Research Article

Cancer Prevention Research

Mortality in the Randomized, Controlled Lung Intergroup Trial of Isotretinoin

J. Jack Lee[1], Lei Feng[1], Daniel S. Reshef[5], Anita L. Sabichi[2], Brendell Williams[3], Waree Rinsurongkawong[1], Ignacio I. Wistuba[2,4], Reuben Lotan[2], and Scott M. Lippman[2]

Abstract

In 2001, we reported that mortality may have been higher with isotretinoin (30 mg/d for 3 years) than with placebo in the subgroup of current smokers among the 1,166 patients with definitively resected early-stage non–small cell lung cancer who participated in the randomized, controlled Lung Intergroup Trial. We report the overall and cause (cancer, cardiovascular disease, or other)-specific mortality associated with long-term isotretinoin after an extended median follow-up of 6.2 years that included the capture of cause-of-death data from 428 deceased patients. Overall mortality was 36.7% in each of the two trial arms, about two thirds related to cancer and one third to other or unknown causes. Overall and cancer deaths increased in current smokers in the isotretinoin arm during the treatment and the extended follow-up period. No mortality end point increased among never smokers and former smokers taking isotretinoin, and cancer deaths decreased marginally in this combined subgroup. Isotretinoin also increased deaths from cardiovascular disease in current smokers. The present analysis supports the safety of protracted isotretinoin use in the combined group of never smokers and former smokers, which has important public health implications, for example, for treating acne in young people. The increased mortality in current smokers in this study is further evidence of the multifaceted danger of active smoking. The overall indications of this study have public health implications for treating acne in young people and other uses of retinoids in smokers. *Cancer Prev Res; 3(6); 738–44. ©2010 AACR.*

Introduction

Despite intensive therapeutic and smoking cessation efforts, death from lung cancer is higher than from any other cancer in the United States (1). Isotretinoin, also called Accutane or 13-*cis*-retinoic acid, belongs to the retinoid class comprising vitamin A and its natural and synthetic analogues. Based on strong clinical and laboratory data supporting the potential of isotretinoin to reduce the risk of smoking-related cancers (2–5), the multicenter Lung Intergroup Trial (LIT) of isotretinoin in patients with definitively resected stage I non–small cell lung cancer was launched in 1992. LIT primary results were reported in April of 2001 (6).

Authors' Affiliations: Departments of [1]Biostatistics, [2]Thoracic/Head and Neck Medical Oncology, [3]Multicenter Clinical Research, and [4]Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; and [5]Hoffmann-La Roche, Inc., Drug Safety Risk Management, Nutley, New Jersey

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

Data collection, management, and analysis were done by M. D. Anderson personnel independent from Hoffmann-La Roche. D.S. Reshef was also involved in the study design and data interpretation.

Corresponding Author: J. Jack Lee, Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, 1400 Pressler Street, Unit 1411, Houston, TX 77030-4009. Phone: 713-794-4158; Fax: 713-563-4243; E-mail: jjlee@mdanderson.org.

doi: 10.1158/1940-6207.CAPR-09-0124

LIT involved 1,166 patients randomized to either isotretinoin (30 mg/d) or placebo for 3 years and followed for up to 4 additional years before the study was closed in February 2000. Although isotretinoin did not influence second primary tumors (SPT), recurrence, or mortality in the overall analysis, subgroup analyses involving treatment-by-smoking interactions suggested a treatment-associated benefit in never smokers (reduced recurrence and mortality versus placebo) and harm in current smokers (increased recurrence and mortality versus placebo). The increased number of deaths in current smokers on isotretinoin was difficult to interpret because of limited follow-up and cause-of-death information. LIT results are consistent with the overall finding of increased lung cancer incidence with retinoids and other agents in subjects who continue to smoke (7–10).

The LIT treatment-by-smoking interaction, especially the significantly increased mortality in current smokers on isotretinoin (versus in current smokers on placebo), raised concerns for the safety of isotretinoin in smokers. Therefore, we conducted the present extended analysis of the causes of death in LIT patients, which has important safety implications for the long-term use of isotretinoin in other settings.

Materials and Methods

The protocol amendment for the extended follow-up of LIT was reviewed and approved by the M. D. Anderson

Institutional Review Board and each participating institution, and written informed consent was signed by patients who were still alive at the time of this follow-up.

The analyzed study population included 1,166 eligible patients, who were properly randomized between February 1993 and June 1997 to receive placebo (n = 577) or isotretinoin (n = 589; ref. 6). In the present follow-up study, cause-of-death data on case report forms for all randomized patients who had died during or following the trial were submitted to the M. D. Anderson Cancer Center. In addition, 150 charts were reviewed during 21 visits to sites reporting five or more deaths. The case report forms did not include treatment group assignments, and the site-visit chart reviews were conducted in a blinded fashion to eliminate potential bias based on treatment group assignment from the cause-of-death determinations. Data collected during the original LIT analyses were updated and augmented by variables including the last follow-up date for patients who are still alive and death date for deceased patients. We reviewed death documents, including the autopsy report, hospital summary report, treating physician report, and death certificate, for each deceased patient. We carefully assessed the cancer status at death and cause of death, and we collected and examined detailed prior histories of cardiovascular events and chronic obstructive pulmonary disease/emphysema during the extended follow-up. Cardiovascular disease (CVD) deaths included death from ischemic heart disease (e.g., myocardial infarction, angina pectoris, and heart failure) and stroke.

We plotted Kaplan-Meier curves and cumulative incidence curves to summarize the time-to-event data. Analysis of the treatment effect on cause-specific death used competing risk models, including the cause-specific hazard rate (under the Cox proportional hazards model) and cumulative incidence rate (applying the K-sample test; refs. 11–13). The main distinction between the two approaches for analyzing treatment effect involves other competing risks, which were excluded from the cause-specific hazard rate analysis and were included in the cumulative incidence rate method. We constructed hazard ratio estimates to estimate the treatment effect over time (14) and applied Gail and Simon's likelihood ratio test to test the qualitative interactions between treatment and smoking status (15).

Results

The study population had an average age of 64 years and median age of 65 years. Fifty-seven percent were male and 92% were white. There were three stratification factors—tumor histology, tumor stage, and smoking status at registration. The overall distributions within these factors were as follows: 32% squamous versus 68% nonsquamous tumors; 54% T_1 versus 46% T_2 lesions; and 8% never smokers versus 53% former smokers (quit ≥1 year) versus 39% current smokers (quit <1 year or actively smoking). The two treatment arms were well balanced with respect to both demographic characteristics and the stratification factors. A total of 428 deceased and 738 living patients were included in the present study. The median follow-up of living patients was 6.2 years, compared with 3.5 years in the previously reported analysis (6). This median follow-up period is more than twice as long as the period of treatment (3 years) stipulated in the original LIT protocol. The updated Kaplan-Meier plots for the SPT-free survival, recurrence-free survival, and overall survival in the isotretinoin and placebo groups by smoking status can be found in Supplementary Fig. S1.

Table 1 lists the overall and cause-specific mortality rates in both trial arms. Overall mortality rates and cancer mortality rates were nearly equivalent between the two arms. Cancer accounted for about 69%, CVD for 11%, and pulmonary disease for 8% of overall mortality. Of a total 295 cancer deaths, all but 1.7% (3 from pancreatic cancer, 1 from bladder cancer, and 1 from lymphoma) were attributable to lung cancer. Table 2 provides the analyses of cause-specific death by smoking status in the isotretinoin

Table 1. Causes of death in each treatment arm

	Placebo (n = 577)	Isotretinoin (n = 589)	Total (N = 1,166)
Deaths	212 (36.7%)	216 (36.7%)	428 (36.7%)
Causes of death			
Cancer	146 (68.9%)	149 (69.0%)	295 (68.9%)
Other	52 (24.5%)	56 (25.9%)	108 (25.2%)
Pulmonary diseases/complications	13 (6.1%)	20 (9.3%)	33 (7.7%)
CVD	23 (10.8%)	24 (11.1%)	47 (11.0%)
Suicide	1 (0.5%)	0 (0%)	1 (0.2%)
Others*	15 (7.1%)	12 (5.6%)	27 (6.3%)
Unknown	14 (6.6%)	11 (5.1%)	25 (5.9%)

*These 27 deaths from other causes include infection (10), hematologic complications (9), Alzheimer's/dementia (2), choking (1), accident (1), renal failure (1), dehydration (1), complication caused by another drug (1), and natural causes (1).

Table 2. Cause-specific deaths for isotretinoin versus placebo by the Cox proportional hazards model and cumulative incidence analysis by the K-sample test

		Cox proportional hazards model				K-sample test
Smoking status	**Cause of death**	**Parameter estimate**	**SE**	***P***	**HR (95% CI)**	***P***
Never smokers	Cancer	−0.70	0.52	0.18	0.50 (0.18-1.37)	0.18
	Cardiovascular	−0.34	1.41	0.81	0.71 (0.04-11.37)	0.89
	Other	−0.95	1.22	0.44	0.39 (0.04-4.26)	0.50
Former smokers	Cancer	−0.19	0.17	0.25	0.83 (0.60-1.15)	0.28
	Cardiovascular	0.28	0.43	0.52	1.33 (0.57-3.10)	0.44
	Other	−0.29	0.29	0.31	0.75 (0.43-1.31)	0.36
Current smokers	Cancer	0.32	0.18	0.07	1.38 (0.98-1.95)	0.10
	Cardiovascular	−0.17	0.42	0.69	0.84 (0.37-1.93)	0.53
	Other	0.47	0.35	0.18	1.60 (0.81-3.17)	0.29
All patients	Cancer	−0.01	0.12	0.96	0.99 (0.79-1.25)	0.93
	Cardiovascular	0.01	0.29	0.97	1.01 (0.57-1.79)	0.97
	Other	−0.04	0.22	0.84	0.96 (0.63-1.46)	0.83

versus placebo arm and in the overall patient population. There was no significant treatment effect on any specific cause of death by either test (Cox proportional hazards model in the setting of cause-specific competing risk and the K-sample test) used in this analysis (Table 2). Isotretinoin showed a nonsignificant protective effect for cancer [hazard ratio (HR), 0.50; 95% confidence interval (95% CI), 0.18–1.37; $P = 0.18$, Cox model] in never smokers. On the other hand, isotretinoin showed a nonsignificant increase in cancer mortality in current smokers [HR, 1.38; 95% CI, 0.98-1.95; $P = 0.07$, Cox model]. Treatment effects on CVD or other causes of death were weaker. Similar results occurred in analyses that included patients' prior disease histories as covariates.

Figure 1 shows the cumulative incidence curves (based on competing risk models) of deaths from cancer, CVD, and other causes for all patients and subgroups of patients defined by smoking status. These curves for males and females are shown in Supplementary Fig. S2A and B, respectively. There was no apparent treatment effect on cancer, CVD, and other deaths in all patients (panel A). The plot for never smokers suggests a protective effect of treatment (versus placebo) against cancer death (panel B), although the cumulative incidence of cancer death was not statistically significantly different ($P = 0.18$, K-sample test). Former smokers on treatment and placebo had substantially overlapping mortality risk curves, indicating no significant differences in cancer mortality (panel C). Current smokers on treatment (versus placebo) had a trend toward increased cancer death ($P = 0.10$, K-sample test; panel D). Noncancer death had lower cumulative incidences than cancer death but, similar to cancer death, was reduced in never smokers or former smokers and was increased in current smokers of the treatment arm (versus placebo).

The previously reported LIT analyses indicated some significant interactions between treatment and patient smoking status. The magnitudes of these interactions were reduced (with tighter CIs) but remained statistically significant in the present analysis, in which the mortality HR of the interaction between treatment and current smoking (versus never smoking) was 2.89 (95% CI, 1.14–7.29; $P = 0.025$; Table 3). The interaction between treatment and former smoking (versus never smoking) was 1.81 (95% CI, 0.72–4.53; $P = 0.21$; Table 3). Previously reported associations in LIT between stage or histology and mortality, however, persisted at similar magnitudes in the follow-up analyses. Multicovariate Cox regression analysis (Table 3) showed that higher mortality was significantly associated with stage T_2 (versus T_1; HR, 1.35; 95% CI, 1.11–1.63; $P = 0.002$) and with squamous histology (versus nonsquamous; HR, 1.39; 95% CI, 1.14–1.69; $P = 0.001$). Table 4 further characterizes the quantitative and qualitative interaction by presenting the treatment effect on mortality across never, former, and current smokers by the Cox model and Gail and Simon's interaction tests after adjustment for stage and histology. Overall survival was significantly worse in current smokers on treatment (versus on placebo; HR, 1.36; 95% CI, 1.02-1.82; $P = 0.035$). The protective effect of isotretinoin in never smokers was marginally significant (HR, 0.46; 95% CI, 0.19-1.12; $P = 0.086$). The test of quantitative interaction showed that isotretinoin had different effects in the three smoking groups ($P = 0.013$). Furthermore, the Gail and Simon's likelihood ratio tests showed a significant qualitative interaction in overall survival; that is, treatment moved overall survival in opposite directions in different subgroups ($P < 0.05$).

We also estimated the HRs of treatment (versus placebo) for cancer death and CVD death at different times, allowing us to compare effects during treatment with those after stopping treatment [Fig. 2; methods of analyses (14) are in the figure caption]. Figure 2 reflects analyses in all patients stratified into two groups—never smokers plus former smokers and current smokers. Isotretinoin had a

slightly protective effect against cancer death in never smokers plus former smokers, with an HR <1 and an upper end of the CI hovering just below 1 between years 3 and 4 (panel A). On the other hand, isotretinoin had a trend of increased cancer death in current smokers (panel C) and increased CVD death in current smokers before 2 years of treatment (panel D). These findings were similar between males and females (Supplementary Fig. S3A and B).

Discussion

The treatment-by-smoking interactions of the original LIT analysis—harm in current smokers and benefit in never smokers—weakened in the extended analysis, which added more events collected after treatment had stopped. For example, the overall mortality HR in current smokers dropped from 4.39 in the original (6) to 2.89 in the extended analysis. This attenuation of treatment effects likely reflects the substantial posttreatment component (44%) of the median follow-up of the extended analysis. Cancer and CVD deaths increased in current smokers in the isotretinoin arm during the treatment period. No mortality end point increased among never smokers and former smokers taking isotretinoin, and cancer deaths decreased marginally in this combined subgroup during the treatment period. We found no benefit or harm in former smokers alone. The slightly negative effect of isotretinoin on SPT-free survival in never smokers likely is an artifact of a very small subgroup analysis; if real, however, this finding could reflect molecular distinctions between recurrent and second primary lung cancer (or certain aspects of retinoid biology, as discussed

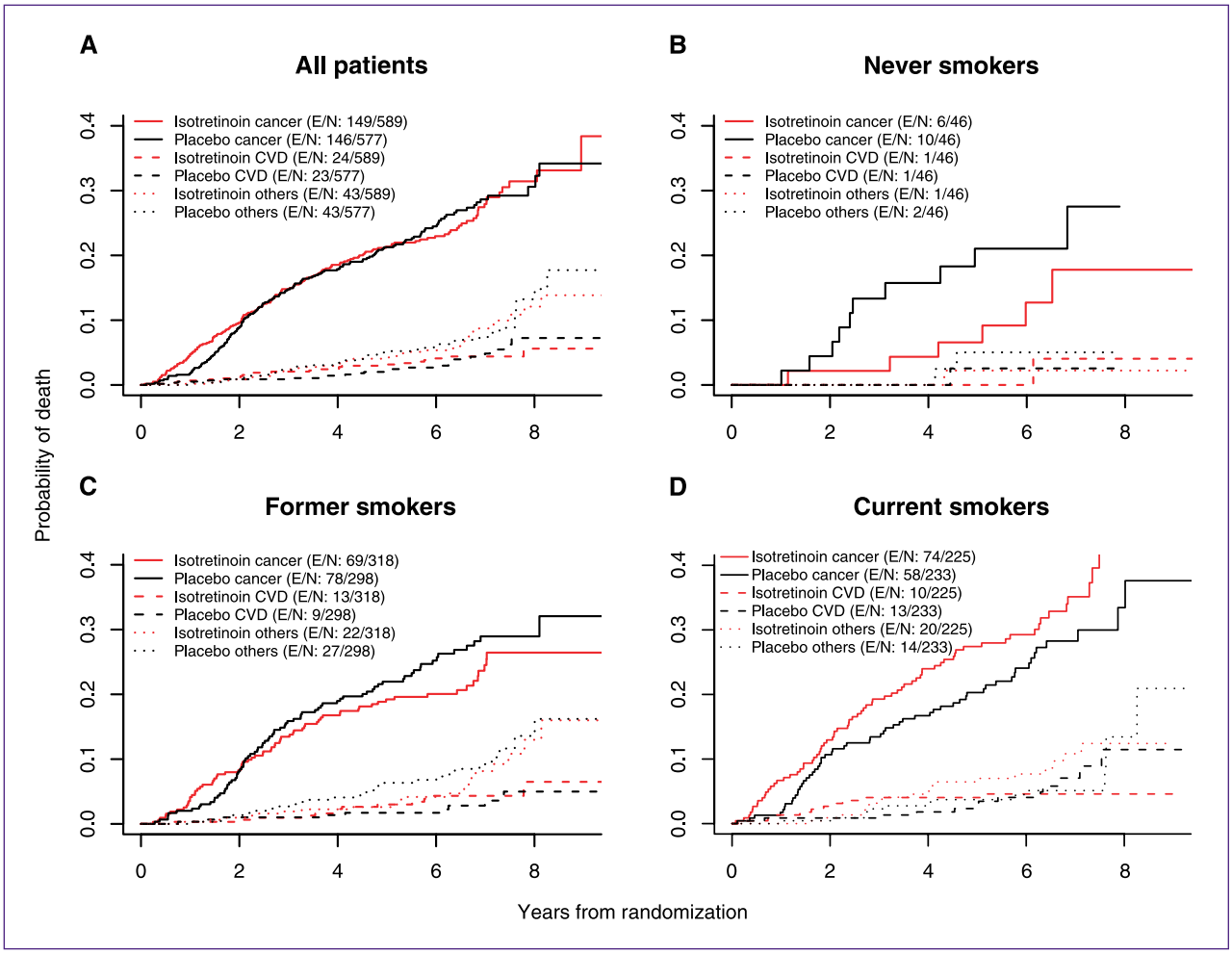

Fig. 1. Cumulative incidence curves of cancer death (solid lines), cardiovascular death (dashed lines), or other death (dotted lines) for the isotretinoin (red lines) and placebo (black lines) groups for all patients (A), never smokers (B), former smokers (C), and current smokers (D). The *X* axes show the number of years after randomization. The numbers of deaths, or events (*E*), from cancer, CVD, and other causes, and the number of patients (*N*) that went into constructing each cause-of-death curve are shown. In A, for example, isotretinoin has 149 cancer, 24 cardiovascular, and 43 noncancer deaths with a *N* of 589, and placebo has 146 cancer, 23 cardiovascular, and 43 noncancer deaths with a *N* of 577. Number of events (*E*) for each cause of death and total number of patients (*N*) are also shown in B, C, and D.

Table 3. Multicovariate mortality analysis (Cox model)

Covariate	Mortality	
	HR (95% CI)	*P*
Treatment		
Isotretinoin	0.47 (0.20-1.14)	0.095
Placebo	1.00	
Stage		
T_2	1.35 (1.11-1.63)	0.002
T_1	1.00	
Histology		
Squamous	1.39 (1.14-1.69)	0.001
Nonsquamous	1.00	
Smoking status		
Current smokers	1.21 (0.67-2.18)	0.52
Former smokers	1.13 (0.63-2.02)	0.68
Never smokers	1.00	
Treatment-by-smoking status		
Current smokers	2.89 (1.14-7.29)	0.025
Former smokers	1.81 (0.72-4.53)	0.21
Never smokers	1.00	

below). The pattern of recurrence-free survival in relation to the smoking status of isotretinoin patients—longer survival with less smoking exposure—also involves small subgroups and nonsignificant findings; however, this pattern is supported by the treatment- by-smoking interactions of the original and follow-up analyses.

Our long-term LIT findings complement long-term follow-up results of two other major lung cancer prevention trials, the Alpha-Tocopherol, Beta-Carotene (ATBC) trial (16) and Beta-Carotene and Retinol Efficacy Trial (CARET; ref. 17), both of which found initial harmful smoking-treatment interactions that dissipated or weakened in long-term follow-up after treatment had stopped. The ATBC trial was conducted in 29,133 male smokers, whereas CARET was conducted in 18,314 men and women smokers and/or asbestos-exposed workers. The three trials differed importantly in clinical parameters (including high-risk noncancer participants in the ATBC trial and CARET versus resected stage I non–small cell lung cancer patients in LIT), interventions, and demographics (including men and women in LIT and CARET versus only men in ATBC). Notwithstanding these and other differences, however, we offer the following comparative observations. The β-carotene–related effect of increased mortality in the all-male ATBC population of smokers was due to CVD and lung cancer during treatment and primarily to CVD during posttreatment follow-up (α-tocopherol was not associated with increased mortality). All-cause, lung cancer, and CVD mortality in CARET increased during treatment, remaining significant for lung cancer in posttreatment follow-up. CARET also detected gender differences in treatment-related mortality. A significant increase in CVD mortality during treatment persisted strongly afterward in women but not in men (especially in the first 3 years posttreatment), and all-cause, lung cancer, or CVD mortality increased far more in women than in men. The LIT and ATBC trials do not necessarily reflect these unexpected gender differences in CARET mortality. A relatively small number of CVD deaths makes them difficult to interpret in LIT.

The beneficial trends of isotretinoin in never smokers are consistent with major biological differences in lung cancer development between smokers and never smokers, which have been detected at histopathologic and molecular levels (18). Most lung cancers in never smokers are adenocarcinomas, whereas squamous cell carcinomas and small-cell carcinomas are associated more strongly with smoking than with never smoking (19). Lung adenocarcinomas in never smokers are characterized by significantly higher frequencies of *EGFR* and *HER2* tyrosine kinase domain mutations, and in smokers by *ALK-EML4* fusion gene abnormalities (20) and expression of the estrogen receptor (21). Lung adenocarcinomas in smokers also have a significantly higher frequency of *KRAS*

Table 4. The treatment effect on all-cause mortality by smoking status as analyzed by the Cox model and Gail and Simon's interaction (adjusted for stage and histology)

	Smoking status		
	Never (*n* = 92)	Former (*n* = 616)	Current (*n* = 458)
Coefficient estimate	–0.785	–0.158	0.310
SE of coefficient	0.457	0.136	0.147
P value for testing no effect	0.086	0.24	0.035
HR (95% CI)	0.46 (0.19-1.12)	0.85 (0.65-1.11)	1.36 (1.02-1.82)
(Estimate/SE)^2	2.943	1.363	4.454
Test of heterogeneity (*H*)	Averaged standard treatment effect = 0.019		
	Test statistics H = 8.72 (2 *df*), P = 0.013		
Test of qualitative (*Q*) interaction between	Q^+ = 4.454; Q^- = 4.306		
treatment effect and smoking status	Min(Q^+, Q^-) = 4.306; threshold = 4.23 for P = 0.05		

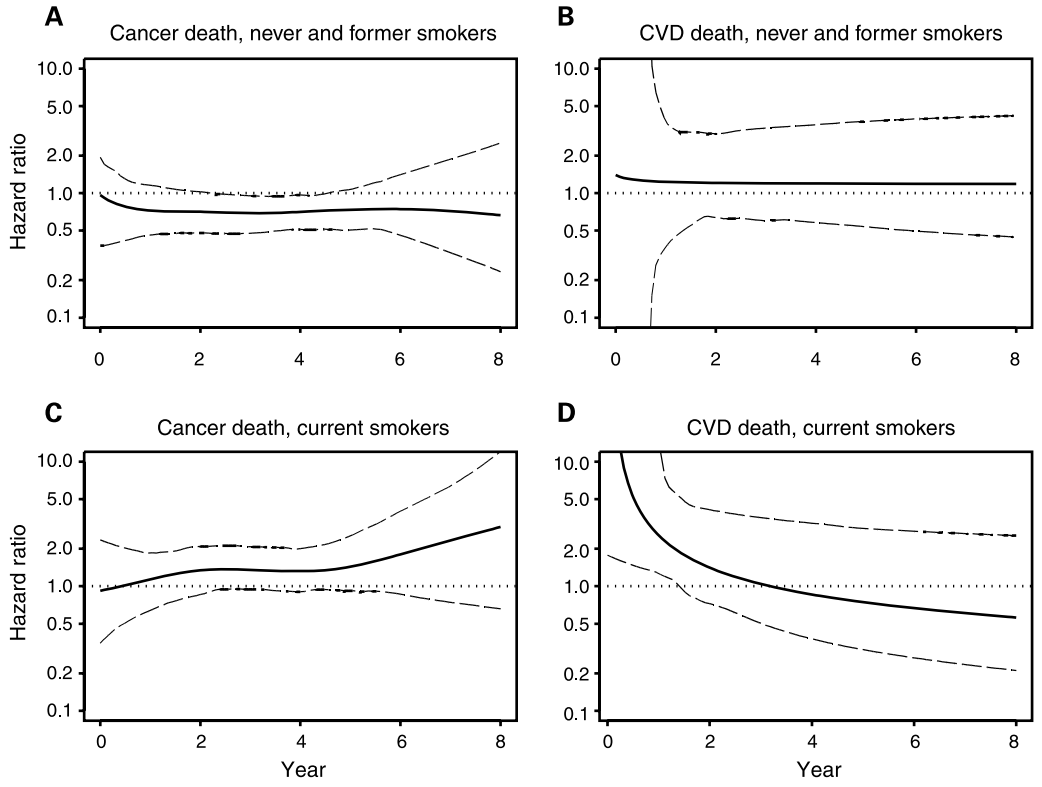

Fig. 2. A to D, HRs of isotretinoin (versus placebo) over time for cancer death and CVD death stratified by never smokers plus former smokers or current smokers. Results are shown for all patients. The hazard rates were estimated by the nonparametric Nelson and Aalen method and triquadratic kernel smoother with boundary corrections at different time points (14). HRs were plotted on the logarithmic scale to provide a symmetrical comparison of the potential protective effect (HR <1) or harmful effect (HR >1). Pointwise 95% confidence intervals (dashed lines) were calculated using the bootstrap method for each smoothed HR curve.

mutations (compared with adenocarcinomas in never smokers), and of note, the *KRAS* mutation profile also differs between these cancers in smokers (mostly G-to-T or G-to-C transversions) and never smokers (G-to-A transition; ref. 22). Also, *TP53* is frequently mutated in lung cancer (19), and the differing pattern of *TP53* point mutations in smokers (G-to-T transversions and A-to-G transitions) and never smokers (G-to-A transitions) with lung cancer is similar to that of *KRAS*.

Since the original 2001 report of LIT (6), some progress has been made in the field of retinoid research that may have implications for the interpretation of this trial. For example, we now can better explain the failure of isotretinoin to prevent SPTs. The majority of non–small cell lung cancer patients may develop a resistance to isotretinoin due to aberrant retinoid receptor expression (23) resulting from the suppression of retinoic acid receptor β2 expression in smokers by tobacco carcinogens (24) or nicotine (25), which silence the gene promoter by methylating CpG islands (26, 27). Also, isotretinoin could protect lung epithelial cells from apoptosis induced by chronic exposure to the high oxygen tension and free radicals associated with cigarette smoking, thus increasing the pool of progenitor cells with DNA damage that can eventually develop into SPTs (27, 28).

The possibility that isotretinoin increased SPT incidence relative to placebo in never smokers is less biologically plausible and more difficult to interpret (than the neutral effects of isotretinoin on SPTs in current smokers). One potential mechanism of such an effect, however, is that isotretinoin can increase levels of the serum marker of DNA oxidative damage 8-hydroxy-2-deoxyguanosine (8-OHdG) and decrease total antioxidant status, as was observed by Georgala et al. in cystic acne patients treated with systemic isotretinoin at 0.5 mg/kg per day (29). The authors suggested that serum levels of 8-OHdG in patients taking isotretinoin may increase from a direct effect of isotretinoin on liver, muscle, and epidermal cells. If isotretinoin caused similar DNA damage resulting in increased 8-OHdG in the lung epithelial cells of nonsmoking LIT patients, this could have led to increased malignant transformation and SPT development. This hypothesis is supported by data indicating that 8-OHdG and other products of oxidative DNA damage and repair are associated with a susceptibility to lung cancer (30). Again, these mechanistic speculations are more academic than

practical because we believe the finding of increased SPTs in never smokers taking isotretinoin very likely was an artifact of a very small subgroup analysis.

Note that isotretinoin as a potential chemopreventive agent is an off-label use. The present analyses indicate that isotretinoin administered over a protracted period of time potentially was harmful in current smokers, although the overall mortality HR lessened in this extended follow-up, and safe in the combined group of never smokers and former smokers. Despite clinical, biological, and dosing differences between our lung cancer population and other retinoid users such as young people taking isotretinoin for severe cystic or nodular acne, our present findings may have public health implications for the use of retinoids.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Susan P. Ackermann Shiff and Xian Zhou for their earlier involvement in the study.

Grant Support

Grants CA45809 and CA16672 from the National Cancer Institute and a contract (CS2002-6792RM) from Hoffmann-La Roche, Inc.

Received 06/22/2009; revised 10/16/2009; accepted 12/08/2009; published OnlineFirst 05/25/2010.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics. CA Cancer J Clin 2008;58:71–96.
2. Hong WK, Endicott J, Itri LM, et al. 13-*cis*-Retinoic acid in the treatment of oral leukoplakia. N Engl J Med 1986;315:1501–5.
3. Hong WK, Lippman SM, Itri LM, et al. Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. N Engl J Med 1990;323:795–801.
4. Lippman SM, Batsakis JG, Toth BB, et al. Comparison of low-dose isotretinoin with β carotene to prevent oral carcinogenesis. N Engl J Med 1993;328:15–20.
5. Lippman SM, Hong WK. Not yet standard: retinoids versus second primary tumors [Editorial]. J Clin Oncol 1993;11:1204–7.
6. Lippman SM, Lee JJ, Karp DD, et al. Randomized Phase III intergroup trial of isotretinoin to prevent second primary tumors in Stage 1 non-small cell lung cancer. J Natl Cancer Inst 2001;93:605–18.
7. Mayne ST, Lippman SM. Retinoids, carotenoids, and micronutrients. In: DeVita VT, Hellman S, Rosenberg SA, editors. Principles and practice of oncology. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
8. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst 1996;88:1550–9.
9. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and β carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–35.
10. van Zanwijk N, Dalesio O, Pastarino U, de Vries N, van Tinteren H. Euroscan, a randomised trial of vitamin A and *N*-acetylcysteine in patients with head and neck cancer or lung cancer. J Natl Cancer Inst 2001;92:977–87.
11. Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. New York: Wiley; 1980.
12. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. Annals of Statistics 1988;16:1141–54.
13. Fine JP, Gray RJ. A proportional hazard model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509.
14. Hess KR, Serachitopol DM, Brown BW. Hazard function estimators: a simulation study. Stat Med 1999;18:3075–88.
15. Gail M, Simon R. Testing for qualitative interactions between treatment effects and patient subsets. Biometrics 1985;41:361–72.
16. Virtamo J, Pietinen P, Huttunen JK, et al. Incidence of cancer and mortality following α-tocopherol and β-carotene supplementation: a postintervention follow-up. JAMA 2003;290:476–85.
17. Goodman GE, Thornquist MD, Balmes J, et al. The Beta-Carotene and Retinol Efficacy Trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping β-carotene and retinol supplements. J Natl Cancer Inst 2004;96:1743–50.
18. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med 2008;359:1367–80.
19. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. Nat Rev Cancer 2007;7:778–90.
20. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561–6.
21. Raso MG, Behrens C, Herynk MH, et al. Immunohistochemical expression of estrogen and progesterone receptors identifies a subset of NSCLCs and correlates with EGFR mutation. Clin Cancer Res 2009;15:5359–68.
22. Riely GJ, Kris MG, Rosenbaum D, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res 2008;14:5731–4.
23. Brabender J, Metzger R, Salonga D, et al. Comprehensive expression analysis of retinoic acid receptors and retinoid X receptors in non-small cell lung cancer: implications for tumor development and prognosis. Carcinogenesis 2005;26:525–30.
24. Song S, Xu XC. Effect of benzo[*a*]pyrene diol epoxide on expression of retinoic acid receptor-β in immortalized esophageal epithelial cells and esophageal cancer cells. Biochem Biophys Res Commun 2001;281:872–7.
25. Chen GQ, Lin B, Dawson MI, Zhang XK. Nicotine modulates the effects of retinoids on growth inhibition and RARβ expression in lung cancer cells. Int J Cancer 2002;99:171–8.
26. Toyooka S, Maruyama R, Toyooka KO, et al. Smoke exposure, histologic type and geography-related differences in the methylation profiles of non-small cell lung cancer. Int J Cancer 2003;103:153–60.
27. Kim JS, Lee H, Kim H, et al. Promoter methylation of retinoic acid receptor β2 and the development of second primary lung cancers in non-small-cell lung cancer. J Clin Oncol 2004;22:3443–50.
28. Moreno-Manzano V, Ishikawa Y, Lucio-Cazana J, Kitamura M. Suppression of apoptosis by all-*trans*-retinoic acid. J Biol Chem 1999;274:20251–8.
29. Georgala S, Papassotiriou I, Georgala C, Demetriou E, Schulpis KH. Isotretinoin therapy induces DNA oxidative damage. Clin Chem Lab Med 2005;43:1178–82.
30. Gackowski D, Speina E, Zielinska M, et al. Products of oxidative DNA damage and repair as possible biomarkers of susceptibility to lung cancer. Cancer Res 2003;63:4899–902.