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LUNG CANCER CHEMOPREVENTION WITH RETINOL PALMITATE

Preliminary data from a randomized trial on stage Ia non small-cell lung cancer

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Abstract

On the basis of epidemiologic and experimental evidence of an anticancer activity of vitamin A, a randomized clinical trial was activated in Milan with the aim of evaluating if retinol palmitate administration (per os, 300 000 I.U. daily) after complete resection of stage Ia non small-cell lung cancer could reduce the occurrence of cancer relapses (within 3 years) and/or the occurrence of new primary tumors (beyond 3 years). By September 1987, 181 patients had entered the trial: 87 in the treatment arm and 94 in the control arm. After a median follow-up of 14 months, the interim analysis was focused on the evaluation of toxicity, compliance, and early recurrences. Although the large majority of patients were affected by skin and mucous membrane desquamation and dryness during treatment, these symptoms were generally mild and well tolerated, and never induced the patient to stop the treatment. Other side effects like headache, hair loss, itching, or dyspepsia were detected at a much lower frequency. Only in 3 patients the treatment was interrupted, because of signs or symptoms potentially related to vitamin A administration. At the time of the analysis, a total of 42 (23%) patients had relapsed; 16 (18%) in the treated arm, and 26 (28%) in the control arm. The largest difference between treated patients and controls was observed for bone metastases (2 vs. 7) and brain metastases (3 vs. 6), and for squamous histology (6 vs. 11). Only 2 cases of new primary cancer were detected, both in the control arm. These results are promising both in terms of tolerance and efficacy of treatment, but given the short median follow-up they must be very cautiously interpreted. A longer follow-up is necessary to establish whether a significant proportion of early recurrences could be prevented, or only delayed, by vitamin A administration.

Key words: Lung neoplasms; non small-cell lung cancer, chemoprevention, retinol.

Chemoprevention of human cancer, i.e. prevention of cancer by the use of pharmacological agents to inhibit or reverse the process of carcinogenesis (70), represents a new promising field of clinical oncology. Data are accumulating on the intrinsic characteristics of human carcinogenesis, and on several agents potentially able to interfere with the various steps of such a process (5). Although the major goal for lung cancer prevention is abstention from smoking, reversal or antagonism of promotion/progression steps may help in reducing lung cancer incidence and mortality in subjects previously exposed to tobacco. Curatively treated lung cancer patients, at high risk of developing new malignancies (20, 51, 66), are an ideal model to test chemopreventive agents, especially substances like retinol, whose anticancer activity might also be tested in an adjuvant setting.

Since the definition of chemical structure of retinol (32), vitamin A has been widely investigated. It is now well known that in higher animals retinol is essential for vision, reproduction, and maintenance of differentiated epithelia and mucus secretion (23, 84). Trans-retinoic acid shares only a part of these functions, being unable to support vision and reproduction and so animals maintained on retinoic acid as the only source of vitamin A are both blind and sterile (17, 76). Due to their peculiar differentiation properties, vitamin A and retinoids were tested as potential anticarcinogenic agents in virtually all the systems available. The literature on the experimental activity of vitamin A and retinoids has been the subject of several extensive reviews (39, 47, 70), exploring all the various mechanisms of action of these substances. Basically, we can summarize this large experience in 6 points: enhancement of the physiological mechanisms of cell differenti-

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Table 1

Results of experimental lung cancer chemoprevention in animals

Animal/carcinogen	Substance	Cancer incidence (treated/controls %)	Reference
Rats/trach. 3-MCH	Retinyl-A	Reduced (19/54)	Cone et al. (13)
Hamsters/trach. BP	Retinyl-P	Reduced (11/32)	Saffiotti et al. (62)
Rats/trach. 3-MCH	Retinyl-A	Reduced (3/42)	Nettesheim et al. (50)
Hamsters/trach. BP	Retinoic A	Unchanged	Smith et al. (68)
Hamsters/trach. BP	13-cis-RA	Reduced (1/10)	Port et al. (59)
Animals/trach. MNU	13-cis-RA	Unchanged	Yarita et al. (87)
Animals/trach. MNU	13-cis-RA	Increased (20/6)	Stinson et al. (72)
Animals/trach. MNU	4-HPR	Unchanged	Grubbs et al.(24)

ation (18, 71, 85), inhibition of malignant transformation (7, 11, 12, 50, 70), suppression of tumor promotion (6–8, 40, 81), inhibition of sarcoma and epidermal growth factors (22, 77), enhancement of cell-mediated (39, 46, 74) and antibody-mediated (15, 19) immune response to cancer, and direct action against neoplastic cells (34, 38, 78, 79). Such a broad spectrum of biological activity suggested a potential effect on all stages of carcinogenesis, as well as on early cancer, and made vitamin A and retinoids the most promising chemopreventive agents.

A summary of lung cancer chemoprevention studies in animals is shown in Table 1. It is evident that in systems where indirect-acting carcinogens (benzo-pyrene, methylcholantrene) were employed, retinol and its derivatives were both active (13, 50, 59, 62). While in experiments using a direct-acting carcinogen like N-methyl-N-nitrosourea, syntectic retinoids were inactive (24, 72, 87). These data are compatible with an essential anti-promotion effect of vitamin A. From the clinical point of view, the dynamics of tumor promotion are especially interesting, since the possibility of interfering with 'late-stage' carcinogenesis is likely to provide a more rapid and effective path to human lung cancer chemoprevention.

Many epidemiological investigations have tried to correlate such experimental evidence with human cancer risks. New studies have been conducted on the association between serum and dietary levels of vitamin A and subsequent risk of cancer, while older studies have been re-analyzed with this specific purpose. In contrast with other sites, for lung cancer there is now a considerable amount of data in support of the hypothetical anticancer activity of vitamin A. Six out of 9 studies on serum retinol, obtained from a recent review of the literature (52), have shown a lower mean value in lung cancer patients, ranging from 1 to 19% of the corresponding value in the controls. On the other hand, the 3 studies available on serum beta-carotene all show a higher risk of cancer related to low beta-carotene levels in the blood (52). Case-control studies on dietary intake of vitamin A and lung cancer are even more impressive: with a broad spectrum of dietary sources and over 4000 cases examined, an average 1.5 to 2-fold increase in the risk of cancer has been associated to low vitamin A intake (52, 58). Theoretically, it may be estimated from the relative risks observed in heavy smokers that 25% of lung cancers are related to low levels of vitamin A (14). So far, the hypothesis of a higher risk of lung cancer related to low vitamin A consumption is strongly supported by the whole epidemiological experience. As far as the clinical anticancer activity of vitamin A is concerned, the most convincing data come from skin cancer. A summary of published results is reported in Table 2. These data clearly demonstrated that retinoids, either topically or orally administered, were able to induce complete remissions in a high proportion of patients with basal cell and advanced squamous cell carcinoma. In other human cancers, the results were by far less convincing. The available data on head and neck or lung cancer treatment are summarized in Table 3. Objective results are difficult indeed to evaluate since most studies were not randomized, retinoids were very often combined with other treatments like surgery or radiotherapy, or given to patients with advanced tumors where it was unlikely to obtain any response. The only conclusion we can draw from this heterogeneous experience is that these compounds deserve better clinical evaluation with an optimal model, i.e. randomized setting, adequate doses, and proper population.

The limiting factor in vitamin A administration for either treatment or prevention are the accompanying side effects. During the last 20 years chemical laboratories have made an enormous effort to give rise to original compounds being more active and less toxic than natural vitamin A. So far, over 1500 new synthetic retinoids have been produced and biologically tested since 1968 (9). Classical biological assays included in vitro reversal of tracheal keratinization on hamsters raised on vitamin A-deficient diet (69) and in vivo ability to reverse skin papillomas in mice (81). According to the model of therapeutic index, defined by Bollag as the ratio between dose causing hypervitaminosis A and dose causing antipapilloma effect

Table 2

Clinical activity of retinoids in basal cell and advanced squamous cell carcinoma of the skin

		No.	CR%	PR%	Reference
Basal Cell					
Retinoic A	Topical	16	42	58	Belisario (3)
Retinoic A	Topical	12	31	62	Bollag (9)
Retinoic A	Topical	15	13	87	Sankowski et al. (63)
13-cis-RA	Oral	11	16	65	Peck et al. (54)
13-cis-RA	Oral	4	0	25	Meyskens et al. (45)
13-cis-RA	Oral	5	0	40	Lippman et al. (37)
Etretinate	Oral	20	10	35	Beretti et al. (4)
Advanced squamous cell					
13-cis-RA	Oral	5	0	60	Meyskens et al. (45)
13-cis-RA	Oral	9	11	33	Lippman et al. (37)
Etretinate	Oral	4	25	25	Grupper et al. (25)

Table 3

Clinical activity of vitamin A and retinoids in head and neck and advanced lung cancer

		No.	CR%	PR%	Reference
Head and neck					
13-cis-RA	Single agent	19	0	16	Meyskens et al. (45)
13-cis-RA	Single agent	19	5	5	Kessler et al. (33)
Retinol	Combination	33	Improved survival		Komiyama et al. (35)
Retinol	Combination	25	Symptomatic		Thatcher et al. (75)
Advanced lung cancer					
13-cis-RA	Single agent	5	0	0	Saccomanno et al. (61)
13-cis-RA	Single agent	22	0	9**	Lippman et al. (37)
Retinol	Combination	9	11	0	Micksche et al. (46)
Etretinate	Combination	46*	No effect		Weber et al. (82)

* randomized trial. ** minor responses.

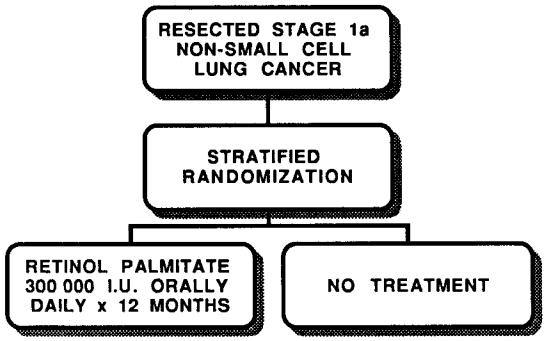

Figure. Design of the trial.

in mice, 13-cis-retinoic acid, etretinate, and arotinoid ethyl ester appeared the most active compounds. In this animal model, those were significantly more active than retinoic acid in terms of absolute concentration, with an increasing therapeutic index from 0.2 to 2.0 (9).

Unfortunately, these favorable data could not be translated into medical practice. Clinical application of synthetic retinoids was associated with a pattern of side effects superimposable to hypervitaminosis A, which very often prevented long-term administration at proper dosage (31, 55, 57). As a matter of fact, natural vitamin A, with or without beta-carotene, is currently being used in most chemoprevention studies on healthy populations (16, 65).

The present paper illustrates the design and preliminary analysis of the randomized trial on chemoprevention of stage Ia lung cancer with retinol palmitate, activated in 1985 at the Istituto Nazionale Tumori of Milan. The main objective of the study was to assess whether vitamin A administration was able to prevent the occurrence of a new primary cancer, and/or recurrence of previous disease. The interim analysis was focused on toxicity, compliance and early recurrences, in order to obtain additional information for the planning of a large cooperative study on head and neck cancer patients.

Material and Methods

The study design is summarized in the Figure. Eligible patients were randomized to treatment with retinol palmitate (300 000 I.U./daily per os for 12 months), or to control

without treatment. Assuming a risk of cancer relapse of about 30%, and a 5-year incidence of new primary cancers close to 20%, the dimension of the trial was set at 200 patients per arm. Patients were recruited from the Thoracic Surgery Departments of the Istituto Nazionale Tumori, Niguarda Hospital, and S. Carlo Hospital in Milan. Randomization was separated in the 3 centres, with stratification according to cell type (squamous vs. non squamous), and previous cancer at another site (absent vs. cured).

Eligibility criteria included non small-cell lung cancer in pathological stage Ia (T1–2, N0) according to the TNM classification (UICC, 1978), radical surgery within 2 months, age <75 years, normal liver and kidney function, incapacity or unwillingness to become pregnant, availability for a minimum 5-year follow-up, and informed consent. The histopathological diagnosis was carried out by pathologists from the respective centres according to the WHO criteria (1978). Patients with serious liver failure, acute or chronic hepatitis, serious ischaemic heart disease, glaucoma, persistent disease (R2), or previous chemotherapy were considered uneligible. The discovery of a microresidual tumor at the margin of the bronchial resection (R1) was not considered a reason for exclusion but those patients had to be submitted to adjuvant radiotherapy at this particular site.

Each individual centre was responsible for the recruitment and out-patient follow-up, according to a standardized procedure. A blood sample was taken from all subjects prior to randomization, in order to assess hemoglobin, hematocrit, WBC, RBC and platelet count, SGOT and SGPT, total bilirubin, alkaline phosphatase, total protein and total albumin, serum amylase, BUN, creatinemia, blood sugar, cholesterol and triglycerides. A complete urinanalysis was also performed.

During their stay in hospital or immediately thereafter in the out-patients' clinic, the base-line status of patients was assessed by physical examination, chest radiogram, sputum cytology, and ECG. Eligible patients were then informed about the aims and management of the study, asked to give their consent, randomized by means of a telephone call to the Data Centre, and eventually provided with the required dose of the drug until the next check-up session (one package per month = 10 million I.U.). Moreover, all subjects were interviewed about their past and present tobacco, alcohol and vitamin A intake and asked to refrain from smoking and—as much as possible—from alcoholic drinks.

The basic follow-up was focused on relapse of disease and occurrence of new primary tumors. Both groups were followed up every 4 months with chest radiogram, sputum cytology, liver, kidney and heart function tests, physical examination and assessment of symptoms, and a brief interview regarding current tobacco intake.

Furthermore, patients belonging to the treatment arm were evaluated for compliance and possible toxicity every 6 weeks during the first 4 months, and subsequently every 2 months up to 12 months. During these sessions the physical condition of the patient was assessed as well as symptoms, adherence to the protocol of drug intake, and results of liver and kidney function tests (SGOT, SGPT, alkaline phosphatase, bilirubin, BUN, creatinemia, cholesterol and triglycerides).

Table 4

Patients' characteristics according to treatment allocation

	Treated	Controls	Total
Age			
0–59	37	47	84
>60	50	47	97
Sex			
Male	82	84	166
Female	5	10	15
Type 1			
Squamous	39	45	84
Other	48	49	97
Previous Cancer			
Absent	83	86	169
Present	4	8	12
Resection			
Sublobar	4	9	13
Lobar	79	79	158
Pneumo	4	6	10
pT			
1	34	34	68
2	53	60	113
R1 (microresidual disease)	4	1	5
Total	87	94	181

A liquid preparation of emulsified retinol palmitate was chosen, in consideration of its absorption and activity features. The drug, which was currently on sale in Germany under the commercial name of 'A-mulsin', was supplied by Mucos Pharma GmbH & Co, Geretsried (Munich). It was planned to administer the drug per os in a daily dose of 300 000 I.U. for 12 months, in order to reach the total dose of 90 million I.U. Based on available data on prolonged administration of the drug at the same dose, mild side effects had to be expected in the majority of patients, essentially consisting in desquamation and dryness of the skin and mucous membranes. Mild subclinical hepatic toxicity (increased SGOT/SGPT) was likely to occur in a limited number of subjects; of a transient nature, and possibly disappearing without treatment suspension. Anyhow, a strict protocol for compliance and toxicity monitoring was activated.

Prior to treatment each patient was carefully informed about the drug features and possible side effects. They received a written memo with instructions for use, dosage and expected side effects. According to the personal hab-

Table 5

Prevalence of symptoms in patients treated with retinol

Time of treatment (months)	Any symptoms (%)			Subjective skin dryness (%)			Objective skin desquamation (%)		
	Light	Moderate	Severe	Light	Moderate	Severe	Light	Moderate	Severe
1	38	13	4	34	13	1	33	12	0
4	51	13	1	41	9	1	39	6	1
6	36	20	4	36	16	3	32	9	2
12	31	17	2	27	17	0	29	8	0

its of each subject, a line of conduct was recommended which favored optimal drug absorption. Drug intake and compliance were indirectly assessed during each check-up by means of a detailed interview and by checking quantity of the drug not used by the patient.

With respect to serum values of liver enzymes, obtained at the moment of entrance into the study, hepatic toxicity was defined as MILD (1.5–2 times the base-line level), moderate (2–3 times) or serious (>3 times).

All subjects showing liver enzyme abnormalities were placed under strict surveillance. In cases of mild toxicity a control was made every 2 weeks. If values had not reverted to normal, the dose was halved until the next check-up, and in any case until values were back at the base-line level. In cases of moderate toxicity the dose was halved until function parameters were back at the starting level. If no normalization was observed, or in case of worsening toxicity, the treatment was discontinued. In cases of serious or recurrent toxicity, administration of retinol palmitate was stopped.

Data managements for all patients (including randomization, collection, input, quality control, and analysis) were centralized at the Istituto Nazionale Tumori. A working group consisting of all researchers involved in the study met every 2 months to evaluate the status of the trial.

Between July 1985 and September 1987, 181 patients, 87 in the treatment arm and 94 in the control arm, had entered the trial and were eligible for interim analysis. Patient characteristics are summarized in Table 4. The 2 groups were comparable in age distribution, size and type of primary tumor. Median follow-up, at the time of the analysis, was 14 months. The number of patients evaluable at the different intervals, for the treatment arm and the control arm, were 87 and 94 respectively at the first month, 81 and 84 at 4 months, 71 and 73 at 6 months, and 54 and 55 respectively at 12 months.

Results

Nearly all treated patients experienced side effects related to the administration of retinol palmitate. In some patients they occurred earlier, usually 2–3 weeks from the beginning of treatment, and disappeared after 3–4 months of regular intake. In other patients the side effects occurred later, after 4–6 months of intake. So, the total prevalence of patients with any subjective or objective symptom at 1, 4, 6, and 12 months from the treatment onset, depicted in Table 5, ranged between 50 and 70%. In most cases the side effects were mild, with a tendency to spontaneous reduction of intensity during treatment. Serious side effects occurred in a minority of patients (below 5%), and were never troublesome. Table 5 illustrates the prevalence of dryness of the skin, as reported by the patient himself, and of objective desquamation, as detected by the physician, at the same follow-up periods. These were by far the commonest symptoms, and again they were only mild in most instances. The total incidence of other, less common side effects was itching (18%), dyspepsia (11%), bleeding (8%), nausea (7%), headache (5%), cutaneous erythema (4%), hair loss (3%), and atopic dermatitis (2%).

Table 6 illustrates the abnormalities observed in those serum values which were used to monitor vitamin A toxicity. The prevalence of liver enzyme (SGOT) elevation at 1, 4, 6, and 12 months from the treatment onset, is depicted in Table 6. From 15 to 27% of treated patients have shown SGOT elevation above the value of 40 i.u.; among these, up to 14% had values exceeding 70 i.u. Serum SGOT elevation was also observed up to 6–14% of controls. Similar figures were observed for SGPT and gamma-GT, and are not presented. Abnormal values for triglycerides are illustrated in Table 6. In treated patients the prevalence of hypertriglyceridemia increased constantly from 3% at the first month to 27% at the 12th month; the corresponding values for controls were 2% to 12% respectively. A similar pattern was evident for hypercholesterolemia (Table 6) with a maximum prevalence of 27% for treated subjects and 10% for controls.

The compliance of the treated group was indirectly assessed on the basis of a detailed interview on drug intake, and measurement of residual drug at each control. This figure was very high (>95%), and was in agreement with the high prevalence of side effects.

Table 6

Laboratory variables in retinol treated patients and controls

Time of treatment (months)	SGOT elevation (%)			Triglyceride elevation (%)		Cholesterol elevation (%)	
	>40 i.u. Retinol	>70 i.u.	>40 i.u. Controls	>150 mg/100 ml		>220 mg/100 ml	
				Retinol	Controls	Retinol	Controls
1	15	5	6	3	2	4	0
4	27	12	14	15	2	16	5
6	21	9	9	17	3	17	8
12	24	9	12	27	12	24	10

So far, 3 patients have stopped treatment because of signs or symptoms potentially related to vitamin A administration. One patient developed acute exacerbation of a pre-existing atopic dermatitis after 4 months of regular treatment. In this patient the acute symptoms disappeared soon after suspension of retinol. The second patient developed hemorrhagic proctitis after 8 months of treatment. Such clinical picture was probably unrelated to vitamin A and did not normalize after stopping retinol administration. The last patient developed acute type B viral hepatitis 4 months after treatment onset. Four months later, bone metastases became evident, and the patient eventually died with disseminated disease.

Table 7 summarizes the absolute number and relative frequency of recurrences, so far observed in the 2 arms after a median follow-up of 14 months, related to stratification parameters and other clinical features. A total of 42 (23%) patients have relapsed; 16 (18%) in the treated arm, and 26 (28%) in the control arm. Regardless of treatment assignment, recurrences were more frequent in non-squamous type (26% vs. 20%), in patients with previous cancer (33% vs. 22%), in pathologic T2 lesions (27% vs. 18%), and in case of microscopic residual tumor (R1) on the resection margin (40% vs. 23%). Within each of these subgroups the relative frequence of relapse was higher in the control arm compared to the treatment arm, with the only exception of R1 patients. The proportion of cases with histological confirmation was 45% in the treated arm and 56% in the control arm. As illustrated in Table 4, of 5 patients with microresidual disease, 1 was assigned to the control arm and 4 to the treatment arm. Two of these (50%) did not undergo the adjuvant radiotherapy, which was planned in the protocol, and developed a local recurrence at the site of bronchial stump 13 and 17 months respectively after lung resection. Considering the small group of patients where a previous cancer had been cured before the lung cancer resection, the relapse figures were 0/4 in the treated arm and 4/8 in the control arm.

Table 8 illustrates the site of recurrence in the 2 arms. The largest difference between treated patients and controls was observed for bone metastases (2 vs. 7) and brain metastases (3 vs. 6). In one of these patients, belonging to the treatment arm, brain metastases occurred immediately after randomization, and before any treatment was actually started. When the analysis was restricted to squamous cell carcinomas (Table 8), the observed differences were

Table 7

Number of observed recurrences in treated patients and controls, according to tumor type and extent, and occurrence of previous cancer

	Treated (%)	Controls (%)	Total (%)
Type			
Squamous	6 (15)	11 (24)	17 (20)
Other	10 (21)	15 (31)	25 (26)
Previous cancer			
Absent	16 (19)	22 (26)	38 (22)
Present	0	4 (50)	4 (33)
pT			
1	4 (12)	8 (24)	12 (18)
2	12 (23)	18 (30)	30 (27)
R1			
Absent	14 (17)	26 (28)	40 (23)
Present	2 (50)	0	2 (40)
Total	16 (18)	26 (28)	42 (23)

Table 8

Site of recurrences in the total series and in squamous cell carcinomas

Site	Total series		Squamous cell carcinoma	
	Retinol	Controls	Retinol	Controls
Bone	2	7	0	4
Brain	3	6	1	1
Lung	6	9	2	6
Other	5	4	3	0
Total	16	26	6	11

slightly larger (6 vs. 11), and became evident even for lung recurrences (2 vs. 6).

At the time of present analysis, only 2 cases of new primary cancer have been detected, both in the control arm. The first was a case of pulmonary adenocarcinoma, occurring 5 months after the previous squamous cell cancer. The other was a squamous cell carcinoma of the larynx, and occurred 15 months after primary lung cancer.

Discussion

There are two basic philosophies in the existing trials on chemoprevention of human lung cancer with vitamin A.

The first one, mainly inspired by epidemiological data, is aimed at interfering with the early phases of carcinogenesis, by counteracting a hypothetical vitamin A deficiency. Low doses of vitamin A and/or provitamin (beta-carotene) are administered, on a long-term basis, to otherwise healthy individuals at high risk of developing a lung cancer due to previous heavy exposure to smoking, asbestos, or other carcinogens. Doses are kept below the threshold of side effect onset, in order to obtain high recruitment and compliance rates, allow a double-blind setting, and reduce the risk of harmful effects for the population under treatment. This is the typical approach of NCI chemoprevention trials (5, 16).

The other philosophy, mainly inspired by clinical data, is aimed at interfering with the promotion and/or progression of cancer, by administering vitamin A as a true anticancer agent. High doses of vitamin A or retinoids are given on a short-term basis to patients at the highest risk of developing a new primary (or recurrent) lung cancer because of precancerous lesions or previously treated cancer. Doses are set at the therapeutic level for dermatological diseases, in order to obtain the highest pharmacological activity against cancer. Being the counterpart of an adjuvant treatment, side effects are better tolerated and easily monitored during the standard patient's follow-up. The present study belongs to the second group, and was designed to evaluate both the adjuvant and the chemopreventive activity of vitamin A.

Stage Ia lung cancer was considered an optimal model, on the basis of the following considerations. Prognosis of resected lung cancer has remained unchanged, and disappointingly low, over the last 20 years, despite all attempts to improve long-term survival by early diagnosis (20), adjuvant chemotherapy (26), radiotherapy (41, 80), or immunotherapy (30). Recent data from the Lung Cancer Study Group have opened new prospectives for multimodality treatments, showing a definite improvement on relapse-free interval in stage II and III lung cancer with adjuvant polichemotherapy (27, 28), but a longer follow-up is mandatory to prove a long-term survival benefit even in those advanced stages. In stage I, 3 main causes of failure have been identified: recurrence of disease, new primary cancer and concomitant non-neoplastic chronic diseases. Recurrence of disease has been reported in 30–40% of resected stage I lung cancer (51), mostly within 2 years of operation. Approximately one third of these events occurred in the lungs, and two thirds in other distant sites. For patients who were alive at the end of the second year, it has been estimated that the annual incidence of a second primary lung cancer from the 3rd to the 13th year after the first pulmonary resection was as high as 10–15% (29, 66). Long-term follow-up of early lung cancer, in the framework of screening programs gave superimposable results, with a 20% incidence of second lung primary tumors after resection of early lung cancer (20). Moreover, other cancers (in the upper aero-digestive tract or bladder) were frequently detected in these patients, related to the same etiologic factors (smoking, alcohol, etc.), thus expanding the potential target of chemoprevention outside the lung. Our experimental model was then focused on 2 different targets: the anticancer activity against residual tumor microfoci, eventually expressed as a reduction of early recurrences (within 3 years), and the chemopreventive activity, expressed as a reduction of the occurrence of new primary tumors (beyond 3 years).

Concerning the study design, a crucial point was the choice of dose and duration of treatment in relation to the expected side effects and toxicity. Forty years of clinical application in ophtalmology and dermatology provided much information on pharmacology, toxicity and activity of vitamin A, but the large heterogeneity in the quality of preparation, daily dose, duration of treatment and underlying disease, prevented reliable assumptions regarding the maximum tolerated dose for long-term administration. While the typical syndrome of vitamin A intoxication was usually described after an acute intake of retinol at doses exceeding 1 million of International Units (I.U.), the clinical picture of chronic hypervitaminosis A was more subtle, eventually appearing after administration of doses as low as 40000 IU/day (49). The only analysis providing quantitative figures about the dose/effect relationship on 100 cases of chronic hypervitaminosis A was published by Korner in 1975 (36). He calculated that for treatments lasting more than 6 months, the daily dose of 5000 I.U. per kg of body-weight should never be exceeded in order to avoid chronic hypervitaminosis. In our trial the dose of 300000 I.U./day was then selected on the basis of 2 parameters: high therapeutic effect in the treatment of dermatological diseases like ichthyosis (60), psoriasis (21, 64) and oral leukoplakia (67), and maximum tolerance (4000 I.U./kg./day) according to Korner (36). The emulsified preparation of retinol palmitate was chosen in consideration of its absorption properties, which guaranteed a 6-fold higher bioavailability in comparison with the equivalent oily solution (36). As from the literature, signs and symptoms greatly differed in quality and quantity from patient to patient (23), though skin or bone problems were generally prominent in chronic toxicity. Out of 579 cases

of so called hypervitaminosis A, collected by Bauernfeind from 195 articles in the literature (2), only 20 cases were finally considered as serious intoxication, none of them lethal. Common mucocutaneous side effects like dryness, desquamation, or cheilitis had to be expected in virtually all treated patients. In adults, mild hair loss and bone pain were also reported after prolonged treatment, followed in order of frequency by headache, nausea and vomiting, due to increased intracranial pressure. Hepatomegaly and hepatic injury were less frequent and usually transient, unless related to pre-existing serious liver disease. A single case of cirrhosis and portal hypertension had been described in a 72-year-old man having taken retinol for 7 years (1).

Overall, our results were superimposable to the literature data in terms of prevalence of side effects, but more favorable than the average reports in terms of subjective tolerance. Although the large majority of patients were affected by skin and mucous membrane desquamation and dryness sometimes during treatment, these symptoms were generally mild and well tolerated, and never induced the patient to stop treatment. Other side effects like headache, hair loss, itching, or dyspepsia were detected at a much lower frequence than expected. Severe worsening of pre-existing atopic disease was observed in one patient, and hemorrhagic proctitis in another patient. Vitamin A administration was stopped in both cases, even though the proctitis was probably unrelated to the treatment. Liver enzyme abnormalities occurred in a slightly higher proportion of cases, compared with reports on dermatological patients (43, 54, 73, 83). However, it has to be taken into account that part of such a toxic effect could be related to previous pulmonary surgery, as serum abnormalities were observed even in untreated patients. In fact, the toxicity attributable to vitamin A (difference between treated patients and controls) is in the expected range of 10–15%. Similar considerations apply to triglycerides and cholesterol, with the main difference of a cumulative effect along treatment. This trend of delayed toxicity will require careful monitoring of long-term effects after completion of treatment (12 months), having major implications on future plans of prolonged treatments with high-dose retinol palmitate for chemoprevention purposes.

The evaluation of a potential benefit of vitamin A, in terms of reduction of new primary malignancies, was clearly beyond the limits of the actual number of patients and duration of follow-up. The observation of 2 cases of new primary cancers, both in the control arm, does not imply any evidence of a positive effect.

As far as recurrences are concerned, since the large majority of these events usually occur within 2 years after the operation, there was a sound rationale to estimate that any substantial modification in the relapse rate induced by the adjuvant treatment could be detected after a relatively short follow-up. In the present analysis, the relapse figures in the control arm were of the same magnitude as that expected on the basis of our (53) and other retrospective series (51). In the meantime, the relapse figures in the treated arm were substantially lower than expected in virtually all prognostic subgroups. Such differences could not be attributable to an information bias, since the clinical follow-up of treated subjects was more frequent and accurate for reasons of toxicity and compliance monitoring. On the contrary, pulmonary relapses were to some extent overestimated in the treatment arm, due to the fact that 4 cases of improper surgical treatment (R1) occurred in this arm, 2 of which refused postoperative radiotherapy until local recurrence took place. Moreover, in another patient brain metastases occurred immediately after randomization and before any treatment was actually started. These 3 cases (including protocol violations) were kept in the analysis even though they did not represent real failures of the adjuvant treatment.

As from the literature, clinical applications of vitamin A appeared more effective in carcinomas of the squamous type. In the attempt to confirm these findings, we stratified the randomization according to histology, and made a separate evaluation of patients with squamous cell lung cancer. Although the numbers are limited present data are compatible with a greater effect in squamous cell, compared to non-squamous cell lung cancer.

We conclude from this preliminary analysis of our ongoing trial, that high dose oral retinol palmitate in emulsion was a well tolerated treatment, with limited subjective and objective toxicity. The results are promising both in terms of tolerance and efficacy of treatment, but given the short median follow-up they must be very cautiously interpreted. A longer follow-up will hopefully clarify whether early recurrences after radical resection of stage Ia lung cancer were actually prevented, or only delayed, by vitamin A administration.

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