THE RETINOIDS

Biology, Chemistry, and Medicine

SECOND EDITION

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The Trustees of Columbia Univ. Ex. 2126 - Page 1 of 36
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15 / Retinoids and Human Cancer

Waun Ki Hong and Loretta M. Itri

Introduction, 597
Epidemiology, 598
Epidemiologic Data on the Relationship Between Retinoids and Human Cancer, 598
Carcinoma of the Lung, 599
Carcinoma of the Esophagus, 600
Carcinoma of the Stomach, 600
Carcinoma of the Pancreas, 601
Carcinoma of the Colon/Rectum, 601
Carcinoma of the Bladder and Urinary Tract, 601
Carcinoma of the Prostate, 601
Carcinoma of the Cervix, 601
Carcinoma of the Breast, 602
Summary, 602
Serum Retinoid and Carotenoid Levels and Cancer Incidence, 603
General Studies, 603
Carcinoma of the Lung, 603
Carcinoma of the Oral Cavity and Oropharynx, 604
Carcinoma of the Esophagus, 604
Carcinoma of the Gastrointestinal Tract, 604
Carcinoma of the Prostate, 604
Carcinoma of the Cervix, 605
Carcinoma of the Ovary, 605
Carcinoma of the Breast, 605
Summary, 605
Safety Aspects of Retinoids, 605
All-*trans* Retinoic Acid, 605
Isotretinoin, 606
Etretinate, 606
Fenretinide, 606
Arotinoids, 607
Summary, 607
Therapeutic Trials, 607
Hematologic Malignancies, 607
Myelodysplastic Syndrome, 607
Acute Promyelocytic Leukemia, 609
Chronic Myelogenous Leukemia, 610
Cutaneous T-Cell Lymphoma/Mycosis Fungoides, 610
Conclusions, 612
Epithelial Malignancies, 612
Skin Cancer, 612
Head and Neck Cancer, 612
Advanced Lung Cancer, 613
Bladder Cancer, 613
Cervical Cancer, 613
Prevention Trials, 614
Chemoprevention Trials in Human Premalignant Lesions, 614
Oral Premalignancy, 614
Bronchial Metaplasia, 615
Laryngeal Papillomatosis, 616
Barrett's Esophagus, 616
Actinic Keratoses, 617
Cervical Dysplasia, 617
Trials of Chemoprevention Against Second Primary Tumors, 618
Head and Neck Cancer, 618
Skin Cancer, 619
Breast Cancer, 620
Lung Cancer, 621
Bladder Cancer, 621
Primary Prevention Trials for Epithelial Cancer, 622
The United States Physicians' Health Study, 622
Subjects with Risk Factors, 622
Summary and Future Directions, 623
References, 624

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INTRODUCTION

The scientific basis for the use of retinoids as preventive and therapeutic agents in human cancer is discussed in detail in this book. The capacity of retinoids to modulate

The Trustees of Columbia Univ. Ex. 2126 - Page 3 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

the differentiation and proliferation of both preneoplastic and neoplastic cells is an important biologic property of these substances (Lotan, 1980). This chapter deals with the use of retinoids in the treatment and prevention of cancer in humans.

Epidemiologic studies have suggested an inverse correlation between the development of cancer and the dietary consumption of vitamin A or beta-carotene. In 1925, Wolbach and Howe noted histologic similarities between the epithelium of vitamin A-deficient organs and neoplastic tissue. Lasnitzki demonstrated in 1955 that a premalignant phenotype of mouse prostate cancer that had been induced with the carcinogen 3-methylcholanthrene could be altered by retinoid treatment. Lasnitzki showed that in prostate cell cultures, retinoid treatment caused the disappearance of atypical epithelial cells that had been induced by the carcinogen, and that the atypical cells were replaced by cells with more normal morphology. Epidemiologic studies and animal experiments prompted researchers to test the efficacy of retinoids in the prevention and treatment of cancer (Goodman, 1984). As investigators have gained experience with retinoid therapy, the methodology for studying these substances has also improved.

The retinoids are a class of over 3,000 naturally occurring and synthetic vitamin A analogues. The pleotropic actions of retinoids include their effects on cell membranes, gap junctions, protein kinase C, and intracellular and extracellular proteins, as well as their modulation of the expression of growth factors such as transforming growth factor-β (TGF-β) and the epidermal growth factor receptor (EGFR), and of programmed cell death. Although it is well known that retinoic acid (RA) modulates gene expression, the precise mechanism by which this occurs is not understood. The interaction between retinoids and the nuclear retinoic acid receptors (RARs) will help explain their effects on cells.

The nuclear RARs appear to mediate the effects of retinoids ranging from the most severe toxicities such as teratogenicity to the beneficial effects in preventing and treating cancer (Giguere et al., 1987; Petkovich et al., 1987). Six human retinoic acid receptors have been cloned: RAR-α, RAR-β, RAR-γ, RXR-α, RXR-β, and RXR-γ. These receptors are strongly homologous to other receptors of the steroid-thyroid hormone superfamily (Evans, 1988).

This chapter discusses the extensive data on the use of retinoids in the prevention and treatment of cancer in humans. Striking clinical successes have been achieved in the use of retinoids in the treatment of skin cancers, as documented elsewhere in this chapter (Lippman et al., 1987a, 1987b). Most recently, retinoids have been shown to hold promise for treating acute promyelocytic leukemia (PML) and for preventing premalignant oral lesions and second primary tumors in cases of head and neck cancer. The latest results of clinical trials of a combination of 13-*cis*-RA and interferon-α in the treatment of advanced carcinomas of the skin and cervix have generated tremendous enthusiasm among retinoid researchers by highlighting the unexplored clinical potential of combinations of retinoids and biologic-response modifiers.

EPIDEMIOLOGY

Epidemiologic Data on the Relationship Between Retinoids and Human Cancer

Epidemiologic studies offer several advantages over laboratory investigations. Because the data are obtained from direct observations of human populations, the interpretation of results is not complicated by problems involved in extrapolating *in vitro* or animal data. Moreover, the degree of environmental exposure can be sufficiently quantified to permit the assessment of dose-response correlations. Finally, the results of such studies may permit intervention in the form of eliminating or reducing risk factors before the actual mechanisms of a disease are fully understood.

Unfortunately, certain limitations are also inherent in epidemiologic trials. Because the data are primarily observational (such as recalled dietary information), imprecise measurement is virtually guaranteed. Moreover, the investigators in such trials must frequently depend on data sources with a high potential for error (e.g., variability in the reporting of cancer in different countries). In studies of diet and cancer, the long latency period between risk exposure and the manifestation of illness has serious implications. In retrospective studies, the investigator must assume that a subject's current food intake reflects previous consumption patterns, or that the trial participant can accurately recall events long past. Although prospective studies are based on current dietary data, the investigator must wait many years for the disease to appear. Although prospective studies yield less error-prone data, they are especially costly because of the large populations and lengthy follow-up periods required to achieve statistically significant results.

Despite their limitations, epidemiologic studies have long pointed to an inverse relationship between cancer risk and vitamin A consumption. With the recent completion of a number of large, long-term prospective studies, the protective effects of vitamin A in relation to certain cancers has become even more apparent. This protective association is most pronounced in the case of β-carotene.

β-Carotene (provitamin A) is one of several naturally occurring precursors of retinol. Because of inefficient intestinal conversion, only one-sixth of the dietary intake of β-carotene is ultimately converted to retinol (Goodman, 1979). Moreover, as the amount of β-carotene in the diet increases, the conversion to retinol

The Trustees of Columbia Univ. Ex. 2126 - Page 4 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

decreases, so that systemic levels of retinol do not become toxic even if massive doses are consumed. A variable amount of β-carotene is also absorbed unchanged from the intestine, enters into circulation, and is stored in adipose tissue. As shown by the studies described below, an inverse association between consumption of this nutrient and the development of cancer has been documented in a range of neoplasms.

Carcinoma of the Lung

In 1975, Bjelke first reported epidemiologic data suggesting that vitamin A may protect against lung cancer. A survey was mailed to 8,278 Norwegian men, asking them to report their smoking and dietary habits. Investigators then calculated a vitamin A index based on the reported consumption of vegetables and dairy products. Thirty-six cases of lung cancer were subsequently diagnosed in these men. After controlling for cigarette smoking, a lower incidence of lung cancer was noted in the patients with high vitamin A index values in all age groups. Although this study, being prospective, was more likely to produce accurate data, the number of lung cancer deaths reported was small, and the magnitude of the association with vitamin A could not be accurately established.

Other studies reported in the late 1970s suggested that vitamin A consumption provided protection against the development of lung cancer. In a case-control study among Chinese women in Singapore, MacLennan et al. (1977) found an inverse association between the consumption of leafy green vegetables (rich in vitamin A) and lung cancer.

Pharmacologic preparations of vitamin A also appeared to confer some benefit. In comparing the use of these preparations among 800 newly diagnosed cancer patients and 3,433 patients with nonmalignant conditions, Smith and Jick (1978) found a negative association between the development of cancer and the frequent use of vitamin A preparations in men.

A retrospective study of the dietary and smoking habits of 292 male patients with lung cancer and 801 control patients with nonrespiratory nonneoplastic diseases suggested that vitamin A might ameliorate some of the carcinogenic effects of smoking (Mettlin et al., 1979). Not only did lung cancer patients in this study have lower values of vitamin A intake than controls, but the reduced relative risk of lung cancer associated with vitamin A intake was most evident among men who smoked heavily.

Hirayama (1979a) prospectively studied 265,118 adults who were interviewed at the time of the Japanese census of 1965. During the initial interview, participants were grouped according to smoking habits, consumption of green and yellow vegetables, and socioeconomic status. Consumers of green and yellow vegetables, both smokers and non-smokers, showed a lower risk of lung cancer. Ex-smokers who ate green and yellow vegetables on a daily basis had a lower cancer risk than those who did not, suggesting that these foods have an effect on the recovery process.

Gregor et al. (1980) obtained estimates of dietary vitamin A intake from 100 patients with lung cancer and 173 controls. The intake of vitamin A was significantly lower in male cancer patients than in male controls, but it was higher in female patients than in female controls. However, the intake of vitamin A supplements was lower in patients than in controls of both sexes.

Early in the 1980s, the specific association between β-carotene and a reduced risk of lung cancer became more evident. In a prospective study of 1,954 middle-aged men followed for 19 years, the intake of dietary β-carotene was inversely related to the incidence of lung cancer both before and after adjustment for cigarette smoking (Shekelle et al., 1981). On the other hand, there was no significant relationship between the intake of preformed vitamin A (retinol) and the risk of lung cancer.

A population-based case-control study in New Jersey of 763 cases and 900 controls also illustrated the more pronounced impact of carotenoid intake on the development of lung cancer in smokers (Ziegler et al., 1986). The investigators asked participants about their consumption 4 years earlier of 44 food items that provide 83% of the vitamin A in the American diet, and about their use of vitamin A supplements. The men in the lowest quartile of carotenoid intake had a relative risk of 1.3 compared to those in the highest quartile after adjusting for smoking. An intake of dark green vegetables, or of dark yellow-orange vegetables was more strongly associated with a protective effect than was the carotenoid index, but the protective effect of vegetables was limited to current and recent cigarette smokers. The reduction in risk with vegetable intake was most apparent for squamous-cell carcinomas, but it extended to adenocarcinomas and most other histologic types of carcinoma when only current and recent smokers were analyzed.

In a case-control study based on questionnaires administered to 1,253 cases and 1,274 controls, Fontham et al. (1988) found a specific association between serum carotene levels and squamous- and small-cell carcinomas. A significant inverse relationship in risk with retinol intake was limited to adenocarcinoma and was more pronounced among blacks (Fontham, 1990).

The results of a Hawaiian study of 230 male and 102 female lung cancer patients and 597 male and 268 female controls (Le Marchand et al., 1989) demonstrated a clear dose-dependent negative association between dietary β-carotene and lung cancer risk in both sexes. All vegetables, dark green vegetables, cruciferous vegetables, and tomatoes showed stronger inverse associations with risk than did β-carotene, leading the authors to suggest

The Trustees of Columbia Univ. Ex. 2126 - Page 5 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

that other constituents of vegetables may also protect against lung cancer in humans. No clear association was noted between retinol and lung cancer.

French investigators who interviewed 106 patients with epidermoid lung cancer and 212 matched controls found that consumption of preformed vitamin A and consumption of β-carotene were significantly and independently associated with a reduced risk of epidermoid lung cancer (Dartigues et al., 1990). Thus, while this study confirmed the protective role of β-carotene, it also suggested that preformed vitamin A might offer distinct and important protection as well.

Harris et al. (1991) estimated the dietary intake of carotene during the year before diagnosis in 969 men with lung cancer, 75 men with other epithelial cancers, and 97 hospital controls. They observed a significant inverse trend between carotene intake and lung cancer and a non-significant trend for other epithelial cancers. Unlike the results of Le Marchand et al. mentioned above, the protective effect of estimated dietary carotene intake in this study was considerably stronger than the effect of total intake of carotene-rich vegetables and fruits, suggesting that the protective factor was carotene itself rather than another component of vegetables and fruits.

Finally, two recently reported large prospective trials offer further evidence of the inverse association of dietary retinoids and the incidence of lung cancer. Knekt et al. (1991) studied the intake of retinoids, carotenoids, vitamin E, vitamin C, and selenium and the subsequent risk of lung cancer in 4,538 initially cancer-free Finnish men. During a 20-year follow-up, 117 cases of lung cancer were diagnosed. There was a significant inverse relationship between the intake of carotenoids and vitamin C and the incidence of lung cancer among nonsmokers, and a non-significant inverse trend between vitamin E intake and lung cancer. In the total cohort, there was a significant inverse association between intake of margarine and fruits and the risk of lung cancer, although these relationships were stronger among nonsmokers.

The most recent prospective study was conducted in a group of white male insurance policy holders over 35 years of age who completed a mail questionnaire on tobacco use, diet, and demographic characteristics (Chow et al., 1992). Two hundred and nineteen of these men died of lung cancer within the 20-year follow-up period. There was an inverse association between lung cancer and intake of fruits, and the risk of lung cancer was lower among men in the highest quintiles of dietary consumption of vitamins A and C.

Carcinoma of the Esophagus

In 1961, 150 men with squamous-cell carcinoma of the esophagus were compared with 150 control patients with cancers of other sites (Wynder and Bross, 1961). Consumption of milk and vegetables was found to be lower in the patients with esophageal cancer. In a 1981 retrospective review, the dietary habits of 147 patients with cancer of the esophagus were compared to those of 264 men of similar age with a diagnosis of noncancerous disease (Mettlin et al., 1981). Even after controlling for possible associations with smoking or ethanol consumption, an overall higher frequency of vegetable and fruit consumption was associated with a significantly lower cancer risk. Progressively higher levels of consumption were associated with progressively lower risks.

In the Caspian Littoral of Iran (a region with a particularly high incidence of esophageal cancer), 38 randomly chosen villages were evaluated for environmental characteristics. An analysis of food intake indicated a higher cancer risk in areas with a low consumption of fruit and green vegetables. Areas of low risk generally had higher intakes of vitamins A and C (Hormozdiari et al., 1975). A subsequent case-control study in this region confirmed the earlier findings and demonstrated a strong association between esophageal cancer and a low consumption of fresh fruit and vegetables (Cook-Mozaffari, 1979; Cook-Mozaffari et al., 1979).

More recent studies also provide evidence for a relationship between foods containing vitamin A and cancer of the esophagus, but the results differ in terms of the direction of the association and form of the vitamin that is important. Findings in one study comparing 178 patients with esophageal cancer and matched controls suggested that the risk of cancer of the esophagus increased with the ingestion of foods containing retinol but not carotene (Graham et al., 1990). Conversely, in another study comparing the dietary intake of 211 men with cancer of the esophagus and 712 matched controls, there was no significant association between the retinol index and the risk of esophageal cancer, but the β-carotene index showed a protective effect (Valsecchi, 1992).

Carcinoma of the Stomach

An early case-control study relating diet and the incidence of cancer of the stomach was conducted by Hirayama (1967) in Japan. The results indicated an inverse association between daily milk consumption and stomach cancer. A later prospective cohort study by the same author (Hirayama, 1977) of more than 26,000 Japanese citizens revealed a similar inverse relationship between the incidence of gastric cancer and milk consumption.

Graham et al. (1972) studied 228 patients with gastric cancer who were matched by age, country of birth, and background nationality with control patients who had no neoplastic or gastrointestinal disease. When the subjects' food intake was analyzed, no relationships were

The Trustees of Columbia Univ. Ex. 2126 - Page 6 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

noted between cancer and fried food preparation or consumption, meat or fish consumption, or alcohol ingestion. However, the groups consumed substantially different amounts of various raw vegetables, and control patients were noted to have eaten larger quantities of raw vegetables than the cancer patients.

A similar association between decreased gastric cancer risk and increased consumption of raw vegetables was noted by Haenszel et al. (1972). In a study of 220 Japanese stomach-cancer patients and 440 hospital-patient controls in Hawaii, consumers of pickled vegetables and dry or salted fish were noted to have an increased cancer risk, whereas consumers of raw vegetables appeared to have a lower risk.

Carcinoma of the Pancreas

A full dietary history was used to estimate the daily intake of calories, fat, protein, carbohydrate, cholesterol, retinol, vitamin C, and fiber in 110 cases of pancreatic cancer and 195 controls in Poland (Zatonski et al., 1991). A significant inverse association was seen between vitamin C intake and cancer of the pancreas, but only weak inverse associations were seen between pancreatic cancer and the intake of either retinol or fiber.

Carcinoma of the Colon/Rectum

Paymaster et al. (1968), in an epidemiologic study of gastrointestinal neoplasias in India, noted that fewer cases of colon and rectum cancers were found in populations consuming a strict vegetarian diet. A case-control study of the diet of 198 patients with colon cancer and two matched control groups was conducted by Modan et al. (1975). Comparison of these groups revealed a significantly lower consumption of fiber and certain vegetables among the cancer patients. In a large, ongoing case-control study of over 40,000 persons in Minnesota and Norway, Bjelke (1980) observed a decreased consumption of several vegetables in colorectal cancer patients. The patients also had a lower index of vitamin A intake that correlated with their vegetable consumption.

Carcinoma of the Bladder and Urinary Tract

Mettlin and Graham (1979) obtained retrospective dietary information from 569 patients with bladder cancer and 1,025 age-matched controls. Using a computed index of vitamin A intake, they found that patients with a lower index had an increased risk of bladder cancer. A similarly increased risk was associated with infrequent milk and carrot consumption.

More recently, Steineck et al. (1990) conducted a population-based case-referent study in which information was obtained in 78% of 418 identified cases of urothelial cancer and from 77% of 511 selected referents. In this Swedish population, the relative risk for patients who took vitamin A supplements was 0.5, and the risk progressively decreased as vitamin A consumption increased. The association between vitamin A and urinary cancer was far less pronounced in a Hawaiian study comparing 195 male and 66 female cases of lower urinary tract cancer with matched controls. Although there was a decrease in risk with an increasing consumption of dark green vegetables in men, the total intake of carotenoids, retinol, and total vitamin A was only weakly and inconsistently related to risk in both sexes (Nomura et al., 1991).

Carcinoma of the Prostate

Schuman et al. (1982) conducted a study of 358 patients with prostate carcinoma and 715 matched controls. Dietary information was obtained by interviews and dietary questionnaires. In addition to other findings, the authors noted a markedly lower consumption of certain vegetables and liver among the patients with prostate cancer. A prospective epidemiologic study of 122,000 Japanese men aged 40 years or older was conducted by Hirayama (1979b). In a 10-year follow-up, men who consumed green and yellow vegetables on a daily basis had a significantly lower age-standardized death rate from prostate cancer.

In two more recent studies, age appeared to be a factor in the association between the risk of prostate cancer and vitamin A consumption. In one report, vitamin A intake was assessed from the dietary histories of 452 men with prostate cancer and 899 controls (Kolonel et al., 1987). In men less than 70 years of age, there were no significant associations between vitamin A intake and the risk of prostate cancer. However, in men 70 years or older, the risk increased directly with the amount of vitamin A consumed. The findings were similar for the various components of vitamin A, but were somewhat stronger for total carotene than for total retinol.

The second study was based on epidemiologic questionnaires mailed to 17,633 men aged 35 years and older (Hsing et al., 1990). During the 20-year follow-up, 149 of the responders developed fatal prostate cancer. No significant associations were found between the risk of prostate cancer and the consumption of meat, dairy products, fruits, or vegetables.

Carcinoma of the Cervix

In a case-control study conducted in 1984 (Wylie-Rosett et al., 1984), 87 women with abnormal cytology in a Pap smear examination were compared to 82 matched controls for their dietary intake of vitamin A.

The Trustees of Columbia Univ. Ex. 2126 - Page 7 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

Relative to the controls, the subset of cases with severe dysplasia or carcinoma *in situ* were significantly more likely to have had a total vitamin A intake or a total β-carotene intake or both that were below the pooled median. Odds ratios revealed an approximately threefold greater risk of severe dysplasia or carcinoma *in situ* in women with a lower vitamin A or β-carotene intake. In addition, retinol-binding protein (RBP) was either absent or undetectable in 78.8% of the dysplastic tissue samples, but in only 23.5% of the normal tissue samples.

Subsequent reports have also documented an inverse association between vitamin A consumption and cervical cancer. In a larger study conducted in Italy, 392 patients with invasive cancer were compared with 392 matched controls hospitalized for acute conditions unrelated to any of the risk factors for cervical cancer, and 247 patients with cervical intraepithelial neoplasia were compared with 247 women with normal Pap smears at the same clinic at which the cases of invasive cancer had been identified (LaVecchia et al., 1988). The estimated β-carotene, but not retinol, intake was inversely and strongly related to the risk of invasive cervical cancer. Allowance for several potential distorting factors, including indicators of social status and the major risk factors for cervical cancer, did not materially modify the risk estimates. In contrast, no association emerged between vitamin A consumption estimates and intraepithelial neoplasia. The authors suggested that these results indicate that β-carotene (or some other correlate of a vegetable-rich diet) may affect the later stages of carcinogenesis, thus influencing the risk of invasive carcinoma but not of its precursors.

In another case-control study (Verreault et al., 1989), the diets of 189 women with cervical cancer were compared to those of 227 controls selected through random-digit dialing. Diet during the year preceding diagnosis was assessed by interview, using a food-item questionnaire. After an adjustment for known risk factors, the frequent consumption of dark green or yellow vegetables and of fruit juices was related to a reduced risk of cervical cancer. A high dietary intake of carotene was also associated with a lower risk of disease, particularly of the squamous-cell type. The intakes of preformed vitamin A and of folic acid, on the other hand, were not related to the risk of cervical cancer.

In the most recently reported study of diet and cervical cancer, investigators interviewed 748 cases and 1,411 hospital and community controls in four Latin American countries about their consumption of 58 food items (Herrero, 1991). After adjustment for factors such as sexual and reproductive behavior, socioeconomic status, screening practices, and infection with the human papillomavirus, a slightly lower risk was observed for subjects in the highest quartiles of consumption of fruit and fruit juices, but no reductions in risk were associated with the consumption of vegetables, foods of animal origin, complex carbohydrates, legumes, or folacin-rich foods. When nutrient indices were derived, however, patients who consumed higher amounts of vitamin C, β-carotene, and other carotenoids had a significantly lower cancer risk.

Carcinoma of the Breast

In 1988, Rohan et al. reported a relationship between diet and breast cancer in a study of 451 cases and age-matched controls taken from the electoral register. Dietary intake was measured by self-administered questionnaires. In this population, risk varied little with the level of retinol intake, but decreased significantly with an increasing intake of β-carotene. A multivariate adjustment for the effects of potentially confounding variables did not alter these patterns.

Canadian researchers investigating the relationship of diet and breast cancer (Hislop et al., 1990) found that the occurrence of proliferative benign breast disease was inversely associated with vitamin A supplementation and with frequent green vegetable consumption. On the other hand, no association was found between vitamin A or vegetable intake and severe atypias or borderline carcinoma *in situ*.

Finally, in a comparison of dietary interviews conducted with 439 postmenopausal breast cancer patients and age-matched controls from the same community (Graham et al., 1991), the risk of cancer, adjusted for potentially confounding factors, was found to be greatest among women with the lowest ingestion of carotene or a substance correlated with vegetable ingestion. Risk was not associated with retinol intake, however.

Summary

Epidemiologic evidence continues to demonstrate an inverse relationship between β-carotene consumption and the development of certain neoplasms. β-Carotene has its strongest protective effect against lung cancer, although it is also related to a reduced risk of esophageal cancer, urinary tract carcinomas, cervical cancer, and carcinoma of the breast.

Retinol, on the other hand, seems to have an inconsistent, nonexistent or, in the case of esophageal cancer, a possibly positive association with the development of cancer. The results of one study of prostate cancer are unique in showing an age-differential in the association between vitamin A intake and the development of disease; results from several trials have contradicted this finding. Two studies (Kolonel et al., 1985; Kolonel et al., 1987) found a direct relationship between vitamin A consumption and increased risk of prostate cancer only in men 70 or 75 years of age and older, while a third study (Hsing et al. 1990) showed an inverse relationship

The Trustees of Columbia Univ. Ex. 2126 - Page 8 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

between vitamin A intake and prostate cancer in older men under 75 years of age. Difficulties in assessing retinol levels may account for some of these inconsistencies. Until more definitive information is available, however, the role of dietary retinol in the development of cancer remains an unresolved issue.

Serum Retinoid and Carotenoid Levels and Cancer Incidence

Several problems are inherent in studies that attempt to examine the relationship between the development of cancer and serum levels of retinol and β-carotene. Because it is difficult to obtain accurate serum levels of these substances, these values are not always reliable. This is particularly true in the case of retinol, a substance that is tightly controlled by physiologic mechanisms. Moreover, unlike carotene, serum retinol levels do not reflect the dietary intake of retinol under normal conditions. Hence, it is not surprising that no consistent relationship has been shown between retinol levels and the risk of cancer. Finally, β-carotene or retinol levels may have become depressed as a result of already established cancers. More studies are needed in individuals with early cancers in whom disease has not already had a major impact on diet or general health.

General Studies

Early studies of retinoid and carotenoid levels and cancer focused on the association between serum levels of these substances and cancer at any site. Several of these studies analyzed retinol levels in stored samples of blood taken some years previously for unrelated research. Wald et al. (1980) prospectively followed 16,000 men who had samples of serum collected and frozen. Retinol levels were analyzed in samples from 86 of these men who subsequently developed cancer and from 172 who did not (controls). Low retinol levels were associated with an increased risk of malignant disease. This association was independent of age, smoking habits, and serum cholesterol levels, and was highest in men who developed lung cancer. The risk of cancer at any site for men with retinol levels in the lowest quintile was 2.2 times greater than the risk for men with levels in the highest quintile. Although the apparent protective effects of retinol are statistically significant, so few cancers were observed in these studies that there is substantial statistical uncertainty about the magnitude of the effect.

Another study utilizing stored serum was conducted by Kark et al. (1981) in Evans County, Georgia. The 3,102 participants were apparently free of cancer at the time the samples were taken. Participants were followed for periods up to 14 years, during which 129 cases of cancer were diagnosed. Retinol levels were measured in the stored sera from 85 cancer patients and 174 matched controls. Persons who eventually developed cancer had significantly lower serum retinol levels than controls at least 12 months before the diagnosis of cancer. The association was noted in all four race-sex groups, and was stronger for males than females. However, an extension of this study to 135 further cases and 237 controls failed to find a significant relationship between serum retinol and case status (Pelag et al., 1984).

Peto et al. (1981) examined the role of β-carotene in cancer prevention. Included in this extensive review were eight retrospective studies from several countries and one region in which the serum retinol levels of patients with recently diagnosed cancer were significantly lower than those of controls. These studies were done in India (Wahi et al., 1962), East Africa (Clifford, 1972), Pakistan (Ibrahim et al., 1977), Britain (Basu et al., 1976; Atukorala et al., 1979; Capel and Williams, 1979), and the United States (Abels et al., 1941; Cohen et al., 1978).

Reviewing several prospective epidemiologic studies in which biochemical variables were monitored, Wald (1987) pointed out that a low serum retinol concentration was associated with a high risk of cancer, but only within the first 3 years of illness, suggesting that reduced levels of retinol may be a metabolic consequence of cancer. Conversely, although a low serum level of β-carotene was also associated with a high risk of cancer, in this case the association persisted for many years before diagnosis, indicating that this relationship probably preceded the development of cancer.

In contrast, Coates et al. (1988) found no relationship between serum retinol and the subsequent development of cancer. In a study using previously stored serum from 156 cases of cancer and 287 matched controls, levels of retinol were not associated with the incidence of cancer at all sites combined, either overall and within subgroups defined by age, sex, smoking status, and family history of cancer.

Results of a recent study showed that levels of serum retinol, β-carotene, vitamin E, and selenium were all lower among individuals who later developed cancer than among controls (Comstock et al., 1991). Low levels of β-carotene were most likely to be associated with subsequent cancer, but there were marked differences by cancer site.

Carcinoma of the Lung

One of the earliest efforts to examine the specific relationship between lung cancer and serum levels of vitamin A was a small study conducted in 28 patients by Basu et al. (1976). They found that patients with either squamous- or oat-cell carcinoma had significantly lower plasma vitamin A levels than those with large-cell undifferentiated carcinomas.

The Trustees of Columbia Univ. Ex. 2126 - Page 9 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

Several years later, Willett et al. (1984a) compared sera collected from 111 individuals who developed cancer within the following 5 years with sera from 210 cancer-free matched controls. The mean values for retinol, RBP, and carotenoids were similar for the two groups, and the mean baseline retinol level in the 18 patients with subsequent lung cancer was actually higher than that in their matched controls.

In another study, Nomura et al. (1985) evaluated stored serum specimens obtained from over 6,800 men of Japanese ancestry from 1971 to 1975. By 1985, 81 colon, 74 lung, 70 stomach, 32 rectum, and 27 urinary bladder cancers had been diagnosed in this cohort. The stored serum from these cases was compared with that of 302 controls. No association was found between retinol and any of the cancers, although there was a significant association between β-carotene and lung cancer.

Other prospective studies have also found a relationship between serum levels of retinoids and lung cancer. Using blood samples collected previously, Menkes et al. (1986) compared sera from 99 lung cancer patients with those from 196 controls matched for age, sex, race, and smoking history. The results demonstrated a strong inverse association between serum β-carotene and the risk of squamous-cell carcinoma of the lung.

The Multiple Risk Factor Intervention Trial (MRFIT) evaluated baseline serum levels of β-carotene, total carotenoids, vitamins A and E, and RBP in 156 initially healthy male participants and 311 matched controls (Connett et al., 1989). Both total carotenoid and β-carotene levels were lower in the 66 patients who died of lung cancer than in their controls, and these relationships persisted after adjusting for smoking, alcohol intake, serum thiocyanate levels, and cholesterol levels. In contrast, there were no significant differences between cases and controls in their intake of total carotenoids or β-carotene and the number of overall cancer deaths. Serum levels of retinol, vitamin E, and RBP were not related to cancer at any site.

In one of the largest prospective epidemiologic studies of retinoids and lung cancer yet conducted, Knekt et al. (1990) stored sera from 36,265 Finnish people, then followed them for an average of 8 years. Sera from 766 patients with cancer diagnosed during the follow-up period were compared with samples from 1,419 matched participants who did not develop cancer. The mean level of serum retinol was 3.3% higher in male cancer patients than in male controls, and 2.8% higher in female controls than in female patients. There was an inverse gradient between serum retinol level and the occurrence of cancer in men, although this association was seen primarily in the first 2 years of follow-up. Differences between patients and controls in serum levels of β-carotene were far more dramatic. Serum levels of β-carotene were 14.0% higher in male controls than in male cancer patients and 5.5% higher in female controls than in female patients. These differences were particularly clear in lung cancer. The investigators suggested that the association between retinol and cancer may be due to preclinical cancer.

Most recently, Stahelin (1991) compared the serum levels of antioxidants and carotene in 204 persons who subsequently died of cancer (lung, 68; stomach, 21; colon, 17; other 99) with those of 2,421 controls assayed at the same time. Significantly lower mean carotene levels were found in all cancer patients, in patients with bronchial cancer, and in patients with stomach cancer than in controls, and the relative risk of lung cancer was significantly increased in subjects with low carotene levels. Low levels of both carotene and retinol were associated with higher risks for all cancers.

Carcinoma of the Oral Cavity and Oropharynx

Two studies compared the mean plasma levels of vitamin A and β-carotene in patients with carcinoma of the oral cavity and oropharynx with those of a control population (Ibrahim et al., 1977; Chaudhy et al., 1980). Both showed decreased levels of β-carotene in the cancer population. Moreover, patients whose disease progressed showed a progressive decrease in plasma vitamin A.

Carcinoma of the Esophagus

Biochemical analysis was performed on the sera of randomly selected individuals from a population characterized by a high incidence of oral and esophageal cancers (Zaridze et al., 1985). The results revealed low levels of riboflavin, vitamin A, and carotenoids.

Carcinoma of the Gastrointestinal Tract

Willett et al. (1984b) reported an inverse association between serum retinol levels and cancer among subjects with low serum selenium levels and subjects with gastrointestinal cancer. They also noted that the risk of cancer was elevated for individuals with both low levels of serum retinol and high levels of RBP.

Carcinoma of the Prostate

The relationship between serum retinol levels determined at the time of a baseline examination and the subsequent development of prostate cancer was explored in a cohort of 2,440 men 50 years of age or older (Reichman et al., 1990). The study participants were followed for a median of 10 years. During this period, 84 men

The Trustees of Columbia Univ. Ex. 2126 - Page 10 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

developed prostate cancer. The mean level of serum retinol was significantly lower in the cases of prostate cancer than in subjects who did not develop cancer, and a statistically significant trend was observed for an increased risk of prostate cancer with decreasing levels of serum retinol.

Carcinoma of the Cervix

Two studies point to the involvement of retinoids in the development of cervical cancer. In the first of these (Bernstein and Harris, 1984), sera drawn from sexually active patients with cervical intraepithelial cancer were compared with sera from matched normal controls. Although the dietary profiles of the two groups were not statistically different, the women with cervical cancer had significantly lower levels of serum retinol.

Potischman et al. (1991) evaluated 387 patients and 670 controls from four Latin American countries to determine whether the serum levels of eight micronutrients were associated with a risk of invasive cervical cancer. The analysis was restricted to patients with stage I or stage II disease in order to minimize the effect of disease on serologic markers. Mean levels of retinol in the cancer patients did not differ significantly from those in controls, but mean levels of β-carotene were lower in the cancer patients. A significant trend toward a decreasing risk was associated with higher levels of β-carotene.

Carcinoma of the Ovary

Das et al. (1987) reported that the mean concentrations of serum retinol and serum total cholesterol of ovarian cancer patients in Singapore were significantly lower than their respective concentrations in noncancerous control subjects. There were moderate direct correlations between retinol and cholesterol levels in the control subjects but not in the cancer patients.

Carcinoma of the Breast

Stored plasma samples from 5,004 women in Guernsey were prospectively studied by Wald et al. in 1984. Retinol, β-carotene, and vitamin E were measured in samples from 39 women who developed breast cancer and 78 controls who did not. Plasma retinol levels were not found to be related to the risk of breast cancer, whereas β-carotene levels showed a non-significant tendency to be lower in women who developed cancer than in controls. Plasma levels of vitamin E, on the other hand, showed a clear negative association with the risk of breast cancer.

Summary

Comparative studies have demonstrated the association between serum levels of retinol and the development of cancer in numerous sites, including the lung, oral cavity/oropharynx, esophagus, gastrointestinal tract, prostate, cervix, ovary, and breast. The evidence for a relationship between tumors in many of these sites and serum levels of β-carotene is quite strong, and several studies indicate that this relationship precedes the development of disease. However, the results for retinol are far less consistent. Although significant correlations between serum levels of retinol and certain tumors have been found, the data suggest that at least in some cases, this association may rather be a consequence than a cause of cancer.

SAFETY ASPECTS OF RETINOIDS

Treatment with retinoids is associated with a distinct toxicity profile typical of hypervitaminosis A, and which includes mucocutaneous reactions, liver toxicity, alopecia, and skeletal abnormalities (David et al., 1988). In children, the chronic use of retinoids may inhibit growth by prematurely closing the epiphyses (David et al., 1988). Both natural and synthetic retinoids may affect the eyes, resulting in blepharoconjunctivitis, pseudotumor cerebri, and, in the cases of isotretinoin and fenretinide, nyctalopia, or night blindness (Gross and Helfgott, 1992). Of all the adverse reactions associated with retinoids, their profound teratogenic effects on the central nervous system, heart, and craniofacial structures of the fetus are of most concern and have limited the use of these compounds in women of childbearing age (Shalita, 1988). With the exception of birth defects and liver and bone toxicities, most of the side effects of excess vitamin A ingestion are reversible following discontinuation of the drug (Yob and Pochi, 1987).

The type and severity of these adverse reactions vary with the retinoid compound used, the dose and duration of treatment, and the characteristics of the patient population receiving the drug.

All-*Trans*-Retinoic Acid

In general, the toxic effects associated with tretinoin, or all-*trans*-retinoic acid, have been those typically seen with retinoids (i.e., skin and mucous membrane effects, including dryness, itching, peeling, cheilitis, and penile excoriations; headache; pseudotumor cerebri; bone pain; digestive symptoms; elevation of liver enzymes; and hypertriglyceridemia and hypercholesterolemia; Huang et al., 1988; Warrell et al., in press).

The Trustees of Columbia Univ. Ex. 2126 - Page 11 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

In addition to these side effects, approximately 25 to 30% of patients who have received retinoic acid for the treatment of acute promyelocytic leukemias have experienced a unique combination of adverse reactions resembling the capillary leak syndrome (Frankel et al., 1992). This "retinoic acid syndrome" is characterized by marked leukocytosis (although many patients have normal leukocyte counts), thrombosis, fever, respiratory distress, radiographic pulmonary infiltrates, pleural effusions, and weight gain. Once established, the syndrome has proven extremely difficult to manage, and several patients have developed progressive hypoxemia and died from multiple organ failure. In most patients, progression of the syndrome can be halted by early intervention with corticosteroids (Warrell et al., 1993).

Isotretinoin

The adverse reactions most often observed in patients receiving isotretinoin (13-*cis*-retinoic acid, or 13-cRA) for the treatment of cancer (Clamon et al., 1985; Peck et al., 1988; Finklestein et al., 1992; Tangrea et al., 1992b), oral leukoplakia (Hong et al., 1986), or dermatologic conditions (Shalita et al., 1988; Bigby and Stern, 1988; McElwee et al., 1991) have been headache (in some cases attributable to pseudotumor cerebri), cheilitis, facial erythema, dry skin, dry eyes, hypertriglyceridemia, conjunctivitis, and myalgia.

In some studies, patients receiving isotretinoin have experienced ocular reactions. Patients receiving this drug for the treatment of acne have experienced a number of toxic effects involving the eye, such as anterior segment inflammation, dry eye syndrome, contact lens intolerance, altered refraction, photosensitivity, and reduced night vision (Caffery and Josephson, 1988). Other investigators have observed more serious ocular side effects in this patient population, including papilledema, pseudotumor cerebri, and white or gray subepithelial corneal opacities (Fraunfelder et al., 1985). All of these adverse reactions were reversible with discontinuation of the drug.

Occasionally, patients receiving 13-cRA for the treatment of myelodysplastic syndrome have developed unexpected thrombocytopenia. Some but not all of these patients had low platelet counts prior to treatment (Greenberg et al., 1985).

Because 13-cRA has been widely used in the treatment of acne, significant information has accumulated about its teratogenic potential, despite warnings in the labeling against use of the drug in women who are likely to become pregnant. Exposure to even a single dose of 13-cRA during any time within the first trimester of pregnancy has been associated with congenital malformations (Dai et al., 1992). Birth defects resulting from exposure to 13-cRA include the Dandy-Walker malformation, microencephaly, hypertelorism, small ear canals, cleft palate, small mouth, prominent frontal bossing, hydrocephaly, microphthalmia, small undifferentiated ears, and congenital heart disease (Benke, 1984).

Etretinate

The side effects most frequently associated with the use of etretinate have been alopecia; mucocutaneous symptoms, such as cheilitis, drying of the mucous membranes, thinning of the skin, and scaling; hypertriglyceridemia; hypercholesterolemia; mild structural changes in the liver and elevations of liver enzymes; and minor skeletal changes, including periosteal thickening, hyperostosis of the vertebral column, disk degeneration, osteoporosis, and calcification of the spinal ligaments and slender long bones (Camuto et al., 1987; Halkier-Sorenson and Andresen, 1989; Ciompi et al., 1988; Pilkington and Brogden, 1992). Less frequent have been extraspinal tendon and ligament calcification (Archer et al., 1989), thrombocytopenia (Liang, 1988), and birth defects (Orfanos et al., 1987).

Unlike other retinoids, etretinate has an extremely long half-life. Because of its high lipophilicity, it accumulates in subcutaneous fat and continues to be released from storage tissue for 6 to 12 months after therapy has been discontinued (Orfanos et al., 1987). Thus, whereas most of the side effects of other retinoids are readily reversible, this is not always the case with etretinate. For example, in rare cases, patients receiving etretinate have developed acute hepatitis that progressed to chronic active hepatitis despite discontinuation of the drug (Weiss et al., 1985). Because of the problems associated with the long half-life of etretinate, its use is strictly limited.

Fenretinide

Although far less clinical data are available on fenretinide (4-hydroxyphenyl retinamide, or 4-HPR), it appears to produce fewer of the usual manifestations of hypervitaminosis A that are commonly seen with all-*trans*-RA, 13-cRA, and etretinate, particularly mucocutaneous consequences. Unlike naturally occurring retinoids, 4-HPR does not accumulate in the liver, and therefore does not appear to be associated with serious hepatotoxicity. To date, the toxic effects specifically associated with 4-HPR have been night blindness, abnormal rod photoreceptor function, and skin rash (Gross and Helfgott, 1992; Modiano et al., 1990; Kaiser-Kupfer et al., 1986). These adverse effects are readily reversible upon cessation of the drug.

Further investigation has revealed that 4-HPR competes with retinol at a retinoid-binding site in the eye. In

The Trustees of Columbia Univ. Ex. 2126 - Page 12 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

ongoing clinical trials of this drug in the prevention of breast cancer, the introduction of a 3-day drug holiday, permitting circulating retinol to bind to the receptor, has significantly reduced the occurrence of nyctalopia.

Arotinoids

There are few clinical data on which to make a judgement about the safety of the new arotinoid series of drugs. However, the published literature makes it clear that these retinoid derivatives are likely to be teratogenic.

Summary

All retinoids are associated to one degree or another with the syndrome of hypervitaminosis A. Although this effect is not usually dose-limiting, the obvious presence of mucocutaneous side effects causes major problems in the design of blinded studies. The hepatic toxicity of individual retinoids varies according to their metabolism and deposition, with some (i.e., etretinate and 13-cRA) exerting more deleterious effects than others. Fenretinide seems to be unique in its propensity to cause nyctalopia, although the introduction of a 3-day drug holiday appears to ameliorate this problem. Finally, the teratogenic potential of all retinoids cannot be understated. The systemic administration of any retinoid should be avoided in women of childbearing potential unless the risks are specifically judged to outweigh the consequences.

THERAPEUTIC TRIALS

Natural and synthetic retinoids have a range of biologic activities that should be clinically exploitable. Retinoids modify immune reactions, exert potent effects on inflammatory processes, and markedly suppress the activity of sebaceous glands (Orfanos et al., 1987). From an oncologic perspective, however, the most important property of retinoids is their effect on cellular differentiation.

Although many retinoids have been synthesized and screened for activity, only a few have been evaluated clinically. When early studies of vitamin A were conducted, therapeutic doses resulted in the syndrome of acute hypervitaminosis A. Efforts were therefore focused on the synthesis of less toxic vitamin A derivatives (Bollag, 1983, 1985).

The first of these synthetic derivatives was all-*trans*-RA, which is formed by a chemical alteration of the polar end group of retinol from CH_2OH to COOH (Boyd, 1989). All-*trans*-RA, 13-cRA and 4-HPR can be considered as comprising the first generation of retinoids.

The second generation of retinoids consist of the aromatic compounds etretinate (Ro 10-9359) and its principal metabolite, etretin (Ro 10-1670). More recently, a third generation of closely related compounds has entered clinical trials. Called arotinoids, these polyaromatic compounds are composed of aromatic rings.

The primary clinical target for retinoids was acne and related skin diseases. Nevertheless, all-*trans*-RA, 13-cRA, etretinate, 4-HPR, and several arotinoids have all been investigated to one degree or another for the treatment of human malignancies.

Hematologic Malignancies

The *in vitro* ability of retinoids to induce differentiation in a variety of malignant cell lines has generated great interest in the use of these compounds in the treatment of hematologic malignancies. Although retinoid therapy has been investigated in myelodysplastic syndrome, chronic myelogenous leukemia, and cutaneous T-cell lymphoma, its greatest success by far has been in the treatment of acute promyelocytic leukemia (APL).

Myelodysplastic Syndrome

Many investigators have attempted to define the role of 13-cRA in the treatment of myelodysplastic syndrome (MDS). However, the results of both single-agent and combination trials were inconsistent; some trials demonstrated improvement related to treatment and others showed no benefit. These discrepancies may be due to the heterogenous clinical presentation of the underlying disease, but without more definitive data it is difficult to make any recommendations about the use of 13-cRA in the routine treatment of MDS. With the exception of a small study of 4-HPR, which demonstrated no activity, no other retinoids have been evaluated as treatment for patients with MDS.

Single-Agent Therapy

A number of investigators have reported positive results using 13-cRA as a single agent in treating MDS. In an early phase I trial of 13-cRA in 17 patients, Gold et al. (1983) observed improvements in hematologic parameters in 5 of 15 evaluable patients; the improvements included normalization of the bone marrow blast count and increases in leukocyte count, platelet count, or hemoglobin concentration.

The Trustees of Columbia Univ. Ex. 2126 - Page 13 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

Following this report, several other small studies showed minor or better responses to therapy with 13-cRA. Two of 18 patients treated with 13-cRA achieved partial responses in a phase I–II study by Greenberg et al. (1985); 8 of 15 patients treated with 13-cRA by Picozzi et al. (1986) had increases in peripheral granulocytes of 20% or more; therapy with 13-cRA led to a complete clinical and cytogenetic remission in a single patient reported by Abrahm et al. (1986); and 4 of 8 patients treated with 13-cRA by Kerndrup et al. (1987) achieved minor responses.

Larger studies also reported improvements in MDS patients treated with 13-cRA. Clark et al. (1987b) conducted a randomized trial involving 70 patients with MDS who had 5% or fewer marrow blast cells. The 1-year survival rate among treated patients with nonsideroblastic disease was 77% (23/30), compared with 39% (7/18) in the control group. There were too few deaths among patients with sideroblastic anemia (4/22) to permit an evaluation of the effect of 13-cRA therapy on survival in these patients.

The same group (1987c) examined the relationship between prognosis and surface antigen expression on peripheral blood granulocytes and monocytes in 37 patients with MDS. Patients who exhibited many abnormalities in antigen expression appeared to have had significantly shorter survival periods than those with few abnormalities. The investigators also compared antigen expression in six additional patients before and after 6 weeks of treatment with 13-cRA with antigen expression in control patients at similar points in time. Five of the six treated patients showed a decrease in abnormal antigen expression, compared to two of the six control patients. The authors concluded that myeloid antigen expression was a useful prognostic indicator, and could be altered by treatment with 13-cRA.

The most recent trial of 13-cRA as single-agent therapy for MDS was conducted by Leoni et al. (1988). In this study, 20 patients with primary MDS received the drug at doses of 50 to 100 mg/m^2/d for a minimum of 4 weeks. Twelve patients achieved an increase in hemoglobin levels of greater than 1 g/dL, and 7 experienced an associated increase in their granulocyte count of more than 50% over baseline values. However, sustained responses were seen only in patients with refractory anemia with or without ringed sideroblasts, and a normal or hypercellular bone marrow. Treatment did not improve the course of MDS in the 4 patients with excess blasts.

In contrast to these reports, other investigators have not found 13-cRA to be of benefit as a single agent in treating MDS. In two trials, one involving 4 patients (Hoffman and Robinson, 1988) and the other a study involving 68 patients (Koeffler et al., 1988), no significant responses to treatment were observed.

Combination Therapy

Several groups have investigated the use of 13-cRA in combination with other agents. Based on the *in vitro* findings that cytarabine (ara-C) and 13-cRA had a synergistic effect on the differentiation of leukemic cells, Ho et al. (1987) initiated a phase II trial of low-dose ara-C and 13-cRA in 14 patients with MDS. The response rate of one partial response and one minor response was no better than that reported for either drug used alone.

In a much larger study, 63 patients were randomized to treatment with either low-dose ara-C alone or low-dose ara-C in combination with both 13-cRA and 1-α-hydroxy-vitamin D_3 (Hellstrom et al., 1990). The addition of 13-cRA and vitamin D had no positive influence on remission rates, duration of remissions, or survival, but the patients who received the combination of these drugs experienced significantly more toxic effects.

Hellstrom et al. (1988) also investigated a four-drug regimen consisting of low-dose ara-C, 13-*cis*-RA, vitamin D, and interferon-α in 62 patients with MDS or acute leukemia. Evaluation of the full combination of these four agents was not possible because of toxicity. The reported overall response rate was 44%, but there was no significant difference noted between the number of patients who responded to the combination regimen (50%) and the number who responded to low-dose ara-C (43%).

Clark et al. (1987a) randomized 98 patients with MDS to treatment or control groups, and gave the treatment group 13-cRA alone if they had fewer than 5% marrow blasts or ara-C with 13-cRA added after 12 weeks if they had 6 to 30% marrow blasts. In low-blast patients, myeloid surface antigens reverted to a normal pattern. After 25 months of treatment there were no significant differences in survival between the treated and control groups, either in the total population or in the high- and low-blast groups considered separately. However, an analysis of 39 patients with non-sideroblastic disease and fewer than 5% blasts showed an increase in survival in the treated group.

In an effort to reduce the toxicity of full-dose (100 mg/m^2/d) 13-cRA in patients with MDS, Besa and colleagues compared patients who received the drug at full-dose with those who received a combination of full-dose 13-cRA and α-tocopherol (800 units/d). In three trials of this combination (Besa et al., 1987, 1988, 1990), the addition of α-tocopherol reduced the toxicity of 13-cRA and enabled patients to tolerate full-dose therapy.

In the single small study of 4-HPR in patients with MDS, the drug appeared to enhance leukemic progression (Garewal et al., 1989). No evidence of efficacy was observed when the drug was administered to 14 patients at 300 mg/d, later escalated to 400 mg/d. Two patients

with longstanding stable sideroblastic anemia experienced leukemic progression. In one of these patients, clinical features of chronic myelomonocytic leukemia appeared. The second patient developed acute myelomonocytic leukemia with cytogenetic progression.

In summary, available data on the use of retinoids in MDS are less than adequate. Most studies in this area have been based on small numbers of patients, have involved multiple agents, or have used complicated study designs, making the role of retinoids in this complex disease difficult to determine. Although there have been a few tantalizing reports suggesting that 13-cRA may have activity in MDS, definitive clinical data are not available.

Acute Promyelocytic Leukemia

Although retinoids have demonstrated some clinical benefit in a number of malignancies, the most compelling and thoroughly documented results have been obtained with all-*trans*-RA in the treatment of APL. The discoveries resulting from trials of all-*trans*-RA have not only led to immediate changes in the management of patients with APL, but have contributed substantially to an understanding of the pathogenesis of this disease, as well as to an elucidation of the mechanism of action of RA in general. Finally, changes observed at the clinical, microscopic, and genetic levels have provided unequivocal documentation that cell differentiation constitutes a potent and practical alternative means for the management of human cancer.

In the mid-1970s, Rowley et al. (1977) observed that APL was consistently associated with a specific translocation between chromosomes 15 and 17. A decade later, RAR-α was shown to map to chromosome 17 (Mattei et al., 1988). Since then, several groups have cloned the t(15;17) breakpoint and found a receptor rearrangement in virtually all examined patients with APL (Borrow et al., 1990; de Thé et al., 1990; Lemmons et al., 1990; Alcalay et al., 1991).

As this work was in progress, other investigators began exploring the use of retinoids in treating APL. Early reports suggested that treatment with 13-cRA could induce the differentiation of APL cells and effect clinical remissions (Flynn et al., 1983; Nilsson, 1984; Daenen et al., 1986; Fontana et al., 1989; Wijermans et al., 1989). Shortly thereafter, Chinese investigators began trials of all-*trans*-RA in the treatment of APL (Huang et al., 1987).

Although *in vitro* studies by Breitman et al. (1980, 1981) initially indicated that 13-cRA and all-*trans*-RA were equally effective in inducing the differentiation of APL cells, later studies have shown that 13-*cis*-RA is less effective than all-*trans*-RA (Chomienne et al., 1990a). Furthermore, recent efforts to treat patients with 13-cRA have not resulted in meaningful clinical responses (Runde et al., 1993), and this drug is not recommended for use in APL.

Single-Agent Therapy

Research groups in Asia, Europe, and the United States have now treated approximately 1,500 APL patients with all-*trans*-RA as a single agent. The results reported in 565 of these cases have been summarized in a recent review article by Warrell et al. (1993). With a usual dose of 45 mg/m^2/d, complete response rates of 50% (22/44) were observed in Bethesda, Maryland (Light et al., 1992), while complete response rates of 70% (18/26) were observed in Japan (Ohno et al., 1992), of 74% (23/31) in Paris (Castaigne et al., 1990), of 77% (45/58) in New York (Warrell et al., 1991), of 85% (340/400) in Shanghai (Huang et al., 1987; Huang et al., 1988; Sun et al., 1992), and of 94% (47/50) in Suzhou (Chen et al., 1991). Combined, these studies demonstrate a mean complete response rate of 84%, comparing favorably with the 60 to 80% complete response rate achieved with conventional chemotherapy in APL (Mayer et al., 1987; Kantarjian et al., 1986; Cummingham et al., 1989; Rodeghiero et al., 1990).

In evaluating the response rates achieved in these studies, two factors should be kept in mind. First, the trials included both patients with newly diagnosed disease and those who had relapsed from or were resistant to chemotherapy. Second, since a clinical response to all-*trans*-RA has been found to be closely correlated with the presence of the (15;17) translocation (Chomienne et al., 1990b; Miller et al., 1990; LoCoco et al., 1991), it is important to note that 10 to 15% of the patients treated probably did not have this translocation (Warrell et al., 1993). Patients with a documented receptor rearrangement have exhibited considerably higher response rates, generally in the 95% range. On the other hand, patients who do not exhibit this translocation have not responded to treatment (Miller et al., 1992).

The period to remission of APL with all-*trans*-RA is reported to be from 1 to 3 months (Sun et al., 1992; Warrell et al., 1992). Unfortunately, these remissions have been of short duration, ranging from 1 to 14+ months, with a median of 4 months (Warrell et al., 1992). Only a few patients treated with all-*trans*-RA alone have maintained remissions for more than 1 year (Huang et al., 1988; Sun et al., 1992; Castaigne et al., 1990; Warrell et al., 1991; Chen et al., 1991).

In vitro studies suggest that relapse and resistance to all-*trans*-RA may be due to its clinical pharmacology. Continuous daily treatment with all-*trans*-RA results in a marked decrease in plasma drug concentrations that

The Trustees of Columbia Univ. Ex. 2126 - Page 15 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

has been associated with relapse (Muindi et al., 1992), leading some researchers to suggest that clinical failure may be due to the inability to sustain therapeutic concentrations of the drug.

Combination Therapy

Efforts to extend the remission of APL with maintenance therapy combining all-*trans*-RA and other chemotherapeutic agents have not been successful. Although patients who relapsed after chemotherapy-induced complete responses could be effectively reinduced to a second complete remission with all-*trans*-RA, the reverse has not been true. In fact, if relapse occurred after a complete response induced by a combination of chemotherapy and RA, sensitivity to RA was greatly reduced; thus, some investigators have suggested that all-*trans*-RA be replaced by conventional chemotherapy as soon as a complete response is reached (Chen et al., 1991).

All-*trans*-RA appears to be more effective than conventional chemotherapy in inducing initial remissions from APL, and this induction is achieved with less morbidity and probably less mortality. There are no data to suggest a role for all-*trans*-RA as a maintenance therapeutic agent or as a means of preventing relapse in APL, however, and there is preliminary evidence that the long-term administration of this agent may actually be disadvantageous (Warrell et al., 1993). Therefore, the current practice is to follow induction with several cycles of consolidation chemotherapy.

Although 13-cRA has no clinical activity in APL, *in vitro* studies indicate that another retinoid analogue, 9-cRA, may be as effective as all-*trans*-RA (R. Warrell, personal communication). If 9-cRA or other retinoids are not followed by the reduction of plasma levels associated with all-*trans*-RA, they may be appropriate for more extended use as maintenance agents. In any event, the seminal studies of all-*trans*-RA in the treatment of APL provide a basis for further work in exploiting the potential of retinoids as antineoplastic agents with a novel mechanism of action.

Chronic Myelogenous Leukemia

Studies of retinoids in chronic myelocytic leukemia (CML) have been scanty, and the results have not represented an improvement over those with conventional chemotherapy.

Presant and Bearman (1981) reported preliminary findings of a randomized trial that used 13-cRA either alone or in combination with busulfan for the treatment of chronic-phase CML. No differences were noted in the time to development of blastic transformation. The addition of the retinoid did not increase the acute toxicity of busulfan.

In an effort to increase the rate and duration of remissions achieved with chemotherapy in patients with chronic-phase CML, Arlin et al. (1987) added 13-cRA to a combination regimen consisting of daunorubicin, ara-C, and thioguanine. Of 17 patients who were evaluable, 1 (6%) patient achieved a complete remission and 7 (41%) achieved partial remissions. The median duration of remission was 1.6 months, which did not represent an improvement over the results with chemotherapy alone in chronic-phase patients.

Cutaneous T-Cell Lymphoma/Mycosis Fungoides

As single agents, several retinoids, including 13-cRA, etretinate, and the arotinoid ethylester Ro 13-6298, have proven beneficial in treating patients with cutaneous T-cell lymphoma (CTCL)/mycosis fungoides (MF). On the other hand, the results of studies using retinoids combined with other modalities have been less consistent. In some trials, the addition of retinoids improved the results obtained with phototherapy, chemotherapy, or interferon-α. In contrast, the combination of retinoids and radiotherapy did not elicit a greater response than radiotherapy alone.

Single-Agent Therapy

In one of the earliest attempts to use retinoids in the treatment of CTCL, Kessler et al. (1983) administered 13-cRA to four patients with refractory tumors. Extensive tumors and plaques were nearly completely cleared in one patient, who remained in partial remission with continued improvement for over 15 months. Two other patients showed an improvement in pruritus and a 50% reduction in plaques at 4 and 6 weeks, respectively. The fourth patient experienced improvement but was forced to discontinue therapy because of dryness and scaling. In another study, two of six patients with MF refractory to electron-beam radiotherapy also showed significant improvements in cutaneous disease when treated with 13-cRA (Warrell et al., 1982).

Thomsen et al. (1984) administered 13-cRA to 20 patients with mycosis fungoides (MF) and 4 with Sézary syndrome. Of the patients with MF, 6 (30%) achieved complete responses and 10 (50%) had partial responses. Among responders, the first sign of remission was seen within 2 to 4 weeks. Although 13-cRA was effective in refractory MF, no convincing responses were seen in the patients with Sézary syndrome.

Kessler et al. (1987) also found encouraging results in a phase II trial of 13-cRA. Of 25 patients with extensive MF, 11 (44%) had objective responses, three of which were complete clinical responses without concomitant evidence of pathologic clearing of the disease. The me-

The Trustees of Columbia Univ. Ex. 2126 - Page 16 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

dian time to response was 2 months and the median duration of response was 8 months or longer.

All six patients with CTCL treated with 13 cRA by Neely et al. (1987) experienced symptomatic relief, with a fading of skin lesions and disappearance of pruritus, although post-treatment biopsy specimens showed no changes from pretreatment specimens.

In a comparison of two different retinoids in the treatment of MF and Sézary syndrome, Molin et al. (1987b) administered 13-cRA to 39 patients and etretinate to another 29 patients. Eight (21%) of the 13-cRA-treated patients experienced complete remission and 15 (38%) experienced partial remissions within 2 months. Comparable responses were seen in patients receiving etretinate; 5 (21%) achieved a complete response and 11 (46%) a partial response. Only one patient with Sézary syndrome had a partial remission.

Hungarian researchers have employed etretinate in patients with CTCL (Dobozy et al., 1987). Two of the three patients treated experienced considerable relief of symptoms, and in the third, total relief was achieved after 4 to 8 weeks of therapy. However, all three patients relapsed.

Other investigators have tried a new polyaromatic arotenoid-ethylester, Ro 13-6298, in the treatment of CTCL. This drug has a much higher antiproliferative activity than other known retinoids. Tousignant et al. (1987) were successful in controlling a case of MF limited to the skin with Ro 13-6298. In a small trial with this arotinoid, Hoting and Meissner (1988) treated six patients with refractory CTCL. Three of the six patients had objective responses, one complete and two partial. The complete response had been maintained for 102 weeks at the time of the report, whereas the mean duration of the partial responses was 43 weeks.

Combination Therapy

In several studies, retinoids have been combined with psoralen plus long-wavelength ultraviolet light (PUVA) in treating MF. Souteyrand et al. (1981) treated 10 patients with MF with etretinate alone or in combination with PUVA. As a sole treatment, etretinate did not cause significant clinical improvement, but when used in combination with PUVA, it effected rapid improvement with complete clearing of lesions in several cases. Similar responses have been reported by Zachariae et al. (1982).

Thomsen et al. (1989) also reported greater success in treating MF when retinoids were combined with PUVA. Sixty-nine patients with MF were treated with PUVA or the combination of etretinate, 13-cRA, and PUVA. The numbers of complete responses achieved with the two regimens were equivalent (72% with PUVA alone; 73% with retinoids plus PUVA). However, remissions were obtained with fewer PUVA sessions and with a lower UVA dosage when PUVA was combined with retinoids; furthermore, the duration of remissions tended to be prolonged if retinoids were given as maintenance therapy.

Sixteen patients (12 with CTCL, one with Sézary syndrome, one with actinic reticuloid, and two with parapsoriasis) were treated with either arotinoid ethylester (Ro 13-6298) alone or a combination of etretinate and PUVA (Mahrle and Thiele, 1987). Most of the patients had minor to distinct responses; more than 50% of the skin lesions cleared in 67% of the patients. After discontinuation of the retinoid therapy, relapses occurred in all cases within 3 to 10 weeks. There was no difference in efficacy between the arotinoid alone and the combination of etretinate and PUVA, but the latter was less toxic.

In an effort to prolong the relapse-free period from the 2 to 4 years typically obtained with radiotherapy in patients with early-stage MF, Jones et al. (1992) treated 23 patients with a combination of radiotherapy and etretinate. After 2 years, the mean relapse-free survival period of complete responders who received the combination regimen was similar to that of stage-matched concurrent and historical controls.

Recently, there has been interest in combining retinoids with interferon-α to treat CTCL. In a small study of 11 patients given recombinant interferon-α-$_{2A}$ either alone or in combination with etretinate, patients in both groups responded, but it was unclear whether the combination was superior to single-agent therapy (Zachariae and Thestrup-Pedersen, 1990).

A larger study by Dreno et al. (1991) illustrated that etretinate could improve the results obtained with interferon-α in CTCL. Forty-five patients with CTCL (32 with MF and 13 with Sézary syndrome) were treated with low-dose recombinant interferon-α_{2A} for 3 months. Seventeen patients (38%) responded to this regimen and continued to receive interferon-α alone. The remaining 28 patients were given a combination of etretinate and interferon. Eleven of these patients achieved complete or partial remissions in response to the combination regimen, raising the response rate of the total study population to 62%.

A few investigators have attempted to combine retinoids and chemotherapy, but the results of these small studies have not been definitive. Molin et al. (1987a) treated 8 patients with progressive-phase MF with a combination of bleomycin, cyclophosphamide, and prednisolone and 12 patients with this three-drug regimen plus etretinate. The objective response rates achieved in the two groups were comparable (5/8 and 7/12, respectively).

In 1987, Zachariae and Thestrup-Pedersen reported the results of a 6-year study of 20 patients with advanced MF who were treated with a combination of etretinate and bleomycin, cyclophosphamide, and prednisone. Sixteen of the patients (85%) had initial complete remis-

The Trustees of Columbia Univ. Ex. 2126 - Page 17 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

sions, lasting an average of 8 months. Seven patients achieved a second complete remission. The overall survival rates were 50% after 2 years and 30% after 4 years. These results indicate that the combination of etretinate and chemotherapy compares favorably with other chemotherapeutic regimens used in treating MF.

Conclusions

In an effort to exploit the effects of retinoids on cellular differentiation, many investigators have attempted to utilize these compounds in treating hematologic malignancies. Unfortunately, the results in studies of MDS and CML have been either unimpressive or inconsistent. In contrast, a number of retinoids have evinced activity in small studies of CTCL, but larger, carefully designed trials are needed to confirm these results.

In the case of APL, however, all-*trans*-RA has proven superior to conventional chemotherapy in inducing initial remissions, and has provided clear evidence that the differentiation-inducing properties of retinoids can be employed in treating certain tumors. The newer retinoid, 9-cRA, may be even more beneficial in this disease, perhaps offering the possibility of long-term maintenance therapy.

Epithelial Malignancies

Retinoids have been studied in the treatment of advanced malignancies, but their significant activity has so far been primarily limited to two epithelial tumors: nonmelanomatous skin cancer and cervical cancer (Smith et al., 1992).

Skin Cancer

Squamous-cell carcinoma of the skin frequently results in severe cosmetic deformity of the face and causes about 3,000 deaths per year in the United States. In the United States alone, approximately 5 to 10%, or tens of thousands, of patients with squamous-cell carcinoma of the skin are not cured by local therapy. There is no standard effective systemic therapy for advanced local, regional, or distant metastatic squamous-cell carcinoma of the skin.

Single-Agent Therapy

A large body of preclinical pharmacologic and clinical data support the use of retinoids in the treatment of skin cancer. Overall response rates to single-agent retinoid therapy range from 45 to 100%. The best-studied single agent is 13-cRA, followed by etretinate and arotinoid therapy. An initial study from the University of Arizona, using 13-cRA in a diverse group of skin cancers, reported that three of five patients with advanced squamous-cell carcinoma of the skin had major responses. Following the initial trial, an additional 11 patients with advanced skin cancer were treated, and 6 patients responded (Meyskens et al., 1982; Lippman and Meyskens, 1987).

Combination Therapy

The rationale for combining 13-cRA and interferon-α is that it has shown additive antitumor and synergistic activity in many *in vitro* systems (Bollag, 1983). *In vivo* work also indicates that the combination of 13-cRA and interferon has an enhanced antitumor effect in solid tumor xenograft models. Chemoprevention data indicate that 13-cRA has activity in premalignancy and in the prevention of invasive cancer in the high-risk patient population. Interferon-α is known to have activity in actinic keratoses. Combinations of biologic-response modifiers such as interferon-α and 13-cRA have been studied at the University of Texas M. D. Anderson Cancer Center. Patients with advanced skin cancer received 13-cRA, 1 mg/kg/d, and interferon-α subcutaneously at 3 million units/d. All patients received the combination treatment for at least 2 months. Of the 32 patients enrolled in the study (28 evaluable), half presented with metastatic disease, and many were of notably advanced age (median, over 65 years). The overall response rate was over 60%, and the complete response rate was 25%. Subset analysis revealed lower response rates (below 30%) in patients with advanced metastatic disease. The major limiting toxic effects were persistent fatigue from the interferon-α and mucocutaneous disorders from 13-cRA (Lippman et al., 1992a). Future clinical studies with laboratory correlates of interferon combinations in advanced skin cancer, in an adjuvant setting or integrated with cytotoxic or other biologic agents, will contribute important information about the multimodality approach to controlling advanced skin cancer.

Head and Neck Cancer

Current regimens for systemic chemotherapy have not significantly prolonged the survival of patients with advanced head and neck cancer. The median survival of patients with recurrent head and neck cancer is 4 to 6 months. The current regimens' inability to prolong survival indicates the urgent need for new drugs for treating these cancers. Retinoids have significant chemopreventive activity in the genesis of squamous-cell carcinoma of the head and neck. Modest activity has been reported with the use of 13-cRA as a single agent against recurrent squamous carcinoma of the head and neck, with a response rate of approximately 20%. A combination of 13-cRA and interferon-α has been used in patients with recurrent head and neck cancer; however, of the 21 patients treated, none had a complete response and only

The Trustees of Columbia Univ. Ex. 2126 - Page 18 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

1 had a partial response. Two patients had a minor response, four patients had stable disease, and 13 patients experienced disease progression. The combination of 13-cRA and interferon-α appears to be ineffective as a treatment for recurrent squamous-cell carcinoma of the head and neck (Voravud et al., 1993). Future studies should focus on locally advanced, unresectable disease rather than on recurrent tumors, and on adjuvant treatment in combination with a cytotoxic agent with or without local radiotherapy. At present, the efficacy of retinoids in the treatment of recurrent head and neck cancer, either as single agents or in combination with interferon-α, is limited.

Advanced Lung Cancer

Lung cancer is the leading cause of cancer mortality in the United States. Approximately 60 to 70% of patients with non-small-cell lung cancer present with advanced, unresectable disease. Retinoids have been reported to reverse sputum atypia in heavy smokers and to decrease squamous metaplasia diagnosed by bronchial biopsy. Several studies have evaluated the correlation between serum retinol and carotenoid levels and the development of lung cancer. Decreased serum retinol levels have been correlated with an increased risk of lung cancer, but the strongest association was between low β-carotene levels and lung cancer. The results of a Phase II study of 13-cRA in the treatment of advanced non-small-cell lung cancer were disappointing; the response rate was less than 10% (Grunberg et al., 1987).

Interferon-α was used in patients with squamous-cell carcinoma of the lung and was found to be ineffective (response rates about 5%). *In vitro* studies of a combination of interferon-α and 13-cRA showed a greater antiproliferative effect on various squamous carcinoma cell lines than did either agent alone. Furthermore, this combination was shown to have substantial activity in patients with squamous-cell carcinoma of the skin or cervix. We performed a prospective Phase II trial of this combination of agents in patients with locally advanced or metastatic squamous-cell cancer of the lung. Twenty-one patients were enrolled and 17 of them were evaluable for response, 4 with locally advanced and 13 with metastatic disease. Disappointingly, only one patient with locally advanced disease had a partial response, and he experienced substantial toxic effects consisting of mucocutaneous side effects, fatigue, and anorexia; these effects caused eight patients to terminate the study. The combination of 13-cRA and interferon-α is unlikely to produce significant clinical activity in patients with metastatic squamous-cell lung cancer (Rinaldi, 1993). Future research should focus on integrating cytotoxic agents with local radiotherapy for advanced non-small-cell lung cancer.

Bladder Cancer

Patients with superficial bladder cancer are at high risk for recurrence of the disease (Bonney and Prout, 1980). The effect of retinoids on recurrent disease has been studied. Among 102 bladder cancer patients, tumor recurrence was not significantly different in the patients with the highest reported dietary vitamin A intake and the remainder of the group (Michalek et al., 1987; Ross et al., 1988). Other investigators have also studied the effects of retinoids on recurrent bladder cancer, with conflicting results. Among the early studies, one trial demonstrated three responses in five patients given etretinate, whereas another trial of 13-cRA had to be stopped because of toxicity. A trial of 13-cRA in combination with 5-fluorouracil and interferon-α was conducted in patients with recurrent bladder cancer. The activity of this combination was no better than that of 5-fluorouracil with interferon-α. Moreover, the retinoid regimen was associated with significant toxicity requiring reduction of the 13-cRA dose (Recondo et al., 1991). The therapeutic value of retinoids in recurrent bladder cancer appears to be limited and needs further investigation. A combination of a cytotoxic agent and one of the newer retinoids, such as 4-HPR or 9-cRA, may be effective.

Cervical Cancer

The use of single-agent retinoid therapy in invasive cervical cancer is limited. Two of five patients with refractory end-stage cervical cancer had minor responses when treated with 13-cRA. A combination of 13-cRA and interferon-α has been tried in advanced cervical cancer. The rationale for using this particular combination in cervical cancer was its ability to inhibit viral carcinogenesis *in vivo* and to suppress papilloma-virus-transformed cell growth. The retinoid was given daily at a dose of 1 mg/kg, and interferon-α was subcutaneously injected at a dose of 6 million units daily. Thirty-two patients were treated; 24 (75%) had stage II or higher disease and 18 (56%) had a primary lesion of more than 10 cm in diameter. The treatment regimen was fairly well tolerated, except for mild cheilitis, conjunctivitis, malaise, and fever, and 16 patients (50%) had major responses. Four patients had a complete clinical response, in 3 of whom the response was pathologically confirmed at hysterectomy. Symptomatic relief correlated dramatically with response. The median time to response was 1 month and the duration of response was approximately 3 months (Kavanagh et al., in press; Lippman et al., 1992b). The presence of nuclear retinoic acid receptors and of human papillomavirus, as well as immunohistochemical staining for nuclear antigen in proliferating cells (PCNA) of the tumor specimen at various points during treatment, are now being studied as possible laboratory correlates of

The Trustees of Columbia Univ. Ex. 2126 - Page 19 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

retinoid efficacy. Ongoing studies are focusing on the use of 13-cRA with interferon-α as neoadjuvant setting followed by radiotherapy, with the simultaneous administration of 13-cRA and interferon-α in advanced cervical cancer. Cervical cancer is a major public health problem in countries with a relatively low standard of living. There is strong evidence that viruses, particularly papillomaviruses, have a role in the development of this cancer. Retinoids, either as single agents or in combination with other biologic substances, represent a unique approach to improving the biologic understanding of and devising new therapeutic modalities for advanced cervical cancer.

PREVENTION TRIALS

The survival rates for patients with many epithelial tumors and the increasing incidences of these tumors remain beyond the control of established preventive and therapeutic modalities (Greenwald et al., 1990). One of the new directions for controlling these tumors is chemoprevention, the systemic use of a specific chemical or natural agent to reverse or suppress the progression of premalignant cells or lesions to the point of invasive cancer (Sporn et al., 1976; 1979; 1991). Data from epidemiologic and animal experiments provide a strong rationale for trials of chemoprevention in humans (Bertram et al., 1987). Experiments in animal models of carcinogenesis have demonstrated that specific chemical agents can prevent premalignant lesions from progressing to invasive cancers. Epidemiologic studies strongly suggest the existence of dietary inhibitors of carcinogenesis in humans.

Two important concepts support the study of chemoprevention. One is the concept of multistep epithelial carcinogenesis. The basic premise of chemoprevention is that the process of multistep carcinogenesis can be stopped or reversed in the preinvasive stage. The other concept is field cancerization (Slaughter et al., 1953). This is the process whereby multiple malignant or premalignant foci form throughout the tissues of a "field," or area repeatedly exposed to a common carcinogenic insult. An example is the exposure of the entire upper aerodigestive tract and lung to tobacco smoke, which induces multiple, diffuse primary premalignant foci in the aerodigestive tract. Cancer chemoprevention trials employ two types of endpoints. One is a definite or long-term endpoint, such as cancer incidence, and the other is a short-term, intermediate biologic endpoint. Currently, the cancer incidence endpoint must be used in phase III studies to definitively establish the efficacy of chemopreventive agents. Intermediate endpoints, including premalignant lesions and biochemical and molecular markers, are used primarily in phase I and II trials to indicate biologic activity and to select an agent for phase III testing. Subjects for chemoprevention trials are drawn from the general population and from special populations, such as individuals at high risk owing to previously cured cancer, those with premalignant lesions, and those at high risk from genetic or acquired risk factors.

Chemoprevention Trials in Human Premalignant Lesions

Oral Premalignancy

Cancer of the upper aerodigestive tract has become an important model for understanding human carcinogenesis. As mentioned above, exposure to known carcinogens such as tobacco and alcohol puts the entire epithelium of the upper aerodigestive tract at risk for the development of cancer. Oral leukoplakia and erythroplakias, consisting of white or reddish plaques of the epithelial lining of the oral cavity, are premalignant lesions that frequently develop into invasive cancer. One long-term follow-up (an average of 7.3 years) demonstrated that 17.5% of 257 patients with leukoplakia developed invasive squamous cancer (Silverman et al., 1984). Surgery has been considered the standard therapy for this condition, but it is not always possible. The effects of retinoids on epithelial differentiation and proliferation led us to investigate the efficacy of retinoids in reversing oral leukoplakia (Shklar et al., 1980). Studies describing the use of retinoids and carotenoids for the treatment of oral leukoplakia are summarized in Table 1. These data clearly demonstrate the ability of retinoid therapy to induce objective responses in oral leukoplakia.

The most effective and least toxic form of retinoid therapy for oral leukoplakia has not yet been established; however, among the studies in this area, the trial reported by Hong et al. (1986) is quite unique. The investigators tested the activity of 13-cRA in a randomized, placebo-controlled, double-blind trial involving 44 leukoplakia patients. The patients were treated with a dose of 1 to 2 mg/kg/d of 13-cRA for 3 months, with an additional 6 months of follow-up. The trial demonstrated several important features of retinoid therapy in oral premalignancy. Sixty-seven percent of the patients had an objective response to the therapy, and 54% had a histologic response. In contrast, the patients receiving placebo had only a 10% objective response rate. The therapy was fairly toxic; two-thirds of the patients experienced mucocutaneous toxicity and hypertriglyceridemia. These toxic effects resolved when the patients stopped taking the drug. Forty-seven percent of patients treated with high-dose 13-cRA required dose reduction.

Another important aspect of this trial was the high rate of relapse following discontinuation of the drug. Within 2 to 3 months after the completion of treatment, 9 of the 16 patients who had experienced an objective response had relapsed. This led us to design a 12-month trial to evaluate the efficacy and toxicity of low-dose 13-cRA in

The Trustees of Columbia Univ. Ex. 2126 - Page 20 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

TABLE 1. *Retinoid and natural-agent chemoprevention trials in oral leukoplakia*

Investigator	Agent	Evaluable patients	Response (complete response plus partial response) (%)
Koch and Schettler (1973)	13-cRA	24	87
	Tretinoin	27	59
	Etretinate	24	91
Shah et al. (1983)	13-cRA	11	82
Koch (1978)	Etretinate	21	71.5
	Etretinate	24	83.5
Hong et al. (1986)	13-cRA	24	67
	Placebo	20	10
Lippman et al. (1993)	13-cRA	60	55
Garewal et al. (1990)	β-carotene	17	71
Toma et al. (1990)	β-carotene	15	26
Stich et al. (1988)	β-carotene	27	14.8
	β-carotene and vitamin A	51	27.5
	Placebo	33	3
Silverman et al. (1963)	Vitamin A	16	44
Silverman et al. (1965)	Vitamin A	6	83
Stich et al. (1991)	Vitamin A	21	51.7
	Placebo	33	3.0
Benner (1993)	α-Tocopherol	43	46

13-cRA, 13-*cis*-retinoic acid.

maintaining remission or non-progression in oral premalignancy. This second trial was conducted in two phases, a 3-month induction phase employing high-dose 13-cRA, followed by 9 months of maintenance with either low-dose 13-cRA (0.5 mg/kg/d) or β-carotene (30 mg/d). Seventy patients were enrolled in this study. We concluded that low-dose 13-cRA, if preceded by high-dose induction, was significantly more active than β-carotene in oral leukoplakia, and was as easily tolerated (Lippman et al., 1993). After the synthetic retinoids, β-carotene is the most-studied agent in oral leukoplakia, followed by vitamin E and selenium. The efficacy and safety of a combination of retinol and β-carotene in oral leukoplakia are under investigation.

Bronchial Metaplasia

The bronchial epithelium is normally lined by pseudostratified ciliated columnar cells. People who are smokers generally exhibit squamous metaplasia with or without atypical cells (Auerbach et al., 1956). Using well-defined criteria, dysplasia can be graded as mild, moderate, or severe. Bronchial metaplasia and dysplasia are generally regarded as premalignant precursors of lung cancer. They can be sampled directly by bronchoscopic biopsy and washing, or indirectly by sputum sampling. Preclinical studies *in vitro* and *in vivo* unequivocally indicate that retinoids can prevent or delay the formation of tumor and effect tumor regression in experimental lung cancer (Saffiotti et al., 1967). This finding is supported by epidemiologic evidence that reduced retinoid or carotenoid intake or reduced serum levels of these substances lead to a higher incidence of lung cancer.

Several clinical studies of retinoids or other micronutrients in reversing bronchial metaplasia have been completed, and other studies are currently underway or in the planning stages (Table 2). Saccomanno et al. (1982) administered a relatively high dose of 13-cRA to 26 patients with documented cytologic abnormalities of the sputum; the duration of treatment ranged from 1 day to 40 weeks. There was no improvement in the degree of atypia after treatment. Mathe and colleagues evaluated the efficacy of etretinate (25 mg/d) for 6 months in heavy smokers with bronchial metaplasia. Bronchial metaplasia was assessed by the evaluation of biopsy specimens obtained at bronchoscopy. Among 106 patients screened with initial bronchoscopy, 54 received treatment based on the presence of bronchial metaplasia (Mathe et al., 1982). Thirty-four patients underwent follow-up bronchoscopy and were considered evaluable. Of these 34 patients, 4 had stopped smoking and had no metaplasia observed on the follow-up examination. The metaplasia index in the 34 evaluable patients dropped from a mean of 35% at baseline to 27% following 6 months of etretinate ($p < 0.001$).

Heimberger et al. (1988) evaluated the effects of folate and vitamin B_{12} on squamous metaplasia in chronic smokers. The hypothesis for this trial was that folate deficiency secondary to cigarette smoking was the most significant factor contributing to the development of bronchial metaplasia. This was based on the observation that smokers with bronchial metaplasia had lower plasma folate levels than smokers without bronchial metaplasia. Seventy-three individuals with bronchial metaplasia were randomized to receive either folate at 10 mg/d and vitamin B_{12}, or placebo for 4 months. Serum folate levels were monitored and were found to markedly increase in the group that received folate and vitamin B_{12}. Interestingly, the number of subjects with improvement in the degree of atypia on sputum cytology was significantly higher in the treatment group than in the placebo group ($p = 0.02$).

Recently, the randomized trial of Arnold et al. (1992) failed to demonstrate that etretinate caused a significant

The Trustees of Columbia Univ. Ex. 2126 - Page 21 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

TABLE 2. *Chemoprevention trials of lung premalignancy*

Trial	Participants in study	Agent	Results
Saccomanno (1974)	26	13-cRA 1.0–2.5 mg/kg/d	No improvement in metaplasia by sputum cytology
Gouveia et al. (1986)	40	Etretinate 25 mg/d	Metaplasia index on bronchoscopic biopsies from 34.57% to 26.96% in 34 evaluable subjects ($p < 0.001$)
Heimberger et al. (1988)	36	10 mg folate + 500 mg vitamin B_{12} vs. placebo	Grade of sputum atypia improved in treatment arm without significant change in placebo arm ($p = 0.02$)
Lee et al. (1993)	87 Randomized	13-cRA 1 mg/kg/d vs. placebo	No improvement in metaplasia in the 13-cRA group
McLarty et al. (1991)	705 Randomized	β-carotene, 50 mg, and retinol, 25,000 IU daily vs. placebo	Increased serum β-carotene levels in treatment group

d, day; IU, international units; 13-cRA, 13-*cis*-retinoic acid.

reversal of sputum atypia. The subjects of this study had a cigarette smoking history of at least 15 pack years and were screened for sputum atypia. One hundred fifty individuals who demonstrated mild atypia in at least two specimens, or who demonstrated moderate or severe atypia in one specimen, were randomized to receive either etretinate (25 mg/d) or placebo. Seventy-five subjects were entered in each arm of the trial. Of the patients who completed 6 months of treatment, 71 of those receiving etretinate and 67 receiving placebo were evaluated for response. Improvement in the degree of sputum atypia was noted in 32% of the individuals who received etretinate and 30% of the individuals who received placebo.

Hong and colleagues at M. D. Anderson Cancer Center are currently performing a randomized, double-blind, placebo-controlled 6-month trial of the efficacy of 13-cRA (1 mg/kg/d) in reversing bronchial metaplasia among chronic smokers. Follow-up bronchoscopy is performed at 6 and 12 months. This trial is based on the evaluation of bronchoscopy specimens for metaplasia index, and will also evaluate biologic markers as intermediate endpoints. Accrual of the study population is complete, with 157 patients screened and 87 randomized. The final data will be unveiled in 1993. Another ongoing, randomized trial by McLarty et al. is evaluating the effects of retinol and β-carotene on sputum atypia in individuals exposed to asbestos. The primary endpoint of this trial is a change in sputum cytology. At the time of the last report, 1,100 individuals had been screened and 758 had been randomized. The trial should be completed in 1993. At present, reports of the efficacy of 13-cRA and etretinate against bronchial premalignant lesions are conflicting. Other retinoids, including 4-HPR, are being investigated for their efficacy in bronchial metaplasia.

Laryngeal Papillomatosis

Laryngeal papillomatosis is a condition characterized by the recurrent development of polypoid lesions on the vocal cords. A lesion in this location may precede the development of squamous carcinoma of the larynx. Treatment of this condition with 13-cRA (0.5–2.0 mg/kg/d) has been fairly successful. Alberts et al. (1986) treated five patients, of whom two achieved a complete remission and one a partial remission. In another study, 67% of 42 patients treated with etretinate (1 mg/kg/d) achieved a complete response (Bichler, 1993). Although these positive results occurred in patients with extensive disease, adjuvant treatment of laryngeal papillomatosis with 13-cRA in a recent small randomized study by Bell et al. (1988) did not prevent recurrence.

Barrett's Esophagus

Barrett's esophagus is a condition in which the esophageal mucosa is lined by metaplastic columnar epithelium rather than normal, stratified squamous epithelium. A major factor contributing to the etiology of this condition is chronic esophageal reflux. Barrett's esophagus is not an uncommon lesion in Western countries. The most recent studies utilizing esophageal endoscopy report that as many as 10% of all patients with symptoms of gastroesophageal reflux, such as heartburn, will develop Barrett's esophagus.

One of the most important considerations in Barrett's esophagus is its malignant potential; patients with this condition are at increased risk of developing adenocarcinoma of the esophagus. Prevalence studies suggest that these lesions develop into invasive cancer at a fairly high rate. Epidemiologic studies show that patients with

The Trustees of Columbia Univ. Ex. 2126 - Page 22 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

Barrett's esophagus have a 30 to 40% higher risk of esophageal cancer than does the general population.

Because Barrett's esophagus is considered a premalignant lesion, it is an excellent model for chemoprevention studies. At present, there is no standard treatment for Barrett's esophagus, since neither aggressive medical treatment nor surgical antireflux therapy have been effective. Clinical trials to assess the efficacy of several chemopreventive agents against the condition were begun by investigators at the University of Arizona. Their first study used 13-cRA (1 mg/kg/d); there were considerable toxic effects, and only 11 of 16 patients completed at least 6 weeks of treatment. All 11 patients were evaluable, but no responses were noted. In addition to the usual toxic effects associated with 13-cRA, ulcerating mucosa of the esophagus also developed in two patients following discontinuation of the treatment (Garewal, 1992). The optimal retinoid agent and duration of treatment with minimum side effects has not yet been established for Barrett's esophagus. Further investigation, especially with the newer retinoids, is indicated.

Actinic Keratoses

Actinic keratoses are premalignant lesions caused by significant, cumulative exposure to the sun, and are particularly common in the older population. They are usually less than 1 cm in size when first manifested, and are red and scaly in appearance. Retinoid treatment for this condition has been studied since the early 1970s, primarily consisting of topical tretinoin at different dose levels. A complete response rate of approximately 50%, significantly higher than the average, was reported. The most recent data come from a Phase III trial by Kligman and Thorne (1991). This trial reported a dose-response relationship, with a decreasing lesion count after tretinoin therapy. Although the study suggests that topical tretinoin has significant dose-related activity in actinic keratoses, some of the data are conflicting. The lesions are generally too small to follow with histopathologic tests, and most of the lesions would be removed by a biopsy, leaving too little to treat. An important endpoint should be whether the treatment of existing actinic keratoses leads to a decreased number of skin cancers.

The use of topical tretinoin for the prevention of skin cancer has not been adequately studied; however, the systemic use of retinoids, especially etretinate, in actinic keratoses has been reported in several studies, including two that were placebo-controlled and randomized, and which demonstrated that retinoid had benefit. Forty-six patients with actinic keratoses were treated with etretinate for 3 months in a nonrandomized study, with an overall response rate of 95%. On follow-up, over 70% of the patients relapsed within 12 months of stopping treatment. Moriarty (1982) reported the first randomized trial of a systemic retinoid for the treatment of actinic keratoses. In this study, 75 mg/d of etretinate was given for 2 months to 40 patients with actinic keratoses. The response rate was significantly higher in the retinoid-treated group (84%) than in the placebo group (5%). The most recent randomized study tested etretinate (1 mg/kg/d) for 4 months in 31 patients with actinic keratoses. Again, a significant retinoid effect was found. The response rate was 93% (14 of 15 patients) in the retinoid-treated group and 13% in the control group.

Cervical Dysplasia

Cervical cancer is the second most common malignancy in women worldwide. Cervical intraepithelial neoplasia (CIN) is considered a precursor of invasive cervical cancer. A series of analyses by Romney et al. (1981) demonstrated a significant, threefold greater risk of severe dysplasia and CIN in women with a low dietary intake of vitamin A, β-carotene, and ascorbic acid.

Romney et al. (1981) reported a Phase I trial of retinyl acetate gel applied to the vagina in doses ranging from 0 to 18 mg/d over a 7-day period. Fifty women with cervical dysplasia were enrolled. No lesions progressed, and several women showed some improvement without severe toxicity.

Investigators at the University of Arizona (Graham et al., 1986; Meyskens and Surwit, 1986; Peng et al., 1986; Loescher et al., 1990) have conducted a series of clinical trials with topical all-*trans*-RA in women with cervical dysplasia. The first trial enrolled 18 women and utilized a collagen sponge to deliver the all-*trans*-RA. The lesions were reduced in 6 of the 18 women, but the toxic effects in the vagina were severe, leading to the design of a second trial of all-*trans*-RA. That trial tested a different delivery system, a cervical cap. Thirty-six patients with mild to severe dysplastic lesions were assessed. Thirty-three percent (12 of 36) of the patients achieved a complete response, and there was a significant dose-response relationship.

This Phase I trial of all-*trans*-RA led to another in which the dose of all-*trans*-RA was 0.372%. The drug was administered daily for 4 days, and then for 2 days every 3 months for 1 year. Fifty percent (10 of 20) of the women had a complete response, and after 1 year of follow-up, only 1 patient with a complete response had had a recurrence of dysplasia.

These data strongly suggest that topical all-*trans*-RA is active in CIN, producing complete responses in 30 to 50% of dysplastic lesions. A recently completed placebo-controlled Phase III trial of locally applied β-*trans*-RA in cervical intraepithelial neoplasia (CIN) has demonstrated significant activity in patients with CIN II but not CIN III (Meyskens et al., 1993).

The Trustees of Columbia Univ. Ex. 2126 - Page 23 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

Trials of Chemoprevention Against Second Primary Tumors

Clinical studies of chemoprevention have recently made advances in several areas, including cancers of the head and neck (primarily in the upper aerodigestive tract), skin, lung, breast, colon, and bladder. Epithelial cancers in these regions pose major challenges. Cancer of the upper aerodigestive tract and lung accounts for roughly 30% of all cancer deaths in the United States. Breast cancer will now afflict one of every nine women in the United States. Colorectal cancer is the second-leading cause of cancer death in the United States, and the survival rate in this disease has not changed significantly. Early-stage cancer of the head and neck, lung, or breast can be cured with surgical resection. However, these cancers confer a risk of second primary tumors. Clinical studies of retinoids in preventing initial and second primary tumors in several types of epithelial malignancies have been reported.

Head and Neck Cancer

The standard treatment for early (stage I or II) head and neck cancer is either surgical excision or radiotherapy (Hong and Bromer, 1983). The major cause of treatment failure in patients with early-stage disease is the development of second primary tumors. It is very unfortunate and ironic that patients who have been successfully treated for cancer of the head and neck are at increased risk of developing additional primary neoplasms. Second primary tumors occur at a rate of 3 to 7% per year in patients previously treated for head and neck cancer (Tepperman et al., 1981; Cooper et al., 1989; Licciardello et al., 1989; McDonald et al., 1989). Although the occurrence of multiple primary tumors may reflect an inherent predisposition toward cancer, it seems likely that the common etiologic risk for the initial tumor is important in the pathogenesis of subsequent primary tumors. The development of second primary tumors in the aerodigestive tract may be due to the process of diffuse field cancerization, in which the epithelium at risk has been exposed to continued carcinogenic insult. The entire epithelial field therefore has a relatively high likelihood of developing multiple premalignant or malignant lesions.

Because of the retinoids' significant activity in reversing premalignant oral lesions, and the relatively high risk of both recurrent disease and second primary tumors in patients with head and neck cancer, a group of these patients received adjuvant retinoid treatment in a randomized, double-blind study (Hong et al., 1990). Following definitive treatment for squamous carcinoma of the head and neck, patients were treated for 1 year with high-dose 13-cRA (50–100 mg/m^2/d) or placebo. One hundred patients were evaluated. Patient characteristics such as age, sex, primary tumor site, tumor differentiation, alcohol and tobacco histories, and initial stage were comparable in the two groups. The types of previous treatment, including surgery and radiotherapy, were stratification variables for this trial. Fifty-nine percent of the patients treated with 13-cRA and 43% of those who received placebo had locally advanced disease, of stage III or IV. Patient characteristics were comparable in both groups, and the results of the trial are also summarized in Table 3.

Treatment with 13-cRA was not associated with a reduction in the incidence of recurrent tumors, but the formation of second primary tumors was dramatically reduced among the retinoid-treated patients. Multiple second primary tumors occurred in four patients, all in the placebo group. The vast majority of second primary tumors were of squamous histologic type and occurred in the upper aerodigestive tract. Toxic effects were significantly more frequent and severe in the 13-cRA group of patients; these consisted of mucocutaneous dryness and hypertriglyceridemia. Many patients were unable to complete a full course of the retinoid therapy, primarily due to toxic effects. At 48 months of follow-up, the rate of second primary tumor development is 6% among the 13-cRA-treated patients, and 28% among patients who received placebo. This randomized trial definitely establishes the efficacy of high-dose 13-cRA in preventing second primary tumors in patients with head and neck cancer. This trial led to a multicenter Phase III trial of low-dose 13-cRA for preventing second primary tumors in patients with early-stage head and neck cancer.

M. D. Anderson/Radiation Therapy Oncology Group Phase III Trial

Ongoing trials in the chemoprevention of second primary tumors in head and neck cancer are being con-

TABLE 3. *High-dose 13-cRA adjuvant trial for second primary tumors*

Occurrence of second primary tumors	Placebo group (n = 50)	13-cRA group (n = 49)
Total number of second primary tumors	14	3
Patients with second primary tumors at multiple sites	4	0
Site of second primary tumor		
Head and neck	10	1
Lung	3	0
Esophagus	2	1
Other	2 (AML, skin)	1 (bladder)

[a] Median follow-up: 48 months.

13-cRA, 13-*cis*-retinoic acid; AML, acute promyelocytic leukemia.

ducted by the M. D. Anderson Cancer Center and the Radiation Therapy Oncology Group (RTOG). The trial by Hong et al. of high-dose 13-cRA demonstrated a marked reduction in the incidence of second primary tumors, but had the major drawback of significant toxicity. A more recent study by our group demonstrated that low-dose 13-cRA was very well tolerated. This led to our current chemoprevention trial, which is specifically designed to determine whether low-dose (30 mg/d) 13-cRA will prevent second primary tumors in patients with early-stage head and neck cancer. The endpoint of this study is reduction in the number of second primary tumors. This cooperative study is randomized, double-blind, and placebo-controlled. Patients will be treated for 3 years and followed for an additional 4 years. This study is much larger than our previous adjuvant trial; 972 patients will be randomized to receive either 13-cRA or placebo. The study was opened by the RTOG in February 1992, and by the end of November 1992, 211 patients had been enrolled.

The Euroscan Trial

Euroscan, another ongoing study of the chemoprevention of second primary tumors in head and neck cancer patients, is being performed through the European Organization for Research and Treatment of Cancer (EORTC). This trial was based on Pastorino's work (1987; 1991) with retinyl palmitate following the resection of early-stage lung cancer. It is simultaneously studying the effects of chemoprevention in two populations of patients at risk for the development of second primary tumors: patients with non-small-cell lung cancer that has been completely resected, and patients who have received curative therapy for head and neck cancer. The agents being used in this trial are *N*-acetylcysteine (600 mg/d) for 2 years and retinyl palmitate at 300,000 IU/d during the first year and at 150,000 IU/d during the second year. This trial is using a 2×2 factorial design; patients are assigned to receive *N*-acetylcysteine alone, retinyl palmitate alone, both regimens, or placebo. The endpoints of this study are the development of second primary tumors, local and distant recurrence, and survival rates. The accrual of patients for this trial is ongoing, and the target sample size for the trial is over 2,000. Toxicity data from the initial 554 patients enrolled suggest that the treatment regimens are well tolerated; side effects have more commonly been noted in the group receiving retinyl palmitate, and usually consist of skin complaints (de Vries et al., 1991).

Northern California Oncology Group/Eastern Cooperative Oncology Group Trial

Another chemoprevention study of 13-cRA was initiated through the Northern California Oncology Group (NCOG) in conjunction with the Eastern Cooperative Oncology Group (ECOG). This randomized study involves patients with stage I or stage II cancer of the head and neck treated with 13-cRA (0.15 mg/kg/d) or placebo for 2 years. The therapy is begun as adjuvant primary therapy shortly after the completion of surgery or radiotherapy.

Yale Trial

A trial of the effectiveness of β-carotene in preventing second primary tumors in patients with head and neck cancer is being conducted by the Cancer Prevention Research Unit of Yale University. This randomized, double-blind, placebo-controlled trial is studying the efficacy of β-carotene (50 mg/d) in reducing the incidence of second primary tumors and local recurrences. The study is actively accruing patients at this time.

There is tremendous enthusiasm over the prospect of effective chemoprevention of head and neck cancer. Understanding the carcinogenic and field-cancerization processes will improve strategies for chemoprevention. Molecular and cellular changes during this multi-step process, and the role of nuclear RA receptors, are now being investigated to aid our understanding of the mechanism of the activity of RA as a chemopreventive agent in head and neck cancer.

Skin Cancer

The rationale for using retinoids for the prevention of skin cancer comes from strong preclinical and clinical data. In most models, chemical- and ultraviolet-induced carcinogenesis is inhibited by RA. Bollag (1983) first demonstrated that all-*trans*-RA had activity in the prevention of DMBA-induced premalignant and malignant squamous cell lesions of the skin. Several controlled studies of actinic keratosis have suggested that various topical and systemic retinoids are active against this condition.

Xeroderma Pigmentosum

Xeroderma pigmentosum is a rare, autosomal-recessive genodermatosis characterized by sun sensitivity and a deficiency in the repair of UV-light-damaged DNA. Patients with xeroderma pigmentosum develop skin cancer at a frequency that is more than 1,000 times greater than in the general population.

The most promising data for preventing new skin cancer was reported by Kraemer et al. (1988) at the National Cancer Institute. Although only five patients were studied, they demonstrated that high-dose 13-cRA (2 mg/kg/d) for 2 years significantly suppressed the development of basal-cell carcinoma. These patients had a rapid

The Trustees of Columbia Univ. Ex. 2126 - Page 25 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

rate of recurrence, possibly due to a rebound phenomenon. In a more recent study by these investigators, lower doses of 13-cRA (0.5 mg/kg/d) were less effective, suggesting a dose-response relationship. The high-dose 13-cRA regimen produced notable mucocutaneous toxic effects in all patients and radiologic skeletal changes in two patients.

Four large-scale, Phase III chemoprevention studies of skin cancer (Table 4) have been conducted in the United States. These studies involved thousands of patients at much lower risk than those in the study by Kraemer et al. (1988), who were followed for many years. One of these studies used a very low dose of 13-cRA (5–10 mg/kg/d), while another employed relatively high doses of β-carotene (50 mg/d). Both studies reported no significant chemopreventive effect (Greenberg et al., 1990; Tangrea et al., 1992a). The other two large-scale studies have not yet been reported, although both studies have been completed and the data are being analyzed. One of these was a placebo-controlled trial of single-agent retinol and single-agent, low-dose 13-cRA, designed as a two-arm study in patients with prior invasive skin cancer. The other study was a placebo-controlled two-arm trial of retinol in patients with prior actinic keratosis and no history of invasive cancer. These two studies will provide critical data on the role of low-dose retinoids in large-scale skin cancer prevention, which will aid in the design of future studies.

Breast Cancer

Breast cancer is the most frequent tumor in women in the Western countries. The incidence of breast cancer is continuously rising. Improved treatment and early diagnosis are known to decrease mortality. Epidemiologic and laboratory data provide a strong rationale for developing studies of breast-cancer chemoprevention. A large-scale Italian trial is using 4-HPR in the prevention of contralateral second breast cancers, which occur at a rate of 0.8% per year in patients who have had a previous resection for early-stage breast cancer. The treatment consists of 4-HPR (200 mg/d), with a 3-day drug "holiday" each month, for a total of 5 years. This study has accrued over 2,500 patients and is continuing (Costa et al., 1989).

Tamoxifen certainly has potential as a chemopreventive agent in breast cancer. Treatment with tamoxifen inhibits the G0 and G1 phases of the cell cycle, with resulting inhibition of growth, and appears to be more cytostatic than cytotoxic. In animal experiments, tamoxifen inhibited the initiation and promotion of DMBA-induced mammary tumors. Tamoxifen is now used for both the palliation and adjuvant treatment of carcinoma of the breast. It has been shown to significantly reduce the rate of contralateral breast cancer in several National Surgical Adjuvant Project for Breast and Bowel Cancer (NSABP) adjuvant trials. The difference in overall contralateral second primary tumor rates between tamoxifen- and placebo-treated women in a group of 30,000 women from over 40 adjuvant studies is highly significant (Early Breast Cancer Trialists' Collaborative Group, 1992). These studies indicate that long-term tamoxifen treatment is necessary to achieve a preventive effect (Moon et al., 1979, 1982; McCormick et al., 1982).

The significance of tamoxifen's benefit in postmenopausal women is quite clear, but it is not as clear in premenopausal women. Tamoxifen's relatively benign toxic profile supports its use in the chemoprevention of primary and secondary breast cancers, but in premenopausal women, tamoxifen produces significant symptoms of estrogen deficiency, such as hot flashes, vaginal dryness, and menstrual irregularities. The significant increase in the rate of hot flashes is the major toxic effect of tamoxifen in postmenopausal women. Tamoxifen acts like estrogen in increasing the rate of endometrial (and possibly other) cancers. Long-term treatment with tamoxifen at high doses is known to produce liver tumors in rats, but data from the adjuvant tamoxifen trials have not indicated an increase in liver tumors in humans. Ta-

TABLE 4. *Phase III skin-cancer chemoprevention trials with retinoids and β-carotene*[a]

Study center	Agent	Dose	Population (sample size)	Results
University of Arizona	13-cRA Retinol	5–10 mg/d 25,000 IU	≥4 BCC/SCC (524)	Accrual complete; under analysis
University of Arizona	Retinol	25,000 IU	≥10 AKS (2,298)	Accrual complete; under analysis
Dartmouth College	β-carotene	50 mg/d	≥1 BCC/SCC (1805)	No effect
National Cancer Institute Intramural	13-cRA	10 mg/d	≥2 BCC/SCC (981)	No effect

[a] All are multicenter, randomized, placebo-controlled, double-blind trials in which invasive skin cancer incidence is the primary study endpoint.

13-cRA, 13-*cis*-retinoic acid; d, day; IU, international units; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; AKS, actinic keratoses.

moxifen's estrogen-like effects have been shown to increase bone-mineral density and reduce total and low-density lipoproteins and cholesterol (Love et al., 1991).

Large-scale trials of tamoxifen for preventing breast cancer in women at high-risk for it are underway throughout the world. The NSABP trial has just begun. This trial will enroll 16,000 women who are at greater risk for developing breast cancer than women in the general population. The eligibility criteria allow any woman over age 60 into the trial. The risk brackets, which depend on age, presence of benign breast disease, number of children, and family history will be assessed by use of the mathematical model of Gail and colleagues. Tamoxifen's effects on the cardiovascular and skeletal systems will be systematically evaluated by the NSABP Tamoxifen Prevention Trial Monitoring Committee. The chemopreventive efficacy of combinations of tamoxifen and retinoids, which has been demonstrated in animals (Ratko et al., 1989), has not yet been adequately studied in a clinical trial.

Lung Cancer

Patients with early-stage non-small-cell lung cancer have an increased risk of developing second primary tumors of the lung that is roughly equivalent to that after head and neck cancer. Pastorino et al. (1991) in Milan, conducted a randomized trial of 307 patients who had undergone complete resections of stage I non-small-cell cancers of the lung. The patients were randomized to receive either retinyl palmitate at 300,000 IU/day or placebo for 12 months. The primary endpoint of this study was either tumor recurrence or the development of a second primary tumor. Although the improvement in disease-free survival for the retinyl palmitate group was not statistically significant, the retinyl palmitate group did show a statistically significant improvement in regard to time-to-new-primary-cancer. Over 70% of second primary tumors developed within the carcinogen-exposed field at risk.

In the United States, a randomized intergroup trial has been developed to test the efficacy of 13-cRA in preventing second primary tumors following the resection of stage I non-small-cell lung cancers. Eligible patients must have had non-small-cell lung cancer classified as either T_1 or T_2, must be lymph-node negative (N_0), and must be from 6 weeks to 36 months into the post-resection period. These patients will receive either 13-cRA (30 mg/d) or placebo for 3 years. The accrual goal for this study is approximately 1,000 patients (Huber et al., in press).

The need for retinoid chemoprevention studies in small-cell lung cancer is under discussion. Cured small-cell lung cancer patients have one of the highest rates of developing second primary tumors of all cancer patients; the rate is more than twice that after head and neck cancer. In this disease setting, second primary tumors are the most common cause of cancer death at 4 years after definitive primary therapy. Second primary tumors in small-cell lung cancer most often occur in the lung, esophagus, and bladder (Heyne et al., 1992).

Bladder Cancer

Transitional-cell carcinoma of the bladder represents an ideal disease for the development of chemopreventive strategies (Lower and Kanarek, 1981). This disease has known carcinogenic promoters associated with multicentric premalignant changes, and a propensity for second primary tumors as well as recurrent tumors. Preclinical work in animal models has shown that retinoids have significant anticarcinogenic activity against transitional-cell carcinomas (Sporn et al., 1977; Croft et al., 1981). Several clinical studies have been conducted of retinoid therapy following the resection of superficial (noninvasive) bladder cancers. A study by Pederson et al. (1984) compared placebo and etretinate (50 mg/d) in 73 patients with recurrent noninvasive bladder tumors. Although the efficacy of etretinate was not significantly different from that of placebo, the short duration of treatment, at only 8 months, and the dropout rate of over 50% in the etretinate group, make this result quite inconclusive. Alfthan et al. (1983) reported the results of a placebo-controlled trial of etretinate in 32 patients whose recurrent bladder tumors had been removed. Thirty of the patients were evaluable, of whom 15 had been treated with etretinate. After the experience of the first 10 patients, the initial daily dose was reduced from 50 to 25 mg because of side effects. The treatment duration ranged from 10 to 26 months. Overall, etretinate had some protective effect in 11 of 15 patients (73%), whereas the placebo had an effect in only 4 of 15 (27%) (Alfthan et al., 1986).

The National Bladder Cancer Collaborative Group initiated a clinical study involving 13-cRA (0.5 mg/kg/d) in patients with recurrent, superficial low-grade lesions. Unfortunately, after studying only 22 patients, this study was prematurely discontinued because of toxic cutaneous and ophthalmologic effects. Studer et al. (1984) randomized 86 patients with recurrent superficial bladder cancer to receive etretinate or placebo. The group receiving etretinate had a significantly lower rate of recurrence than did the placebo group.

The synthetic retinoid 4-HPR appears to be an especially interesting compound for the chemoprevention of bladder cancer. Moon et al. (1979) first demonstrated that 4-HPR can decrease bladder carcinogenesis induced by nitrosamine. Tests in rodent bladder-tumor models

The Trustees of Columbia Univ. Ex. 2126 - Page 27 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

indicate that 4-HPR is one of the strongest anticarcinogenic retinoids. It is also less toxic in humans than etretinate and 13-cRA, which might allow its use in prolonged therapy at higher doses.

In summary, the retinoids have clearly shown activity against tumor recurrences and second primary tumors in the bladder in both preclinical and animal models. The efficacy of retinoids in the prevention and treatment of recurrent and second primary tumors of the bladder needs to be studied further and their exact role in this setting remains to be defined. The most effective retinoid for the prevention and treatment of human bladder cancer is unknown. A limited study of 13-cRA showed this compound to be very toxic at the dose employed, and to have little if any clinical activity. Etretinate has been the most extensively studied retinoid in terms of recurrent and secondary bladder cancer. Randomized studies show it to be active in the chemoprevention of recurrent superficial cancer and to be tolerable at 25 mg/d. At present, the most promising, although untested, compound is 4-HPR (McCormick and Patel, 1985; Moon and Itri, 1984). This compound shows very promising activity in most pre-clinical systems, and trials in patients with tumors at other sites than the bladder have shown that 4-HPR is relatively less toxic than other retinoids yet studied. It is possible that retinoids like 4-HPR could be effective when used intravesically, but this approach has not yet been studied. Clinical studies of 4-HPR incorporating new biomarkers are underway in both Europe and the United States. These trials should better define the role of 4-HPR in the prevention and treatment of bladder cancer.

Primary Prevention Trials for Epithelial Cancer

The most conclusive endpoint of a chemoprevention trial is cancer incidence, which is a valid endpoint for establishing the chemopreventive efficacy of a drug. Unfortunately, the analysis of cancer incidence requires that large Phase III trials be conducted, enrolling thousands of subjects and lasting for many years, with tremendous costs. Therefore, chemoprevention trials present problems in study design, since not all individuals studied have or will develop cancer. Drug toxicity and adherence to the treatment regimen are of major importance in ensuring the success of chemoprevention trials. The occurrence of lung cancer is infrequent in most populations, even smokers. Strategies to decrease the number of subjects or shorten the duration of a trial are imperative from a practical point of view. Nevertheless, several randomized Phase III trials of primary prevention are underway, using subjects at high risk or from the general population.

The United States Physicians' Health Study

This study is a randomized 2×2 factorial trial evaluating the benefit of aspirin and β-carotene in reducing cardiac mortality and cancer incidence, respectively. Following the run-in period, in which they received aspirin and placebo, as well as β-carotene to test compliance, the subjects were randomized to receive either 325 mg of aspirin every other day, 50 mg of β-carotene every other day, both treatments, or two placebos. An accrual of 22,000 male American physicians has been completed. The study was designed to have a better than 90% probability of detecting a 20% reduction in cancer incidence in the β-carotene arm, at an average of 7.5 years of follow-up. The study is in progress, but will not be completed in the near future (Buring et al., 1989).

Subjects with Risk Factors

One of the approaches to decreasing sample size is to identify individuals with a high risk of developing cancer. Cigarette smoke, asbestos exposure, and mining have all been demonstrated to increase the risk of lung cancer (Fiore et al., 1989). Several large studies of high-risk subjects are currently underway to determine whether chemopreventive agents can decrease their incidence of lung cancer.

Beta-Carotene and Retinol Efficacy Trial (CARET)

Cigarette smoking remains the single most important risk factor for the development of lung cancer. Smokers have a relative risk of 13.3% of developing lung cancer. Several randomized studies are evaluating the role of carotenoids and retinoids in preventing the development of lung cancer in smokers. A pilot trial at the University of Pittsburgh randomized 400 smokers to receive either β-carotene or placebo. β-Carotene levels were increased in the group receiving β-carotene, confirming compliance and demonstrating that toxicity was quite minimal. This demonstrated the feasibility of a large randomized trial of β-carotene in smokers.

Recently, investigators at the Fred Hutchinson Cancer Center in Seattle have completed a feasibility study of the safety of retinyl palmitate (25,000 IU) and β-carotene (30 mg) with placebo controls in individuals with a smoking history of 20 pack-years or more. The safety aspects of this study were completed, with an accrual of over 1,000 individuals. Compliance was in excess of 90% as determined by self-report and periodic pill counts. The only toxic effect reported was mild yellowing of the skin in individuals who received β-carotene. Based on this vanguard study, a large randomized trial of more than 13,000 smokers and 4,200 asbestos workers

The Trustees of Columbia Univ. Ex. 2126 - Page 28 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

was initiated in 1989. As of February 1991, 6,245 participants had been randomized (Goodman and Owenn, 1992).

The Finland Study

The Finland Study is the largest randomized trial to evaluate the efficacy of chemoprevention of lung cancer in smokers. It began in Finland in 1984 and was sponsored by the National Public Health Institute of Finland in collaboration with the U.S. National Cancer Institute. A 1-year pilot study evaluated the feasibility of a large-scale trial. Eligible patients completed a 3-month run-in period on placebo, following which they were randomized, using a 2×2 factorial design, to receive β-carotene, alpha-tocopherol, a combination of both agents, or placebo. Two hundred and ninety thousand male subjects were screened in a marked survey; as of 1991, 29,200 subjects had been randomized, and compliance was high: 93% of the subjects took more than 90% of their capsules. Furthermore, the serum levels of β-carotene and α-tocopherol increased significantly in the patients who received those agents. No significant toxic effects have been reported, and the efficacy results are still pending.

Lung cancer is the leading cause of death from cancer in the United States for both men and women. Smoking is a major, well-established risk factor for this cancer, as is exposure to the fibers of asbestos, and these carcinogens act synergistically. The latency period between exposure to either of these carcinogens and the development of lung cancer may be a decade; thus, prevention agents for lung cancer could save many people. Even though the exposure may have occurred and ceased years ago, the need for a cancer chemoprevention program is imperative. Several lines of research in the clinical chemoprevention of lung cancer are underway, and several of the large studies currently in progress will determine whether agents such as RA or β-carotene will be recommended to the public by the end of the decade as chemopreventive agents for lung cancer.

SUMMARY AND FUTURE DIRECTIONS

During the past 10 years, tremendous progress has been made in both basic and clinical research on retinoids. Whereas studies conducted over the past two decades with experimental animals and cultured tumor cells have indicated that retinoids have potential in the treatment and prevention of cancer, the successful application of retinoids in clinical trials is a very recent and exciting development in the field of retinoid research. In therapeutic terms, all-*trans*-RA has most recently been demonstrated to have striking activity in acute promyelocytic leukemia. In addition, the latest of recent trials of 13-cRA combined with interferon-α for the treatment of advanced skin cancer or cervical cancer have generated tremendous excitement and enthusiasm. The only chemopreventive agent to have shown significant preventive activity, albeit in small randomized clinical trials, is 13-cRA. The activity occurred in trials of adjuvant therapy to reverse premalignant oral lesions, which was achieved with both high- and low-dose 13-cRA. The development of second primary tumors was also prevented by treatment with high-dose 13-cRA in head and neck cancer patients. Several randomized, placebo-controlled trials of 13-cRA are now underway in patients with head and neck cancer, lung cancer, skin cancer, or breast cancer. The results of these trials will help to determine future multimodality treatment strategies in epithelial cancers, such as primary and adjuvant therapy followed by long-term chemoprevention.

The major drawback of retinoids in therapeutic and preventive settings are the doses required for therapeutic efficacy, which are complicated by both acute and chronic side effects. These high doses are generally poorly tolerated, often requiring dose reduction or even the discontinuation of retinoid therapy. The long-term use of retinoids may induce calcification of ligaments and hyperostosis. Furthermore, retinoids are teratogenic and contraindicated in women of childbearing potential.

The next few years promise extremely exciting developments in the field of clinical retinoid research. The clinical results currently available provide strong evidence for the potential roles of retinoids as differentiation agents, as chemopreventive agents, and for cancer treatment in general. Retinoids, like cytokines, exert antitumor effects in cultures of human transformed cell lines, in organ cultures, and in transplantable or chemically induced animal tumors. Clinically, they are useful agents in a limited number of neoplastic diseases. Single-agent therapy, either with retinoids alone or with cytokines such as interferon-α alone, shows limited activity. Synergism between cytokines and retinoids has been demonstrated. A combination of a panel of cytokines and a series of retinoids demonstrated a significant synergistic effect in inhibiting proliferation and induction of differentiation. The first clinical trials with interferon-α and 13-cRA have generated promising results in advanced skin cancer and cervical cancer. More clinical investigations will have to be done to determine which cytokine, in combination with which retinoid, will have the greatest therapeutic efficacy, particularly in certain types of tumors.

In the management of advanced cancer, retinoids are most likely to be used in combination with other antineoplastic therapies. The retinoids' relative lack of the severe toxic reactions that are commonly associated with standard anticancer therapy should allow for their ready use

The Trustees of Columbia Univ. Ex. 2126 - Page 29 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

in combination regimens. In fact, two cooperative group studies sponsored by the National Cancer Institute are underway to establish the role of all-*trans*-RA, as either a single agent or in combination with chemotherapy, in the standard management of APL.

Retinoids also represent a unique approach to the problem of adjuvant therapy in several epithelial cancers. Since retinoids have a mechanism of action that differs from that of conventional cytotoxic therapies, they may be effective in situations in which the tumor burden is reduced after surgery or radiotherapy. The need for newer retinoid analogues, mediated by interactions with specific nuclear RA receptors, is clear; analogues with improved therapeutic indexes will be investigated.

It is quite clear that retinoid derivatives chosen for cancer prevention must cause minimal toxicity. It is possible that synergistic combinations of a retinoid with other chemopreventive agents will diminish toxicity and increase therapeutic activity. A combination of high-dose 13-cRA and α-tocopherol has been investigated and found to significantly reduce retinoid toxicity. Since chemopreventive treatment will last a long time, possibly a lifetime, the potential biologic activity of retinoids on lipid metabolism and the skeletal system, as well as on reproductive functions, must be carefully considered. New molecular advances provide unprecedented abilities to determine the mechanism of both desired therapeutic effects and undesired side effects of retinoids. These recent molecular discoveries will allow the design and synthesis of molecules that have equal or greater therapeutic efficacy than the current retinoids, with a reduced incidence and severity of side effects, making them more suitable for cancer prevention trials in healthy patients. The therapeutic activity of all-*trans*-RA in APL, the prevention of second primary tumors in head and neck cancer patients, and the reversal of premalignant oral lesions with 13-cRA clearly represent major advances in public health. Ongoing trials in cancer treatment and prevention with retinoids will provide further valuable information.

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The Trustees of Columbia Univ. Ex. 2126 - Page 33 of 36
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The Trustees of Columbia Univ. Ex. 2126 - Page 35 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313

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The Trustees of Columbia Univ. Ex. 2126 - Page 36 of 36
Gene Company Pty Ltd v. The Trustees of Columbia Univ. - IPR2024-00313