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Cervical Cancer Chemoprevention, Vaccines, and Surrogate Endpoint Biomarkers

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At the Second International Conference on Cervical Cancer, held April 11–14, 2002, experts in cervical cancer prevention, detection, and treatment reviewed the need for more research in chemoprevention, including prophylactic and therapeutic vaccines, immunomodulators, peptides, and surrogate endpoint biomarkers. Investigators and clinicians noted the need for more rigorous Phase I randomized clinical trials, more attention to the risk factors that can affect study results in this patient population, and validation of optical technologies that will provide valuable quantitative information in real time regarding disease regression and progression. They discussed the role of the human papillomavirus (HPV) in cervical cancer development and the importance of developing strategies to suppress HPV persistence and progression. Results in Phase I randomized clinical trials have been disappointing because few have demonstrated statistically significant regression attributable to the agent tested. Researchers recommended using a transgenic mouse model to test and validate new compounds, initiating vaccine and immunomodulator trials, and developing immunologic surrogate endpoint biomarkers. *Cancer* **2003;98(9 Suppl):2044–51.** © *2003 American Cancer Society.*

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Cervical intraepithelial neoplasia (CIN), also known as cervical squamous intraepithelial lesions (SILs), provides an excellent model for various types of research, including chemoprevention trials. The natural history of cervical lesions has been well defined,[1] and the cervix is easily accessible, which makes histologic and pathologic studies more convenient than in other tissues. The progression of cervical lesions takes place over months to years. The Papanicolaou (Pap) smear is a well-known screening test for cervical cancer, and it can provide a cytologic model of disease progression. Cervical histopathology is one of the best validated models of CIN or SIL progression to cervical cancer. Colposcopy, which permits viewing the cervix through a mounted magnifying lens (called a colposcope) and using acetic acid as a contrast agent, provides a visual model of carcinogenic progression (Figs. 1 and 2).

Chemoprevention Agents

Chemoprevention is defined as using micronutrients or pharmaceuticals to prevent or delay the development of cancer. Interest in micronutrients arose from the many epidemiological studies demonstrating that nutrient deficiencies existed in CIN cases but not in controls. Although many micronutrients have been tested (includ-

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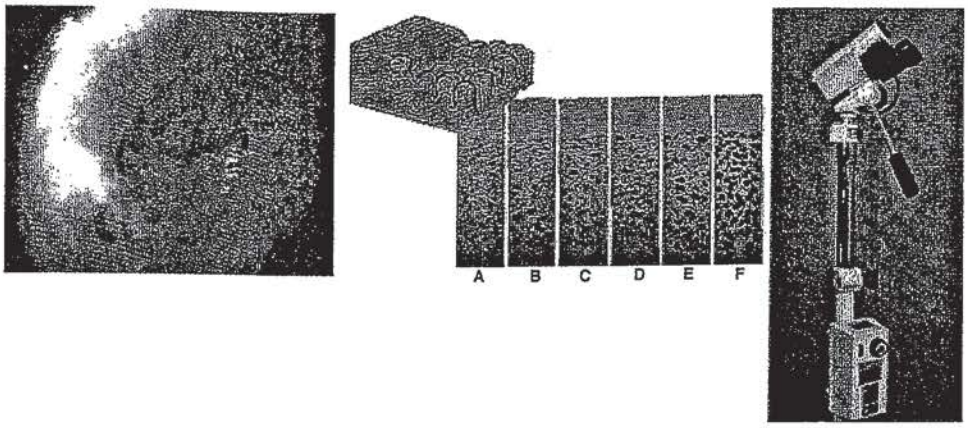

FIGURE 1. Colposcopic evaluation of the cervix may include (left) visual inspection through the colposcope or (center) cytologic evaluation allowing classification into one of six categories. The colposcope itself (right) includes a magnifying lens and light.

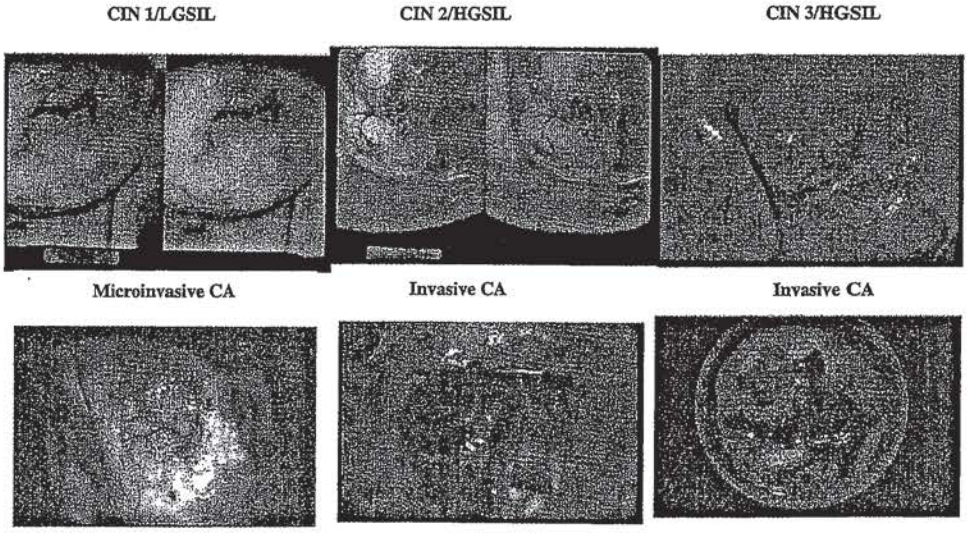

FIGURE 2. Colposcopic view of the cervix, demonstrating progression from cervical intraepithelial neoplasia 1/low-grade squamous intraepithelial lesions (CIN 1/LGSIL) through CIN 2 and CIN 3/high-grade squamous intraepithelial lesions (HGSIL) to invasive cervical cancer (CA).

ing folate, β-carotene, and vitamin C), none has produced a statistically significant regression of lesions in the treated group.[2] Several of these studies have been hampered by their design in that many of the micronutrients were not subjected to Phase I trial design controls meant to determine an effective dose or duration of use; therefore, the dose used in the Phase II study may not have been appropriate.[3,4]

Several pharmaceutical agents have appeared promising (Tables 1, 2).[5–27] Many of these pharmaceuticals have been tested in cell lines and animal models and have effectively suppressed the growth of cancerous or precancerous cells. In addition, because the carcinogenic role of the human papillomavirus (HPV) in cervical cancer has been established both in the

TABLE 1
Studies of Chemoprevention Agents

Chemopreventive agent	Past studies	Ongoing studies
Retinoids	Retinyl acetate gel All-*trans*-retinoic acid 4-HPR	All-*trans*-retinoic acid
Micronutrients	β-carotene Folate Vitamin C	
Polyamine synthesis inhibitors	DFMO	DFMO
Adduct reducers	Indole-3-carbinol	Indole-3-carbinol

4-HPR: N-(4-hydroxyphenyl)retinamide; DFMO: α-difluoromethylornithine.

TABLE 2
Cervical Cancer Chemoprevention Trials by Agent

Chemopreventive and study	Study design	No. of evaluable patients	Disease	Dose and duration of treatment	Results[a]		
					Pilot/Phase I	Phase II/III	
						CR	CR + PR
Retinoids							
Retinyl acetate gel (topical) Rommey et al.[5]	Phase I–II	50	CIN 1–2	Placebo (3 patients), 3 mg (14 patients), 6 mg (14 patients), 9 mg (12 patients), 18 mg (7 patients) 7-day treatment for 3 consecutive treatment cycles	Toxicity: 50% at 3 mg, 21% at 6 mg: 75% at 9 mg, 100% at 18 mg. Response: None reported. Results: Selected 9-mg dose		
All-TRA topical) Surwit et al.[6]	Phase I	18	CIN 2–3	Liquid: 0.05% (8 patients). 0.10% (4 patients). 0.20% (1 patient) Cream: 0.1% (5 patients) 4 consecutive 24-hour applications given once	Toxicity: 55% (10/18) overall. Response: 11% Results: Designed next Phase I study		
All-TRA (topical) Meyskens et al.[7]	Phase I	35	CIN 1–2	Cream: 0.05%, 0.0667%, 0.0833%, 0.1167%, 0.1583%, 0.21%, 0.28%, 0.372%, and 0.484%; 4 patients treated at each dose level for 4 consecutive 24-hour applications	Toxicity: Moderate—24% (5/21) at 0.21%–0.372%; 100% (3/3) at 0.484%. Response: 33% (7/21) CR + PR at 6 month. Results: Selected 0.372% dose as least toxic and probably most active		
All-TRA (topical) Weiner et al.[8]	Phase I	36	CIN 1–3	0.05–0.12% dose: 4 consecutive 24-hour applications; 0.15%–0.48% dose: 4 consecutive 24-hour application	Response: 14% (2/14) at 0.05%–0.12%; 45% (10/22) at 0.15–0.48%		
All-TRA (topical) Weiner et al.[8]	Phase I	36	CIN 1–3	0.05–0.12% dose: 4 consecutive 24-hour applications; 0.15–0.48% dose: 4 consecutive 24-hour applications	Response: 14% (2/14) at 0.05–0.12%; 45% (10/22) at 0.15–0.48%		
All-TRA (topical) Graham et al.[9]	Phase II Single arm	20	CIN 1–3	0.372% dose used daily for 2 days at baseline, 3 mo., 6 mo., and 9 mo.		50% (10/20)	
All-TRA (topical) Meyskens et al.[10]	Phase IIb	141	CIN 2	0.372% dose used daily for 4 days at baseline and for 2 days at 3 mo. and 2 days at 6 mo. versus placebo		TRA: 43% (32/75) Placebo: 27% (18/66)	
All-TRA (topical) Meyskens et al.[10]	Phase IIb	160	CIN 3	0.372% dose used daily for 4 days at baseline and for 2 days at 3 and 2 days at 6 mo. vs. placebo		TRA: 25% (10/40). Placebo: 31% (16/51)	
All-TRA (topical) Ruffin et al.[11]	Phase IIb	180 (proposed)	CIN 2–3			NA	NA

(continued)

TABLE 2
(continued)

Chemopreventive and study	Study design	No. of evaluable patients	Disease	Dose and duration of treatment	Results[a]		
					Pilot/Phase I	Phase II/III	
						CR	CR + PR
4-HPR (oral) Follen et al.[12]	Phase IIb	36	CIN 2–3	200 mg/day with 3-day drug holiday monthly for 6 mo vs. placebo			4-HPR: 25% (5/20). Placebo: 44% (7/16)
9-*cis* retinoic acid Alvarez et al.[15]	Phase II	114	CIN 2–3	50 mg (high-dose group) or 25 mg (low-dose group) daily for 12 weeks vs. placebo		Low-dose 9-CRA : 32%. High-dose 9-CRA : 32%. Placebo: 32%	
Micronutrients							
Vitamin C Romney et al.[14]	Pilot	28	CIN 1–2	1 g/day for 6 mo. vs. placebo	Toxicity: None. Response: Vitamin C slightly favored over placebo (not quantified). Results: Recommendation to proceed to Phase I study		
β-carotene Romney et al.[15,16]	Phase II	74	CIN 1–3	30 mg vs. placebo for 9 mo			β-carotene: 46% (18/39). Placebo: 50% (15/30)
β-carotene Manetta et al.[17]	Phase I-II Single arm	30	CIN 1–2	30 mg per day for 6 mo	β-carotene: 70% (21/30)		
β-carotene Berman[18] and Keefe et al.[19]	Phase III	103	CIN 2–3	30 mg vs. placebo for 6 mo		β-carotene: 32% Placebo: 32%	
β-carotene De Vet et al.[20]	Phase II	137	CIN 1–3	10 mg vs. placebo for 3 mo		β-carotene: 16% (22/137). Placebo: 11% (15/141)	β-carotene: 32% (44/137). Placebo: 32% (45/141)
β-carotene Fairley et al.[21]	Phase II	117	Atypia to CIN 2	30 mg vs. placebo for 12 mo			β-carotene: 63% (37/59). Placebo: 60% (31/52)
β-carotene, vitamin C Mackerras et al.[22]	Phase II	141	Atypia to CIN 1	30 mg β-carotene, 500 mg vitamin C, or both vs. placebo for 6 mo		β-carotene: 44% (16/36). Vitamin C: 26% (9/35). Both: 23% (8/35). Placebo: 29% (10/35)	
Folate, vitamin C Butterworth et al.[23]	Phase II	47	CIN 1–2	10 mg folate vs. placebo for 3 mo		Folate: 14% (3/22). Placebo (vitamin C): 4% (1/25)	Folate: 36% (8/22). Placebo (vitamin C): 16% (4/25)
Folate, vitamin C Butterworth et al.[24]	Phase II	177	CIN 1–2	10 mg folate vs. placebo for 6 mo		Folate: 64% (58/91). Placebo (vitamin C): 52% (45/86)	
Folate, Childers et al.[25]	Phase III	331	HPV CIN 1–2	5 mg folate vs. placebo for 6 mo		Folate: 7% (9/129). Placebo: 6% (7/117)	
Polyamine synthesis inhibitors							
DFMO (oral) Mitchell et al.[26]	Phase I	30	CIN 3, CIS	0.06, 0.125, 0.250, 0.50 and 1.0 mg/m^2, 6 patients at each dose level for 30 days	Response: 50% (15/30) CR + PR. Result: Selected doses of 0.125 and 0.5 g/m^2/day		

(continued)

TABLE 2
(*continued*)

Chemopreventive and study	Study design	No. of evaluable patients	Disease	Dose and duration of treatment	Results[a]		
					Pilot/Phase I	Phase II/III	
						CR	CR + PR
DFMO (oral) Follen et al. [unreported]	Phase II	180 (proposed)	CIN 2–3	0.125 and 0.50 mg/m^2 vs. placebo, 60 patients at each dose level for 30 days			NA
Adduct reducers							
Indole-3-carbinol (oral) Bell et al.[27]	Phase II	27	CIN 2–3	200 mg or 400 mg per day vs. placebo for 3 mo.		200 mg: 50% (4/8) CR.400 mg: 44% (4/9) CR. Placebo: 0% (0/10)	

CR: complete response; CR + PR: complete response + partial response; CIN: cervical intraepithelial neoplasia; all-TRA: all-*trans*-retinoic acid; 4-HPR: N-(4-hydroxyphenyl)retinamide; HPV: human papillomavirus; DFMO: α-difluoromethylornithine; CIS: carcinoma in situ; NA: not applicable.

[a] Published reports do not consistently include toxicity results; response including complete response and partial response data), and decision regarding next phase.

Reprinted with kind permission of Kluwer Academic Publishers from Follen M, Vlastos A-T, Meyskens FL, Atkinson EN, Schottenfeld D. Why phase II trials in cervical chemoprevention are negative: what have we learned? *Cancer Causes Control.* 2002;13:855–873.[4]

field of molecular biology and epidemiology, many of these pharmaceuticals have been tested for their ability to suppress the production of viral oncoproteins.[28] A few of these agents have been subjected to rigorous Phase I study design.[3,4] The only agent that has been demonstrated to cause regression of CIN/SILs in a randomized controlled trial in a statistically significant manner in a trial of sufficient sample size is topical all-*trans*-retinoic acid.[15]

Biomarkers, Vaccines, and Peptides

Although the field of cervical chemoprevention has yielded few successes, much has been learned regarding the carcinogenic process. Surrogate endpoint biomarkers serve as alternative endpoints for cancer incidence and are very helpful in determining the efficacy of chemopreventive agents.[2] The development and validation of these surrogate endpoint biomarkers is critically important to chemoprevention in other organ sites and, more important, in the development of new treatment strategies. Because HPV is a major etiologic agent, the measurement of HPV persistence and viral load should be considered as important as identifying biomarkers. Classes of surrogate endpoint biomarkers are listed in Table 3.[28]

Both vaccines and pharmaceuticals that suppress HPV are of interest. HPV vaccines are being developed following two strategies: preventive and therapeutic.[29,30] Clinical trials of preventive vaccines aimed at creating antibody recognition of HPV capsid proteins are reported to be under way.[31] Similarly, clinical trials of therapeutic vaccines aimed at inducing cytotoxic T-cell recognition of HPV oncoproteins also are in progress. Both the prophylactic and therapeutic vaccines employ a number of strategies including virus-like particles, DNA vaccines, peptide vaccines, heat-sensitive protein fusion vaccines, and chimeric viral-like particle vaccines.

In addition to vaccines, there are other approaches to suppressing HPV, including immunomodulation and peptide drugs. There has been some success in the trial of prophylactic vaccines of virus-like particles.[32] The viral-like particle approach to prophylactic vaccines appears quite promising. Similarly, some success has been reported using therapeutic peptide vaccines.

Imiquimod, a topical agent, is an immune response modifier that is believed to induce local cytokines (including interferon-α) to cause wart regression and currently is an accepted treatment for vulvar and vaginal warts.[33,34] To our knowledge, no reports of randomized clinical trials of its use in the cervix have been published to date. Another compound, cidofovir, which is injected, is a peptide that suppresses viral expression and has been approved by the U.S. Food and Drug Administration as a treatment for laryngeal papillomatosis.[35–38]

Much of the validation of the surrogate endpoint biomarkers that has taken place in the field of chemoprevention can now be used to determine the success of vaccines, immunomodulators, and other antiviral

TABLE 3
Classes of Biomarkers in the Cervical Epithelium

Quantitative histopathologic and cytologic markers
Nuclei (abnormal size, shape, texture, pleomorphism)
Nucleoli (abnormal number, size, shape, position, pleomorphism)
Nuclear matrix (tissue architecture)
Proliferation markers
Proliferating cell nuclear antigen
Ki-67, MIB-1
Labeling indices (thymide, BrdU)
Mitotic frequency (MPM-2)
Regulation markers
Tumor suppressors (p53, Rb)
HPV viral load and oncoprotein expression
Oncogenes (*ras, myc, c-erb,* B2)
Altered growth factors and receptors (epidermal growth factor receptor, transforming growth factor-α, cyclin-dependent kinases, retinoic acid receptors)
Polyamines (ornithine decarboxylase, arginine, ornithine, putrescine, spermine, spermidine)
Arachidonic acid
Differentiation markers
Fibrillar proteins (cytokeratins, involucrin, cornifin, filaggrin, actin microfilaments, microtubules)
Adhesion molecules (cell-cell: gap junctions, desmosomes) (cell-substrate: integrins, cadherins, laminins, fibronectin, proteoglycans, collagen)
Glycoconjugates (lectins, lactoferrin, mucins, blood group substances, glycolipids, CD44)
General genomic instability markers
Chromosome aberrations (AgNORs, micronuclei, three-group metaphases, double minutes, deletions, insertions, translocations, inversions, isochromosomes, FHIT)
DNA abnormalities (DNA hypomethylation, LOH, point mutations, gene amplification)
Aneuploidy (measured by flow cytometry)
Tissue maintenance markers
Metalloproteinases
Telomerases
Apoptosis and antiapoptotic markers

BrdU: bromodeoxyuridine; MPM-2: mitotic protein monoclonal 2; Rb, retinoblastoma; HPV: human papillomavirus; AgNORs: silver-staining nucleolar organizer region protein; FHIT: fragile histidine triad; LOH: loss of heterozygosity. Reprinted with permission from Follen M, Schottenfeld D. Surrogate endpoint biomarkers and their modulation in cervical chemoprevention trials. *Cancer.* 2001;91:1758–1776.[28]

agents. Similarly, many of the lessons learned from the study design of cervical chemoprevention trials can be applied so that the clinical trials of these agents can proceed more quickly. Rigorous attention must be paid to duration of use, dosage, and method of follow-up. Investigators need to be cognizant of risk factors that may modify a patient's response to treatment. Although the best strategy is to stratify patients in the trial by these risk factors at the time of study entry, researchers should at least take these risk factors into account when analyzing response. These include the nutritional status of the patient, smoking status, recurrent as opposed to incident disease, use of hormonal contraception, immunocompetence (human immunodeficiency virus, organ transplantation, connective tissue disorders, or other autoimmune disorders), age, and menopausal status.

Future Directions

Optical technologies may provide a novel biomarker of disease progression and regression. These technologies include such strategies as fluorescence and reflectance spectroscopy, optical coherence tomography, and confocal imaging, which provide real-time information regarding the redox ratio, chromatin distribution, and the nuclear-to-cytoplasmic ratio. An illustration of redox potentials in cervical tissue is shown in Figure 3. Once validated, optical biomarkers could help monitor disease regression, persistence, or progression in patients in real time without biopsy. Although there is much to be done in the development of these optical technologies to validate their use, they provide an exciting opportunity to obtain quantitative information in real time at each visit. Because biopsy itself induces regression, the use of these optical technologies would allow investigators to monitor patients safely throughout clinical trials of these new agents. Optical contrast agents, which target biomarkers, also will provide a novel method of gathering molecular biologic data quantitatively and reproducibly throughout a trial. Optical contrast agents could be designed specifically for HPV or other immunologic or molecular biologic targets that are associated with increased progression of disease.

Some of the new research directions in chemoprevention and vaccine development that were mentioned in discussion included using a transgenic mouse model to test and validate new compounds and conducting Phase I clinical trials of nonsteroidal antiinflammatory drugs. The need for a clinical trial of indole-3-carbinol (with background studies of the role of estrogen in HPV integration, persistence, and expression) also was discussed, as was the need for well-designed vaccine trials in general. The development of immunologic surrogate endpoint biomarkers was another research area mentioned that needs exploring, as do well-designed trials of immunomodulators such as imiquimod and peptide drugs such as cidofovir. Finally, using optical technologies as new biomarkers in randomized clinical trials was discussed as a tool for monitoring chemoprevention and vaccine studies.

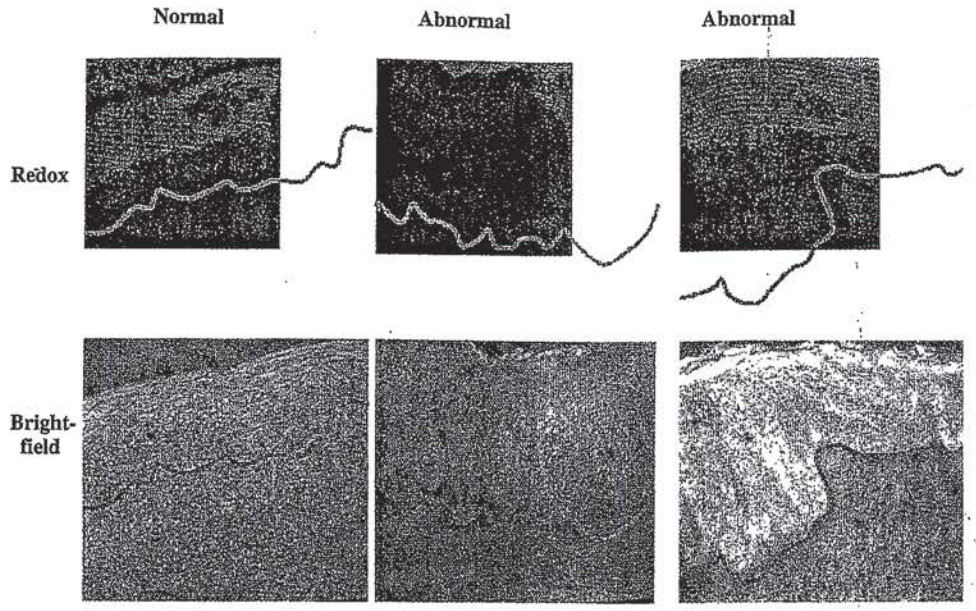

FIGURE 3. Illustration of redox potentials in cervical tissue (with regard to redox values, orange indicates approximately 0.4 and black indicates approximately 0.1).

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