Surrogate Endpoint Biomarkers for Cervical Cancer Chemoprevention Trials

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Abstract   Cervical intraepithelial neoplasia (CIN) represents a spectrum of epithelial changes that provide an excellent model for developing chemopreventive interventions for cervical cancer. Possible *drug effect* surrogate endpoint biomarkers are dependent on the agent under investigation. Published and preliminary clinical reports suggest retinoids and carotenoids are effective chemopreventive agents for CIN. Determination of plasma and tissue pharmacology of these agents and their metabolites could serve as drug effect intermediate endpoints. In addition, retinoic acid receptors could serve as both drug and biological effect intermediate endpoints. Possible *biological effect* surrogate endpoint biomarkers include cytomorphological parameters, proliferation markers, genomic markers, regulatory markers, and differentiation. Given the demonstrated causality of human papillomavirus (HPV) for cervical cancer, establishing the relationship to HPV will be an essential component of any biological intermediate endpoint biomarker. The *pathologic effect* surrogate endpoint biomarker for cervical cancer is CIN, used clinically for years. The desired effect for chemopreventive trials is complete regression or prevention of progression. In planning chemopreventive trials, investigators need to consider spontaneous regression rates, the subjective nature of detecting CIN, and the impact of biopsy on regression.

If intermediate endpoint biomarkers that met the above criteria were available for cervical cancer, then new chemopreventive agents could be rapidly explored. The efficacy of these new agents could be determined with a moderate number of subjects exposed to minimal risk over an acceptable amount of time. The impacts on health care for women would be significant.   © 1995 Wiley-Liss, Inc.

CLINICAL IMPACT OF CERVICAL CANCER

Cervix carcinoma is an important and feasible target for chemoprevention efforts. This common malignancy [1], while not lethal at early stages of the disease in most cases, occurs in young, child-bearing, sexually active women. Carcinoma is thought to result from the progression of cervical intraepithelial neoplasia (CIN).

Cervix carcinoma is an important health problem world-wide [1]. In the U.S., 15,800 new cases of invasive cervix cancer are predicted in 1995 with 4,800 deaths attributable to this disease (1.8% of all cancer-related deaths in women) [2]. These figures do not include the more than 50,000 cases of carcinoma *in situ* (CIS) and many times that number of cases of cervical dysplasia for which we have no estimates for incidence or prevalence. The age-adjusted death rate for cervical carcinoma in the U.S. is 3.2 per 100,000 and remains level [3]. Anecdotally, we have observed in our clinics an increased number of younger women with invasive cervical carcinomas and dysplasia. This shift to younger age groups has been attributed to the growing spread of human papillomavirus (HPV) infections in the population [4].

A number of important epidemiologic risk factors have been identified for developing CIN and invasive cervical cancer. Early sexual experiences, the number of sexual partners, and male partner factors (number of sexual partners, history of venereal disease, early sexual experience) are important risk factors [5–8]. Smoking increases the risk of developing cervical cancer [9–11]. Of key importance is infection with human papillomavirus (HPV). HPV DNA sequences can be recovered from greater than 90% of cases of invasive cervix carcinoma. Whether the links between sexual history, smoking, and squamous intraepithelial lesions influence risk primarily through HPV or whether they are independent risk factors suggesting other contributing causes of invasive carcinoma remains controversial. However, the recent case control trial by Schiffman *et al.* [12] using PCR assays found that the great majority of all grades of CIN can be attributed to HPV infection and that HPV infection meets epidemiologic criteria as a causal agent for cervical cancer.

ISSUES FOR CHEMOPREVENTION TRIALS

Cancer chemoprevention trials pose unique problems in design. Chemoprevention agents are aimed at a healthy population. Therefore, toxicity is intolerable. An *effective* dose must be determined that is not toxic. The dose must be tolerated on a chronic basis over a long period (years). Furthermore, in many premalignant conditions or high-risk individuals, there is no easily identifiable therapeutic endpoint. Endpoints for cancer clinical trials are usually a measured reduction in tumor size or a statistically measured survival in a population whose survival is limited. For most chemopreventive interventions, no such easily measured endpoints exist. Currently, we must rely upon events that happen years later, such as the development of cancer. Complex biostatistical and epidemiologic tools are necessary in massive study populations to prove clinical efficacy. Another approach is to develop new, surrogate endpoint biomarkers.

Criteria for Surrogate Endpoint Biomarkers

An optimal intermediate biological endpoint for an at-risk population will be readily expressed in plasma or in tissues accessible to biopsy, related in some way to the process of neoplastic transformation, may be easily measured from small quantities of tissue, quantifiable as a continuous variable, and may be expected to be modulated by a chemopreventive intervention. The following review describes some of the histological and molecular changes that occur before and/or during the malignant transformation process. Many of these changes are potential surrogate endpoint biomarkers. Our goal is to identify the most likely candidates based on the evidence available to satisfy the above criteria.

BIOCHEMICAL EFFECT SURROGATE ENDPOINT BIOMARKERS

We have defined a biochemical or drug effect surrogate endpoint biomarker as an intervention which modulates a cell's normal biochemical function. Drug effect surrogate endpoint biomarkers address three important aspects of a chemoprevention trial's design. First, they serve as reproducible, quantitative methods for determining whether a pharmacodynamically important drug concentration has been delivered intracellularly to the target site. Second, they can be used to define endpoints for Phase I chemoprevention dose searching trials. The use of a drug effect surrogate endpoint biomarker as an endpoint for a Phase I chemoprevention trial recognizes that the therapeutic index differs for different drug use indications. Third, drug effect surrogate endpoint biomarkers can be used as measures of adequate pharmacodynamic action at the target issues in Phase II or III chemoprevention trials. Such data can be used as part of an adherence assessment.

An optimal drug effect surrogate endpoint biomarker also serves as a biologic surrogate endpoint biomarker. Thus, the drug effect surrogate endpoint biomarker may be a cellular product (protein, carbohydrate, gene expression) of a regulatory aspect of cellular growth control or a product reflecting changes in a cell that has functional but not morphologic transformational changes. In many cases, the usefulness of drug effect surrogate endpoint biomarkers as biologic endpoints is unclear. The drug effect surrogate endpoint biomarker becomes one of a number of potential biologic surrogate endpoint biomarkers that are assessed in Phase IIa and Phase IIb chemoprevention trials.

Potential Surrogates for Retinoid Chemoprevention Trials in Cervix

Drug effect surrogate endpoint biomarkers are defined by the drug being tested in a Phase I or II chemoprevention trial. Retinoids and carotenoids have been the primary chemopreventive agents tested to date in cervical cancer. Drug effect surrogate endpoint biomarkers for these agents would represent evidence of effect in plasma or at the target site, cervix epithelial cells. Fenretinide (4-HPR) suppresses retinol in plasma

(from a normal range of 270–747 ng/ml to 16–188 ng/ml). Retinol is easily measured in plasma using readily adapted high performance liquid chromatography methodology [13]. Cellular retinol concentration in cervical epithelium has not been measured. Extraction procedures from tissue samples have not been validated. Given the accessibility of cervix epithelium, direct measurement of 4-HPR concentrations in target tissue is possible.

Retinoids are known to exert their biological effects by binding to specific nuclear receptor proteins, which are members of the steroid receptor superfamily. These receptors regulate the expression of specific target genes in a ligand-dependent manner. Two general classes of these receptors have been described, and are known as retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Phase I chemoprevention trials of new synthetic retinoids (e.g., 9-cis-retinoic acid, SR11203, SR11217, TTAB, TTNN) may be based upon their specific binding of nuclear RARs or RXRs. Thus, drug effect and optimal chemopreventive doses in the future may be based upon specific drug binding to target sites. The potential for retinoid regulation of cellular proliferation may permit the use of RARs and RXRs as biologic as well as drug effect surrogate intermediate biomarkers.

BIOLOGICAL EFFECT SURROGATE ENDPOINT BIOMARKERS

We define a biological effect surrogate endpoint biomarker as one in which an intervention changes a cellular product. This cellular product must be linked to cancer risk, carcinogenic exposure, carcinogenesis, or tumorigenesis. The following are examples of biologic effect surrogate intermediate endpoints for cervical cancer.

HPV

There is compelling molecular and epidemiological evidence indicating that infection with certain genital HPVs has a critical role in the cellular changes that precede cervical cancer as well as other genital cancers [12,14,15]. Of the more than 70 characterized HPV types, about 30% are associated with genital tract infections. Whereas 90% of cervical cancers contain HPV DNA sequences, only certain genital HPV types

are associated with cervical cancer. Lorincz et al. [15], in their study of 2,624 women, detected HPV DNA in 79.3% of women with confirmed cervical neoplasia by Southern blot methods. In addition, 23.7% of women with borderline atypia and 6.4% of women with a normal cervix were positive for HPV DNA. This study defined four HPV risk groups based on associations with cervical cancer. The "low-risk" group contained HPV types 6, 11, 42, 43 and 44. DNA sequences from these virus types were absent from all cervical cancers but were present in 20.2% of low-grade squamous intraepithelial lesions (SIL). An "intermediate risk" group consisted of HPV types 31, 33, 35, 51, 52 and 58. Viral DNA was detected in 23.8% of high-grade SIL but in only 10.5% of cervical cancers. The "high-risk HPV 16" group was defined by the presence of HPV 16; DNA from this virus type was found in 47.1% of both high-grade SIL and cervical cancers. The "high-risk HPV 18" group contained HPV 18, 45 and 56; DNA from these virus types were found in 26.8% of cervical cancers, but only 6.5% of high-grade SIL.

In most cases of carcinoma, the HPV DNA is integrated into cellular genomes, while in CIN lesions the viral DNA is extrachromosomal [16]. Integration appears to be random as far as the site of integration in the host chromosome is concerned. However, the viral genome integrates near known oncogenes in some cell lines [17]. Integration generally occurs in the E1/E2 region of the viral genome, disrupting the E2 viral transcription regulatory circuit [18]. The E2 open reading frame (ORF) encodes a transcription regulatory protein that is a DNA binding protein. For HPV 16 and HPV 18, E2 appears to act as a repressor of the promoter from which the E6 and E7 genes are transcribed [19]. Thus, it is thought that disruption of the E2 gene provides a selective advantage, leading to uncontrolled proliferation of the cell due to deregulated expression of E6 and E7 genes [20].

In all cell lines examined thus far and in most cervical cancers, the E6 and E7 ORFs are preserved and expressed as mRNAs and proteins. Continued expression of these proteins is critical for maintenance of the malignant phenotype of cervical cancer cells [21]. In tissue cultures, HPV 16 and HPV 18 E6 and E7 can profoundly influence the growth and differentiation of human keratinocytes. Transfection of primary human keratinocytes with virus DNA results in immortalization of nontumorigenic cells [22,23]. Immortalization is dependent on continued expression of E6 and E7 [24].

Knowing that HPV 16 and HPV 18 E6 bind to and promote the degradation of tumor suppresser gene product p53 provides insights into the biochemical basis of cell transformation [25, 26]. Furthermore, the E7 product from these viruses, the most abundant viral protein in cervical cancer cells [27,28], binds to Rb105, another tumor suppresser gene product [29]. The low efficiency of keratinocyte immortalization by HPV 6 and HPV 11 is correlated with the weak binding of their E6 and E7 gene products to p53 and Rb105 [30,31]. Expression of E6 may account for the accumulation of mutations in immortalized cells; p53 is required for G_1 cell-cycle arrest after DNA damage. Kessis et al. [32] have shown that E6 expression perturbs G_1 arrest after DNA damage, thus permitting DNA replication and fixation of mutations.

The potential biologic effects of surrogate intermediate endpoints related to HPV are the presence of viral DNA or the expression of early genes E2, E6 or E7. In a model system of normal human keratinocytes and several independently derived HPV 16 immortalized keratinocyte cell lines, Pirisi et al. [33] have recently demonstrated that the HPV 16 lines are more sensitive than normal keratinocyte cell lines to all-trans-retinoic acid. Incubation in all-trans-retinoic acid at 10^{-7} M caused HPV 16 immortalized keratinocytes to express 2- to 4-fold less viral mRNA from E6 and E7 than untreated cells. The cells did not terminally differentiate; when the retinoic acid was removed, proliferation resumed. Future studies in women with CIN are needed to determine if E6 or E7 expression is related to progression to invasive disease, or whether suppression of E6 or E7 expression is related to disease regression. The other criteria for surrogate endpoint biomarkers have been met.

Cytomorphometry

The cellular morphology used to identify SIL could serve as a surrogate endpoint biomarker. Nuclear and cytological parameters such as size, density, nuclear/cytoplasm ratio, texture and geometry of nuclei have been examined to improve the diagnosis of cervical cancer [34–39]

and other diseases. However, the issue in chemoprevention of cervical cancer is not only diagnosis, but prediction of progression or regression of preinvasive disease states.

Few studies collect data that can address this issue. Rosenthal et al. [34] found that nuclear mean optical density, cytoplasm mean optical density, and nuclear-cytoplasm area ratio, along with autocorrelations of nuclear optical density, were predictive of progression or regression in 23 cervical smears; 7 progressed and 16 regressed without any intervention. Kwikkel et al. [35] found no features predictive of progression versus nonprogression in one set of women (n = 41). In another set of women (n = 20), they found cell area, shape of nucleus, and mean density of nucleus to correctly classify 80% of the specimens as progression or nonprogression [35]. A unique aspect of this study was the emphasis on visually normal intermediate cells rather than visually abnormal cells as the basis for prediction [35]. Thus, the available data on cytometric features that predict progression or regression are based on a small number of women and have inconsistent findings.

It remains unclear whether cellular or nuclear morphology will be useful surrogate endpoint biomarkers for cervical cancer. In contrast, some form of quantitative cytology and histopathology will be essential to assure accurate identification and classification of patients in chemoprevention trials.

Genomic Markers

The chromosomal karyotyping of cervical lesions has shown that the lesions can be diploid or euploid, polyploid or aneuploid. Some authors believe ploidy is a good predictor of biological behavior of cervical cancer [40], but the available data are conflicting and inconsistent. In addition, most studies have been done in women with invasive cervical cancer, which is not the target for chemoprevention. Any findings from these studies are likely not relevant to earlier stages of disease.

Studies among women with earlier stages of disease have some interesting but still inconsistent findings about DNA content or ploidy status. There appears to be a trend of higher percentages of women with aneuploidy with severe dysplasia or CIS compared to CIN I or II [41–46]. However, others have demonstrated no difference in ploidy status between normal cervical tissue and abnormal [47] and 100% aneuploid status in CIN I [48]. The issue of progression versus regression has been associated with ploidy status in two studies [41,49]. HPV has been shown to alter the determination of DNA content or ploidy findings [42,46,48,50] sufficiently that HPV should always be measured. Most of the studies noted above failed to determine the presence of HPV infection. Future studies of genomic markers need larger sample sizes of preinvasive disease states and consistent assays of ploidy along with quantitative histopathology and determination of HPV status and viral type before consideration as a surrogate endpoint biomarker.

Measures of genomic instability may be useful surrogate endpoint biomarkers. The available data is minimal. Kaelbling and colleagues [51] demonstrated loss of heterozygosity of chromosome region 17p13 in HPV-negative tumors. Other studies revealed nonrandom structural changes in chromosomes 1, 3, 11, and 17p, with specific allelic losses on 3p reported in up to 80–90% of tumors, and 30% on 11q [52]. In contrast, Rader and colleagues [52] did not detect loss of heterozygosity in any of 15 early stage cervical cancers, all positive for HPV. As with other genomic markers, a great deal more work needs to be done before considering genomic instability as a surrogate endpoint biomarker.

Proliferation Markers

The carcinogenic transformation of normal cells to cancer is clearly linked to significant changes in cellular kinetics. Proliferation markers and corresponding indices, such as mitotic index, S-phase fraction, Ki-67, etc., have been used to describe the cellular kinetics of normal cells, dysplastic cells, and cancerous cells. Brugal [53] has argued that three types of markers are necessary to describe malignant population growth kinetics: one measures the growth fraction (e.g., Ki-67), a second evaluates cell cycle speed in arbitrary units, and a third assesses the S-phase cell occurrence frequency (e.g., BrdU and PCNA). All of these markers of cellular kinetics have the potential to serve as surrogate endpoint biomarkers.

The monoclonal antibody Ki-67 binds to a nuclear antigen expressed by cycling cells of several human tissues and to the cytoplasm of basal layer cells in squamous epithelia. Among women with invasive squamous cell carcinoma of the cervix, a range of 10–50% Ki-67 staining has been observed, indicating considerable variation in tumor growth rates [54]. In addition, there was no significant relationship between Ki-67 staining and conventional histological parameters [54], but a high Ki-67 score has been associated with early recurrence [55]. Rishi and colleagues [56] have used Ki-67 on 40 benign and malignant tumors, using cytological smears and frozen tissues. The number of Ki-67 positive cells on cytological smears correlated well with Ki-67-cells from corresponding tissue. These studies suggest that Ki-67 could be used on cytological smears from the cervix instead of using biopsy specimens. If the other criteria for surrogate endpoint biomarkers are established for Ki-67, then this collection approach would be quite useful.

With the availability of a monoclonal antibody to bromodeoxyuridine (BrdU), BrdU-containing nuclei can be identified. Very few studies have evaluated the use of BrdU in cervical tissue. Fukuda *et al.* [57] found that BrdU-positive cells were mainly located in the parabasal area of normal epithelia and distributed throughout the epithelium with severe dysplasia and CIS. However, marked intra-tumor heterogeneity has been demonstrated [58] and the delivery method requires involved intravenous administration or intracervical injections [57]. Thus BrdU does not currently look very feasible as a surrogate endpoint biomarker.

Proliferating cell nuclear antigen (PCNA, cyclin), first detected in 1978, has been suggested to be necessary for DNA replication and cellular proliferation. The pattern of PCNA staining in normal cervical epithelium is localized to the parabasal area. Several studies of PCNA have demonstrated increased activity from normal epithelia to condyloma with and without dysplasia to moderate and severe dysplasia, showing increasing disruption of normal regulation of cell proliferation [59,60]. In addition, the loss of negative growth control demonstrated by changes in PCNA appears to be linked to consequences of HPV infection, through either loss of p53 function [60] or epidermal growth factor receptor (EGFR) regulatory mechanisms [61]. PCNA has

good supporting data and is feasible to collect and measure, therefore, it is a strong candidate for use as a surrogate endpoint biomarker.

It remains unclear whether one proliferative index is enough to describe the cellular kinetics of a neoplasm. Given the available data, we recommend incorporation of both Ki-67 and PCNA as possible surrogate endpoint biomarkers.

Regulatory Markers

The transformation of a normal cell into a cancer cell is a complex multistep process resulting in a clone of cells that are no longer under normal regulatory control. Advances in several fields have provided several regulatory markers to investigate the different steps in carcinogenesis related to regulatory control. The potential regulatory markers include transcription factors, oncogenes, and tumor suppresser genes.

Studies in oncogenes and cervical carcinoma have been done primarily in women with invasive disease. The expression of c-*myc* oncogene has been reported to increase from normal tissue to invasive disease [62–66], but the correlation with prognosis or relapse is inconsistent [65–70]. Other potential oncogenes include Ha-*ras* which is expressed primarily in high-grade or invasive lesions [66,67]. In evaluating each of these oncogenes as a potential surrogate endpoint biomarker, the impact of HPV needs to be considered. HPV appears to have significantly more impact on disease state and progression than overexpression of any oncogene in the studies that have measured both HPV and other oncogenes [69,71–74].

The expression rate of EGFR correlates with growth properties in a squamous carcinoma cell line. The presumption is that EGFR could be a useful surrogate endpoint biomarker for cervical cancer. EGFR expression has been noted in intermediate and superficial areas in severely dysplastic lesions and primarily in basal regions in normal epithelium [75,76]. Various clones of the C4-I cervical cancer cell line demonstrated no correlation between growth rate, expression of EGFR, and level of HPV gene products [77]. Among 97 HPV lesions of the cervix, EGFR and c-*erb*B-2 were not associated with specific HPV types, grade of CIN, or the clinical course [78]. Thus, the available data does not strongly sup-

port the potential of EGFR as a surrogate endpoint biomarker for cervical cancer.

Differentiation Markers

Cervical epithelium differentiates along the squamous pathway in carcinogenesis. Thus, markers related to this pathway could be surrogate endpoint biomarkers. Several different potential surrogate endpoint biomarkers related to differentiation include keratins and involucrin. Intermediate filaments, characterized by keratins, are a major cytoskeletal element present in virtually all epithelial cells. Keratins represent many different polypetides ranging in molecular weight from 40–70 kD. They can be further subdivided into two groups according to molecular weight and electrophoretic mobility. At least one keratin from each of these two subgroups is present in all epithelial cells. The ectocervical epithelium contains type II keratins 1, 4, 5 and 6, and type I keratins 13, 14, 15, and 19 with some variable expression of keratins 2, 8, 10, 11, 16, and 17 [79]. There is evidence to suggest that changes take place in the keratin polypeptide distribution from normal cervical epithelium to CIN and cervical cancer [80]. This requires the use of several monoclonal antibodies to detect the differences. Earlier studies, which relied upon a few monoclonal antibodies such as CAM 5.2, may have limited ability to detect changes in the various heterogeneous groups. Keratins may serve as a surrogate endpoint biomarker if the specific phenotype related to CIN and progression to invasive disease could be delineated.

Involucrin is a cytoplasmic protein synthesized in stratified squamous epithelia. It is the major precursor to the crosslinked envelope formed immediately beneath the cellular plasma membrane of maturing squamous epithelial cells. Involucrin is absent from the basal and suprabasal cell layers and only detected at the outer portion of the epithelium where it becomes crosslinked. Therefore, involucrin could serve as a specific marker of normal squamous differentiation and maturation. Involucrin expression is altered in preneoplastic and neoplastic conditions of numerous squamous epithelia, including the cervix. With respect to cervix, involucrin can be considered a highly specific marker of squamous cell differentiation in both normal and patho-

logical epithelium [81,82]. The orderly staining pattern restricted to the outer layers of normal squamous cervical epithelium is generally lost in preinvasive and invasive cervical lesions. However, there is a vast spectrum of staining patterns from normal epithelium to malignant lesions. Recent studies suggest that involucrin appears unable to reliably differentiate between benign and neoplastic conditions [83,84]. Inconsistency among available data could be improved if the technique for interpreting staining patterns could be refined to a more objectively consistent process, such as computerized interpretation/imaging. Involucrin is a potential surrogate endpoint biomarker since it can be done on biopsy specimens, but the technical issues need to be addressed and the relationship to neoplastic disease needs to be clarified.

PATHOLOGICAL EFFECT SURROGATE ENDPOINT BIOMARKERS

We define a pathologic effect surrogate endpoint biomarker as one in which a defined lesion such as CIN or low-grade/high-grade SIL reverses after a defined treatment period. This pathologic endpoint permits the use of a "gold standard" proof of surrogate biomarker efficacy and makes CIN an attractive human model to identify surrogate endpoint biomarkers in cancer chemoprevention [85].

Squamous Cell Carcinoma as a Continuum of CIN

Squamous cell carcinoma usually arises from the squamous-columnar junction of the cervix, and is preceded by, and thought to result from, the progression of cervical dysplasia and CIS [86,87]. Further support for a continuum of disease is provided by the observation that cervical dysplasia is most often diagnosed among women in their 20s, CIS in their 30s, and invasive cancer after the age of 40 [88].

Recent information challenges the assertion that invasive carcinoma of the cervix is part of a continuum from CIN. The alternative view is that CIN I and CIN II–III are distinct disease processes with CIN I being the consequence of a self-limited, sexually transmitted viral infection and CIN II–III being a cervical cancer precursor lesion [89]. Several observations support this as-

sertion. First, the anatomic distribution of CIN I is different (peripheral cervical lesions) from CIN II–III (central cervical lesions) [90]. Second, the mean age of women with CIN I and CIN II–III are comparable in incident case analysis [8] as distinguished from prevalent case analyses [91]. Thus, the concept that younger women with CIN I progress to higher grades of CIN may be erroneous. Third, 61% of women with CIN II–III never had a smear showing CIN I [92]. Fourth, 50–90% of CIN lesions most likely to regress are caused by HPV types other than "high-risk", whereas lesions most likely to progress to invasive carcinoma are caused by high-risk HPV types [12]. Finally, in vitro infection of human keratinocytes with HPV 16 produces a stratified epithelium that resembles CIN II–III; infection with HPV 6 results in a stratification resembling CIN I [93].

A substantial proportion of CIN spontaneously regresses [94]; CIN I regress more frequently than CIN II or III. The relative frequency of spontaneous regression varies widely from 11% to 44% [95–97] among published reports of this phenomenon. Biopsies of the suspected small lesions have either cured the dysplasia or have stimulated an inflammatory response about the biopsy that destroys the residual CIN [98]. Spontaneous regression of CIN III is rare [98].

Use of CIN as the Pathological Effect Surrogate Endpoint Biomarker

Using CIN or SIL as an endpoint for studies is difficult because of high spontaneous regression rates. However, the clinical significance of treating a disease that is likely to regress spontaneously is in doubt. Therefore, some have limited their chemopreventive studies to CIN II and III or high-grade SIL. Historically, the follow-up of pregnant women with CIN III involves an initial assessment with colposcopy and biopsy and cytological follow-up. Treatment is delayed until after delivery as long as the colposcopy is adequate and the lesion and cytology do not change during pregnancy. Therefore, chemoprevention studies using CIN III are possible and have been done without risk to women as long as all subjects receive close observation and follow-up. This reduces the problem with spontaneous regression and reduces the number of women required. The compromise is that CIN III may represent a lesion that is too late for chemopreven-

tive intervention. In addition, far more women suffer from the diagnosis of CIN I or CIN II, so the impact in the U.S. will be less.

SUMMARY

The list of potential surrogate endpoint biomarkers for cervical cancer can be overwhelming. In this review, we have highlighted available data for more commonly noted candidates. We believe that our model of evaluating potential chemopreventive agents based on biochemical effect, biological effect and pathological effect allows for a focused, systematic investigation. Clearly, the most critical issue is selecting valid surrogate endpoint biomarkers. Currently, we are focusing our efforts on retinoid-related chemopreventive agents for CIN, so we have chosen plasma levels and tissue levels of retinoids as a biochemical effect. The RAR and RXR were chosen for both biochemical and biological effects. The other biologic effect surrogate endpoints are E6 and E7 expression of HPV , along with PCNA and Ki-67. Finally, we include the pathological effect of regression, progression or no change of the histological diagnosis.

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