained in the vagina during screening or triage [35,36]. The positive predictive value of a positive Hybrid Capture II test in a woman with otherwise normal cervical cytology in identifying high-grade cervical dysplasia or cancer during screening is less than 10% [37].

Preinvasive lesions and the risk of invasive cervical cancer

Early studies by Broders and others showed that full-thickness noninvasive epithelial cell proliferation can be found adjacent to areas of invasive squamous cell carcinomas; this led to the term "carcinoma in situ" (CIS) [38]. When it was recognized that CIS often preceded cases of invasive squamous cell cancer, the concept that a precursor lesion could be identified and eradicated prior to the development of invasive cervical carcinoma was originated [39]. When other, less severe epithelial cell abnormalities were also found associated with the presence or development of cervical carcinoma, the concept of a natural history encompassing different grades of neoplastic proliferation, labeled "dysplasia," was introduced.

Neoplastic proliferation is defined as the histologic presence of cells with nuclear atypia and nuclear enlargement when compared with the surrounding cytoplasm. To further define dysplasia, these cells (which share features with normal epithelial basal cells) are abnormally distributed away from the basal layer within the epithelium [40]. Later refinements in classification correlated to the severity of the dysplasia (ranging from mild, moderate, or severe) and with the presence of abnormal cells present in the lower one-third, two-thirds, or throughout the cervical epithelium. Severe dysplasia and carcinoma were later considered to be diagnostically indistinguishable; this led to controversy regarding management of all dysplastic lesions because the interpretation from histologic samples suffered from significant interobserver variability [41]. Richart further characterized dysplasia as CIN [42]. Mild dysplasia corresponds with CIN 1, moderate dysplasia with CIN 2, and severe dysplasia or CIS with CIN 3. He theorized that invasive cancer could be prevented if any one of these lesions could be detected and managed in a premalignant state regardless of the grade of the lesion at the time of detection. This hypothesis was later supported by the finding that some dysplasia and virtually all CIS lesions are aneuploid, monoclonal, epithelial cell proliferations [43].

There is a renewed controversy regarding the true malignant potential of low-grade CIN lesions. Evidence linking HPV infection and genomic alteration favoring oncogenic transformation of cervical epithelium has refined the understanding that some low-grade lesions represent a productive viral infection that might not progress toward malignancy, whereas others represent a much higher risk of persistent neoplastic transformation. The oncogenic potential of various types of HPV viruses has been addressed in another section of this chapter. It should be recognized that it is plausible that high-grade lesions (CIN 2 and 3) are consistently aneuploid, highly associated with high-risk HPV types, and represent a clone of cells that could progress toward invasive carcinoma if left untreated. It is also plausible that some—or even most—of the lesions arose from the more prevalent pool of low-grade lesions (CIN 1 or condyloma), which are usually diploid or polyploid, heterogeneous with respect to harboring both low- and high-grade viral subtypes, or possibly arose directly from normal cervical epithelium. This challenges an alternative theory that high-grade lesions always arise from low-grade lesions. Later refinements of histologic classification based on the relationship of HPV infection to the development of productive preinvasive lesions, cervical adenocarcinoma, and squamous cell carcinoma led to the use of the term ''lesion'' in the classification of cytologic and histologic specimens in the development of the Bethesda Classification System [44]. The authors of this system suggested the term ''low-grade squamous intraepithelial lesion'' (LSIL) for lesions previously classified CIN 1 or koilocytotic atypia and ''high-grade squamous intraepithelial neoplasia'' (HSIL) for lesions previously classified as CIN 2, CIN 3, or CIS. The terms LSIL and HSIL are used interchangeably in cytologic and histologic reporting, leading to much confusion. Because cytohistologic correlation is poor, the natural history of lesions cannot be studied accurately unless the Bethesda terminology is restricted to cytology. Retaining the old CIN terminology for histologic specimens has been advocated because this

might prove to be useful in clinical management as clinicians continue to question the risk of progression of CIN 1 and 2 lesions toward malignancy. The implementation of Bethesda system terminology has not proven to be superior to its predecessors in further defining which patients are at higher risk of dying from invasive cervical cancer, but it has led to a more standardized approach in the management of Pap smear abnormalities.

The need to detect, manage, and treat all intraepithelial lesions remains controversial. Some experts believe that clinicians should target detection toward the indisputable high-grade cancer precursors. Even expert pathologists can disagree as to the grade of dysplasia on cytologic and histologic specimens, making the reproducibility and external validity of studies that related grade of intraepithelial neoplasia to the prognosis of the patient questionable [45,46]. This situation might be as much a function of clinicians' inability to find at least half of the cohort who harbor low-grade lesions using conventional Pap screening and the inability to predict which individuals from the cohort are at risk of neoplastic transformation. It has been established that monoclonal aneuploid proliferation occurs in some low-grade epithelial abnormalities, which confers risk toward progression of disease [47]. Clinicians have downplayed the importance of finding and managing patients with low-grade lesions based on studies with limitations. The rate of disease regression in this cohort might be overstated in the interpretation of published studies. Commonly referenced studies either (1) incorrectly defined regression by using cytologic evidence (with its inherent low sensitivity in detecting or correlating with histologic intraepithelial neoplasia) [48], (2) altered the natural history of disease by way of a local inflammatory effect or complete excision of the lesion during procurement of the biopsy [49], or (3) followed patients colposcopically for a short interval (< 12 months), failing to histologically document and correlate the colposcopic findings at the conclusion of the study [50]. The unclear distinction related to cytologic and histologic follow-up data or other limitations in study design have confounded the understanding of the natural history of low-grade disease. In these studies and others that prospectively followed a cohort of patients with histologic evidence of low-grade disease, the documented risk of progression ranged from 15% to 33%, which cannot be ignored [51]. If colposcopy is omitted and patients are followed with cytology alone, the potential of missing microinvasive cancer or high-grade disease has been documented [52]. Because of limitations related to the skills of the colposcopist or the topographical distribution of disease on the cervix, up to 50% of cases of low-grade disease might have an adjacent higher-grade lesion that is missed during colposcopy and biopsy [53,54]. This might be a function of lesion size and distribution. As more of the transformation zone is involved in neoplasia, the probability of error with directed biopsies increases and the chance of missing the highest-grade dysplastic process decreases (as discussed in a subsequent section). The risk associated with high-grade lesions is indisputable, while the risk associated with low-grade lesions remains unclear and might require further subclassification. The discovery of the highly prevalent low-grade lesion should not be ignored as inconsequential during screening, triage, and treatment.

Propensity of dysplastic lesions to exfoliate and risk of progressive neoplasia

New evidence related to the propensity of some dysplastic lesions to reliably exfoliate and provide cells for collection during conventional or liquid-based cytologic testing tempers the expectation that in vitro screening tests can find all women with cervical neoplasia [55]. The error associated with tests such as the Pap smear, which rely on cell exfoliation, can be random or nonrandom. The clinician's inability to sample over the entire ectocervical and endocervical topography and find cells from the precancerous lesion, the efficiency of transfer of collected cells to the slide, and the interpretation of the cells that make it to the slide for analysis are all prone to random error. The ability of sampling from the surface of epithelium wherein the neoplastic process is present in the lower one-third or two-thirds of the epithelium, thus defining mild and moderate dysplasia, might be limited by current screening methodology. In contrast to lower-grade dysplasia, cytologic screening is more sensitive, but it is not infallible in finding high-grade CIN or early carcinoma, wherein abnormal cells reach the epithelial surface. Keratinization, which accompanies some HPV-induced cervical neoplasia, might also serve as a barrier for exfoliation. The ability of cells to exfoliate from the cervix and be collected is related to the distribution of desmosomes and molecules, which bind cells together in the epithelium and to the basement membrane. Adhesion molecules such as the cadherins and integrins are usually distributed in the lower two-thirds of normal cervical epithelium, which allows for normal cell exfoliation at the surface (shedders). Research has shown that adhesion molecule biosynthesis is altered in dysplastic cervical epithelium [56,57]. Adhesion molecules might serve a role in cell differentiation and modification of the immune response to neoplastic transformation. In a recent study, the distribution of E-Cadherin throughout the thickness of the epithelium to the surface was present in the majority of cases of high-grade cervical neoplasia, in which an antecedent Pap smear result was falsely negative (devoid of abnormal cells) [55]. Patients whose lesions do not exfoliate normally (nonshedders) are at higher risk of harboring cervical neoplasia, which can progress if undiscovered. Errors related to the sampling and in vitro evaluation of exfoliated cells suffers from this significant, nonrandom component that might not be overcome with repeated testing. The accuracy of tests for HPV DNA from exfoliated epithelium might suffer from similar limitations. Visual or other in vivo tests performed by clinicians that do not rely on exfoliation can reduce the risk that nonshedding lesions and lesions that reside in the lower portion of the cervical epithelium are missed during a single screening evaluation.

Premalignant lesion size and risk

Although determining lesion size is integral in staging of invasive carcinoma, it also plays a role in prognosis and the risk of metastatic disease. It is uncertain whether or not the size of CIN lesions also affect patients' prognosis related to

subsequent emergence of high-grade cervical neoplasia or malignancy. There is evidence suggesting that the larger the cervical lesion, or the more surface area colposcopically visible in the transformation zone with neoplastic features, the greater the likelihood that part or all of that lesion is comprised of high-grade intraepithelial neoplasia carrying an increased risk of progression toward malignancy. Jarmulowicz and colleagues showed a correlation with increasing lesion size and the probability of diagnosing high-grade CIN. Furthermore, in patients with CIN 3 histologic diagnoses, the larger the lesion, the more likely that the cytologic smear also suggests a high-grade lesion, thus a more consistent correlation. A larger lesion would be a greater target for random sampling, making it more likely the lesion would be contacted during sampling [58]. Kierkegaard and associates studied 689 women undergoing colposcopic evaluation and found a higher likelihood of finding high-grade disease in patients whose lesions occupied greater than 50% of the volume of the cervix or extended beyond the limits of the transformation zone (OR 3.6, CI 2.1–6.3) than in patients with smaller lesions [59]. Finally, Sun and colleagues showed a correlation between lesion size, viral load, and the probability of finding high-grade neoplasia following colposcopic evaluation for a variety of referral indications [60].

Prior history of dysplasia

There are conflicting retrospective data in the published literature regarding the risk of persistent or recurrent cervical disease in patients with a prior diagnosis of cervical dysplasia [61,62]. More likely, changes in other variables such as sexual behavior and other lifestyle choices influenced the initial onset of disease. Regardless of the prior history, if the patient eliminates those variables through preventative measures or health-seeking behavior changes, the risk is avoided. In contrast to recurrence, inadequate treatment most often leads to persistence of the original lesion, which can pose an ongoing risk to the host if undiscovered in follow-up care.

Age, life cycle, sociodemographic issues, and sexual activity

With the onset of menstruation during puberty and the early reproductive years, the female cervix is susceptible to intrinsic and extrinsic agents that can influence neoplastic transformation. Squamous intraepithelial lesions tend to originate in women in their reproductive years, and acquisition of disease is characteristic of a sexually transmitted disease. The exception would be in cases of suspected vertical transmission of HPV from mother to newborn daughters, or in cases in which maternal ingestion of diethylstilbestrol conferred an increased risk of lower genital neoplastic transformation to her female offspring [63–65].

There is good evidence showing that the development of cervical neoplasia is increased in women who began intercourse at an early age or had multiple sexual partners during their lifetime. This might be related to the probability of exposure

to the oncogenic HPV virus and other sexually transmitted agents related to malignant transformation. Early age at first intercourse is linked to a higher incidence of invasive cervical cancer because the transformation zone in the young woman is undergoing significant squamous metaplasia, and intercourse increases the likelihood of atypia [66]. The immune competent woman under 30 years of age who is exposed to the HPV virus during this prone period is more likely to develop a transient, productive, viral, low-grade intraepithelial lesion; however, some patients harbor a clone of immortalized or aneuploid cell lines that can progress to malignancy [67]. Sexual intercourse with multiple partners or high-risk males imparts a significant risk directly related to the likelihood of HPV infection with oncogenic subtypes [68]. In addition to sexual behavior there is an increased probability of cervical cancer in women of lower social class, lower educational level, and pregnancy frequency and parity, though this is probably also attributable to exposure to HPV [69]. The risk of cervical cancer is rare in women younger than age 16 and remains stable in women older than age 40. This fact stresses the importance of cervical cancer screening in sexually active adolescents, premenopausal patients, and postmenopausal patients.

Cultural, geographic, ethnic, and racial demographic risk factors

The risk of developing invasive cervical cancer is related to cultural, ethnic, or racial differences that are exceedingly difficult to extract from other epidemiological and genetic risks already discussed. Differences in disease incidence, prevalence, and death rates do exist related to these variables, but the prognosis of patients who develop and harbor preinvasive disease is related to other factors that cluster in relation to ethnic or geographical groupings, or they are attributable to environmental risks associated with the patients' work or home settings. This explanation might not be entirely true regarding racial predisposition and mortality from invasive cervical cancer. In the US, black women have the highest age-adjusted mortality rate from cervical cancer, followed by Hispanic women and American Indians. The lowest mortality rate is seen in Japanese women [70]. A study of Caucasian, Hispanic, and African American women showed the highest prevalence of oncogenic subtypes of HPV (by PCR) and low- and high-grade squamous intraepithelial changes in Pap smears among Hispanic women attending M.D. Anderson (Texas) colposcopy clinic, followed by Caucasian and African American women [71].

When designing public health measures or screening programs in different settings around the world, the prevalent rate of cancer and deaths from cancer will dictate the technology utilized and the intervals used to screen the population in question. Both low- and high-risk patients in these settings might benefit when more sensitive but more expensive tests are utilized, but cost effectiveness might not be realized. The approach to treatment of preinvasive disease might also differ based on these demographic variables and on the ability to adequately follow patients conservatively in the community.

Tobacco use and risk of cervical malignancy

Most studies linking smoking behavior to an increased risk of cervical malignancy are prone to bias from confounding variables [72]. Nevertheless, the scientific basis behind neoplastic transformation and inhaled and circulating carcinogens found in cigarette smoke and cervical tissues is established, as is the additive effect in patients with coincident HPV infection [73–75]. Many studies have shown an increased risk of finding both high- and low-grade CIN in smokers over nonsmokers, even when adjusted for age of first intercourse, lifetime number of sexual partners, and cervical HPV status [76]. The effect might be dose-related, as evidenced by the number of cigarettes per day smoked [77]. Kjellberg showed a higher risk of cervical cancer in smokers related to the development of CIN 2 and CIN 3 adjusted for the presence of HPV infection [78]. In regard to prognosis, patients with colposcopically documented neoplastic lesions who stop smoking have a higher probability of regression of their disease [79]. The confounding effect of concomitant HPV infection in smokers has led to several studies that are contradictory regarding cigarette smoking as a sole risk factor for the development or progression of cervical neoplasia [80,81].

Immune compromise

Many cervical intraepithelial lesions are detected and cleared by predominantly host cell-mediated immune defenses. When host defenses are compromised, the infection, subsequent alteration of the cell genome, and oncogenic cell proteins and markers are not recognized. This might be the result of downregulation of protective immune regulatory proteins. Lower genital tract neoplasia, CIN, and cervical malignancy are all more common in patients who are immunocompromised. The most common reasons for immune compromise include infection with HIV, treatment with drugs that are immunosuppressive, or pregnancy. The relative risk of high-grade cervical neoplasia is five times higher in patients who are using immunosuppressive drugs compared with untreated control patients [82]. Patients on chronic glucocorticoid therapy are at risk for cervical carcinogenesis because of deregulation of tumor suppression and inappropriate expression of E6 and E7 [83]. The rate of HPV detection is higher in patients who have HIV and low CD4 counts, and patients who are infected are more likely to have multifocal lower genital tract disease and larger cervical lesions documented during colposcopy. Preinvasive lesions are more likely to recur after treatment (with any modality), as are cancers in HIV-infected patients [84,85]. Cervical cancer became an AIDS-defining illness in 1993.

The Pap smear has limitations when employed as the sole screening test for immunosuppressed patients. The false-positive and false-negative rates of screening are elevated in HIV-infected and immunosuppressed renal transplant patients [86,87]. The common approach in screening, triage, and treatment is more aggressive because the risk of progression of precursors towards invasive disease is more likely.

Health service access and health-seeking behavior

Studies in populations have shown a strong correlation between cytologic screening in women worldwide and the decline in mortality from cervical cancer [88]. Screening and health-seeking behavior might be a function of access to health care services or choice (driven by fear or cultural issues). A case–control study showed that when women undergo Pap smears at least every 3 years, their risk of developing cervical cancer is ten times less than in women who are not screened [89]. There is evidence showing that a reduction in deaths from cervical cancer preceded the widespread implementation of Pap smear use in the US, suggesting that changes in lifestyle, preventative health measures, and other unknown factors might be confounding variables [70]. There have been no randomized, controlled studies directly testing the cause and effect relationship between the practice of cervical cytologic screening and the incidence of invasive cervical cancer. Decisions regarding treatment or conservative management of cervical cancer precursors are predicated by the patient's ability or willingness to seek care. Treatment of high-grade CIN is indisputable, but the risk of malignant progression increases when clinicians lose the ability to keep patients with low-grade cervical disease under surveillance. The importance of finding and eradicating lesions in unscreened and underserved patients is also crucial for prevention in this high-risk group, and it might justify the increased risk and expenditure associated with costly technologies that overcome the inherent high false-negative rate of a single Pap smear evaluation for this less predictable cohort.

Endogenous and exogenous hormonal exposure and risk

The use of oral contraceptives has been studied extensively with respect to the risk of cervical cancer. Prior to the availability of PCR methods in the validation of HPV infection, studies showed an increased risk of invasive cervical cancer with oral contraceptive use [90]. This association is questionable when HPV status is controlled in the analysis [91]. One study has shown a persistent risk with oral contraceptives regardless of HPV status [92]. Kjelberg and associates showed no excess risk associated with oral contraceptive use in a case–control study involving 137 women with CIN 2 and CIN 3 compared with healthy age-matched women when HPV infection was taken into account [78]. In regard to endogenous hormonal status, the same study did find that pregnancy appeared to be a risk factor for CIN2 and CIN 3. Tabrizi showed an association between prior pregnancy, HPV-positive status, and the presence of CIN 2 and CIN 3 lesions [93]. Pregnancy does not influence the progression of disease or prognosis of patients with invasive cervical cancer, however [94].

DES, a nonsteroidal estrogen, was used from 1940 to the early 1970s in the US and Europe to improve pregnancy outcomes in women with prior pregnancy loss and gestational diabetes. In a landmark article, Herbst and colleagues reported an increased rate of clear cell adenocarcinoma in the vagina and cervix of women

who were exposed to DES in utero after maternal ingestion (DES daughters) [95]. In such cases, the entire cervical portio and upper third of the vagina remains at risk of neoplastic transformation through adolescence and adulthood, and screening for neoplasia is made problematic by needed cytologic and visual surveillance of the entire "at-risk" epithelium. The American Cancer Society has stated that approximately 1 in 1000 women exposed to DES in utero are at risk for invasive cervical cancer (0.1%). A more aggressive approach in screening, referral to colposcopy, and treatment of any preinvasive lesion is practiced when caring for DES daughters.

Genetic predisposition to cervical malignancy and risk

Studies involving female siblings and twins have suggested a small contribution of familial genetic factors and predisposition to cervical cancer. Cervical cancer was diagnosed more frequently in mothers and sisters of patients with invasive cervical cancer in a prospective study [96]. An interesting study of cervical cancer patients that compared the risk of cervical cancer in biologic mothers and sisters with adoptive relatives showed that first-degree relatives had twice the risk [97]. Some theorize that HPV can be vertically transmitted from mother to daughter and can be responsible for the increased risk, but the risk appears to persist in half-sisters with only the father in common. The effect of a common environment during growth and maturation in families makes the contribution of environmental factors difficult to control in the analysis, and there are no published studies that followed monozygotic female siblings raised apart for the development of cervical neoplasia and malignancy.

There is new and emerging evidence of increased susceptibility to invasive cervical cancer related to immunogenetic factors. Aoki and colleagues showed an increased association with HLA-Bw46 (gene frequency = 6.3%, relative risk = 3.9, $P < 0.025$) in 66 invasive squamous cell cancer cases when compared with 206 normal controls (gene frequency = 1.7%) [98]. One study using PCR showed an increase in the prevalence of the HLA-DRB (DRB1-1501) allele (33% in older cohort, 28% in younger cohort) in patients with in situ and invasive cervical cancer in comparison with the known prevalence of the allele in the US population (19%) [99]. Another study by Krul showed that there might be a genetic predisposition to cervical malignancy in patients with the higher-risk HLA types DR15 and DQ4 [100].

Either through inheritance or mutation, the products of genetically altered cervical cells predispose to malignant transformation by inducing, promoting, or failing to suppress orderly cell replication. The genes responsible, the proteins that they encode, and general markers for cell division and replication have all been touted as potential "markers" for cervical cancer. As such, they are likely targets for molecular analysis in cytologic specimens or tissue obtained during cervical cancer screening or lesion biopsy evaluation. A list of these markers, their relationship to cell proliferation, and their origins is presented in Table 2.

Table 2
Biomarkers, molecular structure, and relation to oncogenicity

#	Marker	Structure	Relation to oncogenecity
1	Telomerase activity [1,34]	Protein/RNA	Increases in CIN 1
2	MIB-1 [1,33,34,37,47]	Protein	Prognostic marker, atrophic cell pattern
3	PCNA [1,33,46]	Protein	Prognostics marker/triage
4	HPV [1]	DNA	Predictive/prognostics marker/triage
5	p53 [1,34]	Protein/DNA	Tumor progress/cell proliferation
6	Ribosomal protein S12 [2]	Protein	Early marker
7	Squamous cell carcinoma-associated antigen (SCCA) [3,48]	Protein, RNA	Tumor marker, metastasis
8	Cancer antigen 125 (CA125) [3]	Protein	Core marker
9	TPS [4,24]	Serum protein	Disease progression marker
10	p16INK4a [5,8,33]	Protein/RNA	Screening and diagnostic marker
11	MUC1 variants C and D [6]	Protein	Carcinogenic marker
12	Methylenetetrahydrofolate reductase (MTHFR) polymorphism [29]	DNA	Tumor progression
13	BCl 2 [10,41]	Protein	Tumor progression
14	C4.8 and C21.7 [12]	RNA	Increased expression in premalignant lesions
15	DAF, CD55 [27,28]	Protein	Ratios in tumor to normal tissue indicates progression of disease
16	Membrane cofactor proteins (MCP, CD46) and CD59 [13]	Protein	Metaplastic indicator
17	Enhanced expression of H-ras, c-myc, B-myb, p53 [19]	Proteins/RNA	Post HPV activation
18	p16INK4 [33]	Protein	Surrogate marker for HPV?
19	CD44 varients [23,42,49]	Protein/RNA	Microinvasion & diagnostic marker
20	MN protein [15,33,35]	Protein	Low expression correlates with adverse prognosis
21	Intestinal alkaline phosphatase (IAP) [30,31]	Protein	Microinvasion marker
22	E6/E7 oncoproteins [25,44,45,50,51]	Protein/ antibody/RNA	Tumor marker
23	P150 protein [11,16,21]	Protein	Progress of invasiveness
24	CDK inhibitors [17]	Protein	Under expression is associated with neoplastic transformation
25	VDK (1,25-dihydroxy- vitamin D3 receptors) [18]	Protein/RNA	Marker for precancerous lesion
26	EGF-R/Her-2/neu [42]	Protein/DNA	Prognostic
27	c-erbB2 gene [20,46]	Protein/RNA	Potential prognostic marker
28	CD3-zeta [22]	Protein	Decreased T-cell marker indicates cancer
29	High mobility Group IHMGI(Y) [32]	Nuclear protein	Intraepithelial lesion and invasive caecinoma
30	E-Cadherin [36,40]	Protein, RNA	Indirect marker of level of exfoliation
31	Laminin-5 [53]	Protein	Tumor marker of early invasion

Table 2 (*continued*)

#	Marker	Structure	Relation to oncogenecity
32	Cytokeratin [52]	Protein	Tumor specific marker
33	TK (thymidineKinase) [26]	Protein	Survival prediction

Courtesy of Dr. Yathi Naidu.

These markers are the subject of several hundred papers published during last three decades; selected few references are listed.

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Prior history of dysplasia

The strength of the evidence related to a prior history of dysplasia is prone to reporting error related to the documentation that the evidence is histologic-based rather than based on an unconfirmed abnormal cytologic result. It is known that the correlation between cytologic and histologic grade is not strong, and prediction of future risk based on this evidence is confusing when patients' anecdotal accounts are relied upon [15]. Patients who do not immunologically clear cells possessing oncogenic HPV DNA or patients who remain exposed to high-risk males are more likely to exhibit persistent or recurrent infections that are then linked to the discovery of CIN and cancer in women who were previously diagnosed. It is important to obtain a comprehensive history regarding the nature of the treatment and documentation of cure, and a thorough sexual history in patients with prior disease.

Other sexually transmitted disease exposure and risk

Herpes Simplex Virus 2 (HSV-2) can be found in cervical carcinoma cells grown in culture or using in situ hybridization, suggesting a role in the development of cervical cancer [101]. The presence of HSV-2 antibodies, which is a marker of prior HSV-2, poses a relative risk of 1.6 for the development of cervical carcinoma [102]. The role of HSV is more likely as a cofactor than inducer of cervical malignant transformation.

There is evidence that prior infection through serologic confirmation of the lower genital tract with *Chlamydia trachomatis* confers an increased risk of cervical neoplasia that is independent of concomitant exposure to HPV [103]. Other researchers have confirmed this finding, especially in cases in which HPV was also discovered, and they theorize that the process of chronic inflammation might serve as a cofactor in the progression of cervical neoplasia [104]. One report from Spain showed an increased risk for invasive cervical cancer in women with antibodies to *Neisseria gonorrhea* after adjustment for HPV DNA prevalence [105].

Nutritional factors

Deficiencies in nutrients and vitamins, specifically vitamin C, carotenoids, vitamin E, and folic acid have been linked to an increased risk of cervical neoplasia and cancer [106]. Lack of circulating antioxidants such as β-carotene,

Notes to Table 2 (*continued*):

[46] Chung Hua Chung Liu Tsa Chih 1997;19:150–2.
[47] J Pathol 2000;190:545–53.
[48] Br J Cancer 2000;82:429–35.
[49] Mol Diagn 1999;4:45–56.
[50] Eur J Gynaecol Oncol 1998;19:46–9.
[51] Lab Invest 1998;78:9–18.
[52] J Clin Pathol 1999;52:41–6.

lutein, and vitamin A was associated with persistent HPV infection in studies that compared patients with normal controls [107,108]. The underlying influence of micronutrients on cancer risk can be confounded by the HPV status of the patient, and early research did not control for this factor. HPV infection can affect the metabolism of these micronutrients, lowering circulating levels. Deficiencies in micronutrients might impair host defenses against HPV. The biologic mechanisms behind the antineoplastic effects of carotenoids, vitamin C, and tochopherols might be caused by their function as antioxidants in scavenging free radicals. In addition, retinoic acid and its precursors and derivatives have an effect on epithelial proliferation and might protect against viral infection [107,109]. In a recent study of Hispanic nonsmokers, deficiencies in serum α- and β-carotene, lutein, lycopene, β-cryptoxanthin, α-tocopherol, γ-tocopherol, and ascorbate were associated with a higher persistence of HPV as measured by Hybrid Capture II testing over a 3-month period and the subsequent discovery of high-grade CIN [108]. Low folate levels are associated with DNA hypomethylation, which has been correlated with the presence of CIN [110,111]. Elevated homocysteine levels are associated with folate deficiency and have been shown to be elevated in women with cervical cancer as opposed to age-matched controls [112]. Supplementation of antioxidants and micronutrients in the prevention of cervical neoplasia and cancer will be addressed in the section related to primary prevention of cervical cancer.

Summary: prevention through risk abatement

Until primary prevention of cervical malignancy is feasible, either through the application of a vaccine or meaningful lifestyle modifications, the goal of clinicians is to identify women at risk of suffering or dying as a result of the development of premalignant lesions of the cervix that are likely to progress toward carcinoma if left untreated. In the end, the majority of such lesions show evidence of high-risk oncogenic HPV infection. A challenge is that many more women enter the continuum from normal cervical epithelium to malignancy, but few ever completely undergo malignant transformation. Testing for HPV infection using exfoliated cells might not efficiently cull out this high-risk cohort in all clinical settings. The rate of positive tests might approach 30% to 50% of the population screened, suggesting that HPV infection is quite prevalent, while the propensity of those infected to develop lesions and progress toward malignancy is rare. This is especially true for young women under age 30 whose lesions might be transient until immune competence eliminates the risk of malignancy. Many women who test positive for high-risk viral subtypes show no evidence of lesions during colposcopy and are difficult to counsel regarding their true risk, making the utility of HPV testing in screening less attractive. Women who have cytologic abnormalities during screening (in vitro) or visible cervical lesions during the cervical inspection (in vivo) are also at a higher risk. Most clinicians begin with gross inspection of the cervix and cytologic sampling of the cervix and lower

genital tract to guide the use of colposcopy in defining risk, using histologic confirmation as the highest standard to guide management. Visualization of the cervix in addition to cytologic sampling during screening enables clinicians to find high-risk lesions that are either missed or do not exfoliate when brushed or scraped and are not represented in cytologic specimens. The highest-risk cohort is patients with lesions that are destined to become malignant but are not recognized because of failure of screening, patients who have lesions that are overlooked by the host's immunologic defenses, or lesions that are never defined because of failure to screen. The next highest risk group is patients in which a lesion is suspected (ASCUS or SIL on cytology, visual lesion seen during screening, positive oncogenic HPV testing) but is not confirmed by biopsy, either through a misdirected colposcopic biopsy, failure to perform colposcopy in patients with low-grade abnormalities, or complacency in the use of colposcopy and biopsy. The decision to treat high-grade neoplastic precursors in situations in which the risk of progression from preinvasive to invasive disease is well defined is free of controversy and is universally recommended [113]. The management of low-grade lesions remains controversial, and the additional risk factors discussed in this chapter can be used to define patients in whom conservative management would not be practical, because of an increased risk that the lesion would not regress without therapy. The identification and use of the risk factors associated with cervical malignancy is therefore paramount in any cervical cancer prevention program. The presence of risk factors in patients undergoing screening can better define the high-risk patient, which helps clinicians decide on the use of limited resources and

- who receives additional technology (new adjuncts) with the increased expense to overcome the inherent false-negative rate of screening, recognizing that some risk factors remain hidden, such as the propensity of neoplastic lesions to exfoliate and provide cells for collection.
- which patients with unconfirmed visual acetowhite cervical changes, minor or low-grade cytologic abnormalities, or evidence of oncogenic HPV subtypes from cervical samples are referred for colposcopy.
- which patients with biopsy-proven CIN I or early HPV infection receive treatment or are conservatively managed to allow for host immunologic recognition and lesion eradication.
- at what intervals and with what technology patients with cervical neoplasia who have been treated or conservatively managed are followed.

Toward a comprehensive risk scoring system

Applying data from populations related to the aforementioned sections to individual cases is challenging. Sophisticated regression analyses related to risk factors are not sufficient to weight each individual factor related to the risk of developing and dying from invasive cervical cancer. In addition, the analysis

would be confounded by the probability of death from other causes related to age. By assigning risk based on the evidence, clinicians move from managing "low-grade" or "high-grade" intraepithelial lesions toward a more holistic secondary prevention approach that takes into account the aforementioned risk factors in context. This more comprehensive approach to patient care helps assure that efforts and investment of time and resources to reduce the risk of malignancy is balanced by the overall likelihood that lesions can appear and regress without clinicians' knowledge or interventions. Use of the Gail Model risk scoring system in the prevention of breast cancer is a potential model to guide the clinical use of screening, triage, and treatment resources in the prevention of invasive cervical cancer [114,115]. Efforts are ongoing to extract the average risk related to clinical findings and epidemiological risk factors to guide patient care.

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Primary prevention of uterine cervix cancer: focus on vaccine history and current strategy

Krishnansu Sujata Tewari, MD, Philip John DiSaia, MD*

Division of Gynecologic Oncology, Chao Family NCI-Designated Comprehensive Cancer Center, University of California, Irvine, Medical Center, 101 The City Drive, Orange, CA 92868, USA

Primary prevention of uterine cervix cancer spans the gamut of human papillomavirus (HPV) vaccine development, dietary adjustment, chemoprevention, and risk reduction. Lifestyle and social behaviors impact on risk for cervical cancer and will be discussed in great detail by Tewari and DiSaia in the future. Before examining the growing body of molecular evidence, animal studies, and phase I clinical trials that suggest that a virus-based vaccine for cervical cancer may soon become a reality, we must reflect on what has gone before in our vaccine-based battle with viral disease.

The conquest of smallpox

Variolation: the genius of China

Smallpox has been called the great scourge of mankind. Over the ages, it has crippled, disfigured, or killed one fourth of all humanity (Fig. 1). Just in the twentieth century alone, nearly 200 million deaths were attributed to this disease. Every corner of the world has felt its grip and known its devastation. Physical anthropologists have speculated that the disease first appeared around 10,000 Before the common era (BCE) among the agricultural settlements in northeastern Africa. Its scars can be found on the mummy of the Egyptian pharaoh Ramses V, who died in 1157 BCE and on other Eighteenth Dynasty mummies [1]. The first known smallpox epidemic was recorded in 1350 BCE, when Egyptian prisoners unwittingly spread the disease to the Hittites. Even the Hittite King Suppiluliumus I and his heir were claimed as victims by the virus. Egyptian merchants eventually brought the smallpox virus to India.

* Corresponding author.
E-mail address: pjdisaia@uci.edu (P.J. DiSaia).

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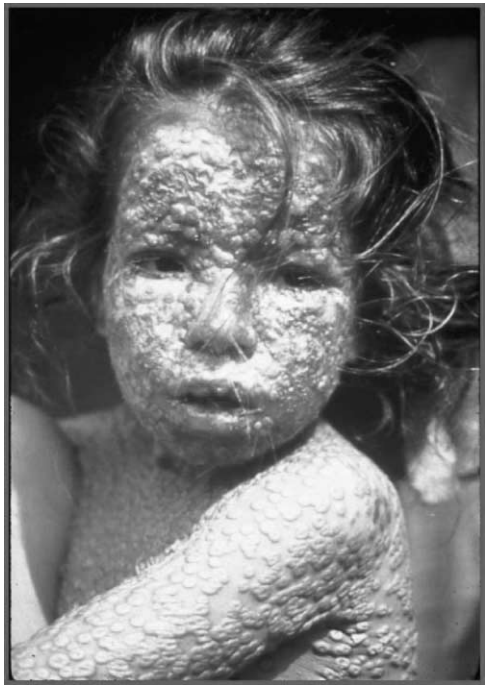


Fig. 1. A young child afflicted with smallpox.

During the Athenian epidemic in 430 BCE, Thucydidus made the curious observation among the ancients that subsequent immunity resulted in people who survived the disease [2]. In 910 AD, Rhazes, the greatest physician of Islam and of the Middle Ages, recorded the first known medical description of smallpox and its transmission and documented postinfection immunity [3].

The origins of inoculation against smallpox are shrouded in mystery. The technique originated in China at the southern province of Szechuan in a famous mountain called O-Mei Shan, which is known for its connection with Buddhism and the native Chinese religion of Taoism. In anticipation that intentional exposure of healthy people to the disease would result in immunity, samples from vesicles, pus from pustules, or scabs were inhaled through the nose or subbed into the cut skin of healthy human beings. The Taoist alchemists lived as hermits in the mountain caves and are reputed to have possessed the secret of smallpox inoculation in the tenth century AD. We will never know how long they had it before that time, but during the period from 1567 to 1572, variolation was widely practiced in China [4]. During the seventeenth century AD, the practice of variolation spread to the Ottoman Empire. It soon became common knowledge in Europe and in the United Kingdom that the ''speckled monsters'' (ie, survivors of smallpox in the eighteenth century vernacular) were immune.

Dr. Edward Jenner

The English country physician, Dr. Edward Jenner (1749–1823), made a valid and fateful observation that laid the foundations for widespread vaccination and the eventual eradication of smallpox [5]. He noticed that farm and dairy workers were afflicted with cowpox, which was prevalent in dairy cattle. The cowpox sores (vaccinia) were similar to those of smallpox (variola). Dr. Jenner also observed that persons with cowpox had only chills and malaise for 1 or 2 days and then recovered quickly without sequelae. He also observed that when smallpox broke out in the area, individuals who had been sick with a mild case of cowpox did not get smallpox [6].

On May 14, 1796, Dr. Jenner deliberately inoculated 8-year-old James Phipps with cowpox-infected material from a local milkmaid. The boy apparently had the expected mild form of the lesions and no serious illness manifested. Several months later, Dr. Jenner inoculated the boy with smallpox and there was no effect at all. He had actually succeeded in vaccinating the boy. He submitted his results to the Royal Society, but after rejection, he published his work privately [7]. Although he was not the first to think of this idea, as Sir William Osler (1849–1919) once said, "in science the credit goes not to the one who first thinks of an idea, but to the one who convinces the world." US President Thomas Jefferson (1743–1826) espoused the concept of vaccination, and French Emperor Napoleon Bonaparte (1769–1821) had his entire army vaccinated in 1805. Clearly, Dr. Jenner is among the great medical giants.

In the late nineteenth century, the French chemist Louis Pasteur (1822–1895) honored Jenner by actually coining the term "vaccination" and used his germ theory of disease to explain how vaccination worked [8]. As an aside, it is curious to note that in contradistinction to Jenner's focused and deliberate investigation into the prophylaxis against viral illnesses, British bacteriologist Sir Alexander Fleming's (1881–1955) discovery of penicillin came about accidentally in 1928, when he observed that the mold that contaminated one of his culture plates destroyed the bacteria in its vicinity.

The eradication of smallpox

The war against smallpox continued for nearly two centuries. On January 1, 1967, the World Health Organization (WHO) launched the Intensified Smallpox Eradication Program [9]. At that time, smallpox afflicted up to 15 million people annually, of whom 2 million died and millions were left disfigured and sometimes blind. The plan at that time was to rely entirely on mass vaccination of susceptible persons in endemic countries. This strategy had been previously successful in Western Europe, North America, and Japan. The WHO Expert Committee on Smallpox in 1964 recommended that the goal should be to vaccinate 100% of the population, based on the observation in India that smallpox persisted in areas in which 80% of the population had been vaccinated.

The best known example of WHO's accomplishments is the eradication of smallpox, which occurred in 1974 [10]. In 1977, the last case of smallpox was reported in Somalia. In 1980, WHO was able to certify that the disease had been eradicated [11]. Had smallpox not been eradicated, the past 20 years would have witnessed some 350 million new victims. Smallpox is the only major human disease to have been eradicated [12].

Vaccinating women against premature death

Human papillomavirus

Human papillomaviruses are icosehedral, non-enveloped viruses that contain double-stranded circular DNA molecules of approximately 8 kb (Fig. 2); the genome contains eight open reading frames and a noncoding region that contains transcription regulatory sequences and the origin of replication [13]. The proteins involved in DNA replication, transcription, and cellular transformation are encoded by the six early open reading frames (E1–E6), whereas the capsid proteins (ie, the major capsid protein L1 and the minor capsid protein L2) are encoded by the late open reading frames and form the icosahedric capsids with 72 capsomeres to enclose the HPV genome (Fig. 3) [14].

Importantly, HPVs are exclusively epitheliotropic [15]. The life history of an epithelium is one in which cells migrate from the basal layer, differentiating as

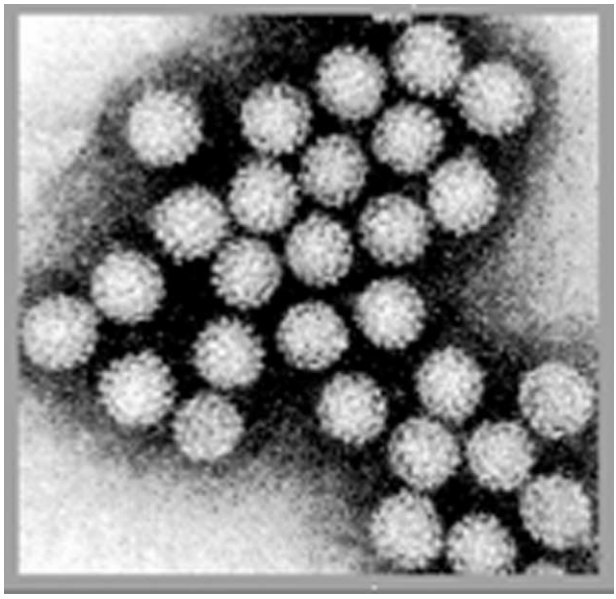


Fig. 2. HPV virions.

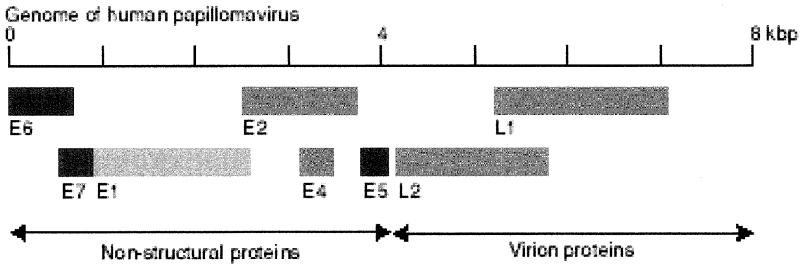


Fig. 3. The linearized DNA genome of HPV.

they make their way toward the surface, at which point they are exfoliated and replaced by cells from below. After minor local trauma, viral particles composed of capsids assess the target cells and facilitate entry of the viral DNA. In neoplastic tissues of the human uterine cervix, oncogenic strains of HPV (eg, HPV subtypes 16 and 18) are found predominantly in the basal or parabasal cells of the transformation zone. The transformation zone is that region of active cellular metaplasia in which the columnar cells of the endocervical canal are replaced by the stratified squamous epithelium of the ectocervix.

The immediate early proteins E1, E2, and E5 are first expressed. E1 and E5 encode DNA-binding proteins, which maintain a stable viral episome [15]. E2 is involved in the positive and negative regulation of viral gene expression [15]. For example, the expression of E2 permits low levels of expression of the viral oncogenes, E6 and E7, which are expressed in the lower spinous layers and are involved in regulating cell proliferation and interfering with the host cell cycle control mechanisms to activate cellular DNA synthesis. Like L1 and L2, the expression of E4 is largely restricted to the upper spinous layers. The precise function of E4 is unknown. There is no HPV equivalent of E3. Mature virions may be released from exfoliating cells.

E6 and E7 are referred to as viral oncogenes because they constitute the major mechanism through which oncogenic HPVs contribute to the development of cervical cancer [16]. E6 encodes a 16 kDa to 19 kDa protein that binds to the tumor suppressor protein, p53, and causes its degradation by the ubiquitin proteolysis pathway, and E7 encodes for a 10 kDa to 14 kDa protein that has transforming activity and binds to retinoblastoma-susceptibility protein. E6 also has been linked to telomerase activation and the immortalization of cells. Specifically, oncogenic HPV E6 binds to p53 with a high affinity, which then results in loss of p53-dependent G_1 arrest and apoptosis. Oncogenic HPV E7 binds to retinoblastoma-susceptibility protein and prevents the sequestration of E2-F transcription factors, which leads to a disruption of cell cycle control [16].

Although HPV exists as an episome during the initial infection, in advanced lesions and invasive cancers, HPV has integrated into the host genome, thereby permitting expression of E6-E7 transcripts that have enhanced stability (most likely in consequence to the disruption of the regulatory viral gene E2 at the point

of integration) [17]. Although viral particles no longer can be produced after integration, the continued E6 and E7 activity prolongs the cell cycle with resultant loss of effective DNA repair mechanisms and the opportunity for accumulation of genetic changes along the multistep road to carcinogenesis.

The immunologic response to human papillomavirus infection

Although an immune response to HPV infection is manifested by the host, the frequent presence of chronic infection suggests that the HPV evades the host's immune surveillance mechanisms [18]. The infected host cellular membrane expresses only the E5 HPV protein, and this paucity of viral surface antigens may allow for attenuation of the immune response. E5 binds and inactivates a protein necessary for processing antigens for presentation in type II major histocompatibility complexes. Why most individuals apparently clear the virus while some fail is not known, but the increased occurrence of HPV lesions in immunosuppressed individuals points to involvement by the host immune system [19]. In immunocompetent individuals, HPV infection is asymptomatic and the virus is normally cleared [20].

Innate and adaptive immune responses may play a role in the natural clearance of HPV infection [21]. The innate immune response is rapidly induced and nonspecific; it does not generate immune memory [22]. The innate immune portfolio is relegated to the epithelial borders and is triggered by danger signals when bacterial or viral infection occurs [23]. Monocytes, macrophages, natural killer cells, and antigen-presenting cells are the immunomodulatory cellular effects operant in the innate immune response [24]. The key effector cytokines include interleukin-1, interferon alpha/beta, and tumor necrosis factor [25]. These molecules activate antigen-presenting cells, such as dendritic cells, which are the sentinels of the host and are responsible for the initiation of adaptive immunity [26].

The adaptive immune response leads to the generation of the antigen-specific effector cells, such as the CD4 T-helper cell, the CD8 T-killer cell, and the B cells, which secrete antibodies [27]. The end result is one in which the pathogens or pathogen-infected cells become targeted and memory cells are formed that prevent or limit subsequent infection with the same organism. The activation of the innate immune response can stimulate the adaptive immune response. In an attempt to understand this immunologic phenomenon, two theories that differ in how the immune response is initiated have been advanced.

In the self/non-self theory, the key concept is the recognition of foreign entities by germline encoded receptors that are able to recognize pathogen-associated molecular patterns, such as the lipopolysaccharide in gram-negative bacteria or the double-stranded RNA in retroviral infections [28]. In contrast, the danger model examines the situation from an evolutionary point of view and attests that what really matters is whether the host is damaged [29]. The signals are endogenous in origin so that any cellular distress or unnatural cell death leads to the induction of innate immunity [30]. The critical assumption is that the danger model offers the possibility that infection by a foreign entity that is noninjurious

does not evoke a response, a scenario that may be analogous to HPV infection of the uterine cervix. The antigen-specific T cells are the fulcrum of the antiviral responses, and the mechanism through which adaptive immunity to HPV infection is generated appears in Fig. 4.

Theoretically, the generation of natural immunity may be recapitulated through a vaccination strategy that uses an attenuated virus to mimic the natural infection process. The existing animal models for vaccination strategies provide a strong theoretical momentum and impetus for the various HPV vaccines in development. To optimize vaccine design, however, we first must understand the key viral targets and immunologic mechanisms through which natural HPV is cleared. The problem lies in the fact that not only is it difficult to study the precise local immune events that occur in the human uterine cervix but also the temporal relationship of these events with the onset of infection is problematic.

Antibodies to capsid proteins and cytotoxic T lymphocytes directed against oncogenes seem to be involved in the serologic and cellular responses that result in the clearance of HPV infection [22]. Investigations with animal papillomavirus have demonstrated a protection against infection conferred by antibodies that recognize conformational epitopes on the virus (ie, virus-like particles, VLPs) [31]. These antibodies also are able to neutralize the animal papillomaviruses.

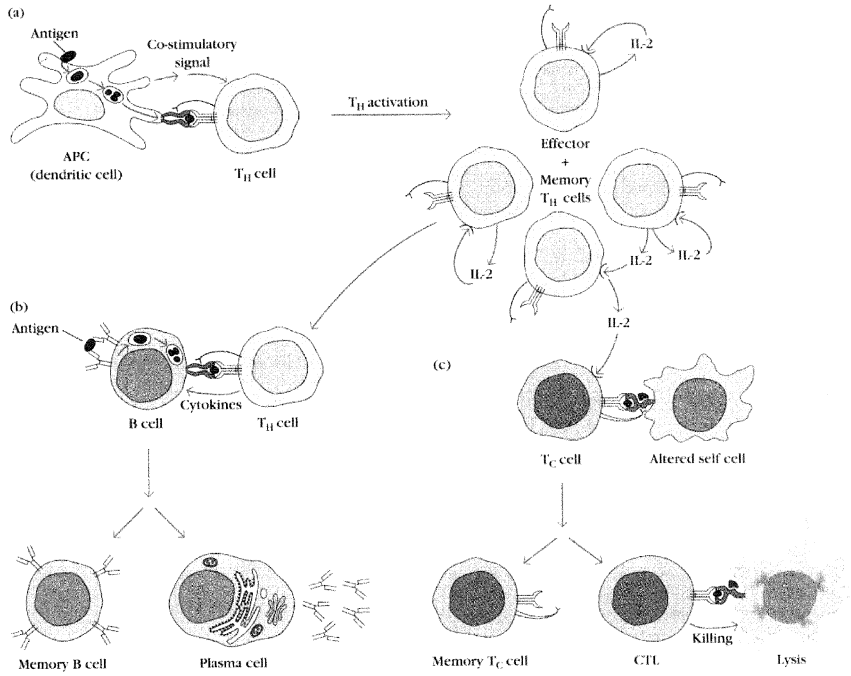


Fig. 4. Adaptive immunity in association with HPV infection of the uterine cervix. (*From* DiSaia PJ, Creasman WT (eds.) Clinical Gynecologic Oncology, 6th edition. Mosby, Inc., 2002; with permission.)

Serologic assays using VLPs have shown that many individuals exposed to the HPV develop antibodies to L1 [32].

Although this serologic response to the HPV capsid proteins is a consequence of exposure to the virus, the absence of these antibodies does not correlate with the lack of infection. HPV lesions do not increase with depression of the humoral immune system, but in individuals with depressed cell-mediated immunity (eg, women infected with HIV and some allograft transplant recipients maintained on immunosuppressive pharmacotherapeutic agents), the number of HPV lesions increases [19,33,34]. The constitutive expression of HPV oncogenes E6 and E7 in cervical tumors has prompted a search for cytotoxic T lymphocytes (CTLs) in women with cervical cancer.

The candidate peptide epitopes from HPV 16 E6 and E7 that could be presented by the most common human leukocyte antigen-A alleles have been identified and used to detect specific CTLs. This approach has led to some interesting observations, one of which is that in women with persistent HPV infection, HPV 16 E6- and E7-specific CTL responses occur [35]. Only E7 CTLs have been found in women who harbor invasive cancers.

Persistent infection correlates with the development and progression of cervical neoplasia, but why do some HPV infections persist? Oncogenic E6 and E7 modulate the α-interferon response pathways of infected cells, which counteracts any protection from interferons induced by innate immunity [36]. Through evolutionary programming, the HPV is a pathogen that has survived through a combination of viral stealth and specific interference with innate immunity.

Human papillomavirus and HIV: ancient virus or zoonosis?

In contemplating a vaccine for HPV, Halpern acknowledges that it is important to consider the evolutionary context in which such a vaccine would be deployed [37]. Inevitably, phylogenetic comparisons to HIV must be made because HIV has been the subject of even more extensive study than HPV and may serve as a model for trouble shooting potential difficulties with HPV vaccination. Fortunately, despite similarities in phylogenetic structure between HPV and HIV, there are also striking differences in the evolutionary potentials and histories of these viruses that permit an optimistic outlook for an HPV vaccination strategy.

When examining the phylogenetic trees of the primate immunodeficiency viruses and the papillomaviruses, certain similarities emerge. For example, viruses that infect humans are found in several separate regions of the tree, intermingled with viruses that infect animals [37]. At the deepest levels of both trees, there also seem to have been periods of rapid diversification [37]. Finally, in both virus families, even more divergent viruses are found in more remote hosts (eg, the finch immunodeficiency virus and the finch papillomavirus) [37].

The differences between HIV and HPV, however, are salient. For example, the differential mutation rates of the two virus families are reflected by differences in the temporal and geographic heterogeneity of sequences. Although there is

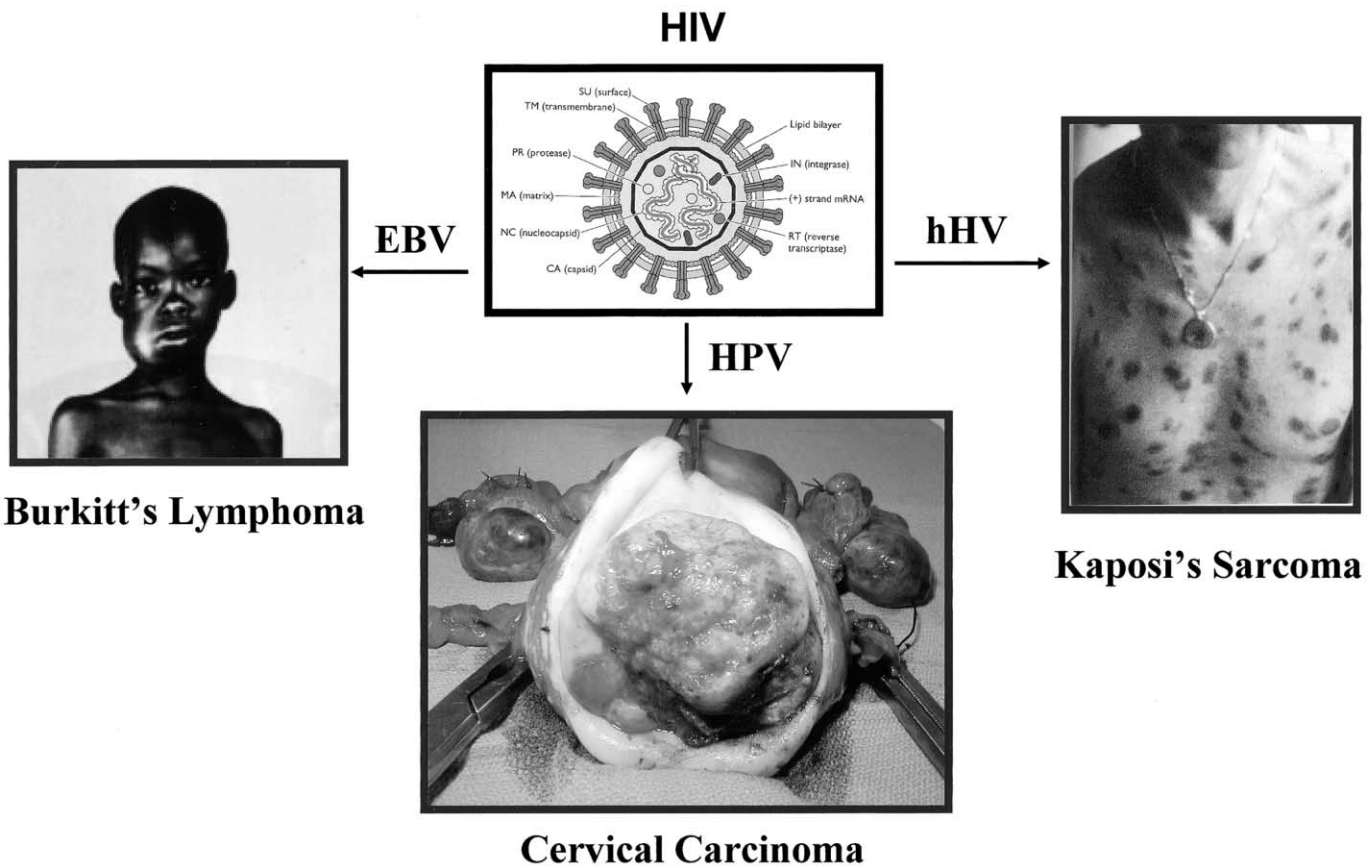
considerable variation among geographic regions in the prevalence of HIV subtypes, HPV types are more uniformly distributed [38–41]. Although HIV and HPV are considered ''emerging'' pathogens, the different patterns of geographic sequence variation suggest that papillomaviruses are endemic rather than epidemic; HIV, on the other hand, is epidemic (or pandemic). This last observation is in agreement with theories regarding adaptation of an infectious agent to the host.

HIV is pathogenic and engages the host immune system with unfortunate efficacy, being highly immunogenic in a natural infection [42]. In contradistinction, papillomaviruses are relatively benign and their persistence results from their ability to evade the host immune system [43]. Cervical cancer is the exceptional outcome. Whereas HIV is capable of repeatedly escaping the immune system through its mutational potential, HPV must avoid fully activating the immune system.

Zoonosis occurs when a disease or infection is naturally transmissible from animals to humans. Zoonotic agents can be a bacterium, virus, parasite, or other biologic entity (eg, a prion). Zoonoses represent a permanent risk for humans. The causes for re-emerging and emerging of zoonoses are inclusive of worldwide tourism and animal trade, the penetration of new geographic and ecologic regions by humans and changing of habitats, the introduction and spread of exotic and other animal species in human populations, the changes in animal husbandry and feeding practices, the changes in eating habits, production and food preparation processing, hygienic constraints (ie, close contact between animals and humans), mutations of infectious agents that permit a change of hosts, and new transmission routes (eg, xenotransplantation). HIV zoonosis is a scenario that has been largely accepted within the scientific community [44].

Within-host or host-linked evolution predicts that the divergence between an animal virus and the most similar human virus should be correlated with the divergence between the animal and human hosts. The divergence of the papillomaviruses is great enough that standard models of sequence evolution begin to fail [37]. Although there are supporters of a papillomavirus zoonosis, the available sequence data suggest that such a conclusion is currently premature. Bernard et al support the theory of an ancient virus and believe that the major clades (supergroups) of papillomaviruses are antiquarian and that the viruses have been restricted to single hosts [45].

The foregoing discussion on phylogenetic systems and evolutionary constraints evokes the cenancestor that the authors briefly commented on in the general theory of cervical carcinogenesis [46]. The history and origins of the papillomavirus and its similarities and differences to HIV must be understood before an effective HPV vaccine can be constructed. An even more interesting aside is the observation that the cancers that arise in patients who suffer from HIV infection and the resultant AIDS are neoplasms for which a viral etiologic agent has been implicated: Burkitt's lymphoma and the Epstein-Barr virus, Kaposi's sarcoma and the human herpes virus, and uterine cervical cancer and HPV (Fig. 5). This observation points to synergism among the viruses.


HIV
SU (surface)
TM (transmembrane)
Lipid bilayer
PR (protease)
IN (integrase)
MA (matrix)
(+) strand mRNA
NC (nucleocapsid)
RT (reverse transcriptase)
CA (capsid)
EBV
hHV
HPV
Burkitt's Lymphoma
Kaposi's Sarcoma
Cervical Carcinoma

Human papillomavirus and cervical cancer: the epidemiologic evidence

Before turning to HPV vaccination strategies, it is important to review the epidemiologic evidence that implicates HPV as the cause of uterine cervical cancer [47]. Before the 1970s, many investigators believed that there was only one type of HPV species and that the specific tissue infected dictated the type of wart that developed. With the advent of recombinant DNA technology and molecular cloning, it later became evident that there were many HPV types and that a specific predilection of these different types for various epithelial surfaces existed. Still, during the early 1970s, Professor Stanley from Cambridge University correctly asserted that few investigators would have believed that genital tract HPV infections could be associated with anything more sinister than exophytic condyloma [13].

In 1976, Meisels and Fortin observed that the pathognomonic histologic feature of papillomavirus infection, koilocytosis, was present in flat intraepithelial lesions of the uterine cervix [48]. This finding was noteworthy because it directly linked HPV infection with precancerous lesions. The seminal series of papers from Harald zur Hausen et al confirmed the cytologic and histologic observations with hard molecular virology and identified new, genital HPVs with enhanced pathogenicity [49].

The DNA sequences of two of the new HPV types, HPVs 6 and 11, were found to be contained in most condyloma acuminatum [50,51]. There were related but not altogether identical sequences present in invasive uterine cervical carcinomas. The watershed event arrived in 1983, when investigators from the zur Hausen laboratory cloned and characterized HPVs 16 and 18 from cervical carcinoma biopsies [52–54]. As Professor Stanley acknowledged, the massive and rapid expansion of data that followed was a result of zur Hausen's generosity to the scientific community to which he freely provided HPV-specific reagents. [13] HPVs 16 and 18 were found to be associated not only with invasive cervical carcinomas but also with cervical intraepithelial neoplasia grade 3 (CIN 3) and high-grade squamous intraepithelial lesions [55].

Stating that HPVs are found in cervical neoplasms reflects observational data in the laboratory only. It is more than a stone's throw to reach the threshold of causality. If one looks to history, one finds that questions regarding causality are not new to the realm of infectious diseases. During the late nineteenth century the two most dangerous diseases were King Cholera and White Death-Tuberculosis (consumption). Dr. Robert Koch (1843–1910) is recognized throughout the world as the Father of Modern Bacteriology, and he achieved remarkable breakthroughs in the field of infectious diseases, including the discovery of the tubercle bacillus [56]. In an attempt to define what an infectious disease actually is, he created his famous *postulates*, which currently bear his name. Basically, *if*

Fig. 5. Examples of virus-associated human neoplasms that occur after infection with HIV and subsequent immune system deregulation. *Right,* Kaposi' sarcoma and the human herpes virus. *Bottom center*, Cervical cancer and the HPV. *Left,* Burkitt's lymphoma and the Epstein-Barr virus.

an organism can be isolated from a host that suffers from the disease and the organism can be cultured in the laboratory and the organism causes the same disease when introduced into another host and the organism can be re-isolated from that host, *then* the organism is the cause of the disease and the disease is an infectious disease. The implicit assumption is that the other host must have a genetic make-up that causes it to react to the organism in the same way as the original host. It must be acknowledged that these steps do not apply to all infectious diseases. For example, the bacterium that causes leprosy, *Mycobacterium leprae*, cannot be cultured in the laboratory. Leprosy is still recognized as an infectious disease, however.

By the mid-to-late 1980s, many investigators asserted that infection with high-risk HPV "caused" cervical cancer. Because the epidemiology of cervical cancer always had suggested that it was a sexually transmitted disease, HPVs turned out to be good candidates for the etiologic infectious agent [57]. Demonstrating viral causality in human cancer is difficult, however, because Koch's postulates can be fulfilled in rabbits (eg, the cottontail rabbit papillomavirus was studied exhaustively in the 1930s) [58,59] but not in people. The presence of viral genomes in carcinoma cells is suggestive of, but not unequivocal proof of, causality.

As one of the authors (P.J.D.) has stated numerous times, HPV may be simply a passenger. It may be that cervical cancer cells are susceptible to infection by HPV or that viral gene expression has no mechanistic relationship to the development and progression of cervical neoplasia. What was lacking were sound epidemiologic data that fulfill the criteria of causality and laboratory observations that supported the oncogenic potential of high-risk HPVs.

The epidemiologic evidence that has amounted over the past 15 years linking HPV infection to invasive cervical cancer is strong. The body of work is founded on a consistent set of case series, case-control studies, and cohort studies, and many investigators consider the association to be causal. The largest study analyzing the prevalence of HPV DNA in squamous cell carcinoma of the uterine cervix was conducted by the International Biological Study on Cervical Cancer [38]. Using a highly sensitive polymerase chain reaction assay with generic primers, direct detection of HPV genomes in fresh or fixed tissue biopsies, tissue scrapings, and exfoliated cells was accomplished through nucleic acid amplification and subsequent molecular hybridization procedures. Importantly, 22 countries contributed more than 1000 specimens and the prevalence rate reached 93%; the prevalence rate was raised to 99.7% when a re-analysis of the negative samples was performed using different primers [60]. HPV 16 was the dominant subtype detected (except in Indonesia, where HPV 18 was more common).

With odds ratios greater than 15 in the methodologically sound case-control series, the strength of the association between oncogenic HPVs and cervical cancer and high-grade squamous intraepithelial lesions effectively rules out the possibility of chance, bias, or confounding [13]. During the 1990s, reliable, large-scale prospective data became available from cohort studies conducted by Ho et al, Koutsky et al, Remmink et al, and Liaw et al, and several noteworthy observations were made [61–64]. First, infection with oncogenic HPVs preceded

the development of high-grade squamous intraepithelial lesions [61–63]. Second, HPV DNA can be detected before cytologic identification of squamous intraepithelial lesions [64]. Finally, infection by oncogenic strains was predictive of high-grade squamous intraepithelial lesions [64].

Herrero et al conducted a population-based study of HPV infection and cervical neoplasia in rural Costa Rica and reported their findings to the National Cancer Institute at the dawn of the new millennium [65]. The investigators found that HPV infection was common in young women but declined rapidly to a low beyond age 35 years. The prevalence of low-grade squamous intraepithelial lesions peaked in women at approximately age 29, whereas that of high-grade squamous intraepithelial lesions occurred in the 30- to 40-year-old age group and then again in the over-65 age group. Nearly every high-grade squamous intraepithelial lesion and invasive cancer contained oncogenic HPVs, with odds ratios of 320 and 710, respectively [65]. As Stanley succinctly stated in her excellent treatise, the epidemiologic evidence clearly shows that HPV infection is the cause of cervical dysplasia and infection with high-risk HPVs is the major risk factor (ie, is necessary) for the subsequent development of cervical cancer [13]. The reader, however, should be advised that the epidemiologic databases cited also demonstrate that infection with oncogenic HPVs is not sufficient.

Vaccination strategies: an epidemiologic theory for vaccination

Successful cervical cancer screening programs are founded on the detection and treatment of precursor lesions, through which the incidence and mortality from cervical cancer has been reduced in some Western countries [66–69]. 80% of cervical cancer deaths worldwide occur in poor regions of the world with insufficient health care resources that are unable to support screening programs for asymptomatic individuals and cannot provide radical surgery or radical pelvic radiotherapy to women with invasive disease [70]. Alternative strategies are needed, especially in the area of primary prevention, because 40% of women treated with invasive disease experience recurrence and die [71]. In the past there have been no specific antiviral treatment modalities for HPV-associated diseases, including anogenital warts, laryngeal papillomatosis, preinvasive lesions of the vulva, vagina, and cervix, and invasive cervical cancer.

To control an infectious disease, one must take into consideration the basic reproductive number of infection, designated R_0, which describes the potential for spread of an infection [72]. The three central components of the basic reproductive number include the duration of infectiousness, the contact pattern of potential hosts, and the likelihood of transmission on contact between an infectious and a susceptible host [73]. These are the parameters that clinicians attempt to reduce through interventions aimed at increasing treatment and cure rates, education or quarantine to reduce contacts, and barriers to reduce infectiousness. Because the basic reproductive number applies to the situation in which the entire population is susceptible, if one considers vaccination, the target

becomes the effective reproductive number, designated R_1 [72]. In other words, through vaccination one attempts to reduce R_1 at a given moment in time. Vaccination reduces the fraction of the susceptible population.

Although most antiviral vaccines are based on the use of virions to induce antivirion antibodies, it is difficult to produce sufficient quantities of HPV virions in cultured cells to induce a host response. Because HPV virions contain oncogenic DNA genomes, attenuated HPV virions are risky candidates for vaccine development. In the early 1990s, however, it was discovered that by inducing expression of the major HPV capsid protein L1 in cultured eukaryotic cells, it was possible to produce HPV VLPs. Specifically, L1 is the most antigenic of the HPV-encoded proteins, and it alone can self-assemble to form the VLP [74]. Morphologically identical to native HPV virions, these VLPs lack the viral DNA core and can be injected safely into a host to induce an antibody response without risk for oncogenic sequelae [75–77]. Because the consensus thinking is that prophylactic vaccines based on HPV capsid proteins L1 and L2 offer the best chance for success, this technology offers the most promise. In addition to eukaryotic cells, VLPs also can be constructed using yeast, recombinant bacteria, and insect cells.

The goal of prophylactic and therapeutic immunization (see later discussion) is a stimulation of the adaptive immune response with memory to produce antigen-specific effector molecules and cells either to prevent infection or eliminate infected or transformed cells. A prophylactic vaccine would aim to prevent HPV infection in individuals at greatest risk of exposure to HPV, including young women at the onset of sexual activity, by generating an effective immune response at the site and time of infection and inhibit the establishment of long-term infection and reinfection.

Currently, 17 viral vaccines are licensed and used in the United States (Table 1) [27]. One should note that the smallpox vaccine has not been administered routinely since the time of virus eradication, and the killed Salk poliomyelitis vaccine has been recommended to replace the live Sabin vaccine. Using a yeast, the surface antigen of plasma-derived hepatitis B virus vaccine is made exclusively by recombinant expression [27].

Ten of the 17 vaccines are live attenuated viruses, 4 are killed whole virus, and the influenza vaccine is an ether-split killed virus [27]. The only subunit vaccine is that for hepatitis B, and it is also the only one that is recombinant expressed. The remaining surface antigen vaccine of hepatitis B remains plasma derived. There has been no encore to follow the recombinant subunit hepatitis B vaccine, because live attenuated and killed viruses remain the models for successful vaccine development. Vaccines against nonpropagable agents, such as hepatitis C and HPV, offer no alternative choice and we must rely on the subunit approach.

Animal and human studies

The collected works on the papillomavirus vaccine studies encompass the principles on which successful vaccines are based [78–89]. Although each of

Table 1
Available and licensed human viral vaccines in the United States

Viral vaccine	Technology
Measles	Live attenuated
Mumps	Live attenuated
Rubella	Live attenuated
MMR combined	Live attenuated
Poliovirus	
Sabin	Killed whole virus
Salk	Live attenuated
Varicella	Live attenuated
Influenza	Ether split whole virus
Adenovirus	Live attenuated
Hepatitis A	Killed / inactivated
Hepatitis B	
hepatitis B	Surface antigen plasma derived
hepatitis B	Subunit recombinant expressed
Japanese B encephalitis	Killed / inactivated
Rabies	Killed / inactivated
Rotavirus	Live, oral, tetravalent[a]
Smallpox (Vaccinia)	Live
Yellowfever	Live attenuated

[a] Contains four live viruses.

the proteins expressed by the HPV would be reasonable with which to design a vaccine, the L1 and L2 capsid proteins evoke antibodies that neutralize the virus, and the E6 and E7 anti-oncogene vaccines theoretically suppress the mutagenic activity of the virus and target infected cells for destruction. It is likely that humoral and cell-mediated responses are required for the prevention and treatment of papillomavirus infections. Antibodies destroy or neutralize toxins and free viruses (ie, soluble substances) through interactions with helper CD4+ T cells and the major histocompatibility complex II algorithm, whereas cytotoxic CD8+ T cells that work through the major histocompatibility complex I pathway destroy infected cells and suppress their transcriptional and translational machinery.

Because infection with papillomaviruses is strictly species specific, the use of various animal papillomaviruses has allowed investigators to study the concept that VLP-derived papillomavirus vaccines can generate virus-neutralizing antibodies. Several laboratory studies that used comparable animal papillomavirus vaccines in animals resulted in good immune responses (Table 2). Recombinant vectors that encode HPV L1 have been developed to produce VLPs in yeast, insect cells, and the bacterium *Escherichia coli*. Through parenteral immunization of cottontail rabbits with microgram doses of purified VLPs followed by booster injections and a challenge with high-dose purified virus to an abraded epithelium, Breitburd et al were able to show that VLPs induce antibodies to conformational capsid epitopes that can neutralize virus particles [90]. Christensen et al confirmed these observations in their own VLP vaccine study using a

Table 2
Animal papillomavirus vaccine studies of immunologic response

Investigator	Model	Infection	Vaccination schedule	Time to challenge[a]
Christensen [91]	CRPV, rabbit	Cutaneous	0, 4, and 8 wk	2 and 12 wk after vaccination
Kirnbauer [92]	BPV4, cow	Mucosal	0 and 4 wk	2 wk after vaccination
Suzich [93]	COPV, dog	Mucosal	0 and 2 wk	2 wk after vaccination
Breitburd [90]	CRPV, rabbit	Cutaneous	0, 2, and 4 wk	1 wk after vaccination

CRPV, cottontail rabbit papillomavirus; BPV, bovine papillomavirus; COPV, canine oral papillomavirus.

[a] More than 90% protection was observed in all four studies when the animals were challenged with homologous virus within 1 month of the last booster vaccination and ELISA measurement of the VLP antibody titers.

fresh group of cottontail rabbits [91]. Kirnbauer et al and Suzich et al also have had good results immunizing cattle and dogs with bovine papillomavirus VLPs and canine oral papillomavirus VLPs, respectively [92,93].

Although the principle that VLP vaccination can induce neutralizing immunity against papillomavirus challenge has been established, Stern et al correctly noted that the lack of a genital transmission model remains problematic because it is not possible to predict the efficacy of an analogous human vaccine in preventing sexually transmitted HPV infection [22]. Efficacious protection from natural venereal transmission of genital HPVs requires a high-titered, local antibody response in the lower reproductive tract mucosa.

The encouraging results from the animal studies have prompted commercial and public institutions to undertake clinical trials of HPV VLP-derived vaccines. The public sector trials are sponsored by the National Cancer Institute in collaboration with the National Institute of Allergies and Infectious Disease and the Johns Hopkins University [94]. With the aim of establishing safety and immunogenicity, phase I trials in humans are in progress using VLP-derived vaccines.

Seventy-two healthy volunteers (58 women and 14 men) at Johns Hopkins University were entered into the first double-blinded, placebo-controlled, phase I trial of dose escalation through a three-inoculation regimen. An enrollment criterion of four or fewer lifetime sexual partners was included to reduce the likelihood that the vaccines would have HPV 16 virion antibodies before vaccination. In a progress report by Schiller and Hildesheim, it was noted that by 1 month after the second vaccination, all subjects who received the VLP had seroconverted, as measured in a VLP-based IgG enzyme-linked immunosorbent assay [94]. By the study's conclusion, Harro et al noted that when given with or without adjuvant, the vaccinated subjects had an increase in their serum antibody titer that was 40-fold higher than those who naturally seroconverted (ie, serologic levels from 6 subjects found to be seropositive to HPV 16 at study initiation were used to delineate the baseline natural serologic level for HPV 16 exposure) [95]. None of the subjects experienced any substantial systemic sequelae. The predominant reaction noted was local pain at the injection site that resolved

spontaneously within a few days. None of the placebo vaccinated subjects seroconverted during the course of the study.

Preliminary data from several other phase I and phase II clinical trials with HPV L1 VLPs given intramuscularly to healthy volunteers have confirmed that the subunit vaccines are tolerated at various dosages and elicit a strong humoral immune response [96–99]. The question remains, however, as to whether anti-VLP antibodies can be detected in the vaginal secretions of immunized women and if the local antibody response can prevent subsequent viral infection. It is unknown whether serum IgG antibodies alone are sufficient for protection against infection at the cervix, because secretory IgA is normally considered to be the first line of antibody defense at mucosal surfaces.

The National Cancer Institute is using the HPV 16 VLP vaccine in a proof of principle trial in the Guanacaste province of Costa Rica [94]. The trial is co-sponsored by the national government of Costa Rica. This site was selected because it is the focus of a large ongoing natural history study of genital HPV infection and its relationship to cervical neoplasia [100]. Three thousand young women will be randomized to either the vaccine or placebo, and the study design will control for risk factors associated with HPV infection and progression of HPV-induced lesions. A three-arm trial is being considered that involves placebo, intramuscular vaccination with HPV 16 L1 VLPs, and a chimeric VLP composed of L1 plus a recombinant protein consisting of HPV 16 L2 fused to nonstructural HPV 16 proteins (most likely E7 and E2). The latter arm would evaluate the possibility that a vaccine that generates cell-mediated immune response to non-structural viral proteins might increase vaccine efficacy by inducing regression of subclinical infections that result from imperfect neutralization of virus by antibodies. In the end, the investigators wish to determine whether a simple parenteral inoculation of VLPs will result in sufficiently high and sustained virion antibody concentrations in the female genital tract to prevent infection by sexually transmitted virions.

Another placebo-controlled, randomized, phase I study is underway in Manchester, UK using an *E. coli*-derived HPV 16 L1 VLP vaccine. The study involves dose escalation over three immunizations, and the goals are to establish safety and measure the magnitude and kinetics of serologic and cell-mediated responses to the vaccine [22]. Future optimization may include adjuvantation, targeting dendritic cells, cytokine patterning, establishing long-term memory, creating chimeric vaccines (fusion genes to stimulate humoral and cell-mediated immunity), and determining optimal mode of delivery using recombinant viral or plasmid DNA vectors.

Socioeconomic implications of a cervical cancer vaccine

The public health paradoxes that exist in the context of sexually transmitted diseases must be addressed when considering vaccine testing and efficacy. A stigma associated with dealing with sexually transmitted diseases remains, which

may have a negative impact in accruing women to participate in an HPV vaccine trial. It is reasonable that asymptomatic and presumably infection-free subjects would not wish to be perceived as belonging to a high-risk group [101]. Advertising or "selling" an effective vaccine to the susceptible populations is exacerbated by the fact that the target population consists predominantly of adolescent girls and young women.

Behavioral changes also must be taken into account if vaccine delivery is to be successful. There may be an increase in risky sexual activities, which may allow for an increase in other sexually transmitted diseases. Garnett and Waddell found even more information concerning the possibility that women who are vaccinated against HPV will believe they are protected fully from ever getting cervical cancer and will no longer seek routine gynecologic care [72]. Because the vaccine does not protect against all HPV subtypes, vaccinated women will still be at risk for developing cervical cancer by nontargeted HPVs or escape mutants. The duration of protection from the vaccine may not be lifelong. Although the frequency of screening may be reduced as dictated by an individual's overall risk, it should not be eliminated altogether.

Economic ramifications of bringing an HPV vaccine into widespread use in developing countries are problematic. Countries are ranked by their gross national product into one of the four income bands, and countries in the lowest bands can purchase vaccines from distributors at reduced costs. Unfortunately, most funding agencies still lean toward therapeutic care, which can provide relatively immediate results when compared to preventive measures, whose results are not evident for many years [102].

Because childhood vaccination programs are easily implemented into existing postnatal care systems and school programs, traditional childhood vaccination programs have been successful, even in the developing world. Vaccination programs for adults in the third world are another story, and proper surveillance systems, education about the link between the HPV and cervical cancer, and accurate data about the global burden of the disease are required before political support for the allocation of resources to an HPV vaccine can be possible [103].

A few words on therapeutic vaccines

An efficacious prophylactic vaccine would prevent the establishment of long-term HPV infection and reinfection by generating an effective immune response at the site and time of initial infection. Such a vaccine would be suitable for young women at the onset of sexual activity because they constitute the population at greatest risk. Therapeutic vaccines, on the other hand, would be directed at the elimination of residual disease after therapy for intraepithelial and invasive disease. Therapeutic vaccines also could be designed to retard progression and induce regression of disease.

Therapeutic vaccines may be based on peptides, proteins, chimeric VLPs that contain nonstructural virus proteins, DNA, viral vectors, bacterial vectors, den-

dritic cells, and modified tumor cells [104]. Therapeutic vaccine strategies that target high-risk HPV E6 and E7 oncogenes could provide an attractive, universally applicable option if such vaccines can have proven efficacy and be affordable.

Unlike attenuated viral vaccines, which enhance immunity through aberrant replication, DNA-based vaccines do not have the ability to propagate, and their ability to produce immunity is limited. Attempts to overcome this obstacle have included designing a full-length HPV 16 E7 gene fused to the *Mycobacterium tuberculosis* heat shock protein 70. Chen et al have tested this hybrid vaccine in a murine model and have observed a 30-fold increase in E7-specific CD8+ cells [105]. The use of E6 and E7, however, is controversial because of the potential for mutagenesis. Discussion of therapeutic vaccines is beyond the scope of this article.

Micronutrients

Nutritional factors may be linked to cervical cancer via cell growth regulation, programmed cell death mechanisms (ie, apoptosis), and immune system dysfunction. It is challenging, however, to demonstrate the impact that various nutrients and dietary constituents may have on the development and progression of cervical cancer, because interactions between these factors and other etiologic agents (eg, the HPV and tobacco) are likely to occur and confound the analyses [106]. Diet is a modifiable risk factor that may allow for effective primary prevention strategies.

Carotenoids are pigments found in plant foods and are not synthesized in animals [107]. They serve as a precursor to vitamin A and other retinoids. Although most case-control series have not implicated dietary preformed vitamin A or plasma retinol concentrations in cervical cancer risk [108–111], other studies have demonstrated a link between low dietary intakes of carotenoids and cervical carcinogenesis [112,113].

Muto et al demonstrated that liposomal beta-carotene at a concentration of 10 mL/L was able to induce apoptosis in preneoplastic cells (ie, dysplastic) of the uterine cervix via downregulation of the epidermal growth factor receptor protein [114]. Two randomized clinical trials that used beta-carotene supplements to promote regression of cervical dysplasia concluded recently and were found to have negative results [115,116]. Additional studies are ongoing, however, including three randomized controlled trials in Australia, one of which examines the effect of beta-carotene (15 and 30 mg, daily) plus vitamin C (300 and 500 mg, daily) in women with CIN 1 [117].

Vitamin C also has been found to be inversely related to the risk of cervical cancer [106,108,118]. Vitamin C ingestion may influence immune system modulators and as an antioxidant it helps protect DNA from reactive oxygen species. Vitamin C may inhibit oncogene transformation and improve the stability and use of folic acid [106].

Epidemiologic studies of dietary folate have demonstrated a stronger link with CIN than with carcinoma in situ or invasive cervical cancer [106]. Similar to the

case with vitamin C, it is crucial that optimal measurements of folic acid involve the red blood cell rather than serum concentrations for any meaningful results to be obtained. Because the cytologic features of megaloblastosis associated with folic acid deficiency are morphologically similar to the changes associated with cervical dysplasia, several investigators are examining the effect of folate supplementation on promoting regression. Two randomized clinical trials, however, have had negative results [119,120]. Folic acid plays a crucial role in DNA methylation and can affect gene expression in laboratory animals [121]. Perhaps a low folate status permits the incorporation of HPV DNA at folate-sensitive fragile sites, and folic acid supplementation would have protective effects early on in the HPV infective process, before the development of intraepithelial neoplasia [119,122].

The epidemiologic evidence that links vitamin E intake to cervical cancer risk is less well established than for dietary carotenoid and vitamin C [106,108]. Although a chemoprotective role for vitamin E may be related to its antioxidant function, this micronutrient also has been shown to enhance cell-mediated immune response and phagocyte-derived functions [123]. There may be a relationship between vitamin E status and the persistence of HPV infection.

The epidemiologic evidence suggests that certain micronutrients may serve as biomarkers of food choices if one were trying to reduce cervical cancer risk. For example, fruits and vegetables are the primary dietary sources of carotenoids, vitamin C, and folic acid [124]. As Rock et al correctly point out, however, biologic mechanisms that determine the optimal timing for intervention have not been worked out [125]. The dietary supplementation trials that have been concluded have been negative because we do not yet know at which stages in the process of carcinogenesis and progression the administration of a micronutrient would be expected to have a biologic effect.

Chemopreventive agents

Chemoprevention was defined by Sporn et al in 1976, when they advanced the concept of prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (ie, retinoids) [126]. Chemoprevention already had proved feasible as evidenced by the decrease in dental decay through fluoridation of water, reduction of heart disease via lipid reduction, and diminution of stroke with daily aspirin intake [127]. In separate reviews, Bertram et al, Boone et al, and Weinstein provided convincing evidence that chemoprevention can reduce the incidence of human cancer [128–130]. Cancer of the uterine cervix should be an obvious target for chemopreventive efforts.

The most extensive experience in the study of chemoprevention for cervical cancer has come from the use of all–trans-retinoic acid. Meyskens et al at the University of California, Irvine, conducted a phase III study using topical applications of all–trans-retinoic acid (1 mL of a 0.375% solution) and demonstrated increased complete histologic regression of CIN 2 (27% placebo, 43% all–trans-retinoic acid) but not for CIN 3 [131].

Polyamines play a critical role in cell growth and transformation. 2-difluoromethylornithine is a specific inhibitor of ornithine decarboxylase, the key enzyme in the biosynthetic pathway of polyamines. Nishioka et al are studying polyamines as potential biomarkers of CIN [132], whereas Boiko et al are investigating the chemoprotective role of 2-difluoromethylornithine in 30 women with CIN 3. Of 25 evaluable patients, there were 11 responders [133]. Additional work examining 2-difluoromethylornithine as a chemoprotective agent for cervical cancer and other human malignancies is currently underway.

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