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(54) Title: TREATMENT OF LYSOSOMAL DISORDERS (IL-1 ANTAGONISTS)

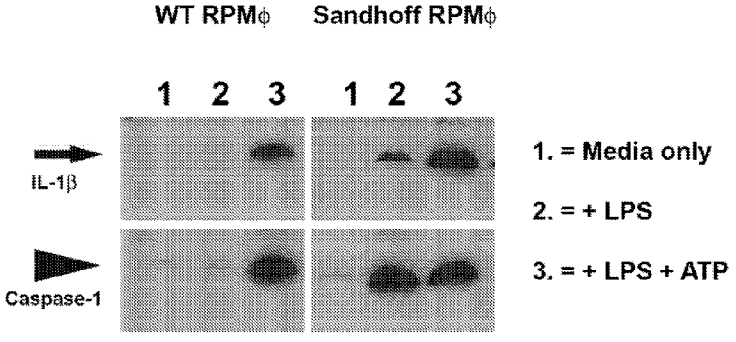


Figure 1

(57) Abstract: The invention relates to methods for treating a Lysosomal storage disease (LSD) in a patient. Kits for use in such methods are also provided.

WO 2016/005747 A1

TREATMENT OF LYSOSOMAL DISORDERS (IL-1 ANTAGONISTS)

Field of the Invention

The invention relates to methods for preventing or treating a lysosomal storage disorder (LSD) in a patient. The invention also provides Interleukin 1-beta (IL-1β) antagonists and kits for preventing or treating a LSD. The inventors have surprisingly shown that IL-1β antagonists can be used for preventing or treating a LSD.

Background of the Invention

Lysosomal storage disorders (LSDs) are a group of inherited metabolic diseases caused by defects in lysosomal homeostasis. To date, LSDs encompass over 60 diseases, with a collective clinical frequency of 1:5000 live births. These diseases can be classified into two main groups: primary storage disorders resulting from a direct deficiency in degradation pathways (typically lysosomal enzyme deficiency disorders); and secondary storage disorders which are caused by malfunctioning downstream lysosomal proteins. In most cases, multiple organs and tissues are involved. Region-specific neurodegeneration and chronic neuroinflammation are featured in the majority of these diseases.

The pathology of LSDs affects many of the body's systems, but mainly the nervous system. Mental retardation is a common symptom. Such disorders are generally severely progressive and unremitting. They tend to present in the first few years of life and the severe progression results in frequent hospitalization. If left untreated, patients often die in their mid-teens.

There is very limited knowledge as to the profile of pro-inflammatory molecules in LSDs.

Current therapeutic approaches for LSDs are limited. There are few, if any, curative treatments and many of the therapeutic options merely improve quality of life. Some LSDs have been responsive to bone marrow transplantation or enzyme replacement therapy. Some benefit has also been reported in a clinical trial using an inhibitor of glycosphingolipid (GSL) biosynthesis: the imino sugar drug, miglustat (Patterson *et al.*, Rev Neurol (separata) 2006; 43: 8). However, there are currently no non-specific treatments that benefit all LSDs.

There is therefore a need to develop improved treatments of LSDs.

Summary of the Invention

The inventors have surprisingly shown that Interleukin 1 beta (IL-1β) antagonists can be used for preventing or treating a lysosomal storage disorder (LSD) in a patient.

The invention provides a method for preventing or treating a LSD in a patient, wherein the method comprises administering to the patient an IL-1β antagonist and thereby treating the LSD.

The invention further provides an IL-1β antagonist for use in a method for preventing or treating a LSD.

The invention further provides an agent for the prevention or treatment of a LSD, comprising an IL-1β antagonist as an active ingredient.

The invention further provides a kit for preventing or treating a LSD, comprising a means for diagnosing or prognosing a LSD and an IL-1β antagonist.

Brief description of the Figures

Figure 1 shows western blots of either wild type macrophages or macrophages taken from Sandhoff disease mice (*hexb*$^{-/-}$).

Figure 2 shows impaired production of the pro-inflammatory cytokine IL-1β by macrophages from Niemann Pick Disease Type C (*NPC*$^{-/-}$) macrophages.

Figures 3A and 3B show that NPC cells display impaired generation of IL-1β, but enhanced production of the pro-inflammatory cytokine TNF.

Figure 4 shows growth curves for *hexb*$^{-/-}$ (Sandhoff mice) with and without Anakinra treatment.

Figure 5 shows a positive effect of Anakinra on unassisted rearing as shown by the number of rearing events over time.

Figure 6 shows a trend towards delayed decline in function in Anakinra treated *hexb*$^{-/-}$ mice (Sandhoff mice) compared to untreated *hexb*$^{-/-}$ mice (Sandhoff mice) using bar crossing assays.

Figure 7 shows a positive impact of Anakinra on life span of *hexb*$^{-/-}$ mice (Sandhoff mice).

Figure 8 shows the chemical structures of LysoTracker®. (***A***) is LysoTracker® blue. (***B***) is LysoTracker® green. (***C***) is LysoTracker® red.

Brief description of the Sequence Listing

SEQ ID NO:1 shows the sequence of IL-1β cDNA, Genbank-NM_000576.2

SEQ ID NO: 2 shows the sequence of IL-1β RNA, Genbank- NM_000576.2

SEQ ID NO: 3 shows the sequence of IL-1β protein, Genbank-NP_000567.1, the active protein after cleavage with CASP1/ICE is amino acids 117-538 of the protein

SEQ ID NO: 4 shows the sequence of IL-1βR type I isoform 1 cDNA, Genbank-NM_000877.3

SEQ ID NO: 5 shows the sequence of IL-1βR type I isoform 1 RNA, Genbank-NM_000877.3

SEQ ID NO: 6 shows the sequence of IL-1βR type I isoform 1 protein, Genbank-NP_000868.1

SEQ ID NO: 7 shows the sequence of IL-1βR type I isoform 2 cDNA, Genbank-NM_001288706.1

SEQ ID NO: 8 shows the sequence of IL-1βR type I isoform 2 RNA, Genbank-NM_001288706.1

SEQ ID NO: 9 shows the sequence of IL-1βR type I isoform 2 protein, Genbank-NP_001275635.1

SEQ ID NO: 10 shows the sequence of IL-1βR type II isoform 1 protein, Genbank-NP_004624.1

SEQ ID NO: 11 shows the sequence of IL-1βR type II isoform 2 protein, Genbank-NP_001248348.1

SEQ ID NO: 12 shows the sequence of IL-1βRAP isoform 1 cDNA, Genbank-NM_002182.3

SEQ ID NO: 13 shows the sequence of IL-1βRAP isoform 1 RNA, Genbank-NM_002182.3

SEQ ID NO: 14 shows the sequence of IL-1βRAP isoform 1 protein, Genbank-NP_002173.1. This isoform is membrane bound.

SEQ ID NO: 15 shows the sequence of IL-1βRAP isoform 2 cDNA, Genbank-NM_134470.3

SEQ ID NO: 16 shows the sequence of IL-1βRAP isoform 2 RNA, Genbank-NM_134470.3

SEQ ID NO: 17 shows the sequence of IL-1βRAP isoform 2 protein, Genbank-NP_608273.1. This isoform is soluble.

SEQ ID NO: 18 shows the sequence of IL-1βRA isoform 1 precursor protein, Genbank-NP_776214.1

SEQ ID NO: 19 shows the sequence of IL-1βRA isoform 2 protein, Genbank-NP_776213.1

SEQ ID NO: 20 shows the sequence of IL-1βRA isoform 3 protein, Genbank-NP_000568.1

SEQ ID NO: 21 shows the sequence of IL-1βRA isoform 4 protein, Genbank- NP_776215.1

Detailed Description of the Invention

It is to be understood that different applications of the disclosed methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

In addition as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "an antagonist" includes "antagonists", reference to "an antibody" includes two or more such antibodies, reference to "a LSD" includes two or more such LSDs, and the like.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

Method of the invention

The present invention relates to a method for treating a LSD in a patient. The method for treating the LSD comprises administering to the patient an IL-1β antagonist. The invention also concerns an IL-1β antagonist for use in a method for preventing or treating a LSD in a patient. The invention also concerns use of an IL-1β antagonist in the manufacture of a medicament for treating or preventing a LSD in a patient.

The patient is preferably a mammal. The mammal may be a commercially farmed animal, such as a horse, a cow, a sheep or a pig, a laboratory animal, such as a mouse or a rat, or a pet, such as a cat, a dog, a rabbit or a guinea pig. The patient is more preferably human.

Lysosomal Storage Disorders (LSDs)

A LSD is any disorder that involves dysfunction or disruption in the late endosomal/lysosomal system.

A LSD is any disorder that involves an increased volume and/or pH of the endosomal/lysosomal system. The LSD may involve increased storage of lipid or non-lipid. The LSD may be a primary lysosomal hydrolase defect, a post-translational processing defect of lysosomal enzymes, a trafficking defect for lysosomal enzymes, a defect in lysosomal enzyme protection, a defect in soluble non-enzymatic lysosomal proteins, a transmembrane

(non-enzyme) protein defect or an unclassified defect.

Primary lysosomal hydrolase defects include, but are not limited to, Gaucher disease (Glucosylceramidase defect), GM1 gangliosidosis (GM1-β-galactosidase defect), Tay-Sachs disease (β -Hexosaminidase A defect), Sandhoff disease (β -Hexosaminidase A+B defect), Fabry disease (α -Galactosidase A defect), Krabbe disease (β-Galactosyl ceramidase defect), Niemann-Pick disease Type A and B (Sphingomyelinase defect), Metachromatic leukodystrophy (Arylsulphatase A defect), MPS IH (Hurler syndrome; α-Iduronidase defect), MPS IS (Scheie syndrome; α-Iduronidase defect), MPS II (Hunter syndrome; Iduronate sulphatase defect), MPS IIIA (Sanfilippo A syndrome; Heparan sulphamidase defect), MPS IIIB (Sanfilippo B syndrome; Acetyl α-glucosaminidase defect), MPS IIIC (Sanfilippo C syndrome; Acetyl CoA: α-glucosaminide N-acetyltransferase defect), MPS IIID (Sanfilippo D syndrome; N-Acetyl glucosamine-6-sulphatase defect), MPS IV A (Morquio A disease; Acetyl galactosamine-6-sulphatase defect), MPS IVB (Morquio B disease; β-Galactosidase defect), MPS V (redesignated MPS IS), MPS VI (Maroteaux Lamy Syndrome; Acetyl galactosamine-4-sulphatase (Arylsulphatase B) defect), MPS VII (Sly Syndrome; β-Glucuronidase defect), MPS IX (Hyaluronidase defect), Wolman disease (WD; Acid lipase defect), Farber disease (Acid ceramidase defect), Cholesteryl ester storage disease (Acid lipase defect), Pompe disease (Type II; α 1,4-Glucosidase defect), Aspartylglucosaminuria (Glycosylasparaginase defect), Fucosidosis (α-Fucosidase defect), α-Mannosidosis (α-Mannosidase defect), β-Mannosidosis (β-Mannosidase defect), Schindler disease (N-Acetylgalactosaminidase defect), Sialidosis (α-Neuraminidase defect), Infantile neuronal ceroid lipofuscinoses (CLN1; Palmitoyl protein thioesterase defect) and Late infantile neuronal ceroid lipofuscinoses (CLN2; Carboxypeptidase defect).

Post-translational processing defects of lysosomal enzymes include, but are not limited to, Mucosulphatidosis (MSD; Multiple sulphatase defect).

Trafficking defects for lysosomal enzymes include, but are not limited to, Mucolipidosis type II (I-cell disease; N-Acetyl glucosamine phosphoryl transferase defect), Mucolipidosis type IIIA (pseudo-Hurler polydystrophy; N-Acetyl glucosamine phosphoryl transferase defect) and Mucolipidosis type IIIC.

Defects in lysosomal enzyme protection include, but are not limited to, Galactosialidosis (Protective protein cathepsin A (PPCA) defect), β-galactosidase defects and neuraminidase defects.

Defects in soluble non-enzymatic lysosomal proteins include, but are not limited to, GM2 activator protein deficiency (Variant AB), Sphingolipid activator protein (SAP) deficiency and Neuronal ceroid lipofuscinoses (NCL) (CLN5).

Transmembrane (non-enzyme) protein defects include, but are not limited to, Danon disease (Lysosome-associated membrane protein 2 (LAMP2) defect), Niemann-Pick Type C (NPC1 and NPC2 defect), Cystinosis (Cystinosin defect), Infantile free sialic acid storage disease (ISSD; Sialin defect), Salla disease (free sialic acid storage; Sialin defect), Juvenile neuronal ceroid lipofuscinoses (CLN3, Batten disease), Neuronal ceroid lipofuscinoses (NCL) (CLN6 and CLN8) and Mucolipidosis type IV (Mucolipin defect).

Unclassified defects include, but are not limited to, Neuronal ceroid lipofuscinoses (NCL) (CLN4 and CLN7).

The ability of lysosomes to fuse with late endosomes relies upon calcium release, specifically from the late endosomal/lysosomal compartment itself. When insufficient calcium is released from acidic stores there is a complete block in late endosome-lysosome fusion. A severe human disease results from calcium deficiency in the acidic compartment, the lysosomal storage disease termed Niemann-Pick disease type C (NPC).

Niemann-Pick diseases are a heterogeneous group of autosomal recessive lysosomal lipid storage disorders. Common cellular features include abnormal sphingomyelin (SM) storage in mononuclear phagocytic cells and parenchymal tissues, as well as (hepato)splenomegaly. Among the three main subgroups (A-C), Niemann-Pick disease type C (previously classified as NPC and NPD and now appreciated to be a single disease) is classified as a fatal neurovisceral LSD caused by abnormal intracellular cholesterol transport-induced accumulation of unesterified cholesterol in late endosome/lysosomal compartments. Clinical manifestations of NPC differ according to age of onset, where typical clinical signs include chronic jaundice in neonates, ataxia, dystonia, vertical supranuclear gaze palsy, dementia and other motor and neurological problems. A previous clinical study has indicated a mean age at diagnosis to be 10.4 years whilst the average age of death is 16.2 years old. Rarer adult-onset forms have also been reported and are probably under-diagnosed.

Outside the CNS, the cellular characteristics of NPC include abnormal accumulation of unesterified cholesterol and other lipids (e.g. GSLs) within late endosome/lysosomal compartments. Conversely, there is no net elevation in cholesterol in the CNS (although it does have an altered distribution) but there are highly elevated levels of GSLs. Progressive neurodegeneration is particularly characterized by sequential degeneration of GABAergic

Purkinje neurons in the cerebellum, which parallels the onset and progression of cerebellar ataxia and other aspects of neurological dysfunctions seen during the course of NPC.

Genetic studies have shown that NPC disease is caused by mutations in either the *Npc1* or *Npc2* genes.

NPC is unusual as it is caused by mutations in two genes, NPC1 or NPC2, that function as part of the same cellular pathway. However, the precise mechanistic link between these two genes remains unknown and the functional roles of these proteins remains enigmatic. NPC1 encodes a multimembrane spanning protein of the limiting membrane of the late endosome/lysosome where as NPC2 is a soluble cholesterol binding protein of the lysosome.

When NPC1 is inactivated, sphingosine is the first lipid to be stored, suggesting that NPC1 plays a role in the transport of sphingosine from the lysosome, where it is normally generated as part of sphingolipid catabolism. Elevated sphingosine in turn causes a defect in calcium entry into acidic stores resulting in greatly reduced calcium release from this compartment. This then prevents late endosome-lysosome fusion, which is a calcium dependent process, and causes the secondary accumulation of lipids (cholesterol, sphingomyelin and glycosphingolipids) that are cargos in transit through the late endocytic pathway. Other secondary consequences of inhibiting NPC1 function include defective endocytosis and failure to clear autophagic vacuoles. It has been shown that the NPC1/NPC2 cellular pathway is targeted by pathogenic mycobacteria to promote their survival in late endosomes.

The term "Niemann-Pick type C disease like cellular phenotype" as used herein, means a cellular phenotype which includes: (a) abnormal cholesterol metabolism and trafficking; (b) abnormal sphingolipid storage and trafficking; and (c) defective endocytosis. Typically, the abnormal sphingolipid storage (b) involves the majority of sphingolipids in the cell being present at abnormally elevated levels. Typically, (c) comprises defective endocytosis of substantially all biomolecules in the endocytic pathway, including lipids and biomolcules other than lipids, for instance proteins.

Any LSD with secondary inhibition of the NPC disease pathway would benefit from being treated by the method of the invention.

The LSD to be treated by the method of the invention is preferably any of Niemann-Pick type C (NPC1), NPC2, Smith-Lemli-Opitz Syndrome (SLOS), an inborn error of cholesterol synthesis, Tangier disease, Pelizaeus-Merzbacher disease, the Neuronal Ceroid Lipofuscinoses, primary glycosphingolipidoses (i.e. Gaucher, Fabry, GM1, GM2

gangliosidoses, Krabbe and MLD), Farber disease and Multiple Sulphatase Deficiency.

Interleukin 1β (IL-1β)

IL-1β is probably the best known of the 11 members that constitute the IL-1 family of ligands that includes both pro-inflammatory and anti-inflammatory (inhibitory) species. IL-1β is a potent pro-inflammatory molecule, primarily produced by macrophages and dendritic cells and has multiple effects on not only other cells of the innate immune system but also adaptive immune cells, such as T cells.

IL-1β is generated through the activity of a cellular protein complex called the inflammasome. The generation of bioactive IL-1β requires two signals. The first signal (e.g. lipopolysaccharide from Gram negative bacteria) causes the generation of pro-proteins such as pro-IL-1β (the cytokine) and pro-caspase-1 (an enzyme). These pro-proteins are not biologically active. A second signal (e.g. ATP) triggers activation of the protein complex that causes auto-cleavage of pro-caspase-1 to generate active caspase-1 (CASP1/ICE), which then acts on pro-IL-1β to generate active IL-1β, which is secreted by the cell.

IL-1β is produced in response to inflammatory stimuli and mediates various physiologic responses, including inflammatory and immunologic reactions.

There are two IL-1β receptors (IL-1βR), types I and II. Both types have several isoforms. IL-1βR type I is responsible for mediating the signalling of IL-1β. IL-1βR type II acts as a decoy receptor for IL-1β, preventing its interaction with IL-1βR type I. Another inhibitor of IL-1β signalling is the IL-1βR antagonist protein (IL-1βRA). IL-1βRA competitively binds to IL-1βR and prevents the binding of IL-1β to IL-1βR. IL-1βRA competitively binds to IL-1R but does not trigger a pro-inflammatory response. Also required for IL-1β signalling is the IL-1β receptor accessory protein (IL-1βRAP). This protein has two isoforms. The first isoform is membrane-bound. The second isoform is soluble. IL-1βRAP forms a complex with IL-1β and IL-1βR type I, and is a necessary part of the active IL-1βR complex.

The cDNA of IL-1β is shown in SEQ ID NO: 1. The RNA sequence of IL-1β is shown in SEQ ID NO: 2. The amino acid sequence of the IL-1β precursor protein is shown in SEQ ID NO: 3.

IL-1βR is found in two types, both of which have several isoforms. The cDNA of IL-1βR type I isoform 1 is shown in SEQ ID NO: 4. The corresponding RNA and amino acid

sequences are shown in SEQ ID NOs: 5 and 6. The cDNA of IL-1βR type I isoform 2 is shown in SEQ ID NO: 7. The corresponding RNA and amino acid sequences are shown in SEQ ID NOs: 8 and 9. The amino acid sequences of IL-1βR type II isoform 1 and IL-1βR type II isoform 2 are shown in SEQ ID NOs: 10 and 11.

IL-1βRAP is found in several isoforms. The cDNA of IL-1βRAP isoform 1 is shown in SEQ ID NO: 12. The corresponding RNA and amino acid sequences are shown in SEQ ID NOs: 13 and 14. The cDNA of IL-1βRAP isoform 2 is shown in SEQ ID NO: 15. The corresponding RNA and amino acid sequences are shown in SEQ ID NOs: 16 and 17.

IL-1βRA is found in several isoforms. The amino acid sequences of IL-1βRA isoforms 1 to 4 are shown in SEQ ID NOs: 18, 19, 20 and 21.

All isoforms and allelic variants of IL-1β are encompassed by "IL-1β". Any mention of IL-1βR herein encompasses all isoforms and allelic variants of IL-1βR type I and/or IL-1βRAP. Any mention of IL-1βR herein does not encompass IL-1βR type II. In other words, decreasing IL-1βR activity may involve decreasing the activity of IL-1βR type I and/or IL-1βRAP. Similarly, decreasing IL-1βR expression may involve decreasing the expression of IL-1βR type I and/or IL-1βRAP. All isoforms and allelic variants of IL-1βR type II are encompassed by "IL-1βR type II". All isoforms and allelic variants of IL-1βRA are encompassed by "IL-1βRA".

An allelic variant of IL-1β, IL-1βR, IL-1βR type II, or IL-1βRA is a naturally occurring variant in the nucleic acid sequence of IL-1β, IL-1βR, IL-1βR type II, or IL-1βRA. The invention therefore concerns antagonising an isoform or allelic variant of IL-1β and/or IL-1βR.

The present inventors have surprisingly found that that IL-1β antagonists can be used for treating a LSD in a patient.

Interleukin 1β (IL-1β) antagonists

IL-1β antagonists block the function of IL-1β. Blocking the function of IL-1β encompasses any reduction in its activity and/or expression. Blocking the function of IL-1β encompasses any reduction in the activity and/or expression of IL-1βR. Blocking the function of IL-1β encompasses any reduction in the activity and/or expression of IL-1βRAP. Blocking the function of IL-1β encompasses any reduction in the activity and/or expression of IL-1β, IL-1βR and IL-1βRAP. Blocking the function of IL-1β encompasses any increase

in the activity and/or expression of IL-1βR type II. Blocking the function of IL-1β encompasses any increase in the activity and/or expression of IL-1βRA. Blocking the function of IL-1β typically results in reduced inflammation.

The IL-1β antagonist preferably decreases the activity of IL-1β. The IL-1β antagonist preferably decreases the activity of IL-1β by from about 1 to about 100%, such as at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99%.

The IL-1β antagonist preferably binds to IL-1β. The IL-1β antagonist preferably specifically binds to IL-1β. For example, the IL-1β antagonist preferably binds to IL-1β, but not to IL-1α. Specific binding is discussed in more detail below. The IL-1β antagonist preferably reduces the ability of IL-1β to bind to IL-1βR. The IL-1β antagonist preferably prevents binding of IL-1β to IL-1βR.

The IL-1β antagonist preferably binds to the portion of IL-1β that interacts with IL-1βR. The IL-1β antagonist preferably reduces the ability of IL-1 βto activate IL-1βR. The IL-1β antagonist preferably prevents IL-1β from activating IL-1βR.

The IL-1β antagonist preferably reduces the bioavailability of IL-1β. The IL-1β antagonist preferably reduces the bioavailability of IL-1β by from about 1 to about 100%, such as at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99%. The IL-1β antagonist preferably sequesters the IL-1β such that it is not available to bind to IL-1βR.

The IL-1β antagonist preferably decreases the activity of IL-1βR. The IL-1β antagonist preferably decreases the activity of IL-1βR by from about 1 to about 100%, such as at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99%.

The IL-1β antagonist preferably binds to IL-1βR. The IL-1β antagonist preferably specifically binds to IL-1βR. The IL-1β antagonist preferably reduces the ability of IL-1βR to be activated by IL-1β. The IL-1β antagonist preferably prevents IL-1βR being activated by IL-1β. The IL-1β antagonist preferably reduces the ability of IL-1βR to carry out intracellular signalling. The IL-1β antagonist preferably prevents IL-1βR from carrying out intracellular signalling.

The IL-1β antagonist preferably reduces the bioavailability of IL-1βR. The IL-1β antagonist preferably reduces the bioavailability of IL-1βR by from about 1 to about 100%,

such as at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99%. The IL-1β antagonist preferably sequesters the IL-1βR such that it is not available to bind to IL-1β.

The IL-1β antagonist preferably binds to the IL-1β-IL-1βR complex. The IL-1β antagonist preferably prevents the activation of the IL-1β-IL-1βR complex.

Decreasing the activity of IL-1β and/or IL-1βR may take place via any suitable mechanism, depending for example on the nature (see below) of the IL-1β antagonist used, e.g. steric interference in any direct or indirect IL-1β IL-1βR interaction or knockdown of IL-1β expression.

The IL-1β antagonist preferably decreases the expression of IL-1β. Decreasing the expression of IL-1β encompasses decreasing the transcription of IL-1β mRNA. Decreasing the expression of IL-1β encompasses decreasing the trafficking of IL-1β mRNA out of the nucleus. Decreasing the expression of IL-1β encompasses decreasing the translation of IL-1β protein. Decreasing the expression of IL-1β encompasses decreasing the post-translational modification of IL-1β protein. Decreasing the expression of IL-1β encompasses decreasing the trafficking of IL-1β protein. Decreasing the expression of IL-1β encompasses decreasing the cleavage of IL-1β protein to its active form. Decreasing the expression of IL-1β encompasses increasing the degradation of IL-1β.

The IL-1β antagonist preferably decreases the expression of IL-1β by from about 1 to about 100%, such as at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99%.

The IL-1β antagonist preferably decreases the expression of IL-1βR. Decreasing the expression of IL-1βR encompasses decreasing the transcription of IL-1βR mRNA. Decreasing the expression IL-1βR encompasses decreasing the trafficking of IL-1βR mRNA out of the nucleus. Decreasing the expression of IL-1βR encompasses decreasing the translation of IL-1βR protein. Decreasing the expression of IL-1βR encompasses decreasing the post-translational modification of IL-1βR protein. Decreasing the expression of IL-1βR encompasses decreasing the trafficking of IL-1β protein to the cell membrane. Decreasing the expression of IL-1βR encompasses decreasing the expression of IL-1βR protein at the cell membrane. Decreasing the expression of IL-1βR encompasses increasing the degradation of IL-1βR.

The IL-1β antagonist preferably decreases the expression of IL-1βR by about 1 to about 100%, such as at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99%.

Decreasing the expression of IL-1β and/or IL-1βR may take place via any suitable mechanism, depending for example on the nature (see below) of the IL-1β antagonist used.

The IL-1β antagonist preferably (a) decreases the activity of IL-1β, (b) decreases the activity of IL-1βR, (c) decreases the expression of IL-1β or (d) decreases the expression of IL-1βR. The IL-1β antagonist may perform any combination of (a) to (d), such as (a); (b); (c); (d); (a) and (b); (a) and (c); (a) and (d); (b) and (c); (b) and (d); (c) and (d); (a), (b) and (c); (a), (b) and (d); (a), (c) and (d); (b), (c) and (d); or (a), (b), (c) or (d).

Decreasing the activity of IL-1β and/or IL-1βR and/or the expression of IL-1β and/or IL-1βR can be measured by any suitable means. For example, prevention of the IL-1β-IL-1βR interaction can be determined by an IL-1β-IL-1βR binding assay. Expression of IL-1β and/or IL-1βR may be measured using immunohistochemistry, western blotting, mass spectrometry, fluorescence-activated cell sorting (FACS) or quantitative reverse transcription polymerase chain reaction (qRT-PCR), such as real time qRT-PCR. Other methods for determination of IL-1β include specific ELISA (enzyme-linked immunoabsorbant assay) and bioassay.

Any suitable antagonist may be used according to the invention. For example a small molecule, a polypeptide, a peptide or peptidomimetic, a polynucleotide, an oligonucleotide, an antisense RNA, small interfering RNA (siRNA) or small hairpin RNA (shRNA), an aptamer, or an antibody or antibody fragment may be used. Preferred antagonists include, but are not limited to, peptide fragments of IL-1β or IL-1βR, double-stranded RNA, aptamers and antibodies.

Small molecules

Small molecule IL-1β antagonists for use in the invention encompass any known in the art.

Peptides and Polypeptides

The IL-1β antagonist for use in the invention can comprise a peptide. The IL-1β antagonist for use in the invention can comprise a peptidomimetic. The IL-1β antagonist for use in the invention can comprise a polypeptide. A peptide is a polymer comprising from

about two to about 50 amino acids. A polypeptide is a polymer comprising about 50 or more amino acids. The amino acids can be naturally occurring or artificial. One or more of the amino acids in the peptide or polypeptide may be D amino acids. Said peptides and polypeptides may be linear or cyclic.

Peptide and polypeptide IL-1β antagonists will typically comprise fragments of IL-1β. Such fragments may compete with full-length IL-1β for binding to IL-1βR and hence antagonise IL-1β. Peptide and polypeptide antagonists will typically comprise fragments of IL-1βR that bind to IL-1β and hence antagonise IL-1β. Peptide and polypeptide antagonists will typically comprise fragments of IL-1βR type II. Peptide and polypeptide antagonists will typically comprise fragments of IL-1βR type II that bind to IL-1β and hence antagonise IL-1β. Peptide and polypeptide antagonists will typically comprise fragments of IL-1βRA. Peptide and polypeptide antagonists will typically comprise fragments of IL-1βRA that bind to IL-1βR and so prevent the binding of IL-1β to IL-1βR. Peptide and polypeptide IL-1β antagonists may bind to IL-1β and so prevent the binding of IL-1β to IL-1βR. Peptide and polypeptide IL-1β antagonists may bind to IL-1βR and so prevent the binding of IL-1β to IL-1βR. Peptide and polypeptide IL-1β antagonists may bind to and sequester IL-1β. Peptide and polypeptide IL-1β antagonists may bind to and sequester IL-1βR.

The IL-1β antagonist preferably comprises at least about 10 consecutive amino acids from SEQ ID NO: 3 or a variant thereof having at least about 80%, at least about 90%, at least about 95%, at least about 98% or at least about 99% amino acid sequence identity to SEQ ID NO: 3 over its entire length. The IL-1β antagonist preferably comprises at least 10 consecutive amino acids from SEQ ID NOs: 6, 9, 10, 11, 14, 17, 18, 19, 20 or 21 or a variant thereof having at least about 80%, at least about 90%, at least about 95%, at least about 98% or at least about 99% amino acid sequence identity to SEQ ID NOs: 6, 9, 10, 11, 14, 17, 18, 19, 20 or 21 over its entire length. The IL-1β antagonist may comprise at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90 or at least about 100 consecutive amino acids from SEQ ID NOs: 3, 6, 9, 10, 11, 14, 17, 18, 19, 20 or 21 or a variant thereof having at least about 80%, at least about 90%, at least about 95%, at least about 98% or at least about 99% amino acid sequence identity to SEQ ID NOs: 3, 6, 9, 10, 11, 14, 17, 18, 19, 20 or 21 over its entire length.

Peptide and polypeptide IL-1β antagonists can be identified in any suitable manner, for example, by systematic screening of contiguous or overlapping peptides spanning part or

all of the IL-1β or IL-1βR sequences. Peptidomimetics may also be designed to mimic such peptide antagonists.

The ability of a peptide or polypeptide to bind IL-1β and/or IL-1βR can be determined using any method. Binding assays suitable for IL-1β are known in the art. For instance, dissociation constants may be measured using radioactively labelled protein and/or surface plasmon resonance techniques (see for example Grell *et al.,* 1998, *Eur. J. Immunol.*)

The above mentioned sequence identity is calculated on the basis of amino acid identity. The UWGCG Package provides programs including GAP, BESTFIT, COMPARE, ALIGN and PILEUP that can be used to calculate sequence identity or to line up sequences (for example used on their default settings). The BLAST algorithm can also be used to compare or line up two sequences, typically on its default settings. Software for performing a BLAST comparison of two sequences is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm is further described below. Similar publicly available tools for the alignment and comparison of sequences may be found on the European Bioinformatics Institute website (http://www.ebi.ac.uk), for example the ALIGN and CLUSTALW programs.

A BLAST analysis is preferably used for calculating identity. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al.*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.* USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.* USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two polynucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Similar sequences typically differ by at least 1, 2, 5, 10, 20 or more mutations (which may be substitutions, deletions or insertions of amino acids). These mutations may be measured across any of the regions mentioned above in relation to calculating identity. In the case of proteins as above, the substitutions are preferably conservative substitutions. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

Any of the peptide and polypeptide IL-1β antagonists for use in the invention may be in the form of a dimer. Any of the peptide and polypeptide IL-1β antagonists may further be chemically-modified to form a derivative. Derivatives include peptides or polypeptides that have lipid extensions or have been glycosylated. Derivatives also include peptides or polypeptides that have been detectably labelled. Detectably labelled peptides or polypeptides have been labelled with a labelling moiety that can be readily detected. Examples of labelling moieties include, but are not limited to, radioisotopes or radionucleotides, fluorophores such as green fluorescent protein (GFP), electron-dense reagents, quenchers of fluorescence, enzymes, affinity tags and epitope tags. Preferred radioisotopes include tritium and iodine. Affinity tags are labels that confer the ability to specifically bind a reagent onto

the labelled molecule. Examples include, but are not limited to, biotin, histidine tags and glutathione-S-transferase (GST). Labels may be detected by, for example, spectroscopic, photochemical, radiochemical, biochemical, immunochemical or chemical methods that are known in the art.

Any of the peptide and polypeptide IL-1β antagonists for use in the invention may also comprise additional amino acids or polypeptide sequences. The peptide and polypeptide IL-1β antagonists may comprise additional polypeptide sequences such that they form fusion proteins. The additional polypeptide sequences may be fused at the amino terminus, carboxy terminus or both the amino terminus and the carboxy terminus. Examples of fusion partners include, but are not limited to, GST, maltose binding protein, alkaline phosphatates, thiorexidin, GFP, histidine tags and epitope tags (for example, Myc or FLAG).

An example of a peptide and polypeptide IL-1β antagonist for use in the invention is Anakinra, also known as Kineret®. Anakinra is a recombinant form of IL-1RA. Anakinra blocks the biologic activity of IL-1β, including inflammation and cartilage degradation associated with rheumatoid arthritis, by competitively inhibiting the binding of IL-1β to IL-1βR. Anakinra is used for the treatment of auto-inflammatory diseases, including Muckle-Wells Syndrome, Familial Mediterranean fever etc. It is prescribed for chronic inflammatory conditions, in particular rheumatoid arthritis.

Peptide and polypeptide IL-1β antagonists for use in the invention may also comprise soluble IL-1βR chimeras.

Peptide and polypeptide IL-1β antagonists for use in the invention may also comprise IL-1βR type II chimeras.

Peptide and polypeptide IL-1β antagonists for use in the invention may also comprise IL-1βRA chimeras.

An example of a peptide and polypeptide IL-1β antagonist for use in the invention is Rilonacept (trade name Arcalyst®). Rilonacept is a fusion protein comprising fragments of IL-1βR type 1 and IL-1βRAP.

Peptides and polypeptides may be made using standard methods. The polynucleotide sequence encoding a peptide or polypeptide may be cloned into any suitable expression vector. In an expression vector, the polynucleotide sequence encoding the peptide or polypeptide is typically operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell. Such expression vectors can be used to express the peptide or polypeptide.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. Multiple copies of the same or different polynucleotide may be introduced into the vector.

The expression vector may then be introduced into a suitable host cell. Thus, the peptide or polypeptide can be produced by inserting a polynucleotide sequence encoding a construct into an expression vector, introducing the vector into a compatible bacterial host cell, and growing the host cell under conditions which bring about expression of the polynucleotide sequence.

The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide sequence and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene. Promoters and other expression regulation signals may be selected to be compatible with the host cell for which the expression vector is designed. A T7, *trc, lac*, *ara* or λ_L promoter is typically used.

The host cell typically expresses the peptide or polypeptide at a high level. Host cells transformed with a polynucleotide sequence encoding the peptide or polypeptide will be chosen to be compatible with the expression vector used to transform the cell. The host cell is typically bacterial and preferably *E. coli.* Any cell with a λ DE3 lysogen, for example C41 (DE3), BL21 (DE3), JM109 (DE3), B834 (DE3), TUNER, Origami and Origami B, can express a vector comprising the T7 promoter.

Polynucleotides and oligonucleotides

The IL-1β antagonist for use in the invention can comprise a polynucleotide. The IL-1β antagonist for use in the invention can comprise an oligonucleotide. The IL-1β antagonist for use in the invention can comprise an antisense RNA. The IL-1β antagonist for use in the invention can comprise a small interfering RNA (siRNA). The IL-1β antagonist for use in the invention can comprise a small hairpin RNA (shRNA).

Polynucleotide and oligonucleotide IL-1β antagonists for use in the invention may decrease the expression of IL-1β or IL-1βR. Polynucleotide and oligonucleotide IL-1β antagonists for use in the invention may bind to or hybridise to IL-1β or IL-1βR mRNAs.

Polynucleotide and oligonucleotide IL-1β antagonists for use in the invention may bind to or hybridise to IL-1β mRNA as shown in SEQ ID NO: 2 or IL-1βR mRNA, as shown in SEQ ID NO: 5 or 8, or IL-1βRAP mRNA, as shown in SEQ ID NO: 13 or 16 or any allelic variants thereof.

The terms "nucleic acid molecule" and "polynucleotide" are used interchangeably herein and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides, ribonucleotides or analogs thereof. The terms "nucleic acid molecule" and "oligonucleotide" are used interchangeably and refer to a polymeric form of nucleotides either deoxyribonucleotides or ribonucleotides, or analogs thereof, of fewer than about 100 or about 50 nucleotides. A nucleic acid may comprise conventional bases, sugar residues and inter- nucleotide linkages, but may also comprise modified bases, modified sugar residues or modified linkages. A nucleic acid molecule may be single stranded or double stranded.

The nucleotides can be naturally occurring or artificial. A nucleotide typically contains a nucleobase, a sugar and at least one linking group, such as a phosphate, 2'O-methyl, 2' methoxy-ethyl, phosphoramidate, methylphosphonate or phosphorothioate group. The nucleobase is typically heterocyclic. Nucleobases include, but are not limited to, purines and pyrimidines and more specifically adenine (A), guanine (G), thymine (T), uracil (U) and cytosine (C). The sugar is typically a pentose sugar. Nucleotide sugars include, but are not limited to, ribose and deoxyribose. The nucleotide is typically a ribonucleotide or deoxyribonucleotide. The nucleotide typically contains a monophosphate, diphosphate or triphosphate. Phosphates may be attached on the 5' or 3' side of a nucleotide.

Nucleotides include, but are not limited to, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), guanosine monophosphate (GMP), guanosine diphosphate (GDP), guanosine triphosphate (GTP), thymidine monophosphate (TMP), thymidine diphosphate (TDP), thymidine triphosphate (TTP), uridine monophosphate (UMP), uridine diphosphate (UDP), uridine triphosphate (UTP), cytidine monophosphate (CMP), cytidine diphosphate (CDP), cytidine triphosphate (CTP), 5-methylcytidine monophosphate, 5-methylcytidine diphosphate, 5-methylcytidine triphosphate, 5-hydroxymethylcytidine monophosphate, 5-hydroxymethylcytidine diphosphate, 5-hydroxymethylcytidine triphosphate, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), deoxyadenosine monophosphate (dAMP), deoxyadenosine diphosphate (dADP), deoxyadenosine triphosphate (dATP), deoxyguanosine monophosphate (dGMP), deoxyguanosine diphosphate (dGDP),

deoxyguanosine triphosphate (dGTP), deoxythymidine monophosphate (dTMP), deoxythymidine diphosphate (dTDP), deoxythymidine triphosphate (dTTP), deoxyuridine monophosphate (dUMP), deoxyuridine diphosphate (dUDP), deoxyuridine triphosphate (dUTP), deoxycytidine monophosphate (dCMP), deoxycytidine diphosphate (dCDP) and deoxycytidine triphosphate (dCTP), 5-methyl-2'-deoxycytidine monophosphate, 5-methyl-2'-deoxycytidine diphosphate, 5-methyl-2'-deoxycytidine triphosphate, 5-hydroxymethyl-2'-deoxycytidine monophosphate, 5-hydroxymethyl-2'-deoxycytidine diphosphate and 5-hydroxymethyl-2'-deoxycytidine triphosphate. The nucleotides are preferably selected from AMP, TMP, GMP, UMP, dAMP, dTMP, dGMP or dCMP.

The nucleotides may contain additional modifications. In particular, suitable modified nucleotides include, but are not limited to, 2'amino pyrimidines (such as 2'-amino cytidine and 2'-amino uridine), 2'-hyrdroxyl purines (such as , 2'-fluoro pyrimidines (such as 2'-fluorocytidine and 2'fluoro uridine), hydroxyl pyrimidines (such as 5'-α-P-borano uridine), 2'-O-methyl nucleotides (such as 2'-O-methyl adenosine, 2'-O-methyl guanosine, 2'-O-methyl cytidine and 2'-O-methyl uridine), 4'-thio pyrimidines (such as 4'-thio uridine and 4'-thio cytidine) and nucleotides have modifications of the nucleobase (such as 5-pentynyl-2'-deoxy uridine, 5-(3-aminopropyl)-uridine and 1,6-diaminohexyl-N-5-carbamoylmethyl uridine).

One or more nucleotides in the polynucleotide or oligonucleotide can be oxidized or methylated. One or more nucleotides in the polynucleotide or oligonucleotide may be damaged. For instance, the polynucleotide or oligonucleotide may comprise a pyrimidine dimer. Such dimers are typically associated with damage by ultraviolet light.

The nucleotides in the polynucleotide or oligonucleotide may be attached to each other in any manner. The nucleotides may be linked by phosphate, 2'O-methyl, 2' methoxy-ethyl, phosphoramidate, methylphosphonate or phosphorothioate linkages. The nucleotides are typically attached by their sugar and phosphate groups as in nucleic acids. The nucleotides may be connected via their nucleobases as in pyrimidine dimers.

The polynucleotide or oligonucleotide can be a nucleic acid, such as deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA). The polynucleotide or oligonucleotide may be any synthetic nucleic acid known in the art, such as peptide nucleic acid (PNA), glycerol nucleic acid (GNA), threose nucleic acid (TNA), locked nucleic acid (LNA), morpholino nucleic acid or other synthetic polymers with nucleotide side chains. The polynucleotide or oligonucleotide may be single stranded or double stranded.

The IL-1β antagonist may comprise an oligonucleotide or polynucleotide which specifically hybridises to a part of SEQ ID NO: 2 (IL-1β mRNA), or any allelic variant thereof. The oligonucleotide is preferably RNA.

The IL-1β antagonist may comprise an oligonucleotide or polynucleotide which specifically hybridises to a part of SEQ ID NOs: 5 or 8 (IL-1βR mRNA), or any allelic variant thereof. The oligonucleotide is preferably RNA.

The IL-1β antagonist may comprise an oligonucleotide or polynucleotide which specifically hybridises to a part of SEQ ID NOs: 13 or 16 (IL-1βRAP mRNA), or any allelic variant thereof. The oligonucleotide is preferably RNA.

A "*part*" can be defined as any number of consecutive nucleotides from IL-1β or IL-1βR mRNA, such as SEQ ID NOs: 2, 5, 8, 13 or 16. A part may be about 10, 15, 20, 25, 30, 40, 50, 100, 150, 200 or more consecutive nucleotides. A part may be about 20, 21, 22, 23, 24 or 25 consecutive nucleotides of IL-1β or IL-1βR mRNA. A part may be about 10, 11, 12, 13, 14 or 15 consecutive nucleotides of IL-1β or IL-1βR mRNA.

The length of the oligonucleotide or polynucleotide is not fixed, as long as the oligonucleotide or polynucleotide is capable of binding to IL-1β or IL-1βR mRNA. The oligonucleotide may comprise a nucleic acid sequence that is complementary to a part of the IL-1β or IL-1βR mRNA sequence and may further comprise additional nucleotides at the 5' and/or 3' ends of that complementary sequence.

The polynucleotide IL-1β antagonist may be about 500, 400, 300, 200 or 100 nucleotides/nucleotide pairs or fewer in length.

The oligonucleotide or polynucleotide IL-1β antagonist may be about 100, 90, 80, 70, 60, 50, 40, 30, 25 or 20 nucleotides/nucleotide pairs or fewer in length. The oligonucleotide or polynucleotide may be at least about 20, 21, 22, 23, 24, 25, 26 or 27 nucleotides/nucleotide pairs in length, preferably at least about 23, 24, 25 or 26 nucleotides/nucleotide pairs in length, especially about 25 nucleotides/nucleotide pairs in length. The oligonucleotide or polynucleotide IL-1β antagonist may be preferably about 20, 21 or 22 nucleotides/nucleotide pairs in length.

The IL-1β antagonist may be capable of specifically binding, or specifically hybridising, to the mRNA sequence of IL-1β or IL-1βR as set forth in SEQ ID NOs: 2, 5, 8, 13 and 16 or allelic variants, but not other mRNA sequences.

An oligonucleotide or polynucleotide "*specifically hybridises*" to a target sequence when it hybridises with preferential or high affinity to the target sequence but does not substantially hybridise, does not hybridise or hybridises with only low affinity to other sequences.

An oligonucleotide or polynucleotide "*specifically hybridises*" if it hybridises to the target sequence with a melting temperature (T_m) that is at least 2 °C, such as at least 3 °C, at least 4 °C, at least 5 °C, at least 6 °C, at least 7 °C, at least 8 °C, at least 9 °C or at least 10 °C, greater than its T_m for other sequences. More preferably, the oligonucleotide or polynucleotide hybridises to the target sequence with a T_m that is at least 2 °C, such as at least 3 °C, at least 4 °C, at least 5 °C, at least 6 °C, at least 7 °C, at least 8 °C, at least 9 °C, at least 10 °C, at least 20 °C, at least 30 °C or at least 40 °C, greater than its T_m for other nucleic acids. Preferably, the portion hybridises to the target sequence with a T_m that is at least 2 °C, such as at least 3 °C, at least 4 °C, at least 5 °C, at least 6 °C, at least 7 °C, at least 8 °C, at least 9 °C, at least 10 °C, at least 20 °C, at least 30 °C or at least 40 °C, greater than its T_m for a sequence which differs from the target sequence by one or more nucleotides, such as by 1, 2, 3, 4 or 5 or more nucleotides. The portion typically hybridises to the target sequence with a T_m of at least 90 °C, such as at least 92 °C or at least 95 °C. T_m can be measured experimentally using known techniques, including the use of DNA microarrays, or can be calculated using publicly available T_m calculators, such as those available over the internet.

Conditions that permit the hybridisation are well-known in the art (for example, Sambrook et al., 2001, Molecular Cloning: a laboratory manual, 3rd edition, Cold Spring Harbour Laboratory Press; and Current Protocols in Molecular Biology, Chapter 2, Ausubel et al., Eds., Greene Publishing and Wiley-lnterscience, New York (1995)). Hybridisation can be carried out under low stringency conditions, for example in the presence of a buffered solution of 30 to 35% formamide, 1 M NaCl and 1 % SDS (sodium dodecyl sulfate) at 37 °C followed by a 20 wash in from 1X (0.1650 M Na^+) to 2X (0.33 M Na^+) SSC (standard sodium citrate) at 50 °C. Hybridisation can be carried out under moderate stringency conditions, for example in the presence of a buffer solution of 40 to 45% formamide, 1 M NaCl, and 1 % SDS at 37 °C, followed by a wash in from 0.5X (0.0825 M Na^+) to 1X (0.1650 M Na^+) SSC at 55 °C. Hybridisation can be carried out under high stringency conditions, for example in the presence of a buffered solution of 50% formamide, 1 M NaCl, 1% SDS at 37 °C, followed by a wash in 0.1X (0.0165 M Na^+) SSC at 60 °C.

The oligonucleotide or polynucleotide may comprise a sequence which is substantially complementary to the target sequence. Typically, the oligonucleotides or

polynucleotides are 100% complementary. However, lower levels of complementarity may also be acceptable, such as 95%, 90%, 85% and even 80%. Complementarity below 100% is acceptable as long as the oligonucleotides specifically hybridise to the target sequence. An oligonucleotide or polynucleotide may therefore have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mismatches across a region of 5, 10, 15, 20, 21, 22, 30, 40 or 50 nucleotides.

Using known techniques and based on a knowledge of the sequence of IL-1β or IL-1βR, double-stranded RNA (dsRNA) molecules can be designed to antagonise IL-1β by sequence identity-based targeting of IL-1β RNA, as set forth in SEQ ID NO: 2, or IL-1βR RNA, as set forth in SEQ ID NOs: 5 or 8, or IL-1βRAP RNA, as set forth in SEQ ID NOs: 13 or 16. Such dsRNAs will typically be small interfering RNAs (siRNAs), usually in a stem-loop ("hairpin") configuration, or micro-RNAs (miRNAs). The sequence of such dsRNAs will comprise a portion that corresponds with that of a portion of the mRNA encoding IL-1β or IL-1βR. This portion will usually be 100% complementary to the target portion within the IL-1β or IL-1βR mRNA but lower levels of complementarity (e.g. at least about 85%, 90% or more or 95% or more) may also be used.

The IL-1β antagonist may comprise an oligonucleotide which comprises about 50 or fewer consecutive nucleotides, such as about 40 or fewer, about 30 or fewer or about 25 or fewer consecutive nucleotides, from SEQ ID NO: 1 or a variant thereof which has at least about 90% identity, such as at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity or at least about 99% identity, to SEQ ID NO: 1 over its entire sequence. The oligonucleotide is preferably about 20, 21, 22, 23, 24 or 25 nucleotides/nucleotide pairs in length. The oligonucleotide is preferably RNA.

The IL-1β antagonist may comprise an oligonucleotide which comprises about 50 or fewer consecutive nucleotides, such as about 40 or fewer, about 30 or fewer or about 25 or fewer consecutive nucleotides, from SEQ ID NOs: 4, 7, 12 or 15 or a variant thereof which has at least about 90% identity, such as at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity or at least about 99% identity, to SEQ ID NOs: 4, 7, 12 or 15 over its entire sequence. The oligonucleotide is preferably about 20, 21, 22, 23, 24 or 25 nucleotides/nucleotide pairs in length.

Oligonucleotide or polynucleotide sequences may be isolated and replicated using standard methods in the art. The gene encoding an IL-1β antagonist of the invention may be amplified using PCR involving specific primers. The amplified sequences may then be incorporated into a recombinant replicable vector such as a cloning vector. The vector may

be used to replicate the polynucleotide in a compatible host cell. Thus polynucleotide or oligonucleotide sequences encoding the IL-1β antagonist of the invention may be made by introducing a polynucleotide or oligonucleotide encoding the IL-1β antagonist of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells for cloning of polynucleotides and oligonucleotides are known in the art and described in more detail above.

Aptamers

Aptamers are generally nucleic acid molecules that bind a specific target molecule. The aptamers for use in the present invention preferably bind to specific target molecules and therefore block the function of IL-1β. The aptamers for use in the present invention preferably bind to IL-1β or IL-1βR.

Aptamers can be engineered completely in vitro, are readily produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications. These characteristics make them particularly useful in pharmaceutical and therapeutic utilities.

As used herein, "aptamer" refers in general to a single or double stranded oligonucleotide or a mixture of such oligonucleotides, wherein the oligonucleotide or mixture is capable of binding specifically to a target. Oligonucleotide aptamers will be discussed here, but the skilled reader will appreciate that other aptamers having equivalent binding characteristics can also be used, such as peptide aptamers.

In general, aptamers may comprise oligonucleotides that are at least 5, at least 10 or at least 15 nucleotides in length. Aptamers may comprise sequences that are up to 40, up to 60 or up to 100 or more nucleotides in length. For example, aptamers may be from 5 to 100 nucleotides, from 10 to 40 nucleotides, or from 15 to 40 nucleotides in length. Where possible, aptamers of shorter length are preferred as these will often lead to less interference by other molecules or materials.

Non-modified aptamers are cleared rapidly from the bloodstream, with a half-life of minutes to hours, mainly due to nuclease degradation and clearance from the body by the kidneys. Such non-modified aptamers have utility in, for example, the treatment of transient conditions such as in stimulating blood clotting. Alternatively, aptamers may be modified to improve their half life. Several such modifications are available, such as the addition of 2'-fluorine-substituted pyrimidines or polyethylene glycol (PEG) linkages.

Aptamers may be generated using routine methods such as the Systematic Evolution of Ligands by Exponential enrichment (SELEX) procedure. SELEX is a method for the in vitro evolution of nucleic acid molecules with highly specific binding to target molecules. It is described in, for example, US 5,654,151, US 5,503,978, US 5,567,588 and WO 96/38579.

The SELEX method involves the selection of nucleic acid aptamers and in particular single stranded nucleic acids capable of binding to a desired target, from a collection of oligonucleotides. A collection of single-stranded nucleic acids (e.g., DNA, RNA, or variants thereof) is contacted with a target, under conditions favourable for binding, those nucleic acids which are bound to targets in the mixture are separated from those which do not bind, the nucleic acid-target complexes are dissociated, those nucleic acids which had bound to the target are amplified to yield a collection or library which is enriched in nucleic acids having the desired binding activity, and then this series of steps is repeated as necessary to produce a library of nucleic acids (aptamers) having specific binding affinity for the relevant target.

Antibodies

The IL-1β antagonist preferably comprises an antibody or antibody fragment. Antibody IL-1β antagonists may bind to IL-1β and so prevent the binding of IL-1β to IL-1βR. Peptide and polypeptide IL-1β antagonists may bind to IL-1βR and so prevent the binding of IL-1β to IL-1βR. Antibody IL-1β antagonists may bind to and sequester IL-1β. Antibody IL-1β antagonists may bind to and sequester IL-1βR. The IL-1β antagonist preferably comprises an antibody or antibody fragment which specifically binds to IL-1β, IL-1βR or any allelic variant thereof. The IL-1β antagonist preferably comprises an antibody or antibody fragment which specifically binds IL-1β and IL-1βR or any allelic variants thereof. IL-1β preferably comprises the sequence shown in SEQ ID NO: 3. IL-1βR preferably comprises the sequences shown in SEQ ID NOs: 6 or 9. IL-1βRAP preferably comprises the sequences shown in SEQ ID NOs: 14 or 17.

An antibody, or other compound, "specifically binds" to a polypeptide when it binds with preferential or high affinity to the protein for which it is specific, but does substantially bind, not bind or binds with only low affinity to other polypeptides. The IL-1β antagonist may be capable of specifically binding the amino acid sequence of IL-1β or IL-1βR as set forth in SEQ ID NOs: 3, 6, 9, 14 and 17, or allelic variants, but not other proteins.

For example, an antibody specifically binds to an antigen if it binds to the antigen with preferential or high affinity, but does not bind or binds with only low affinity to other or

different antigens. An antibody binds with preferential or high affinity if it binds with a Kd of 1 x 10^{-6} M or less, more preferably 1 x 10^{-7} M or less, 5 x 10^{-8} M or less, more preferably 1 x 10^{-8} M or less or more preferably 5 x 10^{-9} M or less. An antibody binds with low affinity if it binds with a Kd of 1 x 10^{-6} M or more, more preferably 1 x 10^{-5} M or more, more preferably 1 x 10^{-4} M or more, more preferably 1 x 10^{-3} M or more, even more preferably 1 x 10^{-2} M or more.

A variety of protocols for competitive binding or immunoradiometric assays to determine the specific binding capability of an antibody are well known in the art (see for example Maddox *et al*, J. Exp. Med. 158, 1211-1226, 1993). Such immunoassays typically involve the formation of complexes between the specific protein and its antibody and the measurement of complex formation.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An antibody refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR).

The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include a Fab fragment, a F(ab')2 fragment, a Fab' fragment, a Fd fragment, a Fv fragment, a dAb fragment and an isolated complementarity determining region (CDR). Single chain antibodies such as scFv antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody

fragments may be obtained using conventional techniques known to those of skill in the art, and the fragments may be screened for utility in the same manner as intact antibodies.

Antibodies for use in the invention can be produced by any suitable method. Means for preparing and characterising antibodies are well known in the art, see for example Harlow and Lane (1988) "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. For example, an antibody may be produced by raising an antibody in a host animal against the whole polypeptide or a fragment thereof, for example an antigenic epitope thereof, hereinafter the "immunogen". The fragment may be any of the fragments mentioned herein (typically at least 10 or at least 15 amino acids long).

An antibody for use in the invention may be a monoclonal antibody or a polyclonal antibody, and will preferably be a monoclonal antibody. An antibody of the invention may be a chimeric antibody, a CDR-grafted antibody, a nanobody, a human or humanised antibody or an antigen binding portion of any thereof. For the production of both monoclonal and polyclonal antibodies, the experimental animal is typically a non-human mammal such as a goat, rabbit, rat or mouse but may also be raised in other species such as camelids.

A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the animal's serum. The animal may therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* **256**, 495-497).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

Monoclonal antibodies (mAbs) for use in the invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.,* the standard somatic cell hybridization technique of Kohler and Milstein. The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure and can be achieved using techniques well known in the art.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat, mouse, guinea pig, chicken, sheep or horse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

An antibody for use in the invention may be produced by a method comprising: immunising a non-human mammal with an immunogen comprising full-length IL-1β or a peptide fragment of IL-1β, obtaining an antibody preparation from said mammal; and deriving therefrom monoclonal antibodies that specifically recognise said epitope.

An antibody for use in the invention may be prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for the immunoglobulin genes of interest or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody of interest, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences.

An antibody for use in the invention may be a human antibody or a humanised antibody. The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

Such a human antibody may be a human monoclonal antibody. Such a human monoclonal antibody may be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

Human antibodies may be prepared by in vitro immunisation of human lymphocytes followed by transformation of the lymphocytes with Epstein-Barr virus.

The term "human antibody derivatives" refers to any modified form of the human antibody, e.g., a conjugate of the antibody and another agent or antibody.

The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

Antibodies for use in the invention will have IL-1β antagonist properties as discussed above. In one embodiment, a monoclonal antibody specifically recognises an epitope within IL-1β and blocks the activity of IL-1β. In anther embodiment, a monoclonal antibody specifically recognises an epitope within IL-1βR and blocks the activity of IL-1βR. In one embodiment, the monoclonal antibody specifically recognises an epitope within IL-1β and blocks the interaction between IL-1β and IL-1βR. In one embodiment, the monoclonal antibody specifically recognises an epitope within IL-1βR and blocks the interaction between IL-1β and IL-1βR.

Antibodies for use in the invention can be tested for binding to IL-1β or IL-1βR by, for example, standard ELISA or Western blotting. An ELISA assay can also be used to screen for hybridomas that show positive reactivity with the target protein. The binding specificity of an antibody may also be determined by monitoring binding of the antibody to cells expressing the target protein, for example by flow cytometry.

Examples of antibodies for use in the invention include canakinumab and gevokizumab. Canakinumab (trade name Ilaris®) is a human monoclonal antibody that selectively binds IL-1β. Gevokizumab is a humanized monoclonal antibody that binds IL-1β.

In another preferred aspect of the invention, the IL-1β antagonist comprises a neutralising antibody to IL-1βR. Typically, the neutralising antibody to IL-1βR is a neutralising antibody to IL-1βR as set forth in SEQ ID NOs: 6, 9, 14 or 17.

Method of treating a LSD

The present invention relates to a method of treating a LSD in a patient, wherein the method comprises administering to the patient an IL-1β antagonist and thereby treating the LSD.

Methods of administration and conditions to be treated are described below. The dose of the IL-1β antagonist to be used in accordance with the invention will depend upon the nature of the LSD. A suitable dose can be determined by a skilled practitioner based on his common general knowledge, taking into account, for example, the mode of administration. For example, a suitable dose may be selected to reflect the level of a therapeutic agent that would be present in the blood circulatory system of a patient after *in vivo* administration.

The IL-1β antagonist can be administered to the patient by any suitable means. The IL-1β antagonist can be administered by enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intra-arterial, intramuscular, intraperitoneal, intraarticular, topical or other appropriate administration routes.

The formulation will depend upon the IL-1β antagonist. The IL-1β antagonist may be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), parenterally, subcutaneously, intravenously, intramuscularly, intrasternally, transdermally or by infusion techniques. IL-1β antagonist may also be administered as a suppository. A physician will be able to determine the required route of administration for each particular patient.

The IL-1β antagonist can be formulated for use with a pharmaceutically acceptable carrier or diluent and this may be carried out using routine methods in the pharmaceutical art. The pharmaceutical carrier or diluent may be, for example, an isotonic solution. For example, solid oral forms may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tabletting, sugar-coating, or film coating processes.

Liquid dispersions for oral administration may be syrups, emulsions and suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Solutions for intravenous or infusions may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1% to 2%.

Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95% of active ingredient, preferably 25% to 70%. Where the pharmaceutical composition is lyophilised, the lyophilised material may be reconstituted prior to administration, e.g. a suspension. Reconstitution is preferably effected in buffer.

Capsules, tablets and pills for oral administration to a patient may be provided with an enteric coating comprising, for example, Eudragit “S”, Eudragit “L”, cellulose acetate, cellulose acetate phthalate or hydroxypropylmethyl cellulose.

Pharmaceutical compositions suitable for delivery by needleless injection, for example, transdermally, may also be used.

The method of the invention may be for treating an LSD. In the case of treating, the patient typically has the LSD, i.e. has been diagnosed as having the LSD, or is suspected as having the LSD, i.e. shows the symptoms of the LSD. As used herein, the term “treating” includes any of following: the prevention of the LSD or of one or more symptoms associated with the LSD; a reduction or prevention of the development or progression of the LSD or symptoms; and the reduction or elimination of an existing LSD or symptoms. Symptoms as described herein include neurological symptoms. Examples of neurological symptoms include ataxia, dystonia, vertical supranuclear gaze palsy and dementia.

The method may be for preventing the disease. In this embodiment, the patient can be asymptomatic. The patient can have a genetic predisposition to the LSD. The patient may have one or more family member(s) with an LSD. As used herein, the term "preventing" includes the prevention of the onset of the disease or of one or more symptoms associated with the disease. Symptoms as described herein include neurological symptoms. Examples of neurological symptoms include ataxia, dystonia, vertical supranuclear gaze palsy and dementia.

Therapy and prevention includes, but is not limited to, preventing or eliciting an effective anti-IL-1β response and/or preventing, alleviating, reducing, curing or at least partially arresting symptoms and/or complications resulting from or associated with an LSD. When provided therapeutically, the therapy is typically provided at or shortly after the onset of a symptom of the LSD. Such therapeutic administration is typically to prevent or ameliorate the progression of, or a symptom of the LSD or to reduce the severity of such a symptom or LSD. When provided prophylactically, the treatment is typically provided before the onset of a symptom of an LSD. Such prophylatic administration is typically to prevent the onset of symptoms of the LSD. Symptoms as described herein include neurological symptoms. Examples of neurological symptoms include ataxia, dystonia, vertical supranuclear gaze palsy and dementia.

Specific routes, dosages and methods of administration of IL-1β antagonists for use in the invention may be routinely determined by the medical practitioner. Typically, a therapeutically effective or a prophylactically effective amount of the IL-1β antagonist is administered to the patient. A prophylactically effective amount is an amount which prevents the onset of one or more symptoms of the LSD. A therapeutically effective amount of the compound is an amount effective to ameliorate one or more symptoms of the LSD.

A therapeutically or prophylactically effective amount of the IL-1β antagonist is administered. The dose may be determined according to various parameters, especially according to the compound used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50mg per kg, preferably from about 0.1mg/kg to 10mg/kg of body weight, according to the activity of the specific inhibitor, the age, weight and conditions of the patient to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 5mg to 2g.

The IL-1β antagonist may be a polynucleotide. Preferably, such polynucleotides are provided in the form of an expression vector, which may be expressed in the cells of the patient to be treated. The polynucleotides maybe naked nucleotide sequences or be in combination with cationic lipids, polymers or targeting systems. The polynucleotides may be delivered by any available technique. For example, the polynucleotide may be introduced by needle injection, preferably intradermally, subcutaneously or intramuscularly. Alternatively, the polynucleotide may be delivered directly across the skin using a polynucleotide delivery device such as particle-mediated gene delivery. The polynucleotide may be administered topically to the skin, or to mucosal surfaces for example by intranasal, oral, intravaginal or intrarectal administration.

Uptake of polynucleotide constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of these agents includes cationic agents, for example, calcium phosphate and DEAE-Dextran and lipofectants, for example, lipofectam and transfectam. The dosage of the nucleic acid to be administered can be altered. Typically the polynucleotide is administered in the range of 1pg to 1mg, preferably to 1pg to 10μg nucleic acid for particle mediated gene delivery and 10μg to 1mg for other routes.

The IL-1β antagonist may be employed alone as part of a composition, such as but not limited to a pharmaceutical composition or a vaccine composition or an immunotherapeutic composition to prevent and/or treat a LSD.

Combination therapies

An IL-1β antagonist may be used in combination with one or more other therapies intended prevent and/or to treat the LSD in the same patient. By a combination is meant that the therapies may be administered simultaneously, in a combined or separate form, to a patient. The therapies may be administered separately or sequentially to a patient as part of the same therapeutic regimen. The other therapy may be a general therapy aimed at treating or improving the condition of a patient with a LSD. For example, treatment with methotrexate, glucocorticoids, salicylates, nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics, other DMARDs, aminosalicylates, corticosteroids, and/or immunomodulatory agents (e.g., 6-mercaptopurine and azathioprine) may be combined with an IL-1β antagonist. Data from animal models of some LSDs have show modest efficacy of NSAIDs in increasing life span and improving neurological function.

The other therapy may be a specific treatment directed at the particular disease or condition suffered by the patient, or directed at a particular symptom of such a disease or condition. For example, where the patient has rheumatoid arthritis, the treatment may comprise treatment with an IL-1β antagonist, and also treatment with a further therapy specifically intended to treat, prevent or reduce the symptoms of the LSD.

Kit

Also provided is a kit that comprises a means for diagnosing or prognosing a LSD and an IL-1β antagonist. In particular, such means may include a specific binding agent, probe, primer, pair or combination of primers, enzyme, or antibody, including an antibody fragment, as defined herein which is capable of detecting or aiding in the detection of a LSD, as defined herein. The kit may comprise LysoTracker®, which is a fluorescent marker and is commercially-available from both Invitrogen and also Lonza. The LysoTracker® may be blue, blue-white, yellow, green or red. The chemical structures of LysoTracker® blue, green and red are shown in Figure 8.

The kit also comprises an IL-1β antagonist. The kit may contain any of the IL-1β antagonists defined above. The kit may further comprise buffers or aqueous solutions. The kit may further comprise instructions for using the IL-1β antagonist in a method of the invention.

The invention is illustrated by the following Example:

Example

Materials & Methods

Animals

A breeding colony of *hexb* mice was maintained as heterozygote breeding pairs and affected ($hexb^{-/-}$) animals were identified using an established PCR genotyping method. Cohorts (5-10 animals) of age (3 week) and sex matched $hexb^{-/-}$ mice and appropriate genotype ($hexb^{+/-}$) controls were set up. All procedures were conducted according to Animals (Scientific Procedures) Act 1986 under a valid Home Office License. Life span was determined and disease progression monitored in all experimental groups until the humane end point was reached, defined as being when the affected mice were unable to right themselves within 30 seconds when laid on their side, as designated in the Home Office License. In untreated $hexb^{-/-}$ animals this occurred at ~ 15 weeks of age.

Isolation and culture of peritoneal macrophages

Peritoneal macrophages from adult wild type and *hexb*$^{-/-}$ mice were obtained by peritoneal lavage, centrifuged, resuspended in RPMI-1640 supplemented with 10% (v/v) fetal bovine serum, 1% penicillin/streptomycin and 1% glutamine and plated into 24 well tissue culture plates and cultured at 37°C, 5% CO_2.

Treatments, processing of supernatants and western blotting

Culture medium was replaced with either Optimem (Life technologies) alone or primed with Optimem containing 200 ng/ml lipopolysaccharide (from *Equus abortus*) and incubated for 6 hr. Where indicated, LPS containing supernatants were removed and replaced with Optimem containing 5 mM ATP and incubated for a further 60 mins. Supernatants were collected, proteins precipitated with methanol/ chloroform, resuspended and run on SDS-PAGE and then transferred to nylon membrane. Blots were incubated with goat anti-mouse IL-1b antibody (R&D Systems) and then HRP-conjugated anti-goat IgG. Blots were developed with chemiluminescent substrate (Thermo Scientific).

Measurement of cytokines by ELISA

Culture supernatants were generated as described above and levels of IL-1β or TNFα were measured using specific ELISAs (e-Bioscience) according to the manufacturer's instructions.

Drug treatments

Anakinra/Kineret or vehicle were administered to *hexb*$^{-/-}$ and control mice via a peripheral route and drug dosage was according to published rodent studies (and under advice from Biovitrum) at twice weekly intervals.

Behavioural Analyses

Mouse behavioural analyses were performed as described previously (Jeyakumar M. et al (2004) Ann Neurol 56(5); 642-649). Body weights of individual mice were recorded on a weekly basis. Two other quantitative measurements were used for quantifying loss of motor strength and coordination and so monitoring disease progression in Sandhoff mice. The first was rearing analysis. The second was bar crossing analysis. These behavioral assessments were performed on individual animals at weekly intervals.

Example 1- Aberrant signalling for activation IL-1β in Sandhoff disease mice when compared with wild-type mice

Sandhoff disease, a lysosomal storage disease that is caused by mutations in the hexosaminidase B protein is characterized by inflammation that promotes disease progression. The pro-inflammatory cytokine IL-1β is generated through the activity of a cellular protein complex called the inflammasome. Generation of bioactive IL-1β requires two signals. Signal 1 (e.g. lipopolysaccharide from Gram negative bacteria) causes the generation of pro-proteins such as pro-IL-1β (the cytokine) and pro-caspase-1 (an enzyme). These pro-proteins are not biologically active. A second signal (signal 2 e.g. ATP) triggers activation of the protein complex that causes auto-cleavage of pro-caspase1 to generate active caspase-1, which then acts on pro-IL-1β to generate active IL-1β, which is secreted by the cell.

Western blots of either wild type macrophages or macrophages taken from Sandhoff disease mice (*hexb*$^{-/-}$) demonstrate that whilst wild type cells require exposure to the two signals (lane 3 in left hand panels) to produce active IL-1β and caspase-1, Sandhoff disease cells require only a single signal – LPS alone (lane 2 in right hand panels) to generate active IL-1β and caspase-1, Figure 1. These data indicate that Sandhoff disease cells produce active IL-1β via an aberrantly regulation.

Example 2- Impaired production of the pro-inflammatory cytokine IL-1β by macrophages from Niemann Pick Disease Type C (*NPC*$^{-/-}$) macrophages

Figure 2 shows impaired production of the pro-inflammatory cytokine IL-1β by macrophages from Niemann Pick Disease Type C (*NPC*$^{-/-}$) macrophages. Niemann Pick Disease Type C (NPC) is caused by mutations in the NPC protein and is characterized by inflammation that promotes disease progression. Production of IL-1β by wild type and NPC macrophages after exposure to LPS (from Gram negative bacteria) and peptidoglycan (Gram positive bacteria) was investigated. IL-1β production was measured using a specific ELISA.

Whilst LPS plus ATP or peptidoglycan plus ATP triggered generation of IL-1β, macrophages from *NPC*$^{-/-}$ mice produced significantly less when exposed to LPS (left panel) or peptidoglycan (right panel), independent of the dose of LPS or peptidoglycan.

Example 3- NPC cells display impaired generation of IL-1β, but enhanced production of the pro-inflammatory cytokine TNF.

Further to Example 2 the effect of LPS on cells (a murine macrophage cell line J774) that had been pharmacologically treated with the drug U18666A to induce an NPC disease

phenotype was investigated. Figure 3 shows that NPC cells displayed impaired generation of IL-1β, but enhanced production of the pro-inflammatory cytokine TNF.

Figure 3A shows a western blot probed for IL-1β of J774 cells treated with or without U18666A and then exposed to different doses of LPS (signal 1) followed by ATP (signal 2). The western blot can resolve the bands that correspond to pro-IL-1β (p35) and mature IL-1β (p17). As can be seen, pharmacological induction of NPC disease resulted in significantly less production of both pro-IL-1β and mature IL-1β across a wide range of doses of LPS.

Figure 3B shows generation of IL-1β and TNF by J774 cells exposed to LPS and ATP measured by specific ELISA. The lower panel confirms the IL-1β data shown in the western blot with significantly impaired IL-1β production by U18666A treated cells exposed to LPS. The upper panel shows that unlike IL-1β, levels of the pro-inflammatory cytokine TNF produced by U18666A-treated J774 cells exposed to LPS are significantly greater than controls. This indicates that the impairment of IL-1β production by NPC disease cells is selective and there is not a generalised inhibition of pro-inflammatory cytokine production.

Example 4- Anakinra treated *hexb*$^{-/-}$ (Sandhoff mice) have an improved growth curve when compared with untreated *hexb*$^{-/-}$ (Sandhoff mice).

Untreated (PBS) mice had a shallow growth curve that never exceeded a maximal body weight of 20 grams, whereas Anakinra treated mice had an improved growth curve achieved a maximal body weight of 25 grams, Figure 4.

Example 5-Anakinra has a positive effect on rearing activity of *hexb*$^{-/-}$ (Sandhoff mice).

Rearing activity in mice is an indicator of muscle strength and motor coordination. Rearing is measured in two ways; assisted (against the cage wall) and unassisted (without support). Anakinra had a positive effect on unassisted rearing as shown by the number of rearing events over time, Figure 5.

Example 6- Effect of Anakinra on bar crossing of *hexb*$^{-/-}$ (Sandhoff mice).

Bar crossing is another functional test for muscle strength and motor coordination. There was no statistical difference between treated and untreated animals in this task. There was a trend towards delayed decline in function, Figure 6.

Example 7-Improved survival of Anakinra treated *hexb*$^{-/-}$ (Sandhoff mice).

Survival curves show a positive impact of Anakinra on life span, Figure 7.

References

Patterson *et al.*, (2006) Rev. Neurol. **43**, 8

Grell *et al.,* (1998), Eur. J. Immunol. **28**, 257-263

Altschul *et al.*, (1990) J Mol Biol. **215**, 403-410

Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA **89**, 10915-10919

Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA **90**, 5873-5787

Maddox *et al*, (1993) J. Exp. Med. **158**, 1211-1226

Kohler and Milstein (1975) Nature **256**, 495-497

Zettlitz *et al.* (2010) MAbs **2**, 639-647

Jeyakumar M. et al (2004) Ann Neurol **56**, 642-649

CLAIMS

1. A method for preventing or treating a lysosomal storage disorder (LSD) in a patient, wherein the method comprises administering to the patient an Interleukin 1β (IL-1β) antagonist and thereby treating the LSD.

2. The method according to claim 1, wherein the IL-1β antagonist comprises a small molecule, a polypeptide, a peptide or peptidomimetic, a polynucleotide, an oligonucleotide, an antisense RNA, small interfering RNA (siRNA) or small hairpin RNA (shRNA), an aptamer, or an antibody or antibody fragment.

3. The method according to claim 1 or 2, wherein the IL-1β antagonist reduces the activity of IL-1β and/or Interleukin 1β Receptor (IL-1βR) and/or Interleukin 1β Receptor Accessory Protein (IL-1βRA), or any allelic variant thereof.

4. The method according to claim 2 or 3, wherein the IL-1β antagonist comprises an antibody or antibody fragment which specifically binds to IL-1β, IL-1βR, IL-1βRAP or any allelic variant thereof.

5. The method according to claim 4, wherein IL-1β comprises the sequence as shown in SEQ ID NO: 3, wherein IL-1βR comprises the sequence as shown in SEQ ID NOs: 6 or 9 or wherein IL-1βRA comprises the sequence as shown in SEQ ID NOs: 14 or 17.

6. The method according to claim 4, wherein the antibody is a monoclonal antibody.

7. The method according to claim 6, wherein the monoclonal antibody is Canakinumab.

8. The method according to claim 2 or 3, wherein the IL-1β antagonist comprises a fragment of IL-1β, IL-1βR, IL-1βR type II, IL-1βRA and/or IL-1βRAP.

9. The method according to claim 8, wherein the IL-1β antagonist is Anakinra.

10. The method according to claim 8, wherein the IL-1β antagonist is Rilonacept.

11. The method according to claim 1 or 2, wherein the IL-1β antagonist decreases the expression of IL-1β and/or IL-1βR and/or IL-1βRA.

12. The method according to claim 11, wherein the IL-1β antagonist comprises:

(a) an oligonucleotide or polynucleotide which specifically hybridises to a part of SEQ ID NO: 2, SEQ ID NOs: 5 or 8, or SEQ ID NOs: 13 or 16 or any allelic variant thereof or (b) an oligonucleotide which comprises about 50 or fewer consecutive nucleotides from SEQ ID NO: 1, SEQ ID NOs: 4 or 7 or SEQ ID NOs: 12 or 15, or a variant thereof which has at least about 90% identity to SEQ ID NO: 1 or SEQ ID NOs: 4, 7, 12 or 15 over its entire sequence.

13. The method according to any one of the preceding claims, wherein the LSD is any of Niemann-Pick type C (NPC1), NPC2, Smith-Lemli-Opitz Syndrome (SLOS), an inborn error of cholesterol synthesis, Tangier disease, Pelizaeus-Merzbacher disease, the Neuronal Ceroid Lipofuscinoses, primary glycosphingolipidoses (i.e. Gaucher, Fabry, GM1, GM2 gangliosidoses, Krabbe and MLD), Farber disease and Multiple Sulphatase Deficiency.

14. The method according to any one of the preceding claims, wherein the method further comprises administering another therapy or agent intended to prevent or treat the LSD.

15. An IL-1β antagonist for use in a method for preventing or treating a LSD.

16. An agent for the prevention or treatment of a LSD, comprising an IL-1β antagonist as an active ingredient.

17. A kit for preventing or treating a LSD, comprising a means for diagnosing or prognosing a LSD and an IL-1β antagonist.

18. A kit according to claim 16, wherein the means is LysoTracker® .

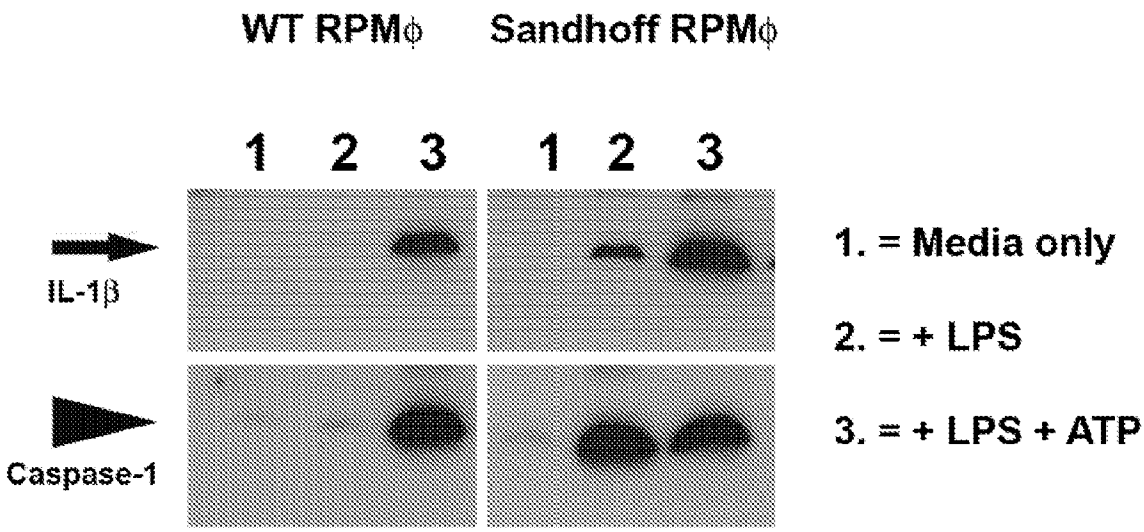

WT RPMϕ
Sandhoff RPMϕ
1 2 3 1 2 3
IL-1β
Caspase-1
1. = Media only
2. = + LPS
3. = + LPS + ATP

Figure 1

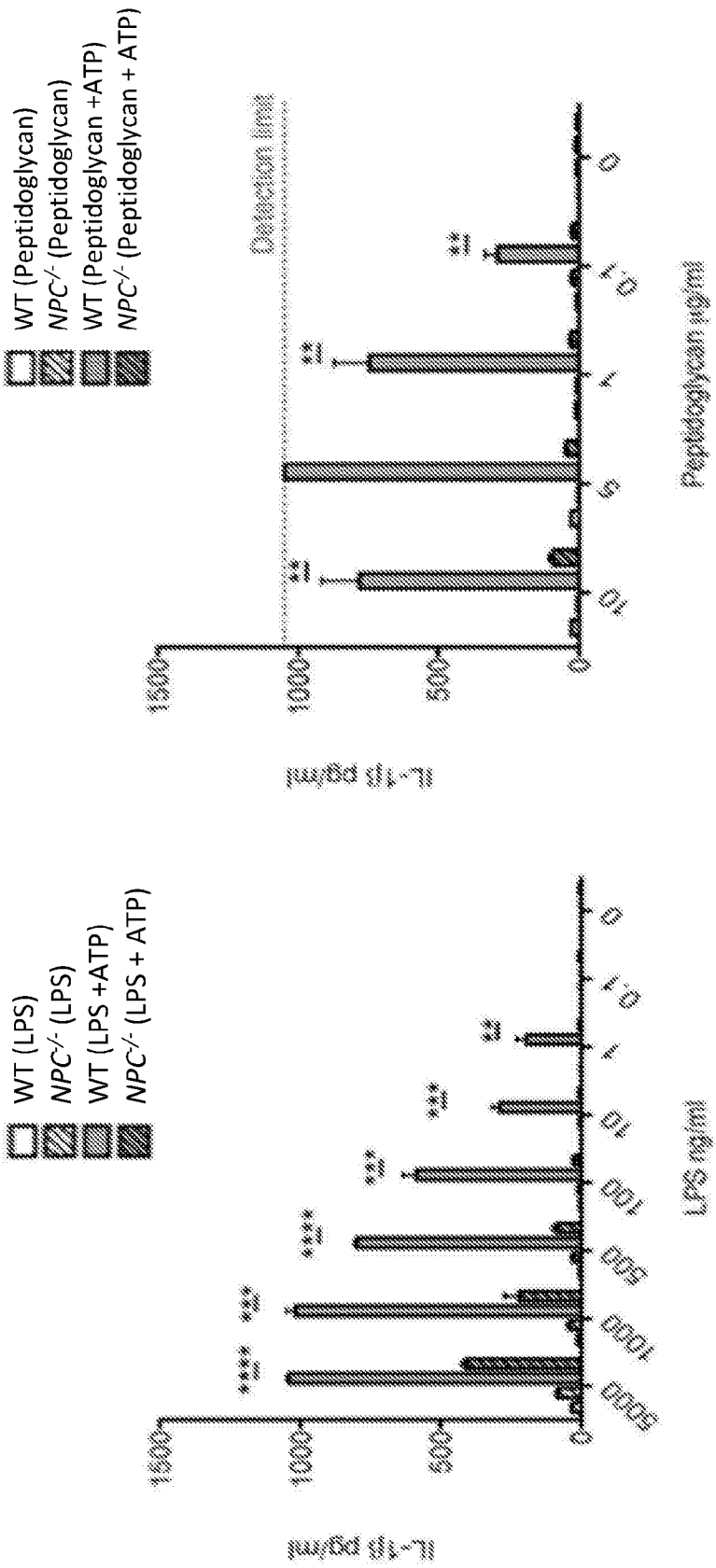

WT (Peptidoglycan)
NPC-/- (Peptidoglycan)
WT (Peptidoglycan +ATP)
NPC-/- (Peptidoglycan + ATP)
Detection limit
IL-1β pg/ml
1500
1000
500
0
Peptidoglycan µg/ml
0
0.1
1
5
10
WT (LPS)
NPC-/- (LPS)
WT (LPS +ATP)
NPC-/- (LPS + ATP)
LPS ng/ml
0
0.1
1
10
100
500
1000
1500

Figure 2

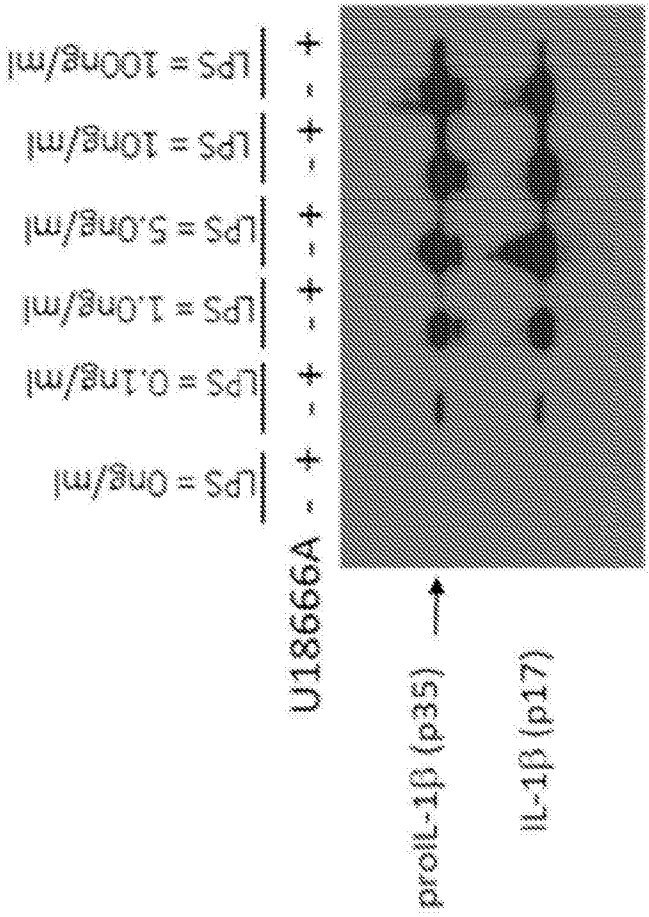

proIL-1β (p35)
IL-1β (p17)
U18666A - + - + - + - + - + - +
LPS = 0ng/ml
LPS = 0.1ng/ml
LPS = 1.0ng/ml
LPS = 5.0ng/ml
LPS = 10ng/ml
LPS = 100ng/ml

Figure 3A

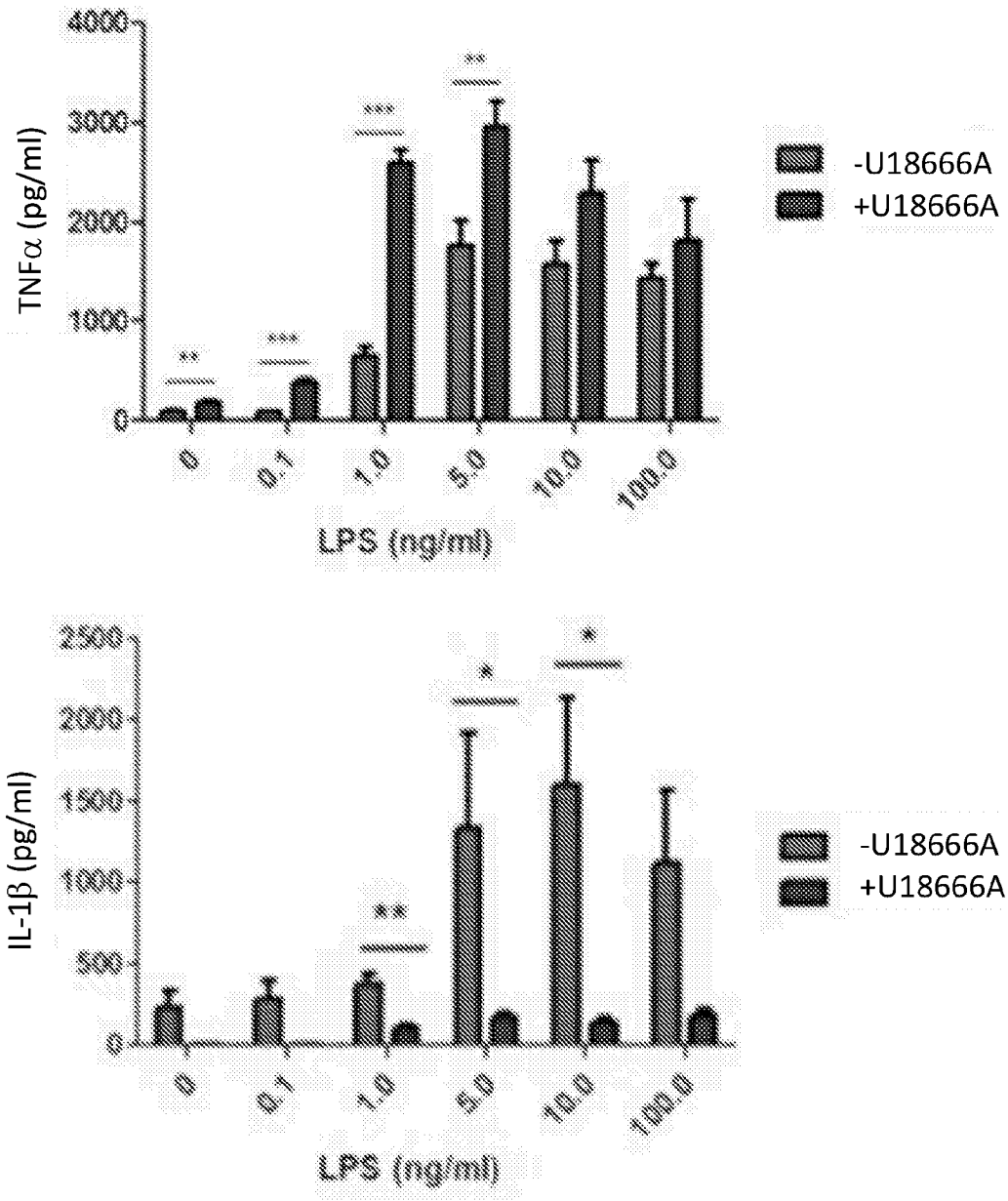

TNFα (pg/ml)
4000
3000
2000
1000
0
**
***
***
**
0
0.1
1.0
5.0
10.0
100.0
LPS (ng/ml)
-U18666A
+U18666A
IL-1β (pg/ml)
2500
2000
1500
1000
500
0
**
*
*
0
0.1
1.0
5.0
10.0
100.0
LPS (ng/ml)
-U18666A
+U18666A

Figure 3B

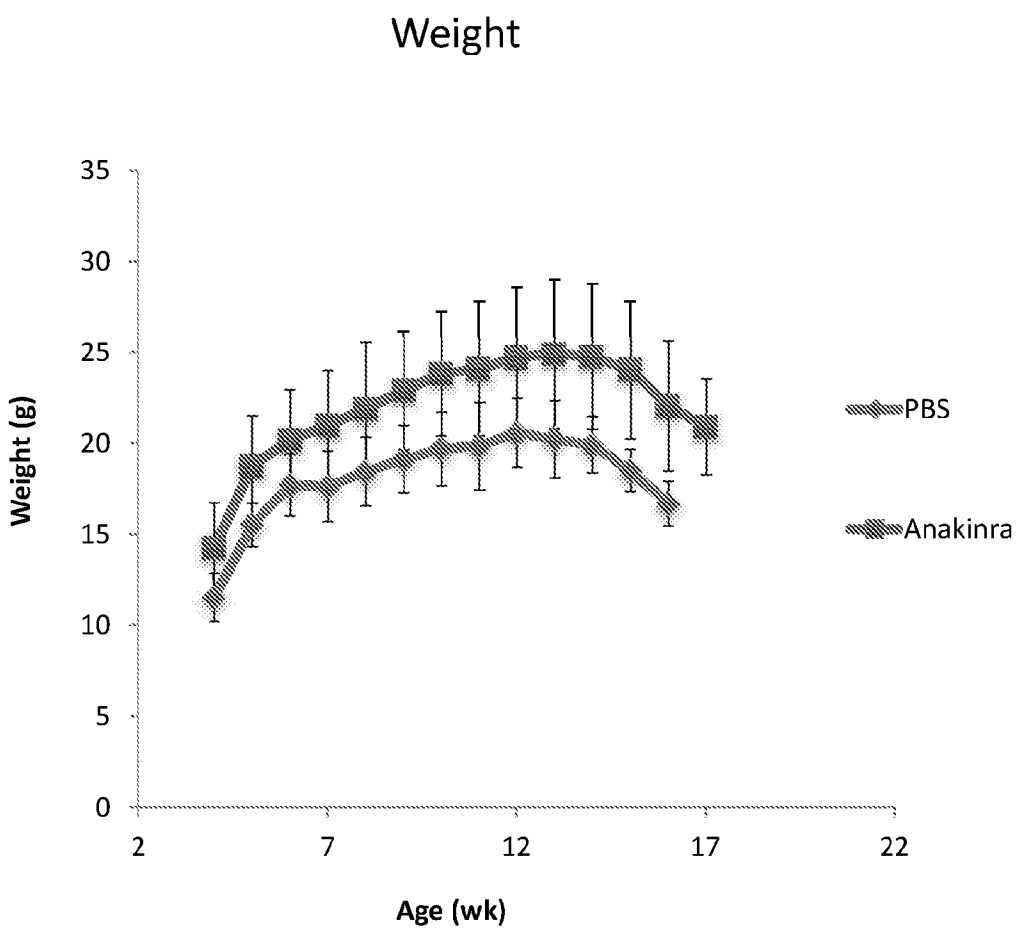
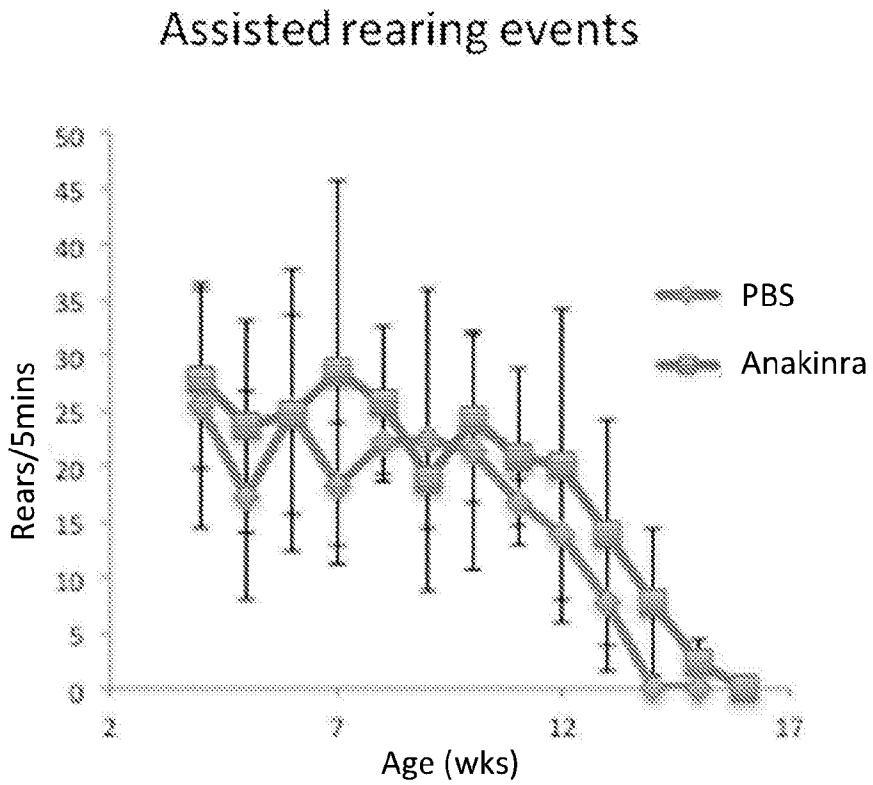

Weight
35
30
25
20
15
10
5
0
Weight (g)
2
7
12
17
22
Age (wk)
PBS
Anakinra

Figure 4


Assisted rearing events
Rears/5mins
50
45
40
35
30
25
20
15
10
5
0
2
7
12
17
Age (wks)
PBS
Anakinra

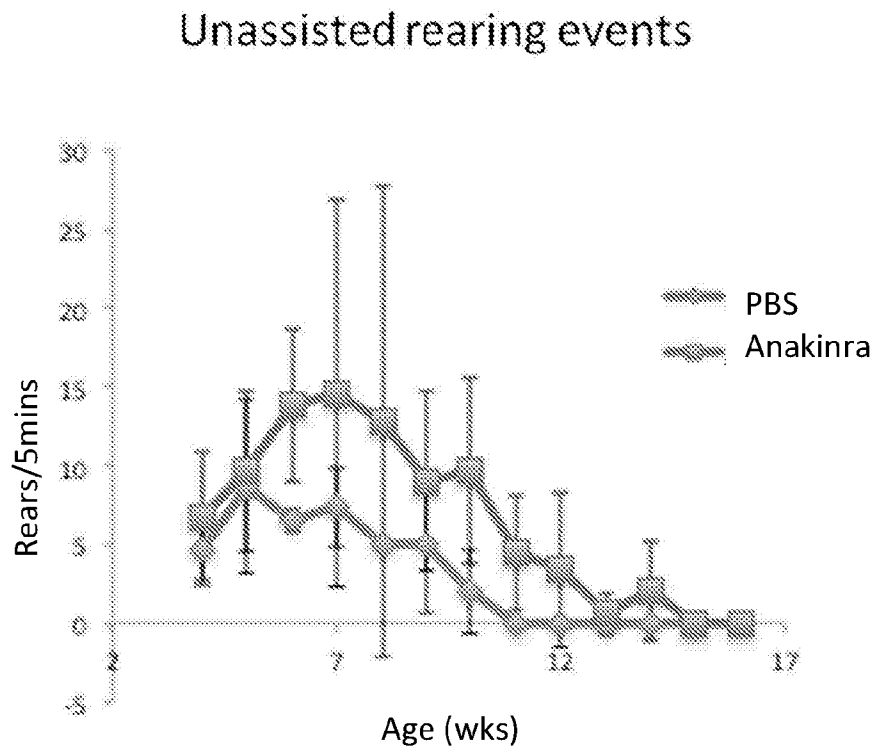

Unassisted rearing events
Rears/5mins
30
25
20
15
10
5
0
-5
2
7
12
17
Age (wks)
PBS
Anakinra

Figure 5

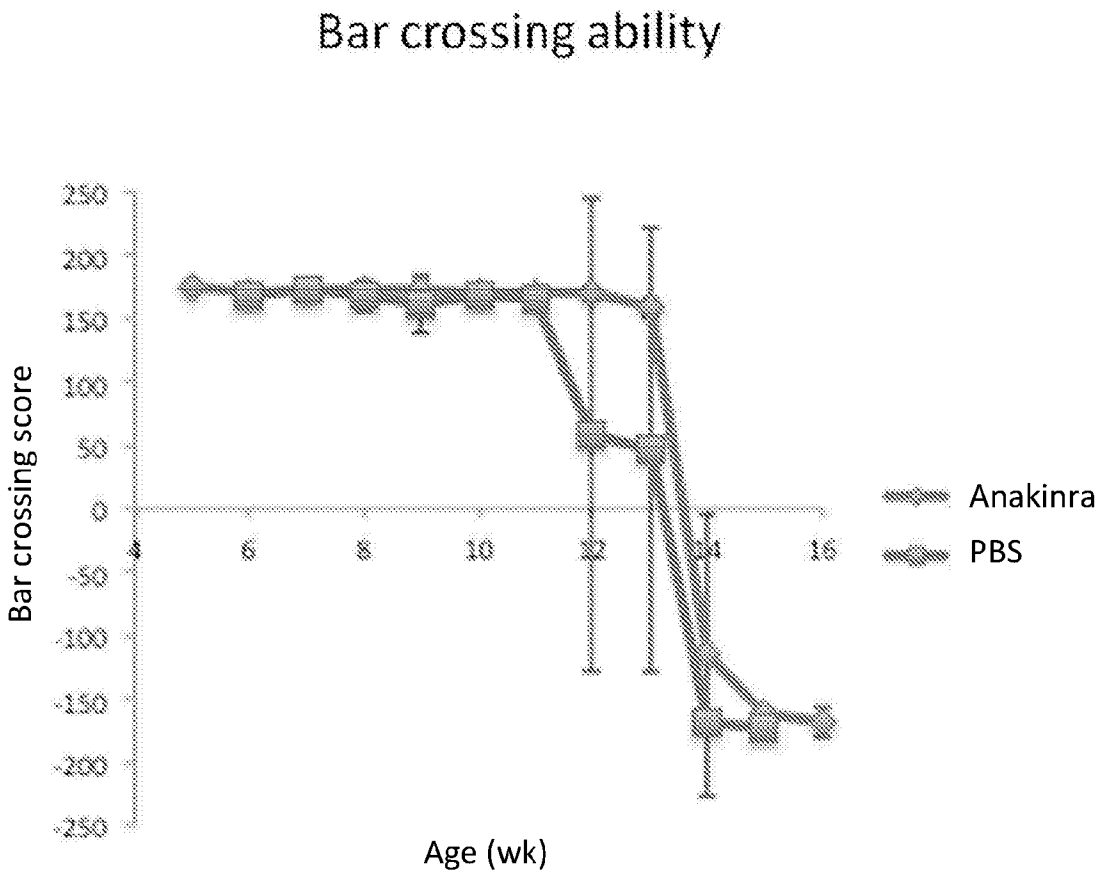

Bar crossing ability
Bar crossing score
250
200
150
100
50
0
-50
-100
-150
-200
-250
4
6
8
10
12
14
16
Age (wk)
Anakinra
PBS

Figure 6

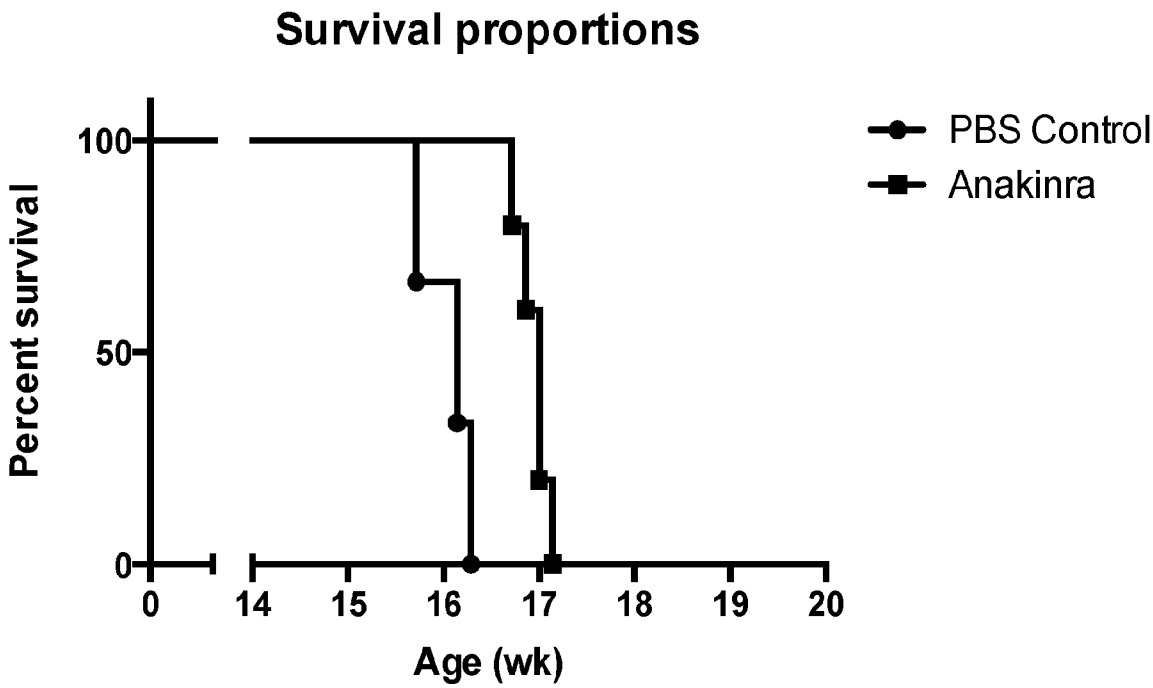
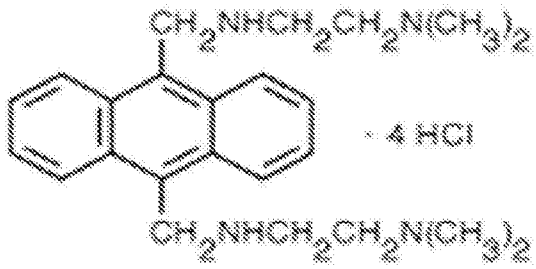

Survival proportions
Percent survival
100
50
0
0
14
15
16
17
18
19
20
Age (wk)
PBS Control
Anakinra

Figure 7

A


CH2NHCH2CH2N(CH3)2
· 4 HCl
CH2NHCH2CH2N(CH3)2

B


H3C
N
B
N
F
F
H3C
CH2CH2
C
O
NHCH2CH2N(CH3)2

C

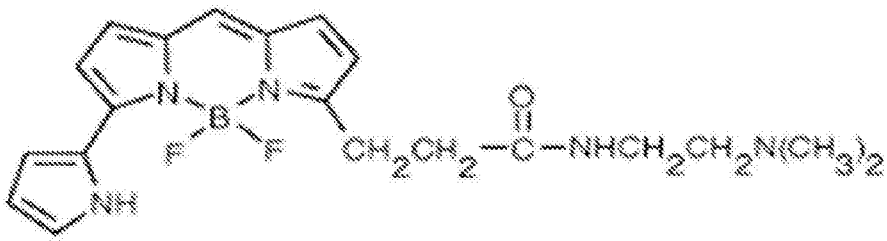

N
B
N
F
F
NH
CH2CH2
C
O
NHCH2CH2N(CH3)2

Figure 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/051979

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7088 A61K39/395 A61K38/20 A61P3/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/36152 A1 (AMGEN INC [US]) 10 May 2002 (2002-05-10) abstract paragraphs [0045], [0065], [0069], [0070], [0111] -----	1-18
X	WO 01/87328 A2 (IMMUNEX CORP [US]; SIMS JOHN E [US]; NEAL LARRY F O [US]; CONNOR TIMOT) 22 November 2001 (2001-11-22) abstract page 4, lines 15-18 page 7, line 14 ----- -/--	16

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
9 September 2015	23/10/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weisser, Dagmar

3

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. HOEGEN ET AL: "The NLRP3 Inflammasome Contributes to Brain Injury in Pneumococcal Meningitis and Is Activated through ATP-Dependent Lysosomal Cathepsin B Release", THE JOURNAL OF IMMUNOLOGY, vol. 187, no. 10, 15 November 2011 (2011-11-15), pages 5440-5451, XP055210888, ISSN: 0022-1767, DOI: 10.4049/jimmunol.1100790 abstract figure 2	16
A	SAFYAN ET AL.: "An association study of inflammatory cytokine gene polymorphisms in Fabry disease", EUROPEAN CYTOKINE NETWORK, vol. 17, no. 4, December 2006 (2006-12), pages 271-275, XP002744254, DOI: 10.1684/ecn.2006.0043 the whole document	1-18
A	XIONG ET AL.: "Microglia in juvenile neuronal ceroid lipofuscinosis are primed toward a pro-inflammatory phenotype", JOURNAL OF NEUROCHEMISTRY, vol. 127, no. 2, 22 August 2013 (2013-08-22), pages 245-258, XP002744255, DOI: 10.1111/jnc.12385 the whole document	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2015/051979

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0236152 A1	10-05-2002	AU 2017802 A	15-05-2002
		AU 2006228063 A1	02-11-2006
		CA 2425842 A1	10-05-2002
		EP 1330260 A1	30-07-2003
		ES 2484715 T3	12-08-2014
		JP 4516718 B2	04-08-2010
		JP 2004512373 A	22-04-2004
		JP 2008231106 A	02-10-2008
		JP 2012046519 A	08-03-2012
		JP 2014012742 A	23-01-2014
		JP 2015143284 A	06-08-2015
		MX PA03003808 A	28-07-2003
		US 2002077294 A1	20-06-2002
		US 2005106148 A1	19-05-2005
		US 2010303815 A1	02-12-2010
		WO 0236152 A1	10-05-2002
WO 0187328 A2	22-11-2001	AU 5975801 A	26-11-2001
		EP 1282435 A2	12-02-2003
		EP 1712239 A2	18-10-2006
		EP 2241328 A1	20-10-2010
		EP 2329842 A2	08-06-2011
		US 2001053764 A1	20-12-2001
		US 2004023869 A1	05-02-2004
		US 2009022733 A1	22-01-2009
		WO 0187328 A2	22-11-2001