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(54) Title: COMPOSITIONS AND METHODS RELATING TO MULTIMERIC AND OLIGOMERIC SOLUBLE FRAGMENTS OF THE TWEAK RECEPTOR

(57) Abstract: The present invention provides methods and compositions relating to fusion proteins comprising multimeric soluble TWEAK receptor fragments and an oligomerization domain. Such fusion proteins are useful for antagonizing the TWEAK receptor and for treating diseases or conditions mediated by angiogenesis, such as solid tumors and inflammatory conditions.

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TITLE

COMPOSITIONS AND METHODS RELATING TO MULTIMERIC AND OLIGOMERIC SOLUBLE FRAGMENTS OF THE TWEAK RECEPTOR

BACKGROUND OF THE INVENTION

Angiogenesis is a multi-step developmental process that results in the formation of new blood vessels off of existing vessels. This spatially and temporally regulated process involves loosening of matrix contacts and support cell interactions in the existing vessels by proteases, followed by coordinated movement, morphological alteration, and proliferation of the smooth muscle and endothelial cells of the existing vessel. The nascent cells then extend into the target tissue followed by cell-cell interactions in which the endothelial cells form tubes that the smooth muscle cells surround. In a coordinated fashion, extracellular matrix proteins of the vessel are secreted and peri-endothelial support cells are recruited to support and maintain structural integrity (see, *e.g.*, Daniel *et al.*, Ann. Rev. Physiol. 2000(62):649, 2000). Angiogenesis plays important roles in both normal and pathological physiology.

Under normal physiological conditions, angiogenesis is involved in fetal and embryonic development, wound healing, organ regeneration, and female reproductive remodeling processes including formation of the endometrium, corpus luteum, and placenta. Angiogenesis is stringently regulated under normal conditions, especially in adult animals, and perturbation of the regulatory controls can lead to pathological angiogenesis.

Pathological angiogenesis has been implicated in the manifestation and/or progression of inflammatory diseases, certain eye disorders, and cancer. In particular, several lines of evidence support the concept that angiogenesis is essential for the growth and persistence of solid tumors and their metastases (see, *e.g.*, Folkman, N. Engl. J. Med. 285:1182, 1971; Folkman *et al.*, Nature 339:58, 1989; Kim *et al.*, Nature 362:841, 1993; Hori *et al.*, Cancer Res., 51:6180, 1991). Angiogenesis inhibitors are therefore useful for the prevention (*e.g.*, treatment of premalignant conditions), intervention (*e.g.*, treatment of small tumors), and regression (*e.g.*, treatment of large tumors) of cancers (see, *e.g.*, Bergers *et al.*, Science 284:808, 1999).

There is a need for additional compositions and methods of modulating angiogenesis for the prevention, abrogation, and mitigation of disease.

The TWEAK protein, which has also been called TREPA and Apo3L, is a member of the tumor necrosis factor (TNF) family and is expressed in a wide variety of human tissues (Chicheportiche *et al.*, J. Biol. Chem., 272(51):32401, 1997; see also Wiley, PCT Publication No. WO 98/35061, 13 August 1998). Like most TNF family members, TWEAK is a Type II membrane protein with an extracellular C-terminal domain. Although TWEAK was originally described as a weak inducer of apoptosis, this induction of cell death was later shown to be indirect (Schneider *et al.*, Eur. J. Immunol. 29:1785, 1999).

Lynch *et al.* demonstrated that TWEAK directly induces endothelial cell proliferation and angiogenesis (J. Biol. Chem., 274(13):8455, 1999). Picomolar concentrations of recombinant soluble TWEAK induce proliferation in multiple endothelial cell lines and in aortic smooth muscle cells, and reduce the requirement for serum and growth factors in culture. Moreover, TWEAK induces a strong angiogenic response in a rat corneal pocket assay. Since TNF family members initiate biological responses by signaling through members of the TNF receptor family, there has been great interest in identifying and characterizing the TWEAK receptor.

Marsters *et al.* reported that TWEAK binds to and signals through a death-domain containing receptor known variously as DR3, Apo3, WSL-1, TRAMP, or LARD (Marsters *et al.*, Current Biology 8(9):525, 1998). Schneider *et al.*, however, showed that TWEAK binds to and signals in Kym-1 cells but that Kym-1 cells do not express the receptor DR3 (Schneider *et al.*, Eur. J. Immunol. 29:1785, 1999). Wiley subsequently identified the primary TWEAK receptor and described certain soluble fragments and variants of it that antagonize the wild-type TWEAK receptor (PCT Pub. No. WO 01/45730).

Because TWEAK induces angiogenesis *in vivo*, there is a particular need for antagonists of the major functional TWEAK receptor. Such TWEAK receptor antagonists would be useful for reducing angiogenesis and treating human diseases, including cancers and inflammatory diseases.

A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was, in Australia, known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

Throughout the description and the claims of this specification the word "comprise" and variations of the word, such as "comprising" and "comprises" is not intended to exclude other additives, components, integers or steps.

SUMMARY OF THE INVENTION

The present invention is based upon the identification and characterization of polypeptides comprising multimeric soluble fragments of the major functional TWEAK receptor (TWEAKR) and an oligomerization domain. Surprisingly, these polypeptides have a higher binding affinity for TWEAK and/or are better competitors for TWEAK binding than would be expected from the TWEAK binding and competition properties of polypeptides comprising soluble monomeric TWEAKR fragments and an oligomerization domain or comprising soluble multimeric TWEAKR fragments without an oligomerization domain.

The invention provides, for example, compositions and methods for inhibiting angiogenesis in a mammal in need of such treatment comprising administering a therapeutically effective amount of a composition comprising a TWEAK receptor antagonist. The composition preferably comprises a pharmaceutically acceptable carrier and the mammal is preferably a human.

In one aspect, the present invention provides a polypeptide comprising a first soluble fragment of a TWEAK receptor, a second soluble fragment of a TWEAK receptor, and an oligomerization domain,

In one aspect, the present invention provides a polypeptide comprising a first soluble fragment of a TWEAK receptor, a second soluble fragment of a TWEAK receptor, and an oligomerization domain, wherein said polypeptide binds to TWEAK, and said first soluble fragment consists of a sequence that is at least 90%, 95% or 100% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In one embodiment, said first soluble fragment consists of a sequence that is at least 95% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid

sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment consists of a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment and said second soluble fragment each independently consists of a sequence that is at least 90% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment and said second soluble fragment each independently consists of a sequence that is at least 90% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment and said second soluble fragment each independently consists of a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

In another embodiment, said polypeptide comprises a third soluble fragment of a TWEAK receptor. In another embodiment, said first soluble fragment, second soluble fragment, and third soluble fragment each independently consists of a sequence that is at least 90% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment, second soluble fragment, and third soluble fragment each independently consists of a sequence that is at least 95% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment, second soluble fragment, and third soluble fragment each independently consists of a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

In another embodiment, said polypeptide comprises a fourth soluble fragment of a TWEAK receptor. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, and fourth soluble fragment each independently consists of a sequence that is at least 90% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In

another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, and fourth soluble fragment each independently consists of a sequence that is at least 95% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, and fourth soluble fragment each independently consists of a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

In another embodiment, said polypeptide comprises a fifth soluble fragment of a TWEAK receptor. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, and fifth soluble fragment each independently consists of a sequence that is at least 90% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, and fifth soluble fragment each independently consists of a sequence that is at least 95% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, and fifth soluble fragment each independently consists of a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

In another embodiment, said polypeptide comprises a sixth soluble fragment of a TWEAK receptor. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, fifth soluble fragment, and sixth soluble fragment each independently consists of a sequence that is at least 90% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, fifth soluble fragment, and sixth soluble fragment each independently consists of a sequence that is at least 95% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said

first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, fifth soluble fragment, and sixth soluble fragment each independently consists of a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

In another embodiment, said polypeptide comprises a seventh soluble fragment of a TWEAK receptor. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, fifth soluble fragment, sixth soluble fragment, and seventh soluble fragment each independently consists of a sequence that is at least 90% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, fifth soluble fragment, sixth soluble fragment, and seventh soluble fragment each independently consists of a sequence that is at least 95% identical to a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7. In another embodiment, said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, fifth soluble fragment, sixth soluble fragment, and seventh soluble fragment each independently consists of a sequence selected from the group consisting of: residues 29 through 70 of the amino acid sequence of SEQ ID NO:7; residues 28 through 79 of the amino acid sequence of SEQ ID NO:7; residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

In another embodiment, said polypeptide comprises a linker. In another embodiment, said linker joins said first soluble TWEAKR fragment and said second soluble TWEAKR fragment. In another embodiment, said linker joins said second soluble TWEAKR fragment and said oligomerization domain. In another embodiment, said polypeptide comprises a first linker and a second linker, wherein said first linker joins said first soluble TWEAKR fragment and said second soluble TWEAKR fragment, and said second linker joins said second soluble TWEAKR fragment and said oligomerization domain. In another embodiment, said linker comprises the amino acid sequence GGGGG (SEQ ID NO:45).

In another embodiment, said first soluble fragment and said second soluble fragment are joined together without an intervening polypeptide sequence. In another embodiment, said first soluble fragment and said oligomerization domain are joined together without an intervening polypeptide sequence. In another embodiment, said first soluble fragment, said second soluble fragment, and said oligomerization domain are joined together, without an intervening polypeptide sequence, in a linear and contiguous polypeptide.

In another embodiment, said oligomerization domain is N-terminal to said first soluble TWEAKR fragment and to said second soluble TWEAKR fragment. In another embodiment, said oligomerization domain is C-terminal to said first soluble TWEAKR fragment and to said second soluble TWEAKR fragment. In another embodiment, said oligomerization domain comprises a leucine zipper. In another embodiment, said

oligomerization domain comprises a fragment of an antibody. In another embodiment, said fragment of an antibody comprises an Fc domain.

In another embodiment, said polypeptide comprises a sequence that is at least 90% identical to a sequence selected from the group consisting of: SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; SEQ ID NO:15; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:21; SEQ ID NO:23; SEQ ID NO:25; SEQ ID NO:27; SEQ ID NO:29; SEQ ID NO:31; SEQ ID NO:33; SEQ ID NO:35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; SEQ ID NO:43; and SEQ ID NO:44. In another embodiment, said polypeptide comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of: SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; SEQ ID NO:15; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:21; SEQ ID NO:23; SEQ ID NO:25; SEQ ID NO:27; SEQ ID NO:29; SEQ ID NO:31; SEQ ID NO:33; SEQ ID NO:35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; SEQ ID NO:43; and SEQ ID NO:44. In another embodiment, said polypeptide comprises a sequence selected from the group consisting of: SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; SEQ ID NO:15; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:21; SEQ ID NO:23; SEQ ID NO:25; SEQ ID NO:27; SEQ ID NO:29; SEQ ID NO:31; SEQ ID NO:33; SEQ ID NO:35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; SEQ ID NO:43; and SEQ ID NO:44.

In another aspect, the present invention provides a protein comprising a first said polypeptide and a second said polypeptide, wherein said first and second polypeptides are oligomerized to each other. In another embodiment, the amino acid sequence of said first polypeptide is identical to the amino acid sequence of said second polypeptide. In another embodiment, the amino acid sequence of said first polypeptide is not identical to the amino acid sequence of said second polypeptide.

In another aspect, the present invention provides a method of inhibiting a TWEAK receptor in a subject comprising administering to said subject said polypeptide.

In another aspect, the present invention provides a method of inhibiting angiogenesis in a subject comprising administering to said subject a therapeutically-effective amount of a composition comprising said polypeptide. In another embodiment, said composition further comprises a pharmaceutically acceptable carrier. In another embodiment, said subject is a mammal. In another embodiment, said mammal is a human. In another embodiment, said subject has a disease or condition mediated or exacerbated by angiogenesis. In another embodiment, said disease or condition is characterized by ocular neovascularization. In another embodiment, said disease or condition is a solid tumor. In another embodiment, said method further comprises treating said subject with radiation. In another embodiment, said method further comprises treating said subject with a second chemotherapeutic agent. In another embodiment, said second chemotherapeutic agent is selected from the group consisting of: an alkylating agent, an antimetabolite, a vinca alkaloid, a plant-derived chemotherapeutic, a nitrosourea, an antitumor antibiotic, an antitumor enzyme, a topoisomerase inhibitor, a platinum analog, an adrenocortical suppressant, a hormone, a hormone agonist, a hormone antagonist, an antibody, an immunotherapeutic, a blood cell factor, a radiotherapeutic, and a biological response modifier. In another embodiment, said second chemotherapeutic agent is selected from the group consisting of cisplatin, cyclophosphamide, mechloretamine, melphalan, bleomycin, carboplatin, fluorouracil, 5-fluorodeoxyuridine, methotrexate, taxol, asparaginase, vincristine, vinblastine, a lymphokine, a cytokine, an

interleukin, an interferon, alpha interferon, beta interferon, delta interferon, TNF, chlorambucil, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, cytarabine, mercaptopurine, thioguanine, vindesine, etoposide, teniposide, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, hydroxyurea, methylhydrazine, mitotane, tamoxifen, and fluoxymesterone. In another embodiment, said disease or condition is an inflammatory disease or condition. In another embodiment, said method further comprises treating said subject with a second therapeutic agent. In another embodiment, said second therapeutic agent inhibits a cytokine or a cytokine receptor that promotes inflammation. In another embodiment, said second therapeutic agent comprises a soluble fragment of said cytokine receptor, an antibody that binds said cytokine, or an antibody that binds said cytokine receptor. In another embodiment, said second therapeutic agent activates a receptor that inhibits inflammation. In another embodiment, said second therapeutic agent is selected from the group consisting of Flt3 ligand, CD40 ligand, interleukin-2, an interleukin-4 antagonist, an IL-13 antagonist, interleukin-12, 4-1BB ligand, an anti-4-1BB antibody, a TNF antagonist, a TNF receptor antagonist, TRAIL, a CD148 agonist, a VEGF antagonist, a VEGF receptor antagonist, an IgE antagonist, and a Tek antagonist.

In another aspect, the present invention provides a nucleic acid, or its complement, comprising a sequence that encodes said polypeptide. In another embodiment, said nucleic acid, or its complement, hybridizes under moderately stringent hybridization conditions to a second nucleic acid, and said second nucleic acid comprises a sequence selected from the group consisting of: SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:14; SEQ ID NO:16; SEQ ID NO:20; SEQ ID NO:22; SEQ ID NO:24; SEQ ID NO:26; SEQ ID NO:28; SEQ ID NO:30; SEQ ID NO:32; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; and SEQ ID NO:42. In another embodiment, said nucleic acid, or its complement, comprises a sequence that is at least 90% identical to a sequence selected from the group consisting of: SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:14; SEQ ID NO:16; SEQ ID NO:20; SEQ ID NO:22; SEQ ID NO:24; SEQ ID NO:26; SEQ ID NO:28; SEQ ID NO:30; SEQ ID NO:32; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; and SEQ ID NO:42. In another embodiment, said nucleic acid, or its complement, comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of: SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:14; SEQ ID NO:16; SEQ ID NO:20; SEQ ID NO:22; SEQ ID NO:24; SEQ ID NO:26; SEQ ID NO:28; SEQ ID NO:30; SEQ ID NO:32; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; and SEQ ID NO:42. In another embodiment, said nucleic acid, or its complement, comprises a sequence selected from the group consisting of: SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:14; SEQ ID NO:16; SEQ ID NO:20; SEQ ID NO:22; SEQ ID NO:24; SEQ ID NO:26; SEQ ID NO:28; SEQ ID NO:30; SEQ ID NO:32; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; and SEQ ID NO:42. In another embodiment, said nucleic acid encodes a polypeptide sequence selected from the group consisting of: SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; SEQ ID NO:15; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:21; SEQ ID NO:23; SEQ ID NO:25; SEQ ID NO:27; SEQ ID NO:29; SEQ ID NO:31; SEQ ID NO:33; SEQ ID NO:35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; SEQ ID NO:43; and SEQ ID NO:44.

In another aspect, the present invention provides a vector comprising said nucleic acid. In another embodiment, said vector is an expression vector.

In another aspect, the present invention provides a host cell comprising said nucleic acid.

In another aspect, the present invention provides a method of producing a polypeptide comprising culturing said host cell under conditions promoting expression of said polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a sequence alignment of the human and murine TWEAK receptor polypeptide sequences. The top sequence is the murine TWEAK receptor polypeptide (SEQ ID NO:5), and the bottom sequence is the human TWEAK receptor polypeptide (SEQ ID NO:4).

Figure 2 shows the effect of TWEAKR-Fc on PMA-induced HRMEC wound closure.

Figure 3 shows the effect of TWEAKR-Fc on EGF-induced HRMEC wound closure.

Figure 4 shows the effect of human TWEAKR-Fc on TWEAK-induced (100 ng/ml) HUVEC proliferation.

Figure 5 shows the effect of human TWEAKR-Fc on FGF-2-induced (10 ng/ml) HUVEC proliferation.

Figure 6 shows the binding of TWEAKR:Fc (SEQ ID NO:7; "monomer"), TWEAKR:DR5:Fc (SEQ ID NO:9, "DR5"), di-TWEAKR:Fc (SEQ ID NO:11, "dimer"), and tri-TWEAKR:Fc (SEQ ID NO:13, "trimer") to TWEAK-FLAG in an ELISA binding assay.

Figure 7 shows the ability of TWEAKR:Fc (SEQ ID NO:7; "hu+eu"), TWEAKR:DR5:Fc (SEQ ID NO:9, "DR5+eu"), di-TWEAKR:Fc (SEQ ID NO:11, "di+eu"), and tri-TWEAKR:Fc (SEQ ID NO:13, "tri+eu") to compete with europium labeled TWEAKR for binding to TWEAK in a competition binding assay.

Figure 8 shows the binding of TWEAKR:Gly5:Fc (SEQ ID NO:15; black circles), TWEAKR:1KPEG:Fc (SEQ ID NO:17, asterisks), TWEAKR:1KPEG:TWEAKR:Gly5:Fc (SEQ ID NO:18; black triangles), and TWEAKR:Gly5:TWEAKR:Gly5:Fc (SEQ ID NO:19; white circles) to TWEAK using an ELISA-style assay.

Figure 9 shows a comparison of TweakR-Fc oligomers binding to soluble TWEAK at 50 ng/ml using huTWEAKR:Fc (SEQ ID NO:7) (black squares), TWEAKR 43 monomer (SEQ ID NO:31) (white squares), TWEAKR 43 dimer (SEQ ID NO:33) (white triangles), TWEAKR 43 trimer (SEQ ID NO:35) (crosses), TWEAKR 43 tetramer (SEQ ID NO:37) (white diamonds), and TWEAKR 43 pentamer (SEQ ID NO:39) (white circles).

Figure 10 shows a comparison of TweakR-Fc oligomers binding to soluble TWEAK at 50 ng/ml using TWEAKR 43 tetramer (SEQ ID NO:37) (white diamonds), TWEAKR 43 pentamer (SEQ ID NO:39) (white circles), TWEAKR 43 hexamer (SEQ ID NO:41) (white triangles), and TWEAKR 43 heptamer (SEQ ID NO:43) (asterisks).

Figure 11 shows a comparison of TweakR-Fc oligomers binding to soluble TWEAK at 33 ng/ml using CHO TANDEM (SEQ ID NO:19 expressed in stably transfected CHO cells), TWEAKR 43 pentamer (SEQ ID NO:39) (white circles), TWEAKR 43 hexamer (SEQ ID NO:41) (white triangles), and TWEAKR 43 heptamer (SEQ ID NO:43) (asterisks).

Figure 12 shows a comparison of TweakR-Fc oligomers binding to soluble TWEAK at 50 ng/ml

using huTWEAKR:Fc (SEQ ID NO:7) (black squares), TWEAKR 40 monomer (SEQ ID NO:21) (white squares), TWEAKR 40 dimer (SEQ ID NO:23) (white triangles), TWEAKR 40 trimer (SEQ ID NO:25) (crosses), TWEAKR 40 tetramer (SEQ ID NO:27) (white diamonds), and TWEAKR 40 pentamer (SEQ ID NO:29) (white circles).

Figure 13 shows a comparison of TweakR-Fc oligomers vs Europium TweakR binding to immobilized TWEAK at 50 ng/ml using huTWEAKR:Fc (SEQ ID NO:7) (black squares), TWEAKR 40 monomer (SEQ ID NO:21) (white squares), TWEAKR 40 dimer (SEQ ID NO:23) (white triangles), TWEAKR 40 trimer (SEQ ID NO:25) (crosses), TWEAKR 40 tetramer (SEQ ID NO:27) (white diamonds), TWEAKR 40 pentamer (SEQ ID NO:29) (white circles).

Figure 14 shows a comparison of TweakR-Fc oligomers vs Europium TweakR binding to immobilized TWEAK at 50 ng/ml using huTWEAKR:Fc (SEQ ID NO:7) (black squares), TWEAKR 43 monomer (SEQ ID NO:31) (white squares), TWEAKR 43 dimer (SEQ ID NO:33) (white triangles), TWEAKR 43 trimer (SEQ ID NO:35) (crosses), TWEAKR 43 tetramer (SEQ ID NO:37) (white diamonds), TWEAKR 43 pentamer (SEQ ID NO:39) (white circles), TWEAKR 43 hexamer (SEQ ID NO:41) (vertical lines), and TWEAKR 43 heptamer (SEQ ID NO:43) (asterisks).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions and methods relating to polypeptides comprising multimers of soluble fragments of TWEAKR and an oligomerization domain.

Abbreviations and Terminology Used in the Specification

"4-1BB" and **"4-1BB ligand"** (4-1BB-L) are polypeptides described, *inter alia*, in U.S. Patent No. 5,674,704, including soluble forms thereof.

"bFGF" is basic fibroblast growth factor.

"BSA" is bovine serum albumin.

"CD40 ligand" (CD40L) is a polypeptide described, *inter alia*, in U.S. Patent No. 5,716,805, including soluble forms thereof.

"CHO" is a Chinese hamster ovary cell line.

"DMEM" is Dulbecco's Modified eagle Medium, a commercially available cell culture medium.

"ELISA" is Enzyme-Linked Immunosorbent Assay.

"Flt3L" is Flt3 ligand, a polypeptide described, *inter alia*, in U.S. Patent No. 5,554,512, including soluble forms thereof.

"HRMEC" are primary human renal microvascular endothelial cells.

"HUVEC" is a line of human umbilical vein endothelial cells.

"PBS" is phosphate buffered saline.

"PMA" is phorbol 12-myristate-13-acetate.

"RTKs" are receptor tyrosine kinases.

"Tek," which has also been called Tie2 and ork, is an RTK that is predominantly expressed in vascular endothelium. The molecular cloning of human Tek (ork) has been described by Ziegler, U.S. Patent

No. 5,447,860. **"Tek antagonists"** are described, *inter alia*, in Cerretti *et al.*, PCT Publication No. WO 00/75323, 14 December 2000.

"TNFR" is a tumor necrosis factor receptor, including soluble forms thereof. **"TNFR/Fc"** is a tumor necrosis factor receptor-Fc fusion polypeptide.

"TRAIL" is TNF-related apoptosis-inducing ligand, a type II transmembrane polypeptide in the TNF family described, *inter alia*, in U.S. Patent No. 5,763,223, including soluble forms thereof.

"VEGF" is vascular endothelial growth factor, also known as VPF or vascular permeability factor.

Soluble TWEAK Receptor Polypeptides

Native human TWEAK receptor cDNA has the sequence SEQ ID NO:3, which encodes a 129 residue polypeptide (SEQ ID NO:4). Examination of the DNA sequence predicts a polypeptide having an approximately 78 amino acid extracellular domain (residues 1-78 of SEQ ID NO:4, including the signal peptide), an approximately 23 amino acid transmembrane domain (residues 79-101 of SEQ ID NO:4), and an approximately 28 amino acid intracellular domain (residues 102-129 of SEQ ID NO:4). The TWEAK receptor sequence has also been reported by Kato *et al.*, PCT Publication No. WO 98/55508, 10 December 1998 and by Incyte, PCT Publication No. WO 99/61471, 02 December 1999. As used herein, "TWEAKR" includes polypeptides having these sequences, and in particular comprising amino acids 28-79 of SEQ ID NO:7, as well as naturally occurring variants thereof.

In one aspect of the invention, a polypeptide comprising a soluble TWEAK receptor fragment and an oligomerization domain is used as a TWEAKR antagonist to inhibit angiogenesis and/or to inhibit the binding of TWEAK ligand to TWEAKR.

Soluble polypeptides are capable of being secreted from the cells in which they are expressed. The use of soluble forms of polypeptides is advantageous for certain applications. Purification of the polypeptides from recombinant host cells is facilitated since the polypeptides are secreted, and soluble proteins are generally suited for parenteral administration. A secreted soluble polypeptide may be identified (and distinguished from its non-soluble membrane-bound counterparts) by separating intact cells which express the desired polypeptide from the culture medium, *e.g.*, by centrifugation, and assaying the medium (supernatant) for the presence of the desired polypeptide. The presence of the desired polypeptide in the medium indicates that the polypeptide was secreted from the cells and thus is a soluble form of the polypeptide. Soluble polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence encoding a desired soluble polypeptide may be subcloned into an expression vector for production of the polypeptide, or the desired encoding DNA fragment may be chemically synthesized.

Soluble TWEAKR polypeptides comprise all or part of the TWEAKR extracellular domain, but generally lack the transmembrane domain or a fragment thereof that would cause retention of the polypeptide at the cell surface. Soluble polypeptides may include part of the transmembrane domain or all or part of the cytoplasmic domain as long as the polypeptide is secreted from the cell in which it is produced. Soluble TWEAKR polypeptides advantageously comprise a native or heterologous signal peptide when initially synthesized, to promote secretion from the cell, but the signal sequence is cleaved upon secretion. The term "TWEAKR extracellular domain" is intended to encompass all or part of the native TWEAKR extracellular

domain, as well as related forms including but not limited to: (a) fragments, (b) variants, (c) derivatives, and (d) fusion polypeptides. The ability of these related forms to inhibit angiogenesis or other TWEAKR-mediated responses may be determined *in vitro* or *in vivo*, using methods such as those exemplified below or using other assays known in the art. Examples of soluble TWEAKR polypeptides are provided below.

Multimeric soluble TWEAKR fragments are dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, nonamers, decamers, or higher multimers of a soluble fragment of TWEAKR. Multimers may be linked together by any means known in the art. For example, they can be part of a continuous polypeptide chain, wherein each monomer can be linked to its neighboring monomer(s) directly via peptide bond(s), or indirectly, through one or more intervening amino acids and peptide bonds, *e.g.*, through a linker. Multimers also can be joined to each other by, for example, other types of covalent bonds, *e.g.*, by disulfide bonds formed between cysteine residues on different soluble TWEAKR polypeptides.

Oligomerization domains are polypeptides that cause polypeptides comprising them to oligomerize, *i.e.*, to form covalent and/or non-covalent associations with another polypeptide comprising a corresponding oligomerization domain. Thus, two or more polypeptides are "oligomerized" if they are bound to each other *via* their oligomerization domains. Any oligomerization domain known in the art can be used. Examples include leucine zippers and certain polypeptides derived from antibodies, *e.g.*, Fc domains, as described in more detail below. The polypeptides in an oligomer can have identical polypeptide sequences, similar polypeptide sequences, or different polypeptide sequences. In particular embodiments, the oligimerized polypeptides of the present invention comprise from four to fourteen soluble TWEAKR fragments.

In some embodiments, a polypeptide comprising a multimeric soluble TWEAKR fragment and an oligmerization domain is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi *et al.* (Proc. Natl. Acad. Sci. USA 88:10535, 1991); Byrn *et al.* (Nature 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1-10.19.11, 1992).

One embodiment of the present invention is directed to a TWEAKR-Fc oligomer comprising two polypeptides, each polypeptide comprising two soluble TWEAKR fragments and an Fc domain (diTWEAKR-Fc). A polynucleotide encoding the diTWEAKR-Fc polypeptide is inserted into an appropriate expression vector. diTWEAKR-Fc polypeptides are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield tetravalent soluble TWEAKR. The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included.

One suitable Fc polypeptide, described in PCT application WO 93/10151, is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and by Baum *et al.*, EMBO J. 13:3992, 1994. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino

acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors. Fusion polypeptides comprising Fc moieties, and multimers formed therefrom, offer an advantage of facile purification by affinity chromatography over Protein A or Protein G columns, and Fc fusion polypeptides may provide a longer *in vivo* half life, which is useful in therapeutic applications, than unmodified polypeptides.

In other embodiments, a multimeric soluble TWEAKR fragment may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a soluble TWEAKR oligomer with eight or more soluble TWEAKR fragments.

In another embodiment, the oligomerization domain comprises a leucine zipper domain. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz *et al.*, Science 240:1759, 1988), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe *et al.* FEBS Lett. 344:191, 1994. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow *et al.*, Semin. Immunol. 6:267, 1994. Recombinant fusion proteins comprising a soluble TWEAKR polypeptide fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble TWEAKR multimer that forms is recovered from the culture supernatant.

Alternatively, the polypeptides of the invention comprise peptide linkers (spacers). A linker is a sequence of one or more amino acids whose amino terminal end is peptide bonded to a first polypeptide and whose carboxy terminal end is peptide bonded to a second polypeptide such that the first polypeptide, the linker, and the second polypeptide form a contiguous sequence of amino acids. Such a linker is said to "join" the first polypeptide and the second polypeptide, in contrast to a first polypeptide and a second polypeptide that are joined together without an intervening polypeptide sequence (*i.e.*, without a linker). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180, 4,935,233, and 5,073,627. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, for example, the DNA sequences encoding TWEAKR fragments, and/or between the polynucleotide sequences encoding the TWEAKR fragments and the oligomerization domain, using conventional techniques known in the art. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between sequences encoding soluble TWEAKR fragments. In particular embodiments, a polypeptide of the invention comprises from two to four soluble TWEAKR fragments, separated by peptide linkers, and an oligomerization domain.

The present invention encompasses the use of various forms of oligomerized soluble TWEAKR multimers that inhibit angiogenesis and/or other TWEAKR-mediated responses. The term "oligomerized soluble TWEAKR multimer" is intended to encompass oligomerized multimers containing all or part of the native TWEAKR extracellular domain, as well as related forms including, but not limited to, oligomerized multimers of, fragments, variants, derivatives, and fusion polypeptides of soluble TWEAKR. The ability of these related forms to inhibit angiogenesis or other TWEAKR-mediated responses may be determined *in vitro*

or *in vivo*, using methods such as those exemplified in the examples or using other assays known in the art.

Among the polypeptides, multimers and oligomers useful in practicing the present invention are polypeptides comprising TWEAKR variants that retain the ability to bind ligand and/or inhibit angiogenesis or other TWEAKR-mediated responses. Such TWEAKR variants include polypeptides that are substantially homologous to native TWEAKR, but which have an amino acid sequence different from that of a native TWEAKR because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, TWEAKR polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native TWEAKR sequence. Included as variants of TWEAKR polypeptides are those variants that are naturally occurring, such as allelic forms and alternatively spliced forms, as well as variants that have been constructed by modifying the amino acid sequence of a TWEAKR polypeptide or the nucleotide sequence of a nucleic acid encoding a TWEAKR polypeptide.

Generally, substitutions for one or more amino acids present in the native polypeptide should be made conservatively. Examples of conservative substitutions include substitution of amino acids outside of the active domain(s), and substitution of amino acids that do not alter the secondary and/or tertiary structure of TWEAKR. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn, or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are known in the art.

In some preferred embodiments the TWEAKR variant is at least about 70% identical in amino acid sequence to the amino acid sequence of native TWEAKR; in some preferred embodiments the TWEAKR variant is at least about 80% identical in amino acid sequence to the amino acid sequence of native TWEAKR. In some more preferred embodiments the TWEAKR variant is at least about 90% identical in amino acid sequence to the amino acid sequence of native TWEAKR; in some more preferred embodiments the TWEAKR variant is at least about 95% identical in amino acid sequence to the amino acid sequence of native TWEAKR. In some most preferred embodiments the TWEAKR variant is at least about 98% identical in amino acid sequence to the amino acid sequence of native TWEAKR; in some most preferred embodiments the TWEAKR variant is at least about 99% identical in amino acid sequence to the amino acid sequence of native TWEAKR. Percent identity, in the case of both polypeptides and nucleic acids, may be determined by visual inspection. Percent identity may also be determined using the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970) as revised by Smith and Waterman (Adv. Appl. Math 2:482, 1981. Preferably, percent identity is determined by using a computer program, for example, the GAP computer program version 10.x available from the Genetics Computer Group (GCG; Madison, WI, see also Devereux *et al.*, *Nucl. Acids Res.* 12:387, 1984). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979 for amino acids; (2) a penalty of 30 (amino acids) or 50 (nucleotides) for each gap and an additional 1 (amino acids) or 3 (nucleotides) penalty for each symbol in each gap; (3) no penalty

for end gaps; and (4) no maximum penalty for long gaps. Other programs used by one skilled in the art of sequence comparison may also be used. For fragments of TWEAKR, the percent identity is calculated based on that portion of TWEAKR that is present in the fragment.

The present invention further encompasses the use of soluble TWEAKR polypeptides with or without associated native-pattern glycosylation. TWEAKR expressed in yeast or mammalian expression systems (*e.g.*, COS-1 or COS-7 cells) may be similar to or significantly different from a native TWEAKR polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of TWEAKR polypeptides in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Different host cells may also process polypeptides differentially, resulting in heterogeneous mixtures of polypeptides with variable N- or C-termini.

The primary amino acid structure of soluble TWEAKR polypeptides may be modified to create derivatives by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of TWEAKR may be prepared by linking particular functional groups to TWEAKR amino acid side chains or at the N-terminus or C-terminus of a TWEAKR polypeptide.

Fusion polypeptides of soluble TWEAKR that are useful in practicing the invention also include covalent or aggregative conjugates of a TWEAKR polypeptide with other polypeptides added to provide novel polyfunctional entities.

Recombinant Production of TWEAK Receptor Polypeptides

TWEAKR polypeptides, including soluble TWEAKR polypeptides, fragments, and fusion polypeptides, used in the present invention may be prepared using a recombinant expression system. Host cells transformed with a recombinant expression vector ("recombinant host cells") encoding the TWEAKR polypeptide are cultured under conditions that promote expression of TWEAKR and the TWEAKR is recovered. TWEAKR polypeptides can also be produced in transgenic plants or animals, or by chemical synthesis.

The invention encompasses nucleic acid molecules encoding the TWEAKR polypeptides used in the invention, including: (a) nucleic acids that encode residues 28-79 of SEQ ID NO:7 and fragments thereof that bind TWEAK; (b) nucleic acids that are at least 70%, 80%, 90%, 95%, 98%, or 99% identical to a nucleic acid of (a), and which encode a polypeptide capable of binding TWEAK; and (c) nucleic acids that hybridize at moderate stringency to a nucleic acid of (a), and which encode a polypeptide capable of binding TWEAK.

Due to degeneracy of the genetic code, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. Included as embodiments of the invention are nucleic acid sequences capable of hybridizing under moderately stringent conditions (*e.g.*, prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) to the DNA sequences encoding TWEAKR. The skilled artisan can determine additional combinations of salt and temperature that constitute moderate hybridization stringency (see also, Sambrook, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1989; Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1982; and Ausubel, *Current Protocols in Molecular Biology*,

Wiley and Sons, 1989 and later versions, which are incorporated herein by reference). Conditions of higher stringency include higher temperatures for hybridization and post-hybridization washes, and/or lower salt concentration. Percent identity of nucleic acids may be determined using the methods described above for polypeptides, *i.e.*, by methods including visual inspection and the use of computer programs such as GAP.

Any suitable expression system may be employed for the production of recombinant TWEAKR. Recombinant expression vectors include DNA encoding a TWEAKR polypeptide operably linked to suitable transcriptional and translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the TWEAKR DNA sequence. Thus, a promoter nucleotide sequence is operably linked to a TWEAKR DNA sequence if the promoter nucleotide sequence controls the transcription of the TWEAKR DNA sequence. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. A sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (referred to by a variety of names including secretory leader, leader peptide, or leader) may be fused in frame to the TWEAKR sequence so that the TWEAKR polypeptide is initially translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the TWEAKR polypeptide. The signal peptide is cleaved from the TWEAKR polypeptide upon secretion of TWEAKR from the cell.

Suitable host cells for expression of TWEAKR polypeptides include prokaryotes, yeast and higher eukaryotic cells, including insect and mammalian cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, insect, and mammalian cellular hosts are described, for example, in Pouwels *et al. Cloning Vectors: A Laboratory Manual,* Elsevier, New York, 1985.

Prokaryotes include gram negative or gram positive organisms, for example, *E. coli* or *Bacilli.* Suitable prokaryotic host cells for transformation include, for example, *E. coli, Bacillus subtilis, Salmonella typhimurium,* and various other species within the genera *Pseudomonas, Streptomyces,* and *Staphylococcus.* In a prokaryotic host cell, such as *E. coli*, TWEAKR polypeptides may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant polypeptide.

Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker gene(s). A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. An appropriate promoter and a TWEAKR DNA sequence are inserted into the pBR322 vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA).

Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include

β-lactamase (penicillinase), lactose promoter system (Chang *et al.*, Nature 275:615, 1978; Goeddel *et al.*, Nature 281:544, 1979), tryptophan (trp) promoter system (Goeddel *et al.*, Nucl. Acids Res. 8:4057, 1980; EP-A-36776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful prokaryotic host cell expression system employs a phage λ P_L promoter and a cI857ts thermolabile repressor sequence. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2 (resident in *E. coli* strain JMB9, ATCC 37092) and pPLc28 (resident in *E. coli* RR1, ATCC 53082).

TWEAKR polypeptides may also be expressed in yeast host cells, preferably from the *Saccharomyces* genus (*e.g.*, *S. cerevisiae*). Other genera of yeast, such as *Pichia* or *Kluyveromyces*, may also be employed. Yeast vectors will often contain an origin of replication sequence from a 2μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem. 255:2073, 1980) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg. 7:149, 1968; Holland *et al.*, Biochem. 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phospho-glucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657. Another alternative is the glucose-repressible ADH2 promoter described by Russell *et al.* (J. Biol. Chem. 258:2674, 1982) and Beier *et al.* (Nature 300:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

The yeast α-factor leader sequence may be employed to direct secretion of recombinant polypeptides. The α-factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. *See, e.g.*, Kurjan *et al.*, Cell 30:933, 1982; Bitter *et al.*, *Proc. Natl. Acad. Sci. USA 81*:5330, 1984. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen *et al.*, Proc. Natl. Acad. Sci. USA 75:1929, 1978. The Hinnen *et al.* protocol selects for Trp^+ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μg/ml adenine and 20 μg/ml uracil.

Yeast host cells transformed by vectors containing an ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μg/ml adenine and 80 μg/ml uracil. Derepression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Insect host cell culture systems also may be employed to express recombinant TWEAKR polypeptides, including soluble TWEAKR polypeptides. Bacculovirus systems for production of heterologous

polypeptides in insect cells are reviewed by Luckow and Summers, Bio/Technology 6:47, 1988.

Mammalian cells are particularly preferred for use as host cells. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman *et al.*, Cell *23*:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan *et al.* (EMBO J. 10: 2821, 1991). For the production of therapeutic polypeptides it is particularly advantageous to use a mammalian host cell line which has been adapted to grow in media that does not contain animal proteins.

Established methods for introducing DNA into mammalian cells have been described (Kaufman, R.J., *Large Scale Mammalian Cell Culture*, 1990, pp. 15-69). Additional protocols using commercially available reagents, such as Lipofectamine (Gibco/BRL) or Lipofectamine-Plus, can be used to transfect cells (Felgner *et al.*, Proc. Natl. Acad. Sci. USA 84:7413, 1987). In addition, electroporation can be used to transfect mammalian cells using conventional procedures, such as those in Sambrook *et al. Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1-3, Cold Spring Harbor Laboratory Press, 1989). Selection of stable transformants can be performed using methods known in the art, such as, for example, resistance to cytotoxic drugs. Kaufman *et al.*, Meth. in Enzymology 185:487, 1990, describes several selection schemes, such as dihydrofolate reductase (DHFR) resistance. A suitable host strain for DHFR selection can be CHO strain DX-B11, which is deficient in DHFR (Urlaub and Chasin, Proc. Natl. Acad. Sci. USA *77*:4216, 1980). A plasmid expressing the DHFR cDNA can be introduced into strain DX-B11, and only cells that contain the plasmid can grow in the appropriate selective media. Other examples of selectable markers that can be incorporated into an expression vector include cDNAs conferring resistance to antibiotics, such as G418 and hygromycin B. Cells harboring the vector can be selected on the basis of resistance to these compounds.

Transcriptional and translational control sequences for mammalian host cell expression vectors can be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from polyoma virus, adenovirus 2, simian virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites can be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment, which can also contain a viral origin of replication (Fiers *et al.*, Nature 273:113, 1978; Kaufman, Meth. in Enzymology, 1990). Smaller or larger SV40 fragments can also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Additional control sequences shown to improve expression of heterologous genes from mammalian expression vectors include such elements as the expression augmenting sequence element (EASE) derived from CHO cells (Morris *et al.*, *Animal Cell Technology*, 1997, pp. 529-534) and the tripartite leader (TPL) and VA gene RNAs from Adenovirus 2 (Gingeras *et al.*, J. Biol. Chem. 257:13475, 1982). The internal ribosome entry site (IRES) sequences of viral origin allows dicistronic mRNAs to be translated efficiently (Oh and Sarnow, Current Opinion in Genetics and Development 3:295, 1993; Ramesh *et al.*, Nucleic Acids Research 24:2697, 1996). Expression of a heterologous cDNA as part of a dicistronic mRNA followed by the gene for

a selectable marker (*e.g.* DHFR) has been shown to improve transfectability of the host and expression of the heterologous cDNA (Kaufman, Meth. in Enzymology, 1990). Exemplary expression vectors that employ dicistronic mRNAs are pTR-DC/GFP described by Mosser *et al.*, Biotechniques 22:150, 1997, and p2A5I described by Morris *et al.*, *Animal Cell Technology*, 1997, pp. 529-534.

A useful high expression vector, pCAVNOT, has been described by Mosley *et al.*, Cell 59:335, 1989. Other expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (Mol. Cell. Biol. 3:280, 1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman *et al.* (Mol. Immunol. 23:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman *et al.*, Nature 312:768, 1984, has been deposited as ATCC 39890. Additional useful mammalian expression vectors are known in the art.

Regarding signal peptides that may be employed in producing TWEAKR polypeptides, the native TWEAKR signal peptide may used or it may be replaced by a heterologous signal peptide or leader sequence, if desired. The choice of signal peptide or leader may depend on factors such as the type of host cells in which the recombinant TWEAKR is to be produced. Examples of heterologous signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195, the signal sequence for interleukin-2 receptor described in Cosman *et al.*, Nature 312:768 (1984); the interleukin-4 receptor signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

Using the techniques of recombinant DNA including mutagenesis and the polymerase chain reaction (PCR), the skilled artisan can produce DNA sequences that encode TWEAKR polypeptides comprising various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences, including TWEAKR fragments, variants, derivatives, and fusion polypeptides.

Transgenic animals, including mice, goats, sheep, and pigs, and transgenic plants, including tobacco, tomato, legumes, grasses, and grains, may also be used as bioreactors for the production of TWEAKR polypeptides, including soluble TWEAKR polypeptides. In the case of transgenic animals, it is particularly advantageous to construct a chimeric DNA including a TWEAKR coding sequence operably linked to cis-acting regulatory sequences that promote expression of the soluble TWEAKR in milk and/or other body fluids (see, *e.g.*, U.S. Patent No. 5,843,705; U.S. Patent No. 5,880,327). In the case of transgenic plants it is particularly advantageous to produce TWEAKR in a particular cell type, tissue, or organ (see, *e.g.*, US Patent No. 5,639,947; U.S. Patent No. 5,889,189).

The skilled artisan will recognize that the procedure for purifying expressed soluble TWEAKR polypeptides will vary according to the host system employed, and whether or not the recombinant polypeptide is secreted. Soluble TWEAKR polypeptides may be purified using methods known in the art, including one or more concentration, salting-out, ion exchange, hydrophobic interaction, affinity purification, HPLC, or size exclusion chromatography steps. Fusion polypeptides comprising Fc moieties (and multimers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

Methods of Treatment

Described below are methods and compositions employing the TWEAK receptor or ligand, or the genes encoding the TWEAK receptor or ligand, to promote or suppress angiogenesis in a target tissue or group of cells. The terms "treat," "treating," "treatment," "therapy," "therapeutic," and the like are intended to include preventative therapy, prophylactic therapy, ameliorative therapy, and curative therapy.

The disclosed polypeptides, compositions, and methods are used to inhibit angiogenesis or other TWEAKR-mediated responses in a mammal in need of such treatment. The term "TWEAKR-mediated response" includes any cellular, physiological, or other biological response that is caused at least in part by the binding of TWEAK ligand to TWEAKR, or which may be inhibited or suppressed, in whole or in part, by blocking TWEAK from binding to TWEAKR. The treatment is advantageously administered in order to prevent the onset or the recurrence of a disease or condition mediated by angiogenesis, or to treat a mammal that has a disease or condition mediated by angiogenesis. Diseases and conditions mediated by angiogenesis include but are not limited to ocular disorders, malignant and metastatic conditions, and inflammatory diseases.

Among the ocular disorders that can be treated according to the present invention are eye diseases characterized by ocular neovascularization including, but not limited to, diabetic retinopathy (a major complication of diabetes), retinopathy of prematurity (this devastating eye condition, that frequently leads to chronic vision problems and carries a high risk of blindness, is a severe complication during the care of premature infants), neovascular glaucoma, retinoblastoma, retrolental fibroplasia, rubeosis, uveitis, macular degeneration, and corneal graft neovascularization. Other eye inflammatory diseases, ocular tumors, and diseases associated with choroidal or iris neovascularization can also be treated according to the present invention.

The present invention can also be used to treat malignant and metastatic conditions such as solid tumors. Solid tumors include both primary and metastatic sarcomas and carcinomas.

The present invention can also be used to treat inflammatory diseases including, but not limited to, arthritis, rheumatism, and psoriasis.

Other diseases and conditions that can be treated according to the present invention include benign tumors and preneoplastic conditions, myocardial angiogenesis, hemophilic joints, scleroderma, vascular adhesions, atherosclerotic plaque neovascularization, telangiectasia, and wound granulation.

Disease states that are angiogenic-dependent include coronary or peripheral atherosclerosis and ischemia of any tissue or organ, including the heart, liver, brain, and the like. These types of diseases can be treated by compositions that promote angiogenesis.

The methods according to the present invention can be tested in *in vivo* animal models to confirm the desired prophylactic or therapeutic activity, as well as to determine the optimal therapeutic dosage, prior to administration to humans.

The amount of a particular TWEAKR antagonist that will be effective in a particular method of treatment depends upon age, type and severity of the condition to be treated, body weight, desired duration of treatment, method of administration, and other parameters. Effective dosages are determined by a physician

or other qualified medical professional. Typical effective dosages are about 0.01 mg/kg to about 100 mg/kg body weight. In some preferred embodiments the dosage is about 0.1-50 mg/kg; in some preferred embodiments the dosage is about 0.5-10 mg/kg. The dosage for local administration is typically lower than for systemic administration. In some embodiments a single administration is sufficient; in some embodiments the TWEAKR antagonist is administered as multiple doses over one or more days.

The TWEAKR antagonists are typically administered in the form of a pharmaceutical composition comprising one or more pharmacologically acceptable carriers. Pharmaceutically acceptable carriers include diluents, fillers, adjuvants, excipients, and vehicles which are pharmaceutically acceptable for the route of administration, and may be aqueous or oleaginous suspensions formulated using suitable dispersing, wetting, and suspending agents.

Pharmaceutically acceptable carriers are generally sterile and free of pyrogenic agents, and may include water, oils, solvents, salts, sugars and other carbohydrates, emulsifying agents, buffering agents, antimicrobial agents, and chelating agents. The particular pharmaceutically acceptable carrier and the ratio of active compound to carrier are determined by the solubility and chemical properties of the composition, the mode of administration, and standard pharmaceutical practice.

The compositions as described herein may be contained in a vial, bottle, tube, syringe inhaler or other container for single or multiple administrations. Such containers may be made of glass or a polymer material such as polypropylene, polyethylene, or polyvinylchloride, for example. Preferred containers may include a seal, or other closure system, such as a rubber stopper that may be penetrated by a needle in order to withdraw a single dose and then re-seal upon removal of the needle. All such containers for injectable liquids, lyophilized formulations, reconstituted lyophilized formulations or reconstitutable powders for injection known in the art or for the administration of aerosolized compositions are contemplated for use in the presently disclosed compositions and methods.

The TWEAKR antagonists are administered to the patient in a manner appropriate to the indication. Thus, for example, a TWEAKR antagonist, or a pharmaceutical composition thereof, may be administered by intravenous, transdermal, intradermal, intraperitoneal, intramuscular, intranasal, epidural, oral, topical, subcutaneous, intracavity, sustained release from implants, peristaltic routes, or by any other suitable technique. Parenteral administration is preferred.

In certain embodiments of the claimed invention, the treatment further comprises treating the mammal with one or more additional chemotherapeutic agents. The additional chemotherapeutic agent(s) may be administered prior to, concurrently with, or following the administration of the TWEAKR antagonist. The use of more than one chemotherapeutic agent is particularly advantageous when the mammal that is being treated has a solid tumor. In some embodiments of the claimed invention, the treatment further comprises treating the mammal with radiation. Radiation, including brachytherapy and teletherapy, may be administered prior to, concurrently with, or following the administration of the second chemotherapeutic agent(s) and/or TWEAKR antagonist.

When the mammal that is being treated has a solid tumor, the method preferably includes the administration of, in addition to a TWEAKR antagonist, one or more chemotherapeutic agents selected from the group consisting of alkylating agents, antimetabolites, vinca alkaloids and other plant-derived

chemotherapeutics, nitrosoureas, antitumor antibiotics, antitumor enzymes, topoisomerase inhibitors, platinum analogs, adrenocortical suppressants, hormones, hormone agonists and antagonists, antibodies, immunotherapeutics, blood cell factors, radiotherapeutics, and biological response modifiers.

In some preferred embodiments the method includes administration of, in addition to a TWEAKR antagonist, one or more chemotherapeutic agents selected from the group consisting of cisplatin, cyclophosphamide, mechloretamine, melphalan, bleomycin, carboplatin, fluorouracil, 5-fluorodeoxyuridine, methotrexate, taxol, asparaginase, vincristine, and vinblastine, lymphokines and cytokines such as interleukins, interferons (including alpha, beta, or delta), and TNF, chlorambucil, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, cytarabine, mercaptopurine, thioguanine, vindesine, etoposide, teniposide, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, hydroxyurea, methylhydrazine, mitotane, tamoxifen, and fluoxymesterone.

In some preferred embodiments the method includes administration of, in addition to a TWEAKR antagonist, one or more chemotherapeutic agents, including various soluble forms thereof, selected from the group consisting of Flt3 ligand, CD40 ligand, interleukin-2, interleukin-12, 4-1BB ligand, anti-4-1BB antibodies, TNF antagonists and TNF receptor antagonists, TRAIL, VEGF antagonists, VEGF receptor (including VEGF-R1 and VEGF-R2, also known as Flt1 and Flk1 or KDR) antagonists, Tek antagonists, and CD148 (also referred to as DEP-1, ECRTP, and PTPRJ, see Takahashi *et al.*, J. Am. Soc. Nephrol. 10:2135-45, 1999) agonists. In some preferred embodiments the TWEAKR antagonists of the invention are used as a component of, or in combination with, "metronomic therapy," such as that described by Browder *et al.* and Klement *et al.* (Cancer Research 60:1878, 2000; J. Clin. Invest. 105(8):R15, 2000; see also Barinaga, Science 288:245, 2000).

The polypeptides, compositions, and methods of the present invention may be used as a first line treatment, for the treatment of residual disease following primary therapy, or as an adjunct to other therapies including chemotherapy, surgery, radiation, and other therapeutic methods known in the art.

When the nucleic acid sequences of the present invention are delivered according to the methods disclosed herein, it is advantageous to use a delivery mechanism so that the sequences will be incorporated into a cell for expression. Delivery systems that may advantageously be employed in the contemplated methods include the use of, for example, viral delivery systems such as retroviral and adenoviral vectors, as well as non-viral delivery systems. Such delivery systems are well known by those skilled in the art.

Methods of Screening

The TWEAK receptor as described herein may be used in a variety of methods of screening to isolate, for example, TWEAKR agonists and antagonists. TWEAKR agonists are compounds that promote the biological activity of TWEAKR and TWEAKR antagonists are compounds that inhibit the biological activity of TWEAKR. Compounds identified via the following screening assays can be used in compositions and methods for modulating angiogenesis to treat a variety of disease states. The present invention provides methods of screening for compounds that (1) modulate TWEAK receptor or ligand gene expression in a target tissue or cell, (2) modulate the TWEAK receptor-ligand interaction to regulate angiogenesis; (3) bind to the TWEAK receptor or ligand to influence angiogenesis; or (4) interfere with or regulate the bound TWEAK

receptor-ligand complex's influence on downstream events such as angiogenesis.

The present invention contemplates the use of assays that are designed to identify compounds that modulate the activity of a TWEAK receptor or ligand gene (*i.e.*, modulate the level of TWEAK gene expression and/or modulate the level of TWEAK gene product activity). Assays may additionally be utilized that identify compounds that bind to TWEAK gene regulatory sequences (*e.g.*, promoter sequences; see *e.g.*, Platt, 1994, J. Biol. Chem. 269, 28558-28562), and that may modulate the level of TWEAK gene expression.

Such an assay may involve, for example, the use of a control system, in which transcription and translation of the TWEAK receptor or ligand gene occurs, in comparison to a system including a test compounds suspected of influencing normal transcription or translation of a TWEAK gene. For example, one could determine the rate of TWEAK receptor RNA produced by cardiac cells, and use this to determine if a test compound influences that rate. To assess the influence of a test compound suspected to influence this normal rate of transcription, one would first determine the rate of TWEAK receptor RNA production in a cardiac cell culture by, for example, Northern Blotting. One could then administer the test compound to a cardiac cell culture under otherwise identical conditions as the control culture. Then the rate of TWEAK receptor RNA in the culture treated with the test compound could be determined by, for example, Northern Blotting, and compared to the rate of TWEAK receptor RNA produced by the control culture cells. An increase in the TWEAK receptor RNA in the cells contacted with the test compound relative to control cells is indicative of a stimulator of TWEAK receptor gene transcription and/or translation in cardiac cells, while a decrease is indicative of an inhibitor of TWEAK receptor gene transcription and/or translation in cardiac cells.

There are a variety of other methods that can be used to determine the level of TWEAK receptor or ligand gene expression as well, and may further be used in assays to determine the influence of a test compound on the level of TWEAK receptor or ligand gene expression. For example, RNA from a cell type or tissue known, or suspected, to express the TWEAK receptor or ligand gene, such as cardiac, may be isolated and tested utilizing hybridization or PCR techniques. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells to be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the TWEAK receptor or ligand gene. Such analyses may reveal both quantitative and qualitative aspects of the expression pattern of the TWEAK receptor or ligand gene, including activation or inactivation of TWEAK receptor or ligand gene expression.

In one embodiment of such a detection scheme, a cDNA molecule is synthesized from an RNA molecule of interest (*e.g.*, by reverse transcription of the RNA molecule into cDNA). A sequence within the cDNA is then used as the template for a nucleic acid amplification reaction, such as a PCR amplification reaction, or the like. The nucleic acid reagents used as synthesis initiation reagents (*e.g.*, primers) in the reverse transcription and nucleic acid amplification steps of this method are chosen from among the TWEAK receptor or ligand gene nucleic acid segments described above. The preferred lengths of such nucleic acid reagents are at least 9-30 nucleotides. For detection of the amplified product, the nucleic acid amplification may be performed using radioactively or non-radioactively labeled nucleotides. Alternatively, enough amplified product may be made such that the product may be visualized by standard ethidium bromide staining or by utilizing any other suitable nucleic acid staining method.

Additionally, it is possible to perform such TWEAK receptor or ligand gene expression assays "in situ", *i.e.*, directly upon tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections, such that no nucleic acid purification is necessary. TWEAK receptor or ligand gene nucleic acid segments described above can be used as probes and/or primers for such in situ procedures (see, for example, Nuovo, G. J., 1992, "PCR In Situ Hybridization: Protocols And Applications", Raven Press, NY).

Compounds identified via assays such as those described herein may be useful, for example, in modulating angiogenesis influenced by the TWEAK receptor-ligand interaction. Such methods of stimulating or inhibiting TWEAK-influenced angiogenesis are discussed herein.

Alternatively, assay systems may be designed to identify compounds capable of binding the TWEAK receptor or ligand polypeptide of the invention and thereby influencing angiogenesis resulting from this interaction. Compounds identified may be useful, for example, in modulating the vascularization of target tissues or cells, may be utilized in screens for identifying compounds that disrupt normal TWEAK receptor-ligand interactions, or may in themselves disrupt such interactions.

The principle of the assays used to identify compounds that bind to the TWEAK receptor or ligand involves preparing a reaction mixture of the TWEAK receptor or ligand and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected in the reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay screening for compounds that bind to the TWEAK receptor, would involve anchoring the TWEAK receptor or the test substance onto a solid phase and detecting TWEAK receptor/test compound complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, the TWEAK receptor may be anchored onto a solid surface, and the test compound, which is not anchored, may be labeled, either directly or indirectly. Alternatively, these same methods could be used to screen for test compounds that bind to the TWEAK ligand rather than receptor.

In practice, microtiter plates may conveniently be utilized as the solid phase. The anchored component may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be immobilized may be used to anchor the protein to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the previously non-immobilized component (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for the TWEAK

receptor or ligand or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for the other component of the possible complex to detect anchored complexes.

Those compounds identified as binding agents for either the TWEAK receptor or the TWEAK ligand may further be assessed for their ability to interfere with TWEAK receptor-ligand interaction, as described below, and thereby suppress or promote angiogenesis resulting from TWEAK receptor-ligand interaction. Such compounds may then be used therapeutically to stimulate or inhibit angiogenesis.

The TWEAK receptor and ligand polypeptides of the present invention may also be used in a screening assay to identify compounds and small molecules which specifically interact with the disclosed TWEAK receptor or ligand to either inhibit (antagonize) or enhance (agonize) interaction between these molecules. Thus, for example, polypeptides of the invention may be used to identify antagonists and agonists from cells, cell-free preparations, chemical libraries, and natural product mixtures. The antagonists and agonists may be natural or modified substrates, ligands, enzymes, receptors, *etc.* of the polypeptides of the instant invention, or may be structural or functional mimetics of the polypeptides. Potential antagonists of the TWEAK receptor-ligand interaction of the instant invention may include small molecules, peptides, and antibodies that bind to and occupy a binding site of the polypeptides, causing them to be unavailable to interact and therefore preventing their normal ability to modulate angiogenesis. Other potential antagonists are antisense molecules which may hybridize to mRNA *in vivo* and block translation of the mRNA into the polypeptides of the instant invention. Potential agonists include small molecules, peptides and antibodies which bind to the instant TWEAK polypeptides and influence angiogenesis as caused by the disclosed interactions of the TWEAK polypeptides of the instant invention.

Small molecule agonists and antagonists are usually less than 10K molecular weight and may possess a number of physiochemical and pharmacological properties that enhance cell penetration, resist degradation and prolong their physiological half-lives. (Gibbs, "Pharmaceutical Research in Molecular Oncology," *Cell*, Vol. 79, (1994).) Antibodies, which include intact molecules as well as fragments such as Fab and F(ab')2 fragments, may be used to bind to and inhibit the polypeptides of the instant invention by blocking the commencement of a signaling cascade. It is preferable that the antibodies are humanized, and more preferable that the antibodies are human. The antibodies of the present invention may be prepared by any of a variety of well-known methods.

Specific screening methods are known in the art and many are extensively incorporated in high throughput test systems so that large numbers of test compounds can be screened within a short amount of time. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, cell based assays, *etc.* These assay formats are well known in the art. The screening assays of the present invention are amenable to screening of chemical libraries and are suitable for the identification of small molecule drug candidates, antibodies, peptides and other antagonists and agonists.

One embodiment of a method for identifying molecules which antagonize or inhibit TWEAK receptor-ligand interaction involves adding a candidate molecule to a medium which contains cells that express the polypeptides of the instant invention; changing the conditions of said medium so that, but for the presence of the candidate molecule, the polypeptides would interact; and observing the binding and inhibition

of angiogenesis. Binding of the TWEAK receptor and ligand can be determined according to competitive binding assays outlined above, and well known in the art. The angiogenic effect of this binding can be determined *via* cell proliferation assays such as, for example, cell density assays, or other cell proliferation assays that are also well-known in the art. The activity of the cells contacted with the candidate molecule may then be compared with the identical cells which were not contacted and agonists and antagonists of the TWEAK polypeptide interactions of the instant invention may be identified. The measurement of biological activity may be performed by a number of well-known methods such as measuring the amount of protein present (*e.g.* an ELISA) or of the protein's activity. A decrease in biological stimulation or activation would indicate an antagonist. An increase would indicate an agonist.

Screening assays can further be designed to find molecules that mimic the biological activity resulting from the TWEAK polypeptide interactions of the instant invention. Molecules which mimic the biological activity of a polypeptide may be useful for enhancing the biological activity of the polypeptide. To identify compounds for therapeutically active agents that mimic the biological activity of a polypeptide, it must first be determined whether a candidate molecule binds to the polypeptide. A binding candidate molecule is then added to a biological assay to determine its biological effects. The biological effects of the candidate molecule are then compared to those of the polypeptide.

Additionally, complex formation within reaction mixtures containing the test compound and normal TWEAK receptor or ligand gene protein may also be compared to complex formation within reaction mixtures containing the test compound and a mutant TWEAK receptor or ligand gene protein. This comparison may be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal TWEAK receptor or ligand gene proteins.

The assay for compounds that interfere with the interaction of the TWEAK receptor or ligand gene products and binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the TWEAK receptor or ligand gene product or the binding partner onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the TWEAK receptor or ligand gene products and the binding partners, *e.g.*, by competition, can be identified by conducting the reaction in the presence of the test substance; *i.e.*, by adding the test substance to the reaction mixture prior to or simultaneously with the TWEAK receptor and ligand gene products. Alternatively, test compounds that disrupt preformed complexes, *e.g.*, compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the TWEAK receptor or ligand gene product, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The anchored species may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished simply by coating the solid surface with a solution of the TWEAK receptor or ligand gene product and drying. Alternatively, an immobilized antibody

specific for the species to be anchored may be used to anchor the species to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the TWEAK receptor or ligand gene product is prepared in which either the TWEAK receptor or ligand gene product or its binding partners is labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Pat. No. *4,109,496* by Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt TWEAK receptor or ligand gene product interaction can be identified.

In a particular embodiment, the TWEAK receptor or ligand gene product can be prepared for immobilization using recombinant DNA techniques. For example, the TWEAK receptor or ligand coding region can be fused to a glutathione-S-transferase (GST) gene using a fusion vector, such as pGEX-5X-1, in such a manner that its binding activity is maintained in the resulting fusion protein. The interactive binding partner can be purified and used to raise a monoclonal antibody, using methods routinely practiced in the art. This antibody can be labeled with the radioactive isotope <125> I, for example, by methods routinely practiced in the art. In a heterogeneous assay, *e.g.*, the GST-TWEAK receptor or ligand fusion protein can be anchored to glutathione-agarose beads. The TWEAK receptor or ligand gene product can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal antibody can be added to the system and allowed to bind to the complexed components. The interaction between the TWEAK receptor and ligand gene products can be detected by measuring the amount of radioactivity that remains associated with the glutathione-agarose beads. A successful inhibition of the interaction by the test compound will result in a decrease in measured radioactivity.

Alternatively, a GST-TWEAK receptor gene fusion protein and TWEAK ligand gene product (or *vice versa*) can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound can be added either during or after the species are allowed to interact. This mixture can then be added to the glutathione-agarose beads and unbound material is washed away. Again the extent of inhibition of the TWEAK receptor-ligand gene product interaction can be detected by adding the labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of the TWEAK receptor and/or ligand protein, in place of one or both of the full length proteins. Any number of methods routinely practiced in the art can be used to identify and isolate the binding sites. These methods include, but are not limited to, mutagenesis of the gene encoding one of the proteins and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the gene encoding the second species in the complex can then be selected. Sequence analysis of the genes encoding the respective proteins will reveal the mutations that correspond to the region of the protein involved in interactive binding. Alternatively, one protein can be anchored to a solid surface using methods described in this Section above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide comprising the binding domain may remain associated with the solid material, which can be isolated and identified by amino acid sequencing. Also, once the gene coding for the segments can be engineered to express peptide fragments of the protein, which can then be tested for binding activity and purified or synthesized.

For example, and not by way of limitation, a TWEAK receptor or ligand gene product can be anchored to a solid material as described, above, in this Section by making a GST-TWEAK receptor or ligand fusion protein and allowing it to bind to glutathione agarose beads. The interactive binding partner obtained can be labeled with a radioactive isotope, such as <35> S, and cleaved with a proteolytic enzyme such as trypsin. Cleavage products can then be added to the anchored GST-TWEAK receptor fusion protein or TWEAK ligand fusion protein and allowed to bind. After washing away unbound peptides, labeled bound material, representing the binding partner binding domain, can be eluted, purified, and analyzed for amino acid sequence by well-known methods. Peptides so identified can be produced synthetically or fused to appropriate facilitative proteins using recombinant DNA technology.

The TWEAK receptor-ligand interactions of the invention, *in vivo*, initiate a cascade of events that either stimulate or suppress angiogenesis in a target group of cell or tissue. Molecules, such as nucleic acid molecules, proteins, or small molecules may, in turn, influence this cascade. Compounds that disrupt the TWEAK receptor-ligand interaction effects in this way may be useful in regulating angiogenesis.

The basic principle of the assay systems used to identify compounds that interfere with the angiogenic or anti-angiogenic effect of TWEAK receptor-ligand interaction involves preparing a reaction mixture containing the TWEAK receptor and ligand under conditions and for a time sufficient to allow the two to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity of the effect of this interaction, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or may be added at a time subsequent to the

addition of the TWEAK receptor-ligand complex. Control reaction mixtures are incubated without the test compound or with a placebo. The inhibition or potentiation of any effect of the TWEAK complex on vascularization is then detected. Normal angiogenic response in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the cascade of events initiated by the TWEAK receptor-ligand interaction. Enhanced angiogenesis in the test compounds-containing culture indicates a stimulator of the TWEAK receptor-ligand complex effect.

The following examples are intended to illustrate particular embodiments and do not to limit the scope of the invention.

EXAMPLE 1

This example presents the cloning and identification of the TWEAK Receptor.

Expression Cloning of TWEAK Receptor cDNA

To clone TWEAK Receptor cDNA, an expression vector encoding a growth hormone leader, a leucine zipper multimerization domain, and the C-terminal extracellular domain of human TWEAK (see Chicheportiche *et al.*, J. Biol. Chem. 272(51):32401, 1997) was constructed. This expression vector, which was named pDC409-LZ-TWEAK, comprised the DNA sequence SEQ ID NO:1 and encoded the polypeptide SEQ ID NO:2. pDC409-LZ-TWEAK conditioned supernatants were produced by transient transfection into CV1-EBNA cells. These supernatants were incubated with magnetic beads coated with polyclonal goat anti-mouse antibody that had previously been incubated with a mouse monoclonal antibody against the leucine zipper. Control beads were produced by mixing the coated beads with supernatants from cells transfected with empty vector.

A monolayer of COS cells grown in a T175 flask was transfected with 15 μg of DNA pools of complexity of 100,000 from a HUVEC cDNA expression library. After 2 days these cells were lifted from the flask, and incubated in 1.5 mls of binding media plus 5% non-fat dried milk for 3 hours at 4 degrees C on a rotator wheel. Cells were pre-cleared by adding control beads and rotated at 4 degrees C for an additional 45 minutes after which bead bound cells were removed with a magnet. Pre-clearing was repeated 2-3 times, then TWEAK coated beads were added to the cells and rotated 30 minutes at 4 degrees C. Cells binding the TWEAK beads were separated by use of a magnet and washed 4x in PBS. Plasmid DNA was extracted from these cells by lysing in 0.1% SDS, and electroporating the supernatants in DH101B cells. Colonies were grown overnight on ampicilin selective media. Transformants were pooled and used as a source of plasmid DNA for a further round of panning. After 2 rounds of panning, positive clones were picked from the resulting pool based on their ability to bind TWEAK using a slide binding protocol like that described in Part B, below.

The human TWEAK receptor (also called TWEAKR) cDNA was determined to have the sequence SEQ ID NO:3, which encodes a 129 residue polypeptide (SEQ ID NO:4). Examination of the sequence predicts a polypeptide having an approximately 78 amino acid extracellular domain (residues 1-78 of SEQ ID NO:4, including the signal peptide), an approximately 23 amino acid transmembrane domain (residues 79-101 of SEQ ID NO:4), and an approximately 28 amino acid intracellular domain (residues 102-129 of SEQ ID NO:4). TWEAKR is the smallest known TNF receptor family member. It has a single cysteine-rich repeat

region in the extracellular domain, as compared to the 3-4 repeats of other TNF receptor family members. The TWEAKR polypeptide was previously described as a transmembrane protein encoded by a human liver cDNA clone (WO 98/55508, see also WO 99/61471), but had not been identified as the TWEAK receptor. A murine homolog, the FGF-inducible Fn14 (Meighan-Mantha *et al.*, J. Biol. Chem. 274(46):33166, 1999), is approximately 82% identical to the human protein, as shown by the alignment in Figure 1.

The newly identified TWEAK receptor was tested side by side with DR3 (which had been identified as the TWEAK receptor by Marsters *et al.*, Current Biology 8:525, 1998) for the ability to bind to TWEAK.

B. The TWEAK Receptor Binds to TWEAK

Slides of COS cells were transfected with expression vectors containing TWEAKR, DR3, or vector without insert (control). After two days the cells were incubated with concentrated supernatants from CV-1 cells transfected with a vector encoding the leucine zipper TWEAK extracellular domain fusion protein. One hour later the cells were washed and probed with an I-125 labeled antibody against the leucine-zipper domain. The slides were washed, fixed, and autoradiography was performed using x-ray film. The TWEAKR transfected cells bound significant amounts of TWEAK. TWEAK did not bind to the cells transfected with DR3 or the control cells. This experiment confirmed that the TWEAKR polypeptide identified in part A above, rather than DR3, is the major receptor for TWEAK. After discovery of the functional TWEAK receptor, other investigators also reported that DR3 is not the major receptor for TWEAK (Kaptein *et al.*, FEBS Lett., 485(2-3):135, 2000. The TWEAK-TWEAKR binding interaction was further characterized by Scatchard analysis.

CV-1 cells were transfected with human full length TWEAK and mixed 1:30 with Raji cells, which do not express TWEAK. The cells were incubated with serial dilutions of 125-I labeled human TWEAK receptor-Fc for 2 hours at 4 degrees Celsius. Free and bound probe was separated by microfuging the samples through a phalate oil mixture in plastic tubes. Supernatants and pellets were gamma-counted. Scatchard analyses of TWEAK ligand binding the TWEAK receptor showed a binding affinity constant (Ka) of approximately $4.5 \times 10^8\ M^{-1}$.

C. The TWEAK Receptor is Strongly Expressed in Cardiac Tissue

To determine the expression pattern of the TWEAK receptor, Northern blot analyses were performed. Human multiple tissue northern blots were purchased from Clontech (Palo Alto, CA) and probed with P-32 labeled random primed DNA from the TWEAK receptor coding region. The blots were washed and autoradiography was performed using x-ray film. Results showed that in the adult TWEAKR is strongly expressed in heart, placenta, and some skeletal muscle samples. Strong expression in heart tissue further supports the utility of TWEAKR in the diagnosis and treatment of cardiac disease. In contrast to the adult, the fetal tissues expressed TWEAKR more ubiquitously; TWEAKR transcripts were seen in the lung and liver.

EXAMPLE 2

This example presents the recombinant production of soluble TWEAK Receptor Fc (TWEAKR-Fc) fusion polypeptides.

To construct a nucleic acid encoding the TWEAKR extracellular domain fused to Fc, a nucleic acid encoding the N-terminal 79 amino acids from TWEAKR, including the leader (signal peptide), was joined to a nucleic acid encoding an Fc portion from human IgG1. Sequences for this construct are shown as SEQ ID NO:6 (nucleic acid) and SEQ ID NO:7 (amino acid). In SEQ ID NO:7, residues 1-27 are the predicted signal peptide (predicted to be cleaved upon secretion from the cell; the actual cleavage site was identified by N-terminal sequence analysis, see below), residues 28-79 are from the cysteine-rich TWEAKR extracellular domain, residues 80-81 are from a BglII cloning site, and the remainder is the Fc portion. Upon insertion into a mammalian expression vector, and expression in and secretion from a mammalian host cells, this construct produced a polypeptide designated TWEAKR-Fc. N-terminal sequence analysis determined that the secreted polypeptide designated TWEAKR-Fc had an N-terminus corresponding to residue 28 (Glu) of SEQ ID NO:7. Anti-angiogenic activity of TWEAKR-Fc was demonstrated using assays such as those described in the following examples. An analogous Fc-fusion construct was prepared using the murine TWEAKR extracellular domain.

EXAMPLE 3

This example presents a planar endothelial cell migration (wound closure) assay useful for measuring the activity of TWEAK receptor antagonists. In this assay, endothelial cell migration is measured as the rate of closure of a circular wound in a cultured cell monolayer. The rate of wound closure is linear, and is dynamically regulated by agents that stimulate and inhibit angiogenesis *in vivo*.

Primary human renal microvascular endothelial cells, HRMEC, were isolated, cultured, and used at the third passage after thawing, as described in Martin *et al.*, *In vitro* Cell Dev Biol 33:261, 1997. Replicate circular lesions, "wounds," (600-800 micron diameter) were generated in confluent HRMEC monolayers using a silicon-tipped drill press. At the time of wounding the medium (DMEM + 1% BSA) was supplemented with 20 ng/ml PMA (phorbol-12-myristate-13-acetate), EGF (4 ng/ml), and 0.150 to 5 μg/ml TWEAKR-Fc, or a combination of 40 ng/ml EGF and 0.150 to 5 μg/ml TWEAKR-Fc. The residual wound area was measured as a function of time (0-12 hours) using a microscope and image analysis software (Bioquant, Nashville, TN). The relative migration rate was calculated for each agent and combination of agents by linear regression of residual wound area plotted over time. The results are shown in Figures 2-3.

Compared to huIgG or media+BSA, TWEAKR-Fc inhibited PMA-induced endothelial migration in a dose responsive manner, reducing the rate of migration to unstimulated levels at 5 μg/ml (Figure 2). Neither huIgG nor TWEAKR-Fc inhibited basal (uninduced) migration. When HRMEC migration was induced by EGF, TWEAKR-Fc inhibited endothelial migration in a dose-dependent manner, reducing the rate of migration to unstimulated levels at 5 μg/ml (Figure 3).

EXAMPLE 4

This example presents a mouse corneal pocket assay useful for measuring the activity of TWEAK receptor antagonists. In this assay, agents to be tested for angiogenic or anti-angiogenic activity are immobilized in a slow release form in a hydron pellet, which is implanted into micropockets created in the

corneal epithelium of anesthetized mice. Vascularization is measured as the appearance, density, and extent of vessel ingrowth from the vascularized corneal limbus into the normally avascular cornea.

Hydron pellets, as described in Kenyon *et al.*, Invest Opthamol. & Visual Science 37:1625, 1996, incorporated sucralfate with bFGF (90 ng/pellet), bFGF and IgG (14 μg/pellet, control), or bFGF and TWEAKR-Fc (14 μg). The pellets were surgically implanted into corneal stromal micropockets created by micro-dissection 1mm medial to the lateral corneal limbus of 6-8 week old male C57BL mice. After five days, at the peak of neovascular response to bFGF, the corneas were photographed, using a Zeiss slit lamp, at an incipient angle of 35-50° from the polar axis in the meridian containing the pellet. Images were digitized and processed by subtractive color filters (Adobe Photoshop 4.0) to delineate established microvessels by hemoglobin content. Image analysis software (Bioquant, Nashville, TN) was used to calculate the fraction of the corneal image that was vascularized, the vessel density within the vascularized area, and the vessel density within the total cornea.

As shown in Table 1, TWEAKR-Fc (100 pmol) inhibited bFGF (3 pmol)-induced corneal angiogenesis, reducing the vascular density to 50% of that induced by FGF alone or FGF+IgG.

Table 1
Effect of TWEAKR-Fc on FGF-induced Angiogenesis in the Mouse Corneal Pocket Assay

Treatment	Greater than 50% Reduction in Number and Length of Vessels n/total n (%)
FGF alone	0/2 (0%)
FGF+IgG	0/2 (0%)
FGF+TWEAKR-Fc	6/9 (67%)

EXAMPLE 5

This example presents an endothelial cell proliferation assay useful for measuring the activity of a TWEAK receptor antagonist. In this assay, endothelial cell proliferation is measured after 4 days of cell growth in microtiter wells using a cell labeling molecule called calcein AM. Esterases expressed by the cells cleave the calcein and cause it to fluoresce when excited at 485 nm. Uncleaved calcein does not fluoresce. The amount of fluorescence is directly related to the number of endothelial cells in the culture well. Endothelial cell proliferation is often regulated by agents that stimulate and/or inhibit angiogenesis *in vivo*.

Primary HUVEC (human umbilical vein endothelial cells) were obtained from a commercial source (Clonetics, Walkersville, MD), cultured, and used at passage 2 to 7. Replicate cultures were set up by adding 3000 HUVEC to each microtiter well in endothelial cell basal media (EBM, an endothelial cell basal media that contains no growth factors or serum and is based on the media formulations developed by Dr. Richard Ham at the University of Colorado, Clonetics) plus 0.05% FBS (fetal bovine serum). At the time of culture initiation FGF-2 (fibroblast growth factor-2, 10 ng/ml) or human TWEAK (100 ng/ml) was added to the cultures in the presence of human IgG (huIgG, control) or human TWEAKR-Fc at concentrations ranging from 0.08 μg/ml to 20 μg/ml (0.25 to 20 μg/ml for TWEAK-induced and 0.08 to 6.7 μg/ml for FGF-2-induced). The HUVEC containing cultures were incubated for 4 days at 37 degrees C, 5% $C0_2$. On the fourth day of culture 4 μM calcein-AM was added to the cultures and 2 hours later the wells were evaluated for

fluorescence. The results, expressed as the average fluorescence (485-530 nm) counts for replicate wells plus or minus the SEM, are shown in Figures 4 and 5.

TWEAKR-Fc specifically inhibited TWEAK-induced HUVEC proliferation in a dose-dependent manner when compared to huIgG which did not effect TWEAK-induced proliferation (Figure 4). In addition, TWEAKR-Fc inhibited the basal proliferation of HUVEC observed during culture in EBM plus 0.05% FBS, as compared to huIgG which did not. Interestingly, TWEAKR-Fc also inhibited FGF-2 mediated HUVEC proliferation at concentrations of greater than 2 μg/ml, as compared to huIgG which did not effect the FGF-2 induced HUVEC proliferative response (Figure 5). These results show that TWEAKR-Fc inhibits HUVEC proliferation induced by the addition of exogenous recombinant human TWEAK. That TWEAKR-Fc partially inhibits serum -induced HUVEC-proliferation indicates HUVEC produce endogenous TWEAK that promotes growth/survival of the EC (endothelial cell) via the TWEAKR. TWEAKR-Fc attenuation of FGF-2 induced proliferation indicates that at least part of the EC response to FGF-2 is dependent on endogenous TWEAK/TWEAKR interaction.

EXAMPLE 6

This example presents a murine cardiac ischemia/engraftment model useful for measuring the activity of aTWEAK receptor antagonist.

Survival of heterotopically transplanted cardiac tissue from one mouse donor to the ear skin of another genetically similar mouse requires adequate neovascularization by the transplanted heart and the surrounding tissue, to promote survival and energy for cardiac muscle function. Inadequate vasculature at the site of transplant causes excessive ischemia to the heart, tissue damage, and failure of the tissue to engraft. Agents that antagonize factors involved in endothelial cell migration and vessel formation can decrease angiogenesis at the site of transplant, thereby limiting graft tissue function and ultimately engraftment itself. A murine heterotopic cardiac isograft model is used to demonstrate the effects of TWEAKR antagonists, including antibodies and TWEAKR-Fc, on neovascularization.

Female BALB/c (≈12 weeks of age) recipients are given neonatal heart grafts from donor mice of the same strain. The donor heart tissue is grafted into the left ear pinnae of the recipient on day 0 and the mice are divided into two groups. The control group receives human IgG (Hu IgG) while the other group receives the TWEAKR antagonist, both intraperitoneally. The treatments are continued for five consecutive days. The functionality of the grafts is determined by monitoring visible pulsatile activity on days 7 and 14 post-engraftment. The inhibition of functional engraftment, as a function of the dose of TWEAKR antagonist, is determined. The histology of the transplanted hearts is examined is order to visualize the effects of the TWEAKR antagonist on edema at the site of transplant and host and donor tissue vasculature (using, *e.g.*, Factor VIII staining).

EXAMPLE 7

This example presents a method of treating tumors with a TWEAK receptor antagonist.

TWEAKR antagonists are tested in animal models of solid tumors. The effect of the TWEAKR antagonists is determined by measuring tumor frequency and tumor growth.

EXAMPLE 8

This example presents an ELISA-based assay useful for determining the binding properties of TWEAK binding molecules, for example, monomeric and multimeric TWEAKR-Fc oligomers. For this example, huTWEAKR:Fc (SEQ ID NO:7), di-TWEAKR:Fc (SEQ ID NO:11), tri-TWEAKR:Fc (SEQ ID NO:13) and TWEAKR:DR5:Fc (SEQ ID NO:9) were used.

Ninety-six well LINBRO®/TITERTEK™ enzyme immunoassay plates (ICN Biochemicals, Aurora, OH) were coated with TWEAKR:Fc (SEQ ID NO:7) at a concentration of 1 mg/ml in PBS, 50µl/well, plate sealer applied, and incubated overnight at 4° C. Each well was washed three times with PBST (PBS + 0.1 % TWEEN 20; 200µl/well, then incubated for one hour at 37° C in PBST + 3% nonfat dry milk (NFDM).

In separate reactions, FLAG-tagged TWEAK (TWEAK-FLAG) was pre-incubated with each of the above TWEAKR polypeptides at ambient temperature for 30 min., in DMEM + 0.5% low Ig serum, in 96 well U-bottom plates at a final concentration of TWEAK-FLAG of 50ng/ml and a final concentration of each TWEAKR polypeptide of from 9,000 to 4 ng/ml, in 3-fold serial dilutions.

The EIA plate was again washed three times with PBST + 3% NFDM. 50µl/well of ligand/receptor mixture was added, incubated at ambient temperature for 30 min., then washed three times with PBST + 3% NFDM.

FLAG-M2 biotinylated antibody, diluted 1:500 in PBST + 3% NFDM, was added at 50µl/well, incubated for 45 min. at ambient temperature, and washed three times with PBST + 3% NFDM.

SA-HRP, diluted 1:2000 in PBST + 3% NFDM, was added at 50µl/well, then incubated for 45 min. at ambient temperature.

The plates were washed five times, 100µl/well 3,3',5,5'-tetramethylbenzidine (TMB) was added, and the plates were incubated at ambient temperature for 5-20 minutes.

The reaction was stopped with 50µl/well of 1M H_3PO_4 and absorbances read at $A_{450/570}$.

As shown in Figure 6, TWEAKR:Fc showed the weakest binding, followed by TWEAKR:DR5:Fc, then di-TWEAKR:Fc (SEQ ID NO:11), then tri-TWEAKR:Fc (SEQ ID NO:13). Thus, the more soluble TWEAKR domains the fusion protein comprised, the stronger it bound to TWEAK. Moreover, the increase in binding was more than additive, as shown by the difference in binding between TWEAKR:Fc and di-TWEAKR:Fc.

EXAMPLE 9

This example presents a competition binding assay using Europium labeled TWEAKR:Fc useful for determining the binding properties of TWEAK binding molecules, for example, monomeric and multimeric TWEAKR-Fc oligomers. For this example, huTWEAKR:Fc (SEQ ID NO:7), di-TWEAKR:Fc (SEQ ID NO:11), tri-TWEAKR:Fc (SEQ ID NO:13) and TWEAKR:DR5:Fc (SEQ ID NO:9) were used.

M2 anti-flag antibody was diluted to a concentration of 4 µg/ml in 0.1M $NaHCO_3$. 100µl/well was used to coat 96 well flat bottom plates. Plate sealer was applied and the plates were incubated at 4° C overnight.

Each well was washed five times with PBST (PBS + 0.1% TWEEN 20), then 200µl/well of PBST + 3% NFDM was added. The plates were incubated for one hour at 37° C, then washed five times with PBST.

FLAG-TWEAK was diluted to 50 ng/ml in PBS and added to 100µl/well. The plates were incubated at ambient temperature, with shaking, for one hour. The plates were washed five more times in PBST.

Unlabeled and europium labeled receptors were pre-mixed in 96 well U-bottom plates, diluted in PBST + 3% NFDM to a final concentration of 35ng/ml for europium-labled TWEAKR, three fold dilutions of unlabeled receptors, to final concentrations of 9000-12ng/ml.

To each well was added 100µl of pre-mixed receptors. Plates were incubated for one hour at ambient temperature with shaking, then washed ten times as before. 100µl/well of enhancement solution (Perkin Elmer, 1244-105) was added and the plates were incubated for five minutes with shaking. Absorbances were read at A_{615} on a Wallac plate reader.

As shown in Figure 7, monomeric TWEAKR showed the poorest ability to compete with europium labeled TWEAKR:Fc for binding to TWEAK, followed by dimeric TWEAKR and trimeric TWEAKR.

EXAMPLE 10

This example presents an ELISA-style assay useful for determining the binding properties of TWEAK binding molecules, for example, monomeric and multimeric TWEAKR-Fc oligomers.

TWEAKR:Gly5:Fc (SEQ ID NO:15) is a fusion protein comprising an N-terminal methionine residue, residues 29 through 70 of the TWEAK receptor, five glycine residues, and an Fc fragment-derived peptide. TWEAKR:1KPEG:Fc (SEQ ID NO:17) is a fusion protein comprising an N-terminal methionine residue, residues 29 through 70 of the TWEAK receptor, a linker, and an Fc fragment-derived peptide. TWEAKR:1KPEG:TWEAKR:Gly5:Fc (SEQ ID NO:18) is a fusion protein comprising an N-terminal methionine residue, residues 29 through 70 of the TWEAK receptor, a linker, residues 29 through 70 of the TWEAK receptor, five glycine residues, and an Fc fragment-derived peptide. TWEAKR:Gly5:TWEAKR:Gly5:Fc (SEQ ID NO:19) is a fusion protein comprising an N-terminal methionine residue, residues 29 through 70 of the TWEAK receptor, five glycine residues, residues 29 through 70 of the TWEAK receptor, five glycine residues, and an Fc fragment-derived peptide.

Using an ELISA-style assay similar to that described in Example 8, it was determined that monomeric oligomers (TWEAKR:Gly5:Fc and TWEAKR:1KPEG:Fc) bind to TWEAK about equally well, but much less well than dimeric oligomeric constructs (TWEAKR:1KPEG:TWEAKR:Gly5:Fc and TWEAKR:Gly5:TWEAKR:Gly5:Fc) (Figure 8).

EXAMPLE 11

This example presents the results of an ELISA-based TWEAK-binding assay using huTWEAKR:Fc (SEQ ID NO:7), TWEAKR 40mono-3 (SEQ ID NO:21), TWEAKR 40dimer-1 (SEQ ID NO:23), TWEAKR 40trimer-5 (SEQ ID NO:25), TWEAKR 40quad-2 (SEQ ID NO:27), TWEAKR 40quint-1 (SEQ ID NO:29), TWEAKR 43mono-F4 (SEQ ID NO:31), TWEAKR 43dimer-F2 (SEQ ID NO:33), TWEAKR 43trimer-1 (SEQ ID NO:35), TWEAKR 43quad-2 (SEQ ID NO:37), TWEAKR 43quint-3 (SEQ ID NO:39), TWEAKR

43hex-1 (SEQ ID NO:41), and TWEAKR 43sept-1 (SEQ ID NO:43). The protocol described in Example 8 was used except that in the present example BSA was omitted from the wash buffers. The results are presented in Figures 9-12.

EXAMPLE 12

This example presents the results of a competition binding assay using Europium labeled TWEAKR:Fc as described in Example 9. In the present example, huTWEAKR:Fc (SEQ ID NO:7), TWEAKR 40mono-3 (SEQ ID NO:21), TWEAKR 40dimer-1 (SEQ ID NO:23), TWEAKR 40trimer-5 (SEQ ID NO:25), TWEAKR 40quad-2 (SEQ ID NO:27), TWEAKR 40quint-1 (SEQ ID NO:29), TWEAKR 43mono-F4 (SEQ ID NO:31), TWEAKR 43dimer-F2 (SEQ ID NO:33), TWEAKR 43trimer-1 (SEQ ID NO:35), TWEAKR 43quad-2 (SEQ ID NO:37), TWEAKR 43quint-3 (SEQ ID NO:39), TWEAKR 43hex-1 (SEQ ID NO:41), and TWEAKR 43sept-1 (SEQ ID NO:43) were used. The results are presented in Figures 13 and 14.

The relevant disclosures of publications cited herein are specifically incorporated by reference. The examples presented above are not intended to be exhaustive or to limit the scope of the invention. The skilled artisan will understand that variations and modifications and variations are possible in light of the above teachings, and such modifications and variations are intended to be within the scope of the invention.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A polypeptide comprising a first soluble fragment of a TWEAK receptor, a second soluble fragment of a TWEAK receptor, and an oligomerization domain, wherein said polypeptide binds to TWEAK, and said first soluble fragment consists of a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. residues 29 through 70 of the amino acid sequence of SEQ ID NO:7;

b. residues 28 through 79 of the amino acid sequence of SEQ ID NO:7;

c. residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and

d. residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

2. The polypeptide of Claim 1, wherein said first soluble fragment and said second soluble fragment each independently consists of a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. residues 29 through 70 of the amino acid sequence of SEQ ID NO:7;

b. residues 28 through 79 of the amino acid sequence of SEQ ID NO:7;

c. residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and

d. residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

3. The polypeptide of Claim 1 or claim 2, further comprising a third soluble fragment of a TWEAK receptor.

4. The polypeptide of Claim 3, wherein said first soluble fragment, second soluble fragment, and third soluble fragment each independently consists of a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. residues 29 through 70 of the amino acid sequence of SEQ ID NO:7;

b. residues 28 through 79 of the amino acid sequence of SEQ ID NO:7;

c. residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and

d. residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

5. The polypeptide of Claim 4, further comprising a fourth soluble fragment of a TWEAK receptor.

6. The polypeptide of Claim 5, wherein said first soluble fragment, second soluble fragment, third soluble fragment, and fourth soluble fragment each independently consists of a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. residues 29 through 70 of the amino acid sequence of SEQ ID NO:7;

b. residues 28 through 79 of the amino acid sequence of SEQ ID NO:7;

c. residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and

d. residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

7. The polypeptide of Claim 6, further comprising a fifth soluble fragment of a TWEAK receptor.

8. The polypeptide of Claim 7, wherein said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, and fifth soluble fragment each independently consists of a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. residues 29 through 70 of the amino acid sequence of SEQ ID NO:7;

b. residues 28 through 79 of the amino acid sequence of SEQ ID NO:7;

c. residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and

d. residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

9. The polypeptide of Claim 8, further comprising a sixth soluble fragment of a TWEAK receptor.

10. The polypeptide of Claim 9, wherein said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, fifth soluble fragment, and sixth soluble fragment each independently consists of a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. residues 29 through 70 of the amino acid sequence of SEQ ID NO:7;

b. residues 28 through 79 of the amino acid sequence of SEQ ID NO:7;

c. residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and

d. residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

11. The polypeptide of Claim 10, further comprising a seventh soluble fragment of a TWEAK receptor.

12. The polypeptide of Claim 11, wherein said first soluble fragment, second soluble fragment, third soluble fragment, fourth soluble fragment, fifth soluble fragment, sixth soluble fragment, and seventh soluble fragment each independently consists of a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. residues 29 through 70 of the amino acid sequence of SEQ ID NO:7;

b. residues 28 through 79 of the amino acid sequence of SEQ ID NO:7;

c. residues 30 through 73 of the amino acid sequence of SEQ ID NO:7; and

d. residues 30 through 70 of the amino acid sequence of SEQ ID NO:7.

13. The polypeptide of any one of Claims 1 to 12, further comprising a linker.

14. The polypeptide of any one of Claims 1to 12, further comprising a first linker and a second linker, wherein said first linker joins said first soluble TWEAKR fragment and said second soluble TWEAKR fragment, and said second linker joins said second soluble TWEAKR fragment and said oligomerization domain.

15. The polypeptide of Claim 1, wherein said oligomerization domain is N-terminal to said first soluble TWEAKR fragment and to said second soluble TWEAKR fragment.

16. The polypeptide of Claim 1, wherein said oligomerization domain is C-terminal to said first soluble TWEAKR fragment and to said second soluble TWEAKR fragment.

17. The polypeptide of Claim 1, wherein said oligomerization domain comprises a leucine zipper.

18. The polypeptide of Claim 1, wherein said oligomerization domain comprises a fragment of an antibody.

19. The polypeptide of Claim 38, wherein said fragment of an antibody comprises an Fc domain.

20. The polypeptide of Claim 1, wherein said polypeptide comprises a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. SEQ ID NO:9;
b. SEQ ID NO:11;
c. SEQ ID NO:13;
d. SEQ ID NO:15;
e. SEQ ID NO:17;
f. SEQ ID NO:18;
g. SEQ ID NO:19;
h. SEQ ID NO:21;
i. SEQ ID NO:23;
j. SEQ ID NO:25;
k. SEQ ID NO:27;
l. SEQ ID NO:29;
m. SEQ ID NO:31;
n. SEQ ID NO:33;
o. SEQ ID NO:35;
p. SEQ ID NO:37;
q. SEQ ID NO:39;
r. SEQ ID NO:41;
s. SEQ ID NO:43; and
t. SEQ ID NO:44.

21. A protein comprising a first polypeptide of Claim 1 and a second polypeptide of Claim 1, wherein said first and second polypeptides are oligomerized to each other.

22. The protein of Claim 21 wherein the amino acid sequence of said first polypeptide is identical to the amino acid sequence of said second polypeptide.

23. The protein of Claim 21 wherein the amino acid sequence of said first polypeptide is not identical to the amino acid sequence of said second polypeptide.

24. A method of inhibiting a TWEAK receptor in a subject comprising administering to said subject the polypeptide of Claim 1.

25. A method of inhibiting angiogenesis in a subject comprising administering to said subject a therapeutically-effective amount of a composition comprising the polypeptide of Claim 1.

26. The method of Claim 25 wherein said composition further comprises a pharmaceutically acceptable carrier.

27. The method of Claim 25 wherein said subject is a mammal.

28. The method of Claim 27 wherein said mammal is a human.

29. The method of Claim 25 wherein said subject has a disease or condition mediated or exacerbated by angiogenesis.

30. The method of Claim 29 wherein said disease or condition is characterized by ocular neovascularization.

31. The method of Claim 29 wherein said disease or condition is a solid tumor.

32. The method of Claim 31 wherein said method further comprises treating said subject with radiation.

33. The method of Claim 31 wherein said method further comprises treating said subject with a second chemotherapeutic agent.

34. The method of Claim 33 wherein said second chemotherapeutic agent is selected from the group consisting of: an alkylating agent, an antimetabolite, a vinca alkaloid, a plant-derived chemotherapeutic, a nitrosourea, an antitumor antibiotic, an antitumor enzyme, a topoisomerase inhibitor, a platinum analog, an adrenocortical suppressant, a hormone, a hormone agonist, a hormone antagonist, an antibody, an immunotherapeutic, a blood cell factor, a radiotherapeutic, and a biological response modifier.

35. The method of Claim 33 wherein said second chemotherapeutic agent is selected from the group consisting of cisplatin, cyclophosphamide, mechloretamine, melphalan, bleomycin, carboplatin, fluorouracil, 5-fluorodeoxyuridine, methotrexate, taxol, asparaginase, vincristine, vinblastine, a lymphokine, a cytokine, an interleukin, an interferon, alpha interferon, beta interferon, delta interferon, TNF, chlorambucil, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, cytarabine, mercaptopurine, thioguanine, vindesine, etoposide, teniposide, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, hydroxyurea, methylhydrazine, mitotane, tamoxifen, and fluoxymesterone.

36. The method of Claim 29 wherein said disease or condition is an inflammatory disease or condition.

37. The method of Claim 39 wherein said method further comprises treating said subject with a second therapeutic agent.

38. The method of Claim 37 wherein said second therapeutic agent inhibits a cytokine or a cytokine receptor that promotes inflammation.

39. The method of Claim 38 wherein said second therapeutic agent comprises a soluble fragment of said cytokine receptor, an antibody that binds said cytokine, or an antibody that binds said cytokine receptor.

40. The method of Claim 37 wherein said second therapeutic agent activates a receptor that inhibits inflammation.

41. The method of Claim 37 wherein said second therapeutic agent is selected from the group consisting of Flt3 ligand, CD40 ligand, interleukin-2, an interleukin-4 antagonist, an IL-13 antagonist, interleukin-12, 4-1BB ligand, an anti-4-1BB antibody, a TNF antagonist, a TNF receptor antagonist, TRAIL, a CD148 agonist, a VEGF antagonist, a VEGF receptor antagonist, an IgE antagonist, and a Tek antagonist.

42. A nucleic acid, or its complement, comprising a sequence that encodes said polypeptide of Claim 1.

43. The nucleic acid of Claim 42, wherein said nucleic acid, or its complement, hybridizes under moderately stringent hybridization conditions to a second nucleic acid, and said second nucleic acid comprises a sequence selected from the group consisting of:

a. SEQ ID NO:10;
b. SEQ ID NO:12;
c. SEQ ID NO:14;
d. SEQ ID NO:16;
e. SEQ ID NO:20;
f. SEQ ID NO:22;
g. SEQ ID NO:24;
h. SEQ ID NO:26;
i. SEQ ID NO:28;
j. SEQ ID NO:30;
k. SEQ ID NO:32;
l. SEQ ID NO:34;
m. SEQ ID NO:36;
n. SEQ ID NO:38;
o. SEQ ID NO:40; and
p. SEQ ID NO:42.

44. The nucleic acid of Claim 42, wherein said nucleic acid, or its complement, comprises a sequence that is at least 90%, 95%, or 100% identical to a sequence selected from the group consisting of:

a. SEQ ID NO:10;

b. SEQ ID NO:12;
c. SEQ ID NO:14;
d. SEQ ID NO:16;
e. SEQ ID NO:20;
f. SEQ ID NO:22;
g. SEQ ID NO:24;
h. SEQ ID NO:26;
i. SEQ ID NO:28;
j. SEQ ID NO:30;
k. SEQ ID NO:32;
l. SEQ ID NO:34;
m. SEQ ID NO:36;
n. SEQ ID NO:38;
o. SEQ ID NO:40; and
p. SEQ ID NO:42.

45. The nucleic acid of Claim 42 wherein said nucleic acid encodes a polypeptide sequence selected from the group consisting of:

a. SEQ ID NO:9;
b. SEQ ID NO:11;
c. SEQ ID NO:13;
d. SEQ ID NO:15;
e. SEQ ID NO:17;
f. SEQ ID NO:18;
g. SEQ ID NO:19;
h. SEQ ID NO:21;
i. SEQ ID NO:23;
j. SEQ ID NO:25;
k. SEQ ID NO:27;
l. SEQ ID NO:29;
m. SEQ ID NO:31;
n. SEQ ID NO:33;
o. SEQ ID NO:35;

p. SEQ ID NO:37;

q. SEQ ID NO:39;

r. SEQ ID NO:41;

s. SEQ ID NO:43; and

t. SEQ ID NO:44.

46. A vector comprising said nucleic acid of Claim 42.

47. The vector of Claim 46, wherein said vector is an expression vector.

48. A host cell comprising said nucleic acid of Claim 42.

49. A method of producing a polypeptide comprising culturing the host cell of Claim 48 under conditions promoting expression of said polypeptide.

50. A polypeptide of claim 1, substantially as hereinbefore described with reference to the Figures and/or Examples

```
  1 MAPGWPRSLPQILVLGFGLVLMRAAAGEQAPGTSPCSSGSSWSADLDKCM 50
    ||  |   | |  .:||||   | |:|. ||||||||.||| ||||||||||||
  1 MARGSLRRLLRLLVLGLWLALLRSVAGEQAPGTAPCSRGSSWSADLDKCM 50

                .          .          .          .          .
 51 DCASCPARPHSDFCLGCAAAPPAHFRLLWPILGGALSLVLVLALVSSFLV 100
    |||||  ||||||||||||||||| ||||||||||||||   || |.| |||
 51 DCASCRARPHSDFCLGCAAAPPAPFRLLWPILGGALSLTFVLGLLSGFLV 100

                .          .
101 WRRCRRREKFTTPIEETGGEGCPGVALIQ 129
    |||||||||||||||||||||||| |||||
101 WRRCRRREKFTTPIEETGGEGCPAVALIQ 129
```

Fig. 1

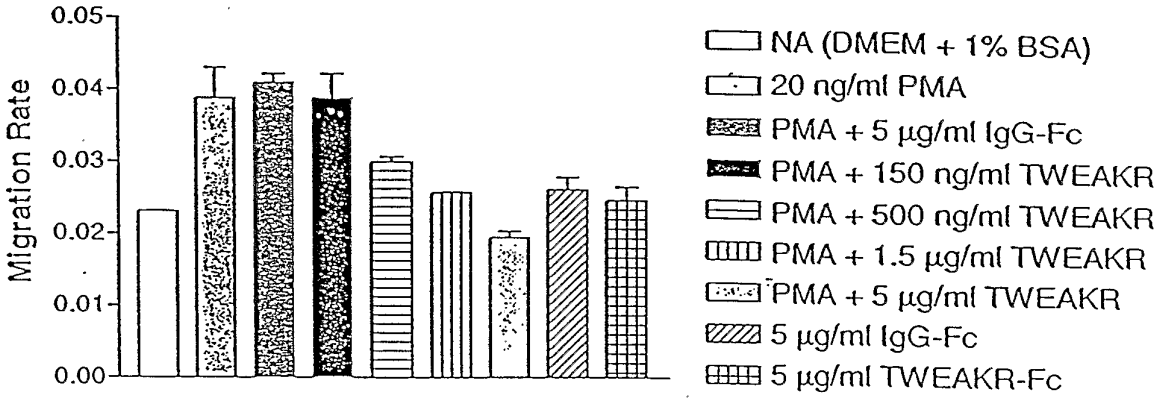

Migration Rate
0.05
0.04
0.03
0.02
0.01
0.00
NA (DMEM + 1% BSA)
20 ng/ml PMA
PMA + 5 µg/ml IgG-Fc
PMA + 150 ng/ml TWEAKR
PMA + 500 ng/ml TWEAKR
PMA + 1.5 µg/ml TWEAKR
PMA + 5 µg/ml TWEAKR
5 µg/ml IgG-Fc
5 µg/ml TWEAKR-Fc

Fig. 2

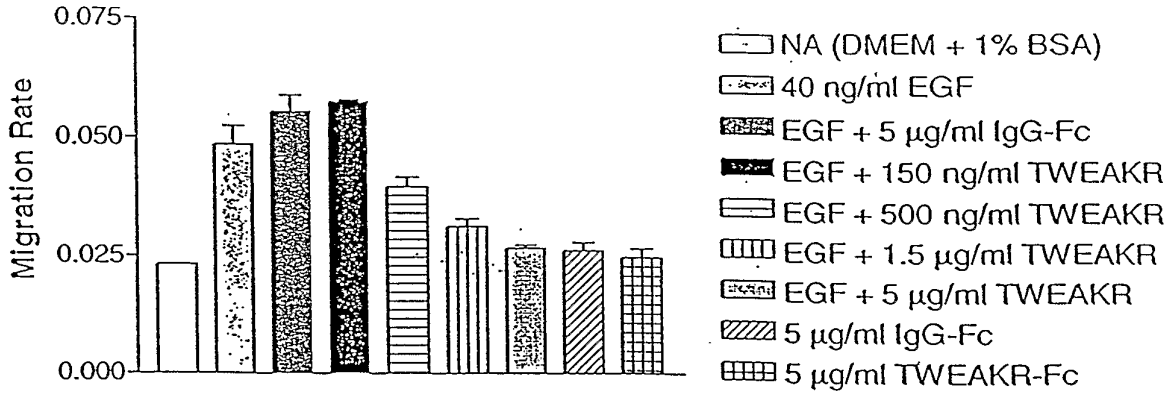

Migration Rate
0.075
0.050
0.025
0.000
NA (DMEM + 1% BSA)
40 ng/ml EGF
EGF + 5 µg/ml IgG-Fc
EGF + 150 ng/ml TWEAKR
EGF + 500 ng/ml TWEAKR
EGF + 1.5 µg/ml TWEAKR
EGF + 5 µg/ml TWEAKR
5 µg/ml IgG-Fc
5 µg/ml TWEAKR-Fc

Fig. 3

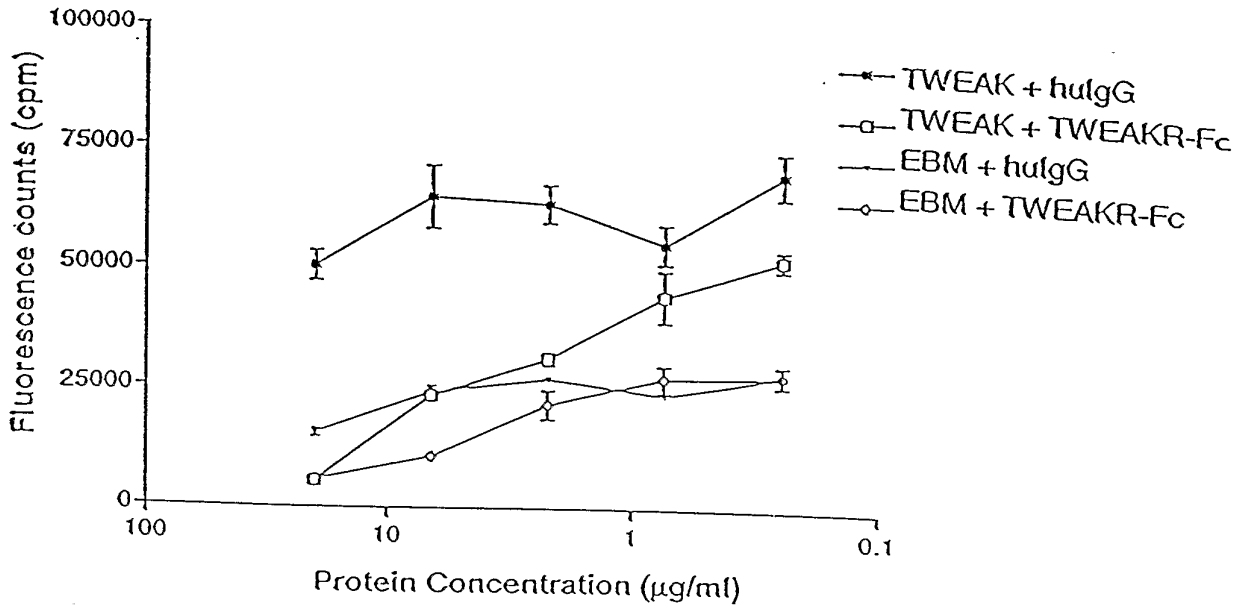

100000
75000
50000
25000
0
Fluorescence counts (cpm)
100
10
1
0.1
Protein Concentration (µg/ml)
TWEAK + huIgG
TWEAK + TWEAKR-Fc
EBM + huIgG
EBM + TWEAKR-Fc

Fig. 4

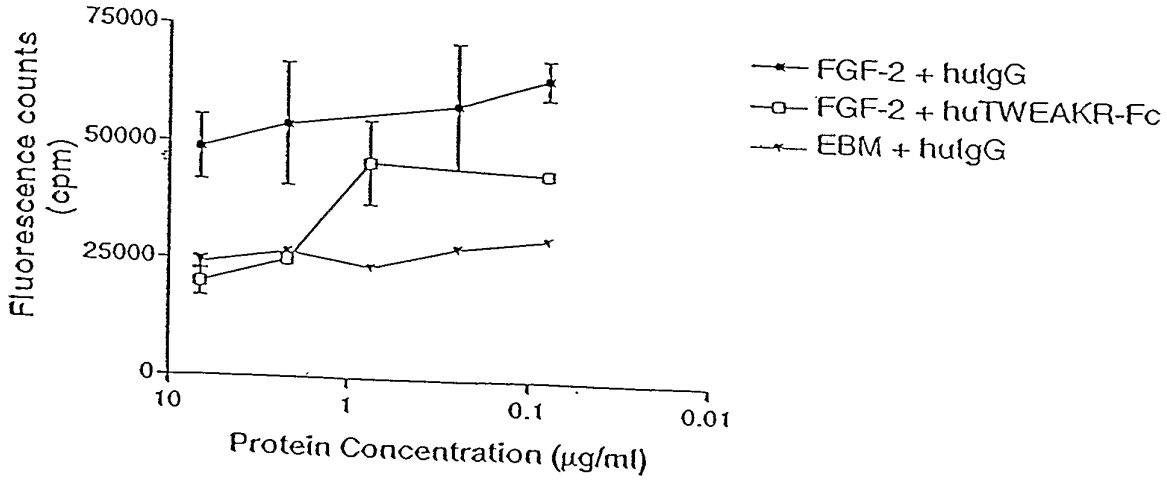

75000
50000
25000
0
Fluorescence counts (cpm)
10
1
0.1
0.01
Protein Concentration (µg/ml)
FGF-2 + huIgG
FGF-2 + huTWEAKR-Fc
EBM + huIgG

Fig. 5

Fig. 6

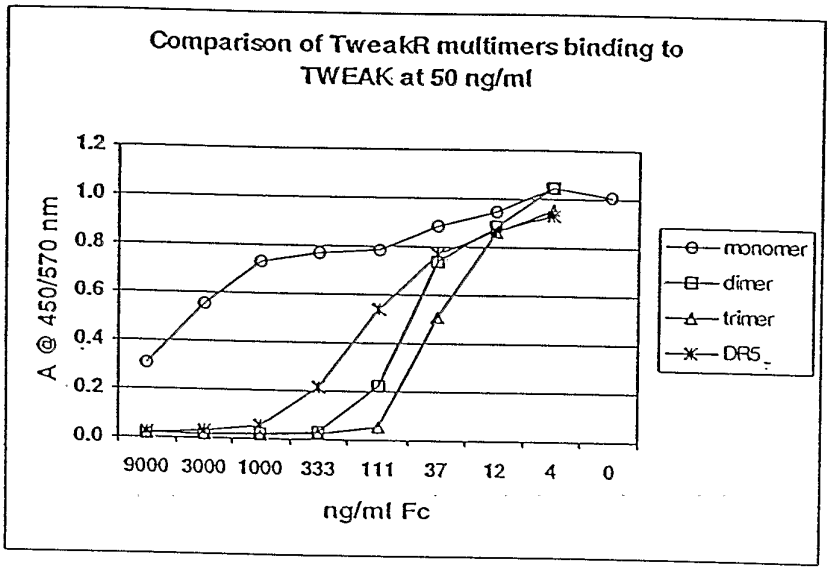

Comparison of TweakR multimers binding to TWEAK at 50 ng/ml
A @ 450/570 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
9000 3000 1000 333 111 37 12 4 0
ng/ml Fc
monomer
dimer
trimer
DR5

Fig. 7

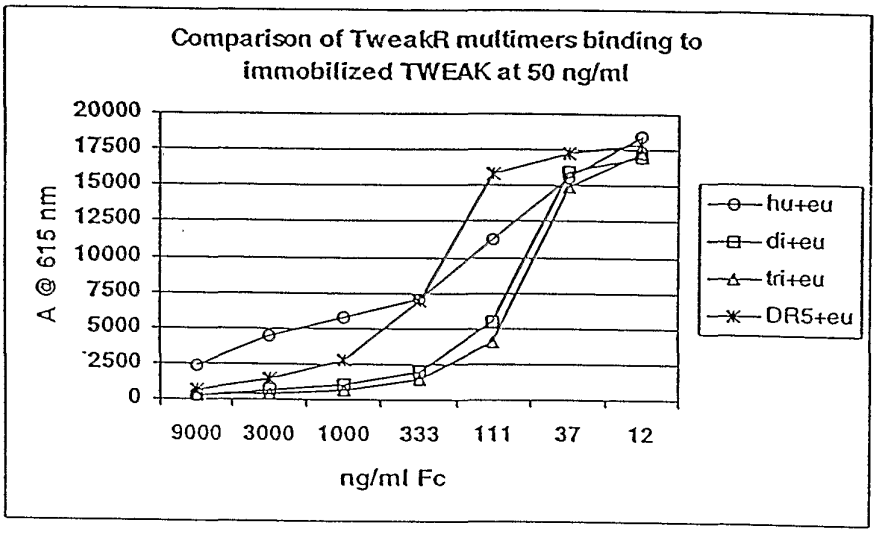
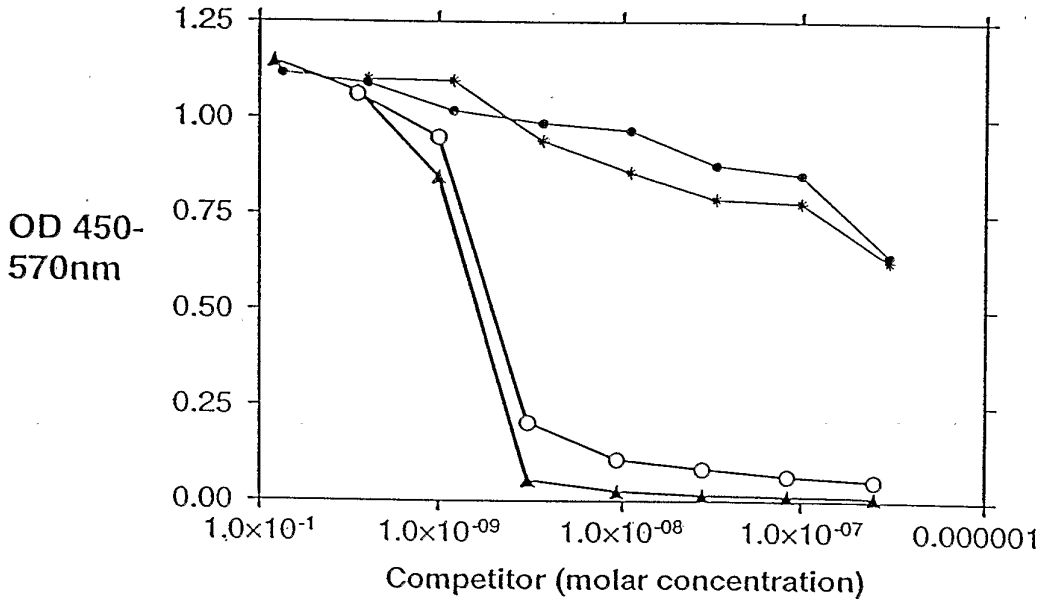

Comparison of TweakR multimers binding to immobilized TWEAK at 50 ng/ml
A @ 615 nm
20000
17500
15000
12500
10000
7500
5000
2500
0
9000
3000
1000
333
111
37
12
ng/ml Fc
hu+eu
di+eu
tri+eu
DR5+eu

Fig. 8


1.25
1.00
0.75
0.50
0.25
0.00
OD 450-570nm
1.0×10-1
1.0×10-09
1.0×10-08
1.0×10-07
0.000001
Competitor (molar concentration)

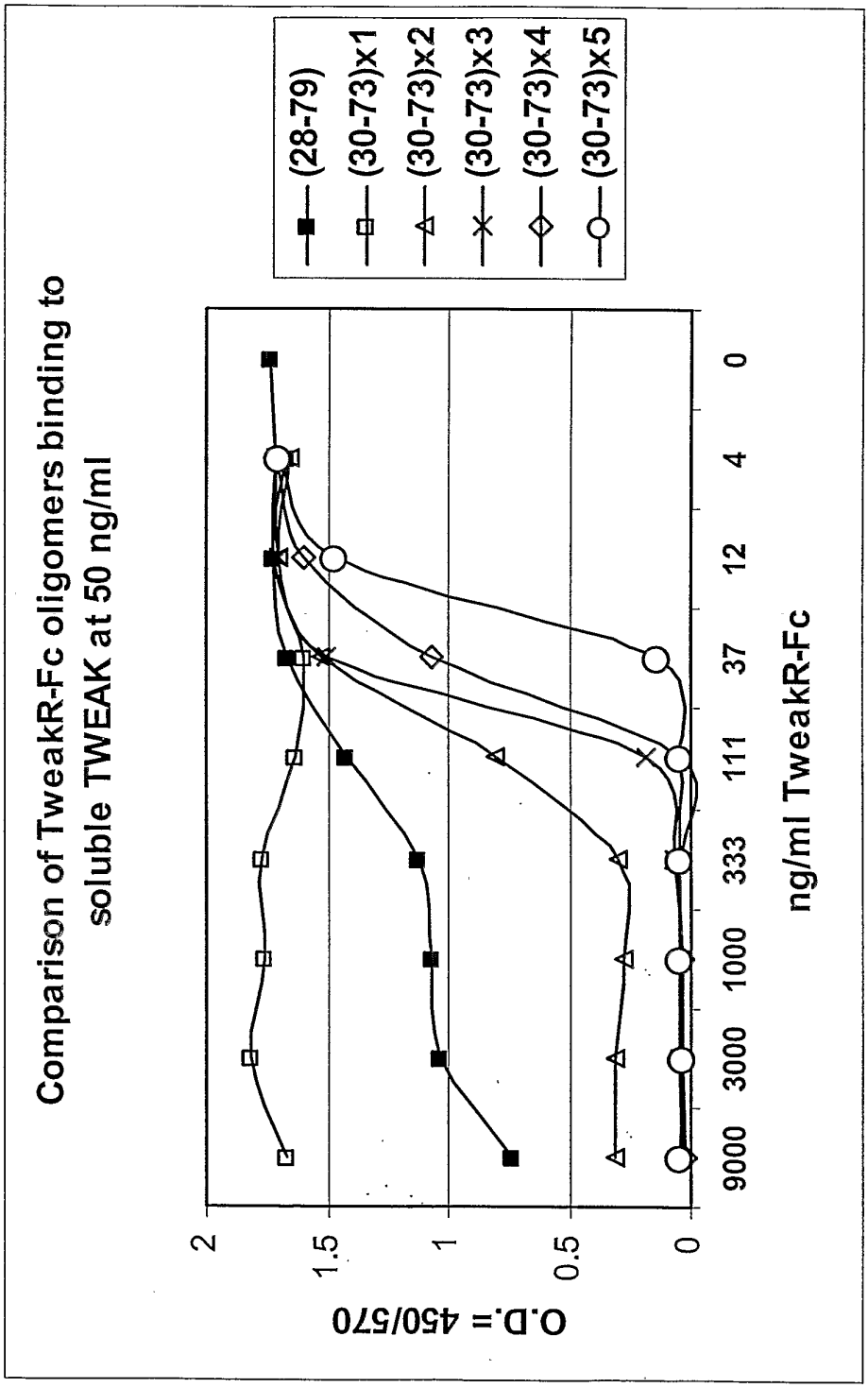

Comparison of TweakR-Fc oligomers binding to soluble TWEAK at 50 ng/ml
O.D.= 450/570
2
1.5
1
0.5
0
9000
3000
1000
333
111
37
12
4
0
ng/ml TweakR-Fc
(28-79)
(30-73)x1
(30-73)x2
(30-73)x3
(30-73)x4
(30-73)x5

FIGURE 9

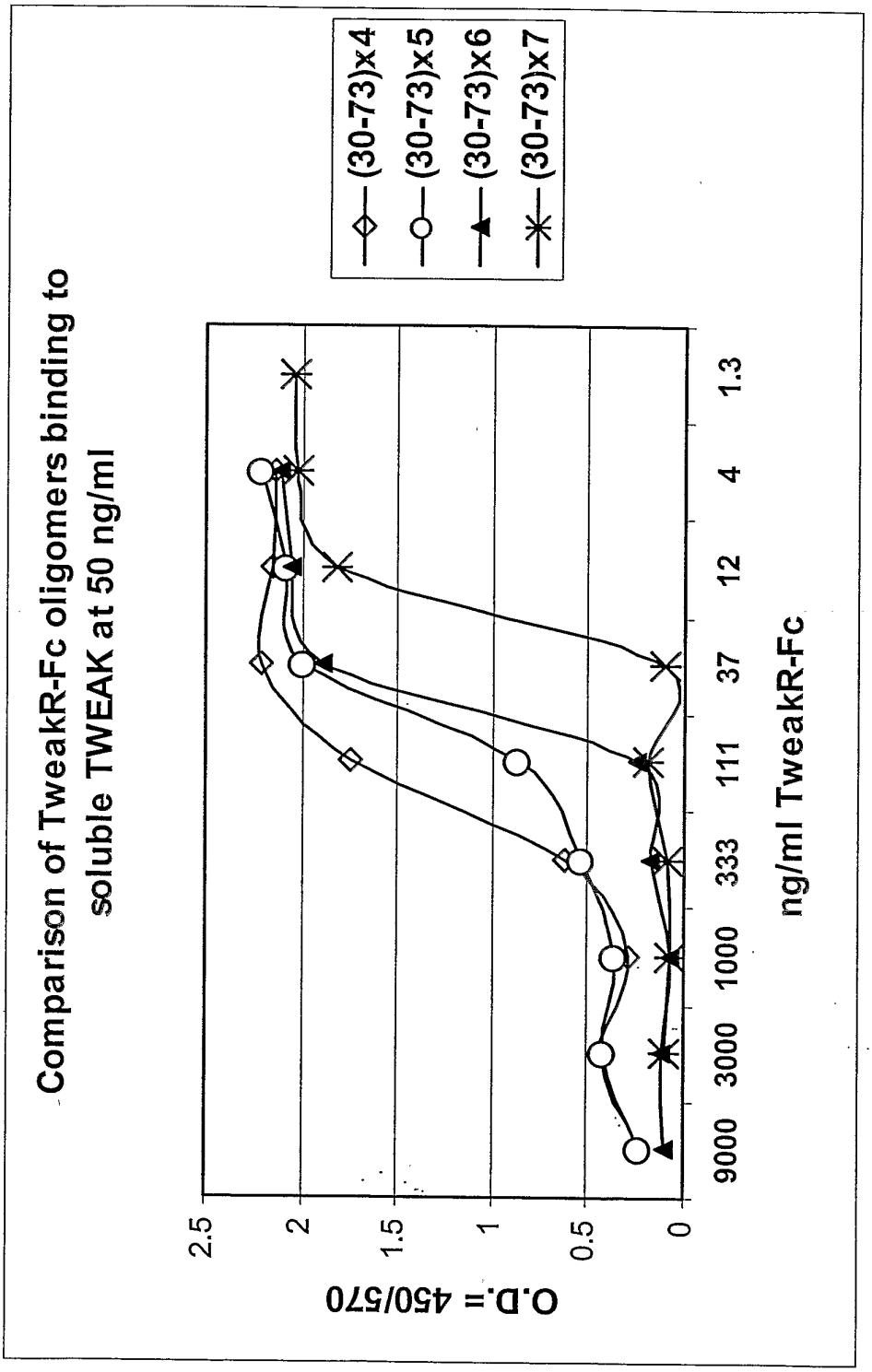

Comparison of TweakR-Fc oligomers binding to soluble TWEAK at 50 ng/ml
(30-73)x4
(30-73)x5
(30-73)x6
(30-73)x7
O.D.= 450/570
2.5
2
1.5
1
0.5
0
9000
3000
1000
333
111
37
12
4
1.3
ng/ml TweakR-Fc

FIGURE 10

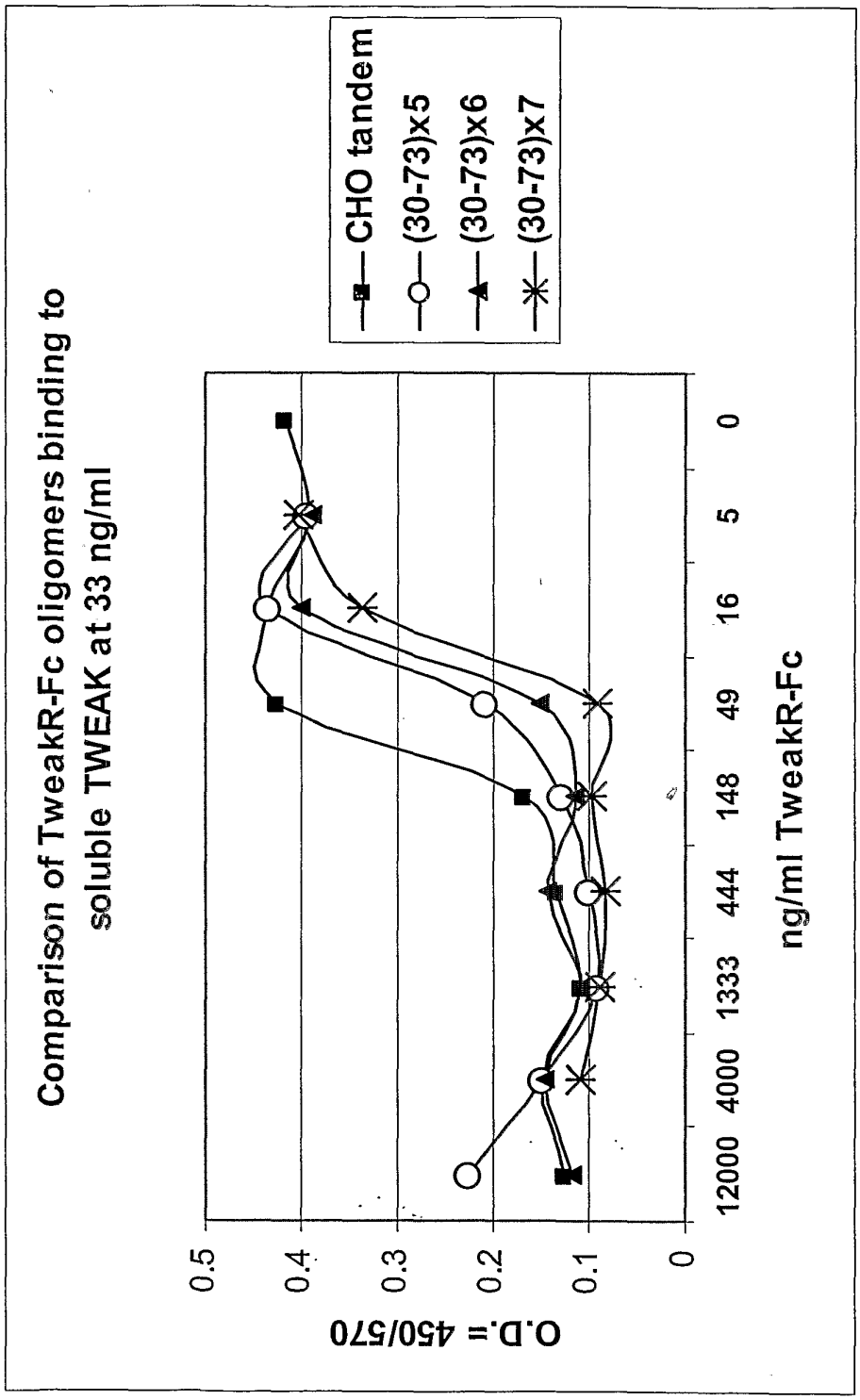

Comparison of TweakR-Fc oligomers binding to soluble TWEAK at 33 ng/ml
CHO tandem
(30-73)x5
(30-73)x6
(30-73)x7
O.D.= 450/570
0.5
0.4
0.3
0.2
0.1
0
12000 4000 1333 444 148 49 16 5 0
ng/ml TweakR-Fc

FIGURE 11

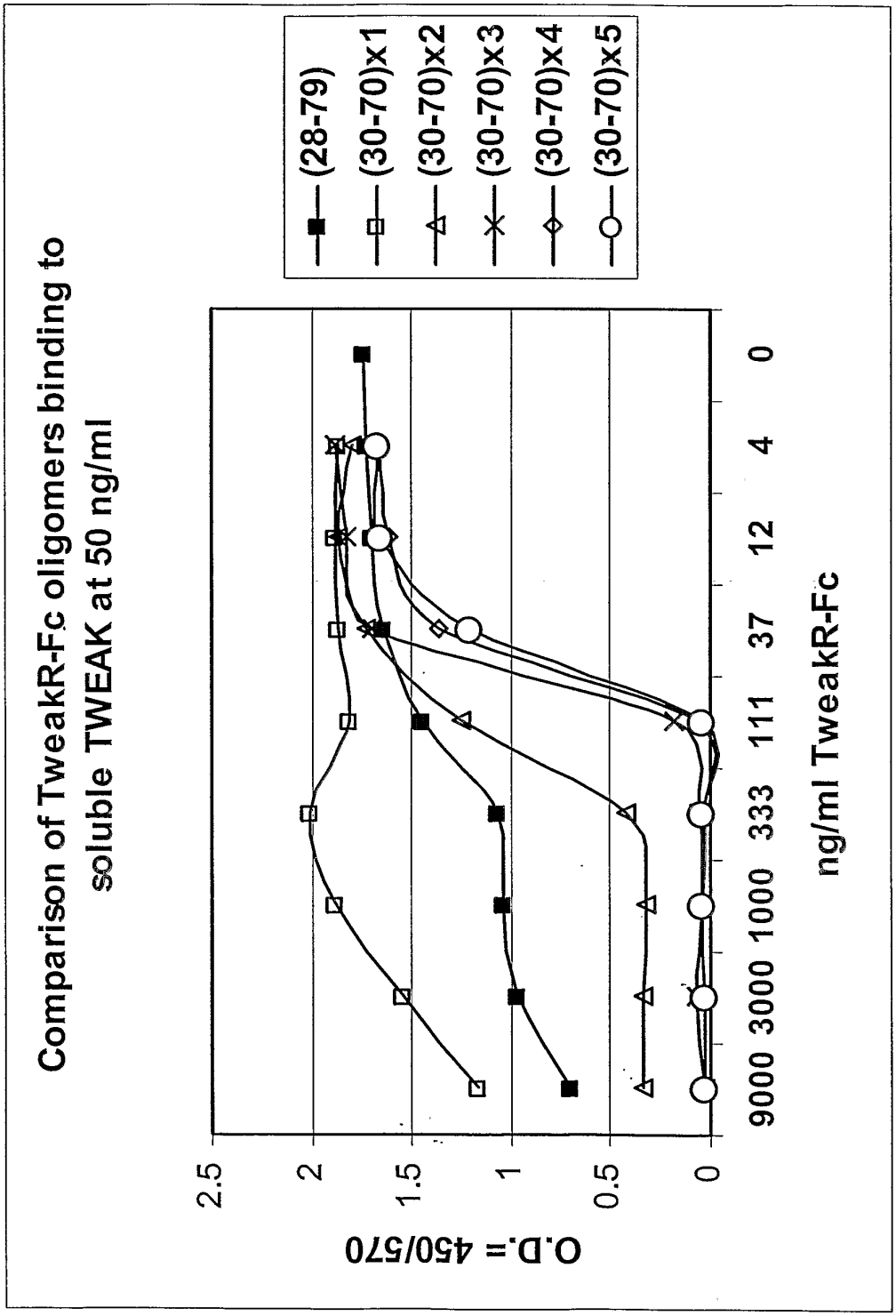

Comparison of TweakR-Fc oligomers binding to soluble TWEAK at 50 ng/ml
O.D.= 450/570
2.5
2
1.5
1
0.5
0
9000 3000 1000 333 111 37 12 4 0
ng/ml TweakR-Fc
(28-79)
(30-70)x1
(30-70)x2
(30-70)x3
(30-70)x4
(30-70)x5

FIGURE 12

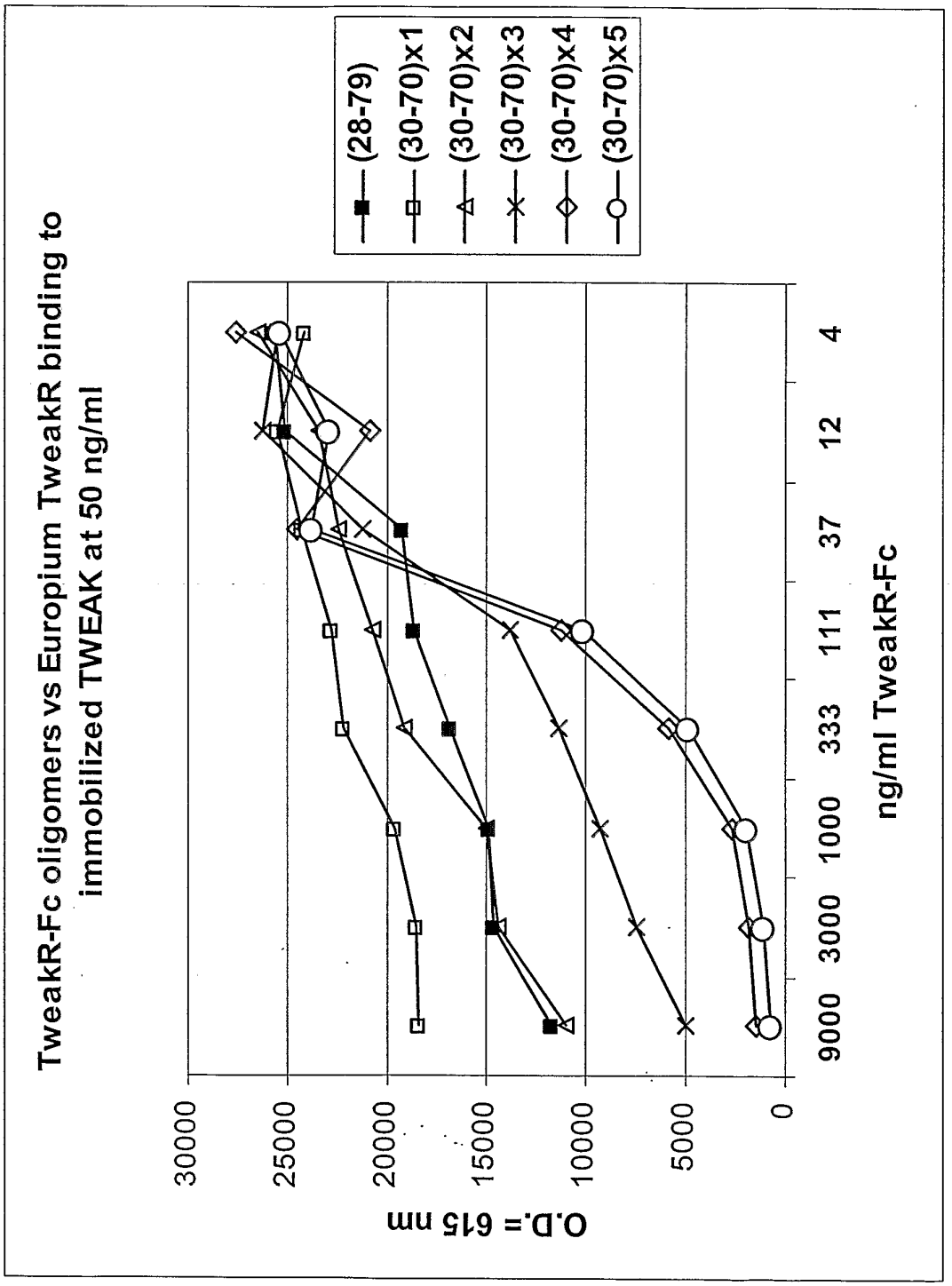

TweakR-Fc oligomers vs Europium TweakR binding to immobilized TWEAK at 50 ng/ml
O.D.= 615 nm
30000
25000
20000
15000
10000
5000
0
9000 3000 1000 333 111 37 12 4
ng/ml TweakR-Fc
(28-79)
(30-70)x1
(30-70)x2
(30-70)x3
(30-70)x4
(30-70)x5

FIGURE 13

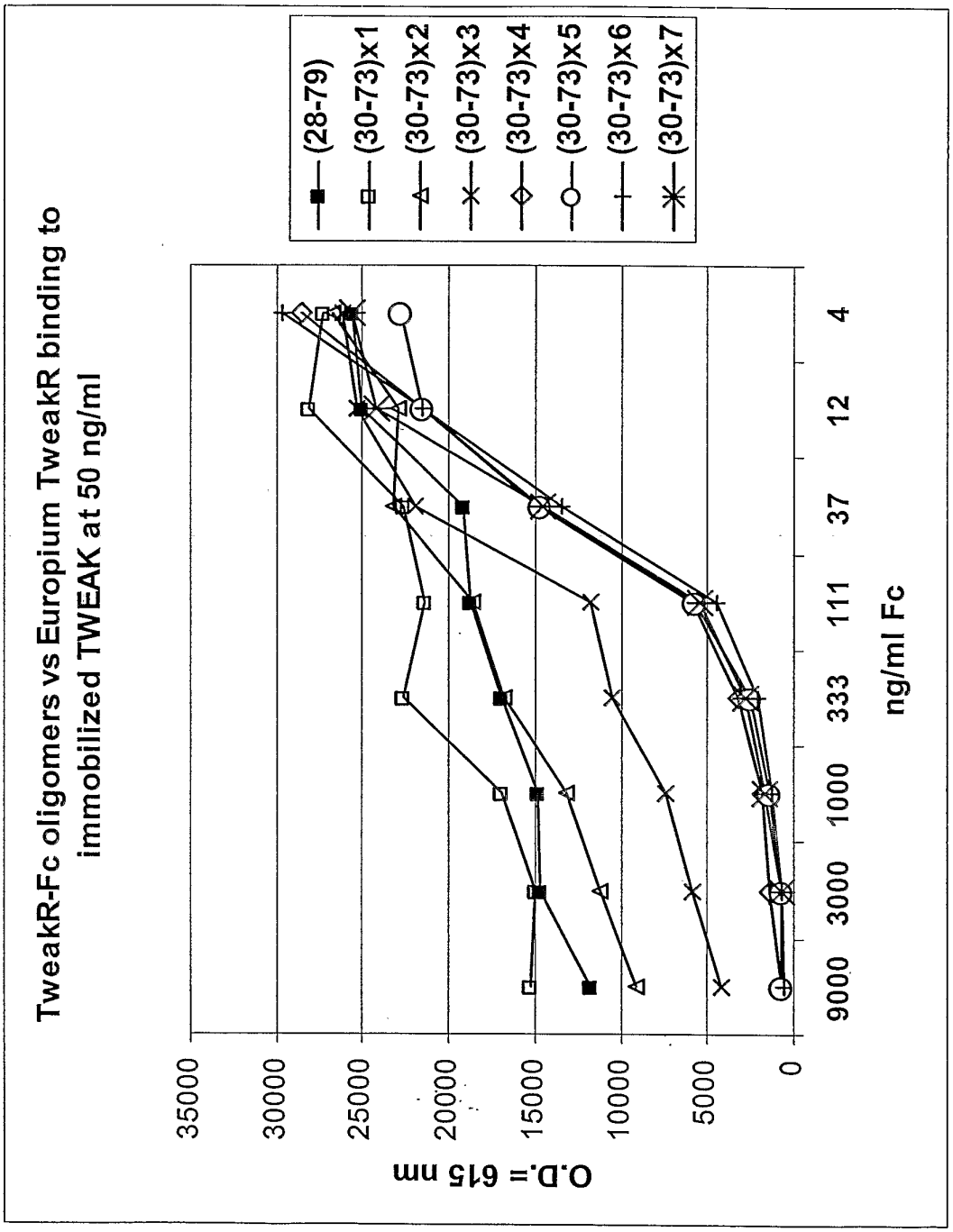

TweakR-Fc oligomers vs Europium TweakR binding to immobilized TWEAK at 50 ng/ml
O.D.= 615 nm
35000
30000
25000
20000
15000
10000
5000
0
9000
3000
1000
333
111
37
12
4
ng/ml Fc
(28-79)
(30-73)x1
(30-73)x2
(30-73)x3
(30-73)x4
(30-73)x5
(30-73)x6
(30-73)x7

FIGURE 14

SEQUENCE LISTING

<110> AMGEN INC.
Wiley, Steven R.

<120> COMPOSITIONS AND METHODS RELATING TO MULTIMERIC AND OLIGOMERIC SOLUBLE FRAGMENTS OF THE TWEAK RECEPTOR

<130> 3430-WO

<140> --to be assigned--
<141> 2004-07-23

<150> US 60/490,036
<151> 2003-07-24

<160> 45

<170> PatentIn version 3.2

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1

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Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu
5 10 15

ccc tgg ctt caa gag ggc agt gca act agt tct gac cgt atg aaa cag 153
Pro Trp Leu Gln Glu Gly Ser Ala Thr Ser Ser Asp Arg Met Lys Gln
20 25 30

ata gag gat aag atc gaa gag atc cta agt aag att tat cat ata gag 201
Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr His Ile Glu
35 40 45 50

aat gaa atc gcc cgt atc aaa aag ctg att ggc gag cgg act aga tct 249
Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Arg Thr Arg Ser
55 60 65

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Ser Leu Gly Ser Arg Ala Ser Leu Ser Ala Gln Glu Pro Ala Gln Glu
70 75 80

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    100                 105                 110

cct cgc aga agt gca cct aaa ggc cgg aaa aca cgg gct cga aga gcg      441
Pro Arg Arg Ser Ala Pro Lys Gly Arg Lys Thr Arg Ala Arg Arg Ala
115                 120                 125                 130

atc gca gcc cat tat gaa gtt cat cca cga cct gga cag gac gga gcg      489
Ile Ala Ala His Tyr Glu Val His Pro Arg Pro Gly Gln Asp Gly Ala
                135                 140                 145

cag gca ggt gtg gac ggg aca gtg agt ggc tgg gag gaa gcc aga atc      537
Gln Ala Gly Val Asp Gly Thr Val Ser Gly Trp Glu Glu Ala Arg Ile
            150                 155                 160

aac agc tcc agc cct ctg cgc tac aac cgc cag atc ggg gag ttt ata      585
Asn Ser Ser Ser Pro Leu Arg Tyr Asn Arg Gln Ile Gly Glu Phe Ile
        165                 170                 175

gtc acc cgg gct ggg ctc tac tac ctg tac tgt cag gtg cac ttt gat      633
Val Thr Arg Ala Gly Leu Tyr Tyr Leu Tyr Cys Gln Val His Phe Asp
    180                 185                 190

gag ggg aag gct gtc tac ctg aag ctg gac ttg ctg gtg gat ggt gtg      681
Glu Gly Lys Ala Val Tyr Leu Lys Leu Asp Leu Leu Val Asp Gly Val
195                 200                 205                 210

ctg gcc ctg cgc tgc ctg gag gaa ttc tca gcc act gcg gcc agt tcc      729
Leu Ala Leu Arg Cys Leu Glu Glu Phe Ser Ala Thr Ala Ala Ser Ser
                215                 220                 225

ctc ggg ccc cag ctc cgc ctc tgc cag gtg tct ggg ctg ttg gcc ctg      777
Leu Gly Pro Gln Leu Arg Leu Cys Gln Val Ser Gly Leu Leu Ala Leu
            230                 235                 240

cgg cca ggg tcc tcc ctg cgg atc cgc acc ctc ccc tgg gcc cat ctc      825
Arg Pro Gly Ser Ser Leu Arg Ile Arg Thr Leu Pro Trp Ala His Leu
        245                 250                 255

aag gct gcc ccc ttc ctc acc tac ttc gga ctc ttc cag gtt cac tga      873
Lys Ala Ala Pro Phe Leu Thr Tyr Phe Gly Leu Phe Gln Val His
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            20                  25                  30

Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr His
        35                  40                  45

Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Arg Thr
    50                  55                  60

Arg Ser Ser Leu Gly Ser Arg Ala Ser Leu Ser Ala Gln Glu Pro Ala
65                  70                  75                  80

Gln Glu Glu Leu Val Ala Glu Glu Asp Gln Asp Pro Ser Glu Leu Asn
                85                  90                  95

Pro Gln Thr Glu Glu Ser Gln Asp Pro Ala Pro Phe Leu Asn Arg Leu
            100                 105                 110

Val Arg Pro Arg Arg Ser Ala Pro Lys Gly Arg Lys Thr Arg Ala Arg
        115                 120                 125

Arg Ala Ile Ala Ala His Tyr Glu Val His Pro Arg Pro Gly Gln Asp
    130                 135                 140

Gly Ala Gln Ala Gly Val Asp Gly Thr Val Ser Gly Trp Glu Glu Ala
145                 150                 155                 160

Arg Ile Asn Ser Ser Ser Pro Leu Arg Tyr Asn Arg Gln Ile Gly Glu
                165                 170                 175

Phe Ile Val Thr Arg Ala Gly Leu Tyr Tyr Leu Tyr Cys Gln Val His
            180                 185                 190

Phe Asp Glu Gly Lys Ala Val Tyr Leu Lys Leu Asp Leu Leu Val Asp
        195                 200                 205

Gly Val Leu Ala Leu Arg Cys Leu Glu Glu Phe Ser Ala Thr Ala Ala
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Ser Ser Leu Gly Pro Gln Leu Arg Leu Cys Gln Val Ser Gly Leu Leu
225                 230                 235                 240

Ala Leu Arg Pro Gly Ser Ser Leu Arg Ile Arg Thr Leu Pro Trp Ala
                245                 250                 255
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Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly Leu Trp
        5                   10                  15

ctg gcg ttg ctg cgc tcc gtg gcc ggg gag caa gcg cca ggc acc gcc      154
Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly Thr Ala
    20                  25                  30

ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc atg      202
Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met
35                  40                  45                  50

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Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly
                55                  60                  65

tgc gct gca gca cct cct gcc ccc ttc cgg ctg ctt tgg ccc atc ctt      298
Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Pro Ile Leu
            70                  75                  80

ggg ggc gct ctg agc ctg acc ttc gtg ctg ggg ctg ctt tct ggc ttt      346
Gly Gly Ala Leu Ser Leu Thr Phe Val Leu Gly Leu Leu Ser Gly Phe
        85                  90                  95

ttg gtc tgg aga cga tgc cgc agg aga gag aag ttc acc acc ccc ata      394
Leu Val Trp Arg Arg Cys Arg Arg Arg Glu Lys Phe Thr Thr Pro Ile
    100                 105                 110

gag gag acc ggc gga gag ggc tgc cca gct gtg gcg ctg atc cag tga      442
Glu Glu Thr Gly Gly Glu Gly Cys Pro Ala Val Ala Leu Ile Gln
115                 120                 125

caatgtgccc cctgccagcc ggggctcgcc cactcatcat tcattcatcc attctagagc    502

cagtctctgc ctcccagacg cggcgggagc caagctcctc caaccacaag gggggtgggg    562

ggcggtgaat cacctctgag gcctgggccc agggttcagg ggaaccttcc aaggtgtctg    622

gttgccctgc ctctggctcc agaacagaaa gggagcctca cgctggctca cacaaaacag    682
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ctgacactga ctaaggaact gcagcatttg cacaggggag gggggtgccc tccttcctag 742

aggccctggg ggccaggctg acttgggggg cagacttgac actaggcccc actcactcag 802

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agaggg 868

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Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
35 40 45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
50 55 60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Pro
65 70 75 80

Ile Leu Gly Gly Ala Leu Ser Leu Thr Phe Val Leu Gly Leu Leu Ser
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Gly Phe Leu Val Trp Arg Arg Cys Arg Arg Arg Glu Lys Phe Thr Thr
100 105 110

Pro Ile Glu Glu Thr Gly Gly Glu Gly Cys Pro Ala Val Ala Leu Ile
115 120 125

Gln

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<213> Mus sp.

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Thr Ser Pro Cys Ser Ser Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
        35                  40                  45

Cys Met Asp Cys Ala Ser Cys Pro Ala Arg Pro His Ser Asp Phe Cys
    50                  55                  60

Leu Gly Cys Ala Ala Ala Pro Pro Ala His Phe Arg Leu Leu Trp Pro
65                  70                  75                  80

Ile Leu Gly Gly Ala Leu Ser Leu Val Leu Val Leu Ala Leu Val Ser
                85                  90                  95

Ser Phe Leu Val Trp Arg Arg Cys Arg Arg Arg Glu Lys Phe Thr Thr
            100                 105                 110

Pro Ile Glu Glu Thr Gly Gly Glu Gly Cys Pro Gly Val Ala Leu Ile
        115                 120                 125

Gln
```

<210> 6
<211> 932
<212> DNA
<213> Artificial Sequence

<220>
<223> TWEAKR:Fc

<220>
<221> CDS
<222> (1)..(930)

<400> 6

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atg gct cgg ggc tcg ctg cgc cgg ttg ctg cgg ctc ctc gtg ctg ggg       48
Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1               5                   10                  15

ctc tgg ctg gcg ttg ctg cgc tcc gtg gcc ggg gag caa gcg cca ggc       96
Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
            20                  25                  30

acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag      144
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
        35                  40                  45
```

```
tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc      192
Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
    50                  55                  60

ctg ggc tgc gct gca gca cct cct gcc ccc ttc cgg ctg ctt tgg aga      240
Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Arg
65                  70                  75                  80

tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc      288
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
                85                  90                  95

gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc      336
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            100                 105                 110

ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg      384
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        115                 120                 125

agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg      432
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
    130                 135                 140

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc      480
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
145                 150                 155                 160

acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg      528
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                165                 170                 175

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc      576
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
            180                 185                 190

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca      624
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        195                 200                 205

cag gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag      672
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
    210                 215                 220

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc      720
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
225                 230                 235                 240

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg      768
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                245                 250                 255

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tat agc aag ctc      816
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            260                 265                 270

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc      864
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        275                 280                 285
```

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc 912
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
290 295 300

ctg tct ccg ggt aaa tga ac 932
Leu Ser Pro Gly Lys
305

<210> 7
<211> 309
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 7

Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1 5 10 15

Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
20 25 30

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
35 40 45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
50 55 60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Arg
65 70 75 80

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
85 90 95

Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
100 105 110

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
115 120 125

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
130 135 140

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
145 150 155 160

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
165 170 175

```
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
            180                 185                 190

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        195                 200                 205

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
    210                 215                 220

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
225                 230                 235                 240

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                245                 250                 255

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            260                 265                 270

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        275                 280                 285

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    290                 295                 300

Leu Ser Pro Gly Lys
305
```

<210> 8
<211> 1089
<212> DNA
<213> Artificial Sequence

<220>
<223> TWEAKRDr5-Fc

<220>
<221> CDS
<222> (1)..(1086)

<400> 8

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atg gct cgg ggc tcg ctg cgc cgg ttg ctg cgg ctc ctc gtg ctg ggg       48
Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1               5                   10                  15

ctc tgg ctg gcg ttg ctg cgc tcc gtg gcc ggg gag caa gcg cca ggc       96
Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
            20                  25                  30

acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag      144
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
        35                  40                  45
```

```
tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc      192
Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
    50                  55                  60

ctg ggc tgc gct gca gca cct cct gcc ccc ttc cgg ctg ctt tgg gag      240
Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Glu
65                  70                  75                  80

caa gcg cca ggc acc gcc ccc tgc tcc cgc ggc agc act cac tgg aat      288
Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Thr His Trp Asn
                85                  90                  95

gac ctc ctt ttc tgc ttg cgc tgc acc agg tgt gat tca ggt gaa gtg      336
Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val
            100                 105                 110

gag cta agt ccc tgc acc acg acc gct gca gca cct cct gcc ccc ttc      384
Glu Leu Ser Pro Cys Thr Thr Thr Ala Ala Ala Pro Pro Ala Pro Phe
        115                 120                 125

cgg ctg ctt tgg aga tct tgt gac aaa act cac aca tgc cca ccg tgc      432
Arg Leu Leu Trp Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
    130                 135                 140

cca gca cct gaa gcc gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca      480
Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro
145                 150                 155                 160

aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc      528
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
                165                 170                 175

gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg      576
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
            180                 185                 190

tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag      624
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
        195                 200                 205

gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg      672
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
    210                 215                 220

cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac      720
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
225                 230                 235                 240

aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg      768
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                245                 250                 255

cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gag gag      816
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
            260                 265                 270

atg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat      864
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
        275                 280                 285
```

```
ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac      912
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    290                 295                 300

aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc      960
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
305                 310                 315                 320

ctc tat agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac     1008
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
                325                 330                 335

gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg     1056
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
            340                 345                 350

cag aag agc ctc tcc ctg tct ccg ggt aaa tga                        1089
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360
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<210> 9
<211> 362
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 9

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Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1               5                   10                  15

Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
            20                  25                  30

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
        35                  40                  45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
    50                  55                  60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Glu
65                  70                  75                  80

Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Thr His Trp Asn
                85                  90                  95

Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val
            100                 105                 110

Glu Leu Ser Pro Cys Thr Thr Thr Ala Ala Ala Pro Pro Ala Pro Phe
        115                 120                 125
```

```
Arg Leu Leu Trp Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
    130                 135                 140

Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro
145                 150                 155                 160

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
                165                 170                 175

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
            180                 185                 190

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
        195                 200                 205

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
    210                 215                 220

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
225                 230                 235                 240

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                245                 250                 255

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
            260                 265                 270

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
        275                 280                 285

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    290                 295                 300

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
305                 310                 315                 320

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
                325                 330                 335

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
            340                 345                 350

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360
```

<210> 10
<211> 1086
<212> DNA
<213> Artificial Sequence

<220>
<223> Di TWEAKR-Fc

<220>
<221> CDS
<222> (1)..(1083)

<400> 10

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atg gct cgg ggc tcg ctg cgc cgg ttg ctg cgg ctc ctc gtg ctg ggg      48
Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1               5                   10                  15

ctc tgg ctg gcg ttg ctg cgc tcc gtg gcc ggg gag caa gcg cca ggc      96
Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
            20                  25                  30

acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag     144
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
        35                  40                  45

tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc     192
Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
    50                  55                  60

ctg ggc tgc gct gca gca cct cct gcc ccc ttc cgg ctg ctt tgg gag     240
Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Glu
65                  70                  75                  80

caa gcg cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg     288
Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala
                85                  90                  95

gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac     336
Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His
            100                 105                 110

agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc ccc ttc cgg     384
Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg
        115                 120                 125

ctg ctt tgg aga tct tgt gac aaa act cac aca tgc cca ccg tgc cca     432
Leu Leu Trp Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
    130                 135                 140

gca cct gaa gcc gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa     480
Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys
145                 150                 155                 160

ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg     528
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
                165                 170                 175

gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac     576
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
            180                 185                 190
```

```
gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag      624
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
        195                 200                 205

cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac      672
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
    210                 215                 220

cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa      720
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
225                 230                 235                 240

gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag      768
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
                245                 250                 255

ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gag gag atg      816
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
            260                 265                 270

acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc      864
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
        275                 280                 285

agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac      912
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
    290                 295                 300

tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc      960
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
305                 310                 315                 320

tat agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc     1008
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
                325                 330                 335

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag     1056
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
            340                 345                 350

aag agc ctc tcc ctg tct ccg ggt aaa tga                            1086
Lys Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360
```

<210> 11
<211> 361
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 11

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Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1               5                   10                  15

Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
            20                  25                  30
```

```
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
        35                  40                  45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
    50                  55                  60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Glu
65                  70                  75                  80

Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala
                85                  90                  95

Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His
            100                 105                 110

Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg
        115                 120                 125

Leu Leu Trp Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
    130                 135                 140

Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys
145                 150                 155                 160

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
                165                 170                 175

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
            180                 185                 190

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
        195                 200                 205

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
    210                 215                 220

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
225                 230                 235                 240

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
                245                 250                 255

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
            260                 265                 270
```

```
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
        275                 280                 285

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
    290                 295                 300

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
305                 310                 315                 320

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
                325                 330                 335

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
            340                 345                 350

Lys Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360
```

<210> 12
<211> 1242
<212> DNA
<213> Artificial Sequence

<220>
<223> Tri TWEAKR-Fc

<220>
<221> CDS
<222> (1)..(1239)

<400> 12

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atg gct cgg ggc tcg ctg cgc cgg ttg ctg cgg ctc ctc gtg ctg ggg       48
Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1               5                   10                  15

ctc tgg ctg gcg ttg ctg cgc tcc gtg gcc ggg gag caa gcg cca ggc       96
Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
            20                  25                  30

acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag      144
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
        35                  40                  45

tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc      192
Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
    50                  55                  60

ctg ggc tgc gct gca gca cct cct gcc ccc ttc cgg ctg ctt tgg gag      240
Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Glu
65                  70                  75                  80

caa gcg cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg      288
Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala
                85                  90                  95
```

```
gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac      336
Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His
            100                 105                 110

agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc ccc ttc cgg      384
Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg
        115                 120                 125

ctg ctt tgg gag caa gcg cca ggc acc gcc ccc tgc tcc cgc ggc agc      432
Leu Leu Trp Glu Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser
    130                 135                 140

tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg      480
Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg
145                 150                 155                 160

gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct      528
Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro
                165                 170                 175

gcc ccc ttc cgg ctg ctt tgg aga tct tgt gac aaa act cac aca tgc      576
Ala Pro Phe Arg Leu Leu Trp Arg Ser Cys Asp Lys Thr His Thr Cys
            180                 185                 190

cca ccg tgc cca gca cct gaa gcc gag ggc gcg ccg tca gtc ttc ctc      624
Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu
        195                 200                 205

ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag      672
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
    210                 215                 220

gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag      720
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
225                 230                 235                 240

ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag      768
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
                245                 250                 255

ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc      816
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
            260                 265                 270

acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag      864
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
        275                 280                 285

gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa      912
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
    290                 295                 300

gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc      960
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
305                 310                 315                 320

cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa     1008
Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                325                 330                 335
```

```
ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag     1056
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
            340                 345                 350

ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc     1104
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
        355                 360                 365

tcc ttc ttc ctc tat agc aag ctc acc gtg gac aag agc agg tgg cag     1152
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
    370                 375                 380

cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac     1200
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
385                 390                 395                 400

cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga             1242
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                405                 410
```

<210> 13
<211> 413
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 13

```
Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1               5                   10                  15

Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
            20                  25                  30

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
        35                  40                  45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
    50                  55                  60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Glu
65                  70                  75                  80

Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala
                85                  90                  95

Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His
            100                 105                 110

Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg
        115                 120                 125
```

```
Leu Leu Trp Glu Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser
    130                 135                 140

Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg
145                 150                 155                 160

Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro
                165                 170                 175

Ala Pro Phe Arg Leu Leu Trp Arg Ser Cys Asp Lys Thr His Thr Cys
            180                 185                 190

Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu
        195                 200                 205

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
    210                 215                 220

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
225                 230                 235                 240

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
                245                 250                 255

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
            260                 265                 270

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
        275                 280                 285

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
    290                 295                 300

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
305                 310                 315                 320

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                325                 330                 335

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
            340                 345                 350

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
        355                 360                 365
```

```
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
    370                 375                 380

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
385                 390                 395                 400

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                405                 410
```

<210> 14
<211> 828
<212> DNA
<213> Artificial

<220>
<223> HuTWEAKr 29-70:Gly5:Fc

<220>
<221> CDS
<222> (1)..(825)

<400> 14

```
atg caa gct cca ggt act gca cca tgt tct cgt ggt tct tct tgg tct      48
Met Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser
1               5                   10                  15

gct gat ctt gat aaa tgt atg gat tgt gct tct tgt cgt gct cgt cca      96
Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro
            20                  25                  30

cat tct gat ttt tgt ctg ggt tgt gct gct gct ggt ggt ggt ggt ggt     144
His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Gly Gly Gly Gly Gly
        35                  40                  45

gac aaa act cac aca tgt cca ccg tgc cca gca cct gaa ctc ctg ggg     192
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
    50                  55                  60

gga ccg tca gtt ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg     240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
65                  70                  75                  80

atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac     288
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
                85                  90                  95

gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg     336
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
            100                 105                 110

cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac     384
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
        115                 120                 125

cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc     432
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
    130                 135                 140
```

```
aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc      480
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
145                 150                 155                 160

gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg      528
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
                165                 170                 175

tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc      576
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
            180                 185                 190

ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag      624
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
        195                 200                 205

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc      672
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
    210                 215                 220

gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg      720
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
225                 230                 235                 240

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg      768
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                245                 250                 255

cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct      816
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
            260                 265                 270

ccg ggt aaa taa                                                     828
Pro Gly Lys
        275
```

<210> 15
<211> 275
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 15

```
Met Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser
1               5                   10                  15

Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro
            20                  25                  30

His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Gly Gly Gly Gly Gly
        35                  40                  45

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
    50                  55                  60
```

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
65 70 75 80

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
85 90 95

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
100 105 110

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
115 120 125

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
130 135 140

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
145 150 155 160

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
165 170 175

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
180 185 190

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
195 200 205

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
210 215 220

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
225 230 235 240

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
245 250 255

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
260 265 270

Pro Gly Lys
275

<210> 16
<211> 879
<212> DNA
<213> Artificial

<220>
<223> HuTWEAKr 29-70:1KPEG:Fc

<220>
<221> CDS
<222> (1)..(876)

<400> 16

```
atg caa gct cca ggt act gca cca tgt tct cgt ggt tct tct tgg tct       48
Met Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser
1               5                  10                  15

gct gat ctt gat aaa tgt atg gat tgt gct tct tgt cgt gct cgt cca       96
Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro
            20                  25                  30

cat tct gat ttt tgt ctg ggt tgt gct gct gct ggt tcc gga tct gct      144
His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Gly Ser Gly Ser Ala
        35                  40                  45

acc ggt ggt tcc ggt tct acc gct tct tct gga tcc ggt tcc gct acc      192
Thr Gly Gly Ser Gly Ser Thr Ala Ser Ser Gly Ser Gly Ser Ala Thr
    50                  55                  60

ggt gac aaa act cac aca tgt cca ccg tgc cca gca cct gaa ctc ctg      240
Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
65                  70                  75                  80

ggg gga ccg tca gtt ttc ctc ttc ccc cca aaa ccc aag gac acc ctc      288
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                85                  90                  95

atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc      336
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            100                 105                 110

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag      384
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        115                 120                 125

gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg      432
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
    130                 135                 140

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat      480
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
145                 150                 155                 160

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc      528
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                165                 170                 175

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag      576
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            180                 185                 190

gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc      624
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
        195                 200                 205
```

```
agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg      672
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    210                 215                 220

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct      720
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
225                 230                 235                 240

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc      768
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                245                 250                 255

gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg      816
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            260                 265                 270

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg      864
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        275                 280                 285

tct ccg ggt aaa taa                                                 879
Ser Pro Gly Lys
    290
```

<210> 17
<211> 292
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 17

```
Met Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser
1               5                   10                  15

Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro
            20                  25                  30

His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Gly Ser Gly Ser Ala
        35                  40                  45

Thr Gly Gly Ser Gly Ser Thr Ala Ser Ser Gly Ser Gly Ser Ala Thr
    50                  55                  60

Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
65                  70                  75                  80

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                85                  90                  95

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            100                 105                 110
```

```
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        115                 120                 125

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
    130                 135                 140

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
145                 150                 155                 160

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                165                 170                 175

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            180                 185                 190

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
        195                 200                 205

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    210                 215                 220

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
225                 230                 235                 240

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                245                 250                 255

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            260                 265                 270

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        275                 280                 285

Ser Pro Gly Lys
    290
```

<210> 18
<211> 339
<212> PRT
<213> Artificial sequence

<220>
<223> TWEAKR:1KPEG:TWEAKR:Gly5:Fc

<400> 18

```
Met Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser
1               5                   10                  15
```

```
Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro
            20                  25                  30

His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Gly Ser Gly Ser Ala
        35                  40                  45

Thr Gly Gly Ser Gly Ser Thr Ala Ser Ser Gly Ser Gly Ser Ala Thr
    50                  55                  60

His Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser
65                  70                  75                  80

Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro
                85                  90                  95

His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Gly Gly Gly Gly Gly
            100                 105                 110

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
        115                 120                 125

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
    130                 135                 140

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
145                 150                 155                 160

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
                165                 170                 175

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
            180                 185                 190

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
        195                 200                 205

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
    210                 215                 220

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
225                 230                 235                 240

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
                245                 250                 255
```

```
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
            260                 265                 270

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
        275                 280                 285

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
    290                 295                 300

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
305                 310                 315                 320

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
                325                 330                 335

Pro Gly Lys
```

<210> 19
<211> 322
<212> PRT
<213> Artificial sequence

<220>
<223> TWEAKR:Gly5:TWEAKR:Gly5:Fc

<400> 19

```
Met Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser
1               5                   10                  15

Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro
            20                  25                  30

His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Gly Gly Gly Gly Gly
        35                  40                  45

Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala
    50                  55                  60

Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His
65                  70                  75                  80

Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Gly Gly Gly Gly Gly Asp
                85                  90                  95

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            100                 105                 110
```

```
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        115                 120                 125

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    130                 135                 140

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
145                 150                 155                 160

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                165                 170                 175

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            180                 185                 190

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        195                 200                 205

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    210                 215                 220

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
225                 230                 235                 240

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                245                 250                 255

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            260                 265                 270

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        275                 280                 285

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    290                 295                 300

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
305                 310                 315                 320

Gly Lys
```

<210> 20
<211> 867
<212> DNA
<213> Artificial sequence

<220>

<223> TWEAKR 40mono-3

<220>
<221> CDS
<222> (1)..(867)

<400> 20

```
atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca       48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc       96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg      144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca gac aaa act      192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Asp Lys Thr
    50                  55                  60

cac aca tgt cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca      240
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
65                  70                  75                  80

gtt ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg      288
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                85                  90                  95

acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct      336
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            100                 105                 110

gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc      384
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        115                 120                 125

aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc      432
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    130                 135                 140

agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac      480
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
145                 150                 155                 160

aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc      528
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                165                 170                 175

atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg      576
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            180                 185                 190

ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc      624
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
        195                 200                 205
```

```
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc      672
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    210                 215                 220

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac      720
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
225                 230                 235                 240

tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc      768
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                245                 250                 255

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct      816
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            260                 265                 270

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa      864
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        275                 280                 285

taa                                                                  867
```

<210> 21
<211> 288
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 21

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Asp Lys Thr
    50                  55                  60

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
65                  70                  75                  80

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                85                  90                  95

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            100                 105                 110
```

```
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        115                 120                 125

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    130                 135                 140

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
145                 150                 155                 160

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                165                 170                 175

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            180                 185                 190

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
        195                 200                 205

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    210                 215                 220

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
225                 230                 235                 240

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                245                 250                 255

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            260                 265                 270

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        275                 280                 285
```

<210> 22
<211> 990
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 40dimer-1

<220>
<221> CDS
<222> (1)..(990)

<400> 22
atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca 48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1   5   10   15

```
ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc        96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg       144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca gca cca ggc       192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly
    50                  55                  60

acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag       240
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
65                  70                  75                  80

tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc       288
Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
                85                  90                  95

ctg ggc tgc gct gca gca gac aaa act cac aca tgt cca ccg tgc cca       336
Leu Gly Cys Ala Ala Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro
            100                 105                 110

gca cct gaa ctc ctg ggg gga ccg tca gtt ttc ctc ttc ccc cca aaa       384
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
        115                 120                 125

ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg       432
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
    130                 135                 140

gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac       480
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
145                 150                 155                 160

gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag       528
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
                165                 170                 175

cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac       576
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
            180                 185                 190

cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa       624
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
        195                 200                 205

gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag       672
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
    210                 215                 220

ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg       720
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
225                 230                 235                 240

acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc       768
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
                245                 250                 255
```

```
agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac      816
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
            260                 265                 270

tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc      864
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
        275                 280                 285

tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc      912
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
    290                 295                 300

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag      960
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
305                 310                 315                 320

aag agc ctc tcc ctg tct ccg ggt aaa taa                             990
Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325
```

<210> 23
<211> 329
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 23

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly
    50                  55                  60

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
65                  70                  75                  80

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
                85                  90                  95

Leu Gly Cys Ala Ala Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro
            100                 105                 110

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
        115                 120                 125
```

```
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
    130                 135                 140

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
145                 150                 155                 160

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
                165                 170                 175

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
            180                 185                 190

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
        195                 200                 205

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
    210                 215                 220

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
225                 230                 235                 240

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
                245                 250                 255

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
            260                 265                 270

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
        275                 280                 285

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
    290                 295                 300

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
305                 310                 315                 320

Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325
```

<210> 24
<211> 1113
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 40trimer-5

<220>
<221> CDS
<222> (1)..(1113)

<400> 24

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atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca       48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc       96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg      144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca gca cca ggc      192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly
    50                  55                  60

acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag      240
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
65                  70                  75                  80

tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc      288
Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
                85                  90                  95

ctg ggc tgc gct gca gca gca cca ggc acc gcc ccc tgc tcc cgc ggc      336
Leu Gly Cys Ala Ala Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly
            100                 105                 110

agc tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc      384
Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys
        115                 120                 125

agg gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca gac      432
Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Asp
    130                 135                 140

aaa act cac aca tgt cca ccg tgc cca gca cct gaa ctc ctg ggg gga      480
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
145                 150                 155                 160

ccg tca gtt ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc      528
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                165                 170                 175

tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa      576
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            180                 185                 190

gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat      624
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        195                 200                 205

aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt      672
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    210                 215                 220
```

```
gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag      720
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
225                 230                 235                 240

gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag      768
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                245                 250                 255

aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac      816
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            260                 265                 270

acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg      864
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        275                 280                 285

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg      912
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    290                 295                 300

gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg      960
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
305                 310                 315                 320

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac     1008
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                325                 330                 335

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat     1056
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            340                 345                 350

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg     1104
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        355                 360                 365

ggt aaa taa                                                        1113
Gly Lys
    370
```

<210> 25
<211> 370
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 25

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45
```

```
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly
    50                  55                  60

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
65                  70                  75                  80

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
                85                  90                  95

Leu Gly Cys Ala Ala Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly
            100                 105                 110

Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys
        115                 120                 125

Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Asp
    130                 135                 140

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
145                 150                 155                 160

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                165                 170                 175

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            180                 185                 190

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        195                 200                 205

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    210                 215                 220

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
225                 230                 235                 240

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                245                 250                 255

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            260                 265                 270

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        275                 280                 285
```

```
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    290                 295                 300

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
305                 310                 315                 320

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                325                 330                 335

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            340                 345                 350

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        355                 360                 365

Gly Lys
    370
```

<210> 26
<211> 1236
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 40quad-2

<220>
<221> CDS
<222> (1)..(1236)

<400> 26

```
atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca       48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc       96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg      144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca gca cca ggc      192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly
    50                  55                  60

acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag      240
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
65                  70                  75                  80

tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc      288
Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
                85                  90                  95
```

```
ctg ggc tgc gct gca gca gca cca ggc acc gcc ccc tgc tcc cgc ggc      336
Leu Gly Cys Ala Ala Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly
            100                 105                 110

agc tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc      384
Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys
        115                 120                 125

agg gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca gca      432
Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala
    130                 135                 140

cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg      480
Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu
145                 150                 155                 160

gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac      528
Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp
                165                 170                 175

ttc tgc ctg ggc tgc gct gca gca gac aaa act cac aca tgt cca ccg      576
Phe Cys Leu Gly Cys Ala Ala Ala Asp Lys Thr His Thr Cys Pro Pro
            180                 185                 190

tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtt ttc ctc ttc ccc      624
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
        195                 200                 205

cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca      672
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
    210                 215                 220

tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac      720
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
225                 230                 235                 240

tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg      768
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
                245                 250                 255

gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc      816
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
            260                 265                 270

ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc      864
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
        275                 280                 285

aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa      912
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
    290                 295                 300

ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat      960
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
305                 310                 315                 320

gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc     1008
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
                325                 330                 335
```

```
tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag     1056
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
            340                 345                 350

aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc     1104
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
        355                 360                 365

ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg     1152
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
    370                 375                 380

aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac     1200
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
385                 390                 395                 400

acg cag aag agc ctc tcc ctg tct ccg ggt aaa taa                     1236
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                405                 410
```

<210> 27
<211> 411
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 27

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly
    50                  55                  60

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
65                  70                  75                  80

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
                85                  90                  95

Leu Gly Cys Ala Ala Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly
            100                 105                 110

Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys
        115                 120                 125
```

```
Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala
    130                 135                 140

Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu
145                 150                 155                 160

Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp
                165                 170                 175

Phe Cys Leu Gly Cys Ala Ala Ala Asp Lys Thr His Thr Cys Pro Pro
            180                 185                 190

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
        195                 200                 205

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
    210                 215                 220

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
225                 230                 235                 240

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
                245                 250                 255

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
            260                 265                 270

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
        275                 280                 285

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
    290                 295                 300

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
305                 310                 315                 320

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
                325                 330                 335

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
            340                 345                 350

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
        355                 360                 365
```

```
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
    370                 375                 380

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
385                 390                 395                 400

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                405                 410
```

<210> 28
<211> 1359
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 40quint-1

<220>
<221> CDS
<222> (1)..(1359)

<400> 28

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atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca      48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc      96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg     144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca gca cca ggc     192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly
    50                  55                  60

acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag     240
Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
65                  70                  75                  80

tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc     288
Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
                85                  90                  95

ctg ggc tgc gct gca gca gca cca ggc acc gcc ccc tgc tcc cgc ggc     336
Leu Gly Cys Ala Ala Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly
            100                 105                 110

agc tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc     384
Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys
        115                 120                 125

agg gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca gca     432
Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala
    130                 135                 140
```

```
cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg      480
Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu
145                 150                 155                 160

gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac      528
Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp
                165                 170                 175

ttc tgc ctg ggc tgc gct gca gca gca cca ggc acc gcc ccc tgc tcc      576
Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly Thr Ala Pro Cys Ser
            180                 185                 190

cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg      624
Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
        195                 200                 205

tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca      672
Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
    210                 215                 220

gca gac aaa act cac aca tgt cca ccg tgc cca gca cct gaa ctc ctg      720
Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225                 230                 235                 240

ggg gga ccg tca gtt ttc ctc ttc ccc cca aaa ccc aag gac acc ctc      768
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255

atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc      816
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            260                 265                 270

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag      864
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        275                 280                 285

gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg      912
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
    290                 295                 300

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat      960
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305                 310                 315                 320

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc     1008
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                325                 330                 335

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag     1056
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            340                 345                 350

gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc     1104
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
        355                 360                 365

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg     1152
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    370                 375                 380
```

```
gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct     1200
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385                 390                 395                 400

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc     1248
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                405                 410                 415

gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg     1296
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            420                 425                 430

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg     1344
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        435                 440                 445

tct ccg ggt aaa taa                                                 1359
Ser Pro Gly Lys
    450
```

<210> 29
<211> 452
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 29

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly
    50                  55                  60

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
65                  70                  75                  80

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
                85                  90                  95

Leu Gly Cys Ala Ala Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly
            100                 105                 110

Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys
        115                 120                 125
```

```
Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Ala
    130                 135                 140

Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu
145                 150                 155                 160

Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp
                165                 170                 175

Phe Cys Leu Gly Cys Ala Ala Ala Ala Pro Gly Thr Ala Pro Cys Ser
            180                 185                 190

Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
        195                 200                 205

Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
    210                 215                 220

Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225                 230                 235                 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            260                 265                 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        275                 280                 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
    290                 295                 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305                 310                 315                 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                325                 330                 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            340                 345                 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
        355                 360                 365
```

```
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    370                 375                 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385                 390                 395                 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                405                 410                 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            420                 425                 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        435                 440                 445

Ser Pro Gly Lys
    450
```

<210> 30
<211> 876
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 43mono-F4

<220>
<221> CDS
<222> (1)..(876)

<400> 30

```
atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca      48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc      96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg     144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc     192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

gac aaa act cac aca tgt cca ccg tgc cca gca cct gaa ctc ctg ggg     240
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
65                  70                  75                  80

gga ccg tca gtt ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg     288
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                85                  90                  95
```

```
atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac      336
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            100                 105                 110

gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg      384
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
        115                 120                 125

cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac      432
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
    130                 135                 140

cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc      480
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
145                 150                 155                 160

aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc      528
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                165                 170                 175

gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg      576
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
            180                 185                 190

tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc      624
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
        195                 200                 205

ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag      672
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    210                 215                 220

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc      720
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
225                 230                 235                 240

gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg      768
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                245                 250                 255

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg      816
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            260                 265                 270

cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct      864
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        275                 280                 285

ccg ggt aaa taa                                                      876
Pro Gly Lys
    290
```

<210> 31
<211> 291
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 31

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                  10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
65                  70                  75                  80

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                85                  90                  95

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            100                 105                 110

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
        115                 120                 125

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
    130                 135                 140

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
145                 150                 155                 160

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                165                 170                 175

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
            180                 185                 190

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
        195                 200                 205

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    210                 215                 220

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
225                 230                 235                 240
```

```
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                245                 250                 255

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            260                 265                 270

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        275                 280                 285

Pro Gly Lys
    290
```

<210> 32
<211> 1008
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 43dimer-F2

<220>
<221> CDS
<222> (1)..(1008)

<400> 32

```
atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca      48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc      96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg     144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc     192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac     240
Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc     288
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc gac aaa act cac     336
Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Asp Lys Thr His
            100                 105                 110

aca tgt cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtt     384
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
        115                 120                 125
```

```
ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc      432
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
    130                 135                 140

cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag      480
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
145                 150                 155                 160

gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag      528
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
                165                 170                 175

aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc      576
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
            180                 185                 190

gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag      624
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
        195                 200                 205

tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc      672
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
    210                 215                 220

tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc      720
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
225                 230                 235                 240

cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg      768
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                245                 250                 255

gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat      816
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
            260                 265                 270

ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc      864
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
        275                 280                 285

gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg      912
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
    290                 295                 300

tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg      960
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
305                 310                 315                 320

cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa taa     1008
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330                 335
```

<210> 33
<211> 335
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 33

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                  10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Asp Lys Thr His
            100                 105                 110

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
        115                 120                 125

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
    130                 135                 140

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
145                 150                 155                 160

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
                165                 170                 175

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
            180                 185                 190

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
        195                 200                 205

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
    210                 215                 220

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
225                 230                 235                 240
```

```
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                245                 250                 255

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
            260                 265                 270

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
        275                 280                 285

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
    290                 295                 300

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
305                 310                 315                 320

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330                 335
```

<210> 34
<211> 1140
<212> DNA
<213> Artificial

<220>
<223> TWEAKR 43trimer-1

<220>
<221> CDS
<222> (1)..(1140)

<400> 34

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atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca      48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc      96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg     144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc     192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac     240
Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc     288
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95
```

```
gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc gca cca ggc acc      336
Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
            100                 105                 110

gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc      384
Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
        115                 120                 125

atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg      432
Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
    130                 135                 140

ggc tgc gct gca gca cct cct gcc gac aaa act cac aca tgt cca ccg      480
Gly Cys Ala Ala Ala Pro Pro Ala Asp Lys Thr His Thr Cys Pro Pro
145                 150                 155                 160

tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtt ttc ctc ttc ccc      528
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
                165                 170                 175

cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca      576
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
            180                 185                 190

tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac      624
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
        195                 200                 205

tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg      672
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
    210                 215                 220

gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc      720
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
225                 230                 235                 240

ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc      768
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
                245                 250                 255

aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa      816
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
            260                 265                 270

ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat      864
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
        275                 280                 285

gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc      912
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
    290                 295                 300

tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag      960
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
305                 310                 315                 320

aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc     1008
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
                325                 330                 335
```

```
ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg     1056
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
            340                 345                 350

aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac     1104
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
        355                 360                 365

acg cag aag agc ctc tcc ctg tct ccg ggt aaa taa                     1140
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    370                 375
```

<210> 35
<211> 379
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 35

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
            100                 105                 110

Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
        115                 120                 125

Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
    130                 135                 140

Gly Cys Ala Ala Ala Pro Pro Ala Asp Lys Thr His Thr Cys Pro Pro
145                 150                 155                 160
```

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
165 170 175

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
180 185 190

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
195 200 205

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
210 215 220

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
225 230 235 240

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
245 250 255

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
260 265 270

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
275 280 285

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
290 295 300

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
305 310 315 320

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
325 330 335

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
340 345 350

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
355 360 365

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375

<210> 36
<211> 1272
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 43quad-2

<220>
<221> CDS
<222> (1)..(1272)

<400> 36

```
atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca       48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc       96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg      144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc      192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac      240
Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc      288
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc gca cca ggc acc      336
Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
            100                 105                 110

gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc      384
Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
        115                 120                 125

atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg      432
Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
    130                 135                 140

ggc tgc gct gca gca cct cct gcc gca cca ggc acc gcc ccc tgc tcc      480
Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser
145                 150                 155                 160

cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg      528
Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
                165                 170                 175

tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca      576
Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
            180                 185                 190

gca cct cct gcc gac aaa act cac aca tgt cca ccg tgc cca gca cct      624
Ala Pro Pro Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
        195                 200                 205
```

```
gaa ctc ctg ggg gga ccg tca gtt ttc ctc ttc ccc cca aaa ccc aag      672
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
    210                 215                 220

gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg      720
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
225                 230                 235                 240

gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac      768
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
                245                 250                 255

ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac      816
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
            260                 265                 270

aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac      864
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
        275                 280                 285

tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc      912
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
    290                 295                 300

cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga      960
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
305                 310                 315                 320

gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag     1008
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
                325                 330                 335

aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac     1056
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
            340                 345                 350

atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag     1104
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
        355                 360                 365

acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc     1152
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
    370                 375                 380

aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca     1200
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
385                 390                 395                 400

tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc     1248
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
                405                 410                 415

ctc tcc ctg tct ccg ggt aaa taa                                    1272
Leu Ser Leu Ser Pro Gly Lys
            420
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<210> 37
<211> 423
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 37

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
20 25 30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
35 40 45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
50 55 60

Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65 70 75 80

Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
85 90 95

Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
100 105 110

Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
115 120 125

Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
130 135 140

Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser
145 150 155 160

Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
165 170 175

Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
180 185 190

Ala Pro Pro Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
195 200 205

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
210 215 220

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
225 230 235 240

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
245 250 255

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
260 265 270

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
275 280 285

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
290 295 300

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
305 310 315 320

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
325 330 335

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
340 345 350

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
355 360 365

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
370 375 380

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
385 390 395 400

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
405 410 415

Leu Ser Leu Ser Pro Gly Lys
420

<210> 38
<211> 1404
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 43quint-3

<220>
<221> CDS
<222> (1)..(1404)

<400> 38

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atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca       48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc       96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg      144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc      192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac      240
Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc      288
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc gca cca ggc acc      336
Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
            100                 105                 110

gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc      384
Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
        115                 120                 125

atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg      432
Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
    130                 135                 140

ggc tgc gct gca gca cct cct gcc gca cca ggc acc gcc ccc tgc tcc      480
Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser
145                 150                 155                 160

cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg      528
Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
                165                 170                 175

tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca      576
Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
            180                 185                 190

gca cct cct gcc gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc      624
Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
        195                 200                 205

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg      672
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
    210                 215                 220
```

```
cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc      720
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
225                 230                 235                 240

gac aaa act cac aca tgt cca ccg tgc cca gca cct gaa ctc ctg ggg      768
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                245                 250                 255

gga ccg tca gtt ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg      816
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac      864
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg      912
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac      960
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc     1008
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc     1056
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg     1104
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc     1152
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    370                 375                 380

ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag     1200
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc     1248
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg     1296
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg     1344
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct     1392
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460
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ccg ggt aaa taa 1404
Pro Gly Lys
465

<210> 39
<211> 467
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 39

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
20 25 30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
35 40 45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
50 55 60

Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65 70 75 80

Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
85 90 95

Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
100 105 110

Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
115 120 125

Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
130 135 140

Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser
145 150 155 160

Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
165 170 175

Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
180 185 190

```
Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
        195                 200                 205

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
    210                 215                 220

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
225                 230                 235                 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270
                                                    -

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430
```

```
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Lys
465
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<210> 40
<211> 1536
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 43hex-1

<220>
<221> CDS
<222> (1)..(1536)

<400> 40

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atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca      48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc      96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg     144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc     192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac     240
Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc     288
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc gca cca ggc acc     336
Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
            100                 105                 110

gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc     384
Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
        115                 120                 125

atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg     432
Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
    130                 135                 140
```

```
ggc tgc gct gca gca cct cct gcc gca cca ggc acc gcc ccc tgc tcc      480
Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser
145                 150                 155                 160

cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg      528
Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
                165                 170                 175

tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca      576
Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
            180                 185                 190

gca cct cct gcc gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc      624
Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
        195                 200                 205

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg      672
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
    210                 215                 220

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc      720
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
225                 230                 235                 240

gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac      768
Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
                245                 250                 255

ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc      816
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
            260                 265                 270

gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc gac aaa act cac      864
Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Asp Lys Thr His
        275                 280                 285

aca tgt cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtt      912
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
    290                 295                 300

ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc      960
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
305                 310                 315                 320

cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag     1008
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
                325                 330                 335

gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag     1056
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
            340                 345                 350

aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc     1104
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
        355                 360                 365

gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag     1152
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
    370                 375                 380
```

```
tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc     1200
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
385                 390                 395                 400

tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc     1248
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
                405                 410                 415

cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg     1296
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
            420                 425                 430

gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat     1344
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
        435                 440                 445

ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc     1392
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
    450                 455                 460

gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg     1440
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
465                 470                 475                 480

tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg     1488
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
                485                 490                 495

cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa taa     1536
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            500                 505                 510
```

<210> 41
<211> 511
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 41

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80
```

```
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
            100                 105                 110

Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
        115                 120                 125

Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
    130                 135                 140

Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser
145                 150                 155                 160

Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
                165                 170                 175

Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
            180                 185                 190

Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
        195                 200                 205

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
    210                 215                 220

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
225                 230                 235                 240

Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
                245                 250                 255

Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
            260                 265                 270

Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Asp Lys Thr His
        275                 280                 285

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
    290                 295                 300

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
305                 310                 315                 320
```

```
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
                325                 330                 335

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
            340                 345                 350

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
        355                 360                 365

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
    370                 375                 380

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
385                 390                 395                 400

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
                405                 410                 415

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
            420                 425                 430

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
        435                 440                 445

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
    450                 455                 460

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
465                 470                 475                 480

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
                485                 490                 495

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            500                 505                 510
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<210> 42
<211> 1668
<212> DNA
<213> Artificial sequence

<220>
<223> TWEAKR 43-sept-1

<220>
<221> CDS
<222> (1)..(1668)

<400> 42

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atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca        48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                  10                  15

ggt tcc acc ggt gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc        96
Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg       144
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc       192
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac       240
Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc       288
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc gca cca ggc acc       336
Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
            100                 105                 110

gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc       384
Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
        115                 120                 125

atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg       432
Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
    130                 135                 140

ggc tgc gct gca gca cct cct gcc gca cca ggc acc gcc ccc tgc tcc       480
Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser
145                 150                 155                 160

cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg       528
Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
                165                 170                 175

tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca       576
Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
            180                 185                 190

gca cct cct gcc gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc       624
Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
        195                 200                 205

tgg agc gcg gac ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg       672
Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
    210                 215                 220

cga ccg cac agc gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc       720
Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
225                 230                 235                 240
```

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gca cca ggc acc gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac      768
Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
                245                 250                 255

ctg gac aag tgc atg gac tgc gcg tct tgc agg gcg cga ccg cac agc      816
Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
            260                 265                 270

gac ttc tgc ctg ggc tgc gct gca gca cct cct gcc gca cca ggc acc      864
Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
        275                 280                 285

gcc ccc tgc tcc cgc ggc agc tcc tgg agc gcg gac ctg gac aag tgc      912
Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
    290                 295                 300

atg gac tgc gcg tct tgc agg gcg cga ccg cac agc gac ttc tgc ctg      960
Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
305                 310                 315                 320

ggc tgc gct gca gca cct cct gcc gac aaa act cac aca tgt cca ccg     1008
Gly Cys Ala Ala Ala Pro Pro Ala Asp Lys Thr His Thr Cys Pro Pro
                325                 330                 335

tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtt ttc ctc ttc ccc     1056
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
            340                 345                 350

cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca     1104
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
        355                 360                 365

tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac     1152
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
    370                 375                 380

tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg     1200
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
385                 390                 395                 400

gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc     1248
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
                405                 410                 415

ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc     1296
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
            420                 425                 430

aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa     1344
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
        435                 440                 445

ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat     1392
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
    450                 455                 460

gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc     1440
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
465                 470                 475                 480
```

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tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag      1488
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
                485                 490                 495

aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc      1536
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
            500                 505                 510

ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg      1584
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
        515                 520                 525

aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac      1632
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
    530                 535                 540

acg cag aag agc ctc tcc ctg tct ccg ggt aaa taa                     1668
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
545                 550                 555
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<210> 43
<211> 555
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 43

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
            20                  25                  30

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
        35                  40                  45

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
    50                  55                  60

Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
65                  70                  75                  80

Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
                85                  90                  95

Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
            100                 105                 110

Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
        115                 120                 125
```

Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
130 135 140

Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser
145 150 155 160

Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala
165 170 175

Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala
180 185 190

Ala Pro Pro Ala Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser
195 200 205

Trp Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala
210 215 220

Arg Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala
225 230 235 240

Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp
245 250 255

Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser
260 265 270

Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Ala Pro Gly Thr
275 280 285

Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys Cys
290 295 300

Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu
305 310 315 320

Gly Cys Ala Ala Ala Pro Pro Ala Asp Lys Thr His Thr Cys Pro Pro
325 330 335

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
340 345 350

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
355 360 365

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
370 375 380

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
385 390 395 400

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
405 410 415

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
420 425 430

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
435 440 445

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
450 455 460

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
465 470 475 480

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
485 490 495

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
500 505 510

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
515 520 525

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
530 535 540

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
545 550 555

<210> 44
<211> 282
<212> PRT
<213> Artificial Sequence

<220>
<223> huTWEAKr 28-76:gly5:Fc

<400> 44

Met Glu Gln Ala Pro Gly Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp
1 5 10 15

Ser Ala Asp Leu Asp Lys Cys Met Asp Cys Ala Ser Cys Arg Ala Arg
20 25 30

Pro His Ser Asp Phe Cys Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro
35 40 45

Phe Arg Gly Gly Gly Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys
50 55 60

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
65 70 75 80

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
85 90 95

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
100 105 110

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
115 120 125

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
130 135 140

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
145 150 155 160

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
165 170 175

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
180 185 190

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
195 200 205

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
210 215 220

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
225 230 235 240

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
245 250 255

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
260 265 270

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
275 280

<210> 45
<211> 5
<212> PRT
<213> Artificial sequence

<220>
<223> linker

<400> 45

Gly Gly Gly Gly Gly
1 5