Journal of Cellular Biochemistry, Supplement 23:1-9 (1995)

Strategies for Phase II Cancer Chemoprevention Trials: Cervix, Endometrium, and Ovary

Gary J. Kelloff, MD,[1] Charles W. Boone, MD, PhD,[1] James A. Crowell, PhD,[1] Susan G. Nayfield, MD,[1] Ernest Hawk, MD,[1] Vernon E. Steele, PhD,[1] Ronald A. Lubet, PhD,[1] and Caroline C. Sigman, PhD[2]

[1] Chemoprevention Branch (CB), Division of Cancer Prevention and Control (DCPC), National Cancer Institute (NCI), National Institutes of Health, Bethesda, MD 20892
[2] CCS Associates, Mountain View, CA 94043

Abstract Well-designed and conducted Phase II clinical trials are very important to cancer chemoprevention drug development. Three critical aspects govern the design and conduct of these trials—well-characterized agents, suitable cohorts, and reliable biomarkers for measuring efficacy that can serve as surrogate endpoints for cancer incidence.

Requirements for the agent are experimental or epidemiological data showing chemopreventive efficacy, safety on chronic administration, and a mechanistic rationale for the chemopreventive activity observed. Agents that meet these criteria for chemoprevention of cervical cancer include antiproliferative drugs (*e.g.*, 2-difluoromethylornithine), retinoids, folic acid, antioxidant vitamins and other agents that prevent cellular oxidative damage. Because of the significant cervical cancer risk associated with human papilloma virus (HPV) infection, agents that interfere with the activity of HPV products may also prove to be effective chemopreventives. In endometrium, unopposed estrogen exposure has been associated with cancer incidence. Thus, pure antiestrogens and progestins may be chemopreventive in this tissue. Ovarian cancer risk is correlated to ovulation frequency; therefore, oral contraceptives are potentially chemopreventive in the ovary. Recent clinical observations also suggest that retinoids, particularly all-*trans*-*N*-4-hydroxyphenylretinamide, may be chemopreventive in this tissue.

The cohort should be suitable for measuring the chemopreventive activity of the agent and the intermediate biomarkers chosen. In the cervix, patients with cervical intraepithelial neoplasia (CIN) and in endometrium, patients with atypical hyperplasia, fit these criteria. Defining a cohort for a Phase II trial in the ovary is more difficult. This tissue is less accessible for biopsy; consequently, the presence of precancerous lesions is more difficult to confirm.

The criteria for biomarkers are that they fit expected biological mechanisms (*i.e.*, differential expression in normal and high-risk tissue, on or closely linked to the causal pathway for the cancer, modulated by chemopreventive agents, and short latency compared with cancer), may be assayed reliably and quantitatively, measured easily, and correlate to decreased cancer incidence. They must occur in sufficient incidence to allow their biological and statistical evaluation relevant to cancer.

Since carcinogenesis is a multipath process, single biomarkers are difficult to validate as surrogate endpoints, perhaps appearing on only one or a few of the many possible causal pathways. Panels of biomarkers, particularly those representing the range of carcinogenesis pathways, may prove more useful as surrogate endpoints. It is important to avoid relying solely on biomarkers that do not describe cancer but represent isolated events that may or may not be on the causal pathway or otherwise associated with carcinogenesis. These include markers of normal cellular processes that may be increased or

Send correspondence to Dr. Gary J. Kelloff, Chief, Chemoprevention Branch, DCPC, National Cancer Institute/NIH, 9000 Rockville Pike, EPN Suite 201, Bethesda, MD 20892.

expressed during carcinogenesis. Chemoprevention trials should be designed to evaluate fully the two or three biomarkers that appear to be the best models of the cancer. Additional biomarkers should be considered only if they can be analyzed efficiently and the sample size allows more important biomarkers to be evaluated completely.

Two types of biomarkers that stand out regarding their high correlation to cancer and their ability to be quantified are measures of intraepithelial neoplasia and indicators of cellular proliferation. Measurements made by computer-assisted image analysis that are potentially useful as surrogate endpoint biomarkers include nuclear polymorphism comprising nuclear size, shape (roundness), and texture (DNA distribution patterns); nucleolar size and number of nucleoli/nuclei; DNA ploidy; and proliferation biomarkers such as S-phase fraction and PCNA. CIN and atypical endometrial hyperplasia are both examples of intraepithelial neoplasia that meet the biomarker criteria and are the basis for quantifiable surrogate endpoints for Phase II chemoprevention trials.

Key words: Cervical cancer, cervical intraepithelial neoplasia (CIN), chemoprevention, computer-assisted *image* analysis, endometrial cancer, intermediate biomarkers, ovarian cancer, Phase II trials

Well-designed and conducted Phase II clinical trials that support claims of chemopreventive efficacy are of primary importance to cancer chemoprevention drug development, and, ultimately, marketing approval [1,2]. As described previously [2,3] and outlined in Table I, three critical aspects govern the design and conduct of these trials—well-characterized agents, suitable cohorts, and reliable biomarkers for measuring efficacy that can serve as surrogate endpoints for cancer incidence. The following discussion will consider the significant factors pertaining to the selection of agents, cohorts, and biomarkers for Phase II trials particularly in uterine cervix, endometrium, and ovary.

AGENTS POTENTIALLY EFFECTIVE IN CERVIX, ENDOMETRIUM, AND OVARY

Of the three agent criteria for successful Phase II clinical trials, the first is evidence of chemopreventive efficacy, particularly in preventing cancer at the target site. The second criterion is sufficient prior clinical use or preclinical efficacy, toxicity, and pharmacodynamics data to allow estimation of an efficacy/safety ratio. Often, dose-titration studies to determine the optimal dose and dosing regimen are performed as part of the Phase II trials (for example, see Nishioka *et al.* in these proceedings [4]). The third criterion is that there is a logical, presumed mechanism of chemopreventive activity of the agent. Such mechanisms guide the selection of both cohorts and endpoints for clinical trials. Specifically the agent should be able to modulate the intermediate endpoints chosen as well as the cancer itself. Some agents with chemopreventive potential which meet these criteria in cervix, endometrium, and ovary are listed in Table II. For example, a potent antiproliferative agent like 2-difluoromethylornithine (DFMO) may be effective against the proliferative component of cervical cancers. Quantitative proliferation measures in cervix (*e.g.*, proliferating cell nuclear antigen (PCNA) and S-phase fraction) may prove useful as chemopreventive endpoints for studies with DFMO. Also, DFMO is an irreversible inhibitor of the enzyme ornithine decarboxylase (ODC). It apparently exerts its antiproliferative activity by inhibiting this enzyme and, consequently, the formation of cellular polyamines [5,6]. Nishioka has provided preliminary evidence that precancerous cervical intraepithelial neoplasia (CIN) contains detectable levels of ODC and polyamines [4], which may be a target for DFMO. Thus, ODC activity and polyamine levels may be useful drug effect measurements for evaluating the potential effect of DFMO in cervix. A Phase II chemoprevention study of DFMO in patients with severe CIN (CIN III) is in progress.

Epidemiological [*e.g.*, 7] and limited clinical [8] evidence indicate that vitamin A and its derivatives (retinoids) may also prevent cervical cancer. Retinoids have multiple interrelated mechanisms of action; they modulate signal transduction including hormonal and growth factor activity, promote differentiation, and induce apoptosis [reviewed in 9]. Applied topically in a collagen sponge inserted in a cervical cap, all-*trans*-retinoic acid caused regression of moderate CIN (CIN II) lesions [8]. Recent evidence (Oridate *et al.*, these proceedings, [10]) suggests that apop-

TABLE I. Requirements for Successful Phase II Trials

Agent	
•	Experimental and/or epidemiological data supporting chemopreventive activity (efficacy)
•	Safety on chronic administration at multiple of efficacious dose
•	Mechanistic rationale for chemopreventive activity
Cohort	
•	Suitable for chemopreventive activity of agent
•	Suitable for measurement of biomarkers
•	Risk/benefit analysis acceptable
Biomarkers	
•	Likely to be affected by agent
•	Accessible and measurable by test with adequate performance criteria
•	Modulation supports chemopreventive activity hypothesis

TABLE II. Phase II/III Chemoprevention Trials in Cervix, Endometrium and Ovary: Agents and Cohorts

Target	Agents	Cohorts
Cervix	DFMO Folic Acid β-Carotene 4-HPR Oltipraz all-*trans*-Retinoic Acid Vitamin C	CIN I, II, III Patients
Endometrium	Pure Antiestrogen Megestrol or Other Progestin	Atypical endometrial hyperplasia patients
Ovary	Oral contraceptives Retinoids (4-HPR)	High-risk (not likely feasible for Phase II trial)

tosis contributes to the inhibitory effect of retinoids in cervical neoplasia. All-*trans*-*N*-(4-hydroxyphenyl)retinamide (4-HPR), 13-*cis*-retinoic acid, and all-*trans*-retinoic acid inhibited proliferation in several cervical carcinoma cell lines. 4-HPR, unlike the other retinoids, did not induce growth of the cell lines, but did inhibit apoptosis in these cells as measured by DNA fragmentation. The investigators suggested that similar effects might be seen in CIN; thus, apoptosis might be an intermediate biomarker for chemopreventive effects of retinoids in cervix. A Phase II chemoprevention trial of 4-HPR in patients with CIN III is in progress.

Folic acid deficiency and low cervical folate levels have been associated with cervical dysplasia; likewise, several studies have indicated a protective role for folic acid against cervical neoplasia [reviewed in 11]. Thus far, results of chemoprevention studies have not demonstrated a clear protective effect. For example, a previous Phase II trial showed that supplementation with 10 mg folic acid/day po for three months caused a significant improvement in cervical cytology among oral contraceptive users with CIN [12]. In this study the oral contraceptive users had lower blood folate levels than other women. Reportedly, blood levels in oral contraceptive users

with CIN are lower still [13]. In contrast, two Phase III trials showed no significant effect of folate. In one, 10 mg folic acid/day po for six months failed to improve CIN I or CIN II [14]. In the second, 5 mg folic acid/day po for six months failed to improve atypical cervical cytology (CIN I or koilocytic atypia) as demonstrated by Pap smear or coloposcopy [15], despite increased blood folate levels in the folate-treatment group. It should be noted that unlike the study in oral contraceptive users, these two trials were not limited to women likely to have low or deficiency levels of blood folate. The potential of folic acid as a chemopreventive agent is indicated by its importance in maintaining normal cellular methylation levels and in gene expression [11,16]. Additional studies to evaluate its role in preventing cervical neoplasia are warranted.

In these proceedings, Romney describes the epidemiological association of low plasma levels of antioxidants—β-carotene, vitamin C, vitamin E—with CIN and cervical cancer [17]. Phase II and Phase III chemoprevention trials of β-carotene in CIN II and III and vitamin C alone and combined with β-carotene in CIN II are on-going. Smoking is a well-known risk factor for cervical cancer [18] and is also known to deplete antioxidants that may protect cells from electrophilic carcinogens and other oxidant damage. Higher levels of polycyclic aromatic hydrocarbon-DNA adducts have been found in cervical biopsy specimens from smokers than from nonsmokers [19]. Thus, besides the antioxidant vitamins, agents such as oltipraz that promote detoxification of oxidants may prove beneficial as chemopreventives [9,20].

The highest risk factor for cervical neoplasia, both cancers and precursor lesions, is human papilloma virus (HPV) infection, especially HPVs 16 and 18. Vaccination against the virus is one preventive strategy. In fact, a vaccine against E6 and E7 protein products of the virus has been proposed [21]; E6 and E7 expression is associated with transformation and maintenance of the malignant phenotype in virus-infected cells. Studies on the molecular mechanisms of E6 and E7 have suggested additional strategies for preventing progression of HPV-associated neoplasia. As described by Münger in these proceedings [22], E6 and E7 protein products result in the complexation and degradation of cellular regulatory proteins. For example, E6 proteins trigger the ubiquitin pathway that inactivates the p53 tumor suppressor gene product [23,24]. Agents that prevent these interactions or restore or replace the function of the regulatory proteins may reduce the risk of HPV-associated neoplasia. In this regard, polyclonal antibodies and antisense peptides to components of the ubiquitin transfer pathway recently have been reported to prevent E6-induced deactivation of p53 [25].

In the endometrium and ovary, fewer potential chemopreventive strategies and agents have emerged. However, one clear possibility in the endometrium is inhibition of estrogenic activity. Endometrioid carcinomas, which account for the majority of endometrial cancers, are strongly associated with unopposed estrogen stimulation (reviewed by Schottenfeld in these proceedings [26]). Also, tamoxifen, which is antiestrogenic in breast, has estrogen-agonist activity in endometrium. Tamoxifen treatment in breast cancer patients has been linked to increased risk of endometrial cancer as has unopposed estrogen replacement therapy in postmenopausal women [reviewed in 27]. This evidence suggests the possibility of pure antiestrogens (*i.e.*, those without estrogen-agonist effects) and progestins as chemopreventive agents in this tissue.

In ovary, there is compelling epidemiological evidence of a direct correlation of cancer risk to ovulation frequency (reviewed by Tortolero-Luna in these proceedings [28]). Thus, oral contraceptive use is linked to reduced risk; hence, oral contraceptives are a potential chemopreventive agent in ovary. Interestingly, a Phase III trial of 4-HPR in preventing second breast cancers found reduced ovarian cancer incidence in patients treated with 4-HPR compared with placebo controls [29]. This observation suggests retinoids as possible chemopreventives in ovary.

SUITABLE COHORTS IN CERVIX, ENDOMETRIUM AND OVARY

The first criterion for a cohort is that it be matched to the chemopreventive agent being evaluated. That is, a chemopreventive agent is likely to be most effective in subjects whose disease or risk of disease can be modulated by the presumed mechanism of the agent within the relatively short duration of the trials (one month to three years). In cervix, patients with CIN [*e.g.*,

8,30] and in endometrium, patients with atypical hyperplasia [31,32] fit this criterion. Cohorts at high risk for cancer are not good candidates for Phase II chemoprevention trials. Examples are subjects at risk because of germline mutations who do not also have detectable premalignant lesions, such as BRCA1 carriers who are at high risk for ovarian cancer [33,34]. Practically, the chemopreventive effect should also be easily measurable in the subject population. More accessible tissues that can be monitored relatively non-invasively provide better sites for definitive efficacy trials than less accessible tissues. Cervical tissue is readily available with relatively non-invasive procedures such as the Pap smear and coloposcopy for routine evaluation; biopsies are also easily done in this tissue. Endometrium is also relatively attainable. On the other hand, ovarian tissue is not accessible without invasive surgery. This is not to say that chemopreventive agents will not be effective in such difficult setting, but that initial demonstration of chemopreventive activity may be best carried out where fewer obstacles to measurement exist.

POTENTIAL SURROGATE ENDPOINT BIOMARKERS IN CERVIX, ENDOMETRIUM AND OVARY

The selection and evaluation criteria for those biomarkers that can serve as surrogate endpoints for cancer in Phase II chemoprevention trials

TABLE III. Potential Surrogate Endpoint Biomarkers for Chemoprevention Trials in Cervix, Endometrium, and Ovary

	Cervix	Endometrium	Ovary
First Priority: On causal pathway; modulatable, measurable, and quantifiable	Histological: Cervical intraepithelial neoplasia (CIN)	Atypical hyperplasia	Surface epithelial dysplasia
	Possible CIA Measurements: Nuclear morphometry (area, shape, texture), nucleolar morphometry (size, shape, number/nucleus), nuclear texture, DNA ploidy		
	Proliferation: S-phase fraction, Ki-67 antigen expression (using MIB-1 antibody), nuclear organizer regions (AgNORs)	S-phase fraction, Ki-67 antigen expression, PCNA, AgNORs	AgNORs
	Possible CIA Measurements: Cytometry with DNA or immunohistochemical labelling		
Second Priority: May or may not be directly on causal pathway, modulatable, or quantifible	Differentiation: Involucrin	Altered Lewis[b] blood group antigen (increased MSN-1 antibody)	
	Genetic/Regulatory: Epidermal growth factor receptor expression (EGFR), altered oncogene expression (*e.g.*, *ras* expression and interaction with HPV), altered tumor suppressors (*e.g.*, p53), loss of heterozygosity (*e.g.*, chromosomes 3p14, 3p25)	EGFR expression, fibroblast growth factor expression, transforming growth factor (TGF)-α expression, TGF-β expression, plasminogen activator expression, altered oncogene expression (*e.g.*, K-*ras*, c-*myc*)	Altered tumor suppressors (*e.g.*, p53), altered oncogene expression (*e.g.*, *ras*)
	Biochemical: ODC activity and polyamine levels		

have been described in detail previously [2], and so are summarized only briefly in the following, particularly as they pertain to cervix, endometrium, and ovary. It was noted that biomarkers causally related to subsequent cancer are generally more easily validated as surrogate endpoints than biomarkers for which the relationship to cancer is indirect. Carcinogenesis is considered to be progressive. Thus, biomarkers appearing close in time to the cancer and those exhibiting increasing/decreasing incidence or potency during carcinogenesis are most likely to be reliable surrogate endpoints. As we discussed [2,35], intraepithelial neoplasia (IEN) is an intermediate biomarker that meets these requirements, and is the standard against which other intermediate biomarkers are evaluated as potential surrogate endpoints. CIN is a well-known example of IEN; atypical hyperplasia in endometrium may be another (reviewed by Sherman in these proceedings [36]).

Also, because of the multiple possible causal pathways to cancer, the validation of single biomarkers as surrogate endpoints is complicated. Consequently, panels of biomarkers, particularly those representing the range of carcinogenesis pathways, may prove more useful as surrogate endpoints.

As we emphasized, to meet the criterion of high correlation to cancer when designing Phase II clinical trials with biomarkers, "molecular reductionism"—reliance solely on biomarkers that do not describe cancer but represent isolated events that may or may not be on the causal pathway or otherwise associated with carcinogenesis—should be avoided. Examples are biomarkers of normal cellular processes that may be increased or expressed during carcinogenesis. Unfortunately, biomarkers of some of the most potentially interesting control mechanisms in carcinogenesis fall into this category—*e.g.*, expression of receptors and growth factors, activities of carcinogen detoxifying enzymes such as glutathione *S*-transferase, and indicators of cellular proliferation involved in signal transduction such as ODC. These biomarkers have the disadvantage of nonspecificity for cancer and are not likely to be easily validated as surrogate endpoints. Freedman and his associates [37,38] described mathematically the concept of attributable proportion—that is, the higher the percentage of cancers that can be attributed to a biomarker or panel of biomarkers, the better this biomarker or panel of biomarkers serves as a surrogate endpoint.

Two other points should be made. First, it is critical to have evidence that a chemopreventive agent can modulate the biomarker(s) chosen as the surrogate endpoint. As noted above, Meyskens and colleagues [8] have shown that CIN is modulatable. Hyperproliferation is another example of a biomarker that can be inhibited by chemopreventive agents, as demonstrated by calcium in colon [39,40]. Mitchell and colleagues have proposed evaluation of several biomarkers (*e.g.*, PCNA, S-phase fraction, Ki-67) related to proliferation in chemoprevention studies with CIN [30].

The second point is that surrogate endpoints should have short latency compared with cancer incidence—ideally, months or a few years compared with the many years and decades required for cancers to develop. For example, Meyskens *et al.* were able to show improvement in CIN within six months [8].

Mitchell *et al.* have previously reviewed potential biomarkers in cervix [30] and Brenner *et al.* provides a review in these proceedings [41]; Baker [42,43] and Berchuk [44,45] have provided similar comprehensive reviews of biomarkers in endometrium and ovary in these proceedings. Table III lists biomarkers of interest in these targets.

STRATEGIES FOR PHASE II CHEMOPREVENTION TRIALS WITH BIOMARKERS IN CERVIX, ENDOMETRIUM AND OVARY

A few more highly important factors contribute to successful Phase II chemoprevention trials with biomarker endpoints. First, the biomarkers must occur in sufficient incidence to allow their biological and statistical evaluation relevant to cancer. Since not all alterations of a specific biomarker will develop into cancer, its incidence is necessarily higher than that of the target cancer. Second, the ultimate goal is to conduct Phase II trials with surrogate endpoints that will allow a claim for chemopreventive efficacy to be made [1]. A claim can be made most persuasively by modulating biomarkers that are on the causal pathway and, with rare exceptions, are the histological lesions directly preceding cancers. Fur-

ther, without a precursor lesion, the evaluation and validation of earlier biomarkers within a time frame less than that for induction of the cancer is difficult. In cervix, CIN is an appropriate precursor lesion, and Phase II trials using CIN as an endpoint are already in progress. Atypical endometrial hyperplasia holds similar promise as a surrogate endpoint. Currently, the situation in ovary is less favorable. While ovarian surface epithelial dysplasia and inclusion cysts may be potential cancer precursors (reviewed in these proceedings by Baker [43] and Sculley [46]), it may not be feasible to identify a cohort with these lesions, since the ovary is relatively inaccessible to biopsy.

The greatest effort should be devoted to the most valuable biomarkers. We feel that trials should be designed to evaluate fully the two or three biomarkers that appear to be the best models of the cancer. It is likely that additional biomarkers will have diminishing value in establishing efficacy, and should be considered only if they can be analyzed efficiently and the sample size allows the more important biomarkers to be evaluated completely. Most importantly, biomarkers chosen should be quantifiable and assayed according to well-defined and validated methodologies. We have said before [1–3] and have shown in this discussion that two types of biomarkers stand out in regard to their close correlation to cancer and carcinogenesis and their ability to be quantified. These are morphologic and cytological measures of IEN and indicators of cellular proliferation. In these proceedings, several speakers address the quantification of these measures by computer-assisted image analysis (CIA). Baak provides an overview of quantitative morphometric measurements and how they may be applied in all three tissues, particularly endometrium [47]. Mitchell *et al.* discuss an approach for quantitative evaluation of histological and proliferation biomarkers in cervix [48]. Palcic *et al.* describe the evaluation of cervical smears using quantitative image cytometry [49]. Tezuka *et al.* [31,32] and Takahashi [50] describe quantitative morphometric measurements of atypical endometrial hyperplasia. The changes analyzed in both cervix and endometrium include nuclear polymorphism comprising nuclear size, shape (roundness), and texture (DNA distribution patterns). Further, nucleolar size and number of nucleoli/nucleolus are cited as potential surrogate endpoint biomarkers in endometrium [50]. Besides CIA, Richards-Kortum *et al.* have described a fluorescence spectroscopy methodology for quantitative evaluation of CIN [51]. Table III shows a strategy for prioritizing biomarkers in evaluating chemopreventive efficacy in Phase II clinical trials.

As this discussion and other articles in these proceedings indicate, there are promising chemopreventive agents for cervical, endometrial and ovarian cancer. Most importantly, quantifiable biomarkers have been identified, particularly in cervix and endometrium, that can be used to evaluate these agents in Phase II clinical trials.

REFERENCES

1. Kelloff GJ, Johnson JJ, Crowell JA, Boone CW, DeGeorge JJ, Steele VE, Mehta MU, Temeck JW, Schmidt WJ, Burke G, Greenwald P, Temple RJ: Approaches to the development and marketing approval of drugs that prevent cancer. Cancer Epidemiol Biomarkers Prev 4: 1–10, 1995.
2. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Doody LA: Surrogate endpoint biomarkers for Phase II cancer chemoprevention trials. J Cell Biochem Suppl 19:1–9, 1994.
3. Kelloff GJ, Boone CW, Steele VE, Crowell JA, Lubet R, Sigman CC: Progress in cancer chemoprevention: Perspectives on agent selection and short-term clinical intervention trials. Cancer Res 54:2015s–2024s, 1994.
4. Nishioka K, Melgarejo A, Lyon R, Mitchell MF: Polyamines as biomarkers of cervical intraepithelial neoplasia. J Cell Biochem 23 (Suppl), 1995 (this issue).
5. Pegg AE: Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res 48:759–774, 1988.
6. Nishioka K: International Symposium on Polyamines in Cancer. Critical role of polyamines in cancer: Basic mechanisms and clinical approaches. Cancer Res 53: 2689–2692, 1993.
7. Schneider A, Shah K: The role of vitamins in the etiology of cervical neoplasia: An epidemiological review. Arch Gynecol Obstet 246:1–13, 1989.
8. Meyskens FL Jr, Surwit E, Moon TE, Childers JM, Davis JR, Dorr RT, Johnson CS, Alberts DS: 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, 1994.
9. Kelloff GJ, Boone CW, Steele VE, Fay JR, Lubet RA, Crowell JA, Sigman CC: Mechanistic considerations in chemopreventive drug development. J Cell Biochem 20 (Suppl):1–24, 1994.
10. Oridate N, Lotan D, Hong W-K, Mitchell MF, Lotan R: Inhibition of proliferation and induction of apop-

tosis in cervical carcinoma cells by retinoids: Implications for chemoprevention trials. J Cell Biochem 23 (Suppl), 1995 (this issue).
11. Glynn SA, Albanes D: Folate and cancer: A review of the literature. Nutr Cancer 22:101–119, 1994.
12. Butterworth CE Jr, Hatch KD, Gore H, Mueller H, Krumdrick C: Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. Am J Clin Nutr 35:73–82, 1982.
13. Whitehead N, Reyner F, Lindenbaum J: Megaloblastic changes in the cervical epithelium. Association with oral contraceptive therapy and reversal with folic acid. JAMA 226:1421–1424, 1973.
14. Butterworth CE Jr, Hatch KD, Soong SJ, Cole P, Tamura T, Sauberlich HE, Borst M, Macaluso M, Baker V: Oral folic acid supplementation for cervical dysplasia: A clinical intervention trial. Am J Obstet Gynecol 166:3803–3809, 1992.
15. Childers JM, Chu J, Voigt LF, Feigl P, Tamimi HK, Franklin EW, Alberts DS, Meyskens FL Jr: Chemoprevention of cervical cancer with folic acid: A phase III Southwest Oncology Group Intergroup Study. Cancer Epidemiol Biomarkers Prev 4:155–159, 1995.
16. Huennekens FM, Duffy TH, Vitols KS: Folic acid metabolism and its disruption by pharmacologic agents. Natl Cancer Inst Monogr 5:1–8, 1987.
17. Romney SL: Prevention and pathogenesis of cervical dysplasia/cancer: Role of antioxidants. J Cell Biochem 23 (Suppl), 1995 (this issue).
18. Brinton L, Fraumeni JF: Epidemiology of uterine cervical cancer. J Chronic Dis 39:1051–1065, 1986.
19. Simmons AM, Phillips DH, Coleman DV: Damage to DNA in cervical epithelium related to smoking tobacco. Br J Med 306:1444–1448, 1993.
20. Kensler TW, Trush MA, Guyton KZ: Free radicals as targets for cancer chemoprevention: Prospects and problems. In: Steele VE, Stoner GD, Boone CW, Kelloff GJ (eds): "Cellular and Molecular Targets for Chemoprevention." Boca Raton: CRC Press, 1992, pp 173–191.
21. Lowy DR, Kirnbauer R, Schiller JT: Genital human papillomavirus infection. Proc Natl Acad Sci USA 91:2436–2440, 1994.
22. Münger K: Molecular biology of cervical cancer. J Cell Biochem 23 (Suppl), 1995 (this issue).
23. Liang XH, Volmann M, Klein R, Herman B, Lockett SJ: Co-localization of the tumor-suppressor protein p53 and human papillomavirus E6 protein in human cervical carcinoma cell lines. Oncogene 8:2645–2652, 1993.
24. Herrington CS: Human papillomaviruses and cervical neoplasia. II. Interaction of HPV with other factors. J Clin Pathol 48:1–6, 1995.
25. Dimond PF: Tumor suppressor breakdown inhibited in cell cultures. Gen Eng News 15:1,36, 1995.
26. Schottenfeld D: Epidemiology of endometrial neoplasia. J Cell Biochem 23 (Suppl), 1995 (this issue).
27. Kelloff GJ, Crowell JA, Boone CW, Steele VE, Lubet RA, Greenwald P, Alberts DS, Covey JM, Doody LA, Knapp GG, Nayfield S, Parkinson DR, Prasad VK, Prorok PC, Sausville EA, Sigman CC: Strategy and planning for chemopreventive drug development: Clinical development plans I—tamoxifen. J Cell Biochem 20 (Suppl):252–267, 1994.
28. Tortolero-Luna G: Epidemiology of ovarian cancer. J Cell Biochem 23 (Suppl), 1995 (this issue).
29. De Palo G, Veronesi U, Camerini T, Formelli F, Mascotti G, Boni C, Fosser V, Del Vecchio M, Campa T, Costa A, Marubini E: Can fenretinide protect women against ovarian cancer? J Natl Cancer Inst 87:146–147, 1995.
30. Mitchell MF, Hittelman WN, Hong WK, Lotan R, Schottenfeld D: The natural history of cervical intraepithelial neoplasia: An argument for intermediate endpoint biomarkers. Cancer Epidemiol Biomarkers Prev 3:619–626, 1994.
31. Tezuka F, Chiba R, Takahashi T: Morphometric and multivariate statistical detection of cancer cells in endometrial cytology. Anal Quant Cytol Histol 165: 332–338, 1994.
32. Tezuka F, Chiba R, Takahashi T: Quantitative pathology of endometrial lesions. J Cell Biochem 23 (Suppl), 1995 (this issue).
33. Goldgar DE, Fields P, Lewis CM, Tran TD, Cannon-Albright LA, Ward JH, Swensen J, Skolnick MH: A large kindred with 17q-linked breast and ovarian cancer: Genetic, phenotypic, and genealogical analysis. J Natl Cancer Inst 86:200–209, 1994.
34. Easton DF, Ford D, Bishop DT: Breast and ovarian cancer incidence in BRCA1-mutation carriers. Am J Hum Genet 56:265–271, 1995.
35. Boone CW, Kelloff GJ, Steele VE: Natural history of intraepithelial neoplasia in humans with implications for cancer chemoprevention strategy. Cancer Res 52: 1651–1659, 1992.
36. Sherman ME: Pathology of endometrial cancer precursors. J Cell Biochem 23 (Suppl), 1995 (this issue).
37. Schatzkin A, Freedman LS, Schiffman MH, Dawsey SM: Validation of intermediate endpoints in cancer research. J Natl Cancer Inst 82:460–468, 1990.
38. Freedman LS, Schatzkin A, Schiffman MH: Statistical validation of intermediate markers of precancer for use as endpoints in chemoprevention trials. J Cell Biochem 16G (Suppl):27–32, 1992.
39. Lipkin M, Newmark H: Effect of dietary calcium on colonic epithelial-cell proliferation in subjects at high risk for familial colonic cancer. N Engl J Med 313: 381–1384, 1985.
40. Buset M, Lipkin M, Winawer S, Swaroop S, Friedman E: Inhibition of human colonic epithelial cell proliferation *in vivo* and *in vitro* by calcium. Cancer Res 46:5426–5430, 1986.
41. Brenner DE, Ogaily M, Johnston CM, Lancaster WD, Gregoire L, Ruffin MT: Surrogate biomarker endpoints for cervical cancer chemoprevention trials. J Cell Biochem 23 (Suppl), 1995 (this issue).
42. Baker VV: Potential criteria for cohort selection in chemoprevention trials of uterine adenocarcinoma. J Cell Biochem 23 (Suppl), 1995 (this issue).
43. Baker VV: Potential criteria for cohort selection in

chemoprevention trials of epithelial ovarian cancer. J Cell Biochem 23 (Suppl), 1995 (this issue).
44. Berchuk A: Biomarkers in the endometrium. J Cell Biochem 23 (Suppl), 1995 (this issue).
45. Berchuk A: Biomarkers in the ovary. J Cell Biochem 23 (Suppl), 1995 (this issue).
46. Sculley RE: Pathology of ovarian cancer precursors. J Cell Biochem 23 (Suppl), 1995 (this issue).
47. Baak JPA: The role of computerized morphometric and cytometric feature analysis in endometrial cancer prognosis. J Cell Biochem 23 (Suppl), 1995 (this issue).
48. Mitchell MF, Hittelman WK, Lotan R, Nishioka K, Tortolero-Luna G, Hong WK, Richards-Kortum R: Chemoprevention trials in the cervix: Design, feasibility, and recruitment. J Cell Biochem 23 (Suppl), 1995 (this issue).
49. Palcic B, Garner DM, MacAulay CE: Image cytometry and chemoprevention in cervical cancer. J Cell Biochem 23 (Suppl), 1995 (this issue).
50. Takahashi T: Computer-assisted pathology of intraepithelial neoplasms. J Cell Biochem 23 (Suppl), 1995 (this issue).
51. Richards-Kortum R, Ramanajam N, Yazdi Y, Mahadevan A, Malpica A, Thomsen S, Mitchell MF: Fluorescence spectroscopy for screening and diagnosis of cervical intraepithelial neoplasia. J Cell Biochem 23 (Suppl), 1995 (this issue).