NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

CALCIUM

DRUG IDENTIFICATION

CAS Registry No.: 7440-70-2

CAS Name (9CI): Calcium

Synonyms:
Calcium Acetate (62-54-4)
Calcium Carbonate (471-34-1)
Caltrate®
Os-Cal 500®
Tums®
Calcium Chloride (10043-52-4)
Calcium Citrate (813-94-5)
Citrical®
2-Hydroxy-1,2,3-propanetricarboxylic Acid, Calcium
Calcium *d*-Gluconate (299-28-5)
Calcium Lactate (814-80-2)
2-Hydroxypropanoic Acid, Calcium
Calcium Phosphate, Dibasic (7757-93-9)
Calcium Phosphate, Tribasic (12167-74-7)

Related Compounds:
Calcium *d*-Glucarate (5793-88-4)
d-Glucaric Acid, Calcium Salt (1:1)
Calcium *d*-Saccharate

Structure: Ca^{+2}

EXECUTIVE SUMMARY

Calcium is an essential dietary mineral in humans, and represents the fifth most abundant element in the body [reviewed in 1]. The current recommended daily allowance (RDA=800–1,200 mg/day) is based primarily on nerve, muscle and cardiac function, and blood coagulation. The chemopreventive activity of the element was suggested by epidemiological studies associating high dietary calcium or milk intake (a major source of calcium) with decreased colon cancer risk or mortality [2–7]. Subsequent animal studies have demonstrated inhibition of colon carcinogenesis by calcium salts. The first mechanism to explain these effects was based on the epidemiological association between high-fat diets and increased risk of colon cancer [reviewed in 8]. Animal and *in vitro* studies showed that excess amounts of free bile acids (*e.g.*, cholic acid, deoxycholic acid) and unabsorbed fatty acids promote carcinogenesis by irritation and damage of the colon epithelium, which induces compensatory proliferation and expansion of the proliferative compartment [9–15]. Calcium intervention was

shown to decrease the proliferative stimulus by binding with the lipid products to form insoluble calcium soaps [9,12,13,16–18].

In addition to the physiologic functions outlined previously, calcium is also involved in membrane integrity, cellular differentiation, proliferation and death [reviewed in 20], and intra- and intercellular signalling [19,20]. Although the calcium soap hypothesis remains the most plausible and demonstrable explanation for prevention of cancer, alterations in differentiation- and proliferation-related biochemistry (*e.g.*, ornithine decarboxylase (ODC) activity) as seen in other tissues (*e.g.*, skin, esophagus, liver, mammary gland) have also been suggested as mechanisms [reviewed in 21–24]. In normal cells, these functions are dependent on closely regulated intracellular concentrations of the ionized form. Calcium entry into cells is restricted, since cytosolic concentrations are maintained at much lower levels (0.1–1 μM) than in extracellular fluid (1.3–2.3 mM) [reviewed in 25,26]. In the colon lumen, however, calcium levels fluctuate over a greater range (2–20 mM); *in vitro* data have shown that these same levels inhibit the growth of normal crypt cells [27]. This demonstrates a direct effect of the element on proliferation of normal colon cells. Dr. Martin Lipkin and co-workers were [28,29] the first to demonstrate a response of the colon crypt proliferation profile to calcium in familial colon cancer patients. However, as cells progress through neoplasia, a gradual loss of the normal response to calcium, or "field defect," is observed [30]. For example, proliferation measured *in vitro* as [^{3}H]-thymidine labeling was calcium-sensitive in normal-appearing colon cells from high-risk subjects (*e.g.*, previous colon neoplasm, familial association) and some early premalignant lesions (*e.g.*, tubular adenoma), but not from villous adenomas or carcinomas [31–34]. Distribution of proliferating cells over the height of the crypt (*i.e.*, proliferative compartment) may also be altered, especially in individuals with inherited familial adenomatous polyposis (FAP); differential responses to calcium between the crypt compartments have been demonstrated. Similarly, signal transduction pathways appear to be altered during colon carcinogenesis. Bile salts and TPA activate protein kinase C in premalignant human colonic cells, but not in normal cells [reviewed in 35]. Treatment with calcium salts has been shown to decrease total protein kinase C, tyrosine kinase, and ODC activities in rat colon cancer models [36–39]; decreased ODC activity has been demonstrated following calcium treatment of adenoma patients [reviewed in 26]. Due to the low toxicity and demonstrated chemopreventive efficacy in both preclinical and clinical settings, calcium (as the citrate and carbonate salts) was considered by the CB for development as a chemopreventive drug for colon cancer.

Calcium glucarate is also being considered because of its chemopreventive efficacy in several target organs, including breast and colon. This activity may result from properties of both components, calcium and glucaric acid. Glucaric acid is converted to *d*-glucaro-1,4-lactone, a β-glucuronidase inhibitor [40–42]. Many endogenous (*e.g.*, estrogen) and exogenous compounds (*e.g.*, carcinogens) are conjugated to glucuronic acid, which enhances elimination from the body. Inhibition of subsequent cleavage by β-glucuronidase in tissue or by gut bacteria would further enhance elimination of larger conjugated molecules. Thus, calcium glucarate has been shown to inhibit both mammary gland and colon carcinogenesis in animal models; calcium may be acting to inhibit cell proliferation and glucarate may reduce circulating estrogen as a result of β-glucuronidase inhibition. Because of the low toxicity and demonstrated preclinical efficacy, calcium glucarate was also considered for development as a chemopreventive drug, primarily for breast and colon cancer.

In preclinical testing, all the calcium salts discussed appear to act primarily as antipromoters in colon carcinogenesis models, with and without proliferative stimuli such as high-fat diet or small bowel resection. Calcium glucarate also inhibited rat mammary gland tumorigenesis; however, synergistic combinations with retinoids have been demonstrated at doses that were ineffective alone. Other effective combinations include calcium glucarate with vitamin D_3 in mouse bladder tumorigenesis.

A significant effort in the CB program is to identify and validate intermediate biomarkers of cancer, and evaluate the potential of chemopreventive agents to modulate these endpoints. In the colon, calcium salts have been shown to alter histological (aberrant crypt foci, adenomatous polyps), proliferation-related (ODC activity, expansion of the proliferative compartment, [^{3}H]-thymidine labeling index, mitotic figures), and genetic (K-*ras* expression) biomarkers. The combination of vitamin D_3 analogs with calcium glucarate suggested an effect against aberrant crypt foci in rat colon; however, significant toxicity requiring reduction of the original doses limited interpretation of the data. Finally, various calcium salts have also been shown to modulate premalignant lesions in mouse

lung and skin, and rat stomach and liver.

Limited preclinical toxicity data suggest that calcium salts have low toxicity, especially by the oral route. The CB has funded 90-day oral toxicity studies of calcium glucarate (tetrahydrate) in rats and dogs. No adverse effects related to doses up to 50,000 mg/kg diet were observed in either species. Due to the wide human experience with calcium salts in general and the fact that long-term clinical trials are in progress, preclinical carcinogenicity and reproductive toxicity testing are not likely to be required for long-term clinical trials.

Orally administered calcium compounds generally exhibit low human toxicity; however, at high doses or in certain disease states, hypercalcemia may be produced. Symptoms include nausea, muscle weakness, loss of renal concentrating capacity, coma, and, in extreme cases, cardiac arrest. At elemental calcium doses above the maximum recommended by the FDA for OTC antacid products (3,200 mg Ca^{+2} qd as the carbonate or phosphate salts), CB-funded Phase I trials of the carbonate and citrate salts (4,000 mg Ca^{+2} qd for 16 weeks) found increased serum calcium, with a low incidence of nausea, vomiting, and weakness.

Doses of 1,000–2,000 mg elemental calcium appear to inhibit colon proliferation in high-risk individuals; these levels are similar to the RDA recommended by some clinicians for postmenopausal women. Administration of 2–4 calcium carbonate tablets (Caltrate®) provides doses of calcium below the maximum approved for OTC antacid products (≤3,200 mg Ca^{+2}, or 8,000 mg carbonate salt). In contrast, achieving a clinical dose of calcium glucarate which inhibits serum β-glucuronidase may be problematic. Doses up to 12 g salt did not produce a consistent enzyme response; however, doses comparable to the effective rodent dose would require 70 g calcium glucarate.

Three NCI, DCPC-funded Phase II trials and one Phase III trial of calcium carbonate in colon polyp patients are in progress; an additional Phase II trial will begin this year. The endpoints in these studies include modulation of polyp incidence and proliferation biomarkers. Combinations of agents with calcium carbonate are the major strategy for proposed trials. This year, a Phase II study of calcium carbonate with vitamin D_3 will begin in colon polyp patients. Studies planned for 1995 include modulation of biomarkers in the breast and colon by calcium plus vitamin D_3, and a short-term Phase II trial of calcium carbonate in breast cancer patients scheduled for surgery is also planned.

Calcium is available in the form of various salts which have been used as food additives (as the acetate, gluconate, lactate, or citrate), antacids (as carbonate or phosphate), for electrolyte replacement or hypocalcemia (as the chloride, gluconate, lactate, or phosphate), or in mineral supplements (as carbonate, phosphate, citrate, lactate, or gluconate) [43]. Calcium glucarate has been used in the pharmaceutical industry as a stabilizer, and also occurs naturally in plants consumed as food and in mammals during the catabolism of glucuronic acid [44]. The carbonate is the calcium salt of choice for clinical development as a cancer chemopreventive drug because of the following: (1) GRAS status as a nutritional supplement, food additive and antacid product; (2) the amount of pharmacokinetic and safety data; (3) the highest calcium content of the salts under consideration; and (4) sufficient preclinical efficacy data. Lederle produces calcium carbonate as the Caltrate® formulation, and no supply problems are foreseen.

PRECLINICAL EFFICACY STUDIES

Except for calcium glucarate, calcium salts have been primarily tested for efficacy in the colon in both published and CB-funded preclinical testing. In these studies, calcium appears to act primarily as an antipromoter in colon carcinogenesis. In the CB testing program, calcium chloride and calcium glucarate are currently on test in the AOM-induced rat colon carcinogenesis model. In published studies, calcium administration decreased colon tumor development in the AOM-induced rat (as the carbonate, lactate or glucarate) [45–47] and mouse (as the glucarate) [48] models and the DMH-induced rat model (as carbonate, lactate or gluconate) [38, 49–52]. The element was also effective against AOM-induced carcinogenesis promoted by a high fat diet (as calcium NOS or lactate) [46,53], high-corn oil diet (as the lactate) [46], jejunal transection (as the lactate) [54,55], or small bowel resection (as the lactate) [54,55]. In related studies, duodenal adenocarcinomas induced by AOM and promoted by jejunal transection or small bowel resection were inhibited by calcium lactate [55].

Calcium salts have also inhibited carcinogenesis in other target organs. In CB testing, 20,000 mg calcium glucarate/kg diet (*ca.* 37.5 mg Ca^{+2}/day) inhibited MNU-induced rat mammary carcinogenesis. *In vitro*, the salt at 0.001 μM decreased formation of hyperplastic alveolar nodules (HAN) in the DMBA-induced mouse mammary organ culture model. In published studies, calcium glucarate was

effective against DMBA- and MNU-induced rat mammary carcinogenesis [40,56–58]. Finally, calcium chloride inhibited bracken fern/thiamine HCl-induced rat urinary bladder and intestine carcinogenesis [59].

A significant effort in the CB program is to identify and validate intermediate biomarkers of cancer, and evaluate the potential of chemopreventive agents to modulate these endpoints. Such studies in animals contribute to the development of more efficient screens for identifying new chemopreventive agents, as well as identifying biomarkers to be used as surrogate endpoints for cancer incidence in clinical trials [reviewed in 60]. In CB-funded histological biomarker studies, the chloride (at 11,500 mg salt/kg diet, or *ca.* 5.2 mmol/kg-bw/day), glucarate (at 25,000 mg salt/kg diet, or *ca.* 5.0 mmol/kg-bw/day), carbonate (at 7,825 mg salt/kg diet, or *ca.* 3.9 mmol/kg-bw/day) and lactate (at 34,140 mg salt/kg diet, or *ca.* 7.8 mmol/kg-bw/day) salts of calcium inhibited formation of a putative premalignant lesion—aberrant crypt foci—in the AOM-induced rat colon. In published studies, calcium gluconate inhibited the incidence of colon adenomatous polyps in the DMH-induced rat model [61]. In other organs, various calcium salts inhibited MNNG-induced rat glandular stomach hyperplasia (chloride) [62], enzyme-altered foci in DEN-exposed rat liver (glucarate) [63], nickel or lead acetate-induced mouse lung adenomas (acetate) [64], B(*a*)P-induced mouse lung adenomas (glucarate) [44], and skin papillomas in the DMBA-induced/TPA-promoted mouse (glucarate) [40].

Because bile acids and high fat promote tumorigenesis by irritation and mitogenesis [65,66], published studies of calcium have investigated modulation of proliferation biomarkers in the colon. Measures of proliferation (*e.g.*, [^{3}H]-thymidine labeling index, mitotic figures/crypt) induced in the colon by oleic acid and lauric acid [10], cholic acid [14], and deoxycholic acid [16,67] were decreased by dietary administration of the carbonate, dibasic phosphate, and lactate salts, respectively. In addition, raising the calcium level in the rodent nutritional stress diet (40% calories from fat, high phosphate, low calcium and vitamin D) abolishes induction of the hyperproliferative response [19,68]. In chemical-induced carcinogenesis models, the labeling index was decreased in the MNNG-exposed rat colon by calcium lactate and carbonate [69,70].

Hyperproliferation of crypt cells, which occurs early in colon carcinogenesis, is accompanied by elevated ODC activity [71,72]. Modulation of this proliferation biomarker by calcium carbonate has been demonstrated in the AOM-induced rat colon [37]. In related studies, gastrin-, MAM- or EGF-stimulated ODC activity in rat colon mucosal explants was abolished by calcium chloride [reviewed in 53].

A single study has reported modulation of a genetic biomarker by a calcium salt. K-*ras* G→A transversions were abolished in DMH-induced rat colon tumors by dietary calcium carbonate supplementation [73]; the same regimen reduced tumor size and multiplicity [38]. Since cells in S-phase are more sensitive to mutagens, modulation may be related to the antiproliferative effect of calcium.

Combinations of agents with calcium salts have been investigated as a method to decrease the dose and potential toxicity of each individual agent, while retaining efficacy. In CB-funded studies, an effective combination has been calcium glucarate with vitamin D_3. The bioactive metabolite of the vitamin promotes transport of calcium from the intestinal lumen and maintains serum calcium homeostasis. In *in vivo* assays, 2,500 mg calcium glucarate/kg diet (*ca.* 1.3 mmol glucarate/kg-bw/day, or 1.8 mg Ca^{+2}/day) plus 1.25 mg vitamin D_3/kg diet (*ca.* 0.42 μmol/kg-bw/day) was effective against OH-BBN-induced mouse bladder tumorigenesis. *In vitro*, this combination (0.01 μM calcium glucarate + 0.26 μM vitamin D_3) reduced development of HAN in DMBA-induced/TPA-promoted mouse mammary organ culture at a concentration of glucarate that was ineffective alone. Currently, the calcium glucarate and vitamin D_3 combination is on test in the MNU-induced rat mammary carcinogenesis model. Also, combinations of vitamin D_3 analogs (*e.g.*, Ro 24-5531) with calcium glucarate appeared to inhibit formation of aberrant crypt foci in the AOM-induced rat colon. Unfortunately, significant toxicity requiring reduction of the original doses limits interpretation of the data.

In published studies, combinations of calcium glucarate with retinoids have demonstrated synergistic chemopreventive activity [reviewed in 74]. Inhibition of DMBA-induced rat mammary carcinogenesis has been obtained with dietary combinations of the salt with 13-*cis*-retinoic acid and with 4-HPR; the individual agents were ineffective or slightly enhanced tumorigenesis. At the doses used, inhibition of β-glucuronidase in tissue microsomes was slight, although the gut bacterial enzyme was significantly inhibited. Although enterohepatic circulation of estrogens might be affected, other mechanisms have been suggested.

These include formation of a long-acting retinoid glucuronide [74], or induction of cAMP and TGF-β and suppression of protein kinase C by both agents [75].

PRECLINICAL SAFETY STUDIES

Limited preclinical toxicology and pharmacokinetic data are available for calcium salts; however, oral administration in humans suggests that calcium salts in general exhibit low toxicity. The CB has completed 90-day subchronic toxicity studies of calcium glucarate in rats and dogs, as well as several genotoxicity studies. Information from published studies has suggested that some calcium salts given iv or ip are carcinogenic or genotoxic; however, this may not be relevant for evaluating oral toxicity of these compounds.

Safety Published acute toxicity values suggest that calcium salts are fairly nontoxic. The oral LD_{50}s for the acetate, carbonate and chloride salts are 4,280, 6,450, and 1,000 mg/kg-bw, respectively [76]. When administered ip and iv, these substances are 10-fold more potent. As part of a CB-funded subchronic toxicity study, the LD_{50} for calcium glucarate was found to be >5,000 mg/kg-bw in rats.

Limited subchronic studies of calcium in the literature also suggest low toxicity. Sheep administered approximately 200 mmol Ca^{+2}/day as calcium chloride for 6 weeks showed only elevation of urinary calcium [77]. In contrast, iv administration produced changes in heart rate, blood pressure, plasma calcium, and urinary sodium and potassium. Calcium lactate given orally to rabbits at 2,500 mg/kg-bw/day for 15 days caused hyperplasia and increased RNA content in the thyroid [78].

In published studies, carcinogenic and genotoxic effects of calcium acetate and chloride have been suggested, but only by non-oral administration. As part of a chemoprevention study, calcium acetate (total dose: 11 mmol/kg-bw, ip) for 30 weeks significantly increased lung adenoma multiplicity in strain A mice [64]. Also, calcium chloride has been shown to induce unscheduled DNA synthesis when administered ip to rats at 2.5 mmol/kg-bw [79]. The mechanism in both cases may be a proliferative response resulting from direct irritation of the tissue by calcium.

The CB has funded 90-day oral toxicity studies of calcium glucarate (tetrahydrate) in rats and dogs. No adverse effects on body weight gain, food consumption, clinical parameters, hematology, or histopathology related to doses up to 50,000 mg/kg diet were observed in either species. This dose is approximately equivalent to 7 mmol salt/kg-bw/day (280 mg Ca^{+2}/kg-bw/day) in both species. Finally, no genotoxic effects were found in the mouse bone marrow micronucleus test, the Chinese hamster ovary cell sister chromatid exchange assay, and the Ames *Salmonella* test.

ADME No information on preclinical pharmacokinetics of calcium salts was found, except for calcium glucarate. According to published information, insoluble calcium glucarate undergoes slow dissolution and conversion to *d*-glucaro-1,2-lactone at low stomach pH [reviewed in 41]. In the CB-funded subchronic study of calcium glucarate in rats and dogs, plasma glucaric acid levels were dose-related. In contrast, inhibition of serum β-glucuronidase activity was not related to calcium glucarate dose, and urinary 17-ketosteroid excretion showed no significant difference from control. In published studies on rats, 1% and 5% dietary calcium glucarate (*ca.* 2 and 10 mmol salt/kg-bw/day) decreased serum β-glucuronidase activity by 47% and 60% of control, respectively [56]. In a study of 4% salt-supplemented diet, bacterial enzyme activity in the proximal and distal intestinal contents was decreased 70 and 54%, respectively [80].

CLINICAL SAFETY: PHASE I STUDIES

Orally administered calcium compounds generally exhibit low toxicity, except in certain disease states that produce symptomatic hypercalcemia. Three CB-funded Phase I trials of calcium are in progress to investigate the safety and pharmacokinetics of orally administered salts—carbonate, citrate and glucarate (see Table I). Because of their use as antacids, the pharmacokinetics of orally administered calcium salts (primarily calcium carbonate) have been described in the literature. Published information also exists on some additional salts that have been used in electrolyte replacement, such as calcium acetate, gluconate and chloride. These data are summarized below.

Drug Effect Measurement For calcium salts in general, the most obvious measure of drug effect is serum calcium levels. For calcium glucarate, the obvious drug effect measurement is serum β-glucuronidase activity. However, no consistent changes were observed in serum and urinary activities or in

urinary glucarate levels in the CB-funded Phase I trial (Dr. C. Young, Memorial Sloan-Kettering Cancer Center). It should be noted that the doses used were 20-fold lower than the dietary level in rats which has been shown to decrease serum β-glucuronidase by 60%. However, in preclinical studies, chemopreventive efficacy has been obtained at calcium glucarate doses which inhibited gut bacterial β-glucuronidase activity, but not serum activity.

Safety Orally administered doses of calcium compounds producing moderate elevations of the element in extracellular concentrations generally exhibit low toxicity. However, certain disease states (uremia, hypothyroidism, adrenocortical insufficiency) can produce clinically relevant hypercalcemia [1]. Adverse effects include muscle weakness, loss of renal concentrating capacity, coma, and, in extreme cases, cardiac arrest. In the gastrointestinal tract, calcium can produce acid rebound at doses as low as 500 mg [81]. This is a local effect of the cation on the small intestine to release gastrin, which in turn produces continued HCl hypersecretion. Also, increased basicity of the urine from chronic use of antacids such as calcium carbonate predisposes to calcium phosphate stones. When exposure is by subcutaneous, intravenous or topical routes, some calcium salts (*e.g.*, chloride) are irritating to tissue and can cause painful necrosis and sloughing [1].

In a CB-funded Phase I trial (Dr. R.G. Winn, University of Texas, M.D. Anderson Cancer Center), evaluation of the toxicity of the calcium salts of carbonate and citrate (2,000–4,000 Ca^{+2} qd for 16 wks) has been completed [82,83]. Although the two lowest oral doses of either compound were well-tolerated, 4,000 mg Ca^{+2} qd increased serum calcium in a third of the patients. A low incidence of minor symptoms was also reported, such as nausea, vomiting, and weakness. Urinary calcium excretion increased at all doses (hypercalciuria), but it is unknown if this causes kidney stones. The clinically acceptable dose for either salt was 2,000 mg Ca^{+2} qd; the main complaint at this level was constipation.

Possible adverse effects of calcium glucarate administered orally for 1 and 3 months are under study in a second CB-sponsored Phase I trial (Dr. C. Young) [83]. The one-month dose-escalation study in progress evaluated a total of 9,000–24,000 mg calcium glucarate per day in divided doses. The highest dose represents the maximum amount of calcium that the FDA recommends for supplement use (3,000 mg Ca^{+2} qd). The only effect at the two lowest dose levels (9,000 and 12,000 mg salt qd) was neck and back muscle discomfort in one subject, which resolved after a oneweek interruption. A three-month study will use the two highest nontoxic doses determined from the one-month study in progress.

Published sources were used for toxicity information on calcium acetate, gluconate, and chloride. Oral calcium acetate at 8,000–10,700 mg qd is indicated for control of primary phosphate retention and secondary hyperparathyroidism in renal failure [84]. The primary toxicity on chronic treatment is hypercalcemia. Mild hypercalcemia (10.5 mg Ca^{+2}/dl) may be asymptomatic or manifest as constipation, anorexia, nausea and vomiting; more severe cases (>12 mg Ca^{+2}/dl) can precipitate confusion, delirium, coma and cardiac arrhythmias.

Calcium gluconate (500–2,000 mg) and calcium chloride (500–1,000 mg) are available as injectable solutions for treatment of conditions arising from calcium deficiency or hyperkalemia [84]. Minor adverse reactions have been reported, including tingling sensations, sense of heat wave, and chalky taste. Rapid injection may cause vasodilation, decreased blood pressure, bradycardia, cardiac arrhythmias, or cardiac arrest. Tissue injection causes severe necrosis and sloughing. Oral administration of calcium chloride is not recommended due to gastrointestinal hemorrhage [85].

ADME The general pharmacokinetics of calcium salts have been described in the literature [1], however, calcium carbonate will be discussed as a representative orally administered compound [81]. Approximately 10–16% of calcium from the carbonate salt is absorbed from the intestines in the soluble ionized form, depending on vitamin D, lumen pH, dietary protein, and the presence of binding factors (phytate, fiber, oxalates) [85,86]. Uptake into the mucosa may be carrier-mediated, with no further increases at doses ≥20,000 mg. After a single dose of 4,000 mg calcium carbonate (1,600 mg Ca^{+2}), the plasma concentration of calcium rises and may be maintained for nearly 3 hrs; after an 8,000 mg dose, the increase is more persistent. Although plasma calcium is regulated to about 10 mg/dl (normal range: 8.5–10.5 mg/dl, with 50% as the free cation) by the actions of calcitonin, vitamin D and parathyroid hormone, steady-state may not be achieved for several months after beginning calcium carbonate as an antacid treatment. Secretion of calcitonin can be stimulated by the rise in ionic calcium; the hormone compensates for the increased calcium by decreasing mineral resorption from bone and by increasing renal excretion. Normal renal excretion

is about 7% of a daily dose of 50 mg Ca^{+2}/kg-bw. Total dose elimination is primarily fecal (80%), including the remaining insoluble antacid and unabsorbed calcium (as the ion or insoluble hydroxides, soaps or phosphates) [86].

Limited published information on the pharmacokinetics of other calcium salts is available. One study compared intestinal absorption of calcium as the carbonate and as the lactogluconate [87]. Plasma radioactivity from a small amount of co-administered $^{47}Ca^{+2}$ was significantly higher with calcium carbonate. Two other studies compared plasma and urinary calcium following ingestion of the citrate and carbonate salts [88,89]. The increments in both serum and urinary calcium were higher following the citrate than the carbonate. Other studies have shown no difference in absorption between 500 mg Ca^{+2} doses given as the acetate, lactate, gluconate, citrate, and carbonate salts [*e.g.*, 90]. Thus, the effect of the anion on calcium bioavailability is unclear.

Limited pharmacokinetic parameters have been determined in the CB-sponsored Phase I trial of calcium carbonate and calcium citrate (Dr. R. Winn) [82]. Following daily intake of 2,000 mg Ca^{+2} as either compound for 16 weeks, serum calcium was unchanged (*ca.* 9.39 mg/dl); however, 3,000 mg Ca^{+2} increased serum levels significantly over baseline (9.79 mg/dl). Twenty-four hour urinary excretion of the element increased >44% after 8 and 16 weeks on both doses.

Single-dose pharmacokinetics have been determined for calcium glucarate in a CB-sponsored Phase I trial (Dr. C. Young). Serum glucarate increased 3- to 20-fold over baseline in all patients. At 3,000 mg salt (*ca.* 0.17 mmol/kg-bw), C_{max}= 1.80 μg/ml and t_{max}=1 hr; at 6,000 mg salt (*ca.* 0.34 mmol/kg-bw), C_{max}=1.28 μg/ml and t_{max}= 2 hrs. Excretion was highly variable, but <1% of the administered dose appeared in the urine after 24 hrs in most patients (8/9). At the doses tested, calcium glucarate had no effect on β-glucuronidase activity measured in the urine or serum. Preliminary data from administration of 9,000 and 12,000 mg salt qd (*ca.* 0.5, 0.7 mmol/kg-bw qd) for one month show no consistent changes in serum and urinary β-glucuronidase activities or urinary glucarate levels. In contrast, an early study demonstrated significant inhibition of urinary β-glucuronidase activity in bladder cancer patients using other glucarate salts or the lactone metabolite [91]. However, these patients tend to have higher urinary enzyme activity even after the tumor is removed [92].

CLINICAL EFFICACY: PHASE II/III STUDIES

The NCI has funded one Phase III and three Phase II trials investigating the effectiveness of calcium carbonate in colon polyp patients; an additional trial will begin this year. In these studies, FAP patients are specifically excluded due to the lack of evidence for a calcium effect on colon crypt proliferation in this cohort. An additional Phase II study of calcium carbonate in breast is planned for 1995. As listed in Table I, combinations of agents with calcium salts are also under consideration. A Phase II trial of calcium carbonate with vitamin D_3 in polyp patients will begin this year. Studies planned for 1995 include modulation of biomarkers by calcium combined with vitamin D_3 in the colon and breast. Many published studies have investigated the effect of calcium salts on measures of colonic proliferation in various populations at risk for colon cancer.

The NCI-funded Phase III trial (Dr. J.A. Baron, Dartmouth College) is testing prevention of colon polyp recurrence by calcium carbonate in patients with a recent history of sporadic tumors [83]. Accrual of 850 patients to receive 3,000 mg calcium carbonate qd (1,200 mg Ca^{+2}) or placebo for 4 years has been completed. Study endpoints include recurrence of polyps, incidence of new polyps, histologic assessment, adverse effects, and analysis of dietary calcium intake and colon polyp risk; however, no results are available at this time.

The objective of Phase II trials is to titrate the agent dose against intermediate biomarkers and other measures of efficacy, as well as toxicity. Two NCI-funded Phase II trials are investigating the effect of calcium carbonate on proliferative indices in patients with previous sporadic colon polyps. A Phase II trial (Dr. J.A. Baron) is investigating the effects of the same treatment protocol as the Phase III trial above (3,000 mg calcium carbonate qd for 4 years) on colon mucosal cell proliferation in patients with prior sporadic colon polyps [83]. The trial is also comparing proliferative indices measured by BrdU and PCNA, and relating risk factors (*e.g.*, high-fat diet, age) to labeling indices. Accrual to the treatment and placebo arms is closed; no further information is available.

The endpoints in a second Phase II trial (Dr. J.D. Potter, University of Minnesota) are the effects of 3,000 and 5,000 mg calcium carbonate qd on colon epithelium proliferation, variability of colon cell proliferation, blood pressure, serum cholesterol, and toxicity. The results of an 8-week feasibility study were published recently [93]. In 21 sporadic

adenoma patients who consumed a Western-style diet, the pooled baseline [^{3}H]-thymidine labeling index in biopsies of flat colon mucosa was 4.7% of crypt cells. Calcium at 1,200 mg qd (3,000 mg calcium carbonate qd) had no effect on proliferation after the short treatment interval. In contrast, a higher dose of 2,000 mg Ca^{+2} qd (as the carbonate or citrate salt) for the same interval in the CB-funded Phase I trial (Dr. R. Winn) significantly decreased the BrdU labeling index in cultured rectal crypts. The reduction in PCNA labeling index was not significant.

The third Phase II trial of calcium carbonate in progress is funded by the Cooperative Community Oncology Program at NCI, and is being carried out by the Southwest Oncology Group (Dr. D.Z.J. Chu, City of Hope National Medical Center) [83]. Prevention of polyps and carcinomas in patients with a previously resected colon carcinoma by treatment with 1,800 mg calcium carbonate qd (720 mg Ca^{+2} qd) for 5 years is being compared with a placebo group (100 patients/arm). Other endpoints include treatment and colonoscopy compliance, and feasibility of measuring proliferation by BrdU and PCNA in adenomas and uninvolved mucosa.

As shown in Table I, two NCI-funded Phase II trials will begin this year. The first will compare the effects of vitamin D_3, calcitriol (active metabolite of vitamin D_3), and calcium carbonate on proliferation indices in patients with previously resected colon polyps or cancer. A second Phase II trial involves the combination of 2,400 mg calcium carbonate with 400 IU vitamin D_3 qd in patients with colon polyps <6 mm in diameter. The endpoints are modulation of polyp growth and other intermediate biomarkers. Three additional Phase II studies are planned for 1995: one trial of calcium in the breast and the combination of calcium and vitamin D_3 in the breast and colon.

A published double-blind, placebo-controlled trial in Norway (Norwegian Cancer Society) with a daily combination of 1,600 mg Ca^{+2} (NOS), 101 μg selenium (NOS), 15 mg β-carotene, 75 mg vitamin E, and 150 mg vitamin C for 3 years began in July 1989 [94]. Over 18 months, a total of 116 patients with colon polyps were accrued and allocated to separate groups based on the size of the largest polyp (<5 mm, 5–9 mm, >9 mm); each group was then randomized to a placebo or treatment arm. The endpoints are modulation of histological biomarkers (*e.g.*, changes in polyp diameter, number of polyps reaching 5 or 10 mm, new polyp incidence) and colon cancer incidence.

Certain high-risk areas for esophageal cancer in China are correlated with nutrient deficiencies, including calcium [95]. Since both rat and human esophageal cells tend to differentiate in high calcium medium *in vitro*, a trial investigating modulation of precancerous lesions (*e.g.*, basal cell hyperplasia, dysplasia) in this tissue by calcium carbonate (1,200 mg Ca^{+2}) took place in Huixian Province, China [96]. Following 11 months of treatment, the proportion of histologically improved cases was greater than the placebo group (30% versus 20%), but not significantly.

A number of small studies have investigated the effect of calcium on proliferative intermediate biomarkers of colon carcinogenesis. Methods for measuring proliferation in biopsies included [^{3}H]-thymidine labeling index (*i.e.*, [^{3}H]-thymidine labeled cells per crypt/total cells per crypt) and the crypt cell production rate (*i.e.*, mitotic figures/crypt after vincristine treatment). Patients treated with calcium show different responses depending on their risk status for colon cancer. In general, inhibition of proliferation by calcium was seen in patients at high risk due to previous sporadic colonic adenoma/carcinoma or familial association. Effective protocols included 1,000–2,000 mg Ca^{+2} as calcium carbonate for 1–3 months [28,29,97–100] or 1,250 mg Ca^{+2} as calcium gluconate for 2 months [101]. Although one study has shown a decrease in mitotic figures/crypt in FAP patients after calcium treatment [102], this group tended not to show reduction in whole crypt proliferation following 1,200–1,250 mg calcium as the carbonate for 3–9 months [29,103]. However, when proliferation in the longitudinal regions of crypts was analyzed in these patients, the proliferative compartment had contracted toward the crypt base; this pattern is typical of individuals at decreased risk for colon cancer [29].

A published study also investigated calcium modulation of a colonic differentiation biomarker, binding of the lectin soybean agglutinin (SBA) to terminal *N*-acetylgalactosamine residues. In preliminary work, archived biopsies of colon adenocarcinomas, adjacent transitional mucosa, and distal normal mucosa from the same patient demonstrated that immunoperoxidase staining for SBA decreased from normal/transitional mucosa to adenocarcinoma; thus, risk was inversely proportional to staining level [104]. In the resulting chemoprevention study, diets of patients at increased risk for colon cancer due to previous adenoma or familial association were supplemented with 1,500 mg calcium as the carbonate or citrate. After >3 months, SBA reactivity increased significantly (35% toward

normal) in individuals who had below-normal SBA levels before receiving calcium supplementation.

PHARMACODYNAMICS

Effective doses for inhibition of proliferation in patients at high risk for colon cancer appear to be 1,000–2,000 mg Ca^{+2} qd as calcium carbonate [28, 29,97–100]. These doses can be achieved by daily administration of 3–5 tablets of the OTC antacid product Caltrate®, as shown in Table II. This is below the maximum daily allowable dose of elemental calcium as an antacid, which is 3,200 mg Ca^{+2}. However, the comparable calcium carbonate dose (0.46 mmol calcium carbonate/kg-bw qd) is almost 9-fold less than the effective dose of 7,825 mg salt/kg diet (*ca.* 3.9 mmol/kg-bw/day) in the rat colon aberrant crypt assay. However, limited human data suggest that inhibition of colon proliferation occurs at lower doses. The ongoing NCI-funded Phase III and II trials (Dr. J. Baron) should provide relevant human data on prevention of colon premalignancy and modulation of proliferation at the lower daily dose of 1,200 mg Ca^{+2} (3,000 mg calcium carbonate).

In both CB-funded and published studies, the effective doses of calcium glucarate in rat mammary gland carcinogenesis models were 20–100 g/kg diet (*ca.* 4–20 mmol/kg-bw/day). A published study found that the same doses significantly decreased serum β-glucuronidase levels [56]. In contrast, the CB-funded toxicology study in rats and dogs at doses up to 50 g/kg diet (*ca.* 7 mmol/kg-bw/day in the rat) found no effect on the serum enzyme activity or urinary excretion of 17-ketosteroids. The salt appeared to be bioavailable, since a dose-dependent increase in the circulating glucarate metabolites was also observed in both species. Since the serum β-glucuronidase appears to vary widely over the course of a day in glucarate-treated animals [80], the sampling time may not have been optimal. Additionally, the Phase I trial (Dr. C. Young) of up to 12 g calcium glucarate has also failed to find a consistent decrease in serum β-glucuronidase; however, the clinical dose (0.7 mmol/kg-bw qd) was approximately 5-fold lower than the rodent dose (4 mmol/kg-bw/day). The required human dose could be 70 g calcium glucarate qd, a very large dose of compound.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

The obvious drug effect measurement for calcium glucarate is decreased serum β-glucuronidase activity. However, no consistent changes were observed in serum and urinary β-glucuronidase activities or urinary glucarate levels after doses of 9 and 12 g qd (*ca.* 0.4 and 0.53 mmol/kg-bw qd) for 1 month in an CB-funded Phase I trial (Dr. C. Young). It should be noted that these doses are 20-fold lower than the dietary level which has been shown to decrease the serum enzyme by 60% in rats (10 mmol/kg-bw/day). Also, the animal study measured enzyme activity using a commercially available diagnostic kit (Sigma Chemical). The Phase I study used a method adapted from an assay for β-glucuronidase activity in cerebrospinal fluid. These two assays should be run in parallel to demonstrate equivalence.

In preclinical studies, chemopreventive efficacy has been obtained at calcium glucarate doses which did not inhibit serum β-glucuronidase; in contrast, gut bacterial enzyme was inhibited [80]. It has been suggested that glucarate is not as accessible to the lysosomal enzyme in the plasma as it is to gut flora [105]. Inhibition of the intestinal enzyme could in turn affect enterohepatic circulation of glucuronide conjugates, such as estrogen. Gut bacterial enzyme inhibition and urinary 17-ketosteroid levels should be investigated as alternate drug effect measurements.

For the other calcium salts, plasma calcium is the most obvious systemic drug effect measurement; however, this is also a measure of toxicity. For oral administration, alternate measures need to be identified. Administration of a non-absorbed form of calcium (*i.e.*, active only in the colon) would avoid this problem.

Safety Issues

Although combinations with calcium glucarate are being evaluated in preclinical efficacy studies, clinical development would require animal toxicology studies on each combination before initiating Phase I trials. For this reason, comparisons between combinations using the glucarate and the carbonate salts as the source of calcium should be considered to investigate their equivalence. If the carbonate salt produces the same result, the effective combinations could be evaluated in Phase II trials without further work.

Pharmacodynamics Issues

The maximum recommended allowance for OTC antacid products is 3,200 mg Ca^{+2} qd (*ca.* 1.14

mmol Ca^{+2}/kg-bw qd) as calcium carbonate or phosphate. Effective doses for inhibition of proliferation in patients at high risk for colon cancer (1,000–2,000 mg Ca^{+2} qd as calcium carbonate) appear to be attainable under this limit. However, the clinical calcium carbonate dose (0.46 mmol calcium carbonate/kg-bw qd) is almost 9-fold less than the effective dose of 7,825 mg salt/kg diet (3.9 mmol/kg-bw/day) in the rat colon aberrant crypt assay. Hopefully, the ongoing NCI-funded Phase III trial will determine the effect of 1,200 mg Ca^{+2} as a daily 3,000 mg calcium carbonate dose on prevention of human colon premalignancy.

In both CB and published studies, the effective doses of calcium glucarate in rat mammary gland carcinogenesis models were 20–100 g/kg diet (*ca.* 4–20 mmol/kg-bw/day). However, conflicting data on the ability of the salt to inhibit serum β-glucuronidase activity at these doses exists. Additionally, the Phase I trial of up to 12 g calcium glucarate has also failed to find a consistent decrease in serum β-glucuronidase; however, the clinical dose (0.7 mmol/kg-bw qd) was approximately 5-fold lower than the rodent dose (4 mmol/kg-bw/day). The required human dose could be 70 g calcium glucarate qd, a very large dose of compound, which may be less than the chemopreventive dose.

Regulatory Issues

Various forms of calcium are available to consumers as single ingredients or in combinations with other nutritional agents. The calcium carbonate and phosphate salts have been evaluated by the FDA as safe and effective OTC antacid products. The only anticipated issue that would prevent a Phase II study on calcium carbonate would be selection of a high chronic dose which has shown toxicity or the lack of adequate data to evaluate the risk. In contrast, calcium glucarate is a "New Chemical Entity," and as such requires significant developmental work.

Supply and Formulation Issues

Currently marketed oral forms of calcium include calcium carbonate, calcium citrate, calcium gluconate, calcium lactate, and dibasic and tribasic calcium phosphate. Calcium carbonate is available in the greatest variety of strengths and formulations. In an ongoing NCI-funded trial, Lederle is supplying both Caltrate® and placebo tablets, and no problems are foreseen. Calcium glucarate for Dr. Young's trial was purchased as bulk drug from Akzo and repackaged in 1–6 g doses packed in light-resistant glass containers. Clinical development would require formulation of both product and placebo tablets.

Intermediate Biomarker Issues

In several clinical trials involving the colon, proliferation-related biomarkers are being investigated as surrogate endpoints to demonstrate chemopreventive efficacy. Methods include BrdU, PCNA and [^{3}H]-thymidine labeling index. As noted at a recent conference, the procedures need to be standardized, reproducible and precise [106].

The colon crypts of FAP patients tend to show expansion of the proliferative compartment. In several studies, FAP patients appeared to be non-responders to calcium treatment, since whole crypt proliferation was not altered. However, one study demonstrated a response in these patients as a change in the distribution and size of the proliferative compartment. The relevance of measuring individual intermediate biomarkers in specific patient populations needs to be assessed.

Clinical Studies Issues

Clinical development of calcium glucarate as a cancer chemopreventive agent in the breast and colon will not be pursued. First, the calcium moiety can be delivered more efficiently as the carbonate salt to inhibit colon carcinogenesis. Second, based on the effective preclinical dose, the amount of glucarate salt necessary to inhibit breast carcinogenesis in humans (70 g daily) would deliver more elemental calcium (8,750 mg) than the maximum allowed for OTC antacid use. Additional preclinical safety and pharmacokinetics data would be required. Finally, the Phase I trial of calcium glucarate did not demonstrate a consistent drug effect, *i.e.*, decreased serum β-glucuronidase.

The calcium salt most appropriate for development as a cancer chemopreventive drug is the carbonate. It possesses more of the characteristics of the ideal calcium salt, including low toxicity in both preclinical and human studies, FDA recognition of safety as an OTC antacid product, high proportion of elemental calcium by weight, positive animal efficacy results, and clinical evidence for modulation of cancer incidence or intermediate biomarkers. Thus, it is the current position that future CB-supported chemoprevention studies be limited to the use of calcium carbonate. To decrease the number of tablets required for treatment,

the clinical trial effort will specifically involve combinations of calcium carbonate with aspirin (colon), with vitamin D_3 (breast and colon), and with retinoids (breast).

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Table I. Clinical Trials of Calcium Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME)					
NO1-CN-15337-01 Phase I and Pharmacokinetic Studies of Calcium Glucarate (Dr. Charles Young, Memorial Sloan-Kettering Cancer Center) 6/91– IND 40,062	----	Patients with previous breast cancer Single-dose: 9 patients total; 4 in 3 g and 5 in 6 g, 1x groups (1 patient on Ensure Plus Diet) Subchronic and chronic: 16 patients each	Single dose: Oral 3 and 6 g calcium glucarate Subchronic: Oral 9, 12, 18, and 24 g qd for 1 month Chronic: Oral, 2 non-toxic subchronic doses for 3 months	Single- and multidose pharmacokinetics and toxicity Drug effect measurements: Serum and urine β-glucuronidase	Single-dose study completed. Serum glucurate increased 3- to 20-fold above baseline in all patients; t_{max} 1.5–2 hours. Excretion was highly variable, but 8/9 patients had less than 1% of the administered drug in urine after 24 hours. The drug had no effect on β-glucuronidase levels in urine and serum Multidose (subchronic): In progress; 5 patients accrued thus far Multidose (chronic): Has not been initiated

Table I. Clinical Trials of Calcium Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME) *(continued)*					
NO1-CN-85108-02 Phase I and Pharmacokinetic Studies of Calcium Carbonate (Dr. Rodger G. Winn, Univ. of Texas, M.D. Anderson Cancer Center) 6/90–7/94 IND 36,640	---	Patients with previous colon cancer, free of disease 46 total: 19 each at 2,000 mg and 3,000 mg; 8 at 4,000 mg	2000, 3000, 4000 mg Ca^{+2} qd as carbonate for 16 weeks 6 months	Chronic pharmacokinetics and safety Intermediate biomarkers: Proliferation biomarkers such as PCNA and BrdU in colon mucosa	Study completed Hypercalcemia (≥10.6 mg/dl) occurred in 38% of patients at 4,000 mg, 16% at 3,000 mg, and 5% at 2,000 mg. Six patients withdrew from the highest dose group, necessitating substitution of the 3,000 mg dose. Clinically acceptable dose of 2,000 mg determined Rectal crypt BrdU labeling index significantly decreased after 8 and 16 weeks of 2,000 mg Ca^{+2}. Reduction in PCNA labeling was not significant Published report: [82]

Table I. Clinical Trials of Calcium Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME) *(continued)*					
NO1-CN-85108-03 Phase I and Pharmacokinetic Studies of Calcium Lactate (Citrate) (Dr. Rodger G. Winn, Univ. of Texas, M.D. Anderson Cancer Center) 6/90–7/94 IND 36,640	----	Patients with previous colon cancer, free of disease 28 total: 19 each at 2,000 mg; 9 at 4,000 mg	2000, 3000, 4000 mg Ca^{+2} qd as carbonate for 16 weeks 6 months	Chronic pharmacokinetics and safety Intermediate biomarkers: Proliferation biomarkers such as PCNA and BrdU in colon mucosa	Study completed Hypercalcemia (≥10.6 mg/dl) occurred in 38% of patients at 4,000 mg, and none at 2,000 mg. Clinically acceptable dose of 2,000 mg determined Rectal crypt BrdU labeling index significantly decreased after 8 and 16 weeks of 2,000 mg Ca^{+2}. Reduction in PCNA labeling was not significant Published report: [82]
Phase II (Dose-titration, efficacy, intermediate biomarkers)					
RO1-CA-53827 Rectal Mucosal Proliferation Indices: Effect of Calcium Carbonate (Dr. John A. Baron, Dartmouth College) 8/91–2/94	Colon	Previous colon polyp patients 300 patients	3000 mg calcium carbonate qd (1,200 mg Ca^{+2}) for 4 years	Intermediate biomarkers: Polyp recurrence, new polyp incidence, BrdU, PCNA	No further information available

Table I. Clinical Trials of Calcium Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
RO1-CA-51932 Phase II and Pilot Study Calcium and Colorectal Epithelial Cell Proliferation (Dr. John D. Potter, University of Minnesota) 9/90–2/94 Investigator IND	Colon	Pilot study: Patients with previous colon polyps consuming a high-fat Western- style diet (35-69 years of age) Pilot study: 21 patients, 11 placebo and 10 on calcium supplement Phase II Study: 80 patients with previous colon polyps	Pilot study: 3,000 mg calcium carbonate qd (1,200 mg Ca^{+2}) for 8 weeks Phase II study: 3,000 or 5,000 mg calcium carbonate qd	Pilot study: Intermediate biomarkers assay methods Intermediate biomarkers: [^{3}H]-Thymidine labeling index in colon crypts; toxicity	Pilot study completed. Calcium carbonate did not decrease colon cell proliferation; scoring methods determined for Phase II Phase II has not been initiated Published Report: [93]
PO1-CA-41108 Phase IIa Colon Cancer Prevention Program Project (Fiber and Calcium) (Dr. David S. Alberts, Univ. of Arizona) 9/86–4/95 (Program Project) IND 29,294	Colon	Previous colon polyp patients 95 patients	0.25, 1.5 g calcium carbonate qd; 2, 13.5 g wheat bran qd for 9 months	Intermediate biomarker: [^{3}H]-Thymidine labeling	Preliminary report found no significant difference in whole crypt labeling following either treatment due to high variability in the placebo group Published report: [107]

Table I. Clinical Trials of Calcium Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
NCI-P92-0031/SWOG-9041 Phase II Pilot Randomized, Placebo-Controlled Study of Calcium for Chemoprevention of Colorectal Adenomas and New Primary Carcinomas in Surgically Treated Patients (Dr. David Z.J. Chu, City of Hope National Medical Center)	Colon	Histologically proven, completely resected colorectal carcinoma patients (Stage 0/I/II colon cancer except T4, Stage 0/I rectal cancer) 200 patients; 50 in the biomarkers study	1,800 mg calcium carbonate qd for 5 years	Efficacy: Recurrence rates of polyps and carcinomas, disease-free survival, overall survival Intermediate biomarkers: BrdU, PCNA in adenomas and uninvolved rectal mucosa Safety Other: Patient accrual rate, medication compliance, dropout and completion rate of yearly surveillance colonoscopy	Study in progress

Table I. Clinical Trials of Calcium Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
NO1-CN-25439-01 Phase II Clinical Trials of Calcium and Vitamin D in Patients with Colorectal Adenomatous Polyps. Modulation of Polyp Growth and Associated Surrogate Endpoint Biomarkers (SEB) (Dr. Peter R. Holt, St. Luke's-Roosevelt Institute of Health Science) 9/94- IND 36,670	Colon	Patients with colon adenomatous polyps <6 mm diameter	2,400 mg calcium carbonate + 400 IU vitamin D_3 qd for 6 months 3 years	Efficacy: New polyps and colon cancer incidence Other intermediate biomarkers: To be determined	New Study
NO1-CN-25439-02 Phase II Clinical Trials of Calcium and Vitamin D in Patients with High Probability of Hyperproliferation Based on a History of Previous Adenomatous Polyps Resected Within 2 Years, or of Colorectal Cancer. Modulation of Surrogate Endpoint Biomarkers (SEB) (Dr. Peter R. Holt, St. Luke's-Roosevelt Institute of Health Sciences) 9/94-	Colon	Patients with adenomatous polyps resected within 2 years or colorectal cancer	1,500 mg Ca^{+2} qd; or 0.25 µg calcitriol bid; or 400 IU vitamin D_3 qd for 6 months 18 months	Efficacy: Proliferation indices (total labeling index, median proliferation peak along the column, fraction of total proliferation located in the upper 2/5 of the crypt column) Intermediate biomarkers Differentiation, other biomarkers	New study

Table I. Clinical Trials of Calcium Sponsored/Funded by NCI, DCPC *(continued)*

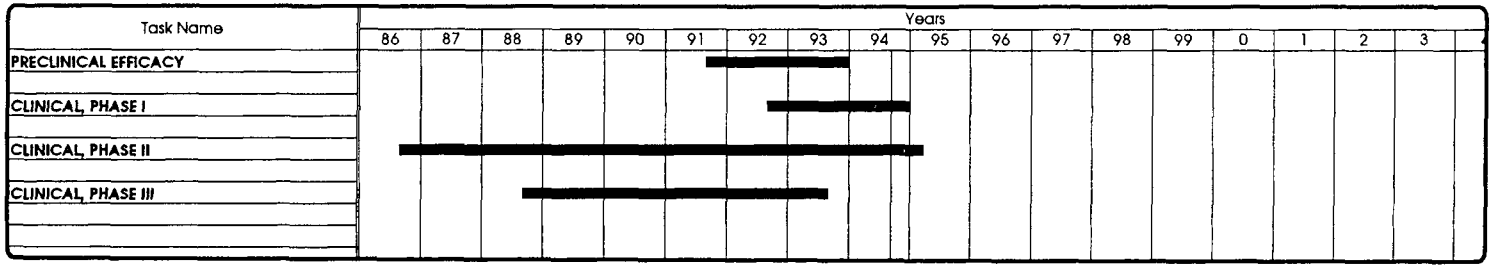
Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
Planned Study 1995	Breast	Patients scheduled for biopsy/surgery	Calcium carbonate 1 wk–2 months	Intermediate biomarkers	Study not yet designed
Planned Study 1995	Breast	Patients at high risk of breast cancer	Calcium + vitamin D_3 6 months	---	Study not yet designed
Planned Study 1995	Colon	Patients with previously resected polyps	Calcium + vitamin D_3 for 2–3 years	Efficacy: New polyps and other intermediate biomarkers	Study not yet designed
Phase III (Efficacy, intermediate biomarkers)					
UO1-CA-46927 Calcium in the Prevention of Neoplastic Polyps (Dr. John A. Baron, Dartmouth College) 9/88–7/93 IND 31,866	Colon	Previous colon adenoma patients 850 patients	3,000 mg calcium carbonate qd (1,200 mg Ca^{+2}) for 4 years 5 years	Efficacy: Colon polyp recurrence, new polyp incidence; risk analysis Safety	No further information available

Table II. Calcium Dosing Requirements[a]

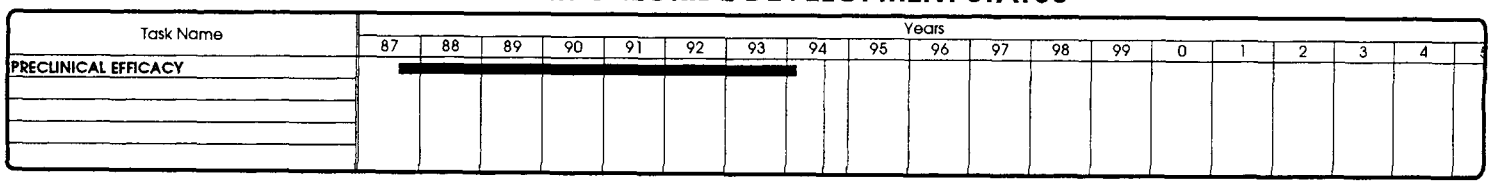
Ca^{+2} Salt	mg Ca^{+2}/ g Salt	% Ca^{+2}	mg Salt/ Tablet	# Tablets for 3 g Ca^{+2}
Carbonate[b]	400	40	1,500	5
Chloride	272	27.2	—	—[c]
Citrate	211	21.1	950	15
Glucarate	125	12.5	—	—[d]
Gluconate	90	9	1,000	33.3
Lactate	130	13	650	35.5
Phosphate				
Dibasic	230	23	500	26
Tribasic	380	38	1600	5

[a]Reference: [86]; [b]Caltrate® 500 (Lederle); [c]Injection only; [d]Investigational drug provided in packaged containers.

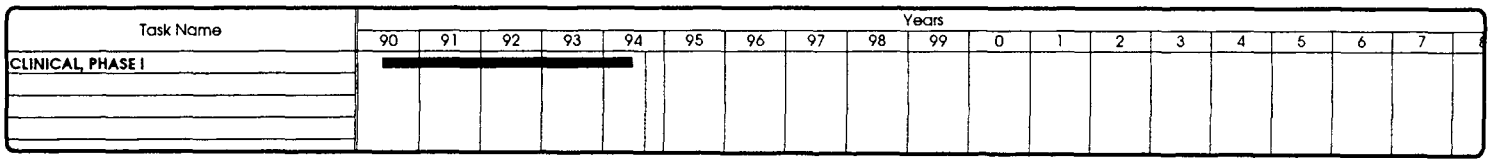
CALCIUM CARBONATE DEVELOPMENT STATUS



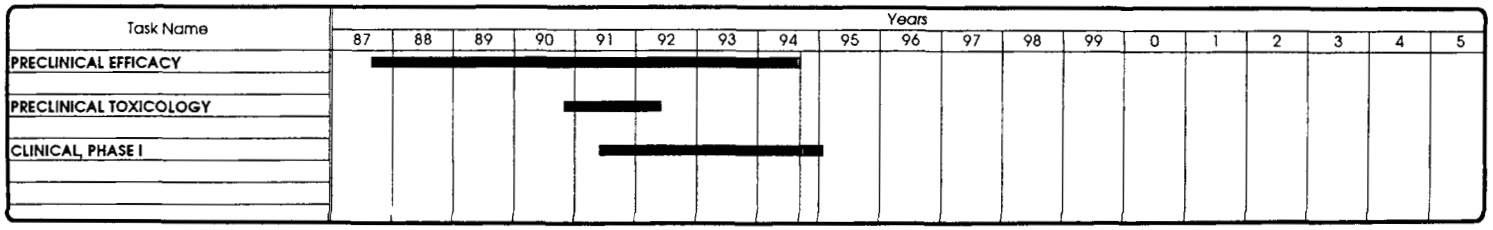
CALCIUM CHLORIDE DEVELOPMENT STATUS



CALCIUM CITRATE DEVELOPMENT STATUS



CALCIUM GLUCARATE DEVELOPMENT STATUS



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CALCIUM LACTATE DEVELOPMENT STATUS