NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

VITAMIN D_3 and ANALOGS

DRUG IDENTIFICATION

CAS Registry No.: 67-97-0

CAS Name (9CI): (3β,5Z,7E)-9,10-Secocholesta-5,7,10(19)-triene-3-ol

Synonyms: Cholecalciferol

Related Compounds:

25-Hydroxyvitamin D_3 (CAS No. 19356-17-3)
Calcifediol

1α,25-Dihydroxyvitamin D_3 (CAS No. 32222-06-3)
(1α,3β,5Z,7E)-9,10-Secocholesta-5,7,10(19)-triene-1,3,25-ol
Calcijex® (Active ingredient)
Calcitriol
1,25-Dihydroxycholecalciferol
1,25-Dihydroxyvitamin D_3
Rocaltrol® (Active ingredient)

Vitamin D_2 (CAS No. 59-14-6)
(3β,5Z,7E,22E)-9,10-Secoergosta-5,7,10(19)-tetraen-3-ol
Calciferol
Ergocalciferol

Dihydrotachysterol
Dychysterol

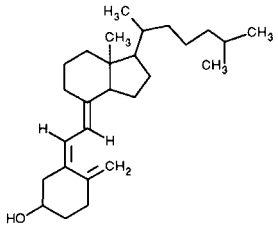
Analogs:

1α,25-Dihydroxy-16-ene-vitamin D_3 (CAS No. 124409-58-1)
1α,25-Dihydroxy-16-ene-cholecalciferol
Ro 24-2637

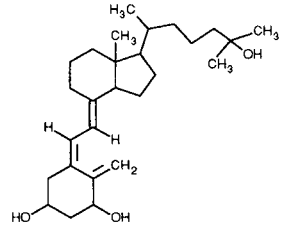
1α,25-Dihydroxy-16-ene-23-yne-vitamin D_3 (CAS No. 118694-43-2)
Ro 23-7553

1α,25-Dihydroxy-16-ene-23-yne-26,27-hexafluorovitamin D_3
(CAS No. 137102-93-3)
Ro 24-5531

Structures:



Vitamin D_3



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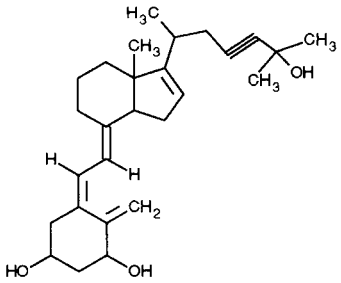
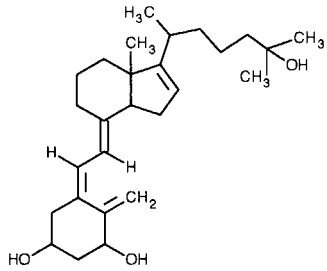
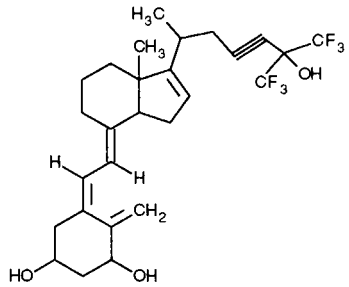


Ro 23-7553



Ro 24-2637



Ro 24-5531

EXECUTIVE SUMMARY

Vitamins D_3 and D_2 can be obtained from the diet; only vitamin D_3 can also be synthesized from 7-dehydrocholesterol in skin exposed to sunlight [1]. The term vitamin D applies to both substances (vitamins D_2 and D_3) that have the ability to prevent or cure rickets. The inverse association between vitamin D and cancer was derived from the geographical variation in prostate, breast, and colon cancer death rates, which increase with increasing latitude and decreasing sunlight intensity [2–7]. Subsequent epidemiological studies suggested inverse relationships between dietary vitamin D intake (including marine fish and vitamin D_2-fortified milk products) or serum 25-hydroxyvitamin D_3 levels and colon cancer risk [8–14]. Vitamin D_3 is metabolized in the liver to 25-hydroxyvitamin D_3 ($t_{½}$ in weeks), and the kidney converts this compound to the physiologically active hormone 1α,25-dihydroxyvitamin D_3 [15]. The latter binds to the intracellular vitamin D receptor, heterodimerizes with the retinoid X receptor, and binds DNA. Protein transcription is initiated in the appropriate cell types, producing the classic net increase in circulating calcium and phosphorus by increasing intestinal absorption and mobilization from bone, and decreasing urinary excretion [reviewed in 16,17]. Receptors are also found in tissues unrelated to these functions, such as mammary glands, colon, prostate, and skin [reviewed in 18,19]. Although the function of the receptors is unknown, vitamin D_3, and therefore the active metabolite, has been shown to possess chemoprevention-related activities in these and other tissues, including inhibition of proliferation and DNA synthesis [20–22], modulation of signal transduction by calcium and protein kinase C [reviewed in 23], modulation of c-*myc*, c-*fos* and c-*jun* oncogene expression [24–28], inhibition of ODC induction [29], lipid peroxidation [30] and angiogenesis [31], and induction of differentiation [32–35], TGF-β expression [23,36,37] and, possibly, apoptosis [38]. Unfortunately, the concentrations of 1α,25-dihydroxyvitamin D_3 necessary to suppress cell growth in culture would produce severe hypercalcemia *in vivo* [39]. This has led to the development of vitamin D_3 analogs (deltanoids) which retain the chemopreventive activities of the hormone with less calcium toxicity; they appear to have decreased affinity for the vitamin D receptor, although some have greater potency as inducers of

transcription *in vitro* [40]. The analogs which were considered for further development as cancer chemopreventive drugs by the CB are Ro 23-7553, Ro 24-5531, and Ro 24-2637.

In vitro studies first demonstrated that 1α,25-dihydroxyvitamin D_3 inhibited proliferation and induced differentiation in human leukemia cell lines and cells from human prostate, skin, breast, and colon cancers. However, the analogs were found to be more potent in leukemia cell lines. In rodent-derived systems, the vitamin and all the analogs reduced carcinogen-induced morphologic transformation of mouse mammary organ cultures and rat tracheal epithelial cells. *In vivo*, vitamin D_3 inhibited rat bladder and colon carcinogenesis; 1α,25-dihydroxyvitamin D_3 was effective against rat colon and mouse skin cancer models. Only the most potent analog—Ro 24-5531—has been tested in a carcinogen-induced animal model; it inhibited rat mammary gland carcinoma development. The response was further increased by treatment in combination with tamoxifen. Deltanoids Ro 23-7553 and Ro 24-5531 are currently on test in rat mammary gland and colon carcinogenesis models.

A significant effort in the CB program is to identify intermediate biomarkers of cancer and validate them as surrogate endpoints for clinical chemoprevention trials. In preclinical studies, 1α,25-dihydroxyvitamin D_3 decreased development of precancerous lesions (*i.e.*, histological biomarkers) in mouse skin; the hormone also reduced induction of ODC activity in rat colon and mouse skin. Inhibition of histological biomarkers by the deltanoids, alone and in combination with calcium, has been suggested by studies in rat colon; however, significant toxicity limited interpretation of these data.

No preclinical toxicity studies of vitamin D_3, its active metabolite, or the analogs have been undertaken by the CB. Published information demonstrates that 1α,25-dihydroxyvitamin D_3 produces significant toxicity in rodent studies, primarily hypercalcemia, weight loss and tissue calcification. Some evidence for embryotoxicity and teratogenicity was also found; however, maternal toxicity was often a contributing factor. The analogs of vitamin D_3 were synthesized to reduce toxicity while retaining chemopreventive efficacy. The analogs appear to have less potential for calcium-related adverse effects in both chicken and rodent models.

In humans, the primary response to vitamin D_3 is an increase in calcium absorption from intestines and bone, resulting in increased blood calcium levels. Thus, at pharmacologic doses, the most common adverse effects are hypercalcemia and hypercalciurea. Early symptoms of hypercalcemia include headache, nausea, vomiting, muscle and bone pain and constipation; late symptoms are polyuria, polydipsia, pancreatitis, elevated BUN, hypercholesterolemia, soft tissue calcification, hypertension and cardiac arrhythmia.

No CB-contracted clinical trials of vitamin D_3, related compounds or analogs have been completed. Because of preclinical efficacy data, the colon is one of the major target organs for clinical cancer chemoprevention with vitamin D_3. One strategy to decrease the vitamin dose is to use the combination of vitamin D_3 with calcium carbonate; this takes advantage of the demonstrated preclinical efficacy of each agent in the colon, as well as the physiological relationship between them. Thus, one Phase II trial in the colon with vitamin D_3 in combination with calcium carbonate began in 1994. The endpoints are modulation of growth of colorectal polyps and other of intermediate biomarkers in a cohort of patients with previously resected polyps. Two additional trials are planned for 1995 to evaluate the effect of the combination on intermediate biomarkers in breast and colon. An additional Phase II trial of the effects of 1α,25-dihydroxyvitamin D_3 on colon biomarkers began in 1994. A second strategy is the clinical development of vitamin D_3 analogs, which will be considered when sufficient preclinical efficacy and toxicity data are available.

Calcitriol (synthetic 1α,25-dihydroxyvitamin D_3) is the active ingredient in two pharmaceuticals, Calcijex® (Abbot Laboratories) and Rocaltrol® (Hoffman-La Roche), indicated for the management of hypocalcemia resulting from chronic kidney dialysis and hypoparathyroidism. Hoffman-La Roche is supplying the vitamin D_3 analogs, and no problems are foreseen.

PRECLINICAL EFFICACY STUDIES

Published *in vitro* studies first demonstrated that vitamin D_3 and 1α,25-dihydroxyvitamin D_3 had cancer-inhibiting properties in various cell systems. For example, the active metabolite has been shown to inhibit proliferation and induce differentiation in human leukemia cell lines [26,41,42], human prostate [43], skin [44], breast [21] and colon cancer cells [20,45,46], and human malignant shwannoma cells [47]. Subsequent studies showed that the analogs share some of the same properties with

1α,25-dihydroxyvitamin D_3. Ro 24-5531 [37,48,49], Ro 23-7553 [22,41,48,50–53], and Ro 24-2637 [52] also inhibited proliferation and enhanced differentiation in various human leukemia cell lines; however, as demonstrated in human leukemia HL-60 cells, the analogs were more potent. The fluorinated analog Ro 24-5531 was 80-fold more effective (ED_{50}=0.2 nM) than bioactive vitamin D_3 in inhibiting growth, making it the most active of the deltanoids under discussion [48]. Ro 23-7553 was the second most potent at 40-fold the activity of the hormone; Ro 24-2637 was 10.7-fold more effective. The rank order for differentiation enhancement was the same.

The CB-funded *in vitro* testing of vitamin D_3, 1α,25-dihydroxyvitamin D_3, and the deltanoids has been primarily in combination with calcium glucarate. *In vitro*, each deltanoid, alone and in combination, inhibited anchorage-independent growth of human lung tumor A427 cells and morphological transformation of B(*a*)P-induced rat tracheal epithelial (RTE) cell primary cultures. As single agents, vitamin D_3 and Ro 23-7553 decreased development of hyperplastic alveolar nodules (HAN) in the DMBA-induced/TPA-promoted mouse mammary organ culture assay (MMOC); the remaining deltanoids were effective in the MMOC assay without promotion. All analog combinations with calcium glucarate were also effective, except Ro 23-7553. Vitamin D_3, 1α,25-dihydroxyvitamin D_3, Ro 23-7553, and Ro 24-5531 are currently on test in the MNU-induced rat mammary gland carcinogenesis model *in vivo*; vitamin D_3 is also on test in combination with calcium glucarate.

In completed CB-funded *in vivo* studies, 1.25 mg vitamin D_3/kg diet (*ca.* 0.42 μmol/kg-bw/day) in combination with 2.5 mg calcium glucarate/kg diet decreased bladder carcinogenesis in the OH-BBN-exposed mouse; however, body weight gain was also significantly reduced (12%). Efficacy assays of Ro 23-7553 and Ro 24-5531 in the AOM-induced rat colon carcinogenesis model are in progress.

In published studies, both vitamin D_3 and its bioactive metabolite were effective in the DMH-induced rat colon model of carcinogenesis [54–56]. 1α,25-Dihydroxyvitamin D_3 was also shown to decrease induction of the proliferation biomarker ODC activity in the same system [56], as well as in promoter-induced mouse skin [57]. Vitamin D_3 inhibited ODC induction by cholic acid [29]. In other target organs, the metabolite decreased development of both cancerous and precancerous (papilloma) lesions in the DMBA-induced/TPA-promoted mouse skin model [57,58] and retinoblastomas in transgenic SV40 T-antigen mice [59].

Only limited animal efficacy data have been published for the analogs. Ro 24-5531, the most potent analog in HL-60 cells, has been tested in the MNU-induced rat mammary carcinogenesis model [49,60]. Doses of 1.25 nmol/kg diet (*ca.* 0.06 nmol/kg-bw/day) for 6 months significantly inhibited tumors initiated by a low dose of carcinogen (15 mg MNU/kg-bw, 1x). In the standard assay (50 mg MNU/kg-bw, 1x) however, doses up to 2.5 nmol Ro 24-5531/kg diet were ineffective [60, 61]. The most effective chemopreventive strategy was potentiation of Ro 24-5531 (1.25, 2.5 nmol/kg diet) by combination with tamoxifen (0.25, 0.5 mg/kg diet); the response was measured as total tumor weight/rat and incidence of tumor-free rats [61]. It should be noted that these tumors are estrogen-dependent; in related experiments, bioactive vitamin D_3 has been shown to inhibit estrogen-induced growth and gene transcription (pS2) in human breast carcinoma MCF-7 cells [62]. The mechanism for this antiestrogenic effect may be related to the existence of a superfamily of structurally homologous nuclear receptors for steroid and thyroid hormones, and vitamins D and A [63]. The receptor-ligand complexes are transcription factors that modulate the expression of specific target genes. Induction of TGF-β in particular may serve as a common pathway for growth inhibition or differentiation induction [64].

A second published *in vivo* study has investigated the response of leukemia cells to Ro 23-7553. Doses of 0.8 μg of the analog on alternate days (ip) significantly increased the survival of mice injected with myeloid leukemia cells; in contrast, bioactive vitamin D_3 was ineffective [65].

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 markers. Such studies in animals contribute to the development of more efficient screens to identify new chemopreventive agents, as well as identifying biomarkers to be used as surrogate endpoints in clinical trials [66]. Results from CB-contracted histological biomarker studies suggest that the hormone and all analogs inhibited formation of a putative premalignant lesion—aberrant crypt foci—in the AOM-induced rat colon when tested alone and in combination with calcium glucarate; only Ro 24-2637 was ineffective alone at the same dose. Unfortunately, significant toxicity requiring reduction of the original doses limits interpretation of the data.

Besides inhibition of premalignant lesions, the potential for modulation of proliferation and genetic biomarkers have been suggested by *in vitro* studies. In HL-60 cells, 1α,25-dihydroxyvitamin D_3 decreased expression of c-*myc*, a measure of proliferative potential [24,25,67]. Ro 23-7553 (0.1 nM) and Ro 24-5531 (0.5 nM) required even lower doses than the hormone (2 nM) to decrease c-*myc* mRNA by 95% in the same cell line [48,50]. In two other human leukemia cell lines (U937, THP-1), Ro 24-5531 induced differentiation, as well as expression of TGF-β and its type II receptor [37].

PRECLINICAL SAFETY STUDIES

The CB has not funded toxicity studies of vitamin D_3, 1α,25-dihydroxyvitamin D_3, or any of the deltanoids under discussion. Sufficient data exist to undertake Phase II trials of vitamin D_3 or the active metabolite; however, combinations may require additional toxicity testing. Similarly, clinical development of the analogs would require completion of subchronic and chronic toxicity studies in two species. The information on preclinical toxicity and pharmacokinetics included below was obtained primarily from the open literature.

Safety Vitamin D_3, and therefore the active metabolite, produces significant toxicity, primarily hypercalcemia, weight loss, and tissue calcification. Oral (ig) LD_{50} values for 1α,25-dihydroxyvitamin D_3 are very low: 1.35 μg in mice and >5 μg in rats [68]; the compound is even more potent given subcutaneously.

In subchronic studies, 1α,25-dihydroxyvitamin D_3 given to female rats at 0.5 μg/kg-bw on alternate days (0.6 nmol/kg-bw/day) for 2 weeks significantly increased serum calcium (20%) and urinary excretion (>4-fold) [21]. In transgenic SV40 T-antigen mice, 0.025 and 0.5 μg 1α,25-dihydroxyvitamin D_3 given 5x/week (ip) for 5 weeks resulted in significantly increased serum calcium (+3–5 mg/dl) and weight loss [59]. At the highest dose, serum calcium increased almost 50%. In the preliminary range-finding study, 0.05, 0.1 and 0.2 μg 1α,25-dihydroxyvitamin D_3 on the same dosing schedule resulted in mortality rates of 25%, 50% and 50%, respectively.

Some evidence of cancer promotion has been reported for 1α,25-dihydroxyvitamin D_3; however, no long-term studies to specifically evaluate carcinogenic potential have been conducted [45]. In a published chemoprevention study, treatment of mice increased the formation of DMBA-induced skin tumors [69]; however, the metabolite was negative in the Ames/*Salmonella* mutagenicity assay [70]. *In vitro*, 1α,25-dihydroxyvitamin D_3 enhanced chemical- and radiation-induced transformation of mouse BALB 3T3 cells [71–73].

Limited evidence for teratogenic effects of 1α,25-dihydroxyvitamin D_3 has been published; however, maternal toxicity was often observed. In rabbits, 0.3 μg/day on gestation days 7–18 resulted in 19% maternal mortality, decreased fetal body weights, and a reduction in the number of offspring surviving 24 hours [70,74]. In a second rabbit study, all fetuses in three litters had external and skeletal abnormalities following maternal doses 4- and 15-fold higher than the recommended human exposure; however, none of the remaining 23 litters showed significant abnormalities [70]. No explanation of these results was offered. In a study of peri- and postnatal development, 0.08 μg 1α,25-dihydroxyvitamin D_3/day to rats resulted in hypercalcemia in the pups and dams; 0.3 μg/day also resulted in increased serum urea nitrogen and reduced weight gain in the dams.

The analogs of vitamin D_3 were synthesized to retain chemopreventive efficacy while decreasing toxicity. Based on induction of intestinal calcium absorption and bone mobilization in vitamin D-deficient chickens given a calcium-free diet for 36 hours, bioactive vitamin D_3 has the greatest potential for induction of hypercalcemia [48]. The calcium-enhancing properties of Ro 24-5531 and Ro 23-7553 were 6.7–10.4% and 2–3.3%, respectively, of those displayed by 1α,25-dihydroxyvitamin D_3. Thus, the analogs appear to have much less potential for calcium toxicity [75].

In rodent studies, pups of female rats given 1 μg bioactive vitamin D_3/kg-bw (2.4 nmol/kg-bw; ig) on lactation days 3–6 displayed significant soft tissue calcification and growth impairment, whereas Ro 23-7553 at a 10-fold higher dose lacked adverse effects [51]. The neonatal calcium-related toxicity appeared to correlate with receptor-mediated effects in adult animals. In adult rats, the analog produced only a fraction of receptor-related effects compared with the active metabolite at the same dose (12.5 ng/kg-bw, iv) when measured as bone calcium mobilization (0%), intestinal calcium absorption (53%), and competitive receptor binding (47%). In mice, Ro 23-7553 was 10–25-fold less potent than 1α,25-dihydroxyvitamin D_3 in causing calcium-related toxicity [65]. Animals receiving ≥0.1 μg 1α,25-dihydroxyvitamin D_3 ip on alternate days for 2–6 weeks had hypercalcemia (≥12 mg Ca^{+2}/dl); among the analogs, only mice receiving

≥2.4 μg Ro 23-7553 on the same schedule displayed hypercalcemia.

Some toxicity information was available from chemoprevention studies. In a CB-sponsored intermediate biomarker study, 1α,25-dihydroxyvitamin D_3 and the analogs produced significant toxicity after subchronic oral intake. After 35 or 45 days on a range of doses, the major effect was soft tissue mineralization in the heart, aorta, kidney, stomach, and lung, indicative of hypercalcemia. In a published study, 2.5 nmol Ro 24-5531/kg diet (*ca.* 0.13 nmol/kg-bw/day) had no effect on serum calcium when fed to female rats for 5–7 months as part of an MNU-induced mammary cancer chemoprevention assay [49].

ADME The pharmacokinetics of intravenous 1α,25-dihydroxyvitamin D_3 have been determined in published studies. In rats given a single dose of 10 μg/kg-bw, the parameters were as follows: elimination $t_{½}$=3.8 hr, AUC=309 ng/ml·hr, and Cl= 6 ml/hr [76]. In dogs, the plasma parameters after a bolus dose of unknown magnitude were $t_{½}$= 7.0 hrs and Cl=6.8 ml/min [77].

CLINICAL SAFETY: PHASE I STUDIES

No Phase I trials of deltanoids have been funded by the CB. The human data on vitamin D_3 and its active metabolite have been taken from published sources. No information on the safety and pharmacokinetics of the analogs was found.

Drug Effect Measurement The most obvious drug effect measurement would be serum calcium levels. However, since this is also a measure of toxicity, the chemopreventive doses used would presumably produce little change. An appropriate measurement needs to be determined and standardized. Possibilities include plasma alkaline phosphatase, serum creatinine phosphokinase, erythrocyte 2,3-diphosphoglycerate, and tissue TGF-β levels [63,78].

Safety The primary response to vitamin D_3 is an increase in calcium absorption from the intestine and bone, and a resulting increase in blood calcium levels. With chronic exogenous treatment with the active metabolite, the most common adverse effects are hypercalcemia and hypercalciurea [33,74]. Early symptoms of hypercalcemia include headache, nausea, vomiting, muscle and bone pain, and constipation; late symptoms are polyuria, polydipsia, pancreatitis, elevated BUN, hypercholesterolemia, soft tissue calcification, hypertension, and cardiac arrhythmia. The recommended dose of 1α,25-dihydroxyvitamin D_3 in the treatment of hypocalcemia resulting from chronic renal dialysis is 0.01 μg/kg-bw, 3x/week; the dose can be increased by 0.005–0.01 μg/kg-bw at 2–4 week intervals based on serum calcium levels. In a clinical study of osteoporosis treatment in patients with normal serum calcium, doses of 0.75 μg qd produced hypercalcemia; however, in countries with low calcium intake, higher doses have been tolerated. In a published clinical chemoprevention study, half of the patients (9/18) treated for preleukemic myelodysplastic syndrome developed hypercalcemia following doses of 2 μg 1α,25-dihydroxyvitamin D_3 qd for ≥12 weeks [79]. Serum calcium increased from a median of 8.95 mg/dl to a median of 10.3 mg/dl at the end of the study.

It should be noted that the vitamin D_3 metabolite can enter fetal circulation and may be excreted in human milk. Administration of 17–36 μg 1α,25-dihydroxyvitamin D_3 qd during one woman's pregnancy resulted in mild hypercalcemia in the first two days of the infant's life [70].

ADME Vitamin D (as D_3 and D_2) is rapidly absorbed from the gastrointestinal tract if fat absorption is normal and bile is present [80]. It enters the blood via chylomicrons, and then associates with a specific α-globulin. In the liver, vitamin D is converted to the 25-hydroxy derivative, the major circulating form. Normal serum concentrations of 25-hydroxyvitamin D_3 are 10–80 ng/ml. When given orally, 25-hydroxyvitamin D_3 has an elimination $t_{½}$ of 16 days, although 30 days has also been reported. The 25-hydroxylated derivative is stored in fat and muscles, or further hydroxylated in the kidneys to the active form, 1α,25-dihydroxyvitamin D. The normal range of 1α,25-dihydroxyvitamin D_3 in plasma is 0.015–0.06 ng/ml [10]. The metabolites of vitamin D are excreted primarily in bile and feces [80].

1α,25-Dihydroxyvitamin D_3 (as Rocaltrol®) was rapidly absorbed from the intestine [68]; peak serum values were reached 3–6 hrs after administration of single doses of 0.25–1 μg. The plasma elimination $t_{½}$ ranged from 3–6 hrs.

CLINICAL EFFICACY: PHASE II STUDIES

Two CB-funded Phase II trials with vitamin D_3 will begin this year. One trial will compare the efficacy of daily calcium (1,500 mg as calcium carbonate), vitamin D_3 (400 IU), or 1α,25-dihydroxyvitamin D_3 (0.5 μg) in modulating intermediate biomarkers in patients with previously resected

colorectal polyps or cancer. The second trial involves administration of vitamin D_3 (10 μg) combined with calcium (2,400 mg as calcium carbonate) to suppress the growth of existing colorectal polyps. Two additional trials are planned for 1995 to investigate modulation of biomarkers in the breast and colon by vitamin D_3 in combination with calcium.

In a small published study, cells removed from patients (n=18) with a preleukemic condition, myelodysplastic syndrome, and exposed to the hormone showed decreased proliferation and induction of myeloblast and premyelocyte differentiation into monocytes, macrophages, metamyelocytes and granulocytes [81]. However, oral administration of 1α,25-dihydroxyvitamin D_3 to the same patients did not result in a clinical effect on blood parameters [79]. A second study in seven patients treated with 2.5 μg qd for 8–24 weeks had similar results [reviewed in 82].

In a small proliferation-related biomarker study, a differential effect of bioactive vitamin D_3 on colon biopsies from normal and FAP subjects was demonstrated [81]. A concentration of 0.1 nM 1α,25-dihydroxyvitamin D_3 reduced the crypt cell production rate (mitotic figures/crypt/hour after vincristine arrest) in normal colon explant by 2.2; however, the same concentration reduced the value by 4.5 in FAP colon explants.

PHARMACODYNAMICS

In the MNU-induced rat mammary gland cancer model, the effective 6-month dietary dose for the analog Ro 24-5531 was 0.06 nmol/kg-bw/day [49, 60], 10% of the dose of 1,25α-dihydroxyvitamin D_3 (0.6 nmol/kg-bw/day) which produces a significant increase in plasma calcium after 2 weeks [21]. Interestingly, Ro 24-5531 had approximately 7–10% of the potential for induction of hypercalcemia as 1,25α-dihydroxyvitamin D_3 in the chicken model [48]. Using this ratio, the effective dose of the analog in humans may be 0.3 nmol/kg-bw qd, or 10% of the 1,25α-dihydroxyvitamin D_3 dose producing hypercalcemia (0.75 μg qd, or *ca.* 0.03 nmol/kg-bw).

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

The most obvious drug effect measurement would be serum calcium levels. However, since this is also a measure of toxicity, the chemopreventive doses used would presumably produce little change. An appropriate measurement needs to be determined and standardized before beginning clinical trials.

Safety Issues

Preclinical studies suggest that 1α,25-dihydroxyvitamin D_3 has embryotoxic, teratogenic, and cancer-promoting activities. The adverse reproductive effects appear to be related to hypercalcemia, which is less of a concern for the analogs. The carcinogenic potential of the analogs needs to be determined in chronic studies in two species before long-term clinical development is undertaken.

Pharmacodynamics Issues

With a range of effective analog doses in the AOM-induced aberrant crypt focus model, the chronic toxicity information (see **Toxicology Issues**) could be used to calculate a therapeutic ratio in the rat. Using the rodent therapeutic ratio, the potential for chemopreventive efficacy without calcium toxicity could be estimated for human use. This information may be available from Hoffman-La Roche.

Regulatory Issues

Sufficient preclinical toxicity data exist to undertake Phase II trials of vitamin D_3 or the active metabolite; however, combinations may require additional toxicity testing. Similarly, clinical development of the analogs would require completion of subchronic and chronic toxicity studies in two species before Phase I trials can be initiated. This information may be available from Hoffman-La Roche. From the experience with the CB-funded aberrant crypt focus assay in the rat colon, the chronic NOELs have not been adequately defined for the analogs.

Supply and Formulation Issues

Hoffman-La Roche is supplying the vitamin D_3 analogs; no problems are foreseen. The company holds European patents (1989) on Ro 24-2637 and Ro 23-7553 for treatment of dermal sebaceous gland (*e.g.*, acne, seborrheic dermatitis) and hyperproliferative (*e.g.*, psoriasis) diseases, and skin neoplasms. A U.S. patent on vitamin D_3, 1α,25-dihydroxyvitamin D_3, and Ro 23-7553 for prevention

and treatment of chemotherapy-induced alopecia was issued (1993) to the University of Miami.

Intermediate Biomarker Issues

It would be useful to investigate several types of intermediate biomarkers in preclinical and clinical studies with deltanoids. Proliferation (*e.g.*, expansion of the proliferative compartment, ODC activity induction), genetic (*e.g.*, c-*myc* expression), and histological (precancerous lesions) biomarkers have been suggested in both *in vitro* and *in vivo* studies. It is important that sampling procedures and analytical methods be standardized and quality controlled so that the best biomarkers can serve as surrogate endpoints in Phase II trials.

Clinical Studies Issues

Because of the preclinical efficacy data, the colon is one of the major target organs for clinical cancer chemoprevention with vitamin D_3. One strategy to decrease the vitamin dose is to use the combination of vitamin D_3 with calcium carbonate; this takes advantage of the demonstrated preclinical efficacy of each agent in the colon, as well as the physiological relationship between them. Thus, one Phase II trial in the colon with vitamin D_3 in combination with calcium carbonate began in 1994. The endpoints are modulation of growth of colorectal polyps and other intermediate biomarkers in a cohort of patients with previously resected polyps. Two additional trials are planned for 1995 to evaluate the effect of the combination on intermediate biomarkers in breast and colon.

A second strategy is the development of vitamin D_3 analogs, which will be considered when sufficient preclinical efficacy and toxicity data are available. Although the vitamin D analogs appear to have less potential for calcium-related adverse effects, this needs to be explored in Phase I trials. If the therapeutic ratio from preclinical and clinical toxicity studies is favorable, Phase II trials will be undertaken in the breast and colon.

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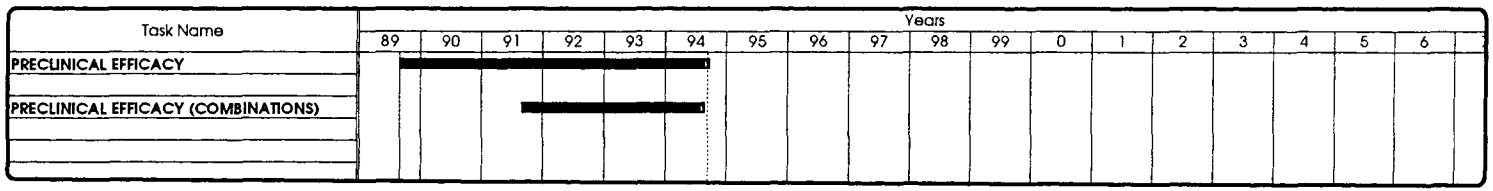
Table I. Clinical Trials of Vitamin D_3 Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers)					
Planned Study Chemoprevention of Breast Cancer with Calcium and Vitamin D in High-risk Subjects 1995	Breast	Women at high risk for breast cancer	Vitamin D_3 + calcium 6 months		Study not yet designed
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 Sciences) 9/94–	Colon	Patients with colon adenomatous polyps <6 mm in diameter	400 IU vitamin D_3 + 2,400 mg Ca^{+2} qd for 6 months 3 years	Efficacy: Development of new polyps and colon cancer 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 previous resected adenomatous polyps within 2 years or colorectal cancer	0.25 μg 1α,25-dihydroxy-vitamin D_3 (calcitriol) bid; or 400 IU vitamin D_3 qd; or 1,500 mg Ca^{+2} qd for 6 months 18 months	Intermediate biomarkers: 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); differentiation; other	New study

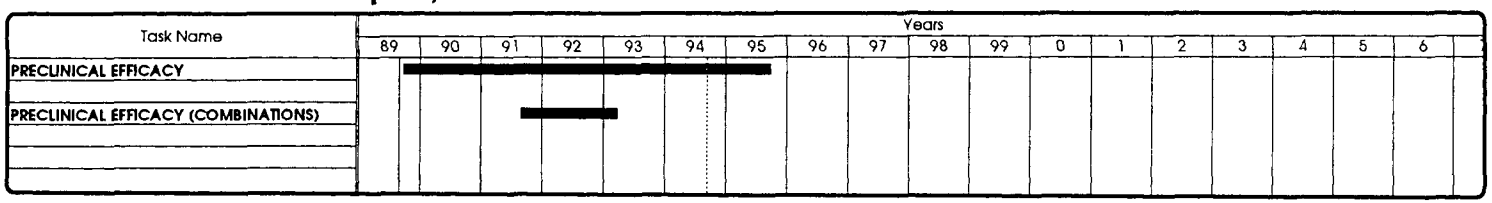
Table I. Clinical Trials of Vitamin D_3 Sponsored/Funded by NCI, DCPC *(continued)*

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 Prevention of Colorectal Polyps and Modulation of Surrogate Endpoint Biomarkers (SEBs) with Calcium and Vitamin D 1995	Colon	Patients with previously resected colon polyps	Vitamin D_3 + calcium 3 years	Efficacy: New polyps and other intermediate biomarkers	Study not yet designed

VITAMIN D3 DEVELOPMENT STATUS



1alpha,25-DIHYDROXYVITAMIN D3 DEVELOPMENT STATUS



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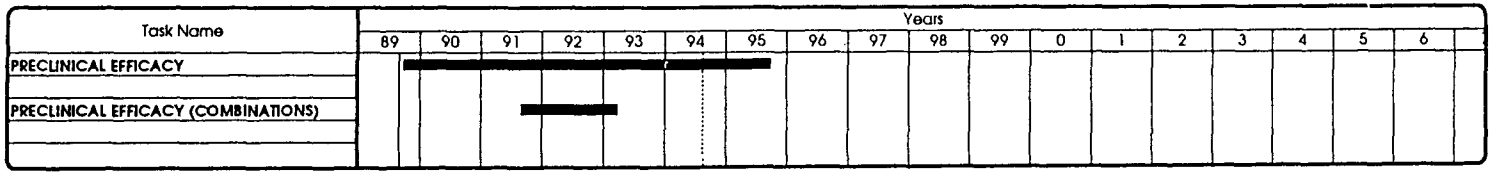

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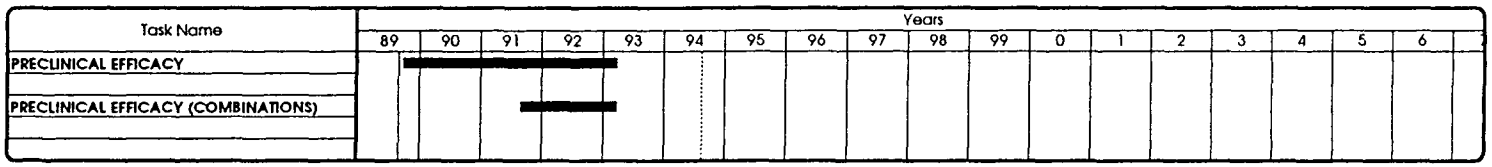

Ro 23-7553 DEVELOPMENT STATUS