Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).
This invention relates to reducing the incidence, inhibition, suppression, prevention and treatment of androgen-deprivation induced osteoporosis and/or bone fractures and/or loss of bone mineral density (BMD) in men suffering from prostate cancer. More particularly, this invention provides a method of treating, preventing, suppressing, inhibiting, or reducing the risk of developing androgen-deprivation induced osteoporosis and/or bone fractures and/or loss of BMD in men suffering from prostate cancer, comprising administering to a male subject suffering from prostate cancer an anti-estrogen agent and/or its analog, derivative, isomer, metabolite, pharmaceutically acceptable salt, pharmaceutical product, hydrate, N-oxide, or any combination thereof.

BACKGROUND OF THE INVENTION

It is well established that the bone mineral density of males decrease with age. Decreased amounts of bone mineral content and density correlates with decreased bone strength and predispose to fracture. The molecular mechanisms underlying the pleiotropic effects of sex-hormones in non-reproductive tissues are only beginning to be understood, but it is clear that physiologic concentrations of androgens and estrogens play an important role in maintaining bone homeostasis throughout the life-cycle. Consequently, when androgen or estrogen deprivation occurs, there is a resultant increase in the rate of bone remodeling that tilts the balance of resorption and formation in the favor of resorption, contributing to an overall loss of bone mass. In males, the natural decline in sex-hormones at maturity (direct decline in androgens as well as lower levels of estrogens derived from peripheral aromatization of androgens) is associated with the frailty of bones. This effect is also observed in males who have been castrated.

Prostate cancer is one of the most frequently diagnosed noncutaneous cancers among men in the United States. One of the approaches to the treatment of prostate cancer is by androgen deprivation. The male sex hormone, testosterone, stimulates the growth of cancerous prostatic cells and, therefore, is the primary fuel for the growth of prostate cancer. The goal of androgen deprivation is to decrease the stimulation by testosterone of the cancerous prostatic cells. Testosterone normally is produced by the testes in response to stimulation from a hormonal signal called luteinizing hormone (LH) which in turn is stimulated by luteinizing-hormone releasing hormone (LH-RH). Androgen deprivation is accomplished either surgically by bilateral orchidectomy or chemically by LH-RH agonists (LHRH) with or without nonsteroidal antiandrogens.

Current studies suggest that early androgen deprivation in patients with micrometastatic disease may indeed prolong survival [Messing EM, et al (1999), N Engl J Med 34, 1781-1788; Newling (2001), Urology 58(Suppl 2A), 50-55]. Moreover, androgen deprivation is being employed in numerous new clinical settings, including neoadjuvant therapy prior to radical prostatectomy, long-term adjuvant therapy for patients at high risk for recurrence following radiation or surgery, neoadjuvant therapy for radiation, and treatment of biochemical recurrence following radiation or surgery [Carroll, et al (2001), Urology 58, 1-4; Horwitz EM, et al (2001), Int J Radiat Oncol Biol Phys 15;49(4), 947-56]. Thus, more prostate cancer patients have become candidates for and are being treated by androgen ablation. Moreover, these prostate cancer patients are being treated earlier and longer than in the past, which in some cases may be as long as 10 or more years of androgen deprivation therapy.

Unfortunately, androgen deprivation therapy is fraught with significant side effects, including hot flashes, gynecomastia, osteoporosis, decreased lean muscle mass, depression and other mood changes, loss of libido, and erectile dysfunction [Stege R (2000), Prostate Suppl 10,38-42]. Consequently, complications of androgen blockade now contribute significantly to the morbidity, and in some cases the mortality, of men suffering from prostate cancer.

Given that more, patients today are being treated by long-term androgen deprivation, osteoporosis has become a clinically important side effect in men suffering from prostate cancer undergoing androgen deprivation. Loss of bone mineral density (BMD) occurs in the majority of patients being treated by androgen deprivation by 6 months. New innovative approaches are urgently needed at both the basic science and clinical levels to decrease the Incidence of androgen-deprivation induced osteoporosis in men suffering from prostate cancer.

US-5,610,150-A discloses a method of treatment of androgen-related diseases such as prostate cancer in susceptible male animals, including humans, novel antiandrogens and/or novel sex steroid biosynthesis inhibitors as part of a combination therapy. Sex steroid biosynthesis inhibitors, especially those capable of inhibiting conversion of dehydroepiandrosterone (DHEA) or 4-androstenedione (DELA...sup.4 -dione) to natural sex steroids (and testosterone into dihydrotestosterone) in peripheral tissues, are used in combination with antiandrogens usually after blockade of testicular hormonal secretions. Antiestrogens can also be part of the combination therapy.

WO-00/07576-A describes novel methods of treating and/or inhibiting development of prostatic cancer, benign prostatic hyperplasia, prostatitis, acne, seborrhea, hirsutism or androgenic alopecia utilize inhibitors of type 3 3α-hydroxysteroid dehydrogenase alone or in combination with other active pharmaceuticals such as inhibitors of type 5-
17-β-hydroxysteroid dehydrogenase. Furthermore, novel inhibitors and pharmaceutical products are also disclosed.

SUMMARY OF THE INVENTION

[0009] This invention relates to the use of a Toremifene in the manufacture of a medicament for the treatment, prevention, suppression or inhibition of, or for reducing the risk of developing: androgen-deprivation induced osteoporosis; androgen deprivation induced loss of bone mineral density (BMD); or androgen-deprivation induced bone fractures; in a male subject having prostate cancer.

BRIEF DESCRIPTION OF THE FIGURES

[0010] The present invention will be understood and appreciated more fully from the following detailed description taken in conjunction with the appended figures which depict

Figure 1: Effect of Toremifene on C-telopeptide of rat collagen I (RatLaps ELISA).
Figure 2: Effect of Toremifene on serum osteocalcin levels. A) 10 and 30 days; B) 60 and 120 days.

DETAILED DESCRIPTION OF THE INVENTION

[0011] This invention provides: The use of Toremifene in the manufacture of a medicament for 1) treating androgen-deprivation induced osteoporosis in a male subject suffering from prostate cancer; 2) preventing androgen-deprivation induced osteoporosis in a male subject suffering from prostate cancer; 3) suppressing or inhibiting androgen-deprivation induced osteoporosis in a male subject suffering from prostate cancer; 4) reducing the risk of developing androgen-deprivation induced osteoporosis in a male subject suffering from prostate cancer; 5) treating androgen-deprivation induced loss of BMD in a male subject suffering from prostate cancer; 6) preventing androgen-deprivation induced loss of BMD in a male subject suffering from prostate cancer; 7) suppressing or inhibiting androgen-deprivation induced loss of BMD in a male subject suffering from prostate cancer; 8) reducing the risk of developing androgen-deprivation induced loss of BMD in a male subject suffering from prostate cancer; 9) treating androgen-deprivation induced bone fractures in a male subject suffering from prostate cancer; 10) preventing androgen-deprivation induced bone fractures in a male subject suffering from prostate cancer; 11) suppressing or inhibiting androgen-deprivation induced bone fractures in a male subject suffering from prostate cancer; 12) reducing the risk of developing androgen-deprivation induced bone fractures in a male subject suffering from prostate cancer.

[0012] As provided herein, the results demonstrate that administration of Toremifene, is bone sparing. This is determined by measuring the levels of bone-specific serum markers that indicate bone resorption and formation. Further, this invention demonstrates that Toremifene (and/or 17-β-Estradiol), increases bone mineral density.

[0013] The anti-estrogen that treats, prevents, suppresses, inhibits or reduces the risk of developing androgen-deprivation induced osteoporosis and/or loss of BMD is a Toremifene

[0014] Toremifene is an example of a triphenylalkylene compound described in US. Patent Nos. 4,696,949 and 5,491,173 to Tolvola et al. The parenteral and topical administration to mammalian subjects of formulations containing Toremifene is described in U.S. Patent No. 5,571,534 to Jalonen et al. and in U.S. Patent No. 5,605,700 to DeGregorio et al.

[0015] Osteoporosis is a systemic skeletal disease, characterized by low bone mass and deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. In osteoporotic patients, bone strength is abnormal, with a resulting increase in the risk of fracture. Osteoporosis depletes both the calcium and the protein collagen normally found in the bone, resulting in either abnormal bone quality or decreased bone density. Bones that are affected by osteoporosis can fracture with only a minor fall or injury that normally would not cause a bone fracture. The fracture can be either in the form of cracking (as in a hip fracture) or collapsing (as in a compression fracture of the spine). The spine, hips, and wrists are common areas of osteoporosis bone fractures, although fractures can also occur in other skeletal areas.

[0016] BMD is a measured calculation of the true mass of bone. The absolute amount of bone as measured by bone mineral density (BMD) generally correlates with bone strength and its ability to bear weight. By measuring BMD, it is possible to predict fracture risk in the same manner that measuring blood pressure can help predict the risk of stroke.

[0017] BMD in one embodiment can be measured by known bone-mineral content mapping techniques. Bone density from the hip, spine, wrist, or calcaneus may be measured by a variety of techniques. The preferred method of BMD measurement is dual-energy X-ray densitometry (DXA). BMD of the hip, antero-posterior (AP) spine, lateral spine, and wrist can be measured using this technology. Measurement at any site predicts overall risk of fracture, but information from a specific site is the best predictor of fracture at that site. Quantitative computerized tomography (QCT) is also used to measure BMD of the spine. See for example, "Nuclear Medicine: "Quantitative Procedures" by Wahner H W,
Pharmaceutical Compositions

[0021] In one embodiment, the pharmaceutical composition of the present invention comprise Toremifene and a pharmaceutically acceptable carrier. The pharmaceutical composition is used for treating and/or preventing androgen-deprivation induced osteoporosis and/or loss of BMD; for suppressing or inhibiting androgen-deprivation induced osteoporosis and/or loss of BMD; and/or for reducing the risk of developing androgen-deprivation induced osteoporosis and/or loss of BMD.

[0022] As used herein, “pharmaceutical composition” means a “therapeutically effective amount” of the active ingredient, i.e. the Toremifene together with a pharmaceutically acceptable carrier or diluent. A “therapeutically effective amount” as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen.

[0023] The pharmaceutical compositions containing the Toremifene is to be administered to a subject by any method known to a person skilled in the art, such as parenterally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermally, subcutaneously, intraperitoneally, intravenicularly, intracranially, intravaginally or intratumorally.

[0024] In one embodiment, the pharmaceutical compositions are to be administered orally, and are thus formulated in a form suitable for oral administration, i.e. as a solid or a liquid preparation. Suitable solid oral formulations include tablets, capsules, pills, granules, pellets and the like. Suitable liquid oral formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In one embodiment, the pharmaceutical compositions are to be administered intravenously, intradermally, subcutaneously, intraperitoneally, intravenicularly, intracranially, intravaginally or intratumorally.

[0025] Further, in another embodiment, the pharmaceutical compositions are to be administered by intravenous, intraarterial, or intramuscular injection of a liquid preparation. Suitable liquid formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In one embodiment the pharmaceutical compositions are to be administered intravenously, and are thus formulated in a form suitable for intravenous administration. In another embodiment, the pharmaceutical compositions are to be administered intraarterially, and are thus formulated in a form suitable for intravenous administration. In another embodiment the pharmaceutical compositions are to be administered intramuscularly, and are thus formulated in a form suitable for intramuscular administration.

[0026] Further, in another embodiment, the pharmaceutical compositions are to be administered topically to body surfaces, and are thus formulated in a form suitable for topical administration. Suitable topical formulations include gels, ointments, creams, lotions, drops and the like. For topical administration, the Toremifene is prepared and applied as solutions, suspensions, or emulsions in a physiologically-acceptable diluent with or without a pharmaceutical carrier.

[0027] Further, in another embodiment, the pharmaceutical compositions are to be administered as a suppository, for example a rectal suppository or a urethral suppository. Further, in another embodiment, the pharmaceutical compositions are to be administered by subcutaneous implantation of a pellet. In a further embodiment, the pellet provides for controlled release of an estrogen agent over a period of time.

[0028] In another embodiment, the active compound can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid).

[0029] As used herein “pharmaceutically acceptable carriers or diluents” are well known to those skilled in the art. The carrier or diluent may be a solid carrier or diluent for solid formulations, a liquid carrier or diluent for liquid formulations, or mixtures thereof.
Solid carriers/diluents include, but are not limited to, a gum, a starch (e.g., corn starch, pregeletanized starch), a sugar (e.g., lactose, mannitol, sucrose, dextrose), a cellulose material (e.g., microcrystalline cellulose), an acrylate (e.g., polymethylacrylate), calcium carbonate, magnesium oxide, t alc, or mixtures thereof.

For liquid formulations, pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, and fish-liver oil.

Parenteral vehicles (for subcutaneous, intravenous, intraaerial, or intramuscular injection) include sodium chloride solution, Ringer’s dextrose, dextrose and sodium chloride, lactated Ringer’s and fixed oils. Intravenous vehicles include fluid and nutrient replacers, electrolyte replacers such as those based on Ringer’s dextrose, and the like. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions. Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, and fish-liver oil.

In addition, the compositions may further comprise binders (e.g., acacia, cornstarch, gelatin, carborner, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g. cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate), buffers (e.g., Tris-HCl, acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g., sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., glycerol, polyethylene glycol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g., hydroxypropyl cellulose, hydroxypropylmethyl cellulose), viscosity increasing agents (e.g., carborner, colloid sodium dihydrate, ethyl cellulose, guar gum), sweeteners (e.g. aspartame, citric acid), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), lubricants (e.g. stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g. colloid sodium dioxide), plasticizers (e.g. diethyl phthalate, triethyl citrate), emulsifiers (e.g. carborner, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g. ethyl cellulose, acrylates, polymethacrylates) and/or adjuvants.

In one embodiment, the pharmaceutical compositions provided herein are controlled-release compositions, i.e. compositions in which the Toremifene compound is released over a period of time after administration. Controlled- or sustained-release compositions include formulation in lipophilic depot (e.g. fatty acids, waxes, oils). In another embodiment, the composition is an immediate-release composition, i.e. a composition in which all of the Toremifene compound is released immediately after administration.

In yet another embodiment, the pharmaceutical composition can be delivered in a controlled release system. For example, the agent may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i.e., the brain, thus allowing only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984). Other controlled-release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

The compositions may also include incorporation of the active material into or onto particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts.) Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance.

Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to lipids of tissue-specific receptors.

Also comprehended by the invention are compounds modified by the covalent attachment of water soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrolidone or polyproline. The modified compounds are known to exhibit substantially longer half-lives in blood following Intravenous injection than do the corresponding unmodified compounds (Abuchowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound’s solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result the desired in vivo biological activity may be achieved by the administration of such polymer-compound abducts less frequently or in lower doses than with the unmodified compound.
The preparation of pharmaceutical compositions which contain an active component is well understood in the art, for example by mixing, granulating, or tablet-forming processes. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. For oral administration, the Toremifene is mixed with additives customary for this purpose, such as vehicles, stabilizers, or inert diluents, and converted by customary methods into suitable forms for administration, such as tablets, coated tablets, hard or soft gelatin capsules aqueous, alcoholic or oily solutions. For parenteral administration, the Toremifene is converted into a solution, suspension, or emulsion, if desired with the substances customary and suitable for this purpose, for example, solubilizers or other.

An active component can be formulated into the composition as neutralized pharmaceutical acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule), which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaaine, and the like.

For use in medicine, the salts of Toremifene are pharmaceutically acceptable salts. Other salts may, however, be useful in the preparation of the compounds, according to the invention or of their pharmaceutically acceptable salts. Suitable pharmaceutically acceptable salts of the compounds of this invention include acid addition salts which may, for example, be formed by mixing a solution of the compound according to the invention with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulphuric acid, methanesulphonic acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, oxalic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid.

As defined herein, "contacting" means that the Toremifene of the present invention is introduced into a sample containing the enzyme in a test tube, flask, tissue culture, chip, array, plate, microplate, capillary, or the like, and incubated at a temperature and time sufficient to permit binding of the anti-estrogen to the enzyme. Methods for contacting the samples with the Toremifene or other specific binding components are known to those skilled in the art and may be selected depending on the type of assay protocol to be run. Incubation methods are also standard and are known to those skilled in the art.

In another embodiment, the term "contacting" means that the Toremifene of the present invention is introduced into a subject receiving treatment, and the Toremifene compound is allowed to come in contact with the androgen receptor in vivo.

As used herein, the term "treating" includes preventative as well as disorder remitative treatment. As used herein, the terms "reducing", "suppressing" and "inhibiting" have their commonly understood meaning of lessening or decreasing. As used herein, the term "progression" means increasing in scope or severity, advancing, growing or becoming worse. As used herein, the term "recurrence" means the return of a disease after a remission.

As used herein, the term "administering" refers to bringing a subject in contact with an anti-estrogen compound of the present invention. As used herein, administration can be accomplished in vitro, i.e. in a test tube, or in vivo, i.e. in cells or tissues of living organisms, for example humans. In one embodiment, the present invention encompasses administering the compounds of the present invention to a subject.

In one embodiment, the present invention comprise the use of Toremifene as the sole active ingredient which is to be administered. However, also encompassed within the scope of the present invention are methods for hormone therapy, for treating prostate cancer, for delaying the progression of prostate cancer, and for preventing and/or treating the recurrence of prostate cancer, which comprise administering the anti-estrogen compounds in combination with one or more therapeutic agents. These agents include, but are not limited to: LHRH analogs, reversible antiandrogens (such as bicalutamide or flutamide), additional anti-estrogens, anticancer drugs, 5-alpha reductase inhibitors, aromatase inhibitors, progestins, selective androgen receptor modulators (SARMs) or agents acting through other nuclear hormone receptors.

Thus, in one embodiment, the present invention include using compositions and pharmaceutical compositions comprising Toremifene in combination with an LHRH analog. In another embodiment, the present invention include using compositions and pharmaceutical compositions comprising Toremifene, in combination with a reversible antiandrogen. In another embodiment, the present invention include using compositions and pharmaceutical compositions comprising Toremifene, in combination with an additional anti-estrogen. In another embodiment, the present invention include using compositions and pharmaceutical compositions comprising Toremifene, in combination with an additional anti-estrogen and another anticancer drug. In another embodiment, the present invention include using compositions and pharmaceutical compositions comprising Toremifene, in combination with a 5-alpha reductase inhibitor. In another embodiment, the present invention include using compositions and pharmaceutical compositions comprising Toremifene, in combination with an aromatase inhibitor. In another embodiment, the present invention include using compositions and pharmaceutical compositions comprising Toremifene, in combination with a progestin. In another embodiment, the present Invention Include using compositions and pharmaceutical compositions comprising Toremifene in combination with a SARM.
bodiment, the present invention include using compositions and pharmaceutical compositions comprising Toremifene, in combination with an agent acting through other nuclear hormone receptors.

[0048] Various embodiments of dosage ranges are contemplated by this invention. The dosage may be in the range of 1-80 mg/day. The dosage may be in the range of 5-80 mg/day. In another embodiment the dosage is in the range of 35-66 mg/day. In another embodiment the dosage is in the range of 20-60 mg/day. In another embodiment the dosage is in the range of 40-60 mg/day. In another embodiment the dosage is in the range of 45-60 mg/day. In another embodiment the dosage is in the range of 15-25 mg/day. In another embodiment the dosage is in the range of 55-65 mg/day. In one embodiment, the dosage is 20 mg/day. In another embodiment, the dosage is 40 mg/day. In another embodiment, the dosage is 60 mg/day.

[0049] The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention.

EXPERIMENTAL DETAILS SECTION

EXAMPLE 1

Effect of Toremifene on bone turnover in human male subjects

[0050] In a Phase IIa clinical trial to determine whether Toremifene has chemopreventive activity against prostate cancer, 18 men with high-grade prostatic intraepithelial neoplasia (HGPIN) were treated with 60mg/d of Toremifene for 4 months. At Day 120 there was a significant reduction from baseline in serum calcium (mean -0.12, p=0.005) and at both day 60 and day 120, alkaline phosphatase was significantly decreased compared to baseline (mean= -18.7 at Day 60 and -21.0 at Day 120, and p<0.001 for both visits).

[0051] These clinical data demonstrate that the anti-estrogen Toremifene showed estrogenic effects on bone favourably affecting bone turnover markers in men.

EXAMPLE 2: EFFECT OF TOREMIFENE ON BONE IN MALE RATS

Drug Delivery System

[0052] The test article, positive control and placebo were delivered by ALZA pumps manufactured by Durect Corporation (Cupertino, CA). Pumps were implanted in a subcutaneous pocket using appropriate surgical technique. The pumps employed in this study deliver a continuous rate of drug over a 30-day period with Toremifene formulated to release 1.8 mg/day (2 mL pump) and 17-β-Estradiol (positive control) is released at 70 μg/day. Data provided by the manufacturer of the pumps validates the constant rate of drug delivery over a 28 day period, and suggests that the constant rate can be expected for several additional days. Animals were anesthetized and pump replacement was performed for each dosage group on days 31, 61, and 91 to provide drug administration over a 120 day period. Every animal on study had a pellet implanted to control for potential confounding variables associated with surgery for implantation.

Study Groups:

[0053] Adult male Sprague-Dawley rats (14-weeks old at start of study), weighing approximately 0.35 kg, were used in the experiments. This study employed five test groups of 12 rats. Treatment groups represent placebo control (castrated and sham operated), 17-β-estradiol (castrated) and Toremifene (castrated and sham operated). 5 animals from each treatment group were sacrificed at day 60 and day 120 and bone metabolism markers were measured and bone was harvested for biomechanical strength and density testing.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Sham</th>
<th>Castrated</th>
<th># of animals</th>
<th>Sacrifice Schedule (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Placebo control</td>
<td></td>
<td></td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>Placebo control</td>
<td></td>
<td></td>
<td>5</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td>Toremifene (1.75 mg/day)</td>
<td>✔️</td>
<td></td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>Toremifene (1.75 mg/day)</td>
<td>✔️</td>
<td></td>
<td>5</td>
<td>120</td>
</tr>
<tr>
<td>5</td>
<td>Estradiol (0.07 mg/day)</td>
<td>✔️</td>
<td></td>
<td>5</td>
<td>60</td>
</tr>
</tbody>
</table>
Clinical Observations: Cage-side observations were made and recorded once weekly (daily cage checks note any exceptions). Effects on vital functions were observed. Any animals found moribund or presenting with signs of acute toxicity were anesthetized by an intramuscular ketamine/xylazine (87/13) injection and euthanized by abdominal aorta exsanguinations.

Body Weight Body weights were taken in triplicate and averaged once per week. Sacrifices were made at 60 and 120-days. Each sacrifice involved 5 rats from each treatment group, and 2 rats at 30, 60, 90, and 120 days to verify drug in plasma (refer to Table 1 for group allocation to treatment and sacrifice). Animals were anesthetized by an injection of ketamine/xylazine (87/13) and sacrificed by abdominal aorta exsanguinations. Blood collected at exsanguinations was processed to collect serum.

Bone remodeling was assessed per serum markers to analyze bone resorption and bone formation of treatment rats. The tests listed in Table 2 indicate the bone remodeling category and the amounts of serum required for analysis. Serum chemistry analysis of blood calcium, phosphorus, bilirubin and creatinine levels, and bone-specific alkaline phosphatase, were performed at AniLytics, Inc. (Gaithersburg, MD).

**Table 2. Assays for serum markers of bone remodeling in mouse**

<table>
<thead>
<tr>
<th>Remodeling Category</th>
<th>Tests Run</th>
<th>Test Supplier</th>
<th>Minimum Serum Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Formation</td>
<td>Rat-MID Osteocalcin ELISA</td>
<td>OSTEOMETER</td>
<td>20ul</td>
</tr>
<tr>
<td></td>
<td>Chemistry Analysis of Calcium, Phosphorus, Bilirubin, Creatine</td>
<td>AniLytics, Inc.</td>
<td>70ul</td>
</tr>
<tr>
<td></td>
<td>Bone Spec. Phosphatase</td>
<td>AniLytics, Inc.</td>
<td>50ul</td>
</tr>
<tr>
<td>Bone Resorption</td>
<td>Serum CrossLaps One Step ELISA</td>
<td>OSTEOMETER</td>
<td>20ul</td>
</tr>
<tr>
<td></td>
<td>(Deoxy)Pyridinoline Crosslink in serum</td>
<td>METRA</td>
<td>25ul</td>
</tr>
<tr>
<td></td>
<td>RatTRAP ELISA (tartrate-resistant Acid P)</td>
<td>SBA Sciences</td>
<td>50ul</td>
</tr>
</tbody>
</table>

 Specimen collection included femoral bones in addition to blood. Blood was processed for serum. Serum was aliquoted and frozen at -80°C until analysis. Serum tests were performed at GTx, Inc. for osteocalcin levels (bone formation marker) and c-telopeptide (bone resorption marker). After the femur was removed from each animal, it was stripped of extraneous tissue, and stored at -20°C until biomechanical strength and bone mineral density analysis.

**RANDOMIZATION/ASSIGNMENT TO GROUPS:**

Prior to study, 10 groups were randomized to treatments, then sixty (60) 12 week-old rats were ordered, ear-tagged and weighed by the Manager of Animal Resources. Sixty animals were then randomized to the 10 treatment groups of 6 animals each. An ANOVA was performed to establish the presence or absence of significant difference in body weight (within 10% of average body weight is acceptable). The population was already restricted and could not be restricted further. If there was a significant difference, it was duly noted, but changes in assignment were not made. Bone parameters were not normalized to body weight. Values for bone parameters are reported as absolute and evaluated based on group means compared to the appropriate controls.
COMPOUNDS

[0060] Test Article 1:
Identification: Toremifene
Description: White crystalline powder, that is extracted from Toremifene tablets as active ingredient.

[0061] Test Article 2 (Positive Control):
Identification: 17-β-Estradiol (Sigma Aldrich, for laboratory research use).

EXAMPLE 3

EFFECT OF TOREMIFENE ON BONE DENSITY AND SERUM MARKERS FOR BONE REMODELING IN MALE RATS

[0062] The purpose of this study is to determine whether administration of Toremifene to mature male rats is bone sparing as can presently be measured by the levels of bone-specific serum markers that indicate bone resorption and formation (where 17-β-Estradiol is used as a positive control). The effect of Toremifene (and 17-β-Estradiol) on androgen deprivation-induced bone loss was also determined through bone density and mechanical strength testing.

[0063] It is well established that the bone mineral density of males, similar to that of females, decreases with age. Decreased amounts of bone mineral content and density correlates with decreased bone strength, and predisposes to fracture. The molecular mechanism underlying the pleiotropic effects of sex-hormones in non-reproductive tissues are only beginning to be understood, but it is clear that physiologic concentrations of androgens and estrogens play an important role in maintaining bone homeostasis throughout the life-cycle. Consequently, when androgen and/or estrogen deprivation occurs, there is a resultant increase in the rate of bone remodeling that tilts the balance of resorption and formation in the favor of resorption, contributing to the overall loss of bone mass. In males, the natural decline in sex-hormone at maturity (direct decline in androgens as well as lower levels of estrogens derived from peripheral aromatization of androgens) is associated with the frailty of bones. This effect is also observed in males who have been castrated. Previous studies demonstrate in mice that pharmacological therapy with selective estrogen receptor modulator (SERM) compounds in androgen deficient males has a positive effect on bone mineral density and that bone can be maintained or even increased (Broulik, Horm Metab Res, 2000; 32: 181-184). It is suggested that in humans, this intervention would halt the onset of osteoporosis or at least reduce its severity. Importantly, recent human data indicates that estrogen loss in elderly males is more critical for short-term bone changes than loss of androgens. It is believed that the selective estrogen receptor modulator Toremifene (trade name is Acapodene™ and generic name is toremifene) will have similar bone-building properties when compared with other nonsteroidal anti-estrogens (e.g. Tamoxifene, (Broulik, Horm Metab Res, 2000; 32: 181-184), and will be a clinically useful drug for preventing osteoporosis and maintaining bone density in aging males. The model used herein is an orchidectomy model, which is an experimental model used to mimic the type of androgen deprivation that would be caused by, for example, LHRH agonist therapy in prostate cancer.

MATERIALS AND METHODS

Study Design

[0064] Male Sprague-Dawley rats (Harlan Sprague Dawley) were placed on study at 14-weeks of age. They were randomized and divided into five treatment groups: vehicle only (placebo, or P) after sham operation, vehicle only after orchidectomy (Orx), Toremifene (5 mg/kg/day) after sham-operation, and Toremifene after orchidectomy, and 17-β-estradiol (0.5 mg/kg/day) after orchidectomy. Test articles were delivered subcutaneously by Alzet pumps. Pumps were re-implanted every 30 days until the end of the study. Five to six animals from each group were sacrificed after 60 and 120 days of treatment, and femurs were harvested, stripped from soft tissue and muscle, and stored individually in polypropylene vials at -80°C until analysis of bone density and mechanical strength testing. Additionally, serum was collected on days 15, 30, 60, 90 and 120, to measure markers of bone metabolism and to perform serum chemistry.

[0065] After collection, serum was divided into 3 aliquots and stored at -80°C until analysis. 125 µl for serum biochemistry performed by AniLytics, Gaithersburg, MD (Bone-Specific Alkaline Phosphatase, Calcium, Phosphorus, Creatinine and Bilirubin). 100 µl for serum Rat Osteocalcin ELISA and Serum RatLaps ELISA for C-terminal telopeptides determination. The rest of the collected serum was left for repeated test if necessary.

[0066] Bones were stripped from soft tissue and muscles and stored individually in 15 ml vials at -80 C, until further testing.
Analysis of Bone Turnover Markers from Rat Serum Serum RatLaps ELISA, Osteometer BioTech A/S, Denmark

[0067] The assay was performed in duplicates, each 20 μl of serum as well as standards and control. Briefly, 100 μl of Biotinylated RatLaps Antigen was incubated 30 min in each well, strips were washed and 20 μl of Standard, Control and unknown samples were added to appropriate wells, followed by 100 μl of primary antibody (polyclonal Ab raised against a synthetic peptide EKSQDGGR specific for a part of the C-terminal telopeptide alpha1 chain of rat type I collagen). After overnight incubation at 4 C, the wells were washed and 100 μl of Peroxidase conjugated Goat anti-Rabbit IgG Antibody was added to each well and incubated 60 min. After washing the strips 100 μl of the Chromogenic Substrate Solution was added to each well, and after 15 min the reaction was stopped and the absorbance was measured at 450 nm. The means of the duplicate absorbance determination were calculated, and a standard curve was constructed using 4-parametric logistic curve fit. Sample RatLaps concentrations were determined by interpolation.

Rat Osteocalcin EIA, Biomedical Technologies, MA

[0068] This assay is specific for rat Osteocalcin and both carboxylated and decarboxylated rat Osteocalcin are recognized. A monoclonal antibody against the N-terminal region of Osteocalcin is bound to the polystyrene wells. The assay was performed in duplicates. 100 μl of standards, control and 1 to 20 diluted samples were added to appropriate wells and incubated overnight at 4 C. After washing the strips, 100 μl of Goat polyclonal antibody of high specificity for C-terminus of rat Osteocalcin was added and incubated 60 min at 22 C. After washing, 100 μl of Donkey anti-goat IgG peroxidase conjugated antibody was added and incubated 60 min at 22 C in the dark. The reaction was stopped, and the absorbance was measured at 450 nm. A standard curve was constructed using 4-parametric logistic curve fit. Sample osteocalcin concentrations were determined by interpolation.

Bone Analysis Methodology

Peripheral Quantitative Computed Tomography (pQCT)

[0069] Right femurs were subjected to pQCT analysis using a Stratec XCT-RM with associated software (Stratec Medizintechnik GmbH, Pforzheim, Germany. Software version 5.40 C). Femurs were scanned at two sites, 20% and 50% of the total femoral length measured from the distal femur. The position was verified using scout views, and scan results from two 0.5 mm slices perpendicular to the long axis of the femur shaft were recorded.

Mechanical Testing

[0070] Using a Material Testing System (Model 5501 R Instron Corp., Canton, MA), two types of mechanical testing were performed on the right femur. The load and extension curves were collected by the machine software (Merlin II, Instron Corp.). All tests were conducted using a 5 kN load cell at a constant loading rate of 6 mm/min. The general applications of the tests were as described in Turner and Burr (Bone, 1993; 14: 595-608) and Ke et al (Bone, 1998; 23, 249-255).

Compression Test of the Distal Femur

[0071] A compression test was used to determine the mechanical properties of the distal femur. The distal femur test specimen was obtained by removing a 3 mm segment directly proximal to the distal condyle using a slow speed diamond saw with constant saline irrigation. An electronic caliper was used to measure the average anterior/posterior diameter (a), medial/lateral diameter (b), and height (h) of the bone. The extrinsic parameters, maximal load (F_u), stiffness (S) and energy (W), were obtained from the load and extension curve. The following intrinsic parameters were calculated from the measured values: Cross sectional area (CSA) = (π * a * b) / 4; Ultimate Strength (σ) = F_u / CSA; Elastic modulus (E)=S / (CSA / h); Toughness (T) = W / (CSA * h).

RESULTS/DISCUSSION

Analysis of Serum Markers of Bone Turnover

[0072] Bone turnover markers have been demonstrated as an effective, validated tool for the clinical scientist to monitor bone activity. Ultimately, the data of greatest consequence to evaluate novel therapeutics aimed at osteoporosis treatment and prevention is a demonstrated improvement in the quality of bone itself. However, because changes in bone turnover
markers correlate well with bone strength testing, in the present study we analyzed C-telopeptide and osteocalcin levels for interim analysis and supplementary data to support of the effectiveness of treatment.

**C-telopeptide of Type I collagen:**

[0073] As demonstrated in Figure 1, C-telopeptide levels in orchidectomized animals were slightly increased by 22 and 9.5% over the placebo 15 and 30 day groups, respectively, indicating that, after castration, bone resorptive activity is increased and type I collagen is being degraded, with fragments containing the cross-linked molecules released into the blood. Further, treatment of orchidectomized animals with toremifene and the positive control, 17-β-estradiol, reduced the C-telopeptide levels to at or below control values (i.e. unorchidectomized, placebo-treated animals), with the 30 day treatment trending to significance for the 17-β-estradiol group.

[0074] Because toremifene and 17-β-estradiol decrease the level of C-telopeptide in the serum, the data indicate that these agents are acting to prevent the resorption of bone induced by androgen deprivation.

**Osteocalcin:**

[0075] Similar to other published literature, Applicants found that osteocalcin levels were increased by castration. Toremifene significantly reduced the osteocalcin levels in castrated animals to intact control levels (p<0.05 at 15 days, and p<0.02 at 30 days, Figure 2A). The increase in osteocalcin levels were most pronounced 15 days after castration, although Toremifene and 17-β-estradiol continued to significantly reduce osteocalcin levels to below that of intact control rats for up to 120 days (Figure 2B). These results indicate that the rate of bone formation in males is upregulated following orchidectomy to compensate for increased resorptive activity. 17-β-Estradiol, and the selective estrogen receptor modulator toremifene, stabilize the bone resorption and formation processes, hence decreasing overall osteocalcin levels that are detectable in serum.

**Biomechanical Analysis of Bone**

[0076] Androgen deficiency, induced by castration, has been used as a model of male osteoporosis. In this model, most of the bone loss occurs in cancellous bone. To further understand the effects of toremifene on bone mineral density and mechanical strength, bones were harvested upon completion of the in-life study phase and sent to SkeleTech (Bothell, WA) for testing. All bones were thawed in physiological saline prior to analysis. Statistical analysis was performed using SAS software (SAS Institute, Cary, NC). One-way analysis of variance (group) was performed. Individual group differences were ascertained with Dunnett’s procedure using treatment group 2 (Castrated + Placebo) as the reference group. A p value of < 0.05 was considered significant. Where appropriate, a p value of < 0.1 is noted as a trend (when the treatment results are in the direction of the positive control).

**Distal Right Femur Cancellous pQCT:**

[0077] Total bone mineral content and density were lower, though not statistically significant, in Orx animals when compared to Sham-operated animals. Both Toremifene and 17-β-Estradiol treatment appeared to reduce total bone area and increase total bone mineral density. Cancellous bone mineral content and density in Orx animals were 34% less than Sham-operated animals. Toremifene partially prevented this decrease whereas 17-β-Estradiol was able to fully prevent this cancellous bone loss. None of the parameters produced statistically significant differences from the Orx group due to the small sample size and the large variations in the measured results. The results are summarized in Table 3.

<table>
<thead>
<tr>
<th>Table 3: Summary of the Distal Right Femur Cancellous pQCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Group</td>
</tr>
<tr>
<td>Sham (n=6)</td>
</tr>
<tr>
<td>S.E.M</td>
</tr>
<tr>
<td>p&lt; 0.05</td>
</tr>
</tbody>
</table>
Mechanical strength was tested at the distal femur, a site rich in cancellous bone. Maximum load, stiffness, ultimate strength, and elastic modulus were lower in Orx animals when compared to Sham-operated animals. Toremifene treatment of Orx animals improved various parameters to a level that was better than Sham-operated animals. 17-β-Estradiol treatment showed statistically significant improvements in these parameter values. Cross-sectional area was decreased in toremifene and 17-β-Estradiol -treated Orx animals as expected from pQCT data. The results are summarized in Table 3.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Total Bone Content (mg/mm)</th>
<th>Total Bone Area (mm²)</th>
<th>Total Bone Density (mg/cm³)</th>
<th>Trabecular Content (mg/mm)</th>
<th>Trabecular Area (mm²)</th>
<th>Trabecular Density (mg/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx (n = 5)</td>
<td>Mean 11.07</td>
<td>17.38</td>
<td>639.50</td>
<td>1.08</td>
<td>7.82</td>
<td>138.38</td>
</tr>
<tr>
<td></td>
<td>S.E.M 0.50</td>
<td>1.26</td>
<td>47.07</td>
<td>0.22</td>
<td>0.57</td>
<td>31.88</td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.05</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
</tr>
<tr>
<td>Cx + Toremifene (n = 5)</td>
<td>Mean 10.69</td>
<td>15.96</td>
<td>669.74</td>
<td>1.13</td>
<td>7.18</td>
<td>157.56</td>
</tr>
<tr>
<td></td>
<td>S.E.M 0.48</td>
<td>0.67</td>
<td>21.95</td>
<td>0.22</td>
<td>0.30</td>
<td>32.77</td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.05</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>Cx + EST (n = 5)</td>
<td>Mean 11.01</td>
<td>15.95</td>
<td>690.74</td>
<td>1.52</td>
<td>7.17</td>
<td>210.28</td>
</tr>
<tr>
<td></td>
<td>S.E.M 0.76</td>
<td>0.98</td>
<td>26.68</td>
<td>0.50</td>
<td>0.44</td>
<td>62.69</td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.05</td>
<td>n.s</td>
<td>n.s</td>
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<td>n.s</td>
</tr>
</tbody>
</table>

p<0.05 vs. TG2.
n.s. = not significant
n.a. = not applicable
### Table 4: Summary of the Distal Right Femur Compression Test

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Maximum Load (N)</th>
<th>Stiffness (N/mm)</th>
<th>Energy (mJ)</th>
<th>Ultimate Strength (N/mm²)</th>
<th>Elastic Modulus (MPa)</th>
<th>Toughness (MJ/mm³)</th>
<th>CSA (mm²)</th>
<th>Height (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>420.04</td>
<td>3400.22</td>
<td>43.10</td>
<td>18.95</td>
<td>481.38</td>
<td>0.62</td>
<td>22.32</td>
<td>3.13</td>
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<tr>
<td></td>
<td>123.75</td>
<td>944.50</td>
<td>13.09</td>
<td>5.86</td>
<td>152.38</td>
<td>0.18</td>
<td>1.64</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.05</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Cx (n = 5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>386.80</td>
<td>2538.96</td>
<td>49.97</td>
<td>16.73</td>
<td>341.02</td>
<td>0.68</td>
<td>23.19</td>
<td>3.14</td>
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<tr>
<td></td>
<td>70.43</td>
<td>887.56</td>
<td>18.45</td>
<td>3.33</td>
<td>109.52</td>
<td>0.23</td>
<td>1.28</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.05</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Cx + Toremifene (n = 5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>467.01</td>
<td>3856.71</td>
<td>56.96</td>
<td>22.13</td>
<td>579.07</td>
<td>0.86</td>
<td>21.30</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>125.89</td>
<td>1303.56</td>
<td>17.07</td>
<td>6.34</td>
<td>211.70</td>
<td>0.30</td>
<td>1.43</td>
<td>0.15</td>
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<tr>
<td></td>
<td>p &lt; 0.05</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Cx + EST (n = 5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>613.61</td>
<td>4842.67</td>
<td>58.28</td>
<td>31.23</td>
<td>753.44</td>
<td>0.98</td>
<td>19.75</td>
<td>3.06</td>
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<td></td>
<td>72.10</td>
<td>740.18</td>
<td>18.39</td>
<td>4.76</td>
<td>136.97</td>
<td>0.34</td>
<td>1.14</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.05</td>
<td>*</td>
<td>n.s.</td>
<td>*</td>
<td>*</td>
<td>n.s.</td>
<td>*</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

p<0.05 vs. TG2.
n.s. = not significant
n.a. = not applicable
SUMMARY

[0079] Androgen deficiency model resulted in animals that had increased levels of the bone resorption marker C-telopeptide as well as osteocalcin in serum. Treatment with toremifen and 17-β-estradiol significantly reduced the levels of these serum markers following castration. Further, androgen deficiency resulted in a 34% loss of cancellous bone mineral content and density. Importantly, the use of toremifen partially prevented this loss. As predicted, estrogen was very effective in preventing cancellous bone loss due to androgen deficiency. Additionally, the compression test of the distal femur showed improved strength parameters in orchidectomized groups treated with toremifen, and statistically significant improvements with estrogen treatment. These measurements are partially correlated with total bone mineral density at that site. In conclusion, the data presented herein suggests that the selective estrogen receptor modulator toremifen would have a positive effect for bone improvement in men undergoing androgen-deprivation therapy for prostate cancer.

[0080] It will be appreciated by a person skilled in the art that the present invention is not limited by what has been particularly shown and described hereinabove.

Claims

1. Use of Toremifen in the manufacture of a medicament for the treatment, prevention, suppression or inhibition of, or for reducing the risk of developing:
   androgen-deprivation induced osteoporosis;
   androgen-deprivation induced loss of bone mineral density (BMD); or
   androgen-deprivation induced bone fractures; in a male subject having prostate cancer.

2. Use according to claim 1, wherein the medicament is for the treatment of androgen-deprivation induced osteoporosis in a male subject having prostate cancer.

3. Use according to claim 1, wherein the medicament is for the prevention of androgen-deprivation induced osteoporosis in a male subject having prostate cancer.

4. Use according to claim 1, wherein the medicament is for the suppression or inhibition of androgen-deprivation induced osteoporosis in a male subject having prostate cancer.

5. Use according to claim 1, wherein the medicament is for reducing the risk of developing androgen-deprivation induced osteoporosis in a male subject having prostate cancer.

6. Use according to claim 1, wherein the medicament is for the treatment of androgen-deprivation induced loss of bone mineral density (BMD) in a male subject having prostate cancer.

7. Use according to claim 1, wherein the medicament is for the prevention of androgen-deprivation induced loss of bone mineral density (BMD) in a male subject having prostate cancer.

8. Use according to claim 1, wherein the medicament is for the suppression or inhibition of androgen-deprivation induced loss of bone mineral density (BMD) in a male subject having prostate cancer.

9. Use according to claim 1, wherein the medicament is for reducing the risk of developing androgen-deprivation induced loss of bone mineral density (BMD) in a male subject having prostate cancer.

10. Use according to claim 1, wherein the medicament is for the treatment of androgen-deprivation induced bone fractures in a male subject having prostate cancer.

11. Use according to claim 1, wherein the medicament is for the prevention of androgen-deprivation induced bone fractures in a male subject having prostate cancer.

12. Use according to claim 1, wherein the medicament is for the suppression or inhibition of androgen-deprivation induced bone fractures in a male subject having prostate cancer.
13. Use according to claim 1, wherein the medicament is for reducing the risk of developing androgen-deprivation induced bone fractures in a male subject having prostate cancer.

14. The use according to any of claims 1-13, wherein the medicament is a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

15. The use according to claim 14, wherein the pharmaceutical composition is in a liquid form for intravenous, intraarterial, or intramuscular injection to said subject.

16. The use according to claim 14, wherein the pharmaceutical composition is contained in a pellet for subcutaneous implantation in the subject.

17. The use according to claim 14, wherein the pharmaceutical composition is in a liquid or solid form for oral administration to the subject.

18. The use according to claim 14, wherein the pharmaceutical composition is for topical application to the skin surface of the subject.

19. The use according to claim 14, wherein the pharmaceutical composition is a pellet, a tablet, a capsule, a solution, a suspension, an emulsion, an elixir, a gel, a cream, a suppository or a parenteral formulation.

Patentansprüche

1. Verwendung von Toremifen zur Herstellung eines Medikaments zur Behandlung, Vorbeugung, Unterdrückung oder Hemmung, oder zur Verringerung des Risikos der Entwicklung, von:
   durch Androgenmangel ausgelöster Osteoporose,
   durch Androgenmangel ausgelösten Verlust an Knochenmineraldichte (bone material density, BMD); oder
   durch Androgenmangel ausgelösten Knochenfrakturen;
   in einem männlichen Subjekt mit Prostatakrebs.

2. Verwendung gemäß Anspruch 1, wobei das Medikament eines zur Behandlung von durch Androgenmangel ausgelöster Osteoporose in einem männlichen Subjekt mit Prostatakrebs ist.

3. Verwendung gemäß Anspruch 1, wobei das Medikament eines zur Vorbeugung von durch Androgenmangel ausgelöster Osteoporose in einem männlichen Subjekt mit Prostatakrebs ist.

4. Verwendung gemäß Anspruch 1, wobei das Medikament eines zur Unterdrückung oder Hemmung von durch Androgenmangel ausgelöster Osteoporose in einem männlichen Subjekt mit Prostatakrebs ist.

5. Verwendung gemäß Anspruch 1, wobei das Medikament eines zur Verringerung des Risikos der Entwicklung von durch Androgenmangel ausgelöster Osteoporose in einem männlichen Subjekt mit Prostatakrebs ist.

6. Verwendung gemäß Anspruch 1, wobei das Medikament eines zur Behandlung eines durch Androgenmangel ausgelösten Verlusts an Knochenmineraldichte (BMD) in einem männlichen Subjekt mit Prostatakrebs ist.

7. Verwendung gemäß Anspruch 1, wobei das Medikament eines zur Vorbeugung eines durch Androgenmangel ausgelösten Verlusts an Knochenmineraldichte (BMD) in einem männlichen Subjekt mit Prostatakrebs ist.

8. Verwendung gemäß Anspruch 1, wobei das Medikament eines zur Unterdrückung oder Hemmung eines durch Androgenmangel ausgelösten Verlusts an Knochenmineraldichte (BMD) in einem männlichen Subjekt mit Prostatakrebs ist.

9. Verwendung gemäß Anspruch 1, wobei das Medikament eines zur Verringerung des Risikos der Entwicklung eines durch Androgenmangel ausgelösten Verlusts an Knochenmineraldichte (BMD) in einem männlichen Subjekt mit Prostatakrebs ist.
Revendications

1. Utilisation de torémifène dans la fabrication d’un médicament pour le traitement, la prévention, la suppression ou l’inhibition de, ou pour réduire le risque d’un développement de:

- ostéoporose induite par une carence en androgène;
- une perte induite par une carence en androgène de la densité minérale osseuse (BMD); ou
- de fractures d’os induites par une carence en androgène;
- dans un sujet mâle ayant un cancer de la prostate.

2. Utilisation selon la revendication 1, où le médicament est destiné au traitement de l’ostéoporose induite par une carence en androgène dans un sujet mâle ayant un cancer de la prostate.

3. Utilisation selon la revendication 1, où le médicament est destiné à empêcher une ostéoporose induite par une carence en androgène dans un sujet mâle ayant un cancer de la prostate.

4. Utilisation selon la revendication 1, où le médicament est destiné à supprimer ou inhiber une ostéoporose induite par une carence en androgène dans un sujet mâle ayant un cancer de la prostate.

5. Utilisation selon la revendication 1, où le médicament est destiné à réduire le risque d’un développement d’une ostéoporose induite par une carence en androgène dans un sujet mâle ayant un cancer de la prostate.

6. Utilisation selon la revendication 1, où le médicament est destiné au traitement d’une perte induite par une carence en androgène de la densité minérale osseuse (BMD) dans un sujet mâle ayant un cancer de la prostate.

7. Utilisation selon la revendication 1, où le médicament est destiné à éviter une perte induite par une carence en androgène de la densité minérale osseuse (BMD) dans un sujet mâle ayant un cancer de la prostate.
8. Utilisation selon la revendication 1, où le médicament est destiné à supprimer ou inhiber une perte induite par une carence en androgène de la densité minérale osseuse (BMD) dans un sujet mâle ayant un cancer de la prostate.

9. Utilisation selon la revendication 1, où le médicament est destiné à réduire le risque d’un développement d’une perte induite par une carence en androgène de la densité minérale osseuse (BMD) dans un sujet mâle ayant un cancer de la prostate.

10. Utilisation selon la revendication 1, où le médicament est destiné au traitement de fractures d’os induites par une carence en androgène dans un sujet mâle ayant un cancer de la prostate.

11. Utilisation selon la revendication 1, où le médicament est destiné à éviter des fractures d’os induites par une carence en androgène dans un sujet mâle ayant un cancer de la prostate.

12. Utilisation selon la revendication 1, où le médicament est destiné à supprimer ou inhiber des fractures d’os induites par une carence en androgène dans un sujet mâle ayant un cancer de la prostate.

13. Utilisation selon la revendication 1, où le médicament est destiné à réduire le risque d’un développement de fractures d’os induite par une carence en androgène dans un sujet mâle ayant un cancer de la prostate.

14. Utilisation selon l’une des revendications 1 à 13, où le médicament est une composition pharmaceutique comprenant un support pharmaceutiquement acceptable.

15. Utilisation selon la revendication 14, où la composition pharmaceutique se présente sous forme liquide pour une injection intraveineuse, intra-artérielle ou intramusculaire dans le sujet.

16. Utilisation selon la revendication 14, où la composition pharmaceutique est renfermée dans une pastille en vue d’une implantation sous-cutanée dans le sujet.

17. Utilisation selon la revendication 14, où la composition pharmaceutique se présente sous forme liquide ou solide en vue d’une administration orale au sujet.

18. Utilisation selon la revendication 14, où la composition pharmaceutique est destinée à une application topique à la surface de la peau du sujet.

19. Utilisation selon la revendication 14, où la composition pharmaceutique est une pastille, un comprimé, une capsule, une solution, une suspension, une émulsion, un élixir, un gel, une crème, un suppositoire ou une formulation parentérale.
Figure 1. Effect of Toremifene on C-telopeptide of rat collagen I (RatLaps ELISA)

- Placebo
- Orx
- Toremifene
- Estradiol

- 15 days
- 30 days

# p<0.01 vs. P
Figure 2A. Effect of Toremifene on serum osteocalcin levels at 15 and 30 days

* p<0.05; # p<0.02
Figure 2B. Effect of Toremifene on serum osteocalcin levels at 60 and 120 days

*=p<0.01 vs. Orx
#=p<0.01 vs. P

60 days
120 days

ng/ml

P Orx Toremifene E
REFERENCES CITED IN THE DESCRIPTION

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