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Description

[0001] The present invention relates to specific binding members directed to tenascin-C, in particular human antibodies against human tenascin-C. These specific binding members have a range of therapeutic applications, for example in the diagnosis and treatment of cancer.

[0002] Tenascin-C is a large hexameric glycoprotein of the extracellular matrix which modulates cellular adhesion. It is involved in processes such as cell proliferation and cell migration and is associated with changes in tissue architecture as occurring during morphogenesis and embryogenesis as well as under tumorigenesis or angiogenesis.

[0003] The schematic domain structure of tenascin-C is depicted in Figure 1. Several isoforms of tenascin-C can be generated as a result of alternative splicing which may lead to the inclusion of (multiple) domains in the central part of this protein, ranging from domain A1 to domain D [Borsi L et al Int J Cancer 1992; 52:688-692, Carnemolla B et al. Eur J Biochem 1992; 205:561-567]. It had previously been assumed that domains A1 - D could be inserted or omitted "in block" in the tenascin-C molecule by a mechanism of alternative splicing, leading to "tenascin-C large" and "tenascin-C small" molecules [Borsi L et al J Biol Chem 1995; 270:6243-6245]. A strong over-expression of the large isoform of tenascin-C has been reported for a number of tumors [Borsi 1992 supra], and two monoclonal antibodies specific for domains A1 and D, respectively, have been extensively characterised in the clinic [Riva P et al. Int J Cancer 1992; 51: 7-13, Riva P et al. Cancer Res 1995; 55:5952s-5956s, Paganelli G et al Eur J Nucl Med 1994; 21:314-321, Reardon DA et al. J Clin Oncol 2002; 20:1389-1397, Bigner DD et al. J Clin Oncol 1998; 16:2202-2212].

[0004] However, it has recently become clear that a more complex regulation of the alternative splicing mechanism takes place, leading to an increased molecular heterogeneity among the large isoforms of tenascin-C. For example, it has been reported that the extra domain C of tenascin-C displays a more restricted pattern of expression compared with the other alternatively spliced domains of tenascin-C [Carnemolla B et al. Am J Pathol 1999; 154:1345-1352], with a predominantly perivascular staining as depicted with immunohistochemistry. The C domain of tenascin-C is undetectable in most normal adult tissues, but is over-expressed in high-grade astrocytomas [Carnemolla B et al. Am J Pathol 1999; 154:1345-1352] and other tumor types. Further support for the heterogeneity between large tenascin-C isoforms comes from transcriptional analyses, which confirmed that large tenascin-C transcripts feature a heterogeneous composition [Katenkamp K et al. J Pathol 2004; 203:771-779]. An additional level of complexity is provided by the presence or absence of post-translational modifications (e.g. glycosylation), which may modify certain epitopes on the surface of individual protein domains and make them unavailable to a specific molecular recognition *in vitro* or *in vivo* to specific monoclonal antibodies.

[0005] Even though the rapid isolation of antibodies specific to virtually any protein of interest can be accomplished with existing methodologies *in vitro*, it is not obvious that such antibodies recognize the epitope in biological specimens or in animal models of disease. Possible reasons for lack of binding *in vivo* include post-translational modifications of the epitope, masking of the epitope and insufficient antibody specificity or stability. It is therefore difficult to assess the suitability of monoclonal antibodies for practical applications based solely on their reactivity with recombinant antigens (or antigen fragments) in typical solid-phase assays, such as enzyme-linked immunosorbent assays (ELISA), which are routinely used for the screening of monoclonal antibodies.

[0006] Monoclonal antibodies to the individual domains of the tenascin-C large isoforms therefore need to be analysed individually, in order to evaluate their suitability for diagnostic and therapeutic applications.

[0007] The present inventors have isolated human monoclonal antibody fragments specific to domain A1 within the alternatively spliced region of tenascin-C. These antibodies are characterized by their ability to recognize large tenascin-C isoforms in biological specimens, as well as by a highly specific binding in ELISA assays, with a striking differentiation among closely related antigens.

[0008] An aspect of the invention provides a specific binding member which binds human tenascin-C, in particular tenascin-C large isoform.

[0009] Preferred specific binding members are tumour specific and bind preferentially to tumour tissue relative to normal tissue. Specific binding members may, for example, bind to stroma and/or neo- and peri-vascular structures of tumour tissue preferentially to normal tissue.

[0010] A specific binding member may bind preferentially to tenascin-C large isoform relative to tenascin-C small isoform. The invention provides specific binding members as defined in any of claims 1 to 15.

[0011] For example, a suitable specific binding member may comprise the 4A1-F16/3A1-D5 VL domain of SEQ ID NO. 4 or SEQ ID NO: 50 and/or the 4A1-F16 VH domain of SEQ ID NO. 2 or the 3A1-D5 VH domain of SEQ ID NO: 12. 4A1-F16 is also referred to herein as F16.

[0012] Generally, a VH domain is paired with a VL domain to provide an antibody antigen-binding site.

[0013] In one preferred embodiment, a VH domain described herein (i.e. SEQ ID NOS 2 or 12) is paired with the corresponding VL domain (i.e. SEQ ID NO: 4 or SEQ ID NO: 50), so that an antibody antigen-binding site is formed comprising both the VH and VL domains.

[0014] CDRs are generally defined as per Kabat. Preferably a VH domain and/or a VL domain comprises a CDR1, a

CDR2 and a CDR3. 4A1-F16 VH CDR1 is shown in SEQ ID NO: 5. 3A1-D5 VH CDR1 is shown in SEQ ID NO: 13. 4A1-F16 and 3A1-D5 VH CDRs 2 and 3 are shown in SEQ ID NOS 6 and 7, respectively. 4A1-F16 and 3A1-D5 VL CDR's 1, 2 and 3 are shown in SEQ ID NOS 8, 9 and 10, respectively.

[0015] In some embodiments, a specific binding member may comprise an antibody VH domain comprising a CDR3 with the amino acid sequence of SEQ ID NO. 7, a CDR2 with the amino acid sequence of SEQ ID NO. 6, and a CDR1 with the amino acid sequence of SEQ ID NO. 5 or, more preferably, SEQ ID NO. 13.

[0016] Preferably the specific binding member is an scFv, as described in more detail elsewhere herein. The VH and VL domains may be joined through a peptide linker, for example a linker having an amino acid sequence as set out in SEQ ID NO: 37. Normally, the linker has an amino acid sequence comprising one or more tandem repeats of a motif. Typically the motif is a five residue sequence, and preferably at least 4 of the residues are Gly or Ser. Where four of the five residues is Gly or Ser, the other residue may be Ala. More preferably each of the five residues is Gly or Ser. Preferred motifs are GGGGS, SSSSG, GSGSA and GSGGG (SEQ ID NOS: 76, 77, 78 and 79, respectively). Preferably, the motifs are adjacent in the sequence, with no intervening nucleotides between the repeats. The linker sequence may comprise or consist of between one and five, preferably three or four, repeats of the motif. For example, a linker with three tandem repeats may have one of the following amino acid sequences:

GGGSGGGSGGGGS - SEQ ID NO: 39

SSSSGSSSSGSSSSG - SEQ ID NO: 41

GSGSAGSGSAGSGSA - SEQ ID NO: 42

GGSGGGSGGGSGGG - SEQ ID NO: 43.

[0017] Variants of the VH and VL domains and CDRs of which the sequences are set out herein and which can be employed in specific binding members for tenascin-C can be obtained by means of methods of sequence alteration or mutation and screening. Such methods are also provided by the present invention.

[0018] Variable domain amino acid sequence variants of any of the VH and VL domains whose sequences are specifically disclosed herein may be employed in accordance with the present invention, as discussed. Particular variants may include one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue), wherein there are less than 5 alterations, 4, 3, 2 or 1. Alterations may be made in one or more framework regions and/or one or more CDRs. In particular, alterations may be made in VH CDR1, VH CDR2 and/or VH CDR3, especially VH CDR3.

[0019] A specific binding member according to the invention may be one which competes for binding to antigen with any specific binding member which both binds Tenascin-C large isoform, in particular the A1 or C domains thereof, and comprises a specific binding member, VH and/or VL domain disclosed herein, or VH CDR disclosed herein, or variant of any of these. Competition between binding members may be assayed easily in vitro, for example using ELISA and/or by tagging a specific reporter molecule to one binding member which can be detected in the presence of other untagged binding member (s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope.

[0020] Thus, a further aspect of the present invention provides a specific binding member comprising a human antibody antigen-binding site which competes with 4A1-F16 or 3A1-D5 for binding to tenascin-C.

[0021] Various methods are available in the art for obtaining antibodies against tenascin-C large isoform which may compete with 4A1-F16 or 3A1-D5. Preferably, such antibodies bind preferentially to tenascin-C large isoform relative to tenascin-C small isoform.

[0022] A method of obtaining one or more specific binding members able to bind the antigen may comprise bringing into contact a library of specific binding members according to the invention and said antigen, and selecting one or more specific binding members of the library able to bind said antigen.

[0023] The library may be displayed on the surface of bacteriophage particles, each particle containing nucleic acid encoding the antibody VH variable domain displayed on its surface, and optionally also a displayed VL domain if present.

[0024] Following selection of specific binding members able to bind the antigen and displayed on bacteriophage particles, nucleic acid may be taken from a bacteriophage particle displaying a said selected specific binding member. Such nucleic acid may be used in subsequent production of a specific binding member or an antibody VH variable domain (optionally an antibody VL variable domain) by expression from nucleic acid with the sequence of nucleic acid taken from a bacteriophage particle displaying a said selected specific binding member.

[0025] An antibody VH variable domain with the amino acid sequence of an antibody VH variable domain of a said selected specific binding member may be provided in isolated form, as may a specific binding member comprising such a VH domain.

[0026] Ability to bind tenascin-C may be further tested, also ability to compete with 4A1-F16 or 3A1-D5 for binding to tenascin-C.

[0027] A specific binding member according to the present invention may bind tenascin-C with the affinity of 4A1-F16 or 3A1-D5 or with a greater or lesser affinity.

[0028] Binding affinity of different specific binding members can be compared under appropriate conditions.

[0029] In addition to antibody sequences, a specific binding member according to the present invention may comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen.

[0030] Specific binding members of the invention may carry a detectable label, for example an agent which facilitates tumor detection such as a radionuclide or fluorophore, or may be conjugated to an agent capable of triggering a biocidal event, such as a radionuclide, photosensitiser, drug, cytokine, pro-coagulant factor, toxin or enzyme (e.g. via a peptidyl bond or linker), for use in a method of therapy.

[0031] In further aspects, the invention provides an isolated nucleic acid which comprises a sequence encoding a specific binding member, VH or VL domains according to the present invention, and methods of preparing a specific binding member, a VH domain and/or a VL domain of the invention, which comprise expressing said nucleic acid under conditions to bring about production of said specific binding member, VH domain and/or VL domain, and recovering it.

[0032] Specific binding members described herein may be used in a method of treatment or diagnosis of the human or animal body, such as a method of treatment (which may include prophylactic treatment) of a disease or disorder, in particular a proliferative disorder such as cancer, in a human patient which comprises administering to said patient an effective amount of a specific binding member.

[0033] A further aspect of the present invention provides nucleic acid, generally isolated, encoding an antibody VH variable domain and/or VL variable domain disclosed herein.

[0034] A further aspect provides a host cell transformed with nucleic acid of the invention.

[0035] A yet further aspect provides a method of production of an antibody VH variable domain, the method including causing expression from encoding nucleic acid. Such a method may comprise culturing host cells under conditions for production of said antibody VH variable domain.

[0036] Analogous methods for production of VL variable domains and specific binding members comprising a VH and/or VL domain are provided as further aspects of the present invention.

[0037] A method of production may comprise a step of isolation and/or purification of the product.

[0038] A method of production may comprise formulating the product into a composition including at least one additional component, such as a pharmaceutically acceptable excipient.

[0039] These and other aspects of the invention are described in further detail below.

TERMINOLOGY

Specific binding member

[0040] This describes a member of a pair of molecules that have binding specificity for one another. The members of a specific binding pair may be naturally derived or wholly or partially synthetically produced. One member of the pair of molecules has an area on its surface, or a cavity, which specifically binds to and is therefore complementary to a particular spatial and polar organisation of the other member of the pair of molecules. Thus the members of the pair have the property of binding specifically to each other. Examples of types of specific binding pairs are antigen-antibody, biotin-avidin, hormone-hormone receptor, receptor-ligand, enzyme-substrate. This application is concerned with antigen-antibody type reactions.

Antibody

[0041] This describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any polypeptide or protein having a binding domain which is, or is substantially homologous to, an antibody binding domain. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies. It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

[0042] As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

[0043] It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al, Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993). Fv, scFv or diabody molecules may be stabilised by the incorporation of disulphide bridges linking the VH and VL domains (Y. Reiter et al. Nature Biotech 14 1239-1245 1996). Minibodies comprising an scFv joined to a CH3 domain may also be made (S. Hu et al, Cancer Res. 56 3055-3061 1996).

[0044] Diabodies are multimers of polypeptides, each polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (e.g. by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are formed by the association of the first domain of one polypeptide within the multimer with the second domain of another polypeptide within the multimer (WO94/13804).

[0045] Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. Current Opinion Biotechnol. 4, 446-449 (1993)), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

[0046] Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E. coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against antigen X, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected. Bispecific whole antibodies may be made by knobs-into-holes engineering (J. B. B. Ridgeway et al, Protein Eng. 9 616-621, 1996).

Antigen binding domain

[0047] This describes the part of an antibody which comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be provided by one or more antibody variable domains (e.g. a so-called Fd antibody fragment consisting of a VH domain). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

Specific

[0048] This may be used to refer to the situation in which one member of a specific binding pair will not show any significant binding to molecules other than its specific binding partner(s). For example, an antibody specific for Tenascin-C may show little or no binding to other components of the extracellular matrix such as fibronectin. Similarly, an antibody specific for Tenascin-C large isoform may show little or no binding to Tenascin-C small isoform. The term is also applicable where e.g. an antigen binding domain is specific for a particular epitope which is carried by a number of antigens, in which case the specific binding member carrying the antigen binding domain will be able to bind to the various antigens carrying the epitope.

Comprise

[0049] This is generally used in the sense of include, that is to say permitting the presence of one or more features or components.

Isolated

[0050] This refers to the state in which specific binding members of the invention, or nucleic acid encoding such binding members, will be in accordance with the present invention. Members and nucleic acid will be free or substantially free of material with which they are naturally associated such as other polypeptides or nucleic acids with which they are found in their natural environment, or the environment in which they are prepared (e.g. cell culture) when such preparation

is by recombinant DNA technology practised *in vitro* or *in vivo*. Members and nucleic acid may be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the members will normally be mixed with gelatin or other carriers if used to coat microtitre plates for use in immunoassays, or will be mixed with pharmaceutically acceptable carriers or diluents when used in diagnosis or therapy. Specific binding members may be glycosylated, either naturally or by systems of heterologous eukaryotic cells (e.g. CHO or NS0 (ECACC 85110503) cells, or they may be (for example if produced by expression in a prokaryotic cell) unglycosylated.

[0051] By "substantially as set out" it is meant that the relevant CDR or VH or VL domain of the invention will be either identical or highly similar to the specified regions of which the sequence is set out herein. By "highly similar" it is contemplated that from 1 to 5, preferably from 1 to 4 such as 1 to 3 or 1 or 2, or 3 or 4, substitutions may be made in the CDR and/or VH or VL domain.

[0052] The structure for carrying a CDR of the invention will generally be of an antibody heavy or light chain sequence or substantial portion thereof in which the CDR is located at a location corresponding to the CDR of naturally occurring VH and VL antibody variable domains encoded by rearranged immunoglobulin genes. The structures and locations of immunoglobulin variable domains may be determined by reference to (Kabat, E.A. et al, Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (<http://immuno.bme.nwu.edu>)).

[0053] Preferably, a CDR amino acid sequence substantially as set out herein is carried as a CDR in a human variable domain or a substantial portion thereof.

[0054] Variable domains employed in the invention may be obtained from any germline or rearranged human variable domain, or may be a synthetic variable domain based on consensus sequences of known human variable domains. A CDR sequence of the invention (e.g. CDR1, CDR2 or CDR3) may be introduced into a repertoire of variable domains lacking a CDR (e.g. the corresponding CDR1, CDR2 or CDR3), using recombinant DNA technology.

[0055] For example, Marks et al (Bio/Technology, 1992, 10:779-783) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks *et al* further describe how this repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR-derived sequences of the present invention may be shuffled with repertoires of VH or VL domains lacking a CDR, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide specific binding members of the invention. The repertoire may then be displayed in a suitable host system such as the phage display system of WO92/01047 so that suitable specific binding members may be selected. A repertoire may consist of from anything from 10⁴ individual members upwards, for example from 10⁶ to 10⁸ or 10¹⁰ members.

[0056] Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (Nature, 1994, 370:389-391), who describes the technique in relation to a β -lactamase gene but observes that the approach may be used for the generation of antibodies.

[0057] A further alternative is to generate novel VH or VL regions carrying CDR-derived sequences of the invention using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. Such a technique is described by Gram et al (1992, Proc. Natl. Acad. Sci. USA, 89:3576-3580), who used error-prone PCR.

[0058] Another method which may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed by Barbas et al, (1994, Proc. Natl. Acad. Sci., USA, 91:3809-3813) and Schier et al (1996, J. Mol. Biol. 263:551-567).

[0059] All the above-described techniques are known as such in the art and in themselves do not form part of the present invention. The skilled person will be able to use such techniques to provide specific binding members of the invention using routine methodology in the art.

[0060] A substantial portion of an immunoglobulin variable domain will comprise at least the three CDR regions, together with their intervening framework regions. Preferably, the portion will also include at least about 50% of either or both of the first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with naturally occurring variable domain regions. For example, construction of specific binding members of the present invention made by recombinant DNA techniques may result in the introduction of NB or C-terminal residues encoded by linkers introduced to facilitate cloning or other manipulation steps. Other manipulation steps include the introduction of linkers to join variable domains of the invention to further protein sequences including immunoglobulin heavy chains, other variable domains (for example in the production of diabodies) or protein labels as discussed in more details below.

[0061] Specific binding members of the present invention may further comprise antibody constant regions or parts thereof. For example, a VL domain may be attached at its C-terminal end to antibody light chain constant domains including human C κ or C λ chains, preferably C λ chains. Similarly, a specific binding member based on a VH domain

may be attached at its C-terminal end to all or part of an immunoglobulin heavy chain derived from any antibody isotype, e.g. IgG, IgA, IgE and IgM and any of the isotype sub-classes, particularly IgG1 and IgG4.

[0062] Specific binding members of the invention may be labelled with a detectable or functional label.

[0063] Detectable labels may include radionuclides, such as iodine-131, yttrium-90, indium-111 and technicium-99, which may be attached to antibodies of the invention using conventional chemistry known in the art of antibody imaging. A specific binding member labelled with a radioactive isotope may be used to selectively deliver radiation to a specific target, such as a tumour. This may be useful in imaging the tumour or in delivering a cytotoxic dose of radiation, as described below.

[0064] Other detectable labels may include enzyme labels such as horseradish peroxidase, chemical moieties such as biotin which may be detected via binding to a specific cognate detectable moiety, e.g. labelled avidin, fluorochromes such as fluorescein, rhodamine, phycoerythrin and Texas Red and near infrared fluorophores, including cyanine dye derivatives such as Cy7 (Amersham Pharmacia) and Alexa750 (Molecular probes).

[0065] In other embodiments, a detectable label may comprise a microbubble derivative, which is detectable by ultrasound (Joseph S et al Pharm Res. 2004 Jun;21(6):920-6), or a magnetic particle (Schellenberger EA et al. Bioconjug. Chem. 2004 Sep-Oct; 15(5): 1062-7).

[0066] A functional label may include an agent which is capable of triggering a biocidal event or has an anti-cancer effect. Suitable labels include radionuclides, photosensitisers, toxin polypeptides, toxic small molecules and other drugs, cytokines (e.g. IL2, IL12, TNF), chemokines, pro-coagulant factors (e.g. tissue factor), enzymes, liposomes, and immune response factors (see, for example, D. Neri (2004) CHIMIA "Tumor Targeting" vol. 58, pages 723-726).

[0067] Radionuclides include iodine-131, yttrium-90, indium-111 and technicium-99 and are described in more detail above.

[0068] A toxin polypeptide or peptide has cytotoxic or apoptotic activity and may be derived from a microbial, plant, animal or human source. In some embodiments, a toxin polypeptide may be inserted directly into the constant regions of a specific binding member. Examples of toxin polypeptides include *Pseudomonas* exotoxin, ricin α -chain and angiotenin.

[0069] Toxic small molecules include chemical compounds with cytotoxic activity, including, for example, DNA-complexing agents or cell cycle inhibitors. In some embodiments, the toxic molecule may be liberated in the vicinity of the target cell by cleavage of a pH- or enzyme-sensitive linker (e.g. linkers containing imine bonds). Examples of toxic small molecules include maytansine, calicheamicin, epothilone and tubulysin and derivatives thereof.

[0070] Immune response factors may include specific binding members which bind to immune effector cells. The binding of the specific binding member may invoke a cell-mediated immune response against the target cell.

[0071] In preferred embodiments, a specific binding member of the invention is conjugated with a cytokine. A fusion protein comprising the specific binding member or a polypeptide component thereof (e.g. a heavy chain or a light chain of an antibody or multi-chain antibody fragment, such as a Fab) and the cytokine may be produced. Thus, for example, a VH domain or VL domain of a specific binding member of the invention may be fused to the cytokine. Typically the specific binding member, or component thereof, and cytokine are joined via a peptide linker, e.g. a peptide of about 5-25 residues, e.g. 10-20 residues, preferably about 15 residues. Suitable examples of peptide linkers are given herein. Preferably the cytokine is IL2, more preferably human IL2. The cytokine may be fused upstream (N-terminal) or downstream (C-terminal) of the specific binding member or polypeptide component thereof. A preferred embodiment is a fusion protein comprising specific binding member (especially an antibody molecule, e.g. scFv molecule) of the invention and IL2. Amino acid sequences of such fusion proteins, and nucleic acids comprising nucleotide sequences encoding them, form part of the invention.

[0072] Specific binding members of the present invention may be useful in methods of diagnosis, such as tumour imaging, or in the treatment in human or animal subjects, for example for cancer conditions.

[0073] Accordingly, further aspects of the invention provide pharmaceutical compositions comprising a specific binding member, and use of such a specific binding member in the manufacture of a medicament for administration, for example in a method of making a medicament or pharmaceutical composition comprising formulating the specific binding member with a pharmaceutically acceptable excipient.

[0074] A specific binding member for use in a method of treatment is preferably conjugated with or linked to a functional label which elicits an anti-tumour effect. In preferred embodiments, as noted above, the specific binding member is conjugated with or linked to a cytokine e.g. IL2.

[0075] Clinical indications in which a specific binding member as described herein may be used to provide therapeutic benefit include proliferative disorders such as pre-malignant and malignant neoplasms and tumours, (e.g., histiocytoma, glioma, astrocytoma, osteoma), cancers (e.g., lung cancer, small cell lung cancer, gastrointestinal cancer, bowel cancer, colon cancer, breast carcinoma, ovarian carcinoma, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreas cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, melanoma), leukaemias and angiogenic diseases.

[0076] A pre-malignant or malignant condition may occur in any cell-type, including but not limited to, lung, colon,

breast, ovarian, prostate, liver, pancreas, brain, and skin.

[0077] A proliferative disorder suitable for treatment as described herein may be characterised by the presence of cells or tissue in which expression of tenascin-C large isoforms, in particular large isoforms comprising the A1 or C domains, is increased or elevated.

[0078] Compositions provided by the invention may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, eg decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors. Appropriate doses of antibody are well known in the art; see Ledermann J.A. et al. (1991) *Int J. Cancer* 47: 659-664; Bagshawe K.D. et al. (1991) *Antibody, Immunoconjugates and Radiopharmaceuticals* 4: 915-922.

[0079] The precise dose will depend upon a number of factors, including whether the antibody is for diagnosis or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment or diabody), and the nature of any detectable label or other molecule attached to the antibody. A typical antibody dose will be in the range 0.5mg to 100g for systemic applications, and 10µg to 1mg for local applications. Typically, the antibody will be a whole antibody, preferably the IgG1 or IgG4 isotype. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician.

[0080] Specific binding members of the present invention will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the specific binding member.

[0081] Thus pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. intravenous.

[0082] Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

[0083] For intravenous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

[0084] A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Other treatments may include the administration of suitable doses of pain relief drugs such as non-steroidal anti-inflammatory drugs (e.g. aspirin, paracetamol, ibuprofen or ketoprofen) or opiates such as morphine, or anti-emetics.

[0085] Specific binding members of the invention may be useful in a method of detecting and/or imaging tumour cells comprising administering an antibody as described herein to an individual and detecting the binding of said antibody to tumour cells in said individual.

[0086] Preferred antibodies for use in such methods may be conjugated or linked to a detectable label such as a radionuclide or fluorophore.

[0087] A method of the invention may comprise causing or allowing binding of a specific binding member as provided herein to tenascin-C *in vitro*.

[0088] The amount of binding of specific binding member to tenascin-C may be determined. In some embodiments, the binding of the specific binding member to a sample obtained from an individual may be determined.

[0089] Quantitation may be related to the amount of the antigen, which may be of diagnostic interest.

[0090] The binding of antibodies may be determined by any appropriate means. For example, the antibody may be linked or conjugated to a reporter molecule or detectable label and the presence, amount or localisation of the label or reporter on the sample determined.

[0091] Binding of an antibody *in vivo*, for example in a method of molecular imaging, may be determined by radioactive detection (e.g. PET, SPECT), near infrared fluorescence imaging (e.g. diffuse optical tomography, endoscopy), ultrasound (e.g. with targeted microbubble derivatives) and MRI (with targeted magnetic particles).

[0092] In other embodiments, binding of the antibody may take place *in vitro*, for example in ELISA, Western blotting, immunocytochemistry, immuno-precipitation or affinity chromatography.

[0093] A method of detecting and/or imaging tumour cells may thus comprise contacting an antibody as described herein with a sample obtained from an individual and detecting the binding of said antibody to tumour cells in said sample.

[0094] Preferred antibodies for use in such in vitro methods may be conjugated or linked to a reporter molecule. The reporter molecule may be a radionuclide, fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine. For *in vivo* imaging, radionuclides or fluorphors are preferred.

[0095] Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

[0096] The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge.

[0097] Competition between binding members may be assayed easily in vitro, for example by tagging a specific reporter molecule to one binding member which can be detected in the presence of other untagged binding member(s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope. Competition may be determined using standard techniques such as ELISA.

[0098] In testing for competition a peptide fragment of the antigen may be employed, especially a peptide including an epitope of interest. A peptide having the epitope sequence plus one or more amino acids at either end may be used. Such a peptide may be said to "consist essentially" of the specified sequence. Specific binding members according to the present invention may be such that their binding for antigen is inhibited by a peptide with or including the sequence given. In testing for this, a peptide with either sequence plus one or more amino acids may be used. Suitable peptides may be obtained from sequences of the D, C and/or A1 domains of tenascin-C.

[0099] Specific binding members which bind a specific peptide may be isolated for example from a phage display library by panning with the peptide(s).

[0100] The present invention further provides an isolated nucleic acid encoding a specific binding member of the present invention. Nucleic acid includes DNA and RNA. In a preferred aspect, the invention provides a nucleic acid which codes for a CDR or VH or VL domain of the invention as defined above.

[0101] The present invention also provides constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise at least one polynucleotide as above.

[0102] The present invention also provides a recombinant host cell which comprises one or more constructs as above. A nucleic acid encoding any CDR, VH or VL domain, or specific binding member as provided itself forms an aspect of the present invention, as does a method of production of the encoded product, which method comprises expression from encoding nucleic acid therefor. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression a VH or VL domain, or specific binding member may be isolated and/or purified using any suitable technique, then used as appropriate.

[0103] Specific binding members, VH and/or VL domains, and encoding nucleic acid molecules and vectors according to the present invention may be provided isolated and/or purified, e.g. from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes origin other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may comprise DNA or RNA and may be wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

[0104] Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells and many others. A common, preferred bacterial host is *E. coli*.

[0105] The expression of antibodies and antibody fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Plückthun, A. Bio/Technology 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a specific binding member, see for recent reviews, for example Ref, M.E. (1993) Curr. Opinion Biotech. 4: 573-576; Trill J.J. et al. (1995) Curr. Opinion Biotech 6: 553-560.

[0106] Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory

Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

[0107] Thus, a further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. A still further aspect provides a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

[0108] The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

[0109] In one embodiment, the nucleic acid of the invention is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques.

[0110] The present invention also provides a method which comprises using a construct as stated above in an expression system in order to express a specific binding member or polypeptide as above.

[0111] Other methods of the invention comprise conjugating or linking a specific binding member as described herein with a detectable label or anti-cancer agent.

[0112] Suitable labels and agents are described above. Labels and agents may be conjugated with a specific binding member using standard chemical means.

[0113] Aspects and embodiments of the present invention will now be illustrated by way of example with reference to the following experimentation.

[0114] Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

[0115] Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures described above and tables described below.

Figure 1 - Schematic representation of the small (A) and large (B) tenascin-C isoform. Several fibronectin type III like domains are subject to alternative splicing, either being included (B) or omitted (A) in the molecule.

Figure 2 shows the biodistribution of 4A1-F16-SIP antibody in U87 tumor xenograft bearing nude mice. (A) Values represent the average percent injected dose per gram of tissue within a group of three mice, three (upper) and six (lower) hours after injection. Error bars represent standard deviation.

Figure 3 shows the biodistribution of 4A1-F16-SIP antibody in U87 tumor xenograft bearing nude mice. (A) Values represent the average percent-injected dose per gram of tissue within a group of three mice, 24 (upper) and 48 hours (lower) hours after injection. Error bars represent standard deviation.

Figure 4 shows an alternative presentation of the biodistribution data shown in Figures 2 and 3.

Example 1

[0116] The gene encoding domain A1 of human tenascin-C was cloned by RT-PCR from mRNA isolated from normal human dermal fibroblasts (NHDF) cultured at pH 7.5 in the absence of serum using PCR oligos *TnC-A1 BamHI ba* (cgggatcctccactgaacaagccctgag) and *TnC-A1 BglII for* (ggagatcttcccctgtggaggcctcagc)[16]. The gene was then cloned into pQE12 bacterial expression vector (Qiagen) and expressed in E.coli TG-1. Purification was performed by means of the His-tag using Ni-NTA sepharose resin loaded with Nickel (Qiagen).

[0117] The purified domain A1 was biotinylated prior to selections, using sulfo-NHS-SS-biotin (Pierce). Biopanning was performed as described in (Viti F, et al Methods Enzymol 2000; 326:480-505). Briefly, biotinylated protein (final concentration 10^{-7} M) was incubated with 600 μ l preblocked ETH-2 library phage for 30 minutes. Bound phages were captured by addition of 5.3×10^7 streptavidin-coated magnetic beads (Dyna). After extensive washing, selected phage was eluted by reducing the disulphide bond in the biotin linker. Isolated phage was amplified in TG-1 and concentrated from the supernatant by polyethylene glycol precipitation. After three rounds of panning, 144 isolated antibody clones were screened by ELISA performed as described in Viti F, et al Methods Enzymol 2000; 326:480-505. Streptavidin-coated ELISA plates (StreptaWell High Bind, Roche) were incubated with biotinylated antigen, supernatant of induced monoclonal E.coli TG-1 cultures expressing scFv antibody fragments were added and bound antibody were detected with the M2 monoclonal antibody, followed by anti-mouse Immunoglobulin G - HRP conjugate. The antibody clones with

the highest signal were analyzed by real-time interaction analysis using a BIAcore 3000 instrument. The three best clones were purified on a protein A-sepharose column and tested by immunohistochemistry on various tumor cryosections.

[0118] One of the clones which exhibited a selective staining pattern in immunohistochemistry, scFv (3A1-D5), was chosen for affinity maturation. An affinity maturation library was constructed in a phagemid vector, pDN322 (Pini A et al. J Biol Chem 1998; 273:21769-21776), by inserting random mutations at positions 31-33 within the complementarity-determining region 1 of the variable heavy chain (VH CDR1) using PCR oligonucleotides *LMB3 long ba* (caggaaacagctatgacatgattac, priming upstream of the 5' end of the antibody gene) and *DP47CDR1mut* for (agcctggcgaccagctcgcmnmnmnmngctaaagggtgaatccagaggctgc). The 3' end of the antibody gene was amplified using primers *DP47CDR1 ba* (gagctgggtccgccaggctcc) and *fdseq long for* (gacgttagtaaatgaatttctgtatgagg). The two fragments were assembled by PCR using primers *LMB3 long ba* and *fdseq long for*.

[0119] Biopanning of the affinity maturation library was performed with biotinylated antigen. After two rounds of panning (as described above, but using 10⁻⁸M biotinylated antigen), a total of 382 antibody clones were screened by ELISA. 69 clones which were positive in ELISA were further characterized by M2-ELISA (Scheuermann J et al J Immunol Methods 2003; 276:129-134) to evaluate k_{off} values of the individual antibody clones. Briefly, supernatant containing scFv antibodies were added onto a surface coated with anti-Flag M2 monoclonal antibody (SIGMA). After addition of biotinylated antigen and incubation to the equilibrium, an excess of unbiotinylated antigen was added as competitor. After competition times of 0, 30, 60, 90 and 120 minutes, respectively, the remaining fraction of biotinylated antigen was detected using Streptavidin-HRP conjugate.

[0120] BIAcore was used to rank different ELISA-positive clones based on their dissociation profile. The five best clones were chosen for purification on an antigen-coated sepharose column. Purified antibodies were subject to size exclusion chromatography and resulting monomeric scFv antibody fragment fractions were used to determine affinity constants and kinetic parameters using BIAcore 3000.

[0121] The antibody with the best dissociation constant (K_D), 4A1-F16, was cloned into a bivalent minibody format, by genetically fusing the scFv(4A1-F16) sequence in front of the 5'-end of the gene encoding the CH4 domain of human immunoglobulin E, yielding a small immune protein (SIP) (Borsi L et al Int J Cancer 2002; 102:75-85), termed 4A1-F16-SIP. The CH4 domain promotes homodimerization, increasing functional affinity of the antibody due to the higher avidity.

[0122] Immunohistochemistry was performed with the anti-A1 domain of tenascin-C antibody fragment scFv(3A1-D5) on human head-and-neck cancer tissues. Primary antibody was detected by means of the peptidic FLAG-tag with was appended to the C-terminus of the scFv antibody, using the monoclonal anti-FLAG antibody M2 (SIGMA) followed by the APAAP system (DAKO). scFv(3A1-D5) and scFv(4A1-F16) were observed to strongly stain the tumor stroma and neovascular structures on cryosections of various head-and-neck cancers, whereas they did not react with normal mouth mucosa of a healthy donor.

[0123] The tumor targeting ability of 4A1-F16-SIP was assessed as follows:

[0124] Tumors were induced in Balb/C nu/nu mice by subcutaneous injection of 3x10⁶ U87 human glioblastoma cells per mouse (ATCC). 20 to 25 days post injection, when tumors reached a size of 300-1500 mm³, radiolabeled antibody was injected.

[0125] The preparation of the antibody included further purification of affinity-purified 4A1-F16-SIP antibody by size exclusion chromatography on superdex 75. The fraction representing the homodimeric form (75kDa) was collected and subsequently labeled with iodine-125 (Amersham) using iodogen (Pierce).

[0126] Approximately 5 µg of antibody were injected intravenously into the tail vein of tumor bearing mice. After 3, 6, 24 and 48 hours, respectively, three mice were sacrificed, the organs excised and accumulation of I-125 determined in a γ-counter.

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Sequences

[0128]

SEQ ID NO: 1. 4A1-F16 VH domain nucleotide sequence

GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG
 GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC
 CGG TAT GGT GCG AGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG
 GAG TGG GTC TCA GCT ATT AGT GGT AGT GGT GGT AGC ACA TAC TAC
 GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC
 AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC
 ACG GCC GTA TAT TAC TGT GCG AAA GCG CAT AAT GCT TTT GAC TAC
 TGG GGC CAG GGA ACC CTG GTC ACC GTG TCG AGA

SEQ ID NO: 2 4A1-F16 VH domain amino acid sequence
 EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYGASWVRQA PGKGLEWVSA
 ISGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKAH
 NAFDYWGQGT LTVTSR

SEQ ID NO: 3 4A1-F16 & 3A1-D5 VL domain nucleotide sequence

TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA
CAG ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT
TAT GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT
GTC ATC TAT GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA
TTC TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT
GGG GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TCT
GTT TAT ACT ATG CCG CCC GTG GTA TTC GGC GGA GGG ACC AAG CTG
ACC GTC CTA GGC

SEQ ID NO: 4 4A1-F16 & 3A1-D5 VL domain amino acid sequence

SSELTQDPVAV SVALGQTVRI TCQGDSLRSY YASWYQQKPG QAPVLVIYVK
NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSS VYTMPPVVFG
GGTKLTVLG

SEQ ID NO: 5 4A1-F16 VH CDR1 amino acid sequence

RYGAS

SEQ ID NO: 6 4A1-F16 & 3A1-D5 VH CDR2 amino acid sequence

AISGSGGSTYYADSVKG

SEQ ID NO: 7 4A1-F16 & 3A1-D5 VH CDR3 amino acid sequence

AHNAFDY

SEQ ID NO: 8 4A1-F16 & 3A1-D5 VL CDR1 amino acid sequence

QGDSLRSYYAS

SEQ ID NO: 9 4A1-F16 & 3A1-D5 VL CDR2 amino acid sequence

GKNNRPS

SEQ ID NO: 10 4A1-F16 & 3A1-D5 VL CDR3 amino acid sequence

NSSVYTMPPVV

SEQ ID NO: 11 3A1-D5 VH domain nucleotide sequence

GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG
GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC
AGC TAT GCC GCG AGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG
GAG TGG GTC TCA GCT ATT AGT GGT AGT GGT GGT AGC ACA TAC TAC
GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC
AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC
ACG GCC GTA TAT TAC TGT GCG AAA GCG CAT AAT GCT TTT GAC TAC
TGG GGC CAG GGA ACC CTG GTC ACC GTG TCG AGA

SEQ ID NO: 12 3A1-D5 VH domain amino acid sequence
 EVQLLES GGG LVQPGGSLRL SCAASGFTFS SYAASWVRQA PGKGLEWVSA
 5 ISGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKAH
 NAFDYWGQGT LVTVSR

10 SEQ ID NO : 13 3A1-D5 VH CDR1 amino acid sequence
 SYAAS

SEQ ID NO: 14 E10 VH domain nucleotide sequence
 15 GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG
 GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC
 20 GGT AGT CGT ATG GGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG
 GAG TGG GTC TCA GCT ATT AAT GAG GAG GGT GGT CAG ACA TAC TAC
 25 GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC
 AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC
 ACG GCC GTA TAT TAC TGT GCG AAA CAT CCG CCG CAT CGG CCG TTT
 30 GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTG TCG AGA

SEQ ID NO: 15 E10 VH domain amino acid sequence
 35 EVQLVESGGG LVQPGGSLRL SCAASGFTFS GSRMGWVRQA PGKGLEWVSA
 INEEGGQTYT ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKHP
 PHRPFDYWGQ GTLVTVSR

SEQ ID NO: 16. E10 & A12 VL domain nucleotide sequence
 45 TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA
 CAG ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT
 TAT GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT
 GTC ATC TAT GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA
 50 TTC TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT
 GGG GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TCT
 CAT GGG CCC CGT AGG CCT GTG GTA TTC GGC GGA GGG ACC AAG CTG
 55 ACC GTC CTA GGC

SEQ ID NO: 17 E10 & A12 VL domain amino acid sequence
 SSELTDPAV SVALGQTVRI TCQGDSLRSY YASWYQQKPG QAPVLVIYGK
 5 NNRPSGIPDRFSGSSSGNTA SLTITGAQAE DEADYYCNSS HGPRRPVVFG
 GGTKLTVLG

10 SEQ ID NO : 18 E10, F4 & G11 VH CDR1 amino acid sequence
 GSRMG
 SEQ ID NO : 19 E10, F4 & G11 VH CDR1 amino acid sequence
 AINEEGGQTYADSVKG
 SEQ ID NO : 20 E10, A12, F4 & G11 VH CDR3 amino acid sequence
 15 HPPHRPFDY
 SEQ ID NO : 21 E10 & A12 VL CDR1 amino acid sequence
 QGDSLRSYYAS
 SEQ ID NO : 22 E10, A12 & G11 VL CDR2 amino acid sequence
 GKNNRPS
 20 SEQ ID NO : 23 E10 & A12 VL CDR3 amino acid sequence
 NSSHGPRRPVV

25 SEQ ID NO: 24 A12 VH domain nucleotide sequence
 GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG
 GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC
 AGC TAT GCC ATG AGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG
 30 GAG TGG GTC TCA GCT ATT AGT GGT AGT GGT GGT AGC ACA TAC TAC
 GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC
 AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC
 35 ACG GCC GTA TAT TAC TGT GCG AAA CAT CCG CCG CAT CGG CCG TTT
 GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTG TCG AGA

40 SEQ ID NO 25 A12 VH domain amino acid sequence
 EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYAMSWVRQA PGKGLEWVSA
 45 ISGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKHP
 PHRPFDYWGQ GTLVTVSR

50 SEQ ID NO : 26 A12 VH CDR1 amino acid sequence
 SYAMS
 SEQ ID NO : 27 A12 VH CDR2 amino acid sequence
 AISGSGGSTYYADSVKG
 55

SEQ ID NO: 28 F4 & G11 VH domain nucleic acid sequence

GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG
 GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC
 GGT AGT CGT ATG GGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG
 GAG TGG GTC TCA GCT ATT AAT GAG GAG GGT GGT CAG ACA TAC TAC
 GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC
 AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

ACG GCC GTA TAT TAC TGT GCG AAA CAT CCG CCG CAT CGG CCG TTT
 GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCG AGA

SEQ ID NO: 29 F4 & G11 VH domain amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSGSRMGWVRQAPGKGLEWVSAINEEGGQTTY
 ADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKHPPHRPFDYWGQGLTVTSR

SEQ ID NO: 30 F4 VL domain nucleic acid sequence

TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA
 CAG ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA CTT TAT
 TAT GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT
 GTC ATC TAT GGT AAA TCT AGT CGG CCC TCA GGG ATC CCA GAC CGA
 TTC TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT
 GGG GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TCT
 CAT GGG CCC CGT AGG CCT GTG GTA TTC GGC GGA GGG ACC AAG CTG
 ACC GTC CTA GGC

SEQ ID NO: 31 F4 VL domain amino acid sequence

SSELTQDPAVSVALGQTVRITCQGDSLRLYYASWYQQKPGQAPVLVIYGKSSRPSGIPDR
 FSGSSSGNTASLTITGAQAEDEADYYCNSSHGPRRPVVFGGGTKLTVLG

SEQ ID NO : 32 F4 & G11 VL CDR1 amino acid sequence

QGDSLRLYYAS

SEQ ID NO : 33 F4 VL CDR2 amino acid sequence

GKSSRPS

SEQ ID NO: 34 G11 VL domain nucleic acid sequence

5 TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA
CAG ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA CTT TAT
TAT GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT
GTC ATC TAT GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA
10 TTC TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT
GGG GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TCT
CAT GGG CCC CGT AGG CCT GTG GTA TTC GGC GGA GGG ACC AAG CTG
15 ACC GTC CTA GGC

20 SEQ ID NO: 35 G11 VL domain amino acid sequence

SSELTQDPAVSVALGQTVRITCQGDSLRLYYASWYQQKPGQAPVLVIYGKNNRPSGIPDR
FSGSSSGNTASLTITGAQAEDEADYCNSSHGPRRPVVFGGGTKLTVLG

25 SEQ ID NO : 36 Peptide linker nucleic acid sequence

GGT GGA GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA

SEQ ID NO : 37 Peptide linker amino acid sequence

GGGGSGGGSGGGG

30 SEQ ID NO : 38 peptide linker nucleic acid sequence

GGT GGA GGC GGT TCA GGC GGA GGT GGT TCT GGC GGT GGC GGA TCG

SEQ ID NO : 39 Peptide linker amino acid sequence

GGGGSGGGSGGGGS

SEQ ID NO : 40 Peptide linker nucleic acid sequence

35 TCT TCC TCA TCG GGT AGT AGC TCT TCC GGC TCA TCG TCC AGC GGC

SEQ ID NO : 41 Peptide linker amino acid sequence

SSSSGSSSSGSSSSG

SEQ ID NO : 42 Peptide linker amino acid sequence

GSGSAGSGSAGSGSA

40 SEQ ID NO : 43 Peptide linker amino acid sequence

GGSGGGSGGGSGG

45 SEQ ID NO: 44 New F4 & G11 VH domain nucleic acid sequence

GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG
GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC
GGT AGT CGT ATG GGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG
50 GAG TGG GTC TCA GCT ATT AAT GAG GAG GGT GGT CAG ACA TAC TAC
GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC
AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAA GAC
55 ACG GCC GTA TAT TAC TGT GCG AAA CAT CCG CCG CAT CGG CCG TTT
GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCG AGT

SEQ ID NO: 45 New F4 & G11 VH domain amino acid sequence

EVQLLESGGGLVQPGGSLRLSCAASGFTFSGSRMGWVRQAPGKGLEWVSAINEEGGQTYAD
SVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKHPPHRPFDYWGQGLTVTVSS

SEQ ID NO: 46 New G11 VL domain nucleic acid sequence

TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA CTT TAT TAT
GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC
ATC TAT GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC
TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG
GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TCT CAT
GGG CCC CGT AGG CCT GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC
GTC CTA GGC

SEQ ID NO: 47 New G11 VL domain amino acid sequence

SELTQDPAVSVALGQTVRITCQGDSLRLYYASWYQQKPGQAPV LVIYGKNNRPSGIPDR
FSGSSSGNTASLTITGAQAEDEADYYCNS SHGPRRPVVFGGGTKLTVLG

SEQ ID NO: 48 New E10 VH domain amino acid sequence

EVQLLESGGG LVQPGGSLRL SCAASGFTFS GSRMGWVRQA PGKGLEWVSA
INEEGGQTYY ADSVKGRFTI SRDN SKNTLY LQMNSLRAED TAVYYCAKHP
PHRPFDYWGQ GTLVTVSS

SEQ ID NO: 49 New 4A1-F16 & 3A1-D5 VL domain nucleotide
sequence

5 TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT
GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC
10 ATC TAT GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC
TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG
GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TCT GTT
15 TAT ACT ATG CCG CCC GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC
GTC CTA GGC

20

SEQ ID NO: 50 New 4A1-F16 & 3A1-D5 VL domain amino acid
sequence

25 SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDR
FSGSSSGNTASLTITGAQAEDEADYYCNSSVYTMPPVVFGGGTKLTVLG

30

SEQ ID NO: 51 P12 VH domain nucleic acid sequence

35 GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTC
CTGTGCAGCCTCTGGATTACCTTTGGCCAGTATTCTATGAGCTGGGTCCGCCAGGCTCCAG
GGAAGGGGCTGGAGTGGGTCTCAGCTATTACGGGGACTGGTGGTGAGACATACTACGCAGAC
TCCGTGGAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAAAT
40 GAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGGGCGGCGGATTTTTC
ACTACTGGGGCCAGGGAACCCTGGTCACCGTGTGCGAGA

45

SEQ ID NO: 52 P12 VH domain amino acid sequence

50 EVQLVESGGGLVQPGGSLRLSCAASGFTFGQYSMSWVRQAPGKGLEWVSAITGTGGETYY
ADSVEGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGRRIFDYWGQGLVTVSR

SEQ ID NO: 53 P12 and D11 VL domain nucleic acid sequence

TCGAGTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCAC
 ATGCCAAGGAGACAGCCTCAGACGGCAGCCTGCAAGCTGGTACCAGCAGAAGCCAGGACAGG
 CCCCTGTACTTGTTCATCTATTATAAAAAGCTGCGGCCCTCAGGGATCCCAGACCGATTCTCT
 GGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATGAGGC
 TGACTATTACTGTAACCTCTTTTCGCCCAAGCCGAAGCCTGTGGTATTTCGGCGGAGGGACCA
 AGCTGACCGTCCTAGGC

SEQ ID NO: 54 P12 and D11 VL domain amino acid sequence

SSELTQDPAVSVALGQTVRITCQGDSLRRQPASWYQQKPGQAPVLVIYYKKLRPSGIPDR
 FSGSSSGNTASLTITGAQAEDEADYYCNSFSPKPKPVVFGGGTKLTVLG

SEQ ID NO: 55 D11 VH domain nucleic acid sequence

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTC
 CTGTGCAGCCTCTGGATTACCTTTGGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAG
 GGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGAC
 TCCGTGGAGGGCCGGTTACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAAT
 GAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGGGCGGCGGATTTTTG
 ACTACTGGGGCCAGGGAACCCTGGTCACCGTGTGCGAGA

SEQ ID NO: 56 D11 VH domain amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYAMSWVRQAPGKGLEWVSAISGSGGSTYY
 ADSVEGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGRRIFDYWGQGTLVTVSR

SEQ ID NO: 57 F4S VH domain nucleic acid sequence

GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG
 GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC
 AGC TAT GCC ATG AGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG
 GAG TGG GTC TCA GCT ATT AGT GGT AGT GGT GGT AGC ACA TAC TAC
 GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC
 AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC
 ACG GCC GTA TAT TAC TGT GCG AAA GGG CGG CGG ATT TTT GAC TAC
 TGG GGC CAG GGA ACC CTG GTC ACC GTG TCG AGA

SEQ ID NO: 58 F4S VH domain amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY
 ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGRRIFDYWGQGTLLVTVSR

SEQ ID NO: 59 F4S VL domain nucleic acid sequence

TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA
 CAG ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT
 TAT GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT
 GTC ATC TAT GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA
 TTC TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT
 GGG GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TTT
 TCG CCC AAG CCG AAG CCT GTG GTA TTC GGC GGA GGG ACC AAG CTG
 ACC GTC CTA GGC

SEQ ID NO: 60 F4S VL domain amino acid sequence

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDR
 FSGSSSGNTASLTITGAQAEDEADYYCNSFSPKPKPVVFGGGTKLTVLG

SEQ ID NO : 61 P12 VH CDR1 amino acid sequence

QYSMS

SEQ ID NO : 62 D11 and F4S VH CDR1 amino acid sequence

SYAMS

SEQ ID NO : 63 P12 VH CDR2 amino acid sequence

AITGTGGETYYADSVGE

SEQ ID NO : 64 D11 VH CDR2 amino acid sequence

AISGSGGSTYYADSVGE

SEQ ID NO : 65 F4S VH CDR2 amino acid sequence

AISGSGGSTYYADSVKG

SEQ ID NO : 66 P12, D11 and F4S VH CDR3 amino acid sequence

GRRIFDY

SEQ ID NO : 67 P12 and D11 VL CDR1 amino acid sequence

QGD SLRRQPAS

SEQ ID NO : 68 F4S VL CDR1 amino acid sequence

QGD SLRSYYAS

SEQ ID NO : 69 P12 and D11 VL CDR2 amino acid sequence

YKKLRPS

SEQ ID NO : 70 F4S VL CDR2 amino acid sequence

GKNNRPS

SEQ ID NO : 71 P12, D11 and F4S VL CDR3 amino acid sequence

NSFSPKPKPVV

SEQ ID NO : 72 PCR oligonucleotide primer *TnC-D BamHI* ba

cgggatccgttacagaagccgaaccggaa

SEQ ID NO : 73 PCR oligonucleotide primer *TnC-D BglII* for

cgggatccgttacagaagccgaaccggaa

SEQ ID NO : 74 PCR oligonucleotide primer mmTnC-A1 EcoBa

agaattcattaaagaggagaaattaactatgagaggatcctccacggaagaagtccttc

SEQ ID NO : 75 PCR oligonucleotide primer mmTnC-A1 BglFo

tgagatctgtccctgtgaggtctcggc

SEQ ID NO : 76 Peptide linker motif

GGGS

SEQ ID NO : 77 Peptide linker motif

SSSSG

SEQ ID NO : 78 Peptide linker motif

GSGSA

SEQ ID NO : 79 Peptide linker motif

GGSGG

SEQ ID NO: 80. New E10 & A12 VL domain nucleotide sequence

TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG

ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT

GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC

ATC TAT GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC

TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG

GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TCT CAT

GGG CCC CGT AGG CCT GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC

GTC CTA GGC

SEQ ID NO: 81 New E10 & A12 VL domain amino acid sequence

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVPLVIYGKNNRPSGIPDR

FSGSSSGNTASLTITGAQAEDEADYYCNSSHGPRRPVVFGGGTKLTVLG

SEQ ID NO: 82 New F4 VL domain nucleic acid sequence

TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA CTT TAT TAT
 GCA AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC
 ATC TAT GGT AAA TCT AGT CGG CCC TCA GGG ATC CCA GAC CGA TTC
 TCT GGC TCC AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG
 GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC TCC TCT CAT
 GGG CCC CGT AGG CCT GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC
 GTC CTA GGC

SEQ ID NO: 83 New F4 VL domain amino acid sequence

SELTQDPAVSVALGQTVRITCQGDSLRLYYASWYQQKPGQAPVTLVIYGKSSRPSGIPDR
 FSGSSSGNTASLTITGAQAEDEADYYCNSSHGPRRPVVFEGGTKLTVLG

Claims

1. An isolated specific binding member that binds human tenascin-C and which comprises;

an antibody VH domain comprising VH CDRs 1, 2 and 3 wherein:

VH CDR1 is SEQ ID NO: 5 or SEQ ID NO: 13;
 VH CDR2 is SEQ ID NO: 6; and
 VH CDR3 is SEQ ID NO: 7;

and

an antibody VL domain comprising VL CDRs 1, 2 and 3 wherein:

VL CDR1 is SEQ ID NO. 8;
 VL CDR2 is SEQ ID NO. 9; and
 VL CDR3 is SEQ ID NO. 10.

2. A specific binding member according to claim 1, wherein VH CDR1 is SEQ ID NO: 5.
3. A specific binding member according to claim 1 or claim 2, comprising the antibody VH domain of SEQ ID NO: 2, or a VH domain having less than 5 amino acid alterations in SEQ ID NO: 2.
4. A specific binding member according to claim 1, comprising the VH domain sequence of SEQ ID NO: 12, or a VH domain having less than 5 amino acid alterations in SEQ ID NO: 12.
5. A specific binding member according to any of the preceding claims, comprising the VL domain sequence of SEQ ID NO: 50, or a VL domain having less than 5 amino acid alterations in SEQ ID NO: 4 or SEQ ID NO: 50.
6. A specific binding member according to claim 3, comprising the VH domain sequence of SEQ ID NO: 2 and the VL domain sequence of SEQ ID NO: 50.
7. An isolated specific binding member that binds human tenascin-C, comprising an antibody VH domain with amino acid sequence SEQ ID NO: 2 or an amino acid sequence having less than 5 amino acid sequence alterations in SEQ ID NO: 2 and

an antibody VL domain with amino acid sequence SEQ ID NO: 50 or an amino acid sequence having less than 5 amino acid sequence alterations in SEQ ID NO: 50, wherein the binding member binds tenascin-C with an affinity of or greater than the affinity of an antibody comprising D5 VH domain SEQ ID NO: 12 and VL domain SEQ ID NO: 50.

8. A specific binding member according to claim 6 or claim 7, comprising a small immune protein (SIP), wherein the SIP comprises an scFv molecule comprising the VH domain sequence of SEQ ID NO: 2 and the VL domain sequence of SEQ ID NO: 50, fused to the CH4 domain of human immunoglobulin E.
9. A specific binding member according to claim 8, wherein the SIP is homodimeric.
10. A specific binding member according to any one of claims 1 to 7 that comprises an scFv antibody molecule.
11. A specific binding member according to any one of claims 1 to 7 that comprises an antibody constant region.
12. A specific binding member according to claim 11 that comprises a whole antibody.
13. A specific binding member according to any one of claims 1 to 12 conjugated to a detectable label or to a cytokine.
14. A specific binding member according to claim 13, wherein a VH domain or VL domain of the specific binding member is conjugated to a cytokine via a peptide linker as a fusion protein.
15. A specific binding member according to claim 13 or claim 14, wherein the cytokine is IL2.
16. A specific binding member according to any one of claims 1 to 13 conjugated to a cytotoxic agent.
17. An isolated nucleic acid which comprises a nucleotide sequence encoding a specific binding member or antibody VH or VL domain of a specific binding member according to any one of claims 1 to 12.
18. A host cell transformed with nucleic acid according to claim 17.
19. A method of producing a specific binding member or antibody VH or VL domain, the method comprising culturing host cells according to claim 18 under conditions for production of said specific binding member or antibody VH or VL domain.
20. A method according to claim 19 further comprising isolating and/or purifying said specific binding member or antibody VH or VL variable domain.
21. A method according to claim 20 comprising conjugating the specific binding member or antibody VH or VL variable domain with a detectable label or cytotoxic agent.
22. A method according to claim 21 wherein the label is a radionuclide or flurophor.
23. A method according to any one of claims 19 to 22 further comprising formulating the specific binding member or antibody VH or VL variable domain into a composition including at least one additional component.
24. A method according to any one of claims 19 to 23 further comprising binding the specific binding member with tenascin-C or a fragment of tenascin-C *in vitro*.
25. A method comprising binding a specific binding member according to any one of claims 1 to 16 to tenascin-C or a fragment of tenascin-C *in vitro*.
26. A method according to claim 24 or claim 25 comprising determining the amount of binding of specific binding member.
27. A specific binding member according to any one of claims 1 to 16 for use in a method of treatment or diagnosis.
28. Use of specific binding member according to any one of claims 1 to 16 in the manufacture of a medicament for use

in a method of treatment or *in vivo* diagnosis of a proliferative disorder.

Patentansprüche

1. Isoliertes spezifisches Bindungselement, das menschliches Tenascin-C bindet und welches Folgendes umfasst:

eine Antikörper-VH-Domäne, umfassend VH-CDRs 1, 2 und 3, worin:

VH-CDR1 Seq.-ID Nr. 5 oder Seq.-ID Nr. 13 ist;
VH-CDR2 Seq.-ID Nr. 6 ist; und
VH-CDR3 Seq.-ID Nr. 7 ist;
und

eine Antikörper-VL-Domäne, umfassend VL-CDRs 1, 2 und 3, worin:

VL-CDR1 Seq.-ID Nr. 8 ist;
VL-CDR2 Seq.-ID Nr. 9 ist; und
VL-CDR3 Seq.-ID Nr. 10 ist.

2. Spezifisches Bindungselement nach Anspruch 1, worin VH-CDR1 Seq.-ID Nr. 5 ist.

3. Spezifisches Bindungselement nach Anspruch 1 oder Anspruch 2, umfassend die Antikörper-VH-Domäne aus Seq.-ID Nr. 2 oder eine VH-Domäne mit weniger als 5 Aminosäure-Veränderungen in Seq.-ID Nr. 2.

4. Spezifisches Bindungselement nach Anspruch 1, umfassend die VH-Domänen-Sequenz aus Seq.-ID Nr. 12 oder eine VH-Domäne mit weniger als 5 Aminosäure-Veränderungen in Seq.-ID Nr. 12.

5. Spezifisches Bindungselement nach einem der vorangegangenen Ansprüche, umfassend die VL-Domänen-Sequenz aus Seq.-ID Nr. 50 oder eine VL-Domäne mit weniger als 5 Aminosäure-Veränderungen in Seq.-ID Nr. 4 oder Seq.-ID Nr. 50.

6. Spezifisches Bindungselement nach Anspruch 3, umfassend die VH-Domänen-Sequenz aus Seq.-ID Nr. 2 und die VL-Domänen-Sequenz aus Seq.-ID Nr. 50.

7. Isoliertes spezifisches Bindungselement, das menschliches Tenascin-C bindet und welches Folgendes umfasst:

eine Antikörper-VH-Domäne mit Aminosäure-Sequenz Seq.-ID Nr. 2 oder einer Aminosäure-Sequenz mit weniger als 5 Aminosäuresequenzveränderungen in Seq.-ID Nr. 2 und
eine Antikörper-VL-Domäne mit Aminosäure-Sequenz Seq.-ID Nr. 50 oder einer Aminosäure-Sequenz mit weniger als 5 Aminosäuresequenzveränderungen in Seq.-ID Nr. 50,
worin das Bindungselement Tenascin-C mit einer Affinität von oder größer der Affinität eines Antikörpers bindet, der die D5-VH-Domäne Seq.-ID Nr. 12 und die VL-Domäne Seq.-ID Nr. 50 umfasst.

8. Spezifisches Bindungselement nach Anspruch 6 oder Anspruch 7, umfassend ein kleines Immunprotein (SIP), worin das SIP ein scFv-Molekül umfasst, das die VH-Domänen-Sequenz von Seq.-ID Nr. 2 sowie die VL-Domänen-Sequenz von Seq.-ID Nr. 50 umfasst, fusioniert an die CH4-Domäne von menschlichem Immunglobulin E.

9. Spezifisches Bindungselement nach Anspruch 8, worin das SIP homodimer ist.

10. Spezifisches Bindungselement nach einem der Ansprüche 1 bis 7, das ein scFv-Antikörper-Molekül umfasst.

11. Spezifisches Bindungselement nach einem der Ansprüche 1 bis 7, das eine konstante Antikörper-Region umfasst.

12. Spezifisches Bindungselement nach Anspruch 11, das einen ganzen Antikörper umfasst.

13. Spezifisches Bindungselement nach einem der Ansprüche 1 bis 12, konjugiert an eine detektierbare Markierung oder an ein Zytokin.

14. Spezifisches Bindungselement nach Anspruch 13, worin eine VH-Domäne oder eine VL-Domäne des spezifischen Bindungselements durch einen Peptidlinker als ein Fusionsprotein an ein Zytokin konjugiert ist.
15. Spezifisches Bindungselement nach Anspruch 13 oder Anspruch 14, worin das Zytokin IL2 ist.
16. Spezifisches Bindungselement nach einem der Ansprüche 1 bis 13, konjugiert an ein zytotoxisches Mittel.
17. Isolierte Nucleinsäure, umfassend eine Nucleotidsequenz, die für ein spezifisches Bindungselement oder eine Antikörper-VH- oder -VL-Domäne eines spezifischen Bindungselements nach einem der Ansprüche 1 bis 12 kodiert.
18. Wirtszelle, die mit Nucleinsäure nach Anspruch 17 transformiert ist.
19. Verfahren zur Produktion eines spezifischen Bindungselements oder einer Antikörper-VH- oder -VL-Domäne, wobei das Verfahren das Züchten von Wirtszellen nach Anspruch 18 unter Bedingungen zur Produktion des spezifischen Bindungselements oder der spezifischen Antikörper-VH- oder -VL-Domäne umfasst.
20. Verfahren nach Anspruch 19, weiters umfassend das Isolieren und/oder das Reinigen des spezifischen Bindungselements oder der variablen Antikörper-VH- oder -VL-Domäne.
21. Verfahren nach Anspruch 20, umfassend das Konjugieren des spezifischen Bindungselements oder der spezifischen variablen Antikörper-VH- oder -VL-Domäne mit einer detektierbaren Markierung oder einem zytotoxischen Mittel.
22. Verfahren nach Anspruch 21, worin die Markierung ein Radionuclid oder ein Fluorophor ist.
23. Verfahren nach einem der Ansprüche 19 bis 22, weiters umfassend das Formulieren des spezifischen Bindungselements oder der variablen Antikörper-VH- oder -VL-Domäne in eine Zusammensetzung, die zumindest eine zusätzliche Komponente umfasst.
24. Verfahren nach einem der Ansprüche 19 bis 23, weiters umfassend das In-vitro-Binden des spezifischen Bindungselements mit Tenascin-C oder einem Fragment von Tenascin-C.
25. Verfahren, umfassend das In-vitro-Binden eines spezifischen Bindungselements nach einem der Ansprüche 1 bis 16 an Tenascin-C oder ein Fragment von Tenascin-C.
26. Verfahren nach Anspruch 24 oder Anspruch 25, umfassend das Bestimmen der Bindungsmenge des spezifischen Bindungselements.
27. Spezifisches Bindungselement nach einem der Ansprüche 1 bis 16 zur Verwendung in einem Behandlungs- oder Diagnoseverfahren.
28. Verwendung eines spezifischen Bindungselements nach einem der Ansprüche 1 bis 16 zur Herstellung eines Medikaments zur Verwendung in einem Behandlungsverfahren oder zur In-vivo-Diagnose einer proliferativen Störung.

Revendications

1. Élément de liaison spécifique isolé qui se lie à une tenascin-C humaine et qui comprend:

un domaine VH des anticorps comprenant des VH CDRs 1, 2 et 3 dans lequel:

VH CDR1 est SEQ ID NO: 5 ou SEQ ID NO: 13;
 VH CDR2 est SEQ ID NO: 6; et
 VH CDR3 est SEQ ID NO: 7;
 et

un domaine VL des anticorps comprenant des VL CDRs 1, 2 et 3 dans lequel:

VL CDR1 est SEQ ID NO. 8;

VL CDR2 est SEQ ID NO. 9; et
VL CDR3 est SEQ ID NO. 10.

2. Elément de liaison spécifique selon la revendication 1, où le VH CDR1 est SEQ ID NO: 5.
3. Elément de liaison spécifique selon la revendication 1 ou la revendication 2, comprenant le domaine VH des anticorps de la SEQ ID NO: 2 ou bien un domaine VH ayant moins de 5 altérations des acides aminés dans la SEQ ID NO: 2.
4. Elément de liaison spécifique selon la revendication 1, comprenant la séquence de domaine VH de la SEQ ID NO: 12, ou un domaine VH ayant moins de 5 altérations des acides aminés dans la SEQ ID NO: 12.
5. Elément de liaison spécifique selon l'une quelconque des revendications précédentes, comprenant la séquence de domaine VL de la SEQ ID NO: 50 ou bien un domaine VL ayant moins de 5 altérations des acides aminés dans la SEQ ID NO: 4 ou la SEQ ID NO: 50.
6. Elément de liaison spécifique selon la revendication 3, comprenant la séquence de domaine VH de la SEQ ID NO: 2 et la séquence de domaine VL de la SEQ ID NO: 50.
7. Elément de liaison spécifique isolé qui se lie à une tenascine-C humaine, comprenant un domaine VH des anticorps avec une séquence d'acides aminés SEQ ID NO: 2 ou bien une séquence d'acides aminés ayant moins de 5 altérations de la séquence des acides aminés dans SEQ ID NO: 2 et un domaine VL des anticorps avec une séquence des acides aminés SEQ ID NO: 50 ou bien une séquence des acides aminés ayant moins de 5 altérations de la séquence des acides aminés dans SEQ ID NO: 50, où l'élément de liaison se lie à la tenascine-C avec une affinité égale ou supérieure à l'affinité d'un anticorps comprenant un domaine VH D5 SEQ ID NO: 12 et un domaine VL SEQ ID NO: 50.
8. Elément de liaison spécifique selon la revendication 6 ou la revendication 7, comprenant une petite protéine immune (SIP), où la SIP comprend une molécule scFv comprenant la séquence de domaine VH de la SEQ ID NO: 2 et la séquence de domaine VL de la SEQ ID NO: 50, fusionnée au domaine CH4 de l'immunoglobuline humaine E.
9. Elément de liaison spécifique selon la revendication 8, où la SIP est homodimère.
10. Elément de liaison spécifique selon l'une quelconque des revendications 1 à 7, qui comprend une molécule d'anticorps scFv.
11. Elément de liaison spécifique selon l'une quelconque des revendications 1 à 7, qui comprend une région constante d'anticorps.
12. Elément de liaison spécifique selon la revendication 11, qui comprend un anticorps entier.
13. Elément de liaison spécifique selon l'une quelconque des revendications 1 à 12, conjugué à un traceur détectable ou à une cytokine.
14. Elément de liaison spécifique selon la revendication 13, où un domaine VH ou domaine VL de l'élément de liaison spécifique est conjugué à une cytokine par un lien peptidique comme une protéine de fusion.
15. Elément de liaison spécifique selon la revendication 13 ou la revendication 14, où la cytokine est IL2.
16. Elément de liaison spécifique selon l'une quelconque des revendications 1 à 13 conjugué à un agent cytotoxique.
17. Acide nucléique isolé qui comprend une séquence de nucléotide codant pour un élément de liaison spécifique ou VH des anticorps ou domaine VL d'un élément de liaison spécifique selon l'une quelconque des revendications 1 à 12.
18. Cellule hôte transformée avec de l'acide nucléique selon la revendication 17.
19. Méthode de production d'un élément de liaison spécifique ou VH des anticorps ou domaine VL, la méthode comprenant la culture de cellules hôtes selon la revendication 18 sous des conditions pour la production dudit élément de liaison spécifique ou du VH des anticorps ou domaine VL.

20. Méthode selon la revendication 19, comprenant en outre l'isolation et/ou la purification dudit élément de liaison spécifique ou VH des anticorps ou domaine variable VL.

5 21. Méthode selon la revendication 20, comprenant la conjugaison de l'élément de liaison spécifique ou du VH des anticorps ou du domaine variable VL avec un traceur détectable ou agent cytotoxique.

22. Méthode selon la revendication 21, où le traceur est un radionucléide ou fluorophore.

10 23. Méthode selon l'une quelconque des revendications 19 à 22, comprenant en outre la formulation de l'élément de liaison spécifique ou du VH des anticorps ou du domaine variable VL en une composition incluant au moins un composant additionnel.

15 24. Méthode selon l'une quelconque des revendications 19 à 23, comprenant en outre la liaison de l'élément de liaison spécifique à la tenascine-C ou un fragment de la tenascine-C *in vitro*.

25 25. Méthode comprenant la liaison d'un élément de liaison spécifique selon l'une quelconque des revendications 1 à 16 à la tenascine-C ou un fragment de la tenascine-C *in vitro*.

20 26. Méthode selon la revendication 24 ou la revendication 25, comprenant la détermination de la quantité de liaison de l'élément de liaison spécifique.

27. Élément de liaison spécifique selon l'une quelconque des revendications 1 à 16 pour utilisation dans une méthode de traitement ou de diagnostic.

25 28. Utilisation d'un élément de liaison spécifique selon l'une quelconque des revendications 1 à 16 dans la fabrication d'un médicament pour utilisation dans une méthode de traitement ou de diagnostic *in vivo* d'un trouble de prolifération.

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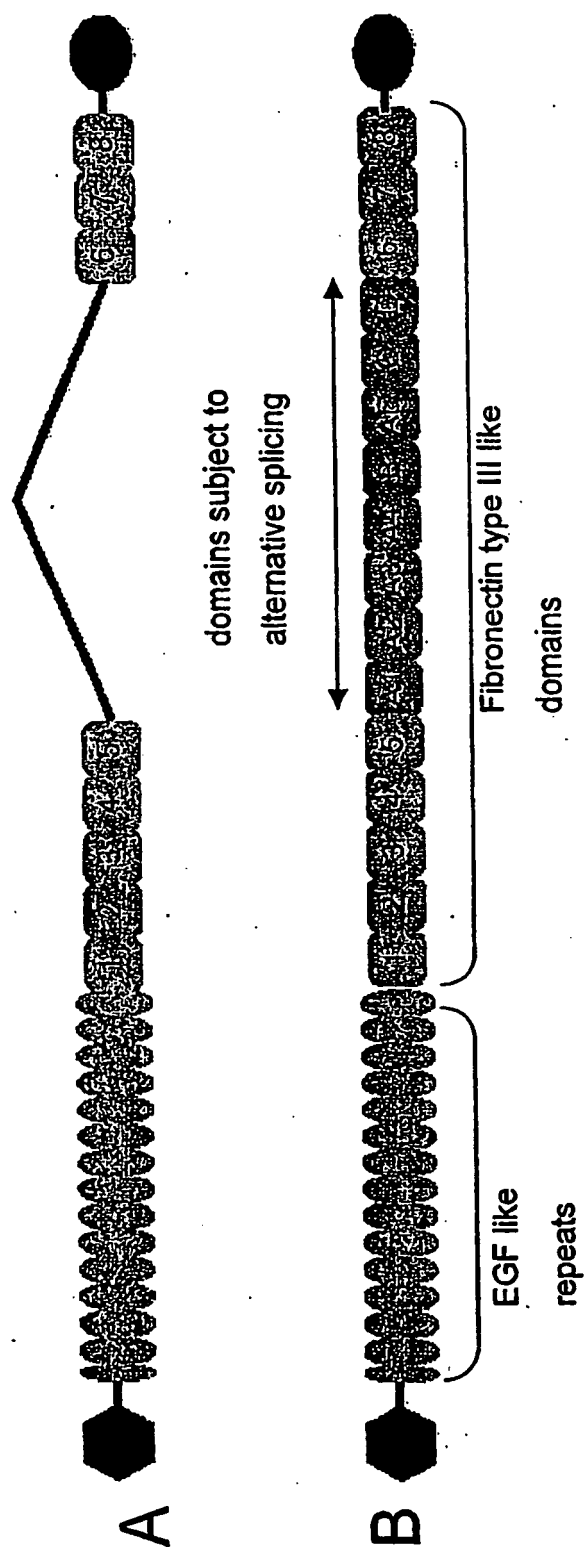


Figure 1

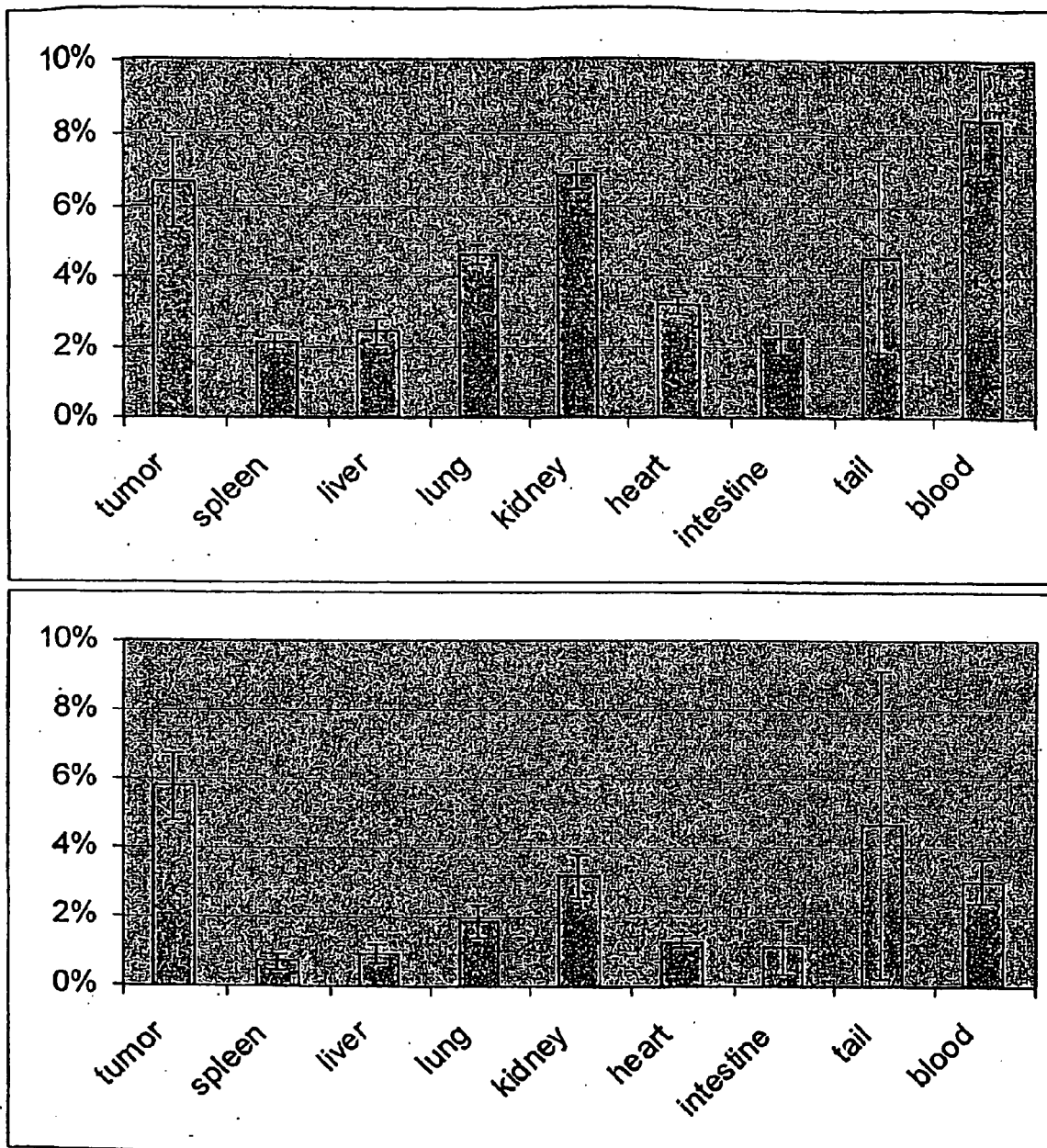


Figure 2

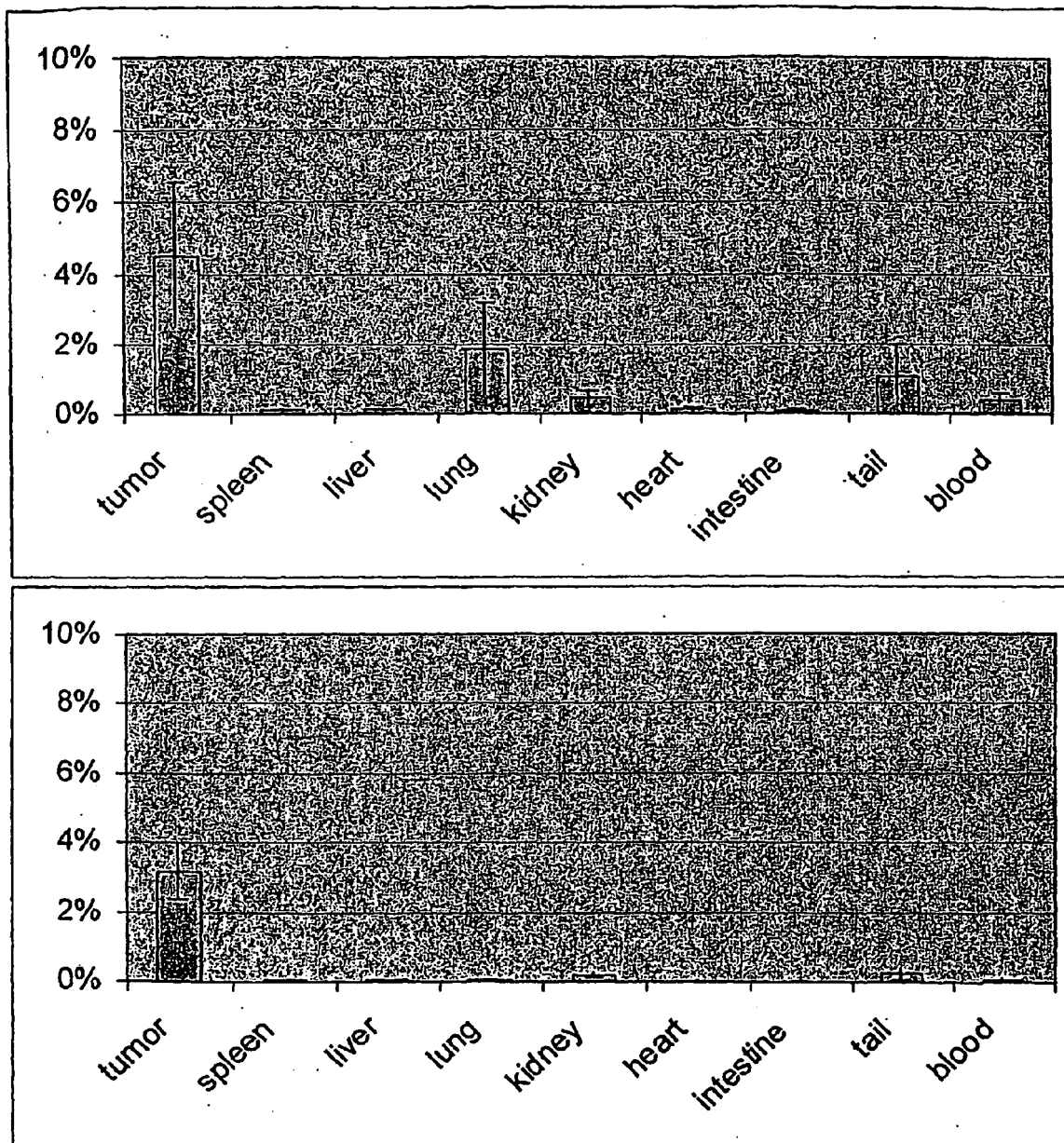


Figure 3

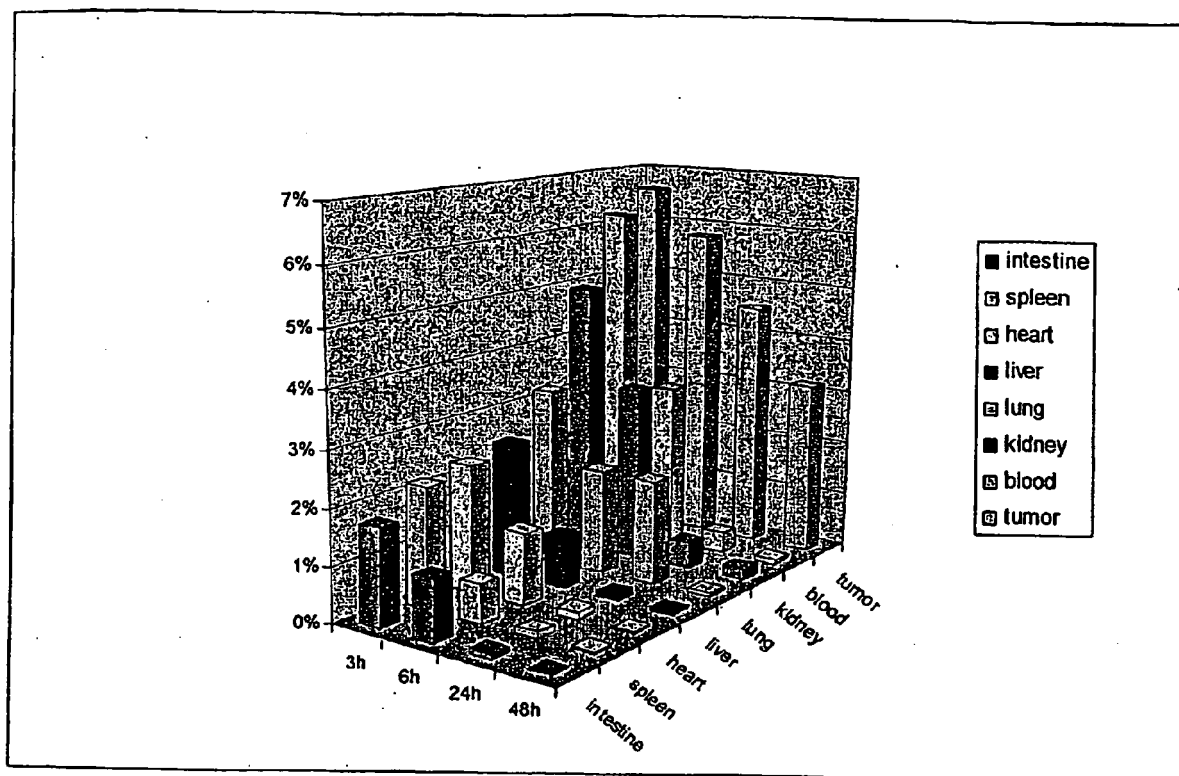


Figure 4

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