The invention regards the modulation of a proteolytic enzyme, Cathepsin L that results in increased bone production by bone-forming cells. Drugs that preferentially target this enzyme within bone forming cells and inhibit its activity represent a new class of anabolic, or bone building, therapies. Such drugs are useful in the treatment of diseases or disorders such as osteoporosis, Paget’s disease, metastatic bone cancer, myeloma bone disease, bone fractures, etc.
DESCRIPTION

USE OF CATHEPSIN L ANTAGONISTS IN THE TREATMENT OF BONE DISEASE

BACKGROUND OF THE INVENTION

The present application claims benefit of priority to U.S. Provisional Application Serial No. 61/012,954 filed December 12, 2007, and U.S. Provisional Application Serial No. 61/025,939 filed February 4, 2008, the entire contents of both are hereby incorporated by reference.

I. Field of the Invention

The present invention relates to the fields of molecular biology and medicine. More particularly, it relates to the fields of bone disease & injury, bone repair, bone implants, bone grafts, periodontal disease and cancer. Specifically, it deals with the use of Cathepsin L antagonists to promote bone formation and to treat bone diseases such as osteoporosis, as well as bone trauma and cancers with bone involvement, including multiple myeloma and myeloma bone disease.

II. Related Art

Over 200 million people worldwide suffer from bone disorders such as osteoporosis, bone fractures, and periodontal (gum) disease (where the teeth loose surrounding bone). Osteoporosis represents a large and rapidly growing health care problem with an unmet medical need for therapies that stimulate bone formation. Most current drugs for osteoporosis retard bone degradation but do not stimulate bone formation to replace already lost bone. Compounds that stimulate bone formation thus represent an unmet need in the area of bone disease. Osteoporosis is known to affect approximately 100 million people worldwide - 35 million of whom live in the U.S., Western Europe and Japan. Moreover, over 25 million individuals suffer bone fractures yearly, 60 million have periodontal disease (in which the tooth loosens from the jaw bone), and another 18 million have other bone disorders such as bone cancer.

Most current therapies for osteoporosis patients focus on prevention of bone loss, not bone formation. This remains an important consideration as significant
morbidity and mortality are associated with prolonged bed rest in the elderly that occurs post bone fracture, particularly those who have suffered hip fractures. Complications of bed rest include blood clots and pneumonia. These complications are recognized and measures are usually taken to avoid them, but these are hardly the best approach to therapy.  

Yet another bone-related health issues is bone reconstruction and, specifically, the ability to reconstruct defects in bone tissue that result from traumatic injury, as a consequence of cancer or cancer surgery, as a result of a birth defect, or as a result of aging. There is a significant need for more frequent orthopedic implants, and cranial and facial bone are particular targets for this type of reconstructive need. The availability of new implant materials, e.g., titanium, has permitted the repair of relatively large defects. Titanium implants provide excellent temporary stability across bony defects. However, experience has shown that a lack of viable bone bridging the defect can result in exposure of the appliance, infection, structural instability and, ultimately, failure to repair the defect.  

Autologous bone grafts are another possibility to deal with bone injury, but they have several demonstrated disadvantages in that they must be harvested from a donor site such as iliac crest or rib, they usually provide insufficient bone to completely fill the defect, and the bone that does form is sometimes prone to infection and resorption. Partially purified xenogeneic preparations are not practical for clinical use because microgram quantities are purified from kilograms of bovine bone, making large scale commercial production both costly and impractical. Allografts and demineralized bone preparations are therefore often employed. Microsurgical transfers of free bone grafts with attached soft tissue and blood vessels can close bony defects and allow an immediate source of blood supply to the graft. However, these techniques are time consuming, have been shown to produce a great deal of morbidity, and can only be used by specially trained individuals.  

Another form of bone disease is that resulting from cancer. A number of cancers metastasize to bone and can result in bone weakening, and some are even associated with bone destruction and bone loss, such as breast, lung, thyroid, kidney and prostate cancer. In addition, Multiple Myeloma and its associated myeloma bone disease (MBD) is not a metastatic cancer. Rather, myeloma cells are derived from the B-cells of the immune system that normally reside in the bone marrow and are therefore intimately associated with bone. Indeed, the bone marrow
microenvironment plays an important role in the growth, survival and resistance to chemotherapy of the myeloma cells, which, in turn, regulate the increased bone loss associated with this disorder (world-wide-web at multiplemyeloma.org). Over 90% of myeloma patients have bone involvement, versus 40-60% of cancer patients who have bone metastasis, and over 80% of these MBD patients have intractable bone pain. Additionally, approximately 30% of myeloma patients have hypercalcemia that is a result of the increased osteolytic activity associated with this disease (Cavo et al., 2006).

Unlike the osteolysis associated with other bone tumors, the MBD lesions are unique in that they do not heal or repair, despite the patients' having many years of complete remission (world-wide-web at multiplemyeloma.org; Terpos et al, 2005). Mechanistically, this seems to be related to the inhibition and/or loss of the bone-forming osteoblast during disease progression. Indeed, bone marker studies and histomorphometry indicate that both the bone-resorbing osteoclast and osteoblast activity are increased, but balanced early in the disease, whereas overt MBD shows high osteoclast activity and low osteoblast activity (world-wide-web at multiplemyeloma.org). Thus, MBD is a disorder in which bone formation and bone loss are uncoupled and would benefit from therapies that both stimulate bone formation and retard its loss. To date, no such therapies exist.

Therefore, there continues to be a need for improved methods of stimulating bone formation in vivo to treat bone disease and injury, including cancer, and for producing bone ex vivo in the production and use of implants.
SUMMARY OF THE INVENTION

Thus, in accordance with the present invention, there is provided a method of promoting bone formation comprising (i) selecting a first Cathepsin L inhibitor; and (ii) administering to a subject the first inhibitor of Cathepsin L. The inhibitor may be an intracellular or extracellular inhibitor of Cathepsin L, or both. It may be biological or an organopharmaceutical small molecule. The biological may be a peptide, an siRNA, an antisense molecule or a single-chain antibody. The organopharmaceutical small molecule may be a structural variant of a known Cathepsin L inhibitor based on hydrophilic and lipophilic characteristics, spatial properties, or other information available from medicinal chemistry and chemical enzymology studies on these compounds. The first inhibitor may also prevent bone resorption. The subject may suffer from a bone-related pathology or injury, such as osteoporosis, vitamin D deficiency, primary bone cancer, metastatic bone cancer, multiple myeloma or myeloma bone disease, bone fracture, periodontal disease, Paget’s disease, osteoporosis secondary to other diseases, or in need of bone grafts or implants. The subject is a human or a non-human animal. The compound may possess or lack bone resorption inhibition.

The method may further comprise contacting the subject with a second agent. The second agent may be a compound that inhibits bone loss such, as bisphosphonate, a SERM or a second inhibitor of Cathepsin L that is distinct from the first inhibitor, and may be distinct in its mode (extracellular versus intracellular) of action. The second agent may be an inhibitor of Cathepsin K. The second agent may be a promoter of bone formation such as PTH or an analogue thereof. The first and/or second agent may be formulated to increase transmembrane delivery. The first and/or second agent may be formulated with a cell permeability factor. The first and/or second agent may be formulated in a lipid delivery vehicle. The first and/or second agent may be formulated to enhance its bioavailability. The first and/or second agent may be administered to the subject more than once. The first and/or second agent is administered to the subject by oral or intravenous routes.

In another embodiment, there is provided a method of producing bone from an isolated bone cell comprising (i) selecting a Cathepsin L inhibitor; (ii) contacting the bone cell with the inhibitor of Cathepsin L; and (iii) culturing the cell under conditions promoting bone formation. The method may further comprise implanting
the bone cell or bone derived therefrom into a subject. The bone cell may have been obtained, prior to step (ii) from the subject or an allogeneic donor. The obtained cell may have been derived from a stem cell prior to step (ii). The stem cell may have been derived or cultured under conditions promoting the formation of an osteogenic cell prior to step (ii). The compound may possess or lack bone resorption inhibition.

In still another embodiment, there is provided a method of identifying an agent that promotes bone formation comprising (i) providing a candidate compound; (ii) admixing the candidate compound with an isolated Cathepsin L enzyme, or a cell expressing Cathepsin L, or an experimental animal, cells of which express Cathepsin L; (iii) measuring Cathepsin L activity or expression or bone formation; and (iv) comparing the characteristic measured in step (iii) with that observed in the absence of the candidate modulator, wherein a difference between the measured characteristic indicates that the candidate compound is, indeed, a modulator of Cathepsin L. The compound may possess or lack bone resorption inhibition.

Further, there is provided a method of treating a subject with multiple myeloma comprising (i) selecting a first Cathepsin L inhibitor; and (ii) administering to a subject the first inhibitor of Cathepsin L. The inhibitor may be a biological or an organopharmaceutical small molecule. The biological may be a peptide, an siRNA, an antisense molecule or a single-chain antibody. The organopharmaceutical small molecule may be a structural variant of a known Cathepsin L inhibitor based on hydrophilic and lipophilic characteristics, spatial properties, or other information available from medicinal chemistry and chemical enzymology studies on these compounds. The first inhibitor may also prevent bone resorption or stimulate bone formation.

The method may further comprise contacting the subject with a second agent. The second agent may be a compound that inhibits bone loss such, as bisphosphonate, a SERM or a second inhibitor of Cathepsin L that is distinct from the first inhibitor. The second inhibitor of Cathepsin L may be distinct in its mode (extracellular versus intracellular) action as the first inhibitor of Cathepsin L. The second agent may be an inhibitor of Cathepsin K. The second agent may be a promoter of bone formation such as PTH or an analogue thereof, or an inhibitor of Cathepsin K such as VEL-0230 or an analogue thereof. The second agent may also be an anti-cancer agent, such as a chemotherapy, a radiotherapy, an immunotherapy, a cytokine therapy, a toxin therapy
or a gene therapy. The multiple myeloma may be recurrent, metastatic or drug resistant.

The first and/or second agent may be formulated to increase transmembrane delivery. The first and/or second agent may be formulated with a cell permeability factor. The first and/or second agent may be formulated in a lipid delivery vehicle. The first and/or second agent may be formulated to enhance its bioavailability. The first and/or second agent may be administered to the subject more than once. The first and/or second agent may be administered to the subject by oral or intravenous routes, injection into the tumor or tumor vasculature, or administration local or regional to a tumor.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions and kits of the invention can be used to achieve methods of the invention.

Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

**FIG 1 - Inhibition of Cathepsin L Stimulates Bone Mineralization In Vitro.** Cultures of human bone osteoblasts stimulated with Bone Morphogenetic
Protein (BMP)-2, a positive control, a Cathepsin L inhibitor (A) or unstimulated (control) and stained with Alizarin (red).

FIG. 2 - Quantitative Analysis of Cathepsin L Inhibitor Stimulation of Human Bone Cell Mineralization.

FIG. 3 - Exemplary Cathepsin L Inhibitors. Cathepsin Inhibitor 4 = C_{27}H_{29}N_{2}O_{4}S; Cathepsin Inhibitor 6 = C_{19}H_{48}N_{7}O_{4}F.
DETAILED DESCRIPTION OF THE INVENTION

The inventor previously discovered a unique mechanism of action for Cathepsin K, a proteolytic enzyme previously thought to be involved only in bone degradation. Its role in modulating bone resorption is that of a classic proteolytic enzyme, functioning to degrade components of the extra-cellular compartment, particularly collagen (Bossard et al., 1996). Moreover, the prevalent viewpoint is that this enzyme functions solely and/or selectively in osteoclasts to degrade extracellular proteins and, as such, is targeted by anti-catabolic therapies that function to inhibit the bone-resorbing osteoclast (Yasuda et al., 2005; Grabowskal et al., 2005; Troen, 2004; Dodds, 2003; Zaidi et al., 2003). Recently, Cathepsin K was shown to be expressed by osteoblasts; however, this report still subscribed to the concept that Cathepsin K has an extracellular role and, further, did not test enzymatic function of this enzyme within osteoblasts (Mandelin et al., 2006).

An important feature of this previous invention is that within bone-forming cells Cathepsin K plays a role in bone formation as inhibition of the enzyme unexpectedly results in an increase in bone formation, as described below. The role of Cathepsin K in bone formation intracellular signaling networks is supported by three observations: (1) the inventor's Cathepsin K inhibitors stimulate bone formation both in vivo and in vitro; (2) RNA interference studies of Cathepsin K (RNAi; performed via small-interfering RNA (siRNA) treatment) demonstrate that inhibition of this message results in a bone-formation phenotype in treated human osteoblasts; and (3) stimulation of bone formation with multiple osteogenic growth factors decreases Cathepsin K message and activity within the osteoblast.

Numerous patents exist on the structure of Cathepsin K inhibitors and their use in treating the extracellular activity of the enzyme. The inventors’ prior work, by way of contrast, proposes the use of intracellular inhibition of Cathepsin K to various forms of bone disease and to treat multiple myeloma, including myeloma bone disease. Here, they extend their work on cathepsins to Cathepsin L, and show that similar benefits may accrue in the areas of promoting bone growth, inhibition bone resorption, promoting bone repair and grafting, and treating multiple myeloma and myeloma bone disease through the administration of Cathepsin L preferential or selective inhibitors.
I. Cathepsin L

Cathepsins are proteases that function in the normal physiological as well as pathological degradation of connective tissue. Cathepsins play a major role in intracellular protein degradation and turnover, bone remodeling, and prohormone activation (Marx, 1987). Cathepsin B, H, L and S are ubiquitously expressed lysosomal cysteine proteinases that belong to the papain superfamily. They are found at constitutive levels in many tissues in the human including kidney, liver, lung and spleen. Some pathological roles of cathepsins include an involvement in glomerulonephritis, arthritis, and cancer metastasis (Sloan and Honn, 1984). Greatly elevated levels of cathepsin L and B mRNA and protein are seen in tumor cells. Cathepsin L mRNA is also induced in fibroblasts treated with tumor promoting agents and growth factors (Kane and Gottesman, 1990).

Cathepsins, a cysteine protease family, also play an important role in bone turnover. Cathepsin K, a member of this protease family, is considered the primary protease responsible for the degradation of the bone matrix (Jones, 2003). Cathepsin K was discovered in 1994, is synthesized as a 37 kDa pre-pro enzyme, and is presumably autoactivated to the mature 27 kDa enzyme at low pH (McQueney et al., 1997; Littlewood-Evans et al., 1997; U.S. Patent 5,861,298). It is abundantly and relatively selectively expressed in osteoclasts, where it is localized in lysosomes in the ruffled border and in resorption lacunae on the bone surface. This cellular and extracellular localization suggests an important role in bone resorption, as does its recently characterized functional activity profile. The unique ability of Cathepsin K to degrade type I collagen both within and outside the helical regions and also type II collagens at the N-terminal of the triple helix, to act at an acidic and neutral pH, shows that Cathepsin K plays an important role in bone and cartilage break down.

The cysteine protease, Cathepsin L, is produced and secreted by osteoclasts where its primary activity is extracellular (Goto, 1993; Hill, 1994; Kakegawa, 1995). However, the degree expression is variable (Goto, 1993; Hill, 1994; Kakegawa, 1993; Drake, 1996). Cathepsin L appears to function in the degradation of the bone matrix by augmenting that of Cathepsin K during inflammation or other cytokine-mediated events. Thus, collagen degradation by osteoclasts stimulated with inflammatory cytokines is inhibited in response to Cathepsin L inhibition, whereas untreated cells are not (Furuyama, 2000) and may mediate acute versus chronic bone effects (Millest, 1997). Cathepsin L activity is also seen in primary bone cancers and metastatic bone
disease (Park, 1996). It also functions to a degree to maintain bone volume in vivo (Potts, 2004).

II. Bone Diseases

A. Diseases and Conditions Requiring Bone Repair

The following is a brief discussion of human conditions that exemplify the variety of diseases and disorders that would benefit from the use of Cathepsin L antagonists or combinations of Cathepsin L antagonists and second agents as described above or cells treated therewith in promoting bone formation and/or bone repair. In addition to the following, several other conditions, such as, for example, vitamin D deficiency, exists.

Fracture. The first example is the otherwise healthy individual who suffers a fracture. Often, clinical bone fracture is treated by casting to alleviate pain and allow natural repair mechanisms to repair the wound. There has been progress in the treatment of fracture in recent times, however, even without considering the various complications that may arise in treating fractured bones, any new procedures to increase bone healing in normal circumstances would represent a great advance.

Periodontal Disease. Progressive periodontal disease leads to tooth loss through destruction of the tooth's attachment to the surrounding bone. Approximately 5 - 20% of the U.S. population (15-60 million individuals) suffers from severe generalized periodontal disease, and there are 2 million related surgical procedures. Moreover, if the disease is defined as the identification of at least one site of clinical attachment loss, then approximately 80% of all adults are affected, and 90% of those aged 55 to 64 years. If untreated, approximately 88% of affected individuals show moderate to rapid progression of the disease which shows a strong correlation with age. The major current treatment for periodontal disease is regenerative therapy consisting of replacement of lost periodontal tissues. The lost bone is usually treated with an individual's own bone and bone marrow, due to their high osteogenic potential. Bone allografts (between individuals) can also be performed using stored human bone. Although current periodontal cost analyses are hard to obtain, the size of the affected population and the current use of bone grafts as a first-order therapy strongly suggest that this area represents an attractive target for bone-building therapies.
Osteopenia/osteoporosis. The terms osteopenia and osteoporosis refer to a heterogeneous group of disorders characterized by decreased bone mass and fractures. Osteopenia is a bone mass that is one or more standard deviations below the mean bone mass for a population; osteoporosis is defined as 2.5 SD or lower. An estimated 20-25 million people are at increased risk for fracture because of site-specific bone loss. Risk factors for osteoporosis include increasing age, gender (more females), low bone mass, early menopause, race (Caucasians in general; Asian and Hispanic females), low calcium intake, reduced physical activity, genetic factors, environmental factors (including cigarette smoking and abuse of alcohol or caffeine), and deficiencies in neuromuscular control that create a propensity to fall.

More than a million fractures in the U.S. each year can be attributed to osteoporosis. In economic terms, the costs (exclusive of lost wages) for osteoporosis therapies are $35 billion worldwide. Demographic trends (i.e., the gradually increasing age of the U.S. population) suggest that these costs may increase to $62 billion by the year 2020. Clearly, osteoporosis is a significant health care problem.

Osteoporosis, once thought to be a natural part of aging among women, is no longer considered age or gender-dependent. Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. Bone strength reflects the integration of two main features: bone density and bone quality. Bone density is expressed as grams of mineral per area or volume and in any given individual is determined by peak bone mass and amount of bone loss. Bone quality refers to architecture, turnover, damage accumulation (e.g., microfractures) and mineralization. A fracture occurs when a failure-inducing force (e.g., trauma) is applied to osteoporotic bone.

Current therapies for osteoporosis patients focus on fracture prevention, not for promoting bone formation or fracture repair. This remains an important consideration because of the literature, which clearly states that significant morbidity and mortality are associated with prolonged bed rest in the elderly, particularly those who have suffered hip fractures. Complications of bed rest include blood clots and pneumonia. These complications are recognized and measures are usually taken to avoid them, but these is hardly the best approach to therapy. Thus, the osteoporotic patient population would benefit from new therapies designed to strengthen bone and speed up the fracture repair process, thus getting these people on their feet before the complications arise.
**Bone Reconstruction/Grafting.** A fourth example is related to bone reconstruction and, specifically, the ability to reconstruct defects in bone tissue that result from traumatic injury; as a consequence of cancer or cancer surgery; as a result of a birth defect; or as a result of aging. There is a significant need for more frequent orthopedic implants, and cranial and facial bone are particular targets for this type of reconstructive need. The availability of new implant materials, *e.g.*, titanium, has permitted the repair of relatively large defects. Titanium implants provide excellent temporary stability across bony defects and are an excellent material for bone implants or artificial joints such as hip, knee and joint replacements. However, experience has shown that a lack of viable bone binding to implants the defect can result in exposure of the appliance to infection, structural instability and, ultimately, failure to repair the defect. Thus, a therapeutic agent that stimulates bone formation on or around the implant will facilitate more rapid recovery.

Autologous bone grafts are another possibility, but they have several demonstrated disadvantages in that they must be harvested from a donor site such as iliac crest or rib, they usually provide insufficient bone to completely fill the defect, and the bone that does form is sometimes prone to infection and resorption. Partially purified xenogeneic preparations are not practical for clinical use because microgram quantities are purified from kilograms of bovine bone, making large scale commercial production both costly and impractical. Allografts and demineralized bone preparations are therefore often employed, but suffer from their devitalized nature in that they only function as scaffolds for endogenous bone cell growth.

Microsurgical transfers of free bone grafts with attached soft tissue and blood vessels can close bony defects with an immediate source of blood supply to the graft. However, these techniques are time consuming, have been shown to produce a great deal of morbidity, and can only be used by specially trained individuals. Furthermore, the bone implant is often limited in quantity and is not readily contoured. In the mandible, for example, the majority of patients cannot wear dental appliances using presently accepted techniques (even after continuity is established), and thus gain little improvement in the ability to masticate.

In connection with bone reconstruction, specific problem areas for improvement are those concerned with treating large defects, such as created by trauma, birth defects, or particularly, following tumor resection; and also the area of artificial joints. The success of orthopaedic implants, interfaces and artificial joints
could conceivably be improved if the surface of the implant, or a functional part of an implant, were to be coated with a bone stimulatory agent. The surface of implants could be coated with one or more appropriate materials in order to promote a more effective interaction with the biological site surrounding the implant and, ideally, to promote tissue repair.

Primary Bone Cancer and Metastatic bone Disease. Bone cancer occurs infrequently while bone metastases are present in a wide range of cancers, including thyroid, kidney, and lung. Metastatic bone cancer is a chronic condition; survival from the time of diagnosis is variable depending on tumor type. In prostate and breast cancer and in multiple myeloma, survival time is measurable in years. For advanced lung cancer, it is measured in months. Cancer symptoms include pain, hypercalcemia, pathologic fracture, and spinal cord or nerve compression. Prognosis of metastatic bone cancer is influenced by primary tumor site, presence of extra-osseous disease, and the extent and tempo of the bone disease. Bone cancer/metastasis progression is determined by imaging tests and measurement of bone specific markers. Recent investigations show a strong correlation between the rate of bone resorption and clinical outcome, both in terms of disease progression or death.

Multiple Myeloma. Multiple myeloma (MM) is a B-lymphocyte malignancy characterized by the accumulation of malignant clonal plasma cells in the bone marrow. The clinical manifestations of the disease are due to the replacement of normal bone marrow components by abnormal plasma cells, with subsequent overproduction of a monoclonal immunoglobulin (M protein or M component), bone destruction, bone pain, anemia, hypercalcemia and renal dysfunction.

As distinct from other cancers that spread to the bone (e.g., breast, lung, thyroid, kidney, prostate), myeloma bone disease (MBD) is not a metastatic disease. Rather, myeloma cells are derived from the B-cells of the immune system that normally reside in the bone marrow and are therefore intimately associated with bone. Indeed, the bone marrow microenvironment plays an important role in the growth, survival and resistance to chemotherapy of the myeloma cells, which, in turn, regulate the increased bone loss associated with this disorder (world-wide-web at multiplemyeloma.org). Over 90% of myeloma patients have bone involvement, versus 40-60% of cancer patients who have bone metastasis, and over 80% have intractable bone pain. Additionally, approximately 30% of myeloma patients have
hypercalcemia that is a result of the increased osteolytic activity associated with this disease (Cavo et al, 2006).

Common problems in myeloma are weakness, confusion and fatigue due to hypercalcemia. Headache, visual changes and retinopathy may be the result of hyperviscosity of the blood depending on the properties of the paraprotein. Finally, there may be radicular pain, loss of bowel or bladder control (due to involvement of spinal cord leading to cord compression) or carpal tunnel syndrome and other neuropathies (due to infiltration of peripheral nerves by amyloid). It may give rise to paraplegia in late presenting cases.

Myeloma Bone Disease. As discussed above, unlike the osteolysis associated with other bone tumors, the MBD lesions are unique in that they do not heal or repair, despite the patients' having many years of complete remission. Mechanistically, this seems to be related to the inhibition and/or loss of the bone-forming osteoblast during disease progression. Indeed, bone marker studies and histomorphometry indicate that both the bone-resorbing osteoclast and osteoblast activity are increased, but balanced early in the disease, whereas overt MBD shows high osteoclast activity and low osteoblast activity. Thus, MBD is a disorder in which bone formation and bone loss are uncoupled and would benefit from therapies that both stimulate bone formation and retard its loss.

A number of therapeutic approaches have been used in MBD, with the endpoints of treating pain, hypercalcemia, or the reduction of skeletal related events (SRE). Many of these may present serious complications. Surgery, such as vertebroplasty or kyphoplasty, that is performed for stability and pain relief has the attendant surgical risks (e.g., infection) made worse by a compromised immune system and does not reverse existing skeletal defects. Radiation therapy and radioisotope therapy are both used to prevent/control disease progression and have the typical risks of irradiation therapies. More recently, drugs such as the bisphosphonates that inhibit osteoclast activity have become a standard of therapy for MBD, despite the fact that they work poorly in this disorder. In 9 major double-blind, placebo-controlled trials on bisphosphonates, only 66% of patients showed an effective reduction in pain; 56% showed a reduction in SRE and only 1 of the 9 demonstrated a survival benefit.
III. Treatment of Bone Diseases

A. Antagonists

A Cathepsin L antagonist of the present invention is one that is capable of inhibiting either or both of the intracellular and extracellular function of Cathepsin L and also promoting the formation of bone \textit{in vitro} and/or \textit{in vivo} by the inhibition of intracellular Cathepsin L. In addition, as compared to other Cathepsins, the inhibitor of Cathepsin L will have preferential inhibitor activity against Cathepsin L of at least 3-fold over that of other cathepsins in enzymatic assays, in particular over Cathepsin K.

The only known activity of Cathepsin L antagonists is to modulate the extracellular activity of Cathepsin L in certain physiological states of osteoclasts. An agent of the present invention is one that is capable of inhibiting the function of Cathepsin L and in so doing promoting the formation of bone \textit{in vitro} and/or \textit{in vivo}. The agent may also inhibit bone resorption. The effect of these molecular and cellular actions will be to treat bone disease and injury and to improve the function in the clinical situation (reduction in pain, increase in mobility, \textit{etc.}) of a subject suffering from bone-related pathologies, such as osteoporosis, bone cancer and metastasis, multiple myeloma, malignant hypercalcemia, Paget's Disease, and arthritis.


B. Bone Disease

In one aspect, the present invention provides for the treatment of bone disease and bone trauma by stimulating the production of new bone tissue, both \textit{in vivo} and \textit{ex vivo} for transplant purposes. An agent of the present invention is one that is capable of inhibiting the function of Cathepsin L and also promoting the formation of bone \textit{in vitro} and/or \textit{in vivo} by the inhibition of intracellular Cathepsin L within the osteoblast. The agent may also inhibit bone resorption. The effect of these molecular and cellular actions will be to treat bone disease and injury and to improve the patient's ability to
function (reduction in pain, increase in mobility, etc.) of a subject suffering from bone-related pathologies.

Other agents may be used in combination with intracellular Cathepsin L inhibitors. More generally, these agents would be provided in a combined amount (along with the intracellular Cathepsin L inhibitor) to produce any of the effects discussed above. This process may involve contacting the cell or subject with both agents at the same time. This may be achieved by contacting the cell with a single composition or pharmacological formulation that includes both agents, or by contacting the cell or subject with two distinct compositions or formulations, at the same time, wherein one composition includes the intracellular inhibitor and the other includes the second agent.

Alternatively, one agent may precede or follow the other by intervals ranging from minutes to weeks. In embodiments where the agents are applied separately to the cell or subject, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agents would still be able to exert an advantageously combined effect on the cell or subject. In such instances, it is contemplated that one may contact the cell or subject with both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Various combinations may be employed, the intracellular inhibitor of Cathepsin L is "A" and the other agent is "B":

\[
\begin{align*}
A/B/A & \quad B/A/B & \quad B/B/A & \quad PJPJB & \quad PJBIB & \quad BIPJK & \quad PJBIB & \quad BIPJBIK \\
BIBIBIK & \quad BIBIPJB & \quad PJPJIBB & \quad PJBJIPJ & \quad PJBIK & \quad BIBIPJK \\
PJPJIBK & \quad BIPJPJB & \quad PJPJPJB & \quad BIPJPJK & \quad PJBPJK & \quad PJJPBIK
\end{align*}
\]

Administration protocols and formulation of such agents will generally follow those of standard pharmaceutical drugs, as discussed further below. Combination agents include bisphosphonates (Didronel™, Fosamax™ and Actonel™), SERMs (Evista) or other hormone derivatives, and Parathyroid Hormone (PTH) analogs. In addition, other combination agents may be Cathepsin K inhibitors. U.S. Patents 6,642,239, 6,531,612, 6,462,076 and 6,274,336, as well as U.S. Publication Nos. 2006/0074092,

C. **Osteoporosis, Bone Trauma, Bone Cancer, Bone Metastasis and Myeloma Bone Disease (herein: "Bone Disease")**

In another one aspect, treatment of Bone Disease by compounds of the present invention addresses bone loss by stimulating the production of new bone tissue. However, data also show that Cathepsin L inhibitors inhibit the function of osteoclasts without killing them. This is distinct from the effects of bisphosphonates that stimulate apoptosis, or from those resulting from Densoamab™, an antibody against RANK ligand that inhibits osteoclast formation. Both of those agents reduce osteoclast numbers, which can also have adverse effects.

Thus, agents of the present invention will stimulate bone formation to restore lost bone in multiple myeloma, and inhibit osteoclasts in Bone Disease via their anabolic action. The agent may also inhibit bone resorption and in so doing may inhibit further Bone Disease progression. The compounds of the present invention do not reduce osteoclast number, so these important cells can still contribute to bone homeostasis by signaling osteoblasts. Additionally, compounds of the present invention prevent osteoclasts from forming "bone-resorption pits" where the myeloma and/or cancer cells locate themselves, thus reducing the area in which tumor cells can live. Therefore, the physical presence of the non-resorbing osteoclasts, plus the lack of osteoclast-pits, both impeded myeloma cell growth as well as promoting bone growth and/or reducing bone loss since myeloma cells both inhibit the osteoblast and stimulate the osteoclasts. As such, disease progression (defined as myeloma growth, metastasis, bone disease, bone loss, and bone resportion) may slow, stop, and even be reversed. U.S. Patent Publn. US2007/069211, incorporated herein by reference, describes the use of Cathepsin K inhibitors of the present invention in treating various bone disease.

Other agents may be used in combination with Cathepsin L inhibitors to effect greater inhibition of Cathepsin L, and thus provide a more effective therapy for Bone Diseases. More generally, these agents would be provided in a combined amount (along with other agents) to produce or increase any of the effects discussed herein. This process may involve contacting the cell or subject with both agents at the same time. This may be achieved by contacting the cell or subject with a single
composition or pharmacological formulation that includes both agents, or by contacting the cell or subject with two distinct compositions or formulations, at the same time, wherein one composition includes the intracellular Cathepsin L inhibitor and the other includes the second agent.

Alternatively, one agent may precede or follow the other by intervals ranging from minutes to weeks. In embodiments where the agents are applied separately to the cell or subject, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agents would still be able to exert an advantageously combined effect on the cell or subject. In such instances, it is contemplated that one may contact the cell with both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Another type of combination therapy involves the use of Cathepsin L inhibitors with tradition cancer therapies, including chemotherapy, radiotherapy, immunotherapy, hormone therapy, cytokine therapy, gene therapy, or toxin therapy.

Various combinations may be employed, the intracellular inhibitor of Cathepsin L is "A" and the other agent is "B":

\[
\begin{align*}
A/B/A & \quad B/A/B & \quad B/B/A & \quad A/A/B & \quad A/B/B & \quad B/A/B/B \\
B/A/B/B & \quad B/B/B/A & \quad B/B/A/B & \quad A/A/B/B & \quad A/B/A/B & \quad A/B/B/A \\
B/B/A/A & \quad B/A/B/A & \quad B/A/A/B & \quad A/A/A/B & \quad B/A/A/A & \quad A/B/A/A \\
A/A/B/A & \\
\end{align*}
\]

Administration protocols and formulation of such agents will generally follow those of standard pharmaceutical drugs, as discussed further below. Combination agents include bisphosphonates (Didronel™, Fosamax™ and Actonel™), SERMs (Evista) or other hormone derivatives, and Parathyroid Hormone (PTH) analogs. In addition, U.S. Patents 6,642,239, 6,531,612, 6,462,076 and 6,274,336, as well as U.S. Publication Nos. 2006/0074092, 2006/0020001, 2005/0245596, 2005/0107616, 2005/0054819, and 2004/0249153, disclose other Cathepsin K inhibitors that work in
an extracellular fashion. Other agents include, thalidomide, cyclophosphamide, VAD
(vincristine, doxorubicin, and dexamethasone), low-dose therapy with melphalan
combined with prednisone, plasmapheresis, dialysis, high-dose chemotherapy with
melphalan combined with bone marrow transplant, thalidomide combined with
reduced-intensity chemotherapy (melphalan and prednisone), cyclophosphamide,
5 dexamethasone, bortezomib (or Velcade®) and lenalidomide (or Revlimid®).

IV. Pharmaceutical Formulations and Delivery

A. Compositions and Routes

Pharmaceutical compositions of the present invention comprise an effective
amount of one or more Cathepsin L antagonists dissolved or dispersed in a
10 pharmaceutically acceptable carrier. The phrases "pharmaceutical or
pharmacologically acceptable" refer to molecular entities and compositions that do
not produce an adverse, allergic or other untoward reaction when administered to an
animal, such as, for example, a human, as appropriate. The preparation of a
pharmaceutical composition that contains at least one Cathepsin L antagonist, and
optionally an additional active ingredient, will be known to those of skill in the art in
light of the present disclosure, as exemplified by Remington's Pharmaceutical
Moreover, for animal (e.g., human) administration, it will be understood that
preparations should meet sterility, pyrogenicity, general safety and purity standards as
required by FDA Office of Biological Standards.

As used herein, "pharmaceutically acceptable carrier" includes any and all
solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g.,
antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents,
salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration
agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and
combinations thereof, as would be known to one of ordinary skill in the art (see, for
example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company,
1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any
conventional carrier is incompatible with the active ingredient, its use in the
pharmaceutical compositions is contemplated.

The Cathepsin L antagonist may be admixed with different types of carriers
depending on whether it is to be administered orally or by injection. The present
invention can be administered buccally, intravenously, intradermally, transdermally, intrathecally, intraarterially, intraperitoneally, intranasally, intravaginally, intrarectally, topically, intramuscularly, intratumorally, into tumor vasculature, subcutaneously, mucosally, orally, topically, locally, inhalation (e.g., aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (e.g., nanoparticles, liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference). In particular, the Cathepsin L antagonist is formulated into a syringeable composition for use in intravenous administration.

The Cathepsin L antagonist may be formulated into a composition in a free base, neutral or salt form or ester. It may also be synthesized/formulated in a prodrug form. Pharmaceutically acceptable salts, include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, fumaric, or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

Further in accordance with the present invention, the composition of the present invention suitable for administration is provided in a pharmaceutically acceptable carrier with or without an inert diluent. The carrier should be assimilable and includes liquid, semi-solid, i.e., pastes, or solid carriers. Except insofar as any conventional media, agent, diluent or carrier is detrimental to the recipient or to the therapeutic effectiveness of the composition contained therein, its use in administrable composition for use in practicing the methods of the present invention is appropriate. Examples of carriers or diluents include fats, oils, water, saline solutions, lipids, liposomes, resins, binders, fillers and the like, or combinations thereof. The composition may also comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents,
including but not limited to parabens (e.g., methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

In a specific embodiment of the present invention, the composition is combined or mixed thoroughly with a semi-solid or solid carrier. The mixing can be carried out in any convenient manner such as grinding. Stabilizing agents can be also added in the mixing process in order to protect the composition from loss of therapeutic activity, i.e., denaturation in the stomach. Examples of stabilizers for use in the composition include buffers, amino acids such as glycine and lysine, carbohydrates such as dextrose, mannose, galactose, fructose, lactose, sucrose, maltose, sorbitol, mannitol, etc.

In further embodiments, the present invention may concern the use of a pharmaceutical lipid vehicle compositions that include Cathepsin L antagonist, one or more lipids, and an aqueous solvent. As used herein, the term "lipid" will be defined to include any of a broad range of substances that are characteristically insoluble in water and extractable with an organic solvent. This broad class of compounds are well known to those of skill in the art, and as the term "lipid" is used herein, it is not limited to any particular structure. Examples include compounds which contain long-chain aliphatic hydrocarbons and their derivatives. A lipid may be naturally-occurring or synthetic (i.e., designed or produced by man). Lipids are well known in the art, and include for example, neutral fats, phospholipids, phosphoglycerides, steroids, terpenes, lysolipids, glycosphingolipids, glycolipids, sulphatides, lipids with ether and ester-linked fatty acids and polymerizable lipids, and combinations thereof.

One of ordinary skill in the art would be familiar with the range of techniques that can be employed for dispersing a composition in a lipid vehicle. For example, the Cathepsin L antagonist may be dispersed in a solution containing a lipid, dissolved with a lipid, emulsified with a lipid, mixed with a lipid, combined with a lipid, covalently bonded to a lipid, contained as a suspension in a lipid, contained or complexed with a micelle or liposome, or otherwise associated with a lipid or lipid structure by any means known to those of ordinary skill in the art. The dispersion may or may not result in the formation of liposomes.

The actual dosage amount of a composition of the present invention administered to an animal patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the
route of administration. Depending upon the dosage and the route of administration, the number of administrations of a preferred dosage and/or an effective amount may vary according to the response of the subject. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

In certain embodiments, Cathepsin L antagonist pharmaceutical compositions may comprise, for example, at least about 0.1% of the antagonist, about 0.5% of the antagonist, or about 1.0% of the antagonist. In other embodiments, the antagonist may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. Naturally, the amount of the antagonist in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

In other non-limiting examples, a dose of a Cathepsin L antagonist may also comprise from about 0.1 microgram/kg/body weight, about 0.2 microgram/kg/body weight, about 0.5 microgram/kg/body weight, about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above.
In particular embodiments of the present invention, the Cathepsin L antagonists are formulated to be administered via an alimentary route. Alimentary routes include all possible routes of administration in which the composition is in direct contact with the alimentary tract. Specifically, the pharmaceutical compositions disclosed herein may be administered orally, buccally, rectally, or sublingually. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft- shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

In certain embodiments, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz et al., 1997; Hwang et al., 1998; U.S. Patents 5,641,515, 5,580,579 and 5,792, 451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof; an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or combinations thereof; a lubricant, such as, for example, magnesium stearate; a sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. When the dosage form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Gelatin capsules, tablets, or pills may be enterically coated. Enteric coatings prevent denaturation of the composition in the stomach or upper bowel where the pH is acidic. See, e.g., U.S. Patent 5,629,001.

Upon reaching the small intestines, the basic pH therein dissolves the coating and permits the composition to be released and absorbed by specialized cells, e.g., epithelial enterocytes and Peyer's patch M cells. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as
preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

For oral administration, such as in the treatment of periodontal disease, the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, gel or sublingual orally-administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet, gel or solution form that may be placed under the tongue, along the gum line, brushed on to teeth surfaces, or otherwise dissolved in the mouth. U.S. Patents 6,074,674 and 6,270,750, both incorporated by reference, describe topical, sustained release compositions for periodontal procedures.

In further embodiments, Cathepsin L antagonist may be administered via a parenteral route. As used herein, the term "parenteral" includes routes that bypass the alimentary tract. Specifically, the pharmaceutical compositions disclosed herein may be administered for example, but not limited to intravenously, intradermally, intramuscularly, intraarterially, intrathecally, subcutaneous, or intraperitoneally U.S. Patents 6,537,514, 6,613,308, 5,466,468, 5,543,158; 5,641,515; and 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Patent 5,466,468, specifically incorporated herein by reference in its
entirety). In all cases the form must be sterile and must be fluid to the extent that easy injectability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (i.e., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologies standards.

Sustained release formulations for treating of bone conditions include U.S. Patents 4,722,948, 4,843,112, 4,975,526, 5,085,861, 5,162,114, 5,741,796 and 6,936,270, all of which are incorporated by reference. Methods and injectable compositions for bone repair are described in U.S. Patents 4,863,732, 5,531,791,
5,840,290, 6,281,195, 6,288,043, 6,485,754, 6,662,805 and 7,008,433, all of which are incorporated by reference.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. A powdered composition is combined with a liquid carrier such as, e.g., water or a saline solution, with or without a stabilizing agent.

B. Devices

In addition to providing Cathepsin L inhibitors and pharmaceuticals for administration by routes discussed above, such inhibitors, alone or in combination, maybe used in the context of devices, such as implants. A variety of bone related implants are contemplated, including dental implants, joint implants such as hips, knees, and elbows, vertebral/spinal implants, and others. The Cathepsin L inhibitor may be impregnated in a surface of the implant, including in a bioactive matrix or coating. The inhibitor may further formulated to sustained, delayed, prolonged or time release. The coating may comprise polymers, for example, such as those listed below. The following is a list of U.S. patents relating to bone implants and devices which may be utilized in accordance with this embodiment of the invention:

### TABLE 1 - BONE IMPLANT PATENTS

<table>
<thead>
<tr>
<th>U.S. Patent*</th>
<th>Patent Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>7,044,972</td>
<td>Bone implant, in particular, an inter-vertebral implant</td>
</tr>
<tr>
<td>7,022,137</td>
<td>Bone hemi-lumbar interbody spinal fusion implant having an asymmetrical leading end and method of installation thereof</td>
</tr>
<tr>
<td>7,001,551</td>
<td>Method of forming a composite bone material implant</td>
</tr>
<tr>
<td>6,994,726</td>
<td>Dual function prosthetic bone implant and method for</td>
</tr>
<tr>
<td>Patent Number</td>
<td>Description</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------</td>
</tr>
<tr>
<td>6,989,031</td>
<td>Hemi-interbody spinal implant manufactured from a major long bone ring or a bone composite</td>
</tr>
<tr>
<td>6,988,015</td>
<td>Bone implant</td>
</tr>
<tr>
<td>6,981,975</td>
<td>Method for inserting a spinal fusion implant having deployable bone engaging projections</td>
</tr>
<tr>
<td>6,981,872</td>
<td>Bone implant method of implanting, and kit for use in making implants, particularly useful with respect to dental implants</td>
</tr>
<tr>
<td>6,929,662</td>
<td>End member for a bone fusion implant</td>
</tr>
<tr>
<td>6,923,830</td>
<td>Spinal fusion implant having deployable bone engaging projections</td>
</tr>
<tr>
<td>6,921,264</td>
<td>Implant to be implanted in bone tissue or in bone tissue supplemented with bone substitute material</td>
</tr>
<tr>
<td>6,918,766</td>
<td>Method, arrangement and use of an implant for ensuring delivery of bioactive substance to the bone and/or tissue surrounding the implant</td>
</tr>
<tr>
<td>6,913,621</td>
<td>Flexible implant using partially demineralized bone</td>
</tr>
<tr>
<td>6,899,734</td>
<td>Modular implant for fusing adjacent bone structure</td>
</tr>
<tr>
<td>6,860,884</td>
<td>Implant for bone connector</td>
</tr>
<tr>
<td>6,852,129</td>
<td>Adjustable bone fusion implant and method</td>
</tr>
<tr>
<td>6,802,845</td>
<td>Implant for bone connector</td>
</tr>
<tr>
<td>6,786,908</td>
<td>Bone fracture support implant with non-metal spacers</td>
</tr>
<tr>
<td>6,767,367</td>
<td>Spinal fusion implant having deployable bone engaging projections</td>
</tr>
<tr>
<td>6,761,738</td>
<td>Reinforced molded implant formed of cortical bone</td>
</tr>
<tr>
<td>6,755,832</td>
<td>Bone plate implant</td>
</tr>
<tr>
<td>6,730,129</td>
<td>Implant for application in bone, method for producing such an implant, and use of such an implant</td>
</tr>
<tr>
<td>6,689,167</td>
<td>Method of using spinal fusion device, bone joining implant, and vertebral fusion implant</td>
</tr>
<tr>
<td>6,689,136</td>
<td>Implant for fixing two bone fragments to each other</td>
</tr>
<tr>
<td>6,666,890</td>
<td>Bone hemi-lumbar interbody spinal implant having an asymmetrical leading end and method of installation thereof</td>
</tr>
<tr>
<td>6,652,592</td>
<td>Segmentally demineralized bone implant</td>
</tr>
<tr>
<td>6,648,917</td>
<td>Adjustable bone fusion implant and method</td>
</tr>
<tr>
<td>6,607,557</td>
<td>Artificial bone graft implant</td>
</tr>
<tr>
<td>6,599,322</td>
<td>Method for producing undercut micro recesses in a surface, a surgical implant made thereby, and method for fixing an implant to bone</td>
</tr>
<tr>
<td>6,562,074</td>
<td>Adjustable bone fusion implant and method</td>
</tr>
<tr>
<td>6,562,073</td>
<td>Spinal bone implant</td>
</tr>
<tr>
<td>D473.944</td>
<td>Bone implant</td>
</tr>
<tr>
<td>6,540,770</td>
<td>Reversible fixation device for securing an implant in bone</td>
</tr>
<tr>
<td>Patent Number</td>
<td>Description</td>
</tr>
<tr>
<td>---------------</td>
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</tr>
<tr>
<td>6,537,277</td>
<td>Implant for fixing a bone plate</td>
</tr>
<tr>
<td>6,506,051</td>
<td>Bone implant with intermediate member and expanding assembly</td>
</tr>
<tr>
<td>6,478,825</td>
<td>Implant, method of making same and use of the implant for the treatment of bone defects</td>
</tr>
<tr>
<td>6,458,136</td>
<td>Orthopaedic instrument for sizing implant sites and for pressurizing bone cement and a method for using the same</td>
</tr>
<tr>
<td>6,447,545</td>
<td>Self-aligning bone implant</td>
</tr>
<tr>
<td>6,436,146</td>
<td>Implant for treating ailments of a joint or a bone</td>
</tr>
<tr>
<td>6,417,617</td>
<td>Spinal fusion device, bone joining implant, and vertebral fusion implant</td>
</tr>
<tr>
<td>6,370,418</td>
<td>Device and method for measuring the position of a bone implant</td>
</tr>
<tr>
<td>6,364,880</td>
<td>Spinal implant with bone screws</td>
</tr>
<tr>
<td>6,350,126</td>
<td>Bone hemi-lumbar interbody spinal implant having an asymmetrical leading end and method of installation thereof</td>
</tr>
<tr>
<td>6,350,283</td>
<td>Bone implant and method of securing</td>
</tr>
<tr>
<td>6,214,050</td>
<td>Expandable implant for inter-bone stabilization and adapted to extrude osteogenic material, and a method of stabilizing bones while extruding osteogenic material</td>
</tr>
<tr>
<td>6,213,775</td>
<td>Method of fastening an implant to a bone and an implant therefor</td>
</tr>
<tr>
<td>6,206,923</td>
<td>Flexible implant using partially demineralized bone</td>
</tr>
<tr>
<td>6,203,545</td>
<td>Implant for fixing bone fragments after an osteotomy</td>
</tr>
<tr>
<td>6,149,689</td>
<td>Implant as bone replacement</td>
</tr>
<tr>
<td>6,149,688</td>
<td>Artificial bone graft implant</td>
</tr>
<tr>
<td>6,149,686</td>
<td>Threaded spinal implant with bone ingrowth openings</td>
</tr>
<tr>
<td>6,126,662</td>
<td>Bone implant</td>
</tr>
<tr>
<td>6,083,264</td>
<td>Implant material for replacing or augmenting living bone tissue involving thermoplastic syntactic foam</td>
</tr>
<tr>
<td>6,058,590</td>
<td>Apparatus and methods for embedding a biocompatible material in a polymer bone implant</td>
</tr>
<tr>
<td>6,018,094</td>
<td>Implant and insert assembly for bone and uses thereof</td>
</tr>
<tr>
<td>5,976,147</td>
<td>Modular instrumentation for bone preparation and implant trial reduction of orthopedic implants</td>
</tr>
<tr>
<td>5,906,488</td>
<td>Releasable holding device preventing undesirable rotation during tightening of a screw connection in a bone anchored implant</td>
</tr>
<tr>
<td>5,899,939</td>
<td>Bone-derived implant for load-supporting applications</td>
</tr>
<tr>
<td>5,895,425</td>
<td>Bone implant</td>
</tr>
</tbody>
</table>
5,890,902 Implant bone locking mechanism and artificial periodontal ligament system
5,885,287 Self-tapping interbody bone implant
5,819,748 Implant for use in bone surgery
5,810,589 Dental implant abutment combination that reduces crestal bone stress
5,759,035 Bone fusion dental implant with hybrid anchor
5,720,750 Device for the preparation of a tubular bone for the insertion of an implant shaft
5,709,683 Interbody bone implant having conjoining stabilization features for bony fusion
5,674,725 Implant materials having a phosphatase and an organophosphorus compound for in vivo mineralization of bone
5,658,338 Prosthetic modular bone fixation mantle and implant system
5,639,402 Method for fabricating artificial bone implant green parts
5,624,462 Bone implant and method of securing
D38 1,080 Combined metallic skull base surgical implant and bone flap fixation plate
5,607,430 Bone stabilization implant having a bone plate portion with integral cable clamping means
5,571,185 Process for the production of a bone implant and a bone implant produced thereby
5,456,723 Metallic implant anchorable to bone tissue for replacing a broken or diseased bone
5,441,538 Bone implant and method of securing
5,405,388 Bone biopsy implant
5,397,358 Bone implant
5,383,935 Prosthetic implant with self-generated current for early fixation in skeletal bone
5,364,268 Method for installing a dental implant fixture in cortical bone
5,312,256 Dental implant for vertical penetration, adapted to different degrees of hardness of the bone

* - The preceding patents are all hereby incorporated by reference in their entirety.

i. Bone Precursor Cells (Osteoprogenitor cells)

Human bone precursor cells are characterized as small-sized cells that express low amounts of bone proteins (osteocalcin, osteonectin, and alkaline phosphatase) and have a low degree of internal complexity (Long et al., 1995). When stimulated to differentiate, these preosteoblast cells become osteoblast in their appearance, size,
antigenic expression, and internal structure. Although these cells are normally present at very low frequencies in bone marrow, a process for isolating these cells has been described (Long et al, 1995). U.S. Patent 5,972,703 further describes methods of isolating and using bone precursor cells, and is specifically incorporated herein by reference.

A number of studies indicate that bone marrow derived cells have osteogenic potential. The majority of these investigations point to mesenchymal stem cells (MSC) as undergoing differentiation into osteoblasts when cultured in the presence of bone-active cytokines (Jaiswal et al, 2000; Phinney et al, 1999; Aubin, 1998; Zohar et al., 1997). Mesenchymal stem cells are a pluripotent population capable of generating multiple stromal cell lineages. MSC, as currently used, are a heterogeneous population of cells isolated by plastic adherence, and propagated by low-density passage. Nonetheless, a recent publication indicates the clonal nature of cell fate outcomes in MSC indicating that a single MSC cell can give rise two or three mesenchymal lineages one of which is usually bone cells (Pitenger et al., 1999). These studies are consistent with earlier reports that demonstrated the osteogenic potential of bone marrow stromal cells, in particular the so-called CFU-f from both mice and human (Friedenstein et al., 1968; Reddi and Huggins, 1972; Friedenstein et al, 1982; Ashton et al, 1985; Bleiberg, 1985; Gronthos et al, 1994; Gronthos et al, 1999).

Single-cell isolation of human MSC generated clones that express the same surface phenotype as unfractionated MSC (Pitenger et al, 1999). Interestingly, of the 6 MSC clones evaluated, 2 retained osteogenic, chondrogenic and adipogenic potential; others were bipotent (either osteo- plus chondrogenic potential, or osteo-adipocytic potential) or were uni-lineage (chondrocyte). This suggests that MSC themselves are heterogeneous in nature (although culture conditions also may have led to loss of lineage potential). To date, the self-renewal capacity of MSC remains in question. Nonetheless, these in vitro studies and other in vivo studies (Kadiyala et al, 1997; Petite et al, 2000; Krebsbach et al, 1999) show that MSC can commit to the bone cell lineage and develop to the state of matrix mineralization in vitro, or bone formation in vivo.
ii. Preosteoblasts

Preosteoblasts are intermediate between osteoprogenitor cells and osteoblasts. The show increasing expression of bone phenotypic markers such as alkaline phosphatase (Kale et al., 2000). They have a more limited proliferative capacity, but nonetheless continue to divide and produce more preosteoblasts or osteoblasts.

iii. Osteoblasts

Osteoblasts are differentiated cells of the bone-forming cell lineage. They are large cells, possessing a eccentric nucleus, and produce of the extracellular proteins required for bone formation. They can be obtained from bone as populations of both preosteoblasts and osteoblasts as described in U.S. Serial No. 09/753,043, which is specifically incorporated herein by reference.

iv. Serum-Free Media

Cell culture may be employed using serum-free media for growth of bone-producing cells, and the subsequent conversion of stem cells into bone-forming osteogenic or osteoprogenitor cells. The following section describes attributes and conditions for using serum-free media. See also U.S. Patent 6,811,776.

The use of serum-free culture for the manufacture of recombinant biopharmaceuticals from mammalian cells has been thoroughly reviewed (Barnes, 1987; Barnes & Sam, 1980; Broad et al., 1991; Jayme, 1991). The list of the main additives which are used as supplements for serum-free media is summarized by Barnes (1987) and Barnes & Sam (1980). Most commercially available serum-free media contain a carrier protein such as albumin. The presence of carrier protein might be required for protection of the cell viability.

An example of serum free culture medium can be found in U.S. Patent 5,063,157, herein incorporated by reference. The media comprises, in addition to the base medium, transferrin, insulin, a peptone, a β-D-xylopyranose derivative, selenite and a biological polyamine. Another serum free cell growth medium for mammalian cells is disclosed in U.S. Patent 4,443,546. This growth medium, in addition to the basic medium, contains seven ingredients. EPA 481 791 discloses a culture medium for CHO cells comprising water, an osmolality regulator, a buffer, an energy source, amino acids, an iron source, a growth factor and other optional components. The two
media exemplified contain 19 and 17 components, respectively. Examples of potential additives to serum free media follow below.

**Albumin.** Albumin is preferably supplied in the form of bovine (BSA) or human serum albumin (HSA) in an effective amount for the growth of cells. Albumin provides a source of protein in the media. Albumin is thought to act as a carrier for trace elements and essential fatty acids. Preferably, the albumin used in the present formulations is free of pyrogens and viruses, and when necessary, is approved regulatory agencies for infusion into human patients. The HSA may be deionized using resin beads prior to use. The concentration of human serum albumin is 1-8 mg/ml, preferably 3-5 mg/ml, most preferably 4 mg/ml.

**Soluble Carrier/Fatty Acid Complex.** The albumin mentioned above could be substituted by a soluble carrier/essential fatty acid complex and a soluble carrier cholesterol complex which can effectively deliver the fatty acid and cholesterol to the cells. An example of such a complex is a cyclodextrin/linoleic acid, cholesterol and oleic acid complex. This is advantageous as it would allow for the replacement of the poorly characterized albumin with a well defined molecule. The use of cyclodextrin removes the need for the addition of human/animal serum albumin, thereby eliminating any trace undesired materials which the albumin would introduce into the media. The use of cyclodextrin simplifies the addition of specific lipophilic nutrients to a serum-free culture.

The lipophilic substances which can be complexed with cyclodextrin include unsaturated fatty acids such as linoleic acid, cholesterol and oleic acid. The linoleic acid, cholesterol and oleic acid are present in effective amounts and can be present in equal proportions such that the total amount is 0.001 to 100 µg/ml, preferably 0.1 to 10 µg/ml. The preparation of such complexes is known in the art and is described, for example, in U.S. Patent 4,533,637, the entire contents of which is hereby incorporated by reference.

**Iron Source.** A source of iron in an effective amount and in a form that can be utilized by the cells can be added to the media. The iron can be supplied by saturating transferrin, its carrier molecule, in an effective amount. The transferrin may be derived from animal sera or recombinantly synthesized. It is understood that when transferrin is derived from an animal source, it is purified to remove other animal proteins, and thus is usually at least 99% pure. The transferrin concentration is
usually between 80 and 500 µg/ml, preferably between 120 and 500 µg/ml, more preferably between 130 and 500 µg/ml, even more preferably between 275 and 400 µg/ml and most preferably 300 µg/ml. An iron salt, preferably a water soluble iron salt, such as iron chloride (e.g., FeCl$_3$-OH$_2$O) dissolved in an aqueous solution such as an organic acid solution (e.g., citric acid) is used to supply the iron to transferrin. One mole of iron chloride is usually used for every mole of citric acid. The concentration of iron chloride is 0.0008 to 8 µg/ml, preferably 0.08 to 0.8 µg/ml, most preferably 0.08 µg/ml.

**Insulin Growth Factor.** Insulin also may be added to the media of the present invention in an effective amount. The insulin concentration is between 0.25 and 2.5 U/ml, more preferably 0.4-2.1 U/ml, most preferably 0.48 U/ml. In the conversion of Units to mass, 27 U=1 mg. Therefore, incorporating the conversion, the insulin concentration is approximately between 9.26 µg/ml and 92.6 µg/ml, more preferably 14.8 µg/ml-77.8 µg/ml, most preferably 17.7 µg/ml. It is again understood that human insulin is more preferable than animal insulin. Highly purified recombinant insulin is most preferred. An insulin like growth factor such as insulin like growth factor 1 and insulin like growth factor 2 may be used in place of or in addition to insulin in an amount which provides substantially the same result as a corresponding amount of insulin. Thus, the term "insulin growth factor" includes both insulin and insulin like growth factors.

**Additional Components.** The addition of other lipids to the above essential reagents could enhance the proliferative potential of precursor cells. These components, however, are preferably not added unless they are necessary for a particular experiment or to grow a particular type of cell. Optionally, triglycerides and/or phospholipids may be included as additional sources of lipid. A preferable source of lipid contains a mixture of neutral triglycerides of predominantly unsaturated fatty acids such as linoleic, oleic, palmitic, linolenic, and stearic acid. Such a preparation may also contain phosphatidylethanolamine and phosphatidylcholine. Another source of lipid is a human plasma fraction precipitated by ethanol and preferably rendered virus free by pasteurization.

Other ingredients which can optionally be added to the media are cited in the following references: WO 95/061 12, U.S. Patent 4,533,637, U.S. Patent 5,405,772. The entire contents of all of these references are incorporated by reference.
Undesired Components. When the media is to be used to grow cells for introduction into a human patient, the media preferably does not contain ingredients such as bovine serum albumin, mammalian serum, and/or any natural proteins of human or mammalian origin (as explained above). It is preferable that recombinant or synthetic proteins, if they are available and of high quality, are used. Most preferably, the amino acid sequences of the recombinant or synthetic proteins are identical to or highly homologous with those of humans. Thus, the most preferable serum-free media formulations herein contain no animal-derived proteins and do not have even a non-detectable presence of animal protein.

In the most ideal system, optional components which are not necessary are preferably not added to the medium. Such optional components are described in the prior art cited above and may be selected from the group consisting of meat extract, peptone, phosphatidylcholine, ethanolamine, anti-oxidants, deoxyribonucleosides, ribonucleosides, soybean lecithin, corticosteroids, myoinositol, monothioglycerol, and bovine or other animal serum albumin.

V. Polymers for Implanting of Bone Cells

As discussed above, bone cells stimulated with Cathepsin L antagonists and/or second agents as discussed above of the present invention may be used for autologous or heterologous transplant into subjects in need thereof. In many embodiments, supports will be used to provide a scaffold or matrix over with bone cells and grown and deposit bone. Over the last decade there has been a tremendous increase in applications for polymeric materials, including in vivo medical applications. These materials are well suited to implantation as they can serve as a temporary scaffold to be replaced by host tissues, degrade by hydrolysis to non-toxic products, and be excreted, as described by Kulkarni et al. (1971) and Hollinger and Battistone (1986).

Either natural or synthetic polymers can be used to form the matrix, although synthetic polymers are preferred for reproducibility and controlled release kinetics. Synthetic polymers that can be used include bioerodible polymers such as poly(lactide) (PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycolide) (PLGA), and other poly(alpha-hydroxy acids), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates and degradable polyurethanes, and non-erodible polymers such as polyacrylates,
ethylene-vinyl acetate polymers and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifms, polyethylene oxide, polyvinyl alcohol, and nylon. Although non-degradable materials can be used to form the matrix or a portion of the matrix, they are not preferred. Examples of natural polymers include proteins such as albumin, fibrin or fibrinogen, collagen, synthetic polyamino acids, and prolamines, and polysaccharides such as alginate, heparin, and other naturally occurring biodegradable polymers of sugar units.

Four polymers widely used in medical applications are poly(paradioxanone) (PDS), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and PLAGA copolymers. Copolymerization enables modulation of the degradation time of the material. By changing the ratios of crystalline to amorphous polymers during polymerization, properties of the resulting material can be altered to suit the needs of the application. These polymers, including poly(lactide-co-glycolic) acid (PLGA), have been used as polymer composites for bone replacement as reported by Elgendy et al. (1993). Substituted polyphosphazenes have been shown to support osteogenic cell growth, as reported by Laurencin et al. (1993). Poly(organophosphazenes) are high molecular weight polymers containing a backbone of alternating phosphorus and nitrogen atoms. There are a wide variety of polyphosphazenes, each derived from the same precursor polymer, poly(dichlorophosphazene). The chlorine-substituted species can be modified by replacement of the chlorine atoms by different organic nucleophiles such as o-methylphenoxide along with amino acids. The physical and chemical properties of the polymer can be altered by adding various ratios of hydrolytic sensitive side chains such as ethyl glycinate, as described by Wade et al. (1978) and Allcock and Fuller (1981). This will affect the degradation of the polymer as an implantable and biodegradable material as well as vary the support of osteogenic cells for bone and tissue implants, as shown by Laurencin et al. (1993).

PLA, PGA and PLA/PGA copolymers are particularly useful for forming the biodegradable matrices. PLA polymers are usually prepared from the cyclic esters of lactic acids. Both L(+) and D(-) forms of lactic acid can be used to prepare the PLA polymers, as well as the optically inactive DL-lactic acid mixture of D(-) and L(+) lactic acids. Methods of preparing polylactides are well documented in the patent literature. The following U.S. Patents, the teachings of which are hereby incorporated by reference, describe in detail suitable polylactides, their properties and their
preparation: U.S. Patents 1,995,970; 2,703,316; 2,758,987; 2,951,828; 2,676,945; 2,683,136; and 3,531,561. PGA is the homopolymer of glycolic acid (hydroxyacetic acid). In the conversion of glycolic acid to poly(glycolic acid), glycolic acid is initially reacted with itself to form the cyclic ester glycolide, which in the presence of heat and a catalyst is converted to a high molecular weight linear-chain polymer. PGA polymers and their properties are described in more detail in "Cyanamid Research Develops World's First Synthetic Absorbable Suture," Chemistry and Industry, 905 (1970).

The erosion of the matrix is related to the molecular weights of PLA, PGA or PLA/PGA. The higher molecular weights, weight average molecular weights of 90,000 or higher, result in polymer matrices which retain their structural integrity for longer periods of time; while lower molecular weights, weight average molecular weights of 30,000 or less, result in both slower release and shorter matrix lives. Poly(lactide-co-glycolide) (50:50), degrades in about six weeks following implantation.

All polymers for use in the matrix must meet the mechanical and biochemical parameters necessary to provide adequate support for the cells with subsequent growth and proliferation. The polymers can be characterized with respect to mechanical properties such as tensile strength using an Instron tester, for polymer molecular weight by gel permeation chromatography (GPC), glass transition temperature by differential scanning calorimetry (DSC) and bond structure by infrared (IR) spectroscopy, with respect to toxicology by initial screening tests involving Ames assays and in vitro teratogenicity assays, and implantation studies in animals for immunogenicity, inflammation, release and degradation studies.

These polymers are particularly useful in forming fibrous or sponge type matrices for implantation. Polymers can also be used to form hydrogels in which the cells are suspended and then implanted.

A. **Other Matrix Materials**

Another class of materials for making the matrix is hydroxyapatite, or a similar ceramic formed of tricalcium phosphate (TCP) or calcium phosphate (CaPO₄). Calcium hydroxyapatites occur naturally as geological deposits and in normal biological tissues, principally bone, cartilage, enamel, dentin, and cementum of vertebrates and in many sites of pathological calcifications such as blood vessels and
skin. Synthetic calcium hydroxyapatite is formed in the laboratory either as pure $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ or hydroxyapatite that is impure, containing other ions such as carbonate, fluoride, chloride for example, or crystals deficient in calcium or crystals in which calcium is partly or completely replaced by other ions such as barium, strontium and lead. Essentially none of the geological and biological apatites are "pure" hydroxyapatite since they contain a variety of other ions and cations and may have different ratios of calcium to phosphorous than the pure synthetic apatites.

In general, the crystals of pure synthetic apatites, geological apatites and many impure synthetically produced apatites are larger and more crystalline than the biological crystals of bone, dentin, cementum and cartilage. The crystals of bone, dentin and cementum are very small, irregularly shaped, very thin plates whose rough average dimensions are approximately 10 to 50 angstroms in thickness, 30 to 150 angstroms in width, and 200 to 600 angstroms in length. The synthetic materials are highly diverse, as reported in the literature. For example, the characterization of four commercial apatites was reported by Pinholt et al. (1992); Marden et al. (1990) reports on a protein, biodegradable material; Pinholt et al. (1991) reports on the use of a bovine bone material called Bio-Oss™; Friedman et al. (1991) and Costantino et al. (1991) report on a hydroxyapatite cement; Roesgen (1990) reports on the use of calcium phosphate ceramics in combination with autogenic bone; Ono et al. (1990) reports on the use of apatite-wollastonite containing glass ceramic granules, hydroxyapatite granules, and alumina granules; Passuti et al. (1989) reports on macroporous calcium phosphate ceramic performance; Harada (1989) reports on the use of a mixture of hydroxyapatite particles and tricalcium phosphate powder for bone implantation; Ohgushi et al. (1989) reports on the use of porous calcium phosphate ceramics alone and in combination with bone marrow cells; Pochon et al. (1986) reports on the use of beta-tricalcium phosphate for implantation; and Glowacki et al. (1985), reports on the use of demineralized bone implants.

As used herein, all of these materials are generally referred to as "hydroxyapatite." In the preferred form, the hydroxyapatite is particles having a diameter between approximately ten and 100 µm in diameter, most preferably about 50 µm in diameter. Another form of hydroxyapatite scaffold is devitalized coral as a scaffold. Le Guehenneec et al. (2004); Chen et al (2004); Devecioglu et al (2004); Kujala et al (2004).
Calcium phosphate ceramics can be used as implants in the repair of bone defects because these materials are non-toxic, non-immunogenic, and are composed of calcium and phosphate ions, the main constituents of bone (Frame, 1987; Parsons et al., 1988). Both tricalcium phosphate (TCP) \( \text{Ca}_3(\text{PO}_4)_2 \) and hydroxyapatite (HA) \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) have been widely used. Calcium phosphate implants are osteoinductive, and have the apparent ability to become directly bonded to bone. As a result, a strong bone-implant interface is created.

Calcium phosphate ceramics have a degree of bioresorbability which is governed by their chemistry and material structure. High density HA and TCP implants exhibit little resorption, while porous ones are more easily broken down by dissolution in body fluids and resorbed by phagocytosis. However, TCP degrades more quickly than HA structures of the same porosity \textit{in vitro}. HA is relatively insoluble in aqueous environments. However, the mechanical properties of calcium phosphate ceramics make them ill-suited to serve as a structural element under load bearing circumstances. Ceramics are not preferred since they are brittle and have low resistance to impact loading.

B. Polymers for Forming Hydrogels

Polymers that can form ionic hydrogels which are malleable can also be used to support the cells. Injecting a suspension of cells in a polymer solution may be performed to improve the reproducibility of cell seeding throughout a device, to protect the cells from shear forces or pressure induced necrosis, or to aid in defining the spatial location of cell delivery. The injectable polymer may also be utilized to deliver ells and promote the formation of new tissue without the use of any other matrix. In a preferred embodiment, the hydrogel is produced by cross-linking the ionic salt of a polymer with ions, whose strength increases with either increasing concentrations of ions or polymer. The polymer solution is mixed with the cells to be implanted to form a suspension, which is then injected directly into a patient prior to polymerization of the suspension. The suspension subsequently polymerizes over a short period of time due to the presence \textit{in vivo} of physiological concentrations of ions such as calcium in the case where the polymer is a polysaccharide such as alginate.

A hydrogel is defined as a substance formed when an organic polymer (natural or synthetic) is cross-linked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure which entraps water molecules to form a gel.
Examples of materials which can be used to form a hydrogel include polysaccharides such as alginate, polyphosphazenes, and polyacrylates such as hydroxyethyl methacrylate (HEMA), which are cross-linked ionically, or block copolymers such as Pluronics™ or Tetronics™, polyethylene oxide-polypropylene glycol block copolymers which are cross-linked by temperature or pH, respectively. Other materials include proteins such as fibrinogen, collagen, polymers such as polyvinylpyrrolidone, hyaluronic acid and collagen.

In general, these polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions, that have charged side groups, or a monovalent ionic salt thereof. Examples of polymers with acidic side groups that can be reacted with cations are poly(phosphazenes), poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), and sulfonated polymers, such as sulfonated polystyrene. Copolymers having acidic side groups formed by reaction of acrylic or methacrylic acid and vinyl ether monomers or polymers can also be used. Examples of acidic groups are carboxylic acid groups, sulfonic acid groups, halogenated (preferably fluorinated) alcohol groups, phenolic OH groups, and acidic OH groups. Examples of polymers with basic side groups that can be reacted with anions are poly(vinyl amines), poly(vinyl pyridine), poly(vinyl imidazole), and some imino substituted polyphosphazenes. The ammonium or quaternary salt of the polymers can also be formed from the backbone nitrogens or pendant imino groups. Examples of basic side groups are amino and imino groups.

Alginate can be ionically cross-linked with divalent cations, in water, at room temperature, to form a hydrogel matrix. Due to these mild conditions, alginate has been the most commonly used polymer for hybridoma cell encapsulation, as described, for example, in U.S. Patent 4,352,883. Described therein is an aqueous solution containing the biological materials to be encapsulated is suspended in a solution of a water soluble polymer, the suspension is formed into droplets which are configured into discrete microcapsules by contact with multivalent cations, then the surface of the microcapsules is cross-linked with polyamino acids to form a semi-permeable membrane around the encapsulated materials.

The polyphosphazenes suitable for cross-linking have a majority of side chain groups which are acidic and capable of forming salt bridges with di- or trivalent cations. Examples of preferred acidic side groups are carboxylic acid groups and
sulfonic acid groups. Hydrolyrically stable polyphosphazenes are formed of monomers having carboxylic acid side groups that are cross-linked by divalent or trivalent cations such as Ca$^{2+}$ or Al$^{3+}$. Polymers can be synthesized that degrade by hydrolysis by incorporating monomers having imidazole, amino acid ester, or glycerol side groups. Bioerodible polyphosphazenes have at least two differing types of side chains, acidic side groups capable of forming salt bridges with multivalent cations, and side groups that hydrolyze under in vivo conditions, e.g., imidazole groups, amino acid esters, glycerol and glucosyl.

The water soluble polymer with charged side groups is cross-linked by reacting the polymer with an aqueous solution containing multivalent ions of the opposite charge, either multivalent cations if the polymer has acidic side groups or multivalent anions if the polymer has basic side groups. The preferred cations for cross-linking of the polymers with acidic side groups to form a hydrogel are divalent and trivalent cations such as copper, calcium, aluminum, magnesium, strontium, barium, and tin, although di-, tri- or tetra-functional organic cations such as alkylammonium salts can also be used. Aqueous solutions of the salts of these cations are added to the polymers to form soft, highly swollen hydrogels and membranes. The higher the concentration of cation, or the higher the valence, the greater the degree of cross-linking of the polymer. Concentrations from as low as 0.005M have been demonstrated to cross-link the polymer. Higher concentrations are limited by the solubility of the salt. The preferred anions for cross-linking of the polymers to form a hydrogel are divalent and trivalent anions such as low molecular weight dicarboxylic acids, for example, terephthalic acid, sulfate ions and carbonate ions. Aqueous solutions of the salts of these anions are added to the polymers to form soft, highly swollen hydrogels and membranes, as described with respect to cations.

A variety of polycations can be used to complex and thereby stabilize the polymer hydrogel into a semi-permeable surface membrane. Examples of materials that can be used include polymers having basic reactive groups such as amine or imine groups, having a preferred molecular weight between 3,000 and 100,000, such as polyethyleneimine and polylysine. These are commercially available. One polycation is poly(L-lysine), examples of synthetic polyamines are polyethyleneimine, poly(vinylamine), and poly(allyl amine). There are also natural polycations such as the polysaccharide, chitosan. Polyanions that can be used to form a semi-permeable membrane by reaction with basic surface groups on the polymer.
hydrogel include polymers and copolymers of acrylic acid, methacrylic acid, and other derivatives of acrylic acid, polymers with pendant SO₃H groups such as sulfonated polystyrene, and polystyrene with carboxylic acid groups.

5 VI. Screening Assays

In still further embodiments, the present invention provides methods identifying new and useful inhibitors of Cathepsin L for use in stimulating bone production. These assays may comprise random screening of large libraries of candidate substances; alternatively, the assays may be used to focus on particular classes of compounds selected with an eye towards structural attributes that are believed to make them more likely to modulate the function of Cathepsin L.

By function, it is meant that one may assay for Cathepsin L enzyme activity, expression or bone formation. To identify a Cathepsin L modulator, one generally will determine the function of Cathepsin L in the presence and absence of the candidate substance, a modulator defined as any substance that alters function. For example, a method generally comprises:

(a) providing a candidate modulator;
(b) admixing the candidate modulator with an isolated enzyme, cell lysate or cell, or a suitable experimental animal;
(c) measuring Cathepsin L activity, expression or bone formation; and
(d) comparing the characteristic measured in step (c) with that observed in the absence of the candidate modulator,

wherein a difference between the measured characteristic indicates that said candidate modulator is, indeed, a modulator of Cathepsin L.

Assays may be conducted in cell free systems, in isolated cells, or in organisms including transgenic animals. Bone formation can be identified by the von Koassa or Alzarin Red stains, FTIR or Raman spectrometric analysis, or by fluorochromes linked to compounds that bind bone. U.S. Patent 6,346,373 describes a whole cell based assay for Cathepsin K activity.

It will, of course, be understood that all the screening methods of the present invention are useful in themselves notwithstanding the fact that effective candidates may not be found. The invention provides methods for screening for such candidates, not solely methods of finding them.
VII. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1 — IN VITRO INHIBITION OF CATHEPSIN L STIMULATES BONE CELL MINERALIZATION

Treatment of isolated human osteoblasts with inhibitors of Cathepsin L results in a powerful mineralization response. As indicated by Alizarin Red staining, inhibition by Cathepsin L Inhibitory Compound A (CLIC; 1 - 10 µM) demonstrates a significant increase in mineralization (FIG. 1). Quantitation of the degree of mineralization shows that CLIC stimulates a 7-fold increase in mineralization, compared to that seen with the biological regulator BMP-2 (FIG. 2).

Examination of Cathepsin L activity in bone-forming cells reveals that, like Cathepsin K, this cysteine protease plays a role in bone formation. Thus, Cathepsin L antagonists stimulate osteoblast development and the formation of bone. These observations demonstrate that such antagonists, alone, or in conjunction with other agents may be beneficial therapies in a wide variety of bone diseases.

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All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents.
described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.
V. References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

5
U.S. Patent 1,995,970
U.S. Patent 2,676,945
U.S. Patent 2,683,136
U.S. Patent 2,703,316

10 U.S. Patent 2,758,987
U.S. Patent 2,951,828
U.S. Patent 3,531,561
U.S. Patent 4,443,546
U.S. Patent 4,507,232

15 U.S. Patent 4,533,637
U.S. Patent 4,533,637
U.S. Patent 4,722,948
U.S. Patent 4,843,112
U.S. Patent 4,863,732

20 U.S. Patent 4,975,526
U.S. Patent 5,063,157
U.S. Patent 5,085,861
U.S. Patent 5,162,114
U.S. Patent 5,328,909

25 U.S. Patent 5,395,958
U.S. Patent 5,399,363
U.S. Patent 5,405,772
U.S. Patent 5,416,117
U.S. Patent 5,422,359

30 U.S. Patent 5,424,325
U.S. Patent 5,466,468
U.S. Patent 5,506,243
U.S. Patent 5,531,791
U.S. Patent 5,543,158
U.S. Patent 5,556,853
U.S. Patent 5,580,579
U.S. Patent 5,629,001
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5 U.S. Patent 5,639,783
U.S. Patent 5,641,515
U.S. Patent 5,698,519
U.S. Patent 5,716,980
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10 U.S. Patent 5,792,451
U.S. Patent 5,840,290
U.S. Patent 5,843,508
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20 U.S. Patent 6,242,239
U.S. Patent 6,270,750
U.S. Patent 6,274,336
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25 U.S. Patent 6,462,076
U.S. Patent 6,485,754
U.S. Patent 6,531,612
U.S. Patent 6,537,514
U.S. Patent 6,613,308

30 U.S. Patent 6,662,805
U.S. Patent 6,811,776
U.S. Patent 6,936,270
U.S. Patent 7,008,433

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U.S. Serial No. 09/753,043


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CLAIMS

1. A method of promoting bone formation comprising:
   (i) selecting a first Cathepsin L inhibitor; and
   (ii) administering to a subject said first inhibitor of Cathepsin L.

2. The method of claim 1, wherein said first inhibitor is a biological or an organopharmaceutical small molecule.

3. The method of claim 2, wherein said first inhibitor is a biological.

4. The method of claim 3, wherein said biological is a peptide, an siRNA, an antisense molecule or a single-chain antibody.

5. The method of claim 2, wherein said first inhibitor is an organopharmaceutical small molecule.

6. The method of claim 1, wherein said first inhibitor is an extracellular inhibitor of Cathepsin L.

7. The method of claim 1, wherein said first inhibitor is a intracellular inhibitor of Cathepsin L.

8. The method of claim 1, wherein said first inhibitor is a extracellular and intracellular inhibitor of Cathepsin L.

9. The method of claim 1, further comprising contacting said subject with a second agent.

10. The method of claim 9, wherein said second agent is a bisphosphonate, a PTH analog, an inhibitor of Cathepsin K or a second inhibitor of Cathepsin L that is distinct from said first inhibitor.

II. The method of claim 10, wherein the agents are a Cathepsin L inhibitor an intracellular Cathepsin K inhibitor.

12. The method of claim 1, wherein said first inhibitor is formulated to increase transmembrane delivery.
13. The method of claim 12, wherein said first inhibitor is modified to increase cell permeability or formulated with a cell permeability factor.

14. The method of claim 12, wherein said first inhibitor is formulated in a lipid delivery vehicle.

15. The method of claim 12, wherein said first inhibitor is formulated or modified to enhance its bioavailability.

16. The method of claim 1, wherein said subject is a human.

17. The method of claim 1, wherein said subject suffers from a bone-related pathology or injury.

18. The method of claim 17, wherein said bone-related pathology is osteoporosis, vitamin D deficiency, bone cancer, bone fracture, periodontal disease, Paget’s disease, osteoporosis secondary to other diseases.

19. The method of claim 1, wherein said first inhibitor also prevents bone resorption.

20. The method of claim 1, wherein said first inhibitor is administered to said subject more than once.

21. The method of claim 1, wherein said first inhibitor is administered to said subject by topical, oral or intravenous routes.

22. The method of claim 1, wherein the subject is a non-human animal.

23. A method of producing bone in an isolated bone cell comprising:

   (i) selecting an intracellular Cathepsin L inhibitor;

   (ii) contacting said bone cell with said inhibitor of Cathepsin L; and

   (iii) culturing said cell under conditions promoting bone formation.

24. The method of claim 23, further comprising implanting said bone cell in a subject.

25. The method of claim 24, wherein said bone cell was obtained, prior to step (ii) from said subject.

26. The method of claim 25, wherein said obtained cell was a stem cell prior to step (ii).
27. The method of claim 26, wherein said stem cell was cultured under conditions promoting the formation of an osteogenic cell prior to step (ii).

28. A method of identifying an agent that modulates bone formation comprising:
   (i) providing a candidate compound;
   (ii) admixing the candidate compound with an isolated Cathepsin L enzyme, or a cell expressing Cathepsin L, or an experimental animal, cells of which express Cathepsin L;
   (iii) measuring Cathepsin L activity or expression or bone formation; and
   (iv) comparing the characteristic measured in step (iii) with that observed in the absence of the candidate modulator, wherein a difference between the measured characteristic indicates that said candidate compound is, indeed, a modulator of Cathepsin L.

29. The method of claim 28, wherein said modulator promotes bone formation.

30. The method of claim 28, wherein said modulator inhibits bone formation.

31. A method of treating a subject with multiple myeloma comprising:
   (i) selecting an intracellular Cathepsin L inhibitor; and
   (ii) administering to said subject said first inhibitor of Cathepsin L.

32. The method of claim 31, wherein said inhibitor is a biological or an organopharmaceutical small molecule.

33. The method of claim 32, wherein said first inhibitor is a biological.

34. The method of claim 33, wherein said biological is a peptide, an siRNA, an antisense molecule or a single-chain antibody.

35. The method of claim 32, wherein said inhibitor is an organopharmaceutical small molecule.

36. The method of claim 31, wherein said first inhibitor is an extracellular inhibitor of Cathepsin L.
37. The method of claim 31, wherein said first inhibitor is a intracellular inhibitor of Cathepsin L

38. The method of claim 31, wherein said first inhibitor is a extracellular and intracellular inhibitor of Cathepsin L

39. The method of claim 31, further comprising contacting said subject with a second agent.

40. The method of claim 39, wherein said second agent is a bisphosphonate, an osteoclast inhibitor such as a PTH analog, an inhibitor of Cathepsin K, or a second inhibitor of Cathepsin L that is distinct from said first inhibitor.

41. The method of claim 40, wherein the second inhibitor of Cathepsin L is an extracellular inhibitor of Cathepsin L.

42. The method of claim 31, wherein said first inhibitor is formulated to increase transmembrane delivery.

43. The method of claim 42, wherein said first inhibitor is modified to enhance permeability or formulated with a cell permeability factor.

44. The method of claim 42, wherein said first inhibitor is formulated in a lipid delivery vehicle.

45. The method of claim 42, wherein said first inhibitor is modified or formulated to enhance its bioavailability.

46. The method of claim 31, wherein said subject is a human.

47. The method of claim 31, wherein the subject is a non-human animal.

48. The method of claim 39, wherein said second agent is an anti-cancer agent.

49. The method of claim 48, wherein said anti-cancer agent is a chemotherapeutic, a radiotherapeutic, an immunotherapeutic, a hormone therapy, a cytokine therapy, a toxin therapy or a gene therapy.

50. The method of claim 31, wherein said first inhibitor also prevents bone resorption.

51. The method of claim 31, wherein said first inhibitor is administered to said subject more than once.
52. The method of claim 31, wherein said first inhibitor also stimulates bone formation.

53. The method of claim 52, wherein said first inhibitor is administered to said subject more than once.

54. The method of claim 31, wherein said first inhibitor is administered to said subject by oral, intravenous, intra-tumoral, or tumor vasculature routes.

55. The method of claim 31, wherein the multiple myeloma is recurrent, metastatic or drug resistant.
FIG. 1

Control

BMP-2

Cathepsin L Inhibitor A (1μM)

Cathepsin L Inhibitor A (10μM)
FIG. 2
Cathepsin L inhibitor 4

Calbiochem Cat. # 219433
Formula $C_{27}H_{29}N_{2}O_{4}S$

References:

Cathepsin L inhibitor 6

Calbiochem Cat. # 219495
Formula $C_{19}H_{48}N_{7}O_{4}F$

An end protected tripeptide that acts as a highly selective, potent, and reversible inhibitor of human recombinant cathepsin-L ($K_i = 19 \text{ nM}$). Resists proteolysis by cathepsin-L and mimics the mode of autoinhibition of procathepsin-L. Displays $\sim$310- and $\sim$210-fold greater selectivity for cathepsin-L over cathepsin-K ($K_i = 5.9 \mu M$, human recomb.) and cathepsin-B ($K_i = 4.1 \mu M$, human recomb.), respectively. Purity: $\geq 97\%$ by HPLC.

References:
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 9/64 (2009.01)

USPC - 435/226

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)

IPC(8) C12N 9/64 (2009.01)

USPC 435/226

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC 435/226, 435/183, 435/212, 435/320 1, 514/1, 514/525

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWest, Google Scholar, Google Patent cathepsin, cathepsin L, cathepsin K, intracellular, extracellular, bone resorption, bone formation, cancer, transmembrane, peptide, inhibitor, biological, delivery

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<tbody>
<tr>
<td>Y</td>
<td>&quot;participation of Cathepsin L on bone resorption&quot; (KAKEGAWAA H et al) 09 Mar 1993 (09 03 1993) REBS 321 247-250, page 249, para 3</td>
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</tbody>
</table>

Further documents are listed in the continuation of Box C

D. Date of the actual completion of the international search

09 Feb 2009 (09 02 2009)

Date of mailing of the international search report

23 FEB 2009

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