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APPENDICES:

- A. Study Manual Table of Contents
- B. Forms Manual Table of Contents
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- D. NCI Smoking Cessation Pamphlets and Fred Hutchinson Cancer Research Center Pamphlet ("Quit Smoking")
- E. CARET Centralized Follow-up Manual (CCFM) Table of Contents
- F. Data Collection Forms (CCFM Appendix C)
- G. Sample CARET *Reporter*

1.0 OBJECTIVES

- 1.1 To recruit a total of 4,000 participants with significant asbestos exposure and 13,000 heavy smokers and to randomize them to two intervention arms: (1) beta-carotene 30 mg and retinyl palmitate 25,000 IU orally each day or (2) placebo orally each day. (DONE: goals exceeded)
- 1.2 To document the occurrence of lung cancer in both intervention arms during intervention (DONE) and post-intervention. (ONGOING)
- 1.3 To document the occurrence of other cancers and causes of death in both intervention arms during intervention (DONE) and post-intervention. (ONGOING)
- 1.4 To collect data on participant self-reported cataract diagnosis and cataract extractions in both intervention arms. (ONGOING)
- 1.5 To assess the safety of long-term daily oral administration of 30 mg beta-carotene and 25,000 IU retinyl palmitate in the target high-risk populations. (DONE)
- 1.6 To determine medication rates and estimate total vitamin A and beta-carotene intake in each participant. (DONE)
- 1.7 To obtain serum from all Efficacy participants biennially and from all Pilot participants annually, for long-term storage and retrospective case/control analysis. (DONE)
- 1.8 To obtain one whole blood sample from participants for future DNA studies. (DONE)
- 1.9 To obtain tumor and non-tumor tissue from participants diagnosed with lung cancer, mesothelioma, or cancer of an unknown primary site for future studies of biomarkers potentially related to lung cancer. (DONE)
- 1.10 To maintain the CARET serum bank for additional hypothesis-driven studies by CARET investigators and by other investigators with the Coordinating Center as the gatekeeper. (ONGOING)

2.0 HYPOTHESES AND STUDY ENDPOINTS

- 2.1 Primary Hypothesis: Daily oral supplementation with beta-carotene and retinyl palmitate will decrease the incidence of lung cancer in these high-risk populations.
- 2.2 Secondary hypotheses:
 - 2.21 Baseline serum concentrations and total daily intake of beta-carotene and/or retinoids will correlate inversely with risk of developing lung cancer.
 - 2.22 Oral supplementation with beta-carotene and retinyl palmitate will decrease the incidence of other cancers and mortality.
 - 2.23 Oral supplementation with beta-carotene and retinyl palmitate will decrease the rate of cataract extractions.
- 2.3 The study endpoints are:
 - 2.31 Primary: incidence of lung cancer
 - 2.32 Secondary: incidence of other cancers
 - 2.33 Secondary: incidence and causes of death
 - 2.34 Ancillary: incidence and rates of cataract extractions

3.0 MILESTONES AND SUMMARY OF INTERVENTION

- | | |
|-----------|---|
| May 1985 | First asbestos-exposed pilot participants randomized in Seattle |
| July 1985 | First heavy smoker pilot participants randomized in Seattle |

1988	Full recruitment/randomization to Pilot studies
1988	Initiation of expanded full scale recruitment at Seattle, Portland, San Francisco, New Haven, and Baltimore
1990	Further expansion at Portland, and addition of Irvine
1993-94	Completion of recruitment/randomization at Baltimore (November 1993), Portland (December 1993), Seattle (February 1994), San Francisco (June 1994), New Haven (August 1994), and Irvine (September 1994).
August 1994	First interim analysis
September 1995	Second interim analysis
January 1996	Termination of active intervention
May 2, 1996	CARET initial results paper published (<i>New England Journal of Medicine</i>)
November 6, 1996	CARET detailed lung cancer results published (<i>Journal of the National Cancer Institute</i>)
April 1, 2000	Centralize CARET activities to the Coordinating Center

3.1 INTERVENTION SUMMARY - EFFICACY PARTICIPANTS

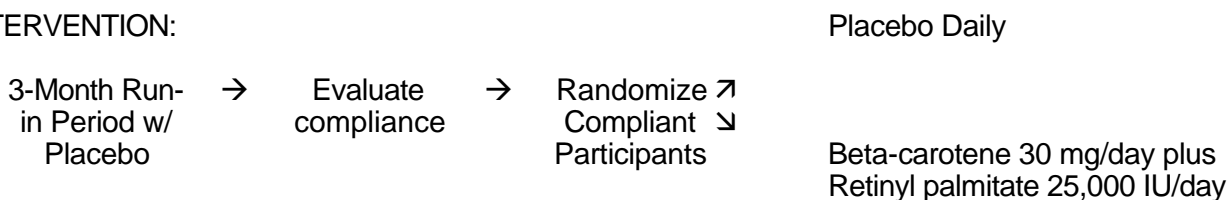
ELIGIBILITY - ASBESTOS-EXPOSED PARTICIPANTS:

- 1) Age 45-69, men only
- 2) Occupational exposure to asbestos beginning at least 15 years prior to enrollment
- 3) Have chest radiograph changes compatible with asbestos exposure (ILO criteria) and/or completed at least five years of employment at least ten years previously in one of nine high-risk trades: plumbers and pipefitters; steam fitters; shipyard boilermakers; shipyard electricians; shipscalers; insulators; plasterboard workers; sheetmetal workers; and non-shipyard boilermakers
- 4) Current smoker or stopped smoking \leq 15 years prior to enrollment
- 5) No history of cancer (other than non-melanoma skin cancer) within last 5 years
- 6) No history of hepatitis or cirrhosis within the last 12 months
- 7) SGOT and alkaline phosphatase less than 2.5X and 1.5X upper normal limits, respectively
- 8) Willingness to limit vitamin A supplementation to \leq 5,500 units daily and no beta-carotene supplementation
- 9) Take $>50\%$ of study vitamins during the placebo run-in period

ELIGIBILITY - HEAVY SMOKERS:

- 1) Age 50-69
- 2) Current smoker or stopped smoking \leq 6 years prior to enrollment
- 3) \geq 20 pack-years smoking history
- 4) No history of cancer (other than non-melanoma skin cancer) within the last 5 years
- 5) No history of hepatitis or cirrhosis within the last 12 months
- 6) SGOT and alkaline phosphatase less than 2.5X and 1.5X upper normal limits, respectively
- 7) Willingness to limit vitamin A supplementation to \leq 5,500 IU daily and no beta-carotene supplementation
- 8) Women must be post-menopausal or no longer having menstrual periods
- 9) Take $>50\%$ of study vitamins during the placebo run-in period

INTERVENTION:



ENDPOINTS:

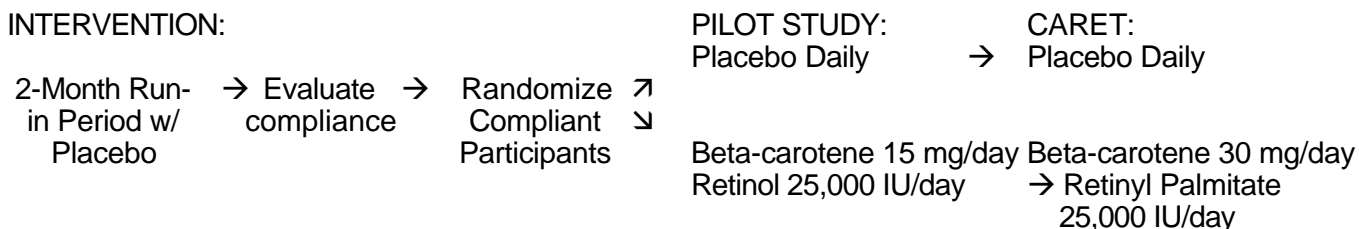
- Primary: incidence of lung cancer
- Secondary: incidence of any other cancer, death
- Ancillary: incidence of cataract diagnosis and extraction

3.2 INTERVENTION SUMMARY - PILOT PARTICIPANTS

ELIGIBILITY - ASBESTOS-EXPOSED PARTICIPANTS:

- 1) Age 45-74, men only
- 2) Occupational exposure to asbestos beginning at least 15 years prior to enrollment
- 3) Have chest radiograph changes compatible with asbestos exposure (ILO criteria) and/or completed at least five years of employment at least ten years previously in one of eight high-risk trades: plumbers and pipefitters; steam fitters; shipyard boilermakers; shipyard electricians; shipscalers; insulators; plasterboard workers; sheetmetal workers
- 4) No history of cancer (other than non-melanoma skin cancer) within last 5 years
- 5) No history of hepatitis or cirrhosis within the last 12 months
- 6) SGOT and alkaline phosphatase less than the 99% upper limits of laboratory normal
- 7) Willingness to limit vitamin A supplementation to $\leq 5,500$ units daily and no beta-carotene supplementation
- 8) Karnofsky performance status ≥ 70
- 9) Take $>70\%$ of study vitamins during the placebo run-in period.

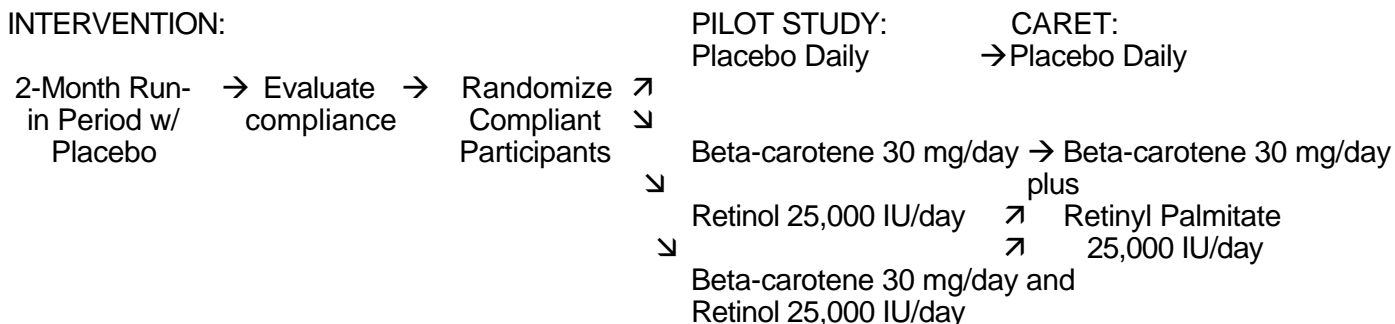
INTERVENTION:



ELIGIBILITY - HEAVY SMOKERS:

- 1) Age 50-69
- 2) Current smoker or stopped smoking ≤ 6 years prior to enrollment
- 3) ≥ 20 pack-years smoking history
- 4) No history of cancer (other than non-melanoma skin cancer) within the last 5 years
- 5) No history of hepatitis or cirrhosis within the last 12 months
- 6) SGOT and alkaline phosphatase less than the 99% upper limit of laboratory normal
- 7) Willingness to limit vitamin A supplementation to $\leq 5,500$ IU daily and no beta-carotene supplementation
- 8) Women must be post-menopausal or no longer having menstrual periods
- 9) Karnofsky performance status > 70
- 10) Take $>70\%$ of study vitamins during the placebo run-in period

INTERVENTION:



ENDPOINTS - ASBESTOS-EXPOSED AND HEAVY SMOKERS:

- Primary: incidence of lung cancer
- Secondary: incidence of any other cancer, death
- Ancillary: incidence of cataract diagnosis and extraction

4.0 INTRODUCTION AND BACKGROUND

4.1 Lung cancer is the leading cause of death from cancer among both men and women in the United States, accounting for approximately 28 percent of cancer deaths and 5 percent of all deaths. Approximately 149,000 Americans died of this disease in 1993. Five-year survival remains approximately 15 percent, despite aggressive therapy (1). National Cancer Institute (NCI) Cancer Control goals for lung cancer are focused almost entirely on prevention (2).

Primary prevention of lung cancers is eminently feasible. The two leading risk factors, cigarette smoking and exposures to respirable fibers of asbestos, are well-established and interact synergistically (3-8). Obviously, dose, dose rates, years from first exposure to asbestos, individual susceptibility factors, and other variables influence the absolute rates and individual risks. The latency period is at least 10 years and often 30-40 years.

Smoking cessation and prevention of relapse to smoking have a deservedly prominent place in the NCI Cancer Control goals to reduce age-adjusted cancer death rates by 25 to 50 percent by the year 2000 (2). In parallel, occupational exposure standards, protective equipment, and efforts to substitute other materials for asbestos are aimed at primary prevention of asbestos-related lung cancer. However, in concert with those efforts, cancer control strategies aimed at preventing the development and/or progression of tumors in persons who already have histories of cigarette smoking and/or occupational exposures to asbestos are essential. At the time CARET was begun, approximately 80 percent of men and 50 percent of women in the 55-64 age group were current or former smokers (9) and an estimated 4 million workers were exposed significantly to asbestos in shipyards and related activities during the period of World War II alone. Nicholson et al. (10) predicted that those already exposed to asbestos will suffer asbestos-related cancer deaths at an excess rate of 8,000 to 10,000 per year at least to the end of the century; a consensus figure is 4,000-6,000 lung cancer deaths per year (11). The latency period offered a major opportunity to intervene to prevent cancers in these high-risk persons.

4.2 By the time CARET was proposed and initiated, the chemopreventive activity of vitamin A and its synthetic and naturally occurring analogues, the retinoids, had attracted wide interest (12-14). Many animal studies had shown these compounds could prevent and reverse chemically induced pulmonary neoplasia (15-17). Most importantly, some studies had demonstrated that substantial reductions in tumor incidence and size could be obtained when the retinoids were administered long after the exposures to inducing carcinogens. For example, the incidence of breast cancers at 20 weeks decreased by about 50 percent when rats were given retinyl acetate for a 6-week period starting two weeks prior to exposure to DMBA. When retinyl acetate was given starting 12 weeks after exposure to DMBA, there was a 40 percent decrease in incidence of breast tumors at 20 weeks (18). In cultures of hamster tracheal cells, retinyl methyl ether has inhibited the metaplastic transformation induced by crocidolite or amosite asbestos (19).

In humans, retinoids have reversed cigarette-smoking-induced pre-neoplastic bronchial lesions and reduced the incidence of micronuclei in buccal smear cells. Mathe et al. (20) administered etretinate for 6 months at 30 mg/day to 67 heavy cigarette smokers who underwent serial bronchoscopies with biopsies. In the 34 participants with severe metaplasia at the start of the study, treatment decreased the degree of metaplasia ($p < 0.01$). In studies with betel nut chewers by Stich et al. (21) the proportion of buccal mucosal cells with micronuclei decreased to normal during 3 months of treatment with the combination of retinyl palmitate and beta-carotene. Bollag (22) has reviewed the beneficial effects of retinoids on other precancerous lesions in humans.

Epidemiologic investigations generally have shown a striking inverse relationship between intake of beta-carotene and/or vitamin A (20-26) and lung cancer risk, and between serum concentrations of beta-carotene and lung cancer risk (27-30). Such epidemiologic associations, of course, could not establish that increasing the intake or blood concentrations of vitamin A and beta-carotene would actually decrease the incidence of lung cancers. Only the intervention studies supported under the NCI cancer control program could address that crucial question.

- 4.3 Establishing the safety of any chemopreventive agent is important, especially since participants in the trials are volunteers without cancers, not patients (31). Even high-risk participants have less than one chance of lung cancer per 100 per year. Thus, it is essential to avoid significant side effects, both subjective and objective. The pilot studies to CARET and CARET itself addressed this issue directly through extensive monitoring of symptoms, signs, and laboratory values potentially attributable to vitamin A or beta-carotene.

The combination of beta-carotene and retinyl palmitate employed in CARET had the dual attraction of avoiding possible dose-related toxicity from higher doses of either agent, especially retinyl palmitate, and of gaining the complementary anti-tumor actions of the two agents. Beta-carotene is thought to function as an electron scavenging anti-oxidant (12,32), as well as a precursor of retinol. Retinyl palmitate functions to maintain or enhance the differentiated state of epithelial cells, including bronchial epithelium (12,32). Retinol in vivo inhibits cell growth possibly via changes in cell surface glycolipids/proteins (33,34). We recognize that the mechanisms of these effects and their chemopreventive significance in humans are currently speculative. We have demonstrated that the doses chosen are capable of substantially increasing serum beta-carotene and serum retinyl palmitate concentrations without any early signs of liver toxicity or marked increases in triglycerides.

- 4.4 The costs of full-scale chemoprevention trials even for common cancers are very high. The experience of our colleagues in planning the Women's Health Trial of reduction of dietary fat intake to prevent breast cancer helped in the planning for CARET. We devised a strategic plan to pool the two high-risk populations in ways that made project management more efficient, that moderated costs, that permitted pooled as well as separate analyses of efficacy, and that generated toxicity and compliance observations from identical protocols. It is likely that the underlying carcinogenic mechanisms for these two high-risk populations have much in common; asbestos fibers retained in the pulmonary parenchyma are thought to act as co-carcinogens with the carcinogens in cigarette smoke (and in other inhaled pollutants) (10,11). The distribution of histologic types of lung cancers is comparable between asbestos-exposed and non-asbestos-exposed smokers (35,36), and there is little or no basis in either population to exclude adenocarcinomas (11,20). Furthermore, the retinoids have demonstrated chemopreventive activity in animals against adenocarcinomas of breast and colon. It is likely that the chemoprevention of bronchogenic carcinoma in smokers and in asbestos-exposed individuals (with smoking and also without known smoking) would be exercised against similar tumor promotion and tumor progression steps.

BACKGROUND

- 4.5 Phase I/II investigations of the toxicity and clinical pharmacology of retinol were carried out by Goodman in Arizona and Seattle, as described in the Vanguard study proposal (37-39).
- 4.6 Between 1985 and 1988, Goodman and associates in the FHCRC Cancer Prevention Research Program conducted a 4-arm phase II pilot trial of beta-carotene and retinol as potential cancer prevention agents in high-risk smokers (40). The objectives were to address the feasibility of a larger intervention trial, participant adherence, and the potential side effects of these agents. Participants were recruited from a local health insurer, King County Blue Shield. A mailing to 29,927 subscribers described the trial and elicited responses about eligibility and interests in participating. Potentially eligible participants were contacted and an initial study center visit was scheduled. At this first appointment, participants' informed consent was obtained, a dietary questionnaire was administered, a baseline side effect evaluation was made, a limited physical

examination was done, and blood samples were obtained. At the end of a two-month placebo run-in, participants returned for randomization to: (1) beta-carotene placebo and retinol placebo; (2) beta-carotene 30 mg/day and retinol placebo; (3) beta-carotene placebo and retinol 25,000 IU/day; or (4) beta-carotene 30 mg/day and retinol 25,000 IU/day. Participants received their study vitamins in monthly blister packs, with three capsules per day. In follow-up, phone contacts were alternated with study center visits at two-month intervals to evaluate side effects and adherence. A total of 1,029 participants were randomized, exceeding the recruitment goal of 920.

Subjective and objective side effects were measured with a standardized questionnaire and side effect grading scale. The only side effect showing statistically significant differences across intervention arms was yellow coloration of the skin, which was more common in the groups receiving beta-carotene. Subjective side effect ratings in all groups were higher at the initial visits, decreasing as the participants became more familiar with the study center and the questionnaire, and as anxiety about taking the daily capsules lessened. There were no statistically significant changes in SGOT or alkaline phosphatase over time or across the four intervention arms.

During the pilot study, 234 participants inactivated, of whom 76 subsequently reactivated into CARET. Many inactivations were due to non-specific side effects (40). Initially, the protocol for this pilot inactivated participants whenever any of the symptoms being monitored exceeded threshold levels, regardless of whether there was a known reason for the symptom.

Adherence was evaluated by pill counts and by participant reports. The estimates obtained by these two methods did not differ significantly. The extent of adherence was stable over time, with over 90% adherence among active participants. There was no statistically significant change in serum retinol, but serum retinyl palmitate concentrations increased in both arms receiving retinol. Mean serum beta-carotene concentrations in the arms receiving beta-carotene increased from 200 to 2400 ng/ml by 4 months and stayed at that level thereafter.

- 4.7 Also from 1985 to 1988, Omenn and associates in the FHCR/CUW Cancer Prevention Research Program conducted a two-arm trial of 15 mg/day beta-carotene plus 25,000 IU/day retinol versus placebos in men aged 45-74 occupationally exposed to asbestos (41). Vitamins were distributed in monthly blister packs with two capsules per day. Recruitment involved federal and state workers' compensation programs, selected occupational medicine pulmonary physicians, plaintiffs attorneys, the Navy Asbestos Medical Surveillance Program at Bremerton, WA, and major unions in the Puget Sound area. The enrollment strategy was directly parallel to that described above for the smokers, with a two-month placebo run-in (at which time a chest X-Ray was obtained) and follow-up every 2 months. A total of 816 participants was randomized. In this cohort the baseline prevalence of asbestos-related radiographic abnormalities (fibrosis and/or pleural changes) was 62 percent, compared to previous studies of shipyard insulators with 52 percent prevalence and of workers from all crafts with 46 percent prevalence (42). In these other studies, the relative risk (RR) for mortality from lung cancer among the shipyard insulators exposed to asbestos versus those not exposed was 3.9 (42). Based upon the radiographic findings, it is reasonable to conclude that the study cohort has asbestos exposure and lung cancer risks similar to those of the asbestos insulators (43). Doll & Peto (7) and Nicholson et al. (10) have utilized a relative risk model in which lung cancer risk is independent of age and smoking status and increases with duration and intensity of exposure. The RR rose from 3.4 for those with 15-19 years from first exposure to a peak RR of 6.0 for those with 30-34 years, declining to 3.9 for those greater than 45 years since first exposure. The mean time from first occupational exposure to asbestos for the participants in this pilot was 35 years, with a mean of 25 years cumulative duration of exposure in the respective high-risk trades.

Subjective and objective toxicity were evaluated exactly as in the smokers study. There were no statistically significant differences in subjective reports or in objective ratings between the two intervention arms, other than the expected skin yellowing. During this pilot study, 211 participants had 296 instances of threshold grade symptoms and signs requiring dosage reduction according to the protocol. A fully anticipated problem arose from insistence by the FDA in 1983 that the "upper limit of normal" for SGOT and alkaline phosphatase be used to exclude participants or to define toxicity once randomized, rather than the 2.5x upper limit of normal used in other studies

and in a Tyler, Texas, study with principal investigator Jerry McLarty. In all, 22 potential participants were excluded, 19 randomized participants underwent symptom management, and 9 were permanently inactivated before this situation was redressed in July 1987.

In all, 193 participants were incomplete enrollments (i.e., completed the first visit but were not randomized): 2 died, 95 were not interested, and 96 were ineligible. One hundred twenty participants inactivated during the pilot study after randomization, of whom 34 reactivated into CARET. As in the smokers pilot, the primary reasons were non-specific side effects. Adherence to taking the study vitamins was similar to that in the smokers pilot. Given the medico-legal complications dominating the lives of many men suffering asbestos-related illnesses, it was anticipated that there might be difficulties with adherence. The study center staff built admirable relationships with the participants through personal interest, professional performance, and timely sharing of relevant results.

- 4.8 These pilot studies gave considerable information on the side effects of beta-carotene and retinol alone and in combination. The transition of Pilot Study randomized participants to the Vanguard protocol began in July 1988. A total of 1,566 (85%) of the 1,845 randomized Pilot participants made the transition to the Vanguard protocol. These participants are called the Vanguard cohort in CARET. A total of 856 heavy smokers and 710 asbestos-exposed Vanguard participants were active participants in CARET by the end of active intervention in CARET. Because toxicity of the study vitamins was expected to depend on the duration of dose, it was anticipated that any significant side effects would become evident in this group prior to their development in the full-scale cohort. As a result, side effect monitoring was more intensive in the Vanguard cohort, resulting in a more cost-efficient trial.
- 4.9 CARET, the full-scale efficacy trial, began in July 1988 for additional recruitment at the Seattle Study Center and new study centers in Baltimore, New Haven, Portland, and San Francisco. Final expansion in southern California began mid-1991 at the Irvine Study Center. Participants randomized after July 1988 into CARET are referred to as the Efficacy cohort. Accrual into the Efficacy cohort was completed on September 10, 1994, with a total of 18,314 participants randomized (including those randomized during the pilot studies). All five of the original study centers exceeded their accrual goals, and Irvine, which began randomizations in January 1992, achieved 98% of its overall goal. Irvine halted randomization since the overall accrual goal for the trial was achieved. The remainder of this protocol presents the recruitment, enrollment, and follow-up activities for the full-scale efficacy trial.
- 4.10 In September 1995, CARET's Safety and Endpoints Monitoring Committee (SEMC) reviewed the second interim analysis of CARET endpoint data. Under the original monitoring plan for CARET, there were to be two interim analyses with the second interim analysis taking place in the fall of 1996. Because of concern over the Alpha-Tocopherol, Beta-Carotene (ATBC) trial results, in August 1994 the SEMC requested that an additional interim analysis be added in fall 1995.

The SEMC reviewed statistical analyses of differences between arms in the incidence of lung cancer and mortality. The planned primary analysis was a weighted log-rank test on lung cancer cases confirmed by the Endpoints Review Committee (ERC), stratified on study center, population, and cohort, with the first two years after randomization weighted linearly. The p -value for this test was 0.053 (with the active arm having the excess of lung cancers), which did not cross the O'Brien-Fleming stopping boundary for this interim analysis, $p = 0.007$. For deaths, the stratified weighted log-rank test p -value was 0.0036 for confirmed deaths and 0.0052 for all reported deaths. Additional analyses included intervention arm relative risk estimates and p -values for lung cancer and mortality from Cox proportional hazard models, with and without weighting, overall and within participant subgroups; an evaluation of the sensitivity of results to the choice of weighting period; intervention arm differences in survival after the diagnosis of lung cancer; the association of beta-carotene serum concentrations at baseline and one year post-randomization with lung cancer rates; and the conditional power of the trial.

After a thorough review of the data on September 9, the SEMC concluded that there was very little chance that, if carried to completion, the trial would show a clear benefit from taking the study

vitamins. There was extensive discussion of the likelihood of showing adverse effects. Although all other subgroups showed an excess of endpoints in the participants receiving active vitamins, there was a relative risk (RR) of 0.895% confidence interval (CI) = 0.5 - 1.3 in favor of the intervention in the former heavy smokers subgroup. After a preliminary vote to recommend termination of the active intervention in the trial, there was re-consideration.

Following the SEMC meeting, the NCI Director of the Division of Cancer Prevention and Control convened a conference call with a panel of leading cancer prevention trial researchers on September 26, 1995, to review the CARET results and the analysis and interpretation of the interim results. The group raised the issue of adjusting p-values for multiple endpoints, and recommended methods which were pursued by the CARET statisticians. On December 18, 1995, an ad hoc National Cancer Institute review committee reviewed the CARET results analyses and concluded that, considered together with the data from the ATBC trial, the data from CARET provided sufficient evidence of a harmful effect of the intervention on both lung cancer incidence and total mortality. The committee could not justify continuation of the intervention even in former smokers.

By conference call on January 5, 1996, the CARET Coordinating Center Director alerted the Steering Committee members of the recommendations to terminate active intervention. The Steering Committee convened in Seattle on January 11, reviewed the data intensively, and voted to stop the intervention. The Steering Committee drafted a letter to CARET participants informing them of the decision; the Coordinating Center mailed the letters on January 12-13, and most participants received them by January 16. Participants were asked to confirm receipt of the letter by returning a postcard to the Coordinating Center.

Simultaneous press conferences were held at the NCI and at the study centers on January 18. On January 19, the Coordinating Center sent each participant a second mailing, consisting of a letter which stated the participant's intervention arm and a mailer for the return of all remaining vitamin bottles to the study center. The Coordinating Center and study centers monitored the return of vitamin bottles that had been dispensed to participants prior to January 12, as well as participant-initiated telephone calls to the study centers and Coordinating Center following the stopping of the intervention.

- 4.11 By January 1996, four NCI-sponsored major randomized trials involving beta-carotene had been completed. In 1993, the Linxian trial (44) in 29,584 presumed vitamin and mineral-deficient Chinese adults was published. This trial tested a daily combination of three antioxidants: 15 mg beta-carotene, 50 mcg selenium, and 30 mg alpha-tocopherol, plus three other combinations of micronutrients in various combinations in a fractional factorial design. Those receiving the antioxidants had 9% fewer deaths than those not receiving the antioxidants. In 1994, the Alpha-Tocopherol Beta-Carotene (ATBC) trial (45) in 29,133 male smokers in Finland, which tested 20 mg/day beta-carotene and 50 mg/day alpha-tocopherol in a factorial design, reported that those participants randomized to receive beta-carotene had 18% more lung cancers and 8% more deaths than those receiving placebo. In 1996, CARET (46) and the Physicians' Health Study (PHS) (47) simultaneously reported their results. CARET, which tested the combination of 30 mg/day beta-carotene plus 25,000 IU/day retinyl palmitate in 18,314 smokers and asbestos-exposed participants, reported 28% more lung cancers and 17% more deaths in those receiving the active vitamins; PHS, which tested beta-carotene 50 mg every other day in 22,071 male physicians reported no effect, either beneficial or adverse. This combination of trials convincingly demonstrated no benefit of high-dose supplementation of beta-carotene in individuals with adequate dietary intake of vitamins and minerals, and raised a significant possibility of harm from beta-carotene in those at high risk for lung cancer.
- 4.12 On November 6, 1996, ATBC and CARET both published in the *Journal of the National Cancer Institute* detailed analyses of the lung cancer results. According to CARET's pre-specified analysis, there was a RR of 1.36 (95% CI = 1.07 - 1.73) for weighted lung cancer incidence for the active intervention group compared with the placebo group, and a RR = 1.59 (95% CI = 1.13 - 2.23) for weighted lung cancer mortality.

5.0 RECRUITMENT SOURCES AND SITES

5.1 Asbestos-Exposed Participants

5.11 Participants were identified through workers' compensation claims, union rolls, records of lawyers, medical subspecialty study centers, and organizations of retired military personnel.

5.12 Recruitment study centers

The asbestos-exposed participant recruitment sites are in Groton, Connecticut; San Francisco, California; Seattle, Washington; Baltimore, Maryland; and Portland, Oregon.

5.2 Heavy Smokers

5.21 Recruitment sources

A variety of recruitment sources were used to recruit smokers including medical insurance carriers and health maintenance organizations such as Blue Shield and Blue Cross of Washington and Oregon, Group Health Cooperative of Seattle, and Kaiser Permanente of Oregon. Study centers also accepted self-referrals, and advertised in local newsletters such as the American Association of Retired Persons (AARP), local newspapers, and radio. All recruitment sources were assigned codes for tracking purposes. This information is kept as part of a Status Report for each study center maintained by the Coordinating Center.

5.22 Recruitment study centers

The smoker recruitment sites are in Seattle, Washington; Portland, Oregon; and Irvine, California. To increase retention of asbestos-exposed participants, the three asbestos-exposed participant recruitment sites recruited household members who were smokers. The smoker household member recruitment sites are in Baltimore, Maryland; Groton, Connecticut; and San Francisco, California.

6.0 INTERVENTION ASSIGNMENT

6.1 Randomization of eligible participants occurred at the end of the Second Visit. The staff verified eligibility and ran the computerized randomization program which assigned a unique study identification number to the participant. Each study identification number had an intervention assignment (active or placebo) previously associated to it by the Coordinating Center. The study center staff remained blinded to participants' intervention assignments until active intervention was ended in January 1996. Efficacy randomization was stratified by risk population and study center. Balance on all other non-intervention variables was left to chance; the arms were well balanced on potential confounding variables.

Randomization in the asbestos Pilot study was stratified by smoking history (never smoked, former smoker, or current smoker), years since first exposure to asbestos (15-30 or >30 years), parenchymal fibrosis (chest X-Ray ILO grade profusion [$<1/0$ or $\geq 1/0$], and recruitment source (medical, workers' compensation agencies, Navy, unions, or other). Randomization in the heavy smoker Pilot study was stratified by age (50-59 and 60-69 years) and smoking history (20-39 and 40 or more pack-years).

6.2 The active intervention agents were beta-carotene at a dose of 30 mg/day and retinyl palmitate 25,000 IU/day, combined in a single capsule. Study vitamins were dispensed in bottles of 200 capsules. Participants took one capsule per day.

7.0 PARTICIPANT ENROLLMENT AND FOLLOW-UP

7.1 Recruitment and Enrollment (see Study Manual, *Section 3 - Recruitment* and *Section 4 - Enrollment*)

This section describes the events from the time of the first contact of a potential participant to the time of randomization.

The initial contact with potential smoker participants was made by mail. This mailing contained the introductory cover letter, heavy smokers interest survey, a fact sheet, and a business reply. Potential asbestos-exposed participants were recruited directly at occupational health clinics or were contacted by mail via an introductory cover letter and an asbestos-exposed participants interest survey.

7.11 The returned interest survey provided information on:

- 7.111 Age range
- 7.112 Smoking history
- 7.113 Employment and asbestos exposure history
- 7.114 Willingness to participate
- 7.115 Contact information

7.12 Participants determined to be potentially eligible based on a review of the returned interest survey were then sorted by best time to call. A screening phone call (See *Form 3 - Screening Phone Contact Record* in the Forms Manual) was then completed, which obtained the following information:

- 7.121 Clarification or completion of missing answers on the interest survey
- 7.122 Confirmation of whether a doctor ever told them they had cancer or liver disease
- 7.123 Determination of whether they were enrolled in any other studies
- 7.124 Willingness to limit personal intake of vitamin A to 5,500 IU per day and no beta-carotene
- 7.125 Confirmation of post-menopausal status
- 7.126 Interest in participating

Participants potentially eligible and interested were scheduled for a First Visit and were sent a confirmation letter confirming their appointment time and date and including information on directions and parking. Participants were also sent in the same envelope a health and work history questionnaire and contact information form (see *Form 11 - Questionnaire #1* and *Form 10 - Contact Information* in the Forms Manual) to complete. A reminder phone call was made or a reminder postcard was sent a few days before the scheduled visit and participants were reminded to bring in their completed forms.

7.13 When the participants reported to the study center for their First Visit, the following took place:

- 7.131 The purpose and timetable of the study were explained.
- 7.132 If participants agreed, they signed the informed consent form. Each study center had its own IRB-approved consent form (See Study Manual *Appendix B - Sample Consent Form*).
- 7.133 Participants' health and work history questionnaire was reviewed; and they were asked to complete a dietary intake questionnaire (see *Form 15 - Food Questionnaire*, Forms Manual), and a pulmonary symptom history (see *Form 8 - Respiratory History Questionnaire*, Forms Manual). Asbestos-exposed participants had spirometry performed, and a chest X-Ray ordered (see Study Manual, *Section 8 - Special Procedures-Asbestos-Exposed Participants Only*).

- 7.134 For asbestos-exposed participants, posterior-anterior (PA) chest radiograph was taken at maximal inspiration and interpreted at each site by a NIOSH-certified A or B reader. This reading was used to determine eligibility. Readers were blinded to asbestos exposure history, clinical status and age, and radiologist interpretation. Each chest PA X-Ray was also read by a radiologist for clinical purposes. Interpretation was according to the International Labor Organization classification of radiographs for pneumoconiosis. For quality assurance, sample X-rays across sites and across time were reviewed by Coordinating Center readers to determine comparability of readings.
- 7.135 For asbestos-exposed participants, maximum expiratory volume curves were obtained in a sitting position on an American Thoracic Society-approved spirometer. Attempts to obtain three acceptable curves from each participant were undertaken with the largest forced expiratory volume at one second (FEV) and forced vital capacity (FVC) used as observed values. Acceptability of all tracings was determined at each study center, using approved criteria of shape of tracing and variability of values between tracings.
- 7.136 Baseline symptoms and signs were evaluated by questionnaire and physical exam (see Study Manual, *Section 9 - Symptom Monitoring*).
- 7.137 Pre-randomization blood samples were obtained and sent for analysis to determine eligibility (SGOT and alkaline phosphatase). Additional serum was frozen for retrospective analysis (four 2.0 ml aliquots of serum). Samples were recorded on a blood collection and processing form (see *Form 71 - Blood Collection and Processing*, Forms Manual). Participants with a birthday on the 1st to 4th day of a month were selected for an additional quality control blood sample (one additional 2.0 ml aliquot).
- 7.138 Smoking cessation packets were distributed to participants at the First Visit only upon request. Study interviewers provided a brief counseling session on smoking cessation which encourages smokers to stop smoking and former smokers to maintain their smoking cessation (see Study Manual, *Section 2 - Study Center Guidelines*). Smokers who requested additional assistance or information on smoking cessation were given the NCI pamphlets "Why Do You Smoke?" and "Clearing the Air, A Guide to Quitting Smoking" (see Appendix D) and were given information about resources in the community.
- 7.139 All participants were given one bottle containing 200 capsules of placebo and a vitamin information sheet (see Appendix E - Sample Vitamin Information Sheet), and an Orientation Booklet (See Study Manual *Appendix C*). They were then scheduled for a Second Visit three to six months later, when randomization would take place.
- 7.2 Second Visit (see Study Manual *Section 4 - Enrollment*). When participants reported to the study center for the Second (randomization) Visit, the following took place:
- 7.21 Eligibility criteria were confirmed.
- 7.22 Data for exclusion criteria were reviewed.
- 7.23 Symptom assessment and physical examination were repeated (see *Form 52 - Visit Assessment and Summary* and *Form 14 - Questionnaire #4*) as part of the baseline assessment. Asbestos-exposed participants had a repeat spirometry (see *Form 76 - Spirometry*).
- 7.24 Level of vitamin adherence was determined by comparing the weight of the returned vitamin bottle to the number of weeks between the First and Second Visits (see table in Forms Manual for *Form 21 - Second Visit Eligibility Review*). Participants who had taken \geq 50 percent of the prescribed agents were eligible for randomization.

- 7.25 Eligible participants were randomized.
- 7.26 A six month supply of the study vitamins was distributed.
- 7.3 Follow-up visits: Efficacy participants returned to the study center at 6 months and 1 year from randomization, then yearly thereafter. Vanguard participants had visits every 6 months. Study Manual *Section 5 - Follow-Up* references all follow-up activity. Phone substitutions for visits were offered to participants at potential risk for inactivating from the study. At each Annual Visit and Vanguard Semiannual Visit, the following activities were performed:
 - 7.31 Evaluation of symptoms
 - 7.32 Encouragement to continue participating in the study and to adhere to the protocol.
 - 7.33 Update of health information
 - 7.34 Review and update of contact information
 - 7.35 Assessment of vitamin adherence and supplemental vitamin intake
 - 7.36 Dispensement of study vitamins (two bottles at Efficacy Annual Visits, one bottle at Vanguard Semiannual Visits)
 - 7.37 Collection of a blood sample (three 2.0 ml and four 0.5 ml aliquots for banking, one 10 ml tube for analysis for SGOT, alkaline phosphatase, cholesterol, and triglycerides) from Vanguard participants (not done at Vanguard Semiannual Visit)
 - 7.38 Collection of cataract diagnosis and extraction information (not done at Vanguard Semiannual Visit)
 - 7.39 At each even-numbered Annual Visit the following additional activities were performed:
 - 7.391 Review of the self-administered dietary intake questionnaire
 - 7.392 Collection of respiratory information
 - 7.393 Performance of spirometry by asbestos-exposed participants
 - 7.394 Collection of a blood sample from Efficacy participants (three 2.0 ml and four 0.5 ml aliquots for banking)
- 7.4 Routine Phone Contact of Active Participants: Study center staff contacted active participants by phone twice per year. In the first year after randomization, Efficacy participants were called 3 and 9 months after the Second Visit. In subsequent years, Efficacy participants were called 4 and 8 months after the anniversary of the Second Visit. Vanguard participants were called 3 and 9 months after the anniversary of their randomization visit. At each phone call the following activities were performed:
 - 7.41 Update of health information
 - 7.42 Review and update of participant contact information
 - 7.43 Assessment of vitamin adherence
 - 7.44 Evaluation of symptoms
- 7.5 Routine Phone Contact of Inactive Participants: Participants who stopped taking their study vitamins are referred to as inactive. Study center staff contacted inactive participants by phone twice a year, on and 6 months after the anniversary of their randomization visit. At each phone call the following activities were performed:
 - 7.51 Update of health information
 - 7.52 Review and update of participant contact information
- 7.6 One time activities: The following activity was attempted until either it was successfully completed, following specific additional informed consent, or the participant refused to complete the activity.

- 7.61 Collection of whole blood sample for future DNA studies.
- 7.7 Transition Visits: Beginning January 22, 1996, study centers contacted all participants, those who were active at the end of intervention as well as those who had previously inactivated, to conduct a final Transition Visit. Phone substitutions for Transition Visits are offered to participants who are unable or unwilling to come in to the study center. Prior to the Transition Visit, study centers complete a quality assurance chart audit checklist to ensure that the chart contains all required information. At the Transition Visit, the following activities are performed:
- 7.71 Update of health information
 - 7.72 Review and update of contact information
 - 7.73 Confirmation of stoppage of study vitamins and final assessment of supplemental vitamin intake
 - 7.74 Collection of a blood sample (three 2.0 ml and four 0.5 ml aliquots for banking, plus a whole blood sample for future DNA studies, if not previously obtained [see 7.61])
 - 7.75 Review of the self-administered dietary questionnaire
 - 7.76 Collection of respiratory information
 - 7.77 Performance of spirometry by asbestos-exposed participants
 - 7.78 Collection of cataract diagnosis and extraction information
 - 7.79 Review of self-administered physical activity and immunization questionnaires
- 7.8 Interim Phone Calls: Beginning January 22, 1996, and continuing until a participant has a Transition Visit, study centers place routine Interim Phone Calls. These calls are made according to the schedule in 7.4. At each Interim Phone Call, the following activities are performed:
- 7.81 Update of health information
 - 7.82 Review and update of contact information
 - 7.83 Confirmation of stoppage of study vitamins and supplemental vitamin intake
- 7.9 Post-Intervention Follow-Up (PIF) Phone Call: Study centers contact all participants by phone once per year, on the anniversary of the randomization date, until the post-intervention follow-up phase of CARET ends. At the PIF call, the following activities are performed:
- 7.91 Update of health information
 - 7.92 Review and update of contact information
 - 7.93 Collection of cataract diagnosis and extraction information
- 7.10 PATH Recruitment
- CARET will mail letters introducing the Physical Activity for Total Health (PATH) to female CARET participants from the Seattle Study Center to invite them to participate in a randomized clinical trial of the effect on endogenous sex hormones. No data on CARET participants will be released to PATH with this activity. If CARET participate in PATH, their participation will be covered under PATH's IR file #4290.
- 7.11 Baltimore Study Center Closure
- The CARET Baltimore Study Center plans to close in April 1999. A letter from the Baltimore Study Center Principle investigator, Dr. James Keogh, will be sent to the Baltimore participants informing them of the closing of the study center. A letter from the CARET Coordinating Center Principle Investigator will be sent to the Baltimore participants which informs the participants of the future direction of the study. On April 1, 1999, Baltimore participants will begin to receive follow-up telephone calls conducted by Gilmore Research Group in Seattle, WA.
- 7.12 Passive Consent

The Irvine Study Center, New Haven Study Center, Portland Study Center, and San Francisco Study Center plan to close their study centers as of April 1, 2000. A letter from each study centers investigator will be sent to their participants informing them of the closing of the study center.

7.12.1 The Portland Study Center participant charts will remain at Kaiser Permanente in Portland, Oregon. The letter from the Portland Study Center Investigator omits the mention of transferring participant charts to the Coordinating Center

7.13 Centralization of participant follow-up from local study centers to the Coordinating Center.

Beginning April 1, 1999 through March 31, 2000 the Irvine Study Center, New Haven Study Center, Portland Study Center, and San Francisco Study Center will conduct one final telephone contact with their participants. The following activities will be performed:

7.13.1 Update of health information

7.13.2 Review and update of contact information

7.13.3 Confirm receipt of letter informing participant of change of status from local study center contact to centralized contract by the Coordinating Center (see Passive Consent 7.12)

7.13.4 Participant charts (originals), including “do not contacts” and “difficult to locate” will be sent to the Coordinating Center to assure accessibility of the hard data. The Portland Study Center institution (Kaiser Permanente in Portland, Oregon) requires that their participant charts stay within their institution. If the Coordinating Center requires hard data from Portland Study Center participants charts, staff from Kaiser Permanente will copy the appropriate information and forward the information to the Coordinating Center.

7.14 Centralized participant follow-up at the Coordinating Center through annual mailings

Beginning April 1, 2000 the Coordinating Center will conduct annual participant follow-up by mailing a standard questionnaire to each participant once per year around the date of the participants randomization date. The questionnaire will:

7.14.1 Update health information

7.14.2 Update contact information

7.15 CARET Data Collection Forms to Comply with Confidentiality Guideline

In compliance to guidelines resulting from the December 1999 NCI workshop relating to participant confidentiality, procedure form changes were made. The questionnaire for the December 2000 mailing has been redesigned to comply with the guidelines. The current *Form 517* will be separated into three documents which are cover memo, *Form 588* and *Form 517*. *Form 588* – Contact Information form is a two-sided form that requests updated participant contact information. The form will be returned in one of two postage-paid envelopes. The other envelope is for *Form 517*, which will still be a 4-page booklet style form. Its content is restricted to questions about participants' health, smoking history, bone fractures, and use of pain medications. The participant's study ID number is pre-printed on the form.

7.16 A risk prediction model for the early detection of lung cancer has been developed based on published reports. The goal is to validate the model on an independent cohort of individuals. Two sets of analyses are planned based on those presented by Costantino et al. The sample will include CARET participants aged 50 years and older with a positive smoking history. The variables for longitudinal risk factor data will include: age at enrollment, sex, number of years smoking, number of cigarettes/packs per day, variables containing information on quitting and other alterations in smoking habits, annual follow-up data on smoking habits, study arm. The variables for longitudinal incidence data will include: date of cancer diagnosis, characteristics of diagnosis, stage, cell type, symptomatic or pre-symptomatic. This study will utilize deidentified data with defined endpoints that will not require further information from subjects. Therefore, consent is not needed.

CARET deidentified data used in the prediction model noted above will be sent to Ruwan Gunaratne, working under Dr. Melvyn Tockman of the Lee Moffitt Cancer Center of University of Southern Florida (USF). Gunaratne plans to compare the model noted above to one derived using neural networks. Data variables used in his data sharing are: age, duration of smoking, duration of abstinence, average amount smoked per day, sex, asbestos exposure, diagnosis after each year of follow-up and survival after each year of follow-up.

USF IRB approval for study received February 17, 2005.

7.17 SELECT Recruitment

CARET participants are invited to join SELECT. Information about SELECT will be included in the mailing as well as a survey for participants to fill and return.

7.18 Scripts in response to JNCI press release for CARET manuscript: A script has been drafted (attached in the modification) that will be used to address questions to CARET participants and the public in response to a CARET manuscript to be published in the December 2004 issue of JNCI and scheduled for a press release on December 1, 2004.

7.19 CARET Coordinating Center stopped collecting participant information and participant follow up ended on June 30, 2005. An end of study letter was sent to all active participants and End of Study scripts were drafted to be used to address questions from CARET participants in response to the End of Study Letter. The CARET 1-800 number will remain active through December 31, 2005 to accommodate participant questions.

7.20 Final CARET Reporter was sent to CARET participants explaining why CARET is ending.

8.0 ADHERENCE

During active intervention, CARET carefully monitored participants' adherence to their visit schedule and to taking study vitamins daily. The study centers are responsible for the promotion and maintenance of participant adherence to the study protocol. The Coordinating Center is responsible for monitoring participant adherence and advising the study centers on methods of promoting adherence.

8.1 Adherence to Study Protocol during Active Intervention

Participant adherence to visit schedules and vitamin schedules was critical to the quality of data collected during the active intervention phase of the study.

8.1.1 Convenient parking, comfortable waiting facilities, supportive staff, and appointment reminders contribute to the participant's willingness to adhere to visit schedules. Staff certification and quality assurance checklists monitor study center compliance to study protocol designed to encourage adherence to visit schedule (See Study Manual *Section 2.7 - Certification*). Feedback reports inform study centers of their performance as related to adherence (see Study Center Data System Manual *Section 10 - Central Database Reports*).

8.1.2 Participant's randomization to the study determined the vitamin dispensing schedule. Vitamin adherence was monitored in several ways: Participants were asked about vitamin adherence at all visits and phone calls. Quality control procedures monitored study vitamin consumption (see *Form 83 - Vitamin Adherence*). Analysis of serum samples determined vitamin levels in participants' blood.

8.2 Reactivation of Inactive Participants

During active intervention, any participant who decided to stop taking the study vitamins or who failed to report to the study center and could not be reached by phone for a period a six months or

more was inactivated. The study center called these inactive participants twice a year to follow-up on their health status and to try to reactivate the participant to the study.

8.3 Adherence to Study Protocol during the Post-Intervention Follow-up

8.3.1 Participant adherence to the routine post-intervention follow-up telephone call schedule is essential for continued monitoring of participants' health and periodic analysis of endpoint data by intervention arm. During post-intervention follow-up to February 2000, convenient scheduling of phone calls, skilled interviewing technique, and informative mailings are expected to contribute to participants' adherence.

8.3.2 Study centers attempt to maintain contact with all participants throughout the post-intervention follow-up period, regardless of the participant's study status prior to the end of intervention. For participants who are no longer willing to receive routine telephone calls, study centers, guided by applicable state or local requirements, attempt to obtain permission to obtain follow-up information from a physician or personal contact. Routine mailings are withheld from those participants who are not willing to receive them.

8.3.3 Staff certification and quality assurance checklists continue to be required during post-intervention follow-up, to ensure that all staff members collect follow-up data in a standardized and skilled manner (see Transition Bulletin 23).

8.3.4 The Coordinating Center continues to monitor participant adherence to the telephone contact schedule and to produce regular feedback reports to study centers on their performance.

9.0 SYMPTOM MONITORING AND MANAGEMENT

9.1 This trial was preceded by two pilot trials (see sections 3.6 and 3.7) which found no evidence of adverse effects from the pilot study vitamins (beta-carotene and/or retinol), except for mild yellowing of the skin in the intervention arms receiving beta-carotene. At the end of the pilot studies, the participants were merged into a single cohort (the Vanguard cohort) and were closely followed for the development of side effects. Since side effects of these agents are related to the duration of use, any significant side effects should have appeared in the Vanguard group first. Because of the close monitoring of the Vanguard cohort, side effect evaluation in the Efficacy cohort was streamlined for cost effectiveness. Symptom monitoring and management was stopped for both cohorts in January 1996, when participants were instructed to stop taking study vitamins.

9.2 The known side effects from oral beta-carotene and retinyl palmitate are listed below:

9.2.1 Beta-carotene

9.2.1.1 Yellow coloration of skin has been the only noted side effect.

9.2.1.2 Up to 200 mg/day for 5-10 years has been given to participants with erythropoietic protoporphyria with no reported side effect other than carotenemia.

9.2.2 Retinyl palmitate

9.2.2.1 Central Nervous System: Irritability, headache, increased intracranial pressure, papilledema, exophthalmos.

9.2.2.2 Dermatologic: Fissures of the lips, cheilosis, drying and cracking of the skin, alopecia, rashes, erythema, scaling of the skin, desquamation.

9.2.2.3 Hepatic: abnormal liver-spleen scans, hepatosplenomegaly, cirrhosis, abnormal liver function, jaundice, hepatic failure.

9.2.2.4 Psychiatric: Emotional instability, change in personality, depression, change in libido.

9.2.2.5 Reproductive: Menstrual changes, impotency, oligospermia, teratogenicity.

9.2.2.6 Other: Elevation of plasma triglycerides.

9.2.2.7 Retinyl palmitate side effects are related to both dosage and duration of administration.

9.2.2.8 Hypervitaminosis A is uncommon at doses of vitamin A <100,000 IU/day. Side effects have been reported at an oral dose probably greater than 2×10^6 units (as a single dose). Case reports of side effects have been reported at doses of 500,000 IU/day for 2-3 months, and one case has been reported at 25,000 IU/day for 7-10 years.

9.3 Symptom Assessment

9.3.1 The early side effects of excess retinyl palmitate are non-specific and are common conditions for people in the age range of CARET participants. Symptoms were assessed in a consistent manner in all participants receiving study vitamins (either active or placebo) to 1) evaluate the amount of symptoms attributable to the active vitamins and 2) identify any severe symptoms which may have been related to the study vitamins and which would have necessitated a change in the vitamin dosage.

9.3.2 Following standardized procedures, study center staff assessed participants for the incidence and severity of the following 13 symptoms: skin redness, dryness, itching, and yellowing; chapping of the lips; bone pain; headaches; anxiety; depression; nosebleeds; vomiting; frequency of bowel movements; and weight loss. The grading system used to determine the severity of symptoms is described in Study Manual *Section 9 - Symptom Monitoring*, Figure 9-1-1. To be graded above the baseline grade (either 0 or 1) for the symptom, the symptom needed to be present every day for the past two weeks. For the Efficacy cohort, symptoms were assessed at the First (enrollment), Second (randomization), Third (six month), and thereafter annual study center visits and twice a year by telephone. For the Vanguard cohort, symptoms were assessed at each semiannual study center visit and twice a year by telephone, and SGOT and alkaline phosphatase levels were obtained annually.

9.3.3 Symptoms were assessed at each study center visit using *Form 12 - Questionnaire #2* (at First Visit), *Form 14 - Questionnaire #4*, *Form 96 - Health Questionnaire #6* (at subsequent visits), and *Form 52 - Visit Assessment and Summary*. During routine active follow-up phone calls, telephone call substitutions for visits, and participant-initiated phone calls, *Form 17 - Contact Summary* and 13 symptom-specific questionnaires were used.

9.3.4 The threshold grade for each monitored symptom, except for skin yellowing for which there was no threshold grade, was 4. The threshold levels for SGOT and alkaline phosphatase were 2.5 times and 1.5 times the laboratory's upper limits of normal, respectively. All instances of threshold grade symptoms were reviewed by the study center Principal Investigator or medical officer at case presentations.

9.3.5 If participants complained of symptoms, other than the 13 monitored symptoms, that were thought to be significant by the study center staff, the participant was referred to his or her primary care physician. Questions about any non-monitored symptoms that might have been related to the intervention agents were brought to the attention of the study center Principal Investigator or medical officer by the study center staff. These symptoms, as well as newly diagnosed medical problems, were recorded to permit analysis of occurrence of previously undescribed side effects.

9.4 Symptom Management (See Study Manual *Section 9.2*)

- 9.4.1 If a participant experienced a monitored symptom (except for skin yellowing) that did not have an explainable cause and was at or above the participant's action grade for the symptom, the participant was placed on symptom management. Action grades were defined separately for each participant and symptom. For most participants, the action grade for a symptom was 4. Under two conditions the action grade was 5: 1) the symptom was grade 4 at baseline (First or Second Visit) or 2) at a post-randomization visit, the participant had a grade 4 or 5 symptom that did not resolve during symptom management. There was no symptom management for skin yellowing. For depression, no explainable cause was allowed.
- 9.4.2 The CARET symptom management procedure followed the standard drug challenge philosophy. It involved two withdrawals of the study vitamins, with one intervening rechallenge. If the occurrence of a symptom seemed to be temporally related to the study vitamins, the participant either had the dosage of the study vitamins permanently reduced to one-half (if that dose was not associated with symptoms), or was withdrawn from study vitamins permanently (if half dose of the study vitamins was associated with recurrent symptoms). The failure of symptoms to resolve after study vitamin withdrawal was considered evidence that symptoms were not related to the intervention, and the participant was instructed to resume taking the study vitamins. A symptom was considered resolved if the current symptom grade was below the participant's action grade for the symptom. A symptom was considered recurrent if the current symptom grade was at or above the participant's action grade for the symptom.
- 9.4.3 Efficacy Full-Dose Symptom Management
- Figure 9-5 in Study Manual *Section 9* shows the flow chart followed for Efficacy participants who were receiving a full dose of study vitamins at the start of symptom management. The study center Principal Investigator or medical officer reviewed each symptom management step.
- 9.4.3.1 First, a participant was asked to continue the study vitamins for a two-week evaluation. If the participant agreed, the study center staff re-contacted the participant in two weeks and reassessed the symptom. If the action grade symptom had resolved, the participant continued on full dose of the study vitamins and symptom management was ended.
- 9.4.3.2 If the participant refused to continue on study vitamins for two weeks or the symptom had not resolved at the two week assessment, the participant stopped taking the study vitamins. At the end of 30 days and 60 days (if symptoms had not resolved at 30 days), the participant was re-contacted by phone and the symptom assessed. If the symptom had not resolved, the participant was restarted on full-dose study vitamins and symptom management was ended with the presumption that the symptom was not due to the study vitamins. If appropriate, the participant was asked to contact his or her personal physician for evaluation of the symptom.
- 9.4.3.3 If the symptom resolved, the participant was restarted on full-dose study vitamins and re-evaluated at 30 days and 60 days (if symptom had not recurred at 30 days). If at 60 days the symptom had not recurred, the participant was continued on full-dose study vitamins and symptom management was ended.
- 9.4.3.4 If the symptom was recurrent at either 30 or 60 days, a second withdrawal of study vitamins was initiated. See Figure 9-5 for details of subsequent steps.
- 9.4.4 Vanguard Full-Dose Symptom Management
- Vanguard full-dose symptom management was similar to the Efficacy procedure except that 1) participants were immediately withdrawn from study vitamins and 2) participants were challenged with half-dose earlier in the procedure (see Figure 9-7 in Study Manual *Section 9*).

9.4.5 Half-Dose Symptom Management

If action grade symptoms occurred after participants had been placed on half dose (as a result of prior symptom management), the participant went through symptom management as outlined in Figure 2 for Efficacy participants and Figure 2 for Vanguard participants.

9.4.6 Symptom Management for SGOT and Alkaline Phosphatase (Vanguard only)

Figure 9-9 shows the symptom management steps followed when a Vanguard participant had a threshold grade SGOT or alkaline phosphatase level. Participants were immediately withdrawn from the study vitamins and had follow-up blood draws at 60 day intervals.

9.4.7 If, while the participant was on symptom management, threshold grade symptoms developed which were different from those which initiated symptom management, these new findings were not managed during that round of symptom management. If the symptoms persisted after conclusion of the symptom management, it triggered a new round of symptom management.

9.5 External Monitoring

The Safety and Endpoints Monitoring Committee (SEMC) closely monitored the symptom data on all participants by intervention arm and would have recommended protocol changes if an excess in symptoms had been observed. Semiannually, the committee reviewed summaries by coded intervention arm of reported symptom grades and the numbers of participants who had completed symptom management. The committee was unblinded as to the intervention arm codes in March 1994.

10.0 ENDPOINT DETERMINATION

10.1.1 The primary endpoint of this trial is the diagnosis of lung cancer. Secondary endpoints are the diagnosis of malignant mesothelioma (pleural and peritoneal), other malignancies, and deaths from all causes. Ancillary endpoints are self-reported cataract extractions. Study centers begin collecting the endpoint materials required for confirmation upon notification of a primary or secondary endpoint and after obtaining a signed release for medical information from the participant (See Study Manual, *Section 10 - Endpoints*).

10.1.2 Beginning April 1, 1999 for the Baltimore Study Center and April 1, 2000 for the Irvine Study Center, New Haven Study Center, Portland, Study Center, and San Francisco Study Center, endpoint data collection activities will go from being performed by the local study centers to centralized at the CARET Coordinating Center.

10.1.3 Beginning in November 2003, the Coordinating Center will be notified of new cancers and/or deaths via the mailed questionnaires or a participant-initiated telephone call. Upon notification, each self-reported cancer will be compared to previous reports to determine if the cancer is a re-report, recurrence or metastasis. If the cancer endpoint question is unclear, the participant will be called back for clarification. We will match our databases annually to cancer registries in the areas containing our study participants to determine the occurrence of cancers that may not have been reported to us. A death certificate will be obtained for all reported deaths either from the State or from the next-of-kin. Review of the death certificate will assist in clarifying "cause of death" and may be the sole report of an additional cancer endpoint. In addition, in order to detect unreported deaths we will annually match to the National Death Index our lists of participants from whom we do not have confirmation of vital status within the previous 21 months.

10.2 Endpoints are ascertained by active and passive follow-up. The exact methods of passive follow-up vary between study centers.

10.2.1 Active follow-up

10.2.1.1 Information on general health and the diagnosis of cancer is obtained at each routine visit and phone contact.

10.2.1.2 Participants who did not return to the study center for scheduled visits were contacted by phone or post card.

10.2.1.3 If the participant cannot be contacted, another contact source such as a relative, neighbor, friend, or local physician is contacted.

10.2.2 Passive follow-up

10.2.2.1 In areas covered by a SEER registry (Seattle, Irvine and San Francisco, and the state of Connecticut) or other centralized registry (Portland and the state of Maryland), periodic scans of registry databases are made to learn of new cancer endpoints and forward this confirmation to the Endpoints Review Committee for review.

10.2.2.2 We periodically search national death indexes such as the National Death Index and the Social Security Death Index to confirm vital status of randomized participants.

10.2.2.3 In December, 1997, CARET requested permission from the IRB to use the FHCRC Tracking Resource Center to obtain an updated vital status on CARET Seattle Study Center participants who had been difficult to locate and for those who had a contact status of 'do not contact.' Fourteen of the participants who were located had not received the CARET results letter described in Section 4.10, paragraph 5. Of these fourteen, 3 of them had had their last contact within five years of the CARET results. CARET sent the results letter to these 3 participants.

10.2.2.4 The CARET Coordinating Center will use the FHCRC Tracking Resource Center to obtain an updated "vital status" on those participants who are "difficult to locate" (last date known to be alive greater than 21 months) and for those who have a contact status of "do not contact". For those participants who have not limited our contact, we would use all sources available and make further attempts to reach the participant directly. The following script would be used if the participant asks how we located them "*We used public sources and databases to obtain a recent address or phone number.*" For those participants who have limited our contact, we would use the Public Records to update "vital status" only and no further attempts to reach the participant would be made.

10.3 Endpoints Review Committee

The Endpoints Review Committee provides a mechanism for specialty physicians to review endpoint materials to confirm all primary or secondary study endpoints in a standardized manner. Primary and secondary endpoints are confirmed according to the extent and reliability of the endpoint materials sent from the study center to the Coordinating Center.

10.4 Endpoint Review and Confirmation

10.4.1 All reported endpoints are reviewed and confirmed by a member of the Endpoints Review Committee, or by the entire committee if the reviewer requests assistance. For reported cases of lung cancer, mesothelioma, and cancers of unknown primary site, the Coordinating Center pathologist reviews the pathology report from the diagnosing hospital, as well as any tissue specimens obtained.

10.4.2 Because of the low concordance rates in diagnosing the sub-type of non-small cell lung cancer, the main categories for analysis are

10.4.2.1 Lung cancer (all types)

10.4.2.2 Non-small cell lung cancer

10.4.2.3. Small cell lung cancer

10.4.2.4 Subtypes of non-small cell lung cancer are examined with the understanding that pathology review is required to obtain meaningful results.

10.4.3 Beginning March 1, 1997, cause of death is determined from the death certificate.

10.4.4 Survival is measured from:

10.4.4.1 Time of randomization until death

10.4.4.2 Time of diagnosis of lung cancer until death

10.4.4.3 Time of diagnosis of cancer until death

10.5 Endpoints Quality Assurance

The Endpoints Review Committee routinely re-reviews a random sample of previously closed endpoints cases as a quality control check of the review process. The sampling scheme provides for a re-review of 10% of lung cancers and mesotheliomas, 5% of other cancers, 10% of cardiovascular deaths, 5% of other deaths, and 5% of reported endpoints that are closed as non-CARET endpoints.

10.6 The CARET Tissue Bank stores tumor and non-tumor tissue blocks and slides for future biomarker analysis.

10.6.1 Pathology specimens from submitting institutions are requested for each endpoint reported as a lung cancer or a cancer of unknown primary site. With permission from the submitting institution and a signed release from the participant, the Coordinating Center pathologist determines what specimens to retain for the Tissue Bank.

10.6.2 The Biomarkers Committee determines the analyses to be performed using the tissue.

11.0 TIMETABLE FOR STUDY CENTER PROCEDURES AND DATA COLLECTION

Figure 1 - Timeline for Routine Participant Contacts summarizes the data collection procedures for CARET, from recruitment through active follow-up and post-intervention follow-up (see page 23).

Figure 2 - Forms Completed at Participant Contacts lists the forms completed at each participant contact, by type of contact (see page 24).

FIGURE 1
Timeline for Routine Participant Contacts

<u>Stage in Study</u>	<u>Timing</u>	<u>Type of Contact</u>	<u>Procedures</u>
• Recruitment	Variable	Initial Mailing	Screen for smoking exposure, age,[asbestos exposure, sex]
	2-4 weeks after Initial Mailing	Screening Phone Call	Screen for exclusions: cancer, liver disease, lack of interest; schedule First Visit
	Visit is scheduled	First Visit	Screen for exclusions, collect blood sample and analyses for SGOT and alkaline phosphatase levels,
• Enrollment	1-4 weeks after Screening		[X-Ray and work history eligibility], complete questionnaires, assess symptoms and record baseline
	Phone Call		symptom grades, dispense enrollment vitamins [perform spirometry]
	3-6 months after First	Second Visit	Screen for exclusions: cancer, liver disease;, complete questionnaires, assess symptoms and record baseline

Visit			symptom grades, [pre- and post-bronchodilator spirometry]. Randomize, assign Study ID and dispense study vitamins
• First year Active 3 months Follow-up		Phone Call	Record symptom grades, document vitamin adherence
	6 months	Third Visit	Complete questionnaires, assess symptoms and record grades, dispense vitamins, and document vitamin adherence
	9 months	Phone Call	Record symptom grades, vitamin adherence
• Subsequent Active Follow-up (Vanguard only)	every 6 months	Semiannual Visit	Complete questionnaires, assess symptoms, record symptom grades, dispense vitamins, and document vitamin adherence
	12 months post-randomization & every other year after	Annual Visit odd-numbered	Complete questionnaires, assess symptoms and record symptom grades, dispense vitamins, and document vitamin adherence
	24 months post-randomization & every other year after	Annual Visit even-numbered	VANGUARD ONLY: Collect blood sample and analyze for SGOT and alkaline phosphatase levels Complete questionnaires, assess symptoms and record symptom grades, collect blood samples, dispense vitamins, and document vitamin adherence, (perform pre-bronchodilator spirometry) VANGUARD ONLY: Collect blood sample and analyze for SGOT and alkaline phosphatase levels [pre-bronchodilator spirometry]
	4 & 8 months (3 & 9 months for Vanguard) after each expected Annual Visit until Transition Visit	Phone calls	Assess general health and, prior to January 22, 1996, record symptom grades and document vitamin adherence
• Inactive Follow-up	Every 6 months	Phone calls	Collect updated health data and participant contact information
• Transition to Post-Intervention Follow-up	First routine visit January 22, 1996	Transition Visit scheduled after	Complete all activities of an even-numbered Annual Visit except vitamin dispensing, and confirm stoppage of study vitamins
• Post-Intervention Follow-up		Every 12 months	Phone calls Collect updated health data and participant contact information
• Last local study center contact	Every 12 months	Phone calls	Collect updated health data and participant contact information; discuss centralization
• Centralization Follow-up	Every 12 months	Mailings Coordinating Center	from the Collect updated health data and participant contact information

NOTE: procedures enclosed in [] indicate procedures for asbestos-exposed participants only

FIGURE 2

Forms Completed at Participant Contacts

	Screening		First Visit	Second Visit	Third Visit	Odd Ann. Visit	Even Ann. Visit	Semi-Ann. Visit	Routine Phone Call	Tran-sition Visit	Inter-	Post	Cent. Foll Up
	Mail	Phone Call									im	Int. Foll Up	
Self-Administered Forms:													
Form 01 - Interest Survey	X												
Form 08 - Respiratory History Questionnaire			X				X			X			
Form 10 - Contact Information			X	X									
Form 11 - Questionnaire #1			X										
Form 12 - Questionnaire #2			X										
Form 14 - Questionnaire #4				E	E	E	E				E		
Form 15 - Food Questionnaire			X				X				X		
Form 96 - Health Questionnaire #6						V	V	V			V		
Form 150 - Physical Activity Questionnaire											X		
Form 151 - Immunization Questionnaire											X		
Form 153 - Transition Visit Chart Audit Checklist									X				
Form 517 – CARET Annual Health Questionnaire													
	X												
Form 588 – CARET Contact Information													X
Interviewer-Completed Forms:													
Form 03 - Screening Phone Contact Record		X											
Form 21 - Second Visit Eligibility Review			X										
Form 22 - Enrollment				X									
Form 41 or 42 - First Visit Eligibility Review	X												
Form 17 - Contact Summary			X	X	X	X	X	V	X	X	X		
Form 17 - Contact Summary (Section E)			X	X	X	X	X	V		X			
Form 17 - Contact Summary (Sections C/D)										X	X		
Form 09 - Contact Information Update					X	X	X	V	X	X	X		
Form 52 - Visit Assessment and Summary			X	X	X	X	X	V					
Form 76 - Spirometry			A	A			A			A			
Form 77 - Study Center X-Ray Review			A										
Form 71 - Blood Collection and Processing			X			V	X			X			
Form 81 - Vitamin Dispensing History	X	X	X	X	X	X	X	V					
Form 83 - Vitamin Adherence				X	X	X	X	V		X	X		
Form 317 - Post Intervention Contact Summary												X	
Form 309 - Post Intervention Participant Contact Information Update												X	
Form 320 – Passive Consent												X	X
Form 518 – Centralized Telephone Health Questionnaire													X
Form 589 – Telephone Contact Information													X
As Needed:													
Form 17 - Contact Summary- Section A - Inactive Follow-up													
Form 17 - Contact Summary- Section B - Symptom Management													
Form 18 - Notice of Incomplete Enrollment													
Form 26 - Inactivation Contact													
Form 27 -Vital Status or Missed Contact													
Form 31 - Initial Notice of Endpoint													
Form 53 - Symptom Management - Full Dose (Efficacy) (Log Sheet only)													
Form 54 - Symptom Management - Half Dose (Efficacy)													
Form 93 - Symptom Management - Full Dose (Vanguard)													
Form 94 - Symptom Management - Half Dose (Vanguard)													
Form 326 - Reasons for Not Calling Participant													
Form 526 – Reason for No Further Contact with Participant													X

E = Efficacy participants only

V = Vanguard participants only

A = Asbestos-exposed workers only

12.0 STATISTICAL METHODS AND ANALYSIS

12.1 In the design of CARET, a protective effect of the study vitamins was considered to be of public health importance if the vitamins could prevent 33% of the lung cancers in fully-adherent individuals. Because of less-than-full adherence to the study vitamins and a likely time lag between beginning to receive study vitamins and the achievement of full effect of the study vitamins, CARET's statisticians estimated that the effect that could actually be obtained in CARET corresponding to this maximal potential effect of 33% was a 23% reduction. If CARET had continued active intervention to its planned completion date of November 1997, the trial was projected to have had 84% power to detect such an effect. The design parameters include a sample size of 4060 asbestos-exposed participants and 14,254 heavy smokers, randomized between 1985 and 1994, with follow-up through November 1997 for a mean of 6 years.

12.2 The following additional variables were considered:

12.2.1 The rate of lung cancer in the target population was computed from 1985 SEER data, 1986 smoking prevalence, 1980 attributable risk data, and relative risks for smoking and exposure to asbestos. Monitoring during the trial indicated that, while the lung cancer incidence rates in heavy smokers matched the projections well, the rates in asbestos-exposed participants did not, likely at least in part due to the baseline chest X-Ray detecting and screening out latent lung cancers. For the power calculations, the projections of lung cancer incidence for the asbestos-exposed participants were reduced by 20% to bring them into line with the observed rates.

12.2.2 Loss of participants to death from causes other than lung cancer was computed from 1980 mortality data, 1986 smoking prevalence data, and excess mortality data from the Surgeon General's Report on Smoking and Health. The observed rates during the trial were 60% of projected; in fact, the mortality rates in CARET were less than those seen in the general population for CARET's age range, even though all CARET participants had extensive histories of cigarette smoking and/or asbestos exposure. For the power calculations, the rates of non-lung cancer mortality were reduced by 40% from the initial projections.

12.2.3 The mean capsule consumption rate was initially estimated from three similar prevention intervention trials, and then as data accumulated by the observed capsule consumption rates in CARET, with linear projections for future years.

12.2.4 Time lag to full vitamin effectiveness was assumed to be two years.

12.2.5 Loss of participants to follow-up (i.e., inability to determine vital status at the conclusion of the trial) was projected to be 2%.

12.3 The primary endpoint is lung cancer incidence.

12.3.1 At the end of active intervention in the trial, the effect of the study vitamins was assessed by testing the difference between the arms in this incidence rate by a weighted log-rank statistic, stratified on population (asbestos-exposed or heavy smoker), cohort (Pilot/Vanguard or Efficacy), and study center (six centers), with weight function that is zero at the time of randomization, rising linearly to one at two years after randomization, then remaining one. The weight function was chosen to give less weight to early developing tumors, before the retinyl palmitate and beta-carotene full effect occurs.

12.3.2 Tests of the primary endpoint were scheduled to be performed twice during the course of active intervention in the trial, when one-third and when two-thirds of the projected total number of weighted endpoints had been closed by the Endpoints Review Committee, with the possibility of stopping the trial early if there were strong evidence in favor of or against the hypothesis. Critical p-values from O'Brien-Fleming monitoring boundaries (48) for the two interim analyses were 0.0006 at the first and 0.015 at the second. The p-value for the final analysis at the end of the trial would then be 0.047. After the first interim analysis, completed per this schedule, CARET's external Safety and Endpoints Monitoring

Committee requested an additional interim analysis in July 1995. Using the O'Brien-Fleming spending function, the critical p-value for this analysis was 0.007.

- 12.3.3 The primary analysis during the post-intervention phase of CARET will continue to be the stratified, weighted log-rank statistic, but with time measured from January 12, 1996, when the letter informing participants to stop their study vitamins was mailed. A secondary analysis will downweight linearly to the date when average active arm serum beta-carotene levels fall to 10% of the baseline (and placebo) values. Failure to find a statistically significant difference may imply that the excess lung cancer incidence in the active arm has not persisted. To quantify the magnitudes and time courses of relative risks consistent with the post-intervention data, we will use Kaplan-Meier and related estimators of incidence and cumulative incidence, as well as placing greater emphasis on relative risk estimates and associated confidence intervals than on formal test results. We model the effects of potential covariates on the lung cancer incidence through Cox regression models, using time-dependent covariates as appropriate, or through further stratification of the log-rank test. Among the important variables for which to adjust the analysis are the length of time active in the trial (as a proxy for the amount of vitamin stores in the body), the clearance time for beta-carotene in our study of these parameters, and lung cancer risk factors.
- 12.3.4 The estimates of the power of CARET were updated every six months with data on achieved accrual, loss of participants to deaths other than lung cancer, capsule consumption rates, and incidence rates of lung cancer. These updates consistently showed CARET doing better than originally planned, so that no remedial actions were necessary to maintain the trial's power.
- 12.3.5 Detailed analyses of all study parameters (except blood analyses) were done on all trial participants. The blood analyses for retinol, retinyl palmitate, beta-carotene, alpha-carotene, and alpha-tocopherol were done on all active participants in the Pilot/Vanguard, 10% of active participants in Efficacy, and retrospectively on participants reporting lung cancer. Analysis of the serum data used the case-cohort approach (49). An expanded blood analysis, including a more extensive set of carotenoids (lutein, zeaxanthin, gamma-tocopherol, cryptoxanthin, trans-lycopene, and total lycopene), has begun. The purpose of the complex carotenoid panel is to determine if beta-carotene given during active intervention affects levels of other carotenoids in the serum. We reported in 1994 (50) that beta-carotene levels in the active arm had no influence on serum alpha-tocopherol levels.
- 12.3.6 Secondary, explanatory, and ancillary analyses have been and are being performed, including but not restricted to the following:
- 12.3.6.1 Tests of lung cancer rates adjusted for potential covariates
 - 12.3.6.2 Tests of the effectiveness of the vitamins on the rate of mesothelioma, all epithelial cancers, and specific cancers
 - 12.3.6.3 Tests of the association between smoking and fibrosis
 - 12.3.6.4 Tests of fibrosis as an independent risk factor for lung cancer (in the asbestos-exposed sub-population)
 - 12.3.6.5 Tests of correlation of baseline serum beta-carotene and retinol and of baseline total daily intake of beta-carotene and retinol with subsequent lung cancer and total cancer risk.

13.0 DATA COLLECTION AND MANAGEMENT

13.1 Data Collection

Beginning April 1, 1999 for the Baltimore Study Center and April 1, 2000 for the Irvine Study Center, New Haven Study Center, Portland Study Center, and the San Francisco Study Center,

the collection of all data collection activities will go from local study centers to centralized at the Coordinating Center.

13.1.1 The scientific data collected originate at the study centers:

- 13.1.1.1 Questionnaires and forms completed by the study center staff during telephone and in-person interviews of study participants
- 13.1.1.2 Questionnaires completed by the study participants
- 13.1.1.3 Results of tests such as blood testing, x-ray readings, and spirometry
- 13.1.1.4 Blood samples and vitamin dispensing and adherence information
- 13.1.1.5 Participant contact information such as participant address and phone number, and names and phone numbers of personal contacts and primary physician.

13.1.2 Administrative data needed to manage the study center operation, such as appointment schedules, recruiting sources, recruiting names and addresses, budgetary items, mass mailings, and local correspondence, are maintained exclusively on the study center personal computers. If the proper facilities were not available, the Coordinating Center assisted the study centers in preparing mass mailings for their recruiting efforts.

13.1.3 Scientific data are collected on both written forms and mark-sense forms. Most written forms are key-entered at the study centers. Mark-sense forms and certain written forms are forwarded to the Coordinating Center for scanning and key-entry. Endpoint materials are forwarded to the Coordinating Center. Endpoint review data are collected and key-entered at the Coordinating Center.

13.1.4 In addition to the Study Manual, the Coordinating Center developed a Forms Manual containing all the data collection instruments for all data items and a Study Center Data System Manual containing data collection procedures. These Manuals also contain: 1) information on confidentiality; 2) details regarding form coding and key-entry procedures; 3) guidelines for compliance, timeliness, completeness, and accuracy of data; 4) instructions and guidelines for data submission and examples of batch reports; 5) information on computerized edit checks; and 6) information on data monitoring reports sent from the Coordinating Center to the study centers.

13.1.5 For the post-intervention follow-up phase of CARET, the Coordinating Center will develop a comprehensive manual to include all forms, operational procedures, and data management procedures.

13.1.6 Data collection forms will reflect the changes in the endpoints determination (see Section 10.1.3) and the stopping of collecting data on bone fractures and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). The smoking question has also been reformatted.

13.2 Data Quality Control

Several quality control and quality assessment methods are used to ensure that the data are accurate. These methods include: development of detailed data collection guidelines; interviewer and data collection staff training; two-pass key-entry and verification of data; computerized edit checks; routine feedback and quality control reports; and periodic audits of study center records compared with data in the central database.

13.3 Data Processing

13.3.1 In the study centers

- 13.3.1.1 Administrative data are collected and processed within the study centers. Recruitment data were maintained on the study center personal computer (PC), including lists of potential participants, phone numbers, call attempts, and interview

disposition. For eligible participants, additional data were collected. Recruitment data were summarized and transmitted to the Coordinating Center on a monthly basis. Study center appointment information is not sent to the Coordinating Center (CC).

13.3.1.2 Scientific data about the participant are key-entered, independently verified, and edited using a stand-alone key-entry package. These data are transmitted to the Coordinating Center for processing once per week. Lengthy mark-sense questionnaires (health history, dietary assessment, occupational exposure, and baseline side effects data) are reviewed at the study center and sent to the Coordinating Center for processing.

13.3.1.3 Methods

Each study center uses one or more personal computers (PCs) to maintain its administrative data and to prepare scientific data for transmission to the Coordinating Center using commercially available software packages. Each study center has a staff person designated as Data Coordinator who is responsible for the accurate and timely key-entry and maintenance of these data. Quality control procedures are established by the Coordinating Center, and training sessions are conducted to ensure that each study center understands the importance and proper procedures for producing quality scientific data.

13.3.2 At the Coordinating Center

13.3.2.1 Scientific Data

13.3.2.1.1 These data have the highest priority at the Coordinating Center for key-entry, processing, and quality control. Administrative and recruiting data are processed at least weekly, but on a less urgent basis.

13.3.2.1.2 Data on randomization, which was performed at the study centers, were subjected to routine, rigorous quality control checks at the Coordinating Center.

13.3.2.1.3 Enrollment and routine follow-up data are transmitted to the Coordinating Center weekly by the study centers.

13.3.2.1.4 The Coordinating Center maintains a scientific database of participant data captured at the study centers. This database is updated on a routine schedule. There are routine quality control measures to ensure data integrity. Routine data reports are sent to the study centers to verify data quality.

13.3.2.2 Administrative data

The Coordinating Center does not maintain an electronic database on study center administrative data.

13.3.2.3 Recruitment data

The Coordinating Center assisted the study centers in preparing mass mailings for their recruiting efforts. Although the individual study centers initiated and administered the recruiting process, it is beyond the scope of most study center operations to coordinate data processing for computer lists containing many thousands of names and addresses. The Coordinating Center provided a standard service for processing contents of computer tapes, ordering laser-printed mailings, selecting valid random samples from large lists, and comparing new computer tapes to lists already processed. Selected mailing data were returned to the study centers so that response information could be recorded in their administrative databases.

13.4 Communication between study centers and the Coordinating Center

The study design relies heavily on timely and accurate communications between the study centers and the Coordinating Center, which necessitates good choices in selecting computer hardware, software and adequate training of users. We modeled our design on the successful CASS clinical trials, as well as taking advantage of local expertise from the Women's Health Trial Statistical Coordinating Unit and the Southwest Oncology Group Statistical Center.

13.4.1 Data transfer

Data are transferred from the study centers to the Coordinating Center by mail on diskette, or via electronic mail (MCI Mail) on a weekly basis.

13.4.2 Document transmission

The following items are sent by mail between the study centers and Coordinating Center:

- Updates to study documentation

- Study memoranda, Study Center Inquiries, routine correspondence, Requests for Information

- Routine data reports

- Data error reports

Certain inquiries and memoranda are sent by electronic mail. Study Center Data System updates are sent by the Coordinating Center to study centers on diskette in the mail.

13.4.3 Methods

The same microcomputer that is used in the study centers for scientific key-entry and administrative data maintenance is used for electronic document transmission.

13.4.4 Protection of Confidentiality

The Coordinating Center has implemented procedures related to the protection of confidentiality of participants.

13.4.4.1 Transport of confidential and identifying information outside of the Coordinating Center

Procedures have been implemented for telecommuters who work at home using CARET participant data. All unnecessary identifying information is removed from forms being transported outside the office. All documents are kept in lock boxes in the home office area. The supervisor is kept apprised of exactly what work is being taken home by documenting this via a handwritten note or by e-mail. In addition, all telecommuters sign a Telecommuter's Agreement and Confidentiality Pledge.

13.4.4.2 Eliminate unnecessary confidential and identifying information on forms that are transported.

Documents containing confidential information are retrieved and returned on a regularly scheduled basis. All documents containing confidential information are kept secured while being transported to and from FHCR.

13.4.4.3 IRB Training

All employees must complete IRB training before the end of their probation. Employees can either attend a two-hour tutorial presented by IRO staff at the Fred Hutchinson Cancer Research Center or complete a web-based training course.

14.0 TRIAL ORGANIZATION

The institutional participants in this study include six study centers and a Coordinating Center. The six study centers are Baltimore Study Center, University of Maryland, Baltimore, Maryland; Irvine Study Center, University of California, Orange, California; New Haven Study Center, Yale University and Lawrence and Memorial Hospital, Groton, Connecticut; Portland Study Center, Kaiser Permanente Center for Health Research, Portland, Oregon; San Francisco Study Center, University of California at San Francisco Division of Occupational and Environmental Medicine, San Francisco, California; and Seattle Study Center, Fred Hutchinson Cancer Research Center, Seattle, Washington. Scientific guidance is provided by a steering committee and its subcommittees, consisting of the Principal Investigators and co-investigators from the participating institutions (see Study Manual *Section I - Organization*).

Beginning April 1, 1999 for the Baltimore Study Center and April 1, 2000 for the Irvine Study Center, New Haven Study Center, Portland Study Center, and the San Francisco Study Center, participant follow-up and endpoint data collection activities went from the local study centers to centralized at the CARET Coordinating Center. Beginning April 1, 1999 the Seattle Study Center was folded into the Coordinating Center to perform all participant follow-up and endpoint data collection activities on all CARET participants.

14.1 Study centers

Each study center recruited, enrolled, evaluated, and conducted in-person and telephone follow-up of participants in one or both of the high-risk populations under study, heavy smokers and asbestos-exposed workers. Each study center is directed by a Principal Investigator, assisted by a team, which includes a study center manager, data coordinator, interviewers, endpoints specialists, and support staff. Each asbestos study center had access to a radiologist for the reading of X-rays to determine participant eligibility. Specifically, each study center:

- 14.1.1 Performed all activities in conformity with the protocol, including maintaining the blinding and confidentiality of intervention procedures and medical and trial data
- 14.1.2 Implemented the agreed upon timetable for participant accrual
- 14.1.3 Delivered chemopreventive agents in double blind fashion and monitored compliance as indicated in the protocol, including drawing of periodic blood samples
- 14.1.4 Monitored all participants for potential side effects by means of regular in-person and telephone interviews
- 14.1.5 Worked cooperatively with other study units in the implementation of the study
- 14.1.6 Collected data on eligibility, follow-up, and endpoints and entered these into the trial database according to uniform standards.

14.2 Coordinating Center

The Coordinating Center, located at the Fred Hutchinson Cancer Research Center in Seattle, consists of administrative, data management, statistical, and quality assurance staff plus a blood laboratory. The Coordinating Center is responsible for implementing all modifications to the design of the study and this protocol, as approved by the Steering Committee. The Coordinating Center is also responsible for the collection, editing, quality control, storage, and analysis of data generated by the study centers; the storage and analyses of blood samples and procedures for routine communications. During the intervention phase of the trial, it also managed the randomization of participants and the distribution of chemopreventive agents. Specifically, the Coordinating Center has the following functions:

- 14.2.1 Development of the Study Manual, Forms Manual, Study Center Data System Manual, and all data collection instruments
- 14.2.2 Procurement of all chemopreventive agents and placebos and distribution to study centers

- 14.2.3 Development of a system for distributed randomization of participants at the study centers, while maintaining the double blind nature of this trial
 - 14.2.4 Training of study center managers and interviewers on follow-up and retention procedures
 - 14.2.5 Training of study center data coordinators in the use of study center personal computers, data collection and review, and key-entry and data transfer
 - 14.2.6 Establishment and maintenance of a data quality control program for regular review of data transmitted by the study centers
 - 14.2.7 Development and implementation of data capture and transmission schemes and arrangement for long term storage of all trial data
 - 14.2.8 Establishment of procedures for shipping blood samples from the study centers to the Coordinating Center and for storage and analysis of the samples
 - 14.2.9 Arrangement for multiple readings of X-rays for asbestos-exposed participants for reliability purposes
 - 14.3.0 Development of appropriate methods of analysis and presentation of study data
 - 14.3.1 Scheduling of regular meetings of study investigators and staff members as appropriate, for scientific consideration of study progress and procedures
 - 14.3.2 Preparation of regular scientific reports describing study progress and preparation of papers for publication in collaboration with study investigators
- 14.3 Committees
- 14.3.1 The Steering Committee and subcommittees are responsible for tracking study performance, assuring good communications between study centers and Coordinating Center, approving ancillary studies, and reviewing all protocols, publications and policies.
 - 14.3.2 The external Safety and Endpoints Monitoring Committee (SEMC) regularly reviewed data on endpoints and monitored symptoms, as summarized by the Coordinating Center, conducted two formal interim analyses of trial data, and set study termination and code-break rules. After the end of CARET intervention, the SEMC's role is limited to the review of endpoints data.

15.2 Pharmaceutical Data

Tishcon Corporation encapsulated and shipped the CARET vitamins to the Coordinating Center:

15.2.1 Placebo capsules contained the following inactive ingredients: gelatin, sucrose food starch, tribasic calcium phosphate, BHA, and BHT. The finished placebo capsule is identical to the active capsules but totally devoid of the two active ingredients: beta-carotene and retinyl palmitate

15.2.2 Active capsules contained 30 mg of beta-carotene (raw materials supplied by Hoffman-LaRoche), 25,000 IU of retinyl palmitate, and the same inactive ingredients found in the placebo capsule

16.0 INFORMED CONSENT

After CARET wide discussion and preparation of draft informed consent forms, each study center completed its own consent form for the trial, in accordance with the requirements of its respective Institutional Review Board (IRB). In addition, as directed by the Coordinating Center and required by the IRB, study centers developed specialized consent forms for new trial activities and ancillary studies. See *Appendix B* of the Study Manual for a sample consent form. Study centers also developed release of information (ROI) forms to obtain medical records and pathology specimens for endpoints adjudication and for banking of pathology specimens. See Study Manual *Appendix D, Bulletin #65*, for a sample ROI.

17.0 CARET Biological Specimen Bank

Approval for the designation of the CARET Coordinating Center as the gatekeeper for the CARET specimen bank was obtained in April 1996 under IR File #4239. CARET began centralizing activities at the Fred Hutchinson Cancer Research Center in April of 2000 as part of the continuation grant (4/99 – 3/04). As part of this transition to centralization, the IR File 4239 – CARET Coordinating and IR File #H636 – CARET Seattle Study Center were merged into one file. Thus, the CARET Coordinating Center is the gatekeeper serum samples collected from CARET participants.

The CARET Biological Specimen Bank consists of biologic materials from participants recruited to the two pilot studies during 1985-1987 and the efficacy cohort from 1988-1994. Attachment 1_ shows the history of CARET's specimen collection schedule. Samples which are included in the specimen bank are: whole blood, serum, plasma, DNA spot cards, and tissue. How these samples were collected are described in other sections of this protocol.

17.1 Continued follow-up of CARET participants will continue until 2008.

CARET stopped collecting tissue samples in April 2003.

17.2 Ancillary Studies

CARET has a formal process for reviewing proposals utilizing its data and specimen samples (Attachment 2). The investigators must submit a 3-5 page proposal outlining the background information, results of pilot studies, and the specific proposal. A statistical review of the proposal should be included. See Attachment 3 which shows a template we send to investigators for developing their proposals. The CARET Principal Investigator selects a primary and two secondary reviewers from the CARET Steering Committee. If the proposed area of study is not consistent with any of the CARET investigators' expertise, an independent outside reviewer may be suggested as the primary reviewer.

The primary reviewer is responsible for summarizing the proposal and presenting his or her critique to the CARET Steering committee. The two secondary reviewers are responsible for

presenting their critiques. All members of the Steering Committee and CARET Co-investigators discuss the proposals and vote to approve or disapprove and prioritize the proposal relative to other currently approved proposals.

17.2.1 Procedure and checklist for CARET Ancillary Studies

The investigator will prepare a proposal and submit to the CARET Coordinating Center (CC). The Coordinating Center will obtain any necessary documents including the Confidentiality Pledge, Certificate of Privilege and documentation of Human Subjects Training. The proposal will then be sent to the NCI Project Director for approval. The Coordinating Center will produce a status report showing the impact of a study on the specimen bank inventory.

The Coordinating Center will forward the proposal to the CARET Steering Committee for review and approval. After it has been approved, the CARET Principal Investigator will provide the investigator with a letter of intent that CARET supports their proposal. At that time, the investigator will submit their proposal to their own IRB for review.

The Coordinating Center will submit the proposal to the FHCRC IRB for review, along with IRB approval from the investigator institution. The Coordinating Center will notify the investigator of IRB approval. After it has been approved at the FHCRC IRB, copies will be sent to CARET study center's IRBs for their files.

The Coordinating Center will work with the investigator in setting up a timetable for obtaining the necessary specimens (if applicable) or data files needed for the study. If specimens are to be sent out, the Certificate of Privilege will also be sent again with the specimens.

See attachment 4 for the CARET Ancillary Studies procedures and checklist.

17.3 Listed below are CARET ancillary studies and their corresponding IR File numbers. The main CARET grant (NCI UO1 CA 63673) allows for the funding of these additional ancillary studies.

17.3.1 IR file #4208 "BC Clear-Determining the Plasma Disappearance and Pharmacokinetics of Beta-Carotene and Long Term Oral Supplementation". Objectives: To determine the serum concentrations of beta-carotene, retinol, retinyl palmitate, and alpha-tocopherol over time after CARET participants discontinued oral supplementation with beta-carotene. This study began after CARET intervention ceased.

17.3.2 IR File # 4284 "Publication of Anonymous Research Date-Baseline Serum Concentrations of Beta-Carotene in Participants in CARET". Objectives:

17.3.3 IR File #4242 "Smoker Spiros-The Effect of the CARET Vitamins on Ventilatory Function in the CARET Smoker Cohort". Objectives: To determine if the administration of the CARET vitamins had a protective effect on the age-related loss of lung function in current and former smokers.

17.3.4 IR File # 4363 "P-53 Prevalence of Anti-P53 antibodies and P53 Mutations in CARET Participants with Lung or Prostate Cancer". Objectives: To determine the incidence of measurable concentrations of antibodies against the mutant P53 protein in CARET participants with and without lung cancer (This study has been closed.)

17.3.5 IR File #4364 "PSA Correlation between the increase in PSA and the Diagnosis of Prostate Cancer and Histologic Grade". Objectives: To retrospectively determine the slope of the increase in serum PSA (protein bound and free) in CARET participants diagnosed with prostate cancer, and to compare this increase with measurements from a set of healthy CARET participants and matched controls.

- 17.3.6 IR File #4381 “Immunization-Vitamin Enhancement in Pneumococcal Vaccine Response”. Objectives: To examine the effects of the combination of beta-carotene and retinyl palmitate on antibody responses to immunization for Streptococcus Pneumoniae and influenza infections among CARET participants.
- 17.3.7 IR File #4391 “Fatty Acids/Prostate-Association between plasma phospholipid Fatty Acid Levels and Prostate, breast, and Colon Cancer”. Objectives: To determine the relationship between serum phospholipid fatty acid levels, biomarkers of recent fat intake (4-6 weeks), and prostate cancer using existing serum samples from selected male CARET participants. The second objective is to also determine the degree of correlation between assessment of dietary fat intake from the food frequency questionnaire on file and the biomarker fatty acids.
- 17.3.8 IR File #4393 “Determining the Plasma Disappearance and Pharmacokinetics of Beta-Carotene after Long-Term Oral Supplementation “ Objectives: To determine the effects of long-term supplementation of Beta-carotene and retinyl palmitate on lipoproteins (HDL, LDL, VLDL) and other carotenoids and retinoids by analysis of existing serum samples on 52 New Haven CARET participants stored at the CARET Coordinating Center. These results will be compared to existing chart data on CARET Seattle participants.
- 17.3.9 IR File #4392 “Consent Waiver for Deceased participants. DNA Protocol and Consent Mailing-Association between Genetic Factors and Risk of Lung Cancer and other Diseases”. Objectives: To test stored serum from deceased participants for markers approved under IR File #4406.
- 17.3.10 IR file #4399 “Folate/Homocysteine-Association Between Serum Homocysteine and Folate Levels and Cardiovascular Disease”. Objectives: To investigate associations and relative risks for the folic acid-homocysteine-cardiovascular disease endpoints cascade in CARET participants, testing whether low folic acid generation and ingestion correlate with serum folate levels; serum folate with serum total homocysteine levels; and the relationship between homocysteine levels and cardiovascular disease and coronary heart disease.
- 17.3.11 IR File #4406 “Associations Between Genetic Factors and Risk of Lung Cancer and Other Diseases”. Objectives: To determine possible associations between genetic factors and risk of lung cancer and other diseases. Existing participant samples from CARET serum bank will be sent to collaborating laboratories for analysis.
- 17.3.12 IR File #4541 “Diet and Genetic Risk for Lung and Prostate Cancers”. Objectives: This proposal is for an investigation into the associations between the incidence of lung cancer and prostate cancer and diet, and modification of other potential risk factors by diet (This file was closed and re-opened as 5053).
- 17.3.13 IR File #4618 “Homocysteine Studies Collaboration: Release of CARET Data for Meta-Analysis”. Objectives: To release data on CARET participants in the study entitled “Association Between Serum Homocysteine and Folate Levels and Cardiovascular Disease” (IR File #4399) to the investigators of the Homocysteine Studies Collaboration based at the University of Oxford, England, to use in a meta-analysis.
- 17.3.14 IR File #4629 “Pilot Study to Determine the Utility of the Washington Cancer Registry for CARET Endpoint Ascertainment”. Objectives: To search the Washington State Cancer Registry (CSS & Eastern Washington) for cancer data on 6,750 CARET Seattle Study Center participants. We are evaluating how effective CARET is at capturing cancer incidence compared to the registry system.

- 17.3.15IR File #4667 “Association Between Genetic Risk Factors and Smoking Behavior”. Objectives: The goal of this study is to examine genetic influences on smoking behavior, with an emphasis on successful quitting.
- 17.3.16IR File #4668 “Pilot of Poly2000 GeneChip for Scanning Polymorphisms in CARET Participants”. Objectives: The goal of this study was to examine the usefulness of the Poly2000 GeneChip technology in scanning CARET samples for single nucleotide polymorphisms (this study has been closed.)
- 17.3.17IR File # 4669 “Association Between Flavonols and Risk of Lung Cancer Measurement of Plasma and/or Serum Quercetin Concentrations in CARET Participants”. Objectives: To examine the association of baseline plasma and/or serum quercetin concentrations in lung cancer participants and controls. The hypothesis is that baseline plasma and/or serum quercetin concentrations will be lower in CARET participants who developed lung cancer compared to controls.
- 17.3.18IR File #4670 “Association Between Chlamydia Pneumoniae Infection and Risk of Lung Cancer”. Objectives: To determine whether chronic infection with Chlamydia Pneumoniae may increase risk of lung cancer.
- 17.3.19IR file #4671 “Association Between Growth Factors and Risk of Lung Cancer and Other Diseases”. Objectives: To determine in the CARET population if the following growth factors are associated with the incidence of lung cancer and cardiovascular disease.
- 17.3.20IR file #4768 “CARET Ancillary Study: Association Between Serum Concentrations of Selenium and the Incidence of Lung and Prostate Cancer”. Objective: To investigate the association between the serum concentrations of selenium and the incidence of lung and prostate cancer.
- 17.3.21IR File #4860 “Hepatotoxicity-Hepatic Effects of Low Dose Vitamin A Supplementation”. Objective: To assess the technical feasibility of quantitatively determining procollagen levels from frozen banked serum on 10 participants from the CARET intervention group and compare them to 10 participants from the CARET placebo group. The specific aims of this data was to provide an estimate of mean values, variance, and distribution to utilize in sample size calculations for a larger, comprehensive study (This file has been closed.)
- 17.3.22IR File #4942 “Release of Tabular Data to External Party” Objective: Dr. Michal Freedman of NCI has analyzed data from the Alpha-tocopherol-Beta-carotene (ATBC) trial and has found a disparity in the occurrence of amyotrophic lateral sclerosis (ALS) between those receiving beta-carotene and those receiving placebo. To help her interpret this finding, she has requested information on the incidence of ALS by intervention group IN CARET.
- 17.3.23IR File #5028 “Association between Folate and B12 Levels and Lung and Prostate Cancers”. Objective: To investigate the relationship between serum folate and B12 levels and lung and prostate cancer incidence in CARET participants. To determine the effect of intervention on the serum folate and B12 levels in CARET participants with and without the diagnoses of lung and prostate cancers.
- 17.3.24IR File #5040 “The Relationship between Plasma IGF-1, IGF-2, and IGFBP-3 and Lung Cancer Risk”. Objective: To examine the relationship between serum IGF-1, IGF-2, and IGFBP-3 and lung cancer risk by conducting a nested lung cancer case-control study within the CARET trial cohort.
- 17.3.25IR File #5082 “Association Between S100 Proteins and Lung Cancer – A Pilot Study” Objective: The short-term goals of the pilot study are to test for S100 proteins (MRP8 and MRP14) in stored CARET serum. The long-term goals of this

research are to investigate whether these biomarkers are correlated with disease and may help explain the effect of the intervention in the CARET study.

17.3.26IR File #5154 "Detection of Aberrant Hypermethylation of Cancer-Related Genes in Serum as a Screening Tool for Early Detection of Lung Cancer". Objective: Pilot study to validate ability to use CARET samples to confirm the possibility of performing a study looking at aberrant promoter hypermethylation of cancer related genes (p16, MGMT, DAP-kinase, GSTP1, and APC) in serum/plasma samples as tool for the early detection of lung cancer in high-risk populations and/or monitoring patients with lung cancer during treatment utilizing CARET specimens (This study has been closed).

17.3.27IR File #5171 "Serum Concentrations of Mesothelin in the Early Diagnosis of Mesothelioma". Objective: The aim of this pilot study was to determine if mesothelin is elevated in patients with malignant mesothelioma. An initial phase II trial has been added to measure the concentration of MPF/Mesothelin in the serum of 10 patients with a diagnosis of malignant mesothelioma and 10 matched healthy controls. This modification to IR file #5171 was approved on February 7, 2003.

17.3.28IR File #5259 "The Association Between Physical Activity and Cancer Incidence and Mortality and Identifying the Correlates of Physical Activity". Objective: This study aims to investigate physical activity in relation to all-site cancer and lung cancer incidence and mortality in the CARET study. A second purpose is to investigate how total time spent in physical activity and intensity of that time are related to cancer outcomes in this sample. Third, the study seeks to identify when in the lifespan physical activity is most important in terms of cancer outcomes.

17.3.29IR File # 5474 "Validation of Protein Markers for Lung Cancer Using CARET Sera and Proteomics Techniques". Objectives: The objectives of this study are to (1) validate the finding from pilot studies with CARET sera of autoantibodies annexins I and II and PGP9.5 as potential biomarkers for lung cancers before the clinical diagnosis, evaluating sensitivity and specificity by time before diagnosis, treatment arm, gender, histologic type, and smoking status; (2) identify additional antigens and antibodies in sera from CARET participants, evaluating sensitivity and specificity by time before diagnosis, treatment arm, gender, histologic type, and smoking status, and, (3) compare the findings for individual biomarker candidates in participants who were current smokers versus former smokers. This ancillary study was approved by the IRB on September 30, 2002.

17.4 The following studies utilize CARET data or specimens but have their own separate funding. These studies with their own independent funding are listed below.

17.4.1 IR File #4534 "Endogenous Sex Hormones, Genetics, and Prostate Cancer". Objectives: To examine the association of prostate cancer risk with (1) serum concentrations of testosterone, sex-hormone binding globulin (SHBG), androstenedione, dehydroepiandrosterone sulfate (DHEAS), 3 α -androstane-20-one-3 β -diol glucuronide (3 α -diol G), and estradiol; and (2) polymorphisms of the 5 α reductase type II (SRD5A2) gene and of the androgen receptor gene. This protocol has been modified to include analysis of A49t polymorphism of the SRD5A2 gene. This study is funded through grant number RO1CA788 O1A1.

Data from this study will be released to the Dr. Andrew Roddam of the Cancer Research UK Epidemiology Unit/Radcliffe Infirmary in Oxford UK. Pooled deidentified data will be used in a pooled analysis (see attached protocol and data request form). IR File #4534 to be modified as well to show this data analysis.

17.4.2 IR File #4588 "Analyzing CARET Specimens to Model Serum Markers for Cost Effective Ovarian Cancer Screening". Objective: The aim of this study was provide serum samples from the CARET Serum Bank for seven female CARET participants previously diagnosed with

ovarian cancer, and matched controls, in order to 1) pilot methods to conduct a case-control study of ovarian cancer serum markers for early detection, and 2) obtain preliminary estimates of the mean concentration and change in concentration over time in each marker and the correlation between markers, separately in cases and controls. Funding for this study is pending.

- 17.4.3 IR File #5053 “Diet and Genetic Risks for Lung Cancer”. Objective: To better understand the association of fruit and vegetable intake with the reduced risk of lung cancer and to examine whether the associations between fruit and vegetable intake with lung cancer risk differs among participants grouped by their genetic risk. This study is funded through grant number CA89734-02.
- 17.4.4 IR File #5524 “CARET Ancillary Study: SNPs in Lung Cancer Risk and Therapeutic Response. The objective of this study is to utilize CARET specimens to refine experimental techniques for DNA extraction that will be used in a larger study. This study was approved by the IRB on November 30, 2002.
- 17.4.5 IR File #5609 “Diet and Genetic Risks for Prostate Cancer”. Objective: This proposal will investigate associations of dietary influences on oxidative balance (fat, fruit and vegetable intakes) and polymorphisms in oxidative stress regulatory enzymes with the risk of prostate cancer. The hypothesis is that dietary factors that increase oxidative stress (e.g., dietary fat) are associated with increased risk of prostate cancer; dietary factors that decrease oxidative stress (e.g., fruits and vegetables) are associated with decreased risk of prostate cancer; and the magnitude of these risks will vary by cancer susceptibility genetic profile. This study utilizing CARET specimens is funded through grant number RO1 CA 097678901A1
- 17.4.6 IR File #5608 “SNPs and Cancer Risk and Response. Objective: This proposal will develop PCR arrays for a panel of 20 cytokine gene SNPs and apply the method to test a large clinical population of cancer patients and cancer-free individuals using samples from CARET. Investigators will collaborate with CARET and will genotype cytokine gene polymorphisms in genomic DNA that has been extracted from the archived blood spots, and will correlate the risk of cancer to each SNP using the cancer-free individuals as controls. We will investigate whether among cancer patients, the presence of certain SNPs correlates with survival. The specific aims of the study are to: 1) Develop and apply PCR arrays for genotyping SNPs in human genomic DNA; 1a) Design PCR arrays to detect SNPs encoded by cytokine genes; b) Define the extent and nature of CGPs in a clinical population of patients with lung cancer and in cancer-free individuals; 2) Define the frequency of CGPs in patients with lung cancer and in healthy individuals; 3) Determine the effect of CGPs on lung cancer survival. This study was approved by the IRB on November 3, 2004.
- 17.4.7 IR File #6000 “Pilot Study Genetic Association Study of Diabetes Candidate Genes and Pancreatic Cancer in the CARET Cohort”. Objective: This proposal will investigate associations of single nucleotide polymorphisms (SNPs) and haplotypes in six diabetes candidate genes with risk for pancreatic cancer. DNA extraction from all samples will be completed in Year 1, using banked blood samples for all participants. Genotyping will be performed at the Functional Genomics Laboratory at the University of Washington with strict quality control procedures. Samples will be genotyped for half of the SNPs in Year 1, and the remaining SNPs will be genotyped in Year 2. Also in Year 2, haplotypes will be constructed using recently developed software, and both univariate and multivariate statistical approaches will be used to assess the genetic associations. The results of these analyses will not be disclosed to participants, because the genetic tests to be performed are not clinically relevant. This study was approved by the IRB on March 25, 2005.
- 17.4.8 IR File #6258 “Molecular Epidemiology of Lung Cancer”. Objective: In a nested case-control study, this proposal is to determine whether polymorphisms of enzymes involved in the repair of smoking-induced DNA damage, namely those from the base excision (BER) and nucleotide excision repair (NER) pathways, are associated with risk of lung cancer. Genotyping of the functional single nucleotide polymorphisms (SNPs) as well as haplotype-

tagging SNPs of 26 DNA repair genes, a total of 236 polymorphisms, that have been resequenced by the Seattle Variation Discovery Resource for the Environmental Genome Project. The lung cancer cases (N = 900) and controls (n = 1800) for this study will come from the CARET specimen repository. Genotyping of cases and controls will utilize DNA obtained from leukocytes extracted from frozen whole blood samples. Detailed quantitative information on smoking and dietary history (using a food frequency questionnaire), obtained prior to the diagnosis of lung cancer, is available through CARET records. Cases and controls will be compared with respect to the prevalence of putative "high risk" genotypes, alone and in combination with other putative "high risk" genotypes within each pathway and in the two pathways combined. Results will be interpreted with multiple comparisons taken into account. The proposed study has sufficient statistical power to identify interactions between some of the high-risk genotypes, and to investigate whether the risk associated with a particular genotype varies by other risk factors, such as intake of antioxidant-rich fruits (e.g. Rosaceae fruits) and vegetables (e.g. Cruciferae vegetables), and food-derived nutrients, such as alpha-carotene, beta-carotene, lycopene, and tocopherols.

17.4.9 "Integrated Biomarker Profiles for Lung Cancer and COPD". Objective: 1) Determine whether integrating circulating biomarkers discriminate between high-risk individuals who will not develop lung cancer. 2) Determine whether integrating biomarkers of the host response to cigarette smoke discriminate between CARET participants with and without COPD and whether the integrated biomarker predicts reapid rate of airflow decline. (Attachment A). CARET will supply specimen samples for this study.

17.5 The following studies are reviewed as "Non-human" studies

18.0 REFERENCES

1. Silverberg E. CA 1986; 36:9-25.
2. Greenwald P and Sondik EJ, eds. NCI Monograph, #2, 1-105, 1986. Appendix B, Tables B-2,3,4,5.
3. Hammond EC and Horn D. JAMA 1958; 166:1159-1172.
4. Hammond EC and Horn D. JAMA 1958; 166:1294-1308.
5. Doll R and Hill AB. Brit Med J 1956; 2:1071-1081.
6. Hammond EC, Selikoff IJ, and Seidman H. Ann NY Acad Sci 1979; 330:473-490.
7. Doll R and Peto J. London, 1985.
8. Berry G, Newhouse ML, and Antonis P. Brit J Ind Med 1985; 42:12-18.
9. Havlik RJ. IN Health Statistics on Older Persons, United States, 1986, p. 40. DHHS Publication No. (PHS) 87-1409.
10. Nicholson Wi, Perkel G, and Selikoff IJ. Amer J Ind Med 1982; 3:259-311.
11. Omenn GS, Merchant J, Boatman E, et al. Evtl Health Perspect 1986; 70:51-56.
12. Peto R, Doll R, Buckley JD, et al. Nature 1981; 290:201-209.
13. Sestili MA and Dell JG, eds. May 17-18, 1984, National Cancer Institute. NIH Publication No. 85-2715.
14. Bertram JS, Kolonel LN, Meyskens FL. Cancer Research 1987; 47:3017-3031.
15. Saffiotti UK, Montesano R, Sellakumar AR, et al. Cancer 1967; 20:857-864.
16. Port C, Sporn M, Kaufman D. Proc Am Assoc Cancer Res 1975; 16:21.
17. Lasnitzki I and Bollag W. Cancer Treat Rep 1982; 66:1375-1380.
18. McCormick DL, Burns Fi, and Albert RE. Cancer Res 1980; 40:1140-1143.
19. Mossman BT, Craighead JE, and MacPherson BV. Science 1980; 207:311-313.
20. Temple NJ and Basu TK. JNCI 1987; 78:1211-1214.
21. Mathe G, Gouveia J, et al. First Int Conf Modulation and Mediation of Cancer by Vitamins, Tuscon, Ariz., Feb. 23-26, 1982.
22. Stich H, Rosin M, and Vallejera M. Lancet 1984; 1:1204-1206.
23. Bollag W and Matter A. Ann NY Acad Sci 1981; 351:9-23.
24. Bjelke E. Int J Can 1975; 15:561-565.
25. Shekele RB, Shuguey L, William WJ, et al. Lancet ii 1977; 1185-1190.
26. Zeigler RG, Mason RJ, Stemhagen A, et al. J Nat Ca Inst 1984; 73:1429-1434.
27. Hinds MW, Kolonel LN, Hankin JN, et al. Am J Epid 1984; 119:227-237.
28. Nomura AMY, Stemmermann GN, Heilbrun LK, et al. Cancer Res May 1986; 45:2369-2372.
29. Kark JD, Smith AH, Switzer BR, et al. JNCI January 1981; 66:7-16, No. 1.
30. Wald N, Idle M, Boreham J, and Bailey A. Lancet October 18, 1980; 813-815.
31. Omenn GS, Goodman GE, Kleinman GD, et al. Ann NY Acad Sci (in press).
32. Goodman DS. N Engl J Med 1984; 310:1023-1031.
33. Patt LM, Itaya K, and Hakomori S-I. Nature 1978; 273:379-381.
34. Lotan R, Lotan D, and Deutsch V. Cancer Res 1987; 47:3152-3158.

35. Churg J. JAMA 1985; 253:2984-85.
36. Kannerstein M and Churg J. Cancer 1972; 30:14-21.
37. Goodman GE, Einsphar JG, Alberts DS, et al. Cancer Res 1982; 42:2087-2091.
38. Goodman GE, Alberts DS, Peng YM, et al. Cancer Treatment Rep 1984; 68:1125-1133.
39. Goodman GE, Christofferson E, Kaplan HG, et al. Cancer Treatment Reports, August 1986; 70(8):1023-1024.
40. Goodman GE, Omenn GS, Thornquist MD, et al. Cancer Epidemiol Biomarkers Prev 1993; 2:389-396.
41. Omenn GS, Goodman GE, Thornquist MD, et al. Cancer Epidemiol Biomarkers Prev 1993; 2:381-387.
42. Selikoff I, Lilis R, and Nicholson W. Ann NY Acad Sci 1979; 330:295-312.
43. Selikoff IJ, Hammond EC, and Seidman H. Ann NY Acad Sci 1979; 330:91-116.
44. Blot WJ, Li J-Y, Taylor PR, et al. J Natl Cancer Inst 1993; 85:1483-92.
45. The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group. N Engl J Med 1994; 330:1029-35.
46. Omenn GS, Goodman GE, Thornquist MD, et al. N Engl J Med 1996;334:1150-55.
47. Hennekens CH, Buring JE, Manson JE, et al. N Engl J Med 1996; 334:1145-49.
48. O'Brien PC, Fleming TR. Biometrics 1979; 35:549-556.
49. Prentice RL. Biometrika 1986; 73:1-11.
50. Goodman GE, Metch BJ, Omenn GS. Cancer Epidemiol Biomarkers Prev 1994; 3:429-432.

19.0 APPENDIX

- A. Post Intervention Follow-up Study Manual Table of Contents
- B. Forms Manual Table of Contents
- C. Study Center Data System Manual Table of Contents
- D. NCI Smoking Cessation Pamphlets and Fred Hutchinson Cancer Research Center Pamphlet ("Quit Smoking")

- E. CARET Centralized Follow-up Manual (CCFM) Table of Contents
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To Protocol

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NCI Smoking Cessation Pamphlets and Fred Hutchinson Cancer Research Center Pamphlet
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CARET Centralized Follow-up Manual (CCFM)
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PROTOCOL FOR THE FULL SCALE EFFICACY TRIAL - CARET

APPENDIX G
To Protocol

Data Collection Forms
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Appendix C

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2. CARET Forms Manual Table of Contents

3. CARET Study Center Data System Manual Table of Contents

4. NCI Smoking Cessation Pamphlets and FHCRC Pamphlet

5. Sample Newsletter

**CARET Review Process for Ancillary Studies requesting the use of
CARET specimen and/or data**

1. All investigators requesting to utilize CARET specimens or data will submit their proposals (using the template given to them by the Coordinating Center) to the CARET Coordinating Center.
2. The Coordinating Center will review the proposals for completeness and thoroughness. The Coordinating Center is responsible for performing a preliminary determination of specimen availability and assessment of the impact that the proposed study would have on the CARET specimen bank; this information will be submitted with the proposal to the Steering Committee.
3. The CARET Principal Investigator will select a primary and two secondary reviewers from the CARET Steering Committee. If the proposed area of study is not consistent with any of the CARET investigators' expertise, an independent reviewer may be suggested as the primary reviewer.
4. The Coordinating Center will send the proposal to the primary and two secondary reviewers and to the Steering Committee for their review, as well as to all the CARET investigators for their review.
5. The Coordinating Center will set up a conference call to discuss the proposal.
6. The primary reviewer will be responsible for summarizing the proposal and presenting his or her critique to the Steering Committee.
7. The two secondary reviewers will be responsible for presenting their critiques.
8. All members of the Steering Committee and CARET co-investigators will then discuss the proposal and vote to approve or disapprove and prioritize the proposal relative to other currently approved proposals.
9. The Coordinating Center will summarize the discussion and the CARET Principal Investigator will send the results to the requesting investigator. If the study is approved, a final determination of specimen availability using the priority assigned in Step 8 will be made.
10. Once a proposal has been approved by the CARET investigators, the proposal must be approved by the CARET NCI Project Director and receive IRB review and approval. The requesting investigator must sign an agreement which lists all agreed to assays, confirm that not additional assays will be added without prior approval by the CARET NCI Project Director, the CARET Steering Committee, and all participating IRBs.
11. Funding is the responsibility of the requesting investigator.

CONFIDENTIALITY PLEDGE

In consideration of my access to the CARET Serum Bank and information described below and maintained at or belonging to Fred Hutchinson Cancer Research Center (FHCRC), I agree as follows:

1. "Confidential information" means the following records, data, biological specimens, and information to be provided by the CARET Coordinating Center for the study **"NAME OF STUDY"**.

2. I agree not to make use of, disseminate, disclose, or in any way circulate any confidential information except as expressly permitted by this Confidentiality Pledge. Confidential information may be published or otherwise disclosed in connection with the study entitled **"NAME OF STUDY"**, Institutional Review number **pending**; provided, however, that no disclosure may be made which permits identification of any individual participant or the participant's contacts unless permitted by applicable law and approved by an Institutional Review Board of FHCRC. Confidential information may also be disclosed to other persons working on the study who have signed a confidentiality pledge.

3. I agree not to provide access to confidential information to any unauthorized person.

4. I agree to indemnify, defend and hold FHCRC harmless from any causes of action, claims, damages or liabilities arising or alleged to arise from my failure to comply with any of the provisions of this Confidentiality Pledge.

5. I agree to maintain appropriate procedures to ensure that confidential information remains confidential to the extent required by this confidentiality pledge.

6. I agree to destroy all individual identifiers contained in any confidential information which would serve to identify a CARET participant or participant contact as soon as the purposes of the research for which I have been given access to the confidential information have been accomplished and to notify The CARET Coordinating Center to this effect in writing.

7. I agree to return all unused samples, portions of samples, or derivatives (including DNA) to the CARET Coordinating Center.

8. I agree to comply with all applicable laws and regulations regarding the confidentiality of individually identifiable health care information, including, without limitation, the Washington version of the Uniform Health Care Information Act, RCW Chapter 70.03.

9. I understand and acknowledge that this agreement may not be amended and that use of confidential information in a manner not permitted by this Confidentiality Pledge is not permitted without the prior written consent of the chair of the approving Institutional Review Board and CARET Principal Investigator Gary Goodman, M.D.

Dated: _____

Name of Individual (Print): _____ Phone Number:

Title: _____

Signature: _____

[ORIGINAL TO CARET COORDINATING CENTER attn: Cim Edelstein M1-B514]