A Phase I Pharmacokinetic and Pharmacodynamic Study of Intravenous Calcitriol in Combination with Oral Gefitinib in Patients with Advanced Solid Tumors

Marwan G. Fakih,[1] Donald L. Trump,[1] Josephia R. Muindi,[1] Jennifer D. Black,[3] Ronald J. Bernardi,[4] Patrick J. Creaven,[1] James Schwartz,[1] Michael G. Brattain,[3] Alan Hutson,[2] Renee French,[1] and Candace S. Johnson[3]

Abstract

Purpose: In preclinical models, calcitriol and the tyrosine kinase inhibitor gefitinib are synergistic and modulate extracellular signal-regulated kinase (Erk) and Akt pathways. Therefore, we conducted a phase I study of calcitriol and gefitinib to determine the maximum tolerated dose (MTD) of this combination.

Experimental Design: Calcitriol was given i.v. over 1 h on weeks 1, 3, and weekly thereafter. Gefitinib was given at a fixed oral daily dose of 250 mg starting at week 2 (day 8). Escalation occurred in cohorts of three patients until the MTD was defined. Pharmacokinetic studies were done for calcitriol and gefitinib. Serial skin biopsies were done to investigate epidermal growth factor receptor (EGFR) pathway pharmacodynamic interactions.

Results: Thirty-two patients were treated. Dose-limiting hypercalcemia was noted in two of four patients receiving 96 μg/wk of calcitriol. One of seven patients developed dose-limiting hypercalcemia at the MTD 74 μg/wk calcitriol dose level. The relationship between calcitriol dose and peak serum calcitriol (C_{max}) and systemic exposure (AUC) was linear. Mean (±SD) serum calcitriol C_{max} at the MTD was 6.68 ± 1.42 ng/mL. Gefitinib treatment inhibited EGFR, Akt, and Erk phosphorylation in the skin. Calcitriol did not have consistent effects on skin EGFR or its downstream elements. The combination of gefitinib and calcitriol did not modulate tumor EGFR pathway in patients with serial tumor biopsies.

Conclusions: High doses of weekly i.v. calcitriol can be administered safely in combination with gefitinib. Calcitriol concentrations achieved at the MTD 74 μg calcitriol exceed *in vivo* concentrations associated with antitumor activity in preclinical models.

Calcitriol (1,25-dihydroxyvitamin-D_3) has both tumor differentiating and antiproliferative activities *in vitro* and *in vivo* in hematologic and solid tumors (1–10). Numerous mechanisms for these effects have been postulated, including decreased retinoblastoma protein phosphorylation, p21 overexpression, G_0-G_1 cell arrest, Akt down-regulation, mitogen-activated protein kinase down-regulation, and induction of the proapoptotic protein mitogen-activated protein kinase kinase kinase-1 (11–13). More recent evidence suggests that calcitriol may exert antitumor activity by targeting the epidermal growth factor receptor (EGFR) pathway (14, 15). Studies in our laboratory show that calcitriol was synergistic when added to the tyrosine kinase inhibitor gefitinib *in vitro* and *in vivo* in murine and human squamous cell model systems (16). These preclinical findings provided the rationale to clinically investigate the combination of calcitriol and gefitinib.

This phase I study was designed to identify the maximum tolerated dose (MTD) of weekly i.v. calcitriol with gefitinib at 250 mg/d. Clinical data from lung cancer studies at the time of activation of this study suggested a clinical benefit in association with gefitinib treatment at a daily dose of 250 mg/d; this benefit was equivalent to the one seen at higher doses of 500 mg/d (17). Thus, a fixed dose of daily gefitinib of 250 mg/d was selected. Because calcitriol causes hypercalcemia when given daily or every other day but not with weekly oral dosing, we elected to incorporate a weekly calcitriol schedule in our study (18–20). I.v. administration of calcitriol was elected due to the lack of dose-dependent increases in calcitriol serum concentrations with commercially available formulations at oral dose levels exceeding 0.48 μg/kg/wk (20). A weekly

Authors' Affiliations: Departments of [1]Medicine and [2]Biostatistics, Roswell Park Cancer Institute and the University at Buffalo; [3]Department of Pharmacology, Roswell Park Cancer Institute, Buffalo, New York; and [4]Department of Pediatrics, Baylor College of Medicine, Houston, Texas

Received 5/15/06; revised 9/27/06; accepted 11/17/06.

Grant support: NIH grants CA67267, CA85142, CA63502, and CA95045, and American Cancer Society Career Development Award MRSG-04-270-01 (M.G. Fakih).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: This article was presented in part as a poster presentation at AACR Annual Meeting, 2005.

Requests for reprints: Marwan G. Fakih, Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263. Phone: 716-845-8189 or 716-845-3362; Fax: 716-845-8008; E-mail: marwan.fakih@roswellpark.org.

doi:10.1158/1078-0432.CCR-06-1165

schedule of (i.v.) calcitriol was chosen for patient convenience and to minimize risk of hypercalcemia.

Secondary end points included the evaluation of calcitriol and gefitinib pharmacokinetics and to investigate the possibility of pharmacokinetic interaction between these agents. Serial skin biopsies were done before treatment, after calcitriol treatment, after gefitinib treatment, and after the combination of gefitinib and calcitriol to explore the effects of these drugs and their combination on the EGFR pathway. Skin biopsies were scheduled around 8 h from calcitriol and/or gefitinib dosing. Biopsy timing was selected based on preclinical data supporting maximum EGFR phosphorylation inhibition after 8 h from treatment with a calcitriol analogue (15).

Materials and Methods

Patient criteria. Patients with histologically or cytologically confirmed solid tumors that were metastatic or unresectable and for which standard curative or palliative measures did not exist were eligible for the trial. The last chemotherapeutic or radiation treatment was at least 4 weeks (6 weeks for nitrosoureas or mitomycin C) before trial enrollment. Other criteria included age ≥18 years, Eastern Cooperative Oncology Group performance status ≤2, estimated life expectancy >12 weeks, no central nervous system involvement, adequate bone marrow function (neutrophils ≥1,500/μL, hemoglobin ≥8.0 g/dL, and platelets ≥100,000/μL), adequate hepatic function (serum bilirubin ≤ upper limit of the reference range; serum aspartate aminotransferase and alanine aminotransferase ≤2.5 × upper limit of the reference range), and adequate renal function (creatinine ≤1.5 upper limit of the reference range or creatinine clearance ≥60 mL/min). The study excluded patients unable to receive oral medications; patients with brain metastases; patients with history of hypercalcemia or genitourinary stones; patients on digoxin, thiazide, calcium supplements, and supraphysiologic doses of glucocorticoids (exceeding the equivalent of 15 mg of hydrocortisone per day); and patients with prior allergic reaction to calcitriol or gefitinib. HIV-positive patients were not eligible because of possible pharmacokinetic interaction with antiretroviral drugs. Patients with reproductive potential had to agree to use adequate contraception before study entry and for the duration of study participation. The study and consent form was approved by the Institutional Scientific and Review Committee and the Institutional Review Board before its activation. All patients provided signed informed consent before study entry. The study was conducted in accordance with the Good Clinical Practice Guidelines as issued by the International Conference on Harmonization and the Declaration of Helsinki.

Study design and treatment plan. Three patients were entered at each dose level. In the absence of dose-limiting toxicity (DLT), the next dose level was explored. If DLT was seen in one patient, three further patients were added at that dose level and, if no additional DLT was seen, escalation to the next dose level occurred. If at least two patients had DLT at a given dose level, accrual to that dose level was stopped; this was the maximally administered dose. Further patients were then added, as required, to the previous dose level (and if necessary to lower dose levels) to establish the highest dose at which less than two of six patients had DLT. This was the MTD.

Patients received calcitriol i.v. over 60 min once weekly except for week 2. Calcitriol (Calcijex) was supplied in 1 μg vials by Abbott Laboratories (Abbott Park, IL). The first dose level of calcitriol was 10 μg. Subsequent dose escalation levels were 15, 20, 26, 34, 44, 57, 74, and 96 μg/wk. No intrapatient escalation was allowed. Gefitinib (Iressa) was supplied by AstraZeneca (London, United Kingdom) in 250 mg tablets and was given once daily starting 1 week after the first dose of calcitriol (treatment schema; Fig. 1).

A DLT was any of the following toxicities that were attributable to study treatment on cycle 1 (first 4 weeks of treatment): any grade 3 or 4 toxicity except for grade 3 anemia, any grade 2 or above hypercalcemia (corrected calcium >11.5 mg/dL) if confirmed on repeat blood draw (>72 h), any grade 2 or above hypercalcemia that is associated with serious hypercalcemic symptoms, any treatment interruption that lasts for >2 weeks that is related to treatment toxicity, sustained increase (>72 h) in creatinine to >2× baseline and >2 g/dL, and clinical or radiological evidence of new genitourinary stones.

No dose modifications were allowed for gefitinib; however, treatment interruptions were allowed for periods not exceeding 2 weeks for grade 3 and above skin toxicity, grade 3 and above diarrhea, grade 3 and above nausea unrelated to hypercalcemia, and grade 4 neutropenia. In case of dose interruption secondary to toxicity, treatment was resumed upon improvement to grade 1 or less with the exception of neutropenia, in which case retreatment was allowed when the count exceeded 500 cells/mm^3. No dose modifications were allowed for DLTs related to calcitriol, in which case patients had to be discontinued from study. No growth factors were allowed on the study with the exception of recombinant erythropoietin.

Clinical evaluation and follow-up. A complete medical history, physical examination, pregnancy test for women with reproductive potential, complete blood count, and comprehensive chemistry profile (electrolytes, blood urea nitrogen, creatinine, magnesium, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, bilirubin) were obtained within 1 week before treatment initiation. Baseline computed tomography scans of the chest, abdomen, and pelvis were obtained within 4 weeks before initiation of treatment. Complete blood count and comprehensive chemistry were repeated on a weekly basis. Calcium, phosphorous, and creatinine levels were measured before as well as 3 days after each calcitriol infusion. Medical history, physical examination, and toxicity assessment as per National Cancer Institute Common Toxicity Criteria 2.0 were done on the first day of the 1st and 3rd weeks of cycle 1 and on day 1 of week 1 of each subsequent cycle. Corrected calcium was used to grade hypercalcemia [corrected calcium = serum calcium + (4 − serum albumin) × 0.8]. Computed tomography scans of chest, abdomen, and pelvis were repeated every two cycles (8 weeks) to assess response. Responses were categorized according to the Response Evaluation Criteria in Solid Tumors (21).

Skin and tumor biopsies. Skin biopsy specimens (4-mm wide, 8-mm deep) were obtained from the suprascapular areas using a punch biopsy to the level of the s.c. tissue after local anesthesia with 1% lidocaine. Serial biopsies were obtained from the same regions (Fig. 1). The first skin biopsy was obtained before treatment (baseline). The second

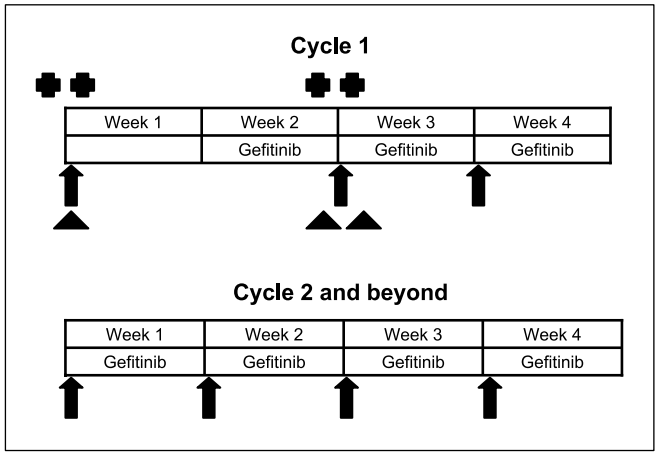

Fig. 1. Treatment schema. On cycle 1, calcitriol (*black arrow*) was given on days 1 and 15 and weekly thereafter. Gefitinib started on day 8. Skin biopsies (*black cross*) were done at baseline, after the first dose of calcitriol, after 7 d of gefitinib (day 14), and after the combination calcitriol + gefitinib (day 15). Pharmacokinetics (*black triangle*) were done on days 1 and 15 for calcitriol and on day 15 for gefitinib. On cycles 2 and beyond, calcitriol was administered weekly along with daily gefitinib.

biopsy was obtained ~6 to 8 h after the first calcitriol infusion (day 1) to determine the effects of calcitriol alone on the EGFR pathway. The third biopsy was obtained after 1 week of treatment with gefitinib (day 14) and before the second infusion of calcitriol to determine the effects of gefitinib alone on the EGFR pathway. The last skin biopsy was done 6 to 8 h after the second dose of calcitriol (day 15) to determine the effects of calcitriol and gefitinib combination on the EGFR pathway.

In selected consenting patients with easily accessible tumors, tumor biopsies were obtained before treatment and 6 to 8 h after the second dose of calcitriol to determine the effects of calcitriol and gefitinib combination on the tumor EGFR pathway.

Immunohistochemistry studies. Levels of EGFR, phosphorylated (p)EGFR, Erk, pErk, Akt, and pAkt in the skin and tumor biopsies were assayed by immunohistochemistry procedures using antibodies to EGFR (Zymed, San Francisco, CA), pEGFR (Calbiochem, San Diego, CA), Erk (Cell Signaling, Beverly, MA), pErk (Cell Signaling), Akt (Santa Cruz Biotechnology, Santa Cruz, CA), and pAkt (Cell Signaling). The methodology was previously described (22, 23).

Immunohistochemistry analysis. The staining intensity was measured semiquantitatively by a researcher (J.D.B.) with extensive experience in this field. Each sample was scored from 0 (no staining), 1+ (weakly positive), 2+ (moderately positive), to 3+ (strongly positive) for EGFR, pEGFR, Erk, pErk, Akt, and pAkt.

Pharmacokinetics. Ten milliliters of blood were collected in a nonheparinized (red-top) tube at baseline; at 30 and 45 min; and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, and 72 h after calcitriol infusion on days 1 and 15 of treatment for calcitriol pharmacokinetics. Pharmacokinetic sampling at dose <57 μg/wk was up to 24 h; it was extended after a protocol amendment to 72 h for patients treated at 57, 74, and 96 μg/wk calcitriol doses. Serum samples prepared immediately by a 10-min centrifugation at 2,000 × *g* were batched and stored in 1 to 2 mL aliquots at −20°C until assayed for calcitriol levels. Serum calcitriol concentrations were determined using 1,25-dihydroxyvitamin D_3-[I^{125}] RIA kit from DiaSorin Co. (Stillwater, MN). The analytic characteristics of this assay have previously been described (18).

On days 8 and 15, the 1st and 8th days of gefitinib administration, 4 mL of blood were collected at (0, 0.5, 1, 2, 4, 6, 8, and 24 h) into tubes containing lithium heparin anticoagulant. Trough levels were also obtained 1 week later (day 22). Within 30 to 60 min of collection, blood samples were centrifuged at 1,000 × *g* for 10 min to provide plasma for evaluation of gefitinib levels. Plasma samples were stored at −20°C before analysis by high-performance liquid chromatography with tandem mass spectrometry. Analysis was done by Analytico Medinet B.V., AstraZeneca (Breda, the Netherlands), as previously described (24). Plasma drug concentrations were used to determine whether steady state had been achieved and to provide a measure of exposure.

Results

Demographics. Between December 2003 and April 2005, 34 patients (32 evaluable) were entered on the study. Two patients never received treatment secondary to progressive disease and early withdrawal. Patient characteristics are detailed in Table 1.

Treatment administration. Thirty-two patients received treatment on study. Nine dose levels were evaluated. The median number of cycles administered was 2 (8 weeks). Five patients received four or more cycles. All patients without DLT were able to receive all intended dosage on cycle 1.

Toxicity. All 32 treated patients were evaluated for toxicity. Nonhematologic toxicities are outlined in Table 2.

Hematologic toxicity

The combination of calcitriol and gefitinib did not result in any significant bone marrow suppression. None of the patients treated on this study developed >grade 2 neutropenia or thrombocytopenia. Two patients developed grade 2 anemia on cycles 2 and beyond treatment. One patient developed grade 3 anemia during cycle 7 of treatment, which was attributed to chronic disease and anticoagulation therapy. One patient developed grade 4 anemia related to nonsteroidal anti-inflammatory drug–induced gastrointestinal bleeding on cycle 4 of treatment.

Nonhematologic toxicity

Rash and gastrointestinal toxicities. The most common nonhematologic adverse events were rash, diarrhea, nausea, and vomiting. Twenty patients developed rash on study, none of which was grade 3 (13 patients grade 1; 7 patients grade 2). The rash typically developed in the first 3 weeks of treatment of gefitinib (cycle 1) and did not seem to worsen with continuation of treatment. Diarrhea occurred in 18 patients and was limited to grade 1 (13 patients) and grade 2 (5 patients); it was manageable in all patients with loperamide use. Nausea and vomiting were less common. Only four patients developed grade 2 nausea and vomiting during treatment. Rash and gastrointestinal toxicities are summarized in Table 2.

Hypercalcemia. Hypercalcemia was limited to grade 1 (greater than the upper limit of the reference range to 11.5 mg/dL) in 12 patients on the first 7 dose levels (10-57 μg). Seven patients (one patient was replaced because of early progression) were enrolled at the eight dose level of 74 μg calcitriol per week: four patients developed grade 1, one grade 2 (>11.5 to 12.5 mg/dL), and one grade 3 hypercalcemia (>12.5 to 13.5 mg/dL). Four patients were enrolled at the ninth dose level of 96 μg calcitriol per week: one patient developed grade 1 and two patients developed grade 2 hypercalcemia. The results are summarized in Table 3.

The mean increase in cycle 1 calcium levels between days 1 and 4 of each calcitriol week (calcium D4-calcium D1) was more pronounced at the highest two dose levels investigated (Fig. 2A). The mean increase was higher than 2 mg/dL in three patients only: two patients at the 96 μg calcitriol dose level and

Table 1. Patient characteristics

Patient characteristics (*N* = 32 evaluable)	
Gender (male/female)	21/11
Age (median/range), y	65 (36-87)
Primary tumor	
Colorectal	11
Prostate	4
Non–small lung cancer	3
Head and neck	3
Pancreatic	2
Sarcoma	2
Mesothelioma	1
Breast	1
Gastrointestinal stromal tumor	1
Anal	1
Esophageal	1
Penile	1
Unknown primary	1
ECOG performance status 0	9
ECOG performance status 1	21
ECOG performance status 2	2
Prior chemotherapy	32

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2. Nonhematologic toxicities: rash, diarrhea, and nausea/vomiting (cycle 1 and all cycles)

	DL1 10 μg (3 pts)	DL2 15 μg (3 pts)	DL3 20 μg (3 pts)	DL4 26 μg (3 pts)	DL5 34 μg (3 pts)	DL6 44 μg (3 pts)	DL7 57 μg (3 pts)	DL8* 74 μg (7 pts)	DL9† 96 μg (4 pts)
Cycle 1									
Rash grade 1	2	2	1	1	0	1	3	3	1
Rash grade 2	1	1	1	0	0	1	0	1	0
Diarrhea grade 1	0	0	1	1	1	1	0	3	1
Diarrhea grade 2	0	1	2	0	1	0	0	0	0
N/V grade 1	0	0	0	0	0	0	0	0	0
N/V grade 2	0	1	0	0	0	0	0	0	0
All cycles									
Rash grade 1	2	2	1	1	0	1	3	2	1
Rash grade 2	1	1	2	0	0	1	0	2	0
Diarrhea grade 1	1	0	1	2	1	1	3	3	1
Diarrhea grade 2	0	1	2	0	1	0	0	1	0
N/V grade 1	0	0	0	0	0	0	0	0	0
N/V grade 2	0	1	0	0	1	0	0	0	2

NOTE: All patients received the same dose of 250 mg/d gefitinib. Only the calcitriol dose level (in μg) is listed.
Abbreviations: Pts, patients; N/V, nausea and vomiting.
*One DLT (refer to Results).
†Two DLTs (refer to Results).

one patient at the 74 μg calcitriol level. Hypercalcemia always resolved completely within 7 days from calcitriol dosing.

Other toxicities. Phosphorous and creatinine levels did not vary between day 4 and day 1 or between cycles, even at higher doses of calcitriol. One patient developed grade 3 corneal toxicity (abrasion) on cycle 4, which was attributed to gefitinib and resulted in treatment discontinuation.

DLT, MTD, and recommended dose. No DLTs were noted on dose levels 1 to 7. At dose level 8 (74 μg/wk of calcitriol), no DLTs were noted in the first three patients and escalation proceeded to dose level 9 (96 μg/wk). A total of four patients were enrolled at dose level 9, two of whom developed DLTs attributed to calcitriol and occurring within the first 3 days after the first infusion. Both DLTs consisted of symptomatic grade 2 hypercalcemia: corrected calcium of 11.7 mg/dL with hallucinations in one patient and corrected calcium of 12.4 mg/dL with ataxia in the second. Central nervous system toxicities were resolved within 24 to 48 h in both patients with resolution of hypercalcemia. Dose level 9 was declared a nontolerable dose level, and dose level 8 (calcitriol 74 μg/wk) was expanded to a total of seven evaluable patients (one replaced before completing cycle 1 secondary to disease progression). One of seven patients at dose level 8 developed an asymptomatic grade 3 DLT hypercalcemia (corrected calcium of 13.4 mg/dL) on day 4 of week 4 of cycle 1. Dose level 8 was declared the MTD.

Antitumor activity. Thirty patients were evaluable for response. Two of the 32 treated patients were nonevaluable for response because they were taken off study secondary to DLT hypercalcemia. None of the patients had an objective response. Three patients had a confirmed disease stabilization lasting 8 months in two (penile cancer and prostate) and 4 months (prostate) in the third.

Pharmacokinetics

Calcitriol pharmacokinetics. There was a substantial interpatient variation in day 1 pretreatment serum calcitriol levels; the median (range) concentration was 39 pg/mL (13-145 pg/mL). Day 1 concentration over time plots showed substantial interpatient variability in serum calcitriol levels achieved at all dose levels. Figure 2B shows concentration over time plots at two low calcitriol doses (10 and 20 μg), the MTD of 74 μg, and 96 μg (the highest calcitriol doses administered). Peak serum levels were observed half way through the calcitriol infusion and started decreasing immediately after the infusion. Calcitriol was cleared from serum in a biexponential fashion, remained elevated 24 and 48 h posttreatment, and were within the pretreatment range at 72 h. A summary of the serum calcitriol pharmacokinetic variables at all calcitriol dose levels is shown in Table 4. C_{max} serum calcitriol concentrations in the range of 10 nmol/L (≥4.0 ng/mL) were achieved in patients treated with at 74 and 96 μg calcitriol doses. Figure 2C shows that the

Table 3. Hypercalcemia (cycle 1)

	DL1 (3 pts)	DL2 (3 pts)	DL3 (3 pts)	DL4 (3 pts)	DL5 (3 pts)	DL6 (3 pts)	DL7 (3 pts)	DL8 (7 pts)	DL9 (4 pts)
Hypercalcemia grade 1 (<ULN to 11.5 mg/dL)	0	0	3	2	2	2	3	4	1
Hypercalcemia grade 2 (>11.5 to 12.5 mg/dL)	0	0	0	0	0	0	0	1	2
Hypercalcemia grade 3 (>12.5 to 13.5 mg/dL)	0	0	0	0	0	0	0	1	0

Abbreviation: ULN, upper limit of the reference range.

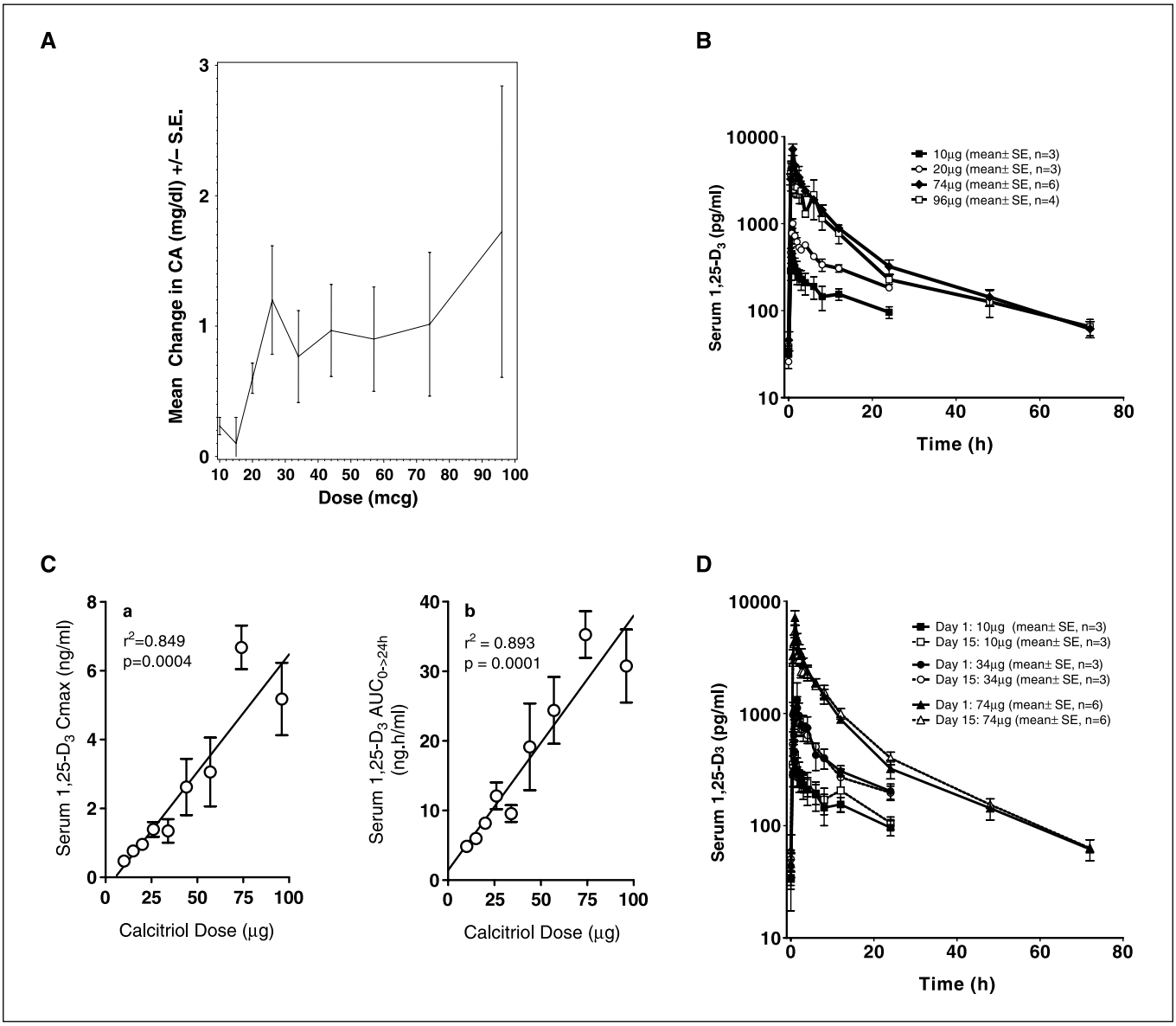

Fig. 2. *A,* increase in calcium (day 4 minus day 1) at all dose levels of calcitriol. *B,* concentration over time plots for low calcitriol doses (10 and 20 μg) and high doses of calcitriol (MTD of 74 and at 96 μg). *C,* relationship between calcitriol dose administered and either C_{max} or $AUC_{0\text{-}24\ h}$. *D,* effect of gefitinib on serum calcitriol concentration over time plots at 10 μg (lowest dose), 34 μg (intermediate dose), and 74 μg (the calcitriol MTD). Day 1, calcitriol alone. Day 15, calcitriol + gefitinib.

relationship between calcitriol dose administered and either C_{max} or $AUC_{0\text{-}24\ h}$ achieved was linear.

Day 15 repeat pharmacokinetic studies after administration of calcitriol with gefitinib showed no effect on serum calcitriol concentration-time profiles. Figure 2D shows the effect of gefitinib on serum calcitriol concentration over time plots at 10 μg (lowest dose), 44 μg (intermediate dose), and 74 μg (the MTD of calcitriol). Gefitinib administration had no effect on serum calcitriol pharmacokinetic variables at all calcitriol dose levels ($P > 0.05$, data not shown).

Gefitinib pharmacokinetics. Intensive gefitinib pharmacokinetic sampling was done on the 1st and 8th day of gefitinib. A repeat trough level was also obtained 1 week later. Gefitinib pharmacokinetic results are summarized in Table 5. Intensive gefitinib pharmacokinetic sampling on day 15 (day 8 of gefitinib) and trough levels from day 22 of cycle 1 (day 15 of gefitinib) were consistent with prior steady-state levels from prior studies using single-agent gefitinib at 250 mg/d on IDEAL 1 study (17).[5]

Immunohistochemistry (skin and tumor). Skin biopsies were done (*a*) prestudy; (*b*) after 6 to 8 h from first calcitriol infusion; (*c*) on day 14, after 7 days of gefitinib (after being 2-week calcitriol-free); and (*d*) on day 15 after 6 to 8 h from second infusion of calcitriol (concurrently with gefitinib). Significant interpatient variability was noted in skin EGFR pathway staining for all tested variables (data not shown). In general, total EGFR, Erk, and Akt levels were unchanged with calcitriol, gefitinib, or combination therapy. Gefitinib alone (day 14 biopsy) decreased pEGFR, pErk, and pAkt staining. This

[5] A.J. Barge, personal communication, AstraZeneca data on file.

effect on Akt was less obvious when calcitriol and gefitinib were given in combination (Fig. 3). No definite effects of calcitriol on EGFR signaling could be confirmed; specifically, no pAkt effects could be delineated. Furthermore, no calcitriol dose-effect trends on EGFR pathway were detected.

Two patients (breast cancer and gastrointestinal stromal tumor) treated at dose level 4 of calcitriol agreed to tumor biopsies at baseline and on day 15 of treatment to evaluate the effect of the combination calcitriol and gefitinib on intratumor EGFR pathway. In contrast to the inhibitory effects noted in the skin, no effects were noted with combination therapy on tumor EGFR, Erk, and Akt phosphorylation/activation.

Discussion

Calcitriol, 1,25-dihydroxyvitamin-D_3, has antitumor and differentiating activities in preclinical models. Preclinical data indicate that antitumor activity is calcitriol concentration dependent—antitumor activity increases as concentrations are escalated from 1 to 100 nmol/L (25, 26). Multiple trials of calcitriol using a variety of doses and schedules have been conducted. Daily and every-other-day schedules have been hindered by dose-limiting hypercalcemia (18). Intermittent dosing (once weekly or daily for 3 consecutive days repeated every week) was not associated with any calcitriol DLTs (20, 27). However, intermittent oral administration of the commercial oral formulations of calcitriol formulations was associated with limited bioavailability, considerable interpatient variability in AUC and C_{max}, and loss of linear relationship between dose and exposure (AUC, C_{max}; refs. 20, 27).

This phase I clinical trial is the first to investigate i.v. calcitriol escalation. The study was designed to identify the MTD dose of i.v. weekly calcitriol in combination with oral daily gefitinib based on previously described synergy data (16). The MTD of i.v. calcitriol in combination with oral gefitinib (250 mg/d) was 74 μg/wk. DLTs, in the form of hypercalcemia, were noted at 96 μg/wk in two of four patients enrolled at that dose level. It is of interest that all patients with dose-limiting hypercalcemia on this study had head and neck cancers and suffered from cachexia, suggesting the possible susceptibility of this group of patients to hypercalcemia. Parathyroid hormone–related peptide (two patients) and parathyroid hormone (one patient) levels were slightly elevated in all DLT patients, but similar findings were also noted in other patients without hypercalcemia at various dose levels on this study. Thus, a paraneoplastic component of hypercalcemia in our DLT patients cannot be ruled out or confirmed at this point.

Table 4. Day 1 i.v. serum calcitriol pharmacokinetic variables

Calcitriol (μg)	*n*	C_{max} (ng/mL)	$AUC_{0\text{-}24\ h}$ (ng h/mL)	$T_{1/2}$ (h)
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

Table 5. Pharmacokinetic variables for gefitinib after single (day 8) and multiple (days 15 and 22) doses

Variable	Day 8	Day 15	Day 22
C_{max} (ng/mL)	166	335	NC
T_{max} (h)	4	6	NC
$AUC_{(0\text{-}24\ h)}$ (ng h/mL)	2,323	5,988	NC
C_{min} (ng/mL)	68	198	202

Gefitinib, with or without calcitriol, inhibited the skin EGFR pathway as measured by pEGFR, pErk, and pAkt. This is consistent with previously published data with single-agent gefitinib (28, 29). However, a high degree of interpatient variability in staining limits the utility of this model. Furthermore, there was no correlation between pEGFR inhibition in the skin and tumor. This is highlighted by the lack of EGFR pathway inhibition in tumors of two patients who agreed to tumor biopsies (both had EGFR pathway inhibition in skin). This lack of tumor EGFR modulation by gefitinib plus calcitriol is in line with a recently reported phase II study of gefitinib in metastatic colorectal cancer in which neither the 250 nor 500 mg/d dose levels inhibited tumor EGFR pathway (30). It is noteworthy that calcitriol did not positively modulate the effects of gefitinib on skin or tumor EGFR pathway. Amelioration of pAkt inhibition by gefitinib was observed in several patients. These results contrast with the preclinical data generated by our group in a preclinical squamous cell cancer model (16). It is unclear if the lack of correlation between our clinical and preclinical data is due to inadequacy of skin as a surrogate marker for calcitriol activity or due to a divergence between preclinical and clinical effects of calcitriol. It is possible that intradermal concentrations of calcitriol, even at the higher dose levels, do not reach a threshold needed to exert biological activity. It is also possible that the variations made on the clinical schedule compared with the preclinical schedule may have affected the positive interaction between calcitriol and gefitinib that was documented in the laboratory. Finally, we cannot rule out that the timing of the skin and tumor biopsies (8 h after calcitriol dosing) may have failed to capture the effects of calcitriol on the EGFR pathway, especially that calcitriol C_{max} was achieved ~0.5 h after calcitriol administration.

Gefitinib did not affect the pharmacokinetics of calcitriol; pharmacokinetic variables were similar on days 1 (without gefitinib) and 15 (with gefitinib). The study design did not permit the evaluation of the effects of calcitriol on gefitinib. However, intensive gefitinib pharmacokinetic sampling on day 15 (day 8 of gefitinib) and trough levels (serum collected before a.m. dose) from day 22 of cycle 1 (day 15 of gefitinib) were consistent with steady-state levels from prior studies using single-agent gefitinib at 250 mg/d (e.g., on IDEAL 1 study; ref. 17).[5] Thus, gefitinib pharmacokinetic data from this study are entirely consistent with historical gefitinib data. This suggests that there is no marked effect of calcitriol on gefitinib pharmacokinetics. However, given the high interpatient variability in the pharmacokinetics of gefitinib, it is unlikely that this type of comparison would rule out mild to moderate interactions.

Calcitriol C_{max} and AUC at the MTD (74 μg/wk) were 6.68 ± 1.42 ng/mL and 35.65 ± 8.01 ng h/mL, respectively. It is surprising that C_{max} and AUC were lower at the nontolerable calcitriol dose of 96 μg/wk than the MTD. The lack of increase in AUC and C_{max} at 96 μg may be related to interpatient variability in calcitriol clearance or induction of calcitriol clearance at higher dose levels. The occurrence of DLT hypercalcemia at the 96 μg dose level despite lower C_{max} and AUC than the MTD could be related to patient selection at that dose level (cachectic with head and neck cancer). Other contributing factors in need of further investigation include variations in vitamin D receptor polymorphism, activity of metabolizing enzymes such as CYP24, and baseline calcium and vitamin D balance.

C_{max} of calcitriol at the MTD (74 μg/wk i.v.) was markedly higher than 10 nmol/L, the concentration associated with calcitriol single-agent activity in preclinical *in vivo* models (31, 32). The C_{max} of the MTD 74 μg/wk i.v. calcitriol (6.68 ng/mL; C_{max} 16 nmol/L) was also markedly higher than the C_{max} achieved at 75 μg of a novel weekly oral formulation of calcitriol (DN101, C_{max} 3.8 nmol/L); however, AUC was similar for both formulations (35.6 and 38.4 for calcitriol and DN101, respectively; ref. 33). C_{max} achieved at our MTD of 74 μg/wk i.v. calcitriol was comparable with the C_{max} achieved with 165 μg/wk of oral DN101 (33). Higher C_{max} levels with i.v. formulation of calcitriol compared with DN101 are expected given the longer T_{max} of 1 to 2 h with DN101 in comparison with 0.5 h with i.v. calcitriol (achieved half way through infusion; ref. 33). The lack of significant hypercalcemia at DN101 doses of 165 to 180 μg/wk contrasts with the dose-limiting hypercalcemia noted in our study (33, 34). It is unclear if the pharmacokinetic characteristics of i.v. administration versus oral administration may factor in this difference in hypercalcemic effects. It is possible that the higher C_{max} levels in the presence of a threshold AUC have resulted in the higher hypercalcemic effects in our studies. This hypothesis will require further investigation in upcoming studies. Alternative explanations may be related to patient characteristics (such as parathyroid hormone or parathyroid hormone–related peptide baseline or primary tumor), especially that all DLT patients on our study were patients with head and neck cancer.

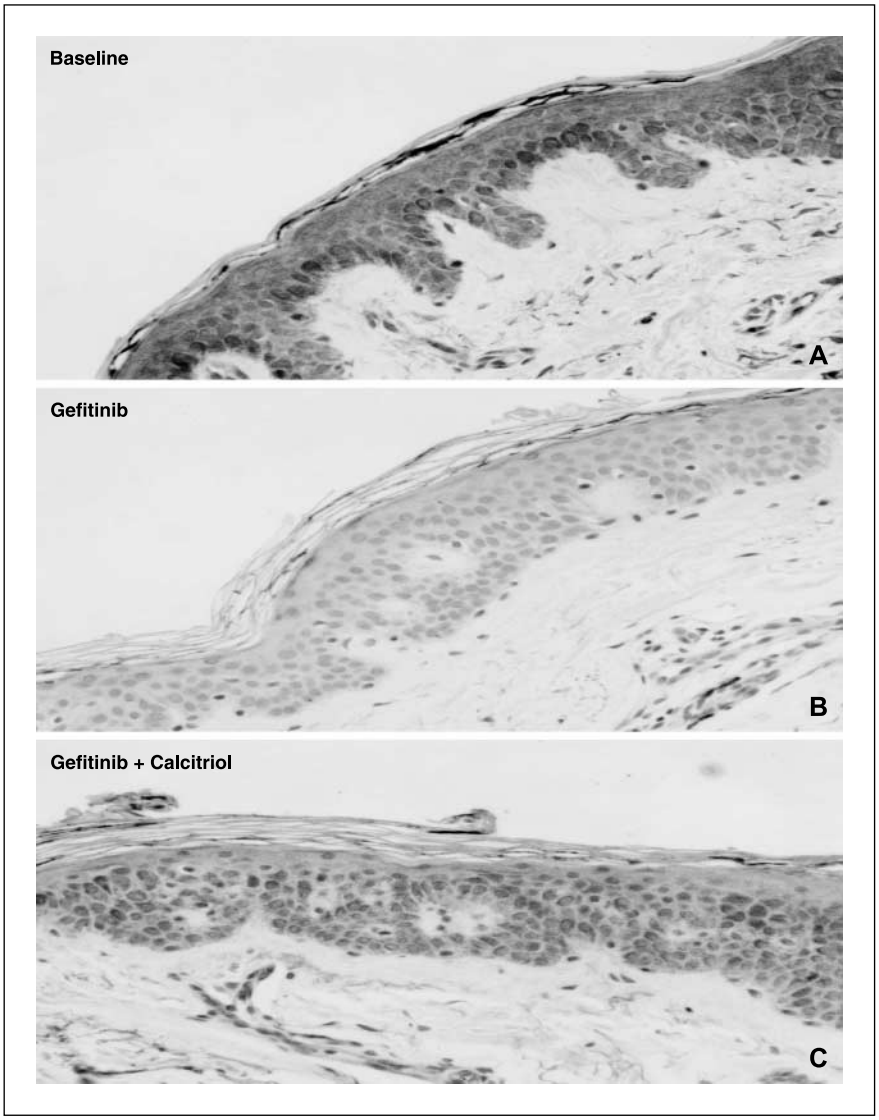

Fig. 3. pAkt immunohistochemistry in serial skin biopsies in a representative patient at the MTD (74 μg calcitriol). *A,* pAkt at baseline. *B,* pAkt after gefitinib only. *C,* pAkt after gefitinib plus calcitriol. The addition of calcitriol resulted in an attenuation of gefitinib inhibitory effects on Akt phosphorylation.

We have shown the feasibility of safely administering high i.v. weekly doses of calcitriol up to 74 μg in a refractory cancer population in combination with oral gefitinib. The lack of pharmacokinetic interaction with gefitinib suggests that similar doses of calcitriol can be administered as a single agent or in combination with other agents that have no pharmacokinetic interaction with calcitriol. For example, the investigation of high doses of i.v. calcitriol may be appealing in combination with taxanes, in which preclinical synergy has been established (35). This is supported further by recent data that suggest a survival advantage to combining DN101 to docetaxel compared with docetaxel alone in patients with hormone refractory prostate cancer (36). The formulation of i.v. calcitriol that is clinically available is a disadvantage (1 μg/ampul). Ideally, further development of i.v. calcitriol should be accompanied by formulation of calcitriol in higher dose ampules.

The clinical development of high doses of calcitriol with chemotherapy continues to be under development in our institute based on favorable preclinical studies. No further clinical studies of calcitriol and gefitinib are planned given the withdrawal of gefitinib from the market and the lack of signs of activity in this large phase I study. Development of future calcitriol and anti-EGFR combinations should take into account the limitations of the skin pharmacodynamic model and should follow more extensive preclinical investigations.

Acknowledgments

We thank Mary Vaughan and Donna Oleszek for their assistance with the immunostaining analysis and Kristie Engler for serum calcitriol measurements.

References

1. Colston K, Colston MJ, Feldman D. 1,25-Dihydroxyvitamin D3 and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. Endocrinology 1981;108:1083–6.
2. Chouvet C, Vicard E, Devonec M, Saez S. 1,25-Dihydroxyvitamin D3 inhibitory effect on the growth of two human breast cancer cell lines (MCF-7, BT-20). J Steroid Biochem 1986;24:373–6.
3. Cross HS, Pavelka M, Slavik J, Peterlik M. Growth control of human colon cancer cells by vitamin D and calcium *in vitro*. J Natl Cancer Inst 1992;84:1355–7.
4. Skowronski RJ, Peehl DM, Feldman D. Vitamin D and prostate cancer: 1,25 dihydroxyvitamin D3 receptors and actions in human prostate cancer cell lines. Endocrinology 1993;132:1952–60.
5. Tsuchiya H, Morishita H, Tomita K, Ueda Y, Tanaka M. Differentiating and antitumor activities of 1α,25-dihydroxyvitamin D3 *in vitro* and 1 α-hydroxyvitamin D3 *in vivo* on human osteosarcoma. J Orthop Res 1993; 11:122–30.
6. Testa U, Masciulli R, Tritarelli E, et al. Transforming growth factor-β potentiates vitamin D3-induced terminal monocytic differentiation of human leukemic cell lines. J Immunol 1993;150:2418–30.
7. Eisman JA, Barkla DH, Tutton PJ. Suppression of *in vivo* growth of human cancer solid tumor xenografts by 1,25-dihydroxyvitamin D3. Cancer Res 1987;47:21–5.
8. Miller GJ, Stapleton GE, Ferrara JA, et al. The human prostatic carcinoma cell line LNCaP expresses biologically active, specific receptors for 1 α,25-dihydroxyvitamin D3. Cancer Res 1992;52:515–20.
9. Thomas MG, Tebbutt S, Williamson RC. Vitamin D and its metabolites inhibit cell proliferation in human rectal mucosa and a colon cancer cell line. Gut 1992;33: 1660–3.
10. Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cells. Cancer Res 1994;54:805–10.
11. Hershberger PA, Modzelewski RA, Shurin ZR, Rueger RM, Trump DL, Johnson CS. 1,25-Dihydroxycholecalciferol (1,25-D3) inhibits the growth of squamous cell carcinoma and down-modulates p21 (Waf1/Cip1) *in vitro* and *in vivo*. Cancer Res 1999;59:2644–9.
12. Scaglione-Sewell BA, Bissonnette M, Skarosi S, Abraham C, Brasitus TA. A vitamin D3 analog induces a G1-phase arrest in CaCo-2 cells by inhibiting cdk2 and cdk6: roles of cyclin E, p21Waf1, and p27Kip1. Endocrinology 2000;141:3931–9.
13. McGuire TF, Trump DL, Johnson CS. Vitamin D(3)-induced apoptosis of murine squamous cell carcinoma cells. Selective induction of caspase-dependent MEK cleavage and up-regulation of MEKK-1. J Biol Chem 2001;276:26365–73.
14. Tong WM, Kallay E, Hofer H, et al. Growth regulation of human colon cancer cells by epidermal growth factor and 1,25-dihydroxyvitamin D3 is mediated by mutual modulation of receptor expression. Eur J Cancer 1998;34:2119–25.
15. Lee E, Jeon SH, Yi JY, Jin YJ, Son YS. Calcipotriol inhibits autocrine phosphorylation of EGF receptor in a calcium-dependent manner, a possible mechanism for its inhibition of cell proliferation and stimulation of cell differentiation. Biochem Biophys Res Commun 2001;284:419–25.
16. Bernardi R, Johnson C, Trump D. ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor (EGDR-TKI), enhances the antiproliferative effects of calcitriol (1,25-dihydroxyvitamin D3) in squamous cell carcinoma: effects on ERK and Akt [abstract 3883]. Proceedings of the American Association for Cancer Research. Volume 43; 2002. p. 783.
17. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. J Clin Oncol 2003;21:2237–46.
18. Smith DC, Johnson CS, Freeman CC, Muindi J, Wilson JW, Trump DL. A Phase I trial of calcitriol (1,25-dihydroxycholecalciferol) in patients with advanced malignancy. Clin Cancer Res 1999;5: 1339–45.
19. Mellibovsky L, Diez A, Perez-Vila E, et al. Vitamin D treatment in myelodysplastic syndromes. Br J Haematol 1998;100:516–20.
20. Beer TM, Munar M, Henner WD. A Phase I trial of pulse calcitriol in patients with refractory malignancies: pulse dosing permits substantial dose escalation. Cancer 2001;91:2431–9.
21. Therasse P, Arbuck SG, Eisenhauer EA, et al.; European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
22. Malik SN, Siu LL, Rowinsky EK, et al. Pharmacodynamic evaluation of the epidermal growth factor receptor inhibitor OSI-774 in human epidermis of cancer patients. Clin Cancer Res 2003;9:2478–86.
23. Malik SN, Brattain M, Ghosh PM, et al. Immunohistochemical demonstration of phospho-Akt in high Gleason grade prostate cancer. Clin Cancer Res 2002;8:1168–71.
24. Jones HK, Stafford LE, Swaisland HC, Payne R. A sensitive assay for ZD1839 (Iressa) in human plasma by liquid-liquid extraction and high performance liquid chromatography with mass spectrometric detection: validation and use in Phase I clinical trials. J Pharm Biomed Anal 2002;29:221–8.
25. Kane KF, Langman MJ, Williams GR. Antiproliferative responses to two human colon cancer cell lines to vitamin D3 are differently modified by 9-*cis*-retinoic acid. Cancer Res 1996;56:623–32.
26. Akhter J, Goerdel M, Morris DL. Vitamin D3 analogue (EB 1089) inhibits *in vitro* cellular proliferation of human colon cancer cells. Br J Surg 1996;83:229–30.
27. Muindi JR, Peng Y, Potter DM, et al. Pharmacokinetics of high-dose oral calcitriol: results from a phase 1 trial of calcitriol and paclitaxel. Clin Pharmacol Ther 2002;72:648–59.
28. Albanell J, Rojo F, Averbuch S, et al. Pharmacodynamic studies of the epidermal growth factor receptor inhibitor ZD1839 in skin from cancer patients: histopathologic and molecular consequences of receptor inhibition. J Clin Oncol 2002;20:110–24.
29. Baselga J, Rischin D, Ranson M, et al. Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. J Clin Oncol 2002;20: 4292–302.
30. Rothenberg ML, LaFleur B, Levy DE, et al. Randomized phase II trial of the clinical and biological effects of two dose levels of gefitinib in patients with recurrent colorectal adenocarcinoma. J Clin Oncol 2005; 23:9265–74.
31. Trump DL, Hershberger PA, Bernardi RJ, et al. Antitumor activity of calcitriol: pre-clinical and clinical studies. J Steroid Biochem Mol Biol 2004;89–90:519–26.
32. Muindi JR, Modzelewski RA, Peng Y, Trump DL, Johnson CS. Pharmacokinetics of 1α,25-dihydroxyvitamin D3 in normal mice after systemic exposure to effective and safe antitumor doses. Oncology 2004; 66:62–6.
33. Beer TM, Javle M, Lam GN, Henner WD, Wong A, Trump DL. Pharmacokinetics and tolerability of a single dose of DN-101, a new formulation of calcitriol, in patients with cancer. Clin Cancer Res 2005;11:7794–9.
34. Chan J, Beer T, Quinn D, Garzotto M, Sokoloff M, Ryan C. Phase II study of DN-101 (high dose calcitriol), mitoxantrone, and prednisone in androgen-independent prostate cancer (AIPC) [abstract 233]. Prostate Cancer Symposium Program Proceedings; 2006. p. 210.
35. Hershberger PA, Yu WD, Modzelewski RA, Rueger RM, Johnson CS, Trump DL. Calcitriol (1,25-dihydroxycholecalciferol) enhances paclitaxel antitumor activity *in vitro* and *in vivo* and accelerates paclitaxel-induced apoptosis. Clin Cancer Res 2001;7:1043–51.
36. Beer T, Ryan C, Venner P, et al. Interim results from ASCENT: A double-blinded randomized study of DN-101 (high-dose calcitriol) plus docetaxel versus placebo plus docetaxel in androgen-independent prostate cancer (AIPC) [abstract 4516]. American Society of Clinical Oncology Annual Meeting Proceedings. Volume 23; 2005. p. 382.