BIOLOGICAL PRODUCTS

The present invention relates to an antibody molecule having specificity for antigenic determinants of the B lymphocyte antigen, CD22. The present invention also relates to the therapeutic uses of the antibody molecule and methods for producing the antibody molecule.

In a natural antibody molecule, there are two heavy chains and two light chains. Each heavy chain and each light chain has at its N-terminal end a variable domain. Each variable domain is composed of four framework regions (FRs) alternating with three complementarity determining regions (CDRs). The residues in the variable domains are conventionally numbered according to a system devised by Kabat *et al.* This system is set forth in Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat *et al.* (*supra*)"). This numbering system is used in the present specification except where otherwise indicated.

The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering.

Construction of CDR-grafted antibodies is described in European Patent Application EP-A-0239400, which discloses a process in which the CDRs of a mouse monoclonal antibody are grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The

CDRs determine the antigen binding specificity of antibodies and are relatively short peptide sequences carried on the framework regions of the variable domains.

The earliest work on humanising monoclonal antibodies by CDR-grafting was carried out on monoclonal antibodies recognising synthetic antigens, such as NP. However, examples in which a mouse monoclonal antibody recognising lysozyme and a rat monoclonal antibody recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen *et al.* (Science, 239, 1534-1536, 1988) and Riechmann *et al.* (Nature, 332, 323-324, 1988), respectively.

Riechmann *et al.*, found that the transfer of the CDRs alone (as defined by Kabat (Kabat *et al.* (*supra*) and Wu *et al.*, J. Exp. Med., 132, 211-250, 1970)) was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. It was found that a number of framework residues have to be altered so that they correspond to those of the donor framework region. Proposed criteria for selecting which framework residues need to be altered are described in International Patent Application No. WO 90/07861.

A number of reviews discussing CDR-grafted antibodies have been published, including Vaughan *et al.* (Nature Biotechnology, 16, 535-539, 1998).

Malignant lymphomas are a diverse group of neoplasms. The majority of cases occur in older people. Non-Hodgkins Lymphoma (NHL) is a disease that currently affects 200,000 to 250,000 patients in the U.S. It is the second fastest rising cancer in the U.S., rising at a rate of about 55,000 new cases per year. The incidence is rising at a rate that is greater than can be accounted for simply by the increasing age of the population and exposure to known risk factors.

The classification of lymphoma is complex, and has evolved in recent decades. In 1994 the Revised European-American Lymphoma (REAL) classification was introduced. This classification organises lymphomas of B cell (the most frequently identified), T cell and unclassifiable origin into agreed subtypes. In everyday practice, the grouping of NHLs into low, intermediate and high-grade categories on the basis of their general histological appearance, broadly reflects their clinical behaviour.

NHL predominantly affects the lymph nodes but, in individual patients, the tumour may involve other anatomical sites such as the liver, spleen, bone marrow, lung, gut and skin. The disease commonly presents as a painless enlargement of lymph nodes.

Extranodal lymphoma most frequently affects the gut, although primary lymphoma of virtually every organ has been documented. Systemic symptoms include fever, sweats, tiredness and weight loss.

Until recently, the Ann Arbor staging system, based entirely upon the anatomical extent of disease, was the major determinant of therapy in NHL. This information may be refined by incorporating additional prognostic pointers, including age, serum lactate dehydrogenase levels and performance status. Even so, knowledge of the Ann Arbor staging system, together with the histological and immunological subtype of the tumour, is still the major determinant of treatment.

Low grade NHL has an indolent course, with a median patient survival of 8 to 10 years. Survival is little impacted by currently available therapy, although irradiation of local disease and chemotherapy for systemic symptoms improves patients' quality of life. Combination chemotherapy may be reserved for relapsed disease. Intermediate disease and, especially, high grade disease is extremely aggressive and tends to disseminate. Disease of this grade requires urgent treatment. Radiotherapy may be a useful component of treatment in patients with very bulky disease. Many different chemotherapy regimens have been employed, and long-term disease-free survival may be obtained in more than half of patients. High dose therapy with stem cell support was introduced initially for patients with relapsed or refractory disease, but is now increasingly finding a place in first line therapy for patients with poor-risk disease. The tendency in recent years for an increasingly aggressive therapeutic approach must be balanced against the generally elderly age and relative debility of many patients with NHL, and by the need to match the toxicity of treatment to the individual prognosis of each patient's disease.

Improved treatments, that are more effective and better tolerated, are needed. Agents recently introduced include new cytotoxic drugs, progressively incorporated into combinations, and the introduction of antibody-based therapies.

Non-Hodgkin's lymphoma encompasses a range of B cell lymphomas. B cell antigens therefore represent suitable targets for antibody therapy.

CD22 is a 135 kDa membrane glycoprotein belonging to a family of sialic acid binding proteins called sialoadhesins. It is detected in the cytoplasm early in B cell development, appears on the cell surface simultaneously with IgD and is found on most mature B cells. Expression is increased following B cell activation. CD22 is lost with

terminal differentiation and is generally reported as being absent on plasma cells. Thus this internalising antigen is present on the surface of pre-B cells and mature B cells but not stem cells or plasma cells.

Two isoforms of CD22 exist in man. The predominant form (CD22β) contains 7 immunoglobulin-like (Ig-like) domains in the extracellular region. The CD22α variant lacks Ig-like domain 4 and may have a truncated cytoplasmic domain. Antibodies which block CD22 adhesion to monocytes, neutrophils, lymphocytes and erythrocytes have been shown to bind within the first or second Ig-like domain.

The cytoplasmic domain of CD22 is tyrosine phosphorylated upon ligation of the B cell antigen receptor and associates with Lyk, Syk and phosphatidyl inositol 3-kinase. The function of CD22 is to down-modulate the B cell activation threshold. It can also mediate cell adhesion through interaction with cells bearing the appropriate sialoglycoconjugates.

CD22 is expressed in most B cell leukaemias and lymphomas, including NHL, acute lymphoblastic leukaemia (B-ALL), chronic lymphocytic leukaemia (B-CLL) and especially acute non-lymphocytic leukaemia (ANLL).

Monoclonal antibodies against CD22 have been described in the prior art. WO 98/41641 describes recombinant anti-CD22 antibodies with cysteine residues at V_H44 and V_L100. WO 96/04925 describes the V_H and V_L regions of the anti-CD22 antibody LL2. US 5686072 describes combinations of anti-CD22 and anti-CD19 immunotoxins. WO 98/42378 describes the use of naked anti-CD22 antibodies for the treatment of B-cell malignancies.

A number of antibody-based therapeutics have either been recently licensed, eg. Rituxan (an unlabelled chimeric human γ1 (+mγ1V-region) specific for CD20), or are in clinical trials for this disease. These rely either on complement- or ADCC- mediated killing of B cells or the use of radionuclides, such as ^{131}I or ^{90}Y, which have associated preparation and use problems for clinicians and patients. There is a need for an antibody molecule to treat NHL which can be used repeatedly and produced easily and efficiently. There is also a need for an antibody molecule, which has high affinity for CD22 and low immunogenicity in humans.

Summary of the invention

In a first aspect, the present invention provides an antibody molecule having specificity for human CD22, comprising a heavy chain wherein the variable domain comprises a CDR (as defined by Kabat *et al.*, (*supra*)) having the sequence given as H1 in Figure 1 (SEQ ID NO:1) for CDR-H1, as H2 in Figure 1 (SEQ ID NO:2) or an H2 from which a potential glycosylation site has been removed, or an H2 in which the lysine residue at position 60 (according to the Kabat numbering system) has been replaced by an alternative amino acid, or an H2 in which both the glycosylation site and the reactive lysine at position 60 have been removed for CDR-H2 or as H3 in Figure 1 (SEQ ID NO:3) for CDR-H3.

The antibody molecule of the first aspect of the present invention comprises at least one CDR selected from H1, H2 and H3 (SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3) for the heavy chain variable domain. Preferably, the antibody molecule comprises at least two and more preferably all three CDRs in the heavy chain variable domain.

In a second aspect of the present invention, there is provided an antibody molecule having specificity for human CD22, comprising a light chain wherein the variable domain comprises a CDR (as defined by Kabat *et al.*, (*supra*)) having the sequence given as L1 in Figure 1 (SEQ ID NO:4) for CDR-L1, L2 in Figure 1 (SEQ ID NO:5) for CDR-L2 or L3 in Figure 1 (SEQ ID NO:6) for CDR-L3.

The antibody molecule of the second aspect of the present invention comprises at least one CDR selected from L1, L2 and L3 (SEQ ID NO:4; SEQ ID NO:5 and SEQ ID NO:6) for the light chain variable domain. Preferably, the antibody molecule comprises at least two and more preferably all three CDRs in the light chain variable domain.

The antibody molecules of the first and second aspects of the present invention preferably have a complementary light chain or a complementary heavy chain, respectively.

Preferably, the antibody molecule of the first or second aspect of the present invention comprises a heavy chain wherein the variable domain comprises a CDR (as defined by Kabat *et al.*, (*supra*)) having the sequence given as H1 in Figure 1 (SEQ ID NO:1) for CDR-H1, as H2 in Figure 1 (SEQ ID NO:2) or an H2 from which a potential glycosylation site has been removed, or an H2 in which the lysine residue at position 60

(according to the Kabat numbering system) has been replaced by an alternative amino acid, or an H2 in which both the glycosylation site and the reactive lysine at position 60 have been removed for CDR-H2 or as H3 in Figure 1 (SEQ ID NO:3) for CDR-H3 and a light chain wherein the variable domain comprises a CDR (as defined by Kabat *et al.*, (*supra*)) having the sequence given as L1 in Figure 1 (SEQ ID NO:4) for CDR-L1, as L2 in Figure 1 (SEQ ID NO:5) for CDR-L2 or as L3 in Figure 1 (SEQ ID NO:6) for CDR-L3.

The CDRs given in SEQ IDS NOS:1 to 6 and in Figure 1 referred to above are derived from a mouse monoclonal antibody 5/44.

The complete sequences of the variable domains of the mouse 5/44 antibody are shown in Figure 2 (light chain) (SEQ ID NO:7) and Figure 3 (heavy chain) (SEQ ID NO:8). This mouse antibody is also referred to below as "the donor antibody" or the "murine monoclonal antibody".

A first alternatively preferred embodiment of the first or second aspect of the present invention is the mouse monoclonal antibody 5/44 having the light and heavy chain variable domain sequences shown in Figure 2 (SEQ ID NO:7) and Figure 3 (SEQ ID NO:8), respectively. The light chain constant region of 5/44 is kappa and the heavy chain constant region is IgG1.

In a second alternatively preferred embodiment, the antibody according to either of the first and second aspects of the present invention is a chimeric mouse/human antibody molecule, referred to herein as the chimeric 5/44 antibody molecule. The chimeric antibody molecule comprises the variable domains of the mouse monoclonal antibody 5/44 (SEQ ID NOS:7 and 8) and human constant domains. Preferably, the chimeric 5/44 antibody molecule comprises the human C kappa domain (Hieter *et al.*, Cell, 22, 197-207, 1980; Genebank accession number J00241) in the light chain and the human gamma 4 domains (Flanagan *et al.*, Nature, 300, 709-713, 1982) in the heavy chain, optionally with the serine residue at position 241 replaced by a proline residue.

Preferably, the antibody of the present invention comprises a heavy chain wherein the variable domain comprises as CDR-H2 (as defined by Kabat *et al.*, (*supra*)) an H2' in which a potential glycosylation site sequence has been removed and which unexpectedly increased the affinity of the chimeric 5/44 antibody for the CD22 antigen and which preferably has as CDR-H2 the sequence given as H2' (SEQ ID NO:13).

Alternatively or additionally, the antibody of the present invention may comprise a heavy chain wherein the variable domain comprises as CDR-H2 (as defined by Kabat et al., (supra)) an H2'' in which a lysine residue at position 60, which is located at an exposed position within CDR-H2 and which is considered to have the potential to react with conjugation agents resulting in a reduction of antigen binding affinity, is substituted for an alternative amino acid to result in a conserved substitution. Preferably CDR-H2 has the sequence given as H2'' (SEQ ID NO:15).

Alternatively or additionally, the antibody of the present invention may comprise a heavy chain wherein the variable domain comprises as CDR-H2 (as defined by Kabat et al., (supra)) an H2''' in which both the potential glycosylation site sequence and the lysine residue at position 60, are substituted for alternative amino acids. Preferably CDR-H2 has the sequence given as H2''' (SEQ ID NO:16).

In a third alternatively preferred embodiment, the antibody according to either of the first and second aspects of the present invention is a CDR-grafted antibody molecule. The term "a CDR-grafted antibody molecule" as used herein refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, a modified CDR) from a donor antibody (e.g. a murine monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody).

Preferably, such a CDR-grafted antibody has a variable domain comprising human acceptor framework regions as well as one or more of the donor CDRs referred to above.

When the CDRs are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al. (supra)*). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used. The preferred framework region for the light chain is the human germline sub-group sequence (DPK9+JK1) shown in Figure 5 (SEQ ID NO:17). The preferred framework region for the heavy chain is the human sub-group sequence (DP7+JH4) shown in Figure 6 (SEQ ID NO:21).

In a CDR-grafted antibody of the present invention, it is preferred to use as the acceptor antibody one having chains which are homologous to the chains of the donor antibody. The acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

Also, in a CDR-grafted antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody or to a residue that is a conservative substitution for the residue found at the same position in the donor antibody. Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967.

Preferably, in a CDR-grafted antibody molecule according to the present invention, if the acceptor light chain has the human sub-group DPK9+JK1 sequence (shown in Figure 2) (SEQ ID NO:17) then the acceptor framework regions of the light chain comprise donor residues at positions 2, 4, 37, 38, 45 and 60 and may additionally comprise a donor residue at position 3 (according to Kabat *et al.* (*supra*)).

Preferably, in a CDR-grafted antibody molecule of the present invention, if the acceptor heavy chain has the human DP7+JH4 sequence (shown in Figure 3) (SEQ ID NO:21), then the acceptor framework regions of the heavy chain comprise, in addition to one or more donor CDRs, donor residues at positions 1, 28, 48, 71 and 93 and may additionally comprise donor residues at positions 67 and 69 (according to Kabat *et al.* *(supra)*).

Donor residues are residues from the donor antibody, i.e. the antibody from which the CDRs were originally derived.

Preferably, the antibody of the present invention comprises a heavy chain wherein the variable domain comprises as CDR-H2 (as defined by Kabat *et al.*, (*supra*)) an H2' in which a potential glycosylation site sequence has been removed in order to increase the

affinity of the chimeric 5/44 antibody for the CD22 antigen and which preferably has as CDR-H2 the sequence given as H2' (SEQ ID NO:13).

Alternatively or additionally, the antibody of the present invention may comprise a heavy chain wherein the variable domain comprises as CDR-H2 (as defined by Kabat et al., (supra)) an H2'' in which a lysine residue at position 60, which is located at an exposed position within CDR-H2 and which is considered to have the potential to react with conjugation agents resulting in a reduction of antigen binding affinity, is substituted for an alternative amino acid. Preferably CDR-H2 has the sequence given as H2'' (SEQ ID NO:15).

Alternatively or additionally, the antibody of the present invention may comprise a heavy chain wherein the variable domain comprises as CDR-H2 (as defined by Kabat et al., (supra)) an H2''' in which both the potential glycosylation site sequence and the lysine residue at position 60, are substituted for alternative amino acids. Preferably CDR-H2 has the sequence given as H2''' (SEQ ID NO:16).

The antibody molecule of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, modified Fab, Fab', $F(ab')_2$ or Fv fragment; a light chain or heavy chain monomer or dimer; a single chain antibody, e.g. a single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker. Similarly, the heavy and light chain variable regions may be combined with other antibody domains as appropriate.

The antibody molecule of the present invention may have an effector or a reporter molecule attached to it. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, procedures of recombinant DNA technology may be used to produce an antibody molecule in which the Fc fragment (CH2, CH3 and hinge domains), the CH2 and CH3 domains or the CH3 domain of a complete immunoglobulin molecule has (have) been replaced by, or has (have) attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

The antibody molecule of the present invention preferably has a binding affinity of at least 0.85×10^{-10} M, more preferably at least 0.75×10^{-10} M and most preferably at least 0.5×10^{-10} M.

Preferably, the antibody molecule of the present invention comprises the light chain variable domain 5/44-gL1 (SEQ ID NO:19) and the heavy chain variable domain 5/44-gH7 (SEQ ID NO:27). The sequences of the variable domains of these light and heavy chains are shown in Figures 5 and 6, respectively.

The present invention also relates to variants of the antibody molecule of the present invention, which have an improved affinity for CD22. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang *et al.*, J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks *et al.*, Bio/Technology, 10, 779-783, 1992), use of mutator strains of *E. coli* (Low *et al.*, J. Mol. Biol., 250, 359-368, 1996), DNA shuffling (Patten *et al.*, Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson *et al.*, J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Crameri *et al.*, Nature, 391, 288-291, 1998). Vaughan *et al.* (*supra*) discusses these methods of affinity maturation.

The present invention also provides a DNA sequence encoding the heavy and/or light chain(s) of the antibody molecule of the present invention.

Preferably, the DNA sequence encodes the heavy or the light chain of the antibody molecule of the present invention.

The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences of the present invention. Preferably, the cloning or expression vector comprises two DNA sequences, encoding the light chain and the heavy chain of the antibody molecule of the present invention, respectively.

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

DNA sequences which encode the antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA

sequences coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

DNA coding for acceptor framework sequences is widely available to those skilled in the art and can be readily synthesised on the basis of their known amino acid sequences.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody molecule of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used, in part, for expression of antibody fragments such as Fab and $F(ab')_2$ fragments, and especially Fv fragments and single chain antibody fragments, for example, single chain Fvs. Eukaryotic, e.g. mammalian, host cell expression systems may be used for production of larger antibody molecules, including complete antibody molecules. Suitable mammalian host cells include CHO, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell containing a vector of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

The present invention also provides a therapeutic or diagnostic composition comprising an antibody molecule of the present invention in combination with a pharmaceutically acceptable excipient, diluent or carrier.

The present invention also provides a process for preparation of a therapeutic or diagnostic composition comprising admixing the antibody molecule of the present invention together with a pharmaceutically acceptable excipient, diluent or carrier.

The antibody molecule may be the sole active ingredient in the therapeutic or diagnostic composition or may be accompanied by other active ingredients including other antibody ingredients, for example anti-T cell, anti-IFNγ or anti-LPS antibodies, or non-antibody ingredients such as xanthines.

The pharmaceutical compositions preferably comprise a therapeutically effective amount of the antibody of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any antibody, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, an effective dose will be from 0.01 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 20 mg/kg, more preferably about 15 mg/kg.

Compositions may be administered individually to a patient or may be administered in combination with other agents, drugs or hormones.

The dose at which the antibody molecule of the present invention is administered depends on the nature of the condition to be treated, the grade of the malignant lymphoma

or leukaemia and on whether the antibody molecule is being used prophylactically or to treat an existing condition.

The frequency of dose will depend on the half-life of the antibody molecule and the duration of its effect. If the antibody molecule has a short half-life (e.g. 2 to 10 hours) it may be necessary to give one or more doses per day. Alternatively, if the antibody molecule has a long half life (e.g. 2 to 15 days) it may only be necessary to give a dosage once per day, once per week or even once every 1 or 2 months.

A pharmaceutical composition may also contain a pharmaceutically acceptable carrier for administration of the antibody. The carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Preferred forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, it is preferred that the compositions are adapted for administration to human subjects.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

It is also envisaged that the antibody of the present invention will be administered by use of gene therapy. In order to achieve this, DNA sequences encoding the heavy and light chains of the antibody molecule under the control of appropriate DNA components are introduced into a patient such that the antibody chains are expressed from the DNA sequences and assembled *in situ*.

The present invention also provides the antibody molecule of the present invention for use in treating a disease mediated by cells expressing CD22.

The present invention further provides the use of the antibody molecule according to the present invention in the manufacture of a medicament for the treatment of a disease mediated by cells expressing CD22.

The antibody molecule of the present invention may be utilised in any therapy where it is desired to reduce the level of cells expressing CD22 that are present in the human or animal body. These CD22-expressing cells may be circulating in the body or be present in an undesirably high level localised at a particular site in the body. For example, elevated levels of cells expressing CD22 will be present in B cell lymphomas and leukaemias. The antibody molecule of the present invention may be utilised in the therapy of diseases mediated by cells expressing CD22.

The antibody molecule of the present invention is preferably used for treatment of malignant lymphomas and leukaemias, most preferably NHL.

The present invention also provides a method of treating human or animal subjects suffering from or at risk of a disorder mediated by cells expressing CD22, the method comprising administering to the subject an effective amount of the antibody molecule of the present invention.

The antibody molecule of the present invention may also be used in diagnosis, for example in the *in vivo* diagnosis and imaging of disease states involving cells that express CD22.

The present invention is further described by way of illustration only in the following examples, which refer to the accompanying Figures, in which:

Figure 1 shows the amino acid sequence of the CDRs of mouse monoclonal antibody 5/44 (SEQ ID NOS:1 to 6);

Figure 2 shows the complete sequence of the light chain variable domain of mouse monoclonal antibody 5/44;

Figure 3 shows the complete sequence of the heavy chain variable domain of mouse monoclonal antibody 5/44;

Figure 4 shows the strategy for removal of the glycosylation site and reactive lysine in CDR-H2;

Figure 5 shows the graft design for the 5/44 light chain sequence;

Figure 6 shows the graft design for the 5/44 heavy chain sequence;

Figure 7 shows the vectors pMRR14 and pMRR10.1;

Figure 8 shows the Biacore assay results of the chimeric 5/44 mutants;

Figure 9 shows the oligonucleotides for 5/44 gH1 and gL1 gene assemblies;

Figure 10 shows the intermediate vectors pCR2.1(544gH1) and pCR2.1(544gL1);

Figure 11 shows the oligonucleotide cassettes used to make further grafts;

Figure 12 shows the competition assay between fluorescently labelled mouse 5/44 antibody and grafted variants; and

Figure 13 shows the full DNA and protein sequence of the grafted heavy and light chains.

Detailed description of the invention

Example 1: Generation of Candidate Antibodies

A panel of antibodies against CD22 were selected from hybridomas using the following selection criteria: binding to Daudi cells, internalisation on Daudi cells, binding to peripheral blood mononuclear cells (PBMC), internalisation on PBMC, affinity (greater than 10^{-9}M), mouse γ1 and production rate. 5/44 was selected as the preferred antibody.

Example 2: Gene Cloning and Expression of a Chimeric 5/44 Antibody Molecule

Preparation of 5/44 Hybridoma Cells and RNA Preparation therefrom

Hybridoma 5/44 was generated by conventional hybridoma technology following immunisation of mice with human CD22 protein. RNA was prepared from 5/44 hybridoma cells using a RNEasy kit (Qiagen, Crawley, UK; Catalogue No. 74106). The RNA obtained was reverse transcribed to cDNA, as described below.

Distribution of CD22 on NHL tumours

An immunohistochemistry study was undertaken to examine the incidence and distribution of staining using the 5/44 anti-CD22 monoclonal antibodies. Control anti-CD20 and anti-CD79a antibodies were included in the study to confirm B cell areas of tumours.

A total of 50 tumours were studied and these were categorised as follows by using the Working Formulation and REAL classification systems:

- 7 B lymphoblastic leukaemia/lymphoma (High/I)
- 4 B-CLL/small lymphocytic lymphoma (Low/A)
- 3 lymphoplasmacytoid/Immunocytoma (Low/A)
- 1 Mantle cell (Int/F)
- 14 Follicle center lymphoma (Low to Int/D)
- 13 Diffuse large cell lymphoma (Int to High/G,H)
- 6 Unclassifiable (K)
- 2 T cell lymphomas

40 B cell lymphomas were positive for CD22 antigen with the 5/44 antibody at 0.1 μg/ml and a further 6 became positive when the concentration was increased to 0.5 μg/ml. For the remaining 2 B cell tumours that were negative at 0.1 μg/ml, there was insufficient tissue remaining to test at the higher concentration. However, parallel testing with another Celltech anti-CD22 antibody 6/13, which gave stronger staining than 5/44, resulted in all 48 B cell lymphomas staining positive for CD22.

Thus, it is possible to conclude that the CD22 antigen is widely expressed on B cell lymphomas and thus provides a suitable target for immunotherapy in NHL.

PCR Cloning of 5/44 V_H and V_L

cDNA sequences coding for the variable domains of 5/44 heavy and light chains were synthesised using reverse transcriptase to produce single stranded cDNA copies of the mRNA present in the total RNA. This was then used as the template for amplification of the murine V-region sequences using specific oligonucleotide primers by the Polymerase Chain Reaction (PCR).

a) cDNA Synthesis

cDNA was synthesised in a 20 µl reaction volume containing the following reagents: 50mM Tris-HCl pH 8.3, 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$, 0.5 mM each deoxyribonucleoside triphosphate, 20 units RNAsin, 75 ng random hexanucleotide primer, 2 µg 5/44 RNA and 200 units Moloney Murine Leukemia Virus reverse transcriptase. After incubation at 42°C for 60 minutes, the reaction was terminated by heating at 95°C for 5 minutes.

b) PCR

Aliquots of the cDNA were subjected to PCR using combinations of primers specific for the heavy and light chains. Degenerate primer pools designed to anneal with the conserved sequences of the signal peptide were used as forward primers. These sequences all contain, in order, a restriction site (V_L SfuI; V_H HindIII) starting 7 nucleotides from their 5' ends, the sequence GCCGCCACC (SEQ ID NO:50), to allow optimal translation of the resulting mRNAs, an initiation codon and 20-30 nucleotides based on the leader peptide sequences of known mouse antibodies (Kabat *et al.*, Sequences of proteins of immunological interest, 5th Edition, 1991, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health).

The 3' primers are designed to span the framework 4 J-C junction of the antibody and contain a restriction site for the enzyme BsiWI to facilitate cloning of the V_L PCR fragment. The heavy chain 3' primers are a mixture designed to span the J-C junction of the antibody. The 3' primer includes an ApaI restriction site to facilitate cloning. The 3' region of the primers contains a mixed sequence based on those found in known mouse antibodies (Kabat *et al.*, 1991, *supra*).

The combinations of primers described above enable the PCR products for V_H and V1 to be cloned directly into an appropriate expression vector (see below) to produce

chimeric (mouse-human) heavy and light chains and for these genes to be expressed in mammalian cells to produce chimeric antibodies of the desired isotype.

Incubations (100 µl) for the PCR were set up as follows. Each reaction contained 10 mM Tris-HCl pH 8.3, 1.5 mM MgC12, 50 mM KC1, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 10 pmoles 5' primer mix, 10 pmoles 3' primer, 1 µl cDNA and 1 unit Taq polymerase. Reactions were incubated at 95°C for 5 minutes and then cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, aliquots of each reaction were analysed by electrophoresis on an agarose gel.

For the heavy chain V-region, an amplified DNA product was only obtained when a primer pool annealing within the start of framework I replaced the signal peptide primer pool. The fragments were cloned into DNA sequencing vectors. The DNA sequence was determined and translated to give a deduced amino acid sequence. This deduced sequence was verified by reference to the N-terminal protein sequence determined experimentally. Figures 2 and 3 shows the DNA/protein sequence of the mature light and heavy chain V-regions of mouse monoclonal 5/44 respectively.

c) Molecular Cloning of the PCR Fragments

The murine v-region sequences were then cloned into the expression vectors pMRR10.1 and pMRR14 (Figure 7). These are vectors for the expression of light and heavy chain respectively containing DNA encoding constant regions of human kappa light chain and human gamma-4 heavy chain. The V_L region was sub-cloned into the expression vector by restriction digest and ligation from the sequencing vector, using SfuI and BsiWI restriction sites, creating plasmid pMRR10(544cL). The heavy chain DNA was amplified by PCR using a 5' primer to introduce a signal peptide, since this was not obtained in the cloning strategy – a mouse heavy chain antibody leader from a different in-house hybridoma (termed 162) was employed. The 5' primer had the following sequence:

$^{5'}$GCGCGCAAGCTTGCCGCCACCATGGACTTCGGATTCTCTCTCGTGTTCCTGGC ACTCATTCTCAAGGGAGTGCAGTGTGAGGTGCAGCTCGTCGAGTCTGG$^{3'}$ (SEQ ID NO:51).

The reverse primer was identical to that used in the original V_H gene cloning. The resultant PCR product was digested with enzymes HindIII and ApaI, was sub-cloned, and its DNA sequence was confirmed, creating plasmid pMRR14(544cH). Transient co-

transfection of both expression vectors into CHO cells generated chimeric c5/44 antibody. This was achieved using the Lipofectamine reagent according to the manufacturer's protocols (InVitrogen:Life Technology, Groningen, The Netherlands. Catalogue no. 11668-027).

Removal of Glycosylation Site and Reactive Lysine

A potential N-linked glycosylation site sequence was observed in CDR-H2, having the amino acid sequence N-Y-T (Figure 3). SDS-PAGE, Western blotting and carbohydrate staining of gels of 5/44 and its fragments (including Fab) indicated that this site was indeed glycosylated (not shown). In addition, a lysine residue was observed at an exposed position within CDR-H2, which had the potential to reduce the binding affinity of the antibody by providing an additional site for conjugation with an agent with which the antibody may be conjugated.

A PCR strategy was used to introduce amino acid substitutions into the CDR-H2 sequence in an attempt to remove the glycosylation site and/or the reactive lysine, as shown in Figure 4. Forward primers encoding the mutations N55Q, T57A or T57V were used to remove the glycosylation site (Figure 4) and a fourth forward primer containing the substitution K60R, was generated to remove the reactive lysine residue (Figure 4). A framework 4 reverse primer was used in each of these PCR amplifications. The PCR products were digested with the enzymes XbaI and ApaI and were inserted into pMRR14(544cH) (also cleaved with XbaI and ApaI) to generate expression plasmids encoding these mutants. The N55Q, T57A and T57V mutations ablate the glycosylation site by changing the amino acid sequence away from the consensus N-X-T/S whilst the K60R mutation replaces the potentially reactive lysine with the similarly positively charged residue arginine. The resultant cH variant plasmids were co-transfected with the cL plasmid to generate expressed chimeric antibody variants.

Evaluation of Activities of Chimeric Genes

The activities of the chimeric genes were evaluated following transient transfection into CHO cells .

c) Determination of Affinity constants by BiaCore analysis.

The affinities of chimeric 5/44 or its variants, which have had their glycosylation site or their reactive lysine removed, were investigated using BIA technology for binding to CD22-mFc constructs. The results are shown in Figure 8. All binding measurements were performed in the BIAcore™ 2000 instrument (Pharmacia Biosensor AB, Uppsala, Sweden). The assay was performed by capture of CD22mFc via the immobilised anti-mouse Fc. The antibody was in the soluble phase. Samples, standard, and controls (50ul) were injected over immobilised anti-mouse Fc followed by antibody in the soluble phase. After each cycle the surface was regenerated with 50ul of 40mM HCl at 30ul/min. The kinetic analysis was performed using the BIAevaluation 3.1 software (Pharmacia).

Removal of the glycosylation site in construct T57A resulted in a slightly faster on-rate and a significantly slower off-rate compared to the chimeric 5/44, giving an affinity improvement of approximately 5-fold. The N55Q mutation had no effect on affinity. This result was unexpected as it suggests that the removal of the carbohydrate itself apparently has no effect on binding (as with the N55Q change). The improved affinity was observed only with the T57A change. One possible explanation is that, regardless of the presence of carbohydrate, the threonine at position 57 exerts a negative effect on binding that is removed on conversion of threonine to alanine. The hypothesis that the small size of alanine is important, and that the negative effect of threonine is related to its size, is supported from the result obtained using the T57V mutation: that replacement with valine at position 57 is not beneficial (results not shown).

Removal of the lysine by the K60R mutation had a neutral effect on affinity, i.e. the introduction of arginine removes a potential reactive site without compromising affinity.

The mutations for removal of the glycosylation site and for removal of the reactive lysine were therefore both included in the humanisation design.

Example 2: CDR-Grafting of 5/44

The molecular cloning of genes for the variable regions of the heavy and light chains of the 5/44 antibody and their use to produce chimeric (mouse/human) 5/44 antibodies has been described above. The nucleotide and amino acid sequences of the mouse 5/44 V_L and V_H domains are shown in Figures 2 and 3 (SEQ ID NOS:7 and 8),

respectively. This example describes the CDR-grafting of the 5/44 antibody onto human frameworks to reduce potential immunogenicity in humans, according to the method of Adair et al., (WO91/09967).

CDR-Grafting of 5/44 Light Chain

Protein sequence alignment with consensus sequences from human sub-group I kappa light chain V region indicated 64% sequence identity. Consequently, for constructing the CDR-grafted light chain, the acceptor framework regions chosen corresponded to those of the human VK sub-group I germline O12,DPK9 sequence. The framework 4 acceptor sequence was derived from the human J-region germline sequence JK1.

A comparison of the amino acid sequences of the framework regions of murine 5/44 and the acceptor sequence is given in Figure 5 and shows that there are 27 differences between the donor and acceptor chains. At each position, an analysis was made of the potential of the murine residue to contribute to antigen binding, either directly or indirectly, through effects on packing or at the V_H/V_L interface. If a murine residue was considered important and sufficiently different from the human residue in terms of size, polarity or charge, then that murine residue was retained. Based on this analysis, two versions of the CDR-grafted light chain, having the sequences given in SEQ ID NO:19 and SEQ ID NO:20 (Figure 5), were constructed.

CDR-Grafting of 5/44 Heavy Chain

CDR-grafting of 5/44 heavy chain was accomplished using the same strategy as described for the light chain. The V-domain of 5/44 heavy chain was found to be homologous to human heavy chains belonging to sub-group I (70% sequence identity) and therefore the sequence of the human sub-group I germline framework VH1-3,DP7 was used as an acceptor framework. The framework 4 acceptor sequences were derived from human J-region germline sequence JH4.

A comparison of 5/44 heavy chain with the framework regions is shown in Figure 6 where it can be seen that the 5/44 heavy chain differs from the acceptor sequence at 22 positions. Analysis of the contribution that any of these might make to antigen binding led

to 5 versions of the CDR-grafted heavy chains being constructed, having the sequences given in SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO:27 (Figure 6).

Construction of genes for grafted sequences.

Genes were designed to encode the grafted sequences gH1 and gL1, and a series of overlapping oligonucleotides were designed and constructed (Figure 9). A PCR assembly technique was employed to construct the CDR-grafted V-region genes. Reaction volumes of 100 ul were set up containing 10 mM Tris-HCl pH8.3, 1.5 mM MgCl2, 50 mM KCl, 0.001 % gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 1 pmole each of the 'internal' primers (T1, T2, T3, B1, B2, B3), 10 pmole each of the 'external' primers (F1, R1), and 1 unit of Taq polymerase (AmpliTaq, Applied BioSystems, catalogue no. N808-0171). PCR cycle parameters were 94 °C for 1 minute, 55 °C for 1 minute and 72 °C for 1 minute, for 30 cycles. The reaction products were then run on a 1.5 % agarose gel, excised and recovered using QIAGEN spin columns (QIAquick gel extraction kit, cat no. 28706). The DNA was eluted in a volume of 30 µl. Aliquots (1 µl) of the gH1 and gL1 DNA were then cloned into the InVitrogen TOPO TA cloning vector pCR2.1 TOPO (catalogue no. K4500-01) according to the manufacturer's instructions. This non-expression vector served as a cloning intermediate to facilitate sequencing of a large number of clones. DNA sequencing using vector-specific primers was used to identify correct clones containing gH1 and gL1, creating plasmids pCR2.1 (544gH1) and pCR2.1(544gL1) (Figure 10).

An oligonucleotide cassette replacement method was used to create the humanised grafts gH4,5,6 and 7, and gL2. Figure 11 shows the design of the oligonucleotide cassettes. To construct each variant, the vector (pCR2.1(544gH1) or pCR2.1(544gL1)) was cut with the restriction enzymes shown (XmaI/SacII for the heavy chain, XmaI/BstEII for the light chain). The large vector fragment was gel purified from agarose and was used in ligation with the oligonucleotide cassette. These cassettes are composed of 2 complementary oligonucleotides (shown in Figure 11), mixed at a concentration of 0.5 pmoles/µl in a volume of 200 µl 12.5 mM Tris-HCl pH 7.5, 2.5 mM $MgCl_2$, 25 mM NaCl, 0.25 mM dithioerythritol. Annealing was achieved by heating to 95 °C for 3 minutes in a waterbath (volume 500 ml) then allowing the reaction to slow-cool to room temperature. The annealed oligonucleotide cassette was then diluted ten-fold in water before ligation

into the appropriately cut vector. DNA sequencing was used to confirm the correct sequence, creating plasmids pCR2.1 (5/44-gH4-7) and pCR2.1(5/44-gL2). The verified grafted sequences were then sub-cloned into the expression vectors pMRR14 (heavy chain) and pMR10.1 (light chain).

CD22 binding activity of CDR-grafted sequences

The vectors encoding grafted variants were co-transfected into CHO cells in a variety of combinations, together with the original chimeric antibody chains. Binding activity was compared in a competition assay, competing the binding of the original mouse 5/44 antibody for binding to Ramos cells (obtained from ATCC, a Burkitt's lymphoma lymphoblast human cell line expressing surface CD22). This assay was considered the best way to compare grafts in their ability to bind to cell surface CD22. The results are shown in Figure 8. As can be seen, there is very little difference between any of the grafts, all performing more effectively than the chimeric at competing against the murine parent. The introduction of the 3 additional human residues at the end of CDR-H3 (gH5 and gH7) does not appear to have affected binding.

The graft combination with the least number of murine residues was selected, gL1gH7. The light chain graft gL1 has 6 donor residues. Residues V2, V4, L37 and Q45 are potentially important packing residues. Residue H38 is at the V_H/V_L interface. Residue D60 is a surface residue close to the CDR-L2 and may directly contribute to antigen binding. Of these residues, V2, L37, Q45 and D60 are found in germline sequences of human kappa genes from other sub-groups. The heavy chain graft gH7 has 4 donor framework residues (Residue R28 is considered to be part of CDR-H1 under the structural definition used in CDR-grafting (se Adair et al (1991 WO91/09967)). Residues E1 and A71 are surface residues close to the CDR's. Residue I48 is a potential packing residue. Residue T93 is present at the V_H/V_L interface. Of these residues, E1 and A71 are found in other germline genes of human sub-group I. Residue I48 is found in human germline sub-group 4, and T73 is found in human germline sub-group 3.

The full DNA and protein sequence of both the light chain and heavy chain, including approximate position of introns within the constant region genes provided by the vectors, are shown in Figure 13 and are given in SEQ ID NO:29 and SEQ ID NO:28

respectively for the light chain and SEQ ID NO: 31 and SEQ ID NO:30 respectively for the heavy chain.

DNA encoding these light and heavy chain genes was excised from these vectors. Heavy chain DNA was digested at the 5' HindIII site, then was treated with the Klenow fragment of *E. coli* DNA polymerase I to create a 5' blunt end. Cleavage at the 3' EcoRI site resulted in the heavy chain fragment which was purified from agarose gels. In the same way, a light chain frament was produced, blunted at the 5' SfuI site and with a 3' EcoRI site. Both fragments were cloned into DHFR based expression vectors and used to generate stable cell lines in CHO cells.

CLAIMS

1. An antibody molecule having specificity for human CD22, comprising a heavy chain wherein the variable domain comprises a CDR having at least one of the sequences given as H1 in Figure 1 (SEQ ID NO:1) for CDR-H1, as H2 in Figure 1 (SEQ ID NO:2) or H2' (SEQ ID NO:13) or H2'' (SEQ ID NO:15) or H2''' (SEQ ID NO:16) for CDR-H2 or as H3 in Figure 1 (SEQ ID NO:3) for CDR-H3.

2. An antibody molecule having specificity for human CD22, comprising a light chain wherein the variable domain comprises a CDR having at least one of the sequences given as L1 in Figure 1 (SEQ ID NO:4) for CDR-L1, as L2 in Figure 1 (SEQ ID NO:5) for CDR-L2 or as L3 in Figure 1 (SEQ ID NO:6) for CDR-L3.

3. The antibody molecule of claim 1 or claim 2 comprising a heavy chain wherein the variable domain comprises a CDR having at least one of the sequences given in SEQ ID NO:1 for CDR-H1, SEQ ID NO:2 or SEQ ID NO:13 or SEQ ID NO:15 or SEQ ID NO:16 for CDR-H2 or SEQ ID NO:3 for CDR-H3 and a light chain wherein the variable domain comprises a CDR having at least one of the sequences given in SEQ ID NO:4 for CDR-L1, SEQ ID NO:5 for CDR-L2 or SEQ ID NO:6 for CDR-L3.

4. The antibody molecule of claim 3, which comprises SEQ ID NO:1 for CDR-H1, SEQ ID NO: 2 or SEQ ID NO:13 or SEQ ID NO:15 or SEQ ID NO:16 for CDR-H2, SEQ ID NO:3 for CDR-H3, SEQ ID NO:4 for CDR-L1, SEQ ID NO:5 for CDR-L2 and SEQ ID NO:6 for CDR-L3.

5. The antibody molecule of any one of claims 1 to 4, which is a CDR-grafted antibody molecule.

6. The antibody molecule of claim 5, wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

7. The antibody molecule of claim 6, wherein the human acceptor framework regions of the variable domain of the heavy chain are based on a human sub-group I consensus

sequence and comprise donor residues at positions 1, 28, 48, 71 and 93 that correspond to the residues at those positions in SEQ ID NO:8.

8. The antibody molecule of claim 7, additionally comprising donor residues at positions 67 and 69 that correspond to the residues at those positions in SEQ ID NO:8.

9. The antibody molecule of any one of claims 6 to 8, wherein the human acceptor framework regions of the variable domain of the light chain are based on a human sub-group I consensus sequence and comprise donor residues at positions 2, 4, 37, 38, 45 and 60 that correspond to the residues at those positions in SEQ ID NO:7.

10. The antibody molecule of claim 9, additionally comprising a donor residue at position 3 that corresponds to the residue at that position in SEQ ID NO:7.

11. An antibody molecule having specificity for human CD22, comprising a heavy chain according to either claim 7 or claim 8, and a light chain according to either claim 9 or claim 10.

12. The antibody molecule of any one of claims 1 to 11, comprising the light chain variable region 5/44-gL1 (SEQ ID NO:19) and the heavy chain variable region 5/44-gH7 (SEQ ID NO:27).

13. An antibody molecule having specificity for human CD22, comprising a light chain, wherein the sequence of the light chain comprises the sequence given in SEQ ID NO:28.

14. An antibody molecule having specificity for human CD22, comprising a light chain, wherein the sequence of the light chain consists of the sequence given in SEQ ID NO:28.

15. An antibody molecule having specificity for human CD22, comprising a heavy chain, wherein the sequence of the heavy chain comprises the sequence given in SEQ ID NO:30.

16. An antibody molecule having specificity for human CD22, comprising a heavy chain, wherein the sequence of the heavy chain consists of the sequence given in SEQ ID NO:30.

17. An antibody molecule having specificity for human CD22, having a light chain comprising the sequence given in SEQ ID NO:28 and a heavy chain comprising the sequence given in SEQ ID NO:30.

18. An antibody molecule having specificity for human CD22, having a light chain consisting of the sequence given in SEQ ID NO:28 and a heavy chain consisting of the sequence given in SEQ ID NO:30.

19. A variant of the antibody molecule of any one of claims 1 to 18, which has an improved affinity for CD22.

20. The variant of claim 19 which is obtained by an affinity maturation protocol.

21. The antibody of any one of claims 1 to 4, which is murine anti-CD22 monoclonal antibody 5/44, wherein the variable domain of the light chain has the sequence given in SEQ ID NO: 7 and the variable domain of the heavy chain has the sequence given in SEQ ID NO: 8.

22. The antibody molecule of any one of claims 1 to 4, which is a chimeric antibody molecule comprising the sequences of the light and heavy chain variable domains of the monoclonal antibody of claim 21, recited in SEQ ID NO:7 and SEQ ID NO:8 respectively.

23. An antibody molecule comprising a hybrid CDR comprising a truncated donor CDR sequence wherein the missing portion of the donor CDR is replaced by a different sequence and forms a functional CDR.

24. The antibody molecule of claim 23, wherein the missing part of the CDR sequence is from the antibody from which the framework regions of the antibody molecule are derived.

25. The antibody molecule of claim 24, wherein the missing part of the CDR sequence is derived from a germline antibody having consensus framework regions.

26. The antibody molecule of any one of claims 23 to 25, wherein CDR-H2 of the heavy chain is hybrid in the antibody molecule.

27. The antibody molecule of any one of claims 23 to 26, wherein the truncation of the donor CDR is from 1 to 8 amino acids.

28. The antibody molecule of claim 27, wherein the truncation is from 4 to 6 amino acids.

29. The antibody molecule of any one of claims 23 to 28, wherein the truncation is made at the C-terminus of the CDR.

30. A DNA sequence encoding the heavy chain and/or the light chain of an antibody molecule according to any one of claims 1 to 29.

31. A cloning or expression vector comprising a DNA sequence according to claim 30.

32. A host cell comprising a cloning or expression vector according to claim 31.

33. An antibody molecule according to any one of claims 1 to 29 or a DNA sequence according to claim 30 for use in therapy.

34. The antibody molecule of any one of claims 1 to 29 or 33, having specificity for human CD22, or a DNA sequence according to claim 30 for use in treating a pathology mediated by cells expressing CD22.

35. An antibody molecule according to claim 33 or claim 34 or a DNA sequence according to claim 33 or claim 34, for use in treating malignant lymphoma.

36. The antibody molecule or DNA sequence of claim 35, wherein the malignant lymphoma is Non-Hodgkin's lymphoma.

37. Use of the antibody molecule of any one of claims 1 to 29, having specificity for human CD22, or use of a DNA sequence according to claim 30 in the manufacture of a medicament for the treatment of a pathology mediated by cells expressing CD22.

38. The use of claim 37, wherein the pathology is malignant lymphoma.

39. Claim 38, wherein the malignant lymphoma is Non-Hodgkin's lymphoma.

40. A therapeutic or diagnostic composition comprising the antibody molecule of any one of claims 1 to 29 or the DNA sequence of claim 30.

41. A therapeutic or diagnostic composition according to claim 40, comprising a pharmaceutically acceptable excipient, diluent or carrier.

42. A therapeutic or diagnostic composition according to claim 40 or claim 41, additionally comprising anti-T cell, anti-IFNγ or anti-LPS antibodies, or non-antibody ingredients such as xanthines.

43. A process for the production of an antibody molecule according to any one of claims 1 to 29 comprising culturing a host cell according to claim 32 under conditions suitable for leading to expression of protein from DNA encoding said antibody molecule and isolating said antibody molecule.

44. A process for the preparation of a therapeutic or diagnostic composition according to any one of claims 40 to 42, comprising admixing an antibody molecule

according to any one of claims 1 to 29 together with a pharmaceutically acceptable excipient, diluent or carrier.

45. A polypeptide having the amino acid sequence given in any one of SEQ ID NOs:1 to 28 or SEQ ID NO:30.

SEQUENCE LISTING

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<211> 11
<212> PRT
<213> Homo sapiens

<220>
<221> JK1

<400> 18
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

<210> 19
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223>

<220>
<221> gL1

<400> 19
Asp Val Gln Val Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Leu Ala Asn Ser
20 25 30

Tyr Gly Asn Thr Phe Leu Ser Trp Tyr Leu His Lys Pro Gly Lys Ala
35 40 45

Pro Gln Leu Leu Ile Tyr Gly Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75 80

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Gly
85 90 95

Thr His Gln Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 20
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223>

<220>
<221> gL2

<400> 20
Asp Val Val Val Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Leu Ala Asn Ser
20 25 30

Tyr Gly Asn Thr Phe Leu Ser Trp Tyr Leu His Lys Pro Gly Lys Ala
35 40 45

```
Pro Gln Leu Leu Ile Tyr Gly Ile Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65                  70                  75                  80

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Gly
                85                  90                  95

Thr His Gln Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg
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<210> 21
<211> 80
<212> PRT
<213> Homo sapiens

<220>
<221> DP7

<400> 21

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Trp Val
            20                  25                  30

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Lys Phe Gln Gly
        35                  40                  45

Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr Met Glu
    50                  55                  60

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
65                  70                  75                  80
```

<210> 22
<211> 11
<212> PRT
<213> Homo sapiens

<220>
<221> JH4

<400> 22

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Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1               5                   10
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<210> 23
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223>

<220>

<221> gH1

<400> 23
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Arg Phe Thr Asn Tyr
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Gly Asn Gln Tyr Thr Thr Tyr Lys Arg Asn Leu
50 55 60

Lys Gly Arg Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Thr Arg Glu Gly Tyr Gly Asn Tyr Gly Ala Trp Phe Ala Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 24
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223>

<220>
<221> gH4

<400> 24
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Arg Phe Thr Asn Tyr
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Gly Asn Asn Tyr Ala Thr Tyr Arg Arg Asn Leu
50 55 60

Lys Gly Arg Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Thr Arg Glu Gly Tyr Gly Asn Tyr Gly Ala Trp Phe Ala Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser

115 120

<210> 25
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223>

<220>
<221> gH5

<400> 25
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Arg Phe Thr Asn Tyr
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Gly Asn Asn Tyr Ala Thr Tyr Arg Arg Asn Leu
50 55 60

Lys Gly Arg Val Thr Met Thr Ala Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Thr Arg Glu Gly Tyr Gly Asn Tyr Gly Ala Trp Phe Ala Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 26
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223>

<220>
<221> gH6

<400> 26
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Arg Phe Thr Asn Tyr
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asn Pro Gly Asn Asn Tyr Ala Thr Tyr Arg Arg Lys Phe
50 55 60

```
Gln Gly Arg Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80

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

Thr Arg Glu Gly Tyr Gly Asn Tyr Gly Ala Trp Phe Ala Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120

<210>   27
<211>   121
<212>   PRT
<213>   Artificial Sequence

<220>
<223>

<220>
<221>   gH7

<400>   27
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Arg Phe Thr Asn Tyr
            20                  25                  30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Gly Ile Asn Pro Gly Asn Asn Tyr Ala Thr Tyr Arg Arg Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Ala Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80

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

Thr Arg Glu Gly Tyr Gly Asn Tyr Gly Ala Trp Phe Ala Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120

<210>   28
<211>   239
<212>   PRT
<213>   Artificial Sequence

<220>
<223>

<220>
<221>   Full sequence of grafted light chain

<400>   28
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Met Lys Leu Pro Val Arg Leu Leu Val Leu Leu Leu Phe Trp Ile Pro
1               5                  10                  15

Ala Ser Arg Gly Asp Val Gln Val Thr Gln Ser Pro Ser Ser Leu Ser
            20                  25                  30

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser
        35                  40                  45

Leu Ala Asn Ser Tyr Gly Asn Thr Phe Leu Ser Trp Tyr Leu His Lys
    50                  55                  60

Pro Gly Lys Ala Pro Gln Leu Leu Ile Tyr Gly Ile Ser Asn Arg Phe
65                  70                  75                  80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
                85                  90                  95

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
            100                 105                 110

Cys Leu Gln Gly Thr His Gln Pro Tyr Thr Phe Gly Gln Gly Thr Lys
        115                 120                 125

Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
    130                 135                 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                 150                 155                 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
                165                 170                 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
            180                 185                 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
        195                 200                 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
    210                 215                 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235

<210>   29
<211>   781
<212>   DNA
<213>

<220>
<221>   Full DNA sequence of grafted light chain

<400>   29
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cctgcttccc ggggtgacgt tcaagtgacc cagagcccat ccagcctgag cgcatctgta   120
ggagaccggg tcaccatcac ttgtagatcc agtcagagtc ttgcaaacag ttatgggaac   180
acctttttgt cttggtatct gcacaaacca ggtaaagccc cacaattgct catctacgga   240
atctctaaca gatttagtgg tgtaccagac aggttcagcg gttccggaag tggtactgat   300
ttcaccctca cgatctcgtc tctccagcca gaagatttcg ccacttatta ctgtttacaa   360
ggtacacatc agccgtacac attcggtcag ggtactaaag tagaaatcaa acgtacggta   420
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gcggccccat ctgtcttcat cttcccgcca tctgatgagc agttgaaatc tggaactgcc 480
tctgttgtgt gcctgctgaa taacttctat cccagagagg ccaaagtaca gtggaaggtg 540
gataacgccc tccaatcggg taactcccag gagagtgtca cagagcagga cagcaaggac 600
agcacctaca gcctcagcag caccctgacg ctgagcaaag cagactacga gaaacacaaa 660
gtctacgcct gcgaagtcac ccatcagggc ctgagctcgc ccgtcacaaa gagcttcaac 720
aggggagagt gttagaggga gaagtgcccc cacctgctcc tcagttccag cctgggaatt 780
c 781

<210> 30
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223>

<220>
<221> Full sequence of grafted heavy chain

<400> 30
Met Asp Phe Gly Phe Ser Leu Val Phe Leu Ala Leu Ile Leu Lys Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Arg Phe
35 40 45

Thr Asn Tyr Trp Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Ile Gly Gly Ile Asn Pro Gly Asn Asn Tyr Ala Thr Tyr Arg
65 70 75 80

Arg Lys Phe Gln Gly Arg Val Thr Met Thr Ala Asp Thr Ser Thr Ser
85 90 95

Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Thr Arg Glu Gly Tyr Gly Asn Tyr Gly Ala Trp Phe Ala
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn
210 215 220

```
Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser
225                 230                 235                 240

Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Leu Gly Lys
465

<210>   31
<211>   2160
<212>   DNA
<213>   Artificial Sequence

<220>
<223>

<220>
<221>   Full DNA sequence of grafted heavy chain

<400>   31
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aagcttgccg ccaccatgga cttcggattc tctctcgtgt tcctggcact cattctcaag 60
ggagtgcagt gtgaggtgca attggtccag tcaggagcag aggttaagaa gcctggtgct 120
tccgtcaaag tttcgtgtaa ggctagcggc tacaggttca caaattattg gattcattgg 180
gtcaggcagg ctccgggaca aggcctggaa tggatcggtg gcattaatcc cgggaataac 240
tacgctacat ataggagaaa attccagggc agagttacga tgaccgcgga cacctccaca 300
agcactgtct acatggagct gtcatctctg agatccgagg acaccgcagt gtactattgt 360
actagagaag gctacggtaa ttacggagcc tggttcgcct actggggcca gggtacccta 420
gtcacagtct cctcagcttc tacaaagggc ccatccgtct tccccctggc gccctgctcc 480
aggagcacct ccgagagcac agccgccctg ggctgcctgg tcaaggacta cttccccgaa 540
ccggtgacgg tgtcgtggaa ctcaggcgcc ctgaccagcg gcgtgcacac cttcccggct 600
gtcctacagt cctcaggact ctactccctc agcagcgtgg tgaccgtgcc ctccagcagc 660
ttgggcacga agacctacac ctgcaacgta gatcacaagc ccagcaacac caaggtggac 720
aagagagttg gtgagaggcc agcacaggga gggagggtgt ctgctggaag ccaggctcag 780
ccctcctgcc tggacgcacc ccggctgtgc agccccagcc cagggcagca aggcatgccc 840
catctgtctc ctcacccgga ggcctctgac caccccactc atgcccaggg agagggtctt 900
ctggattttt ccaccaggct ccgggcagcc acaggctgga tgcccctacc ccaggccctg 960
cgcatacagg ggcaggtgct gcgctcagac ctgccaagag ccatatccgg gaggaccctg 1020
cccctgacct aagcccaccc caaaggccaa actctccact ccctcagctc agacaccttc 1080
tctcctccca gatctgagta actcccaatc ttctctctgc agagtccaaa tatggtcccc 1140
catgcccacc atgcccaggt aagccaaccc aggcctcgcc ctccagctca aggcgggaca 1200
ggtgccctag agtagcctgc atccagggac aggccccagc cgggtgctga cgcatccacc 1260
tccatctctt cctcagcacc tgagttcctg gggggaccat cagtcttcct gttccccccа 1320
aaacccaagg acactctcat gatctcccgg acccctgagg tcacgtgcgt ggtggtggac 1380
gtgagccagg aagaccccga ggtccagttc aactggtacg tggatggcgt ggaggtgcat 1440
aatgccaaga caaagccgcg ggaggagcag ttcaacagca cgtaccgtgt ggtcagcgtc 1500
ctcaccgtcc tgcaccagga ctggctgaac ggcaaggagt acaagtgcaa ggtctccaac 1560
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gtgcgagggc cacatggaca gaggtcagct cggcccaccc tctgccctgg gagtgaccgc 1680
tgtgccaacc tctgtcccta cagggcagcc ccgagagcca caggtgtaca ccctgccccc 1740
atcccaggag gagatgacca agaaccaggt cagcctgacc tgcctggtca aaggcttcta 1800
ccccagcgac atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac 1860
cacgcctccc gtgctggact ccgacggctc cttcttcctc tacagcaggc taaccgtgga 1920
caagagcagg tggcaggagg ggaatgtctt ctcatgctcc gtgatgcatg aggctctgca 1980
caaccactac acacagaaga gcctctccct gtctctgggt aaatgagtgc cagggccggc 2040
aagcccccgc tccccgggct ctcggggtcg cgcgaggatg cttggcacgt accccgtcta 2100
catacttccc aggcacccag catggaaata aagcacccac cactgccctg gctcgaattc 2160

<210> 32
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gH1 T1

<400> 32
agtgtgaggt gcaattggtc cagtcaggag cagaggttaa gaagcctggt gcttccgtca 60
aagtttcgtg taaggctagc ggctacaggt tcac 94

<210> 33
<211> 96
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>

<221> 544gH1 T2

<400> 33
gtggcattaa tcccgggaat cagtacacta catataaaag aaatctaaag ggcagagcaa 60
cgctgaccgc ggacacctcc acaagcactg tctaca 96

<210> 34
<211> 95
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gH1 T3

<400> 34
agagaaggct acggtaatta cggagcctgg ttcgcctact ggggccaggg taccctagtc 60
acagtctcct cagcttctac aaagggccca agaaa 95

<210> 35
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544 gH1 B1

<400> 35
ggaccaattg cacctcacac tgcactccct tgagaatgag tgccaggaac acgagagaga 60
atccgaagtc catggtggcg gcaagctttt attc 94

<210> 36
<211> 97
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gH1 B2

<400> 36
gattcccggg attaatgcca ccgatccatt ccaggccttg tcccggagcc tgcctgaccc 60
aatgaatcca ataatttgtg aacctgtagc cgctagc 97

<210> 37
<211> 93
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gH1 B3

<400> 37
cgtaattacc gtagccttct ctagtacaat agtacactgc ggtgtcctcg gatctcagag 60
atgacagctc catgtagaca gtgcttgtgg agg 93

<210> 38
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gH1 F1

<400> 38
gaataaaagc ttgccgccac c 21

<210> 39
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gH1 R1

<400> 39
tttcttgggc cctttgtaga ag 22

<210> 40
<211> 87
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544 gL1 T1

<400> 40
gcttcccggg gtgacgttca agtgacccag agcccatcca gcctgagcgc atctgtagga 60
gaccgggtca ccatcacttg tagatcc 87

<210> 41
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544 gL1 T2

<400> 41
tatctgcaca aaccaggtaa agccccacaa ttgctcatct acggaatctc taacagattt 60

agtggtgtac cagacaggtt cagcggttcc 90

<210> 42
<211> 91
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gL1 T3

<400> 42
agatttcgcc acttattact gtttacaagg tacacatcag ccgtacacat tcggtcaggg 60
tactaaagta gaaatcaaac gtacggcgtg c 91

<210> 43
<211> 88
<212> DNA
<213>

<220>
<221> 544gL1 B1

<400> 43
gaacgtcacc ccgggaagca ggaatccaga acaacagaag caccaacagc ctaacaggca 60
acttcatggt ggcggcttcg aatcatcc 88

<210> 44
<211> 88
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gL1 B2

<400> 44
ctttacctgg tttgtgcaga taccaagaca aaaaggtgtt cccataactg tttgcaagac 60
tctgactgga tctacaagtg atggtgac 88

<210> 45
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gL1 B3

<400> 45
aacagtaata agtggcgaaa tcttctggct ggagagacga gatcgtgagg gtgaaatcag 60
taccacttcc ggaaccgctg aacctgtctg 90

<210> 46
<211> 20

<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> 544gL1 F1

<400> 46
ggatgattcg aagccgccac 20

<210> 47
<211> 21
<212> DNA
<213>

<220>
<221> 544gL1 R1

<400> 47
gcacgccgta cgtttgattt c 21

<210> 48
<211> 339
<212> DNA
<213> mouse

<220>
<221> DNA sequence of mouse monoclonal 5/44 VL

<400> 48
gatgttgtgg tgactcaaac tccactctcc ctgcctgtca gctttggaga tcaagtttct 60
atctcttgca ggtctagtca gagtcttgca aacagttatg ggaacacctt tttgtcttgg 120
tacctgcaca agcctggcca gtctccacag ctcctcatct atgggatttc caacagattt 180
tctggggtgc cagacaggtt cactggcagt ggttcaggga cagatttcac actcaagatc 240
agcacaataa agcctgagga cttgggaatg tattactgct tacaaggtac acatcagccg 300
tacacgttcg gaggggggac caagctggaa ataaaacgt 339

<210> 49
<211> 363
<212> DNA
<213> mouse

<220>
<221> DNA sequence of mouse monoclonal 5/44 VH

<400> 49
gaggtccaac tgcagcagtc tgggactgta ctggcaaggc ctggggcttc cgtgaagatg 60
tcctgcaagg cttctggcta caggtttacc aactactgga ttcactgggt aaaacagagg 120
cctgggcagg gtctagaatg gattggtggt attaatcctg gaaataatta tactacgtat 180
aagaggaact tgaagggcaa ggccacactg actgcagtca catccgccag cactgcctac 240
atggacctca gcagcctgac aagtgaggac tctgcggtct attactgtac aagagagggc 300
tatggtaact acggggcctg gtttgcttac tggggccagg ggactctggt caccgtctcc 360
tca 363

<210> 50
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence within oligonucleotide primer

<400> 50
gccgccacc 9

<210> 51
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' oligonucleotide primer

<400> 51
gcgcgcaagc ttgccgccac catggacttc ggattctctc tcgtgttcct ggcactcatt 60
ctcaagggag tgcagtgtga ggtgcagctc gtcgagtctg g 101