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(54) Title: MOLECULES THAT ARE ABLE TO INHIBIT THE BINDING BETWEEN NGF AND THE TrkA RECEPTOR AS ANALGESICS WITH PROLONGED EFFECT

(57) Abstract: Use of an anti-NGF antibody capable of inhibiting the binding between NGF and TrkA, capable of blocking the biological activity of TrkA for the preparation of a medicament for treating and/or preventing chronic pain.



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BACKGROUND TO THE INVENTION

The present invention relates to the use of molecules that are capable of inhibiting the binding between NGF and its receptor, TrkA. In particular, it relates to anti-NGF antibodies that, by blocking the biological activity of NGF, have a prolonged analgesic effect. Owing to the enduring analgesic effect thereof, they are an advantageous therapy for pathologies with persistent forms of pain, known also as chronic pain, such as but not limited to neuropathic or oncological pain.

STATE OF THE ART

The nociceptive signals afferent to the spinal cord are carried by the fibres A δ and C, the cell bodies of which (primary sensitive neurons) are located in the spinal dorsal ganglia (DRG). The primary sensitive neurons release glutamate together with ATP as an excitatory neurotransmitter, and various other substances such as substance P and CGRP (calcitonin-gene-related-peptide), (Hunt and Mantyh, 2001). The release of these excitatory neurotransmitters is controlled by various classes of receptors present on the afferent terminals, including those that are sensitive to capsaicin (vanilloid receptors, VR1), those activated by GABA, those activated by ATP itself and those activated by cannabinoids (CB1) (Sivilotti and Nistri, 1991; Hunt and Mantyh, 2001; Khakh, 2001; Morisset et al., 2001). One of the physiopathological mechanisms whereby chronic pain occurs is allodynia, i.e. the transformation of stimuli that are not normally painful into painful sensations. This phenomenon involves various ionic currents and thus various channels of the "ligand-gated" type, including the receptor for the capsaicin, VR1, and the ionotropic receptors for ATP (Khakh, 2001). The simultaneous activation of the receptors for VR1 and of those for ATP on spinal nociceptive interneurons generates a considerable accumulation of the excitatory synaptic signals with reinforcement of the transmission of the painful stimulus (Nakatsuka et al., 2002). On this basis, it is therefore clear that the ATP receptors (especially those belonging to the class P2X3) play a fundamental role in the pain paths (Burnstock, 2001). These receptors are present

on the peripheral nerve terminals activated by algogenic stimuli, on the cell bodies of the neurons in the DRGs and on the presynaptic terminals thereof, as well as naturally on postsynaptic terminals in the spinal cord (Khakh, 2001). There is considerable evidence that shows that the system constituted by the nerve growth factor (NGF) and
5 by the high-affinity receptor thereof TrkA (Levi-Montalcini, 1987; Levi-Montalcini et al., 1996; Frade and Barde, 1998; Kaplan, 1998) plays a fundamental role in the molecular processes underlying the main forms of "persistent" pain. This indicates a main therapeutical area (the one of pain, with particular reference to the "tonic" forms), for the antibodies that block the NGF/TrkA system (Levine, 1998). The development of
10 sensitive nociceptive neurons depends greatly on NGF, and the responses of the adult nociceptors are modulated by the same factor (Julius and Basbaum, 2001). In particular, NGF exerts acute sensitisation of the capsaicin algogenic stimulus (Shu and Mendell, 1999). From a functional point of view, the nociceptive neurons, following chronic inflammation, develop alterations in the frequency and duration of their action potential.
15 These phenomena regress by blocking endogenous NGF leading to a significant attenuation of the hyperexcitability that is typical of chronic painful states (Djouhri et al., 2001). NGF action in defining the pain threshold in adult nociceptors is mediated by the TrkA receptor, also through modulation of the response mediated by the VR1 receptor present on the nociceptive terminals. The TrkA-dependent potentiation of the
20 VR1 response is thought to occur through the intracellular transduction pathway of the gamma version of phospholipase C (PLCgamma, Chuang et al., 2001). The levels of peripheral NGF are increased in inflammatory processes, while the administration of exogenous NGF has a hyperalgesic effect on rats and produces muscular pain in humans. Furthermore, NGF produces hypersensitisation to heat stimulation in humans
25 and mammals in general. NGF is released by mastocytes, fibroblasts and other cell types in the peripheral sites where inflammatory processes occur. In particular the mastocytes appear to play a fundamental role (Woolf et al., 1996). As these cells produce NGF and at the same time express functional TrkA receptors on their surface (Nilsson et al., 1997), they are able to respond to NGF itself, in the presence of lysophosphatidylserine
30 (Horigome et al., 1993; Kawamoto et al., 2002). As a result, the system NGF/TrkA appears to mediate mastocyte activation through a positive feedback autocrine mechanism allowing the local amplification of the algogenic inflammatory signal.

High levels of NGF are also found in neurons, where this neurotrophin is apparently responsible for the modifications of nerve fibres, associated with pain (Harpf et al., 2002). In certain forms of cancer, the excess of NGF facilitates the growth and infiltration of nerve fibres with induction of oncological pain (Zhu et al., 1999). Recent
5 experimental studies show that, by blocking NGF, it could be possible to significantly reduce the formation of neuromas, responsible for neuropathic pain, without damaging the cell bodies of the lesioned neurons (Kryger et al., 2001). These results elicited significant interest in therapeutic approaches based on the reduction of the effects of NGF for the treatment of chronic pain (Saragovi and Gehring, 2000). In recent years, the
10 involvement of the NGF/TrkA system in the molecular processes of pain transduction has also been demonstrated on a genetic base. In particular, mutations of the TrkA gene (localised on the chromosome 1q21-q22) are responsible for a hereditary recessive autosomic syndrome known as CIPA ("congenital insensitivity to pain with anhydrosis"), characterised by recurrent episodic fever, anhydrosis, absence of reaction
15 to stimuli that cause pain, mental retardation and a tendency to self-mutilation (Indo et al., 1996; Saragovi and Gehring, 2000; Indo, 2001; Indo et al., 2001). Further confirmation of the involvement of NGF in the nociceptive response was recently obtained by the work on phenotype characterisation of anti-NGF transgenic mice (AD11). In these animals, the ectopic expression of the anti-NGF antibody α D11
20 produces a functional block of NGF in adult. This block translates in a consistent manner into an increase in the latency time of the response to harmful heat stimuli (Capsoni et al., 2000; Ruberti et al., 2000). Antibodies that are able to neutralise the biological activity of the NGF/TrkA system by blocking the ligand or the receptor may represent an important resource in pain therapy, in particular for persistent forms of
25 pain. In this context, a very recent publication demonstrates that treatment with a neutralising anti-NGF antibody produces significant pain reduction in a murine oncological pain model (Sevcik et al., 2005). Nevertheless, in the administration protocol used by Sevcik et al., the maximum time lapse between the last injection of anti-NGF and observation of the behaviour does not exceed 4 days, so it is not a long-
30 term effect.

A long-term effect can be defined as an effect which is still evident for at least 1-2 week(s), after the last administration of the antibody, implying that there is no necessary correlation between the effect and the bloodstream concentration of the antibody itself. A long-term effect may require new gene expression and may represent a permanent or prolonged modification of the original physiopathological state. In many cases, the drug that is capable of producing a long-term effect, may be defined as a "disease-modifying" active principle, i.e. capable of modifying in depth the course of the disease, unlike products displaying a simple pharmacological effect on the symptoms.

The authors of the present invention dispose of a panel of antibodies (directed against the NGF ligand) that are able to block the biological effects of NGF that are mediated by the TrkA ligand. α D11 (anti-NGF) is of particular interest. The comparison between this antibody, directed against the ligand and others directed against the TrkA receptor, is of significant interest as the inhibition of the NGF ligand is not functionally equivalent to the inhibition of the TrkA receptor. Three points must in fact be considered:

- i) stoichiometric reasons, in the context of the same system, the availability of ligand and of receptor may vary greatly and vary in a different fashion, over time;
- ii) the presence of a second receptor for NGF (p75) that is shared by all the neurotrophins and mediates distinct biological functions with respect to TrkA (Hempstead, 2002);
- iii) the presence, in nature, of "immature" forms of NGF (pre-pro-NGF), characterised by distinct properties, in terms of bioactivity, and binding preferably to the p75 receptor (Lee et al., 2001).

α D11 is a rat monoclonal antibody directed against mouse NGF (but also able to recognise rat and human NGF). Its interaction with NGF inhibits the binding thereof with TrkA, blocking the physiological action thereof (Cattaneo et al., 1988). α D11 also inhibits the binding of NGF to the p75 receptor. This anti-NGF antibody is absolutely unique in terms of its specificity of binding to its antigen (as compared to all the other neurotrophins), through the affinity of binding with the antigen (picomolar) and through neutralising features, showed both *in vitro* and *in vivo* (Cattaneo et al., 1988; Berardi et al., 1994; Molnar et al., 1997; Mohiar et al., 1998). The α D11 epitope is located at

the level of NGF loop I and/or NGF loop II that are exposed to the outer part of the molecule and spatially very close to each other. Moreover, the conserved reactivity of alphaD11 in different species is consistent with the epitope assignment, since amino acid residues of these two loops are highly conserved. The potent neutralising activity of alphaD11 shows that the recognised epitope is very close to the NGF receptor binding site. Moreover, the lack of cross reactivity of alphaD11 with other members of the neurotrophin family suggests that: i) the epitope is located in NGF regions that are not shared with other neurotrophins, ii) the epitope itself may be involved in the "specificity path" mediating NGF-TrkA recognition. The epitope recognized by the alphaD11 antibody on the NGF molecule was identified by testing the binding activity of the antibody towards an extensive panel of NGF mutants. On the basis of this systematic screening, a region (aa. 41-49, loop I) of the NGF molecule was identified that is highly expressed on top of NGF molecule and that is responsible (though not exclusively) for the binding of the antibody to its antigen (Gonfloni, 1995). As a matter of fact also the NGF aa. region 23-35 (loop IT) may contribute to the binding.

On the basis of such in-depth knowledge around structure, by means of an innovative method, a humanised version of α D11 was generated (Hu- α D11), displaying the same antigen binding features of the parental version (patent application WO 05/061540).

The therapies that are currently available for treating pain of neuropathic origin (caused by a primary lesion or by a dysfunction of the nervous system, for example the pain associated by a lesion of the spinal cord), for treating oncological pain, and for numerous other forms of persistent pain (also of inflammatory nature) have been found to be of limited effectiveness. There is therefore an obvious need to identify and develop new molecules that have an analgesic activity and which work through a different action mechanism as compared to currently used analgesic drugs, in order to solve side effect related problems. The international patent application WO 02/20479 discloses small synthesis molecules that inhibit the TrkA receptor, having a potential analgesic activity. Nevertheless, the effect of these molecules on certain pain models has not been demonstrated. Furthermore, as compared to antibodies, the small molecules have the drawback of being more likely to penetrate the haematoencephalic barrier, with the possibility of serious side effects. In fact, the cholinergic neurons of the basal forebrain, a

neuronal population that is affected by various forms of progressive neurodegeneration, including Alzheimer's disease (Saper et al., 1985), express the TrkA receptor and depend on NGF for correct functioning (Holtzman et al., 1992). The international patent application WO 01/78698 proposes the use of an NGF antagonist for preventing or
5 treating chronic visceral pain, but not for neuropathic or oncological pain. Even if the application states that the antagonist can bind both NGF and the TrkA receptor, it is not demonstrated that, upon binding of the antagonist to the TrkA receptor, the receptor is functionally blocked. On the basis of the capacity of the antibody α D11 to block the biological activity of NGF/TrkA, the antibody α D11 and its humanised version were
10 tested in various (rodent) animal models of persistent pain, in particular in the model CCI ("Chronic Constriction Injury", chronic constriction injury of the sciatic nerve), one of the models available for assessment of chronic pain of a neuropathic nature (Bennett and Xie, 1988).

15 SUMMARY OF THE INVENTION

The present invention relates to the use of an anti-NGF molecule that is able to inhibit the binding between NGF and TrkA, for the preparation of a medicament for the treatment of chronic pain.

20 An anti-NGF molecule that blocks the biological activity of TrkA is defined as a molecule that acts as an antagonist in terms of the NGF binding with the TrkA receptor and comprises: synthetic molecule or monoclonal antibody or a biological/synthetic derivative thereof that:

- i) binds to TrkA;
- 25 ii) inhibits the binding of NGF to the "native" TrkA receptor expressed on the surface of living cells ("native" meaning "in the natural *in vivo* conformation"); and
- iii) blocks the biological activity that derives from the NGF binding with the same TrkA receptor.

30 The term "blocking the biological activity" does not simply mean blocking activation of the receptor, defined as blocking the conversion process of the receptor itself into an "active" state but also functional neutralisation of the biological consequences that are downstream of this activation process: second messengers, new gene expression,

phenotypical and functional modifications. The molecule is not only able to block TrkA in a classic *in vitro* test (test of neuritic growth in PC12 cells), but also *in vivo* (functional block of the cholinergic neurons of the basal forebrain and block of the nociception in a classic "hot plate" test).

5

Accordingly, in one aspect the present invention provides use of an anti-NGF antibody capable of inhibiting the binding between NGF and TrkA for the preparation of a medicament for effecting a permanent or prolonged modification of the pathophysiological state underlying chronic pain in the treatment and/or prevention of chronic pain.

10

In another aspect, the present invention provides a method for treating and/or preventing chronic pain in a subject in need of such treatment, and which said method comprises administration to said subject of an effective amount of an anti-NGF antibody capable of inhibiting the binding between NGF and TrkA so as to effect a permanent or prolonged modification of the pathophysiological state underlying chronic pain.

15

Preferably the antibody is able to recognise and bind to a NGF molecule domain containing the aa. 41-49 region of human or rat NGF: EVNINNSVF (SEQ ID No. 9), more preferably the domain contains also the aa 23-35 region: GDKTTATDIKGKE (SEQ ID No. 10). More preferably the antibody is capable of blocking the biological activity of TrkA.

20

In a preferred aspect the variable region of the antibody light chain comprises at least one, more preferably two, most preferably three of the complementarity determining

25

regions (CDRs) having the sequence selected from aa. 24 to aa. 34 of SEQ ID No.1; from aa. 50 to aa. 56 of SEQ ID No. 1; from aa. 89 to aa. 97 of SEQ ID No. 1.

In a further preferred aspect the variable region of the antibody light chain comprises essentially the sequence of SEQ ID No. 1.

5

(VL, SEQ ID No. 1):

L CDR1 L CDR2

DIQMTQSPASLSASLGGETVTIECRASEDIYNALAWYQQKPGKSPQLLIYNTDTLHTGVP

L CDR3

10 *SRFSGSGSGTQYSLKINSLSQSEDVASYFCQHYFHYPRTFGGGTKLELK*

In a preferred aspect the variable region of the antibody heavy chain comprises at least one, more preferably two, most preferably three of the complementarity determining regions (CDRs) having the sequence selected from aa. 26 to aa. 35 of SEQ ID No. 2; from aa. 50 to aa. 65 of SEQ ID No. 2; from aa. 98 to aa. 111 of SEQ ID No. 2.

15

In a further preferred aspect the variable region of the antibody heavy chain comprises essentially the sequence of SEQ ID No. 2.

(VH, SEQ ID NO 2):

20 H CDR1 H CDR2

QVQLKESGPGGLVQPSQTLSTCTVSGFSLTNNNVNWRQATGRGLEWMGGVWAGGATDY

H CDR3

NSALKSRLTITTRDTSKSQVFLKMHSLSQSEDATYYCARDGGYSSSTLYAMDAWGQGTTV

25 *TVSA*

The antibody may be in single chain form and comprises a light chain variable region and a heavy chain variable region joined by a linker.

Alternatively the antibody may comprise two light chains and two heavy chains.

30

In a preferred aspect of the invention the anti-NGF antibody is a human or humanised antibody. The skilled in the art shall select the proper humanisation method to design the antibody, a preferred method is the method as disclosed in WO 2005/061540.

Briefly, a "humanized" variant of the antibody variable region was obtained by grafting the Complementarity Determining Regions (CDRs) of the rat antibody on to a human immunoglobulin framework. The complete structural information obtained from X-ray diffraction studies concerning the Fab fragment of the α D11 antibody was exploited to select an acceptor framework of human origin. Two different criteria were adopted to minimize structural differences between the rat α D11 and the acceptor human antibody: i) level of primary structure homology, ii) level of tridimensional structure similarity. After choosing the framework, the replacement of human residues by rat counterparts was minimized to reduce the potential immunogenicity of the resulting humanized antibody.

Exemplary humanised antibodies comprise a light chain variable region which is a humanised derivative of SEQ ID No 1 (a rat origin sequence). Exemplary humanised antibodies comprise a heavy chain variable region which is a humanised derivative of SEQ ID No 2 (a rat origin sequence). In a preferred aspect of the invention the variable region of the humanised antibody light chain comprises essentially the sequence of SEQ ID No. 3.

SEQ ID NO 3 (VL, variable region of the light chain of Hu- α D11):

L CDR1 L CDR2

DIQMTQSPSSLSASVGDRVTITCRASEDIYNALAWYQOKPGKAPKLLIYNTDTLHTGVP

L CDR3

SRFSGSGSGTDYTLTISSLQPEDFATYFCQHYFHYPRTEFGQGTKVEIK

In a preferred aspect of the invention the variable region of the humanised antibody heavy chain comprises essentially the sequence of SEQ ID No. 4.

SEQ ID No. 4 (VH, variable region of the heavy chain of Hu- α D11):

H CDR1 H CDR2

EVQLVESGGGLVQPGGSLRLSCAASGFSLTNNNVNWRQAPGKGLEWVGGVWAGGATDY

H CDR3

NSALKSRFTISRDN SKNTAYLQMNSLRAEDTAVYYCARDGGYSSSTLYAMDAGWGQGTLV

TVSS

The above described humanized variable regions were cloned into appropriate expression vectors into a human IgG1 or IgG4 isotype format and transfected into mammalian cell lines to allow for expression, purification and pharmacological characterization.

Different variants of Hu- α D11 (complete IgG: heavy chain + light chain) were finally produced (differing because of different constant parts).

In a preferred aspect of the invention the humanised antibody light chain has essentially the sequence of SEQ ID No. 8.

SEQ ID NO 8, Hu- α D11 Vk human Ck

DIQMTQSPSSLSASVGVDRVITTCRASEDIYNALAWYQCKPGKAPKLLIYNTDTLHTGVP
SRFSGSGSGTDYTLTISSLPEDFATYFCQHYFHYPRTEFGQCTKVEIKRTVAAPSVEIF
 PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSS
 TLTLISKADYEKKHKVYACEVTHQGLSSPVTKSFNRGEC

(Italics= variable regions; Bold=mutations in the rat sequence in the humanization process; Underlined=CDRs)

In a preferred embodiment, the humanised anti-NGF heavy chain has essentially one of the following 3 sequences:

SEQ ID NO 5, Hu-antiNGF (VH) human IgG1

EVQLVESGGGLVQPGGSLRLSCAASGFSLTNNNVNWRQAPGKGLEWVGGVWAGGATDY
NSALKSRFTISRDN SKNTAYLQMNSLRAEDTAVYYCARDGGYSSSTLYAMDANGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
 VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHTCPPCPA
 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
 PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
 TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
 KLTVDKSRWQQGNV FSCSVMEALHNHYTQKSLSLSPGK

SEQ ID NO 6, Hu- α D11 (VH) human IgG1* (IgG1 with N297A mutation, as described by Bolt et al., 1993)

EVQLVESGGGLVQPGGSLRLSCAASG*FSLTNNNVNWRQ****APGKGLEWVGGVWAGGATDY***
NSALKSRFTISRDN*SKNTAYLQMNSLRAEDTAVYYCARDGGYSSSTLYAMDAWGQ****GT****LV***
 5 ***TVSSAST**KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
 VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
 PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
 TLPSSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
 10 KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK*

SEQ ID NO 7, Hu- α D11 (VH) human IgG4

EVQLVESGGGLVQPGGSLRLSCAASG*FSLTNNNVNWRQ****APGKGLEWVGGVWAGGATDY***
NSALKSRFTISRDN*SKNTAYLQMNSLRAEDTAVYYCARDGGYSSSTLYAMDAWGQ****GT****LV***
 15 ***TVSSAST**KGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
 VLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPPEF
 LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPRE
 EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLF
 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLT
 20 VDKSRWQEGNVFCFSVMHEALHNHYTQKSLSLGLGK*

(Italics= variable regions; Bold=mutations in the rat sequence in the humanization process; Underlined=CDRs; N297A mutation to abolish glycosilation site).

In a preferred aspect the molecules of the invention are used for the preparation of a
 25 medicament for the pain of the chronic inflammatory type, preferably caused by
 pancreatitis, kidney stones, headaches, dysmenorrhoea, musculoskeletal pain, sprains,
 visceral pain, ovarian cysts, prostatitis, cystitis, interstitial cystitis, post-operative pain,
 migraine, trigeminal neuralgia, pain from burns and/or wounds, pain associated with
 trauma, neuropathic pain, pain associated with musculoskeletal diseases, rheumatoid
 30 arthritis, osteoarthritis, ankylosing spondylitis, periarticular pathologies, oncological
 pain, pain from bone metastases, pain from HIV.

Alternatively the pain is a neuropathic pain or an oncological pain.

According to International Association for the Study of Pain (IASP, www.iasp-pain.org <<http://www.iasp-pain.org>>), pain is generally defined as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage or both". The essential element in all forms of pain is the activation of specialized high-threshold receptors and nerve fibers to warn the organism of potential tissue damage. The involvement of inflammatory cells and processes is a common element in many pain states. The term "acute pain" means immediate, generally high threshold, pain brought about by injury such as a cut, crush, burn, or by chemical stimulation. The term "chronic pain," as used herein, means pain other than acute pain. It is understood that chronic pain often is of relatively long duration, for example, months or years and can be continuous or intermittent.

The anti-NGF antibody is suitably administered systemically. Systemic administration of the anti-NGF antibody can be performed by injection, e.g. continuous intravenous infusion, bolus intravenous infusion, subcutaneous or intramuscular injection. Alternatively other forms of administration (e.g. oral, mucosal, via inhalation, sublingually, etc.) may also be used. Local delivery of the antibody can be performed by local administration eg intra-articular injection or subcutaneous, intramuscular injection in the vicinity of affected tissues.

The anti-NGF antibody will suitably be formulated in a pharmaceutical composition appropriate for the intended route of administration. Solutions for injection will suitably contain the antibody dissolved or dispersed in an aqueous medium (eg water for injection) containing appropriate buffers and molarity modifiers eg phosphate, salt and/or dextrose.

Treatment regimen i.e. dose, timing and repetition, can be represented by single or repeated administrations (eg injections) of the product by the chosen administration route. The interval of dose administration can be subject to modifications depending on the extent and duration of the clinical response, as well as the particular individual and the individual clinical history. Suitably the anti-NGF antibody has a long duration of action. In particular the clinical effect of the antibody extends following administration as long as 21 days as determined from animal studies. Furthermore, preliminary data imply that anti-NGF antibodies may manifest clinical benefit for a longer period than

that in which its presence can be detected in a relevant biological matrix such as serum or plasma following its administration.

In light of the intended long duration of action (i.e. an effect suitably lasting at least one week, or preferably at least two weeks e.g. at least three weeks or at least four weeks), suitably the antibody may be administered to subjects at a frequency of not more than once per week e.g. not more than once per two weeks or once per three weeks or once per four weeks.

A suitable dose of the anti-NGF antibody will typically range from 0.1mg/kg to 10mg/kg body weight.

Non-limitative embodiments of the present invention will now be disclosed, with particular reference to the following figures:

FIGURE 1: BIAcore analysis of the binding of the α D11 anti-NGF antibody to mouse NGF (m-NGF) and recombinant mouse proNGF (rm-proNGF). The α D11 anti-NGF antibody was immobilized on flow cell 2, while flow cell 1 was left as a blank. Each curve is obtained subtracting the background signal (measured in cell 1) to the signal measured in cell 2. The Surface Plasmon Resonance signal gives the amount of surface-bound component at each stage and is expressed in resonance units (RU).

For m-NGF binding, the immobilization of the antibody was of 3000 resonance units (RU) in the experiment of panel A and of 6000 RU in the experiment of panel B. The injected concentrations of the m-NGF are indicated on top of each curve. From a complete analysis of the data, the affinity parameters were evaluated, and resulted to be the following: $K_A = 3,55 \cdot 10^{11}$ l/M; $K_D = 2,81 \cdot 10^{-12}$ M (χ^2 value of 0.123).

For rm-proNGF binding (panel C), the immobilization of the antibody was of 3000 RU. The injected concentrations of the rm-proNGF are indicated on top of each curve. A kinetic analysis of the data allowed to evaluate the following parameters: $K_A = 1,2 \cdot 10^9$ l/M; $K_D = 1,9 \cdot 10^{-9}$ M (χ^2 value of 0.09).

FIGURE 2: Effect of Fab α D11 (alphaD11) and Fab Hu- α D11 (Hu-alphaD11) anti-NGF antibodies on the formalin-evoked pain (phase 2 of the formalin test: 15-40 min. Phase 2 corresponds to inflammation-related pain). Mice were subcutaneously injected with 5% formalin in the dorsal portion of the right hindpaw.

5

Treatment consisted in antibody injection (Fab alphaD11 or Fab Hu-alphaD11 vs either mock Fab or saline) performed (in the same paw as for formalin) 45 min, before formalin injection and testing (single dose of each antibody: 12.5 μ g). Each experimental group included at least 8 animals. Statistical analysis of data showed a significant analgesic effect of anti-NGF treatment (both for the parental and the humanized version of the antibody) that was clearly specific for the second phase (inflammatory) of pain response (time spent in licking). The effect of anti-NGF antibodies (both parental and humanized versions) is statistically different (ANOVA) with respect to either saline (** $p < 0.01$) or mock Fab treatment (# $p < 0.05$).

15

FIGURE 3: Effect of the anti-NGF monoclonal antibody α D11 (1.4 mg/kg) on neuropathic pain: mechanical allodynia measured by means of a plantar dynamic aesthesiometer; CD1 mice subjected to chronic constriction of the sciatic nerve; the antibodies are injected I.P. at days 3, 4, 5, 6 after lesion of the sciatic nerve. Observation period: from day 3 to day 14. As a negative control, both saline (sal) and mouse immunoglobulins (IgG, 1.4 mg/kg) were used. Results were expressed in terms of absolute value (grams) of the threshold force for the hindpaw ipsilateral to lesion. The values were subjected to statistical analysis by means of an analysis of the variance (ANOVA) for repeated measurements, in which both the "treatment" factor and the repeated measurement (days) were significant with $p < 0.01$. The animals treated with anti-TrkA or anti-NGF are significantly different from the controls from day 4 to day 14.

FIGURE 4: Effect of the anti-NGF α D11 antibody (1.4 mg/kg) on neuropathic pain: mechanical allodynia measured by means of a plantar dynamic aesthesiometer; CD1 mice subjected to chronic constriction of the sciatic nerve; the antibodies were injected I.P. at days 3, 4, 5, 6 after lesion of the sciatic nerve. Observation period: from day 3 to day 14. As a control, both saline (sal) and mouse immunoglobulins (IgG, 1.4 mg/kg) were used. Results were expressed as a percentage, % (ratio between the threshold force of the

hindpaw ipsilateral to lesion and that corresponding to the contralateral hindpaw). The corresponding absolute values were subjected to statistical analysis by means of an analysis of the variance (ANOVA) for repeated measurements, in which both the "treatment" factor and the repeated measurement (days) were significant with $p < 0.01$ (at least). The animals treated with anti-NGF were significantly different from the controls from day 4 to day 14.

FIGURE 5: Effects of the anti-NGF monoclonal antibody α D11 (2 mg/kg dose), on neuropathic pain: mechanical allodynia measured by means of a plantar dynamic aesthesiometer; CD1 mice subjected to chronic constriction of the sciatic nerve; the antibodies were injected I.P. at days 3, 4, 5, 6, 7, 8, 9, 10 after lesion of the sciatic nerve. Observation period: from day 3 to day 31. As a negative control, mouse immunoglobulins were used (IgG, 2 mg/kg). Results were expressed as a percentage % (ratio between the threshold force of the hindpaw ipsilateral to lesion and that corresponding to the contralateral hindpaw). The corresponding absolute values were subjected to statistical analysis by means of an analysis of the variance (ANOVA) for repeated measurements, in which both the "treatment" factor and the repeated measurement (days) were significant with $p < 0.01$ (at least). The animals treated with α D11 were significantly different from the controls up to the last day of observation (31), from day 4 to day 14, and from day 21 to day 31.

FIGURE 6: Comparison between the effects of the parental (α D11) and humanized (Hu- α D11, human IgG4 format) version of the anti-NGF neutralizing antibody (1 dose tested: 2 mg/Kg) on neuropathic pain: mechano-allodynia measured by means of dynamic plantar aesthesiometer; CD1 mice subject to CCI (chronic constriction injury) of the sciatic nerve; antibodies I.P. injected at days 3, 4, 5, 6, 7, 8, 9, 10, following lesion of the sciatic nerve. Observation period: from day 3 to day 31. Rat Immunoglobulins were employed for negative control (IgG, 2 mg/kg). Results expressed as % (ratio between the threshold force for the hindpaw ipsilateral to lesion and the contralateral hindpaw). Analysis of variance (ANOVA) for repeated measures on corresponding absolute values in which both the factor "treatment" and the repeated measure (days) were significant with (at least) $p < 0.01$. Animals treated with either α D11 or Hu- α D11 were significantly different

from controls up to the last observation day (31), from day 4 to day 14, as well as from day 21 up to day 31.

METHODS

5 **Production of monoclonal antibodies**

The monoclonal antibody α D11 is produced from a hybridoma supernatant, according to standard methods, disclosed above (Galfre and Milstein, 1981; Cattaneo et al., 1988; Cattaneo et al., 1999). The supernatant containing the antibody was subjected to precipitation (29% ammonium sulphate), followed by dialysis against PBS 1X (Spectra-
10 Por 12/14K membrane, Spectrum) and affinity chromatography on sepharose protein G column (4-Fast Flow, Amersham Biosciences). Elution occurred by means of a low pH (HCl 5 mM) solution that was neutralised upon collection. The final eluate was concentrated (Amicon Ultra-15, 50K, Millipore) to obtain preparations of purified antibody in concentrations between 1 and 5mg/ml.

15

The Fab (Fragments Antigen binding) version of the α D11 antibody was produced as previously described (patent application WO 05/061540, Covaceuszach et al., 2004). Briefly, Fab fragments were obtained from the correspondent whole monoclonal antibodies (IgG format) by papain proteolysis, followed by an ion exchange
20 chromatography purification step and concentration of the Fab fragments collected in the flow-through. In order to separate the Fab fragments from the quite low amount of uncleaved IgG that was still present, size exclusion chromatography on a Superdex G75 column (Pharmacia) was performed using an FPLC system (Pharmacia), followed by a final concentration step.

25

As far as the humanised versions (IgG1/IgG1*/IgG4) of the antibody (Hu- α D11) are concerned, they were also purified as disclosed above, starting from the supernatants of stably transfected cell lines, which were stable cotransfectants for the heavy chain (pVH/CMVexpress) and the light chain (pVL/CMVexpress) of each antibody. The
30 vectors used have been disclosed previously (patent application WO 05/061540). The stable co-transfected clones were obtained through double selection with G418 and mycophenolic acid. In order to produce the IgG4 variant of Hu- α D11,

since the pVH/CMVexpress vector comprises the constant part of human IgG1, this was replaced by the corresponding Fc region of IgG4 (cloned by RT-PCR from human lymphocyte RNA). The IgG1* variant (=IgG1 with the N297A mutation described by Bolt et al., 1993) was generated by site directed mutagenesis.

5 **Surface plasmon resonance studies**

Experiments were performed on CM5 chips with amine coupling, using a BIAcore 2000 machine. Coupling was performed with a specific kit purchased at BIAcore and the coupling reaction was carried out according to manufacturer's instructions.

10 Anti-NGF antibodies were immobilized on chip, while mouse NGF (m-NGF, Alomone) or recombinant mouse proNGF (rm-proNGF) were injected at decreasing concentrations to obtain binding curves.

The flow used in the experiments was of 30 μ l/min, unless otherwise indicated. The regeneration of the chip was carried out in all cases with a pulse (10 μ L) of 10 mM Glycine pH 1.5. Data collected were analyzed using the Package BIAevaluation 3.0.

15 The apparent equilibrium constant K_D is defined as the k_a/k_d ratio.

Experiments in murine pain models

The animals were treated and handled in accordance with the guidelines of the IASP Ethical Committee and the Italian national law (DL116/92, application of European Direction 86/609/EEC) on the use of animals in research. Every necessary effort was
20 made to minimise the suffering of the animals and to use the minimum amount of animals required to produce reliable scientific data.

Formalin test

For the preliminary formalin tests (Porro and Cavazzuti, 1993), CD1 male mice (Charles River Labs, Como, Italy) were used, weighing 35-40g at the beginning of the
25 experiments. Upon their arrival in the laboratory (at least 2 weeks before the experiments), mice were housed in standard transparent plastic cage (4 for cage) at constant temperature (22 \pm 1°C) and relative humidity (60%), under a regular light/dark schedule (light 7.00-19.00). Food and water were unlimited. The experiments were carried out between 09.00 and 14.00 hours. For the formalin test, one animal at a time
30 was placed in a transparent plexiglass cage (30 x 12 x 13 cm), and allowed to move freely for 30 min before the beginning of the test. After this adaptation period, 20 μ l of

formalin solution (5% in saline) were subcutaneously (sc) injected into the dorsal surface of the right hind paw of mice using a microsyringe equipped with a 26-gauge needle and the observation period started. A mirror was placed behind the cage and a videocamera in front of the cage to allow an unimpeded view of the animal's hind-paws. The licking activity, i.e. the total amount of time the animal spent licking and/or biting the injected paw, was taken as index of pain. The licking activity was recorded continuously for 40 min and calculated in blocks of consecutive 5-min periods (phase 2 corresponds to the block 15-40 min and can be identified with inflammation-related behaviours, general activity (time spent exploring the environment during walking, rearing and leaning), and self-grooming (time spent for face and body cleaning) during the formalin test were also continuously recorded for 40 min. No significant differences following treatment with anti-NGF antibodies were observed for these parameters. In this set of experiments, antibodies were administered as Fabs (fragments antigen binding; single dose of each antibody: 12.5 µg per animal). Each mouse was subcutaneously (sc) injected 45 min before the test with anti-NGF antibody (either parental or humanized) or irrelevant Fab into the dorsal surface of the right hind paw using a Hamilton micro-syringe with a 26-gauge needle (volume injected = 20µl). Each animal underwent only one treatment. Testing was performed blind as for treatment group to which each subject belonged. The two phases characterizing the formalin test were separately analysed by one-way ANOVAs.

Sciatic nerve surgery

Male CD1 mice, weighing approximately 35 g, were anaesthetised (intraperitoneal injection with 500 mg/kg chloral hydrate), the sciatic nerve of the right hind leg was exposed to undergo loose ligature by means of stitching thread according to the chronic constriction lesion model (CCI) of the sciatic nerve, disclosed by Bennett and Xie (1988). The loose ligature of the sciatic nerve, at level of the upper thigh, induced peripheral mononeuropathy characterised by thermal/mechanical allodynia and hyperalgesia. By ligation of the nerve at 3 different but near points, the neuropathy was fully developed 3 days following the lesion and lasted for 2-3 months.

Pharmacological treatment

Starting from the third day following the lesion, anti-NGF (α D11) blocking antibodies were administered in an entire form (Mab) that were diluted in saline solution (vehicle), as indicated in Table I. As controls, mouse or rat irrelevant immunoglobulins used (IgG), in the same dose as the blocking antibodies (at the greater dose if 2 doses were used), or saline solution. Each experimental group included N=10 animals (unless explicitly stated otherwise).

Table I: Administration protocols and measurement of mechanical allodynia.

Antibody	Dose	Administration i.p.	Allodynia measurement
α D11	50 μ g/mouse = 1.4 mg/kg	4, at days 3, 4, 5, 6 after lesion	Days 3 to 14
α D11	70 μ g/mouse = 2 mg/kg	8, at days 3, 4, 5, 6, 7, 8, 9, 10 after lesion	Days 3 to 31

Mechanical allodynia was measured by means of a plantar dynamic aesthesiometer (Ugo Basile), as indicated in Table I. Day 3 was considered the baseline.

The same protocols were used to assess the analgesic action of the humanised versions of α D11.

Statistical analysis of results (CCI experiments)

The results were expressed in 2 different ways, both as an absolute value of the threshold force value (in grams) that was sufficient for the animal to retract the hind leg that is ipsilateral to the lesion, or in percentage value, as the ratio between the absolute values of the hind legs (ipsilateral/contralateral). The values were subjected to statistical analysis by means of an analysis of the variance (ANOVA) for repeated measurements, in which both the "treatment" factor and the repeated measurement (days) were significant with $p < 0.01$.

RESULTS

Binding

A BIACORE study was performed, aimed at further characterising the binding properties of the α D11 anti-NGF antibody (and its humanized variant) by evaluating the binding

affinity of this antibody for mouse NGF and recombinant mouse pro-NGF. Fig. 1 shows the results of these experiments: the α D11 antibody binds with different kinetics on NGF and proNGF. Similar results were also obtained with Hu- α D11.

- 5 The very small dissociation constant from NGF is representative of a very tight binding of the antibody to its antigen, and is quite a unique example among antibodies binding kinetics. By comparing the anti-NGF antibody binding to NGF and to proNGF, it is possible to assess that, in the latter case, the affinity is almost three orders of magnitude lower (nanomolar instead of picomolar). Considering that proNGF differs from NGF
10 only by a short stretch of additional amino acids, this difference in binding affinities is absolutely unexpected and surprising.

As proNGF preferably binds to p75 (Lee, 2001), whereas mature NGF has a higher affinity for the TrkA receptor, α D11 and Hu- α D11 can be considered as novel selective
15 inhibitors of the TrkA-mediated pathway, a remarkable property that has a particular relevance in view of the clinical use of anti-NGF neutralizing antibodies.

Inflammatory pain

A first set of *in vivo* experiments, performed in mice and concerning formalin-evoked
20 pain (inflammatory pain), demonstrated that:

- (i) the α D11 anti-NGF antibody (in Fab format) was able to significantly reduce the pain response (formalin test: phase 2), as compared to an irrelevant Fab;
- (ii) the same result could be obtained by replacing α D11 with its humanized variant (Hu- α D11, Fig.2).

25 This means that Hu- α D11 displays as powerful analgesic properties as α D11 in a relevant model of inflammatory pain.

Neuropathic pain

The results on the CCI model showed that the blocking antibody α D11 (Fig. 3 and Fig. 4)
30 had a significant analgesic effect. As shown in Fig. 3 and Fig. 4, the antibody started to have an analgesic effect from the second day of administration (day 4), reaching the maximum effect around day 6, keeping substantially the same analgesic efficacy for the entire duration of the observation until day 14. Expressing the result in percentage terms

(ratio between the threshold force of the hindpaw ipsilateral to lesion and that corresponding to the contralateral hindpaw), as in Fig. 4, it can be stated that for the blocking antibody, the maximum percentage value was around 60%, being around 40% for the control groups (IgG and saline).

5

When the animals were observed for 4 weeks, up to day 31, administration of the antibodies blocking the NGF-TrkA system (Fig. 5 and Fig. 6) revealed a two-phase effect. The first phase of analgesic efficacy (from day 3 to day 17, i.e. until a week after the last injection) was characterised by a maximum effect around days 11-12. After a reduction
10 of the effect (up to day 17), a second analgesic phase was observed with an increase in the effect up to day 31. Two phases in the analgesic action of NGF/TrkA blocking antibodies can therefore be distinguished: the first ("pharmacological" effect), that comprises the treatment period and the first week after the last injection of antibody (the week during which the effect diminishes, parallel to the haematic concentration of the antibody); the
15 second, which identifies a long-term effect, probably requiring new gene expression, which is an effect that gives these antibodies the unique feature (in the field of neuropathic pain) of being a "disease-modifying" active principle, i.e. capable of modifying in depth the course of the disease, unlike the products currently used in this therapeutic context, which demonstrate a simple pharmacological effect on the
20 symptoms. Fig.5 shows the analgesic effect of α D11 (2 mg/kg). The results are expressed in percentage terms. At day 17, the animals treated with α D11 were indistinguishable from the controls (IgG). From day 21 α D11 recovered analgesic effect, which reached a final level (day 31) (greater than 60%, as compared to 40% of controls).

25 Substantially identical results to those illustrated above were obtained when instead of the α D11 antibody, the variants of its humanised version (Hu- α D11) were employed (dose used: 2 mg/kg for each antibody), confirming that the latter have the same analgesic properties as the parental version. The antibody was humanised with the

method of WO2005/061540, both at the light (SEQ ID No. 3) and the heavy chain (SEQ ID No. 4) variable regions. To construct whole humanised antibodies, different constant regions were utilised, as above described (SEQ ID No. 5-8).

As typical example of the equivalence in terms of analgesic activity (CCI) of parental and humanized antibodies, Fig. 6 shows the comparison between α D11 and Hu- α D11 (IgG4 format).

On this basis, it is possible to state that Hu- α D11 has the same long-term effect as its parental version.

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The claims defining the invention are as follows:

1. Use of an anti-NGF antibody capable of inhibiting the binding between NGF and TrkA for the preparation of a medicament for effecting a permanent or prolonged modification of the pathophysiological state underlying chronic pain in the treatment
5 and/or prevention of chronic pain.
2. A method for treating and/or preventing chronic pain in a subject in need of such treatment, which said method comprises administration to said subject of an effective amount of an anti-NGF antibody capable of inhibiting the binding between NGF and TrkA so as to effect a permanent or prolonged modification of the pathophysiological
10 state underlying chronic pain.
3. Use according to claim 1 or the method according to claim 2 wherein the pain is of the chronic inflammatory type.
4. Use or method according to claim 3 wherein the chronic pain is caused by pancreatitis, kidney stones, headaches, dysmenorrhea, musculoskeletal pain, sprains,
15 visceral pain, ovarian cysts, prostatitis, cystitis, interstitial cystitis, post-operative pain, migraine, trigeminal neuralgia, pain from burns and/or wounds, pain associated with trauma, neuropathic pain, pain associated with musculoskeletal diseases, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, periarticular pathologies, oncological pain, pain from bone metastases, pain from HIV.
- 20 5. Use or method according to any one of claims 1 to 4 wherein the pain is neuropathic pain.
6. Use or method according to any one of claims 1 to 4 wherein the pain is oncological pain.
7. Use or method according to any one of the preceding claims, wherein the antibody
25 is able to recognise and bind to a NGF molecule domain containing the amino acid 41-49 region of human or rat NGF: EVNINNSVF (SEQ ID No. 9).
8. Use or method according to any one of the preceding claims, wherein the antibody is able to recognise and bind to a NGF molecule domain containing the amino acid 23-35 region: GDKTTATDIKGKE (SEQ ID No. 10).

9. Use or method according to any one of the preceding claims, wherein the antibody is capable of blocking the biological activity of TrkA.

10. Use or method according to any one of the preceding claims, wherein the variable region of the antibody light chain comprises at least one of the complementarity
5 determining regions (CDRs) having the following sequences: from amino acid 24 to amino acid 34 of SEQ ID No. 1; from amino acid 50 to amino acid 56 of SEQ ID No. 1; and from amino acid 89 to amino acid 97 of SEQ ID No. 1.

11. Use or method according to any one of the preceding claims, wherein the variable
10 region of the antibody light chain comprises at least two of the complementarity determining regions (CDRs) having the following sequences: from amino acid 24 to amino acid 34 of SEQ ID No. 1; from amino acid 50 to amino acid 56 of SEQ ID No. 1; and from amino acid 89 to amino acid 97 of SEQ ID No. 1.

12. Use or method according to any one of the preceding claims, wherein the variable
15 region of the antibody light chain comprises all of three complementarity determining regions (CDRs) having the following sequences: from amino acid 24 to amino acid 34 of SEQ ID No. 1; from amino acid 50 to amino acid 56 of SEQ ID No. 1; and from amino acid 89 to amino acid 97 of SEQ ID No. 1.

13. Use or method according to any one of the preceding claims, wherein the variable region of the antibody light chain comprises the sequence of SEQ ID No. 1.

20 14. Use or method according to any one of the preceding claims, wherein the variable region of the antibody heavy chain comprises at least one of the complementarity determining regions (CDRs) having the following sequences: from amino acid 26 to amino acid 35 of SEQ ID No. 2; from amino acid 50 to amino acid 65 of SEQ ID No. 2; and from amino acid 98 to amino acid 111 of SEQ ID No. 2.

25 15. Use or method according to any one of the preceding claims, wherein the variable region of the antibody heavy chain comprises at least two of the complementarity determining regions (CDRs) having the following sequences: from amino acid 26 to amino acid 35 of SEQ ID No. 2; from amino acid 50 to amino acid 65 of SEQ ID No. 2; and from amino acid 98 to amino acid 111 of SEQ ID No. 2.

16. Use or method according to any one of the preceding claims, wherein the variable region of the antibody heavy chain comprises all of three of the complementarity determining regions (CDRs) having the following sequences: from amino acid 26 to amino acid 35 of SEQ ID No. 2; from amino acid 50 to amino acid 65 of SEQ ID No. 2;
5 and from amino acid 98 to amino acid 111 of SEQ ID No. 2.

17. Use or method according to any one of the preceding claims, wherein the variable region of the antibody heavy chain comprises the sequence of SEQ ID No. 2.

18. Use or method according to any one of the preceding claims, wherein the antibody is in single chain form and comprises a light chain variable region and a heavy chain
10 variable region joined by a linker.

19. Use or method according to any one of claims 1 to 17 wherein the antibody comprises two light chains and two heavy chains.

20. Use or method according to any one of the preceding claims, wherein the anti-NGF antibody is a human or humanised antibody.

15 21. Use or method according to claim 20 wherein the variable region of the humanised antibody light chain comprises the sequence of SEQ ID No. 3.

22. Use or method according to claim 20 or claim 21 wherein the variable region of the humanised antibody heavy chain comprises the sequence of SEQ ID No. 4.

23. Use or method according to any one of claims 20 to 22 wherein the humanised
20 antibody light chain has the sequence of SEQ ID No. 8.

24. Use or method according to any one of claims 20 to 23 wherein the humanised antibody heavy chain has a sequence selected from SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7.

25. Use or method according to any one of claims 21 to 24 which is an IgG1 isotype
25 antibody.

26. Use or method according to any one of claims 21 to 24 which is an IgG4 isotype antibody.

27. Use or method according to any one of the preceding claims wherein said antibody has a duration of action which is evident for at least one week after the last administration of the antibody.

28. Use or method according to any one of the preceding claims wherein said antibody
5 is administered at a frequency of not more than once per week.

29. Use according to claim 1 or method according to claim 2 substantially as hereinbefore described with reference to any one of the Examples.

Dated 3 November, 2011

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PanGenetics 110 BV

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

15

1/6

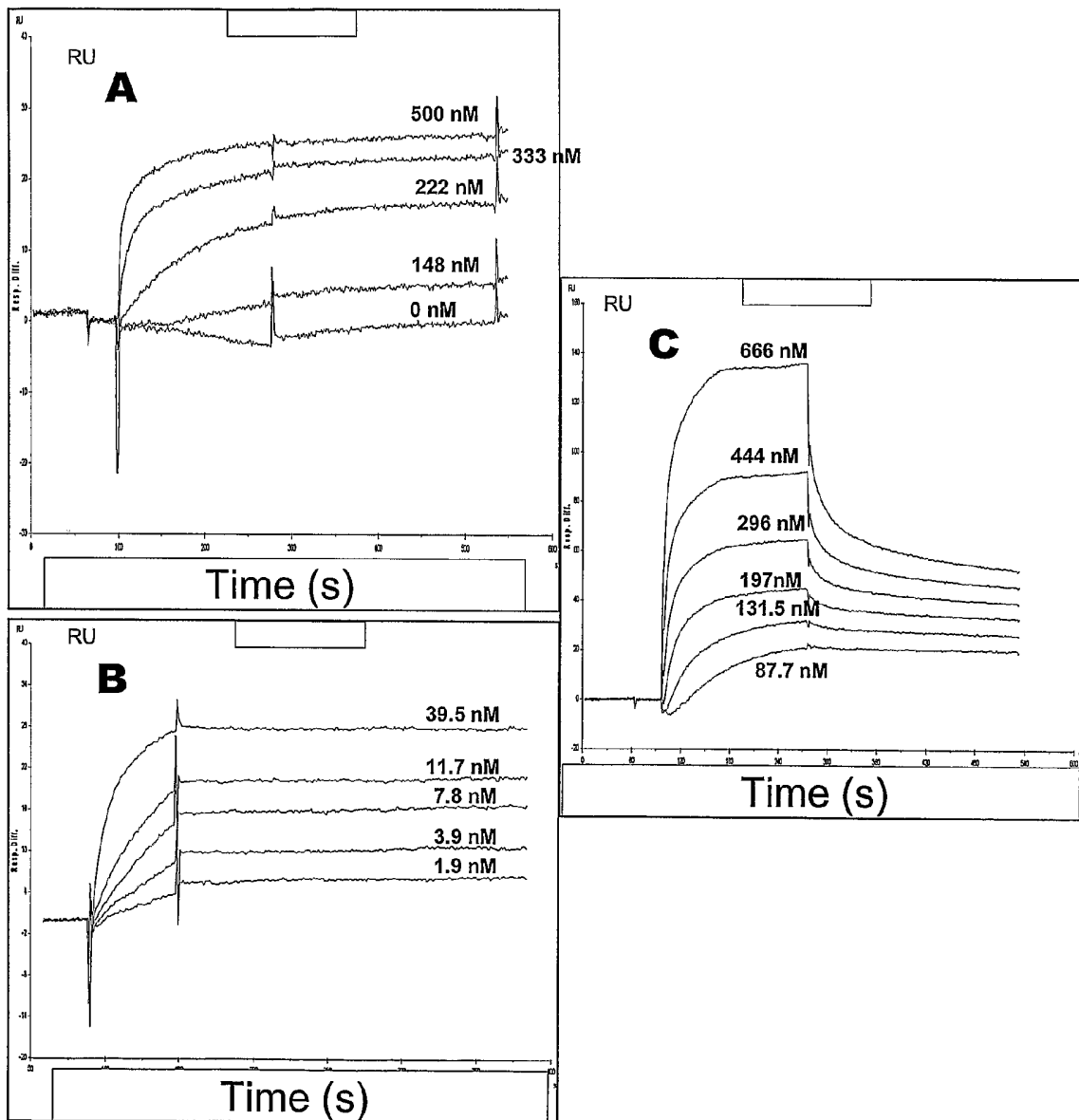


Fig. 1

2/6

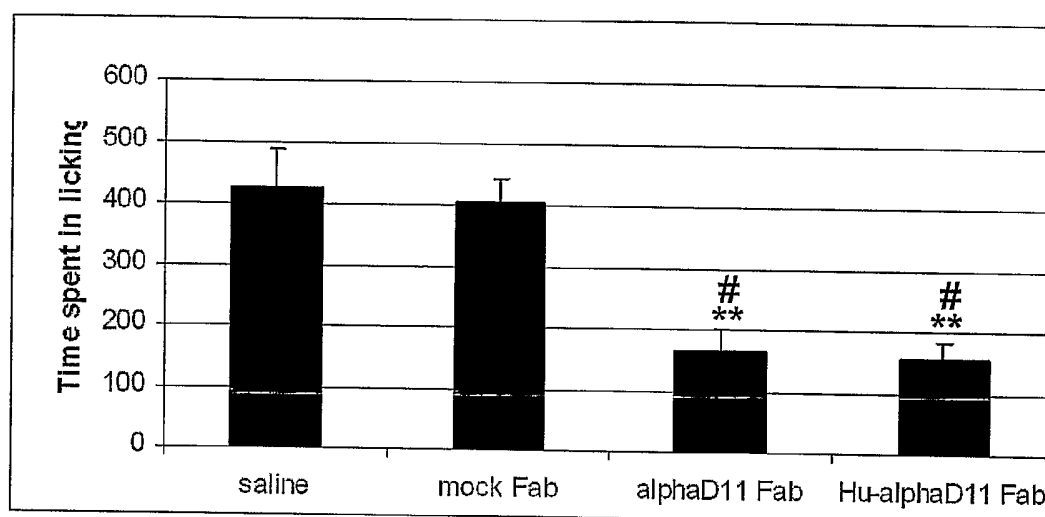


Fig. 2

3/6

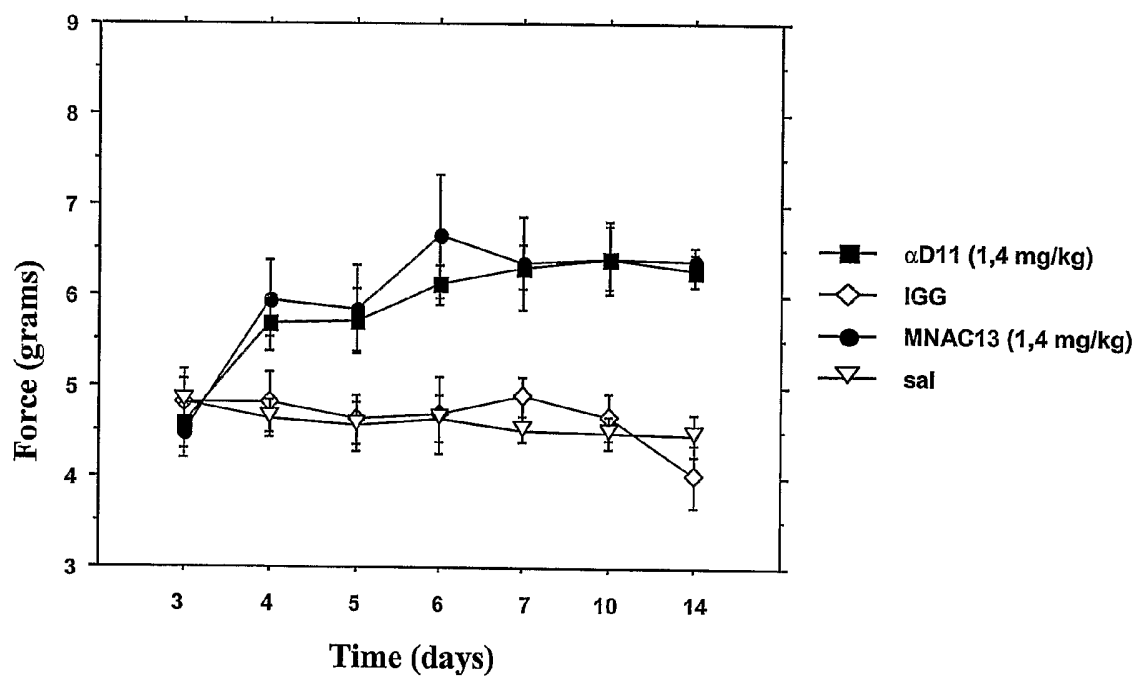


Fig. 3

4/6

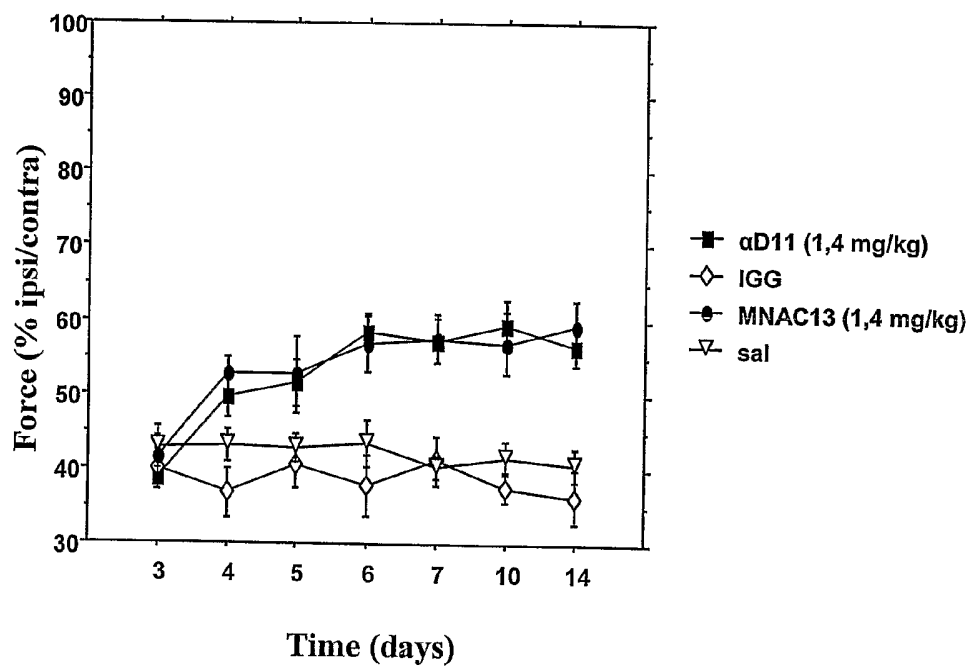


Fig. 4

5/6

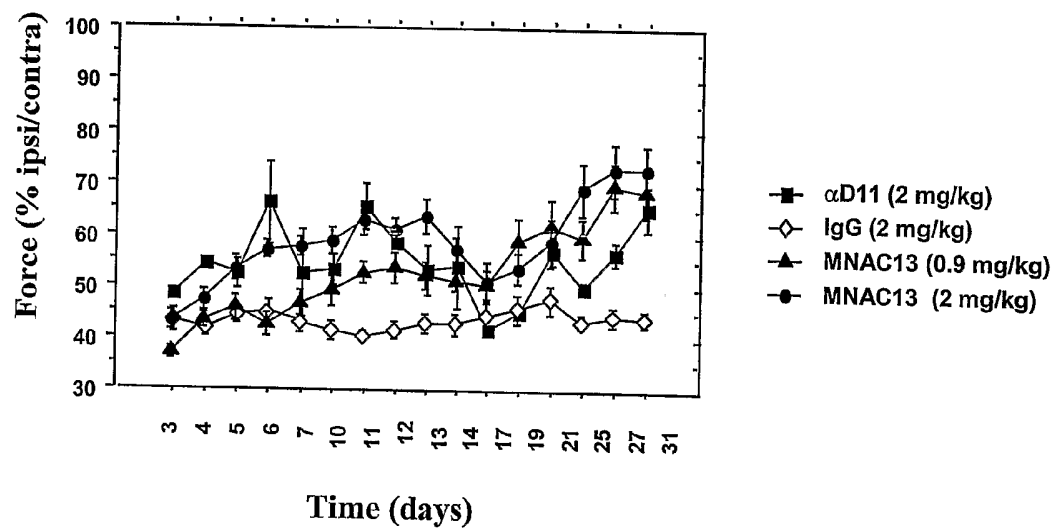


Fig. 5

6/6

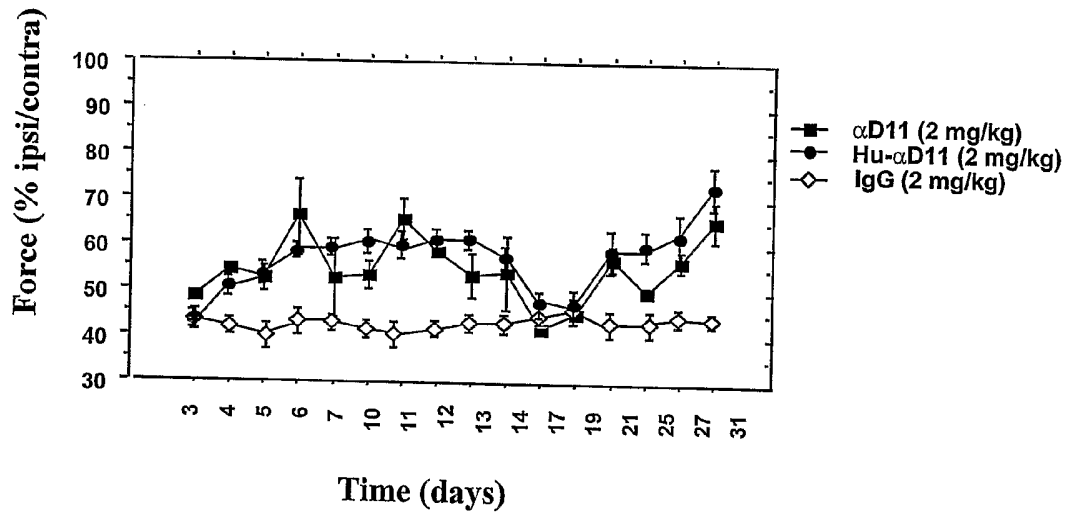


Fig. 6

837308Seq
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 <150> PCT/IT2006/000426
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				20					25					30	Ala
Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ser	Pro	Gln	Leu	Leu	Ile
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Tyr	Asn	Thr	Asp	Thr	Leu	His	Thr	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
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Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Ser	Leu	Lys	Ile	Asn	Ser	Leu	Gln	Ser
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Glu	Asp	Val	Ala	Ser	Tyr	Phe	Cys	Gln	His	Tyr	Phe	His	Tyr	Pro	Arg
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Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Leu	Lys					
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Asn Val Asn Trp Val Arg Gln Ala Thr Gly Arg Gly Leu Glu Trp Met
35 40 45

Gly Gly Val Trp Ala Gly Gly Ala Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Thr Arg Asp Thr Ser Lys Ser Gln Val Phe Leu
65 70 75 80

Lys Met His Ser Leu Gln Ser Glu Asp Thr Ala Thr Tyr Tyr Cys Ala
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Arg Asp Gly Gly Tyr Ser Ser Ser Thr Leu Tyr Ala Met Asp Ala Trp
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Gly Gln Gly Thr Thr Val Thr Val Ser Ala
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr Asn Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asn Thr Asp Thr Leu His Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Glu Asp Phe Ala Thr Tyr Phe Cys Gln His Tyr Phe His Tyr Pro Arg
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Asn Asn
20 25 30

Asn Val Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Gly Val Trp Ala Gly Gly Ala Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp Gly Gly Tyr Ser Ser Ser Thr Leu Tyr Ala Met Asp Ala Trp
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Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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20 25 30

Asn Val Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

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G y G y Val Trp Al a G y G y Al a Thr Asp Tyr Asn Ser Al a Leu Lys
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 Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Al a Tyr Leu
 65 70 75 80
 G n Met Asn Ser Leu Arg Al a G u Asp Thr Al a Val Tyr Tyr Cys Al a
 85 90 95
 Arg Asp G y G y Tyr Ser Ser Ser Thr Leu Tyr Al a Met Asp Al a Trp
 100 105 110
 G y G n G y Thr Leu Val Thr Val Ser Ser Al a Ser Thr Lys G y Pro
 115 120 125
 Ser Val Phe Pro Leu Al a Pro Ser Ser Lys Ser Thr Ser G y G y Thr
 130 135 140
 Al a Al a Leu G y Cys Leu Val Lys Asp Tyr Phe Pro G u Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser G y Al a Leu Thr Ser G y Val His Thr Phe Pro
 165 170 175
 Al a Val Leu G n Ser Ser G y Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu G y Thr G n Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val G u Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Al a Pro G u Leu Leu
 225 230 235 240
 G y G y Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro G u Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His G u Asp Pro G u Val Lys Phe Asn Trp Tyr Val Asp G y Val G u
 275 280 285
 Val His Asn Al a Lys Thr Lys Pro Arg G u G u G n Tyr Asn Ser Thr
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 Tyr Arg Val Val Ser Val Leu Thr Val Leu His G n Asp Trp Leu Asn
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G y Lys G u Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

I l e G u Lys Thr I l e Ser Lys Ala Lys G y G n Pro Arg G u Pro G n
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp G u Leu Thr Lys Asn G n Val
355 360 365

Ser Leu Thr Cys Leu Val Lys G y Phe Tyr Pro Ser Asp I l e Ala Val
370 375 380

G u Trp G u Ser Asn G y G n Pro G u Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp G y Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp G n G n G y Asn Val Phe Ser Cys Ser Val
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Ser Pro G y Lys
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20 25 30

Asn Val Asn Trp Val Arg G n Ala Pro G y Lys G y Leu G u Trp Val
35 40 45

G y G y Val Trp Ala G y G y Ala Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Phe Thr I l e Ser Arg Asp Asn Ser Lys Asn Thr Ala Tyr Leu
65 70 75 80

G n M e t Asn Ser Leu Arg Ala G u Asp Thr Ala Val Tyr Tyr Cys Ala
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 Ser Val 130 Phe Pro Leu Ala Pro 135 Ser Ser Lys Ser Thr 140 Ser Gly Gly Thr
 Ala 145 Ala Leu Gly Cys Leu 150 Val Lys Asp Tyr Phe 155 Pro Gu Pro Val Thr 160
 Val Ser Trp Asn Ser 165 Gly Ala Leu Thr Ser 170 Gly Val His Thr Phe 175 Pro
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 Val Pro Ser 195 Ser Ser Leu Gly Thr 200 Gln Thr Tyr Ile Cys 205 Asn Val Asn
 His Lys 210 Pro Ser Asn Thr Lys 215 Val Asp Lys Arg Val 220 Gu Pro Lys Ser
 Cys 225 Asp Lys Thr His Thr 230 Cys Pro Pro Cys Pro 235 Ala Pro Gu Leu Leu 240
 Gly Gly Pro Ser Val 245 Phe Leu Phe Pro Pro 250 Lys Pro Lys Asp Thr 255 Leu
 Met Ile Ser Arg 260 Thr Pro Gu Val Thr 265 Cys Val Val Val Asp 270 Val Ser
 His Gu Asp 275 Pro Gu Val Lys Phe 280 Asn Trp Tyr Val Asp 285 Gly Val Gu
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 Gly Lys Gu Tyr Lys 325 Cys Lys Val Ser Asn 330 Lys Ala Leu Pro Ala 335 Pro
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Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
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 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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 35 40 45
 Gly Gly Val Trp Ala Gly Gly Ala Thr Asp Tyr Asn Ser Ala Leu Lys
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 Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Tyr Leu
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 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
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 Arg Asp Gly Gly Tyr Ser Ser Ser Thr Leu Tyr Ala Met Asp Ala Trp
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Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr
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Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys
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