

A Phase I/II Clinical Trial of intravenous (IV) Calcitriol with fixed dose of Cisplatin and Docetaxel in Advanced Non-Small Cell Lung Cancer.

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Protocol Synopsis

A Phase I/II study of Intravenous Calcitriol in Combination with fixed dose of cisplatin and docetaxel in advanced non-small cell lung cancer

Number of Centers: 4

Estimated number of patients:

Phase I – minimum 18 patients.

Phase II – minimum 39 patients.

Study Period: October 2006 – September 2011

Accrual Duration: 5 years

Objectives

Primary Objectives

1. To conduct a phase I study to determine the maximum tolerated dose (MTD) and the dose limiting toxicity (DLT) of intravenous calcitriol when administered prior to fixed dose cisplatin 75mg/m² and docetaxel 75 mg/m², every 3 weeks in patients with advanced non-small cell lung cancer (NSCLC)
2. To conduct a phase II study using the MTD of calcitriol (determined from the phase I study) in combination with fixed dose of cisplatin (75mg/m²) and docetaxel (75 mg/m²) administered every 3 weeks in patients with advanced NSCLC and to characterize the toxicity and response of the combination in these patients

Secondary Objectives

1. To assess pharmacokinetics (PK) of intravenous (IV) calcitriol in combination with cisplatin and docetaxel during cycle 1 of the phase II part of the study using a validated limited sampling technique.
2. To correlate the pharmacokinetic parameters of systemic calcitriol exposure (AUC) with SNPs of the 24-hydroxylase (CYP24), the major vitamin D₃ inactivating enzyme.

Trial Design: Open labeled, multi institution, non-randomized, phase I/II study

Background and rationale: 1, 25 dihydroxycholecalciferol (vitamin D or calcitriol), a central factor in bone and mineral metabolism, is a potent antiproliferative, differentiating and apoptotic agent in a wide variety of malignant cell types. Calcitriol has significant antitumor activity *in vitro* and *in vivo* in murine squamous cell carcinoma (SCC). Dexamethasone (dex) potentiates the antitumor effect of calcitriol, enhances calcitriol-mediated cell cycle arrest and apoptosis and decreases calcitriol-induced hypercalcemia. (1, 2) Both *in vitro* and *in vivo*, dexamethasone significantly increases vitamin D receptor (VDR) ligand binding in the tumor while decreasing binding in intestinal mucosa. (2) Dexamethasone, in combination with calcitriol, results in a significant time-dependent increase in VDR protein. Calcitriol significantly enhances the *in vitro* and *in vivo* antitumor efficacy of the platinum analogues, cisplatin and carboplatin as well as the taxanes, paclitaxel and docetaxel. (3, 4) Our preclinical studies demonstrate that the combination of calcitriol/dex/cisplatin results in marked enhancement of antitumor activity both *in vitro* and *in vivo* in a murine squamous cell

carcinoma model system when compared to these agents alone or in two-drug combinations. In *in vitro* culture systems, cisplatin and calcitriol are synergistic by median dose effect. While calcitriol does not increase total intracellular platinum content or formation of GG or AG adducts, calcitriol pre-treatment reduces the cellular capacity to repair cisplatin damaged DNA. In addition, we have established a correlation between the ability of calcitriol to decrease expression of p53 and p21 and increase cisplatin cytotoxicity. Therefore, calcitriol-mediated suppression of p53 and its downstream targets may compromise repair of platinum: DNA adducts and enhance cisplatin cytotoxicity. Based on these, we have initiated **studies in dogs with spontaneous epithelial as well as sarcomatoid cancers. Using escalating doses of calcitriol and fixed dose of cisplatin we have shown complete responses in 3/6 dogs at calcitriol doses and serum levels achievable in humans.** The dog studies are continuing. These preclinical data lend a strong rationale to perform clinical studies of the combination of calcitriol with cisplatin and docetaxel. The starting dose of calcitriol is based on the dog studies where DLT was noted in 1/6 dogs at 1.5mcg/kg body weight; In addition, a phase I study done with the IV formulation showed the MTD to be 74µg total dose; this dose was tolerated on a weekly schedule. At present this study has enrolled patients at calcitriol doses of 163µg in combination with dexamethasone. Three patients at this level have no DLTs; this is equal to a cumulative dose of 489µg of calcitriol in 3 weeks. Therefore, we believe that a SD of 30µg/m² is both a safe starting dose as well as a dose that is close to one that will achieve the target plasma concentrations.

Patient Population and Inclusion/Exclusion Criteria

Eligibility

1. Proven histological or cytological diagnosis of stage IIIB (malignant pleural effusion)/ IV NSCLC.
2. Age > 18 years
3. Performance status must be ECOG 0-1.
4. No prior or concurrent malignancy, except non-melanoma skin cancer, or CIS of the cervix, unless documented disease-free for > 2 years.
5. No prior use of chemotherapy for stage IV NSCLC; Adjuvant therapy is permitted.
6. Adequate bone marrow, hepatic, and renal function, as evidenced by the following: WBC >3.0 x 10⁹/L, neutrophils ≥ 1.5 x 10⁹/L; platelet count ≥ 100 x 10⁹/L; Hgb ≥ 10 g/dL (may be transfused to 10g/dL); total bilirubin within the upper limit of the institutional normal range; (transaminases SGOT or SGPT) ≤ 1.5 times the upper limit of the institutional normal range. Creatinine within the upper limit of the institutional normal range; creatinine clearance ≥50 ml/min
7. Patients must have measurable or evaluable disease (not required for the phase I part of the study)
8. Normal cardiac function with no history of uncontrolled heart disease
9. Female patients must not be pregnant; they must be post-menopausal or practicing an accepted form of birth control. If pregnancy is a possibility, a pregnancy test will be required prior to initiation of therapy.
10. Life expectancy of at least 12 weeks.
11. Patient and investigator signed study-specific consent form, indicating the investigational nature of the study

12. Patients must be accessible for treatment and follow-up.
13. No chemotherapy or radiotherapy within 3 weeks of study entry defined here as day 1 of therapy with calcitriol plus chemotherapy (6 weeks for mitomycin C or a nitrosourea).
14. No treatment with investigational drugs within 3 weeks of study entry.
15. No other serious illness or medical condition including unstable cardiac disease requiring treatment, new onset crescendo or rest angina; history of significant neurological or psychiatric disorders including psychotic disorders, dementia, or seizures; or active infection are permitted. No evidence of grade ≥ 2 peripheral neuropathy. No history of severe hypersensitivity reaction to docetaxel or other drugs formulated with polysorbate 80
16. Palliative radiation is permitted as long as there has been at least 1 week since the last palliative XRT. Use of growth factors is strongly recommended for these patients.
17. Treated brain metastasis allowed with no waiting period following gamma knife and at least 2 weeks after whole brain XRT as long as neurologically stable.

Exclusion Criteria:

1. Known hypersensitivity to Vitamin D, docetaxel, cisplatin
2. Hypercalcemia (patients with serum albumin corrected calcium* > 10.7 mg/dL)
3. History of renal/bladder stones over the past 10 years
4. History of nephrectomy.
5. Uncontrolled heart disease, unstable angina, heart failure, current digoxin therapy
6. Thiazide, Digoxin or glucocorticoid therapy (except the pre-medication Dexamethasone used in the study as prescribed)
7. Unwillingness to stop calcium supplementation
8. Concurrent use of Phenytoin, Barbiturates, Rifampin, Carbamazepine, Phenobarbital or St John's wort.
9. Treatment with any investigational drug within 3 weeks before Day 1 of protocol
10. Any unresolved toxicity (NCI CTCAE version 3.0, ≥ 2) (Please see appendix V for link)
11. Pregnancy/Lactation
12. Patients with IIIB NSCLC who are eligible for definitive chemoradiation.

* Ca corrected = Ca (measured) + (0.8 x (4 - albumin))

Investigational Product, Standard drugs, Dosage and Method of administration

1. Calcitriol (generic IV calcitriol 1mcg/ml), dosage per cohort on the schedule chart, given intravenously every 21 days). Commercially available intravenous calcitriol will be used.
2. Dexamethasone 4mg p.o bid for 3 days, starting day before, day of and day after chemotherapy; commercially available as 2/4 mg tablets
3. Cisplatin 75mg/m² every 3 weeks. Available as an aqueous solution of cisplatin 100 mg/100ml.
4. Docetaxel 75 mg/m² every 3 weeks; available as a sterile solution containing 40 mg/ml of docetaxel (80 mg/2ml vial and 20 mg/0.5 ml vial)

Schema of Dosing

Phase I: Dose escalation of calcitriol in combination with fixed dose of cisplatin and docetaxel.

Dose Level (DL)	Calcitriol (mcg)/m ² IV q21 days	Cisplatin (mg/m ²) IV Q 21 days	Docetaxel (mg/m ²) IV Q 21 days
DL 1	30	75	75
DL 2	45	75	75
DL 3	60	75	75
DL 4	80	75	75
DL 5*	100	75	75

*Further dose escalation may continue beyond DL 5 by increments of 30% until a dose limiting level.

Schedule: Docetaxel will be administered as a one hour intravenous infusion Day 1 of each cycle followed by Cisplatin every 21 days. Calcitriol will be given intravenously every 3 weeks on day 1 of each cycle prior to docetaxel infusion. **Only in the first cycle of the phase II study, calcitriol will be given on the day before the chemotherapy.** Dexamethasone will be given 4 mg orally B.I.D for six doses, starting the day before docetaxel.

Treatment Schedule for Phase I

	D-1	D1	D2	D21	D22	D23
Docetaxel		X			X	
Cisplatin		X			X	
Dexamethasone	X	X	X	X	X	X
Calcitriol		X			X	

Treatment Schedule for Phase II⁺

	D-1	D1	D2	D21	D22	D23
Docetaxel		X			X	
Cisplatin		X			X	
Dexamethasone	X	X	X	X	X	X
Calcitriol	X@	X			X	
Pharmacokinetics⁺	X					

+ The calcitriol dose for the phase II study will be the MTD from the phase I study
@Only in the first cycle of the phase II study, calcitriol will given on the day before the chemotherapy for the purpose of PK⁺ studies using the limited sampling technique (baseline; immediately following calcitriol infusion; 2 and 4h after start of infusion)

Phase I dose escalation: doses of calcitriol will be escalated in patient cohorts of three, until the MTD is reached, according to the following scheme:

# patients experiencing DLT/ cohort size	Action
0/3	Increase to next dose level
1/3	Accrue 3 more patients at same dose level
1/3+0/3	Increase to next dose level
1/3+ ≥1/3	Stop: evaluate previous dose for MTD

MTD will be based on the first cycle of therapy administered; to be evaluable a patient must complete 1 cycle of therapy.

Dose limiting toxicities are defined as follows (using the NCI common toxicity criteria 3.0):

1. Hypercalcemia:
 - (a) Corrected serum calcium* ≥ 12 mg/dL persisting >7 days or <7 days if symptomatic
 - (b) Any corrected calcium ≥ 13 mg/dL

(symptomatic or asymptomatic)

Or

2. Development of nephrolithiasis – symptomatic or radiographic

Or

3. Renal Dysfunction: The serum creatinine will be measured prior to each cycle of therapy. If the entry serum creatinine was <1.4 mg/dL, an increase of 0.5 mg/dL will mandate interruption of calcitriol until creatinine returns to within 10% of baseline level. A creatinine increase to ≥ 2 mg/dL will mandate interruption of calcitriol until creatinine returns to within 10% of baseline level. In the event where creatinine levels increase ≥ 1.5 x baseline, calcitriol therapy will be withheld until creatinine decreases back to <1.5 x baseline. Sustained increases (>72 hours) in creatinine of more than double the baseline and >2.5 mg/dL will constitute a DLT and will result in removal from study even in the absence of hypercalcemia. When the MTD is established, the phase II portion of this study will be initiated using the MTD obtained from the phase I portion of this trial.

*Ca corrected = Ca (measured) + (0.8 x (4 - albumin))

The definition of DLT as related to hypophosphatemia will be as follows:

Hypophosphatemia will constitute a DLT if:

- $PO_4 < 1.5$ mg/dL persisting for > 10 days
- or
- $PO_4 < 1.5$ mg/dL associated with symptoms unequivocally related to hypophosphatemia: profound weakness, ventilatory compromise
- Or
- $PO_4 < 0.75$ mg/dL

4. In addition, the following will also constitute a DLT

-Any grade 3 or greater non-hematological toxicity other than nausea, vomiting, alopecia.

-Any grade 4 hematological toxicity lasting greater than 7 days

-All grade 5 events except if related to disease progression.

-Any hospitalization/removal from study related to toxicities/adverse events from the study drugs.

Duration of Administration: Patients are to be treated until there is unacceptable toxicity, evidence of progressive disease, or a total of up to four cycles in the case of stable/responding disease, at the discretion of the investigator.

Number of Patients:

Phase I – minimum 18 patients.

Phase II – minimum 39 patients.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS
Abbreviations or Terms Definitions

AE- Adverse Event
ALAT- Serum Alanine Aminotransferase
ANC- Absolute Neutrophil Count
ASAT- Serum Aspartate Aminotransferase
BID-Twice a day
BSA- Body Surface Area
CBC- Complete Blood Count
CDDP- Cisplatin
CI- Confidence Interval
CR- Complete Response
CRF-Case Report Form
CRS- Clinical Research Service
CTC-Common Terminology Criteria for Adverse Events v3.0
CTO- Clinical Trials Office
D- Day of cycle (e.g.: D1 is the first day of the treatment cycle)
D5W- 5 percent dextrose in water
DNA- Deoxyribonucleic Acid
ECG- Electrocardiogram
ECOG-Eastern Cooperative Oncology Group
FDA- Food & Drug Administration
FSR- Final Study Report
GCP- Good Clinical Practice
G-CSF- Granulocyte Colony Stimulating Factor
GILT- Genotypic International Lung Trial
I.M. - Intramuscular
I.V. - Intravenous
ICH- International Conference on Harmonization
IEC- Independent Ethics Committee
IRB- Institutional Review Board
ITT- Intent-To-Treat
MRI- Magnetic Resonance Imaging
NC- No Change (Stable Disease)
NCI- National Cancer Institute
NE- Not Evaluable
NS- Normal Saline
NSCLC- Non-Small Cell Lung Cancer
PD- Progressive Disease
PET- Positron Emission Tomography
PO- *Per Os* (by mouth)
PR- Partial Response
PS- Performance Status
RECIST- Response Evaluation Criteria in Solid Tumors
RNA- Ribonucleic Acid
SAE- Serious Adverse Event
SAERF- Serious Adverse Event Report Form
SAP- Statistical Analysis Plan

SGOT- Serum Glutamic Oxalo-acetic Transaminase

SGPT- Serum Glutamic Pyruvic Transaminase

SWOG- Southwest Oncology Group

TID- Three Times a Day

UNL- Upper Normal Limit for that institution

Vs Versus

WBC- White Blood Cell Count

WHO- World Health Organization

1.0 STUDY OBJECTIVES

1.1 Primary Objectives

1. To conduct a phase I study to determine the maximum tolerated dose (MTD) and the dose limiting toxicity (DLT) of intravenous calcitriol when administered prior to fixed dose cisplatin and docetaxel every 3 weeks in patients with advanced non-small cell lung cancer (NSCLC)
2. To conduct a phase II study using the MTD of calcitriol (determined from the phase I study) in combination with fixed dose of cisplatin (75mg/m²) and docetaxel (75 mg/m²) administered every 3 weeks in patients with advanced NSCLC and to characterize the toxicity and response of the combination in these patients

1.2 Secondary Objectives

1. To assess pharmacokinetics of IV calcitriol in combination with cisplatin and docetaxel during cycle 1 of the phase II part of the study using a validated limited sampling technique.
2. To correlate the pharmacokinetic parameters of systemic calcitriol exposure (AUC) with SNPs of the 24-hydroxylase (CYP24), the major vitamin D₃ inactivating enzyme.

2.0 BACKGROUND AND RATIONALE

2.1 Cisplatin: Cis-diamminedichloroplatinum (II), (cisplatin, CDDP) and its analogues form the cornerstone of combination therapy of advanced NSCLC. Lung cancer, with 120,000 deaths each year in the United States alone, is the biggest cancer killer claiming more lives than breast, prostate and colon cancers combined. These cancers are often advanced at presentation and cures are uncommon. The response rates to platinum – based combinations are low (20-40%) and cures rare. Limited activity and moderate toxicity are limitations of current approaches. Attempts to enhance response through the use of new agents such as the receptor tyrosine kinase inhibitors (RTKis) or other targeted drugs (example monoclonal antibodies to the vascular endothelial factor receptor) have yielded only modest improvements in few patients. The majority of patients with advanced NSCLC are dead from their disease in less than 1 year. Given the morbidity and mortality associated with NSCLC, it is imperative to continue to look at ways to improve the efficacy of platinum based chemotherapy regimens.

2.11: The cellular response to DNA damage mediated by cisplatin. Cisplatin cytotoxicity results from the formation of covalent, bifunctional intrastrand adducts between adjacent guanine residues in DNA. Through the actions of DNA-PK, ATM or ATR, DNA damage results in a cellular response involving phosphorylation and stabilization of the tumor suppressor p53. (5) Phosphorylation increases the sequence specific DNA binding activity of p53, resulting in increased transcription of target genes including (but not limited to) the cyclin-dependent kinase inhibitor, p21^{Waf1/Cip1} (6), Gadd45 (7) and the pro-apoptotic Bcl-2 family member, Bax. (8) The induction of p53 and its downstream targets p21 and Gadd45 prevents replication of damaged DNA by blocking cell cycle progression, thus allowing time for DNA repair. As part of the G1 checkpoint, p21 binds to and inhibits the activity of cyclin: cdk complexes required for entry into S phase. (9) Similarly, p21 participates in G2/M checkpoint control by inhibiting cdc2: cyclin B activity, required for progression into mitosis. (10) Gadd45 dissociates cdc2 from cyclin B1 (10) thus reinforcing the G2/M checkpoint.

Additionally, p21 (11) and Gadd45 (12) via their interactions with proliferating cell nuclear antigen (PCNA), may modulate nucleotide excision repair, the process by which platinum:DNA adducts are corrected. The importance of p21 and Gadd45 to the cellular response to cisplatin induced DNA damage is evidenced by the increase in sensitivity that is observed when expression of these molecules is decreased. (13, 14)

2.2 Docetaxel:

Docetaxel (Taxotere[®]) is a semi-synthetic taxane that is FDA-approved for the treatment of metastatic breast cancer as well as platinum-refractory non-small cell lung cancer. (15) Docetaxel, a taxane, is a semi synthetic agent derived from 10-deacetyl Baccatin III. It differs from paclitaxel in several ways, including greater uptake into tumor cells and greater affinity for microtubules, as compared with paclitaxel. (16) Docetaxel acts by disrupting the microtubular network in cells that is essential for mitotic and interphase cellular functions. Docetaxel binds to free tubulin and promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly. This leads to the production of microtubule bundles without normal function and to the stabilization of microtubules, which results in the inhibition of mitosis in cells. The inactivation of Bcl-2 (17) and the induction of cyclin-dependent kinase p27/Kip-1 expression have also been suggested as possible mechanisms of docetaxel's antitumor effect.

2.21: Docetaxel in lung cancer: As a single agent docetaxel has yielded response rates of 21% and 63% in advanced NSCLC, with one-year survival rates of 21% and 39% in separate phase II studies. Docetaxel has been approved as a single agent for second line treatment of NSCLC. This followed the results of an international phase III study of docetaxel compared with best supportive care in previously untreated patients with NSCLC. An overall survival advantage at 25% at 1 year compared with 16% at 1 year was observed for the docetaxel arm compared with best supportive care. (18)

2.3: Cisplatin and Docetaxel in metastatic NSCLC: With best supportive care alone, patients with metastatic NSCLC have a median survival of 4-5 months and 1 –year survival of 10%. (19) Chemotherapy, particularly cisplatin based chemotherapy provided a survival benefit in metastatic NSCLC. The hazard ratio for death was 0.73 in favor of chemotherapy ($p < 0.0001$). This equaled a 1.5 month increase in median survival and a 10% increase in survival at 1 year. (20) Addition of newer agents such as the taxanes to cisplatin has increased response rates with modest improvements in median survival. ECOG 1594 randomized 1,207 patients to 3 platinum based doublet chemotherapy regimens (carboplatin/paclitaxel, cisplatin/docetaxel and cisplatin/gemcitabine). The median survival as well as overall survival was not significantly different in the 3 arms (MS of about 8 months and 1 year survival of 32%) (21) The largest phase III trial to date (TAX 326), involving 1220 patients showed that chemotherapy with cisplatin/docetaxel had a significantly higher response rate (32%) and a higher median survival of 11.3 months compared with cisplatin/vinorelbine. (22) Based on this, we have chosen to study if addition of calcitriol will increase the response rates afforded by cisplatin/docetaxel doublet in a phase I/II study.

2.4 Calcitriol: The active form of vitamin D has antiproliferative activity both in vitro and in vivo at supraphysiologic doses. This activity is mediated through the vitamin D receptor (VDR). The VDR is found, not only in classic target organs (intestinal tract, kidney, bone),

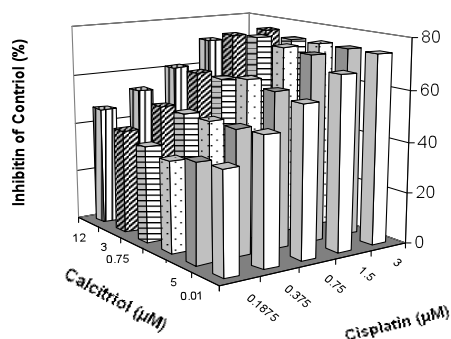
but also in many other epithelial and mesenchymal cells as well as leukemic cells, osteosarcoma, breast, lung, prostate and colon carcinoma, melanoma, squamous cell carcinoma, glioma, and other malignant cell types. (23, 24) Calcitriol can induce differentiation, cell cycle arrest and/or apoptosis in leukemic and tumor cells. Progression through the cell cycle is regulated by cyclins and their associated cyclin dependent kinases (cdk). The cdk inhibitors p21^{Waf1/Cip1} and p27^{Kip} are implicated in G₁ phase arrest. (25, 26) In HL-60 cells, a human myelomonocytic leukemia cell line, calcitriol arrests cells in G₁; this effect is mediated through an increase in p27. (27) Calcitriol mediated arrest in G₀/G₁ is also observed in human breast cancer lines. (28) In the human cell line SCC25, calcitriol and the analogue, EB1089 modulates p21 and p27 and induces Gadd45, the growth arrest and DNA damage gene. (29) Apoptosis is mediated by biochemically diverse stimuli that activate caspases and bring about cellular destruction through specific cleavage of key cellular proteins. (30) Drs Trump and Johnson's laboratory has shown significant effects of calcitriol and its analogues on both the cell cycle and the apoptotic signaling pathway with potential for modulation of therapeutic efficacy. (2, 31, 32)

2.4.1 Preclinical studies of calcitriol and cytotoxic chemotherapy including combination with cisplatin and taxanes.

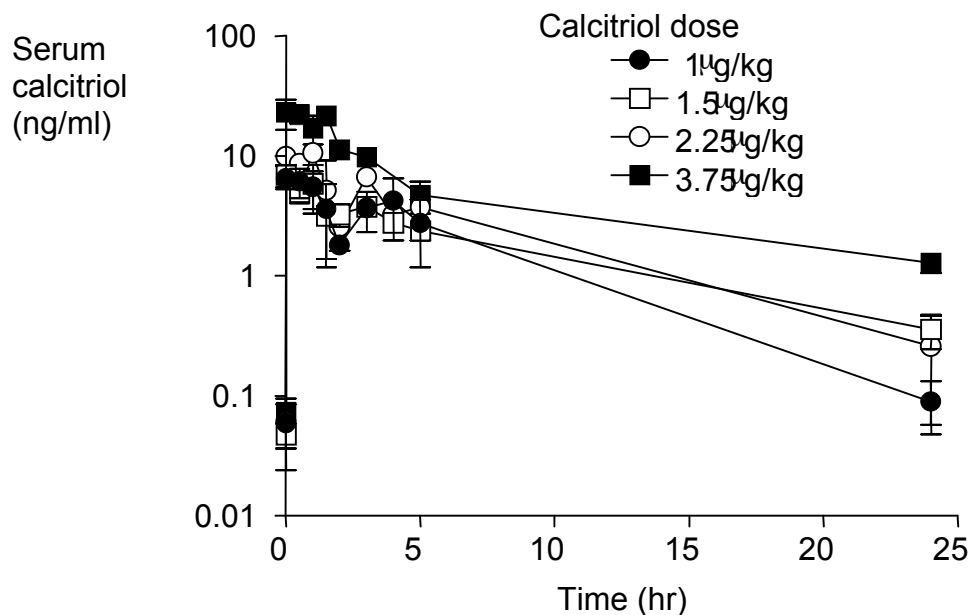
Studies in our laboratory demonstrate that vitamin D (1,25 dihydroxycholecalciferol or calcitriol), has significant antitumor activity *in vitro* and *in vivo* in murine squamous cell carcinoma (SCC), human xenograft prostatic adenocarcinoma (PC-3) and rat metastatic prostatic adenocarcinoma Dunning (MLL) model systems. (3, 33) Calcitriol induces G₀/G₁ arrest, modulates p27^{Kip1} and p21^{Waf1/Cip1}, the cyclin dependent kinase (cdk) inhibitors implicated in G₁ arrest (4, 33, 34) and induces cleavage of caspase 3, PARP and the mitogen-activated protein kinase (MEK) in a caspase-dependent manner. (31) Calcitriol also decreases phospho-Erk (P-Erk) and phospho-Akt (P-Akt), kinases that regulate cell survival pathways and up-regulates the pro-apoptotic signaling molecule, MEKK-1. (3) Calcitriol significantly enhances the *in vitro* and *in vivo* antitumor efficacy of the platinum analogues and taxanes. (4, 34, 35) Enhancement of drug-mediated apoptosis is associated with an increase in PARP-MEK- and caspase3-cleavage, the expression of p73 and MEKK-1 and a decrease in P-Erk and P-Akt. (3, 35)

Glucocorticoids enhance calcitriol-mediated activities pre-clinically (*in vitro* and *in vivo*) and clinically. We have demonstrated that dexamethasone (dex) significantly potentiates the antitumor effect of calcitriol and decreases calcitriol-induced hypercalcemia. (2, 32) Both *in vitro* and *in vivo*, dex increases vitamin D receptor (VDR) ligand binding in the tumor while decreasing binding in intestinal mucosa (32), the site of calcium absorption. (36) P-Erk and P-Akt are also decreased with calcitriol/dex, as compared to either agent alone. (2) When the dex is added to calcitriol and a number of cytotoxic drugs, a greater antitumor effect is observed than with each drug alone or any two drug combination. (34, 37) Dex enhances VDRE transcriptional activity through a potential effect on coactivator stimulation of transcription. No convincing differences between dex, prednisolone or hydrocortisone have been detected at equivalent glucocorticoids doses.

2.4.2:Pre-clinical studies in dogs: The objective of the study was to determine if calcitriol enhances cisplatin cytotoxicity in canine cancer cell lines and determine a safe combination of calcitriol and cisplatin for use in tumor-bearing dogs. Calcitriol and cisplatin were found to be synergistic by isobologram median dose effect analysis for canine osteosarcoma, mast cell tumor and breast cancer cell lines.

Figure 1:**Isobologram Analysis OS 2.4 cells treated with
Calcitriol+Cisplatin**

Based on these, a phase I study of calcitriol and cisplatin was initiated. Escalating doses of IV calcitriol were given along with fixed dose ($60\text{mg}/\text{m}^2$) of cisplatin to dogs. Between February 2005 and October 2006, 22 dogs were entered into the phase I trial and received calcitriol at 8 dose levels. Five of 22 (23%) were mixed-breed dogs and 17 (77%) were purebred including 3 Golden Retrievers, 3 Labrador Retrievers, 2 Rottweilers, 2 Australian Shepherds; 1 each were Boxer, Borzoi, Doberman Pinscher, German Shepherd, Gordon Setter, Greyhound, and Vizla. The majority (11 of 22, 50%) of dogs had osteosarcoma; tumors originated from the appendicular skeleton in 10 dogs and 1 had extraskelatal osteosarcoma of soft parts. Three (14%) dogs had chondrosarcoma, 2 (9%) squamous cell carcinoma, 2 melanoma, 2 soft tissue sarcoma, and 1 (4%) each had thyroid carcinoma and undifferentiated carcinoma. Fifteen dogs had previously undergone only surgery, 2 had received docetaxel chemotherapy, 1 had surgery and radiotherapy, 1 had surgery followed by was persistent when re-evaluated on days 14 and 21. Dose level 3 was expanded to a total of six dogs and there were no further episodes of DLT. At dose level 7 ($3.75\text{ mcg}/\text{kg}$), one dog had hypercalcemia (serum calcium $14.5\text{ mg}/\text{dL}$) with adverse gastrointestinal signs including grade 4 vomiting and abdominal pain (3 days after treatment). Dose level 7 was expanded to a total of seven dogs and there were no further episodes of DLT. At dose level 8 ($5.5\text{ mcg}/\text{kg}$), two of four dogs had DLT consisting of hypercalcemia (serum calcium $13.7\text{ mg}/\text{dL}$) and grade 4 vomiting in one dog (day 1 after treatment) and hypercalcemia (serum calcium $18.0\text{ mg}/\text{dL}$) and grade 3 vomiting in the other dog (day 2 after treatment). This met the nontolerable dose level criterion of C2 of six affected dogs at a dosage. Thus, we concluded that i.v. calcitriol at a dosage of $3.75\text{ mcg}/\text{kg}$ in combination with cisplatin at $60\text{ mg}/\text{m}^2$ was determined to be the MTD. 7 of the 18 dogs had measurable tumors, and 3 had complete response as characterized by a 100% reduction in tumor volume. (# 48 Rassnick et al. *Cancer Chemother Pharmacol* (2008) 62:881–891)

Figure 2: Serum 1, 25-dihydroxyvitamin D₃ (calcitriol) pharmacokinetics in dogs

Plots of serum calcitriol concentration over time: Shown in figure 2, (i) C_{max} is achieved at the end of infusion (ii) Calcitriol doses $\geq 1 \mu\text{g/kg}$ achieved biologically effective serum calcitriol levels ($>10\text{nM}$ or $>4.0\text{ng/ml}$) sustained for up to 5 hours (iii) calcitriol levels still above baseline at 24h.

Calcitriol Dose ($\mu\text{g/kg}$)	N	C _{max} (ng/ml)	AUC _{0-24h} (ng.hr/ml)
0.10	3	0.600 \pm 0.023	5.540 \pm 0.324
0.25	2	1.129 \pm 0.045	12.68 \pm 2.580
0.50	4	2.417 \pm 0.472	10.89 \pm 1.269
1.00	2	6.555 \pm 1.301	46.47 \pm 22.43
1.50	3	7.262 \pm 1.810	44.22 \pm 6.330
2.25	2	11.53 \pm 0.015	65.65 \pm 5.390
3.75	4	29.01 \pm 3.811	118.8 \pm 16.92

Table 2: PK of calcitriol in dogs.

C_{max} = peak serum calcitriol levels

AUC_{0-24h} = Area under the concentration x time curve over 24hrs

Serum calcitriol levels measured by radioimmunoassay

AUC calculated by trapezoidal estimation

2.4.3 Clinical Studies with Calcitriol:

Several studies using calcitriol in various formulations and schedules have been conducted. The following paragraphs will be limited to those that have utilized oral and intravenous formulations in patients with cancer.

A) Studies utilizing oral formulations of calcitriol:

Based on pre-clinical data, two phase I clinical trials of calcitriol with either carboplatin or paclitaxel were initiated. (38) Patients with advanced cancer were treated with carboplatin (AUC=5) Q28 days + escalating doses of calcitriol QDX3 Q28 days. Calcitriol starting dose was 4 μ g QDX3. Studies were designed such that in each patient, carboplatin was given on day 1 before calcitriol in one of the first two cycles of treatment and on day 3 after two days of high dose calcitriol on the other. Dose-limiting toxicity was not encountered in this trial. The AUC of carboplatin was higher in each patient following calcitriol than before calcitriol (mean AUC = 7.6 μ g/ml.hr \pm 1.8, carboplatin day 3 [DDDC] vs. AUC = 6.6 μ g/ml.hr \pm 1.4, carboplatin day 1 [CDDD], p= 0.04). This increase in AUC was observed with no change with increasing calcitriol dose. While no dose limiting toxicity was seen, myelosuppression (%change in platelet count) following the sequence carboplatin \rightarrow calcitriol was less than that following calcitriol \rightarrow carboplatin, consistent with the change in AUC. No clinically detectable renal impairment has been seen with either sequence. In another trial, patients with advanced cancer were treated with paclitaxel (80mg/m² weekly x 6) + escalating doses of calcitriol, QDX3 weeklyX6. The starting dose of calcitriol was 4 μ g p.o QDX3, weekly and we entered patients through the 38 μ g dose level where it appears that we reached saturable concentrations at 16-20 μ g. No dose limiting toxicity was encountered. No changes in peak concentration, AUC or T 1/2 were noted. To investigate the issue of bioavailability of calcitriol, patients were given escalating doses of calcitriol starting at 14 μ g using a liquid formulation of calcitriol with potentially greater bioavailability. (39) Pharmacokinetic (pk) data following oral administration indicate that the serum calcitriol AUC is not proportional to calcitriol dose suggesting a decrease in bioavailability and calcitriol exposure. (40) Oral administration of calcitriol at the highest dose studied results in an AUC in man (7.5ng/ml.hr) that is significantly lower than the AUC in mice (38.1ng/ml.hr) at doses that correlate with a significant antitumor effect *in vivo*. (41)

In a phase II trial in androgen independent prostate cancer (AIPC) oral calcitriol (12 μ g/day QDx3, weekly) and dex (4 mg QDx4, weekly), a 50% reduction in prostate specific antigen (PSA) in 28% of the patients was seen, no hypercalcemia noted (38) and molecular changes in peripheral blood monocytes (PBM) similar to those observed in cell lines observed. Antitumor effects were also noted following calcitriol/dex in men with localized disease with a rising PSA following prostatectomy or irradiation. These trials utilized oral calcitriol – a route of administration where drug exposure and bioavailability may be an issue and limit response. (40) Induction of CYP24, the enzyme primarily responsible for calcitriol catabolism (42), may be a factor in bioavailability and calcitriol exposure; therefore, studies were initiated to investigate the effect of ketoconazole, a CYP24 inhibitor (43), on *in vitro* and *in vivo* anti-tumor effects of calcitriol/dex in the PC-3 prostate tumor model. Ketoconazole enhanced the anti-tumor activity of calcitriol/dex and decreased CYP24 activity. It is to be noted that the decrease in CYP24 activity was seen only in kidney tissues and not in PC3 tumor.

Calcitriol plasma Pharmacokinetics (using oral formulation). Pharmacokinetic 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 pharmacokinetic studies. 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 (C_{max}) for each patient studied at each dose level is portrayed in Figure 3A. As shown in Fig3B, baseline-subtracted serum calcitriol $\text{AUC}_{0\text{-}24\text{hr}}$ (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 ($\text{AUC} = a \times \text{dose} / (1 + b \times \text{dose})$) indicates that $\text{AUC}_{0\text{-}24\text{hr}}$ is not proportional to dose ($a = 540 \pm 140 \text{ pg}\cdot\text{hr}/\text{ml}\cdot\mu\text{g}$; if AUC were proportional to dose, b would equal 0).

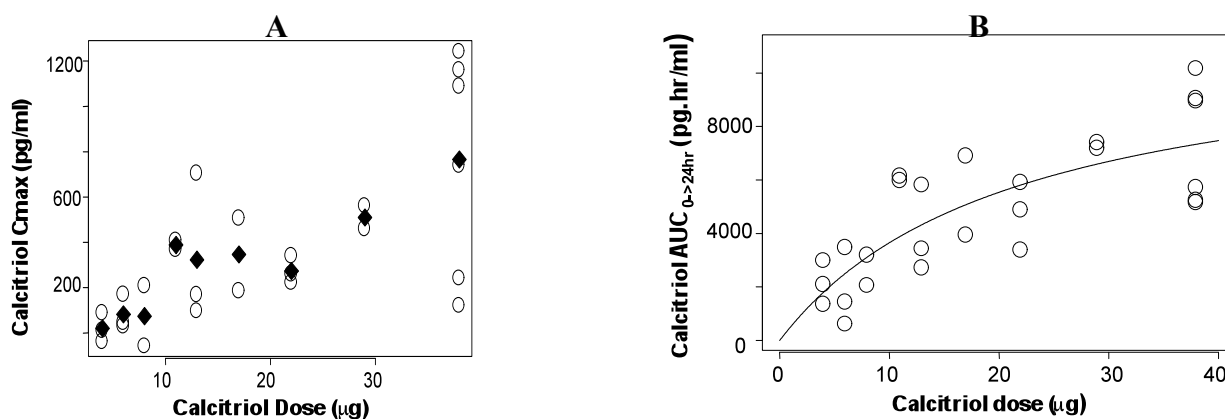


Figure 3A Scatter plot of the maximum serum calcitriol concentration (C_{max}) vs. calcitriol doses. Closed symbols represent mean values at each dose level. **(3B)** Baseline-subtracted serum calcitriol $\text{AUC}_{0\text{-}24\text{hr}}$ (area under the concentration-time curve for the 24 hour period after calcitriol administration) plotted against dose, and a fit of the Michaelis-Menten function, p -value of 0.0014. The effect of the nonlinearity over the range of doses studied is large: the fit value of $\text{AUC}_{0\text{-}24\text{hr}}$ 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.

In sharp contrast to the above study that used commercially available oral calcitriol, Beer et al in a more recent study using DN101, a proprietary formulation of oral calcitriol (Novacea), showed a dose-proportional increase in both C_{max} (linear regression $r = 0.86$, $P < 0.0001$) and AUC (linear regression $r = 0.89$, $P < 0.0001$) across the full range of DN-101 doses tested. Absorption was rapid, with a T_{max} of ~ 1 to 2 hours. Terminal $T_{1/2}$ values were dose independent and ranged from 5.5 to 16.2 hours. In this study, thirty-eight patients with advanced cancer were enrolled in 2002 and 2003. The median age was 70 years (range, 44-91 years). Dose escalation was stopped at the 165 μg level when the number of capsules required at one time reached 11. No dose-limiting toxicities occurred. Transient and self-limited grade 3 toxicities were hyponatremia (2) and proteinuria (1). A dose-proportional increase in peak concentration (C_{max}) and area under the concentration curve (AUC) was seen across the full range of DN-101

doses tested. At the 165 μg dose, C_{max} was 6.21 ± 1.99 ng/mL, AUC (0-24) was 41.3 ± 9.77 ng h/mL, AUC (0- ∞) was 55.4 ± 8.44 , and half-life ($T_{1/2}$) was 16.2 hours. At doses between 15 and 165 μg , DN-101 exhibits linear pharmacokinetics. At 165 μg , DN-101 achieves systemic exposure that is 5 to 8-fold higher than that achieved with commercial formulations of calcitriol, which makes DN-101 comparable to that required for antitumor activity *in vivo* in a murine squamous cell carcinoma model. (44)

Studies of calcitriol in combination with a taxane or with platinum compounds: Phase I trials of calcitriol in combination with carboplatin as well as with paclitaxel have been performed.(38) Dose limiting toxicities were not noted in this trial in which calcitriol was given as an oral formulation at 4mcg QD X3 and carboplatin either immediately before or after (2 schedules in the same patient). No clinically detectable renal impairment was noted with either schedule. In a similar manner, escalating doses of calcitriol was combined with weekly paclitaxel (80mg.m2) and no DLTs were noted. More recently, calcitriol (oral formulation, DN-101) was combined with docetaxel in patients with hormone refractory prostate cancer (the ASCENT study) (47); The primary endpoint for the ASCENT study was the percentage of patients experiencing a reduction of 50 percent or more in prostate specific antigen (PSA response). Secondary endpoints of the study include overall survival, skeletal morbidity-free survival, tumor response rate in patients with measurable disease, time to PSA response and safety. The data from this study demonstrated that the specified primary endpoint of PSA response occurred more frequently in patients on study receiving DN-101 plus Docetaxel (58%) than those patients in the control group (49%), but was not statistically significant. Overall survival, as measured by the multivariate hazard ratio, indicated an estimated 49% improvement in survival time for patients receiving the combination treatment versus those receiving Docetaxel plus placebo. This survival improvement meets the criteria for statistical significance. The median survival in patients treated with DN-101 plus Docetaxel was estimated at 23.5 months, as compared to an observed median survival of 16.4 months for patients treated in the control group. Importantly, the DN-101 and Docetaxel combination had a favorable safety profile when compared to Taxotere alone. Overall Serious Adverse Events were 27% in patients receiving the combination treatment and 41% in patients receiving Docetaxel plus placebo. Grade 3 or 4 Adverse Events were 58% in patients receiving the DN-101 and Docetaxel combination and 70% in patients receiving Docetaxel plus placebo.

B. Clinical studies with IV formulation of calcitriol: At RPCI, a phase I study of escalating dose IV calcitriol in combination with the EGFRtki, gefitinib was initiated. Patients (pts) with refractory solid tumors were eligible for study provided they had normal organ function, ECOG performance status 0-2, and no history of hypercalcemia. IV calcitriol was infused over 1 hour on day (D) 1 and 15 and weekly thereafter. Gefitinib 250 mg was given orally once a day starting Day 8. Calcitriol was escalated in 3 + 3 design (dose levels (DL)): 10, 15, 20, 26, 34, 44, 57, 74, and 96 mcg). No inpatient escalation was allowed. Calcitriol PKs were obtained on D1 and 15 (Table 3 and Figures 4A and 4B) Gefitinib PKs were obtained D15. Dose-limiting toxicity (DLT) was defined as any grade (G) 3 toxicity (except for anemia), sustained (>72 hours) G 2 hypercalcemia or symptomatic G 2 hypercalcemia, genitourinary stones, or increase in creatinine to 2 x baseline. Of 30 pts treated and evaluated, first cycle calcitriol-related toxicity was limited to hypercalcemia and at dose levels ≥ 20 mcg/week. Only grade (G) 1 hypercalcemia (<11.5 mg/dl) was noted at dose levels 20 to 57mcg/week. G2 (>11.5 –12.5 mg/dl) and G3 (>12.5-13.5 mg/dl) hypercalcemia were encountered at the doses of 74mcg/week (1/7 G2 and 1/7 G3) and

96mcg/week (2/4 G2). DLT occurred in 3 pts: 1 pt with G3 hypercalcemia at 74mcg/week and 2 pts with G2 hypercalcemia associated with central nervous system toxicity at 96mcg/week. Calcitriol at 74mcg/week and gefitinib 250mg/day was the maximum tolerated dose (MTD). Stable disease was noted in 1 pt each with NSCLC, GIST, penile, and prostate cancer. The relationship between calcitriol dose and AUC was linear over the dose range studied (DL 1-9). At the recommended dose level (74mcg), the AUC and Cmax were 31 ng.hr/ml and 6.68 ng/ml (16.0nM). Neither AUC nor Cmax was predictive of dose-limiting hypercalcemia. (#49 Fakhri et al, Clin Cancer Res 2007;1223 13(4) February 15, 2007). The increase in Cmax and AUC of calcitriol exhibited a linear association with the dose of calcitriol. Calcitriol Cmax and AUC levels increased with dose-escalation and approached biologically effective levels at higher doses (54mcg and above).

Pharmacokinetic correlates in above intravenous study:

Table 3: Serum PK parameters of intravenously administered calcitriol

Calcitriol (µg)	N	Cmax (ng/ml)	AUC 0-24hr (ng.h/ml)	T _{1/2} (hr)
10	3	0.46 ± 0.21	4.59 ± 0.91	13.5 ± 2.9
15	3	0.77 ± 0.37	5.92 ± 1.00	12.3 ± 0.9
20	3	1.01 ± 0.22	8.32 ± 1.04	12.5 ± 1.9
26	3	1.45 ± 0.47	12.43 ± 3.64	11.6 ± 1.4
34	3	1.44 ± 0.84	9.89 ± 3.05	13.3
44	3	2.72 ± 1.39	17.87 ± 10.72	19.0 ± 1.5
57	3	3.80 ± 2.38	24.15 ± 8.62	20.9 ± 3.6
74	6	6.68 ± 1.42	35.65 ± 8.01	16.1 ± 4.3
96	4	4.23 ± 1.12	25.85 ± 4.41	18.2 ± 1.9

The table and graphs show that the target Cmax and AUC is achieved at calcitriol doses ≥ 74µg

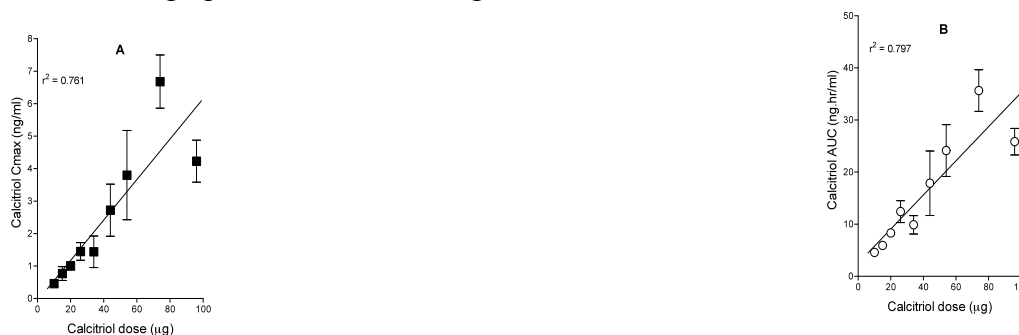


Figure 4A and B

3.0 RATIONALE FOR IV FORMULATION OF CALCITRIOL:

While there are no apparent differences in PKs between the oral and IV formulation (please see graph below), we will use the commercially available I.V. formulation (Generic calcitriol 1mcg/ml) as we do not have access to oral formulation. In addition, our pre-clinical data in dogs was obtained using the I.V. formulation.

Comparison of I.V. and PO PK The data shown in the graphs below (Figure 5A and B) are an attempt to superimpose the IV and oral studies. This graph compares RPCI IV calcitriol studies (unpublished data) and DN 101(Published data-Beer, T. M. et al. Clin Cancer Res 2005;11:7794-7799) oral calcitriol study

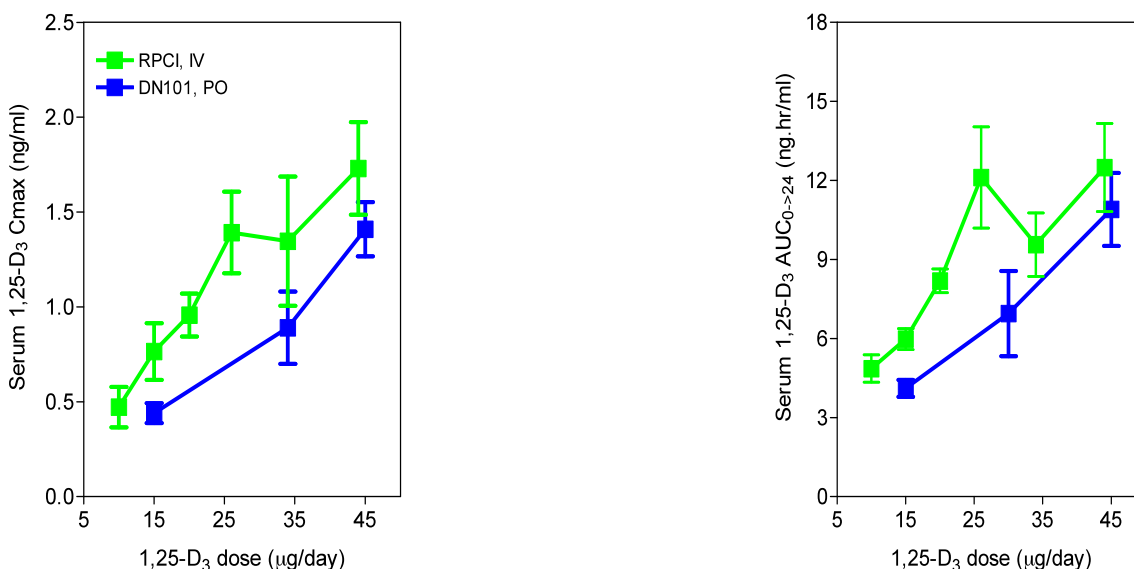


Figure 5A and B

3.1a: Rationale for combining calcitriol with cisplatin:

Calcitriol modulation of cisplatin cytotoxicity: In *in vitro* and *in vivo* systems, cisplatin and calcitriol are synergistic by median dose effect analysis. While calcitriol does not increase total intracellular platinum content or formation of GG or AG adducts, calcitriol pre-treatment reduces the cellular capacity to repair cisplatin damaged DNA. In addition, we have established a correlation between the ability of calcitriol to decrease expression of p53 and p21 and increase cisplatin cytotoxicity. Therefore, calcitriol-mediated suppression of p53 and

its downstream targets may compromise repair of platinum: DNA adducts and enhance cisplatin cytotoxicity. Our ongoing phase I trial of CDDP plus calcitriol in dogs with spontaneous tumors yields a striking complete response rate (3/6) in preliminary analysis.

3.1b: Rationale for using dexamethasone: Our preliminary data has shown that dexamethasone increases the anti-tumor activity of calcitriol (both by increasing the expression of VDR-vitamin D receptors as well as by down regulation P-AKT and related downstream targets of apoptosis); in addition dexamethasone has abrogated the hypercalcemic effects of calcitriol, one of the dose limiting toxicities of supraphysiologic doses of calcitriol. Dexamethasone also abrogates potential hypersensitivity reactions caused by docetaxel.

3.2: Rationale for Starting dose: The dog studies (see above) showed that the MTD was 3.75 mcg/kg. At that DL, there was 1 DLT consisting of increase in serum creatinine. The target levels in the plasma (corresponding to the biologically effective dose) was noted at 2.25 mcg/kg, giving a Cmax of around 16nM; at this dose there was no DLT noted. Using a conversion factor described by Freireich et al (45), we believe that a SD of 30 mcg/m² is safe. ; In addition, a phase I study done with the IV formulation showed the MTD to be 74µg total dose; this dose was tolerated on a weekly schedule. At present this study has enrolled patients at calcitriol doses of 163µg in combination with dexamethasone. Three patients at this level have no DLTs; this is equal to a cumulative dose of 489µg of calcitriol in 3 weeks. Therefore, we believe that a SD of 30 µg/m² is both a safe starting dose as well as a dose that is close to one that will achieve the target plasma concentrations. The reason to pick per m^{2 dosing} for calcitriol is for uniformity of dosing. (with the proposed chemotherapy) as well as to make a more feasible correlation with the dog data.

3.3: Rationale for combining calcitriol with cisplatin and docetaxel: While the preliminary data in dogs is with cisplatin and calcitriol, there is data (please see above) on the combination of calcitriol and taxanes as well. In patients with advanced lung cancer, the standard of care is a platinum doublet; hence we have chosen to study the effects of calcitriol on the combination of cisplatin and docetaxel. By doing a phase I study, we will study the safety of this combination prior to proceeding to a phase II study.

3.4: Rationale for studying calcitriol PKs in the phase II part of the study.

The pharmacokinetics (PKs) of calcitriol has been studied extensively (please see above) both as a single agent as well as in combination with carboplatin and paclitaxel. There were no interactions when calcitriol was combined with chemotherapy. (38)

We will study the PK of intravenous calcitriol to determine if there is a correlation between AUC, t1/2 and the polymorphisms of the CYP gene (please see below). If we demonstrate in this patient population that polymorphisms of the CYP gene correlate with PK parameters, the dose of calcitriol could be individualized not only in patients with lung cancer, but potentially in patients with other diseases where calcitriol has therapeutic benefits.

3.5: Rationale to study polymorphisms of the CYP gene

CYP24 gene encodes for Vitamin D 24-hydroxylase, the main catabolizing enzyme for calcitriol. This gene is located on the long arm of chromosome 20. An over expression of this gene has been seen in breast and esophagus cancers. In esophageal cancer, over expression of

this gene is associated with poorer survival; also the same authors showed that there was an inverse correlation between VDR expression and CYP24 (in other words, patients with high CYP24 expression had a tendency to poorer survival). (46)

Our laboratory has analyzed blood samples from patients enrolled in phase I clinical trials with calcitriol. There were no SNPs identified in CYP24 exons 6, 8 and 9. However, SNPs have been identified in the introns between exons 9 and 10 positions and between 24 and 46. from the c-terminal end of exon 9. SNPs in these regions could potentially contribute to the differences in PKs and therefore activity of calcitriol.

To determine the relationship between CYP24 SNPs and calcitriol clearance, the elimination half life, T_{1/2}, values were dichotomized using the corresponding median values. Then, the new binary variables between T/T and T/C were compared using Fisher's exact tests due to the limited sample sizes. The results of this analysis show a significant relationship between CYP24 SNPs and elimination T_{1/2}. (P-value = 0.0377 (one-sided test). T/T type tends to have lower T_{1/2}. These results are supportive of the hypothesis CYP24 SNPs may contribute variable systemic exposure after calcitriol treatment. We would like to validate this hypothesis in this proposed larger study.

The rationale for the study can be thus summarized:

- 1. Both calcitriol and the chemotherapy agents have anti-tumor activity.**
- 2. Calcitriol is synergistic with cisplatin in both in vitro as well as in vivo pre-clinical models.**
- 3. Current therapy for advanced lung cancer with platinum based combination is associated with modest responses and no cures, with a median survival in responders, of about 8 months.**

4.0 PATIENT SELECTION:

4.1 Inclusion:

1. Proven histological or cytological diagnosis of advanced NSCLC (**Stage IIIB due to malignant pleural effusions** and Stage IV) incurable with other modalities. (Please see Appendix I for staging system).
2. Age > 18 years
3. Performance status (PS) must be ECOG 0-1. (Please see Appendix II for PS)
4. No prior or concurrent malignancy, except non-melanoma skin cancer, or CIS of the cervix, unless documented disease-free for > 2 years.
5. No prior use of chemotherapy for stage IV NSCLC; adjuvant therapy is permitted.
6. Adequate bone marrow, hepatic, and renal function, as evidenced by the following: WBC > 3.0 x 10⁹/L, neutrophils ≥ 1.5 x 10⁹/L; platelet count ≥ 100 x 10⁹/L; Hgb ≥ 10g/dL (may be transfused to 10g/dL); total bilirubin within the upper limit of the institutional normal range; (transaminases SGOT or SGPT) ≤ 1.5 times the upper limit of the institutional normal range. Creatinine within the upper limit of the institutional normal range; creatinine clearance ≥ 50 ml/min (using Cockcroft Gault equation with actual body weight, Appendix III)
7. Patients must have measurable disease (not required for the phase I part of the study)
8. Normal cardiac function or compensated heart disease with no history of unstable angina or CHF in 6 weeks prior to study entry.

9. Female patients must not be pregnant; they must be post-menopausal or practicing (either they or the spouse) an accepted form of birth control. If pregnancy is a possibility, a pregnancy test will be required prior to initiation of therapy.
10. Life expectancy of at least 12 weeks.
11. Patient and investigator signed study-specific consent form, indicating the investigational nature of the study
12. Patients must be accessible for treatment and follow-up.
13. No chemotherapy or radiotherapy within 3 weeks of study entry, defined here as the date of day 1 of calcitriol plus chemotherapy. (6 weeks for mitomycinC or a nitrosourea).
14. No treatment with investigational drugs (either on phase I, II or III trials) within 3 weeks of study entry.
15. No other serious illness or medical condition including unstable cardiac disease requiring treatment, new onset crescendo or rest angina; history of significant neurological or psychiatric disorders including psychotic disorders, dementia, or seizures; or active infection are permitted. No evidence of grade ≥ 2 peripheral neuropathy. No history of severe hypersensitivity reaction to docetaxel or other drugs formulated with polysorbate 80
16. Palliative radiation is permitted as long as there has been at least 1 week since the last palliative XRT. Use of growth factors is strongly recommended for these patients.
17. Treated brain metastasis allowed with no waiting period following gamma knife and at least 2 weeks after whole brain XRT as long as neurologically stable.

4.2 Exclusion Criteria:

1. Known hypersensitivity to Vitamin D, docetaxel, cisplatin
2. Hypercalcemia (patients with serum albumin corrected calcium > 10.7 mg/dL)
3. History of renal/bladder stones over the past 10 years
4. History of nephrectomy.
5. Uncontrolled heart disease, unstable angina, heart failure, current digoxin therapy
6. Thiazide, Digoxin or glucocorticoid therapy (except the pre-medication Dexamethasone used in the study as prescribed)
7. Unwillingness to stop calcium supplementation
8. Concurrent use of Phenytoin, barbiturates, rifampin, Carbamazepine, Phenobarbital or St John's wort.
9. Treatment with any investigational drug within 30 days before Day 1 of protocol treatment.
10. Any unresolved toxicity (NCI CTCAE version 3.0, ≥ 2) (Please see appendix V for link)
11. Pregnancy/Lactation
12. Patients with IIIB NSCLC who are eligible for definitive chemoradiation.

4.3 Inclusion of women and minorities

Both men and women of all ethnic groups are eligible for this trial.

5.0 TREATMENT PLAN:

5.1 Pretreatment Evaluation

The following will be conducted or obtained prior to initial test drug dosing. The timing of these evaluations is presented in Table 1.

1. Signed written informed consent.
2. Complete medical history, including diagnosis of NSCLC, prior chemotherapy, concurrent illness, concomitant medications, and allergy history.
3. Physical examination, including weight, height, focused neurological exam, ECOG performance status, temperature, vital signs (blood pressure, pulse and respiration rate), and clinical tumor locations and measurements.
4. Hematology: complete blood count with white blood cell differential and platelet counts.
5. Serum chemistries: alkaline phosphatase, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), total serum bilirubin, serum creatinine and calculated creatinine clearance, electrolytes, calcium, phosphorous, total protein, albumin, glucose, BUN; in patients with squamous histology, PTH and iPTH levels will be assessed.
6. Imaging studies for potential evaluable disease sites as clinically indicated
7. Baseline toxicity evaluation to document baseline symptoms

Table 1 – Baseline and On-Study Evaluations

Investigations	Baseline	On-Study	End of Treatment (no less than 30 days after the patient's last day of treatment) ^e	Follow up
Patient Informed Consent	X	-	-	-
Medical history /Physical Exam, including a focused neurological exam	Within 7 days prior to infusion	One week after chemo and pre-chemo during treatment ^a +/- 2 days	X	-
Vital Signs	Prior to infusion of each drug	One week after chemo and pre-chemo during treatment +/- 2 days	X	-
Hematology ^b	Within 14 days of D1 of therapy	One week after chemo and pre-chemo during treatment +/- 2 days	X	-
Blood chemistry ^{b,d} *including phosphorous	Within 14 days of D1 of therapy	One week after chemo and pre-chemo during treatment ^c +/- 2 days (Biochemistry)	X	-
Imaging: Tumor assessments	Within 4 weeks prior to study entry	X (Radiology and Tumor assessment) Every 2 cycles	X	-
Toxicity/Baseline Symptoms	Within 3 days prior to infusion	1 week after chemo and more frequently with signs and symptoms +/- 2 days	Toxicity Evaluation	Study related adverse events and survival
Blood for calcitriol PK		Starting day before chemotherapy (only in the first cycle of phase II portion of the study) 0hrs; immediately following calcitriol infusion; 2, and 4 hrs after start of calcitriol infusion	-	-
Blood for CYP24 polymorphism (for Ph I & Ph II)	X	-	-	-

a If a treatment is delayed ≥ 1 week, to be performed on day 1 of the delayed course prior to treatment.

- b. May be performed outside of the study center but always in the same laboratory provided that the normal ranges are outlined clearly in the laboratory report.
- c. One additional blood chemistry (Ca electrolytes) checked 3-4 days after cycle 1 alone
- d. In patients with squamous cell carcinoma, blood will be drawn for iPTH and PTH at pre-treatment and if day 4 Calcium is above normal.
- e. Evaluations should be done prior to patient starting a new therapy. Tumor assessments may be done at the discretion of the treating physician if patient is starting a new therapy less than 30 days after stopping the study medication. Imaging studies are not mandatory if there is unequivocal clinical evidence of progression, example: new neck lymph node appearing on therapy.

5.2: Treatment Schedule

Calcitriol IV (see below for dose levels) followed by docetaxel administered at a dose of 75 mg/m² infused over 1 hour followed by cisplatin 75 mg/m² IV repeated every 3 weeks. To limit sensitivity reactions to docetaxel and to potentiate calcitriol effect and reduce calcitriol-induced hypercalcemia, give dexamethasone 4 mg orally BID for six doses; starting the day before docetaxel.

Antiemetics will be given per Institute standards.

In the phase II portion of the study, growth factors may be given per Institute standards at the discretion of the treating physician in order to avoid hematologic toxicities. Growth factor use is strongly recommended in patients who have had palliative or whole brain radiation.

Calcitriol: Starting Dose = 30 mcg/m² every 3 weeks given IV immediately prior to each q 3 weekly dose of docetaxel. Following docetaxel, pre-cisplatin hydration should be started. Cisplatin will be followed by additional hydration. Calcitriol (intravenous formulation); for cycle 1 of the phase II study; calcitriol will be given 24h prior to docetaxel infusion.

Dose Escalation: 30 mcg/m² every 3 weeks, 45 mcg/m² every 3 weeks
60 mcg/m² every 3 weeks, 80 mcg/m² every 3 weeks 100 mcg/m² every 3 weeks. Further dose escalation will occur at 30% increments

After the maximum tolerated dose (MTD) of calcitriol has been determined, the phase II portion of the trial will begin. The calcitriol dose for the phase II portion of this trial will be at the MTD determined from the phase I study. Patients who received calcitriol dose higher than the MTD during the phase I portion of the trial and who had no adverse effects should continue this higher dose for the duration of their therapy.

5.3: Duration of Administration

Patients are to be treated until there is unacceptable toxicity or evidence of progressive disease. Patients with stable disease may be treated for a total of four cycles, at the discretion of the investigator. This may be carried out for phase I and II portions of the study.

5.4: Dose Modification Rules

In the phase I portion of the study, the three patients treated at a given dose will be observed for dose-limiting toxicity for a minimum of 21 days before accrual to the next dose is permitted. If the patient's tumor is stable, or an objective tumor regression is observed, the patient will be continued on calcitriol and dexamethasone and q 21 day dosing of docetaxel and cisplatin. If acute toxicity attributable to docetaxel and cisplatin is noted, the patient must

have recovered from the toxicity before receiving subsequent doses of docetaxel and cisplatin. Side effects most commonly associated with docetaxel administration include hypersensitivity reactions, fluid retention, and bone marrow suppression. Side effects most commonly associated with cisplatin administration include nausea, vomiting, tinnitus, hearing loss, renal toxicity, electrolyte imbalances, neuropathy and bone marrow suppression. Previously described side effects for calcitriol include hypercalcemia or nephrolithiasis; and side effects of dexamethasone are fluid retention, glucose intolerance, weight gain, increased appetite.

Dose limiting toxicities are defined as follows (using the NCI CTCAE version 3.0):

Hypercalcemia: corrected serum calcium ≥ 12 mg/dL persisting >7 days or <7 days if symptomatic

Or

\geq Any corrected serum calcium 13mg/dL or greater (with/ without symptoms secondary to hypercalcemia)

Or

Development of nephrolithiasis – symptomatic or radiographic

***Ca corrected = Ca (measured) + (0.8 x (4 - albumin))**

Or

Renal Dysfunction: The serum creatinine will be measured prior to each cycle of therapy. If the entry serum creatinine was <1.4 mg/dL, an increase of 0.5 mg/dL will mandate interruption of calcitriol until creatinine returns to within 10% of baseline level. A creatinine increase to ≥ 2 mg/dL will mandate interruption of calcitriol until creatinine returns to within 10% of baseline level. In the event where creatinine levels increase ≥ 1.5 x baseline, calcitriol therapy will be withheld until creatinine decreases back to <1.5 x baseline. Sustained increases (>72 hours) in creatinine of more than double the baseline and >2.5 mg/dL will constitute a DLT and will result in removal from study even in the absence of hypercalcemia. Mild to moderate hypophosphatemia has been seen in 10-20% of patients treated with high dose calcitriol. The etiology of hypophosphatemia following calcitriol administration is not clear. Hypervitaminosis D is not a commonly described cause of hypophosphatemia. Recognized causes of hypophosphatemia include hyperparathyroidism, carbohydrate loading, calcitonin, glucagons or catecholamine administration, respiratory alkalosis, diminished phosphate absorption (e.g. vitamin D deficiency) and renal phosphate wasting (diuretics, hypercalcemia, PTHrP, metabolic acidosis) Whether PTHrP production by patients with cancer or transient hypercalcemia or Hypercalciuria associated with high dose calcitriol administration contribute to the observed hypophosphatemia is unclear. Hypophosphatemia is generally asymptomatic unless profound (<1.5 mg/dL) and persistent. [Severe hypophosphatemia: pathophysiologic implications, presentations and treatment. Subramanian, R and Khardori, R *Medicine* 79(1) p1-8, 2000] Since hypophosphatemia is relatively common and has not been clinically significant in with our experience in >300 patients treated with high dose calcitriol or other D analogues, nor in the experience of Beer et al on the recent Novacea randomized trial.(47)

The definition of DLT as related to hypophosphatemia will be as follows:

Hypophosphatemia will constitute a DLT if:

- $PO_4 < 1.5$ mg/dL persisting for > 10 days

or

- PO4 <1.5 mg/dL associated with symptoms unequivocally related to hypophosphatemia: profound weakness, ventilatory compromise
- Or
- PO4 <0.75 mg/dL

In addition, the following will also constitute a DLT

- Any grade 3 or greater non-hematological toxicity other than nausea, vomiting, alopecia.
- Any grade 4 hematological toxicity lasting greater than 7 days
- All grade 5 events except if related to disease progression.
- Any hospitalization/removal from study related to toxicities/adverse events from the study drugs.

The appropriate treatment will be used to ameliorate signs and symptoms including antiemetics for nausea and vomiting, antidiarrheals for diarrhea, and antipyretics, antihistamines, and/or steroids for drug fever before toxicity grade is determined. During at least the first and second infusions of docetaxel and cisplatin, a careful evaluation of general sense of well being will be done and blood pressure and heart rate measured as described below so that immediate intervention can occur in response to symptoms of an untoward reaction. Facilities and equipment for resuscitation will be immediately available, including antihistamine, steroids, and epinephrine. If a reaction occurs, the specific treatment medically indicated for a given symptom (e.g., epinephrine in case of anaphylactic shock) will be instituted. In addition, it is recommended to take the measures outlined in Table 3.

Table 2. Measures for Anaphylactoid/Hypersensitivity Reactions

Severity of Symptoms	Treatment Guidelines
Mild symptoms: localized cutaneous reactions such as mild pruritus, flushing, rash	consider decreasing the rate of infusion until recovery from symptoms, stay at bedside and monitor patient, then, complete docetaxel infusion at the initial planned rate
Moderate symptoms: any symptom that is not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP > 80 mm Hg	interrupt docetaxel infusion give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV; monitor patient until resolution of symptoms resume docetaxel infusion after recovery of symptoms; depending on the physician’s assessment of the patient, docetaxel infusion should be resumed at a slower rate, then increased incrementally to the initial planned rate, (eg. infuse at an 8 hour rate for 5 minutes, then at a 4-h rate for 5 minutes, then at a 2-h rate for 5 minutes, then finally, resume at the 1-h infusion rate) depending on the intensity of the reaction observed, additional oral or IV premedication

	with an antihistamine should also be given for the next cycle of treatment, and the rate of infusion should be decreased initially and then increased back to the recommended 1-hour infusion, (<i>eg. infuse at an 8 hour rate for 5 minutes, then at a 4-h rate for 5 minutes, then at a 2-h rate for 5 minutes, and finally, administer at the 1-h infusion rate</i>)
Severe symptoms: any reaction such as bronchospasm, generalized urticaria, systolic BP \leq 80mm Hg, angioedema	immediately discontinue docetaxel infusion give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV and/or epinephrine as needed; monitor patient until resolution of symptoms The same treatment guidelines outlined under moderate symptoms should be followed.
Anaphylaxis (NCI grade 4 reaction)	NO FURTHER STUDY DRUG THERAPY

If symptomatic, patients developing edema may be treated. Recommended treatment is as follows:

- (1) Furosemide 20mg 1 tablet daily.
 - (2) If fluid retention progresses, increase furosemide 40 mg PO qd.
 - (3) If fluid retention continues to progress, treat with furosemide 40 mg PO qd and additional diuretic such as spirinolactone.
 - (4) Further therapy should be customized depending upon the clinical situation.
- All other toxic effects should be managed symptomatically if possible. For \geq grade 3 non-hematological toxicities, study drugs should be held until resolution to \leq grade 1, then reinstated, if medically appropriate. All ancillary treatment will be recorded on the appropriate case report form (CRF). The average amount of prn medication taken per day should be recorded (for example, "morphine sulfate 60 mg/day, not "morphine sulfate prn".) Ongoing supportive and palliative care, e.g., nutritional support and pain control, will be provided as necessary throughout the study.

5.5. Evaluations during Study

The following evaluations will be made at the times designated in Table 1

1. Medical history update, including documentation of concurrent conditions and concomitant medications.
2. Physical examination including weight, ECOG performance status, neurological exam, and clinical tumor measurement (Refer to section 7.1).
3. Vital signs (blood pressure and pulse) will be recorded prior to infusion of each drug. If bradycardia (less than 50 beats/min) or other significant cardiac findings occur, then continuous ECG monitoring will be established for the next infusion of the offending drug, for that patient.
4. Hematology, including complete blood count with white blood cell differential and platelet counts.
5. Serum chemistry, including alkaline phosphatase, AST/SGOT, ALT/SGPT, total serum bilirubin, serum creatinine, electrolytes, calcium, phosphorous, total protein, albumin, glucose

and BUN. Total bilirubin, ALT, and AST should be reviewed the day of treatment, prior to infusion. Serum PTH and iPTH will be drawn in case of hypercalcemia on day 4 in patients with squamous cell carcinoma.

6. Imaging studies as indicated

7. Toxicity/symptom evaluation

5.6 Criteria for Removal of Patients from the Study

Patients may be discontinued from trial treatment and assessments at any time at the discretion of the investigator(s). Specific reasons for discontinuing a patient from this trial are the following:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event: eg: Development of life-threatening and/or significant irreversible toxicity not manageable by symptomatic care, dose reduction and/or delay.
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Administration of radiotherapy (except for palliation of pain) or of any other chemotherapeutic or experimental drug during the trial. Palliative radiotherapy cannot encompass more than 20% of marrow (please see Appendix IV for reference); Patient must wait at least 1 week before resuming therapy on the protocol.
- Withdrawal of patient consent.

Deaths due unequivocally to progression are not SAEs. All trial treatment-related toxicities and SAEs must be followed up until resolution. All patients who have new or worsening CTCAE Grade 3 or 4 laboratory values at the time of withdrawal must have further tests performed, and the results must be recorded appropriately until the laboratory values have returned to CTCAE Grade 1 or 2, unless these values are not likely to improve because of the underlying disease. In these cases, the investigators must record their opinions in the patients' medical records. Laboratory abnormalities that constitute DLTs must be recorded. Any laboratory abnormality CTCAE Grade 3 or 4, or CTCAE Grade 1 or 2 hematology or biochemistry laboratory values that are considered not due to tumor progression should be recorded as AEs. At withdrawal all ongoing study-related toxicities and SAEs must be followed until resolution, unless in the investigator's opinion, the condition is unlikely to resolve due to the patient's underlying disease. After withdrawal from treatment, patients must be followed up for existing AEs for 30 calendar days after administration of the last dose of trial drug. Collection of new AEs will occur for 30 days following the last dose of investigational product or until the patient starts new treatment for progression of disease whichever comes first. All SAEs occurring during that period must be followed up until resolved, unless in the investigator's opinion the condition is unlikely to resolve because of the patient's underlying disease.

5.7 End-of-Study Evaluation

The following assessments will be repeated no less than 30 days after the patient's last day of treatment: history, physical examination, including neurological examination; hematology, biochemistry; toxicity evaluation. In the event a patient discontinues study treatment and begins a new regimen, these evaluations should be done prior to starting the new treatment. Radiology

and tumor assessment should be reassessed no less than 30 days after the patient's last day of treatment unless second line therapy has been started, in which case it should be done at the discretion of the treating physician.

5.8. Follow-Up

Patients will be followed until death to assess study-related adverse events and survival.

6.0A CALCITRIOL PHARMACOKINETICS:

An exploratory pharmacokinetic substudy will be carried out in the phase II portion of the study using a limited sampling technique (described previously). Calcitriol levels will be drawn at 0, 2, and 4h. pharmacokinetics will reflect the pharmacokinetics of calcitriol and its metabolites when calcitriol is given alone. A minimum of seven (7) mL of whole blood will be drawn at PreRx and at each of the subsequent points listed above. Serum calcitriol assays will be performed under the direction of Dr. JR Muindi. All samples should be stored at -70⁰F and protected from light. Samples will be immediately centrifuged, batched and frozen in 1-2 cc aliquots of serum for assay of calcitriol and metabolite levels. Serum calcitriol concentrations will be determined using 1, 25-dihydroxyvitamin D₃- [I¹²⁵] RIA kit from DiaSorin Co. (Stillwater, Minnesota). The analytical characteristics of this assay have previously been described

Collection of Biological Samples: If indicated clinically and, at the discretion of the investigator (e.g., for unusual or severe AEs), an additional blood sample (10cc) may be collected.

Documentation

It is essential the following information is recorded:

- Trial number, patient number, date of sample, and the sample collection time;
- The date and actual time the blood sample was taken
- The time the dose of calcitriol was given.

6.0 B: CYP 24 Polymorphisms: CYP 24 Polymorphisms: Five ml of blood for CYP24 polymorphism should be collected before treatment in EDTA tubes and sent to Dr. Candace Johnson's laboratory. DNA will be extracted from the blood using a QIAamp Blood kit. (QIAGEN, Hilden, Germany).

7.0 : SAFETY AND EFFICACY

A.Safety Parameters

(i). Clinical Measurements

Clinical safety measurements will include findings on medical history/update; physical examination, including ECOG performance status, vital signs, height, weight, neurological exam; and assessment of toxicity/symptoms/adverse events. Toxicity at baseline, for each cycle and worst toxicity for a given patient/cycle will be noted. Patients' data will be analyzed for evidence of cumulative toxicity with repeated cycles of therapy. Dose-limiting toxicity for dose escalation is defined above. Toxicities will be assessed and graded, where appropriate, according-to the NCI Common Toxicity Criteria (see Appendix IV). When toxicities cannot be graded according to NCI-CTCAE, they will be graded as mild (asymptomatic), moderate (symptomatic but not interfering significantly with function), or severe (causing significant interference with function) or life threatening.

Cardiovascular toxicity will be monitored by blood pressure and pulse recording prior to and after infusion. ECGs will be performed as clinically indicated. For the purposes of toxicity evaluation, "fluid retention" will be defined as the development of edema or cytologically negative pleural effusion, ascites or pericardial effusion and will be graded as mild, moderate or severe. The definition of "edema" for this study will be edema > trace.

(ii) Laboratory Measurements

Laboratory studies will be performed as described

Myelosuppressive toxicity shall be reported as lowest observed white blood cell (WBC) count, polymorphonuclear neutrophil (PMN) count, and platelet count. Erythropoietic toxicity will be reported as lowest observed hemoglobin. Red blood cell and platelet transfusions will be noted. Every reasonable effort will be made to determine day of onset, day of nadir, and day of recovery for hematotoxicity. Renal and hepatic toxicity will be reported as changes in blood urea nitrogen (BUN), creatinine, AST/SGOT, bilirubin, alkaline phosphatase, and creatinine clearance (as clinically indicated) during a cycle of therapy. Toxicity will be graded according to NCI Common Toxicity Criteria (version 3.0)

7.1 Assessment of Efficacy

Patient Evaluability

Patients who do not receive a full cycle of chemotherapy for reasons other than drug toxicity will not be included in the determination of the MTD and will be replaced. Patients will be considered evaluability for toxicity from the time of the first dose of calcitriol. In the phase II part of the study, eligible patients who receive two courses of treatment and completion of tumor assessment scans after the 2nd course will be considered evaluable for efficacy. Efficacy measurements: will be done by the Response Evaluation Criteria in Solid Tumors (RECIST) (please see appendix VI)

Overall responses for all possible combinations of tumor responses in target and nontarget lesions with or without the appearance of new lesions*

Target lesions	Nontarget lesions	New lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; and PD = progressive disease. See Appendix B for more details.

Responses should be confirmed at a follow-up visit at least 4 weeks later

Primary Efficacy Parameter

The primary efficacy parameter for the phase II trial is tumor response. The same method of assessment and the same staging technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Definition of Measurable and Non-Measurable Lesions: The RECIST criteria of evaluation will be used for all tumor assessment.

At baseline, tumor lesions will be categorized as:

Measurable: lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan or non-measurable: all other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with spiral CT scan) and truly non-measurable lesions. All measurements should be recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

Lesions that are considered as truly non-measurable include the following:

1. Bone lesions
2. Leptomeningeal disease
3. Ascites
4. Pleural / pericardial effusion
5. Inflammatory breast disease
6. Lymphangitis cutis / pulmonis
7. Abdominal masses that are not confirmed and followed by imaging techniques;
8. Cystic lesions

Tumor Response Evaluation**Assessment of Overall Burden and Measurable Disease**

To assess objective response, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in this protocol (except for the phase I part of the study). Measurable disease is defined by the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature could be confirmed by cytology/histology at the discretion of the Investigator.

Baseline Documentation of “Target” and “Non-Target” Lesions

All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all targets

Lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease. All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as “present” or “absent”.

Evaluation of Target Lesions

Complete Response (CR): disappearance of all target lesions.

Partial Response (PR): at least a 30% decrease in the sum of LD of target lesions taking as reference the baseline sum LD.

Progression (PD): at least a 20% increase in the sum of LD of target lesions taking as references the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Stable Disease (SD): neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

Evaluation of Non-Target Lesions

Complete Response (CR): disappearance of all non-target lesions.

Non-Complete Response (non-CR) / Non-Progression (non-PD): persistence of one or more nontarget lesion or/and maintenance of tumor marker level above the normal limits.

Progression (PD): appearance of one or more new lesions. Unequivocal progression of existing nontarget lesions.

Confirmatory Measurement and Duration of Response**Confirmation**

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed at least 4 (anytime between 4 to 8 weeks) weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 4 weeks

Duration of Overall Response

The duration of overall response is measured from the time that measurement criteria are met for complete response or partial response (whichever status is recorded first) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall complete response is measured from the time measurement criteria are first met for complete response until the first date that recurrent disease is objectively documented.

Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for disease progression is met (taking as reference the smallest measurements recorded since the treatment started).

8. 0 DOSE MODIFICATIONS**A) Myelosuppression**

Treatment day parameters: Prior to receiving any dose of docetaxel and cisplatin, patients must have an absolute neutrophil count $\geq 1,500/\text{mm}^3$ and a platelet count of $> 100,000/\text{mm}^3$.

See table below for nadir count dose modifications:

Grade 4 neutropenia associated with fever (one reading of oral temperature $> 38.5^\circ \text{C}$, or three readings of oral temperature $> 38.0^\circ \text{C}$ in a 24-hour period) should be retreated after recovery (according to table below), along with G-CSF at the discretion of the treating physician. G-

CSF is commercially available, and patients requiring G-CSF on this protocol are expected to purchase the agent commercially.

Dose Modification for Cisplatin and Docetaxel: based on nadir hematologic values for preceding cycle.

ANC nadir		PLATELETS	Percent of previous dose
≥500	and	≥50,000	100%
<500	and	≥ 50,000	75%
any	and	<50,000	50%
Recurrence of gr 3 or 4 neutropenia or thrombocytopenia after 2 dose reductions			No further Cisplatin

Any dose reductions are permanent and all future doses of Cisplatin and Docetaxel would be at the modified dose.

B) Abnormal Liver Function Tests (Taxotere®)

Patients who develop abnormal liver function tests while on the study, for any reason, will have the following dose reductions:

Bilirubin		Alkaline phosphatase		SGOT	Action
> ULN	o r	> 5 x ULN	o r	> 5 x ULN	Wait ≤ 3 weeks. If recovered*, reduce docetaxel dose by 25%. If not, off study.
≤ ULN	a n d	≤ 5 x ULN	a n d	1.6 – 5 x ULN	Reduce docetaxel dose by 25%

*Bilirubin ≤ ULN and alkaline phosphatase ≤ 5 x ULN and SGOT ≤ 5 x ULN.

Note: A maximum of two dose reductions per patient are allowed.

ULN = upper limit of normal for institution

C) Stomatitis

If stomatitis is present on day 1 of any cycle, treatment should be withheld until stomatitis has resolved. If Grade 3/4 stomatitis occurs, the dose of docetaxel should be reduced 25% for subsequent cycles.

D) Other non-Hematologic Toxicities

For Grade 3 and 4 toxicities, treatment should be withheld until the toxicity resolves to Grade 1 or less, then reinstated (if medically appropriate) at a 25% dose reduction (for cisplatin, follow table below). If treatment is withheld for longer than three weeks due to Grade 3/4 toxicity, the patient will be withdrawn from the study.

Dose modification for Cisplatin: Other non hematological toxicity

Grade	Cisplatin
0-2	100% of previous dose
3-4	50% of previous dose
Recurrence of gr 3 or 4 after 2 dose reductions	Discontinue treatment

E) Neurosensory toxicity

Grade	Dose for Cisplatin (mg/m ²)
0-1	100% Of previous dose
2	50% of previous dose
3-4	Discontinue Cisplatin*

- If significant tinnitus or hearing loss experienced by pt, Cisplatin should be stopped or reduced according to investigator discretion

Calcitriol dose modifications: Any DLT related to hypercalcemia will result in treatment discontinuation and removal from study. In addition, any grade 3 or greater toxicity attributable to calcitriol alone will warrant a dose modification of calcitriol by 50% and this dose will be the one used for subsequent cycles for that individual patient.

Renal Dysfunction: The serum creatinine will be measured prior to each cycle of therapy. If the entry serum creatinine was <1.4mg/dL, an increase of 0.5 mg/dL will mandate interruption of calcitriol until creatinine returns to within 10% of baseline level. **In the event where creatinine levels increase ≥ 1.5 x baseline, calcitriol therapy will be withheld until creatinine decreases back to < 1.5 x baseline.** A creatinine increase to ≥ 2 mg/dL will mandate interruption of calcitriol until creatinine returns to within 10% of baseline level. Sustained increases (>72 hours) in creatinine of more than double the baseline and >2.5mg/dL will constitute a DLT.

Other Concomitant Therapy with Calcitriol

Caution should be exercised when calcitriol is co-administered with calcium supplements as this might enhance hypercalcemia. All patients will be instructed to discontinue calcium supplementation. Thiazides decrease urinary excretion of calcium and will be a contraindication to this treatment. Since hypercalcemia increases the risk of arrhythmias patients on digoxin will not be allowed on study.

9.0 ANCILLARY THERAPY

A: Required Concomitant medications

Dexamethasone 4mg will be taken orally twice on the day before, the day of and the day after each dose of cisplatin/docetaxel.

Anti-emetics: see section 5.2

Pre and post Cisplatin: Hydration will be according to individual institutional guidelines

B: Use of growth factors: Growth factors will be allowed in the phase II portion of the study per Institutional guidelines.

10.0: STATISTICAL CONSIDERATIONS

10.1: Study Design/Endpoints

The basic design of this Phase I trial utilizes a standard 3-3 dose-finding scheme (shown below). In this scheme, the dose is escalated using cohorts of 3 patients until 2 or more DLTs are observed at a dose level. At this point escalation stops and doses are de-escalated until no more than 1 of 6 patients experiences a DLT. There will be no dose escalation within a patient. The MTD will be determined for calcitriol in combination with fixed doses of cisplatin (75mg/m²) and docetaxel (75mg/m²)

10.2: Sample Size/Accrual Rate: Calcitriol will be dose escalated along with a fixed dose of cisplatin (75mg/m²) and docetaxel (75mg/m²) until DLT is reached and a MTD is defined. Accrual rate will be expected at a rate of 2-3 patients/months or 24-30 patients/year. The dose escalation scheme for this phase I trial is as outlined in the following table.

# patients experiencing DLT/ cohort size	Action
0/3	Increase to next dose level
1/3	Accrue 3 more patients at same dose level
1/3+0/3	Increase to next dose level
1/3+ ≥1/3	Stop: evaluate previous dose for MTD
# patients experiencing DLT/ cohort size	Action
0/3	Increase to next dose level
1/3	Accrue 3 more patients at same dose level
1/3+0/3	Increase to next dose level

Using this dose escalation scheme, the probability of escalating to the next dose level, based on the true probability of dose-limiting toxicity (DLT) at the current dose is given in the following table.

Probability of escalating past dose “i” given dose “i” reached	Probability of Toxicity at the “i” Dose Level for a single patient							
	10%	20%	30%	40%	50%	60%	70%	80%
	0.906	0.709	0.494	0.309	0.172	0.082	0.032	0.009

Assuming all 5 dose levels will be studied, the minimum number of patients to be accrued to this study will be 18.

10.3 Phase II end-points: The goal is to conduct a phase II study to assess activity of Calcitriol, CDDP and Docetaxel in a two-stage sequential design. Early closure is considered only for no evidence of activity (futility). The endpoint is response (CR+PR). The phase II portion of the trial is based on the hypothesis:

$$H_0: P_R \leq 0.25$$

$$H_a: P_R \geq 0.45$$

where P_r is the probability of response. The test statistic is simply the number of responses (S_1 and S_2 , subscript represents the accrual stage) among the number of patients evaluable for response. The null hypothesis is the range of probabilities that are associated with an “inactive” therapeutic effect and the alternative hypothesis is the range of probabilities that are associated with an “active” therapeutic effect. The decision rules for each stage of accrual are described in the table below.

Accrual Stage	Total Accrual Goal	Decision		
		Accept H_0 “Declare Inactive”	Continue to second stage of accrual	Accept H_a “Declare Active”
1	20	≤ 3	4-20	-
2	39 [§]	≤ 13	-	≥ 14

§ Second stage of accrual will require 19 additional patients.

The operating characteristics for these rules are as follows: $\Pr\{\text{reject } H_0|H_0\} = 0.0858$ (i.e., size) and $\Pr\{\text{reject } H_0|H_a\} = 0.904$ (i.e., power). The expected sample size is 34.7 and 38.9 if P_r is equal to 0.25 and 0.45, respectively. The decision rule is the most powerful test as described by Kepner and Chang. *Statistics & Probability Letters* 62:87-92, 2003. The operating characteristics are based on the exact probabilities determined from the joint binomial distribution of the two accrual stages.

10.4 Polymorphism Objective: This secondary objective is to correlate CYP24 polymorphism (major or minor allele) and serum calcitriol exposure. We will use relative odds as a measure of association and calculate the 90% confidence interval. No formal hypothesis testing will be conducted. If both stages of accrual are completed it will provide 39 patients treated at the MTD for the 2x2 table analysis. Polymorphisms with uncommon minor alleles" will be quite limited and the analyses of these alleles can be considered only exploratory.

11.0 ADVERSE EVENTS

Definitions: The definitions of AEs, SAEs, and other significant AEs (OAEs) are given below. It is of the utmost importance that all staff involved in the study is familiar with the content of this section. The principal investigator is responsible for ensuring this.

11.1: Adverse Event (AE) An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g.,

tachycardia, enlarged liver). In clinical trials an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered. If any of the above events occurs, information will be required as to if it is an event possibly due to adverse effect of the study drug or an infection possibly caused with the study drug.

11.2: Serious Adverse Event

A SAE is an AE occurring during any trial phase (i.e., run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfills one or more of the following criteria:

- Results in death
- Is immediately life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent 1 of the outcomes listed above

Any events or hospitalizations that are unequivocally due to progression of disease should not be reported as a SAE. The causality of SAEs (i.e., their relationship to study treatment) will be assessed by the investigators, who in completing the relevant case report form must answer “yes” or “no” to the question: “Do you consider that there is a reasonable possibility that the event may have been caused by the drug?”

11.3: Other Significant Adverse Event: (OAE) will be identified by the DSMB of each institute and if applicable also by the Clinical Study Team Physician during the evaluation of safety data for the Clinical Study Report (CSR). Significant AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the patient from study treatment will be classified as OAEs. Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. For each OAE, a narrative may be written and included in the CSR.

11.4: Recording of Adverse Events using NCI CTCAE version 3.0: Any detrimental change in a patient’s condition, subsequent to entering the trial and during the 30-day follow-up period after the final treatment, should be considered an AE. The development of a new cancer should be regarded as an AE. New cancers are those that are not the primary reason for the administration of the study treatment and have been identified after the patient’s inclusion in this study. Any CTCAE Grade 3 or 4, or CTCAE Grade 1 or 2 hematology or biochemistry laboratory values that are considered not due to tumor progression should be recorded as AEs. Abnormal laboratory tests and other objective measures that meet the criteria for an SAE or result in discontinuation of the trial drug should be recorded. All patients who have CTCAE Grade 3 or 4 laboratory values at the time of withdrawal must be followed up until the laboratory values have returned to CTCAE Grade 1 or 2 or until 30 days after the date of withdrawal (whichever comes first), unless these values are not likely to improve because of the underlying disease. For an AE to be a suspected drug-related event there should be at least a reasonable possibility of a causal relationship between the trial drug and the AE. It is important to distinguish between serious and severe AEs. Severity is a measure of intensity. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not

an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE.

All AEs should be documented. A description of the event, including its date of onset and resolution, whether it constitutes a SAE, any action taken (e.g., changes to study treatment, other treatment given, and follow-up tests) and outcome, should be provided along with the investigator's assessment of causality (the relationship to the study treatment[s]). AEs will also be graded according to the NCI CTCAE, and changes documented.

For an AE to be a suspected drug-related event there should be at least a reasonable possibility of a causal relationship between the study medicinal product and the AE

11.5: Disease Progression: Any events that are unequivocally due to progression of disease must not be reported as AEs.

11.6 Lack of Efficacy: When there is deterioration in the condition for which the study treatment is being used (i.e., solid tumors) there may be uncertainty as to whether this is lack of efficacy or an AE. In such cases, unless the reporting physician considers that the study treatment contributed to the deterioration or local regulations state to the contrary, the deterioration should be considered a lack of efficacy and not an AE.

11.7: Abnormal Vital Signs: If an abnormal laboratory value for vital sign is associated with clinical signs and symptoms, the sign/symptom should be reported as an AE and the associated vital sign should be considered additional information that must be collected on the relevant CRF.

11.8: Pregnancy: Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even if the patient was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

11.9: Handling of Unresolved AEs/SAEs at Completion/Withdrawal: All study-related toxicities and SAEs must be followed until resolution, unless, in the investigator's opinion, the condition is unlikely to resolve due to the patient's underlying disease.

11.91: **Reporting of SAEs**

All AE reports for all clinical trials conducted are reported to the Data Safety Monitoring Board (DSMB) (monthly), and the Institutional Review Board (IRB). For SAEs, reports are faxed to the CRS/CTO at the time of their occurrence or within 24 hours of awareness. Serious unexpected AEs are reported immediately to the IRB as appropriate, in accordance with the UMCCC reporting requirements.

Any clinical study event that is judged to be an AE must be recorded in the medical record during the study. The clinical investigator must evaluate the AE for severity, causal relationship to the test article under study, action taken and outcome. The clinical investigator will sign and date the medical record page. All supportive information is filed in the source documents. Within 5 working days, the research nurse or designee will submit a MedWatch report to the CTO/Clinical Research Services. Routine AEs will be reported in the annual report to the IRB. All SAEs will be reported by the clinical investigator to the CTO/CRS (within 24 hours of awareness) for accurate and timely recording and reporting. The SAE is also recorded on the medical chart, managed medically as appropriate, and the

event is followed until resolution. The clinical investigator is responsible for grading the seriousness of the event and for immediately notifying the Coordinating Center as well as the CRS office for referral to the IRB as appropriate. All information available on the event (hospital records, lab tests, discharge summaries, etc.) is forwarded to the CTO/CRS as applicable.

A complete written SAE report must be sent with a cover page indicating the following:

- Drug Name; Calcitriol
- The investigator's name and address
- The trial name

All SAEs will be documented. The investigator is responsible for informing the Coordinating Center, and the site IRB as per local requirements.

12.0: DATA SAFETY MONITORING PROCEDURES

The Data and Safety Monitoring Board (DSMB) of The University of Michigan Comprehensive Cancer Center is the DSMB for this study responsible for monitoring the study's scientific progress, accrual and any serious adverse events.

Each participating site is required to have its own Data and Safety Monitoring Committee (DSMC) for the study composed of *the local site principal investigator, site co-investigators, site data managers and other members of the study staff involved in the conduct of the trial.* During the committee's *monthly or more frequently depending on the activity of the study* meeting, the principal investigator will discuss matters related to:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (AE reporting)
- Adherence to protocol (potential or real protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

These meetings are to be documented by the *site coordinator or Data Manager* using the Protocol Specific Data and Safety Monitoring Report (DSMR), signed by the site principal investigator *or co-investigator* and submitted to the Clinical Trials Office of The University of Michigan Comprehensive Cancer Center on a quarterly basis together with other pertinent documents.

Similarly, protocol deviations are to be documented using the *Notice of Protocol Deviation Form* and requires the signatures of both the *site coordinator or Data Manager* and the site principal investigator *or co-investigator*. These reports are to be sent to the Clinical Trials Office *within 5 business days of the event and on a monthly basis with the Protocol Specific Data and Safety Monitoring Report.*

13.0.: TRIAL MANAGEMENT

13A. Subject Screening and Registration Procedure

Patient registration for this trial will be centrally managed by the Clinical Trials Office of The University of Michigan Comprehensive Cancer Center as described below:

A potential study subject who has been screened for the trial and who has signed the Informed Consent document will be initially documented by the participating site on the Screening and Enrollment Log provided by the Clinical Trials Office.

It is the responsibility of the local site investigator to determine patient eligibility prior to submitting patient registration request to the Clinical Trials Office. After patient eligibility has been determined, a copy of the **completed** Eligibility Worksheet together with all the pertinent source documents will be submitted by the requesting site to the Clinical Trials Office, either by fax or by email.

Multi-site Coordinator Fax: 734-936-9582

Multi-site Email: CTO-Multisite@med.umich.edu

A Multi-Site Coordinator of the Clinical Trials Office, who acts as the registrar, will review the submitted documents and process the registration. Subsequently, an email will be sent by the registrar to the requesting site registrar to confirm patient registration and to provide the study identification number that has been assigned to the patient. In addition, a copy of the completed Section Two of the Eligibility Worksheet signed and dated by the registrar, will be faxed back to the requesting site registrar.

Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log. These patients will not have study identification number assigned to them, and will not receive study treatment.

13B: Clinical Monitoring Procedures

This study will be monitored by a representative of the Clinical Trials Office of The University of Michigan Comprehensive Cancer Center. Monitoring visits will be made during the conduct of the study and at study close-out.

Prior to subject recruitment, a participating site will undergo site initiation meeting to be conducted by the Clinical Trials Office. This meeting can be done either in an actual site visit, teleconference, videoconferencing, or web-based meeting. As a general rule, a site initiation meeting is conducted when the Study Reference Binder has been made and completed by the site. The site's principal investigator and his study staff should make every effort in attending the site initiation meeting.

During the entire conduct of the study, monitoring visits will be made depending on the rate of enrollment, time frame between subject visits, number of subjects enrolled in the study, and data entry cut-off dates. The purpose of these visits is to verify:

- Adherence to the protocol
- Completeness and accuracy of study data collected
- Proper storage, dispensing and inventory of study medication
- Compliance with regulations

These visits may be in the form of a site visit or a review of the documents at the Clinical Trials Office. Thus, during a monitoring visit to a site, access to relevant hospital or clinical records must be given by the site investigator to the Clinical Trials Office representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. In the latter case, a site will be required to ship or fax study documents to be reviewed to the Clinical Trials Office.

The Clinical Trials Office expects the relevant investigational staff to be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents, especially for monitoring visits conducted at the site. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site.

Participating site will also undergo a site close-out upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and that the site investigator is aware of his/her ongoing responsibilities. In general, a site close-out is conducted during a site visit; however, site close-out can occur without a site visit if all of the following apply:

- No patient has signed the Informed Consent Form and has enrolled into the study
- Investigational agent has not been dispensed
- All investigational agent and materials have been returned as defined for the study or destroyed and accounted for properly.

13 C: Quality Assurance and Audits

In addition to the clinical monitoring procedures, the Quality Assurance Review Committee (QARC) of The University of Michigan Comprehensive Cancer Center provides assurance that trials are conducted and study data are collected, documented and reported in compliance with the protocol, Good Clinical Practices (GCP) Guidelines and regulatory requirements by performing annual quality assurance audits. These audits may be conducted in one of two ways:

- On-site audit of study records, including source documents
- Audit of study records and source documents at the Clinical Trials Office

In the latter case, participating sites would be required to ship the documents to be audited to the Clinical Trials Office.

All audit findings are reported by QARC to the Data and Safety Monitoring Board. These findings are followed-up by the DSMB until they have been resolved.

The DSMB can also request QARC for a ‘for cause’ audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the Clinical Trials Office that such a request has been made.

Changes to the Protocol

If it is necessary for the trial protocol to be amended, the amendment or a new version of the trial protocol must be approved by the IRB. If a protocol amendment requires a change to a particular center’s Written Informed Consent Form. The IRB must be notified. Approval of the revised Written Informed Consent Form by the IRB is required before the revised form is used.

Ethics

The principal investigator(s) is also responsible for providing the IRB with reports of any SAEs from any other trial conducted with the investigational product. The trial will be performed in accordance with Good Clinical Practice.

Procedures in Case of a Medical Emergency

The principal investigator(s) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study.

14.0: STUDY MEDICATIONS

Drug handling precautions for cytotoxic drugs should be followed. Avoid contact or inhalation.

A. Docetaxel (Taxotere®)

Docetaxel (RP 56976, Taxotere®) is a semi-synthetic novel drug derived from a precursor extracted from needles of the European yew tree, *Taxus baccata*. This compound belongs to a class of antimitotic agents, the taxanes, and acts as a spindle poison. In vitro, docetaxel promotes microtubule assembly and inhibits disassembly, thus stabilizing microtubules. This mode of action is essentially opposite to that of the vinca alkaloids.

HOW SUPPLIED:

TAXOTERE for Injection Concentrate is supplied in a single-dose vial as a sterile, pyrogen-free, non-aqueous, viscous solution with an accompanying sterile, non-pyrogenic, diluent (13% ethanol in Water for Injection) vial. The following strengths are available: Taxotere will be obtained from commercial supply and will not be reimbursed by the study

TAXOTERE 80 MG (NDC 0075-8001-80)

TAXOTERE (docetaxel) 80 mg Concentrate for Infusion: 80 mg docetaxel in 2 mL polysorbate 80 and diluent for TAXOTERE 80 mg. 13% (w/w) ethanol in Water for Injection. Both items are in a blister pack in one carton.

TAXOTERE 20 MG (NDC 0075-8001-20)

TAXOTERE (docetaxel) 20 mg Concentrate for Infusion: 20 mg docetaxel in 0.5 mL polysorbate 80 and diluent for TAXOTERE 20 mg. 13% (w/w) ethanol in Water for Injection. Both items are in a blister pack in one carton.

TAXOTERE® infusion solution, if stored between 2 and 25°C (36 and 77°F) is stable for 4 hours. Fully prepared TAXOTERE® infusion solution (in either 0.9% Sodium Chloride solution or 5% Dextrose solution) should be used within 4 hours (including the 1 hour i.v. administration).

TAXOTERE for Injection Concentrate requires two dilutions prior to administration. Please follow the preparation instructions provided below. **Note:** Both the TAXOTERE for Injection Concentrate and the diluent vials contain an overfill.

Preparation of the Initial Diluted Solution

1. Gather the appropriate number of vials of TAXOTERE for Injection Concentrate and diluent (13% Ethanol in Water for Injection). If the vials were refrigerated, allow them to stand at room temperature for approximately 5 minutes.
2. Aseptically withdraw the contents of the appropriate diluent vial into a syringe and transfer it to the appropriate vial of TAXOTERE for Injection Concentrate. **If the procedure is followed as described, an initial diluted solution of 10mg docetaxel/mL will result.**
3. Gently rotate the initial diluted solution for approximately 15 seconds to assure full mixture of the concentrate and diluent.
4. The initial diluted TAXOTERE solution (10 mg docetaxel/mL) should be clear; however, there may be some foam on top of the solution due to the polysorbate 80. Allow the solution to stand for a few minutes to allow any foam to dissipate. It is not required that all foam dissipate prior to continuing the preparation process.

The initial diluted solution may be used immediately or stored either in the refrigerator or at room temperature for a maximum of 8 hours.

Preparation of the Final Dilution for Infusion

1. Aseptically withdraw the required amount of initial diluted TAXOTERE solution (10 mg docetaxel/mL) with a calibrated syringe and inject into a 250 mL infusion bag or bottle of either 0.9% Sodium Chloride solution or 5% Dextrose solution to produce a final concentration of 0.3 to 0.74 mg/mL.

If a dose greater than 200 mg of TAXOTERE is required, use a larger volume of the infusion vehicle so that a concentration of 0.74 mg/mL TAXOTERE is not exceeded.

2. Thoroughly mix the infusion by manual rotation.
3. As with all parenteral products, TAXOTERE should be inspected visually for particulate matter or discoloration prior to administration whenever the solution and container permit. If the TAXOTERE for Injection initial diluted solution or final dilution for infusion is not clear or appears to have precipitation, these should be discarded.

The final TAXOTERE dilution for infusion should be administered intravenously as a 1-hour infusion under ambient room temperature and lighting conditions.

Visual inspection: As with all parenteral products, docetaxel should be inspected visually for particulate matter or discoloration prior to administration whenever the solution and container permit. If docetaxel premix solution or infusion solution is not clear or appears to have precipitation, the solution should be discarded.

Recommendations for safe handling: Docetaxel is an antineoplastic agent and, as with other potentially toxic compounds, caution should be exercised when handling it and preparing

docetaxel solutions. The use of gloves is recommended. If docetaxel concentrate, premix solution or infusion solution should come into contact with skin, wash immediately and thoroughly with soap and water. If docetaxel concentrate, premix solution or infusion solution should come into contact with mucous membranes, wash immediately and thoroughly with water.

Note: In order to minimize patient exposure to the plasticizer DEHP [di-(2-ethylhexyl) phthalate] which may be leached from PVC, docetaxel should be mixed in non-PVC IV bags and administered through non-PVC lined administration sets.

B. CISPLATIN

Description

A platinum-containing inorganic complex that contains a platinum atom surrounded in a plane by 2 chloride atoms and 2 ammonia molecules in the cis position (DDP; cis-DDP; Cis-Diamminedichloroplatinum; cis-Platinum II).

Source

Cisplatin will be obtained from commercial supply and will not be reimbursed by the study. A total dose of 75 mg/m² of cisplatin will be diluted to a volume of 500 mL with 0.9% sodium chloride prior to infusion. Once reconstituted, the cisplatin solution should not be refrigerated. Prior to the administration of Cisplatin, the patient will be adequately hydrated.

Side Effects

Very Common Side Effects:

nausea, vomiting, mild to moderate decreases in kidney function, and imbalances in the level of some minerals in the blood (especially magnesium);
decreased white blood cells which can lead to infection; decreased red blood cells which can lead to anemia (feelings of being tired and loss of energy) and decreased platelet count, which can lead to bruising and bleeding after injury;

Common Side Effects:

Numbness and loss of taste

Less Common Side Effects:

heart problems, thinning or loss of body hair, mouth sores;
abnormal liver function tests (blood tests to show how your liver is working);
hearing loss, and eye problems

Rare but Life-threatening Side Effects:

allergic reactions, kidney failure, and possible death

C. Calcitriol (Generic calcitriol 1mcg/ml) is a commercially available source of Vitamin D. Calcitriol works by binding to VDR which subsequently triggers cellular pathways that may stop cancer cell growth, leading to cancer cell death. The IV formulation can result in higher therapeutic blood levels than those achieved with previous forms of calcitriol. **Expected Toxicities of Calcitriol:** Commonly seen side effects with high dose calcitriol include hypercalcemia, hypercalciuria, and hyperphosphatemia. Clinical toxicity has been mostly linked to the level and duration of associated hypercalcemia. Early signs of hypercalcemia include weakness, headache, somnolence, nausea, vomiting, dry mouth, constipation, muscle pain, bone pain, metallic taste, and anorexia. Late signs include polyuria, polydipsia, anorexia, weight loss, nocturia, conjunctivitis (calcific), pancreatitis, photophobia, rhinorrhea, pruritus,

hyperthermia, decreased libido, elevated blood urea nitrogen (BUN), albuminuria, hypercholesterolemia, elevated aspartate transaminase (AST) and alanine transaminase (ALT), ectopic calcification, nephrocalcinosis, hypertension, cardiac arrhythmias, dystrophy, sensory disturbances, dehydration, apathy, arrested growth, urinary tract infections, and, rarely, overt psychosis. Chronic hypercalcemia has also been associated with an increase in creatinine levels in patients with normal baseline renal function. Hypersensitivity reactions to calcitriol are rare but have been reported. One case of allergic reaction and another case of erythema multiforme have been confirmed by rechallenge.

Availability: IV Calcitriol is commercially available. It will be provided free of charge to the patients for this protocol.

Drug ordering: Calcitriol will be ordered by the Investigational Drug Service through the appropriate grant. IDS will send a request to the purchasing department. **Agent inventory records:** The IDS will maintain inventory and dispensing records on the NIH2564DARF.

Preparation: Add calcitriol to 0.9% sodium chloride (non-PVC) to achieve a final concentration < 0.5 mcg/ml. Final preparation is stable for 3 hours (personal communication Johnson lab).

Administration: Calcitriol will be infused IV over 1 hour.

D. Dexamethasone: Dexamethasone – oral tab, available in 4 mg tablets is commercially available. The dexamethasone dose in this trial is intended to prevent allergic reactions to docetaxel, as part of the anti-emetic regimen as well as to mitigate calcitriol associated hypercalcemia

Expected Toxicities: Adverse reactions to dexamethasone are those associated with glucocorticoid and mineralocorticoid use. Most occur with prolonged and high dose therapy and are somewhat reduced in patients receiving a short 3 day course around chemotherapy. The dexamethasone dose in this trial is intended to prevent allergic reactions to docetaxel, as part of the anti-emetic regimen as well as to mitigate calcitriol associated hypercalcemia. It is anticipated to have limited potential to cause any of the following side effects: electrolyte disturbances, fluid retention, and exacerbation of congestive heart failure increased risk of infection (esp. mucocutaneous candidiasis), polyuria, fluid retention, “moon facies”, centripetal obesity, mood alterations (depression or anxiety). Long term use may cause myopathy, osteoporosis, impaired wound healing, fragile skin, and Cushingoid habitus. Patients with mild glucose intolerance may develop frank diabetes, and glaucoma can be precipitated in patients with increased intraocular pressure. Mood disturbances and increased appetite are also commonly reported.

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**APPENDIX I:
INTERNATIONAL STAGING SYSTEM FOR LUNG CANCER (1997)**

TNM Definitions

Primary Tumor (T) N = Regional Lymph Nodes M = Distant Metastasis

TX: Tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized roentgenographically or bronchoscopically, or primary tumor cannot be assessed.

T0: No evidence of primary tumor.

Tis: Carcinoma *in situ*.

T1: A tumor = 3.0 cm in greatest dimension, surrounded lung or visceral pleura, without evidence of invasion proximal to a lobar bronchus at bronchoscopy.*

T2: A tumor more than 3.0 cm in greatest dimension, or a tumor of any size that either invades the visceral pleura or has associated atelectasis or obstructive pneumonitis extending to the hilar region. At bronchoscopy, the proximal extent of demonstrable tumor must be within a lobar bronchus or at least 2.0 cm distal to the carina. Any associated atelectasis or obstructive pneumonitis must involve less than an entire lung.

T3: A tumor of any size with direct extension into the chest wall (including superior sulcus tumors), diaphragm, or the mediastinal pleura or pericardium without involving the heart, great vessels, trachea, esophagus or vertebral body, or a tumor in the main bronchus within 2 cm of the carina without involving the carina.

T4: A tumor of any size with invasion of the mediastinum or involving heart, great vessels, trachea, esophagus, vertebral body or carina or presence of malignant pleural effusion.*

NOTE:

T1* The uncommon superficial tumor of any size with its invasive component limited to the bronchial wall which may extend proximal to the main bronchus is classified as T1.

T4* Most pleural effusions associated with lung cancer are due to tumor. There are, however, a few patients in whom multiple cytopathologic examinations of pleural fluid are negative for tumor. The fluid is non-bloody and is not an exudate. In such cases where those elements and clinical judgment dictate that the effusion is not related to the tumor, the patient should be staged T1, T2 or T3, excluding effusion as a staging element.

Pericardial effusions are classified according to the same rules.

Nodal Involvement (N)

NX: Regional lymph nodes cannot be assessed.

N0: No metastasis to regional lymph nodes.

N1: Metastasis to lymph nodes in the ipsilateral peribronchial and/or the ipsilateral hilar lymph nodes, and intrapulmonary nodes involved by direct extension to the primary tumor.

N2: Metastasis to ipsilateral mediastinal lymph nodes and/or subcarinal lymph nodes.

N3: Metastasis to contralateral mediastinal lymph nodes, contralateral hilar lymph nodes, ipsilateral or contralateral scalene or supraclavicular lymph nodes.

Distant Metastasis (M)

MX: Presence of distant metastasis cannot be assessed.

M0: No distant metastasis.

M1: Distant metastasis present separate metastatic tumor nodules in the ipsilateral non-primary-tumor

lobe(s) of the lung are classified M1.

Staging Group (1997)

OccultCarcinomaTX N0 M0
Stage 0 *Tis* Carcinoma in situ
Stage 1A T1 N0 M0
Stage IB T2 N0 M0
Stage IIA T1 N0 M0
Stage IIB T2 N1 M0
Stage IIIA T1-3,N1N2M0
Stage IIIB Any T,T4,N3M0
Stage IV Any T Any N M1

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Appendix II

ECOG PERFORMANCE STATUS SCALE

Point Description

0 Fully active, able to carry on all pre-disease performance without restriction

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work

2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours

3 Capable of only limited self-care, confined to bed or chair more than 50 % of waking hours.

4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

APPENDIX III:

Cockcroft Gault formula

$$*CCC = \frac{(140 - \text{age}) \times \text{wt. in kg (Actual Body Weight)}}{72 \times \text{Serum Creatinine}} \times 0.85(\text{female}) \text{ or } 1.00(\text{male})$$

Appendix IV. Common Toxicity Criteria (CTCAE)

This study will utilize the Common Terminology Criteria for Adverse Events v3.0 (CTCAE) for toxicity and Adverse Event reporting. It can be downloaded from the CTEP home page (<http://ctep.nih.gov>). All appropriate treatment areas will have access to a copy of the CTCAE v3.0.

**Appendix V: RECIST (Response Evaluation Criteria in Solid Tumors) CRITERIA
(Therasse, P et al, JNCI, Vol 92, No 3, pp 205)**

Evaluation of target lesions. taking into account the measurement of the longest diameter only for all target lesions:

Complete response—the disappearance of all target lesions;

Partial response—at least a 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter;

Progressive disease—at least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the treatment started or the appearance of one or more new lesions;

Stable disease—neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment started

Appendix VI: Calculation for Dosing Weight

$$\text{Dosing weight} = \text{IBW} + 0.4(\text{TBW} - \text{IBW})$$

$$\text{IBW (male)} = 50 + (0.91 \times \text{ht (cm)} - 152)$$

$$\text{IBW (female)} = 45 + (0.91 \times \text{ht (cm)} - 152)$$

Appendix VII - Laboratory studies

Pharmacogenomics (collection and storage): At the baseline visit, 5 ml of blood will be collected in a EDTA tube. Whole blood should be transferred to a plastic tube and frozen at -80°C. Samples from VA Hospital and UM will be stored at the Brenner laboratory at the UMCC (2150 CC) and then shipped to Dr. Johnson's laboratory every 4 months:

Attn: Candace Johnson, PhD
Roswell Park Cancer Institute
Elm and Carlton Streets
Buffalo, NY 14263

Pharmacogenomics assays: CYP24 pharmacogenomics assays will be performed under the direction of Dr. A. Adjei. Analysis of common variant alleles for CYP 24, the common metabolizing enzyme for calcitriol will be conducted. **CYP 24 Polymorphisms methodology:** Five ml of blood for CYP24 polymorphism should be collected at baseline, before treatment only in EDTA tubes. DNA will be extracted from the blood using a QIAamp Blood kit (QIAGEN, Hilden, Germany). Genomic DNA will be extracted from patient samples using the Trizol Reagent (Invitrogen, Carlsbad, CA) and the exon 9 -intron -exon10 region will be amplified by PCR. Primers used are Forward: **ctg tga tca tgc tgc ctc tt** and Reverse: **ggt tgc caa cat gcg ctg ag**. The amplified DNA band will be visualized on ethidium bromide stained agarose gels, extracted from gel, purified using a Qiagen DNA gel extraction kit (Qiagen, Germantown, MD) and sequenced on an Applied Biosystems 3130xl Genetic Analyzer using BigDye Terminator v3.1 sequencing chemistry. Analysis will be performed using Applied Biosystems Sequencing Analysis v 5.2 software (RPCI Biopolymer facility, Buffalo, NY). SNPs will be identified as double-peaks sequences on the electrophoregram data.

Calcitriol PK sample collection and storage: An exploratory pharmacokinetic sub study will be carried out in the phase II portion of the study using a limited sampling technique. Calcitriol levels will be drawn at 0; immediately following calcitriol infusion; 2 and 4 hours after the start of calcitriol infusion. Day -1 pharmacokinetics will reflect the pharmacokinetics of calcitriol and its metabolites when calcitriol is given alone. A minimum of seven (7) mL of whole blood will be drawn at pretreatment and at each of the subsequent points listed above. All samples should be stored at -70°F and protected from light. Samples will be centrifuged, batched and frozen in 1-2 cc aliquots of serum for assay of calcitriol and metabolite levels. For samples collected at VA Hospital and UM, this will occur at the Brenner Laboratory at UMCC (2150 CC). Batch shipment will occur to Candace Johnson's laboratory.

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Buffalo, NY 14263

Calcitriol PK Methodology: Serum calcitriol assays will be performed under the direction of Dr. JR Muindi in Dr Candace Johnson's laboratory. Serum calcitriol concentrations will be determined using 1, 25-dihydroxyvitamin D₃- [¹²⁵I] RIA kit from Dias Orin Co. (Stillwater, Minnesota). The analytical characteristics of this assay have previously been described.