Journal of Cellular Biochemistry, Supplement 22:1–10 (1995)

Retinoids and Chemoprevention: Clinical and Basic Studies

Scott M. Lippman, MD[1], Richard A. Heyman, PhD[2], Jonathan M. Kurie, MD[1], Steven E. Benner, MD[1], and Waun Ki Hong, MD[1]

[1] University of Texas M.D. Anderson Cancer Center, Houston, TX 77030
[2] Ligand Pharmaceuticals, Inc., San Diego, CA 92121

Abstract Retinoids, which include natural vitamin A (retinol) and its esters and synthetic analogues, are the best-studied class of agents in chemoprevention. There are more than 4,000 different retinoids which have a wide spectrum of preclinical activities, structures, pharmacological profiles, tissue distributions, receptor specificities, and toxicities. A number of retinoids have significant activity in many *in vivo* experimental systems including skin, bladder, lung, breast and oral carcinogenesis. In clinical trials, several retinoids have achieved significant activity in the reversal of head and neck, skin, and cervical premalignancy, and in the prevention of second primary tumors associated with head and neck, skin, and non-small cell lung cancer. Since 1984, our group has conducted a series of clinical trials to explore the chemopreventive potential of 13-*cis*-retinoic acid (13cRA) in the aerodigestive tract. We have conducted two consecutive randomized studies in subjects with premalignant lesions of the oral cavity. These studies showed that high-dose 13cRA alone can achieve significant short-term reversal of oral premalignancy, and that high-dose followed by low-dose 13cRA can maintain suppression of oral carcinogenesis. Three other randomized trials have confirmed significant retinoid activity in this human carcinogenic system. We also developed a randomized, placebo-controlled trial of adjuvant high-dose 13cRA in patients with head and neck cancer. Second primary tumor development was significantly decreased in the 13cRA group, but 13cRA had no impact on primary disease recurrence or survival. This presentation will update the current status of clinical trials and correlative laboratory studies of potential intermediate endpoint biomarkers in retinoid chemoprevention of aerodigestive tract carcinogenesis.

Key words: Breast cancer, carcinogenesis, cervical cancer, chemoprevention, head and neck cancer, lung cancer, skin cancer, retinoids, retinoid receptors

Retinoids are a large family of compounds which includes the natural and synthetic derivatives of vitamin A [1,2]. They have been studied extensively in preclinical and clinical chemoprevention. In animals, various retinoids have significant chemopreventive activity in many sites, including breast, bladder, head and neck, lung, cervix, skin, and prostate carcinogenesis [1,3–5]. Clinical trials have observed significant retinoid chemopreventive activity in head and neck, lung, skin, and cervical carcinogenesis [5]. This paper will review the current status of retinoid clinical chemoprevention, retinoid biology, and new retinoid development strategies based on receptor selectivity.

Address correspondence to Scott M. Lippman, MD, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 80, Houston, TX 77030.

EPITHELIAL CARCINOGENESIS

Two biologic concepts—multistep carcinogenesis and field carcinogenesis—underlie chemoprevention efforts. Multistep carcinogenesis, initially observed microscopically, is now under study at the molecular level. Molecular studies have revealed specific genetic alterations contributing to cancer development at various sites. The best

studied model of human multistep carcinogenesis comes from molecular studies in the colon. Adenoma-carcinoma progression in the colon has been shown to involve *K-ras* mutation and tumor suppressor gene loss on chromosomes 5q, 17p, and 18q [6]. Study of molecular carcinogenesis is proceeding rapidly in other sites, including upper aerodigestive tract cancers, where genetic loss on chromosomes 3p, 9p, 11q, 13q, and 17p are common [7]. The timing of genetic changes in preinvasive carcinogenesis in the upper aerodigestive tract and other epithelial sites is currently unknown. How these molecular changes may signify intermediate endpoints for chemoprevention has recently been reviewed elsewhere [5,8].

The other major concept of chemoprevention, field carcinogenesis, describes the phenomena of carcinogenic changes occurring diffusely in a large epithelial area. These changes often are caused by repeated exposure to specific carcinogens, *e.g.*, UV in the skin or cigarette smoke in the upper aerodigestive tract. The clinical sequelae of field carcinogenesis are the development of second primary tumors (SPTs), which occur at different rates depending on the primary tumor site and various environmental and genetic risk factors. The basic and clinical characteristics of SPTs are best worked out in the aerodigestive tract. Molecular studies of p53 strongly support the basic concept of field carcinogenesis—that SPTs arise from progression of independent premalignant foci [9–13]. The clinical characteristics and importance of SPTs in the aerodigestive tract are well worked out—SPTs occur at an annual rate of 4–8% and are the major cancer-related cause of death in early stage disease [14–16].

CLINICAL TRIALS

Head and Neck Trials

Retinoids have undergone extensive study in the reversal of oral premalignant lesions. Five randomized studies have been reported, all involving natural or synthetic derivatives of vitamin A (retinoids). The first study reported in 1986 included a three-month placebo-controlled study of 13-*cis*-retinoic acid (2 mg/kg/day). The complete plus partial response rate in the 44 evaluable patients was 67% in the retinoid arm and 10% in the placebo arm ($p = 0.0002$). The histologic improvement rate (reversal of cytologic abnormalities) was also higher in the retinoid arm (54% versus 10%, $p = 0.01$). There were two major problems, however, with this high-dose, short-term approach. First, high-dose 13-*cis*-retinoic acid toxicity was substantial and not acceptable in this clinical setting. Second, over 50% of the responders recurred or developed new lesions within 2–3 months of stopping the short-term retinoid intervention [17].

Based on the results of this placebo-controlled trial, a randomized maintenance trial was designed [18]. In this trial, patients initially received a three-month induction course of high-dose 13-*cis*-retinoic acid (1.5 mg/kg/day), followed by a nine-month maintenance course with low-dose 13-*cis*-retinoic acid (0.5 mg/kg/day) or β-carotene (30 mg/kg/day) in patients with responding or stable lesions during induction. The induction phase response rate was 55%. During maintenance, two (8%) of the patients in the retinoid group progressed versus 16 (55%) in the β-carotene group ($p < 0.001$). Maintenance therapy was well tolerated with no patients discontinuing therapy because of toxicity.

This trial included a number of clinical-laboratory translational studies. Standard histologic features, micronuclei, blood-group antigen expression, proliferation markers (*e.g.*, PCNA), squamous differentiation markers, nuclear retinoic acid receptors (RARs and RXRs), p53, and cytogenetic markers were studied and correlated with clinical outcomes [19–25]. Important translational findings from these interactive clinical-laboratory studies include the following: (a) cytogenetic alterations (*e.g.*, polysomy of chromosome 7 and 17) occur in oral premalignant lesions and may be associated with malignant transformation [19,20]; (b) p53 protein accumulation is an early event in oral carcinogenesis and is associated with retinoid resistance [21,22]; and (c) RAR-β expression is suppressed in approximately two-thirds of oral premalignant lesions and is highly upregulated by high-dose 13-*cis*-retinoic acid [23,24].

Three other randomized retinoid studies have been reported, two induction trails [26,27] and one maintenance [28] trial. All three studies observed significant retinoid chemopreventive activity.

In a randomized adjuvant study of high-dose 13-*cis*-retinoic acid in 103 head and neck cancer

patients, Hong *et al.* [29] achieved a significant reduction in the rate of second primary tumors. Following definitive local therapy of stage I–IV disease with surgery and/or radiotherapy, patients were randomly assigned to twelve months of 13-*cis*-retinoic acid (50–100 mg/m^2/day) or placebo. At a median follow-up of 32 months, there were no significant differences in primary disease recurrence (local, regional, or distant) or survival. The rate of SPTs, however, was significantly lower in the retinoid arm than in the placebo group. SPTs had developed in only two (4%) of the 13-*cis*-retinoic acid-treated patients compared with 12 (24%) of the placebo-treated patients ($p = 0.005$). More than 70% of SPTs occurred in the carcinogen-exposed aerodigestive tract fields of the head and neck, lungs, and esophagus. Side effects were substantial and included dry skin, cheilitis, conjunctivitis, and hypertriglyceridemia. Only one-third of the retinoid treated patients were able to complete the 12-month planned intervention.

This trial has been recently reanalyzed with the median follow-up extended to 55 months (versus earlier analysis of 32 months) [30]. The retinoid group continued to have fewer SPTs within the aerodigestive tract, developing in only three (7%) in the retinoid group and 13 (33%) in the placebo group ($p = 0.008$). The results suggest that the retinoid chemopreventive effect persisted well after the 12-month intervention. Furthermore, significant reduction in SPTs occurred in the retinoid arm despite the reduced doses in approximately one-third of the patients. Based on this high-dose adjuvant trial [29] and the recently completed low-dose 13-*cis*-retinoic acid trial in oral premalignancy [18], a multicenter large-scale Phase III NCI trial was designed and is ongoing to evaluate low-dose 13-*cis*-retinoic acid as adjuvant chemoprevention in stage I and II head and neck cancer. A recent report on this large-scale trial indicates that low-dose 13-*cis*-retinoic acid is well tolerated [31], confirming toxicity data from the earlier low-dose trial in oral premalignancy [18].

The synthetic retinoid etretinate underwent a recent French trial designed to prevent SPTs following definitive therapy of early stage squamous cell carcinomas of the oral cavity and oral pharynx [32]. In this randomized, placebo-controlled trial, the etretinate dose was 50 mg/day for one month, then 25 mg/day for two years. The rates of SPT development in the two study arms were not significantly different. Interpretation of this report is hampered by a lack of details regarding study compliance and SPT determination. This trial did, however, confirm other prospective data on the high rate of head and neck cancer-associated SPTs [16,33,34]. At a median follow-up of 41 months, 24% of placebo patients developed SPTs. Furthermore, consistent with field carcinogenesis, approximately 80% of SPTs developed in the head and neck, lungs or esophagus [32].

Lung Trials

A group of French investigators examined the effect of a retinoid directly on the bronchial epithelium [35]. Chronic smokers with squamous metaplasia of the bronchial epithelium detected in initial bronchoscopy specimens were treated with etretinate (25 mg/day) for six months. In this uncontrolled trial, a decline in the extent of squamous metaplasia was observed in most treated patients.

The positive result of this French study led to two randomized trials—one in Canada and one in the US. The Canadian study evaluated the ability of etretinate (25 mg/day) to reverse sputum atypia in chronic smokers. Changes in sputum atypia were assessed at the completion of a six-month treatment period. No difference in the degree of atypia occurred between the etretinate and placebo groups [36]. In the US study, chronic smokers underwent bronchoscopy with endobronchial biopsies taken from six specific anatomic sites within the proximal lung field [37], as reported in the earlier single-arm French study [35]. Ninety-three of the 152 chronic smokers who underwent bronchoscopic biopsies had squamous metaplasia or dysplasia. Eligible smokers with metaplasia or dysplasia were randomized to six months of 13-*cis*-retinoic acid or placebo. Results on the primary histologic study endpoint were recently published. The extent of metaplasia decreased similarly (in approximately 50% of subjects) in both study arms. Only smoking cessation significantly predicted a reduction in metaplasia index during the six-month intervention. Although the 13-*cis*-retinoic acid arm results were consistent with the French findings with etretinate, comparison with a placebo control group in the US retinoid trial

yielded a negative interpretation, in contrast to the uncontrolled French trial. These findings underscore the critical importance of placebo-controlled designs to establish drug activity in chemoprevention trials using intermediate endpoints.

Pastorino *et al.* [38] tested the natural retinoid derivative retinyl palmitate in 307 patients following resection of stage I non-small cell lung cancer. Following surgery, patients were randomly assigned to either treatment with retinyl palmitate (300,000 IU) for one year or observation. Greater than 80% compliance indicated that the high-dose retinyl palmitate was well tolerated. Only three patients dropped out of the treatment arm because of toxicity.

Eighteen patients in the retinyl palmitate arm developed SPTs compared with 29 patients in the control group. Reduction of tobacco-associated SPTs was more pronounced. After a median follow-up of 46 months, tobacco-related SPTs developed in 13 retinyl palmitate-treated patients compared with 25 control patients. The time to development of tobacco-associated SPTs also favored the retinyl palmitate arm ($p = 0.045$).

These encouraging results, which add to those of several other epithelial sites indicating significant retinoic activity in suppressing carcinogenesis, have led to the design of several large-scale Phase III retinoid trials [reviewed in 5]. There are two major ongoing Phase III trials studying retinoid prevention of lung cancer-associated SPTs, one in Europe and one in the US. The European multicenter trial (EUROSCAN) is a randomized 2x2 factorial design, adjuvant chemoprevention study of retinyl palmitate and *N*-acetylcysteine in early stage head and neck and lung cancer. In the US, there is a large multicenter Phase III trial of low-dose isotretinoin after complete resection of stage I non-small cell lung cancer (intergroup NCI I 91-0001).

Breast Trials

Moon and colleagues [3] showed that fenretinide was among the most active chemopreventive agents in early mammary carcinogenesis model studies. This laboratory work led to a large-scale randomized trial of fenretinide (versus no treatment) in more than 3,000 women after definitive local therapy of $T_{1,2}$ breast cancer. The primary endpoint of this study is contralateral breast cancer, which occurs at a rate of 0.8% per year. The study has completed accrual and is under analysis [28].

Current NCI plans are to initiate a Phase III trial of fenretinide plus tamoxifen. Mammary carcinogenesis studies from Moon and others showed that this combination was significantly more active than was either agent alone [3]. Single-agent tamoxifen chemopreventive activity has been shown in several preclinical and clinical studies [5,39]. There has been controversy over tamoxifen use in chemoprevention which is reviewed in detail elsewhere [5].

Further support for the combination of tamoxifen and fenretinide comes from a recent Phase I study in advanced breast cancer patients. This study recently showed that the combination is well tolerated and produces a substantial reduction in insulin-like growth factor (IGF)-I plasma levels [40]. Tamoxifen's suppressive effects on IGF-I are thought to be a mechanism of this agent's anticarcinogenic effects in breast carcinogenesis [41].

Skin Trials

Retinoids have been studied in several Phase II and III randomized trials in the skin. The data suggest that both topical and systemic retinoid therapy have significant activity in reversing premalignant skin lesions [5]. Several small studies found that 13-*cis*-retinoic acid or etretinate can significantly reduce the number of skin cancers in very high risk patients with xeroderma pigmentosum or transplant recipients [5,42]. Large-scale Phase III data in skin cancer are mixed. Trials of high-dose β-carotene [43] and very low-dose 13-*cis*-retinoic acid [44] in patients with prior skin cancer are negative. A large placebo-controlled trial of retinol (25,000 IU/day) in patients with prior skin premalignancy achieved a significant reduction of squamous cell skin cancers in the retinoid arm [45].

Cervical Trials

Many randomized chemoprevention studies have been conducted in cervical dysplasia, including two studies of folic acid, four of interferons, one of β-carotene, and one of all-*trans*-retinoic acid (ATRA) [5]. The only positive cervix

study was a placebo-controlled trial of topical ATRA [46]. This trial achieved a significant reversal of moderate [cervical intraepithelial neoplasia (CIN) 2] but not severe (CIN 3) dysplasia in the retinoid group.

RETINOID BIOLOGY

Retinoids exert their biological effects by binding to and activating specific intracellular receptors (IRs) resulting in a regulation of gene expression within cells [47–50]. The modulation of gene expression by retinoids determines its regulatory role on cell differentiation, cell proliferation and apoptosis. Recent insights into the molecular identification of the receptors for retinoids has occurred, beginning with the cloning and characterization of the human retinoic acid receptor [51,52]. In 1987, Petkovich *et al.* [51] and Giguere *et al.* [52] independently isolated a human orphan receptor gene and using the cotransfection assay, they demonstrated that it encoded the first known retinoic acid-activated transcription factor. This receptor was shown to bind ATRA with high affinity. The retinoic acid receptor (RAR) was found to be structurally and functionally related to other members of the IR superfamily. The discovery of an RAR provided a mechanism for understanding the biological effects of ATRA. Subsequently, additional RAR subtypes were isolated and designated RAR-α, RAR-β, and RAR-γ [53–55]. The ligand-binding and the DNA-binding domains of these receptors are highly conserved. The discovery of multiple RARs raised the question of whether all actions of ATRA were mediated by these receptors and whether additional biologically active vitamin A metabolites and retinoid receptors existed.

In 1990, a second class of retinoid responsive receptors was identified: the retinoid X receptors (RXRs) [56]. Subsequently, three members of the RXR subfamily, designated RXR-α, RXR-β, and RXR-γ, have been identified [50,57]. Although these receptors were identified by ATRA's ability to activate gene expression, this transcriptional response required considerably higher concentrations of ATRA than was needed to activate the RARs. Furthermore, RXRs were unable to bind ATRA with high affinity [56]. These data, as well as the observation that the amino acid sequences of the RXRs were quite different from the RARs, suggested that there may be two distinct retinoid receptor pathways. These observations led to the development of a strategy to identify a metabolite of ATRA that bound to and activated RXRs [58]. In 1992, two groups working independently identified 9-*cis*-retinoic acid as a retinoic acid isomer that binds with high affinity to members of the RXR subfamily [58,59]. Additional studies demonstrated that 9-*cis*-retinoic acid is a high affinity ligand not only for members of the RXR subfamily, but also for the RARs (Fig. 1); thus it functions as a pan agonist in that it binds and activates both receptor pathways [60,61].

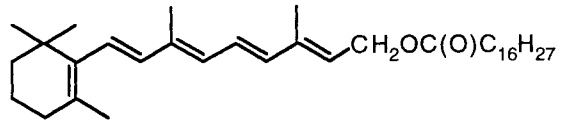
The use of cloned human IRs has allowed the development of technologies designed to identify novel endogenous hormones as well as synthetic compounds which mimic the action of hormones and/or have a greater degree of receptor selectivity. Just as the discovery of receptor subtypes for the neurotransmitters led to the development of more specific and therapeutically improved drugs, *e.g.*, cardioselective β-adrenergic blockers and receptor subtype-selective antihistamines, the discovery of IR subtypes suggests a similar potential for improving the selectivity and therapeutic index of IR-active drugs. Identifying compounds which selectively interact with distinct receptor subtypes should allow the development of drugs with fewer side effects.

Recently a few compounds with unique receptor selectivities have entered into human clinical trials (Fig. 2). These include an RAR-β/γ selective retinoid (Tazarotene) for dermatological uses, and a RAR/RXR pan agonist (9-*cis*-retinoic acid) and an RXR selective compound 4-[1-(3,5,5,8, 8, pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-ethenyl]benzoic acid (LDG1069) for oncological uses [62].

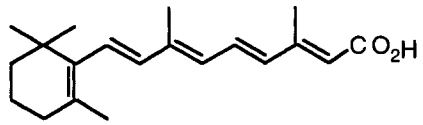
While the discovery of these nuclear retinoid receptors has illuminated cellular mechanisms of retinoid response, there are retinoids that do not bind known receptors yet have potent biologic effects. 4-Hydroxyphenylretinamide (4-HPR) is a synthetic retinoid which does not bind RARs or RXRs, yet it has demonstrated preclinical activity in the prevention of prostate, bladder and breast cancer [63-65], and *in vitro* it induces apoptotic cell death in several cancer cell lines [66]. These studies suggest that there may be additional retinoid pathways involving receptors not yet discovered. Another possibility is that 4-HPR may

Retinoids in Clinical Trials

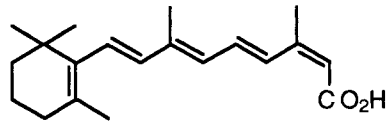
Receptor Selectivity



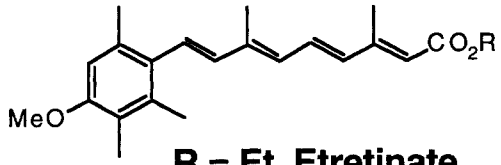
Retinyl palmitate — **prodrug?**



ATRA — **RAR-α,β,γ**



13-*cis* RA — **prodrug?**

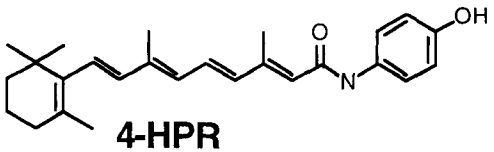


R = Et, Etretinate
R = H, Acitretin — **RAR-α,β,γ**

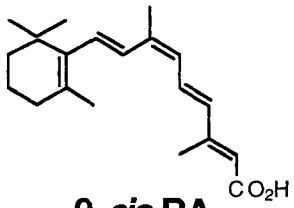
Fig. 1. Retinoid receptor selectivity of agents in Phase II and III clinical trials.

Retinoids in Clinical Trials

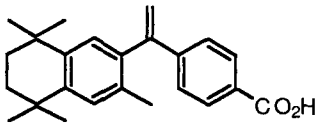
Receptor Selectivity



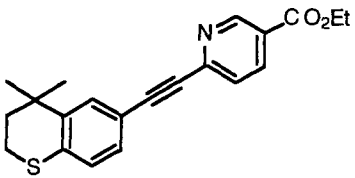
4-HPR — **prodrug?**



9-*cis* RA — **RAR-α,β,γ/RXR-α,β,γ**



LGD 1069 — **RXR-α,β,γ**



Tazarotene — **RAR-β,γ**

Fig. 2. *Retinoid receptor selectivity of new compounds in clinical trials.*

serve as a prodrug to the naturally occurring retinoic acid isomers.

In conclusion, basic scientific studies into the mechanism of retinoid action have enhanced our understanding of cellular pathways that control growth and differentiation. As our understanding grows, we will be challenged to harness this knowledge to augment the usefulness of retinoids as preventive and therapeutic agents. The identification of receptor-selective compounds and our increased understanding of the molecular basis of retinoic action offers promising opportunities to identify specific retinoids for effective chemoprevention and therapy of human cancer. Synthetic retinoids are being designed to target specific tissues and to induce specific biologic effects. This progress will depend on interactions between basic scientific and clinical investigators in order to translate laboratory findings into clinical advances.

REFERENCES

1. Lippman SM, Kessler JF, Meyskens FL: Retinoids as preventive and therapeutic anticancer agents (Part I). Cancer Treat Rep 71:391–405, 1987.
2. Smith MA, Parkinson DR, Cheson BD, Friedman MA: Retinoids in cancer therapy. J Clin Oncol 10:839-864, 1992.
3. Moon RC, Mehta RG: Chemoprevention of experimental carcinogenesis in animals. Prev Med 18:576–591, 1989.
4. Tanaka T: Chemoprevention of oral cancer. Oral Oncol Eur J Cancer 30B, 1994 (in press).
5. Lippman SM, Benner SE, Hong WK: Cancer chemoprevention. J Clin Oncol 12:851–873, 1994.
6. Vogelstein B, Fearon ER, Hamilton SR: Genetic alterations during colorectal tumor development. N Engl J Med 319:525–532, 1988.
7. Nawroz H, van der Riet P, Hruban RH, Koch W, Ruppert JM, Sidransky D: Allelotype of head and neck squamous cell carcinoma. Cancer Res 54:1152–1155, 1994.
8. Lippman SM, Lee JS, Lotan R, Hittelman W, Wargovich MJ, Hong WK: Biomarkers as intermediate end points in chemoprevention trials. J Natl Cancer Inst 82:555–560, 1990.
9. Chung KY, Mukhopadhyay T, Kim J, Casson A, Ro JY, Goepfert H, Hong WK, Roth JA: Discordant p53 gene mutations in primary head and neck cancers and corresponding second primary cancers of the upper aerodigestive tract. Cancer Res 53:1676–1683, 1993.
10. Nees M, Homann N, Discher H, Andl T, Enders C, Herold-Mende C, Schuhmann A, Bosch FX: Expression of mutated p53 occurs in tumor-distant epithelia of head and neck cancer patients: A possible molecular basis for the development of multiple tumors. Cancer Res 53:4189–4196, 1993.
11. Shin DM, Kim J, Ro JY, Hittelman J, Roth JA, Hong WK, Hittelman WN: Activation of p53 gene expression in premalignant lesions during head and neck tumorigenesis. Cancer Res 54:321–326, 1994.
12. Harris CC, Hollstein M: Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 329:1318–1327, 1993.
13. Boyle JO, Hakim J, Koch W, van der Riet P, Hruban RH, Rao RA, Correo R, Eby YJ, Ruppert JM, Sidransky D: The incidence of p53 mutations increases with progression of head and neck cancer. Cancer Res 53:4477–4480, 1993.
14. Hong WK, Lippman SM, Wolf GT: Recent advances in head and neck cancer—larynx preservation and cancer chemoprevention. Cancer Res 53:5113–5120, 1994.
15. Heyne KH, Lippman SM, Lee JJ, Lee JS, Hong WK: The incidence of second primary tumors in long-term survivors of small-cell lung cancer. J Clin Oncol 10:1519–1524, 1992.
16. Benner SE, Lippman SM, Hong WK: Chemoprevention of second primary tumors: A model for intervention trials. Eur J Cancer 30A:727–729, 1994.
17. Hong WK, Endicott J, Itri LM, Doos W, Batsakis JG, Bell R, Fofonoff S, Byers R, Atkinson EN, Vaughan C, Toth BB, Kramer A, Dimery IW, Skipper P, Strong S: 13-*cis*-Retinoic acid in the treatment of oral leukoplakia. N Engl J Med 315:1501–1505, 1986.
18. Lippman SM, Batsakis JG, Toth BB, Weber RS, Lee JJ, Martin JW, Hays GL, Goepfert H, Hong WK: Comparison of low-dose isotretinoin with beta carotene to prevent oral carcinogenesis. N Engl J Med 328:15–20, 1993.
19. Lee JS, Kim SY, Hong WK, Lippman SM, Ro JY, Gay ML, Hittelman WN: Detection of chromosomal polysomy in oral leukoplakia, a premalignant lesion. J Natl Cancer Inst 85:1951–1954, 1993.
20. Kim HJ, Lee JS, Shin DM, Lippman SM, Ro JY, Hong, WK, Hittelman WM: Chromosomal instability, p53 expression, and retinoid response in oral premalignancy. Proc Am Soc Clin Oncol, 1995 (in press).
21. Lippman SM, Shin DM, Lee JJ, Batsakis JG, Lotan R, Tainsky MA, Hittelman WN, Hong WK: p53 and retinoid chemoprevention of oral carcinogenesis. Cancer Res, 1995 (in press).
22. Shin DM, Lippman SM, Lee JJ, Xu X-C, Hittelman WN, Hong WK: Accumulation of p53 protein and retinoic acid receptor-β (RAR-β) in retinoid chemoprevention. Proc Am Soc Clin Oncol, 1995 (in press).
23. Lotan R: Retinoic acid receptors and retinoid-regulated differentiation markers as intermediate endpoints in chemoprevention. Proc Am Assoc Cancer Res 35:684, 1994.
24. Lotan R, Xu XC, Ro JY, Lee JS, Lippman SM, Lee JJ, Hong WK: Decreased retinoic acid receptor-β in human oral premalignant lesions and its induction by retinoic acid in vivo. Proc Am Assoc Clin Oncol

13:170, 1994.
25. Benner SE, Lippman SM, Wargovich MJ, Lee JJ, Velasco M, Martin JW, Toth BB, Hong WK: Micronuclei, a biomarker for chemoprevention trials: Results of a randomized study in oral premalignancy. Int J Cancer 59:457–459, 1994.
26. Stich HF, Hornby AP, Mathew B, Sankaranarayanan R, Krishnan Nair M: Response of oral leukoplakias to the administration of vitamin A. Cancer Lett 40:93–101, 1988.
27. Han J, Lu Y, Sun Z, Gu QM, Scanlon KJ: Evaluation of *N*-4-(hydroxycarbophenyl) retinamide as a cancer prevention agent and as a cancer chemotherapeutic agent. In Vivo 4:153–160, 1990.
28. Costa A, Formelli F, Chiesa F, Decensi A, De Palo G, Veronesi U: Prospects of chemoprevention of human cancers with the synthetic retinoid fenretinide. Cancer Res 54:2032s–2037s, 1994.
29. Hong WK, Lippman SM, Itri LM, Karp DD, Lee JS, Byers RM, Schantz SP, Kramer AM, Lotan R, Peters LJ, Dimery IW, Brown BW, Goepfert H: Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. N Engl J Med 323:795–801, 1990.
30. Benner SE, Pajak TF, Lippman SM, Earley C, Hong WK: Prevention of second primary tumors with isotretinoin in squamous cell carcinoma of the head and neck: Long term follow-up. J Natl Cancer Inst 86: 140–141, 1994.
31. Benner SE, Pajak TF, Stetz J, Lippman SM, Hong WK, Schantz SP: Toxicity of isotretinoin in a chemoprevention trial to prevent second primary tumors following head and neck cancer. J Natl Cancer Inst 86:1799–1801, 1994.
32. Bolla M, Lefur R, Ton Van J, Domenge C, Badet JM, Koskas Y, Laplanche A: Prevention of second primary tumors with etretinate in squamous cell carcinoma of the oral cavity and oropharynx. Results of a multicentric double-blind randomized study. Eur J Cancer 30A:767–772, 1994.
33. Vikram B: Changing patterns of failure in advanced head and neck cancer. Arch Otolaryngol 110:564–565, 1984.
34. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK: Head and neck cancer. N Engl J Med 328:184–194, 1993.
35. Misset JL, Mathé G, Santelli G, Gouveia J, Homasson JP, Sudre MC, Gaget H: Regression of bronchial epidermoid metaplasia in heavy smokers with etretinate treatment. Cancer Detect Prev 9:167–170, 1986.
36. Arnold AM, Browman GP, Levine MN, D'Souza T, Johnstone B, Skingley P, Turner-Smith L, Cayco R, Booker L, Newhouse M, Hryniuk WM: The effect of the synthetic retinoid etretinate on sputum cytology: Results from a randomized trial. Br J Cancer 65:737–743, 1992.
37. Lee JS, Lippman SM, Benner SE, Lee JJ, Lukeman JM, Morice RC, Peters EJ, Pang AC, Fritsche HA Jr, Hong WK: Randomized placebo-controlled trial of isotretinoin in chemoprevention of bronchial squamous metaplasia. J Clin Oncol 12:937–945, 1994.
38. Pastorino U, Ifante M, Maioli M, Chiesa G, Buyse M, Firket P, Rosmentz N, Clerici M, Soresi E, Valente M, Belloni PA, Ravasi G: Adjuvant treatment of stage I lung cancer with high-dose vitamin A. J Clin Oncol 11:1216–1222, 1993.
39. Early Breast Cancer Trialists' Collaborative Group: Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. Lancet 339:1–15, 71–84, 1992.
40. Cobleigh MA, Oleske DM, Nickerson T, Pollak M: Serum IGF-I levels in stage IV breast cancer treated with tamoxifen and fenretinide. Proc Am Assoc Cancer Res, 1995 (in press).
41. Yee D: The insulin-like growth factor system as a target in breast cancer. Breast Cancer Res Treat 32: 85–95, 1994.
42. Kraemer KH, DiGiovanna JJ, Moshell AN, Tarone RE, Peck GL: Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin. N Engl J Med 318:1633–1637, 1988.
43. Greenberg ER, Baron JA, Stukel TA, Stevens MM, Mandel JS, Spencer SK, Elias PM, Lowe N, Nierenberg DW, Bayrd G, Vance JC, Freeman DH Jr, Clendenning WE, Kwan T, Skin Cancer Prevention Study Group: A clinical trial of beta carotene to prevent basal-cell and squamous-cell cancers of the skin. N Engl J Med 323:789–795, 1990.
44. Tangrea JA, Edwards BK, Taylor PR, Hartman AM, Peck GL, Salasche SJ, Menon PA, Benson PM, Mellette JR, Guill MA, Robinson JK, Guin JD, Stoll HL, Grabski WJ, Winton GB, other members of the Isotretinoin-Basal Cell Carcinoma Study Group: Long-term therapy with low-dose isotretinoin for prevention of basal cell carcinoma: A multicenter clinical trial. J Natl Cancer Inst 84:328–332, 1992.
45. Moon TE, Cartmel B, Levine N, Bangert J, Peng YM, The Nevada-Arizona Skin Cancer Study Group: Chemoprevention and etiology of non-melanoma skin cancers. Proc Am Soc Prev Oncol, 1993 (abstract).
46. Meyskens FL Jr, Surwit E, Moon TE, Childers JM, Davis JR, Door RT, Johnson CS, Alberts DS: Enhancement of regression of cervical intraepithelial neoplasia II (moderate dysplasia) with topically applied all-*trans*-retinoic acid: A randomized trial. J Natl Cancer Inst 86:539–543, 1994.
47. Evans R: The steroid and thyroid hormone receptor superfamily. Science 240:889, 1988.
48. O'Malley B: The steroid receptor superfamily: More excitement predicted for the future. Mol Endocrinol 4:363–369, 1990.
49. Leid M, Kastner P, Chambon P: Multiplicity generates diversity in the retinoic acid signalling pathways. TIBS 17:427, 1992.
50. Mangelsdorf DJ, Umesono K, Evans RM: The retinoid receptors. In MB Sporn, AB Roberts, DS Goodman (eds): "The Retinoids." New York: Raven Press, 1994, pp 319–349.
51. Petkovich M, Brand NJ, Krust A, Chambon P: A human retinoic acid receptor which belongs to the

family of nuclear receptors. Nature 330:444–450, 1987.
52. Giguere V, Ong ES, Segui P, Evans RM: Identification of a receptor for the morphogen retinoic acid. Nature (London) 330:624–629, 1987.
53. Brand N, Petkovich M, Krust A, Chambon P, de The H, Marchio A, Tiollais P, Dejean A: Identification of a second human retinoic acid receptor. Nature 332: 850–853, 1988.
54. Benbrock D, Lemhardt E, Pfahl M: A new retinoic acid receptor identified from hepatocarcinoma. Nature 333:669–672, 1988.
55. Ishikawa T, Umesono K, Mangelsdorf DJ, Aburatani H, Stanger BZ, Shibasaki Y, Imawari M, Evans RM, Takaku F: A functional retinoic acid receptor encoded by the gene on human chromosome 12. Mol Endocrinol 4:837–844, 1990.
56. Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM: Nuclear receptor that identifies a novel retinoic acid response pathway. Nature 345:224–229, 1990.
57. Mangelsdorf DJ, Borgmeyer U, Heyman RA, Zhou JY, Ong ES, Oro AE, Kakizuka A, Evans RM: Characterization of three RXR genes that mediate the action of 9-*cis* retinoic acid. Genes Dev 6:329–344, 1992.
58. Heyman RA, Mangelsdorf DJ, Dyck JA, Thaller C: 9-*cis* Retinoic acid is a high affinity ligand for the retinoid X receptor. Cell 68:397–406, 1992.
59. Levin AA, Sturzenbecker LJ, Kazmer S, Bosakowski T, Huselton C, Allenby G, Speck J, Kratzeisen Cl, Rosenberger M, Lovey A, Grippo JF: 9-*cis* Retinoic acid stereoisomer binds and activates the nuclear receptor of RXRα. Nature 355:359–361, 1992.
60. Allegretto EA, McClurg MR, Lazarchik SB, Clemm DL, Kerner SA, Elgort MG, Boehm MF, White SK, Pike PW, Heyman RA: Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: Correlation with hormone binding and effects of metabolism. J Biol Chem 268:26625–26633, 1993.
61. Allenby G, Janocha R, Kazmer S, Speck J, Grippo JF, Levin AA: Binding of 9-*cis*-retinoic acid and all-*trans*-retinoic acid to retinoic acid receptors α, β, and γ. J Biol Chem 269:16689–16695, 1994.
62. Boehm M, McClurg M, Pathirana C, Mangelsdorf D, White SK, Herbert J, Winn D, Goldman ME, Heyman RA: Synthesis of high specific activity [^{3}H]-9-*cis*-retinoic acid and β application for identifying retinoids with unusual binding properties. J Med Chem (in press).
63. Moon RC, Thompson HJ, Becci PJ, Grubbs CJ, Gander RJ, Newton DL, Smith JM, Phillips SL, Henderson WR, Mullen LT, Brown CC, Sporn MB: *N*-(4-Hydroxyphenyl)retinamide, a new retinoid for prevention of breast cancer in the rat. Cancer Res 39:1339–1346, 1979.
64. Malone WF, Kelloff GJ, Pierson H, Greenwald P: Chemoprevention of bladder cancer. Cancer 60:650–657, 1987.
65. Slawin K, Kadmon D, Park SH, Scardino PT, Anzano M, Sporn MB, Thompson TC: Dietary fenretinide, a synthetic retinoid, decreases the tumor incidence and the tumor mass of *ras+myc*-induced carcinomas in the mouse prostate reconstitution model system. Cancer Res 53:4461–4465, 1993.
66. Delia D, Aiello A, Lombardi L, Pelicci PG, Grignani F, Grignani F, Formelli F, Menard S, Costa A, Veronesi U, Pierotti MA: *N*-(4-Hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid. Cancer Res 53:6036–6041, 1993.