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(54) Title: METHOD FOR PREVENTING OR TREATING BREAST CANCER USING A VITAMIN D ANALOG

(57) Abstract: The present invention relates to methods of using the vitamin D analog 1ß-hydroxymethyl-3-epi-16-ene-24,24-difluoro-27a,27a-bishomo-25(OH)D3, also referred to as QW-1624F2-2, in the prevention or treatment of breast cancer whereby the analog decreases the expression of CYP24 and c-Jun and inhibits cell proliferation and cell invasion and induces apoptosis.

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METHOD FOR PREVENTING OR TREATING BREAST CANCER USING A VITAMIN D ANALOG

Background of the Invention

Breast cancer is the most frequently occurring cancer and the leading cause of death due to malignancy in women. A majority of breast cancer-related deaths is due to the metastatic spread of tumor cells to distant areas of the body by way of the lymphatics and circulation. Therefore, the treatment of metastatic disease represents a continuing therapeutic challenge.

The hormone 1,25-dihydroxyvitamin D3 (calcitriol) has been shown to inhibit breast tumor cell growth, to promote breast tumor cell differentiation, as well to induce apoptotic cell death (Hansen, et al. (2001) *Front. Biosci.* 6:D820-48; Hansen, et al. (2000) *Curr. Pharm. Desc.* 6:803-28). The presence of both the 1-alpha and the 25-hydroxyl groups are generally thought to be essential for effective binding of 1,25-dihydroxyvitamin D_3 ligand to the nuclear vitamin D receptor (VDR) (Peleg and Posner (2003) *Curr. Top. Med. Chem.* 3:1555-72). Hybrid vitamin D analog, QW-1624F2-2, which was prepared by incorporating the calcium ablating 1-hydroxmethyl alteration along with the potentiating C, D ring 16-unsaturation, side chain 24,24-difluorination and 26,27-homologation, has been shown to have potent anti-proliferative effects in a skin tumor model (Posner, et al. (1998) *J. Med. Chem.* 41:3008-14), indicating that growth inhibition may not require the 1-alpha and 25-dihydroxy groups (Kensler, et al. (2000) *Carcinogenesis* 21:1341-5).

CYP24, which codes for the enzyme 24-hydroxylase, has been identified as a candidate oncogene present in clinical breast cancer samples (Albertson, et al. (2000) *Nature Genet.* 25:144-6). This enzyme catabolizes 1,25-dihydroxyvitamin D_3 to

the less active 1,24,15-trihydroxyvitamin D_3 analog and ultimately into calcitrioc acid that is excreted (Jones, et al. (1998) *Physiol. Rev.* 78:1193-231). It has been proposed that the progression of breast carcinoma is associated not only with changes in VDR expression but also with the pre-receptor control of ligand availability through vitamin D_3 degradation by the vitamin D hydroxylases CYP27b and CYP24 (Townsend, et al. (2003) *Endocrine Abst.* 7:OC23). The clinical significance of the over-expression of CYP24 has been correlated with poor prognosis and overall survival in other tumor types such as colon (Cross, et al. (2003) *Rec. Results Canc. Res.* 164:413-25), esophageal (Mimori, et al. (2004) *Ann. Oncol.* 15:236-41) and non-small cell lung carcinomas (Jones, et al. (1999) *Endocrinology* 140:3303-10).

Miettinen et al. ((2004) *Int. J. Cancer* 108:367-73) teach the enhanced growth-inhibiting effects of CYP24 inhibition by VID400 followed by vitamin D_3 treatment in ovarian cancer cells. Similarly, Christensen, et al. ((2004) *Breast Cancer Res. Treat.* 85:53-63) disclose that an anti-estrogen and vitamin D analog combination therapy is synergistic, abrogating the development of resistance in MCF-7 breast tumor cells *in vitro*. Further, Katzburg, et al. (2004) *J. Ster. Biochem. Mol. Biol.* 88:213-4) have demonstrated that treatment with nonhypercalcemic calcitriol analogs such as QW-1624F2-2 increase the responsiveness of primary human osteoblasts to 17β-estradiol, dihydrotestosterone or raloxifene.

U.S. Patent Application Serial No. 10/270,158 teaches 16-ene-C25-oxime and 16-ene-C-25-oxime ether analogs of 1 alpha,25-dihydroxy vitamin D_3, compositions comprising these compounds and methods of using these compounds as inhibitors of CYP24 activity for treating diseases such as breast cancer, lung cancer and prostate cancer.

Summary of the Invention

The present invention relates to the finding that the 1-hydroxymethyl-24-difluorinated low-calcemic hybrid QW-1624F2-2 analog of 1,25-dihydroxyvitamin D_3 (calcitriol) has anti-proliferative, anti-invasive and apoptosis promoting properties in breast tumor cells. Further, this analog further enhances the response to anticancer agents in metastatic human breast tumor cells and suppresses CYP24 and c-jun expression.

Thus, the present invention provides for a method for preventing or treating breast cancer via administering an effective amount of vitamin D analog 1β-hydroxymethyl-3-epi-16-ene-24,24-difluoro-27a,27a-bishomo-25(OH)D_3 to a subject having or suspected of having a breast cancer so that said cancer is prevented or treated. In one embodiment, the vitamin D analog decreases the expression of CYP24 or c-Jun. In another embodiment, the vitamin D analog is co-administered with another anti-cancer agent.

The present invention further provides for a method of inhibiting proliferation or invasion or inducing apoptosis of a breast cancer cell via contacting a breast cancer cell with an effective amount of vitamin D analog 1β-hydroxymethyl-3-epi-16-ene-24,24-difluoro-27a,27a-bishomo-25(OH)D_3 so that proliferation or invasion by said cell is inhibited or apoptosis of said cell is induced.

Detailed Description of the Invention

Although studies have suggested that vitamin D analog 1β-hydroxymethyl-3-epi-16-ene-24,24-difluoro-27a,27a-bishomo-25(OH)D_3, also known by the name QW-1624F2-2, may be useful in treating cancers of the colon, prostate, and breast, there has previously been no experimental evidence in humans or any model system that would definitively link the use of QW-1624F2-2 in the prevention or treatment of breast cancer in

humans. In order to provide such definitive evidence of a therapeutic effect in humans, studies in model systems are routinely performed. It has now been shown that QW-1624F2-2 has anti-proliferative, anti-invasive and apoptosis promoting properties in breast tumor cells. QW-1624F2-2 has been found to be a potent inhibitor of CYP24 expression in a metastatic sub-line of human MCF-7 breast tumor cells over-expressing the angiogenic factor-fgf-4.

Microarray analysis was performed on total RNA isolated from the metastatic breast tumor cells using a chip consisting of more than 22,000 probe sets with approximately 14,500 well-characterized genes. After subtraction of background and normalization of genes, treatment of metastatic breast tumor cells with 100 nM of QW-1624F2-2 for 48 hours resulted in a nine-fold suppression of CYP24 mRNA expression and a fifteen-fold suppression of Kallikrein 6 (KLK6), a serine protease that cleaves amyloid precursor protein. RT-PCR analysis of CYP24 expression confirmed suppression of CYP24A showing that the CYP24 transcript was almost undetectable. Further, QW-1624F2-2 was uniquely different from the 1,25-dihydroxyvitamin D_3 analog EB1089 (Peleg and Posner (2003) *supra*), in that EB1089, stimulated rather than suppressed CYP24A expression. Similarly, U.S. Patent Application Serial No. 10/270,158 teaches that not all analogs of calcitriol inhibit CYP24 expression.

Although QW-1624F2-2 is a structurally modified derivative of 1,25-dihydroxyvitamin D_3, the studies provided herein indicate that QW-1624F2-2 does indeed interact with the VDR.

In addition, the QW-1624F2-2 compound also exerted antiproliferative effects in MKL-4 cells as determined by a trypan blue exclusion assay, reducing growth by 30 ± 2% alone and additively with tamoxifen (by 54 ± 5%) while tamoxifen by

itself exerted little or no effect. Further, the QW-1624F2-2 promoted apoptosis both alone and in combination with tamoxifen.

Using another metastatic cell line, MCF7cJun, which overexpresses the proto-oncogene c-jun (Smith, et al. (1999) *Oncogene* 18:6063-70) it was demonstrated that QW-1624F2-2 repressed the expression of the c-jun transcript by five-fold (approximately 80%). RT-PCR analysis substantiated the results obtained by microarray analysis, with an approximately 58% decrease in JUN expression in cells treated with QW-1624F2-2. Increased expression of the proto-oncogene c-jun has been widely implicated both with endocrine failure and with disease spread in clinical breast tumors (Gee, et al. (2000) *Int. J. Cancer* 89:177-86; Johnston, et al. (1999) *Clin. Cancer Res.* 5:251-6).

The lack of detectable suppression of CYP24A in the MCF7cjun cells may be related to constitutively low CYP24A expression in this cell line due to the overexpression of the proto-oncogene c-jun. It has been demonstrated that overexpression of wild-type c-jun enhances the repression of another cytochrome P450 isozyme, CYP7A1, by taurocholate in rat hepatocytes (Gupta, et al. (2001) *J. Biol. Chem.* 276:15816-22).

Unlike MKL-4 cells whose invasive potential could only be assessed by FGF-induced alterations in the blood vessel formation during tumor growth *in vivo*, MCF7cJun cells have been demonstrated to be invasive *in vitro* (Smith, et al. (1999) *supra*). Thus, using MCF7cJun cells it was determined that treatment with analog QW-1624F2-2 decreased the invasion and migration of these cells by more than 50% as evaluated by a MATRIGEL™ invasion assay. Further evaluation of the QW-1624F2-2 analog over a concentration range of 50-200 nM (for 24 hours) demonstrated a clear dose response effect on the

invasiveness of metastatic MCF7cJun cells, while no significant effect was observed on the invasive potential of similar cells harboring empty-vector control clones (7-1 neo).

These results indicate that the less-calcemic analog QW-1624F2-2 significantly down-regulates the oncogene CYP24 as well as the oncogene c-jun, inhibits breast tumor cell growth and invasion, and promotes apoptotic cell death. This is the first demonstration that a synthetic deltanoid can act as a highly specific inhibitor of CYP24 and c-jun in human breast tumor cells. This effect of blocking CYP24 by QW-1624F2-2 results in prolonging the biological lifetime of calcitriol and its analogs, and thus allows for smaller amounts to be used with or without other chemotherapeutic agents for effective treatment of breast cancer. Accordingly, QW-1624F2-2 is useful for breast cancer adjuvant therapy or for modulating the growth of breast cancer cells.

Therefore, the present invention is a method for preventing or treating breast cancer in a subject having or at risk of having a breast cancer. A subject having or suspected of having breast cancer may exhibit one or more of the typical signs or symptoms associated with the disease including a lump, an area of thickening, or a dimple in the breast or the less common signs include breast swelling and redness or an enlarged underarm lymph node. Patients at risk of having breast cancer include those with a family member or family history of having breast cancer or who have inherited an abnormal breast cancer gene.

A subject having or at risk of having a breast cancer is administered an effective amount of vitamin D analog 1β-hydroxymethyl-3-epi-16-ene-24,24-difluoro-27a,27a-bishomo-25(OH)D_3 (QW-1624F2-2) to have a beneficial or desired clinical result. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or

more symptoms or conditions, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. Treatment can also mean prolonging survival as compared to expected survival if not receiving treatment. As will be understood by the skilled artisan, the signs or symptoms of the breast cancer can vary with the stage of the cancer and the signs or symptoms associated with various stages are well-known to the skilled clinician. See, for example, The American Joint Committee on Cancer Staging Manual, Sixth Edition.

An effective amount of QW-1624F2-2 is an amount sufficient to effect beneficial or desired results, including clinical results, and, as such, an effective amount depends upon the context in which it is being applied. For example, in the context of administering an agent that decreases CYP24 or c-Jun expression, an effective amount of an agent is, for example, an amount sufficient to achieve such a decrease in CYP24 or c-Jun expression as compared to the response obtained without administration of the QW-1624F2-2. An effective amount of QW-1624F2-2 required to achieve the desired outcome of preventing, eliminating, stabilizing or alleviating a sign or symptom of a breast cancer will be dependent on the patient and the condition of the patient, the mode of administration, and the stage of the cancer being prevented or treated.

A reduction or decrease in CYP24 or c-Jun expression is intended to mean a 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 100% decrease in expression when compared to otherwise same conditions wherein QW-1624F2-2 is not present.

Having shown that QW-1624F2-2 inhibits breast tumor cell growth and invasion, and promotes apoptotic cell death, the

present invention also relates to a method for using QW-1624F2-2 to modulate the growth of a breast cancer cell either *in vitro* or *in vivo*. The method involves contacting a breast cancer cell with an effective amount of QW-1624F2-2 so that proliferation or invasion of said cell is inhibited or reduced and apoptosis of said cell is induced or promoted. A reduction or inhibition of cell proliferation or invasion is intended to mean a 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 100% decrease when compared to otherwise same conditions wherein QW-1624F2-2 is not present. As one of skill in the art can appreciate, means for determining cell proliferation and invasion can vary depending on whether the cell is *in vitro* or *in vivo*.

Cell proliferation measurements *in vitro* can be determined by counting cells before and after the addition of QW-1624F2-2 and comparing the number of cells present after addition of QW-1624F2-2 to similar cells not contacted with QW-1624F2-2. Similarly, cell proliferation measurements *in vivo* can be determined by monitoring the size of a tumor before and after contacting cells of the tumor with QW-1624F2-2.

Cancer cell invasion *in vitro* can be determined as exemplified herein using a MATRIGEL™ invasion assay or using other well-established methods, *e.g.*, using a CHEMICON® Cell Invasion Assay Kit (CHEMICON® International, Temecula, CA) in the presence or absence of QW-1624F2-2. Similarly, cell invasion measurements *in vivo* can be determined by monitoring tumor location and growth before and after contacting cells of the tumor with QW-1624F2-2.

Induction or promotion of apoptosis of a breast cancer cell is intended to mean a 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 100% increase in cell death when compared to otherwise same conditions wherein QW-1624F2-2 is not present.

Measurements of cell death can be performed using methods well-known to those of skill in the art, *e.g.*, measuring a reduction in tumor size, an decrease in cell number or the expression of apoptosis marker proteins (*e.g.*, caspases).

For use in accordance with the present invention, QW-1624F2-2 can generally be formulated into a pharmaceutical composition for administration to human subjects in a biologically compatible form suitable for administration *in vivo*. A pharmaceutical composition containing QW-1624F2-2 can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective amount of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Generally, a pharmaceutical composition contains, for example, 0.1 to 99.5%, or more suitably 0.5 to 90%, of active ingredient in combination with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro, editor, 20th ed. Lippincott Williams & Wilkins: Philadelphia, PA, 2000. Such compositions include, albeit not exclusively, solutions of QW-1624F2-2 in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

QW-1624F2-2 can be used in accordance with the method of the present invention in the form of a salt, solvate or as hydrate. All forms are within the scope of the invention.

In accordance with the methods of the invention, QW-1624F2-2 can be administered to a subject in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. QW-1624F2-2 can be administered, for example, by oral, parenteral, buccal,

sublingual, nasal, rectal, patch, pump or transdermal administration and the pharmaceutical compositions formulated accordingly. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal and topical modes of administration. Parenteral administration can be by continuous infusion over a selected period of time.

QW-1624F2-2 can be orally administered, for example, with an inert diluent or with an assimilable edible carder, or it can be enclosed in hard or soft shell gelatin capsules, or it can be compressed into tablets, or it can be incorporated directly with the food of the diet. For oral therapeutic administration, QW-1624F2-2 can be incorporated with an excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

QW-1624F2-2 can also be administered parenterally. Solutions of QW-1624F2-2 can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. A person skilled in the art would know how to prepare suitable formulations using conventional procedures and ingredients for the selection and preparation of suitable formulations.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists.

Compositions for nasal administration can conveniently be formulated as aerosols, drops, gels and powders. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomizing device. Alternatively, the sealed container can be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form comprises an aerosol dispenser, it will contain a propellant which can be a compressed gas such as compressed air or an organic propellant such as fluorochlorohydrocarbon. The aerosol dosage forms can also take the form of a pump-atomizer.

Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, wherein the active ingredient is formulated with a carrier such as sugar, acacia, tragacanth, or gelatin and glycerine.

QW-1624F2-2 can be administered to a subject alone or in combination with pharmaceutically acceptable carriers, as noted above, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

The dosage of QW-1624F2-2 can vary depending on many factors such as the mode of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the frequency of the treatment and the type of concurrent treatment, if any and the clearance rate of the compound in the subject to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. QW-1624F2-2 can be administered initially in a

suitable dosage that can be adjusted as required, depending on the clinical response.

Actual dosage levels and time course of administration of QW-1624F2-2 in a pharmaceutical composition can vary so as to obtain an amount of QW-1624F2-2 which is effective to achieve the desired therapeutic response for a particular patient and mode of administration, without being toxic to the patient. In general, a dose range for QW-1624F2-2 is from 0.1 to 10 mg or more per day.

QW-1624F2-2 can be used alone or in combination with a second anticancer agent that modulates CYP24 or c-Jun expression or in combination with other types of treatment (which may or may not modulate CYP24 or c-Jun) for breast cancer. For example, QW-1624F2-2 can be administered in combination with calcitriol or other vitamin D receptor agonists. The use of QW-1624F2-2 will avoid, or at least minimize, the hypercalcemic toxicity associated with medicinal use of calcitriol or other vitamin D receptor agonists. Other treatments which can be combined with the use of QW-1624F2-2 include, but are not limited to, Doxorubicin, Paclitaxel, Methotrexate, 5-Fluorouracil, Docetaxel, Thiotepa, Cis-platin, Estrogen receptor modulators such as Tamoxifen and Toremifene, Estrogens (*e.g.*, diethylstilbestrol), Androgens (*e.g.*, fluoxymesterone), Gonadotropin-Releasing Hormone (GnRH), Anastrozole, Aromatase inhibitors (antineoplastics), Vinorelbine tartrate, Gemcitabine hydrochloride, Progestins (*e.g.*, Medroxyprogesterone acetate, Megestrole acetate), Trastuzumab (HERCEPTIN®), or Cyclophosphamide.

QW-1624F2-2 can be administered prior to the administration of the second agent, simultaneously with the second agent, or after the administration of the second agent. Furthermore, QW-1624F2-2 can be administered in a proform

which is converted into its active metabolite, or more active metabolite *in vivo*.

The invention is described in greater detail by the following non-limiting examples.

Example 1: CYP24 and c-Jun Expression

Microarray analysis. MKL-4 or MCF7cJun cells treated for 48 hours with vehicle (isopropanol) or QW-1624F2-2 analog were harvested and total RNA was isolated using standard RNA isolation methods. Levels of CYP24 and c-Jun expression were determined by probing an AFFYMATRIX® chip (Human Genome U133 Set; AFFYMATRIX®, Santa Clara, CA) in accordance with manufacturer's instructions. Data relating to individual genes of both treatment groups were plotted using the log of non-normalized signal intensity for Cy3-labeled vehicle-treated control (X-axis) and Cy5-labeled QW-1624F2-2 analog-treated cells (Y-axis). Regression analysis was performed using standard methods.

Semi-quantitative RT-PCR. RNA was isolated from control and QW-1624F2-2 treated cells using well-established methods. Under standard conditions in combination with CYP24 primers: Left - 5'-GCA GCC TAG TGC AGA TTT CC-3' (SEQ ID NO:1) and Right - 5'-CCA GAA CTG TTG CCT TGT CA-3' (SEQ ID NO:2), or c-Jun primers: Left - 5'-TGA CTG CAA AGA TGG AAA CG-3' (SEQ ID NO:3) and Right - 5'-CCT GCT CAT CTG TCA CGT TC-3' (SEQ ID NO:4), the respective genes were PCR amplified, separated by gel electrophoresis and quantified.

Example 2: Cell Growth Assays

Light microscopic analysis and TUNEL analysis on cytospin samples of MKL-4 cells following a 48 hour treatment with vehicle (control), QW-1624F2-2 alone, Tamoxifen alone (1 μM),

or Tamoxifen combined with QW-1624F2-2 were conducted monitor cell proliferation and apoptosis.

In vitro invasion assays were conducted by plating cells on day 1 and treating with 200 nM QW-1624F2-2 on day 2. Approximately 48 hours following treatment, cells were labeled with fluorescent probe, Dil (Molecular Probes, Eugene, OR), trypsinized, counted using a hemocytometer and diluted using serum-free culture medium to 1.25 x 10^5 cells/mL and applied in the rehydrated MATRIGEL™-coated inserts of 6-well invasion chambers. Medium with the chemo-attractant (FBS) was placed in the lower chamber of the wells and the cells were allowed to migrate for approximately 20 hours at 37°C. Cells that migrated or invaded to the apical side of the insert were visualized using a fluorescent microscope. A count of the stained cells was later determined from multiple digital images. Data (percent invasion) was expressed as a ratio of cells invading through the MATRIGEL™-coated membrane relative to the migration through the control membrane with no MATRIGEL™ coating. The data was further converted to an Invasion Index where the ratio of the invasive capacity of the treated versus untreated cells was calculated.

What is claimed is:

1. A method for preventing or treating breast cancer comprising administering an effective amount of vitamin D analog 1β-hydroxymethyl-3-epi-16-ene-24,24-difluoro-27a,27a-bishomo-25(OH)D_3 to a subject having or at risk of having a breast cancer so that said cancer is prevented or treated.

2. The method of claim 1, wherein the effective amount of vitamin D analog decreases the expression of CYP24 or c-Jun.

3. The method of claim 1, further comprising co-administering a second anti-cancer agent.

4. A method for modulating the growth of a breast cancer cell comprising contacting a breast cancer cell with an effective amount of vitamin D analog 1β-hydroxymethyl-3-epi-16-ene-24,24-difluoro-27a,27a-bishomo-25(OH)D_3 so that proliferation of said cell is inhibited; invasion of said cell is inhibited; or apoptosis of said cell is induced.

SEQUENCE LISTING

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Sundaram, Sujatha

<120> METHOD FOR PREVENTING OR TREATING BREAST CANCER USING A VITAMIN D ANALOG

<130> DC-0323

<150> US 60/600,676
<151> 2004-08-11

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INTERNATIONAL SEARCH REPORT

PCT/US05/28382

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A 61 K 31/59
US CL : 514/167
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN DATABASE SEARCH, REGISTRY, TEXT, STMEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,329,538 B1 (BATCHO ET AL.) 11 December 2001, lines 5-65 in column 15, lines 1-67 in column 16, Tables I to V.	1-4
Y	SWAMI et al., 1alpha,25-dihydroxyvitamin D3 Down-Regulators, Estrogen Receptor Abundance and Suppresses Estrogen Actions in MCF-7 Human Breast Cancer Cells, Clinical Cancer Research, August 2000, Vol 6, 3371-3379. See the entire document especially abstract of the inven tion, introduction on page 3371, lines 1-17 in first column on page 3372Table I and results on pages 3373 and 3374.	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
10 December 2005 (10.12.2005)

Date of mailing of the international search report
11 JAN 2006

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Int

PCT/US05/28382

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☐ on paper

   ☒ in electronic form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☐ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: