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Randomized Trial of Supplemental β-Carotene to Prevent Second Head and Neck Cancer[1]

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ABSTRACT

β-carotene has established efficacy in animal models of oral carcinogenesis and has been shown to regress oral precancerous lesions in humans. The purpose of this study was to see whether these effects extended to the prevention of oral/pharyngeal/laryngeal (head and neck) cancer in humans. The subject population for this randomized, placebo-controlled, double-blinded clinical trial included 264 patients who had been curatively treated for a recent early-stage squamous cell carcinoma of the oral cavity, pharynx, or larynx. Patients were assigned randomly to receive 50 mg of β-carotene per day or placebo and were followed for up to 90 months for the development of second primary tumors and local recurrences. After a median follow-up of 51 months, there was no difference between the two groups in the time to failure [second primary tumors plus local recurrences: relative risk (RR), 0.90; 95% confidence interval (CI), 0.56–1.45]. In site-specific analyses, supplemental β-carotene had no significant effect on second head and neck cancer (RR, 0.69; 95% CI, 0.39–1.25) or lung cancer (RR, 1.44; 95% CI, 0.62–3.39). Total mortality was not significantly affected by this intervention (RR, 0.86; 95% CI, 0.52–1.42). Whereas none of the effects were statistically significant, the point estimates suggested a possible decrease in second head and neck cancer risk but a possible increase in lung cancer risk. These effects are consistent with the effects observed in trials using intermediate end point biological markers in humans, in which β-carotene has established efficacy in oral precancerous lesions but has no effect or slightly worsens sputum cytology, and in animal carcinogenicity studies, in which β-carotene has established efficacy in buccal pouch carcinogenesis in hamsters but not in animal models of respiratory tract/lung carcinogenesis, with some suggestions of tumor-promoting effects in respiratory tract/lung. If our results are replicated by other ongoing/completed trials, this suggests a critical need for mechanistic studies addressing differential responses in one epithelial site (head and neck) *versus* another (lung).

INTRODUCTION

Squamous cell carcinoma of the oral cavity, pharynx, and larynx, collectively referred to as head and neck cancer, is a major health problem in the United States and throughout the world. It is estimated that 40,400 new cases were identified and that 12,300 patients died of these cancers in the United States in 1999 (1). These cancers also assume a high relative importance because of the functional impairment and cosmetic deformity associated with the cancer and its treatment.

With advances in early detection, nearly one-half of these patients present early with stage I or II disease. Although treatment given at this stage is relatively successful, failure remains common, and is manifested primarily by local recurrences and second primary cancers, the latter of which generally occur in other sites within the head and neck, or in the esophagus or lung. The expected incidence of second primaries varies by site of the initial cancer (2–4), and by current tobacco habits (5), but a reasonable estimate is that 4% of stage I/II patients will develop second primary cancers yearly (6). These second primary cancers are a consequence of field cancerization of the upper aerodigestive tract, usually associated with chronic exposure to tobacco and alcohol. Second cancers of the head and neck are the leading cause of death in patients diagnosed with early-stage head and neck cancers (7). Prevention of second cancers is a model for chemoprevention of epithelial carcinogenesis and has great clinical relevance.

Several agents are known to induce regression of precancerous lesions of the oral cavity, particularly β-carotene and various retinoids. As reviewed elsewhere (8), at least three randomized and six nonrandomized trials of β-carotene and seven of various retinoids have reported significant chemopreventive efficacy in oral precancerous lesions. Retinoids have also been evaluated as potential chemopreventive agents in head and neck cancer, with one trial reporting significant benefit with regard to second primary tumors (9) but two others reporting a lack of benefit (10, 11).

Because β-carotene has been demonstrated to have efficacy in the regression of oral precancerous lesions and lacks the side effects typically seen with higher-dose retinoids, we initiated a trial to evaluate β-carotene in the prevention of second head and neck cancers. The primary objective of this randomized, double-blinded, placebo-controlled trial was to determine whether supplemental β-carotene (50 mg/day) reduces failure attributable to second primary tumors (head and neck, esophagus, and lung) and local recurrences in patients curatively treated for early-stage cancers of the head and neck. A secondary objective was to evaluate the effect of this intervention on overall mortality.

SUBJECTS AND METHODS

Recruitment and Eligibility. The design of this trial has been reported previously (12), and is summarized in Fig. 1. Patients were recruited from two sites, one based at Yale University that recruited from the entire state of Connecticut, and the second based at the University of Miami that recruited from 14 hospitals in south Florida. In Connecticut, patients were identified using the Yale Cancer Center's Rapid Case Ascertainment Shared Resource, which allows for population-based identification of patients with newly diagnosed malignancies. Patients were identified by reviewing pathology reports at all 35 of the state's general acute care hospitals and surveying the Connecticut Tumor Registry on a regular basis. Connecticut participates in the National Cancer Institute SEER (Surveillance, Epidemiology and End Results) Program. IRB[3] approval was obtained from all of the 49 hospitals from which patients were recruited.

To be eligible, patients had to have a recently diagnosed stage I or stage II

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[3] The abbreviations used are: IRB, institutional review board; ICD, International Classification of Disease; ATBC, α-Tocopherol and β-Carotene (trial); CARET, Carotene and Retinol Efficacy Trial; RR, relative risk; CI, confidence interval.

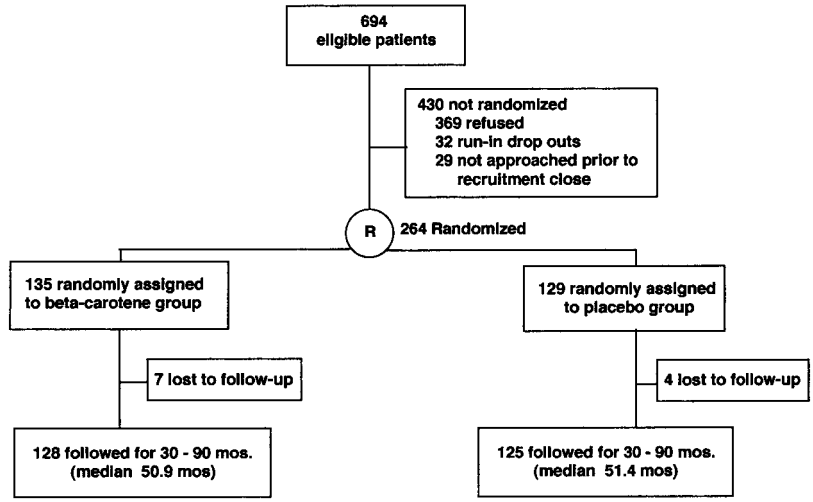

Fig. 1. Study profile and design: recruitment, eligibility, run-in, and follow-up status of patients enrolled in the β-carotene chemoprevention trial of second primary tumors in patients with early-stage squamous cell carcinoma of the head and neck.

squamous cell carcinoma of one of the following sites: tongue (ICD no. 141), gum or mouth (ICD no. 143–145), oropharynx (ICD no. 146), hypopharynx (ICD no. 148), pharynx (ICD no. 149) or larynx (ICD no. 161). Patients with carcinoma *in situ* at the above sites were also eligible. Patients had to be between 20 and 79 years of age, have completed their treatment for the first cancer (generally radiation therapy or surgical excision), be considered free of cancer at any site at entry into the trial, have no significant comorbidities, and not have taken supplements of retinol, β-carotene, vitamin E, or selenium within the past year (multivitamin use allowed). Patients with prior upper aerodigestive tract cancers within the past 5 years, or with synchronous cancers of the esophagus or lung were not eligible to participate. The target sample size was 400+ patients, based on the approach of Lakatos (13), which allows for the adjustment of event rates in the presence of time-dependent rates of losses, noncompliance, drop-ins, and lag in the effectiveness of treatment.

Protocol and Treatment Assignment. The patient's physician was contacted to determine eligibility and to obtain physician consent prior to contacting potential participants. Participants were approached for participation by letter and then by phone; those who agreed were subsequently visited by a trained nurse- or physician-interviewer/phlebotomist (usually in the participant's home), who obtained signed consent prior to proceeding. Participants were enrolled in a 1-month placebo run-in period. Patients who were able to complete the run-in protocol (consumed >75% of the capsules and did not drop out) were randomized to receive either supplemental β-carotene (50 mg/day, Lurotin; BASF, Wyandotte, MI) or a corresponding identical placebo. Study medications were blister packed (one capsule per day) into monthly calendar packs to facilitate compliance with the intervention. A stratified randomization was used, with patients stratified on the basis of tumor site (oral cavity *versus* pharynx *versus* larynx) and smoking history (<25 pack-years *versus* ≥25 pack-years). The randomization list was prepared by the study biostatistician (J. B.) using random permuted blocks with a block size of four within each stratum, and an allocation ratio of 1:1. The Yale and University of Miami Research Pharmacies administered the randomization list, and all of the study personnel remained blinded to the treatment allocation throughout the trial.

Visits were made to subjects' homes by nurse- or physician-interviewer/phlebotomists at the following time points: −1 month (baseline, start of run-in), time 0 (randomization, post-run-in), 3 months, 12 months, and annually thereafter. New calendar packs (up to 6-month supply) were given at these time points; used calendar packs were retrieved to estimate compliance (pill counts). In between these visits, calendar packs were mailed to the participant by the research pharmacies, and participants were sent prepaid return mailers to facilitate the return of used calendar packs.

Patients were contacted every 3 months by phone or visit. Patients who stopped taking the capsules were contacted every 6 months. Information on second cancers was obtained in several ways. Patients were asked to report any possible second cancers, and pathology reports were obtained from the diagnosing institution. Because both Connecticut and south Florida are covered by population-based tumor registries (Connecticut Tumor Registry, Florida Cancer Data System), searches were made of the registries to identify any newly diagnosed malignancies. Death certificates were obtained for deceased participants, and coded by a trained nosologist.

End Point Review. All of the suspected clinical end points were evaluated by an End Point Review Committee comprised of three study physicians. Briefly, an end point review packet that included pathology reports for the first and second cancers for each subject with a suspected end point was assigned to a study physician. That study physician then contacted the patient's primary physician to seek additional clinical information, which was documented in the patient's end point review packet. That same study physician then categorized clinical end points into local recurrences, new primary tumors in the field of prevention (head and neck, esophagus, lung), other incident cancers, regional recurrences, or metastases. A second study physician was then given the end point review packet and classified the end point without knowledge of the first reviewer's classification. If the two agreed, the end point was accepted. End point review meetings involving all three of the study physicians were convened to discuss end points when there was disagreement. Tumors were considered to be second primaries if they occurred >2 cm away from the site of the initial tumor in a patient treated by irradiation, >2 cm away from the suture line in a patient treated by surgery, or >5 years after diagnosis of the first primary tumor. This definition is used clinically but is thought to misclassify some new primary tumors as local recurrences. Patients classified as having curatively treated local recurrences were allowed to continue on the supplement and were followed for second primary tumors; patients with any other end point were taken off the supplement but were followed for the duration of the trial.

Other Data Collection. All of the subjects were asked to report potential side effects at the end of the run-in and at the end of each 3-month interval, using standard toxicity grading scales. Dose-reduction procedures were in place for patients with perceived side effects.

Approximately 20 ml of venous blood was obtained in heparinized vacuum tubes at each of the visits. After centrifugation, plasma was aliquotted and stored at −70°C pending analysis. Samples from Miami were shipped in batches to the Yale Micronutrient Analysis laboratory for analysis. Plasma β-carotene, carotenoids, retinol, and α-tocopherol were analyzed by reverse-phase high-performance liquid chromatography as described previously (14). Laboratory technicians were blinded to treatment assignment. Formal quality control procedures were in place, including use of internal standards, external standards, plasma pools, and participation in the micronutrient measurement proficiency testing program of the National Institute of Standards and Technology. Non-laboratory study personnel did not have access to the plasma β-carotene concentration data from the laboratory, to maintain the blinding.

Patients with newly diagnosed head and neck cancers may temporarily quit smoking at the time of diagnosis. Thus, for these analyses, baseline smoking status is defined as the smoking status immediately prior to the diagnosis of the first cancer. Smoking status was updated annually thereafter for use as a time-dependent covariate in the analysis.

Monitoring. Recruitment for the trial was initiated in late 1990, with the first patients randomized at the Connecticut site in early 1991 and at the Florida site in 1993. In 1994, the ATBC trial, which was a two-by-two factorial trial of α-tocopherol and β-carotene in the primary prevention of lung cancer in smokers, reported a significant excess of lung cancer in β-carotene-supplemented smokers (15). The external data and safety monitoring committee for our trial was convened, and recommended continuation of this trial. All of the participants were notified in writing of the ATBC results immediately prior to public release of the results. In January 1996, investigators from CARET, a clinical trial of β-carotene plus retinyl palmitate in patients at high risk for lung cancer, announced that CARET was being terminated early because of an excess of lung cancer in the intervention group, with the results published in May of 1996 (16). As before, all of the participants in our trial were notified in writing of the CARET results immediately prior to public release; patients who continued to take capsules were reconsented. The external data and safety monitoring committee for our trial was again convened and was given unblinded end point data from this trial, along with unblinded confidential end point data for head and neck cancers provided generously by the three other major β-carotene trials [ATBC, CARET, and the Physicians' Health Study (17)]. Our monitoring committee recommended unanimously that the trial be continued in all of the participants including smokers. However, recruitment of new subjects to the protocol was terminated in January 1996 by the investigators because of concerns about the feasibility of recruiting additional subjects. Thus, recruitment was closed prior to reaching the target sample size. A conditional power analysis was conducted to explore the likely gain from extending the duration of intervention and follow-up in the trial. This analysis indicated that it would not be cost-effective to extend intervention beyond the planned ending date of June 30, 1998.

Statistical Analysis. All of the analyses were performed with SAS (SAS Institute, Cary, NC), using an intention-to-treat analysis. Differences between the two groups in selected demographic characteristics were evaluated using Student's *t* test or χ^2 analyses as appropriate. Survival curves were generated according to the method of Kaplan and Meier, and the log-rank test was used to compare survival distributions between the two groups. All of the *P*s were two-tailed. Cox proportional hazards models were used to calculate the RR of having an end point in the β-carotene group relative to those taking placebo, adjusted for other covariates. Several covariates were examined, including several indices of tobacco exposure, plasma β-carotene levels at baseline, gender, and age. Two time-dependent variables were also incorporated into the models: annual smoking status during the period the subject was active in the trial, and annual mean compliance based on the pill counts. Interaction terms were used in the model to assess whether the treatment effect varied according to smoking status and compliance level. The interaction between age and treatment assignment was also investigated, although the mean age in each treatment arm was essentially the same.

The primary end point of the trial was the combined end point of local recurrence and second primary cancers of the head and neck, esophagus, and lung. The combined end point was selected *a priori* because of diagnostic difficulties in discriminating second primary tumors from local recurrences. Secondary end points included the following: local recurrence; second primary cancers in the field of prevention (head and neck, esophagus, lung); and local recurrence plus second primary cancers in the head and neck. In addition, all-cause mortality was a prespecified secondary end point. Lung cancer was added as an additional end point of interest after the release of the results of CARET and ATBC. For patients with multiple primary tumors, the date of diagnosis of the first primary tumor after randomization was used in the survival analysis.

The Cox proportional hazards model was fitted two ways: no weighting and linear down-weighting for events occurring in the first 12 months after randomization. The results were essentially unchanged; therefore, we present the results based on the nonweighted analysis. Of the patients who were randomized, 6 were later identified as protocol violators: 4 were found to have had a previous head and neck cancer within the past 5 years and 2 were found to have had stage III cancer at the time of randomization. All of the analyses were run with and without protocol violators. The results were not altered; therefore, we present the results based on the full population of 264 randomized subjects.

RESULTS

Recruitment in Connecticut was population-based; therefore, the following numbers from Connecticut reflect the actual recruitment rates from the entire population of potentially eligible patients in this state. These data may be particularly useful for recruitment projections for others planning chemoprevention trials in head and neck cancer. A total of 1994 potentially eligible patients were identified on the basis of hospital pathology reports. Eligibility could not be determined for 313 patients because of physician nonresponse or physician refusal, leaving a total of 1681 patients who were evaluated for clinical eligibility. Of these, 527 were deemed eligible and 1154 ineligible. The primary reasons for ineligibility included late stage at diagnosis ($n = 741$), previous/recurrent head and neck cancer ($n = 141$), death ($n = 64$), taking supplemental vitamins ($n = 39$), or other reasons including synchronous cancers, other severe comorbidities, or non-squamous cell tumors ($n = 169$). Recruitment in Florida was not population based but included a total of 348 potentially eligible subjects, 331 of whom completed full eligibility evaluation. Of these, 164 were ineligible, leaving 167 eligible patients from Florida.

The flow of the 694 eligible patients is shown in Fig. 1. A total of 264 patients were randomized. The characteristics of the 264 randomized patients are shown in Table 1. The two treatment groups were well balanced with no significant differences in any known prognostic factors.

During the period from 1991 to January 1996, a total of 40 patients stopped taking study medications: 14 lost interest, 10 stopped because of perceived side effects, 9 stopped because of comorbid conditions, and 7 stopped for other reasons. With the release of the CARET results in January 1996, an additional 76 patients stopped supplementation. All of the patients continued to be followed for the duration of the trial. The median follow-up for all of the patients was 51.1 months from the date of randomization (50.9 months for β-carotene; 51.4 for

Table 1 *Demographic characteristics[a] of study participants at baseline*

Groups were well balanced as shown. Data are mean ± SD or number of patients and relevant percentage by treatment arm, as indicated.

Characteristic	β-Carotene $n = 135$	Placebo $n = 129$	P^b
Age (mean ± SD)	67.8 ± 9.8	67.9 ± 9.3	0.93
Sex			
Male	113 (84%)	101 (78%)	0.26
Female	22 (16%)	28 (22%)	
Race			
White/non-Hispanic	121 (90%)	114 (88%)	0.72
Hispanic/Puerto Rican	6 (4%)	9 (7%)	
Nonwhite	8 (6%)	6 (5%)	
Tumor site			
Oral cavity	34 (25%)	31 (24%)	0.90
Pharynx	11 (8%)	9 (7%)	
Larynx	90 (67%)	89 (69%)	
Stage[c]			
Carcinoma *in situ*	6 (4%)	7 (5%)	0.87
I	83 (62%)	82 (64%)	
II	45 (34%)	40 (31%)	
Clinical site			
Connecticut	106 (79%)	101 (78%)	0.97
Florida	29 (21%)	28 (22%)	
Baseline smoking status			
Current	65 (48%)	70 (54%)	0.41
Former	61 (45%)	48 (37%)	
Never	9 (7%)	11 (9%)	
Pack-years smoked (mean ± SD)[d]	45.5 ± 30.6	45.5 ± 30.9	0.99
Plasma β-carotene (μg/ml)	0.16 ± 0.14	0.19 ± 0.19	0.24

[a] Demographic characteristics at baseline of the 264 patients randomized in the Carotene Prevention Trial, which allocated patients with first primary head or neck cancer to either 50 mg of β-carotene/day or placebo.
[b] From χ^2 analysis or Student's *t* test as appropriate.
[c] Detailed stage category missing for one early-stage patient.
[d] Never smokers included.

Table 2 *Compliance in taking capsules, by treatment assignment and study year*

Data show proportion of patients taking various categories of supplement by year after randomization and by treatment arm (pill counts).

	β-Carotene	Placebo	Total
Year 1			
>90.0%	105	92	197
75.0–89.9%	15	16	31
50.0–74.9%	5	6	11
<50.0%	1	2	3
Total	126	116	242
Year 2			
>90.0%	76	67	143
75.0–89.9%	5	4	9
50.0–74.9%	3	4	7
<50.0%	1	0	1
Total	85	75	160
Year 3			
>90.0%	57	46	103
75.0–89.9%	3	4	7
50.0–74.9%	1	2	3
<50.0%	0	1	1
Total	61	53	114
Year 4			
>90.0%	42	31	73
75.0–89.9%	3	3	6
50.0–74.9%	3	1	4
<50.0%	0	1	1
Total	48	36	84
Year 5			
>90.0%	26	23	49
75.0–89.9%	2	0	2
50.0–74.9%	0	0	0
<50.0%	1	1	2
Total	29	24	53
Year 6			
>90.0%	13	11	24
75.0–89.9%	0	0	0
50.0–74.9%	2	1	3
<50.0%	0	0	0
Total	15	12	27
Year 7			
>90.0%	6	7	13
75.0–89.9%	0	0	0
50.0–74.9%	0	0	0
<50.0%	1	0	1
Total	7	7	14
Year 8			
>90.0%	2	4	6
75.0–89.9%	0	0	0
50.0–74.9%	0	0	0
<50.0%	0	0	0
Total	2	4	6

placebo) up to a maximum of 90 months. Among the active patients, compliance as assessed by pill counts was excellent (Table 2).

The effect of this intervention on plasma concentrations of β-carotene, other carotenoids, retinol, and α-tocopherol in this population has been reported previously (14). Supplementation with β-carotene produced a persistent 9- to 10-fold increase in median plasma β-carotene concentrations and a persistent 2-fold increase in median plasma α-carotene concentrations [small quantities of α-carotene were detected in the supplement (14)]. Plasma β-carotene concentrations were stable over the multiyear intervention in the placebo group. This intervention did not alter concentrations of lycopene, lutein/zeaxanthin, retinol, or α-tocopherol.

A total of nine patients (five randomized to β-carotene and four to placebo) reported having grade 2 or higher toxicity for one or more of the following symptoms: depression, headache, bone pain, cheilitis, dry skin, and diarrhea. The frequency of reporting of each of these side effects was similar in the two groups. As was expected, skin yellowing was reported more frequently in patients randomized to β-carotene as compared with placebo, with 25 (18%) of 135 in the β-carotene group reporting skin yellowing on at least one occasion as compared with 8 (6%) of 129 participants in the placebo group.

End points by treatment arm, and adjusted RR estimates are shown in Table 3. None of the differences between the two groups were statistically significantly different, although the β-carotene supplemented group had reduced risks for all of the end points with the exception of lung cancers (RR, 1.44; 95% CI, 0.62–3.39). Because lung was the most common site for second primary tumors, this resulted in an overall increased risk of total second primary tumors (RR, 1.20; 95% CI, 0.59–2.45). The relatively low number of second primary tumors in the head and neck region is partially a result of diagnostic misclassification, in that some tumors classified as local recurrences were most likely new primary tumors. However, it is difficult to differentiate local recurrences from new primary tumors in the head and neck region in the absence of a detailed molecular analysis.

Considering all of the cancers in the head and neck region, β-carotene supplementation had no statistically significant effect on risk (RR, 0.69; 95% CI, 0.39–1.25). Although this risk estimate and the lung cancer risk estimate (RR, 1.44) were not statistically significant, the data suggest that this intervention resulted in discordant effects in the head and neck sites *versus* lung.

Total mortality was nonsignificantly decreased by 14% with β-carotene supplementation, primarily because of fewer cardiovascular disease end points in the β-carotene supplemented group. Patients randomized to β-carotene also were significantly ($P = 0.01$) older at their time of death compared with patients randomized to placebo (mean, 69.7 years *versus* 64.4 years; corresponding medians are 70.4 years in the β-carotene group and 66.3 years in the placebo group).

The survival curves for the primary end point and for total mortality, the end points with the greatest number of events, are shown in Figs. 2 and 3. Supplemental β-carotene had no significant effect on time to failure for the primary end point (Fig. 2, $P = 0.59$, log-rank test) or time to death (Fig. 3, $P = 0.41$, log-rank test). Further stratification of the mortality distribution by tobacco use at the time of diagnosis is shown in Fig. 4, and the log-rank test revealed significant differences in the survival distributions ($P = 0.03$). The cumulative probability of survival for both smokers and nonsmokers was nonsignificantly better for those in the β-carotene group. In a model that included variables for age, treatment arm, and smoking status as

Table 3 *Primary and secondary clinical end points, by treatment arm*

Results show number of subjects experiencing a given end point, by treatment arm. RR and 95% CIs are shown and are adjusted for age and smoking status at baseline.

End point	β-Carotene	Placebo	RR	(95% CI)
Local recurrence + second primary tumor	33	34[a]	0.90	0.56–1.45
Local recurrence	16	21	0.72	0.37–1.39
Second primary tumor	17	14	1.20	0.59–2.45
Site of Second Cancer				
Head and neck	3	4		
Esophagus	1	1		
Lung	13	9	1.44	0.62–3.39
Any second head and neck cancer (local recurrence or new primary)	19	25	0.69	0.39–1.25
Total deaths	29	33	0.86	0.52–1.42
Cause of death				
Head/Neck/Esophagus/Lung cancer	15	14		
Cardiovascular disease	6	11		
Other cancer	5	2		
Miscellaneous	3	6		
Age at death (mean ± SD)	69.7 ± 6.4	64.4 ± 8.8		$P = 0.01$

[a] One patient had both a local recurrence and a second primary tumor. This patient was included in the RR calculation for second primary tumors but not for local recurrences.

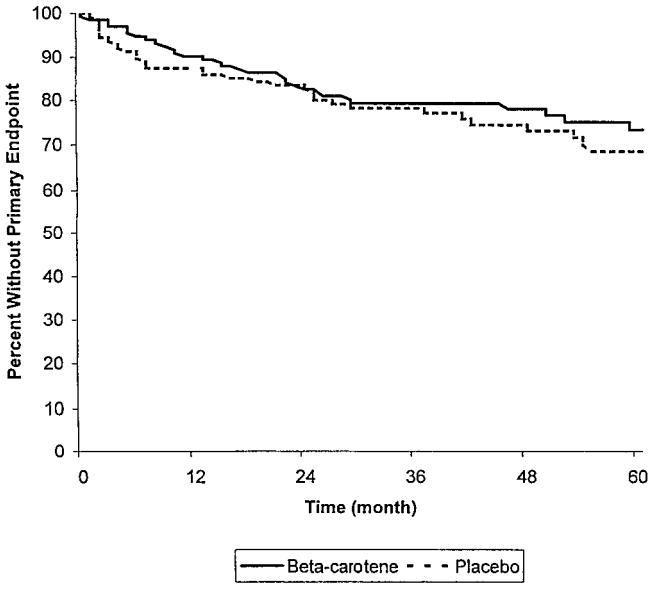

Fig. 2. Cumulative probability of remaining free of second primary tumors in the oral cavity, pharynx, larynx, or lung, or of local recurrences, according to treatment assignment ($P = 0.59$, log-rank test). Number at risk: for β-carotene, 135, 85, and 41 at baseline, 30 months, and 60 months, respectively; for placebo, 129, 75, and 33 at baseline, 30 months, and 60 months, respectively.

predictors of mortality, the interaction of age with treatment arm was statistically significant ($P = 0.02$). None of the other *P*s from the log-rank tests for the other end points were statistically significant.

Subsequent analyses were done to examine the effect of the intervention on mortality by baseline β-carotene plasma concentration. The population was stratified into two groups: lowest quartile of plasma β-carotene at entry (<0.066 μg/dl), or second through fourth quartiles of plasma β-carotene, based on the distribution in the full population. The lowest quartile was selected as an *a priori* cut point, based on the report of Greenberg *et al.* (18), which showed that persons in the lowest quartile of plasma β-carotene were at significantly greater risk for mortality. β-carotene supplementation similarly and nonsignificantly reduced the risk of death in both groups; the RR estimates comparing those randomized with β-carotene *versus* those randomized with placebo were 0.82 for those in the low-baseline β-carotene stratum *versus* 0.81 for those in the high-baseline stratum.

DISCUSSION

This randomized, double-blind, placebo-controlled multicenter trial was initiated to evaluate the efficacy of supplemental β-carotene in the prevention of second head and neck cancers. Whereas a number of β-carotene trials have been initiated over the past two decades, the evidence supporting a potential role for β-carotene in human cancer prevention was arguably stronger and more consistent for oral cancers than for any other tumor site. Supplemental β-carotene had been shown to regress oral precancerous lesions in several clinical trials (8), and β-carotene had established chemopreventive activity in rodent models of oral carcinogenesis. The IARC (19) concluded in 1998 that "there is sufficient evidence for cancer-preventive activity of β-carotene in experimental animals," with this designation based in part on efficacy in buccal pouch carcinogenesis in hamsters. Suggested efficacy in oral cancer prevention was also consistent with a large body of epidemiological literature, demonstrating that individuals with higher intake of β-carotene in the diet or higher levels of β-carotene in blood are at reduced risk for the development of several tobacco-related cancers including oral and laryngeal cancers, as reviewed elsewhere (19, 20).

A trial aimed at the primary prevention of oral cancer would have required tens of thousands of randomized patients; however, recruitment of patients with a prior malignancy provided a cost-effective alternative for evaluating efficacy. This is a consequence of the substantial risk that these patients face in terms of the development of second cancers. Also, efficacy in this patient setting would have direct clinical relevance, leading to our decision to evaluate this agent in secondary prevention.

Chemoprevention trials generally require several years to complete recruitment, followed by several years of intervention and follow-up. During this process, results of other trials may become available that impact the conduct of ongoing trials. In our case, results of two primary prevention trials of lung cancer were released (15, 16), indicating that supplemental β-carotene, alone or in combination with retinyl palmitate, could increase rather than reduce the risk of lung cancer. At the same time, a third report suggested no benefit and a slight worsening of abnormal sputum cytology in asbestos-workers supplemented with β-carotene plus retinol (21).

The safety of the participants enrolled in this trial was of utmost concern; therefore, all of the patients were informed of the findings, as were all of the hospital IRBs. The data and safety monitoring committee for this trial reviewed all of the pertinent results, as described previously. Continuation of the trial was recommended, largely because of the observation that the participants randomized to β-carotene were doing better, not worse. At the time of the interim analysis in June 1996, the rate ratio comparing the rate of incident events in the β-carotene group *versus* the placebo group was 0.81 for the primary end point, 0.65 for local recurrence, and 0.61 for mortality. The rate ratio for lung cancer at that time was 1.29. Recruitment was terminated because of feasibility concerns because all of the interim data were confidential and could not be shared with either patients or hospital IRBs. Many patients stopped supplementation after the release of the CARET results, so that additional follow-up would add very little in terms of study power, considering the number of participants who had already experienced an end point in addition to those who had stopped supplementation.

The results, although not significant, were in the direction hypothesized with regard to head and neck cancers (RR, 0.69), and all of the

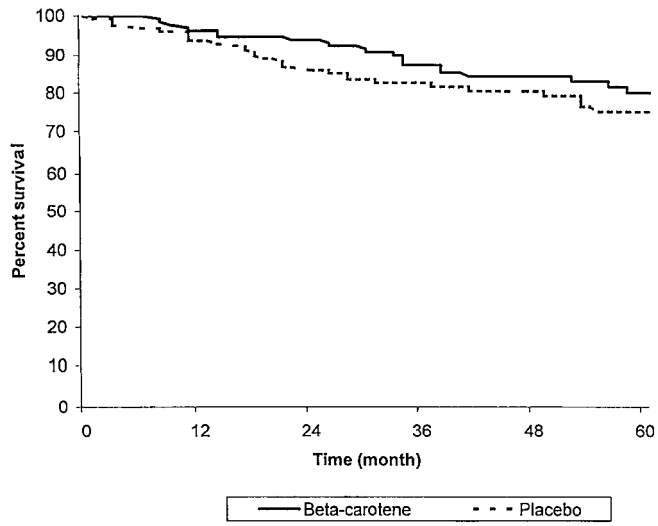

Fig. 3. Cumulative probability of overall survival, according to treatment assignment ($P = 0.41$, log-rank test). Number at risk: for β-carotene, 135, 97, and 49 at baseline, 30 months, and 60 months, respectively; for placebo, 129, 88, and 45 at baseline, 30 months, and 60 months, respectively.

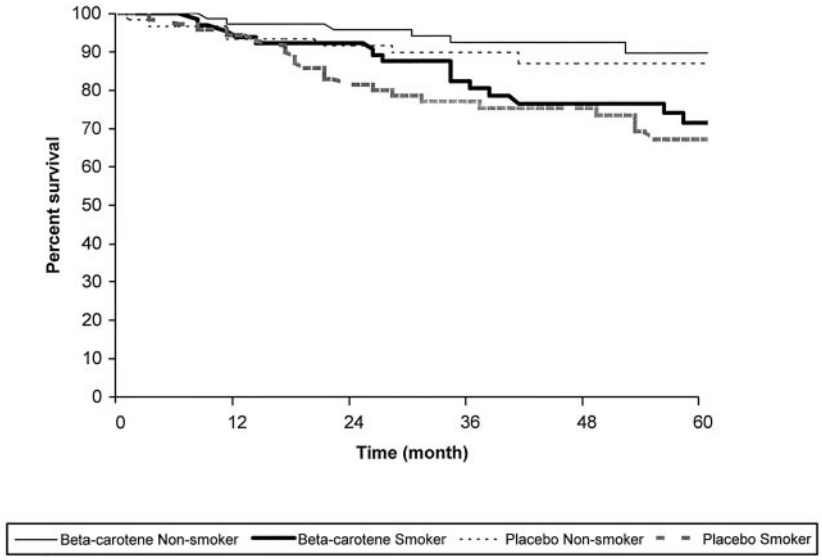

Fig. 4. Cumulative probability of overall survival, according to treatment assignment and baseline smoking status ($P = 0.03$, log-rank test). Number at risk: for β-carotene nonsmokers, 70, 51, and 23, at baseline, 30 months, and 60 months, respectively; for β-carotene smokers, 65, 45, and 26, at baseline, 30 months, and 60 months, respectively; for placebo nonsmokers, 59, 41, and 19, at baseline, 30 months, and 60 months, respectively; and for placebo smokers, 70, 46, and 26, at baseline, 30 months, and 60 months, respectively.

point estimates for head and neck cancers throughout the interim and final analyses were consistently beneath 1.0. However, the CIs (0.39–1.25) surrounding this estimate do not exclude the possibility of a null or even adverse effect on head and neck cancers. Despite the small sample size as compared with primary prevention trials, our results are consistent with other findings suggesting that supplemental β-carotene increased the risk of lung cancer, although the CIs around this estimate are also wide (0.62–3.39). In support of possible differential site-specific effects are data from intermediate end point biological marker trials, in which β-carotene regresses oral precancerous lesions (8) but has no effect on, or slightly worsens, sputum cytology (21), a putative intermediate end point for lung cancer. Retinoids, structurally related to carotenoids and β-carotene, similarly have been shown to have efficacy in trials of oral precancerous lesions but not in trials using intermediate end points for lung cancer [*e.g.,* bronchial metaplasia (8)]. Animal data also support site-specific effects: β-carotene was effective in preventing buccal pouch carcinogenesis in hamsters in about 20 studies (19); it was ineffective in only one study when also given after tumor development. In contrast, β-carotene was ineffective in three studies in respiratory tract carcinogenesis in hamsters and two studies of lung carcinogenesis in mice, and there were indications of weak promotional effects in some of these studies (19). The mechanistic basis for why β-carotene is effective in oral precancerous lesions/hamster buccal pouch carcinogenesis but not in intermediate end points for lung cancer/animal models of respiratory tract/lung carcinogenesis is not known.

The possibility that a chemopreventive agent might have differential site-specific effects is not new; tamoxifen, *e.g.,* is known to reduce the risk of breast cancer while increasing the risk of endometrial cancer (22). In such a situation, it is imperative to consider the effects of the agent on total mortality. In this patient population, persons randomized to β-carotene had a mortality advantage at the two interim analyses and in the final analysis. Also, both mean and median age at death significantly favored persons randomized to β-carotene as compared with persons on placebo.

The strength of this study lies in the fact that, to our knowledge, this trial is the only randomized, double-blind, placebo-controlled trial of β-carotene in head and neck cancer prevention. Italian investigators randomized 211 patients with early-stage head and neck cancer to β-carotene [75 mg/day for 3-month cycles with 1-month intercycle intervals) *versus* follow-up (23)]. The final results of that trial have not yet been reported; we anxiously await a final report from that trial to see if these findings are replicated. A meta-analysis of the data from the two trials may help to rectify the lack of statistical power found in each of the individual trials. Also, an examination of incident oral, pharyngeal, and laryngeal cancers in persons in ATBC and in the Physicians' Health Study, the two other major trials that used β-carotene as a single agent, might also provide insight into the plausibility of a possible reduction in head and neck cancers but not lung cancers with supplemental β-carotene.

Another strength of this trial is that we successfully randomized 38% of eligible patients in these two regions into a multiyear trial, increasing the generalizability of the results. In comparison, only about 3% of adults with cancer are enrolled in therapeutic trials (24).

Although this is the only randomized, placebo-controlled trial of β-carotene in this patient population, this trial also had some important limitations, particularly the compromised sample size and study power after reports of adverse effects on lung cancer and no overall benefit to β-carotene in other major trials. Seventy-six participants stopped taking study medication in early 1996; however, many of these persons had already been on supplement for several years. Furthermore, β-carotene is lipid-soluble, and blood levels of β-carotene remain significantly elevated above baseline even at 12 months after ceasing supplementation.[4,5]

Considering our overall results along with the results of other clinical trials involving β-carotene, reviewed elsewhere (8), we cannot recommend supplemental β-carotene for the prevention of second head and neck cancers. Although there were some suggestions of benefit, these were not statistically significant and should be considered against the increasingly consistent and statistically significant findings from other trials of an increased risk of lung cancer with supplemental β-carotene; in CARET, increased risk was particularly noted for large-cell carcinomas but was also observed for squamous cell carcinomas and adenocarcinomas (25). The suggested benefit that we observed in head and neck cancer is consistent with intermediate end point trials of β-carotene, and with animal carcinogenesis studies. This finding, if confirmed in ongoing/completed trials or in a meta-analysis using data from our trial combined with the Italian trial (23), could be the basis for mechanistic studies addressing differential responses in one epithelial site (head and neck) *versus* another (lung).

In the meantime, the search for other more efficacious agents for

[4] Unpublished data from CARET.
[5] Dr. Carrie Redlich, personal communication.

the prevention of these cancers continues. A large, intergroup trial is investigating the efficacy of low-dose 13-*cis*-retinoic acid in this clinical setting (26). Results of that trial are anticipated in the next few years. Pending results of that study, efforts to encourage tobacco cessation in patients who have been cured of an early-stage head and neck cancer should be intensified, given our findings that continuing tobacco exposure adversely affects the risk of all-cause mortality.

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