Randomized Phase III Intergroup Trial of Isotretinoin to Prevent Second Primary Tumors in Stage I Non-Small-Cell Lung Cancer

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Background: **Promising data have suggested that retinoid chemoprevention may help to control second primary tumors (SPTs), recurrence, and mortality of stage I non-small-cell lung cancer (NSCLC) patients.** ***Methods:*** **We carried out a National Cancer Institute (NCI) Intergroup phase III trial (NCI #I91–0001) with 1166 patients with pathologic stage I NSCLC (6 weeks to 3 years from definitive resection and no prior radiotherapy or chemotherapy). Patients were randomly assigned to receive a placebo or the retinoid isotretinoin (30 mg/day) for 3 years in a double-blind fashion. Patients were stratified at randomization by tumor stage, histology, and smoking status. The primary endpoint (time to SPT) and the secondary endpoints (times to recurrence and death) were analyzed by log-rank test and the Cox proportional hazards model. All statistical tests were two-sided.** ***Results:*** **After a median follow-up of 3.5 years, there were no statistically significant differences between the placebo and isotretinoin arms with respect to the time to SPTs, recurrences, or mortality. The unadjusted hazard ratio (HR) of isotretinoin versus placebo was 1.08 (95% confidence interval [CI] = 0.78 to 1.49) for SPTs, 0.99 (95% CI = 0.76 to 1.29) for recurrence, and 1.07 (95% CI = 0.84 to 1.35) for mortality. Multivariate analyses showed that the rate of SPTs was not affected by any stratification factor. Rate of recurrence was affected by tumor stage (HR for T_2 versus T_1 = 1.77 [95% CI = 1.35 to 2.31]) and a treatment-by-smoking interaction (HR for treatment-by-current-versus-never-smoking status = 3.11 [95% CI = 1.00 to 9.71]). Mortality was affected by tumor stage (HR for T_2 versus T_1 = 1.39 [95% CI = 1.10 to 1.77]), histology (HR for squamous versus nonsquamous = 1.31 [95% CI = 1.03 to 1.68]), and a treatment-by-smoking interaction (HR for treatment-by-current-versus-never-smoking = 4.39 [95% CI = 1.11 to 17.29]). Mucocutaneous toxicity ($P<.001$) and noncompliance (40% versus 25% at 3 years) were higher in the isotretinoin arm than in the placebo arm.** ***Conclusions:*** **Isotretinoin treatment did not improve the overall rates of SPTs, recurrences, or mortality in stage I NSCLC. Secondary multivariate and subset analyses suggested that isotretinoin was harmful in current smokers and beneficial in never smokers. [J Natl Cancer Inst 2001;93:605–18].**

Lung cancer is the most common cancer in the world *(1).* It is by far the greatest cause of cancer-related death in the United States, where more than 157 000 lung cancer deaths are predicted for 2001 *(1).* Even after 30 years of improving therapeutic approaches, the 5-year mortality rate of lung cancer remains an alarmingly high 86%.

Stage I lung cancer ($T_{1-2}N_0$) constitutes 10%–20% of all non-small-cell lung cancer (NSCLC) cases. The 5-year survival rates following surgical treatment of stage I disease are 67% for pathologic stage T_1N_0 NSCLC and 57% for stage T_2N_0 *(2–4).* These poor survival rates are due primarily to recurrences and second primary tumors (SPTs); SPT rates in this setting have been reported to be between 2% and 3% per year *(5,6).*

These bleak incidence and mortality data for lung cancer and its associated SPTs have led to intensive efforts to develop new strategies for controlling this disease, including chemoprevention. A major focus of lung cancer chemoprevention is the prevention of SPTs in early-stage disease *(7).* The retinoids, which regulate the growth and differentiation of normal bronchial epithelial cells *(8),* are a class of agents that have shown promise for lung cancer prevention.

Preclinical, epidemiologic, and early clinical studies in head and neck and lung carcinogenesis *(7–20)* provided the rationale for studying the efficacy of retinoids in lung cancer prevention. Squamous metaplasia occurs in the lungs of smokers, and the early findings of Wolbach and Howe *(13)* paved the way for later preclinical studies *(8,9,14,15)* showing that vitamin A treatment could reverse the squamous metaplasia that developed in vitamin A-deficient rodents. Other experimental animal models subsequently indicated that retinoids have cancer preventive effects in other organ systems *(9,14,15).*

Supported by the preclinical data, the primary rationale for our present study of the retinoid isotretinoin (13-*cis*-retinoic acid) came from rigorously controlled trial data showing that

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See "Notes" following "References."

retinoids statistically significantly reversed oral premalignancy and prevented SPTs associated with head and neck cancer *(16–19).* We suspected that these findings for head and neck cancer would be relevant to lung cancer in part because both types of cancer are caused by tobacco smoke carcinogens *(21,22).* Indeed, another trial had suggested that tobacco-related SPTs associated with lung cancer were reduced by the natural retinoid retinyl palmitate *(20).* On the basis of these preliminary data and the known risk of SPTs in patients with early-stage head and neck or lung cancer *(7,23),* we launched separate large-scale, National Cancer Institute (NCI) Intergroup phase III placebo-controlled trials to investigate the efficacy of isotretinoin in preventing SPTs associated with $T_{1-2}N_0$ head and neck cancer and $T_{1-2}N_0$ NSCLC. Here we report the primary results of the lung cancer trial.

Subjects and Methods

This multicenter NCI Intergroup trial (NCI #I91–0001), which was led by the University of Texas M. D. Anderson Cancer Center Community Clinical Oncology Program (CCOP) research base, included patient recruitment within affiliated institutions of the Eastern Cooperative Oncology Group, the Cancer and Leukemia Group B, the Southwest Oncology Group, the Radiation Therapy Oncology Group, the North Central Cancer Treatment Group, and The University of Texas M. D. Anderson Cancer Center (MDACC).

Patient Eligibility

To be eligible for participation in the trial, patients had to meet the following requirements: have had complete resection of a histologically proven squamous, adenocarcinoma, large-cell, or bronchioalveolar NSCLC between 6 weeks and 3 years before study entry; be currently free of disease; have a postoperative diagnosis of stage I ($T_{1-2}N_0$) NSCLC; have no previous or current chemotherapy or radiotherapy; be at least 18 years of age; and provide signed institutional review board-approved informed consent forms indicating awareness of the investigational nature of this study. Eligible patients were also required to have adequate bone marrow function (defined by a white blood cell count of $\geqslant 3000/mm^3$ and a platelet count of $\geqslant 100\,000/mm^3$), hepatic function (defined by a total bilirubin level of $\leqslant 1.5$ mg/dL, alanine aminotransferase of $\leqslant 56$ IU/L, or aspartate aminotransferase of $\leqslant 40$ IU/L, or by values considered to be normal by the participating institution), and a fasting triglyceride level of less than or equal to 320 mg/dL. Patients were required to have a life expectancy of at least 12 months and a Zubrod performance status of 0, 1, or 2 *(24).*

Women of childbearing potential were not eligible for this trial before July 19, 1995. Effective July 19, 1995, the study protocol was amended to allow women of childbearing potential to register for the trial if they received counseling about the potential teratogenic effects of isotretinoin, including oral and written warnings of the hazards of taking isotretinoin during pregnancy and of the possibility and risk of contraception failure. Eligibility for such women also required written acknowledgment of the understanding of these warnings, the agreement to practice some form of contraception during the study, and a negative serum pregnancy test within 2 weeks of study entry.

Patients were not eligible for the trial if they had taken vitamin A supplements at doses of greater than 25 000 IU/day or β-carotene supplements at doses of greater than 30 mg/day within 3 months of study entry. Patients were also not eligible if they had had any other cancer(s) concurrently with stage I NSCLC or any other non-NSCLC, except localized nonmelanoma skin cancer, within 5 years of study entry. Finally, patients with metastatic disease (even if resectable) or a history of more than one primary lung tumor, including synchronous lesions, at any time were also not eligible.

Study Design and Treatment Plan

Potentially eligible patients had to complete a comprehensive pretreatment evaluation within 4 weeks of potential registration to determine eligibility. This evaluation included a complete physical examination and medical history (including details of tobacco and alcohol use, Zubrod performance status, recent weight loss, and concurrent nonmalignant disease), laboratory studies (complete blood cell count, platelet count, SMA-12 chemistry profile, and fasting triglyceride level), and chest posterior–anterior (PA) and lateral radiographs. Women of childbearing potential also had to complete a serum pregnancy test within 2 weeks of study entry.

Before randomization, there was an 8-week placebo run-in period, after which each patient was evaluated for compliance and disease status. Only those patients who had taken at least 75% of the prescribed number of capsules (by an actual capsule count) and who remained disease free were eligible for randomization.

Patients were randomly assigned to receive either isotretinoin or a placebo to be taken orally once a day for 3 years. Isotretinoin and the placebo were manufactured as matched soft-gel capsules by Hoffmann-La Roche Inc. (Nutley, NJ) and were distributed by the Drug Management and Authorization Section, NCI. At randomization, patients were stratified by tumor histology (squamous versus nonsquamous), stage [T_1 versus T_2, by use of the tumor–node–metastasis staging system *(2)*], and smoking status at registration. These stratification factors were selected because of their potential independent impact on the major study endpoints. Current smokers were defined as active smokers and those who had stopped smoking less than 1 year before registration; former smokers were defined as those who had not smoked for 1 or more years; and never smokers were defined as those who smoked 100 or fewer cigarettes ever. The treatment allocation was done via a randomized permuted block (size = 4) within strata to balance patient factors.

Periodic study evaluations, which included a standardized patient history and physical examination, were conducted at randomization, at 3 months, at 6 months, and then every 6 months for the remainder of the study. Periodic laboratory tests included a complete blood cell count (every 6 months for the first 3 years and annually thereafter) and SMA-12 and triglyceride evaluations (at 3 months and then every 6 months for 3 years; after 3 years, only SMA-12 evaluation continued annually). Chest PA and lateral radiographs were obtained every 6 months or more frequently if clinically indicated. Protocol compliance was measured by capsule count at each clinic visit. Telephone interviews by the study nurse or data manager to encourage adherence and retention were scheduled for 9, 15, 21, 27, and 33 months after randomization.

The following four different isotretinoin dose levels, from the initial dose to three different modifications based on toxicity, were used: 0 (30 mg/day), −1 (20 mg/day), −2 (10 mg/day), and −3 (10 mg every other day). With the exception of hypertriglyceridemia, toxicity was graded by use of the Common Toxicity Criteria (NCI) (http://ctep.info.nih.gov/CTC3/default.htm). If a patient had a toxic effect of grade 2, 3, or 4, treatment was suspended and the specific toxicity was reassessed weekly via telephone or clinic visit until it subsided to grade 0 or 1. At that point, isotretinoin treatment was restarted at the next lower dose level. If the patient was taking isotretinoin at less than 30 mg/day and had a grade 0 or 1 toxicity for 3 consecutive months, the dose was increased to the next higher level. No patient received more than 30 mg/day of isotretinoin. Triglyceride toxicity was graded as follows: grade 1 toxicity was defined as more than 2.5 times but less than or equal to five times the normal level; grade 2 toxicity was defined as more than five times but less than or equal to 10 times the normal level; and grade 3 toxicity was defined as more than 10 times the normal triglyceride level or if a patient experienced complications (e.g., pancreatitis) at any grade of triglyceride toxicity. At their physician's discretion, patients with grade 2 or 3 triglyceride toxicity were either treated with a lipid-lowering medication (e.g., gemfibrozil) or taken off the study capsules. If taken off the study capsules, patients were rechecked after 1 month; if they had a toxicity grade of 0 or 1, treatment was resumed at the next lower dose level.

The primary study endpoint was the development of SPTs. All cancer events were verified histologically and classified as either an SPT or a recurrence. The Endpoint Review Committee (from MDACC CCOP and MDACC) used the following guidelines to provide consistency in determining which cancer events were lung SPTs. New lung lesions were considered to be SPTs if they met at least one of the following criteria: having a different histology from the primary tumor, occurring in a different lobe than the primary tumor, occurring in the contralateral lung, or occurring more than 5 years after the primary tumor. Recurrence was a secondary endpoint, and patients who developed recurrences that were successfully treated by surgery (with or without radiotherapy or chemotherapy) could continue on the study capsules. All endpoints were reviewed and confirmed formally by the Endpoint Review Committee, whose members were blinded to treatment assignments and side-effect results.

Statistical Analyses

We initially sought to accrue 1260 patients to enter the run-in period, of whom 1134 would be randomly assigned to one of the study arms (this latter figure accounts for a 10% assumed loss to randomization during the run-in period).

These goals were later revised after our discovery, in December 1994, of an error in the randomization program that had resulted in the nonrandom assignment of 107 patients to the isotretinoin arm of the study. This error was discovered during a quality-control check involving unblinded verification of drug assignments and confirmation of errors by the statistician and programmer who wrote the randomization program. The randomization program performed a balance check for the small accruing centers (with an expected accrual of fewer than 12 patients each) as a group and for each of the large accruing centers (with an expected accrual of 12 or more patients each). This approach had several advantages, such as facilitating efficient drug distribution and balancing the two study arms. Large centers kept a drug supply on site, whereas small centers received drug only at the times of patient registrations (avoiding shelf wastage). Each patient who registered at a large accruing center was randomly assigned consecutively within his or her center's group, whereas patients registered at small accruing centers were pooled and then randomized from the communal pool. This pooling allowed the study-arm assignments of patients from small accrual centers to be balanced with respect to the three stratification factors. The 107 patients affected by the randomization error were consecutively registered patients from the small accrual centers. Although the random assignment of these patients had, in fact, been correct, a subsequent program error at the data-management center conveyed the wrong electronic instructions to the drug-distribution center to ship isotretinoin to the approximately one half of those 107 patients who should have received placebo.

It was decided by the NCI Intergroup Steering Committee, with the approval of the Data Monitoring Committee (DMC) and the NCI program director, to exclude the 107 affected patients from the final analyses and to accrue 107 additional patients. The revised accrual goals were, therefore, 1379 patients at run-in and 1241 at randomization. Under the assumptions indicated below, 1241 randomly assigned patients would permit the detection of a 50% reduction in the incidence rate of SPTs with at least 80% power and a 5% level for the two-sided test of statistical significance. The 50% reduction in SPT incidence was based on our earlier head and neck cancer trial, in which a higher dose of isotretinoin resulted in an 80% reduction in SPT incidence rate *(17).* The present study was designed as a 7-year trial with a uniform 4-year accrual. The sample size calculations used the method and computer program of Lachin and Foulkes *(25)* and incorporated the interim analysis proposed by Geller and Pocock *(26)* and O'Brien and Fleming *(27).*

We assumed an annual SPT rate of 3%, a 5-year survival rate of 60%, and a 2% annual loss to follow-up. In addition, we assumed that no patients on placebo would start taking isotretinoin (i.e., drop-in) because it must be prescribed by a health professional. Drop-out rates after randomization were predicted to be 10% on the basis of data from randomized prevention trials of isotretinoin in head and neck carcinogenesis *(16–18).*

The primary endpoint was time to SPT. The statistical analyses were based on the intent-to-treat method. All patients randomly assigned to a treatment were included in the comparison, regardless of how much of or how long they took their assigned treatment, and were grouped by the assigned treatment. Patients who were either lost to follow-up or died without an SPT were included in the time-to-event analysis with a censored status on the last day of contact. The primary hypothesis of treatment effect was tested by use of the Cox proportional hazards model, with the stratification factors included as covariates. Kaplan–Meier curves were generated to estimate the probability of no event at any given time for the time-to-event data. Both the Cox model and the log-rank test were applied in the analyses of censored data.

Interim reports with summary statistics were prepared every 6 months during the 7-year study and included patient accrual information, clinical data quality and control issues, compliance rate, and frequency and severity of toxic effects and adverse drug reactions. The Intergroup Steering Committee (from participating groups, including MDACC CCOP, MDACC, cooperative groups, and the NCI) met annually for the first 5 years of the study to monitor these issues.

The first and second formal interim analyses and the final analysis were planned for 3, 5, and 7 years, respectively, after the first patient was randomly assigned to treatment. The criteria used for early termination of the trial were P values of less than .0005 and less than .014 (two-sided test) for the first and second formal interim analyses, respectively. These P values were selected according to the criteria proposed by Geller and Pocock *(26)* and O'Brien and Fleming *(27)* to preserve an overall statistical significance level of .05 for the study. The significance level was .045 when the final analysis was performed.

The prespecified secondary endpoints of the study included time-to-recurrence and overall survival. A *post hoc* analysis on the basis of the data suggested that there might be a treatment-by-smoking interaction. A small simulation study was conducted to evaluate the statistical power for testing this interaction. The secondary endpoint, *post hoc,* and subset analyses were performed by use of a two-sided statistical test with a 5% significance level. However, because of the multiple testing problem, these findings need to be interpreted with care.

RESULTS

A total of 1486 registered patients were accrued from December 1992 to April 1997. The trial flow diagram in Fig. 1 shows the percentages of total accrual contributed by each cooperative group and information about the progress of patients throughout this phase III two-group, parallel-design, randomized controlled trial. Forty-two patients were found to be ineligible during their placebo run-in periods for the following reasons: evidence of synchronous lesions (20 patients) or active lung

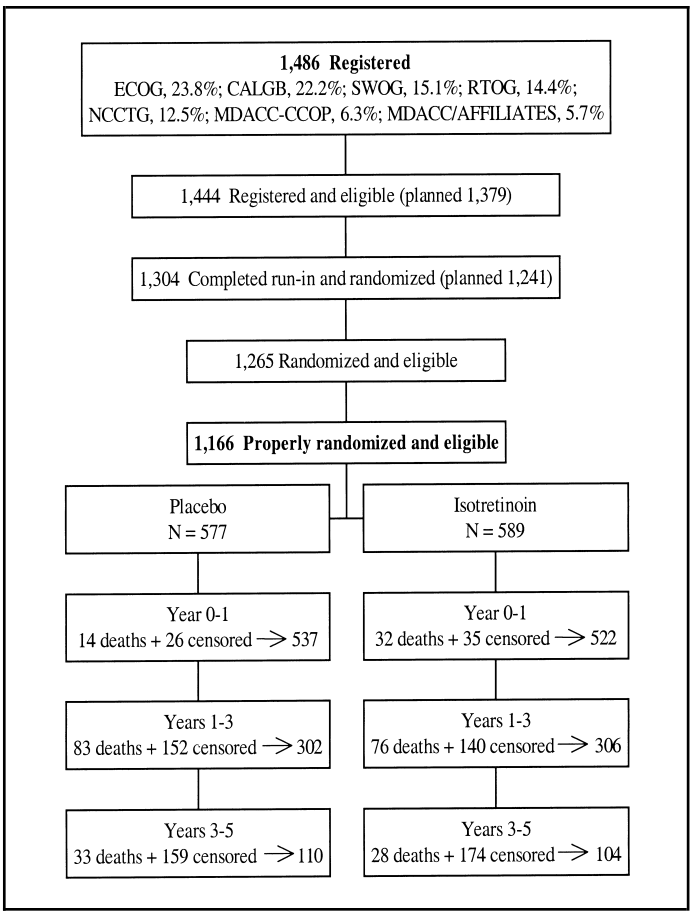

Fig. 1. Trial flow diagram. **Top box** includes the percentages of registered patients from the Eastern Cooperative Oncology Group (ECOG), the Cancer and Leukemia Group B (CALGB), the Southwest Oncology Group (SWOG), the Radiation Therapy Oncology Group (RTOG), the North Central Cancer Treatment Group (NCCTG), The University of Texas M. D. Anderson Cancer Center Community Clinical Oncology Program (MDACC-CCOP), and the MDACC and affiliates. The 1166 properly randomly assigned and eligible patients exclude 99 patients with a randomization error. In the **boxes** beneath each treatment arm, the **numbers after the arrows** represent the numbers of patients still at risk after the intervals specified in the boxes. Patients at risk included all of those still in treatment/follow-up, including patients with second primary tumors or recurrence and still at risk for mortality. Censored patients included those lost to follow-up, those who refused to participate, or those who were censored administratively but were still in treatment or follow-up at the protocol-mandated completion of the study. Substantial portions of the censored patients in their 1- to 3-year and 3- to 5-year intervals were censored administratively, which is consistent with the 3.5-year median patient follow-up of our present analyses.

cancer (six) at the time of registration, a prior disqualifying cancer history (three), less than 6 weeks or more than 36 months from resection (two), required prestudy testing was not completed (four), improper institutional procedures (mistaken randomizations during the placebo run-in period) (six), and women of childbearing potential before July 19, 1995 (one). Of the 1444 registered and eligible patients, 1304 (90%) completed the run-in period and were randomly assigned to one of the study arms. The first and last patients were randomly assigned on February 5, 1993, and June 23, 1997, respectively. One hundred forty eligible patients were not randomly assigned for the following reasons: refusal (59 patients), noncompliance (51), disease discovered during the placebo run-in (26), and intercurrent illness (four). During the run-in period, 28 episodes of grade 2 and three episodes of grade 3 toxicity were reported.

After central review at the coordinating data-management center (MDACC-CCOP), 39 of the 1304 randomly assigned patients were found to be ineligible because of synchronous lung cancer (23 patients), low run-in compliance (five), prior lymph node or T_3 disease (three), and other isolated causes (eight) that had been missed earlier because of reporting or entry errors involving ineligibility data. Of the remaining 1265 randomly assigned, eligible patients, 577 were assigned to receive placebo and 688 were assigned to receive isotretinoin. The size discrepancy between the two treatment arms was caused by the computer randomization error (*see* "Statistical Analyses") that resulted in the nonrandom assignment of 107 patients to isotretinoin. Of the 107 improperly randomly assigned patients, 99 were eligible for the study and eight subsequently became ineligible for various reasons after randomization. The main analyses reported here exclude the 99 improperly randomly assigned (but eligible) patients and so include a total of 1166 randomly assigned patients—577 in the placebo arm and 589 in the isotretinoin arm. Secondary analyses that included the 99 improperly randomly assigned patients on isotretinoin produced results similar to those from the main analyses.

Table 1 lists the patient characteristics overall and by treatment arm. The study population had a median age of 65 years and was predominantly male (57%) and white (92%). The two arms were well balanced with respect to these demographic characteristics and the three stratification factors (histology, tumor stage, and smoking status). Among the overall study population, the most frequently found categories with respect to the three stratification factors were as follows: 68% had tumors with nonsquamous histology, 54% had stage T_1 lesions, and 53% were former smokers. Current and former smokers had smoked for a median of 40 years (range, 1–70 years). The percentages of the overall study population that used chewing tobacco or snuff and smoked pipes or cigars were 6% and 17%, respectively, and did not differ substantially by treatment arm. The first, second (median), and third quartile times between surgery and registration were 2.1, 4.5, and 11.6 months, respectively, for the placebo arm and 2.3, 5.1, and 12.5 months, respectively, for the isotretinoin arm.

The toxicity and compliance results included all 1166 randomly assigned patients. Three randomly assigned patients (one in the placebo arm and two in the isotretinoin arm) had no follow-up visits, and so all time-to-event analyses were based on 1163 patients. The median follow-up time for living patients was 3.5 years. There were 174 patients lost to follow-up after beginning the study. We defined "lost to follow-up" as patients who missed their scheduled appointments for 18 months or longer. Of the 174 such patients, 31 were lost during the first year, 39 during the second year, 63 between years 2 and 3, and 41 after 3 years.

Table 1. Characteristics of randomly assigned, eligible patients by study arm*

Characteristic	Placebo (n = 577)	Isotretinoin (n = 589)	Total (n = 1166)
Age, y			
Median (range)	66.0 (34–81)	65.0 (31–86)	65.0 (31–86)
Mean (SD)	64.1 (8.9)	64.3 (8.6)	64.2 (8.8)
Sex, No. (%)			
Female	251 (43.5)	248 (42.1)	499 (42.8)
Male	326 (56.5)	341 (57.9)	667 (57.2)
Race, No. (%)			
White	525 (91.0)	552 (93.7)	1077 (92.4)
Black	41 (7.1)	31 (5.3)	72 (6.2)
Other	11 (1.9)	6 (1.0)	17 (1.5)
Histology, No. (%)			
Squamous	191 (33.1)	187 (31.8)	378 (32.4)
Nonsquamous	386 (66.9)	402 (68.3)	788 (67.6)
T stage, No. (%)			
T_1	315 (54.6)	316 (53.7)	631 (54.1)
T_2	262 (45.4)	273 (46.3)	535 (45.9)
Smoking status, No. (%)			
Current smoker	231 (40.0)	225 (38.2)	456 (39.1)
Former smoker	301 (52.2)	319 (54.2)	620 (53.2)
Never smoker	45 (7.8)	45 (7.6)	90 (7.7)

*SD = standard deviation; T = tumor.

The overall event rates and Kaplan–Meier curves of time to SPT (the primary endpoint) and of time to recurrence and mortality (the major secondary endpoints), by treatment and by stratification factor, are presented in Table 2 and Figs. 2–4, respectively. The results of the univariate and multivariate analyses by use of the Cox proportional hazards model are presented in Tables 3 and 4, respectively. These analyses revealed that there were no statistically significant differences between the isotretinoin and placebo arms with regard to SPTs, recurrences, or mortality as measured by event rates, Kaplan–Meier estimates, or univariate or multivariate hazard ratios (HRs). No treatment-effect differences in the primary or major secondary endpoints occurred among the patients recruited from the different cooperative groups (data not shown).

The overall univariate analyses of the effects of the three stratification factors (tumor stage, histology, and smoking status) on the three major endpoints produced the following results (Table 3): SPTs were not associated with any stratification factor; recurrence was statistically significantly associated with tumor stage (HR of T_2 versus T_1 = 1.74; 95% confidence interval [CI] = 1.33 to 2.27); and mortality was statistically significantly associated with tumor stage (HR of T_2 versus T_1 = 1.42; 95% CI = 1.12 to 1.80), histology (HR of squamous versus nonsquamous = 1.42; 95% CI = 1.11 to 1.80), and smoking status (HRs of current and former smokers versus nonsmokers = 2.44 [95% CI = 1.32 to 4.53] and 1.94 [95% CI = 1.05 to 3.58], respectively).

We examined the treatment effects within the stratification-factor subsets (Figs. 3 and 4). Only smoking status interacted with treatment in affecting any major clinical endpoints. The survival curves based on Kaplan–Meier analysis (Fig. 4) indicate that current smokers in the isotretinoin arm had statistically nonsignificantly higher rates of recurrence (P = .15, log-rank test)

Table 2. Event rates (95% confidence intervals) according to stratification factors*

Event	Tumor stage		Squamous		Smoking status			Overall
	T_1	T_2	Yes	No	Never smoker	Former smoker	Current smoker	
SPT								
Isotretinoin	3.8 (2.8 to 5.3)	4.6 (3.3 to 6.3)	4.5 (3.1 to 6.6)	4.0 (3.0 to 5.3)	4.1 (1.8 to 9.1)	4.1 (3.0 to 5.5)	4.3 (2.9 to 6.2)	4.2 (3.3 to 5.2)
Placebo	3.3 (2.4 to 4.7)	4.6 (3.3 to 6.4)	5.1 (3.6 to 7.4)	3.3 (2.4 to 4.5)	1.3 (0.3 to 5.3)	4.4 (3.3 to 5.9)	3.7 (2.5 to 5.4)	3.9 (3.1 to 4.9)
Recurrence								
Isotretinoin	5.0 (3.8 to 6.6)	7.7 (6.0 to 9.9)	6.5 (4.7 to 9.1)	6.0 (4.8 to 7.6)	3.5 (1.4 to 8.3)	5.7 (4.4 to 7.4)	7.6 (5.7 to 10.1)	6.2 (5.1 to 7.5)
Placebo	4.3 (3.2 to 5.8)	8.8 (6.9 to 11.2)	6.1 (4.4 to 8.5)	6.3 (5.0 to 7.9)	7.7 (4.3 to 13.9)	6.5 (5.1 to 8.4)	5.5 (4.0 to 7.6)	6.2 (5.2 to 7.5)
Death								
Isotretinoin	6.5 (5.1 to 8.2)	8.8 (7.0 to 11.0)	9.9 (7.6 to 12.7)	6.4 (5.2 to 7.9)	2.0 (0.6 to 6.1)	6.3 (4.9 to 8.0)	10.6 (8.5 to 13.4)	7.5 (6.4 to 8.8)
Placebo	5.9 (4.6 to 7.6)	8.6 (6.8 to 10.8)	8.3 (6.3 to 10.9)	6.5 (5.2 to 8.0)	5.2 (2.6 to 10.4)	7.6 (6.0 to 9.4)	6.8 (5.2 to 9.0)	7.1 (6.0 to 8.4)

*Event rates were calculated as the total number of cases divided by the total number of follow-up years and are expressed as percentages.
T = tumor; SPT = second primary tumor.

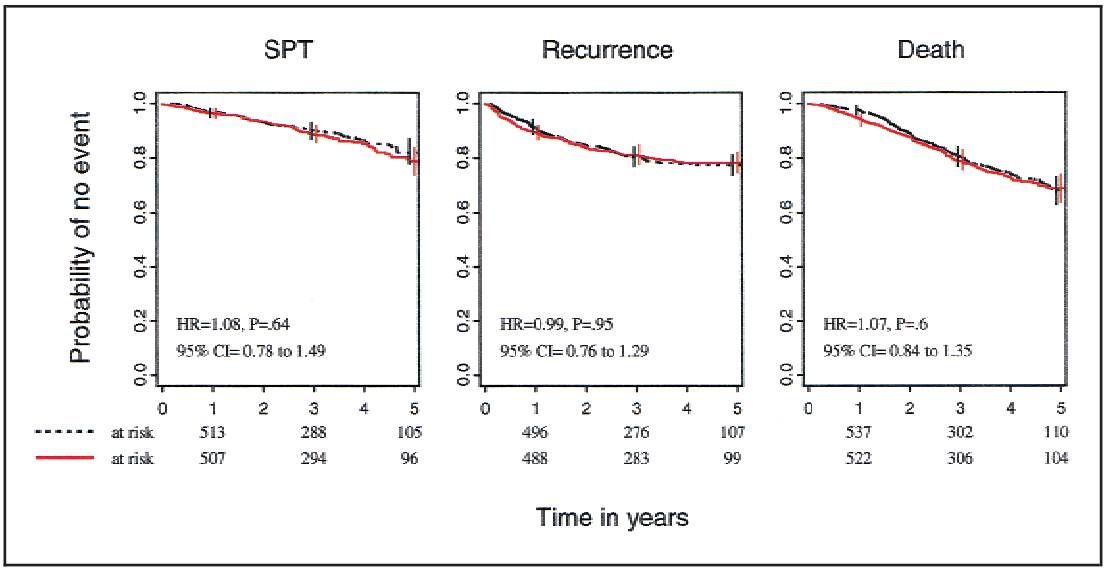

Fig. 2. Kaplan–Meier plots of time-to-event by treatment. SPT = second primary tumor; HR = hazard ratio; CI = confidence interval. **Dotted black line** is the placebo group; the **solid red line** is the isotretinoin group. The ***y*-axis** indicates the probability of no event. A total of 1163 patients were randomly assigned, eligible, and evaluable for survival endpoints. The HRs represent the rate of an event in the isotretinoin arm relative to this rate in the placebo arm. The numbers of patients in each arm who were at risk for an SPT, recurrence, or death in years 1, 3, and 5 are presented below the appropriate graphs.

and statistically significantly higher rates of mortality ($P = .01$) than current smokers in the placebo arm, whereas never smokers in the isotretinoin arm had lower rates of recurrence ($P = .12$) and mortality ($P = .14$) than never smokers in the placebo arm, although the differences were not statistically significant. Although the treatment-related benefits in recurrence and mortality in never smokers were greater in magnitude than the adverse effects in current smokers, the benefits were not statistically significant because of the smaller numbers of never smokers (Fig. 4). Mortality in current smokers was the only statistically significant subset finding. Overall, there were 123 deaths among current smokers; 73 occurred in the isotretinoin arm, and 50 occurred in the placebo arm. Most of these 123 deaths were cancer related. The proportions of non-cancer-related deaths among current smokers in the isotretinoin and placebo arms were 32% and 26%, respectively ($P = .51$). Although we examined the pattern of these non-cancer-related deaths, it was difficult to meaningfully interpret this pattern because the prespecified mortality endpoints of this study did not include any disease or condition other than SPT or recurrence. Moreover, secondary subset analyses (noncancer deaths) within another subset (current smokers) involve small numbers of patients and are, therefore, likely to produce results that are chance occurrences.

The multivariate analyses that evaluated the effects of treatment with regard to the three stratification factors indicated that isotretinoin had no effect on SPTs, recurrences, or mortality ($P = .66$, $P = .94$, and $P = .56$, respectively; data not shown). However, further examination of the data suggested that there might be a treatment-by-smoking interaction with respect to recurrence and mortality. The results of the multivariate analyses incorporating treatment, the three stratification factors, and the treatment-by-smoking-status interaction are presented in Table 4. These analyses showed that the HR for SPT was not associated with a treatment effect, with any of the three stratification factors, or with a treatment-by-smoking interaction. By contrast, the HR for recurrence was statistically significantly associated with tumor stage (HR of T_2 versus $T_1 = 1.77$; 95% CI = 1.35 to 2.31) and with treatment-by-smoking interaction (HR of treatment-by-current-versus-never-smoking = 3.11; 95% CI = 1.00 to 9.71). The HR for mortality was statistically significantly associated with tumor stage (HR of T_2 versus $T_1 = 1.39$; 95% CI = 1.10 to 1.77), histology (HR of squamous versus nonsquamous = 1.31; 95% CI = 1.03 to 1.68), and with treatment-by-smoking interaction (HR of treatment-by-current-versus-never-smoking = 4.39; 95% CI = 1.11 to 17.29). The multivariate analysis of treatment-by-current-versus-never-smoking involved

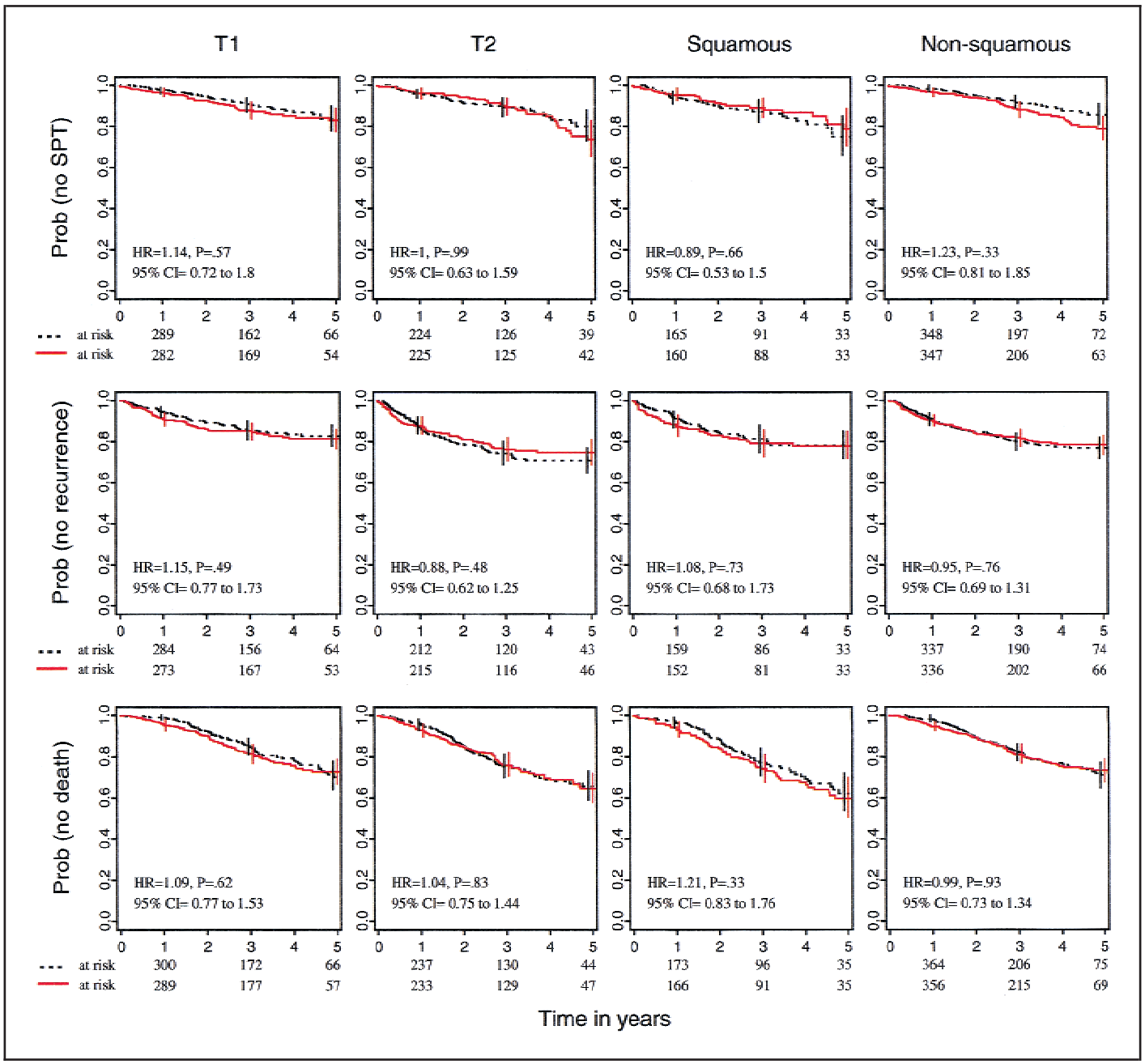

Fig. 3. Kaplan–Meier plots of time-to-event data stratified by tumor stage (T_1 or T_2) and histology (squamous or nonsquamous). SPT = second primary tumor; Prob = probability; HR = hazard ratio; CI = confidence interval; T = tumor. The ***y*-axis** indicates the probability of no event. **Dotted black line** represents patients on placebo; **solid red line** represents patients on isotretinoin. The numbers of stratified patients in each arm who were at risk for an SPT, recurrence, or death in years 1, 3, and 5 are presented below the appropriate graphs.

statistically significant differential treatment effects with respect to recurrence and mortality: harmful in current smokers and beneficial in never smokers. We found no effects of treatment-by-histology or treatment-by-tumor-stage interactions on the major endpoints. The *P* values for assessments of treatment-by-tumor-stage effects on SPTs, recurrences, and mortality were $P = .77$, $P = .29$, and $P = .73$, respectively, and for assessments of treatment-by-histology effects were $P = .37$, $P = .50$, and $P = .37$, respectively.

To facilitate the interpretation of the study outcome, a small simulation study was carried out, and the statistical power for testing the treatment-by-smoking interaction was evaluated. On the basis of the data, we assumed that the overall survival time follows a Weibull distribution and that the censoring time follows a uniform distribution. Parameters were chosen to match the survival distribution and censoring rate in the data. With 1000 simulation trials, we had 61.3% power to detect the treatment-by-smoking interaction with a two-sided 5% type I error rate.

We also assessed the patterns of failure in our patients to better understand the natural history of stage I NSCLC. In the placebo arm only, the annual rate of SPTs was 3.9% (95% CI = 3.1% to 4.9%) and of recurrence was 6.2% (95% CI = 5.2% to 7.5%) (Table 2). Overall, recurrences were more frequent than SPTs (totals of 221 and 147, respectively), and recurrences had a threefold to fourfold greater impact on mortality than did SPTs

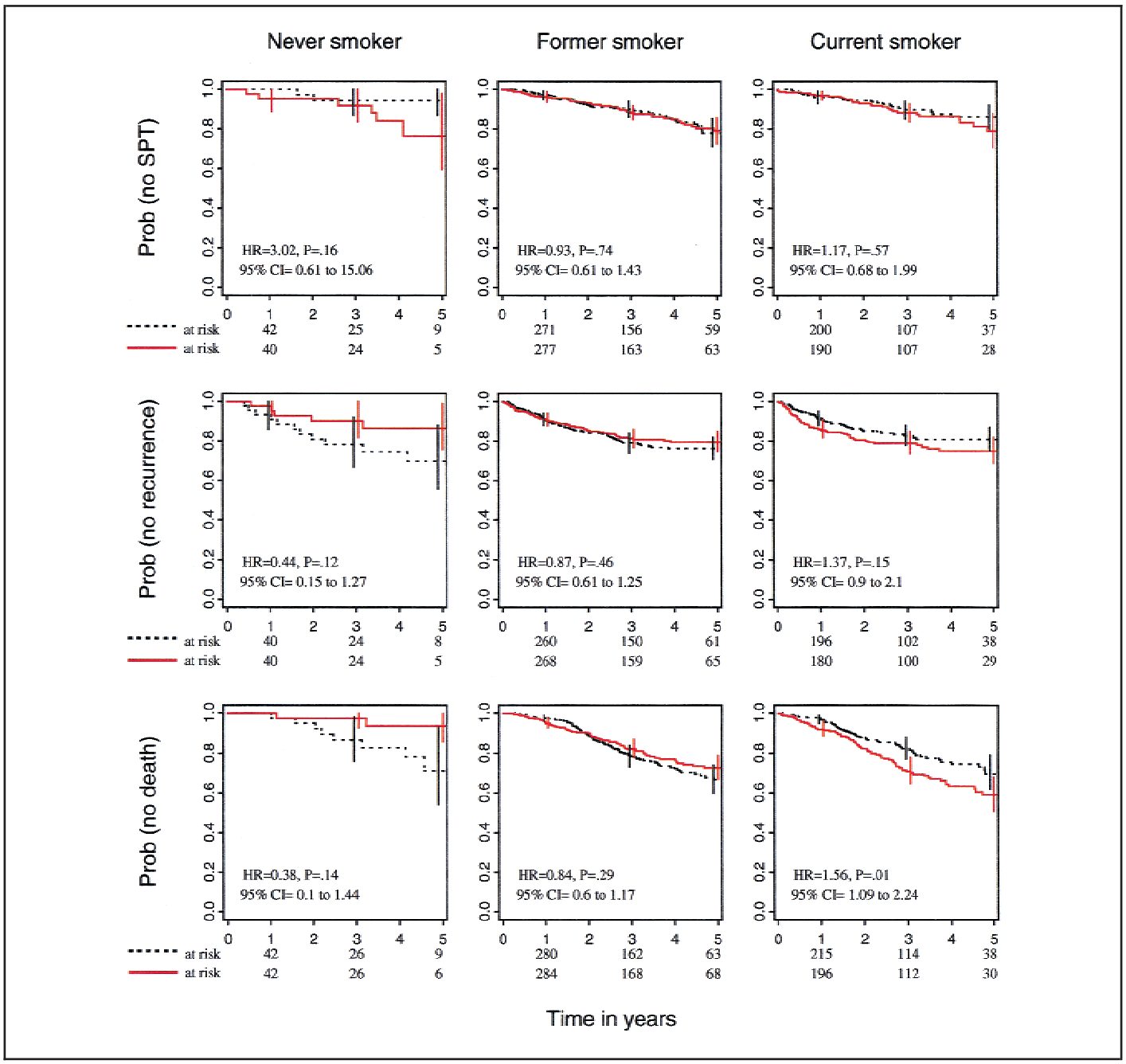

Fig. 4. Kaplan–Meier plots of time-to-event data stratified by smoking status. SPT = second primary tumor; Prob = probability; HR = hazard ratio; CI = confidence interval. The ***y*-axis** indicates the probability of no event. **Dotted black line** represents patients on placebo; **solid red line** represents patients on isotretinoin. The numbers of stratified patients in each arm who were at risk for an SPT, recurrence, or death in years 1, 3, and 5 are presented below the appropriate graphs.

(165 recurrence deaths versus 47 SPT deaths). The different incidence and mortality patterns of recurrence and SPT were seen even in patients with the earliest disease stage (T_1); among these patients, there were 94 cases of and 76 deaths from recurrences and 74 cases of and 23 deaths from SPTs.

We also analyzed smoking-related SPTs, which were a prespecified secondary endpoint. Of the 147 total SPTs, 62% overall (47 in the isotretinoin arm versus 44 in the placebo arm) were considered to be smoking related because they developed in the lung, head and neck, esophagus, or bladder, all common sites of tobacco-related carcinogenesis. In the multivariate Cox proportional hazard model, smoking-related SPT development was not statistically significantly associated with current smoking (HR = 3.90; 95% CI = 0.52 to 29.02) or former smoking (HR = 3.09; 95% CI = 0.42 to 22.87), although the HRs were greater than 1.0. Moreover, there was no statistically significant association between smoking-related SPT development and treatment or tumor stage or a treatment-by-smoking interaction. Smoking-related SPT development was statistically significantly associated with squamous histology (HR = 1.54; 95% CI = 1.01 to 2.35; P = .047). The most common non-smoking-related SPTs—gastrointestinal (15 cases), prostate (14), and breast (13) tumors—occurred at similar frequencies in the two arms of the study.

Table 3. Univariate analyses of effects of treatment and stratification factors on rates of SPT, recurrence, and mortality by use of the Cox proportional hazards model*

	SPT				Recurrence				Mortality			
Covariate	Events†	No.‡	HR (95% CI)	*P*§	Events†	No.‡	HR (95% CI)	*P*§	Events†	No.‡	HR (95% CI)	*P*§
Treatment												
Isotretinoin	76	587	1.08 (0.78 to 1.49)	.64	110	587	0.99 (0.76 to 1.29)	.95	142	587	1.07 (0.84 to 1.35)	.60
Placebo‖	71	576	1.00		111	576	1.00		135	576	1.00	
Stage												
T_2	73	533	1.28 (0.93 to 1.77)	.13	127	533	1.74 (1.33 to 2.27)	<.0001	145	533	1.42 (1.12 to 1.80)	.004
T_1‖	74	630	1.00		94	630	1.00		132	630	1.00	
Histology												
SCC	56	377	1.32 (0.95 to 1.84)	.10	70	377	1.01 (0.76 to 1.34)	.94	110	377	1.42 (1.11 to 1.80)	.005
Non-SCC‖	91	786	1.00		151	786	1.00		167	786	1.00	
Smoking												
Current smoker	54	455	1.51 (0.72 to 3.16)	.28	86	455	1.14 (0.67 to 1.94)	.63	123	455	2.44 (1.32 to 4.53)	.005
Former smoker	85	618	1.56 (0.76 to 3.22)	.23	119	618	1.10 (0.66 to 1.86)	.71	143	618	1.94 (1.05 to 3.58)	.034
Never smoker‖	8	90	1.00		16	90	1.00		11	90	1.00	

*SPT = second primary tumor; No. = number of patients; HR = hazard ratio; CI = confidence interval; T = tumor; SCC = squamous cell carcinoma.

†Number of events that occurred within 5 years of randomization.

‡Three of the 1166 randomly assigned patients (one in the placebo arm and two in the isotretinoin arm) had no follow-up visits; therefore, all time-to-event analyses were based on 1163 patients.

§*P* values were based on the two-sided Wald test.

‖Reference category.

Table 4. Multivariate analyses of effects of treatment, stratification factors, and treatment-by-smoking interaction on the risk of SPT, recurrence, and mortality by use of the Cox proportional hazards model*

	SPT		Recurrence		Mortality	
Covariate	HR (95% CI)	*P*†	HR (95% CI)	*P*†	HR (95% CI)	*P*†
Treatment						
Isotretinoin	3.14 (0.63 to 15.55)	.16	0.44 (0.15 to 1.27)	.13	.036 (0.10 to 1.37)	.13
Placebo‡	1.00		1.00		1.00	
Stage						
T_2	1.24 (0.89 to 1.72)	.20	1.77 (1.35 to 2.31)	<.001	1.39 (1.10 to 1.77)	.007
T_1‡	1.00		1.00		1.00	
Histology						
Squamous	1.24 (0.88 to 1.74)	.22	0.93 (0.70 to 1.25)	.64	1.31 (1.03 to 1.68)	.03
Nonsquamous‡	1.00		1.00		1.00	
Smoking status						
Current smoker	2.72 (0.64 to 11.51)	.17	0.73 (0.37 to 1.44)	.36	1.21 (0.57 to 2.56)	.62
Former smoker	3.10 (0.75 to 12.84)	.12	0.87 (0.46 to 1.67)	.68	1.30 (0.63 to 2.71)	.48
Never smoker‡	1.00		1.00		1.00	
Treatment-by-smoking status						
Current smoker	0.37 (0.07 to 2.01)	.25	3.11 (1.00 to 9.71)	.05	4.39 (1.11 to 17.29)	.04
Former smoker	0.30 (0.06 to 1.55)	.15	1.96 (0.64 to 5.96)	.15	2.28 (0.58 to 8.92)	.24
Never smoker‡	1.00		1.00		1.00	

*SPT = second primary tumor; HR = hazard ratio; CI = confidence interval; T = tumor.

†*P* values were based on the two-sided Wald test.

‡Reference category.

Statistically significant treatment-related toxic effects included cheilitis (*P*<.001), skin dryness (*P*<.001), conjunctivitis (*P*<.001), and arthralgia (*P* = .047) (Table 5). Elevated triglyceride levels also were found more frequently in the isotretinoin arm than in the placebo arm, but the difference was not statistically significant. The 3-, 6-, 12-, and 18-month noncompliance rates (documented by capsule count that <75% of the capsules were taken) in the placebo arm were 4.3%, 8.4%, 15.0%, and 21.5%, respectively. This rate stabilized at about 25% after 24 months. The 3-, 6-, 12-, 24-, and 36-month noncompliance rates in the isotretinoin arm were 8.4%, 16.3%, 24.4%, 34.3%, and 39.8%, respectively. Noncompliance in the isotretinoin arm was related to increased toxicity.

The trial was completed in February 2000, 7 years after the first patient was randomly assigned, as mandated by the trial protocol. At that time, the DMC directed that all of the study patients be notified that isotretinoin was not effective, that there was a possible isotretinoin–smoking interaction, and that any current smokers who were still taking isotretinoin should stop taking the drug. All of the patients in either arm who had not reached the end of their 3-year treatment were advised that the study was complete and to stop treatment. This advice affected

Table 5. Frequent toxicity of randomized eligible patients by study arm*

	Placebo (n = 577)†				Isotretinoin (n = 589)†				
Toxic effect	G1	G2	G3	G4	G1	G2	G3	G4	P‡
Cheilitis	83	8	0	0	392	141	14	0	<.001
Skin dryness	171	38	1	0	373	128	23	1	<.001
Conjunctivitis	50	4	1	0	152	41	15	0	<.001
Headache	14	3	4	0	11	0	0	0	.007§
Arthralgia	30	10	0	0	49	17	5	0	.047
Hypertriglyceridemia	24	4	0	0	83	10	1	0	.12
Abnormal vision	12	2	2	1	12	3	1	0	.75
Abnormal LFTs	20	2	6	1	32	5	1	0	.45
GI symptoms	29	10	2	0	28	9	3	0	1.00
Fatigue	19	6	2	0	20	5	3	0	1.00

*G = grade; LFTs = liver function tests; GI = gastrointestinal.

†The numbers in these columns include multiple toxic incidents in single patients; multiple incidents of the same toxicity were counted as only one incident (at the highest grade).

‡Two-sided *P* values by Fisher's exact test based on the proportion of grades 2, 3, and 4 toxic effects in the isotretinoin versus placebo arm.

§This toxic effect was statistically significantly higher in the placebo arm.

only 41 patients on isotretinoin, all of whom were near the end of their 3-year treatment, and did not affect any of the primary or major secondary statistical analyses.

Discussion

We found no statistically significant differences between oral isotretinoin (30 mg/day) and placebo with respect to the rates of all SPTs (the primary endpoint), smoking-related SPTs, recurrence, or survival in this large phase III randomized controlled trial involving 1166 randomly assigned patients with pathologic stage I NSCLC. Isotretinoin-related increases in arthralgia and mucocutaneous toxic effects were consistent with previously reported data *(10–12,16–18,28–31),* and the 3-year noncompliance rates in the isotretinoin and placebo arms were 40% and 25%, respectively. The high annual rates of SPTs (3.9%) and recurrence (6.2%) in the placebo arm highlight the need for novel chemoprevention, screening, and adjuvant approaches for the control of stage I NSCLC.

Our overall (in isotretinoin plus placebo patients) secondary univariate analyses involving the three stratification factors (tumor stage, histology, and smoking status) showed that stage T_2 was associated with increased recurrence and increased mortality, squamous histology was associated with increased mortality, and current or former smoking (versus never smoking) was associated with increased mortality. In multivariate analyses, there were no interactions between treatment and either tumor stage or histology. However, these analyses suggested that there were statistically significant interactions between treatment and smoking status: Mortality and recurrence were increased in current smokers but were decreased in never smokers in the isotretinoin arm (versus in the placebo arm). The (unadjusted) Kaplan–Meier estimates also suggested that mortality (statistically significantly) and recurrence (not statistically significantly) were increased in current smokers in the isotretinoin arm and that mortality and recurrence were decreased (not statistically significantly) in never smokers in the isotretinoin arm (versus in the placebo arm). A provocative statistically nonsignificant beneficial trend in recurrence and mortality occurred with longer follow-up in the former smokers (the overall largest subgroup) in the isotretinoin arm (*see* Fig. 4). The results of our *post hoc* analyses of a smoking–isotretinoin interaction and the other secondary/subset findings need to be interpreted with care because they are susceptible to chance occurrence *(32).* As with the other two stratification factors, smoking status was selected because of its likely independent effects on the major clinical endpoints, not because of any anticipated interaction with treatment. There was no smoking–isotretinoin interactive effect on SPT development, which is an important finding that relates to the purely preventive primary hypothesis of our trial.

Our assessments of smoking status in the placebo arm (Table 2) provide important preliminary data on the independent effects of this stratification factor in stage I NSCLC patients. The average annual SPT, recurrence, and mortality rates in patients on placebo were consistently, but not statistically significantly, lower in current smokers than in former smokers. The lowest SPT rate occurred in never smokers on placebo. These data must be interpreted cautiously, however, because of their subset nature and the relatively short 3.5-year median follow-up. We plan longer follow-up studies to help clarify the associations between smoking status and clinical outcomes of patients with early-stage NSCLC.

The clinical study of isotretinoin and other retinoids is decades old. Retinoids are approved by the U.S. Food and Drug Administration (FDA) for use in several malignant and nonmalignant disease settings. These agents have also produced promising preclinical and clinical data in a number of other settings, including cancer prevention and therapy and treatment of other serious diseases, such as diabetes, hypertension, heart disease, and emphysema *(9–12,28,33–46).* Isotretinoin and other retinoids have well-established short- and long-term toxicity profiles *(10–12,28,29),* neither of which includes interactions with smoking. The limited clinical data on smoking–retinoid interactions come from the European Study on Chemoprevention With Vitamin A and *N*-Acetylcysteine (EUROSCAN), a large phase III trial that tested the efficacy of retinyl palmitate and *N*-acetylcysteine in preventing tobacco-related SPTs associated with both lung and head and neck cancer *(47).* The EUROSCAN trial included open-label drug distribution and a 2 × 2 factorial design and enrolled approximately 250 patients with NSCLC in each arm. The rates of overall, event-free, and tobacco-related SPT-free survival associated with retinyl palmitate in the EUROSCAN trial were not lower in never smokers (6% of the trial population) than they were in current and former smokers combined. The EUROSCAN smoking analyses did not distinguish the results of current versus former smokers or of lung cancer patients versus head and neck cancer patients. The major differences in study design, lung cancer population (stage, prior therapy, histology), form of retinoid tested, and other aspects make it difficult, if not impossible, to directly compare the EUROSCAN trial with our trial.

Two small, short-term, randomized, placebo-controlled studies in lung premalignancy have produced additional results concerning smoking status and retinoid treatment. A trial of isotretinoin in bronchial metaplasia *(48)* found differential treatment trends by smoking status. Active smokers on isotretinoin had slightly worse metaplasia outcomes than did active smokers on placebo, whereas patients who had recently quit smoking had slightly better metaplasia outcomes on isotretinoin than recent quitters on placebo. A trial of fenretinide, which has both retinoic acid receptor (RAR)-dependent and -independent effects

(49), found no effect of treatment on bronchial metaplasia in active smokers *(50).*

Two previous large-scale randomized, controlled trials of β-carotene or β-carotene plus vitamin A in the prevention of primary lung cancer found adverse effects in smokers. The primary analyses of both trials found that lung cancer incidence and mortality increased in current smokers in the treatment arms *(51,52).* Results from *in vitro* and animal studies *(53–59)* have elucidated a biologically plausible mechanism for the interaction between smoking and β-carotene. An animal study *(58)* showed that the combination of β-carotene and smoking is associated with suppression of the RAR-β and overexpression of the transcription factor AP-1 in the lung. In contrast, isotretinoin increases RAR-β expression, which frequently is suppressed by tobacco-related carcinogenesis of the lung and head and neck *(60–62).*

However, it is important to note two substantial differences between our results with isotretinoin and these earlier findings with β-carotene with respect to smoking. First, the effects of β-carotene on the primary endpoint, lung cancer incidence, are definitive, whereas the effects of isotretinoin on secondary endpoints within subsets in our study are uncertain. Second, β-carotene was shown to increase development of new cancers, whereas our findings revealed no adverse smoking–treatment interaction involving new cancers (SPTs).

Earlier evidence suggested that retinoids are beneficial in treating oral premalignancy and in preventing SPTs associated with head and neck cancer. However, despite these findings and the common etiology of tobacco-induced field carcinogenesis shared by cancers of the lung and head and neck, the two types of cancer appear to differ substantially in their biology, particularly in their responses to retinoids. Retinoic acid treatment inhibits growth and suppresses AP-1 expression in cell lines derived from head and neck cancer but not in those derived from lung cancer *(63–66).* Isotretinoin increases RAR-β expression to a far greater extent in head and neck carcinogenesis than it does in lung carcinogenesis *(60,61).* Whereas the vast majority of head and neck cancers are squamous cell cancers, only approximately 30% of NSCLCs are of squamous histology. These cancers also show opposite patterns of SPTs and recurrence: Relatively more recurrences occur in T_{1-2} N_0 NSCLC, and relatively more SPTs occur in T_{1-2} N_0 head and neck cancer *(67).* Finally, the results of randomized clinical trials of retinoids, including trials of isotretinoin, have been positive in suppressing head and neck (oral) premalignancy *(16–19,68,69)* but negative in suppressing lung premalignancy *(48,50,70).* The differences between lung and head and neck carcinogenesis also are reflected in a recent DMC review of our ongoing phase III SPT-prevention trial of isotretinoin in head and neck cancer patients. On the basis of the present lung study's secondary smoking data, the head and neck trial DMC examined unblinded data of that trial and opted to continue it.

The molecular mechanisms of retinoid actions in lung carcinogenesis are very complex and not well understood. Consequently, it is not clear why isotretinoin failed to prevent premalignant epithelial cells from developing into NSCLC-associated SPTs. Previous studies *(61,62)* have shown that isotretinoin treatment leads to a modest increase in the expression of RAR-β in the lungs of smokers who do not have lung cancer. Although this finding indicates that the bronchial lung epithelium of smokers may retain some ability to respond to retinoid signaling, this ability is far less than that of oral premalignant lesions *(60).* It is, therefore, possible that the lack of isotretinoin activity in preventing SPTs in our trial was due to defects in the genes that are regulated by retinoic acid via RAR-β. This theory is supported by a study using an *in vitro* model of human lung carcinogenesis that showed that premalignant cells exhibit a decreased response to growth inhibition by retinoic acid compared with normal lung cells, despite having normal RAR-β expression *(71).* Alternatively, it is possible that the systemic delivery of isotretinoin in our present study did not enable sufficiently high concentrations of retinoic acid to reach the lung tissue. In this regard, recent animal studies have found that the direct administration of isotretinoin by inhalation increased expression of RARs *(72)* and had chemopreventive activity *(73).*

The mechanism responsible for the overall resistance of NSCLC recurrence to isotretinoin is also unclear. One possible mechanism for this resistance may be related to defective retinoid signaling, as manifested by the resistance to the growth-inhibitory effects of retinoic acid *(63,64,66,71,74,75)* that is exhibited by most cell lines derived from NSCLC. This resistance to growth inhibition may be related to the suppression of RAR-β expression in the lung, which has been shown *in vitro (75–78)* and *in vivo (61,62,79).* The possible causes of this RAR-β suppression *(80–83)* include the loss of heterozygosity of the 3p24 RAR-β locus *(80),* the silencing of the RAR-β gene by methylation of its p2 promoter *(81),* and the aberrant retinoid signaling despite the presence of other retinoid receptors *(71).* However, it is also possible that other nuclear retinoid receptors are suppressed in the lung *(79,84).* Also, there may be defects downstream of nuclear receptors that block retinoid activity *(85).* Abnormalities in the balance of the orphan receptors COUP and Nur77 *(86)* or co-factors essential for retinoid signaling *(87,88)* could also explain aberrant retinoid signaling in NSCLC.

We can offer only tentative molecular explanations to account for our secondary findings that isotretinoin had a strong differential effect on lung cancer recurrence in the different smoking status groups. Several findings have suggested potential adverse interactions of retinoic acid with tobacco smoke that could mediate lung cancer recurrence. Tobacco carcinogens can suppress RAR-β expression *(89).* In human esophageal cancer, RAR-β expression is selectively lost during carcinogenic progression *in vivo (90)* and is related to retinoid activity *in vitro (91).* Therefore, the recent *in vitro* finding that RAR-β expression is lost in human esophageal cancer cells exposed to the tobacco carcinogen benzo[*a*]pyrene diol epoxide *(89)* may relate to the adverse interaction that we observed between smoking and isotretinoin. Tobacco carcinogens can induce retinoic acid metabolism [e.g., by cytochrome P450 enzymes *(92,93)*] and DNA methylation *(81,93).* Retinoic acid and smoking can increase gastrin-releasing peptide (GRP) expression *(94),* and smoking can increase GRP receptor expression *(95).* Finally, the tobacco carcinogen benzo[*a*]pyrene and retinoic acid can induce NF-κB activation *(96,97).* These smoking-related genetic and epigenetic changes are more prevalent in the lungs of active smokers than in the lungs of former smokers, which may help explain the difference in recurrence between these two subgroups. The possible persistence of these changes in the lungs of former smokers may help to explain the difference in recurrence between former and never smokers. We plan further analyses to determine if the duration of smoking cessation affected the recurrence rate in the treatment arm.

Large-scale, randomized, controlled trials provide valuable information in addition to answering their major endpoint questions. Our trial has provided the most definitive prospective data available to date on the natural history of pathologic stage I NSCLC. Two surprising findings involved our secondary endpoint of recurrence. First, recurrence was a substantially greater problem with respect to incidence and mortality impact than our primary endpoint of SPT prevention, even in the earliest stage pathologic T_1N_0 NSCLC patients. This finding confirmed the earlier preliminary combined-arm data of our trial reported in 1998 *(98)*. Second, we found a provocative isotretinoin–smoking interaction, which requires further basic study. We also plan to study the molecular differences between the SPTs and recurrences *(99)* in our patients with respect to this treatment–smoking interaction.

Cancer chemoprevention has matured tremendously over the past few years *(100–102)*. The positive effects of tamoxifen in breast cancer prevention and celecoxib in controlling familial adenomatous polyposis generated the first approvals by the FDA of cancer chemopreventive agents and led to the discovery of novel molecular targets/models for studies of cancer prevention *(100–113)*. Equally important data are provided when the field definitively tests promising agents that do not prove their hypotheses *(100)* because this ultimately saves limited resources by refocusing research efforts and provides physicians, patients, and the public with hard facts about what cannot reduce cancer risk in specific settings. Paralleling decades of lung cancer therapy survival-outcome results, the disappointing results of all of the completed randomized prevention trials in this site indicate that lung cancer prevention is possibly cancer chemoprevention's most difficult challenge. Although no completed randomized chemoprevention trial has shown a benefit in active smokers, the present secondary findings of a potential benefit in never and former smokers support future chemoprevention studies in this setting.

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NOTES

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