METHODS OF VITAMIN D TREATMENT

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application No. 62/314,359 filed March 28, 2016, is hereby claimed, and the disclosure thereof is incorporated herein by reference.

BACKGROUND

[0002] The Vitamin D metabolites known as 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 (collectively referred to as "25-hydroxyvitamin D") are fat-soluble steroid prohormones that contribute to the maintenance of adequate levels of Vitamin D hormones, calcium and phosphorus in the bloodstream. The prohormone 25-hydroxyvitamin D_2 is produced from Vitamin D_2 (ergocalciferol), and 25-hydroxyvitamin D_3 (calcifediol) is produced from Vitamin D_3 (cholecalciferol), primarily by one or more enzymes located in the liver. The two prohormones also can be produced outside of the liver from Vitamin D_2 and Vitamin D_3 (collectively referred to as "Vitamin D") in certain cells, such as enterocytes, which contain enzymes identical or similar to those found in the liver.

[0003] The Vitamin D prohormones are further metabolized in the kidneys by the 1α-hydroxylase enzyme CYP27B1 into potent hormones. The prohormone 25-hydroxyvitamin D_2 is metabolized into a hormone known as 1α,25-dihydroxyvitamin D_2 (ercalcitriol); likewise, 25-hydroxyvitamin D_3 is metabolized into 1α,25-dihydroxyvitamin D_3 (calcitriol). Production of these hormones from the prohormones also can occur outside of the kidney in cells which contain the required enzyme(s). Rapidly rising or excessive intracellular levels of Vitamin D hormones stimulate the expression of the 24-hydroxylase enzyme CYP24A1 in cells which contain the Vitamin D receptor (VDR). CYP24A1 catabolizes 25-hydroxyvitamin D to form 24,25-dihydroxyvitamin D as the major catabolite.

[0004] The Vitamin D hormones have essential roles in human health which are mediated by intracellular Vitamin D receptors (VDRs). The Vitamin D hormones participate in the regulation of cellular differentiation and growth, parathyroid hormone (PTH) secretion by the parathyroid glands, and normal bone formation and metabolism. In particular, the Vitamin D hormones regulate blood calcium levels by controlling the absorption of dietary calcium and phosphorus by the small intestine and the reabsorption of calcium by the kidneys. Under normal conditions,

actions of Vitamin D on stimulating intestinal calcium absorption predominate, such that dietary calcium is the main source of serum calcium. However, if dietary calcium or Vitamin D is insufficient, the parathyroid gland increases secretion of PTH to enhance calcium mobilization from bone to maintain serum calcium levels. Excessive Vitamin D hormone levels, whether transient or prolonged, can lead to side effects including abnormally elevated urine calcium (hypercalciuria), blood calcium (hypercalcemia), blood phosphorus (hyperphosphatemia), and adynamic bone disease. Vitamin D hormones are also required for the normal functioning of the musculoskeletal, immune and renin-angiotensin systems. Numerous other roles for Vitamin D hormones are being postulated and elucidated, based on the documented presence of intracellular VDRs in nearly every human tissue.

[0005] Left untreated, inadequate Vitamin D supply can cause serious bone disorders, including rickets and osteomalacia, and may contribute to the development of many other disorders including osteoporosis, non-traumatic fractures of the spine and hip, obesity, diabetes, muscle weakness, immune deficiencies, hypertension, psoriasis, and various cancers.

[0006] The Institute of Medicine (IOM) of the National Academy of Sciences has concluded that an Adequate Intake (AI) of Vitamin D for a healthy individual ranges from 200 to 600 IU per day, depending on the individual's age and sex (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, *Dietary reference intakes: calcium, phosphorus, magnesium, vitamin D, and fluoride.* Washington, DC: National Academy Press (1997), incorporated by reference). The AI for Vitamin D was defined primarily on the basis of a serum 25-hydroxyvitamin D level sufficient to prevent Vitamin D deficiency rickets or osteomalacia (or greater than or equal to 11 ng/mL). The IOM also established a Tolerable Upper Intake Level (UL) for Vitamin D of 2,000 IU per day, based on evidence that higher doses are associated with an increased risk of hypercalciuria, hypercalcemia and related sequelae, including cardiac arrhythmias, seizures, and generalized vascular and other soft-tissue calcification. An accepted upper limit for serum 25-hydroxyvitamin D in healthy subjects is about 100 ng/mL or 250 nmol/L (see, e.g., Jones, G, *Am. J. Clin. Nutr.* 88(suppl): 582S-6S, 2008; Holick, MF, *Ann. Epidemiol.* 19(2): 73-78, 2009).

[0007] However, the definition of Vitamin D insufficiency (VDI) in general, and specifically in subjects having Chronic Kidney Disease (CKD), is controversial. The IOM has defined VDI

as total 25-hydroxyvitamin D below 20 ng/mL, whereas the Kidney Disease Outcomes Quality Initiative (KDOQI), Kidney Disease Improving Global Outcomes, (KDIGO) and Endocrinology clinical practice guidelines have defined VDI as 25-hydroxyvitamin D below 30 ng/mL (National Kidney Foundation KDOQI Guidelines, 2003; National Kidney Foundation KDIGO Guidelines, 2009; Holick et al., *J Clin Endocrinol Metab* 96(7):1911-30, 2011). The appearance of the 25-hydroxyvitamin D ($25(OH)D_3$) catabolite, 24,25-dihydroxyvitamin D ($24,25(OH)_2D$), may be a useful measure of sufficiency, with VDI defined by a ratio of $25(OH)D_3$:$24,25(OH)_2D_3$ of greater than 20 (Kaufmann et al., *J Clin Endocrinol Metab* 99(7):2567-2574, 2014). Serum levels of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D are strongly correlated, with the catabolism of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D increasing as serum 25-hydroxyvitamin D rises in normal (i.e., non-CKD) populations (Berg et al., *Clin Chem* 61(6):877-884, 2015; Wagner at al., *J Ster Biochem Mol Bio* 126:72-22, 2011). While CYP24 is typically decreased in normal subjects having VDI, in subjects with CKD, elevated levels of CYP24 have been observed (Helvig et al., *Kidney Int* 78(5):463-72, 2010; International Patent Application No. PCT/US2009/39355).

[0008] Regardless of how VDI is defined, conventional oral Vitamin D supplements are far from ideal for achieving and maintaining optimal blood 25-hydroxyvitamin D levels. These preparations typically contain 400 IU to 5,000 IU of Vitamin D_3 or 50,000 IU of Vitamin D_2 and are formulated for quick or immediate release in the gastrointestinal tract. When administered at chronically high doses, as is often required for Vitamin D repletion in CKD, these products have significant, and often severe, limitations. Additionally, prior uses of immediate-release formulations of calcifediol have not been demonstrated to be effective for suppressing elevated PTH in clinical studies conducted with patients with CKD Stage 3 or Stage 4.

[0009] Controlled release oral formulations and gradually administered parenteral (e.g., slow push IV) formulations of 25-hydroxyvitamin D_2 and/or 25-hydroxyvitamin D_3 can be administered to treat 25-hydroxyvitamin D insufficiency and deficiency without supraphysiological surges in intraluminal, intracellular and blood levels of 25-hydroxyvitamin D and their consequences; without causing substantially increased catabolism of the administered 25-hydroxyvitamin D; and, without causing serious side effects associated with Vitamin D supplementation. The controlled release and gradually administered formulations effectively lower PTH levels without undesirable increases in serum calcium and serum phosphorus and are

therefore useful for treating secondary hyperparathyroidism, for example in CKD patients. *See* International Patent Application Nos. PCT/US2007/061521, PCT/US2008/061579, PCT/US2014/028132, and PCT/EP2015/068219, incorporated herein by reference.

[0010] The controlled released compositions provide substantially increased absorption of 25-hydroxyvitamin D via transport on Vitamin D binding protein (DBP) and decreased absorption via transport in chylomicrons. The compositions also provide maintenance of substantially constant blood levels of 25-hydroxyvitamin D during a 24-hour post-dosing period. By providing a gradual, sustained and direct release of 25-hydroxyvitamin D_2/25-hydroxyvitamin D_3 and absorption preferentially to circulating DBP (rather than to chylomicrons), blood, intraluminal and intracellular 25-hydroxyvitamin D concentration spikes, i.e., supraphysiologic levels and related unwanted catabolism can be mitigated or eliminated. Furthermore, by providing a gradual and sustained release, serum levels of 25-hydroxyvitamin D can be increased and maintained more predictably than by administration of immediate release formulations, allowing for a consistent dosage and reducing or eliminating the need for frequent patient monitoring.

SUMMARY

[0011] The present disclosure relates to methods of treating vitamin D-responsive diseases by raising 25-hydroxyvitamin D (e.g., as serum total 25-hydroxyvitamin D, or calcifediol) to high levels, and concomitantly raising 1,25-dihydroxyvitamin D (e.g., as serum total 1,25-dihydroxyvitamin D, or calcitriol) to high levels, without inducing hypercalcemia.

[0012] The present disclosure also relates to methods of treating vitamin D insufficiency and secondary hyperparathyroidism in patients having CKD. In one aspect, a method of treating secondary hyperparathyroidism in a patient having CKD comprises administering to the patient repeat doses of 25-hydroxyvitamin D effective to raise the patient's serum 25-hydroxyvitamin D level to greater than 90 ng/ml. In another aspect, a method of treating vitamin D insufficiency in a patient having CKD comprises administering to the patient repeat doses of 25-hydroxyvitamin D effective to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20. In still another aspect, a method of treating secondary hyperparathyroidism in a patient having CKD comprises administering to the patient repeat doses of 25-hydroxyvitamin D effective to raise the patient's serum 25-hydroxyvitamin D level

to greater than 90 ng/ml and to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20.

[0013] The present disclosure also relates to methods of administering an extended release dosage form of 25-hydroxyvitamin D, optionally including a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle, and a stabilizing agent; to a patient in need thereof, comprising administering repeat doses of the extended release oral dosage form to the subject effective to achieve one or more of the following: (1) a baseline-adjusted Cmax for 25-hydroxyvitamin D_3 of about 5 ng/mL to about 150 ng/mL; (2) a baseline-adjusted Tmax for 25-hydroxyvitamin D_3 of about 5 days to about 60 days; (3) a baseline-adjusted $AUC_{0\text{-}6\ weeks}$ for 25-hydroxyvitamin D_3 of about 100 ng·d/mL to about 3300 ng·d/mL; (4) a baseline-adjusted Cmax for 1,25-dihydroxyvitamin D of about 0.1 pg/mL to about 50 pg/mL; (5) a baseline-adjusted Tmax for 1,25-dihydroxyvitamin D of about 1 days to about 44 days; (6) a baseline-adjusted $AUC_{0\text{-}6\ weeks}$ for 1,25-dihydroxyvitamin D of about 1 g·d/mL to about 1300 g·d/mL; (7) an increase in mean serum 25-hydroxyvitamin D of about 1 ng/mL/week to about 7 ng/mL/week; (8) an increase in mean serum 1,25-dihydroxyvitamin D of about 1 to about 10 pg/mL/week; (9) a mean steady state serum 25-hydroxyvitamin D level of about 50 ng/mL to about 55 ng/mL; (10) a reduction in plasma intact PTH from baseline of about 30 pg/mL to about 80 pg/mL; (12) a steady state serum 25-hydroxyvitamin D level of greater than about 50 ng/mL to about 100 ng/mL; (13) an increase in serum 1,25-dihydroxyvitamin D from baseline of at least about 10 pg/mL; (14) an increase in serum calcium from baseline of 0 to about 0.3 mg/dL; (15) an increase in serum phosphorous from baseline of 0 to about 0.3 mg/dL; (16) a decrease in serum BSAP from baseline of at least about 10 U/L; (17) a decrease in serum CTX-1 from baseline of at least about 100 pg/mL; (18) a decrease in serum P1NP from baseline of at least about 30 ng/mL; and (19) a decrease in plasma iPTH from baseline of about 15% to about 40%.

[0014] In any of the methods described herein, the patient optionally has CKD Stage 1, 2, 3, 4, or 5. In various embodiments, the method comprises administering repeat doses of 25-hydroxyvitamin D in an amount effective to increase the patient's serum 25-hydroxyvitamin D level to greater than 100 ng/mL, greater than 125 ng/mL, greater than 150 ng/mL, greater than 175 ng/mL, or greater than 200 ng/mL, without causing hypercalcemia, hyperphosphatemia, and/or hypercalciuria. The method can include repeat dosing to achive a serum 25-D level in a range of about 120 ng/mL to about 200 ng/mL, or about 120 ng/mL to about 160 ng/mL, or

about 150 ng/mL to about 200 ng/mL, e.g. in Stage 5 CKD patients on dialysis. Optionally, a method of the disclosure comprises administering repeat doses of 25-hydroxyvitamin D in an amount effective to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, or less than 10.

[0015] For the methods described herein, optional features, including but not limited to components, compositional ranges thereof, substituents, conditions, and steps, are contemplated to be selected from the various aspects, embodiments, and examples provided herein. Further aspects and advantages will be apparent to those of ordinary skill in the art from a review of the following detailed description, taken in conjunction with the drawings. While the methods are susceptible of embodiments in various forms, the description hereafter includes specific embodiments with the understanding that the disclosure is illustrative and is not intended to limit the invention to the specific embodiments described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **Figure 1A** shows the mean (±SE) change from baseline in plasma iPTH in subjects administered a placebo (open circles) or an extended release formulation of 25-hydroxyvitamin D_3 (closed circles) for 26 weeks. **Figure 1B** shows the mean (±SE) change from baseline in plasma iPTH in CKD Stage 3 subjects administered a placebo (open circles), CKD Stage 3 subjects administered an extended release formulation of 25-hydroxyvitamin D_3 (open squares), CKD Stage 4 subjects administered a placebo (closed circles), and CKD Stage 4 subjects administered an extended release formulation of 25-hydroxyvitamin D_3 (closed squares) for up to 62 weeks. **Figure 1C** shows the mean (±SE) plasma iPTH level (pg/mL) compared to the serum total 25-hydroxyvitamin D level (ng/mL) in subjects having CKD Stage 3 or Stage 4 (top graph), CKD Stage 3 (middle graph) or CKD Stage 4 (bottom graph). Asterisks denote statistical significance from the group having serum total 25-hydroxyvitamin D of 0-20 ng/mL.

[0017] **Figure 2A** shows the mean (±SE) serum calcifediol (ng/mL) in subjects administered a placebo (squares) or an extended release formulation of 25-hydroxyvitamin D_3 (diamonds) for 26 weeks. Triple asterisks (***) denote a significant difference from placebo, $p < 0.0001$. **Figure 2B** shows the mean (±SE) serum total 1,25-dihydroxyvitamin D level (pg/mL) compared to the serum total 25-hydroxyvitamin D level (ng/mL) in subjects having CKD Stage 3 or Stage 4 (top

graph), CKD Stage 3 (middle graph) or CKD Stage 4 (bottom graph). Asterisks denote statistical significance from the group having serum total 25-hydroxyvitamin D of 0-20 ng/mL.

[0018] **Figure 3A** shows mean (±SE) serum 24,25-dihydroxyvitamin D_3 (ng/mL) in subjects administered a placebo (squares) or an extended release formulation of 25-hydroxyvitamin D_3 (diamonds) for 26 weeks. Triple asterisks (***) denote a significant difference from placebo, $p < 0.0001$. **Figure 3B** shows the ratio of serum calcifediol (25-hydroxvitamin D_3) to serum 24,25-dihydroxyvitamin D_3 in subjects administered a placebo (squares) or an extended release formulation of 25-hydroxyvitamin D_3 (diamonds) for 26 weeks. Asterisks denote a significant difference from placebo (*=$p < 0.05$, **=$p < 0.001$,***=$p < 0.0001$). **Figure 3C** and **Figure 3D** show the ratio of calcifediol to 24,25-dihydroxyvitamin D_3 compared to serum calcifediol at baseline (**Figure 3C**) and at the end of the efficacy assessment period (EAP) (**Figure 3D**) for CKD Stage 3 and Stage 4 patients administered a placebo for 26 weeks. **Figure 3E** and **Figure 3F** show the ratio of calcifediol to 24,25-dihydroxyvitamin D_3 compared to serum calcifediol at baseline (**Figure 3E**) and at the end of the efficacy assessment period (EAP) (**Figure 3F**) for CKD Stage 3 and Stage 4 patients administered an extended release formulation of 25-hydroxyvitamin D_3 for 26 weeks.

[0019] **Figure 4A** to **Figure 4C** show the ratio of calcifediol to 24,25-dihydroxyvitamin D_3 compared to serum calcifediol at Visit 6/Day 57 (**Figure 4A**), Visit 10/Day 141 (**Figure 4B**) and Visit 13/Day 183 (**Figure 4C**) for CKD Stage 3 patients administered an extended release formulation of 25-hydroxyvitamin D_3 for 26 weeks.

[0020] **Figure 5A** to **Figure 5C** show the ratio of calcifediol to 24,25-dihydroxyvitamin D_3 compared to serum calcifediol at Visit 6/Day 57 (**Figure 5A**), Visit 10/Day 141 (**Figure 5B**) and Visit 13/Day 183 (**Figure 5C**) for CKD Stage 4 patients administered an extended release formulation of 25-hydroxyvitamin D_3 for 26 weeks.

[0021] **Figure 6A** shows the mean (±SE) serum calcium (target < 9.8 mg/dL) and serum phosphorus (target < 5.5 mg/dL) in subjects administered a placebo (squares) or an extended release formulation of 25-hydroxyvitamin D_3 (diamonds) for 26 weeks. **Figure 6B** shows the mean (±SE) serum calcium (mg/dL), and **Figure 6C** shows the mean (±SE) serum phosphorus, compared to the serum total 25-hydroxyvitamin D level. **Figure 6D** shows the mean (±SE) urine calcium/creatinine ratio (Ca/Cr) and **Figure 6E** shows the mean urine tubular reabsorption of

phosphate (TRP) compared to the serum total 25-hydroxyvitamin D level for CKD Stage 3 and Stage 4 patients administered an extended release formulation of 25-hydroxyvitamin D_3 for 26 weeks.

[0022] **Figure 7A** shows the mean (±SE) serum bone-specific alkaline phosphatase (BSAP) (U/L) compared to the serum total 25-hydroxyvitamin D level (ng/mL) in subjects having CKD Stage 3 or Stage 4 (top graph), CKD Stage 3 (middle graph) or CKD Stage 4 (bottom graph). **Figure 7B** shows the mean (±SE) serum collagen type 1 cross-linked C-telopeptide (CTX-1) (pg/mL) (top graph) and **Figure 7C** shows the mean (±SE) serumtype 1 procollagen N-terminal (P1NP) (ng/mL) (bottom graph) compared to the serum total 25-hydroxyvitamin D level (ng/mL) in subjects having CKD Stage 3 or Stage 4. Asterisks denote statistical significance compared to the group having serum total 25-hydroxyvitamin D of 0-20 ng/mL.

[0023] **Figures 8** and **9** show serum calcifediol, serum calcitriol, serum calcium, serum phosphorous, and serum 24,25-dihydroxyvitamin D_3 values resulting from dosing subjects with increasing doses of extended-release, oral calcifediol over a period of up to one year.

DETAILED DESCRIPTION

[0024] The present disclosure relates to methods of administering vitamin D compounds, and methods of treating vitamin-D responsive diseases.

[0025] The present disclosure also relates to methods of treating Vitamin D insufficiency and secondary hyperparathyroidism in patients having Chronic Kidney Disease (CKD). In various embodiments, the methods are used to treat patients having CKD Stage 1, Stage 2, Stage 3, Stage 4, or Stage 5, or a mixed patient population having a combination of any of the foregoing. In one aspect, a method of the disclosure is used to treat a patient having CKD Stage 3 or Stage 4. In another aspect, a method of the disclosure is used to treat a patient having CKD Stage 5, e.g., a hemodialysis patient. Optionally, the patient having CKD has a serum total 25-hydroxyvitamin D concentration of less than 30 ng/mL.

[0026] The following definitions may be useful in aiding the skilled practitioner in understanding the disclosure. Unless otherwise defined herein, scientific and technical terms used in the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art.

[0027] As used herein, the term "comprising" indicates the potential inclusion of other agents, elements, steps, or features, in addition to those specified.

[0028] As used herein, the term "25-hydroxyvitamin D" refers to one or more of 25-hydroxyvitamin D_2, 25-hydroxyvitamin D_3 (also known as calcifediol, calcidiol, and 25-hydroxycholecalciferol), 25-hydroxyvitamin D_4, 25-hydroxyvitamin D_5, 25-hydroxyvitamin D_7, analogs of the foregoing, and combinations thereof. It is specifically contemplated that in any embodiment described herein, 25-hydroxyvitamin D can include 25-hydroxyvitamin D_3, 25-hydroxyvitamin D_2, or a combination of 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2. For example, it is specifically contemplated that in any embodiment described herein, 25-hydroxyvitamin D can include 25-hydroxyvitamin D_3. Serum 25-hydroxyvitamin D and serum total 25-hydroxyvitamin D refer to the total of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 measured in serum by a laboratory assay, unless a reference is made to a particular 25-hydroxyvitamin D form, e.g., 25-hydroxyvitamin D_3.

[0029] As used herein, the term "1,25-dihydroxyvitamin D" refers to one or more of 1,25-dihydroxyvitamin D_2, 1,25-dihydroxyvitamin D_3, 1,25-dihydroxyvitamin D_4, 1,25-dihydroxyvitamin D_5, 1,25-dihydroxyvitamin D_7, analogs of the foregoing, and combinations thereof. For example, 1,25-dihydroxyvitamin D can include 1,25-dihydroxyvitamin D_2, 1,25-dihydroxyvitamin D_3, or a combination of 1,25-dihydroxyvitamin D_2 and 1,25-dihydroxyvitamin D_3. Serum 1,25-dihydroxyvitamin D and serum total 1,25-dihydroxyvitamin D will be understood to refer to the total of 1,25-dihydroxyvitamin D_2 and 1,25-dihydroxyvitamin D_3 measured in serum by a laboratory assay, unless a reference is made to a particular 1,25-dihydroxyvitamin D form.

[0030] As used herein, the term "Vitamin D repletion therapy" refers to the administration to a patient of one or more of a Vitamin D prehormone (e.g., cholecalciferol or ergocalciferol) or an analog thereof , or a Vitamin D prohormone (e.g., 25-hydroxyvitamin D_2 or 25-hydroxyvitamin D_3) or analog thereof. Examples of compounds suitable for use in Vitamin D repletion therapy include ergocalciferol, cholecalciferol, 25-hydroxyvitamin D_2, and 25-hydroxyvitamin D_3.

[0031] As used herein, the term "Vitamin D hormone replacement therapy" refers to the administration to a patient of an effective amount of one or more of active vitamin D hormones, which include active Vitamin D hormone metabolites, and active Vitamin D hormone analogs,

including 1α-hydroxylated Vitamin D compounds. Examples of compounds suitable for use in Vitamin D hormone replacement therapy include the Vitamin D hormones 1,25-dihydroxyvitamin D_2, 1,25-dihydroxyvitamin D_3, 1,25-dihydroxyvitamin D_4, and analogs thereof, including doxercalciferol and paricalcitol.

[0032] As used herein, "co-administration" refers to the administration of two or more compounds, e.g., more than one compound for Vitamin D repletion therapy, or a compound for Vitamin D repletion therapy and a compound for Vitamin D hormone replacement therapy, to the same patient. For example, co-administration encompasses (a) simultaneous administration of a first and second compound and (b) administration of a first compound, followed by administration of a second compound. For example, the first and second compounds can be administered within 24 hours, 8 hours, 4 hours, 2 hours, or 1 hour of each other. In other embodiments, different time periods of between administration of first and second compounds may be applicable.

[0033] As used herein, the terms "controlled release," "modified release," "sustained release," and "extended release" refer to the release of the administered 25-hydroxyvitamin D compound from a composition in a way that deviates from immediate release. For example, an oral extended release formulation makes the 25-hydroxyvitamin D compound available over an extended period of time following ingestion via the extended release formulation design (see USP 24 <1151>). The foregoing terms optionally include delayed release characteristics, wherein the release of 25-hydroxyvitamin D from an oral formulation is delayed until the capsule has passed through the stomach (see USP 24 <1151>).

[0034] As used herein, the term "hypercalcemia" refers to a condition in a patient wherein the patient has two consecutive serum calcium measurements in a course of treatment (e.g., within one month or two weeks apart) above about 10.3 mg/dL.

[0035] As used herein, the term "hyperphosphatemia" refers to a condition wherein a patient has two consecutive serum phosphorus measurements in a course of treatment (e.g., within one month or two weeks apart) above about 5.5 mg/dL.

[0036] As used herein, the term "hypercalciuria" refers to a condition wherein a patient has a ratio of urine calcium to urine creatinine (Ca/Cr) of > 0.2.

[0037] As used herein, the term "bioequivalent" refers to a formulation having *in vivo* biological equivalence to a formulation described herein, if the 90% confidence intervals (90% CI) of the ratios of the Cmax and $AUC_{(0\text{-}inf)}$ between the bioequivalent formulation and the reference formulation are both in the range of 80% to 125% (0.8-1.25), when tested with equivalent methods (including an equivalent dose and fasted conditions).

[0038] It is specifically understood that any numerical value recited herein includes all values from the lower value to the upper value, i.e., all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application. For example, if a concentration range or a beneficial effect range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. As another example, a stated concentration of about 20% is intended to include values from 19.5% up to 20.5%. These are only examples of what is specifically intended.

[0039] In jurisdictions that forbid the patenting of methods that are practiced on the human body, the meaning of "administering" a composition to a human subject shall be restricted to prescribing a controlled substance that a human subject will self-administer by any technique (e.g., orally, inhalation, topical application, injection, insertion, etc.) and to the manufacture of a medicament for use in the methods described herein. The broadest reasonable interpretation that is consistent with laws or regulations defining patentable subject matter is intended. In jurisdictions that do not forbid the patenting of methods that are practiced on the human body, "administering" compositions includes both methods practiced on the human body and also the foregoing activities.

[0040] The compositions and methods of the invention are useful for prophylactic or therapeutic treatment of vitamin D-responsive diseases, i.e., diseases where vitamin D, 25-hydroxyvitamin D or active vitamin D (e.g., 1,25-dihydroxyvitamin D) prevents onset or progression of disease, or reduces signs or symptoms of disease. Such vitamin D-responsive diseases include cancer (e.g., breast, lung, skin, melanoma, colon, colorectal, rectal, prostate and bone cancer). 1,25-dihydroxyvitamin D has been observed to induce cell differentiation and/or inhibit cell proliferation in vitro for a number of cells. Vitamin D-responsive diseases also include autoimmune diseases, for example, type I diabetes, multiple sclerosis, rheumatoid

arthritis, polymyositis, dermatomyositis, scleroderma, fibrosis, Grave's disease, Hashimoto's disease, acute or chronic transplant rejection, acute or chronic graft versus host disease, inflammatory bowel disease, Crohn's disease, systemic lupus erythematosis, Sjogren's Syndrome, eczema and psoriasis, dermatitis, including atopic dermatitis, contact dermatitis, allergic dermatitis and/or chronic dermatitis. Vitamin D-responsive diseases also include other inflammatory diseases, for example, asthma, chronic obstructive pulmonary disease, polycystic kidney disease, polycystic ovary syndrome, pancreatitis, nephritis, hepatitis, and/or infection. Vitamin D-responsive diseases have also been reported to include hypertension and cardiovascular diseases. Thus, the invention contemplates prophylactic or therapeutic treatment of subjects at risk of or suffering from cardiovascular diseases, for example, subjects with atherosclerosis, arteriosclerosis, coronary artery disease, cerebrovascular disease, peripheral vascular disease, myocardial infarction, myocardial ischemia, cerebral ischemia, stroke, congestive heart failure, cardiomyopathy, obesity or other weight disorders, lipid disorders (e.g. hyperlipidemia, dyslipidemia including associated diabetic dyslipidemia and mixed dyslipidemia hypoalphalipoproteinemia, hypertriglyceridemia, hypercholesterolemia, and low HDL (high density lipoprotein)), metabolic disorders (e.g. Metabolic Syndrome, Type II diabetes mellitus, Type I diabetes mellitus, hyperinsulinemia, impaired glucose tolerance, insulin resistance, diabetic complication including neuropathy, nephropathy, retinopathy, diabetic foot ulcer and cataracts), and/or thrombosis.

[0041] Diseases which can benefit from a modulation in the levels of vitamin D compounds, include, but are not limited to: (i) in the parathyroid-- hypoparathyroidism, Pseudohypo-parathyroidism, secondary hyperparathyroidism; (ii) in the pancreas--diabetes; (iii) in the thyroid--medullary carcinoma; (iv) in the skin--psoriasis; wound healing; (v) in the lung--sarcoidosis and tuberculosis; (vi) in the kidney--chronic kidney disease, hypophosphatemic VDRR, vitamin D dependent rickets; (vii) in the bone--anticonvulsant treatment, fibrogenisis imperfecta ossium, osteitis fibrosa cystica, osteomalacia, osteoporosis, osteopenia, osteosclerosis, renal osteodytrophy, rickets; (viii) in the intestine--glucocorticoid antagonism, idopathic hypercalcemia, malabsorption syndrome, steatorrhea, tropical sprue; and (ix) autoimmune disorders.

[0042] In embodiments of the invention, the disease that benefits from a modulation in the levels of vitamin D compounds are selected from cancer, dermatological disorders (for example

psoriasis), parathyroid disorders (for example hyperparathyroidism and secondary hyperparathyroidism), bone disorders (for example osteoporosis) and autoimmune disorders.

[0043] In one type of embodiment, the patient has osteoporosis. The patient can suffer from postmenopausal osteoporosis. The patient can suffer from senile ostoporosis.

[0044] In one type of embodiment, the patient is one who has previously been treated with a bisphosphonate. For example, the bisphosphonate can be etidronate, pamidronate, alendronate, risedronate, zolendronate, or ibandronate. The bisphosphonate can be one having a long half-life in bone, e.g. at least 3 years, or at least 5 years.

[0045] In one aspect, the disclosure provides a method of treating a vitamin-D responsive disease by raising the patient's serum 25-hydroxyvitamin D level as described herein, and concomitantly raising the patient's serum 1,25-dihydroxyvitamin D level to a high level described herein, without causing hypercalcemia. For example, the patient's serum 25-hydroxyvitamin D level can be raised using an extended release, oral dosage form containing 25-hydroxyvitamin D_3, 25-hydroxyvitamin D_2, or a combination of 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2.

[0046] Repeat doses of 25-hydroxyvitamin D can be given, for example, daily, every other day, two or three times per week, weekly, every other week, or monthly. Optionally, the repeat doses of 25-hydroxyvitamin D are administered for an extended period, for example, at least one week, at least two weeks, at least three weeks, one month, at least two months, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least ten months, at least eleven months, at least one year, or longer. It is contemplated that an escalating dosing schedule can be implemented wherein repeat doses are administered on a first schedule for a first period of time, and then at a higher dose on the same schedule for a second period of time. For example, the dose can be an initial dose daily for one month, then an increased dose daily for a second month, then a further increased dose daily for the third month. In another embodiment the dose can be an initial dose 3 times per week for one month, then an increased dose 3 times week for a second month, and then a further increased dose 3 times per week for a third month.

[0047] In one type of embodiment, the 25-hydroxyvitamin D (e.g. calcifediol) can be administered in the fasted state. In one type of embodiment, the 25-hydroxyvitamin D (e.g. calcifediol) is administered at bedtime.

[0048] In one aspect the 25-hydroxyvitamin D can be administered while controlling the patient's total dietary calcium intake (from both food sources and supplements combined) to 1000 mg/day, or less than 1000 mg/day, or less than 900 mg/day, or less than 800 mg/day, or less than 700 mg/day, or less than 600 mg/day, or less than 500 mg/day, for example in a range of 400-1000 mg/day, or 400-900 mg/day, or 400-800 mg/day, or 400-700 mg/day, or 400-600 mg/day, or 500-700 mg/day.

[0049] The methods of the disclosure are effective to raise the patient's serum 25-hydroxyvitamin D to levels previously considered unsafe, but without causing one or more side effects associated with Vitamin D toxicity. Recent data suggest a treatment target for serum 25-hydroxyvitamin D in CKD patients of 40 to 50 ng/mL to lower PTH levels (Ennis et al., *J Nephrol* 29(1):63-70, 2015). While a serum 25-hydroxyvitamin D level of 100 ng/mL has been considered an acceptable upper limit of normal, the methods of the disclosure can be used to raise serum 25-hydroxyvitamin D to significantly higher levels, without causing, for example, one or more of hypercalcemia, hyperphosphatemia, hypercalciuria, and adynamic bone disease. In one aspect, the method is effective to raise the patient's serum 25-hydroxyvitamin D level to greater than about 100 ng/mL, for example, greater than about 110 ng/mL, greater than about 120 ng/mL, greater than about 130 ng/mL, greater than about 140 ng/mL, greater than about 150 ng/mL, greater than about160 ng/mL, greater than about 170 ng/mL, greater than about 180 ng/mL, greater than about 190 ng/mL, greater than about 200 ng/mL, greater than about 210 ng/mL, greater than about 220 ng/mL, greater than about 230 ng/mL, greater than about 240 ng/mL, greater than about 250 ng/mL, greater than about 300 ng/mL, or greater than about 350 ng/mL. In another aspect, the method is effective to raise the patient's serum 25-hydroxyvitamin D level to up to 500 ng/mL, up to 450 ng/mL, up to 400 ng/mL, up to 350 ng/mL, up to 300 ng/mL, up to 250 ng/mL, or up to 240 ng/mL, or up to 230 ng/mL, or up to 220 ng/mL, or up to 210 ng/mL, or up to 200 ng/mL, or up to 190 ng/mL, or up to 180 ng/mL, for example. Optionally, the patient's serum 25-hydroxyvitamin D level is raised to a value in a range of about 90 ng/mL to about 120 ng/mL, or in a range of about 100 ng/mL to about 150 ng/mL, or in a range of about 120 ng/mL to about 160 ng/mL, or in a range of about 140 ng/mL to about 180

ng/mL, or in a range of about 150 ng/mL to about 200 ng/mL, or in a range of about 180 ng/mL to about 200 ng/mL, or in a range of about 190 ng/mL to about 220 ng/mL, or in a range of about 150 ng/mL to about 350 ng/mL or in a range of about 200 ng/mL to about 300 ng/mL, or in a range of about 250 ng/mL to about 300 ng/mL, or in a range of about 150 ng/mL to about 300 ng/mL.

[0050] The Vitamin D prohormones 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 are metabolized primarily in the kidneys by the 1α-hydroxylase enzyme CYP27B1 into the potent, active hormones (25-hydroxyvitamin D_2 is metabolized into 1α,25-dihydroxyvitamin D_2 and 25-hydroxyvitamin D_3 is metabolized into 1α,25-dihydroxyvitamin D_3). It was observed that with repeat dosing of extended release oral 25-hydroxyvitamin D_3 in stage 3 and 4 CKD patients, a dose of 30 mcg/day raised serum 1,25-dihydroxyvitamin D, but a dose of 60 mcg/day provided levels of serum 1,25-dihydroxyvitamin D approximately equal to the 30 mcg/day dose; yet, a dose of 90 mcg/day did further raise serum 1,25-dihydroxyvitamin D. It is believed that at a lower dose, 1,25-dihydroxyvitamin D resulted primarily from renal 1-hydroxylase, but that as serum 1,25-dihydroxyvitamin D levels rose, renal 1-hydroxylase was suppressed. With increased dosing of the extended release oral 25-hydroxyvitamin D_3 (e.g. 90 mcg/day) it is believed that extrarenal production of 1,25-dihydroxyvitamin D was contributing to serum 1,25-dihydroxyvitamin D levels to a more significant extent.

[0051] It has also been observed that with increasing, repeat doses of extended release oral 25-hydroxyvitamin D_3, serum 1,25-dihydroxyvitamin D levels can be raised to very high levels, without inducing hypercalcemia. It is believed that the gradual rise in serum 25-hydroxyvitamin D, and the gradual rise in serum 1,25-dihydroxyvitamin D, allow for a physiological adaptation under which intestinal calcium absorption is not induced or is induced to a significantly lesser extent, compared to methods which raise serum 25-hydroxyvitamin D and/ serum 1,25-dihydroxyvitamin D more rapidly.

[0052] In another aspect, the disclosure provides a method of treating secondary hyperparathyroidism in a patient having CKD, comprising administering to the patient repeat doses of 25-hydroxyvitamin D effective to raise the patient's serum 25-hydroxyvitamin D level to greater than 90 ng/ml. In another aspect, the disclosure provides a method of treating secondary hyperparathyroidism in a patient having CKD, comprising administering to the patient

repeat doses of 25-hydroxyvitamin D effective to raise the patient's serum 25-hydroxyvitamin D level to greater than 90 ng/ml and to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20, or less than 15.

[0053] In another aspect, a method of the disclosure comprises administering repeat doses of a compound for Vitamin D repletion therapy, for example, 25-hydroxyvitamin D and/or ergocalciferol or cholecalciferol, in an amount effective to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20, or less than 18, or less than 16, or less than 15, or less than 14, or less than 12, or less than 10. For example, in one aspect, the disclosure provides a method of treating vitamin D insufficiency in a patient having CKD, comprising administering to the patient repeat doses of 25-hydroxyvitamin D effective to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20. In another aspect, the disclosure provides a method of treating vitamin D insufficiency in a patient having CKD, comprising administering to the patient repeat doses of a compound for Vitamin D repletion therapy, for example, 25-hydroxyvitamin D and/or ergocalciferol or cholecalciferol, in an amount effective to increase the patient's serum 25-hydroxyvitamin D level to greater than about 100 ng/mL and to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20. In normal subjects, a plot of the serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D (25(OH)D3:24,25(OH)2D3) versus serum 25-hydroxyvitamin D (25(OH)D3) reached a plateau when 25(OH)D3:24,25(OH)2D3 was less than 20, indicating Vitamin D sufficiency (Kaufmann et al., *supra*). In CKD patients, a ratio of 25(OH)D3:24,25(OH)2D3 of less than 25 was not achieved even as serum 25-hydroxyvitamin D values approached and exceeded 100 ng/mL (Figure 3F), indicating that the ratio of 25(OH)D3:24,25(OH)2D3 corresponding to Vitamin D sufficiency in CKD patients would be achieved at a much higher concentration of serum 25-hydroxyvitamin D for defining Vitamin D sufficiency in CKD patients could be significantly less than 20. Optionally, the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D is lowered to or maintained at a ratio of less than 20, less than 15, or less than 10, for example, the ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D is about 25, about 24, about 23, about 22, about 21, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, or about 5. Quantification of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D can be performed using a suitable assay. Suitable assays are known

in the art, for example, radioimmunoassay, mass spectrometry, HPLC, and LC-MS/MS, e.g., as described in Kaufmann et al., *supra,* Wagner et al., *supra*, and Berg et al., *supra*.

[0054] Administration of a compound for Vitamin D hormone replacement therapy alone can reduce serum 25-hydroxyvitamin D while increasing catabolism by CYP24A, thereby increasing serum 24,25-dihydroxyvitamin D and resulting in a decrease in the serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D, but without achieving Vitamin D sufficiency. It may be possible that administration of a compound for Vitamin D hormone replacement therapy alone could result in the serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to 20 or less. Accordingly, it is contemplated that in one category of embodiments of the methods described herein, patients will and will be treated to control the patient's ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20 and simultaneously will have a serum level of 25-hydroxyvitamin D of at least 30 ng/ml. In another category of embodiments of the methods described herein, patients will be treated to control the patient's ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20 and will receive vitamin D repletion therapy. Optionally, the patients can be treated in the absence of administration of a compound for Vitamin D hormone replacement therapy. In one type of embodiment, a method of treating secondary hyperparathyroidism and Vitamin D insufficiency in a patient having Chronic Kidney Disease comprises administering to the patient repeat doses of a compound for Vitamin D repletion therapy effective to raise the patient's serum 25-hydroxyvitamin D level to greater than 90 ng/ml and to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20, wherein the patient is not receiving Vitamin D hormone replacement therapy. In another type of embodiment, a method of treating secondary hyperparathyroidism and Vitamin D insufficiency in a patient having Chronic Kidney Disease comprises co-administering to the patient repeat doses of a compound for Vitamin D repletion therapy and a compound for Vitamin D hormone replacement therapy effective to raise the patient's serum 25-hydroxyvitamin D level to greater than 30 ng/ml and to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20.

[0055] In another aspect, the disclosure provides a method of treating vitamin D insufficiency in a patient having CKD, comprising administering to the patient repeat doses of 25-hydroxyvitamin D effective to increase the patient's serum 1,25-dihydroxyvitamin D level to greater than about 40 pg/mL, optionally to also control the patient's serum ratio of 25-

hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20. Optionally, the patient's serum 1,25-dihydroxyvitamin D level is increased to greater than 40 pg/mL, greater than 45 pg/mL, greater than 50 pg/mL, greater than 55 pg/mL, greater than 60 pg/mL, greater than 65 pg/mL, greater than 70 pg/mL, greater than 75 pg/mL, greater than 80 pg/mL, greater than 90 pg/mL, greater than 100 pg/mL, greater than 110 pg/mL, greater than 120 pg/mL, greater than 130 pg/mL, greater than 140 pg/mL, greater than 150 pg/mL, greater than 160 pg/mL, greater than 170 pg/mL, greater than 180 pg/mL, greater than 190 pg/mL, greater than 200 pg/mL, greater than 210 pg/mL, greater than 220 pg/mL, greater than 230 pg/mL, greater than 240 pg/mL, greater than 250 pg/mL, greater than 260 pg/mL, greater than 270 pg/mL, greater than 280 pg/mL, greater than 290 pg/mL, or greater than 300 pg/mL. Optionally, the patient's serum 1,25-dihydroxyvitamin D level is increased to a value within a range of 150 pg/mL to 350 pg/mL, or 150 pg/mL to 300 pg/mL, or 200 pg/mL to 300 pg/mL, for example. Optionally, the patient's serum 1,25-dihydroxyvitamin D level is increased from baseline by at least 25 pg/mL, at least 50 pg/mL, at least 75 pg/mL, at least 100 pg/mL, at least 125 pg/mL, at least 150 pg/mL, at least 175 pg/mL, or at least 200 pg/mL.

[0056] In any of the methods of the disclosure, repeat doses of 25-hydroxyvitamin D are optionally administered to the subject in an amount effective to have one or more effects including to (a) lower PTH, (b) increase serum 25-hydroxyvitamin D, (c) increase serum 1,25-dihydroxyvitamin D, or (d) control the ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D as described herein. The dosing can also be performed to avoid one or more negative effects, including (a) without increasing serum calcium, (b) without increasing serum phosphorus, (c) without increasing a marker of bone turnover, (d) without inducing hypercalcemia, or (e) without inducing hyperphosphatemia. In one aspect, repeat doses of 25-hydroxyvitamin D are optionally administered to the subject in an amount effective to lower the patient's blood PTH level (e.g., plasma intact PTH) by at least about 15%, for example, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%, compared to its pre-treatment level. In another aspect, repeat doses of 25-hydroxyvitamin D are optionally administered to a patient population in an amount effective to lower the mean plasma intact PTH level of the patient population by at least about 15%, for example, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least

about 35%, at least about 40%, at least about 45%, or at least about 50%, compared to its pre-treatment level.

[0057] In one aspect, repeat doses of 25-hydroxyvitamin D are administered in an amount effective to maintain serum calcium in a range of about 9.0 mg/dL to about 10.0 mg/dL, for example in a range of about 9.0 mg/dL to about 9.5 mg/dL, in a range of about 9.2 mg/dL to about 9.6 mg/dL, or in a range of about 9.3 mg/dL to about 9.8 mg/dL. In another aspect, repeat doses of 25-hydroxyvitamin D are administered in an amount effective to increase serum calcium in an amount in the range of 0 to about 0.3 mg/dL. In one aspect, repeat doses of 25-hydroxyvitamin D are administered in an amount effective to maintain serum phosphorous in a range of about 3.5 mg/dL to about 4.5 mg/dL, for example in a range of about 3.5 mg/dL to about 4.0 mg/dL, in a range of about 3.6 mg/dL to about 4.2 mg/dL, or in a range of about 3.7 mg/dL to about 4.1 mg/dL. In another aspect, repeat doses of 25-hydroxyvitamin D are administered in an amount effective to increase serum phosphorous in an amount in the range of 0 to about 0.3 mg/dL. In one aspect, repeat doses of 25-hydroxyvitamin D are administered in an amount effective to maintain or decrease a marker of bone turnover selected from urine Ca/Cr, urine TRP, serum BSAP, serum CTX-1, serum P1NP, and combinations thereof.

[0058] In any of the methods of the disclosure, 25-hydroxyvitamin D is optionally administered in a modified release composition, for example, an extended-release oral dosage form comprising 25-hydroxyvitamin D_3. In one type of embodiment, modified release compositions intended for oral administration in accordance with the present invention are designed to contain a concentration of 25-hydroxyvitamin D (e.g. 25-hydroxyvitamin D_3, or a combination of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3) of 1 to 1000 mcg per unit dose, or 1 to 500 mcg per unit dose or 1 to 100 mcg per dose, or 1 to 50 mcg per dose, or 10 to 40 mcg per dose, for example 30 mcg per dose,60 mcg per dose, 90 mcg per dose, 150 mcg per dose, 300 mcg per dose, 450 mcg per dose, 600 mcg per dose, 750 mcg per dose, or 900 mcg per dose, and are prepared in such a manner as to effect controlled or substantially constant release of the 25-hydroxyvitamin D into the gastrointestinal tract of a subject over an extended period of time. In one embodiment, the 25-hydroxyvitamin D is 25-hydroxyvitamin D_3. In another embodiment, the 25-hydroxyvitamin D is a combination of 25-hydroxyvitamin D_3 and 25-hydroxy vitamin D_2 and are useful in supporting both the Vitamin D_3 and Vitamin D_2 endocrine systems. Currently available oral Vitamin D supplements and the previously marketed oral

formulation of 25-hydroxyvitamin D_3 have supported just one or the other system. In one type of embodiment, the release can be in the ileum or later, for example in the colon. In another type embodiment, the composition can result in a substantially increased absorption of 25-hydroxyvitamin D via transport on DBP and decreased absorption via transport in chylomicrons. Examples of modified release compositions of 25-hydroxyvitamin D are described in U.S. Patent Nos. 8,207,149; 8,361,488; 8,426,391; 8,778,373; and 8,906,410; and U.S. Patent Application No. 14/213,285, incorporated herein by reference.

[0059] In one type of embodiment, the 25-hydroxyvitamin D is administered orally. For example, the 25-hydroxyvitamin D can be administered in an oral modified release formulation. In the alternative, the 25-hydroxyvitamin D can be administered in an oral immediate release formulation in multiple daily doses in order to produce a pharmacokinetic profile of serum 25-hydroxyvitamin D that is similar to that achieved by an oral modified or sustained release formulation.

[0060] The preparation of a modified release form of 25-hydroxyvitamin D suitable for oral administration can be carried out according to many different techniques. For example, one or more 25-hydroxyvitamin D compounds can be dispersed within a matrix, i.e., a mixture of rate controlling constituents and excipients in carefully selected ratios within the matrix, and optionally encased with a coating material. In another alternative, various coating techniques can be utilized to control the rate and/or the site of the release of the 25-hydroxyvitamin D from the pharmaceutical formulation. For example, the dissolution of the coating may be triggered by the pH of the surrounding media, and the resulting gradual dissolution of the coating over time exposes the matrix to the fluid of the local environment. In one type of embodiment, after the coating becomes permeable, 25-hydroxyvitamin D diffuses from the outer surface of the matrix. When this surface becomes exhausted or depleted of 25-hydroxyvitamin D, the underlying stores begin to be depleted by diffusion through the disintegrating matrix to the external solution. In another type of embodiment, release of 25-hydroxyvitamin D is by gradual disintegration or erosion of the matrix, e.g., via solubility of one or more components of the matrix and/or by lack of physical integrity.

[0061] In one aspect, a formulation in accordance with the disclosure provides one or more 25-hydroxyvitamin D compounds within a matrix that releasably binds the ingredients for extended release, e.g., when exposed to the contents of the ileum and/or colon.

[0062] Optionally, the 25-hydroxyvitamin D-containing dosage form or matrix can be suitably covered with a coating that is resistant to disintegration in gastric juices. The coated modified release formulation of 25-hydroxyvitamin D is then administered orally to subjects, e.g., animals or human patients. As the formulation travels through the proximal portion of the small intestine, the enteric coating becomes progressively more permeable but, in a suitable embodiment, it provides a persisting structural framework around the 25-hydroxyvitamin D-containing matrix. The 25-hydroxyvitamin D-containing matrix becomes significantly exposed to intestinal fluids in the ileum through the permeable overcoating, and the 25-hydroxyvitamin D is then gradually released by simple diffusion and/or slow disintegration of the matrix.

[0063] Once released into the lumen of the ileum, the 25-hydroxyvitamin D is absorbed into the lymphatic system or into the portal bloodstream, where it is bound to and transported by the DBP. In a delayed release embodiment, the 25-hydroxyvitamin D is primarily absorbed at a point beyond the duodenum and jejunum. These proximal portions of the small intestine can respond to high intralumenal levels of 25-hydroxyvitamin D and in the process, can catabolize significant quantities of the 25-hydroxyvitamin D. By substantially delaying 25-hydroxyvitamin D release until the ileum and/or colon, the pharmaceutical composition described herein can virtually eliminate these potential first-pass effects in the proximal intestine and reduces unwanted catabolism. Significant catabolism of administered 25-hydroxyvitamin D prior to absorption into the bloodstream significantly lowers its bioavailability. Elimination of first-pass effects reduces the risk of Vitamin D toxicity. Substantially delayed release of 25-hydroxyvitamin D (i.e., beyond the duodenum and jejunum) markedly decreases the amount of 25-hydroxyvitamin D that is incorporated and absorbed from the small intestine via chylomicrons (since chylomicron formation and absorption occurs primarily in the jejunum) and correspondingly increases the amount of 25-hydroxyvitamin D that is absorbed directly through the intestinal wall and onto DBP circulating in lymph or portal blood.

[0064] In one embodiment of the invention, a controlled release oral formulation of 25-hydroxyvitamin D is prepared generally according to the following procedure. A sufficient

quantity of 25-hydroxyvitamin D is completely dissolved in a minimal volume of USP-grade absolute ethanol (or other suitable solvent) and mixed with appropriate amounts and types of pharmaceutical-grade excipients to form a matrix which is solid or semi-solid at both room temperature and at the normal temperature of the human body. The matrix is completely or almost entirely resistant to digestion in the stomach and upper small intestine, and it gradually disintegrates in the lower small intestine and/or colon.

[0065] In a suitable formulation, the matrix binds the 25-hydroxyvitamin D compound(s) and permits a slow, relatively steady, e.g. substantially constant, release of 25-hydroxyvitamin D over a period of four to eight hours or more, by simple diffusion and/or gradual disintegration, into the contents of the lumen of the lower small intestine and/or colon. The formulation optionally further has an enteric coating that partially dissolves in aqueous solutions having a pH of about 7.0 to 8.0, or simply dissolves slowly enough that significant release of 25-hydroxyvitamin D is delayed until after the formulation passes through the duodenum and jejunum.

[0066] The means for providing the controlled release of 25-hydroxyvitamin D may be selected from any suitable controlled release delivery system, including any of the known controlled release delivery systems of an active ingredient, including the wax matrix system, and the EUDRAGIT RS/RL system (Rohm Pharma, GmbH, Weiterstadt, Germany).

[0067] The wax matrix system provides a lipophilic matrix. The wax matrix system may utilize, for example, natural or synthetic waxes, digestible or non-digestible waxes, e.g. beeswax, white wax, cachalot wax or similar compositions, or mixtures of waxes. In one type of embodiment the wax is a non-digestible wax. The active ingredient(s) are dispersed in the wax binder which slowly disintegrates in intestinal fluids to gradually release the active ingredient(s). The wax binder that is impregnated with 25-hydroxyvitamin D can be loaded into capsules, e.g. softgel capsules. A softgel capsule may comprise one or more gel-forming agents, e.g., gelatin, starch, carrageenan, and/or other pharmaceutically acceptable polymers. In one embodiment, partially crosslinked soft gelatin capsules are used. As another option, vegetable-based capsules can be used. The wax matrix system disperses the active ingredient(s) in a wax binder which softens at body temperature and slowly disintegrates in intestinal fluids to gradually release the active ingredient(s). The system suitably can include a mixture of waxes, with the optional addition of oils, to achieve a melting point which is higher than body temperature, but lower than

the melting temperature of the selected formulations used to create the shell of a soft or hard capsule, or vegetable capsule shell, or other formulation used to create a shell casing or other coating.

[0068] In one type of embodiment, the wax matrix comprises a controlled release agent, an emulsifier, and an absorption enhancer. Examples of controlled release agents suitable for use include, but are not limited to, waxes, including synthetic waxes, microcrystalline wax, paraffin wax, carnauba wax, and beeswax; polyethoxylated castor oil derivatives, hydrogenated vegetable oils, glyceryl mono-, di- or tribehenates; long-chain alcohols, such as stearyl alcohol, cetyl alcohol, and polyethylene glycol; and mixtures of any of the foregoing. Non-digestible waxy substances, e.g. hard paraffin wax, are particularly contemplated. The controlled release agent can be present in an amount of at least 5 wt% of the formulation, or greater than about 5 wt% of the formulation. For example, depending on the controlled release agent used, the controlled release agent can comprise at least 5 wt% of the formulation or at least 10 wt% of the formulation, or at least 15 wt% of the formulation, or at least 20 wt% of the formulation, or at least 25 wt% of the formulation, or greater than 5 wt% of the formulation, or greater than 10 wt% of the formulation, or greater than 15 wt% of the formulation, or greater than 20 wt% of the formulation, and or greater than 25 wt% of the formulation. The controlled release agent can be present in an amount 50 wt% or less, 40 wt% or less, 35 wt% or less, or 30 wt% or less. Suitable ranges include 5 wt% to 40 wt%, 10 wt% to 30 wt% and 15 wt% to 25 wt%. Examples include about 15 wt%, about 16 wt%, about 17 wt%, about 18 wt%, about 19 wt%, about 20 wt%, about 21 wt%, about 22 wt%, about 23 wt%, about 24 wt%, and about 25 wt%.

[0069] Examples of emulsifiers suitable for use in the formulation include, but are not limited to, lipophilic agents having an HLB of less than 7, such as mixed fatty acid monoglycerides; mixed fatty acid diglycerides; mixtures of fatty acid mono- and diglycerides; lipophilic polyglycerol esters; glycerol esters including glyceryl monooleate, glyceryl dioleate, glyceryl monostearate, glyceryl distearate, glyceryl monopalmitate, and glyceryl dipalmitate; glyceryl-lacto esters of fatty acids; propylene glycol esters including propylene glycol monopalmitate, propylene glycol monostearate, and propylene glycol monooleate; sorbitan esters including sorbitan monostearate, sorbitan sesquioleate; fatty acids and their soaps including stearic acid, palmitic acid, and oleic acid; and mixtures thereof glyceryl monooleate, glyceryl dioleate, glyceryl monostearate, glyceryl distearate, glyceryl monopalmitate, and glyceryl dipalmitate;

glyceryl-lacto esters of fatty acids; propylene glycol esters including propylene glycol monopalmitate, propylene glycol monostearate, and propylene glycol monooleate; sorbitan esters including sorbitan monostearate, sorbitan sesquioleate; fatty acids and their soaps including stearic acid, palmitic acid, and oleic acid; and mixtures thereof.

[0070] One type of lipophilic agent is selected from glycerides and derivatives thereof. Glycerides are selected from the group consisting of medium or long chain glycerides, caprylocaproyl macrogolglycerides, and mixtures thereof, for example. Medium chain glycerides can include, but are not limited to, medium chain monoglycerides, medium chain diglycerides, caprylic/capric triglyceride, glyceryl monolaurate, glyceryl monostearate, caprylic/capric glycerides, glycerylmonocaprylate, glyceryl monodicaprylate, caprylic/capric linoleic triglyceride, and caprylic/capric/succinic triglyceride. Monoglycerides having a low melting point are specifically contemplated for making the formulation. Monoglycerides can include, but are not limited to, glyceryl monostearate, glyceryl monopalmitate, glyceryl monooleate, glyceryl monocaprylate, glyceryl monocaprate, glyceryl monolaurate, etc.. Glycerol monostearate (GMS) is specifically contemplated. GMS is a natural emulsifying agent. It is oil soluble, but poorly soluble in water. GMS has an HLB value of 3.8. The lipophilic emulsifier can be present in an amount in a range of about 10 wt% to about 40 wt%, or about 20 wt% to about 25 wt%, for example. Other examples include about 20 wt%, about 21 wt%, about 22 wt%, about 23 wt%, about 24 wt%, and about 25 wt%.

[0071] Examples of suitable absorption enhancers include, but are not limited to, caprylocaproyl macrogolglycerides such as polyethylene glycosylated glycerides, also known as polyglycolized glycerides or PEGylated glycerides. PEGylated glycerides which may be employed in the composition include, but are not limited to, mixtures of monoglycerides, diglycerides, and triglycerides and monoesters and diesters of polyethylene glycol, polyethylene glycosylated almond glycerides, polyethylene glycosylated corn glycerides, and polyethylene glycosylated caprylic/capric triglyceride. The absorption enhancer can have an HLB value from 13 to 18, or from 13 to 15. One class of absorption enhancers is known under the trade name GELUCIRE (Gattefosse Corporation, Paramus, New Jersey, USA). GELUCIRE is a well-known excipient which is a family of fatty acid esters of glycerol and PEG esters, also known as polyglycolized glycerides. GELUCIRE is used in various applications including preparing sustained release pharmaceutical compositions. GELUCIRE compounds are inert, semi-solid

waxy materials which are amphiphilic and are available with varying physical characteristics such as melting point, HLB, and solubilities in various solvents. They are surface active in nature and disperse or solubilize in aqueous media forming micelles, microscopic globules or vesicles. They are identified by their melting point/HLB value. The melting point is expressed in degrees Celsius. One or a mixture of different grades of GELUCIRE excipient may be chosen to achieve the desired characteristics of melting point and/or HLB value. GELUCIRE 44/14 (lauroyl macrogolglycerides/lauroyl polyoxylglycerides) has a melting point of 44 °C and a HLB of 14, and is particularly contemplated. The absorption enhancer can be present in an amount of about 5 wt% to about 20 wt%, or about 8 wt% to about 15 wt%, for example. Other examples include about 8 wt%, about 9 wt%, about 10 wt%, about 11, wt% about 12 wt%, about 13 wt%, about 14 wt%, and about 15 wt%.

[0072] Examples of other lipid matrices suitable for use with the methods of the invention include one or more of glycerides, fatty acids and alcohols, and fatty acid esters.

[0073] In one embodiment, a formulation may comprise an oily vehicle for the 25-hydroxyvitamin D compound. Any pharmaceutically-acceptable oil can be used. Examples include animal (e.g., fish), vegetable (e.g., soybean), and mineral oils. An oil can be selected to readily dissolve the 25-hydroxyvitamin D compound used. Oily vehicles can include non-digestible oils, such as mineral oils, particularly liquid paraffins, and squalene. The ratio between the wax matrix and the oily vehicle can be optimized in order to achieve the desired rate of release of the 25-hydroxyvitamin D compound. Thus, if a heavier oil component is used, relatively less of the wax matrix can be used, and if a lighter oil component is used, then relatively more wax matrix can be used. In one embodiment, the particular choice of oily vehicle provides a controlled release so that absorption of 25-hydroxyvitamin D is delayed until the formulation reaches the ileum and/or colon.

[0074] In one embodiment, a formulation may comprise a stabilizing agent such as a cellulose compound. Examples of cellulose compounds and stabilizing agents include, but are not limited to, celluloronic acid, carboxy methyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl cellulose, hydroxyl propyl methyl cellulose (hypromellose), methylcellulose, polyanionic cellulose, and combinations thereof. Also contemplated are one or more of poloxamers (e.g., polaxamer 407), poly (ethylene oxide) polymers (e.g., Dow's POLYOX

polymers), povidones, and fumed silicas (e.g., AEROSIL 200, Evonik Industries AG, Essen, Germany). The stabilizing agent, e.g., a cellulosic compound, can be present in an amount of at least about 5 % of the formulation, based on the total weight of the formulation excluding any additional coatings or shells (wt%), for example. For example, the cellulosic compound can be present in an amount of at least 5 wt% of the formulation, or at least 10 wt% of the formulation, or at least 15 wt% of the formulation, or greater than 5 wt% of the formulation, or greater than 10 wt% of the formulation, or greater than 15 wt% of the formulation. Suitable ranges include 5 wt% to 30 wt%, 10 wt% to 20 wt%, 10 wt% to 15 wt%, 5 wt% to 15 wt%, and 7.5 wt% to 12.5 wt.%. Examples include about 5 wt%, about 6 wt%, about 7 wt%, about 8 wt%, about 9 wt%, about 10 wt%, about 11 wt%, about 12 wt%, about 13 wt%, about 14 wt%, and about 15 wt%. It will be understood that the stabilizing agent referred to herein is an agent that stabilizes the dissolution release profile (and thus also the *in vivo* release profile) against substantial change over time during storage conditions, e.g., typical shelf storage conditions. Other agents which are known in the art as preservatives for preventing degradation of the active component itself are not intended to be encompassed within the terms "stabilizing agent" and "stabilizer" although such preservatives are also contemplated for use in the formulations of the disclosure.

[0075] Specifically, in one suitable type of embodiment, the waxes selected for the matrix are melted and thoroughly mixed, optionally at a temperature of about 80 °C. The desired quantity of oil(s), lauroyl polyoxylglycerides, mono- and di-glycerides, butylated hydroxytolulene, and hypromellose, are subsequently added, followed by sufficient mixing for homogenization. The waxy mixture is then gradually cooled to a temperature just above its melting point, optionally to a temperature in the range of about 50 °C to about 62 °C. The mixing steps are optionally performed under vacuum. The desired amount of 25-hydroxyvitamin D, dissolved in ethanol, is uniformly distributed into the molten matrix, optionally under vacuum and/or at a temperature in a range of about 59 °C to about 62 °C. The matrix is loaded into capsules, for example vegetable-based or gelatin-based capsules, optionally under vacuum and/or at a temperature in a range of about 63 °C to about 70 °C.

[0076] The filled capsules optionally are treated for appropriate periods of time with a solution containing an aldehyde, such as acetaldehyde, to partially crosslink a polymer, e.g., gelatin, in the capsule shell, when used. The capsule shell becomes increasingly crosslinked, over a period of several weeks and, thereby, more resistant to dissolution in the contents of stomach and upper

intestine. When properly constructed, this gelatin shell will gradually dissolve after oral administration and become sufficiently porous (without fully disintegrating) by the time it reaches the ileum to allow the 25-hydroxyvitamin D to diffuse slowly from the wax matrix into the contents of the lower small intestine and/or colon.

[0077] Another suitable controlled-release oral drug delivery system is the EUDRAGIT RL/RS system in which the active 25-hydroxyvitamin D ingredient is formed into granules having a dimension of 25/30 mesh. The granules are then uniformly coated with a thin polymeric lacquer, which is water-insoluble but slowly water-permeable. The coated granules can be mixed with optional additives including one or more of antioxidants, stabilizers, binders, lubricants, processing aids and the like. The mixture may be compacted into a tablet which, prior to use, is hard and dry and can be further coated, or it may be poured into a capsule. After the tablet or capsule is swallowed and comes into contact with the aqueous intestinal fluids, the thin lacquer begins to swell and slowly allows permeation by intestinal fluids. As the intestinal fluid slowly permeates the lacquer coating, the contained 25-hydroxyvitamin D is slowly released. By the time the tablet or capsule has passed through the small intestine, about four to eight hours or more later, the 25-hydroxyvitamin D will have been slowly, but completely, released. Accordingly, the ingested tablet will release a stream of 25-hydroxyvitamin D_3 as well as any other active ingredient.

[0078] The EUDRAGIT system is comprised of high permeability lacquers (RL) and low permeability lacquers (RS). RS is a water-insoluble film former based on neutral swellable methacrylic acids esters with a small proportion of trimethylammonioethyl methacrylate chlorides; the molar ratio of the quaternary ammonium groups to the neutral ester group is about 1:40. RL is also a water insoluble swellable film former based on neutral methacrylic acid esters with a small portion of trimethylammonioethyl methacrylate chloride, the molar ratio of quaternary ammonium groups to neutral ester groups is about 1:20. The permeability of the coating and thus the time course of drug release can be titrated by varying the proportion of RS to RL coating material. For further details of the Eudragit RL/RS system, reference is made to technical publications available from Rohm Tech, Inc. 195 Canal Street, Maiden, Mass., 02146 and Lehmann et al., *Int. J. Pharm. Tech. & Prod. Mfr.* 2(r), 31-43, 1981, incorporated herein by reference.

[0079] Other examples of insoluble polymers include polyvinyl esters, polyvinyl acetals, polyacrylic acid esters, butadiene styrene copolymers and the like.

[0080] In one embodiment, once the coated granules are either formed into a tablet or put into a capsule, and the tablet or capsule is coated with an enteric-coating material which dissolves at a pH of 7.0 to 8.0. One such pH-dependent enteric-coating material is EUDRAGIT L/S which dissolves in intestinal fluid, but not in the gastric juices. Other enteric-coating materials may be used such as cellulose acetate phthalate (CAP), which is resistant to dissolution by gastric juices, but readily disintegrates due to the hydrolytic effect of the intestinal esterases.

[0081] In one embodiment, the particular choice of enteric-coating material and controlled release coating material provides a controlled and substantially constant release over a period of 4 to 8 hours or more so that substantial release is delayed until the formulation reaches the ileum. Optionally, a controlled release composition in accordance with the present disclosure, when administered once a day, can suitably provide substantially constant intralumenal, intracellular and blood 25-hydroxyvitamin D levels compared to an equal dose of an immediate release composition of 25-hydroxyvitamin D administered once a day.

[0082] The dosage forms may also contain adjuvants, such as preserving adjuvants. Formulations according to the invention may also contain other therapeutically valuable substances or may contain more than one of the compounds specified herein and in the claims in admixture.

[0083] As an alternative to oral 25-hydroxyvitamin D, intravenous administration of 25-hydroxyvitamin D is also contemplated. In one embodiment, the 25-hydroxyvitamin D is administered as a sterile intravenous injection, optionally a bolus injection of a composition that results in a sustained release profile. In another embodiment, the 25-hydroxyvitamin D is administered via gradual injection/infusion, e.g., over a period of 1 to 5 hours, to effect controlled or substantially constant release of the 25-hydroxyvitamin D directly to DBP in the blood of the patient. For example, the composition may be injected or infused over a course of at least about 1 hour, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, or at least about 6 hours, and up to 24 hours, for example. In one embodiment, the composition intended for intravenous administration in accordance with disclosure is designed to contain a concentration of the 25-hydroxyvitamin D compound(s) of 1 to 100 mcg

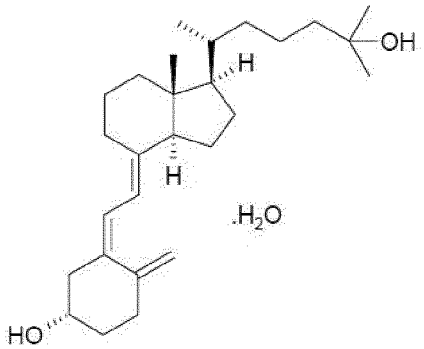
per unit dose. Sterile, isotonic formulations of 25-hydroxyvitamin D may be prepared by dissolving 25-hydroxyvitamin D in absolute ethanol, propylene glycol or another suitable solvent, and combining the resulting solution with one or more surfactants, salts and preservatives in appropriate volumes of water for injection. Such formulations can be administered slowly from syringes, for example, via heparin locks, or by addition to larger volumes of sterile solutions (e.g., saline solution) being steadily infused over time.

[0084] A particular formulation of 25-hydroxyvitamin D_3 for use in the methods of the disclosure is an extended release oral formulation comprising 25-hydroxyvitamin D (e.g., about 30 mcg, about 60 mcg, about 90 mcg, about 150 mcg, about 300 mcg, about 450 mcg, about 600 mcg, about 750 mcg, or about 900 mcg 25-hydroxyvitamin D_3), about 2 wt% (e.g., 2.32 wt%) anhydrous/dehydrated ethanol, about 10 wt% (e.g., 9.75 wt%) lauroyl polyoxylglycerides (e.g., GELUCIRE 44/14), about 20 wt% (e.g., 20.00 wt.%) hard paraffin, about 23 wt% (e.g., 22.56 wt%) mono- and di-glycerides (e.g., GMS), about 35 wt% (e.g., 35.34 wt%) liquid paraffin or mineral oil, about 10 wt% hypromellose, and optionally a small amount of preservative (e.g., 0.02 wt% BHT). The formulation is optionally encased in a soft capsule shell, for example, a capsule shell comprising modified starch, carrageenan (e.g., iota and/or kappa), sodium phosphate, dibasic, sorbitol sorbitan solution, dye (e.g., FD&C Blue #1), titanium dioxide and purified water. In one aspect, the 90% confidence interval (90% CI) of the ratio of the mean Cmax between a test formulation and a formulation of the disclosure is in the range of 80% to 125% (0.8-1.25). In another aspect, the 90% confidence interval (90% CI) of the ratio of the mean $AUC_{(0\text{-inf})}$ between a test formulation and a formulation of the disclosure is in the range of 80% to 125% (0.8-1.25). Optionally, the 90% confidence interval (90% CI) of the ratio of the Tmax between a test formulation and a formulation of the disclosure is in the range of 80% to 125% (0.8-1.25). A formulation which is bioequivalent to a formulation described herein is also specifically contemplated for use in the methods of the disclosure.

[0085] In one aspect, the disclosure provides a method of treating secondary hyperparathyroidism in a patient with Chronic Kidney Disease Stage 3 or Stage 4 and serum total 25-hydroxyvitamin D levels less than 30 ng/mL comprising administering an oral extended release formulation of calcifediol. Optionally, the patient has a serum calcium level less than 9.8 mg/dL and/or a serum phosphorus level less than 5.5 mg/dL. Optionally, the method comprises administering an oral extended release formulation of 25-hydroxyvitamin D_3 comprising 30 mcg

calcifediol once daily, for example, at bedtime. In one embodiment, the method comprises administering 60 mcg calcifediol to raise serum total 25-hydroxyvitamin D into the range of 30 ng/mL to 100 ng/mL and to lower plasma intact parathyroid hormone (iPTH) to the iPTH treatment goal provided that serum calcium is within the normal reference range. In various embodiments, the method comprises administering from about 30 mcg to about 60 mcg calcifediol daily. In some embodiments, the method comprises administering 25-hydroxyvitamin D in a range of about 300 mcg to about 900 mcg per week, e.g. 600 mcg per week, optionally divided into two or three doses per week, e.g. three times per week at dialysis treatment.

[0086] In one embodiment, calcifediol is synthetically manufactured as calcifediol monohydrate. Calcifediol monohydrate is a white crystalline powder, has a calculated molecular weight of 418.65 and is soluble in alcohol and fatty oils but practically insoluble in water. Chemically, calcifediol monohydrate is (3β,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,25-diol monohydrate and its structural formula is:



[0087] The present invention is further explained by the following examples which should not be construed by way of limiting the scope of the present invention.

Example 1

Phase III Clinical Trial of Extended Release 25-Hydroxyvitamin D_3 in Patients with CKD Stage 3 or 4

[0088] The efficacy and safety of an extended release formulation of 25-hydroxyvitamin D_3 (RAYALDEE, Opko Ireland Global Holdings Ltd.) were evaluated in two identical multicenter, randomized, placebo-controlled, double-blind studies involving patients with secondary hyperparathyroidism (iPTH >85 pg/mL), stage 3 or 4 chronic kidney disease and associated serum total 25-hydroxyvitamin D levels ≥10 ng/mL and ≤30 ng/mL. Subjects were stratified by disease stage and randomized in a 2:1 ratio to receive a once daily 30 mcg oral dose of RAYALDEE (or matching placebo) at bedtime for 12 weeks followed by an additional 14 weeks of treatment with a once daily oral dose of either 30 or 60 mcg of RAYALDEE (or placebo) at bedtime. Each capsule contained the following excipients: mineral oil, monoglycerides and diglycerides, paraffin, hypromellose, lauroyl polyoxylglycerides, dehydrated alcohol and butylated hydroxytoluene. The capsule shells contained modified starch, carrageenan, sodium phosphate, dibasic, sorbitol sorbitan solution, FD&C Blue #1, titanium dioxide and purified water. Medium chain triglyceride (fractionated coconut) oil was used as a lubricant during manufacture, and trace amounts may have been present in the final formulation.

[0089] A total of 213 subjects were randomized in one study (72 received placebo and 141 received RAYALDEE), and 216 subjects were randomized in the other (72 received placebo and 144 received RAYALDEE). The subjects' mean age was 66 years (range 25-85), 50% were male, 65% White, 32% African-American or Black and 3% Other. At baseline, subjects had secondary hyperparathyroidism, Stage 3 (52%) or Stage 4 (48%) CKD without macroalbuminuria and serum total 25-hydroxyvitamin D levels less than 30 ng/mL. The most common causes of CKD were diabetes and hypertension and the mean estimated GFR at baseline was 31 mL/min/1.73m^2. At baseline, mean serum intact PTH was 148 pg/mL, mean serum calcium was 9.2 mg/dL, mean serum phosphorus was 3.7 mg/dL and mean serum 25-hydroxyvitamin D was 20 ng/mL. Mean baseline iPTH was 130 pg/mL for subjects with Stage 3 disease (n=222) and 166 pg/mL for subjects with Stage 4 disease (n=207). Of the 429 subjects, 354 (83%) completed the studies and 298 (69%) elected to continue treatment for an additional 6 months with RAYALDEE during an open-label extension study. All subjects started on a dose of 30 mcg, administered once daily at bedtime, and most (210, 74%) increased to 60 mcg after 12 weeks if the plasma intact PTH level was greater than 70 pg/mL, the serum 25-hydroxyvitamin D level was less than 65 ng/mL and the serum calcium level was less than 9.8 mg/dL.

[0090] The primary analysis compared the proportion of individuals who experienced an at least 30% reduction in plasma intact PTH from baseline to end of trial (average of weeks 20, 22, 24 and 26). A larger proportion of patients randomized to RAYALDEE experienced an at least 30% reduction in plasma intact PTH from baseline compared to placebo in both trials (33% versus 8% in the first trial ($P<0.001$) and 34% versus 7% in the second trial ($P<0.001$)).

[0091] Mean plasma iPTH gradually declined to near normal levels over 1 year of treatment with RAYALDEE, regardless of CKD stage, but increased with placebo treatment. At the end of 26 weeks of treatment, mean pooled plasma iPTH had declined by 22±32 % with RAYALDEE treatment, but increased by 9±36% with placebo treatment (Figure 1A), with comparable efficacy in CKD Stage 3 and Stage 4 (Figure 1B). iPTH suppression was directly proportional to elevation of serum total 25-hydroxyvitamin D (Figure 1C) and was sustained during the 6-month extension study.

[0092] Serum total 25-hydroxyvitamin D levels gradually increased to ≥30 ng/mL in 80% and 83% of subjects treated with RAYALDEE versus 3% and 7% of subjects treated with placebo ($p<0.001$) in the two studies. Average steady-state levels were 50±20 and 56±19 ng/mL for subjects receiving 30 mcg daily, and 69±22 and 67±21 ng/mL for subjects receiving 60 mcg daily, in the first and second studies, respectively (Figure 2A). Serum 25-hydroxyvitamin D levels as high as about 185 ng/mL were achieved without any adverse reactions. The results were comparable to a prior repeat-dose clinical study, wherein 30 mcg daily doses of RAYALDEE produced incremental increases in mean serum 25-hydroxyvitamin D of up to a total of 5.8±1.2 (SE) ng/mL/week and a corresponding increase in mean serum 1,25-dihydroxyvitamin D of up to a total of 6.7±3.1 pg/mL/week. Reductions in circulating plasma intact PTH were observed within the first two weeks of RAYALDEE treatment.

[0093] Plasma iPTH and serum total 25-hydroxyvitamin D response rates were similar in Stages 3 and Stage 4 CKD, as were increases in serum total 25-hydroxyvitamin D and 1,25 dihydroxyvitamin D. Mean serum total 1,25D significantly increased by the end of the treatment period, regardless of CKD stage (Figure 2B).

[0094] Of the 285 subjects treated with RAYALDEE, 179 had $24,25(OH)_2D_3$ levels at baseline that were above LOQ (<0.51 ng/mL). Over 26 weeks of RAYALDEE treatment, mean 25(OH)D3 and 24,25(OH)2D3 increased significantly ($p<0.0001$ vs. placebo) from baseline of

29 to 87 ng/mL and 1.0 to 4.1 ng/mL (Figure 3A), respectively; mean iPTH decreased significantly ($p<0.0001$) from 147 to 111 pg/mL compared to a mean increase in placebo subjects. The 25(OH)D3:24,25(OH)2D3 ratios were variable (RSD ~30%), but decreased with RAYALDEE treatment from a mean of 30 to 25 (Figure 3B), and after 26 weeks were significantly lower than with placebo ($p<0.0001$). The results indicated that the ratio of $25(OH)D_3$:$24,25(OH)_2D_3$ is useful for diagnosing VDI in CKD patients. The data further indicated that reducing this ratio to <20 would require $25(OH)D_3$ levels higher than 85 ng/mL, demonstrating that the target for 25(OH)D is higher than expected in CKD patients compared to normal subjects.

[0095] No differences in urine calcium or phosphorus were observed between treatment groups. Mean serum Ca and P levels in subjects treated with extended release calcifediol were generally similar to placebo-treated subjects (Figure 6A); slight elevations were noted at several time points. Mean±SD serum calcium corrected for albumin increased in pooled data from a baseline of 9.2±0.3 mg/dL by 0.2±0.3 mg/dL with RAYALDEE compared to 0.1±0.3 mg/dL with placebo treatment. Serum phosphorus increased with RAYALDEE by 0.2±0.5 mg/dL from a baseline of 3.7±0.6 mg/dL with RAYALDEE compared to 0.1±0.4 mg/dL from a baseline of 3.8±0.5 with placebo treatment. No exposure-response relationship was observed for serum calcium (Figure 6B) or serum phosphorus (Figure 6C) based on the amount of 25-hydroxyvitamin D_3 administered. A total of 6 subjects (2%) in the RAYALDEE treatment group required dose reductions after meeting the protocol-defined criterion of albumin-corrected hypercalcemia (two consecutive serum calcium values >10.3 mg/dL), compared to no subjects in the placebo group. Five of these subjects had a history of serum calcium ≥9.8 mg/dL prior to treatment, and the sixth subject had received concomitant thiazide therapy. A total of 4.2% of RAYALDEE subjects and 2.1% of placebo subjects experienced ≥1 elevation in serum calcium above the upper limit of normal (10.5 mg/dL). Adverse events of "blood calcium increased" or "hypercalcemia" were reported in 0.7% of subjects in the RAYALDEE and 1.4% in the placebo treatment groups. One subject (0.4%) in the RAYALDEE treatment group met the protocol-defined criterion of hyperphosphatemia (two consecutive serum phosphorus values >5.5 mg/dL deemed to be study drug related) and required dose reduction, compared to no subjects in the placebo group. A total of 45.6% of RAYALDEE subjects and 44.4% of placebo subjects experienced ≥1 elevation in serum phosphorus above the upper limit of normal (4.4 mg/dL).

Adverse events of "blood phosphorus increased" or "hyperphosphatemia" were reported in 1.8% of subjects in the RAYALDEE and 2.8% in the placebo treatment groups.

[0096] Markers of bone turnover decreased during treatment with RAYALDEE. Urine calcium/creatinine ratio (Ca/Cr) and Tubular Reabsorption of Phosphorus (TRP) were unchanged (Figures 6D and 6E). Serum total alkaline phosphatase decreased from 93 to 87 U/L, serum bone-specific alkaline phosphatase (BSAP) decreased from 38 to 27 U/L (Figure 7A), serum C-Terminal Telopeptide-1 (CTX-1) decreased from 734 to 612 pg/mL (Figure 7B), and serum Procollagen-1 Amino-terminal Propeptide (P1NP) decreased from 99 to 89 ng/mL (Figure 7C). During placebo administration, these bone markers either increased or remained relatively unchanged.

[0097] A food effect study with a supratherapeutic dose of 450 mcg in healthy subjects showed an approximately 5-fold increase in maximum serum calcifediol concentration (C_{max}) and a 3.5-fold increase in AUC_{0-t} when RAYALDEE was administered with a high fat, high calorie meal compared to fasting.

[0098] A single-dose study with a supratherapeutic dose of 900 mcg in healthy subjects produced maximum blood calcifediol concentrations in the range of 21 to 32 hours after dosing in the fasted state.

Example 2

Pharmacokinetic Studies

[0099] The pharmacokinetics of RAYALDEE were assessed in healthy subjects and subjects with CKD Stage 3 or Stage 4. Exposure to calcifediol increased proportionally over the dose range of 30 to 90 mcg following repeated daily administration of RAYALDEE at bedtime to subjects with secondary hyperparathyroidism, chronic kidney disease and vitamin D insufficiency. Steady-state levels of serum total 25-hydroxyvitamin D were reached after approximately 3 months. Following multiple dose administration of RAYALDEE, the average steady state concentrations of serum total 25-hydroxyvitamin D were 53 ng/mL and 68 ng/mL for doses of 30 mcg and 60 mcg dose group, respectively. With respect to distribution, calcifediol is extensively bound to plasma proteins (>98%). The mean apparent volume of distribution was 8.8 L in healthy subjects following a single oral dose of RAYALDEE, and 30.1 L in subjects with Stage 3 or Stage 4 chronic kidney disease following repeated dosing.

With respect to elimination, the mean elimination half-life of calcifediol was approximately 11 days in healthy individuals following a single dose of RAYALDEE, and approximately 25 days in patients with Stage 3 or Stage 4 chronic kidney disease following repeated once daily dosing. With respect to metabolism, production of calcitriol from calcifediol is catalyzed by the 1α-hydroxylase enzyme, CYP27B1, located in the kidney, parathyroid gland, and other tissues. CYP24A1, located in all vitamin D responsive tissues, catabolizes both calcifediol and calcitriol to inactive metabolites. Calcitriol suppresses CYP27B1 and upregulates CYP24A1. Excretion of calcifediol occurs primarily through the biliary fecal route. No effect of CKD stage on steady-state calcifediol concentrations was evident following daily RAYALDEE administration, in an analysis of population pharmacokinetics in subjects with CKD Stage 3 or Stage 4. Table 1 shows a summary of baseline-adjusted pharmacokinetic parameters for calcifediol by treatment group.

TABLE 1

	Placebo	**30 µg**	**60 µg**	**90 µg**
Baseline (ng/mL)				
Mean (SD)	16.4 (8.2)	16.2 (7.3)	19.8 (8.7)	18.4 (9.8)
Median	12.9	17.2	21.2	16.7
Minimum, Maximum	4.4, 30.4	5.0, 25.8	5.8, 32.5	6.7, 38.9
C_{max} (ng/mL)				
Mean (SD)	4.1 (3.5)	27.8 (8.2)	60.3 (19.0)	85.7 (26.9)
Median	3.1	28.1	60.8	76.0
Minimum, Maximum	0.6, 13.8	10.8, 43.4	30.3, 89.5	55.4, 146.4
$AUC_{0\text{-}6\ Weeks}$ (ng·d/mL)				
Mean (SD)	45.9 (60.0)	709.2 (246.3)	1531.4 (374.8)	2134.3 (584.3)
Median	32.1	6843.0	1573.0	1963.8
Minimum, Maximum	-60.1, 222.3	307.8, 1249.0	712.7, 2221.8	1377.5, 3207.3
t_{max} (d)				
Mean (SD)	NA	37.8 (10.4)	41.1 (5.2)	42.6 (5.3)
Median	NA	42.50	43.0	43.0
Minimum, Maximum	NA	8.0, 44.0	29.0, 45.0	35.0, 57.0
$t_{1/2}$ (d)				
Mean (SD)	NA	25.8 (16.3)	33.1 (9.3)	50.1 (51.0)
Median	NA	24.1	31.6	37.7
Minimum, Maximum	NA	5.2, 52.6	17.4, 52.3	23.2, 224.0

[0100] Table 2 shows a summary of baseline-adjusted pharmacokinetic parameters for 1,25-dihydroxyvitamin D by treatment group.

TABLE 2

	Placebo N=23	30 µg N=12	60 µg N=16	90 µg N=14
Baseline (pg/mL)				
Mean (SD)	20.8 (10.11)	18.3 (7.53)	20.6 (7.62)	20.6 (7.29)
Median	17.0	17.0	18.0	21.0
Minimum, Maximum	7.0, 41.4	5.1, 30.7	8.2, 33.6	9.3, 34.5
C_{max} (pg/mL)				
Mean (SD)	7.6 (5.71)	6.4 (7.66)	18.4 (6.24)	19.9 (14.30)
Median	4.9	5.0	18.4	18.9
Minimum, Maximum	1.9, 22.6	-6.3, 21.0	7.3, 29.9	-11.6, 48.3
$AUC_{0\text{-}6\ Weeks}$(g·d/mL)				
Mean (SD)	11.5 (112.97)	100.6 (185.38)	249.9 (198.83)	371.1 (290.81)
Median	16.2	23.0	298.7	352.2
Minimum, Maximum	-267.1, 219.8	-145.4, 452.3	-191.7, 563.6	-5.8, 1235.8
t_{max} (d)				
Mean (SD)	24.4 (15.55)	16.8 (16.09)	26.4 (11.52)	25.5 (13.88)
Median	23.0	12.0	23.0	23.00
Minimum, Maximum	2.0, 45.0	1.0, 44.0	8.0, 44.0	1.0, 44.0

Example 3

Clinical Trial of Extended Release 25-hydroxyvitamin D_3 in Patients with CKD Stage 5

[0101] A multi-center, randomized, double-blind, placebo-controlled study clinical study is conducted to evaluate the safety and efficacy of three weekly doses of an extended release formulation of 25-hydroxyvitamin D_3 versus placebo in reducing serum intact parathyroid hormone (iPTH) by at least 30% from pre-treatment baseline in subjects with secondary hyperparathyroidism, vitamin D insufficiency and CKD Stage 5, who are undergoing hemodialysis three times per week. The extended release formulation of 25-hydroxyvitamin D_3 is provided as a capsule having the same formulation as RAYALDEE, but containing 150 mcg 25-hydroxyvitamin D_3. Approximately 600 subjects are screened to randomize approximately 280 eligible subjects balanced for severity of SHPT in a 1:1:1:1 ratio into four parallel groups receiving the following treatments for 52 weeks: (a) extended release formulation of 25-hydroxyvitamin D_3 at 300 mcg per week, (b) extended release formulation of 25-hydroxyvitamin D_3 at 600 mcg per week, (c) extended release formulation of 25-hydroxyvitamin D_3 at 900 mcg per week, or (d) matching placebo.

[0102] To be enrolled in the study, subjects must be at least 18 years of age and diagnosed with CKD Stage 5 requiring in-center hemodialysis three times a week for the preceding 9 months, as confirmed by medical history. During the screening visit, subject must exhibit a serum iPTH level ≥150 pg/mL and <600 pg/mL, if receiving calcitriol or active vitamin D analogue, or a serum iPTH level ≥400 pg/mL and <900 pg/mL, if not receiving calcitriol or active vitamin D analogue. Subjects receiving treatment with cinacalcet, etelcalcetide, calcitriol or other 1-hydroxylated vitamin D analogues, or vitamin D supplementation at a rate about 1,000 IU/day forgo further dosing with these agents for the duration of the study and complete a 4-week wash-out period prior to the 52-week treatment period, to achieve serum iPTH ≥400 pg/mL and <900 pg/mL, serum calcium <9.8 mg/dL, and serum total 25-hydroxyvitamin D <30 ng/mL.

[0103] Blood samples are collected at the start of the mid-week dialysis session from all subjects for safety and efficacy assessments at weekly or biweekly intervals during the pre-treatment washout period, the 52-week treatment period and the 6-week post-treatment follow-up period. Enrolled subjects in the 600 mcg or 900 mcg group receive one or two capsules containing 150 mcg 25-hydroxyvitamin D_3 or placebo three times per week (e.g., on a Monday, Wednesday, Friday schedule or a Tuesday, Thursday, Saturday schedule) for 52 weeks. Subjects in the 300 mcg group receive one capsule containing 150 mcg 25-hydroxyvitamin D_3 two times per week (e.g., on Monday/Tuesday and Friday/Saturday). The capsules are administered in the dialysis center at the end of regularly scheduled hemodialysis to achieve the total cumulative weekly dose. Subjects undergo hemodialysis during the study with a dialysate calcium concentration of ≤ 2.5 mEq/L. Additional blood samples are collected in a subset of subjects from each treatment group during the post-treatment follow-up period in order to establish the pharmacokinetic profile of serum 25-hydroxyvitamin D_3 after the last administered dose.

[0104] Subjects are eligible for dose titration at the start of week 26 provided that (a) iPTH has not decreased by at least 40% from pretreatment baseline and remains >300 pg/mL, (b) serum calcium is <9.8 mg/dL, (c) serum phosphorus is <6.0 mg/dL, and (d) the currently assigned dosage in the trial is less than 900 mcg/week. Dose titration will occur in increments of 300 mcg/week unless a lower increment is necessary in order to keep the maximum dose from exceeding 900 mcg/week. Subjects reduce the dose by 150 mcg per week in the event that iPTH is confirmed to be <150 pg/mL, serum calcium is confirmed to be >10.3 mg/dL, or serum

phosphorus is confirmed to be >6.5 mg/dL, provided that that the investigator deems the elevated serum phosphorus to be related to study drug administration and has taken appropriate and persistent actions to control serum phosphorus by initiating or adjusting phosphate binder therapy. Subjects suspend dosing if iPTH is confirmed to be <100 pg/mL or serum calcium is confirmed to be >11.0 mg/dL, and resume when iPTH is ≥150 pg/mL and serum calcium is ≤9.8 mg/dL at a dose that has been reduced by 150 mcg per week. Subjects who experience more than a 100% increase in iPTH from pre-treatment baseline or exhibit iPTH above 1,200 pg/mL on two consecutive visits (if at least 2 weeks apart) terminate dosing with study drugs and further participation in the treatment period and immediately enter the post-treatment follow-up period.

[0105] Key parameters monitored at regular intervals during the study include: iPTH, serum calcium, serum phosphorus, serum total 25-hydroxyvitamin D, serum total 1,25-dihydroxyvitamin D, serum 25-hydroxyvitamin D_3; serum 1,25-dihydroxyvitamin D_3, serum 24,25-dihydroxyvitamin D_3,vital signs (VS), and adverse events (AEs). Electrocardiograms are conducted at baseline and during the last month of treatment. Additional exploratory parameters, including levels of fibroblast growth factor 23 (FGF-23), serum bone-specific alkaline phosphatase (BAP), serum C-terminal telopeptide of type 1 collagen (CTx), serum procollagen type 1 N-terminal propeptide (P1NP), T50 calcification propensity score, other bone markers, and immune function, are also monitored.

[0106] A majority of the subjects exhibit a mean decrease in serum iPTH of between 30% and 50% from pre-treatment baseline during the last 6 weeks of treatment (weeks 47-52). After 12 weeks of treatment, steady-state conditions are nearly reached (median time to reach steady state is approximately 16 weeks), with subjects treated with the extended release formulation of 25-hydroxyvitamin D_3 exhibiting a serum 25-hydroxyvitamin D_3 concentration between about 50 ng/mL and about 90 ng/mL for the group treated with 300 mcg/week, between about 120 ng/mL and about 160 ng/mL for the group treated with 600 mcg/week, and between about 150 ng/mL and about 200 ng/mL for the group treated with 900 mcg/week.

Example 4

Safely Raising Serum 1,25-dihydroxyvitamin D To Supraphysiologic Levels

[0107] Figures 8 and 9 show early results, in two subjects, from a trial involving dosing subjects with extended-release, oral calcifediol over a period of up to one year.

[0108] The following protocol was applied. Subjects were to receive extended-release, oral calcifediol capsules at an initial daily dose of 30 micrograms, for four weeks, dosed at bedtime. Following the initial dose, the daily dose was allowed to be increased in 30 microgram increments at four-week intervals, to a maximum of 300 micrograms per day. During the dose escalation, if serum calcium was greater than 10.3 mg/dL for two consecutive visits then dosing was to be discontinued until serum calcium was 10.0 mg/dL or lower, at which time the subject was to recommense dosing at a dose of 30 micrograms lower than the previous dose, and to remain at that dosing level for twelve weeks. Dosing was to be stopped if serum calcium was greater than 10.3 mg/dL for a third time.

[0109] The subjects in the trial had advanced breast or prostate carcinomas with metastases to bone, and were receiving ongoing therapy with denosumab or zolendronic acid. Subjects had initial serum calcium less than 9.8 mg/dL and plasma iPTH of greater than 70 pg/mL.

[0110] Figures 8 and 9 show that serum calcifediol levels in these subjects rose to about 300 ng/ml or greater, while serum calcitriol levels rose to greater than 250 pg/mL. Serum calcium and serum phosphate levels were generally flat. The ratio of 25-hydroxyvitamin D_3 to serum 24,25-dihydroxyvitamin D_3 in these subjects was less than 20 and was generally about 10:1 or less.

[0111] The foregoing description is given for clearness of understanding only, and no unnecessary limitations should be understood therefrom, as modifications within the scope of the invention may be apparent to those having ordinary skill in the art. Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise" and variations such as "comprises" and "comprising" will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0112] Throughout the specification, where compositions are described as including components or materials, it is contemplated that the compositions can also consist essentially of, or consist of, any combination of the recited components or materials, unless described otherwise. Likewise, where methods are described as including particular steps, it is contemplated that the methods can also consist essentially of, or consist of, any combination of the recited steps, unless described otherwise. The invention illustratively disclosed herein

suitably may be practiced in the absence of any element or step which is not specifically disclosed herein.

[0113] The practice of a method disclosed herein, and individual steps thereof, can be performed manually and/or with the aid of electronic equipment. Although processes have been described with reference to particular embodiments, a person of ordinary skill in the art will readily appreciate that other ways of performing the acts associated with the methods may be used. For example, the order of the various steps may be changed without departing from the scope or spirit of the method, unless described otherwise. In addition, some of the individual steps can be combined, omitted, or further subdivided into additional steps.

[0114] All patents, publications and references cited herein are hereby fully incorporated by reference. In case of conflict between the present disclosure and incorporated patents, publications and references, the present disclosure should control.

What is Claimed:

1. A method of treating secondary hyperparathyroidism in a patient having Chronic Kidney Disease Stage 3 or 4, comprising administering to the patient repeat doses of 25-hydroxyvitamin D effective to raise the patient's serum 25-hydroxyvitamin D level to greater than 90 ng/ml.

2. The method of claim 1, further comprising administering the repeat doses in an amount effective to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20.

3. A method of treating secondary hyperparathyroidism in a patient having Chronic Kidney Disease Stage 3 or 4, comprising administering to the patient repeat doses of 25-hydroxyvitamin D effective to raise the patient's serum 25-hydroxyvitamin D level to greater than 90 ng/ml and to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20.

4. A method of treating vitamin D insufficiency in a patient having Chronic Kidney Disease Stage 3 or 4, comprising administering to the patient repeat doses of 25-hydroxyvitamin D effective to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 20.

5. The method of any of claims 1-4, wherein the repeat doses are administered for at least 3 months.

6. The method of any of claims 1-5, wherein the repeat doses are effective to raise the patient's serum 25-hydroxyvitamin D level to greater than 100 ng/ml.

7. The method of any of claims 1-6, wherein the amount is effective to control the patient's serum ratio of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D to less than 15.

8. The method of any of claims 1-7, wherein the doses of 25-hydroxyvitamin D are administered as controlled release oral dosages.

9. The method of any of claims 1-8, wherein the patient having Chronic Kidney Disease Stage 3 or 4 also has a serum total 25-hydroxyvitamin D concentration of less than 30 ng/ml.

10. A method of treating secondary hyperparathyroidism in a patient with Chronic Kidney Disease Stage 3 or Stage 4 and serum total 25-hydroxyvitamin D levels less than 30 ng/mL, comprising administering an oral extended release formulation of calcifediol.

11. An extended release dosage form of 25-hydroxyvitamin D, or use thereof, comprising about 30 mcg of 25-hydroxyvitamin D_3, a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle, and a stabilizing agent, wherein said dosage form, when administered to a patient having CKD Stage 3 or Stage 4, has one or more of the following baseline-adjusted pharmacokinetic parameters for 25-hydroxyvitamin D_3 after repeat dosing: (1) Cmax of about 10 ng/mL to about 45 ng/mL and (2) $AUC_{0\text{-}6\ weeks}$ of about 300 ng·d/mL to about 1300 ng·d/mL; and further optionally a Tmax of about 8 days to about 45 days.

12. An extended release dosage form of 25-hydroxyvitamin D, or use thereof, comprising about 60 mcg of 25-hydroxyvitamin D_3, a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle, and a stabilizing agent, wherein said dosage form, when administered to a patient having CKD Stage 3 or Stage 4, has one or more of the following baseline-adjusted pharmacokinetic parameters for 25-hydroxyvitamin D_3 after repeat dosing: (1) Cmax of about 30 ng/mL to about 90 ng/mL and (2) $AUC_{0\text{-}6\ weeks}$ of about 700 ng·d/mL to about 2300 ng·d/mL; and further optionally a Tmax of about 29 days to about 45 days.

13. An extended release dosage form of 25-hydroxyvitamin D, or use thereof, comprising about 30 mcg of 25-hydroxyvitamin D_3, a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle, and a stabilizing agent, wherein said dosage form, when administered to a patient having CKD Stage 3 or Stage 4, has one or more of the following baseline-adjusted pharmacokinetic parameters for 1,25-dihydroxyvitamin D after repeat dosing: (1) Cmax of about 1 pg/mL to about 21 pg/mL and (2) $AUC_{0\text{-}6\ weeks}$ of about 1 g·d/mL to about 460 g·d/mL, further optionally a Tmax of about 1 day to about 44 days.

14. An extended release dosage form of 25-hydroxyvitamin D, or use thereof, comprising about 60 mcg of 25-hydroxyvitamin D_3, a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle, and a stabilizing agent, wherein said dosage form, when administered to a patient having CKD Stage 3 or Stage 4, has one or more of the following baseline-adjusted pharmacokinetic parameters for 1,25-dihydroxyvitamin D after

repeat dosing: (1) Cmax of about 7 pg/mL to about 30 pg/mL and (2) $AUC_{0\text{-}6\ weeks}$ of about 1 g·d/mL to about 570 g·d/mL, further optionally a Tmax of about 8 days to about 44 days.

15. A method of administering an extended release dosage form of 25-hydroxyvitamin D comprising about 10 mcg to about 90 mcg 25-hydroxyvitamin D_3, a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle, and a stabilizing agent to a patient having CKD Stage 3 or Stage 4, comprising administering repeat doses of the extended release oral dosage form to the patient effective to achieve one or more of the following baseline-adjusted pharmacokinetic parameters for 25-hydroxyvitamin D_3: (1) Cmax of about 5 ng/mL to about 150 ng/mL; (2) Tmax of about 5 days to about 60 days; (3) $AUC_{0\text{-}6\ weeks}$ of about 100 ng·d/mL to about 3300 ng·d/mL.

16. A method of administering an extended release dosage form of 25-hydroxyvitamin D comprising about 10 mcg to about 90 mcg 25-hydroxyvitamin D_3, optionally including a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle, and a stabilizing agent, to a patient in need thereof, optionally having CKD Stage 3 or Stage 4, comprising administering repeat doses of the extended release oral dosage form to the subject effective to achieve one or more of the following baseline-adjusted pharmacokinetic parameters for 1,25-dihydroxyvitamin D: (1) Cmax of about 0.1 pg/mL to about 50 pg/mL; (2) Tmax of about 1 days to about 44 days; (3) $AUC_{0\text{-}6\ weeks}$ of about 1 g·d/mL to about 1300 g·d/mL.

17. A method of administering an extended release dosage form of 25-hydroxyvitamin D comprising about 30 mcg 25-hydroxyvitamin D_3, optionally including a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle, and a stabilizing agent; to a patient in need thereof, optionally having CKD Stage 3 or Stage 4, comprising administering repeat doses of the extended release oral dosage form to the subject effective to achieve one or more of the following: (1) an increase in mean serum 25-hydroxyvitamin D of about 1 ng/mL/week to about 7 ng/mL/week; (2) an increase in mean serum 1,25-dihydroxyvitamin D of about 1 to about 10 pg/mL/week; (3) a mean steady state serum 25-hydroxyvitamin D level of about 50 ng/mL to about 55 ng/mL.

18. A method of administering an extended release dosage form of 25-hydroxyvitamin D comprising about 30 mcg to about 60 mcg 25-hydroxyvitamin D_3, optionally including a waxy controlled release agent, an emulsifier, an absorption enhancer, an oily vehicle,

and a stabilizing agent; to a patient in need thereof, optionally having CKD Stage 3 or Stage 4, comprising administering repeat doses of the extended release oral dosage form to the subject effective to achieve one or more of the following: (1) a reduction in plasma intact PTH from baseline of about 30 pg/mL to about 80 pg/mL; (2) a steady state serum 25-hydroxyvitamin D level of greater than about 50 ng/mL to about 100 ng/mL; (3) an increase in serum 1,25-dihydroxyvitamin D from baseline of at least about 10 pg/mL; (4) an increase in serum calcium from baseline of 0 to about 0.3 mg/dL; (5) an increase in serum phosphorous from baseline of 0 to about 0.3 mg/dL; (6) a decrease in serum BSAP from baseline of at least about 10 U/L; (7) a decrease in serum CTX-1 from baseline of at least about 100 pg/mL; and (8) a decrease in serum P1NP from baseline of at least about 30 ng/mL.

19. The method or use of any of claims 1-18, wherein the repeat doses are effective to lower the patient's plasma PTH level by at least 15%, optionally by at least 30%, compared to baseline.

20. The method or use of any of claims 1-19, wherein the repeat doses are administered at a frequency of one dose per day.

21. The method or use of any of claims 1-20, wherein the repeat doses are administered for an extended period of at least one month, optionally at least six months.

22. A method of treating a vitamin-D responsive disease in a patient, comprising administering to the patient repeated doses of 25-hydroxyvitamin D effective to raise the patient's serum 25-hydroxyvitamin D level to a range of 100 ng/ml to 500 ng/ml, and concomitantly raise the patient's serum 1,25-dihydroxyvitamin D level to a range of 50 pg/ml to 350 pg/ml, without inducing hypercalcemia.