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RETINOIDS AS ANTICANCER AGENTS

Scott M. Lippman and Frank L. Meyskens, Jr.

Section of Hematology/Oncology, Arizona Cancer Center, Tucson, Arizona 85724

HISTORICAL PROSPECTIVE

Burt Wolbach and Percy Howe (1925) were the first investigators to discover a relationship between vitamin A and neoplasia. In their pioneering report, they made the following scientifically historical statements: "The specific tissue changes which follow the deprivation of fat-soluble vitamin A....suggests the acquisition of neoplastic properties....We conclude that the deficiency results in loss of specific (chemical) functions of the epitheliums concerned, while the power of growth becomes augmented....The study of the reverse changes that follow in the rapid amelioration when the rats are restored to an adequate diet has been begun and will be reported later." Wolbach and Howe's classic paper followed the recognition in 1909 and naming in 1920 of the fat-soluble substance essential for normal growth we now call vitamin A.

Many other early studies, including that by Abels et al. in 1941 which first associated vitamin A deficiencies with established human malignancy, further supported the link between vitamin A and neoplastic disease. Recognition that vitamin A deficiency leads to hyperkeratosis of the skin in humans and to squamous metaplasia in animals spurred an initial rush to treat cutaneous disorders with this new wonder drug; however, early excitement had to be tempered because of unacceptable toxic effects in many patients, especially acute CNS and mucocutaneous toxicities and major chronic reproductive, skeletal, liver and lipid toxicities (Lippman et al., 1987b). The symptoms of

acute hypervitaminosis A were first reported over 100 years ago, many years before vitamin A had been identified. These early reports involved the ingestion of polar bear and seal livers by Eskimos and Arctic explorers. The acute toxic symptoms included severe headache, drowsiness, irritability, nausea, and vomiting, followed 24 hours later by erythema and desquamation of the face, trunk, palms and soles. These symptoms generally resolved in 7-10 days. The earliest pathologically documented changes consistent with chronic hypervitaminosis A were reported in a partial homoerectus skeleton found in Kenya.

These severe side effects, especially the hepatotoxicity, led to the search for vitamin A derivatives with improved therapeutic ratios. New drugs began to be synthesized in the 1950s and were called "retinoids"--a generic term encompassing all the natural (excluding carotenoids) and synthetic compounds having some or all of the biological activities of vitamin A "retinol."

Retinoids have a basic molecular structure consisting of a cyclic end group, a polyene side chain and polar end group (Bollag, 1983). Chemical manipulation of the polar end group and the polyene side chain produced the first-generation synthetic retinoids. The most widely studied of these molecules are tretinoin in vitro and isotretinoin in vivo. The second-generation retinoids were developed by altering the cyclic end group. The prototype second-generation molecule is etretinate, which has been extensively studied in Europe and only recently approved in the U.S. The retinamides and arotinoids are two new classes of retinoids with high therapeutic indexes, which are now entering clinical trial. The retinamides, such as 4HPR, are a group of first-generation retinoids in which the terminal carboxyl group of retinoic acid is replaced by an end substituted carboxyamide group. This group of drugs was relatively nontoxic in phase I trials. Cyclization of the polyene side chain has produced the arotinoids, which are potent third-generation retinoids. The arotinoid TTNPB and its ethylester (Ro13-6298) are less toxic and over 1,000 times more potent than first- or second-generation retinoids in several standard screening tests. Modifying any part of the retinoid molecule can have dramatic effects on its distribution to the organs and on the drug's anticancer activity. For example, isotretinoin is highly effective in inhibiting phorbol ester-promoted mouse skin carcinogenesis and rodent bladder cancer, but

is ineffective in inhibiting rat mammary carcinogenesis. On the other hand, retinyl acetate is extremely active in the rat mammary model but ineffective in skin carcinogenesis models. Differences in storage, distribution, and metabolism account, in part, for differing degrees of toxicities of various retinoids. For example, etretinate may cause more serious teratogenic effects than those of isotretinoin, since its long half-life is 80-100 days compared to only a 12 to 20 hours with isotretinoin.

ANIMAL MODEL STUDIES

To better understand the anticarcinogenic activity of retinoids, one must review the elegantly constructed animal models of chemical carcinogenesis, such as the two-stage carcinogenesis system first described in the mouse skin model and later shown to apply to many other model systems, including bladder, lung and mammary gland carcinogenesis in various experimental animals. The initiation phase of carcinogenesis is irreversible and occurs with only a single application of the carcinogen at a subthreshold dose. The promotion phase is a multistep process, which is initially reversible and requires repetitive promoter treatments after initiation. At some point during the promotion phase, this process becomes irreversible and enters the progression phase. This two-stage system is invaluable for studying the potential effects of carcinogenesis inhibitors on both initiation and promotion (Bertram et al., 1987). Agents which prevent the initiation phase include beta-carotene. Retinoids act primarly in the promotion and progression phase after exposure to initiating and promoting agents. In contrast to anti-initiators, antipromoting agents, such as retinoids, must be continually present to retain their anticarcinogenic effect. Discontinuing these agents usually results in the complete loss of their anticancer effect and a return of the uncontrolled neoplastic process.

Studies employing the mouse skin carcinogenesis model have supplied much of the preclinical evidence for retinoids' effects in the chemoprevention of skin cancer. In 1967, Davies was the first investigator to show that vitamin A supplementation in mice could reduce the number and induce regression of DMBA-induced papillomas. Bollag (1983) amplified Davies data through a series of studies in which he administered tretinoin intragastrically during

the promotion phase of mouse skin carcinogenesis. He achieved a reduction of papilloma and carcinoma multiplicity and a decrease in papilloma volume. He then used tretinoin to produce an impressive regression of established papillomas in a dose-response fashion. A similar result was observed in mice with histologically established carcinoma. By retarding papilloma growth and enhancing its regression, Bollag inferred that retinoic acid not only prevented carcinogenesis but also had a therapeutic effect. In other experiments, he delayed the administration of the natural retinoid until after the appearance of several well-established papillomas and still achieved regression, thus confirming the drugs' therapeutic activity. Bollag went on to develop the standard mouse skin assay system for testing the therapeutic indexes of synthetic retinoids. His standard assay measures the ability of retinoids to cause regression of established carcinogen-induced papillomas. Using this assay, he defined the therapeutic index of a drug as the ratio between the ip dose per day causing a defined degree of hypervitaminosis A in a 14-day period and the ip dose causing 50% reduction of all papillomas.

Many of the most salient features of retinoids' preventive effects were illustrated by Moon's excellent studies of chemical carcinogenesis in rat mammary glands (Moon et al., 1983). Moon's group tested several vitamin A derivatives in the rat mammary carcinogenesis model and observed that retinyl acetate and 4HPR were highly effective in reducing the incidence and increasing the latency of MNU-induced rat mammary cancer. In contrast, isotretinoin had no anticancer activity in this model system. These data further demonstrate that minor alterations in the basic molecular structure can significantly affect retinoids' anticarcinogenic activities. Although the authors showed that continuous therapy with retinoid was needed to maintain the protective effect, therapy could be delayed for 16 weeks after initiation and still prevent cancer promotion. Combining retinoid treatment with other chemopreventive modalities can augment tumor inhibition in this tumor model system. Ovariectomy combined with 4HPR was significantly more effective than either treatment alone in preventing rat mammary cancer. Similarly, combining retinoid with the antiestrogen tamoxifen was synergistic in preventing rat mammary carcinogenesis. When begun at the time of the removal of the rat's first mammary tumor, the combination of retinyl acetate and ovari-

ectomy is also more effective as adjuvant therapy than either therapy alone. Based on these and other data, Veronesi's group in Milan have begun a large-scale trial using 4HPR as adjuvant therapy for patients with stage-I breast cancer. These data also suggest that 4HPR, with its extremely high therapeutic index in the rat mammary carcinogenesis model, may be an important drug for preventing breast cancer in high-risk patients. Many of these earlier studies in mammary cancer were confirmed by the study of carcinogen-induced bladder cancer in rats. Bladder cancer studies include the following data: retinoid exposure can be effective when started within a fairly broad time spectrum during the promotion phase; the retinamides, for example 4HPR, had the highest therapeutic ratio; and the retinoid restraint was overcome in time despite continued therapy, which led to the incidence of cancer eventually reaching the same level as that with controls. Nevertheless, treated rats had significantly prolonged life spans, which if extrapolated into human terms would correspond to a 5-10-year increase in symptom-free life. These results may be significant for patients (generally elderly) at highest risk of developing recurrent bladder cancer.

The many animal carcinogenesis models clearly document in a controlled experimental setting that retinoids can be powerfully active in the prevention and therapy of cancer. A vast amount of data from in vitro work with transformed human and animal cell lines also substantiate the anticancer activity of retinoids. Both the animal model and transformed cell line studies provide a strong part of the rationale for using retinoids to treat established malignancies. Although not as clear cut, several epidemiologic studies also support retinoids for human cancer therapy and contribute to the basis on which clinicians design clinical trials (Lippman and Meyskens, 1987a). Unfortunately, dietary studies have not clearly distinguished between the relative protective effects of retinol and carotenoids (which the body converts in part to retinol). Also, a positive relationship between cancer risk and serum retinol levels has not been uniformly reported, and some unconfirmed epidemiologic studies even suggest that retinoids may increase risk in some cancers, such as prostate cancer. At least in part, the possible anticancer effects of dietary and serum levels of vitamin A depend on interactions with other micronutrients, such as selenium and zinc, which also help to confound the inter-

pretation of epidemiologic studies. Since the epidemiologic and lab work alone strongly suggest but may never firmly establish the link between cancer risk and dietary and serum levels of vitamin A, carefully controlled clinical trials are the best hope of clarifying this issue.

The most widely employed methods for preclinical retinoid screening are Bollag's mouse papilloma assay and Sporn's hamster tracheal organ culture system (Bollag, 1983). Although these screens have predicted retinoid efficacy for malignant epithelial disorders, additional systems are needed for screening retinoid effects on other tissue types and disease states. It would be unfortunate if we failed to identify retinoids that could be effective in nonepithelial diseases simply because we relied exclusively on one or two epithelial oriented screens. Therefore, many other experimental systems have been explored for retinoid activity. These include in vitro cell culture, in vitro organ culture, and other in vivo approaches. Interest is growing in the site-specific screening of retinoids' growth-inhibition and differentiation-inducing activity in many murine and human transformed cell lines, the most notable of which are the murine (F9) teratocarcinoma and melanoma cell lines and the human (HL60) promyelocytic and neuroblastoma cell lines (Lippman et al., 1987a). Researchers use these lines to test retinoids alone and combined with other agents, such as hormones, cytotoxic drugs, and other biological modi- fiers, and combined with other modalities, such as irradiation.

MECHANISM

Despite extensive investigation, the molecular mechanisms of action which cause the diverse cellular changes modulated by retinoids remain incompletely understood. However, recent research has made it possible to hypothesize a basic underlying mechanism and to present a unifying concept of retinoid anticarcinogenic activity. This involves the Protein Kinase-C cascade system (Nishizuka, 1986). Phorbol esters such as TPA have been established as major tumor promoting agents. Therefore, protein kinase-C (PK-C), the phorbol ester receptor, plays a critical role in the carcinogenic process and has recently been implicated in the mediation of many phorbol ester-promoted actions such as ODC induction and EGF receptor down regulation. Activated (membrane-bound) PK-C may

transmit signals to the nucleus to regulate gene or oncogene transcription and may act by nontranscriptional actions, such as phosphorylation of the EGF receptor. Recent work by Cope and others supports the theory that retinoids modulate PK-C activity (Cope et al., 1986). Studying mouse brain PK-C, Cope's group observed that although unbound or apo-RBPs are substrates for PK-C, the holo-forms with bound retinoid inhibit PK-C activity. Although the precise way the retinoids interact with PK-C at the molecular level is not known, Cope showed that the retinoids do not inhibit phorbol ester binding to PK-C. Cope also showed that modulation of PK-C alters phosphorylation of retinoid-binding proteins and other specific cytosolic and membrane substrates, which could explain the myriad of reported retinoid actions. These include the regulation of enzyme synthesis, membrane structure, growth factors, binding proteins, gene transcription, postgenomic effects, extracellular actions, and the immune system (Jetten, 1984; Lippman et al., 1987a; Lotan, 1980; Sporn and Roberts, 1984; Wolf, 1984). The PK-C cascade may also modulate retinoids' antagonism towards phorbol esters, synergistic activity with other agents (e.g., antiestrogens and selenium) and ability to reverse cytotoxic drug resistance (Lippman et al., 1987a).

HUMAN STUDIES

Vitamin A or retinol intervention trials in prevention and therapy at clinically tolerable doses appear to have only limited efficacy in human cancer. Taken together, the epidemiologic and clinical studies with systemic natural vitamin A are disappointing (Goodman, 1986). Of course, hyper- and hypo-vitaminosis A are obvious health problems, however, whether vitamin A supplementation helps prevent cancers in people whose vitamin A levels are within physiologic range is unknown. The immediate future of vitamin A in human cancer prevention and therapy clearly lies in the use of synthetic retinoids with higher therapeutic ratios.

An early phase II study from the Arizona Cancer Center (ACC) documented significant activity of systemic isotretinoin in histologically established premalignancies and malignancies, especially of epithelial origin (Meyskens et al., 1982). This and other early studies led to the widespread clinical investigation of several synthetic retinoids in human neoplasia. Isotretinoin has been used

mainly in the U.S. and etretinate and the arotinoid Ro13-6298 have been studied more widely in Europe.

CUTANEOUS PRENEOPLASIA

Because they accumulate primarily in the skin, initially retinoids were used most widely to manage preneoplastic skin diseases. Several types of premalignant skin lesions have responded significantly to retinoids. These include actinic keratosis, keratoacanthoma, basal cell carcinomas, epidermodysplasia verruciformis and dysplastic nevus syndrome (Lippman and Meyskens, 1987b).

Actinic keratoses occur in Caucasians exposed to high levels of ultraviolet light, and approximately 5% of these lesions progress to nonmelanomatous skin cancer. Local destructive procedures are the current standard therapy. Both topically applied tretinoin and orally administered etretinate and arotinoid Ro13-6298 have produced significant results with these lesicns. In the six studies totalling 331 patients reported to date, complete responses of 46% to topical and 49% to oral retinoids have been achieved. The initial positive studies reported by Belisario and Bollag and Ott in the early 1970s have been confirmed by two recent randomized placebo-controlled trials. The drugs were generally well tolerated, with side effects consisting mainly of chelitis and scaling. These studies demonstrate uniquivocally that retinoids are effective in treating actinic keratosis, although high posttherapy relapse rates indicate that maintenance retinoid therapy is required to prevent disease progression.

Although not studied as extensively as actinic keratosis, the three other cutaneous premalignancies keratoacanthomas, epidermodysplasia verruciformis and dysplastic nevus syndrome also have produced promising results. Keratoacanthomas are hyperkeratotic skin lesions with a propensity to malignant transformation especially when multiple lesions are present. In studies of 11 patients (5 with multiple lesions), impressive responses to both etretinate and isotretinoin occurred within 2 to 3 months of treatment and have been sustained for more than one year after cessation of retinoid therapy.

Epidermodysplasia verruciformis is an unusual disorder characterized by multiple flat, wart-like lesions beginning in childhood and developing into squamous cell skin

cancers. These lesions usually contain the oncogenic human papilloma virus type 5. Although limited to several isolated case reports, the use of etretinate has produced significant clinical improvement. This improvement has occurred in some cases despite the persistence of the virus in the lesions. As in the other retinoid responsive preneoplastic diseases discussed above, relapse in these patients follows reducing or discontinuing the drug.

The dysplastic nevus syndrome may occur either familially or sporadically and frequently undergoes malignant change to melanoma. Treatment with surgical excision applies only to patients with a limited number of lesions and may be of only temporary benefit. Results with topical 5FU therapy have been variable. We treated three patients with topical tretinoin (0.05%). After 10-12 weeks, dysplastic histologic features underwent marked reduction, a result which has never been reported to occur spontaneously. Repeated skin biopsies from treated lesions showed changes to benign nevocellular nevi without dysplasia. Unfortunately, oral isotretinoin was relatively ineffective. The results with topical tretinoin are exciting and require further study with more patients to assess the actual efficacy of retinoids in the resolution of the dysplastic nevi and in preventing transformation of these nevi to melanoma.

Although the efficacy of retinoids is established in many cutaneous preneoplastic disorders, future study is required to determine the role of retinoids in preventing the onset of cutaneous malignancies. Prevention trials at the Arizona Cancer Center, Memorial Sloan-Kettering Cancer Center and National Cancer Institute are currently investigating this issue (Bertram et al., 1987). At the Arizona Cancer Center, we are conducting two stratified randomized prospective studies evaluating the efficacy of retinoids in the prevention of cutaneous malignancy. Begun in 1984, the first study includes actinic keratosis patients with greater than 10 prior lesions which had been surgically removed. They are randomized to receive either placebo or retinol (25,000 IU/day). The primary goal is to determine the number of new basal and squamous cell carcinomas. Our secondary goal is to determine the number of new actinic keratoses. To date, this study has accrued 1800 of a planned 2400 patients. Also begun in 1984, a second study includes patients with 8 or more prior documented and removed cutaneous squamous or basal cell can-

cers. They are randomized to receive placebo, retinol (25,000 IU/day), or isotretinoin (0.1 mg/kg/day) in divided doses. To date, 150 of a planned 350 patients have been accrued. Our goal in this second study is also to determine the effect of retinoids on the development of new basal and squamous cell cancers. Basal cell carcinoma of the skin is also the subject of several β-carotene prevention studies at the Muhimbili Medical Center in Tanzania, the Brigham Womans Hospital, Dartmouth University and the National Cancer Institute (Bertram et al., 1987). The results of prevention studies such as those discussed in this section could have a major public health impact.

NONCUTANEOUS PRENEOPLASIA

Currently, several noncutaneous premalignancies, including oral leukoplakia, cervical dysplasia, myelodysplastic syndromes, superficial bladder cancer, bronchial metaplasia, and laryngeal papillomatosis are receiving more attention in retinoid trials (Lippman et al., 1987b).

Retinyl acetate and retinol have been effective in experimental animals and in human epidemiological leukoplakia studies. Precancerous leukoplakia lesions probably do not regress spontaneously and can be safely monitored, thus presenting an excellent opportunity for the six clinical studies with retinoids involving a total of 163 patients and producing an overall response rate of 79% (CR = 12%). In comparing etretinate to isotretinoin and tretinoin, Koch achieved greater results with less toxicity using etretinate which produced a response rate of 91%, including more sustained remissions and fewer relapses and disease progressions. Relapses occurred within 1 or 2 months after completion of therapy. Koch then compared results in a second study between oral etretinate alone and oral etretinate plus topical etretinate paste. Response rates were high in both groups, with relapses again occurring within 2 months of stopping the drug. A controlled, randomized study by Hong et al. also achieved positive results in 67% of isotretinoin-treated patients, but relapses with lesion progression usually occurred within 2-3 months after stopping the drug.

Retinoids' anti epithelial cancer effects in other areas of the body and the association of dietary deficiencies of vitamin A with cervical dysplasia and carcinoma suggested these drugs for treating these disorders

in situ. An initial ACC study used a collagen sponge and standard diaphragm to deliver tretinoin to the affected tissues. This method resulted in unacceptable vaginal toxicity. Results were promising, however, and led to our phase-I study in which a cervical cap delivered the drug. Of the high- and low-dose groups, those patients in the former showed a higher response rate (complete response = 45%), clearly demonstrating the efficacy of the retinoid. Two other studies have also produced significant results, including a phase-II study at the ACC employing tretinoin in 20 patients, 10 of which achieved a complete response with 9 complete responders remaining disease free more than 12 months post-therapy. Although their clinical use in cervical dysplasia may be limited currently by the embriopathic potential of tretinoin and the availability of other successful modalities, retinoids have produced significant results in this disorder and newer, less-toxic retinoids continue to be developed. These results may have important implications for the long-term management of cervical preneoplasia as well as for the field of chemoprevention in general.

Since approximately 40% of patients with myelodysplastic syndromes (MDS) progress to acute leukemia, MDS are considered to be preleukemic states. Vitamin A deficiency leads to anemia in humans and reduced bone marrow precursors in animals. Both abnormalities revert to normal upon vitamin A replacement. These effects, along with the in-vitro antiproliferative and differentiation-inducing activities of retinoids in preleukemic and myeloid leukemic cells, led to six clinical retinoid studies totalling 75 MDS patients. An overall response rate of 34% with four well-documented complete responses indicates the efficacy of isotretinoin in this disorder. This conclusion is further supported by a recent randomized trial of isotretinoin reported by Clark et al (1987) with 70 patients. This study showed a significantly improved one year survival in retinoid-treated non-sideroblastic patients (77% vs 36%, $p = 0.004$ by log-rank test). These data are especially encouraging in light of the poor response of MDS to intensive cytotoxic chemotherapy and to low-dose Ara-C (which had at first seemed promising).

Data from many studies--in vivo animal models, in vitro and epidemiological--suggested retinoids for treating bladder cancer. In 1972, Evard and Bollag

observed a prophylactic effect of tretinoin in human bladder papilloma. Five clinical trials have involved retinoid therapy following resection of superficial bladder tumors, which normally recur in approximately 40%-90% of cases without drug intervention. In several studies, mucocutaneous toxicity was a significant limiting factor. In two randomized, double-blind trials, however, prolonged, low-dose etretinate appeared effective and compared well to therapy with intravesical cytotoxic agents.

Positive epidemiological studies and the laboratory observations that vitamin A deficiency leads to bronchial squamous metaplasia, which vitamin A and its analogs can reverse, gave rise to several studies in patients with high risks of developing lung cancer. In 1986, Misset et al. conducted one of the most significant of these studies involving 40 heavy smokers. The index of metaplasia of 36 evaluable patients treated with etretinate for six months showed a significant decrease from 34.5% to 26.96%. These impressive results, in addition to the complete resolutions of metaplasia in 4 patients who had ceased smoking during therapy, demonstrate that retinoids may play an active role in reversing this disease.

Laryngeal papillomatosis is a proliferation of polypoid lesions on the vocal cords which may precede squamous cell carcinoma. Interferon controls the disease in approximately 50% of patients, but unfortunately also has significant toxicity. We have used isotretinoin (1-2 mg/kg/d) to treat six patients with recurrent progressive papillomatosis of the larynx. Three patients achieved complete remissions. Bichler reported similar results using etretinate (1 mg/kg/d) in 42 patients. Twenty-eight, or 67%, achieved complete resolutions of their disease. These results suggest that retinoids may be an effective and relatively nontoxic alternative to interferon or surgery for the treatment of this disorder.

THERAPEUTIC ANTICANCER ACTIVITY

In addition to retinoids' more recognized preventive effects, these drugs have also shown antitumor activity in several preclinical studies, which led to growing clinical trials with retinoid therapy in established malignancies. Nearly 500 patients have undergone therapy, producing significant results in many cancers such as basal cell carcinoma, cutaneous squamous cell carcinoma, mycosis fun-

goides and acute promyelocytic leukemia.

Topical and systemic retinoids have been used to treat basal cell carcinoma. A total of 49 patients were treated in several different studies of topical retinoic acid, which produced a 99% response rate with 33% CRs (Lippman and Meyskens, 1987b). Bollag's observations that therapeutic effect occurred after 6 to 8 weeks of treatment and that a significant number of patients recurred at 10-months follow-up were characteristic of all these topical studies. Three other studies totalling 55 patients employed oral retinoids, either isotretinoin or etretinate. Data from these studies are also promising with a 51% objective response rate (9% CR), although complete and partial responses required maintenance therapy to prevent recurrences, especially in patients with multiple lesions. This preliminary work has not yet defined optimal preparation, dose and treatment schedules.

Retinoid treatment for cutaneous squamous cell cancer in humans has been limited. In-vivo results in animals have been promising and 4 studies involving 14 patients with advanced squamous cell cancer of the skin have also netted significant results of a 71% overall response rate, including 3 complete sustained remissions (Lippman and Meyskens, 1987b). These patients also tolerated the retinoids well. Since no standard effective systemic therapy for advanced or metastatic squamous cell carcinoma of the skin currently exists, these results are very encouraging.

Primarily involving the skin for most of its natural history, mycosis fungoides is an uncommon T-cell lymphoproliferative disorder (Lippman and Meyskens, 1987b). Single-agent retinoid treatments of this disease with either isotretinoin, etretinate or the arotinoid Ro13-6298 include 92 patients and have produced impressive results with an overall response rate of 62% (21% CR). Responses in these studies generally required maintenance retinoid treatment to avoid relapse. Other studies of combination modalities also indicate retinoid activity. Etretinate combined with PUVA produced 4 rapid improvements including 1 complete response and 1 failure (in a patient with Sezary's syndrome) out of 5 treated patients. Etretinate combined with cytotoxic antineoplastic agents, PUVA and prednisone produced 8 complete and 2 partial responses in 10 treated patients. And etretinate combined with meth-

oxypsoralen and PUVA also produced favorable results.

In general, extensive human in-vitro and animal in-vivo data encourage the use of retinoids in leukemia. Acute promyelocytic leukemia (APL) is the most promising leukemic disorder to undergo clinical retinoid trial (Lippman et al., 1987b). In 1983, Flynn et al. described the first APL patient treated with isotretinoin. After a 2-week course, this patient demonstrated a marked elevation of his peripheral granulocyte count. In all, only 5 cases have been studied, all of which involved previously refractory patients. Remarkable responses have been documented in 4 of these patients, including 2 patients with prolonged ($\geq$ 1 year) CRs.

Several other malignancies, including malignant eccrine poroma, choriocarcinoma, melanoma, head and neck cancer and several other leukemias, have also responded significantly to retinoids (Lippman et al., 1987b). In addition to documenting retinoid-responsive neoplasias, the identification of unresponsive disorders is also important. We have accumulated data on many malignancies that appear to be unresponsive to isotretinoin, including colon cancer, non-small cell lung cancer and sarcoma.

FUTURE DIRECTIONS

Unequivocally, retinoids have proved their anticancer activity in vitro and in animal models. Clinical study has clearly demonstrated that retinoids, especially the synthetics, have efficacy as single agents in human malignant and premalignant cutaneous disorders. There is now a growing interest in retinoids for the treatment of noncutaneous disorders as well, such as oral leukoplakia, bronchial metaplasia, laryngeal papillomatosis, cervical dysplasia, preleukemia, superficial bladder cancer, and acute promyelocytic leukemia. The future of retinoids depends in part on clinical testing of the retinamides (e.g., 4HPR, which is just entering a broad phase-II trial in the U.S.) and the arotinoids (e.g., Ro13-6298), which have higher therapeutic indexes. Testing combinations of retinoids with other modalities, cytotoxics, and other biological modifiers (including other retinoids with different spectra of activity and toxicity) is also an important area for future clinical research. Unfortunately, the rush to institute combination studies has led to poorly designed, uncontrolled studies, which will need

confirmation by better-designed trials. Another area for future clinical study involves retinoids as effective but less-toxic alternatives to standard therapies with cytotoxic agents. Many centers are also planning to use retinoids as adjuvant and neoadjuvant therapy in cutaneous malignancies such, as squamous and basal cell carcinomas. A recurrent theme from the many clinical trials is that most retinoid-responsive cutaneous malignant and premalignant disorders require maintenance therapy to prevent relapse. Therefore, the future of retinoids may depend less on screening for efficacy than on screening for toxic effects.

Although retinoids have already shown great promise in human cancer, many in-vitro questions remain, and clinical work is just beginning.

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