THERAPEUTIC USE OF P75NTR NEUROTROPHIN BINDING PROTEIN

FIELD OF THE INVENTION

The present invention relates to a new therapeutic use of a p75NTR neurotrophin binding protein and related molecules in the treatment of osteoarthritis.

BACKGROUND TO THE INVENTION

Osteoarthritis is a group of conditions in which the joints are degraded, leading to pain, tenderness, stiffness and locking of joints. It is the most common form of arthritis, affecting millions of people around the world.

The condition is generally accepted to be the result of mechanical damage to cartilage in joints with inadequate self repair. In addition to leading to inflammation, pain and swelling, the loss of cartilage can lead to the formation of bone outgrowths (osteophytes) which exacerbate symptoms and can lead to narrowing and distortion of the joint. The precise mechanisms of cartilage damage and loss are not precisely understood and may be the result of a combination of factors.

Treatment of osteoarthritis is limited to management of the condition, with no curative treatment options available. There are also no reported treatments which halt progression of this degenerative disease. Management options include physiotherapy, administration of pain killers and/or administration of anti-inflammatory drugs and, in some cases, joint replacement via surgery.

The neurotrophins, neurotrophic growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/ 5 (NT-4/5) act via four receptors: the low affinity p75 neurotrophin receptor (p75NTR), and the high affinity tyrosine kinase receptors; TrkA, TrkB, and TrkC. The low affinity receptor p75NTR binds and is activated by all four neurotrophins and has been reported to function independently from the other receptors. However, the Trk receptors are more selectively activated i.e. NGF is the selective ligand for TrkA, BDNF the ligand for TrkB and NT-3, 4/5 the ligands for TrkC. In addition it has been reported, when p75NTR and Trk proteins are co-expressed, they form complexes, which alter the signalling of both receptors (Huang and Reichardt, 2003). Indeed, it has been suggested that p75NTR facilitates the selectivity of each of the neurotrophins for their respective Trk receptor.

The p75NTR is a member of the tumor necrosis factor receptor superfamily (TNFR-SF) and was the first member of this superfamily to be characterized fully. The superfamily (encoded by some 30 genes in humans) is defined by ligand-binding domains consisting of one or more (typically four) repeats of a 40 amino acid cysteine-rich domain (CRD) that was first identified in p75NTR (Johnson et al., 1986; Radeke et al., 1987). In contrast, no sequence motif is shared by the intracellular domains of all TNFR-SF family members. Consequently, signalling mechanisms of TNFR-SF proteins vary significantly.

An unusual feature of p75NTR structure is the existence of a disulfide-linked p75NTR dimer, formed via cysteinyl residues within the transmembrane domains. This disulfide linkage is required for effective neurotrophin-dependent signalling by p75NTR and plays

an important role in the formation of an intracellular and extracellular domain (Vilar et al., 2009b). Neurotrophins exist physiologically as noncovalently associated dimers (Bothwell and Shooter, 1977) with a distribution half-life of approximately 5 min (Tria et al., 1994). Neurotrophin-dependent p75NTR activation involves association of a neurotrophin dimer with CRDs 2-4 of the two extracellular domains of a p75NTR dimer (He and Garcia, 2004). Recent studies support a model in which neurotrophin binding causes the two extracellular domains of p75NTR dimers to move closer together, forcing the intracellular domains to splay apart in a snail-tong-like motion centered on the disulfide bond and permitting association of the intracellular domains with the signalling adapter proteins, NRIF and TRAF6 (Vilar et al., 2009a, 2009b). Intra-transmembrane domain disulfide bonds, such as are present in p75NTR, have not been described previously in other TNFR-SF family members, or in any other membrane protein.

p75NTR undergoes sequential proteolytic cleavage by alpha-secretase and gamma-secretase activities and matrix metalloproteinases (MMPs), releasing its intracellular domain (ICD) into the cytoplasm, in a manner analogous to the cleavage-dependent signalling pathway of Notch and beta-amyloid precursor protein (Jung et al., 2003; Kanning et al., 2003). Cytoplasmic release of the p75NTR ICD by this pathway promotes signalling by associated NRIF (Kenchappa et al., 2006). The role of the extracellular domain of p75NTR, following the proteolytic cleavage by alpha-secretase and gamma-secretase activities and MMPs is not fully understood.

It has been documented that NGF and other neurotrophins (BDNF, NT-3 and NT-4/5) play a significant role in pathology for example pain due to osteoarthritis, pancreatitis, rheumatoid arthritis, psoriasis, pruritis and multiple sclerosis (Watanabe et al., 2010; Raychaudhuri et al., 2011; Barthel et al., 2009; Truzzi et al., 2011; McDonald et al., 2011; Yamaoka et al., 2007). It was been demonstrated that selective antibodies to any of the neurotrophins; either NGF or BDNF, NT-3 and NT-4/5 significantly reduce pain. Furthermore, antibodies directed to the neurotrophin receptors p75NTR Trk A, Trk B or Trk C have also been demonstrated to be efficacious in models of pain (Orita S et al., 2010; Svensson P et al., 2010; Iwakura et al., 2010; Cirilio et al., 2010; Pezet et al., 2010; Hayashi et al., 2011; Chu et al., 2011; Ueda et al., 2010; Ghilardi et al., 2010; Fukui et al., 2010). Fukui et al., (2010) in a model of pain (mechanical allodynia following sciatic nerve crush) demonstrated significant efficacy on pain related endpoints following treatment with an anti-p75NTR antibody. It was concluded from this study that the treatment with a p75NTR inhibitory antibody reduced CGRP and p75NTR expression resulting in a significant reduction in pain.

BRIEF DESCRIPTION OF THE INVENTION

The current invention demonstrates the extracellular domain of p75NTR is useful in the treatment of osteoarthritis. The extracellular domain of p75NTR has been shown to halt and even reverse the progression of the disease.

Accordingly, there is provided in a first aspect a p75NTR neurotrophin binding protein (p75NTR(NBP)) for use in the treatment of osteoarthritis.

In preferred embodiments, the treatment of osteoarthritis includes relief from the symptoms of osteoarthritis. Preferably relief from the symptoms of osteoarthritis include,

but are not limited to reduction in pain, inflammation, swelling, tenderness, joint stiffness or increase in joint mobility or any combination of these.

In a particularly preferred embodiment, treatment of osteoarthritis includes slowing or arresting of disease progression and/or reduction in cartilage loss. Preferably treatment of osteoarthritis includes reversal of disease progression, regrowth of cartilage and/or curative treatment. Preferably disease progression is determined by the rate of cartilage loss or regrowth. In other preferred embodiments, disease progression may be monitored by determining the number of chondrocytes present in a joint.

In other preferred embodiments, the treatment of osteoarthritis includes prophylactic treatment.

In certain preferred embodiments the p75NTR(NBP) is a human p75NTR(NBP).

In other preferred embodiments the p75NTR(NBP) comprises a p75NTR(NBP) connected to one or more auxiliary molecules. Preferably, the one or more auxiliary molecules are selected from: (a) transferrin or a portion thereof; (b) albumin or a portion thereof; (c) an immunoglobulin Fc or a portion thereof; or (d) a polyethylene glycol polymer chain. In still other preferred embodiments the p75NTR(NBP) is connected to the one or more auxiliary molecules via one or more linkers.

In an especially preferred embodiment the p75NTR(NBP) has the amino acid sequence according to SEQ ID NO 3.

In a particularly preferred embodiment the p75NTR(NBP) binds to any of NGF, BDNF, NT3 or NT4/5 with a binding affinity (K_d) of between about 5 pM to about 5 nM as measured by surface plasmon resonance at 20 °C.

In a preferred embodiment the p75NTR(NBP) is for separate, sequential or simultaneous use in a combination combined with a second pharmacologically active compound. Preferably the second pharmacologically active compound of the combination is selected from an opioid analgesic, a nonsteroidal anti-inflammatory drug (NSAID), a barbiturate sedative, a benzodiazepine having a sedative action, an H1 antagonist having a sedative action, a sedative such as glutethimide, meprobamate, methaqualone or dichloralphenazone; a skeletal muscle relaxant; an NMDA receptor antagonist, an alpha-adrenergic, a tricyclic antidepressant, an anticonvulsant, a tachykinin (NK) antagonist, particularly an NK-3, NK-2 or NK-1 antagonist, a muscarinic antagonist, a COX-2 selective inhibitor, a coal-tar analgesic, in particular paracetamol; a neuroleptic a vanilloid receptor agonist or antagonist; a beta-adrenergic; a local anaesthetic; a corticosteroid; a 5-HT receptor agonist or antagonist; a 5-HT2A receptor antagonist; a cholinergic (nicotinic) analgesic; Tramadol®; a PDEV inhibitor; a cannabinoid; metabotropic glutamate subtype 1 receptor (mGluR1) antagonist; a serotonin reuptake inhibitor; a noradrenaline (norepinephrine) reuptake inhibitor; a dual serotonin-noradrenaline reuptake inhibitor; an inducible nitric oxide synthase (iNOS) inhibitor; an acetylcholinesterase inhibitor; a prostaglandin E2 subtype 4 (EP4) antagonist; a leukotriene B4 antagonist; a 5-lipoxygenase inhibitor; a sodium channel blocker; or a 5-HT3 antagonist, and the pharmaceutically acceptable salts and solvates thereof.

Preferably the p75NTR(NBP) is formulated for oral, sublingual, buccal, topical, rectal, inhalation, transdermal, subcutaneous, intravenous, intra-arterial, intramuscular, intracardiac, intraosseous, intrasynovial, intradermal, intraperitoneal, transmucosal, vaginal, intravitreal, intra-articular, peri-articular, local or epicutaneous administration.

In a further aspect of the present invention there is provided a nucleic acid encoding a p75NTR(NBP), for use in the treatment of osteoarthritis as defined above.

In another aspect of the present invention there is provided a replicable expression vector for transfecting a cell, comprising a nucleic acid encoding a p75NTR(NBP), for use in the treatment of osteoarthritis as defined above.

In yet another aspect of the present invention there is provided a host cell expressing a p75NTR(NBP), for use in the treatment of osteoarthritis as defined above.

In a still further aspect of the present invention there is provided a pharmaceutical composition, comprising the p75NTR(NBP), the nucleic acid molecule, the replicable expression vector, or the host cell described above, and a pharmaceutically acceptable carrier and/or an excipient.

Another aspect of the invention pertains to a kit comprising:

a. the p75NTR(NBP), the nucleic acid molecule, the replicable expression vector, the host cell, or the pharmaceutical composition described above; and
b. instructions for the administration of an effective amount of the p75NTR(NBP), nucleic acid molecule, replicable expression vector or pharmaceutical composition to an individual for any one or more of the prevention or treatment of osteoarthritis and/or a symptom of osteoarthritis or for ameliorating, controlling, reducing incidence of, or delaying or reversing the development or progression of osteoarthritis and/or a symptom of osteoarthritis.

In another aspect of the present invention there is provided a method of treating and/or preventing osteoarthritis and/or a symptom of osteoarthritis in an individual comprising administering to said individual a therapeutically effective amount of the p75NTR(NBP), the nucleic acid molecule, the replicable expression vector, the host cell, or the pharmaceutical composition described above, optionally further comprising a pharmaceutically acceptable carrier.

DESCRIPTION OF FIGURES

The present invention will be further understood by reference to the attached figures, in which:

Figure 1: Progression of loss of cartilage area following injection of MIA or ETF-PBS. Data points are mean ± SEM, n=6

Figure 2: Medial aspect of the rat knee stained with haematoxylin and eosin from animals treated with control antibody (A,B) or p75NTR 0.3 mg/kg (C,D), 1 mg/kg (E,F), 3 mg/kg (G,H). Experimental osteoarthritis was induced in the left knee (A,C,E,G) of each animal by intra-articular injection of MIA. The right knee was injected with ETF-PBS

(control; B,D,F,H). (X4 magnification) Each set of left and right knee images are taken from an individual animal to show the contralateral control.

Figure 3: Medial aspect of the rat knee stained with safranin O from animals treated with control antibody (A,B) or p75NTR 3 mg/kg (C,D). Experimental osteoarthritis was induced in the left knee (A and C) of each animal by intra-articular injection of MIA. The right knee was injected with ETF-PBS (control; B and D). (X4 magnification) Each set of left and right knee images are taken from an individual animal to show the contralateral control

Figure 4: Total cartilage area (from cartilage surface down to the border between the calcified cartilage and subchondral bone) in rear knee joints on Day 26 . Significant difference compared to control antibody are denoted thus * $P<0.1$ and ** $P<0.05$.

Figure 5: Cartilage area stained by safranin O at threshold absorption wavelength of 130nm in rear knee joints following 26 days of treatment. Data are mean ± SEM, n=6 Significant difference compared to control antibody are denoted thus ** $P<0.05$.

Figure 6: Amino acid sequence of a p75NTR(NBP)-Fc fusion protein (SEQ ID No. 1). The alpha and gamma secretase cleavage sites are shown in bold type. The IgG1 Fc portion is shown in italics.

Figure 7: Translation product (SEQ ID No. 2), from start to stop codons, of the nucleic acid sequence set forth in Figure 9 (SEQ ID No. 4).

Figure 8: Amino acid sequence of a preferred p75NTR(NBP)-Fc fusion protein (SEQ ID No. 3). The IgG1 Fc portion is shown in italics. The linker sequence between the p75NTR(NBP) and Fc portions is shown underlined.

Figure 9: Nucleic acid sequence of full product gene from 5′ cloning site to 3′ cloning site (SEQ ID No. 4)

Figure 10: p75-NTR(NBP)-Fc fusion protein variants: 1: p75_NTR - The p75-NTR sequence (SEQ ID No. 6); 2: Commercially available p75-NTR-Fc fusion protein (SEQ ID No. 7); 3: p75_Fc - The commercially available p75-NTR-Fc fusion protein with the Fc sequence modified to that of the Lonza constant region of IgG1za (SEQ ID No. 8); 4: p75_Fc_C222S - The commercially available p75-NTR-Fc fusion protein with the Fc sequence modified to that of the Lonza constant region of IgG1za and an additional cysteine to serine mutation at position 222 (SEQ ID No. 9); 5: p75_Fc_G4x1 – Variant 1, a proposed p75-NTR-Fc fusion protein with a four residue glycine linker (SEQ ID No. 10); 6: p75_Fc_G4Sx1 – variant 2, a proposed p75-NTR-Fc fusion protein with a single tetra-glycine serine linker (SEQ ID No. 11); 7: p75_Fc_G4Sx2 – variant 3, a proposed p75-NTR-Fc fusion protein with two tetra-glycine serine linkers (SEQ ID No. 12); 8: Lonza constant region of IgG1za (SEQ ID No. 13).

In this alignment a formatting scheme is used to highlight regions of similarity between the putative receptors, the Fc-fusion protein and the Fc constant region: Boxed type is used to indicate regions of identical sequence between the variant proteins and the p75-NTR; Single underlining is used to indicate regions of identical sequence between all of the Fc-fusion proteins and the Lonza IgG1za Fc; Italics are used to indicate linker regions at the junction of the p75-NTR and the Fc constant region; Double-underlining and bold

type are used to indicate the position of non-identical sequence outside the linker region, at the position equivalent to 222 in the parental p75-NTR Fc-fusion protein.

Figure 11: SEQ ID NO. 14 Human p75NTR full amino acid sequence

Figure 12: SEQ ID NO. 15 Human p75NTR extracellular domain including signal sequence

Figure 13: SEQ ID NO. 16 Human p75NTR extracellular domain without signal sequence

Figure 14: SEQ ID NO. 17 Human p75NTR(NBP) neurotrophin binding domain 1

Figure 15: SEQ ID NO. 18 Human p75NTR(NBP) neurotrophin binding domain 2

Figure 16: SEQ ID NO.19 Human p75NTR(NBP) neurotrophin binding domain 3

Figure 17: SEQ ID NO. 20 Human p75NTR(NBP) neurotrophin binding domain 4

Figure 18: SEQ ID NO. 21 Human p75NTR(NBP) neurotrophin binding domain 5

Figure 19: SEQ ID NO. 22 Human Transferrin

Figure 20: SEQ ID NO. 23 Human Albumin

Figure 21: SEQ ID NO. 24 Human Fc IgG1

Figure 22: SEQ ID NO. 25 Human Fc IgG2

Figure 23: SEQ ID NO. 26 Human Fc IgG3

Figure 24: SEQ ID NO. 27 Human Fc IgG4

Figure 25: SEQ ID NO. 28 Human Fc Fragment Engineered For Extended Serum Half-Life

Figure 26: SEQ ID NO. 29 Human Fc Fragment Engineered For Lack Of Effector Functions

Figure 27: SEQ ID NO. 30 p75NTR(NBP)-Fc linker

Figure 28: SEQ ID NO. 31 p75NTR(NBP)-Fc linker

Figure 29: SEQ ID NO. 32 p75NTR(NBP)-Fc linker

Figure 30: Mean body weight at day 0. Weight in grams are plotted as mean ± standard deviations. Animals in Group 1 were treated with 1 mg/kg p75NTR-Fc, Group 2 with 3 mg/kg p75NTR-Fc, Group 3 with 0.3 mg/kg p75NTR-Fc, Group 4 with 3 mg/kg PG-007 and Group 5 with 3 mg/kg Control IgG Fc.

Figure 31: Mean body weight of rats during the eight week study described in Example 3. Weight in grams are plotted as mean ± standard deviations. Animals in Group 1 were treated with 1 mg/kg p75NTR-Fc, Group 2 with 3 mg/kg p75NTR-Fc, Group 3 with 0.3 mg/kg p75NTR-Fc, Group 4 with 3 mg/kg PG-007 and Group 5 with 3 mg/kg Control IgG Fc.

Figure 32: Spontaneous pain measurements determined with time following treatment with test agents. Data is shown for each animal at each timepoint (from day 35 to day 56). Animals in Group 1 were treated with 1 mg/kg p75NTR-Fc, Group 2 with 3 mg/kg p75NTR-Fc, Group 3 with 0.3 mg/kg p75NTR-Fc, Group 4 with 3 mg/kg PG-007, Group 5 with 3 mg/kg Control IgG Fc, Group 6 with ETF-PBS and Group 7 were naïve. *p<0.05 and **<0.01 for one sample t-test against the theoretical mean of 0.5.

Figure 33: Spontaneous pain measurements determined with time following treatment with test agents. Data is shown for each animal at each timepoint (from day 35 to day 56). Animals in Group 1 were treated with 1 mg/kg p75NTR-Fc, Group 2 with 3 mg/kg p75NTR-Fc, Group 3 with 0.3 mg/kg p75NTR-Fc, Group 4 with 3 mg/kg PG-007, Group 5 with 3 mg/kg Control IgG Fc, Group 6 with ETF-PBS and Group 7 were naïve. *p<0.05 and **<0.01 for one sample t-test against the theoretical mean of 0.5.

Figure 34: Medial aspect of the rat knee stained with Safranin O Fast Green from animals treated with 3 mg/kg control IgG-Fc from day 28 to day 56 following a low MIA or ETF-PBS injection on day 0 into the knee (X4 magnification). Representative images are taken from animals treated with 3 mg/kg control IgG-Fc. Each set of left and right knee image are taken from one individual animal to show the contralateral control.

Figure 35: Medial aspect of the rat knee stained with Safranin O Fast Green from animals treated with 3 mg/kg PG-007 from day 28 to day 56 following a low MIA or ETF-PBS injection on day 0 into the knee (X4 magnification). Representative images are taken from animals treated with 3 mg/kg PG-007. Each set of left and right knee image are taken from one individual animal to show the contralateral control.

Figure 36: Medial aspect of the rat knee stained with Safranin O Fast Green from animals treated with 3 mg/kg p75NTR-Fc from day 28 to day 56 following a low MIA or ETF-PBS injection on day 0 into the knee (X4 magnification). Representative images are taken from animals treated with 3 mg/kg p75NTR-Fc. Each set of left and right knee image are taken from one individual animal to show the contralateral control.

Figure 37: Medial aspect of the rat knee stained with Safranin O Fast Green from animals treated with 1 mg/kg p75NTR-Fc from day 28 to day 56 following a low MIA or ETF-PBS injection on day 0 into the knee (X4 magnification). Representative images are taken from animals treated with 1 mg/ p75NTR-Fc. Each set of left and right knee image are taken from one individual animal to show the contralateral control.

Figure 38: Medial aspect of the rat knee stained with Safranin O Fast Green from animals treated with 0.3 mg/kg p75NTR-Fc from day 28 to day 56 following a low MIA or ETF-PBS injection on day 0 into the knee (X4 magnification). Representative images are taken from animals treated with 0.3 mg/kg p75NTR-Fc. Each set of left and right knee image are taken from one individual animal to show the contralateral control.

Figure 39: Cartilage pathology grades. Administration of PG007 showed no histologically significant efficacy effects. P75NTR-Fc showed marked efficacy on chondroprotection at 0.3 and 1.0 mg/kg. The efficacy profile at 3.0mg/kg was more variable – with 50% of the group showing overlay with the control group.

Figure 40: Sub-chondral bone grades. Administration of PG007 was associated with histologically significant increase in sub-chondral bone pathology compared to the control group – similar to the profile observed with 3.0mg/kg P75NTRFc. By contrast,

administration of P75NTR-Fc at 0.3 and 1.0 mg/kg was associated with marked improvement in sub-chondral bone histology.

Figure 41: Stromal cavity grades. Administration of PG007 was not associated with any histologically significant efficacy effects. By contrast, administration of P75NTR-Fc at all does was associated with reduction in osteolytic pathology and expansion of stromal cavities, although the effects at 0.3 and 1.0 mg/kg were most marked – both groups reducing pathology to almost normal levels.

Figure 42: Cancellous bone grades. Administration of PG007 was associated with cancellous bone pathology similar to controls – with two samples exceeding control ranges. By contrast, P75NTR-Fc markedly reduced cancellous bone pathology at the 0.3 and 1.0 mg/kg dose levels. P75NTF-Fc at 3.0 mg/kg presented a more variable profile, with the majority of samples showing overlay with the control group.

Figure 43: Bone marrow hyper-cellularity. Administration of PG007 was associated with expansion of myeloid cells in the bone marrow, with four samples either at the top or exceeding the control range. P75NTR-Fc reduced marrow cellularity – with reduced myeloid expansion being a prominent feature – at the 0.3 and 1.0 mg/kg doses. The 3.0 mg/kg dose, although showing a trend towards inhibition, showed overlay with low level responders in the control group.

Figure 44: Multi-focal bone sclerosis. There were no histologically significant differences between the study groups – although the P75NTR-Fc samples did show a clustering towards the top end of the control range.

Figure 45: Osteoblast hyperplasia. Administration of P75NTR-Fc at 0.3 and 1.0 mg/kg was associated with histologically significant osteoblast proliferation – with palisading and osteoblastic plate formation being especially prominent in the epiphyseal zone, at the sub-chondral bone/ cartilage zone. In addition, although not graded, there were prominent 'fibroblast-like' cells in this zone. PG007 and P75NTR-Fc (3.0 mg/kg) were histologically similar to controls (Figure 45).

DETAILED DESCRIPTION

Osteoarthritis symptoms include inflammation, pain and swelling. In addition, the loss of cartilage can lead to the formation of bone outgrowths (osteophytes) which exacerbate symptoms and can lead to narrowing and distortion of the joint. Treatment options are currently limited to disease management options including physiotherapy, administration of pain killers and/or administration of anti-inflammatory drugs and, in some cases, joint replacement via surgery. Until now, no curative treatment options for osteoarthritis have been reported. There are also no reported treatments which halt progression of this degenerative disease. Accordingly, the present invention seeks to address the need for a curative osteoarthritis treatment, or at least to provide a treatment capable of halting degeneration in osteoarthritis.

Surprisingly, the present inventors have discovered that administration of a p75NTR neurotrophin binding protein (p75NTR(NBP)) in an accepted animal model of osteoarthritis not only halted disease progression, but also resulted in significant reversal of damage attributable to osteoarthritis progression.

Accordingly, there is provided in a first aspect a p75NTR neurotrophin binding protein (p75NTR(NBP)) for use in the treatment of osteoarthritis.

In preferred embodiments, the treatment of osteoarthritis includes relief from the symptoms of osteoarthritis. Preferably relief from the symptoms of osteoarthritis include, but are not limited to reduction in pain, inflammation, swelling, tenderness, joint stiffness or increase in joint mobility or any combination of these.

In a particularly preferred embodiment, treatment of osteoarthritis includes slowing or arresting of disease progression and/or reduction in cartilage loss. Preferably treatment of osteoarthritis includes reversal of disease progression, regrowth of cartilage and/or curative treatment. Preferably disease progression is determined by the rate of cartilage loss or regrowth. The rate of cartilage loss may be monitored by a variety of methods, including but not limited to Magnetic Resonance Imaging (MRI) or X-ray computed tomography (x-ray CT). In other preferred embodiments, disease progression may be monitored by determining the number of chondrocytes present in a joint.

The term "curative treatment" as used herein is intended to encompass treatments which restore a patient to their pre-disease state. Such treatments may require continued administration of the active compound in order to maintain a pre-disease state. Alternatively, curative treatments may be halted once a pre-disease state is reached.

In other preferred embodiments, the treatment is of either primary or secondary osteoarthritis. Treatment of primary osteoarthritis includes treatment of both primary generalized nodal osteoarthritis and erosive osteoarthritis (EOA, also called inflammatory osteoarthritis).

Treatment of osteoarthritis is also intended to include amelioration of disease symptoms as classified under the WOMAC grading or Outerbridge classification systems. In preferred embodiments, the treatment of osteoarthritis results in reversal of disease progression, leading to a change of disease stage (WOMAC grading) or grade (Outerbridge classification).

In other preferred embodiments, the treatment of osteoarthritis includes prophylactic treatment.

In certain preferred embodiments the p75NTR(NBP) is a human p75NTR(NBP).

In other preferred embodiments the p75NTR(NBP) comprises a p75NTR(NBP) connected to one or more auxiliary molecules. Preferably, the one or more auxiliary molecules are selected from: (a) transferrin or a portion thereof; (b) albumin or a portion thereof; (c) an immunoglobulin Fc or a portion thereof; or (d) a polyethylene glycol polymer chain. In still other preferred embodiments the p75NTR(NBP) is connected to the one or more auxiliary molecules via one or more linkers. Preferably the linker is selected from: (a) a covalent bond; (b) a non-covalent bond; (c) a peptide bond; or (d) one amino acid or a plurality of amino acids comprising a peptide. In a particularly preferred embodiment, the p75NTR(NBP) comprises a p75NTR(NBP) connected to an immunoglobulin Fc or a portion thereof, optionally via a linker.

Where the p75NTR(NBP) is connected to more than one auxiliary molecule, optionally each auxiliary molecule is either the same or different or a mixture of the same and different. Similarly, where the p75NTR(NBP) is connected to more than one auxiliary molecule via one or more linkers, optionally each linker is either the same or different or a mixture of the same and different

In an especially preferred embodiment the p75NTR(NBP) has the amino acid sequence according to SEQ ID NO 3.

In a particularly preferred embodiment the p75NTR(NBP) binds to any of NGF, BDNF, NT3 or NT4/5 with a binding affinity (K_d) of between about 5 pM to about 5 nM as measured by surface plasmon resonance at 20 °C.

In a preferred embodiment the p75NTR(NBP) is for separate, sequential or simultaneous use in a combination combined with a second pharmacologically active compound. Preferably the second pharmacologically active compound of the combination is selected from an opioid analgesic, a nonsteroidal anti-inflammatory drug (NSAID), a barbiturate sedative, a benzodiazepine having a sedative action, an H1 antagonist having a sedative action, a sedative such as glutethimide, meprobamate, methaqualone or dichloralphenazone; a skeletal muscle relaxant; an NMDA receptor antagonist, an alpha-adrenergic, a tricyclic antidepressant, an anticonvulsant, a tachykinin (NK) antagonist, particularly an NK-3, NK-2 or NK-1 antagonist, a muscarinic antagonist, a COX-2 selective inhibitor, a coal-tar analgesic, in particular paracetamol; a neuroleptic a vanilloid receptor agonist or antagonist; a beta-adrenergic; a local anaesthetic; a corticosteroid; a 5-HT receptor agonist or antagonist; a 5-HT2A receptor antagonist; a cholinergic (nicotinic) analgesic; Tramadol®; a PDEV inhibitor; a cannabinoid; metabotropic glutamate subtype 1 receptor (mGluR1) antagonist; a serotonin reuptake inhibitor; a noradrenaline (norepinephrine) reuptake inhibitor; a dual serotonin-noradrenaline reuptake inhibitor; an inducible nitric oxide synthase (iNOS) inhibitor; an acetylcholinesterase inhibitor; a prostaglandin E2 subtype 4 (EP4) antagonist; a leukotriene B4 antagonist; a 5-lipoxygenase inhibitor; a sodium channel blocker; or a 5-HT3 antagonist, and the pharmaceutically acceptable salts and solvates thereof.

Preferred opioid analgesics include, but are not limited to, morphine, heroin, hydromorphone, oxymorphone, levorphanol, levallorphan, methadone, meperidine, fentanyl, cocaine, codeine, dihydrocodeine, oxycodone, hydrocodone, propoxyphene, nalmefene, nalorphine, naloxone, naltrexone, buprenorphine, butorphanol, nalbuphine or pentazocine.

Preferred nonsteroidal anti-inflammatory drugs (NSAIDs) include, but are not limited to, aspirin, diclofenac, diflusinal, etodolac, fenbufen, fenoprofen, flufenisal, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamic acid, mefenamic acid, meloxicam, nabumetone, naproxen, nimesulide, nitroflurbiprofen, olsalazine, oxaprozin, phenylbutazone, piroxicam, sulfasalazine, sulindac, tolmetin or zomepirac.

Preferred barbiturate sedatives include, but are not limited to, amobarbital, aprobarbital, butabarbital, butabital, mephobarbital, metharbital, methohexital, pentobarbital, phenobartital, secobarbital, talbutal, theamylal or thiopental.

Preferred benzodiazepines having a sedative action include, but are not limited to, chlordiazepoxide, clorazepate, diazepam, flurazepam, lorazepam, oxazepam, temazepam or triazolam.

Preferred H1 antagonists having a sedative action include, but are not limited to, diphenhydramine, pyrilamine, promethazine, chlorpheniramine or chlorcyclizine.

Preferred sedatives include, but are not limited to, glutethimide, meprobamate, methaqualone or dichloralphenazone.

Preferred skeletal muscle relaxants include, but are not limited to, baclofen, carisoprodol, chlorzoxazone, cyclobenzaprine, methocarbamol or orphrenadine.

Preferred NMDA receptor antagonists include, but are not limited to, dextromethorphan ((+)-3-hydroxy-N-methylmorphinan) or its metabolite dextrorphan ((+)-3-hydroxy-N-methylmorphinan), ketamine, memantine, pyrroloquinoline quinine, cis-4-(phosphonomethyl)-2-piperidinecarboxylic acid, budipine, EN-3231 (MorphiDex®, a combination formulation of morphine and dextromethorphan), topiramate, neramexane or perzinfotel including an NR2B antagonist, e.g. ifenprodil, traxoprodil or (−)-(R)-6-{2-[4-(3-fluorophenyl)-4-hydroxy-1-piperidinyl]-1-hydroxyethyl-3,4-dihydro-2(1H)-quinolinone.

Preferred alpha-adrenergics include, but are not limited to, doxazosin, tamsulosin, clonidine, guanfacine, dexmetatomidine, modafinil, or 4-amino-6,7-dimethoxy-2-(5-methane-sulfonamido-1,2,3,4-tetrahydroisoquinol-2-yl)-5-(2-pyridyl) quinazoline.

Preferred tricyclic antidepressants include, but are not limited to, desipramine, imipramine, amitriptyline or nortriptyline.

Preferred anticonvulsants include, but are not limited to, carbamazepine, lamotrigine, topiratmate or valproate.

Preferred tachykinin (NK) antagonists include, but are not limited to, (alphaR,9R)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7H-[1,4]diazocino[2,1-g][1,7]-naphthyridine-6-13-dione (TAK-637), 5-[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy-3-(4-fluorophenyl)-4-morpholinyl]-methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one (MK-869), aprepitant, lanepitant, dapitant or 3-[[2-methoxy-5-(trifluoromethoxy)phenyl]-methylamino]-2-phenylpiperidine (2S,3S).

Preferred muscarinic antagonists include, but are not limited to, oxybutynin, tolterodine, propiverine, tropsium chloride, darifenacin, solifenacin, temiverine and ipratropium.
Preferred COX-2 selective inhibitors include, but are not limited to, celecoxib, rofecoxib, parecoxib, valdecoxib, deracoxib, etoricoxib, or lumiracoxib.

Preferred coal-tar analgesics include, but are not limited to, paracetamol.

Preferred neuroleptics include, but are not limited to,droperidol, chlorpromazine, haloperidol, perphenazine, thioridazine, mesoridazine, trifluoperazine, fluphenazine, clozapine, olanzapine, risperidone, ziprasidone, quetiapine, sertindole, aripiprazole, sonepiprazole, blonanserin, iloperidone, perospirone, raclopride, zotepine, bifeprunox,

asenapine, lurasidone, amisulpride, balaperidone, palindore, eplivanserin, osanetant, rimonabant, meclinertant, Miraxion® or sarizotan.

Preferred vanilloid receptor agonists include, but are not limited to, resinferatoxin. Preferred vanilloid receptor antagonists include, but are not limited to, capsazepine.

Preferred beta-adrenergics include, but are not limited to, propranolol. Preferred local anaesthetics include, but are not limited to, mexiletine. Preferred corticosteroids include, but are not limited to, dexamethasone.

Preferred 5-HT receptor agonists or antagonists, particularly 5-HT1B/1D agonists include, but are not limited to, eletriptan, sumatriptan, naratriptan, zolmitriptan or rizatriptan; Preferred 5-HT2A receptor antagonists include, but are not limited to, R(+)-alpha-(2,3-dimethoxy-phenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol (MDL-100907).

Preferred cholinergic (nicotinic) analgesics include, but are not limited to, ispronicline (TC-1734), (E)-N-methyl-4-(3-pyridinyl)-3-buten-1-amine (RJR-2403), (R)-5-(2-azetidinylmethoxy)-2-chloropyridine (ABT-594) or nicotine.

Preferred PDEV inhibitors include, but are not limited to, 5-[2-ethoxy-5-(4-methyl-1-piperazinyl-sulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (sildenafil), (6R,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)-pyrazino[2',1':6,1]-pyrido[3,4-b]indole-1,4-dione (IC-351 or tadalafil), 2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one (vardenafil), 5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, 5-(5-acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-isopropyl-3-azetidinyl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, 5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, 4-[(3-chloro-4-methoxybenzyl)amino]-2-[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]-N-(pyrimidin-2-ylmethyl)pyrimidine-5-carboxamide, 3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-N-[2-(1-methylpyrrolidin-2-yl)ethyl]-4-propoxybenzenesulfonamide.

Preferred cannabinoids include, but are not limited to, tetrahydrocannabinol, cannabinol, cannabidiol, cannabigerol, tetrahydrocannabivarin, cannabidivarin and cannabichromene.

Preferred serotonin reuptake inhibitors include, but are not limited to, sertraline, sertraline metabolite demethylsertraline, fluoxetine, norfluoxetine (fluoxetine desmethyl metabolite), fluvoxamine, paroxetine, citalopram, citalopram metabolite desmethylcitalopram, escitalopram, d,l-fenfluramine, femoxetine, ifoxetine, cyanodothiepin, litoxetine, dapoxetine, nefazodone, cericlamine and trazodone.

Preferred noradrenaline (norepinephrine) reuptake inhibitors include, but are not limited to, maprotiline, lofepramine, mirtazepine, oxaprotiline, fezolamine, tomoxetine, mianserin, buproprion, buproprion metabolite hydroxybuproprion, nomifensine and viloxazine (Vivalan®), especially a selective noradrenaline reuptake inhibitor such as reboxetine, in particular (S,S)-reboxetine.

Preferred dual serotonin-noradrenaline reuptake inhibitors include, but are not limited to, venlafaxine, venlafaxine metabolite O-desmethylvenlafaxine, clomipramine, clomipramine metabolite desmethylclomipramine, duloxetine, milnacipran and imipramine.
Preferred inducible nitric oxide synthase (iNOS) inhibitors include, but are not limited to, S-[2-[(1-iminoethyl)amino]ethyl]-L-homocysteine, S-[2-[(1-iminoethyl)-amino]ethyl]-4,4-dioxo-L-cysteine, S-[2-[(1-iminoethyl)amino]ethyl]-2-methyl-L-cysteine, (2S,5Z)-2-amino-2-methyl-7-[(1-iminoethyl)amino]-5-heptenoic acid, 2-[[(1R,3S)-3-amino-4-hydroxy-1-(5-thiazolyl)-butyl]thio]-5-chloro-3-pyridinecarbonitrile; 2-[[(1R,3S)-3-amino-4-hydroxy-1-(5-thiazolyl)butyl]thio]-4-chlorobenzonitrile, (2S,4R)-2-amino-4-[[2-chloro-5-(trifluoromethyl)phenyl]thio]-5-thiazolebutanol, 2-[[(1R,3S)-3-amino-4-hydroxy-1-(5-thiazolyl) butyl]thio]-6-(trifluoromethyl)-3 pyridinecarbonitrile, 2-[[(1R,3S)-3- amino-4-hydroxy- 1 -(5-thiazolyl)butyl]thio]-5-chlorobenzonitrile, N-[4-[2-(3-chlorobenzylamino)ethyl]phenyl]thiophene-2-carboxamidine, or guanidinoethyldisulfide.

Preferred acetylcholinesterase inhibitors include, but are not limited to, donepezil.
Preferred prostaglandin E2 subtype 4 (EP4) antagonists include, but are not limited to, N-[({2-[4-(2-ethyl-4,6-dimethyl-1H-imidazo[4,5-c]pyridin-1-yl)phenyl]ethyl}amino)-carbonyl]-4-methylbenzenesulfonamide or 4-[(1S)-1-({[5-chloro-2-(3-fluorophenoxy)pyridin-3-yl]carbonyl}amino)ethyl]benzoic acid.

Preferred leukotriene B4 antagonists include, but are not limited to, 1-(3-biphenyl-4-ylmethyl-4-hydroxy-chroman-7-yl)-cyclopentanecarboxylic acid (CP-105696), 5-[2-(2-Carboxyethyl)-3-[6-(4-methoxyphenyl)-5E- hexenyl]oxyphenoxy]-valeric acid (ONO-4057) or DPC-11870.

Preferred 5-lipoxygenase inhibitors include, but are not limited to, zileuton, 6-[(3-fluoro-5-[4-methoxy-3,4,5,6-tetrahydro-2H-pyran-4-yl])phenoxy-methyl]-1-methyl-2-quinolone (ZD-2138), or 2,3,5-trimethyl-6-(3-pyridylmethyl),1,4-benzoquinone (CV-6504).

Preferred sodium channel blockers include, but are not limited to, lidocaine. Preferred 5-HT3 antagonists include, but are not limited to, ondansetron.

Preferably the p75NTR(NBP) is formulated for oral, sublingual, buccal, topical, rectal, inhalation, transdermal, subcutaneous, intravenous, intra-arterial, intramuscular, intracardiac, intraosseous, intrasynovial, intradermal, intraperitoneal, transmucosal, vaginal, intravitreal, intra-articular, peri-articular, local or epicutaneous administration.

In a further aspect of the present invention there is provided a nucleic acid encoding a p75NTR(NBP), for use in the treatment of osteoarthritis as defined above.

In another aspect of the present invention there is provided a replicable expression vector for transfecting a cell, comprising a nucleic acid encoding a p75NTR(NBP), for use in the treatment of osteoarthritis as defined above.

In yet another aspect of the present invention there is provided a host cell expressing a p75NTR(NBP), for use in the treatment of osteoarthritis as defined above.

In a still further aspect of the present invention there is provided a pharmaceutical composition, comprising the p75NTR(NBP), the nucleic acid molecule, the replicable expression vector, or the host cell described above, and a pharmaceutically acceptable carrier and/or an excipient.

Preferred pharmaceutically acceptable carriers include, but are not limited to, […]

Another aspect of the invention pertains to a kit comprising:

a. the p75NTR(NBP), the nucleic acid molecule, the replicable expression vector, the host cell, or the pharmaceutical composition described above; and
b. instructions for the administration of an effective amount of the p75NTR(NBP), nucleic acid molecule, replicable expression vector or pharmaceutical composition to an individual for any one or more of the prevention or treatment of osteoarthritis and/or a symptom of osteoarthritis or for ameliorating, controlling, reducing incidence of, or delaying or reversing the development or progression of osteoarthritis and/or a symptom of osteoarthritis.

In another aspect of the present invention there is provided a method of treating and/or preventing osteoarthritis and/or a symptom of osteoarthritis in an individual comprising administering to said individual a therapeutically effective amount of the p75NTR(NBP), the nucleic acid molecule, the replicable expression vector, the host cell, or the pharmaceutical composition described above, optionally further comprising a pharmaceutically acceptable carrier.

EXAMPLES

EXAMPLE 1 – Affinity measurements of p75NTR using biacore

Methods

The kinetics and affinity of p75NTR are determined by surface plasmon resonance technology using a Biacore T200 (GE Healthcare, Sweden). The Biacore methods are based on those recommended by Abdiche and colleagues. (Abdiche, et al., 2008) Protein A (10 µg/mL in 10 mM sodium acetate buffer) is immobilised on the surface of a CM5 biosensor chip by the amine coupling method using 1-ethyl-3-(3-dimethylaminopropyl carbodiimide) (EDC) and N-hydroxy-succinimide (NHS) and ethanolamine as provided in the amine coupling kit.

Briefly, the steps involved in the amine-coupling immobilisation wizard on the Biacore instrument are:

- EDC and NHS are mixed 1:1
- EDC/NHS mixture is injected over each flow cell of a CM5 chip for 420 seconds at 10 µL/min
- Protein A [10 µg/mL] in 10 mM sodium acetate buffer, pH 4.5 is injected over the same flow cell for 420 seconds at 10 µL/min

- Ethanolamine is injected over the same flow cell for 420 seconds at 10 µL/min

Approximately 2200–2900 response units (RU) are immobilised. One flow cell is set as the blank control. p75NTR-Fc is captured onto the other flow cell of the biosensor chip at 15oC using a 30 second injection of p75NTR (10 µg/mL in HBS-EP [0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20]) at a flow rate of 10 µL/min to achieve the desired p75NTR RU level (~400 RU; calculated using the molecular weights of the p75NTR, the relevant neurotrophin and stoichiometric ratio). Immobilising p75NTR rather than the ligand helps ensure the neurotrophins are in their native states.

A single-cycle kinetics study is performed by injecting increasing concentrations of neurotrophin in HBS-EP buffer over the flow cells for 120 seconds at 30 µL/min per neurotrophin concentration. Following the final neurotrophin injection HBS-EP is flowed over the chip for 600 seconds to determine dissociation rates. Chip sensor surfaces are regenerated back to their Protein A surface by injecting 10 mM Glycine HCl, pH 2 for 60 seconds at 30 µL/min prior to every new injection cycle.

Multi-cycle kinetics studies are performed by injecting the lowest neurotrophin oncentration over the flow cells for 300 seconds at 30 µL/min, followed by HBS-EP buffer for 300 seconds at 30 µL/min. The chip is then regenerated by 2 x 60 second injections of 10 mM Glycine, HCl pH 2 and the cycle repeated for each increasing neurotrophin concentration.

Results

p75NTR binds reversibly to the neurotrophins NGF, BDNF, NT-3 and NT-4. The affinities measured using Biacore are given in Table 1.

Table 1: Affinity of p75NTR for the four neurotrophins

Neurotrophin	Affinity (pM)	Affinity Range(pM)
NGF	554	50–5000
BDNF	41.8	5–500
NT-3	14.2	1–100
NT-4	181	10–1000

EXAMPLE 2 - Monoiodoacetate-induced osteoarthritis study

Experimental osteoarthritis induced by intra-articular injection of monoiodoactetate (MIA) in the rear knee of rats is a well-recognised model of osteoarthritis. The pathological progression of the disease and pain behaviour have been reported (Guzman, et al., 2003) (Fernihough, et al., 2004). MIA disrupts glycolysis by inhibition of glyceraldehyde-3-phosphate dehydrogenase, leading to chondrocyte death (Harvey & Dickenson, 2009). The structural integrity of cartilage relies on the normal functioning of chondrocytes, thus MIA-induced loss of chondrocytes leads to cartilage degeneration and changes of

the subchondral bone consistent with the clinical histopathology of OA (Janusz, et al., 2001; Kobayashi, et al., 2003; Naveen, et al., 2014). Injection of 0.3 mg MIA induces loss of cartilage over a 10-week period. Cartilage loss is greatest between Week 3 and Week 6 (Figure 1).

44 male Wistar rats (Charles River, UK) housed in pairs and weighing 200–250 g on Day -2, are randomly assigned by pair to treatment such that the mean body weight of each treatment group is similar. On Day 0 the rats receive treatment as indicated in Table 1. Freshly prepared human IgG (3.25 mg/mL in endotoxin free phosphate buffered saline [ETF-PBS]) and p75NTR (3.25 mg/mL in EF-PBS) are administered subcutaneously. The laboratory personnel are blinded to the treatment of the animals throughout the study.

Table 2: Treatment groups. MIA, monoiodiacetate; IgG, immunoglobulin G

Treatment group (n)	MIA	Antibody
1 (6)	0.3 mg	Human IgG 3.0 mg/kg
2 (6)	0.3 mg	p75NTR 0.3 mg/kg
3 (6)	0.3 mg	p75NTR 1.0 mg/kg
4 (6)	0.3 mg	p75NTR 3.0 mg/kg

Three hours later all animals are anaesthetised with isofluorane. Each anaesthetised animal receives an intra-articular injection of 50 µL EF-PBS containing 0.3 mg MIA in either the right or left knee, according to the randomisation schedule. The contralateral knee of each anaesthetised animal is injected with 50 µL EF-PBS. A further respective antibody or p75NTR treatment is administered on Day 5 and 15.

The study terminates on Day 26, when cartilage loss is evident and active (Figure 1). Animals are anaesthetised using isoflourane, terminal blood samples are taken by cardiac puncture and plasma samples prepared. The skin on the lower hind legs is removed and the muscle bundles separated from the bones but left intact with the knee. The femur, fibula and tibia are severed and the knee with attached muscle is placed in 10% neutral buffered formalin. Tissue samples are kept in buffered formalin for 48–72 hours before processing for histological analysis.

Tissue samples are prepared for light microscopy using standard procedures as soon as possible after collection to minimise damage caused from the formalin fixation. The samples are decalcified in 8% formic acid for 10 days, processed using a Shandon Citadel tissue processer and embedded into molten paraffin wax. At least four sections (10 µm) from each rat knee tissue block are processed for standard Haematoxylin and Eosin (H&E) staining using an automated linear staining machine (Leica ST4040). Further sections are stained using Safranin O. Slides are viewed at either times four or ten magnification and image analysis is performed using a computerised system. The total cartilage area, from cartilage surface down to the border between the calcified cartilage and subchondral bone is measured. Light absorption of stain bound to tissue sections is quantified under monochromatic light with digital densitometry. The intensity

of the red staining of the glycosaminoglycans by Safranin O is quantitated by measuring the cartilage area stained when the absorption threshold is set at 130 nm.

The concentration of control antibody and exogenous p75NTR in plasma is estimated by measuring human IgG using an enzyme - linked immunosorbent assay (ELISA).

Results - Effect of p75NTR on histological changes in the knee following MIA-induced OA

No tissue degeneration nor features of OA were observed in knees that had been injected with ETF-PBS in animals from all treatment groups (Figure 2B,D,F,H, Figure 3B,D,F,H). In contrast, MIA-injected knees in animals treated with control antibody showed areas of mild chondrocyte degeneration, which frequently involved the entire thickness of the articular cartilage (Figure 2A). There was evidence of a recent inflammatory response in some areas, marked by leukocyte accumulation. Treatment with p75NTR did not accelerate the progression of OA (Figure 2C,E,G). There were fewer histological changes in the architecture of low MIA treated knees treated with p75NTR compared with animals treated with control antibodies, indicating protection from injury or repair of damage. This was evident at all p75NTR concentrations tested (0.3–3.0 mg/kg). Indeed, animals treated with the highest dose of p75NTR-Fc had significant ($P<0.05$) increase in cartilage area (evidence of p75NTR-Fc induced repair in OA see Fig 4).

Effect of p75NTR on proteoglycan density in the knee following MIA-induced OA

The overall cartilage area in MIA-treated knees was improved in animals treated with p75NTR-Fc: this was significantly different ($P<0.05$) for animals treated with p75NTR-Fc at 3.0 mg/kg compared to corresponding control animals on Day 26 post intra-articular injection (Figure 4).

The intensity of safranin O staining, as assessed by image analysis, which is proportional to the glycosaminoglycan content of cartilage, showed marked differences ($P<0.05$) between the treatment groups (see Figure 5). Using an absorption threshold of 130 nm , only 20% of the cartilage from MIA-treated knee was red-stained above the 130 nm threshold in animals treated with control antibodies compared with the contralateral knees, indicating a substantial loss of glycosaminoglycans. In contrast, approximately 50% of the cartilage in the MIA-treated knees of animals receiving p75NTR-Fc was above the 130 nm threshold (Figure 5). This indicates that p75NTR-Fc is preventing the ongoing loss of structural integrity of the cartilage following MIA injection and moreover disease modification in OA.

EXAMPLE 3 - Effects of p75NTR-Fc on regression of osteoarthritis in rat compared to PG-007

The aim of this study is to assess whether ascending doses of p75NTR-Fc can cause regression of OA once the disease has been established. In addition the efficacy of the treatment in terms of the pain associated with MIA induced arthritis in the rat has been assessed and compared with treatment with the Tanezumab like anti-Neurotrophin Growth Factor (NGF) antibody, PG-007.

Protocol

Reagents

Reagent	Source and catalogue number	Batch number
Monosodium Iodoacetate (MIA)	Sigma I2512	#SLBB6147V
Endotoxin free PBS (ETF-PBS)	Promocell C-40240	359P109
10% neutral buffered formalin solution	Sigma HT501320	090M4370
EDTA (dipotassium salt dihydrate)	Sigma ED2P	BCBF9021V

Table 3. Details of reagents used in Example 3

Preparation of MIA

MIA was prepared at a concentration of 0.3 mg / 50 µl ETF-PBS (the volume used for each intra-articular injection) which is equivalent to 6 mg/ml MIA stock solution. 68 mg of MIA was weighed out and dissolved in 11.3 ml ETF-PBS. The MIA was prepared a day in advance and was stored at 4°C in the dark until required. All animals received the MIA prepared from the same batch.

Test Agents

Test agent	Source and catalogue number	Batch number
PG-007	Lonza at 3.25mg/ml,	
0.026 EU/mg	Batch 1 (provided by Levicept)	
p75NTR-Fc	2.90 mg/ml stock	
Human IgG Fc fragment full-length protein	AbCam AB90285 prepared at 2.15 mg/ml in ETF-PBS	GR196868-1

Table 4. Details of test agents used in Example 3

Preparation of test agents

The test agents used in this study were prepared fresh on the specific day that the animals were dosed (see Table 4). Wherever possible the volumes of each antibody prepared were equal so that the *in vivo* scientist injecting the antibody was unaware of what treatment was in each vial (see Table 5).

Antibody	Dose(mg/kg)	Stock Concentration (mg/ml)	Stock volume (ml)	Vehicle volume (ml)	Final volume (ml)	Final concentration(mg/ml)	Dose volume (ml/kg)
PG-007	3	3.25	3.30	14.70	18	0.60	5
p75NTR-Fc	3	2.90	3.73	14.27	18	0.60	5
p75NTR-Fc	1	2.90	1.24	16.76	18	0.20	5
p75NTR-Fc	0.3	2.90	0.37	17.63	18	0.06	5
IgG-Fc	3	2.15	5.00	13.00	18	0.60	5

Table 5. Typical preparation of test agents used in Example 3

Animals

39 male Wistar rats (from Charles River, UK) weighing 120-150g on arrival were used in Example 3. Each animal was checked on arrival and appeared outwardly healthy. They were randomly assigned to a cage of three and each rat was allocated a unique identification number by a tattoo on the tail. Animals were acclimatised to the animal unit for at least ten days prior to the start of the study on day 0.

Once the rats had acclimatised to their environment they were transferred to a stock/procedure room, where all the *in vivo* procedures were carried out. Animals were kept illuminated by fluorescent lights set to give a 12 hour light-dark cycle (on 07.00 off 19.00) as recommended in the Home Office Animals (Scientific Procedures) Act 1986. The rooms were air-conditioned and the air temperature (21°C +/- 2°C) and relative humidity were routinely measured.

Rats were fed R105-25 irradiated complete diet for rats (Scientific Animal Food and Engineering, Augy, France) and autoclaved water was available *ad libitum*. Each batch of diet was checked and screened routinely for composition and contaminants. Nesting and cages were autoclaved and each cage was individually ventilated (IVC system).

Experimental Design

Following the induction of arthritis by the intraarticular injection of MIA in one knee and once were significant differences were observed in the weight bearing (incapacitance measurements) of the limbs treated with MIA compared to its contralateral control limb treated with ETF-PBS, animals were randomised into five treatment groups of equivalent nociceptive behaviour (n=6 animals per group) and one vehicle control group (n=3). Six naïve animals were included as negative controls.

Intra-articular injection in one knee (n)	Treatment (group) by the subcutaneous route	Treatment days	Terminal day
MIA 0.3 mg (6)	PG-007 3 mg/kg	30, 35, 39, 45 and 50	58
MIA 0.3 mg (6)	P75NTR-Fc 0.3 mg/kg	30, 35, 39, 45 and 50	58
MIA 0.3 mg (6)	P75NTR-Fc 1 mg/kg	30, 35, 39, 45 and 50	58
MIA 0.3 mg (6)	P75NTR-Fc 3 mg/kg	30, 35, 39, 45 and 50	58
MIA 0.3 mg (6)	Human IgG FC fragment full length protein	30, 35, 39, 45 and 50	58
MIA 0.3 mg (3)	PBS	30, 35, 39, 45 and 50	58
Naïve (6)	None	None	58

Table 6. Overview of experimental design for Example 3

Body weight was regularly recorded through the study and assessment of spontaneous pain was measured at weekly intervals.

As anti NGF anti-neurotrophins have been shown to cause rats to scratch around the face and neck, animals were regularly observed for signs of skin lesions and any lesion scores recorded, particularly after day six, to ensure that we were not reaching the humane clinical end-point as determined in previous studies (animals will be killed if the maximum area of total skin lesions exceeds 10 cm^2 or if any one lesion was greater than 2 cm x 3 cm in size and became deeper and wet with no signs of healing within a 24 hour period).

On the terminal day a blood sample was taken by cardiac puncture and plasma prepared. The knees were removed and placed in 10% neutral buffered formal saline and processed for histology.

Randomisation of treatment(s)

Injection of MIA

Randomisation was carried out so that either the left or right knee of each rat was injected with MIA (with the contralateral knee from each rat injected with ETF-PBS). The allocation of which knee received MIA or saline for each rat was produced using a random number generator in Microsoft Excel for the Mac (Version 14.1.1). Personnel who had no contact with the animals carried out the randomisation procedure and allocation (see Appendix 1 for the schedule). Two 7 ml polypropylene vials were labelled for each animal to denote the left or right knee (total of 66 vials). Two people (one scoring and checking to the master randomisation sheet and one aliquoting the solution for the intra-articular injection) prepared the 66 vials. The aliquoting was carried out in sequence so that the MIA vials were filled first followed with the remaining vials being filled with ETF-PBS (this was the contralateral knee vial for each animal). The vials for the naïve animals were left empty.

Dosing of test agents

Animals were randomised into treatment groups of equal differences in the nociceptive behaviour of the knee that had been treated with MIA compared to the knee treated with vehicle control. This was performed on day 28 following the induction of OA.

Animal Procedures

Intra-articular injection of the knee

Rats were anaesthetised by inhalation of Isoflurane using a Boyles Apparatus. The hairs on both knees of each animal were clipped and the knees swabbed with ethanol. Each knee was injected through the infra-patellar ligament with 50µl of either 0.3 mg MIA in ETF-PBS or ETF-PBS alone using a 0.5 ml sterile Becton Dickinson Micro-Fine insulin syringe with an attached 27 G needle. The six naïve animals were anaesthetised and the hairs on both knees were clipped only. Throughout the study *in vivo* scientists were blind to the treatment status of all animals.

Dosing of test agents

Test agents were dosed by the subcutaneous route in the scruff of the neck or flank using a dose volume of 5 ml/kg.

Assessment of spontaneous pain

Spontaneous pain was determined for each animal at weekly intervals by measuring the weight bearing of the left and right hind limbs using an Incapacitance tester (Linton Instruments, U.K.). Rats were placed in an appropriately sized perspex animal box on the incapacitance tester so that their hind feet sat on separate sensors. The size of the box chosen was based on the body weight of the rat (small rat holder for rats up to 450 g and large rat/guinea pig holder for rats over 450g), which allowed the rat to sit comfortably without being squashed, but similarly did not offer too much space so that the rat could turn around. Once the rat was steady and calm, the weight bearing of each limb was recorded over 5 seconds and the average force in grams exerted by both hind limbs was recorded. The weight distribution of the hind paws was determined five times for each rat at each time point, and the mean of the five readings calculated. The individual weight bearing data was converted into a weight distribution by dividing the weight of the right limb by the total weight for both hind limbs.

Terminal blood sample

Terminal blood samples were taken by cardiac puncture under Isoflurane anaesthetic with a Terumo 2 ml syringe and 21G needle that had been flushed with 1% potassium EDTA in MilliQ water. The blood collected was put into 2 ml polypropylene tubes. Animals were then killed by cervical dislocation.

Plasma preparation

Terminal blood samples were centrifuged at 2700 x g for 10 minutes and the plasma aliquoted into polypropylene tubes (four aliquots per animal) and frozen at -80 °C.

Removal of the rat knees

The skin on the lower leg was removed and the muscle bundles separated from the bones but left intact with the knee. The femur, fibula and tibia were severed and the knee with attached muscle was removed and placed in approximately 80 ml of 10% neutral buffered formalin in a 125 ml screw cap container. The knees were kept in buffered formalin from 48 to 72 hours before being processed for histological analysis.

Processing for histology

Tissue samples were prepared for light microscopy using standard procedures and carried out externally at the University of Cambridge veterinary school. Briefly, after the knee joints were fixed, the tissues were rinsed with PBS and subsequently decalcified in 8% formic acid for 10 days. Following decalcification the tissue was processed using a Shandon Citadel tissue processer which dehydrated the tissue through a series of graded ethanol concentrations (six changes of 4 hour ranging from 75% to 100% ethanol), rinsed with 100% chloroform (three changes of 4 hour) and finally embedded into

molten paraffin wax to form a tissue block (two changes of 4 hour). The tissues were embedded into cassettes using a Surgipath PEC 3001 machine with molten paraffin wax. The tissue was processed as soon as possible after collection to minimise damage caused from the formalin fixation so that tissue sections could be potentially used for immunohistochemistry.

At least four 10 µm sections were cut from each rat knee tissue block using a Leica RM2135 wax microtome. Two sections were placed on a glass slide and the slides were processed for standard Haematoxylin and Eosin (H&E) and Safranin O fast Green (Safranin O F/G) staining using an automated linear staining machine (Leica ST4040). Briefly, the linear staining machine has 23 reagent stations and four water stations arranged in a configuration for staining slides, which follow a standard H&E or Safranin O F/G protocol. For H&E slides were exchanged between the 27 stations after one minute in each and follow a xylene, ethanol, water, haematoxylin, water, acid alcohol, water, eosin, water, ethanol and xylene series. (all solvents from Fisher Scientific and H&E from Leica). The sections were allowed to air dry, were mounted and covered with a coverslip ready to be viewed under a microscope.

Image Analysis

Slides were viewed at either times 2, 4 or 10 X magnification using an Olympus AX70 microscope using Image-Pro Plus image analysis software (suite v7.0, Media Cybernetics, U.K.). An initial informal analysis of the stained tissue sections for OA-like features was performed to show the overall gross changes of the medial knee joint of each rat.

Image analysis was conducted on the SO/FG slides using the X2 objective where the total area of the cartilage (in mm^2) was determined for all the rat knees (MIA and ETF-PBS treated knees). The depth of the cartilage layer (a minimum of 6 measurements was made per medial knee joint) and this was compared to the depth of the sub-chondral bone from the same knee joint.

Data Analysis

Image analysis (such as cartilage area) and pain assessment data (for example, weight distribution imbalance) was analysed for each animal using classical statistics. Multiple measurements were collected and averaged from animals treated with either p75-NTR-Fc (at different doses) or PG-007 and values were compared to control animals using the appropriate classical statistical tests. For all analyses, $p<0.05$ was taken to indicate statistical significance.

Results

Body Weight

The body weights of the rats in the different groups were compared to test for differences in the sizes of the animals. At day 0, there was no statistically significant

difference between the body weights of the animals in the different treatment groups (p = 0.76 n.s., one-way ANOVA, see Figure 30).

The mean body weight between the six treatment groups (rats treated with p75NTR-Fc or PG-007 compared to rats treated with control antibody) was not statistically significant from each other during the course of the study (p = n.s., two way ANOVA, see Figure 31).

Spontaneous Pain Measurements

Effects of p75NTR-Fc and PG-007 on the spontaneous pain provoked by OA

Spontaneous pain was assessed using an incapacitance tester to measure the distribution of weight through the rear limbs. Assessments were made at baseline (except for group 7, naïve animals) and again on days 15, 21 and 28 following the injection of MIA into one knee (contralateral knees were injected with ETF-PBS). The data is shown in Figure 32 and is illustrated as the proportion of the total weight over the rear limbs that are being supported by the MIA-treated rear limb. For naïve animals the proportion of weight that is passing through the left rear limb is shown.

For all the animals there was no statistically significant difference between the proportion of weight on the treated rear limb and the theoretical expectation of 0.5 (even distribution across both rear limbs at baseline on day 0 (p=0.379, Kruskal Wallis test; see Figure 32). By day 28, all five treatment groups were significantly different to the theoretical mean of 0.5 and were essentially taking the weight of their MIA injected knee (see Figure 32). Based on previous studies this indicated that the extent of OA pathology was significant enough to start dosing the rats.

Dosing was started in rats with the respective treatment regimen from day 30 onwards until the end of the study on day 56 (treatment every 5 days via the subcutaneous route). During the course of the study further assessment of spontaneous pain were measured (made on days 35, 42, 49 and 56, see Figure 4). Throughout the study up until day 49, animals treated with control IgG Fc remained significantly different between the proportion of weight on the treated rear limb and the theoretical expectation of 0.5, indicating that the MIA treated knee was causing pain to the animal (see Figure 33). In contrast, in animals treated with anti-NGF PG-007 and those treated with p75NTR-Fc (from 0.3 to 3 mg/kg) there was no significant between the proportion of weight on the MIA treated rear limb and the theoretical expectation of 0.5 (ie even distribution of weight on the rear limbs) as time progressed.

Histological Changes in the Knee Following Mia-Induced Oa

Effects of p75NTR-Fc and PG007 on the regression of OA

Knees injected with MIA from animals treated with control antibodies showed areas of chondrocyte degeneration, which in some areas has led to full cartilage loss (Figure 34). This is highlighted by the associated loss of Safranin O fast green staining in the

cartilage. In addition, there were significant pathological changes in the sub chondral bone underlying the areas of the greatest cartilage damage (Figure 34). There is a spectrum of changes in the subchondral bone, which reflect both reactive changes and progression driven from remodelling through to sclerotic changes (Figure 34).

Animals treated with 3 mg/kg PG-007 for 28 days after significant pathology has been established (identified by a pain assessment where the proportion of weight on the treated rear limb and the theoretical expectation of 0.5 were significantly different) showed significant histological changes compared to animals treated with control antibody with loss of the overall integrity of the rat knee and no histological evidence of efficacy (Figure 35). This pathology is consistent with associated loss of cartilage and bone marrow and evidence of bone necrosis, which overall leads to an overall rapid progression and in some animals a worsening of the OA compared to animals treated with control antibodies (figure 35). In contrast, animals treated with p75NTR-Fc (0.3 to 3 mg/kg) exhibit a very different histopathology and there is evidence of significant chondroprotection and an associated significant increase in subchondral bone pathology compared to animals treated with control antibodies, which leads to an overall reduced severity of the bone pathology and OA (Figures 36-38). This is phenotypically represented by a reduced myeloid expansion in the bone marrow, which diminishes the inflammatory mediated osteolytic drive.

Animals treated with the lower doses of p75NTR-Fc (0.3 and 1 mg/kg) show the most marked changes in the histopathology (see Figures 37 and 38), and in both treatment groups the pathology was almost at normal levels. Taking into consideration the histological results at 3 mg/kg p75NTR-Fc (see Figure 36) the overall profile suggests that p75NTR-Fc exhibits a "bell-shaped" dose response curve.

Histopathology assessment

Histopathology was performed on Safranin O Fast Green stained sections of decalcified knees. In general, the quality of both tissue processing and staining was excellent, with only three slides rejected on quality grounds. The slides were organised into treatment groups and so the initial pathology assessment was not blinded. However, for the purposes of grading, all groups (with the exception of vehicle) were randomised - using a simple random number sequence – in order to reduce observer bias and diagnostic drift.

Descriptive cartilage histopathology phenotypes

Control Group

There was a clear distinction between paired slides. Slides with most marked cartilage pathology are: 4RA, 5LA, 6LB, 10RA, 11LB (insufficient cartilage to grade on slide 11RA) and 12RB. The majority of cartilage changes were full thickness erosions with obvious peripheral chondrocyte necrosis.

Group 4 – PG007, 3mg/kg

There was a clear distinction between paired slides. Slides with most marked cartilage pathology are: 1RA, 2RB, 3LB, 34LA, 35LA, 36RB. Overall, the cartilage pathology was histologically similar to the control group.

Group 3 – P75NTR-Fc, 0.3mg/kg

The distinction between paired slides was not so marked compared to the control group. Administration of P75NTR-Fc was associated with marked inhibition of MIA-induced cartilage pathology – indeed near normal in two cases. Three slides were rejected from this group on quality grounds.

Group 1 – P75NTR-Fc, 1.0mg/kg

The distinction between paired slides was not so marked compared to the control group. Slides with most marked cartilage pathology (although modest compared to control lesions) are: 7LA, 8LA, 13RB, 15RA, 31RB, 33LB. Administration of P75NTR-Fc was associated with marked inhibition of MIA-induced cartilage pathology.

Group 2 – P75NTR-Fc, 3.0mg/kg

This group presented with marked variability in terms of efficacy effects on cartilage pathology – with a spectrum of effects from control-type lesions to those resembling Group 1 (50%). Slides with the most marked cartilage pathology are: 16LB, 17RA, 18RB, 22RB, 23LA and 24LA. Administration of P75NTR-Fc at the top dose did not show the unequivocal effects associated with 0.3 and 1.0mg/kg.

Histopathology grade criteria

Allocation of grade is based upon the most frequent lesion observed within each anatomical zone. Based upon the initial assessment, key histological features were identified in the control group and which showed evident changes following 1.0 mg/kg P75NTR-Fc administration. Grade criteria were those which have been employed in previous MIA study pathology assessment (MLF).

Cartilage pathology

Many publications detail the use of the Mankin Score for grading human cartilage pathology. The original Mankin Score (or HHGS score) ranges from 0 (normal) to 14 (severe pathology) and suffers from marked inter-observer bias. In addition, this scoring system underestimates the involvement of pannus pathology and often conflates early changes with normal variation. Importantly, the Mankin Score expands the grade range for moderate to severe pathology, and markedly conflates mild to moderate changes. Thus, the Mankin Score, applied directly, has questionable utility in pre-clinical studies as it rarely describes a pharmacodynamic range and is insensitive to the differences in cell turnover between rodents and man. The grading system used in this study is a modification of the OARSI system using the descriptive scheme of Pelletier:

0 - no significant abnormalities; 1 – minimal superficial fibrillation; 2 -superficial erosion; loss of superficial zone chondrocytes; 3 – deep zone erosion; 4 – full thickness erosion; multi-focal exposure of sub-chondral bone, < 50% area; 5 – loss of cartilage plate; detritic forms in articular space, > 50% area

Sub-chondral bone

The sub-chondral bone plate reacts to loss of cartilage integrity across the spectrum of cartilage grades before undergoing degenerative changes – essentially osteolysis mediated. 0 – no significant abnormalities; 1 – superficial zone disorganisation (erratic osteoid zones); 2 – full thickness disorganisation; 3- multi-focal osteolysis; 4 – multi-focal osteopenia; 5 – confluent osteopaenic zones

Stromal cavities

The stromal cavities are the marrow cavities within and immediately distal to the subchondral plate. 0 – no significant abnormalities; 1 – minimal osteolysis; 2 – moderate osteolysis; 3 – marked osteolysis with evidence of multiple lytic pits in majority of cavities; 4 – post-osteolytic fusion of cavities; 5 – fused cavities breach sub-chondral bone and/ or periosteum

Cancellous bone

The cancellous bone system undergoes reactive changes in minimal cartilage pathology due to changes in joint biomechanics – progressing to a more degenerative phenotype secondary to inflammation

0 – no significant abnormalities; 1 – multi-focal areas of superficial bone resorption/ osteolysis; 2 – multiple areas of marked osteolysis; 3 – multiple areas of osteolysis with loss of normal tide-mark zonation; 4 – multi-focal zones of osteolysis with evident osteocyte loss; 5 – cancellous bone breach with/without bone fusion

Bone marrow hyper-cellularity

0 – no significant abnormalities; 1 – multiple focal condensed foci of cells within marrow stroma; 2 – multiple focal condensed foci of cells within marrow stroma and periosteal pits; 3 – diffuse marrow hyper-cellularity – zonal; 4 – expansion of bone marrow into stromal cavities; 5 – breach of marrow compartment into sub-chondral plate or periosteal with/ without pannus mixing

Bone sclerosis

Bone sclerosis is usually seen as areas of fibroplastic expansion from the stromal cavities. 0 – no significant abnormalities; 1 – occasional, small, foci; 2 – multiple foci; 3 – multiple confluent foci; 4 – multiple confluent foci with associated bone degeneration; 5 – multiple confluent foci with bone cysts

Osteoblast hyperplasia

0 – no significant abnormalities; 1 – multiple osteoblast plates; 2- multiple hypertrophic osteoblast plates; 3 – obvious osteoblast palisading; 4 – hypertrophic osteoblast plates - zonal; 5 – hypertrophic osteoblast plates – multi-zonal.

Histology grade results (most severe cartilage pathology sample from paired sets plotted)

Cartilage pathology

Administration of PG007 showed no histologically significant efficacy effects. P75NTR-Fc showed marked efficacy on chondroprotection at 0.3 and 1.0 mg/kg. The efficacy profile

at 3.0mg/kg was more variable – with 50% of the group showing overlay with the control group (Figure 39).

Sub-chondral bone

Administration of PG007 was associated with histologically significant increase in sub-chondral bone pathology compared to the control group – similar to the profile observed with 3.0mg/kg P75NTRFc. By contrast, administration of P75NTR-Fc at 0.3 and 1.0 mg/kg was associated with marked improvement in sub-chondral bone histology (Figure 40).

Stromal cavities

Administration of PG007 was not associated with any histologically significant efficacy effects. By contrast, administration of P75NTR-Fc at all does was associated with reduction in osteolytic pathology and expansion of stromal cavities, although the effects at 0.3 and 1.0 mg/kg were most marked – both groups reducing pathology to almost normal levels (Figure 41).

Cancellous bone

Administration of PG007 was associated with cancellous bone pathology similar to controls – with two samples exceeding control ranges. By contrast, P75NTR-Fc markedly reduced cancellous bone pathology at the 0.3 and 1.0 mg/kg dose levels. P75NTF-Fc at 3.0 mg/kg presented a more variable profile, with the majority of samples showing overlay with the control group (Figure 42).

Bone marrow hyper-cellularity

Administration of PG007 was associated with expansion of myeloid cells in the bone marrow, with four samples either at the top or exceeding the control range. P75NTR-Fc reduced marrow cellularity – with reduced myeloid expansion being a prominent feature – at the 0.3 and 1.0 mg/kg doses. The 3.0 mg/kg dose, although showing a trend towards inhibition, showed overlay with low level responders in the control group (Figure 43).

Bone sclerosis

There were no histologically significant differences between the study groups – although the P75NTR-Fc samples did show a clustering towards the top end of the control range (Figure 44).

Osteoblast hyperplasia

Administration of P75NTR-Fc at 0.3 and 1.0 mg/kg was associated with histologically significant osteoblast proliferation – with palisading and osteoblastic plate formation being especially prominent in the epiphyseal zone, at the sub-chondral bone/ cartilage zone. In addition, although not graded, there were prominent 'fibroblast-like' cells in this zone. PG007 and P75NTR-Fc (3.0 mg/kg) were histologically similar to controls (Figure 45).

Adverse Effects

Two rats treated with 3 mg/kg PG-007 developed skin lesions. Very minor skin lesions began to appear around the face, neck and shoulder region in some rats by day 45 (15 days following treatment) but became more apparent from day 49 (19 days following treatment) onwards in one rat. These lesions were not associated with the injection site. Rats were checked regularly and the number, size and extent of each lesion was quantified to ensure we remained within the humane clinical end-point of the study. No rats treated with p75NTR-Fc at any concentration developed skin lesions.

Discussion

Histopathology

PG007 was not associated with histological evidence of efficacy. In contrast, P75NTR-Fc at 0.3 and 1.0 mg/kg was associated with histologically significant chondroprotection, with reduced severity of bone pathology and osteolysis-mediated bone erosion. The finding of reduced bone marrow hyper-cellularity (notably myeloid expansion) supports this phenotype, and suggests that limitation of an inflammation-mediated osteolytic drive is involved in the efficacy response. Importantly, these doses were also associated with an expansion of the osteoblast pool – suggestive that the mechanism of efficacy of P75NTR-Fc is a dual mechanism, limiting osteolysis (thus limitation of myeloid cell activation) whilst expanding the osteoblast pool (thus augmenting the mesenchyme cell pool). The histological profile of the 3.0 mg/kg P75NTR-Fc group suggests that P75NTR-Fc exhibits a 'bellshaped' dose response curve. Although in many respects the histological profile of 3.0 mg/kg P75NTR-Fc and PG007 are similar, the exact phenotype suggests that the osteolytic drive of P75NTRFc is less than that of PG007 on a dose equivalency basis. Classically, histopathology assessment of MIA models is based upon a linear relationship from primary cartilage pathology resulting in reactive bone changes. The bone marrow changes are often deemed consequential, secondary to a reactive synovitis arising from biomechanical changes due to cartilage loss. There is accumulating data from osteoimmunology suggesting that this linear relationship is naïve, and that the bone compartment can exert an important role in conditioning both the temporal development and phenotype of the cartilage response. Indeed, this research supports imaging data from human studies which show bone remodelling even in minimal grade cartilage lesions. Thus it is likely that the cartilage and sub-chondral bone should be viewed as an integrated functional unit – with the latter providing conditioning to support chondrocyte proliferation and survival and so influencing structural outcomes in osteoarthritis. The data from the present study offers support to the hypothesis that manipulation of the NGF pathway, whilst offering possibilities for reducing osteolysis, also may augment mesenchyme cell mediated osteoprotection – and thus suppressing the drive from superficial chondronecrosis to deep zone lesions and hence full thickness cartilage erosions and cartilage loss.

Conclusions

In this study the effect of a therapeutic dosing regimen using p75NTR-Fc (from 0.3 to 3 mg/kg) was investigated to determine whether regression of the OA pathology following MIA injection could be observed. OA was allowed to develop for 30 days before dosing

commenced, which was identified by pain measurements being significantly different from the theoretical mean of 0.5.

Animals treated with lower doses of p75NTR-Fc (0.3 and 1 mg/kg) were both analgesic and efficacious and showed significantly less OA pathology compared to animals treated with control antibodies. In contrast, despite rats treated with 3 mg/kg PG-007 showing analgesia this was not associated with histological evidence of efficacy and any improvement in the OA pathology. Animals treated with the higher dose of p75NTR-Fc (3 mg/kg) were analgesic however; there were some histopathological similarities to animals treated with 3 mg/kg PG-007.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. Moreover, all embodiments described herein are considered to be broadly applicable and combinable with any and all other consistent embodiments, as appropriate.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

Claims

1. A p75NTR neurotrophin binding protein (p75NTR(NBP)) for use in the treatment of osteoarthritis.

2. The p75NTR(NBP) for use according to claim 1, wherein treatment of osteoarthritis includes relief from the symptoms of osteoarthritis.

3. The p75NTR(NBP), wherein relief from the symptoms of osteoarthritis includes reduction in pain, inflammation, swelling, tenderness, joint stiffness or increase in joint mobility or any combination of these.

4. The p75NTR(NBP) for use according to claim 1, wherein treatment of osteoarthritis includes slowing or arresting of disease progression.

5. The p75NTR(NBP) for use according to claim 1, wherein treatment of osteoarthritis includes reversal of disease progression, regrowth of cartilage and/or curative treatment.

6. The p75NTR(NBP) for use according to claim 4 or 5, wherein disease progression is determined by the rate of cartilage loss or regrowth.

7. The p75NTR(NBP) for use according to claim 1, wherein treatment of osteoarthritis includes prophylactic treatment.

8. The p75NTR(NBP) for use according to any preceding claim, wherein the p75NTR(NBP) comprises a p75NTR(NBP) connected to one or more auxiliary molecules.

9. The p75NTR(NBP) for use according to claim 8, wherein the one or more auxiliary molecules are selected from: (a) transferrin or a portion thereof; (b) albumin or a portion thereof; (c) an immunoglobulin Fc or a portion thereof; or (d) a polyethylene glycol polymer chain.

10. The p75NTR(NBP) for use according to either claim 8 or 9, the p75NTR(NBP) is connected to the one or more auxiliary molecules via one or more linkers.

11. The p75NTR(NBP) for use according to either claim 9 or 10, wherein the p75NTR(NBP) comprises a p75NTR(NBP) connected to an immunoglobulin Fc or a portion thereof.

12. The p75NTR(NBP) for use according to any preceding claim, wherein the p75NTR(NBP) has the amino acid sequence according to SEQ ID NO 3.

13. The p75NTR(NBP) for use according to any preceding claim wherein the p75NTR(NBP) binds to any of NGF, BDNF, NT3 or NT4/5 with a binding affinity (K_d) of between about 5 pM to about 5 nM as measured by surface plasmon resonance at 20 °C.

14. The p75NTR(NBP) for use according to any preceding claim, wherein the p75NTR(NBP) is for separate, sequential or simultaneous use in a combination combined with a second pharmacologically active compound.

15. The p75NTR(NBP) for use according to claim 14 wherein the second pharmacologically active compound of the combination is selected from an opioid analgesic, a nonsteroidal anti-inflammatory drug (NSAID), a barbiturate sedative, a benzodiazepine having a sedative action, an H1 antagonist having a sedative action, a sedative such as glutethimide, meprobamate, methaqualone or dichloralphenazone; a skeletal muscle relaxant; an NMDA receptor antagonist, an alpha-adrenergic, a tricyclic antidepressant, an anticonvulsant, a tachykinin (NK) antagonist, particularly an NK-3, NK-2 or NK-1 antagonist, a muscarinic antagonist, a COX-2 selective inhibitor, a coal-tar analgesic, in particular paracetamol; a neuroleptic a vanilloid receptor agonist or antagonist; a beta-adrenergic; a local anaesthetic; a corticosteroid; a 5-HT receptor agonist or antagonist; a 5-HT2A receptor antagonist; a cholinergic (nicotinic) analgesic; Tramadol®; a PDEV inhibitor; a cannabinoid; metabotropic glutamate subtype 1 receptor (mGluR1) antagonist; a serotonin reuptake inhibitor; a noradrenaline (norepinephrine) reuptake inhibitor; a dual serotonin-noradrenaline reuptake inhibitor; an inducible nitric oxide synthase (iNOS) inhibitor; an acetylcholinesterase inhibitor; a prostaglandin E2 subtype 4 (EP4) antagonist; a leukotriene B4 antagonist; a 5-lipoxygenase inhibitor; a sodium channel blocker; or a 5-HT3 antagonist, and the pharmaceutically acceptable salts and solvates thereof.

16. The p75NTR(NBP) for use according to any preceding claim, wherein the p75NTR(NBP) is formulated for oral, sublingual, buccal, topical, rectal, inhalation, transdermal, subcutaneous, intravenous, intra-arterial, intramuscular, intracardiac, intraosseous, intrasynovial, intradermal, intraperitoneal, transmucosal, vaginal, intravitreal, intra-articular, peri-articular, local or epicutaneous administration.

17. A nucleic acid encoding a p75NTR(NBP), for use in the treatment of osteoarthritis as defined in any of claims 1 to 16.

18. A replicable expression vector for transfecting a cell, comprising a nucleic acid encoding a p75NTR(NBP), for use in the treatment of osteoarthritis as defined in any of claims 1 to 16.

19. A host cell expressing a p75NTR(NBP), for use in the treatment of osteoarthritis as defined in any of claims 1 to 16.

20. A pharmaceutical composition, comprising the p75NTR(NBP) according to any one of claims 1 to 16 or the nucleic acid molecule according to claim 17, or the replicable expression vector according to claim 18, or the host cell according to claim 19, and a pharmaceutically acceptable carrier and/or an excipient.

21. A kit comprising:
    a. the p75NTR(NBP) according to any one of claims 1 to 16 or the nucleic acid molecule according to claim 17, or the replicable expression vector according to claim 18, or the host cell according to claim 19, or the pharmaceutical composition of claim 20; and

b. instructions for the administration of an effective amount of the p75NTR(NBP), nucleic acid molecule, replicable expression vector or pharmaceutical composition to an individual for any one or more of the prevention or treatment of osteoarthritis and/or a symptom of osteoarthritis or for ameliorating, controlling, reducing incidence of, or delaying or reversing the development or progression of osteoarthritis and/or a symptom of osteoarthritis.

22. A method of treating and/or preventing osteoarthritis and/or a symptom of osteoarthritis in an individual comprising administering to said individual a therapeutically effective amount of the p75NTR(NBP) according to any one of claims 1 to 16 or the nucleic acid molecule according to claim 17, or the replicable expression vector according to claim 18, or the host cell according to claim 19, or the pharmaceutical composition of claim 20, optionally further comprising a pharmaceutically acceptable carrier.

23. The method according to claim 22, wherein the the p75NTR(NBP) comprises a p75NTR(NBP) connected to an immunoglobulin Fc or a portion thereof.