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(54) **Title:** NEW USE FOR JNK INHIBITOR MOLECULES FOR TREATMENT OF VARIOUS DISEASES

(57) **Abstract:** The present invention relates to the use of novel JNK inhibitor molecules and their use in a method of treatment of the human or animal body by therapy.



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Xigen Inflammation Ltd.

New Use for JNK inhibitor molecules for treatment of various diseases

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The present invention relates to the field of enzyme inhibition, in particular to (poly-)peptide inhibitors of c-Jun amino terminal kinase (JNK). In particular, the present invention relates to using these JNK inhibitors in the treatment of various diseases.

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The c-Jun amino terminal kinase (JNK) is a member of the stress-activated group of mitogen-activated protein (MAP) kinases. These kinases have been implicated in the control of cell growth and differentiation, and, more generally, in the response of cells to environmental stimuli. The JNK signal transduction pathway is activated in response to environmental stress and by the engagement of several classes of cell surface receptors. These receptors can include cytokine receptors,

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serpentine receptors and receptor tyrosine kinases. In mammalian cells, JNK has been implicated in biological processes such as oncogenic transformation and mediating adaptive responses to environmental stress. JNK has also been associated with modulating immune responses, including maturation and differentiation of immune cells, as well as effecting programmed cell death in cells identified for destruction by the immune system. This unique property makes JNK signaling a

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promising target for developing pharmacological intervention. Among several neurological disorders, JNK signaling is particularly implicated in ischemic stroke and Parkinson's disease, but also in other diseases as mentioned further below. Furthermore, the mitogen-activated protein kinase (MAPK) p38alpha was shown to negatively regulate the cell proliferation by antagonizing the

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JNK-c-Jun-pathway. The mitogen-activated protein kinase (MAPK) p38alpha therefore appears to be active in suppression of normal and cancer cell proliferation and, as a further, demonstrates the involvement of JNK in cancer diseases (see e.g. Hui *et al.*, Nature Genetics, Vol 39, No. 6, June 2007). It was also shown, that c-Jun N-terminal Kinase (JNK) is involved in neuropathic pain produced by spinal nerve ligation (SNL), wherein SNL induced a slow and persistent activation of JNK, in particular JNK1, whereas p38 mitogen-activated protein kinase activation was found in

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spinal microglia after SNL, which had fallen to near basal level by 21 days (Zhuang *et al.*, The Journal of Neuroscience, March 29, 2006, 26(13):3551-3560)). In 2007 (Biochemica et Biophysica Acta, pp. 1341-1348), Johnson *et al.* discussed in a review the c-Jun kinase/stress-activated pathway, the involvement of JNK signalling in diseases such as the involvement in excitotoxicity of

hippocampal neurons, liver ischemia, reperfusion, neurodegenerative diseases, hearing loss, deafness, neural tube birth defects, cancer, chronic inflammatory diseases, obesity, diabetes, in particular insulin-resistant diabetes, and proposed that it is likely that selective JNK inhibitors are needed for treatment of various diseases with a high degree of specificity and lack of toxicity.

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Inhibition or interruption of the JNK signalling pathway is thus a promising approach in combating disorders strongly related to JNK signalling. However, there are only a few inhibitors of the JNK signaling pathway known so far.

10 Inhibitors of the JNK signaling pathway as already known in the prior art include e.g. upstream kinase inhibitors (for example, CEP-1347), small chemical inhibitors of JNK (SP600125 and AS601245), which directly affect kinase activity e.g. by competing with the ATP-binding site of the protein kinase, and peptide inhibitors of the interaction between JNK and its substrates (see e.g. Kuan et al., Current Drug Targets – CNS & Neurological Disorders, February 2005, vol. 4, no. 1, pp.
15 63-67; WO 2007/031280; all incorporated herewith by reference). WO 2007/031280 discloses small cell permeable fusion peptides, comprising a so-called TAT transporter sequence derived from the basic trafficking sequence of the HIV-TAT protein and an amino acid inhibitory sequence of IB1.

WO 2007/031280 discloses in particular two specific sequences, L-TAT-IB1
20 (GRKKRRQRRRPPRKRPTTLNLFQVPRSQD, herein SEQ ID NO: 196) and D-TAT-IB1 (dqsrpvpqpfInltprkprprrrqrkkrq; herein SEQ ID NO: 197), the latter being the retro-inverso sequence of L-TAT-IB1. Due to the HIV TAT derived transporter sequence, these fusion peptides are more efficiently transported into the target cells, where they remain effective until proteolytic degradation.

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Since ATP independent peptide inhibitors of JNK are usually more specific inhibitors, they are frequently the first choice if it comes to inhibiting JNK. However, even the peptide inhibitors disclosed in WO 2007/031280 are not optimal for all purposes. For example, compound L-TAT-IB1 (herein SEQ ID NO: 196) which consists of L amino acids only, is quickly proteolytically degraded.

30 In order to overcome this problem the inventors of WO 2007/031280 also suggested D-TAT-IB1 (herein SEQ ID NO: 197), which comprises D amino acids. To be more precise, D-TAT-IB1 exhibits the retro-inverso sequence of L-TAT-IB1. Incorporation of D-amino acids is made difficult by the fact that the change in stereochemistry may lead to a loss of function. The retro-inverso approach may be employed to reduce said risk because the use of i) only D-amino acids ii) but in the inverse
35 peptide sequence may more likely yield an acceptable conformational analogue to the original peptide than incorporating one or more D-amino acids into the original sequence. In the case of

WO 2007/031280 this approach resulted nevertheless in a significant decrease in inhibitory capacity in comparison to L-TAT-IB1 (see Fig. 4). Additionally, the retro-inverso peptide is extremely stable towards proteolytic digestion with the consequence that controlled digestions, for example in time sensitive experiments, are hardly possible.

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JNK inhibitors have been discussed, proposed and successfully tested in the art as treatment for a variety of disease states. Already in 1997, Dickens et al. described the c-Jun amino terminal kinase inhibitor JIP-1 and proposed JIP-1 as candidate compounds for therapeutic strategies for the treatment of for example chronic myeloid leukaemia, in particular, in the context of Bcr-Abl caused transformation of pre-B-cells (Science; 1997; 277(5326):693-696).

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In 2001, Bonny and co-workers published that cell-permeable peptide inhibitors of JNK confirm long term protection to pancreatic β -cells from IL-1 β -induced apoptosis and may, thus, preserve β -cells in the autoimmune destruction in the course of diabetes (Diabetes, 50, 2001, p. 77 – 82).

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Bonny et al. (Reviews in Neurosciences, 2005, p. 57 – 67) discussed also the inhibitory action of the JNK inhibitor D-JNKI-1 and other JNK inhibitors in the context of excitotoxicity, neuronal cell death, hypoxia, ischemia, traumatic brain damage, epilepsy, neurodegenerative diseases, apoptosis of neurons and inner ear sensory auditory cells etc.

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In WO 98/49188 JIP-1 derived inhibitors of JNK signalling are proposed for the treatment of neurodegenerative diseases, such as Parkinson's disease or Alzheimer's disease; stroke and associated memory loss, autoimmune diseases such as arthritis; other conditions characterized by inflammation; malignancies, such as leukemias, e.g. chronic myelogenous leukemia (CML); oxidative damage to organs such as the liver and kidney; heart diseases; and transplant rejections.

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Borsello et al. (Nat Med, 2003, (9), p. 1180 – 1186) published that a peptide inhibitor of c-Jun-N-terminal kinase protects against excitotoxicity and cerebral ischemia.

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Assi et al. have published that another specific JNK-inhibitor, SP600125, targets tumor necrosis factor- α production and epithelial cell apoptosis in acute murine colitis. The authors concluded that inhibition of JNK is of value in human inflammatory bowel disease treatment (Immunology; 2006, 118(1):112-121).

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In Kennedy et al. (Cell Cycle, 2003, 2(3), p. 199 – 201), the role of JNK signalling in tumor development is discussed in more detail.

Lee Yong Hee et al. (J Biol Chem 2003, 278(5), P. 2896 – 2902) showed that c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signalling cascade and have proposed that inhibition of JNK signalling is a good therapeutic approach to reduce insulin resistance in diabetic patients.

Milano et al. (Am J Physiol Heart Circ Physiol 2007; 192(4): H1828 – H1835) discovered that a peptide inhibitor of c-Jun NH₂-terminal kinase reduces myocardial ischemia-reperfusion injury and infarct size in vivo. The authors of said study used a peptide inhibitor, D-JNKI-I, a two domain peptide containing a 20 amino acid sequence of the minimal JNK-binding domain of islet-brain-1/JNK-interacting protein-1, linked to a 10 amino acid TAT sequence of the human immuno deficiency virus TAT protein that mediates intracellular translocation. The authors have concluded that a reduction in JNK activity and phosphorylation due to the presence of said inhibitor is important in the preservation of cardiac function in rats in the phase of ischemia and apoptosis.

A further group has published that small peptide inhibitors of JNKs protect against MPTP-induced nigral dopaminergic injury via inhibiting the JNK-signalling pathway (Pan et al., Laboratory investigation, 2010, 90, 156 – 167). The authors concluded that a peptide comprising residues 153 – 163 of murine JIP-1 fused to TAT peptide offers neuroprotection against MPTP injury via inhibiting the JNK-signalling pathway and provides a therapeutic approach for Parkinson's disease.

For hearing damage, Pirvola et al. (The Journal of Neuroscience, 2000, 20(1); 43 – 50) described the rescue of hearing, auditory hair cells and neurons by CEP-1347/KT7515, an inhibitor of c-Jun-N-terminal kinase activation. The authors suggested in general that therapeutic intervention in the JNK signalling cascade may offer opportunities to treat inner ear injuries. Treatment of hearing loss by means of administering JNK-inhibitory peptides is also disclosed for example in WO 03/103698.

For retinal diseases and age-related macula degeneration in particular, Roduit et al. (Apoptosis, 2008, 13(3), p. 343 – 353) have likewise suggested to use JNK-inhibition as therapeutic approach. Similar considerations relying on JNK-inhibition are disclosed for example in WO 2010/113753 for the treatment of age-related macular degeneration, diabetic macular edema, diabetic retinopathy, central exudative chorioretinopathy, angioid streaks, retinal pigment epithelium detachment, multifocal choroiditis, neovascular maculopathy, retinopathy of prematurity, retinitis pigmentosa, Leber's disease, retinal artery occlusion, retinal vein occlusion, central serous chorioretinopathy, retinal macroaneurysm, retinal detachment, proliferative vitreoretinopathy, Stargardt's disease,

choroidal sclerosis, chorioderemia, vitelliform macular dystrophy, Oguchi's disease, fundus albipunctatus, retinitis punctata albescens, and gyrate atrophy of choroid and retina.

5 Zoukhri et al. (Journal of Neurochemistry, 2006, 96, 126 – 135) identified that c-Jun NH₂-terminal kinase mediates interleukin-1 β -induced inhibition of lacrimal gland secretion. They concluded that JNK plays a pivotal role in IL-1 β -mediated inhibition of lacrimal gland secretion and subsequent dry eye.

10 For uveitis, Touchard et al. (Invest Ophthalmol Vis Sci, 2010, 51(9); 4683 – 4693) have suggested to use D-JNKI 1 as effective treatment.

For IBD (inflammatory bowel disease) Roy et al. (World J Gastroenterol 2008, 14(2), 200 – 202) have highlighted the role of the JNK signal transduction pathway therein and have proposed to use peptidic JNK inhibitors for the treatment of said disease state.

15 Beckham et al (J Virol. 2007 Jul;81(13):6984-6992) showed that the JNK inhibitor D-JNKI-1 is effective in protecting mice from viral encephalitis, and suggest thus JNK inhibition as promising and novel treatment strategy for viral encephalitis.

20 Palin et al. (Psychopharmacology (Berl). 2008 May;197(4):629-635) used the same JNK inhibitor, D-JNKI-1, and found that pre-treatment with D-JNKI-1 (10 ng/mouse), but not D-TAT, significantly inhibited all three indices of sickness induced by central TNF α and suggested that JNK inhibition as means for treating major depressive disorders that develop on a background of cytokine-induced sickness behaviour.

25 In WO 2010/151638 treatment of the neurodegenerative disease spinal muscular atrophy by way of JNK inhibition was proposed.

30 The above introductory section highlights on the basis of selected publications the usefulness of JNK inhibitors in the treatment of various diseases. Thus, there is a constant need in the art for JNK inhibitors for use in the treatment of human (and animal) diseases.

Thus, the problem to be solved by the present invention was to provide further (peptide) inhibitors of JNK for the treatment of specific diseases.

The object of the present invention is solved by the inventor by means of the subject-matter set out below and in the appended claims.

Brief description of the Figures

In the following a brief description of the appended figures will be given. The figures are intended to illustrate the present invention in more detail. However, they are not intended to limit the subject matter of the invention in any way.

Fig. 1: Illustration of the inhibitory efficacy of several JNK inhibitors according to the present invention, which was investigated by in vitro AlphaScreen assay (Amplified Luminescence Proximity Homogeneous-Screen Assay).

Fig.1A: Inhibition of JNK1 by SEQ ID NOs: 193, 2, 3, 5, 6, and 7.

Fig.1B: Inhibition of JNK2 by SEQ ID NOs: 193, 2, 3, 5, 6, and 7.

Fig.1C: Inhibition of JNK3 by SEQ ID NOs: 193, 2, 3, 5, 6, and 7.

Fig. 2: Table illustrating the inhibitory efficacy of several JNK inhibitors (SEQ ID NOs: 193, 2, 3, 5, 6, and 7) according to the present invention. Given are the IC₅₀ values in the nM range, the respective standard error of the mean and the number of experiments performed (n).

Fig. 3: Illustration of the inhibitory efficacy of several JNK inhibitors according to the present invention, which are fusion proteins of a JNK inhibitory (poly-)peptide sequence and a transporter sequence. The inhibitory efficacy was determined by means of in vitro AlphaScreen assay (Amplified Luminescence Proximity Homogeneous-Screen Assay).

Fig.3A: Inhibition of JNK1 by SEQ ID NOs: 194, 195, 172, 200, 46, 173, 174, 175, 176, 177, 178, 179, 180, 181 and 197.

Fig.3B: Inhibition of JNK2 by SEQ ID NOs: 194, 195, 172, 200, 46, 173, 174, 175, 176, 177, 178, 179, 180, 181 and 197.

Fig.3C: Inhibition of JNK3 by SEQ ID NOs: 194, 195, 172, 200, 46, 173, 174, 175, 176, 177, 178, 179, 180, 181 and 197.

Fig.3D: Inhibition of JNK1 by SEQ ID NOs: 194, 195, 172, 200, 46, 182, 183, 184, 185, 186, 187, 188, 189, 190 and 197.

Fig.3E: Inhibition of JNK2 by SEQ ID NOs: 194, 195, 172, 200, 46, 182, 183, 184, 185, 186, 187, 188, 189, 190 and 197.

Fig.3F: Inhibition of JNK3 by SEQ ID NOs: 194, 195, 172, 200, 46, 182, 183, 184, 185, 186, 187, 188, 189, 190 and 197.

Fig. 4: Table illustrating the inhibitory efficacy of several JNK inhibitors according to the present invention, which are fusion proteins of a JNK inhibitory (poly-)peptide sequence and a transporter sequence. Given are the IC₅₀ values in the nM range, the respective standard error of the mean (SEM) and the number of experiments performed (n).

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Fig. 5: Stability of JNK inhibitors with SEQ ID NOs: 172, 196 and 197 in 50% human serum. The JNK inhibitor with SEQ ID NO: 196 was totally degraded into amino acids residues within 6 hours (A). The JNK inhibitor with SEQ ID NO: 172 was completely degraded only after 14 days (B). The JNK inhibitor with SEQ ID NO: 197 was stable at least up to 30 days (B).

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Fig. 6: shows internalization experiments using TAT derived transporter constructs with D-amino acid/L-amino acid pattern as denoted in SEQ ID NO: 30. The transporter sequences analyzed correspond to SEQ ID NOs: 52-94 plus SEQ ID NOs: 45, 47, 46, 43 and 99 (Fig 6a) and SEQ ID NOs: 100-147 (Fig. 6b). As can be seen, all transporters with the consensus sequence rXXXrXXXr (SEQ ID NO: 31) showed a higher internalization capability than the L-TAT transporter (SEQ ID NO: 43). Hela cells were incubated 24 hours in 96well plate with 10mM of the respective transporters. The cells were then washed twice with an acidic buffer (0.2M Glycin, 0.15M NaCl, pH 3.0) and twice with PBS. Cells were broken by the addition of RIPA lysis buffer. The relative amount of internalized peptide was then determined by reading the fluorescence intensity (Fusion Alpha plate reader; PerkinElmer) of each extract followed by background subtraction.

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Fig. 7 The JNK inhibitor with the sequence of SEQ ID NO: 172 blocks LPS-induced cytokine and chemokine release in THP1-PMA-differentiated macrophages. Fig. 7A: TNF release (THP1pma 6h 3ng/ml LPS); Fig. 7B: TNF- α release (THP1pma 6h 10ng/ml LPS); Fig. 7C: IL 6 release (THP1pma 6h 10ng/ml LPS); Fig. 7D: MCP1 release (THP1pma 6h 3ng/ml LPS).

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Fig. 8 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced IL6 release in THP1 differentiated macrophages with higher potency than D-TAT-IB1 (SEQ ID NO: 197), dTAT (SEQ ID NO: 45) and SP 600125. LPS was added for 6h (10 ng/ml).

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Fig. 9 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced TNF α release in THP1 differentiated macrophages with higher potency than D-TAT-IB1 (SEQ ID NO: 197), dTAT (SEQ ID NO: 45) and SP 600125. LPS was added for 6h (10 ng/ml).

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Fig. 10 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced IL-6 release in PMA differentiated macrophages with higher potency than D-TAT-IB1 (SEQ ID NO: 197) and L-TAT-IB1 (SEQ ID NO: 196). LPS was added for 6h.

5 Fig. 11 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced TNF α release in PMA differentiated macrophages with higher potency than D-TAT-IB1 (SEQ ID NO: 197) and L-TAT-IB1 (SEQ ID NO: 196).

10 Fig. 12 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced TNF α release in Primary Rat Whole Blood Cells at 3 ng/ml. Given are the results for the control, 1 μ M of SEQ ID NO: 172, 3 μ M of SEQ ID NO: 172, and 10 μ M of SEQ ID NO: 172 at different levels of LPS (ng/ml).

15 Fig. 13 The JNK inhibitor of SEQ ID NO: 172 blocks IL-2 secretion by primary human T-cells in response to PMA/Ionomycin.

Fig. 14 The JNK inhibitor of SEQ ID NO: 172 blocks IL-2 secretion by primary human T-cells in response to CD3/CD28 stimulation. The JNK inhibitors used are indicated by their SEQ ID NO: 172 and 197.

20 Fig. 15 Dose-dependent inhibition by JNK inhibitor with SEQ ID NO: 172 of CD3/CD28-induced IL-2 release in primary rat lymph-nodes purified T cells. Control rat were sacrificed and lymph-nodes were harvested. T cells further were purified (using magnetic negative selection) and plated into 96-well plates at 200.000 cells/well. Cells were treated with anti-rat CD3 and anti-rat CD28 antibodies (2 μ g/mL). JNK inhibitor with SEQ ID NO: 172 was added to the cultures 1h before CD3/CD28 treatment and IL-2 release was assessed in supernatant 24h after treatment.

25 Fig. 16 Dose-dependent inhibition of CD3/CD28-induced IL-2 release in primary rat lymph nodes purified T cells: Comparison of several JNK inhibitors, namely SEQ ID NOs: 172, 197 and SP600125.

30 Fig. 17 Dose dependent inhibition of IL-2 release in rat whole blood stimulated with PMA + ionomycin. JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 μ M 1h before stimulation with PMA + ionomycin. Three doses of activators were added (25/500 ng/mL, 50/750 ng/mL and 50/1000 ng/mL) for 4h. IL-2 release

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was assessed in supernatant. JNK inhibitor with SEQ ID NO: 172 at 10 μ M did efficiently reduce PMA-iono-induced IL-2 release at the three tested activator concentrations.

Fig. 18 JNK inhibition and IL-6 release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 μ M 1h before whole blood stimulation with LPS (0.02 ng/mL) for 4 hours. The JNK inhibitor with SEQ ID NO: 172 did reduce the LPS-induced IL-6 release in a dose-dependent manner.

Fig. 19 JNK inhibition and IL-2 release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 μ M 1h before whole blood stimulation with PMA+ionomycin (25/700 ng/mL, 50/800 ng/mL and 50/1000 ng/mL) for 4 hours. The JNK inhibitor with SEQ ID NO: 172 did reduce the PMA+ionomycin-induced IL-2 release in a dose-dependent manner.

Fig. 20 JNK inhibition and IFN- γ release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 μ M 1h before whole blood stimulation with PMA+ionomycin (25/700 ng/mL, 50/800 ng/mL and 50/1000 ng/mL) for 4 hours. The JNK inhibitor with SEQ ID NO: 172 did reduce the PMA+ionomycin-induced IFN- γ release in a dose-dependent manner.

Fig. 21 JNK inhibition and TNF- α release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 μ M 1h before whole blood stimulation with PMA+ionomycin (25/700 ng/mL, 50/800 ng/mL and 50/1000 ng/mL) for 4 hours. The JNK inhibitor with SEQ ID NO: 172 did reduce the PMA+ionomycin-induced TNF- α release in a dose-dependent manner.

Fig. 22 JNK inhibition and TNF- α release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 μ M 1h before whole blood stimulation with PHA-L (5 μ g/mL) for 3 days. The JNK inhibitor with SEQ ID NO: 172 did reduce the PHA-L-induced TNF- α release in a dose-dependent manner.

Fig. 23 JNK inhibition and IL-2 release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 μ M 1h before whole blood stimulation with PHA-L (5 μ g/mL) for 3 days. The JNK inhibitor with SEQ ID NO: 172 did reduce the PHA-L-induced IL-2 release in a dose-dependent manner.

Fig. 24 JNK inhibition and TNF- α release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 μ M 1h before whole blood stimulation with CD3 +/- CD28 antibodies (2 μ g/mL) for 3 days. The JNK inhibitor with SEQ ID NO: 172 did reduce the CD3/CD28-induced TNF- α release in a dose-dependent manner.

Fig. 25 Photographic illustration of in vivo anti-inflammatory properties of the JNK inhibitors with SEQ ID NO: 197 (10 μ g/kg) and SEQ ID NO: 172 (10 μ g/kg) after CFA (complete Freund's adjuvant) induced paw swelling. Paw swelling was induced in the left hind paw, the right hind paw was not treated.

Fig. 26 Graphical representation of in vivo anti-inflammatory properties of the JNK inhibitors with SEQ ID NO: 197 (10 μ g/kg, n=4) and SEQ ID NO: 172 (10 μ g/kg, n=3) after CFA (complete Freund's adjuvant) induced paw swelling. Indicated is the measured circumference of the left hind paw after treatment.

Fig. 27 Graphical representation of in vivo anti-inflammatory properties of the JNK inhibitors with SEQ ID NO: 197 (10 μ g/kg) and SEQ ID NO: 172 (10 μ g/kg) after CFA (complete Freund's adjuvant) induced paw swelling. Indicated is the measured in vivo cytokine release one hour after CFA induced paw swelling.

Fig. 28 Clinical evaluation of administration of different amounts of the JNK inhibitor according to SEQ ID NO: 172 in albino rats after intravenous administration (endotoxin-induced uveitis model, EIU). From left to right: Vehicle, 0.015 mg/kg (i.v.) of SEQ ID NO: 172; 0.18 mg/kg (i.v.) of SEQ ID NO: 172; 1.8 mg/kg (i.v.) of SEQ ID NO: 172, 2 mg/kg (i.v.) of SEQ ID NO: 197 and 20 μ g dexamethasone (administered directly by subconjunctival injection to the eye). Indicated is the clinical score (mean and the SEM).

Fig. 29 Responsive effects of the JNK inhibitor of SEQ ID NO: 172 after daily intravenous administration in 14 day rat chronic established Type II collagen arthritis (RTTC/SOL-1). Shown is the body weight change from day 0 to day 14. From left to right: Normal control + Vehicle (NaCl), Disease Control + Vehicle (NaCl), 5 mg/kg (i.v.) of SEQ ID NO: 172; 1 mg/kg (i.v.) of SEQ ID NO: 172; 0.1 mg/kg (i.v.) of SEQ ID NO: 172, 0.01 mg/kg (i.v.) of SEQ ID NO: 172, 0.05 mg/kg (i.v.) of dexamethasone. Indicated is the clinical score (mean and the SEM). n= 4/normal group, n=8/treatment group; *p $\leq$ 0.05 1-way ANOVA to disease control + Vehicle (NaCl)

Fig.30 Responsive effects of the JNK inhibitor of SEQ ID NO: 172 after daily intravenous administration in 14 day rat chronic established Type II collagen arthritis (RTTC/SOL-1). Shown is the ankle diameter (in) over time. n= 4/normal group, n=8/treatment group; *p ≤0.05 2-way RM ANOVA to disease control + Vehicle (NaCl).

Fig. 31 Responsive effects of the JNK inhibitor of SEQ ID NO: 172 after daily intravenous administration in 14 day rat chronic established Type II collagen arthritis (RTTC/SOL-1). Illustrated are the ankle histopathology scores regarding inflammation, pannus, cartilage damage and bone resorption. n=8 in the treatment group. *p ≤0.05 Mann-Whitney U test to disease control + Vehicle (NaCl).

Fig. 32 Responsive effects of the JNK inhibitor of SEQ ID NO: 172 after daily intravenous administration in 14 day rat chronic established Type II collagen arthritis (RTTC/SOL-1). Illustrated are the knee histopathology scores regarding inflammation, pannus, cartilage damage and bone resorption. n=8 in the treatment group. *p ≤0.05 Mann-Whitney U test to disease control + Vehicle (NaCl).

Fig. 33 Clinical scoring by slit lamp 24 hours after EIU induction and administration of JNK inhibitor according to SEQ ID NO: 172 (1 mg/kg i.v.) at different times prior to EIU induction. From left to right: Vehicle (0 hours); SEQ ID NO: 172 4 weeks prior to EIU induction; SEQ ID NO: 172 2 weeks prior to EIU induction; SEQ ID NO: 172 1 week prior to EIU induction; SEQ ID NO: 172 48 hours prior to EIU induction; SEQ ID NO: 172 24 hours prior to EIU induction; SEQ ID NO: 172 0 hours prior to EIU induction; Dexamethasone (2 mg/kg i.v.) 0 hours prior to EIU induction. Mean ± SEM. *p<0.05 versus vehicle, **p<0.01 versus vehicle.

Fig. 34 Number of PMN cells per section quantified 24 hours after EIU induction and administration of JNK inhibitor according to SEQ ID NO: 172 (1 mg/kg i.v.) at different times prior to EIU induction. From left to right: Vehicle (0 hours); SEQ ID NO: 172 4 weeks prior to EIU induction; SEQ ID NO: 172 2 weeks prior to EIU induction; SEQ ID NO: 172 1 week prior to EIU induction; SEQ ID NO: 172 48 hours prior to EIU induction; SEQ ID NO: 172 24 hours prior to EIU induction; SEQ ID NO: 172 0 hours prior to EIU induction; Dexamethasone (2 mg/kg i.v.) 0 hours prior to EIU induction. Mean ± SEM. *p<0.05 versus vehicle, **p<0.01 versus vehicle.

Fig. 35 shows the mean calculated TBUT AUC values for animals with scopolamine-induced dry eye syndrome. Shown are the results for animals treated with vehicle, 3 different concentrations of an all-D-retro-inverso JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 197, 3 different concentrations of a JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 172, and the results for animals treated with cyclosporine.

Fig. 36 shows the mean calculated PRTT AUCs for animals with scopolamine induced Dry Eye (Day 7-21). Shown are the results for animals treated with vehicle, 3 different concentrations of an all-D-retro-inverso JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 197, 3 different concentrations of a JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 172, and the results for animals treated with cyclosporine.

Fig. 37 shows the mean histological Cornea Lesion Scores for animals with scopolamine induced dry eye syndrome. Shown are the results for animals treated with vehicle, 3 different concentrations of an all-D-retro-inverso JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 197, 3 different concentrations of a JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 172, and the results for animals treated with cyclosporine.

Fig. 38 shows the renal function assessed by protidemia (A) and urea level (B) of rats in an Adriamycin (ADR)-induced nephropathy model on Days 8, 14, 29, 41 and 56 after ADR administration. Groups No. 1 ("ADR") and No. 2 ("ADR + JNK inhibitor SEQ Id NO: 172") have been treated on Day 0 with ADR to induce necropathy, whereas group No. 3 ("NaCl") received 0.9% NaCl. Moreover, group No. 2 ("ADR + JNK inhibitor SEQ Id NO: 172") has been treated on Day 0 with the JNK inhibitor SEQ ID NO: 172, whereas groups No. 1 and 3 received vehicle (0.9% NaCl).

Fig. 39 shows kidney sections of the rats in the Adriamycin (ADR)-induced nephropathy model stained with periodic acid-Schiff (PAS) (original magnification x40). For the sections shown in the left column, rats were sacrificed at Day 8 following ADR administration, whereas for the sections shown in the right column, rats were sacrificed at Day 56. ADR has been administered only to the groups "ADR" and "ADR + XG104", whereas the group "NaCl" received 0.9% NaCl only. The group "ADR + XG104" has been treated on Day 0 with the JNK inhibitor SEQ ID NO: 172 (i.e. "XG104" refers to the JNK inhibitor SEQ ID NO: 172), whereas the other groups ("ADR" and "NaCl") received vehicle (0.9% NaCl).

Fig. 40 shows the kidney fibrosis in ADR nephropathy evaluated with Masson's trichrome (blue) on Days 8 (left four panels) and 56 (right four panels) following ADR administration for the group "ADR" (upper panel), which has been treated with ADR and vehicle at Day 0 and for the group "ADR + XG104" (lower panel), which has been treated with ADR and the JNK inhibitor SEQ ID NO: 172 at Day 0. The original magnification x10 is depicted in the left panels for the respective day and the original magnification x40 is depicted in the right panels for the respective day.

Fig. 41 shows the average group grade for inflammation of the ear in an imiquimod-induced psoriasis-model in mice after six consecutive days of imiquimod application. The "average grade" refers to the microscopic histopathology end-points (cf. Example 14). Three doses (0.02, 0.2 and 2 mg/kg) of the JNK inhibitor of SEQ ID NO: 172 have been tested (groups "XG-104 0.02 mg/kg, XG-104 0.2 mg/kg, and XG-104 2 mg/kg, respectively). Prednisolone and dexamethasone served as positive controls. The groups XG-104 0.2 mg/kg, prednisolone and dexamethasone showed significant differences from the vehicle control group.

Fig. 42 Renal ischemia was induced in rats of group G2 and group G3 by clamping both renal pedicles with atraumatic clamp for 40 min, whereas in group G1 rats no ischemia was induced. Rats of group G3 received a single dose of 2 mg/kg of the JNK inhibitor according to SEQ ID NO. 172 ("XG-104") (in 0.9% NaCl as vehicle) and rats of groups G1 and G2 received vehicle, respectively, by IV injection in the tail vein on Day 0, one hour after clamping period (after reperfusion) both renal pedicles with atraumatic clamp. Serum creatinine and urea were increased in vehicle-treated ischemic rats (G2) 24h following ischemia, as compared to vehicle-treated controls rats without ischemia (G1). On the other hand, XG-104-treated-ischemic rats (G3) exhibited lower serum creatinine and lower urea, relatively to untreated ischemic rats (G2).

Fig. 43 shows for Example 20 that XG-104 blocks the development of a psoriatic phenotype in vivo in the K5.STAT3c model for psoriasis. Wild type or K5STAT3c mice were treated with either vehicle (Saline) or XG-104 compound prior to tape stripping, followed by daily treatments of vehicle or XG-104. After five days, mice were sacrificed and biopsies taken from the lesion sites in order to quantify acanthosis (thickening of the epidermis). Quantification of acanthosis is shown for two independent experiments separately (A) and combined (B). Dots represent mean values from individual mice (A&B). A one-way ANOVA test was performed to analyze significance.

Fig. 44 shows for Example 20 representative histologies of K5.STAT3c mice treated with either Saline or XG-104, indicating that XG-104 blocks the development of a psoriatic phenotype in vivo in the K5.STAT3c model for psoriasis.

- 5 Fig. 45 shows for Example 19 the results of the determination of the cytotoxic activity of XG-104 against HepG2 (A) and PLC/PRF/5 (B) tumour cell lines using MTS assay.

Fig. 46 shows for Example 23 the study design.

- 10 Fig. 47 shows for Example 23 the effects of vehicle and XG-104 (2 mg/kg, *i.v.*) on tubular damages in a rat model of bilateral IR. *** $P < 0.001$ *versus* Group 1 (Sham/Vehicle) by a Student *t*-test ns; + $P < 0.05$ *versus* Group 2 (IR/Vehicle) by a one way ANOVA followed by a Bonferroni's post test.

- 15 Fig. 48 shows for Example 23 the effects of vehicle and XG-104 (2 mg/kg, *i.v.*) on total tubular histological scores in a rat model of bilateral IR. Total tubular score represents all tubular changes including degeneration and necrosis, tubular cast, tubular epithelial vacuolation and regeneration (basophilic tubules). *** $P < 0.001$ *versus* Group 1 (Sham/Vehicle) by a Student *t*-test; + $P < 0.05$ *versus* Group 2 (IR/Vehicle) by a one way ANOVA followed by a Bonferroni's
20 post test.

- Fig. 49 shows for Example 23 representative images of hematoxylin/eosin stained kidney sections: comparison between Groups 2 (IR/Vehicle) and 3 (IR/XG-104). Animal 53 (Top Left), Animal 15 (Top Right), Animal 46 (Bottom left), and Animal 18 (Bottom right): 10x. Representative
25 photomicrographs of tubular degeneration/necrosis and tubular casts in Group 2 (vehicle) and 3 (XG-104). Animals having scores from 1 to 4 are represented. The main difference between groups is that the severity of tubular necrosis and cast in Group 2 is generally higher than that observed in Group 3. In Group 2, lesions are extended partially or to the majority of the cortex. Comparatively, in Group 3, lesions are limited to the cortico-medullary
30 junction. Lesions consist of a mixture of active necrosis, cellular tubular casts, hyaline casts, and occasional basophilic tubules.

Fig. 50 shows for Example 24 the study design (A) and the AUCs method to assess allodynia and hyperalgesia (B).

Fig. 51 shows for Example 24 the effect of XG-104 (50 mg/mL, i.v.) and ibuprofen (50 mg/mL, i.v.) treatments on nociceptive parameters 24h post-CYP injection. Nociceptive threshold (A), nociceptive scores (B), AUC 1-8 g (C) or AUC 8-60 g (D) 24h after CYP injection. Results are expressed as mean $\pm$ s.e.m. (n=10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs Vehicle-treated group, Mann Whitney test (A and C), Two-way RM ANOVA (B), and Unpaired t test and Mann Whitney test (D).

Fig. 52 shows for Example 24 the effect of XG-104 (50 mg/mL, i.v.) and ibuprofen (50 mg/mL, i.v.) treatments on urinary bladder wall thickness as well as on oedema and haemorrhage scores 24h post-CYP injection. Urinary bladder wall thickness (A), oedema scores (B), or haemorrhage scores (C) 24h after CYP injection. Results are expressed as mean $\pm$ s.e.m. (n=10). ns= $p > 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs Vehicle-treated group, Mann Whitney test and Unpaired t test (A) or Mann Whitney test (B and C).

Fig. 53 shows for Example 25 the effect of XG-104 (2 mg/kg, i.v.) and ibuprofen (10 mg/kg, i.v.) treatments on nociceptive parameters 24h post-CYP injection. Nociceptive threshold (A), nociceptive scores (B), AUC 1-8 g (C) or AUC 8-60 g (D) 24h after CYP injection. Results are expressed as mean $\pm$ s.e.m. (n=10). ** $p < 0.01$, *** $p < 0.001$ vs Vehicle-treated group, Mann Whitney test (A), Two-way RM ANOVA (B), Mann Whitney test and Unpaired t test (C) and Unpaired t test (D).

Fig. 54 shows for Example 26 the effect of XG-104 on neuronal apoptosis. (A) Immunoblot analysis of primary mouse cortical neuron cultures exposed to 2 μ M or 25 μ M of A β 1-42 (A β ₄₂) during 5 hours. Neurons were pre-treated with or without 5 μ M or 10 μ M of the specific inhibitor, XG-104. (B) Corresponding histogram showing no modification of JNK activity with 2 μ M of A β ₄₂. Pre-treatment with 5 μ M and 10 μ M of XG-104 decreased JNK activity by respectively 29.2% and 60%. 25 μ M A β ₄₂ treatment of the neurons increased JNK activity by 14%. Pre-treatment with 5 μ M and 10 μ M XG-104 decreased JNK activity by, 17.5% and 59.6%, respectively. In both A β ₄₂ cell stress conditions, 10 μ M XG-104 concentration was more effective to decrease JNK activity. (C) Neuronal apoptosis is measured by the level of cleaved PARP protein, which increases during apoptosis. Both A β ₄₂ stress conditions did not significantly exacerbate spontaneous apoptosis. Pre-treatment with 5 μ M and 10 μ M XG-104 decreased PARP cleavage by 46.8% and 80.2%,

respectively, with 2 μM $\text{A}\beta_{42}$ and decreased by 69% and 80.6%, respectively, with 25 μM $\text{A}\beta_{42}$.

Fig. 55 shows for Example 26 the decrease of neuronal apoptosis after PKR down-regulation and/or JNK inhibition with XG-104, referred to as "JNKi" (in Fig. 55). (A) Immunoblot results of the levels of JNK and c-Jun activation, caspase 3 and PARP cleaved activated fragments in primary neuronal cultures of WT and $\text{PKR}^{-/-}$ mice, treated by 2 μM of $\text{A}\beta_{42}$ after or not pre-inhibition of JNK with 10 μM JNKi compound. (B-D) Corresponding histograms of JNK activity (B), phospho c-Jun (C), and total c-Jun (D). (E-G) Apoptosis is measured by the level of cleaved caspase 3 (E), caspase 3 activity measured in the cell culture supernatant (F) and cleaved PARP (G). Data are means $\pm$ SEM ($n \geq 3$ per condition). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

JNK inhibitors

In a first aspect the present invention relates to a JNK inhibitor, which comprises an inhibitory (poly-)peptide sequence according to the following general formula:

X1-X2-X3-R-X4-X5-X6-L-X7-L-X8 (SEQ ID NO: 1),

wherein X1 is an amino acid selected from amino acids R, P, Q and r,
 wherein X2 is an amino acid selected from amino acids R, P, G and r,
 wherein X3 is an amino acid selected from amino acids K, R, k and r,
 wherein X4 is an amino acid selected from amino acids P and K,
 wherein X5 is an amino acid selected from amino acids T, a, s, q, k or is absent,
 wherein X6 is an amino acid selected from amino acids T, D and A,
 wherein X7 is an amino acid selected from amino acids N, n, r and K; and
 wherein X8 is an amino acid selected from F, f and w,

with the proviso that at least one, at least two, at least three, at least four, at least five or six of the amino acids selected from the group consisting of X1, X2, X3, X5, X7 and X8 is/are a D-amino acid(s), preferably with the proviso that at least one, at least two, at least three or four of the amino acids selected from the group consisting of X3, X5, X7 and X8 is/are a D-amino acid(s),

for use in a method for treatment of the human or animal body by therapy, in particular for the treatment of the diseases/disorders disclosed herein.

The inhibitory (poly-)peptide sequence of the JNK inhibitor according to the present invention comprises L-amino acids and in most embodiments D-amino acids. Unless specified otherwise, L-amino acid residues are indicated herein in capital letters, while D amino acid residues are indicated in small letters. Glycine may be indicated in capital or small letters (since there is no D- or L-glycine). The amino acid sequences disclosed herein are always given from N- to C-terminus (left to right) unless specified otherwise. The given amino acid sequence may be modified or unmodified at the C- and/or N-terminus, e.g. acetylation at the C-terminus and/or amidation or modification with cysteamide at the N-terminus. Such conceivable, but optional modifications at the C- and/or N-terminus of the amino acid sequences disclosed herein are - for sake of clarity - not specifically indicated.

The JNK inhibitors of the present invention are (poly-)peptide inhibitors of the c-Jun N-terminal kinase (JNK). Said inhibitors inhibit the kinase activity of c-Jun N-terminal kinase (JNK), i.e. prevent or reduce the extent of phosphorylation of JNK substrates, such as c-Jun, ATF2 and/or Elk-1 by e.g. blocking the JNK activity. A person skilled in the art will understand that the term "inhibitor", as used herein, does not comprise compounds which irreversibly destroy the c-Jun N-terminal kinase (JNK) molecule and/or kinase activity. Accordingly, the JNK inhibitory activity of the inhibitors of the present invention typically refers to compounds which bind in a competitive or non-competitive manner to JNK. Furthermore, the term "inhibiting JNK activity" as used herein, refers to the inhibition of the kinase activity of c-Jun N-terminal kinase (JNK).

Furthermore, as used herein, a JNK inhibitor comprises at least one functional unit of a polymer of amino acids, i.e. a (poly-)peptide sequence. Moreover, this at least one functional polymer of amino acids provides for inhibition of JNK activity. The amino acid monomers of said inhibitory (poly-)peptide sequence are usually linked to each other via peptide bonds, but (chemical) modifications of said peptide bond(s) or of side chain residues may be tolerable, provided the inhibitory activity (inhibition of JNK activity) is not totally lost, i.e. the resulting chemical entity still qualifies as JNK inhibitor as functionally defined herein. The term "(poly-)peptide" shall not be construed as limiting the length of the (poly-)peptide unit. Preferably, the inhibitory (poly-)peptide sequence of the JNK inhibitors of the present invention is less than 500, 490, 480, 470, 460, 450, 440, 430, 420, 410, 400, 390, 380, 370, 360, 350, 340, 330, 320, 310, 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24,

23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, or less than 12 amino acids long. Preferably, the inhibitory (poly-)peptide sequence does not have less than 10 amino acid residues, more preferably not less than 11 amino acid residues.

5 Furthermore, a "JNK inhibitor" of the present invention inhibits JNK activity, e.g. exhibits with regard to the inhibition of human JNK mediated phosphorylation of a c-Jun substrate (SEQ ID NO: 198) an IC 50 value of:

- 10 a) less than 3000 nM, more preferably less than 2000 nM, even more preferably less than 1000 nM, even more preferably less than 500 nM, even more preferably less than 250 nM, even more preferably less than 200 nM, even more preferably less than 150 nM, most preferably less than 100 nM with regard to inhibition of human JNK1,
- 15 b) less than 3000 nM, more preferably less than 2000 nM, even more preferably less than 1000 nM, even more preferably less than 500 nM, even more preferably less than 250 nM, even more preferably less than 200 nM, even more preferably less than 150 nM, most preferably less than 100 nM with regard to inhibition of human JNK2, and/or
- 20 c) less than 3000 nM, more preferably less than 2000 nM, even more preferably less than 1000 nM, even more preferably less than 500 nM, even more preferably less than 250 nM, even more preferably less than 200 nM, even more preferably less than 150 nM, most preferably less than 100 nM with regard to inhibition of human JNK3.

For some applications, it is preferred that the inhibitor inhibits human JNK2 and/or human JNK3 according to the above definition, but not JNK1 according to the above definition.

25 Whether JNK activity is inhibited or not, may easily be assessed by a person skilled in the art. There are several methods known in the art. One example is a radioactive kinase assay or a non-radioactive kinase assay (e.g. Alpha screen test; see for example Guenat et al. J Biomol Screen, 2006; 11: pages 1015-1026).

30 A JNK inhibitor according to the present invention may thus for example comprise an inhibitory (poly-)peptide sequence according to any of SEQ ID NOs: 2 to 27 (see table 1).

Table 1: Examples for inhibitory (poly-)peptide sequences of JNK-inhibitors according to the present invention
--

Amino acid sequence	SEQ ID NO:
rPKR <u>P</u> TT <u>L</u> N <u>L</u> F	2
RPkR <u>P</u> TT <u>L</u> N <u>L</u> F	3
RPKR <u>P</u> aT <u>L</u> N <u>L</u> F	4
RPKR <u>P</u> TT <u>L</u> n <u>L</u> F	5
RPKR <u>P</u> TT <u>L</u> r <u>L</u> F	6
RPKR <u>P</u> TT <u>L</u> N <u>L</u> f	7
RPkR <u>P</u> aT <u>L</u> N <u>L</u> f	8
RPkR <u>P</u> TT <u>L</u> N <u>L</u> f	9
RPkR <u>P</u> TT <u>L</u> r <u>L</u> f	10
RRrR <u>P</u> TT <u>L</u> N <u>L</u> f	11
QRrR <u>P</u> TT <u>L</u> N <u>L</u> f	12
RPkR <u>P</u> TT <u>L</u> N <u>L</u> w	13
RPkR <u>P</u> TD <u>L</u> N <u>L</u> f	14
RRrR <u>P</u> TT <u>L</u> r <u>L</u> w	15
QRrR <u>P</u> TT <u>L</u> r <u>L</u> w	16
RRrR <u>P</u> TD <u>L</u> r <u>L</u> w	17
QRrR <u>P</u> TD <u>L</u> r <u>L</u> w	18
RRrR <u>P</u> aT <u>L</u> N <u>L</u> f	19
QRrR <u>P</u> aT <u>L</u> N <u>L</u> f	20
RrKR <u>P</u> aT <u>L</u> N <u>L</u> f	21
RPkR <u>P</u> sT <u>L</u> N <u>L</u> f	22
RPkR <u>P</u> qT <u>L</u> N <u>L</u> f	23
RPkR <u>P</u> kT <u>L</u> N <u>L</u> f	24
rGKR <u>K</u> AL <u>K</u> <u>L</u> f	25
rGKR <u>K</u> AL <u>r</u> <u>L</u> f	26
RRrR <u>K</u> AL <u>r</u> <u>L</u> f	27

The JNK inhibitor according to the present invention may also be a JNK inhibitor (variant) which comprises an inhibitory (poly-)peptide sequence sharing at least 50%, more preferably at least 55%, more preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, most preferably at least 90%, more preferably at least 95% sequence identity with a sequence selected from SEQ ID NOs: 1-27, in particular with SEQ ID NO: 8,

preferably with the proviso that with regard to the respective sequence selected from SEQ ID NOs: 1-27, such inhibitory (poly-)peptide sequence sharing sequence identity

- a) maintains the L-arginine (R) residue on position 4,
- b) maintains the two L-leucine (L) residues at position 8 and 10 (positions 7 and 9 with regard to SEQ ID NOs: 25-27),
- c) exhibits one, two, three, four, five or six D-amino acid(s) at the respective positions corresponding to the amino acids selected from the group consisting of X1, X2, X3, X5, X7 and X8 of SEQ ID NO: 1 and respective positions in SEQ ID NOs: 2-27, more preferably exhibits one, two, three or four D-amino acid(s) at the positions corresponding to the amino acids selected from the group consisting of X3, X5, X7 and X8 of SEQ ID NO: 1 and respective positions in SEQ ID NOs: 2-27, and
- d) still inhibits JNK activity (i.e. is a JNK inhibitor as defined herein).

Certainly, variants disclosed herein (in particular JNK inhibitor variants comprising an inhibitory (poly-)peptide sequence sharing - within the above definition – a certain degree of sequence identity with a sequence selected from SEQ ID NOs: 1-27), share preferably less than 100% sequence identity with the respective reference sequence.

In view of said definition and for sake of clarity the residues which may preferably not be altered variants of JNK inhibitors comprising SEQ ID NOs: 1-27 (see a) and b) in the above definition) are underlined in table 1.

The non-identical amino acids are preferably the result of conservative amino acid substitutions.

Conservative amino acid substitutions, as used herein, may include amino acid residues within a group which have sufficiently similar physicochemical properties, so that a substitution between members of the group will preserve the biological activity of the molecule (see e.g. Grantham, R. (1974), *Science* 185, 862-864). Particularly, conservative amino acid substitutions are preferably substitutions in which the amino acids originate from the same class of amino acids (e.g. basic amino acids, acidic amino acids, polar amino acids, amino acids with aliphatic side chains, amino acids with positively or negatively charged side chains, amino acids with aromatic groups in the side chains, amino acids the side chains of which can enter into hydrogen bridges, e.g. side chains which have a hydroxyl function, etc.). Conservative substitutions are in the present case for example substituting a basic amino acid residue (Lys, Arg, His) for another basic amino acid residue (Lys, Arg, His), substituting an aliphatic amino acid residue (Gly, Ala, Val, Leu, Ile) for another aliphatic amino acid residue, substituting an aromatic amino acid residue (Phe, Tyr, Trp) for another aromatic

amino acid residue, substituting threonine by serine or leucine by isoleucine. Further conservative amino acid exchanges will be known to the person skilled in the art. The isomer form should preferably be maintained, e.g. K is preferably substituted for R or H, while k is preferably substituted for r and h.

5

Further possible substitutions within the above definition for JNK inhibitor variants are for example:

- a) one, two or more of X1, X2, X3, X4, X5, X6, X7 and/or X8 of SEQ ID NO: 1 or the corresponding positions within the respective sequence selected from SEQ ID NOs: 2-27 are substituted for A or a,
- 10 b) X1 or X8 of SEQ ID NO: 1 or the corresponding position within the respective sequence selected from SEQ ID NOs: 2-27 is deleted;
- c) X5 of SEQ ID NO: 1 or the corresponding position within the respective sequence selected from SEQ ID NOs: 2-27 is E, Y, L, V, F or K;
- d) X5 of SEQ ID NO: 1 or the corresponding position within the respective sequence
15 selected from SEQ ID NOs: 2-27 is E, L, V, F or K; or
- e) one, two or three of X1, X2, X3 of SEQ ID NO: 1 or the corresponding positions within the respective sequence selected from SEQ ID NOs: 2-27 are neutral amino acids.

- 20 As used herein, the term "% sequence identity", has to be understood as follows: Two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may then be determined over the whole length of each of the sequences being compared (so-called global alignment), that is particularly suitable for sequences of the same or similar length, or over
25 shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length. In the above context, an amino acid sequence having a "sequence identity" of at least, for example, 95% to a query amino acid sequence, is intended to mean that the sequence of the subject amino acid sequence is identical to the query sequence except that the subject amino acid sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid
30 sequence. In other words, to obtain an amino acid sequence having a sequence of at least 95% identity to a query amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the subject sequence may be inserted or substituted with another amino acid or deleted. For purposes of determining sequence identity, the substitution of an L-amino acid for a D-amino acid (and vice versa) is considered to yield a non-identical residue, even if it is merely the D- (or L-isomer) of the
35 very same amino acid.

Methods for comparing the identity and homology of two or more sequences are well known in the art. The percentage to which two sequences are identical can for example be determined by using a mathematical algorithm. A preferred, but not limiting, example of a mathematical algorithm which can be used is the algorithm of Karlin *et al.* (1993), PNAS USA, 90:5873-5877. Such an algorithm is integrated in the BLAST family of programs, e.g. BLAST or NBLAST program (see also Altschul *et al.*, 1990, J. Mol. Biol. 215, 403-410 or Altschul *et al.* (1997), Nucleic Acids Res, 25:3389-3402), accessible through the home page of the NCBI at world wide web site ncbi.nlm.nih.gov) and FASTA (Pearson (1990), Methods Enzymol. 183, 63-98; Pearson and Lipman (1988), Proc. Natl. Acad. Sci. U. S. A 85, 2444-2448.). Sequences which are identical to other sequences to a certain extent can be identified by these programmes. Furthermore, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux *et al.*, 1984, Nucleic Acids Res., 387-395), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of (Smith and Waterman (1981), J. Mol. Biol. 147, 195-197.) and finds the best single region of similarity between two sequences.

Certainly, the JNK inhibitor according to the present invention may comprise – in addition to the inhibitory (poly-)peptide sequence mentioned above – additional sequences or sequence elements, domains, labels (e.g. fluorescent or radioactive labels), epitopes etc., as long as the ability to inhibit JNK activity as defined herein is not lost. For example, the JNK inhibitor according to the present invention may also comprise a transporter sequence. A "transporter sequence" as used herein, is a (poly-)peptide sequence providing for translocation of the molecule it is attached to across biological membranes. Accordingly, a JNK inhibitor according to the present invention comprising a transporter sequence is preferably capable of translocating (e.g. the conjugated cargo compound) across biological membranes. Thus, such JNK inhibitors of the present invention may more readily enter into a cell, a cellular subcompartment and/or into the nucleus of a cell.

Said transporter sequence may be joined for example (e.g. directly) N-terminally or (e.g. directly) C-terminally to the inhibitory (poly-)peptide sequence of the JNK inhibitor, preferably by a covalent linkage. The transporter sequence and the inhibitory (poly-)peptide sequence may also be spaced apart, e.g. may be separated by intermediate or linker sequences. It is also contemplated that the transporter sequence may be positioned entirely elsewhere in the JNK inhibitor molecule than the inhibitory (poly-)peptide sequence, in particular if the JNK inhibitor is a more complex molecule (e.g. comprising several domains, is a multimeric conjugate etc.). It is also contemplated that the transporter sequence and the inhibitory (poly-)peptide sequence may overlap. However, the JNK inhibitory activity of the JNK inhibitory portion needs to be maintained. Examples for such overlapping instances are given further below.

Transporter sequences for use with the JNK inhibitor of the present invention may be selected from, without being limited thereto, transporter sequences derived from HIV TAT (HIV), e.g. native proteins such as e.g. the TAT protein (e.g. as described in U.S. Patent Nos. 5,804,604 and 5,674,980, each of these references being incorporated herein by reference), HSV VP22 (*Herpes simplex*) (described in e.g. WO 97/05265; Elliott and O'Hare, Cell 88 : 223-233 (1997)), non-viral proteins (Jackson et al, Proc. Natl. Acad. Sci. USA 89 : 10691-10695 (1992)), transporter sequences derived from Antennapedia, particularly from *Drosophila antennapedia* (e.g. the antennapedia carrier sequence thereof), FGF, lactoferrin, etc. or derived from basic peptides, e.g. peptides having a length of at least 5 or at least 10 or at least 15 amino acids, e.g. 5 to 15 amino acids, preferably 10 to 12 amino acids, Such transporter sequences preferably comprise at least 50%, more preferably at least 80 %, more preferably 85 % or even 90 % basic amino acids, such as e.g. arginine, lysine and/or histidine, or may be selected from e.g. arginine rich peptide sequences, such as RRRRRRRRR (R₉; SEQ ID NO: 152), RRRRRRRR (R₈; SEQ ID NO: 153), RRRRRRR (R₇; SEQ ID NO: 154), RRRRRR (R₆, SEQ ID NO: 155), RRRRR (R₅, SEQ ID NO: 156) etc., from VP22, from PTD-4 proteins or peptides, from RGD-K₁₆, from PEPT1/2 or PEPT1/2 proteins or peptides, from SynB3 or SynB3 proteins or peptides, from PC inhibitors, from P21 derived proteins or peptides, or from JNKI proteins or peptides.

Examples of transporter sequences for use in the JNK inhibitor of the present invention are in particular, without being limited thereto, basic transporter sequences derived from the HIV-1 TAT protein. Preferably, the basic transporter sequence of the HIV-1 TAT protein may include sequences from the human immunodeficiency virus HIV-1 TAT protein, e.g. as described in, e.g., U.S. Patent Nos. 5,804,604 and 5,674,980, each incorporated herein by reference. In this context, the full-length HIV-1 TAT protein has 86 amino acid residues encoded by two exons of the HIV TAT gene. TAT amino acids 1-72 are encoded by exon 1, whereas amino acids 73-86 are encoded by exon 2. The full-length TAT protein is characterized by a basic region which contains two lysines and six arginines (amino acids 49-57) and a cysteine-rich region which contains seven cysteine residues (amino acids 22-37). The basic region (i.e., amino acids 49-57) was thought to be important for nuclear localization. Ruben, S. *et al.*, J. Virol. 63: 1-8 (1989); Hauber, J. *et al.*, J. Virol. 63 1181-1187 (1989). The cysteine-rich region mediates the formation of metal-linked dimers in vitro (Frankel, A. D. et al, Science 240: 70-73 (1988); Frankel, A. D. *et al.*, Proc. Natl. Acad. Sci USA 85: 6297-6300 (1988)) and is essential for its activity as a transactivator (Garcia, J. A. *et al.*, EMBO J. 7: 3143 (1988); Sadaie, M. R. *et al.*, J. Virol. 63:1 (1989)). As in other regulatory proteins, the N-terminal region may be involved in protection against intracellular proteases (Bachmair, A. *et al.*, Cell 56: 1019-1032 (1989)). Preferred TAT transporter sequences for use in the JNK inhibitor of the present

invention are preferably characterized by the presence of the TAT basic region amino acid sequence (amino acids 49-57 of naturally-occurring TAT protein); the absence of the TAT cysteine-rich region amino acid sequence (amino acids 22-36 of naturally-occurring TAT protein) and the absence of the TAT exon 2-encoded carboxy-terminal domain (amino acids 73-86 of naturally-occurring TAT protein). More preferably, the transporter sequence in the JNK inhibitor of the present invention may be selected from an amino acid sequence containing TAT residues 48-57 or 49 to 57 or variants thereof.

Preferably, the transporter sequence in a given JNK inhibitor of the present invention also exhibits D-amino acids, for example in order to improve stability towards proteases. Particularly preferred are transporter sequences which exhibit a specific order of alternating D- and L-amino acids. Such order of alternating D- and L-amino acids (the motif) may follow –without being limited thereto – the pattern of any one of SEQ ID NOs: 28-30:

$d_l L L L_x d_m L L L_y d_n$ (SEQ ID NO: 28);

$d L L L d (L L L d)_a$ (SEQ ID NO: 29); and/or

$d L L L d L L L d$ (SEQ ID NO: 30);

wherein:

d	is a D-amino acid;
L	is a L-amino acid;
a	is 0 – 3, preferably 0-2, more preferably 0, 1, 2 or 3, even more preferably 0, 1, or 2 and most preferably 1;
l, m and n	are independently from each other 1 or 2, preferably 1;
x and y	are independently from each other 0, 1 or 2, preferably 1.

Said order of D- and L-amino acids (motif) becomes relevant when the transporter sequence is synthesized, i.e. while the amino acid sequence (i.e. the type of side chain residues) remains unaltered, the respective isomers alternate. For example, a known transporter sequence derived from HIV TAT is RKKRRQRRR (SEQ ID NO: 43). Applying the D-/L amino acid order of SEQ ID NO: 30 thereto would yield rKKRrQRRr (SEQ ID NO: 46).

In a particular embodiment the transporter sequence of the JNK inhibitor of the present invention may comprise at least one sequence according to rXXXrXXXr (SEQ ID NO: 31), wherein:

r represents an D-enantiomeric arginine;

X is any L-amino acid (including glycine);

- and wherein each X may be selected individually and independently of any other X within SEQ ID NO: 31. Preferably at least 4 out of said 6 X L-amino acids within SEQ ID NO: 31 are K or R. In another embodiment the JNK inhibitor according to the present invention comprises the transporter sequence $rX_1X_2X_3rX_4X_5X_6r$ (SEQ ID NO: 32), wherein X_1 is K, X_2 is K, X_3 is R and X_4 , X_5 , and X_6 are any L-amino acid (including glycine) selected independently from each other. Similarly, the transporter sequence of the JNK inhibitor according to the present invention may comprise the sequence $rX_1X_2X_3rX_4X_5X_6r$ (SEQ ID NO: 33), wherein X_4 is Q, X_5 is R, X_6 is R and X_1 , X_2 , and X_3 are any L-amino acid (including glycine) selected independently from each other. The inventive JNK inhibitor may also comprise the sequence $rX_1X_2X_3rX_4X_5X_6r$ (SEQ ID NO: 34), wherein one, two, three, four, five or six X amino acid residues are chosen from the group consisting of: X_1 is K, X_2 is K, X_3 is R, X_4 is Q, X_5 is R, X_6 is R, while the remaining X amino acid residues not selected from above group may be any L-amino acid (including glycine) and are selected independently from each other. X_1 is then preferably Y and/or X_4 is preferably K or R.
- Examples of transporter sequences for use in the inventive JNK inhibitor molecule may be selected, without being limited thereto, from sequences as given in table 2 below, (SEQ ID NOs: 31-170) or from any fragment or variant or chemically modified derivative thereof (preferably it retains the function of translocating across a biological membrane).

Table 2:

Examples for transporter (poly-)peptide sequences for use in the JNK-inhibitors according to the present invention

SEQUENCE/PEPTIDE NAME	SEQ ID NO	AA	SEQUENCE
r3 (generic)	31	9	rXXXrXXXr
r3 (generic; right half)	32	9	rKKRrX ₄ X ₅ X ₆ r
r3 (generic; left half)	33	9	rX ₁ X ₂ X ₃ rQRRr
r3 (generic; individual)	34	9	rX ₁ X ₂ X ₃ rX ₄ X ₅ X ₆ r
TAT (1-86)	35	86	MEPVDPRLEP WKHPGSQPKT ACTNCYCKKC CFHCQVCFIT KALGISYGRK KRRQRRRPPQ GSQTHQVSLK KQPTSQSRGD PTGPKE
TAT (37-72)	36	36	CFITKALGIS YGRKKRRQRR RPPQGSQTHQ VLSKQ
TAT (37-58)	37	22	CFITKALGIS YGRKKRRQRR RP
TAT (38-58)GGC	38	24	FITKALGISY GRKKRRQRRR PGGC
TAT CGG(47-58)	39	15	CGGYGRKKRR QRRRP
TAT (47-58)GGC	40	15	YGRKKRRQRR RPGGC
TAT (1-72) Mut Cys/Ala 72	41	56	MEPVDPRLEP WKHPGSQPKT AFITKALGIS YGRKKRRQRR RPPQGSQTHQ VLSKQ
L-TAT (s1a)	42	10	GRKKRRQRRR (NH ₂ -GRKKRRQRRR-COOH)
L-TAT (s1b)	43	9	RKKRRQRRR (NH ₂ -GRKKRRQRRR-COOH)
L-TAT (s1c)	44	11	YDRKKRRQRRR
D-TAT	45	9	rrrqrrkkrr
r ₃ -L-TAT	46	9	rKKRrQRRr
r ₃ -L-TATi	47	9	rRRQrRKKr
βA-r ₃ -L-TAT	48	9	βA-rKKRrQRRr (βA: beta alanine)
βA-r ₃ -L-TATi	49	9	βA-rRRQrRKKr (βA: beta alanine)
FITC-βA-r ₃ -L-TAT	50	9	FITC-βA-rKKRrQRRr (βA: beta alanine)
FITC-βA-r ₃ -L-TATi	51	9	FITC-βA-rRRQrRKKr (βA: beta alanine)
TAT(s2-1)	52	9	rAKRrQRRr
TAT(s2-2)	53	9	rKARrQRRr
TAT(s2-3)	54	9	rKKArQRRr
TAT(s2-4)	55	9	rKKRrARRr
TAT(s2-5)	56	9	rKKRrQARr
TAT(s2-6)	57	9	rKKRrQARr
TAT(s2-7)	58	9	rDKRrQRRr
TAT(s2-8)	59	9	rKDRrQRRr
TAT(s2-9)	60	9	rKKDrQRRr

TAT(s2-10)	61	9	rKKRrDRRr
TAT(s2-11)	62	9	rKKRrQDRr
TAT(s2-12)	63	9	rKKRrQRDr
TAT(s2-13)	64	9	rEKrRrQRRr
TAT(s2-14)	65	9	rKERrQRRr
TAT(s2-15)	66	9	rKKErQRRr
TAT(s2-16)	67	9	rKKRrERRr
TAT(s2-17)	68	9	rKKRrQERr
TAT(s2-18)	69	9	rKKRrQREr
TAT(s2-19)	70	9	rFKRrQRRr
TAT(s2-20)	71	9	rKFRrQRRr
TAT(s2-21)	72	9	rKKFrQRRr
TAT(s2-22)	73	9	rKKRrFRRr
TAT(s2-23)	74	9	rKKRrQFRr
TAT(s2-24)	75	9	rKKRrQFRr
TAT(s2-25)	76	9	rKKRrQRRr
TAT(s2-26)	77	9	rKRRrQRRr
TAT(s2-27)	78	9	rKKKrrQRRr
TAT(s2-28)	79	9	rKKRrRRRr
TAT(s2-29)	80	9	rKKRrQKRr
TAT(s2-30)	81	9	rKKRrQKRr
TAT(s2-31)	82	9	rHKRrQRRr
TAT(s2-32)	83	9	rKHRrQRRr
TAT(s2-33)	84	9	rKKHrQRRr
TAT(s2-34)	85	9	rKKRrHRRr
TAT(s2-35)	86	9	rKKRrQHRr
TAT(s2-36)	87	9	rKKRrQRHr
TAT(s2-37)	88	9	rIKRrQRRr
TAT(s2-38)	89	9	rKIRrQRRr
TAT(s2-39)	90	9	rKKIrQRRr
TAT(s2-40)	91	9	rKKRrIRRr
TAT(s2-41)	92	9	rKKRrQIRr
TAT(s2-42)	93	9	rKKRrQRIr
TAT(s2-43)	94	9	rLKRrQRRr
TAT(s2-44)	95	9	rKLrRrQRRr

TAT(s2-45)	96	9	rKKLrQRRr
TAT(s2-46)	97	9	rKKRrLRRr
TAT(s2-47)	98	9	rKKRrQLRr
TAT(s2-48)	99	9	rKKRrQRLr
TAT(s2-49)	100	9	rMKRrQRRr
TAT(s2-50)	101	9	rKMRrQRRr
TAT(s2-51)	102	9	rKKMrQRRr
TAT(s2-52)	103	9	rKKRrMRRr
TAT(s2-53)	104	9	rKKRrQMRr
TAT(s2-54)	105	9	rKKRrQMRr
TAT(s2-55)	106	9	rNKRrQRRr
TAT(s2-56)	107	9	rKNRrQRRr
TAT(s2-57)	108	9	rKKNrQRRr
TAT(s2-58)	109	9	rKKRrNRRr
TAT(s2-59)	110	9	rKKRrQNRr
TAT(s2-60)	111	9	rKKRrQRNr
TAT(s2-61)	112	9	rQKRrQRRr
TAT(s2-62)	113	9	rQQRrQRRr
TAT(s2-63)	114	9	rKKQrQRRr
TAT(s2-64)	115	9	rKKRrKRRr
TAT(s2-65)	116	9	rKKRrQQRr
TAT(s2-66)	117	9	rKKRrQRQr
TAT(s2-67)	118	9	rSKRrQRRr
TAT(s2-68)	119	9	rKSRrQRRr
TAT(s2-69)	120	9	rKKSrQRRr
TAT(s2-70)	121	9	rKKRrSRRr
TAT(s2-71)	122	9	rKKRrQSRr
TAT(s2-72)	123	9	rKKRrQRSr
TAT(s2-73)	124	9	rTKRrQRRr
TAT(s2-74)	125	9	rKTRrQRRr
TAT(s2-75)	126	9	rKKTrQRRr
TAT(s2-76)	127	9	rKKRrTRRr
TAT(s2-77)	128	9	rKKRrQTRr
TAT(s2-78)	129	9	rKKRrQRTTr
TAT(s2-79)	130	9	rVKRrQRRr

TAT(s2-80)	131	9	rKVRrQRRr
TAT(s2-81)	132	9	rKKVrQRRr
TAT(s2-82)	133	9	rKKRrVRRr
TAT(s2-83)	134	9	rKKRrQVRr
TAT(s2-84)	135	9	rKKRrQRVr
TAT(s2-85)	136	9	rWKRrQRRr
TAT(s2-86)	137	9	rKWRrQRRr
TAT(s2-87)	138	9	rKKWrQRRr
TAT(s2-88)	139	9	rKKRrWRRr
TAT(s2-89)	140	9	rKKRrQWRr
TAT(s2-90)	141	9	rKKRrQRWr
TAT(s2-91)	142	9	rYKRrQRRr
TAT(s2-92)	143	9	rKYRrQRRr
TAT(s2-93)	144	9	rKKYrQRRr
TAT(s2-94)	145	9	rKKRrYRRr
TAT(s2-95)	146	9	rKKRrQYRr
TAT(s2-96)	147	9	rKKRrQRYr
TAT(s2-97)	148	8	rKKRrQRr
TAT(s2-98)	149	9	rKKRrQRrK
TAT(s2-99)	150	9	rKKRrQRrR
r ₃ R ₆	151	9	rrrrrrrrr
L-R ₉	152	9	RRRRRRRRR
L-R ₈	153	8	RRRRRRRR
L-R ₇	154	7	RRRRRRR
L-R ₆	155	6	RRRRRR
L-R ₅	156	5	RRRRR
r ₉	157	9	rrrrrrrrr
r ₅ R ₄ (D/L)	158	9	rRrRrRrRr
r ₅ R ₄ (DD/LL)	159	9	rrRRrrRRr
PTD-4	160	11	YARAAARQARA
PTD-4 (variant 1)	161	11	WARAAARQARA
PTD-4 (variant 2)	162	11	WARAQRAAARA
L-P1 Penetratin	163	16	RQVKVWFQNRRMKWKK
D-P1 Penetratin	164	16	KKWKMRNRNQFWVKVQR
JNKI, bestfit	165	17	WKRAAARKARAMSLNLF

JNK1, bestfit (variant 1)	166	17	WKRAAARAARAMSLNLF
MDCK transcytose sequence	167	9	RYRGDLGRR
YKGL	168	4	YKGL
P1	169	4	RRTK
P66	170	4	RRPK

As mentioned above, transporter sequences may also be selected from fragments or variants of the above sequences of table 2 (with the proviso that such fragment or variant retain preferably the function to provide for translocation across biological membranes). In this specific context, variants and/or fragments of those transporter sequences preferably comprise a peptide sequence sharing at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 85%, preferably at least 90%, more preferably at least 95% and most preferably at least 99% sequence identity over the whole length of the sequence with such a transporter sequence as defined in Table 2. In this specific context, a "fragment" of a transporter sequence as defined in Table 2, is preferably to be understood as a truncated sequence thereof, i.e. an amino acid sequence, which is N-terminally, C-terminally and/or intrasequentially truncated compared to the amino acid sequence of the original sequence.

Furthermore, a "variant" of a transporter sequence or its fragment as defined above, is preferably to be understood as a sequence wherein the amino acid sequence of the variant differs from the original transporter sequence or a fragment thereof as defined herein in one or more mutation(s), such as one or more substituted, (or, if necessary, inserted and/or deleted) amino acid(s). Preferably, variants of such a transporter sequence as defined above have the same biological function or specific activity compared to the respective original sequence, i.e. provide for transport, e.g. into cells or the nucleus. In this context, a variant of such a transporter sequence as defined above may for example comprise about 1 to 50, 1 to 20, more preferably 1 to 10 and most preferably 1 to 5, 4, 3, 2 or 1 amino acid alterations. Variants of such a transporter sequence as defined above may preferably comprise conservative amino acid substitutions. The concept of conservative amino acid substitutions is known in the art and has already been set out above for the JNK inhibitory (poly-)peptide sequence and applies here accordingly.

The length of a transporter sequence incorporated in the JNK inhibitor of the present invention may vary. It is contemplated that in some embodiments the transporter sequence of the JNK inhibitor according to the present invention is less than 150, less than 140, less than 130, less than 120, less than 110, less than 100, less than 90, less than 80, less than 70, less than 60, less than 50, less than 40, less than 30, less than 20, and/or less than 10 amino acids in length.

Whether a specific transporter sequence is still functional in the context of the JNK inhibitor according to the present invention may readily be determined by a person skilled in the art. For instance, the JNK inhibitor comprising a transporter domain may be fused to a label, e.g. a fluorescent protein such as GFP, a radioactive label, an enzyme, a fluorophore, an epitope etc. which can be readily detected in a cell. Then, the JNK inhibitor comprising the transporter sequence and the label is transfected into a cell or added to a culture supernatant and permeation of cell membranes can be monitored by using biophysical and biochemical standard methods (for example flow cytometry, (immuno)fluorescence microscopy etc.).

- 10 Specific examples of JNK inhibitors according to the present invention comprising a transporter sequence are given in table 3:

Table 3: Examples for JNK inhibitors comprising an inhibitory (poly-)peptide sequence and a transporter sequence		
Amino acid sequence	AA	SEQ ID NO:
rKKRrQRRrRPkRPTTLNlf	20	171
rKKRrQRRrRPkRPaTLNlf	20	172
rKKRrQRRrRPkRPTTLrLf	20	173
rKKRrQRRrRPTTLNlf	17	174
rKKRrQRrRPTTLNlf	16	175
rKKRrQRRrRPkRPTTLNlw	20	176
rKKRrQRRrRPkRPTDLNlf	20	177
rKKRrQRRrRPTTLrLw	17	178
rKKRrQRrRPTTLrLw	16	179
rKKRrQRRrRPTDLrLw	17	180
rKKRrQRrRPTDLrLw	16	181
rKKRrQRRrRPaTLNlf	17	182
rKKRrQRrRPaTLNlf	16	183
rKKRrQRrRPaTLNlf	17	184
rKKRrQRRrRPkRPsTLNlf	20	185
rKKRrQRRrRPkRPqTLNlf	20	186
rKKRrQRRrRPkRPaTLNlf	20	187
rKKRrQRRrGKRKALKlf	18	188
rKKRrQRRrGKRKALrLf	18	189
rKKRrQRRrRKALrLf	16	190

As mentioned above, in a particular embodiment of the present invention the transporter sequence and the inhibitory (poly-)peptide sequence may overlap. In other words, the N-terminus of the transporter sequence may overlap with the C-terminus of the inhibitory (poly-)peptide sequence or the C-terminus of the transporter sequence may overlap with the N-terminus of the inhibitory (poly-)peptide sequence. The latter embodiment is particularly preferred. Preferably, the transporter sequence overlaps by one, two or three amino acid residues with the inhibitory (poly-)peptide sequence. In such scenario, a given transporter sequence may overlap with SEQ ID NO:1 or the respective variants thereof at position 1 (X1), position 1 and 2 (X1, X2), positions 1, 2 and 3 (X1, X2, X3).

SEQ ID NOs: 174, 175, 178, 179, 180, 181, 182, 183, 184, 188, 189 and 190 are examples for JNK inhibitors according to the present invention, wherein transporter sequence and the inhibitory (poly-)peptide sequence overlap, e.g. rKKRrQRRrRPTTLNlf (SEQ ID NO: 174) is an overlap of SEQ ID NO: 46 (underlined) and SEQ ID NO: 11 (italics).

The JNK inhibitor according to the present invention may also be selected from JNK inhibitors, which are a variant of any one of the JNK inhibitors according to SEQ ID NOs: 171-190. Preferably, such variant shares at least 50%, more preferably at least 55%, more preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, most preferably at least 95% sequence identity with the sequence of SEQ ID NOs: 171-190, in particular with SEQ ID NO: 172,

with the proviso that with respect to the inhibitory (poly-)peptide sequence within said sequences of SEQ ID NOs: 171-190 (see for reference inhibitory (poly-)peptide sequence of SEQ ID NO: 1 and specific examples of SEQ ID NOs: 2-27)) such sequence sharing sequence identity

- a) maintains the L-arginine (R) residue on position 4 within the inhibitory (poly-)peptide sequence,
- b) maintains the two L-leucine (L) residues at position 8 and 10 (positions 7 and 9 with regard to SEQ ID NOs: 25-27) within the inhibitory (poly-)peptide sequence,
- c) exhibits at least one, at least two, at least three, at least four, at least five or six D-amino acid(s) at the respective positions corresponding to the amino acids selected from the group consisting of X1, X2, X3, X5, X7 and or X8 of SEQ ID NO: 1 and respective positions in SEQ ID NOs: 2-27, more preferably exhibits at least one, at least two, at least three or four D-amino acid(s) at the positions corresponding to the amino acids selected from the group consisting of X3, X5, X7 and X8 of SEQ ID NO: 1 and respective positions in SEQ ID NOs: 2-27, and

d) inhibits JNK activity (i.e. is a JNK inhibitor as defined herein).

In view of said definition and for sake of clarity the residues which may preferably not be altered in variants of JNK inhibitors comprising SEQ ID NOs: 171-190 (see a) and b) in the above definition) are underlined in table 3.

The non-identical amino acids in the variants of JNK inhibitors comprising SEQ ID NOs: 171-190 are preferably the result of conservative amino acid substitutions (see above). Certainly, the further possible substitutions mentioned above are also contemplated for variants of JNK inhibitors comprising SEQ ID NOs: 171-190. Likewise, the present invention certainly also contemplates variants of any one of the JNK inhibitors according to SEQ ID NOs: 171-190, which deviate from the original sequence not or not exclusively in the inhibitory (poly-)peptide sequence, but exhibits variant residues in the transporter sequence. For variants and fragments of transporter sequences, the respective disclosure herein is pertinent.

As mentioned previously, the transporter sequence and the JNK inhibitory (poly-)peptide sequence of the JNK inhibitors according to the present invention need not necessarily be directly linked to each other. They may also be linked by e.g. an intermediate or linking (poly-)peptide sequences. Preferred intermediate or linking sequences separating the inhibitory (poly-)peptide sequences and other (functional) sequences such as transporter sequences consist of short peptide sequences of less than 10 amino acids in length, like a hexamer, a pentamer, a tetramer, a tripeptide or a dipeptide or a single amino acid residue. Particularly preferred intermediate sequence are one, two or more copies of di-proline, di-glycine, di-arginine and/or di-lysine, all either in L-amino acid form only, or in D-amino acid form only, or with mixed D- and L-amino acids. Alternatively, other known peptide spacer or linker sequences may be employed as well.

A particularly preferred JNK inhibitor according to the present invention comprises SEQ ID NO: 8 (or a sequence sharing sequence identity with SEQ ID NO: 8 with the scope and limitations defined further above) and a transporter sequence. The transporter sequence is preferably selected from any one of SEQ ID Nos: 31-170 or variants thereof as defined herein, even more preferably from any one of SEQ ID NOs: 31-34 and 46-151. A particularly preferred embodiment of a JNK inhibitor according to the present invention is a JNK inhibitor comprising SEQ ID NO: 8 and SEQ ID NO: 46 (or sequences sharing respective sequence identity thereto within the scope and limitations defined above). A preferred example is a JNK inhibitor comprising the sequence of SEQ ID NO: 172 or respective variants thereof varying in the transporter sequence and/or the inhibitory (poly-)peptide sequence as defined herein.

In a further aspect, the present invention relates to a JNK inhibitor comprising

- a) an inhibitory (poly-)peptide comprising a sequence from the group of sequences consisting of RPTTLNLF (SEQ ID NO: 191), KRPTTLNLF (SEQ ID NO: 192), RRPTTLNLF and/or RPKRPTTLNLF (SEQ ID NO: 193), and
- b) a transporter sequence, preferably a transporter sequence selected from the transporter sequences disclosed in table 2 or variants/fragments thereof, even more preferably selected from SEQ ID NOs: 31-34 and 46-151 or respective variants or fragments thereof.

The transporter sequence and the inhibitory (poly-)peptide sequence may overlap. Preferred transporter sequences for said embodiment of the invention are particularly the transporter sequence of SEQ ID NO: 46, preferably (covalently) linked (e.g. directly) to the N-terminus of the inhibitory (poly-)peptide sequence.

A JNK inhibitor of the present invention may also be a JNK inhibitor comprising or consisting of the sequence GRKKRRQRRRPPKRPTTLNLFQVPRSQD (SEQ ID NO: 194), or the sequence GRKKRRQRRRPTTLNLFQVPRSQD (SEQ ID NO: 195).

In a further aspect, the present invention relates to a (poly-)peptide comprising a transporter sequence selected from the group of sequences consisting of rKKRrQRr (SEQ ID NO: 148), rKKRrQRrK (SEQ ID NO: 149), and/or rKKRrQRrR (SEQ ID NO: 150).

As used herein, "comprising" a sequence or a given SEQ ID NO as disclosed herein usually implies that (at least) one copy of said sequence is present, e.g. in the JNK inhibitor molecule. For example, one inhibitory (poly-)peptide sequence will usually suffice to achieve sufficient inhibition of JNK activity. However, it is contemplated according to the invention to use two or more copies of the respective sequence (e.g. two or more copies of an inhibitory (poly-)peptide sequence of different or same type and/or two or more copies of a transporter sequence of different or the same type) may also employed for the inventive (poly)peptide, as long as the overall ability of the resulting molecule to inhibit JNK activity is not abolished (i.e. the respective molecule is still a JNK inhibitor as defined herein).

The inventive JNK inhibitors may be obtained or produced by methods well-known in the art, e.g. by chemical synthesis via solid-phase peptide synthesis using Fmoc (9-fluorenylmethyloxycarbonyl) strategy, i.e. by successive rounds of Fmoc deprotection and Fmoc-amino acid coupling cycles. A

commercial service offering such peptide synthesis is provided by many companies, for example the company PolyPeptide (Straßbourg, France).

The JNK inhibitors for use according to the present invention may optionally be further modified, in particular at the amino acid residues of the inhibitory (poly-peptide) sequence. Possible modifications may for example be selected from one or more of items (i) to (xiii) of the group consisting of:

- (i) radioactive labels, i.e. radioactive phosphorylation or a radioactive label with sulphur, hydrogen, carbon, nitrogen, etc.;
- (ii) colored dyes (e.g. digoxigenin, etc.);
- (iii) fluorescent groups (e.g. fluorescein, etc.);
- (iv) chemoluminescent groups;
- (v) groups for immobilization on a solid phase (e.g. His-tag, biotin, strep-tag, flag-tag, antibodies, epitopes, etc.);
- (vi) pegylation,
- (vii) glycosylation,
- (viii) hesylation,
- (ix) protease cleavage sites (e.g. for controlled release of the JNK inhibitor)
- (x) peptide backbone modifications (e.g. (Ψ CH₂-NH) bonds)
- (xi) protection of amino acid side chain residues,
- (xii) protection of N- and/or C-terminus (e.g. N-terminal amidation or C-terminal acetylation)
- (xiii) a combination of elements of two or more of the elements mentioned under (i) to (xii).

Particularly preferred are modifications selected from (i) to (xi) and combinations of elements of two or more of the elements mentioned under (i) to (xi). In this context, the present invention relates in a further aspect to a JNK inhibitor as disclosed herein modified with modifications selected from (i) to (xi) or modified with a combination of two or more of the elements mentioned under (i) to (xi), and a pharmaceutical composition (see below) comprising such modified JNK inhibitor.

Pharmaceutical compositions

The JNK inhibitors as defined according to the invention can be formulated in a pharmaceutical composition, which may be applied in the prevention or treatment of any of the diseases as defined herein. Typically, such a pharmaceutical composition used according to the present invention includes as an active component a JNK inhibitor as defined herein, in particular a JNK inhibitor

comprising or consisting of an inhibitory (poly-)peptide sequence according to SEQ ID NO: 1, as defined herein. Preferably, the active compound is a JNK inhibitor comprising or consisting of an inhibitory (poly-)peptide sequence according to any one of SEQ ID NOs: 2-27, optionally in (covalent) conjugation (via or without a linker sequence) with any suitable transporter sequence; if a transporter sequence is attached, any of the sequences according to any one of SEQ ID NOs: 171-190, or a variant thereof as defined herein, preferably a sequence according to SEQ ID NOs: 172, or a variant thereof as defined herein, may be selected.

The inventors of the present invention additionally found that the JNK-inhibitors as defined herein, in particular if fused to a transporter sequence; exhibit a particularly pronounced uptake rate into cells involved in the diseases of the present invention. Therefore, the amount of a JNK-inhibitor in the pharmaceutical composition to be administered to a subject, may – without being limited thereto – be employed on the basis of a low dose within that composition. Thus, the dose to be administered may be much lower than for peptide drugs known in the art, such as DTS-108 (Florence Meyer-Losic et al., Clin Cancer Res., 2008, 2145-53). Thereby, for example a reduction of potential side reactions and a reduction in costs is achieved by the inventive (poly)peptides.

Preferably, the dose (per kg body weight), e.g. to be administered on a daily basis to the subject, is in the range of up to about 10 mmol/kg, preferably up to about 1 mmol/kg, more preferably up to about 100 µmol/kg, even more preferably up to about 10 µmol/kg, even more preferably up to about 1 µmol/kg, even more preferably up to about 100 nmol/kg, most preferably up to about 50 nmol/kg.

Thus, the dose range may preferably be from about 0,01 pmol/kg to about 1 mmol/kg, from about 0,1 pmol/kg to about 0,1 mmol/kg, from about 1,0 pmol/kg to about 0,01 mmol/kg, from about 10 pmol/kg to about 1 µmol/kg, from about 50 pmol/kg to about 500 nmol/kg, from about 100 pmol/kg to about 300 nmol/kg, from about 200 pmol/kg to about 100 nmol/kg, from about 300 pmol/kg to about 50 nmol/kg, from about 500 pmol/kg to about 30 nmol/kg, from about 250 pmol/kg to about 5 nmol/kg, from about 750 pmol/kg to about 10 nmol/kg, from about 1 nmol/kg to about 50 nmol/kg, or a combination of any two of said values.

In this context, prescription of treatment, e.g. decisions on dosage etc. when using the above pharmaceutical composition is typically within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to

practitioners. Examples of the techniques and protocols mentioned above can be found in REMINGTON'S PHARMACEUTICAL SCIENCES, 16th edition, Osol, A. (ed), 1980. Accordingly, a "safe and effective amount" for components of the pharmaceutical compositions as used according to the present invention means an amount of each or all of these components, that is sufficient to significantly induce a positive modification of diseases or disorders strongly related to JNK signalling as defined herein. At the same time, however, a "safe and effective amount" is small enough to avoid serious side-effects, that is to say to permit a sensible relationship between advantage and risk. The determination of these limits typically lies within the scope of sensible medical judgment. A "safe and effective amount" of such a component will vary in connection with the particular condition to be treated and also with the age and physical condition of the patient to be treated, the severity of the condition, the duration of the treatment, the nature of the accompanying therapy, of the particular pharmaceutically acceptable carrier used, and similar factors, within the knowledge and experience of the accompanying doctor. The pharmaceutical compositions according to the invention can be used according to the invention for human and also for veterinary medical purposes.

The pharmaceutical composition as used according to the present invention may furthermore comprise, in addition to one or more of the JNK inhibitors, a (compatible) pharmaceutically acceptable carrier, excipient, buffer, stabilizer or other materials well known to those skilled in the art.

In this context, the expression "(compatible) pharmaceutically acceptable carrier" preferably includes the liquid or non-liquid basis of the composition. The term "compatible" means that the constituents of the pharmaceutical composition as used herein are capable of being mixed with the pharmaceutically active component as defined above and with one another component in such a manner that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the composition under usual use conditions. Pharmaceutically acceptable carriers must, of course, have sufficiently high purity and sufficiently low toxicity to make them suitable for administration to a person to be treated.

If the pharmaceutical composition as used herein is provided in liquid form, the pharmaceutically acceptable carrier will typically comprise one or more (compatible) pharmaceutically acceptable liquid carriers. The composition may comprise as (compatible) pharmaceutically acceptable liquid carriers e.g. pyrogen-free water; isotonic saline, i.e. a solution of 0.9 % NaCl, or buffered (aqueous) solutions, e.g. phosphate, citrate etc. buffered solutions, vegetable oils, such as, for example, groundnut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil from theobroma; polyols, such

as, for example, polypropylene glycol, glycerol, sorbitol, mannitol and polyethylene glycol; alginic acid, etc. Particularly for injection and/or infusion of the pharmaceutical composition as used herein, a buffer, preferably an aqueous buffer, and/or 0.9 % NaCl may be used.

5 If the pharmaceutical composition as used herein is provided in solid form, the pharmaceutically acceptable carrier will typically comprise one or more (compatible) pharmaceutically acceptable solid carriers. The composition may comprise as (compatible) pharmaceutically acceptable solid carriers e.g. one or more compatible solid or liquid fillers or diluents or encapsulating compounds may be used as well, which are suitable for administration to a person. Some examples of such
10 (compatible) pharmaceutically acceptable solid carriers are e.g. sugars, such as, for example, lactose, glucose and sucrose; starches, such as, for example, corn starch or potato starch; cellulose and its derivatives, such as, for example, sodium carboxymethylcellulose, ethylcellulose, cellulose acetate; powdered tragacanth; malt; gelatin; tallow; solid glidants, such as, for example, stearic acid, magnesium stearate; calcium sulphate, etc.

15 The precise nature of the (compatible) pharmaceutically acceptable carrier or other material may depend on the route of administration. The choice of a (compatible) pharmaceutically acceptable carrier may thus be determined in principle by the manner in which the pharmaceutical composition as used according to the invention is administered. Various possible routes of administration are
20 listed in the list "Route of Administration" of the FDA (cf. FDA: Data Standards Manual – Drug Nomenclature Monographs – Monograph Number: C-DRG-00301; Version Number 004), which is incorporated by reference herein. Further guidance for selecting an appropriate route of administration, in particular for non-human animals, can be found in Turner PV et al. (2011) Journal of the American Association for Laboratory Animal Science, Vol. 50, No 5, p. 600 – 613, which is
25 also incorporated by reference herein. Preferred examples for routes for administration include, for example, parenteral routes (e.g. via injection), such as intravenous, intramuscular, subcutaneous, intradermal, or transdermal routes, etc., enteral routes, such as oral, or rectal routes, etc., topical routes, such as nasal, or intranasal routes, etc., or other routes, such as epidermal routes or patch delivery. Also contemplated (in particular for eye related diseases) are instillation, intravitreal, and
30 subconjunctival administration. Likewise, administration may occur intratympanical, for example, whenever ear related diseases are treated.

The pharmaceutical composition as used according to the invention can be administered, for example, systemically. In general, routes for systemic administration include, for example,
35 parenteral routes (e.g. via injection and/or infusion), such as intravenous, intra-arterial, intraosseous, intramuscular, subcutaneous, intradermal, transdermal, or transmucosal routes, etc., and enteral

routes (e.g. as tablets, capsules, suppositories, via feeding tubes, gastrostomy), such as oral, gastrointestinal or rectal routes, etc.. By systemic administration a system-wide action can be achieved and systemic administration is often very convenient, however, depending on the circumstances it may also trigger unwanted "side-effects" and/or higher concentrations of the JNK inhibitor according to the invention may be necessary as compared to local administration. Systemic administration is in general applicable for the prevention and/or treatment of the diseases/disorders mentioned herein due to its system-wide action. Preferred routes of systemic administration are intravenous, intramuscular, subcutaneous, oral and rectal administration, whereby intravenous and oral administration are particularly preferred.

The pharmaceutical composition as used according to the invention can also be administered, for example, locally, for example topically. Topical administration typically refers to application to body surfaces such as the skin or mucous membranes, whereas the more general term „local administration" additionally comprises application in and/or into specific parts of the body. Topical application is particularly preferred for the treatment and/or prevention of diseases and/or disorders of the skin and/or subcutaneous tissue as defined herein as well as for certain diseases of the mouth and/or diseases relating to or are accessible by mucous membranes.

Routes for local administration include, for example, inhalational routes, such as nasal, or intranasal routes, ophtalmic and otic drugs, e.g. eye drops and ear drops, administration through the mucous membranes in the body, etc., or other routes, such as epidermal routes, epicutaneous routes (application to the skin) or patch delivery and other local application, e.g. injection and/or infusion, into the organ or tissue to be treated etc.. In local administration side effects are typically largely avoided. It is of note, that certain routes of administration may provide both, a local and a systemic effect, for example inhalation.

Routes for administration for the pharmaceutical composition as used according to the invention can be chosen according to the desired location of the application depending on the disorder/disease to be prevented or treated.

For example, an enteral administration refers to the gastrointestinal tract as application location and includes oral (p.o.), gastrointestinal and rectal administration, whereby these are typically systemic administration routes, which are applicable to the prevention/treatment of the diseases mentioned herein in general. In addition, enteral administration is preferred to prevent and/or treat diseases/disorders of the gastrointestinal tract as mentioned herein, for example inflammatory diseases of the gastrointestinal tract, metabolic diseases, cancer and tumor diseases, in particular of

the gastrointestinal tract etc.. For example, the oral route is usually the most convenient for a patient and carries the lowest cost. Therefore, oral administration is preferred for convenient systemic administration, if applicable. Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier as defined above, such as gelatin, and optionally an adjuvant. Liquid pharmaceutical compositions for oral administration generally may include a liquid carrier as defined above, such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

Furthermore, enteral administration also includes application locations in the proximal gastrointestinal tract without reaching the intestines, for example sublingual, sublabial, buccal or intragigival application. Such routes of administration are preferred for applications in stomatology, i.e. disease/disorders of the mouth which may be treated and/or prevented with the JNK inhibitors as disclosed herein, for example pulpitis in general, in particular acute pulpitis, chronic pulpitis, hyperplastic pulpitis, ulcerative pulpitis, irreversible pulpitis and/or reversible pulpitis; periimplantitis; periodontitis in general, in particular chronic periodontitis, complex periodontitis, simplex periodontitis, aggressive periodontitis, and/or apical periodontitis, e.g. of pulpal origin; periodontosis, in particular juvenile periodontosis; gingivitis in general, in particular acute gingivitis, chronic gingivitis, plaque-induced gingivitis, and/or non-plaque-induced gingivitis; pericoronitis, in particular acute and chronic pericoronitis; sialadenitis (sialoadenitis); parotitis, in particular infectious parotitis and autoimmune parotitis; stomatitis in general, in particular aphthous stomatitis (e.g., minor or major), Bednar's aphthae, periadenitis mucosa necrotica recurrens, recurrent aphthous ulcer, stomatitis herpetiformis, gangrenous stomatitis, denture stomatitis, ulcerative stomatitis, vesicular stomatitis and/or gingivostomatitis; mucositis, in particular mucositis due to antineoplastic therapy, due to (other) drugs, or due to radiation, ulcerative mucositis and/or oral mucositis; cheilitis in general, in particular chapped lips, actinic cheilitis, angular cheilitis, eczematous cheilitis, infectious cheilitis, granulomatous cheilitis, drug-related cheilitis, exfoliative cheilitis, cheilitis glandularis, and/or plasma cell cheilitis; cellulitis (bacterial infection), in particular of mouth and/or lips; desquamative disorders, in particular desquamative gingivitis; and/or temporomandibular joint disorder. Particularly preferred diseases to be treated and/or prevented according to the invention by these routes of administration are selected from periodontitis, in particular chronic periodontitis, mucositis, oral desquamative disorders, oral lichen planus, pemphigus vulgaris, pulpitis, stomatitis, temporomandibular joint disorder, and peri-implantitis.

For example, intragingival administration, e.g. by injection into the gums (gingiva), is preferred in stomatology applications, for example for preventing and/or treating periodontitis. For example, disorders/diseases of the mouth, in particular periodontitis, may be prevented or treated by sublingual, sublabial, buccal or intragingival application, in particular intragingival application, of the pharmaceutical composition as defined above comprising a dose (per kg body weight) of 100 ng/kg to 100 mg/kg, preferably 10 µg/kg to 10 mg/kg, more preferably of the JNK inhibitor according to the present invention.

Alternatively, the diseases of the mouth mentioned herein may also be treated and/or prevented by systemic and, preferably, topical administration of the JNK inhibitor as disclosed herein or the respective pharmaceutical composition.

In addition, enteral administration also includes strictly enteral administration, i.e. directly into the intestines, which can be used for systemic as well as for local administration.

Moreover, the JNK inhibitor according to the present invention, used in the prevention and/or treatment of diseases and/or disorders according to the present invention may be administered to the central nervous system (CNS). Such routes of administration include in particular epidural (peridural), intra-CSF (intra-cerebrospinal fluid), intracerebroventricular (intraventricular), intrathecal and intracerebral administration, for example administration into specific brain regions, whereby problems relating to the blood-brain-barrier can be avoided. Such CNS routes of administration are preferred if the disease/disorder to be treated is a neural, a neurological and/or a neurodegenerative disease as specified herein.

In addition, the JNK inhibitor according to the present invention, used in the prevention and/or treatment of diseases and/or disorders according to the present invention may be administered at, in or onto the eye. Such routes of administration include eye drops applied topically, for example onto the conjunctiva, and, intravitreal (IVT), subconjunctival, and posterior juxtasceral administration, e.g. by injection, infusion and/or instillation and/or localized, sustained-release drug delivery (for example in case of the subconjunctival route), whereby eyedrops (for topical application), intravitreal (IVT) and subconjunctival routes of administration are particularly preferred. The subconjunctival route is safer and less invasive than the intravitreal route, however, the intravitreal route involves less systemic exposure than the subconjunctival route due to the presence of conjunctival and orbital blood vessels and tissue.

Local administration onto/in the eye is particularly preferred for eye-related diseases/disorders to be treated and/or prevented as disclosed herein, for example age-related macular degeneration (AMD), in particular in the wet and dry form; angioid streaks; anterior ischemic optic neuropathy; anterior uveitis; cataract, in particular age related cataract; central exudative chorioretinopathy; central serous chorioretinopathy; chalazion; chorioderemia; chorioiditis; choroidal sclerosis; conjunctivitis; cyclitis; diabetic retinopathy; dry eye syndrome; endophthalmitis; episcleritis; eye infection; fundus albipunctatus; gyrate atrophy of choroid and retina; hordeolum; inflammatory diseases of the blephara; inflammatory diseases of the choroid; inflammatory diseases of the ciliary body; inflammatory diseases of the conjunctiva; inflammatory diseases of the cornea; inflammatory diseases of the iris; inflammatory diseases of the lacrimal gland; inflammatory diseases of the orbital bone; inflammatory diseases of the sclera; inflammatory diseases of the vitreous body; inflammatory diseases of the uvea; inflammatory diseases of the retina; intermediate uveitis; iritis; keratitis; Leber's disease; multifocal choroiditis; myositis of the eye muscle; neovascular maculopathy (e.g. caused by high myopia, tilted disc syndrome, choroidal osteoma or the like); NMDA induced retinotoxicity; non-chronic or chronic inflammatory eye diseases; Oguchi's disease; optic nerve disease; orbital phlegmon; panophthalmitis; panuveitis; post capsule opacification; posterior capsule opacification (PCO) (a cataract after-surgery complication); posterior uveitis; intraocular inflammation, in particular post-surgery intraocular inflammation; proliferative vitreoretinopathy; retinal artery occlusion; retinal detachment, retinal diseases; retinal injuries; retinal macroaneurysm; retinal pigment epithelium detachment; retinal vein occlusion; retinitis; retinitis pigmentosa; retinitis punctata albescens; retinopathy, in particular retinopathy of prematurity and diabetic retinopathy; scleritis; Stargardt's disease; treatment of inflamed ocular wounds and/or ocular wound edges; treatment of intraocular inflammation after eye surgery or trauma; uveitis; vitelliform macular dystrophy; etc.

For the treatment of dry eye, it is preferred to address aqueous tear-deficient dry or evaporative dry eye diseases. Aqueous tear-deficient dry eye may refer to Sjögren syndrome dry eye or Non-Sjögren syndrome dry eye. Non-Sjögren syndrome dry eye may be caused by primary or secondary lacrimal gland dysfunction or obstruction of the lacrimal gland ducts. Evaporative dry eye may have intrinsic, e.g. Meibomian gland dysfunction, low blink rate or disorders of lid aperture, or extrinsic causes, e.g. ocular surface disorder, lens wear or allergic rhinitis. In particular Sjögren's or non-Sjögren's dry eye syndrome are to be treated by the present invention.

In particular, dry eye syndrome, uveitis, in particular anterior and/or posterior uveitis, age-related macular degeneration (AMD), in particular the wet and the dry form of AMD, retinopathy, in particular retinopathy of prematurity and diabetic retinopathy, and post-surgery or post-trauma eye

inflammation, in particular post-surgery or post-trauma intraocular inflammation, are prevented and/or treated by the JNK inhibitor used according to the present invention by local administration in and/or onto the eye, preferably by instillation, e.g. eye drops, and/or intravitreal and/or subconjunctival administration, e.g. by injection or instillation. Instillation, e.g. eyedrops, and/or subconjunctival administration, e.g. by injection, are thereby preferred routes of administration

For these routes of administration, in particular for instillation, e.g. eyedrops, intravitreal and/or subconjunctival administration, the respective pharmaceutical composition according to the present invention, preferably comprises a dose per eye in the range of 10 ng to 100 mg, more preferably in the range of 100 ng to 10 mg, even more preferably in the range of 1 µg to 5 mg, and particularly preferable in the range of 100 µg to 1 mg, for example 0.1, 0.2, or 0.4 mg, of the JNK inhibitor according to the present invention, preferably of the JNK inhibitor according to a sequence of SEQ ID NO. 172. One single administration or more administrations, in particular two, three, four or five, administrations of such dose(s) are preferred, whereby subsequent dose(s) may be administered on different days of the treatment schedule.

For example for intravitreal and/or subconjunctival administration in humans a single dose (per eye) of the JNK inhibitor is preferably in the range of 1 µg to 5 mg, preferably 50 µg to 1,5 mg, more preferably 500 µg to 1 µg, most preferably 800 µg to 1 mg. The injection volume, in particular for subconjunctival injection, may be for example 100 µl to 500 µl, e.g. 250 µl.

For instillation, e.g. eye drops, in humans a single dose (per eye) of the JNK inhibitor is preferably in the range of 1 µg to 5 mg, preferably 10 µg to 1,5 mg, more preferably 50 µg to 1 mg, most preferably 100 µg to 600 µg. In the treatment and/or prevention by way of instillation, a single dose or repeated doses may be administered, preferably daily, for example daily 2 to 4 times per day, preferably daily 3 times a day, for several weeks, preferably 2 to 4 weeks, more preferably 3 weeks. Such an administration is for example particularly useful to treat and/or prevent dry eye syndrome.

For topical ocular administration, in particular as eyedrops, which may be applied to both eyes or to one eye only, depending on the need, the pharmaceutical composition comprising the JNK inhibitor according to the invention is typically a solution, preferably an ophthalmic solution, e.g. comprising (sterile) 0.9 % NaCl. Such a pharmaceutical composition comprises in particular 0.001 % - 10 % of the JNK inhibitor as described herein, preferably 0.01 % - 5 % of the JNK inhibitor as described herein, more preferably 0.05 % - 2 % of the JNK inhibitor as described herein, even more preferably 0.1 % - 1 % of the JNK inhibitor as described herein. The eyedrops may be administered once or repeatedly, whereby repeated administration is preferred. In general, the administration

depends on the need and may for example be on demand. In repeated administration, subsequent dose(s) may be administered on different days of the treatment schedule, whereby on the same day a single dose or more than one single doses, in particular two, three, four or five, preferably two or three doses may be administered, whereby such repeated administration is preferably spaced by intervals of one or more hour(s), e.g. two, three, four, five, six, seven or eight hours.

In addition, eye diseases as described herein may of course also be treated and/or prevented by systemic application of the JNK inhibitor according to the invention (which also applies to the other diseases/disorders as described herein). The dose for systemic administration in eye diseases, in particular for intravenous administration, ranges preferably from 0.001 mg/kg to 10 mg/kg, more preferably from 0.01 mg/kg to 5 mg/kg, even more preferably from 0.1 mg/kg to 2 mg/kg. Such doses are for example particularly useful to treat and/or prevent uveitis, whereby the treatment schedule may comprises a single dose or repeated doses, whereby subsequent dose(s) may be administered on different days of the treatment schedule.

For example, if more than a single dose is applied, in particular intravenously, in the treatment and/or prevention of uveitis, the doses are typically spaced by intervals of at least one day, preferably by intervals of at least two days, more preferably by intervals of at least three days, even more preferably by intervals of at least four days, at least five days, or at least six days, particularly preferably by intervals of at least a week, most preferably by intervals of at least ten days.

Other routes of administration for the use of the JNK inhibitor according to the present invention, which are typically chosen according to the disease to be prevented and/or treated and the respective pharmacokinetics, include – but are not limited to – epicutaneous application (onto the skin) and/or intralesional application (into a skin lesion), for example for skin diseases as defined herein (mentioned herein), in particular selected from psoriasis, eczema, dermatitis, acne, mouth ulcers, erythema, lichen plan, sarcoidose, vasculitis, and adult linear IgA disease; nasal administration, for example for diseases of the respiratory system and in particular lung diseases, for example acute respiratory distress syndrome (ARDS), asthma, chronic illnesses involving the respiratory system, chronic obstructive pulmonary disease (COPD), cystic fibrosis, inflammatory lung diseases, pneumonia, and pulmonary fibrosis; intraarticular administration (into a joint space), for example in arthritis, in particular juvenile idiopathic arthritis, psoriatic arthritis and rheumatoid arthritis, and arthrosis, and osteoarthritis; intravesical administration (i.e. into the urinary bladder), for example for diseases of the urinary system, in particular the urinary bladder; intracardiac administration, intracavernous administration, intravaginal administration, and intradermal administration.

In general, the method of administration depends on various factors as mentioned above, for example the selected pharmaceutical carrier and the nature of the pharmaceutical preparation (e.g. as a liquid, tablet etc.) as well as the route of administration. For example, the pharmaceutical composition comprising the JNK inhibitor according to the invention may be prepared as a liquid, for example as a solution of the JNK inhibitor according to the invention in 0.9 % NaCl. A liquid pharmaceutical composition can be administered by various methods, for example as a spray (e.g., for inhalational, intranasal etc. routes), as a fluid for topical application, by injection, including bolus injection, by infusion, for example by using a pump, by instillation, but also p.o., e.g. as drops or drinking solution, in a patch delivery system etc.. Accordingly, for the administration different devices may be used, in particular for injection and/or infusion, e.g. a syringe (including a pre-filled syringe); an injection device (e.g. the INJECT-EASET™ and GENJECT™ device); an infusion pump (such as e.g. Accu-Chek™); an injector pen (such as the GENPENT™); a needleless device (e.g. MEDDECTOR™ and BIOJECTOR™); or an autoinjector.

The suitable amount of the pharmaceutical composition to be used can be determined by routine experiments with animal models. Such models include, without implying any limitation, for example rabbit, sheep, mouse, rat, dog, gerbil, pig, and non-human primate models. Preferred unit dose forms for administration, in particular for injection and/or infusion include sterile solutions of water, physiological saline or mixtures thereof. The pH of such solutions should be adjusted to about 7.4. Suitable carriers for administration, in particular for injection and/or infusion include hydrogels, devices for controlled or delayed release, polylactic acid and collagen matrices. Suitable pharmaceutically acceptable carriers for topical application include those, which are suitable for use in lotions, creams, gels and the like. If the compound is to be administered per orally, tablets, capsules and the like are the preferred unit dose form. The pharmaceutically acceptable carriers for the preparation of unit dose forms, which can be used for oral administration are well known in the prior art. The choice thereof will depend on secondary considerations such as taste, costs and storability, which are not critical for the purposes of the present invention, and can be made without difficulty by a person skilled in the art.

For intravenous, intramuscular, intraperitoneal, cutaneous or subcutaneous injection and/or infusion, or injection and/or infusion at the site of affliction, i.e. local injection/infusion, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, in particular 0.9 % NaCl, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers,

5 buffers, antioxidants and/or other additives may be included, as required. Whether it is a polypeptide, peptide, or nucleic acid molecule, other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is preferably in a "prophylactically effective amount or a "therapeutically effective amount" (as the case may be), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. For example, for i.v. administration in humans, single doses of up to 1 mg per kg body weight are preferred, more preferably up to 500 µg per kg body weight, even more preferably up to 100 µg per kg body weight, for example in the range of 100 ng to 1 mg per kg body weight, more specifically in the range of 1 µg to 500 µg per kg body weight, even more specifically in the range of 5 µg to 100 µg per kg body weight. Such doses may be administered for example as injection and/or infusion, in particular as infusion, whereby the duration of the infusion varies for example between 1 to 90 min, preferably 10 to 70 min, more preferably 30 to 60 min.

15 In addition, the pharmaceutical composition as used according to the present invention may additionally – i.e. in addition to any one or more of the JNK inhibitors as defined herein, and/or variants, fragments or derivatives thereof, nucleic acids, cells or cells transfected with a vector and/or nucleic acids as defined above – also comprise optionally a further "active component", which is also useful in the respective disease. In this context, the pharmaceutical composition according to the present invention may also combined in the therapy of the diseases according to the present invention with a further pharmaceutical composition comprising a further "active component". For example, a pharmaceutical composition comprising a JNK inhibitor according to the present invention may be used in post-surgery intraocular inflammation as stand-alone therapy or in combination with corticosteroids, preferably glucocorticoids, e.g. dexamethasone. Moreover, e.g. a pharmaceutical composition comprising a JNK inhibitor and/or chimeric peptide according to the present invention may preferably be used in the prevention and/or treatment of Alzheimer's Disease and/or Mild Cognitive Impairment, in particular MCI due to Alzheimer's disease, as stand-alone therapy or in combination with PKR inhibitors and, optionally, in addition to the JNK inhibitor according to the present invention and the PKR inhibitor with a amyloid lowering agent. PKR inhibitors are in particular peptides, e.g. "SC1481" by Polypeptide Group. Amyloid lowering agents include β-secretase (BACE1) inhibitors, γ-secretase inhibitors (GSI) and modulators (GSM). Non-limiting examples of such amyloid lowering agents, which are currently in clinical trials may be retrieved from Vassar R. (2014) BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. *Alzheimers Res Ther.*;6(9):89 and/or from Jia Q, Deng Y, Qing H (2014) Potential therapeutic strategies for Alzheimer's disease targeting or beyond β-amyloid: insights from clinical trials. *Biomed Res Int.* 2014;2014:837157; for example Pioglitazone, CTS-21166, MK8931, LY2886721,

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AZD3293, E2609, NIC5-15, Begacestat, CHF 5074, EVP-0962, Atorvastatin, Simvastatin, Etazolate, Epigallocatechin-3-gallate (EGCg), Scyllo-inositol (ELND005/AZD103), Tramiprosate (3 APS), PBT2, Affitope AD02, and Affitope AD03. In the case of a combination therapy, separate pharmaceutical compositions for the active components to be combined are preferred for better individual dosing, however for convenience also a single pharmaceutical composition comprising the active components to be combined is conceivable. In the case of separate pharmaceutical compositions for the active components to be combined the administration of the JNK inhibitor according to the present invention may be before, during (concomitant or overlapping administration) or after the administration of the other active component comprised in a separate pharmaceutical composition, for example the PKR inhibitor, the amyloid lowering agent or the glucocorticoid. Administration “before” the administration of the JNK inhibitor preferably means within 24 h, more preferably within 12 h, even more preferably within 3 h, particularly preferably within 1 h and most preferably within 30 min before the administration of the JNK inhibitor starts. Administration “after” the administration of the JNK inhibitor preferably means within 24 h, more preferably within 12 h, even more preferably within 3 h, particularly preferably within 1 h and most preferably within 30 min after the administration of the JNK inhibitor is finished.

Particularly preferred embodiments of the use of the JNK inhibitor according to the present invention – for example a JNK inhibitor comprising or consisting of an inhibitory (poly)peptide sequence according to any of sequences of SEQ ID NOs: 2 to 27, potentially comprising an additional transporter sequence, whereby any of the sequences according to any one of SEQ ID NOs: 171-190, or a variant thereof as defined herein, are preferred and the sequence according to SEQ ID NO: 172, or a variant thereof as defined herein, are particularly preferred – include (but are not limited to) the prevention and/or treatment of the following diseases/disorders:

(i) diseases of the mouth and/or the jaw bone, in particular inflammatory diseases of the mouth and/or the jaw bone selected from (i) pulpitis in general, in particular acute pulpitis, chronic pulpitis, hyperplastic pulpitis, ulcerative pulpitis, irreversible pulpitis and/or reversible pulpitis; (ii) periimplantitis; (iii) periodontitis in general, in particular chronic periodontitis, complex periodontitis, simplex periodontitis, aggressive periodontitis, and/or apical periodontitis, e.g. of pulpal origin; periodontosis, in particular juvenile periodontosis; (iv) gingivitis in general, in particular acute gingivitis, chronic gingivitis, plaque-induced gingivitis, and/or non-plaque-induced gingivitis; (v) pericoronitis, in particular acute and chronic pericoronitis; sialadenitis (sialoadenitis); parotitis, in particular infectious parotitis and autoimmune parotitis; (vi) stomatitis in general, in particular aphthous stomatitis (e.g., minor or major), Bednar’s aphthae, periadenitis mucosa necrotica recurrens, recurrent aphthous ulcer, stomatitis herpetiformis, gangrenous stomatitis,

denture stomatitis, ulcerative stomatitis, vesicular stomatitis and/or gingivostomatitis; (vii) mucositis, in particular mucositis due to antineoplastic therapy, due to (other) drugs, or due to radiation, ulcerative mucositis and/or oral mucositis; (viii) cheilitis in general, in particular chapped lips, actinic cheilitis, angular cheilitis, eczematous cheilitis, infectious cheilitis, granulomatous cheilitis, drug-related cheilitis, exfoliative cheilitis, cheilitis glandularis, and/or plasma cell cheilitis; and (ix) cellulitis (bacterial infection), in particular of mouth and/or lips; desquamative disorders, in particular desquamative gingivitis; and/or temporomandibular joint disorder, whereby periodontitis, periimplantitis, gingivitis, stomatitis and mucositis are preferred and periodontitis is particularly preferred; wherein for the treatment and/or prevention of the diseases of the mouth and/or the jaw bone the JNK inhibitor is preferably applied in doses (per kg body weight) in the range of 100 µg/kg to 100 mg/kg, more preferably 1 mg/kg to 10 mg/kg, even more preferably 2 mg/kg to 5 mg/kg, and which is preferably applied intragingivally or topically, particularly preferably intragingivally;

(ii) nephrological diseases (kidney diseases), in particular selected from (i) glomerulonephritis, for example nonproliferative glomerulonephritis, in particular minimal change disease, focal segmental glomerulosclerosis, focal segmental glomerular hyalinosis and/or sclerosis, focal glomerulonephritis, membranous glomerulonephritis, and/or thin basement membrane disease, and proliferative glomerulonephritis, in particular membrano-proliferative glomerulonephritis, mesangio-proliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, mesangiocapillary proliferative glomerulonephritis, dense deposit disease (membranoproliferative glomerulonephritis type II), extracapillary glomerulonephritis (crescentic glomerulonephritis), rapidly progressive glomerulonephritis (RPGN), in particular Type I RPGN, Type II RPGN, Type III RPGN, and Type IV RPGN, acute proliferate glomerulonephritis, post-infectious glomerulonephritis, and/or IgA nephropathy (Berger's disease); acute nephritic syndrome; rapidly progressive nephritic syndrome; recurrent and persistent hematuria; chronic nephritic syndrome; nephrotic syndrome; proteinuria with specified morphological lesion; glomerulitis; glomerulopathy; glomerulosclerosis; (ii) acute kidney injury ("AKI", also called "acute renal failure" or "acute kidney failure") in general, in particular prerenal AKI, intrinsic AKI, postrenal AKI, AKI with tubular necrosis for example acute tubular necrosis, renal tubular necrosis, AKI with cortical necrosis for example acute cortical necrosis and renal cortical necrosis, AKI with medullary necrosis, for example medullary (papillary) necrosis, acute medullary (papillary) necrosis and chronic medullary (papillary) necrosis, or other AKI; or (iii) nephropathy, in particular selected from membranous nephropathy, diabetic nephropathy, IgA nephropathy, hereditary nephropathy, analgesic nephropathy, CFHR5 nephropathy, contrast-induced nephropathy, amyloid nephropathy, reflux nephropathy and/or Mesoamerican nephropathy; diabetic nephropathy, whereby preferably the disorder/disease to be prevented and/or treated is glomerulonephritis or acute kidney injury; wherein

for the treatment and/or prevention of the nephrological diseases (kidney diseases) the JNK inhibitor is preferably applied in doses (per kg body weight) in the range of 10 µg/kg to 100 mg/kg, more preferably 100 µg/kg to 10 mg/kg, even more preferably 1 mg/kg to 5 mg/kg, if applicable repeatedly, for example daily or weekly for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10 days and/or weeks, and
5 which is preferably applied systemically, e.g. i.v. or s.c.;

(iii) diseases of the eye, in particular (i) dry eye syndrome; (ii) uveitis, in particular anterior, intermediate and/or posterior uveitis, sympathetic uveitis and/or panuveitis, preferably anterior and/or posterior uveitis; (iii) age-related macular degeneration (AMD), including exudative and/or
10 non-exudative age-related macular degeneration, preferably the wet or the dry form of age-related macular degeneration; (iv) retinopathy, in particular selected from diabetic retinopathy, (arterial hypertension induced) hypertensive retinopathy, exudative retinopathy, radiation induced retinopathy, sun-induced solar retinopathy, trauma-induced retinopathy, e.g. Purtscher's retinopathy, retinopathy of prematurity (ROP) and/or hyperviscosity-related retinopathy, non-
15 diabetic proliferative retinopathy, and/or proliferative vitreo-retinopathy, whereby diabetic retinopathy and retinopathy of prematurity (ROP) are preferred and diabetic retinopathy is particularly preferred; and/or (v) post-surgery inflammation of the eye, in particular after the surgery performed on and/or in the eye, for example after cataract surgery, laser eye surgery, glaucoma surgery, refractive surgery, corneal surgery, vitreo-retinal surgery, eye muscle surgery, oculoplastic
20 surgery, and/or surgery involving the lacrimal apparatus, in particular after complex eye surgery and/or after uncomplicated eye surgery, whereby post-surgery intraocular inflammation is preferred; wherein for the treatment and/or prevention of the diseases of the eye the JNK inhibitor is preferably applied in doses in the range of 0.01 µg/eye to 10 mg/eye, more preferably 0.1 µg/eye to 5 mg/eye, even more preferably 1 µg/eye to 2 mg/eye, particularly preferably 50 µg/eye to 1.5 mg/eye, most
25 preferably 100 µg/eye to 1 mg/eye, preferably by a single application, e.g. injection or instillation, however, if necessary repeatedly, for example daily, every 2 or 3 days or weekly, for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, weeks, and which is preferably applied in or onto the eye, preferably intravitreally or subconjunctivally, more preferably subconjunctivally; and/or the JNK inhibitor is preferably applied as eye drops, which may be applied to both eyes or to one eye only, wherein the
30 pharmaceutical composition comprising the JNK inhibitor according to the invention is typically a solution, preferably an ophthalmic solution, e.g. comprising (sterile) 0.9 % NaCl and wherein a pharmaceutical composition comprises in particular 0.001 % - 10 % of the JNK inhibitor as described herein, preferably 0.01 % - 5 % of the JNK inhibitor as described herein, more preferably 0.05 % - 2 % of the JNK inhibitor as described herein, even more preferably 0.1 % - 1 % of the JNK
35 inhibitor as described herein, in particular for treating and/or preventing dry eye syndrome; and/or the JNK inhibitor is preferably applied systemically, in particular intravenously, whereby the dose

ranges preferably from 0.001 mg/kg to 10 mg/kg, more preferably from 0.01 mg/kg to 5 mg/kg, even more preferably from 0.1 mg/kg to 2 mg/kg, whereby such administration is for example particularly useful to treat and/or prevent uveitis, whereby the treatment schedule may comprises a single dose or repeated doses, whereby subsequent dose(s) may be administered on different days of the treatment schedule.

(iv) diseases of the skin, in particular papulosquamous disorders, in particular selected from psoriasis in general, for example psoriasis vulgaris, nummular psoriasis, plaque psoriasis, generalized pustular psoriasis, impetigo herpetiformis, Von Zumbusch's disease, acrodermatitis continua, guttate psoriasis, arthropathis psoriasis, distal interphalangeal psoriatic arthropathy, psoriatic arthritis mutilans, psoriatic spondylitis, psoriatic juvenile arthropathy, psoriatic arthropathy in general, and/or flexural psoriasis; parapsoriasis in general, for example large-plaque parapsoriasis, small-plaque parapsoriasis, retiform parapsoriasis, pityriasis lichenoides and lymphomatoid papulosis; pityriasis rosea; lichen planus and other papulosquamous disorders for example pityriasis rubra pilaris, lichen nitidus, lichen striatus, lichen ruber moniliformis, and infantile popular acrodermatitis. Preferably, the disorder/disease to be prevented and/or treated is psoriasis, for example psoriasis vulgaris, nummular psoriasis, plaque psoriasis, generalized pustular psoriasis, impetigo herpetiformis, Von Zumbusch's disease, acrodermatitis continua, guttate psoriasis, arthropathis psoriasis, distal interphalangeal psoriatic arthropathy, psoriatic arthritis mutilans, psoriatic spondylitis, psoriatic juvenile arthropathy, psoriatic arthropathy in general, and/or flexural psoriasis; wherein for the treatment and/or prevention of the skin diseases the JNK inhibitor is preferably applied in doses (per kg body weight) in the range of 1 µg/kg to 100 mg/kg, more preferably 10 µg/kg to 10 mg/kg, even more preferably 50 µg/kg to 5 mg/kg, particularly preferably 100 µg/kg to 1 mg/kg, if applicable repeatedly, for example daily or weekly, preferably daily, for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10 days and/or weeks, and which is preferably applied systemically, e.g. i.v., p.o. or s.c., and/or topically, epicutaneously and/or intralesionally (e.g. into skin lesion).

(v) arthritis and diseases/disorders of the joint, in particular selected from arthritis in general, osteoarthritis (degenerative joint disease), septic arthritis, rheumatoid arthritis, psoriatic arthritis, and related autoimmune diseases and arthritis; wherein for the treatment and/or prevention of the skin diseases the JNK inhibitor is preferably applied in doses (per kg body weight) in the range of 1 µg/kg to 100 mg/kg, more preferably 10 µg/kg to 50 mg/kg, even more preferably 50 µg/kg to 10 mg/kg, particularly preferably 100 µg/kg to 5 mg/kg, if applicable repeatedly, for example daily or weekly for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days and/or weeks, and which is preferably applied systemically, e.g. i.v., p.o. or s.c., particularly preferably i.v..

(vi) cancer and tumor diseases, in particular selected from (i) liver cancer and liver carcinoma in general, in particular liver metastases, liver cell carcinoma, hepatocellular carcinoma, hepatoma, intrahepatic bile duct carcinoma, cholangiocarcinoma, hepatoblastoma, angiosarcoma (of liver), and other specified or unspecified sarcomas and carcinomas of the liver; (ii) prostate cancer and/or prostate carcinoma; and/or (iii) colon cancer and colon carcinoma in general, in particular cecum carcinoma, appendix carcinoma, ascending colon carcinoma, hepatic flexure carcinoma, transverse colon carcinoma, splenic flexure carcinoma, descending colon carcinoma, sigmoid colon carcinoma, carcinoma of overlapping sites of colon and/or malignant carcinoid tumors of the colon, wherein for the treatment and/or prevention of the cancer and tumor diseases the JNK inhibitor is preferably applied in doses (per kg body weight) in the range of 1 µg/kg to 100 mg/kg, more preferably 10 µg/kg to 50 mg/kg, even more preferably 0.1 mg/kg to 20 mg/kg, particularly preferably 0.1 mg/kg to 5 mg/kg [doses mice!], if applicable repeatedly, for example daily, every 2 or 3 days or weekly, for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, weeks, and which is preferably applied systemically, e.g. p.o., i.v. or s.c..

(vii) diseases and/or disorders of the urinary system, in particular ureteritis; urinary tract infection (bladder infection, acute cystitis); cystitis in general, in particular interstitial cystitis, Hunner's ulcer, trigonitis and/or hemorrhagic cystitis; urethritis, in particular nongonococcal urethritis or gonococcal urethritis; painful bladder syndrome; IC/PBS; urethral syndrome; and/or retroperitoneal fibrosis; preferably IC/PBS; wherein for the treatment and/or prevention of the diseases and/or disorders of the urinary system, preferably for the treatment and/or prevention of IC/PBS, the JNK inhibitor is preferably applied (i) systemically, more preferably intravenously, e.g. by intravenous injection, in doses of (per kg body weight) in the range of 100 ng/kg to 10 mg/kg, more preferably 1 µg/kg to 5 mg/kg, even more preferably 10 µg/kg to 2 mg/kg, particularly preferably 0.1 mg/kg to 1 mg/kg, most preferably 0.2 mg/kg to 0.5 mg/kg, preferably administered in one single dose, however, if applicable also preferably administered repeatedly, for example daily, every 2 or 3 days or weekly, for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, weeks; or the JNK inhibitor is also preferably applied (ii) intravesically, more preferably by intravesical infusion, preferably at a concentration of 10 µg/ml – 1000 mg/ml, more preferably 50 µg/ml – 500 mg/ml, even more preferably 100 µg/ml – 100 mg/ml, and particularly preferably 0.5 mg/ml – 50 mg/ml, preferably in single doses of 0.1 – 1000 mg, more preferably 0.5 – 500 mg, even more preferably 1 – 100 mg, and particularly preferably 2 – 10 mg, preferably administered in one single dose, however, if applicable also preferably administered repeatedly, for example daily, every 2 or 3 days or weekly, for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, weeks.

(viii) neural, neuronal or neurodegenerative disorders, in particular neurodegenerative disease, preferably Alzheimer's disease, for example Alzheimer's disease with early onset, Alzheimer's disease with late onset, Alzheimer's dementia senile and presenile forms, and/or Mild Cognitive Impairment, in particular Mild Cognitive Impairment due to Alzheimer's Disease, wherein for the treatment and/or prevention of the neural, neuronal or neurodegenerative disorders the JNK inhibitor is preferably applied in doses (per kg body weight) in the range of 1 µg/kg to 100 mg/kg, more preferably 10 µg/kg to 50 mg/kg, even more preferably 100 µg/kg to 10 mg/kg, and particularly preferably 500 µg/kg to 1 mg/kg, whereby the JNK inhibitor is preferably administered, if applicable, once or repeatedly, preferably weekly (once per week) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more weeks, every second week (once per two weeks) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more weeks, monthly (once per month) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more months, every sixth week (once per every six weeks) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more months, every second month (once per two months) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more months or every third month (once per three months) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more weeks, more preferably weekly (once per week) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more weeks, every second week (once per two weeks) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more weeks, monthly (once per month) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more months, even more preferably monthly (once per month) for several, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more months, and which is preferably applied systemically, e.g. i.v., p.o., i.m., s.c. or intra-CSF (intra-cerebrospinal fluid) moreover, for treating and/or preventing neural, neuronal or neurodegenerative disorders, in particular neurodegenerative disease, preferably Alzheimer's disease, for example Alzheimer's disease with early onset, Alzheimer's disease with late onset, Alzheimer's dementia senile and presenile forms, and/or Mild Cognitive Impairment, in particular Mild Cognitive Impairment due to Alzheimer's Disease, the JNK inhibitors of the present invention may be administered as stand-alone therapy, however, the JNK inhibitors of the present invention may also be administered in combination with other medications, e.g. with a PKR inhibitor, e.g. "SC1481" by Polypeptide Group, and, optionally, in addition to the JNK inhibitor according to the present invention and the PKR inhibitor with a amyloid lowering agent, whereby amyloid lowering agents include β-secretase (BACE1) inhibitors, γ-secretase inhibitors (GSI) and modulators (GSM) and examples of such inhibitors, which are currently in clinical trials may be retrieved from Vassar R. (2014) BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. *Alzheimers Res Ther.*;6(9):89 or from Jia Q, Deng Y, Qing H (2014) Potential therapeutic strategies for Alzheimer's disease targeting or beyond β-amyloid: insights from clinical trials. *Biomed Res Int.* 2014;2014:837157.

Prevention and/or treatment of a disease as defined herein typically includes administration of a pharmaceutical composition as defined above. The JNK inhibitors of the present invention will modulate the JNK activity in the subject. The term "modulate" includes in particular the suppression of phosphorylation of c-jun, ATF2 or NFAT4 in any of the diseases disclosed herein, for example, by using at least one JNK inhibitor comprising or consisting of an inhibitory (poly)peptide sequence according to any of sequences of SEQ ID NOs: 2 to 27, potentially comprising an additional transporter sequence, whereby – if a transporter sequence is attached – any of the sequences according to any one of SEQ ID NOs: 171-190, or a variant thereof as defined herein, are preferred, and the sequence according to SEQ ID NO: 172, or a variant thereof as defined herein, is particularly preferred, as a competitive inhibitor of the natural c-jun, ATF2 and NFAT4 binding site in a cell. The term "modulate" also includes suppression of hetero- and homomeric complexes of transcription factors made up of, without being limited thereto, c-jun, ATF2, or NFAT4 and their related partners, such as for example the AP-1 complex that is made up of c-jun, AFT2 and c-fos.

Treatment of a subject with the pharmaceutical composition as disclosed above may be typically accomplished by administering (*in vivo*) an ("therapeutically effective") amount of said pharmaceutical composition to a subject, wherein the subject may be e.g. a human subject or an animal, whereby a human is particularly preferred. The animal is preferably a non-human mammal, e.g., a non-human primate, mouse, rat, dog, cat, cow, horse or pig. The term "therapeutically effective" means that the active component of the pharmaceutical composition is of sufficient quantity to ameliorate the diseases and disorders as discussed herein.

According to another preferred embodiment, the JNK inhibitor of the present invention, for example, a JNK inhibitor comprising or consisting of an inhibitory (poly)peptide sequence according to any of sequences of SEQ ID NOs: 2 to 27, potentially comprising an additional transporter sequence, whereby – if a transporter sequence is attached – any of the sequences according to any one of SEQ ID NOs: 171-190, or a variant thereof as defined herein, are preferred, and the sequence according to SEQ ID NO: 172, or a variant thereof as defined herein, is particularly preferred, may be utilized for the treatment of a tissue or organ prior to its transplantation. Preferably, a solution for the isolation, transport, perfusion, implantation or the like of an organ and/or tissue to be transplanted comprises the JNK inhibitor according to the present invention, preferably in a concentration in the range of 1 to 1000 μ M, more preferably in the range of 10 to 500 μ M, even more preferably in the range of 50 to 150 μ M. For this aspect of the invention, the transplant is a kidney, heart, lung, pancreas, in particular pancreatic islets (also called islets of Langerhans), liver, blood cell, bone marrow, cornea, accidental severed limb, in particular fingers, hand, foot, face, nose, bone, cardiac valve, blood vessel or intestine transplant, preferably a kidney, heart, pancreas, in particular

pancreatic islets (also called islets of Langerhans), or skin transplant. For example, the JNK inhibitor according to the invention may be contained in the solution for the isolation of pancreatic islets. Such a solution may be for example injected into the pancreatic duct prior to isolation. Moreover, it is preferred if a solution containing the JNK inhibitor according to the invention is applied in isolation, transport, perfusion, transplantation or the like of an organ and/or tissue, in particular if the time of ischemia exceeds 15 min, more preferably, if the time of ischemia exceeds 20 min, even more preferably if the time of ischemia is at least 30 min. These ischemia times may apply to warm and/or cold ischemia time, however, it is particularly preferred if they apply exclusively to warm ischemia time (WIT), whereby WIT refers to the length of time that elapses between a donor's death, in particular from the time of cross-clamping or of asystole in non-heart-beating donors, until cold perfusion is commenced and to ischemia during implantation, from removal of the organ from ice until reperfusion.

Diseases and disorders

The present invention is directed to specific uses (or methods of use) of the above disclosed JNK inhibitors or pharmaceutical compositions containing the same in a method for treatment of the human or animal body by therapy, in particular of the human body. As mentioned above JNK signalling is involved in a multitude of diverse disease states and disorder and inhibition of said signalling has proposed and successfully tested for many of these. The inventors of the present invention found that the JNK inhibitors disclosed herein are effective JNK inhibitors for the treatment of the diseases as disclosed in the following.

Treatment of a human or animal body by therapy, as used herein, refers to any kind of therapeutic treatment of a respective subject. It includes for example prevention of onset of the disease or symptoms (prophylaxis), i.e. typically prior to manifestation of the disease in the patient. The term also includes the "mere" treatment of symptoms of a given disease, i.e. the treatment will ameliorate pathogenesis by reducing disease-associated symptoms, without necessarily curing the underlying cause of the disease and symptoms. Certainly, curing the underlying cause of the disease is also encompassed by the term. The term also encompasses a treatment which delays or even stops progression of the respective disease.

In one embodiment the JNK inhibitors according to the present invention may be administered for example prophylactically prior to potential onset of a foreseeable disorder, e.g. prior to a planned surgical intervention or planned exposure to stressful stimuli. A surgical intervention could for example bear the risk of inflammation of the respective wound or neighbouring tissue. Exposure to stressful stimuli like radiation could lead to apoptosis of affected tissue and cells. In such scenario,

the JNK inhibitors according to the present invention may, for example, be administered at least once up to about 4 weeks in advance. The JNK inhibitors may for example be administered at least 24 hours, at least 48 hours, at least 1 week, at least 2 weeks or 4 weeks in advance.

- 5 The diseases and disorders to be treated and/or prevented with the JNK inhibitors as disclosed herein may be acute or chronic.

While the JNK inhibitors of the present invention may be used in general for the treatment and/or prevention of diseases of various organs, such as diseases of the eye, diseases of the bone, neural
10 diseases, neuronal diseases, neurodegenerative diseases, diseases of the skin, immune and/or autoimmune diseases, diseases of the eye, diseases of the mouth, diseases of the kidney, diseases of the urinary system, inflammatory diseases, metabolic diseases, cardiovascular diseases, proliferative diseases (in particular cancers and tumors), diseases of the ear, diseases of the intestine, diseases of the respiratory system (e.g. lung diseases), infectious diseases, and various other diseases, the present
15 invention specifically refers to the following diseases:

Among the disease to be treated and/or prevented by the inventive molecules, skin diseases and diseases of the subcutaneous tissue are to be mentioned, in particular inflammatory skin diseases, more specifically skin diseases selected from the group consisting of eczema, Psoriasis, dermatitis,
20 acne, mouth ulcers, erythema, Lichen plan, sarcoidosis, vascularitis and adult linear IgA disease,. Dermatitis encompasses e.g. atopic dermatitis or contact dermatitis. In particular, the skin diseases and diseases of the subcutaneous tissue to be treated and/or prevented with the JNK inhibitor as described herein can be selected from papulosquamous disorders in general, in particular psoriasis in general, for example psoriasis vulgaris, nummular psoriasis, plaque psoriasis, generalized pustular
25 psoriasis, impetigo herpetiformis, Von Zumbusch's disease, acrodermatitis continua, guttate psoriasis, arthropathis psoriasis, distal interphalangeal psoriatic arthropathy, psoriatic arthritis mutilans, psoriatic spondylitis, psoriatic juvenile arthropathy, psoriatic arthropathy in general, and/or flexural psoriasis, parapsoriasis in general, for example large-plaque parapsoriasis, small-plaque parapsoriasis, retiform parapsoriasis, pityriasis lichenoides and lymphomatoid papulosis;
30 pityriasis rosea; lichen planus and other papulosquamous disorders for example pityriasis rubra pilaris, lichen nitidus, lichen striatus, lichen ruber moniliformis, and infantile popular acrodermatitis; eczema; dermatitis in general, in particular atopic dermatitis for example Besnier's prurigo, atopic or diffuse neurodermatitis, flexural eczema, infantile eczema, intrinsic eczema, allergic eczema, other atopic dermatitis, seborrheic dermatitis for example seborrhea capitis, seborrheic infantile
35 dermatitis, other seborrheic dermatitis, diaper dermatitis for example diaper erythema, diaper rash and psoriasiform diaper rash, allergic contact dermatitis, in particular due to metals, due to

adhesives, due to cosmetics, due to drugs in contact with skin, due to dyes, due to other chemical products, due to food in contact with skin, due to plants except food, due to animal dander, and/or due to other agents, irritant contact dermatitis, in particular due to detergents, due to oils and greases, due to solvents, due to cosmetics, due to drugs in contact with skin, due to other chemical products, due to food in contact with skin, due to plants except food, due to metal, and/or due to other agents, unspecified contact dermatitis, exfoliative dermatitis, dermatitis for example general and localized skin eruption due to substances taken internally, in particular due to drugs and medicaments, due to ingested food, due to other substances, nummular dermatitis, dermatitis gangrenosa, dermatitis herpetiformis, dry skin dermatitis, factitial dermatitis, perioral dermatitis, radiation-related disorders of the skin and subcutaneous tissue, stasis dermatitis, Lichen simplex chronicus and prurigo, pruritus, dyshidrosis, cutaneous autosensitization, infective dermatitis, erythema intertrigo and/or pityriasis alba; cellulitis (bacterial infection involving the skin); lymphangitis, in particular acute or chronic lymphangitis; panniculitis in general, in particular lobular panniculitis without vasculitis, for example acute panniculitis, previously termed Weber-Christian disease and systemic nodular panniculitis, lobular panniculitis with vasculitis, septal panniculitis without vasculitis and/or septal panniculitis with vasculitis; lymphadenitis, in particular acute lymphadenitis; pilonidal cyst and sinus; pyoderma in general, in particular pyoderma gangrenosum, pyoderma vegetans, dermatitis gangrenosa, purulent dermatitis, septic dermatitis and suppurative dermatitis; erythrasma; omphalitis; pemphigus, in particular pemphigus vulgaris, pemphigus vegetans, pemphigus foliaceus, Brazilian pemphigus, pemphigus erythematosus, drug-induced pemphigus, IgA pemphigus, for example subcorneal pustular dermatosis and intraepidermal neutrophilic IgA dermatosis, and/or paraneoplastic pemphigus; acne in general, in particular acne vulgaris, acne conglobata, acne varioliformis, acne necrotica miliaris, acne tropica, infantile acne acné excoriée des jeunes filles, Picker's acne, and/or acne keloid; mouth and other skin ulcers; urticaria in general, in particular allergic urticaria, idiopathic urticarial, urticarial due to cold and heat, dermatographic urticarial, vibratory urticarial, cholinergic urticarial, and/or contact urticarial; erythema in general, in particular erythema multiforme for example nonbullous erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis (Lyell), and Stevens-Johnson syndrome-toxic epidermal necrolysis overlap syndrome, erythema nodosum, toxic erythema, erythema annulare centrifugum, erythema marginatum and/or other chronic figurate erythema; sunburn and other acute skin changes due to ultraviolet radiation; skin changes due to chronic exposure to nonionizing radiation; radiodermatitis; folliculitis; perifolliculitis; pseudofolliculitis barbae; hidradenitis suppurativa; sarcoidose; vascularitis; adult linear IgA disease; rosacea, in particular perioral dermatitis, rhinophyma, and other rosacea; and/or follicular cysts of skin and subcutaneous tissue, in particular epidermal cyst, pilar cyst, trichodermal cyst, steatocystoma multiplex, sebaceous cyst and/or other follicular cysts.

Examples for preferred skin diseases which can be treated with the JNK inhibitors of the present invention are psoriasis and lupus erythematosus. In more general terms, skin diseases and diseases of the subcutaneous tissue, which can preferably be treated and/or prevented with the JNK inhibitors as disclosed herein are papulosquamous disorders. These include psoriasis, parapsoriasis, pityriasis rosea, lichen planus and other papulosquamous disorders for example pityriasis rubra pilaris, lichen nitidus, lichen striatus, lichen ruber moniliformis, and infantile popular acrodermatitis. Preferably the disease to be treated and/or prevented by the JNK inhibitor according to the invention is selected from the group of psoriasis and parapsoriasis, whereby psoriasis is particularly preferred. Examples for psoriasis include psoriasis vulgaris, nummular psoriasis, plaque psoriasis, generalized pustular psoriasis, impetigo herpetiformis, Von Zumbusch's disease, acrodermatitis continua, guttate psoriasis, arthropathis psoriasis, distal interphalangeal psoriatic arthropathy, psoriatic arthritis mutilans, psoriatic spondylitis, psoriatic juvenile arthropathy, psoriatic arthropathy in general, and/or flexural psoriasis. Examples for parapsoriasis include large-plaque parapsoriasis, small-plaque parapsoriasis, retiform parapsoriasis, pityriasis lichenoides and lymphomatoid papulosis.

(Anti-inflammatory) treatment upon tissue or organ transplantation, is treatable by the inventive molecules in particular upon heart, kidney, and skin (tissue), lung, pancreas, liver, blood cells (e.g. any kind of blood cell, such as platelets, white blood cells, red blood cells), bone marrow, cornea, accidental severed limbs (fingers, hand, foot, face, nose etc.), bones of whatever type, cardiac valve, blood vessels, segments of the intestine or the intestine as such. Such a treatment is e.g. considered appropriate whenever e.g. a graft vs. host or host vs graft reaction occurs upon organ/tissue transplantation. The use of the inventive molecules may also be employed whenever transplantation surgery is carried, in particular in case of skin (or, pancreas, liver, lung, heart, kidney) graft vs. host or host vs. skin (or, pancreas, liver, lung, heart, kidney) graft reaction.

Among neurodegenerative diseases, in particular those associated with chronic inflammation, tauopathies and amyloidoses and prion diseases are addressed by the inventive molecules. Other such neurodegenerative disease refer to the various forms of dementia, e.g. frontotemporal dementia and dementia with lewy bodies, schizophrenia and bipolar disorder, spinocerebellar ataxia, spinocerebellar atrophy, multiple system atrophy, motor neuron disease, corticobasal degeneration, progressive supranuclear palsy or hereditary spastic paraparesis. Another field of indication is pain (e.g. neuropathic, incident, breakthrough, psychogenic, phantom, chronic or acute forms of pain). Another field of use is the treatment of bladder diseases, in particular for treating loss of bladder function (e.g. urinary incontinence, overactive bladder, interstitial cystitis or bladder cancer) or stomatitis.

The inventive molecules are used for the treatment of fibrotic diseases or fibrosis as well, in particular lung, heart, liver, bone marrow, mediastinum, retroperitoneum, skin, intestine, joint, and shoulder fibrosis.

5 While inflammatory diseases of the mouth and the jaw/mandible are treatable in general by the inventive molecules, gingivitis, osteonecrosis (e.g. of the jaw bone), peri-implantitis, pulpitis, and periodontitis are particularly suitable for the use of these inventive molecules for therapeutic purposes. In particular, diseases and/or disorders of the mouth or the jaw bone to be treated and/or prevented with the JNK inhibitor as described herein can be selected from pulpitis in general, in particular acute pulpitis, chronic pulpitis, hyperplastic pulpitis, ulcerative pulpitis, irreversible pulpitis and/or reversible pulpitis; periimplantitis; periodontitis in general, in particular chronic periodontitis, complex periodontitis, simplex periodontitis, aggressive periodontitis, and/or apical periodontitis, e.g. of pulpal origin; periodontosis, in particular juvenile periodontosis; gingivitis in general, in particular acute gingivitis, chronic gingivitis, plaque-induced gingivitis, and/or non-plaque-induced gingivitis; pericoronitis, in particular acute and chronic pericoronitis; sialadenitis (sialoadenitis); parotitis, in particular infectious parotitis and autoimmune parotitis; stomatitis in general, in particular aphthous stomatitis (e.g., minor or major), Bednar's aphthae, periadenitis mucosa necrotica recurrens, recurrent aphthous ulcer, stomatitis herpetiformis, gangrenous stomatitis, denture stomatitis, ulcerative stomatitis, vesicular stomatitis and/or gingivostomatitis; mucositis, in particular mucositis due to antineoplastic therapy, due to (other) drugs, or due to radiation, ulcerative mucositis and/or oral mucositis; cheilitis in general, in particular chapped lips, actinic cheilitis, angular cheilitis, eczematous cheilitis, infectious cheilitis, granulomatous cheilitis, drug-related cheilitis, exfoliative cheilitis, cheilitis glandularis, and/or plasma cell cheilitis; cellulitis (bacterial infection), in particular of mouth and/or lips; desquamative disorders, in particular desquamative gingivitis; and/or temporomandibular joint disorder.

In addition, polypes are effectively treatable by using the inventive molecules.

Also inflammatory or non-inflammatory pathophysiologies of the kidney are effectively treated by using the inventive molecules. In particular, the disease is selected from the group consisting of glomerulonephritis in general, in particular membrano-proliferative glomerulonephritis, mesangio-proliferative glomerulonephritis, rapidly progressive glomerulonephritis, acute kidney injury ("AKI", also called "acute renal failure" or "acute kidney failure") in general, in particular prerenal AKI, intrinsic AKI, postrenal AKI, AKI with tubular necrosis for example acute tubular necrosis, renal tubular necrosis, AKI with cortical necrosis for example acute cortical necrosis and renal cortical necrosis, AKI with medullary necrosis, for example medullary (papillary) necrosis, acute medullary

(papillary) necrosis and chronic medullary (papillary) necrosis, or other AKI; nephropathies in general, in particular membranous nephropathy or diabetic nephropathy, nephritis in general, in particular lupus nephritis, pyelonephritis, interstitial nephritis, tubulointerstitial nephritis, chronic nephritis or acute nephritis, and minimal change disease and focal segmental glomerulosclerosis.

Moreover, diseases and/or disorders of the kidney (nephrological diseases) to be treated and/or prevented with the JNK inhibitor as described herein can be selected from glomerulonephritis in general, for example nonproliferative glomerulonephritis, in particular minimal change disease, focal segmental glomerulosclerosis, focal segmental glomerular hyalinosis and/or sclerosis, focal glomerulonephritis, membranous glomerulonephritis, and/or thin basement membrane disease, and proliferative glomerulonephritis, in particular membrano-proliferative glomerulonephritis, mesangio-proliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, mesangiocapillary proliferative glomerulonephritis, dense deposit disease (membranoproliferative glomerulonephritis type II), extracapillary glomerulonephritis (crescentic glomerulonephritis), rapidly progressive glomerulonephritis (RPGN), in particular Type I RPGN, Type II RPGN, Type III RPGN, and Type IV RPGN, acute proliferate glomerulonephritis, post-infectious glomerulonephritis, and/or IgA nephropathy (Berger's disease); acute nephritic syndrome; rapidly progressive nephritic syndrome; recurrent and persistent hematuria; chronic nephritic syndrome; nephrotic syndrome; proteinuria with specified morphological lesion; glomerulitis; glomerulopathy; glomerulosclerosis; acute kidney injury ("AKI", also called "acute renal failure" or "acute kidney failure") in general, in particular prerenal AKI, intrinsic AKI, postrenal AKI, AKI with tubular necrosis for example acute tubular necrosis, renal tubular necrosis, AKI with cortical necrosis for example acute cortical necrosis and renal cortical necrosis, AKI with medullary necrosis, for example medullary (papillary) necrosis, acute medullary (papillary) necrosis and chronic medullary (papillary) necrosis, or other AKI; chronic kidney disease; nephropathies in general, in particular membranous nephropathy, diabetic nephropathy, IgA nephropathy, hereditary nephropathy, analgesic nephropathy, CFHR5 nephropathy, contrast-induced nephropathy, amyloid nephropathy, reflux nephropathy and/or Mesoamerican nephropathy; nephritis in general, in particular lupus nephritis, pyelonephritis, interstitial nephritis, tubulointerstitial nephritis, chronic nephritis or acute nephritis, diffuse proliferative nephritis, and/or focal proliferative nephritis, tubulo-interstitial nephritis, infectious interstitial nephritis, pyelitis, pyelonephritis, interstitial nephritis; tubulopathy, tubulitis, in particular RTA (RTA1 and RTA2), Fanconi syndrome, Bartter syndrome, Gitelman syndrome, Liddle's syndrome, nephrogenic diabetes insipidus, renal papillary necrosis, hydronephrosis, pyonephrosis and/or acute tubular necrosis chronic kidney disease (CKD); Goodpasture syndrome (anti-glomerular basement antibody disease); granulomatosis with polyangiitis; microscopic polyangiitis; and/or Churg–Strauss syndrome.

Glomerulonephritis refers to several renal diseases, whereby many of the diseases are characterised by inflammation either of the glomeruli or small blood vessels in the kidneys, but not all diseases necessarily have an inflammatory component. Acute kidney injury ("AKI", also called "acute renal failure" or "acute kidney failure") is an abrupt loss of kidney function, which is often investigated in a renal ischemia/ reperfusion injury model. Nephropathies, i.e. damage to or disease of a kidney, includes also nephrosis, which is non-inflammatory nephropathy, and nephritis, which is inflammatory kidney disease.

Among the diseases or disorders which are effectively treated by the inventive molecules, a larger number of diseases or disorders may be linked to inflammatory processes, but do not necessarily have to be associated with such inflammatory processes. The following diseases or disorders are specifically disclosed in this regard as being treatable by the use of the inventive molecules: Addison's disease, Agammaglobulinemia, Alopecia areata, Amyotrophic lateral sclerosis, Antiphospholipid syndrome, Atopic allergy, Autoimmune aplastic anemia, Autoimmune cardiomyopathy, Autoimmune enteropathy, Autoimmune hemolytic anemia, Autoimmune inner ear, disease, Autoimmune lymphoproliferative syndrome, Autoimmune polyendocrine syndrome, Autoimmune progesterone dermatitis, Idiopathic thrombocytopenic purpura, Autoimmune urticaria, Balo concentric sclerosis, Bullous pemphigoid, Castleman's disease, Cicatricial pemphigoid, Cold agglutinin disease, Complement component 2 deficiency associated disease, Cushing's syndrome, Dago's disease, Adiposis dolorosa, Eosinophilic pneumonia, Epidermolysis bullosa acquisita, Hemolytic disease of the newborn, Cryoglobulinemia, Evans syndrome, Fibrodysplasia ossificans progressive, Gastrointestinal pemphigoid, Goodpasture's syndrome, Hashimoto's encephalopathy, Gestational pemphigoid, Hughes-stovin syndrome, Hypogammaglobulinemia, Lambert-eaton myasthenic syndrome, Lichen sclerosus, Morphea, Pityriasis lichenoides et varioliformis acuta, Myasthenia gravis, Narcolepsy, Neuromyotonia, Opsoclonus myoclonus syndrome, Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria, Parry-romberg syndrome, Pernicious anemia, POEMS syndrome, Pyoderma gangrenosum, Pure red cell aplasia, Raynaud's phenomenon, Restless legs syndrome, Retroperitoneal fibrosis, Autoimmune polyendocrine syndrome type 2, Stiff person syndrome, Susac's syndrome, Febrile neutrophilic dermatosis, Sydenham's chorea, Thrombocytopenia, and vitiligo.

While any kind of inflammatory eye disease may be treated by the use of the inventive molecules, the following eye-related diseases are specifically disclosed: inflammation after corneal surgery, non-infective keratitis, chorioretinal inflammation, and sympathetic ophthalmia. In more general terms, the JNK inhibitors as described herein can be used to treat and/or prevent inflammatory and non-inflammatory diseases of the eye, in particular selected from uveitis, in particular anterior,

intermediate and/or posterior uveitis, sympathetic uveitis and/or panuveitis; scleritis in general, in particular anterior scleritis, brawny scleritis, posterior scleritis, and scleritis with corneal involvement; episcleritis in general, in particular episcleritis periodica fugax and nodular episcleritis; retinitis; conjunctivitis in general, in particular acute conjunctivitis, mucopurulent conjunctivitis, 5 atopic conjunctivitis, toxic conjunctivitis, pseudomembraneous conjunctivitis, serous conjunctivitis, chronic conjunctivitis, giant pupillary conjunctivitis, follicular conjunctivitis vernal conjunctivitis, blepharoconjunctivitis, and/or pingueculitis; non-infectious keratitis in general, in particular corneal ulcer, superficial keratitis, macular keratitis, filamentary keratitis, photokeratitis, punctate keratitis, keratoconjunctivitis, for example exposure keratoconjunctivitis, Dry Eye Syndrome 10 (keratoconjunctivitis sicca), neurotrophic keratoconjunctivitis, ophthalmia nodosa, phlyctenular keratoconjunctivitis, vernal keratoconjunctivitis and other keratoconjunctivitis, interstitial and deep keratitis, sclerosing keratitis, corneal neovascularization and other keratitis; iridocyclitis in general, in particular acute iridocyclitis, subacute iridocyclitis and chronic iridocyclitis, primary iridocyclitis, recurrent iridocyclitis and secondary iridocyclitis, lens-induced iridocyclitis, Fuchs' heterochromic 15 cyclitis, Vogt-Koyanagi syndrome; iritis; chorioretinal inflammation in general, in particular focal and disseminated chorioretinal inflammation, chorioretinitis, chorioiditis, retinitis, retinochoroiditis, posterior cyclitis, Harada's disease, chorioretinal inflammation in infectious and parasitic diseases; post-surgery inflammation of the eye, in particular whereby the surgery was performed on and/or in the eye, for example after cataract surgery, laser eye surgery, glaucoma surgery, refractive surgery, 20 corneal surgery, vitreo-retinal surgery, eye muscle surgery, oculoplastic surgery, and surgery involving the lacrimal apparatus, in particular post-surgery intraocular inflammation, preferably post-surgery intraocular inflammation after complex eye surgery and/or after uncomplicated eye surgery, for example inflammation of postprocedural bleb; inflammatory diseases damaging the retina of the eye; retinal vasculitis, in particular Eales disease and retinal perivasculitis; retinopathy 25 in general, in particular diabetic retinopathy, (arterial hypertension induced) hypertensive retinopathy, exudative retinopathy, radiation induced retinopathy, sun-induced solar retinopathy, trauma-induced retinopathy, e.g. Purtscher's retinopathy, retinopathy of prematurity (ROP) and/or hyperviscosity-related retinopathy, non-diabetic proliferative retinopathy, and/or proliferative vitreo-retinopathy; blebitis; endophthalmitis; sympathetic ophthalmia; hordeolum; chalazion; 30 blepharitis; dermatitis and other inflammations of the eyelid; dacryoadenitis; canaliculitis, in particular acute and chronic lacrimal canaliculitis; dacryocystitis; inflammation of the orbit, in particular cellulitis of orbit, periostitis of orbit, tenonitis of orbit, granuloma of orbit and orbital myositis; purulent and parasitic endophthalmitis; and diseases and/or disorders relating to degeneration of macula and/or posterior pole in general, in particular age-related macular 35 degeneration (AMD), in particular the wet or the dry form of age-related macular degeneration, exudative and/or non-exudative age-related macular degeneration, and cataract.

The "dry" form of advanced AMD, results from atrophy of the retinal pigment epithelial layer below the retina, which causes vision loss through loss of photoreceptors (rods and cones) in the central part of the eye. Neovascular, the "wet" form of advanced AMD, causes vision loss due to abnormal blood vessel growth (choroidal neovascularization) in the choriocapillaris, through Bruch's membrane, ultimately leading to blood and protein leakage below the macula. Bleeding, leaking, and scarring from these blood vessels eventually cause irreversible damage to the photoreceptors and rapid vision loss, if left untreated. The inventive molecules are suitable for treating both forms of AMD.

In particular, the JNK inhibitors of the present invention can be used to treat and/or prevent Dry eye syndrome (DES). Dry eye syndrome (DES), also called keratitis sicca, xerophthalmia, keratoconjunctivitis sicca (KCS) or cornea sicca, is an eye disease caused by eye dryness, which, in turn, is caused by either decreased tear production or increased tear film evaporation. Typical symptoms of dry eye syndrome are dryness, burning and a sandy-gritty eye irritation. Dry eye syndrome is often associated with ocular surface inflammation. If dry eye syndrome is left untreated or becomes severe, it can produce complications that can cause eye damage, resulting in impaired vision or even in the loss of vision. Untreated dry eye syndrome can in particular lead to pathological cases in the eye epithelium, squamous metaplasia, loss of goblet cells, thickening of the corneal surface, corneal erosion, punctate keratopathy, epithelial defects, corneal ulceration, corneal neovascularization, corneal scarring, corneal thinning, and even corneal perforation. The JNK inhibitors according to the present invention may be utilized in treatment and/or prevention of dry eye syndrome, e.g. due to aging, diabetes, contact lenses or other causes and/or after eye surgery or trauma, in particular after Lasik (laser-assisted in situ keratomileusis), commonly referred to simply as laser eye surgery.

The standard treatment of dry eye may involve the administration of artificial tears, cyclosporine (in particular cyclosporine A; e.g. Restasis®); autologous serum eye drops; lubricating tear ointments and/or the administration of (cortico-)steroids, for example in the form of drops or eye ointments. Therefore, the present invention also relates to the use of the JNK inhibitor as described herein in a method of treatment of dry eye syndrome, wherein the method comprises the combined administration of the JNK inhibitor as defined herein together with a standard treatment for dry eye, in particular with any one of the above mentioned treatments. Particularly preferred is the combination with cyclosporine A and most preferably with artificial tears. Combined administration comprises the parallel administration and/or subsequent administration (either first the JNK inhibitor described herein and then the (cortico)steroids or vice versa). Certainly, subsequent and parallel

administration may also be combined, e.g. the treatment is started with JNK inhibitors described herein and at a later point in time in the course of the treatment (cortico)steroids are given in parallel, or vice versa.

5 In particular, the JNK inhibitors of the present invention can be used to treat and/or prevent inflammatory diseases of the sclera, the cornea, the iris, the ciliary body, the retina and/or the choroid of the eye. Preferably, the JNK inhibitors of the present invention can be used to treat and/or prevent uveitis, i.e. an inflammation of the uvea. The uvea consists of the middle, pigmented vascular structures of the eye and includes the iris, the ciliary body, and the choroid. Typically, 10 uveitis is classified as anterior uveitis, intermediate uveitis, posterior uveitis, and/or panuveitis, whereby the latter is the inflammation of all the layers of the uvea. Furthermore, uveitis includes sympathetic ophthalmia (sympathetic uveitis), which is a bilateral diffuse granulomatous uveitis of both eyes following trauma to one eye. Anterior uveitis, which is particularly preferred to be treated with the JNK inhibitors of the present invention, includes iridocyclitis and iritis. Iritis is the 15 inflammation of the anterior chamber and iris. Iridocyclitis presents the same symptoms as iritis, but also includes inflammation in the vitreous cavity. Examples of iridocyclitis to be prevented and/or treated with the JNK inhibitors of the present invention include – but are not limited to – acute iridocyclitis, subacute iridocyclitis and chronic iridocyclitis, primary iridocyclitis, recurrent iridocyclitis and secondary iridocyclitis, lens-induced iridocyclitis, Fuchs' heterochromic cyclitis, 20 and Vogt-Koyanagi syndrome. Intermediate uveitis, also known as pars planitis, in particular includes vitritis, which is inflammation of cells in the vitreous cavity, sometimes with "snowbanking" or deposition of inflammatory material on the pars plana. Posterior uveitis includes in particular chorioretinitis, which is the inflammation of the retina and choroid, and chorioiditis (choroid only). In more general terms, the JNK inhibitors as disclosed herein can be used to treat and/or prevent 25 chorioretinal inflammation in general, for example focal and/or disseminated chorioretinal inflammation, chorioretinitis, chorioiditis, retinochoroiditis, posterior cyclitis, Harada's disease, chorioretinal inflammation in infectious and parasitic diseases and/or retinitis, i.e. an inflammation of the retina. Inflammatory diseases damaging the retina of the eye in general are included, in addition to retinitis in particular retinal vasculitis, for example Eales disease and retinal perivasculitis. 30 Further inflammatory diseases of the sclera, the cornea, the iris, the ciliary body, the retina and/or the choroid of the eye to be treated and/or prevented with the JNK inhibitors as disclosed herein include scleritis, i.e. an inflammation of the sclera, for example anterior scleritis, brawny scleritis, posterior scleritis, scleritis with corneal involvement and scleromalacia perforans; episcleritis, in particular episcleritis periodica fugax and nodular episcleritis; and keratitis, which is an 35 inflammation of the cornea, in particular corneal ulcer, superficial keratitis, macular keratitis, filamentary keratitis, photokeratitis, punctate keratitis, keratoconjunctivitis, for example exposure

keratoconjunctivitis, keratoconjunctivitis sicca (dry eyes), neurotrophic keratoconjunctivitis, ophthalmia nodosa, phlyctenular keratoconjunctivitis, vernal keratoconjunctivitis and other keratoconjunctivitis, interstitial and deep keratitis, sclerosing keratitis, corneal neovascularization and other keratitis.

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In addition, the JNK inhibitors as disclosed herein are particularly useful to treat and/or prevent post-surgery (or "post-procedural") or post-trauma inflammation of the eye. "Post-surgery" refers in particular to a surgery performed on and/or in the eye, for example cataract surgery, laser eye surgery, glaucoma surgery, refractive surgery, corneal surgery, vitreo-retinal surgery, eye muscle surgery, oculoplastic surgery, and/or surgery involving the lacrimal apparatus. Preferably, the surgery referred to in "post-surgery" is a complex eye surgery and/or an uncomplicated eye surgery. Particularly preferred is the use of JNK inhibitors as disclosed herein to treat and/or prevent post-surgery or post-trauma intraocular inflammation, which may be for example (but not limited to) inflammation of postprocedural bleb.

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Another particularly preferred eye disease to be treated and/or prevented with the JNK inhibitors according to the invention is retinopathy. Non-limiting examples of retinopathy include diabetic retinopathy, hypertensive retinopathy (e.g., arterial hypertension induced), exudative retinopathy, radiation induced retinopathy, sun-induced solar retinopathy, trauma-induced retinopathy, e.g. Purtscher's retinopathy, retinopathy of prematurity (ROP) and/or hyperviscosity-related retinopathy, non-diabetic proliferative retinopathy, and/or proliferative vitreo-retinopathy. The JNK inhibitors as disclosed herein are particularly preferred for the treatment and/or prevention of diabetic retinopathy and retinopathy of prematurity, respectively.

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Retinopathy of prematurity (ROP), previously known as retrolental fibroplasia (RLF), is a disease of the eye affecting prematurely-born babies generally having received intensive neonatal care. It is thought to be caused by disorganized growth of retinal blood vessels which may result in scarring and retinal detachment. ROP can be mild and may resolve spontaneously, but it may lead to blindness in serious cases. As such, all preterm babies are at risk for ROP, and very low birth weight is an additional risk factor. Both oxygen toxicity and relative hypoxia can contribute to the development of ROP. The inventive molecules are suitable for treating ROP.

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Furthermore, the inventive molecules are particularly suitable to treat all forms of retinopathy, in particular diabetes mellitus induced retinopathy, arterial hypertension induced hypertensive retinopathy, radiation induced retinopathy (due to exposure to ionizing radiation), sun-induced solar

retinopathy (exposure to sunlight), trauma-induced retinopathy (e.g. Purtscher's retinopathy) and hyperviscosity-related retinopathy as seen in disorders which cause paraproteinemia).

In addition, the JNK inhibitors as disclosed herein are particularly useful to treat and/or prevent arthritis and related disease and/or disorders of joint. Arthritis is a form of joint disorder that involves inflammation of one or more joints. There are over 100 different forms of arthritis. The most common form, osteoarthritis (degenerative joint disease), is a result of trauma to the joint, infection of the joint, or age. Other arthritis forms are rheumatoid arthritis, psoriatic arthritis, and related autoimmune diseases. Septic arthritis is caused by joint infection. There are several diseases where joint pain is primary, and is considered the main feature. Generally when a person has "arthritis" it means that they have one of these diseases, which include osteoarthritis, rheumatoid arthritis, gout and pseudogout, septic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, Still's disease. Joint pain can also be a symptom of other diseases. In this case, the arthritis is considered to be secondary to the main disease; these include psoriasis (Psoriatic arthritis), reactive arthritis, Ehlers-Danlos Syndrome, haemochromatosis, hepatitis, Lyme disease, Sjogren's disease, Hashimoto's Thyroiditis, Inflammatory bowel disease (including Crohn's disease and ulcerative colitis), Henoch-Schönlein purpura, Hyperimmunoglobulinemia D with recurrent fever, Sarcoidosis, Whipple's disease, TNF receptor associated periodic syndrome, Wegener's granulomatosis (and many other vasculitis syndromes), Familial Mediterranean fever and sSystemic lupus erythematosus. An undifferentiated arthritis is an arthritis that does not fit into well-known clinical disease categories, possibly being an early stage of a definite rheumatic disease.

In particular, diseases and/or disorders arthritis relating to athritis, which may be treated and/or prevented with the JNK inhibitors as disclosed herein, can be selected from pyogenic arthritis, in particular staphylococcal arthritis and polyarthritis, pneumococcal arthritis and polyarthritis, other streptococcal arthritis and polyarthritis, and arthritis and polyarthritis due to other bacteria; direct infections of joint in infectious and parasitic diseases in general; postinfective and reactive arthropathies, in particular arthropathy following intestinal bypass, postdysenteric arthropathy, postimmunozation arthropathy, Reiter's disease, and other reactive arthropathies; inflammatory polyarthropathies, in particular rheumatoid arthritis with rheumatoid factor, for example Felty's syndrome, rheumatoid lung disease with rheumatoid arthritis, rheumatoid vasculitis with rheumatoid arthritis, rheumatoid heart disease with rheumatoid arthritis, rheumatoid myopathy with rheumatoid arthritis, rheumatoid polyneuropathy with rheumatoid arthritis, rheumatoid arthritis with involvement of other organs and systems, rheumatoid arthritis with rheumatoid factor without organ or systems involvement; other rheumatoid arthritis, for example rheumatoid arthritis without rheumatoid factor, Adult-onset Still's disease, rheumatoid bursitis, rheumatoid nodule, inflammatory polyarthropathy; enteropathic arthropathies; juvenile arthritis, for example unspecified juvenile

rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile rheumatoid arthritis with systemic onset, juvenile rheumatoid polyarthritis (seronegative), and pauciarticular juvenile rheumatoid arthritis; chronic gout, for example idiopathic chronic gout, lead-induced chronic gout, drug-induced chronic gout, chronic gout due to renal impairment; gout, for example idiopathic gout, lead-induced gout, drug-induced gout, gout due to renal impairment; other crystal arthropathies, for example familial and other chondrocalcinosis; other arthropathies for example Kaschin-Beck disease, Villonodular synovitis (pigmented), palindromic rheumatism, intermittent hydrarthrosis, traumatic arthropathy; other arthritis, for example polyarthritis and monoarthritis; other arthropathies, for example Charcôt's joint; osteoarthritis, in particular polyosteoarthritis, for example primary generalized (osteo)arthritis, Heberden's nodes, Bouchard's nodes, secondary multiple arthritis and erosive arthritis, osteoarthritis of the hip, osteoarthritis of the knee, osteoarthritis of first carpometacarpal joint, primary, secondary and post-traumatic osteoarthritis; and other joint disorders, in particular acquired deformities of fingers and toes, for example Mallet finger, Boutonniere deformity, swan-neck deformity, Hallux valgus, disorders of patella, internal derangement of knee, ankylosis of joint, protrusio acetabuli; and other joint disorders, for example hemarthrosis, fistula of joint, flail joint, and osteophyte.

A further class of inflammatory-associated diseases to be treated by the use of the inventive molecules is the following: acute disseminated encephalomyelitis, antisynthetase syndrome, autoimmune hepatitis, autoimmune peripheral neuropathy, pancreatitis, in particular autoimmune pancreatitis, Bickerstaff's encephalitis, Blau syndrome, Coeliac disease, Chagas disease, chronic inflammatory demyelinating polyneuropathy, osteomyelitis, in particular chronic recurrent multifocal osteomyelitis, Churg-Strauss syndrome, Cogan syndrome, giant-cell arteritis, CREST syndrome, vasculitis, in particular cutaneous small-vessel vasculitis or urticarial vasculitis, dermatitis, in particular dermatitis herpetiformis, dermatomyositis, systemic scleroderma, Dressler's syndrome, drug-induced lupus erythematosus, discoid lupus erythematosus, enthesitis, eosinophilic fasciitis, gastroenteritis, in particular, eosinophilic gastroenteritis, erythema nodosum, idiopathic pulmonary fibrosis, gastritis, Grave's disease, Guillain-barré syndrome, Hashimoto's thyroiditis, Henoch-Schönlein purpura, Hidradenitis suppurativa, idiopathic inflammatory demyelinating diseases, myositis, in particular inclusion body myositis, cystitis, Kawasaki disease, Lichen planus, lupoid hepatitis, Majeeb syndrome, Ménière's disease, Microscopic polyangiitis, mixed connective tissue disease, myelitis, in particular neuromyelitis, e.g. neuromyelitis optica, thyroiditis, in particular Ord's thyroiditis, rheumatism, in particular palindromic rheumatism, Parsonage-Turner syndrome, perivenous encephalomyelitis, polyarteritis nodosa, polymyalgia rheumatica, polymyositis, cirrhosis, in particular primary biliary cirrhosis, cholangitis, in particular primary sclerosing cholangitis, progressive inflammatory neuropathy, Rasmussen's encephalitis, chondritis,

in particular polychondritis, e.g. relapsing polychondritis, reactive arthritis (Reiter disease), rheumatic fever, sarcoidosis, Schnitzler syndrome, serum sickness, spondylitis, in particular ankylosing spondylitis, spondyloarthropathy, Takayasu's arteritis, Tolosa-Hunt syndrome, transverse myelitis, and granulomatosis, in particular Wegener's granulomatosis.

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In the most preferred embodiment of the present invention, the inventive molecules are used for the treatment of the following diseases or disorders: persistent or acute inflammatory diseases of the skin, in particular psoriasis, dry eye disease (Dry Eye Syndrome), uveitis, persistent or acute inflammatory diseases damaging the retina of the eye, retinopathy, in particular diabetic retinopathy or retinopathies caused by other diseases, age-related macular degeneration (AMD), in particular the wet or the dry form of age-related macular degeneration, retinopathy of prematurity (ROP), persistent or acute inflammatory diseases of the mouth, in particular peri-implantitis, pulpitis, periodontitis, anti-inflammatory treatment upon tissue or organ transplantation, in particular upon heart, kidney, and skin (tissue) transplantation, graft rejection upon heart, kidney or skin (tissue) transplantation, inflammatory brain diseases and/or tauopathies, in particular for the treatment of Alzheimer's disease in general, for example Alzheimer's disease with early onset, Alzheimer's disease with late onset, Alzheimer's dementia senile and presenile forms, metabolic disorders, diseases of the kidney, in particular glomerulonephritis and acute kidney injury, and arthrosis/arthritis, in particular reactive arthritis, rheumatoid arthrosis, juvenile idiopathic arthritis, and psoriatic arthritis.

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Thus, in a particularly preferred embodiment, the disorder/disease to be prevented and/or treated is a neurodegenerative disease, in particular tauopathies, preferably Alzheimer's disease, for example Alzheimer's disease with early onset, Alzheimer's disease with late onset, Alzheimer's dementia senile and presenile forms.

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder that leads to progressive cognitive decline with memory loss and dementia. Neuropathological lesions are characterized by extracellular deposition of senile plaques, formed by β -amyloid (A β) peptide, and intracellular neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau proteins (Duyckaerts et al., 2009, Acta Neuropathol 118: 5-36). According to the amyloid cascade hypothesis, neurodegeneration in AD could be linked to an abnormal amyloid precursor protein (APP) processing through the activity of the beta-site APP cleaving enzyme 1 (BACE1) and presenilin 1, leading to the production of toxic A β oligomers that accumulate in fibrillar A β peptides before forming A β plaques. A β accumulations can lead to synaptic dysfunction, altered kinase activities resulting in NFTs formation, neuronal loss and dementia (Hardy and Higgins, 1992, Science 256:

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184-5). AD pathogenesis is thus believed to be triggered by the accumulation of A β , whereby A β self-aggregates into oligomers, which can be of various sizes, and forms diffuse and neuritic plaques in the parenchyma and blood vessels. A β oligomers and plaques are potent synaptotoxins, block proteasome function, inhibit mitochondrial activity, alter intracellular Ca²⁺ levels and stimulate inflammatory processes. Loss of the normal physiological functions of A β is also thought to contribute to neuronal dysfunction. A β interacts with the signalling pathways that regulate the phosphorylation of the microtubule-associated protein tau. Hyperphosphorylation of tau disrupts its normal function in regulating axonal transport and leads to the accumulation of neurofibrillary tangles (NFTs) and toxic species of soluble tau. Furthermore, degradation of hyperphosphorylated tau by the proteasome is inhibited by the actions of A β . These two proteins and their associated signalling pathways therefore represent important therapeutic targets for AD.

C-Jun N-terminal kinases (JNKs) are serine-threonine protein kinases, coded by three genes JNK1, JNK2, and JNK3, expressed as ten different isoforms by mRNA alternative splicing, each isoforms being expressed as a short form (46 kDa) and a long form (54 kDa) (Davis, 2000, Cell 103: 239-52). While JNK1 and JNK2 are ubiquitous, JNK3 is mainly expressed in the brain (Kyriakis and Avruch, 2001, Physiol Rev 81: 807-69). JNKs are activated by phosphorylation (pJNK) through MAPKinase activation by extracellular stimuli, such as ultraviolet stress, cytokines and A β peptides and they have multiple functions including gene expression regulation, cell proliferation and apoptosis (Dhanasekaran and Reddy, 2008, Oncogene 27: 6245-51).

According to the present invention, it is assumed that the JNK inhibitors according to the present invention reduce tau hyperphosphorylation and, thus, neuronal loss. Therefore, the JNK inhibitors according to the present invention can be useful for treating and/or preventing tauopathies. Tauopathies are a class of neurodegenerative diseases associated with the pathological aggregation of tau protein in the human brain. The best-known tauopathy is Alzheimer's disease (AD), wherein tau protein is deposited within neurons in the form of neurofibrillary tangles (NFTs), which are formed by hyperphosphorylation of tau protein. The degree of NFT involvement in AD is defined by Braak stages. Braak stages I and II are used when NFT involvement is confined mainly to the transentorhinal region of the brain, stages III and IV when there is also involvement of limbic regions such as the hippocampus, and V and VI when there is extensive neocortical involvement. This should not be confused with the degree of senile plaque involvement, which progresses differently. Thus, the JNK inhibitors can be used according to the present invention for treating and/or preventing tauopathies, in particular Alzheimer's disease with NFT involvement, for example AD with Braak stage I, AD with Braak stage II, AD with Braak stage III, AD with Braak stage IV and/or AD with Braak stage V.

Further tauopathies, i.e. conditions in which neurofibrillary tangles (NFTs) are commonly observed, and which can thus be treated and/or prevented by the JNK inhibitors according to the present invention, include progressive supranuclear palsy although with straight filament rather than PHF (paired helical filaments) tau; dementia pugilistica (chronic traumatic encephalopathy); frontotemporal dementia and parkinsonism linked to chromosome 17, however without detectable β -amyloid plaques; Lytico-Bodig disease (Parkinson-dementia complex of Guam); tangle-predominant dementia, with NFTs similar to AD, but without plaques; ganglioglioma and gangliocytoma; meningoangiomatosis; subacute sclerosing panencephalitis; and/or lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, and lipofuscinosis. Further tauopathies, which can be treated and/or prevented by the JNK inhibitors according to the present invention, include Pick's disease; corticobasal degeneration; Argyrophilic grain disease (AGD); frontotemporal dementia and frontotemporal lobar degeneration. In Pick's disease and corticobasal degeneration tau proteins are deposited in the form of inclusion bodies within swollen or "ballooned" neurons. Argyrophilic grain disease (AGD), another type of dementia, which is sometimes considered as a type of Alzheimer disease and which may co-exist with other tauopathies such as progressive supranuclear palsy, corticobasal degeneration, and also Pick's disease, is marked by the presence of abundant argyrophilic grains and coiled bodies on microscopic examination of brain tissue. The non-Alzheimer's tauopathies are sometimes grouped together as "Pick's complex".

It is also preferred according to the present invention, that the disorder/disease to be prevented and/or treated by the JNK inhibitor according to the present invention is Mild Cognitive Impairment (MCI), in particular MCI due to Alzheimer's Disease. Typically, Mild Cognitive Impairment (MCI) is different from Alzheimer's Disease, i.e. Mild Cognitive Impairment (MCI) is typically not Alzheimer's Disease, but is a disease on its own classified by ICD-10 in F06.7. In ICD-10 (F06.7), MCI is described as a disorder characterized by impairment of memory, learning difficulties, and reduced ability to concentrate on a task for more than brief periods. There is often a marked feeling of mental fatigue when mental tasks are attempted, and new learning is found to be subjectively difficult even when objectively successful. None of these symptoms is so severe that a diagnosis of either dementia (F00-F03) or delirium (F05.-) can be made. The disorder may precede, accompany, or follow a wide variety of infections and physical disorders, both cerebral and systemic, but direct evidence of cerebral involvement is not necessarily present. It can be differentiated from postencephalitic syndrome (F07.1) and postconcussional syndrome (F07.2) by its different etiology, more restricted range of generally milder symptoms, and usually shorter duration. Mild cognitive impairment (MCI), in particular MCI due to Alzheimer's Disease, causes a slight but noticeable and measurable decline in cognitive abilities, including memory and thinking skills. MCI involves the onset and evolution

of cognitive impairments whatever type beyond those expected based on the age and education of the individual, but which are not significant enough to interfere with their daily activities. The diagnosis of MCI is described for example by Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease; *Alzheimers Dement.*;7(3):270-9. MCI may be at the onset of whatever type of dementia or represents an ephemerical form of cognitive impairment which may disappear over time without resulting in a clinical manifestation of dementia. A person with MCI is at an increased risk of developing Alzheimer's or another dementia, in particular at an increased risk of developing Alzheimer's Disease, without however necessarily developing dementia, in particular Alzheimer's Disease. No medications are currently approved by the U.S. Food and Drug Administration (FDA) to treat Mild Cognitive Impairment. Drugs approved to treat symptoms of Alzheimer's Disease have not shown any lasting benefit in delaying or preventing progression of MCI to dementia.

The JNK inhibitors of the present invention may also be used for the treatment of diseases and/or disorders of the urinary system in particular selected from ureteritis; urinary tract infection (bladder infection, acute cystitis); cystitis in general, in particular interstitial cystitis, Hunner's ulcer, trigonitis and/or hemorrhagic cystitis; urethritis, in particular nongonococcal urethritis or gonococcal urethritis; painful bladder syndrome; IC/PBS; urethral syndrome; and/or retroperitoneal fibrosis, preferably cystitis in general, in particular interstitial cystitis. In this context it is noted that interstitial cystitis (IC) varies very much in symptoms and severity and, thus, most researchers believe it is not one, but several diseases. In recent years, scientists have started to use the terms "bladder pain syndrome" (BPS) or "painful bladder syndrome" (PBS) to describe cases with painful urinary symptoms that may not meet the strictest definition of IC. The term "IC/PBS" includes all cases of urinary pain that can't be attributed to other causes, such as infection or urinary stones. The term interstitial cystitis, or IC, is typically used alone when describing cases that meet all of the IC criteria, for example as established by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

The JNK inhibitors of the present invention may also be used for the treatment of metabolic disorders, for example for the treatment of diabetes (type 1 or type 2, in particular type 1), Fabry disease, Gaucher disease, hypothermia, hyperthermia hypoxia, lipid histiocytosis, lipidoses, metachromatic leukodystrophy, mucopolysaccharidosis, Niemann-Pick disease, obesity, and Wolman's disease. More generally, metabolic disorders may be of hereditary form or may be acquired disorders of

carbohydrate metabolism, e.g., glycogen storage disease, disorders of amino acid metabolism, e.g., phenylketonuria, maple syrup urine disease, glutaric acidemia type 1, Urea Cycle Disorder or Urea Cycle Defects, e.g., Carbamoyl phosphate synthetase I deficiency, disorders of organic acid metabolism (organic acidurias), e.g., alcaptonuria, disorders of fatty acid oxidation and mitochondrial metabolism, e.g., Medium-chain acyl-coenzyme A dehydrogenase deficiency (often shortened to MCADD.), disorders of porphyrin metabolism, e.g. acute intermittent porphyria, disorders of purine or pyrimidine metabolism, e.g., Lesch-Nyhan syndrome, Disorders of steroid metabolism, e.g., lipoid congenital adrenal hyperplasia, or congenital adrenal hyperplasia, disorders of mitochondrial function, e.g., Kearns-Sayre syndrome, disorders of peroxisomal function. e.g., Zellweger syndrome, or Lysosomal storage disorders, e.g., Gaucher's disease or Niemann Pick disease.

The JNK inhibitors of the present invention may also be used for the treatment of neoplasms in particular cancer (malignant neoplasms) and/or tumor diseases, in particular selected from solid tumors in general; hematologic tumors in general, in particular leukemia, for example acute lymphocytic leukemia (L1, L2, L3), acute lymphoid leukaemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukaemia (CLL), chronic myeloid leukaemia (CML), promyelocytic leukemia (M3), monocytic leukemia, myeloblastic leukemia (M1), myeloblastic leukemia (M2), megakaryoblastic leukemia (M7) and myelomonocytic leukemia (M4); myeloma, for example multiple myeloma; lymphomas, for example non-Hodgkin's lymphomas, mycosis fungoides, Burkitt's lymphoma, and Hodgkin's syndrome; pancreatic cancer, in particular pancreatic carcinoma; ovarian cancer, in particular ovarian carcinoma; liver cancer and liver carcinoma in general, in particular liver metastases, liver cell carcinoma, hepatocellular carcinoma, hepatoma, intrahepatic bile duct carcinoma, cholangiocarcinoma, hepatoblastoma, angiosarcoma (of liver), and other specified or unspecified sarcomas and carcinomas of the liver; skin cancer; melanoma, in particular malignant melanoma; squamous cell carcinoma; glioblastoma; colon cancer and colon carcinoma in general, in particular cecum carcinoma, appendix carcinoma, ascending colon carcinoma, hepatic flexure carcinoma, transverse colon carcinoma, splenic flexure carcinoma, descending colon carcinoma, sigmoid colon carcinoma, carcinoma of overlapping sites of colon and/or malignant carcinoid tumors of the colon; prostate cancer and prostate tumours, in particular prostate carcinoma; and further cancer and/or tumor diseases, in particular selected from acoustic neuroma lung carcinomas; adenocarcinomas; anal carcinoma; bronchial carcinoma; cervix carcinoma; cervical cancer; astrocytoma; basalioma; cancer with Bcr-Abl transformation; bladder cancer; blastomas; bone cancer; brain metastases; brain tumours; breast cancer; carcinoids; cervical cancer; corpus carcinoma; craniopharyngeomas; CUP syndrome; virus-induced tumours; EBV-induced B cell lymphoma; endometrium carcinoma; erythroleukemia (M6); esophagus cancer;

gallbladder cancer; gastrointestinal cancer; gastrointestinal stromal tumors; gastrointestinal tumours; genitourinary cancer; glaucoma; gliomas; head/neck tumours; hepatitis B-induced tumours; hepatocell or hepatocellular carcinomas; hepatocarcinomas; hepatomas; herpes virus-induced tumours; HTLV-1-induced lymphomas; HTLV-2-induced lymphomas; insulinomas; intestinal cancer; Kaposi's sarcoma; kidney cancer; kidney carcinomas; laryngeal cancer; leukemia; lid tumour; lung cancer; lymphoid cancer; mammary carcinomas; mantle cell lymphoma; neurinoma; medulloblastoma; meningioma; mesothelioma; non-small cell carcinoma; non-small cell carcinoma of the lung; oesophageal cancer; oesophageal carcinoma; oligodendroglioma; papilloma virus-induced carcinomas; penis cancer; pituitary tumour; plasmocytoma; rectal tumours; rectum carcinoma; renal-cell carcinoma; retinoblastoma; sarcomas; Schneeberger's disease; small cell lung carcinomas; small intestine cancer; small intestine tumours; soft tissue tumours; spinalioma; squamous cell carcinoma; stomach cancer; testicular cancer; throat cancer; thymoma; thyroid cancer; thyroid carcinoma; tongue cancer; undifferentiated AML (MO); urethral cancer; uterine cancer; vaginal cancer; Von Hippel Lindau disease; vulval cancer; Wilms' Tumor; Xeroderma pigmentosum.

A person skilled in the art will readily realize that the above mentioned disease states and disorders may belong to more than one of the above mentioned disease classes. For example, bronchial carcinoma is certainly not only a proliferative disease but would also belong in the group of diseases of the respiratory system including lung diseases. Thus, the above mentioned classification of individual diseases is not considered to be limiting or concluding but is considered to of exemplary nature only. It does not preclude that individual disease states recited in one class are factually also suitable examples for the application of the JNK inhibitors of the present invention as treatment in another class of disease states. A person skilled in the art will readily be capable of assigning the different disease states and disorders to matching classifications.

Finally, as mentioned above, the present invention contemplates the use of a JNK inhibitor as defined herein for the treatment and/or prevention of various diseases states and disorders. The present invention does not contemplate to use the JNK inhibitors as defined herein for immunizing non-human animals, e.g. for the production of monoclonal antibodies. Such methods are herein not considered to be methods for treatment of the animal body by therapy.

Tissue and organ transplantation

According to another aspect the present invention provides a JNK inhibitor as defined herein for the (in vitro) treatment of a tissue or organ transplant prior to its transplantation. The term "prior to its transplantation" comprises the time of isolation and the time of perfusion/transport. Thus, the treatment of a tissue or organ transplant "prior to its transplantation" refers for example to treatment during the isolation and/or during perfusion and/or during transport. In particular, a solution used for isolation of of a tissue or organ transplant as well as a solution used for perfusion, transport and/or otherwise treatment of a tissue or organ transplant can preferably contain the JNK inhibitor according to the invention.

In transplantation the tolerable cold ischemia time (CIT) and the tolerable warm ischemia time (WIT) play critical roles. CIT is the length of time that elapses between an organ being removed from the donor, in particular the time of perfusion/treatment of an organ by cold solutions, to its transplantation into the recipient. WIT is in general a term used to describe ischemia of cells and tissues under normothermic conditions. In particular WIT refers to the length of time that elapses between a donor's death, in particular from the time of cross-clamping or of asystole in non-heart-beating donors, until cold perfusion is commenced. Additionally, WIT may also refer to ischemia during implantation, from removal of the organ from ice until reperfusion. In allotransplantation usually, a transplant originating from a brain-dead donor is typically not subjected to WIT, but has 8-12 hrs of CIT (time needed for transportation from the procurement hospital to the isolation lab), whereas a transplant from a non-heart beating donor is typically exposed to a longer WIT and also 8-12 hrs of CIT. However, such transplantation is currently not used routinely because of concerns about damage due to the WIT. In autotransplantation, WIT may occur, however, CIT is usually limited (typically 1 – 2 hrs, for example in islet autotransplantation in patients with chronic pancreatitis).

Depending on the donor, the organ and/or tissue is not perfused with blood for a variable amount of time prior to its transplantation, leading to ischemia. Ischemia is an inevitable event accompanying transplantation, for example kidney transplantation. Ischemic changes start with brain death, which is associated with severe hemodynamic disturbances: increasing intracranial pressure results in bradycardia and decreased cardiac output; the Cushing reflex causes tachycardia and increased blood pressure; and after a short period of stabilization, systemic vascular resistance declines with hypotension leading to cardiac arrest. Free radical-mediated injury releases proinflammatory cytokines and activates innate immunity. It has been suggested that all of these changes-the early innate response and the ischemic tissue damage play roles in the development of adaptive responses, which in turn may lead to transplant rejection. Hypothermic storage of the organ and/or tissue of various durations before transplantation add to ischemic tissue damage. The final

stage of ischemic injury occurs during reperfusion. Reperfusion injury, the effector phase of ischemic injury, develops hours or days after the initial insult. Repair and regeneration processes occur together with cellular apoptosis, autophagy, and necrosis; the fate of the organ depends on whether cell death or regeneration prevails. The whole process has been described as the ischemia-reperfusion (I-R) injury. It has a profound influence on not only the early but also the late function of a transplanted organ or tissue. Prevention of I-R injury can thus already be started before organ recovery by donor pretreatment.

It was found that transplants may be (pre-)treated by the JNK inhibitors according to the present invention in order to improve their viability and functionality until transplanted to the host. For that aspect of the invention, the transplant is in particular a kidney, heart, lung, pancreas, in particular pancreatic islets (also called islets of Langerhans), liver, blood cell, bone marrow, cornea, accidental severed limb, in particular fingers, hand, foot, face, nose, bone, cardiac valve, blood vessel or intestine transplant, preferably a kidney, heart, pancreas, in particular pancreatic islets (also called islets of Langerhans), or skin transplant.

Moreover, in a further aspect, the present invention provides a JNK inhibitor as defined herein for the treatment of a tissue or organ transplant, or an animal or human who received a tissue or organ transplant during or after transplantation. The term "after transplantation" refers in particular to reperfusion of the organ or tissue, for example a kidney, whereby reperfusion begins for example by unclamping the respective blood flow. The treatment with a JNK inhibitor according to the present invention after transplantation refers in particular to the time interval of up to four hours after reperfusion, preferably up to two hours after reperfusion, more preferably up to one hour after reperfusion and/or at the day(s) subsequent to transplantation. For the treatment after transplantation, for example after kidney transplantation, the JNK inhibitor according to the present invention may be administered for example to an animal or human who received a tissue or organ transplant as pharmaceutical composition as described herein, for example systemically, in particular intravenously, in a dose in the range of 0.01 – 10 mg/kg, preferably in the range of 0.1 – 5 mg/kg, more preferably in the range of 0.5 – 2 mg/kg at a single dose or repeated doses.

For that aspect of the invention, the transplant is in particular a kidney, heart, lung, pancreas, in particular pancreatic islets (also called islets of Langerhans), liver, blood cell, bone marrow, cornea, accidental severed limb, in particular fingers, hand, foot, face, nose, bone, cardiac valve, blood vessel or intestine transplant, preferably a kidney, heart, pancreas, in particular pancreatic islets (also called islets of Langerhans), or skin transplant.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such
5 modifications fall within the scope of the appended claims.

All references cited herein are herewith incorporated by reference.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as
10 commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated
15 by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Examples

20 In the following, particular examples illustrating various embodiments and aspects of the invention are presented. However, the present invention shall not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become readily apparent to those skilled in the art from the foregoing description, accompanying figures and the examples below. All such modifications fall within the
25 scope of the appended claims.

Example 1: Synthesis of JNK inhibitor SEQ ID NO: 172

As illustrative example, synthesis of the JNK inhibitor with SEQ ID NO: 172 is set out below. A person skilled in the art will know that said synthesis may also be used for and easily adapted to the
30 synthesis of any other JNK inhibitor according to the present invention.

The JNK inhibitor with SEQ ID NO: 172 was manufactured by solid-phase peptide synthesis using the Fmoc (9-fluorenylmethyloxycarbonyl) strategy. The linker between the peptide and the resin was the Rink amide linker (p-[Fmoc-2,3-dimethoxybenzyl]-phenoxyacetic acid). The peptide was
35 synthesized by successive Fmoc deprotection and Fmoc-amino acid coupling cycles. At the end of the synthesis, the completed peptide was cleaved by trifluoroacetic acid (TFA) directly to yield the

crude C-terminal amide, which was then purified by preparative reverse phase HPLC. The purified fractions were pooled in a homogeneous batch that is treated by ion exchange chromatography to obtain its acetate salt. The peptide was then freeze-dried.

5 1.1 Solid Phase Synthesis of the Peptide

Except when noted, the manufacturing took place at room temperature ($22^{\circ}\text{C} \pm 7^{\circ}\text{C}$) in an air-filtered environment. The scale of synthesis was 0.7 mmoles of the starting amino acid on the resin, for an expected yield of about 1g of purified peptide. Synthesis was performed manually in a 30–50 mL reactor equipped with a fritted disk with mechanical stirring and/or nitrogen bubbling.

10

1.2 Preparation of the resin

The p-methylbenzhydrylamide resin (MBHA-resin) was first washed with dichloromethane/dimethylformamide/diisopropylethylamine under nitrogen. The washed resin was then coupled to the Rink amide linker (p-[Fmoc-2,4-dimethoxybenzyl]-phenoxyacetic acid) in PyBOB(benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate)/ diisopropyl-ethylamine/1-hydroxybenzotriazole to yield Fmoc-Rink amide-MBHA resin.

15

1.3 Coupling of Amino Acids

Amino acids were coupled to the resin using the following cycle:

20 The Fmoc-Rink amide-MBHA resin was deprotected by washing it in 35% (v/v) piperidine/dimethylformamide, followed by dimethylformamide. The deprotection reaction took approximately 16 minutes. Fmoc-protected amino acids (e.g., 2 eq of amino acid and HOBt (1-hydroxybenzotriazole) in dimethylformamide/dichloromethane (50/50) were added to the resin followed by addition of 2 eq of the coupling agent diisopropylcarbodiimide (DIC). The coupling
25 reaction took from one hour to overnight depending upon the respective amino acid being added. Volumes were calculated on a basis of 0.5 mL/100mg of peptide-resin and adjusted after each cycle. After coupling, the resin was washed 3 times with DMF. Completeness of coupling was tested by the ninhydrin test (or Kaiser test 1) on primary amines and the chloranil test 2 on secondary amines. On some occasions, the chloranil test may be associated with a ninhydrin test as a security control.
30 In case the coupling test indicated incompleteness of reaction, coupling was repeated with a lower excess (0.5-1 eq) of amino acid, PYBOP, HOBt in dimethylformamide/dichloromethane and diisopropylethylamine. Functionality of the resin was measured and generally 0.6–0.2 meq/g, depending on the original loading of the resin. After the last amino acid has been coupled, the peptide-resin was deprotected as usual and then washed 5 times with DCM before drying in an oven
35 under vacuum at 30°C . After the peptide-resin had dried, the yield of the solid-phase synthesis was

calculated as the ratio of the weight increase of the peptide resin compared to the theoretical weight increase calculated from the initial loading of the resin. The yield may be close to 100%.

1.4 Cleavage And Deprotection

- 5 The peptide was cleaved from the resin in a mixture of trifluoroacetic acid/1,2-ethanedthiol/thioanisole/water/phenol (88/2.2/4.4/4.4/7 v/v), also called TFA/K reagent, for 4 hours at room temperature. The reaction volume was 1mL/100mg of peptide resin. During addition of the resin to the reagent, the mixture temperature was regulated to stay below 30°C.

10 1.5 Extraction of the peptide from the resin:

The peptide was extracted from the resin by filtration through a fritted disc. After concentration on a rotavapor to 1/3 of its volume, the peptide was precipitated by cold t-butyl methyl ether and filtered. The crude peptide was then dried under vacuum at 30°C.

15 1.6 Preparative HPLC Purification:

The crude peptide was then purified by reverse-phase HPLC to a purity of $\geq 95\%$. The purified fractions were concentrated on a rotavaporator and freeze-dried.

1.7 Ion Exchange Chromatography

- 20 The concentrated freeze-dried pools of purified peptide with the sequence of SEQ ID NO: 172 was dissolved in water and purified by ion exchange chromatography on Dowex acetate, 50–100 mesh resin.

25

The required starting reagents for the synthesis were :

	CAS Registry Number	Chemical Name	Molecular Weight
Fmoc-Rink amide linker	145069-56-3	p-[Fmoc-2,4-dimethoxybenzyl]-phenoxyacetic acid	539.6
Fmoc-D-Ala-OH, H ₂ O	79990-15-1	N-alpha-Fmoc-D-alanine	311.3

Fmoc-Arg(Pbf)-OH	154445-77-9	N-alpha-Fmoc-N [2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl]-arginine	648.8
Fmoc-D-Arg(Pbf)-OH	187618-60-6	N-alpha-Fmoc-N [2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl]-D-arginine	648.8
Fmoc-Asn(Trt)-OH	132388-59-1	N-alpha-Fmoc-N-beta-trityl-asparagine	596.7
Fmoc-Gln(Trt)-OH	132327-80-1	N-alpha-Fmoc-N-delta-trityl-glutamine	610.7
Fmoc-Leu-OH	35661-60-0	N-alpha-Fmoc-leucine	353.4
Fmoc-Lys(Boc)-OH	71989-26-9	N-alpha-Fmoc-N epsilon-Boc-L-lysine	468.5
Fmoc-D-Lys(Boc)-OH	143824-78-6	N-alpha-Fmoc-N epsilon-Boc-D-lysine	468.5
Fmoc-D-Phe-OH	86123-10-6	N-alpha-Fmoc-D-phenylalanine	387.4
Fmoc-Pro-OH	71989-31-6	N-alpha-Fmoc-proline	337.4
Fmoc-Thr(tBu)-OH	71989-35-0	N-alpha-Fmoc-O-t-butyl-threonine	397.5

Other JNK inhibitors of the present invention may be prepared in similar manner.

5

Example 2: Inhibitory efficacy of selected JNK inhibitors according to the present invention

10

In the following a standard operating procedure will be set forth describing how the Inhibitory efficacy of JNK inhibitors according to the present invention was measured. The method allows to measure in vitro, in a non radioactive standardized assay, the ability of a candidate compound to decrease the phosphorylation of the c-Jun specific substrate by JNK. Moreover, it will be illustrated how to determine the inhibitory effect (IC50) and the Ki of a chosen compound for JNK. The method is suitable to verify whether a candidate compound does or does not inhibit JNK activity. And a

person skilled in the art will certainly understand how to adapt the below methods for his specific purposes and needs.

2.1 Material

AlphaScreen reagent and plate:

- His-JNK1 (ref 14-327, Upstate, 10 µg in 100 µl: concentration: 2.2 µM) 5nM final
- His-JNK2 (ref 14-329, Upstate, 10 µg in 100 µl: concentration: 2 µM) 5nM final
- His-JNK3 (ref 14-501, Upstate, 10 µg in 100 µl: concentration: 1.88 µM) 5nM final
- Anti-Phospho-cJun (ref 06-828, Upstate, lot DAM1503356, concentration: 44.5 µM) 10nM final
- Biotin-cJun (29-67):
sequence: Biotin – SNPKILKQSM TLNLADPVGSLKPHLR AKNSDLLTSPDVG (SEQ ID NO: 198), lot 100509 (mw 4382.11, P 99.28%) dissolved in H₂O, concentration: 10 mM) 30nM final
- ATP (ref AS001A, Invitrogen, lot 50860B, concentration 100 mM)) 5 µM final
- SAD beads (ref 6760617M, PerkinElmer, lot 540-460-A, concentration 5mg/ml) 20 µg/ml final
- AprotA beads (ref 6760617M, PerkinElmer, lot 540-460-A, concentration 5mg/ml) 20 µg/ml final
- Optiplate 384well white plate (ref 6007299, PerkinElmer, lot 654280/2008)
- 96well plate for peptide dilution (ref 82.1581, Sarstedt)
- TopSeals-A (ref 6005185, Perkin Elmer, Lot 65673)
- Bioluminescent energy transfer reading
- The bioluminescent energy transfer was read on the Fusion Alpha Plate reader (Perkin Elmer).

Pipette:

- An electronic EDP3 pipette 20-300 (Ref 17007243; Rainin) was used to fill in the plate with the Enzyme-Antibody mix, the Substrate-ATP mix and the Beads.
- A PIPETMAN® Ultra multichannel 8X20 (Ref 21040; Gilson) was used to fill in the plate with the inhibitory compounds.

Buffer and solutions

- Kinase Buffer: 20mM Tris-base pH 7.4, 10mM MgCl₂, 1mM DTT, 100μM Na₃VO₄, 0.01% Tween, (1% DMSO)
- Stop Buffer: 20mM Tris-base pH 7.4, 200mM NaCl, 80mM EDTA-K (pH de 8 with KOH instead of NaOH), 0.3% BSA
- JNK dilution Kinase buffer: 50mM Tris-base pH 7.4, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.1% β-mercaptoethanol.

2.2 Method

To assess inhibitory effect of the peptides, a standard AlphaScreen assay (see for example Guenat et al. J Biomol Screen, 2006; 11: pages 1015-1026) was performed. The different components were prepared and subsequently mixed as indicated. The plates were sealed and incubated as following:

5 μl	JNK + Antibody	
5 μl	TP kinase + / - inhibitor	Pre-incubation 30 min
5 μl	Biotin-cJun + ATP	Incubation 60 min at 24°C
10 μl	Beads SAD + A protA	Incubation 60 min in the dark at 24°C

To avoid contamination, the mixes were added with the pipette in different corner of the well. After the filling in of the plate with each mix, the plate was tapped (Keep one side fix and let the opposite side tap the table) to let the mix go down the walls of the wells.

The bioluminescent energy transfer was read on the Fusion Alpha Plate reader (Perkin Elmer).

All compounds should at least be tested in triplicate in 3 independent experiments for each isoform of JNK. Possibly concentrations of the compounds to be tested were 0, 0.03 nM, 0.1 nM, 0.3 nM, 1 nM, 3 nM, 10 nM, 30 nM, 100 nM, 300 nM, 1 μM, 3 μM, 10 μM, 30 μM, and 100 μM. Controls were samples either without JNK or without substrate (c-Jun).

Mix preparation

JNK1, JNK2 and JNK3 5nM

Biotin-cJun 30 nM

ATP 5 μM; Anti phospho-cJun (S63) 10nM

Bille SAD/AprotA 20 μg/ml

Antibody [final] = 10nM (anti Phospho cJun (S63))

Detection part : [Mix] X5 (5 μl in final volume of 25 μl)

[Stock] = 44.5 μM (ref 06-828, Upstate, Lot DAM1503356)

10 nM → 50nM in Kinase Buffer

JNK1, JNK2 and JNK3 [final] = 5nM

Reaction part : [Mix] X3 (5 µl in final volume of 15 µl)

- 5 [Stock] = 2.2 µM for JNK1 (ref 14-327, Upstate, lot D7KN022CU)
 2.0 µM for JNK2 (ref 14-329, Upstate, lot 33221CU)
 1.88 µM for JNK3 (ref 14-501, Upstate, lot D7CN041CU)
 5 nM → 15nM in Antibody Buffer

10 Inhibitor:

Reaction part : [Mix] X3 (5 µl in final volume of 15 µl)

[Stock] = 10 mM

- 100 µM → 300 µM in Kinase Buffer
 30 µM → 90 µM in Kinase Buffer
 15 10 µM → 30 µM in Kinase Buffer
 ...
 0.03 nM → 0.09 nM in Kinase Buffer
 And 0 nM → Kinase Buffer

- 20 Two series of 10 times serial dilutions were performed in a 96 well plate, one beginning with 300 µM to 0 nM, the second with 90 µM to 0.03 nM. The peptides are added in the 384 plates with an 8 channels multipipette (ref F14401, Gilson, 8X20).

ATP [final] = 5 µM

- 25 Reaction part : [Mix] X3 (5 µl in final volume of 15 µl)
 [Stock] = 100 mM (ref AS001A, Invitrogen, lot 50860B)
 5 µM → 15 µM in Kinase Buffer

Biotin c-Jun [final] = 30nM

- 30 Reaction part : [Mix] X3 (5 µl in final volume of 15 µl)
 [Stock] = 10 mM
 30 nM → 30nM in ATP Buffer

Beads SAD / A ProtA [final] = 20 µg/ml (Light sensitive)

- 35 Detection part : [Mix] X 2.5 (10 µl in final volume of 25 µl)
 [Stock] = 5 mg/ml → 20 µg/ml 50 µg/ml in STOP Buffer

Mix in the dark room (green Light) or in the darkness.

Analysis of the IC50 curves:

The analysis was performed by the GraphPad Prism4 software with the following equation:

5 Sigmoidal dose-response (No constraint).

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC50} - X))})$$

The outliers data were avoided using Grugg's test.

10 Comparison of the IC50:

The analysis was performed by the GraphPad Prism4 software with the following test: One way ANOVA test followed by a Tukey's Multiple Comparison Test. $P < 0.05$ was considerate as significant.

15 The Km of the ATP for JNK and the Km of biotin-cJun specific peptide were determined in the report AlphaScreen standardization assay

The mathematical relation between Ki and IC50 ($K_i = \text{IC}_{50} / (1 + ([\text{Substrate}] / K_m \text{ of the substrate}))$) may be used to calculate the Ki values.

20

Example 3: Internalization experiments and analysis

3.1 Materials and Methods for uptake experiments

a) Cell line:

25 The cell line used for this experiment was HL-60 (Ref CCL-240, ATCC, Lot 116523)

b) Culture medium and plates

RPMI (Ref 21875-091, Invitrogen, Lot 8296) or DMEM (Ref 41965, Invitrogen, Lot 13481) complemented with:

30 10% FBS (Ref A64906-0098, PAA, Lot A15-151): decomplexed at 56°C, 30 min, on 04.04.2008.

1mM Sodium Pyruvate (Ref S8636, Sigma, Lot 56K2386)

Penicillin (100 unit/ml)/Streptomycin (100µg/ml) (Ref P4333, Sigma, Lot 106K2321)

35 PBS 10X (Ref 70011, Invitrogen, Lot 8277): diluted to 1X with sterile H₂O

Trypsine-0.05% EDTA (Ref L-11660, PAA, Lot L66007-1194)

6 well culture plates (Ref 140675, Nunc, Lot 102613)

24 well culture plates (Ref 142475, Nunc, Lot 095849)

5 96 well culture plates (Ref 167008, Nunc, Lot 083310)

96 well plates for protein dosing (Ref 82.1581, Sarstedt)

96 well plates for fluorescence measurement (Ref 6005279, Perkin Elmer)

10

c) **Solutions**

Poly-D-lysine coating solution (Sigma P9011 Lot 095K5104): 25µg/ml final diluted in PBS 1x

15 Acidic wash buffer: 0.2M Glycin, 0.15M NaCl, pH 3.0

Ripa lysis buffer: 10mM NaH₂PO₄ pH 7.2, 150mM NaCl, 1% Triton X-100, 1mM EDTA pH 8.0, 200µM Na₃VO₂, 0.1% SDS, 1X protease inhibitor cocktail (Ref 11873580001, Roche, Lot 13732700)

20

d) **Microscopy and fluorescence plate reader**

Cells were observed and counted using an inverted microscope (Axiovert 40 CFL; Zeiss; 20X).

The fluorescence was read with the Fusion Alpha Plate reader (Perkin Elmer).

25

e) **Method**

FITC marked peptide internalization was studied on suspension cells. Cells were plated into poly-DL-lysine coated dishes at a concentration of 1×10^6 cells/ml. Plates were then incubated for 24 h at 37 °C, 5 % CO₂ and 100% relative humidity prior to the addition of a known concentration of peptide. After peptide addition, the cells were incubated 30 min, 1, 6 or 24 h at 37 °C, 5 % CO₂ and 100 % relative humidity. Cells were then washed twice with an acidic buffer (Glycin 0.2 M, NaCl 0.15 M, pH 3.0) in order to remove the cell-surface adsorbed peptide (see Kameyama et al., (2007), *Biopolymers*, **88**, 98-107). The acidic buffer was used as peptides rich in basic amino acids adsorb strongly on the cell surfaces, which often results in overestimation of internalized peptide. The cell wash using an acidic buffer was thus employed to remove the cell-surface adsorbed peptides.

30

35

The acid wash was carried out in determining cellular uptake of Fab/cell-permeating peptide conjugates, followed by two PBS washes. Cells were broken by the addition of the RIPA lysis buffer. The relative amount of internalized peptide was then determined by fluorescence after background subtraction and protein content normalization.

- 5 The steps are thus:
1. Cell culture
 2. Acidic wash and cellular extracts
 3. Analysis of peptide internalization with a fluorescence plate reader

10 f) Cell culture and peptide treatment

The 6 well culture plates are coated with 3 ml of Poly-D-Lys (Sigma P9011; 25 µg/ml in PBS), the 24 well plates with 600 µl and the 96 well plates with 125 µl and incubated for 4 h at 37°C, CO₂ 5 % and 100 % relative humidity.

After 4 hours the dishes were washed twice with 3.5ml PBS, 700 µl or 150 µl PBS for the 6, 24 or 15 96 well plates, respectively.

The cells were plated into the dishes in 2.4 ml medium (RPMI) at plating densities of 1'000'000 cells/ml for suspension cells. After inoculation, the plates were incubated at 37°C, 5 % CO₂ and 100 % relative humidity for 24 hours prior to the addition of the peptide. Adherent cells should be at a density of 90-95% the day of treatment and were plated in DMEM :

20

well	Surface of culture (cm ²)	Medium	Nb adherent cells	Nb suspension cells
96 well	0.3	100 – 200 µl	8'000 - 30'000	100'000
24 well	2	500 – 1000 µl	100'000 - 200'000	500'000-1'000'000
35mm (P35) / 6 well	10	2,4 ml	250'000 - 2'100'000	2'400'000
60mm (P60)	20	3,5 ml	15 * 10 ⁵	1'000'000/ml
10cm (P100)	60	10 ml	15-60 * 10 ⁵	

The cells were treated with the desired concentration of FITC labeled peptide (stock solution at a concentration of 10 mM in H₂O).

Following peptide addition, the cells were incubated 0 to 24 hours (e.g. 30 min, 1, 6 or 24 hours) at 37 °C, CO₂ 5 % and 100 % relative humidity.

25

Acidic wash and cellular extracts :

The extracts were cooled on ice.

Suspension cells (or cells, which don't attach well to the dish):

Transfer the cells in « Falcon 15 ml ». To recover the maximum of cells, wash the dish with 1 ml of PBS.

Harvest the cells 2 min at 2400 rpm max.

- 5 Suspend the cells in 1 ml cold PBS.

Transfer the cells into a coated "Eppendorf tube" (coated with 1ml of poly D-Lys for 4 hours and washed twice with 1ml PBS).

Wash three times with 1 ml of cold acidic wash buffer and centrifuge 2 min at 2400 rpm max.

Beware of the spreading of the cells in the "eppendorf".

- 10 Wash twice with 1 ml cold PBS to neutralize.

Add 50 µl of lysis RIPA Buffer.

Incubate 30 min-1h on ice with agitation.

Adherent cells:

- 15 Wash three times with 3 ml, 1 ml or 200 µl (for 6, 24 or 96 well plates, respectively) of cold acidic wash buffer. Beware of the cells who detach from the dish.

Wash twice with 1 ml cold PBS (for 6, 24 or 96 well plates, respectively) to neutralize.

Add 50 µl of lysis RIPA buffer.

Incubate 30 min-1h on ice with agitation.

- 20 Scrap the cells with a cold scraper. The 24 and 96 well plates were directly centrifuged at 4000rpm at 4° for 15min to remove the cellular debris. Then the supernatants (100 or 50ml respectively for the 24 or 96 well plates) were directly transferred in a dark 96 well plate. The plates were read by a fluorescence plate reader (Fusion Alpha, Perkin Elmer).

Transfer the lysate in a coated "eppendorf" (coated with 1ml of poly D-Lys for 4 hours and wash twice with 1ml PBS).

- 25 The lysed cells were then centrifuged 30 min at 10000 g at 4 °C to remove the cellular debris.

Remove the supernatant and store it at -80 °C in a coated "Eppendorf tube" (coated with 1 ml of poly D-Lys for 4 hours and washed twice with 1 ml PBS).

Analysis of peptide internalization with a fluorescence plate reader :

- 30 The content of each protein extract was determined by a standard BCA assay (Kit N°23225, Pierce), following the instructions of the manufacturer.

The relative fluorescence of each sample is determined after reading 10 µl of each sample in a fluorescence plate reader (Fusion Alpha, Perkin Elmer), background subtraction and normalization by protein concentration.

35

3.2 Uptake experiments

The time dependent internalization (uptake) of FITC-labeled TAT derived transporter constructs into cells of the HL-60 cell line was carried out as described above using sequences transporter peptides of SEQ ID NOs: 52-96, 43, and 45-47. These sequences are listed below in Table 4.

5

Table 4: Transporter sequence tested in uptake experiments												
SEQ ID NO:	peptide No: abbreviation in Figure 6											
46	r3-L-TAT	H2N	dR	K	K	R	dR	Q	R	R	dR	CONH2
52	1	H2N	dR	A	K	R	dR	Q	R	R	dR	CONH2
53	2	H2N	dR	K	A	R	dR	Q	R	R	dR	CONH2
54	3	H2N	dR	K	K	A	dR	Q	R	R	dR	CONH2
55	4	H2N	dR	K	K	R	dR	A	R	R	dR	CONH2
56	5	H2N	dR	K	K	R	dR	Q	A	R	dR	CONH2
57	6	H2N	dR	K	K	R	dR	Q	R	A	dR	CONH2
58	7	H2N	dR	D	K	R	dR	Q	R	R	dR	CONH2
59	8	H2N	dR	K	D	R	dR	Q	R	R	dR	CONH2
60	9	H2N	dR	K	K	D	dR	Q	R	R	dR	CONH2
61	10	H2N	dR	K	K	R	dR	D	R	R	dR	CONH2
62	11	H2N	dR	K	K	R	dR	Q	D	R	dR	CONH2
63	12	H2N	dR	K	K	R	dR	Q	R	D	dR	CONH2
64	13	H2N	dR	E	K	R	dR	Q	R	R	dR	CONH2
65	14	H2N	dR	K	E	R	dR	Q	R	R	dR	CONH2
66	15	H2N	dR	K	K	E	dR	Q	R	R	dR	CONH2
67	16	H2N	dR	K	K	R	dR	E	R	R	dR	CONH2
68	17	H2N	dR	K	K	R	dR	Q	E	R	dR	CONH2
69	18	H2N	dR	K	K	R	dR	Q	R	E	dR	CONH2
70	19	H2N	dR	F	K	R	dR	Q	R	R	dR	CONH2
71	20	H2N	dR	K	F	R	dR	Q	R	R	dR	CONH2
72	21	H2N	dR	K	K	F	dR	Q	R	R	dR	CONH2
73	22	H2N	dR	K	K	R	dR	F	R	R	dR	CONH2
74	23	H2N	dR	K	K	R	dR	Q	F	R	dR	CONH2
75	24	H2N	dR	K	K	R	dR	Q	R	F	dR	CONH2
76	25	H2N	dR	R	K	R	dR	Q	R	R	dR	CONH2
77	26	H2N	dR	K	R	R	dR	Q	R	R	dR	CONH2
78	27	H2N	dR	K	K	K	dR	Q	R	R	dR	CONH2
79	28	H2N	dR	K	K	R	dR	R	R	R	dR	CONH2
80	29	H2N	dR	K	K	R	dR	Q	K	R	dR	CONH2
81	30	H2N	dR	K	K	R	dR	Q	R	K	dR	CONH2
82	31	H2N	dR	H	K	R	dR	Q	R	R	dR	CONH2
83	32	H2N	dR	K	H	R	dR	Q	R	R	dR	CONH2
84	33	H2N	dR	K	K	H	dR	Q	R	R	dR	CONH2
85	34	H2N	dR	K	K	R	dR	H	R	R	dR	CONH2
86	35	H2N	dR	K	K	R	dR	Q	H	R	dR	CONH2
87	36	H2N	dR	K	K	R	dR	Q	R	H	dR	CONH2
88	37	H2N	dR	I	K	R	dR	Q	R	R	dR	CONH2
89	38	H2N	dR	K	I	R	dR	Q	R	R	dR	CONH2

90	39	H2N	dR	K	K	I	dR	Q	R	R	dR	CONH2
91	40	H2N	dR	K	K	R	dR	I	R	R	dR	CONH2
92	41	H2N	dR	K	K	R	dR	Q	I	R	dR	CONH2
93	42	H2N	dR	K	K	R	dR	Q	R	I	dR	CONH2
94	43	H2N	dR	L	K	R	dR	Q	R	R	dR	CONH2
45	44 (D-TAT)	H2N	dR	dR	dR	dQ	dR	dR	dK	dK	dR	CONH2
47	45 (r3-L-TATi)	H2N	dR	R	R	Q	dR	R	K	K	dR	CONH2
46	46 (r3-L-TAT)	H2N	dR	K	K	R	dR	Q	R	R	dR	CONH2
43	47 (L-TAT)	H2N	R	K	K	R	R	Q	R	R	R	CONH2
99	48	H2N	dR	K	K	R	dR	Q	R	L	dR	CONH2
100	49	H2N	dR	M	K	R	dR	Q	R	R	dR	CONH2
101	50	H2N	dR	K	M	R	dR	Q	R	R	dR	CONH2
102	51	H2N	dR	K	K	M	dR	Q	R	R	dR	CONH2
103	52	H2N	dR	K	K	R	dR	M	R	R	dR	CONH2
104	53	H2N	dR	K	K	R	dR	Q	M	R	dR	CONH2
105	54	H2N	dR	K	K	R	dR	Q	R	M	dR	CONH2
106	55	H2N	dR	N	K	R	dR	Q	R	R	dR	CONH2
107	56	H2N	dR	K	N	R	dR	Q	R	R	dR	CONH2
108	57	H2N	dR	K	K	N	dR	Q	R	R	dR	CONH2
109	58	H2N	dR	K	K	R	dR	N	R	R	dR	CONH2
110	59	H2N	dR	K	K	R	dR	Q	N	R	dR	CONH2
111	60	H2N	dR	K	K	R	dR	Q	R	N	dR	CONH2
112	61	H2N	dR	Q	K	R	dR	Q	R	R	dR	CONH2
113	62	H2N	dR	K	Q	R	dR	Q	R	R	dR	CONH2
114	63	H2N	dR	K	K	Q	dR	Q	R	R	dR	CONH2
115	64	H2N	dR	K	K	R	dR	K	R	R	dR	CONH2
116	65	H2N	dR	K	K	R	dR	Q	Q	R	dR	CONH2
117	66	H2N	dR	K	K	R	dR	Q	R	Q	dR	CONH2
118	67	H2N	dR	S	K	R	dR	Q	R	R	dR	CONH2
119	68	H2N	dR	K	S	R	dR	Q	R	R	dR	CONH2
120	69	H2N	dR	K	K	S	dR	Q	R	R	dR	CONH2
121	70	H2N	dR	K	K	R	dR	S	R	R	dR	CONH2
122	71	H2N	dR	K	K	R	dR	Q	S	R	dR	CONH2
123	72	H2N	dR	K	K	R	dR	Q	R	S	dR	CONH2
124	73	H2N	dR	T	K	R	dR	Q	R	R	dR	CONH2
125	74	H2N	dR	K	T	R	dR	Q	R	R	dR	CONH2
126	75	H2N	dR	K	K	T	dR	Q	R	R	dR	CONH2
127	76	H2N	dR	K	K	R	dR	T	R	R	dR	CONH2
128	77	H2N	dR	K	K	R	dR	Q	T	R	dR	CONH2
129	78	H2N	dR	K	K	R	dR	Q	R	T	dR	CONH2
130	79	H2N	dR	V	K	R	dR	Q	R	R	dR	CONH2
131	80	H2N	dR	K	V	R	dR	Q	R	R	dR	CONH2
132	81	H2N	dR	K	K	V	dR	Q	R	R	dR	CONH2
133	82	H2N	dR	K	K	R	dR	V	R	R	dR	CONH2
134	83	H2N	dR	K	K	R	dR	Q	V	R	dR	CONH2
135	84	H2N	dR	K	K	R	dR	Q	R	V	dR	CONH2
136	85	H2N	dR	W	K	R	dR	Q	R	R	dR	CONH2
137	86	H2N	dR	K	W	R	dR	Q	R	R	dR	CONH2
138	87	H2N	dR	K	K	W	dR	Q	R	R	dR	CONH2
139	88	H2N	dR	K	K	R	dR	W	R	R	dR	CONH2
140	89	H2N	dR	K	K	R	dR	Q	W	R	dR	CONH2

141	90	H2N	dR	K	K	R	dR	Q	R	W	dR	CONH2
142	91	H2N	dR	Y	K	R	dR	Q	R	R	dR	CONH2
143	92	H2N	dR	K	Y	R	dR	Q	R	R	dR	CONH2
144	93	H2N	dR	K	K	Y	dR	Q	R	R	dR	CONH2
145	94	H2N	dR	K	K	R	dR	Y	R	R	dR	CONH2
146	95	H2N	dR	K	K	R	dR	Q	Y	R	dR	CONH2
147	96	H2N	dR	K	K	R	dR	Q	R	Y	dR	CONH2

In the above table D amino acids are indicated by a small "d" prior to the respective amino acid residue (e.g. dR = D-Arg).

- 5 For a few sequences synthesis failed in the first approach due to technical reasons. These sequences are abbreviated in Figure 6 as 1, 2, 3, 4, 5, 6, 7, 8, 43, 52, 53, 54, 55, 56, 57, 85, 86, 87, 88, 89, and 90. All the remaining sequences were used in the internalization experiments.

The results are shown in Figure 6.

10

As can be seen in Figure 6, after 24 hours of incubation, all transporters with the consensus sequence rXXXrXXXr (SEQ ID NO: 31) showed a higher internalization capability than the L-TAT transporter (SEQ ID NO: 43). Hela cells were incubated 24hours in 96well plate with 10mM of the r3-L-TAT-derived transporters. The cells were then washed twice with an acidic buffer (0.2M Glycin, 0.15M NaCl, pH 3.0) and twice with PBS. Cells were broken by the addition of RIPA lysis buffer. The relative amount of internalized peptide was then determined by reading the fluorescence intensity (Fusion Alpha plate reader; PerkinElmer) of each extract followed by background subtraction

15

As can be seen in Figure 6, one position appears to be critical for highest transporter activity and for improved kinetics of transport activity: Y in position 2 (peptide N°91 corresponding to SEQ ID NO: 142).

20

The conclusion from the results of this experiment is as follows:

- After 24 hours incubation, all transporters with the consensus sequence rXXXrXXXr (SEQ ID NO: 31) (see Table 2 for a selection of possible sequences) showed a higher internalization capability than the L-TAT transporter (SEQ ID NO: 43) (Figure 6). Those results fully validate the consensus sequence rXXXrXXXr (SEQ ID NO: 31).
- One position is critical for highest transporter activity and (Figure 6): Y in position 2 (sequence 91 corresponding to SEQ ID NO: 142).

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30

Accordingly, such TAT derived sequences as shown in Table 4 are preferred, which exhibit an Y in position 2, particularly when the sequence exhibits 9 aa and has the consensus sequence rXXXrXXXr (SEQ ID NO: 31).

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Example 4: Measurement of cytokine and chemokine release

10 In the following the procedure will be set forth describing how the released amount of several human cytokines after ligand induced secretion from human cells (Blood, WBC, PBMC, purified primary lymphocytes, cell lines, ...) was measured.

The technique used is a Sandwich ELISA, which allows measuring the amount of antigen between two layers of antibodies (i.e. capture and detection antibody). The antigen to be measured must contain at least two antigenic sites capable of binding to antibody, since at least two antibodies act
15 in the sandwich. Either monoclonal or polyclonal antibodies can be used as the capture and detection antibodies in Sandwich ELISA systems. Monoclonal antibodies recognize a single epitope that allows fine detection and quantification of small differences in antigen. A polyclonal is often used as the capture antibody to pull down as much of the antigen as possible. The advantage of Sandwich ELISA is that the sample does not have to be purified before analysis, and the assay can
20 be very sensitive (up to 2 to 5 times more sensitive than direct or indirect).

The method may be used to determine the effect of the JNK inhibitors of the present invention in vitro/ cell culture. At non toxic doses, compound efficacy is indicated by the decrease of the cytokine levels (the variation of optical density (absorbance at 450 nm)) as compared to non-treated samples
25 and is monitored by ELISA. Results are express in ng/ml.

4.1 Material

- 30
- 96 well plate:
for collecting the supernatants (Ref 82.1581, Sarstedt)
for ELISA (F96 maxisorp, Ref 442404, Nunc)
 - TopSeal-A: 96well microplate seals (Ref 600585, PerkinElmer).
 - ELISA reagent

Coating buffer ELISA: 0.1M NaCarbonate pH 9.5 (= 7.13g NaHCO₃ (ref 71627, Fluka) + 1.59g Na₂CO₃ (ref 71345, Fluka) in 1 litre H₂O, pH to 9.5 with NaOH concentrated)

Wash buffer ELISA: PBS 1X + 0.01% Tween20. Prepare 1 litre PBS 1X (PBS10X: ref 70011, GIBCO) and add 100ul of Tween20 (ref P1379, Sigma) slowly while mixing with magnetic agitator)

Assay diluent: PBS 1X + 10% FBS (Ref A15-151, PAA, decompemented at 56°C, 30 min).

DAKO TMB (ref S1599, DAKO): commercial substrate solution

Stop Solution: 1M H₃PO₄ (→ for 200ml = 177ml H₂O + 23ml H₃PO₄ 85% (ref 345245, Aldrich).

- ELISA Kit (reagent for 20 plates)

IFN-γ: Human IFN-γ ELISA set, BD OptEIA™ (ref 555142, DB).

IL-1β: Human IL-1β ELISA set II, BD OptEIA™ (ref 557953, BD)

IL-10 : Human IL-10 ELISA set II, BD OptEIA™ (ref 555157, DB).

IL-12 : Human IL-12 (p70) ELISA set, BD OptEIA™ (ref 555183, DB).

IL-15 : Human IL-15 ELISA Set, BD OptEIA™ (ref 559268, DB).

IL-2: Human IL-2 ELISA set, BD OptEIA™ (ref 555190, DB).

IL-4 : Human IL-4 ELISA set, BD OptEIA™ (ref 555194, DB).

IL-5 : Human IL-5 ELISA set, BD OptEIA™ (ref 555202, DB).

IL-6: Human IL-6 ELISA setI, BD OptEIA™ (ref 555220, DB).

IL-8: Human IL-8 ELISA set, BD OptEIA™ (ref 555244, DB).

MCP-1: Human MCP-1 ELISA set, BD OptEIA™ (ref 555179, BD)

TNF-α: Kit human TNF ELISA set, BD OptEIA™ (ref 555212, DB).

- Absorbance reading: The absorbance was read on the Fusion Alpha Plate reader (Perkin Elmer).
- Repeating pipettes, digital pipettes or multichannel pipettes.

4.2 Method

Preparation of the samples

The samples are culture medium supernatant from cultured human cells (typically whole blood, WBC, PBMC, Purified subtype of WBC, cancerous cell lines). Remove any particulate material by centrifugation (400g 5min 4°C) and assay immediately or store samples at -20°C. Avoid repeated freeze-thaw cycles.

One hour before using, defrost the samples on ice and centrifuge them. At step 11, dilute the samples in assay diluent directly into the plate (add first assay diluent, then the samples and pipette up and down):

5 Preparation of Standard

After warming lyophilized standard to room temperature, carefully open vial to avoid loss of material. Reconstitute lyophilized standard with the proposed volume of deionized water to yield a stock standard. Allow the standard to equilibrate for at least 15 minutes before making dilutions. Vortex gently to mix. After

10

reconstitution, **immediately** aliquot standard stock in polypropylene vials at 50 µl per vial and freeze at -20°C for up to 6 months. If necessary, store at 2-8° C for up to 8 hours prior to aliquotting/freezing. Do not leave reconstituted standard at room temperature.

15

Immediately before use, prepare a ten point standard curve using 2-fold serial dilutions in reagent Diluent. A high standard of 4000 pg/ml is recommended.

Preparation of Detector Mix

One-step incubation of Biotin/SAv reagents. Add required volume of Detection Antibody to Assay Diluent. Within 15 minutes prior to use, add required quantity of Enzyme Reagent, vortex or mix well. For recommended dilutions, see lot-specific Instruction/Analysis Certificate. Discard any remaining Working Detector after use.

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Coating with Capture Antibody

1. Coat the wells of a PVC microtiter plate with 100 µL per well of Capture Antibody diluted in Coating Buffer. For recommended antibody coating dilution, see lot-specific Instruction/Analysis Certificate.
2. Cover the plate with an adhesive plastic and incubate **overnight at 4°C**.
3. Remove the coating solution and wash the plate by filling the wells with 150µl wash buffer.
4. The solutions or washes are removed by flicking the plate over a sink.
5. Repeat the process two times for a total of three washes.
6. After the last wash, remove any remaining wash buffer by patting the plate on a paper towel.

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Blocking

7. Block the remaining protein-binding sites in the coated wells by adding 100µl reagent Diluent per well.
8. Cover the plate with an adhesive plastic and incubate for 1 h at room temperature.
9. During the incubation, start preparing the standard.

5

Adding samples

10. Do one wash as in step 3 with 150µl of wash buffer. The plates are now ready for sample addition.
11. Add 50 µl of appropriately diluted samples in assay diluent to each well. For accurate quantitative results, always compare signal of unknown samples against those of a standard curve. Standards (triplicates) and blank must be run with each cytokine to ensure accuracy.
12. Cover the plate with an adhesive plastic and incubate for 2 h at room temperature.

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Incubation with Detection Antibody and Secondary Antibody

13. Wash the plate four times with 150µl wash buffer like step 3.
14. Add 50 µl of detector MIX (detection antibody +Secondary Streptavidin-HRP antibody in assay diluent) to each well at recommended dilutions (see lot-specific Instruction/Analysis Certificate).
15. Cover the plate with an adhesive plastic and incubate for 1 h at room temperature light protect.
16. Wash the plate six times with 150µl wash buffer as in step 3.
17. Add 50 µl DAKO TMB solution to each well, incubate for 15-20 min at room temperature, in the dark, not sealed.
18. Add 50 µl of stop solution to each well. Gently tap the plate to ensure thorough mixing.
19. Mix the plate 5min at 500rpm on a plate mixer.
20. Read the optical density at 450 nm. (Program: Cytokine_ELISA on Fusion Alpha Plate reader).

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Data analysis

Average the triplicate readings for each standard control and each sample. Subtract the average zero standard optical density (O.D). Create a standard curve plotting the log of the cytokine concentration

versus the log of the O.D and the best fit line can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. A standard curve should be generated for each set of samples assayed. The outliers data were avoided using Grugg's test. Then the data which weren't in the interval of two times the SD, were
5 discard. The independent experiments are taken into account if the positive control showed data as previously observed. The independent experiments are pooled ($N > 3$).

The data are presented in pg/ml of cytokine release or in %, compared to the induced condition without inhibitor treatment.

10

Example 5: THP1 differentiation – stimulation for cytokine release

In the following the procedure will be set forth describing how cytokine production from human PMA differentiated THP1 cells challenged by LPS for 6h was induced in order to test the ability of
15 JNK inhibitors of the present invention, in particular of a JNK inhibitor with SEQ ID NO: 172, to reduce stimulation-induced cytokine release. THP1 cells were stimulated ex-vivo by different ligands for the readout of cytokine release. At non toxic doses, JNK inhibitor efficacy is indicated by the decrease of the cytokine levels as compared to non-treated samples and is monitored by ELISA. The toxicity of the compound are evaluated by the reduction of a tretazolium salt (MTS) to
20 formazan, giving a purple colour.

Procedure:

a. Material

- Cell Line: THP-1 (Ref TIB-202, ATCC, lot 57731475)
- 25 • Culture medium, reagent and plates
RPMI (Ref 21875-091, Invitrogen) complemented with:
10% FBS (Ref A15-151, PAA): decomplexed at 56°C, 30 min.
10mM Hepes (Ref H0887, Sigma)
50 M β -mercaptoethanol (Ref 63690, Fluka : stock at 14.3M): add 560 μ l of 50mM aliquots
30 in PBS stocked at -20°C)
1mM Sodium Pyruvate (Ref S8636, Sigma)
Penicilline (100unit/ml) / Streptomycine (100 g/ml) (Ref P4333, Sigma)
The RPMI medium is then filtrated with a 0.22 μ m filter (Ref SCGPU05RE, Millipore).
PBS 10X (Ref 70011, Invitrogen): diluted to 1X with sterile H₂O
35 DMSO: Ref 41444, Fluka

PMA (phorbol 12-myristate 13-acetate, Ref P1585, Sigma, concentration 1mM = 616.8ug/ml in DMSO at -20°C). Use directly at a final concentration of 100nM in RPMI (1ul in 10ml of medium).

LPS ultrapure (Lipopolysaccharide, Ref tlr1-eklps, Invivogen, concentration 5mg/ml): Stock solution of LPS: 3 g/ml in PBS at 4°C. Use directly to prepare a 4X concentrated solution of 40ng/ml in RPMI medium (min 1800 µl /plate; for 5 plates: 125 µl of LPS 3 g/ml + 9250 µl RPMI).

96 well plate:

for adherent cell culture (Ref 167008, Nunc)

for collecting the supernatants (Ref 82.1581, Sarstedt)

for ELISA (F96 maxisorp, Ref 442404, Nunc)

Coating solutions: poly-D-lysine (Ref P9011, Sigma): 25 g/ml final diluted in PBS 1x

- ELISA reagent and kits

Coating buffer ELISA: 0.1M NaCarbonate pH 9.5 (= 7.13g NaHCO₃ (ref 71627, Fluka) + 1.59g Na₂CO₃ (ref 71345, Fluka) in 1 liter H₂O, pH to 9.5 with NaOH concentrated)

Wash buffer ELISA: PBS 1X + 0.01% Tween20 (ref P1379, Sigma, lot 094K0052)(= prepare 1 liter PBS 1X and add 100ul of Tween20 slowly while mixing with magnetic agitator)

Assay diluent: PBS 1X + 10% FBS (Ref A15-151, PAA, decomplexed at 56°C, 30 min).

DAKO TMB (ref S1599, DAKO): commercial substrate solution

Stop Solution: 1M H₃PO₄ (→ for 200ml = 177ml H₂O + 23ml H₃PO₄ 85% (ref 345245, Aldrich).

TNF- : Kit human TNF ELISA set, BD OptEIA (ref 555212, DB).

- Cytotoxicity measurement: CellTiter 96 reagent (ref G3581, Promega)
- Control compound: SP600125 (ref ALX-270-339-M025, Alexis, concentration: 20mM DMSO)
- Absorbance reading: The absorbance was read on the Fusion Alpha Plate reader (Perkin Elmer).
- Repeating pipettes, digital pipettes or multichannel pipettes.
- TopSeal-A: 96well microplate seals (Ref 600585, PerkinElmer).

b. Method

Well coating

The plates had been coated with 200 µl of poly D-Lysine (1x) and incubated 2 hours at 37°C, CO₂ 5% and 100% relative humidity.

5 Cell plating

After 2 hours the wells were washed twice with 200 l PBS 1X (use immediately or leave with 200 l of PBS 1X at 37°C till use, but no more than 3 days).

The cells were counted. The desired number of cells was taken and resuspended in the amount of media necessary to get a dilution of 1'000'000 cells/ml. 100nM of PMA was added to induce the
10 differentiation of the THP1 from suspension monocytes to adherent macrophages. The cells were plated into the wells in 100 l medium at plating densities of 100'000cells/well. After inoculation, the plates were incubated at 37°C, 5% CO₂ and 100% relative humidity 3 days to let them differentiate, prior to the addition of experimental drugs.

Cell treatment

15 After 3 days, the adherent cells were observed with the microscope. The media containing PMA was aspirated and replaced by 100 l of fresh RPMI media without PMA (no washing step with PBS 1X).

Experimental drug were prepared at the concentration of 10 mM in H₂O or DMSO and stored at -80°C. Prior to each daily use, one aliquot of JNK inhibitor was defrost and diluted to reach a 4X
20 concentrated solution (120 M) in RPMI medium and then to the desired concentration in RPMI. The SP600125 was diluted to reach a 4X concentrated solution (40 M) in RPMI medium and then to the desired concentration in RPMI containing 0.8% DMSO.

The plates were treated with 50 µl of medium or a solution of 4X the final desired drug concentration
25 (0, 100nM, 1, 3, 10 or 30 M final for JNK compound or at 0, 10, 100nM, 1, 3 or 10 M final for the SP600125 positive control). Following drug addition, the plates were incubated for an additional 1h at 37°C, 5% CO₂ and 100% relative humidity.

After 1 hours, the secretion of TNF was induced by the addition of 50 l of a 4X concentrated dilution
30 of LPS ultrapure (3ng/ml final).

Assay

After 6 hours, 100 l of the supernatant were transferred to new 96well plates. Those plates were sealed and stored at -20° till the analysis by ELISA (e.g. see example 4) of the secretion of the
35 cytokines.

The cytotoxic effect of the compounds was evaluated by MTS absorbance (e.g. see example 4) and cells were observed using an inverted microscope (Axiovert 40 CFL; Zeiss; 10X).

Data analysis

- 5 Analyses of the data are performed as indicated in the ELISA (see example 4). Briefly, for ELISA: Average the triplicate readings for each standard control and each sample. Subtract the average zero standard optical density (O.D). Create a standard curve plotting the log of the cytokine concentration versus the log of the O.D and the best fit line can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution
- 10 factor. A standard curve should be generated for each set of samples assayed. The outliers data were avoid using Grugg's test. Then the data which weren't in the interval of two times the SD, were discard. The independent experiments are taken into account if the positive control showed data as previously observed. The independent experiments are pooled ($N > 3$).
- 15 For the Cytotoxicity effect evaluation: on each plate of each independent experiment taken into account for the cytokine release experiment analysis, the average of the absorbance of the medium alone was considerate as the background and subtracted to each absorbance value. The average of triplicate of the non treated cells of each compound was considerate as the 100% viability. The average of triplicate of each compound was normalized by its 100%. The outliers data were avoid
- 20 using Grugg's test. Then the data which weren't in the interval of two times the SD, were discard. The independent experiments are pooled ($N > 3$).

All statistical comparisons of conditions were performed by the GraphPad Prism4 software with the following test: One way ANOVA test followed by a Tukey's Multiple Comparison Test. $P < 0.05$ was

25 considerate as significant..

Example 6: JNK inhibitor of SEQ ID NO: 172 and TNF α release in Primary Rat or human whole blood cells

- 30 Whole blood is collected from anesthetized rat or human healthy volunteers using a venipuncture connected to a pre-labeled vacuum tube containing sodium citrate. Tubes are gently mixed by inversion 7-8 times; and are then kept at RT until stimulation. JNK inhibitor of SEQ ID NO: 172 is prepared 6 times concentrated in PBS, and 30 μ l/well of mix is added into 96-well plate. Whole
- 35 blood is diluted by 1:2 in PBS and 120 μ l of diluted blood is added in each well where either PBS alone or JNK inhibitor of SEQ ID NO: 172 has been previously added. Whole blood is incubated at

- 37°C; 85 rpm (Stuart Orbital incubator SI500) for 60 min. Activators (LPS) are the prepared, 30µl/well of LPS, 6 times concentrated. After 60min incubation, LPS is added to the blood, blood is mixed by pipetting up and down, and then kept for 4h under agitation (85rpm), at 37°C. After the 4h incubation, the plates are centrifuged at about 770g, 4°C for 15 min in a pre-cooled centrifuge.
- 5 Supernatants are finally collected and kept at -20°C until cytokine measurement. Cytokine (IL-6, IL-2, IFN γ and TNF α) were then measured using standard Elisa kits (e.g. from R&D Systems: DuoSet Elisas; or from BD Biosciences: BD Opteia Set Elisa). Results are expressed as pg/ml of supernatant of the measured cytokine.
- 10 A similar experiment was conducted with PMA+ionomycin instead of LPS as activator/stimulant.

Example 7: Half-life of specific JNK inhibitors disclosed herein

- 15 The JNK inhibitors with the sequence of SEQ ID NOs: 196, 197, and 172 (0.1mM final concentration) were digested in human serum (10 and 50% in PBS 1x). The experiment was performed as described by Tugyi et al. (Proc Natl Acad Sci U S A, 2005, 413-418). The remaining intact peptide was quantified by UPLC-MS. Stability was assessed for SEQ ID NOs: 196, 197, and 172 identically but in two separate assays. While the JNK inhibitor with SEQ ID NO: 196 was totally
- 20 degraded into amino acids residues within 6 hours, the JNK inhibitor with SEQ ID NO: 172 was completely degraded only after 14 days. The JNK inhibitor with SEQ ID NO: 197 was still stable after 30 days.

- 25 **Example 8: Dose-dependent inhibition by JNK inhibitor with sequence of SEQ ID NO: 172 of CD3/CD28-induced IL-2 release in rat primary T-cells**

- Control animal were sacrificed, lymph nodes (LN) were harvested and kept in complete RPMI medium. LN were smashed with complete RPMI on 70µm filter using a 5ml piston. A few drops of
- 30 media were added to keep strainer wet. Cells were centrifuged for 7 min at 450g and 4°C. Pellet was resuspended in 5 ml fresh medium. Cells were passed again through cell strainer. An aliquot of cells was counted, while cells were centrifuged again 10min at 1400 rpm and 4°C. Cells were resuspended in MACS buffer (80µl of MACS buffer per 10⁷ cells). 10µl of anti-rat MHC microbeads were added per 10million cells, cells were incubated for 15min at 4°-8°C. Cells were washed with 15ml MACS
- 35 buffer and centrifuge for 7 min at 700g and 4°C. Pellet was resuspended in 500µl MACS buffer per 10⁸ cells. One LS column was placed in the magnetic field of the MACS separator per animal.

Column was first rinsed with 3 ml of MACS buffer. One tube was placed below the column in ice to collect cells = T cells (negative selection so we collect what is eluted). Cell suspension was added and elute was collected on ice. Column was washed 3 times with 3mL MACS buffer. Eluted T cells were centrifuges for 7 min at 700g and 4°C. Resuspended cells were counted and plated at density of 200000cells/well in 100µl of complete medium. Plates were pre-coated the day before experiment with 2µg/mL of CD3 antibody, and the day of experiment plates were washed three times with PBS. Cells were treated with 100µl of (poly-)peptide JNK inhibitor (SEQ ID NO: 172), two times concentrated for 1h before ligand activation. After 1h of pre-treatment with (poly-)peptide JNK inhibitor (SEQ ID NO: 172), cells were then stimulated with 2µg/mL of anti CD28 antibody for 24h. After 24h of stimulation, supernatant were collected and stored at -20°C until analysis. Cytokines were then measured using standard Elisa kits. Results are expressed as pg/ml of supernatant of the measured cytokine.

In a further experiment, essentially the same protocol as set forth above was used, but in addition to the (poly-)peptide JNK inhibitors with SEQ ID NO: 172, JNK inhibitors with the sequence of SEQ ID NO: 197 and the drug molecule SP600125 were also tested thus allowing to compare the effects of these inhibitors on the inhibition of CD3/CD28-induced IL-2 release.

Example 9: JNK inhibitor and TNFα/IL-2 release in human whole blood:

Whole blood from human healthy volunteers was collected using a venipuncture connected to a pre-labeled vacuum tube containing sodium citrate. Tubes are gently mixed by inversion 7-8 times; and are then kept at RT until stimulation. 350µl of RPMI + P/S were added into 1,2 ml-96-well plate. 10 times concentrated of SEQ ID NO: 172 was prepared in RPMI+P/S (50µl per well). 50µl was added into 1.2ml- 96 well plates. 50µl of whole blood was then added in each well where either medium alone or JNK inhibitor has been previously added. Whole blood was incubated at 37°C, 5% CO2 for 60 min. 50µl / well of ligands diluted in RPMI+ P/S was prepared, corresponding to the final dilution 10 times concentrated. After 60min of incubation, ligand was added; wells were then mixed by pipetting up and down the blood. Whole blood was incubated for 3 days at 37°C (wells were mixed by pipetting each well up and down once per day). At the end of incubation, plates were mixed and then centrifuged at 2500rpm, 4°C for 15 min in a pre-cooled centrifuge. Cytokine were then measured using standard Elisa kits. Results are expressed as pg/ml of supernatant of the measured cytokine.

A similar experiment was carried out with slight modifications. In the case of CD3/CD8 stimulation, CD3 antibody was coated at 2µg/mL in PBS overnight at 4°C. The day of experiment, wells were washed three times with PBS and left in PBS until use at 37°C. CD28 antibody was added 1h after SEQ ID NO: 172 at final concentration of 2µg/mL; supernatants were collected after 3 days of stimulation.

Example 10: Anti-Inflammatory potency in a rat model of endotoxins induced uveitis (EIU)

The anti-inflammatory potency of the JNK inhibitor of SEQ ID NO: 172 was tested in albino rats following intravenous administration (EIU/LPS model). The aim of this study was to determine the effects of single intravenous injections of SEQ ID NO: 172 (0.015, 0.18, and 1.80 mg/kg) on the inflammatory response in an endotoxins-induced uveitis albino rat model and to compare these affects to those obtained with prior art JNK inhibitor of SEQ ID NO: 197 (2 mg/kg). As a further control served phosphate sodic dexamethasone ("reference") and vehicle (0.9 % NaCl).

Sixty (60) male Lewis rats were randomly divided into six (6) groups of ten (10) animals each. 0.9 % NaCl (vehicle, "control"), SEQ ID NO: 197 at 2 mg/kg and SEQ ID NO: 172 at three concentrations (1.80 mg/kg, 0.18 mg/kg and 0.015 mg/kg) were administered by intravenous injection just before EIU induction (on the day of induction). Accordingly, phosphate sodic dexamethasone (20 µg/eye, 5 µl; "reference") was administered by a single sub-conjunctival injection in both eyes just before EIU induction (on the day of induction). EIU was induced by footpad injection of lipopolysaccharide (LPS, 1 mg/kg). 24 hours after LPS injection, inflammatory response was evaluated by clinical scoring.

The intensity of clinical ocular inflammation was scored on a scale from 0 to 4 for each eye:

- | | |
|---------|--|
| Grade 0 | no inflammation |
| Grade 1 | slight iris and conjunctival vasodilation |
| Grade 2 | moderate iris and conjunctival vasodilation with flare |
| Grade 3 | intense iris and conjunctival vasodilation with flare |
| Grade 4 | intense inflammatory reaction |
| (+1) | fibrin formation and seclusion of pupils |

Twenty-four hours after LPS induction, clinical scores for the vehicle-treated rats were 3.6 ± 0.2 (mean $\pm$ SEM, n = 20) with a median of 4 (range, 2-5). A significant reduction ($p < 0.001$) in the severity of the ocular inflammation was detected 24 hours after induction and intravenous treatment with SEQ ID NO: 197 (2 mg/kg) (mean score: 2.2 ± 0.3 , median: 2), corresponding to a 40%

decrease of EIU scores compared with the score observed in vehicle group. Intravenous treatment with SEQ ID NO: 172, at approximately the same dose (1.80 mg/kg) reduced also significantly the severity of the ocular inflammation by 42% (mean score: 2.1 ± 0.3 , median: 2, $p = 0.001$). The lower doses (0.18 and 0.015 mg/kg) reduced by 33% (mean score: 2.4 ± 0.3 , median: 2) and 36% (mean score: 2.3 ± 0.3 , median: 2) the inflammation, respectively. The reduction was significant with $p < 0.001$.

A sub-conjunctival treatment with dexamethasone (20 $\mu\text{g}/\text{eye}$), used as positive control drug also significantly reduced the clinical scores by 79% (mean score: 0.8 ± 0.2 , median: 0.5, $p < 0.001$).

Under these experimental conditions, it can be stated that a single intravenous injection of SEQ ID NO: 197 at 2 mg/kg partially prevented the endotoxin-induced inflammation observed in the anterior chamber. In comparison, SEQ ID NO: 172 intravenously injected at 0.015, 0.18, 1.80 mg/kg also reduced the endotoxin-induced inflammation in the anterior chamber.

Example 11: Dose-responsive effects after intravenous administration of JNK inhibitor after 14 days in a rat model of chronic established type II collagen arthritis

Rat collagen arthritis is an experimental model of polyarthritis that has been widely used for preclinical testing of numerous anti-arthritic agents that are either under preclinical or clinical investigation or are currently used as therapeutics in this disease. The hallmarks of this model are reliable onset and progression of robust, easily measurable polyarticular inflammation, marked cartilage destruction in association with pannus formation, and mild to moderate bone resorption and periosteal bone proliferation.

Intravenous (IV) efficacy of the JNK inhibitor of SEQ ID NO: 172 administered daily (QD) for 14 days (arthritis d1–14) for inhibition of the inflammation (paw swelling), cartilage destruction, and bone resorption that occurs in established type II collagen arthritis in rats was determined in said experimental model.

Animals (8/group for arthritis) were anesthetized with isoflurane and injected with 300 μl of Freund's Incomplete Adjuvant (Difco, Detroit, MI) containing 2 mg/ml bovine type II collagen (Elastin Products, Owensville, Missouri) at the base of the tail and 2 sites on the back on days 0 and 6. On day 10 of the study (arthritis d0), onset of arthritis occurred and rats were randomized into treatment

groups. Randomization into each group was done after ankle joint swelling was obviously established in at least one hind paw.

Female Lewis rats with established type II collagen arthritis were treated daily (QD) on arthritis days 1-14 by the intravenous (IV) route with vehicle (0.9% NaCl), SEQ ID NO: 172 (0.01, 0.1, 1, or 5 mg/kg), or the reference compound dexamethasone (Dex, 0.05 mg/kg). Animals were terminated on arthritis day 14. Efficacy evaluation was based on animal body weights, daily ankle caliper measurements, ankle diameter expressed as area under the curve (AUC), terminal hind paw weights, and histopathologic evaluation of ankles and knees of selected groups.

Scoring of Joints Collagen arthritic ankles and knees are given scores of 0-5 for inflammation, pannus formation and bone resorption according to the following criteria:

Knee and/or Ankle Inflammation

15	0	Normal
	0.5	Minimal focal inflammation
	1	Minimal infiltration of inflammatory cells in synovium/periarticular tissue
	2	Mild infiltration
	3	Moderate infiltration with moderate edema
20	4	Marked infiltration with marked edema
	5	Severe infiltration with severe edema

Ankle Pannus

	0	Normal
25	0.5	Minimal infiltration of pannus in cartilage and subchondral bone, affects only marginal zones and affects only a few joints
	1	Minimal infiltration of pannus in cartilage and subchondral bone, primarily affects marginal zones
	2	Mild infiltration (<1/4 of tibia or tarsals at marginal zones)
30	3	Moderate infiltration (1/4 to 1/3 of tibia or small tarsals affected at marginal zones)
	4	Marked infiltration (1/2 to 3/4 of tibia or tarsals affected at marginal zones)
	5	Severe infiltration (>3/4 of tibia or tarsals affected at marginal zones, severe distortion of overall architecture)

35 Knee Pannus

0	Normal
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- 0.5 Minimal infiltration of pannus in cartilage and subchondral bone, affects only marginal zones and affects only a few joints
- 1 Minimal infiltration of pannus in cartilage and subchondral bone, approximately 1–10% of cartilage surface or subchondral bone affected
- 5 2 Mild infiltration (extends over up to 1/4 of surface or subchondral area of tibia or femur), approximately 11–25% of cartilage surface or subchondral bone affected
- 3 Moderate infiltration (extends over >1/4 but < 1/2 of surface or subchondral area of tibia or femur) approximately 26–50% of cartilage surface or subchondral bone affected
- 10 4 Marked infiltration (extends over 1/2 to 3/4 of tibial or femoral surface) approximately 51–75% of cartilage surface or subchondral bone affected
- 5 Severe infiltration approximately 76–100% of cartilage surface or subchondral bone affected

15 Ankle Cartilage Damage (emphasis on small tarsals)

- 0 Normal
- 0.5 Minimal decrease in T blue staining, affects only marginal zones and affects only a few joints
- 1 Minimal = minimal to mild loss of toluidine blue staining with no obvious chondrocyte loss or collagen disruption
- 20 2 Mild = mild loss of toluidine blue staining with focal mild (superficial) chondrocyte loss and/or collagen disruption
- 3 Moderate = moderate loss of toluidine blue staining with multifocal moderate (depth to middle zone) chondrocyte loss and/or collagen disruption, smaller tarsals affected
- 25 to 1/2 to 3/4 depth with rare areas of full thickness loss
- 4 Marked = marked loss of toluidine blue staining with multifocal marked (depth to deep zone) chondrocyte loss and/or collagen disruption, 1 or 2 small tarsals surfaces have full thickness loss of cartilage
- 5 Severe = severe diffuse loss of toluidine blue staining with multifocal severe (depth to tide mark) chondrocyte loss and/or collagen disruption affecting more than 2
- 30 cartilage surfaces

Knee Cartilage Damage

- 0 Normal
- 35 0.5 Minimal decrease in T blue staining, affects only marginal zones

- 1 Minimal = minimal to mild loss of toluidine blue staining with no obvious chondrocyte loss or collagen disruption
- 2 Mild = mild loss of toluidine blue staining with focal mild (superficial) chondrocyte loss and/or collagen disruption, may have few small areas of 50% depth of cartilage affected
- 5 3 Moderate = moderate loss of toluidine blue staining with multifocal to diffuse moderate (depth to middle zone) chondrocyte loss and/or collagen disruption, may have 1–2 small areas of full thickness loss affecting less than 1/4 of the total width of a surface and not more than 25% of the total width of all surfaces
- 10 4 Marked = marked loss of toluidine blue staining with multifocal to diffuse marked (depth to deep zone) chondrocyte loss and/or collagen disruption or 1 surface with near total loss and partial loss on others, total overall loss less than 50% of width of all surfaces combined
- 15 5 Severe = severe diffuse loss of toluidine blue staining with multifocal severe (depth to tide mark) chondrocyte loss and/or collagen disruption on both femurs and/or tibias, total overall loss greater than 50% of width of all surfaces combined

Ankle Bone Resorption

- 0 Normal
- 20 0.5 Minimal resorption affects only marginal zones and affects only a few joints
- 1 Minimal = small areas of resorption, not readily apparent on low magnification, rare osteoclasts
- 2 Mild = more numerous areas of resorption, not readily apparent on low magnification, osteoclasts more numerous, <1/4 of tibia or tarsals at marginal zones resorbed
- 25 3 Moderate = obvious resorption of medullary trabecular and cortical bone without full thickness defects in cortex, loss of some medullary trabeculae, lesion apparent on low magnification, osteoclasts more numerous, 1/4 to 1/3 of tibia or tarsals affected at marginal zones
- 30 4 Marked = Full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts, 1/2 to 3/4 of tibia or tarsals affected at marginal zones
- 35 5 Severe = Full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts, >3/4 of tibia or tarsals affected at marginal zones, severe distortion of overall architecture

Knee Bone Resorption

0 Normal

0.5 Minimal resorption affects only marginal zones

5 1 Minimal = small areas of resorption, not readily apparent on low magnification, approximately 1–10% of total joint width of subchondral bone affected

2 Mild = more numerous areas of resorption, definite loss of subchondral bone, approximately 11–25% of total joint width of subchondral bone affected

10 3 Moderate = obvious resorption of subchondral bone approximately 26–50% of total joint width of subchondral bone affected

4 Marked = obvious resorption of subchondral bone approximately 51–75% of total joint width of subchondral bone affected

5 Severe = distortion of entire joint due to destruction approximately 76–100% of total joint width of subchondral bone affected

15

Results:

Disease severity in the disease control group increased from days 1 to 5 with day 4–5 having the greatest daily increase. Then the incremental increases were smaller to the peak at day 7. From that point forward, acute swelling generally decreased and calliper measures were decreased. The treatment groups followed this general pattern as well.

20

Body weight loss was observed in all disease groups whereas the normal control group had a weight increase. Body weight loss was significantly (25%, $p < 0.05$ by ANOVA) inhibited for rats treated with 5 mg/kg SEQ ID NO: 172 as compared to vehicle treated disease controls. When compared to disease controls using a Student's t-test, inhibition of body weight loss was also significant for rats treated with 1 mg/kg SEQ ID NO: 172 (21%, $p < 0.05$) or Dex (21%, $p < 0.05$). Results of treatment with SEQ ID NO: 172 were dose responsive for this parameter.

25

Daily ankle diameter measurements were significantly ($p < 0.05$ by 2-way RM ANOVA) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 ($p < 0.05$ days 4–12) or Dex ($p < 0.05$ d3–14) as compared to disease controls.

30

Ankle diameter AUC was significantly ($p < 0.05$ by ANOVA) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 (43% reduction), 1 mg/kg SEQ ID NO: 172 (27%), or Dex (97%) as compared to disease controls. Results of treatment with SEQ ID NO: 172 were dose responsive for this parameter.

35

Final paw weights were significantly ($p < 0.05$ by ANOVA) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 (26% reduction) or Dex (114%) as compared to disease controls. Results of treatment with SEQ ID NO: 172 were dose responsive for this parameter.

5

Relative liver weights were not significantly (by ANOVA) affected for rats in any treatment group as compared to disease controls.

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Spleen weights relative to body weight were significantly ($p < 0.05$ by ANOVA) reduced for rats treated with Dex as compared to disease controls. Relative spleen weights for Dex treated rats were also significantly reduced as compared to normal controls. Relative spleen weights were not significantly affected for rats treated with SEQ ID NO: 172.

15

Thymus weights relative to body weight were significantly ($p < 0.05$ by ANOVA) reduced for rats treated with Dex as compared to disease controls. Relative thymus weights for Dex treated rats were also significantly reduced as compared to normal controls. Relative thymus weights were not significantly affected for rats treated with SEQ ID NO: 172.

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All ankle histopathology parameters were significantly (by Mann-Whitney U test) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 (25% reduction of summed scores) as compared to disease controls.

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All knee histopathology parameters were significantly (by Mann-Whitney U test) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 (73% reduction of summed scores) as compared to disease controls.

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Results of this study indicated that daily intravenous treatment with SEQ ID NO: 172 (5 mg/kg) had significant beneficial effect on the clinical and histopathology parameters associated with established type II collagen arthritis in rats. Treatment with SEQ ID NO: 172 (1 mg/kg) resulted in significantly reduced ankle diameter AUC. The beneficial effect on ankle diameter was observed up to day 12 despite the reduction of swelling after day 7 in disease control animals. Results of treatment with SEQ ID NO: 172 were dose responsive.

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Treatment with SEQ ID NO: 172 had no adverse effect on organ weights unlike dexamethasone.

Example 12: Effect of the all-D-retro-inverso JNK-inhibitor (poly-)peptide of SEQ ID NO: 197 and the JNK inhibitor (poly-)peptide of SEQ ID NO: 172 at three doses in a Scopolamine-Induced Model of Dry Eye in Mice

5 Study concept

The objective of this study was to assess the effects of two different compounds, the all-D-retro-inverso JNK-inhibitor (poly-)peptide of SEQ ID NO: 197 and the JNK inhibitor (poly-)peptide of SEQ ID NO: 172, at three dose levels in a mouse model of scopolamine-induced dry eye.

- 10 The peptides of SEQ ID NO: 197 and SEQ ID NO: 172 were tested for efficacy in this murine model of dry eye. The peptides were both tested at a low, medium and a high dose. For the peptide of SEQ ID NO: 197 the concentrations measured in the formulation samples for low, medium and high dose levels were 0.06% (w/v), 0.25% (w/v) and 0.6% (w/v), respectively, and for SEQ ID NO: 172 the concentrations measured in the formulation samples for the low, medium and high dose levels, were 0.05% (w/v), 0.2% (w/v) and 0.6% (w/v), respectively. The vehicle, which also served as the negative control, was 0.9% Sodium Chloride for Injection USP.

The study consisted of a total of 9 groups of female C57BL/6 mice, comprising 8 groups of 12 mice each and an additional group of 4 mice. Bilateral short-term dry eye was induced by a combination of scopolamine hydrobromide (Sigma-Aldrich Corp., St. Louis, MO) injection (subcutaneous (SC), four times daily, 0.5 mg/dose, Days 0-21) and by exposing mice to the drying environment of constant air draft. Starting on Day 1, mice of Groups 1–8 were treated three times daily (TID) for 21 days with bilateral topical ocular (oculus uterque; OU) administration (5 µL/eye/dose) of vehicle (0.9% sterile saline; negative control article); the peptide of SEQ ID NO: 197 (0.06%, 0.25% and 0.6%), the peptide of SEQ ID NO: 172 (0.05%, 0.2% and 0.6%); or cyclosporine (0.05%; positive control, an immunosuppressant drug used to reduce the activity of the immune system). Mice of Group 9 were maintained as un-induced, (no dry eye) untreated controls.

During the in-life (treatment) period, clinical observations were recorded once daily; slit-lamp examination (SLE) with corneal fluorescein staining, tear break-up time test (TBUT), and phenol red thread test (PRTT) were performed three times per week. Necropsies were performed on Day 22; eyes, eye lids, conjunctivae, and lacrimal glands were collected from both eyes of each animal. Tissues from the right eyes (oculus dexter, OD) were fixed and then evaluated microscopically. Tissues from the left eyes (oculus sinister; OS) were flash-frozen in liquid nitrogen and stored frozen at -80 °C for possible subsequent analyses.

Table 5: Experimental Design

Group	Number of animals (females)	Induction of Dry Eye (QID, SC) Days 0 to 21	Treatment (TID, OU, 5 µL/eye) Days 1 to 21
1	12	Scopolamine (200 µL of 2.5 mg/mL sol., 0.5 mg/dose)	Vehicle
2	12		SEQ ID NO: 197 (0.06%)
3	12		SEQ ID NO: 197 (0.25%)
4	12		SEQ ID NO: 197 (0.6%)
5	12		SEQ ID NO: 172 (0.05%)
6	12		SEQ ID NO: 172 (0.2%)
7	12		SEQ ID NO: 172 (0.6%)
8	12		Restasis®* (0.05%)
9	4	No dry eye induction	No treatment

* Cyclosporine

5 Methods

1. Dose preparation

The (poly-)peptide of SEQ ID NO: 197 was obtained from Polypeptide Laboratories (France) as a 1.5-mL clear plastic microfuge vial containing 300.65 mg of dry powder.

- 10 The (poly-)peptide of SEQ ID NO: 172 was obtained from Polypeptide Laboratories (France) as a 1.5-mL clear plastic microfuge vial containing 302.7 mg of dry powder.

Prior to the start of the study, the (poly-)peptides of SEQ ID NO: 172 and of SEQ ID NO: 197 were formulated in sterile saline (vehicle). Dosing solutions at each concentration were sterilized using 0.2-µm filters, aliquoted to multiple pre-labeled vials, and frozen at -20 °C. The concentrations measured in the formulation samples for the peptide of SEQ ID NO: 197 were 0.058%, 0.25% and 0.624%, rounded to 0.06%, 0.25% and 0.6%. The concentrations measured in the formulation samples for the peptide of SEQ ID NO: 172 were 0.053%, 0.217% and 0.562%, rounded to 0.05, 0.2% and 0.6%.

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On each day of dosing, one set of dosing solutions was thawed and used for that day's dose administrations. The controls (vehicle, cyclosporine) were provided ready to dose; no dose preparation was necessary.

2. Slit-Lamp Examinations (SLE)

Prior to entry into the study, each animal underwent a SLE and indirect ophthalmic examination using topically-applied fluorescein. Ocular findings were recorded using the Draize scale ocular scoring. SLE and Draize scoring were repeated three times a week during the in-life period.

3. Tear Break-Up Time (TBUT) Test and Subsequent Corneal Examination

The TBUT test was conducted three times weekly by measuring the time elapsed in seconds between a complete blink after application of fluorescein to the cornea and the appearance of the first random dry spot in the tear film. To perform the TBUT, 0.1% liquid sodium fluorescein was dropped into the conjunctival sac, the eyelids were manually closed three times and then held open revealing a continuous fluorescein-containing tear film covering the cornea, and the time (in seconds) required for the film to break (appearance of a dry spot or streak) was recorded. At least ninety seconds later, corneal epithelial damage was graded using a slit-lamp with a cobalt blue filter after another drop of 0.1% fluorescein was reapplied to the cornea; the cornea then was scored per the Draize ocular scale.

4. Phenol Red Thread Tear Test (PRTT)

Tear production was measured three times a week in both eyes using PRTT test strips (Zone-Quick; Menicon, Nagoya, Japan). Prior to the first treatment of the day, a thread was applied to the lateral canthus of the conjunctival fornix of each eye for 30 seconds under slit-lamp biomicroscopy. Tear migration up the thread (i.e., the length of the wetted cotton thread) was measured using a millimeter scale.

5. Necropsy and Pathology

At necropsy on Day 22, both eyes from each animal, including the globes, lacrimal glands, eyelids, and conjunctivae, were excised. The right eye and associated tissues were fixed by overnight submersion in modified Davidson's solution followed by transfer to 10% neutral buffered formalin (NBF). The fixed tissues of the right eye were dehydrated, embedded in paraffin, sectioned at 3 to 5- μ m thicknesses, and slide-mounted tissues were stained with hematoxylin and eosin (H & E). Stained slides were evaluated via light microscopy. Detailed and complete histopathologic assessment was conducted on all parts of the eye, with at least two section levels being examined histopathologically for each right eye. Special attention was paid to the cornea, epithelia (including goblet cells) of the conjunctiva and cornea, as well as the lacrimal gland. These tissues were scored for injury based upon a 0–4 scale, with 0 being normal, 1 being minimal, 2 being mild, 3 being moderate, and 4 being severe. For each cornea, scores were based on corneal epithelium thickness, and corneal

inflammation. Conjunctivae were scored for erosion and inflammation as well as presence or absence of goblet cells.

RESULTS

Four-times daily SC administration of scopolamine (0.5mg/dose) induced a dry eye syndrome in female C57BL/6 mice characterized by a decrease in the volume of aqueous tear production and changes in the physiochemical properties of the tears rendering them less capable of maintaining a stable tear film able to effectively lubricate and protect the eye.

1. Tear Break-Up Time (TBUT) Test and Corneal Examination

The tear break-up time tests (TBUTs) were performed prior to the induction of dry eye, and again on Days 2, 4, 7, 9, 11, 14, 16, 18 and 21 after dry eye induction. After initiation of dosing with scopolamine (dry eye induction) TBUT mean values began to decrease in all animals, but appeared to decrease more slowly in Group 6 (mid-dose of SEQ ID NO: 172). The TBUT mean nadir for Groups 5, 6, 7 (low, mid and high-dose of the peptide of SEQ ID NO: 172), and Group 8 (cyclosporine) occurred on Day 7, reaching similar values (6.6 ± 0.4 , 6.7 ± 0.4 , 6.7 ± 0.3 , and 6.4 ± 0.4 s, respectively). Subsequently, the TBUT means of these groups increased to a peak on Day 9. Groups 6 and 7 (SEQ ID NO: 172 mid and high-dose groups) TBUT means rose to higher values (10.0 ± 0.7 s and 9.9 ± 0.8 s, respectively) than Group 8, the cyclosporine group (8.5 ± 0.3 s), while the peak TBUT mean of Group 5, the low-dose of SEQ ID NO: 172 (8.0 ± 0.4 s) was slightly below that of Group 8 (cyclosporine). TBUT means for the mid and high-dose of SEQ ID NO: 197-treated animals, Groups 3 and 4, continued to decline after onset of dosing, reaching a nadir on Day 9, while the low-dose Group 2 increased on Day 9. The low, medium and high-dose TBUT means of SEQ ID NO: 172-treated animals (Groups 2, 3 and 4, respectively) were above the vehicle group and generally below the low, mid and high-dose group means of SEQ ID NO: 172-treated animals.

When the area under the curve (AUC) for TBUT values from Day 7 to Day 21 was used to compare the various treatments with the vehicle control, treatment with mid, low and high-dose of the peptide of SEQ ID NO: 172 (0.05%, 0.2% and 0.6%, respectively), Groups 5, 6, and 7, as well as animals treated with cyclosporine (0.05%), Group 8, showed significant increases in the TBUT AUC (Kruskal-Wallis nonparametric ANOVA). The peptide of SEQ ID NO: 172 appeared to produce a dose-dependent increase in TBUT, with the mid and high-doses often producing similar effects. Furthermore, there were no significant differences in TBUT AUC between the cyclosporine-treated group, the groups treated with three dose levels of SEQ ID NO: 172 and the un-induced group (Groups 5, 6, 7, 8, and 9). This finding suggests that all three doses of the peptide of SEQ ID NO:

172 and cyclosporine were approximately equally effective in improving or reversing the ophthalmological changes that underlie the TBUT changes in this dry eye model.

Groups treated with low, mid and high dose levels of the peptide of SEQ ID NO: 197 (Groups 2-4) showed slight generally dose-dependent increases in TBUT which started to increase approximately two days later than animals treated with SEQ ID NO: 172 or cyclosporine.

Table 6: Mean Calculated TBUT AUC Values:

Group	TBUT AUC
Group 1	71.19
Group 2	88.54
Group 3	91.19
Group 4	89.98
Group 5	102.98
Group 6	119.08
Group 7	119.31
Group 8	116.1
Group 9	124.54

10

2. Phenol Red Thread Tear Test (PRTT)

PRTT tests were performed prior to the induction of dry eye, and again on Days 2, 4, 7, 9, 11, 14, 16, 18 and 21. PRTT values from Day 0 to Day 4 decreased in all mice that had dry eye induced, indicating a decrease in tear production after the administration of scopolamine and exposure to a drying environment of increased air draft created by the blowers. The nadir in PRTT in most groups occurred at approximately Day 7. PRTT kept decreasing in the vehicle control group (Group 1) reaching a nadir on Day 14. After the nadir, there was an increase in all dry eye groups. These findings indicate that initiation of scopolamine treatment one day earlier than initiation of compound treatment was sufficient to initiate physiological changes in the eye associated with dry eye syndrome. Even the cyclosporine-treated group showed a decrease in PRTT similar to other groups through approximately Day 7, then increased to a peak on Days 11-14, followed by a slight decrease. In the last PRTT test (Day 21) cyclosporine (Group 8), and Groups 6 and 7 all had similar PRTT values suggesting that both the mid and high-dose of the peptide of SEQ ID NO: 172 treatments have therapeutic effects similar to cyclosporine in increasing the aqueous tear production in this murine dry eye model.

25

Animals treated with the low, mid or high-dose of the peptide of SEQ ID NO: 172 produced significantly more aqueous tears compared to vehicle-treated animals. Thus, similar to TBUT, the peptide of SEQ ID NO: 172 produced generally dose-related significant increases in the production of aqueous tears in this model.

Groups treated with low, mid and high dose levels of the peptide of SEQ ID NO: 197 (0.06%, 0.25% and 0.6%, Groups 2, 3 and 4, respectively) showed generally dose-dependent increases in PRTT.

Table 7: Mean PRTT AUC Values

Group	PRTT AUC
Group 1	35.02
Group 2	39.96
Group 3	42.79
Group 4	43.17
Group 5	44.38
Group 6	44.85
Group 7	46.10
Group 8	49.44
Group 9	113.63

3. Histopathology

In this study histologic changes were generally confined to the cornea. Findings in the cornea consisted of increased keratinization of the corneal epithelial surface, increased thickness of the corneal epithelium, increased cellularity of the corneal epithelium, mildly increased incidence of mitosis of the basal epithelial layer consistent with increased epithelial cell turnover. These findings are indicative of a physiologic adaptive response to corneal drying and corneal surface irritation. Surface ulceration, corneal stromal edema and inflammatory infiltrate into the cornea were not seen in this study. The eyes in Group 9, the untreated group (normal mice, no scopolamine treatment), were within normal limits. There was some minimal nonsuppurative inflammation of the eye lids scattered throughout all groups, but the conjunctiva, retina, lacrimal glands and other parts of the eye were within normal limits. Goblet cells appeared to be within limits in all groups. Goblet cells are a primary producer of mucin which helps the tears form a stronger more adhesive film.

Mild to moderate corneal changes were noted in all groups except the untreated normal eye group (Group 9) and were slightly more severe in Group 1, the vehicle-treated group and Group 2, the low dose of the peptide of SEQ ID NO: 197, in comparison to the other treatment groups. These findings were consistent with the positive beneficial effects of increased tear production on the cornea.

When histological scores of the various treatment groups were compared to the histological scores in the cyclosporine group to determine if any other treatments produced "similar score reductions" to cyclosporine, Groups 4, 6, and 7 were found to be not significantly different than the cyclosporine group scores. Thus, these three treatments, mid and high-dose of the peptide of SEQ ID NO: 172 and the high-dose of the peptide of SEQ ID NO: 197, were the most effective, after cyclosporine, in reducing/ameliorating the corneal changes associated with this murine dry eye model.

Example 13: Effect of a JNK inhibitor on Adriamycin-induced nephropathy in rats

Adriamycin treatment induces glomerular disease in rat and mice mimicking human focal segmental and glomerular sclerosis (FSGS). In this model, tubular and interstitial inflammatory lesions occur during the disease course, partly due to heavy proteinuria. In the absence of therapy, kidney disease progresses to terminal renal failure within eight weeks. Podocyte injury is one of the initial steps in the sequences leading to glomerulosclerosis. The aim of the study was to investigate whether a JNK inhibitor could prevent the development of renal lesions and the renal failure.

Methods

30 male Sprague-Dawley rats (Charles River) were used in this study (divided into 3 groups of ten rats). Nephropathy was induced by a single intravenous injection of Adriamycin 10 mg/kg on Day 0. The JNK inhibitor of SEQ ID NO: 172 (2 mg/kg; in NaCl 0.9%) or vehicle was administered intravenously into the tail vein on Day 0. The administration volume was 0.2 ml.

The table below summarizes the random allocation:

Group N°	ADR (Day 0)	Treatment (Day 0)	Dose volume / Route of administration	Dose concentration	Number of animals
1	10 mg/kg	NaCl 0.9%	0.2 ml, IV	0	10
2	10 mg/kg	JNK inhibitor of SEQ ID NO: 172 2 mg/kg	0.2 ml, IV	1 mg/ml	10

3	NaCl 0.9%	NaCl 0.9%	0.2 ml, IV	0	10
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Each day, the general behavior and the appearance of all animals were observed. The health of the animals was monitored (moribund animals, abnormal important loss of weight, major intolerance of the substance, etc...). No rats were removed.

5

Retroorbital blood was collected at Days 7, 14, 28, 42 and 56 from 4 rats per group. Serum creatinine concentrations, blood urea and protidemia were measured using appropriate kits from Advia Chemistry 1650 (Bayer Healthcare AG, Leverkusen, Germany).

10 Two rats per group were sacrificed on Days 7, 14, 28, 42 and 56 after anesthesia. After animal sacrifice, both kidneys were collected. For histopathological examination fixed tissue specimens were dehydrated in graded alcohol solutions, cleared in toluene, and embedded in paraffin. Sections (4 μ m) were stained with periodic acid-Schiff (PAS), and Masson's trichrome staining was performed to detect collagen deposition. Glomerular and tubulointerstitial sclerosis were quantified under
15 microscope.

Results were expressed in the form of individual and summarized data tables using Microsoft Excel® Software. Numerical results were expressed as mean $\pm$ standard error of the mean (SEM). Due to the small number of animal tested, no statistical analyses was performed.

20

Results:

Effect of the JNK inhibitor of SEQ ID NO: 172 on renal function during the progression of the disease: Urea and creatinine serum levels were measured to study the renal function during the kidney
25 disease course. Because creatinine interferes with the calorimetric dosage, only urea that is a fine indicator of renal function was analyzed. Whereas urea serum levels were remarkably stable in untreated rats (below 5 mmol/l), ADR induced progressive increase of urea levels, which sharply raised from Day 28 up to 25 mmol/l at Day 41, then 48 mmol/l at Day 56 reflecting terminal renal failure (Figure 38 B). On the other hand, JNK inhibitor of SEQ ID NO: 172-treated rats exhibited an
30 urea serum level below 10 mmol/l throughout the course of the disease (Figure 38 B). These results suggest that JNK inhibitor of SEQ ID NO: 172 prevents the progression to renal disease and renal failure.

Histopathological findings (PAS and Masson trichrome staining):

ADR-induced structural changes were evaluated under light microscope. Saline-treated control rats showed morphologically normal glomeruli and tubules. On Day 8, light microscopic examination showed some areas with focal segmental glomerulosclerosis and proteinaceous casts in the ADR nephrosis group. In contrast, although some tubules were filled with proteins in JNK inhibitor of SEQ ID NO: 172-treated rats, glomeruli exhibited a normal architecture with absence or discrete mesangial hypercellularity, while the tubular structures and interstitium did not display pathological changes (Figure 39). By Day 14, ADR treated rats exhibited progressive glomerulosclerosis, hyaline deposits, tubular dilation and cast formation. The degree of glomerulosclerosis was dramatically worsened in this group and became diffuse with obvious adhesion between the glomerular tufts and the Bowman's space in most glomeruli by Day 29 and 41, associated with severe tubular atrophy and interstitial fibrosis. At Day 56, diffuse glomerular sclerosis was observed in all glomeruli (Figure 40). However, JNK inhibitor of SEQ ID NO: 172-treated rats had a relatively normal appearance at Day 8, and develop few focal and segmental glomerulosclerosis and tubulointerstitial fibrosis at Day 56 compared with ADR-treated rats. Altogether, these results strongly suggest that the JNK inhibitor of SEQ ID NO: 172 prevents the development of glomerular and tubulointerstitial fibrosis and may explain the preservation of renal function in this group.

The study results provide evidence that the JNK inhibitor of SEQ ID NO: 172 prevents the progression of glomerular and tubulointerstitial injuries induced by ADR. Moreover, this molecule preserves renal function.

Example 14: Evaluation of a JNK inhibitor on Imiquimod-induced psoriasis in mice

Imiquimod (IMQ), a ligand for TLR7 and TLR8, is a potent immune response modifier. It has been demonstrated for potent antiviral and antitumor effects in many animal models. Van der Fits et al. (The Journal of Immunology 2009, 182, P. 5836 - 5845) have demonstrated that the topical application of IMQ in BALB/c mice induced psoriasis and closely resemble human psoriasis lesion.

Methods

Female BALB/cAnNCrl mice (Charles River, age 8 to 10 weeks at study start) have been assigned to the following groups (treatment schedule):

Group	Dose (mg/kg)	Dose Volume (ml/kg)	Prep Conc. (mg/ml)	Dosing Duration (# Days)	Prep Frequency	Route	No. of animals
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Vehicle	N/A	5	N/A	Days 1, 4, 7	Days 1, 4, 7	IV	8
SEQ ID NO: 172	0.02	5	0.004	Days 1, 4, 7	Days 1, 4, 7	IV	8
SEQ ID NO: 172	0.2	5	0.04	Days 1, 4, 7	Days 1, 4, 7	IV	8
SEQ ID NO: 172	2	5	0.4	Days 1, 4, 7	Days 1, 4, 7	IV	8
Prednisolone	10	10	1	7	Daily	PO	8
Dexamethasone	0.5	5	0.1	Days 1, 4, 7	Days 1, 4, 7	IV	6

Additionally, a group of five animals has not been treated ("Naïve" group).

To demonstrate whether topical application of IMQ induced skin inflammation is accompanied by structural features characteristic for psoriasis, IMQ cream (approx. 62.5 mg Imiquimod Cream 5%) has been applied on the back of shaved skin and to the right ear of the BALB/c mice for 6 consecutive days (days 2 through 7).

In this experiment, two positive controls have been utilized. Firstly, Prednisolone at 10 mg/kg (vehicle: 1% Hydroxyethylcellulose, 0.25% Polysorbate 80, and 0.05% Antifoam in purified water) has been dosed daily and orally (group "Prednisolone"). Secondly, Dexamethasone has been administered at 0.5 mg/kg (vehicle: sterile 0.9% NaCl) on days 1, 4 and 7 via intravenous route.

The JNK inhibitor of SEQ ID NO: 172 ("SEQ ID NO: 172") has been dissolved in 0.9% NaCl. To receive three different doses (cf. above, groups table) it has been serially diluted (1:10 fold). The JNK inhibitor of SEQ ID NO: 172 was readily soluble and did not fall out of solution. The three different doses of the JNK inhibitor of SEQ ID NO: 172 (0.02, 0.2 and 2 mg/kg) have been administered to the respective groups intravenously on days 1, 4 and 7.

On day 8, animals have been sacrificed and the tissue (ear) has been fixed in 10% neutral buffered formalin. For histopathology hematoxylin-and-eosin-stained sections (cross cut) have been prepared and microscopic evaluation on the collected tissues from all animals has been performed. Methods and end-points for histopathology were similarly described in the van der Fits (2009) paper in that inflammation, epidermal hyperplasia, epidermal hyperkeratosis (rather than parakeratosis) were observed and recorded for severity grade, whereby the respective methodology from Van der Fits et al. (The Journal of Immunology 2009, 182, P. 5836 - 5845) is hereby incorporated by reference. Histopathology grading scores were excluded for either skin or ear in animals with secondary inflammatory processes (full thickness epidermal ulcers). Scores were averaged by group and

standard deviation and statistical significance were calculated. The graph in Figure 41 shows group averages (+/-) standard deviation (SD) are depicted below. Formalin-fixed, paraffin embedded skin from the dorsal surface of the mouse (BALB/c) was stained with hematoxylin and eosin (H&E) stain and assessed microscopically. An important difference from the above reference and to describe in more detail the observations of the present study: Hyperkeratosis can be defined specifically as orthokeratotic (no retained nuclei) or parakeratotic (retained nuclei). Either can occur normally in various anatomical locations and depending on species; however, both conditions are well defined in particular disease states. The van der Fits paper describes their Imiquimod (IMQ)-induced psoriasis model as causing parakeratotic hyperkeratosis similar to what is seen in the human condition, and that was a defined end-point for this study. However, the Danilenko et al. (Veterinary Pathology 2008 45:563) has shown that many rodent psoriasis models have orthokeratotic hyperkeratosis. In reality, the same lesion can sometimes exhibit both types of hyperkeratosis, and the rodents in this study had primarily orthokeratotic hyperkeratosis with rare, multifocal parakeratosis. The more general term 'hyperkeratosis' was used for grading end-points and describe in the text what type was seen (primarily orthokeratotic). Another difference from the van der Fits paper, is that they describe human patients as having decreased granulation in their stratum granulosum layer of the epidermis (and in their study, the rodent skin was reportedly similar); however, in this study, and the Danilenko review, many rodent models of psoriasis exhibit increased (hypergranulosis) granulation in this layer or the layer itself is hyperplastic.

Microscopic Histopathology end-points were graded as such:

1=MI = minimal

2=SL = slight

3=MO = moderate

4=MA = marked

5=SE = severe

Results

The JNK inhibitor of SEQ ID NO: 172 mid-dose group (statistically significant) and the JNK inhibitor of SEQ ID NO: 172 high-dose group had decreased inflammation of the ear compared to the vehicle-IMQ dose group (Fig. 41). Also the positive control groups, i.e. the Prednisolone group and the Dexamethasone group, showed decreased inflammation of the ear compared to the vehicle-IMQ dose group (both statistically significant, Fig. 41). In general, inflammation that was present in the dermis consisted of lymphocytes and macrophages admixed with fewer neutrophils. Inflammation in the epidermis, which was much less common, was primarily neutrophilic and was present in

intracorneal layers (of orthokeratotic layers) and in the intraepidermis as Munro's microabscesses. Inflammation was not present in the naïve group.

Minimal decreases in epidermal hyperplasia of the ear were also observed for the JNK inhibitor of SEQ ID NO: 172 mid-dose group that was slightly below that observed for the Prednisolone and Dexamethasone groups. Although the JNK inhibitor of SEQ ID NO: 172 mid-dose and prednisolone groups were below that of the vehicle-IMQ dose group, they were not statistically significant. No overt differences were exhibited as a dose-response treated with JNK inhibitor of SEQ ID NO: 172 for ear with regards to epidermal hyperkeratosis, however the JNK inhibitor of SEQ ID NO: 172 low-dose group, Prednisolone, and Dexamethasone groups had minimally decreased average grades compared to the vehicle-IMQ dose group. The naïve group was microscopically normal.

Example 15: Effects of a JNK inhibitor on Renal Ischemia/Reperfusion Lesions

Renal Ischemia/Reperfusion (Renal I/R) injury is a commonly employed model of acute kidney injury (AKI), also known as acute renal failure. In addition to the clinical relevance of studies that examine renal I/R injury to acute kidney injury, experimental renal I/R injury is also an important model that is used to assess the conditions that occur in patients receiving a kidney transplant. Depending upon the donor, transplanted kidneys are not perfused with blood for a variable amount of time prior to transplantation. Because AKI has such serious effects in patients, and all transplanted kidneys experience renal I/R injury to some extent, the clinical relevance and translational importance of this type of research to human health is extremely high. The aim of this study is thus to investigate the influence of the JNK inhibitor of SEQ ID NO: 172 on experimental renal ischemia/reperfusion in rats.

To this end, 26 male Wistar rats (age 5 to 6 weeks, Charles River) are assigned to the following groups:

Group N°	Pretreatment (1 hour before clamping)	Treatment (1 hour after clamping)	Dose volume / Route of administration	Concentration	Renal Ischemia time (min)	Number of animals
1	Heparine (5000 IU/kg)	NaCl 0.9%	2 ml/kg, IV	0		6

2	Heparine (5000 IU/kg)	JNK inhibitor SEQ ID NO: 172 2000 µg/kg	2 ml/kg, IV	1 mg/ml	40	10
3	Heparine (5000 IU/kg)	NaCl 0.9%	2 ml/kg, IV	0	40	10

Renal ischemia will be induced by clamping both renal pedicles with atraumatic clamp (induction of necropathy). One unique dose of the JNK inhibitor of SEQ ID NO: 172 (2000 µg/kg) will be administered intravenously (IV) into the tail vein on Day 0, one hour after clamping period (after reperfusion) both renal pedicles with atraumatic clamp. The administration volume will be 2 ml/kg. Heparin (5000 IU/kg) will be administered intraperitoneally 1 hour before clamping.

Each day, the general behavior and the appearance of all animals is observed. If animal health is not compatible with the continuation of the study (moribund animals, abnormal important loss of weight, major intolerance of the substance, etc...), animals will be ethically sacrificed under the responsibility of the Study Director. Individual rats are housed in metabolic cages (Techniplast, France). Urine is collected every 24 hours up to 72 hours. Blood samples are obtained from tail vein before, then at 24 and 72 hours after reperfusion. At the end of both periods (24 and 72 hours), 5 rats per group (3 for group 1) are sacrificed. After animal sacrifice, both kidneys are collected. Five rats per group (3 for group 1) are used at each time point (24 and 72 hours after reperfusion). For the evaluation of the renal function, serum creatinine (µmol/ml) or urea concentrations (mmol/mL) are measured with the appropriate kits (Bayer Healthcare AG, Leverkusen, Germany). For the evaluation of proteinuria and albuminuria, proteinuria and albuminuria are performed using appropriate kits from Advia Chemistry 1650 (Bayer Healthcare AG, Leverkusen, Germany).

Evaluation of histological lesions is performed 24 and 72 hours after reperfusion. For light microscopy, kidneys are incubated for 16 hours in Dubosq-Brazil, dehydrated, embedded in paraffin, cut into sections and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) reagent. Three sections will be analyzed for each staining.

For immunohistochemistry analysis, kidney samples are fixed for 16 hours in Dubosq Brazil, and subsequently dehydrated and embedded in paraffin. Antigen retrieval is performed by immersing the slides in boiling 0.01 M citrate buffer in a 500 W microwave oven for 15 min. The endogenous peroxidase activity is blocked with 0,3% H₂O₂ in methanol for 30 min. Slides are incubated with the blocking reagents consisting of the Avidin-biotin solution for 30 min and the normal blocking serum for 20 min. For immunodetection, the slides are incubated overnight with an antibody, then with a biotinylated secondary antibody. An avidin-biotinylated horseradish peroxidase complex

(Vectastain ABC Reagent, Vector Laboratories; Burlingame, CA) and 3,3'-diaminobenzidine (Sigma Biochemicals; St Louis, MO) as a chromogen is applied for visualization of the immunoreaction. Slides are counterstained with hematoxylin. Omission of the primary antibody is considered as a negative control.

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Immunofluorescence labeling is carried out on 4 mm thick cryostat sections of kidney tissue fixed in acetone for 10 min, air-dried for 30 min at room temperature, then incubated in PBS for 3 min and blocked in 1% BSA in PBS. The sections are incubated with the indicated antibodies for 1 hour at room temperature, washed in PBS and incubated with Red Texas-conjugated secondary antibodies. Sections are examined by fluorescence microscopy (Zeiss) for immunofluorescence analysis.

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The expression of several markers specific of podocyte damage, inflammation and renal fibrosis (RelA, TGF β , TNF α , Masson trichrome) is evaluated by immunohistochemistry and immunofluorescence. Quantitative transcription profile of TNF, IL6, CXCL1 (KC), CXCL2 (MIP-2) and MCP1 in kidneys are determined.

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Example 16: Inhibitory effects of a JNK inhibitor on the inflammatory response in a rat periodontitis model

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The aim of this study is to investigate the influence of the JNK inhibitor of SEQ ID NO: 172 on inflammation induced in a periodontitis model in the rat.

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30 Sprague-Dawley rats (male, 42 – 56 days old) are used in this study (divided into 4 groups of ten rats). Experimental periodontitis is induced by a ligature placed around the 1st molar (one molar per animal) on Day 0 for 10 days. One dose of 4 mg/kg of the JNK inhibitor of SEQ ID NO: 172 (in 0.9% NaCl as vehicle) is administered intragingivally (IGV) on day 10. The administration volume is 10 μ l. Administrations are performed IGV in the attached gingiva surrounding the first molar.

30

The table below summarizes the random allocation:

Group N°	Ligature (Day 0)	Treatment	Route of administration	Number of animals
1	-	-	IGV	10
2	Yes	NaCl 0.9%	IGV	10
3	Yes	SEQ ID NO: 172 4 mg/kg	IGV	10

Each day, the general behavior and the appearance of all animals is observed. If animal health is not compatible with the continuation of the study (moribund animals, abnormal important loss of weight, major intolerance of the substance, etc...), animals are ethically sacrificed under the responsibility of the Study Director. Periodontitis inflammation aspect are analyzed by macroscopic observation of gingival tissue on days 0, 10 and 17. Plaque index and gingival inflammation index are measured on day 0, 10 and 17 as periodontal clinical indices using clinical scoring.

On day 17 the animals are sacrificed and samples are collected. Gingival tissue is excised for bio-molecular analysis on all animals. After euthanasia, mandibles are excised for histological evaluation. For the evaluation of inflammatory cells, quantification of inflammatory cells is performed by histomorphometric measurements. For the evaluation of inflammatory protein levels, the level of inflammatory proteins (p-JNK, TNF, IL-1, IL-10, MMP-8, MMP-9) are measured from gingival tissue homogenates. For the evaluation of tissue destruction, bone tissue destruction is evaluated on 3 animals per group by radiological analysis (micro-CT). Periodontal complex destruction is evaluated by histological analysis. For the evaluation of bone microarchitecture, bone trabecular measurements (thickness, separation) are evaluated by radiological analysis (micro-CT) on 3 animals per group on days 0, 10 and 17. For the identification of oral bacteria, bacterial population in dental pockets are identified by DNA probes (real time PCR) on 9 periodontopathogens on days 0, 10 and 17. For the collagen framework, measurements of total collagen amount are performed using Polarized-light microscopy. The collagen I/collagen III ratio is evaluated by histomorphometrical analysis.

Example 17: Evaluation of the action duration of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") in the endotoxin-induced uveitis (EIU) model in rats

Twenty four (18) female Lewis rats (36 eyes) were randomly divided into 6 groups of 3 animals each. EIU was induced by a single footpad injection of 100 µl sterile pyrogen-free saline containing 200 µg of LPS (Lipopolysaccharides from *Salmonella typhimurium*, Sigma-Aldrich, France)(2 mg/ml).

Animals were treated 48 hours, 1 week, 2 weeks or 4 weeks before EIU induction by intravenous injection of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") at a single dose of 1 mg/kg (dose volume 1 ml/kg) in the tail vein. Dexamethasone 2 mg/kg or vehicle (NaCl 0.9 %) were injected intravenously immediately before EIU induction.

The effect of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") on EIU was evaluated using clinical scoring and PMN cells quantification 24 hours after induction. Ocular examinations were performed by slit lamp at 24 hours *i.e.* at the clinical peak of the disease in this model. The intensity of clinical ocular inflammation was scored on a scale from 0 to 5 for each eye:

- 5 Grade 0: no inflammation,
- Grade 1: presence of a minimal iris and conjunctival vasodilatation but without the observation of flare or cells in the anterior chamber (AC),
- Grade 2: presence of moderate iris and conjunctival vessel dilation but without evident flare or cells in the AC,
- 10 Grade 3: presence of intense iris vessels dilation, flare and less than 10 cells per slit lamp ,field in the AC,
- Grade 4: presence of more severe clinical signs than grade 3, with more than 10 cells in the AC with or without the formation of a hypopyon,
- Grade 5: presence of intense inflammatory reaction, fibrin formation in the AC and total
- 15 seclusion of the pupil.

Clinical evaluation was performed in a blinded manner.

For histology, eighteen eyeballs (one per animal) were collected and fixed for 1h at room temperature in phosphate buffered saline (PBS) containing 4% paraformaldehyde before being
20 rinsed overnight in PBS. The next day, samples were embedded in optimal cutting temperature (OTC) compound (Tissue-Tek®, Sakura Finetek, Zoeterwoude, Netherland) and the optic nerve level using a cryostat (Leica CM 3050S, France) and mounted on super-frost slides for histology. After nuclei staining with DAPI (Sigma-Aldrich, France), sections were mounted in PBS/Glycerol (1/1) and observed by fluorescence photomicroscopy (FXA Microphot, Nikon, USA). Digitized micrographs
25 were obtained using a digital camera (Spot, BFI Optilas, France). PMN cells identified by the shape of their nuclei stained with DAPI, were quantified on histological sections. The analysis was performed with 2 different sections per eye at the optic nerve head level.

Twenty-four hours after LPS induction, clinical scores for the vehicle-treated rats were 4.6 ± 0.2
30 (mean $\pm$ SEM, n = 8). Reduction was calculated as (grade in vehicle-treated eye – grade in test item treated eye)/(grade in vehicle-treated eye). A significant reduction (*p < 0.05, **p < 0.01) in the severity of the ocular inflammation was detected 24 hours after induction and intravenous treatment with the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") (1 mg/kg) administered 48 hours before EIU induction (mean score: 2.8 ± 0.3), corresponding to a 40% (**) decrease of EIU scores
35 compared with the score observed in vehicle group, 24 hours after induction and intravenous treatment with the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") (1 mg/kg) administered

1 week before EIU induction (mean score: 3.3 ± 0.3), corresponding to a 27% (*) decrease of EIU scores compared with the score observed in vehicle group, and 24 hours after induction and intravenous treatment with the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") (1 mg/kg) administered 2 weeks before EIU induction (mean score: 3.0 ± 0.2), corresponding to a 35% (**) decrease of EIU scores compared with the score observed in vehicle group. However, no significant reduction was observed 24 hours after induction and intravenous treatment with the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") (1 mg/kg) administered 4 weeks before EIU induction (mean score: 4.2 ± 0.1), corresponding to a 9% decrease of EIU scores compared with the score observed in vehicle group.

A single intravenous treatment with dexamethasone (2 mg/kg) immediately before EIU induction used as positive control drug also significantly reduced the clinical scores by 69% (mean score: 1.4 ± 0.2 , $p < 0.01$).

In the histological investigation the number of PMN cells was significantly decreased when the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") was administered 4 weeks ($p < 0.05$) and 2 weeks ($p < 0.01$) before EIU induction. Accordingly, the number of PMN cells was significantly decreased when dexamethasone was administered immediately before EIU induction.

Conclusion: The aim of this study was to evaluate the action duration of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") (1 mg/kg) as an anti-inflammatory in the Endotoxin-Induced Uveitis (EIU) model in rats. The effect of XG-104 on EIU was evaluated using clinical scoring and PMN cells quantification 24 hours after induction. The mean clinical score of XG-104 treated eyes 48 hours, 1 week and 2 weeks after LPS challenge was statistically different from the mean score of vehicle group with 40%, 27% and 35% reduction, respectively. The number of PMN cells was significantly decreased when XG-104 was administered 2 and 4 weeks, respectively, before EIU induction with 88% and 69% reduction compared to vehicle treated group, respectively. Dexamethasone significantly reduced the clinical score and the PMN cells number when administered immediately before LPS challenge. The action duration of XG-104 was therefore demonstrated to be between 2 and 4 weeks.

Example 18: Effects of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") on Renal Ischemia/Reperfusion Lesions

Renal Ischemia/Reperfusion (Renal I/R) injury is a commonly employed model of acute kidney injury (AKI), also known as acute renal failure. In addition to the clinical relevance of studies that examine renal I/R injury to acute kidney injury, experimental renal I/R injury is also an important model that is used to assess the conditions that occur in patients receiving a kidney transplant. Depending upon the donor, transplanted kidneys are not perfused with blood for a variable amount of time prior to transplantation. Because AKI has such serious effects in patients, and all transplanted kidneys experience renal I/R injury to some extent, the clinical relevance and translational importance of this type of research to human health is extremely high. The aim of this study is thus to investigate the influence of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") on experimental renal ischemia/reperfusion in rats.

Twenty-six (26) male Wistar rats (age 5 – 6 weeks) were used in this study (divided into 2 groups of 10 rats and 1 group of 6 rats). Rats were housed in standard cages and had free access to food and tap water. Each day, the general behavior and the appearance of all animals were observed. The health of the animals was monitored (moribund animals, abnormal important loss of weight, major intolerance of the substance, etc...). No rats were removed.

Renal ischemia was induced by clamping both renal pedicles with atraumatic clamp. A single dose of 2 mg/kg of the JNK inhibitor according to SEQ ID NO: 172 (in 0.9% NaCl as vehicle) or vehicle, respectively, was administered by IV injection in the tail vein on Day 0, one hour after clamping period (after reperfusion) both renal pedicles with atraumatic clamp. The administration volume was 2 ml/kg. Heparin (5000 UI/kg) was administered intraperitoneally 1 hour before clamping (in all groups).

The table below summarizes the random allocation:

Group N°	Treatment (1 hour after clamping)	Dose volume / Route of administration	Concentration	Renal Ischemia time (min)	Number of animals
1	NaCl 0.9%	2 mL/kg, IV	0		6
2	NaCl 0.9%	2 mL/kg, IV	0	40	10
3	XG-104 (2 mg/kg)	2 mL/kg, IV	1 mg/mL	40	10

For sample collection, rats were housed individually in metabolic cages (Techniplast, France). Urine was collected at 72 hours. Blood samples were obtained from tail vein before and at 24 hours after reperfusion. After animal sacrifice, both kidneys were collected.

For evaluation of proteinuria and albuminuria appropriate kits from Advia Chemistry 1650 (Bayer Healthcare AG, Leverkusen, Germany) were used.

For evaluation of renal function, blood was collected from the tail vein at 24 hours after reperfusion. Serum creatinine ($\mu\text{mol/mL}$) and urea concentrations (mmol/mL) were measured using appropriate kits (Bayer Healthcare AG, Leverkusen, Germany).

5 Evaluation of histological lesions was performed at 24 and 72 hours after reperfusion.

For light microscopy, kidneys were be incubated for 16 hours in Dubosq-Brazil, dehydrated, embedded in paraffin, cut into sections and stained with hematoxylin and eosin (H&E) or with
10 periodic acid-Schiff (PAS).

For immunohistochemistry, kidney samples were fixed for 16 hours in Dubosq Brazil, and subsequently dehydrated and embedded in paraffin. Antigen retrieval was performed by immersing the slides in boiling 0.01 M citrate buffer in a 500 W microwave oven for 15 min. The endogenous
15 peroxidase activity was blocked with 0,3% H_2O_2 in methanol for 30 min. Slides were incubated with the blocking reagents consisting of the Avidin- biotin solution for 30 min and the normal blocking serum for 20 min. For immunodetection, the slides were incubated overnight with an antibody, then with a biotinylated secondary antibody. An avidinbiotinylated horseradish peroxidase complex (Vectastain ABC Reagent, Vector Laboratories; Burlingame, CA) and 3,3'-
20 diaminobenzidine (Sigma Biochemicals; St Louis, MO) as a chromogen were applied for visualization of the immunoreaction. Slides were counterstained with hematoxylin. Omission of the primary antibody was considered as a negative control.

Immunofluorescence labeling was carried out on 4 mm thick cryostat sections of kidney tissue fixed
25 in acetone for 10 min, air-dried for 30 min at room temperature, then incubated in PBS for 3 min and blocked in 1 % BSA in PBS. The sections were incubated with the indicated antibodies for 1 hour at room temperature, washed in PBS and incubated with Red Texas-conjugated secondary antibodies. Sections will be examined by fluorescence microscopy (Zeiss).

30 Moreover, expression of several markers specific of podocyte damage, inflammation and renal fibrosis (RelA, TGF β , TNF α , Masson trichrome) were evaluated by immunohistochemistry and immunofluorescence. Quantitative transcription profile of TNF α , IL6, CXCL 1 (KC), CXCL2 (MIP-2) and MCP1 in kidneys were determined.

35 Results:

Results are shown in Fig. 42. Serum creatinine and urea were increased in vehicle-treated ischemic rats (G2) 24h following ischemia, as compared to vehicle-treated controls rats without ischemia (G1). On the other hand, ischemic rats treated with of the JNK inhibitor according to SEQ ID NO: 172 (G3) exhibited lower serum creatinine and lower urea, relatively to untreated ischemic rats (G2).
5 These results suggest that the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") may prevent the ischemia-induced renal failure.

Example 19: Antitumour activity of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104")
10 against human liver tumour cell lines

The aim of this study is to determine the cytotoxic activity of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") against human hepatocarcinoma and human hepatoma cell lines using MTS assay.

15 The human hepatocarcinoma cell line HepG2 (origin: American Type Culture Collection, Manassas, Virginia, USA; the HepG2 cell line was established from the tumor tissue of a 15-year old Argentine boy with a hepatocellular carcinoma in 1975, there is no evidence of a Hepatitis B virus genome in this cell line) and the human hepatoma cell line PLC/PRF/5 (origin: American Type Culture
20 Collection, Manassas, Virginia, USA; the PLC/PRF/5 cell line secrete hepatitis virus B surface antigen (HBsAg)) are used. Tumor cells are grown as monolayer at 37°C in a humidified atmosphere (5% CO₂, 95% air). The culture medium is EMEM (ref: BE12-611F, Lonza) supplemented with 10% fetal bovine serum (ref: 3302, Pan), 0.1 mM NEAA (ref: BE13-114E, Lonza) and 1 mM NaPyr (ref: BE13-115E, Lonza). The cells are adherent to plastic flasks. For experimental use, tumor cells are detached
25 from the culture flask by a 5-minute treatment with trypsin-versene (ref: BE02-007E, Lonza), in Hanks' medium without calcium or magnesium (ref: BE10-543F, Lonza) and neutralized by addition of complete culture medium. The cells are counted in a hemocytometer and their viability is assessed by 0.25% trypan blue exclusion assay.

30 Tumor cells are plated at the optimal seeding density in flat-bottom microtitration 96-well plates (ref 167008, Nunc, Dutscher, Brumath, France) and incubated in 190 µL drug-free culture medium at +37°C in a humidified atmosphere containing 5% CO₂ for 24 hours before treatment.

35 Dilutions of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") as well as distribution to plates containing cells are performed manually. At treatment start 10 µL of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") dilutions are added to wells at the following final

concentrations (for both cell lines): 0, 3.8×10^{-4} , 1.5×10^{-3} , 6.1×10^{-3} , 2.4×10^{-2} , 9.8×10^{-2} , 0.4, 1.6, 6.3, 25 and 100 μM . Then cells are incubated for 72 hours in 200 μL final volume of culture medium containing the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") at + 37°C in a humidified atmosphere containing 5% CO_2 . At the end of treatments, the cytotoxic activity is evaluated by a MTS assay.

The *in vitro* cytotoxic activity of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") is revealed by a MTS assay using tetrazolium compound (MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) and an electron coupling reagent named PMS (phenazine methosulfate). Like MTT, MTS is bio-reduced by cells into a formazan product that is directly soluble in culture medium without processing, unlike MTT. At the end of cell treatment, 40 μL of a 0.22 μM freshly filtered combined solution of MTS (20 mL at 2 mg/mL, ref: G11, Promega, Charbonnières, France) and PMS (1 mL at 0.92 mg/mL, ref: P9625, Sigma) in Dulbecco's Phosphate Buffered Saline (DPBS, ref: 17-513F, Cambrex), are added in each well. Absorbance (Optical Density, OD) is measured at 490 nm in each well using a VICTOR3™ 1420 multilabeled counter (Wallac, PerkinElmer, Courtaboeuf, France).

Individual OD values of MTS assays are provided. Dose response for index of cytotoxicity (IC) is expressed as follows:

$$\text{IC} = (\text{OD}_{\text{drug-exposed wells}} / \text{OD}_{\text{vehicle-exposed wells}}) \times 100$$

whereby IC_{50} refers to the drug concentration to obtain a 50% inhibition of cell proliferation. IC_{50} represent drug concentration required to obtain 50% of cellular cytotoxicity. Dose-response curves are plotted using XLFit5 (IDBS, United Kingdom) and provided. The IC_{50} determination values are calculated using the XLFit5 software from semi-log curves. Each individual IC_{50} determination values are provided as well as mean $\pm$ SD IC_{50} values.

Figure 45 shows the results of the determination of the cytotoxic activity of XG-104 against HepG2 and PLC/PRF/5 tumour cell lines using MTS assay.

Example 20: Effects of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") in a mouse model of psoriasis

The aim of this study is to evaluate the effects of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") in the BK5.STAT3C mouse model of psoriasis. This model uses heterozygous transgenic mice whose keratinocytes express a constitutively active form of Stat3.

5 Psoriasis is a chronic inflammatory skin disorder characterized by accelerated growth and altered differentiation of keratinocytes and angiogenesis with marked ectasia of blood vessels. It develops through interactions between the skin and immune system mediated by T cells, dendritic cells, and inflammatory cytokines. *In vivo* modelling of the disease has been achieved with varying degrees of success. While xenotransplantation models reflect the human disease the best, they are more elaborate and complex. One of the transgenic mouse models resembling human psoriasis the most is the K5.STAT3C model. K5.STAT3C mice express the constitutively active form of the transcription factor Stat3 in basal keratinocytes, and upon tape stripping develop skin lesions that histologically recapitulate hallmarks of psoriasis. As in human psoriasis, T cells played a critical role for the induction of the psoriatic phenotype. Here, an evaluation of efficacy for compound XG-104 was performed in the K5.STAT3C mouse model of psoriasis.

To this end, two independent experiments are performed, each with at least 15 mice per experiment (5 mice per group). In each experiment, BK5.STAT3C heterozygous transgenic mice (*Tg(KRT5-Stat3*^{A661C}*N663C)1/dg*; henceforth referred to as K5.STAT3C mice) were randomly allocated to the following experimental groups 1 and 2, and wild-type littermates were allocated to group 3:

Group	n	Treatment	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Vol. (mL/kg)	Freq.	Route	Treatment Schedule
1	5 (tg)	Vehicle	n/a	n/a	10	QD	IP	D0-D5
2	5 (tg)	XG-104	5	0.5	10	QD	IP	D0-D5
3	5 (wt)	Vehicle	n/a	n/a	10	QD	IP	D0-D5

Group 1 (Vehicle, BK5.STAT3C transgenic mice):

Vehicle (0.9% NaCl) is given once a day (QD) by intraperitoneal injection from Day 0 to Day 5.

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Group 2 (XG-104, BK5.STAT3C transgenic mice):

The JNK inhibitor according to SEQ ID NO: 172 ("XG-104") is given once a day (QD) by intraperitoneal injection from Day 0 to Day 5.

30 Group 3 (Vehicle, FVB wild type mice)

Vehicle (0.9% NaCl) is given once a day (QD) by intraperitoneal injection from Day 0 to Day 5.

On Day 0, psoriasis is induced by tape stripping (12-18 times) the back of the mouse after depilation. To this end, mice were anaesthetized using a Ketamine/Xylazine cocktail (100mg/kg and 10mg/kg respectively) intraperitoneally, shaved, depilated and skin injury was applied by 15 gentle strokes of tape stripping. Mice were treated with either vehicle (saline) or XG-104 right before anaesthesia on day 0 and once a day thereafter for 5 days. Mice were sacrificed after 5 days of experimentation by dislocation and lesions were excised for histological evaluation. Two independent experiments were performed with 5 mice per group (wt vehicle; tg vehicle; tg XG-104).

Animals are weighed once on day 0. All animals are observed for signs of ill health daily throughout the study. After termination of the study at day 5, approximately 1cm² skin biopsy (lesions) is collected on the back of each mouse, embedded with Optimal Cutting Temperature (OCT)-containing moulds, frozen on dry ice, sectioned in 6µm slices using a cryotome and stained with H&E. Epidermal thickness (acanthosis) is histologically measured blinded by two independent experimenters. One slide per mouse and 10 different zones of the sections are measured.

As compared to wild-type littermates, tape stripping induced a thickened epidermis (acanthosis) on all K5.STAT3C mice treated with vehicle, with prominent sites of parakeratosis (Figure 43 A). Treatment with XG-104 significantly reduced the acanthosis in both experiments (Figure 43 A). Pooling of the values of both experiments (Figure 43 B), adds further power to the observations by increasing the statistical significance ($p < 0.001$). Additionally, we observed absence of any parakeratosis (retention of nuclei in the corneal layer) over the extent of the skin biopsies in XG-104 treated mice as compared to mice treated with vehicle (Figure 44). Taken together, these data show that XG-104 is able to efficiently inhibit the induction of a psoriatic phenotype in the K5.STAT3C mouse.

Thus, the results confirm that XG-104 treatment is sufficient to block the development of a psoriatic phenotype in the K5.STAT3C mouse for psoriasis.

Example 21: Safety, tolerability and pharmacokinetics of single and repeated topical doses of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") administered to healthy male volunteers in a randomized, double blind, placebo controlled at each dose level Phase I study

The primary objective of the study was to assess determine the tolerability and safety of single and repeated instillations of ascending doses of XG-104 (the JNK inhibitor according to SEQ ID NO: 172) in healthy male volunteers. The secondary objective of the study was to assess the systemic pharmacokinetics of single and repeated instillations of ascending doses of XG-104 in healthy male
5 volunteers.

XG-104 was prepared as solution for instillations (in NaCl 0.9%). NaCl 0.9% served as placebo. A total of 49 subjects were included in this study, 28 in the "Single dose part" of this study and 21 in the "Multiple dose part" of this study. In the single dose part, the 28 subjects included were
10 randomised between one of the 5 groups: 5 subjects in each of the 4 XG-104 groups (0.1 mg, 0.2 mg, 0.4 mg, 0.8 mg) (0.2, 0.4, 0.8 and 1.6%, respectively) and 8 subjects in the placebo group. In the multiple dose part, the 21 subjects included were randomised between one of the 4 groups: 5 subjects in each of the 3 XG-104 groups (0.1 mg, 0.2 mg, 0.4 mg) and 6 subjects in the placebo group. All subjects completed the study.

In the "Single dose part" of this study, single topical doses of 0.1, 0.2, 0.4 and 0.8 mg (0.2, 0.4, 0.8
and 1.6%, respectively) of XG-104 were tested. A single administration of XG-104 or placebo was
performed on day 1 (D1) (one drop in the right eye). In the single dose part, subjects were
hospitalized for approximately 36h, namely from day -1 (D-1) evening to D2 morning. On day 3 an
20 ambulatory visit was performed and the study ended with a visit at D4.

In the "Multiple dose part" of this study, three dose levels were tested. Doses were chosen according to the results of the single dose part as follows: first group: 0.1 mg (0.2%), second group: 0.2 mg (0.4%), and third group: 0.4 mg (0.8%). XG-104 or placebo was administered tid ("*ter in die*" – three
25 times daily), each time 1 drop in the right eye, from D1 to D21. The administration took place at around 8 a.m., 12 a.m. and 8 p.m.. In the multiple dose part, subjects were hospitalized from D-1 evening to D22 morning, whereby the end of the study was a visit at D36 (± 2). For more flexibility, subjects had the possibility to perform some visits in ambulatory. In that case visits were the following: hospitalization for approximately 36h, D-1 evening to D2 morning, ambulatory visits
30 from D2 to D7, hospitalization for approximately 36h, D7 evening to D9 morning, ambulatory visits from D9 to D14, hospitalization for approximately 36h, D14 evening to D16 morning, ambulatory visits from D16 to D20, and hospitalization for approximately 36h, D20 evening to D22 morning. The end of the study was a visit at D36 (± 2), accordingly.

The safety parameters evaluated included: physical examination; vital signs (BP, PR); 12-lead ECG (electrocardiogram); fundus of the eye, intraocular pressure, slit lamp examination, best-corrected
35

visual acuity, redness; Schirmer's tear test, TBUT (tear film break-up time); clinical laboratory tests (haematology, haemostasis, clinical chemistry and urinalysis); adverse events; and assessment of tolerability.

- 5 The pharmacokinetic parameters evaluated included the following plasma parameters: $C_{\max}$, $T_{\max}$, AUC_{0-12} , AUC_{0-24} , AUC_t , AUC_{inf} , Kel , $t_{1/2}$, $\%AUC_{extra}$, V_d/F , Cl/F .

Results:

10 1. Pharmacokinetic Results:

In this study, six hundred human plasma samples were analyzed to quantify XG-104. All standard samples. QC samples and calibration curve parameters met the acceptance criteria. However, all concentration measured in specimen samples were below the LLOQ (lower limit of quantification: < 40 ng/mL).

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2. Safety Results:

2.1 Single dose part

During the overall study period, 15 out of 28 subjects reported the occurrence of 34 adverse events. 32 of these were treatment emergent adverse events (TEAEs) and 2 were non-emergent (blood creatine phosphokinase increased and neck pain). Among the TEAEs, 21 were experienced after XG-104 administration and 11 after placebo administration. All were of mild intensity. The most reported TEAEs were eye disorders: 17 reported on the right eye (treated) and 13 reported on the left eye (non-treated). Among TEAEs reported on the right eye, 3 were probably related (all in the XG-104 0.8 mg (1.6%) group) to study drug administration (conjunctivitis (2) and conjunctival hyperaemia (1)) and 14 were unrelated (punctate keratitis (12): 5 in the placebo group, 2 in the XG-104 0.1 mg (0.2%) group, 2 in the XG-104 0.2 mg (0.4%) group, 2 in the XG-104 0.4 mg (0.8%) group, 1 in the XG-104 0.8 mg (1.6%) group; and eye pruritus (1) in 0.1mg (0.2%) and conjunctival hyperaemia (1) in 0.2mg (0.4%) group). All TEAEs reported on the left eye were unrelated to study drug administration (punctate keratitis (12), conjunctival hyperaemia (1)). No serious adverse events were reported during this study. No clinically relevant findings were observed in clinical examination, vital signs or ECG parameters.

A single dose of XG-104 0.1 mg, 0.2 mg, 0.4 mg was well tolerated and a single dose of XG-104 0.8 mg (1.6%) was rather well tolerated (2 episodes of conjunctivitis). The choice for the three dose-levels in repeated doses was: 0.1 mg, 0.2 mg and 0.4 mg (0.2, 0.4 and 0.8%, respectively).

35

2.2 Multiple dose part

During the overall study period, 17 out of 21 subjects reported the occurrence of 66 adverse events. 65 of these were treatment emergent adverse events (TEAEs) and 1 was non-emergent (punctate keratitis). Among the TEAEs, 61 were experienced after XG-104 administration and 4 after placebo administration. All were of mild to moderate intensity. The most reported TEAEs were eye disorders: 44 reported on the right eye (treated) and 18 reported on the left eye (non-treated). Among TEAEs reported on the right eye, 3 were probably related to study drug administration (conjunctivitis (2) in the XG-104 0.1 mg (0.2%) group and eyelid irritation (1) in the XG-104 0.2 mg (0.4%) group); 39 were unlikely related (conjunctival hyperaemia (21): 6 in the XG-104 0.1 mg (0.2%) group, 5 in the XG-104 0.2 mg (0.4%) group and 10 in the XG-104 0.4 mg (0.8%) group; punctate keratitis (17): 6 in the XG-104 0.1 mg (0.2%) group, 6 in the XG-104 0.2 mg (0.4%) group and 5 in the XG-104 0.4 mg (0.8%) group; conjunctivitis (1) in the XG-104 0.4 mg (0.8%) group); 2 were unrelated (punctate keratitis (2): 1 in the placebo group and 1 in the XG-104 0.2 mg (0.4%) group). Among TEAEs reported on the left eye, 12 were unlikely related to study drug administration (conjunctival hyperaemia (7): 2 in the XG-104 0.1 mg (0.2%) group, 3 in the XG-104 0.2 mg (0.4%) group and 2 in the XG-104 0.4 mg (0.8%) group; punctate keratitis (5): 2 in the XG-104 0.1 mg (0.2%) group, 1 in the XG-104 0.2 mg (0.4%) group and 2 in the XG-104 0.4 mg (0.8%) group); 6 were unrelated to study administration (punctate keratitis (6): 1 in the placebo group, 1 in the XG-104 0.1 mg (0.2%) group and 4 in the XG-104 0.2 mg (0.4%) group). Ophthalmic examination confirmed a global dose effect (lower abnormal examination in the placebo group) increasing between day 3 and day 20.

No serious adverse events were reported during this study. No clinically relevant findings were observed in clinical examination, biological parameters, vital signs or ECG parameters. A repeated dose t.i.d. of XG-104 (0.1 mg, 0.2 mg, 0.4 mg) in 21 healthy male subjects was well tolerated.

Example 22: Efficacy and safety of a JNK inhibitor for the treatment of dry eye (Clinical Phase II)

A multicenter, randomized, double-masked, placebo-controlled, clinical phase II study served to assess the efficacy and safety of ophthalmic solutions of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104") in the environment and during challenge in the controlled adverse environment (CAESM) model for the treatment of dry eye. The purpose of this study is to assess the efficacy and safety of ophthalmic solutions of the JNK inhibitor according to SEQ ID NO: 172 compared to placebo for the treatment of the signs and symptoms of dry eye after a four week TID treatment period.

The "Controlled Adverse Environment" model is an environment designed and constructed to provide an environmental challenge to aggravate a clinical condition under study. In general, a controlled adverse environment (CAE) design can be used to control the environment, the subjects' activities, or a combination of both during the clinical trial, thereby providing a stressful environment to exacerbate clinical symptoms and signs of dry eye. Such a stress test is especially valuable in establishing a pharmacological effect in a short period of time. Humidity, temperature, and air-flow are environmental variables that can be monitored and manipulated. Activities can include visual tasks, and the blink rate and tear film stability can be monitored. Thus, the controlled adverse environment (CAESM) model is a clinical model that reproduces a standard ocular challenge (by regulation of humidity, temperature, airflow, lighting conditions, and visual tasking) for the investigation of treatments for dry eye. A key aspect of the CAE is its utility in distinguishing subpopulations of dry eye patients. Subjects challenged by environmental changes (such as those presented by the CAE) normally respond with some degree of physiological compensation, and previous studies have shown that the ability of these mechanisms to adequately compensate for environmental challenges is reduced in those with dry eye.

In this study the subjects are subjects who were diagnosed with dry eye (have a reported history for dry eye for at least six months prior to Visit 1, have a history of use or desire to use eye drops for dry eye symptoms within 6 months of Visit 1, report a score of 2 or higher in at least one symptom on the Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire assessed pre-CAESM, at Visits 1 and 2, have a conjunctival redness score = 1 on the Ora Calibra™ scale at Visits 1 and 2, have a total corneal fluorescein staining score = 2 in at least one region according to the Ora Calibra™ Scale at Visits 1 and 2, pre-CAESM etc.).

Three different concentration of an ophthalmic solution of the JNK inhibitor according to SEQ ID NO: 172 ("XG-104"), namely XG-104 0.2 %, XG-104 0.4 %, and XG-104 0.8 % are compared versus placebo (vehicle: NaCl 0.9%) eye drops efficacy. Visits # 1, 2, 3, and 4 are planned at days -7, 1, 15, and 29 respectively.

In particular corneal fluorescein staining in the inferior region change from Pre-CAESM to Post-CAESM, as measured by the Ora Calibra™ Scale, in the worst eye at baseline, preferably at visit 4 at day 29, and the worst dry eye symptom determined from subject diary data recorded preferably during the 1-week run-in period between Visit 1 and Visit 2, evaluated over the 7 days preceding Visit 4 (not including day of visit) during the treatment period, serve as primary outcome measures.

Secondary outcome measures are in particular (i) fluorescein staining (using Ora Calibra™ Scale and NEI Scale), preferably at visits 3 and 4 (pre- and post-CAESM; regions: central, superior, inferior (visit 3 preferably only as visit 4 is the primary endpoint), temporal, nasal, corneal sum, conjunctival sum and total), (ii) Lissamine green staining (using Ora Calibra™ Scale and NEI Scale), preferably at
5 visits 3 and 4 (pre- and post-CAESM and change from pre- to post-CAESM; regions: central, superior, inferior, temporal, nasal, corneal sum, conjunctival sum, and total), (iii) tear film break-up time, preferably at visits 3 and 4 (pre- and post-CAESM), (iv) conjunctival redness using Ora Calibra™ Scale, preferably at visits 3 and 4 (pre- and post-CAESM), (v) lid margin redness using Ora Calibra™ Scale, preferably at visits 3 and 4 (pre- and post-CAESM), (vi) tear osmolarity, preferably at visits 2
10 and 4 (post-CAESM), (vii) blink rate, preferably at visits 3 and 4 (pre-CAESM) using Ora Calibra™ methodology, (viii) ocular protection index (OPI 2.0), preferably at visits 3 and 4 (pre-CAESM) using Ora Calibra™ methodology, (ix) unanesthetized Schirmer's Test, preferably at visits 3 and 4 (pre-CAESM), drop comfort and symptom assessment using Ora Calibra™ Scales after randomization, preferably at visits 2 and 3, (x) ocular surface disease index (OSDI), preferably at visits 3 and 4 (pre-
15 CAESM), (xi) ocular discomfort using Ora Calibra™ Scale, preferably at visits 3 and 4 (pre and post-CAESM), (xii) ocular discomfort using Ora Calibra™ Discomfort and 4-Symptom Questionnaire, preferably at visits 3 and 4 (pre- and post-CAESM), (xiii) ocular discomfort using Ora Calibra™ Scale, preferably at visits 3 and 4 (during CAESM exposure), and (xiv) daily diary.

20 Other pre-specified outcome measures include in particular visual acuity (ETDRS), preferably at visits 1, 2, 3 and 4 (Days -7, 1, 15 and 29) (pre-CAESM), slit-lamp biomicroscopy, preferably at visits 1, 2, 3 and 4 (Days -7, 1, 15 and 29) (pre- and post-CAESM), adverse event query, dilated funduscopy biomicroscopy, preferably at visits 1 and 4 (Days -7 and 29), and intraocular pressure, preferably at visits 1 and 4 (Days -7 and 29).

25

Example 23: Effects of XG-104 (the JNK inhibitor according to SEQ ID No. 172) in a rat model of kidney bilateral ischemia reperfusion

30 This study is based on the previous study of XG-104 in renal ischemia/reperfusion (Example 15). The aim of the study was to evaluate the effect of XG-104 on histological damages in a rat model of kidney bilateral ischemia reperfusion.

35 Ischemia reperfusion (IR) injury is a complex phenomenon, which is often encountered in vascular surgery, organ procurement and transplantation in humans. The experimental model of kidney bilateral ischemia reperfusion (IR) in rodents leads to an acute tubular injury characterized by

impaired kidney function and tubular degeneration. The present model is frequently used for providing a rapid proof of concept for the use of drug candidates in preventing renal IR damages.

Male Sprague-Dawley rats weighing 200-250 g at delivery were used (Charles River Laboratories, L'Arbresle, France). Animals were delivered to the laboratory at least 5 days before the experiments during which time they were acclimatized to laboratory conditions. This study included 3 groups of 11-12 rats each, as follow:

Group	IR surgery	Treatment (<i>i.v.</i>)	Number of animals/group
1	Sham-operated	vehicle	12
2	yes	vehicle	11
3	yes	XG-104 (2 mg/kg)	11

The study design is shown in Figure 46.

The protocol of warm renal ischemia was similar to that previously described (Pechman KR *et al.*, 2009). Briefly, under general anesthesia (pentobarbital; 60 mg/kg, *i.p.* and atropine; 1 mg/kg, *i.p.*), both renal pedicles were isolated and clamped for 40 minutes using atraumatic clamps. After this time, clamps were released to start reperfusion. Animals were maintained at 37°C using a thermo-regulated system (TCAT-2LV Controller, Physitemp Instruments, Clifton, NJ, USA) during the surgery. All the animals were sacrificed 24 hours after the release of both vascular clamps (reperfusion). Sham-operated animals underwent the same surgical procedure without clamping of the kidney vessels.

XG-104 or vehicle (0.9% NaCl) were administered into the tail vein (*i.v.*) at the dose of 2 mg/kg twenty minutes after the release of the second vascular clamp. Intravenous administrations into the tail vein were performed using the volume of 1 mL/kg.

After sacrifice, kidneys were removed, cleaned from all connective tissue and capsule and weighted on an electronic balance (VWR, France). One kidney was transferred in formalin solution 10% (Sigma Aldrich, France) for at least 24 h and then transferred in ethanol 70% for further histological analysis performed by Histalim (Montpellier, France). Right and left kidneys were randomly chosen. Kidney samples were fixed in 10% formalin during 72 hours, transferred into 70 % ethanol, then

embedded in paraffin blocks by Histalim (Montpellier, France). One longitudinal section (3 to 5 μ m) was made per block. Kidney sections of paraffin embedded tissue were stained by hematoxylin and eosin (H&E). All the slides were digitalized at X20 magnitude using Nanozoomer 2.0 HT from Hamamatsu (Hamamatsu, Japan). Each tissue section was examined histologically in a blinded manner to determine if tubular changes were present. The severity of each finding was then graded as follows:

Tubular damage score consisted of either degeneration/necrosis, tubular epithelial vacuolation, regeneration (basophil tubules), and tubular cast:

0: <5% tubules affected (background)

1: 5-20% of tubules affected

2: 21-40% of tubules affected

3: 41-75% of tubules affected

4: >75% of tubules affected

As shown in Figure 47, Group 2 (IR/Vehicle) animals showed a significant increase of tubular damages including tubular degeneration and necrosis, tubular cast formation, and basophilic tubules compared to Sham/Vehicle animals. XG-104 showed significant beneficial effects on tubular damages, specifically on tubular degeneration, necrosis and tubular cast formation (Figure 47) and on the total tubular score (Figure 48). The main difference in term of tubular degeneration and necrosis between animals from XG-104 treated rats (Group 3) and vehicle (Group 2) animals is that the number of tubules affected was lower, and the lesions were mostly limited to the cortico-medullary junction and not extended to the superficial cortex. Kidneys from Group 3 (IR/XG-104) presented also a less severe score for tubular casts when compared to Group 2 (IR/Vehicle). Representative images of these histologicals changes are included in Figure 49.

In particular, tubular changes in Group 1 (Sham/Vehicle) were limited to the presence of single to a few basophilic tubules (Score 1) in 3/12 animals (Figure 47). This incidence is within expected normal limits in naïve young adult control rats and was considered as incidental in origin. Comparatively, all animals in Group 2 (IR/Vehicle) presented moderate to marked (Score 3 and 4) tubular epithelial degeneration and necrosis (3.45 ± 0.52). The most affected tubules were concentrated at the cortico-medullary junction and were histologically characterized by tubules containing large clumps of sloughed and necrotic epithelial cells. Tubular degenerative lesions were also present in most of the cortex in animals with the most severe lesions (Score 4). In addition to tubular degeneration, all animals showed a large number of tubular casts in lumen (Score 3). The presence of small to moderate number of basophilic tubules (Score 1 and 2, mean= 1.36 ± 0.67) was also observed throughout the cortex in 10/11 animals of Group 2 (IR/Vehicle). The basophilic

tubules were indicative of early epithelial regeneration in tubules. For Group 3 (IR/XG-104), tubular lesions were essentially of the same nature and appearance to that observed in Group 2 (IR/Vehicle), but were generally less severe in distribution.

5 More specifically, the mean tubular epithelial degeneration / necrosis score were 2.67 ± 0.65 and 2.18 ± 0.75 in Group 3 (IR/XG-104), respectively. The main difference between Group 2 (IR/Vehicle) and Group 3 (IR/XG-104) was that several animals in the latter group showed a score of 2 (5/12 in Group 3 and 0/11 in Group 2). Finally, only 1/12 animal in Group 3 had a score of 4 comparatively to 5/11 for Group 2. Histologically, the main difference in term of tubular degeneration and necrosis
10 between animals from Group 3 (IR/XG-104) in comparison to Group 2 (IR/Vehicle) was that the number of tubules affected was lower, and the lesions were mostly limited to the cortico-medullary junction and were not extended to the superficial cortex. Group 3 (IR/XG-104) and kidneys presented also a less severe score for tubular casts when compared to Group 2 (IR/Vehicle). Actually, tubular cast scores were 2.50 ± 0.52 in Group 3 (IR/XG-104). In comparison, Group 2 (IR/vehicle)
15 tubular cast score was 3.00 ± 0.00 . The number of basophilic tubules in Group 3 (IR/XG-104) were very comparable to that observed in Group 2. The mean basophilic tubule score for Group 3 (IR/XG-104) were respectively, 1.33 ± 0.65 and 1.73 ± 0.47 ; the score for Group 2 was 1.36 ± 0.67 (Figure 97).

20 More specifically, the mean tubular epithelial degeneration / necrosis score was 2.18 ± 0.75 in Group 3 (IR/XG-104), respectively. The main difference between Group 2 (IR/Vehicle) and Group 3 (IR/XG-104) was that several animals in the latter group showed a score of 2 (8/11 in Group 3 and 0/11 in Group 2). In addition, 1/11 had a score of 1 in Group 3. Finally, only 1/11 animal in Group 3 had a score of 4 comparatively to 5/11 for Group 2. Histologically, the main difference in term of
25 tubular degeneration and necrosis between animals from Group 3 (IR/XG-104) in comparison to Group 2 (IR/Vehicle) was that the number of tubules affected was lower, and the lesions were mostly limited to the cortico-medullary junction and were not extended to the superficial cortex. Group 3 (IR/XG-104) kidneys presented also a less severe score for tubular casts when compared to Group 2 (IR/Vehicle). Actually, tubular cast scores was 2.09 ± 0.54 in Group 3 (IR/XG-104), respectively. In
30 comparison, Group 2 (IR/vehicle) tubular cast score was 3.00 ± 0.00 . The number of basophilic tubules in Group 3 (IR/XG-104) was very comparable to that observed in Group 2. The mean basophilic tubule score for Group 3 (IR/XG-104) was 1.73 ± 0.47 ; the score for Group 2 was 1.36 ± 0.67 (Figure 97).

35 There was no tubular vacuolation observed in any of the four experimental groups. Accordingly, the total tubular score in Group 1 (Sham/Vehicle) was very low as expected (0.25 ± 0.45) since only

few animals presented basophilic tubules without any other tubular changes. In Group 2, the total tubular score was the highest among the four experimental groups, and ranged from 6 to 9 (7.82 ± 0.98). Group 3 total tubular score was relatively lower to that observed in Group 2 (IR/vehicle) with scores ranging from 4 to 8 (6.00 ± 1.26). The differences observed between Group 2 (IR/vehicle) and Group 3 (IR/XG-104) were considered to be biologically significant.

Taken together, XG-104 showed significant beneficial effects on tubular damages and specifically on tubular degeneration, necrosis and tubular cast formation. The main difference in term of tubular degeneration and necrosis between animals from XG-104 treated rats (Group 3) and vehicle (Group 2) IR animals is that the number of tubules affected was lower, and the lesions were mostly limited to the cortico-medullary junction and not extended to the superficial cortex. Kidneys from Group 3 (IR/XG-104) presented also a less severe score for tubular casts when compared to Group 2 (IR/Vehicle).

Example 24: Effects of XG-104 (the JNK inhibitor according to SEQ ID No. 172) administered intravesically on acute cystitis model induced by cyclophosphamide in conscious rats: Evaluation of visceral pain and urinary bladder inflammation

The aim of the present study was to evaluate the effects of intravesical treatment with XG-104 (50 mg/mL) on urinary bladder pain and inflammation in acute CYP-induced cystitis in female Sprague-Dawley rats. This preclinical model is well-used to test therapeutic approaches for the treatment of interstitial cystitis / painful bladder syndrome (IC/PBS).

Adult female Sprague-Dawley rats (Janvier Labs, Le Genest Saint Isle, France), weighing 215 ± 20 g at the beginning of the experiments, were used. Animals were acclimatized to the laboratory conditions for at least 3 days before the start of any experiments. The animals were allocated to the following four experimental groups (n=10 animals per group):

Group	Injection (i.p.)	Treatment (i.ves.)	n
1	Saline	Vehicle (500 μ L, i.ves.)	10
2	CYP	Vehicle (500 μ L, i.ves.)	10
3	CYP	XG-104 (50 mg/mL, i.ves.)	10
4	CYP	Ibuprofen (50 mg/mL, i.ves.)	10

To induce acute cystitis, a single i.p. injection of CYP at a dose of 150 mg/kg in a final volume of 5 mL/kg was performed. Control rats received physiological saline under the same experimental conditions as CYP (final volume of 5 mL/kg, i.p.).

- 5 On the day of each experiment, weight of rats was recorded. Then, in a randomized manner, 500 µL of XG-104 (50 mg/mL), ibuprofen (50 mg/mL) or vehicle were intravesically infused during 30 min under isoflurane anesthesia (2% - 3%).

Assessment of referred visceral pain using von Frey filaments:

- 10 Standardized conditions including fixed time-of-day (a.m. to minimize the potential circadian variations in the behaviours responses) and single-experimenter testing of all animals were applied to minimize variability behavior-based pain testing. Visceral pain including allodynia and hyperalgesia was evaluated by applying to the lower abdomen, close to the urinary bladder, a set of 8 calibrated von Frey filaments of increasing forces (1, 2, 4, 6, 8, 10, 26 and 60 g) with an
15 interstimulus interval of 5 seconds. Prior testing, the abdominal area designed for mechanical stimulation of each animal was shaved. Animals were then placed on a raised wire mesh floor under individual transparent Plexiglas box and acclimatized for at least 30 minutes before starting the von Frey test. Filaments were then applied 1-2 seconds through the mesh floor with enough strength to cause the filament to slightly bend. Each filament was tested 3 times. Care was taken to stimulate
20 different areas within the lower abdominal region in the vicinity of the urinary bladder to avoid desensitization.

Nociceptive behaviors were scored for each animal and each filament as follows:

Score	Behavior
0	no response
1	reaction of the animal (<i>e.g.</i> retraction of the abdomen)
2	reaction of the animal and change of position
3	reaction of the animal, change of position and licking of the site stimulated with von Frey filaments and / or

25 The study design is schematically shown in Fig. 50 A. Briefly, acute cystitis was induced by CYP injection (i.p.) at D0 (as described above). XG-104, ibuprofen or vehicle was intravesically administrated once just after CYP injection (as described above). Von Frey testing was performed in a non-blinded manner as follow:

- At D-1, rats were acclimatized to the individual Plexiglas box for a minimum of 30 min and to the von Frey filaments application, in order to decrease the level of stress due to the new environment.
- At D0, von Frey testing was performed 15 min before CYP or saline injection in order to obtain basal values (D0, T=-15min).
- At D1, von Frey testing was performed 24 hours after CYP or saline injection in order to analyze test compounds effect on CYP-induced visceral pain (D1, T=+24h).
- Just after von Frey testing (+24h), rats were anesthetized for blood samples collection, then sacrificed and urinary bladders were collected as described below.

At the end of the experiment, rats were sacrificed by injection of pentobarbital (54.7 mg/mL, 0.5 mL/rat, i.p.) followed by cervical dislocation. Urinary bladders were rapidly collected and cleaned from lipid tissue. Urinary bladders were weighed, cut at the bladder neck as well as oedema and haemorrhage scoring was performed (see table below). Finally, wall thickness was measured using a digital caliper by placing the bladder wall between the two outside jaws. Urinary bladder oedema and haemorrhage scores were adapted from Gray's criteria (Gray *et al.*, 1986) as follows:

Scores	Oedema	Haemorrhage
0	absent - normal aspect	absent - normal aspect
1	mild - between normal and moderate	telangiectasia - dilatation of the mucosal blood vessels
2	moderate - fluid confined to the internal mucosa	petechial haemorrhages - mucosal pinpoint red dots (glomerulation)
3	severe - fluid seen inside and outside bladder walls	Hemorrhagic spots with blood clots

Nociceptive parameters are expressed as follows:

Parameters	Expression	Description
nociceptive threshold	g	von Frey filament for which a first score of at least 1 (for 3 applications) is obtained
nociceptive scores	%	% of the maximal response (maximum score = 9) for 3 pooled applications

area under the curve (AUC) 1-8 g (allodynia)	% scores x g	plot of individual percentage of nociceptive scores against von Frey forces from : 1 to 8 g or 8 to 60g
area under the curve (AUC) 8- 60 g (hyperalgesia)		

AUCs were calculated using GraphPad Prism® (GraphPad Software Inc., La Jolla, CA, USA). The AUCs method to assess allodynia and hyperalgesia is schematically shown in Figure 50 B.

5 Macroscopic parameters are expressed as follows:

Parameters	Expression
whole urinary bladder weight	mg and % of body weight
oedema	scores
haemorrhage	scores
urinary wall thickness	mm

Results:

10 Before CYP injection, no significant difference in the nociceptive parameters were observed between the 3 different CYP-injected groups. In order to analyse effect of XG-104 on CYP-induced visceral pain, nociceptive parameters were compared between the Vehicle- and the XG-104-treated groups. Twenty-four hours after CYP injection, nociceptive threshold was significantly increased by XG-104 treatment as compared to vehicle ($p < 0.01$, Figure 51 A). XG-104 treatment also significantly
15 decreased nociceptive scores in CYP-injected rats as compared to vehicle ($p < 0.001$, Figure 51 B). In addition, AUC 1-8 g was significantly decreased by XG-104 treatment as compared to vehicle ($p < 0.001$, Figure 51 C). Similarly, AUC 8-60 g was reduced by XG-104 treatment as compared to vehicle ($p < 0.01$, Figure 51 D). In order to analyse the effects of ibuprofen on CYP-induced visceral pain, nociceptive parameters were compared between the Vehicle- and the Ibuprofen-treated
20 groups. Nociceptive threshold was significantly increased by ibuprofen treatment as compared to vehicle in CYP-injected rats ($p < 0.01$, Figure 51 A). Similarly in the Ibuprofen group significant decrease of nociceptive scores was observed as compared to vehicle ($p < 0.01$, Figure 51 B). In addition, AUC 1-8 g and AUC 8-60 g were significantly decreased by ibuprofen treatment as compared to vehicle ($p < 0.001$ and $p < 0.05$, Figures 51 C and 51 D, respectively).

25

Moreover, urinary wall thickness was significantly decreased in XG-104-treated rats ($p < 0.01$, Figure 52 A). XG-104-treatment induced urinary wall thickness decrease was associated with a significant

decrease in oedema scores whereas no significant change in haemorrhage scores was observed as compared to vehicle ($p < 0.05$ and $p > 0.05$ for oedema and haemorrhage scores respectively, Figure 52 B and 52 C). For ibuprofen, also a significant decrease was observed in urinary bladder wall thickness and in oedema scores ($p < 0.001$ and $p < 0.05$, Figure 52 A, B). However, no significant change was observed regarding haemorrhage scores ($p > 0.05$, Figure 52 C) in the Ibuprofen-treated group. It is noteworthy that reddish urine was noticed for some animal in the Ibuprofen-treated group.

Taken together, intravesical treatment of XG-104 (50 mg/mL) significantly reversed visceral pain induced by CYP, 24h after its injection. XG-104 efficiently inhibited both allodynia and hyperalgesia. On analyzed inflammatory parameters, XG-104 decreased urinary bladder inflammation (wall thickness) and oedema scores. In conclusion, administered intravesically, XG-104 displayed strong antinociceptive effects and significant anti-inflammatory properties in an experimental model of IC/PBS.

Example 25: Effects of XG-104 (the JNK inhibitor according to SEQ ID No. 172) administered intravenously on acute cystitis model induced by cyclophosphamide in conscious rats: Evaluation of visceral pain

The aim of the present study was to evaluate the effects of intravenous treatment with XG-104 (2 mg/kg) on urinary bladder pain in acute CYP-induced cystitis in female Sprague-Dawley rats. This preclinical model is well-used to test therapeutic approaches for the treatment of interstitial cystitis / painful bladder syndrome (IC/PBS).

Adult female Sprague-Dawley rats (Janvier Labs, Le Genest Saint Isle, France), weighing 215 ± 20 g at the beginning of the experiments, were used. Animals were acclimatized to the laboratory conditions for at least 3 days before the start of any experiments. The animals were allocated to the following four experimental groups (n=10 animals per group):

Group	Injection (i.p.)	Treatment (i.ves.)	n
1	Saline	Vehicle (1 mL/kg, i.v.)	10
2	CYP	Vehicle (1 mL/kg, i.v.)	10
3	CYP	XG-104 (2 mg/kg, i.v.)	10

4	CYP	Ibuprofen (10 mg/kg, i.v.)	10
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To induce acute cystitis, a single i.p. injection of CYP at a dose of 150 mg/kg in a final volume of 5 mL/kg was performed. Control rats received physiological saline under the same experimental conditions as CYP (final volume of 5 mL/kg, i.p.).

5

On the day of each experiment, weight of rats was recorded. Then, in a randomized manner, XG-104 (2 mg/kg), ibuprofen (10 mg/kg) or vehicle were intravenously administered at a volume of 1 mL/kg.

10 Assessment of referred visceral pain using von Frey filaments:

Standardized conditions including fixed time-of-day (a.m. to minimize the potential circadian variations in the behaviours responses) and single-experimenter testing of all animals were applied to minimize variability behavior-based pain testing. Visceral pain including allodynia and hyperalgesia was evaluated by applying to the lower abdomen, close to the urinary bladder, a set of 8 calibrated von Frey filaments of increasing forces (1, 2, 4, 6, 8, 10, 26 and 60 g) with an interstimulus interval of 5 seconds. Prior testing, the abdominal area designed for mechanical stimulation of each animal was shaved. Animals were then placed on a raised wire mesh floor under individual transparent Plexiglas box and acclimatized for at least 30 minutes before starting the von Frey test. Filaments were then applied 1-2 seconds through the mesh floor with enough strength to cause the filament to slightly bend. Each filament was tested 3 times. Care was taken to stimulate different areas within the lower abdominal region in the vicinity of the urinary bladder to avoid desensitization.

Nociceptive behaviors were scored for each animal and each filament as follows:

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Score	Behavior
0	no response
1	reaction of the animal (<i>e.g.</i> retraction of the abdomen)
2	reaction of the animal and change of position
3	reaction of the animal, change of position and licking of the site stimulated with von Frey filaments and / or

The study design differs from that of Example 24 (cf. Figure 50 A) only in the route of administration (intravenously instead of intravesically) and the doses as specified above. Briefly, acute cystitis was

induced by CYP injection (i.p.) at D0 (as described above). XG-104, ibuprofen or vehicle was intravenously administrated once just after CYP injection (as described above). Von Frey testing was performed in a non-blinded manner as follow:

- At D-1, rats were acclimatized to the individual Plexiglas box for a minimum of 30 min and to the von Frey filaments application, in order to decrease the level of stress due to the new environment.
- At D0, von Frey testing was performed 15 min before CYP or saline injection in order to obtain basal values (D0, T=-15min).
- At D1, von Frey testing was performed 24 hours after CYP or saline injection in order to analyze test compounds effect on CYP-induced visceral pain (D1, T=+24h).
- Just after von Frey testing (+24h), rats were anesthetized for blood samples collection, then sacrificed and urinary bladders were collected as described below.

Nociceptive parameters are expressed as follows:

Parameters	Expression	Description
nociceptive threshold	g	von Frey filament for which a first score of at least 1 (for 3 applications) is obtained
nociceptive scores	%	% of the maximal response (maximum score = 9) for 3 pooled applications
area under the curve (AUC) 1-8 g (allodynia)	% scores x g	plot of individual percentage of nociceptive scores against von Frey forces from : 1 to 8 g or 8 to 60g
area under the curve (AUC) 8-60 g (hyperalgesia)		

AUCs were calculated using GraphPad Prism® (GraphPad Software Inc., La Jolla, CA, USA). The AUCs method to assess allodynia and hyperalgesia is schematically shown in Figure 50 B.

Results:

Before CYP injection, no significant difference in the nociceptive parameters was observed between the 3 different CYP-injected groups ($p > 0.05$). In order to analyse the effects of XG-104 on CYP-induced visceral pain, nociceptive parameters were compared between the Vehicle- and the XG-104-treated groups independently. Twenty-four hours after CYP injection, nociceptive threshold was significantly increased by XG-104 treatment as compared to vehicle ($p < 0.01$, Figure 53 A). XG-104 treatment significantly decreased nociceptive scores in CYP-injected rats as compared to vehicle

($p < 0.001$, Figure 53 B). In addition, AUC 1-8 g was significantly decreased by XG-104 treatment as compared to vehicle ($p < 0.001$, Figure 53 C). Similarly, AUC 8-60 g was significantly reduced by XG-104 treatment as compared to vehicle ($p < 0.01$, Figure 53 D).

5 Taken together, intravenous treatment of XG-104 (2 mg/kg) thus significantly reversed visceral pain induced by CYP, 24h after its injection. XG-104 efficiently inhibited both allodynia and hyperalgesia. Similar effects were observed with intravenous administration of ibuprofen (10 mg/kg). In conclusion, in the experimental cystitis preclinical model, XG-104 displayed significant anti-nociceptive properties.

10 **Example 26: Effects of XG-104 (the JNK inhibitor according to SEQ ID No. 172) on β -amyloid-induced neuronal apoptosis (Alzheimer's disease model)**

15 In this study, the effects of the JNK inhibitor XG-104 on JNK activation and on neuronal apoptosis after $A\beta_{42}$ cell stress was determined.

To this end, primary mouse cortical neuron cultures were exposed to either 2 μ M or 25 μ M of β -amyloid 1-42 ($A\beta_{42}$) for 5 hours to induce $A\beta_{42}$ cell stress. Neurons were pre-treated with or without
20 10 μ M of the specific inhibitor of JNK, XG-104 (SEQ ID No. 172). Levels of phosphorylated JNK (pJNK), total JNK (JNK), cleaved PARP and Tubulin (control) were determined. The ratio of pJNK/JNK served as a measure of JNK activity. The level of cleaved protein PARP, which is known to increase during apoptosis, served as a measure of neuronal apoptosis.

25 Results of the immunoblot analysis of the primary mouse cortical neuron cultures pre-treated with or without 10 μ M of XG-104 and exposed to either 2 μ M or 25 μ M of β -amyloid 1-42 ($A\beta_{42}$) during 5 hours are shown in Figure 54 (A). In Figure 54 (B and C), the corresponding histograms are depicted showing the ratio of phosphorylated JNK on total JNK (pJNK/JNK) for the different experimental groups (B) and the level of cleaved protein PARP (C). Interestingly, in the condition of 2 μ M of $A\beta_{42}$,
30 no modification of JNK activity was observed. Pre-treatment with 5 μ M and 10 μ M of XG-104 decreased JNK activity by respectively 29.2% and 60% (Fig. 54 B). 25 μ M $A\beta_{42}$ treatment of the neurons increased JNK activity by 14% (Fig. 54 B). Thus, pre-treatment with 5 μ M and 10 μ M XG-104 decreased JNK activity by, 17.5% and 59.6%, respectively. In both $A\beta_{42}$ cell stress conditions, 10 μ M XG-104 concentration was more effective to decrease JNK activity (Fig. 54 B).

Neuronal apoptosis was measured by the level of cleaved protein PARP, which is increased during apoptosis (Fig. 54 C). Pre-treatment with 5 μ M and 10 μ M XG-104 decreased PARP cleavage by 46.8% and 80.2%, respectively, with 2 μ M A β ₄₂ and decreased by 69% and 80.6%, respectively, with 25 μ M A β ₄₂.

5

Taken together, XG-104 thus decreased neuronal apoptosis induced by A β ₄₂. XG-104 10 μ M concentration was 1.7 more efficient than XG-104 5 μ M concentration to reduce apoptosis.

10 **Example 27: Effects of XG-104 (the JNK inhibitor according to SEQ ID No. 172) alone or in combination with PKR down-regulation on β -amyloid-induced neuronal apoptosis (Alzheimer's disease model)**

15 To obtain primary cortical neuronal cultures, E15.5 mice embryos were dissected in PBS (Phosphate Buffered Saline) 6% glucose, on ice. Embryos cortices were minced into small pieces and treated with PBS glucose trypsin (Sigma Aldrich, Saint-Louis, USA) for 20 min at 37°C. Dissociated cortical cells were cultured in Neurobasal media complemented with B27, Glutamax and penicillin-streptomycin (Gibco). Neurons were cultured at 37°C, 5% CO₂ on pre-coated with poly-L-lysine (Sigma Aldrich) petri dishes. Neurons were cultured to maturity (7 days) before use.

20

To induce A β ₄₂ stress 2 μ M of A β 1-42 (Thermo Fisher Scientific, MA, USA) were used during 5h on cortical neurons. A β 42-1 inversed peptide (Thermo Fisher Scientific) was used as negative control. A β 1-42 and A β 42-1 were dissolved in pure water and incubated at 37°C for 48h before use.

25 To inhibit JNK, cortical neurons were pre-treated with 10 μ M of XG-104 1h before cell-stress treatment.

30 For immunoblot analysis cells were lysed on ice in a lysis buffer containing 10nM NaPi pH 7.8, 59nM NaCl, 1% Triton, 0.5% DOC, 0.1% SDS, 10% glycerol, 0.1 μ M calyculin A, 1mM Na₃VO₄ and 1X of a protease inhibitor cocktail (Sigma Aldrich). Lysates were sonicated and centrifugated 10 min at 15000g at 4°C. The supernatant protein concentration was determined with the Micro BCA protein assay kit (Thermo Scientific). Thirty micrograms of proteins were resolved on SDS-PAGE and transferred onto nitrocellulose membrane. After blocking with TBS 5% skim milk, the membranes were probed with primary antibodies to JNK full, c-Jun, PKR, eIF2 α (Santa Cruz, Danvers, USA),

pJNK (Millipore, Billerica, USA), phosphor eIF2 α (Thermo Fisher Scientific), PARP and tubulin (Cell Signaling, Danvers, USA). IR Dyes 800 and 700 (Rockland Immunochemical Inc, Gilbertsville, USA) antibodies were used as secondary antibodies. Blots were revealed with Odyssey imaging system (LI-COR Biosciences, Lincoln, USA).

5

For caspase 3 activity analysis culture cell supernatants containing degenerating and dead neurons, and cell medium were collected in parallel of adhesive neurons lysis. Culture cell supernatants were centrifugated 10 min at 15000g at 4°C. Pellets were then resuspended in lysis buffer and caspase 3 activity was measured by using the Caspase 3 Assay kit reagents and protocol (Abcam, Cambridge, UK).

10

Results:

Decrease of JNK and c-JNK activations with XG-104 in A β ₄₂-stressed WT and PKR^{-/-} neurons

15 In the neuronal cultures stressed by A β ₄₂ peptides, the efficacy of XG-104 was investigated. XG-104 was used at 10 μ M, and added to cell medium 1 hour before the induction of A β ₄₂ stress. In WT neurons, JNK activation is reduced after XG-104 exposure (-60%, Fig. 55 A) in A β ₄₂ stressed cultures. XG-104 showed -29% efficacy in order to decrease c-Jun phosphorylation (Fig. 55 C) and -62% efficacy in order to decrease c-Jun expression (Fig. 55 D), compared to stressed WT neurons without peptides. In PKR^{-/-} neurons, JNK activation is reduced by XG-104 (-60%, Fig. 55 A) in A β ₄₂ stressed cultures. In PKR^{-/-} cultures, the use of XG-104 does not modify c-Jun activation (Fig. 55 C), but the use of XG-104 showed a decrease by 62% of c-Jun protein expression after A β ₄₂ stress induction (Fig. 55 D).

20

Decrease of neuronal apoptosis after JNK inhibition in A β ₄₂-stressed WT neurons

25

In WT neuronal cultures treated by A β ₄₂ peptides, the use of XG-104 decreased apoptosis. With XG-104 it was noted a 61% reduction of cleaved caspase 3 expression level (Fig. 55 E), a 78% decrease of caspase 3 activity (Fig. 55 F), and a 77% decrease of cleaved PARP expression level (Fig. 55 G) compared to A β ₄₂ treated WT neurons.

30

Neuronal death due to A β ₄₂ drastically reduced after dual inhibition of PKR and JNK in neurons

In PKR^{-/-} neurons treated by A β ₄₂ and XG-104, the efficacy of the dual inhibition of PKR and JNK was assessed for neuronal apoptosis. The use of XG-104 on A β ₄₂-stressed PKR^{-/-} neurons showed a 42% decrease of cleaved caspase 3 expression level (Fig. 55 E), a 61% decrease of caspase 3 activity (Fig. 55 F), and a 86% decrease of cleaved PARP expression level (Fig. 55 G) compared to A β ₄₂ treated PKR^{-/-} neurons. In neurons dually inhibited for PKR and JNK, cleaved caspase 3, caspase 3 activity and PARP expression levels decreased respectively by 83%, 87% and 93% compared to treated WT neurons.

Claims

1. JNK inhibitor, selected from the group consisting of:

a) a JNK inhibitor, which comprises an inhibitory (poly-)peptide sequence according to the following general formula:

X1-X2-X3-R-X4-X5-X6-L-X7-L-X8 (SEQ ID NO: 1),

wherein X1 is an amino acid selected from amino acids R, P, Q and r,

wherein X2 is an amino acid selected from amino acids R, P, G and r,

wherein X3 is an amino acid selected from amino acids K, R, k and r,

wherein X4 is an amino acid selected from amino acids P and K,

wherein X5 is an amino acid selected from amino acids T, a, s, q, k or is absent,

wherein X6 is an amino acid selected from amino acids T, D and A,

wherein X7 is an amino acid selected from amino acids N, n, r and K; and

wherein X8 is an amino acid selected from F, f and w, and

wherein an amino acid residue given in capital letters indicates an L-amino acid, while an amino acid residue given in small letters indicates a D amino acid residue,

with the proviso that at least one of the amino acids selected from the group consisting of X1, X2, X3, X5, X7 and X8 is/are a D-amino acid(s), and

b) a JNK inhibitor which comprises an inhibitory (poly-)peptide sequence sharing at least at least 80% sequence identity with SEQ ID NO: 1 as defined in a), with the proviso that with respect to SEQ ID NO: 1 such inhibitory (poly-)peptide sequence sharing sequence identity with SEQ ID NO: 1 maintains the L-arginine (R) residue of SEQ ID NO: 1 at position 4 and the two L-leucine (L) residues of SEQ ID NO: 1 at positions 8 and 10 and that at least one of the remaining amino acids in said sequence sharing at least at least 80% sequence identity with SEQ ID NO: 1 is a D-amino acid,

for use in the treatment of the human or animal body of diseases.

2. JNK inhibitor for use according to claim 1, wherein at least one of the amino acids selected from the group consisting of X3, X5, X7 and X8 is/are a D-amino acid(s).

3. JNK inhibitor for use according to claim 1 or 2, wherein the JNK inhibitor comprises an inhibitory (poly-)peptide sequence sharing at least 80% sequence identity with a sequence selected from any one of SEQ ID NOs: 2-27.
- 5 4. JNK inhibitor for use according to anyone of the preceding claims, wherein the inhibitory (poly-)peptide sequence is selected from anyone of SEQ ID NOs: 2-27.
5. JNK inhibitor for use according to anyone of the preceding claims, wherein the JNK inhibitor comprises SEQ ID NO: 8 or an inhibitory (poly-)peptide sequence sharing at least 80%
10 sequence identity with SEQ ID NO: 8.
6. JNK inhibitor for use according to anyone of the preceding claims, wherein the JNK inhibitor comprises a transporter sequence.
- 15 7. JNK inhibitor for use according to claim 6, wherein the inhibitory (poly-)peptide sequence and the transporter sequence overlap.
8. JNK inhibitor for use according to claim 6 or 7, wherein the transporter sequence comprises a sequence of alternating D- and L-amino acids according to anyone of SEQ ID NOs: 28-30.
20
9. JNK inhibitor for use according to anyone of claims 6-8, wherein said transporter sequence is selected from any one of SEQ ID NOs: 31-170.
10. JNK inhibitor for use according to anyone of claims 6-9, wherein said transporter sequence is
25 selected from any one of SEQ ID NOs: 31-34, 46, 47 and 52-151.
11. JNK inhibitor for use according to anyone of claims 6-10, wherein said transporter sequence is positioned directly N-terminal or directly C-terminal of the inhibitory (poly-)peptide sequence.
- 30 12. JNK inhibitor for use according to anyone of claims 6-11, wherein the JNK inhibitor comprises
 - a) a sequence according to any one of SEQ ID NOs: 171-190, or
 - b) a sequence sharing at least 50% sequence identity with at least one of SEQ ID NOs: 171-190, with the proviso that said sequence sharing sequence identity anyone of SEQ ID NOs: 171-190:
35
 - i) maintains the L-arginine (R) residue on position 4 in its sequence stretch corresponding to SEQ ID NO: 1,

- ii) maintains the two L-leucine (L) in its sequence stretch corresponding to SEQ ID NO: 1, and
- iii) exhibits at least one D-amino acid at positions X1, X2, X3, X5, X7 or X8 in its sequence stretch corresponding to SEQ ID NO: 1.

5

13. JNK inhibitor for use according to anyone of claims 6-12, wherein the JNK inhibitor comprises

- a) the sequence of SEQ ID NO: 172 or
- b) a sequence sharing 50% sequence identity with SEQ ID NO: 172, with the proviso that said sequence sharing 50% sequence identity with SEQ ID NO: 172

10

- i) maintains the L-arginine (R) residue on position 4 in its sequence stretch corresponding to SEQ ID NO: 1,
- ii) maintains the two L-leucine (L) in its sequence stretch corresponding to SEQ ID NO: 1, and
- iii) exhibits at least one D-amino acid at positions X1, X2, X3, X5, X7 or X8 in its sequence stretch corresponding to SEQ ID NO: 1.

15

14. JNK inhibitor comprising:

- a) an inhibitory (poly-)peptide comprising a sequence selected from the group of sequences consisting of RPTTLNLF (SEQ ID NO: 191), KRPTTLNLF (SEQ ID NO: 192), RRPTTLNLF and/or RPKRPTTLNLF (SEQ ID NO: 193), and
- b) a transporter sequence selected from SEQ ID NOs: 31-34 and 46-151,

20

for use in a method for treatment of the human or animal body by therapy.

25

15. JNK inhibitor comprising the sequence of SEQ ID NO: 194 or 195 for use in a method for treatment of the human or animal body by therapy.

16. JNK inhibitor for use according to any one of claims 1 to 15, wherein said method is for treatment of the human body by therapy.

30

17. JNK inhibitor for use according to any one of claims 1 to 16, wherein said JNK inhibitor is administered intravenously, intramuscularly, subcutaneously, intradermally, transdermally, enterally, orally, rectally, topically, nasally, locally, intranasally, epidermally, by patch delivery, by instillation, intravitreally, subconjunctivally and/or intratympanically.

35

18. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of kidney diseases and/or disorders in particular selected from glomerulonephritis in general, in particular membrano-proliferative glomerulonephritis, mesangio-proliferative glomerulonephritis, rapidly progressive glomerulonephritis, nephrophathies in general, in particular membranous nephropathy or diabetic nephropathy, nephritis in general, in particular lupus nephritis, pyelonephritis, interstitial nephritis, tubulointerstitial nephritis, chronic nephritis or acute nephritis, and minimal change disease and focal segmental glomerulosclerosis.
19. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of diseases and/or disorders selected from skin diseases, in particular inflammatory skin diseases, more specifically skin diseases selected from the group consisting of eczema, psoriasis, dermatitis, acne, mouth ulcers, erythema, Lichen plan, sarcoidosis, vascularitis and adult linear IgA disease, in particular atopic dermatitis or contact dermatitis.
20. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of diseases and/or disorders selected Addison's disease, Agammaglobulinemia, Alopecia areata, Amyotrophic lateral sclerosis, Antiphospholipid syndrome, Atopic allergy, Autoimmune aplastic anemia, Autoimmune cardiomyopathy, Autoimmune enteropathy, Autoimmune hemolytic anemia, Autoimmune inner ear, disease, Autoimmune lymphoproliferative syndrome, Autoimmune polyendocrine syndrome, Autoimmune progesterone dermatitis, Idiopathic thrombocytopenic purpura, Autoimmune urticaria, Balo concentric sclerosis, Bullous pemphigoid, Castleman's disease, Cicatricial pemphigoid, Cold agglutinin disease, Complement component 2 deficiency associated disease, Cushing's syndrome, Dagos disease, Adiposis dolorosa, Eosinophilic pneumonia, Epidermolysis bullosa acquisita, Hemolytic disease of the newborn, Cryoglobulinemia, Evans syndrome, Fibrodysplasia ossificans progressive, Gastrointestinal pemphigoid, Goodpasture's syndrome, Hashimoto's encephalopathy, Gestational pemphigoid, Hughes-stovin syndrome, Hypogammaglobulinemia, Lambert-eaton myasthenic syndrome, Lichen sclerosus, Morphea, Pityriasis lichenoides et varioliformis acuta, Myasthenia gravis, Narcolepsy, Neuromyotonia, Opsoclonus myoclonus syndrome, Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria, Parry-romberg syndrome, Pernicious anemia, POEMS syndrome, Pyoderma gangrenosum, Pure red cell aplasia, Raynaud's phenomenon, Restless legs syndrome, Retroperitoneal fibrosis, Autoimmune polyendocrine syndrome type 2, Stiff person

syndrome, Susac's syndrome, Febrile neutrophilic dermatosis, Sydenham's chorea, Thrombocytopenia, and vitiligo.

21. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment
of inflammatory diseases and/or disorders selected from acute disseminated encephalomyelitis,
antisyndetase syndrome, autoimmune hepatitis, autoimmune peripheral neuropathy,
pancreatitis, in particular autoimmune pancreatitis, Bickerstaff's encephalitis, Blau syndrome,
Coeliac disease, Chagas disease, chronic inflammatory demyelinating polyneuropathy,
osteomyelitis, in particular chronic recurrent multifocal osteomyelitis, Churg-Strauss syndrome,
Cogan syndrome, giant-cell arteritis, CREST syndrome, vasculitis, in particular cutaneous small-
vessel vasculitis or urticarial vasculitis, dermatitis, in particular dermatitis herpetiformis,
dermatomyositis, systemic scleroderma, Dressler's syndrome, drug-induced lupus
erythematosus, discoid lupus erythematosus, enthesitis, eosinophilic fasciitis, gastroenteritis, in
particular, eosinophilic gastroenteritis, erythema nodosum, idiopathic pulmonary fibrosis,
gastritis, Grave's disease, Guillain-barré syndrome, Hashimoto's thyroiditis, Henoch-Schonlein
purpura, Hidradenitis suppurativa, idiopathic inflammatory demyelinating diseases, myositis,
in particular inclusion body myositis, cystitis, Kawasaki disease, Lichen planus, lupoid
hepatitis, Majeed syndrome, Ménière's disease, Microscopic polyangiitis, mixed connective
tissue disease, myelitis, in particular neuromyelitis, e.g. neuromyelitis optica, thyroiditis, in
particular Ord's thyroiditis, rheumatism, in particular palindromic rheumatism, Parsonage-
Turner syndrome, perivenous encephalomyelitis, polyarteritis nodosa, polymyalgia
rheumatica, polymyositis, cirrhosis, in particular primary biliary cirrhosis, cholangitis, in
particular primary sclerosing cholangitis, progressive inflammatory neuropathy, Rasmussen's
encephalitis, chondritis, in particular polychondritis, e.g. relapsing polychondritis, reactive
arthritis (Reiter disease), rheumatic fever, sarcoidosis, Schnitzler syndrome, serum sickness,
spondylitis, in particular ankylosing spondylitis, spondyloarthropathy, Takayasu's arteritis,
Tolosa-Hunt syndrome, transverse myelitis, and granulomatosis, in particular Wegener's
granulomatosis.
22. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment
of diseases and/or disorders selected from tauopathies and amyloidoses and prion diseases.
23. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment
of polypes.

24. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of diseases and/or disorders selected from gingivitis, osteonecrosis (e.g. of the jaw bone), peri-implantitis, pulpitis, and periodontitis.
- 5 25. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of fibrotic diseases and/or disorders particularly selected from lung, heart, liver, bone marrow, mediastinum, retroperitoneum, skin, intestine, joint, and shoulder fibrosis.
- 10 26. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of diseases and/or disorders selected from various forms of dementia, e.g. frontotemporal dementia and dementia with lewy bodies, schizophrenia, spinocerebellar ataxia, spinocerebellar atrophy, multiple system atrophy, motor neuron disease, corticobasal degeneration, progressive supranuclear palsy or hereditary spastic paraparesis.
- 15 27. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of eye-related diseases and/or disorders selected from inflammation after corneal surgery, non-infective keratitis, chorioretinal inflammation, and sympathetic ophthalmia.
- 20 28. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of diseases and/or disorders resulting from tissue or organ transplantation, in particular selected from heart, kidney, and skin (tissue), lung, pancreas, liver, blood cells, bone marrow, cornea, accidental severed limbs (fingers, hand, foot, face, nose etc.), bones of whatever type, cardiac valve, blood vessels, and segments of the intestine transplantation.
- 25 29. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of psoriasis.
30. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment and/or prevention of Alzheimer's disease, for example Alzheimer's disease with early onset, Alzheimer's disease with late onset, Alzheimer's dementia senile and presenile forms.
- 30 31. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment and/or prevention of Mild Cognitive Impairment, in particular Mild Cognitive Impairment due to Alzheimer's Disease.

32. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of dry eye disease, in particular Sjögren syndrome dry eye or Non-Sjögren syndrome dry eye, most preferably Sjögren syndrome dry eye.
- 5 33. JNK inhibitor for use according to claim 32, wherein the JNK inhibitor is applied in doses in the range of 0.01 µg/eye to 10 mg/eye, more preferably 0.1 µg/eye to 5 mg/eye, even more preferably 1 µg/eye to 2 mg/eye, particularly preferably 50 µg/eye to 1.5 mg/eye, most preferably 100 µg/eye to 1 mg/eye.
- 10 34. JNK inhibitor for use according to claim 32 or 33, wherein the JNK inhibitor is applied by instillation.
35. JNK inhibitor for use according to any of claims 32 to 34, wherein the JNK inhibitor is applied repeatedly, for example daily, every 2 or 3 days or weekly, for several, e.g. 2, 3, 4, 5, 6, 7,
15 8, 9, or 10, weeks.
36. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of retinopathy, in particular diabetic retinopathy, arterial hypertension induced hypertensive retinopathy, radiation induced retinopathy, sun-induced solar retinopathy, trauma-induced
20 retinopathy, e.g. Purtscher's retinopathy, retinopathy of prematurity (ROP) and hyperviscosity-related retinopathy.
37. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of age-related macular degeneration (AMD), in particular the wet or the dry form of age-
25 related macular degeneration.
38. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of organ transplantation, in particular upon heart, kidney, and skin (tissue) transplantation, graft rejection upon heart, kidney or skin (tissue) transplantation,.
30
39. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of arthrosis/arthritis, in particular reactive arthritis, rheumatoid arthrosis, juvenile idiopathic arthritis, and psoriatic arthritis.
- 35 40. JNK inhibitor for use according to any one of claims 1 to 17, wherein said use is for treatment of glomerulonephritis.

41. JNK inhibitor for use according to any one of the preceding claims, wherein the JNK inhibitor consists of the sequence of SEQ ID NO: 172.
- 5 42. Pharmaceutical composition comprising a JNK inhibitor as defined in any of claims 1 to 15 and a pharmaceutically acceptable carrier.
43. Pharmaceutical composition according to claim 42 for use for the treatment of any of the diseases/disorders of claims 18 to 40.

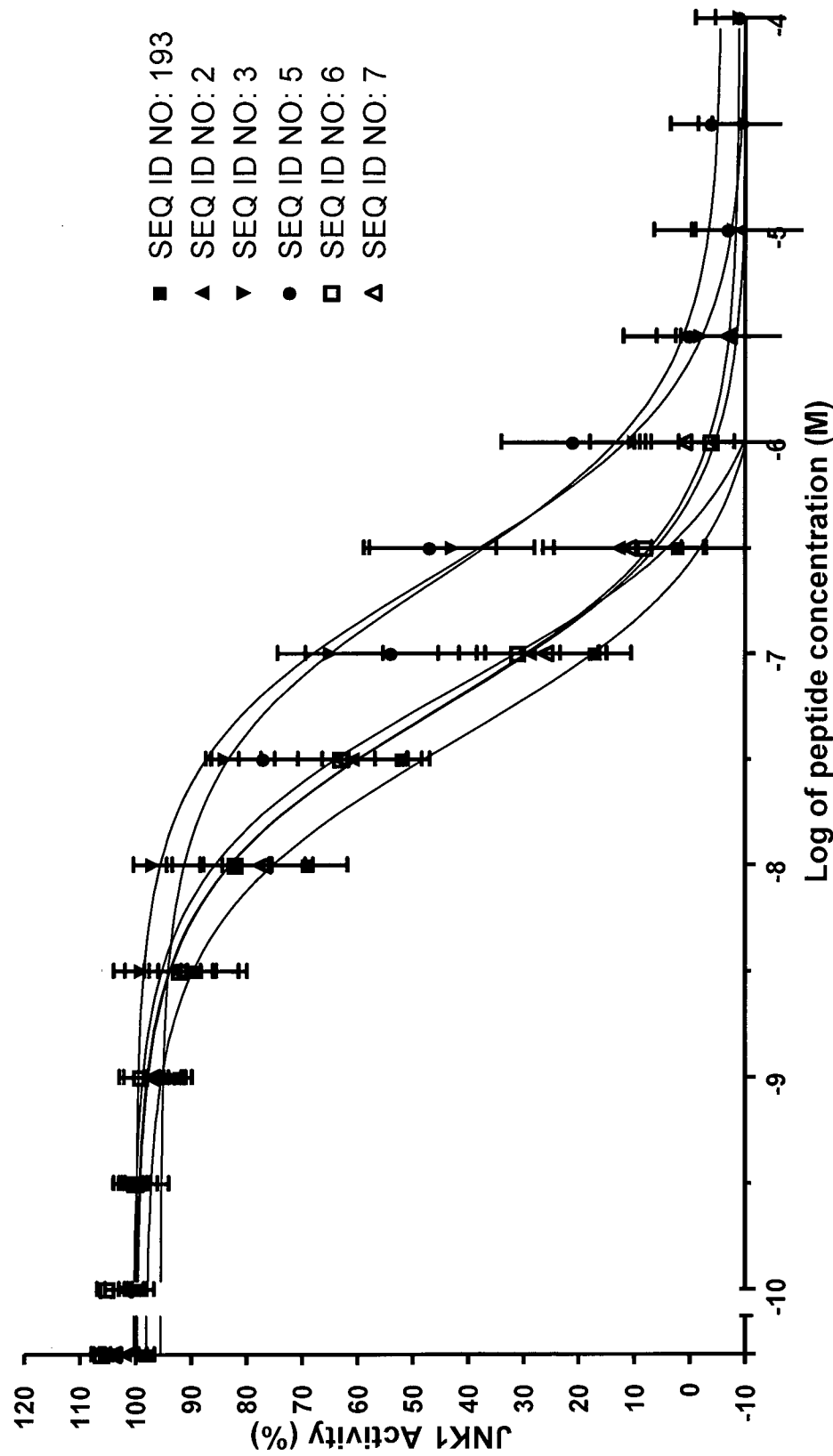


Fig. 1a

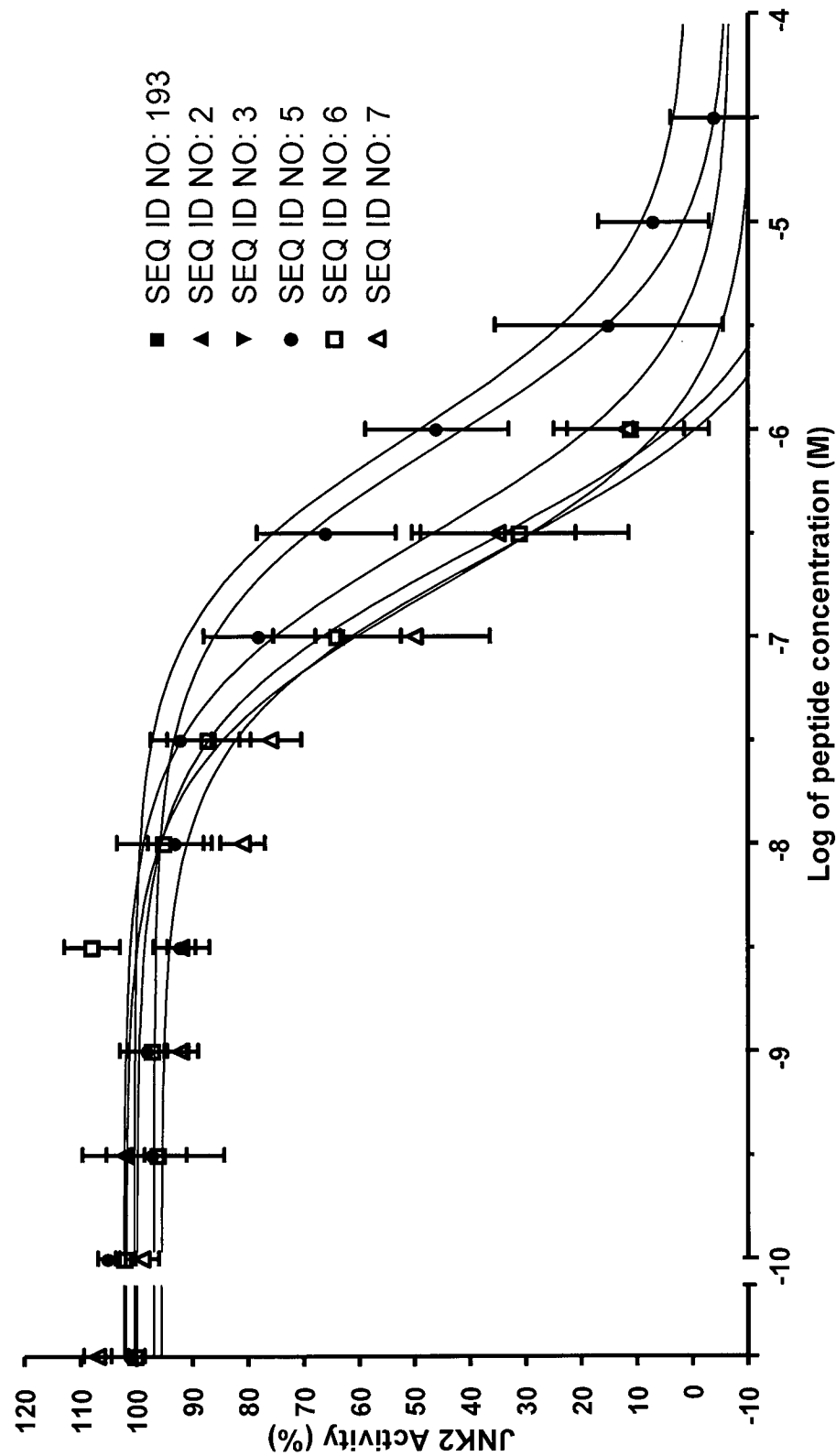


Fig. 1b

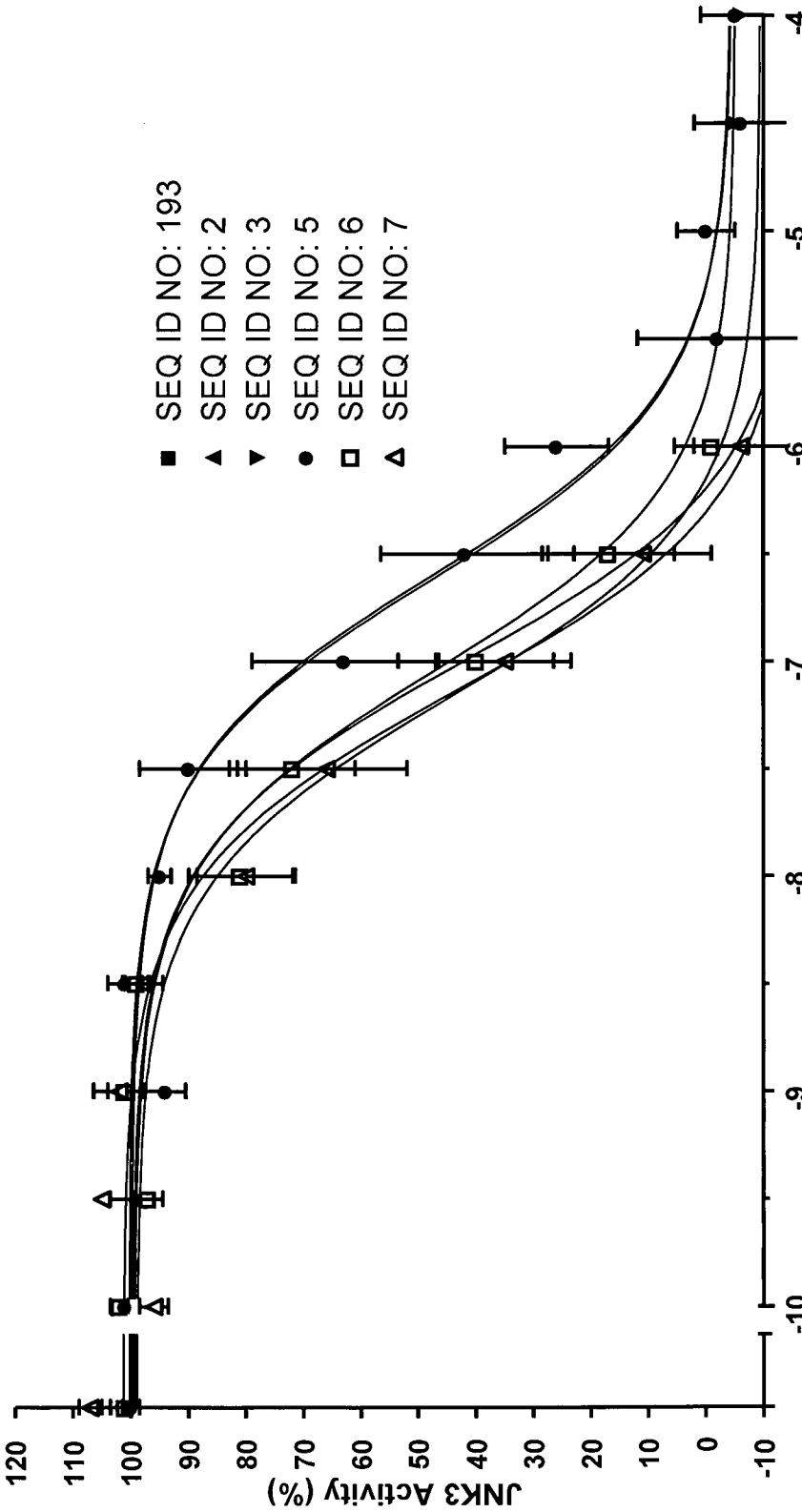


Fig. 1c

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SEQ ID NO.	Sequence			hJNK1			hJNK2			hJNK3		
				IC50	SEM	n	IC50	SEM	n	IC50	SEM	n
193	NH2	R P K R P T T L N L F	CONH2	39,52	0,57	2	183,85	50,45	2	67,68	13,92	2
2	NH2	r P K R P T T L N L F	CONH2	65,55	26,03	3	423,53	241,45	3	103,32	36,53	3
3	NH2	R P k R P T T L N L F	CONH2	311,63	99,86	4	1213,53	437,87	4	359,47	161,02	4
5	NH2	R P K R P T T L n L F	CONH2	347,55	174,17	4	1501,88	701,33	4	387,15	179,51	4
6	NH2	R P K R P T T L r L F	CONH2	90,50	29,63	4	358,75	105,28	4	119,50	39,82	4
7	NH2	R P K R P T T L N L f	CONH2	69,53	21,75	4	278,18	51,43	4	88,97	26,72	4

Fig. 2

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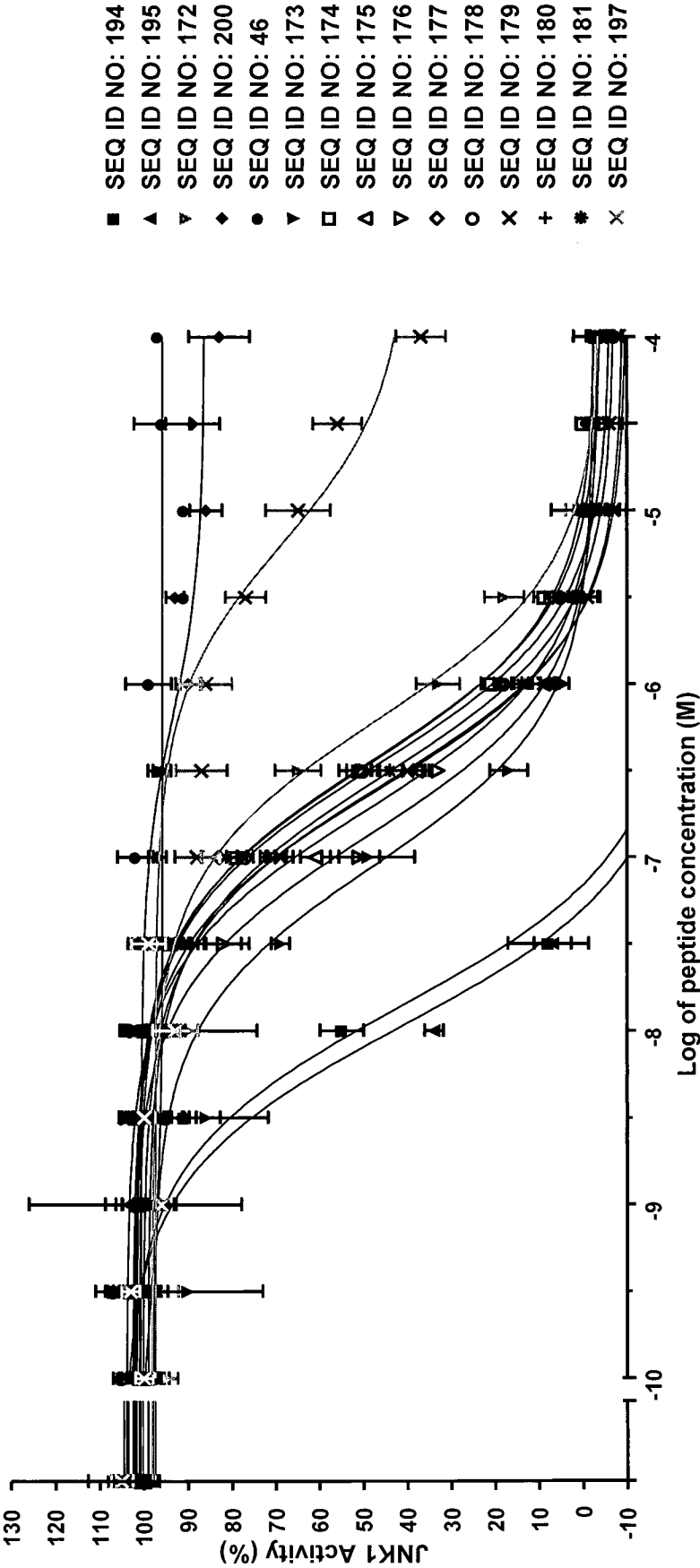


Fig. 3a

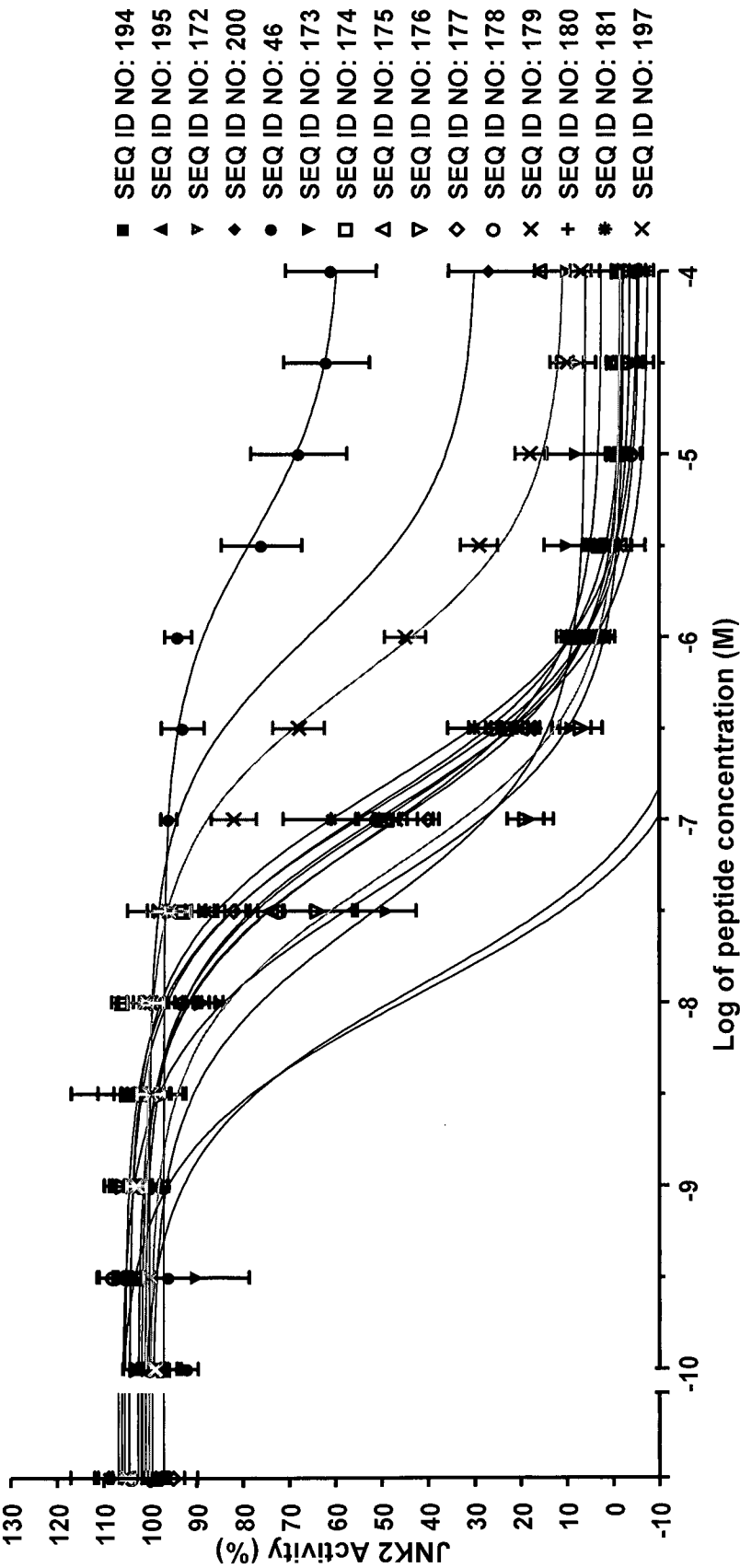


Fig. 3b

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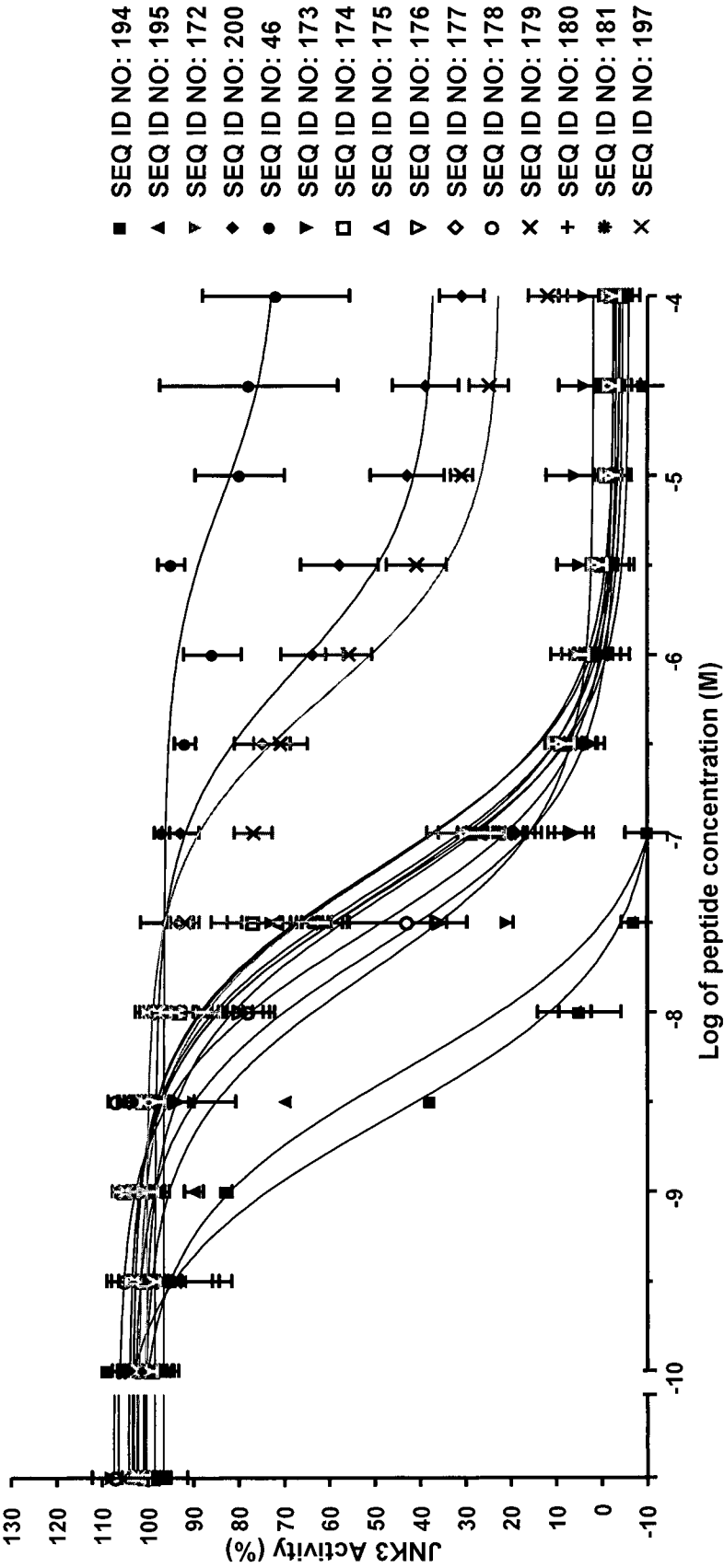


Fig. 3c

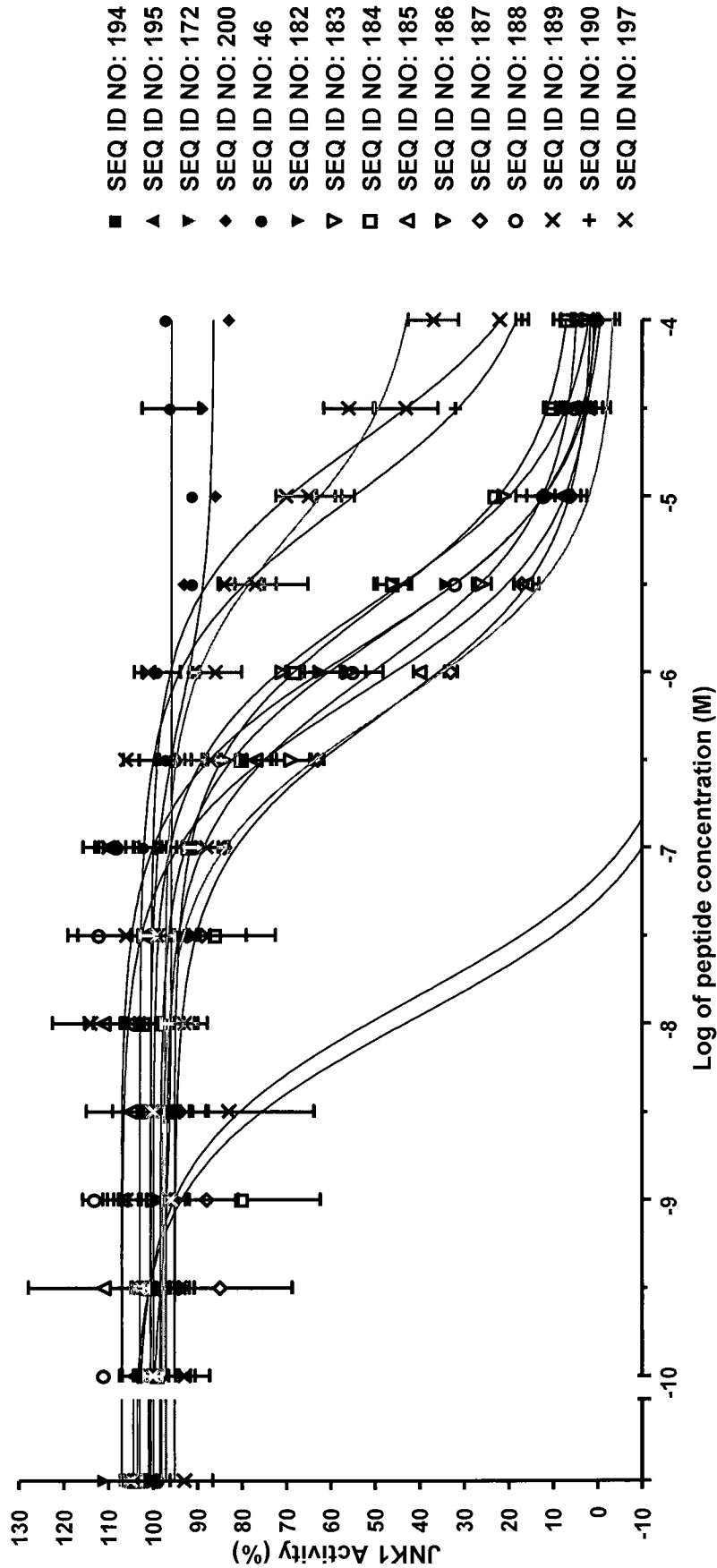


Fig. 3d

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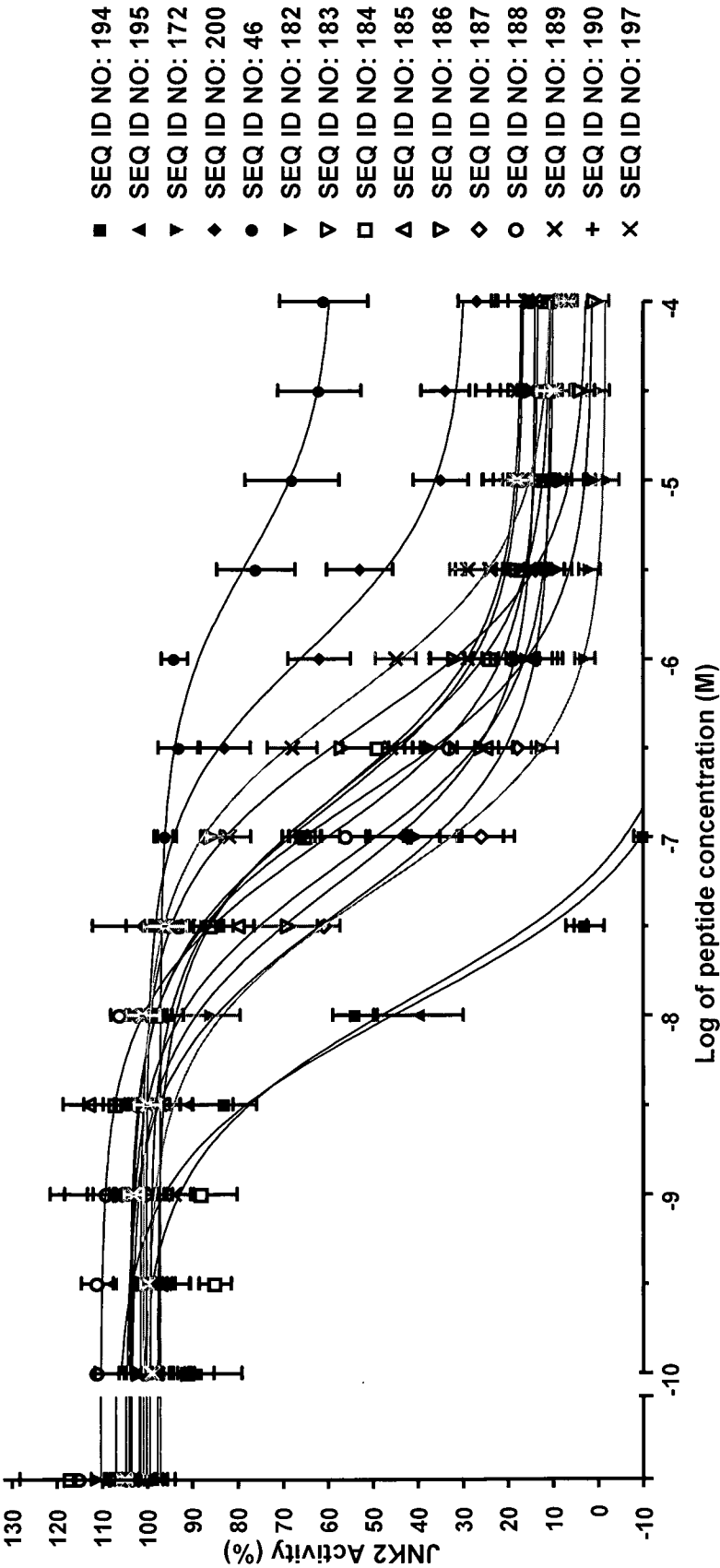


Fig. 3e

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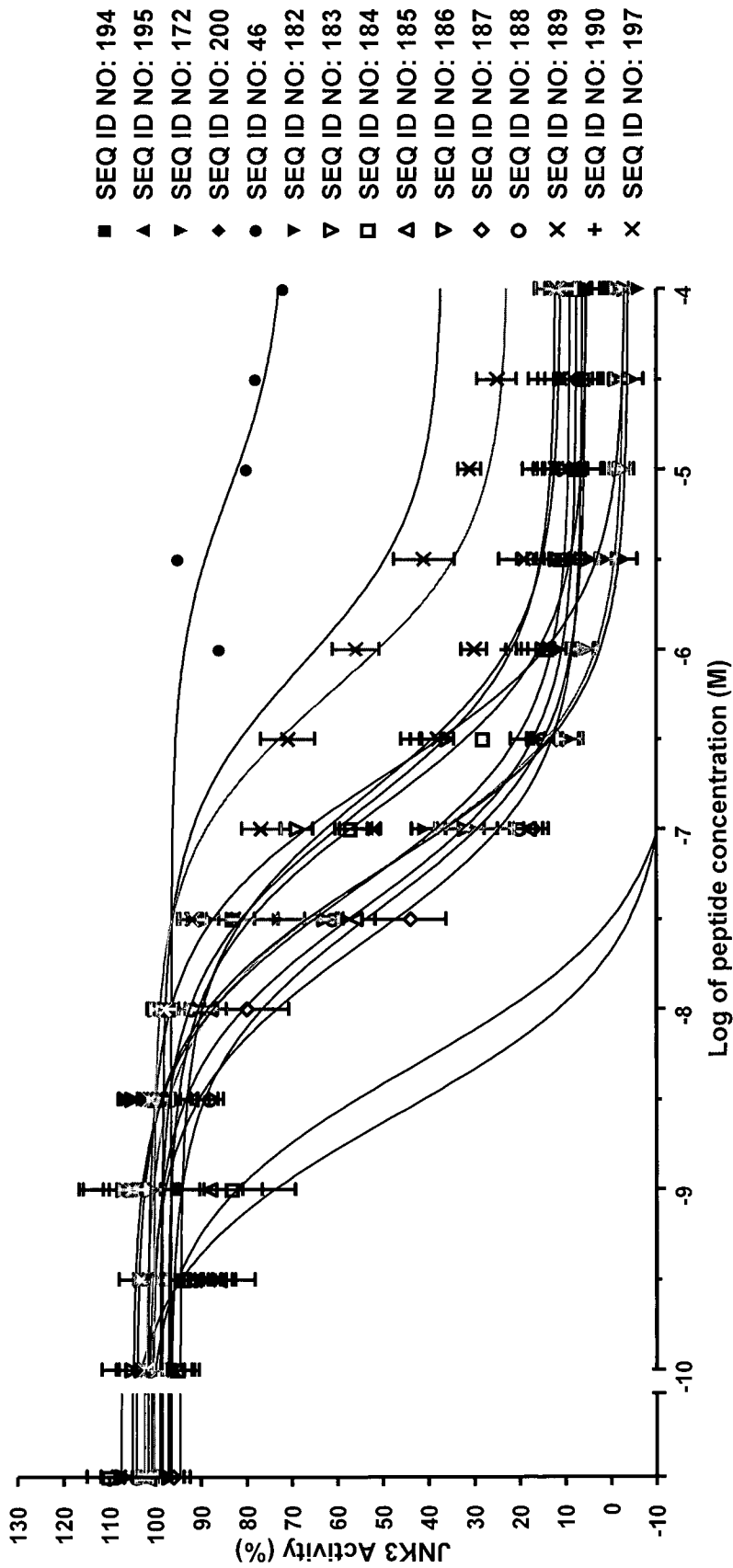


Fig. 3f

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SEQ ID NO:	Sequence		hJNK1		hJNK2		hJNK3	
			IC50	SEM	IC50	SEM	IC50	SEM
196	NH2	G R K K R R Q R R R P P P K R P T T L N L F P Q V P R S Q D	42,20	8,17	8,43	2,01	5,22	0,71
197	CONH2	G r k k r r q r r p p p r p k r p t t l n l f p q v p r s q d	24358,50	10019,91	801,77	114,66	1294,24	255,51
194	NH2	G R K K R R Q R R R P P K R P T T L N L F P Q V P R S Q D	13,99	0,06	12,70	0,48	2,59	0,08
195	NH2	G R K K R R Q R R R P T T L N L F P Q V P R S Q D	10,77	1,83	11,26	0,56	4,92	0,27
172	NH2	r K K R r Q R R r R P k R P a T L N L f	722,49	124,58	54,66	13,04	102,32	47,81
200	NH2	r K K R r Q R R r R P k A A A A N A f	NA	NA	3324,00	2469,99	3820,81	3190,08
46	NH2	r K K R r Q R R r	NA	NA	5340,33	1803,08	8130,86	5323,73
173	NH2	r K K R r Q R R r R P k R P T T L r L f	88,36	4,02	30,03	0,16	16,76	2,03
174	NH2	r K K R r Q R R r R P T T L N L f	333,73	36,46	120,13	4,53	63,12	6,04
175	NH2	r K K R r Q R R r R P T T L N L f	185,30	18,10	82,30	9,26	60,60	6,01
176	NH2	r K K R r Q R R r R P k R P T T L N L w	131,17	12,28	40,33	4,60	22,38	1,60
177	NH2	r K K R r Q R R r R P k R P T D L N L f	355,10	34,02	87,20	7,12	45,38	6,70
178	NH2	r K K R r Q R R r R P T T L r L w	329,33	12,26	108,60	42,64	30,41	5,81
179	NH2	r K K R r Q R R r R P T T L r L w	249,47	22,35	122,11	20,73	45,66	3,79
180	NH2	r K K R r Q R R r R P T D L r L w	265,20	34,65	117,65	10,58	46,99	8,21
181	NH2	r K K R r Q R R r R P T D L r L w	293,70	9,79	160,22	40,13	47,56	5,77
182	NH2	r K K R r Q R R r R P a T L N L f	1677,50	34,50	168,40	20,80	59,36	2,35
183	NH2	r K K R r Q R R r R P a T L N L f	2588,00	494,00	427,30	25,00	199,20	3,90
184	NH2	r K K R r Q R R r K R P a T L N L f	2426,00	129,00	205,95	8,25	129,45	9,65
185	NH2	r K K R r Q R R r R P k R P s T L N L f	765,65	78,15	72,09	2,85	35,52	6,34
186	NH2	r K K R r Q R R r R P k R P q T L N L f	1021,30	100,70	52,59	2,73	44,24	4,80
187	NH2	r K K R r Q R R r R P k R P k T L N L f	594,45	40,45	37,88	5,47	25,41	8,95
188	NH2	r K K R r Q R R r G K R K A L K L f	1421,00	98,00	98,14	27,26	36,12	2,46
189	NH2	r K K R r Q R R r G K R K A L r L f	22270,00	5090,00	175,60	1,30	127,72	31,88
190	NH2	r K K R r Q R R r R K A L r L f	8969,50	2070,50	148,20	9,70	159,35	13,45

Fig. 4

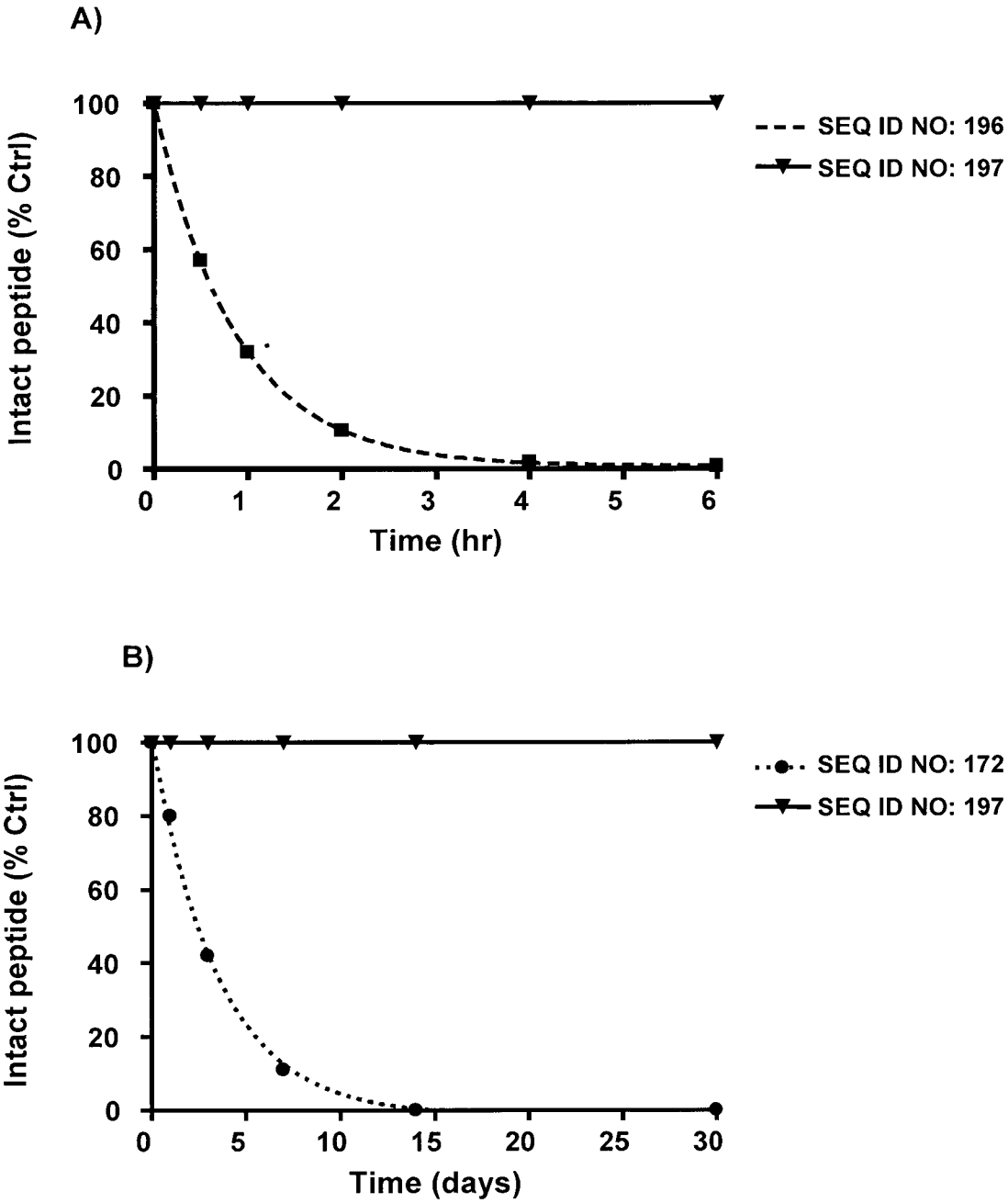


Fig. 5

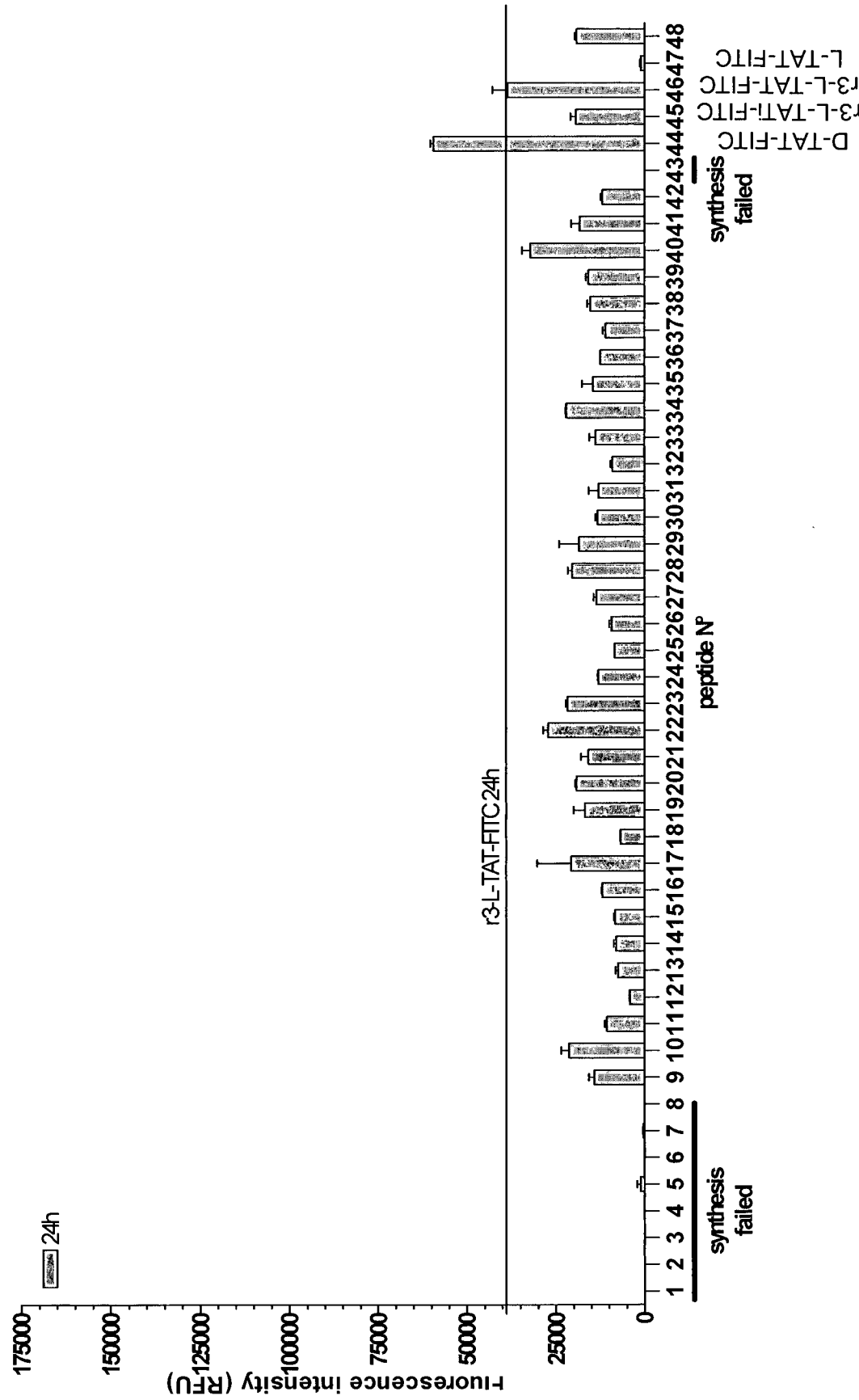


Fig. 6a

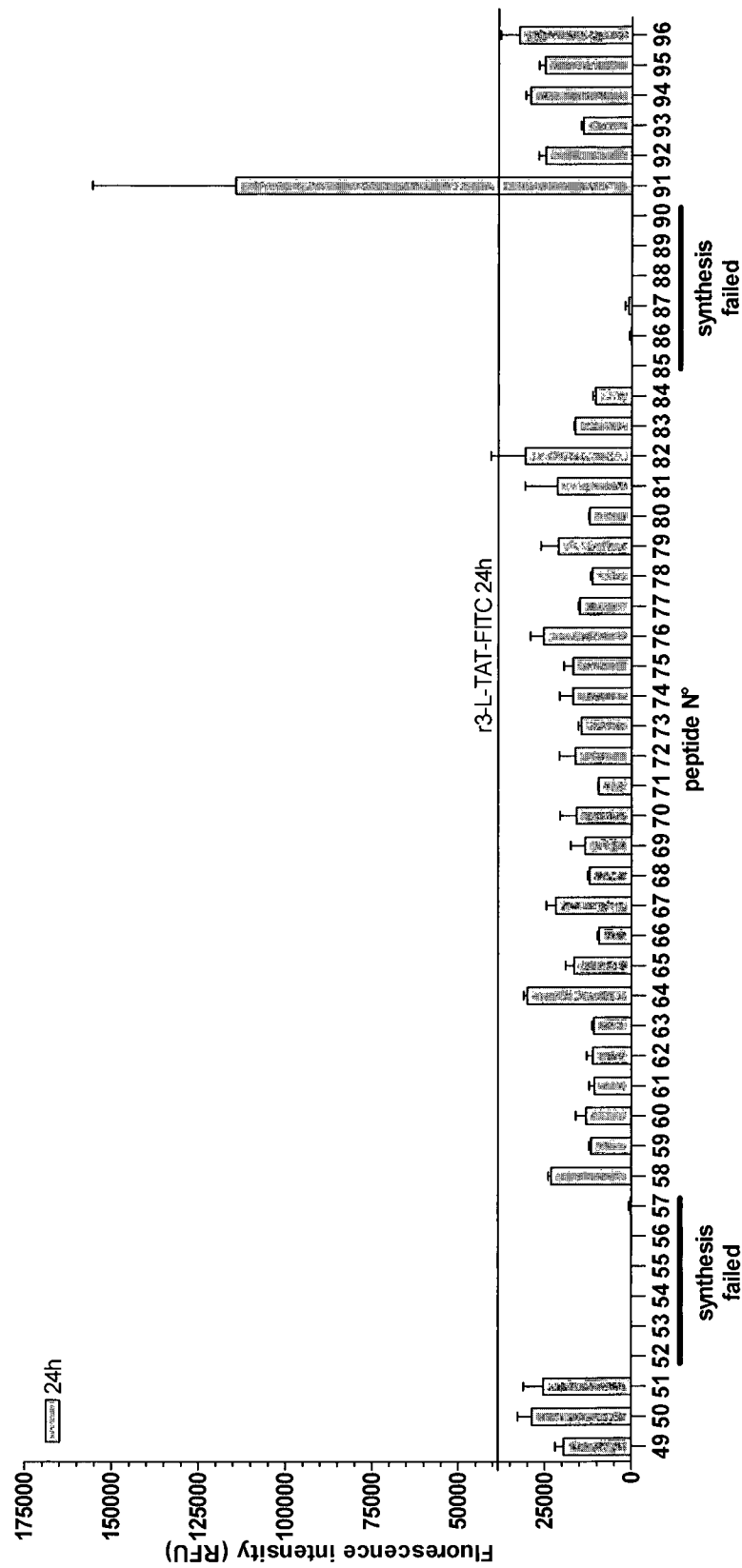


Fig. 6b

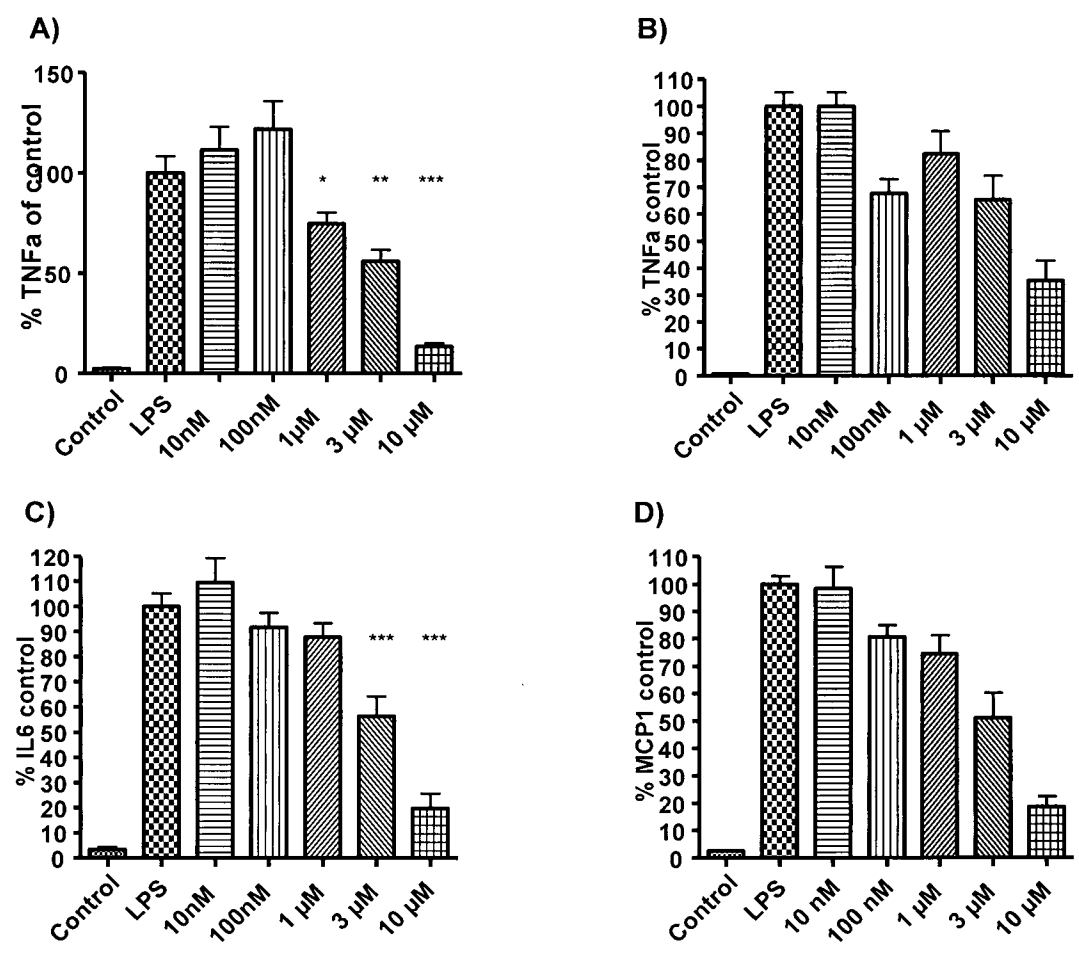


Fig. 7

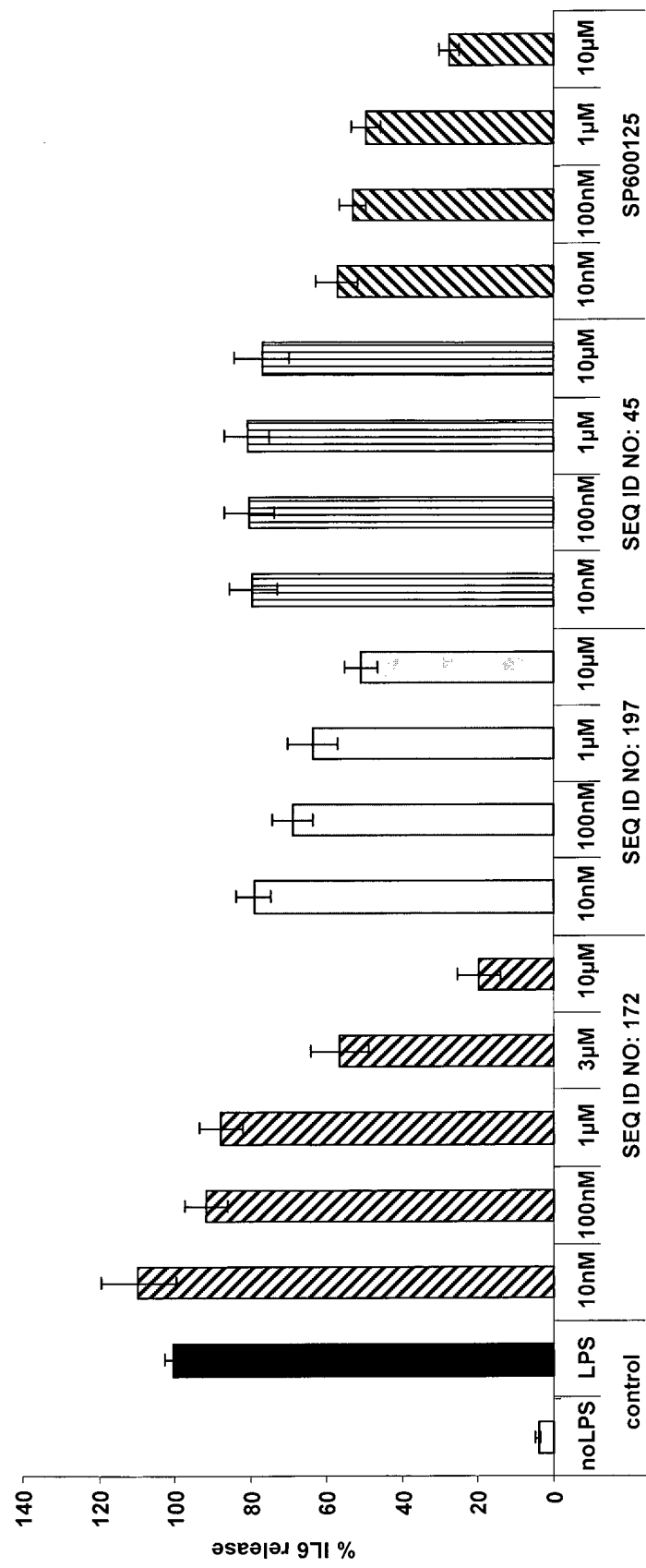


Fig. 8

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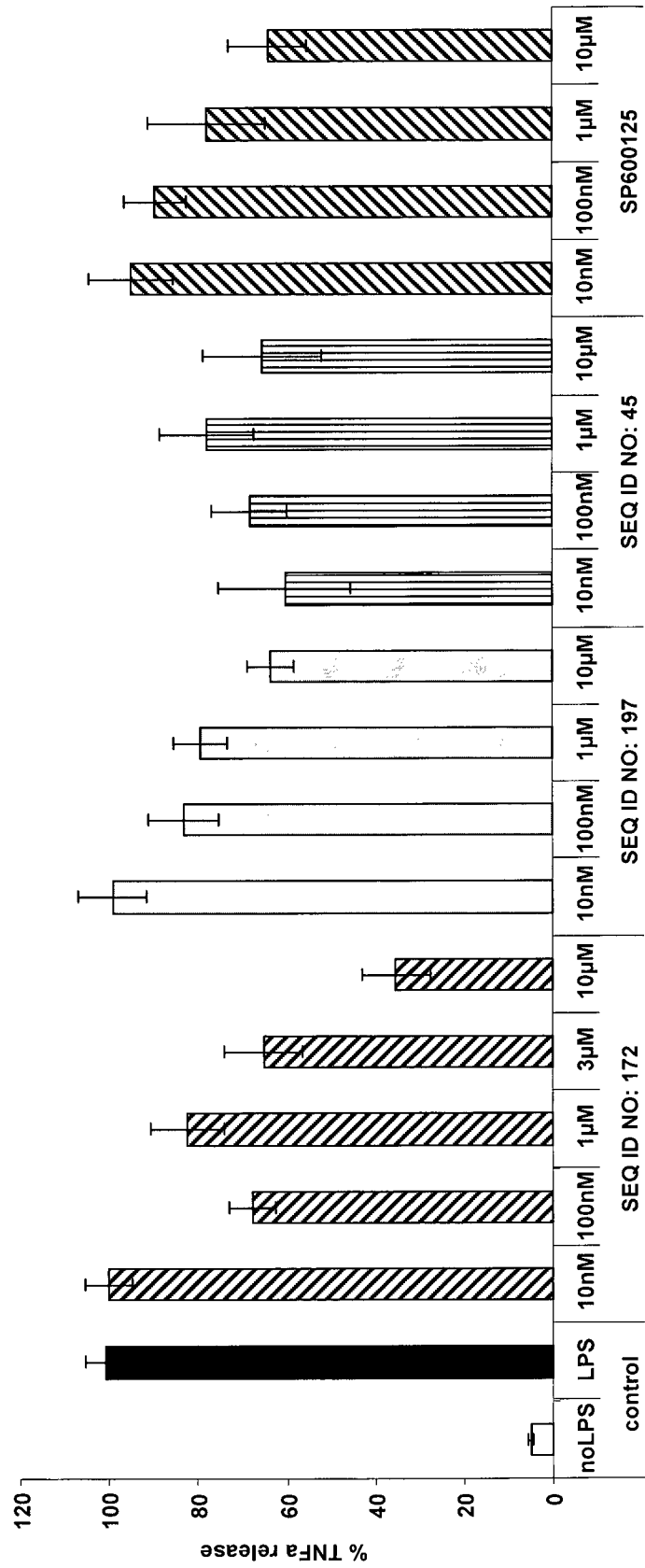


Fig. 9

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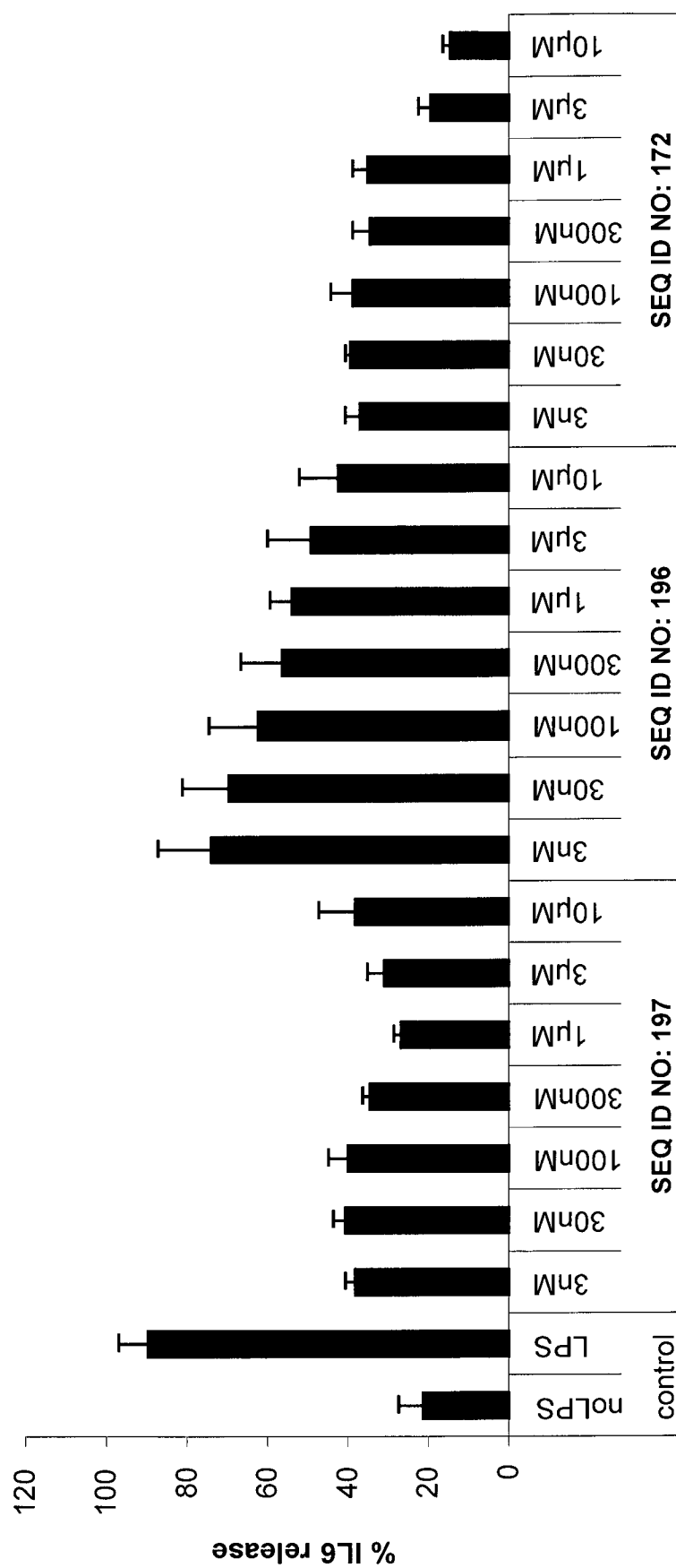


Fig. 10

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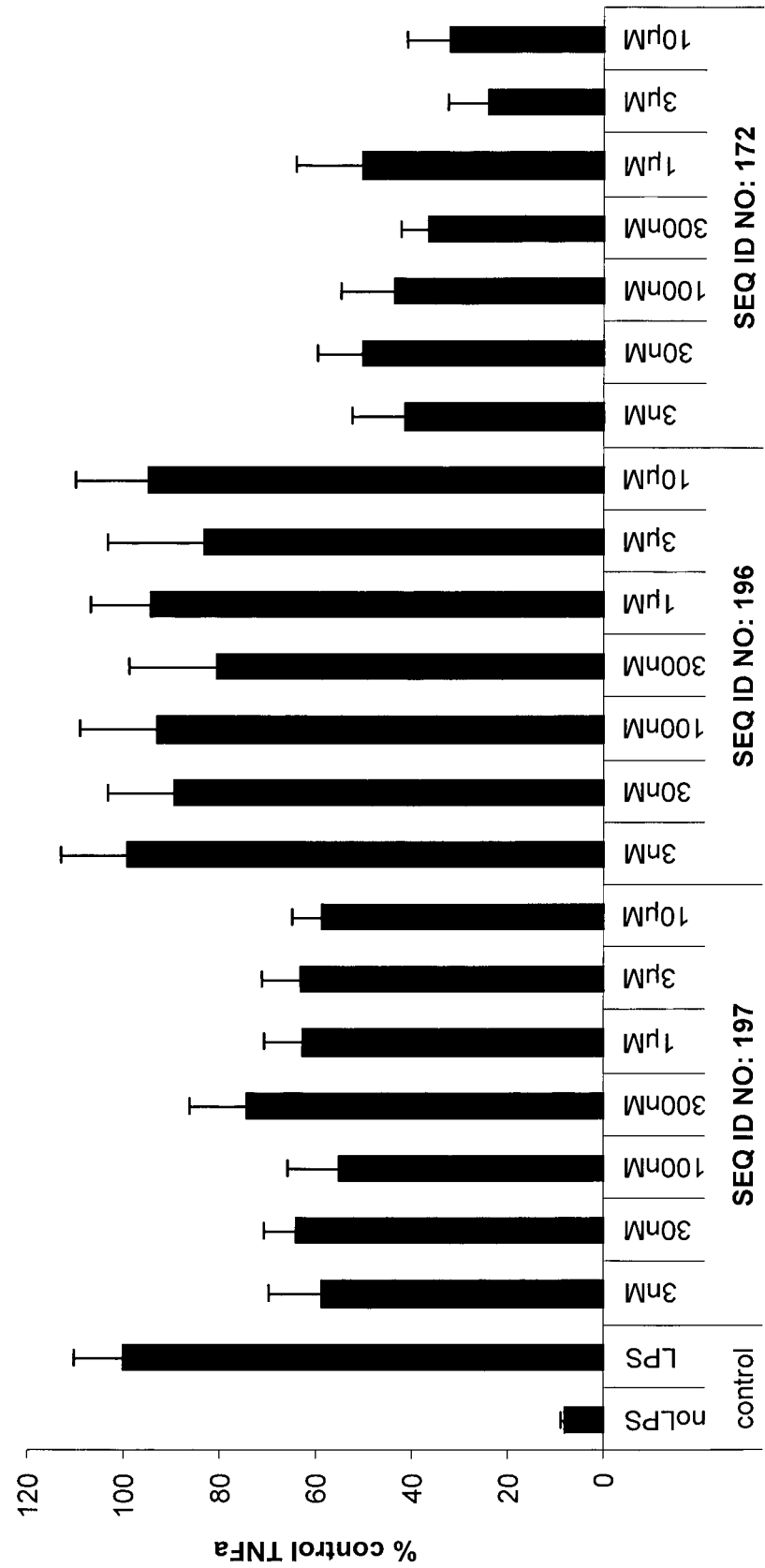


Fig. 11

20/63

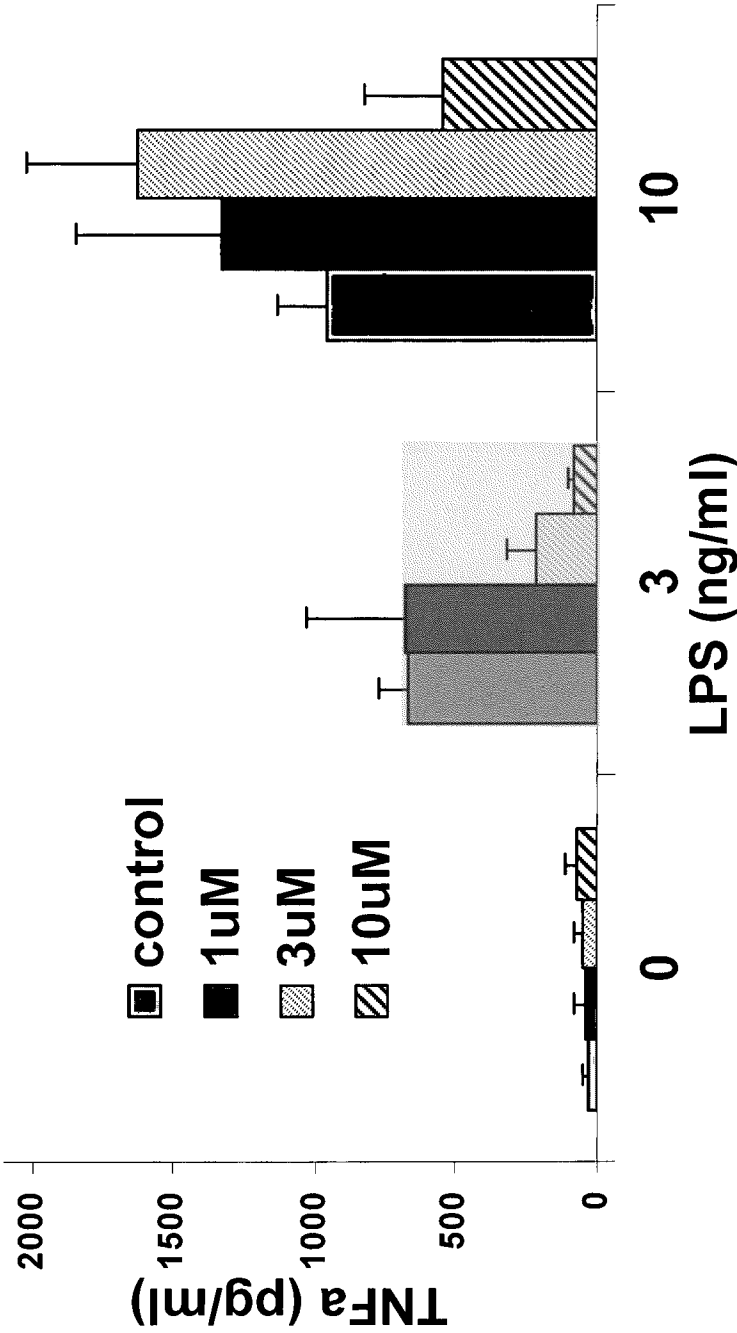


Fig. 12

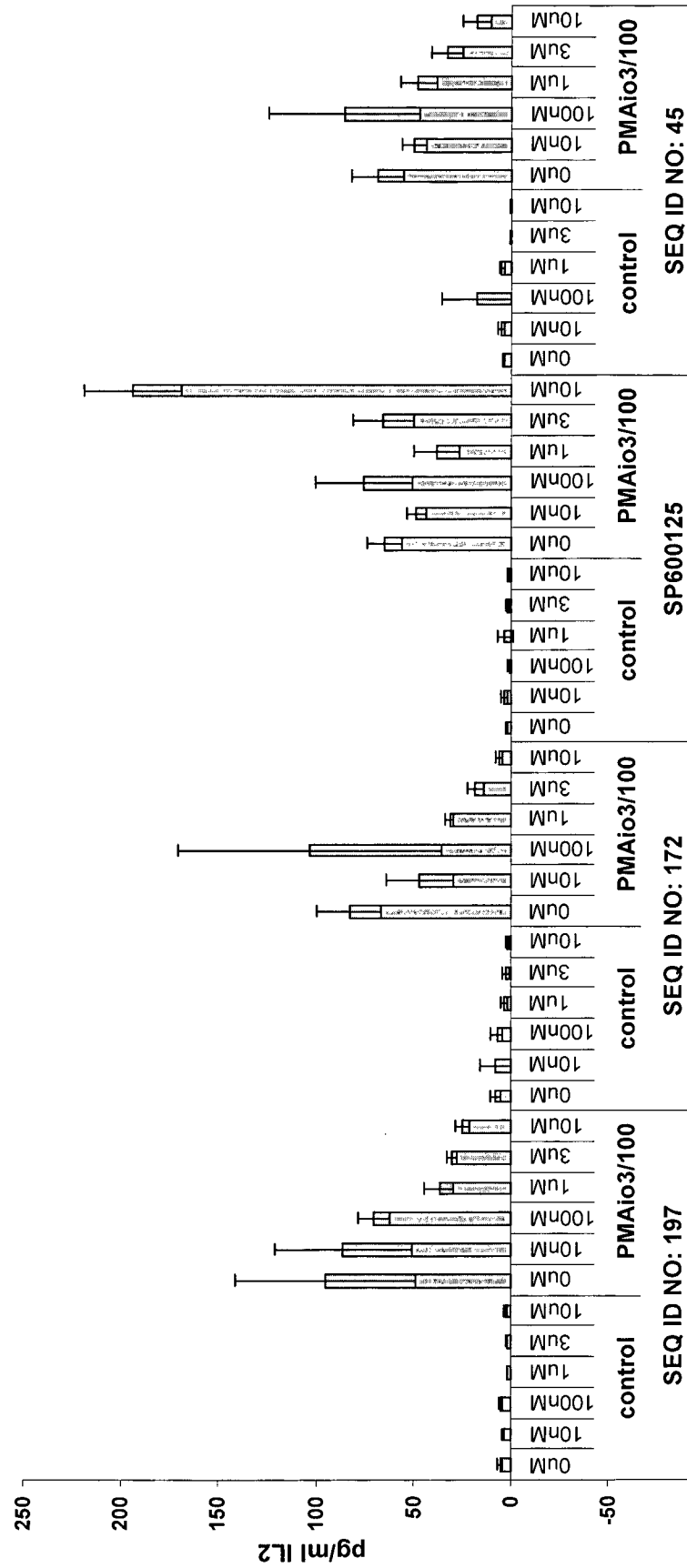


Fig. 13

22/63

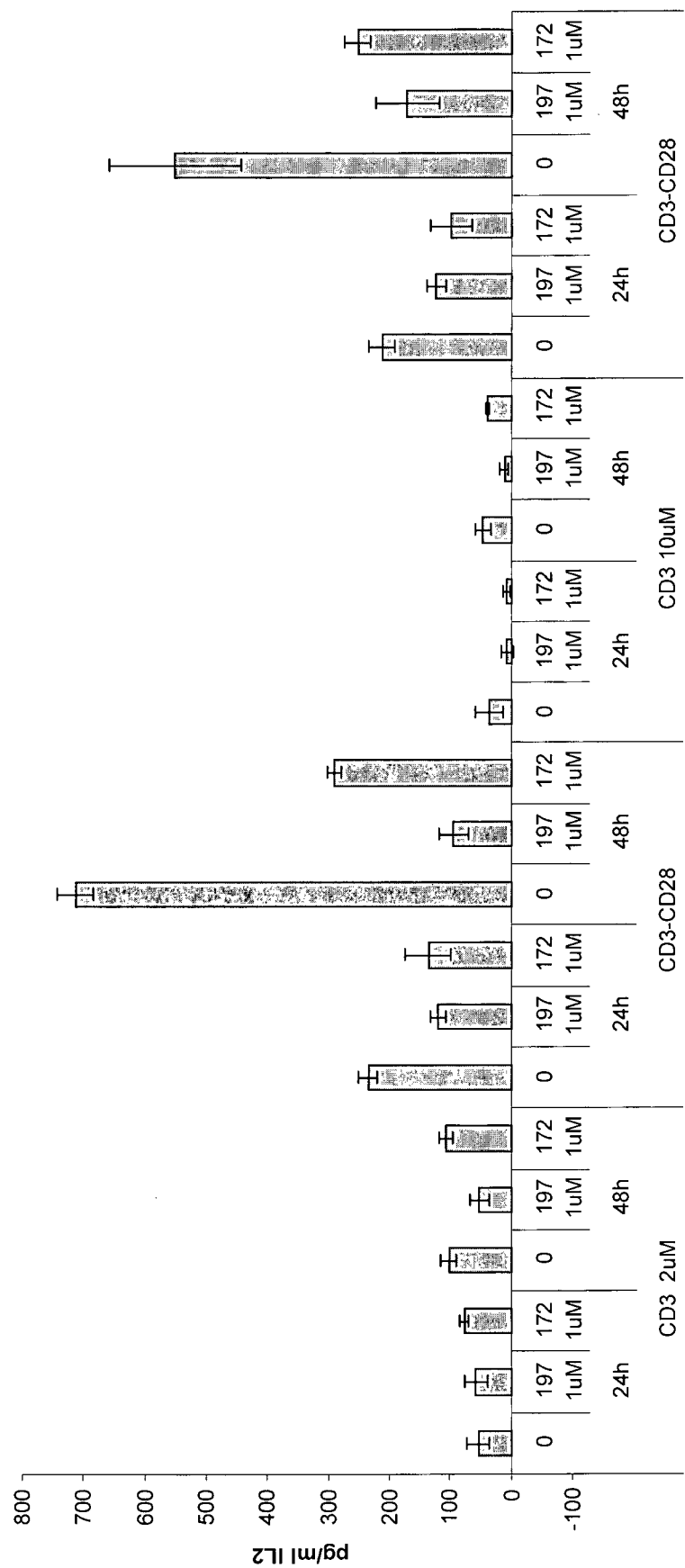


Fig. 14

23/63

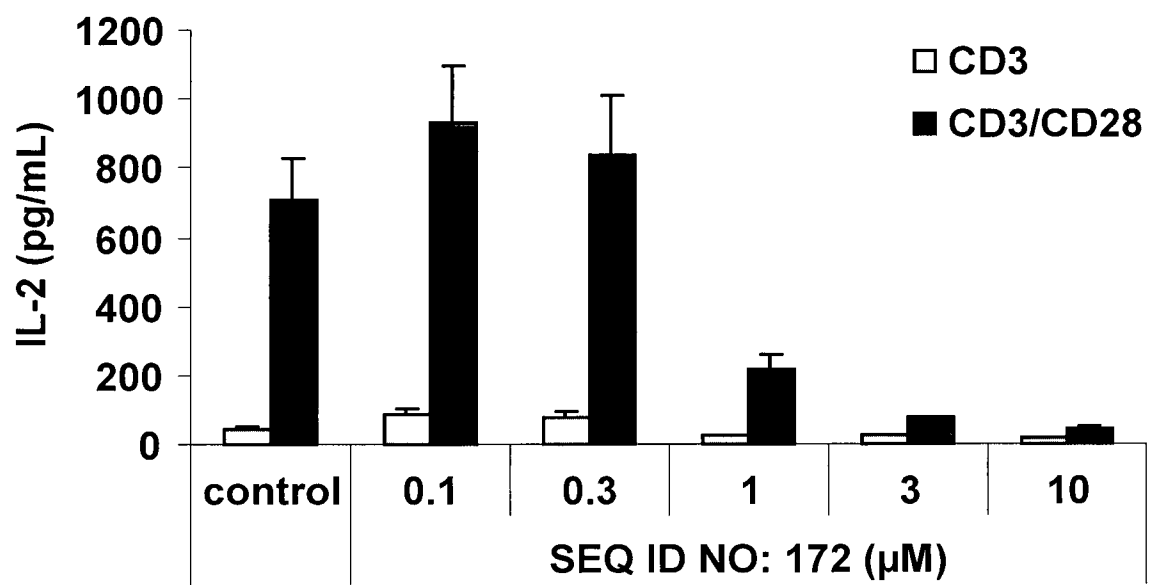


Fig. 15

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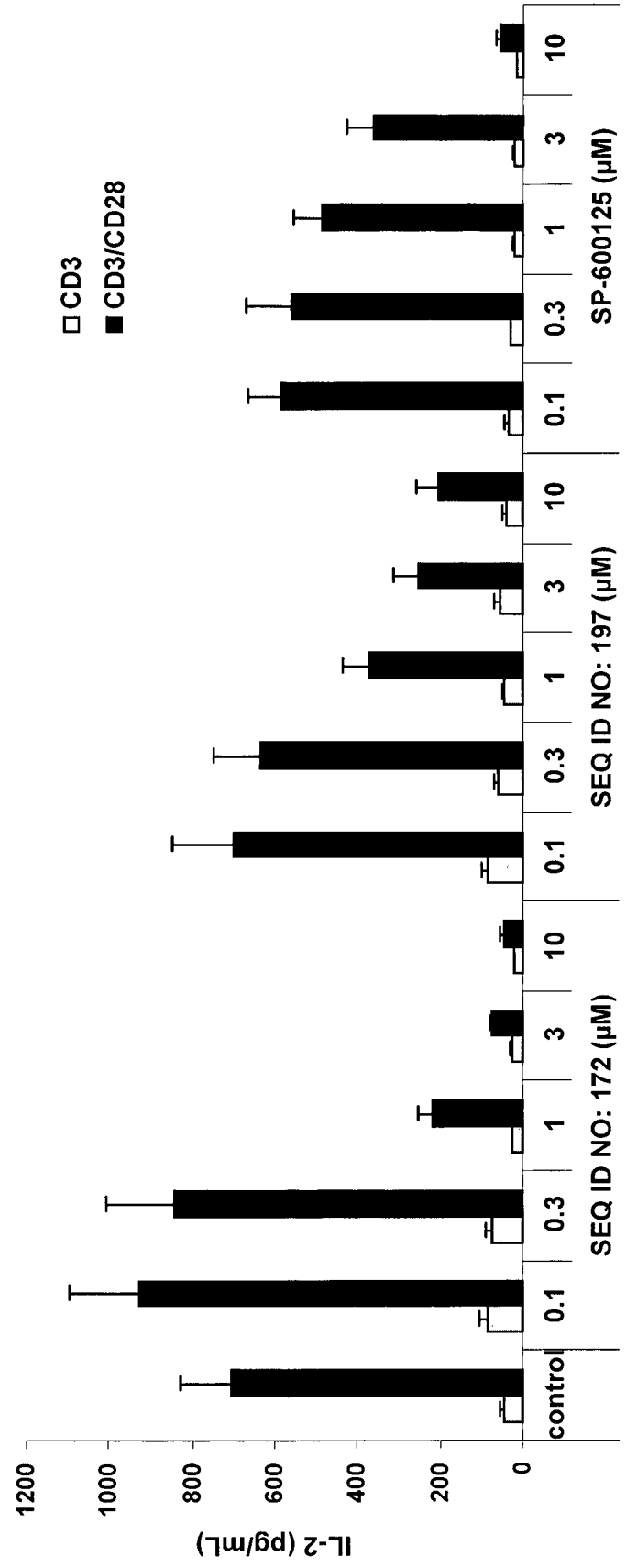


Fig. 16

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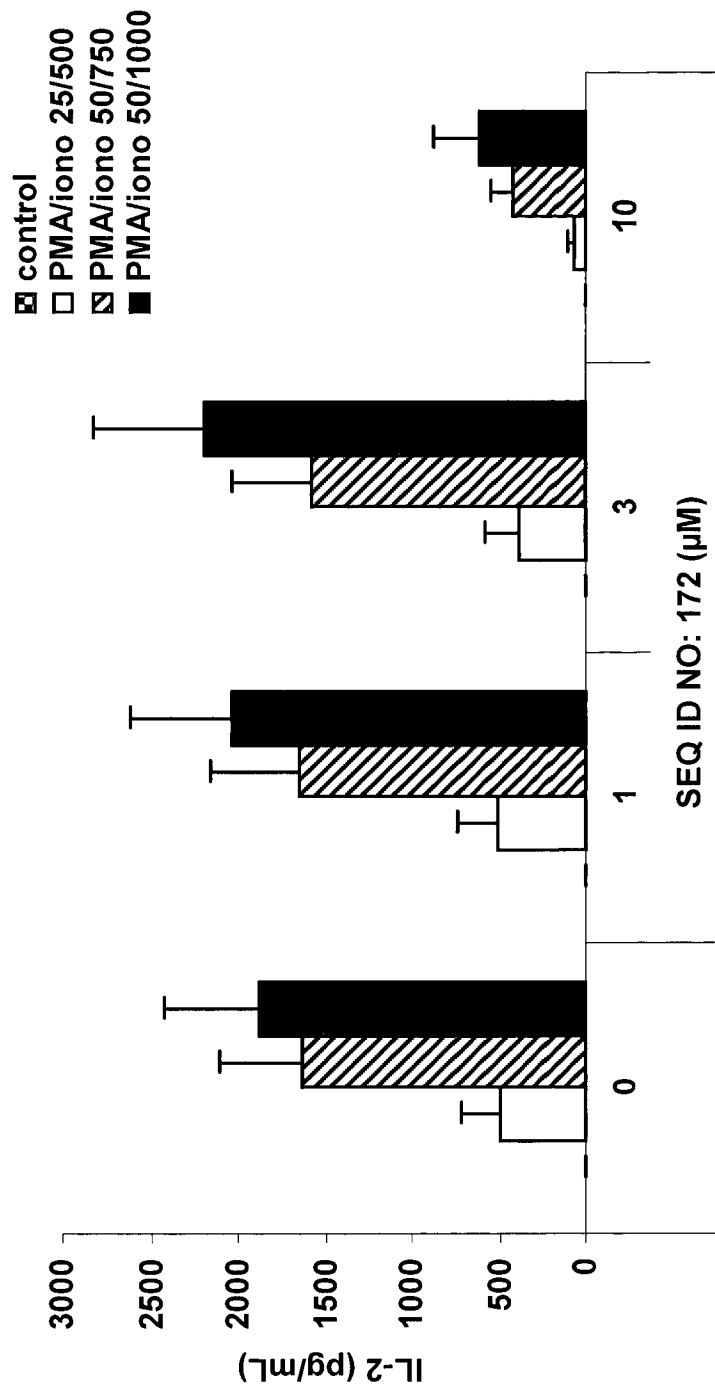


Fig. 17

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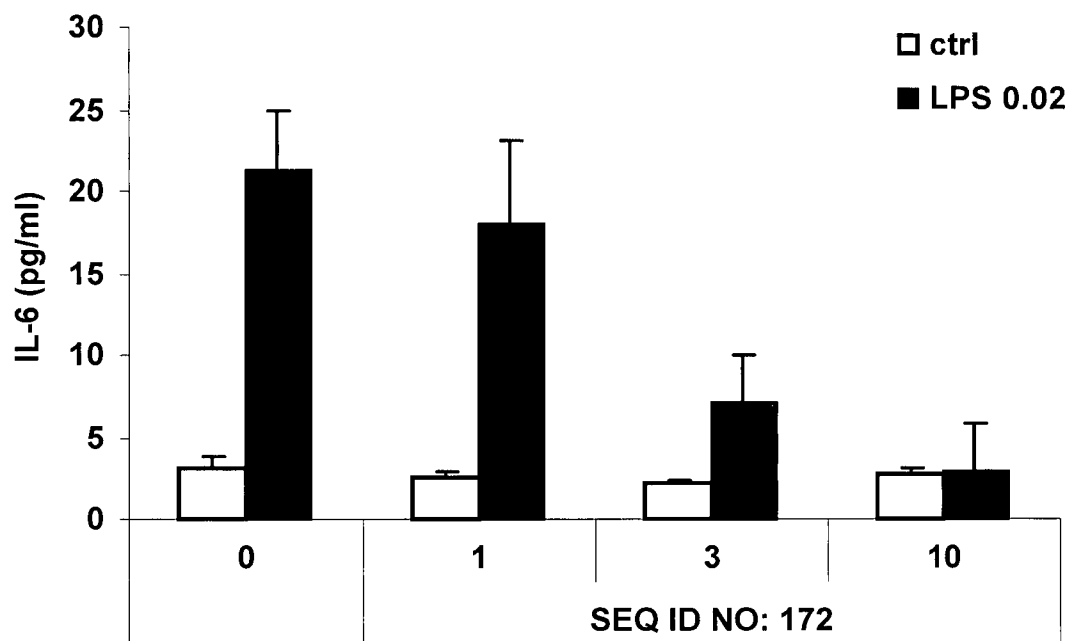


Fig. 18

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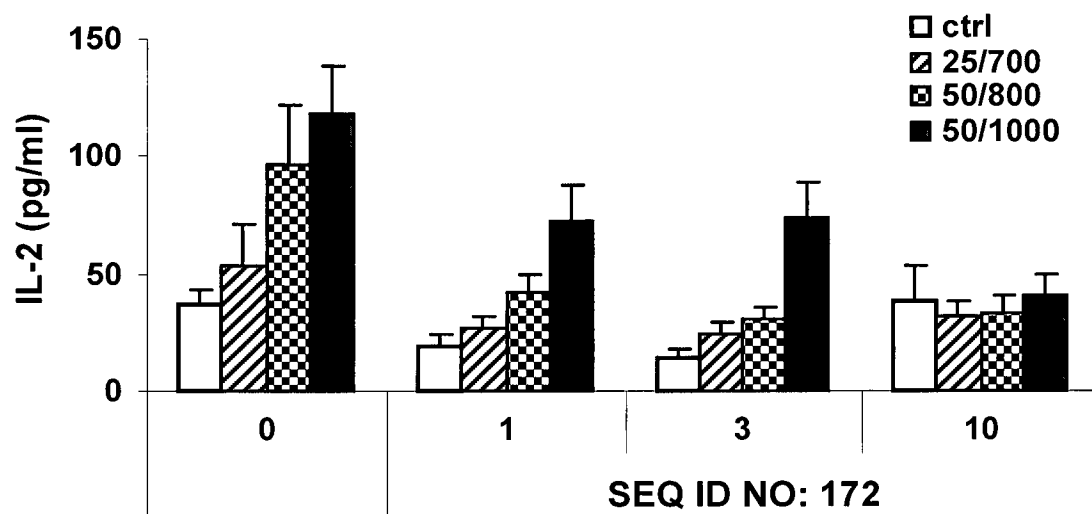


Fig. 19

28/63

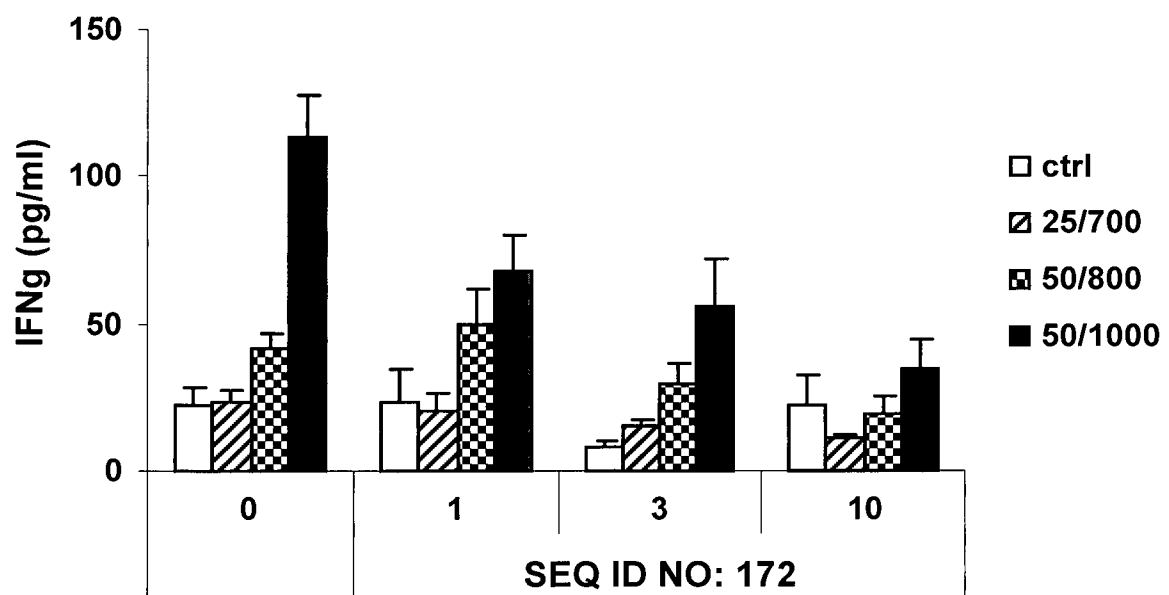


Fig. 20

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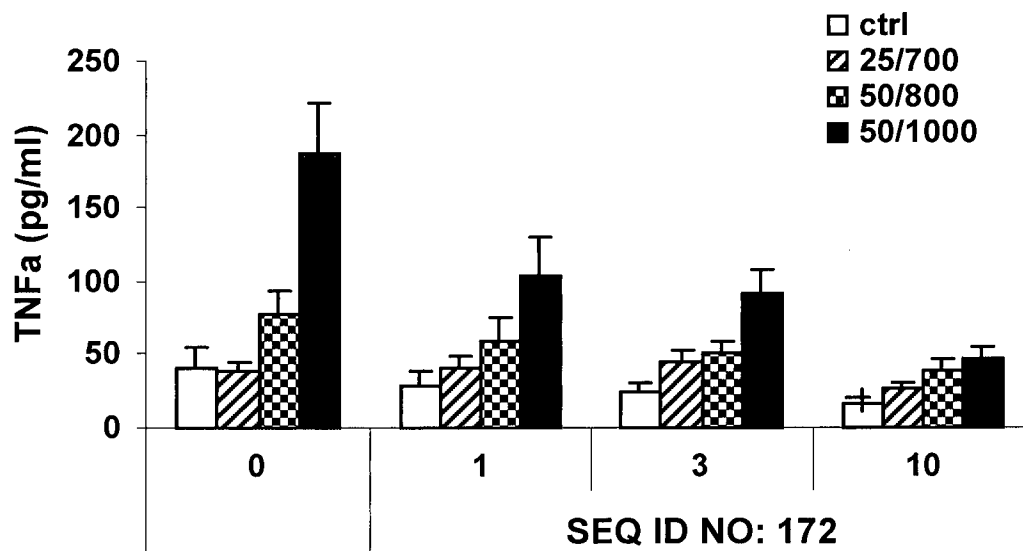


Fig. 21

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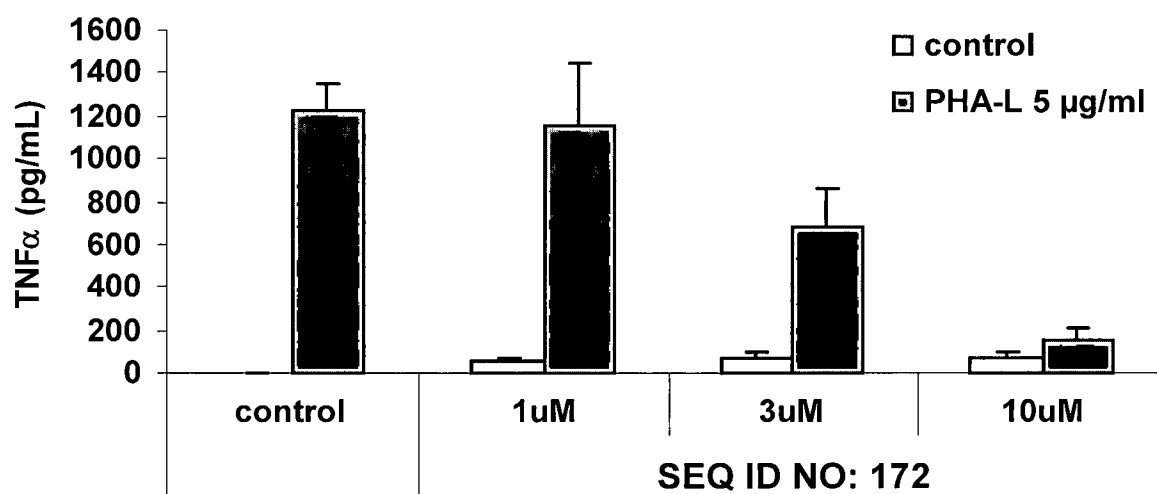


Fig. 22

31/63

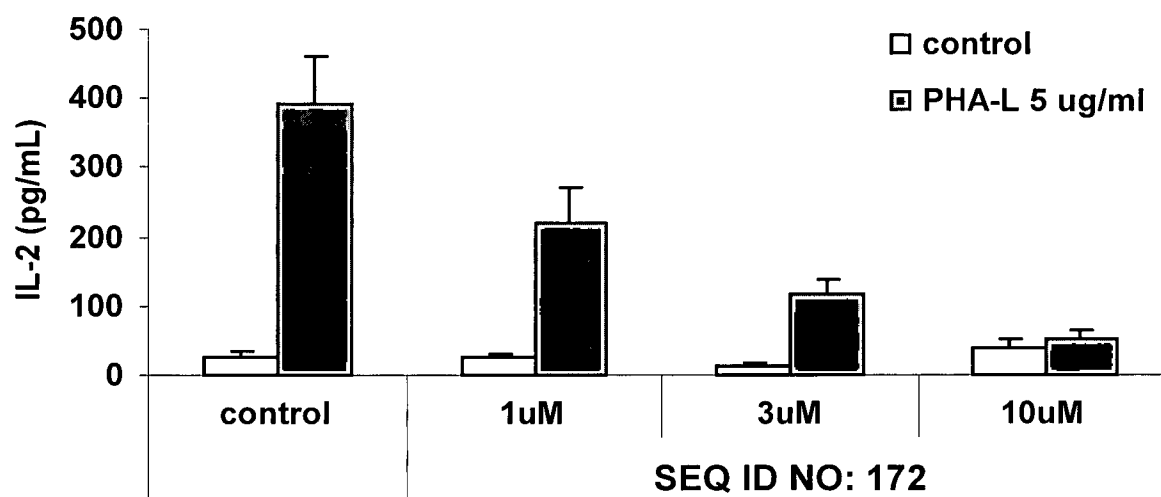


Fig. 23

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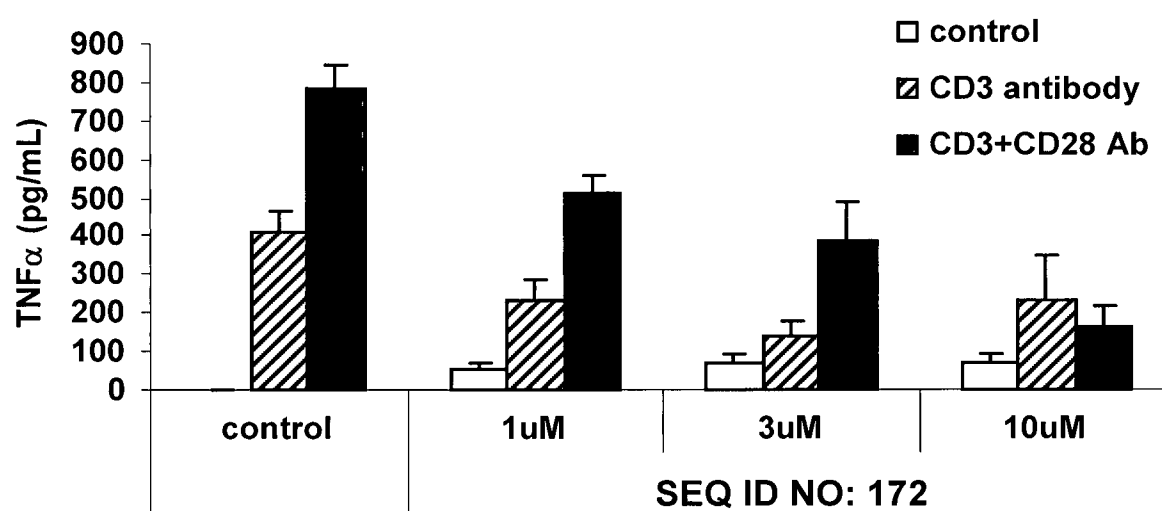


Fig. 24

33/63

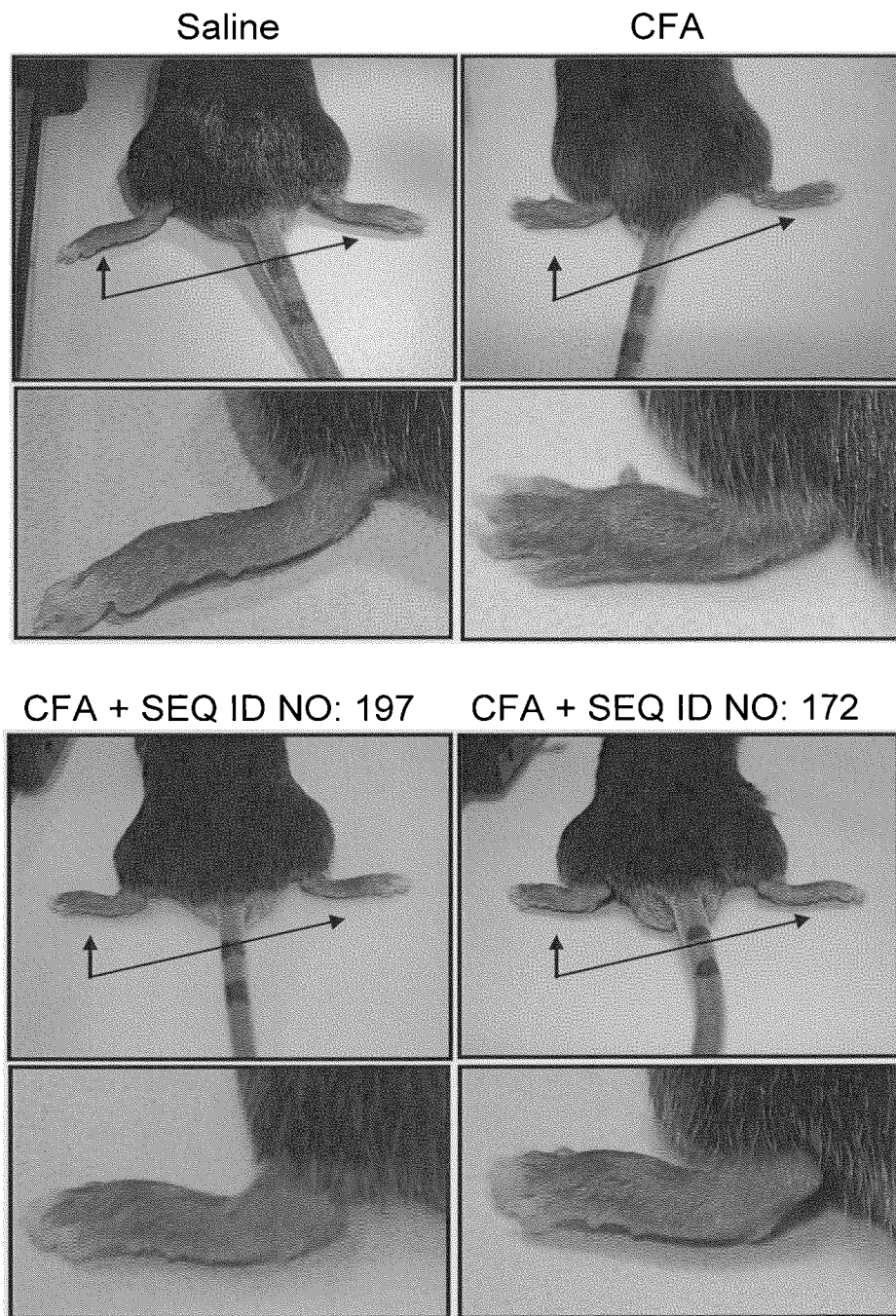


Fig. 25

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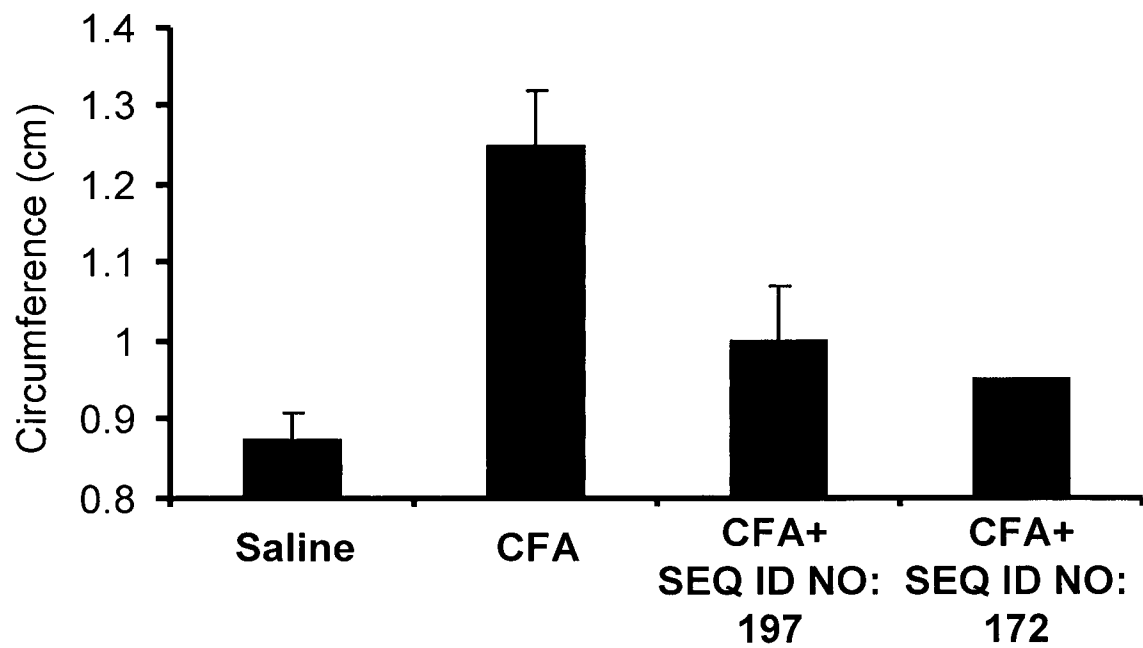


Fig. 26

35/63

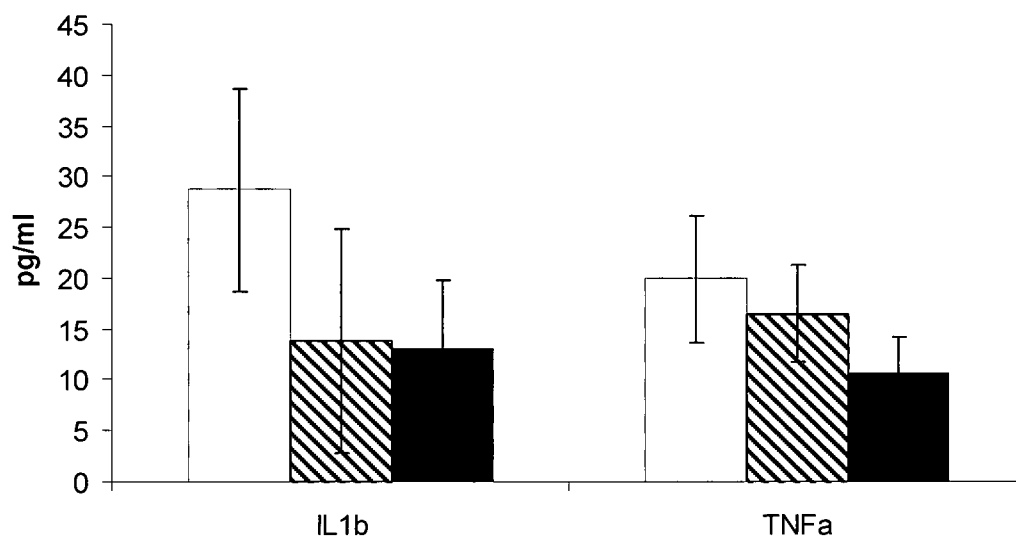


Fig. 27

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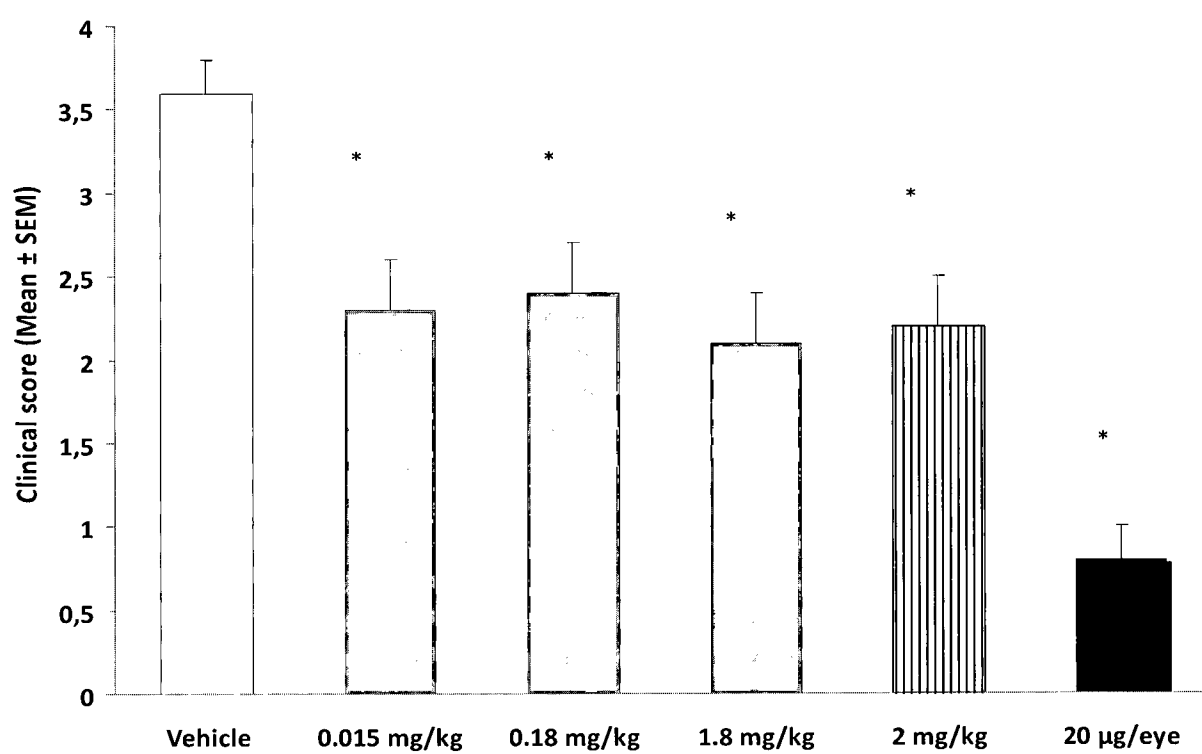


Fig. 28

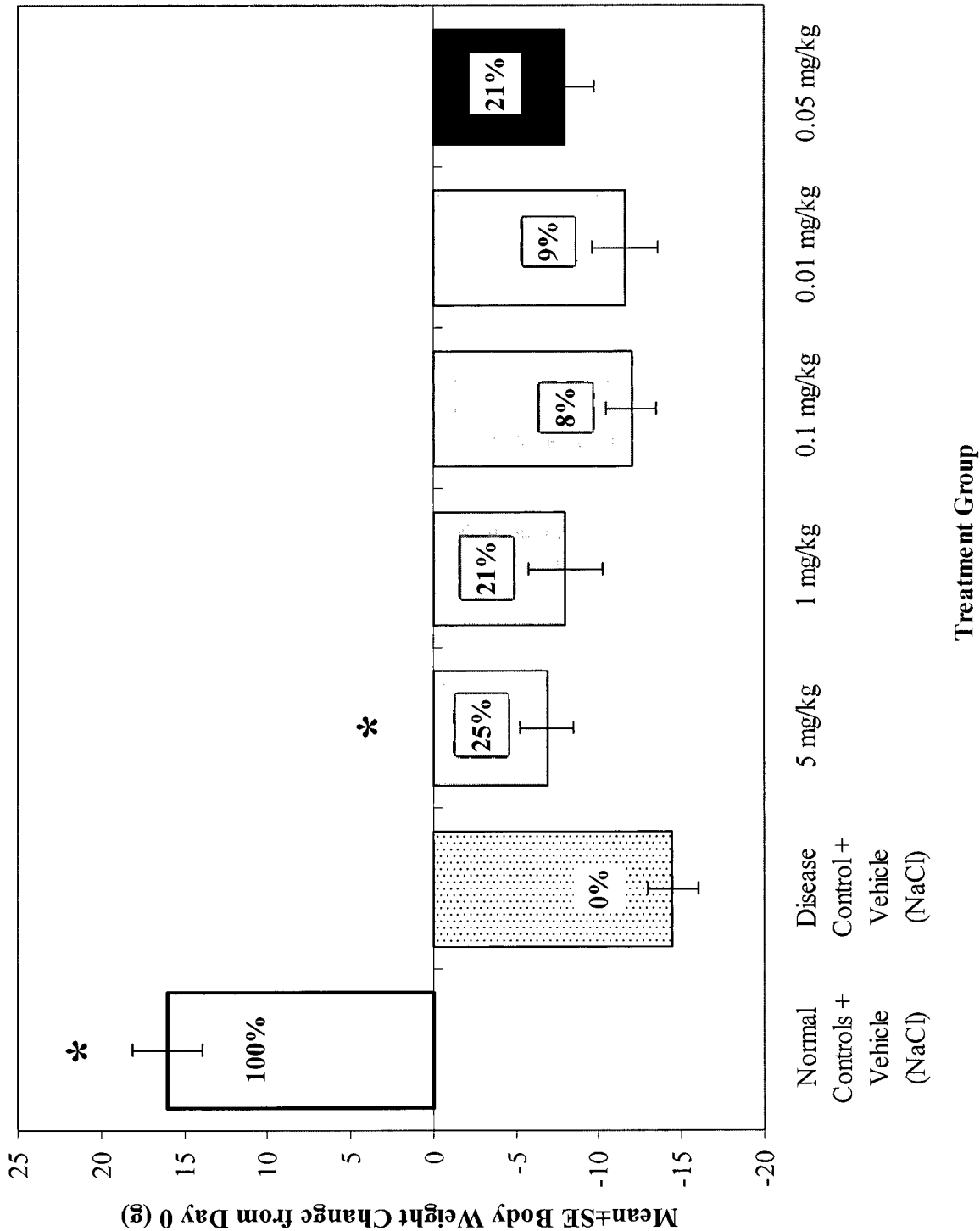


Fig. 29

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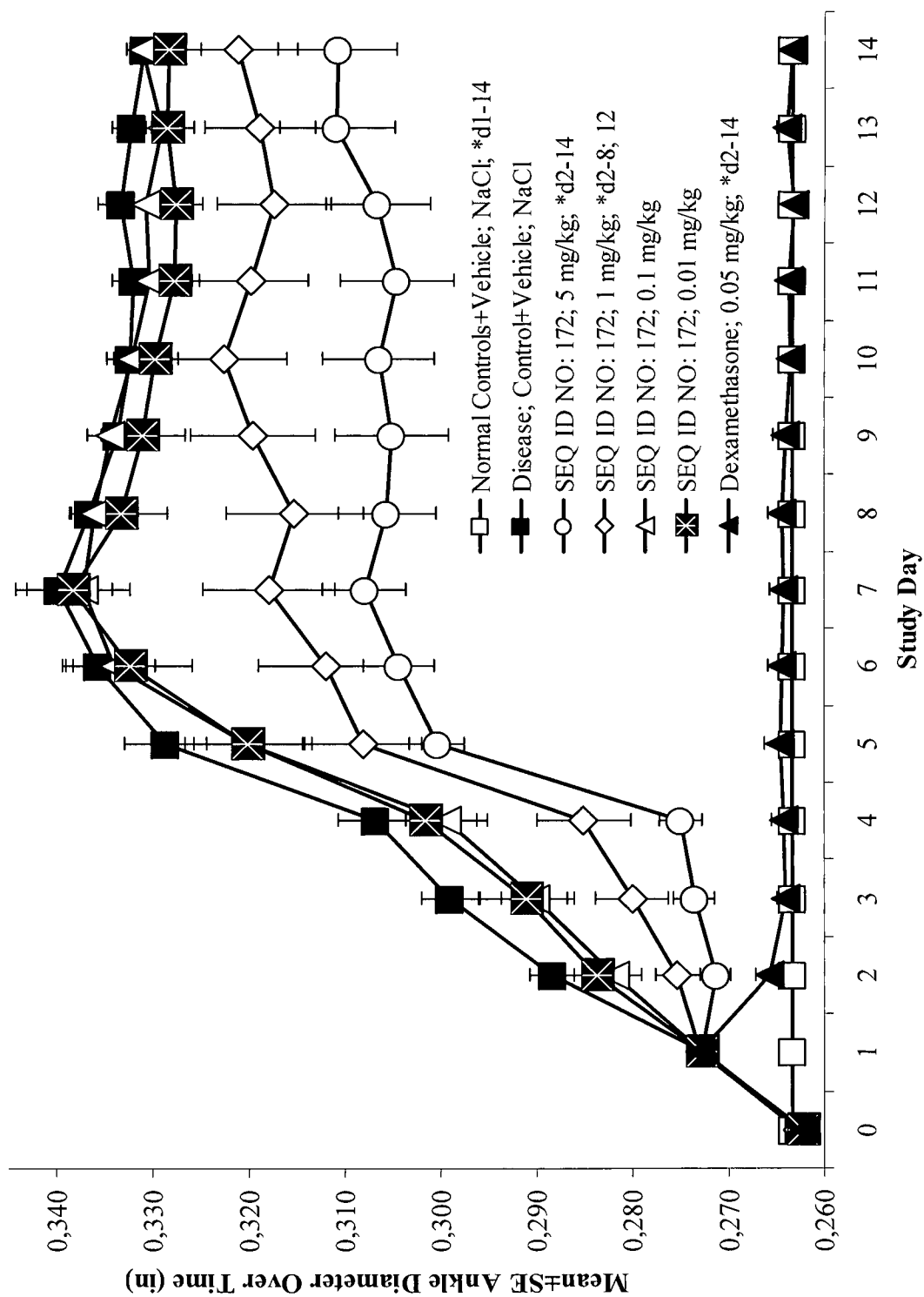


Fig. 30

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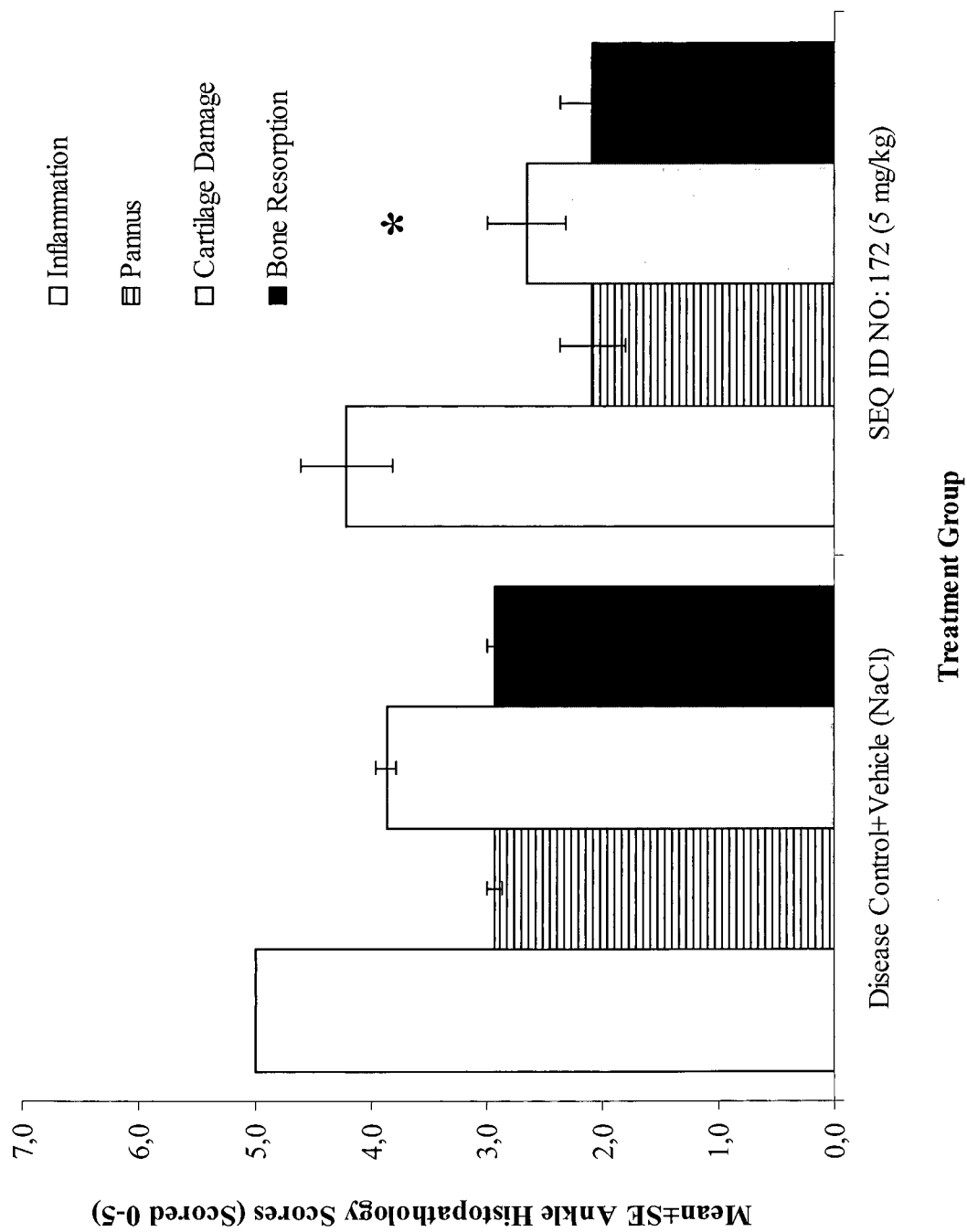


Fig. 31

40/63

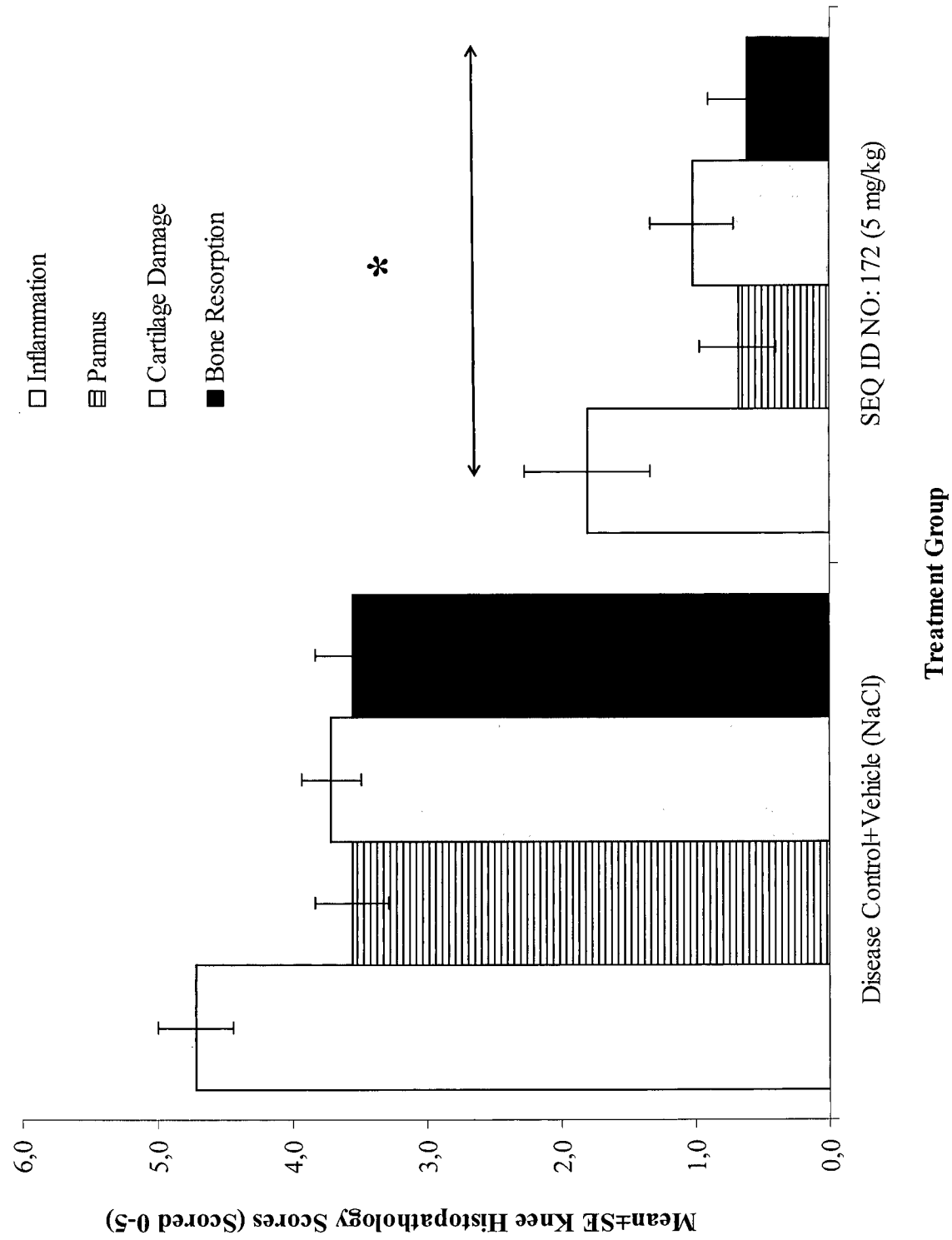


Fig. 32

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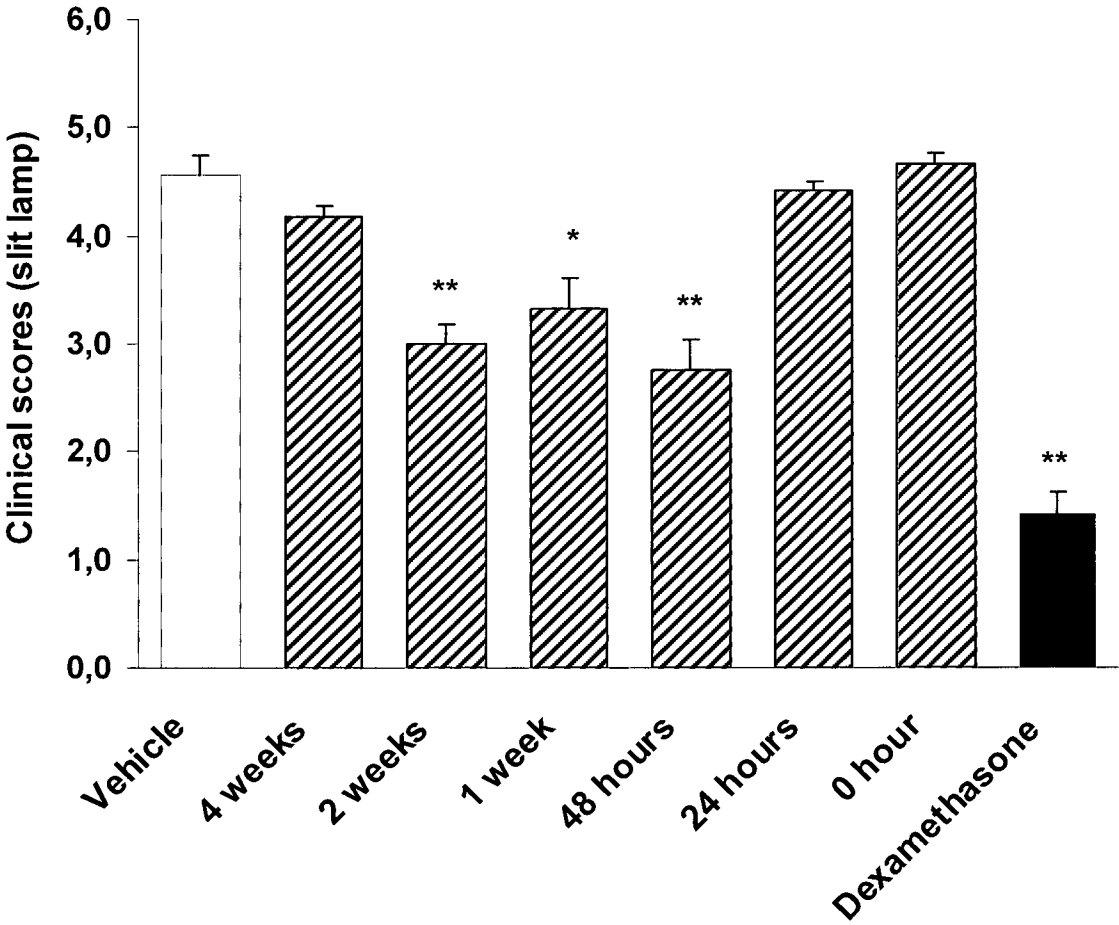


Fig. 33

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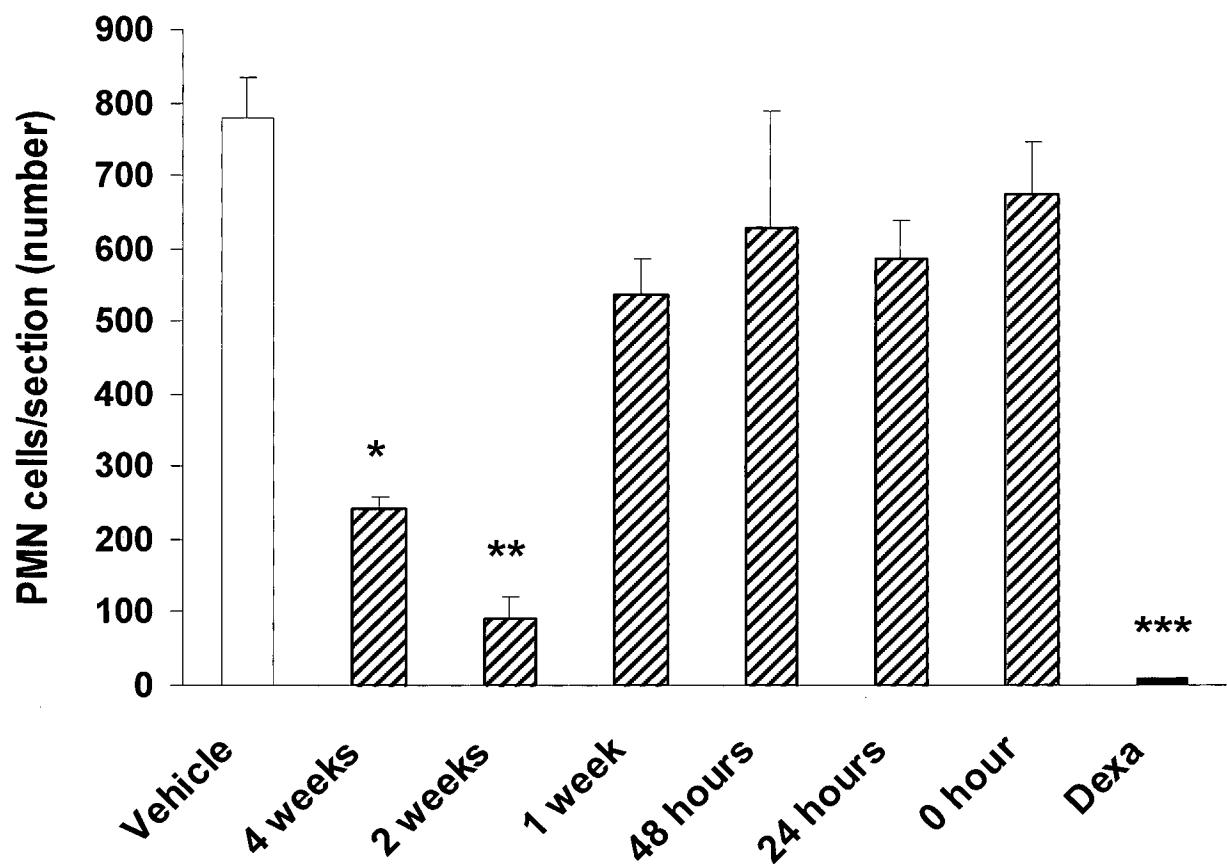


Fig. 34

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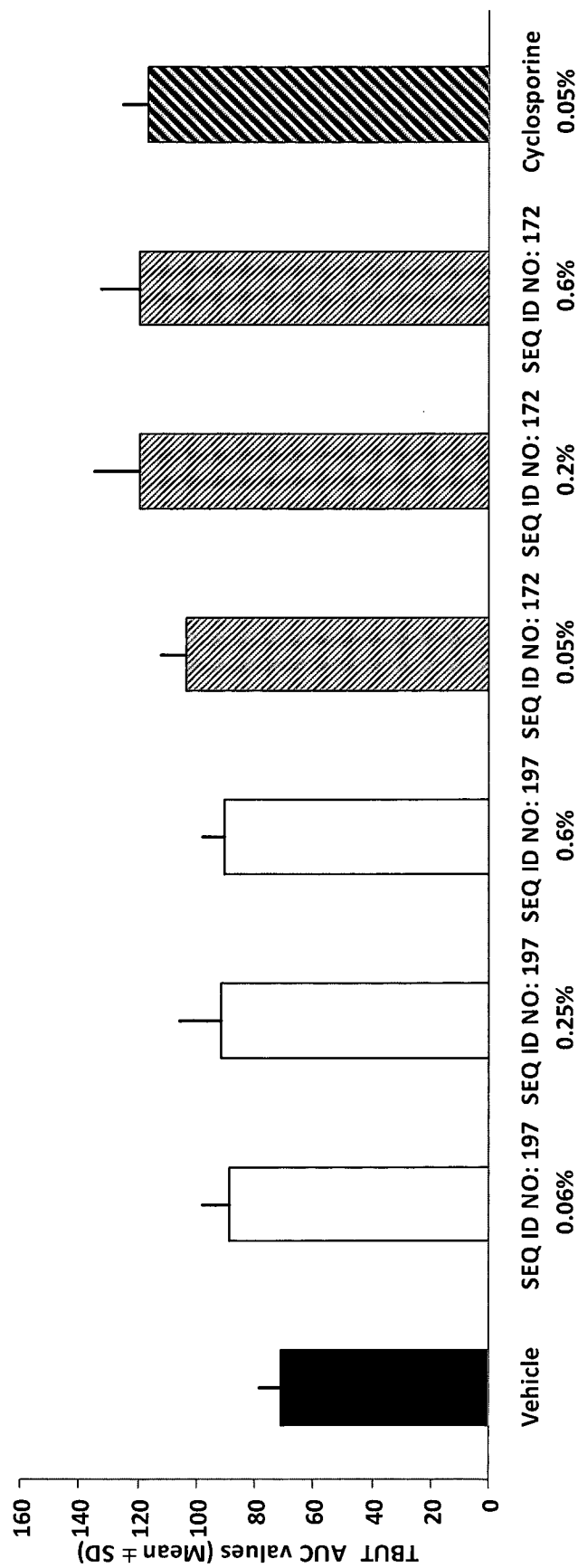


Fig. 35

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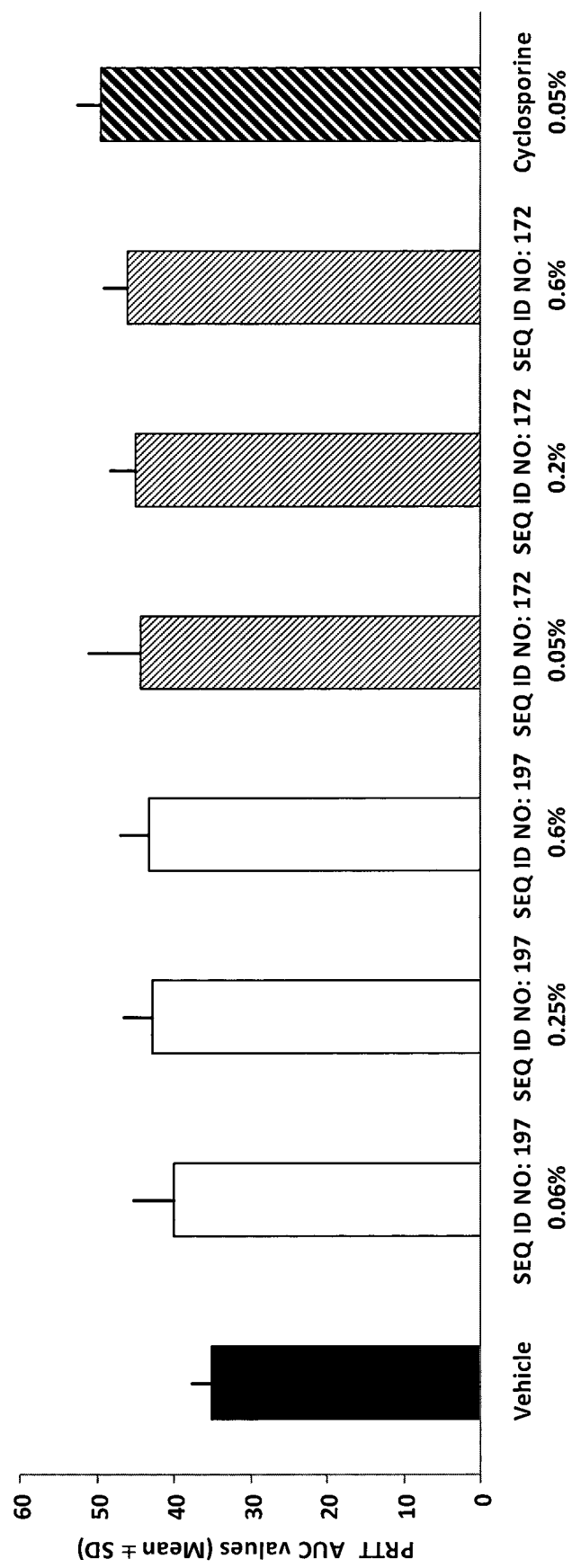


Fig. 36

45/63

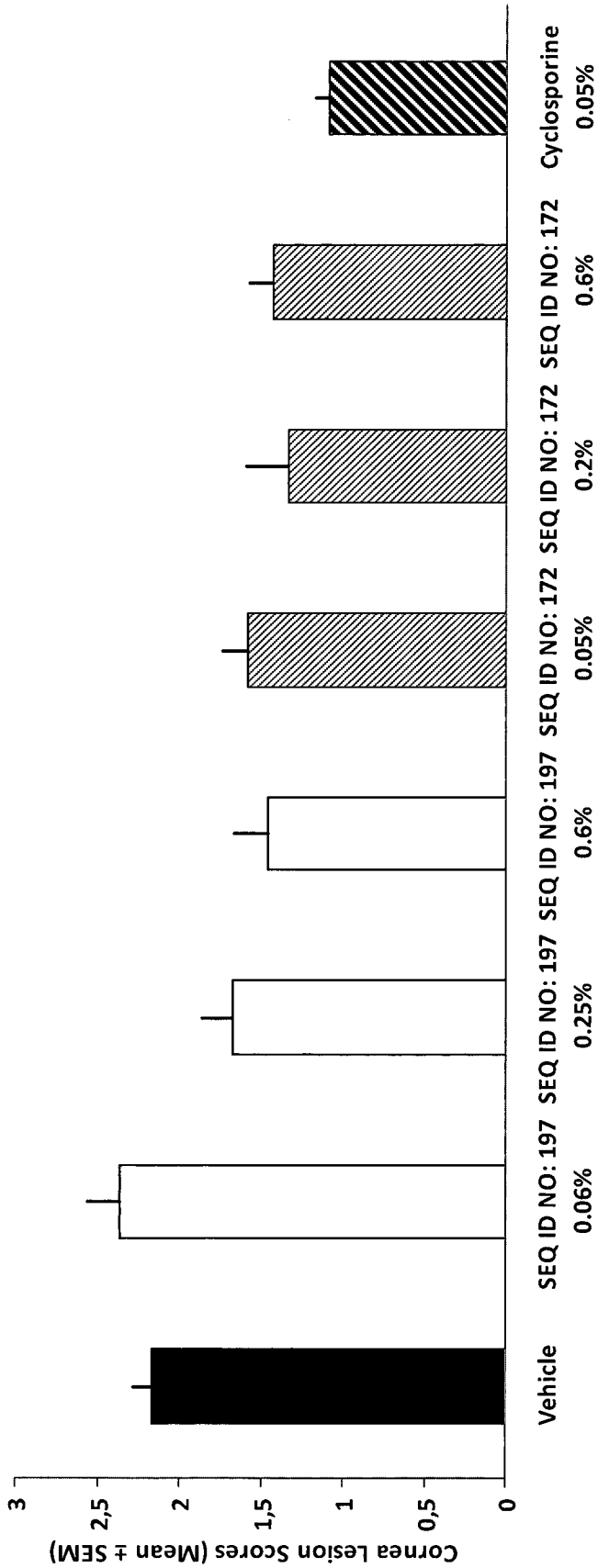
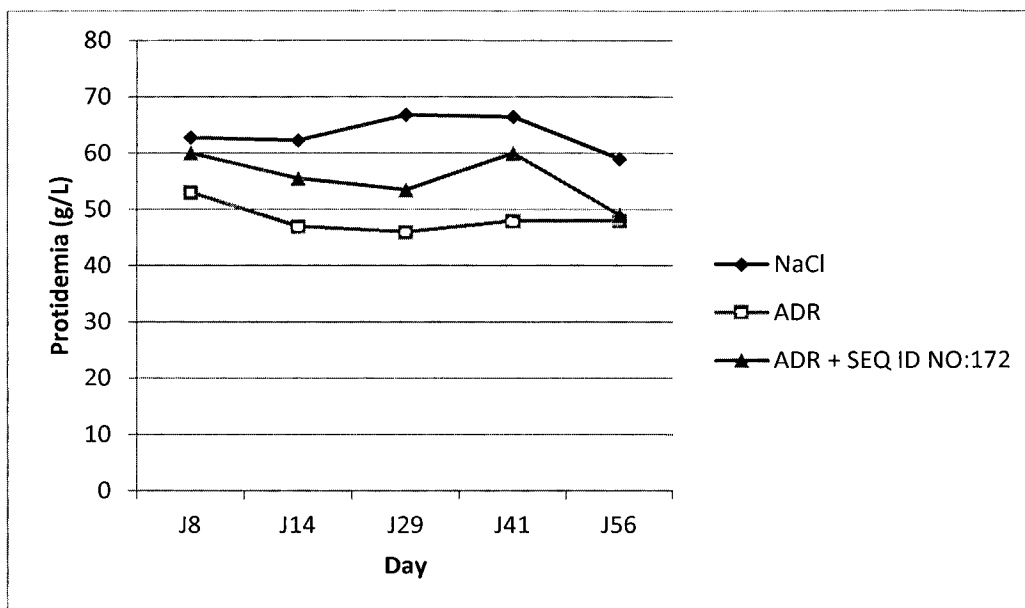
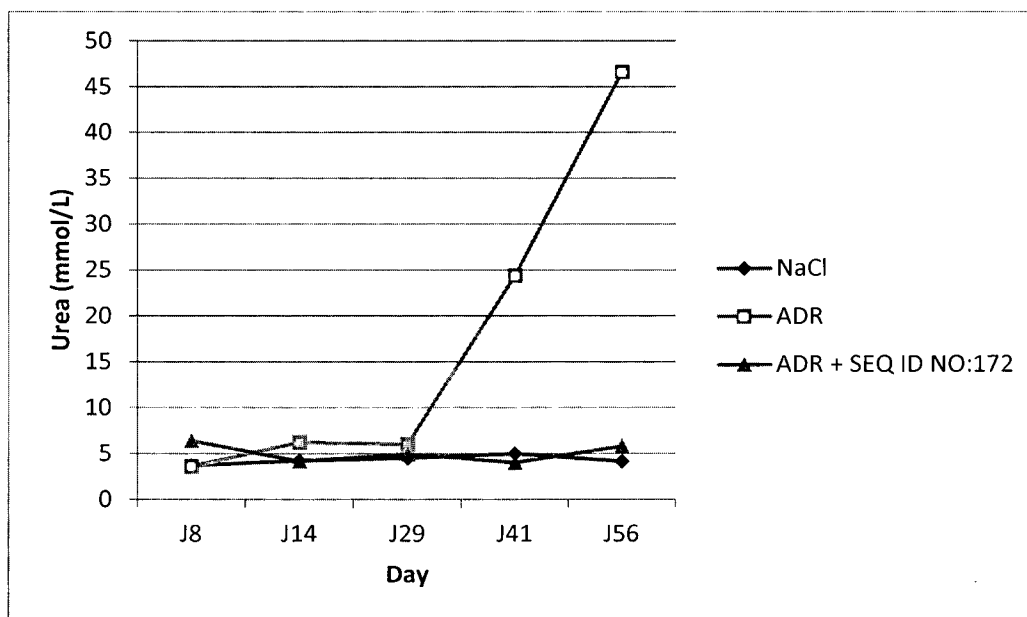


Fig. 37

46/63



A



B

Fig. 38

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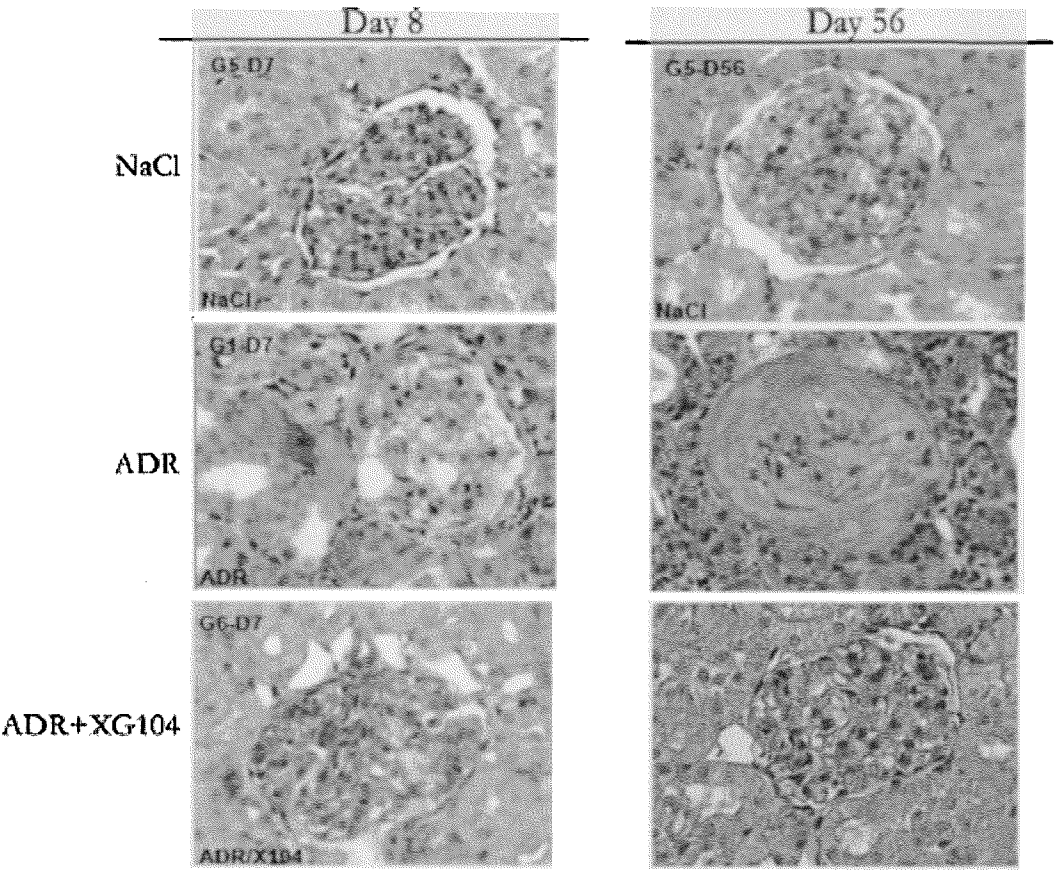


Fig. 39

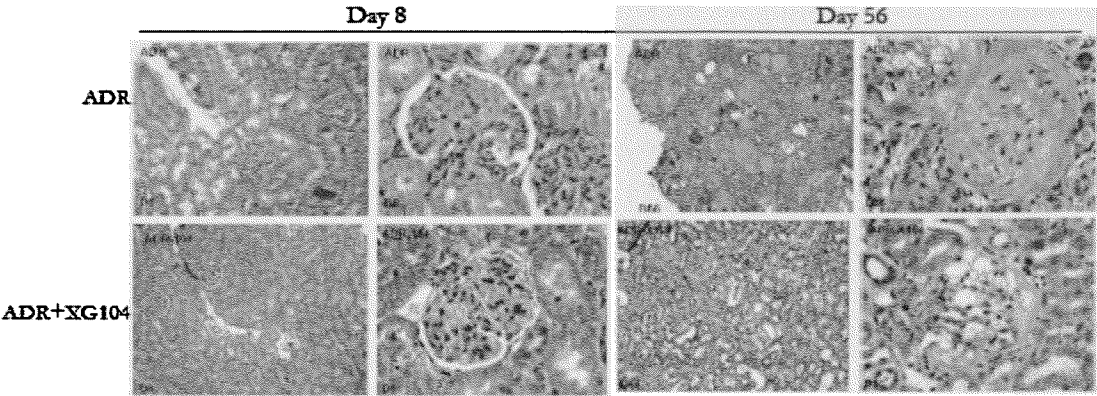


Fig. 40

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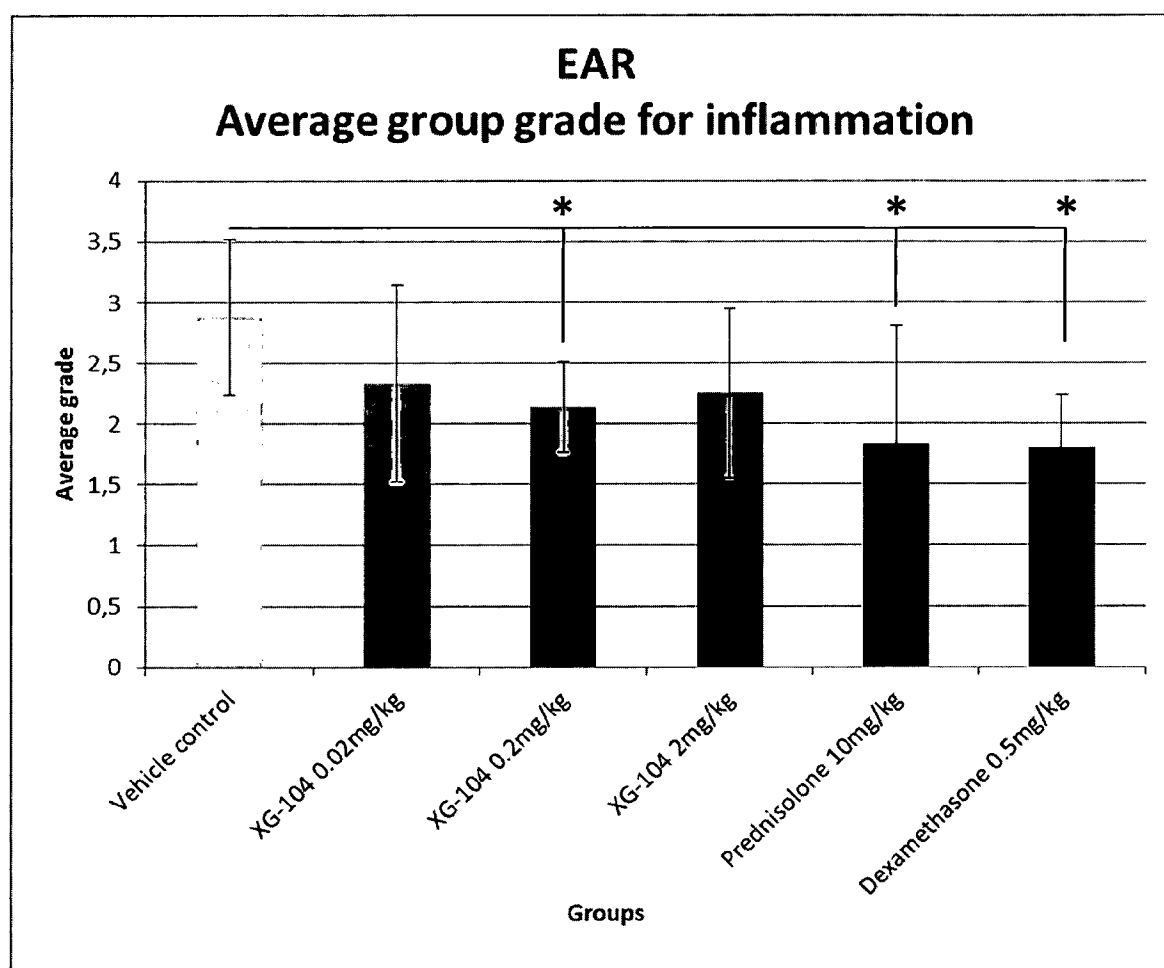


Fig. 41

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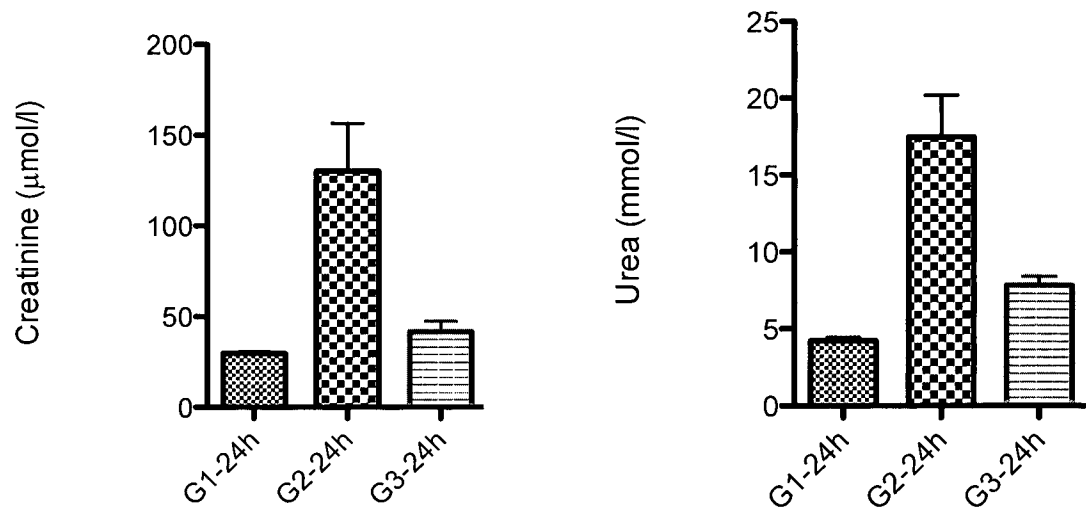
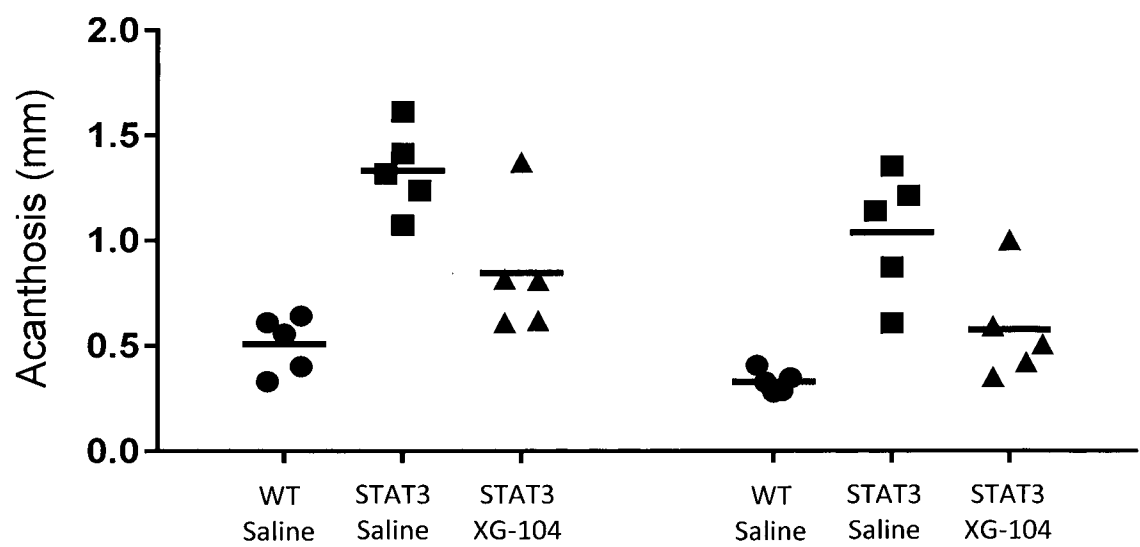


Fig. 42

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A



B

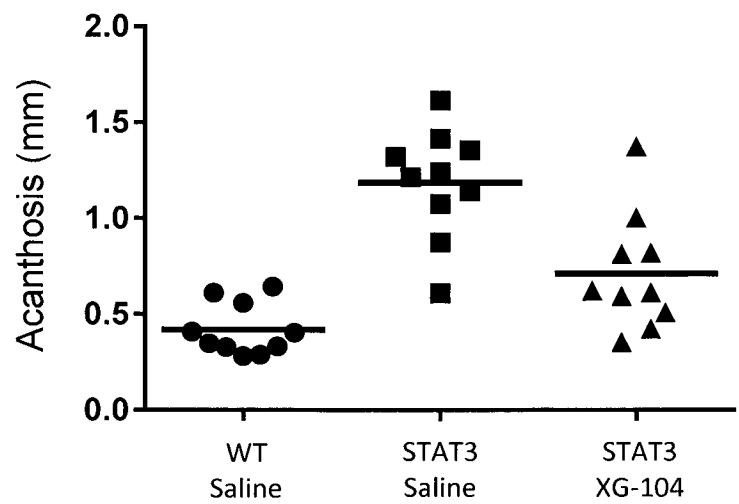


Fig. 43

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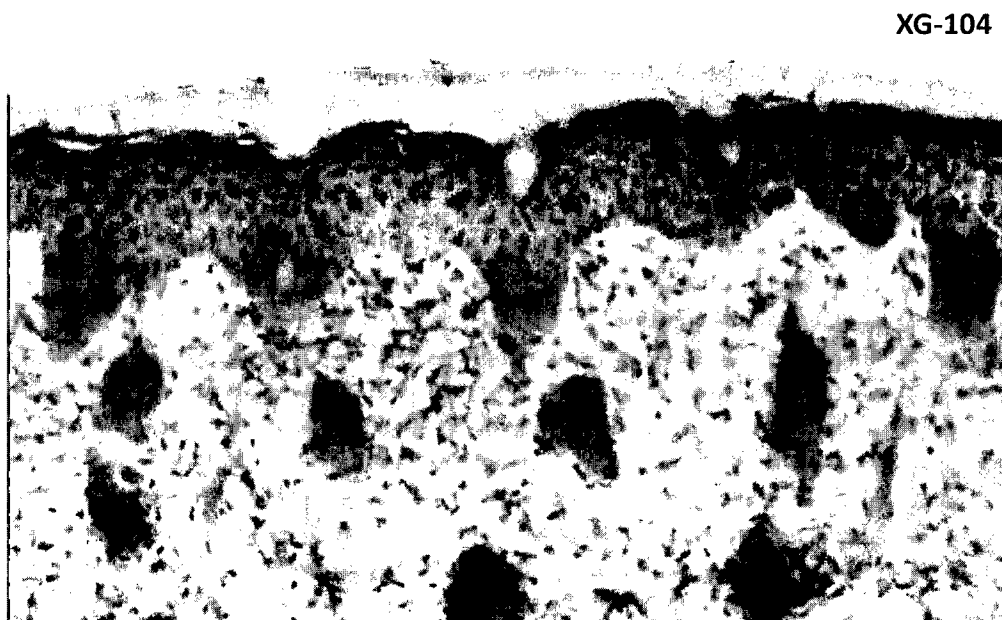
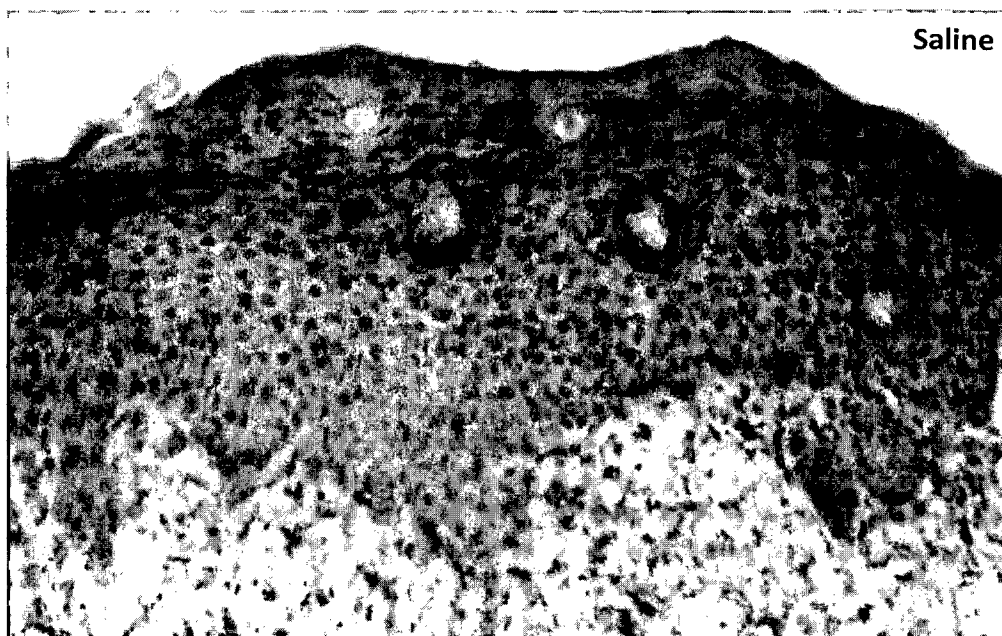
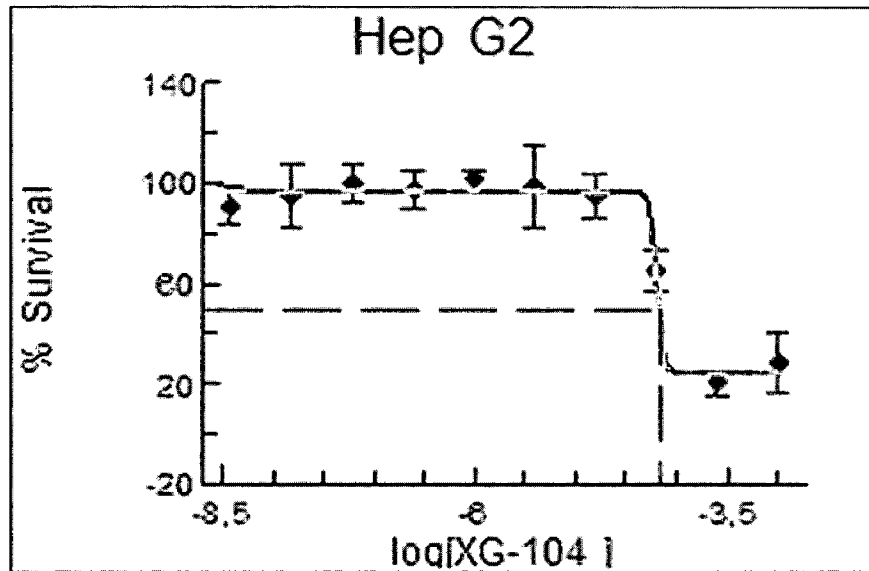


Fig. 44

53/63

A



B

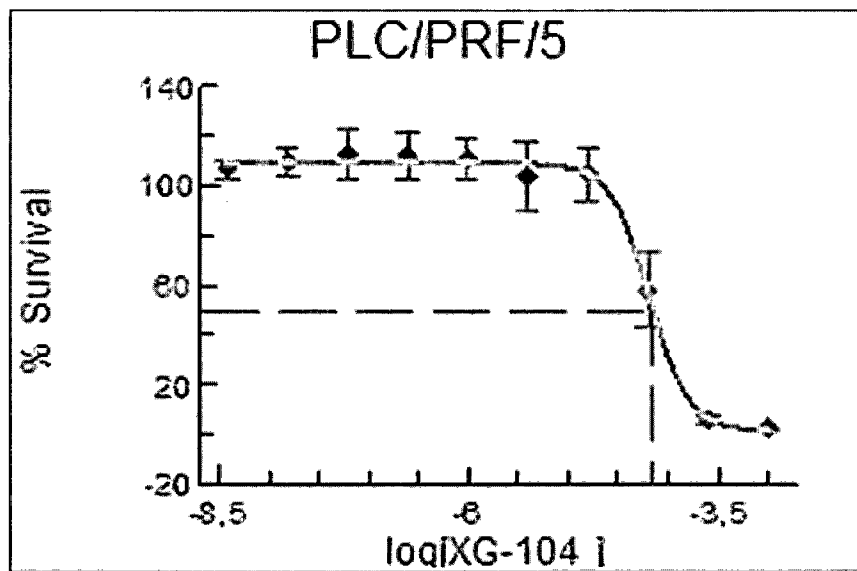


Fig. 45

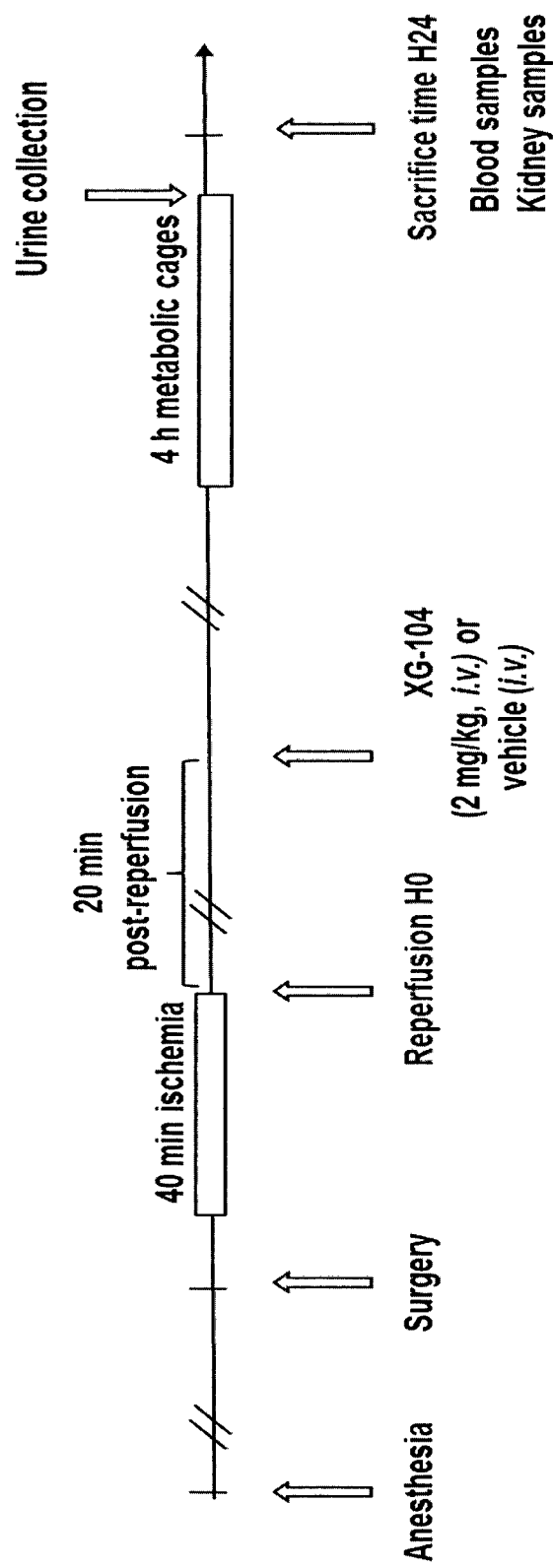


Fig. 46

55/63

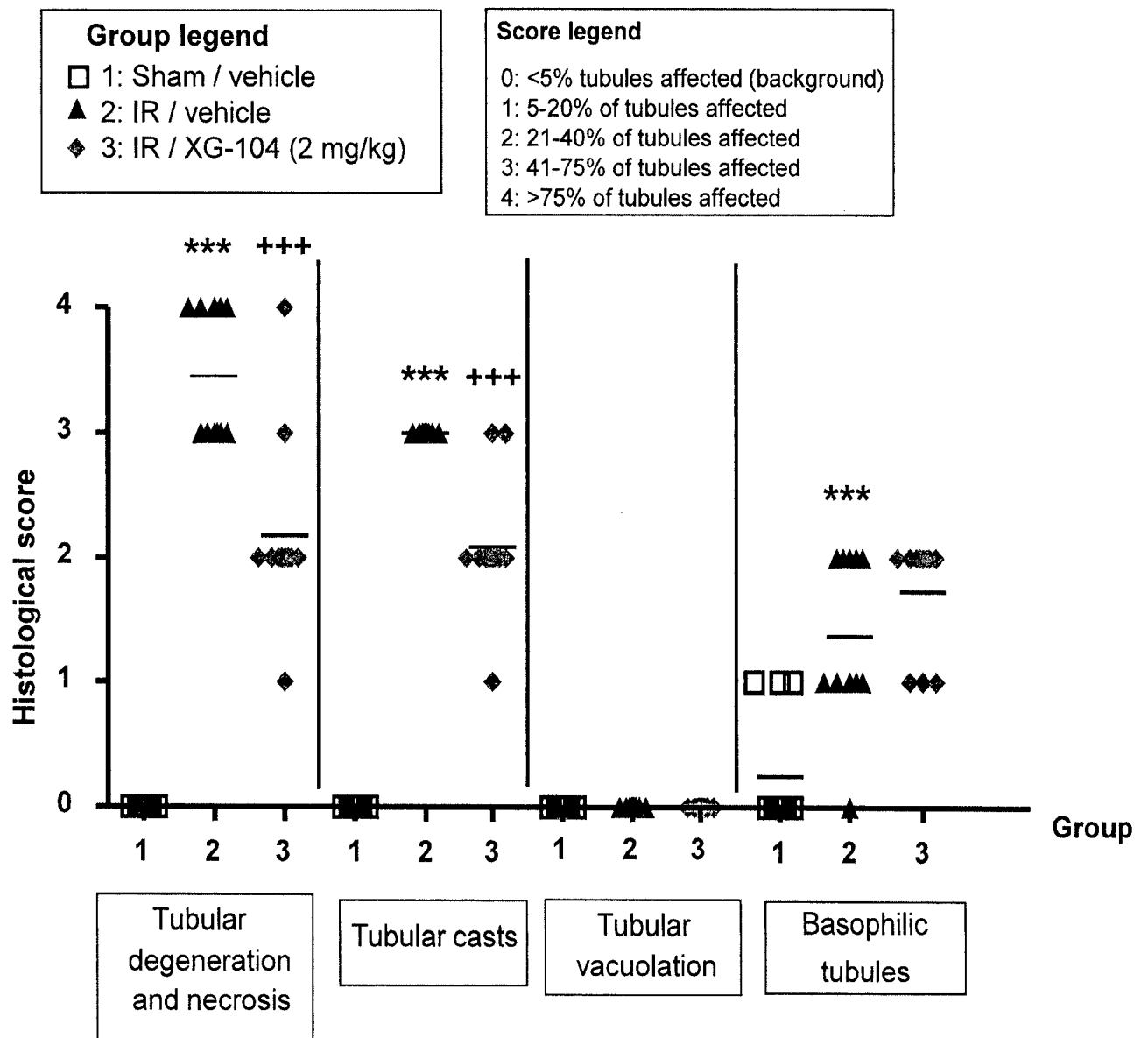


Fig. 47

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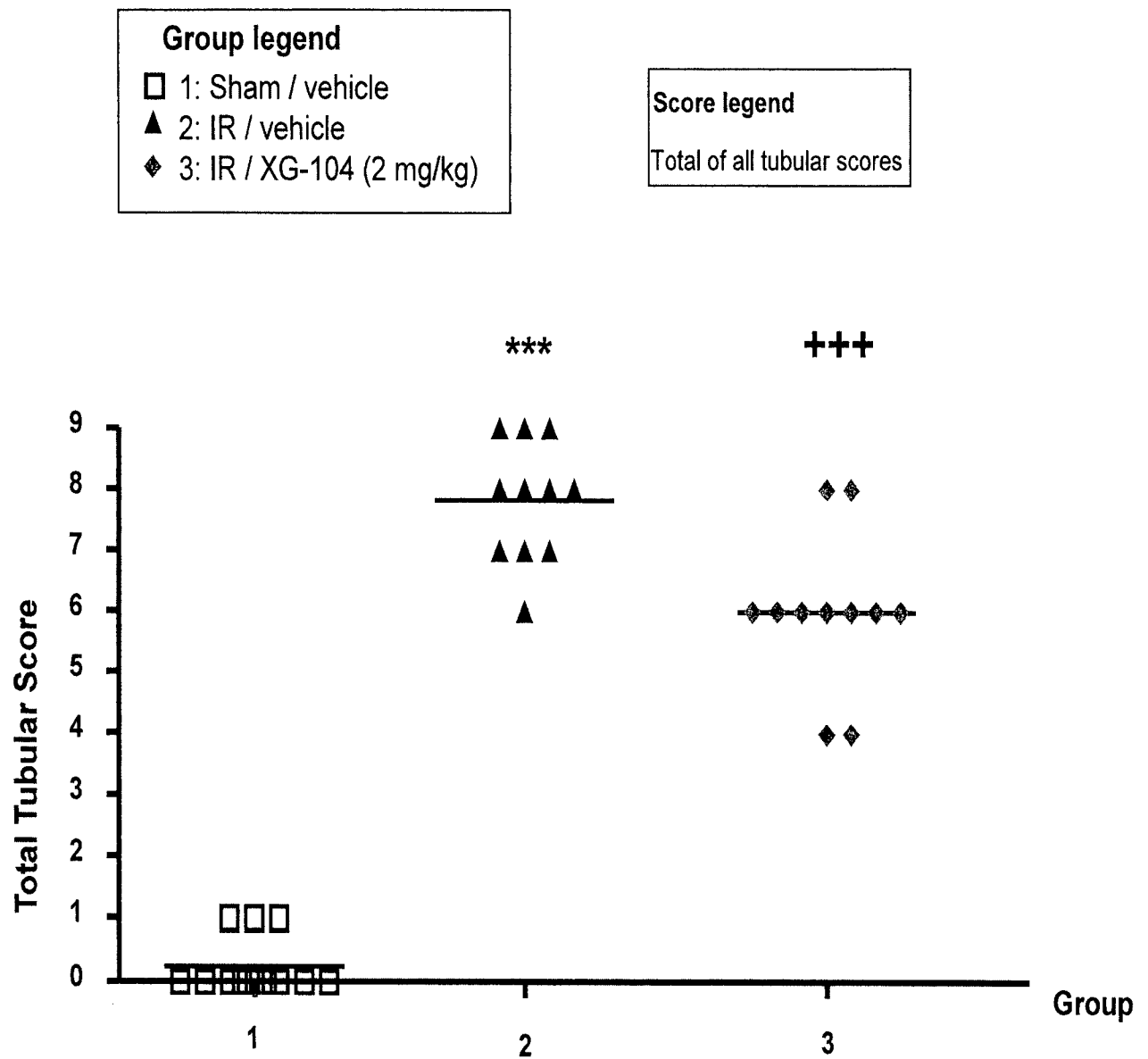


Fig. 48

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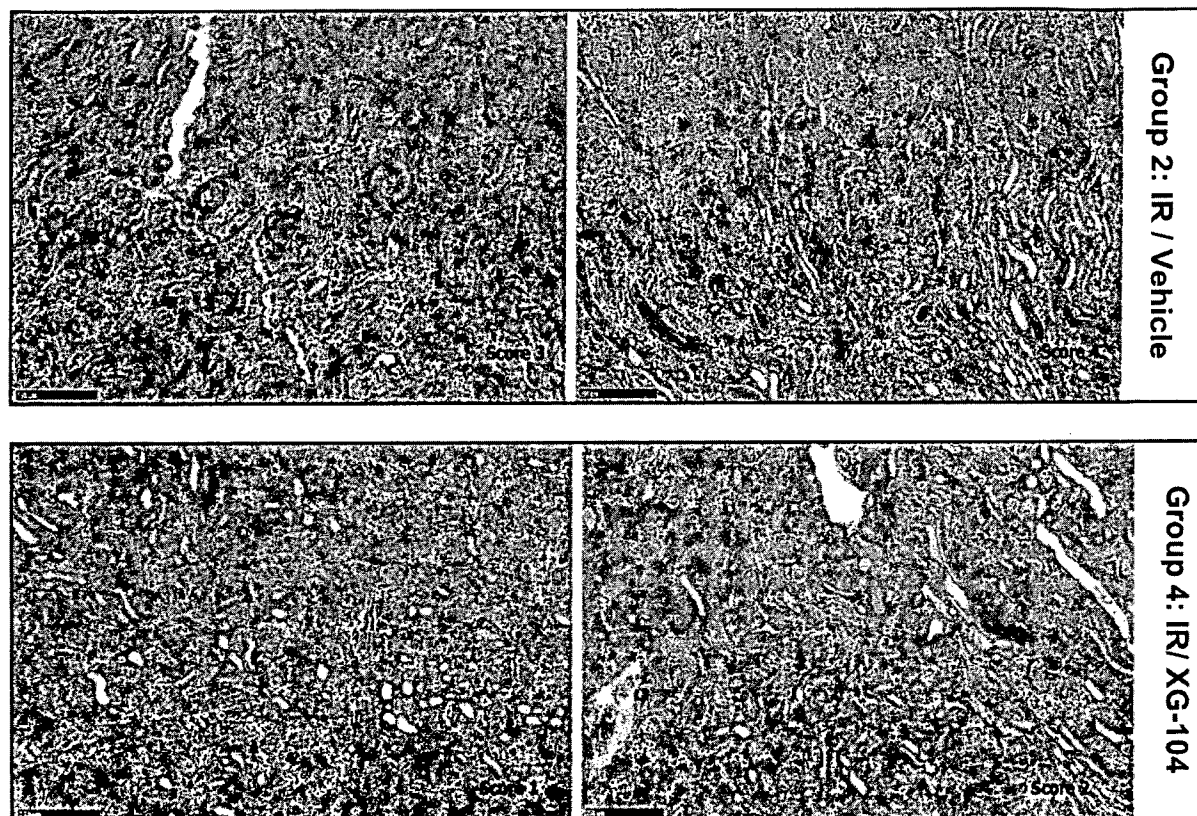
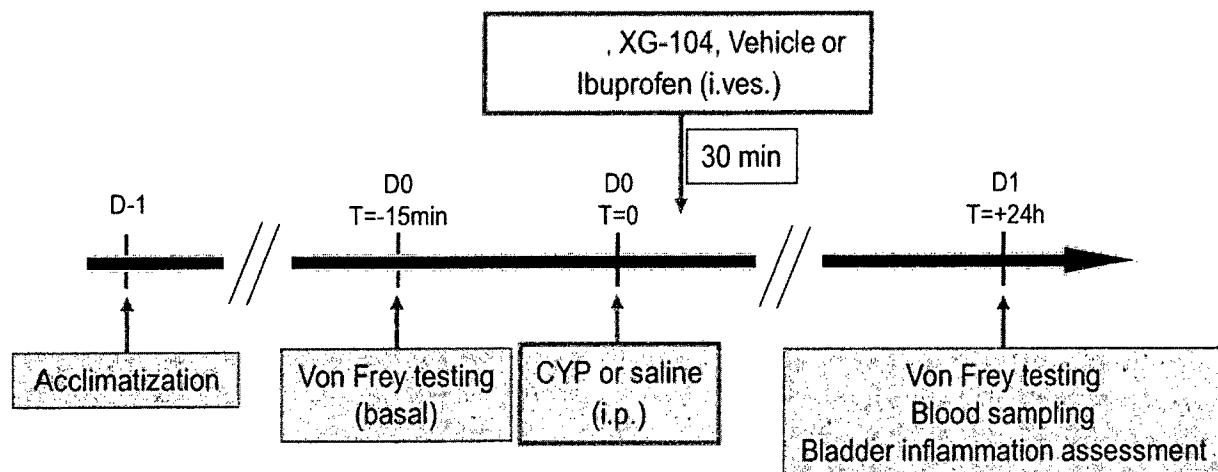


Fig. 49

58/63

A



B

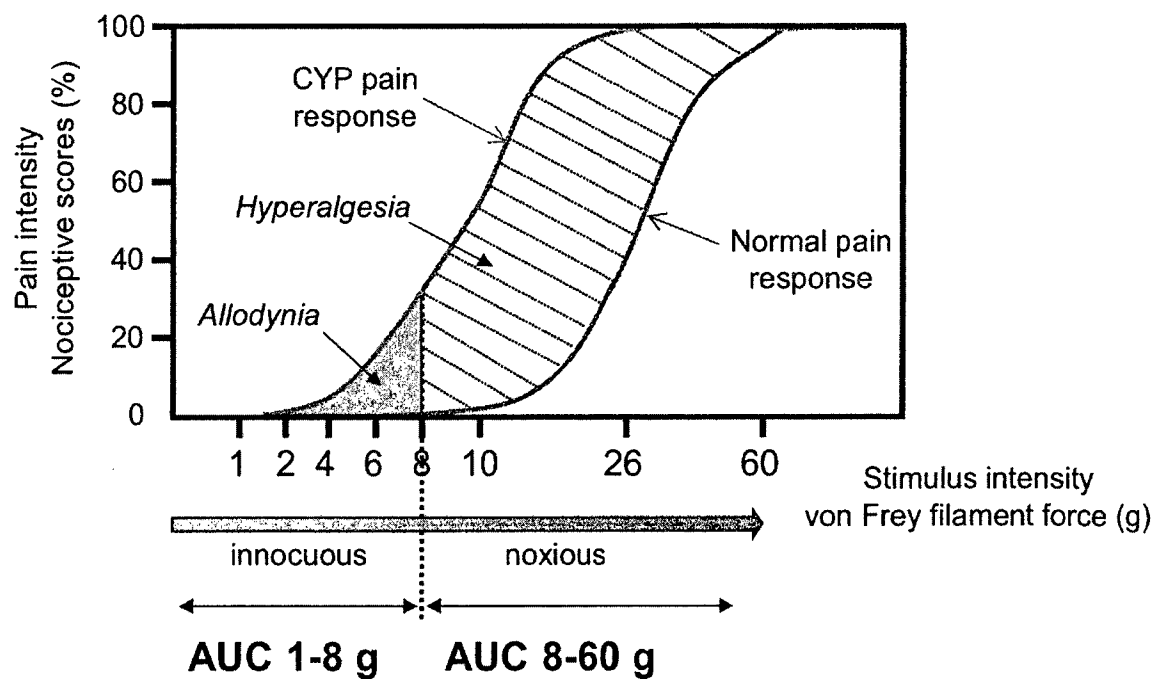
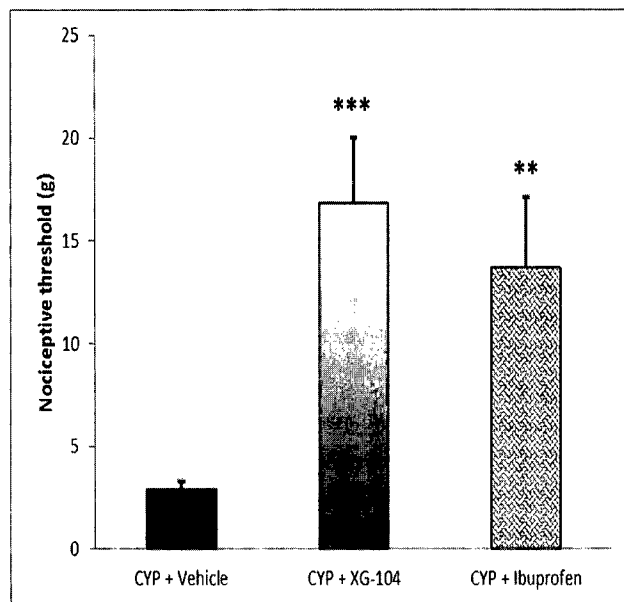


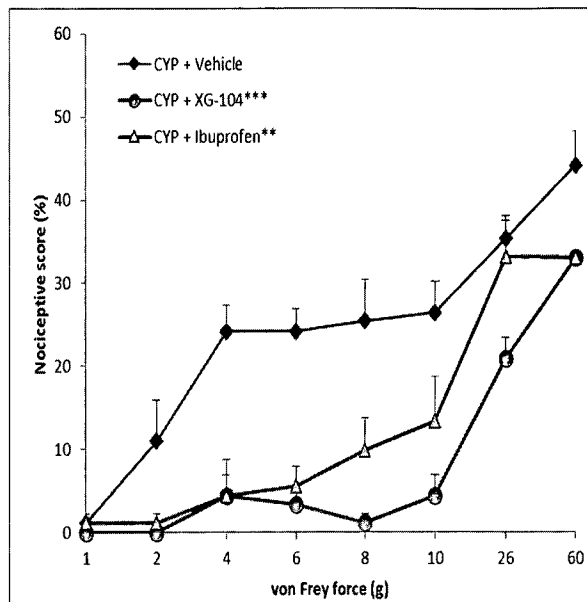
Fig. 50

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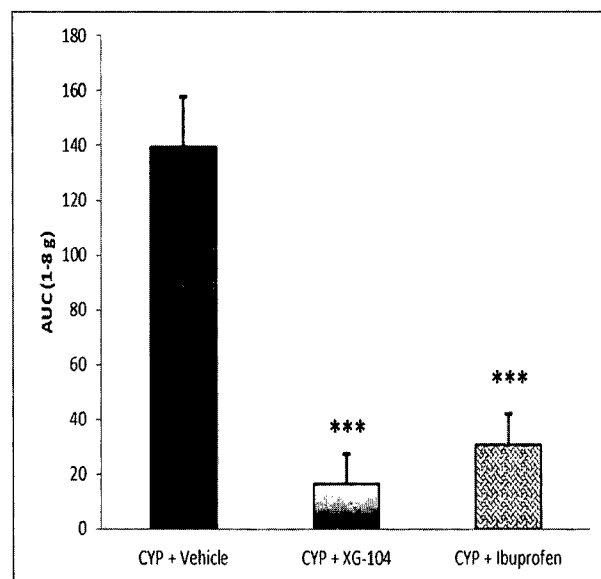
A



B



C



D

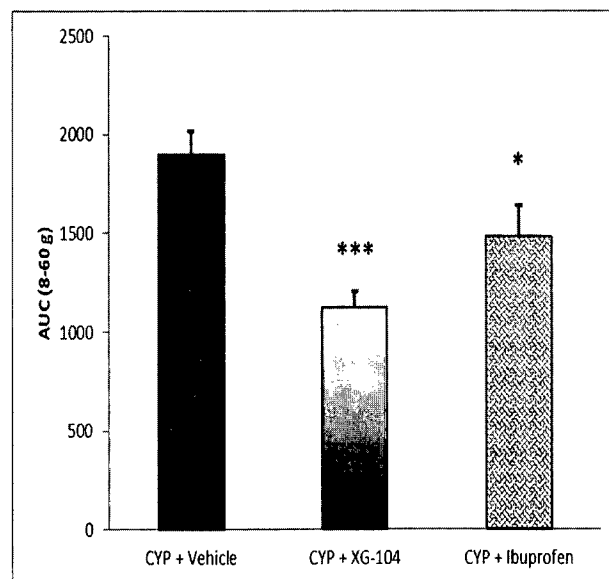
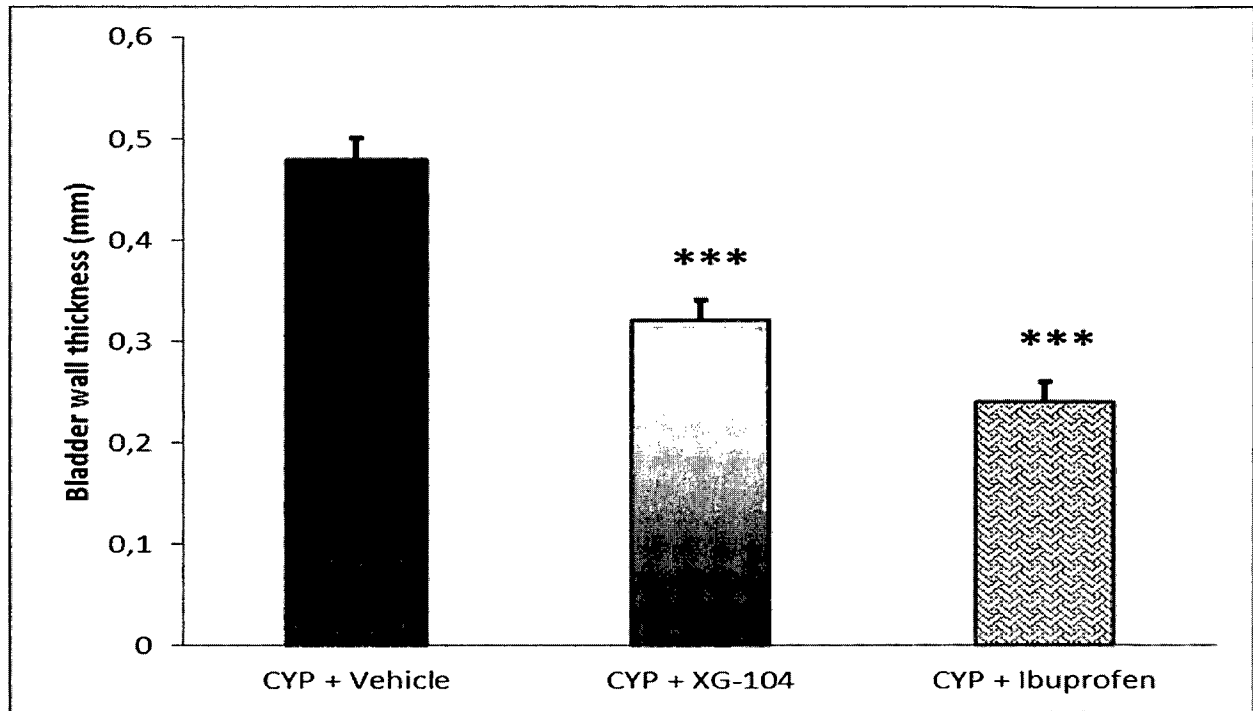


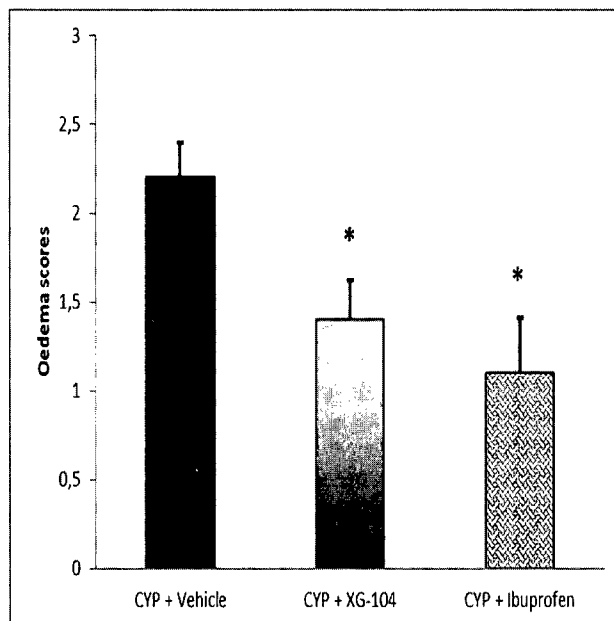
Fig. 51

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A



B



C

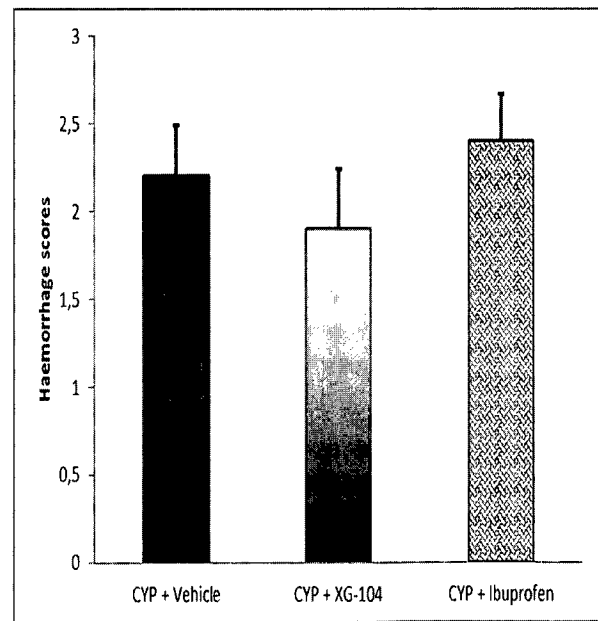


Fig. 52

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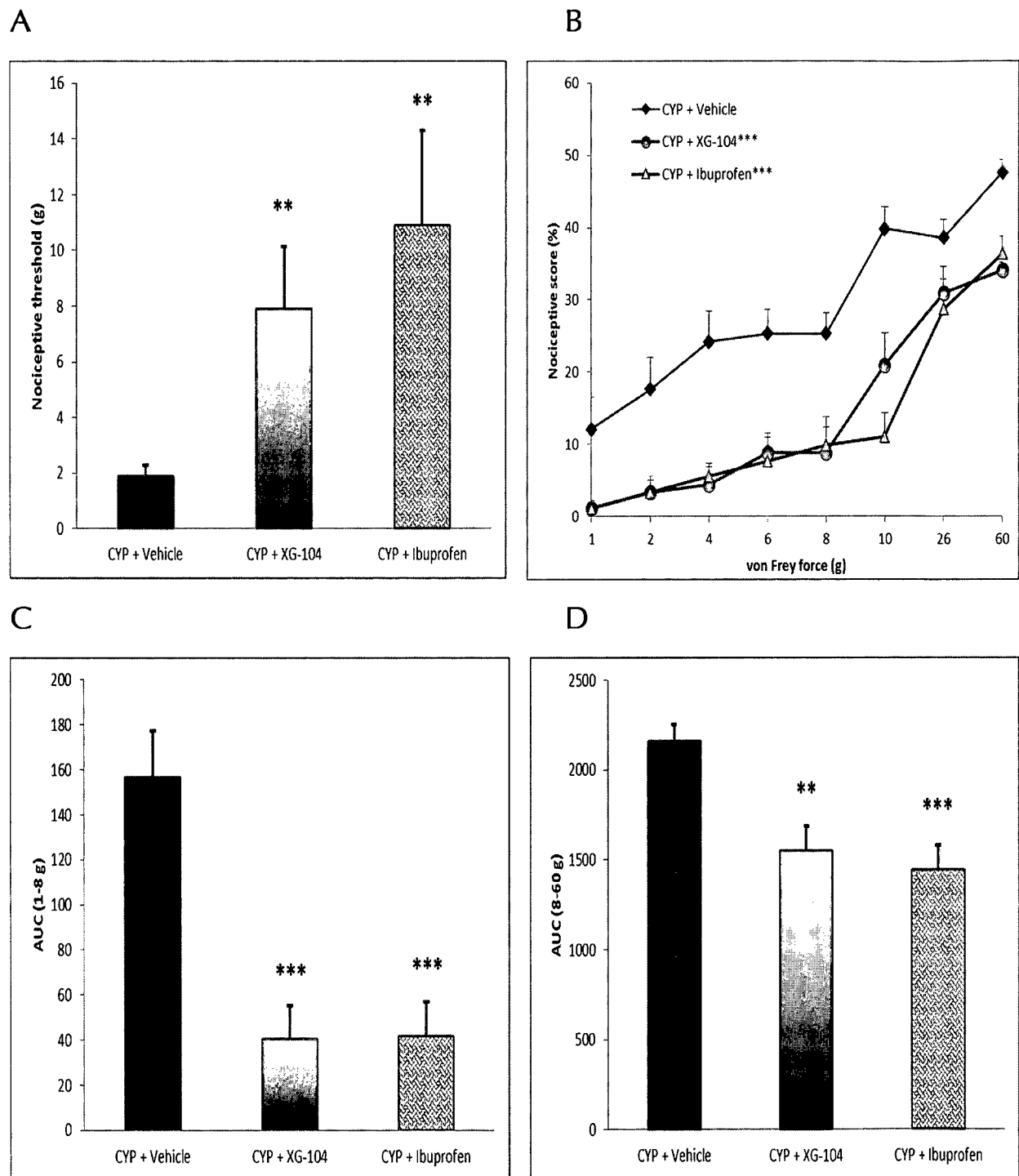


Fig. 53

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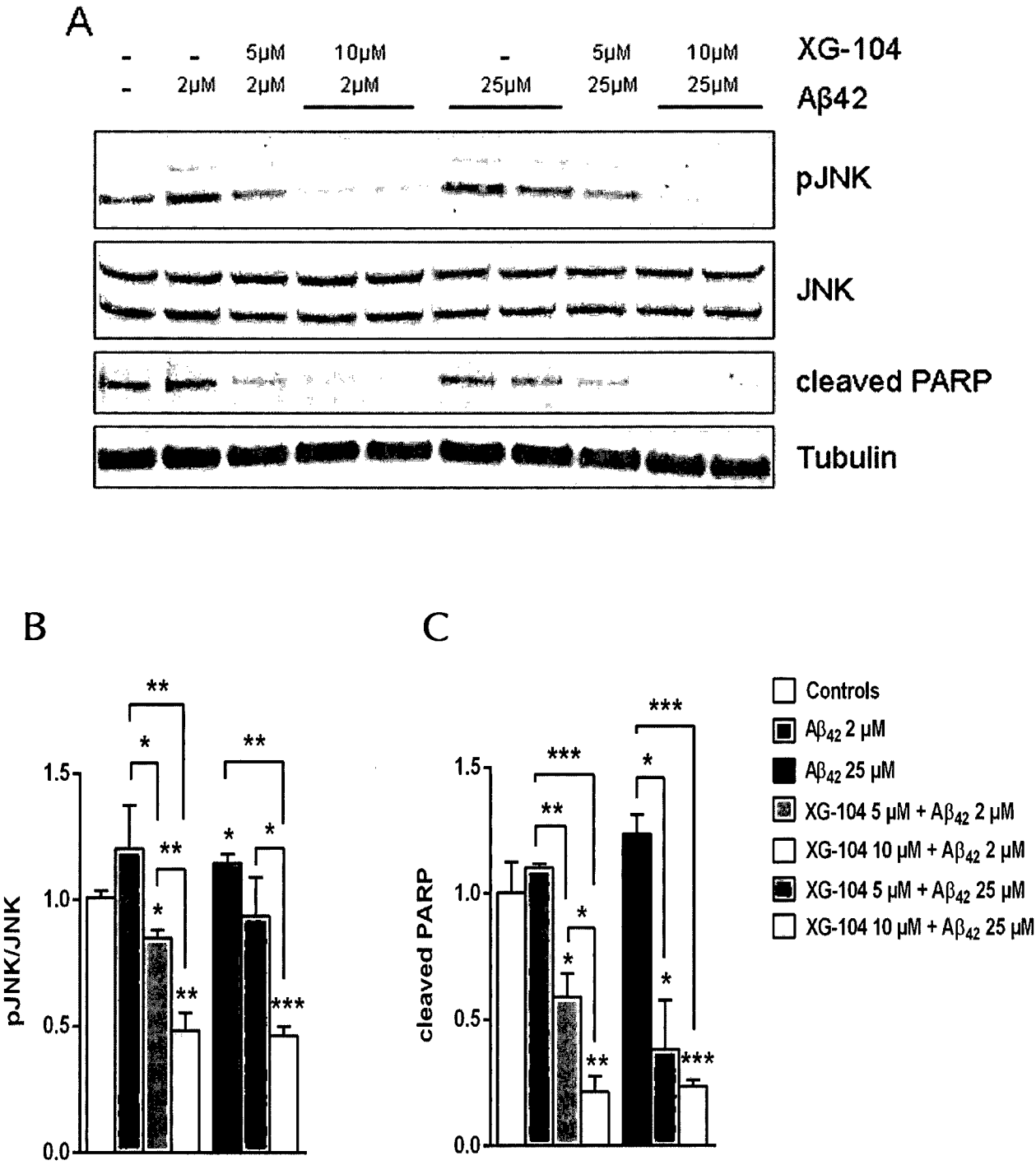


Fig. 54

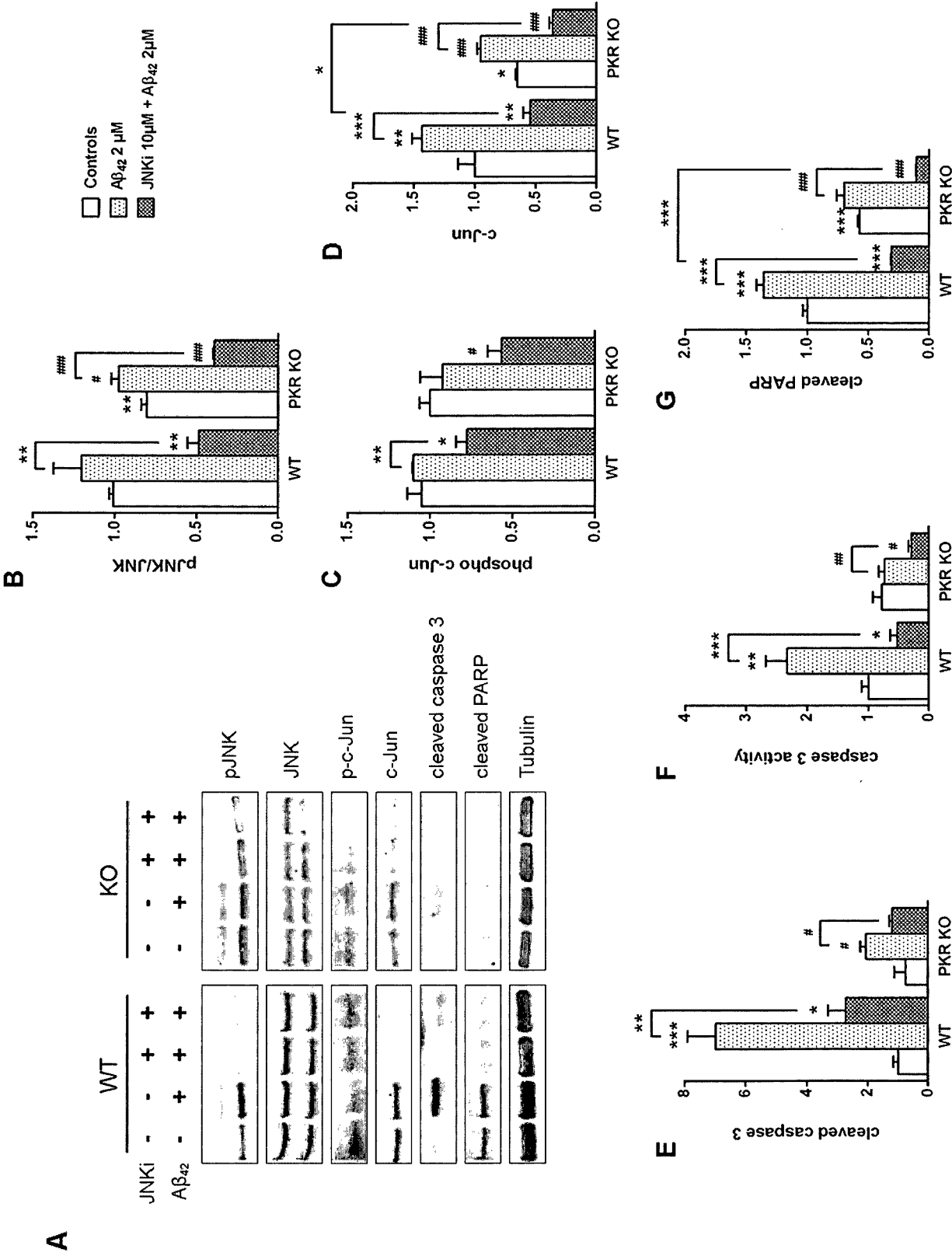


Fig. 55