Overexpression of OPG in transgenic mice leads to profound osteopetrosis characterized by a near complete lack of osteoclasts in the mice. Conversely, ablation of the OPG gene causes severe osteoporosis in mice, indicating an important physiological role of OPG in regulating bone resorption. The secretion of OPG and RANKL from osteoblasts and stromal cells is regulated by numerous hormones and cytokines. The relative levels of OPG and RANKL production are thought to control the extent of bone resorption: expression of RANKL increases bone resorption, whereas excess OPG has the opposite effect. Recombinant OPG blocks the effects of the vast majority of the factors which stimulate osteoclasts, in vitro and in vivo. OPG also inhibits bone resorption in a variety of animal disease models, including ovariectomy, induced osteoporosis, humoral hypercalcemia of malignancy, and experimental bone metastasis. Therefore, OPG might represent an effective therapeutic option for diseases associated with excessive osteoclast activity (Kostenuik PJ, Shalhoub V., Curr Pharm Des. 2001 May;7(8):613-35).

Denosumab is a high affinity fully human monoclonal antibody that binds to human RANKL and inhibits its interactions with RANK, thus having a similar to OPG mode of action. Denosumab under the trade name Prolia was approved by U.S. Food and Drug Administration (FDA) for prevention and treatment of osteoporosis in postmenopausal women. Denosumab under the trade name Xgeva was approved by U.S. Food and Drug Administration (FDA) for the prevention of skeletal-related events in patients with bone metastases from solid tumors. Further clinical trials of denosumab for other bone remodeling related conditions are currently under way, i.e. for bone metastases from other forms of cancer.

It would therefore be desirable to have a therapeutic composition that is capable of binding to RANKL and is based on the naturally occurring OPG molecule, which, while having an acceptable pharmacological profile, has a broader therapeutic potential.

SUMMARY ~~OF THE INVENTION~~

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

In certain aspects, the present ~~invention~~ disclosure provides for a polypeptide that binds human RANKL. The polypeptide comprises a first amino acid sequence comprising amino acids 1 through 215 of human osteoprotegerin (GenBank: U94332.1). The polypeptide further comprises a second amino acid sequence comprising amino acids 103 through 329 of human immunoglobulin gamma-1 Fc (GenBank: J00228.1). The polypeptide may be capable of inhibiting RANKL-induced osteoclast differentiation in an in vitro cell-based assay with an EC50 of about 50 ng/ml or to no more than 10% at a concentration of 100 ng/ml. The polypeptide may comprise amino acid sequence of SEQ ID NO. 1.

In certain aspects, the present ~~invention~~ disclosure provides for a therapeutic composition. The composition comprises a polypeptide that binds to human RANKL. The polypeptide comprises a biologically active portion of human osteoprotegerin and a Fc portion of human immunoglobulin gamma-1. The polypeptide is substantially purified to a homo-dimeric form in solution. The polypeptide may exhibit a half-life in systemic circulation in mice of at least 80 hours after a subcutaneous administration at a dose of 5 mg/kg. The polypeptide may be capable of substantially inhibiting bone lysis induced by human breast cancer in a murine bone lytic model for at least 6 weeks in a course of subcutaneous administration of said composition at a dose of no more than 1 mg/kg three times weekly.

In certain aspects, the present ~~invention~~ disclosure provides for a therapeutic composition. The composition comprises a first amino acid sequence comprising amino acids 1 through 215 of human osteoprotegerin. The polypeptide further comprises a second amino acid sequence comprising amino acids 103 through 329 of human immunoglobulin gamma-1 Fc. The polypeptide may be in a substantially purified to a homo-dimeric form in solution. The polypeptide may exhibit a half-life in systemic circulation in mice of at least 80 hours after a subcutaneous administration at a dose of 5 mg/kg. The polypeptide may be capable of substantially inhibiting bone lysis induced by human breast cancer in a murine bone lytic model for at least 6 weeks in a course of subcutaneous administration of said composition at a dose of no more than 1 mg/kg three times weekly. The polypeptide may comprise amino acid sequence of SEQ ID NO. 1.

In certain aspects, the present ~~invention~~ disclosure provides for an isolated nucleic acid encoding a polypeptide that binds to human RANKL. The polypeptide comprises a first amino acid sequence comprising amino acids 1 through 215 of human osteoprotegerin. The polypeptide further comprises a second amino acid sequence comprising amino acids 103 through 329 of human immunoglobulin

gamma-1 Fc. The first amino acid sequence in the polypeptide may precede the second amino acid sequence. The polypeptide may comprise amino acid sequence of SEQ ID NO. 1. The nucleic acid may have its codon usage optimized for high expression of the polypeptide in a mammalian cell. The nucleic acid may comprise the sequence of SEQ ID NO. 2. The nucleic acid may comprise an expression vector.

In certain aspects, the present ~~invention~~ disclosure provides for a heterologous expression system. The expression system harbors an expression vector comprising a nucleic acid sequence encoding a polypeptide that binds to human RANKL. The polypeptide comprises a first amino acid sequence comprising amino acids 1 through 215 of human osteoprotegerin. The polypeptide further comprises a second amino acid sequence comprising amino acids 103 through 329 of human immunoglobulin gamma-1 Fc. The first amino acid sequence in the polypeptide may precede the second amino acid sequence. The polypeptide may comprise amino acid sequence of SEQ ID NO. 1. The expression vector of the expression system may be harbored in a mammalian cell. The mammalian cell may be a Chinese hamster ovaries (CHO) cell. The expression level of the polypeptide in the expression system may be at least 125 mg per liter of cell culture.

In certain aspects, the present ~~invention~~ disclosure provides for a use of a substance for manufacture of a medicament for the treatment or prevention of a disease associated with bone resorption or remodeling. The substance comprises a polypeptide that binds to human RANKL. The polypeptide comprises a first amino acid sequence comprising amino acids 1 through 215 of human osteoprotegerin. The polypeptide further comprises a second amino acid sequence comprising amino acids 103 through 329 of human immunoglobulin gamma-1 Fc. The first amino acid sequence in the polypeptide may precede the second amino acid sequence. The polypeptide may comprise amino acid sequence of SEQ ID NO. 1. The disease associated with bone resorption or remodeling may be a carcinoma, a breast cancer, a prostate cancer, multiple myeloma, a bone sarcoma, bone metastases due to solid tumors, osteoporosis, rheumatoid arthritis, or psoriatic arthritis.

In certain aspects, the present ~~invention~~ disclosure provides for a method of treating or preventing a disease associated with bone resorption or remodeling. The method comprises administering to a patient in need for treating or preventing a disease associated with bone resorption or remodeling a therapeutically effective amount of a pharmaceutical composition comprising a polypeptide that binds to human RANKL. The polypeptide comprises a first amino acid sequence comprising amino acids 1 through 215 of human osteoprotegerin. The polypeptide further comprises a second amino acid

sequence comprising amino acids 103 through 329 of human immunoglobulin gamma-1 Fc. The first amino acid sequence in the polypeptide may precede the second amino acid sequence. The polypeptide may comprise amino acid sequence of SEQ ID NO. 1. The disease associated with bone resorption or remodeling may be a carcinoma, a breast cancer, a prostate cancer, multiple myeloma, a bone sarcoma, bone metastases due to solid tumors, osteoporosis, rheumatoid arthritis, or psoriatic arthritis.

In another aspect, the present disclosure provides the use of a therapeutically effective amount of a pharmaceutical composition comprising a polypeptide comprising the sequence of SEQ ID NO. 1 in the manufacture of medicament for treating or preventing a disease or condition associated with bone remodeling.

These and other aspects and advantages of the ~~invention~~ disclosure described herein will become apparent upon consideration of the Figures and detailed description below.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings and descriptions are provided to aid in the understanding of the invention:

Figure 1 schematically shows the map of pCDNA4-Fc plasmid and annotated sequence used in the cloning of the polypeptides of the present invention;

Figure 2 schematically shows the map of pKN002 plasmid and annotated sequence used in the cloning of the polypeptides of the present invention;

Figure 3 shows representative transfection growth curves obtained in the process of generating stable cell lined for expressing the polypeptides of the present invention;

Figure 4 shown a size-exclusion HPLC analytical chromatogram of the sample containing polypeptide of SEQ ID NO. 1 after the anion exchange chromatography purification step;

Figure 5 shown a SDS-PAGE analysis (under reducing conditions) of the sample containing polypeptide of SEQ ID NO. 1 after the anion exchange chromatography purification step;

Figure 6 shown a hydroxylapatite chromatography elution profiled showing superior resolution of the sample containing polypeptide of SEQ ID NO. 1;

Figure 7 shown a size-exclusion HPLC analytical chromatogram of the sample of the main peak of the eluate containing polypeptide of SEQ ID NO. 1 from the hydroxylapatite chromatography;

Figure 8 shows X-ray images of tibiae of representative mice from group 1 (s.c. administration of formulation buffer) at eight weeks post-inoculation used for assessment of bone lysis (assigned scores are indicated below each image);

18. The expression system of claim 17, wherein said mammalian cell is a CHO cell.

19. The expression system of claim 17, wherein the level of said polypeptide expression is at least 125 mg per liter of cell culture.

20. Use of a substance for manufacture of a medicament for the treatment or prevention of a disease associated with bone remodeling, the substance comprising a polypeptide comprising the amino acid sequence of SEQ ID NO. 1.

21. The use according to claim 20, wherein said disease is a carcinoma.

22. The use according to claim 20, wherein said disease is a breast cancer.

23. The use according to claim 20, wherein said disease is a prostate cancer.

24. The use according to claim 20, wherein said disease is multiple myeloma.

25. The use according to claim 20, wherein said disease is a bone sarcoma.

26. The use according to claim 20, wherein said disease is bone metastases due to solid tumors.

27. The use according to claim 20, wherein said disease is osteoporosis.

28. The use according to claim 20, wherein said disease is rheumatoid arthritis.

29. The use according to claim 20, wherein said disease is psoriatic arthritis.

30. ~~A method of treating or preventing a disease or condition associated with bone remodeling, the method comprising administering to a patient in need for treating or preventing a disease associated with bone remodeling~~Use of a therapeutically effective amount of a pharmaceutical composition comprising a polypeptide comprising the sequence of SEQ ID NO. 1 in the manufacture of medicament for treating or preventing a disease or condition associated with bone remodeling.

31. The ~~method~~ use of claim 30, wherein said disease is a metastatic carcinoma.

32. The use according to claim 30, wherein said disease is a carcinoma.