HUMANISED ANTIBODIES TO THE EPIDERMAL GROWTH FACTOR RECEPTOR

The present invention relates to humanised antibodies and fragments thereof, and in particular, to humanised antibodies specific for the epithelial growth factor receptor (EGFR).

EGFR is a tumour-associated cell surface antigen, and hence a well-known target for antibodies. Durrant *et al.* (*Prenatal Diagnosis*, 14, 131-140, 1994) describe a mouse monoclonal antibody, known as "340", which binds to EGFR with high specificity. A cell line expressing these antibodies is deposited with the ECACC under accession number 97021428. Monoclonal antibody 340 was raised against the osteosarcoma cell line 791T. Immunoprecipitation studies showed that 340 recognised a membrane glycoprotein of molecular weight 170 kDa from both osteosarcoma tumours and placental tissues. Terminal amino acid sequencing of the purified antigen showed sequence identity to the epidermal growth factor receptor. To confirm that 340 antigen was EGF receptor, radiolabelled EGF and EGF receptor antibodies were shown to bind to the S340 antibody antigen. Furthermore, 340 could compete with EGF binding to its receptor, and EGF could compete with 340 for binding to its antigen. Extensive studies have shown that 340 binds to colorectal, gastric, ovarian, osteosarcoma tumour cell lines. The antibody also recognises foetal trophoblasts and has been used to sort foetal cells from maternal blood.

Several other mouse monoclonal antibodies have been shown to recognise EGF receptor. They fall broadly into two categories: antibodies that bind to the receptor but do not inhibit binding of EGF, and antibodies that bind to the receptor and do inhibit binding of EGF. 340 monoclonal antibodies belong to the latter group.

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The use of rodent, especially mouse, monoclonal antibodies for therapeutic and *in vivo* diagnostic applications in man has been found to be limited by immune responses elicited by patients to the rodent antibody. The development of so-called "HAMA" (human anti-mouse antibody) responses in patients has been shown to limit the ability of antibodies to reach their antigenic targets, resulting in reduced effectiveness of the antibodies. In order to reduce the HAMA response, chimaeric antibodies have been developed in which the mouse variable (V) regions are joined to the human constant (C) regions. Such antibodies have proved clinically useful, although the mouse V region component still provides the basis for generating immunogenicity in patients (LoBuglio *et al.*, *Proc. Nat. Acad. Sci. USA*, **86**, 4220-4224, 1989). Therefore technology for humanised antibodies has been developed whereby the complementarity determining regions (CDRs) from the rodent antibody are grafted onto human V regions and joined to human C regions, to create antibodies where the only "non-human" components are the CDRs which are adjacent to human framework regions. However, it was soon realised that simple transplantation of the CDRs often resulted in reduced affinity of the humanised antibody and consequently that the introduction of certain non-human amino acids in the human V region framework was required to restore the affinity.

The common aspect of these methods for the production of humanised antibodies is to create antibodies which are essentially non-immunogenic in humans. However, the means by which this is achieved has been by the introduction of as much human sequence as possible into the rodent antibody. It is known that certain short peptide sequences or "epitopes" can be immunogenic in humans. Accordingly, techniques have been developed in which such epitopes are identified by computer analysis and amino acids are replaced to produce non-immunogenic peptides (WO98/52976 and WO00/34317).

The inventors have produced humanised versions of the 340 antibody (known as either as "340Ch" (in the case of the mouse human chimera) or "SC100" (in the case of deimmunised antibodies) with reduced immunogenicity with a view to providing a clinically useful therapeutic tool. Unexpectedly, they have found that such humanised antibodies showed similar binding to cells expressing EGFR to the original murine antibody but had an increased ability to inhibit the growth of such cells.

Thus, according to a first aspect of the present invention, there is provided a humanised form of the antibody 340 obtainable from the cell line deposited with the ECACC under accession number 97021428.

It is preferred if the antibody of the present invention comprises the CDRH3 of antibody 340 provided in a human antibody framework. The antibody may further comprise one or more of the other CDRs of the heavy or light chains of 340. Such CDRs are shown in Figure 2 of the accompanying drawings. It may comprise the hypervariable region of antibody 340. The variable region other than the hypervariable region may be derived from the variable region of a human antibody. Methods for making such antibodies are known in the art, for example, in Winter, U.S. Patent No. 5,225,539.

The variable region of the antibody outside of the hypervariable region may also be derived from monoclonal antibody 340. Methods for making such antibodies are known in the art, including, for example, those described in U.S. Patents Nos. 4,816,397 by Boss (Celltech) and 4,816,567 by Cabilly (Genentech). Thus, in a preferred embodiment, the antibody comprises the human antibody framework and a substantial portion of the variable region of antibody 340, preferably the variable region as shown in Figure 2.

In another embodiment, the antibody comprises the human antibody framework and one of V_Hb, c, d or e as disclosed in Figure 6, and one of V_Kb, c or d, as disclosed in Figure 7. Preferred antibodies comprise a human antibody framework with V_Hd and V_Kd or V_Hd or V_Kb.

The human antibody framework is preferably all or a part of the constant region of a human antibody. For example, humanised antibodies based on all or a part of the V_L region shown in Figure 2 may be attached at their C-terminal end to antibody light chain constant domains including human Cκ or Cλ chains. Similarly, antibodies based on all or a part of the V_H region shown in Figure 2 may be attached at their C-terminal end to all or part of an immunoglobulin heavy chain derived from any antibody isotype, e.g. IgG, IgA, IgE and IgM and any of the isotype sub-classes, particularly IgG1 and IgG4. IgG1 is preferred.

Monoclonal antibodies can block ligands by binding near the ligand binding site of the ligand's receptor and sterically blocking access to the ligand. Alternatively, the antibody may molecularly mimic the ligand and interact at the ligand binding site. The inventors show herein that the 340 antibody and its SC100 derivatives are unique as they not only bind at the ligand binding site but show amino acid homology with two distinct areas of the ligand that are brought together by secondary structure. Furthermore, the importance of the CDRH3 region was confirmed in cell binding studies as, when a single amino acid was changed within this region, binding of SC100 to EGFr was considerably reduced. This implies that an antibody with CDR regions showing homology with peptide ligands can effectively compete with ligand for binding to a receptor. They can therefore block intracellular signalling associated with ligand/receptor interaction. In this example, as EGF is both a growth and a survival factor, blocking its interaction with its

receptors results in inhibition of cell growth and apoptosis. As EGF receptor are widely over-expressed in malignancy, blocking EGF receptor by either drug or antibodies inhibits tumour growth. These reagents have been shown to be particularly effective in combination chemotherapy at causing tumour regression in animal models.

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. *et al.*, *Nature* **341**:544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments; (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird *et al.*, *Science* **242**:423-426 (1988); Huston *et al.*, *PNAS USA* **85**:5879-5883 (1988)); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* **90**:6444-6448 (1993)).

Diabodies are multimers of polypeptides, each polypeptide comprising a first domain

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

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Hollinger & Winter, *Current Opinion Biotechnol.* 1993 4:446-449), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. It may be preferable to use scFv dimers or diabodies rather than whole antibodies. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction. Other forms of bispecific antibodies include the single chain "Janusins" described in Traunecker *et al.*, *EMBO Journal* **10**:3655-3659 (1991).

Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E. coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against antigen X, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected.

A substantial portion of an immunoglobulin variable domain will comprise at least the three CDR regions, together with their intervening framework regions. Preferably, the portion will also include at least about 50% of either or both of the

first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with naturally occurring variable domain regions. For example, construction of antibodies of the present invention made by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers introduced to facilitate cloning or other manipulation steps, including the introduction of linkers to join variable domains of the invention to further protein sequences including immunoglobulin heavy chains, other variable domains (for example in the production of diabodies) or protein labels as discussed in more detail below.

Although in one embodiment of the invention, antibodies comprising a pair of binding domains based on the amino acid sequences for the V_L and V_H regions substantially as set out in Figure 2 are preferred, single binding domains based on either of these sequences form further aspects of the invention. In the case of the binding domains based on the amino acid sequence for the V_H region substantially set out in Figure 2, such binding domains may be used as targeting agents since it is known that immunoglobulin V_H domains are capable of binding target antigens in a specific manner.

In the case of either of the single chain specific binding domains, these domains may be used to screen for complementary domains capable of forming a two-domain specific binding member which has *in vivo* properties as good as or equal to the antibodies disclosed herein. This may be achieved by phage display screening methods using the so-called hierarchical dual combinatorial approach as disclosed in WO92/01047 in which an individual colony containing either an H or L chain clone is used to infect a complete library of clones encoding the other chain (L or H) and

the resulting two-chain specific binding member is selected in accordance with phage display techniques such as those described in that reference.

It will be appreciated by those skilled in the art that the sequences of the CDR, hypervariable and variable regions can be modified without losing the ability to bind EGFR. For example, CDR regions of the invention will be either identical or highly homologous to the specified regions of Figures 1 and 2. By "highly homologous" it is contemplated that from 1 to 5, preferably from 1 to 4, such as 1 to 3 or 1 or 2 substitutions may be made in the CDRs. In addition, the hypervariable and variable regions may be modified so that they show substantial homology with the regions specifically disclosed herein. Preferably the degree of homology (between respective CDRs, hypervariable regions or variable regions and their non-modified counterparts) will be at least 60%, more preferably 70%, further preferably 80%, even more preferably 90% or most preferably 95%. Such modified sequences fall within the scope of the present invention, provided of course that they have the ability to bind EGFR and to inhibit the growth of cells at a greater rate than antibody 340. The term "antibody" is to be construed accordingly.

The percent identity of two amino acid sequences or of two nucleic acid sequences is determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in the first sequence for best alignment with the sequence) and comparing the amino acid residues or nucleotides at corresponding positions. The "best alignment" is an alignment of two sequences which results in the highest percent identity. The percent identity is determined by the number of identical amino acid residues or nucleotides in the sequences being compared (*i.e.*, % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. The NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410 have incorporated such an algorithm. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilised as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilising BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. *See* http://www.ncbi.nlm.nih.gov.

Another example of a mathematical algorithm utilised for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the CGC sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis and Robotti (1994) *Comput. Appl. Biosci., 10* :3-5; and FASTA described in Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. 85*:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search.

As shown herein, the CDRH3 of SC100 shows amino acid homology with the growth factor EGF. These results described below suggest that SC100 and fragments and derivatives thereof can be used as cancer therapeutics to inhibit the growth or induce apoptosis of tumour cells as exemplified by inhibition of growth of tumour cell lines, apoptosis of tumour cell lines, *in vivo* inhibition of tumour xenografts in nude mice. Accordingly, the invention further includes the use of "fragments" or "derivatives" of either SC100 or other polypeptides of the "SC100" family which are capable of inhibition of binding of EGF. A preferred group of fragments are those which include all or part of the CDR regions of monoclonal antibodies SC100. A fragment of SC100 or of a polypeptide of the SC100 families means a stretch of amino acid residues of at least 5 to 7 contiguous amino acids. Often at least about 7 to 9 contiguous amino acids, typically at least about 9 to 13 contiguous amino acids, more preferably at least about 20 to 30 or more contiguous amino acids and most preferably at least about 30 to 40 or more consecutive amino acids. A "derivative" of SC100 or of a polypeptide of the SC100 family, or of a fragment of SC100 family polypeptide, means a polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve insertion, addition, deletion and/or substitution of one or more amino acids, while providing a peptide capable of inducing an anti-tumour T-cell response. Preferably such derivatives involve the insertion, addition, deletion and/or substitution of 25 or fewer amino acids, more preferably of 15 or fewer, even more preferably of 10 or fewer, more preferably still of 4 or fewer and most preferably of 1 or 2 amino acids only.

The invention also provides the antibodies mentioned above linked to a coupling partner, e.g. an effector molecule, a label, a drug, a toxin and/or a carrier or transport molecule. Techniques for coupling the antibodies of the invention to both

peptidyl and non-peptidyl coupling partners are well known in the art. In one embodiment, the carrier molecule is a 16 amino acid peptide derived from the homeodomain of *Antennapedia* (e.g. as sold under the name "Penetratin"), which can be coupled to a peptide via terminal Cys residue. The "Penetratin" molecule and its properties are described in WO 91/18981.

Thus, antibodies of the invention may be labelled with a detectable label, for example a radiolabel such as ^{131}I or ^{99}Tc, which may be attached to using conventional chemistry known in the art of antibody imaging. Labels also include enzyme labels such as horseradish peroxidase. Labels further include chemical moieties such as biotin which may be detected via binding to a specific cognate detectable moiety, e.g. labelled avidin. Therefore, antibodies of the present invention may be labelled with a functional label. Such functional labels include toxins such as ricin and enzymes such as bacterial carboxypeptidase or nitroreductase, which are capable of converting prodrugs into active drugs at the site of cancer.

The antibodies of the present invention may be generated wholly or partly by chemical synthesis. The antibodies of the present invention can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if desired and appropriate, after

removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulphonic acid or a reactive derivative thereof.

Another convenient way of producing an antibody according to the present invention (peptide or polypeptide) is to express nucleic acid encoding it, by use of nucleic acid in an expression system.

Accordingly, the present invention also provides nucleic acid encoding the antibodies of the invention.

Generally, nucleic acid according to the present invention is provided as an isolate, in isolated and/or purified form, or free or substantially free of material with which it is naturally associated, such as free or substantially free of nucleic acid flanking the gene in the human genome, except possibly one or more regulatory sequence(s) for expression. Nucleic acid may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA. Where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

Nucleic acid sequences encoding a polypeptide or peptide in accordance with the present invention can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see Sambrook, Fritsch and Maniatis, "Molecular Cloning", A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989, and Ausubel et al, Short Protocols in Molecular Biology, John Wiley and Sons, 1992), given the nucleic acid sequences and clones available. These techniques include (i) the use of the polymerase chain reaction (PCR) to amplify samples of such nucleic acid, e.g. from genomic sources, (ii) chemical synthesis, or (iii) preparing cDNA sequences. DNA encoding SC100

fragments may be generated and used in any suitable way known to those of skill in the art, including by taking encoding DNA, identifying suitable restriction enzyme recognition sites either side of the portion to be expressed, and cutting out said portion from the DNA. The portion may then be operably linked to a suitable promoter in a standard commercially available expression system. Another recombinant approach is to amplify the relevant portion of the DNA with suitable PCR primers. Modifications to the sequences can be made, e.g. using site directed mutagenesis, to lead to the expression of modified peptide or to take account of codon preferences in the host cells used to express the nucleic acid.

In order to obtain expression of the nucleic acid sequences, the sequences can be incorporated into a vector having one or more control sequences operably linked to the nucleic acid to control its expression. The vectors may include other sequences such as promoters or enhancers to drive the expression of the inserted nucleic acid, nucleic acid sequences so that the polypeptide or peptide is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide produced in the host cell is secreted from the cell. Polypeptide can then be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the polypeptide is produced and recovering the polypeptide from the host cells or the surrounding medium. Prokaryotic and eukaryotic cells are used for this purpose in the art, including strains of *E. coli*, yeast, and eukaryotic cells such as COS or CHO cells.

Thus, the present invention also encompasses a method of making an antibody of the present invention, the method including expression from nucleic acid encoding the antibody (generally nucleic acid according to the invention). This may conveniently be achieved by growing a host cell in culture, containing such a vector, under appropriate conditions which cause or allow expression of the antibody.

Polypeptides and peptides may also be expressed in *in vitro* systems, such as reticulocyte lysate.

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

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manuel: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley and Sons, 1992.

Thus, a further aspect of the present invention provides a host cell containing heterologous nucleic acid as disclosed herein.

The nucleic acid of the invention may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome in accordance with

standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell, or otherwise identifiably heterologous or foreign to the cell.

A still further aspect of the invention provides a method which includes introducing the nucleic acid into a host cell. The introduction, which may (particularly for *in vitro* introduction) be generally referred to without limitation as "transformation", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. As an alternative, direct injection of the nucleic acid could be employed.

Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) under conditions for expression of the gene, so that the encoded polypeptide (or peptide) is produced. If the polypeptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into the culture medium. Following production by expression, a polypeptide or peptide may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in the formulation of a composition which may include one or more additional components, such as a pharmaceutical composition

which includes one or more pharmaceutically acceptable excipients, vehicles or carriers (e.g. see below).

As mentioned previously, the polypeptide may also be conjugated to an effector molecule. The effector molecule performs various useful functions such as, for example, inhibiting tumour growth, permitting the polypeptide to enter a cell such as a tumour cell, and directing the polypeptide to the appropriate location within a cell.

The effector molecule, for example, may be a cytotoxic molecule. The cytotoxic molecule may be a protein, or a non-protein organic chemotherapeutic agent. Some examples of suitable chemotherapeutic agents include, for example, doxorubicin, taxol and cisplatin.

Some additional examples of effector molecules suitable for conjugation to the polypeptides of the invention include signal transduction inhibitors, ras inhibitors, and cell cycle inhibitors. Some examples of signal transduction inhibitors include protein tyrosine kinase inhibitors, such as quercetin (Grazieri *et al., Biochim. Biophs. Acta* **714**, 415 (1981)); lavendustin A (Onoda *et al., J. Nat. Produc.* **52**, 1252 (1989)); and herbimycin A (Ushara *et al., Biochem. Int.*, **41**: 831 (1988)).

Proteins and non-protein chemotherapeutic agents may be conjugated to the antibodies of the invention by methods that are known in the art. Such methods include, for example, that described by Greenfield *et al., Cancer Research* **50**, 6600-6607 (1990) for the conjugation of doxorubicin and those described by Arnon *et al.*, Adv. Exp. *Med. Biol.* **303**, 79-90 (1991) and by Kiseleva *et al., Mol. Biol.* (USSR) **25**, 508-514 (1991) for the conjugation of platinum compounds. Doxorubicin, taxol and cisplatin are preferred.

The antibodies of the invention can be formulated in pharmaceutical compositions with a pharmaceutically acceptable carrier,. These compositions may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes. The formulation is preferably liquid, and is ordinarily a physiologic salt solution containing non-phosphate buffer at pH 6.8-7.6, or may be lyophilised powder.

The compositions comprising or for the delivery of the antibodies of the present invention are preferably administered to an individual in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

The antibodies of the invention are particularly relevant to the treatment of existing cancer and in the prevention of the recurrence of cancer after initial treatment or surgery. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980. Thus, the invention also provides (a) the use of an antibody or nucleic acid of the invention in the manufacture of a medicament for the treatment or prophylaxis of cancer, and (b) a method for the treatment or prophylaxis of cancer, comprising administering to a

subject a therapeutically effective amount of an antibody or nucleic acid of the invention.

The antibodies of the invention can significantly inhibit the growth of tumour cells when administered to a human in an effective amount. The optimal dose can be determined by physicians based on a number of parameters including, for example, age, sex, weight, severity of the condition being treated, the active ingredient being administered and the route of administration. In general, a serum concentration of polypeptides and antibodies that permits saturation of EGF receptors is desirable. A concentration in excess of approximately 0.1 nM is normally sufficient. For example, a dose of 100mg/m^2 of antibody provides a serum concentration of approximately 20nM for approximately eight days.

As a rough guideline, doses of antibodies may be given weekly in amounts of 10-300mg/m^2. Equivalent doses of antibody fragments should be used at more frequent intervals in order to maintain a serum level in excess of the concentration that permits saturation of EGF receptors.

Some suitable routes of administration include intravenous, subcutaneous and intramuscle administration. Intravenous administration is preferred.

The antibodies of the invention may be administered along with additional pharmaceutically acceptable ingredients. Such ingredients include, for example, immune system stimulators and chemotherapeutic agents, such as those mentioned above.

A composition may be administered alone or in combination with other treatments, either separately, simultaneously or sequentially, dependent upon the condition to be

treated. Other cancer treatments include other monoclonal antibodies, other chemotherapeutic agents, other radiotherapy techniques or other immuno therapy known in the art. One particular application of the compositions of the invention are as an adjunct to surgery, i.e. to help to reduce the risk of cancer reoccurring after a tumour is removed.

It is envisaged that injections (iv) will be the primary route for therapeutic administration of the antibodies of this invention, intravenous delivery, or delivery through a catheter or other surgical tubing is also used. Liquid formulations may be utilised after reconstitution from powder formulations.

The antibody may also be administered via microspheres, liposomes, other microparticulate delivery systems or sustained release formulations placed in certain tissues including blood. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of shared articles, e.g. suppositories or microcapsules. Implantable or microcapsular sustained release matrices include polylactides (US Patent No. 3, 773, 919, EP-A-0058481) copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al, Biopolymers* **22**(1): 547-556, 1985), poly (2-hydroxyethyl-methacrylate) or ethylene vinyl acetate (Langer *et al, J. Biomed. Mater. Res.* **15**: 167-277, 1981, and Langer, *Chem. Tech.* **12**:98-105, 1982). Liposomes containing the polypeptides are prepared by well-known methods: DE 3,218, 121A; Epstein et al, PNAS USA, 82: 3688-3692, 1985; Hwang et al, PNAS USA, 77: 4030-4034, 1980; EP-A-0052522; E-A-0036676; EP-A-0088046; EP-A-0143949; EP-A-0142541; JP-A-83-11808; US Patent Nos 4,485,045 and 4,544,545. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal rate of the polypeptide leakage.

The antibodies of the present invention may be administered in a localised manner to a tumour site or other desired site or may be delivered in a manner in which it targets tumour or other cells.

The dose of antibodies will be dependent upon the properties of the agent employed, e.g. its binding activity and *in vivo* plasma half-life, the concentration of the polypeptide in the formulation, the administration route, the site and rate of dosage, the clinical tolerance of the patient involved, the pathological condition afflicting the patient and the like, as is well within the skill of the physician. For example, doses of 300μg of polypeptide per patient per administration are preferred, although dosages may range from about 10μg to 1 mg per dose. Different dosages are utilised during a series of sequential inoculations; the practitioner may administer an initial inoculation and then boost with relatively smaller doses of antibody.

The antibodies of the invention can be administered in a variety of ways and to different classes of recipients. Examples of types of cancer that can be treated with the antibody include colorectal cancer, lung , breast, gastric and ovarian cancers.

This invention is also directed to optimise immunisation schedules for enhancing a protective immune response against cancer.

Preferred features of each aspect of the present invention are as for each other aspect *mutatis mutandis*. The prior art documents mentioned herein are incorporated by reference to the fullest extent permitted by law.

The invention will be described further with reference to the following non-limiting examples. Reference is made to the accompanying drawings in which:

Figure 1 shows the DNA sequences of the mouse monoclonal antibody 340 V_h and H_k sequences;

Figure 2 shows the translated protein sequence of the mouse monoclonal antibody 340 V_H and V_K sequences. The emboldened and underlined sequences represent the CDRs which are sequential, i.e. CDR1 is the first sequence shown and CDR3 is the last sequence shown for each chain;

Figure3 shows the sequences of primers used to amplify murine V_h and V_k sequences;

Figure 4 is a diagrammatic representation of an expression vector used for expression of a heavy chain;

Figure 5 is a diagrammatic representation of an expression vector used for expression of a light chain;

Figure 6 shows the alignment of deimmunised heavy chain sequences, including the location of the MHC binding epitopes in the deimmunised heavy chain variants;

Figure 7 shows the alignment of deimmunised light chain sequences including the location of the MHC binding epitopes in the deimmunised light chain;

Figure 8 shows the results of an A431 cell binding assay with a340 chimerised and a340 mouse monoclonal antibodies;

Figure 9 shows the results of an A431 cell binding assay of Vhd deimmunised variants;

Figure 10 shows the results of an A431 cell binding assay of Vhe deimmunised variants;

Figure 11 shows the results of an A431 cell binding assay of Vhb deimmunised variants;

Figure12 illustrates the amino acid homology between the SC100 Monoclonal Antibody and EGF. The amino acid sequence deduced for the complementarily determining region 3 of the immunoglobulin heavy chain of SC100 antibody shows distinct homology with specific areas of the published sequence for Epidermal Growth Factor;

Figure 13 is a molecular model comparing EGF and SC100. These illustrations represent SC100 Heavy chain and EGF respectively and illustrate the structural similarity between the two regions of amino acid homology represented in Figure 6. In particular, these diagrams show how the two areas of similar sequence are brought into close proximity in the EGF structure and how they then are mimicked by the homologous sequence in the CDRH3 of SC100 antibody; and

Figure 14a shows the % inhibition of growth of A431 cells for deimmunised SC100 antibody (clone VhdVkd) (a) and for deimmunised SC100 (clone VhdVkb) (b) compared with mouse 340 monoclonal antibody.

EXAMPLES

Example 1 - Construction of Chimaeric Antibody derived from α340

Total RNA was isolated from 5 x 10^6 hybridoma α340 cells (Durrant *et al.*, *Prenatal Diagnostics*, **14**, 131, 1994) using Qiagen RNeasy kit following manufacturers instructions. The RNA was converted into cDNA using Promega (Southampton, UK) reverse transcriptase, buffer and dNTPs. Variable region heavy (V_H) and light (V_L) chain cDNAs were amplified using primer sets of the method of Jones and Bendig (*Bio/Technology*, **9**, 188, 1991). The amplified DNAs were gel-purified and cloned into the vector pGem® T Easy (Promega). These PCR products were sequenced in both directions using the Applied Biosystems automated sequencer model 373A (Applied Biosystems, Warrington, UK). Resultant V_H and V_L DNA sequences are shown in Figure 1 and the protein sequences in Figure 2 (as used herein V_L is the same as V_K).

The location of the complementarity determining regions (CDRs) was determined with reference to other antibody sequences (Kabat EA *et al.*, US Department of Health and Human Services, 1991). The α340 V_H can be assigned to Mouse Heavy Chains Subgroup III(B) (Kabat *et al.*, 1991) and α340 V_K can be assigned to Mouse Kappa Chains Subgroup II (Kabat *et al*, 1991).

The chimaeric antibody consists of murine variable regions linked to human constant regions. The chimaeric antibody also provides a useful control with the same human constant regions when testing the DeImmunised antibodies (se below). The vectors V_H-PCR1 and V_K-PCR1 (Riechmann *et al.*, Nature, **332**, 323, 1988) were used as templates to introduce 5' flanking sequence including the leader signal peptide, leader intron and the murine immunoglobulin promoter, and 3' flanking sequence

including the splice site and intron sequences, around the murine V_H and V_K genes. The murine V_H and V_K sequences were amplified by primers overlapping with the V_H/V_K-PCR1 vector sequences (see Figure 3) using *pfu* proof-reading polymerase (*pfu* turbo, Stratagene, La Jolla, California). This enabled construction of the full-length chimaeric heavy and light chains. These PCR primers were used to amplify the V_H/V_K regions and the 5' and 3' human regions from the V_H/V_KPCR-1 vectors.

The 5' $Hu_{H/K}$, $V_{H/K}$ and 3' $Hu_{H/K}$ regions were connected and amplified using flanking primers (VH/K13, VH/K14) recognising the ends of VH/K1 and VH/K12 PCR primers giving a complete chimaeric antibody expression cassette. This product was cleaved with BamHI and HindIII restriction enzymes and ligated into BamHI/HindIII cut pUC19 (Enzymes by Promega, pUC19 by Amersham Pharmacia). The sequence of the expression cassette was then confirmed by sequencing as described previously.

The murine V_H and V_L expression cassettes were excised from pUC19 as *Hin*dIII to *Bam*HI fragments, which include the murine heavy chain immunoglobulin promoter, the leader signal peptide, leader intron, the V_H or V_L sequence and the splice site. These were transferred to the expression vectors pSV*gpt* and pSV*hyg* (Figures 4 and 5), which include human IgG1 or κ constant regions respectively and markers for selection in mammalian cells. The DNA sequence was confirmed to be correct for the V_H and V_L in the expression vectors.

Example 2 - Design of α340 DeImmunised Sequences

The following example describes the method by which the human immune response elicited by a pre-existing therapeutic antibody is reduced. There are two steps by which this was achieved, initially the murine heavy and light chain sequences were compared to a database of human germ-line sequences. The most similar germ-line

sequences were chosen as the human template for the deimmunised sequence and changes necessary to convert the murine to the human germ-line sequence were introduced. Residues which were considered to be critical for antibody structure and binding were excluded from this process and not altered. The murine residues that were retained at this stage were largely non-surface, buried residues, apart from residues at the N-terminus for instance, which are close to the CDRs in the final antibody. This process produces a sequence that is broadly similar to a "veneered" antibody as the surface residues are mainly human and the buried residues are as in the original murine sequence.

In the current example, the variable region protein sequences for the α340 antibody have been individually compared to the human germ-line $V_{H/K}$ sequences. In the case of α340, the α340V_H chain was seen to be most similar to germ-line sequences V_HDP42 and J_H6. The α340V_K chain showed most similarity with V_KDP15 and J_K2.

The second step involves the identification of antibody $V_{H/K}$ epitopes responsible for the immune response, and modification of the antibody sequence to remove such sequences. α340 was analysed by a novel process of peptide threading. Analysis was conducted by computer using MPT ver1.0 software (Biovation, Aberdeen, UK). This software package conducts peptide threading according to the methods disclosed in WO98/52976. The software is able to provide an index of potential peptide binding to 18 different MHC class II DR alleles covering greater than 96% of the HLA DR allotypes extant in the human population.

The α340 antibody chains, designed to mimic the human germ-line sequences described previously, were threaded by this method and potential MHC class II epitopes identified. Such epitopes were mutated by substitutions of amino acids responsible for MHC class II binding, by similar residues. These substitutions are

designed to maintain general antibody structure and antigen binding capacity but remove the MHC epitope.

Several variants of each chain were designed to give a range of antibodies with differing levels of MHC binding chains and mutations from the original murine α340. Three DIV_K and four DIV_H variants were designed (V_Kb, V_Kc, V_Kd, V_Hb, V_Hc, V_Hd and V_He) and can be seen in the respective alignments to show the MHC epitopes and mutations introduced to remove them (see Figures 6 and 7).

The mutations introduced include two in the CDRs of the α340 antibody. The I-L mutation of V_Kb is contained in CDR1 of the V_K region. The V-A mutation of V_He is similarly contained in the CDR3 of the V_H region. These substitutions could have a considerable effect on the antigen binding capacity of the α340 antibody and illustrate the importance of producing other variants with differing mutations.

Example 3 - Construction of DeImmunised Antibody Sequences

The DeImmunised variable regions were constructed by the method of overlapping PCR recombination. The cloned murine V_H and V_K genes were used as templates for mutagenesis of the framework regions to the required DeImmunised sequences. Sets of mutagenic primer pairs were synthesised encompassing the regions to be altered.

The use of mutagenic primer pairs dictated the use of annealing temperatures of 48°C-50°C. *pfu* turbo proof-reading polymerase (Stratagene, La Jolla, California) was used for all amplifications. The α340 chimaeric V_H and V_K constructs were used as templates to introduce 5' flanking sequence including the leader signal peptide, leader intron and the murine immunoglobulin promoter, and 3' flanking

sequence including the splice site and intron sequences as well as the variable regions to be modified. Overlapping PCRs were performed using the same flanking primers (VH/K13, VH/K14) as to create the chimaeric expression cassette. The DeImmunised V regions produced were cloned into pUC19 and the entire DNA sequence was confirmed to be correct for each DeImmunised V_H and V_L.

The DeImmunised heavy and light chain V-region genes were excised from pUC19 as *Hin*dIII to *Bam*HI fragments, which include the murine heavy chain immunoglobulin promoter, the leader signal peptide, leader intron, the V_H or V_L sequence and the splice site. These were transferred to the expression vectors pSV*gpt* and pSV*hyg*, which include human IgG1 or κ constant regions respectively and markers for selection in mammalian cells. The DNA sequence was confirmed to be correct for the DeImmunised V_H and V_L in the expression vectors. To prepare the constructs for transformation, approximately 50μg of V_K and 25μg of V_H plasmid (per transformation) was digested with pvuI (Promega, Southampton, UK) to completion. This DNA was then ethanol precipitated and the DNA pellet dried. Prior to transformation the pellet was resuspended in 10μl of molecular biology grade water (per transformation).

Example 4 - Expression of α340 DeImmunised Antibodies

The host cell line for antibody expression was NS0, a non-immunoglobulin producing mouse myeloma, obtained from the European Collection of Animal Cell Cultures, Porton, UK (ECACC No 85110505). The heavy and light chain expression vectors were co-transfected in a variety of combinations into NS0 cells by electroporation. Each DIV_H chain was transfected with each DIV_K chain to give 12 deimmunised variant antibodies. In addition, the chimaeric V_H and V_K regions were transfected to produce a cell line expressing the chimaeric α340 antibody (α340Ch). The α340Ch

antibody was used as a control to show the binding of the antibody before deimmunisation.

NS0 cells were grown in a 75cm^3 flask (NalgeNunc Int., Rochester, NY, USA) in 20mls of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 5ml antibiotics/antimycotics solution (Gibco BRL, Paisley, UK. Cat no. 15240-062), 2.5mls gentamicin (Gibco BRL, Paisley, UK. Cat no. 15710-049) and 5mls sodium pyruvate (Gibco BRL, Paisley, UK. Cat no. 11360-039) per 500mls of media. Once confluent, these cells were centrifuged into a pellet and resuspended in 0.5mls (per transformation) of identical media. This 0.5ml of cells was then added to the DNA previously digested, precipitated and resuspended and incubated on ice for 5 minutes. The cells were then aliquoted into a 2mm cuvette (Biorad, Hercules, CA, USA) and pulsed at 170 volts and 975μF in a Biorad Genepulser II, and then placed on ice for 20 minutes to recover. The cells were then aliquoted into 20mls of DMEM/10%FBS as described above and grown overnight at 37°C, 5% CO_2.

Twenty-four hours later, the cells were centrifuged and resuspended in 85mls of selective DMEM/10%FBS (as described plus 5mls of 25mg/ml xanthine and 160μl of 2.5mg/ml mycophenolic acid per 500mls of media. These are selective agents for the *gpt* gene of the psv heavy chain vector). This was then aliquoted into 4 x 96 well plates in 200μl aliquots per well. These plates were grown for 10 days, until resistant colonies developed.

Production of human antibody by transfected cell clones was measured by ELISA for human IgG. Cell lines secreting antibody were selected and expanded, initially into 24 well plates. These were then expanded up into 25cm^3 flasks and 75cm^3 flasks. Antibody production was assayed by ELISA by comparison with known concentrations of human control antibody. The best antibody producing clones from each transfection

were then expanded into 175cm^3 flasks and the other clones frozen down in liquid nitrogen.

No antibody was produced from cells transfected with V_H version C. It is unclear as to why but considerable numbers of transfectant colonies were produced on three separate occasions and no antibody producing colonies found. The production of variants carrying V_Hb, V_Hd and V_He was continued as described, but V_Hc was not continued.

Example 5 – Production and testing of DeImmunised α340 antibodies

Antibody was purified from 500 ml to 1 litre static cultures by stirring with 0.5ml of ProSepA (Bioprocessing Ltd) overnight. The Prosep A was then isolated by affinity chromatography. Antibody was eluted with 0.1M glycine pH3.0, neutralised and dialysed against PBS overnight. Purified antibody preparations were filter sterilised by filtration and stored at 4°C. The concentration of purified antibody was determined by ELISA for Human IgG.

The α340 DI antibody variants were tested in an ELISA for binding to A431 cells (ECCAC No. 85090402). These epithelial monolayer cells over express the epithelial growth factor receptor (EGFR) and are therefore suitable for assaying binding of α340 to the EGFR antigen. A431 cells were grown to confluence in 96 well plates in DMEM/10% FBS media and 1% non-essential amino acids (NEAA, Gibco BRL, Cat no. 11140-035). The media was then removed and the cells washed 3 times in PBS. They were then incubated in Immunoassay Stabiliser (Quadratech) for 1 hour at room temperature. The solution was then discarded and the plates left to dry for at room temperature. Plates were then stored at -4°C.

Assays comparing the α340Ch antibody with the original murine α340 showed comparable binding of the chimaeric form (Figure 9). Washes were performed with PBS with 0.05% Tween (Sigma). Detection was with horseradish peroxidase conjugated goat anti-human IgG (The Binding Site, Cambridge, UK) and sheep anti-mouse (The Binding Site, Cambridge, UK) for chimaeric and mouse antibodies respectively. Colour was developed with o-phenylene diamine substrate (Sigma, Poole, Dorset, UK).

The results are not directly comparable due to the use of different secondary antibodies with potentially differing binding capacities. However, this clearly showed that α340Ch bound the EGFR of A431 cells and could be used as a positive control to compare the binding affinities of the DI α340 variants.

To assay the DI α340 variants, doubling dilutions (from 100ng per well) of the DI variants and α340 chimaeric antibody (also from 100ng per well) as the positive control were applied across an immunassay plate. An ELISA was performed to show which variants showed binding of a comparable capacity to the α340Ch antibody.

The results of the binding assays for the 3 DeImmunised α340 heavy chains combined with the 3 DeImmunised α340 light chains are shown in Figures 10 to 12. The antibody composed of DeImmunised heavy chain versions b and d combined with DeImmunised light chain version b, c and d showed equivalent binding to A431 compared to the chimaeric antibody, indicating that the mutations introduced to the V_K region do not compromise the EGFR binding of α340. However, the introduction of the mutation unique to V_He (V-A in CDR3 of V_H) resulted in a loss of binding activity towards EGFR of around 3 to 4-fold.

The deimmunised version V_Hd.V_Kb was selected as lead deimmunised α340 antibody as it contained only one potential MHC epitope. Removal of this last epitope led to a considerable loss of EGFR binding by the antibody.

Example 6 – Amino acid Homology Between SC100 Monoclonal Antibody and EGF

The Gene Jockey II software package for Macintosh by Biosoft was used for pairwise sequence comparison of protein sequences to identify areas of similarity/homology. When the CDRH3 region of SC100 was compared to the protein sequence of EGF, amino acid homology was observed in two distinct regions of EGF. However, when these regions were highlighted on the NMR resolved structure of EGF, they were found to be brought together by secondary structure and resemble SC100 CDRH3.

Example 7 – Demonstration of Inhibition of In Vitro Tumour Growth

A431 cells were maintained in RPMI 1640 supplemented with 10% foetal calf serum at 37°C and 5% Carbon dioxide. Confluent cultures of viable cells were harvested with trypsin/EDTA, washed and re-suspended at 5 x 10^4 cells/ml.

100 µl aliquots of cell suspension were then dispensed into flat-bottomed 96-well plates together with increasing amounts of 340 mouse antibody, deimmunised SC100 (V_HdV_Kd or V_Hd,V_Kb) antibody. These cultures were left for 5 days and the number of remaining viable cells determined by crystal violet staining. The results are the mean +SE for quadruplicate wells. Where error bars are not seen, this is because they are so small they are covered by the data point.

Both the V_HdV_Kb and the V_HdV_Kd SC100 clones were able to inhibit the growth of A431 cells more effectively than the mouse monoclonal antibody 340. Indeed, the V_HdV_Kb clone showed 70% inhibition of cell growth.

Claims

1. A humanised form of the antibody 340 obtainable from the cell line deposited with the ECACC under accession number 97021428.

2. An antibody as claimed in claim 1, comprising the CDRH3 of antibody 340 provided in a human antibody framework.

3. An antibody as claimed in claim 2, further comprising one or more of the CDRL1, CDRL2, CDRL3, CDRH1 and CDRH2 of antibody 340.

4. An antibody as claimed in claim 3, comprising the hypervariable region of antibody 340.

5. An antibody as claimed in any preceding claim, comprising a substantial portion of the variable region of antibody 340.

6. An antibody as claimed in claim 5, comprising the variable region as shown in Figure 2.

7. An antibody as claimed in claim 1 or claim 2, comprising one of V_Hb, c, d or e, and one of V_Kb, c or d.

8. An antibody as claimed in claim 7, comprising V_Hd and V_Kd or V_Hd and V_Kb.

9. An antibody as claimed in any one of claims 2 to 8, wherein the human antibody framework is all or a part of the constant region of a human antibody.

10. An antibody as claimed in any preceding claim linked to a coupling partner or effector molecule.

11. Nucleic acid encoding an antibody as claimed in any preceding claim.

12. A vector having one or more control sequences operably linked to a nucleic acid as claimed in claim 11 to control its expression.

13. A cell containing nucleic acid as claimed in claim 11 or a vector as claimed in claim 12.

14. A method of making an antibody, the method including expression from nucleic acid as claimed in claim 11.

15. A method as claimed in claim 14, comprising growing a host cell as claimed in claim 13 in culture under appropriate conditions which cause or allow expression of the antibody.

16. A pharmaceutical composition comprising an antibody as claimed in any one of claims 1 to 10 and a pharmaceutically acceptable carrier.

17. The use of an antibody as claimed in any one of claims 1 to 10, or of a nucleic acid as claimed in claim 11, in medicine.

18. The use of an antibody as claimed in any one of claims 1 to 10, or of a nucleic acid as claimed in claim 11, in the manufacture of a medicament for the treatment or prophylaxis of cancer.