Drugs of the Future 1998, 23(6): 621-634

CCC: 0377-8282/98

Review Article

Retinoids as chemopreventive and therapeutic agents

Shi-Yong Sun and Reuben Lotan*

*Departments of Tumor Biology and Clinical Cancer Prevention, The University of Texas M.D. Anderson Cancer Center Houston, TX 77030, USA. *Correspondence*

CONTENTS

Summary

Retinoids, naturally occurring and synthetic vitamin A metabolites and analogs, exhibit promising antitumor effects in a variety of *in vitro* and *in vivo* model systems and in clinical trials. They inhibit carcinogenesis in various tissues in animal models, suppress premalignant human epithelial lesions and prevent second primary tumors following curative therapy for epithelial malignancies such as head and neck and lung cancer. Laboratory and clinical studies also indicate that retinoids have a potential as therapeutic agents. Retinoids inhibit cell proliferation and induce cell differentiation and apoptosis in various types of tumor cells. Significant therapeutic activity has been observed with all-*trans*-retinoic acid in acute promyelocytic leukemia. The mechanisms underlying the anticarcinogenic and antitumor activities of retinoids appear to be associated with their ability to modulate the growth and differentiation of normal, premalignant and malignant cells *in vitro* and *in vivo*. Most of these effects are mediated by nuclear retinoid receptors; however, other mechanisms may also be involved. This review summarizes the studies which indicate that retinoids are potentially useful agents for cancer chemoprevention and therapy.

Introduction

The term retinoids, first coined by Sporn in 1976 (1), generally refers to the entire group of compounds, including both naturally occurring and synthetic vitamin A (retinol) metabolites and analogs that play important roles in several diverse cellular processes, including embryonic development, vision, reproduction, bone formation, hematopoiesis, metabolism and cellular differentiation, proliferation and apoptosis (2-5). The basic structure of a retinoid molecule consists of a cyclic end group, a polyene side chain and a polar end group (Fig. 1). The chemical manipulation or modification of any part of the molecule produces different retinoids with different therapeutic indices and distinct side effects. The profound effects of vitamin A metabolites on cellular differentiation and proliferation and, in particular, the hope to use them clinically, have spurred the synthesis of a large variety of vitamin A analogs. Since the mid-1950s, more than 4000 vitamin A analogs have been synthesized to produce drugs with improved therapeutic indices and potential uses in a variety of skin disorders and malignant diseases (6-8). More recently, the discovery that two nuclear retinoid receptor subfamilies, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), were the mediators of major functions of retinoids on gene expression has led to the synthesis of novel retinoids with receptor subtype selectivity (9-19). The major limitation for the widespread clinical use of the currently available retinoids is their undesirable side effects and toxicities. These include mucocutaneous irritation, elevation of plasma triglyceride levels, headache, bone toxicity and teratogenicity (6). It is thought that the many diverse actions of retinoids, both desirable and undesirable, arise through activation of multiple retinoid receptor subtypes. Therefore, it is plausible to assume that retinoids with receptor subtype selectivity, such as RAR- and RXR-selective agonists as well as RAR subtype-specific antagonists (Fig. 1), may exhibit improved therapeutic indices.

Presently, the naturally occurring retinoids (Fig. 1) all-*trans*-retinoic acid (ATRA) and 13-*cis*-retinoic acid (13CRA, isotretinoin) are used to treat severe acne, and synthetic etretinate is prescribed for severe, refractory psoriasis. More recently, ATRA and 13CRA, given as single agents or in combination with other agents such as interferon-α (IFN-α), have shown promise in the control of cancers or precancers such as acute promyelocytic leukemia (APL) (20, 21), head and neck cancer (22, 23), skin cancer (24) and cervical cancer (25, 26). Encouragingly, ATRA was recently approved by the Food and Drug Administration in the United States for use in patients with APL who have relapsed after other chemotherapy. Other synthetic retinoids are in various stages of development for the treatment or prevention of

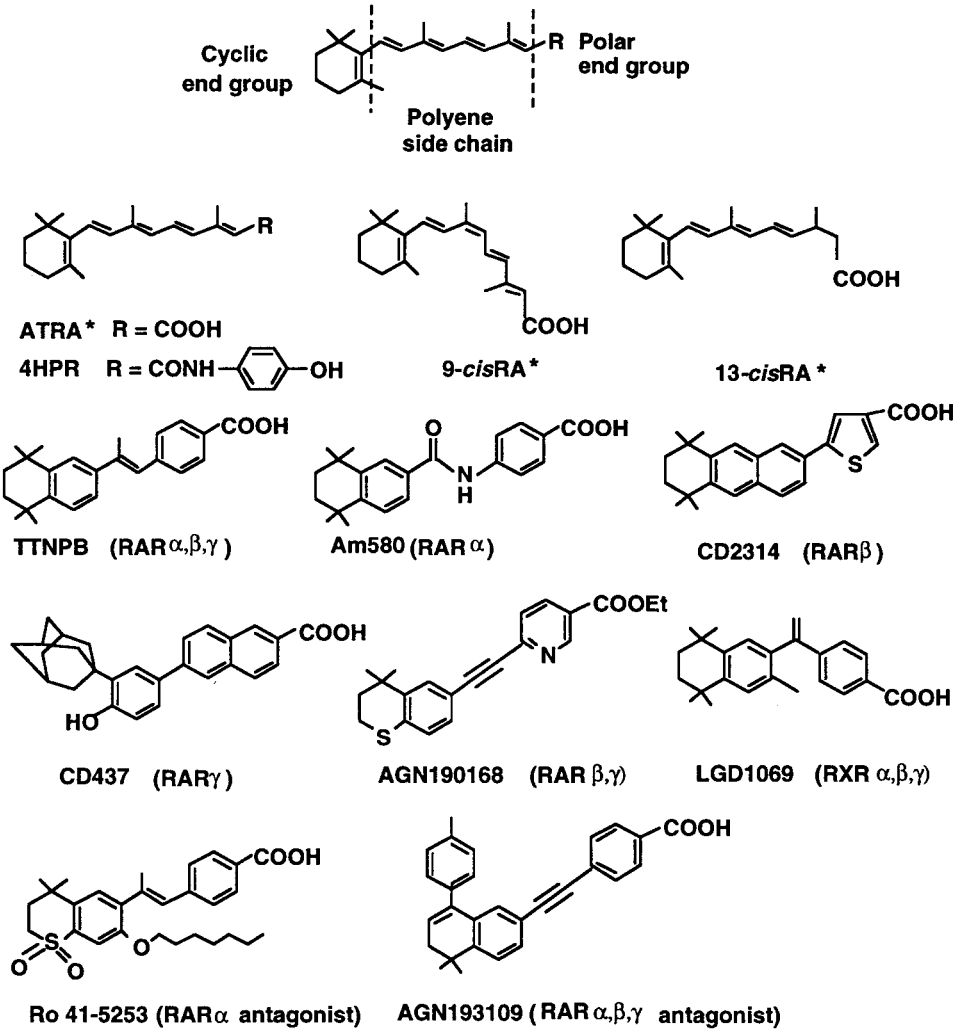

Fig. 1. Chemical structure of selected retinoids and their retinoid receptor selectivity. The naturally occurring retinoic acids are indicated by an asterisk.

cancer and skin diseases. *N*-(4-Hydroxyphenyl) retinamide (4HPR) is in phase III trials in previously treated breast cancer patients to evaluate chemoprevention of second primary breast cancer and in phase II trials as a chemopreventive agent in breast (alone and in combination with tamoxifen), cervix, lung, prostate, bladder, skin (actinic keratosis patients) and oral cavity (27) cancers. Phase III clinical trials for topical treatment of acne and psoriasis have been completed using AGN-190168 (tazarotene), a novel RARβ- and RARγ-selective retinoid (28).

LGD1069 (Targretin®), the first RXR-selective retinoid, has also entered clinical trials for the treatment of cancer (29). CD437, a RARγ-selective retinoid, was recently found to be a very potent inducer of apoptosis in cell lines derived from solid tumors of the breast (30), cervix (31), skin (melanoma) (32), lung (33) and prostate (our unpublished data). Antitumor effects of CD437 were also observed *in vivo* in a human melanoma xenograft in nude mice with minimal toxicity (32), showing its potential for treatment of solid tumors. In addition to receptor agonists, several RAR-selective antagonists (Fig. 1) which bind to the receptors but fail to enhance gene transcription have also been described. Ro-41-5253, which exhibits selectivity for the RARα subtype, was shown to antagonize the teratogenic effects of the RARα-selective agonist AM-580 in rat limb bud cell cultures and in mice (34). These retinoid antagonists may have potential use in the clinic to neutralize or decrease the side effects and toxicity of retinoids.

The diverse effects of retinoids on cell growth, differentiation and apoptosis spurred numerous basic studies and clinical investigations evaluating retinoids for cancer chemotherapy as well as cancer chemoprevention. Over the last decade, these studies have led to exciting progress, which is summarized in this review. Although

progress has also been made in the application of retinoids in several skin diseases (35-37), this review is focused on their potential for chemoprevention and treatment of cancer.

Retinoids as chemopreventive agents

Retinoids are one of the prominent chemopreventive agents that have reached clinical trials (38-40). A strong relationship between vitamin A and cancer development has been established by numerous investigations over the last couple of decades. Vitamin A deficiency in experimental animals has been associated with a higher incidence of cancer and with increased susceptibility to chemical carcinogens (41). Further, epidemiological studies have indicated that individuals with a lower dietary vitamin A intake are at a higher risk to develop cancer (38). These observations have led to the hypothesis that physiological levels of retinoids guard the organism against the development of premalignant and malignant lesions.

Preclinical studies

Extensive studies conducted during the last decade have highlighted the anticarcinogenetic effects of retinoids in cell or tissue culture systems *in vitro* and in animal models *in vivo*. Retinoids suppress the transformation of cells or tissues induced by chemical, physical and viral carcinogens (42). More importantly, retinoids have been found to be effective in suppressing tumor development in several carcinogenesis models, including those of the skin, breast, oral cavity, lung, prostate, bladder, liver and pancreas (41, 42). They have been administered either topically or systemically, in the diet or intragastrically, and before, concurrently or after a carcinogen or a tumor-promoting agent to determine whether they affect tumor initiation, promotion, or both. Many studies have demonstrated that certain retinoids possess antipromotion activity. In some studies, in which multiple tumors developed in control animals asynchronously, retinoids were administered after the first tumor had already appeared and been excised and were found to suppress the development of second primary tumors. The effects of retinoids were reversible when retinoid treatment was started after the carcinogenic insult and discontinued after a few weeks. This finding led to the conclusion that retinoids may have to be used continuously to achieve long-term suppression of carcinogenesis. Initial studies used naturally occurring retinoids such as retinyl palmitate, ATRA or 13CRA. However, with the increase in the availability of synthetic retinoids, more active compounds have been identified that have lower toxicity and/or improved pharmacokinetics than the natural retinoids. Some retinoids were found to be active in certain animal models of carcinogenesis but not in others. The effect of retinoids was not restricted to a specific carcinogen but rather to the type of tissue involved, suggesting that some retinoids exhibit tissue selectivity. It is noteworthy that several studies have demonstrated clearly that certain retinoids that are active inhibitors of carcinogenesis in certain tissues can act as enhancers of carcinogenesis in the same tissue in other strains of mice or in another carcinogenesis model (43-45). This could be due to different tissue distribution and metabolism of some carcinogens.

Clinical trials

Many of the major cancers (*e.g.*, lung, breast, colon) continue to cause severe morbidity and mortality, and the overall survival of patients has not improved significantly over the last few decades. Therefore, efforts are underway to develop new strategies for early intervention in the onset of malignant disease (38-40). Currently, most clinical trials for chemoprevention target individuals at an increased risk of developing cancer such as patients who have premalignant lesions or patients who have been treated for an early-stage cancer but remain at a high risk to develop a second primary cancer. Retinoids have been implicated in the prevention of various epithelial cancers on the basis of epidemiological studies that demonstrated an inverse relationship between vitamin A intake and cancer incidence (38).

1) Effects on premalignant lesions

a) Cutaneous actinic keratoses

Retinoids have been used in the treatment and prevention of a variety of cutaneous premalignant and malignant lesions. Actinic keratoses are premalignant lesions that are prevalent in older people following years of sun damage. Topical ATRA was effective in reducing the number of such lesions with a response rate of about 50%. Further, a randomized trial with 40 patients treated with systemic etretinate (75 mg/day) *versus* placebo for 2 months showed that the drug was effective in reversing lesions in 84% of the patients compared with only 5% in the placebo group. Similar results were obtained in a more recent randomized study with 31 patients (38).

Numerous actinic keratoses often develop in renal transplant recipients. Forty-four such patients with more than 10 keratotic lesions on the hands and forearms were enrolled in a randomized, double-blind, placebo-controlled trial of 6 months treatment with acitretin (30 mg/day) *versus* placebo. Eleven of the 38 evaluable patients developed squamous cell carcinomas, which were distributed unequally between the two groups: 2 of the 19 retinoid-treated group compared to 9 of the 19 placebo group. The treatment also prevented keratotic lesion development inasmuch as the number of lesions decreased by 13.4% in the treatment group and increased by 28.2% in the placebo group (46). Topical ATRA with or without low-dose systemic etretinate (10 mg/day) was also effective in suppressing the develop-

ment of new skin tumors and in reducing the number of existing neoplastic lesions in renal transplant recipients (47).

b) Dysplastic nevi

Patients with dysplastic nevus syndrome were treated with topical ATRA on one-half of their back for 6 months followed by excision of nevi from both the treated and untreated sides of the back and clinical and histological evaluation. The treatment resulted in clinical and histological improvement, including decreased clinical atypia of treated lesions and disappearance of many treated nevi in some patients (48, 49).

c) Oral premalignant lesions

Oral premalignant lesions (OPLs) are either white (leukoplakia) or red (erythroplakia) mucosal patches in the oral cavity or oropharynx that progress to malignant lesions in 6-20% of cases. Because surgery is not usually an option for patients with extensive or multiple lesions, such patients are good candidates for chemoprevention. 13CRA has been used in several randomized, placebo-controlled studies in patients with OPLs. In one study, 44 subjects were randomized to either high-dose 13CRA (1-2 mg/kg/day) or placebo for 3 months with a 6-month follow-up. Major clinical responses were observed in 67% of the treatment arm compared with 10% in the placebo arm (38). This study clearly demonstrated that OPLs are responsive to retinoids. However, there were two unfavorable aspects of the study: high-dose 13CRA had an unacceptable toxicity and resulted in patients leaving the study and OPLs recurred in 50% of the responding patients within 3 months of drug discontinuation. Therefore, a second trial was designed to address these problems as follows: 70 patients were treated with high-dose 13CRA (1.5 mg/kg/day) for 3 months (induction phase). This resulted in a clinical response rate of 55%. The patients were then randomized to two groups for a maintenance phase. One group received low-dose 13CRA (0.5 mg/kg/day) and the other received β-carotene (64 mg/day) for 9 months. The 13CRA group showed an 8% progression rate whereas the β-carotene group showed a 55% progression rate. These results demonstrated the feasibility of maintaining the initial clinical response with low-dose 13CRA but not with β-carotene (38, 40). Interestingly, recent studies with heavy smokers demonstrated that β-carotene can enhance the incidence of lung cancer and suggested that this compound should no longer be considered for future cancer prevention trials (50, 51). In a recent study, 4 of 9 patients treated with 13CRA (1 mg/kg/day) for 3 months had a complete resolution of their OPLs. Transforming growth factor-α expression, which was elevated in the premalignant lesion relative to adjacent normal before treatment, showed a marked decreased after treatment, suggesting that it can be an intermediate biomarker in prevention studies of OPLs (52).

4HPR was used to treat 8 patients with diffuse non-operable OPLs (*e.g.*, leukoplakia) by topical application twice daily. After 1 month of therapy, 2 patients had complete remission and the other 6 had a greater than 75% response rate (53, 54). A much larger study was conducted to evaluate the efficacy of 4HPR in preventing recurrences, new localizations and the development of carcinomas in patients who had been treated surgically for oral leukoplakia. Data from 137 randomized patients who received either 200 mg 4HPR daily for 52 weeks or no intervention with a 1 year follow-up showed 8 recurrences, 12 new occurrences and 1 second primary cancer in the control group, whereas 7 recurrences, 2 new occurrences and no carcinomas developed in the 4HPR group (53, 54).

d) Bronchial metaplasia

Squamous metaplasia is frequently seen in biopsies of bronchial epithelium from heavy smokers. Although a reversal of such a metaplasia was reported in an uncontrolled trial with etretinate, a more recent randomized, placebo-controlled trial with 13CRA (1 mg/kg/day for 6 months) failed to demonstrate a specific retinoid-induced reversal of metaplasia because complete reversal of metaplasia was observed in both arms of the study when 69 individuals were reevaluated at the completion of the study. The reversal of metaplasia was associated with smoking cessation and was not observed in those who continued to smoke (55). Several other studies have also failed to demonstrate that retinoids have an effect on the reversal of bronchial metaplasia or on sputum atypia in chronic smokers (38).

e) Laryngeal papillomatosis

This disease is a benign growth of polypoid lesions on the vocal cords that requires frequent surgical intervention and may precede the development of squamous carcinoma. Treatment of patients with extensive growth with 13CRA (0.5-2 mg/kg/day) or with etretinate (1 mg/kg/day) resulted in 50-67% response rates. However, adjuvant treatment of patients failed to prevent recurrence (38).

f) Cervical dysplasia

Cancer of the cervix develops in a multistep fashion through a series of premalignant lesions of increasing severity called cervical intraepithelial neoplasia I, II and III (CIN I, II, III). A placebo-controlled, randomized trial examined the efficacy of ATRA applied topically as a 1 ml of 0.372% cream on a collagen sponge within a cervical cap to reverse CIN in 301 women. Patients were treated daily for 4 days at the beginning of the trial and then for 2

days at months 3 and 6. The results showed that ATRA induced the regression rate of CIN II lesions (moderate dysplasia) in 43% of the patients compared with a spontaneous regression of 27% in the control group but had no effect on more severe dysplasia (56). A study in China used *N*-(4-carboxyphenyl)retinamide (RII) administered intravaginally in a suppository containing 20 mg (RII) once daily for 2 courses of 50 days each. The treatment caused regression of premalignant lesions in 68% of the patients (57).

2) Prevention of second primary tumors

a) *Xeroderma pigmentosum*

Afflicted with a rare recessive disease of defective DNA repair, patients with *Xeroderma pigmentosum* have a 1000-fold increased risk of developing skin cancers (basal cell carcinoma, squamous cell carcinoma, melanoma). A group of 5 patients who had a total of 121 basal or squamous cell carcinomas 2 years before treatment were rid of all existing tumors surgically and then treated with oral 13CRA at a high dose (2 mg/kg/day) for 2 years and followed for 1 year off drug. During the treatment period, only 25 new tumors developed in these patients. However, after cessation of treatment, there was an 8.5-fold increase in tumor occurrence. These results indicated that the treatment only suppressed the expression of the premalignant lesions and their conversion into malignant lesions but failed to inhibit the initiation of new lesions (58). The same patients were included in a second study with 13CRA at a lower dose (0.5 mg/kg/day) for 1 year and monitored for the incidence of new tumors. The frequency of new tumors decreased in most patients, even at this lower dose. These promising results are a clear proof of principle that chemoprevention may be an important strategy even for patients with a genetic predisposition for cancer development.

b) Basal cell carcinoma

In a randomized, double-blind, placebo-controlled trial, 981 patients with a history of at least two basal cell carcinomas in the 5 years preceding the trial were treated with low-dose 13CRA (0.14 mg/kg/day) or placebo for 3 years. In contrast to the studies with high-dose 13CRA, the low dose was ineffective in decreasing the incidence of basal cell carcinomas (58).

c) Head and neck cancer

A randomized study in which 13CRA was evaluated as adjuvant treatment for recurrence in head and neck cancer resulted in the unexpected observation that the incidence of second primary tumors was reduced in the treatment group. After surgery or radiotherapy of stage I-IV head and neck cancer, 103 patients were randomized to receive 13CRA (50-100 m^2/day) or placebo for 1 year. After a median follow-up of 32 months, second primary tumors had developed in 4% of the treatment group compared with 24% in the control group. However, 13CRA had no effect on the rate of recurrence or metastasis (38-40). A more recent analysis revealed that at 55 months follow-up, the rate of second primary tumor development was 7% and 33% in the treatment and control arms, respectively. To confirm these findings in a larger group of patients, another study is under way to compare the effect of low-dose 13CRA *versus* placebo on the incidence of second primary cancers in about 1200 patients with stage I and II head and neck cancer after surgery or radiotherapy.

In another trial, etretinate (50 mg/day for the first month and 25 mg/day subsequently for a total of 24 months) was compared with placebo in 316 patients who had been treated with surgery or radiation therapy for early-stage head and neck squamous cell carcinoma and followed for 5 years. There was no difference in survival, disease-free survival and the incidence of second primary cancers between the two groups. Thus, etretinate was ineffective in preventing second primary tumors in the oral cavity and oropharynx (59).

d) Non-small cell lung cancer

Vitamin A was used in a trial with 307 patients who had undergone resection of stage I non-small cell lung cancer. The patients were randomized to receive either retinyl palmitate (300,000 IU/day) or placebo. After 1 year, second primary tumors developed in 29 patients in the control compared with 18 in the treatment group. When the smoking status was taken into account, it was found that, after a median follow-up of 46 months, 25 tumors developed in the control group compared with only 13 in the retinyl palmitate group (60).

e) Ovarian cancer

An incidental finding made during the breast cancer prevention trial with 4HPR was that ovarian cancer developed in 6 women and all of them were in the placebo group (61). These findings suggest that 4HPR can prevent the development of ovarian cancer.

f) Bladder cancer

A trial of 13CRA (initially administered at 0.5 mg/kg/day and then increased to 1 mg/kg/day) as prevention of recurrent early-stage bladder cancer in 20 eligible patients showed that the compound was toxic, resulting in 8 patients dropping out of the study before 3 months and 4 before 6 months. Most of the patients had a relapse within 1 year. The study was terminated because of toxicity and lack of positive results (62).

Seventy-nine patients with superficial papillary bladder tumors stages T-a and T-1 entered a prospective, randomized, double-blind trial of etretinate (25 mg/day) *versus* placebo. The time to first recurrence was the same in both groups, but the mean interval to subsequent recurrence was increased from 12.7 months in the placebo group to 20.3 months in the treatment group. Thus, the number of annual transurethral resections in the treatment group decreased from 2.1 to 0.95, whereas in the control group this number decreased from 1.7 to only 1.3 (63).

Retinoids as therapeutic agents

Although retinoids have attracted attention primarily as cancer chemopreventive agents, considerable laboratory and clinical evidence supports their therapeutic effects as anticancer agents. For example, the successful application of ATRA for differentiation therapy of APL (20, 21) has rekindled interest in the use of retinoids for the treatment of established malignancies.

In vitro *effects*

1) Effects on cell proliferation

The growth and differentiation of various normal and malignant cells in culture is modulated (stimulated or inhibited) by retinoids (64). The most frequently observed effect of retinoids on tumor cells *in vitro* is the inhibition of anchorage-dependent growth. Many tumor cell types, including melanoma, neuroblastoma, glioma, retinoblastoma, embryonal carcinoma, carcinomas of the lung, breast, prostate, bladder, colon, skin, head and neck and cervix, and various types of sarcoma that grow as adherent monolayers on plastic tissue culture dishes, often exhibit a decrease in growth rate and saturation density after exposure to retinoids (64). These effects occur in the retinoid concentration range between 1 nM and 10 μM and are dependent on the dose and duration of treatment. DNA synthesis is suppressed within 12-24 h of treatment initiation, and the cells are either arrested or accumulate in the G_1 or S phase of the cell cycle and growth inhibition can be detected within 24-72 h, depending on the doubling time of the cells. Maintenance of the growth inhibited state usually requires the continuous presence of retinoids because their removal from the culture medium often results in a reversal of these effects within 24-72 h. The growth of several types of cells, in particular many hematopoietic cells such as lymphomas, leukemias, myelomas, premonocytic and premyelocytic leukemias, that normally grow in suspension can be inhibited by retinoids. Some solid tumor cells can grow as tightly packed multicellular spheroids suspended in liquid medium. The growth of such spheroids is also suppressed by retinoids (65). Many tumor cells exhibit an anchorage-independent growth as colonies in agar, agarose or methyl cellulose, and this growth is considered to be a hallmark of malignant transformation because it distinguishes malignant cells from normal cells. Treatment of various transformed and tumor cells with retinoids restores anchorage dependence and inhibits the ability of the cells to grow in suspension. The effective concentrations of retinoids for inhibition of tumor cell growth in culture are often pharmacological and not physiological. However, quite a large number of tumor cells are inhibited considerably even at physiological doses (1 μM for retinol and 0.01 μM for ATRA). Often, cells that are only marginally inhibited in a monolayer culture show marked inhibition of anchorage-independent growth in agarose, suggesting that the inhibition of anchorage-independent growth is a more sensitive assay for the suppression of the growth of tumor cells by retinoids (64, 65). This assay has also been performed with cells dissociated from fresh tumor biopsies and has demonstrated sensitivity of different tumor cells to different retinoids (66-68).

2) Cytotoxicity of retinoids and their effect on apoptosis

In addition to the cytostatic effects of retinoids, these compounds exert nonspecific cytotoxic effects and induce apoptosis (programmed cell death). At concentrations higher than 50 μM, many retinoids exhibit cytotoxic effects on most cells. The toxicity of retinoids is probably due to their detergent-like effects at high concentrations. The cytotoxicity is enhanced in serum-free medium, possibly because of the absence of serum albumin, which binds retinoids.

Retinoids also induce apoptosis in various types of cancer cells. The natural retinoids ATRA and 9-*cis*-RA (9CRA) induce apoptosis in some types of cells such as leukemia cells (69-71), but they are not very effective at inducing apoptosis in many solid tumors. More recently, a large number of studies focused on the induction of apoptosis by synthetic retinoids such as 4HPR and CD437, which exhibit much more potent activity in induction of apoptosis than ATRA. 4HPR has been proved be an effective inducer of apoptosis in various types of cancer cells, including leukemia cells (72, 73), neuroblastoma cells (74-76) and lung (77), head and neck (78), cervical (79), breast (80, 81) and prostate (82, 83) cancer cells. Compared with 4HPR, CD437 has an even more potent effect in inducing apoptosis in several solid tumors and is very active in inducing apoptosis in human breast (30), cervical (31), melanoma (32), lung (33) and prostate (our unpublished data) cancer cells.

The induction of apoptosis by retinoids is related to cell growth and differentiation in various ways, depending on the cell type. Growth arrest by retinoids can lead to either terminal differentiation or apoptosis. There are several patterns of relationships between the effects of retinoids on cell differentiation and apoptosis: (i) retinoids first induce differentiation, and then the differentiated cells undergo apoptosis as exemplified by HL-60 myeloid leukemia cells that differentiate into neutrophils and then

die (71); (ii) retinoids induce differentiation and apoptosis concurrently as in F9 embryonal carcinoma cells (84) and P39 myelomonocytic leukemia cells (85); and (iii) retinoids induce apoptosis in a process that is independent of differentiation, as in neuroblastoma cells (69, 76). The induction of apoptosis by 4HPR in various tumor cell types followed the latter pattern in that it was independent of differentiation in several human malignant hematopoietic (71) and neuroblastoma cells (74, 76). In fact, in some cells, 4HPR induced the characteristic DNA fragmentation as early as 6 h after treatment before any differentiated cells appeared (72). CD437 is more potent than 4HPR and induction of apoptosis is easily detected 24 h after treatment, even at low concentrations (1 μM or less) (31, 33, our unpublished data). We, therefore, believe that its action falls into the third category.

The mechanism of apoptosis induction by retinoids in general in tumor cells is still not well defined. The likely mediators of this effect such as nuclear retinoid receptors, the enzyme tissue transglutaminase (tTG) and bcl-2 oncoprotein have been explored. tTG, an enzyme involved in protein cross-linking, accumulates in many cells undergoing apoptosis and may play a role in formation of apoptotic bodies. In some cell types, induction of tTG by retinoids is associated with the induction of apoptosis. ATRA treatment of human cervical carcinoma HeLa-TV or neuroblastoma SK-N-BE resulted in 6- to 12-fold increase in tTG and a parallel increase in the apoptotic index (69). 9CRA induces tTG activity in HL-60 cells and is more potent than ATRA. This enzyme induction is also accompanied by changes in morphology and by DNA fragmentation consistent with features of cells undergoing apoptosis (70). Bcl-2 is a membrane-associated protein whose expression has been linked to the suppression of apoptosis in many cells. Interestingly, 4HPR (73, 81) and other retinoids (30, 86) have been reported to downregulate bcl-2 expression in certain cells. In addition, dysregulated bcl-2 expression has been reported to inhibit apoptosis but not differentiation of retinoic acid-treated HL-60 myelomonocytic leukemia cells (87). These results suggest a possible role of bcl-2 in apoptosis by retinoids in some cell types. More recently, the role of nuclear retinoid receptors in the induction of apoptosis by retinoids has been investigated (86-94). Depending on cell types or different retinoids, RAR- and RXR-dependent and -independent mechanisms are involved in the mediation of apoptosis by retinoids. Ligand activation of RXRs was found to be essential for the induction of apoptosis in HL-60 cells (87, 94), whereas induction of apoptosis by retinoids in T-cell hybridomas and P19 and F9 embryonal carcinoma cells required both RARs and RXRs to form heterodimers as the functional unit (88, 91, 92). In breast cancer cells, RARβ was identified as a mediator of retinoic acid-induced apoptosis (89, 90). Importantly, the downregulation of bcl-2 and induction of tTG by retinoids was found to be related to RARs and/or RXRs, although the results were not very consistent (86, 93). Nagy *et al.* (86) reported that ligand activation of RARs in HL-60 cells resulted in a global suppression of bcl-2 expression, whereas ligand activation of both RARs and RXRs triggered the selective accumulation of tTG in the apoptotic HL-60 cells. However, results reported by Agarwal *et al.* (93) showed that activation of RXRα resulted in apoptosis via downmodulation of bcl-2 mRNA as well as its gene product expression.

Different from natural retinoids, synthetic retinoids such as 4HPR and CD437 have more complicated mechanisms. Recently, 4HPR was reported to be a highly selective activator of retinoid receptors (95, 96), but this was challenged by the report that 4HPR-mediated biological functions involve retinoid receptor-independent pathway in human breast cancer (80), which was supported by the result that 4HPR is effective in inducing apoptosis in cells that are resistant to retinoic acid that activates nuclear receptors efficiently (72). Using RAR-specific antagonists, we found that apoptosis induced by 4HPR can be significantly reversed by the antagonists in some human cancer cells but not in others (our unpublished data). We think that this retinoid may function by a receptor-independent mechanism or by a combination of receptor-dependent and -independent mechanisms depending on the cell type. Recent studies indicate that reactive oxygen species (ROS) are involved in the mediation of apoptosis induced by 4HPR in some cancer cells (97, 98). To address the importance of ROS in the mediation of apoptosis by 4HPR, we performed a study using different antioxidants and 13 different human cancer cell lines, including non-small cell lung cancer, head and neck cancer and prostate cancers that are responsive to 4HPR treatment. We found that several antioxidants dramatically reversed the apoptosis induced by 4HPR in only three of the cell lines that were relatively more sensitive to 4HPR treatment and show a higher level of ROS production by 4HPR. The rest of the cell lines generated relatively lower levels of ROS when incubated with 4HPR, and the apoptosis induced by 4HPR in these cell lines was not reversed by antioxidants. Therefore, we conclude that increased ROS generation is not a general mechanism of apoptosis induction by 4HPR and only plays a critical role in a few specific cancer cell lines that generate high levels of ROS when exposed to 4HPR. The 4HPR-induced apoptosis in many cancer cells may involve additional unknown mechanisms.

Although CD437 is a RARγ-selective retinoid, its action in induction of apoptosis in human breast (30), lung (33) and prostate (our unpublished data) cancer cells is proved to be independent of a RAR-mediated pathway. Interestingly, we found that the effect of CD437 in human head and neck cancer cells is reversible by different RAR-specific antagonists (99), demonstrating that CD437 functions through the RAR pathway in head and neck cancer cells. Therefore, both RAR-dependent and -independent mechanisms may be involved in CD437-induced apoptosis depending on the cell type.

3) Effects of retinoids on cell differentiation

The physiological functions of naturally occurring retinoids include regulation of embryonal development

and maintenance of the proper differentiation of many epithelial and mesenchymal tissues in adults (4). Retinoids also act pharmacologically to restore regulation of differentiation in certain malignant cells *in vitro*. The effects of retinoids on cell differentiation have been studied extensively in a few defined systems primarily in cultured cell lines derived from embryonal carcinoma (EC), normal and malignant keratinocytes, premonocytic and myeloid leukemia, neuroblastoma and melanoma. In most of these cell types, retinoids enhance differentiation. However, in cultured keratinocytes and squamous cell carcinomas, retinoids inhibit squamous differentiation (100). Because in many cases the squamous differentiation of normally nonkeratinizing epithelial cells is aberrant, the effect of retinoids can be viewed as restoration of the normal nonkeratinizing phenotype (100).

The ability of retinoids to induce distinct pathways of differentiation indicates that they do not determine the direction of differentiation but rather enhance predetermined programs in cells that have the potential to undergo differentiation along one or more specific pathways. For example, in F9 EC cells, retinoids induce endodermal differentiation (101), whereas in human embryonal carcinoma cells, retinoids induce a neuronal differentiation (102). In P19 EC cells, retinoids can induce both myogenic and neuronal differentiation depending on the concentration of retinoid used (103). In HL-60 myeloid leukemia cells that have the potential to undergo either myeloid or monocytoid differentiation, retinoids can only induce the myeloid pathway (104). In contrast, ATRA was able to induce three different pathways – ectodermal, mesodermal and endodermal – in a developmentally pluripotent germ cell tumor (105). Thus, the effect of retinoids on the differentiation pathway appears to depend on other cellular factors that are either expressed in the cells constitutively (*e.g.*, certain transcription factors) or are induced by retinoic acid and then turn on a specific differentiation pathway (*e.g.*, Hox genes and AP-2) (106, 107).

4) Effects in combination with other agents

Extensive studies were carried out with the myeloid leukemia cell line HL-60 to define the efficacy of combinations of retinoids and other agents (108). Such studies have demonstrated additive or synergistic effects of retinoids and the following agents: dimethylsulfoxide, dimethylformamide, hexamethylenebisacetamide, butyrate, tiazofurin, tumor necrosis factor-α, 6- thioguanine, 5-aza-2'deoxycytidine, actinomycin, granulocyte colony-stimulating factor and interleukin-6. Studies with embryonal carcinoma have shown that a combination of ATRA and cAMP and cAMP-elevating agents induces a distinct pathway of endodermal differentiation from the one induced by ATRA alone. The combination of ATRA and tamoxifen was found to be more effective in suppressing the growth of human breast carcinoma cells than each agent alone (109). In neuroblastoma cells, ATRA augmented the differentiation-inducing effect of cAMP and IFN-α and showed enhanced activity with cisplatin. Because the combination of 13CRA has been used successfully in several clinical trials (24-26), the nature of their interaction is of particular interest.

The efficacy of the combination of IFNs (mostly IFN-α and IFN-γ) and retinoids (mostly ATRA) in induction of differentiation and growth inhibition has been detected initially *in vitro* in established leukemia cell lines (*e.g.*, HL-60, U937). The effects of IFNs and retinoids were either additive or synergistic, depending on the cell system evaluated and distinct differentiation pathways could be stimulated in different cell types. Furthermore, IFN-α restored responsiveness to ATRA in ATRA-resistant HL-60 cells (110). Likewise, IFN-γ restored responsiveness to ATRA in ATRA-resistant v-myc-expressing U937 cells. Further studies with fresh leukemic cells from acute myelogenous leukemia (AML) patients treated in short-term cultures with IFNs and ATRA also demonstrated synergistic growth inhibition and differentiation induction. In addition, increased inhibition of clonal growth and differentiation was observed after *in vitro* ATRA treatment of cells from patients treated with IFN-α *in vivo*. Additive or synergistic growth inhibition of various human tumor cell lines *in vitro* were reported for the combination of IFN-α or IFN-γ and ATRA or other retinoids. These effects were sometimes also associated with differentiation induction, particularly in neuroblastoma cells. Tumor cell types in which the combination of the two agents showed greater growth inhibition than each agent alone included breast carcinoma, osteosarcoma, ovarian carcinoma, squamous cell carcinoma, neuroblastoma, non-small cell lung carcinoma and cervical carcinoma (111, 112). The combined effects of IFNs and retinoids may result from activation of the same genes or distinct genes, depending on the cell type. For example, both agents induce 2-5-oligoadenylate synthetase (2-5-OASE) gene transcription in some cells. In other cells, one agent can potentiate the effect of the other by different mechanisms. For example, ATRA increased the binding of ^{125}I-labeled IFN-α to various tumor cell lines by increasing the number of receptors. ATRA also stabilized 2-5-OASE mRNA induced by IFN-α (113). In contrast, IFN-γ did not alter the level of cellular retinoic acid-binding proteins and IFN-α did not affect the levels of nuclear RARs in several tumor cell lines. The agents may work through different mechanisms as well. For example, human neuroblastoma cells are induced to differentiate by either agent alone, but the combination of agents leads to synergistic effects probably via distinct mechanisms as indicated by the finding that ATRA increases inositol 1,4,5-triphosphate and 1,2-diacylglycerol (protein kinase C activator), whereas IFN-γ has no effect on the level of these second messengers. In breast cancer cells, IFN-γ increased the level of RARγ (114), and in embryonal carcinoma cells, pretreatment of the cells with ATRA rendered the cells responsive to induction of gene expression by IFN by increasing the level of STAT-1, a mediator of IFN action (115).

In vivo *effects*

The *in vivo* therapeutic effects of retinoids on various cancers have been investigated in various model systems. These studies undoubtedly provide more information on the effectiveness and safety for the application of retinoids in the clinic as therapeutic agents than do *in vitro* studies. 9CRA was reported to induce complete tumor regression of an early-passage human lip squamous cell carcinoma xenograft (70). In vehicle-treated animals, tumor attained a mean volume of 31 mm^3 at 4 weeks posttransplantation and grew steadily to a mean volume of 96 mm^3 at 9 weeks. In contrast, animals treated with 9CRA (60 mg/kg p.o.) for 4 weeks had tumors that were 40% the size of control values, with a mean tumor volume of 12 mm^3. After 6 weeks of treatment, all tumors in the 9CRA-treated group regressed to nonmeasurable volumes. At the conclusion of the experiment, no visible or histological evidence of tumor was found in 9CRA-treated animals (70). In another study using head and neck squamous carcinoma cell line 1483 as xenografts in nude mice, 9CRA, ATRA and 13CRA were not found to universally decrease tumor growth but were found to profoundly suppress squamous cell differentiation (116). Different from 9CRA, high-dose vitamin A was not found to significantly affect the morphology and growth rate of xenografted squamous cell carcinoma of the head and neck (117).

When human neuroblastoma LA-N-5 cells were treated with 4 μM ATRA *in vitro*, there was a marked reduction in the number of mice developing tumors when compared with solvent-treated controls. *In vivo* treatment with ATRA reduced tumor formation when the retinoid was given for 5 days before tumor injection and continued for 14 days thereafter. In established tumors, ATRA inhibited progressive tumor growth. There was no demonstrable effect of ATRA *in vivo* on the morphology of the tumor cells (118). In addition, the effect of ATRA in combination with IFN-γ on the growth of human neuroblastoma cells in nude mice was also studied (119). Tumor growth was significantly inhibited in IFN-γ ($p < 0.005$) and ATRA ($p < 0.05$) treated mice grafted with GI-LI-N. The combination of the two agents, however, did not enhance *in vivo* doubling time further. Tumor growth inhibition was not statistically significant in LA-N-5-bearing mice treated with ATRA or IFN-γ alone, but a synergistic effect between the two drugs was observed ($p < 0.05$) (119).

When 13CRA was administered to nude mice bearing established tumors (LNCaP human prostate tumor xenografts), the tumor size (0.65 ± 0.06 cm^3) was significantly reduced compared with that of untreated controls (1.63 ± 0.12 cm^3). About 50% of the animals in this group showed xenograft necrosis followed by complete regression of tumors by 5 months. The combination of androgen ablation and 13CRA treatment to nude mice bearing tumors showed a synergistic effect in decreasing the tumor size (120). 4HPR administered orally at 1.5 and 15 mg/kg/day significantly inhibited the growth of implanted rat prostate tumor without apparent gross toxicity (121). It should be noted that ATRA was found to increase invasiveness of human prostate cancer PC-3 cells via induction of urinary plasminogen activator (uPA) (122).

Most studies on *in vivo* growth inhibitory effects of retinoids in the past concentrated on the effects of ATRA, 9CRA, 13CRA and 4HPR. In addition to the results described above, these retinoids were also reported to exert growth inhibitory effects *in vivo* in other types of tumors, including breast (123), pancreatic (124, 125), gastric (126) and ovarian (127) cancers and melanoma (128). More importantly, recent studies have demonstrated that some novel synthetic retinoids with RAR selectivity exhibit potent growth inhibitory activity against some human solid tumors such as those of the head and neck (129), and lung cancer (130) and melanoma (32) *in vivo*. the RAR-selective retinoid, ALRT1550, was reported to have potent antitumor activity against human oral squamous carcinoma xenografts in nude mice (129). Xenografts of the head and neck carcinoma cell line UMSCC-22B in nude mice formed well-differentiated squamous carcinomas, and oral administration of ALRT1550 (daily, 5 days/week), beginning 3 days after implanting tumor cells, inhibited tumor growth by up to 89% in a dose-dependent manner over the range of 3-75 μg/kg. ALRT1550 (30 μg/kg) also inhibited the growth of the established tumor by $72 \pm 3\%$ when tumors were allowed to grow to about 100 mm^3 before treatment. In comparison, 9CRA at 30 mg/kg inhibited growth of established tumors by $73 \pm 5\%$ (129). This indicates that ALRT1550 is much more potent than 9CRA in this model system. CD437, a RARγ-selective retinoid, was reported to exhibit a strong growth inhibitory effect on MeWo melanoma cells in a xenograft model (32). Preexisting tumors of 0.4 cm in diameter were treated for 3 weeks with CD437 (10 and 30 mg/kg) in comparison with a control group of vehicle-treated animals. Tumors in CD437-treated mice stopped growing, an effect which was already statistically significantly ($p < 0.01$) at day 13, 3 days after the first administration of CD437, and was maintained for more than 3 weeks after discontinuation of treatment. No difference was observed on the basis of administration route (intratumorally or orally). Apoptotic melanoma cells were identified in tissue sections of CD437-treated MeWo tumors from these animals, which indicated that the growth inhibitory effects of CD437 in these cells involved induction of apoptosis (32). More recently, MX-3350-1, another RARγ-selective retinoid, was proven to exert potent growth inhibitory activity against human lung cancer *in vivo* (130). The tumors in animals treated with MX-3350-1 essentially did not grow significantly once drug administration (90 mg/kg i.p., every other day) had begun ($p < 0.001$) (130). Most importantly, no major signs of toxicity were observed at the effective doses of these retinoids (32, 130). Therefore, these retinoids with different RAR selectivity present a new class of retinoids which may have potential to be developed as more effective therapeutic agents for some solid tumors.

Clinical trials

1) Therapy of hematological malignancies

In 1988 scientists from Shanghai, China reported that patients with a rare form of leukemia – acute promyelocytic leukemia (APL) – were in remission following treatment with ATRA (131). Since then, many studies in China, Europe and the U.S. have confirmed and extended the original observation (20, 21, 131-134).

In the first trial of ATRA as treatment for APL, the efficacy of variable doses of ATRA (30-100 mg/m^2/day) was evidenced by 23 complete remissions (CR) among 24 patients newly diagnosed with APL (131). French trials on the first disease relapse recorded 95% CR (19 of 20 patients) using ATRA at a dose of 45 mg/m^2/day (132). Similar results were also reported by the Memorial Sloan Kettering Cancer Institute in 11 patients (20). By now, more than 2000 patients worldwide with a clinical diagnosis of APL have been treated with ATRA (133). Combined data indicate that the mean CR rate is 84% and the time to achieve CR is 1-3 months in most series (133). At the cellular level, considerable data indicate that induction of remission by ATRA is associated with differentiation of immature neoplastic cells to mature granulocytes, followed by the emergence of normal granulocytic cells as remission begins (133). The successful treatment of APL with ATRA is considered one of the most important advances in the field of retinoid research. Therefore, ATRA became the first retinoid to be approved by the Food and Drug Administration in the U.S. as a treatment for APL relapsed after chemotherapy.

In addition to the treatment of APL, retinoids also have been used as therapeutic agents in the treatment of other hematological malignancies such as myelodysplastic syndromes, non-APL acute myelodysplastic leukemia, juvenile chronic myelogenous leukemia, multiple myeloma and mycosis fungoides. Retinoids such as retinoic acid are generally inactive as single agents in other leukemias. This aspect has been well reviewed by Parkinson and Warrell (134).

2) Therapy of solid tumors

A number of phase II trials of retinoids used alone or in combination are currently under way in a range of solid tumors, including skin, head and neck, lung, cervical, breast, bladder and prostate cancers, as well as neuroblastoma (135). Unfortunately, few responses were observed among patients with established solid tumors after treatment with certain retinoids as single agents or in combination with other agents (135). 13CRA or ATRA, when topically or systemically used, have produced partial responses in skin cancers, including squamous cell carcinoma, basal cell carcinoma and malignant melanoma. The response rates in patients with squamous cell carcinoma of the skin and cervical cancer treated with 13CRA and IFN-α have been 68% and 58%, respectively (24-26). But these results were not observed in patients with malignant melanoma, head and neck and lung squamous cell carcinomas (135). The synthetic retinoid 4HPR has been shown to be inactive when used as a single agent in a phase I trial in patients with advanced melanoma and in patients with stage 1 breast carcinoma (135). However, studies of retinoids in various pediatric cancers have indicated some success. ATRA in combination with IFN-α induced partial remission for 6 months in a 7-year-old boy with neuroblastoma childhood cancer of the autonomic nervous system and adrenal glands (8). In another study, a 3-year-old child with Wilms' tumor caused by the overgrowth of undifferentiated embryonic cells, when treated with ATRA and IFN-α for 18 months, remains disease free (8), although the child had experienced several relapses after surgery and chemotherapy.

Conclusions

So far, the clinical successes in the use of several retinoids have been observed in chemoprevention of certain malignancies and prevention of second primary tumors by adjuvant treatment of patients whose first primary tumor had been treated. The most exciting clinical success in the treatment of malignancy has been in the case of APL. However, results of the few retinoids tested thus far as single agents in treatment of leukemias other than APL and in solid tumors are not encouraging. Future studies should focus on new retinoids, particularly those that induce apoptosis in tumor cells, and on combinations of retinoids with other biological agents (*e.g.*, vitamin D_3, IFN, TNFα), radiotherapy and cytotoxic agents. Of the 38 trials of several retinoids in a variety of cancers sponsored currently by the National Cancer Institute in the U.S., at least 18 are of combination therapy of various retinoids with IFN-α (8). Clinical evaluation is available for only a few of the more than 4000 different retinoids that have been synthesized. It is expected that among the new retinoids some will have greater efficacy and lower toxicity than those analyzed to date.

References

1. Sporn, M., Dunlop, N., Newton, D., Smith, J. *Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids).* Fed Proc 1976, 35: 1332-8.

2. Lotan, R. *Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells.* Biochem Biophys Acta 1980, 605: 33-91.

3. Gudas, L.J., Sporn, M.B., Roberts, A.B. *Cellular biology and biochemistry of the retinoids.* In: The Retinoids. Sporn, M.B., Roberts, A.B., Goodman, D.S. (Eds.). Raven Press: New York 1994, 443-520.

4. DeLuca, L.M. *Retinoids and their receptors in differentiation, embryogenesis and neoplasia.* FASEB J 1991, 5: 2924-33.

5. Lotan, R. *Retinoids and apoptosis: Implication for cancer chemoprevention and therapy.* J Natl Cancer Inst 1995, 87: 1655-7.

6. Lippman, S.M., Kessler, J., Meyskens, F. *Retinoids as preventive and therapeutic anticancer agents (part 1).* Cancer Treat Rep 1987, 71: 391-405.

7. Rosen, J., Day, A., Jones, T.K., Jones, E.T.T., Nadzan, A.M., Stein, R.B. *Intracellular receptors and signal transducers and activators of transcription superfamilies: Novel targets for small-molecule drug discovery*. Med Chem 1995, 38: 4855-74.

8. Steinberg, J. *In cancer, retinoic acid brings hope, but no more miracles.* J NIH Res 1996, 8: 28-9.

9. Delescluse, C., Cavey, M.T., Martin, B. et al. *Selective high affinity retinoic acid receptor beta-gamma ligands.* Mol Pharmacol 1991, 40: 556-62.

10. Lehmann, J.M., Dawson, M.I., Hobbs, P.D., Husmann, M., Pfahl, M. *Identification of retinoids with nuclear receptor subtype-selective activities.* Cancer Res 1991, 51: 4804-9.

11. Lehmann, J.M., Jong, L., Fanjul, A. et al. *Retinoids selective for retinoid X receptor response pathways.* Science 1992, 258: 1944-6.

12. Bernard, B.A., Bernardon, J-M., Delescluse, C. et al. *Identification of synthetic retinoids with selectivity for human nuclear retinoic acid receptor γ.* Biochem Biophys Res Commun 1992, 186: 977-83.

13. Boehm, M.F., Zhang, L., Badea, B.A. et al. *Synthesis and structure-activity relationships of novel retinoid X receptor-selective retinoids.* J Med Chem 1994, 37: 2930-41.

14. Fanjul, A., Dawson, M.I., Hobbs, P.D. et al. *A new class of retinoids with selective inhibition of AP-1 inhibits proliferation*. Nature 1994, 372: 107-10.

15. Dawson, M.I., Jong, L., Hobbs, P.D. et al. *Conformational effects on retinoid receptor selectivity. 2. Effects of retinoid bridging group on retinoid X receptor activity and selectivity.* J Med Chem 1995, 38: 3368-83.

16. Charpentier, B., Bernardon, J-M., Eustache, J. et al. *Synthesis, structure-affinity relationships, and biological activities of ligands binding to retinoic acid receptor subtypes.* J Med Chem 1995, 38: 4993-5006.

17. Eyrolies, L., Kagechika, H., Kawachi, E. et al. *Retinobenzoic acids. 6. Retinoid antagonists with a heterocyclic ring.* J Med Chem 1994, 37: 1508-17.

18. Johnson, A.T., Klein, E.S., Gillett, S.J. et al. *Synthesis and characterization of a highly potent and effective antagonist of retinoic acid receptors. J Med Chem 1996, 38: 4764-7.*

19. Johnson, A.T., Klein, E.S., Wang, L., Pino, M.E., Chandrartna, R.A.S. *Identification of a retinoic acid receptor beta subtype specific agonist.* J Med Chem 1997, 39: 5027-30.

20. Warrell, R. Frankel, S., Miller, W. et al. *Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans retinoic acid).* New Engl J Med 1991, 324: 1385-93.

21. Chen, Z-X., Xue, Y-Q., Zang, R. et al. *A clinical and experimental study on all-trans retinoic acid-treated acute promyelocytic leukemia patient.* Blood 1991, 78: 1413-9.

22. Hong, W.K., Endicott, J., Iri, L.M. et al. *13-cis-Retinoic acid in the treatment of oral leukoplasia.* New Engl J Med 1986, 315: 1501-5.

23. Hong, W.K., Lippman, S.M., Itri, L.M. et al. *Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck.* New Engl J Med 1990, 323: 795-801.

24. Lippman, S.M., Parkinson, D.R., Itri, L.M. et al. *13-cis-Retinoic acid and interferon alpha$_{2a}$: Effective combination therapy for advanced squamous cell carcinoma of the skin.* J Natl Cancer Inst 1992, 84: 235-41.

25. Lippman, S.M., Kavanagh, J.J., Paredes-Espinoza, M. et al. *13-cis-Retinoic acid plus interferon α_{2a}: Highly active systemic therapy for squamous cell carcinoma of the cervix.* J Natl Cancer Inst 1992, 84: 241-5.

26. Lippman, S.M., Kavanagh, J.J., Paredes-Espinoza, M. et al. *13-cis-Retinoic acid plus interferon α_{2a} in locally advanced squamous cell carcinoma of the cervix.* J Natl Cancer Inst 1993, 85: 499-500.

27. Kelloff, G.J., Boone, C.W., Crowell, J.A. et al. *New agents for cancer chemoprevention.* J Cell Biochem 1996, 26S: 1-28.

28. Esgleyes-Ribot, T., Chandraratna, R.A.S., Lew-Kaya, D., Sefton, J., Duvic, M.J. *Response of psoriasis to a new topical retinoid, AGN190168.* J Am Acad Dermatol 1994, 30: 581-90.

29. Rizvi, N.A., Marshall, J.L., Loewen, G.R. et al. *A phase I study of Targretin, an RXR-selective retinoid agonist.* Proc Am Cancer Res 1996, 37: 165.

30. Shao, Z-M., Dawson, M.I., Li, X-S. et al. *p53-Independent G_0/G_1 arrest and apoptosis induced by a novel retinoid in human breast cancer cells.* Oncogene 1995, 11: 493-504.

31. Oridate, N., Higuchi, M., Suzuki, S., Shroot, B., Hong, W. K., Lotan, R. *Rapid induction of apoptosis in human C33A cervical carcinoma cells by the synthetic retinoid 6-[3-(1-adamantyl)-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437).* Int J Cancer 1997, 70: 484-7.

32. Schadendorf, D., Kern, M.A., Artuc, M. et al. *Treatment of melanoma cells with the synthetic retinoid CD437 induces apoptosis via activation of AP-1 in vitro, and causes growth inhibition in xenografts in vivo.* J Cell Biol 1996, 135: 1889-98.

33. Sun, S-Y., Yue, P., Shroot, B., Hong, W.K., Lotan, R. *Induction of apoptosis in human non-small cell lung carcinoma cells by the novel synthetic retinoid CD437.* J Cell Physiol 1997, 173: 279-84.

34. Apfel, C., Bauer, F., Creftaz, M. et al. *Retinoic acid receptor alpha antagonist selectivity counteracts retinoic acid effects.* Proc Natl Acad Sci USA 1992, 89: 7129-33.

35. Wieder, J.M., Lowe, N.J. *Systemic retinoids for psoriasis.* Dermatol Clin 1995, 13: 891-6.

36. Tutoryan, T., Gilchrest, B.A. *Retinoids and the skin*. Nutr Res 1994, 52: 299-310.

37. Biro, D.E., Shalita, A.R. *Clinical aspects of topical retinoids.* Skin Pharmacol 1993, 6(Suppl. 1): 53-60.

38. Hong, W.K., Itri, L.M. *Retinoids and human cancer.* In: The Retinoids. Sporn, M.B., Roberts, A.B., Goodman, D.S. (Eds.). Raven Press: New York 1994, 597-658.

39. Hong, W.K., Lippman, S.M., Hittelman, W.N., Lotan, R. *Retinoid chemoprevention of aerodigestive cancer from basic research to the clinic.* Clin Cancer Res 1995, 1: 677-861.

40. Lippman, S.M., Benner, S.E., Hong, W.K. *The chemoprevention of cancer.* In: Cancer Prevention and Control. Greenwald, P., Kramer, R.S., Weed, D.L. (Eds.). Marcel Dekker: New York 1995, 329-52.

41. Moon, R.C., Mehta, R.G., Rao, K.J.V.N. *Retinoids and cancer in experimental animals.* In: The Retinoids. Sporn, M.B., Roberts, A.B., Goodman, D.S. (Eds.). Raven Press: New York 1994, 573-95.

42. Lotan, R. *Retinoids in chemoprevention.* FASEB J 1996, 10: 1031-9.

43. Hennings, H., Wenk, M.L.M., Donahoe, R. *Retinoic acid promotion of papilloma formation in mouse skin.* Cancer Lett 1982, 16: 1-5.

44. Verma, A.K., Conrad, E.A., Boutwell, R.K. *Differential effects of retinoic acid and 7,8-benzoflavone on the induction of mouse skin tumors by the complete carcinogenesis process and by the initiation-promotion regimen.* Cancer Res 1982, 42: 3519-25.

45. Fischer, S.M., Klein-Szanto, A.J.P., Adamas, L.M. et al. *The first stage and complete promoting activity of retinoic acid but not the analog Ro-10-9359.* Carcinogenesis 1985, 6: 575-8.

46. Bavinck, J.N., Tieben, L.M., Van der Woude, F.J. et al. *Prevention of skin cancer and reduction of keratotic skin lesions during acitretin therapy in renal transplant recipients: A double-blind, placebo-controlled study.* J Clin Oncol 1995, 13: 1933-8.

47. Rook, A.H., Jaworsky, C., Nguyen, T. et al. *Beneficial effect of low-dose systemic retinoid in combination with topical tretinoin for the treatment and prophylaxis of premalignant and malignant skin lesions in renal transplant recipients.* Transplantation 1995, 59: 714-9.

48. Edwards, L., Jaffe, P. *The effects of topical tretinoin on dysplastic nevi.* Arch Dermatol 1990, 126: 494-9.

49. Halpern, A.C., Schuchter, L.M., Elder, D.E. et al. *Effects of topical tretinoin on dysplastic nevi.* J Clin Oncol 1994, 12: 1028-35.

50. Omenn, G.S., Goodman, G.E., Thomquist, M.D. et al. *Risk factors for lung cancer and for intervention effects in CARET, the β-carotene and retinol efficacy trial.* J Natl Cancer Inst 1996, 88: 1550-9.

51. Albanes, D., Heinonen, O.P., Taylor, P.R. et al. *α-Tocopherol and β-carotene supplements and lung cancer incidence in the α-tocopherol, β-carotene cancer prevention study: Effects of baseline characteristics and study compliance.* J Natl Cancer Inst 1996, 88: 1560-70.

52. Beenken, S.W., Huang, P., Sellers, M. et al. *Retinoid modulation of biomarkers in oral leukoplakia/dysplasia.* J Cell Biochem 1994, 19(Suppl.): 270-7.

53. Chiesa, F., Tradati, N., Marazza, M. et al. *Fenretinide (4-HPR) in chemoprevention of oral leukoplakia.* J Cell Biochem 1993, 17(Suppl.): 255-61.

54. Costa, A., Formelli, F., Chiesa, F. et al. *Prospects of chemoprevention of human cancers with the synthetic retinoid fenretinide.* Cancer Res 1994, 54(Suppl.): 2032s-7s.

55. Lee, J.S., Lippman, S.M., Benner, S.E. et al. *Randomized placebo-controlled trial of isotretinoin in chemoprevention of bronchial squamous metaplasia.* J Clin Oncol 1994, 12: 937-45.

56. Meyskens, F.L., Surwit, E., Moon, T.E. et al. *Enhancement of regression of cervical intraepithelial neoplasia II (moderate dysplasia) with topically applied all-trans-retinoic acid: A randomized trial.* J Natl Cancer Inst 1994, 86: 539-43.

57. Han, J. *Highlights of the cancer chemoprevention studies in China.* Prev Med 1993, 22: 712-22.

58. Peck, G.L., DiGiovanna, J.J. *Synthetic retinoids in dermatology.* In: The Retinoids. Sporn, M.B., Roberts, A.B., Goodman, D.S. (Eds.). Raven Press: New York 1994, 631-58.

59. Bolla, M., Lefur, R., Ton Van, J. et al. *Prevention of second primary tumours with etretinate in squamous cell carcinoma of the oral cavity and oropharynx. Results of a multicentric double-blind randomised study.* Eur J Cancer 1994, 30A: 767-72.

60. Pastorino, U., Infante, M., Maioli, M. et al. *Adjuvant treatment of stage I lung cancer with high-dose vitamin A.* J Clin Oncol 1993, 11: 1216-22.

61. De Palo, G., Veronesi, U., Camerini, T. et al. *Can fenretinide protect women against ovarian cancer?* J Natl Cancer Inst 1995, 87: 146-7.

62. Prout, G.R. Jr., Barton, B.A. *13-cis-Retinoic acid in chemoprevention of superficial bladder cancer. The National Bladder Cancer Group.* J Cell Biochem 1992, 161(Suppl.): 148-52.

63. Studer, U.E., Jenzer, S., Biedermann, C. et al. *Adjuvant treatment with vitamin A analogue (etretinate) after transurethral resection of superficial bladder tumors - Final analysis of a prospective randomized multicenter trial in Switzerland.* Eur Urol 1995, 28: 284-90.

64. Amos, B., Lotan, R. *Retinoid sensitive cells and cell lines.* In: Methods in Enzymology: Retinoids. Packer, L. (Ed.). Academic Press: Orlando 1991, 190: 217-25.

65. Lotan, R., Lotan, D., Sacks, P. *Inhibition of tumor cell growth by retinoids.* In: Methods in Enzymology: Retinoids. Packer, L. (Ed.). Academic Press: Orlando 1991, 190: 100-10.

66. Meyskens, F.L. Jr., Salmon, S.E. *Inhibition of human melanoma colony formation by retinoids.* Cancer Res 1979, 39: 4055-7.

67. Meyskens, F.L. Jr., Alberts, D.S., Salmon, S.E. *Effect of 13-cis-retinoic acid and 4-hydroxyphenyl-all-trans-retinamide on human tumor colony formation in soft agar.* Int J Cancer 1983, 32: 295-9.

68. Cowan, J.D., Von Hoff, D.D., Dinesman, A., Clark, G. *Use of a human tumor cloning system to screen retinoids for antineoplastic activity.* Cancer 1983, 51: 92-6.

69. Placentini, M., Annicchiarico-Petruzzelli, M., Olivedo, S. et al. *Phenotype-specific "tissue" transglutaminase regulation in human neuroblastoma cells in response to retinoic acid: Correlation with cell death by apoptosis.* Int J Cancer 1992, 52: 271-8.

70. Gottardis, M.M., Lamph, W.W., Shalinsky, D.R., Wellstein, A., Heyman, R.A. *The efficacy of 9-cis-retinoic acid in experimental models of cancer.* Breast Cancer Res Treat 1996, 38: 85-96.

71. Martin, S.J., Bradley, J.G., Cotter, T.G. *HL-60 cells induced to differentiate towards neutrophils subsequently die via apoptosis.* Clin Exp Immunol 1990, 79: 448-53.

72. Delia, D., Aiello, A., Lombardi, L. et al. *N-(4-Hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid.* Cancer Res 1993, 53: 6036-41.

73. Delia, D., Aiello, A., Fornelli, F. et al. *Regulation of apoptosis induced by the retinoid N-(4-hydroxyphenyl)retinamide and effect of deregulated bcl-2.* Blood 1995, 85: 359-67.

74. Madfti, A., Marcora, E., Bunone, G. et al. *N-(4-hydroxyphenyl)retinamide, a potent inducer of apoptosis in human neuroblastoma cells.* J Natl Cancer Inst 1994, 86: 1245-7.

75. Di Vinci, A., Geido, E., Infusini, E., Giaretti, W. *Neuroblastoma cells apoptosis induced by the synthetic retinoid N-(4-hydroxyphenyl)retinamide.* Int J Cancer 1994, 59: 422-6.

76. Ponzoni, M., Bocca, P., Chiesta, V. et al. *Differential effects of N-(4-hydroxyphenyl)retinamide and retinoic acid on neuroblastoma cells: Apoptosis versus differentiation.* Cancer Res 1995, 55: 853-61.

77. Kalemkerian, G.P., Slusher, R., Ramalingam, S., Gadgeel, S., Mabry, M. *Growth inhibition and induction of apoptosis by fenretinide in small-cell lung cancer cell lines.* J Natl Cancer Inst 1995, 87: 1674-80.

78. Oridate, N., Lotan, D., Xu, X-C., Hong, W.K., Lotan, R. *Differential induction of apoptosis by all-trans-retinoic acid and N-(4-hydroxyphenyl)retinamide in human head and neck squamous carcinoma cell lines.* Clin Cancer Res 1996, 2: 855-63.

79. Oridate, N., Lotan, D., Mitchell, M.F., Hong, W.K., Lotan, R. *Induction of apoptosis by retinoids in human cervical carcinoma cell lines.* Int J Oncol 1995, 7: 433-41.

80. Sheikh, M.S., Shao, Z-M., Li, X-S. et al. *N-(4-Hydroxyphenyl)retinamide (4-HPR)-mediated biological actions involve retinoid receptor-independent pathways in human breast carcinoma.* Carcinogenesis 1995, 16: 2477-86.

81. Wang, T.T., Phang, J.M. Effect of *N-(4-hydroxyphenyl)retinamide on apoptosis in human breast cancer cells.* Cancer Lett 1996, 107: 65-71.

82. Hsieh, T.C., Ng, C., Wu, J.M. *The synthetic retinoid N-(4-hydroxyphenyl)retinamide (4-HPR) exerts antiproliferative and apoptosis-inducing effects in the androgen-independent human prostatic JCA-1 cells.* Biochem Mol Biol Int 1995, 37: 499-506.

83. Roberson, K.M., Penland, S.N. Padilla, G.M. et al. *Fenretinide: Induction of apoptosis and endogenous transforming growth factor beta in PC-3 prostate cancer cells.* Cell Growth Differ 1997, 8: 101-11.

84. Atencia, R., Garcia-Sanz, M., Unda, F., Arechaga, J. *Apoptosis during retinoic acid-induced differentiation of F9 embryonal carcinoma cells.* Exp Cell Res 1994, 214: 663-7.

85. Anzai, N., Kawabata, H., Hirama, T. et al. *Marked apoptosis of human myelomonocytic leukemia cell line P39: Significance of cellular differentiation.* Leukemia 1994, 8: 446-53.

86. Nagy, L., Thomazy, V.A., Chandraratna, R.A., Heyman, R.A., Davies, P.J. *Retinoid-regulated expression of Bcl-2 and tissue transglutaminase during the differentiation and apoptosis of human myeloid leukemia (HL-60) cells.* Leukemia Res 1996, 20: 499-505.

87. Nagy, L., Thomazy, V.A., Shipley, G.L. et al. *Activation of retinoid X receptors induces apoptosis in HL-60 cell lines.* Mol Cell Biol 1995, 15: 3540-51.

88. Bissonnette, R.P., Brunner, T., Lazarchik, S.B. et al. *9-cis-Retinoic acid inhibition of activation-induced apoptosis is mediated via regulation of fas ligand and requires retinoic acid receptor and retinoid X receptor activation. Mol Cell Biol 1995, 15: 5576-85.*

89. Seewaldt, V.L., Johnson, B.S., Parker, M.B., Collins, S.J., Swisshelm, K. *Expression of retinoic acid receptor β mediates retinoic acid-induced growth inhibition and apoptosis in breast cancer cells.* Cell Growth Differ 1995, 6: 1077-88.

90. Liu, Y., Lee, M.O., Wang, H.G. et al. *Retinoic acid receptor β mediates the growth-inhibitory effects of retinoic acid by promoting apoptosis in human breast cancer cells.* Mol Cell Biol 1996, 16: 1138-49.

91. Horn, V., Minucci, S., Ogryzko, V.V. et al. *RAR and RXR selective ligands cooperatively induce apoptosis and neuronal differentiation in P19 embryonal carcinoma cells.* FASEB J 1996, 10: 1071-7.

92. Clifford, J., Chiba, H., Sobieszczuk, D., Metzger, D., Chambon, P. *RXRα-null F9 embryonal carcinoma cells are resistant to the differentiation, anti-proliferative and apoptotic effects of retinoids.* EMBO J 1996, 15: 4142-55.

93. Agarwal, N., Mehta, K. *Possible involvement of bcl-2 pathway in retinoin X receptor α-induced apoptosis of HL-60 cells.* Biochem Biophys Res Commun 1997, 230: 251-3.

94. Mehta, K., McQueen, T., Neamati, N., Collins, S., Andreeff, M. *Activation of retinoid receptor RAR α and RXR α induce differentiation and apoptosis, respectively, in HL-60 cells.* Cell Growth Differ 1997, 7: 179-86.

95. Fanjul, A.N., Delia, D., Pierotti, M.A., Rideout, D., Qiu, J., Pfahl, M. *4-Hydroxyphenylretinamide is a highly selective activator of retinoid receptors.* J Biol Chem 1996, 271: 22441-6.

96. Supino, R., Crosti, M., Clerici, M. et al. *Induction of apoptosis by fenretinide (4HPR) in human ovarian carcinoma cells and its association with retinoic acid receptor expression.* Int J Cancer 1996, 65: 491-7.

97. Delia, D., Aiello, A., Meroni, L. et al. *Role of antioxidants and intracellular free radicals in retinamide-induced cell death.* Carcinogenesis 1997, 18: 943-8.

98. Oridate, N., Suzuki, S., Higuchi, M. et al. *Involvement of reactive oxygen species in N-(4-hydroxyphenyl)retinamide-induced apoptosis in cervical carcinoma cells.* J Natl Cancer Inst 1997, 89: 1191-8.

99. Sun, S-Y., Yue, P., Hong, W.K., Lotan, R. *Differential growth inhibitory effects of RAR-/RXR-selective retinoids in human head and neck cancer cells.* Proc Am Assoc Cancer Res 1997, 38: 174.

100. Lotan, R. *Retinoids and squamous cell differentiation.* In: Retinoids in Oncology. Hong, W.K., Lotan, R. (Eds). Marcel Dekker: New York 1993, 43-72.

101. Strickland, S., Smith, K.K., Marotti, K.R. *Hormonal induction of differentiation in teratocarcinoma stem cells: Generation of parietal endoderm by retinoic acid and dibutyryl cAMP.* Cell 1980, 21: 347-55.

102. Andrews, P.W. *Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro.* Dev Biol 1984, 103: 285-93.

103. McBurney, M.W., Jones-Villeneuve, E.M., Edwards, M.K., Anderson, P.J. *Control of muscle and neuronal differentiation in a cultured embryonal carcinoma cell line.* Nature 1982, 299: 165-7.

104. Breitman, T.R., Selonick, S.E., Collins, S.J. *Induction of differentiation of the human promyelocytic leukemia cell line (HL60) by retinoic acid.* Proc Natl Acad Sci USA 1980, 77: 2936-40.

105. Damjanov, I., Horvat, B., Gibas, Z. *Retinoic acid-induced differentiation of the developmentally pluripotent human germ cell tumor-derived cell line, NCCIT.* Lab Invest 1993, 68: 220-32.

106. Boncinelli, E., Simeone, A., Acampora, D., Mavilio, F. *HOX gene activation by retinoic acid.* Trends Genet 1991, 7: 329-34.

107. Luscher, B., Mitchell, P.J., Williams, T., Tjian, R. *Regulation of transcription factor AP-2 by the morphogen retinoic acid and by second messengers.* Gene Dev 1989, 3: 1507-17.

108. Breitman, T.R., He, R.Y. *Combinations of retinoic acid with either sodium butyrate, dimethyl sulfoxide, or hexamethylene bisacetamide synergistically induce differentiation of the human myeloid leukemia cell line HL60.* Cancer Res 1990, 50: 6268-73.

109. Fontana, J.A. *Interaction of retinoids and tamoxifen on the inhibition of human mammary carcinoma cell proliferation.* Exp Cell Biol 1987, 55: 136-44.

110. Grant. S., Bhalia, K., Weinstein, I.B., Pestka, S., Mileno, M.D., Fisher, P.B. *Recombinant human interferon sensitizes resistant myeloid leukemic cells to induction of terminal differentiation.* Biochem Biophys Res Commun 1985, 130: 379-88.

111. Bollag, W., Peck, R., Frey, J.R. *Inhibition of proliferation by retinoids and cytokines and their combination in four human transformed epithelial cell lines.* Cancer Lett 1992, 62: 167-72.

112. Bollag, W. *Experimental basis of cancer combination chemotherapy with retinoids, cytokines, 1,25-dihydroxyvitamin D_3 and analogs.* J Cell Biochem 1994, 56: 427-35.

113. Higuchi, T., Hannigan, G.E., Malkin, D., Yeger, H., Williams, B.R. *Enhancement by retinoic acid and dibutyryl cyclic adenosine 3':5'-monophosphate of the differentiation and gene expression of human neuroblastoma cells induced by interferon.* Cancer Res 1991, 51: 3958-64.

114. Widschwendter, M., Daxenbichler, G., Gulig, Z. et al. *Activity of retinoic acid receptor-gamma selective binding retinoids alone and in combination with interferon-gamma in breast cancer cell lines.* Int J Cancer 1997, 71: 497-504.

115. Kolla, V., Weihua, X., Kalvakolanu, D.V. *Modulation of interferon action by retinoids. Induction of murine STAT1 gene expression by retinoic acid.* J Biol Chem 1997, 272: 9742-8.

116. Shalinsky, D.R., Bischoff, E.D., Gregory, M.L. et al. *Retinoid-induced suppression of squamous cell differentiation in human oral squamous cell carcinoma xenografts (line 1483) in athymic nude mice.* Cancer Res 1995, 55: 3183-91.

117. Mourad, W.A., Bruner, J.M., Vallieres, E. et al. *The effect of high dose vitamin A on the morphology and proliferative activity of xenograft lung and head and neck cancer.* In Vivo 1996, 10: 329-33.

118. Abemayor, E., Chang, B., Sidell, N. *Effects of retinoic acid on the in vivo growth of human neuroblastoma cells.* Cancer Lett 1990, 55: 1-5.

119. Comaglia-Ferraris, P., Mariottini, G.L., Ponzoni, M. *γ-Interferon and retinoic acid synergize in inhibiting the growth of human neuroblastoma cells in nude mice.* Cancer Lett 1992, 61: 215-20.

120. Dahiya, R., Zhang, D.Y., Ho, R.J. et al. *Regression of LNCaP human prostate tumor xenografts in athymic nude mice by 13-cis- retinoic acid and androgen ablation.* Biochem Mol Biol Int 1995, 35: 487-98.

121. Pienta, K.J., Nguyen, N.M., Lehr, J.E. *Treatment of prostate cancer in the rat with the synthetic retinoid fenretinide. Cancer Res 1993, 53: 224-6.*

122. Liu, D.F., Rabbani, S.A. *Induction of urinary plasminogen activator by retinoic acid results in increased invasiveness of human prostate cancer cells PC-3.* Prostate 1995, 27: 269-79.

123. Halter, S.A., Fraker, L.D., Adcock, D., Vick, S. *Effect of retinoids on xenotransplanted human mammary carcinoma cells in athymic mice.* Cancer Res 1988, 48: 3733-6.

124. Rosewicz, S., Stier, U., Brembeck, F. et al. *Retinoids: Effects on growth, differentiation, and nuclear receptor expression in human pancreatic carcinoma cells.* Gastroenterology 1995, 109: 1646-60.

125. Bold, R.J., Ishizuka, J., Townsend, C.M. Jr., Thompson, J.C. *All-trans-retinoic acid inhibits growth of human pancreatic cancer cell lines.* Pancreas 1996, 12: 189-95.

126. Jiang, S.Y., Shyu, R.Y., Chen, H.Y. et al. *In vitro and in vivo growth inhibition of SC-M1 gastric cancer cells by retinoic acid.* Oncology 1996, 53: 334-40.

127. Formelli, F., Cleris, L. *Synthetic retinoid fenretinide is effective against a human ovarian carcinoma xenograft and potentiates cisplatin activity.* Cancer Res 1993, 53: 5374-6.

128. Schleicher, R.L., Moon, R.C., Patel, M.K., Beattie, C.W. *Influence of retinoids on growth and metastasis of hamster melanoma in athymic mice.* Cancer Res 1988, 48: 1465-9.

129. Shalinsky, D.R., Bischoff, E.D., Lamph, W.W. et al. *A novel retinoic acid receptor-selective retinoid, ALRT1550, has potent antitumor activity against human oral squamous carcinoma xenografts in nude mice.* Cancer Res 1997, 57: 162-8.

130. Lu, X-P., Fanjul, A., Picard, N. et al. *Novel retinoid-related molecules as apoptosis inducers and effective inhibitors of human lung cancer cells in vivo.* Nat Med 1997, 3: 686-90.

131. Huang, M.E., Ye, Y.C., Chair, J.R. et al. *All-trans-retinoic acid in the treatment of acute promyelocytic leukemia.* Blood 1988, 72: 567-72.

132. Degos, L., Chomienne, C., Deniel, M.T. et al. *Treatment of first relapse in acute promyelocytic leukemia with all-trans-retinoic acid.* Lancet 1990, 336: 1440-1.

133. de The, H., Degos, L., Warrell, R.P. Jr. *Clinical and molecular advances in acute promyelocytic leukemia.* In: Retinoids in Oncology. Gegos, L., Parkinson, D.R. (Eds.). Springer-Verlag: New York 1995, 73-88.

134. Parkinson, D.R., Warrell, R.P. Jr. *Retinoid therapy of other haematological malignancies.* In: Retinoids in Oncology. Degos, L., Parkinson, D.R. (Eds.). Springer-Verlag: New York 1995, 89-92.

135. Pastorino, U., Parkinson, D.R., Chiesa, F. *Retinoids in the prevention and therapy of solid tumors.* In: Retinoids in Oncology. Degos, L., Parkinson, D.R. (Eds.). Springer-Verlag: New York 1995, 93-111.