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(54) Title: IL-18 BINDING PROTEIN (IL-18BP) IN INFLAMMATORY DISEASES

(57) Abstract: The present invention provides means and methods for treating Interleukin 18 (IL-18)- associated diseases and disorders. In particular, the present invention discloses antibodies specific for free IL-18 and IL-18 Binding Protein (IL-18BP) for use in such treatments and for the diagnosis of the indications.

IL-18 BINDING PROTEIN (IL-18BP) IN INFLAMMATORY DISEASES

TECHNICAL FIELD

The present invention provides means and methods for treating Interleukin 18 (IL-18)-associated diseases and disorders. In particular, the present invention discloses antibodies specific for free IL-18 and IL-18 Binding Protein (IL-18BP) for use in such treatments and for the diagnosis of the indications.

BACKGROUND ART

Interleukin-18 (IL-18), also known as interferon-gamma inducing factor is a cytokine, which is produced by activated macrophages, Kupffer cells and other cells. IL-18 binds to the IL-18 receptor and induces cell-mediated immunity. Defects (e.g. knock-out) of the IL-18 cytokine receptor or IL-18 cytokine lead to impaired natural killer (NK) cells activity and TH1 responses. Apart from its physiological role, IL-18 may also induce severe inflammatory disorders. For the purpose of early diagnosis of such disorders it therefore would be necessary to quantify the levels of free IL-18 in body fluids of a subject, expected to have such a disorder.

However, at present, the quantification of IL-18 levels in body fluids is usually performed by using ELISA assays, which comprise antibodies that are unspecific for the detection of free IL-18. The result achieved by ELISA assays is limited by the specificity of the used primary antibody, which binds the target antigen. Up to date it is merely possible to detect total IL-18 levels by using the commercially available antibodies, but no antibodies to free IL-18 are known so far. The detection of total IL-18 is inadequate for the assessment of free IL-18 levels, since IL-18 bound in a complex, e.g. bound to its natural antagonist IL-18 binding protein (IL-18BP) has a reduced affinity to IL-18 receptor. Further, it is known, that increased IL-18 levels often are associated with elevated IL-18BP levels.

In virtue of the reasons described above, the determination of total IL-18 it is insufficient to make an adequate diagnosis of IL-18 associated diseases. That means, in order to being able to assess the levels of free IL-18 in body fluids of a subject and to make an adequate diagnosis of IL-18 associated disease, a detection means would be required which specifically bind to free IL-18, but not to IL-18 bound in a complex. Accordingly, there exists at present no effective treatment for IL-18 associated diseases or disorders.

The present invention now provides such detection means in form of an IL-18 binding molecule, particularly an IL-18 binding protein (IL-18BP) or an antibody, which specifically binds to free IL-18, but not to IL-18 bound in a complex. Therefore, the present invention satisfies the need for an adequate means for the detection of free IL-18 and for diagnosis of diseases or disorders, which are associated with free IL-18 in the body fluids.

This opens the door for an efficient personalized medicine approach. In particular, it is now for the first time possible to identify the population of patients that are suffering from diseases or disorders, which are associated with free IL-18 in the body tissues, but particularly in the body fluids and to effectively treat said patients by administration of binding molecules which specifically bind free-IL18.

The present invention thus further provides effective therapeutic means for the treatment and prevention of IL-18 associated diseases or disorders in the population of patients that are suffering from diseases or disorders, which are associated with free IL-18 in the body tissues, but particularly in the body fluids. The present invention also satisfies the need for an effective treatment of diseases or disorders, which are associated with free IL-18 in the body tissues, particularly in the body fluids by providing IL-18 binding molecules, particularly (1) IL-18BP, and/or (2) antibodies, which are specific for free IL-18 and do not cross-react with IL-18 bound in a complex.

The IL-18 binding molecules, but particularly the IL-18BP and the IL-18 specific antibodies according to the present invention are able to reduce and/or abrogate the binding of free IL-18 to its receptor and to provide therapeutic benefits to patient suffering from an IL-18 associated disease or disorder.

Recent non-clinical and clinical investigations have defined a prominent role of the pro-inflammatory cytokine Interleukin 18 (IL-18) in the pathogenesis of Chronic Obstructive Pulmonary Disease, and suggest that IL-18 acts as the master regulator of destructive and remodeling processes.

Chronic obstructive pulmonary disease (COPD), also known as chronic obstructive lung disease (COLD), chronic obstructive airway disease (COAD), chronic airflow limitation (CAL) and chronic obstructive respiratory disease (CORD), is the occurrence of chronic bronchitis or emphysema, a pair of commonly co-existing diseases of the lungs in which the airways narrow over time. This leads to a limited airflow to and from the lung, which is considered as not fully reversible, but becomes rather becomes progressively worse over time. Smoking is responsible for 90% of COPD in the Europe and in the United States. Although not all tobacco smokers will develop COPD, it is estimated that 20% will. Smokers with COPD have higher death rates than nonsmokers with COPD. They also have more

frequent respiratory symptoms (coughing, shortness of breath, etc.) and more deterioration in lung function than non-smokers. Other risk factors are, for instance, genetic susceptibility (e.g. α1-anti-trypsin deficiency as well as regions on chromosome 4 near HHIP and in FAM13A and on chromosome 15 in CHRNA and IREB2), previous tuberculosis, air pollution, occupational exposure to dusts and fumes (airborne particles), exposure to second-hand smoke, and biomass smoke inhalation. COPD is comprised primarily of two related diseases: chronic bronchitis and emphysema. Chronic bronchitis is the inflammation and eventual scarring of the lining of the bronchial tubes. When the bronchi are inflamed and/or infected, less air is able to flow to and from the lungs and a heavy mucus or phlegm is coughed up. Emphysema begins with the destruction of alveoli (air sacs in the lungs where oxygen from the air is exchanged for carbon dioxide in the blood) due in part, by an abnormal inflammatory response of the lung to noxious particles or gases, chiefly tobacco smoke. The walls of the air sacs are thin and fragile. Damage to the air sacs is irreversible and results in permanent "holes" in the tissues of the lower lungs. As air sacs are destroyed, the lungs are able to transfer less and less oxygen to the bloodstream, causing shortness of breath. The lungs also lose their elasticity, which is important to keep airways open. As a result, the patient experiences great difficulty exhaling. In both chronic bronchitis and emphysema the obstruction and tissue destruction is generally permanent and progressive. COPD patients often experience exacerbations. The term "exacerbation" refers to the aggravation of the symptoms or an increase in the severity of the disease. The duration of an exacerbation can vary greatly - from hours to several days. Exacerbations may cause symptoms specific to the respiratory process to increase. The patient may experience increased dyspnea, a productive cough with an altered sputum, and fever. The sputum may increase or be more purulent and change color. The patient may also experience nonspecific symptoms such as malaise, fatigue, insomnia, sleepiness, or depression. Exacerbations of COPD are usually caused by an infection of the lower respiratory tract. The most common causes of infection are: aerobic Gram- positive and Gram-negative bacteria, atypical bacteria, respiratory virus, rhinovirus, influenza virus, RSV, or a combination of pathogens. Viral exacerbations are more severe, last longer, and are associated with greater levels of inflammation and loss of lung function than exacerbations due to other causes (Wedzicha, 2004, PATS; Seemungel et al., 2001, AM. J. RCCM; Tan et al., 2003, Am. J. Med. Donaldson et al., 2000, Thorax). Each COPD patient is likely to experience 1 to 4 exacerbations a year. While many patients experience these exacerbations, it is estimated that they only report about 50% of all episodes to physicians. Frequent exacerbations have been associated with a poor quality of life and a high economic burden.

Studies in COPD disease models have addressed IL-18 - induced pulmonary inflammation in cigarette smoke (CS)-induced and in second hand smoke-induced pulmonary emphysema and inflammation and its association with COPD in smokers, demonstrating that IL-18 and IL-18 signaling pathways via IL-18R are significantly activated by cigarette smoke exposure in animal models and in human lung inflammation and airspace enlargement in cigarette smoke-induced pulmonary emphysema. The results corroborate the important role of alveolar macrophages as the main cellular source of IL-18 release

IL-18 was shown to induce airway and vascular remodeling in lung-specific, inducible IL-18-transgenic mice as well as tissue inflammation, emphysema, mucus metaplasia, and cardiac right ventricle hypertrophy.

IL-18 was further shown to induce emphysema and the cytotoxic response via an IFNγ-dependent mechanism, fibrotic airway remodeling, mucus metaplasia, and vascular remodeling via an IL-17A- and IL-13-dependent pathway. There are important interactions between these pathways with IL-18- inducing IL-13 via an IL-17A–dependent mechanism and the IFNγ and the IL-17A/IL-13 responses counter-regulating one another. Consequently, IL-18 is central to the modulation of multiple inflammatory cascades

Systemic IL-18 levels in patients with COPD suggest alveolar macrophages as the source of circulating IL-18 in COPD and have shown that IL-18 is elevated in circulation and in induced sputum of COPD patients.

Elevated serum IL-18 levels in comorbidities to COPD are suggested to be associated with systemic inflammation.

Further, a strong correlation was found between serum IL-18 levels and lung function.

Overall, the non-clinical and clinical results clearly advocate for inhibiting/neutralizing IL-18 as a potential upstream target, thereby preventing or limiting both the destructive and remodeling processes typically leading to COPD disease manifestation and progression.

It can be concluded from the results of recent clinical investigations on IL-18 levels in circulation and in sputum of COPD patients, that IL-18 lung levels are significantly elevated in COPD patients in association with disease severity.

Data on IL-18 in sputum from patients with COPD suggests that alveolar macrophages are the predominant source of IL-18 in COPD and that IL-18 is significantly overexpressed in the lungs of COPD patients vs. controls.

Recent studies on the role of the NLRP3 inflammasome in stimulating caspase-1 activation followed by the release of the mature form of the pro-inflammatory cytokines IL-1b and IL-

18 have contributed to further elucidate the effects of tobacco smoke in airway inflammation (Rastrick et al., 2013).

In mice exposed to cigarette smoke twice daily, the caspase-1 activation and IL-18 release was examined (Eltom et al., 2011).

The smoke-induced up-regulation of IL-18 via caspase-1 activation is demonstrated by comparing the effects of tobacco smoke and the exposure to normal air.

Altogether, IL-18 presents as the favorite upstream target for future COPD therapeutics capable to interfere with the destructive and remodeling processes in COPD lungs, thereby being a promising candidate for a disease-modifying treatment modality in COPD.

It was therefore suggested in WO2008/150431 A1 to treat COPD and associated comorbidities resulting from elevation of IL-18, IFN-γ, or PKR in subjects suffering from COPD and associated comorbidities by administering to said subjects an IL- 18 Inhibitor, an IL-18Rα Inhibitor, and IFNγ Inhibitor, a PKR Inhibitor, and any combination thereof.

Inhibition of IL-18 by, for example, monoclonal antibodies, which target IL-18 signaling by receptor blockade leads to a long duration of action due the prolonged half-life of these agents, thereby acting not only on the deleterious IL-18 activities but also interfere with the beneficial effects for host defense thus leading to undesired side effects in terms of the response driven to pathogens (viruses, bacteria, fungi and other parasites) by IFN-gamma suppression and lymphocyte T helper type 1.

It was now surprisingly found within the context of the present invention that these undesired side effects can be avoided by taking an alternative approach, i.e., administration of the naturally occurring IL-18 Binding Protein (IL-18BP), which has a high binding affinity to Interleukin 18 (IL-18) and a fundamentally different mode of action of targeting IL-18 as compared, for example, to the art-known monoclonal antibodies.

In a specific embodiment of the invention, the IL-18 Binding Protein (IL-18BP) has a binding affinity of between 20 pM and 30 pM, when determined in a BIAcore setup as shown in Example 4.4.2.

The present invention thus relates in one embodiment to an IL-18 binding molecule, which specifically binds to free IL-18 without cross-reacting with IL-18 bound in a complex (referred to in the following as “free IL-18 specific binding molecule”), particularly a free IL-18 specific binding molecule which is an IL-18 inhibitor, which reduces and/or abrogates the binding of free IL-18 to its receptor (referred to in the following as “IL-18 inhibitor”), particularly an IL-18BP, for use in the treatment of an IL-18 associated disease or disorder in a subject diagnosed of having abnormal levels of free IL-18 and/or an abnormal ratio of

free IL-18/IL-18BP in the body fluids compared to the levels in body fluids of a healthy control subject.

In particular, said abnormal level of free IL-18 in the body fluids exceeds the level in body fluids of a healthy control subject by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%.

In another embodiment the present invention provides the free IL-18 specific binding molecule, particularly the IL-18 inhibitor, particularly the IL-18BP, for use according to the present invention and as disclosed herein, wherein said abnormal levels of free IL-18 in the body samples, particularly in body fluids, have been determined by use of an IL-18 binding molecule, particularly an IL-18 binding protein (IL-18BP) or an antibody, which specifically binds to free IL-18, but not to IL-18 bound in a complex according to the invention and as disclosed herein in certain embodiments.

Further, in one embodiment, the present invention provides the free IL-18 specific binding molecule, particularly the IL-18 inhibitor, particularly the IL-18BP, for use as disclosed in any one of the preceding embodiments, wherein the subject to be treated belongs to a group of subjects which have been determined to have elevated levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP in body samples, particularly in a sample selected from the group consisting of broncho-alveolar lavage fluid (BALF) circulation fluids, secretion fluids, biopsy and homogenized tissue, particularly serum, urine, tear, saliva, bile, sweat, exhalation, expiration, sputum, bronchoalveolar fluid, sebum, cellular, gland, mucosa, and tissue secretion compared to the levels in samples taken from healthy subjects.

Said elevated levels of free IL-18 in a sample form a diseased patient or subject are ≥5 pg/mL and up to10000 pg/mL or higher. In particular, said elevated levels of free IL-18 in a sample form a diseased patient or subject are in the range of ≥5 pg/mL to 10000 pg/mL, particularly in the range of 100 pg/mL to 10000 pg/mL, particularly in the range of 200 pg/mL to 10000 pg/mL, particularly in the range of 300 pg/mL to 10000 pg/mL, particularly in the range of 400 pg/mL to 10000 pg/mL, particularly in the range of 500 pg/mL to 10000 pg/mL, particularly in the range of 600 pg/mL to 10000 pg/mL, particularly in the range of 700 pg/mL to 10000 pg/mL, particularly in the range of 800 pg/mL to 10000 pg/mL, particularly in the range of 900 pg/mL to 10000 pg/mL, particularly in the range of 1000 to 10000 pg/mL, particularly in the range of 1500 pg/mL to 10000 pg/mL, particularly in the range of 2000 pg/mL to 10000 pg/mL, particularly in the range of 3000 pg/mL to 10000 pg/mL, particularly in the range of 4000 pg/mL to 10000 pg/mL, particularly in the range of

5000 pg/mL to 10000 pg/mL. The amount of free IL-18 in serum of healthy subject, particularly a healthy human is ≤5 pg/mL, particularly ≤4 pg/mL, particularly ≤1 pg/mL, particularly ≤0.5 pg/m, particularly below detection level.

Yet another object of the present invention is to provide the free IL-18 specific binding molecule, particularly the IL-18 inhibitor, particularly the IL-18BP, for use as disclosed in any one of the preceding embodiments, wherein said IL-18 associated disease or disorder is one selected from the group consisting of Adult Still's disease, juvenile Still's disease, chronic obstructive pulmonary disease (COPD), transfusion-related lung injury, bronchopulmonary dysplasia (BPD), adult respiratory distress syndrome (ARDS), interstitial lung disease (ILD), idiopathic pulmonary fibrosis, cystic fibrosis, pulmonary arterial hypertension, asthma, bronchiectasis, heart failure, amyotrophic lateral sclerosis (ALS), dry eye disease (DED), keratitis, corneal ulcer and abrasion, corneal neovascularization, pathological intraocular neovascularization, iritis, glaucoma, macular degeneration, Sjögren's syndrome, autoimmune uveitis, Behçet's disease, conjunctivitis, allergic conjunctivitis, dermatitis of eyelid, diabetes type 2, non-alcoholic fatty liver disease (NAFLD), steato hepatitis, solid organ and hematologic transplantation, ischemia reperfusion injury, familial Mediterranean fever, tumor necrosis factor receptor 1-associated periodic syndromes, cryopyrin-associated periodic fever syndromes, hyper-IgD syndromes, gout, Schnitzler syndrome, Wegener's granulomatosis also called granulomatosis with polyangitis (GPA), Hashimoto's thyroiditis, Crohn's disease, ulcerative colitis, immunoglobulin-4 (IgG4)-related diseases and stem cell therapies.

In a particular embodiment, the present invention provides the free IL-18 specific binding molecule, particularly the IL-18 inhibitor, particularly the IL-18BP, for use as disclosed in any one of the preceding embodiments, wherein said IL-18 associated disease or disorder is induced by smoking or second-hand smoke exposure, in particular tobacco smoke exposure.

In another particular embodiment, the present invention provides the free IL-18 specific binding molecule, particularly the IL-18 inhibitor, particularly the IL-18BP, for use as disclosed in any one of the preceding embodiments, wherein said IL-18 associated disease or disorder is induced by viral infection.

Yet another object of the present invention is to provide the free IL-18 specific binding molecule, particularly the IL-18 inhibitor, particularly the IL-18BP, for use as disclosed in any one of the preceding embodiments, wherein said IL-18 associated disease or disorder is an IL-18 induced systemic manifestation of inflammation and associated comorbidities

selected from the group consisting of emphysema, tissue inflammation, tissue destruction, lung resection, disappearance of the vasculature, apoptosis of endothelial cells, mucos metaplasia, cardiac hypertrophy, decrease of VEGF in the lung tissue, pulmonary vessel loss, vessel muscularization, vascular remodeling, collagen deposition, aberrant elastin layers in the lung, fibrotic airway remodeling, airspace enlargement, chronic remodeling of the airways and pulmonary vessels and decreased pulmonary function.

Another object of the present invention is to provide a free IL-18 specific binding molecule, particularly an IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, which is an antibody, particularly an antibody specific for free IL-18, particularly an antagonistic antibody, which prevents binding of free IL-18 to IL-18 receptor, especially free IL-18 binding to IL-18Rα.

The IL-18 specific antibody according to the present invention including any functionally equivalent antibody or parts thereof, binds to IL-18 at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, or is a conformational antibody, which binds to at least two epitopes on the IL-18 molecule, which are comprised of discontinuous amino acids that come together in three-dimensional conformation and interact with the receptor's paratope such that the binding site of IL-18BP on the IL-18 molecule is blocked.

In one embodiment, the IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to the present invention and as disclosed herein in the various embodiments binds free IL-18 protein, but not IL-18/IL-18BP complexes.

In particular, the IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to the present invention shows cross-reactivity with the IL-18/IL-18BP complex of between ≤0.01% and ≤0.05%, particularly of between ≤0.1% and ≤0.2%, particularly between ≤0.2% and ≤0.5%, particularly of between ≤0.5% and ≤1%, particularly of between ≤1% and ≤2% as determined by competitive ELISA.

In a specific embodiment, the IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to the present invention shows cross-reactivity with the IL-18/IL-18BP complex of ≤0.1% as determined by competitive ELISA.

In one embodiment, the IL-18 specific antibody including any functionally equivalent antibody or parts thereof as disclosed in any one of the preceding embodiments sterically hinders the binding of IL-18BP to IL-18.

In still another embodiment, the IL-18 specific antibody including any functionally equivalent antibody or parts thereof as disclosed in any one of the preceding embodiments specifically

binds to a single epitope, a combination of two epitopes or a combination of 3 epitopes comprised in a sequence selected from a group of sequences depicted in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, respectively.

In a specific embodiment, the antibody of the invention specifically binds to a single epitope, comprised in a sequence selected from a group of sequences depicted in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

In another specific embodiment, the antibody of the invention specifically binds to two epitopes, comprised in a sequence selected from a group of sequences depicted in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, respectively.

In particular, the antibody binds to two epitopes comprised in a sequence of (a) SEQ ID NO: 1 and SEQ ID NO: 2, respectively, (b) SEQ ID NO: 1 and SEQ ID NO: 3, respectively, (c) SEQ ID NO: 2 and SEQ ID NO: 3, respectively.

In another specific embodiment, the antibody of the invention specifically binds to three epitopes, comprised in a sequence of SEQ ID NO:1, SEQ ID NO: 2 and SEQ ID NO: 3, respectively.

In one embodiment, the present invention provides the IL-18 specific antibody including any functionally equivalent antibody or parts thereof as disclosed in any one of the preceding embodiments, which antibody specifically binds to a single epitope, a combination of two epitopes or a combination of 3 epitopes selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

In particular, the present invention further relates to said IL-18 specific antibody including any functionally equivalent antibody or parts thereof, wherein said epitope has a sequence which has 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the sequence depicted in SEQ ID NO: 4, SEQ ID NO:5 or SEQ ID NO: 6.

In a specific embodiment, the antibody of the invention specifically binds to a single epitope, comprised in a sequence selected from a group of sequences depicted in SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

In another specific embodiment, the antibody of the invention specifically binds to two epitopes, comprised in a sequence selected from a group of sequences depicted in SEQ ID NO:4, SEQ ID NO: 5 and SEQ ID NO: 6.

In particular, the antibody binds to two epitopes comprised in a sequence of (a) SEQ ID NO: 4 and SEQ ID NO: 5, respectively, (b) SEQ ID NO: 4 and SEQ ID NO: 6, respectively, (c) SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

In another specific embodiment, the antibody of the invention specifically binds to three epitopes, comprised in a sequence of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

In a specific embodiment, the antibody according to the present invention as disclosed herein in the various embodiments including any functionally equivalent antibody or an antigen-binding portion thereof comprises at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NOs: 10, 12, 14, 16, 17, 19, 22, 24 and 26, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NOs: 9, 11, 13, 15, 18, 20, 21, 23, and 25, respectively , wherein said antibody, equivalent antibody or antigen-binding portion thereof binds free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but does not bind IL-18/IL-18BP complexes.

In particular, the antibody according to the present invention as disclosed herein in the various embodiments including any functionally equivalent antibody or an antigen-binding portion thereof comprises the complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NOs: 10, 12, 14, 16, 17, 19, 22, 24 and 26, respectively, and the complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NOs: 9, 11, 13, 15, 18, 20, 21, 23, and 25, respectively, wherein said antibody, equivalent antibody or antigen-binding portion thereof binds free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but does not bind IL-18/IL-18BP complexes to any significant extent.

In a specific embodiment, the antibody according to the present invention as disclosed herein in the various embodiments including any functionally equivalent antibody or an antigen-binding portion thereof comprises at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in Figure 11, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in Figure 11, wherein said antibody, equivalent antibody or antigen-binding portion thereof binds free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but does not bind IL-18/IL-18BP complexes.

In particular, the antibody according to the present invention as disclosed herein in the various embodiments including any functionally equivalent antibody or an antigen-binding portion thereof comprises the complementarity determining regions (CDRs) of the light chain variable region as shown in as shown in Figure 11, and the complementarity determining regions (CDRs) of the heavy chain variable region as shown in as shown in Figure 11, wherein said antibody, equivalent antibody or antigen-binding portion thereof binds free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but does not bind IL-18/IL-18BP complexes to any significant extent.

In a specific embodiment of the invention, the complementarity determining regions (CDRs) are determined according to the variable domain residue numbering as in Kabat.

In another specific embodiment of the invention, the complementarity determining regions (CDRs) are determined according to the variable domain residue numbering as in Chothia.

In another specific embodiment of the invention, the complementarity determining regions (CDRs) are determined by the IMGT system..

The antibody according to the present invention as disclosed herein in the various embodiments including any functionally equivalent antibody or an antigen-binding portion thereof comprises the complementarity determining regions (CDRs) as follows:

a. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32, respectively; or

b. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 33, SEQ ID NO: 34, and SEQ ID NO: 35, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38, respectively; or

c. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 39, SEQ ID NO: 40, and SEQ ID NO: 41, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 42, SEQ ID NO: 43, and SEQ ID NO: 44, respectively; or

d. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 48, SEQ ID NO: 49, and SEQ ID NO: 50, respectively; or

e. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 51, SEQ ID NO: 52, and SEQ ID NO: 53, respectively; or

f. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 57, SEQ ID NO: 58, and SEQ ID NO: 59, respectively; or

g. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 60, SEQ ID NO: 61, and SEQ ID NO: 62, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 66, SEQ ID NO: 67, and SEQ ID NO: 68, respectively; or

h. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 63, SEQ ID NO: 64, and SEQ ID NO: 65, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 66, SEQ ID NO: 67, and SEQ ID NO: 68, respectively; or

i. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 69, SEQ ID NO: 70, and SEQ ID NO: 71, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 72, SEQ ID NO: 73, and SEQ ID NO: 74, respectively; or

j. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 75, SEQ ID NO: 78, and SEQ ID NO: 77, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 78, SEQ ID NO: 79, and SEQ ID NO: 80, respectively.

k. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in figure 11; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in Figure 11.

In one embodiment of the invention, the antibody shows variation in one or more of the CDR sequences to an extent that the antibody incorporating said variant CDRs still has the specific binding activity of an antibody according to the present invention comprising binding of free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, without binding IL-18/IL-18BP complexes.

In another specific embodiment of the invention, said antibody is a human or humanized antibody, in particular a human or humanized antibody, wherein the CDRs have been inserted into a human antibody "scaffold" being derived from one (or more) human immunoglobulin(s).

In still another specific embodiment, the invention provides an antibody including any functionally equivalent antibody or an antigen-binding portion thereof comprising at least a light chain variable region having 75%, 80%, 82%, 83%,84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the sequence shown in SEQ ID NOs: 10, 12, 14, 16, 17, 19, 22, 24, 26, and figure 11, respectively and/or at least a heavy chain variable region having 75%, 80%, 82%, 83%,84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the sequence shown in SEQ ID NOs: 9, 11, 13, 15, 18, 20, 21, 23, 25 and figure 11, respectively, wherein said antibody, equivalent antibody or antigen-binding portion thereof binds free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but does not bind IL-18/IL-18BP complexes.

In still another specific embodiment, the invention provides an antibody including any functionally equivalent antibody or an antigen-binding portion thereof comprising at least a light chain variable region having 75%, 80%, 82%, 83%,84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the sequence shown in SEQ ID NOs: 10, 12, 14, 16, 17, 19, 22, 24. 26, and figure 11, respectively, and/or at least a heavy chain variable region having 75%, 80%, 82%, 83%,84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the sequence shown in SEQ ID NOs: 9, 11, 13, 15, 18, 20, 21, 23, 25 and figure 11, respectively, with the proviso that the sequences of the CDRs of the light chain variable region and/or of the heavy chain variable region remain unchanged

and wherein said antibody, equivalent antibody or antigen-binding portion thereof binds free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but does not bind IL-18/IL-18BP complexes.

In one embodiment, the present invention provides the IL-18 specific antibody including any functionally equivalent antibody or parts thereof for use as disclosed in any one of the preceding embodiments, wherein said antibody or part thereof is a monoclonal antibody or a polyclonal antibody.

In a particular embodiment, the present invention provides the IL-18 specific antibody including any functionally equivalent antibody or parts thereof for use as disclosed in any one of the preceding embodiments, wherein said antibody or part thereof is a chimeric, single chain, bispecific, simianized, human and humanized antibody.

In particular, said antibody is a humanized antibody, particularly a humanized antibody, wherein certain amino acids in the framework and constant domains of the heavy and light chain variable regions and/or the heavy and light chain constant regions have been mutated so as to avoid or abrogate an immune response in humans.

In particular, the IL-18 specific antibody including any functionally equivalent antibody or antigen-binding portion thereof according to the present invention shows cross-reactivity with the IL-18/IL-18BP complex of between ≤0.01% and ≤0.05%, particularly of between ≤0.1% and ≤0.2%, particularly between ≤0.2% and ≤0.5%, particularly of between ≤0.5% and ≤1%, particularly of between ≤1% and ≤2% as determined by competitive ELISA.

In a specific embodiment, the IL-18 specific antibody including any functionally equivalent antibody or antigen-binding portion thereof according to the present invention shows cross-reactivity with the IL-18/IL-18BP complex of ≤0.1% as determined by competitive ELISA.

In another particular embodiment the present invention provides the IL-18 specific antibody including any functionally equivalent antibody or parts thereof as disclosed in any one of the preceding embodiments, wherein said antibody or part thereof binds to human IL-18.

Yet another object of the present invention is to provide the IL-18 specific antibody including any functionally equivalent antibody or parts thereof as disclosed in any one of the preceding embodiments, wherein binding of IL-18 to IL-18 receptor subunit alpha (IL-18Rα) and beta (IL-18Rβ), particularly binding to IL-18Rα is reduced by at least 5%, particularly by at least 10%, particularly by at least 15%, particularly by at least 20%, particularly by at least 25%, particularly by at least 30%, particularly by at least 40%, particularly by at least 45%, particularly by at least 50%, particularly by at least 55%, particularly by at least 60%,

particularly by at least 65%, particularly by at least 70, particularly by at least 75, particularly by at least 80, particularly by at least 85%, particularly by at least 90%, particularly by at least 95%, particularly by 96%, particularly by 97%, particularly by 98%, particularly by 99%,particularly by 100%.

A further object of the present invention is to provide the free IL-18 specific binding molecule, particularly the IL-18 inhibitor, particularly the IL-18BP, particularly the IL-18 specific antibody including any functionally equivalent antibody or parts thereof as disclosed in any one of the preceding embodiments for use in the treatment of an IL-18 associated disease or disorder in a population of subjects diagnosed of having abnormal levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP in body samples, particularly in body fluids, compared to the levels in body fluids of a healthy control subject, wherein said free IL-18 specific binding molecule, inhibitor, IL-18BP or IL-18 specific antibody or part thereof neutralizes the effect of free IL-18 by restricting or preventing IL-18 binding to IL-18 receptor (IL-18R), especially free IL-18 binding to IL-18Rα.

In one embodiment, the present invention provides the IL-18 specific antibody including any functionally equivalent antibody or parts thereof as disclosed in any one of the preceding embodiments, wherein said antibody or parts thereof

a) specifically binds to a single epitope, a combination of two epitopes or a combination of 3 epitopes comprised in a sequence selected from a group of sequences depicted in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3; and/or

b) specifically binds to an epitope, which has a sequence identity of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% to the sequence depicted in SEQ ID NO: 4, SEQ ID NO:5 or SEQ ID NO: 6; and

c) specifically binds to IL-18 at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP; and

d) specifically binds to free IL-18 protein, but not IL-18/IL-18BP complexes; and

e) sterically hinders the binding of IL-18BP to IL-18; and

f) reduces binding of IL-18 to IL-18 receptor, particularly binding to IL-18Rα by at least 5%, particularly by at least 10%, particularly by at least 15%, particularly by at least 20%, particularly by at least 25%, particularly by at least 30%, particularly by at least 40%, particularly by at least 45%, particularly by at least 50%, particularly by at least 55%, particularly by at least 60%, particularly by at least 65%, particularly by at least 70, particularly by at least 75, particularly by at

least 80, particularly by at least 85%, particularly by at least 90%, particularly by at least 95%, particularly by 100%.

In particular, the above specifically defined antibody shows a cross-reactivity with IL-18/IL-18BP complex of between 0.01% and ≤0.05%, particularly of between ≤0.1% and ≤0.2%, particularly between ≤0.2% and ≤0.5%, particularly of between ≤0.5% and ≤1%, particularly of between ≤1% and ≤2% as determined by competitive ELISA.

In certain embodiments of the invention, the IL-18BP and/or the free IL-18 specific antibody as disclosed in any one of the various embodiments can be used as an IL-18 inhibitor.

In certain other embodiment of the invention, the IL-18BP and/or the free IL-18 specific antibody as disclosed in any one of the various embodiments can be used as a capturing molecule, in an assay for detecting free IL-18 in a body sample, particularly in a sample selected from the group consisting of broncho-alveolar lavage fluid (BALF) circulation fluids, secretion fluids, biopsy and homogenized tissue, particularly serum, urine, tear, saliva, bile, sweat, exhalation, expiration, sputum, bronchoalveolar fluid, sebum, cellular, gland, mucosa, and tissue secretion.

In one embodiment, the invention relates to a polynucleotide encoding an antibody according to the invention as disclosed herein in the various embodiments.

In one embodiment, the polynucleotide encodes the variable heavy chain shown in SEQ ID NOs: 9, 11, 13, 15, 18, 20, 21, 23, 25 and figure 11 .

In one embodiment, the polynucleotide encodes the variable light chain shown in SEQ ID NOs: 10, 12, 14, 16, 17, 19, 22, 24. 26, and figure 11.

In one embodiment, the polynucleotide encodes the CDR regions as shown in SEQ ID NOs: 27 – 80.

In particular, the invention relates to a polynucleotide encoding the heavy chain and/or the light chain variable region of the antibody according to the invention having a sequence that has 75%, 80%, 82%, 83%,84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the sequence shown in figure 11.

Moreover, the present invention also provides the IL-18 inhibitor according to the present invention for use as disclosed in any one of the preceding embodiments, wherein the inhibitor is IL-18 Binding Protein (IL-18BP), particularly human IL-18BP (hIL-18 BP), particularly IL-18BP including any functionally equivalent or parts thereof, particularly an IL-18BP as shown in SEQ ID NO: 7.

Also included are transcript variants encoding the IL-18BP.

In one embodiment, the present invention provides the IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, wherein the inhibitor is an IL-18 Binding Protein (IL-18BP) which has a sequence identity of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% to the sequence depicted in SEQ ID NO: 7.

Yet another object of the present invention is to provide the IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, wherein treatment comprises prevention, halting, alleviation or reversion of symptoms associated with said disease or disorder.

In a further embodiment, the present invention provides the IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, wherein IL-18 binding is restricted or inhibited, particularly binding of free IL-18 to IL-18R, but especially binding of free IL-18 to IL-18Rα.

In another embodiment, the present invention provides the IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, wherein IL-18-dependent downstream signaling pathways are modified, particularly inhibited.

In still another embodiment, the present invention provides the IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, wherein increased expression of IFNγ, IL-13 or IL-17A is modified, particularly inhibited, compared to untreated subjects suffering from said disease or disorder.

It is still another object of the present invention to provide the IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, wherein the IL-18 inhibitor compensates the IL-18/IL-18BP imbalance by trapping the excess of free IL-18 in tissue and circulation.

In one embodiment, the present invention provides the IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, wherein the IL-18 inhibitor inhibits infiltration of neutrophils into the lung, particularly through mitigation of G-CSF release in the lung airways.

Yet another embodiment of the present invention is to provide the IL-18 inhibitor for use as disclosed in any one of the preceding embodiments, which is a full-length protein or a mutein, functional derivative, functional fragment, biologically active peptide, fraction, circularly permuted derivative, fused protein, isoform or a salt thereof.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of chronic obstructive pulmonary disease (COPD), heart disease, dry eye disease and/or diabetes type II.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of chronic obstructive pulmonary disease (COPD).

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of heart disease.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of dry eye disease.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of amyotrophic lateral sclerosis (ALS),

The present invention also provides the IL-18 inhibitor, particularly the antagonistic antibody, particularly the IL-18BP, as disclosed in any one of the preceding embodiments, for use in the treatment of diabetes type II.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of Adult Still's disease

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of transfusion-related lung injury.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of juvenile Still's disease.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of bronchopulmonary dysplasia (BPD).

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of acute respiratory distress syndrome (ARDS).

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of interstitial lung disease (ILD).

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of idiopathic pulmonary fibrosis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of cystic fibrosis.

The present invention also provides the IL-18 inhibitor, particularly the antagonistic antibody, particularly the IL-18BP, as disclosed in any one of the preceding embodiments, for use in the treatment of pulmonary arterial hypertension

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of asthma.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of bronchiectasis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of heart failure.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of keratitis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of corneal ulcer.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of corneal neovascularization.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of pathological intraocular neovascularization.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of iritis.

The present invention also provides the IL-18 inhibitor, particularly the antagonistic antibody, particularly the IL-18BP, as disclosed in any one of the preceding embodiments, for use in the treatment of glaucoma.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of macular degeneration.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of Sjögren's syndrome.

The present invention also provides the IL-18 inhibitor, particularly the antagonistic antibody, particularly the IL-18BP, as disclosed in any one of the preceding embodiments, for use in the treatment of autoimmune uveitis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of Behçet's disease.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of conjunctivitis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of dermatitis of eyelid.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of non-alcoholic fatty liver disease (NAFLD).

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of steato hepatitis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of solid organ and hematologic transplantation.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of ischemia reperfusion injury.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of familial Mediterranean fever.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of tumor necrosis factor receptor 1-associated periodic syndromes.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of cryopyrin-associated periodic fever syndromes.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of hyper-IgD syndromes.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of gout.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of Schnitzler syndrome.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of Wegener's granulomatosis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of Hashimoto's thyroiditis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of Crohn's disease.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of ulcerative colitis.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of immunoglobulin-4 (IgG4)-related diseases.

The present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use in the treatment of stem cell therapies.

In another embodiment, the present invention provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use as disclosed in any one of the preceding embodiments, wherein said disease or disorder is induced by smoking or second-hand smoke exposure, in particular tobacco smoke exposure.

In another embodiment, the present invention provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use as disclosed in any one of the preceding embodiments, wherein said disease or disorder is induced by viral infection.

Further, the present invention also provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use as disclosed in any one of the preceding embodiments, wherein said disease or disorder is an IL-18 induced systemic manifestation of inflammation and associated comorbidities selected from the group consisting of emphysema, tissue inflammation, tissue destruction, lung resection, disappearance of the vasculature, mucos

metaplasia, cardiac hypertrophy, decrease of VEGF in the lung tissue, pulmonary vessel loss, vessel muscularization, collagen deposition, aberrant elastin layers in the lung, fibrotic airway remodeling, airspace enlargement, chronic remodeling of the airways and pulmonary vessels and decreased pulmonary function.

Yet another object of the present invention is to provide the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use as disclosed in any one of the preceding embodiments, wherein IL-18 binding is restricted or inhibited, particularly binding of free IL-18 to IL-18R, but especially free IL-18 binding to IL-18Rα.

It is yet another object of the present invention to provide the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use as disclosed in any one of the preceding embodiments, wherein IL-18-dependent downstream signaling pathways are modified, particularly inhibited.

It is yet another object of the present invention to provide the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use as disclosed in any one of the preceding embodiments, wherein increased expression of IFNγ, IL-13 or IL-17A is modified, particularly inhibited, compared to untreated subjects suffering from said disease or disorder.

In one embodiment, the present invention provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use as disclosed in any one of the preceding embodiments, wherein the IL-18 inhibitor compensates the IL-18/IL-18BP imbalance by trapping the excess of free IL-18 in tissue and circulation.

In another embodiment, the present invention provides the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic antibody, as disclosed in any one of the preceding embodiments, for use as disclosed in any one of the preceding embodiments, wherein treatment comprises prevention, halting, alleviation or reversion of symptoms associated with said disease or disorder.

Further, the present invention also provides a pharmaceutical composition for use in the treatment of the disease or disorder as defined in any one of the preceding embodiments in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder as defined in any one of the preceding embodiments, wherein said composition comprises the IL-18 inhibitor, particularly the IL-18BP, particularly the

antagonistic antibody, as disclosed in any one of the preceding embodiments, particularly together with a pharmaceutically acceptable carrier and/or excipient, particularly in a prophylactically and/or therapeutically effective amount.

In particular, the present invention provides the pharmaceutical composition of the preceding embodiment, wherein said pharmaceutical composition optionally further provides another inhibitor of a pro-inflammatory cytokine or functional fragment thereof, or a regulatory factor, which induces in-situ expression of said inhibitor of pro-inflammatory cytokine or functional fragment thereof, co-therapeutic agents such as anti-inflammatory, bronchodilatory, antihistamine, decongestant or anti-tussive drug substances.

In one embodiment, the present invention provides the pharmaceutical composition as disclosed in any one of the preceding embodiments, comprising a pharmaceutically acceptable carrier and/or excipient.

In a specific embodiment, the present invention provides a pharmaceutical composition for use in the treatment of the disease or disorder as defined in any one of the preceding embodiments in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder as defined in any one of the preceding embodiments, wherein said composition comprises the Interleukin-18 Binding Protein (IL-18BP) as disclosed in one or more of the preceding embodiments, particularly together with a pharmaceutically acceptable carrier and/or excipient, particularly in a prophylactically and/or therapeutically effective amount.

In another specific embodiment, the present invention provides a pharmaceutical composition for use in the treatment of the disease or disorder as defined in any one of the preceding embodiments in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder as defined in any one of the preceding embodiments, wherein said composition comprises the antagonistic free IL-18 specific antibody as disclosed in any one of the preceding embodiments, particularly the antagonistic free IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to the present invention, which shows cross-reactivity with the IL-18/IL-18BP complex of ≤0.01% and ≤0.05%, particularly of between ≤0.1% and ≤0.2%, particularly between ≤0.2% and ≤0.5%, particularly of between ≤0.5% and ≤1%, particularly of between ≤1% and ≤2 as determined by competitive ELISA, particularly together with a pharmaceutically acceptable carrier and/or excipient, particularly in a prophylactically and/or therapeutically effective amount.

In a particular embodiment, the present invention provides the pharmaceutical composition of the preceding embodiment, wherein said composition optionally further provides another inhibitor of a pro-inflammatory cytokine or functional fragment thereof, or a regulatory factor, which induces in-situ expression of said inhibitor of pro-inflammatory cytokine or functional fragment thereof, co-therapeutic agents such as anti-inflammatory, bronchodilatory, antihistamine, decongestant or anti-tussive drug substances.

Yet another object of the present invention is to provide the pharmaceutical composition as disclosed in any one of the preceding embodiments, comprising a pharmaceutically acceptable carrier and/or excipient.

The present invention further discloses an expression vector comprising a coding sequence of the IL-18 inhibitor or an IL-18 antisense expressing vector as disclosed in any one of the preceding embodiments, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in the preceding embodiments leads to in situ expression of IL-18 inhibitor for use in the treatment of the disease or disorder as disclosed in any one of the preceding embodiments.

The present invention further discloses an expression vector comprising an IL-18 antisense expressing vector, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in the embodiments of the present invention, leads to in situ inhibition of the expression of IL-18 for use in the treatment of the disease or disorder as defined in any one of the preceding embodiments.

The present invention further discloses an expression vector comprising the coding sequence of a regulatory factor, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as disclosed in any one of the preceding embodiments, leads to in situ expression of said regulatory factor, which modulates upstream signaling pathways that control the expression of the IL-18 inhibitor as disclosed in any one of the preceding embodiments, particularly said regulatory factor induces the cellular expression of IL-18 inhibitor for use in the treatment of the disease or disorder as disclosed in any one of the preceding embodiments.

In particular, said expression vector as disclosed in any one of the preceding embodiments for use in the treatment of the disease or disorder as defined in any one of the preceding embodiment is administered to a subject suffering from such a disease or disorder as disclosed in any one of the preceding embodiments, or having a predisposition to develop such a disease or disorder, alone or in combination with the IL-18 inhibitor as disclosed in

any one of the preceding embodiments, the Interleukin-18 Binding Protein (IL-18BP) as disclosed in any one of the preceding embodiments or the pharmaceutical composition as disclosed in any one of the preceding embodiments.

The present invention further discloses an expression vector comprising the coding sequence of IL-18BP as disclosed in any one of the preceding embodiments, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in the preceding embodiments, leads to in situ expression of IL-18BP for use in the treatment of the disease or disorder as defined in any one of the preceding embodiments.

The present invention further discloses an expression vector comprising the coding sequence of a regulatory factor, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in any one of the preceding embodiments, leads to in situ expression of said regulatory factor, which modulates upstream signaling pathways that control the expression of the IL-18BP as disclosed in any one of the preceding embodiments, particularly said regulatory factor induces the cellular expression of IL-18BP for use in the treatment of the disease or disorder as defined in any one of the preceding embodiments.

In particular, said expression vector as disclosed in any one of the preceding embodiments for use in the treatment of the disease or disorder as defined in any one of the preceding embodiments is administered to a subject suffering from such a disease or disorder as defined in any one of the preceding embodiments, or having a predisposition to develop such a disease or disorder, alone or in combination with the IL-18 inhibitor as disclosed in any one of the preceding embodiments, the Interleukin-18 Binding Protein (IL-18BP) as disclosed in any one of the preceding embodiments or the pharmaceutical composition as disclosed in any one of the preceding embodiments.

Yet another object of the present invention is to provide the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic free IL-18 specific antibody, or the pharmaceutical composition comprising the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic free IL-18 specific antibody, or the expression vector, for use as disclosed in any one of the preceding embodiments, , wherein they are administered to a subject in a prophylactically and/or therapeutically effective amount by systemic, intranasal, intraocular, intravitral, eye drops, buccal, oral, transmucosal, intratracheal, intravenous, subcutaneous, intraurinary tract, intrarectal, intravaginal, sublingual, intrabronchial, intrapulmonary, transdermal or intramuscular administration, in particular broncho-pulmonary administration.

In particular, said subject is a mammal, particularly said subject is a human.

The present invention further relates to a method for treating the disease or disorder as defined in any one of the preceding embodiments in a subject suffering from such a disease or disorder, or having a predisposition to develop such a disease or disorder as defined in any one of the preceding embodiments, comprising administering to said subject a therapeutically or prophylactically effective amount of the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic free IL-18 specific antibody, or the pharmaceutical composition comprising the IL-18 inhibitor, particularly the IL-18BP, particularly the antagonistic free IL-18 specific antibody, or the expression vector, as disclosed in any one of the preceding embodiments,

The present invention further relates to a method of determining the amount of free IL-18 in a sample or *in situ* comprising detecting the specific binding of the free IL-18 specific binding molecule of any one of the preceding embodiments to free IL-18 protein in the sample or *in situ* which includes the steps of:

a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with of the free IL-18 specific binding molecule of any one of the preceding embodiments, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

b) allowing the free IL-18 specific binding molecule to bind to free IL-18;

c) detecting the binding of IL-18 to the free IL-18 specific binding molecule and determining the amount of free IL-18 in the sample.

In another embodiment, the invention provides a method of diagnosing the diseases or disorder as defined in any one of the preceding embodiments in a patient comprising detecting the specific binding of the free IL-18 specific binding molecule of any one of the preceding embodiments to free IL-18 protein in a sample or *in situ* which includes the steps of:

a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with of the free IL-18 specific binding molecule of any one of the preceding embodiments, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

b) allowing the free IL-18 specific binding molecule to bind to free IL-18;

c) detecting the binding of IL-18 to the free IL-18 specific binding molecule and determining the amount of free IL-18 in the sample.

d) comparing the amount of free IL-18 in the sample of the subject suffering from the diseases or disorder as defined in any one of the preceding embodiments to the amount in the sample of a healthy subject.

In still another embodiment, the invention provides a method for diagnosing a predisposition to the diseases or disorder as defined in any one of the preceding embodiments in a patient comprising detecting the specific binding of the free IL-18 specific binding molecule of any one of the preceding embodiments to free IL-18 protein in a sample or *in situ* which includes the steps of:

a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with of the free IL-18 specific binding molecule of any one of the preceding embodiments, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

b) allowing the free IL-18 specific binding molecule to bind to free IL-18;

c) detecting the binding of IL-18 to the free IL-18 specific binding molecule and determining the amount of free IL-18 in the sample.

d) comparing the amount of free IL-18 in the sample of the patient suffering from the diseases or disorder as defined in any one of the preceding embodiments to the amount in the sample of a healthy patient;

wherein an increase in the amount of said free-IL-18 in the sample compared to a normal control value obtained from a healthy patient indicates that said patient is suffering from or is at risk of developing a disease or disorder as defined in any one of the preceding embodiments.

Further comprised herein is a method for monitoring minimal residual disease in a patient following treatment with the IL-18 inhibitor, the pharmaceutical composition, or the expression vector of any one of the preceding embodiments, wherein said method comprises:

a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with of the free IL-18 specific binding molecule of any one of the preceding embodiments, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

b) allowing the free IL-18 specific binding molecule to bind to free IL-18;

c) detecting the binding of IL-18 to the free IL-18 specific binding molecule and determining the amount of free IL-18 in the sample.

d) comparing the amount of free IL-18 in the sample of the patient suffering from the diseases or disorder as defined in any one of the preceding embodiments to the amount in the sample of a healthy patient;

wherein an increase in the amount of said free-IL-18 in the sample compared to a normal control value obtained from a healthy patient indicates that said patient is still suffering from a minimal residual disease.

The invention further relates to a method for predicting responsiveness of a patient to a treatment with the IL-18 inhibitor, the pharmaceutical composition, or the expression vector of any one of the preceding embodiments, wherein said method comprises:

a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with of the free IL-18 specific binding molecule of any one of the preceding embodiments, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

b) allowing the free IL-18 specific binding molecule to bind to free IL-18;

c) detecting the binding of IL-18 to the free IL-18 specific binding molecule and determining the amount of free IL-18 in the sample.

d) comparing the amount of free IL-18 in the sample of the patient suffering from the diseases or disorder as defined in any one of the preceding embodiments to the amount in the sample of a healthy patient;

wherein a decrease in the amount of said free-IL-18 in the sample indicates that said patient has a high potential of being responsive to the treatment.

Any of the methods may comprise the additional step of using in step a) an IL-18BP specific binding molecule, which binds to a different site of IL-18BP than the capturing molecule, particularly wherein one of said molecules binds to the IL-18 binding site of IL-18BP.

Further, any of the above methods may further comprise the additional step of determining in the sample the presence of free IL-18BP by using in step a) an IL-18BP specific capturing molecule and an IL-18BP specific detection molecule, which binds to a different site of IL-18BP than the capturing molecule, particularly, wherein one of said IL-18BP specific molecules binds to the IL-18 binding site of IL-18BP, by determining in step c) the

amount of free and total IL-18 and of free and total IL-18BP bound to the capturing molecule in the sample; and by comparing in step d) the amount of free and/or total IL-18 and free and/or total IL-18BP in the sample of the patient suffering from the diseases or disorder as defined in any one of the preceding embodiments to the amount in the sample of a healthy patient.

The capturing molecule used in any of the above methods may be the free IL-18 specific binding molecule according to any one of the preceding embodiments, particularly the IL-18 BP as described herein or the free IL-binding antibody according to the invention and as described herein. .

The sample used in any of the above methods may be a sample selected from the group consisting of broncho-alveolar lavage fluid (BALF) circulation fluids, secretion fluids, biopsyand homogenized tissue, particularly serum, urine, tear, saliva, bile, sweat, exhalation, expiration, sputum, bronchoalveolar fluid, sebum, cellular, gland, mucosa and tissue secretion etc.

In one embodiment, the amount of free IL-18 in the sample of a subject, particularly a human, suffering from any of the diseases disclosed herein are ≥5 pg/mL and up to10000 pg/mL or higher. In particular, said elevated levels of free IL-18 in a sample form a diseased patient or subject are in the range of ≥5 pg/mL to 10000 pg/mL, particularly in the range of 100 pg/mL to 10000 pg/mL, particularly in the range of 200 pg/mL to 10000 pg/mL, particularly in the range of 300 pg/mL to 10000 pg/mL, particularly in the range of 400 pg/mL to 10000 pg/mL, particularly in the range of 500 pg/mL to 10000 pg/mL, particularly in the range of 600 pg/mL to 10000 pg/mL, particularly in the range of 700 pg/mL to 10000 pg/mL, particularly in the range of 800 pg/mL to 10000 pg/mL, particularly in the range of 900 pg/mL to 10000 pg/mL, particularly in the range of 1000 to 10000 pg/mL, particularly in the range of 1500 pg/mL to 10000 pg/mL, particularly in the range of 2000 pg/mL to 10000 pg/mL, particularly in the range of 3000 pg/mL to 10000 pg/mL, particularly in the range of 4000 pg/mL to 10000 pg/mL, particularly in the range of 5000 pg/mL to 10000 pg/mL. The amount of free IL-18 in serum of healthy subject, particularly a healthy human is ≤5 pg/mL, particularly ≤4 pg/mL, particularly ≤1 pg/mL, particularly ≤0.5 pg/m, particularly below detection level.

In particular, the amount of free IL-18 in isolated sample of a subject, particularly a human, suffering from any of the diseases disclosed herein are ≥5 pg/mL and, particularly, up to 10000 pg/mL, whereas the amount of free IL-18 in sample of a healthy subject, particularly a healthy human, is ≤4 pg/mL.

In another embodiment of the invention, a set of biomarkers is provided for use in any of the above detection method for further specifying the diseases or disorder as defined in any one of the preceding embodiments, for diagnosing a predisposition to the disease or disorder as defined in any one of the preceding embodiments, for monitoring minimal residual disease in a subject, or for predicting responsiveness of a subject to a treatment with IL-18 inhibitor, the pharmaceutical composition, or the expression vector of any one of the preceding embodiments comprising determining a biomarker profile and correlating the obtained profile with a specific disease or disorder.

In particular, the biomarker may be used in a method for diagnosis of the diseases or disorder as defined in any one of the preceding embodiments, for diagnosing a predisposition to the disease or disorder as defined in any one of the preceding embodiments or for monitoring minimal residual disease in a subject, or for predicting responsiveness of a subject to a treatment with IL-18 inhibitor as disclosed in any one of the preceding embodiments, the IL-18BP as disclosed in any one of the preceding embodiments or the pharmaceutical composition comprising IL-18 inhibitor as disclosed in any one of the preceding embodiments comprising the steps of:

a) obtaining a biomarker profile of a subject to be tested by taking a sample of a body fluid from said subject;

b) obtaining a biomarker profile of a healthy reference population;

c) obtaining a biomarker profile from a population which suffers from said disease or disorder and

d) comparing the biomarker profile obtained in step a) with the profile obtained in step b) and step c).

Yet another object of the present invention is to provide a set of biomarkers for use in the diagnosis of the diseases or disorder as defined in any one of the preceding embodiments, for use in the diagnosing a predisposition to the disease or disorder as defined in any one of the preceding embodiments or for use in monitoring minimal residual disease in a subject, or for predicting responsiveness of a subject to a treatment with the IL-18 inhibitor as disclosed in any one of the preceding embodiments, the IL-18BP as disclosed in any one of the preceding embodiments or the pharmaceutical composition comprising IL-18 inhibitor as disclosed in any one of the preceding embodiments, comprising a) IL-18, IL-18BP, IL-13, IL-17A, IL-8, IL-1β, IL-2, IL-12, IL-4, IL-6, INF-γ, TNF-α, VEGF, EGF, HB-EGF, TGF-α, MMP-9, MMP-12, myeloperoxidase, calprotectin measured by immunoassays, TGF-β, Tissue inhibitor of metalloproteinases (TIMP-1), hepatocyte growth factor (HGF), hypoxia induced

factor 1 alpha (HIF-1α), von Willebrand factor (vWF), EN-RAGE, S-RAGE, surfactant protein D, HsCRP, fibrinogen, endothelial microparticles, and b) gases comprising NO, CO, alkanes, pentanes, ethanes measured by exhaled air composition.

Yet another object of the present invention is to provide a pharmaceutical kit comprising IL-18 inhibitor as disclosed in any one of the preceding embodiments, Interleukin-18 Binding Protein (IL-18BP) as disclosed in any one of the preceding embodiments or a pharmaceutical composition comprising IL-18 inhibitor as disclosed in any one of the preceding embodiments and a pharmaceutically acceptable carrier and/or excipient according to the present invention in separate unit dosage forms, said forms being suitable for administration in effective amounts.

In one embodiment, the present invention provides a diagnostic kit for detecting free IL-18, comprising the free IL-18 specific binding molecule of any one of the preceding embodiments as the capturing molecule, and a second IL-18 specific binding molecule as the detection molecule and, optionally, a second IL-18 specific capturing molecule, wherein the detection molecule binds to different sites of IL-18 than the capturing molecule.

In another embodiment, a diagnostic kit is provided for detecting total IL-18 or total IL-18BP, comprising a first IL-18BP specific binding molecule, which does not bind to the IL-18 binding site of IL-18BP and a second IL-18 specific binding molecule, which does not bind to the IL-18BP binding site of IL-18.

Also comprised herein is a diagnostic kit for detecting free IL-18BP, comprising a first IL-18BP specific binding molecule as the capturing molecule and second IL-18 specific binding molecule as the detection molecule, wherein said detection molecule binds to a different site of IL-18BP than the capturing molecule.

In one embodiment, the diagnostic kit incorporates a combination of some or all the binding molecules contained in the above defined diagnostic kits.

It is yet another object of the present invention to provide a diagnostic kit for detecting free IL-18, comprising an IL-18-specific antibody as disclosed in any one of the preceding embodiments as capturing antibody or the IL-18BP as alternative capturing molecule, and a second IL-18 specific detection antibody or an IL-18-specific antibody as disclosed in any one of the preceding embodiments as detection antibody and a second IL-18 specific capturing antibody, wherein the detection antibody bind to different sites of IL-18 than the capturing molecule.

Yet another object of the present invention is to provide a diagnostic kit for detecting total IL-18 or total IL-18BP, comprising a first monoclonal IL-18BP specific antibody which does

not bind to the IL-18 binding site of IL-18BP and a second IL-18 specific antibody, which does not bind to the IL-18BP binding site of IL-18.

It is yet another object of the present invention to provide a diagnostic kit for detecting free IL-18BP, comprising a first monoclonal IL-18BP specific capturing antibody and an IL-18BP specific detection antibody, which binds to a different site of IL-18BP than the capturing antibody.

In another embodiment, the present invention provides a diagnostic kit, which comprises all diagnostic kits as disclosed in any one of the preceding embodiments.

The present invention now provides IL-18BP, for use in the treatment of an IL-18 associated disease or disorder in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder, by administering to said subject a therapeutically effective amount of at least one IL-18BP.

IL-18BP is understood within the scope of the present invention to also include muteins of IL-18BP, functional parts or derivatives of IL-18BP, circularly permuted derivatives of IL-18BP, fused proteins comprising IL-18BP, isoforms of IL-18BP or salts thereof.

IL-18BP may be provided as such or in form of a composition, particularly a pharmaceutical composition. Said compositions may comprise additional medicinal agents, pharmaceutical agents, carriers, buffers, dispersing agents, diluents, co-therapeutic agents such as anti-inflammatory, bronchodilatory, antihistamine, decongestant or anti-tussive drug substances and the like depending on the intended use and application.

Thus, the present invention provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for use in the treatment of an IL-18 associated disease or disorder in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder.

Further provided is an IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient according to the present invention, for treatment of an IL-18 associated disease or disorder in a subject in need of such a treatment, wherein the IL-18 associated disease or disorder is caused by excess expression of IL-18 in specific tissues and/or body compartments, which leads to an IL-18/IL-18BP imbalance in said tissues and/or compartments. For example, the enhanced expression of IL-18 as described herein leads to elevated levels of IL-18 in lung, serum, sputum, broncho-alveolar lavage fluid (BALF) or circulation of said subject compared to healthy control subjects, in particular the levels of IL-18 in sputum and/or in serum are elevated.

The IL-18 associated disease or disorder as described herein in various embodiments of the present invention is caused by excess expression of IL-18. Accordingly, the enhanced expression of IL-18 as described herein leads to elevated levels of IL-18 in lung, serum, sputum, broncho-alveolar lavage fluid (BALF) and/or circulation of a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder, compared to healthy control subjects, especially IL-18 levels in sputum and/or serum are elevated. Further, the elevated levels of IL-18 lead to an IL-18/IL-18BP imbalance in a subject suffering from such a disease or disorder.

In a specific embodiment, the present invention provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for use in compensation of an IL-18/IL-18BP imbalance in a subject suffering a IL-18 associated disease or disorder as described in the various embodiments of the present invention or having a predisposition for such a disease or disorder, by trapping the excess of IL-18. In particular IL-18BP reduces the levels of IL-18 compared to an untreated subject.

In a further embodiment, the present invention provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for use in the treatment of IL-18 associated disease or disorder according to the present invention, wherein IL-18BP leads to an inhibition of the expression of IL-18. In particular IL-18BP reduces the levels of IL-18 towards those of an untreated subject.

The present invention further provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for the use in the treatment of IL-18 induced local and systemic manifestations of inflammation.

The present invention further provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for the use in the treatment of IL-18 induced local and systemic manifestations of inflammation and associated comorbidities such as emphysema, tissue inflammation, tissue destruction, lung resection, disappearance of the vasculature, mucous metaplasia, cardiac hypertrophy, decrease of VEGF in the lung tissue, pulmonary vessel loss, vessel muscularization, collagen deposition, aberrant elastin layers in the lung, fibrotic airway remodeling, airspace enlargement, chronic remodeling of the airways and pulmonary vessels and/or decreased pulmonary function.

In another aspect of the present invention, the increased levels of IL-18 as disclosed by the present invention, trigger an enhanced expression of IFNγ, IL-13 or IL-17A in subjects suffering from said IL-18 associated disease or disorder compared to healthy control subjects. The present invention, thus, provides IL-18BP or a pharmaceutical composition

comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for modulating, particularly for reducing, the expression and/or production of IFNγ, IL-13 or IL-17A in a subject.

In certain embodiments of the present invention, IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient lead to inhibition of IL-18 binding to the IL-18 Receptor (IL-18R), particularly IL-18 binding to the IL-18 Receptor-α (IL-18Rα).

The manifestation of IL-18 associated disease or disorder is triggered by a Th1 cytokine response and/or a Th2 cytokine response. The IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient of the present invention thus leads to inhibition of Th1 cytokine response and/or Th2 cytokine response.

In certain embodiments of the present invention, IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient leads to modulation of IL-18-dependent downstream signaling pathways, e.g. like pathways which regulate TNF-alpha, IL-1beta, IL-8, macrophage inflammatory protein-alpha (MIP-alpha), IL-12, IL-15 and nitric oxide production and/or release. In particular, said signaling pathways are inhibited.

In one embodiment of the present invention, IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient prevents caspase activation. In particular, said caspase is caspase-1.

In one embodiment the present invention provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for use in the treatment of IL-18-associated disease, such as chronic obstructive pulmonary disease (COPD), heart disease and diabetes type 2.

In one embodiment the present invention provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for use in the treatment of IL-18-associated disease, such as chronic obstructive pulmonary disease (COPD), transfusion-related lung injury, bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), Adult Still's disease, juvenile Still's disease, interstitial lung disease (ILD), idiopathic pulmonary fibrosis, cystic fibrosis, pulmonary arterial hypertension, asthma, bronchiectasis, heart failure, amyotrophic lateral sclerosis (ALS), dry eye disease (DED), keratitis, corneal ulcer and abrasion, corneal neovascularization, pathological intraocular neovascularization, iritis, glaucoma, macular degeneration, Sjögren's syndrome, autoimmune uveitis, Behçet's disease, conjunctivitis,

allergic conjunctivitis, dermatitis of eyelid, diabetes type 2, non-alcoholic fatty liver disease (NAFLD), steato hepatitis, solid organ and hematologic transplantation, ischemia reperfusion injury, familial Mediterranean fever, tumor necrosis factor receptor 1-associated periodic syndromes, cryopyrin-associated periodic fever syndromes, hyper-IgD syndromes, gout, Schnitzler syndrome, Wegener's granulomatosis also called granulomatosis with polyangitis (GPA), Hashimoto's thyroiditis, Crohn's disease, ulcerative colitis, immunoglobulin-4 (IgG4)-related diseases and stem cell therapies.

In a particular embodiment, the present invention provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for use in the treatment of IL-18-associated lung disease or disorder, heart disease or disorder or diabetes mellitus type 2 as defined herein.

More particular, said IL-18 associated disease or disorder to be treated is manifested in the lung of the subject and may lead to the development of chronic obstructive pulmonary disease (COPD) associated with systemic manifestations of inflammation and associated comorbidities such as emphysema, tissue inflammation, tissue destruction, lung resection, disappearance of the vasculature, mucous metaplasia, cardiac hypertrophy, decrease of VEGF in the lung tissue, pulmonary vessel loss, pulmonary vessel muscularization, collagen deposition in the lung, aberrant elastin layers in the lung, fibrotic airway remodeling, airspace enlargement, chronic remodeling of the airways and pulmonary vessels and/or decreased pulmonary function. In particular, said manifestation is smoke-induced pulmonary inflammation. In a specific embodiment, the present invention provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient according to the present invention for use in the treatment of chronic obstructive pulmonary disease (COPD).

The observed IL-18/IL-18BP imbalance in a subject and the resulting disease or disorder as described herein, such as COPD may be caused by smoking or second-hand smoke exposure, in particular tobacco smoke exposure and/or a viral infection. In particular, cigarette smoke exposure may lead to the development of smoke-induced pulmonary emphysema and/or inflammation.

In another aspect of the present invention, the IL-18 associated disease or disorder to be treated is induced by long-term exposure to air pollution.

The present invention, thus, further provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient, for treating IL-18 induced airway and vascular remodeling, thus, preventing COPD disease manifestation and progression.

In one aspect of the present invention, alveolar macrophages are an important source of increased IL-18 level. Thus, the IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient, as provided by the present invention, reduces the expression and/or production of IL-18 by alveolar macrophages.

The present invention further provides IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient for preventing and/or inhibiting a smoke-induced form of cell death of lung tissue cells and/or epithelial cells affected by the IL-18 associated disease or disorder as described herein. In particular, said smoke-induced form of cell death is apoptosis.

In one embodiment of the present invention, the pharmaceutical composition of the invention and as disclosed herein in the various embodiments is administered prophylactically.

In another embodiment of the present invention, the pharmaceutical composition of the invention and as disclosed herein in the various embodiments is administered therapeutically.

In one embodiment of the present invention, the pharmaceutical composition of the invention and as disclosed herein in the various embodiments is administered to a subject suffering from IL-18 associated disease or disorder, or having a predisposition to develop such a disease or disorder by systemic, intranasal, intraocular, intravitral, eye drops, buccal, oral, transmucosal, intratracheal, intravenous, subcutaneous, intraurinary tract, intrarectal, intravaginal, sublingual, intrabronchial, intrapulmonary, transdermal or intramuscular administration. In particular, the pharmaceutical composition of the invention and as disclosed herein in the various embodiments is administered by broncho-pulmonary administration.

The pharmaceutical composition of the invention and as disclosed herein in the various embodiments may be provided as a liquid, liquid spray, microspheres, semisolid, gel, or powder for transmucosal administration, e.g. intranasal, buccal, oral transmucosal, intratracheal, intraurinary tract, intravaginal, sublingual, intrabronchial, intrapulmonary and/or transdermal administration. Further, the composition may be in a solid dosage form for buccal, oral transmucosal and/or sublingual administration. Intranasal, buccal, oral intratracheal, intraurinary tract, intravaginal, transmucosal and sublingual administrations lead to the disintegration of the composition as described herein in an oral cavity at body temperature and optionally may adhere to the body tissue of the oral cavity. Additionally, the composition as disclosed herein further may include one or more excipient, diluent,

binder, lubricant, glidant, disintegrant, desensitizing agent, emulsifier, mucosal adhesive, solubilizer, suspension agent, viscosity modifier, ionic tonicity agent, buffer, carrier, surfactant, flavor, or mixture thereof.

In a specific aspect of the present invention, the composition is formulated as a parenteral, intravenous, tablet, pill, bioadhesive patch, drops, sponge, film, lozenge, hard candy, wafer, sphere, lollipop, disc-shaped structure, suppository or spray.

Transmucosal administration is generally rapid because of the rich vascular supply to the mucosa and the lack of a stratum corneum in the epidermis. Such drug transport typically provides a rapid rise in blood concentrations, and similarly avoids the enterohepatic circulation and immediate destruction by gastric acid or partial first- pass effects of gut wall and hepatic metabolism. Drugs typically need to have prolonged exposure to a mucosal surface for significant drug absorption to occur.

The transmucosal routes can also be more effective than the oral route in that these routes can provide for relatively faster absorption and onset of therapeutic action. Further, the transmucosal routes can be preferred for use in treating patients who have difficulty in swallowing tablets, capsules, or other oral solids, or those who have disease-compromised intestinal absorption. Accordingly, there are many advantages to transmucosal administration of IL-18BP or a pharmaceutical composition comprising IL-18BP and a pharmaceutically acceptable carrier and/or excipient.

In either of the intranasal or buccal routes, drug absorption can be delayed or prolonged, or uptake may be almost as rapid as if an intravenous bolus were administered. Because of the high permeability of the rich blood supply, the sublingual route can provide a rapid onset of action.

The intranasal compositions can be administered by any appropriate method according to their form. A composition including microspheres or a powder can be administered using a nasal insufflator device. Examples of these devices are well known to those of skill in the art, and include commercial powder systems such as Fisons Lomudal System. An insufflator produces a finely divided cloud of the dry powder or microspheres. The insufflator is preferably provided with a mechanism to ensure administration of a substantially fixed amount of the composition. The powder or microspheres can be used directly with an insufflator, which is provided with a bottle or container for the powder or microspheres. Alternatively, the powder or microspheres can be filled into a capsule such as a gelatin capsule, or other single dose device adapted for nasal administration. The insufflator preferably has a mechanism to break open the capsule or other device. Further, the composition can provide an initial rapid release of the active ingredient followed by a

sustained release of the active ingredient, for example, by providing more than one type of microsphere or powder. Further, alternative methods suitable for administering a composition to the nasal cavity will be well known by the person of ordinary skill in the art. Any suitable method may be used. For a more detailed description of suitable methods reference is made to EP2112923, EP1635783, EP1648406, EP2112923 (the entire contents of which are incorporated by reference herein).

In one embodiment of the present invention, the pharmaceutical composition of the invention and as disclosed herein in the various embodiments is may be further administered intranasally, i.e. by inhalation and, thus, may be formulated in a form suitable for intranasal administration, i.e. as an aerosol, dry powder formulation or a liquid preparation.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include, but are not limited to, a gum, a starch (e.g. corn starch, pregeletanized starch), a sugar (e.g., lactose, mannitol, sucrose, dextrose), a cellulosic material (e.g. microcrystalline cellulose), an acrylate (e.g. polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

Pharmaceutically acceptable carriers for liquid formulation are aqueous or non-aqueous solutions, suspensions, dry powder formulations, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Examples of oils are those of animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, olive oil, sunflower oil, fish-liver oil, another marine oil, or a lipid from milk or eggs.

The present invention also relates to transpulmonary administration by inhalation of the pharmaceutical composition of the invention and as disclosed herein in the various embodiments is in dry powder, gaseous or volatile formulations into systemic circulation via the respiratory tract. Absorption is virtually as rapid as the formulation can be delivered into the alveoli of the lungs, since the alveolar and vascular epithelial membranes are quite permeable, blood flow is abundant and there is a very large surface for adsorption. For instance, aerosols my be delivered from pressure-packaged, metered-dose inhalers (MDIs).

The the pharmaceutical composition of the invention and as disclosed herein in the various embodiments is will generally be administered in a mixture with a suitable pharmaceutical excipient, diluent or carrier selected with regard to the chosen means of inhalation and standard pharmaceutical practice.

In another embodiment of the invention, the IL-18BP formulation or the formulation of a pharmaceutical composition comprising IL-18BP is a dry powder, optionally together with at

least one particulate pharmaceutically acceptable carrier, which may be one or more materials known as pharmaceutically acceptable carriers, preferably chosen from materials known as carriers in dry powder inhalation compositions, for example saccharides, including monosaccharides, disaccharides, polysaccharides and sugar alcohols such as arabinose, glucose, fructose, ribose, mannose, sucrose, trehalose, lactose, maltose, starches, dextran, mannitol or sorbitol. An especially preferred carrier is lactose, for example lactose monohydrate or anhydrous lactose. The dry powder may be contained as unit doses in capsules of, for example, gelatin or plastic, or in blisters (e.g. of aluminium or plastic), for use in a dry powder inhalation device, which may be a single dose or multiple dose device, preferably in dosage units together with the carrier in amounts to bring the total weight of powder per capsule to from 5 mg to 50 mg. Alternatively, the dry powder may be contained in a reservoir in a multi-dose dry powder inhalation (MDDPI) device adapted to deliver.

Any other therapeutically efficacious route of administration can be used, for example absorption through epithelial or endothelial tissues or by gene therapy wherein a DNA molecule encoding the active agent is administered to the patient (e.g. via an expression vector), which causes the active agent to be expressed and secreted *in vivo*.

In the body, expression of IL-18BP can be induced by modulating upstream signaling pathways, which control the expression of IL-18BP. For instance, IL-18BP is specifically induced by IFN-gamma as part of a negative feedback loop that regulates the induction of IFN-gamma by IL-18. Other known factors which are reported to regulate IL-18BP expression are IL-18, IL-27, IFN-alpha and STAT1.

Thus, in one embodiment of the present invention, cellular expression of IL-18BP is indirectly induced by modification of one or more upstream signaling pathways, which control the expression of IL-18BP. In particular, expression of IL-18BP is indirectly induced by modification of at least one upstream signaling pathway.

The invention further relates to an expression vector comprising the coding sequence of IL-18BP in the preparation of a medicament for the treatment of IL-18 associated disease or disorder as described herein in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder.

In a specific embodiment, the present invention relates to an expression vector comprising the coding sequence of a regulatory factor, which modulates upstream signaling pathways that regulate the expression of IL-18BP. Thus, said regulatory factor induces the expression of IL-18BP by modulating at least one upstream signaling pathway.

The invention further relates to an expression vector comprising the coding sequence of a regulatory factor, which modulates upstream signaling pathways that regulate the expression of IL-18BP in the preparation of a medicament for the treatment of IL-18 associated disease or disorder as described herein in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder.

The invention further relates to an expression vector comprising the coding sequence of a regulatory factor, which induces the expression of IL-18BP for the treatment of IL-18 associated disease or disorder as described herein in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder. In particular it relates to an expression vector comprising the coding sequence of a regulatory factor, which induces the expression of IL-18BP in the lung for the treatment of IL-18 associated disease or disorder as described herein in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder.

Optionally the present invention provides a second expression vector comprising the coding sequence of a second naturally occurring proinflammatory cytokine inhibitor or a regulatory factor which modulates at least one upstream signaling pathway that regulates the expression of said proinflammatory cytokines. In particular, said regulatory factor induces the expression of said cytokine inhibitor.

In particular, the expression of IL-18 is modulated by RNA interference (RNAi) or an IL-18 antisense expressing vector. More particular, the expression of IL-18 is modulated by RNA interference (RNAi), wherein the expression of IL-18 is downregulated by post transcriptional gene silencing (PTGS). In particular, the expression of IL-18 is downregulated in the lung of the subject suffering from the disease or disorder as disclosed herein. The mechanism of RNA interference comprises any post transcriptional gene silencing event, particularly any post transcriptional gene silencing event induced by microRNA (miRNA) or small interfering RNA (siRNA). MicroRNAs (miRNAs) and small interfering RNAs (siRNAs) can be expressed by the expression vector according to the invention and as described herein.

A gene therapeutical approach may thus be used for treating an IL-18 associated disease or disorder as described herein and as disclosed in the various embodiments. Accordingly, the expression of IL-18BP occurs *in situ*, hence, directly neutralizing IL-18 in the tissue or cells affected by said disease or disorder. In particular, the expression of IL-18BP as disclosed herein is induced in the lung.

The pharmaceutical composition of the invention and as disclosed herein in the various embodiments may be used for treatment of an IL-18 associated disease or disorder as

described herein in the various embodiments in human and veterinary medicine for treating humans and animals, including avians, non-human primates, dogs, cats, pigs, goats, sheep, cattle, horses, mice, rats and rabbits.

In a specific embodiment, the present invention provides the pharmaceutical composition of the invention as disclosed herein in the various embodiments for use in the treatment of IL-18 associated disease or disorder as described herein in the various embodiments, wherein the subject is a mammal, in particular the subject is a human.

In another specific embodiment, the pharmaceutical composition of the invention as disclosed herein in the various embodiments is administered in a therapeutically effective amount with a suitable dose of at least a second proinflammatory cytokine inhibitor. In particular said inhibitor is specific for IL-1, IL-6, IL-13, IL-17A, IFNγ or TNFα.

Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media such as phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. Suitable carriers may comprise any material which, when combined with the biologically active compound of the invention, retains the biological activity.

Efforts have been made in the art to chemically modify the barrier properties of skin to permit the penetration of certain agents, enhance the effectiveness of the agent being delivered, enhance delivery times, reduce the dosages delivered, reduce the side effects from various delivery methods, reduce patient reactions, and so forth.

In this regard, penetration enhancers have been used to increase the permeability of the dermal surface to drugs, and are often proton accepting solvents such as dimethyl sulfoxide (DMSO) and dimethylacetamide. Other penetration enhancers that have been studied and reported as effective include 2-pyrrolidine, N,N-diethyl-m-toluamide (Deet), 1-dodecal-azacycloheptane-2-one, N,N-dimethylformamide, N-methyl-2-pyrrolidine, calcium thioglycolate, hexanol, fatty acids and esters, pyrrolidone derivatives, derivatives of 1,3-dioxanes and 1,3-dioxolanes, 1-N-dodecyl-2-pyrrolidone-5-carboxylic acid, 2-pentyl-2-oxo-pyrrolidineacetic acid, 2-dodecyl-2-oxo-1-pyrrolidineacetic acid, 1-azacycloheptan-2-one-2-dodecylacetic acid, and aminoalcohol derivatives, including derivatives of 1,3-dioxanes, among others.

Preparations for transmucosal administration may include sterile aqueous or non-aqueous solutions, suspensions, dry powder formulations and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water,

alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Transmucosal vehicles may include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Preservatives and other additives may also be present including, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. In addition, the pharmaceutical composition of the present invention might comprise proteinaceous carriers, like, e.g., serum albumin or immunoglobulin, preferably of human origin.

The pharmaceutical composition of the invention as disclosed herein in the various embodiments may be administered topically to body surfaces and, thus, be formulated in a form suitable for topical administration. Suitable topical formulations include gels, ointments, creams, lotions, drops and the like. For topical administration, the pharmaceutical composition of the invention as disclosed herein in the various embodiments is prepared and applied as a solution, suspension, or emulsion in a physiologically acceptable diluent with or without a pharmaceutical carrier.

The pharmaceutical composition of the invention and as disclosed herein in the various embodiments may also be administered as controlled-release compositions, i.e. compositions in which the active ingredient is released over a period of time after administration. Controlled- or sustained-release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). In another embodiment, the composition is an immediate-release composition, i.e. a composition in which all the active ingredient is released immediately after administration.

Further examples for suitable formulations are provided in WO 2006/085983, the entire contents of which are incorporated by reference herein. For example, the pharmaceutical composition of the invention and as disclosed herein in the various embodiments is of the present invention may be provided as liposomal formulations. The technology for forming liposomal suspensions is well known in the art. The lipid layer employed can be of any conventional composition and can either contain cholesterol or can be cholesterol-free. The liposomes can be reduced in size, as through the use of standard sonication and homogenization techniques. Liposomal formulations containing the pharmaceutical composition of the invention as disclosed herein in the various embodiments can be lyophilized to produce a lyophilizate which can be reconstituted with a pharmaceutically acceptable carrier, such as water, to regenerate a liposomal suspension. The pharmaceutical composition of the invention as disclosed herein in the various embodiments can be administered to the subject at a suitable dose. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one subject depend upon many factors, including the

subject's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently.

Furthermore, it is envisaged that the pharmaceutical composition of the invention might comprise further biologically active agents, depending on the intended use of the pharmaceutical composition. These further biologically active agents may be e.g. antibodies, antibody fragments, hormones, growth factors, enzymes, binding molecules, cytokines, chemokines, nucleic acid molecules and drugs. In a preferred embodiment, the pharmaceutical composition of the present invention is to be co-administered with long-acting beta-adrenoceptor agonist (LABA), long-acting muscarinic antagonists (LAMA), steroids, corticosteroid, glucocorticoid and glucocorticoid agonists phosphodiesterase inhibitors, kinase inhibitors, cytokine and chemokine inhibitors or antagonists or protease inhibitors or combinations thereof.

The dosage of the pharmaceutical composition of the invention as disclosed herein in the various embodiments will depend on the condition being treated, the particular composition used, and other clinical factors such as weight, size and condition of the subject, body surface area, the particular compound or composition to be administered, other drugs being administered concurrently, and the route of administration.

The pharmaceutical composition of the invention as disclosed herein in the various embodiments may be administered in combination with other biologically active substances and procedures for the treatment of symptoms associated with IL-18 associated disease, such as chronic obstructive pulmonary disease (COPD), transfusion-related lung injury, bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), Adult Still's disease, juvenile Still's disease, interstitial lung disease (ILD), idiopathic pulmonary fibrosis, cystic fibrosis, pulmonary arterial hypertension, asthma, bronchiectasis, heart failure, amyotrophic lateral sclerosis (ALS), dry eye disease (DED), keratitis, corneal ulcer and abrasion, corneal neovascularization, pathological intraocular neovascularization, iritis, glaucoma, macular degeneration, Sjögren's syndrome, autoimmune uveitis, Behçet's disease, conjunctivitis, allergic conjunctivitis, dermatitis of eyelid, diabetes type 2, non-alcoholic fatty liver disease (NAFLD), steato hepatitis, solid organ and hematologic transplantation, ischemia reperfusion injury, familial Mediterranean fever, tumor necrosis factor receptor 1-associated periodic syndromes, cryopyrin-associated periodic fever syndromes, hyper-IgD syndromes, gout, Schnitzler syndrome, Wegener's granulomatosis also called granulomatosis with polyangitis (GPA), Hashimoto's thyroiditis, Crohn's disease, ulcerative colitis, immunoglobulin-4 (IgG4)-related diseases and stem cell therapies.. The other biologically active substances may be part of the same composition already

comprising the composition according to the invention, in form of a mixture, wherein the composition of the invention and the other biologically active substance are intermixed in or with the same pharmaceutically acceptable solvent and/or carrier or may be provided separately as part of a separate compositions, which may be offered separately or together in form of a kit of parts.

The pharmaceutical composition of the invention as disclosed herein in the various embodiments may be administered concomitantly with the other biologically active substance or substances, intermittently or sequentially. For example, the composition according to the invention may be administered simultaneously with a first additional biologically active substance or sequentially after or before administration of said composition. If an application scheme is chosen where more than one additional biologically active substance are administered and at least one composition according to the invention, the compounds or substances may be partially administered simultaneously, partially sequentially in various combinations.

It is thus another object of the present invention to provide for mixtures of the pharmaceutical composition of the invention as disclosed herein in the various embodiments, optionally comprising one or more further biologically active substances in a therapeutically or prophylactically effective amount, as well as to methods of using such a composition according to the invention, or mixtures thereof for the prevention and/or therapeutic treatment and/or alleviation of the effects of chronic obstructive pulmonary disease (COPD), heart disease and diabetes type 2.

It is thus another object of the present invention to provide for mixtures of the pharmaceutical composition of the invention as disclosed herein in the various embodiments, optionally comprising, one or more further biologically active substances in a therapeutically or prophylactically effective amount, as well as to methods of using such a composition according to the invention, or mixtures thereof for the prevention and/or therapeutic treatment and/or alleviation of the effects of chronic obstructive pulmonary disease (COPD), transfusion-related lung injury, bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), Adult Still's disease, juvenile Still's disease, interstitial lung disease (ILD), idiopathic pulmonary fibrosis, cystic fibrosis, pulmonary arterial hypertension, asthma, bronchiectasis, heart failure, amyotrophic lateral sclerosis (ALS), dry eye disease (DED), keratitis, corneal ulcer and abrasion, corneal neovascularization, pathological intraocular neovascularization, iritis, glaucoma, macular degeneration, Sjögren's syndrome, autoimmune uveitis, Behçet's disease, conjunctivitis, allergic conjunctivitis, dermatitis of eyelid, diabetes type 2, non-alcoholic fatty liver disease (NAFLD), steato hepatitis, solid organ and hematologic transplantation, ischemia

reperfusion injury, familial Mediterranean fever, tumor necrosis factor receptor 1-associated periodic syndromes, cryopyrin-associated periodic fever syndromes, hyper-IgD syndromes, gout, Schnitzler syndrome, Wegener's granulomatosis also called granulomatosis with polyangitis (GPA), Hashimoto's thyroiditis, Crohn's disease, ulcerative colitis, immunoglobulin-4 (IgG4)-related diseases and stem cell therapies.

The other biologically active substance or compound may exert its biological effect by the same or a similar mechanism as the composition according to the invention or by an unrelated mechanism of action or by a multiplicity of related and/or unrelated mechanisms of action.

Generally, the other biologically active compound may include antibodies raised against and binding to INF-gamma, IL-17A, IL-13, IL-1beta, IL-6, IL-2, IL-4, IL-12, TNF-alpha In particular, the mixture according to the invention may comprise IL-18BP (IL-18BP) or a pharmaceutical composition comprising IL-18BP (IL-18BP) and a pharmaceutically acceptable carrier and/or excipient according to the invention and as described herein.

Suitable dosages of the pharmaceutical composition of the invention as disclosed herein in the various embodiments will vary depending upon the condition, age and species of the subject, and can be readily determined by those skilled in the art. The total daily dosages of the employed in both veterinary and human medicine will suitably be in the range 0,01-2000 mg/kg body-weight, preferably from 0,1-1000 mg/kg body-weight, preferably from 1-100 mg/kg and these may be administered as single or divided doses, and in addition, the upper limit can also be exceeded when this is found to be indicated. Such dosage will be adjusted to the individual requirements in each particular case including the specific compound(s) being administered, the route of administration, the condition being treated, as well as the subject being treated. However, the compounds can also be administered as depot preparations (implants, slow-release formulations, etc.) weekly, monthly or at even longer intervals. In such cases the dosage will be much higher than the daily one and has to be adapted to the administration form, the body weight and the concrete indication. The appropriate dosage can be determined by conducting conventional model tests, preferably animal models. An effective dose of active ingredient(s) depends at least on the nature of the condition being treated, toxicity, whether the compound(s) is being used prophylactic ally or against an active infection or condition, the method of delivery, and the pharmaceutical formulation, and will be determined by the clinician using conventional dose escalation studies. It can be expected to be from about 0,01 mg to about 1 g/kg body weight per day. For example, for topical delivery the daily candidate dose for an adult human of approximately 70 kg body weight will range from about 1 mg to about 500 mg, generally between about 5 mg and about 40 mg, and may take the form of single or multiple doses or

administration sites. For intranasal delivery the candidate dose can be expected to be from about 0.01 mg to about 1 g/kg body weight per day.

Further, functional derivatives of IL-18BP may be conjugated to polymers in order to improve the properties of the protein, such as the stability, half-life, bioavailability, tolerance by the human body, or immunogenicity. To achieve this goal, IL18-BP may be linked e.g. to Polyethlyenglycol (PEG). PEGylation may be carried out by known methods, described in WO 92/13095, for example.

Therefore, in another embodiment of the present invention, IL-18BP is PEGylated.

In still another embodiment of the invention, IL-18BP is a fused protein comprising all or part of an IL-18BP, which is fused to all or part of an immunoglobulin.

In a further embodiment of the invention, the IL-18BP is PEGylated, fused to all or part of an immunoglobulin, preferably to the constant region of an immunoglobulin, and wherein the fused protein is still capable of binding to IL-18. More specifically, the immunoglobulin may be of the IgG1 or IgG2 isotype.

The person skilled in the art will understand that the resulting fusion protein retains the biological activity of IL-18BP, in particular the binding to IL-18. The fusion may be direct, or via a short linker peptide which can be as short as 1 to 3 amino acid residues in length or longer, for example, 13 amino acid residues in length. Said linker may be a tripeptide of the sequence E-F-M (Glu-Phe-Met) (SEQ ID NO: 9), for example, or a 13-amino acid linker sequence comprising Glu-Phe-Gly-Ala-Gly-Leu-Val-Leu-Gly-Gly-Gln-Phe-Met (SEQ ID NO: 8) introduced between the IL-18BP sequence and the immunoglobulin sequence. The resulting fusion protein has improved properties, such as an extended residence time in body fluids (half-life), increased specific activity, increased expression level, or the purification of the fusion protein is facilitated.

Preferably, it is fused to heavy chain regions, like the CH2 and CH3 domains of human IgG1, for example. The generation of specific fusion proteins comprising IL-18BP and a portion of an immunoglobulin are described in example 11 of WP99/09063, for example. Other isoforms of Ig molecules are also suitable for the generation of fusion proteins according to the present invention, such as isoforms IgG2 or IgG4, or other Ig classes, like IgM or IgA, for example. Fusion proteins may be monomeric or multimeric, hetero or homomultimeric.

In certain further embodiments, the present invention provides a method for treating a subject suffering or having a predisposition to develop a disease or disorder associated with excess expression of IL-18 as described herein in various embodiments of the present

invention, comprising administering to said subject a therapeutically effective amount of the pharmaceutical composition of the invention as disclosed herein in the various embodiments.

Serological assays are well known in the art and have become a useful tool for the detection of antigens in body fluids, such as serum, bronchial alveolar lavage (BAL) fluid and sputum. However, until now no functional diagnostic assay for the detection of IL-18 by specific antibodies exists. Thus, there is a specific need for a diagnostic method for the detection of IL-18 in body fluid, particularly in serum.

The present invention thus discloses methods and kits for the detection and diagnosis of IL-18-associated diseases or conditions as described herein, for diagnosing a predisposition to an IL18-associated disease or condition as described herein or for monitoring minimal residual disease in a subject or for predicting responsiveness of a subject to a treatment with the pharmaceutical composition of the invention as disclosed herein in the various embodiments is and as described herein before. These methods include known immunological methods commonly used for detecting or quantifying substances in biological samples or in an in situ condition.

In one embodiment the present invention further discloses a method for diagnosis of IL-18-associated disease as described herein, or for diagnosing a predisposition to an IL18-associated disease as described herein, or for monitoring minimal residual disease in a subject or for predicting responsiveness of a subject to a treatment with IL-18BP or a pharmaceutical composition comprising IL-18BP (IL-18BP) and a pharmaceutically acceptable carrier and/or excipient according to any one of the preceding embodiments, comprising the steps:

a) obtaining a sample of body fluid from a subject suffering from such a disease;

b) testing said sample for the presence of IL-18 by using the IL-18BP as disclosed herein or the IL-18-specific antibody according to the present invention as capturing molecule;

c) determining the amount of IL-18 bound to the capturing molecule in the sample;

d) comparing the amount of IL-18 in the sample of the subject suffering from such a disease to the amount in the sample of a healthy subject.

The amount of free IL-18 in isolated serum of a subject, particularly a human, suffering from said disease ranges from 5 to 10000 pg/mL, particularly in the range of 100 to 10000 pg/mL, particularly in the range of 200 to 10000 pg/mL, particularly in the range of 300 to 10000 pg/mL, particularly in the range of 400 to 10000 pg/mL, particularly in the range of

500 to 10000 pg/mL, particularly in the range of 600 to 10000 pg/mL, particularly in the range of 700 to 10000 pg/mL, particularly in the range of 800 to 10000 pg/mL, particularly in the range of 900 to 10000 pg/mL, particularly in the range of 1000 to 10000 pg/mL, particularly in the range of 1500 to 10000 pg/mL, particularly in the range of 2000 to 10000 pg/mL, particularly in the range of 3000 to 10000 pg/mL, particularly in the range of 4000 to 10000 pg/mL, particularly in the range of 5000 to 10000 pg/mL. The amount of free IL-18 in serum of healthy subject, particularly a healthy human is ≤40 pg/mL, particularly ≤30 pg/mL, particularly ≤25 pg/mL, particularly ≤20 pg/mL, particularly ≤10 pg/mL, particularly ≤5 pg/mL, particularly ≤1 pg/mL, particularly ≤0.5 pg/mL. Thus, a subject having a detectable IL-18 concentration in the serum between 5 to 10000 pg/mL suffers from the IL-18-associated disease as disclosed herein. The amount of IL-18 in serum and other body fluids can be determined by the diagnostic method as disclosed herein by using a linear standard curve, which is calculated for predefined IL-18 concentrations within the range of 5 to 200 pg/mL.

Diagnosis of an IL-18-associated disease or condition or of a predisposition to an IL-18-associated disease or condition as described herein in a subject may be achieved by detecting the binding of IL-18BP as disclosed herein to IL-18 or the immunospecific binding of a monoclonal antibody or an active fragment thereof as disclosed herein to an epitope of IL-18 in a sample or *in situ,* which includes bringing the sample or a specific body part or body area suspected to contain the IL-18 antigen into contact with IL-18BP and/or an antibody which binds an epitope of the IL-18 protein or a fragment thereof, allowing the IL-18BP or the antibody to bind to the IL-18 antigen to form an immunological complex, detecting the formation of the immunological complex and correlating the presence or absence of the immunological complex with the presence or absence of IL-18 antigen in the sample or specific body part or area, optionally comparing the amount of said immunological complex to a normal control value, wherein an increase in the amount of said aggregate compared to a normal control value indicates that said subject is suffering from or is at risk of developing an IL-18-associated disease or condition.

Monitoring minimal residual disease in a subject following treatment with IL-18BP or a pharmaceutical composition comprising IL-18BPand a pharmaceutically acceptable carrier and/or excipient according to any one of the preceding embodiments and as described herein before may be achieved by detecting the binding of IL-18BP as disclosed herein to IL-18 or the immunospecific binding of a monoclonal antibody or an active fragment thereof as disclosed herein to an epitope of the IL-18 protein or a fragment thereof in a sample or *in situ,* which includes bringing the sample or a specific body part or body area suspected to contain the IL-18 antigen into contact with the IL-18BP and/or the antibody as disclosed

herein which binds an epitope of the IL-18 protein or a fragment thereof, allowing the IL-18BP and/or the antibody to bind to the IL-18 antigen to form an immunological complex, detecting the formation of the immunological complex and correlating the presence or absence of the immunological complex with the presence or absence of IL-18 antigen in the sample or specific body part or area, optionally comparing the amount of said immunological complex to a normal control value, wherein an increase in the amount of said aggregate compared to a normal control value indicates that said subject may still suffer from a minimal residual disease.

Predicting responsiveness of a subject to a treatment with the pharmaceutical composition of the invention as disclosed herein in the various embodiments may be achieved by detecting the binding of IL-18BP as disclosed herein to IL-18 or the immunospecific binding of a monoclonal antibody or an active fragment thereof as disclosed herein to an epitope of the IL-18 protein or a fragment thereof in a sample or *in situ,* which includes bringing the sample or a specific body part or body area suspected to contain the IL-18 antigen into contact with the IL-18BP and/or the antibody which binds an epitope of the IL-18 protein or a fragment thereof, allowing the IL-18BP and/or antibody to bind to the IL-18 antigen to form an immunological complex, detecting the formation of the immunological complex and correlating the presence or absence of the immunological complex with the presence or absence of IL-18 antigen in the sample or specific body part or area, optionally comparing the amount of said immunological complex before and after onset of the treatment, wherein an decrease in the amount of said aggregate indicates that said subject has a high potential of being responsive to the treatment.

Biological samples that may be used in the diagnosis of an IL-18-associated disease or condition as described herein, for diagnosing a predisposition to an IL-18-associated disease or condition or for monitoring minimal residual disease as described herein in a subject or for predicting responsiveness of a subject to a treatment with the pharmaceutical composition of the invention as disclosed herein in the various embodiments are, for example, fluids such as serum, plasma, saliva, gastric secretions, mucus, cerebrospinal fluid, lymphatic fluid and the like or tissue or cell samples obtained from an organism such as neural, brain, lung, cardiac or vascular tissue. For determining the presence or absence of the IL-18 antigen, particularly of free IL-18 antigen, in a sample any immunoassay known to those skilled in the art may be used. (See Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York 1988 555-612) may be used such as, for example, assays which utilize indirect detection methods using secondary reagents for detection, ELISA's and immunoprecipitation and agglutination assays. A detailed

description of these assays is, for example, given in WO96/13590 to Maertens and Stuyver, Zrein et al. (1998) and WO96/29605.

For *in situ* diagnosis, the IL-18BP as disclosed herein and/or the IL-18-specific antibody or any active and functional part thereof as disclosed herein may be administered to the organism to be diagnosed by methods known in the art such as, for example, intravenous, intranasal, intraperitoneal, intracerebral, intraarterial injection such that a specific binding between the IL-18BP and/or the specific antibody with an eptitopic region on the IL-18 antigen may occur. The IL18BP/antigen or antibody/antigen complex may be detected through a label attached to the antibody or a functional fragment thereof.

The immunoassays used in diagnostic applications or in applications for diagnosing a predisposition to an IL-18-associated disease or condition as described herein or for monitoring minimal residual disease in a subject or for predicting responsiveness of a subject to a treatment with the pharmaceutical composition of the invention as disclosed herein in the various embodiments is typically relying on labelled antigens, antibodies, or secondary reagents for detection. These proteins or reagents can be labelled with compounds generally known to those skilled in the art including enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances including colored particles, such as colloidal gold and latex beads. Of these, radioactive labelling can be used for almost all types of assays and with most variations. Enzyme-conjugated labels are particularly useful when radioactivity must be avoided or when quick results are needed. Fluorochromes, although requiring expensive equipment for their use, provide a very sensitive method of detection. Antibodies useful in these assays include monoclonal antibodies, polyclonal antibodies, and affinity purified polyclonal antibodies.

Alternatively, the antibody may be labelled indirectly by reaction with labelled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. The antibody may be conjugated with a second substance and detected with a labelled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibody may be conjugated to biotin and the antibody-biotin conjugate detected using labelled avidin or streptavidin. Similarly, the antibody may be conjugated to a hapten and the antibody-hapten conjugate detected using labelled anti-hapten antibody.

Those of ordinary skill in the art will know of these and other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy, J. H., et al.,1976 (Clin. Chim. Acta 70:1-31), and Schurs, A. H. W. M., et al. 1977 (Clin. Chim

Acta 81:1-40). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, and others, all of which are incorporated by reference herein.

Current immunoassays utilize a double antibody method for detecting the presence of an analyte, wherein the capture antibody is labeled indirectly by reactivity with a second antibody that has been labeled with a detectable label. The second antibody is preferably one that binds to antibodies of the animal from which the monoclonal antibody is derived. In other words, if the monoclonal antibody is a mouse antibody, then the labeled, second antibody is an anti-mouse antibody. For the monoclonal antibody to be used in the assay described below, this label is preferably an antibody-coated bead, particularly a magnetic bead. For the polyclonal antibody to be employed in the immunoassay described herein, the label is preferably a detectable molecule such as a radioactive, fluorescent or an electrochemiluminescent substance.

An alternative double antibody system, often referred to as fast format systems because they are adapted to rapid determinations of the presence of an analyte, may also be employed within the scope of the present invention. The system requires high affinity between the antibody and the analyte. According to one embodiment of the present invention, the presence of the IL-18 antigen is determined using a pair of antibodies, each specific for IL-18 antigen. One of said pairs of antibodies is referred to herein as a "detector antibody" and the other of said pair of antibodies is referred to herein as a "capture antibody". The monoclonal antibody of the present invention can be used as either a capture antibody or a detector antibody. The monoclonal antibody of the present invention can also be used as both capture and detector antibody, together in a single assay. One embodiment of the present invention thus uses the double antibody sandwich method for detecting IL-18 antigen in a sample of biological fluid. In this method, the analyte (IL-18 antigen) is sandwiched between the detector antibody and the capture antibody, the capture antibody being irreversibly immobilized onto a solid support. The detector antibody would contain a detectable label, in order to identify the presence of the antibody-analyte sandwich and thus the presence of the analyte.

Exemplary solid phase substances include, but are not limited to, microtiter plates, test tubes of polystyrene, magnetic, plastic or glass beads and slides which are well known in the field of radioimmunoassay and enzyme immunoassay. Methods for coupling antibodies to solid phases are also well known to those skilled in the art. More recently, a number of porous material such as nylon, nitrocellulose, cellulose acetate, glass fibers and other porous polymers have been employed as solid supports.

The present invention also relates to a diagnostic kit for detecting IL-18 antigen in a biological sample. Moreover, the present invention relates to the latter diagnostic kit which, in addition to a composition as defined above, also comprises a detection reagent as defined above. The term "diagnostic kit" refers in general to any diagnostic kit known in the art. More specifically, the latter term refers to a diagnostic kit as described in Zrein et al. (1998).

It is still another object of the present invention to provide novel immunoprobes and test kits for detection and diagnosis of IL-18-associated diseases and conditions as described herein comprising IL-18BP as disclosed herein before or specific IL-18BP antibodies as disclosed herein before. For immunoprobes, the IL-18BP or the antibodies are directly or indirectly attached to a suitable reporter molecule, e.g., an enzyme or a radionuclide. The test kit includes a container holding the IL-18BP and/or one or more antibodies and instructions for using the IL-18BP and/or the antibodies for the purpose of binding to IL-18 antigen to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of IL-18 antigen.

In accordance with the above, the invention also provides a pharmaceutical kit comprising the pharmaceutical composition of the invention as disclosed herein in the various embodiments in separate unit dosage forms, said forms being suitable for administration in effective amounts. Such a kit suitably further comprises one or more inhalation devices for administration of the pharmaceutical composition of the invention as disclosed herein in the various embodiments. For example, the kit may comprise one or more dry powder inhalation devices adapted to deliver dry powder from a capsule, together with capsules containing a dry powder comprising a dosage unit of the pharmaceutical composition of the invention as disclosed herein in the various embodiments. In another example, the kit may comprise a multi- dose dry powder inhalation device containing in the reservoir thereof a dry powder comprising a multidose dry powder inhalation device containing in the reservoir thereof a dry powder comprising the pharmaceutical composition of the invention as disclosed herein in the various embodiments.

DEFINITIONS

The technical terms and expressions used within the scope of this application are generally to be given the meaning commonly applied to them in the pertinent art if not otherwise indicated herein below.

As used in this specification and the appended embodiments, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a compound" includes one or more compounds.

The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease and/or adverse effects attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a subject and includes: (a) preventing a disease, i.e. related to an undesired immune response from occurring in a subject which may be predisposed to the disease; (b) inhibiting the disease, i.e. arresting its development; or (c) relieving the disease, i.e. causing regression of the disease (d) reversing the disease symptoms, i.e. leading to recovery of damaged tissue.

The expression “IL-18 Binding Protein (IL-18BP)” as used herein includes the full-length protein, a mutein, fragment, peptide, functional derivative, functional fragment, fraction, circularly permuted derivative, fused protein, isoform or a salt thereof.
The term “free IL-18” as used herein means monomeric, soluble and non-complexed interleulin-18 protein.

An "immunoglobulin" is a tetrameric molecule. In a naturally-occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as [kappa] and [lambda] light chains. Heavy chains are classified as [micro], [Delta], [gamma], [alpha], or [epsilon], and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 2 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

Immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by

the framework regions, enabling binding to a specific epitope. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR.2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk J. Mol. Biol, 196:901-917 (1987); Chothia et al. Nature 342:878-883 (1989).

The terms "antibody" or "antibodies" as used herein are art recognized term and are understood to refer to molecules or active fragments of molecules that bind to known antigens, particularly to immunoglobulin molecules and to immunologically active portions of immunoglobulin molecules, i.e. molecules that contain a binding site that immunospecifically binds an antigen. The immunoglobulin according to the invention can be of any type (IgG, IgM, IgD, IgE, IgA and IgY) or class (IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclasses of immunoglobulin molecule.

[0241] The term "Antibody" refers for the purpose of the present invention to an intact immunoglobulin or to an antigen-binding portion thereof that competes with the intact antibody for specific binding. In particular, "Antibodies" are intended within the scope of the present invention to include monoclonal, polyclonal, chimeric, single chain, bispecific or bi-effective, simianized, human and humanized antibodies.

Examples of Antigen-binding portions include, inter alia, Fab, Fab', F(ab')2, scFv, dAb and Fv fragments, including the products of an Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above. Further examples of Antigen-binding portions include complementarity determining region (CDR) fragments, diabodies and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

[0242] Such active fragments can be derived from an antibody of the present invention by a number of art-known techniques. For example, purified monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see for example, Khaw, B. A. ct al. J. Nucl. Med. 23:1011-1019 (1982); Rousseaux et al. Methods Enzymology, 121:663-69, Academic Press, 1986 .

[0243] A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin. In one embodiment, certain amino acids in the framework and constant domains of the heavy and light chains have been mutated so

as to avoid or abrogate an immune response in humans. In an alternative embodiment, a humanized antibody may be produced by fusing the constant domains from a human antibody to the variable domains of a non-human species. Examples of how to make humanized antibodies may be found in United States Patent Nos. 6,054,297 , 5,886,152 and 5,877,293.

In still another embodiment of the invention, a "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin inserted into the a human antibody “scaffold” being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity. Methods to obtain "humanized antibodies" are well known to those of ordinary skill in the art. (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

A "humanized antibody" may also be obtained by a novel genetic engineering approach that enables production of affinity-matured humanlike polyclonal antibodies in large animals such as, for example, rabbits (see, e.g., U.S. Patent No. 7,129,084).

The term "monoclonal antibody" is also well recognized in the art and refers to an antibody that is mass produced in the laboratory from a single clone and that recognizes only one antigen. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell to a fast-growing cell, such as a cancer cell (sometimes referred to as an "immortal" cell). The resulting hybrid cell, or hybridoma, multiplies rapidly, creating a clone that produces large quantities of the antibody. For the purpose of the present invention, "monoclonal antibody" is also to be understood to comprise antibodies that are produced by a mother clone which has not yet reached full monoclonality.

The term "CDRs" refers to the hypervariable region of an antibody. The term "hypervariable region", "HVR", or "HV", when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions are based on sequence variability and are the most commonly used (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)).

The letters "HC" and "LC" preceding the term "CDR" refer, respectively, to a CDR of a heavy chain and a light chain, Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol.196:901-917 (1987)). The AbM hypervariable regions

represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures.

The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

"Functionally equivalent antibody" is understood within the scope of the present invention to refer to an antibody which substantially shares at least one major functional property with an antibody, for example functional properties herein described including, but not limited to: binding specificity to the free IL-18 protein. The antibodies can be of any class such as IgG, IgM, or IgA, etc or any subclass such as IgG1, IgG2a, etc and other subclasses described herein or known in the art, but particularly of the IgG4 class. Further, the antibodies can be produced by any method, such as phage display, or produced in any organism or cell line, including bacteria, insect, mammal or other type of cell or cell line which produces antibodies with desired characteristics, such as humanized antibodies. Antibodies can also be formed by combining a Fab portion and an Fc region from different species.

Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie et al. Science 253:164(1991).

The term "human antibody" includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In a preferred embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). These antibodies may be prepared in a variety of host cells such as a prokaryotic cell, for example, E. coli. In another embodiment, the host cell is a eukaryotic cell, for example, a protist cell, an animal cell, a plant cell, plants or a fungal cell. In an embodiment, the host cell is a mammalian cell including, but not limited to, CHO, COS, NS0, SP2, PER.C6, or a fungal cell, such as

Saccharomyces cerevisiae, or an insect cell, such as Sf9. In another embodiment, cells producing human antibodies can be grown in bioreactors or for plants in green houses and fields(see, for example, in: Riechmann L, et al (1988). *Nature* **332** (6162): 332–323; Queen C, et al. (Dec 1989). *Proc Natl Acad Sci U S A.* **86** (24): 10029–33; Kashmiri SV, et al. (May 2005).. *Methods* **36** (1): 25–34; Hou S, et al (July 2008).. *J Biochem* **144** (1): 115–20).

in host.

A "patient" or "subject" for the purposes of the present invention is used interchangeably and meant to include both humans and other animals, particularly mammals, and other organisms. Thus, the methods are applicable to both human therapy and veterinary applications. In the preferred embodiment the patient or subject is a mammal, and in the most preferred embodiment the patient or subject is a human.

The expressions "pharmaceutical composition" and “therapeutical composition” are used herein interchangeably in the widest sense. They are meant to refer, for the purposes of the present invention, to a therapeutically effective amount of the active ingredient, i.e. the IL-18BP and, optionally, a pharmaceutically acceptable carrier or diluent.

It embraces compositions that are suitable for the curative treatment, the control, the amelioration, an improvement of the condition or the prevention of a disease or disorder in a human being or a non-human animal. Thus, it embraces pharmaceutical compositions for the use in the area of human or veterinary medicine. Such a “therapeutic composition” is characterized in that it embraces at least one IL-18BP compound or a physiologically acceptable salt thereof, and optionally a carrier or excipient whereby the salt and the carrier and excipient are tolerated by the target organism that is treated therewith.

A "therapeutically effective amount" refers to that amount which provides a therapeutic effect for a given condition and administration regimen. In particular, "therapeutically effective amount" means an amount that is effective to prevent, reverse, alleviate or ameliorate symptoms of the disease or prolong the survival of the subject being treated, which may be a human or non-human animal. Determination of a therapeutically effective amount is within the skill of the person skilled in the art. In particular, in the present case a "therapeutically or prophylactically effective amount" refers to the amount of protein or peptide, mutein, functional derivative, fraction, circularly permuted derivative, fused protein, isoform or a salt thereof, and compound or pharmaceutical composition which, when administered to a human or animal, leads to a therapeutic or prophylactic effect in said human or animal. The effective amount is readily determined by one of skill in the art following routine procedures. The therapeutically effective amount or dosage of a compound according to this invention can vary within wide limits and may be determined in

a manner known in the relevant art. The dosage can vary within wide limits and will, of course, have to be adjusted to the individual requirements in each particular case.

The term “transmucosal” administration refers to various administration routes wherein the compound is absorbed by the mucosa of any part of the body. Transmucosal administration comprises, but is not limited to, i.e. intranasal, buccal, oral transmucosal, intratracheal, intraurinary tract, intrarectal, intravaginal, sublingual, intrabronchial, intrapulmonary and transdermal administration.

The definition "pharmaceutically acceptable" is meant to encompass any carrier, excipient, diluent or vehicle, which does not interfere with effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which it is administered.

The term "fused protein" refers to a polypeptide comprising an IL-18BP, or a viral IL-18BP, or a mutein or fragment thereof, fused with another protein, which, e. g., has an extended residence time in body fluids. An IL-18BP or a viral IL-18BP may thus be fused to another protein, polypeptide or the like, e. g., an immunoglobulin or a fragment thereof.

These isoforms, muteins, fused proteins or functional derivatives retain the biological activity of IL-18BP, in particular the binding to IL-18, and preferably have essentially at least an activity similar to IL-18BP. Ideally, such proteins have a biological activity which is even increased in comparison to unmodified IL-18BP. Preferred active fractions have an activity which is better than the activity of IL-18BP, or which have further advantages, like a better stability or a lower toxicity or immunogenicity, or they are easier to produce in large quantities, or easier to purify.

The term "interleukin-18 binding protein" comprises also an IL-18BP mutein, functional derivative, fraction, biologically active peptide, circularly permuted derivative, fused protein, isoform and a salt thereof.

As used herein the term "muteins" refers to analogs of an IL-18BP, or analogs of a viral IL-18BP, in which one or more of the amino acid residues of a natural IL-18BP or viral IL-18BP are replaced by different amino acid residues, or are deleted, or one or more amino acid residues are added to the natural sequence of an IL-18BP, or a viral IL-18BP, without changing considerably the activity of the resulting products as compared with the wild type IL-18BP or viral IL-18BP. These muteins are prepared by known synthesis and/or by site-directed mutagenesis techniques, high throughput mutagenesis, DNA shuffling, protein evolution techniques, or any other known technique suitable therefore.

Any such mutein preferably has a sequence of amino acids sufficiently duplicative of that of an IL-18BP, or sufficiently duplicative of a viral IL-18BP, such as to have substantially

similar activity to IL-18BP. One activity of IL-18BP is its capability of binding IL-18. As long as the mutein has substantial binding activity to IL-18, it can be used in the purification of IL-18, such as by means of affinity chromatography, and thus can be considered to have substantially similar activity to IL-18BP. Thus, it can be determined whether any given mutein has substantially the same activity as IL-18BP by means of routine experimentation comprising subjecting such a mutein, e. g. to a simple sandwich competition assay to determine whether or not it binds to an appropriately labeled IL-18, such as radioimmunoassay or ELISA assay.

Muteins of IL-18BP polypeptides or muteins of viral IL-18BPs, which can be used in accordance with the present invention, or nucleic acid coding therefore, include a finite set of substantially corresponding sequences as substitution peptides or polynucleotides which can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein.

Preferred changes for muteins in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of IL-18BP polypeptides or proteins or viral IL-18BPs, may include synonymous amino acids within a group which have sufficiently similar physicochemical properties that substitution between members of the group will preserve the biological function of the molecule (Grantham, 1974). It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, e. g. under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, e. g. , cysteine residues. Proteins and muteins produced by such deletions and/or insertions come within the purview of the present invention.

"Functional derivatives" as used herein cover derivatives of IL-18BPs or a viral IL-18BP, and their muteins and fused proteins, which may be prepared from the functional groups which occur as side chains on the residues or the N-or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i. e. they do-not destroy the activity of the protein which is substantially similar to the activity of IL-18BP, or viral IL-18BPs, and do not confer toxic properties on compositions containing it.

These derivatives may, for example, include polyethylene glycol side-chains, which may mask antigen sites and extend the residence of an IL-18BP or a viral IL-18BP in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of

free amino groups of the amino acid residues formed with acyl moieties (e. g. alkanol or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl or threonyl residues) formed with acyl moieties.

As "functional fragment" of an IL-18BP, or a viral IL-18BP, mutein and fused protein, the present invention covers any fragment or precursors of the polypeptide chain of the IL-18BP protein molecule alone or together with associated molecules or residues linked thereto, e. g., sugar or phosphate residues, or aggregates of the protein molecule or the sugar residues by themselves, provided said fraction has substantially similar activity to IL-18BP.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of the IL-18BP molecule or analogs thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids, such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids, such as, for example, acetic acid or oxalic acid. Of course, any such salts must retain the biological activity of IL-18BP, e. g. the ability to bind IL-18.

"Isoforms" of IL-18BP are proteins capable of binding IL-18 or fragment thereof, which may be produced by alternative splicing.

The term "circularly permuted derivatives" as used herein refers to a linear molecule in which the termini have been joined together, either directly or through a linker, to produce a circular molecule, and then the circular molecule is opened at another location to produce a new linear molecule with termini different from the termini in the original molecule. Circular permutations include those molecules whose structure is equivalent to a molecule that has been circularized and then opened. Thus, a circularly permuted molecule may be synthesized de novo as a linear molecule and never go through a circularization and opening step. The preparation of circularly permutated derivatives is described in W095/27732.

The expression “abnormal levels of free IL-18” refers to increased or decreased levels of IL-18 compared to the values detected in body fluids of a healthy control subject. In particular, these abnormal levels mean increased values of IL-18. In particular, said abnormal level of free IL-18 in the body fluids exceeds the level in body fluids of a healthy control subject by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%. In certain embodiments of the invention the reference or control value is the normal, non-pathologic base value for free IL-18 determined in the patient to be treated.

The expression “abnormal ratio of free IL-18/IL-18BP” refers to an increased ratio of IL-18 to IL-18BP compared to values found in body fluids of a healthy control subject. In particular, said abnormal ratio of free IL-18 to IL-18BP in the body fluids exceeds the ratio in body fluids of a healthy control subject by 1%, 2.5%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%. In certain embodiments of the invention the reference or control value is the normal, non-pathologic base value for free IL-18 determined in the patient to be treated.

The expressions “gene silencing” and “post transcriptional gene silencing” mean the suppressive regulation of gene expression by mechanisms others than genetic modification. The silencing occurs by mRNA neutralization on the post transcriptional level, wherein mRNA translation is prevented to form an active gene product, which is in most cases a protein.

The term “predisposition” means the increased susceptibility of a subject for developing a specific disease. In the present case a subject is classified as predisposed if for instance elevated IL-18 level appear in the lung, serum, sputum, broncho-alveolar lavage fluid (BALF) or circulation.

The expressions “smoke”, “smoke-induced”, “cigarette smoke” or “cigarette smoke induced” refer to tobacco smoke.

“Alveolar macrophages” are a subtype of macrophages found in the pulmonary alveolus. They often contain granules of exogenous material that they have picked up from the respiratory surfaces. Such black granules are especially common in people, which are long-time exposed to fine dust, fine particles, e.g. like smoker or long-term city dwellers.

A “Th2 cytokine response” mediated by IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, and/or IL-17A, particularly IL-4 and/or IL-8 and/or IL-17A, whereas a “Th1 cytokine response” is mediated by interferon-gamma (IFN-γ), IL-2, and tumor necrosis factor-alpha (TNF-α).

The expression “IL-18/IL-18BP imbalance” relates to the dysregulation of mutual interaction of IL-18 and IL-18BP, which finally leads to an elevated level of unbound IL-18.

A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

A disease or disorder is "alleviated" if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a subject, or both, are reduced.

The terms "dysregulated" or "dysregulation," as used herein, refer to an impairment in a biological process which in turn may lead to deleterious physiological sequela, or abnormal expression of a gene, nucleic acid, protein, peptide, or other biological molecule. In the case where expression of a gene, nucleic acid, protein, peptide, or other biological molecule is dysregulated, the gene, nucleic acid, protein, peptide, or other biological molecule is expressed, processed, or maintained at levels that are outside what is considered the normal range for that of that gene, nucleic acid, protein, peptide, or other biological molecule as determined by a skilled artisan. Dysregulation of a gene, nucleic acid, protein, peptide, or other biological molecule in a mammal may be determined by measuring the level of a gene, nucleic acid, protein, peptide, or other biological molecule in the mammal and comparing the level measured in that mammal to level measured in a matched population known not to be experiencing dysregulation of that gene, nucleic acid, protein, peptide, or other biological molecule dysregulated. Alternatively, the level may be compared to one measured in the same individual at a different time.

The terms “heart disease” or “cardiovascular disease” as used herein comprises diseases and disorders that affect the heart muscle or the blood vessels of the heart and the body. Heart diseases may lead to cardiac failure and eventually are one of the most frequent causes of death in industrial societies. Examples for heart diseases induced by IL-18/IL-18BP imbalance comprise, but are not limited to obstructive heart disease, thrombolytic dysfunction, alcoholic cardiomyopathy, aortic valve prolapse, aortic valve stenosis, arrhythmias, cardiogenic shock, congenital heart disease, dilated cardiomyopathy, heart attack, heart failure, heart tumor, heart valve pulmonary stenosis, hypertrophic cardiomyopathy, idiopathic cardiomyopathy, ischemic heart disease, ischemic cardiomyopathy, mitral regurgitation, mitral valve prolapse, peripartum cardiomyopathy, stable angina.

The term “diabetes mellitus type 2” as used herein is the most common form of diabetes. This disease or disorder is characterized that either the body does not or only insufficiently produce the enzyme insulin or cells have defects in their response to insulin. Such defects are believed to involve the insulin receptor.

As used herein "endogenous" refers to any material from or produced inside an organism, cell, tissue or system. The term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "expression" as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter. The term "expression vector" as used herein refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules, siRNA, ribozymes, and the like. Expression vectors can contain a variety of control sequences, which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operatively linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well. The expression vector according to the present invention can be used in gene therapy for the treatment of the disease or disorder as disclosed herein. In particular, said expression vector is a viral vector. The viruses that can be used as a vehicle to deliver the expression vector is selected from the group of retrovirus, adenovirus, lentivirus, herpes simplex virus, vaccinia, pox virus, and adeno-associated virus.

The terms "inhibit", “neutralize” or “block” as used herein, have to be understood as synonyms which mean reducing a molecule, a reaction, an interaction, a gene expression, an mRNA, and/or a protein's expression, stability, function or activity by a measurable amount or to prevent entirely. Inhibitors are compounds that, e.g., bind to, partially or totally block stimulation, decrease, prevent, delay activation, inactivate, desensitize, or down regulate a protein, a gene, and an mRNA stability, expression, function and activity, e.g., antagonists.

The term “antisense expression vector” refers to an expression vector, which encodes for single-stranded or double-stranded RNA that is complementary to a messenger RNA (mRNA) strand and which inhibits translation of said mRNA into amino acids. The term antisense RNA comprises asRNA, siRNA, shRNA, microRNA.

The term “gene therapy” as used herein means the use of DNA, e.g. an expression vector, as a pharmaceutical agent to treat a disease as disclosed herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Comparison of total and free IL-18 in individual sepsis patients. Adapted from Novick et al 2001. The level of free IL-18 (closed circles) in sera of sepsis patients upon admission was calculated based on the concentration of total IL-18 (open circles) and IL-18BPa, taking into account a 1:1 complex of IL-18 and IL-18BPa and a calculated KD of 400 pM. Each vertical line links total and free IL-18 in an individual serum sample. The

above ELISA assays are performed with the pair of antibodies developed by Taniguchi et al 1997 1, namely antibodies 125-2H as primary/ capture antibody and 159-12B as secondary/ developing antibody.

Figure 2: Detection of total IL-18 with antibodies 125-2H and 159-12B. The data indicates that both antibodies quantify total IL-18.

Figure 3: Titration of 400pg/ml IL-18 as a function of IL-18BP level

Figure 4: Mouse IL-18 induction in the lung airway space at day 5 after 1) air exposure, 2) tobacco smoke (TS), 3) p[I:C] alone, 4) p[I:C] combined to tobacco smoke at day 4 (induction of exacerbation). Dotted line indicates lower limit of detection. Statistical analyses were performed using either the unpaired t-test.

Figure 5: Inhibition of total cell infiltration in the mouse lung airway space at day 5 after 1) air exposure, 2) tobacco smoke (TS), 3) p[I:C] alone, 4) p[I:C] combined to tobacco smoke at day 4 (induction of exacerbation), 5-7) p[I:C] combined to tobacco smoke at day 4 under IL-18BP treatment at either 1, 3 or 10 mg/kg, 8) dexamethasone treatment at 10 mg/kg. Statistical analyses were performed using either the unpaired t-test.

Figure 6: Inhibition of total cell infiltration in the mouse lung airway space at day 5 after 1) air exposure, 2) tobacco smoke (TS), 3) p[I:C] alone, 4) p[I:C] combined to tobacco smoke at day 4 (induction of exacerbation), 5) p[I:C] combined to tobacco smoke at day 4 under IL-18BP treatment at 10 mg/kg, 6) dexamethasone treatment at 10 mg/kg. Statistical analyses were performed using ANOVA test (post-test Sidak's).

Figure 7: Inhibition of neutrophil infiltration by IL-18BP. Neutrophil infiltration in the mouse lung airway space was monitored at day 5 after 1) air exposure, 2) tobacco smoke (TS), 3) p[I:C] alone, 4) p[I:C] combined to tobacco smoke at day 4 (induction of exacerbation), 5-7) p[I:C] combined to tobacco smoke at day 4 under IL-18BP treatment at either 1, 3 or 10 mg/kg, 8) dexamethasone treatment at 10 mg/kg. Statistical analyses were performed using ANOVA test (post-test Sidak's).

Figure 8: Inhibition of neutrophil infiltration by IL-18BP. Neutrophil infiltration in the mouse lung airway space was monitored at day 5 after 1) air exposure, 2) tobacco smoke (TS), 3) p[I:C] alone, 4) p[I:C] combined to tobacco smoke at day 4 (induction of exacerbation), 5) p[I:C] combined to tobacco smoke at day 4 under IL-18BP treatment at 10 mg/kg, 6) dexamethasone treatment at 10 mg/kg. Statistical analyses were performed using ANOVA test (post-test Sidak's).

Figure 9: Inhibition of G-CSF pathway by IL-18BP. The presence of G-CSF (pg/ml) was monitored in the mouse lung airway space by ELISA at day 5 after 1) air exposure, 2)

tobacco smoke (TS), 3) p[I:C] alone, 4) p[I:C] combined to tobacco smoke at day 4 (induction of exacerbation), 5-7) p[I:C] combined to tobacco smoke at day 4 under IL-18BP treatment at either 1, 3 or 10 mg/kg, 8) dexamethasone treatment at 10 mg/kg. Dotted line indicates lower limit of detection. Statistical analyses were performed using Students t-test.

Figure 10: Mitigation of weight loss by IL-18BP. Mouse weight loss was monitored at day 5 after 1) air exposure, 2) tobacco smoke (TS), 3) p[I:C] alone, 4) p[I:C] combined to tobacco smoke at day 4 (induction of exacerbation), 5) p[I:C] combined to tobacco smoke at day 4 under IL-18BP treatment at 10 mg/kg, 7) dexamethasone treatment at 10 mg/kg. Statistical analyses were performed using ANOVA test (post-test Sidak's).

Figure 11: shows the amino acid sequences of the variable heavy chain (VH) and the variable light chain (VK) of antibodies produced by different clones. The complementary determining regions CDR 1, CDR 2 and CDR 3 are identified by underlining the respective sequences as determined by the IMGT numbering system (Lefranc, M.-P. et al., Nucleic Acids Research, 27, 209-212 (1999)). From left to right, the first underlined sequence in each of the VH and VK sequences shown represents CDR1, the second underlined sequence represents CDR 2 and the third underlined sequence represents CDR 3. The variable domain is highlighted in **BOLD.**

SEQUENCES

SEQ ID NO 1: IL-18 Epitope 1: Tyr-Phe-Gly-Lys-Leu-Glu-Ser-Lys-Leu-Ser-Val-Ile-Arg-Asn

SEQ ID NO 2: IL-18 Epitope 2: Phe-Ile-Ile-Ser-Met-Tyr-Lys-Asp-Ser-Gln-Pro-Arg-Gly-Met-Ala-Val-Thre-Ile-Ser-Val-Lys

SEQ ID NO 3: IL-18 Epitope 3: Glu-Met-Asn-Pro-Pro-Asp-Asn-Ile-Lys-Asp-Thr-Lys-Ser-Asp-Ile-Ile-Phe

SEQ ID NO 4: IL-18 Epitope 4: Tyr-Phe-Gly-Lys-Leu-Glu-Ser

SEQ ID NO 5: IL-18 Epitope 5: Tyr-Lys-Asp-Ser-Gln-Pro-Arg-Gly-Met-Ala

SEQ ID NO 6: IL-18 Epitope 6: Asp-Asn-Ile-Lys-Asp-Thr-Lys

SEQ ID NO 7: IL-18 Binding Protein (IL-18BP)

SEQ ID NO: 8: 13-amino acid Linker Sequence: Glu-Phe-Gly-Ala-Gly-Leu-Val-Leu-Gly-Gly-Gln-Phe-Met

SEQ ID NO: 9: Antibody 107C6 VH sequence

SEQ ID NO:10: Antibody 107C6 VK sequence

SEQ ID NO: 11: Antibody 108F8 VH sequence

SEQ ID NO: 12: Antibody 108F8 VK sequence

SEQ ID NO: 13: Antibody 109A6 VH sequence

SEQ ID NO: 14: Antibody 109A6 VK sequence

SEQ ID NO: 15: Antibody 111A6 VH sequence
SEQ ID NO: 16: Antibody 111A6 VK sequence 1
SEQ ID NO: 17: Antibody 111A6 VK sequence 2
SEQ ID NO: 18: Antibody 131B4 VH sequence
SEQ ID NO: 19: Antibody 131B4 VK sequence
SEQ ID NO: 20: Antibody 131E8 VH sequence 1
SEQ ID NO: 21: Antibody 131E8 VH sequence 2
SEQ ID NO: 22: Antibody 131E8 VK sequence
SEQ ID NO: 23: Antibody 132H4 VH sequence
SEQ ID NO: 24: Antibody 132H4 VK sequence
SEQ ID NO: 25: Antibody 133A6 VH sequence
SEQ ID NO: 26: Antibody 133A6 VK sequence
SEQ ID NO: 27: Antibody 107C6 VH sequence CDR1: Gly Tyr Thr Phe Thr Asn Tyr Gly
SEQ ID NO: 28: Antibody 107C6 VH sequence CDR2; Ile Asn Thr Tyr Ser Gly Val Pro
SEQ ID NO: 29: Antibody 107C6 VH sequence CDR3: Ala Arg Glu Gly Tyr Ser Thr Thr Arg Ser Met Asp Tyr
SEQ ID NO: 30: Antibody 107C6 VK sequence CDR1: Gln Ser Leu Leu Asp Ser Arg Thr Arg Lys Asn Tyr
SEQ ID NO: 31: Antibody 107C6 VK sequence CDR2: Trp Ala Ser
SEQ ID NO: 32: Antibody 107C6 VK sequence CDR3: Lys Gln Ser Tyr Asn Leu Arg Thr
SEQ ID NO: 33: Antibody 108F8 VH sequence CDR1: Gly Tyr Thr Phe Thr Asn Tyr Gly
SEQ ID NO: 34: Antibody 108F8 VH sequence CDR2: Ile Asn Thr Tyr Ser Gly Val Pro
SEQ ID NO: 35: Antibody 108F8 VH sequence CDR3: Ala Arg Glu Gly Tyr Ser Thr Thr Arg Ser Met Asp Tyr
SEQ ID NO: 36: Antibody 108F8 VK sequence CDR1: Gln Ser Leu Leu Asp Ser Arg Thr Arg Lys Asn Tyr
SEQ ID NO: 37: Antibody 108F8 VK sequence CDR2: Trp Ala Ser
SEQ D NO: 38: Antibody 108F8 VK sequence CDR3: Lys Gln Ser Tyr Asn Leu Arg Thr
SEQ ID NO: 39: Antibody 109A6 VH sequence CDR1: Gly Phe Lys Ile Lys Asp Thr Tyr
SEQ ID NO: 40: Antibody 109A6 VH sequence CDR2: Ile Asp Pro Ala Asn Gly Asn Thr
SEQ ID NO: 41: Antibody 109A6 VH sequence CDR3: Ala Gly Tyr Val Trp Phe Ala Tyr
SEQ ID NO: 42: Antibody 109A6 VK sequence CDR1: Gln Arg Leu Val His Ser Asn Gly Asn Thr Tyr
SEQ ID NO: 43:: Antibody 109A6 VK sequence CDR2: Thr Val Ser
SEQ D NO: 44: Antibody 109A6 VK sequence CDR3: Ser Gln Ser Thr Leu Val Pro Trp Thr
SEQ ID NO: 45: Antibody 111A6 VH sequence CDR1: Gly Phe Lys Ile Lys Asp Thr Tyr
SEQ ID NO: 46 Antibody 111A6 VH sequence CDR2: Ile Asp Pro Ala Asn Gly Asn Thr

SEQ ID NO: 47: Antibody 111A6 VH sequence CDR3: Ala Gly Tyr Val Trp Phe Ala Tyr
SEQ ID NO: 48: Antibody 111A6 VK sequence 1 CDR1: Ser Ser Val Ser Ser Ser Tyr
SEQ ID NO: 49: Antibody 111A6 VK sequence 1 CDR2: Ser Thr Ser
SEQ ID NO 50: Antibody 111A6 VK sequence 1 CDR3: Gln Gln Tyr Ser Gly Tyr Pro Leu Thr
SEQ ID NO: 51: Antibody 111A6 VK sequence 2 CDR1: Gln Arg Leu Val His Ser Asn Gly Asn Thr Tyr
SEQ ID NO: 52: Antibody 111A6 VK sequence 2 CDR2: Thr Val Ser
SEQ ID NO: 53: Antibody 111A6 VK sequence 2 CDR2: Ser Gln Ser Thr Leu Val Pro Trp Thr
SEQ ID NO: 54: Antibody 131B4 VH sequence CDR1: Gly Phe Lys Ile Lys Asp Thr Tyr
SEQ ID NO: 55: Antibody 131B4 VH sequence CDR2: Ile Asp Pro Ala Asn Gly Asn Thr
SEQ ID NO: 56: Antibody 131B4 VH sequence CDR3: Ala Gly Tyr Val Trp Phe Ala Tyr
SEQ ID NO: 57: Antibody 131B4 VK sequence CDR1: Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr
SEQ ID NO: 58: Antibody 131B4 VK sequence CDR2: Lys Val Ser
SEQ ID NO: 59: Antibody 131B4 VK sequence CDR3: Ser Gln Ser Ser Leu Val Pro Trp Thr
SEQ ID NO: 60: Antibody 131E8 VH sequence 1 CDR1: Gly Phe Ser Leu Pro Asn Tyr Gly
SEQ ID NO: 61: Antibody 131E8 VH sequence 1 CDR2: Ile Trp Ser Gly Gly Ser Thr
SEQ ID NO: 62: Antibody 131E8 VH sequence 1 CDR3: Ala Arg Asn Phe Tyr Ser Lys Tyr Asp Tyr Ala Met Asp Tyr
SEQ ID NO: 63: Antibody 131E8 VH sequence 2 CDR1: Gly Tyr Thr Phe Thr Ser Tyr Trp
SEQ ID NO: 64: Antibody 131E8 VH sequence 2 CDR2: Ile Asn Pro Asn Ser Gly Ser Thr
SEQ ID NO: 65: Antibody 131E8 VH sequence 2 CDR3: Ala Arg Leu Gly Asp Tyr
SEQ ID NO: 66: Antibody 131E8 VK sequence CDR1: Ser Ser Val Ser Tyr
SEQ ID NO: 67: Antibody 131E8 VK sequence CDR2: Asp Thr Ser
SEQ ID NO: 68: Antibody 131E8 VK sequence CDR3: Phe Gln Gly Ser Gly Tyr Pro Leu Thr
SEQ ID NO: 69: Antibody 132H4 VH sequence CDR1: Gly Phe Thr Phe Ser Asn Tyr Ala
SEQ ID NO: 70: Antibody 132H4 VH sequence CDR2: Ile Ser Ser Gly Gly Ala Asn Ile
SEQ ID NO: 71: Antibody 132H4 VH sequence CDR3: Ala Arg Gly Asp Tyr Phe Asn His Phe Trp Phe Ala Tyr
SEQ ID NO: 72: Antibody 132H4 VK sequence CDR1: Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr
SEQ ID NO: 73: Antibody 132H4 VK sequence CDR2: Lys Val Ser
SEQ ID NO: 74: Antibody 132H4 VK sequence CDR3: Phe Gln Gly Ser His Val Pro Trp Thr
SEQ ID NO: 75: Antibody 133A6 VH sequence CDR1: Gly Phe Thr Phe Ser Asn Tyr Ala
SEQ ID NO: 76: Antibody 133A6 VH sequence CDR2: Ile Ser Ser Gly Gly Gly Asn Ile

SEQ ID NO: 77: Antibody 133A6 VH sequence CDR3: Ala Arg Gly Asp Tyr Ser Asn Tyr Phe Trp Phe Ala Tyr
SEQ ID NO: 78: Antibody 133A6 VK sequence CDR1: Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr
SEQ ID NO: 79: Antibody 133A6 VK sequence CDR2: Lys Val Ser
SEQ ID NO: 80: Antibody 133A6 VK sequence CDR3: Phe Gln Gly Ser His Val Pro Trp Thr

EXAMPLES

A. Detection of free IL-18 versus complex IL-18/IL-18BP

1. Common detection of IL-18 in patients

Human IL-18 quantification in patients is performed with ELISA assays detecting total IL-18 (both free form and IL-18BP complex). The ELISA comprises commercially available antibodies (see Table 8 below). Most common ELISA assays are performed with the pair of anti-IL-18 antibodies developed by Taniguchi et al 1997 and sold by different suppliers, namely monoclonal mouse antibody 125-2H as primary/ capture antibody and monoclonal rat 159-12B as secondary/ developing antibody.

Table 8: Scientific publications reporting IL-18 quantifications in human patients

References	Assay, disease	Antibodies and commercial source
Wong CK et al 2000	IL-18 and IL-12 levels in plasma, Systemic Lupus Erythematosus	1. Human IL-18 ELISA kit from MBL, #7620 2. Human IL-12 ELISA kit from R&D Systems, #DP400
Park MC et al 2004	IL-18 level in serum, Systemic Lupus Erythematosus	Human IL-18 ELISA kit from R&D Systems same as MBL kit #7620

Table 8: Scientific publications reporting IL-18 quantifications in human patients		
References	Assay, disease	Antibodies and commercial source
Novick D et al 2001	IL-18 and IL-18BP in serum, Sepsis	1. Two human IL-18 antibodies from R&D systems (mouse monoclonal biotinylated as capture # N/A and rabbit polyclonal ruthenylated as detection # N/A) 2. Two IL-18BP antibodies developed by Interpharm and Serono that are not commercially available, clone MAb No. 582.10 as capture antibody (see above, paragraph 2.2. IL-18BP detection in human serum and urine) and rabbit polyclonal antibody for detection
Novick D et al 2010	IL-18 and IL18BP levels in serum, Systemic Lupus Erythematosus	Same as Novick et al 2001, see previous row
Chen DY et al 2004	IL-18 levels in serum, Adult Still's Disease	Human IL-18 ELISA kit from Bender MedSystems (now eBioscience) comprising 2 human IL-18 antibodies called BMS267/2MST: 1. Monoclonal capture antibody # N/A 2. Monoclonal detection antibody labeled with biotin # N/A and reaction revealed with streptavidin-HRP

2. Estimations of free IL-18 levels

To date, there are no reports of measured levels of free IL-18. Estimations of free IL-18 are made by extrapolation using the calculation described by Novick et al 2004 (see below). The data compares levels of IL-18 and IL-18BP in human. In these studies, researchers

used the pair of commercial monoclonal anti-IL-18 antibodies 125-2H and 159-12B, where antibody 125-2H is used for capture and is known to bind the IL-18/IL-18BP complex (Argiradi et al 2009).. To calculate free IL-18 in patient sera, they applied the Law of Mass Action assuming that the binding of IL-18 antibodies is reversible. The calculation is performed as follow:

K_D = 0.4 nM = ([IL-18] x [IL-18BP]) / [IL-18-IL18BP]
or [IL-18] in nM = (0.4 x [IL-18-IL18BP]) / [IL-18BP]
Where:
IL-18-IL-18BP is a complex
Dissociation constant as calculated by Kim et al 2000, K_D = 0.4 nM
Stoichiometry 1:1 in the complex IL-18-IL-18BP
Concentration of IL-18 is determined by electro-chemiluminescence
Concentration of IL-18BP is determined by ELISA

It is important to note that the authors find large variations of free IL-18 versus the total IL-18 between patients that do not reflect the ratio of IL-18 versus IL-18BP. Interestingly, this IL18/IL-18BP ratio is not reported in the cited publications. Furthermore, anti-IL18 antibodies are not able to distinguish between free IL-18 and the complex form IL-18/IL-18BP. Finally, as described by Novick et al 2001, the anti-IL-18BP antibodies do not detect IL-18BP free form but total IL-18BP since they were reported not to block the interaction between IL-18BP and IL-18, respectively monoclonal antibodies 582.10 and 657.27. Consequently, the calculation of free IL-18 using the concentration of IL-18BP lacks accuracy. Even though encouraging, the data variation indicates that free IL-18 detection could be improved with a more appropriate assay combining antibodies specifically targeting the region of IL-18 that binds to IL-18BP.

3. Confirmation that commonly used commercially antibodies do not detect free IL-18

Eleven commercially available anti-IL-18 monoclonal antibodies were tested for their ability to prevent any IL-18 interaction with IL-18BP. The below data demonstrates that this is not the case and that none of the antibodies tested bind to the site of interaction between IL-18 and IL-18BP. Consequently, the detection of free IL-18 in human samples requires specific design and approaches targeting for example the IL-18 binding site/ epitope to IL-18BP.

The commonly used 125-2H and 159-12B antibodies were tested for both as capture and developing antibodies (see Figure 2). The data indicates that both antibodies do not recognize the IL-18 epitope for IL-18BP and consequently provide only a quantification of total IL-18 (both forms free and complex to IL-18BP).

In parallel to antibodies 125-2H and 159-12G, nine other commercial monoclonal antibodies were tested for their potential to detect free IL-18 in the same conditions as above. As described above, such antibody will be valuable to detect free IL-18 in biological samples. The list of tested commercial antibodies is given in the table 9 below.

Table 9: Tested monoclonal anti-IL-18 antibodies

Company	**Antibody name**
MBL International	D043-3, clone 25-2G
	D-045-6 159-12B biotin
Santa Cruz Biotechnologies	sc-13602 (1.51E3E1)
	sc-133127 (E-8)
Abnova	MAB 1308, clone mxsghk-18
	MAB8223, clone SB116c1
	MAB8224, cone SB116b1
	MAB9935, clone 2
Millipore	04-1503 Anti-Interleukin 18 (clone CPTC-IL18-1)
Lifespan	LS-C137620 (clone 50008-2)

The collected data indicates that none of the commercially available antibodies was able to distinguish the free IL-18 from its complex with IL-18BP.

4. ELISA set up to detect free IL-18

4.1. Capture of free IL-18 with IL-18BP

Microplate wells are coated with an appropriate volume phosphate buffer saline solution containing recombinant human IL-18BP. Plates are incubated for a period of time at 4°C and then stabilized with a blocking buffer containing bovine serum albumin or other appropriate blocking agents. Once the reaction is finished, microplates are sealed and stored at 4°C until used for detection of free IL-18. Microplates can also be dried in a stabilizing solution allowing storage at room temperature and then be reconstituted by hydration when needed for assay.

As an example, for a final reaction volume of 100 µl, dispense first 80 µl of biotin/ antibody conjugate. Samples or biological fluids containing free IL-18 are tested with the IL-18BP coated microplates. After that, 20 µl sample volume containing biological fluid or standard is

dispensed per microplate well. Non-diluted or diluted biological fluid can be but is not restricted to serum, urine, tear, saliva, bile, sweat, exhalation or expiration, sputum, bronchoalveolar fluid, sebum, cellular, gland, mucosa or tissue secretion, biopsy, homogenized tissue. The free IL-18 standard concentrations range between 4.2 pg/ ml to 3000 pg/ ml. Standard and concentrations were prepared from commercially available recombinant human IL-18. The plates are sealed and then incubated under gentle shaking for free IL-18 capture. A suitable period of time is allowed for the reaction ranging from minutes to hours at room temperature, 37°C or other temperatures that do not affect the stability of the samples and reagents. The microplate wells are the washed extensively with the appropriate buffer and then, 100 µl buffer developing mixture is added to each well. The developing mixture contains a streptavidin-conjugated enzyme such as peroxidase or alkaline phosphatase. The microplate wells are sealed and the reaction is allowed for a period of time at A suitable period of time ranging from minutes to hours at room temperature, 37°C or other temperatures that do not affect the stability of the samples and reagents. The resulting reactions are then monitored with a microplate reader at an appropriate nanometer wavelength for absorbance or fluorescence of the produced reagent.

4.2. Capture of free IL-18 with anti-IL-18 antibody

Microplate wells are coated with an appropriate volume phosphate buffer saline solution containing Antibody X. Plates are incubated for a period of time at 4°C and then stabilized with a blocking buffer containing bovine serum albumin or other appropriate blocking agents. Once the reaction is finished, microplates are sealed and stored at 4°C until used for detection of free IL-18. Microplates can also be dried in a stabilizing solution allowing storage at room temperature and then be reconstituted by hydration when needed for assay.

As an example, for a final reaction volume of 100 µl, dispense first 80 µl of biotin/ antibody conjugate. Samples or biological fluids containing free IL-18 are tested with the IL-18BP coated microplates. After that, 20 µl sample volume containing biological fluid or standard is dispensed per microplate well. Non-diluted or diluted biological fluid can be but is not restricted to serum, urine, tear, saliva, bile, sweat, exhalation or expiration, sputum, bronchoalveolar fluid, sebum, cellular, gland, mucosa or tissue secretion, biopsy, homogenized tissue. The free IL-18 standard concentrations range between 4.2 pg/ ml to 3000 pg/ ml. Standard and concentrations were prepared from commercially available recombinant human IL-18. The plates are sealed and then incubated under gentle shaking for free IL-18 capture. A suitable period of time is allowed for the reaction ranging from

minutes to hours at room temperature, 37°C or other temperatures that do not affect the stability of the samples and reagents. The microplate wells are the washed extensively with the appropriate buffer and then, 100 µl buffer developing mixture is added to each well. The developing mixture contains a streptavidin-conjugated enzyme such as peroxidase or alkaline phosphatase. The microplate wells are sealed and the reaction is allowed for a period of time at A suitable period of time ranging from minutes to hours at room temperature, 37°C or other temperatures that do not affect the stability of the samples and reagents. The resulting reactions are then monitored with a microplate reader at an appropriate nanometer wavelength for absorbance or fluorescence.

4.3. Titration of free IL-18 as a function of IL-18BP level

A constant quantity of recombinant IL-18 was titrated as a function of different and well defined quantities of IL-18BP in order to understand when free IL-18 is not any more detectable. A PBS solution of 400 pg/mL IL-18 supplemented by 5% BSA was spiked with defined quantities of IL-18BP ranging from 0 to 10'000 pg/mL. The molar ratios were calculated according to the respective molecular weight of IL-18 and IL-18BP. The free IL-18 detection was performed with ELISA using IL-18BP for IL-18 as described above. The collected data presented in Figure 1 indicates that 400 pg/mL IL-18 detection are near background detection level when IL-18BP concentration is equal or higher to 6000 pg/mL representing a molar ratio IL-18BP/ IL-18 of ~15 fold higher IL-18BP. In contrast, when molar ratio is lower than 15, free IL-18 is easily detectable.

4.4. Revised calculation of dissociation constant (K_D) between human IL-18 and IL-18BP

4.4.1 K_D calculation by titration

A K_D of 400 pM is reported in the literature based on BIAcore measurements (Kim et al 2000 [8]). However, due to the above results, the K_D was revisited with the above ELISA set up. Titration of 10 pM IL-18 was performed with increasing concentrations of IL-18BP (60 pM – 3 nM) in either a) healthy volunteer sera depleted in endogenous IL-18BP or b) PBS supplemented by 5% BSA. The free IL- 18 ELISA in addition to commercially available assays for total IL-18 and total IL-18BP allows the determination of K_D in solution which should reflect better the affinity of IL-18 to its binding protein in body fluids than data from solid-phase BIAcore method. Example of results are exposed in Table 10.

Table 10: Titration of IL-18 in serum or 5% BSA solution containing 1.87 nM IL-18BP

Standard curve			**IL-18 Titration**				
pg/mL IL-18	OD450 nm		Final IL18 spiked ng/mL	IL-18 spiked into	IL-18 spiked into 5%	nM IL-18	nM IL-18BP

				serum OD450 nm	BSA OD450 nm		
2000	2.894		24	0.474	1.151	1.3953	1.87
666.7	2.292		20	0.342	0.897	1.1628	1.87
222.2	0.875		16	0.286	0.735	0.9302	1.87
74.1	0.303		12	0.200	0.511	0.6977	1.87
24.7	0.114		8	0.157	0.348	0.4651	1.87
8.2	0.061		4	0.091	0.188	0.2326	1.87
2.7	0.042		2	0.065	0.155	0.1163	1.87
0	0.039		0	0.040	0.037	0	1.87

K_D was calculated based on the following formula:

K_D = [free IL-18] x [free IL-18BP] / [IL-18/IL-18BP complex]

[free IL-18BP] = [total IL-18BP] – [free IL-18]

[IL-18/IL-18BP complex] = [total IL-18] – [free IL-18]

Result: K_D = 50 pM (Serum diluent) ; 35 pM (5% BSA diluent)

The titration result indicates a K_D of respectively 50 pM in serum diluent and 35 pM in PBS supplemented by 5% BSA. In contrast to the previous estimations of the K_D between human IL-18BP and IL-18, the newly calculated K_D indicates that previous estimations of free IL-18 based on the K_D of 400 pM reported by Kim et al 2000 are not accurate.

4.4.2 K_D estimation by BIAcore

Following the above K_D results obtain by titration, we tested the binding affinity of IL-18BP to IL-18 with a simpler BIAcore setup consisting of binding IL-18BP to the BIAcore chip and then testing its affinity to IL-18. The method setup is the contrary of Kim et al 2000 [8], who bound IL-18 to the BIAcore chip with a monoclonal antibody and then tested the affinity of the complex antibody-IL-18 to IL-18BP. Importantly, the new BIAcore setup collected data that are aligned completely to the above titration findings, i.e. a K_D ranging between 20 and 30 pM. The data is presented in Table 11 below.

Table 11: New BIAcore estimation of human IL-18BP affinity to human IL-18

K_a (10^{+5}/ Ms)	K_d (10^{-6} 1/s)	K_D (10^{-11} M)
5.3 ± 1.2	13.3 ± 2.7	25.9 ± 4.8

4.5. Titration of spiked IL-18 in serum or 5% BSA solution containing IL-18BP

Human serum contains significant levels of endogenous as well as complexed IL-18 to IL-18BP, respectively at ng/mL and pg/ mL levels. Both are detectable with commercially available antibodies. However, no commercially available assays are available to detect free IL-18. In order to verify the above ELISA setup for the detection of free IL-18, we spiked recombinant human IL-18 in human serum to find levels of detection. For this, nanograms of IL-18 were spiked in either serum containing endogenous 35 ng/mL IL-18BP or PBS solution supplemented by 5% BSA and 35 ng/mL IL-18BP. Resulting free IL-18 was monitored with the ELISA procedure described above. Results are presented in Table 12 below.

Table 12: Spiked IL-18 detection in serum or 5% BSA containing 35 ng/ml IL-18BP

Standard curve			**IL-18 Titration**		
pg/mL IL-18	OD450 nm		Final IL18 spiked ng/mL	IL-18 spiked into serum OD450 nm	IL-18 spiked into 5% BSA OD450 nm
2000	3.171		100	3.5	3.5
666.7	1.388		80	3.5	3.5
222.2	0.477		70	2.37	3.5
74.1	0.183		60	0.99	3.37
24.7	0.085		50	0.68	2.05
8.2	0.050		40	0.46	1.17
2.7	0.043		30	0.298	0.75
0	0.043		20	0.185	0.44
			10	0.11	0.16
			5	0.06	0.09
			2	0.05	0.07
			0	0.04	0.04

5. Detection of free IL-18 in patients

5.1. Detection of free IL-18 in serum and synovial fluid from patients suffering from different inflammatory diseases

Samples coming from patients suffering of different inflammatory diseases were tested with the ELISA described above. For this, we selected different disease and stress conditions reported with higher levels of IL-18 such as rheumatoid arthritis, psoriasis, systemic lupus erythematosus and intensive care unit. To our knowledge, no free IL-18 has been identified in those patients, only by calculation with the Law of Mass Action and the K_D of 400 pM reported by Kim et al 2000. According to the above data, it was expected that possible levels of free IL-18 will be difficult to detect due to the total IL-18 levels ranging in serum

below or close to 1000 pg/mL as reported in scientific publications. Furthermore, we tested samples from healthy age-matched controls to verify the performance of our ELISA setup. As expected and contrary to the reported Law of Mass Action estimations, the levels of free IL-18 were not detectable neither in serum nor synovial fluid whereas total IL-18 and IL-18BP were (see Table 13).

Table 13: Detection of free IL-18 in patients from intensive care unit, with psoriasis, lupus and rheumatoid arthritis

Patient #	Patient condition/ disease	Biological fluid	Total IL18 pg/ml	Free IL18 pg/ ml	Calculated free IL-18 pg/ml $K_D = 4 \times 10^{-10}$M	IL18BP ng/ml
1	Healthy	Serum	209.7	-	40.4	29.7
2	Healthy	Serum	125.5	-	24.7	28.9
3	Healthy	Serum	189.7	-	28.2	40.6
4	Healthy	Serum	284.7	-	65.9	23.6
5	Healthy	Serum	227.2	-	55.5	22.0
6	Healthy	Serum	319.7	-	56.4	33.2
7	Healthy	Serum	145.5	-	26.4	32.0
8	Healthy	Serum	206.3	-	59.4	17.6
9	Healthy	Serum	323.0	-	56.5	33.5
10	Healthy	Serum	208.0	-	49.6	22.7
11	Intensive care**	Serum	1158.8	-	52	151.3
12	Intensive care**	Serum	3769.0	-	170.9	151.9
13	Intensive care**	Serum	623.8	-	27.9	151.3
14	Intensive care**	Serum	1978.8	-	104.7	128.0
15	Intensive care	Serum	611.3	-	66.9	57.9
16	Intensive care	Serum	434.7	-	34.3	82.7
17	Psoriasis arthritis serum	Serum	713.8	-	70.6	64.9
17	Psoriasis arthritis synovial fluid	Synovial fluid	533.0	-	78.1	41.5
18	Lupus serum	Serum	510.5	-	123.4	22.5
18	Lupus synovial fluid	Synovial fluid	820.5	-	158.9	30.0
19	Lupus serum	Serum	503.8	-	66.4	46.9
19	Lupus synovial fluid	Synovial fluid	236.3	-	24.9	60.1

20	Rheumatoid arthritis	Plasma	416.3	-	39.9	66.8
21	Rheumatoid arthritis	Serum	281.3	-	67.5	22.6
22	Rheumatoid arthritis	Serum	490.5	-	42.7	74.4
23	Rheumatoid arthritis	Serum	337.2	-	52.2	38.8
24	Rheumatoid arthritis	Serum	342.2	-	53.5	38.3
25	Rheumatoid arthritis	Serum	677.2	-	90.6	46.2
26	Rheumatoid arthritis	Serum	238.8	-	41	34.2
27	Rheumatoid arthritis	Serum	183.8	-	41	24.7
28	Rheumatoid arthritis	Serum	385.5	-	41.6	58.6
29	Rheumatoid arthritis	Serum	345.5	-	42.5	50.6

- : not detectable, levels comparable to the background signal
**: High IL-18BP levels not within standard curve

5.2. Detection of free IL-18 in serum from patients suffering from Adult onset Still's Disease

Following the results and in contrast to the above indications having reasonably low levels of total IL-18, we tested Adult onset Still's Disease patient samples which is known for its elevated levels of total IL-18 in serum (Kawashima et al 2001 and Chen et al 2004). As described by Kawashima et al 2001 and elsewhere, elevated total IL-18 serum levels correlate with Adult onset Still's Disease activity such as a) pyrexia, arthralgia, arthritis, cartilage damage, b) higher levels of Ferritin and c) liver enzymes (LDH). Thanks to the above ELISA set up, we report for the first time free IL-18 levels in Adult onset Still's Disease patients (see Table 14). As for the other tested indications, calculated free IL-18 levels do not correspond to the detected free IL-18 levels. The collected data indicates at least 70% of patients were positive to free IL-18.

Table 14: Detection of free IL-18 in ASD patient serum and synovial fluid

Patient number	**Sample collection date**	**Biological fluid**	**Total IL-18 pg/ml**	**Free IL-18 pg/ml**	**Calculated Free IL-18 pg/ml $K_D = 4 \times 10^{-10}$M**	**IL-18BP ng/ml**
1		Serum	6699	9.6	1366.5	32.6
1		Synovial fluid	439	15.8	439	-
2		Serum	713	22.5	564.3	2.0

3		Serum	106026	3.2*	59030	50.4
4		Serum	225456	24.9	157207	68.1
5		Serum	175589	23.6	139614	36.1
6		Serum	35045	2.5*	8908	45.6
7		Serum	17714	22.4	634.8	206.0
7		Synovial fluid	133325	21.3	11162	193.6
8		Serum	25020	21.1	1277.4	153.7
9		Serum	3625	24.9	394.7	60.8
10	17.02.2006	Serum	11401	7.7	6062	11.3
10	11.06.2007	Serum	79942	31.6	62035	19.1
10	06.04.2009	Serum	37372	18.9	22252	19.2
10	06.08.2010	Serum	185157	12.1	10566	282.9
10	06.06.2012	Serum	131561	11.2	4091	341.2
11	03.01.2006	Serum	150669	34.3	114012	37.2
11	04.04.2007	Serum	106026	26.2	63543	45.2
11	20.10.2008	Serum	225456	23.6	70633	163.0
11	21.04.2010	Serum	175589	23.3	116583	59.8
12	02.06.2009	Serum	3625	8.0	1633	10.5
13	10.03.2010	Serum	439	4.8**	151.2	13.7
14	17.07.2009	Serum	133325	19.3	21118	144.4
15	24.07.2006	Serum	35045	14.3	14628	29.3
16	25.04.2007	Serum	17714	8.0	4075	36.6
16	10.06.2010	Serum	25020	6.4	2592	82.4

* : Level comparable to the background signal
** : Level comparable to the lower limit of detection
- : not detectable, level comparable to the background signal

6. Conclusions

The data in both publications and the above experimental setup demonstrate that commercial monoclonal antibodies detect total IL-18 but not free IL-18. Furthermore, the most commonly used antibodies to quantify IL-18, namely 125-2H and 159-12B, are confirmed as well in detecting total IL-18.

The estimation of free IL-18 using the Law of Mass Action is an interesting approach. Nevertheless, the large error bars obtained do not support its use in clinical monitoring. Furthermore, the anti-IL-18BP antibodies detect total IL-18BP and not the free form. Consequently, the calculation of free IL-18 using the concentration of IL-18BP lacks accuracy.

The proposed approach to quantify free IL-18 by targeting IL-18 binding site to IL-18BP seems more appropriate and is demonstrated for the first time to be more accurate than extrapolated quantifications with the Law of Mass Action. In addition, the affinity of IL-18BP is higher than reported by Kim et al 2000 with a K_D ranging near 50 pM in serum and 20-30 pM with a new BIAcore setup.

Finally, patients suffering of Adult onset Still's Disease were diagnosed as positive to free IL-18 for the first time with the ELISA approach and a set up is presented in the present invention. The data support earlier findings on total IL-18 for Adult onset Still's Disease as reported by Kawashima et al 2001 and Chen et al 2004 reporting high levels of total IL-18. For the first time, the new ELISA approach presented in this application demonstrates presence of free, not complexed and biologically active pro-inflammatory IL-18 in Adult onset Still's Disease patients.

B. IL-18BP efficacy in COPD exacerbation mouse model

The aim of the study was to determine the effect of IL-18BP, administered at three dose levels, by the sub-cutaneous route, on Polyinosinic:polycytidylic acid-induced exacerbation of tobacco-smoke induced pulmonary inflammation, in C57BL/6J mice. High level of dexamethasone, dosed orally, was included in the study as a reference agent.

1. General methodology: Four-day exacerbation/ tobacco smoke mouse model

Mice received either vehicle (PBS) or IL-18BP. IL-18BP was given subcutaneously to 3 groups of animals respectively at 1, 3 or 10 mg/kg 2h prior to the initial tobacco smoke exposure from Day 1 to Day 4. Mice received orally either vehicle or dexamethasone (10mg/kg) 1h prior to each twice daily exposure. Mice received by intranasal administration either the vehicle or Polyinosinic:polycytidylic acid (2mg/kg) 2h prior to the initial air or tobacco smoke exposure on Day 4 to induce lung inflammation exacerbation. Tobacco smoke exposure was performed during the morning and afternoon as follow: Day 1 for 15 min, Day 2 for 25 min, Day 3 for 30 min and Day 4 for 30 min.

Animal groups and their respective treatment regimes are summarized in Table 1.

Table 15: Treatment regimes for tobacco smoke mouse model

Exposure	Treatment s.c. / oral	Treatment Code	n	Dose mg/kg	Challenge	Frequency
Air	Veh/ Veh	A	10	-/-	Veh	Sub-cutaneous 2h prior to initial TS on each day Oral 1h prior to each TS exposure
TS	Veh/ Veh	B	10	-/-	Veh	
Air	Veh/ Veh	C	10	-/-	p[I:C] 2mg/kg	
TS	Veh/ Veh	D	10	-/-	p[I:C] 2mg/kg	
TS	IL-18BP/ Veh	E	10	1/-	p[I:C] 2mg/kg	

TS	IL-18BP/ Veh	F	10	3/-	p[I:C] 2mg/kg	on each day p[I:C] intranasal 2h prior to TS exposure on day 4
TS	IL-18BP/ Veh	G	10	10/-	p[I:C] 2mg/kg	
TS	Veh/ Dex	H	10	-/10	p[I:C] 2mg/kg	

TS: Tobacco smoke;
Veh: Vehicule;
Dex: Dexamethazone,
p[I:C]: Polyinosinic:polycytidylic acid

Following the above treatments, animals were terminally anaesthetised on Day 5. After that, a blood sample was taken via the sub-clavian artery (plasma) and the animals were bronchoalveloar lavaged with 3 X 0.4ml of PBS for further cellular and cytokine/ mediator analysis. Bronchoalveolar lavage supernatants were stored at -80°C for cytokine/mediator analysis. Cells recovered from the BALF were counted using the Sysmex cell counter. Finally, the collected data was statistically analyzed by Students t-test and ANOVA (Sidak's was used in the case of data passed normality test or Kruskal Wallis test if data did not pass normality test).

2. Confirmation of IL-18 pathway activation in the four-day exacerbation/ tobacco smoke mouse model

Mouse IL-18 was tested in the BAL using a commercial ELISA in order to confirm the mouse model for IL-18 pathway activation. The collected data indicates a clear induction of IL-18 in the lung airway space (see Figure 5). IL-18 is not detectable in the control (air only). Interestingly, IL-18 is expressed under smoke exposure but is not significantly induced under poly[I:C] alone (under the lower limit of detection). In contrast and as expected, the combination of smoke and poly[I:C] raises considerably IL-18 to much higher levels in the BAL than smoke or poly[I:C] alone.

3. Exacerbated inflammation mitigation by IL-18BP in exacerbation/ tobacco smoke mouse model

3.1. Inhibition of total cell infiltration and exacerbated inflammation in the lung airway space by IL-18BP

Mice treated by IL-18BP had a significant mitigation of total cell infiltration in the lung following induction of exacerbated inflammation. Doses of either 3 and 10 mg/kg indicated statistically valuable efficacy compared to the positive control dexamethasone (see Figure 5). It is important to note that dexamethasone had no sign of efficacy at 3 mg/kg doses in

the mouse model (data not shown), indicating that the high dexamethasone dose of 10 mg/kg is potentially inducing apoptosis in certain cell types such as macrophages, eosinophils and lymphocytes (data not shown). Similar observation was made with Roflumilast [3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide] in the mouse model where no hint of cell infiltration inhibition was observed with 2.5 mg/kg dose (data not shown). Figure 6 shows clear and statistically relevant efficacy of IL-18BP at 10 mg/kg in exacerbated inflammation inhibition in the current mouse model.

3.2. Inhibition of neutrophil infiltration in the lung airway space by IL-18BP

Neutrophil infiltration was inhibited by IL-18BP in tobacco smoke-exacerbated lungs. Doses of either 3 and 10 mg/kg IL-18BP indicated statistically valuable efficacy compared to the positive control dexamethasone (see Figure 7). In the current mouse model conditions, IL-18BP 10 mg/kg dose seems to have the best statistical efficacy (see Figure 8).

3.3. Inhibition of granulocyte colony-stimulating factor (G-CSF) pathway in the lung airway space by IL-18BP

G-CSF is well acknowledged as key cytokine stimulating the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. Consequently, mitigation of G-CSF pathway-induced by smoke-p[I:C] is an significant factor demonstrating an effect of IL-18BP on neutrophil recruitment in the mouse lung airway space. The presence of G-CSF in the BALF was monitored with a commercially available ELISA kit. Figure 9 demonstrates that administration of IL-18BP mitigates G-CSF release in the lung airways, thereby confirming the inhibition of neutrophil infiltration. The three tested IL-18BP doses have a statistically relevant effect in the mouse model.

3.4. IL-18BP safety: Effect on weight loss in exacerbation/ tobacco smoke mouse model

IL-18BP administration appeared to be well tolerated by exacerbation/ tobacco smoke mouse model. As an example, weight loss was mitigated by IL-18BP even though both Students t-test and ANOVA statistical analyses were not significant (see Figure 10). A large majority of mice receiving 3 and 10 mg/kg IL-18BP lost respectively 6-7% weight in contrast to the control exposed to tobacco smoke and p[I:C] that lost about 9%. Hence, weight loss alleviation data indicates that IL-18BP is not providing additional stress to the animal model. It is interesting to note that mice receiving only p[I:C] did not lose weight compared to mice receiving the combination of p[I:C] and tobacco smoke [see Figure 8, treatment 3) and 4)].

C. Generation of anti-IL-18 monoclonal antibodies

1. Mouse immunization and monoclonal antibody screening

Mice were vaccinated aginst human interleukin-18 using a technology allowing immunization with properly folded proteins. Prior to immunization, genetically modified mice were selected for major histocompatibility complexes supposedly sensitive to IL-18 surface area epitopes binding IL-18BP. Following immunization, B cells were isolated from spleen and hybridized following standard hybridoma technology. Hybridoma were sorted onto microplates and then tested for expression of monoclonal anti-IL-18 antibodies targeting IL-18 epitopes included in IL-18BP binding site. The screening was performed in 3 sequential and selective steps:

First step. Positive antibody screening attempt was performed with IL-18 attached to Luminex beads confirming cell expressing monoclonal anti-IL-18 antibodies.

Second step. Potential antibodies targeting IL-18 on IL-18BP binding site were rescreened in competition with IL-18BP. For this, monoclonal antibodies were bound to Luminex beads carrying IL-18. The complex was then exposed to biotinylated IL-18BP in order to identify interference to previously identified anti-IL-18 antibodies (see Table 1, Column #2). The second screening carried more than 300 positive antibody candidates (see Table 1, Column #3). The number of positive candidates was surprisingly high suggesting an excellent mouse immunization to the targeted epitope area. However, inhibitions were not sufficient due to diminished but still persistent fluorescence signals, thus indicating binding of IL-18BP to the complexed antibody IL-18. Nevertheless and importantly, such standard screening method reported elsewhere does not take into account a potential steric hindrance of the large antibody molecule (about 160 kDa) against the much smaller IL-18BP (about 18 kDa, peptide only).

Third step. A third screening program was undertaken with Luminex beads linked to IL-18BP and then complexed to interleukin-18, assuring the presentation of properly folded recombinant IL-18 to positive antibody candidates. The resulting screening was considerably more selective because most of the above antibodies still bound the Luminex-IL-18 beads thereby indicating that their previous inhibitory effect to IL-18BP was due to steric hindrance. Finally, a total of 12 antibodies were finally considered as targeting IL-18 on the IL-18BP protein due to their very low fluorescence signal after binding IL-18 in the presence of IL-18BP, namely clone # 107C6, 108F8, 109A6, 111A6, 129C3, 131B4, 131E8, 131H1, 132C12, 132H4, 133A6 and 134B2 (see Table

16, Column #4, selected clones representing inhibition means of more than 500 fold compared to Column #2). The positive antibodies versus a set of negatives are presented in Table 16 below.

The collected data from third screening step (Table 16, Column #4) promoted further mRNA sequencing and clone dilution work to enrich positive monoclonal cells out of # 107C6, 108F8, 109A6, 111A6, 129C3, 131B4, 131E8, 131H1, 132C12, 132H4, 133A6 and 134B2. All of these monoclonal antibodies were confirmed to bind to IL-18 on the IL-18BP binding site.

Table 16: Screening of monoclonal antibodies targeting IL-18 on the IL-18BP binding site

Clone name	Column #1 Monoclonal antibodies binding on IL-18	Column #2 IL-18BP binding on IL-18 previously complexed to monoclonal antibody	Column #3 Monoclonal antibody binding on IL-18 previously complexed to IL-18BP
	Fluorescence intensity		
Examples of negative antibodies not following selection criteria			
101D2	26 963	1 226	1 544
104H10	26 508	1 199	2 499
105A2	21 528	1 886	1 840
106H1	27 178	1 011	1 324
108F3	23 496	1 964	2 383
108G6	25 652	1 137	2 507
115E6	25 752	1 604	2 649
119E9	25 420	1 307	2 931
Positive antibodies following selection criteria			
107C6	26 250	1 389	33
108F8	25 126	1 292	45
109A6	25 848	913	33
111A6	25 855	1 398	42
131B4	24 838	1 656	41
131E8	25 411	1 389	36
131H1	24 806	1 026	24
132C12	24 541	1 515	48

132H4	23 839	1 488	28
133A6	23 273	1 631	25
134B2	24 278	1 261	48
129C3	25 412	760	44

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Further Embodiments of the Invention

1. An IL-18 inhibitor for use in the treatment of an IL-18 associated disease or disorder in a subject diagnosed of having abnormal levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP in the body fluids compared to the levels in body fluids of a healthy control subject.
2. The IL-18 inhibitor for use according to embodiment 1, wherein said abnormal level of free IL-18 in the body fluids exceeds the level in body fluids of a healthy control subject by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%.
3. The IL-18 inhibitor for use in any one of the preceding embodiments, wherein the subject to be treated belongs to a group of subjects which have been determined to have elevated levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP (IL-18BP) in the body fluids, particularly serum, sputum, broncho-alveolar lavage fluid (BALF), synovial fluid and/or circulation compared to the levels in the body fluids of a healthy subject.
4. The IL-18 inhibitor for use in embodiments 2 or 3, wherein said elevated levels of free IL-18 in serum are in the range of 5 to 10000 pg/mL, whereas the amount of free IL-18 in serum of healthy subject, particularly a healthy human is ≤4 pg/mL.
5. The IL-18 inhibitor for use according to any one of the preceding embodiments, wherein said IL-18 associated disease or disorder is one selected from the group consisting of chronic obstructive pulmonary disease (COPD), transfusion-related lung injury, bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), Adult Still's disease, juvenile Still's disease, interstitial lung disease (ILD), idiopathic pulmonary fibrosis, cystic fibrosis, pulmonary arterial hypertension, asthma, bronchiectasis, heart failure, amyotrophic lateral sclerosis (ALS), dry eye disease (DED), keratitis, corneal ulcer and abrasion, corneal neovascularization, pathological intraocular neovascularization, iritis, glaucoma, macular degeneration, Sjögren's syndrome, autoimmune uveitis, Behçet's disease, conjunctivitis, allergic conjunctivitis, dermatitis of eyelid, diabetes type 2, non-alcoholic fatty liver disease (NAFLD), steato hepatitis, solid organ and hematologic transplantation, ischemia reperfusion injury, familial Mediterranean fever, tumor necrosis factor receptor 1-associated periodic syndromes, cryopyrin-associated periodic fever syndromes, hyper-IgD syndromes, gout, Schnitzler syndrome, Wegener's granulomatosis also called granulomatosis with polyangitis (GPA), Hashimoto's thyroiditis, Crohn's disease, ulcerative colitis, immunoglobulin-4 (IgG4)-related diseases and stem cell therapies.

6. The IL-18 inhibitor for use according to any one of the preceding embodiments, wherein said IL-18 associated disease or disorder is induced by smoking or second-hand smoke exposure, in particular tobacco smoke exposure.

7. The IL-18 inhibitor for use according to any one of the preceding embodiments, wherein said IL-18 associated disease or disorder is induced by viral infection.

8. The IL-18 inhibitor for use according to any one of the preceding embodiments, wherein said IL-18 associated disease or disorder is an IL-18 induced systemic manifestation of inflammation and associated comorbidities selected from the group consisting of emphysema, tissue inflammation, tissue destruction, lung resection, disappearance of the vasculature, apoptosis of endothelial cells, mucos metaplasia, cardiac hypertrophy, decrease of VEGF in the lung tissue, pulmonary vessel loss, vessel muscularization,, vascular remodeling, collagen deposition, aberrant elastin layers in the lung, fibrotic airway remodeling, airspace enlargement, chronic remodeling of the airways and pulmonary vessels and decreased pulmonary function.

9. The IL-18 inhibitor for use in any one of the preceding embodiments, wherein treatment comprises prevention, halting, alleviation or reversion of symptoms associated with said disease or disorder.

10. The IL-18 inhibitor for use in any one of the preceding embodiments, wherein IL-18 binding is restricted or inhibited, particularly binding of free IL-18 to IL-18R, but especially binding of free IL-18 to IL-18Rα.

11. The IL-18 inhibitor for use in any one of the preceding embodiments, wherein IL-18-dependent downstream signaling pathways are modified, particularly inhibited.

12. The IL-18 inhibitor for use in any one of the preceding embodiments, wherein increased expression of IFNγ, IL-13 or IL-17A is modified, particularly inhibited, compared to untreated subjects suffering from said disease or disorder.

13. The IL-18 inhibitor for use in any one of the preceding embodiments, wherein the IL-18 inhibitor compensates the IL-18/IL-18BP imbalance by trapping and neutralizing the excess of free IL-18 in tissue and circulation.

14. An IL-18 specific antibody including any functionally equivalent antibody or parts thereof, which antibody or part thereof binds to IL-18 at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP.

15. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to embodiment 14, which antibody of part thereof binds free IL-18 protein, but not IL-18/IL-18BP complexes.

16. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to embodiment 14 or embodiment 15, wherein said antibody or part thereof sterically hinders or prevents the binding of IL-18BP to IL-18.

17. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to embodiments 14 to 16, wherein said antibody or part thereof specifically binds to a single epitope, a combination of two epitopes or a combination of 3 epitopes comprised in a sequence selected from a group of sequences depicted in SEQ ID NO.,:1, SEQ ID NO: 2 and SEQ ID NO: 3.

18. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to embodiment 17, wherein said epitope has a sequence which has 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to a sequence selected from a group of sequences depicted in SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

19. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to embodiment 18, wherein said epitope is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

20. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding embodiments, wherein said antibody or part thereof is a monoclonal antibody or a polyclonal antibody.

21. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding embodiments, wherein said antibody or part thereof is a chimeric, single chain, bispecific, simianized, human and humanized antibody.

22. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding embodiments, wherein said antibody or part thereof binds to human IL-18.

23. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding embodiments, wherein binding of IL-18 to IL-18 receptor, particularly binding to IL-18Rα is reduced by at least 5%, particularly by at least 10%, particularly by at least 15%, particularly by at least 20%, particularly by at least 25%, particularly by at least 30%, particularly by at least 40%, particularly by at least 45%, particularly by at least 50%, particularly by at least 55%, particularly by at least 60%, particularly by at least 65%, particularly by at least 70, particularly by at least

75, particularly by at least 80, particularly by at least 85%, particularly by at least 90%, particularly by at least 95%, particularly by 100%.

24. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding embodiments, wherein said antibody or part thereof neutralizes free IL-18 by restricting or preventing IL-18 binding to IL-18 receptor (IL-18R), especially free IL-18 binding to IL-18Rα.

25. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding embodiments, wherein said antibody or parts thereof

    a) specifically binds to a single epitope, a combination of two epitopes or a combination of 3 epitopes comprised in a sequence selected from a group of sequences depicted in SEQ ID NO:1, SEQ ID NO: 2 and SEQ ID NO: 3; and/or

    b) specifically binds to an epitope, which has a sequence identity of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% to the sequence depicted in SEQ ID NO: 4, SEQ ID NO:5 or SEQ ID NO: 6; and

    c) specifically binds to IL-18 at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP; and

    d) specifically binds to free IL-18 protein, but not IL-18/IL-18BP complexes; and

    e) sterically hinders the binding of IL-18BP to IL-18; and

    f) reduces binding of IL-18 to IL-18 receptor, particularly binding to IL-18Rα by at least 5%, particularly by at least 10%, particularly by at least 15%, particularly by at least 20%, particularly by at least 25%, particularly by at least 30%, particularly by at least 40%, particularly by at least 45%, particularly by at least 50%, particularly by at least 55%, particularly by at least 60%, particularly by at least 65%, particularly by at least 70, particularly by at least 75, particularly by at least 80, particularly by at least 85%, particularly by at least 90%, particularly by at least 95%, particularly by 100%.

26. The IL-18 inhibitor for use according to any one of embodiments 1 to 13, wherein the inhibitor is an antibody, particularly an antibody specific for free IL-18, particularly an antagonistic antibody, which prevents binding of free IL-18 to IL-18 receptor, especially free IL-18 binding to IL-18Rα.

27. The IL-18 inhibitor for use according to embodiment 26, wherein said antibody is the antibody of any one of embodiments 14-25.

28. The IL-18 inhibitor for use according to any one of embodiments 1-13, wherein said abnormal levels of free IL-18 in the body fluids has been determined by use of an antibody according to any one of embodiments 14-25.

29. The IL-18 inhibitor for use according to any one of the embodiments 1 to 13, wherein the inhibitor is IL-18BP, particularly human IL-18BP (hIL-18BP), particularly IL-18BP including any functionally equivalent or parts thereof, particularly an IL-18BP as shown in SEQ ID NO: 7.

30. The IL-18 inhibitor for use in according to embodiments 26-29, which is a full-length protein or a mutein, functional derivative, functional fragment, biologically active peptide, fraction, circularly permuted derivative, fused protein, isoform or a salt thereof.

31. IL-18BP for use in the treatment of chronic obstructive pulmonary disease (COPD), heart disease, dry eye disease and/or diabetes type II.

32. The IL-18BP for use according to embodiment 31 for the treatment of chronic obstructive pulmonary disease (COPD).

33. The IL-18BP for use according to embodiment 31 for the treatment of heart disease.

34. The IL-18BP for use according to embodiment 31 for the treatment of dry eye disease.

35. The IL-18BP for use according to embodiment 31 for the treatment of diabetes type II.

36. The IL-18BP for use according to embodiments 31 to 35, wherein said disease or disorder is induced by smoking or second-hand smoke exposure, in particular tobacco smoke exposure.

37. The IL-18BP for use according to any one of the preceding embodiments, wherein said disease or disorder is induced by viral infection.

38. The IL-18BP for use according to any one of the preceding embodiments, wherein said disease or disorder is an IL-18 induced systemic manifestation of inflammation and associated comorbidities selected from the group consisting of emphysema, tissue inflammation, tissue destruction, lung resection, disappearance of the vasculature, mucos metaplasia, cardiac hypertrophy, decrease of VEGF in the lung tissue, pulmonary vessel loss, vessel muscularization, collagen deposition, aberrant elastin layers in the lung, fibrotic airway remodeling, airspace enlargement, chronic remodeling of the airways and pulmonary vessels and decreased pulmonary function.

39. The IL-18BP for use according to any one of the preceding embodiments, wherein IL-18 binding is restricted or inhibited, particularly binding of free IL-18 to IL-18R, but especially free IL-18 binding to IL-18Rα.

40. The IL-18BP for use according to any one of the preceding embodiments, wherein IL-18-dependent downstream signaling pathways are modified, particularly inhibited.

41. The IL-18BP for use according to any one of the preceding embodiments, wherein increased expression of IFNγ, IL-13 or IL-17A is modified, particularly inhibited, compared to untreated subjects suffering from said disease or disorder.

42. The IL-18BP for use according to any one of the preceding embodiments, wherein the IL-18 inhibitor compensates the IL-18/IL-18BP imbalance by trapping the excess of free IL-18 in tissue and circulation.

43. The IL-18BP for use according to any one of the preceding embodiments, wherein treatment comprises prevention, halting, alleviation or reversion of symptoms associated with said disease or disorder.

44. A pharmaceutical composition for use in the treatment of the disease or disorder as defined in any one of embodiments 1-13 in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder as defined in any one of embodiments 1-13, wherein said composition comprises the IL-18 inhibitor according to any one of embodiments 1-13 and 26-30, particularly in a prophylactically and/or therapeutically effective amount.

45. The pharmaceutical composition of embodiment 44, wherein said composition optionally further provides another inhibitor of a pro-inflammatory cytokine or functional fragment thereof, or a regulatory factor, which induces in-situ expression of said inhibitor of pro-inflammatory cytokine or functional fragment thereof, co-therapeutic agents such as anti-inflammatory, bronchodilatory, antihistamine, decongestant or anti-tussive drug substances.

46. The pharmaceutical composition of embodiment 44 or 45, comprising a pharmaceutically acceptable carrier and/or excipient.

47. A pharmaceutical composition for use in the treatment of the disease or disorder as defined in any one of embodiments 31 to 43 in a subject suffering from such a disease or disorder or having a predisposition to develop such a disease or disorder as defined in any one of embodiments 31 to 43, wherein said composition comprises the IL-18BP according to embodiments 31 to 43, particularly in a prophylactically and/or therapeutically effective amount.

48. The pharmaceutical composition of embodiment 47, wherein said composition optionally further provides another inhibitor of a pro-inflammatory cytokine or functional fragment thereof, or a regulatory factor, which induces in-situ expression of said inhibitor of pro-

inflammatory cytokine or functional fragment thereof, co-therapeutic agents such as anti-inflammatory, bronchodilatory, antihistamine, decongestant or anti-tussive drug substances.

49. The pharmaceutical composition of embodiment 47 or 48, comprising a pharmaceutically acceptable carrier and/or excipient.

50. An expression vector comprising a coding sequence of the IL-18 inhibitor according to any one of embodiments 1-13 and 26-30, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in the preceding embodiments leads to in situ expression of IL-18 inhibitor for use in the treatment of the disease or disorder as defined in any one of embodiments 1-13.

51. An expression vector comprising an IL-18 antisense expressing vector, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in embodiments 1-13, leads to in situ inhibition of the expression of IL-18 for use in the treatment of the disease or disorder as defined in any one of embodiments 1-13.

52. The expression vector of embodiment 50 or 51 for use in the treatment of the disease or disorder as defined in any one of embodiments 1-13 and 26-43, wherein said expression vector is administered to a subject suffering from such a disease or disorder, or having a predisposition to develop such a disease or disorder, alone or in combination with the IL-18 inhibitor according to any one of embodiments 1-13 and 26-30, the IL-18BP according to embodiments 31-43 or the pharmaceutical composition according to any one of embodiments 44-49.

53. An expression vector comprising the coding sequence of IL-18BP according to embodiments 31-43, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in the preceding embodiments, leads to in situ expression of IL-18BP for use in the treatment of the disease or disorder as defined in any one of embodiments 31-43.

54. The expression vector of embodiment 53 for use in the treatment of the disease or disorder as defined in any one of embodiments 1-13 and 26-43, wherein said expression vector is administered to a subject suffering from such a disease or disorder, or having a predisposition to develop such a disease or disorder, alone or in combination with the IL-18 inhibitor according to any one of embodiments 1-13 and 26-30, the IL-18BP according to embodiments 31-43 or the pharmaceutical composition according to any one of embodiments 44-49.

55. The IL-18 inhibitor for use according to any one of embodiments 1-13 and 26-30, the IL-18BP for use according to any one of embodiments 31-43, the pharmaceutical composition for use according to any one of embodiments 44-49 or the expression vector for use according to any one of embodiments 50-54, comprising administering to a subject in need thereof a prophylactically and/or therapeutically effective amount of said IL-18 inhibitor, IL-18BP, pharmaceutical composition, or expression vector, particularly by systemic, intranasal, buccal, oral, transmucosal, intratracheal, intravenous, subcutaneous, intraurinary tract, intravaginal, sublingual, intrabronchial, intrapulmonary, transdermal or intramuscular administration, in particular broncho-pulmonary administration.

56. The IL-18 inhibitor, the IL-18BP, the pharmaceutical composition or the expression vector for use according to embodiment 55, wherein said subject is a mammal, particularly said subject is a human.

57. A method for treating the disease or disorder as defined in any one of embodiments 1-13 and 26-43 in a subject suffering from such a disease or disorder, or having a predisposition to develop such a disease or disorder, comprising administering to said subject a therapeutically or prophylactically effective amount of the IL-18 inhibitor according to any one of embodiments 1-13 and 26-30, the IL-18BP according to embodiments 31-43 or the pharmaceutical composition according to any one of embodiments 44-49 and/or the expression vector according to any one of embodiments 50-54, particularly by systemic, intranasal, buccal, oral, transmucosal, intratracheal, intravenous, subcutaneous, intraurinary tract, intravaginal, sublingual, intrabronchial, intrapulmonary, transdermal or intramuscular administration, in particular broncho-pulmonary administration.

58. A method for diagnosis of the diseases or disorder as defined in any one of embodiments 1-13 and 26-43, for diagnosing a predisposition to the disease or disorder as defined in any one of embodiments 1-13 and 26-43, for monitoring minimal residual disease in a subject, or for predicting responsiveness of a subject to a treatment with IL-18 inhibitor according to embodiments 1-13 and 26-30, the IL-18BP according to embodiments 31-43 or the pharmaceutical composition comprising IL-18 inhibitor according to embodiments 44-49, comprising the steps:

    a) obtaining a sample of body fluid, particularly serum from a subject;

    b) testing said sample for the presence of free IL-18 by using the IL-18 antibody of any one of embodiments 14-25 or the IL-18BP of any one of embodiments …as capturing molecule and/or testing the said sample for the presence of free IL-18BP

by using a first monoclonal IL-18BP specific capturing antibody and an IL-18BP specific detection antibody, which binds to a different site of IL-18BP than the capturing antibody, particularly one of said antibodies binds to the IL-18 binding site of IL-18BP;

c) determining the amount of free IL-18 and/or free IL-18BP bound to the capturing molecule in the sample;

d) comparing the amount of free IL-18 and/or free IL-18BP in the sample of the subject suffering from such a disease to the amount in the sample of a healthy subject.

59. A method for diagnosis of the diseases or disorder as defined in any one of embodiments 1-13 and 26-43, for diagnosing a predisposition to the disease or disorder as defined in any one of embodiments 1-13 and 26-43, for monitoring minimal residual disease in a subject, or for predicting responsiveness of a subject to a treatment with IL-18 inhibitor according to embodiments 1-13 and 26-30, the IL-18BP according to embodiments 31-43 or the pharmaceutical composition comprising IL-18 inhibitor according to embodiments 44-49 and a pharmaceutically acceptable carrier and/or excipient according to any one of the preceding embodiments, comprising the steps:

a) obtaining a sample of body fluid, particularly sputum and serum from a subject;

b) testing said sample for the presence of free IL-18 by using the IL-18 antibody of embodiments 14-25 or the IL-18BP as capturing molecule and/or testing the said sample for the presence of free IL-18BP by using a first monoclonal IL-18BP specific capturing antibody and an IL-18BP specific detection antibody, which binds to a different site of IL-18BP than the capturing antibody, particularly one of said IL-18BP specific antibodies binds to the IL-18 binding site of IL-18BP;

c) testing said sample for the presence of total IL-18 total and/or total IL-18BP by using a first monoclonal IL-18BP specific antibody which does not bind to the IL-18 binding site of IL-18BP and a second IL-18 specific antibody, which does not bind to the IL-18BP binding site of IL-18;

d) determining the amount of free and total IL-18 and/or free and total IL-18BP bound to the capturing molecule in the sample;

e) comparing the amount of free and/or total IL-18 and/or free and/or total IL-18BP in the sample of the subject suffering from such a disease to the amount in the sample of a healthy subject.

60. The method for diagnosis of any one of the preceding embodiments, wherein the amount of free IL-18 in isolated serum of a subject, particularly a human, suffering from said disease ranges from 5 to 10000 pg/mL, whereas the amount of free IL-18 in serum of healthy subject, particularly a healthy human is ≤4 pg/mL.

61. A set of biomarkers for use in the diagnosis of the diseases or disorder as defined in any one of embodiments 1-13 and 26-43, for use in diagnosing a predisposition to the disease or disorder as defined in any one of embodiments 1-13 and 26-43 or for use in monitoring minimal residual disease in a subject, or for predicting responsiveness of a subject to a treatment with IL-18 inhibitor according to embodiments 1-13 and 26-30, the IL-18BP according to embodiments 31-43 or the pharmaceutical composition comprising IL-18 inhibitor according to embodiments 44-49.

62. A method for diagnosis of the diseases or disorder as defined in any one of embodiments 1-13 and 26-43, for diagnosing a predisposition to the disease or disorder as defined in any one of embodiments 1-13 and 26-43 or for monitoring minimal residual disease in a subject, or for predicting responsiveness of a subject to a treatment with IL-18 inhibitor according to embodiments 1-13 and 26-30, the IL-18BP according to embodiments 31-43 or the pharmaceutical composition comprising IL-18 inhibitor according to embodiments 44-49, comprising the steps:

    e) obtaining a biomarker profile of a subject to be tested by taking a sample of a body fluid from said subject;

    f) obtaining a biomarker profile of a healthy reference population;

    g) obtaining a biomarker profile from a population which suffers from said disease or disorder and

    h) comparing the biomarker profile obtained in step a) with the profile obtained in step b) and step c).

63. A pharmaceutical kit comprising IL-18 inhibitor according to any one of embodiments 1-13 and 26-30, IL-18BP according to embodiments 31-43 or a pharmaceutical composition comprising IL-18 inhibitor according to embodiments 44-49 and a pharmaceutically acceptable carrier and/or excipient according to the invention in separate unit dosage forms, said forms being suitable for administration in effective amounts.

64. A diagnostic kit for detecting free IL-18, comprising an IL-18-specific antibody according to any one of embodiments 14-25 as capturing antibody or the IL-18BP as alternative capturing molecule, and a second IL-18 specific detection antibody or an IL-18-specific

antibody according to any one of embodiments 14-25 as detection antibody and a second IL-18 specific capturing antibody, wherein the detection antibody bind to different sites of IL-18 than the capturing molecule.

65. A diagnostic kit for detecting total IL-18 or total IL-18BP, comprising a first monoclonal IL-18BP specific antibody which does not bind to the IL-18 binding site of IL-18BP and a second IL-18 specific antibody, which does not bind to the IL-18BP binding site of IL-18.

66. A diagnostic kit for detecting free IL-18BP, comprising a first monoclonal IL-18BP specific capturing antibody and an IL-18BP specific detection antibody, which binds to a different site of IL-18BP than the capturing antibody.

67. A diagnostic kit, which comprises all diagnostic kits of embodiments 64 to 66.

CLAIMS

1. An IL-18 inhibitor for use in the treatment of an IL-18 associated disease or disorder in a population of subjects diagnosed of having abnormal levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP in body samples, particularly in body fluids, compared to the levels in body fluids of a healthy control subject.

2. The IL-18 inhibitor for use according to claim 1, wherein said abnormal level of free IL-18 in the body fluids exceeds the level in body fluids of a healthy control subject by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%.

3. The IL-18 inhibitor for use in any one of the preceding claims, wherein the subject to be treated belongs to a group of subjects which have been determined to have elevated levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP (IL-18BP) in 18BP in body samples, particularly in a sample selected from the group consisting of broncho-alveolar lavage fluid (BALF) circulation fluids, secretion fluids, biopsy and homogenized tissue, particularly serum, urine, tear, saliva, bile, sweat, exhalation, expiration, sputum, bronchoalveolar fluid, sebum, cellular, gland, mucosa, and tissue secretion compared to the levels in the body fluids of a healthy subject.

4. The IL-18 inhibitor for use in claims 2 or 3, wherein said elevated levels of free IL-18 in serum are ≥5 pg/mL and, particularly, up to 10000 pg/mL, whereas the amount of free IL-18 in serum of healthy subject, particularly a healthy human is ≤4 pg/mL.

5. The IL-18 inhibitor for use according to any one of the preceding claims, wherein said IL-18 associated disease or disorder is one selected from the group consisting of chronic obstructive pulmonary disease (COPD), transfusion-related lung injury, bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), Adult Still's disease, juvenile Still's disease, interstitial lung disease (ILD), idiopathic pulmonary fibrosis, cystic fibrosis, pulmonary arterial hypertension, asthma, bronchiectasis, heart failure, amyotrophic lateral sclerosis (ALS), dry eye disease (DED), keratitis, corneal ulcer and abrasion, corneal neovascularization, pathological intraocular neovascularization, iritis, glaucoma, macular degeneration, Sjögren's syndrome, autoimmune uveitis, Behçet's disease, conjunctivitis, allergic conjunctivitis,

dermatitis of eyelid, diabetes type 2, non-alcoholic fatty liver disease (NAFLD), steato hepatitis, solid organ and hematologic transplantation, ischemia reperfusion injury, familial Mediterranean fever, tumor necrosis factor receptor 1-associated periodic syndromes, cryopyrin-associated periodic fever syndromes, hyper-IgD syndromes, gout, Schnitzler syndrome, Wegener's granulomatosis also called granulomatosis with polyangitis (GPA), Hashimoto's thyroiditis, Crohn's disease, ulcerative colitis, immunoglobulin-4 (IgG4)-related diseases and stem cell therapies.

6. The IL-18 inhibitor for use according to any one of the preceding claims, wherein said IL-18 associated disease or disorder is induced by smoking or second-hand smoke exposure, in particular tobacco smoke exposure.

7. The IL-18 inhibitor for use according to any one of the preceding claims, wherein said IL-18 associated disease or disorder is induced by viral infection.

8. The IL-18 inhibitor for use according to any one of the preceding claims, wherein said IL-18 associated disease or disorder is an IL-18 induced systemic manifestation of inflammation and associated comorbidities selected from the group consisting of emphysema, tissue inflammation, tissue destruction, lung resection, disappearance of the vasculature, apoptosis of endothelial cells, mucos metaplasia, cardiac hypertrophy, decrease of VEGF in the lung tissue, pulmonary vessel loss, vessel muscularization,, vascular remodeling, collagen deposition, aberrant elastin layers in the lung, fibrotic airway remodeling, airspace enlargement, chronic remodeling of the airways and pulmonary vessels and decreased pulmonary function.

9. The IL-18 inhibitor for use according to any one of the preceding claims, wherein treatment comprises prevention, halting, alleviation or reversion of symptoms associated with said disease or disorder.

10. The IL-18 inhibitor for use according to any one of the preceding claims, wherein IL-18 binding is restricted or inhibited, particularly binding of free IL-18 to IL-18R.

11. The IL-18 inhibitor for use according to claim 10, wherein binding of free IL-18 to IL-18Rα is restricted or inhibited.

12. The IL-18 inhibitor for use according to any one of the preceding claims, wherein IL-18-dependent downstream signaling pathways are modified, particularly inhibited.

13. The IL-18 inhibitor for use according to any one of the preceding claims, wherein increased expression of IFNγ, IL-13 or IL-17A is modified, particularly inhibited, compared to untreated subjects suffering from said disease or disorder.

14. The IL-18 inhibitor for use according to any one of the preceding claims, wherein the IL-18 inhibitor compensates the IL-18/IL-18BP imbalance by trapping and neutralizing the excess of free IL-18 in tissue and circulation.

15. The IL-18 inhibitor for use according to any one of the preceding claims, wherein the IL-18 inhibitor inhibits infiltration of neutrophils into the lung, particularly through mitigation of G-CSF release in the lung airways.

16. The IL-18 inhibitor for use according to any one of the preceding claims for the treatment of chronic obstructive pulmonary disease (COPD), heart disease, amyotrophic lateral sclerosis (ALS), dry eye disease and/or diabetes type II.

17. The IL-18 inhibitor for use according to any one of the preceding claims for the treatment of chronic obstructive pulmonary disease (COPD).

18. The IL-18 inhibitor for use according to any one of the preceding claims for the treatment of heart disease.

19. The IL-18 inhibitor for use according to any one of the preceding claims for the treatment of amyotrophic lateral sclerosis (ALS).

20. The IL-18 inhibitor for use according to any one of the preceding claims for the treatment of dry eye disease.

21. The IL-18 inhibitor for use according to any one of the preceding claims for the treatment of diabetes type II.

22. The IL-18 inhibitor for use according to any one of the preceding claims, wherein said disease or disorder is induced by smoking or second-hand smoke exposure, in particular tobacco smoke exposure.

23. The IL-18 inhibitor of for use according to any one of the preceding claims, wherein said disease or disorder is induced by viral infection.

24. The IL- IL-18 inhibitor for use according to any one of the preceding claims, wherein said disease or disorder is an IL-18 induced systemic manifestation of inflammation and associated comorbidities selected from the group consisting of emphysema, tissue inflammation, tissue destruction, lung resection, disappearance of the vasculature, mucos metaplasia, cardiac hypertrophy, decrease of VEGF in the lung tissue, pulmonary vessel loss, vessel muscularization, collagen deposition, aberrant elastin layers in the lung, fibrotic airway remodeling, airspace enlargement, chronic remodeling of the airways and pulmonary vessels and decreased pulmonary function.

25. The IL-18 inhibitor for use according to any one of the preceding claims, wherein IL-18 binding is restricted or inhibited, particularly binding of free IL-18 to IL-18R, but especially free IL-18 binding to IL-18Rα.

26. The IL-18 inhibitor for use according to any one of the preceding claims, wherein the IL-18 inhibitor reduces binding of IL-18 to IL-18 receptor, particularly binding to IL-18Rα by at least 5%, particularly by at least 10%, particularly by at least 15%, particularly by at least 20%, particularly by at least 25%, particularly by at least 30%, particularly by at least 40%, particularly by at least 45%, particularly by at least 50%, particularly by at least 55%, particularly by at least 60%, particularly by at least 65%, particularly by at least 70, particularly by at least 75, particularly by at least 80, particularly by at least 85%, particularly by at least 90%, particularly by at least 95%, particularly by 100%.

27. The IL-18 inhibitor for use according to any one of the preceding claims, wherein the IL-18 inhibitor neutralizes free IL-18 by restricting or preventing IL-18 binding to IL-18 receptor (IL-18R), especially free IL-18 binding to IL-18Rα.

28. The IL-18 inhibitor for use according to any one of the preceding claims, wherein the inhibitor is an IL-18 binding molecule, particularly an IL-18 binding molecule specifically binding free IL-18, particularly an IL-18 binding molecule which prevents binding of free IL-18 to IL-18 receptor, especially free IL-18 binding to IL-18Rα.

29. The IL-18 inhibitor for use according to any one of the preceding claims, wherein the IL-18 binding molecule is IL-18BP, particularly human IL-18BP (hIL-18BP), particularly IL-18BP including any functional equivalents or parts thereof, particularly an IL-18BP as shown in SEQ ID NO: 7.

30. The IL-18 inhibitor of claim 28 or 29 for use according to any one of the preceding claims, which is a full-length protein or a mutein, functional derivative, functional fragment, biologically active peptide, fraction, circularly permuted derivative, fused protein, isoform or a salt thereof.

31. The IL-18 inhibitor for use according to any one of the preceding claims, wherein the IL-18 binding molecule is an antibody including any functionally equivalent antibody or parts thereof, particularly an antibody specific for free IL-18, particularly an antagonistic antibody, which prevents binding of free IL-18 to IL-18 receptor, especially free IL-18 binding to IL-18Rα.

32. The IL-18 inhibitor of claim 31 or 32 for use according to any one of the preceding claims, wherein the IL-18 specific antibody including any functionally equivalent

antibody or parts thereof binds to IL-18 at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but not IL-18/IL-18BP complexes.

33. The IL-18 inhibitor of claim 31 or 32 for use according to any one of the preceding claims, wherein the IL-18 specific antibody including any functionally equivalent antibody or parts thereof is a monoclonal antibody or a polyclonal antibody.

34. The IL-18 inhibitor of claim 31 or 32 for use according to any one of the preceding claims, wherein the IL-18 specific antibody including any functionally equivalent antibody or parts thereof is a chimeric, single chain, bispecific, simianized, human and humanized antibody.

35. The IL-18 inhibitor of any one of claims 31-34 for use according to any one of the preceding claims, wherein the IL-18 specific antibody including any functionally equivalent antibody or parts thereof binds to human IL-18.

36. The IL-18 inhibitor for use according to any one of the preceding claims, wherein said abnormal levels of free IL-18 in body samples, particularly in body fluids, have been determined by use of an IL-18 binding molecule as defined in any one of claims 28-35.

37. A pharmaceutical composition for use in the treatment of an IL-18 associated disease or disorder in a population of subjects diagnosed of having abnormal levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP in the body fluids compared to the levels in body fluids of a healthy control subject or having a predisposition to develop such a disease or disorder as defined in any one of claims 1-27, wherein said composition comprises the IL-18 inhibitor as defined in any one of the preceding claims, particularly the IL-18 inhibitor as defined in claims 28-35, particularly in a prophylactically and/or therapeutically effective amount.

38. The pharmaceutical composition of claim 37 for use in the treatment of the disease or disorder as defined in any one of claims 1-27, wherein said composition comprises the IL-18 inhibitor as defined in any one of claims 29 and 30, particularly in a prophylactically and/or therapeutically effective amount.

39. The pharmaceutical composition of claim 36 for use in the treatment of the disease or disorder as defined in any one of claims 1-27, wherein said composition comprises the IL-18 inhibitor as defined in any one of claims 31-35, particularly in a prophylactically and/or therapeutically effective amount.

40. The pharmaceutical composition of any one of claims 37-39 for use in the treatment of the disease or disorder as defined in any one of claims 1-27, wherein said composition optionally further provides another inhibitor of a pro-inflammatory cytokine or functional

fragment thereof, or a regulatory factor, which induces in-situ expression of said inhibitor of pro-inflammatory cytokine or functional fragment thereof, co-therapeutic agents such as anti-inflammatory, bronchodilatory, antihistamine, decongestant or anti-tussive drug substances.

41. The pharmaceutical composition of any one of claims 37-40 for use in the treatment of the disease or disorder as defined in any one of claims 1-27, comprising a pharmaceutically acceptable carrier and/or excipient.

42. An expression vector comprising a coding sequence of the IL-18 inhibitor according to any one of claims 28-35, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in the preceding claims leads to in situ expression of IL-18 inhibitor for use in the treatment of the disease or disorder as defined in any one of claims 1-27.

43. The expression vector of claim 42 for use in the treatment of the disease or disorder as defined in any one of claims 1-27, wherein said expression vector is administered to a subject suffering from such a disease or disorder, or having a predisposition to develop such a disease or disorder, alone or in combination with the IL-18 inhibitor according to any one of claims 28-35, or the pharmaceutical composition according to any one of claims 37-41.

44. The expression vector of any one of claims 42-43 comprising the coding sequence of IL-18BP according to claims 29-30, which upon administration to a subject suffering from a disease or disorder or having a predisposition to develop such a disease or disorder as defined in the preceding claims, leads to in situ expression of IL-18BP for use in the treatment of the disease or disorder as defined in any one of claims 1-27.

45. The IL-18 inhibitor of any one of claims 28-35, the pharmaceutical composition of any one of claims 37-41, or the expression vector of any one of claims 42-44 for use according to any one of claims 1-27, comprising administering to a subject in need thereof a prophylactically and/or therapeutically effective amount of said IL-18 inhibitor, IL-18BP, pharmaceutical composition, or expression vector, particularly by systemic, intranasal, buccal, oral, transmucosal, intratracheal, intravenous, subcutaneous, intraurinary tract, intravaginal, sublingual, intrabronchial, intrapulmonary, transdermal or intramuscular administration, in particular broncho-pulmonary administration.

46. The IL-18 inhibitor, the pharmaceutical composition or the expression vector for use according to claim 45, wherein said subject is a mammal, particularly said subject is a human.

47. A method for treating an IL-18 associated disease or disorder in a population of subjects diagnosed of having abnormal levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP in the body fluids compared to the levels in body fluids of a healthy control subject, or having a predisposition to develop such a disease or disorder, comprising administering to said subject a therapeutically or prophylactically effective amount of the IL-18 inhibitor as defined in any one of claims 28-35, the pharmaceutical composition of any one of claims 37-41, or the expression vector of any one of claims 42-44, particularly by systemic, intranasal, buccal, oral, transmucosal, intratracheal, intravenous, subcutaneous, intraurinary tract, intravaginal, sublingual, intrabronchial, intrapulmonary, transdermal or intramuscular administration, in particular broncho-pulmonary administration.

48. A method of determining the amount of free IL-18 in a sample or *in situ* comprising detecting the specific binding of the IL-18 binding molecule as defined in claim 28, particularly the IL-18BP as defined in any one of claims 29-30 or the antibody as defined in any one of claims 31-35 to free IL-18 protein in the sample or *in situ* which includes the steps of:

    a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with of the IL-18 binding molecule as defined in claim 28, particularly the IL-18BP as defined in any one of claims 29-30 or the antibody as defined in any one of claims 31-35, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

    b) allowing the IL- the IL-18 binding molecule, the 18BP or the antibody to bind to free IL-18;

    c) detecting the binding of IL-18 to the IL- the IL-18 binding molecule, 18BP or the antibody and determining the amount of free IL-18 in the sample.

49. A method of diagnosing an IL-18 associated disease or disorder, particularly an IL-18 associated disease or disorder as defined in any one of claims 1-27 in a patient comprising detecting the specific binding of the IL-18 binding molecule as defined in claim 28, particularly the IL-18BP as defined in any one of claims 29-30 or the antibody as defined in any one of claims 31-35 to free IL-18 protein in a sample or *in situ* which includes the steps of:

    a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with the IL-18 binding molecule as defined in claim 28, particularly the IL-18BP as defined in any one of claims 29-30 or the antibody

as defined in any one of claims 31-35, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

b) allowing the IL-18 binding molecule, IL-18BP or the antibody to bind to free IL-18;

c) detecting the binding of IL-18 to the IL-18 binding molecule, IL-18BP or the antibody and determining the amount of free IL-18 in the sample;

d) comparing the amount of free IL-18 in the sample of the subject suffering from the diseases or disorder as defined in any one of claims 1-27 to the amount in the sample of a healthy subject.

50. A method for diagnosing a predisposition to an IL-18 associated disease or disorder, particularly an IL-18 associated disease or disorder as defined in any one of claims 1-27 in a patient comprising detecting the specific binding of IL-18 binding molecule as defined in claim 28, particularly the IL-18BP as defined in any one of claims 29-30 or the antibody as defined in any one of claims 31-35 to free IL-18 protein in a sample or *in situ* which includes the steps of:

a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with the IL-18 binding molecule as defined in claim 28, particularly the IL-18BP as defined in any one of claims 29-30 or the antibody as defined in any one of claims 31-35, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

b) allowing the IL-18 binding molecule, IL-18BP or the antibody to bind to free IL-18;

c) detecting the binding of IL-18 to the IL-18 binding molecule, IL-18BP or the antibody and determining the amount of free IL-18 in the sample;

d) comparing the amount of free IL-18 in the sample of the patient suffering from the diseases or disorder as defined in any one of claims 1-27 to the amount in the sample of a healthy patient;

wherein an increase in the amount of said free-IL-18 in the sample compared to a normal control value obtained from a healthy patient indicates that said patient is suffering from or is at risk of developing a disease or disorder as defined in any one of claims 1-27.

51. A method for monitoring minimal residual disease in a patient following treatment with the IL-18 inhibitor as defined in any one of claims 28-35, the pharmaceutical composition of any one of claims 37-41, or the expression vector of any one of claims 42-44, wherein said method comprises:

    a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with the IL-18 binding molecule as defined in claim 28, particularly the IL-18BP as defined in any one of claims 29-30 or the antibody as defined in any one of claims 31-35, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

    b) allowing the IL-18 binding molecule, IL-18BP or the antibody to bind to free IL-18;

    c) detecting the binding of IL-18 to the IL-18 binding molecule, IL-18BP or the antibody and determining the amount of free IL-18 in the sample;

    d) comparing the amount of free IL-18 in the sample of the patient suffering from the diseases or disorder as defined in any one of claims 1-27 to the amount in the sample of a healthy patient;

    wherein an increase in the amount of said free-IL-18 in the sample compared to a normal control value obtained from a healthy patient indicates that said patient is still suffering from a minimal residual disease.

52. A method for predicting responsiveness of a patient to a treatment with the IL-18 inhibitor of any one of claims 28-35, the pharmaceutical composition of any one of claims 37-41, or the expression vector of any one of claims 42-44, wherein said method comprises:

    a) bringing a sample or a specific body part or body area suspected to contain free IL-18 into contact with IL-18 binding molecule as defined in claim 28, particularly the IL-18BP as defined in any one of claims 29-30 or the antibody as defined in any one of claims 31-35, which specifically binds to free IL-18, but not to IL-18 bound in a complex and functions as the capturing molecule for free IL-18;

    b) allowing the IL-18 binding molecule, IL-18BP or the antibody to bind to free IL-18;

    c) detecting the binding of IL-18 to the IL-18 binding molecule, IL-18BP or the antibody and determining the amount of free IL-18 in the sample;

d) comparing the amount of free IL-18 in the sample of the patient suffering from the diseases or disorder as defined in any one of claims 1-27 to the amount in the sample of a healthy patient;

wherein a decrease in the amount of said free-IL-18 in the sample indicates that said patient has a high potential of being responsive to the treatment.

53. The method of any one of claims 48 to 52 comprising the additional step of using in step a) an IL-18BP specific binding molecule, which binds to a different site of IL-18BP than the capturing molecule, particularly wherein one of said molecules binds to the IL-18 binding site of IL-18BP.

54. The method of any one of claims 48 to 53 comprising the additional step of determining in the sample the presence of free IL-18BP by using in step a) an IL-18BP specific capturing molecule and an IL-18BP specific detection molecule, which binds to a different site of IL-18BP than the capturing molecule, particularly, wherein one of said IL-18BP specific molecules binds to the IL-18 binding site of IL-18BP, by determining in step c) the amount of free and total IL-18 and of free and total IL-18BP bound to the capturing molecule in the sample; and by comparing in step d) the amount of free and/or total IL-18 and free and/or total IL-18BP in the sample of the patient suffering from the diseases or disorder as defined in any one of claims 1-27 to to the amount in the sample of a healthy patient.

55. The method according to any one of claims 48-54, wherein said capturing molecule is the IL-18 binding molecule as defined in claim 28.

56. The method according to any one of claims 48-54, wherein said capturing molecule is

a. the IL-18BP as defined in any one of claims 29-30.

b. the free IL-18 specific antibody as defined in any one of claims 31-35.

57. The method according to any one of claims 48-56, wherein said sample is selected from the group consisting of broncho-alveolar lavage fluid (BALF) circulation fluids, secretion fluids, biopsy, and homogenized tissue, particularly serum, urine, tear, saliva, bile, sweat, exhalation or expiration, sputum, bronchoalveolar fluid, sebum, cellular, gland, mucosa or tissue secretion.

58. The method of any one of claims 48-57, wherein the amount of free IL-18 in isolated serum of a subject, particularly a human, suffering from said disease are ≥5 pg/mL and, particularly, up to 10000 pg/mL, whereas the amount of free IL-18 in serum of healthy subject, particularly a healthy human is ≤4 pg/mL.

59. A set of biomarkers for use in the method according to any one of claims 48-58 for further specifying the diseases or disorder as defined in any one of claims 1-27, for diagnosing a predisposition to the disease or disorder as defined in any one of claims 1-27, for monitoring minimal residual disease in a subject, or for predicting responsiveness of a subject to a treatment with IL-18 inhibitor of any one of claims 28-35, the pharmaceutical composition of any one of claims 37-41, or the expression vector of any one of claims 42-44 comprising determining a biomarker profile and correlating the obtained profile with a specific disease or disorder.

60. A set of biomarkers for use according to claim 59, wherein the method comprises the steps of:

    i) obtaining a biomarker profile of a subject to be tested by taking a sample of a body fluid from said subject;

    j) obtaining a biomarker profile of a healthy reference population;

    k) obtaining a biomarker profile from a population which suffers from said disease or disorder and

    l) comparing the biomarker profile obtained in step a) with the profile obtained in step b) and step c).

61. A pharmaceutical kit comprising the IL-18 inhibitor of any one of claims 28-35, the pharmaceutical composition of any one of claims 37-41, or the expression vector of any one of claims 42-44 and a pharmaceutically acceptable carrier and/or excipient according to the invention in separate unit dosage forms, said forms being suitable for administration in effective amounts.

62. A diagnostic kit for detecting free IL-18, comprising the IL-18 binding molecule as defined in claim 28, the IL-18BP as defined in any one of claims 29-30 or the antibody as defined in any one of claims 31-35 as the capturing molecule, and a second IL-18 specific binding molecule as the detection molecule and, optionally, a second IL-18 specific capturing molecule, wherein the detection molecule binds to different sites of IL-18 than the capturing molecule.

63. A diagnostic kit for detecting total IL-18 or total IL-18BP, comprising a first IL-18BP specific binding molecule, which does not bind to the IL-18 binding site of IL-18BP and a second IL-18 specific binding molecule, which does not bind to the IL-18BP binding site of IL-18.

64. A diagnostic kit for detecting free IL-18BP, comprising a first IL-18BP specific binding molecule as the capturing molecule and second IL-18 specific binding molecule as the

detection molecule, wherein said detection molecule binds to a different site of IL-18BP than the capturing molecule.

65. A diagnostic kit, which incorporates the binding molecules of claims 62 to 64.

66. The diagnostic kit of any one of claims 61-65 for use in a method according to any one of claims 46-58.

67. An IL-18 specific antibody including any functionally equivalent antibody or parts thereof, which antibody or part thereof binds to IL-18 at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but not IL-18/IL-18BP complexes.

68. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to claim 67, wherein said antibody or part thereof sterically hinders or prevents the binding of IL-18BP to IL-18.

69. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to claims 67 or 68, wherein said antibody or part thereof specifically binds to a single epitope, a combination of two epitopes or a combination of 3 epitopes comprised in a sequence selected from a group of sequences depicted in SEQ ID NO.,:1, SEQ ID NO: 2 and SEQ ID NO: 3.

70. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to claim 69, wherein said epitope has a sequence which has 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to a sequence selected from a group of sequences depicted in SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

71. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to claim 70, wherein said epitope is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

72. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding claims, wherein said antibody or part thereof is a monoclonal antibody or a polyclonal antibody.

73. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding claims, wherein said antibody or part thereof is a chimeric, single chain, bispecific, simianized, human and humanized antibody.

74. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding claims, wherein said antibody or part thereof binds to human IL-18.

75. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding claims, wherein binding of IL-18 to IL-18 receptor, particularly binding to IL-18Rα is reduced by at least 5%, particularly by at least 10%, particularly by at least 15%, particularly by at least 20%, particularly by at least 25%, particularly by at least 30%, particularly by at least 40%, particularly by at least 45%, particularly by at least 50%, particularly by at least 55%, particularly by at least 60%, particularly by at least 65%, particularly by at least 70, particularly by at least 75, particularly by at least 80, particularly by at least 85%, particularly by at least 90%, particularly by at least 95%, particularly by 100%.

76. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding claims, wherein said antibody or part thereof neutralizes free IL-18 by restricting or preventing IL-18 binding to IL-18 receptor (IL-18R), especially free IL-18 binding to IL-18Rα.

77. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of the preceding claims, wherein said antibody or parts thereof

g) specifically binds to a single epitope, a combination of two epitopes or a combination of 3 epitopes comprised in a sequence selected from a group of sequences depicted in SEQ ID NO:1, SEQ ID NO: 2 and SEQ ID NO: 3; and/or

h) specifically binds to an epitope, which has a sequence identity of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% to the sequence depicted in SEQ ID NO: 4, SEQ ID NO:5 or SEQ ID NO: 6; and

i) specifically binds to IL-18 at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP; and

j) specifically binds to free IL-18 protein, but not IL-18/IL-18BP complexes; and

k) sterically hinders the binding of IL-18BP to IL-18; and

l) reduces binding of IL-18 to IL-18 receptor, particularly binding to IL-18Rα by at least 5%, particularly by at least 10%, particularly by at least 15%, particularly by at least 20%, particularly by at least 25%, particularly by at least 30%, particularly by at least 40%, particularly by at least 45%, particularly by at least 50%, particularly by at least 55%, particularly by at least 60%, particularly by at

least 65%, particularly by at least 70, particularly by at least 75, particularly by at least 80, particularly by at least 85%, particularly by at least 90%, particularly by at least 95%, particularly by 100%.

78. The IL-18 specific antibody according to any one of claims 67 to 77 including any functionally equivalent antibody or parts thereof or an antigen-binding portion thereof comprising

a. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 9, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 10, respectively, including variants thereof; or

b. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 11, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 12, respectively, including variants thereof; or

c. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 13, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 14, respectively, including variants thereof; or

d. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 15, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 16, respectively, including variants thereof; or

e. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 15, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 17, respectively, including variants thereof; or

f. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 18, respectively, and/or at least one, at least two or all three complementarity

determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 19, respectively, including variants thereof; or

g. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 20, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 22, respectively, including variants thereof; or

h. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 21, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 22, respectively, including variants thereof; or

i. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 23, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 24, respectively, including variants thereof; or

j. at least one, at least two or all three complementarity determining regions (CDRs) of the heavy chain variable region as shown in SEQ ID NO: 25, respectively, and/or at least one, at least two or all three complementarity determining regions (CDRs) of the light chain variable region as shown in SEQ ID NO: 26, respectively, including variants thereof;

wherein said antibody, equivalent antibody or antigen-binding portion thereof binds free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but does not bind IL-18/IL-18BP complexes.

79. The IL-18 specific antibody according to claim 78 comprising all 6 CDRs of the light chain variable region and the heavy chain variable region.

80. The IL-18 specific antibody according to any one of claims 78 and 79, wherein said antibody comprises

a. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32, respectively; or

b. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 33, SEQ ID NO: 34, and SEQ ID NO: 35, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38, respectively; or

c. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 39, SEQ ID NO: 40, and SEQ ID NO: 41, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 42, SEQ ID NO: 43, and SEQ ID NO: 44, respectively; or

d. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 48, SEQ ID NO: 49, and SEQ ID NO: 50, respectively; or

e. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 51, SEQ ID NO: 52, and SEQ ID NO: 53, respectively; or

f. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 57, SEQ ID NO: 58, and SEQ ID NO: 59, respectively; or

g. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 60, SEQ ID NO: 61, and SEQ ID NO: 62, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 66, SEQ ID NO: 67, and SEQ ID NO: 68, respectively; or

h. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 63, SEQ ID NO: 64, and SEQ ID NO: 65, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 66, SEQ ID NO: 67, and SEQ ID NO: 68, respectively; or

i. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 69, SEQ ID NO: 70, and SEQ ID NO: 71, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 72, SEQ ID NO: 73, and SEQ ID NO: 74, respectively; or

j. CDR1, CDR2, and CDR 3 of the heavy chain variable region having the sequence as shown in SEQ ID NO: 75, SEQ ID NO: 78, and SEQ ID NO: 77, respectively; and CDR1, CDR2, and CDR 3 of the light chain variable region having the sequence as shown in SEQ ID NO: 78, SEQ ID NO: 79, and SEQ ID NO: 80, respectively.

81. The IL-18 specific antibody according to any one of the preceding claims, including any functionally equivalent antibody or parts thereof or an antigen-binding portion thereof comprising

a. a light chain variable region that has at least 75%, 80%, 82%, 83%,84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the sequence shown in SEQ ID NOs: 10, 12, 14, 16, 17, 19, 22, 24 and 26, respectively, and/or

a heavy chain variable region that has at least 75%, 80%, 82%, 83%,84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the sequence shown in SEQ ID NOs: 9, 11, 13, 15, 18, 20, 21, 23, and 25, respectively.

82. The IL-18 specific antibody according to any one of claims 67 to 81 including any functionally equivalent antibody or parts thereof or an antigen-binding portion thereof comprising

a. the heavy chain variable region as shown in SEQ ID NO: 9, respectively, and/or the light chain variable region as shown in SEQ ID NO: 10, respectively, including variants thereof; or

b. the heavy chain variable region as shown in SEQ ID NO: 11, respectively, and/or the light chain variable region as shown in SEQ ID NO: 12, respectively, including variants thereof; or

c. the heavy chain variable region as shown in SEQ ID NO: 13, respectively, and/or the light chain variable region as shown in SEQ ID NO: 14, respectively, including variants thereof; or

d. the heavy chain variable region as shown in SEQ ID NO: 15, respectively, and/or the light chain variable region as shown in SEQ ID NO: 16, respectively, including variants thereof; or

e. the heavy chain variable region as shown in SEQ ID NO: 15, respectively, and/or the light chain variable region as shown in SEQ ID NO: 17, respectively, including variants thereof; or

f. the heavy chain variable region as shown in SEQ ID NO: 18, respectively, and/or the light chain variable region as shown in SEQ ID NO: 19, respectively, including variants thereof; or

g. the heavy chain variable region as shown in SEQ ID NO: 20, respectively, and/or the light chain variable region as shown in SEQ ID NO: 22, respectively, including variants thereof; or

h. the heavy chain variable region as shown in SEQ ID NO: 21, respectively, and/or the light chain variable region as shown in SEQ ID NO: 22, respectively, including variants thereof; or

i. the heavy chain variable region as shown in SEQ ID NO: 23, respectively, and/or the light chain variable region as shown in SEQ ID NO: 24, respectively, including variants thereof; or

j. the heavy chain variable region as shown in SEQ ID NO: 25, respectively, and/or the light chain variable region as shown in SEQ ID NO: 26, respectively, including variants thereof; or

wherein said antibody, equivalent antibody or antigen-binding portion thereof binds free IL-18 protein, particularly at the binding site of IL-18BP or in the vicinity of the binding site of IL-18BP, but does not bind IL-18/IL-18BP complexes.

83. The IL-18 specific antibody according to any one of claims 67-82, which is a monoclonal, polyclonal, chimeric, single chain, bispecific or bi-effective, simianized, human and humanized antibodies

84. The IL-18 specific antibody according to claim 67-82 which is a human or humanized antibody.

85. The IL-18 specific antibody including any functionally equivalent antibody or parts thereof according to any one of claims 67 to 84 for use in the treatment of an IL-18 associated disease or disorder in a population of subjects diagnosed of having abnormal levels of free IL-18 and/or an abnormal ratio of free IL-18/IL-18BP in the

body fluids compared to the levels in body fluids of a healthy control subject, or having a predisposition to develop such a disease or disorder.

86. The IL-18 specific antibody of claim 85 for use in the treatment of an IL-18 associated disease or disorder according to any one of claims 2-27.

87. The IL-18 inhibitor for use according to any one of the preceding claims, wherein the abnormal levels of free IL-18 in body samples, particularly in body fluids, have been determined by use of a method according to any one of claims 48-58.

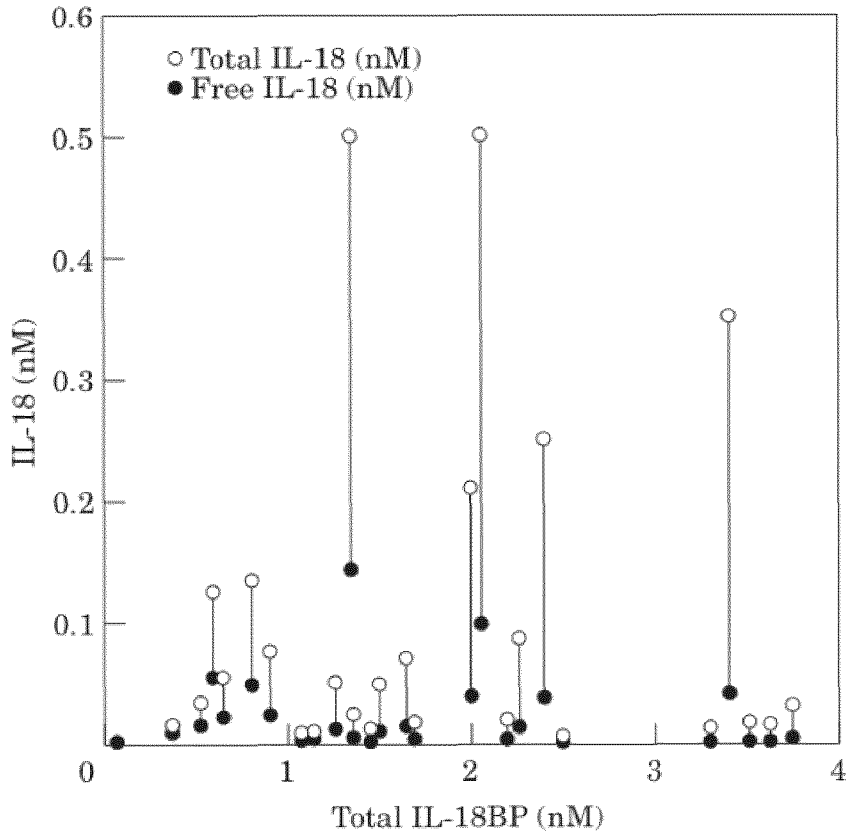
Total IL-18 (nM)
Free IL-18 (nM)
IL-18 (nM)
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
Total IL-18BP (nM)

Figure 1

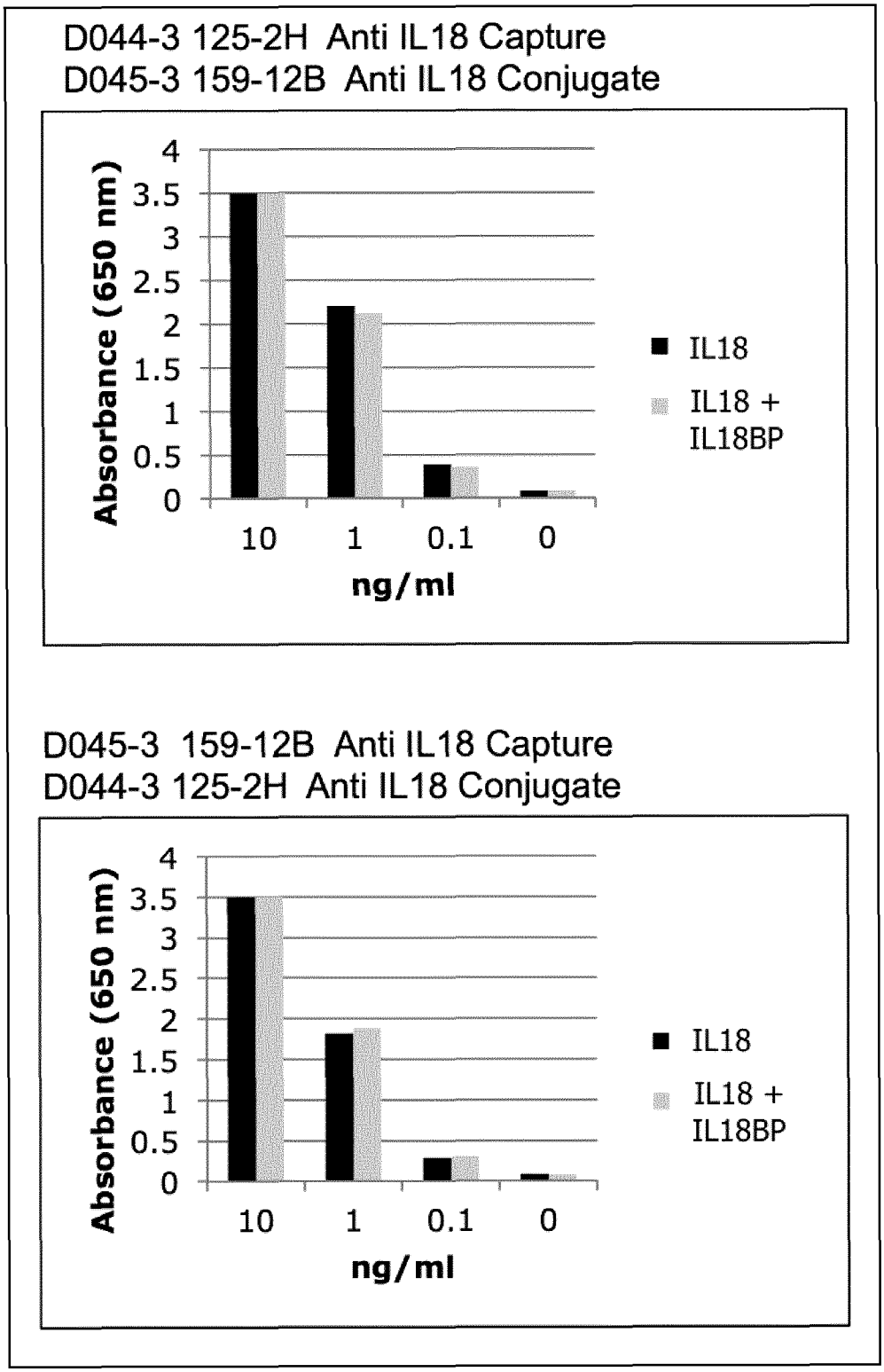
D044-3 125-2H Anti IL18 Capture
D045-3 159-12B Anti IL18 Conjugate
Absorbance (650 nm)
4
3.5
3
2.5
2
1.5
1
0.5
0
10
1
0.1
0
ng/ml
IL18
IL18 + IL18BP
D045-3 159-12B Anti IL18 Capture
D044-3 125-2H Anti IL18 Conjugate
Absorbance (650 nm)
4
3.5
3
2.5
2
1.5
1
0.5
0
10
1
0.1
0
ng/ml
IL18
IL18 + IL18BP

Figure 2

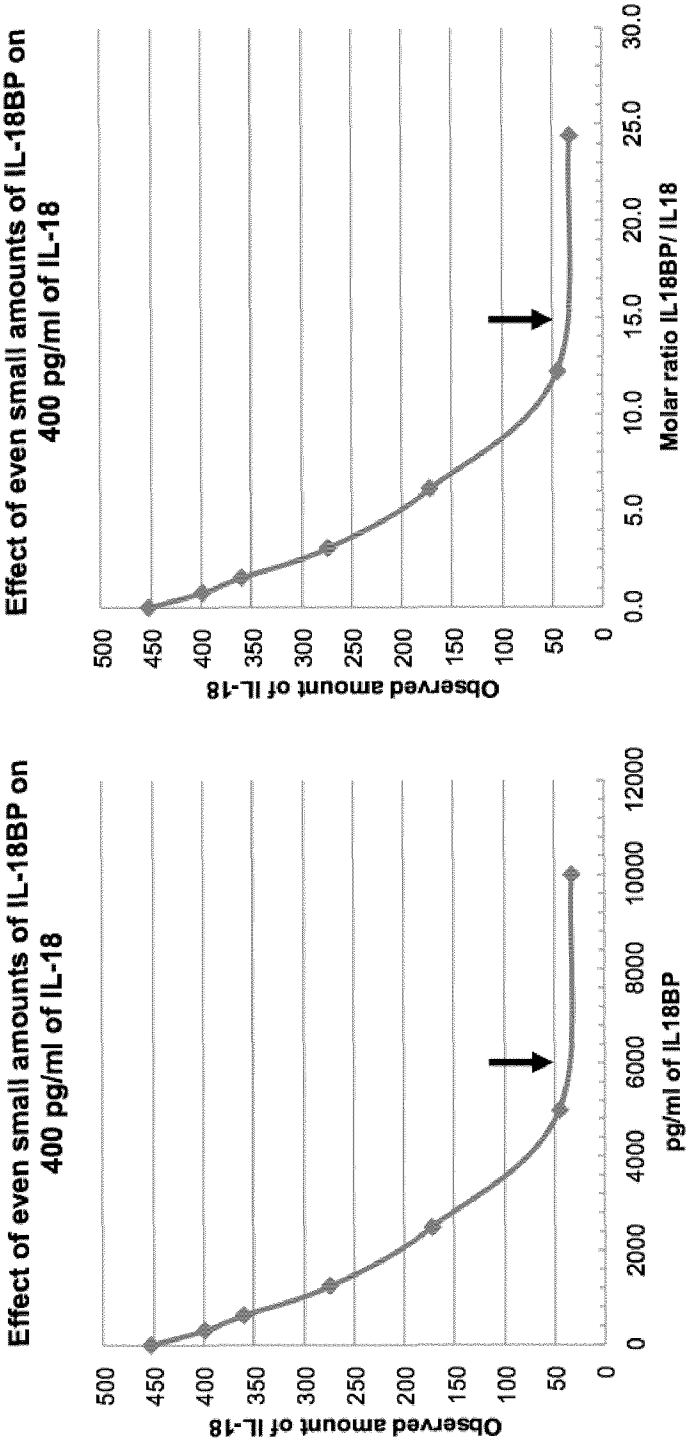
Effect of even small amounts of IL-18BP on 400 pg/ml of IL-18
Observed amount of IL-18
500
450
400
350
300
250
200
150
100
50
0
0
2000
4000
6000
8000
10000
12000
pg/ml of IL18BP
Effect of even small amounts of IL-18BP on 400 pg/ml of IL-18
Observed amount of IL-18
0.0
5.0
10.0
15.0
20.0
25.0
30.0
Molar ratio IL18BP/ IL18

Figure 3

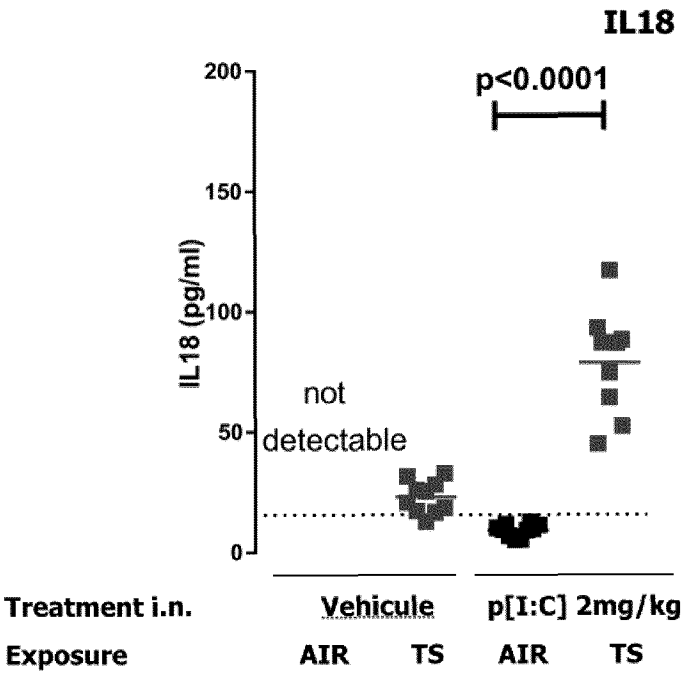
IL18
p<0.0001
200
150
100
50
0
IL18 (pg/ml)
not
detectable
Treatment i.n.
Vehicule
p[I:C] 2mg/kg
Exposure
AIR
TS
AIR
TS

Figure 4

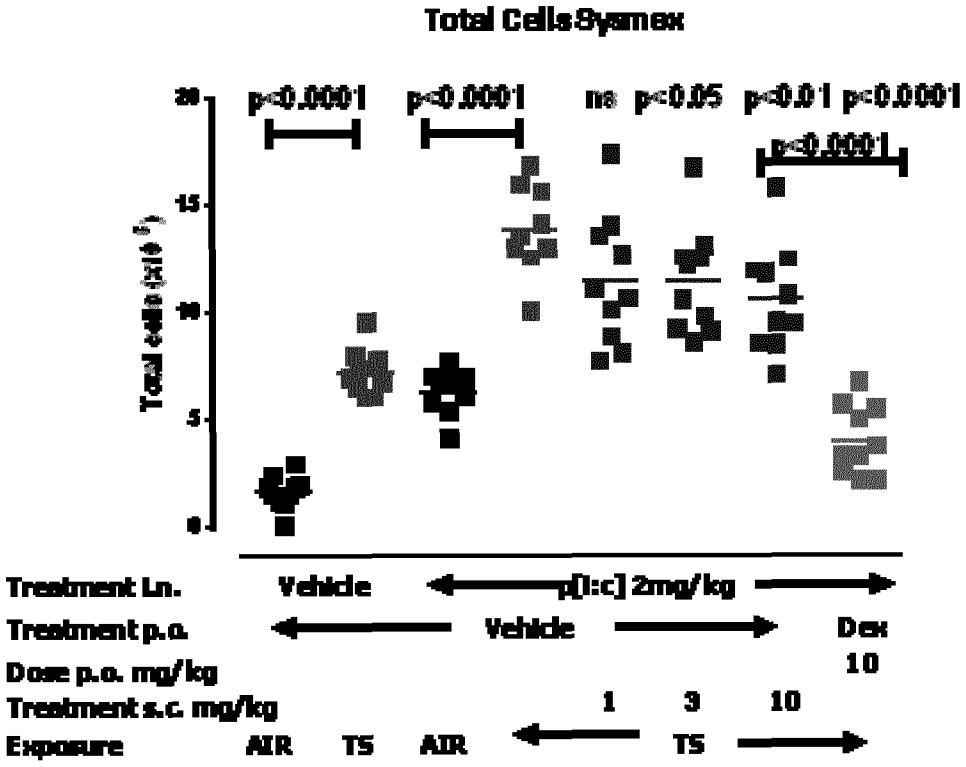
Total Cells Sysmex
p<0.0001
p<0.0001
ns
p<0.05
p<0.01
p<0.0001
p<0.0001
20
15
10
5
0
Total cells (x10 6/)
Treatment i.n.
Vehicle
p[I:c] 2mg/kg
Treatment p.o.
Vehicle
Dex
10
Dose p.o. mg/kg
Treatment s.c. mg/kg
1
3
10
Exposure
AIR
TS
AIR
TS

Figure 5

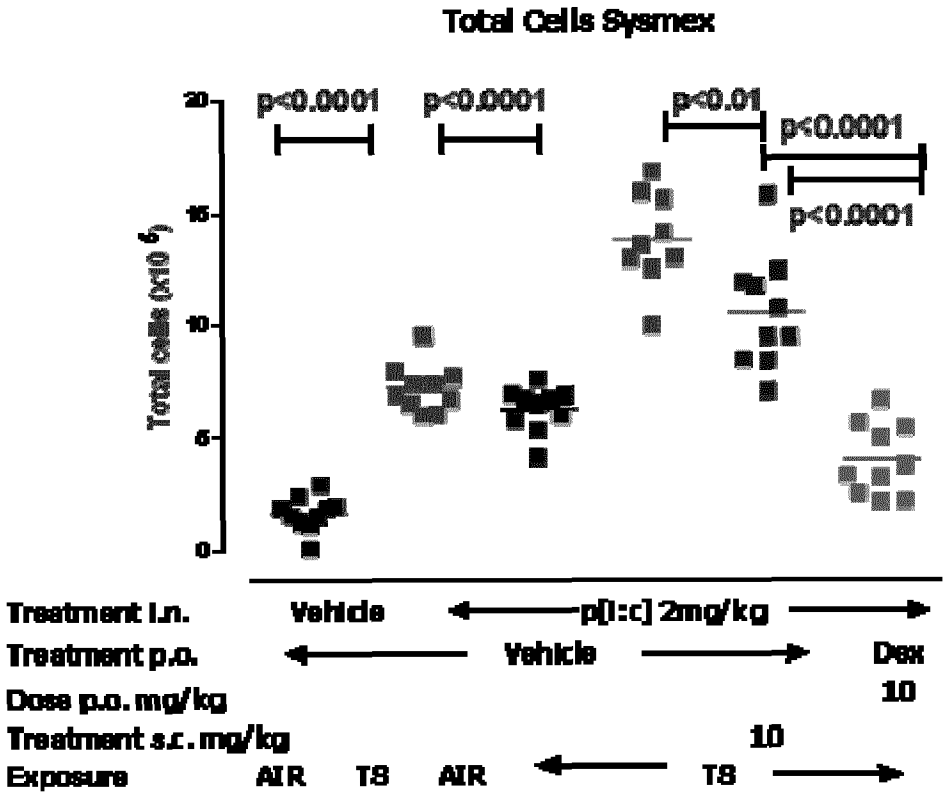
Total Cells Sysmex
p<0.0001
p<0.0001
p<0.01
p<0.0001
p<0.0001
Total cells (x10 6)
20
15
10
5
0
Treatment i.n.
Vehicle
p[I:c] 2mg/kg
Treatment p.o.
Vehicle
Dex
Dose p.o. mg/kg
10
Treatment s.c. mg/kg
10
Exposure
AIR
TS
AIR
TS

Figure 6

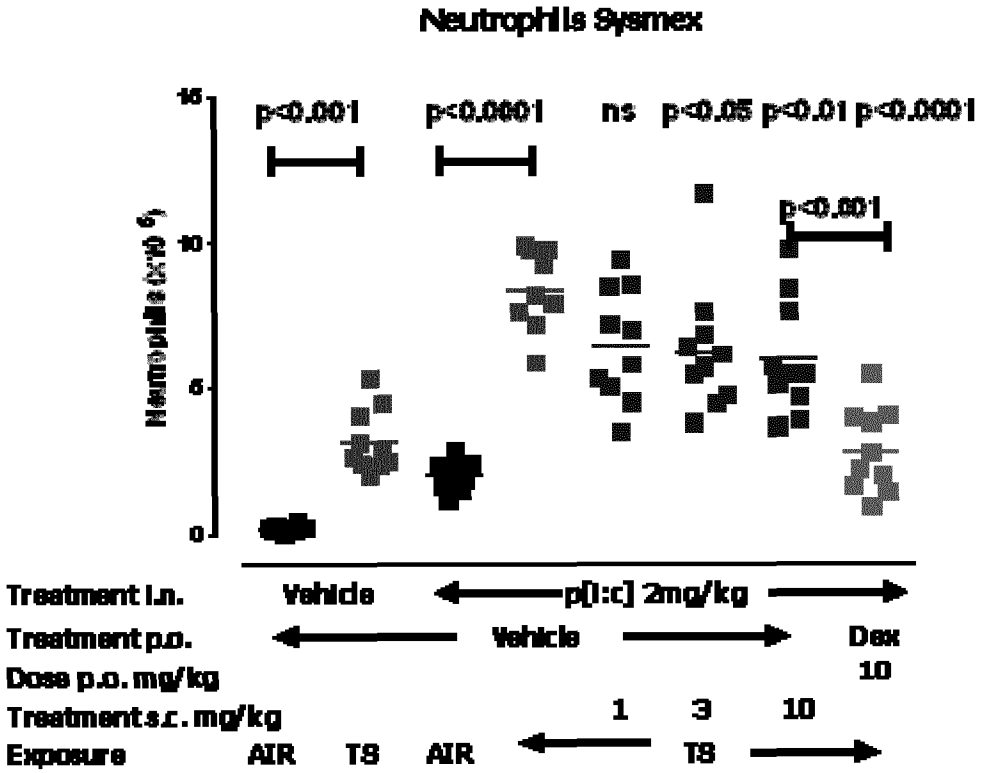
Neutrophils Sysmex
p<0.001
p<0.0001
ns
p<0.05
p<0.01
p<0.0001
p<0.001
15
10
5
0
Treatment i.n.
Vehicle
p[I:c] 2mg/kg
Treatment p.o.
Vehicle
Dex
10
Dose p.o. mg/kg
1
3
10
Treatment s.c. mg/kg
Exposure
AIR
TS
AIR
TS

Figure 7

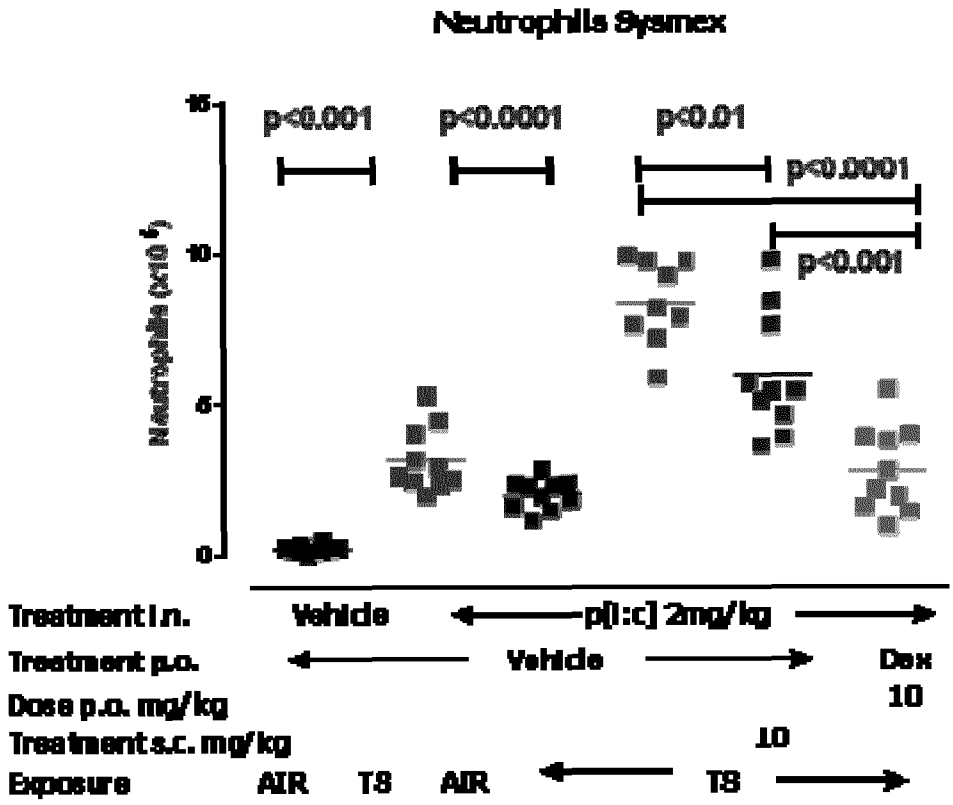
Neutrophils Sysmex
p<0.001
p<0.0001
p<0.01
p<0.0001
p<0.001
Neutrophils (x10 6)
15
10
5
0
Treatment i.n.
Vehicle
p[I:C] 2mg/kg
Treatment p.o.
Vehicle
Dex
Dose p.o. mg/kg
10
Treatment s.c. mg/kg
10
Exposure
AIR
TS
AIR
TS

Figure 8

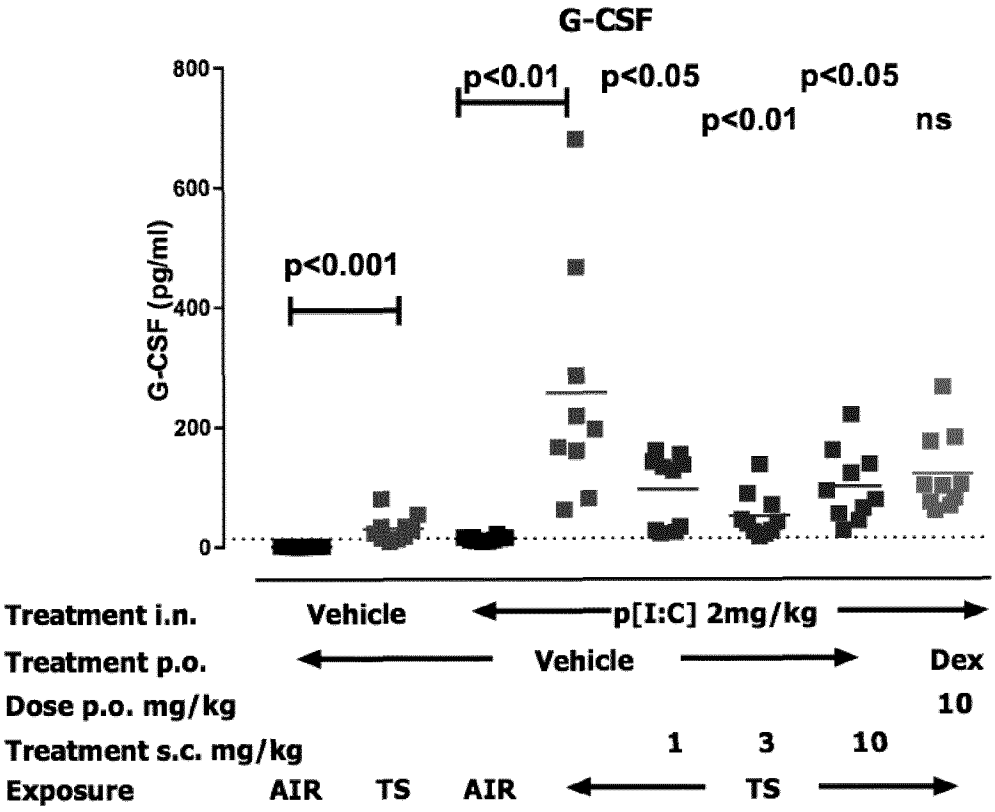
G-CSF
800
600
400
200
0
G-CSF (pg/ml)
p<0.01
p<0.05
p<0.05
p<0.01
ns
p<0.001
Treatment i.n.
Vehicle
p[I:C] 2mg/kg
Treatment p.o.
Vehicle
Dex
Dose p.o. mg/kg
10
Treatment s.c. mg/kg
1
3
10
Exposure
AIR
TS
AIR
TS

Figure 9.

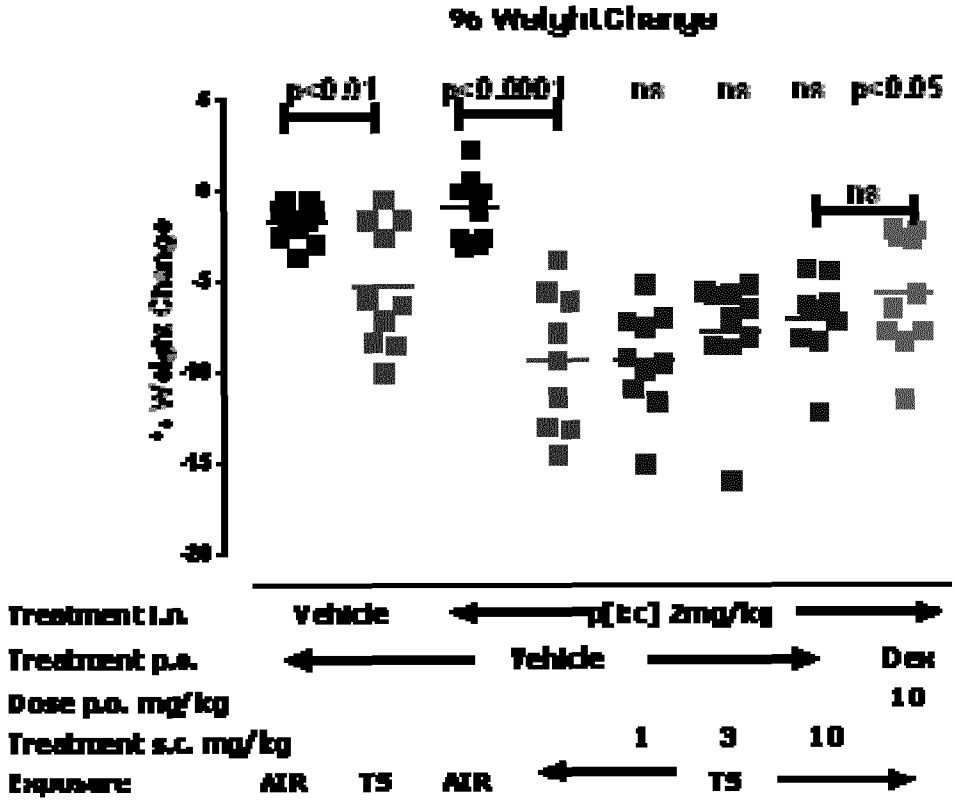
% Weight Change
p<0.01
p<0.0001
ns
ns
ns
p<0.05
ns
Treatment i.n.
Vehicle
p[tc] 2mg/kg
Treatment p.o.
Vehicle
Dex
Dose p.o. mg/kg
10
Treatment s.c. mg/kg
1
3
10
Exposure:
AIR
TS
AIR
TS

Figure 10

107C6

VH DNA Sequence:

ATGGGTTGGGTGTGGACCTTGCCATTCCTGATGGCAGCTGCCCAAAGTATCCA
AGCACAGATCCAGTTGGTGCAGTCTGGTCCTGAACTGAAGAAGCCTGGAGAGA
CAGTCAAGCTCTCCTGCAGGGCTTCTGGATATACATTCACAAACTATGGAATGA
ACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGATAAA
CACCTACTCTGGAGTGCCAACATATGCTGATGACTTCAAGGGACAGTTTGCCTT
CTCTTTGGAAACCTCTGCCGCCACTGCCTTTTTGCAGATCAACAACCTCAAAGA
TGAGGACACGGCTACATATTTTTGTGCAAGAGAGGGATATAGTACTACCAGGT
CTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACG
ACACCCCCATCTGTCTATCCACTGGCC

VH Amino Acid Sequence:

MGWVWTLPFLMAAAQSIQA**QIQLVQSGPELKKPGETVKLSCRASGYTFTNYGMN**
WVKQAPGKGLKWMGWINTYSGVPTYADDFKGQFAFSLETSAATAFLQINNLKD
EDTATYFCAREGYSTTRSMDYWGQGTSVTVSSAKTTPPSVYPLA

VK DNA Sequence:

ATGGAGTCACAGTCTCAGGTTCTTATATTGCTGCTGCTATGGGTATCTGGTACC
TGTGGGGACATTGTGATGTCACAGTCTCCATCCTCCCTGGCTGTGTCAGCAGG
AGAGAAGGTCACTATGAGCTGCAAATCCAGTCAGAGTCTGCTCGACAGTAGAA
CCCGAAAGAACTACTTGGTTTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAA
CTGCTGATCTACTGGGCATCCACTAGGGGATCTGGGGTCCCTGATCGCTTCAC
AGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTG
AAGACCTGGCAGTTTATTACTGCAAACAATCTTATAATCTTCGGACGTTCGGTG
GAGGCACCAAGCTGGAAATCAAACGGGCTGATGCTGCACCAACTGTATCCATC
TTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTT
CTTGAACAACTTCTACCCCAAA

VK Amino Acid Sequence:

MESQSQVLILLLLWVSGTCG**DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLDSRTR**
KNYLVWYQQKPGQSPKLLIYWASTRGSGVPDRFTGSGSGTDFTLTISSVQAEDL
AVYYCKQSYNLRTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFY
PK

Figure 11-1

108F8

VH DNA Sequence:

ATGGGTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTATCCA
ATCACAGATCCAGTTGGTGCAGTCTGGTCCTGATTCGAAGAAGCCTGGAGAGA
CAGTCAAGCTCTCCTGCAGGGCTTCTGGATATACATTCACAAACTATGGAATGA
ACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGATAAA
CACCTACTCTGGAGTGCCAACATATGCTGATGACTTCAAGGGACAGTTTGCCTT
CTCTTTGGAAACCTCTGCCGCCACTGCCTTTTTGCAGATCAACAACCTCAAAGA
TGAGGACACGGCTACATATTTTTGTGCAAGAGAGGGATATAGTACTACCAGGT
CTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACG
ACACCCCCATCTGTCTTCCCCCTGGCACCT

VH Amino Acid Sequence:

MGWVWTLLFLMAAAQSIQS**QIQLVQSGPDSKKPGETVKLSCRASGYTFTNYGMN**
WVKQAPGKGLKWMGWINTYSGVPTYADDFKGQFAFSLETSAATAFLQINNLKD
EDTATYFCAREGYSTTRSMDYWGQGTSVTVSSAKTTPPSVFPLAP

VK DNA Sequence:

ATGGGCTTCAAGATGAAGTCAGTCGACCTGGTTCTTATATTGCTGCTGCTATGG
GTATCTGGTACCTGTGGGGACATTGTGATGTCACAGTCTCCATCCTCCCTGGC
TGTGTCAGCAGGAGAGAAGGTCACTATGAGCTGCAAATCCAGTCAGAGTCTGC
TCGACAGTAGAACCCGAAAGAACTACTTGGTTTGGTACCAGCAGAAACCAGGG
CAGTCTCCTAAACTGCTGATCTACTGGGCATCCACTAGGGGATCTGGGGTCCC
TGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCA
GTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGCAAACAATCTTATAATCTTC
GGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCTGATGCTGCACC
AACTGTATCCATCTTCCCACCATCCAGTGAGC-
AGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCC

VK Amino Acid Sequence:

MGFKMKSVDLVLILLLLWVSGTCG**DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLD**
SRTRKNYLVWYQQKPGQSPKLLIYWASTRGSGVPDRFTGSGSGTDFTLTISSVQ
AEDLAVYYCKQSYNLRTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL
NNFYP

Figure 11-2

109A6

VH DNA Sequence:

ATGAAATGCAGCTGGATTATGTTCTTCCTGATGGCAGTGGTTACAGGGGTCAAT
TCAGAGGTTCAGCTGCAGCAGTCTGGGGCAGAACTTGTGAAGCCAGGGGCCT
CAGTCAAGTTGTCCTGCACAGCTTCTGGCTTCAAAATTAAAGACACCTATATAC
ACTGGGTGATCCAGAGGCCTGCACAGGGCCTGGAATGGATTGGAAGGATTGA
TCCTGCGAATGGTAATACTATTTATGGCTCAAAGTTCCAGGGCAAGGCCACTCT
AACAGCGGACACATCATCCAACACAGCCTACATTCACCTCAGCAGCCTGACAT
CTGGGGACTCTGCCGTCTATTACTGTGCGGGCTACGTTTGGTTTGCTTACTGG
GGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAACAGCCCCATCCGT
CTTCCCCCTGGCACCA

VH Amino Acid Sequence:

MKCSWIMFFLMAVVTGVNS**EVQLQQSGAELVKPGASVKLSCTASGFKIKDTYIH**
WVIQRPAQGLEWIGRIDPANGNTIYGSKFQGKATLTADTSSNTAYIHLSSLTSGDS
AVYYCAGYVWFAYWGQGTLVTVSAATTTAPSVFPLAP

VK DNA Sequence:

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCCTCCAG
CAGTGATGTTGTGATGACCCAAGTTCCACTCTCCCTGCCTGTCAGTCTTGGAG
ATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGACTTGTGCACAGTAATGGAA
ACACCTATTTACATTGGTTCTTACAGAAGCCAGGCCAGTCTCCAAAGCTCCTGA
TCTACACAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGT
GGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCT
GGGAGTTTATTTCTGCTCTCAAAGTACACTTGTTCCGTGGACGTTCGGTGGAG
GCACCAAGCTGGAAATCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTC
CCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTT
GAACAACTTCTACCCAAAG

VK Amino Acid Sequence:

MKLPVRLLVLMFWIPASSS**DVVMTQVPLSLPVSLGDQASISCRSSQRLVHSNGNT**
YLHWFLQKPGQSPKLLIYTVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVY
FCSQSTLVPWTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK

Figure 11-3

111A6

VH DNA Sequence:

ATGAAATGCAGCTGGGTTATGTTCTTCCTGATGGCAGTGGTTACAGGGGTCAA
TTCAGAGGTTCAGCTGCAGCAGTCTGGGGCAGAACTTGTGAAGCCAGGGGCC
TCAGTCAAGTTGTCCTGCACAGCTTCTGGCTTCAAAATTAAAGACACCTATATA
CACTGGGTGATCCAGAGGCCTGCACAGGGCCTGGAATGGATTGGAAGGATTG
ATCCTGCGAATGGTAATACTATTTATGGCTCAAAGTTCCAGGGCAAGGCCACTC
TAACAGCGGACACATCATCCAACACAGCCTACATTCACCTCAGCAGCCTGACA
TCTGGGGACTCTGCCGTCTATTACTGTGCGGGCTACGTTTGGTTTGCTTACTG
GGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAACAGCCCCATCC
GTCTTCCCCCTGGCACCA

VH Amino Acid Sequence:

MKCSWVMFFLMAVVTGVNS**EVQLQQSGAELVKPGASVKLSCTAS<u>GFKIKDTY</u>IH
WVIQRPAQGLEWIGR<u>IDPANGNT</u>IYGSKFQGKATLTADTSSNTAYIHLSSLTSGDS
AVYYC<u>AGYVWFAY</u>WGQGTLVTVSA**ATTTAPSVFPLAP

VK DNA sequence1

ATGGATTTTCAGGTGCAGATTTTCAGCTTCTTGCTAATCAGTGCCTCAGTTGCA
ATGTCCAGAGGAGAAAATGTGCTCACCCAGTCTCCAGCAATCATGTCTGCTTCT
CCAGGGGAGAAGGTCACCATGACCTGCAGGGCCAGGTCAAGTGTAAGTTCCA
GTTACTTGCACTGGTACCAGCAGAAGTCAGGTGCCTCCCCCAAACTCTGGATT
TATAGCACATCCAACTTGGCTTCTGGAGTCCCTACTCGCTTCAGTGGCAGTGG
GTCTGGGACCTCTTACTCTCTCACAATCAGCAGTGTGGAGGCTGAAGATGCTG
CCACTTATTACTGCCAGCAGTACAGTGGTTACCCACTCACGTTCGGTGCTGGG
ACCAAGCTGGAGCTGAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCC
ACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGA
ACAACTTCTACCCCAAG

VK Amino Acid Sequence:

MDFQVQIFSFLLISASVAMSRG**ENVLTQSPAIMSASPGEKVTMTCRAR<u>SSVSSSYL</u>
HWYQQKSGASPKLWIY<u>STS</u>NLASGVPTRFSGSGSGTSYSLTISSVEAEDAATYY
C<u>QQYSGYPLT</u>FGAGTKLELK**RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK

Figure 11-4

VK DNA sequence2

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCCTCCAG
CAGTGATGTTGTGATGACCCAAGTTCCACTCTCCCTGCCTGTCAGTCTTGGAG
ATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGACTTGTGCACAGTAATGGAA
ACACCTATTTACATTGGTTCTTACAGAAGCCAGGCCAGTCTCCAAAGCTCCTGA
TCTACACAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGT
GGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCT
GGGAGTTTATTTCTGCTCTCAAAGTACACTTGTTCCGTGGACGTTCGGTGGAG
GCACCAAGCTGGAAATCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTC
CCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTT
GAACAACTTCTACCCCAAAG

VK Amino Acid Sequence 2:

MKLPVRLLVLMFWIPASSS**DVVMTQVPLSLPVSLGDQASISCRSSQRLVHSNGNT**
YLHWFLQKPGQSPKLLIYTVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVY
FCSQSTLVPWTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK

131B4

VH DNA Sequence:

ATGAAATGCAGCTGGATTATGTTCTTCCTGATGGCAGTGGTTACAGGGGTCAAT
TCAGAGGTTCAGGTGCAGCAGTCTGGGGCAGAGCTTGTGAAGCCAGGGGCCT
CAGTCAAGTTGTCCTGCACAGCTTCTGGCTTCAAAATTAAGGACACCTATATAC
ACTGGTTAAAACAGAGGCCTGAACAGGGCCTGGAATGGATTGGAAGGATTGAT
CCTGCGAATGGTAATACTATATATGGCTCAAAGTTCCAGGGCAAGGCCACTATA
ACAGCAGACACATCATCCAACACAGCCTACATTCAACTCAGCAGCCTGACATCT
GGGGACACTGCCGTCTATTTTTGTGCGGGCTACGTTTGGTTTGCTTACTGGGG
CCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACACCCCCATCCGTCT
TCCCCCTGGCC

VH Amino Acid Sequence:

MKCSWIMFFLMAVVTGVNS**EVQVQQSGAELVKPGASVKLSCTASGFKIKDTYIH**
WLKQRPEQGLEWIGRIDPANGNTIYGSKFQGKATITADTSSNTAYIQLSSLTSGDT
AVYFCAGYVWFAYWGQGTLVTVSAAKTTPPSVFPLA

Figure 11-5

VH DNA Sequence 2:

ATGGCTGTCTTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTCCT
GTCCCAGGTGCAGCTGAAGCAGTCAGGACCTAGCCTAGTGCAGCCCTCACAG
AGCCTGTCCATAACCTGCACAGTCTCTGGTTTCTCATTAACTAGCTATGGTGTA
CACTGGGTTCGCCAGTCTCCAGGAAAGGGTCTGGAGTGGCTGGGAGTGATAT
GGAGAGGTGGAAGCACAGACTACAATGCAGCTTTCATGTCCAGACTGAGCATC
ACCAAGGACAACTCCAAGAGCCAAGTTTTCTTTAAAATGAACAGTCTGCAAGCT
GATGACACTGCCATATACTACTGTGCCAAAAATTGGGAGTATGATGGTTACTGG
GGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGAGAGTCA
GTCCTTCCCAAATGTCTTCCCCCTCGAA

VH Amino Acid Sequence 2:

MAVLGLLFCLVTFPSCVLS**QVQLKQSGPSLVQPSQSLSITCTVSGFSLTSYGVHW**
VRQSPGKGLEWLGVIWRGGSTDYNAAFMSRLSITKDNSKSQVFFKMNSLQADD
TAIYYCAKNWEYDGYWGFAYWGQGTLVTVSAESQSFPNVFPLE

VH DNA Sequence 3:

ATGGCAGTGGTTACAGGGGTCAATTCAGAGGTTCAGCTGCAGCAGTCTGGGG
CTGAGCTTGTGAGGCCAGGGGCCTCAGTCAAGTTGTCCTGCACAGCTTCTGG
CTTTAACATTAAAGACGACTATATGCACTGGGTGAAGCAGAGGCCTGAACAGG
GCCTGGAGTGGATTGGAAGGATTGATCCTGCGAATGGTAATACTAAATATGCC
CCGAAGTTCCAGGACAAGGCCACTATAACTGCAGACACATCCTCCAACACAGC
CTACCTGCAGCTCAGCAGCCTGACATCTGAGGACACTGCCGTCTATTACTGTG
CTAGAAGCTATGATGGTTCTCTGGGGGACTACTGGGGCCAAGGCACCACTCTC
ACAGTCTCCTCAGAGAGTCAGTCCTTCCCAAATGTCTTCCCCCTCGAG

VH Amino Acid Sequence 3:

MAVVTGVNS**EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYMHWVKQRPEQG**
LEWIGRIDPANGNTKYAPKFQDKATITADTSSNTAYLQLSSLTSEDTAVYYCARS
YDGSLGDYWGQGTTLTVSSESQSFPNVFPLE

Figure 11-6

VK DNA Sequence:

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCAGC
AGTGATGCTGTGTTGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGA
TCAAGCCTCCATCTCTTGCACATCTAGTCAGAGCCTTGTACACAGTAATGGAAA
CACCTATTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGA
TCTACAAAGTTTCCGACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGT
GGATCAGGAACAGATTTCACACTCATGATCACCAGAGTGGAGGCTGAGGATCT
GGGAGTTTATTTCTGCTCTCAAAGTTCACTTGTTCCGTGGACGTTCGGTGGAG
GCACCAAGCTGGAAGTCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTC
CCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTT
GAACAACTTCTACCCCAAA

VK Amino Acid Sequence:

MKLPVRLLVLMFWIPASSS**DAVLTQTPLSLPVSLGDQASISCTSSQSLVHSNGNTY**
LHWYLQKPGQSPKLLIYKVSDRFSGVPDRFSGSGSGTDFTLMITRVEAEDLGVYF
CSQSSLVPWTFGGGTKLEVKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK

131E8

VH DNA Sequence 1:

ATGGCTGTTTTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTCCT
ATCCCAGGTGCAGCTGAAGCAGTCAAGACCTGGCCCAGTGCAGCCCTCACAG
AGCCTGTCCATCACCTGCACAGTCTCTGGTTTCTCATTACCTAACTATGGTGTA
CACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAGTGATAT
GGAGTGGTGGAAGCACAGACTATAATGCAGCTTTCAAATCCAGACTGAGCATC
AGCAAGGACAACTCCAAGAGCCAAGTTTTCTTTAAAATGAACAGTCTGCAAGCT
GATGACACAGCCATATACTACTGTGCCAGAAATTTTTATAGTAAGTACGACTAT
GCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAAC
AACACCCCCATCCGTCTTCCCCCTGGC

VH Amino Acid Sequence 1:

MAVLGLLFCLVTFPSCVLS**QVQLKQSRPGPVQPSQSLSITCTVSGFSLPNYGVHW**
VRQPPGKGLEWLGVIWSGGSTDYNAAFKSRLSISKDNSKSQVFFKMNSLQADDT
AIYYCARNFYSKYDYAMDYWGQGTSVTVSSAKTTPPSVFPL

Figure 11-7

VH DNA Sequence 2:

ATGTTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTCCAACTGCA
GCAGCCTGGGTCTGTGCTGGTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC
AAGGCTTCTGGCTACACATTCACCAGCTACTGGATGCACTGGGTGAAGCAGAG
GCCGGGACAAGGCCTTGAGTGGATTGGAAATATTAATCCTAATAGTGGTAGTA
CTAACTACAATGAGAAGTTCAAGGGCAAGGCCACACTGACTGTAGACACATCC
TCCAGCACAGCCTACATGGATCTCAGCAGCCTGACATCTGAGGACTCTGCGGT
CTATTACTGTGCAAGACTGGGTGACTACTGGGGCCAAGGCACCACTCTCACAG
TCTCCTCAAAGAGTCAGTCCTCCCCATCCGTCTTCCCCCTG

VH Amino Acid Sequence 2:

MFFLVATATGVHS**QVQLQQPGSVLVRPGASVKLSCKAS**GYTFTSYW**MHWVKQR**
PGQGLEWIGNINPNSGST**NYNEKFKGKATLTVDTSSSTAYMDLSSLTSEDSAVYY**
CARLGDY**WGQGTTLTVSS**KSQSSPSVFPL

VH DNA Sequence 3:

GCTGTCTTGGGGCTGCTCTTCTGCCTGGTTGCATTTCCAAGCTGTGTCCTGTC
CCAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGC
CTGTCCATCACTTGCACTGTCTCTGGGTTTTCATTAACCAGCTATGGTGTACAC
TGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAGTAATATGGG
CTGGTGGAAGCACAAATTATAATTCGGCTCTCATGTCCAGACTGAGCATCAGC
AAAGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGAT
GACACAGCCATGTACTACTGTGCCAGAGATAGTAACTACTTTGACTACTGGGG
CCAAGGCACCACTCTCACAGTCTCCTCAGAGAGTCAGTCCTTCCCAAATGTCTT
CCCCCTCGTA

VH Amino Acid Sequence 3:

AVLGLLFCLVAFPSCVLS**QVQLKESGPGLVAPSQSLSITCTVS**GFSLTSYG**VHWV**
RQPPGKGLEWLGVIWAGGST**NYNSALMSRLSISKDNSKSQVFLKMNSLQTDDT**
AMYYCARDSNYFDY**WGQGTTLTVSS**ESQSFPNVFPLV

Figure 11-8

VK DNA Sequence:

ATGGATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCCTCAGTCATA
ATGTCCAGAGGAGAAAATGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATCT
CCAGGGGAAAAGGTCACCATGACCTGCAGTGCCAGCTCAAGTGTAAGTTACAT
GCACTGGTACCAGCAGAAGTCAAGCACCTCCCCCAAACTCTGGATTTATGACA
CATCCAAACTGGCTTCTGGAGTCCCAGGTCGCTTCAGTGGCAGTGGGTCTGGA
AACTCTTACTCTCTCACGATCAGCAGCATGGAGGCTGAAGATGTTGCCACTTAT
TACTGTTTTCAGGGGAGTGGGTACCCACTCACGTTCGGCTCGGGGACAAAGTT
GGAAATAAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCA
GTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCT
ACCCCAAA

VK Amino Acid Sequence:

MDFQVQIFSFLLISASVIMSRG**ENVLTQSPAIMSASPGEKVTMTCSASSSVSYMH**
WYQQKSSTSPKLWIYDTSKLASGVPGRFSGSGSGNSYSLTISSMEAEDVATYYC
FQGSGYPLTFGSGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK

131H1

VH DNA Sequence:

ATGGCTGTCTTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTCCT
ATCCCAGGTGCAGCTGAAGCAGTCAGGACCTGGCCTAGTGCAGCCCTCACAG
AGCCTGTCCATCACCTGCACAGTCTCTGGTTTCTCATTAACTAGCTATGGTGTA
CACTGGGTTCGCCAGTCTCCAGGAAAGGGTCTGGAGTGGCTGGGAGTGATAT
GGAGTGGTGGAAGCACAGACTATAATGCAGCTTTCATATCCAGACTGAGCATC
AGCAAGGACAATTCCAAGAGCCAAGTTTTCTTTAAAATGAACAGTCTGCAAGCT
GATGACACAGCCATATATTACTGTGCCAGATCTTATGATTACGACGGGAGGGG
TTACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGAGAGTC
AGTCCTTCCCAAATGTCTTCCCCCTCGTA

VH Amino Acid Sequence:

MAVLGLLFCLVTFPSCVLS**QVQLKQSGPGLVQPSQSLSITCTVSGFSLTSYGVHW**
VRQSPGKGLEWLGVIWSGGSTDYNAAFISRLSISKDNSKSQVFFKMNSLQADDT
AIYYCARSYDYDGRGYFDYWGQGTTLTVSSESQSFPNVFPLV

Figure 11-9

VK DNA Sequence 1:

ATGAGTGTGCTCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGGTGC
CAGATGTGACATCCAGATGACTCAGTCTCCAGCCTCCCTGTCTGCATCTGTGG
GAGAAACTGTCACCATCACATGTCGAGCAAGTGAGAATGTTTACAGATATTTAG
CATGGTATCAGCAGAGACAGGGAAAATCTCCTCAGCTCCTGGTCTATAGTGCA
AAAACCTTAGCAGAAGGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGCAC
ACAGTTTTCTCTGAAGATCAACACCCTGCAGCCTGAAGATTTTGGGACTTATTA
CTGTCAACATCATTATAATACTCCTCTCACGTTCGGTGCTGGGACCAAGCTGGA
GCTGAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTG
AGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACC
CCAAA

VK Amino Acid Sequence 1:

MSVLTQVLGLLLLWLTGARC**DIQMTQSPASLSASVGETVTITCRASENVYRYLAW**
YQQRQGKSPQLLVYSAKTLAEGVPSRFSGSGSGTQFSLKINTLQPEDFGTYYCQ
HHYNTPLTFGAGTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK

VK DNA Sequence 2:

ATGGTTCTTATATGGCTCCTGCTATGGGTATCTGGTACCTGTGGGGACATTGTG
ATGTCACAGTCTCCATCCTCCCTGGCTGTGTCAGCAGGAGAGAAGGTCACTAT
GAGCTGCAAATCCAGTCAGAGTCTGTTCAACAGTAAAACCCGAAAGAACTACTT
GGCTTGGTTTCAGCAAAAACCAGGGCAGTCTCCTGAACTGCTGATCTACTGGG
CATCCACTAGGAAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGG
ACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTA
TTACTGCAAGCAATCTTATAATCTGTGGACGTTCGGCGGAGGCACCAAGCTGG
AAATCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGT
GAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTAC
CCCAAA

VK Amino Acid Sequence 2:

MVLIWLLLWVSGTCG**DIVMSQSPSSLAVSAGEKVTMSCKSSQSLFNSKTRKNYL**
AWFQQKPGQSPELLIYWASTRKSGVPDRFTGSGSGTDFTLTISSVQAEDLAVYY
CKQSYNLWTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK

Figure 11-10

132H4

VH DNA Sequence:

TGAGCTGGGTTTTCCTTGTCCTTATTTTAAAAGGTGTCCAGTGTGAAGTGAAGC
TGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAAACTCTC
CTGTGCAGCCTCTGGATTCACTTTCAGTAACTATGCCATGTCTTGGGTTCGCCA
GAATCCGGCGAAGAGGCTGGAGTGGGTCGCAACCATTAGTAGTGGTGGTGCT
AATATTTACTATCCAGACAGTGTGAAGGGCCGATTCATCATCTCCAGAGACAAT
GCCAGGAACACCCTGTACCTGCAAATGAGCAGTCTGAGGTCTGAGGACACGG
CCATGTATTACTGTGCAAGAGGCGACTATTTTAACCACTTCTGGTTTGCTTACT
GGGGCCAAGGGACTCTTGTCACTGTCTCTGCAGCCAAAACAACAGCCCCATCG
GTCTTCCCCCTGGCA

VH Amino Acid Sequence:

SWVFLVLILKGVQC**EVKLVESGGGLVKPGGSLKLSCAASGFTFSNYAMSWVRQN**
PAKRLEWVATISSGGANIYYPDSVKGRFIISRDNARNTLYLQMSSLRSEDTAMYY
CARGDYFNHFWFAYWGQGTLVTVSAAKTTAPSVFPLA

(missing A at the start of the sequence, should be MSWVF)

Figure 11-11

VK DNA Sequence:

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCAGC
AGTGATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGAT
CAAGCCTCCATCTCTTGTAGATCGAGTCAGAGCATTGTACATAGTAATGGAAAC
ACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGTTCCTGATC
TACAAAGTTTCCAACCGATTTTCAGGGGTCCCAGACAGGTTCAGTGGCAGTGG
ATCAGGGACAGATTTCACACTCAAGATCAACAGAGTGGAGGCTGAGGATCTGG
GAATTTATTACTGCTTTCAGGGTTCACATGTTCCGTGGACGTTCGGTGGAGGC
ACCAAGCTGGAAATCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCC
ACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGA

VK Amino Acid Sequence:

MKLPVRLLVLMFWIPASSS**DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTY**
LEWYLQKPGQSPKFLIYKVSNRFSGVPDRFSGSGSGTDFTLKINRVEAEDLGIYY
CFQGSHVPWTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL

(missing last 6 amino acids usually NNFYPK or NNFYPR)

133A6

VH DNA Sequence:

ATGAACTTTGGGTTGAGATTGGTTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGT
GTGAGGTGAAGCTAGTGGAGTCTGGAGGAGGCTTAGTGAAGCCTGGAGGGTC
CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAACTATGCCATGTC
TTGGGTTCGCCAGACTCCGGCGAAGAGGCTGGAGTGGGTCACAACCATTAGT
AGTGGTGGTGGTAACATCTACTATACAGACAGTGTGAAGGGCCGATTCACCGT
CTCCAGAGACAATGCCAGGAACACCCTGTACCTGCAAATGAGCAGTCTGAGGT
CTGAGGACACGGCCATGTATTACTGTGCAAGAGGCGACTATAGTAACTACTTC
TGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCTCTGTCTCTGAAGCCAAAAC
AACAGCCCCATCGGTCTTCCCCCTGGCACCT

VH Amino Acid Sequence:

MNFGLRLVFLVLVLKGVQC**EVKLVESGGGLVKPGGSLKLSCAASGFTFSNYAMS**
WVRQTPAKRLEWVTTISSGGGNIYYTDSVKGRFTVSRDNARNTLYLQMSSLRSE
DTAMYYCARGDYSNYFWFAYWGQGTLVSVSEAKTTAPSVFPLAP

Figure 11-12

VK DNA Sequence:

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCAGC
AGTGATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGAT
CAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTACATAGTAATGGAAAC
ACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGAT
CTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTG
GATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTG
GGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCGTGGACGTTCGGTGGAGG
CACCAAGCTGGAAATCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCC
CACCATCCAGGGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTG
AACAACTTCTACCCAAAA

VK Amino Acid Sequence:

MKLPVRLLVLMFWIPASSS**DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTY**
LEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYY
CFQGSHVPWTFGGGTKLEIKRADAAPTVSIFPPSREQLTSGGASVVCFLNNFYPK

Figure 11-13

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/069013

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24
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)
C07K

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, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/62285 A1 (APPLIED RESEARCH SYSTEMS [NL]; YEDA RES & DEV [IL]; CHVATCHKO YOLANDE) 30 August 2001 (2001-08-30) examples 3, 6-8, 10-12 -----	1-5, 8-14, 24-30, 37-41, 45,46
X	WO 2004/101617 A1 (APPLIED RESEARCH SYSTEMS [NL]; ALTAROCCA VALTER [IT]; PEZZOTTI ANNA R) 25 November 2004 (2004-11-25) page 17, paragraph 2 - page 19, paragraph 1; claims 15-25 ----- -/--	1-18, 22-30, 37-41, 45,46

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 2014	08/01/2015

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	Kalsner, Inge

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/096456 A1 (ARES TRADING SA [CH]; SHOHAMI ESTHER [IL]) 5 December 2002 (2002-12-05) page 28, last paragraph - page 32, last paragraph	1-4, 31-41
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A	CHARLES A. DINARELLO ET AL: "Interleukin-18 and IL-18 Binding Protein", FRONTIERS IN IMMUNOLOGY, vol. 4, 1 January 2013 (2013-01-01), XP055154394, ISSN: 1664-3224, DOI: 10.3389/fimmu.2013.00289 the whole document	1-46, 67-87
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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.
A	MATTHIAS LOCHNER ET AL: "Anti-Interleukin-18 Therapy in Murine Models of Inflammatory Bowel Disease", PATHOBIOLOGY., vol. 70, no. 3, 1 January 2002 (2002-01-01), pages 164-169, XP055155439, CH ISSN: 1015-2008, DOI: 10.1159/000068149 the whole document -----	1-46, 67-87
A	S. R. THOMPSON ET AL: "Free Interleukin (IL)-18 Levels, and the Impact of IL18 and IL18BP Genetic Variation, in CHD Patients and Healthy Men", ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, vol. 27, no. 12, 1 December 2007 (2007-12-01), pages 2743-2749, XP055154486, ISSN: 1079-5642, DOI: 10.1161/ATVBAHA.107.149245 abstract -----	1-46, 67-87

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2014/069013

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 47-66
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 47-66

Claims 47-66 do not meet the requirements of Art. 5 and 6 PCT for the following reasons:

Claims 47-58 are directed to various methods of treating and diagnosing IL-18 associated diseases wherein neither the diseases not the agents to be used for the treatment or diagnosis are properly and clearly defined.

With respect to the agent to be used in the methods (IL-18 inhibitor) the claim cover limitless and untried downstream developments in relation to yet to be demonstrated /functions/molecular mechanisms/activities. The claims amount to no more than an invitation to set up further research programs for which no guidance is forthcoming and, therefore, it is an undue burden to put the claimed subject-matter into practice. It should be furthermore noted, that the application not provide any indication that at least one of the antibodies characterised (a) does have inhibitory action and (b) would be actually suitable or show a potential effect in therapy.

Claims 47-58 so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Claim 59 is directed to "a set of biomarkers" for use in the methods of preceding claims comprising determining a "biomarker profile". Apart from the fact that the entire application does not disclose any "biomarker" which could be an indication for the skilled person as to what substance/reagent/molecule to use, it is furthermore completely unclear how a (undefined) "set of biomarkers" could possibly be used for determining a profile of exactly such (undefined) "biomarkers".

Claim 60 is equally unclear as the claim does not give any indication as to the nature of the "biomarker" of "biomarker profile".

As neither the claims not the application itself disclose any indication regarding either the biomarkers or the method of determining them the claims are considered to lack disclosure as well as support and clarity in the sense of Art. 5 and 6 to such an extent that a meaningful search and/or examination is not possible.

Claims 61-66 which are directed to diagnostic kits comprising different components wherein each of these components is defined by functional or desirable features lacking any technical (structural) definition. Since, again, the description does not provide any information on the properties of the components of such kits the claims are thus so unclear and so lack disclosure that a meaningful search and examination is not possible.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/069013

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/069013

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