

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020862

MEDICAL REVIEW(S)

MEDICAL OFFICER REVIEW OF NDA

NDA No. 20-862

DRUG: HECTOROL Tablets, 2.5 micrograms
1-alpha-hydroxyvitamin D₂

INDICATION: Secondary hyperparathyroidism of End-Stage Renal Disease

DOSAGE FORM: Capsule, oral, 2.5 mcg

SPONSOR: Bone Care International
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DATE SUBMITTED: March 7, 1998; March 25, 1998; April 27, 1998; June 10, 1998;
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DATE REVIEW COMPLETED: May 20, 1999

RELATED INDS: [REDACTED]

RELATED DRUGS: calcitriol (Rocaltrol[®] oral; Calcijex[®] injection); calcifediol (Calderol[®] oral); ergocalciferol (Calciferol[®] oral); paracalcitol (Zemplar[™] injection)

REVIEWERS:

Medical: Leo Lutwak, M.D., Ph.D.
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SUMMARY	
1. SAFETY	No unusual events were seen in the reported studies. The adverse reactions reported included those expected with the use of vitamin D active agents such as hypercalcemia and hypercalciuria. In addition, cardiac, electrolyte and neurologic problems associated with the diagnosis of end-stage renal disease occurred with expected frequency. The Sponsor states that Hectorol is safer than presently available vitamin D active drugs such as calcitriol and vitamin D ₃ . However, no evidence is presented to substantiate this claim.
2. EFFICACY	
A.	The unusual protocol design leads to questions about the validity of the randomization scheme (see Biometrics Review). The data reported, however, do

demonstrate a statistically significant lowering of serum immune reactive parathyroid hormone levels in the population studied of end-stage renal disease patients on hemodialysis.

B. The data presented do not substantiate a claim for *treatment of secondary hyperparathyroidism of end-stage renal disease* because:

- (1) The relationship of the degree of lowering of IPTH to improvement of hyperparathyroidism has not been demonstrated.
- (2) The duration of the effect on lowering of serum IPTH has not been shown.
- (3) The clinical significance of the efficacy been shown since, although a statistically significant lower IPTH level was achieved with drug than with placebo, the absolute value achieved remained above the normal range.

C. The data presented do not substantiate a claim for *treatment of renal osteodystrophy* because:

- (1) No effects on bone (direct or indirect) have been measured in the reported studies.
- (2) There is no historical evidence for effect on bone of lowering of serum IPTH of the degree demonstrated and for the duration observed in the reported studies.

3. RECOMMENDED ACTION

HECTOROL™ is APPROVED for the indication of lowering of serum immune reactive parathyroid hormone levels in end-stage renal disease patients on hemodialysis.

I. BACKGROUND

The principal human sources of vitamin D are: 1) conversion of 7-dehydrocholesterol in the skin to vitamin D₃ (cholecalciferol) by action of ultraviolet rays and 2) ingestion of either vitamin D₂ (ergocalciferol) or vitamin D₃. Both require further metabolic change in the liver and in the kidney before demonstrating activity on target tissues. The first step is the introduction of an hydroxyl group in the side chain at C-25 by the hepatic enzyme, CYP 27, a vitamin D 25-hydroxylase, producing 25-(OH) D₂ and 25-(OH) D₃ (calcifediol), respectively. These compounds are further hydroxylated at carbon-1 in the proximal nephron by the mitochondrial enzyme, renal 25-hydroxyvitamin D-1 α -hydroxylase, to produce the active forms, 1 α ,25-(OH)₂D₂ and 1 α ,25-(OH)₂D₃ (calcitriol). An alternative metabolic pathway produces 24-hydroxy vitamin D₂ and 24-hydroxy vitamin D₃ in the liver and subsequently, 1 α ,24-(OH)₂D₂ and 1 α ,24-(OH)₂D₃ in the kidney, both also metabolically active to a lesser degree than the 1 α ,25 derivatives.

Calcitriol and 1- α ,25 (OH)₂D₂, the active vitamin D metabolites, regulate intestinal absorption of calcium, tubular reabsorption of calcium by kidney, and, in conjunction with parathyroid hormone, the mobilization of calcium from the skeleton. They act directly on osteoblasts to stimulate bone formation and on parathyroid cells to suppress PTH synthesis and secretion. These functions are mediated by interaction with specific receptor proteins in target tissues.

Of these compounds, two forms of vitamin D₃, calcifediol, and calcitriol are available approved substances in the U.S. In end stage renal disease (ESRD), calcitriol and 1 α -hydroxy vitamin D₃ (alfacalcidol) are appropriate replacement since they do not require

functioning renal tubular tissue for activation. Alfacalcidol has been developed for this purpose in other countries.

The present NDA is for the 1-alpha derivative of vitamin D₂. Calcitriol and 1 α ,25(OH)₂D₂ are considered biologically equivalent since they have been shown to bind to avian and mammalian vitamin D receptors with identical affinities and the precursors, Vitamin D₃ and Vitamin D₂, are used interchangeably in supplements for human consumption. Since alfacalcidol and 1 α -OH D₂ (both "pro-drugs" for the naturally occurring active forms of vitamin D) are bioequivalent in receptor assays and in *in vivo* animal models, the present drug has been developed for use in ESRD patients. It is also claimed that the 1 α -OH D₂ is less toxic than the D₃ derivative.

No clinical studies are reported comparing the D₂ and D₃ derivatives. The basis for the claim of lower toxicity is a study in rats.

Vitamin D₂ is first hydroxylated in the liver at carbon-25 to form 25-hydroxyvitamin D₂ (25-OH-D₂) and then further hydroxylated in the kidneys at carbon-1 to form 1 α ,25(OH)₂D₂. Vitamin D₃ is similarly hydroxylated in the liver and kidneys to form calcitriol.

In renal disease, there is a progressive loss of cells of the proximal nephrons, the primary site for the enzymatic synthesis of the 1-OH derivatives of vitamin D. The loss of functioning nephrons also leads to retention of excess phosphorus which reduces the activity of the responsible enzyme, renal 25-hydroxyvitamin D-1 α -hydroxylase. These two effects account for the low serum levels of dihydroxy vitamin D commonly found in patients with mild to moderate end stage renal disease and contribute to increased secretion of parathyroid hormone (PTH). In severe renal disease, serum levels of active forms of vitamin D are often undetectable.

Since 1 α -OH-D₂ is a pro-drug of 1 α ,25(OH)₂D₂, the pharmacology of 1 α -OH-D₂ and its therapeutic properties in end stage renal disease patients result from hepatic activation of the drug to 1 α ,25(OH)₂D₂. This metabolic activation has been confirmed both *in vitro* with cultured cells derived from a human hepatoma and *in vivo* in rats, monkeys, rabbits and human beings.

The Sponsor claims that Vitamin D₂, unlike vitamin D₃, can be hydroxylated by an alternate pathway in which the initial hydroxylation occurs on carbon-24, producing 1 α ,24-dihydroxyvitamin D₂ (1 α ,24(OH)₂D₂). Similarly, 1 α -OH-D₂ (unlike 1 α -OH-D₃, the analogous pro-drug of calcitriol) can be hydroxylated in this way to produce 1 α ,24(OH)₂D₂. However, no data are presented demonstrating this mechanism in patients or the relative amounts of this and of the 1,25-derivative in normal subjects of ESRD patients.

II. CHEMISTRY

To be reviewed in detail by Chemistry Reviewer.

III. NON-CLINICAL PHARMACOLOGY AND TOXICOLOGY

To be reviewed in detail by Pharmacologist Reviewer.

A. General

Since 1α -OH-D₂ is a pro-drug for $1\alpha,25(\text{OH})_2\text{D}_2$, the activated form of vitamin D. Its pharmacologic effects are those due to regulation of calcium homeostasis by the active substance. Calcium homeostasis involves the interaction of both active vitamin D and parathyroid hormone (PTH) by a feedback mechanism. PTH is secreted by parathyroid glands in response to a decrease in serum calcium. This hormone stimulates the release of calcium from bone, the reabsorption of calcium from the kidney, and the synthesis of $1\alpha,25(\text{OH})_2\text{D}$ in the kidney. $1\alpha,25(\text{OH})_2\text{D}$ stimulates the absorption of calcium from the intestine, the reabsorption of calcium from the kidney, and inhibits the synthesis of PTH. An increase in serum calcium also inhibits secretion of PTH and, indirectly, the production of $1\alpha,25(\text{OH})_2\text{D}$. 1α -OH-D₂ lowers PTH directly by inhibition of PTH synthesis and indirectly by inhibition of PTH synthesis by increased serum calcium.

The side effects of 1α -OH-D₂ are those due to the influx of calcium to the blood. Deleterious effects arise when compensatory mechanisms lowering serum calcium concentration are overwhelmed. If calcium excretion in the urine and/or calcium deposition in bone fail to counter this large influx of calcium, hypercalcemia occurs, which may cause mineralization of soft tissues, principally kidney, heart, and blood vessels.

B. Metabolism

In both rats and monkeys, studies with orally administered radiolabeled compound show that 1α -OH-D₂ is slowly absorbed and is metabolized to $1\alpha,25(\text{OH})_2\text{D}_2$, which circulates in plasma at higher concentrations than the parent 1α -OH-D₂. Studies of blood levels of this metabolite show that the appearance of $1\alpha,25(\text{OH})_2\text{D}_2$ in serum or plasma following administration of 1α -OH-D₂ occurs in a dose-dependent manner, with a maximum concentration attained at 6 to 12 hours after dosing in rats, and at 12 to 24 hours after dosing in monkeys. In rats, serum $1\alpha,25(\text{OH})_2\text{D}_2$ concentrations were approximately twice as high in males as in females after equivalent doses ($\mu\text{g}/\text{kg}$ body weight) of 1α -OH-D₂, whereas in monkeys there were no apparent sex-related differences in serum $1\alpha,25(\text{OH})_2\text{D}_2$ concentrations. This finding is consistent with the reported higher levels of hepatic microsomal 25-hydroxylase in male rats as compared to females.

In rats given doses of 1α -OH-D₂ greater than approximately 2.5 $\mu\text{g}/\text{kg}$, another active metabolite becomes prominent, namely $1\alpha,24(\text{OH})_2\text{D}_2$. The circulating concentrations of this metabolite also increase in a dose-dependent manner; however, unlike $1\alpha,25(\text{OH})_2\text{D}_2$, there are no apparent sex-related differences in the circulating concentrations of $1\alpha,24(\text{OH})_2\text{D}_2$. However, the significance of these observations to the clinical use of the drug has not been demonstrated.

The metabolic activity of the active (1,25 dihydroxy derivative) form of vitamin D₂ is inferred from studies of calcitriol. Calcitriol and $1\alpha,25(\text{OH})_2\text{D}_2$ have been shown by Scatchard analysis to be equal in binding to vitamin D receptors from chick and rat intestine, bovine thymus, pig kidney cells, and human breast cancer cells. These metabolites were also shown to be equivalent in stimulating

intestinal calcium transport in rats. An enzyme present in cells derived from human breast tissue and regulated by the VDR, 25-hydroxyvitamin D-24-hydroxylase, is similarly induced by both 1 α ,25(OH)₂D₂ and calcitriol. Thus, these compounds are considered to be equivalent and the well investigated activities of calcitriol provide an accurate profile of the less studied activities of 1 α ,25(OH)₂D₂.

The primary action of 1 α -OH-D₂ when used as therapy for secondary hyperparathyroidism in renal disease is to reduce elevated levels of PTH in blood. This activity of 1 α -OH-D₂ has been demonstrated in controlled clinical trials, but not in nonclinical studies. On the other hand, animal studies of PTH suppression have been performed only with calcitriol.

Calcitriol decreases PTH levels both directly, by inhibiting the transcription of the parathyroid hormone gene, and indirectly, by increasing serum calcium which in turn decreases secretion of PTH by the parathyroid glands.

The secondary activities of 1 α -OH-D₂ as a therapy for secondary hyperparathyroidism in renal disease are to normalize serum calcium, to augment bone mass, and to suppress endogenous production of calcitriol.

Effects on calcium metabolism were the first reported biological activities of 1 α -OH-D₂ (Lam *et al.*, 1974). Vitamin D-deficient male rats fed a low calcium diet were injected intrajugularly with 0.25 μ g of 1 α -OH-D₂. Intestinal calcium transport was increased within 3 hours, and reached a maximum of approximately 2.5 times the control value within about 12 hours. Mobilization of calcium from bone, as measured by an increase in serum calcium in these same animals, followed a similar pattern.

In the first report of the biological activity of 1 α -OH-D₂ by Lam *et al.* (1974), the authors indicated that 1 α -OH-D₂ had an antirachitic potency three times that of vitamin D₂ in the stimulation of bone calcification.

In the ovariectomized rat, estrogen depletion induces bone loss in the cancellous bone of the vertebrae. This osteopenia can be prevented by treatment with vitamin D metabolites (Erben *et al.*, 1997), probably by diminishing bone turnover through inhibition of PTH secretion. Calcitriol and 1 α ,25(OH)₂D₂ slightly inhibited vertebral cancellous bone loss relative to the control rats; however, 1 α -OH-D₃ and 1 α -OH-D₂ markedly inhibited bone loss in these animals, by 64% and 84%, respectively. The effects of these two compounds on calcium homeostasis differed: 1 α -OH-D₃ produced a 5-fold increase in urinary calcium excretion, whereas 1 α -OH-D₂ produced only a 2-fold increase. The authors concluded, "compared to 1 α -OH-D₃, 1 α -OH-D₂ combined at least equal or higher bone-protective activity in ovariectomized rats with distinctly less pronounced effects on calcium homeostasis".

The Sponsor suggests that on the basis of these observations, 1 α -OH-D₂ might be expected to have a better safety profile than 1 α -OH-D₃. However, no data in normal subjects or patients with ESRD have been obtained to corroborate this claim.

Excessive intakes of all vitamin D compounds are toxic, and in extreme cases can be lethal. The primary cause of toxicity is hypercalcemia. All pathology observed with administration of large amounts of 1α -OH-D₂ can be explained by excessive increases of serum calcium.

The hepatic metabolism of 1α -OH-D₂ differs from that of 1α -OH-D₃, and this difference might account, at least in part, for possible greater therapeutic index.

Strugnell *et al.* (1995) observed that incubation of a human hepatoma cell line with 1α -OH-D₂ produced the expected metabolite, namely $1\alpha,25(\text{OH})_2\text{D}_2$, at low substrate concentrations (1 - 100 nM), whereas at high substrate concentrations (10 μM) there was a predominance of another metabolite, $1\alpha,24(\text{OH})_2\text{D}_2$. In contrast, the predominant metabolite produced from 1α -OH-D₃ at all substrate concentrations was the 25-hydroxylated compound, calcitriol.

The metabolite, $1\alpha,24(\text{OH})_2\text{D}_2$, preserves the physiologic actions of vitamin D while minimizing pharmacologic side effects (Strugnell *et al.*, 1995; Knutson *et al.*, 1997). This metabolite binds to the vitamin D receptor with approximately the same affinity as $1\alpha,25(\text{OH})_2\text{D}_2$ or calcitriol. These three dihydroxylated compounds also induce gene expression to a similar extent in cell cultures containing reporter genes under the regulation of a VDR enhancer sequence. Unlike the $1\alpha,25$ -dihydroxylated compounds, $1\alpha,24(\text{OH})_2\text{D}_2$ possesses an affinity for the serum vitamin D binding protein that is 10 times weaker than that of $1\alpha,25(\text{OH})_2\text{D}_2$.

Comparison of the pharmacokinetics of $1\alpha,24(\text{OH})_2\text{D}_2$ to those of $1\alpha,25(\text{OH})_2\text{D}_2$ and calcitriol following a single oral dose (0.39 $\mu\text{g}/\text{kg}$) revealed similar half-lives in the blood for all three compounds of approximately five hours; however, the C_{max} for $1\alpha,24(\text{OH})_2\text{D}_2$ was considerably lower than the C_{max} for the other compounds (Knutson *et al.*, 1997).

The effect of $1\alpha,24(\text{OH})_2\text{D}_2$ on calcium metabolism was significantly less than the effects of $1\alpha,25(\text{OH})_2\text{D}_2$ and calcitriol. In vitamin D-deficient rats, oral doses of $1\alpha,25(\text{OH})_2\text{D}_2$ and calcitriol produced similar dose-dependent increases in serum calcium, whereas an oral dose 30 times greater was required for $1\alpha,24(\text{OH})_2\text{D}_2$ to produce a similar response. Dose-response curves generated after oral and subcutaneous administration of $1\alpha,24(\text{OH})_2\text{D}_2$ and calcitriol to normal rats indicated that the ED_{50} for urine calcium excretion was 30 times greater for $1\alpha,24(\text{OH})_2\text{D}_2$ (Knutson *et al.*, 1997).

The shift in metabolism of 1α -OH-D₂ to $1\alpha,24(\text{OH})_2\text{D}_2$ seen with increasing dose *in vitro* with hepatoma cell cultures has also been observed *in vivo* in rats (Knutson *et al.*, 1995). Nine hours after the last of seven daily oral doses of 1α -OH-D₂ at 0.39 $\mu\text{g}/\text{kg}$, $1\alpha,25(\text{OH})_2\text{D}_2$ was the predominant metabolite found in the blood with $1\alpha,24(\text{OH})_2\text{D}_2$ being below the limit of detection. In contrast, at a 1α -OH-D₂ dose of 2.5 $\mu\text{g}/\text{kg}/\text{day}$, blood $1\alpha,24(\text{OH})_2\text{D}_2$ levels were the same as calcitriol levels. In another study (Knutson *et al.*, 1995), rats were orally administered 1α -OH-D₂ by gavage at doses of 6, 20 and 100 $\mu\text{g}/\text{kg}/\text{day}$. Twenty-four hours after the last of 14 doses, both $1\alpha,25(\text{OH})_2\text{D}_2$ and $1\alpha,24(\text{OH})_2\text{D}_2$ were detected.

However, there is no evidence to date of circulating $1\alpha,24(\text{OH})_2\text{D}_2$ in patients receiving therapeutic doses of 1α -OH-D₂. The doses used (5 - 20 $\mu\text{g}/\text{day}$) are much lower than the doses administered to rats (2.5 $\mu\text{g}/\text{kg}/\text{day}$).

1 α ,24(OH)₂D₂ has been shown to circulate in the blood of vitamin D-deficient patients receiving pharmacologic doses of vitamin D₂ as therapy (Mawer *et al.*). Patients presenting with vitamin D-deficiency, as evidenced by low 25-OH-D serum levels, were given oral vitamin D₂ (65 μ mol, 10⁶ IU). The circulating levels of this metabolite were approximately one-tenth of those observed for 1 α ,25(OH)₂D₂.

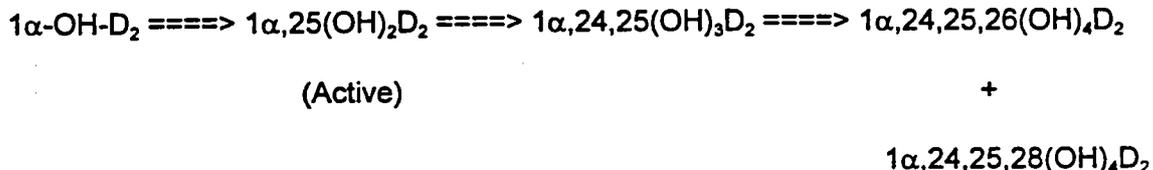
Since no similar studies have been conducted in patients with ESRD receiving Hectorol, these observations are not relevant and cannot be used to claim superiority of Hectorol over D₃ preparations.

No studies of pharmacologic interactions of 1 α -OH-D₂ with other drugs have been conducted.

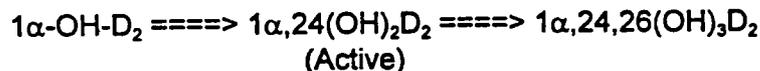
C. *Summary of Metabolism of 1 α -OH-D₂*

The following metabolic pathways have been elucidated for 1 α -OH-D₂.

1. *Via 1 α ,25(OH)₂D₂*



2. *Via 1 α ,24(OH)₂D₂*



Pathway 2, has not been demonstrated to be of any significance in the use of Hectorol in patients with ESRD.

D. *Toxicity*

Acute toxicity studies have been performed in two species, mouse and rat.

1 α -OH-D₂ was toxic by both the oral route and the intraperitoneal (IP) route. Mice appeared more sensitive to the drug than rats, and males of both species more sensitive than the respective females. Clinical findings included reduced activity, dyspnea, ataxia as well as reduced defecation. Deaths occurred 3 to 5 days after dosing by the oral route in rats and 4 to 8 days after dosing in mice. Tan foci on the heart and pale kidneys were seen at necropsy at termination of the study with congestive changes in animals that died during the study.

Multidose toxicity studies have been performed in three species, mouse, rat and cynomolgus monkey, with durations of up to 52 weeks.

The toxicity observed in all the multidose studies was due to increased serum calcium and mineralization principally in kidney, heart, and blood vessels. At necropsy the kidneys were described as discolored, having granular foci, and/or showing a dilated pelvis. Histopathology revealed basophilic tubular epithelium, calcification and dilatation of renal tubules and chronic interstitial nephritis.

Exposure of both rats and monkeys to the drug for 52 weeks produced changes in bones and soft tissues. Hyperostosis was observed in bones, with thickening of the cortical and trabecular bone. Mineralization of multiple organs and tissues, especially the blood vessels, occurred in both species. Other changes noted in hematology, urinalysis, and organ weights were considered secondary to the increased mineralization of tissues. For example, reduction in erythrocytes with increases in reticulocytes was due probably to decreased bone marrow space as a consequence of endosteal bone thickening. A lowered urine pH reflected increased phosphate excretion.

The rat was used as the model for studying the potential effects of oral 1α -OH-D₂ on all three phases of reproduction, including fertility and reproductive performance, development during the period of organogenesis, and perinatal and postnatal development of offspring. In this species, none of the reproductive parameters investigated differed between the groups treated with 1α -OH-D₂ and the study controls and/or historical controls, despite pronounced evidence of maternal toxicity in the high level dosage groups. 1α -OH-D₂ produced no apparent effect on any aspect of reproduction in rats; however, the expected toxic effects on the parent animals were observed. The pregnant rat was more sensitive than the non-pregnant female rat to the toxic effects of 1α -OH-D₂. Rabbits were studied as a second model for effects of oral 1α -OH-D₂ on development during the period of organogenesis. Although fetal weight and incidence of fetal malformations in rabbits in the mid and/or high dose groups were different from the control group, statistical significance was not reached for any parameter.

IV. CLINICAL PHARMACOLOGY

A. General

Administration of 1α -OH-D₂ in humans leads to appearance of $1\alpha,25(\text{OH})_2\text{D}_2$ in blood in a dose- and time-dependent manner, C_{max} for $1\alpha,25(\text{OH})_2\text{D}_2$ occurs from 10 to 12 hours post-dose after an oral dose of 1α -OH-D₂, and at 8 hours after an intravenous dose. These data indicate that the time to reach peak concentration is determined primarily by the rate of metabolic conversion, rather than absorption, of 1α -OH-D₂. **No direct measurements of 1α -OH-D₂ were made and thus no direct estimates of absorption have been performed.** Blood levels of $1\alpha,25(\text{OH})_2\text{D}_2$ in humans resulting from administration of 1α -OH-D₂ are dose-dependent but not dose-proportional; C_{max} and AUC do not increase linearly with dose. Nonlinear pharmacokinetics observed in rats have been attributed in part to increased formation of the secondary metabolite $1\alpha,24(\text{OH})_2\text{D}_2$ at high doses of 1α -OH-D₂. This metabolite has been detected in

plasma from avitaminosis D patients administered large doses of vitamin D₂, but **has not been demonstrated to date in human studies of 1α -OH-D₂**. Bioavailability of $1\alpha,25(\text{OH})_2\text{D}_2$ from orally-dosed 1α -OH-D₂ is about 40% that of a corresponding IV dose. **Estimate of bioavailability is based on pharmacodynamics, rather than on direct measurements. See Biopharmaceutics review.** Steady-state blood concentrations of $1\alpha,25(\text{OH})_2\text{D}_2$ in humans are attained after about four doses when administered every other day. The mean apparent terminal half-life of $1\alpha,25(\text{OH})_2\text{D}_2$ in humans is 32 to 37 hours after an oral dose of 1α -OH-D₂.

Studies in rats and monkeys indicate that urinary excretion of ³H from radiolabeled 1α -OH-D₂ was less than 5% of dose. Human data appear similar; no $1\alpha,25(\text{OH})_2\text{D}_2$ was detected in urine of male subjects following single or repeated doses of 2-6 μg of 1α -OH-D₂. **However, no studies with label were done in humans; since the actual drug was not determined in urine, the absence of $1\alpha,25(\text{OH})_2\text{D}_2$ in the urine is insufficient to rule out excretion of unconverted parent drug.** Protein-binding studies conducted *in vitro* have shown that $1\alpha,25(\text{OH})_2\text{D}_2$ is highly protein-bound. In monkey and rat plasma, and in human serum, approximately 99% of radiolabeled $1\alpha,25(\text{OH})_2\text{D}_2$ was protein-bound. The extent of protein binding appeared to be concentration-independent. Binding of radiolabeled $1\alpha,25(\text{OH})_2\text{D}_2$ to erythrocytes in rat, monkey, and human blood was minimal <5%.

Pharmacokinetic studies are based only on blood levels of the metabolite, $1\alpha,25(\text{OH})_2\text{D}_2$. Two pharmacokinetic studies of 1α -OH-D₂ are reported: a repeated-dose pharmacokinetic study in normal subjects (Protocol No. H-117), and a single-dose pharmacokinetic and bioavailability study in postmenopausal osteoporotic women (Protocol No. H-103). Both studies examined pharmacokinetics of $1\alpha,25(\text{OH})_2\text{D}_2$ after oral administration of 1α -OH-D₂. The oral dosage form was soft gelatin capsules containing 1α -OH-D₂ (2.5 or 1.0 μg) dissolved in fractionated coconut oil (FCO; neutral oil), with 0.03% butylated hydroxyanisole (BHA) as an antioxidant preservative. Protocol No. H-103 also included a determination of the bioavailability of $1\alpha,25(\text{OH})_2\text{D}_2$ from the oral 1α -OH-D₂ dosage form relative to that from an intravenous reference dose (sterile ethanol solution).

An early Phase 1 study of 1α -OH-D₂, conducted in [REDACTED] a former development partner of BCI, included determination of both single-dose and repeated-dose pharmacokinetics of $1\alpha,25(\text{OH})_2\text{D}_2$ following oral administration of 1α -OH-D₂. This study ([REDACTED] Study CPH-001 - CPH-005) used a tablet formulation, which has since been discontinued. Results obtained with this formulation were consistent **(no data are shown, however, to substantiate this claim)** with those obtained with the soft gelatin capsule formulations used in all subsequent studies and so are included, although BCI does not intend to develop a tablet product.

The Phase 3 clinical trials in end-stage renal disease (ESRD) patients, conducted under Protocol No. H-108, included determination of plasma

concentrations of $1\alpha,25(\text{OH})_2\text{D}_2$ at 4-week intervals throughout the 24-week studies. These analyses have provided data on steady-state blood concentrations of $1\alpha,25(\text{OH})_2\text{D}_2$ during long-term oral administration of $1\alpha\text{-OH-D}_2$ in ESRD patients.

1. BCI Protocol No. H-117: Pharmacokinetics of Serum 1-Alpha,25-Dihydroxyvitamin D₂ After Repeated Oral Administration of 1-Alpha-Hydroxyvitamin D₂ in Normal Human Subjects

During repeated administration of oral doses, $1\alpha\text{-OH-D}_2$ was converted to $1\alpha,25(\text{OH})_2\text{D}_2$, whose concentration increased in the serum in a time and dose-dependent manner. Steady-state was attained by the time the fifth dose was administered on Day 9. The maximum serum concentration of $1\alpha,25(\text{OH})_2\text{D}_2$ occurred at 11-12 hours post-dose, consistent with the peak time of 11 hours observed after oral doses in the previous single-dose study. The mean (\pm S.D.) peak concentration after the last 5 μg dose was 29.90 ± 17.65 pg/mL, compared to 67.07 ± 36.33 pg/mL after the last 15 μg dose. The corresponding mean trough concentrations (48 hours post-dose) were 11.05 ± 9.19 , and 26.33 ± 17.14 pg/mL for the 5g and 15- g dose regimens, respectively.

Peak serum concentrations, AUC, and peak-trough concentration differences all increased when the dose was increased from 5 μg administered every other day to 15 μg every other day. However, these parameters did not increase in a linear manner with dose. Adjusted for dose, the AUC, peak concentration, and peak-trough concentration difference for the 15 μg dose were $85.6 \pm 33.5\%$, $85.7 \pm 35.4\%$, and $83.9 \pm 34.4\%$, respectively, of the values expected from the 5 μg regimen based on linear pharmacokinetics.

The terminal elimination rate constant for $1\alpha,25(\text{OH})_2\text{D}_2$ obtained in this study corresponded to a half-life of 32 to 37 hours.

APPEARS THIS WAY ON ORIGINAL

2. **BCI Protocol No. H-103: Pharmacokinetics of $1,25$ -Dihydroxyvitamin D₂ after Oral Administration of 1 -alpha-hydroxyvitamin D₂ in Postmenopausal Osteoporotic Women**

Plasma concentrations of $1\alpha,25(\text{OH})_2\text{D}_2$ increased in a dose- and time-dependent manner following the administration of single oral or intravenous doses of 1α -OH-D₂. The mean time to peak concentration was 8.00 ± 5.89 hours following intravenous administration, and 11.0 ± 4.4 and 11.1 ± 5.0 hours following oral doses of $2 \mu\text{g}$ and $5 \mu\text{g}$, respectively. The peak concentration and area under the curve (AUC) of $1\alpha,25(\text{OH})_2\text{D}_2$ both increased in a dose-dependent manner after oral doses, but not in direct proportion to the administered dose of 1α -OH-D₂. Mean peak concentrations were 9.99 ± 5.29 and 17.38 ± 7.25 pg/mL after the $2 \mu\text{g}$ and $5 \mu\text{g}$ oral doses, respectively, as compared to 51.06 ± 31.45 pg/mL after the $5 \mu\text{g}$ intravenous dose. The Sponsor claims that bioavailability of $1\alpha,25(\text{OH})_2\text{D}_2$ from orally administered 1α -OH-D₂ was approximately 40% of that from an intravenous dose, as determined from their relative AUC (0-48 hour) values. **This assumption is not valid, however, since no direct measurements were made of administered drug and it has been assumed that liver hydroxylation is the same after oral administration as it is after intravenous dosing.**

3. [REDACTED] Study CPH-001 - CPH-005: Report on Phase I Study of [REDACTED]-870

Serum concentrations of $1\alpha,25(\text{OH})_2\text{D}_2$ increased in a dose- and time-dependent manner following the single oral dose of 1α -OH-D₂. The t_{max} was between 8 hours and 12 hours. The area under the curve (AUC) increased in a dose-dependent manner. For the $4 \mu\text{g}$ single dose, the AUC was slightly greater following administration after breakfast as compared with administration under fasting conditions. Serum concentrations of $1\alpha,25(\text{OH})_2\text{D}_2$ increased with repeated administration and reached a plateau after the fourth or fifth dose. The $1\alpha,25(\text{OH})_2\text{D}_2$ disappeared from the serum within 72 hours after the final administration, which indicated no accumulation of $1\alpha,25(\text{OH})_2\text{D}_2$. Endogenous $1\alpha,25(\text{OH})_2\text{D}_3$ decreased with repeated administration of 1α -OH-D₂. No $1\alpha,25(\text{OH})_2\text{D}_2$ was observed in urine.

4. **BCI Protocol No. H-108: A Multicenter, Double-Blind, Placebo-Controlled Study of the Efficacy and Safety of Oral 1α -Hydroxyvitamin D₂ in Reducing Elevated Blood Parathyroid Hormone Levels in End Stage Renal Disease Patients on Hemodialysis.**

Results of these analyses demonstrated a relationship between doses of 1α -OH-D₂ and the plasma concentration of $1\alpha,25(\text{OH})_2\text{D}_2$. Before initiation of treatment with 1α -OH-D₂, the pre-dialysis plasma concentration of $1\alpha,25(\text{OH})_2\text{D}_2$ for most subjects was undetectable (<5 pg/mL; **Some patients had significant measurable levels before drug administration** the mean level for all subjects at Week 0 was 5.04 pg/mL for Study No. H-108-LA and 5.14 pg/mL for Study No. H-108-Memphis. Plasma concentrations of $1\alpha,25(\text{OH})_2\text{D}_2$ increased markedly after 1α -OH-D₂ treatment was begun. The mean pre-dialysis plasma

levels of 1 α ,25(OH)₂D₂ at Week 4 were 44.18 pg/mL for Study No. H-108-LA (n=38) and 35.77 pg/mL for Study No. H-108-Memphis (n=59), both significantly greater than the pre-dose baseline values (P<0.001). At this time 72 of the 99 subjects had received the same 10- μ g dose at the preceding hemodialysis session (44 hours earlier). **The results in the other 27 patients are not discussed.**

In both studies the average maintenance dose during the later weeks of the study stabilized at 15 to 20 μ g/week; at this dosage, the mean plasma concentration of 1 α ,25(OH)₂D₂ stabilized at 20-30 pg/mL. Thus, long-term oral administration of 1 α -OH-D₂ resulted in stable blood levels of 1 α ,25(OH)₂D₂, within a therapeutically effective range. **The definition of "therapeutic effectiveness" is not discussed.**

V. CLINICAL STUDIES

A. BCI Protocol No. H-103: Pharmacokinetics of 1,25-Dihydroxyvitamin D₂ after Oral Administration of 1-alpha-hydroxyvitamin D₂ in Postmenopausal Osteoporotic Women

Fifteen postmenopausal osteoporotic women (ages 58-73 years) participated in this open-label, dose-response study to determine the safety and efficacy of 1 α -OH-D₂. The subjects completed a one-week baseline period, followed successively by a treatment period of up to 7 weeks and a one-week post-treatment observation period. During the treatment period, subjects were administered oral dosages of 1 α -OH-D₂ that were increased stepwise at one-week intervals from a starting dose of 0.5 μ g/day, to 1.0, 2.0, 4.0, and 5.0 μ g/day. Five subjects continued on to doses of 8.0 μ g/day and four to 10.0 μ g/day in the subsequent two weeks. Dietary calcium intake was restricted to 400-600 mg/24 hr. Blood and urine chemistries were determined at baseline and at weekly intervals through one week post-treatment.

Mean serum calcium was significantly (p < 0.05) elevated only at the 4.0 μ g/day dose, but remained within the normal range. Mean urinary calcium excretion was significantly increased at doses of 4.0 and 5.0 μ g/day, from the mean baseline value of 134 \pm 17 mg/24 hr (mean \pm SE), to mean values of 198 \pm 21 and 241 \pm 35 mg/24 hr, respectively (the significance level was not determined for the 8.0 and 10.0 μ g/day doses). None of the subjects had hypercalciuria (> 350 mg/24 hr) at doses less than 5.0 μ g/day. One subject was prematurely removed from the study due to hypercalciuria at the 8.0 μ g/day dose (531 mg calcium/24 hr), and one when intercurrent illness prompted early withdrawal. Urinary calcium excretion for this subject was 360 mg/24 hr at a dose of 2.0 μ g/day. Four other subjects had supranormal levels of urinary calcium by study completion (three at a dose of 5.0 μ g/day, with calcium excretion of 362, 406, and 500 mg/24 hr, and one at 10.0 μ g/day, with calcium excretion of 374 mg/24 hr). Serum creatinine showed a slight and reversible trend toward elevation with dose, and creatinine clearance showed a corresponding slight and reversible trend toward reduction with dose. BUN

showed no meaningful trend. Serum osteocalcin, a marker of osteoblast activity, was significantly elevated at the 2.0 (19%), 4.0 (21%), and 5.0 (28%) $\mu\text{g/day}$ doses ($p < 0.05$), and showed sustained increases relative to baseline of 32% and 55% at the 8.0 and 10.0 $\mu\text{g/day}$ doses, respectively (significance level not determined).

Concentrations of calcitriol and 1 α ,25(OH)₂D₂ were determined in serum obtained 24 hours after the last administered dose at each level. The serum 1 α ,25(OH)₂D₂ level showed a dose-related increase from a mean of 7 pg/mL at baseline to a mean of 23 pg/mL after the 5.0 $\mu\text{g/day}$ dose, while there was a concomitant decrease in serum calcitriol, from a baseline mean of 23 pg/mL to a mean of 14 pg/mL after the 5.0- $\mu\text{g/day}$ dose.

In this short-term study, 1 α -OH-D₂ was well-tolerated with regard to toxicity, as indicated by measurements of serum and urinary calcium and renal function. In addition, 1 α -OH-D₂ administration, at doses greater than 2.0 $\mu\text{g/day}$ was correlated with a significant physiologic response of bone, as determined by - increased serum osteocalcin levels.

B. [REDACTED] Study CPH-001 - CPH-005: Report on Phase I Study of [REDACTED]-870

The safety and pharmacokinetics of 1 α -OH-D₂ ([REDACTED]-870) were evaluated in normal, healthy male subjects following single and repeated-dose administration. The studies were performed at the [REDACTED]. Forty-eight healthy Japanese male subjects, ages 20 to 30 years, participated in the studies. A tablet dosage form (2.0 μg or 4.0 μg of 1 α -OH-D₂) was used. The dose in the single-dose studies ranged from 2 μg to 8 μg of 1 α -OH-D₂. To evaluate the effect of diet on absorption of 1 α -OH-D₂, subjects were dosed with 4 μg of the drug either after breakfast or under fasting conditions, in a cross-over design with a one-week washout period separating the two doses. In the repeated-dose study, a daily dose of 4 μg or 6 μg of 1 α -OH-D₂, or a placebo, was administered after breakfast for seven days. Subjects were monitored for serum calcium, phosphorus, parathyroid hormone (PTH), and bone markers (osteocalcin, calcitonin, and tartrate-resistant acid phosphatase), and for urinary calcium, phosphorus, hydroxyproline, and creatinine. In addition, serum concentrations of calcitriol and 1 α ,25(OH)₂D₂, the active metabolite of 1 α -OH-D₂, were determined.

No adverse events were observed after administration of single doses of 1 α -OH-D₂ ranging from 2 μg to 8 μg , or after repeated doses of 4 μg or 6 $\mu\text{g/day}$. No abnormal values that were attributable to drug administration were observed in renal or hepatic function, or in biochemical parameters, in either the single-dose or the repeated-dose studies.

No problematic abnormal changes such as hypercalcemia were observed. There was a trend toward increased urinary calcium excretion after administration of single and repeated doses of 1 α -OH-D₂, although no apparent dose correlation was observed following administration of 2 μg to 8

μg doses in the single-dose studies. In the repeated-dose study, hypercalciuria (>300 mg/24 hr) occurred in one subject in each of the two treatment groups, as well as in one subject in the placebo group.

Serum PTH levels showed a decreasing trend after administration of 1α -OH-D₂ in the repeated-dose study, with a significant decrease observed in the 6.0 $\mu\text{g}/\text{day}$ group as compared to the placebo group. Other bone metabolism parameters, such as osteocalcin, calcitonin, tartrate-resistant acid phosphatase, and urinary hydroxyproline, showed no significant changes during the treatment period.

Serum concentrations of $1\alpha,25(\text{OH})_2\text{D}_2$ increased in a time-dependent manner following single doses of 1α -OH-D₂. Maximum concentrations occurred between 8 hours and 12 hours postdose, ranging from 17.3 ± 9.9 pg/mL for the 2.0 μg dose to 46.2 ± 22.9 pg/mL for the 8.0 μg dose. The area under the curve (AUC) similarly increased in a dose-dependent manner. For the 4.0 μg single dose, the AUC (0-48 hr) was slightly greater following administration after breakfast as compared with fasting conditions. Serum $1\alpha,25(\text{OH})_2\text{D}_2$ concentrations increased with repeated administration of 1α -OH-D₂, reached a plateau after the 4th-5th administration, and declined to undetectable levels within 72 hours after the final dose of 1α -OH-D₂, indicating no accumulation of $1\alpha,25(\text{OH})_2\text{D}_2$. Endogenous calcitriol decreased with repeated administration of 1α -OH-D₂, however, serum levels of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D did not change. No $1\alpha,25(\text{OH})_2\text{D}_2$ was detected in urine.

C. Overview of Clinical Studies of Efficacy and Safety

Clinical investigations of 1α -OH-D₂ have involved four patient populations: osteoporotic/ osteopenic subjects, ESRD patients, healthy normal subjects, and prostate cancer patients.

Study Identification	Type of Study	Number of Subjects	Duration of Study (treatment)
BCI No. H-101	Open-label; Normal	15	Up to 7 weeks
BCI No. H-102	Double-blind, Placebo-controlled; Osteoporosis	29	52 or 104 weeks
BCI No. H-103	Randomized cross-over, Pharmacokinetic; Osteoporosis	22	Single doses
BCI No. H-106	Open-label, Historical Control; ESRD	27	12 weeks
BCI No. H-107	Randomized, Cross-over, Pharmacokinetic; Normal	8	16 days
BCI Nos. H-108-LA and H-108-Memphis	Double-blind, Placebo-controlled; ESRD	138	16 or 24 weeks
BCI No. H-110	Open-label, Historical Control; ESRD	10	Up to 12 weeks
BCI No. H-112	Randomized, Open-label; Normal	5	Up to 55 days
BCI No. H-113	Open-label; Prostate Cancer	15	12 weeks with optional continued treatment
BCI No. H-117	Randomized, Cross-over, Pharmacokinetic; Normal	25	18 days
CPH-001 through CPH-005	Open-label; Normal	48	Up to 7 days

The patient population of osteoporotic/osteopenic subjects consisted of (1) postmenopausal osteoporotic women between 60 and 71 years of age, and had L2-L3 vertebral bone mineral density between 0.7 and 1.05 g/cm², as determined by dual-energy x-ray absorptiometry (DEXA), and (2) osteopenic men and women between 60 and 100 years of age.

The ESRD patient population consisted of patients of both sexes, between 20 and 75 years of age, with a history of elevated serum PTH values (>400 pg/mL). These patients had been on hemodialysis for at least four months, had a normal (or near normal) serum albumin, and had controlled serum phosphorus. None of the patients had used aluminum-containing dietary phosphate binders during the 12 months preceding the study, or during the conduct of the study. Many had a history of prior treatment with calcitriol.

The healthy normal subjects were men and non-pregnant women between the ages of 20-35.

The prostate cancer patients were males over age 40, who suffered from advanced androgen-independent prostate cancer. All subjects had serum levels of PSA above 10.0 g/mL, with either (1) evaluable or measurable disease or (2) bone scan abnormalities attributable to prostate cancer.

A brief overview of the clinical studies with 1α -OH-D₂ in ESRD patients follows.

1. Initial Phase 2 Trial (Study No. H-106)

This multicenter study was conducted to determine the efficacy of 1α -OH-D₂ in reducing serum PTH levels, and to evaluate its safety with respect to effects on serum calcium and phosphorus levels. The efficacy objectives of the study included determining the effective oral dose range and the response time for reduction of serum PTH levels. The safety objectives included determining the frequency and extent of hypercalcemia and hyperphosphatemia, as well as examining general safety issues such as effects on blood chemistry profiles and CBC parameters. The starting dose in this study was 4.0 μ g of 1α -OH-D₂, administered either daily or three times weekly (after each hemodialysis) for a total weekly dose of 28.0 or 12.0 μ g. Doses were titrated downward as necessary to prevent oversuppression of PTH, to maintain PTH within a target range of 130-250 pg/mL, and to manage incidents of hypercalcemia or hyperphosphatemia.

2. Second Phase 2 Trial (Study No. H-110)

This follow-up study was conducted to refine the pulse dosing regimen for effective PTH reduction in ESRD patients and to confirm the safety of the intended starting dose for the ensuing Phase 3 clinical trials. The patients in this study had all completed the initial Phase 2 study (BCI Protocol No. H-106). The study patients were dosed three times weekly; the starting dose was 10 μ g of 1α -OH-D₂, administered after each hemodialysis, yielding a total weekly dose of 30.0 μ g. Doses were titrated as necessary to manage incidents of hypercalcemia or hyperphosphatemia. Patients completed the study when their plasma PTH fell below 100 pg/mL; no attempt was made to maintain plasma PTH levels within a target range. Safety parameters were serum calcium and phosphorus, blood chemistry profiles, and CBC results.

3. Phase 3 Trials (Study Nos. H-108-LA and H-108-Memphis)

These multicenter trials were conducted using pulse dosing (three times weekly), at a starting dose of 10.0 μ g/hemodialysis (30.0 μ g per week), in order to evaluate efficacy and safety in a larger population of ESRD patients. None of the patients had participated in previous studies with 1α -OH-D₂. An additional objective was to determine the effectiveness of higher doses (up to 20.0 μ g/hemodialysis) in selected patients with unresponsive **(The term "unresponsive" is not defined; if it is used to designate those who did not respond to calcitriol, it was not applied appropriately.)** hyperparathyroidism whose serum calcium and phosphorus were not unduly elevated. All subjects were treated with 1α -OH-D₂ for 16 weeks, then entered an 8-week double-blind, placebo-controlled phase to allow rigorous comparison of efficacy and safety parameters in placebo-dosed subjects vs. those receiving continued treatment with 1α -OH-D₂. Plasma PTH, serum calcium, and serum phosphorus were monitored weekly for all subjects. Doses were titrated as necessary to attempt to control PTH within the target range of 150-300 pg/mL, as well as to manage incidents of hypercalcemia or hyperphosphatemia.

4. Pilot Phase 2 Study in Osteoporotic Women (Study No. H-102)

Subjects received daily treatment with 1α -OH-D₂ for up to 2 years. An initial dose of 1.0 μ g/day was used, and was increased weekly by 1.0 μ g/day to a maximum dose of 5.0 μ g/day. This dose range was chosen on the basis of changes in serum osteocalcin and lack of side effects observed over this range in the Phase 1 study. Safety monitoring of patients for indicators of calcium homeostasis and renal function was done weekly during the initial 6 weeks of treatment and at 3-month intervals thereafter. Measurements included serum and urine calcium, urinary hydroxyproline (marker of bone resorption), creatinine clearance, and BUN. The efficacy parameters monitored were serum osteocalcin (at 3-month intervals), bone density (by dual-energy x-ray absorptiometry, at 6-month intervals), and intestinal calcium absorption (at yearly intervals).

Urinary calcium excretion and serum calcium level were used as the primary indicators for dose titration. The dosage of 1 α -OH-D₂ was increased weekly as noted above until the rate of calcium excretion was elevated to approximately 275-300 mg/24 hours, at which point the dosage was held constant at the highest level attained. Subjects who developed marked hypercalcemia (>10.8 mg/dL), moderate hypercalcemia (10.4-10.8 mg/dL), or marked hypercalciuria (>400 mg/24 hours) immediately suspended treatment until the aberrant parameters were normalized as determined by weekly monitoring, then resumed dosing at a rate 0.5 μ g/day below the previous dose. Subjects who developed mild hypercalcemia (>10.2-10.4 mg/dL) or mild hypercalciuria (350-400 mg/24 hours) which persisted for 2 weeks, had their dosage reduced by 0.5 μ g/day. The incidence of side effects was found to be low, with the principal effects being hypercalcemia and hyperphosphatemia.

VI. Phase 3 Trials in ESRD Patients: Pivotal Trials: STUDY H-108 (LA and Mem)

A. STUDY OBJECTIVE

The objective of this study was to establish the safety and efficacy of pulse dose oral 1 α -OH-D₂ as a therapy for secondary hyperparathyroidism in patients with end stage renal disease (ESRD) on hemodialysis.

Endpoints:

1. Plasma PTH, for evidence of efficacy (No criteria are given.)
2. Serum calcium and phosphorus for evidence of safety.

B. OVERVIEW OF STUDY DESIGN

ESRD subjects undergoing chronic hemodialysis participated in two multicenter, double-blind, placebo-controlled studies conducted using a common protocol (Protocol No. H-108). One study was conducted in the greater Memphis, Tennessee area (sites in Arkansas, Mississippi, and Tennessee), and the other was conducted in the greater Los Angeles, California area (Orange, Inglewood, Newport Beach, Riverside and San Bernardino).

The subjects were between 20 and 75 years of age with a history of secondary hyperparathyroidism. They had been on hemodialysis for at least 4 months, had a normal (or near normal) serum albumin, and had controlled serum phosphorus. The definitions of "near normal" and of "controlled serum phosphorus" are not given.

On admission to the study, each subject was assigned at random, in double-blinded fashion, to one of two treatment groups. One of these groups completed an 8-week washout period and then underwent two consecutive courses of therapy with 1-OH-D₂; the first, open label, lasted 16 weeks and the second, blinded, lasted 8 weeks. The other treatment group completed an identical washout period and then underwent a 16-week open label course of

therapy with 1-OH-D₂ followed by an 8-week course of blinded placebo therapy. The unusual randomization procedure used presents pitfalls in interpretation of final results. Differential rates of drop-out during the first and second periods (16 weeks duration) before actual comparison studies were started may have led to imbalances in the populations undergoing the placebo-controlled phase. Also, the randomization blocks used were quite large for the numbers of subjects recruited at each site. See the Statistician's review for further discussion.

Each subject discontinued 1 α ,25-(OH)₂D₃ therapy for the duration of the study. Throughout the 8-week washout period and the two subsequent treatment periods, subjects underwent routine hemodialysis (3 times per week) using a 2.0 to 3.5 mEq calcium dialysate, and were monitored each week for predialysis levels of serum calcium, serum phosphorus and plasma parathyroid hormone (PTH) at the mid-week hemodialysis. Bone-specific serum markers, plasma 1 α ,25-(OH)₂D₃, plasma 1 α ,25-(OH)₂D₂, blood chemistries and complete blood counts were monitored at selected intervals. The initial dosage of 1 α -OH-D₂ was 10.0 μ g after each hemodialysis session (30.0 μ g/week). This dosage was adjusted, as needed, to bring plasma PTH levels into the targeted range (150 to 300 pg/mL, inclusive). The maximum dosage of 1 α -OH-D₂ in this study was limited to 20.0 μ g after each hemodialysis session (60.0 μ g/week).

Subjects who developed marked hypercalcemia (serum calcium > 11.2 mg/dL) or marked hyperphosphatemia (serum phosphorus > 8.0 mg/dL), immediately suspended 1 α -OH-D₂ treatment and were monitored at each hemodialysis until the serum calcium or phosphorus was sufficiently lowered, and then resumed test drug dosing at a lower rate. Subjects who developed persistent mild hypercalcemia (serum calcium of 10.3 to 11.2 mg/dL) or persistent moderate hyperphosphatemia (serum phosphorus of 7.0 to 8.0 mg/dL) during the treatment periods adjusted their consumption of calcium-based phosphate binders and/or reduced their test drug dosage. Subjects whose plasma PTH fell below 150 pg/mL immediately suspended treatment and then resumed test drug dosing at a lower rate at the mid-week dialysis of the following week.

During the second, blinded, treatment period test drug dosages were adjusted in both treatment groups, as necessary, to achieve and maintain plasma PTH levels in the targeted range and to maintain acceptable serum levels of calcium and phosphorus.

C. MATERIALS AND METHODS

1. Subject Selection

Subjects were recruited from nine community-based hemodialysis units in the area. Subjects qualified for inclusion in the washout period of the study if they were aged 20 to 75 years, had been on hemodialysis treatment for at least four months, had an average serum phosphorus

in the range of 2.5 to \leq 6.9 mg/dL during the previous two months (This criterion is vague; how many determinations were "averaged" and how many actual measurements were above or below this range?), had a history of elevated plasma PTH values (> 400 pg/mL) when not receiving 1 α ,25-(OH)₂D₃ therapy, and had a normal or minimally reduced average serum albumin during the previous two months (not lower than 0.5 g/dL below the normal range (I have the same concerns about the use of "average" here as I stated above.)).

1. Exclusion criteria:

- (1) Did not meet the inclusion criteria specified above.
- (2) Unable to give informed consent.
- (3) Current history of alcohol or drug abuse.
- (4) Partial or total parathyroidectomy within the prior 12 months. Were any patients included who had undergone parathyroidectomy more than 12 months prior to study?
- (5) Use of aluminum-containing preparations as dietary phosphate binders during the previous 12 months, or serum aluminum levels above 40 ng/mL.
- (6) Malignancy requiring ongoing medical treatment, chronic gastrointestinal disease (i.e., malabsorption, surgery affecting absorption, and chronic ulcerative colitis), hepatic impairment, or any other condition which an Investigator believed would interfere with the evaluation of the patient, or put the patient at undue risk.

2. Exclusion from entry into the first treatment period and premature termination of participation in the study:

- (1) Failure to have an average serum phosphorus in the range of 2.5 to \leq 6.9 mg/dL during the washout period.
- (2) Failure to have an average serum calcium of \leq 10.5 mg/dL during the washout period.
- (3) Failure to have an average serum calcium (mg/dL) X serum phosphorus (mg/dL) product of \leq 70.0 during the washout period.
- (4) Renal transplant surgery or partial or complete parathyroidectomy.
- (5) Switch to continuous ambulatory peritoneal dialysis.
- (6) Not having at least one plasma PTH value above 400 pg/mL at enrollment or during the first seven weeks of the washout period (Weeks -8 through -1). The qualifying PTH value must have been

- associated with controlled serum phosphorus (\leq 6.9 mg/dL).
- (7) Not having an average plasma PTH of $>$ 350 pg/mL during the washout period, unless they exhibited at least one plasma PTH value above 400 pg/mL at Week -2 or Week -1 which was associated with controlled serum phosphorus (\leq 6.9 mg/dL).

Enrolled subjects who received 1 α ,25-(OH)₂D₃ therapy (either oral or intravenous) during washout were required to discontinue 1 α ,25-(OH)₂D₃ therapy and to restart the 8-week washout period.

2. Dropouts and Premature Termination

1. Los Angeles Area

One hundred and four subjects were enrolled into the washout period. Of these, 62 (59.6%) qualified for and were admitted into the treatment phase of the study. Thirty-four subjects did not meet the criteria to enter the treatment period and 8 did not enter treatment for other reasons as delineated below:

- (1) Twenty-two subjects did not exhibit plasma PTH levels $>$ 400 pg/mL.
- (2) Four subjects had elevated serum phosphorus levels $>$ 6.9 mg/dL.
- (3) One subject had a serum calcium level $>$ 10.5 mg/dL.
- (4) Two subjects were discontinued for noncompliance with their dialysis schedule.
- (5) Two subjects switched to peritoneal dialysis.
- (6) One subject was unable to change to the required range of dialysate concentration.
- (7) One subject enrolled into the washout period was older than the protocol limit.
- (8) One subject was excluded for regular use of aluminum-based phosphate binders.
- (9) One subject expired during the washout period as a result of illness.
- (10) One subject transferred to another dialysis unit.
- (11) Three subjects were unable to enter treatment because they were too ill.
- (12) Three subjects chose not to participate in the trial after the enrollment visit.

2. Memphis Area

One hundred and eight subjects were enrolled into the washout period. Of these, 76 (70.4%) qualified for and were admitted into the treatment phase of the study. Thirty-two (29.6%) subjects did not meet the criteria to enter the treatment period.

- (1) 18 subjects did not exhibit plasma PTH levels > 400 pg/mL.
- (2) 5 subjects had elevated serum phosphorus levels > 6.9 mg/dL, one subject had a serum calcium level > 10.5 mg/dL.
- (3) 1 had an elevated serum calcium (mg/dL) X serum phosphorus product (mg/dL) > 70.
- (4) 5 subjects expired during the washout period, four as a result of illness, one due to a boating accident.
- (5) 2 subjects voluntarily withdrew during the washout period: one declined for noncompliance with the phosphate binder requirements, and the other no longer chose to participate.

3. Procedures

Routine blood assays (serum chemistry profiles and complete blood counts with differential) were analyzed according to laboratory standard operating procedures at [REDACTED]. Plasma PTH values were assayed at [REDACTED] using a two-site [REDACTED]. Serum osteocalcin and bone-specific alkaline phosphatase were determined by [REDACTED] using [REDACTED].

Serum vitamin D metabolites ($1\alpha,25$ -(OH)₂D₂ and $1\alpha,25$ -(OH)₂D₃) were determined in the Sponsor's laboratory by means of [REDACTED].

[REDACTED] The limits of quantitation were 5.0 pg/mL for $1\alpha,25$ -(OH)₂D₂ and 10.0 pg/mL for $1\alpha,25$ -(OH)₂D₃. For calculation of mean concentrations, a value of 5.0 pg/mL was assigned to plasma $1\alpha,25$ -(OH)₂D₂ concentrations less than the limit of quantitation; likewise, a value of 10.0 pg/mL was assigned to plasma $1\alpha,25$ -(OH)₂D₃ concentrations less than the limit of quantitation.

4. Data Treatment

a. Evaluable Subjects

A subject who received test drug was considered evaluable for statistical purposes provided that:

- (1) The average serum phosphorus was in the range of 2.5 to \leq 6.9 mg/dL during the treatment period.
- (2) At least 80% of the prescribed test dosage was taken, as determined by counting the number of returned capsules.
- (3) Renal transplant surgery, or partial or complete parathyroidectomy had not been performed during the treatment period.
- (4) Treatment with $1\alpha,25$ -(OH)₂D₃ or aluminum-containing products as phosphate binders was not done during the treatment period.
- (5) Analysis of the subject's plasma confirmed a circulating level of $1\alpha,25$ -(OH)₂D₂ which was consistent with the prescribed dosage of test drug.

b. Evaluable Parameters

- (1) Plasma PTH was evaluated for evidence of the test drug's efficacy.
- (2) Serum calcium and phosphorus were evaluated for primary evidence of the test drug's safety.

The definition of "evidence," as applied to either efficacy or safety, was not established *a priori* in the protocol as described in the submission.

c. Adverse Events

All adverse events whether observed by staff or reported by subjects, were recorded, stating the type, onset, duration, severity, relationship to the study medication, and required treatment.

d. Statistical Methods (Discussed in detail in Statistician's Review)

Baseline values for plasma PTH, serum calcium, and serum phosphorus were defined as the average of the data collected during the last three visits during the washout period (Weeks -2, -1 and 0).

Treatment groups were compared with respect to each of the evaluable parameters recorded at baseline using a t-test or a Wilcoxon two-sample test, as appropriate. At each post-baseline visit, the treatment groups were compared with respect to the change from baseline using either a t-test or a Wilcoxon two-sample test, as appropriate. Additionally, the significance of the change from baseline for each of the evaluable parameters at each of the time points was determined using either a paired t-test or a Wilcoxon one-sample test, as appropriate.

For each of the evaluable parameters, an intent-to-treat analysis, using data from all subjects randomized, also was performed. The analysis included an endpoint analysis based on the data collected at the last visit for each subject, determining the change from baseline for each parameter. The treatment groups were compared with respect to the change from baseline using either a t-test or a Wilcoxon two-sample test, as appropriate. Additionally, the significance of the change from baseline for each of the evaluable parameters was determined using either a paired t-test or a Wilcoxon one-sample test, as appropriate.

For the complete blood count and chemistry profile parameters, the treatment groups were compared in two ways: 1) the proportion of subjects for whom the parameter was normal at baseline and then abnormal, post-baseline, using Fisher's exact test; and 2) the mean change from baseline, using a t-test or Wilcoxon two-sample test, as appropriate. Each of these analyses was performed at each sampling time as well as at the end point.

All adverse experiences were recorded. For each type of serious, unexpected adverse event (SAE) or drug-related adverse experience, the treatment groups were compared with respect to the percent of subjects experiencing the adverse effect, using Fisher's exact test.

In addition, the protocol specified that any adverse experiences found to occur with frequency greater than 5% were to be analyzed using the Kaplan-Meier method to estimate the survival curves associated with the time to the occurrence of the adverse effect. The treatment groups were then to be compared with the logrank procedure and the generalized Wilcoxon procedure. Eighteen adverse events with a possible or unknown causality to the test medication were reported in seven

subjects that received 1 -OH-D₂ during the study. These adverse events occurred with a frequency of less than 5% and in too few subjects to be of statistical significance. The statistical analyses described above for analysis of adverse events were therefore not applicable.

All computations were performed using the Statistical Analysis System (SAS). Comparisons were considered to be statistically significant if the two-sided p-value was ≤ 0.05 .

D. SUMMARY OF MAIN FINDINGS

1. Subject Evaluability

a. Los Angeles

(1) Premature Terminations

Twenty-four (38.7%) of the 62 subjects who entered the treatment period were discontinued prematurely from the study or were determined to be non-evaluable.

Twelve subjects were discontinued during Treatment Period 1 as follows: two subjects (Subject Nos. 02103 and 02104) voluntarily withdrew due to experiencing elevated serum phosphorus levels; two subjects (Subject Nos. 08116 and 21105) transferred to another dialysis unit; Subject No. 02118 received a kidney transplant; Subject No. 08104 had a parathyroidectomy performed; Subject No. 20111 received 1 α ,25-(OH)₂D₃; Subject No. 05107 voluntarily withdrew after experiencing leg and thigh pain; Subject No. 20116 voluntarily withdrew after experiencing GI symptoms; Subject No. 08103 was discontinued due to non-compliance with test medication dosing; Subject No. 03105 voluntarily withdrew due to elevated PTH levels; and Subject No. 20104 switched to a twice-a-week dialysis schedule. **Seven of the 12 (previously randomized) discontinued patients in this phase could be considered "treatment failures."**

Five subjects were discontinued during Treatment Period 2 as follows: four subjects (Subject Nos. 08108, 08119, 20105, and 20110) received 1 α ,25-(OH)₂D₃; and one subject (Subject No. 02111) had a parathyroidectomy performed. **All 5 may be considered as "treatment failures."**

Seven subjects completed the 32-week study but were determined to be non-evaluable. Five subjects (Subject Nos. 02107, 02109, 03101, 08120, and 20109) had an average serum phosphorus greater than 6.9 mg/dL during the two treatment periods; and two subjects (Subject Nos. 08101 and 08114) received 1,25-(OH)₂D₃ during Treatment Period 2. **All 7 may be considered as "treatment failures."**