Biomarkers and their use in cervical cancer chemoprevention

Anne-Thérèse Vlastos a, David Schottenfeld b, Michele Follen c,d,*

a Département de Gynécologie et Obstétrique, Hôpitaux Universitaires de Genève, Geneva, Switzerland
b Department of Epidemiology and the Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA
c Department of Gynecologic Oncology and the Biomedical Engineering Center—Box 193, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA
d Department of Obstetrics, Gynecology and Reproductive Sciences, The University of Texas Health Science Center at Houston, Houston, TX, USA

Accepted 19 August 2002

Abstract

Cervical cancer chemoprevention agents under study include diet and micronutrients (particularly β-carotene, folate, and vitamins A, C, and E); medications such as retinoids (retinyl acetate gel, all-trans-retinoic acid, and 4-hydroxyphenylretinamide) that are chemically related to micronutrients; and other chemopreventives meant to affect the carcinogenic process at the cellular level, including such polyamine synthesis inhibitors as α-difluoromethylornithine. Agents become reasonable candidates for study when they have a biologic rationale, they are of low toxicity, and they can be taken for a long period of time. Since the human papillomavirus (HPV) is the major etiologic agent, the medication should show activity against HPV-positive preinvasive and invasive cell lines. The medication needs to be of low toxicity because it may be taken for long periods of time and less toxicity is tolerated in the precancerous setting. Until 1995, none of the studies used surrogate end point biomarkers (SEBs), relying instead on histologic and colposcopic regression as end points. All studies typically included subjects with cervical intraepithelial neoplasia. Conclusions to be drawn from these studies include the following: Though micronutrients are logical candidates for chemoprevention, they haven’t worked consistently, and the reasons remain unclear. Furthermore, SEBs need to be validated in

Contents

1. Chemoprevention trial design ..................................... 262
2. Cohort selection .................................................. 262
3. Selection of agents ................................................ 264
4. Nutritional studies .................................................. 265
5. Chemoprevention trials ............................................. 267
   5.1. Retinoids ...................................................... 267
   5.2. Micronutrients ................................................ 268
      5.2.1. β-carotene and vitamin C ............................. 268
      5.2.2. Folate .................................................. 269
   5.3. Polyamine synthesis inhibitors .............................. 269
   5.4. Nonsteroidal anti-inflammatory drugs ...................... 270
6. Conclusions ....................................................... 270
References .......................................................... 270
Biographies .......................................................... 273

* Corresponding author. Tel.: +1-713-745-2564; fax: +1-713-792-4856.
E-mail address: mfollen@mdanderson.org (M. Follen).

1040-8428/03/$ – see front matter © 2002 Elsevier Science Ireland Ltd. All rights reserved.
doi:10.1016/S1040-8428(02)00107-5
Chemoprevention agents, which block the initiating and promoting events of carcinogenesis, are intended to augment the preventive strategy that includes the avoidance of carcinogens in the environment (referred to as primary prevention) and participation in screening programs (referred to as secondary prevention). Hence, chemoprevention serves as a tertiary preventive measure [1]. Chemoprevention trials have several unique features that distinguish them from therapeutic trials. Among these are ones that call for involving several disciplines in creating trials that weave consideration of the biology of carcinogenesis into the study design [2]. A thorough understanding of epidemiology, study design, statistical analysis, clinical medicine, and molecular biology is necessary to optimize what can be learned from these studies [3–9].

1. Chemoprevention trial design

Chemoprevention studies involve four elements: high-risk cohorts; suitable medications; study designs that include phases I, II, and III; and surrogate end point biomarkers (SEBs). Theoretically, three groups of high-risk patients are eligible for such trials: those who are at high risk for cancer but without a precancerous lesion (eligible for phase III study enrollment), those with a precancerous lesion (phases I, II, III), and those with a previous malignancy who are at high risk for a second primary or for recurrence (phases I, II, III). Risk profiles may be based on genetic factors, lifestyle and environmental exposures, a history of a precursor lesion, or some combination of these [4,9]. For chemoprevention studies related to the cervix uteri, the most suitable groups would be patients with a preinvasive lesion, patients without a lesion but who are positive for the human papillomavirus (HPV) or both HPV and the human immunodeficiency virus, and patients at risk for a recurrence of invasive cervical cancer.

Medications are potentially suitable if their use has a reasonable biologic rationale, they are of low toxicity, and they can be taken for a long period of time. For the cervix, micronutrients, retinoid compounds, α-difluoromethylornithine (DFMO), and nonsteroidal anti-inflammatory drugs are all reasonable candidates.

Phase I chemotherapy trials are designed to evaluate toxicity of a drug at escalating doses. In contrast, phase I chemoprevention trials are often dose de-escalating, seeking the lowest dose at which biologic modulation of the marker takes place and the least toxicity results, though they can also seek appropriate dose levels through dose escalation. The importance of establishing the effective dose for each organ cannot be overemphasized. Tissue levels of the drug should be studied as part of the phase I trial design. In addition to establishing reasonable doses, the phase I trial might also identify which SEBs are modulated by the drug of interest. Preceding a phase I study sometimes is a pilot, or exploratory, study.

Like phase II chemotherapy trials, phase II chemoprevention trials evaluate the effectiveness of a drug in a given organ. In contrast to phase II chemotherapy trials, phase II chemoprevention trials are randomized, require a concurrent blinded control placebo group (because of the spontaneous regression sometimes observed for preneoplastic lesions), and typically evaluate multiple dose levels. Both phase IIa and IIb studies may be short-term (≤1 year) or long-term (1–5 years), but only IIb studies incorporate the use of SEBs. Some investigators do not require phase IIa studies to be randomized, though more can be learned from a placebo-controlled trial. See Table I for a profile of each clinical trial type [10].

Both chemotherapy phase III trials and chemoprevention phase III trials evaluate the cost-benefit ratio of treatments in multicenter settings. In contrast to phase III chemotherapy studies, which compare agents to standard therapies in groups with cancer, chemoprevention studies evaluate cancer incidence reduction in groups at high risk for the development of cancer. The use of SEBs instead of the end point of cancer incidence reduction allows trials to be of shorter duration, to require fewer subjects, to be lower in cost, to use small tissue samples, and to aid in learning more about the carcinogenic process [2,9].

2. Cohort selection

For high-grade dysplastic cervical cancer chemoprevention trials, patients at high risk for cancer include two groups: patients with high-grade lesions and women infected with high-risk oncogenic HPV types. We may later identify patients at high risk genetically, but, at the present, no such designation exists. The rationale for choosing patients with high-grade lesions is that such
<table>
<thead>
<tr>
<th>Design and objective</th>
<th>Type of trial</th>
<th>Pilot*</th>
<th>Phase I</th>
<th>Phase I–II*</th>
<th>Phase IIa</th>
<th>Phase IIb</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition</td>
<td>Exploratory study</td>
<td>Dose escalation study or dose-deescalation study involving a single arm, used to evaluate toxicity and tolerance</td>
<td>Single-arm study used to explore response in the disease of interest, not dose finding</td>
<td>Randomized, double-blinded, placebo-controlled trial, used to evaluate response, may evaluate multiple dose levels</td>
<td>Randomized, double-blinded, placebo-controlled trial used to evaluate biomarkers and response, may evaluate multiple dose levels</td>
<td>Randomized, double-blinded, placebo-controlled trial used to evaluate response in a multicenter setting</td>
<td></td>
</tr>
<tr>
<td>Objective</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td>Maybe</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Recruitment evaluation</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pharmacokinetics evaluation</td>
<td>Maybe</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Efficacy evaluation</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Duration (year)</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>1–5</td>
<td>1–5</td>
<td>1–5</td>
<td>At least 1–5</td>
<td></td>
</tr>
<tr>
<td>Target population</td>
<td>Appropriate target population</td>
<td>Appropriate target population</td>
<td>Appropriate target population</td>
<td>Appropriate target population</td>
<td>Appropriate target population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accrual goal</td>
<td>20</td>
<td>25–100</td>
<td>25–100</td>
<td>100–1000</td>
<td>100–1000</td>
<td>&gt; 1000</td>
<td></td>
</tr>
</tbody>
</table>

Source: adapted from Goodman [10].

* Not defined in Goodman [10] but used by many investigators.
lesions are more likely to progress to invasive cancer: up to 36% of carcinoma in situ (CIS) lesions progressed in a series of studies in which 353 patients were monitored without treatment over periods of 3 months to 30 years [8]. Patients with high-grade cervical lesions are suitable for phase I, II, and III trials of chemopreventive micronutrients and medications. The rationale for selecting patients with oncogenic HPV types comes from the cross-sectional and cohort studies demonstrating greatly increased risk of cervical intraepithelial neoplasia (CIN) and of cervical cancer in women who are HPV positive compared with those who are HPV negative [11]. Patients with oncogenic HPV types are suitable for trials of chemopreventives, antiviral medications, or an HPV vaccine [12–16]. Pathobiologic studies of cervical carcinogenesis will surely contribute to our understanding of the neoplastic process and hence speed the development of new preventive and therapeutic strategies [4].

Chemoprevention may be a treatment of choice in the woman who smokes: has HPV; has multifocal intraepithelial neoplasia of the cervix, vagina, and vulva; or is immunosuppressed. Many patients with cervical neoplasia smoke, up to 60% of patients in our population, and thus may have preneoplastic lesions in the lung [9]. The entry into cervical chemoprevention trials provides an ideal time for a smoking cessation intervention. Since there are known risks to continuing to smoke, whether on a chemoprevention trial or not, every effort should be made to end the smoking. Many of these patients have preneoplastic and neoplastic lesions of the aerodigestive tract. Infection with HPV affects the entire squamous epithelium of the female genital tract, and up to 40% of patients with CIN have lesions of the vagina, vulva, and perianal area [9].

3. Selection of agents

In the last few decades, interest in the relationship between diet and cancer in humans has been very strong [17–19]. Investigators were initially intrigued by international studies in which large differences between countries in cancer incidence rates were found and by the fact that nutritional correlates exist for many cancers [20]. Nutrients are assumed to affect the carcinogenic process at the cellular level; thus, supplementation of nutrients could be expected to prevent or reverse the process of carcinogenesis in the earliest phases. Nutritional studies looking for deficiencies in patients with preinvasive lesions or cancer compared with controls have identified micronutrients of interest for use in chemopreventive studies. Some of the other medications being considered as chemopreventives include synthetic retinoids and alternate forms of vitamin A. Other potential chemopreventives, while not related to micro-nutrients, are believed to interrupt the carcinogenic process in the earliest phases. Few studies have yet focused on the cellular effects of nutritional supplementation on the process of carcinogenesis at the tissue level. Nutritional studies were part of a review by Mitchell et al. that focused on study design, case definition, control selection, and nutritional measurement [21].

Nutritional study methodology and nutritional measures have been the subjects of many excellent reviews [22–26]. It is important to understand both nutritional study methodology and nutritional measures in order to understand the inconsistencies among study results. The most commonly used nutritional measures are the diet record, the 24-h recall, the food frequency questionnaire, and serum levels of nutrients. For a diet record, participants are asked to weigh and record the amounts of food eaten, often for 3- to 7-day periods. The 24-h recall requires participants to recall in an interview what and how much they have eaten in the previous 24 h. Both these measures are often recorded several times throughout the year to include foods eaten in all seasons. The food frequency questionnaire is either an interview or a self-administered tool designed to record a person’s ‘usual’ diet. The items of interest for the study are listed and the participants are asked to record how frequently they consume these foods. On some surveys, the portion size is suggested, and, on others, an average portion size is used for the calculation of nutrients. In general, the diet record is considered the best method. Compared with it, the 24-h recall tends to include underestimations, while the food frequency questionnaire and diet history tend to include overestimations. The food frequency questionnaire is the most suitable instrument for large-scale epidemiologic studies because it can be self-administered and is designed to reflect average intake.

The diet record, the 24-h recall, and the food frequency questionnaire are approximations of the diet over a period of time and thus represent the usual exposure over a longer duration of time. Serum nutrient levels provide an objective, repeatable, biologic measure of a nutrient, but, depending on the nutrient of interest, may reflect an exposure of shorter duration. Values for nutrients in serum studies are often made in quartiles; the highest quartile will be compared to the other three quartiles to generate a risk ratio. Surprisingly often, the diet record, 24-h recall, and food frequency questionnaires correlate poorly with serum nutrient levels. Cohen’s Kappa, a measurement of intrarater agreement, estimates the agreement between serum nutrient levels and each of the three questionnaire methods in the range of 0.2–0.4, which is in the low to moderate range [22–27].

The strongest study designs are those employing food frequency questionnaire data as well as serum and tissue level measures. Study designs including evaluations of
nutrient levels in tissues only became widely available in the 1980s [22–26]. Tissue levels provide another objective, repeatable, biologic measure of a nutrient. Depending on the nutrient, tissue levels may reflect exposure of longer duration.

It has been and will continue to be important to measure nutritional intake in patients enrolled in chemoprevention trials of nutritional supplements. For example, in trials using nutritional supplements, a lack of significant regression of lesions could be due to the fact that patients likely to participate in chemoprevention trials are not as nutritionally depleted as those who participate in nutrition studies, which typically require fewer visits than do chemoprevention studies. Patients sufficiently motivated to participate in chemoprevention trials may be more aware of their diet.

Four studies have appeared in the literature since the aforementioned review [21]. Two are case-control studies of squamous intraepithelial lesions, by Kantesky et al. [28] and Wideroff et al. [29]. In both of these studies, unlike the previously cited studies, HPV measurement was undertaken and controlled for in the analysis. Two studies, those of Peng et al. and Gamboa-Pinto et al., correlated serum and tissue concentrations of micronutrients [30,31]. Both studies showed good correlation between serum and tissue measurements of the carotenoids, suggesting that serum measurements may be a surrogate for tissue measurements. These findings need to be confirmed by other investigators. Tissue nutrient levels serve as a measure that the micronutrient delivered is reaching the tissue and assures us that the level given orally is present at the desired level in tissue. When these findings are clarified, researchers can determine whether having both serum and tissue level measures are imperative for durable study design.

4. Nutritional studies

Many investigators have studied the relationship between nutrition and CIN and cervical cancer. Most studies have focused on vitamin A and its derivatives, vitamin C, vitamin E, and folate. Many chemoprevention trials have been designed to supplement deficiencies of these nutrients. The relevant nutritional studies, described below, provide a rationale for using each nutrient in a chemoprevention trial. The listing of researchers by name or reference number who have led investigations in nutritional chemoprevention is meant to provide a path for further study and an index to the scope of findings. A discussion of the clinical trials by nutrient follows. Of the studies mentioned, only those of Peng et al. [30] included measures of serum and tissue. These were of retinol, β-carotene, and lycopene.

Vitamin A deficiency has been demonstrated to increase risk of squamous epithelial cancer in many organ sites. Vitamin A has been the subject of several case-control studies of CIN and cervical cancer. The vitamin A family includes both retinoids and carotenoids. Natural vitamin A and its esters and synthetic analogs have the potential to inhibit or reverse carcinogenesis. Retinoid compounds control normal cell growth, differentiation, and apoptosis during embryonic development and within epithelial tissues in later life. Carotenoids have been found to interfere with the process of carcinogenesis through antioxidative activity. The mechanism by which carotenoids participate in antioxidation is thought to be oxygen quenching or free-radical scavenging. Serum retinol levels have been found to be statistically significantly lower in CIN cases than in controls by three groups of investigators [32–34], while no significant differences were noted by others [28–30,35–46]. (See section on retinoic acid below.)

β-carotene is the most active and common carotenoid found in the diet and is a remarkably potent source of vitamin A. It is metabolized to retinaldehyde and then converted to retinol. It is thought to be a promising agent based on data from nutritional studies demonstrating β-carotene deficiencies in CIN patients compared with controls. Of all the nutrients studied, the most consistent relationship across the study designs was that between β-carotene deficiency and CIN and cervical cancer, noted by Peng et al. [30] and others [37–39,41–46]. Neither Potischman et al. [46] nor Wideroff [29] found this relationship.

Lycopene is thought to be antioxidative, in particular, to be more efficient in singlet oxygen quenching. Lycopene is not converted to retinol. Lycopene level has been demonstrated to be lower in CIN cases than it is in controls [28,33,41,43], but no significant differences have been noted by others [46].

Vitamin C level has been demonstrated to be lower in cases with CIN than in controls by Verreault et al. [47], Romney et al. [48], Wasserman-Smoller et al. [49], Herrero et al. [45], and Brock et al. [39], but no significant differences were noted by Ziegler et al. [35,36], Basu et al. [37], or Wideroff et al. [29]. Perhaps because of the lack of a good biologic rationale for vitamin C affecting epithelial cell differentiation, it has not been investigated in recent studies as a chemopreventive.

Vitamin E and α- and β-tocopherol are thought to be antioxidative. Vitamin E level was found to be significantly lower in cases than in controls by Verreault et al. [47], Palan et al. [42], and Cuzick et al. [40], but no significant differences were noted by Potischman et al. [46], Batieha et al. [41], Kantesky et al. [28], Wideroff et al. [29], or Peng et al. [30]. Red blood cell folate deficiency was noted in cases compared with controls by Butterworth et al. [50], but no significant differences
Table 2
Cervical cancer chemoprevention trials

<table>
<thead>
<tr>
<th>Investigator (reference)</th>
<th>Study design</th>
<th>Number of patients</th>
<th>Medication</th>
<th>Disease</th>
<th>Results/regression rates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies of retinoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romney et al. [51]</td>
<td>Phase I–II</td>
<td>50</td>
<td>Retinyl acetate gel topically</td>
<td>CIN 1–2</td>
<td>Selected 9-mg dose</td>
</tr>
<tr>
<td>Surwit et al. [52]</td>
<td>Phase I</td>
<td>18</td>
<td>All-TRA topically</td>
<td>CIN 2–3</td>
<td>11% complete response</td>
</tr>
<tr>
<td>Meyskens et al. [53]</td>
<td>Phase I</td>
<td>35</td>
<td>All-TRA topically</td>
<td>CIN 1–2</td>
<td>Selected 0.372% dose</td>
</tr>
<tr>
<td>Weiner et al. [54]</td>
<td>Phase I</td>
<td>36</td>
<td>All-TRA topically</td>
<td>CIN 1–3</td>
<td>0.05%–0.12% dose 14%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.15%–0.48% dose 48%</td>
</tr>
<tr>
<td>Meyskens et al. [55]</td>
<td>Phase III</td>
<td>141 CIN 2</td>
<td>All-TRA topically</td>
<td>CIN 2–3</td>
<td>TRA 43%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placebo 27%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Statistically significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TRA 25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placebo 31%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not statistically significant</td>
</tr>
<tr>
<td>Follen [56]</td>
<td>Phase II</td>
<td>120 proposed</td>
<td>4-HPR Placebo</td>
<td>CIN 2–3</td>
<td>4-HPR 14%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placebo 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Statistically significant</td>
</tr>
<tr>
<td>Ruffin [57]</td>
<td>Phase I</td>
<td>180 proposed</td>
<td>All-TRA</td>
<td>CIN 2–3</td>
<td>NA</td>
</tr>
<tr>
<td>Alvarez [58]</td>
<td>Phase II</td>
<td>60–90 proposed</td>
<td>13-cis-retinoic acid Placebo</td>
<td>CIN 2–3</td>
<td>NA</td>
</tr>
<tr>
<td>Ruffin [59]</td>
<td>Phase II</td>
<td>326 proposed</td>
<td>All-TRA Placebo</td>
<td>CIN 2–3</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Studies of micronutrients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romney et al. [60]</td>
<td>Pilot study</td>
<td>28</td>
<td>Vitamin C, 500 mg Placebo</td>
<td>CIN 1–2</td>
<td>No progression of disease in treated group vitamin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased serum C levels</td>
</tr>
<tr>
<td>Butterworth et al. [61]</td>
<td>Phase II</td>
<td>47</td>
<td>Folate, 10 mg Vitamin C, 10 mg</td>
<td>CIN 1–2</td>
<td>Folate 14%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placebo 41%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not statistically significant</td>
</tr>
<tr>
<td>Butterworth et al. [62]</td>
<td>Phase II</td>
<td>177</td>
<td>Folate, 10 mg Vitamin C, 10 mg</td>
<td>CIN 1–2</td>
<td>Folate 64%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placebo 66%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not statistically significant</td>
</tr>
<tr>
<td>Childers et al. [63]</td>
<td>Phase III</td>
<td>331</td>
<td>Folic acid, 5 mg Placebo</td>
<td>HPV</td>
<td>Folate 7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placebo 6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not statistically significant</td>
</tr>
<tr>
<td>Manetta et al. [64]</td>
<td>Phase I</td>
<td>30</td>
<td>β-carotene</td>
<td>CIN 1–2</td>
<td>Responses</td>
</tr>
<tr>
<td>Romney [65]</td>
<td>Phase I–II</td>
<td>69</td>
<td>β-carotene</td>
<td>CIN 1–3</td>
<td>β-carotene 46%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placebo 50%</td>
</tr>
<tr>
<td>Keefe [66]</td>
<td>Phase II</td>
<td>103</td>
<td>β-carotene</td>
<td>CIN 2–3</td>
<td>β-carotene 47%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placebo 47%</td>
</tr>
<tr>
<td><strong>Studies of polyamine synthesis inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitchell [67]</td>
<td>Phase I</td>
<td>30</td>
<td>DFMO</td>
<td>CIN 3, CIS</td>
<td>Selected doses of 0.125 and 0.5 g/m²/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall 50% regression rate</td>
</tr>
<tr>
<td>Follen (ongoing/unreported)</td>
<td>Phase II</td>
<td>180 proposed</td>
<td>DFMO Placebo</td>
<td>CIN 2–3</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note: All-TRA, all-trans-retinoic acid; 4-HPR, N-(4-hydroxyphenyl)retinamide; DFMO, difluoromethylornithine.
were noted by Ziegler et al. [35,36], Kantesky et al. [28], Herrero et al. [45], Verreault et al. [47], or Wideroff et al. [29].

In summary, β-carotene is the micronutrient most widely recognized in case-control studies as deficient in cases of cervical cancer and CIN as measured by food frequency questionnaires and serum levels. Fewer but equally consistent data exist for lycopene. A good biologic rationale exists for the supplementation of vitamin E, but the nutritional data supporting its deficiency are inconsistent. Additionally, a biologic rationale for its use is not present. Results of retinol, vitamin C, and folate studies have been inconsistent.

5. Chemoprevention trials

Phase I, II, and III chemoprevention trials in the cervix are summarized in Table 2 [51–67]. In all of these studies, patients with CIN 1–3 lesions were chosen as the high-risk cohort. Promising chemopreventive agents that have been or are being investigated in the cervix are retinoids (retinyl acetate gel, all-trans-retinoic acid, and 4-hydroxyphenylretinamide (4-HPR)), micronutrients (β-carotene, folate, and vitamins), and polyamine synthesis inhibitors DFMO. These studies have concentrated on histologic and colposcopic regression as the end points. None of the studies used SEBs or HPV typing until 1995; however, many post-1995 studies did.

5.1. Retinoids

The retinoid group includes vitamin A and its natural and synthetic analogs. Natural vitamin A, its esters, and retinoic acid isomers all-trans-retinoic acid, 9-cis-retinoic acid, and 13-cis-retinoic acid currently are the most widely clinically tested retinoids. These retinoic acids are interconverted in vivo and can activate a wide spectrum of retinoid receptors, including retinoic acid receptors (RARs) and retinoid X receptors. Current systemic therapy with these agents is limited by substantial toxicity [68,69]. In the cervix, most studies using these agents have involved local application with a sponge.

One of the retinoid analogs that seem promising for chemoprevention is 4-HPR. Most of the cellular and molecular mechanisms by which retinoids act are mediated by nuclear RARs; however, 4-HPR may act by means of a distinct mechanism from that of retinoic acid. Support for this contention has come from the studies of Delia et al. [70], who have shown that 4-HPR can induce apoptosis in retinoic acid–resistant cells. Substitution of a carboxamide group at the N position 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 unique mechanism of action in inducing apoptosis in malignant hemopoietic cells, including cells unresponsive to retinoic acid, 4-HPR has lower systemic toxicity and can be given orally. It has been shown consistently to suppress cervical cancer cell growth independent of retinoic acid responsiveness and to involve the production of reactive oxygen species.

In addition, retinoids may negatively affect replication of HPV, making them of particular interest in cervical disease. There are several mechanisms by which retinoic acid may affect the HPV E6 and E7 transforming proteins. Bartsch et al. [71] have demonstrated decreased expression of HPV 18 messenger RNA in the presence of retinoic acid. Retinoic acid has also been shown to increase the secretion of TGF-β in cells immortalized by HPV. Furthermore, in cervical epithelial cells, TGF-β can suppress the expression of the E6 and E7 proteins [71–74]. Thus, the measurement of these factors might serve as markers of responsiveness.

Romney et al. [51] reported on a phase I and II trial using retinyl acetate gel topically in patients with CIN 1 and 2. Patients treated themselves for 7 days for three sequential menstrual cycles, placing the gel intravaginally. Doses included placebo and 3, 6, 9, and 18 mg retinoic acid per 6 g of inert vehicle. No serious side effects were noted. Approximately half of the participants noted vulvar irritation and itching at the 18-mg dose. Only 14% of patients had vaginal burning at any dose during the trial. The study showed that high compliance could be achieved, and after analysis of the side effects at each dose, the researchers identified the 9-mg dose as the best choice for a phase III trial [51]. There is no published report of the phase III trial.

Phase I and II trials by Meyskens et al. [53] demonstrated that all-trans-retinoic acid could be delivered topically to the cervix safely in a cervical sponge and cervical cap. Patients with CIN 1, 2, and 3 were treated by the investigators for 4 days with increasing dosages of topical all-trans-retinoic acid ranging from 0.05 to 0.484%. Patients were seen at 1 week and 1 month for follow-up. Roughly a third of patients experienced vaginal irritation, and roughly half had vaginal burning. Only one patient was asked to discontinue the treatment because of these symptoms. A regression rate of 48% was noted in patients treated with topical doses of 0.15–0.48% all-trans-retinoic acid, compared with a regression rate of 14% in patients treated with lower doses. The optimal dose for a phase III study was determined to be 0.372 [53].

Meyskens et al. [53] reported the results of the randomized phase III trial of topical 0.375% all-trans-retinoic acid in 141 patients with CIN 2 lesions and 160 patients with CIN 3 lesions. Patients with CIS were excluded from the study. Patients were initially treated with 0.375% all-trans-retinoic acid daily for 4 days and then were treated for 2 days each at 3- and 6-month
follow-up visits. Patients were seen for a Pap smear and colposcopy at 9, 12, 15, 21, and 27 months. Biopsies were performed at the 15-month visit. Losses to follow-up were large. Of 151 patients randomized to receive placebo, 81 patients were evaluated at 15 months and 25 patients at 27 months. Of 150 patients randomized to receive all-trans-retinoic acid topically, 88 patients were seen at 15 months and 21 patients at 27 months. There was a statistically significant regression in the CIN 2 lesions but not in the CIN 3 lesions. Sporn and Roberts speculated in an editorial that the reason CIN 2 responded and CIN 3 did not is that it may be harder to induce regression in lesions that are farther along the path toward neoplasia than other lesions and that inducing regression in CIN 3 may require higher doses, longer administration, systemic administration, or two agents instead of one [75]. Though this study was sufficiently powered, high dropout rates in prevention trials impose some limitations on findings.

While no dose has yet been selected from the phase I trial of Ruffin using all-trans-retinoic acid topically [57], an abstract has been published about biomarkers [76]. In the study, 54 women were randomized to one of three 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. So far, 38% of white women and 4% of African-American women are HPV negative, indicating it may not be a useful biomarker in all patients [76]. The rationale for using HPV expression as a biomarker is that high HPV mRNA expression is correlated with active control of HPV gene expression by the cell. This may lead to persistent or progressive disease.

A phase II study of 4-HPR was undertaken at The University of Texas M.D. Anderson Cancer Center [56]. The treatment plan called for either 4-HPR (200 mg/day with a 3-day drug holiday monthly) or placebo to be given orally for 6 months. Crossover from placebo to 4-HPR was to take place if progression was diagnosed by cytology, colposcopy, or biopsy. When an interim analysis at 12 months indicated a significantly worse prognosis for one group, the study was unblinded and researchers discovered that the treated group had the poorer performance. An analysis at 6 months had indicated no significant differences between the groups. Researchers hope an ongoing evaluation of biomarkers may yield an explanation for the findings. SEBs under study in this trial included quantitative cytology and histopathology (nuclear texture, size, and density) and biologic measures of proliferation (proliferating cell nuclear antigen (PCNA)), regulation (epidermal growth factor receptor (EGFR), RAR), differentiation (involucrin, cornifin), and genetic instability (chromosome polysomy, aneuploidy) [56].

5.2. Micronutrients

5.2.1. β-carotene and vitamin C

Romney’s group conducted a phase II trial of vitamin C at 500 mg/day and placebo, with 14 patients in each arm [60]. Significant increases in vitamin C were measured in the serum of patients compared with controls. Enrolled patients had CIN 1 or 2, and no significant progression was noted in the treated arm. The investigators considered this a pilot study and planned a larger trial [60].

Romney’s group recently conducted a phase II trial of β-carotene at 30 mg/day in patients with CIN 2; 98 patients were accrued and 69 were available for analysis of therapy efficacy [65]. The response rate in the β-carotene group was 46% (18/39) compared with 50% (15/30) in the placebo group. The difference was not statistically significant. Two provocative biomarker papers have also been published. Ho et al. [77] reported the use of HPV viral load to predict 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, and HPV typing by Southern blot and PCR. Viral load was most predictive of persistent disease. There was no mention of the effects of β-carotene on viral load in this analysis [77]. Comerci et al. [78] have reported that tissue levels of TGF-β1 were higher after β-carotene treatment than before treatment; however, staining was graded visually but not measured quantitatively, and no mention was made of how many reviewers graded the tissue. Statistically significant increases were seen across parabasal, mid epithelial, and superficial epithelia. No histologic regression rate was reported in this analysis.

Manetta et al. [64] undertook a phase I study of oral β-carotene in 30 patients with CIN 1 or 2 treated daily for 6 months. The authors reported colposcopic regression in 70% (21/30) at 6 months and 33% (10/30) at 12 months. Five of the 11 who were not counted among responders at 12 months who had been responders at 6 months were patients who had been dropped from the study. Five patients were removed from the study for persistent or progressive disease, 3 for pregnancy, and 5 after relocation.

Manetta and Berman [79] have undertaken a phase II study of β-carotene in patients with CIN 2 or 3; 60 patients are expected to be accrued. Brewer et al. [80] have published a biomarker paper from this series. They report the serial changes seen in colposcopic and cervicographic findings in women enrolled in the trial. Data were available for 23 subjects who had regression and 16 with persistent lesions. Small lesions were significantly more likely to regress than larger ones. Patients whose lesions revealed coarse punctuation (usually indicative of a higher grade) were significantly more likely to have persistent disease. The authors noted
a centripetal pattern of regression, a pattern that is interesting but has not been validated in other studies. In an interim analysis, Keefe [66] reported findings in 103 patients (124 enrolled in whom the response rate in the placebo and treated arms were 47% each. (This report was a preliminary report in advance of reporting the final results of the trial.)

5.2.2. Folate

Folic acid acts as a coenzyme in DNA synthesis for normal cellular growth, proliferation, and differentiation. To understand folic acid’s regulation of HPV oncogene expression, Pietrantoni et al. [74] studied the transcription regulators c-fos and 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 by Western blot when concentrations of folate over 100 nmol/l were used. Similarly, E6 protein expression was diminished at concentrations over 100 nmol/l, suggesting that the mechanism by which the c-fos and c-jun were controlled involved diminished viral E6 expression. Folate, specifically red blood cell folate, as well as β-carotene, has been shown to be deficient in CIN patients compared with levels in controls, and these data have supported folate supplementation as a chemopreventive strategy [50]. Red blood cell folate levels below 660 nmol/l have been shown to enhance the susceptibility of patients to HPV-16 infection, which in this trial was identified by Western blot analysis [50].

Butterworth et al. published in 1982 a report that oral contraceptive users taking folate supplements were likely to experience cytologic regression of cervical dysplastic lesions [61]. A phase II randomized trial reported 10 years later by Butterworth et al. studied patients with CIN 1 and 2 lesions who were treated with folate (10 mg) or with vitamin C (10 mg) as a placebo, each for 6 months [62]. In the second report, there were no statistically significant differences in regression of lesions in the 177 evaluable patients treated with folate or vitamin C. A phase III multicenter study of folate supplementation by Childers et al. [63] has had similarly negative results. In this intergroup Southwest Oncology Group study, 331 patients with koilocytic atypia, CIN 1, and CIN 2 were randomized to receive 5 mg folate acid or placebo. While regression was of borderline statistical significance (P = 0.08) at the 3-month visit, no difference in regression between groups was noted at the 6-month visit. So the final conclusion was that at the doses used folic acid was not active.

5.3. 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 [81]. Blocking endogenous ODC prevented transformation of rat fibroblasts by the temperature-sensitive v-src oncogene. The goals of using DFMO to block polyamine-directed transformation are (1) to inhibit transformation under the influence of field cancerization, and (2) to remove cells already transformed by apoptosis [82]. Tumor formation in experimental animals is prevented by inhibitors of ODC such as DFMO [83,84]. DFMO was shown to decrease growth of cervical cancer cell lines, irrespective of HPV positivity, in a study by Hamada et al. that is reported in Mitchell et al. [21].

A phase I study of DFMO was completed at The University of Texas M.D. Anderson Cancer Center [67]. In the phase I study, the medication was given orally as an elixir, thus producing systemic effects. DFMO was administered to patients at five dose levels ranging from 0.06 to 1.0 g/m². Study 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; audiometry; 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 a loop electrocautery excision was performed at study conclusion. This study attempted to determine the dose for the phase II study [67].

Sample size calculations using ODC measurements in the skin indicated that six patients at each dose level would be adequate for determination of a response. Thirty patients were enrolled in the trial and completed all trial assessments. Six patients were treated at 1.0, 0.5, 0.25, 0.125, and 0.06 g/m² daily. One patient took the wrong dose (0.8 g/m²) and was excluded from the analysis. The patients’ ethnic origins were 70% white, 7% African-American, and 23% Hispanic. All patients had histologically confirmed CIN 3 or CIS without suspicion of invasion and none was pregnant. All lesions could be identified colposcopically and were at least one-third the surface area of the cervix. Subsequent review demonstrated that patients were evenly distributed among dose levels by lesion size and grade. Eighty-three percent of patients were HPV positive using PCR.

Of the 29 evaluable patients undergoing loop excision of the cervix, 14 had persistent CIN 3 histologically, 10 had CIN 1 or 2, and 5 had histologically negative specimens. Histologic responses were noted at all dose levels. Polyamine synthesis biomarkers showed significant modulation of the spermidine/spermine ratio at 1.0 g/m²/day. The decrease in the ratio persisted at 0.5 g/m² but was not statistically significant. The arginine level was significantly increased at 1.0 and 0.5 g/m²/day, and this trend continued at 0.125 g/m² but not significantly
so [67]. 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, although they were most significant among responders [85,86]. Quantitative PCNA measurements showed decreased proliferation in all specimens at all dose levels, with the most significant decreases noted in histologic responders [87]. The phosphoprotein antibody MPM-2 was measured in correlation with PCNA, and decreased mitoses correlated well with decreased proliferation [88]. EGFR was measured quantitatively and did not decrease significantly with treatment; however, pretreatment levels of EGFR predicted response [89]. Since EGFR level has been shown to rise as lesions become more dysplastic, EGFR modulation to a lower level, or down regulation, could predict that lesions are responding. Since polyamine biomarkers were significantly modulated at 0.5 g/m², this dose was chosen for the phase II trial. Since trends suggested possible modulation at 0.125 g/m², this dose was also chosen for the phase II trial [67].

After the study, comparisons were made between colposcopically abnormal areas of the cervix and normal areas. The ODC value and spermidine/spermine ratio were found to vary significantly between the two areas in the cervix. The ratio is measured currently with a tissue specimen that is homogenized and therefore unavailable for the assessment and correlation of other biomarkers. An advance in the polyamine trial field would be the development of immunohistochemical markers of polyamine synthesis [90].

In the phase II study, DFMO or placebo will be given for 1 month followed by a loop excision. Patients will be randomized to receive 0.5, 0.125 g/m², or placebo. Eligibility criteria will be expanded to include women older than 18 years old, nonpregnant, with HGSIL (CIN 2 or 3). Because of the inclusion of CIN 2 and anticipation of a higher regression rate, the sample size will be 60 patients per group, or a total of 180 patients. Patients will be monitored for 2 years after loop excision as part of routine care. Since polyamine biomarkers showed such variability, the biomarkers to be used for this trial include quantitative cytology and histopathology, PCNA, and MPM-2. To date, 35 patients of the 180 have been accrued.

5.4. Nonsteroidal anti-inflammatory drugs

The nonsteroidal anti-inflammatory drugs (NSAIDs) are thought to play a role in the control of neoplastic and nonneoplastic cell proliferation and immune function through the inhibition of endogenous prostaglandin biosynthesis. They have been shown to be potential chemopreventives in the colon (reviewed by Kelloff et al.) [5,6]. They are extremely well-tolerated medications, and it may be important to test NSAIDs as chemopreventives in the cervix.

6. Conclusions

While there are several potentially suitable cohorts for cervical chemoprevention trials, those that have been enrolled to date have had preinvasive lesions. Several medications appear suitable, and many have been under investigation. None of the micronutrient trials using β-carotene, folate, or vitamin C have shown responses. A phase I trial of topical all-trans-retinoic acid yielded a recommended dose of 0.372 mg% and a response rate of 48%. This dose was used in a phase III trial, and a significant regression of CIN 2 lesions, but not CIN 3 lesions, occurred. A phase I study of DFMO yielded two doses of interest, 0.125 and 0.5 g/m², and a response rate of 50%. The phase II study is under way. So far, 145 of 180 patients have been recruited, and the investigators remain blinded to the preliminary results. No phase I study of 4-HPR was undertaken in the cervix. The lessons learned from the previous chemoprevention trials in the cervix are: (1) micronutrients are logical candidates for chemoprevention but have not been successful, and the reasons are unclear, (2) studies need to have sufficient sample sizes to account for the regression of preinvasive lesions in the control group, (3) phase I studies need to be conducted in the cervix itself to determine the dose for the phase II trial, (4) SEBs need to be validated in phase I trials, (5) the relationship of the medication to HPV status should be elucidated for the medication to have a long-term impact in this disease, and (6) when possible, the immunobiology of HPV should be studied during chemoprevention trials.

References


Biographies

Anne-Thérèse Vlastos was born in 1965 and graduated in medicine from the University of Geneva, Medical School, Geneva, Switzerland. A visiting scholar when this work was performed, Dr. Vlastos was at the Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, supported by the Swiss National Science Foundation, the Swiss Cancer League, the Cancer and Solidarity Foundation, and the Novartis Foundation.

David Schottenfeld was born in 1931 and graduated in medicine from Cornell University Medical College. He is John G. Searle Professor, Department of Epidemiology, and professor of internal medicine, Department of Internal Medicine, at the University of Michigan in Ann Arbor, Michigan.

Michele Follen was born in 1954 and graduated in medicine from the University of Michigan in 1980. She earned her Ph.D. in 2000 also at the University of Michigan. She is a professor of gynecologic oncology and the director of the Biomedical Engineering Center at The University of Texas M.D. Anderson Cancer Center in Houston, Texas, and a professor in the Department of Obstetrics, Gynecology and Reproductive Sciences at The University of Texas Health Science Center at Houston, Houston, Texas.