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Institutional Review Board

Approval Notice

This institution has an approved assurance of compliance on file with HHS which covers this activity FWA 00006731 Federal Wide Assurance identification number

August 7, 2017

Mary Reid, PhD

Mary.Reid@RoswellPark.org

Dear Mary Reid:

On 8/7/2017, the IRB reviewed the following submission:

Type of Submission:	Modification/Update
Type of Review:	☐ Full Board ☒ Expedited ☐ Exempt ☐ Non-Human Research
Title of Study:	Pilot Study of Oral Calcitriol in Patients at High Risk for Lung Cancer
Investigator:	Mary Reid, PhD
IRB ID:	MOD00001393 / I 90206
Funding:	Name: NATIONAL CANCER INSTITUTE
Grant ID:	None
IND, IDE, or HDE:	IND #74464, HOLDER Roswell Park Cancer Institute
Documents Reviewed:	• I 90206 PROT AMD 18 Clean 7.11.17.pdf, Category: IRB Protocol;

The IRB approved the study from 8/7/2017 to 12/18/2017 inclusive. Before 12/18/2017 or within 30 days of study closure, whichever is earlier, you are to submit a continuing review with required explanations. You can submit a continuing review by navigating to the active study and clicking Create Modification / CR. If continuing review approval is not granted on or before 12/18/2017, approval of this study expires after that date.

The principal investigator is responsible for ensuring that the research complies with all applicable regulations. Any modifications in the research project are subject to approval by the Board prior to initiation by the investigator. The Board reserves the right to stop the research for violations of regulatory or IRB requirements.

A progress report must be submitted to the IRB at least one month prior to the expiration date noted above for continuing review as required by federal regulations and/or institutional requirements.

Please be advised that your research study may be audited periodically by the IRB for compliance.

This activity has been reviewed and approved by an IRB in accordance with the requirements of 45 CFR 46, including its relevant Subparts. This protocol fulfills, when

applicable, requirements for certifying FDA status for each investigational new drug or device.

The study documents have been submitted to Clinical Research Services (CRS) Compliance Office for processing prior to release and protocol implementation. Please contact CRS Compliance for information regarding the protocol implementation release date.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103), including the reporting of Unanticipated Problems and any other Reportable New Information.

Sincerely,
Donald Handley MSc, MBA
Camille P Wicher, PhD, Esq., RN, MSN

Protocol No. I 90206

A Pilot Study of Oral Calcitriol in Patients at High Risk for Lung Cancer

Principal Investigator:
Mary Reid, PhD

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Amendment 15: December 11, 2014
Amendment 16: October 23, 2015
Amendment 17: February 3, 2016
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TABLE OF CONTENTS

ABSTRACT

Title: A Pilot Study of Oral Calcitriol in Patients at High Risk for Lung Cancer

Sponsor: NCI

Phase:

Primary Objectives
To determine the toxicity profile of an oral dose of calcitriol 45 mcg every other week (QOW) in patients at high risk of lung cancer.

Overview of the Study Design: The FDA has requested that a toxicity trial of calcitriol be completed in subjects without cancer using a 45 mcg dose at a lower frequency than was proposed in the previously approved Phase II lung cancer prevention trial. The FDA concerns focus on the lack of evidence of the safety of calcitriol in "healthy" patients who do not have cancer. However, the high risk lung cancer cohort at RPCI is comprised of patients with moderate to severe COPD. In order to document both the toxicity profile of calcitriol at this dose and frequency in high risk subject we have designed a Pilot study. This design incorporates a single-arm toxicity study of 20 patients.

In the proposed "Pilot single-armed pilot study" of 45 mcg of calcitriol, PO, QOW, dosing will continue for 3-months with a defined toxicity monitoring schedule outlined below. A total of 40 patients will be recruited from the High Risk Lung Cancer Screening Clinic and once screened and consented; they will be assigned to calcitriol (n=40). Once 20 patients on calcitriol have completed the 3-month treatment, an interim analysis of adverse events will be performed. Specific criteria for early stopping are outlined below. If the DSMB review of the interim analysis supports proceeding, the remaining 20 patients will be recruited into the trial. A follow-up bronchoscopy will be performed on all subjects after the end of the intervention period according to their routine follow-up schedule.

Eligibility Criteria:

Inclusion Criteria
1. Must have pathologically confirmed squamous metaplasia or squamous dysplasia documented by autofluorescence bronchoscopy within the preceding 60 months.
2. Must be a former or current smoker.
3. Must be between the ages of 40-79 years.
4. Participants must have a total granulocyte count of > 1.5 x 10^9/L, and a platelet count of > 100x10^9/L.
5. Participants must have adequate renal function with a calculated creatinine clearance of >60ml/min with a serum specimen collection at baseline using the Cockcroft-Gault formula: $$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$
6. Participants must have a 24-hour calcium concentration that is ≤ 300 mg/24 hours as measured in a 24-hour urine collection at baseline.

7. Participants must have a total bilirubin less than the upper limit of normal and transaminases/alkaline phosphatase ≤ 2.5 x IULN.
8. Participants must have an albumin of ≥ 2.5 g/dl.
9. Participants must have an ionized serum calcium within normal limits.
10. Must meet ECOG performance status criteria of 0-1 (0 = fully active, must be able to carry out all pre-disease activities without restriction; 1 = restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature).
11. Must be willing to attend all scheduled study visits, complete all study questionnaires, and allow biological specimen collection including a bronchoscopy within 3-4 months after enrollment into the study
12. Women of child-bearing potential (i.e. women who are pre-menopausal or not surgically sterile) must use acceptable contraceptive methods (abstinence, intrauterine device (IUD), oral contraceptives or double barrier device) and must have a negative serum or urine pregnancy test within 1 week prior to beginning treatment on this trial. Sexually active men must also use acceptable contraceptive methods. Pregnant or nursing patients are excluded from participating in this trial. Contraceptive use needs to be continued at least 1 month after the trial has ended.
13. Must be able and willing to sign an informed consent approved by the Institutional Review Board (IRB).

Exclusion Criteria

1. Subjects with life-threatening medical conditions that would preclude bronchoscopy, including: acute cardiac failure, which is unstable despite medication use; uncontrolled hypertension; uncontrolled diabetes mellitus; or unstable coronary artery disease.
2. Patients with severe metabolic disorders that would preclude administration of calcitriol.
3. Evidence of current disease with lung cancer or head and neck cancer.
4. Patients may have a prior history of lung cancer or head and neck cancer treated with curative intent, provided that there has been no evidence of disease (NED) for > 1 year. The qualifying AF bronchoscopy must be negative for malignancy.
5. Patients with a history of any other malignancy within 3 years except non-melanoma skin cancer or cervical CIS.
6. Patients with a history of renal lithiasis within the last 5 years or patients with evidence of kidney stones on entry evaluation.
7. Patients with impaired renal function CRCL ≤ 60 mL/min.
8. Patients with hypercalcemia (using ionized calcium).
9. Subjects taking calcium supplements. If subjects are willing to discontinue these supplements, there must be a two-month wash out period before enrollment.
10. If patients are routinely taking a multivitamin supplement, they will be asked to continue the supplement as long as the amount of Calcium and vitamin D in the supplement is not in excess of the RDA (recommended daily allowance). If they are not taking a multivitamin supplement, they will be asked to not start supplementation while on study.
11. Subjects with a known hypersensitivity to calcitriol.
12. Subjects taking thiazides (which can decrease urinary excretion of calcium)
13. Patients taking phenobarbital, digitalis, thiazides or ketoconazole.

14. Patients taking digoxin or patients who are susceptible to calcium-related dysrhythmias.
15. Patients taking bile acid binding drugs (such as cholestyramine and colestipol).
16. Patients taking Danazol.
17. Patients taking aluminum-based antacids.
18. Oral ketoconazole or other azole antifungals.
19. Women who are pregnant or lactating are excluded from the study.
20. No known allergies to tree nuts (i.e. almonds).

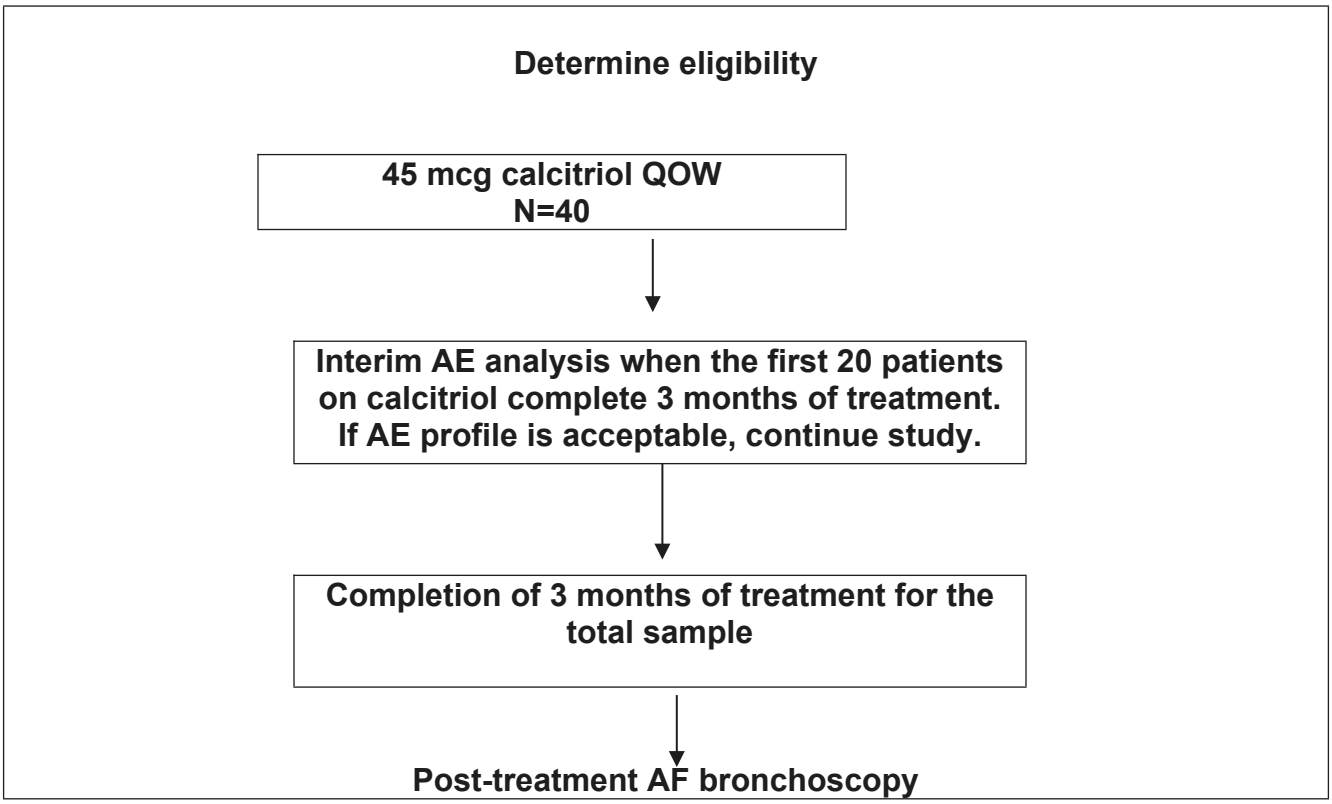
Target Study/Duration: A total of 40 patients will be recruited. The intervention is planned for 3 months and the total duration of the study is expected to last 11 years.

Patient Population: Male or female patients at least 40 years old and younger than 80 years old, former and current smokers, who on a previous bronchoscopy had at least on metaplasia or dysplasia diagnosed, who do not have lung cancer and who are eligible for a second bronchoscopy.

Primary Endpoints:
To determine the number of patients with Grade III-IV toxicities or any Grade II toxicities *that last more than two weeks* associated with a 45 mcg dose of calcitriol administered every other week (QOW) in this population of patients at high risk of lung cancer.

Brief Statistical Section:
We will test the null hypothesis that the proportion of grade 3-4 toxicities and grade 2 toxicities lasting more than 2 weeks will be 10% versus the one-sided alternative that the proportion of grade 3-4 toxicities will be greater than 10%. Given a sample size of n=40, assuming an alpha level 0.05, we will have 80% power to detect the proportion of toxicities of 15% or larger. An interim analysis will be performed after the first 20 subjects on calcitriol have completed the study. The study can be stopped early if the requirements of the null hypothesis (that this dose of calcitriol dose not result in an excess of DTL reports) are met that <= 1 toxicity is observed. After a full review of the data is completed by the DSMB the results will be reported to the FDA. Otherwise, the remaining 20 subjects on calcitriol will be recruited and at treated the end of the study period (3 months), at which point we will either reject the null hypothesis (if 8 or more toxicities are observed) or not reject the null hypothesis if 7 or less toxicities are observed.

STUDY SCHEMA



1. BACKGROUND

The primary aim of this proposal is to establish the safety of calcitriol in this population of high risk lung cancer patients, who do not have cancer. With the safety data generated from this trial, these investigators will be able to proceed with a double-blind placebo controlled clinical trial of 45 mcg of calcitriol QW, as previously approved.

Occurrence of Lung Cancer: Lung cancer is the leading cause of cancer death in the United States, and is a massive public health problem worldwide. It is estimated that 1.2 million people are diagnosed with lung cancer on an annual basis (1). Although there is clinical evidence that the survival of lung cancer dramatically improves with the diagnosis of disease at earlier stages (2-5) and the staging system for lung cancer is based on this relationship (6), the overwhelming majority of lung cancer patients have advanced stage disease at the time of diagnosis. The 5-year survival for lung cancer is only 15%, despite the integration of new chemotherapy agents into surgical and radiotherapy treatment (1).

Target Population for Lung Cancer The increase in lung cancer since 1923 is directly related to cigarette smoking (7). At present, approximately 25% of the US adult population smokes, and 40-50 million Americans are former smokers. Although reduction of cigarette smoking will be helpful to reduce lung cancer incidence, the risk of developing lung cancer for former smokers does not decay for decades after smoking cessation (8, 9). Smokers who develop chronic obstructive lung disease (COPD) have additional risk for the development of lung cancer (10-12). The relative risk of lung cancer in individuals with high levels of exposure to asbestos is 15:1 and the observed risk for those with both tobacco and asbestos exposure is dramatically higher (13). Prior aerodigestive tract cancers may also be thought of as risk factor for the development of a second primary lung cancer (14-16). Patients who are current or former smokers with multiple risk factors including COPD, asbestos exposure, or prior aerodigestive tract cancer are at high risk for lung cancer, and are the most likely to benefit from early detection strategies,(17) and ultimately from chemoprevention interventions (18).

Chemoprevention Trials in Lung Cancer: Primary chemoprevention trials have examined the use of vitamin E and vitamin A (19, 20), which failed to demonstrate benefit in the prevention of lung cancer in smokers. *Tertiary chemoprevention* trials are aimed at the prevention of second primary cancers in individuals who have been previously treated for cancer showed that 13-cis-retinoic acid (isotretinoin) seemed to prevent second primary tumors in resected head and neck cancer patients (21) and retinol palmitate reduced second primary tumors in patients with resected lung cancer. (22). A large randomized follow up study by EUROSCAN failed to confirm benefit from retinol palmitate in the prevention of second primary lung cancers (23). In a subsequent NCI multicenter Phase III placebo-controlled, randomized trial of isotretinoin, the rate of second primary tumors, recurrences, and mortality were not reduced in 1166 patients with Non-Small Cell Lung Cancer (NSCLC)(24). Furthermore, secondary multivariate and subset analyses suggested that isotretinoin was in fact harmful in current smokers and beneficial only in never smokers. Primary and tertiary chemoprevention trials are typically large randomized phase III efforts that require expenditure of considerable resources, and it is reasonable to propose that candidate chemoprevention agents first show activity on a smaller scale. Lee conducted a secondary chemoprevention trial in 152 smokers with biopsy-confirmed metaplasia or dysplasia of the lung epithelium, and found that isotretinoin (13-cRA) (I

mg/kg) did not cause regression of the lesion when compared to placebo (25). A summary of secondary chemoprevention trials for lung cancer is shown in Table 1.

In a more recent study from the same institution (26) 9-cis-retinoic acid (RA) was compared with 13-cRA plus α-tocopherol, or placebo. This group of 226 former smokers were randomly assigned to treatment groups and re-evaluated in 3 months. Six biopsies from predetermined sites were evaluated. The results showed that while neither treatment affected that histology of the sites, 9-cis-RA did restore the RARβ (retinoic acid receptor) in a significant number of lesions. Another generic difficulty with bronchoscopy-based secondary prevention trials is the possibility that the endobronchial biopsy itself may alter the natural history of metaplasia, or even be “curative” of small areas of dysplasia. Biomarkers are keenly sought as surrogate endpoints for chemoprevention trials (27). On the other hand, it is problematic to find agents that activate biomarkers but fail to reverse intraepithelial premalignant lesions (28, 29). For this reason, it is critical to evaluate biomarkers taken from targeted areas of abnormal epithelial proliferation, as detected by AF bronchoscopy (30).

Table 1: Secondary Chemoprevention Trials in Lung Cancer

Author	Endpoint	Method	Compound	Result	N
Heimburger (31)	Metaplasia	Sputum	Folate plus B12	Negative	73
Lee (25)	Dysplasia/metaplasia index	WL bronchoscopy	Isotretinoin	Negative	152
Kurie (28)	Metaplasia and Dysplasia	WL bronchoscopy	4HP retinamide	Negative *	139
Lam (32)	Dysplasia grade	AF bronchoscopy	ADT	Positive **	112
Kurie (26)	Metaplasia and RAR beta expression	WL bronchoscopy	9-Cis retinoic acid	Positive	226
Arnold (33)	Dysplasia	Sputum	Etretinate	Negative	150
Van Poppel (34)	Metaplasia	Sputum	Beta carotene	Negative ***	150
McLarty (35)	Dysplasia	Sputum	Beta carotene	Negative	755
Lam (36)	Dysplasia and MI	AF bronchoscopy	Retinol	Negative	81
Kohlhaufl (37)	Metaplasia and dysplasia	AF bronchoscopy	Inhaled retinol	Positive	11
Ayoub (38, 39)	RAR beta	WL bronchoscopy	13 cis-retinoic acid	Positive ****	44
Lam (37)	Dysplasia	AF bronchoscopy	Budesonide	Negative	112

* Although 4HPR did not reverse bronchial epithelial histology, it did modulate expression of hTERT (29)
** ADT (anethole dithiolethione) did not affect nuclear morphometry index (MI)
*** Only 11% of subjects had metaplasia
**** Effect on dysplasia and metaplasia not reported

Vitamin D (Calcitriol) Characteristics and Metabolism: Vitamin D is a steroid hormone, which modulates calcium homeostasis through actions on kidney, bone and the intestinal tract (40). Vitamin D is synthesized in the skin from 7-dehydro-cholesterol in response to ultraviolet light, is 25-hydroxylated to 25-hydroxycholecalciferol in the liver and 1-hydroxylated to the *active* form, 1,25-dihydroxycholecalciferol (calcitriol) in the kidney (40). In addition to classical effects on bone and mineral metabolism, calcitriol is also involved in the proliferation and differentiation of a variety of different cell types and tissues (41, 42). Physiologic daily production is normally 0.5 to 1.0 mcg and is usually higher during periods of active bone synthesis (40).

Vitamin D as a Chemopreventive Agent: Calcitriol is also a potent anti-proliferative agent in a wide variety of malignant cell types. Calcitriol and other vitamin D analogues have clear chemopreventive and pro-differentiative effects in prostate, breast and leukemic cancer models (43-45) as well as lung cancer cell systems (46, 47). It is well documented that calcitriol is anti-proliferative to numerous tumor cell types, including lung cancer cell lines by our group and others (see references in background of project 2). Tumor cells, especially epithelial tumor types express significant levels of the vitamin D receptor (VDR) and even at relatively low doses of calcitriol (both in vitro and in vivo), cells undergo apoptosis and significant cell cycle arrest. The actions of calcitriol formulations, and not specific to the form used in this trial may be mediated by non-genomic mechanisms (48). Like the retinoids, the actions of vitamin D analogues in general are mediated, in part, through a nuclear receptor, and the vitamin D receptor (VDR) is commonly expressed in lung cancer cell lines (49-52). In the lung, Vitamin D contributes to genomic stability, and also stimulates DNA synthesis in adult alveolar II cells and provides a novel mechanism of modulation of epithelial cell proliferation in the context of lung development and repair against injury (48).

Studies highlight 2 pathways that are important in the metabolism of calcitriol. The first pathway involves the 24-hydroxylase as the first step in catabolism of calcitriol. This enzyme is present in the kidneys as well as in other target organs that express VDR such as the intestine. The second pathway involves the conversion of calcitriol via the stepwise hydroxylation of carbon-26 and carbon-23, and cyclization to yield 1α, 25R- $(OH)_2$-26, 23S-lactone D3. The lactone form is the major circulating metabolite in humans. Enterohepatic recycling and biliary excretion of calcitriol occurs. The metabolites of calcitriol are excreted primarily in feces. After a 1 mcg oral dose of radiolabeled calcitriol was given, 10% of the radiolabeled dose appeared in urine with 24 hours. No data is available on the pharmacokinetics of calcitriol in patients with hepatic dysfunction. The elimination half-life in patients with chronic renal failure increased by at least 2 fold compared to patients with normal renal function.

2. PRELIMINARY DATA

In this section we will describe our work with calcitriol as a potential drug for chemoprevention, including the rationale for the proposed dosage schedule.

Studies with Calcitriol: Our research group has extensive experience in the development of vitamin D-based approaches to cancer therapy. We have recently initiated trials in early prostate cancer with an eye toward chemoprevention and early disease therapy

applications. By employing an intermittent dosing schedule we have been able to administer extremely high doses of calcitriol without dose-limiting toxicity.

Calcitriol and Cancer Biomarkers: The antiproliferative activity of calcitriol in squamous cell carcinoma lines (SCC) is associated with decreased p21 expression in vitro and in vivo (53). Recent studies indicate that p21 suppression increases sensitivity to paclitaxel (54, 55). We have examined the molecular accompaniments of paclitaxel cytotoxicity with and without calcitriol in the human prostatic adenocarcinoma, PC-3. The *in vitro* effects of calcitriol and paclitaxel on p21, Bcl-2, caspase-3, and poly (ADP-ribose) polymerase (PARP) in PC-3 were evaluated by Western blot. Treatment *in vitro* with calcitriol resulted in a decrease in p21 expression in PC-3. Paclitaxel induced apoptosis in PC-3 as evidenced by the time-dependent loss of procaspase-3 and full-length PARP. Paclitaxel caused increase of p21 and loss of Bcl-2. An increase was observed in PARP cleavage in PC-3 treated with calcitriol/paclitaxel as compared to paclitaxel alone (53).

We found that levels of the pro-survival molecule phospho-Akt were reduced by the combination of calcitriol and paclitaxel to a greater extent than either agent alone (46). However, expression of the pro-apoptotic signaling molecule MEKK-1, while being little affected by paclitaxel, was modestly up-regulated by calcitriol alone, and much further up-regulated by the combination. The synergistic increase in MEKK-1 expression for the combination was accompanied by the generation of a slightly faster migrating MEKK-1 species (to form a doublet), as well as multiple MEKK-1 species of lower molecular weight. These species of MEKK-1 appear to result from N-terminal proteolysis and may represent constitutively active forms of MEKK-1 (46).

Calcitriol can modulate the expression of the EGFR (56) and also increases EGF binding. We have observed that treatment of SCC cells with calcitriol leads to an increase in EGFR protein levels, (57) particularly in the population of cells that remain adherent to the tissue culture flask. The up regulation of EGFR protein correlates with a recent report indicating that calcitriol inhibits the degradation of EGFR mRNA (44). In addition, while the levels of p-ERK1/2 and p-Akt, two downstream targets of EGFR signaling, are markedly reduced in the subset of cells that detach from the tissue culture plate and are apoptotic after treatment with calcitriol; these effects are much less pronounced in the cells that remain adherent (45).

The cdk inhibitors p21Waf1/Cip1 and p27Kip are implicated in G_1 phase arrest (58). In HL-60 cells, a human myelomonocytic leukemia cell line, calcitriol arrests cells in G_1; this effect is mediated through an increase in p27 (59). Calcitriol mediated arrest in G_0/G_1 is also observed in human breast cancer lines (45). Calcitriol induces apoptosis in MCF-7 breast cancer cells (55) as well as in HL-60 leukemic cells (42) and the expression of Bcl-2 is down-regulated by calcitriol in HL-60, and in retinoblastoma cells (60).

Animal studies with calcitriol: In multiple model systems (murine/human SCC, metastatic Dunning rat prostate adenocarcinoma (MLL) and the human xenograft PC-3/LnCAP prostate, MV522 lung, Capan-1 pancreatic), calcitriol has significant antiproliferative effects in vitro and in vivo (53, 61-63).

We have shown that an increase in vivo clonogenic cell kill actually results in a decrease in the fractional tumor in a murine model of squamous cell carcinoma. Using a direct measurement of clonogenic cell kill, we have been able to show that the administration of Vitamin D_3 for 3 days in tumor bearing mice resulted in a significant increase in cell kill, which was enhanced by dexamethasone (64). We have also shown similar antitumor activity with Vitamin D_3 analogues, Ro23-7553, and Ro25-6760 in both

murine squamous cell carcinoma, and also in the rat Dunning adenocarcinoma prostate model system (61). When Ro23-7553 is given intraperitoneally (IP) to C3H/HeJ mice bearing squamous cell carcinoma, there is a modest decrease in the surviving fraction of tumor cells, and an increase in the delay of regrowth of the tumor cells, and this antitumor effect is enhanced by cisplatin (65). We have found that even a single dose of D_3 was enough to slow tumor growth significantly in the murine model. When these animals received a daily dose of D_3 for 3 days, even further decreases in fractional tumor volume are observed, when compared with saline control injections (53)

In the prostatic adenocarcinoma cell line, PC-3, in nude mice, neither paclitaxel nor Vitamin D_3 does appear to have independent anti-tumor activity. When given together for prostate adenocarcinoma, the regimen did result in a decreased rate of tumor growth, even in previously treated tumors. In the murine squamous cell carcinoma model, the single-agent anti-tumor activity of D_3 was not only confirmed when compared with saline injections, but also appeared to be synergistic with paclitaxel (63).

The mechanism of antitumor activity may be independent of the Vitamin D receptor. When VDR knockout mice with high endogenous levels of D_3 are transfected with the Lewis lung carcinoma gene, there is a significant inhibition of metastatic growth of lung cancer cells (66). In the metastatic rat Mat-LyLu (MLL) model, calcitriol not only causes inhibition of tumor growth, but significant reduction in the number and size of lung metastasis (62). These findings suggest that D_3 may work as an intrinsic factor for lung cancer prevention in intact animals. Promotion of tumor growth by calcitriol has not been observed in animal models.

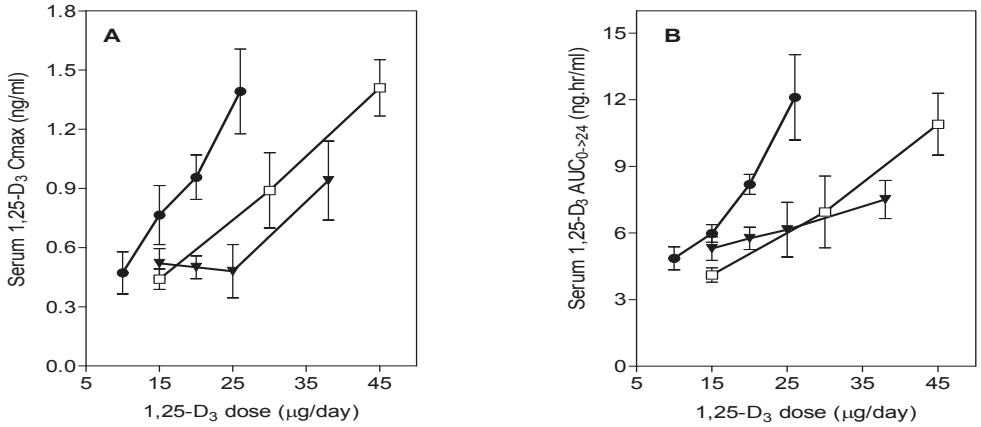
Clinical Trials with calcitriol: The early clinical trials of calcitriol were in leukemia and myelodysplasia and utilized low dose calcitriol QD or QOD. Although some evidence of response was seen, the results were largely disappointing due to hypercalcemia (67-69). Calcitriol causes hypercalcemia by increasing intestinal calcium absorption and mobilizing bone stores (70). Schedule and administration of calcitriol appear to impact in the ability to give larger doses of drug. While substantial dose escalation is possible without toxicity, the oral route of administration is complicated by concerns regarding inter-patient variability and apparent saturability of oral absorption. Since 1999, we initiated six clinical trials and completed five with one ongoing as detailed below. The first trial was a phase I study to evaluate the pharmacokinetics and MTD of calcitriol following subcutaneous (sc) QOD administration (71). Thirty-six patients were entered at doses ranging from 2 μg to 10μg QOD; dose-limiting toxicity (hypercalcemia) occurred in 3 of 3 patients entered at the 10 μg QOD dose. Hypercalcuria occurred at all dose levels examined. No other toxicity was seen. Serum calcitriol levels by radioimmunoassay revealed a decrease in concentration-time curves on the seventh day compared to the first day of therapy. A dose dependent increase in peak serum level and estimated area under the curve (AUC) were seen; the maximum serum levels occurred at the 10 μg QOD dose: 288 ± 74 pg/mL and 321 ± 36 pg/mL days 1 and 7 respectively. The normal range of calcitriol serum concentrations using this assay is 16-56 pg/mL. Serum calcitriol levels were maintained at near peak concentrations for at least 8 hr following sc. injection. Other studies have evaluated oral calcitriol pulse dosing once weekly. In a phase I dose escalation of weekly oral calcitriol, no dose limiting toxicities were noted (72). Dose escalation was halted at the 2.8 mcg/kg level. No increases in serum calcitriol concentrations were noted at dose levels exceeding 0.48mcg/kg suggesting saturation of vitamin D gastrointestinal absorption. This phase I trial demonstrated that patients could tolerate weekly oral dosing of calcitriol at 0.5μg/kg or 40μg per 70kg patient without significant toxicity (72).

In another trial, (73) patients with advanced cancer were treated with paclitaxel (80μg/m2 weekly x 6) + escalating doses of calcitriol, once daily for three days per week (TIW) for 6 weeks. The starting dose of calcitriol was 4μg orally TIW, and we have entered patients through the 38μg dose level where it appears that we have reached saturable concentrations at 16 -20μg. No limiting toxicity has been encountered. The study design called for administration of paclitaxel on day 1 cycle 1 of therapy prior to any calcitriol therapy and on day 3 with the third dose of calcitriol week two and all-subsequent weeks. This permitted the evaluation of the effect of calcitriol on paclitaxel pharmacokinetics – week 1 versus week 2. No changes in peak concentration, AUC or T 1/2 have been noted.

Calcitriol Pharmacokinetics: Pharmacokinetic (PK) studies were required in at least 2 of 3 patients at each dose level of the calcitriol/paclitaxel clinical trial and were performed in 26 of the 36 patients; six patients at the highest dose level (38 μg) underwent PK studies (74). Baseline plasma calcitriol concentrations of the 26 cancer patients resulted in a median concentration of 26 pg/ml (range 13-81). The normal range for this assay is 16-74pg/ml. Serum calcitriol concentrations higher than baseline occurred within an hour of oral calcitriol administration. A scatter plot of the maximum concentration of calcitriol (Cmax) for each patient studied at each dose level is portrayed in Figure 1.

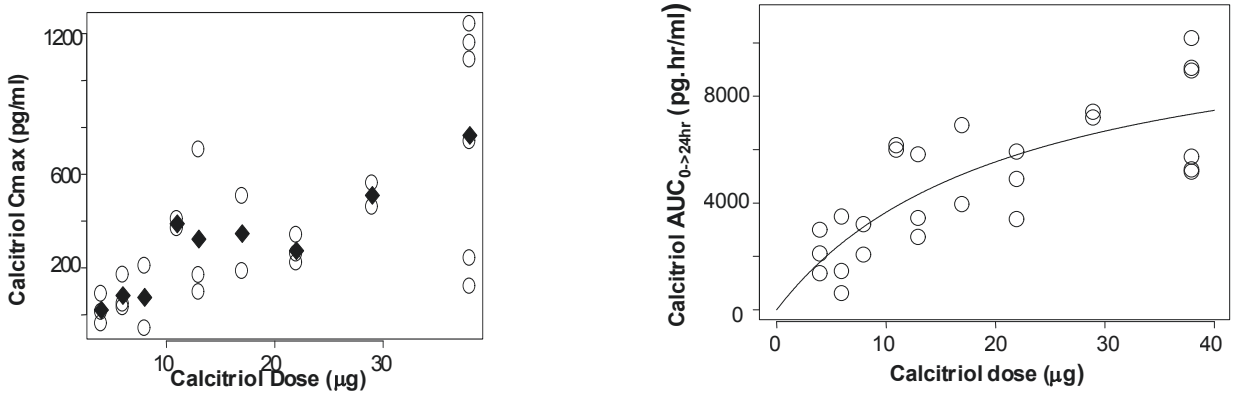
As shown in Figure 2, baseline-subtracted serum calcitriol AUC0->24hr (area under the concentration-time curve for the 24 hour period after calcitriol administration) is plotted against dose. A fit to the Michaelis Menten function (AUC = a×dose/ (1 + b×dose) indicates that AUC0->24hr is not proportional to dose (a = 540 ± 140 pg-hr/ml-μg; if AUC were proportional to dose, b would equal 0). A statistical test for proportionality gives a p-value of 0.0014.

Figures 1 and 2: PK of the oral administration of calcitriol (1) Scatter plot of the maximum serum calcitriol concentration (Cmax) vs. calcitriol doses. Closed symbols represent mean values at each dose level. (2) Baseline-subtracted serum calcitriol AUC0->24hr (area under the concentration-time curve for the 24 hour period after calcitriol



administration) plotted against dose, and a fit of the Michaelis-Menten function.

Figures 3 and 4: Pharmacokinetic (PK) parameters (mean ± SD), Cmax (3) and AUC (4) of calcitriol in patients with advanced cancer treated with IV calcitriol (●), DN101 (□) or oral calcitriol (▼).



The effect of the nonlinearity over the range of doses studies is large: the fit value of $AUC_{0->24hr}$ at 38 μg was only 4 times that at 4 μg, instead of the 9.5 times expected for a proportional relationship. However, no deviation from linearity can be detected up to a dose of 17 μg (p=0.4). In addition, there is insufficient evidence for an association between serum calcium and dose. No patient became hypercalcemic. We initiated a phase I clinical trial of intravenous (IV) calcitriol in combination with the tyrosine kinase inhibitor, gefitinib (Iressa) where patients with advanced cancer are treated in the first week with a single dose of IV calcitriol, in the second week gefitinib alone and subsequent weeks the

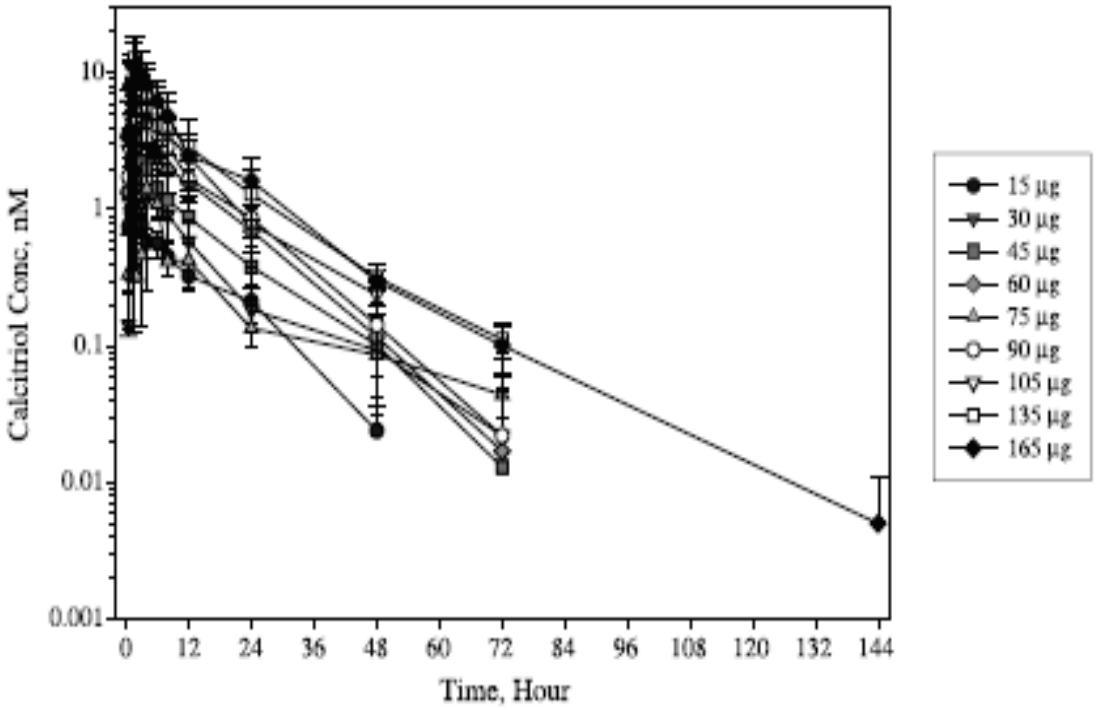
combination of the 2 agents. The dose of calcitriol started at 10μg and was escalated (10, 15, 20, 26,35,47,63 μg) to toxicity (hypercalcemia) with PK performed at each dose level. This study is currently at the 34μg and continues to accrue. To assess the calcitriol bioavailability issue and to determine PK differences with regard to dose and route of administration, we compared the PK data from our oral calcitriol phase I studies, as described above, with the DN101 (an oral reformulation of calcitriol, a product of Novacea) phase I trial (75) and our current ongoing phase I trial using IV calcitriol. As shown in Figures 3 and 4, both Cmax and AUC increased in a dose-dependent manner using both intravenous (IV) calcitriol and DN101 with IV calcitriol resulting in a higher Cmax and AUC at each dose level tested. The administration of oral calcitriol did not increase proportionally at equivalent doses when compared to either IV calcitriol or DN101. Key to clinical trial design is relating serum calcitriol levels in animals that resulted in a significant antitumor effect to human serum levels. In both mice and rats, the AUC for calcitriol from both IV and intraperitoneal (IP) routes of administration was not significantly different with IV resulting in a higher Cmax as compared to IP (76). Peak levels are reached at 1 hr and return to normal at 24 hr following 0.125 and 0.5 μg of calcitriol. Both 0.125 and 0.5 μg when administered to tumor-bearing mice, result in a significant anti-tumor response. No diurnal variation was observed in serum calcitriol levels during the 24 hr sampling period. A similar pattern was obtained for PK parameters of calcitriol in tumor-bearing mice (data not shown). To determine whether the mouse serum calcitriol levels that resulted in an anti-tumor effect could ever be achieved in man, we compared AUC and Cmax levels obtained in the calcitriol/paclitaxel Phase I clinical trial where we have administered 38 μg daily x 3 without toxicity. As shown in Table 3, at 0.125 μg (the lowest dose to consistently result in a significant antitumor effect in mice), the AUC was 37.3 ng/hr/ml and this compares in man at 38 μg to 7.5 ng/hr/ml. Similarly, in mice the Cmax was 9.2 ng/ml and this compared to 1.4 ng/ml in man. At the 0.042 μg dose in mice, an antitumor effect could be seen but was not consistently observed. Therefore, effective serum calcitriol levels are 5-7 times higher in mice than compared to highest oral dose administered in man (38 μg). As described above, this dose, by this route of administration may not result in higher serum calcitriol levels due to decreased bioavailability.

Table 3: Calcitriol Pharmacokinetic Parameters Mice and Man

Mouse (IP)			Man (PO)		
Dose (μg)	AUC (0->24) (ng/hr/ml)	Cmax (ng/ml)	Dose (μg)	AUC (0->24) (ng/hr/ml)	Cmax (ng/ml)
0.042	3.6	0.7	13	3.9 ± 1.4	0.5 ± 0.3
0.125	37.3	9.2	17	5.4±2.1	0.5±2.2
0.5	123.9	43.4	38	7.5±2.1	1.4 ± 0.9

The toxicity from chronic administration of calcitriol 45 mcg PO QW is unknown in a healthy cancer-free population, but it is the dose that has been well-tolerated with little toxicity in other trials. Single dose of calcitriol as DN-101 in healthy volunteers and repeat dosing DN-101 in cancer patients has been well tolerated (77). In the single dose study in patients with advanced malignancies, hyponatremia was observed in 32%. In the same study, when calcitriol was given weekly (78), hyponatremia was only observed in patients receiving docetaxel and was not observed in patients receiving docetaxel with calcitriol (77). The following figure shows the mean calcitriol concentration-time profile following the

administration of a single dose of DN-101. A dose of 45 mcg PO QW was also well-tolerated in a phase III trial for metastatic androgen-independent prostate cancer in a clinical trial (79).



Conclusions regarding calcitriol studies: Calcitriol regulates the transcription of a large number of target genes via its interactions with the vitamin D receptor, and exhibits modulation of many targets including Bcl-2, EGFR, and AKT. Calcitriol exhibits antiproliferative activity in a broad range of tumors, including lung cancer cell lines. Our animal data for lung cancer is based on squamous cell carcinoma models (rather than adenocarcinoma models), and provides a rationale for using calcitriol in the chemoprevention of central squamous-based premalignancy. Oral calcitriol given QD 3X weekly at the 16mcg level provides tissue saturation and has virtually no toxicity. Calcitriol given as 45mcg QW has been well tolerated in cancer patients but the toxicity profile in non-cancer patients must be documented.

Lung Cancer: Early Detection Research: Our research group has extensive with the early detection of lung cancer. We have developed a lung cancer screening clinic for high risk patients, and have implemented an ongoing clinical trial, which utilizes autofluorescence bronchoscopy and low-dose spiral CT of the chest for early detection of lung cancer. We prospectively follow a cohort of high risk patients with premalignant epithelial abnormalities with an eye toward chemoprevention and early disease therapy applications.

Overview: The High Risk Lung Cancer Cohort at RPCI: The lung cancer-screening program at RPCI was established in 1998. This screening program incorporates an epidemiologic questionnaire, physical examination, and chest x-ray, low-dose spiral CT of the chest without contrast, induced sputum, and autofluorescence (AF) bronchoscopy with white light (WL) bronchoscopy. High risk patients have been recruited from several sources: 1) asbestos litigation firms refer asbestos clients with radiographically confirmed asbestosis

for medico-legal evaluation at RPCI. Many of these individuals are smokers with a very high risk of lung cancer 2) patients with surgically treated aerodigestive tract cancers who are disease-free are referred by the department of surgery at RPCI for evaluation in the lung cancer screening program, and 3) Community patients with moderate or severe COPD and are referred by pulmonologists and primary care physicians in the community for evaluation in the lung cancer screening program. The outreach effort associated with this prevention and screening program has included public speaking, press releases, media interviews and the production of a color brochure that is distributed to local pulmonary and oncology offices in the community. Of the high-risk patients who have undergone AF bronchoscopy at RPCI, at least 58% have been identified with bronchial epithelial metaplasia or dysplasia. These individuals are followed in the lung cancer-screening clinic with serial AF bronchoscopy. Based on our experience, there are sufficient numbers of patients currently in the cohort to meet the recruitment needs of this pilot study.

3. METHODS

1. STUDY PURPOSES AND OBJECTIVES

The **Primary Hypothesis** for this study is that high-dose calcitriol 45 mcg. QOW will be well tolerated in a group of patients at high risk of lung cancer.

The **Primary Objectives** for this trial are as follows:

1. To establish the safety of a 45 mcg dose of oral calcitriol QOW in non-cancer patients. Forty patients from the RPCI high risk lung cancer cohort will be recruited to 45 mcg of oral calcitriol QOW (n=40) for a period of 3 months. All adverse events and serious adverse events will be reported to the FDA at the end of the study, regardless of relationship to the expected side effects of calcitriol. Patients will be on the study for 3 months. When the first 20 patients on calcitriol have completed the 3-month treatment, an interim analysis will be performed to determine the degree of dose-limiting toxicities associated with every other week administration.

4.0 ELIGIBILITY/EXCLUSION CRITERIA

Inclusion Criteria

1. Must have pathologically confirmed squamous metaplasia or squamous dysplasia documented by autofluorescence bronchoscopy within the preceding 60 months.
2. Must be a former or current smoker.
3. Must be between the ages of 40-79 years.
4. Participants must have a total granulocyte count of > 1.5x 10^9 /L, and a platelet count of > 100 x 10^9 /L
5. Participants must have adequate renal function with a calculated creatinine clearance of >60ml/min from a serum specimen collection at baseline using the Cockcroft-Gault formula:

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

6. Participants must have a 24-hour calcium concentration that is ≤ 300mg/ 24 hours as measured by 24-hour urine collection at baseline.
7. Participants must have a total bilirubin less than the upper limit of normal and transaminases/alkaline phosphatase ≤ 2.5 x IULN.
8. Participants must have an albumin of ≥ 2.5 g/dl.
9. Participants must have an ionized serum calcium within normal limits.
10. Must meet ECOG performance status criteria of 0-1 (0 = fully active, must be able to carry out all pre-disease activities without restriction; 1 = restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature).
11. Must be willing to attend all scheduled study visits, complete all study questionnaires, and allow biological specimen collection including a bronchoscopy within 3-4 months after enrollment into the study
12. Women of child-bearing potential (i.e. women who are pre-menopausal or not surgically sterile) must use acceptable contraceptive methods (abstinence, intrauterine device (IUD), oral contraceptives or double barrier device) and must have a negative serum or urine pregnancy test within 1 week prior to beginning treatment on this trial. Sexually active men must also use acceptable contraceptive methods. Pregnant or nursing patients are excluded from participating in this trial. Contraceptive use needs to be continued at least 1 month after the trial has ended.
13. Must be able and willing to sign an informed consent approved by the Institutional Review Board (IRB).

Exclusion Criteria

1. Subjects with life-threatening medical conditions that would preclude bronchoscopy, including: acute cardiac failure, which is unstable despite medication use; uncontrolled hypertension; uncontrolled diabetes mellitus; or unstable coronary artery disease.
2. Patients with severe metabolic disorders that would preclude administration of calcitriol.
3. Evidence of current disease with lung cancer or head and neck cancer.
4. Patients may have a prior history of lung cancer or head and neck cancer treated with curative intent, provided that there has been no evidence of disease (NED) for > 1 year. The qualifying AF bronchoscopy must be negative for malignancy.
5. Patient s with a history of any other malignancy within 3 years except non-melanoma skin and cervical CIS.
6. Patients with a history of renal lithiasis within the last 5 years or patients with evidence of kidney stones on entry evaluation.
7. Patients with impaired renal function CRCL ≤ 60 mL/min.
8. Patients with hypercalcemia (using ionized calcium).
9. Subjects taking calcium supplements. If subjects are willing to discontinue these supplements, there must be a 2-month wash out period before enrollment.
10. If patients are routinely taking a multivitamin supplement, they will be asked to continue the supplement as long as the amount of vitamin D in the supplement is not in excess of the RDA (recommended daily allowance). If they are not taking a multivitamin supplement, they will be asked to not start supplementation while on study.
11. Subjects with a known hypersensitivity to calcitriol.
12. Subjects taking thiazides (which can decrease urinary excretion of calcium).
13. Patients taking phenobarbital, digitalis, thiazides or ketoconazole.

14. Patients taking digoxin or patients who are susceptible to calcium-related dysrhythmias.
15. Patients taking bile acid binding drugs (such as cholestyramine and colestipol).
16. Patients taking danazol.
17. Patients taking aluminum-based antacids.
18. Oral ketoconazole or other azole antifungals.
19. Women who are pregnant or lactating are excluded from the study.
20. No known allergies to tree nuts (i.e. almonds).

Study Flow Chart

		MONTH 1						MONTH 2						MONTH 3						
Procedure	**Screening**	**Day 1**	**Day 2**	**Day 14**	**Day 15**	**Day 16**	**Day 28**	**Day 1**	**Day 2**	**Day 14**	**Day 15**	**Day 16**	**Day 28**	**Day 1**	**Day 2**	**Day 14**	**Day 15**	**Day 16**	**Day 28**	**Follow up (1)**
Eligibility Interview	X																			
Informed Consent	X																			
Complete Physical Exam	X																			
Medical History Update	X																			
Toxicity Evaluation (Nurse)	X	X	X			X							X						X	X
Spirometry and FEV1	X																			
Renal Ultrasound	X																			
24 hour urine collection (2)	X																			
Assessment of Supplement Use	X																			
Chemistry Panel (3) and ionized calcium	X		X			X	X						X						X	X
Hematology Panel (3)	X		X			X	X						X						X	X
25-OH vitamin D	X																		X	
Dose 45 mcg Calcitriol (QOW)		X			X			X			X			X			X			
PK Samples for Calcitriol (4)		X	X																	
AF Bronchoscopy	X																			X

Phone call dose reminder				X			X			X			X			X				
Phone call toxicity check								X	X		X	X		X	X		X	X		
Blood for future studies (5)	X	After 24-48 hours and every 3 months while on treatment if the patient signs the DBBR consent																		

1. Follow up labs will be done only for patients with toxicities at the time of cessation of the treatment.
2. 24 hour urine will be collected for the measurement of creatinine and calcium concentration.
3. **Chemistry Panel:** Chloride, CO2, Potassium , sodium, urea nitrogen, Glucose, Calcium, ionized Serum Calcium, Creatinine, Total Protein, Albumin, Aspartate aminotransferase, Alanine aminotransferase, osmolality serum, Anion gap, BUN/Creatinine ratio, Pregnancy test within 1 wk of enrollment (screening only).
   Hematology: CBC will be monitored every other week in the first month then monthly until the end of the intervention.
   The blood collection will occur on the day after the pills are taken. Vitamin D levels (25-OH-Vitamin D) will be drawn twice.
4. PK/PD sampling: Blood will be taken for PK/PD at the following time points, predose, .5, 1, 3, 5 and 24 (day 2) hours post dose.

5.Blood samples will be drawn before treatment, 24-48 hours following initiation of treatment and every three months during treatment alongside clinical blood draws, and frozen for future correlative studies. Samples will be drawn in phlebotomy and sent to the RPCI Data Bank and Bio Repository for storage per DBBR Standard Operating Procedures for Vitamin D Trial banking participants

5.0 Required Studies and Schedule:

Eligibility Determination: Subjects will be seen at the RPCI screening clinic and their eligibility for the pilot study will be determined. If they agree to participate, they will be asked to sign the IRB approved informed consent. At this time (Month 0) they will have blood, collected, complete the study questionnaires. Vitamin D status will be measured at baseline. A medical history, a complete smoking exposure history as per standard clinic routine, enrollment into the DBBR and vitamin/supplement use information as per EMR medication record will also be completed at this time.

Month 0, Day 1 Subjects will receive their 3-month supply of capsules in a labeled blister pack at this time from the study nurse. At this time the PK/PD studies will be initiated.

Pharmacokinetics:
Pharmacokinetics Sampling Times: PK samples will be collected at pre-dose and at hours 0.5, 1, 3, 5, and post-dose 24 hours on Day 2. Samples of blood (4 ml) will be collected in EDTA tubes centrifuged at 4° C and serum is stored frozen at -80°C until analysis by RIA (see attached protocol for analytical details). Derivation of PK parameters and modeling is described below. Serum Chemistry for 25-D3 levels will be measured at baseline. The measurement of the calcitriol levels will be performed in the PK/PD facility at RPCI. One aliquot of buffy coat (lymphocytes) will be collected from these samples and stored at -80C. These aliquots will be sent to the Prevention Laboratory and stored until requested by Dr. Dhillon for pharmacogenomics analysis.

Blood samples will be made available through DBBR Vitamin D (BIOKIT IV) (RPCI approved protocol I 03103), after the patient has been consented for DBBR. All samples and associated data from DBBR will be stripped of PHI and provided with a new study ID prior to receipt. Per protocol, the DBBR will maintain the key between the participant's PHI and study ID. Only the DBBR staff (Principal Investigator and Data Manager) associated with operating the bank have access to this link. The key will never be given to the investigator of this study.
Blood samples will be collected and stored for future studies by the RPCI Data Bank and Bio Repository (DBBR), per Standard Operating Procedures for vitamin D trial banking. The DBBR is a shared resource (RPCI protocol I 03103), where participants are consented to donate blood for research (prior to and following treatments), to complete an epidemiologic questionnaire, and to give permission to have their blood specimens linked to the questionnaire and clinical data including diagnosis and laboratory test results for research. Collected biospecimens and/or data are provided to investigators with RPCI IRB approved research protocols.

Other samples will be made available through Lung Cancer Screening Program (EDR 11003). The samples and associated data from Lung Cancer Screening Program will be stripped of PHI and provided with a new study ID prior to receipt. Lung Cancer Screening Program will maintain the key between the participant's PHI and study ID. Only the Lung Cancer Screening Program staff (Principal Investigator and Data Manager) associated with operating the bank have access to this link.

Once the molecular analysis is finished, the data from molecular analysis and information from DBBR will be linked to Lung Cancer Screening Program database by staff members in Lung Cancer Screening Program.

Weekly Blood Evaluations: After the first dose on calcitriol is taken on Day 1, and the PK study has been done, the patients will return for blood collection on Day 2 (24 hours after the first dose) and on the same day of the week after the pill is taken (Day 16) for the first month of treatment (encompassing two doses) and then monthly for the remainder of the 3-month study period. The patient will not be seen in the clinic but will go directly to phlebotomy. Blood collection will then be done at the end of Months 2 and 3, when the patient is seen by the clinic nurse for toxicity assessment (Weekly blood evaluations may be collected at participating sites).

Phone Calls for Pill Reminders and Toxicity Monitoring: The CRC will call each patient the day before the pill is due to be taken and on the day after the calcitriol is taken, to ask about all potential AE and SAE reactions. If the patient has started taking the treatment on Monday, the nurse may call the patient on Friday before. During these phone calls, the CRC will also assess compliance to the treatment schedule.

Study Clinical Visits and Closeout Visit (Months 1, 2 and 3): Each month that patient will come into the clinic at RPCI (or participating sites) and have a toxicity evaluation performed by the clinic nurse. At the end of the three month period, patients will return to the clinic at RPCI (or participating site) and have a final blood draw and toxicity evaluation. The blood collected will be immediately processed to yield the samples for calcium. All remaining samples will be archived at –86 degrees for future analyses. At each clinical visit, the clinic nurse will interview subjects for the onset of new illnesses or hospitalizations, changes in smoking, vitamin supplement use, and potential symptoms related to calcitriol toxicity as per standard clinic routine. The bronchoscopy will be done after the cessation of the intervention and according to the patient's routine follow-up schedule.

Procedures for Toxicity Monitoring and Reporting: The study nurse will assess potential toxicities during each phone call. If a subject reports changes in any of these symptoms, they will be documented as an AE and graded according to CTCAE 4.0. If the patient reports an AE of $\geq$ 3 grade, it will be reported to the PI or the treating physician to determine if they need to be evaluated at Roswell Park. The complete toxicity information will be reported to the medical director, Dr. Samjot Dhillon, at the time of continuing review. Reports regarding toxicity, patient safety, and study progress will be submitted to the RPCI Institutional Review Board (IRB), FDA and to the NCI at the end of the study. The risks and side effects that have been reported to be associated with calcitriol include:

Likely Side Effects: those that may occur in approximately 10% - 30% of persons who receive this drug.	• Most of the side effects described with calcitriol are related to elevations of calcium referred to as hypercalcemia (33%). • Side effects related to high calcium can occur which include, kidney and bladder stones, weakness, dehydration, constipation, muscle aches, nausea, vomiting.

Unlikely Side Effects: those that occur in approximately 5% to 9% of persons who receive this drug.	• Cardiac arrhythmia (disorders of the regular rhythmic beating of the heart), high or low blood pressure. • Headache, irritability, seizure (rare), somnolence (sleepiness), psychosis (inability to think clearly). • Pruritus (itching of the skin), erythema (reddening of skin), multiforme (allergic reaction that occurs in response to medications). • Hypermagnesemia (excess magnesium), hyperphosphatemia (high level of phosphate in blood), polydipsia (excessive thirst). • Anorexia, constipation, metallic taste, nausea, pancreatitis, vomiting, xerostomia (dry mouth). • Elevated liver function tests • Bone pain, myalgia (muscle pain), dystrophy (weakness of muscles), soft tissue calcification • Conjunctivitis (pink eye), photophobia (sensitivity to light) • Polyuria (increased urination)
Rare but Serious Side Effects: Those that occur in less than 1% of persons who receive this drug/undergo this procedure.	• Rarely, the calcium levels become too high (rarely) then confusion, irregular heartbeats, inflammation of the pancreas and coma can occur.
Unknown Side Effects: Those for which there is no information.	• There is no information on the effects of active smoking when taking calcitriol.

Toxicity Monitoring: Any grade 3 or worse toxicity regardless of attribution or a grade 2 toxicity that persists for more than 2 weeks, will be reported to the FDA and the patient will be removed from the study. In addition, all Grade 1 and short-term 2 toxicities will be reported to the FDA at the end of the study, regardless of attribution.

Removal from Study: Patients will be removed from study for any of the following reasons:

1. Any grade ≥ 3 related to study drug.
2. A grade 2 that persists for more than two weeks.
3. Interim development of new malignancy (lung or extra pulmonary).
4. Withdrawal of consent.

6. STRATIFICATION AND RANDOMIZATION

This is a single armed study and there will not be any randomization.

7. TREATMENT PLAN

Informed consent: All patients prior to enrollment in this study will sign an Informed Consent in conformity to Federal and Institutional guidelines.

Calcitriol Administration: The subjects will receive 45mcg /QOW of oral calcitriol capsules one day every other week. Subjects will take one capsule QOW for 3 months, for a total of 6 capsules. Subjects will be supplied with 3 months' worth of capsules (6 capsules) dispensed by the UB pharmacy.

When the patient has signed the informed consent, eligibility confirmed they will be scheduled to take their first dose and have PK/PD studies. A fax will be sent to Louise Cooper, RPh at the Suny at Buffalo, Buffalo School of Pharmacy. . This fax will include: A completed prescription for the calcitriol (the original will be sent to Louise Cooper by courier that week) and the date of the patient's next scheduled visit.

Her complete contact information is:

Louise Cooper, RPh, MS
SUNY at Buffalo, Buffalo School of Pharmacy
School of Pharmacy and Pharmaceutical Sciences
242 Kapoor Hall
South Campus
Buffalo NY, 14214
Phone 716-645-4806
Fax 716-829-6094

Upon receipt of the fax, Ms. Cooper will label 6 individual blister packs for the calcitriol capsule 45mcg/cap. The label on each blister pack will include the following information:

SUNY at Buffalo, Buffalo School of Pharmacy
New Drug Investigational use
Calcitriol 45 mcg/capsule
645-4806 RPh LMC Date

The 6 blister packs will be placed in an amber zip-lock bag and labeled with patient specific information. The label on each ziplock bag will include the following information:

SUNY at Buffalo, Buffalo School of Pharmacy
Research Pharmacy
242 Kapoor Hall 716-645-4806 RPh: LMC

Subject's Name Subject # Fill Date
Subject's Address

Take one capsule on an empty stomach (at least one hour before or two hours after meals) and drink approximately 4 cups of liquids in addition to your usual intake in the day of taking the pill and for the next two days as directed by project protocol.

Quantity: 6 blister paks
Calcitriol 45mcg/capsule

In case of an emergency, please contact Roswell Park Cancer Institute (716-845-2300) and ask to speak to the Department of Medicine physician on call.

"Caution: New Drug--Limited by Federal (or United States) law to investigational use."

An auxiliary label “Keep in Refrigerator Do not Freeze” will be placed on the amber ziplock bag.

The labeled calcitriol capsules will be sent by courier to Roswell Park and delivered to the Investigational Drug Service (IDS) where the capsules will be held until the patient returns to the clinic to start the trial. The PK/PD studies will start at time 0 and then the patient will take the first capsule. The PK/PD studies will be carried out as described in following sections.

Calcitriol Formulation and Dispensing: In each size 3 opaque capsule there will be 200 microliters of solution containing:

- 45 mcg Calcitriol
- 0.1% of each BHT and BHA (Concentration PCCA uses in their product 12000 nanograms/ml) in Almond oil as the vehicle.

The blister packs are heat sealed and placed in an amber ziplock bag to protect the capsules from sunlight.

Storage: After the completion of the PK/PD studies on Day 0, the patients take the blister pack home and store it in the refrigerator until two weeks later when they take another capsule.

Stability: Shelf life surveillance studies of formulated capsules are ongoing. Current data indicates calcitriol is stable for at least 5 years at room temperature when protected from light.

Method of Administration: Capsules should be taken once every other week. Subjects should take calcitriol on an empty stomach (at least one hour before or two hours after meals) and drink approximately 4 cups of liquids in addition to their usual intake in the day of taking the pill and for the next two days.

8. Specimen and Data Collection and Processing*:* A complete smoking history will be collected as per the clinic routine.

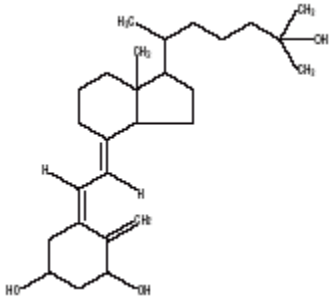
Measurement of Plasma Vitamin D*:* Blood samples for calcitriol determination will be sent to the PK/PD facility at RPCI for analysis. Patients will be coming into to have blood draw the day after taking the tablet. Plasma samples will be separated from 4 ml of blood collected in EDTA tubes for plasma calcitriol measurements. The samples will be centrifuged at 1400rpm at 4^0C for 10 minutes and stored at -80^0C until assayed.

9. DRUG INFORMATION

Calcitriol

Name and Chemical Information
1α, 25 dihydroxycholecalciferol; 1, 25-dihydroxyvitamin D

Chemical Structure



Molecular Formula: C27H44O3
Molecular Weight: 416.64

Mechanism of action
Calcitriol is the primary active metabolite of vitamin D3.

Drug Information

The calcitriol will be supplied by:

PCCA Professional Compounding Centers of America.
9901 South Wilcrest Drive
Houston Texas 77099

The Product Formulation involves making a solution of the following:

Calcitriol 22.5mg
Butylated Hydroxytoluene NF(BHT) 100mg
Butylated Hydroxyanisole NF(BHA) 100mg
in 100 mls of Almond Oil NF

Calcitriol (1, 25-dihydroxycholecalciferol) is the biologically active form of vitamin D. It is commercially available as an oral formulation of calcitriol designed specifically for high-dose, intermittent administration. In this protocol it will be studied in individuals at high-risk to develop lung cancer.

Because of the substantial preclinical data documenting the potent anti-cancer activity of calcitriol, clinical studies were conducted in various solid tumors with the currently marketed formulation of calcitriol using various routes and regimens. While efficacy was suggested in

several studies, hypercalcemia was dose limiting in these studies. Moreover, plasma levels did not reach those that would be predicted to have anti-cancer activity.

Investigators discovered that intermittent dosing with calcitriol permits substantial dose escalation. The maximum dose of calcitriol tested was 2.8 µg/kg PO. Plasma calcitriol reached a plateau at doses ≥ 0.5 µg/kg PO at levels predicted to be associated with anti-cancer activity without causing dose limiting hypercalcemia. The safety of 0.5 µg/kg calcitriol PO qw was supported by clinical data in over 70 subjects with prostate cancer in three Phase 2 studies. The efficacy of calcitriol was suggested by open-label studies in subjects with AIPC when the drug was administered as either a single agent or in combination with docetaxel.

A Phase 1, multicenter, open-label, dose escalation study of DN-101 was carried out to evaluate the safety, tolerability, and PK of DN-101 (another formulation of calcitriol) in 38 subjects with advanced malignancies. An initial dose of up to 165 µg DN-101 PO was not associated with DLTs, so the MTD was not determined. The MTD for repeat weekly dosing in this study was defined as 45 µg PO qw based on the occurrence of transient, asymptomatic Grade 2 hypercalcemia in two subjects who were treated at 60 µg PO qw for nine weeks. The PK of DN-101 was linear and predictable; there was no evidence of saturation of absorption.

Enrollment and treatment in a Phase 2 study of DN-101 in subjects with MDS has been completed. DN-101 was well-tolerated in the 37 subjects treated in this study. The study was closed to further enrollment when the Simon optimal two-stage analysis, which allowed an early study stop if the true rate of erythroid response was ≤ 30%; did not demonstrate the pre-specified number of responses for allowing continuation of the study.

Risks to Subjects Participating in Clinical Studies of Calcitriol Observed Risks: In monotherapy studies in subjects with malignancies, calcitriol has been well-tolerated. Low grade hypercalcemia (generally Grade 1) was the most commonly reported adverse event (AE) in 22% of subjects. Two of 6 subjects treated with 60 µg DN-101 weekly in the Phase I study and 3 of 37 subjects with MDS treated with 45 µg DN-101 weekly developed transient Grade 2 hypercalcemia. Other commonly reported AEs of low grade (Grade 1) include fatigue (14%), nausea (6%), increase in alanine aminotransferase (ALT) (5%), increase in serum creatinine (5%), hyperglycemia (5%), and anorexia (5%). Three different subjects reported a Grade 3 hyponatremia, proteinuria, or hypercholesterolemia, and one subject reported a Grade 4 hypercholesterolemia.

Adverse Events Related to Calcitriol: Based on the available safety data, the following treatment emergent AEs classified by participating Investigators as possibly or probably related to calcitriol have been reported for subjects treated with calcitriol in clinical studies. Most of these AEs were Grade 1 or 2; calcitriol -related Grade 3 or 4 AEs were rare.

1. ***Blood and lymphatic system disorders:*** anemia NOS, leukopenia NOS, neutropenia, thrombocytopenia, disseminated intravascular coagulation
2. ***Ear and labyrinth disorders:*** fortification spectra, ear congestion
3. ***Eye disorders:*** glaucoma NOS, conjunctivitis, lacrimation increased, vision blurred

4. ***Gastrointestinal disorders:*** abdominal pain NOS, constipation, diverticulitis NOS, dyspepsia, nausea, vomiting NOS, diarrhea NOS, stomatitis, abdominal pain upper, loose stools, dry mouth, cheilitis, retching
5. ***General disorders and administration site conditions:*** asthenia, fatigue, pain NOS, chest discomfort, lethargy, edema NOS, edema peripheral, infusion site reaction, mucosal inflammation NOS, pyrexia
6. ***Hepatobiliary disorders:*** hyperbilirubinemia
7. ***Infections and infestations:*** pneumonia NOS, oral candidiasis
8. ***Investigations:*** international normalized ratio increased, alanine aminotransferase increased, AST increased, blood albumin decreased, blood bilirubin increased, blood creatinine increased, blood phosphorous increased, crystal urine, crystal urine present, blood cholesterol increased, hemoglobin decreased, lymphocyte count increased, urine calcium increased, albumin urine present, blood phosphorous decreased, blood urea increased, creatinine renal clearance decreased, platelet count increased, protein total decreased, weight decreased
9. ***Metabolism and nutrition disorders:*** hypercalcemia, hyperglycemia NOS, hyperkalemia, hyperphosphatemia, hyponatremia, hypophosphatemia, hyperuricemia, appetite decreased NOS, polydipsia, dehydration, hypocalcaemia, hypoproteinemia, anorexia, hypercholesterolemia, hypoalbuminemia, hypokalemia
10. ***Musculoskeletal and connective tissue disorders:*** myalgia, arthralgia, joint stiffness, back pain, bone pain, muscle weakness NOS
11. ***Nervous system disorders:*** ataxia, dysphonia, headache, dizziness, carpal tunnel syndrome, dysgeusia, dysaesthesia, hyperaesthesia, hypoaesthesia, paraesthesia
12. ***Psychiatric disorders:*** anxiety, depression
13. ***Renal and urinary disorders:*** nephrolithiasis, polyuria, renal impairment, proteinuria, micturition frequency decreased, urinary incontinence
14. ***Respiratory, thoracic and mediastinal disorders:*** hiccups, pneumonitis NOS, pulmonary embolism, cough, dyspnea exertional, epistaxis, nasal congestion, pharyngolaryngeal pain, postnasal drip, rhinorrhea
15. ***Skin and subcutaneous tissue disorders:*** pruritus, ecchymosis, contusion, rash NOS, dry skin, erythema, nail discolouration, nail disorder NOS, night sweats, rash maculo-papular, sweating increased
16. ***Vascular disorders:*** flushing, hypertension NOS, hypotension NOS

Risks Warranting Further Discussion:

1. ***Hypercalciuria:*** Many subjects treated with calcitriol develop hypercalciuria (elevated urine calcium) but acute nephrolithiasis has been very rare. The risk of nephrolithiasis due to hypercalciuria is minimized by increasing fluid intake.
2. ***Hypercalcemia:*** Hypercalcemia has been identified as the DLT in non-clinical and clinical studies of calcitriol, and occasional subjects taking calcitriol have had Grade 1 or 2 hypercalcemia. In the Phase 1 study of calcitriol in subjects with advanced malignancies, transient asymptomatic hypercalcemia (Grade 2) occurred in two subjects who received 60 µg calcitriol PO qw for nine weeks. The risk of hypercalcemia is minimized by increasing fluid intake, monitoring serum calcium, evaluating symptoms of hypercalcemia, excluding certain medications or supplements, and excluding subjects with a history of cancer-related hypercalcemia. Long-standing hypercalcemia may result in hypercalcemic nephropathy (interstitial

nephritis), a disease characterized by loss of distal tubule and collecting duct function, a reduction in glomerular filtration rate, and tubulointerstitial damage. Early recognition is important because damage can often be arrested or even reversed with discontinuation of the offending agent. Therefore, periodic monitoring of serum creatinine is recommended for subjects receiving high-dose calcitriol, including calcitriol. The interstitial nephritis seen in the 28-day repeated dose toxicity study of calcitriol in dogs was consistent with hypercalcemic nephropathy.

3. ***Transient Renal Insufficiency:*** Occasional subjects have had elevations of BUN or serum creatinine, or decreased creatinine clearance while taking calcitriol. The risk of renal toxicity due to calcitriol is minimized by excluding subjects with ≥ Grade 2 elevations of serum creatinine and monitoring renal function during clinical studies with calcitriol.
4. ***Hyperuricemia:*** The possibility that calcitriol causes hyperuricemia cannot be excluded at this time. In the Phase 2/3 AIPC study (Study DN101-002), Grade 3 or 4 hyperuricemia was reported for two subjects (1.6%) in the group treated with calcitriol and docetaxel; hyperuricemia was not reported for subjects treated with docetaxel and placebo.
5. ***Disseminated Intravascular Coagulation:*** The possibility that calcitriol may cause disseminated intravascular coagulation cannot be excluded at this time. In the Phase 2/3, AIPC study (Study DN101-002) fatal disseminated intravascular coagulation, deemed related to study drug by the investigator occurred in one subject.
6. ***Calcifications of soft tissue*:** The possibility that repeat chronic dosing with high-dose calcitriol causes calcification of soft tissues cannot be excluded at this time.

Overdose: There is no specific antidote to calcitriol. If overdose were suspected, administration of study drug, calcitriol, should be stopped and general supportive measures instituted, including close monitoring of arrhythmias and serum calcium. The risk of overdose is minimized by the standard dose and written dosing instructions for subjects.

Administration: Total duration of therapy will be 3 months. Take one capsule of study drug every other week for a total of 3 months. Patients should be instructed to maintain adequate fluid intake.

Availability: calcitriol is a new formulation of calcitriol. For this study a 45 mcg capsule will be dispensed.

Storage: calcitriol should be protected from light and stored in the refrigerator.

Potential Risks: Potential risks from the intervention are minimal. The most prominent risks are those associated with the potential for hypercalcemia. A bronchoscopy will be performed at the end of the 3-month intervention. This is generally a safe and painless procedure, performed under sedation. The risks associated with bronchoscopy include the following: bleeding (usually mild and self-limiting), drug reaction from sedation, infection – pneumonia or bronchitis (rare), collapse of the lung (pneumothorax) (rare with endobronchial biopsies), and wheezing, breathing trouble or a low oxygen level. These problems are uncommon and are entirely treatable. There is a possible, but rare risk of death with bronchoscopy. Additional minimal risk is associated with blood collection, as

infection and bruising is possible at the point of needle insertion and induction of sputum. These procedures are part of the routine screening for lung cancer. Potential risks of calcitriol administration include hypercalcemia and hypercalcuria.

Procedures for Protecting against Potential Risks: It is unlikely that participants will display symptoms of hypercalcemia. However, all adverse and serious adverse events will be reported to the IRB, FDA and NCI. Minimal risks are associated with blood sample collection. These potential risks will be fully described in approved consent forms.

Anticipated Benefits versus Potential Risks: Benefits from this study include lung cancer screening with bronchoscopy and evaluation of premalignant lesions of the lung. While it is hypothesized that calcitriol will positively modulate the biomarkers for lung cancer (and presumably risk associated with lung cancer), this cannot be viewed as a benefit of the study. Society as a whole will benefit from the knowledge gained in this study, as the toxicity profile in non-cancer patients will be better understood. Given the low level of risk for participants in this trial, the costs and risks are justified.

10. DATA SAFETY MONITORING

This project is a Pilot study and the DSMB will perform the function of data safety monitoring.
The main goals of this monitoring process will be to 1) evaluate the conduct and recruitment progress of the trial and to assure compliance with eligibility requirements; 2) to evaluate all adverse and serious adverse events that were associated with participation in the trial, including events related to procedures and treatment; 3) to oversee the database managements and integrity; 4) to review interim analyses performed by the study statistician regarding effectiveness of randomization and distribution of adverse events.

11. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS REPORTING

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4 of the CTCAE is identified and located at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 4.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs administered to the participant.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.

- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

11.1 Reporting Adverse Events

Table 1 Guidelines for Routine Adverse Event Reporting for Pilot, Phase 2, and Phase 3 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Routine AEs occurring between the start date of intervention until 30 days after the last intervention or until the event has resolved, the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible, will be reported. New information will be reported after it is received.

As requested by the FDA, final reporting of AEs will include all AEs regardless of attribution.

11.2 Serious Adverse Events

11.2.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a participant or participant, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does **NOT** include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.

- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

11.2.2 Reporting Serious Adverse Events

All new SAEs occurring from the date the participant signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. The RPCI SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to Section 11.3 for details on reporting Unanticipated Problems.

11.2.3 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

. Patients will be removed from study for any of the following reasons: Any grade ≥ 3regardless of attribution; a grade 2 that persists for more than two weeks; interim development of new malignancy (lung or extra pulmonary); or withdrawal of consent. Patients will not be given a dose reduction.

11.3 Unanticipated Problems

11.3.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  a) The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
  b) The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed **Serious** per **Section 0**.

11.3.2 Reporting Unanticipated Problems

Unanticipated problem reporting will begin at the time of participant consent. The Unanticipated Problem Form will be submitted to the CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS Compliance with an updated Unanticipated Problem Form. The site Investigator or designated research personnel will report all unanticipated problems, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**.

11.4 FDA Reporting

When RPCI is the IND holder the following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Serious but not fatal or life-threatening;

Or, meets **ANY** of the following criteria:

- A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).
- Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.
- Any findings from animal or in vitro testing that suggest a significant risk for human participants including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.
- Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A MedWatch for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via email to CRSCompliance@RoswellPark.org.

12. STATISTICAL ANALYSIS

PK/PD Modeling - Using a sequential approach (136), a PK/PD structural model will be developed for calcitriol, based on toxicity responses in previous trials. A variety of compartmental population pharmacokinetic structural models will be evaluated to characterize the serum concentration versus time profile following the oral administration of calcitriol. The physiologic pharmacokinetic models explored will be described by the estimation of mean structural model parameters (e.g., plasma volumes of distribution and clearances), the magnitude of inter-individual variability (IIV) in these parameters, and the magnitude of residual variability (RV). To date, a population PK model of calcitriol has not been developed but prior experience of Dr. Muindi revealed that plasma concentrations may either follow a mono- or biexponential decay. Thus, it is anticipated that the appropriate PK model will initially include either a one- or two-compartment model with first-order absorption and (linear) elimination. The PK model could expand to include mechanistic attributes, such as a receptor-mediated clearance mechanisms since calcitriol binds to the VDR receptor and may in part, get internalized as a complex, undergoing receptor-mediated clearance as one of the primary clearance mechanisms. As a result, a series of more complex PK models may be sequentially evaluated if required to adequately describe the pharmacokinetic profile of calcitriol.

According to the basic tenets of target-mediated drug disposition, distribution phases for lower doses are expected to be steeper than that of larger doses. Some compounds that exhibit such behavior also may show regions of convexity in various sections of the plasma concentration-time curve at relatively high doses. If needed, the unique incorporation of receptor-mediated disposition will represent a more mechanistic model to examine the biodisposition of calcitriol following oral administration. Patient demographics, racial group, along with CYP24A1 phenotype, smoking status, and other laboratory measurements will be evaluated as potential patient covariates that contribute to the inter-individual variability in pharmacokinetics. Typical metrics of model predictive performance will be used to assess the accuracy and precision with which the best model predicts the calcitriol concentrations, including: traditional global metrics, such as predicted area under the concentration-time curves, apparent oral clearance, apparent volume of distribution, terminal elimination half-life, and other PK parameters, will be compared to values obtained from a non-compartmental analysis of the raw data. The final model will be used to conduct simulations to predict pharmacokinetic outcomes and the time-course of inaccessible system variables, as well as providing suitable driving functions for the development of mechanism-based pharmacokinetic-pharmacodynamic models of toxicity endpoints (137, 138).

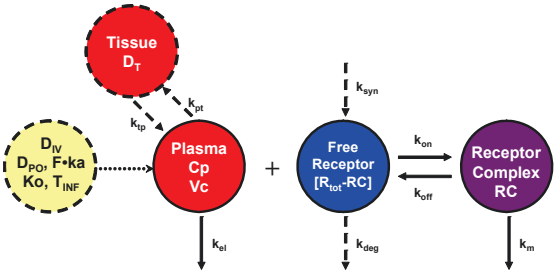


Fig. DM1. General model of target-mediated drug disposition.[2]

Subsequently, a population PK/PD model will be developed to describe the potential relationship between calcitriol exposure and toxicity endpoints, such as hypercalcemia, as indicators of pharmacodynamic response. This analysis will include both structural model development and evaluation of the influence of a limited number of patient covariates, such as patient demographics along with EGFR and smoking status, on the variability in select PK/PD parameters. The relationship between calcitriol concentrations and toxicity endpoints will be explored graphically and various structural models will be applied to the data and evaluated. It is assumed that a sigmoid-E_{max} model or an effect-site PK/PD model, accounting for severity in graded response of the particular toxicity endpoint, may be appropriate to describe the PK/PD relationship. Similar to the PK model development, criteria such as the precision of parameter estimates, magnitude of residual variability and goodness-of-fit will be utilized for PK/PD model selection.

Optimal Biologic Concentration Determination - The optimal biologic concentration will be defined as the steady state concentration range that is associated with less than grade 2 toxicity and leads to target inhibition in 90% or greater of patients. Because there are inter-individual variations in absorption and metabolism of calcitriol, a more rational approach will be to evaluate the feasibility of relating concentrations of calcitriol rather than administered dose, to pharmacodynamic markers and toxicity. Thus, we will examine the relationship between exposure levels (Cp or AUC) of calcitriol with pharmacodynamic endpoints and polymorphisms in CYP24A1 and calcitriol metabolizing enzymes. Determining the strength and direction of the association between genotype(s), PD endpoints and calcitriol exposure levels will allow the construction of a preliminary model that will allow personalized dosing to a target drug exposure range (Cp or AUC) for validation in future chemoprevention studies. Based on these data, patients could be initially started on therapy on a non-toxic median dose that leads to target exposure levels. Eventually, doses could be adjusted during treatment to reach target concentrations, as is done with other drugs that have narrow therapeutic indexes such as vancomycin and busulfan for bone marrow transplantation.

We will test the null hypothesis that the proportion of grade 3-4 toxicities or grade 2 toxicities that persist more than two weeks will be 10% versus the one-sided alternative that the proportion of these toxicities will be greater than 10%. An interim analysis will be performed after the first 20 subjects on calcitriol have completed the study. The study can be stopped early at the interim point and the null hypothesis will not be rejected if <= 1 toxicity is observed, at which time the DSMB will summarize the findings and the results, including all toxicity reports will be submitted to the FDA. Otherwise, the full 40 subjects will be recruited into the study. At the end of the study period, we will again either reject the null hypothesis (if 8 or more DLT toxicities are observed) or not reject the null hypothesis if 7 or less toxicities are observed.

Sample Size Calculations:
Given a sample size of n=40, we will 80% power be able to detect the proportion of toxicities of 15% or larger different from 10%, assuming an alpha level 0.05.

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