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# RESULTS OF THE USE OF VITAMIN A AND RETINOIDS IN CUTANEOUS MALIGNANCIES

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## INTRODUCTION

Vitamin A was identified long ago as an essential agent in the normal differentiation and maturation of epithelial tissues (Moore, 1967; Wolbach and Howe, 1925; Wolf, 1984). Laboratory research has demonstrated clearly that vitamin A deficiency enhances the effect of chemical carcinogens and tumor promoters and that replacing vitamin A reverses neoplastic abnormalities in these tissues (Lippman *et al.*, 1987a; Lotan, 1980; Lotan and Nicolson, 1977; Moon *et al.*, 1983). Clinical study has documented that many preneoplastic and neoplastic disorders respond to vitamin A and its natural and synthetic derivatives (retinoids) (Lippman *et al.*, 1987b). This chapter will present a brief review of the data from *in vitro* and animal skin carcinogenesis model studies that unequivocally demonstrate the anticancer activity of retinoids and a detailed discussion of the growing data from human studies employing retinoids to treat cutaneous neoplastic and preneoplastic processes.

## MECHANISMS

A vast amount of laboratory data has accumulated on the potent antipromoter, antiproliferative and differentiation-inducing effects of vitamin A derivatives on many malignant cell types (Lippman *et al.*, 1987a; Lotan, 1980; Moore, 1967; Wolbach and Howe, 1925; Wolf, 1984). Despite extensive investigation, the mechanism(s) of action which causes the diverse cellular changes modulated by retinoids remains incompletely understood. Recently, however, it has become possible to hypothesize a basic mechanism underlying these other actions and to present a unifying picture of retinoid effects. This involves the protein kinase C/phosphoinositol cascade system. Phorbol esters have been identified as primary promoters of carcinogenesis. Therefore, protein kinase C (PK-C), the phorbol ester receptor, plays a critical role in the promotion phase of the carcinogenic process (Nishizuka, 1986). Recently, PK-C has been implicated in the mediation of many phorbol ester-promoted actions, such as ornithine decarboxylase induction and epidermal growth factor receptor down regulation (Jetten and Shirley, 1986). Membrane-bound (activated) PK-C may transmit signals to the nucleus to regulate gene (or oncogene) transcription by phosphorylating proteins and may act by nontranscriptional actions as well, such as by phosphorylating the EGF receptor.

Recent work by Cope and others proposes the theory that retinoids modulate PK-C activity (Cope *et al.*, 1986; Jetten and Shirley, 1986; Lockyer *et al.*, 1984). Cope *et al.* (1986) have shown through their study of mouse brain PK-C that, although unbound retinoid binding proteins (Apo-cRABP and Apo-cRBP) are substrates for PK-C, the holo-forms (e.g. retinoic acid-cRABP) inhibit PK-C activity. Although the molecular details of retinoids' inhibition of the PK-C cascade are unclear, they do not appear to block phorbol ester binding to PK-C. Cope (1986) also showed that the regulation of PK-C alters phosphorylation of the retinoid binding proteins and other specific cytosolic and

membrane substrates (Cope *et al.*, 1986). These alterations could explain the myriad of reported retinoid actions. These include 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 *et al.*, 1986; Sporn and Roberts, 1984; Wolf, 1984). The PK-C cascade may also modulate the antagonism of retinoids toward phorbol esters, their synergistic activity with other agents (e.g., antiestrogens and selenium) and also their ability to reverse drug resistance (Marsh and Center, 1986; O'Brian *et al.*, 1986; Su *et al.*, 1986).

## SKIN CARCINOGENESIS MODEL

The best-studied model of the multistep process of chemical carcinogenesis is the two-stage carcinogenesis system first established in the mouse skin model and later shown to apply to many other model systems as well (Boutwell *et al.*, 1985; Gensler and Bowden, 1984; Gensler *et al.*, 1987). These include bladder, lung and mammary gland carcinogenesis in experimental animals (Hicks *et al.*, 1985; Moon *et al.*, 1985). The first stage, or initiation phase, is irreversible and occurs after only a single application of the carcinogen at a subthreshold dose. The promotion phase is a multistep process requiring repetitive promoter treatments after initiation. Initially, it is reversible. At some point during the promotion phase, the process becomes irreversible and enters the progression phase. This two-stage system is valuable for investigating the potential effects of inhibitors of carcinogenesis on both initiation and promotion. Primary prevention, or preventing the initiation phase, involves reducing the exposure to recognized initiating agents such as cigarette smoking. The radical-trapping antioxidant action of $\beta$-carotene may act in this phase (Burton and Ingold, 1984). Secondary prevention involves active dietary, pharmacologic or educational intervention after an individual has been exposed to initiators or promoters, but has not yet developed preneoplastic changes. Tertiary prevention involves the use of dietary or pharmacologic inhibitors in situations where histologically identifiable preneoplastic changes have already developed (Bertram *et al.*, 1987; Meyskens, 1984).

Retinoids prevent cancer in the skin, mammary glands, bladder, lung and other sites in experimental animals (Boutwell *et al.*, 1985; Gensler and Bowden, 1984; Hicks *et al.*, 1985; Moon *et al.*, 1985). However, their effects on chemical carcinogenesis are complex and vary with the species, carcinogen, promoter and tissue involved. Studies employing the mouse skin carcinogenesis model have supplied most of the preclinical evidence for the effects of retinoids in the chemoprevention of skin cancer (Boutwell *et al.*, 1985; Gensler and Bowden, 1984). Davies (1967) showed that vitamin A supplementation in mice could reduce the number and induce regression of DMBA (7,12-dimethylbenz[a]-anthracene)-induced papillomas. Advancing Davies' work, Bollag (1979, 1983) conducted a series of studies in which he administered all-*trans*-retinoic acid intragastrically during the promotion phase of mouse skin carcinogenesis. He achieved a reduction in numbers of papillomas and carcinomas, and a decrease in the incidence of carcinoma. Retarding papilloma growth and enhancing its regression led Bollag to infer that retinoic acid not only prevents carcinogenesis but also has a therapeutic effect. In other experiments, he achieved regression even when delaying administration of the natural retinoid until after the appearance of well-established papillomas, thus confirming the drug's therapeutic activity. Bollag went on to develop the standard mouse skin assay systems for testing the therapeutic indexes of synthetic retinoids (Bollag, 1979, 1983). All of the mouse assays exploit three well-studied retinoid effects on experimental skin carcinogenesis. These are, inhibition of papilloma development initiated by DMBA and promoted by 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), regression of established papillomas, and halting the progression of benign papilloma to frank carcinoma. Bollag's standard assay measures the ability of retinoids to cause regression of established carcinogen-induced papillomas. Using this assay, Bollag has defined the therapeutic index of a drug as the ratio between the daily intraperitoneal dose causing a defined degree of

hypervitaminosis A over a 14-day period and the intraperitoneal dose causing a 50% reduction of all papillomas.

Boutwell's group (Boutwell *et al.*, 1985; Loprinzi *et al.*, 1985) pursued a large series of studies into the effects of oral and topical synthetic and natural retinoids on skin tumor promotion. They demonstrated that the induction of epidermal ornithine decarboxylase activity by tumor promoting agents is a critical, possibly obligatory feature of skin tumor promotion and showed further that the topical application of retinoic acid, and several other topical natural and synthetic retinoids, 1 hr prior to applying the promoter TPA clearly inhibited the onset of TPA-induced ornithine decarboxylase activity. This inhibition correlated with each retinoid's inhibitory effect on skin tumor promotion. However, skin carcinogenesis, initiated by the carcinogen DMBA alone, does not respond to retinoic acid, either in inhibition of ornithine decarboxylase induction or prevention of skin papillomas. Although the effects of retinoids on ultraviolet (u.v.) radiation-induced skin carcinogenesis are not clearly understood, some studies indicate that retinoic acid can enhance u.v. tumorigenesis (Lichti *et al.*, 1981). What is clear, however, is that many natural and synthetic retinoids inhibit two-stage phorbol ester-promoted carcinogenesis of the skin.

## EPIDEMIOLOGY

Since vitamin A is critical to epithelial cell growth and differentiation *in vitro* and *in vivo*, great interest has developed in epidemiologic studies of vitamin A and cancer (Bertram *et al.*, 1987; Lippman and Meyskens, 1987; Menkes *et al.*, 1986; Peleg *et al.*, 1984; Peto *et al.*, 1981; Rittenbaugh and Meyskens, 1986; Willet *et al.*, 1984). An estimated 35% of U.S. cancers are due to dietary factors and over 90% of all human cancers are of epithelial origin. Evidence from several epidemiologic studies suggests the possibility that a relationship exists between cancer risk and dietary intake of vitamin A and resulting serum levels. Unfortunately, dietary studies do not clearly distinguish between the relative protective effects of retinol and carotenoids which the body converts in part to retinol. However, a positive cancer risk relationship with serum retinol levels has not been uniformly reported, and some unconfirmed studies even indicate that retinoids may increase risk in some cancers, such as prostate cancer. The possible anticancer effects of dietary and serum levels of vitamin A depend, at least in part on interactions with other micronutrients such as selenium and zinc, which also help to confound the interpretation of epidemiologic studies (Goodwin *et al.*, 1986; Ip and Ip, 1981; Knight *et al.*, 1983; Peng *et al.*, 1986; Thompson *et al.*, 1981; Underwood, 1984). Since neither epidemiologic nor laboratory work will firmly establish the link between cancer risk, and dietary and serum levels of vitamin A, carefully controlled clinical trials are essential to clarify this issue.

## HUMAN CANCER PREVENTION STUDIES

Several intervention trials indicate that natural vitamin A therapy at clinically tolerable doses appears to have only limited efficacy in the prevention and therapy of human cancer (Goodman, 1986; Munoz *et al.*, 1985). Many other studies over the past 40 years of use of vitamin A have detailed its unacceptable toxicity (Bliss and Roels, 1967; DeLuca and Creek, 1986; Korner and Vollin, 1975; Lippman *et al.*, 1987b; Windhorst and Nigra, 1982). Overall, the epidemiologic and clinical studies with systemic natural vitamin A are disappointing. Possibly the use of topical natural vitamin A has some value. While hyper- and hypo-vitaminosis A are obvious health problems, the contribution of vitamin A supplementation to preventing cancers in people whose vitamin A levels are within the physiologic range is not established. The immediate future of this class of drugs in human cancer prevention and therapy lies with careful clinical studies of synthetic retinoids having higher therapeutic ratios (Bollag, 1979; Bollag, 1983; Ellis and Voorhees, 1987; Loeliger *et al.*, 1980).

TABLE 1. *Retinoids in Cutaneous Preneoplasia*

| | Retinoid | Route | Evaluable patients | CR | PR | Minor or NR | Author |
|---|---|---|---|---|---|---|---|
| Actinic keratoses | Retinoic acid | Topical | 60* | 25 | 27 | 8 | Bollag and Ott, 1975 |
| | Retinoic acid | Topical | 93 | 46 | 47 | 0 | Belisario, 1972 |
| | Etretinate | Oral | 46 | 35 | 8 | 3 | Grupper and Berretti, 1983 |
| | Etretinate | Oral | 44 | 10 | 27 | 7 | Moriarty *et al.*, 1982 |
| | Etretinate | Oral | 15 | 0 | 14 | 1 | Watson, 1986 |
| | Arotinoid | Oral | 16 | 0 | 10 | 6 | Kingston *et al.*, 1983 |
| Keratoacanthoma | Etretinate | Oral | 6 | 6 | 0 | 0 | Grupper and Berretti, 1983 |
| | Etretinate | Oral | 1 | 0 | 1 | 0 | Verret *et al.*, 1985 |
| | Etretinate | Oral | 1 | 0 | 1 | 0 | Pichler and Fritsch, 1984 |
| | Etretinate | Oral | 1 | 0 | 1 | 0 | Hayday *et al.*, 1980 |
| | Isotretinoin | Oral | 1 | 1 | 0 | 0 | Shaw and White, 1986 |
| | Isotretinoin | Oral | 1 | 0 | 1 | 0 | Levine *et al.*, 1984b |
| Epidermodysplasia verruciformis | Etretinate | Oral | 2 | 1 | 1 | 0 | Lutzner *et al.*, 1981 |
| | Etretinate | Oral | 3 | 0 | 3 | 0 | Kanerva *et al.*, 1985 |
| | Etretinate | Oral | 2 | 0 | 2 | 0 | Edelson *et al.*, 1981 |
| | Etretinate | Oral | 3 | 0 | 3 | 0 | Jablonska *et al.*, 1981 |
| | Etretinate | Oral | 2 | 0 | 2 | 0 | Guilhou *et al.*, 1980 |
| Dysplastic nevi syndrome | Tretinoin | Topical | 3 | 2 | 1 | 0 | Levine *et al.*, 1984a |
| | Isotretinoin | Oral | 8 | 0 | 1 | 7 | Meyskens *et al.*, 1986 |

*Number of lesions.

## CUTANEOUS PRENEOPLASIA (TABLE 1)

Retinoids accumulate primarily in the skin. Retinoids as cancer prevention agents have therefore been used most widely and effectively in the management of skin disorders (Bollag, 1979; Bollag, 1983; Elias and Williams, 1981; Peck, 1981). The synthetic derivatives in particular, have produced responses in premalignant skin disorders such as actinic keratosis, keratoacanthoma, epidermodysplasia verruciformis and the dysplastic nevus syndrome.

### *Actinic Keratosis*

Actinic keratoses occur commonly in caucasians exposed to high levels of u.v. light. Approximately 5% of these lesions undergo malignant transformation. Topical and oral retinoids have produced impressive results in studies of this condition in man. An early report by Belisario (1972) described 93 patients with actinic keratosis treated with topical retinoic acid cream. Forty-six of these patients experienced a complete clearing of treated lesions, and the other 47 patients had partial responses. Bollag and Ott (1975) saw complete responses in 25 of 60 (40%) evaluable lesions in their study employing topically applied retinoic acid cream (0.1% or 0.3%). They also achieved a partial clearing in 27 (45%) other lesions. Altogether, these impressive responses confirmed those reported by Belisario (1972).

Oral retinoids have also demonstrated activity in four actinic keratoses studies. In the first of these, Grupper and Berretti (1983) utilized an induction dose of etretinate (1 mg/kg per day for 3 months) followed by a maintenance dose of etretinate (0.5 mg/kg per day for 4–5 months). Of the 46 evaluable patients, 35 (76%) experienced complete clearing of their lesions and 8 (17%) had partial clearing. Since 26 of 34 patients (76%) relapsed after 12 months off the retinoid, the authors have begun treating patients with an annual intensification of therapy. Apparently, a 2-month course of etretinate (1 mg/kg per day) has prevented lesion recurrence in most patients.

In a double-blind crossover trial, Moriarty *et al.* (1982) gave patients either oral etretinate (75 mg per day) or placebo. After two months on the drug, 10 complete responses and 27 partial responses occurred in 44 evaluable patients. Only one complete and one partial response occurred in the 44 placebo-treated patients. Watson (1986) also used a double-blind crossover trial to study the effect of etretinate (1 mg/kg per day) or placebo in 15 patients with advanced multiple actinic keratoses. Patients received four months of

treatment before crossover. Fourteen of the 15 retinoid-treated patients had impressive responses. After the 4-months etretinate therapy, nine patients received placebo. One improved and seven remained stable, suggesting prolonged retinoid activity. In a study of 16 patients treated with Ro 13-6298 (1 μg/kg/day for 28 days), Kingston *et al.* (1983) demonstrated that actinic keratoses also respond to this potent oral retinoid of the arotinoid series. Ten patients (63%) had partial improvement of their lesions.

The evidence demonstrates that several vitamin A derivatives (oral and topical) can produce impressive responses in actinic keratoses and that a maintenance schedule is necessary for a continued response. As with most precancerous cutaneous lesions, although the retinoids can cause regression of actinic keratoses, their effect in decreasing the risk of subsequent skin cancers among treated patients is still unknown. This question will require continued study and longer follow-up of large numbers of patients.

### *Keratoacanthoma*

Keratoacanthoma is another preneoplastic skin disorder which has responded to retinoid treatment. These hyperkeratotic lesions have a morphologic resemblance to squamous cell carcinoma of the skin and are capable of undergoing either spontaneous regression or frank malignant change (Schwartz, 1979). Occasionally, keratoacanthomas are multiple and behave more aggressively.

Several studies have reported responses to retinoids in a total of 11 patients with keratoacanthomas (Grupper and Berretti, 1983; Haydey *et al.*, 1980; Levine *et al.*, 1984b; Pichler and Fritsch, 1984; Shaw and White, 1986; Verret *et al.*, 1985). These studies produced impressive sustained responses to isotretinoin or etretinate in five patients with multiple lesions who had not experienced spontaneous regression of lesions for many years (Haydey *et al.*, 1980; Levine *et al.*, 1984b; Pichler and Fritsch, 1984; Shaw and White, 1986; Verret *et al.*, 1985). Six other patients experienced complete responses after treatment with oral etretinate (1 mg/kg per day) (Grupper and Berretti, 1983). Five of these patients have remained in complete remission for more than one year after stopping therapy.

### *Epidermodysplasia Verruciformis*

Epidermodysplasia verruciformis begins in childhood, characterized by familial occurrence, multiple flat wart-like lesions and multiple squamous cell skin cancers. An oncogenic virus, usually human papilloma virus (HPV) type 5, is found in these lesions (Guilhou *et al.*, 1980; Weiner *et al.*, 1985). A 32-year-old man with epidermodysplasia verruciformis for 30 years was treated with oral etretinate (1 mg/kg per day). Within 2 months his lesions had regressed and were HPV-5 negative on rebiopsy (Lutzner and Blanchet-Bardon, 1981). Similar results have been reported in further patients, including those with epidermodysplasia verruciformis induced by HPV-3 (Jablonska *et al.*, 1981; Kanerva *et al.*, 1985). At times, this disorder will respond to retinoid therapy without a corresponding decrease of the virus (Edelson *et al.*, 1981). To confirm these results, further controlled retinoid studies are needed.

### *Dysplastic Nevus Syndrome*

The dysplastic nevus syndrome characteristically displays abnormal melanocytic lesions which have a propensity for undergoing malignant change to cutaneous melanoma (Greene *et al.*, 1978). Dysplastic nevi are found in 5%–8% of the caucasian population. They may occur either as an inherited autosomal dominant trait or in a sporadic form. Patients with few lesions are treated with surgical excision. However, when large numbers of nevi are present, surgery is impractical except in the case of a specific malignant-appearing lesion. Since many cutaneous premalignancies respond to retinoids and dysplastic nevi are often a precursor of malignant melanoma, studies at the University of Arizona have begun to examine the role of retinoids in this precancerous condition.

In an early pilot study, we treated three patients with histologically confirmed multiple dysplastic nevi with a daily topical dose of 0.05% tretinoin (Levine *et al.*, 1984a). After 10–12 weeks of treatment, we rebiopsied the patients and noted marked histologic improvement despite only minimal changes in clinical appearance. In two patients, the post-treatment biopsies showed benign compound nevi without any dysplasia. The third patient's post-treatment biopsy showed benign compound nevi with minimal dysplastic change. These results have not been reported to occur spontaneously. These preliminary data do however need further study since the small number of subjects cannot preclude the possibility of sampling error.

In a subsequent study, we observed that dysplastic nevi were not significantly altered by treatment with oral isotretinoin (40 mg twice a day for 16 weeks) (Meyskens *et al.*, 1986). We rebiopsied eight evaluable patients after treatment. One patient, who was studied by punch biopsy, showed an apparent histologic change from dysplastic nevi to benign nevi. No changes were noted in the other seven patients. These results do not indicate a role for oral retinoids in the dysplastic nevus syndrome. However, several variables may account for the difference in results between this study and those using topical retinoid treatment. For example, the topical retinoid study used only punch biopsies, which have a greater sampling error than that of total surgical excision. Also, the topical route of administration may produce a higher local concentration of retinoic acid in the nevus than the systemic route produces.

Further studies are continuing to examine the effect of topical retinoic acid therapy on a larger number of patients whose dysplastic nevi have been proven histologically by total excision. A study at the University of Arizona is evaluating the effect of topical tretinoin on the clinical and histologic appearance of dysplastic nevi. To date, 11 patients with either the familial or sporadic form of the dysplastic nevus syndrome have been randomized to receive either tretinoin (0.05%) or its vehicle (placebo) in this double-blind study. Results are not yet available.

### *Basal Cell Carcinoma—Prevention*

Retinoids apparently have a role in the prevention, as well as treatment, of basal cell carcinoma. In a study of three patients who had a 6–16-year history of multiple basal cell carcinomas, Peck *et al.* (1982) noted a preventive effect after treatment with isotretinoin. Although, statistically, each of these patients had a 100% chance of developing new lesions within 1 year, no new tumors developed more than 2.5 years after beginning retinoid treatment. Prevention of recurrence seemed to depend on continued treatment with a maintenance oral dose of 1.5 mg/kg/day.

### *Current Skin Cancer Prevention Studies with Retinoids*

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 individuals who have had more than 10 prior actinic keratoses surgically removed. They are randomized to receive either placebo or retinol (25,000 i.u./day). The primary goal is to determine the number of new basal and squamous cell carcinomas. A secondary goal is to determine the number of new actinic keratoses. To date, this study has accrued 1800 of a planned 2400 patients. A second study, also begun in 1984, includes patients with 8 or more prior documented and removed cutaneous squamous or basal cell cancers. They are randomized to receive placebo, retinol (25,000 i.u./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 $\beta$-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.

## Cutaneous Neoplasia (Table 2)

### *Basal Cell Carcinoma*

As in chemoprevention, the greatest use of retinoids in the therapy of established cancers has been for skin disorders. More than 10 years ago, Bollag and Ott (1975) reported that topical tretinoin (0.1%–0.3%) produced a therapeutic effect in 15 of 16 patients with basal cell carcinoma. These effects included five complete responses and ten partial responses. Lesions recurred in two of the complete responders within 10 months after treatment. In the same article they summarized other early studies of retinoic acid conducted by Belisario (1972) and Schumacher and Stuttgen (1971). In all, 49 patients were treated with 14 complete and 19 partial responses. Sankowski *et al.* (1984) utilized topical isotretinoin in 15 patients and achieved similar results. Two complete and 13 partial responses were observed. Therapeutic responses in all four studies occurred within 4–6 weeks and disease usually recurred in the absence of maintenance therapy.

Oral retinoids have also produced objective responses. Using either etretinate or isotretinoin, three studies involving 55 patients demonstrated a 51% objective response rate, which includes a 9% complete response rate. In a study by Peck *et al.* (1979) and Peck (1985), 11 patients with multiple basal cell carcinomas were treated with isotretinoin (4.5 mg/kg per day). Prolonged therapeutic responses depended upon the continued intake of the retinoid. We have reported the results of treating five patients with isotretinoin (2–3 mg/kg/day) for basal cell carcinoma (Lippman *et al.*, 1987b; Meyskens *et al.*, 1982) and noted two partial responses, one lasting 18 months and the other 10 months. In one of the responders, the need for spinal decompression surgery was averted. In a study involving 20 patients with isolated lesions and 20 with multiple lesions, Grupper and Berretti (1983) achieved similar responses with etretinate (1 mg/kg/day). Patients with isolated neoplasms relapsed less often (1 of 10) than those with multiple lesions (12 of 14) at 12-months follow-up. As in the studies with isotretinoin, maintenance etretinate appeared necessary to prevent relapse.

The results using retinoids in the treatment and prevention of basal cell carcinoma are promising. Ongoing studies are now underway to determine the most efficacious retinoid form, dose and treatment schedule for this widespread malignancy.

### *Refractory Squamous Cell Carcinoma (SCCA) of the Skin*

Although the vast majority of patients with SCCA of the skin are cured with local approaches such as surgery and/or irradiation, effective systemic therapy for advanced or refractory local, regional or metastatic disease is lacking. The preliminary results of the limited use of retinoids for squamous cell cancer of the skin are encouraging. Grupper and Berretti (1983) employed etretinate (1 mg/kg/day) to achieve one complete and one minor response in four patients with invasive squamous cell cancer. One complete response, two partial responses and one minor response were seen in five patients with Bowen's disease. Follow-up for one year, revealed no relapses in the responding patients with Bowen's disease or with invasive squamous cell carcinoma who were disease free following treatment either with retinoid alone or combined with local destructive measures. Kingston *et al.* (1983) reported a dramatic partial response to the arotinoid Ro13-6298 (1 $\mu$g/kg/day for 4 weeks) in one of five patients, all with both actinic keratoses and squamous cell skin

TABLE 2. *Retinoids in Cutaneous Neoplasia*

| | Retinoid | Route | Evaluable patients | CR | PR | Minor or NR | Author |
|---|---|---|---|---|---|---|---|
| Basal cell carcinoma | Tretinoin | Topical | 16 | 5 | 10 | 1 | Bollag and Ott, 1975 |
| | Tretinoin | Topical | 12 | 5 | 7 | 0 | Belisario, 1972 |
| | Tretinoin | Topical | 6 | 4 | 2 | 0 | Schumacher and Stuttgen, 1971 |
| | Isotretinoin | Topical | 15 | 2 | 13 | 0 | Sankowski *et al.*, 1984 |
| | Isotretinoin | Oral | 11 (248*) | 39* | 162* | 47* | Peck, 1985 |
| | Isotretinoin | Oral | 4 | 0 | 1 | 3 | Meyskens *et al.*, 1982; Lippman *et al.*, 1987b |
| | Etretinate | Oral | 40 | 3 | 14 | 23 | Grupper and Berretti, 1983 |
| Squamous cell carcinoma | Isotretinoin | Oral | 4 | 1 | 1 | 2 | Grupper and Berretti, 1983 |
| | Isotretinoin | Oral | 5 | 0 | 3 | 2 | Meyskens *et al.*, 1982 |
| | Isotretinoin | Oral | 4 | 1 | 3 | 0 | Lippman and Meyskens, 1987 |
| | Arotinoid | Oral | 1 | 0 | 1 | 4 | Kingston *et al.*, 1983 |
| Melanoma | Tretinoin | Topical | 2 | 1 | 1 | 0 | Levine *et al.*, 1980 |
| | Isotretinoin | Oral | 20 | 0 | 3 | 17 | Meyskens *et al.*, 1982 |
| Myosis fungoides† | Isotretinoin | Oral | 25 | 3 | 8 | 14 | Kessler *et al.*, 1987 |
| | Isotretinoin | Oral | 28 | 6 | 13 | 9 | Molin *et al.*, 1985 |
| | Isotretinoin | Oral | 11 | 1 | 7 | 3 | Claudy and Rouchouse, 1985 |
| | Isotretinoin | Oral | 7 | 0 | 3 | 4 | Warrell *et al.*, 1983 |
| | Isotretinoin | Oral | 1 | 1 | 0 | 0 | Fitzpatrick and Mellette, 1986 |
| | Isotretinoin | Oral | 6 | 0 | 6 | 0 | Neely *et al.*, 1987 |
| | Etretinate/Isotretinoin‡ | Oral | 1 | 0 | 0 | 1 | Jenkinson and Beare, 1984 |
| | Etretinate | Oral | 1 | 1 | 0 | 0 | Wargon and Downie, 1984 |
| | Etretinate | Oral | 1 | 1 | 0 | 0 | Ippolito and Giacalone, 1982 |
| | Etretinate | Oral | 5 | 2 | 0 | 3 | Souteyrand *et al.*, 1981a |
| | Arotinoid | Parenteral | 5 | 3 | 1 | 1 | Mahrle *et al.*, 1983 |
| | Arotinoid | Oral | 1 | 1 | 0 | 0 | Tousignant *et al.*, 1987 |

*Number of lesions.
†For combination studies in mycosis fungoides, see text.
‡This patient first failed etretinate, then failed isotretinoin.

carcinomas. Unfortunately, the responses of the other four SCCA patients were not individually described.

At the University of Arizona, five patients with invasive cutaneous squamous cell carcinoma were treated with isotretinoin (3 mg/kg/day) in a broad phase II study (Meyskens *et al.*, 1982). We observed three responses (one partial and two mixed). More recently, we treated four SCCA patients who were refractory to local therapy, with lower-dose isotretinoin (1 mg/kg/day) (Lippman and Meyskens, 1987). All had major disease regression beginning within three weeks of starting therapy. One patient with extensive involvement of the dorsal aspects of both hands achieved a complete remission continuing at 24 months. Another patient had nearly a complete regression of his massive, 10-cm-by-10-cm fungating neck lesion, with continued response at 9 months. The third patient had a complete resolution of a 4-cm-by-2.5-cm metastatic axillary lesion along with resolution of intractable shoulder pain. The fourth patient, also with multiple lesions on the dorsal aspects of both hands, had an excellent response lasting 2 months but relapsed after his dose was reduced because of side-effects. This patient then required radiation treatment, after which isotretinoin was resumed at protocol dose levels. A partial response in his hand lesions was observed.

Firm conclusions cannot be derived from this limited number of patients. It does appear, however, that retinoids are capable of inducing sustained objective responses. Since no effective standard systemic therapy currently exists for advanced, recurrent cutaneous squamous cell carcinoma, retinoids offer promise. Future retinoid studies should also be directed toward preventing disease recurrence. Prospective studies are being designed to further define retinoid therapy for refractory cutaneous malignancy and to explore its adjuvant and neoadjuvant roles.

### *Melanoma*

Pioneering *in vitro* work by Lotan's group (Lotan and Nicolson, 1977; Lotan, 1979, 1980, 1986; Lotan *et al.*, 1983, 1984) clearly demonstrated retinoids' antiproliferative and differentiation-inducing activity in murine melanoma cells. Although the *in vitro* response of human melanoma cells to retinoids is clearly more heterogeneous, reversible growth-inhibition and, rarely, differentiation-induction have been documented. The continual development and study of animal models should expand our knowledge of retinoid actions on melanoma *in vivo*. In an early series of mouse experiments, Felix *et al.* (1975, 1976) used vitamin A to achieve significant growth inhibition in transplanted S91 murine melanoma from histoincompatible donor mice. They also achieved immune changes by treating the transplanted melanoma with intralesional vitamin A. This cured 79% of these tumors from histoincompatible donor mice, which present an allogeneic tumor system. This result contrasted sharply with the total ineffectiveness of vitamin A treatment of tumors donated by histocompatible mice. These contrasting results and others reported by Patek *et al.* (1979) imply a critical role for cellular immune defenses. The use of this allogeneic tumor system may explain, in part, the discrepancies between these positive results with the generally negative results achieved in other animal melanoma models.

The Arizona Cancer Center has had for some time, a major clinical and laboratory interest in the use of retinoids to control malignant melanoma and its precursor lesion, dysplastic nevus syndrome. Our recent work describes a novel *in vivo* model system for studying retinoid effects on melanoma (Levine, 1985). In this system, S91 murine melanoma cells are inoculated into upper dermal blister cavities created by negative suction pressure in the skin of DBA/2 mice. The resulting tumor nodules resemble cutaneous metastases of melanoma because of their superficial dermal location. We then applied topical tretinoin at varying concentrations in DMSO, beginning one day after inoculation and continuing for 28 days. This prevented the development of visible or palpable tumors and decreased incorporation of [$^{14}$C]-Thiouracil, a measure of melanoma growth, in a dose-dependent manner. This model may also be useful for studying the way retinoids mediate host-tumor immune responses.

Levine and Meyskens (1980) achieved clinical and pathologic responses in several cutaneous melanoma metastases treated with 0.05% tretinoin cream under occlusion. Two patients, who had failed prior cytotoxic therapy, had impressive and prolonged responses to topical tretinoin treatment. Complete clinical and histologic regression of all 21 treated lesions occurred in the first patient. Biopsies of four of these lesions showed dermal extracellular and intracellular melanosis without evidence of tumor. The patient developed no new skin lesions or changes in old lesions during 24 months of follow-up after therapy. The second patient experienced clinical partial responses in 3 of 22 treated lesions. Two biopsy specimens showed regression.

These results led to the inclusion of 20 patients with metastatic melanoma in our broad phase II trial of oral isotretinoin (Meyskens *et al.*, 1982). The overall response rate was 15% (3/20). One patient experienced a 50% reduction in a 10-cm-by-13-cm subcutaneous mass. This response lasted for 4 months with continued treatment. A partial response was noted for 6 months in another patient who had extensive nodal metastases. A third patient's skin metastases showed responses lasting 4 months. Cassidy *et al.* (1981) also reported responses to oral isotretinoin with partial regression in patients with melanoma and cutaneous metastases. This clinical data, added to the *in vitro* data involving the synergy between isotretinoin and alpha interferon in melanoma cell culture and in our human tumor stem cell assay has led us to initiate a prospective two-arm randomized trial comparing recombinant alpha interferon (rαIFN) to rαIFN plus isotretinoin.

In an adjuvant study of patients with stage I and II cutaneous malignant melanoma, we compared recurrence rates of 69 patients receiving BCG (scarification) alone with 50 patients receiving BCG plus oral vitamin A (100,000 i.u./day) (Meyskens *et al.*, 1987). Evaluable totals of 79 Stage I patients and 40 Stage II patients were randomly stratified into either study arm for 18 months. Patient follow-up ranged between 18 and 50 months. No significant difference in disease-free survival was demonstrated between the 69 patients receiving BCG plus vitamin A and the 50 patients receiving only BCG. It is possible that adjuvant therapy with vitamin A in this study was not effective because of either a negative interaction with BCG (which has subsequently been shown to be ineffective) or a vitamin A dose that was too high. Recent experimental work has shown that low-to-moderate doses of vitamin A can enhance the immune response, while higher doses may be deleterious. More recently, Legha *et al.* (1984) began a trial to determine the efficacy of isotretinoin for Stage I and Stage III surgically treated melanoma patients. This adjuvant study group is composed of four control patients and four isotretinoin-treated patients with Stage I disease, and 14 controls and 14 isotretinoin patients with Stage III melanoma. A total of five recurrences in the treated groups and seven recurrences in the control groups have been noted at 1–22-month follow-up periods. We are currently participating in a multi-institutional (Southwest Oncology Group, SWOG) prospective randomized trial comparing surgery alone to surgery plus adjuvant vitamin A. This study design should allow a more definitive assessment of the adjuvant role of vitamin A in melanoma therapy.

Although the results of these studies are modest, the observed responses are encouraging in light of the drug-resistant nature of metastatic melanoma. Our current study utilizing isotretinoin and rαIFN in a two-armed randomized trial and other clinical trials employing combination therapy will be necessary to clarify the proper role of vitamin A and its synthetic derivatives in melanoma.

### *Mycosis Fungoides*

Mycosis fungoides (MF) is an uncommon T-cell lymphoproliferative disorder, which presents mainly in the skin. The predominant lesions are cutaneous plaques and tumors consisting of lymphocytic infiltrates. This disorder can progress to lymph node and visceral involvement, which may lead to death. Current therapies for the management of MF employ any one of several modalities, including immunotherapy, electron-beam radiation therapy, photochemotherapy (psoralen and u.v. A [PUVA]), topical chemotherapy

or systemic chemotherapy. The efficacy of these treatments depends greatly upon the disease stage and extent of extracutaneous involvement. These therapies often cause considerable toxicity, discomfort, inconvenience and expense. Retinoids have been utilized as single agents or in combination with other modalities in the treatment of mycosis fungoides and some impressive results have been reported.

Retinoids as single agents have demonstrated important therapeutic activity. In a phase II trial at the University of Arizona, 25 patients with MF were evaluated after treatment with isotretinoin (1–2 mg/kg/day) (Kessler *et al.*, 1983; Kessler *et al.*, 1987). Three complete and 8 partial responses occurred in this series. Response durations ranged between 1.5 and 24 months, with a median of 8+ months. A minor response occurred in six patients who experienced a decrease in plaque infiltration and erythroderma. As with most disorders, it appears that maintaining MF responses requires continued administration of the retinoid. In a study by Molin *et al.* (1985), 19 out of a series of 28 patients with early to advanced MF had objective responses to isotretinoin. Treatment consisted of isotretinoin (0.1–2.0 mg/kg/day for 2 weeks–9 months). Claudy *et al.* (1982) treated a patient with advanced mycosis fungoides with oral etretinate (1 mg/kg/day) for 4 months. After 1 month, clinical improvement occurred and was sustained by continued treatment. Three weeks after stopping the drug, relapse occurred. Histologic changes corresponded with clinical improvement and then relapse. This response led to a further study by Claudy and Rouchouse (1985) treating parapsoriasis-en-plaque with etretinate. Eleven evaluable patients experienced pathologic clearing of intraepidermal infiltrates. Warrell *et al.* (1983), of Memorial Hospital, reported excellent responses in three of seven MF patients treated with isotretinoin. Treatment consisted of 100 mg/m$^2$ of isotretinoin daily. One patient experienced complete clearing of both erythroderma and an enlarged lymph node. The other two responders exhibited a 50% clearing of plaque lesions, one for 8 months and the other for 7+ months. These responses occurred in plaque-stage disease of erythroderma and lasted 7–9 months. Responses were not noted among four patients with tumor-stage mycosis fungoides.

Neely *et al.* (1987) recently reported six patients treated with isotretinoin (1–2 mg/kg/day) who previously had failed steroid therapy or other forms of standard treatment for MF. Responses occurred within two to eight weeks after starting isotretinoin therapy, and included decreased skin lesions and alleviation of pruritis. The duration of treatment ranged from 8 to 12+ months. No patient experienced a complete response, although all patients had decreased cutaneous erythema and induration. Two other responses of particular interest were a decreased leukocyte and Sezary cell count in one patient with circulating Sezary cells and complete nodular flattening in one patient with early nodular disease. Maintained symptomatic palliation in these patients required continuation of isotretinoin therapy. One patient developed a nodular lesion after 8 months of treatment and had to be removed from study. Mahrle *et al.* (1983) used the arotinoid ethyl ester Ro13-6298 (given parenterally) to treat five patients with mycosis fungoides. Three complete responses and one partial response were observed in patients with plaque- and tumor-stage disease. One patient with Sezary's syndrome exhibited no response.

Jenkinson and Beare (1984) also described one male patient with refractory Sezary's syndrome. His etretinate treatment (1 mg/kg/day for 3 months) produced little improvement and was stopped because of severe cheilitis and peeling skin. This patient had failed whole-body irradiation and experienced an early relapse after wide-field electron beam therapy. Subsequently he was treated with isotretinoin (2 mg/kg/day), also without response. Wargon and Downie (1984) reported a patient with refractory Stage II mycosis fungoides, who was started on oral etretinate (2 mg/kg/day) after failing steroid, cytotoxic and PUVA therapy. This patient had a complete response to the retinoid. After 3 months of therapy, he was placed on a maintenance dose of 1 mg/kg/day. Skin biopsies of previously involved disease sites remain free of atypia, and the patient remains asymptomatic at 5-months follow-up. Ippolito and Giacalone (1982) treated a case of Stage III MF with etretinate (0.75 mg–1.5 mg/kg/day). Regression of the infiltrated plaques and

tumoral masses began after 20 days of therapy. This patient experienced complete resolution of cutaneous manifestations after 45 days of treatment and maintained an excellent response at six months follow-up.

Fitzpatrick and Mellette (1986) reported a patient with treatment-resistant mycosis fungoides who had a complete remission of skin lesions after 6 months of treatment with isotretinoin (1 mg/kg/day). The patient remained free of disease at 16 months follow-up after stopping therapy. Tousignant *et al.* (1987) recently described the efficacy of Ro13-6298 in the treatment of a MF patient. Although this patient had experienced a significant response with prior therapies, some of which incorporated etretinate, he was unable to tolerate the toxicity associated with these treatments. After an initial two-month trial of the arotinoid Ro13-6298 at a dose of 0.002 mg/kg per day, the patient showed no evidence of MF on clinical or histological examination. The therapeutic effect in this patient also required maintenance therapy to avoid relapse. This study and Mahrle's work suggest that the arotinoids are more effective than isotretinoin or etretinate in mycosis fungoides (Mahrle *et al.*, 1983).

Several combination studies have also investigated the effects of retinoids in MF. Souteyrand *et al.* (1981) treated seven patients with etretinate, either alone (two patients) (1 mg/kg/day, which was reduced after 1 month to 0.7 mg/kg/day) or in combination with PUVA (five patients). One of the patients receiving only etretinate experienced clinical improvement without documented histologic change. The other single agent-treated patient did not exhibit treatment-induced improvement at one-month follow-up. Of the five patients who received etretinate plus PUVA, four improved, including one complete response. The fifth patient with Sezary's syndrome had no improvement. Three patients in this series with parapsoriasis en plaques, a precursor to MF, were also treated with etretinate as a single agent. Two of these patients' lesions disappeared, after three months of treatment in one case and four months in the other.

Zachariae *et al.* (1981) reported treatment results using combined modalities in 16 patients with advanced MF. The treatment modalities combined in varying ways were etretinate, PUVA, prednisone, transfer factor and topical and systemic cytotoxic agents. Ten patients received combinations including etretinate and six did not. Results in the etretinate group included eight complete and two partial responses. Of the six non-retinoid treated patients, four had partial responses and two had disease progression while on therapy. Although the responses of etretinate-treated patients in this study are impressive, the contribution of the retinoid is obscured by the other treatments also employed. In studies by Hunzikar *et al.* (1983) and Rusciani *et al.* (1980)—both of which combined methoxypsoralen, PUVA and etretinate—patients experienced a decrease in lesions.

Future work with mycosis fungoides should focus on the potent arotinoids and therapies combining retinoids with other agents and modalities in the treatment of early and advanced disease (including Sezary's syndrome). We have now begun a phase II study to determine the efficacy of combining isotretinoin and recombinant alpha interferon. Also we have started a very promising phase I–II trial combining whole-body electron beam radiation therapy with isotretinoin. This second study is designed to assess the feasibility and efficacy of combining mucocutaneous-active agents.

## FUTURE DIRECTIONS

Clinical studies have 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, including oral leukoplakia, bronchial metaplasia, laryngeal papillomatosis, cervical dysplasia, preleukemia, superficial bladder cancer, and acute promyelocytic leukemia (Lippman *et al.*, 1987). The future of retinoids depends in part on clinical testing of the retinamides such as 4HPR and the arotinoids such as Ro13-6298. Studies of these drugs with their higher therapeutic indices, and on testing combinations of retinoids with other biological response modifiers (including other retinoids with different spectra of activity and toxicity),

cytotoxics, and other modalities are important areas for future clinical research. Unfortunately, the rush to institute combination trials has led to poorly designed, uncontrolled studies. Many centers are also planning to use retinoids as adjuvant and neoadjuvant therapy in cutaneous malignancies such as squamous and basal cell carcinoma. 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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