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Controlled Clinical Trials with Fenretinide in Breast Cancer, Basal Cell Carcinoma and Oral Leukoplakia

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Abstract We are conducting three randomized studies (breast cancer, basal cell carcinoma, oral leukoplakia) and report our methodological approach and accrual here.

The aim of the breast cancer study is prevention of a contralateral primary lesion in women already treated for breast cancer; the aim of the basal cell carcinoma study is prevention of recurrences or new occurrence after surgical resection; and the aim of the oral leukoplakia study is prevention of recurrences and new occurrence after CO_2 laser resection. The studies were planned according to a randomized design with an intervention arm *vs* a no-treatment arm. Patients in the intervention group receive 4-HPR at a dose of 200 mg po. The duration of treatment is five years in the breast cancer study, and one year in the basal cell carcinoma and oral leukoplakia studies. The breast cancer study started in March 1987, closing accrual on July 31, 1993. A total of 2,972 patients entered the study; 2,849 were evaluable (1,422 in the 4-HPR group and 1,427 in the control group). Of 2,849 evaluable patients, 867 completed the first five years, 1,142 are still ongoing, and 840 patients have interrupted the study for various reasons. Follow-up is ongoing. The basal cell carcinoma study started in January 1990. As of January 1994, a total of 786 patients had entered the study; 760 were evaluable (363 in the 4-HPR group and 367 in the control group). Of 760 patients in the study, 568 completed the first year, 62 are ongoing and 130 discontinued for various reasons. The study is ongoing. The oral leukoplakia study started in September 1988, closing accrual on February 1, 1994. A total of 174 patients entered the study; 170 were evaluable (84 in the 4-HPR group and 86 in the control group). The preliminary data of this study have been published. Updated results as of June 1994 were 11 recurrences and three new occurrences in the 4-HPR group; the control group also had 11 recurrences, as well as 12 new occurrences. Follow-up is ongoing.

Key words: 4-HPR, chemoprevention, fenretinide

Chemoprevention has been extensively studied during the last decade, and literature in this field has expanded considerably [1]. Retinoids are an important class of chemical compounds potentially able to inhibit or reverse the process of carcinogenesis. The main factor limiting their clinical use is toxicity, predominantly involving skin and mucosae (dryness, desquamation, peeling, pruritus, urticaria, dermatitis, cutaneous photosensitivity, *etc.*), liver (hepatotoxicity and enhancement of collagen formation), skeleton (ligament calcification, skeletal hyperostosis, decreased bone mass, *etc.*), CNS (headache, ataxia, neuropsychiatric changes), and abnormalities in serum lipids that increase risk of arteriosclerosis and its complications.

Fenretinide [*N*-(4-hydroxyphenyl) retinamide (4-HPR)], a synthetic retinoid described first by Moon *et al.* [2], has proven to be less toxic [3] and

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less teratogenic [4] than other retinoids. 4-HPR does not cause ligament calcification, skeletal hyperostosis or decreases in bone density; it does not cause adverse CNS reactions, modify lipoprotein levels, or accumulate in the liver, thus avoiding hepatoxicity. However, it does cause dermatological side effects, although they are tolerable even after more than three years of administration. It may also lead to impaired dark adaptation, attributed to a reduction of serum retinol. Impaired dark adaptation is dose-related, reversible upon drug interruption, and the incidence is low at a daily dose of 200 mg [reviewed in 5–7].

Our interest in chemopreventive studies at the Istituto Nazionale Tumori of Milan has focused in recent years on three controlled clinical trials with 4-HPR *vs* control: the breast cancer study aimed at preventing a contralateral primary in women already treated for breast cancer; the basal cell carcinoma study aimed at preventing recurrence or new occurrences after surgical resection; and the oral leukoplakia study aimed at preventing recurrence or new occurrences after CO_2 laser resection.

The studies were planned according to a randomized design with two arms: intervention versus no treatment. Patients in the intervention group were treated with 4-HPR at a daily dose of 200 mg po (a dosage that results in minimal adverse effects based on the data of the Phase I study) [reviewed in 5–7]. Since 4-HPR lowers plasma retinol levels [8], and vitamin A deficiency is associated with impaired dark adaptation, the three trials incorporated a three-day drug holiday at the end of each month to allow serum retinol concentrations to recover. Furthermore, it was recommended that 4-HPR be taken after a meal when absorption is higher.

Treatment duration was five years in the breast cancer study and one year in the basal cell carcinoma and oral leukoplakias studies. The five-year treatment duration in the breast cancer study was based on the assumption that the possible efficacy of 4-HPR to halve the risk of contralateral breast cancer may be expected from the third year on (three-year lag time). The one-year treatment durations in the basal cell carcinoma and oral leukoplakia studies were based on the fact that most recurrences in both diseases occur within 18 months from initial treatment.

All three studies use the same methodology. After surgery, eligible patients are invited to participate in the study. If they agree to participate, they sign an informed consent containing all information concerning the trial, and are then randomized by the data center. Chemopreventive treatment starts from the day of randomization.

BREAST CANCER STUDY

Breast cancer is the most common malignant tumor in women of Western countries, with a continuously rising incidence. In Italy (Lombardy Tumor Registry), the incidence rate is 85.9 per 100,000 women per year. The incidence curve rises suddenly at age 45 and at age 70. Breast cancer is the primary cause of cancer death in women 35–55 years old. Early diagnosis and improved treatment are known to increase survival rates, but reducing incidence would be even better, and chemoprevention appears promising.

The high incidence of breast cancer and the above mentioned characteristics of 4-HPR led one of us (U. Veronesi) to the idea of using this compound to prevent contralateral primaries in women already treated for breast cancer whose risk is 0.8% per year within ten years from primary treatment. The final objective is obvious. If 4-HPR can succeed in preventing second primaries in breast cancer patients, it could possibly be useful for a wider group of subjects with high breast cancer risk. The study was supported by a grant from the US National Cancer Institute. Study design and methodological approach have been previously reported [10]. Study participants were breast cancer patients aged 30–70 years treated with ablative or conservative (plus radiotherapy) surgery for T1 (less than 2 cm) or T2 (less than 5 cm) tumors, and all infiltrating types without nodal metastases. In order to be eligible, patients had to have no evidence of local recurrences and/or distant metastases, and no previous treatment with adjuvant chemotherapy and/or hormone therapy. They had to have normal metabolic and liver function tests, and avoid pregnancy during the study. Excluded were those with contemporaneous or previous neoplastic disease (with the exception of basal cell carcinoma of the skin and cervical intraepithelial neoplasia), those with lobular carcinoma *in situ* (LCIS) or Paget's disease, those with geographic inaccessibility, neuropsychiatric difficulties, and those who were familiar with physicians or par-

ticipating in another study. The same applied to patients with ocular or other concomitant diseases. Patients with intraductal carcinoma were considered as protocol deviations and were admitted to the study. Patients were able to enter the study through one of two mechanisms: those identified as potentially eligible through a review of the medical records, and those operated on after March 1, 1987 and proven eligible (Fig. 1).

The following tests are performed at baseline: general objective examination, dermatologic examination, ocular questionnaire, laboratory findings (HB, HT, WBC, PLTS, SGOT, SGPT, BIL, AP, total proteins, BUN, creatinine, blood sugar, cholesterol, triglycerides, retinol blood levels), pregnancy test (for those in childbearing years), mammography, chest X-ray, bone scan, and liver echography. The ocular questionnaire is considered positive if at least two out of three items were positive. If the test showed normal visual function, the subject was considered eligible.

The ocular questionnaire was performed at each control checkpoint. Both groups, intervention and control, were followed with the same tests: physical examination and laboratory determinations every six months, mammography, chest X-ray and liver echography every year, and bone scan at 18, 36 and 60 months. 4-HPR and retinol levels were determined each year in all treated patients.

The sample size was calculated using the procedure discussed by Wu [9], and assuming a three-year lag time needed to obtain full intervention efficacy and a two-year follow-up of all patients after the end of intervention. The total sample size was established at 3,500 subjects for an expected 50% reduction of the incidence rate of contralateral breast cancer and a drop-out rate of 10% [10].

Accrual closed on July 31, 1993, with a total of 2,972 randomized patients (1,496 in the 4-HPR group and 1,476 in the control group). The study closed because Italy introduced neoadjuvant chemotherapy which reduced accrual and because

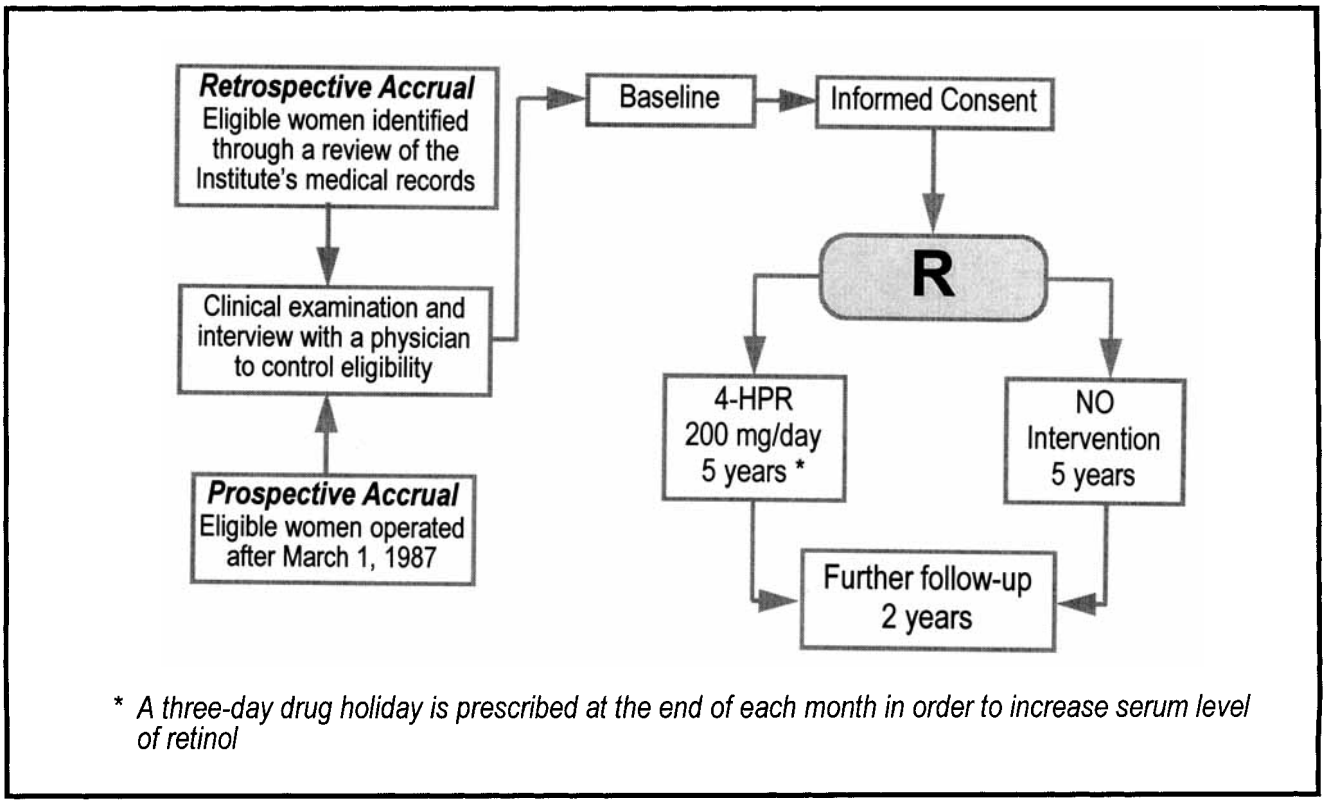

Fig. 1. Design of the 4-HPR breast cancer study.

the NCI Medical Alert recommended adjuvant treatment in all cases (positive or negative axillary nodes). Entry criteria violations were found in 68 patients (2.2%). Twenty-six patients (0.8%) refused to enter the study after signing the informed consent and 29 patients (1.0%) were lost early to follow-up. The total number of evaluable cases is 2,849 (1,422 in the 4-HPR group and 1,427 in the control group). The two groups are well-balanced for menopausal status, primary tumor treatment, histology and tumor size. Of 2,849 evaluable patients, 867 completed the first five years, 1,142 are still ongoing, and 840 patients have interrupted the study for various reasons. A first analysis will be made after June, 1995.

BASAL CELL CARCINOMA STUDY

Basal cell carcinoma (BCC) typically occurs in areas of the body exposed to sunlight. Eighty to 90% of all BCCs occur in the head and neck area, 60% of those at the midline of the face. The natural history of BCC is characterized by local malignancy and high new occurrence and recurrence rates. Approximately 20% of patients develop a local recurrence and 30–40% develop a new BCC within five years [reviewed in 11]. The plurifocality in space and time is the rationale for a chemopreventive post-operative treatment. This is particularly true for lesions on the face in which surgical re-treatment could cause considerable esthetic damage.

During the last decade, several studies have been carried out with different vitamin A derivatives, with controversial results [reviewed in 11]. On the basis of the above-reported data, our clinical trial attempted to evaluate the activity of 4-HPR in preventing recurrences and new occurrences after radical excision. The design of the study is shown in Figure 2. After radical surgery (surgical excision at 3 mm from the macroscopic

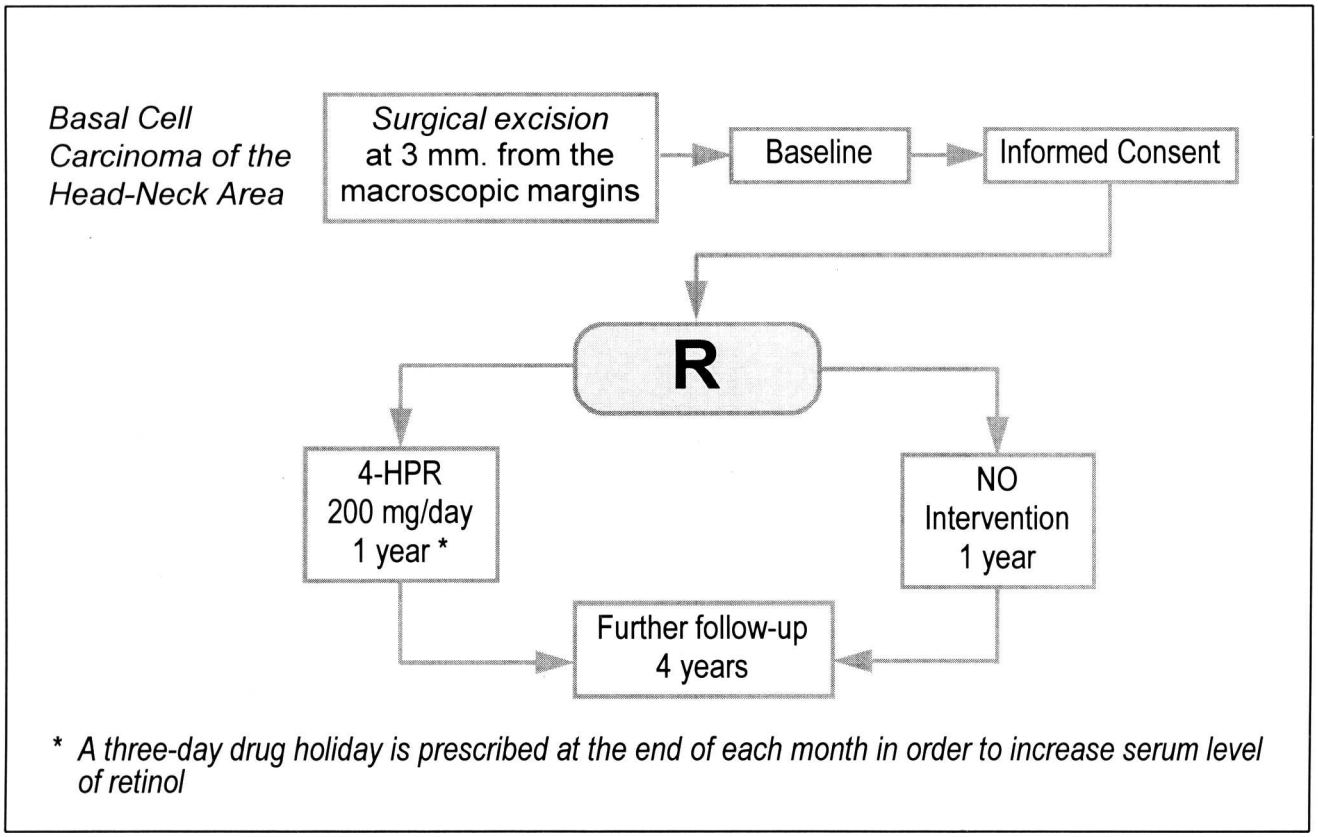

Fig. 2. Design of the 4-HPR basal cell carcinoma study.

margins), patients with a histological diagnosis of BCC were randomized in two arms; 4-HPR for one year *vs* control. Clinical checks and laboratory examinations were performed every six months in both groups during the year of the study. Patients were clinically followed for five years after surgery.

The sample size was calculated assuming a 10% decrease in the probability of an occurring event, with a test efficacy of 0.90 and a significance level of 0.05 (unilateral test). The global required sample size would be about 420 patients for local recurrences, and 720 patients in the case of new occurrences. Since local recurrences and new BCC represent both endpoints of this study, it was decided to accrue 720 patients. However, as the drop-out rate in the course of study is estimated at 20%, recruitment will include as many as 900 patients.

Eleven hospitals participated in the multicentric study starting in January, 1990. By June 30, 1994, a total of 1,252 patients with BCC of the head and neck area had been registered. Three hundred sixty-seven of those were ineligible for various reasons (most for age >80 years, previous neoplastic or cardiovascular diseases), leaving 885 eligible patients. After excluding 69 patients who refused to enter the study, 816 patients were randomized. There were 13 protocol violations and 43 patients lost after randomization, leaving 760 evaluable patients (381 in the 4-HPR group and 379 in the control group). The two groups are well-balanced for clinical characteristics. Of 760 patients in the study, 568 completed the first year and 62 are ongoing. The study was discontinued for various reasons in 130 patients. The study is ongoing and patient entry should be completed in about one year.

ORAL LEUKOPLAKIA STUDY

Leukoplakia is a general term used to describe whitish lesions of the oral mucosa referred to as having an undefined histology. For this reason the World Health Organization Collaborating Center for Oral Precancer defined leukoplakia "as a white patch or plaque that cannot be characterized clinically or pathologically as any other disease" [12]. Leukoplakia is considered a precancerous lesion of the oral cavity; its prevalence varies according to diet and alcohol-tobacco consumption and the clinical picture is not related to the anatomo-pathological one. Recurrences and new occurrences are frequent; invasive carcinoma develops from leukoplakia in about 5–15% of cases. The high recurrence and new occurrence rates and the possibility of developing invasive carcinoma justify chemopreventive trials.

In several non-randomized studies performed in the 1970s–1980s, retinoids have sometimes proven to be efficacious in treating precancerous lesions of the oral cavity [reviewed in 1]. Randomized studies carried out in the 1980s confirmed the activity of retinoids in oral leukoplakia [13,14].

Our study aimed to evaluate the efficacy and tolerability of continuous 4-HPR administration in preventing recurrences and new occurrences of oral leukoplakias after CO_2 laser resection. Study participants had previously untreated homogeneous and non-homogeneous leukoplakia [12]. To be eligible, patients had to be younger than age 75, with no previous or concomitant severe disease (neoplastic, cardiovascular, neurophychiatric, sexually transmitted diseases, *etc.*). Patients had laser microsurgery. The lesion, stained by toluidine blue, was removed under local anaesthesia with at least 0.5 cm margins (in depth and laterally) of normal tissue. After laser surgery, patients without dysplasia who were identified as eligible were asked to sign the informed consent form, then randomized into either the 4-HPR or the control arm for one year (Fig. 3). Both intervention and control patients were asked to stop drinking alcohol and/or smoking, and to carry out dental care; all were given the same examinations and procedures every two months. Patients were followed every three months during the second year, every four months during the third year, and subsequently every six months. The entire duration of follow-up after the year of intervention was four years. Our previous experience [15] showed that the probability of developing recurrences and new occurrences was 40% after three years and 23% within one year of surgery. Assuming a 15% difference in the two arms concentrated in the first year after surgery, 190 patients were needed to detect the difference (α=5%, β=20%, 2-tailed test).

The study started in September 1988 with four hospitals participating; randomization was closed on February 1, 1994, with a considerable reduction in accrual. A total of 307 patients were recruited. One hundred (33%) were ineligible for

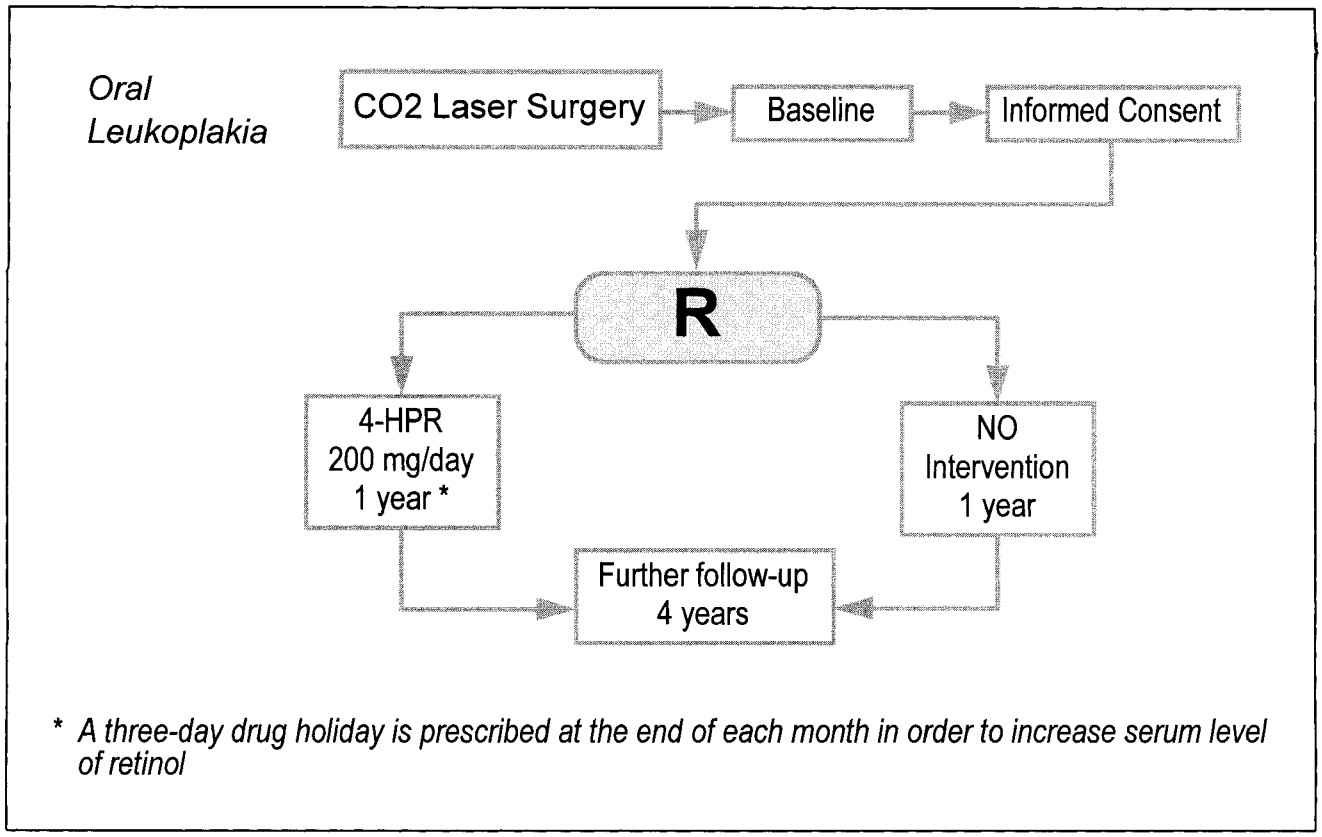

Fig. 3. Design of the 4-HPR oral leukoplakia study.

various reasons (most for synchronous or previous neoplastic diseases or other severe diseases) and 207 were eligible. Since 33 patients refused, 174 patients entered the study, and 170 were evaluable (84 in the 4-HPR group and 86 in the control group). The two arms are well-balanced for clinical characteristics such as age, sex, number and type of lesions, smoking and alcohol habits. The preliminary data of this study have been published [5,16]. The updated results are reported here. By June 30, 1994, 37 of 84 patients (44%) in the 4-HPR group discontinued treatment for various reasons: 19 for complaints, 10 for refusal, 2 for intercurrent events, and 6 for unfavorable events. Compliance with 4-HPR treatment was good: 92% of patients who completed treatment had a compliance between 90–100%. Besides the three new primary tumors in other sites (one in the 4-HPR group and two in the control group), there were 11 recurrences in the 4-HPR group and 11 in the control group; three new occurrences in the 4-HPR group and 12 in the control group. Eight of the 14 unfavorable events in the 4-HPR group occurred after the end of intervention. The median time of unfavorable events after one year of intervention was 32.5 months (range 15–42 months). The first relapse occurred three months after the end of intervention; however, no more unfavorable events appeared for another 25 months.

CONCLUSIONS

Cancer chemoprevention trials are difficult to implement since they require a large number of participants, a long follow-up period, and a high compliance rate. This is particularly true when healthy subjects at high risk for malignancy are compared to healthy subjects at normal risk, and when the endpoint of the study is tumor incidence. But it is also true when the population under study is different from the usual preven-

tion study population. The subjects in our studies are patients with previous treatment for breast cancer, basal cell carcinoma of the head and neck area, or oral leukoplakia. The endpoints are the prevention of contralateral breast cancer, and the recurrence or new occurrence of basal cell carcinoma or leukoplakia. Also in these cases, approaching eligible subjects is not always an easy matter, accrual takes a very long time, the drop-out rate is high, and the follow-up period is very long. For these reasons, no final results are yet available on the efficacy of 4-HPR in the prevention of contralateral breast cancer and recurrences or new occurrences of basal cell carcinoma. Data from the oral leukoplakia study, however, seem to deserve special attention as regards 4-HPR efficacy.

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