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(54) Title: ANTI-INFLAMMATORY DAB

(57) Abstract: The present invention provides a recombinant domain antibody (dAb) which binds to human TNF-α, the dAb comprising an immunoglobulin heavy or light chain variable domain, wherein said variable domain comprises at least one complementarity determining region (CDR) having a sequence derived from a New World primate wherein the CDR is selected from the group consisting of YAATKLQS (SEQ ID No: l), YEASSLQS (SEQ ID No:2), YEASKLQS (SEQ ID No:3) and YSASNLET (SEQ ID No:4). The recombinant domain antibody is used to detect human TNF-α in a sample and to treat a disorder characterized by human TNF-α activity.

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Anti-inflammatory dAb

FIELD OF THE INVENTION

The invention relates to recombinant domain antibodies (dAbs) useful for human therapy. More particularly, the present invention relates to a domain antibody (dAb) which binds to human TNF-α and its use in the treatment of disorders characterised by human TNF-α activity.

BACKGROUND OF THE INVENTION

Tumor necrosis factor alpha (TNF-α) is a cytokine produced by numerous cell types, including monocytes and macrophages, that has been implicated in mediating shock and the pathophysiology of a variety of human diseases and disorders including sepsis, infections, autoimmune diseases, transplant rejection and graft-versus-host disease.

In an effort to counter the harmful effects mediated by human TNF-α, antibodies that bind to and neutralise human TNF-α have been sought as a means to inhibit TNF-α activity. Some of the earliest antibodies directed against human TNF-α were mouse monoclonal antibodies secreted from hybridoma cell lines prepared from lymphocytes harvested from mice immunized with human TNF-α. Although such antibodies were effective in binding to and neutralising human TNF-α, their use in *in vivo* therapy has been limited by problems associated with the administration of mouse antibodies to humans, in particular, elicitation of an unwanted immune response against the mouse antibody in a human, referred to as human anti-mouse antibody (HAMA) reactions.

In an attempt to overcome these problems, murine anti-human TNF-α antibodies have been genetically engineered to be more human-like. For example, human/mouse chimeric antibodies have been created in which antibody variable region sequences from the mouse genome are combined with antibody constant region sequences from the human genome. The chimeric antibodies exhibit the binding characteristics of the parental mouse antibody, and the effector functions associated with the human constant region. Although these chimeric antibodies have been used in human therapy, they still retain some murine sequences and therefore still may elicit anti-chimeric antibody reactions in human recipients, particularly when administered for prolonged periods thus limiting their therapeutic application.

Human monoclonal antibodies against human TNF-α have been developed using human hybridoma techniques. This approach, however, suffers from ethical, clinical and immunological limitations on immunization of human subjects.

It has been postulated that non-human primate antibodies will be tolerated in humans because they are structurally similar to human antibodies (Ehrlich PH et al., Human and primate monoclonal antibodies for *in vivo* therapy. Clin Chem. 34:9 pg 1681-1688 (1988)). Furthermore, because human antibodies are non-immunogenic in Rhesus monkeys (Ehrich PH *et al.*, Rhesus monkey responses to multiple injections of human monoclonal antibodies. Hybridoma 1987; 6:151-60), it is likely that the converse is also applicable and primate antibodies will be non-immunogenic in humans.

Evolutionarily distant primates, such as New World primates, are not only sufficiently different from humans to allow antibodies against human antigens to be generated, but are sufficiently similar to humans to have antibodies similar to human antibodies so that the host does not generate an anti-antibody immune response when such primate-derived antibodies are introduced into a human. New World primates (infraorder- Platyrrhini) comprises at least 53 species commonly divided into two families, the *Callithricidae* and *Cebidae*. The *Callithricidae* consist of marmosets and tamarins. The *Cebidae* includes the squirrel monkey, titi monkey, spider monkey, woolly monkey, capuchin, night or owl monkey and the howler monkey.

Previous studies have characterised the expressed immunoglobulin heavy chain repertoire of the *Callithrix jacchus* marmoset (von Budingen H-C *et al.*, Characterization of the expressed immunoglobulin IGHV repertoire in the New World marmoset *Callithrix jacchus*. Immunogenetics 2001; 53:557-563). Six IGHV subgroups were identified which showed a high degree of sequence similarity to their human IGHV counterparts. The framework regions were more conserved when compared to the complementarity determining regions (CDRs). The degree of similarity between *C. jacchus* and human IGHV sequences was less than between Old World primates and humans.

Domain antibodies

Domain antibodies (dAb) are the smallest functioning binding units of antibodies and correspond to the variable regions of either the heavy (V_H) or light (V_L) chains of antibodies. Domain antibodies have a molecular weight of approximately 13 kDa, or less than one tenth the size of a full antibody.

Immunoglobulin light chains are referred to as either kappa or lambda light chains and the heavy chains as gamma, mu, delta, alpha or epsilon. The variable region gives the antibody its specificity. Within each variable region are regions of hypervariability, otherwise known as complementarity determining regions (CDRs) which are flanked by more conserved regions referred to as framework regions. Within each variable region are three CDRs and four framework regions.

In contrast to conventional antibodies, domain antibodies are well expressed in bacterial, yeast and mammalian systems. Their small size allows for higher molar quantities per gram of product, thus providing a significant increase in potency per dose. In addition, domain antibodies can be used as a building block to create therapeutic products such as multiple targeting dAbs in which a construct containing two or more variable domains bind to two or more therapeutic targets, or dAbs targeted for pulmonary or oral administration.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a recombinant domain antibody (dAb) which binds to human TNF-α, the dAb comprising an immunoglobulin heavy or light chain variable domain, wherein said variable domain comprises at least one complementarity determining region (CDR) having a sequence derived from a New World primate wherein the CDR is selected from the group the group consisting of YAATKLQS (SEQ ID No:1), YEASSLQS (SEQ ID No:2), YEASKLQS (SEQ ID No:3), YSASNLET (SEQ ID No:4).

In a second aspect, the invention provides a pharmaceutical composition comprising an effective amount of the dAb according to the first aspect of the invention, together with a pharmaceutically acceptable carrier or diluent.

In a third aspect, the present invention provides for the use of a dAb according to the first aspect of the invention in a diagnostic application for detecting human TNF-α.

In a fourth aspect, the invention provides a method for treating a disorder characterised by human TNF-α activity in a human subject, comprising administering to the subject a pharmaceutical composition according to the second aspect of the invention.

In a fifth aspect the invention provides a nucleic acid sequence encoding the dAb of the first aspect of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the amino acid (SEQ ID No:6) and nucleotide sequence (SEQ ID No:5) of the acceptor dAb.

Figure 2 shows the nucleotide and amino acid sequences of eleven (11) marmoset and six (6) Owl monkey Vκ gene segments.

Figure 3 shows the acceptor dAb amino acid and nucleotide sequence (both strands). The restriction digest sites for Kpn I and San DI which excises a region including the CDR2 is indicated in the figure. CDR2 residues removed are indicated in underlined.

Figure 4 shows sequence alignments showing oligonucleotides used during cloning and final sequence confirmation of the nucleotide (A) and amino acid (B) sequences shown in Figure 2.

Figure 5 demonstrates the ability of CDR2-grafted dAbs to inhibit the binding of TNF to recombinant TNF receptor. The dAbs tested were as follows: Owl Monkey 1 (CDR= YAATKLQS; SEQ ID No:1), Owl Monkey 2 (CDR=YEASSLQS; SEQ ID No:2), Marmoset 1 (CDR=YEASKLQS; SEQ ID No:3), Marmoset 2 (CDR=YSASNLET; SEQ ID No:4) and Acceptor dAb (CDR=YSASELQS; SEQ ID No:49).

Figure 6 demonstrates the improved ability of Compounds 100 and 123 to neutralise the cytotoxic activity of TNF on mouse L929 fibroblasts relative to acceptor dAb (Compound 145).

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect, the present invention provides a recombinant domain antibody (dAb) which binds to human TNF-α, the dAb comprising an immunoglobulin heavy or light chain variable domain, wherein said variable domain comprises at least one complementarity determining region (CDR) having a sequence derived from a New World primate wherein the CDR is selected from the group the group consisting of YAATKLQS (SEQ ID No:1), YEASSLQS (SEQ ID No:2), YEASKLQS (SEQ ID No:3), YSASNLET (SEQ ID No:4).

Preferably, the CDR is CDR2.

In a preferred embodiment the dAb has a sequence selected from:

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASNLETG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR
[Compound 145; SEQ ID No:7]

DIQMTQSPSSLSASVGDRVTITCRASQAIDSYLHWYQQKPGKAPKLLIYSASNLET
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR
[Compound 123; SEQ ID No:8]

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASNLETG
VPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKVEIKR
[Compound 100; SEQ ID No:9]

DIQMTQSPSSLSASVGDRVTITCRASQAIDSYLHWYQQKPGKAPKLLIYSASNLET
GVPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKVEIKR
[Compound 196; SEQ ID No:10]

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKPPKLLIYSASNLETG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR
[Compound 134; SEQ ID No:50]

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASNLETG
VPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR
[Compound 137; SEQ ID No:51]

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASNLETG
VPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKVEIKR
[Compound 121; SEQ ID No:52]

In a further aspect the invention provides a nucleic acid sequence encoding the dAb of the first aspect of the invention.

The term "binds to" as used herein, is intended to refer to the binding of an antigen by an immunoglobulin variable region with a dissociation constant (K_d) of 1μM or lower as measured by surface plasmon resonance analysis using, for example a BIAcore™ surface plasmon resonance system and BIAcore™ kinetic evaluation software (eg. version 2.1). The affinity or dissociation constant (K_d) for a specific binding interaction is preferably about 500 nM or lower, more preferably about 300 nM or lower and preferably at least 300 nM to 50 pM, 200 nM to 50 pM, and more preferably at least 100 nM to 50 pM, 75 nM to 50 pM, 10 nM to 50 pM.

The term "variable domain" as used herein is meant a folded polypeptide domain which comprises sequences characteristic of immunoglobulin heavy or light chain variable domains and which specifically binds an antigen. A domain antibody or dAb is equivalent to a single variable domain polypeptide.

It will be appreciated by persons skilled in the art that the remainder of the variable domain sequence may be derived from either a human, New World primate or Old World primate variable domain sequence which, because of their evolutionary association with humans, share a high degree of homology with the human sequence. Thus, for example, a CDR selected from the sequences above may be grafted into the human or primate variable region sequence to replace the wild-type CDR.

Accordingly, the invention is further based on a method for amplification of New World primate immunoglobulin variable domain genes, for example by polymerase chain reaction (PCR) from nucleic acid extracted from New World primate lymphocytes using primers specific for heavy and light chain variable domain gene families. For example, information regarding the boundaries of the variable domains of heavy and light chain genes (V_H and V_L respectively) can be used to design PCR primers that amplify the variable domain from a cloned heavy or light chain coding sequence encoding an antibody known to bind a given antigen. The amplified variable domain is then inserted either alone or as a fusion with another polypeptide sequence into a suitable expression vector. The expressed variable domain is then screened for high affinity binding to the desired antigen.

The repertoire of V_H and V_L domains can be a naturally occurring repertoire of immunoglobulin sequences or a synthetic repertoire. A naturally occurring repertoire is one prepared, for example, from immunoglobulin expressing cells harvested from one or more primates. Such repertoires can be naïve ie. prepared from newborn immunoglobulin expressing cells, or rearranged ie. prepared from , for example, adult primate B cells. If desired, clones identified from a natural repertoire, or any repertoire that bind the target antigen are then subject to mutagenesis and further screening in order to produce and select variants with improved binding characteristics.

Synthetic repertoires of single immunoglobulin variable domains are prepared by artificially introducing diversity into a cloned variable domain.

A repertoire of V_H and V_L domains can be screened for desired binding specificity and functional behaviour by, for example phage display. Methods for the construction of bacteriophage display libraries and lambda phage expression libraries are well known in the art. The phage display technique has been described extensively in the art and

examples of methods and compounds for generating and screening such libraries and affinity maturing the products of them can be found in, for example, Barbas et al. (1991) PNAS 88:7978-7982; Clarkson et al. (1991) Nature 352:624:628; Dower et al. PCT. 91/17271, U.S. Patent No. 5,427,908, U.S. Patent No. 5,580,717 and EP 527,839; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Garrad et al. (1991) Bio/Technology 9:1373:1377; Garrard et al. PCT WO 92/09690; Gram et al. (1992) PNAS 89:3576-3580; Griffiths et al. (1993) EMBO J 12:725:734; Griffiths et al. U.S. Patent No. 5,885,793 and EP 589,877; Hawkins et al. (1992) J Mol Biol 226:889-896; Hay et al. (1992) Hum Antibod Hybridomas 3:81-85; Hoogenboom et al. (1991) Nuc Acid Res 19:4133-4137; Huse et al. (1989) Science 246:1275-1281; Knappik et al. (2000) J Mol Biol 296:57-86; Knappik et al. PCT WO 97/08320; Ladner et al. U.S. Patent No. 5,223,409, No. 5,403,484, No. 5,571,698, No. 5,837,500 and EP 436,597; McCafferty et al. (1990) Nature 348:552-554; McCafferty et al. PCT. WO 92/01047, U.S. Patent No. 5,969,108 and EP 589,877; Salfeld et al. PCT WO 97/29131, U.S. Provisional Application No. 60/126,603; and Winter et al. PCT WO 92/20791 and EP 368,684.

Recombinant libraries expressing the repertoire of V_H and V_L domains can be expressed on the surface of microorganisms eg. Yeast or bacteria (see PCT publications WO99/36569 and 98/49286).

The Selective Lymphocyte Antibody Method or SLAM as it is referred to in the state of the art, is another means of generating high affinity antibodies rapidly. Unlike phage display approaches all antibodies are fully divalent. In order to generate New World primate antibodies, New World primates are immunised with a human antigen eg. a TNF-α polypeptide. Following immunisation cells are removed and selectively proliferated in individual micro wells. Supernatants are removed from wells and tested for both binding and function. Gene sequences can be recovered for subsequent manipulations eg. humanisation, Fab fragment, scFv or dAb generation. Thus another example is the derivation of the antibody or antibody species of the invention by SLAM and its derivatives (Babcock, J.S. et al 1996, Proc.Natl. Acad.Sci, USA 93; 7843-7848, US Patent 5,627,052 and PCT publication WO92/02551). Adaptations of SLAM, such as the use of alternatives to testing supernatants such as panning, also lie within the scope of this invention.

In one expression system the recombinant peptide/protein library is displayed on ribosomes (for examples see Roberts, RW and Szostak, J.W.1997. Proc.Natl.Acad.Sci.USA. 94:12297 – 123202 and PCT Publication No. WO98/31700). Thus another example involves the generation and *in vitro* transcription of a DNA library

(eg of antibodies or derivatives preferably prepared from immunised cells, but not so limited), translation of the library such that the protein and "immunised" mRNAs stay on the ribosome, affinity selection (eg by binding to RSP), mRNA isolation, reverse translation and subsequent amplification (eg by polymerase chain reaction or related technology). Additional rounds of selection and amplification can be coupled as necessary to affinity maturation through introduction of somatic mutation in this system or by other methods of affinity maturation as known in the state of the art.

Another example sees the application of emulsion compartmentalisation technology to the generation of the domain antibodies of the invention. In emulsion compartmentalisation, *in vitro* and optical sorting methods are combined with co-compartmentalisation of translated protein and its nucleotide coding sequence in aqueous phase within an oil droplet in an emulsion (see PCT publications no's WO99026711 and WO0040712). The main elements for the generation and selection of antibodies are essentially similar to the *in vitro* method of ribosome display.

The CDR sequences may be obtained from several sources, for example, databases e.g. The National Centre for Biotechnology Information protein and nucleotide databases, The Kabat Database of Sequences of Proteins of Immunological Interest. Alternatively, the CDR regions can be predicted from the V_H and V_L domain repertoire (see for example Kabat EA and Wu TT. Attempts to locate complementarity determining residues in the variable positions of light and heavy chains. Ann. NY Acad. Sci. 190:382-93 (1971)). The CDR sequence may be a genomic DNA or a cDNA.

There are a number of ways in which a replacement CDR may be grafted into a variable domain sequence and such methods will be familiar to those skilled in the art. The preferred method of the present invention involves replacement of the CDR2 in the variable region domain via primer directed mutagenesis. This method consists of annealing a synthetic oligonucleotide encoding a desired mutations to a target region where it serves as a primer for initiation of DNA synthesis *in vitro*, extending the oligonucleotide by a DNA polymerase to generate a double-stranded DNA that carries the desired mutations, and ligating and cloning the sequence into an appropriate expression vector.

Preferably, the domain antibody according to the invention has low immunogenicity in humans.

By reference to the term "low immunogenicity" it is meant that the domain antibody does not raise an antibody response in a human of sufficient magnitude to reduce the

effectiveness of continued administration of the antibody for a sufficient time to achieve therapeutic efficacy.

Preferably, the variable region sequence into which the CDR is grafted is the "dAb acceptor sequence" (designated Compound 128) provided in Figure 1.

The dAb acceptor sequence consists of the amino acid sequence set forth in SEQ ID No:5:

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR (SEQ ID No:6).

This sequence is encoded by the nucleotide sequence set forth in SEQ ID No:5:

GAC ATC CAG ATG ACC CAG TCT CCA TCC TCT CTG TCT GCA TCT GTA GGA GAC CGT GTC ACC ATC ACT TGC CGG GCA AGT CAG AGC ATT GAT AGT TAT TTA CAT TGG TAC CAG CAG AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT AGT GCA TCC GAG TTG CAA AGT GGG GTC CCA TCA CGT TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT CTG CAA CCT GAA GAT TTT GCT ACG TAC TAC TGT CAA CAG GTT GTG TGG CGT CCT TTT ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGG

In one preferred embodiment of the present invention, a marmoset CDR sequence YSASNLET (SEQ ID No:4) is grafted into the dAb acceptor sequence so as to replace the CDR2 sequence (YSASELQS; SEQ ID No:49) of the dAb acceptor sequence to produce the following dAb (designated Compound 145):

Compound 145

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASNLETG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR (SEQ ID No:7)

Thus, in one preferred embodiment, the dAb which binds to human TNF-α comprises the amino acid sequence of SEQ ID No:7.

It is within the scope of the present invention, that the dAb sequence may be further subject to affinity maturation in order to improve its antigen binding characteristics. This may necessitate the modification of certain amino acid residues within CDR1 and CDR3.

For example, the marmoset CDR-grafted dAb set forth in SEQ ID No:7 was affinity matured as set out in the Materials and Methods and tested for TNF-binding. In a further

preferred embodiment, the dAb which binds to human TNF-α comprises the amino acid sequence of SEQ ID No:8 or SEQ ID No:9. These have been designated Compound 123 and Compound 100 respectively and their sequences are shown below:

Compound 123

DIQMTQSPSSLSASVGDRVTITCRASQAIDSYLHWYQQKPGKAPKLLIYSASNLET GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR (SEQ ID No:8)

Compound 100

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASNLETG VPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKVEIKR (SEQ ID No:9)

In a particularly preferred embodiment, the dAb which binds to human TNF-α comprises the amino acid sequence of SEQ ID No:10. This has ben designated Compound 196 and the sequence is provided below:

Compound 196

DIQMTQSPSSLSASVGDRVTITCRASQAIDSYLHWYQQKPGKAPKLLIYSASNLET GVPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKVEIKR (SEQ ID No:10)

The dAb according to the invention may further comprise an immunoglobulin constant region (Fc region) connected thereto. The constant region sequence may be derived from human or primate sequences. The primate sequence may be a New World primate or an Old World primate sequence. Suitable Old World primates include chimpanzee, or other hominid ape eg. gorilla or orang utan, which because of their close phylogenetic proximity to humans, share a high degree of homology with the human constant region sequence.

The dAb (with or without the constant region connected thereto) can be derivatised or linked to another functional molecule. For example, the dAb can be functionally linked by chemical coupling, genetic fusion, noncovalent association or otherwise, to one or more other molecular entities, such as another antibody, a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which the dAb may be derivatised include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. The dAb may also be derivatised with detectable enzymes such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When a dAb is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. A dAb may also be derivatised with biotin, and detected through indirect measurement of avidin or streptavidin binding.

The present invention also extends to PEGylated dAbs (with or without the constant region connected thereto) which provide increased half-life and resistance to degradation without a loss in activity (eg. binding affinity) relative to non-PEGylated antibody polypeptides.

The dAb can be coupled, using methods known in the art, to polymer molecules (preferably PEG) useful for achieving the increased half-life and degradation resistance properties. Polymer moieties which can be utilised in the invention can be synthetic or naturally occurring and include, but or not limited to straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymers, or a branched or unbranched polysaccharide such as a homo-or heteropolysaccharide. Preferred examples of synthetic polymers which can be used in the invention include straight or branched chain poly(ethylene glycol) (PEG), poly(propylene glycol), or poly(vinyl alcohol) and derivatives or substituted forms thereof. Particularly preferred substituted polymers for linkage to dAbs include substituted PEG, including methoxy(polyethylene glycol). Naturally occurring polymer moieties which can be used in addition to or in place of PEG include lactose, amylose, dextran, or glycogen, as well as derivatives thereof which would be recognised by persons skilled in the art.

Derivatized forms of polymer molecules include, for example, derivatives which have additional moieties or reactive groups present therein to permit interaction with amino acid residues of the domain antibody polypeptides described herein. Such derivatives include N-hydroxylsuccinimide (NHS) active esters, succinimidyl propionate polymers, and sulfhydryl-selective reactive agents such as maleimide, vinyl sulfone, and thiol. PEG polymers useful in the invention can be linear molecules, or can be branched wherein multiple PEG moieties are present in a single polymer.

The reactive group (e.g., MAL, NHS, SPA, VS, or Thiol) may be attached directly to the PEG polymer or may be attached to PEG via a linker molecule.

The size of polymers useful in the invention can be in the range of between 500 Da to 60 kDa, for example, between 1000 Da and 60 kDa, 10 kDa and 60 kDa, 20 kDa and 60 kDa, 30 kDa and 60 kDa, 40 kDa and 60 kDa, and up to between 50 kDa and 60 kDa. The polymers used in the invention, particularly PEG, can be straight chain polymers or may possess a branched conformation.

The polymer (PEG) molecules useful in the invention can be attached to a domain antibody using methods which are well known in the art. The first step in the attachment of PEG or other polymer moieties to an antibody polypeptide monomer or multimer of the invention is the substitution of the hydroxyl end-groups of the PEG polymer by electrophile-containing functional groups. Particularly, PEG polymers are attached to either cysteine or lysine residues present in the domain antibody. The cysteine and lysine residues can be naturally occurring, or can be engineered into the antibody polypeptide molecule. For example, cysteine residues can be recombinantly engineered at the C-terminus of a dAb polypeptide, or residues at specific solvent accessible locations in a dAb or other antibody polypeptide can be substituted with cysteine or lysine.

The dAb according to the invention may be linked to one or more molecules which can increase its half-life in vivo. These molecules may be linked to the dAb via a linker so that they do not interfere/sterically hinder the antigen binding site. Alternatively, they may be linked to the constant region. Typically, such molecules are polypeptides which occur naturally in vivo and which resist degradation or removal by endogenous mechanisms. Molecules which increase half life may be selected from the following:

(a) proteins from the extracellular matrix, eg. collagen, laminin, integrin and fibronectin;

(b) proteins found in blood, eg. fibrin α-2 macroglobulin, serum albumin, fibrinogen A, fibrinogen B, serum amyloid protein A, heptaglobin, protein, ubiquitin, uteroglobulin, ß-2 microglobulin, plasminogen, lysozyme, cystatin C, alpha-1-antitrypsin and pancreatic kypsin inhibitor;

(c) immune serum proteins, eg. IgE, IgG, IgM;

(d) transport proteins, eg. retinol binding protein, α-1 microglobulin;

(e) defensins, eg. beta-defensin 1, Neutrophil defensins 1, 2 and 3;

(f) proteins found at the blood brain barrier or in neural tissues, eg. melanocortin receptor, myelin, ascorbate transporter;

(g) transferrin receptor specific ligand-neuropharmaceutical agent fusion proteins (see US5977307); brain capillary endothelial cell receptor, transferrin, transferrin receptor, insulin, insulin- like growth factor 1 (IGF 1) receptor, insulin-like growth factor 2 (IGF 2) receptor, insulin receptor;

(h) proteins localised to the kidney, eg. polycystin, type IV collagen, organic anion transporter K1, Heymann's antigen;

(i) proteins localised to the liver, eg. alcohol dehydrogenase, G250;

(j) blood coagulation factor X;

(k) α-1 antitrypsin;

(l) HNF 1α;

(m) proteins localised to the lung, eg. secretory component (binds IgA);

(n) proteins localised to the Heart, eg. HSP 27;

(o) proteins localised to the skin, eg, keratin;

(p) bone specific proteins, such as bone morphogenic proteins (BMPs) eg. BMP-2, -4, -5, -6, -7 (also referred to as osteogenic protein (OP-1) and -8 (OP-2);

(q) tumour specific proteins, eg. human trophoblast antigen, herceptin receptor, oestrogen receptor, cathepsins eg cathepsin B (found in liver and spleen);

(r) disease-specific proteins, eg. antigens expressed only on activated T- cells: including LAG-3 (lymphocyte activation gene); osteoprotegerin ligand (OPGL) see Nature 402, 304-309, 1999; OX40 (a member of the TNF receptor family, expressed on activated T cells and the only costimulatory T cell molecule known to be specifically up-regulated in human T cell leukaemia virus type-I (HTLV-I)-producing cells - see J. Immunol. 2000 Jul 1;16561):263-70; metalloproteases (associated with arthritis/cancers), including CG6512

Drosophila, human paraplegin, human FtsH, human AFG3L2, murine ftsH; angiogenic growth factors, including acidic fibroblast growth factor (FGF-1), basic fibroblast growth factor (FGF-2), vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), transforming growth factor-α (TGF-α), tumor necrosis factor-alpha (TNF-α), angiogenin, interleukin-3 (IL-3), interleukin-8 (IL-8), platelet derived endothelial growth factor (PD- ECGF), placental growth factor (PlGF), midkine platelet-derived growth factor-BB (PDGF), fractalkine;

(s) stress proteins (heat shock proteins);

(t) proteins involved in Fc transport; and

(u) antibodies, fragments or derivatives directed against endogenous proteins e.g. serum albumin.

In a further embodiment of the present invention, the dAb according to the first aspect may be multimerised, as for example, hetero- or homodimers, hetero- or homotrimers, hetero- or homotetramers, or higher order hetero- or homomultimers. Multimerisation can increase the strength of antigen binding, wherein the strength of binding is related to the sum of the binding affinities of the multiple binding sites.

Thus, the invention provides a domain antibody according to the first aspect, wherein the domain antibody is linked to at least one further domain antibody. Each dAb may bind to the same or different antigens.

The dAb multimers may further comprise one or more dAbs which are linked and wherein each dAb binds to a different antigen, multi-specific ligands including so-called "dual-specific ligands". For example, the dual specific ligands may comprise a pair of V_H domains or a pair of V_L domains. Such dual-specific ligands are described in WO 2004/003019 (PCT/GB2003/002804) in the name of Domantis Ltd.

In a second aspect, the invention provides a pharmaceutical composition comprising an effective amount of the dAb according to the first aspect of the invention, together with a pharmaceutically acceptable carrier or diluent.

A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal antifungal agents, isotonic and absorption delaying agents, and the like which are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline,

dextrose, glycerol, ethanol, and the like as well as combinations thereof. In many cases it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers.

The composition may be in a variety of forms, including liquid, semi-solid and solid dosage forms, such as liquid solutions (eg injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. Preferably, the composition is in the form of an injectable solution for immunization. The administration may be intravenous, subcutaneous, intraperitoneal, intramuscular, transdermal, intrathecal, and intra-arterial.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The compositions can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (ie. dAb) into the required amount in an appropriate solvent with one or a combination of ingredients listed above, followed by filtered sterilisation.

The composition may also be formulated as a sterile powder for the preparation of sterile injectable solutions. The proper fluidity of a solution can be maintained by for example, use of a coating such as lecithin and/or surfactants.

In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Compatible polymers may be used such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters and polylactic acid.

The composition may also be formulated for oral administration. In this embodiment, the dAb may be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet.

The composition may also be formulated for rectal administration.

Supplementary active compounds can also be incorporated into the composition. The domain antibody may be co-formulated with and/or co-administered with one or more additional therapeutic agents eg. anti-inflammatory compounds, soluble TNF-α receptor or a chemical agent that inhibits human TNF-α production, or antibodies that bind other

targets such as cytokines or cell surface molecules. Alternatively, it may be co-administered with a soluble immunochemical reagent such as protein A, C, G or L.

An effective amount may include a therapeutically effective amount or prophylactically effective amount of the dAb of the invention. A therapeutically effective amount refers to an amount effective at dosages and for periods of time necessary, to achieve the desired therapeutic result. A prophylactically effective amount refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result.

In a preferred embodiment the composition is administered to mammals, preferably humans or primates.

In a third aspect, the present invention provides for the use of a dAb according to the first aspect of the invention in a diagnostic application for detecting human TNF-α.

For example, the anti-human TNF-α dAb according to the invention can be used to detect human TNF-α for example in a biological sample, such as serum or plasma using a conventional immunoassay, such as an enzyme linked immunosorbent assay (ELISA), a radioimmunoassay (RIA) or tissue immunohistochemistry. The anti-human TNF-α dAb according to the invention can be assayed in biological fluids by a competition immunoassay using recombinant human TNF-α standards labelled with a detectable substance and an unlabelled anti-human TNF-α antibody.

The anti-human TNF-α dAb according to the invention may also be used to detect TNF-α from species other than humans eg. chimpanzee, marmoset, rhesus, mouse, pig.

The anti-human TNF-α dAb according to the invention may also be used in cell culture applications where it is desired to inhibit TNF-α activity.

In a fourth aspect, the invention provides a method for treating a disorder characterised by human TNF-α activity in a human subject, comprising administering to the subject a pharmaceutical composition according to the second aspect of the invention.

A disorder characterised by human TNF-α activity is intended to include diseases and other disorders in which the presence of TNF-α in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor which contributes to a worsening of the disorder. Preferably, the disorder characterised by human TNF-α activity is selected from the group consisting of inflammation, inflammatory diseases, sepsis, including septic shock, endotoxic shock, gram negative sepsis and toxic shock syndrome; autoimmune disease, including

rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis, allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic syndrome; infectious disease, including fever and myalgias due to infection and cachexia secondary to infection; graft versus host disease; tumour growth or metastasis; pulmonary disorders including adult respiratory distress syndrome, shock lung, chronic pulmonary inflammatory disease, pulmonary sarcoidosis, pulmonary fibrosis and silicosis; inflammatory bowel disorders including Crohn's disease and ulcerative colitis; cardiac disorders; inflammatory bone disorders, hepatitis, coagulation disturbances, burns, reperfusion injury, keloid formation and scar tissue formation.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

All publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia or elsewhere before the priority date of each claim of this application.

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following non-limiting examples.

EXAMPLE 1 Materials and Methods

Isolation of New World primate VL genes

Marmoset (genus Callithrix, species unknown) and Owl monkey (Aotus trivirgatus) genomic DNA were obtained from the European Collection of Cell Cultures (ECACC), catalogue numbers 85011419 and 90110510 respectively. Marmoset DNA was derived from cell line B95-8 while Owl monkey DNA came from cell line OMK 637-69.

Degenerate primers based on human Vκ leader sequences and recombination signal sequences (RSS) were derived from Walter and Tomlinson, Antibody Engineering: A Practical Approach (1996). The primers used for amplification of germline Vκ DNA were as follows:

Primer VK1BL
AATCKCAGGTKCCAGATG (SEQ ID No:11)

Primer VK1BL35a
GTTYRGGTKKGTAACACT (SQ ID No:12)

Primer VK1BL35b
ATGMCTTGTWACACTGTG (SEQ ID No:13)

Genomic PCR (30 cycles) was performed using Taq polymerase with either primer pair VK1BLxVK1BL35a or VK1BLxVK1BL35b. There was overlap between the sequences cloned and the two primer sets used.

PCR products were cloned into Invitrogen's TOPO TA cloning kit (Cat No K4500-01) and sequenced with M13 forward and pUC reverse primers. Sequence was confirmed in forward and reverse directions. In order to further confirm key sequences were not subject to PCR errors, the PCR and cloning process was repeated twice for marmoset sequences. Nucleotide (SEQ ID Nos:14-24 and SEQ ID Nos:36-41) and amino acid (SEQ ID Nos:25-35 and SEQ ID Nos:42-47) are given in Figure 2. Marmoset sequences 1, 2 and 3 were confirmed. Sequences 4, 5, 6, 7 and 8 were seen only in the initial PCR. Sequences 9, 10 and 11 were seen only in the repeat (ie second) PCR and cloning.

Oligo Synthesis and Cloning into Acceptor Sequence

Four CDR sequences, namely YAATKLQS (SEQ ID No:1) from Owl monkey sequence 1 (SEQ ID No:42), YEASSLQS (SEQ ID No:2) from Owl monkey sequence 2 (SEQ ID

No:43), YEASKLQS (SEQ ID No:3) from Marmoset sequence 1 (SEQ ID No:25), and YSASNLET (SEQ ID No:4) from Marmoset sequence 2 (SEQ ID No:26), were chosen from the amino acid sequences shown in Figure 2 as indicated. Owl Monkey sequence 5, YYASSLQS (SEQ ID No:48) was found to be identical to GI6176295 an Aotus nancymaae (Ma's night monkey) cDNA sequence, all other sequences were unique.

An acceptor variable region (anti-TNF domain antibody) sequence in the expression vector (Domantis proprietary vector) was digested (25µg) sequentially with KpnI and SanDI which excises the majority of FR2 as well as CDR2 as indicated on the restriction digest map. The vector was then gel purified to remove the excised wild-type FR2 and CDR2 sequence.

Oligo annealing was performed by incubating oligo pairs (500 pmol of each as shown in Figure 4A and 4B) at 95°C for 5 minutes followed by 65°C for 5 minutes and then allowed to reach room temperature slowly on a hot block. Overlaps were then filled in during a Klenow reaction in the presence of dNTPs.

Affinity Maturation

The marmoset CDR-grafted dAb Compound 145 (SEQ ID No:7) was affinity matured by constructing 14 separate libraries, each a diversification of the sequence of SEQ ID No:7 at a single amino acid residue. The selected residues are shown shaded below.

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLAWYQQKPGKAPKLLIYSASNLETG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSVSWRPSTFGQGTKVEIKR

The selection was based upon residues in CDR1 and CDR3 that are known to be diversified in the mature human Ig repertoire, and framework residues that have been observed to produce functional proteins after mutagenesis in related dAbs. For each of the selected residues, complimentary forward and reverse PCR primer pairs were designed with NKK degeneracy, and two initial PCR reactions were performed each with a single mutagenic primer and flanking primer. After clean-up, the two PCR products were annealed and then amplified using flanking primers alone (splicing by overlap extension of PCR; Lowman H.L. & Clackson T. (eds), Phage Display: A practical approach, Oxford University Press , Oxford, UK). Clones were initially screened by ELIZA using solid-phase TNF, and positive clones were sequenced. dAb protein was purified from the best clones and evaluated for potency in receptor binding assays and L929 cytotoxicity assays. Compounds 100 (SEQ ID No:9) and 123 (SEQ ID No:8) were found to have improved TNF-neutralization relative to the parent dAb, Compound 145 (SEQ ID No:7).

Combination of the affinity-enhancing substitutions of Compounds 100 (SEQ ID No:9) and 123 (SEQ ID No:8), yielded an anti-TNF dAb with further improved potency in the L929 cytotoxicity assay (Compound 196; SEQ ID No:10).

Results

Potency of anti-TNF dAb clones in receptor binding assay (RBA) and cytotoxocity Assay

The ability of the anti-TNF dAbs to inhibit TNF binding to its receptor and to neutralize TNF-mediated cytotoxicity of L929 cells was conducted as follows:

Receptor binding assay

dAbs diversified in the 14 selected positions were tested for the ability to inhibit the binding of TNF to recombinant TNF receptor 1 (p55). Briefly, Maxisorp plates were incubated overnight with 30 mg/ml anti-human Fc mouse monoclonal antibody (Zymed, San Francisco, USA). The wells were washed with phosphate buffered saline (PBS) containing 0.05% Tween-20 and then blocked with 1% BSA in PBS before being incubated with 100 ng/ml TNF receptor 1 Fc fusion protein (R&D Systems, Minneapolis, USA). Each dAb was mixed with TNF which was added to the washed wells at a final concentration of 10 ng/ml. TNF binding was detected with 0.2 mg/ml biotinylated anti-TNF antibody (HyCult biotechnology, Uben, Netherlands) followed by 1 in 500 dilution of horse radish peroxidase labelled streptavidin (Amersham Biosciences, UK) and then incubation with TMB substrate (KPL, Gaithersburg, USA). The reaction was stopped by the addition of HCl and the absorbance was read at 450nm. Anti-TNF dAb activity lead to a decrease in TNF binding and therefore a decrease in absorbance compared with the TNF only control (Figure 5).

L929 Cytotoxicity Assay

Anti-TNF dAbs identified by the minilibrary diversification approach, including Compounds 100 (SEQ ID No:9) and 123 (SEQ ID No:8), were also tested for the ability to neutralise the cytotoxic activity of TNF on mouse L929 fibroblasts (Evans, T. (2000) Molecular Biotechnology 15, 243-248). Briefly, L929 cells plated in microtitre plates were incubated overnight with anti-TNF dAb, 100 pg/ml TNF and 1 mg/ml actinomycin D (Sigma, Poole, UK). Cell viability was measured by reading absorbance at 490nm following an incubation with [3-(4,5-dimethylthiazol-2-yl)-5-(3 - carbboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium (Promega, Madison, USA). Anti-TNF dAb activity lead to a decrease in TNF cytotoxicity and therefore an increase in

absorbance compared with the TNF only control. The results, in comparison with the parent dAb Compound 145 (SEQ ID No:7) are presented in Figure 6.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS

1. A recombinant domain antibody (dAb) which binds to human TNF-α, the dAb comprising an immunoglobulin heavy or light chain variable domain, wherein said variable domain comprises at least one complementarity determining region (CDR) having a sequence derived from a New World primate wherein the CDR is selected from the group the group consisting of YAATKLQS (SEQ ID No:1), YEASSLQS (SEQ ID No:2), YEASKLQS (SEQ ID No:3) and YSASNLET (SEQ ID No:4).

2. A recombinant dAb according to claim 1 wherein the CDR is CDR2.

3. A recombinant dAb according to claim 1 or claim 2 wherein the dAb has a sequence selected from the sequences consisting of:

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSAS NLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKV EIKR (SEQ ID No:7);

DIQMTQSPSSLSASVGDRVTITCRASQAIDSYLHWYQQKPGKAPKLLIYSAS NLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKV EIKR (SEQ ID No:8);

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSAS NLETGVPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKV EIKR (SEQ ID No:9);

DIQMTQSPSSLSASVGDRVTITCRASQAIDSYLHWYQQKPGKAPKLLIYSAS NLETGVPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKV EIKR (SEQ ID No:10);

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKPPKLLIYSAS NLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKV EIKR (SEQ ID No:50);

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSAS NLETGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTK VEIKR (SEQ ID No:51); and

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSAS NLETGVPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKV EIKR (SEQ ID No:52).

4. A recombinant dAb according to claim 3 wherein the dAb has the sequence:

DIQMTQSPSSLSASVGDRVTITCRASQAIDSYLHWYQQKPGKAPKLLIYSAS NLETGVPSRFSGSGSGTDFTLTISSLLPEDFATYYCQQVVWRPFTFGQGTKV EIKR (SEQ ID No:10).

5. A recombinant dAb according to any one of claims 1 to 4 wherein CDR1 and/or CDR3 is modified to improve antigen binding.

6. A recombinant dAb according to any one of claims 1 to 5 wherein the dAb has low immunogenicity in humans.

7. An isolated nucleic acid molecule encoding the dAb of any one of claims 1 to 6.

8. A pharmaceutical composition comprising an effective amount of a recombinant domain antibody (dAb) according to claim 1 to 6, together with a pharmaceutically acceptable carrier or diluent.

9. A method for detecting human TNF-α in a sample comprising contacting the sample with an effective amount of a recombinant dAb according to any one of claims 1 to 6 and detecting the amount of bound dAb.

10. The method according to claim 9 wherein the sample is a biological sample.

11. A method for treating a disorder characterized by human TNF-α activity in a human subject comprising administering to the subject an effective amount of a pharmaceutical composition according to claim 8.

12. A method according to claim 11 wherein the disorder characterized by human TNF-α activity is selected from the group consisting of inflammation, inflammatory diseases, sepsis, including septic shock, endotoxic shock, gram negative sepsis and toxic shock syndrome; autoimmune disease, including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis, allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic syndrome; infectious disease, including fever and myalgias due to infection and cachexia secondary to infection; graft versus host disease; tumour growth or metastasis; pulmonary disorders including adult respiratory distress syndrome, shock lung, chronic

pulmonary inflammatory disease, pulmonary sarcoidosis, pulmonary fibrosis and silicosis; inflammatory bowel disorders including Crohn's disease and ulcerative colitis; cardiac disorders; inflammatory bone disorders, hepatitis, coagulation disturbances, burns, reperfusion injury, keloid formation and scar tissue formation.

FIGURE 1

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1     GAC ATC CAG ATG ACC CAG TCT CCA TCC TCT CTG TCT GCA TCT GTA   45
1     Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val  15
46    GGA GAC CGT GTC ACC ATC ACT TGC CGG GCA AGT CAG AGC ATT GAT   90
16    Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Asp  30
91    AGT TAT TTA CAT TGG TAC CAG CAG AAA CCA GGG AAA GCC CCT AAG   135
31    Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys  45
136   CTC CTG ATC TAT AGT GCA TCC CAG TTG CAA AGT GGG GTC CCA TCA   180
46    Leu Leu Ile Tyr Ser Ala Ser Glu Leu Gln Ser Gly Val Pro Ser  60
181   CGT TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC   225
61    Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile  75
226   AGC AGT CTC CAA CCT GAA GAT TTT GCT ACG TAC TAC TGT CAA CAG   270
76    Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln  90
271   GTT GTG TGG CGT CCT TTT ACG TTC GGC CAA GGG ACC AAG GTG GAA   315
91    Val Val Trp Arg Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu  105
316   ATC AAA CGG   324
106   Ile Lys Arg
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FIGURE 2

Marmoset Sequences

Marmoset nucleotide sequence 1 (SEQ ID No:14)

GACATCCAGATGACCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGGCAAAGTCACCATC
ACTTGCCGGGCGAGTCAGGACATTAACAAGTGGTTAGCCTGGTATCAGCAGAAACCAGGGACA
GTCCCTAAGCCCCTGATCTATGAGGCATCCAAATTGCAAAGTGGGGTCCCATCAAGGTTCAGC
GGCAGTGGATCTGGGACATATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGCTGCA
ACTTATTACTGTCAG

Marmoset nucleotide sequence 2 (SEQ ID No:15)

GACATCCAGATGATCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATC
ACTTGCTGGGCAAGTCAGGGTATTAGCCACTGGTTAGCCTGGTATCAGCAGAAACCAGGGAAA
GCCCCTAAGCTCCTGATCTATAGTGCATCAAATTTAGAAACAGGGGTCCCATCAAGGTTCAGT
GGAAGTGGATCCAGGACAGATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATATTGCA
ACATATTACTGTCAA

Marmoset nucleotide sequence 3 (SEQ ID No:16)

GACATCCAGATGACCCAGACTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATC
ACTTGCCGGGCAAGTCAGGGTATTAGCAGCTGGTTAGCCTGGTATCAGCAGAAACCAGGGAAA
GCCCCTAAGCTCCTGATCTATGGGGCATCAAATTTGGAAACAGGGGTCCCATCAAGATTCAGC
GGAAGTGGATCTGGGACAGATTTTACTCTCACCATCAGCAGTCTGCAGCCTGAAGATATTGCA
ACATATTACTGTCAA

Marmoset nucleotide sequence 4 (SEQ ID No:17)

GACATCCAGATGATCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATC
ACTTGCTGGGCAAGTCAGGGTATTAGCCACTGGTTAGCCTGGTATCAGCAGAAACCAGGGAAA
GCCCCTAAGCTCCTGATCTATAGTGCATCAAATTTAGGAACAGGGGTCCCATCAAGGTTCAGT
GGAAGTGGATCCAGGACAGATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATATTGCA
ACATATTACTGTCAA

Marmoset nucleotide sequence 5 (SEQ ID No:18)

GACATCCAGATGACCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGGCAAAGTCACCATC
ACTTGCCGGGCGTGTCAGGACATTAACAAGTGGTTAGCCTGGTATCAGCAGAAACCAGGGACA
GTCCCTAAGCCCCTGATCTATGAGGCATCCAAATTGCAAAGTGGGGTCCCATCAAGGTTCAGC
GGCAGTGGATCTGGGACATATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGCTGCA
ACTTATTACTGTCAG

Marmoset nucleotide sequence 6 (SEQ ID No:19)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTTACCATC
ACTTGCCGGGCGAGTCAGGGCATTAGTAATTATTTAGCCTGGTATCAGCAGAAACCAGGGAAA
ACTCCTAGGCTCCTGATCTATGCTGCATCCAGTTTACAAACTGGGATTCCCTCTCGGTTCAGC
GGCAGTGGATCTGGGACAGACTACACTCTCACCATCAGCAGCCTGCAGTCTGAAGATGTTGCA
ATTTATTACTGTCAA

Marmoset nucleotide sequence 7 (SEQ ID No:20)

GACATCCAGATGACCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGGCAAAGTCACCATC
ACTTGCCGGGCGAGTCAGGACATTAACAAGTGGTTAGCCTGGTATCAGCAGAAACCAGGGACA
GTCCCTAAGCCCCTGATCTATGAGGCATCCAAATTGCAAAGTGGGGTCCCATCAAGGCTCAGC
GGCAGTGGATCTGGGACATATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGCTGCA
ACTTATTACTGTCAG

Marmoset nucleotide sequence 8 (SEQ ID No:21)

GACATCCAGATGACCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGGCAAAGTCACCATC
ACTTGCCGGGCGAGTCAGGACATTAACAAGTGGTCAGCCTGGTATCAGCAGAAACCAGGGACA
GTCCCTAAGCCCCTGATCTATGAGGCATCCAAATTGCAAAGTGGGGTCCCATCAAGGTTCAGC
GGCAGTGGATCTGGGACATATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGCTGCA
ACTTATTACTGTCAG

Marmoset nucleotide sequence 9 (SEQ ID No:22)

GACATCCAGATGACCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGGCAAAGTCACCGTC
ACTTGCCGGGCGAGTCAGGACATTAACAAGTGGTTAGCCTGGTATCAGCAGAAACCAGGGACA
GTCCCTAAGCCCCTGATCTATGAGGCATCCAAATTGCAAAGTGGGGTCCCATCAAGGTTCAGC
GGCAGTGGATCTGGGACATATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGCTGCA
ACTTATTACTGTCAG

Marmoset nucleotide sequence 10 (SEQ ID No:23)

GACATCCAGATGACCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGGCAAAGTCACCATC
ACTTGCCGGGCGAGTCAGGACATTAACAAGTGGTTAGCCTGGTATCAGCAGAAACCAGGGACA
GTCCTTAAGCCCCTGATCTATGAGGCATCCAAATTGCAAAGTGGGGTCCCATCAAGGTTCAGC
GGCAGTGGATCTGGGACATATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGCTGCA
ACTTATTACTGTCAG

Marmoset nucleotide sequence 11 (SEQ ID No:24)

GACATCCAGATGACCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGGCAAAGTCACCATC
ACTTGCCGGGCGAGTCAGGACATTAACAAGTGGTTAGCCTGGTATCAGCAGAAACCAGGGACA
GTCCCTAAGCCCCTGATCTATGAGGCATCCAAATTGCAAAGTGGGGTCCCATTAAGGTTCAGC
GGCAGTGGATCTGGGACATATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGCTGCA
ACTTATTACTGTCAG

Marmoset amino acid sequence 1 (SEQ ID No:25)

DIQMTQSPSSLTASVGGKVTITCRASQDINKWLAWYQQKPGTVPKPLIYEASKLQSGVPSRFS
GSGSGTYFTLTISSLQPEDAATYYCQ

Marmoset amino acid sequence 2 (SEQ ID No:26)

DIQMIQSPSSLSASVGDRVTITCWASQGISHWLAWYQQKPGKAPKLLIYSASNLETGVPSRFS
GSGSRTDFTLTISSLQPEDIATYYCQ

Marmoset amino acid sequence 3 (SEQ ID No:27)

DIQMTQTPSSLSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYGASNLETGVPSRFS
GSGSGTDFTLTISSLQPEDIATYYCQ

Marmoset amino acid sequence 4 (SEQ ID No:28)

DIQMIQSPSSLSASVGDRVTITCWASQGISHWLAWYQQKPGKAPKLLIYSASNLGTGVPSRFS
GSGSRTDFTLTISSLQPEDIATYYCQ

Marmoset amino acid sequence 5 (SEQ ID No:29)

DIQMTQSPSSLTASVGGKVTITCRACQDINKWLAWYQQKPGTVPKPLIYEASKLQSGVPSRFS
GSGSGTYFTLTISSLQPEDAATYYCQ

Marmoset amino acid sequence 6 (SEQ ID No:30)

DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKTPRLLIYAASSLQTGIPSRFS
GSGSGTDYTLTISSLQSEDVAIYYCQ

Marmoset amino acid sequence 7 (SEQ ID No:31)

DIQMTQSPSSLTASVGGKVTITCRASQDINKWLAWYQQKPGTVPKPLIYEASKLQSGVPSRLS
GSGSGTYFTLTISSLQPEDAATYYCQ

Marmoset amino acid sequence 8 (SEQ ID No:32)

DIQMTQSPSSLTASVGGKVTITCRASQDINKWSAWYQQKPGTVPKPLIYEASKLQSGVPSRFS
GSGSGTYFTLTISSLQPEDAATYYCQ

Marmoset amino acid sequence 9 (SEQ ID No:33)

DIQMTQSPSSLTASVGGKVTVTCRASQDINKWLAWYQQKPGTVPKPLIYEASKLQSGVPSRFS
GSGSGTYFTLTISSLQPEDAATYYCQ

Marmoset amino acid sequence 10 (SEQ ID No:34)

DIQMTQSPSSLTASVGGKVTITCRASQDINKWLAWYQQKPGTVLKPLIYEASKLQSGVPSRFS
GSGSGTYFTLTISSLQPEDAATYYCQ

Marmoset amino acid sequence 11 (SEQ ID No:35)

DIQMTQSPSSLTASVGGKVTITCRASQDINKWLAWYQQKPGTVPKPLIYEASKLQSGVPLRFS
GSGSGTYFTLTISSLQPEDAATYYCQ

Owl Monkey sequences

Owl Monkey nucleotide sequence 1 (SEQ ID No:36)

GACATCCAGATGACCCAGTCTCCATCCTTCCTGTCTGCATCTGCAGGAGACAGAGTCACCATC
ACCTGCCAGGTGAGTCAGGGAATTAGCAGTGAATTACTCTGGTATCAGCAGAAACCAGGGAAA
GCCCCTATGCTCTTGATCTATGCTGCAACCAAATTGCAGTCGGGAATCCCATCTCGGTTCAGT
GGCCATGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCT
ACTTATTACTGTCAA

Owl Monkey nucleotide sequence 2 (SEQ ID No:37)

GACATCCAGATGACCCAGTCTGCATTCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATT
ACTTGCCAGGCGAGTCAGGGCATTACCAGTGATTTAGCCTGGTATCAGCAAAAGCCAGGGAAC
GCCTCTAAGCTCCTGATCTATGAGGCATCCAGTTTACAAAGCGAGGTCCCATCAAGGTTCAGC
GGCAGTGGATCTGGGAGAGATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGTA
ACTTATTACTGTCAA

Owl Monkey nucleotide sequence 3 (SEQ ID No:38)

GACATCCAGATGACCCAGACTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATC
ACTTGCCGGGCGAGTCAAGACATTTACAATTATTTAGCCTGGTATCAGCAGAAACCAGGGAAA
ACTCCTAGGCTCTTGATCTATGCTGCATCCAGTTTGCAAACTGGGATTCCCTCTCGGTTCAGT
GGCAGTGGATCTGGGACAGACTACACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCC
ACTTATTACTGTCAA

Owl Monkey nucleotide sequence 4 (SEQ ID No:39)

GACATCCAGATGACCCAGACTCCATCCTCCCTGCCTGCATCTGTAGGAGACAAAGTCACCATC
ACTTGCCGGGCAAGTCAGGGTATTAGCAGCTGGTTAGCCTGGTATCAGCAGAAACCAGGGAAA
GCCCCTAAGCTCCTGATCCATAAGGCATCAAATTTGGAAACAGGGGTCCCATCAAGGTTCAGT
GGAAGTGGATCTGGGACAGATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATATCGCA
ACATATTACTGTCAA

Owl Monkey nucleotide sequence 5 (SEQ ID No:40)

GACATCCAGATGACCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGACAAAGTCACCATC
ACTTGCCGGGCAAGTCAGGGCATTAGCAATAATTTAGCCTGGTATCAGCAGAAACCAGGGAAA
GCCCCTAAGCCCCTGATCTATTATGCATCCAGTTTGCAAAGCGGGGTCCCATCAAGGTTCAGC
GGCAGTGGATCTGGGGCAGATTACACTCTCACCACCAGCAGCCTGCAGCCTGAAGATTTTGCA
ACTTATTACTGTCAA

Owl Monkey nucleotide Sequence 6 (SEQ ID No:41)

GACAACCAGATGATCCAGTCTCCATCTTCCCTGACTGCATCTGTAGGAGACAGAGTCACCATC
ACTTGCCGAGCCAGTCAGAGTATTAGCAGCTGGTTAGCCTGGTATCAGCAGAAACCAGGGACA
GTCCCTAAGCCTCTGATCTATGACGCATCCAAATTGCTAAGTGGGGTCCCATCAAGGTTCAGT
GGCTGTGGATCTGGGACAGATTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCA
ACTTATTACTGTCAA

Owl Monkey amino acid sequence 1 (SEQ ID No:42)

DIQMTQSPSFLSASAGDRVTITCQVSQGISSELLWYQQKPGKAPMLLIYAATKLQSGIPSRFS
GHGSGTDFTLTISSLQPDDFATYYCQ

Owl Monkey amino acid sequence 2 (SEQ ID No:43)

DIQMTQSAFSLSASVGDRVTITCQASQGITSDLAWYQQKPGNASKLLIYEASSLQSEVPSRFS
GSGSGRDFTLTISSLQPEDFVTYYCQ

Owl Monkey amino acid sequence 3 (SEQ ID No:44)

DIQMTQTPSSLSASVGDRVTITCRASQDIYNYLAWYQQKPGKTPRLLIYAASSLQTGIPSRFS
GSGSGTDYTLTISSLQPDDFATYYCQ

Owl Monkey amino acid sequence 4 (SEQ ID No:45)

DIQMTQTPSSLPASVGDKVTITCRASQGISSWLAWYQQKPGKAPKLLIHKASNLETGVPSRFS
GSGSGTDFTLTISSLQPEDIATYYCQ

Owl Monkey amino acid sequence 5 (SEQ ID No:46)

DIQMTQSPSSLTASVGDKVTITCRASQGISNNLAWYQQKPGKAPKPLIYYASSLQSGVPSRFS
GSGSGADYTLTTSSLQPEDFATYYCQ

Owl Monkey amino acid sequence 6 (SEQ ID No:47)

DNQMIQSPSSLTASVGDRVTITCRASQSISSWLAWYQQKPGTVPKPLIYDASKLLSGVPSRFS
GCGSGTDFTLTISSLQPEDFATYYCQ

FIGURE 3

```
          D  T  Q  M  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T  I  T  C  R  A  S  Q

1         GACATCCAGATGACCCAGTCTCCATCCTCTCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCA   80

1         CTGTAGGTCTACTGGGTCAGAGGTAGGAGAGACAGACGTAGACATCCTCTGGCACAGTGGTAGTGAACGGCCCGTTCAGT   80

          S  I  D  S  Y  L  H  W  Y  Q  Q  K  P  G  K  A  P  K  L  L  I  Y  S  A  S  F

81        GACCATTGATAGTTATTTACATTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATAGTGCATCCGAGT   160

81        CTGGTAACTATCAATAAATGTAACCATGGTCGTCTTTGGTCCCTTTCGGGGATTCGACGACTAGATATCACGTAGGCTCA   160

                                        KpnI

          I  G  S  G  V  P  S  R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P

161       TGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCT   240

161       ACGTTTCACCCCAGGGTAGTGCAAAGTCACCGTCACCTAGACCCTGTCTAAAGTCAGAGTGGTAGTCGTCAGACGTTGGA   240

                                SanDI

          E  D  F  A  T  Y  Y  C  Q  Q  V  V  W  R  P  F  T  F  G  Q  G  T  K  V  E  I  K

241       GAAGATTTTGCTACGTACTACTGTCAACAGGTTGTCTGGCGTCCTTTTACGTTCGGCCAAGGGACCAAGGTGGAAATCAA   320

241       CTTCTAAAACGATGCATGATGACAGTTGTCCAACAGACCGCAGGAAAATGCAAGCCGGTTCCCTGGTTCCACCTTTAGTT   320

          R

321       ACGC   324

321       TGCG   324
```

FIGURE 4A

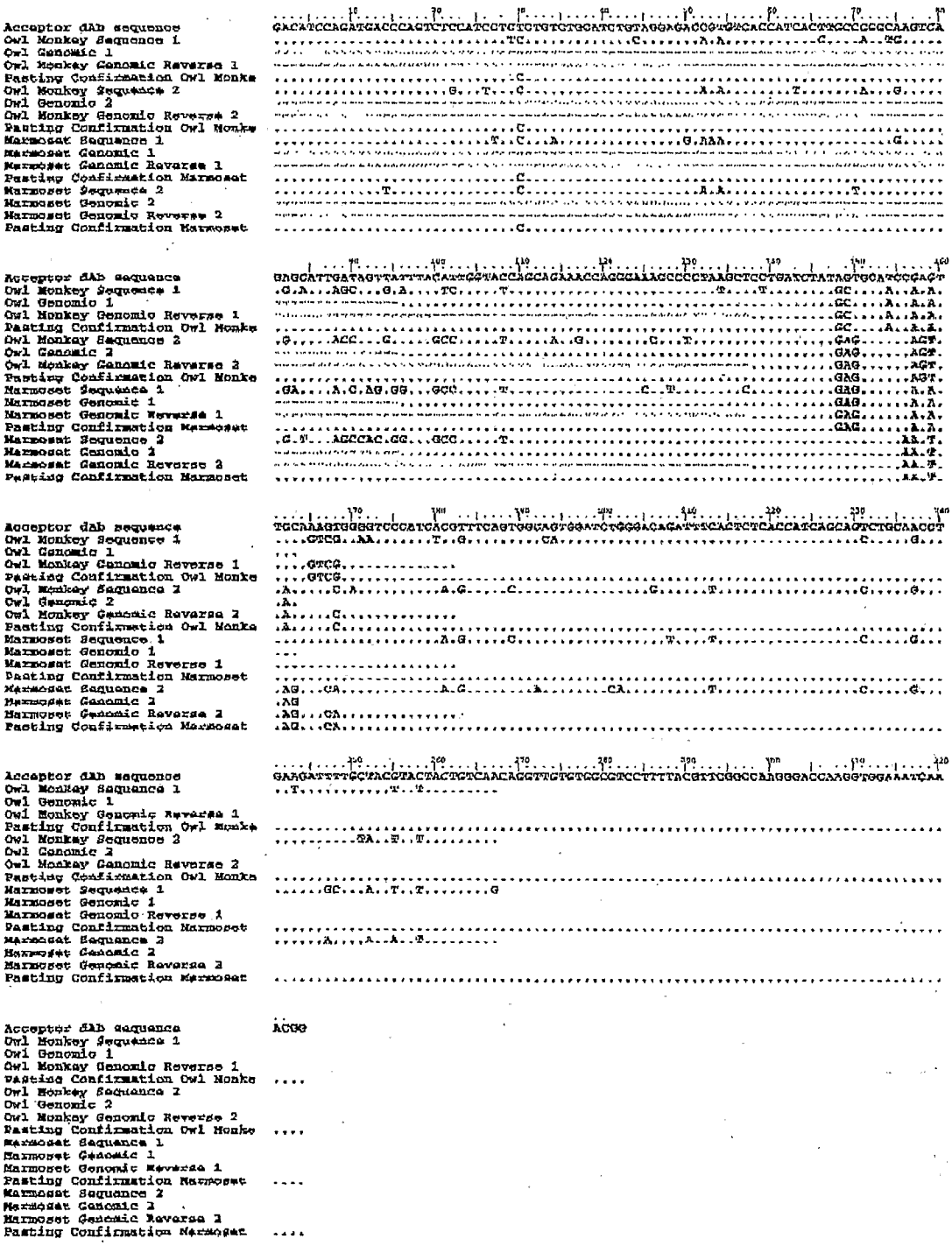

Acceptor dAb sequence
Owl Monkey Sequence 1
Owl Genomic 1
Owl Monkey Genomic Reverse 1
Pasting Confirmation Owl Monke
Owl Monkey Sequence 2
Owl Genomic 2
Owl Monkey Genomic Reverse 2
Pasting Confirmation Owl Monke
Marmoset Sequence 1
Marmoset Genomic 1
Marmoset Genomic Reverse 1
Pasting Confirmation Marmoset
Marmoset Sequence 2
Marmoset Genomic 2
Marmoset Genomic Reverse 2
Pasting Confirmation Marmoset
GACATCCAGATGACCCAGTCTCCATCCTCTGTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGCGCAAGTCA
GAGGATTGATAGTTATTTACATTCGTACCAGCAGAAACCAGGCAAAGCCCCTAAGCTCCTGATCTATAGTGCATCCGAGT
TGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCT
GAAGATTTTGCTACGTACTACTGTCAACAGGTTGTGTGGCGTCCTTTTACGTTCGGCCAAGGGACCAAGGTGGAAATCAA
ACGG

FIGURE 4B

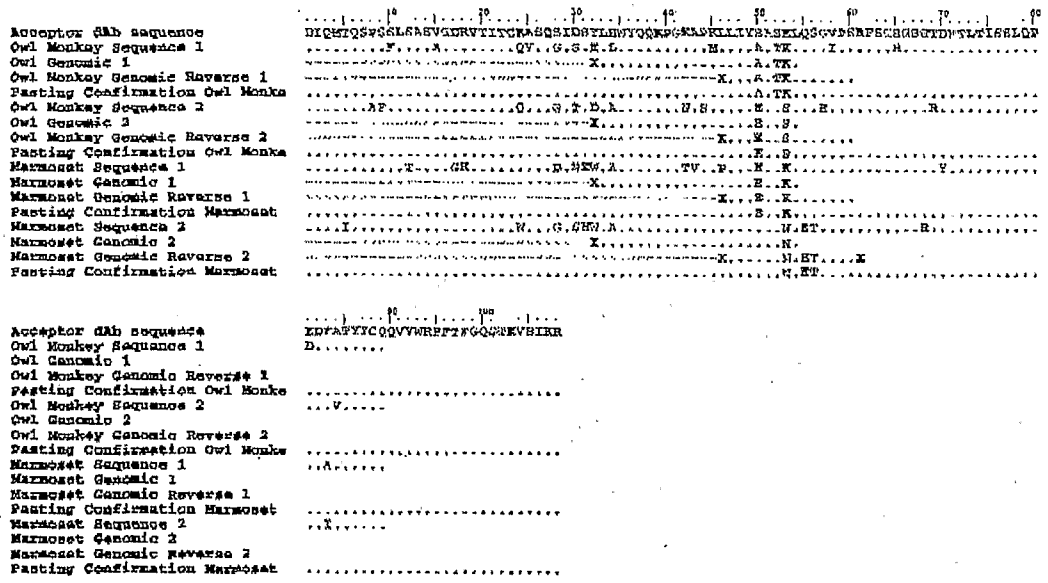

Acceptor dAb sequence
Owl Monkey Sequence 1
Owl Genomic 1
Owl Monkey Genomic Reverse 1
Pasting Confirmation Owl Monke
Owl Monkey Sequence 2
Owl Genomic 2
Owl Monkey Genomic Reverse 2
Pasting Confirmation Owl Monke
Marmoset Sequence 1
Marmoset Genomic 1
Marmoset Genomic Reverse 1
Pasting Confirmation Marmoset
Marmoset Sequence 2
Marmoset Genomic 2
Marmoset Genomic Reverse 2
Pasting Confirmation Marmoset

FIGURE 5

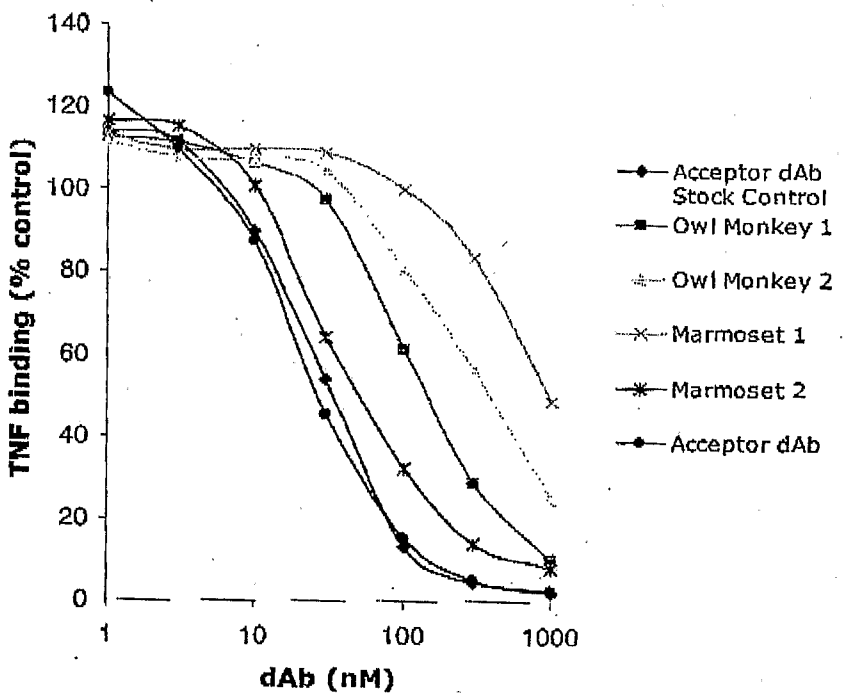
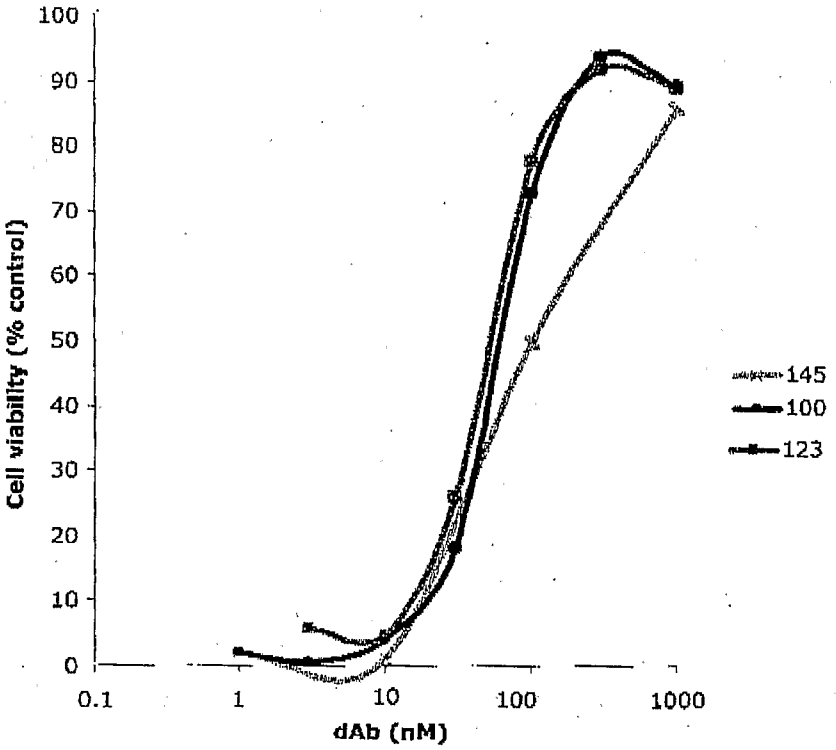

140
120
100
80
60
40
20
0
TNF binding (% control)
1
10
100
1000
dAb (nM)
Acceptor dAb Stock Control
Owl Monkey 1
Owl Monkey 2
Marmoset 1
Marmoset 2
Acceptor dAb

FIGURE 6


100
90
80
70
60
50
40
30
20
10
0
Cell viability (% control)
0.1
1
10
100
1000
dAb (nM)
145
100
123

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/001940

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C07K 16/24 (2006.01) ***A61K 39/395*** (2006.01) ***A61P 29/00*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN Subsequence Search based on SEQ ID Nos 1-4; Files Medline, CA, WPIDS: Keywords: antibody, immunoglobulin, complementarity determining region, marmoset, monkey, Aotus, Callithrix and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/0271663 A1 (IGNATOVICH O et al) 8 December 2005 See especially Fig 23, Sequence TAR1-5-490	1-12
X	US 2005/0118643 A1 (BURGESS T et al) 2 June 2005 See especially SEQ ID No. 177, pages 94-95	1, 2, 5-8, 11-12
X	WO 2003/085089 A2 (Schering Corporation et al) 16 October 2003 See especially Table 3, page 26	1, 2, 5-8, 11-12

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 March 2007	15 MAR 2007
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **R.L. POOLEY** Telephone No : (02) 6283 2242

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/001940

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOLT L. J et al, "Domain antibodies: proteins for therapy", Trends in Biotechnology, Vol. 21, No. 11, November 2003, p 484-490	
A	QIN W et al, "A novel domain antibody rationally designed against TNF-α using variable region of human chain antibody as scaffolds to display antagonistic peptides", Molecular Immunology, Vol 44(9), 2007, p 2355-2361	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2006/001940

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	2005/0271663	AU	2005259006	CA	2571536	CN	1745102
		DE	60305919	EP	1639011	JP	2006512895
		WO	2006/003388				
US	2005/0118643	AU	2004265595	CA	2532027	EP	1648998
		KR	20060054321	NO	20060600	WO	2005/017107
WO	2003/085089	AU	2003220525	CA	2479927	CN	1656122
		EP	1527100	JP	2005530490	US	7141653
		US	2007/025994				

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX