The Clinical Evaluation of Cancer Prevention Agents (44175)

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Abstract. **The clinical development of cancer chemoprevention agents is similar to that of cytotoxic agents. On the basis of promising preclinical studies, agents with potential efficacy are introduced to the clinic as a phase I trial to study the dose-toxicity relationship of the agent and its human pharmacology. If doses projected to have biological activity have acceptable side effects, the agent proceeds to a phase II trial, which enrolls a larger number of participants and administers the agent for a longer time period (up to 5 years). Phase IIb trials test the effect of these agents on potential biomarkers of carcinogenesis to get some indication if the agent has activity. The phase II trial also pilots study design, mechanisms of recruitment, and adherence for future phase III trials. The phase III trial of a chemopreventive agent determines its effect on cancer incidence. Close attention is also paid to long-term side effects.**

The unique features of cancer prevention agents and their evaluation must be considered in this stepwise clinical evaluation. These features include (i) populations recruited to prevention trials are healthy; (ii) in this population there is a low tolerance for side effects; and (iii) the clinical end point of the trial, cancer, is statistically an uncommon event. The evaluation of β-carotene and retinol as studied in the Carotene and Retinol Efficacy Trial (CARET) is used to illustrate this stepwise clinical development. [P.S.E.B.M. 1997, Vol 216]

Our knowledge of the transformation of a normal cell to a cancer cell has greatly expanded over the past decade. The simplistic, stepwise concept of initiation, promotion, and progression has matured to a better understanding of the serial genotypic changes that ultimately lead to cancer (1, 2). The hypothesis has been proposed that it may be possible to intervene pharmacologically during this multistep process and that carcinogenesis may be arrested or reversed. This is the basic premise of cancer chemoprevention. The term chemoprevention can be defined as the use of agents (synthetic or naturally occurring) to reverse, arrest, or slow the process of carcinogenesis.

Epidemiologic studies have suggested that some dietary constituents may be acting as naturally occurring cancer prevention agents and may explain some of the differences in cancer incidence seen in populations with varying dietary intake (3–6). *In vitro* and *in vivo* laboratory studies have supported this concept (7, 8). These areas of research have led to human trials testing the effect of potential cancer prevention agents on cancer incidence and/or potential biomarkers of carcinogenesis. Although the field of clinical cancer chemoprevention is in its infancy and still evolving, there has been a general consensus among investigators on how potential chemoprevention agents identified in epidemiologic and laboratory studies should proceed through clinical evaluation (9–11). In this paper, I will discuss the clinical evaluation of a cancer prevention agent using, as an example, the clinical development of retinol and β-carotene, the agents tested in the Carotene and Retinol Efficacy Trial (CARET).

Phase I, II, and III Trials

The clinical evaluation of a chemopreventive agent should proceed in a fashion parallel to that of a chemotherapeutic agent. The first clinical investigation, the phase I trial, evaluates the dose-toxicity relationship of an agent and explores its pharmacokinetics. As shown in Table I, the phase I objectives of a chemoprevention agent are (i) to determine the short-term (less than 1 year) side effects of an agent; (ii) to evaluate the dose-toxicity relationship of an agent; and (iii) to evaluate the pharmacokinetics of an agent and determine its serum half-life and optimum dosing

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Table I. Phase I Trial

Objectives:
1. To determine the intervention's short-term (<1 year) dose-toxicity relationship
2. To determine the intervention's human pharmacokinetics

Design:
1. Single-arm, nonrandomized
2. Multiple dose levels
3. Less than 1 year in duration
4. Accrual 25–100

schedule. With these limited objectives, phase I trials of chemoprevention agents are similar to those cytotoxic agents.

As with cytotoxic agents, a phase I trial treats groups of individuals using standard Fibronacci dose escalations. Dose escalation is halted when side effects are identified, since higher doses are unlikely to be useful in most target populations. This aspect of drug development differs from that of cytotoxic agents because chemoprevention agents are generally administered to well populations. These agents must be free of side effects, even those that are considered minor. Agents that cause significant side effects in the small number of individuals studied in a phase I trial are unlikely to be sufficiently safe to proceed to further clinical evaluation.

Accrual goals for the phase I trial should be between 25 and 100 participants. This will vary by the number of drug-dose levels studied and the expected incidence of side effects. The populations recruited to these trials will be determined by the known side effects of the agent. Those agents with a low toxicity potential can be tested in normal volunteers or in individuals at risk for malignancy. Other agents with known or unknown toxicity can only be justifiably tested in cancer patients or those at very high risk for malignancy. Hence, the phase I evaluation of the dose-toxicity relationship of an agent is similar to that of a cytotoxic agent with the exception that there is a very low tolerance for side effects.

The goals of the phase II trial are seen in Table II. Since a safe, short-term dose is determined during the phase I trial, the phase II trial evaluates longer-term safety by accruing a larger number of individuals to a longer-term trial and by closely monitoring a wide range of possible toxicities. Phase II trials also begin to address many questions of an anticipated phase III trial. These include an evaluation of study procedures, the methods of participant recruitment, and an estimation of participant retention to both the study itself (since these trials tend to require participation for many years) and taking the prescribed agent.

Although evaluation of efficacy is not a standard goal of the phase II trial, the phase IIb trial attempts to evaluate the efficacy of an agent by studying its effect on chosen biomarkers of carcinogenesis. It is hoped (and must be proven for each biomarker) that an agent's ability to modulate a biomarker will correctly predict its cancer-prevention activity. This concept of an intermediate end point can potentially greatly streamline the often prolonged trials required for the evaluation of chemoprevention agents. However, it is unclear if this concept will be useful for chemoprevention trials (12), and there are currently no validated intermediate end points for the most common cancers now being studied (13).

The design of a phase II trial is usually randomized, double blind, and placebo controlled. These trials are longer than the phase I trial and can run up to 5 years in order to determine side effects reliably. The accrual goal for these trials can be large, ranging from hundreds to thousands of individuals. Since side effects will be uncommon, large populations are needed for adequate statistical power to determine if side effects are more common in the treatment arm than in the placebo arm. The target population for these trials should be the intended population for the specific intervention. This is crucial since recruitment tactics, incidence and types of side effects, biomarker incidence and natural history, and retention results are likely to be specific to individual target populations. The placebo-controlled design will also allow the natural history of biomarkers to be determined and the effect of the intervention agent to be carefully tested.

The phase III trial has the primary objective of determining the effect of the intervention on cancer incidence (Table III). The design of the phase III trial is driven by statistical requirements of the cancer end point. The number of individuals recruited and the length of follow-up will, in large part, be determined by the incidence of cancer in the recruited population and the difference in incidence one hopes to detect between the intervention group and the placebo group.

In addition to determining efficacy, the phase III trial is the only forum where the long-term safety of the agents can be established. Participants in these trials must be closely evaluated for common side effects as well as those that are less common. It also should include a broad evaluation of other diseases and medical problems (other than the study end points) since unexpected results may be seen. The

Table II. Phase II Trial

Objectives:
1. To determine the intervention's side effects
2. To determine optimal recruitment methods of the target population
3. To determine retention of study participants to the study intervention and procedures
4. To determine optimal methods for the conducting of a phase III trial
5. To determine the effect of the intervention on biomarkers of carcinogenesis (phase IIb)

Design:
1. Randomized, double-blind, placebo-controlled
2. Multiple dose levels or agents
3. One to five years in duration
4. Accrual 100s–1000s

Table III. Phase III Trial

Objectives:
1. To determine the effect of the intervention on the incidence of the target cancer
2. To determine the effect of the intervention on total and specific cancer incidence
3. To determine the long-term side effects of the intervention
4. To determine the effect of the intervention on death rate and disease incidence
5. To determine the natural history of specific biomarkers of carcinogenesis (placebo group) and the effect of the intervention agent (treatment group) on these markers

Design:
1. Randomized, double-blind, placebo-controlled
2. Multiple dose levels or agents, alone or in combination
3. Five to ten years in duration
4. Accrual 1000s–10,000s

evaluation should include all causes of death and the incidence of major diseases such as hypertension, diabetes, cardiovascular disease, etc.

These trials are also the only setting in which one can determine the validity of biomarkers of carcinogenesis by studying the relationship between a specific biomarker and cancer in a large population (in the placebo group). It will also determine if the intervention agent is able to modulate the marker (intervention group). The former aspect is crucial since the natural history of many biomarkers is unclear. If a relationship between markers and cancer could be established, and that modulation of this marker predicts a change in cancer incidence, these markers may potentially be used as end points for future phase III intervention trials. Collecting biological samples on individuals during the phase III trial will allow retrospective nested case control studies. However, this aspect of a trial puts an additional burden on trial participants, as well as additional financial costs.

The validation of biomarkers is one goal of a phase III trial that can have a major impact on the conduct of future cancer prevention trials. Once it is shown that a biomarker can reliably predict cancer and that its modulation predicts a change in future cancer incidence, it can act as a surrogate end point for cancer prevention trials.

This outline of objectives for the phase I, II, and III trials of chemoprevention agents is very similar to that of cytotoxic agents. It is applicable provided there is a clear understanding that there are unique aspects of chemoprevention. These include (i) participants are usually healthy or at least "cancer free"; (ii) the degree and incidence of side effects that are acceptable in a healthy population are low; (iii) the end point is disease prevention, not disease response; and (iv) the incidence of the study end point (cancer) is low (for example, the lung cancer incidence in heavy smokers is 5/1000 person-years). These four factors must be considered and will influence every aspect of the design and conduct of these trials.

Cancer Prevention Agents: From Drugs to Vitamins

Currently, a range of compounds, from synthetic drugs to naturally occurring micronutrients in our diet, have been proposed as cancer prevention agents (11, 14). For the former group, it is clear that careful phase I and II trials must be conducted prior to large-phase III trials. However, the latter group represents a unique situation for the standard phase I, II, and III scenario. This group of interventions are frequently vitamins or trace constituents of foods. In a chemoprevention trial, the goal is to augment the diet with these substances. An assumption is made that there is a direct dose-response relationship, and that administering these compounds in doses above those that occur in the diet may result in improved pharmacologic efficacy. This is an assumption that must be tested. In addition, even though these compounds are "natural" when administered in supraphysiologic doses, they must be evaluated like other synthetic compounds since undescribed toxicities or unfavorable interactions with other dietary constituents may occur. This concept must be stressed since many prevention trials administering vitamins or micronutrients in pharmacologic doses have taken a casual attitude toward the evaluation of side effects or toxicity. The dose-toxicity and dose-efficacy relationship of these compounds must be evaluated in a rigorous way analogous to any synthetic drug.

The CARET Experience

As an example of the stepwise development of a clinical chemoprevention agent, presumed to be safe, I will discuss β-carotene and retinol as used in CARET (15). β-Carotene is a natural pigment and precursor to vitamin A which is widely distributed in plants, especially orange and yellow vegetables. Epidemiologic studies have been consistent in suggesting that individuals with low serum concentrations of β-carotene or with diets low in β-carotene–containing foods have a higher incidence of epithelial cancers (16). Although these foods contain many micronutrients, any of which may be responsible for this relationship, β-carotene was thought to be a prime causative candidate. Its mechanisms of action are thought to be through its electron-scavenging antioxidant effect and its conversion to vitamin A (17, 18). Because this compound is poorly absorbed in rodents and is extremely lipophylic, there have been limited *in vitro* and *in vivo* laboratory studies. In 1981, this compound was touted as the most promising cancer chemoprevention agent (19).

Epidemiologic studies had also suggested that the dietary intake and serum concentration of retinol (vitamin A) were also inversely related to cancer (20–22). However, these studies were much less consistent than those with β-carotene. There were, in contrast, laboratory studies that strongly supported the potential use of retinol and synthetic

retinoids as chemoprevention agents. *In vivo* and *in vitro* studies suggested that these compounds could reduce both the number and size of cancers in animals exposed to carcinogens (23, 24). A broad spectrum of activity was found in studies with different animal species, carcinogens, and target organ systems.

There was substantial support for the hypothesis that both supplemental β-carotene and a number of the retinoids may have clinical cancer chemoprevention activity. We felt this evidence was sufficiently strong to initiate clinical trials. Prior to the start of clinical trials, we investigated what was known about the clinical use of these "vitamins."

In the early 1980s, there was limited literature on trials of micronutrients and vitamins. Such was the case with β-carotene and retinol. The published information on the use of β-carotene was limited to individuals with erythropoietic porphyria where it was reported to have a skin-protective effect (25, 26). In these trials, up to 180 mg of β-carotene was given daily for up to a year. There were no reports of toxicity except for yellowing of the skin. There were also numerous case reports of carotenemia in health-food enthusiasts, which suggested that, other than yellow-orange coloration of the skin, this agent was safe (27). The dose that caused carotenemia appeared to be above 30–50 mg/day. However, systematic evaluation of this agent in large populations for long durations was lacking.

The literature on vitamin A toxicity was more comprehensive. This retinoid is well known to accumulate in the liver and can cause both acute and chronic hepatitis and fibrosis, which could lead to cirrhosis and hepatic failure (28–33). The dermatologic literature also reported dermatologic abnormalities and central nervous system/neuropsychiatric toxicity (33).

Because of the concern of toxicity, we conducted a series of phase I–phase II trials of oral retinyl palmitate in cancer patients. We confirmed that a fixed dose of 500,000 IU/day caused reversible neuropsychiatric and hepatic toxicity in five of five cancer patients after 2–3 months (34). We later conducted a phase II trial in 76 cancer patients, with a dose of 200,000 IU/m^2 (35). At the same time the Southwest Oncology Group conducted a phase III trial using a fixed dose of 100,000 IU/day in patients with high-risk melanoma (36). Neither of these latter trials reported significant toxicity after 1–2 years. Based on these findings, we proposed to evaluate a dose of 25,000 units for a phase II chemoprevention trial.

Although formal phase I trials for both β-carotene and retinol were not conducted, most of the goals of a phase I trial had been met by the information available in the literature and the trials we had conducted. The available toxicity profile and the accumulating evidence suggesting efficacy justified the next step, a phase II chemoprevention trial. Our target cancer was lung cancer, the No. 1 cause of cancer deaths in U.S. men and women. This appeared to be an ideal cancer to study since high-risk individuals could easily be identified (cigarette smokers) and the epidemiologic and laboratory evidence suggested that these agents may have activity in this disease.

Two parallel phase II trials were conducted in high-risk populations, one in asbestos workers (employment in a high-risk trade and 12 years since asbestos exposure) and the other in cigarette smokers (20 pack-years or greater smoking history; either current or exsmoker who stopped within 6 years). The smokers trial evaluated β-carotene and retinol in a 2 × 2 design at doses of retinol of 25,000 IU/day and β-carotene 30 mg/day (37). The asbestos workers trial was two-armed and tested the fixed combination of β-carotene 15 mg/day and retinol 25,000 IU/day (38). For the high-risk smokers, we established a direct-mail recruitment program by working with local health insurance programs. Age-selected subscribers were sent a recruitment information packet and questionnaire. Interested individuals returned completed questionnaires directly to the Seattle Study Center. Asbestos workers were recruited from various sources including pulmonary and environmental health clinics, and high-risk trade union membership rosters.

Side effects and retention were evaluated at 4-month intervals with a standardized physical examination and questionnaire designed to evaluate symptoms and signs of retinol and β-carotene toxicity. Participants were contacted by telephone between study center visits and were asked about symptoms. Blood was sampled yearly for liver functions (to evaluate hepatic toxicity). We analyzed serum for the concentration of the administered vitamins to determine the long-term pharmacokinetics of these compounds as well as to study their effect on other serum micronutrients. The asbestos workers trial randomized 814 individuals and the smokers trial randomized 1029 individuals. Participants were followed for a mean of 3 years.

These trials achieved the previously discussed objectives of a phase II trial. The recruitment methods we established were successful and were designed to be easily expandable both locally and to other sites for a future phase III trial. Retention was excellent, with 74% of participants actively taking vitamins at 36 months. We found no difference in the incidence of 13 side effects among any of the treatment groups (including serum liver function profiles). Because of the lack of proven intermediate end points in lung cancer, this trial focused only on phase IIa objectives. There was no evaluation of the effect on biomarkers (a phase IIb objective).

The successful completion of these phase II trials laid the foundation for a phase III trial. This included a confirmation of recruitment methods and an estimation that it would be possible to recruit the number of individuals required for phase III trial. The rate of participant retention was good, and the agents chosen appeared to have a low incidence of toxicity. Trial methods, procedures, and forms were also piloted. Modifications were made to ease future use.

The Vanguard Cohort

Based on the success of our two phase II pilot trials, we felt it was justifiable to proceed with a phase III trial to determine the effect of β-carotene and retinol on the incidence of lung cancer in high-risk individuals. The combination was tested since our phase II trials had not shown any evidence of synergistic toxicity, and there were theoretical complimentary mechanisms of action with β-carotene acting as an "antioxidant" and retinol acting through its retinoid effects. The decision to test a fixed combination was also driven by the population requirements of a two-arm trial versus a 2 × 2 trial (17–18,000 vs 25–27,000).

Based on the experience of our phase II trials, we modified many of the procedures used for the phase III design. Toxicity monitoring was streamlined by developing the concept of a vanguard cohort. This cohort was formed from the two populations who had enrolled in our phase II pilot trials. We requested these participants continue as part of the phase III trial. As part of the transition of the pilot trials to the vanguard population, those individuals randomized to placebo were continued on placebo, while those on any active arm were changed to the combination to be tested in the phase III trial, β-carotene 30 mg/day and retinol 25,000 IU/day. Vanguard participants were seen in the study center twice a year for a comprehenisve evaluation of side effects, which consisted of a physical examination and a self-administered symptom-assessment questionnaire. A telephone questionnaire was administered between each study center visit resulting in a participant contact every 3 months. Since the side effects of these agents are cumulative, any toxicity related to duration of treatment would initially appear in this population prior to appearing in the newly recruited larger cohort. This concept allowed the larger, newly recruited phase III cohort to be less intensively monitored for side effects.

Recruitment

Recruiting 18,000 individuals is a potential obstacle for any prevention trial. However, the recruitment methods we utilized during the phase II trials resulted in a randomization rate of 2% among individuals we contacted by direct mail. This method was easily expandable to other health insurance subscribers and study centers located in other states.

For the phase III trial, participants eligible by smoking criteria were recruited exclusively at study centers located in Seattle, Washington; Portland, Oregon; and Irvine, California. Recruitment methods were identical to those used in the Seattle pilot studies. The cooperating major health insurers in the state of Washington were Blue Cross and Blue Shield, as well as a number of health maintenance organizations (HMOs). In Oregon and in southern California, the major sources of recruitment were the Kaiser Permanente system, Blue Cross and Blue Shield, as well as other HMOs. We also attempted recruitment *via* advertising, by contacting physicians' offices, and by contacting members of the American Association of Retired Persons. In total, the three study centers recruited a total of 14,254 smokers.

The recruitment of asbestos-exposed workers was organized using the model developed in Seattle. Occupational Health Physicians were contacted in areas known to have a large population of asbestos-exposed workers. This included San Francisco, California; Baltimore, Maryland; and Groton, Connecticut. The local investigators in these communities were able to establish recruitment centers in close affiliation with academic occupational medicine programs. Using methods tested in our phase II pilot trial, we were able to recruit studywide a total of 4060 asbestos workers.

The instruments and procedures used for participant follow-up had been tested and modified during the phase II pilots. At the start of the phase III trial, forms were finalized and streamlined. Many of the side-effect assessment forms that had not worked well during the phase II pilot were modified. Physical assessment tools were standardized to obtain comparable data across multiple study centers. Training sessions were conducted for the participant interviewers so that signs and symptoms were assessed and graded in an similar manner. We emphasized self-reporting of symptoms to minimize judgmental evaluations by staff. Standardized telephone interviewer scripts were provided. These procedures assured consistent evaluation of potential side effects across study sites.

The newly recruited participants were seen in the study center yearly. At this visit a standardized physical examination was performed and a self-administered questionnaire was completed. Blood was sampled every other year and stored at −70°C for retrospective evaluation of biomarkers and for the measurement of serum vitamins and related micronutrients. Between the study center visits, participants were contacted twice by telephone and given a standardized telephone health questionnaire.

End Point Evaluation

Although few end points will occur during a phase II pilot, the methods used to determine the study end points (cancer incidence) should be tested. For CARET we established a formal end point ascertainment and evaluation protocol. At each participant contact we inquired about end point incidence. Participants were questioned about whether any physician had made the diagnosis of cancer. In general, participants were so well informed about our need to determine end points that they usually notified their study center if they were diagnosed with cancer. Any reported end point initiated the end point process, which contacted the participant's designated physician and requested medical records. The obtained medical records were reviewed in the study center for completeness and forwarded to the Coordinating Center. An end point review committee was established, which consisted of a panel of four physicians. All lung-cancer cases had histologic review to confirm the diagnosis

Table IV. Stopping Rules for Efficacy Using the O'Brien-Fleming Method with Two Interim Analyses at 1/3 and 2/3 the Expected Number of Total Lung Cancers

	Predicted date	*P* value
First interim analysis	7/94	0.006
Second interim analysis	7/96	0.015
Final analysis	4/98	0.047

and cell type. Agreement of cancer diagnosis between two blinded evaluations confirmed a case. In cases of disagreement, a decision was made by an unblinded discussion among the four members of the committee.

Statistical Analysis

An important part of design of phase III trial is to establish early stopping rules (Table IV). CARET was designed with a planned follow-up of 110,000 person-years, with final analysis occurring in 1998. Three interim analyses were planned at the time one-third and two-thirds of the expected total number of end points had accrued. Stopping rules were based on the O'Brien-Fleming model for multiple analyses.

At the planned second interim analysis, there was statistical evidence of an increased incidence of lung cancer in the population receiving the active study vitamins although the statistical test did not reach the required boundary of the O'Brien-Fleming rule. However, in April of 1994, the Alpha Tocopherol Beta Carotene (ATBC) study had reported that 20 mg/day of β-carotene led to an 18% increased incidence of lung cancer in a population of cigarette smokers with a similar age distribution to the CARET population. Because of our concern about participant safety, an additional statistical analysis was completed in the fall of 1995. Although the O'Brien-Fleming stopping rules again had not been met, the findings were consistent with a result that would show no statistical evidence of benefit for β-carotene supplementation. With a mean follow-up of 4 years, the active treatment groups had a relative risk of lung cancer of 1.28 (95% confidence interval: 1.04–1.57; $P = 0.02$). This result includes relative risks of 1.40 (0.95–2.07) for asbestos workers, 1.42 (1.07–1.87) for current smokers, and 0.80 (0.48–1.31) for exsmokers. Figure 1 shows the cumulative incidence of lung cancer after randomization for the total population.

The incidence in the active and placebo groups was virtually identical for the first 18 months. After the CARET Steering Committee reviewed these results, and given the results of the ATBC trial in April 1994, it was decided that there was adequate evidence to suggest that β-carotene supplement would not be beneficial and indeed appeared to be harmful to the study population. It was decided to halt active intervention. Prior to the public announcement, all individuals were notified of these findings and told to stop taking the study vitamins. Participants will be followed for an additional 5 years for end point determination.

Summary

The development of a cancer chemoprevention agent should be a logical sequence of studies, starting with preclinical epidemiologic studies that suggest a compound may have efficacy, continuing with laboratory studies that show *in vivo* and *in vitro* activity, and leading to early clinic evaluation and, finally, to the testing of the effect on cancer incidence. This scenario took place for β-carotene and vitamin A from the early 1980s until the late 1990s. Although the results with β-carotene in high-risk cigarette smokers have proven to be disappointing, a great deal has been learned about β-carotene and its human physiology. The surprising results of increased incidence of lung cancer in cigarette smokers will hopefully lead to a better understanding of the mechanism of development and progression of cancer and the mechanism of the carotenoids. It also shows the strengths and importance of conducting careful clinical trials even for agents widely accepted as safe.

This scenario also points out one of the major shortfalls of the current clinical evaluation schema: the lack of methods to determine efficacy during pilot studies. Currently, phase III intervention trials are the only mechanism to determine the effect of an intervention agent on cancer incidence. This evaluation requires many years, thousands of participants, and great expense. There is a great need for

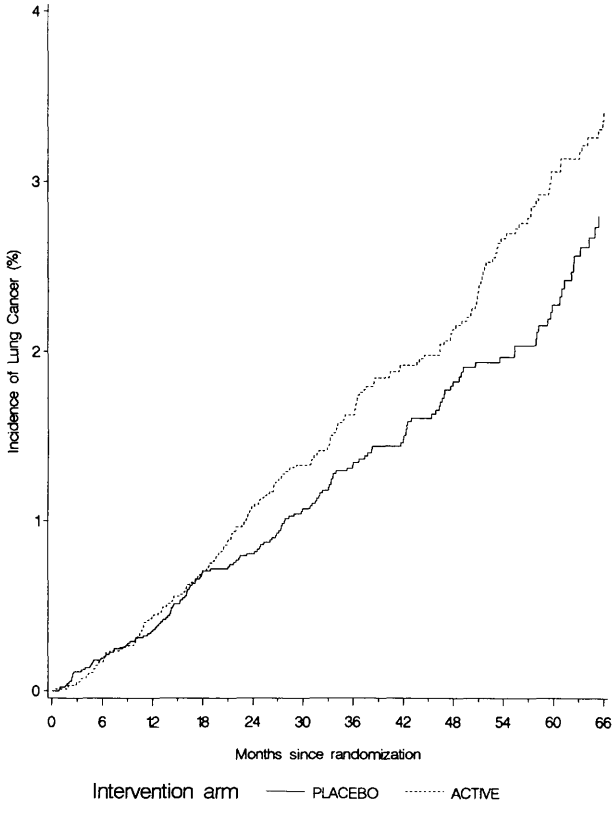

Figure 1. Time course to lung cancer.

early markers of carcinogenesis that can predict the efficacy of an agent in smaller pilot trials. It is hoped that the development of valid intermediate end points will hasten the development of effective cancer chemoprevention agents.

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