

Review

Calcitriol in the Treatment of Prostate Cancer

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Abstract. *Calcitriol, the principal active metabolite of vitamin D and a naturally occurring hormone, showed significant antineoplastic activity in pre-clinical models of prostate cancer and many other tumor types. These antineoplastic effects were observed at calcitriol concentrations substantially above the physiological range. While a number of mechanisms of action have been postulated, the induction of apoptosis and inhibition of proliferation have been most extensively reported. These pre-clinical findings motivated several investigators to pursue a series of clinical trials to examine the potential of targeting the vitamin D receptor for cancer treatment using calcitriol. Initial studies tested daily dosing of calcitriol and showed that substantial dose escalation was not feasible due to hypercalciuria and/or hypercalcemia. In contrast, weekly dosing of calcitriol allowed substantial dose escalation without dose-limiting toxicities. Notably, however, the commercially available formulation of calcitriol exhibited non-linear pharmacokinetics at the highest doses tested. While substantially higher concentrations were achieved, the maximum tolerated dose was not established due to this pharmacological limitation. Intermittently-dosed calcitriol was then combined with several antineoplastic agents, including steroids, bisphosphonates and chemotherapeutic agents. The activity seen in a phase II study of weekly calcitriol plus docetaxel was particularly encouraging and led to the development of DN-101, a proprietary formulation designed for cancer treatment. DN-101 in combination with docetaxel is being evaluated in a placebo-controlled randomized clinical trial that has completed accrual.*

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Calcitriol is the natural ligand for the vitamin D receptor. Extensive pre-clinical work (reviewed in 1-3) has demonstrated the antineoplastic activity of calcitriol. Briefly, the mechanisms for this activity include induction of apoptosis as well as cell cycle arrest and differentiation (4-8). In addition, calcitriol inhibited proliferation, invasion and angiogenesis of tumors in animal studies (9-11). Two important pre-clinical findings have influenced the clinical development of calcitriol-based cancer therapies. First, antineoplastic effects invariably occur at calcitriol concentrations that are substantially above the physiological range. Thus, much of the clinical research has focused on developing methods to safely escalate the dose of calcitriol to achieve potentially therapeutic blood concentrations. Second, a number of investigators have shown that the activity of calcitriol is additive or synergistic with other anticancer agents including steroids, as well as cytotoxic chemotherapy drugs (12-17). As a result, combination therapy has been pursued in clinical trials.

Phase I studies of daily calcitriol in prostate cancer (Table I)

Initial clinical trials primarily focused on testing the feasibility of escalating the dose of calcitriol without substantially altering its usual dosing schedule. Daily oral dosing of calcitriol of 0.5 to 1.5 µg in eleven hormone-refractory prostate cancer patients resulted in no PSA responses, and hypercalcemia was the predominant and dose-limiting toxicity (18). Another study evaluated daily calcitriol in seven early recurrent prostate cancer patients. Doses ranged from 0.5 to 2.5 µg. No PSA responses were seen, although the rate of rise of PSA with therapy was significantly reduced when compared to the pre-treatment rate (19). Dose escalation was limited by hypercalciuria. The effect on PSA kinetics observed in this trial is difficult to interpret in the absence of a control group, but is suggestive of an effect either on the tumor or on PSA production.

Table I. Phase I studies of frequently dosed calcitriol.

Study (Ref.)	Route and schedule	Maximum tolerated dose	Dose-limiting toxicity	Highest peak calcitriol concentration achieved	Comments about activity
Osborn <i>et al.</i> (18)	Daily oral dose of 0.5 – 1.5 µg	1.5 µg	Hypercalcemia	NR	None observed
Gross <i>et al.</i> (19)	Daily oral dose of 0.5 – 2.5 µg	2.5 µg	Hypercalciuria	NR	PSA slope changes
Smith <i>et al.</i> (3)	<i>s.c.</i> inj. alternate days, 2.0 – 10 µg	10 µg	Hypercalcemia	0.7 nM	NR

NR=not reported.

Phase I study of calcitriol administered on alternate days (Table I)

Subcutaneous injection of calcitriol at doses of 2.0 to 10.0 µg to patients with advanced malignancies also resulted in hypercalcemia as the dose-limiting toxicity, and the majority of patients developed progressive disease (3). Peak blood calcitriol concentrations of approximately 0.7 nM were reached with dose escalation to 8 µg on alternate days.

Phase I studies of calcitriol using intermittent dosing (Table II)

Weekly dosing monotherapy. Weekly oral dosing of calcitriol was pursued as a strategy to reduce toxicity while permitting dose escalation to therapeutic levels. Doses that ranged from 0.06 µg/kg to 2.8 µg/kg were tested in a phase I study. This approach allowed significant dose escalation and produced peak blood calcitriol concentrations that ranged from 3.7 to 6.0 nM at the highest doses. Potentially therapeutic peak calcitriol concentrations (C_{max}) were reached, although C_{max} and the area under the concentration curve (AUC) did not increase linearly at doses above 0.48 µg/kg (20). No dose-limiting toxicity was encountered, thus the maximum tolerated dose was not determined. This study demonstrated that substantial dose escalation is feasible with weekly dosing, but also showed that key pharmacokinetic parameters do not increase linearly over a range of doses, identifying a new challenge in the development of calcitriol as a cancer drug.

Dosing three consecutive days out of seven. In an alternative intermittent dosing approach, a phase I trial of calcitriol combined with paclitaxel, evaluated calcitriol at doses up to 38 µg on three consecutive days every seven days without dose-limiting toxicity (21). As in the weekly dosing study, the calcitriol AUC did not increase linearly with increasing dose. Calcitriol C_{max} ranged from 1.4 to 3.5 nM at the highest doses tested.

Dosing three consecutive days out of seven with zoledronate and dexamethasone. Considerable pre-clinical evidence supported

testing the combination of calcitriol with dexamethasone. Dexamethasone enhances the antineoplastic effects of 1,25(OH)₂D₃ *in vitro* and *in vivo* (12, 13). Cell cycle arrest, reduction of phospho-Erk1/2 and phospho-Akt levels, and apoptosis were enhanced when dexamethasone was added to vitamin D (12, 13, 22). Similarly, the inhibition of tumor-derived endothelial cell growth was increased when dexamethasone and calcitriol were combined (23).

Zoledronate is a bisphosphonate approved for the treatment of humoral hypercalcemia of malignancy and was recently approved for reduction of skeletal complications of metastatic hormone-refractory prostate cancer. Thus, a strong rationale existed for testing the combination of calcitriol with dexamethasone and zoledronate (24).

Thirty-one patients were treated with calcitriol doses that ranged from 4 µg to 30 µg per day administered for three consecutive days every seven days along with zoledronate 4 mg *i.v.* monthly (24). Dexamethasone could be added upon disease progression, which occurred in seven patients. Three patients had dose reductions due to hypercalcemia. No maximum tolerated dose was determined. Peak plasma levels ranged from 0.9 to 2.3 nM. No convincing antitumor activity was seen for the combination of calcitriol and zoledronate, however one patient achieved a ≥50% decline in serum PSA after the addition of dexamethasone.

Dosing every three weeks with docetaxel and estramustine. Until the results of TAX327 had been published (25) and docetaxel with prednisone became the standard regimen for androgen-independent prostate cancer, the combination of docetaxel with estramustine was a regimen that was expected to replace mitoxantrone and prednisone. In order to test the safety of calcitriol together with this combination, our group carried out a phase I/II study. Twenty-four patients were treated on day 1 with 60 µg calcitriol orally, 280 mg estramustine orally three times a day on days 1 to 5, and 70 mg/m² docetaxel on day 2 every 21 days for up to 12 cycles (26). The patients also received 325 mg aspirin and 1 or 2 mg warfarin orally daily. A dose de-escalation scheme was planned in the event of higher than expected toxicity, but was not invoked. The regimen was well tolerated with four out of 24 patients developing

Table II. Phase I studies of intermittently-dosed calcitriol.

Study (Ref.)	Dose and schedule of oral calcitriol	Companion drugs	Maximum tolerated dose	Dose-limiting toxicity	Highest peak calcitriol concentration achieved
Beer <i>et al.</i> (20)	0.06 to 2.8 µg/kg weekly	None	Not determined	None	3.7 to 6.0 nM
Muindi <i>et al.</i> (21)	4 to 38 µg daily for 3 days every 7 days	Paclitaxel 80 mg/m ² weekly	Not determined	None	1.4 to 3.5 nM
Morris <i>et al.</i> (24)	4 to 30 µg daily for 3 days every 7 days	Zoledronate 4 mg <i>i.v.</i> monthly; dexamethosone 0.75 mg BID added at progression	Not determined	None	0.9 to 2.3 nM
Tiffany <i>et al.</i> (26)	60 µg every 21 days	Estramustine 280 mg on days 1 to 5, and docetaxel 70 mg/m ² on day 2	Not determined	None	Not determined

asymptomatic and self-limited hypercalcemia. The regimen was active in chemotherapy-naïve patients, but only one out of eleven patients previously treated with docetaxel had a brief response to therapy.

Phase II evaluation of weekly calcitriol as monotherapy

Twenty-two patients with rising PSA after prostatectomy or radiation were treated weekly with 0.5 µg/kg of calcitriol for a median of 10 months in a phase II study (27). In addition to evaluating the safety of the long-term administration of weekly calcitriol, the study examined the impact that pulse calcitriol may have on prostate carcinoma progression as measured by PSA response. No grade 3 or 4 toxicities were seen, and there were no confirmed PSA responses. Three patients had confirmed PSA reductions of 47%, 28% and 10%. An additional three patients had significantly prolonged PSADT increased by 559%, 353% and 143%. Peak calcitriol concentrations, measured in a subset of patients, reached an average of 2 nM. While tantalizing, the meaning of the changes in PSA kinetics observed in this study remains uncertain. A randomized clinical trial would be needed to determine whether this regimen indeed slows the progression of biochemically recurrent prostate cancer.

Phase II studies of calcitriol in combination with cytotoxic chemotherapy (Table III)

Phase II study of calcitriol and carboplatin in metastatic AIPC. Based on pre-clinical data demonstrating synergy between vitamin D receptor ligands and platinum compounds (16), a phase II study of calcitriol and carboplatin administered on an every 4-week schedule was developed. Seventeen patients androgen-independent prostate cancer (AIPC) received oral calcitriol of 0.5 µg/kg on day 1 and intravenous carboplatin at

an AUC dose of 7 (6 in patients with prior radiation) on day 2, repeated every 4 weeks (2). Only one of 17 patients achieved a PSA response. Toxicity associated with the treatment was mild and similar to single-agent carboplatin toxicity. Thus, while this study provided additional safety data for calcitriol, it did not demonstrate an increase in efficacy for the combination over that expected for carboplatin alone. Notably, unlike the subsequent study with docetaxel, calcitriol was administered only once every 4 weeks in this clinical trial. It is not known if such infrequent administration contributed to the lack of efficacy of the combination.

Phase II study of calcitriol and docetaxel in metastatic AIPC. Based on preclinical evidence supporting the combination of calcitriol with taxanes (14, 15), a phase II trial of the combination of high-dose weekly calcitriol and docetaxel in chemotherapy-naïve metastatic AIPC was developed (28). Thirty-seven patients were treated with oral calcitriol on day 1, followed by docetaxel 36 mg/m² intravenously on day 2. Treatment was administered weekly for six consecutive weeks on an 8-week cycle until disease progression, patient request to withdraw, or unacceptable toxicity. Standard dexamethasone premedication was given with docetaxel to prevent allergic reactions and fluid retention; dexamethasone may also reduce hypercalcemia. The study examined the PSA response maintained on two consecutive evaluations at least 4 weeks apart. Secondary end-points included response in measurable disease by RECIST criteria, time to progression, toxicity and survival. Calcitriol and docetaxel pharmacokinetics were carried out in a subset of patients.

The toxicity experienced by patients in this study was similar to that with docetaxel treatment alone. The grade 3 or greater treatment-related toxicities were mostly hematological events: 41% of patients had leukopenia, 24% had neutropenia and 3% had anemia. Twenty-four percent of patients had hyperglycemia that was associated with the

Table III. Phase II studies of weekly calcitriol in combination with chemotherapy.

Study (Ref.)	Dose and schedule of oral calcitriol	Companion drugs	Number of patients	Efficacy results	Interpretation
Beer <i>et al.</i> (28)	0.5 µg/kg administered 24 hours prior to carboplatin	Carboplatin AUC of 7 (6 in patients with prior radiation) every 28 days	17	1 out of 17 patients had a PSA response	Negative phase II study
Beer <i>et al.</i> (23)	0.5 µg/kg administered 24 hours prior to each dose of docetaxel	docetaxel 36 mg/m ² weekly for 6 consecutive weeks repeated every 8 weeks	37	81% had PSA response. 8 out of 15 responded in measurable disease. Median overall survival 19.5 months	Positive phase II study

dexamethasone premedication. Eleven percent of patients had peptic ulcers, and 8% had pneumonia. Three patients experienced hypercalcemia of grade 1 or 2. One patient died of treatment-related pneumonitis.

Eighty-one percent of patients had a confirmed >50% reduction in serum PSA. Fifteen patients had measurable disease, while a confirmed partial response by RECIST criteria was seen in eight. The median time to progression was 11.4 months and the median overall survival was 19.5 months. Overall survival at 1 year was 89%.

The regimen was well tolerated, with no obvious increase in toxicity as compared to phase II trials of docetaxel alone, although there was a higher than expected incidence of gastric and duodenal ulceration. These encouraging phase II results provided the basis for further evaluation of this combination in a randomized double-blinded study.

New calcitriol formulation

The calcitriol formulation used in the previous trials, Rocaltrol, is a commercially available formulation that was not designed for high-dose therapy. In order to administer high doses, patients were required to take large numbers of pills, between 50 to 100 pills over several hours. In addition to the practical limitations inherent in such a dosage, this approach was associated with considerable inter-patient variation in key pharmacokinetic parameters. Further, at higher doses, the pharmacokinetic parameters did not increase in a dose-proportional fashion.

DN-101 (Novacea, Inc., South San Francisco, CA, USA), is a new formulation of calcitriol that was developed to overcome these limitations and designed specifically for high-dose cancer therapy. Preliminary pharmacokinetic data suggests a linear dose-PK relationship not previously seen with the commercially available low-dose formulation. A phase I study showed that the maximum tolerated dose on a weekly schedule was 45 µg when grade 2 hypercalcemia was defined as a dose-limiting toxicity (29). No dose-limiting toxicity was observed at doses up to 165 µg when single-dose administration was tested.

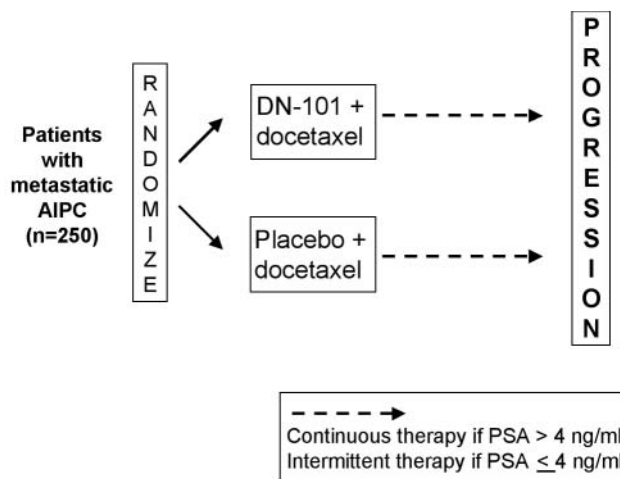


Figure 1. ASCENT study design. Reprinted with permission from Blackwell Publishing. Source: Beer TM, ASCENT: The androgen-independent prostate cancer study of calcitriol enhancing taxotere, *BJU International* 96: 508-513, 2005.

ASCENT: AIPC Study of Calcitriol Enhancing Taxotere

ASCENT was designed to confirm the results of the phase II study of high-dose calcitriol and docetaxel conducted at OHSU, using the DN-101 formulation of calcitriol. ASCENT is a randomized, double-blind, multicenter study of docetaxel and DN-101 or placebo in patients with metastatic AIPC. Patients were randomized to receive either DN-101 (45 µg on day 1 PO) and docetaxel (36 mg/m² on day 2) or placebo plus docetaxel for three consecutive weeks of a 4-week cycle (Figure 1). The study has completed accrual and publication of the final results is awaited.

Conclusion

There is a strong pre-clinical basis for targeting the vitamin D receptor for cancer therapy. Intermittent dosing of calcitriol, the natural ligand for this receptor, has allowed substantial

dose escalation of calcitriol. Encouraging phase II results for the combination of high-dose calcitriol and docetaxel have been published and a double-blinded randomized comparison of this combination to docetaxel has completed accrual.

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