Cancer Causes and Control **13**: 855–873, 2002.

Why phase II trials in cervical chemoprevention are negative: what have we learned?

Michele Follen[1,*,†], Anne-Thérèse Vlastos[1], Frank L. Meyskens Jr.[2], E. Neely Atkinson[1] & David Schottenfeld[3]
[1]*Departments of Gynecologic Oncology and Biomathematics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, USA;* [2]*Chao Family Comprehensive Cancer Center, University of California–Irvine, Orange, California 92868, USA;* [3]*Department of Epidemiology, University of Michigan, School of Public Health, Ann Arbor, Michigan 48104, USA*

Received 21 December 2001; accepted in revised form 1 July 2002

Key words: cervical cancer, cervix, chemoprevention, randomized clinical trials, surrogate endpoint biomarkers.

Abstract

Cervical cancer is an important cause of mortality in women worldwide, and the cervix is a well-established clinical, cytologic, and histopathologic model of carcinogenesis. The cervix is easily accessible for examination and biopsy, and colposcopy improves visualization.

Identifying chemopreventives in cervical cancer requires rigorous study design: dose de-escalating phase I, IIa trials; placebo-controlled phase IIb trials; and multicenter phase III trials. Reduction in disease incidence and surrogate endpoint biomarkers (SEB) may be trial endpoints. The goal of chemoprevention studies is to prevent or delay the development of cancer. Each agent requires a phase I or IIa trial for each organ site.

Phase I, IIa studies of micronutrients, retinoids, α-difluoromethylornithine, and indole-3-carbinol have demonstrated response rates of up to 70%, but results of placebo-controlled phase IIb studies have been disappointing and their findings confounded by the high regression rates in placebo-treated patients.

Enhancement of research methods, including sufficient enrollment guided by power calculations, uniform biopsy at study entry and exit, and strict progression through trial design phases would ensure valid and reliable results. Because human papillomavirus (HPV) is the major etiologic agent, pretrial laboratory and animal studies should have demonstrated the efficacy of the chemopreventive agent to decrease HPV viral protein expression or HPV tumor induction. SEB modulation must be characterized in any trial's earliest phases before use in phases IIb and III. Lessons learned in chemoprevention will serve as a basis for immunoprevention and vaccine trials.

Introduction

Cervical lesions have long been thought by pathologists to be an instructive paradigm of progression from mildly dysplastic lesions to severely dysplastic lesions and potentially to invasive cancer. The accessibility of the cervix allows clinicians to observe cervical lesions over time using the magnifying lens of a colposcope; these lesions show progressive vascular atypia as they advance to neoplasia. This accessibility also allows the cervix to be easily sampled for cells using the Papanicolaou (Pap) smear and for tissue using colposcopically directed biopsy. Just as the biopsy shows predictable changes as lesions progress toward invasion, the Pap smear affords a cytologic model of progression. These factors make the cervix well suited for use in developing screening, diagnostic, and preventive interventions. This review will focus on the incidence and natural history of cervical lesions, discuss study design issues related to chemoprevention trials and phase II randomized clinical trials, review phase II cervical chemoprevention trials, and analyze why many of the trials' findings may be negative. The goal of chemoprevention is to prevent or delay the development of cancer.

* Address correspondence to: Michele Follen, MD, PhD, Department of Gynecologic Oncology and Biomedical Engineering Center, Box 193, The University of Texas M. D. Anderson Cancer Center, 1515 M. D. Anderson Boulevard, Houston, TX 77030, USA. Ph.: (713) 745-2564; Fax: (713) 792-4856; Email: mfollen@mdanderson.org
† Michele Follen published formerly under the name Michele Follen Mitchell.

Incidence of cervical lesions

According to the worldwide cancer incidence database maintained by the World Health Organization, cervical cancer is the third most common malignancy in women worldwide, exceeded only by breast cancer and colorectal cancer. Parkin *et al.* [1] estimated that approximately 371,200 cases of cervical cancer were diagnosed worldwide in 1990, accounting for 10% of all cancers diagnosed in women. When compared with the industrialized countries of North America and western Europe, many developing countries, where approximately 80% of all cases occur, are found to have higher invasive cervical cancer incidence [1, 2]. The global incidence and mortality rates also show wide geographic variation, with a 21-fold difference between the highest and lowest age-standardized rates worldwide. The highest incidences are reported in south-central Asia, southeastern Asia, South America, eastern Europe, and eastern Africa.

In the United States, the Surveillance, Epidemiology, and End Results (SEER) database tracks cervical cancer and carcinoma *in situ* (CIS) incidence based on biopsy results in a 10% population-based sample that is representative of the country's many ethnic groups. According to this database, cervical cancer is the third most common neoplasia of the female genital tract in the United States, following cancers of the endometrium and ovary. It was projected that, in 2002, 13,000 cases of invasive cervical cancer and 65,000 cases of CIS would be diagnosed in women in the United States and approximately 4100 deaths would be attributed to invasive cervical cancer [3, 4].

The precursors to invasive cervical cancer are called squamous intraepithelial lesions (SIL) or cervical intraepithelial neoplasia (CIN). SIL are further classified as high grade (HGSIL), which includes former CIN 2, 3, and CIS, or low grade (LGSIL), which includes HPV-infected tissue and CIN 1. Because SIL/CIN does not have to be reported in the SEER database, its exact incidence is unknown [5]. In the United States, the prevalence of SIL can be estimated by comparing prevalence in different clinical settings. Estimated SIL prevalence ranges from 1.1% in women attending family planning clinics to 13.7% in women attending sexually transmitted disease clinics [5]. Additionally, the National Breast and Cervical Cancer Early Detection Program reported that more than 26,000 cases of SIL were detected in 851,818 Pap smears from medically underserved women, or a prevalence of 3.1% [6]. Kurman *et al.* [7] estimated that 50 million Pap smears are performed annually in the United States. Based on the estimated range of 1.1–13.7% and the projection of 50 million annual Pap smears, the number of SIL cases in the United States may vary from 550,000 to 6,850,000 cases per year.

Kurman *et al.* [7] estimated that, of the 50 million Pap smears performed in the United States annually, 2.5 million showed LGSIL and cervical atypia. (Atypias are not considered CIN or SIL.) However, the prevalence of HGSIL in the United States can be estimated using two large cohorts. In one cohort, which contained 8026 patients in a military setting who underwent screening as part of their annual physical exam, 0.3% of the Pap smears showed HGSIL [8]. In the other cohort, the National Breast and Cervical Cancer Detection Program, in which women are screened annually at health department sites, HGSIL were evident in 1.1% of 100,500 Pap smears [9]. Again, using the percentages of 0.3% and 1.1% and an estimated 50 million annual Pap smears, HGSIL may be incident in 150,000 to 550,000 American women per year.

Thus cervical cancer and its precursors are an important health problem and deserve innovative therapies that are evaluated in rigorously designed clinical trials. Chemoprevention is defined as the use of medications or micronutrients to prevent or delay the onset of cancer. There have been many chemoprevention trials in cervical cancer precursors; they are listed in Table 1 [10–31]. A summary of the problems encountered when analyzing these studies is presented in Table 2.

Choosing an agent with preclinical activity

The epidemiologic evidence linking HPV measured by polymerase chain reaction (PCR) to cervical cancer is consistent across case series, case–control studies, and cohort studies [32–36]. The International Agency for Research on Cancer (IARC) coordinated an international prevalence study of 1000 frozen biopsy specimens from 22 countries and used assays based on PCR to detect 25 HPV types. HPV was eventually detected in 99% of tumors. The most common HPV types were HPV 16 in 49% of tumors, HPV 18 in 12%, HPV 45 in 8%, and HPV 31 in 5%. HPV 16 was the predominant type in all countries except Indonesia. HPV 16 dominated in squamous cell carcinomas and HPV 18 in adenocarcinomas [36]. Muñoz and Bosch [34] summarized the case–control and cohort studies using PCR for detecting the presence and type of HPV. Five PCR-based case–control studies reviewed by Muñoz, Bosch, and colleagues demonstrated remarkably high adjusted odds ratios for HGSIL, ranging from 15.5 to 122.3 for the presence of any HPV type (15.5 [8.2–29.4], 42.0 [15.3–124.3], 20.8 [10.8–40.2], 72.8 [27.6–191.9], 56.9

Table 1. Cervical cancer chemoprevention trials by type of medication

Chemopreventive and study	Study design	Number 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); Romney *et al.* [10]	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.* [11]	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% (2/18) CR. Results: designed next phase I study		
All-TRA (topical); Meyskens *et al.* [12]	Phase I	35	CIN 1–2	Cream: 0.05%, 0.0667%, 0.0833%, 0.1167%, 0.1583%, 0.21%, 0.28%, 0.372%, 0.484%. Four patients treated at each dose level for 4 consecutive 24-h 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 months. Results: selected 0.372% dose as least toxic and probably most active		
All-TRA (topical); Weiner *et al.* [13]	Phase I	36	CIN 1–3	0.05–0.12% dose: 4 consecutive 24-h applications; 0.15–0.48% dose: 4 consecutive 24-h applications	Response: 14% (2/14) at 0.05–0.12%; 45% (10/22) at 0.15–0.48%		
All-TRA (topical); Graham *et al.* [14]	Phase II, single arm	20	CIN 1–3	0.372% dose used daily for 2 days at baseline, 3, 6, and 9 months		50% (10/20)	
All-TRA (topical); Meyskens *et al.* [15]	Phase IIb	141	CIN 2	0.372% dose used daily for 4 days at baseline and for 2 days at 3 months and 2 days at 6 months *versus* placebo		TRA: 43% (32/75), placebo: 27% (18/66)	
All-TRA (topical); Meyskens *et al.* [15]	Phase IIb	160	CIN 3	0.372% dose used daily for 4 days at baseline and for 2 days at 3 months and 2 days at 6 months *versus* placebo		TRA: 25% (10/40), placebo: 31% (16/51)	
All-TRA (topical); Ruffin *et al.* [16]	Phase IIb	180 (proposed)	CIN 2–3			NA	NA
4-HPR (oral); Follen *et al.* [17]	Phase IIb	36	CIN 2–3	200 mg/day with 3-day drug holiday monthly for 6 months *versus* placebo			4-HPR: 25% (5/20), placebo: 44% (7/16)
Micronutrients							
Vitamin C; Romney *et al.* [18]	Pilot	28	CIN 1–2	1 g/day for 6 months *versus* placebo	Toxicity: none. Response: vitamin C slightly favored over placebo (not quantified). Results: recommendation to proceed to phase I study		

Table 1. (Continued)

Chemopreventive and study	Study design	Number of evaluable patients	Disease	Dose and duration of treatment	Results[a]		
					Pilot/phase I	Phase II/III	
						CR	CR + PR
β-Carotene; Romney *et al.* [19, 20]	Phase II	74	CIN 1–3	30 mg *versus* placebo for 9 months			β-Carotene: 46% (18/39); placebo: 50% (15/30)
β-Carotene; Manetta *et al.* [21]	Phase I–II, single arm	30	CIN 1–2	30 mg per day for 6 months	β-Carotene: 70% (21/30)		
β-Carotene; Berman [22] and Keefe *et al.* [23]	Phase III	103	CIN 2–3	30 mg *versus* placebo for 6 months		β-Carotene: 32%, placebo: 32%	
β-Carotene; De Vet *et al.* [24]	Phase II	137	CIN 1–3	10 mg *versus* placebo for 3 months		β-Carotene: 16% (22/137), placebo: 11% (15/141)	β-Carotene: 32% (44/137), placebo: 32% (45/141)
β-Carotene; Fairley *et al.* [25]	Phase II	117	Atypia to CIN 2	30 mg *versus* placebo for 12 months			β-Carotene: 63% (37/59), placebo: 60% (31/52)
β-Carotene, vitamin C; Mackerras *et al.* [26]	Phase II	141	Atypia to CIN 1	30 mg β-carotene, 500 mg vitamin C, or both *versus* placebo for 2 years		β-Carotene: 44% (16/36), vitamin C: 26% (9/35), both: 23% (8/35), placebo: 29% (10/35)	
Folate, vitamin C; Butterworth *et al.* [27]	Phase II	47	CIN 1–2	10 mg folate *versus* placebo for 3 months		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.* [28]	Phase II	177	CIN 1–2	10 mg folate *versus* placebo for 6 months		Folate: 64% (58/91), placebo (vitamin C): 52% (45/86)	
Folate; Childers *et al.* [29]	Phase III	331	HPV CIN 1–2	5 mg folate *versus* placebo for 6 months		Folate: 7% (9/129), placebo: 6% (7/117)	
Polyamine synthesis inhibitors							
DFMO (oral); Mitchell *et al.* [30]	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 per day		
DFMO (oral); Follen *et al.* (unreported)	Phase II	180 (proposed)	CIN 2–3	0.125 and 0.50 mg/m^2 *versus* placebo, 60 patients at each dose level for 30 days			NA
Adduct reducers							
Indole-3-carbinol (oral); Bell *et al.* [31]	Phase II	27	CIN 2–3	200 mg or 400 mg per day *versus* placebo for 3 months		200 mg: 50% (4/8) CR, 400 mg: 44% (4/9) CR, placebo: 0% (0/10)	

Note: CR, complete response; CR + PR, complete response + partial response; CIS, carcinoma *in situ*; all-TRA, all-*trans*-retinoic acid; 4-HPR, N-(4-hydroxyphenyl)retinamide; DFMO, α-difluoromethylornithine.

[a] Published reports do not consistently include toxicity results; response, including CR + PR data; and decision regarding next phase.

Table 2. Problems with current cervical chemoprevention trials

Failure to choose an agent that decreases human papillomavirus (HPV) expression or decreases growth in HPV-positive cell lines or tissue cultures
Failure to choose an agent that in preclinical work has shown promise in producing regression in precancerous lesions
Failure to choose a high-grade cervical intraepithelial neoplasia that is unlikely to regress without therapy (grade 2–3 or carcinoma *in situ*)
Failure to take natural history of the disease into account when calculating the sample size
Failure to perform a phase I trial to determine dose or range of doses prior to the phase II study
Failure to determine duration of dose prior to the phase II study
Failure to set a relevant desired treatment response rate and to design trial with the sample size necessary
Failure to design the trial with the desired difference in response rates between placebo and treated groups in mind
Failure to use colposcopically directed biopsy as an entry and exit test
Failure to define response – partial and complete – and to do so explicitly, quantitatively, and reproducibly

[24.8–130.6], 122.3 [38.5–388.9]) and ranging from 9.9 to 1279.9 for the presence of HPV 16 or 18 (295.5 [44.8–1946.6], 180.0 [49.0–630.0], 9.9 [5.4–18.3], 182.4 [54.0–616.1], 1279.9 [185.5–8829.8]) [33, 35]. For invasive cancer, four PCR-based case–control studies were consistent, demonstrating adjusted odds ratios ranging from 15.6 to 46.2 for the presence of any HPV (46.2 [18.5–111.1], 15.6 [6.9–34.7], 37.1 [19.6–70.4], 32.9 [7.7–141.1]) and ranging from 5.5 to 74.9 for the presence of HPV 16 (14.9 [5.0–49.5], 5.5 [2.4–12.9], 74.9 [32.5–173.0]) [33, 35, 36]. Cohort studies from the United States, the Netherlands, Spain, and Colombia show similar relative risks of more than 15.5 (8.2–29.4) for the presence of HPV detected using PCR-based methods (reviewed in ref. 34). The molecular evidence for the role of HPV in causing cervical cancer is equally compelling [37]. The immune system also plays an important role in the process, and several immunoprevention trials are under way [38]. Since HPV is central, chemoprevention trials should have a biologic rationale that includes decreased expression of HPV.

Choosing a relevant precursor and sample size: the natural history of SIL/CIN

The natural history of SIL/CIN was reviewed initially by Patten in the 1950s, a report discussed and updated recently by Ostor [39] and by Mitchell *et al.* [40]. Table 3 summarizes these reviews. Ostor clustered his review by grade of CIN and described regression rates of 57% for CIN 1, 43% for CIN 2, and 32% for CIN 3. He indicated that overall only 1.7% of CIN 1–3 lesions progressed to invasive cancer. Mitchell *et al.* clustered studies by those followed by Pap only and compared them to those followed by biopsy, separating entirely those that had an entry diagnosis of CIS. CIN 1–3 lesions progressed to invasive cancer in 1–1.4% of cases,

Table 3. Natural history of CIN in cohorts of untreated patients from two reviews

Case classification	Behavior of lesion				
	Regression (%) to lower grade of CIN	Persistence (%) at same grade of CIN	Progression (%)		
			To higher grade of CIN	To carcinoma *in situ*	To invasive cervical cancer
Studies clustered by CIN grade [39]					
CIN 1	57.0	32.0	–	11.0	–
CIN 2	43.0	35.0	–	22.0	–
CIN 3	32.0	56.0	–	12.0	–
Overall all grades of CIN					1.7
Studies clustered by study design [40]					
CIN 1–3 (no carcinoma *in situ*) followed by Papanicolaou smear only	34.0	41.0	25.0	10.0	1.0
CIN 1–3 (no carcinoma *in situ*) followed by Papanicolaou smear and biopsy	45.0	31.0	23.0	14.0	1.4
Carcinoma *in situ* followed by biopsy	–	–	–	–	36.0

Table 4. Number of patients needed per group (treated and placebo) by cervical disease and its expected regression

Cervical disease from natural history study	Disease regression rate (%)[a]	Difference in response rate between treated group and placebo group						
		0.10	0.20	0.30	0.40	0.50	0.60	0.70
CIN 1; Ostor [39]	57	369	85	33	14	–	–	–
CIN 2; Ostor [39]	43	391	97	42	22	12	–	–
CIN 3; Ostor [39]	32	365	95	43	24	14	9	–
CIN 1–3, observed using Pap smear; Mitchell *et al.* [40]	34	372	96	43	23	14	8	–
CIN 1–3, observed using biopsy; Mitchell *et al.* [40]	45	392	96	41	21	11	–	–

Note: Estimates are based on data from natural history studies. Estimates assume a two-sided alpha error of 0.05 and a power of 0.80.
[a] Estimates of regression rates are from studies reported in Table 3.

and if CIS lesions were present, the progression to invasive cancer was much higher – 36%.

Ostor accepted the entry diagnosis as assigned by the pathologist. Mitchell *et al.*, in consideration of the kappa value of 0.40 for both intra- and interobserver agreement among pathologists in interpreting cervical biopsy specimens, divided studies by design rather than pathologic grade. Since CIS had higher intra- and interobserver agreement among pathologists (>0.60), this entity was listed in a separate category.

In each review, the studies included were cohorts of patients followed prospectively with Pap or biopsy. The studies that both authors reviewed ranged in duration from six months to 25 years of follow-up. Some of the studies included colposcopically directed biopsies, while others included blinded biopsies. Very few of the studies included HPV typing. While not all of the studies were of the same caliber, both authors sought to be inclusive rather than exclusive in their reviews. In conclusion, cohorts of patients with LGSIL and HGSIL or CIN 1–3 followed by biopsy or Pap have reasonably high regression rates ranging from 32% to 57%. CIS, followed by biopsy only, has a higher rate of persistence and progression.

How does treatment alter the natural history? Randomized clinical trials of such treatments for SIL/CIN as cryotherapy, laser therapy, and loop excision demonstrate 2-year complete response rates of over 80% regardless of the grade (reviewed in ref. 41). While investigators may choose any level of anticipated benefit in their studies, conventional therapy yields substantial cure rates with minimal complications. Thus a reasonable chemoprevention agent should probably have at least a 40–50% anticipated benefit to provide an advance in treatment to the patient over the natural history and/or conventional therapy.

Based on the reviews by Oster and Mitchell *et al.*, Table 4 shows the number of patients needed for study in each patient group to demonstrate a statistically significant difference, determined by regression rate of the disease based solely on the natural history. Given that study subjects may experience a spontaneous regression, the beneficial outcome of a randomized clinical trial may be higher than predicted. In Table 5, we examine the regression rates noted in the phase II clinical trials and the sample sizes that would be required to obtain a preset desired difference in the response rate of the treated patients ranging from a 10% to 70% difference. Estimates assume an alpha error of 5%, a two-sided measure, and a power of 80%. In Table 6, we see what the minimum detectable statistically significant difference would be for the phase II studies that are reported. Only the sample sizes in the Meyskens *et al.* study [15] and Bell *et al.* study [31] were sufficiently large to detect the anticipated difference desired.

Chemoprevention trials design

Chemoprevention studies involve four elements. First, high-risk cohorts must be identified. Second, suitable medications with low toxicity and reasonable biologic rationale must be selected. Third, the study design should include, in order, phases I, IIa, IIb, and III. Fourth, studies should include the use of surrogate endpoint biomarkers (SEB). Rigorous trial design is critical for the success of these studies [42, 43]. Goodman [44] has outlined the relevant elements in the study design of chemoprevention trials (Table 7). The biology and use of biomarkers are woven into the design of chemoprevention trials.

Phase I chemotherapy trials evaluate toxicity of a drug at escalating doses. In contrast, phase I, IIa chemoprevention trials are often dose de-escalating, seeking the lowest dose at which biologic modulation of the SEB occurs and tolerating little toxicity. The

Table 5. Number of patients needed per group by regression rates and response rate differences between placebo and treatment groups

Chemopreventive, cervical disease, and study	Regression rate in placebo group (%)[a]	Difference in response rate between treated group and placebo group						
		0.10	0.20	0.30	0.40	0.50	0.60	0.70
Retinoids								
All-TRA/CIN 2; Meyskens *et al.* [15]	27	340	90	42	24	15	10	6
All-TRA/CIN 3; Meyskens *et al.* [15]	31	361	94	42	24	15	9	–
Micronutrients								
β-Carotene/CIN 1–3; Romney *et al.* [19, 20]	50	388	93	38	19	–	–	–
β-Carotene/CIN 2–3; Berman [22] and Keefe *et al.* [23]	32	361	95	43	24	14	9	–
β-Carotene/CIN 1–3; De Vet *et al.* [24]	41	389	97	42	22	13	–	–
β-Carotene/atypia to CIN 2; Fairley *et al.* [25]	60	356	81	30	–	–	–	–
β-Carotene/atypia to CIN 1; Mackerras *et al.* [26]	29	351	92	42	24	15	9	5
Folate/CIN 1–2; Butterworth *et al.* [27]	41	389	97	42	22	13	–	–
Folate/CIN 1–2; Butterworth *et al.* [28]	66	322	69	23	–	–	–	–
Folate/HPV to CIN 2; Childers *et al.* [29]	6	146	48	26	16	11	8	6
Adduct reducers								
Indole-3-carbinol/CIN 2–3; Bell *et al.* [31]	0	70	28	17	12	9	7	5

Note: Estimates are based on data from phase II studies in the medical literature. Estimates assume an alpha error of 0.05, a two-sided measure, and a power of 0.80.

[a] Regression rates are complete response rates or complete and partial response rates as reported by authors.

importance of establishing the correct dose for each organ in these trials cannot be overemphasized. Levels of the drug in various tissues of interest should be studied as part of the phase I, IIa trial design. In addition to establishing reasonable doses, the phase I, IIa trials may also be used to identify which SEB are modulated by the drug of interest. Phase II chemoprevention trials, like phase II chemotherapy trials, evaluate the effectiveness of a drug in a given organ. Unlike phase II chemotherapy trials, phase II chemoprevention trials require a placebo group because of the spontaneous regression sometimes observed in preneoplastic lesions. Phase IIa trials are short-term, include a placebo group, look at responses, and may examine SEB modulation. Phase IIb trials are longer, include a concurrent blinded control group receiving a placebo, and incorporate the use of SEB. Both phase III chemotherapy trials and phase III chemoprevention trials evaluate the cost–benefit ratio of treatments in multicenter settings. However, in contrast to phase III chemotherapy studies, which compare investigational agents with standard therapies in groups of patients with cancer, phase III chemoprevention studies evaluate cancer incidence reduction in groups at high risk for cancer. The use of SEB instead of the endpoint of cancer incidence reduction allows these trials to have a shorter duration and a lower cost and to use smaller sample sizes [42, 43].

The importance of the phase I and IIa study designs in chemoprevention cannot be overemphasized. Phase I studies determine the minimally effective and biologically relevant dose of the chemopreventive agent in the organ of interest and the relevant duration of therapy. Phase I, IIa studies can be used not only to validate the SEB but also to demonstrate the modulation of the SEB by the study drug. Levels of the medication in the tissue of interest should be studied as part of the phase I, IIa study design because findings of phase I studies in one organ site may not apply to another. In addition to establishing reasonable doses, the phase I, IIa trials may also be used to validate the SEB modulated by the medication under study in the organ of interest, as SEB modulated in one organ may not be modulated in another organ by the same medication.

Phase II randomized clinical trial design issues

The Consolidated Standards of Reporting Trials (CONSORT) criteria have been designed to improve the reporting of randomized clinical trials. The protocol should prospectively define the hypothesis under study,

Table 6. Minimum delta necessary in completed phase II/III placebo-controlled cervical cancer chemoprevention trials to produce a statistically significant difference between treated and placebo groups

Chemopreventive, cervical disease, and study	Results/regression rate	Subjects (n)	Minimum delta between treated and placebo groups for statistical significance (%)
Retinoids			
All-TRA (topical), CIN 2; Meyskens *et al*. [15]	All-TRA: 43%, placebo: 27%, statistically significant	141	50
All-TRA (topical), CIN 3; Meyskens *et al*. [15]	All-TRA: 25%, placebo: 31%, not statistically significant	160	53
Micronutrients			
Folate, vitamin C, CIN 1–2; Butterworth *et al*. [27]	10 mg folate: 14%, placebo: 41%, not statistically significant	47	80
Folate, CIN 1–2; Butterworth *et al*. [28]	Folate: 64%, placebo: 66%, not statistically significant	177	84
Folic acid, HPV/CIN 1–2; Childers *et al*. [29]	Folate: 7%, placebo: 6%, not statistically significant	331	16
β-Carotene, CIN 1–3; De Vet *et al*. [24]	β-Carotene: 38%, placebo: 41%	137	65
β-Carotene, CIN 1–3; Romney *et al*. [20]	β-Carotene: 46%, placebo: 50%, not statistically significant	74	80
β-Carotene, atypia to CIN 2; Fairley *et al*. [25]	β-Carotene: 63%, placebo: 60%, not statistically significant	117	83
β-Carotene and vitamin C, atypia to CIN 1; Mackerras *et al*. [26]	β-Carotene: 46%, vitamin C: 26%, β-Carotene + vitamin C: 23%, placebo: 29%	141	52
β-Carotene, CIN 2–3; Berman [22] and Keefe *et al*. [23]	β-Carotene: 32%, placebo: 32%	124	53
Adduct reducers			
Indole-3-carbinol, CIN 2–3; Bell *et al*. [31]	200 mg indole-3-carbinol: 50%, 400 mg indole-3-carbinol: 44%, placebo: 0%	27	37

Note: Listed are completed phase II/III placebo-controlled cervical cancer chemoprevention trials with actual numbers of patients, rates of regression, and minimum delta detectable with the current sample size (alpha error, 0.05; two-sided measure; power, 0.80).

describe the study population with inclusion and exclusion criteria, describe the planned interventions and their timing, describe the primary and secondary outcome measures, state the anticipated sample size and rationale for statistical analysis, define stopping rules, and describe the randomization and blinding. In the report of the results of a trial, the participant flow and detailed analyses should be included [45].

The null hypothesis for cervical chemoprevention trials is that the chemopreventive under study will induce no difference in regression rates between treatment and placebo arms. The alternate hypothesis is that the regression rate will be higher in the treatment arm than in the placebo by a given delta error or response. SEB modulation may be a secondary hypothesis, which asserts no change against the alternate hypothesis of an increasing or decreasing measure, depending on the marker.

The study population for chemoprevention trials is drawn from colposcopy clinics where patients are referred for colposcopically directed biopsy after abnormal Pap smear findings. While patients referred to colposcopy clinics have Pap smear diagnoses ranging from atypias to carcinomas, colposcopically directed biopsy is the standard used to establish diagnosis. Most trials exclude patients with findings suggestive of invasive cancer on Pap smear, colposcopy, or colposcopically directed biopsy.

The ideal test at entry is the colposcopically directed biopsy because it is the criterion standard. Using cytology as an entry test incurs substantial false positives and false negatives because the sensitivity and specificity of the Pap smear are both approximately 60% [46]. A meta-analysis indicated that the sensitivity of colposcopically directed biopsy was 96%, while the specificity was 48% [47]. In a separate meta-analysis that examined colposcopy as a screening tool, the sensitivity of colpos-

Table 7. Achievable objectives in study designs for chemoprevention trials

Design and objective	Type of trial					
	Pilot[a]	Phase I	Phase I–II[a]	Phase IIa	Phase IIb	Phase III
Definition	Exploratory study	Dose escalation study or dose-de-escalation study involving a single arm, used to evaluate toxicity and tolerance	Single-arm study used to explore response in the disease of interest, not dose-finding	Randomized, double-blinded, placebo-controlled trial, used to evaluate response, may evaluate multiple dose levels	Randomized, double-blinded placebo-controlled trial used to evaluate biomarkers and response, may evaluate multiple dose levels	Randomized, double-blinded, placebo-controlled trial used to evaluate response in a multicenter setting
Objectives						
Side effect evaluation	Maybe	Short-term: yes; long-term: no	Short-term: yes; long-term: no	Short-term: yes; long-term: yes	Short-term: yes; long-term: yes	Short-term: yes; long-term: yes
Dose/toxicity evaluation	No	Yes	Maybe	Yes	Yes	Yes
Recruitment evaluation	No	No	No	Yes	Yes	Yes
Pharmacokinetics evaluation	Maybe	Yes	No	Yes	Yes	Yes
Efficacy evaluation	No	No	Yes	Yes	Yes	Yes
Duration	<1 year	<1 year	<1 year	1–5 years	1–5 years	At least 1–5 years
Target population	Appropriate target population	Appropriate target population	Appropriate target population	Appropriate target population	Appropriate target population	Appropriate target population
Accrual goal	20	25–100	25–100	100–1000	100–1000	>1000

Source: Adapted from Goodman [44].
[a] Not defined in Goodman [44] but used by many investigators.

copy without biopsy was 86%, while the specificity was 83% [48]. Thus the Pap smear has a false-negative rate of 40% and a false-positive rate of 40%, allowing 40% of patients to be misclassified. Colposcopy without biopsy has a sensitivity and specificity of ~85%, and the false-negative and false-positive proportions are ~15%, resulting in about one-third the chance of misclassification with Pap smear alone. Colposcopically directed biopsy has a false-negative rate of 4%, while the false-positive rate is 52%. This means that, with colposcopy, lesions may be overcalled. Since histology should dictate study entry, lesions that are falsely positive colposcopically would be excluded. Thus the study entry and exit test that would lead to the least misclassification would be colposcopically directed biopsy since its sensitivity is 96% and the lack of specificity is not a problem if histology is used as the criterion for entry.

The planned interventions during the study fall into two categories: the dose and duration of the chemoprevention agent under study and the follow-up and evaluation of re-sponse in the patients. The dose and duration of the che-moprevention agent under study should be determined by a phase I, IIa study in which toxicity, dose, duration, tissue levels, and utility of SEB are evaluated. The phase I, IIa trial should be performed in the organ of interest. Using phase I data from trials in another organ site may mislead the investigator. Similarly, SEB vary among organ sites, and phase I, IIa trials allow investigation of organ-appropriate markers. The primary endpoint for a phase II randomized cervical chemopre-vention trial should be a histopathologic response. The secondary endpoint could be modulation of one or more SEB.

While toxicity, response, or SEB modulation may determine the best dose for the phase II study, the duration is determined with greater difficulty. Once an active dose is determined, a phase I study of duration should be performed. Currently chemopreventives are given for time periods ranging from 1 week to 1 year. The goal with chemoprevention is to use the lowest and best-tolerated dose with activity for the shortest period. Therefore, careful studies of duration and biomarker modulation are equally important as the phase I dose/toxicity trial design.

This study population is characterized by large losses to follow-up. Many investigators are currently studying barriers to participation in these trials. Patients have reported problems – lack of transportation, lack of child care, expense, and fear of developing cancer – as reasons for nonparticipation. The trials of shortest duration and lowest toxicity (those that lower these barriers and maintain accrual) are those most likely to yield meaningful results, though there may be a trade-off if medications take longer than expected to be effective. Since the colposcopically directed biopsy has the highest sensitivity and specificity, it should be used to determine response. The primary endpoint for a phase II randomized cervical chemoprevention trial should be a histopathologic response; the secondary endpoint could be modulation of a SEB.

Defining the response rate carefully is important in any clinical trial. Given the variation in reading of cervical biopsies, well-designed studies should include consensus panel review of biopsies to ascertain response. Quantitative pathology may resolve some of these issues, but it is still not used in many trials. Investigators have chosen many criteria for response. Prior to the establishment of the Bethesda system, a partial response was typically defined as a histologic regression of one grade (e.g., CIN 3 to CIN 2). In some trials, investigators require a two-grade decrease (CIN 3 to CIN 1 or HGSIL to LGSIL). There is no consensus yet on this issue, and quantitative histomorphometry would help considerably.

The secondary endpoint could be modulation of SEB. The SEB of interest will vary according to the effects of the medication under study. Because HPV is a central cause of cervical cancer, the expression of viral load or HPV oncoproteins may be universal markers for these trials.

The anticipated sample size calculation should take into account the natural history of the precursor lesion being studied, the follow-up testing (biopsies during study may induce regression), and the level of anticipated response. The rationale for statistical analysis should be clearly stated and take into account primary and secondary endpoint analyses. Interim analyses generally prove helpful in assessing the trial. Reviewing toxicity and response in blinded fashion protects the patients. Trials should be stopped if they are causing harm.

Review of completed and ongoing cervical cancer chemoprevention trials

Cervical cancer chemoprevention trials to date are summarized in Table 1. In all of these studies, patients having CIN lesions were chosen as the high-risk cohort. Promising cervical cancer chemopreventive agents that have been or are being investigated include the retinoids, retinyl acetate gel, all-*trans*-retinoic acid (all-TRA), and N-(4-hydroxyphenyl)retinamide (4-HPR); micronutrients, including β-carotene, folate, and vitamins; the polyamine synthesis inhibitor, α-difluoromethylornithine (DFMO); and the adduct reducer, indole-3-carbinol. These studies have concentrated on histologic and/or cytologic and colposcopic regression as endpoints. Until 1995, none of the studies systematically used SEB or HPV typing.

Retinoid studies

The retinoids include vitamin A and its natural and synthetic analogs. Natural vitamin A, its esters, and the retinoic acid isomers all-TRA, 9-*cis*-retinoic acid, and 13-*cis*-retinoic acid currently are the most widely clinically tested retinoids. These isomers are interconverted *in vivo* and can activate a wide spectrum of retinoid receptors, both retinoic acid receptors (RAR) and retinoid X receptors. Current systemic therapy using these agents is limited by substantial toxicity [49, 50]. Most cervical studies using these agents have involved local application with a sponge.

One of the retinoid analogs that seems promising in chemoprevention is 4-HPR. Most of the cellular and molecular mechanisms by which retinoids act are mediated by nuclear RAR; however, 4-HPR may act by means of non-RAR mechanism. Support for this contention has come from the study by Delia *et al.* [51], who have shown that 4-HPR can induce apoptosis in retinoic acid–resistant cells. Also, the substitution of an N-substituted carboxyamide group for the terminal carboxyl group is believed to account for the decreased toxicity of 4-HPR compared with other retinoids, making this drug a good choice for long-term use in chemoprevention studies. Because of its different mechanism of action, 4-HPR has low systemic toxicity and can be given orally.

In addition, retinoids have inhibitory effects on the growth of HPV, making such compounds of particular interest in cervical cancer chemoprevention. Specifically, there are several mechanisms by which retinoic acid may affect the HPV E6 and E7 transforming proteins. Bartsch *et al.* [52] have demonstrated decreased expression of HPV messenger RNA in the presence of retinoic acid. Retinoic acid has also been shown to increase the secretion of transforming growth factor α (TGF-α) in cells immortalized by HPV; TGF-α can suppress the expression of the E6 and E7 proteins in cervical epithelial cells [53–55]. Thus, the expression levels of these factors may serve as markers of responsiveness.

Romney *et al.* [10] reported on a phase I and II trial using topical retinyl acetate gel in patients having CIN 1 and CIN 2. Patients treated themselves for 7 days in three sequential menstrual cycles, applying the gel intravaginally. Doses included the placebo and 3, 6, 9, and 18 mg per 6 g of inert vehicle. No serious side effects were noted, but approximately half of the participants noted vulvar irritation and itching with the 18-mg dose. Only 14% of the patients had vaginal burning with any dose during the trial. The study also showed that high compliance could be achieved, and it determined an optimal dose of 9 mg for a phase II trial. There is no published report of the phase II trial.

Phase I and II trials by Surwit *et al.* [11], Meyskens *et al.* [12], and Weiner *et al.* [13] demonstrated that all-TRA could be safely applied topically to the cervix using a cervical sponge and cap. Patients having CIN 1, 2, and 3 received treatment from the investigators for 4 days, using increasing dosages of topical all-TRA ranging from 0.05% to 0.48%. Patients were seen for follow-up at 1 week and 1 month after treatment. Roughly a third of the patients experienced vaginal irritation, and roughly half had vaginal burning. Only one patient discontinued treatment because of these symptoms. A regression rate of 45% was noted in patients who received doses of 0.15–0.48% compared with 14% in those who received lower doses. The optimal dose for a phase III study was determined to be 0.37%.

One of us (F.M.) and his colleagues [15] have reported the results of a randomized phase IIb trial of 0.372% topical all-TRA in 141 patients having CIN 2 lesions and 160 patients having CIN 3 lesions. Patients having CIS were excluded from this study. Patients initially received 0.375% all-TRA daily for 4 days and then for 2 days each at 3- and 6-month follow-up visits. Patients also underwent a Pap smear and colposcopy at 9, 12, 15, 21, and 27 months; biopsies were performed at the 15-month visit. Many of the patients were lost to follow-up. Of 151 patients who received the placebo, 81 were evaluated at 15 months and 25 were evaluated at 27 months. Also, of 150 patients who received all-TRA topically, 88 were seen at 15 months, and 21 were seen at 27 months. There was a statistically significant rate of regression for patients with CIN 2 lesions but not for those with CIN 3 lesions.

While no dose has yet been selected in the phase I trial conducted by Ruffin *et al.* [56] using topical all-TRA, an abstract reporting a study of biomarkers has been published. In that study, 54 women were randomized to one of three all-TRA dose levels. HPV was measured on days 1 and 5 using PCR for HPV presence, semiquantitative PCR for viral load, and reverse transcriptase PCR for E6 and E7 oncoprotein expression. Currently, 38% of white women and 4% of African-American women in the study are HPV negative which, to some, indicates HPV infection may not be a useful biomarker in all patients [16] and, for others, raises questions about the assay.

A phase II study of 4-HPR in chemoprevention of cervical cancer was recently completed at The University of Texas M. D. Anderson Cancer Center [17]. In this study, 4-HPR was given orally to women with biopsy-proven HGSIL; thus, its effects were systemic. Patients underwent a complete medical history survey, nutritional survey, sexual behavior interview, physical examination, colposcopy, colposcopically directed biopsies, HPV testing, blood count measurement, serum chemistry analysis, nyctalopia testing, and smoking cessation counseling. Plans called for patients to receive 4-HPR (200 mg/day with a 3-day drug holiday every month) or placebo for 6 months. Patients were to be monitored at 3-month intervals for 1 year using the aforementioned tests. Crossover from placebo to 4-HPR was to occur if progression was detected using cytology, colposcopy, or biopsy. SEB to be studied in this trial included quantitative cytology and histopathology (nuclear texture, size, and density) and biologic measures of proliferation (proliferating cell nuclear antigen, or PCNA), cellular regulation (epidermal growth factor receptor, or EGFR, and RAR), differentiation (involucrin, cornifin), and genetic instability (chromosome polysomy, aneuploidy). When an interim review indicated at 12 months that one group had a significantly poorer prognosis, researchers broke the code and found that it was the treated group. Analysis showed complete or partial responses in 25% of the treated group and 44% of the placebo group. Since a phase I study of 4-HPR in the cervix had not been performed, all that can be said is that 200 mg/day for 6 months is unfavorable compared with placebo. A higher dose may have been necessary in the cervix.

Micronutrient studies

Carotene and vitamin C

Romney *et al.* [18] conducted a randomized trial of vitamin C in 28 women with CIN 1 or 2. Fourteen women each received vitamin C (1 g/day) in split doses or placebo. Compared with those in controls, serum levels of vitamin C in patients receiving vitamin C were significantly increased. No significant progression was noted in the treatment arm. The investigators considered this a pilot study and planned a larger phase I trial. No true phase I study of vitamin C has been performed.

In a later study, Romney and colleagues [19] conducted a phase II trial of β-carotene given at 30 mg/day

in patients having CIN 2. They expected 138 patients to be accrued. Results for 74 patients have been published, showing no statistically significant difference in the response of 39 patients who received β-carotene and 30 who received a placebo [20]. Additionally, two provocative cervical neoplasia biomarker reports have been published. First, Ho *et al.* [57] reported the use of HPV viral load in predicting persistent disease in an elegant study that controlled for age, ethnicity, education, duration of oral contraceptive use, age at first intercourse, number of sexual partners, smoking status, and HPV typing by Southern blot analysis and PCR. They found that viral load was most predictive of persistent disease; however, there was no mention of the effects of β-carotene on viral load in this analysis. Second, Comerci *et al.* [58] reported that tissue levels of TGF-α_1 were higher after β-carotene treatment than before treatment. Tissue staining in this study was graded visually but not measured quantitatively. No mention was made of how many reviewers graded the tissue samples, but one of the authors is a pathologist. Statistically significant increases in TGF-α_1 were seen across parabasal, midepithelial, and superficial epithelia. Also, no histologic regression rate was reported in this analysis.

Manetta *et al.* [21] undertook a phase I–II study of oral β-carotene taken daily for 6 months by 30 patients having CIN 1 or 2. The dose was not modulated in this study. Five patients were removed from the study after disease progression (two at 3 months, one at 6 months), and three other patients were removed because of pregnancy (one at 3 months, two at 6 months), leaving 27 evaluable patients at 6 months and 22 evaluable patients at 12 months. Twenty-one (78%) of the 27 evaluable patients showed regression colposcopically at 6 months, while 10 (45%) of the 22 patients showed regression colposcopically at 12 months.

In another trial, Berman [22] undertook a phase II study of β-carotene in patients having CIN 2 or 3. They expected to accrue 60 patients. Keefe *et al.* [23] reported a 32% response rate in both β-carotene and placebo arms of the trial. Additionally, Brewer *et al.* [59] published a biomarker report of this series showing the serial changes in colposcopic and cervicographic findings in women enrolled in the trial. Data were available for 23 subjects who had regression and 16 who had persistent lesions. In this study, small lesions were significantly more likely to regress than were larger ones. Also, patients whose lesions had coarse punctation (usually indicative of a higher grade) were significantly more likely to have persistent disease. A centripetal pattern of regression was also noted [59].

In yet another study, De Vet *et al.* [24] conducted a randomized phase II clinical trial of β-carotene in 278 patients having CIN 1–3; patients received β-carotene given at 10 mg/day for 3 months ($n = 137$) or placebo ($n = 141$). They studied both the partial and complete regression rates using colposcopically directed biopsy at study entry and Pap smear or biopsy at study termination. The β-carotene group had a 39% response rate, whereas the placebo group had a response rate of 41%. In a later placebo-controlled phase II study, Fairley *et al.* [25] administered β-carotene at 30 mg/day for 12 months to 117 patients having conditions ranging from cervical atypia to CIN 2. The β-carotene group had a 63% response rate compared with 60% in the control arm. Finally, Mackerras *et al.* [26] conducted a double-blind, placebo-controlled randomized study in which 141 women having cervical atypia to CIN 1 received 30 mg of β-carotene only, 500 mg of vitamin C only, both β-carotene and vitamin C, or neither for 2 years, using Pap smear or biopsy at the end of the study for response. The response rates were as follows: β-carotene only, 46%; vitamin C only, 26%; both, 23%; and neither, 29%. There were no statistically significant differences among these results. Thus all the β-carotene studies are negative. Unfortunately, a true phase I dose-finding study has not been done, so the optimal dose and treatment duration are unknown.

Folate

Like β-carotene, folate, specifically red blood cell folate, has been shown to be deficient in patients with CIN compared with controls. These data have supported folate supplementation as a chemopreventive strategy for CIN [60]. Also, red blood cell folate levels below 660 nmol/L have been shown to enhance the susceptibility of patients to HPV. Because folic acid acts as a coenzyme in DNA synthesis for normal cellular growth, proliferation, and differentiation, Pietrantoni *et al.* [55] studied the regulation of HPV oncogene expression by folic acid. Specifically, they studied c-*fos*, c-*jun*, and HPV E6 expression in CaSki (HPV 16–positive) cell lines treated with folic acid. They found diminished c-*fos* and c-*jun* expression using Western blot analysis when concentrations of folate greater than 100 nmol/L were used. Similarly, E6 protein expression was diminished at folate concentrations of greater than 100 nmol/L, suggesting that the mechanism by which the transcription regulators c-*fos* and c-*jun* were controlled involved diminished viral E6 expression.

In addition, Butterworth *et al.* [27] published an update of a phase II randomized trial in which patients having CIN 1 and 2 lesions received folate (10 mg) or vitamin C (10 mg) as a placebo for 90 days. An initial report on 47 of the patients indicated that those receiving folate were likely to experience cytologic regression of

their lesions; however, in the final report on the 177 evaluable patients there were no statistically significant differences in regression of lesions between those who received folate and those who received vitamin C [28]. A phase II multicenter study of folate supplementation performed by Childers *et al.* [29] had similarly negative results. In this intergroup Southwest Oncology Group study, 331 patients having koilocytotic atypia, CIN 1, or CIN 2 were randomized to receive 5 mg of folic acid or a placebo. There was no difference between the groups in disease regression after treatment for 3 months ($p = 0.08$) or 6 months ($p = 0.23$). Again, no phase I dose-finding study has ever been carried out for folate, so the optimal dose and duration are unknown.

Polyamine synthesis inhibitors

DFMO is an irreversible inhibitor of ornithine decarboxylase (ODC), a key enzyme in the biosynthesis of polyamines (putrescine, spermidine, and spermine) that is now considered a putative proto-oncogene crucial for the regulation of cell growth and transformation [61]. Blocking endogenous ODC prevents transformation of rat fibroblasts by the temperature-sensitive v-src oncogene. The goals of using DFMO to block polyamine-directed transformation are inhibition of transformation under the influence of field cancerization and removal of cells already transformed by apoptosis [62]. Tumor formation in experimental animals is prevented by inhibitors of ODC such as DFMO [63, 64].

A phase I study of DFMO was completed at M. D. Anderson Cancer Center [30]. In this study, the medication was given orally as an elixir; thus, the effects were systemic. DFMO was administered at five dose levels: 1.000, 0.500, 0.250, 0.125, and 0.060 g/m^2. The patients underwent a complete medical history survey; nutritional survey; sexual behavior interview; physical examination; colposcopy; colposcopically directed biopsies; HPV testing; blood counts; serum chemistry analysis; audiogram; plasma DFMO measurement; ornithine and arginine measurements; red blood cell polyamine measurement; tissue DFMO, ODC, and polyamine measurement; and smoking cessation counseling. DFMO was administered for 1 month, and loop electrosurgical excision of the cervix was performed at the study conclusion. Thirty patients were enrolled in the study and completed all studies, but only 29 were evaluable because one patient took the wrong dose. Favorable responses were seen at all dose levels. Overall a 50% response rate was noted (complete and partial responses). The polyamine biomarkers suggested that 0.50 g/m^2 per day and 0.125 g/m^2 per day would be of interest for the phase IIb study, which is ongoing [30]. Quantitative histopathologic biomarkers showed statistically significant decreases in DNA content in all specimens at all dosages. Decreases in DNA content were seen in both histologic responders and nonresponders, though they were most significant in responders [65, 66]. Quantitative PCNA measurements showed decreased proliferation of CIN 3 in all specimens at all dose levels, with the most significant decreases noted in histologic responders [67]. Additionally, MPM-2 was measured in correlation with PCNA. Decreased rates of mitosis correlated well with decreased rates of proliferation of the basal layer [68]. Finally, EGFR was measured quantitatively and did not decrease significantly with treatment; however, pretreatment levels of EGFR were inversely correlated with DFMO response [69]. An advance in the polyamine trial field would be the development of immunohistochemical markers of polyamine synthesis [70].

Adduct reducers

The principal medication in the adduct reducer category currently being studied is indole-3-carbinol [31]. Indole-3-carbinol occurs naturally in vegetables of the genus *Brassica*, such as cabbage, broccoli, and brussels sprouts [71, 72]. This promising anticancer agent induces G_1 cell cycle arrest in human breast cancer cell lines independently of estrogen receptor status [73]. Indole-3-carbinol can also reduce the incidence of spontaneous and carcinogen-induced mammary tumors [73, 74]. Investigators conducted a phase I study of indole-3-carbinol in 60 women at risk for breast cancer, with doses ranging from 50 to 400 mg/day and using the urinary estrogen metabolite ratio of 2-hydroxyestrone to 16-hydroxyestrone (2-OH/16-OH) as determined by enzyme-linked immunosorbent assay as the SEB [75]. With regression analysis, the researchers found that, from baseline, the peak relative change of the ratio was significantly greater in the group of women receiving high-dose therapy (300 or 400 mg/day) than it was in the control group or the group receiving low-dose therapy (50, 100, or 200 mg/day). They concluded that the minimum effective dose for indole-3-carbinol as a breast cancer preventive was 300 mg/day and that at this dose, and within their 4-week study period, the agent presented no significant toxicity.

A phase I study of indole-3-carbinol in women with CIN has not been performed. The biologic rationale for the use of indole-3-carbinol in the cervix is that it has been shown to prevent cervical cancer in HPV type 16 transgenic mice during 6 months of treatment [76]. Nineteen of 25 control mice developed cancer with

estradiol administration, whereas only two of 24 mice fed indole-3-carbinol developed cancer [76].

Bell *et al.* [31] have conducted a phase II study of indole-3-carbinol at two dose levels in the cervix, using doses from the phase I study in the breast. For 12 weeks they treated 27 women having CIN 2 or CIN 3 with a placebo (10 patients), 200 mg/day indole-3-carbinol (eight patients), and 400 mg/day indole-3-carbinol (nine patients). In their study indole-3-carbinol administration produced regression (4 of 8 [50%] at 200 mg/day and 4 of 9 [44%] at 400 mg/day), while placebo administration did not (0 of 10). The differences were statistically significant.

Why phase II cervical chemoprevention trials have been negative?

For all the phase II studies listed, the hypothesis under study was regression of CIN. All the studies recruited patients from the colposcopy clinic. Many studies looked at high-grade lesions, but a few included patients with low-grade lesions and atypias. The major issues in study design in the existing trials are four: underpowering, lack of phase I data for dose and duration of treatment, the use of Pap or Pap and colposcopy for study entry, and inconsistency in tests used at study entry and exit.

Underpowering

Part of the reason that chemoprevention of SIL/CIN trials have not worked has to do with the natural history of CIN and underpowering in studies. Using the estimates derived from natural history and using the regression rate seen in the placebo arm of these phase II studies, sample sizes for CIN studies can be calculated. If one desires a power of 0.80 and allows for an alpha error of 0.05, the estimated sample size for a placebo-controlled trial would be calculated assuming a level of difference in two binomial proportions using the arc sine transformation (ST Plan, Department of Biomathematics, M. D. Anderson Cancer Center, Houston, Texas). The resulting estimates are listed in Tables 4 and 5. The difference in response rate per arm varies from 0.10 to 0.70, meaning, for example, that if the response rate in the placebo arm is 0.30 and there is a delta error, or anticipated difference in response, of 0.20, the response rate in the treatment arm is 0.50. A clinically relevant delta error must be determined by the investigator. If a difference of as little as 10% is expected between the placebo and treated group, then having 350–400 subjects per arm of study generally would be necessary. As the expected differences in response rate increase, the number of subjects needed declines. Thus if a medication is expected to have a high response rate, fewer subjects are needed. The sample sizes and the regression rates in Table 6 indicate that medications would have had to produce at least a 30% response rate in order to produce a statistically significant difference between the two groups. When we calculate the minimum delta that could have been detected given the regression rate and the sample size, most studies, with the exception of the study by Childers *et al.* [29], would have required that the medication be able to induce a 50–80% regression over placebo (Table 6). This effect would have required agents with a high level of activity. This table confirms that only the studies of Meyskens *et al.* [15] and Bell *et al.* [31] were sufficiently powered to detect a difference.

In conclusion, with regard to sample size and power calculations, the following variables must be taken into account: the entry diagnosis (grade of CIN/SIL), how the patients will be observed (biopsy or cytology), the duration of follow-up, and the anticipated difference of interest in response between the placebo and treatment arms. Specifically, the lower the CIN grade, the larger the number of patients required to demonstrate an enhanced rate of regression, given the natural history of the disease. Also, if the patients are to be observed using biopsy, more patients must be studied because there is a slightly higher rate of regression anticipated with biopsies.

Lack of phase I, IIa data

Phase I trials of retinyl acetate gel [10], all-TRA [11–13], and DFMO [30] for cervical neoplasia have been conducted prior to conducting the phase II studies (Table 8). In contrast, there has never been a phase I trial of vitamin C, folate, 4-HPR, or indole-3-carbinol for cervical neoplasia. How were the doses chosen? The doses for vitamin C and folate were known to be safe. The doses for the 4-HPR and indole-3-carbinol trials were taken from breast cancer studies. Lack of a phase I trial may allow for a falsely negative phase II because a biologically inactive dose or an insufficient duration may be chosen.

None of the phase I studies conducted looked carefully at duration of use. Duration needs to be adequately studied. Once a biologically relevant dose is chosen, other phase I trials should be performed to study dose and duration of dose. These studies, done carefully, would produce important biomarker modulation information. Most of the published studies report losses to follow-up, and experience would suggest that the shortest duration possible will enroll and keep the greatest numbers of patients; however, longer duration of use may be necessary when patients have high-grade lesions.

Table 8. Study designs used in cervical cancer chemoprevention trials by agent

Medication	Study design					
	Pilot	Phase I	Phase I–II	Phase IIa	Phase IIb	Phase III
Retinoids						
Topical retinyl acetate gel			Romney *et al.* [10]			
Topical all-TRA		Meyskens *et al.* [12], Weiner *et al.* [13]	Surwit *et al.* [11]	Graham *et al.* [14]	Meyskens *et al.* [15][a], Ruffin *et al.* [16]	
4-Hydoxyphenyl-retinamide					Follen *et al.* [17]	
Micronutrients						
Vitamin C	Romney *et al.* [18]				Mackerras *et al.* [26]	
β-Carotene			Manetta *et al.* [21]		Romney *et al.*, [20], De Vet *et al.* [24], Fairley *et al.* [25], Mackerras *et al.* [26]	Keefe *et al.* [23]
Folate					Butterworth *et al.* [27, 28]	
Polyamine synthesis inhibitors						
α-Difluoromethyl-ornithine		Mitchell *et al.* [30]		Follen *et al.* (ongoing/ unreported)		
Adduct reducers						
Indole-3-carbinol				Bell *et al.* [31][a]		

[a] Statistically significant response rate.

Adequate medication absorption was studied by Romney *et al.* [18] and Meyskens *et al.* [11–13] but not by other investigators. No studies have looked carefully at tissue levels of medication. Some medications will require an additional biopsy for assessment. The need for additional biopsies suggests performing these tissue level tests in phase I studies, in which response is not the primary outcome, but rather tolerance and toxicity.

Study entry criteria, primary endpoint measures, and misclassification

The entry and exit tests are summarized in Table 9. For the test desired at entry, colposcopically directed biopsy is the standard. The phase II studies of Butterworth *et al.* [27, 28] used the Pap smear and colposcopy for entry and exit. This could result in a 15% misclassification of CIN lesions. Fairley *et al.* [25] used the Pap smear alone for entry and exit, which might have resulted in a 40% misclassification of severity of CIN. Using two different tests – one at study entry and one at study termination – permits differential misclassification bias between points of entry and termination. Mackerras *et al.* [26], De Vet *et al.* [24], Butterworth *et al.* [27, 28], and Childers *et al.* [29] chose different tests for study entry and study end. This may partially account for the lack of drug effect noted in these studies.

Misclassification of Pap smears and cervical biopsy specimens has been the subject of several thoughtful reviews [39, 40, 61–67, 77, 78]. The interobserver and intraobserver kappas for the review of Pap smears and occult biopsies are in the 0.4–0.7 range of moderate agreement. Triply blind reviews followed by a consensus panel review are currently the accepted method for obtaining a rigorous gold standard. Quantitative pathology using Feulgen-based stoichiometric stains may soon make quantitative what was previously qualitative. DNA content, chromatin texture features, and tissue architecture are offering algorithms that are both quantitative and reproducible. The field is emerging but promising better mathematical separation of diagnostic categories. Quantitative measure of biomarkers can be correlated with qualitative pathology to create classifications that may be more biologically

Table 9. Study design for phase II studies

Study	Test	
	At entry	At termination
Retinoids		
Meyskens *et al.* [15]	Colposcopically directed biopsy	Colposcopically directed biopsy
Micronutrients		
Butterworth *et al.* [27]	Pap smear and colposcopy	Colposcopically directed biopsy
Butterworth *et al.* [28]	Pap smear and colposcopy	Colposcopically directed biopsy
Childers *et al.* [29]	Colposcopically directed biopsy	Pap smear and colposcopy
De Vet *et al.* [24]	Colposcopically directed biopsy	Pap smear or colposcopy or, if progression, colposcopically directed biopsy
Romney *et al.* [19, 20]	Colposcopically directed biopsy	Colposcopically directed biopsy
Fairley *et al.* [25]	Pap smear	Pap smear
Mackerras *et al.* [26]	Colposcopically directed biopsy	Pap smear and colposcopy or, if progression, colposcopically directed biopsy
Berman [22] and Keefe *et al.* [23]	Colposcopically directed biopsy	Colposcopically directed biopsy
Adduct reducers		
Bell *et al.* [31]	Colposcopically directed biopsy	Colposcopically directed biopsy

at risk than those seen qualitatively with the human eye.

Defining the response rate carefully is important in any clinical trial. Given the variation in reading of cervical biopsies, well-designed studies should include consensus panel review of biopsy specimens to ascertain response. Another issue is that many investigators have reported only partial response rates in their studies, rather than complete responses, partial responses, and total responses. Meyskens *et al.* [15] had well-defined criteria for response and reported a complete response rate. Investigators ideally should report both partial and complete responses in tables, broken down by major covariates such as diagnosis at entry. Meyskens *et al.* [15], De Vet *et al.* [24], and Fairley *et al.* [25] reported response rates by diagnosis; however, only De Vet and colleagues reported both partial and complete responses by diagnosis.

The future: how to conduct cervical cancer chemoprevention studies

Future investigators of cervical cancer chemoprevention need to follow the principles of good study design. Future advances in cervical cancer chemoprevention trials depend on the understanding of factors that have impeded or limited the validity or generalizability of previous interventions [79]. Principles applicable to the design of randomized clinical trials include defining suitable cohorts and carefully selecting the treatment modality in relationship to the anticipated response, primary and secondary outcome measures, and biologic rationale.

Suitable cohorts may have any grade of CIN, but the sample size must account for the rate of regression expected for that grade of CIN as determined by the method of follow-up. The natural history of SIL/CIN is characterized by regression rates of 32–57%, and sample sizes should account for spontaneous regression rates and the stipulated therapeutic objectives.

Chemoprevention trials should be well designed and incorporate the use of biomarkers. Phase I, IIa studies in patients with CIN that are carefully designed and include a placebo group, SEB validation and variability determination, toxicity measures, and similar study entry and termination testing are essential. Phase IIb placebo-controlled studies and multicenter phase III studies should build upon the knowledge obtained in the phase I, IIa studies. The validation and determination of SEB modulation are critical to the success of these studies.

The primary and secondary outcomes measure should be clearly defined. Patients who are enrolled in the study should have a diagnosis based on colposcopically directed biopsy, which is the highest standard. Because this test has the highest sensitivity, all participants should undergo colposcopically directed biopsy at study entry and termination. Response criteria need to be well defined. Because of the large variation in reading of

cervical biopsy specimens, consensus panels of pathologists blinded to study outcome should be used for response evaluation. As quantitative and reproducible measures of pathology emerge, they will add value in assessing response quantitatively rather than qualitatively. In addition, authors should report as much raw data as possible, including at least the entry diagnosis and both partial and complete response rates.

There must be a biologic rationale for the choice of medication, and the incorporation of appropriate SEB will be based on the medication under study. SEB that may be of interest in all studies include viral load and HPV oncoprotein expression. Preclinical laboratory work, including suppression of HPV oncoprotein expression in cell lines or prevention of HPV-induced tumors in mice, would contribute to the biologic rationale.

Cervical cancer chemoprevention studies require of the investigator depth of knowledge, familiarity with biologic and epidemiologic principles, and persistence. Much is now known about the natural history and pathobiology of cervical cancer. This knowledge must be used constructively to refine study designs in the future.

Acknowledgements

A visiting scholar when this work was performed, Dr Vlastos was supported by the Swiss National Science Foundation, the Swiss Cancer League, the Cancer and Solidarity Foundation, and the Novartis Foundation.

This work was supported by National Cancer Institute Grant CN-25483-02.

References

1. Parkin DM, Pisani P, Ferlay J (1999) Estimates of the worldwide incidence of 25 major cancers in 1990. *Int J Cancer* **80**: 827–841.
2. Coleman MP, Esteve J, Damiecki P, Arslan A, Renard H (1993) Trends in cancer incidence and mortality. *IARC Sci Publ*: **121**: 1–806.
3. American Cancer Society (2002) *Cancer Facts & Figures 2002*. Atlanta: American Cancer Society.
4. American Cancer Society (1995) *Cancer Facts & Figures 1995*. Atlanta: American Cancer Society.
5. Paavonen J, Koutsky LA, Kiviat N (1990) Cervical neoplasia and other STD-related genital-anal neoplasias. In: Holmes KK, Mardh PA, Sparling PF, Wiesner PJ, eds. *Sexually Transmitted Diseases*, 2nd edn. New York: McGraw-Hill, pp. 561–592.
6. US Department of Health and Human Services, Centers for Disease Control and Prevention (1999) *The National Breast and Cervical Cancer Early Detection Program At-a-Glance 1999*. Atlanta: Centers for Disease Control and Prevention.
7. Kurman RJ, Henson DE, Herbst AL, Noller KL, Schiffman MH (1994) Interim guidelines for management of abnormal cervical cytology. The 1992 National Cancer Institute Workshop. *JAMA* **271**: 1866–1869.
8. Ollayos CW, Swogger KA (1995) Abnormal cervical smears in the military recruit population. *Mil Med* **160**: 577–578.
9. Center for Disease Control (1994) Results from the national breast and cervical cancer early detection program, October 31, 1991–September 30, 1993. *Morb Mortal Wkly Rep* **43**: 530–534.
10. Romney SL, Dwyer A, Slagle S, *et al.* (1985) Chemoprevention of cervix cancer: phase I–II. A feasibility study involving the topical vaginal administration of retinyl acetate gel. *Gynecol Oncol* **20**: 109–119.
11. Surwit EA, Graham V, Droegemueller W, *et al.* (1982) Evaluation of topically applied *trans*-retinoic acid in the treatment of cervical intraepithelial lesions. *Am J Obstet Gynecol* **143**: 821–823.
12. Meyskens FL Jr, Graham V, Chvapil M, *et al.* (1983) A phase I trial of β-all-*trans*-retinoic acid delivered via a collagen sponge and a cervical cap for mild or moderate intraepithelial cervical neoplasia. *J Natl Cancer Inst* **71**: 921–925.
13. Weiner SA, Surwit EA, Graham VE, *et al.* (1986) A phase I trial of topically applied *trans*-retinoic acid in cervical dysplasia – clinical efficacy. *Invest New Drugs* **4**: 241–244.
14. Graham V, Surwit ES, Weiner S, Meyskens FL (1986) Phase II trial of β-ALL-*trans*-retinoic acid for cervical intraepithelial neoplasia via collagen sponge and cervical cap. *West J Med* **145**: 192–195.
15. Meyskens FL Jr, Surwit E, Moon TE, *et al.* (1994) Enhancement of regression of cervical intraepithelial neoplasia II (moderate dysplasia) with topically applied all-*trans*-retinoic acid: a randomized trial. *J Natl Cancer Inst* **86**: 539–543.
16. Current Clinical Trials Oncology (2000) *National Cancer Institute PDQ*, Vol. 7, No. 1 (January–February) (protocol 13869).
17. Follen M, Atkinson EA, Schottenfeld D, *et al.* (2001) A randomized clinical trial of 4-hydroxyphenylretinamide for high-grade squamous intraepithelial lesions of the cervix. *Clin Cancer Res* **7**: 3356–3365.
18. Romney SL, Basu J, Vermund S, Palan P, Duttagupta C (1987) Plasma reduced and total ascorbic acid in human uterine cervix dysplasias and cancer. *Ann NY Acad Sci* **498**: 132–143.
19. Current Clinical Trials Oncology (1994) *National Cancer Institute PDQ*, Vol. 1, No. 2 (March–April) (protocol 09315).
20. Romney SL, Ho GYF, Palan PR, *et al.* (1997) Effects of β-carotene and other factors on outcome of cervical dysplasia and human papillomavirus infection. *Gynecol Oncol* **65**: 483–492.
21. Manetta A, Schubbert T, Chapman J, *et al.* (1996) β-Carotene treatment of cervical intraepithelial neoplasia: a phase II study. *Cancer Epidemiol Biomarkers Prev* **5**: 929–932.
22. Current Clinical Trials Oncology (1994) *National Cancer Institute PDQ*, Vol. 1, No. 2 (March–April) (protocol 09088).
23. Keefe KA, Schell MJ, Brewer C, *et al.* (1998) A randomized, double blind, phase III trial using oral beta-carotene supplementation for women with high-grade cervical intraepithelial neoplasia. *Cancer Epidemiol Biomarkers Prev* **10**: 1029–1035.
24. De Vet HCW, Knipschild PG, Willebrand D, Schouten HJA, Sturmans F (1991) The effect of beta-carotene on the regression and progression of cervical dysplasia: a clinical experiment. *J Clin Epidemiol* **44**: 273–283.
25. Fairley CK, Tabrizi SN, Chen S, *et al.* (1996) A randomized clinical trial of beta carotene vs. placebo for the treatment of cervical HPV infection. *Int J Gynecol Cancer* **6**: 225–230.
26. Mackerras D, Irwig L, Simpson JM, *et al.* (1999) Randomized double-blind trial of beta-carotene and vitamin C in women with minor cervical abnormalities. *Br J Cancer* **79**: 1448–1453.
27. Butterworth CE, Hatch KD, Soong SJ, *et al.* (1992) Oral folic acid supplementation for cervical dysplasia: a clinical intervention trial. *Am J Obstet Gynecol* **166**: 803–809.

28. Butterworth CE Jr, Hatch KD, Macaluso M, *et al.* (1992) Folate deficiency and cervical dysplasia. *JAMA* **267**: 528–533.
29. Childers JM, Chu J, Voigt LF, *et al.* (1995) Chemoprevention of cervical cancer with folic acid: a phase II Southwest Oncology Group Intergroup study. *Cancer Epidemiol Biomarkers Prev* **4**: 155–159.
30. Mitchell MF, Tortolero-Luna G, Lee JJ, *et al.* (1998) Phase I dose de-escalation trial of α-difluoromethylornithine in patients with grade 3 cervical intraepithelial neoplasia. *Clin Cancer Res* **4**: 303–310.
31. Bell MC, Crowley-Nowick P, Bradlow HL, *et al.* (2000) Placebo controlled trial of indole-3-carbinol in the treatment of CIN. *Gynecol Oncol* **78**: 123–129.
32. Kjaer SK (1998) Risk factors for cervical neoplasia in Denmark. *APMIS* (Suppl.) **80**: 1–41.
33. Muñoz N, Bosch FX (1997) Cervical cancer and human papillomavirus: epidemiological evidence and perspectives for prevention. *Salud Pública de México* **39**: 274–282.
34. Muñoz N, Bosch FX (1992) HPV and cervical neoplasia: review of case-control and cohort studies. *IARC Sci Publ* **119**: 251–261.
35. Bosch FX, Muñoz N, de Sanjosé S, *et al.* (1993) Human papillomavirus and cervical intraepithelial neoplasia grade III/carcinoma in situ: a case–control study in Spain and Colombia. *Cancer Epidemiol Biomarkers Prev* **2**: 415–422.
36. Bosch FX, Manos MM, Muñoz N, *et al.* (1995) Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International Biological Study on Cervical Cancer (IBSCC) Study Group. *J Natl Cancer Inst* **87**: 796–802.
37. zur Hausen H (1999) Yohei Ito Memorial Lecture: Papillomaviruses in human cancers. *Leukemia* **13**: 1–5.
38. Lowy DR, Schiller JT (1998) Papillomaviruses: prophylactic vaccine prospects. *Biochim Biophys Acta* **1423**: M1–8.
39. Ostor G (1993) Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol* **12**: 186–192.
40. Mitchell MF, Hittelman WN, Hong WK, *et al.* (1994) The natural history of cervical intraepithelial neoplasia: an argument for intermediate endpoint biomarkers. *Cancer Epidemiol Biomarkers Prev* **3**: 619–626.
41. Mitchell MF, Tortolero-Luna G, Cook E, *et al.* (1998) A randomized clinical trial of cryotherapy, laser vaporization, and loop electrosurgical excision for the treatment of squamous intraepithelial lesions of the cervix. *Obstet Gynecol* **92**: 737–744.
42. Boone CW, Kelloff GJ, Steele VE (1992) Natural history of intraepithelial neoplasia in humans with implications for cancer chemopreventive strategy. *Cancer Res* **52**: 1651–1659.
43. Mitchell MF, Hittelman WK, Lotan R, *et al.* (1995) Chemoprevention trials in the cervix: design, feasibility, and recruitment. *J Cell Biochem* (Suppl.) **23**: 104–112.
44. Goodman GE. The clinical evaluation of cancer chemoprevention agents: defining and contrasting phase I, II, and III objectives. *Cancer Res* **52** (9 Suppl.): 2752S–2757S.
45. Begg C, Cho M, Eastwood S, *et al.* (1996) Improving the quality of reporting of randomized controlled trials. The CONSORT statement. *JAMA* **276**: 637–639.
46. Fahey MT, Irwig L, Macaskill P (1995) Meta-analysis of Pap test accuracy. *Am J Epidemiol* **141**: 680–689.
47. Mitchell MF, Cantor SB, Ramanujam N, *et al.* (1999) Fluorescence spectroscopy for diagnosis of squamous intraepithelial lesions of the cervix. *Obstet Gynecol* **93**: 462–470.
48. Mitchell MF, Cantor SB, Brookner C, *et al.* (1999) Screening for squamous intraepithelial lesions with fluorescence spectroscopy. *Obstet Gynecol* **94**: 889–896.
49. Evans TRJ, Kaye SB (1999). Retinoids: present role and future potential. *Br J Cancer* **80**: 1–8.
50. Lippman SM, Davies PJA (1997) Retinoids, neoplasia and differentiation therapy. *Cancer Chemother Biol Response Mod* **17**: 349–362.
51. Delia D, Aiello A, Lombardi L, *et al.* (1993) N-(4-hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid. *Cancer Res* **53**: 6036–6041.
52. Bartsch D, Boye B, Baust C, zur Hausen H, Schwarz E (1992) Retinoic acid–mediated repression of human papillomavirus 18 transcription and different ligand regulation of the retinoic acid receptor beta gene in non-tumorigenic and tumorigenic HeLa hybrid cells. *EMBO J* **11**: 2283–2291.
53. Batova A, Danielpour D, Pirisi L, Creek KE (1992) Retinoic acid induces secretion of latent transforming growth factor β1 and β2 in normal and human papillomavirus type 16 – immortalized human keratinocytes. *Cell Growth Differ* **3**: 763–772.
54. Woodworth CD, Notario V, DiPaolo JA (1990) Transforming growth factors α1 and 2 transcriptionally regulate human papillomavirus (HPV) type 16 early gene expression in HPV-immortalized human genital epithelial cells. *J Virol* **64**: 4767–4775.
55. Pietrantoni M, Taylor DD, Gercel-Taylor C, *et al.* (1995) The regulation of HPV oncogene expression by folic acid in cultured human cervical cells. *J Soc Gynecol Invest* **2**: 228.
56. Ruffin MT, Bailey JM, Underwood D, *et al.* (1999) The potential use of HPV copy number and E6/E7 expression as biomarkers in cervical dysplasia. *Proc Am Assoc Cancer Res* **40** (abstract 2850).
57. Ho GY, Burk RD, Klein S, *et al.* (1995) Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst* **87**: 1365–1371.
58. Comerci JT, Runowicz CD, Fields AL, *et al.* (1997) Induction of transforming growth factor α-1 in cervical intraepithelial neoplasia *in vivo* after treatment with β-carotene. *Clin Cancer Res* **3**: 157–160.
59. Brewer CA, Wilczynski SP, Kurosaki T, Daood R, Berman ML (1997) Colposcopic regression patterns in high-grade cervical intraepithelial neoplasia. *Obstet Gynecol* **90**: 617–621.
60. Butterworth CE Jr, Hatch KD, Macaluso M, *et al.* (1992) Folate deficiency and cervical dysplasia. *JAMA* **267**: 528–533.
61. Auvinen M, Paasinen A, Andersson LC, Hölttä E (1992) Ornithine decarboxylase activity is critical for cell transformation. *Nature* **360**: 355–358.
62. Nishioka K, Melgarejo AB, Lyon RR, Mitchell MF (1995) Polyamines as biomarkers of cervical intraepithelial neoplasia. *J Cell Biochem* **23**: 87–95.
63. Verma AK, Boutwell RK (1987) Inhibition of carcinogenesis by inhibitors of putrescine biosynthesis. In: McCann PP, Pegg AE, Sjoerdsma A, eds. *Inhibition of Polyamine Metabolism, Biological Significance and Basis for New Therapies*. Orlando: Academic Press, pp. 249–258.
64. Tempero MA, Nishioka K, Knott K, Zetterman RK (1989) Chemoprevention of mouse colon tumors with difluoromethylornithine during and after carcinogen treatment. *Cancer Res* **49**: 5793–5797.
65. Boiko IV, Mitchell MF, Pandey DK, *et al.* (1997) DNA image cytometric measurement as a surrogate end point biomarker in a phase I trial of α-difluoromethylornithine for cervical intraepithelial neoplasia. *Cancer Epidemiol Biomarkers Prev* **6**: 849–855.
66. Bacus JW, Boone CW, Bacus JV, *et al.* (1999) Image morphometric nuclear grading of intraepithelial neoplastic lesions with applications to cancer chemoprevention trials. *Cancer Epidemiol Biomarkers Prev* **8**: 1087–1094.

67. Hu W, Boiko IV, Mitchell MF, Hittelman WN, *et al.* (1995) Sequential dysregulation of proliferation during cervical carcinogenesis. *Proc Am Assoc Cancer Res* **36**: (abstract 3500).
68. Hu W, Mitchell MF, Boiko IV, *et al.* (1997) Progressive dysregulation of proliferation during cervical carcinogenesis as measured by MPM-2 antibody staining. *Cancer Epidemiol Biomarkers Prev* **6**: 711–718.
69. Boiko IV, Mitchell MF, Hu W, *et al.* (1998) Epidermal growth factor receptor expression in cervical intraepithelial neoplasia and its modulation during an α-difluoromethylornithine chemoprevention trial. *Clin Cancer Res* **4**: 1383–1391.
70. Mitchell MF, Tortolero-Luna G, Lee JJ, *et al.* (1997) Polyamine measurements in the uterine cervix. *J Cell Biochem Suppl* **28–29**: 125–132.
71. Chang YC, Riby J, Chang GH (1999) Cytostatic and antiestrogenic effects of 2-(indol-3-ylmethyl)-3,3′-diindolylmethane, a major *in vivo* product of dietary indole-3-carbinol. *Biochem Pharmacol* **58**: 825–834.
72. Cover CM, Hsieh S, Cram EJ, *et al.* (1999) Indole-3-carbinol and tamoxifen cooperate to arrest the cell cycle of MCF-7 human breast cancer cells. *Cancer Res* **59**: 1244–1251.
73. Katdare M, Osborne MP, Telang NT (1998) Inhibition of aberrant proliferation and induction of apoptosis in pre-neoplastic human mammary epithelial cells by natural phytochemicals. *Oncol Rep* **5**: 311–315.
74. Cover CM, Hsieh SJ, Tran SH, *et al.* (1998) Indole-3-carbinol inhibits the expression of cyclin-dependent kinase-6 and induces a G1 cell cycle arrest of human breast cancer cells independent of estrogen receptor signaling. *J Biol Chem* **273**: 3838–3847.
75. Wong GY, Bradlow L, Sepkovic D, *et al.* (1997) Dose-ranging study of indole-3-carbinol for breast cancer prevention. *J Cell Biochem Suppl* **28–29**: 111–116.
76. Jin L, Oi M, Chen DZ, *et al.* (1999) Indole-3-carbinol prevents cervical cancer in human papilloma virus type 16 (HPV 16) transgenic mice. *Cancer Res* **59**: 3991–3997.
77. Follen M, Schottenfeld D (2001) Surrogate endpoint biomarkers and their modulation in cervical chemoprevention trials. *Cancer* **91**: 1758–1776.
78. Stoler MH, Schiffman M (2001) Atypical squamous cells of undetermined significance—low-grade squamous intraepithelial lesion triage study (ALTS) group. *JAMA* **285**: 2855–2856.
79. Follen M, Meyskens FL Jr, Atkinson EN, Schottenfeld D (2001) Why most randomized phase II cervical cancer chemoprevention trials are uninformative: lessons for the future. *J Natl Cancer Inst* **95**: 1293–1296.