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(54) **SCFV ANTIBODIES AGAINST DISEASE ASSOCIATED MOLECULES**

SCFV ANTIKÖRPER GEGEN KRANKHEITEN-ASSOZIIERTE MOLEKÜLE

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EP 1 242 456 B1

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to canine 5T4.

[0002] In particular, the present invention relates to canine 5T4 polypeptides and nucleotide sequences capable of encoding such polypeptides.

[0003] The present invention also relates to antibodies capable of binding specifically to a canine 5T4 polypeptide.

BACKGROUND TO THE INVENTION

[0004] In certain disease states, a derangement of cellular metabolism can affect the level of expression of one or more disease-associated molecules (DAMs). In some circumstances, this cellular derangement may lead to a change in the levels of expression of the DAM. Thus, each disease causing agent or disease state may have associated with it a DAM which may be crucial in the immune recognition and/or the elimination and/or control of a disease causing agent or disease state in a host organism. In this way, the DAM may be capable of acting as a marker not only for the diagnosis of disease states but also for the accurate staging of the disease profile so that the appropriate therapy may be designed.

[0005] A particular example of DAMs which have been well characterised include the tumour-associated antigens (TAAs). A number of oncofoetal or tumour-associated antigens (TAAs) have been identified and characterised in human and animal tumours.

[0006] These TAAs include carcinoembryonic antigen (CEA), TAG72, c-erB2, (underglycosylated) MUC-1 and p53, epithelial glycoprotein-2 antigen (EGP-2; also known as EGP40, Ep-CAM, KSA, CO17-1A or GA733-2) and the 5T4 antigen. In general, TAAs are antigens which are expressed during foetal development but which are downregulated in adult cells, and are thus normally absent or present only at very low levels in adults. However, during tumourigenesis, tumour cells have been observed to resume expression of TAAs. Thus, it is thought that malignant cells may be distinguished from their non-malignant counterparts by resumption of expression of TAAs. Consequently, application of TAAs for (i) *in vitro* and/or *in vivo*/*ex vivo* diagnosis of tumour disorders; (ii) for imaging and/or immunotherapy of cancer has been suggested and (iii) as indicators of progression of tumour associated disease;

[0007] In order to mount a humoral and/or cellular immune response against a particular disease, the host immune system must come in contact with a DAM. In addition to recognising foreign antigens, T cells often need additional stimulation to become fully activated. It is now becoming apparent that two signals are required for activation of naive T-cells by antigen bearing target cells. One signal is an antigen specific signal, delivered through the T-cell receptor and the second signal is an antigen independent or co-stimulatory signal leading to lymphokine products. These additional signals are delivered through other receptors (such as CD28 and CD40L) on the T cell that interact with ligands (such as B7 and CD40) which are present on professional antigen presenting cells (APCs), such as dendritic cells and macrophages, but which are absent from other cells. These co-stimulatory ligands are often referred to as co-stimulatory molecules.

[0008] By way of example, the B7 family (namely B7.1, B7.2, and possibly B7.3) represent a recently discovered, but important group of co-stimulatory molecules. B7.1 and B7.2 are both member of the Ig gene superfamily. If a T lymphocyte encounters an antigen alone, without co-stimulation by B7, it will respond with either anergy, or apoptosis (programmed cell death). If the co-stimulatory signal is provided it will respond with clonal expansion against the target antigen. No significant amplification of the immune response against a given antigen is thought to occur without co-stimulation (June et al (Immunology Today 15:321-331, 1994); Chen et al(Immunology Today 14:483-486); Townsend et al (Science 259: 368-370)). Freeman et al(J. Immunol._143:2714-2722, 1989). Azuma et al(Nature 366:76-79, 1993). Thus, it has been postulated that one method for stimulating immune recognition of diseased cells which are poorly immunogenic would be to enhance antigen presentation and co-stimulation of lymphocytes in the presence of the DAM.

[0009] By way of example, it has been shown that disease states such as cancer, established tumours may be poorly immunogenic despite the fact that they commonly express DAMs. Transfection of the genes encoding B7-1 and B7-2, either alone or in combination with cytokines, have been shown to enhance the development of immunity to experimental tumours in animal models (e.g. Leong et al. 1997 Int. J. Cancer 71: 476-482; Zitvogel et al. 1996 Eur. J. Immunol. 26: 1335-1341; Cayeux et al. 1997 J. Immunol 158:2834-2841). However, in translating these results into a practical treatment for human cancer, there are a number of significant problems to be overcome. A major problem in such studies has been the need to deliver B7 genes *in vivo* to a large number of cells of the tumour to achieve efficacy. A second problem has been the selective target expression of B7 to the tumour cells to avoid inappropriate immune cell activation directed against other cell types. Some solutions to these problems have been addressed in WO 98/55607 where a tumour interacting protein (TIP) such as a tumour binding protein (TBP) has been used to selectively target a co-stimulatory molecule to tumour cells.

[0010] Recombinant DNA technologies have been applied to develop antibodies that recognise DAMs (Hoogenboom

et al1998 Immunotechnology 4: 1-20; and Winter 1998 FEBS Lett 458: 92-94. Recently, there has been considerable interest in using antibody gene libraries to generating antibodies, such as a single chain antibody (ScFv Abs). It is well known that in certain circumstances, there are advantages of using ScFv Abs; rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved tumour to non-tumour ratios. However, many efforts have failed to produce ScFv Abs of high specificity. Moreover, whole IgGs are regarded as a better format for therapeutic Mabs than ScFc Abs as they are regarded as having an extended serum half life (see Vaughan et al1998, Nature Biotech 16: 535-539).

[0011] The present invention provides a particular DAM, canine 5T4.

[0012] The present invention also provides nucleotide sequences capable of encoding and antibodies capable of binding specifically to a canine 5T4 polypeptide.

DETAILED ASPECTS OF THE INVENTION

[0013] Aspects of the present invention are presented in the accompanying claims and in the following description and drawings. These aspects are presented under separate section headings. However, it is to be understood that the teachings under each section are not necessarily limited to that particular section heading.

5T4 TAA

[0014] The term "tumour associated antigen (TAA)" refers to an antigen which is expressed by the tumour iteself or cells associated with the tumour such as parenchymal cells or those of the associated vasculature. The term "tumour associated antigen (TAA)" includes antigens that distinguish the tumour cells from their normal cellular counterparts where they may be present in trace amounts.

[0015] The TAA 5T4 (see WO 89/07947) has been extensively characterised. It is a 72kDa glycoprotein expressed widely in carcinomas, but having a highly restricted expression pattern in normal adult tissues. It appears to be strongly correlated to metastasis in colorectal and gastric cancer. The full nucleic acid sequence of human 5T4 is known (Myers et al., 1994 J Biol Chem 169: 9319-24).

AMINO ACID SEQUENCES

[0016] As used herein, the term "amino acid sequence" refers to peptide, polypeptide sequences, protein sequences or portions thereof.

VARIANTS /HOMOLOGUES/DERIVATIVES OF AMINO ACID SEQUENCES

[0017] The present invention provides, for the first time, the full canine 5T4 amino acid and nucleic acid sequences (Figure 1 and SEQ ID Nos 14 and 15). Thus the present invention also provides

i) a canine 5T4 polypeptide having the amino acid sequence shown in SEQ ID No 14 or a homologue thereof, having at least 90% identity, or a fragment thereof which comprises amino acids 1-182 and/or 297-420; and
ii) a nucleotide sequence capable of encoding a such canine 5T4 polypeptide. Preferably the nucleotide sequence has the sequence shown as SED ID NO 15 or a homologue thereof having at least 90% identity, or a fragment thereof of at least 50 nucleotides in length.

[0018] Thus, the present invention covers, homologues of the amino acid sequences presented herein, as well as, homologues of the nucleotide sequence coding for those amino acid sequences.

[0019] In the context of the present invention, a homologous sequence is taken to include an amino acid sequence which is at least 90% identical, preferably at least 95 or 98% identical at the amino acid level over at least, for example, the amino acid sequence as set out in SEQ ID No 14 of the sequence listing herein. In particular, homology should typically be considered with respect to those regions of the sequence known to be essential for binding specificity (such as amino acids at positions) rather than non-essential neighbouring sequences. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

[0020] Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

[0021] % homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence,

one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

[0022] Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

[0023] However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

[0024] Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux et al., 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al., 1999 ibid - Chapter 18), FASTA (Atschul et al., 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999 ibid, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program. A new tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequence (see FEMS Microbiol Lett 1999 174(2): 247-50; FEMS Microbiol Lett 1999 177(1): 187-8 and tatiana@ncbi.nlm.nih.gov).

[0025] Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

[0026] Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

[0027] SEQ ID No 14 of the sequence listing herein may be modified for use in the present invention. Typically, modifications are made that maintain the binding specificity of the sequence. Amino acid substitutions may be made, for example from 1, 2 or 3 to 10 or 20 substitutions provided that the modified sequence retains the required binding specificity. Amino acid substitutions may include the use of non-naturally occurring analogues.

[0028] The canine 5T4 sequence of the present invention may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent molecule. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the binding specificity of the canine 5T4 polypeptide is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

[0029] Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	GAP
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R

(continued)

AROMATIC		HFWY

[0030] With regard to a fragment of the canine 5T4 sequence, the fragment conprises amino acids 1-182 and/or 297-420 shown in SEQ ID No 14.

NUCLEOTIDE SEQUENCES

[0031] It will be understood by a skilled person that numerous different nucleotide sequences can encode the same canine 5T4 polypeptide of the present invention as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide encoded by the nucleotide sequence of the present invention to reflect the codon usage of any particular host organism in which the polypeptide of the present invention is to be expressed.

[0032] The terms "variant", "homologue" or "derivative" in relation to the nucleotide sequence set out in SEQ ID No 15 (see Figure 1) of the present invention includes any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for a canine 5T4 polypeptide, preferably a polypeptide as set out in SEQ ID No 14 of the sequence listing of the present invention.

[0033] As indicated above, with respect to sequence homology, preferably there is at least 90%, homology to the sequences shown in the sequence listing herein. More preferably there is at least 95%, more preferably at least 98%, homology. Nucleotide homology comparisons may be conducted as described above. A preferred sequence comparison program is the GCG Wisconsin Bestfit program described above. The default scoring matrix has a match value of 10 for each identical nucleotide and -9 for each mismatch. The default gap creation penalty is -50 and the default gap extension penalty is -3 for each nucleotide.

[0034] The present invention also encompasses nucleotide sequences that are capable of hybridising selectively to the sequences presented herein, or any variant, fragment or derivative thereof, or to the complement of any of the above. Nucleotide sequences are at least 50 nucleotides in length.

[0035] With regard to a fragment of the canine 5T4 sequence, preferably the fragment conprises at least one, preferably some, most preferably all of the nucleic acids 1-546 and/or 890-1263 shown in SEQ ID No 15.

[0036] Where the nucleotide sequence of the invention is double-stranded, both strands of the duplex, either individually or in combination, are encompassed by the present invention. Where the nucleotide sequence is single-stranded, it is to be understood that the complementary sequence of that nucleotide sequence is also included within the scope of the present invention.

[0037] Nucleotide sequences which are not 100% homologous to the sequence shown as SEQ ID No. 15 but fall within the scope of the invention can be obtained in a number of ways. Other variants of the sequences described herein may be obtained for example by probing DNA libraries made from a range of sources. In addition, other viral/bacterial, or cellular homologues particularly cellular homologues found in mammalian cells (e.g. rat, mouse, bovine and primate cells), may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the sequence listing herein. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal species, and probing such libraries with probes comprising all or part of the nucleotide sequence set out in SEQ ID No 15 of the sequence listings of the present invention under conditions of medium to high stringency. Similar considerations apply to obtaining species homologues and allelic variants of the amino acid and/or nucleotide sequences of the present invention.

[0038] Variants and strain/species homologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the variants and homologues encoding conserved amino acid sequences within the sequences of the present invention. Conserved sequences can be predicted, for example, by aligning the amino acid sequences from several variants/homologues. Sequence alignments can be performed using computer software known in the art. For example the GCG Wisconsin PileUp program is widely used. The primers used in degenerate PCR will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences.

[0039] Alternatively, such nucleotide sequences may be obtained by site directed mutagenesis of characterised sequences, such as the nucleotide sequence set out in SEQ ID NO 15 of the sequence listings of the present invention. This may be useful where for example silent codon changes are required to sequences to optimise codon preferences for a particular host cell in which the nucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the binding specificity of the polypeptide encoded by the nucleotide sequences.

[0040] The nucleotide sequences of the present invention may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels, or the nucleotide sequences may be cloned into vectors. Such primers, probes and other fragments will be at least 15, preferably at least 20, for example at least 25, 30 or 40 nucleotides in length.

[0041] The nucleotide sequences such as a DNA polynucleotides according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

[0042] In general, primers will be produced by synthetic means, involving a step wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

[0043] Longer nucleotide sequences will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking a region of the targeting sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction (PCR) under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector

[0044] Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express the polypeptide. As will be understood by those of skill in the art, it may be advantageous to produce the canine 5T4-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray E et al(1989) Nuc Acids Res 17:477-508) can be selected, for example, to increase the rate of the canine 5T4 polypeptide expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

[0045] In one embodiment of the present invention, the canine 5T4 polypeptide is a recombinant polypeptide.

[0046] Preferably the recombinant canine 5T4 polypeptide is prepared using a genetic vector.

VECTOR

[0047] As it is well known in the art, a vector is a tool that allows or faciliates the transfer of an entity from one environment to another. In accordance with the present invention, and by way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a host and/or a target cell for the purpose of replicating the vectors comprising the nucleotide sequences of the present invention and/or expressing the proteins of the invention encoded by the nucleotide sequences of the present invention. Examples of vectors used in recombinant DNA techniques include but are not limited to plasmids, chromosomes, artificial chromosomes or viruses.

[0048] The term "vector" includes expression vectors and/or transformation vectors.

[0049] The term "expression vector" means a construct capable of *in vivo* or *in vitro*/*ex vivo* expression.

[0050] The term "transformation vector" means a construct capable of being transferred from one species to another.

"NAKED DNA"

[0051] The vectors comprising nucleotide sequences encoding polypeptides of the present invention may be administered directly as "a naked nucleic acid construct", preferably further comprising flanking sequences homologous to the host cell genome.

[0052] As used herein, the term "naked DNA" refers to a plasmid comprising a nucleotide sequences encoding a polypeptide of the present invention together with a short promoter region to control its production. It is called "naked" DNA because the plasmids are not carried in any delivery vehicle. When such a DNA plasmid enters a host cell, such as a eukaryotic cell, the proteins it encodes (such as canine 5T4) are transcribed and translated within the cell.

NON-VIRAL DELIVERY

[0053] Alternatively, the vectors comprising nucleotide sequences of the present invention may be introduced into suitable host cells using a variety of non-viral techniques known in the art, such as transfection, transformation, electroporation and biolistic transformation.

[0054] As used herein, the term "transfection" refers to a process using a non-viral vector to deliver a gene to a target mammalian cell.

[0055] Typical transfection methods include electroporation, DNA biolistics, lipid-mediated transfection, compacted

DNA-mediated transfection, liposomes, immunoliposomes, lipofectin, cationic agent-mediated, cationic facial amphiphiles (CFAs) (Nature Biotechnology 1996 14; 556), multivalent cations such as spermine, cationic lipids or polylysine, 1, 2,-bis (oleoyloxy)-3-(trimethylammonio) propane (DOTAP)-cholesterol complexes (Wolff and Trubetskoy 1998 Nature Biotechnology 16: 421) and combinations thereof.

[0056] Uptake of naked nucleic acid constructs by mammalian cells is enhanced by several known transfection techniques for example those including the use of transfection agents. Example of these agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example lipofectam™ and transfectam™). Typically, nucleic acid constructs are mixed with the transfection agent to produce a composition.

VIRAL VECTORS

[0057] Alternatively, the vectors comprising nucleotide sequences of the present invention may be introduced into suitable host cells using a variety of viral techniques which are known in the art, such as for example infection with recombinant viral vectors such as retroviruses, herpes simplex viruses and adenoviruses.

[0058] Preferably the vector is a recombinant viral vectors. Suitable recombinant viral vectors include but are not limited to adenovirus vectors, adeno-associated viral (AAV) vectors, herpes-virus vectors, a retroviral vector, lentiviral vectors, baculoviral vectors, pox viral vectors or parvovirus vectors (see Kestler et al1999 Human Gene Ther 10(10): 1619-32). In the case of viral vectors, gene delivery is mediated by viral infection of a target cell.

RETROVIRAL VECTORS

[0059] Examples of retroviruses include but are not limited to: murine leukemia virus (MLV), human immunodeficiency virus (HTV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV).

[0060] Preferred vectors for use in accordance with the present invention are recombinant viral vectors, in particular recombinant retroviral vectors (RRV) such as lentiviral vectors.

[0061] The term "recombinant retroviral vector" (RRV) refers to a vector with sufficient retroviral genetic information to allow packaging of an RNA genome, in the presence of packaging components, into a viral particle capable of infecting a target cell. Infection of the target cell includes reverse transcription and integration into the target cell genome. The RRV carries non-viral coding sequences which are to be delivered by the vector to the target cell. An RRV is incapable of independent replication to produce infectious retroviral particles within the final target cell. Usually the RRV lacks a functional *gag-pol* and/or env gene and/or other genes essential for replication. The vector of the present invention may be configured as a split-intron vector. A split intron vector is described in PCT patent application WO 99/15683.

[0062] A detailed list of retroviruses may be found in Coffin et al("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763).

LENTIVIRAL VECTORS

[0063] Lentiviruses can be divided into primate and non-primate groups. Examples of primate lentiviruses include but are not limited to: the human immunodeficiency virus (HIV), the causative agent of human auto-immunodeficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV). The non-primate lentiviral group includes the prototype "slow virus" visna/maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV) and the more recently described feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BIV).

[0064] A distinction between the lentivirus family and other types of retroviruses is that lentiviruses have the capability to infect both dividing and non-dividing cells (Lewis et al1992 EMBO. J 11: 3053-3058; Lewis and Emerman 1994 J. Virol. 68: 510-516). In contrast, other retroviruses - such as MLV - are unable to infect non-dividing cells such as those that make up, for example, muscle, brain, lung and liver tissue.

ADENOVIRUSES

[0065] In one embodiment of the present invention, the features of adenoviruses may be combined with the genetic stability of retroviruses/lentiviruses which can be used to transduce target cells to become transient retroviral producer cells capable of stably infect neighbouring cells. Such retroviral producer cells which are engineered to express a 5T4 polypeptide of the present invention can be implanted in organisms such as animals or humans for use in the treatment of disease such as cancer.

POX VIRUSES

[0066] Preferred vectors for use in accordance with the present invention are recombinant pox viral vectors such as fowl pox virus (FPV), entomopox virus, vaccinia virus such as NYVAC, canarypox virus, MVA or other non-replicating viral vector systems such as those described for example in WO 95/30018.

HYBRID VIRAL VECTORS

[0067] In a further broad aspect, the present invention provides a hybrid viral vector system for *in vivo* delivery of a nucleotide sequence encoding a 5T4 polypeptide of the present invention, which system comprises one or more primary viral vectors which encode a secondary viral vector, the primary vector or vectors capable of infecting a first target cell and of expressing therein the secondary viral vector, which secondary vector is capable of transducing a secondary target cell.

[0068] Preferably the primary vector is obtainable from or is based on an adenoviral vector and/or the secondary viral vector is obtainable from or is based on a retroviral vector preferably a lentiviral vector

TARGETED VECTOR.

[0069] The term "targeted vector" refers to a vector whose ability to infect/transfect/transduce a cell or to be expressed in a host and/or target cell is restricted to certain cell types within the host organism, usually cells having a common or similar phenotype.

REPLICATION VECTORS

[0070] The nucleotide sequences encoding the canine 5T4 polypeptide of the present invention may be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleotide sequence in a compatible host cell. Thus in one embodiment of the present invention, the invention provides a method of making the polypeptide of the present invention by introducing a nucleotide sequence of the present invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell.

EXPRESSION VECTOR

[0071] Preferably, a nucleotide sequence of present invention which is inserted into a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence, such as the coding sequence of the canine 5T4 polypeptide of the present invention by the host cell, i.e. the vector is an expression vector. The polypeptide produced by a host recombinant cell may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing the polypeptide coding sequences can be designed with signal sequences which direct secretion of the polypeptide coding sequences through a particular prokaryotic or eukaryotic cell membrane.

EXPRESSION IN VITRO

[0072] The vectors of the present invention may be transformed or transfected into a suitable host cell and/or a target cell as described below to provide for expression of a polypeptide of the present invention. This process may comprise culturing a host cell and/or target cell transformed with an expression vector under conditions to provide for expression by the vector of a coding sequence encoding the canine 5T4 polypeptide and optionally recovering the expressed polypeptide. The vectors may be for example, plasmid or virus vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector. The expression of the polypeptide of the invention may be constitutive such that they are continually produced, or inducible, requiring a stimulus to initiate expression. In the case of inducible expression, polypeptide production can be initiated when required by, for example, addition of an inducer substance to the culture medium, for example dexamethasone or IPTG.

HOST/TARGET CELLS

[0073] Host and/or target cells comprising nucleotide sequences of the present invention may be used to express the

polypeptides of the present invention under *in vitro, in vivo* and *ex vivo* conditions.

[0074] The term" host cell and/or target cell" includes any cell derivable from a suitable organism which a vector is capable of transfecting or transducing. Examples of host and/or target cells can include but are not limited to cells capable of expressing the polypeptides of the present invention under *in vitro, in vivo* and *ex vivo* conditions. Examples of such cells include but are not limited to macrophages, endothelial cells or combinations thereof. Further examples include respiratory airway epithelial cells, hepatocytes, muscle cells, cardiac myocytes, synoviocytes, primary mammary epithelial cess and post-mitotically terminally differentiated non-replicating cells such as macrophages and/or neurons:

[0075] In a preferred embodiment, the cell is a mammalian cell.

[0076] In a highly preferred embodiment, the cell is a human cell.

[0077] The term "organism" includes any suitable organism. In a preferred embodiment, the organism is a mammal. In a highly preferred embodiment, the organism is a human.

[0078] Although the polypeptides of the invention may be produced using prokaryotic cells as host cells, it is preferred to use eukaryotic cells, for example yeast, insect or mammalian cells, in particular mammalian cells. Suitable host cells include bacteria such as *E. coli,* yeast, mammalian cell lines and other eukaryotic cell lines, for example insect Sf9 cells.

[0079] The present invention also provides a method comprising transforming a host and/or target cell with a or the nucleotide sequence(s) of the present invention.

[0080] The term "transformed cell" means a host cell and/or a target cell having a modified genetic structure. With the present invention, a cell has a modified genetic structure when a vector according to the present invention has been introduced into the cell.

[0081] Host cells and/or a target cells may be cultured under suitable conditions which allow expression of the polypeptide of the invention.

[0082] The present invention also provides a method comprising culturing a transformed host cell - which cell has been transformed with a or the nucleotide sequence(s) according to the present invention under conditions suitable for the expression of the polypeptide encoded by said nucleotide sequence(s).

[0083] The present invention also provides a method comprising culturing a transformed host cell - which cell has been transformed with a or the nucleotide sequence(s) according to the present invention or a homologue, or fragment thereof - under conditions suitable for the expression of the polypeptide encoded by said nucleotide sequence(s); and then recovering said polypeptide from the transformed host cell culture.

[0084] The polypeptide of the present invention can be extracted from host cells by a variety of techniques known in the art, including enzymatic, chemical and/or osmotic lysis and physical disruption. The polypeptide may be purified and isolated in a manner known *per se.*

REGULATION OF EXPRESSION IN VITRO/ VIVO/EX VIVO

[0085] The present invention also encompasses gene therapy whereby the canine 5T4-encoding nucleotide sequence (s) of the present invention is regulated *in vitro*/*in vivo*/*ex vivo.* For example, expression regulation may be accomplished by administering compounds that bind to the canine 5T4- encoding nucleotide sequence(s) of the present invention, or control regions associated with the canine 5T4- encoding nucleotide sequence of the present invention, or its corresponding RNA transcript to modify the rate of transcription or translation.

CONTROL SEQUENCES

[0086] Control sequences operably linked to sequences encoding the canine 5T4 polypeptide of the present invention include promoters/enhancers and other expression regulation signals. These control sequences may be selected to be compatible with the host cell and/or target cell in which the expression vector is designed to be used. The control sequences may be modified, for example by the addition of further transcriptional regulatory elements to make the level of transcription directed by the control sequences more responsive to transcriptional modulators.

OPERABLY LINKED

[0087] The term "operably linked" means that the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under condition compatible with the control sequences.

[0088] Preferably the nucleotide sequence of the present invention is operably linked to a transcription unit.

[0089] The term "transcription unit(s)" as described herein are regions of nucleic acid containing coding sequences and the signals for achieving expression of those coding sequences independently of any other coding sequences. Thus, each transcription unit generally comprises at least a promoter, an optional enhancer and a polyadenylation signal.

PROMOTERS

[0090] The term promoter is well-known in the art and is used in the normal sense of the art, e.g. as an RNA polymerase binding site. The term encompasses nucleic acid regions ranging in size and complexity from minimal promoters to promoters including upstream elements and enhancers.

[0091] The promoter is typically selected from promoters which are functional in mammalian, cells, although prokaryotic promoters and promoters functional in other eukaryotic cells may be used. The promoter is typically derived from promoter sequences of viral or eukaryotic genes. For example, it may be a promoter derived from the genome of a cell in which expression is to occur. With respect to eukaryotic promoters, they may be promoters that function in a ubiquitous manner (such as promoters of α-actin, β-actin, tubulin) or, alternatively, a tissue-specific manner (such as promoters of the genes for pyruvate kinase).

HYPOXIC PROMOTERS/ENHANCERS

[0092] The enhancer and/or promoter may be preferentially active in a hypoxic or ischaemic or low glucose environment, such that the canine 5T4-encoding nucleotide sequence(s) is preferentially expressed in the particular tissues of interest, such as in the environment of a tumour, arthritic joint or other sites of ischaemia. Thus, any significant biological effect or deleterious effect of the canine 5T4-encoding nucleotide sequence(s) on the individual being treated may be reduced or eliminated. The enhancer element or other elements conferring regulated expression may be present in multiple copies. Likewise, or in addition, the enhancer and/or promoter may be preferentially active in one or more specific cell types - such as any one or more of macrophages, endothelial cells or combinations thereof. Further examples may include but are not limited to respiratory airway epithelial cells, hepatocytes, muscle cells, cardiac myocytes, synoviocytes, primary mammary epithelial cells and post-mitotically terminally differentiated non-replicating cells such as macrophages and/or neurons.

TISSUE-SPECIFIC PROMOTERS

[0093] The promoters of the present invention may be tissue-specific promoters. Examples of suitable tissue restricted promoters/enhancers are those which are highly active in tumour cells such as a promoter/enhancer from a *MUC*1 gene, a *CEA* gene or a *5T4* antigen gene. Examples of temporally restricted promoters/enhancers are those which are responsive to ischaemia and/or hypoxia, such as hypoxia response elements or the promoter/enhancer of a *grp*78 or a *grp*94 gene. The alpha fetoprotein (AFP) promoter is also a tumour-specific promoter. One preferred promoter-enhancer combination is a human cytomegalovirus (hCMV) major immediate early (MIE) promoter/enhancer combination.

[0094] Preferably the promoters of the present invention are tissue specific. That is, they are capable of driving transcription of a canine 5T4-encoding nucleotide sequence(s) in one tissue while remaining largely "silent" in other tissue types.

[0095] The term "tissue specific" means a promoter which is not restricted in activity to a single tissue type but which nevertheless shows selectivity in that they may be active in one group of tissues and less active or silent in another group. A desirable characteristic of the promoters of the present invention is that they posess a relatively low activity in the absence of activated hypoxia-regulated enhancer elements, even in the target tissue. One means of achieving this is to use "silencer" elements which suppress the activity of a selected promoter in the absence of hypoxia.

[0096] The term "hypoxia" means a condition under which a particular organ or tissue receives an inadequate supply of oxygen.

[0097] The level of expression of a or the canine 5T4-encoding nucleotide sequence(s) under the control of a particular promoter may be modulated by manipulating the promoter region. For example, different domains within a promoter region may possess different gene regulatory activities. The roles of these different regions are typically assessed using vector constructs having different variants of the promoter with specific regions deleted (that is, deletion analysis). This approach may be used to identify, for example, the smallest region capable of conferring tissue specificity or the smallest region conferring hypoxia sensitivity.

[0098] A number of tissue specific promoters, described above, may be particularly advantageous in practising the present invention. In most instances, these promoters may be isolated as convenient restriction digestion fragments suitable for cloning in a selected vector. Alternatively, promoter fragments may be isolated using the polymerase chain reaction. Cloning of the amplified fragments may be facilitated by incorporating restriction sites at the 5' end of the primers.

INDUCIBLE PROMOTERS

[0099] The promoters of the present invention may also be promoters that respond to specific stimuli, for example promoters that bind steroid hormone receptors. Viral promoters may also be used, for example the Moloney murine

leukaemia virus long terminal repeat (MMLV LTR) promoter, the rous sarcoma virus (RSV) LTR promoter or the human cytomegalovirus (CMV) IE promoter.

[0100] It may also be advantageous for the promoters to be inducible so that the levels of expression of the heterologous gene can be regulated during the life-time of the cell.

[0101] Inducible means that the levels of expression obtained using the promoter can be regulated.

ENHANCER

[0102] In addition, any of these promoters may be modified by the addition of further regulatory sequences, for example enhancer sequences. Chimeric promoters may also be used comprising sequence elements from two or more different promoters described above.

[0103] The term "enhancer" includes a DNA sequence which binds to other protein components of the transcription initiation complex and thus facilitates the initiation of transcription directed by its associated promoter.

ANTIBODY

[0104] In one aspect, the present invention provides an antibody capable of binding specifically to a canine 5T4 polypeptide. The antibody may be an ScFv Ab.

Ab SOURCE

[0105] The Ab of the present invention is obtainable from or produced by any suitable source, whether natural or not, or it may be a synthetic Ab, a semi-synthetic Ab, a mimetic, a derivatised Ab, a recombinant Ab, a fermentation optimised Ab, a fusion protein or equivalents, mutants and derivatives thereof as long as it retains the required canine 5T4 binding specificity of the Ab of the present invention. These include a Ab with canine 5T4 binding specificity which may have amino acid substitutions or may have sugars or other molecules attached to amino acid functional groups.

[0106] The term "mimetic" relates to any chemical which may be a peptide, polypeptide, antibody or other organic chemical which has the same binding specificity as the Ab of the present invention.

[0107] The term "derivative" or "derivatised" as used herein includes chemical modification of an Ab. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. Preferably, the Ab includes at least a portion of which has been prepared by recombinant DNA techniques or produced by chemical synthesis techniques or combinations thereof.

[0108] Preferably, the Ab is prepared by the use of chemical synthesis techniques.

CHEMICAL SYNTHESIS METHODS

[0109] The Ab of the present invention or variants, homologues, derivatives, fragments or mimetics thereof may be produced using chemical methods to synthesize the Ab amino acid sequence, in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., Creighton (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, *supra).*

[0110] Direct synthesis of the Ab or variants, homologues, derivatives, fragments or mimetics thereof can be performed using various solid-phase techniques (Roberge JY et al(1995) Science 269: 202-204) and automated synthesis may be achieved, for example, using the ABI 43 1 A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer. Additionally, the amino acid sequences obtainable from the Ab, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with a sequence from other subunits, or any part thereof, to produce a variant Ab.

[0111] In an alternative embodiment of the invention, the coding sequence of the Ab or variants, homologues, derivatives, fragments or mimetics thereof may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers MH et al(1980) Nuc Acids Res Symp Ser 215-23, Horn T et al(1980) Nuc Acids Res Symp Ser 225-232).

[0112] As used herein, the term "ScFv Ab" means an antibody capable of recognising a canine 5T4 polypeptide which has a light chain variable region (VL) and a heavy chain variable (VH) region. The VH and VL partner domains are typically linked/joined via a flexible oligopeptide/peptide linker. The VH and VL partner domains may be connected in the order of VH followed by VL or VL followed by VH. Typically, the the sequences may be connected via a linker sequence in the order VH-linker-VL or VL-linker-VH. As used herein, the term includes fragments of proteolytically-cleaved or recombinantly-prepared portions of an ScFv Ab molecule that are capable of selectively reacting with or

recognising the canine 5T4 polypeptide. Non limiting examples of such proteolytic and/or recombinant fragments include chimeric ScFv antibodies which, for the purposes of this invention, may refer to an ScFv Ab having either a or both heavy and light chain variable regions (VH and VL) encoded by a nucleotide sequence derivable from a mammalian immunoglobulin gene other than a human immunoglobulin gene and either a or both heavy and light chain encoded by a nucleotide sequence derivable from a human immunoglobulin gene. The ScFv Ab may be covalently or non-covalently linked to another entity (such as another ScFv Ab) to form antibodies having two or more binding sites. For example, one ScFv Ab could bind to canine 5T4, and the second ScFv Ab could bind to an immune enhancer molecule.

[0113] In accordance with the present invention, reference to the term "ScFv Ab" includes but is not limited to reference to the peptide *per se* also as well the peptide as part of a fusion protein as well as the nucleotide sequence encoding the peptide and/or the nucleotide sequence encoding the fusion proteins. - The peptide *per se* and/or fusion protein may be a synthetic peptide. Alternatively, the peptide and/or fusion protein may be a genetically expressed/recombinant peptide/fusion protein. For some applications, the term "ScFv Ab means peptide *per se.* The term "ScFv Ab" also includes an ScFv Ab with a secretion leader (L) sequence which is designated herein as LScFv.

[0114] As used herein, the term "variable region" refers to the variable region, or domain, of the light chain (VL) and heavy chain (VH) which contain the determinants for binding recognition specificity and for the overall affinity of the Ab for canine 5T4 polypeptide. The variable domains of each pair of light (VL) and heavy chains (VH) are involved in antigen recognition and form the antigen binding site. The domains of the light and heavy chains have the same general structure and each domain has four framework (FR) regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The FR regions maintain the structural integrity of the variable domain. The CDRs are the polypeptide segments within the variable domain that mediate binding of the canine 5T4.

[0115] Preferably the affinity (K_D) of the ScFv Ab of the present invention for the canine 5T4 antigen is from about 5 x 10^{-10} to about 10 x 10^{-10}.

[0116] Preferably the affinity (K_D) of the ScFv Ab of the present invention for the canine 5T4 antigen is from about 6 x 10^{-10} to about 9 x 10^{-10}.

[0117] Preferably the affinity (K_D) of the ScFv Ab of the present invention for the canine 5T4 antigen is from about 7 x 10^{-10} to about 8 x 10^{-10}.

[0118] Preferably the affinity (K_D) of the ScFv Ab of the present invention for the canine 5T4 antigen is about 7.9 x 10^{-10}. The K_D of the ScFvAb is measured using BIAevaluation software (Pharmacia).

[0119] As used herein, the term "off-rate" means the dissociation rate (k_{off}) of a ScFv Ab from the canine 5T4 antigen. In the context of the present invention, it is measured using BIAevaluation software (Pharmacia). A low off rate is desirable as it reflects the affinity of an Fab fragment for the antigen.

[0120] As used herein, the term "affinity" is defined in terms of the dissociation rate or off-rate (k_{off}) of a ScFv Ab from the canine 5T4 antigen. The lower the off-rate the higher the affinity that a ScFv Ab has for the antigen.

COUPLING

[0121] The canine 5T4 polypeptide or antibody of the present invention can be coupled to other molecules using standard methods. The amino and carboxyl termini of the polypeptide may be isotopically and nonisotopically labeled with many techniques, for example radiolabeling using conventional techniques (tyrosine residues- chloramine T, iodogen, lactoperoxidase; lysine residues- Bolton-Hunter reagent). These coupling techniques are well known to those skilled in the art. The coupling technique is chosen on the basis of the functional groups available on the amino acids including, but not limited to amino, sulfhydral, carboxyl, amide, phenol, and imidazole. Various reagents used to effect these couplings include among others, glutaraldehyde, diazotized benzidine, carbodiimide, and p-benzoquinone.

CHEMICAL COUPLING

[0122] The 5T4 polypeptide or antibody of the present invention may be chemically coupled to isotopes, enzymes, carrier proteins, cytotoxic agents, fluorescent molecules, radioactive nucleotides and other compounds for a variety of applications including but not limited to imaging/prognosis, diagnosis and/or therapy. The efficiency of the coupling reaction is determined using different techniques appropriate for the specific reaction. For example, radiolabeling of a 5T4 polypeptide with ^{125}I is accomplished using chloramine T and $Na^{125}I$ of high specific activity. The reaction is terminated with sodium metabisulfite and the mixture is desalted on disposable columns. The labeled peptide is eluted from the column and fractions are collected. Aliquots are removed from each fraction and radioactivity measured in a gamma counter. In this manner, the unreacted $Na^{125}I$ is separated from the labeled polypeptide. The peptide fractions with the highest specific radioactivity are stored for subsequent use such as analysis of the ability to bind to an anti-canine 5T4 Ab.

IMAGING

[0123] The use of labelled antibodies of the present invention with short lived isotopes enables visualization quantitation of canine 5T4 *in vivo* using autoradiographic, or modem radiographic or other membrane binding techniques such as positron emission tomography in order to locate tumours with 5T4. This application provides important diagnostic and/or prognostic research tools.

CONJUGATES

[0124] In other embodiments, the polypeptide or antibody of the invention is coupled to a scintigraphic radiolabel, a cytotoxic compound or radioisotope, an enzyme for converting a non-toxic prodrug into a cytotoxic drug, a compound for activating the immune system in order to target the resulting conjugate to a disease site such as a colon tumour, or a cell-stimulating compound. Such conjugates have a "binding portion", which consists of the polypeptide/antibody of the invention, and a "functional portion", which consists of the radiolabel, toxin or enzyme. Different linked anti-canine Abs can be synthesized for use in several applications including but not limited to the linkage of an anti-canine Ab to cytotoxic agents for targeted killing of cells that express the canine 5T4.

[0125] The anti-canine 5T4 Ab may alternatively be used alone in order simply to block the activity of 5T4, particularly by physically interfering with its binding of another compound.

[0126] The binding portion and the functional portion of the conjugate (if also a peptide or poypeptide) may be linked together by any of the conventional ways of cross linking polypeptides, such as those generally described in O'Sullivan et al(Anal. Biochem 1979: 100, 100-108). For example, one portion may be enriched with thiol groups and the other portion reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). Amide and thioether bonds, for example achieved with *m*-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

[0127] Alternatively, if the binding portion contains carbohydrates, such as would be the case for an antibody or some antibody fragments, the functional portion may be linked via the carbohydrate portion using the linking technology in EP 0 088 695.

[0128] The functional portion of the conjugate may be an enzyme for converting a non-toxic prodrug into a toxic drug, for example the conjugates of Bagshawe and his colleagues (Bagshawe (1987) Br. J. Cancer 56, 531; Bagshawe et al (Br. J. Cancer 1988: 58, 700); WO 88/07378) or cyanide-releasing systems (WO 91/11201).

[0129] It may not be necessary for the whole enzyme to be present in the conjugate but, of course, the catalytic portion must be present.

[0130] The conjugate may be purified by size exclusion or affinity chromatography, and tested for dual biological activities. The antigen immunoreactivity may be measured using an enzyme-linked immunosorbent assay (ELISA) with immobilised antigen and in a live cell radio-immunoassay. An enzyme assay may be used for β-glucosidase using a substrate which changes in absorbance when the glucose residues are hydrolysed, such as oNPG (*o*-nitrophenyl-β-D-glucopyranoside), liberating 2-nitrophenol which is measured spectrophotometrically at 405 nm.

[0131] The stability of the conjugate may be tested *in vitro* initially by incubating at 37°C in serum, followed by size exclusion FPLC analysis. Stability *in vivo* can be tested in the same way in mice by analysing the serum at various times after injection of the conjugate. In addition, it is possible to radiolabel the polypeptide/Ab with ^{125}I, and the enzyme with ^{131}I before conjugation, and to determine the biodistribution of the conjugate, free polypeptide/Ab and free enzyme in mice.

[0132] Alternatively, the conjugate may be produced as a fusion compound by recombinant DNA techniques whereby a length of DNA comprises respective regions encoding the two portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

[0133] Conceivably, two of the functional portions of the compound may overlap wholly or partly. The DNA is then expressed in a suitable host in known ways.

DIAGNOSTIC KITS

[0134] The present invention also includes diagnostic methods and kits for detection and measurement of canine 5T4 in biological fluids and tissues, and for localization of canine 5T4 in tissues. The antibody of the present invention that possess high binding specificity can be used to establish easy to use kits for rapid, reliable, sensitive, and specific measurement and localization of 5T4 in extracts of plasma, urine, tissues, and in cell culture media. The Ab of the present invention may also be used in a diagnostic method and kit to permit detection of circulating canine 5T4 which, in certain situations, may indicate the progression of a disease state such as the spread of micrometastases by primary tumours *in situ.*

[0135] These kits may include but are not limited to the following techniques; competitive and non-competitive assays, radioimmunoassay, bioluminescence and chemiluminescence assays, fluorometric assays, sandwich assays, immunoradiometric assays, dot blots, enzyme linked assays including ELISA, microtiter plates, antibody coated strips or dipsticks for rapid monitoring of urine or blood, and immunocytochemistry. For each kit the range, sensitivity, precision, reliability, specificity and reproducibility of the assay are established. Intraassay and interassay variation is established at 20%, 50% and 80% points on the standard curves of displacement or activity.

[0136] One example of an assay kit commonly used in research and in the clinic is a radioimmunoassay (RIA) kit. After successful radioiodination and purification of a Ab, the antiserum possessing the highest titer is added at several dilutions to tubes containing a relatively constant amount of radioactivity, such as 10,000 cpm, in a suitable buffer system. Other tubes contain buffer or preimmune serum to determine the non-specific binding. After incubation at 4°C for 24 hours, protein A is added and the tubes are vortexed, incubated at room temperature for 90 minutes, and centrifuged at approximately 2000-2500 times g at 4°C to precipitate the complexes of antiserum bound to the labeled Ab. The supernatant is removed by aspiration and the radioactivity in the pellets counted in a gamma counter. The antiserum dilution that binds approximately 10 to 40 % of the labeled Ab after subtraction of the non-specific binding is further characterized.

IMMUNOHISTOCHEMISTRY

[0137] An immunohistochemistry kit may also be used for localization of in tissues and cells. This immunohistochemistry kit provides instructions, a anti-canine 5T4 Ab, and possibly blocking serum and secondary antiserum linked to a fluorescent molecule such as fluorescein isothiocyanate, or to some other reagent used to visualize the primary antiserum. Immunohistochemistry techniques are well known to those skilled in the art. This immunohistochemistry kit permits localization of canine 5T4 in tissue sections and cultured cells using both light and electron microscopy. It is used for both research and clinical purposes. For example, tumours are biopsied or collected and tissue sections cut with a microtome to examine sites of 5T4 production. Such information is useful for diagnostic and possibly therapeutic purposes in the detection and treatment of diseases such as cancer.

FOETAL CELL ANALYSIS

[0138] The antibody and/or the canine 5T4 sequence of the present invention are also useful in methods for isolating foetal cells from maternal blood. Isolation of foetal cells from maternal blood has been proposed as a non-invasive alternative to aminocentesis (see WO 97/30354).

[0139] 5T4 is known to be expressed at very high levels on trophoblasts. Thus an antibody against 5T4 may be used to isolate trophoblasts from maternal blood.

[0140] Thus the present invention also provides a method for isolating a foetal cell from maternal blood using an an anti-canine 5T4 antibody. The canine 5T4 polypeptide of the present invention is useful for generating such cross-reactive antibodies.

[0141] The foetal cell may, for example, be a trophoblast or an erythrocyte.

[0142] The maternal/foetal cells may be from a human or an animal. Hence, the method of the present invention may be used for medical or veterinary applications. In a preferred embodiment, the mother and foetus are non-human, such that the isolation method is part of a veterinary application.

[0143] The isolation process may form part of a diagnostic method. For example, the foetal cells may then be subject to biochemical or genetic sampling. Such a procedure sould be used to test for foetal abnormalities such as Downs syndrome, or to determine the sex of the foetus.

COMBINATION THERAPY

[0144] The polypeptide and/or antibody of the present invention may be used in combination with other compositions and procedures for the treatment of diseases. By way of example, the polypeptide/antibody may also be used in combination with conventional treatments of diseases such as cancer. By ways of further example, a tumor may be treated conventionally with surgery, radiation or chemotherapy combined with a polypeptide/Ab or a polypeptide/Ab may be subsequently administered to the patient to extend the dormancy of micrometastases and to stabilize any residual primary tumor.

DELIVERY

[0145] The polypeptide/Ab can be delivered with a therapeutically effective agent at the same moment in time and at the same site. Alternatively, the polypeptide/Ab and the therapeutically effective agent may be delivered at a different

time and to a different site. The polypeptide or antibody and the therapeutically effective agent may even be delivered in the same delivery vehicle for the prevention and/or treatment of a disease condition such as cancer.

[0146] Therapeutic strategies based on the use of the Ab include the recruitment and activation of T cells by using a fusion of the canine 5T4 reactive Ab with the bacterial superantigen staphylococcal enterotoxin (Dohlsten *et al*1994) or by using bispecific antibodies, directed to both canine 5T4 and the T-cell CD3 antigen (Kroesen *et al*1994*).* Anti-canine 5T4 antibodies may also be conjugated to different bacterial toxins to yield potent immunotoxins (LeMaistre *et al*1987*;* Zimmermann *et al*1997).

Anti-canine 5T4 Abs may be used in combination with cytotoxic agents for the prevention and/or treatment of disease states such as angiogenesis and/or cancer. Cytotoxic agents such as ricin, linked to the Ab may provide a tool for the destruction of cells that express 5T4. These cells may be found in many locations, including but not limited to, micrometastases and primary tumours.

DOSAGE

[0147] The dosage of the polypeptide or Ab of the present invention will depend on the disease state or condition being treated and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. Depending upon the half-life of the polypeptide/Ab in the particular animal or human, the polypeptide/Ab can be administered between several times per day to once a week. It is to be understood that the present invention has application for both human and veterinary use. The methods of the present invention contemplate single as well as multiple administrations, given either simultaneously or over an extended period of time.

FORMULATIONS

[0148] Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[0149] The polypeptide and/or nucleotide sequence and/or Ab of the present invention may be effective in preventing and/or treating diseases such as cancer related diseases. The present invention includes the method of treating diseases such as cancer related disease with an effective amount of a canine 5T4 nucleotide, polypeptide or anti-canine 5T4 Ab of the present invention. The polypeptide or Ab of the present invention can be provided as a synthetic peptide or an isolated and substantially purified proteins or protein fragments or a combination thereof in pharmaceutically acceptable compositions using formulation methods known to those of ordinary skill in the art. These compositions can be administered by standard routes. These include but are not limited to: oral, rectal, ophthalmic (including intravitreal or intracameral), nasal, topical (including buccal and sublingual), intrauterine, vaginal or parenteral (including subcutaneous, intraperitoneal, intramuscular, intravenous, intradermal, intracranial, intratracheal, and epidural) transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, or parenteral (e.g., intravenous, intraspinal, subcutaneous or intramuscular) routes.

[0150] The polypeptide or Ab formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0151] In addition, the polypeptide/Ab of the present invention may be incorporated into biodegradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired, for example, at the site of a tumor or implanted so that the polypeptide/Ab is slowly released systemically. The biodegradable polymers and their use are described, for example, in detail in Brem et al(J. Neurosurg 1991 74:441-446). Osmotic minipumps may also be used to provide controlled delivery of high concentrations of polypeptide/Ab through cannulae to the site of interest, such as directly into a metastatic growth or into the vascular supply to that tumor.

[0152] The polypeptide/Ab of the present invention may be linked to cytotoxic agents which are infused in a manner designed to maximize delivery to the desired location. For example, ricin-linked high affinity Abs are delivered through a cannula into vessels supplying the target site or directly into the target. Such agents are also delivered in a controlled manner through osmotic pumps coupled to infusion cannulae.

[0153] Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient It should be understood that in addition to the

ingredients, particularly mentioned above, the formulations of the present invention may include other agents conventional in the art having regard to the type of formulation in question.

[0154] The Ab conjugates may be administered in any suitable way, usually parenterally, for example intravenously or intraperitoneally, in standard sterile, non-pyrogenic formulations of diluents and carriers, for example isotonic saline (when administered intravenously). Once the Ab conjugate has bound to the target cells and been cleared from the bloodstream (if necessary), which typically takes a day or so, the pro-drug is administered, usually as a single infused dose, or the tumour is imaged. If needed, because the Ab conjugate may be immunogenic, cyclosporin or some other immunosuppressant can be administered to provide a longer period for treatment but usually this will not be necessary.

[0155] The timing between administrations of the Ab conjugate and pro-drug may be optimised in a routine way since disease/normal tissue ratios of conjugate (at least following intravenous delivery) are highest after about 4-6 days, whereas at this time the absolute amount of conjugate bound to the 5T4, in terms of percent of injected dose per gram, is lower than at earlier times.

[0156] Therefore, the optimum interval between administration of the Ab conjugate and the pro-drug will be a compromise between peak concentration of the enzyme at the disease site and the best distribution ratio between disease and normal tissues. The dosage of the Ab conjugate will be chosen by the physician according to the usual criteria. At least in the case of methods employing a targeted enzyme such as β-glucosidase and intravenous amygdalin as the toxic pro-drug, 1 to 50 daily doses of 0.1 to 10.0 grams per square metre of body surface area, preferably 1.0-5.0 g/m^2 are likely to be appropriate. For oral therapy, three doses per day of 0.05 to 10.0g, preferably 1.0-5.0g, for one to fifty days may be appropriate. The dosage of the Ab conjugate will similarly be chosen according to normal criteria, particularly with reference to the type, stage and location of the disease tissue and the weight of the patient. The duration of treatment will depend in part upon the rapidity and extent of any immune reaction to the Ab conjugate.

[0157] The functional portion of the Ab conjugate, when the the Ab conjugate is used for diagnosis, usually comprises and may consist of a radioactive atom for scintigraphic studies, for example technetium 99m (^{99m}Tc) or iodine-123 (^{123}I), or a spin label for nuclear magnetic resonance (nmr) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-313, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0158] When used in a compound for selective destruction of, for example, the tumour, the functional portion of the Ab may comprise a highly radioactive atom, such as iodine-131, rhenium-186, rhenium-188, yttrium-90 or lead-212, which emits enough energy to destroy neighbouring cells, or a cytotoxic chemical compound such as methotrexate, adriamicin, vinca alkaliods (vincristine, vinblastine, etoposide), daunorubicin or other intercalating agents.

[0159] The radio- or other labels may be incorporated in the Ab conjugate in known ways. For example, the peptide may be biosynthesised or may be synthesised by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as ^{99m}Tc, ^{123}I, ^{186}Rh, ^{188}Rh and ^{111}In can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al(1978) Biochem. Biophys. Res. Commun. 80: 49-57 can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscinigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

PHARMACEUTICAL COMPOSITIONS

[0160] In one aspect, the present invention provides a pharmaceutical composition, which comprises an polypeptide, vector or antibody according to the present invention and optionally a pharmaceutically acceptable carrier, diluent or excipient (including combinations thereof).

[0161] The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier, or excipient. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

[0162] Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

[0163] There may be different composition/formulation requirements dependent on the different delivery systems. By way of example, the pharmaceutical composition of the present invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestable solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by both routes.

[0164] Where the pharmaceutical composition is to be delivered mucosally through the gastrointestinal mucosa, it

should be able to remain stable during transit though the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile.

[0165] Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose or chalk, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

ADMINISTRATION

[0166] Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient and severity of the condition. The dosages below are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited.

[0167] The compositions (or component parts thereof) of the present invention may be administered orally. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by direct injection. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered topically. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by inhalation. In addition or in the alternative the compositions (or component parts thereof) of the present invention may also be administered by one or more of: parenteral, mucosal, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration means, and are formulated for such administration.

[0168] By way of further example, the pharmaceutical composition of the present invention may be administered in accordance with a regimen of 1 to 10 times per day, such as once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

[0169] The term "administered" also includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

[0170] Hence, the pharmaceutical composition of the present invention may be administered by one or more of the following routes: oral administration, injection (such as direct injection), topical, inhalation, parenteral administration, mucosal administration, intramuscular administration, intravenous administration, subcutaneous administration, intraocular administration or transdermal administration.

DISEASES

[0171] Pharmaceutical compositions comprising an effective amount of a polypeptide, vector or antibody of the invention may be used in the treatment of cancer. Specific cancer related disorders include but not limited to: solid tumours; blood born tumours such as leukemias; tumor metastasis; benign tumours, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas.

Figures

[0172]

Figure 1 shows the canine 5T4 coding sequence
In slightly more detail:

[0173] Figure 1 shows the canine 5T4 coding sequence. A mongrel genomic library in λ dash was screened with a probe made from h5T4 cDNA. Positive clones were identified and sequenced.

SEQUENCE LISTING

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Kingsman, Alan

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Tyr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
        35                  40                  45

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

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

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

Ala Arg Ser Thr Met Ile Thr Asn Tyr Val Met Asp Tyr Trp Gly Gln
            100                 105                 110

Val Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125

Gly Thr Gly Gly Gly Gly Ser Ser Ile Val Met Thr Gln Thr Pro Thr
    130                 135                 140

Phe Leu Leu Val Ser Ala Gly Asp Arg Val Thr Ile Thr Cys Lys Ala
145                 150                 155                 160

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Gln Ser Pro Thr Leu Leu Ile Ser Tyr Thr Ser Ser Arg Tyr Ala Gly
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Thr Ile Ser Thr Leu Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln
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Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu
        35                  40                  45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
    50                  55                  60

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
65                  70                  75                  80

Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
                85                  90                  95

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly
            100                 105                 110

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg
        115                 120                 125

Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr
    130                 135                 140

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
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Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
                165                 170                 175

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

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
        195                 200                 205

Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
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Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
225                 230                 235                 240

Asp Gly Gly Gly Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Asp
                245                 250                 255

Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly
            260                 265                 270

Tyr Ser Phe Thr Gly Tyr Tyr Met His Trp Val Lys Gln Ser His Gly
        275                 280                 285

Lys Ser Leu Glu Trp Ile Gly Arg Ile Asn Pro Asn Asn Gly Val Thr
    290                 295                 300

Leu Tyr Asn Gln Lys Phe Lys Asp Lys Ala Ile Leu Thr Val Asp Lys
305                 310                 315                 320

Ser Ser Thr Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp
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Ser Ala Val Tyr Tyr Cys Ala Arg Ser Thr Met Ile Thr Asn Tyr Val
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Met Asp Tyr Trp Gly Gln Val Thr Ser Val Thr Val Ser Ser Gly Gly
        355                 360                 365

Gly Gly Ser Gly Gly Gly Gly Thr Gly Gly Gly Gly Ser Ser Ile Val
    370                 375                 380

Met Thr Gln Thr Pro Thr Phe Leu Leu Val Ser Ala Gly Asp Arg Val
385                 390                 395                 400

Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Ser Asn Asp Val Ala Trp
                405                 410                 415

Tyr Gln Gln Lys Pro Gly Gln Ser Pro Thr Leu Leu Ile Ser Tyr Thr
            420                 425                 430

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

Gln Lys Phe Lys Asp Lys Ala Ile Leu Thr Val Asp Lys Ser Ser Thr
                85                  90                  95

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

Tyr Tyr Cys Ala Arg Ser Thr Met Ile Thr Asn Tyr Val Met Asp Tyr
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Trp Gly Gln Val Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
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Gly Gly Gly Gly Thr Gly Gly Gly Gly Ser Ser Ile Val Met Thr Gln
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Cys Lys Ala Ser Gln Ser Val Ser Asn Asp Val Ala Trp Tyr Gln Gln
            180                 185                 190

Lys Pro Gly Gln Ser Pro Thr Leu Leu Ile Ser Tyr Thr Ser Ser Arg
        195                 200                 205

Tyr Ala Gly Val Pro Asp Arg Phe Ile Gly Ser Gly Tyr Gly Thr Asp
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Phe Thr Phe Thr Ile Ser Thr Leu Gln Ala Glu Asp Leu Ala Val Tyr
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Phe Cys Gln Gln Asp Tyr Asn Ser Pro Pro Thr Phe Gly Gly Gly Thr
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Lys Leu Glu Ile Lys Arg Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
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Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
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Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
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gggaggagca gtacaacagc acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg 1380

actggctgaa tggcaaggag tacaagtgca aggtctccaa caaagccctc ccagccccca 1440

tcgagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg tacaccctgc 1500

ccccatcccg ggatgagctg accaagaacc aggtcagcct gacctgcctg gtcaaaggct 1560

tctatcccag cgacatcgcc gtggagtggg agagcaatgg gcagccggag aacaactaca 1620

agaccacgcc tcccgtgctg gactccgacg gctccttctt cctctatagc aagctcaccg 1680

tggacaagag caggtggcag caggggaacg tcttctcatg ctccgtgatg catgaggctc 1740

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<223> B7-2.5T4.1

<400> 9

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ataacagaaa atgtgtacat aaatttgacc tgctcatcta tacacggtta cccagaacct    480
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ttccctgatg ttacgagcaa tatgaccatc ttctgtattc tggaaactga caagacgcgg    660
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            20                  25                  30

Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln Ser Leu Ser Glu Leu Val
        35                  40                  45

Val Phe Trp Gln Asp Gln Glu Asn Leu Val Leu Asn Glu Val Tyr Leu
    50                  55                  60

Gly Lys Glu Lys Phe Asp Ser Val His Ser Lys Tyr Met Gly Arg Thr
65                  70                  75                  80
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Ser Phe Asp Ser Asp Ser Trp Thr Leu Arg Leu His Asn Leu Gln Ile
                85                  90                  95

Lys Asp Lys Gly Leu Tyr Gln Cys Ile Ile His His Lys Lys Pro Thr
            100                 105                 110

Gly Met Ile Arg Ile His Gln Met Asn Ser Glu Leu Ser Val Leu Ala
        115                 120                 125

Asn Phe Ser Gln Pro Glu Ile Val Pro Ile Ser Asn Ile Thr Glu Asn
    130                 135                 140

Val Tyr Ile Asn Leu Thr Cys Ser Ser Ile His Gly Tyr Pro Glu Pro
145                 150                 155                 160

Lys Lys Met Ser Val Leu Leu Arg Thr Lys Asn Ser Thr Ile Glu Tyr
                165                 170                 175

Asp Gly Ile Met Gln Lys Ser Gln Asp Asn Val Thr Glu Leu Tyr Asp
            180                 185                 190

Val Ser Ile Ser Leu Ser Val Ser Phe Pro Asp Val Thr Ser Asn Met
        195                 200                 205

Thr Ile Phe Cys Ile Leu Glu Thr Asp Lys Thr Arg Leu Leu Ser Ser
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Pro Phe Ser Ile Glu Leu Glu Asp Pro Gln Pro Pro Pro Asp His Ile
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Pro Gly Gly Gly Gly Ser
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<400> 11

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caactgtcca agtcagtgaa agataaggta ttgctgcctt gccgttacaa ctctccgcat 180

gaagatgagt ctgaagaccg aatctactgg caaaaacatg acaaagtggt gctgtctgtc 240

attgctggga aactaaaagt gtggcccgag tataagaacc ggactttata tgacaacact 300

acctactctc ttatcatcct gggcctggtc ctttcagacc ggggcacata cagctgtgtc 360

gttcaaaaga aggaaagagg aacgtatgaa gttaaacact tggctttagt aaagttgtcc 420

atcaaagctg acttctctac ccccaacata actgagtctg gaaacccatc tgcagacact 480

aaaaggatta cctgctttgc ttccgggggt ttcccaaagc ctcgcttctc ttggttggaa 540

aatggaagag aattacctgg catcaatacg acaatttccc aggatcctga atctgaattg 600

tacaccatta gtagccaact agatttcaat acgactcgca accacaccat taagtgtctc 660

attaaatatg gagatgctca cgtgtcagag gacttcacct gggaaaaacc cccagaagac 720

cctcctgata gcaagcccgg gggtggtggg agcggtggtg gcggcagtgg cggcggcgga 780

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cagagccatg gaaagagcct tgagtggatt ggacgtatta atcctaacaa tggtgttact 960

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<400> 12

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ctctctacaa ccagaaattc aaggacaagg ccatattaac tgtagacaag tcatccacca    300
cagcctacat ggagctccgc agcctgacat ctgaggactc tgcggtctat tactgtgcaa    360
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taccagccac caccctcacg ctctctggtc actatgccac catcagcttg ctgaccgtct   1020
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ccgaatggga gcagaaagat gagttcatct gccgtgcagt ccatgaggca gcgagccccc   2040
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caactgtcca agtcagtgaa agataaggta ttgctgcctt gccgttacaa ctctccgcat 180

gaagatgagt ctgaagaccg aatctactgg caaaaacatg acaaagtggt gctgtctgtc 240

attgctggga aactaaaagt gtggcccgag tataagaacc ggactttata tgacaacact 300

acctactctc ttatcatcct gggcctggtc ctttcagacc ggggcacata cagctgtgtc 360

gttcaaaaga aggaaagagg aacgtatgaa gttaaacact tggctttagt aaagttgtcc 420

atcaaagctg acttctctac ccccaacata actgagtctg gaaacccatc tgcagacact 480

aaaaggatta cctgctttgc ttccgggggt ttcccaaagc ctcgcttctc ttggttggaa 540

aatggaagag aattacctgg catcaatacg acaatttccc aggatcctga atctgaattg 600

tacaccatta gtagccaact agatttcaat acgactcgca accacaccat taagtgtctc 660

attaaatatg gagatgctca cgtgtcagag gacttcacct gggaaaaacc cccagaagac 720

cctcctgata gcaagcccgg gggtggtggg agcggtggtg gcggcagtgg cggcggcgga 780

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<210> 14
<211> 1263
<212> DNA
<213> Artificial

<220>
<223> 5T4

<400> 14

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gctgccctcc gagcgggccg cgcgcttcgc gggctgcagt gcctggagct ggccggcaac    660
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<212> PRT
<213> Artificial

<220>
<223> 5T4

<400> 15

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Ser Leu Thr Ser Trp Ala Pro Ser Ala Ala Ala Ser Thr Ser Pro Pro
        35                  40                  45

Ala Ser Ala Ala Ser Ala Pro Pro Pro Leu Pro Gly Gln Cys Pro Gln
    50                  55                  60

Pro Cys Glu Cys Ser Glu Ala Ala Arg Thr Val Lys Cys Val Asn Arg
65                  70                  75                  80

Asn Leu Thr Glu Val Pro Ala Asp Leu Pro Pro Tyr Val Arg Asn Leu
                85                  90                  95

Phe Leu Thr Gly Asn Gln Leu Ala Val Leu Pro Pro Gly Ala Phe Ala
            100                 105                 110

Arg Arg Pro Pro Leu Ala Glu Leu Ala Ala Leu Asn Leu Ser Gly Ser
        115                 120                 125

Ser Leu Arg Glu Val Cys Ala Gly Ala Phe Glu His Leu Pro Ser Leu
    130                 135                 140

Arg Gln Leu Asp Leu Ser His Asn Pro Leu Gly Asn Leu Ser Ala Phe
145                 150                 155                 160

Ala Phe Ala Gly Ser Asp Ala Ser Arg Ser Gly Pro Ser Pro Leu Val
                165                 170                 175

Glu Leu Met Leu Asn His Ile Val Pro Pro Asp Asp Arg Arg Gln Asn
            180                 185                 190

Arg Ser Phe Glu Gly Met Val Ala Ala Ala Leu Arg Ala Gly Arg Ala
        195                 200                 205

Leu Arg Gly Leu Gln Cys Leu Glu Leu Ala Gly Asn Arg Phe Leu Tyr
    210                 215                 220

Leu Pro Arg Asp Val Leu Ala Gln Leu Pro Gly Leu Arg His Leu Asp
225                 230                 235                 240

Leu Arg Asn Asn Ser Leu Val Ser Leu Thr Tyr Val Ser Phe Arg Asn
                245                 250                 255

Leu Thr His Leu Glu Ser Leu His Leu Glu Asp Asn Ala Leu Lys Val
            260                 265                 270

Leu His Asn Ala Thr Leu Ala Glu Leu Gln Ser Leu Pro His Val Arg
        275                 280                 285

Val Phe Leu Asp Asn Asn Pro Trp Val Cys Asp Cys His Met Ala Asp
    290                 295                 300

Met Val Ala Trp Leu Lys Glu Thr Glu Val Val Pro Gly Lys Ala Gly
305                 310                 315                 320

Leu Thr Cys Ala Phe Pro Glu Lys Met Arg Asn Arg Ala Leu Leu Glu
                325                 330                 335

Leu Asn Ser Ser His Leu Asp Cys Asp Pro Ile Leu Pro Pro Ser Leu
```

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                340                 345                 350

Gln Thr Ser Tyr Val Phe Leu Gly Ile Val Leu Ala Leu Ile Gly Ala
        355                 360                 365

Ile Phe Leu Leu Val Leu Tyr Leu Asn Arg Lys Gly Ile Lys Lys Trp
    370                 375                 380

Met His Asn Ile Arg Asp Ala Cys Arg Asp His Met Glu Gly Tyr His
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Tyr Arg Tyr Glu Ile Asn Ala Asp Pro Arg Leu Thr Asn Leu Ser Ser
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Asn Ser Asp Val
            420
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<220>
<223> Flexible linker to join the extracelluar domain of B7.1 and ScFv

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<223> PCR primer

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<210> 18
<211> 30
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<220>
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<210> 19
<211> 29
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<220>
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<400> 19
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<210> 20
<211> 44
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<400> 20
ctcgcggccg cttaccgttt gatttccagc ttggtgcctc cacc 44

<210> 21
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<223> Translation initiation sequence and the human immunoglobulin kapp a light chain signal peptid

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aggtgtccac tccgaggtcc agctgca 87

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atcccatggt ggctcgagt 79

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<212> DNA
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<220>
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<400> 23
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<210> 24
<211> 22
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<220>
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<400> 24
cgtttgattt caagcttggt gc 22

<210> 25
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<220>
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<400> 25
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<210> 26
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<212> DNA
<213> Artificial

<220>
<223> PCR primer

<400> 26
gcgcctcgag tcatttaccc ggagacaggg 30

<210> 27
<211> 40
<212> DNA
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<220>
<223> PCR primer

<400> 27
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<210> 28
<211> 31
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<400> 28
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<210> 29
<211> 29
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<400> 29
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<210> 30
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<220>
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<210> 32
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<400> 32
gcgcgaattc ttaccgtttg atttccagct tggt 34

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<210> 34
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<220>
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<400> 34
gcgcgaattc ttaccgtttg atttccagct tggt 34

<210> 35
<211> 48
<212> DNA

<213> Artificial

<220>
<223> Linker sequence in pBS II

<400> 35
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<210> 36
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Linker sequence in pBS II

<400> 36

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 37
<211> 76
<212> DNA
<213> Artificial

<220>
<223> Leader sequence in pBS II

<400> 37

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acaggtgtac actccc 76

Claims

1. An canine 5T4 polypeptide having the amino acid sequence shown in SEQ ID No 14 or a homologue thereof, having at least 90% identity or a fragment thereof which comprises amino acids 1-182 and/or 297-420.

2. A nucleotide sequence capable of encoding a canine 5T4 polypeptide according to claim 1.

3. A nucleotide sequence according to claim 2, having the sequence shown as SEQ ID NO 15, or a homologue thereof having at least 90% identify, or a fragment thereof of at least 50 nucleotides in length.

4. A nucleotide sequence according to claim 3, having a fragment of the sequence shown as SEQ ID No. 15 which comprises nucleic acids 1-546 and/or 890-1263.

5. An antibody capable of binding specifically to a canine 5T4 polypeptide according to claim 1.

6. A vector comprising a nucleotide sequence according to claim 3 or 4.

7. A retroviral vector according to claim 6.

8. A lentiviral vector according to claim 6.

9. A pox virus vector according to claim 6.

10. A modified vaccinia virus Ankara (MVA) vector according to claim 6.

11. A host cell comprising a nucleotide sequence according to claim 3 or 4.

12. A diagnositic kit comprising an antibody according to claim 5 for detection of canine 5T4.

13. The use of an antibody according to claim 5 to isolate trophoblasts from a maternal blood sample in vitro.

Patentansprüche

1. Hunde-5T4-Polypeptid mit der in SEQ ID NO: 14 gezeigten Aminosäuresequenz oder ein Homologes davon mit wenigstens 90% Identität oder ein Fragment davon, welches die Aminosäuren 1-182 und/oder 297-420 umfaßt.

2. Nukleotidsequenz, die zum Codieren eines Hunde-5T4-Polypeptids nach Anspruch 1 in der Lage ist.

3. Nukleotidsequenz nach Anspruch 2 mit der als SEQ ID NO: 15 gezeigten Sequenz oder ein Homologes davon mit wenigstens 90% Identität oder ein Fragment davon, welches wenigstens 50 Nukleotide lang ist.

4. Nukleotidsequenz nach Anspruch 3 mit einem Fragment der als SEQ ID NO: 15 gezeigten Sequenz, welche die Nukleinsäuren 1-546 und/oder 890-1263 umfaßt.

5. Antikörper, der in der Lage ist, spezifisch an ein Hunde-5T4-Polypeptid nach Anspruch 1 zu binden.

6. Vektor, welcher eine Nukleotidsequenz nach Anspruch 3 oder 4 umfaßt.

7. Retrovirusvektor nach Anspruch 6.

8. Lentivirusvektor nach Anspruch 6.

9. Pockenvirusvektor nach Anspruch 6.

10. Modifizierter Vacciniavirus Ankara (MVA) -Vektor nach Anspruch 6.

11. Wirtszelle, welche eine Nukleotidsequenz nach Anspruch 3 oder 4 umfaßt.

12. Diagnostischer Kit, welcher einen Antikörper nach Anspruch 5 umfaßt, für die Detektion von Hunde-5T4.

13. Verwendung eines Antikörpers nach Anspruch 5 zum Isolieren von Trophoblasten aus einer maternalen Blutprobe in vitro.

Revendications

1. Polypeptide 5T4 canin ayant la séquence d'acides aminés représentée dans SEQ ID No. 14 ou un homologue de cette séquence ayant une identité d'au moins 90 %, ou un fragment de cette séquence qui comprend les acides aminés 1-182 et/ou 297-420.

2. Séquence nucléotidique capable de coder un polypeptide 5T4 canin suivant la revendication 1.

3. Séquence nucléotidique suivant la revendication 2, ayant la séquence représentée comme SEQ ID No. 15 ou un homologue de cette séquence ayant une identité d'au moins 90 %, ou un fragment de cette séquence d'une longueur d'au moins 50 nucléotides.

4. Séquence nucléotidique suivant la revendication 3, ayant un fragment de la séquence représentée comme SEQ ID No. 15 qui comprend les acides nucléiques 1-546 et/ou 890-1263.

5. Anticorps capable de se lier spécifiquement à un polypeptide 5T4 canin suivant la revendication 1.

6. Vecteur comprenant une séquence nucléotidique suivant la revendication 3 ou 4.

7. Vecteur rétroviral suivant la revendication 6.

8. Vecteur lentiviral suivant la revendication 6.

9. Vecteur variolique suivant la revendication 6.

10. Vecteur modifié du virus Ankara (MVA) de la vaccine suivant la revendication 6.

11. Cellule hôte comprenant une séquence nucléotidique suivant la revendication 3 ou 4.

12. Nécessaire pour diagnostic, comprenant un anticorps suivant la revendication 5 pour la détection du 5T4 canin.

13. Utilisation d'un anticorps suivant la revendication 5 pour isoler des trophoblastes d'un échantillon de sang maternel *in vitro.*

FIG. 1

Canine 5T4 Coding Sequence

```
ATGCCTGGGGGGTGCTCCCGGGGCCCCGCCGCCGGGGACGGGCGGTTGCGGCTGGCGCGGCTGGCGCTGGTGCTCCTGGG 80
  M  P  G  G  C  S  R  G  P  A  A  G  D  G  R  L  R  L  A  R  L  A  L  V  L  L

CTGGGTCTCCTCGTCCTCGCTCACCTCCTGGGCGCCCTCCGCCGCCGCCTCCACGTCGCCGCCGGCCTCCGCGGCGTCCG 160
G  W  V  S  S  S  S  S  L  T  S  W  A  P  S  A  A  A  S  T  S  P  P  A  S  A  A  S

CCCCGCCCCCGCTGCCGGGCCAGTGCCCCCAGCCTTGCGAGTGCTCGGAGGCGGCGCGCACGGTCAAGTGCGTTAACCGC 240
 A  P  P  P  L  P  G  Q  C  P  Q  P  C  E  C  S  E  A  A  R  T  V  K  C  V  N  R

AACCTGACCGAGGTGCCCGCGGACCTGCCCCCCTACGTGCGCAACCTCTTCCTCACGGGCAACCAGCTGGCGGTGCTGCC 320
  N  L  T  E  V  P  A  D  L  P  P  Y  V  R  N  L  F  L  T  G  N  Q  L  A  V  L

CCCCGGCGCCTTCGCCCGCCGGCCGCCGCTGGCCGAGCTGGCCGCGCTCAACCTGAGCGGCAGCAGCCTGCGGGAGGTGT 400
P  P  G  A  F  A  R  R  P  P  L  A  E  L  A  A  L  N  L  S  G  S  S  L  R  E  V

GCGCCGGCGCCTTCGAGCACCTGCCCAGCCTGCGCCAGCTCGACCTCAGCCACAACCCGCTGGGCAACCTCAGCGCCTTC 480
 C  A  G  A  F  E  H  L  P  S  L  R  Q  L  D  L  S  H  N  P  L  G  N  L  S  A  F

GCCTTCGCGGGCAGCGACGCCAGCCGCTCGGGCCCCAGCCCCCTGGTGGAGCTGATGCTGAACCACATCGTGCCCCCCGA 560
  A  F  A  G  S  D  A  S  R  S  G  P  S  P  L  V  E  L  M  L  N  H  I  V  P  P

CGACCGGCGGCAGAACCGGAGCTTCGAGGGCATGGTGGCGGCTGCCCTCCGAGCGGGCCGCGCGCTTCGCGGGCTGCAGT 640
D  D  R  R  Q  N  R  S  F  E  G  M  V  A  A  A  L  R  A  G  R  A  L  R  G  L  Q

GCCTGGAGCTGGCCGGCAACCGCTTCCTCTACTTGCCTCGCGACGTCCTGGCCCAGCTACCCGGCCTCCGGCACCTGGAC 720
 C  L  E  L  A  G  N  R  F  L  Y  L  P  R  D  V  L  A  Q  L  P  G  L  R  H  L  D

CTGCGCAACAACTCCCTGGTGAGCCTCACCTACGTGTCCTTCCGCAACCTGACGCACTTGGAGAGCCTCCACCTGGAGGA 800
  L  R  N  N  S  L  V  S  L  T  Y  V  S  F  R  N  L  T  H  L  E  S  L  H  L  E

CAACGCCCTCAAGGTCCTTCACAACGCCACCCTGGCGGAGCTGCAGAGCCTGCCCCACGTCCGGGTCTTCCTGGACAACA 880
D  N  A  L  K  V  L  H  N  A  T  L  A  E  L  Q  S  L  P  H  V  R  V  F  L  D  N

ACCCCTGGGTCTGCGATTGTCACATGGCAGACATGGTGGCCTGGCTCAAGGAGACAGAGGTGGTGCCGGGCAAAGCCGGG 960
 N  P  W  V  C  D  C  H  M  A  D  M  V  A  W  L  K  E  T  E  V  V  P  G  K  A  G

CTCACCTGTGCATTCCCGGAGAAAATGAGGAATCGGGCCCTCTTGGAACTCAACAGCTCCCACCTGGACTGTGACCCTAT 1040
  L  T  C  A  F  P  E  K  M  R  N  R  A  L  L  E  L  N  S  S  H  L  D  C  D  P

CCTCCCTCCATCCCTGCAGACTTCTTATGTCTTCCTAGGTATTGTCTTAGCCCTGATAGGCGCCATCTTCCTACTGGTTT 1120
I  L  P  P  S  L  Q  T  S  Y  V  F  L  G  I  V  L  A  L  I  G  A  I  F  L  L  V

TGTATTTCAACCGCAAGGGGATAAAGAAGTGGATGCATAACATCAGAGATGCCTGCAGGGATCACATGGAAGGGTATCAC 1200
 L  Y  L  N  R  K  G  I  K  K  W  M  H  N  I  R  D  A  C  R  D  H  M  E  G  Y  H

TACAGATACGAAATCAATGCAGACCCCAGGTTAACAAACCTCAGTTCCAATTCGGATGTCTGA                  1263
  Y  R  Y  E  I  N  A  D  P  R  L  T  N  L  S  S  N  S  D  V  .
```

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专利名称(译)	针对疾病相关分子的Scfv抗体		
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摘要(译)

本发明描述了在制备用于预防和/或治疗与DAM相关的疾病的药物中能够识别疾病相关分子（DAM）的ScFv Ab（ScFv Ab）的用途。 ScFv Ab具有治疗，诊断和预后应用。


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

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

Tyr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
        35                  40                  45

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

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

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

Ala Arg Ser Thr Met Ile Thr Asn Tyr Val Met Asp Tyr Trp Gly Gln
            100                 105                 110

Val Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125

Gly Thr Gly Gly Gly Gly Ser Ser Ile Val Met Thr Gln Thr Pro Thr
    130                 135                 140

Phe Leu Leu Val Ser Ala Gly Asp Arg Val Thr Ile Thr Cys Lys Ala
145                 150                 155                 160

Ser Gln Ser Val Ser Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly
                165                 170                 175

Gln Ser Pro Thr Leu Leu Ile Ser Tyr Thr Ser Ser Arg Tyr Ala Gly
            180                 185                 190

Val Pro Asp Arg Phe Ile Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe
        195                 200                 205

Thr Ile Ser Thr Leu Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln
    210                 215                 220

Gln Asp Tyr Asn Ser Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
```