HUMANISED ANTIBODIES WITH ANTI-TUMOUR ACTIVITY

FILING DATA

[0001] This application is associated with and claims priority from Australian patent application no. 2009901129 filed on 16 March 2009 and US patent application no. 61/160682 filed on 16 March 2009 and US patent application no. 61/258517 filed on 5 November 2009, the entire contents of each of these applications is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to humanised antibodies with potent anti-tumour activity. In particular the humanised antibodies have specific binding to and direct killing of human colon tumour cells and display potent immune-mediated cytotoxic activity against human colon cancer cells in antibody-dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) assays. In addition the present invention relates to the use of these humanised antibodies in therapy and diagnosis.

BACKGROUND

[0003] Carbohydrate structures can be tumour-specific or tumour-associated antigens and are thus the focus of many antibody-generating immunization strategies. However, generating anti-carbohydrate specific antibodies is a challenging task as they may lack specificity, affinity or are only of the IgM class (Christensen et al., 2009). Moreover, generating humanised anti-carbohydrate antibodies that have the capacity to kill cancer cells is a challenging task, a fact that is reflected in the rare number of reports on such antibodies. There is only one example of an anti-glycolipid antibody that has been successfully humanised; the antibody recognises the ganglioside GM2 and kills human tumour cells *in vitro* and *in vivo* (US patents 6,423,511 and 6,872,392). Although not humanised there are two other examples of carbohydrate-binding antibodies that have been engineered for human administration. Firstly, anti-carbohydrate antibody RAV-12 is a chimeric mouse-human IgG_1 that shows *in vitro* and *in vivo* efficacy against human colon cancer cells (Loo et al., 2007). Secondly, anti-carbohydrate antibody HMMC-1 has shown *in vitro* efficacy against human ovarian cancer. HMMC-I is a fully human antibody generated by transchromosomal KM mice (Nozawa et al., 2004).

[0004] International patent application No. WO 2005/108430 discloses an anti-cancer mouse monoclonal antibody which is designated SC104. The disclosure of this application is incorporated herein by reference. The exact nature of antigen to which SC104 binds is

unclear but WO2005/108430 suggests that the antigen is a sialyltetraosyl carbohydrate. It is also disclosed that SC104 is capable of directly inducing cell death without the need for immune effector cells.

SUMMARY OF THE INVENTION

[0005] In a first aspect the present invention provides a V_H antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

the sequence of FR1 is X_1VQLQESGPGLVKPSETLSLTCTVX_2GYSX_3X_4 (SEQ ID NO: 95) wherein;
X_1 is Q or E
X_2 is S or T
X_3 is I, L or V
X_4 is S or T
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR2 is WIRQX_5PGKGLX_6WX_7G (SEQ ID NO: 97) wherein
X_5 is S or P
X_6 is Q or E
X_7 is M or I
or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of CDR2 is HIHX_8SGRPTYX_9PSLX_{10}S (SEQ ID NO: 98) wherein
X_8 is F, Y or W
X_9 is N or D
X_{10} is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR3 is R$X_{11}X_{12}$IS$X_{13}X_{14}$TAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 99) wherein
X_{11} is V or I
X_{12} is T or S
X_{13} is R or K

X_{14} is E or D
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto with the proviso that the residue at position 6 must be R or K

the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR4 is WGQGTLVTVSS (SEQ ID NO: 101) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0006] In a second aspect the present invention provides a V_H antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence QVQLQESGPGLVKPSETLSLTCTVSGYSX$_3$SWIRQPPGKGLQWIGRVTISX$_{13}$ETAKN QFSLKLTSMTAADTAVYYCARWGQGTLVTVSS (SEQ ID NO: 102);
wherein X_3 is I, L or V and X_{13} is R or K;
and the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto;
the sequence of CDR2 is HIHX$_8$SGRPTYX$_9$PSLX$_{10}$S (SEQ ID NO: 98) wherein
X_8 is F, Y or W
X_9 is N or D
X_{10} is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto and;
the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0007] In a third aspect the present invention provides a V_L antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

the sequence of FR1 is EX$_{15}$VLTQSPGTLSLSX$_{16}$GERATLSC (SEQ ID NO: 187) wherein,
X_{15} is I or N
X_{16} is A or P
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR3 is GIPDRFSGSGSGX$_{17}$DFTLTISRVEPEDFAVYYC (SEQ ID NO: 191) wherein,
X$_{17}$ is T or N
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR4 is FGQGTKLEIKR (SEQ ID NO: 193) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0008] In a fourth aspect the present invention provides a V_L antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95% identical to the sequence
EIVLTQSPGTLSLSPGERATLSCWYQQKPGQAPRLLIYGIPDRFSGSGSGTDFTLTISR VEPEDFAVYYCFGQGTKLEIKR (SEQ ID NO: 194)
and the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto;
the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto and;
the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical.

[0009] In a fifth aspect the present invention provides a V_H antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

the sequence of FR1 is EVQLQQWGAGLLKPSETLSLTCAVYGYSX$_{18}$X$_{19}$ (SEQ ID NO: 201) wherein;

X_{18} is I, L or V
X_{19} is S or T
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR2 is WIRQPPGKGLEWX_{20}G (SEQ ID NO: 202) wherein
X_{20} is M or I
or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of CDR2 is HIHX_{21}SGRPTYX_{22}PSLX_{23}S (SEQ ID NO: 98) wherein
X_{21} is F, Y or W
X_{22} is N or D
X_{23} is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR3 is R$X_{24}X_{25}$ISX_{26}DTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 203) wherein
X_{24} is V or I
X_{25} is S or T
X_{26} is R or K
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto with the proviso that the residue at position 6 must be R or K

the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR4 is WGQGTLVTVSS (SEQ ID NO: 101) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0010] In a sixth aspect the present invention provides a V_H antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence
EVQLQQWGAGLLKPSETLSLTCAVYGYSX_{18}SWIRQPPGKGLEWIGRVTISX_{26}DTSK NQFSLKLSSVTAADTAVYYCARWGQGTLVTVSS (SEQ ID NO: 204)
wherein X_{18} is I, L or V and X_{26} is R or K
and the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80%

similar, preferably at least 80% identical, thereto;
the sequence of CDR2 is HIHX$_{21}$SGRPTYX$_{22}$PSLX$_{23}$S (SEQ ID NO: 98) wherein
X$_{21}$ is F, Y or W
X$_{22}$ is N or D
X$_{23}$ is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto and;
the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 10) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0011] In a seventh aspect the present invention provides a V_L antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

the sequence of FR1 is EX$_{27}$VLTQSPATLSLSPGERATLSC (SEQ ID NO: 211) wherein,
X$_{27}$ is I or N
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 90% identical, thereto

the sequence of FR3 is GIPDRFSGSGSGX$_{28}$DFTLTISRLEPEDFAVYYC (SEQ ID NO: 212) wherein,
X$_{28}$ is T or N
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR4 is FGGGTKVEIKR (SEQ ID NO: 193) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0012] In an eighth aspect the present invention provides a V_L antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence
EIVLTQSPATLSLSPGERATLSCWYQQKPGQAPRLLIYGIPDRFSGSGSGTDFTLTISR LEPEDFAVYYCFGGGTKVEIKR (SEQ ID NO: 213)
and the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto;
the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto and;
the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical.

[0013] The binding domains of the present invention, when presented as part of an antibody, will bind the human colon cancer cell line Colo205. As will be understood the antibody will consist of two heavy chains, each comprising variable and constant regions, and two light chains each comprising variable and constant regions. When the claimed binding domain is a V_H binding domain the antibody which binds human colon cancer cell line Colo205 will consist of two of the claimed V_H binding domains, two light chains of SEQ ID NO. 7 and two heavy chain constant domains of SEQ ID NO. 92 or SEQ ID NO. 52. In a similar manner when the claimed binding domain is a V_L binding domain the antibody which binds human colon cancer cell line Colo205 will consist of two of the claimed V_L binding domains, two of V_L constant domains of SEQ ID NO. 93 and two heavy chains of SEQ ID NO. 94 or SEQ ID NO.50.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 – Binding (left hand panels) and direct killing (right hand panels) activities of mouse SC104 (variable regions listed in SEQ ID NO:3/SEQ ID NO:1) and chimeric SC104 (SEQ ID NO:4/SEQ ID NO:2) antibodies and the human IgG1 isotype control to SC104 antigen positive (C170, Colo205) and SC104 antigen negative (HCT-116) human colon cancer cell lines as shown by flow cytometry; sequence IDs refer to the sequence listing. Chimeric SC104 and murine SC104 antibodies exhibit comparable selective binding to- and direct killing activity of antigen positive human colon cancer cell lines. Both antibodies do not bind to- and directly kill antigen negative human colon cancer cell lines. Each point represents mean ± SD of three replicate samples.

[0015] Figure 2 – Humanisation of SC104 on human acceptor framework 1U6A or 1QLR incorporating a Kabat-defined CDR-H1 (-□-) yields non functional antibody variants that

do not bind to the SC104 antigen positive human colon cancer cell line C170. The binding activities of humanised SC104 variants using a Kabat-defined CDR-H1 grafted either to the human acceptor 1U6A (SEQ ID NO:7/SEQ ID NO:9, panel A) or 1QLR (SEQ ID NO:8/SEQ ID NO:10, panel B); were assessed by flow cytometry using the antigen positive cell line C170. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

[0016] Figure 3 – Humanisation of SC104 on human acceptor framework 1U6A or 1QLR incorporating an AbM-defined CDR-H1 (-▲-) generates antibody variants that bind to the SC104 antigen positive human colon cancer cell line C170. The binding activities of humanised SC104 variants incorporating an AbM-defined CDR-H1 grafted either to the human acceptor 1U6A (SEQ ID NO:7/SEQ ID NO:11, panel A) or 1QLR (SEQ ID NO:8/SEQ ID NO:12, panel B); were assessed by flow cytometry using the antigen-positive cell line C170. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

[0017] Figure 4 – Analysis of light chain and heavy chain amino acid substitution(s) towards enhancing the binding activity of 1U6A humanised SC104. The influence of the substitutions in each chain was analysed by binding activity assays to the antigen positive cell line C170 by flow cytometry. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

A: Grafts containing Kabat CDR-H1; SEQ ID NO:9 is the unsubstituted V_H, SEQ ID NO:15 contains V_H substitutions Q1E, Q46E, I48M, V67I, T68S, V71R and E72D, SEQ ID NO:7 refers to V_L incorporating a single substitution A15P whilst SEQ ID NO:16 refers to a V_L containing three substitutions I2N, A15P and T69N.

B: Grafts containing AbM CDR-H1; SEQ ID NO:11 refers to a V_H containing no substitutions, SEQ ID NO:13 contains the V_H substitutions Q1E, Q46E, I48M, V67I, T68S, V71R and E72D, SEQ ID NO:7 refers to V_L incorporating a single substitution A15P and SEQ ID NO:16 refers to a V_L containing three substitutions I2N, A15P and T69N.

Heavy chain amino acid substitution(s) (-□- or -○-) enhance the binding activity of 1U6A-humanised SC104 antibody variants to the antigen positive cell line C170. In comparison, the light chain amino acid substitutions appear less important in enhancing the binding activity of humanised SC104.

[0018] Figure 5 – Analysis of six individual V_H substitutions incorporated in 1U6A-humanised antibodies containing and AbM-defined CDR-H1. The influence of the substitutions was analysed by binding activity assays to the antigen positive cell line C170 by flow cytometry. These changes were either Q46E or I48M or V67I or T68S or V71R or E72D and are described by SEQ ID NOs:19 through 24 respectively. Substitution R71 (SEQ ID NO:23) (-○-) was most advantageous in enhancing the binding potency of humanised SC104. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with human IgG_1 isotype as a negative control. Each point represents mean ± SD of three replicate samples.

[0019] Figure 6 – V_H substitution R71 (-□- or -▲-) enhances the binding activity of both AbM- and Kabat-defined CDR-H1 antibody variants to the antigen positive cell line C170 as determined by flow cytometry. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator and the human IgG_1 isotype is used as a negative control. Each point represents mean ± SD of three replicate samples.

A:1QLR-based SC104 variants ± V71R substitution; AbM-defined CDR-H1 grafts: SEQ ID NO:12 (-R) and SEQ ID NO:26 (+R); Kabat-defined CDR-H1 grafts: SEQ ID NO:10 (-R) and SEQ ID NO:27 (+R).

B: 1U6A-based SC104 variants ± V71R substitution. AbM-defined CDR-H1 grafts: SEQ ID NO:11 (-R) and SEQ ID NO:25 (+R); Kabat-defined CDR-H1 grafts: SEQ ID NO:9 (-R) and SEQ ID NO:28 (+R).

[0020] Figure 7 – Effect on binding activity of V_H substitution G27Y in 1QLR-humanised SC104 variants with a Kabat-defined CDR-H1. This substitution does not enhance the binding activity of 1QLR-humanised SC104 (SEQ ID NO:27 (+R) and SEQ ID NO:29 (+R +Y) (-□- or -▲-) vs AbM-CDR-H1 containing V_H SEQ ID NO:26) when examined in flow cytometry-based binding assays to the SC104 antigen positive cell line C170. For comparison, the 1QLR humanised variant incorporating a Kabat-defined CDR-H1 without R71 is shown, SEQ ID NO:10. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator and the human IgG_1 isotype is used as a negative control. Each point represents mean ± SD of three replicate samples.

[0021] Figure 8 – Flow cytometry-based binding assay demonstrating the impact on binding activity upon removal of a predicted strong MHC class II binding peptide sequence from the framework 2:CDR-H2 boundary through substitution of F53 within CDR-H2 of chimeric SC104. Switching F53 to another aromatic residue such as W (SEQ ID NO:30) or Y (SEQ ID NO:31) (-□- or -○-) has negligible impact on binding activity whilst substitution to the preferred residue P (SEQ ID NO:32) dramatically reduces

binding activity to the SC104 antigen positive cell line C170. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

[0022] Figure 9 – Humanised SC104 variants with lower predicted immunogenicity (1U6A V_L/V_H SEQ ID NO:7/SEQ ID NO:50; 1QLR V_L/V_H SEQ ID NO:8/SEQ ID NO:38) are comparable to humanised antibody variants with higher predicted immunogenicity (1U6A V_L/V_H SEQ ID NO:7/SEQ ID NO:25; 1QLR V_L/V_H SEQ ID NO:8/SEQ ID NO:26) in terms of specificity and binding activity to antigen positive (C170, Colo205) or antigen negative (HCT-116) human colon cancer cell lines as determined by flow cytometry. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

[0023] Figure 10 - Flow cytometry-based binding assay demonstrating that residues I (-○-) (SEQ ID NO:50) and L (-□-) (SEQ ID NO:62) but not C (-▲-) (SEQ ID NO:55), at position 29 in 1U6A framework 1 of variable heavy chain confer highest binding activity of humanised SC104 antibody variants with lower predicted immunogenicity to SC104 antigen positive human colon cancer cell line C170. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

[0024] Figure 11- Flow cytometry-based binding assay demonstrating that positive charged residues R (SEQ ID NO:50) (-□-), but not negative charged residue D (SEQ ID NO:75) (-▲-) or neutral residue W (SEQ ID NO:90) (-■ -), at position 71 in 1U6A framework 3 of variable heavy chain confer highest binding activity of humanised SC104 antibody variants with lower predicted immunogenicity to SC104 antigen positive human colon cancer cell line C170. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

[0025] Figure 12 - SC104 antibody variants incorporating the 1U6A framework (V_L/V_H SEQ ID NO:7/SEQ ID NO:50 and SEQ ID NO:7/SEQ ID NO:25) (-□- or -■-) have more potent direct killing activity compared with the corresponding 1QLR-derived SC104 variants (V_L/V_H SEQ ID NO:8/SEQ ID NO:38 and SEQ ID NO:8/SEQ ID NO:26) as determined by flow cytometry using the SC104 antigen positive human colon cancer cell line C170. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

[0026] Figure 13 – Humanised SC104 antibody variants incorporating the 1U6A framework (V_L/V_H SEQ ID NO:7/SEQ ID NO:50 and SEQ ID NO:7/SEQ ID NO:25) (-□- or -■-) have higher antibody-dependent cell-mediated cytotoxicity activity compared with the corresponding 1QLR-derived SC104 variants (V_L/V_H SEQ ID NO:8/SEQ ID NO:38 and SEQ ID NO:8/SEQ ID NO:26) as determined by LDH release assays using the SC104 antigen positive human colon cancer cell line C170. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator and the human IgG_1 isotype is used as a negative control. Each point represents mean ± SD of three replicate samples.

[0027] Figure 14 - SC104 antibody variants incorporating the 1U6A framework (V_L/V_H SEQ ID NO:7/SEQ ID NO:50 and SEQ ID NO:7/SEQ ID NO:25) (-□- or -■-)have more potent complement-dependent cytotoxicity compared with the corresponding 1QLR-derived SC104 antibody variants (V_L/V_H SEQ ID NO:8/SEQ ID NO:38 and SEQ ID NO:8/SEQ ID NO:26) as determined by cell viability assays using the SC104 antigen positive human colon cancer cell line Colo205. The chimeric SC104 antibody (SEQ ID NO:4/SEQ ID NO:2) is shown as a comparator along with a human IgG_1 isotype negative control. Each point represents mean ± SD of three replicate samples.

[0028] Figure 15 - Kifunensin-treated humanised SC104 antibody with lower predicted immunogenicity (SEQ ID NO:7/SEQ ID NO:50) (-○-) has higher antibody-dependent cell-mediated cytotoxicity activity compared with untreated antibody (-▲-) as assessed by LDH release assays using the SC104 antigen positive WiDr human colon cancer cell line. Each point represents mean ± SD of three replicate samples.

[0029] Figure 16 - Potelligent®-engineered humanised SC104 antibody with lower predicted immunogenicity (SEQ ID NO:7/SEQ ID NO:94) (-○-) has higher antibody-dependent cell-mediated cytotoxicity activity compared with untreated (SEQ ID NO:7/SEQ ID NO:50) antibody as assessed by LDH release assays using the SC104 antigen positive Colo201 human colon cancer cell line. Each point represents mean ± SD of three replicate samples.

[0030] Figure 17 - Treatment of HT29 xenograft tumour-bearing mice with humanised SC104 antibody with lower predicted immunogenicity (-○-) (SEQ ID NO:7/SEQ ID NO:50) leads to significant reduced growth compared with vehicle control treatment. Top panel: mean ± SEM tumour volume in groups of 6-10 mice. Asterisks indicate significant differences between treatment groups, $p<0.05$ Mann-Whitney test. Bottom panel: mean and individual tumour weight at end of study. P value as determined by Mann-Whitney test.

[0031] Figure 18 - Treatment of Colo201 xenograft tumour-bearing mice with Potelligent®-engineered humanised SC104 antibody with lower predicted immunogenicity (-○-) (SEQ ID NO:7/SEQ ID NO:94) leads to significant reduced tumour volume compared with vehicle control treatment. Mean ± SEM in groups of 10 mice. Asterisks indicate significant differences between treatment groups, $p<0.05$ t-test.

DETAILED DESCRIPTION OF THE INVENTION

[0032] The present invention relates to humanised anti-cancer binding domains. These binding domains are based on the mouse antibody SC104 (WO 2005/108430) and comprise modified human framework sequences to increase binding and activity, and decrease predicted immunogenicity. As will be appreciated by people skilled in the art, the sequences developed and described herein may be further modified to increase binding by affinity maturation and to increase Fc-mediated effector function. The binding domains described herein can be used either alone or in combination for diagnosis and/or treatment of human cancers, for example colorectal, pancreatic, ovarian and lung cancer.

[0033] In a first aspect the present invention provides a V_H antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

the sequence of FR1 is X_1VQLQESGPGLVKPSETLSLTCTVX_2GYSX_3X_4 (SEQ ID NO: 95) wherein;
X_1 is Q or E
X_2 is S or T
X_3 is I, L or V
X_4 is S or T
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR2 is WIRQX_5PGKGLX_6WX_7G (SEQ ID NO: 97) wherein
X_5 is S or P
X_6 is Q or E
X_7 is M or I
or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of CDR2 is HIHX$_8$SGRPTYX$_9$PSLX$_{10}$S (SEQ ID NO: 98) wherein
X$_8$ is F, Y or W
X$_9$ is N or D
X$_{10}$ is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR3 is RX$_{11}$X$_{12}$ISX$_{13}$X$_{14}$TAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 99) wherein
X$_{11}$ is V or I
X$_{12}$ is T or S
X$_{13}$ is R or K
X$_{14}$ is E or D
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto with the proviso that the residue at position 6 must be R or K

the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR4 is WGQGTLVTVSS (SEQ ID NO: 101) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0034] In a second aspect the present invention provides a V$_H$ antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence QVQLQESGPGLVKPSETLSLTCTVSGYSX$_3$SWIRQPPGKGLQWIGRVTISX$_{13}$ETAKNQFSLKLTSMTAADTAVYYCARWGQGTLVTVSS (SEQ ID NO: 102);
wherein X$_3$ is I, L or V and X$_{13}$ is R or K;
and the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto;
the sequence of CDR2 is HIHX$_8$SGRPTYX$_9$PSLX$_{10}$S (SEQ ID NO: 98) wherein
X$_8$ is F, Y or W
X$_9$ is N or D
X$_{10}$ is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto and;
the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0035] Preferred sequences for FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4 are as follows:-

FR1 is selected from the group consisting of QVQLQESGPGLVKPSETLSLTCTVSGYSIS (SEQ ID NO: 103), QVQLQESGPGLVKPSETLSLTCTVSGYSIT (SEQ ID NO: 104), QVQLQESGPGLVKPSETLSLTCTVSGYSLS (SEQ ID NO: 105), QVQLQESGPGLVKPSETLSLTCTVSGYSLT (SEQ ID NO: 106), QVQLQESGPGLVKPSETLSLTCTVSGYSVS (SEQ ID NO: 107), QVQLQESGPGLVKPSETLSLTCTVSGYSVT (SEQ ID NO: 108), QVQLQESGPGLVKPSETLSLTCTVTGYSIS (SEQ ID NO: 109), QVQLQESGPGLVKPSETLSLTCTVTGYSIT (SEQ ID NO: 110), QVQLQESGPGLVKPSETLSLTCTVTGYSLS (SEQ ID NO: 111), QVQLQESGPGLVKPSETLSLTCTVTGYSLT (SEQ ID NO: 112), QVQLQESGPGLVKPSETLSLTCTVTGYSVS (SEQ ID NO: 113), QVQLQESGPGLVKPSETLSLTCTVTGYSVT (SEQ ID NO: 114), EVQLQESGPGLVKPSETLSLTCTVSGYSIS (SEQ ID NO: 115), EVQLQESGPGLVKPSETLSLTCTVSGYSIT (SEQ ID NO: 116), EVQLQESGPGLVKPSETLSLTCTVSGYSLS (SEQ ID NO: 117), EVQLQESGPGLVKPSETLSLTCTVSGYSLT (SEQ ID NO: 118), EVQLQESGPGLVKPSETLSLTCTVSGYSVS (SEQ ID NO: 119), EVQLQESGPGLVKPSETLSLTCTVSGYSVT (SEQ ID NO: 120), EVQLQESGPGLVKPSETLSLTCTVTGYSIS (SEQ ID NO: 121), EVQLQESGPGLVKPSETLSLTCTVTGYSIT (SEQ ID NO: 122), EVQLQESGPGLVKPSETLSLTCTVTGYSLS (SEQ ID NO: 123), EVQLQESGPGLVKPSETLSLTCTVTGYSLT (SEQ ID NO: 124), EVQLQESGPGLVKPSETLSLTCTVSTGYSVS (SEQ ID NO: 125), and EVQLQESGPGLVKPSETLSLTCTVTGYSVT (SEQ ID NO: 126);

CDR1 is SGYSWH (SEQ ID NO: 96);

FR2 is selected from the group consisting of WIRQSPGKGLQWIG (SEQ ID NO: 127), WIRQSPGKGLQWMG (SEQ ID NO: 128), WIRQSPGKGLEWIG (SEQ ID NO: 129), WIRQSPGKGLEWMG (SEQ ID NO: 130), WIRQPPGKGLQWIG (SEQ ID NO: 131), WIRQPPGKGLQWMG (SEQ ID NO: 132), WIRQPPGKGLEWIG (SEQ ID NO: 133), and WIRQPPGKGLEWMG (SEQ ID NO: 134);

CDR2 is selected from the group consisting of HIHFSGRPTYNPSLSS (SEQ ID NO: 135), HIHFSGRPTYNPSLKS (SEQ ID NO: 136), HIHFSGRPTYNPSLLS (SEQ ID NO: 137), HIHFSGRPTYNPSLHS (SEQ ID NO: 138), HIHFSGRPTYNPSLFS (SEQ ID NO: 139), HIHFSGRPTYNPSLRS (SEQ ID NO: 140), HIHWSGRPTYNPSLSS (SEQ ID NO: 141), HIHWSGRPTYNPSLKS (SEQ ID NO: 142), HIHWSGRPTYNPSLLS (SEQ ID NO: 143), HIHWSGRPTYNPSLHS (SEQ ID NO: 144), HIHWSGRPTYNPSLFS (SEQ ID NO: 145), HIHWSGRPTYNPSLRS (SEQ ID NO: 146), HIHYSGRPTYNPSLSS (SEQ ID NO: 147), HIHYSGRPTYNPSLKS (SEQ ID NO: 148), HIHYSGRPTYNPSLLS (SEQ ID NO: 149), HIHYSGRPTYNPSLHS (SEQ ID NO: 150), HIHYSGRPTYNPSLFS (SEQ ID NO: 151), HIHYSGRPTYNPSLRS (SEQ ID NO: 152), HIHFSGRPTYDPSLSS (SEQ ID NO: 153), HIHFSGRPTYDPSLKS (SEQ ID NO: 154), HIHFSGRPTYDPSLLS (SEQ ID NO: 155), HIHFSGRPTYDPSLHS (SEQ ID NO: 156), HIHFSGRPTYDPSLFS (SEQ ID NO: 157), HIHFSGRPTYDPSLRS (SEQ ID NO: 158), HIHWSGRPTYDPSLSS (SEQ ID NO: 159), HIHWSGRPTYDPSLKS (SEQ ID NO: 160), HIHWSGRPTYDPSLLS (SEQ ID NO: 161), HIHWSGRPTYDPSLHS (SEQ ID NO: 162), HIHWSGRPTYDPSLFS (SEQ ID NO: 163), HIHWSGRPTYDPSLRS (SEQ ID NO: 164), HIHYSGRPTYDPSLSS (SEQ ID NO: 165), HIHYSGRPTYDPSLKS (SEQ ID NO: 166), HIHYSGRPTYDPSLLS (SEQ ID NO: 167), HIHYSGRPTYDPSLHS (SEQ ID NO: 168), HIHYSGRPTYDPSLFS (SEQ ID NO: 169), and HIHYSGRPTYDPSLRS (SEQ ID NO: 170);

FR3 is selected from the group consisting of RVTISRETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 171), RVTISRDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 172), RVTISKETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 173), RVTISKDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 174), RVSISRETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 175), RVSISRDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 176), RVSISKETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 177), RVSISKDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 178), RITISRETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 179), RITISRDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 180), RITISKETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 181), RITISKDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 182), RISISRETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 183),

RISISRDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 184),
RISISKETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 185), and
RISISKDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 186);

CDR3 is KGKGSDDGLNY (SEQ ID NO: 100); and

FR4 is WGQGTLVTVSS (SEQ ID NO: 101).

[0036] In a third aspect the present invention provides a VL antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

the sequence of FR1 is EX_{15}VLTQSPGTLSLSX_{16}GERATLSC (SEQ ID NO: 187) wherein,
X_{15} is I or N
X_{16} is A or P
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR3 is GIPDRFSGSGSGX_{17}DFTLTISRVEPEDFAVYYC (SEQ ID NO: 191) wherein,
X_{17} is T or N
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR4 is FGQGTKLEIKR (SEQ ID NO: 193) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0037] In a fourth aspect the present invention provides a V_L antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95% identical to the sequence
EIVLTQSPGTLSLSPGERATLSCWYQQKPGQAPRLLIYGIPDRFSGSGSGTDFTLTISR VEPEDFAVYYCFGQGTKLEIKR (SEQ ID NO: 194)
and the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto;
the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto and;
the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical.

[0038] Preferred sequences for FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4 are as follows:-

FR1 is selected from the group EIVLTQSPGTLSLSPGERATLSC (SEQ ID NO: 195), EIVLTQSPGTLSLSAGERATLSC (SEQ ID NO: 196), ENVLTQSPGTLSLSPGERATLSC (SEQ ID NO: 197), and ENVLTQSPGTLSLSAGERATLSC (SEQ ID NO: 198).

CDR1 is SASSSLSYIH (SEQ ID NO: 188);

FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189);

CDR2 is DTSNLAS (SEQ ID NO: 190);

FR3 is GIPDRFSGSGSGTDFTLTISRVEPEDFAVYYC (SEQ ID NO: 199) or GIPDRFSGSGSGNDFTLTISRVEPEDFAVYYC (SEQ ID NO: 200);

CDR3 is FQGSEYPLT (SEQ ID NO: 192); and

FR4 is FGQGTKLEIKR (SEQ ID NO: 193).

[0039] In a fifth aspect the present invention provides a VH antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

the sequence of FR1 is EVQLQQWGAGLLKPSETLSLTCAVYGYS$X_{18}X_{19}$ (SEQ ID NO: 201) wherein;

X_{18} is I, L or V
X_{19} is S or T
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR2 is WIRQPPGKGLEWX_{20}G (SEQ ID NO: 202) wherein
X_{20} is M or I
or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of CDR2 is HIHX_{21}SGRPTYX_{22}PSLX_{23}S (SEQ ID NO: 98) wherein
X_{21} is F, Y or W
X_{22} is N or D
X_{23} is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR3 is R$X_{24}X_{25}$ISX_{26}DTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 203) wherein
X_{24} is V or I
X_{25} is S or T
X_{26} is R or K
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto with the proviso that the residue at position 6 must be R or K

the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR4 is WGQGTLVTVSS (SEQ ID NO: 101) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0040] In a sixth aspect the present invention provides a V_H antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence
EVQLQQWGAGLLKPSETLSLTCAVYGYSX_{18}SWIRQPPGKGLEWIGRVTISX_{26}DTSK NQFSLKLSSVTAADTAVYYCARWGQGTLVTVSS (SEQ ID NO: 204)
wherein X_{18} is I, L or V and X_{26} is R or K
and the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80%

similar, preferably at least 80% identical, thereto;
the sequence of CDR2 is HIHX$_{21}$SGRPTYX$_{22}$PSLX$_{23}$S (SEQ ID NO: 98) wherein
X$_{21}$ is F, Y or W
X$_{22}$ is N or D
X$_{23}$ is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto and;
the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0041] Preferred sequences for FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4 are as follows:-

FR1 is selected from the group consisting of
EVQLQQWGAGLLKPSETLSLTCAVYGYSIS (SEQ ID NO: 205),
EVQLQQWGAGLLKPSETLSLTCAVYGYSIT (SEQ ID NO: 206),
EVQLQQWGAGLLKPSETLSLTCAVYGYSLS (SEQ ID NO: 207),
EVQLQQWGAGLLKPSETLSLTCAVYGYSLT (SEQ ID NO: 208),
EVQLQQWGAGLLKPSETLSLTCAVYGYSVS (SEQ ID NO: 209), and
EVQLQQWGAGLLKPSETLSLTCAVYGYSVT (SEQ ID NO: 210);

CDR1 is SGYSWH (SEQ ID NO: 96);

FR2 is WIRQPPGKGLEWIG (SEQ ID NO: 133) or WIRQPPGKGLEWMG (SEQ ID NO: 134);

CDR2 is selected from the group consisting of HIHFSGRPTYNPSLSS (SEQ ID NO: 135), HIHFSGRPTYNPSLKS (SEQ ID NO: 136), HIHFSGRPTYNPSLLS (SEQ ID NO: 137), HIHFSGRPTYNPSLHS (SEQ ID NO: 138), HIHFSGRPTYNPSLFS (SEQ ID NO: 139), HIHFSGRPTYNPSLRS (SEQ ID NO: 140), HIHWSGRPTYNPSLSS (SEQ ID NO: 141), HIHWSGRPTYNPSLKS (SEQ ID NO: 142), HIHWSGRPTYNPSLLS (SEQ ID NO: 143), HIHWSGRPTYNPSLHS (SEQ ID NO: 144), HIHWSGRPTYNPSLFS (SEQ ID NO: 145), HIHWSGRPTYNPSLRS (SEQ ID NO: 146), HIHYSGRPTYNPSLSS (SEQ ID NO: 147), HIHYSGRPTYNPSLKS (SEQ ID NO: 148), HIHYSGRPTYNPSLLS (SEQ ID NO: 149), HIHYSGRPTYNPSLHS (SEQ ID NO: 150), HIHYSGRPTYNPSLFS (SEQ ID NO: 151), HIHYSGRPTYNPSLRS (SEQ ID NO: 152), HIHFSGRPTYDPSLSS (SEQ ID NO: 153), HIHFSGRPTYDPSLKS (SEQ ID NO: 154), HIHFSGRPTYDPSLLS (SEQ ID NO: 155), HIHFSGRPTYDPSLHS (SEQ ID NO: 156), HIHFSGRPTYDPSLFS (SEQ ID NO: 157), HIHFSGRPTYDPSLRS (SEQ ID NO: 158), HIHWSGRPTYDPSLSS (SEQ ID NO: 159), HIHWSGRPTYDPSLKS(SEQ ID NO: 160), HIHWSGRPTYDPSLLS (SEQ ID NO: 161), HIHWSGRPTYDPSLHS (SEQ ID NO: 162),

HIHWSGRPTYDPSLFS (SEQ ID NO: 163), HIHWSGRPTYDPSLRS (SEQ ID NO: 164), HIHYSGRPTYDPSLSS (SEQ ID NO: 165), HIHYSGRPTYDPSLKS (SEQ ID NO: 166), HIHYSGRPTYDPSLLS (SEQ ID NO: 167), HIHYSGRPTYDPSLHS (SEQ ID NO: 168), HIHYSGRPTYDPSLFS (SEQ ID NO: 169), and HIHYSGRPTYDPSLRS (SEQ ID NO: 170);

FR3 is selected from the group consisting of
RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 218),
RVTISKDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 219),
RVSISRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 220),
RVSISKDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 221),
RITISRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 222),
RITISKDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 223),
RISISRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 224),
RISISKDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 225).

CDR3 is KGKGSDDGLNY (SEQ ID NO: 100).

FR4 is WGQGTLVTVSS (SEQ ID NO: 101).

[0042] In a seventh aspect the present invention provides a V_L antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

the sequence of FR1 is EX_{27}VLTQSPATLSLSPGERATLSC (SEQ ID NO: 211) wherein, X_{27} is I or N
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189) or a sequence at least 90% similar, preferably at least 90% identical, thereto

the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 90% identical, thereto

the sequence of FR3 is GIPDRFSGSGSGX_{28}DFTLTISRLEPEDFAVYYC (SEQ ID NO: 212) wherein,

X_{28} is T or N
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto

the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical, thereto

the sequence of FR4 is FGGGTKVEIKR (SEQ ID NO: 193) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

[0043] In an eighth aspect the present invention provides a V_L antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence EIVLTQSPATLSLSPGERATLSCWYQQKPGQAPRLLIYGIPDRFSGSGSGTDFTLTISR LEPEDFAVYYCFGGGTKVEIKR (SEQ ID NO: 213)
and the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto;
the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto and;
the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical.

[0044] Preferred sequences for FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4 are as follows:-

FR1 is selected from the group EIVLTQSPATLSLSPGERATLSC (SEQ ID NO: 214) or ENVLTQSPATLSLSPGERATLSC (SEQ ID NO: 215).

CDR1 is SASSSLSYIH (SEQ ID NO: 188).

FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189).

CDR2 is DTSNLAS (SEQ ID NO: 190).

FR3 is GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC (SEQ ID NO: 216) or GIPDRFSGSGSGNDFTLTISRLEPEDFAVYYC (SEQ ID NO: 217).

CDR3 is FQGSEYPLT (SEQ ID NO: 192).

FR4 is FGGGTKVEIKR (SEQ ID NO: 193).

[0045] In preferred embodiments of the present invention the V_H and V_L antibody binding domains of the present invention further comprise a constant domain. The constant domain may be a human or non-human primate, preferably human, constant region. In one embodiment the sequence of the heavy chain constant domain is

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 52); or

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 92); or

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPEEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 53); and

the sequence of light chain constant domain is

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC (SEQ ID NO: 93).

[0046] In a further preferred embodiment of the present invention there is provided an antibody comprising the V_H of the first aspect and the V_L of the second aspect or the V_H of the third aspect and the V_L of the fourth aspect.

[0047] The binding domains and antibodies of the present invention specifically bind cancer cells which express the antigen bound by SC104, in particular colorectal cancer and non-colorectal cancers such as ovary, pancreas, prostate and lung cancer. Accordingly in another aspect the present invention provides a method of treating cancer in a subject wherein the cancer is selected from colorectal, ovarian, pancreatic, prostate and lung, the method comprising administering to the subject a therapeutically effective amount of the binding domain or antibody according to the present invention.

[0048] The present invention also provides a method of detecting the presence of cancer cells in a sample, the method comprising contacting the cell sample with a binding domain or antibody according to the present invention and detecting binding of the binding domain or antibody according to the present invention to the cells.

[0049] As will be appreciated the sequences developed and described in the present invention may be modified using methods well known in the art to increase binding, by for example, affinity maturation, or to decrease immunogenicity by removing predicted MHC class II-binding motifs. The therapeutic utility of the sequences developed and described herein can be further enhanced by modulating their functional characteristics, such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), serum half-life, biodistribution and binding to Fc receptors or the combination of any of these. This modulation can be achieved by protein-engineering, glyco-engineering or chemical methods. Depending on the therapeutic application required, it could be advantageous to either increase or decrease any of these activities.

[0050] Numerous methods for affinity maturation of antibodies are known in the art. Many of these are based on the general strategy of generating panels or libraries of variant proteins by mutagenesis followed by selection and/or screening for improved affinity. Mutagenesis is often performed at the DNA level, for example by error prone PCR (Thie, Voedisch et al. 2009), by gene shuffling (Kolkman and Stemmer 2001), by use of mutagenic chemicals or irradiation, by use of 'mutator' strains with error prone replication machinery (Greener 1996) or by somatic hypermutation approaches that harness natural affinity maturation machinery (Peled, Kuang et al. 2008). Mutagenesis can also be performed at the RNA level, for example by use of Qβ replicase (Kopsidas, Roberts et al. 2006). Library-based methods allowing screening for improved variant proteins can be based on various display technologies such as phage, yeast, ribosome, bacterial or mammalian cells, and are well known in the art (Benhar 2007). Affinity maturation can be achieved by more directed/predictive methods for example by site-directed mutagenesis or gene synthesis guided by findings from 3D protein modeling (see for example Queen, Schneider et al. 1989 or US patent 6,180,370 or US patent 5,225,539).

[0051] Methods of increasing ADCC have been described by Ferrara, Brunker et al. 2006; Li, Sethuraman et al. 2006; Stavenhagen, Gorlatov et al. 2007; Shields, Namenuk et al. 2001; Shinkawa, Nakamura et al. 2003; and WO 2008/006554. In a preferred form the antibodies of the present invention have a decreased level of fucose.

[0052] Methods of increasing CDC have been described by Idusogie, Wong et al. 2001; Dall'Acqua, Cook et al. 2006; Michaelsen, Aase et al. 1990; Brekke, Bremnes et al. 1993; Tan, Shopes et al. 1990; Norderhaug, Brekke et al. 1991.

[0053] References describing methods of increasing ADCC and CDC include Natsume, In et al. 2008. The disclosure of each of these references is included herein by cross reference.

[0054] A number of methods for modulating antibody serum half-life and biodistribution are based on modifying the interaction between antibody and the neonatal Fc receptor (FcRn), a receptor with a key role in protecting IgG from catabolism, and maintaining high serum antibody concentration. Dall'Acqua et al describe substitutions in the Fc region of IgG1 that enhance binding affinity to FcRn, thereby increasing serum half-life (Dall'Acqua, Woods et al. 2002) and further demonstrate enhanced bioavailability and modulation of ADCC activity with triple substitution of M252Y/S254T/T256E (Dall'Acqua, Kiener et al. 2006). See also U.S Pat. Nos 6,277,375; 6,821,505; and 7,083,784. Hinton et al have described constant domain amino acid substitutions at positions 250 and 428 that confer increased in vivo half-life (Hinton, Johlfs et al. 2004). (Hinton, Xiong et al. 2006). See also U.S Pat. No 7,217,797. Petkova et al have described constant domain amino acid substitutions at positions 307, 380 and 434 that confer increased in vivo half-life (Petkova, Akilesh et al. 2006). See also Shields et al (Shields, Namenuk et al. 2001) and WO 2000/42072. Other examples of constant domain amino acid substitutions which modulate binding to Fc receptors and subsequent function mediated by these receptors, including FcRn binding and serum half-life, are described in U.S Pat. Application Nos 20090142340; 20090068175; and 20090092599.

[0055] The glycans linked to antibody molecules are known to influence interactions of antibody with Fc receptors and glycan receptors and thereby influence antibody activity, including serum half-life (Kaneko, Nimmerjahn et al. 2006; Jones, Papac et al. 2007; and Kanda, Yamada et al. 2007). Hence, certain glycoforms that modulate desired antibody activities can confer therapeutic advantage. Methods for generating engineered glycoforms are known in the art and include but are not limited to those described in U.S. Pat. Nos 6,602,684; 7,326,681; 7,388,081; and WO 2008/006554.

[0056] Extension of half-life by addition of polyethylene glycol (PEG) has been widely used to extend the serum half-life of proteins, as reviewed, for example, by Fishburn (Fishburn 2008).

[0057] As will be recognised it is possible to make conservative amino acid substitutions within the sequences of the current invention. By "conservative substitution" is meant amino acids having similar properties. As used in this specification the following groups of amino acids are to be seen to be conservative substitutions:

H, R and K;
D,E,N and Q;
V, I and L;
C and M;
S, T, P, A and G; and
F, Y and W.

[0058] As will be seen the current specification uses the terms "% similar" and "% identical" to describe a number of sequences. As would be understood the term "% identical" means that in a comparison of two sequences over the specified region the two sequences have the specified number of identical residues in the same position. The term "% similar" has a similar meaning but in addition to the number of identical amino acids between the two sequences regard is also had to where the amino acids are not identical but are conservative substitutions as defined above.

[0059] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0060] All publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia or elsewhere before the priority date of each claim of this application.

[0061] In order that the nature of the present invention may be better understood preferred forms thereof will now be described by reference to the following Examples.

EXAMPLE 1

Generation of chimeric SC104 antibody

[0062] The murine SC104 heavy chain variable region (V_H), SEQ ID NO:1, was formatted *in silico* onto a human IgG1 backbone (human IgG1 heavy chain CH1, hinge, CH2 & CH3 domains such as NCBI accession number AAH72419 and derivatives thereof) to yield the chimeric SC104 heavy chain, SEQ ID NO:2. Similarly, the SC104 light chain variable region (V_L), SEQ ID NO:3, was formatted *in silico* onto a human IgG1 backbone (such as NCBI accession number P01834 and derivatives thereof) to form the corresponding chimeric SC104 light chain, SEQ ID NO:4.

Formatting and production of DNA constructs

[0063] The resulting heavy- and light chain amino acid sequences were back-translated into DNA sequences. The 5'-V_H-specific restriction site AscI was included upstream in the nucleotide sequence encoding the heavy chain polypeptide signal sequence, SEQ ID NO:5. A Tth111I restriction site was introduced into the polynucleotide sequence encoding the 3'-V_H region amino acids TVSS. The 5'-V_L-specific restriction site BsiWI was incorporated upstream of the nucleotide sequence encoding the light chain polypeptide signal sequence, SEQ ID NO:6. An RsrII restriction site was included at the 3'-end of the nucleic acid sequence encoding the amino acids RT located at the C-terminal end of the V_L region. These sequences were then optimised for expression in CHO cells using GeneOptimizer® technology (GeneArt, Germany).

[0064] The variable regions were subsequently synthesised *de novo* by assembly of synthetic oligonucleotides (GeneArt, Germany). The added restriction sites were used to clone the variable regions into the Glutamine Synthetase (GS) Chinese Hamster Ovary (CHO)-based gene expression system vectors (Lonza, UK) containing the appropriate IgG1 constant regions. The resulting heavy chain-containing and light-chain-containing vectors were used to co-transfect CHO-K1SV cells to produce antibodies.

Cell culture and transient expression of antibody constructs

[0065] CHO-K1SV (Lonza, UK) cells were cultured at $2x10^5$ cells/ml - $4x10^6$ cells/ml in Freestyle™ CHO chemically defined cell growth media (Invitrogen™) and cultures supplemented with 6mM final concentration of L-glutamine (Invitrogen™). Cells were cultured at 36.5°C, 10% CO_2 and 140rpm. On the day before transfection, 100ml culture volumes were seeded at $5x10^5$cells/ml in 500ml vented Erlenmeyer flasks (Corning®). The next day 4 mL of OptiPRO™ SFM (Invitrogen™) was prepared in a 15 mL Corning® tube

and mixed with 100µg of each antibody chain. The resulting mixture was filtered through a 0.2µm 13mm syringe-end filter unit (Millipore®) into a fresh 15 mL Corning® tube containing 200µl of Freestyle™ Max reagent (Invitrogen™). This mixture was incubated for 10 minutes at room temperature prior to addition to the CHO-K1SV cells seeded previously. After 7 days the supernatant was harvested by centrifugation at 3000g for 10 mins prior to filtration through a 0.2µm membrane into a sterile receptacle (Corning® filter unit).

Purification of antibodies

[0066] Supernatants harvested from transfected CHO cells were adjusted to pH 7.4 before being loaded onto a HiTrap™ Protein A column (1 mL, GE® Healthcare). The column was washed with 30 mL of 1X PBS (pH 7.4). Elution was performed using 0.1M citric acid pH 3. The eluted antibody was desalted using Zeba™ desalting columns (Pierce®) into 1 X PBS (pH 7.4). Antibody concentration was determined by A_{280} value.

Flow cytometry-based binding assays

[0067] Viable tumour cells and control cells ($2x10^5$, as judged by trypan blue exclusion) were incubated in triplicate with mouse SC104, chimeric SC104 or humanised variants, and human IgG_1 isotype (Sigma-Aldrich®) at various concentrations in 100 µl of buffer (PBS plus 1% FCS) in 96 V-well plates (Eppendorf) for 20min on ice in the dark. Cells were washed twice with buffer before incubation for 20 min in 100 µl of buffer containing goat anti-human IgG (Fc-specific, Sigma-Aldrich®, conjugated to FITC) or goat anti-mouse IgG (Fc-specific, Sigma-Aldrich®, conjugated to FITC) for detecting chimeric or mouse antibodies, respectively. After washing cells were resuspended in buffer and analysed for antibody binding by flow cytometry on a Cell Lab Quanta™ SC MPL (Beckman Coulter) using EV, side scatter and FL-1 gating; during acquisition the cells in the 96-well plate were cooled by underlying a cool pack (Eppendorf). Results were expressed as mean fluorescent intensity (MFI); curve slope values were calculated using non-linear regression analysis by GraphPad Prism® software.

Flow cytometry-based direct killing assays

[0068] Viable tumour cells ($2x10^5$, as judged by trypan blue exclusion) were incubated in triplicate with mouse SC104, chimeric SC104 or humanised variants, and human IgG_1 isotype (Sigma-Aldrich®) at various concentrations in 80 µl of buffer (PBS plus 1% FCS) in 96 V-well plates (Eppendorf) for 2.5 to 3 hours at room temperature in the dark. To each well was added 0.15g of 7AAD (BD® Biosciences) in 20 µl of buffer, and cells were incubated for further 20 min before cell viability was assayed by flow cytometry on a Cell

Lab Quanta™ SC MPL (Beckman Coulter) using EV, side scatter and FL-3 gating; during acquisition the cells in the 96-well plate were cooled by underlying a cool pack (Eppendorf). Results were expressed as percentage of $7AAD^+$ cells; curve slope values were calculated using non-linear regression analysis by GraphPad Prism® software

Chimeric SC104 antibody has potent and specific binding to and direct killing activity of human colon tumour cells

[0069] The chimeric and mouse parental SC104 antibodies were tested for binding to human colon cancer cell lines C170 (ECACC accession number 97071507) and Colo205 (ATCC accession number CCL-222); the mouse SC104 antibody was previously shown to bind strongly to these cell lines using flow cytometry assays (Durrant, Harding et al. 2006). Figure 1 (left hand panels) shows binding of the SC104 antibodies, but not of the human IgG_1 isotype control, to C170 and Colo205, with both the mouse and chimeric SC104 antibody displaying similar binding strength over a range of antibody concentrations. In contrast none of the antibodies bound to the human colon cancer line HCT-116 (ATCC accession number CCL-247) which was thus defined as SC104 antigen negative.

[0070] The mouse SC104 antibody is known to induce direct killing of colon tumour cells without the need for immune effector cells or complement (Durrant, Harding et al. 2006). Indeed both the mouse and chimeric SC104 antibodies, but not the human IgG_1 isotype control, showed potent direct killing of the antigen positive cells C170 and Colo205; in contrast, the antibodies did not show above background killing of the antigen negative cells HCT-116 (Figure 1, right hand panels).

[0071] Other experiments with different antigen negative human colorectal tumour cells (HCT15, ATCC accession number CCL-225), human non-tumour cells (MRC5, ATCC accession number CCL-171) or peripheral blood mononuclear cells from normal human donors displayed no binding and no direct killing activities for mouse and/or chimeric SC104 antibodies (data not shown). Taken together the chimeric SC104 antibody had similar potency and specificity when compared with parental mouse SC104 antibody. Hence chimeric SC104 antibody was used as a reference to test the activity of subsequently generated humanised SC104 antibody variants.

EXAMPLE 2

Humanisation of SC104

Selection of suitable human framework acceptors by 3D modelling

[0072] Independent 3D models of the murine SC104 heavy chain variable region (V_H) and light chain variable region (V_L) were constructed using the database of crystal structures (see for example the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank, http://www.rcsb.org/pdb/home/home.do; part of the Worldwide Protein Data Bank, http://www.wwpdb.org) and software package Discovery Studio v1.7 (Accelrys®, USA). Briefly, the protein data bank database was interrogated by Basic Local Alignment Search Tool (BLAST) searches using either the murine SC104 heavy chain variable region or light chain variable region to identify antibodies of similar polypeptide sequence with accompanying crystal structure information. These structures were subsequently used to build homology models based on the amino acid sequence homology shared by the murine SC104 variable regions and that of the identified crystal structures. Using these murine V_H and V_L models, suitable human V_H and V_L acceptor frameworks were independently identified from the protein data bank that shared framework structural homology with those of the murine antibody. To ensure correct heavy- and light-chain pairing V_H and V_L human acceptor frameworks of same antibody crystal structure were progressed through the humanisation process. Human acceptor antibodies with better murine-human heavy chain framework region structural homology were preferred over those with better murine-human light chain framework homology. These include pdb accession codes 1U6A and 1QLR. Human framework acceptors were assessed to test whether these structures are capable of supporting the SC104 CDRs appropriately.

Formatting of humanised antibody constructs

[0073] Initially, the murine SC104 complementarity determining regions (CDRs) were defined according to the method of Kabat (see (Kabat 1991) given that CDR grafting technologies (see for example (Queen, Schneider et al. 1989) or US patent 6,180,370 or US patent 5,225,539) are based on this categorisation. In later humanisation attempts, the CDR1 of the heavy chain (CDR-H1) was defined using the AbM nomenclature (see for example (Dübel 2007) and references incorporated therein), Table 1. The CDRs were formatted *in silico* onto the selected human frameworks using the DNAStar® software package (version 8; Lasergene®, USA). V_H regions were formatted onto a human IgG1 heavy chain containing CH1, hinge, CH2 & CH3 domains (such as NCBI accession number AAH72419 and derivatives thereof). Concomitantly, V_L regions were formatted

using the corresponding human IgG1 light chain constant region (such as NCBI accession number P01834 and derivatives thereof).

Humanised SC104 variants incorporating a Kabat-defined CDR-H1 unexpectedly yield non functional antibody

[0074] Human acceptor frameworks 1U6A and 1QLR were used for SC104 humanisation. The murine SC104 light chain CDRs were formatted onto the 1U6A and 1QLR light chain framework regions. An uncommon alanine residue at position 15 in the 1U6A framework sequence was removed by substitution with proline (A15P), yielding SEQ ID NO:7. The 1QLR-based humanised SC104 light chain was formatted as described above to yield SEQ ID NO:8. The corresponding 1U6A-based SC104 heavy chains were formatted containing a Kabat-defined CDR-H1, SEQ ID NO:9. Concomitantly the 1QLR-based SC104 heavy chains were formatted containing a Kabat-defined CDR-H1, SEQ ID NO:10. 1U6A-based light- and heavy- chain combinations SEQ ID NO:7 and SEQ ID NO:9 were co-expressed in CHO cells. 1QLR-based light- and heavy- chain combinations SEQ ID NO:8 and SEQ ID NO:10 were also co-expressed in CHO cells. The resulting antibodies were purified by protein A affinity chromatography and tested for live-cell binding activity using flow cytometry. Surprisingly, these humanised antibody variants, both incorporating a Kabat-defined CDR-H1, failed to bind to SC104 antigen positive cells (Figure 2).

Humanised SC104 variants incorporating an AbM-defined CDR-H1 surprisingly yield functional antibody

[0075] Following the unexpected failure upon humanisation incorporating a Kabat-defined CDR-H1, the AbM-defined CDR-H1 nomenclature was applied (Table 1). Subsequently, 1U6A- and 1QLR-based SC104 heavy chains were formatted to incorporate and AbM-defined CDR-H1, SEQ ID NOs:11 and 12 respectively. 1U6A-based light- and heavy- chain combinations SEQ ID NO:7 and SEQ ID NO:11 were co-expressed in CHO cells. 1QLR-based light- and heavy- chain combinations SEQ ID NO:8 and SEQ ID NO:12 were also co-expressed in CHO cells. The resulting antibodies were purified by protein A affinity chromatography and tested for live-cell binding activity using flow cytometry. Surprisingly, these humanised antibody variants, both incorporating an AbM-defined CDR-H1, bound to SC104 antigen positive cells (Figure 3).

Increasing the binding activity of humanised SC104

[0076] Figure 3 indicates that the humanised SC104 antibodies had suboptimal binding activity compared to that observed with the chimeric SC104. These data imply that one or

more framework amino acid substitutions are important in order to humanise SC104 with little resultant loss in binding activity. To address this issue, the framework polypeptide sequences of murine SC104 and 1U6A were compared. Amino acid differences between the frameworks were flagged and scrutinized *in silico* using murine SC104 variable region 3D models. Six V_H and three V_L amino acids (including the substitution A15P to remove an uncommon alanine residue in this position found in the 1U6A acceptor framework) were identified for further *in vitro* investigation to test their contributions to the binding activity of the humanised antibodies. A similar analysis was performed using 1QLR-humanised SC104 whereby four V_H and two V_L amino acids were flagged for testing *in vitro*. All of the positions flagged for *in vitro* investigation in 1QLR-humanised SC104 were identical to those already identified in 1U6A humanised SC104. Subsequently attempts were focussed on identifying the residue(s) critical in enhancing the potency of humanised SC104 using only the 1U6A acceptor.

Predicted substitution(s) required in the humanised V_H frameworks are more important for enhancing binding activity than those predicted in the humanised V_L

[0077] The genes encoding both AbM- and Kabat-defined CDR-H1 variants of the 1U6A-based SC104 heavy chains were resynthesised to incorporate a total of six amino acid residue substitutions – Q46E, I48M, V67I, T68S, V71R, E72D (Kabat numbering). This yielded SEQ ID NOs: 13 (AbM CDR-H1) and 15 (Kabat CDR-H1) respectively. An additional two amino acid substitutions - Q1E and S25T - flagged as lower priority than the aforementioned six by the processes outlined above where also introduced into SEQ ID NO:13 to yield SEQ ID NO:14, an AbM-defined CDR-H1 variant containing a total of eight amino acid substitutions. The corresponding AbM- and Kabat-grafted heavy chain sequences - SEQ ID NOs:11 and 9 respectively were used as non-substituted controls.

[0078] The 1U6A-based light chain was formatted incorporating three substitutions – I2N, A15P, T69N (Kabat numbering) – to create SEQ ID NO:16. The corresponding light chain sequence SEQ ID NO:7 was used as a minimally substituted control. Substituted and non-or minimally substituted combinations of heavy- and light chains were co-expressed and their activity in flow cytometry based cell binding assays assessed. In grafts containing a Kabat-defined CDR-H1, both humanised SC104 antibody variants SEQ ID NO:7/SEQ ID NO:15 and SEQ ID NO:16/SEQ ID NO:15 shared the same heavy chain (SEQ ID NO:15) incorporating the aforementioned six amino acid substitutions. These antibodies displayed similar positive binding activity irrespective of whether paired with either the substitution-containing (SEQ ID NO:16) or minimally-substituted (SEQ ID NO:7) light chains. In contrast, changing the heavy chain to one containing a Kabat-defined CDR-H1 and no amino acid substitutions, such as SEQ ID NO:9, abolished binding irrespective of

whether light chain SEQ ID NO:7 or SEQ ID NO:16 was used, Figure 4A. Similarly, light chains containing either a single or all three substitutions (SEQ ID NOs:7 and 16 respectively) did not markedly affect the potent binding activity of humanised SC104 antibody variants when paired with a heavy chain comprising an AbM-defined CDR-H1 (see for example SEQ ID Nos: 11 and 13 representing unsubstituted and substitution-containing heavy chains respectively), Figure 4B. A similar trend to that of Figure 4B was observed when heavy chain SEQ ID NO:13 was switched for heavy chain SEQ ID NO:14 and an identical flow cytometry-based binding assay with chimeric SC104 (SEQ ID NO:4/SEQ ID NO:2) was performed, results not shown. This data indicated substitutions Q1E and S25T were not responsible for the potent binding activity shown by heavy chain SEQ ID NOs: 13 and 14 compared with their unsubstituted counterpart, SEQ ID NO:11. Consequently substitutions Q1E and S25T were not investigated further. Moreover, these data demonstrate the importance of one or more substitutions in the 1U6A heavy chain to generate a humanised SC104 variant with enhanced binding activity.

[0079] Substitutions in the heavy chain of 1U6A-humanised SC104 were introduced into the Kabat-grafted 1QLR heavy chain sequence (SEQ ID NO:10) at positions - I48M, V67I, T68S and V71R – yielding SEQ ID NO:17. Similarly, substitutions were made in the 1QLR-humanised SC104 light chain – I2N and T69N - to give SEQ ID NO:18. The activity of antibodies containing an AbM-defined CDR-H1 (SEQ ID NO:12) was not significantly affected when expressed with either an unsubstituted (SEQ ID NO:8) or substitution-containing (SEQ ID NO:18) light chain. Expressing the 1QLR-SC104 variant with a Kabat-defined CDR-H1 containing the aforementioned substitutions (SEQ ID NO:17) with the non-substituted 1QLR partner SEQ ID NO:8 yielded a humanised variant with low binding activity. The negligible binding activity observed with the antibody produced upon co-expression of SEQ ID NO:17 with the fully substituted light chain SEQ ID NO:18 is suggestive that the substitutions in the light chain are deleterious to the binding activity of 1QLR-humanised SC104. The binding data of these antibody variants in addition to those described previously is summarised in Table 2.

Presence of R71 in the V_H framework is important to yield highly active humanised SC104

[0080] Individual 1U6A heavy chain variable region framework substitutions Q46E, I48M, V67I, T68S, V71R and E72D were formatted along with Q1E into SEQ ID NO:11, to yield SEQ ID NOs:19 through 24. The resulting heavy chains were co-expressed with light chain SEQ ID NO:7, and the resulting humanised SC104 variants compared by flow cytometry-based binding assays. The humanised SC104 antibody variants with Q46E, I48M, V67I, T68S and E72D displayed similar binding activity to each other but markedly lower activity when compared with chimeric SC104 antibody, Figure 5. In contrast the

humanised SC104 antibody variant carrying the V71R substitution (encoded by SEQ ID NO:23) showed enhanced binding activity similar to the chimeric SC104 antibody. These data indicate that R71 in addition to an AbM-defined CDR-H1 is important for highly potent humanised forms of SC104. Reformatting the R71-containing heavy chain to incorporate the substitution E1Q, SEQ ID NO:25, did not alter the high levels of binding activity of the resulting antibody upon co-expression with light chain SEQ ID NO:7 (not shown).

R71 enhances the binding activity of SC104 humanised variants comprising both AbM- and Kabat-defined CDR-H1

[0081] Substitution V71R, identified as critical for the potent binding activity of SC104 humanised using the human acceptor 1U6A, was introduced into 1QLR-based SC104 variants containing either an AbM- or Kabat-defined CDR-H1 as detailed in SEQ ID Nos:26 and 27 respectively. For comparison, V71R was also introduced into the 1U6A-based SC104 variant SEQ ID NO:10 which comprises a Kabat-defined CDR-H1 to form SEQ ID NO:28. 1QLR-based light and heavy chain combinations SEQ ID NO:8 and SEQ ID NO:26 or SEQ ID NO:27 were co-expressed in CHO cells. 1U6A-based light and heavy chain combination SEQ ID NO:7 and SEQ ID NO:28 was also co-expressed in CHO cells. The resulting antibodies were purified by protein A affinity chromatography and tested for live-cell binding activity to the antigen-positive cell line C170 by flow cytometry. The substitution V71R significantly enhances the live-cell binding activity to the antigen-positive cell line C170 of 1QLR-based SC104 containing an AbM-defined CDR-H1, and to a lesser extent the corresponding Kabat-defined grafted antibody as measured by flow cytometry, Figure 6A. In comparison, the substitution V71R significantly enhances the live-cell binding activity of both Kabat- and AbM- defined CDR-H1 1U6A-based SC104 variants to the antigen-positive cell line C170 as measured by flow cytometry, Figure 6B.

Identification of amino acid substitutions that enhance binding activity of humanised SC104 variants in the region where AbM- and Kabat definitions of CDR-H1 differ

[0082] An AbM-defined heavy chain CDR1 includes an additional five donor amino acid residues at the amino-terminal end of the CDR compared with its Kabat-grafted counterpart, Table 1. The consistent, enhanced potency of SC104 variants containing an AbM-defined CDR-H1 relative to their Kabat-grafted counterparts is surprising. Table 3 compares these five amino acids from donor (included in AbM CDR-H1 grafts), 1U6A and 1QLR acceptor sequences. Comparing the donor and 1U6A sequences, it appears that the non-conservative difference G27Y is most likely responsible for enhancing the potency of

humanised variants containing an AbM-defined CDR-H1 relative to the chimera, since T30S is a conservative change. This substitution, G27Y, was incorporated into the Kabat grafted 1QLR-SC104 variant SEQ ID NO:27 (+R) to yield SEQ ID NO:29 (+R +Y). This heavy chain along with the 1QLR-based light chain SEQ ID NO:8 was co-expressed in CHO cells and the resulting antibody purified as described previously. For comparison, a 1QLR-humanised antibody incorporating a Kabat-defined CDR-H1 without R71 (SEQ ID NO:10) and a second variant incorporating an AbM-defined CDR-H1 with R71 (SEQ ID NO:26) were included in comparative live-cell binding activity to the antigen-positive cell line C170 by flow cytometry, Figure 7. Unexpectedly, the resultant humanised antibody variant produced by light- and heavy chain combination SEQ ID NO:8 and SEQ ID NO:29 had no binding activity.

EXAMPLE 3

SC104 variants with lower predicted immunogenicity

[0083] Murine SC104 variable region amino acid sequences were screened *in silico* for predicted T cell epitopes (Epibase® ,Algonomics, Belgium). Two predicted major histocompatibility complex class II (MHC class II) binding peptides were identified. These are located in the heavy chain at the framework (FR) 2:CDR-H2 and CDR-H2:FR3 boundaries. These were of particular interest, since predictions for their removal involved substituting CDR amino acid residues which may alter antibody binding potency.

Heavy chain V_H region predicted MHC class II-binding peptide removal

[0084] Predictions suggested that the strength of the FR2:CDR-H2 MHC class II binding peptide in the murine V_H region would be reduced on substituting the phenylalanine residue at position 4 of the CDR-H2 sequence HIHFSGRPTYNPSLSS with tryptophan (SEQ ID NO:30), or tyrosine (SEQ ID NO:31), or preferentially proline (SEQ ID NO:32). These heavy chains were co-expressed in CHO cells with light chain SEQ ID NO:4 and the resulting antibodies purified by protein A chromatography as described previously. Surprisingly, comparative flow cytometry-based binding assays with chimeric SC104 revealed that the substitutions F53W and F53Y had negligible effect on the potent binding activity observed by chimeric SC104, whilst the substitution F53P drastically reduced the binding potency of the resulting antibody, Figure 8.

[0085] Further *in silico* immunogenicity predictions (predicted by the software ProPred, Singh,H. and Raghava,G.P.S.(2001) ProPred: Prediction of HLA-DR binding sites. Bioinformatics, 17(12), 1236-37. http://www.imtech.res.in/raghava/propred/) and/or SYFPETHI (Rammensee, Bachmann et al. 1999);

http://www.syfpeithi.de/Scripts/MHCServer.dll/EpitopePrediction.htm) suggested that the strength of this predicted MHC class II binding peptide was diminished upon grafting of the SC104 CDR-H2 into the human acceptor backbone 1U6A or 1QLR.

[0086] The presence of the second predicted MHC class II binding peptide at the CDR-H2:FR3 boundary was predicted to be present in both the murine and humanised SC104 antibody sequences.

SC104 variants with lower predicted immunogenicity

[0087] Based on the acceptor frameworks of 1U6A- and 1QLR-based SC104 novel humanised variants were generated for which potential major histocompatibility complex class II binding sequences were removed. Briefly, the humanised light chain variable regions were only predicted to have tolerated (ie: germline) MHC class II binding sequences, whereas the heavy chain variable regions of 1QLR and 1U6A were predicted to posses one and two strong-binding major histocompatibility complex class II binding sequences respectively. A further weaker-binding putative MHC class II-binding sequence was also predicted immediately prior to CDR-H1 as defined by method of Kabat. A substitution strategy was developed with the aim removing these predicted MHC class II binding epitopes. A panel of 1QLR-based SC104 heavy chain variable region variants with predicted lower immunogenicity as listed in SEQ ID NO:33 through SEQ ID NO:38 was constructed. A panel of 1U6A-based SC104 heavy chain variable region variants with predicted lower immunogenicity as detailed in SEQ ID Nos:39 through 50 was also constructed. Co-expression of 1QLR-based light chain SEQ ID NO:8 with each one of the variants SEQ ID NO:33 through SEQ ID NO:38 in CHO cells was performed and the resulting antibodies purified as detailed previously. Similarly, co-expression of 1U6A-based light chain SEQ ID NO:7 with each one of the variants SEQ ID NO:39 through SEQ ID NO:50 in CHO cells was performed and the resulting antibodies purified as detailed previously.

[0088] Humanised SC104 antibody variants with lower predicted immunogenicity were compared for binding activity to human colon cancer cells that express the SC104 antigen (C170, Colo205) with chimeric SC104 in live-cell flow cytometry assays. Binding activity comparable to that of the chimeric antibody was observed in each case. The SC104 antibody binding was also cell type specific, as the lower predicted immunogenicity SC104 variants did not bind the SC104 antigen negative human colon cancer cells HCT116; examples for the potent and specific binding of lower predicted immunogenicity SC104 variants are shown in Figure 9.

Identification of critical residues in the heavy chain variable region that confer optimal binding activity to SC104 variants with lower predicted immunogenicity

[0089] The sequence $GY_{27}SI_{29}S$ spanning amino acid positions 26 through 30 in the framework region immediately prior to Kabat-defined CDR-H1 conferred high binding activity of SC104 variants with lower predicted immunogenicity when incorporated into acceptor framework 1U6A or 1QLR (Figure 9). In contrast, the sequence $GY_{27}SF_{29}S$ resulted in surprisingly poor binding activity when incorporated in constructs based on the 1QLR acceptor framework (Figure 7), indicating Y27 is not the sole residue involved in conferring high binding activity. The effect of different amino acid residues at position 29 on binding activity was subsequently investigated by varying the amino acid residue present at this position of the 1U6A-humanised heavy chain SEQ ID NO:50. Heavy chain variants were co-expressed in CHO cells with light chain SEQ ID NO:7, purified by protein A affinity chromatography and tested by flow cytometry as described previously. Exemplary binding data is shown in Figure 10, whilst Table 4 summarizes the binding activities of all substitutions tested. Surprisingly, only residues I (SEQ ID NO:50), L (SEQ ID NO:62) or V (SEQ ID NO:70) at position 29 conferred optimal binding activity of humanised SC104 antibody variants with low predicted immunogenicity, whereas the majority of substitutions, including I29F (SEQ ID NO:58), resulted in poor binding activity to SC104 antigen positive human cancer cells C170.

[0090] The importance of R71 in framework 3 of variable heavy chain in yielding a humanised variant possessing optimal high binding activity has previously been demonstrated, Figure 6. To further improve the binding activity of 1U6A-based SC104 variants with lower predicted immunogenicity the residue at position 71 was substituted to generate a panel of heavy chain variants. These variants were subsequently co-expressed in CHO cells with light chain SEQ ID NO:7, purified by protein A affinity chromatography and tested by flow cytometry as described previously. Exemplary binding data is shown in Figure 11. Table 5 summarizes the binding activities of all substitutions tested. Unexpectedly, only the positively charged residues R and K (SEQ ID NOs: 50 and 81 respectively) at position 71 conferred the highest binding activity of humanised SC104 antibody variants to SC104 antigen positive human colon cancer cell line C170.

Modification of N-X-S motif in CDR-H2

[0091] N-linked glycosylation is known to have implications for protein product heterogeneity and potentially impact on immunogenicity. Analysis of the murine variable regions for potential N-linked glycosylation sites was performed (see for example (Ye 2007) or the software prediction algorithm NetNGlyc of

http://www.cbs.dtu.dk/services/NetNGlyc/ by R. Gupta, E. Jung and S. Brunak). This analysis identified the N-X-S motif, NPS, within the CDR-H2 sequence. Typically, the N-X-S motif is indicative of a putative N-linked glycosylation site, however an exception to this rule is when X is a proline residue, as in this case (see for example (Gavel and von Heijne 1990). Nonetheless, an analysis was performed to see if this N residue in CDR-H2 was permissive of change with respect to retaining binding activity. The NPS sequence in the chimeric SC104 sequence was altered to DPS to yield SEQ ID NO:51. Co-expression of light chain SEQ ID NO:4 with the heavy chain SEQ ID NO:51 in CHO cells and subsequent purification of the resulting product (as described previously) was performed. The resulting antibody was compared in flow cytometry-based binding assays to the SC104 antigen-positive cell line C170. Removal of the N-X-S motif by N→ D substitution within CDR-H2 had negligible impact on the binding potency of the resulting antibody upon comparison with chimeric SC104

EXAMPLE 4

Flow cytometry-based direct killing assays

[0092] Viable tumour cells ($2x10^5$, as judged by trypan blue exclusion) were assayed with chimeric SC104, humanised SC104 antibody variants or human IgG_1 isotype (Sigma-Aldrich®) as described in Example 1. 1U6A-based humanised SC104 antibody variants composed of light- and heavy chain combinations SEQ ID NO:7/SEQ ID NO:25 and SEQ ID NO:7/SEQ ID NO:50 were selected for further studies in addition to 1QLR-based variants combinations of SEQ ID NO:8/SEQ ID NO:26 and SEQ ID NO:8/SEQ ID NO:38.

Novel humanised SC104 antibody variants with lower predicted immunogenicity have potent direct killing activity against human colon tumour cells

[0093] The humanised SC104 antibody variants with lower predicted immunogenicity showed potent direct killing activity of human colon cancer cells without the need for immune effector cells. Figure 12 shows an example of direct killing by these humanised SC104 antibody variants, the chimeric SC104 antibody and the human isotype control using C170 tumour cells. When tested similar results were obtained using Colo205 tumour target cells (data not shown). Surprisingly, the 1U6A-derived SC104 antibody variants (SEQ ID NO:7/SEQ ID NO:25 and SEQ ID NO:7/SEQ ID NO:50) showed more potent direct killing activity compared with 1QLR-framework derived SC104 antibody variants (SEQ ID NO:8/SEQ ID NO:28 and SEQ ID NO:8/SEQ ID NO:38). This difference in direct killing activity was unexpected given the similar binding activity of the these SC104 antibody variants (Figure 9)

EXAMPLE 5

Antibody-dependent cell-mediated cytotoxicity (ADCC) assay

[0094] Effector (peripheral blood mononuclear) cells were purified from Buffy Coat preparation of normal human donors (provided by Australian Red Cross Blood Services) using Lymphoprep™ according to the manufacturer's protocol (Axis-Shield PoC AS). Viable effector cells ($5x10^6$/ml) were incubated overnight in RPMI 1640 (Gibco®) plus 10% FCS at 37 °C and 10% CO_2. Tumour target cells and effector cells were washed in PBS followed by media (RPMI1640 w/o phenol red, Gibco®, plus 0.5% FCS), resuspended in media and incubated with various concentration of antibody (chimeric SC104, humanised SC104 or human IgG_1 isotype, Sigma-Aldrich®, # I5154) in triplicate in 96-well U well plates (Corning®) in a 200 µl assay consisting of the following final concentrations: target cells, $1x10^5$ cells/ml; effector cells, $2.5x10^6$ cells/ml; antibody range 10 to 0.001 ug/ml. For controls, target cells only were incubated in the absence (min target) or presence (max target) of 1% Triton®-X (Sigma-Aldrich®), and target and effector cells (background) were incubated in the absence of antibody. Plates were centrifuged for 2 min at 160xg and incubated in humified CO_2 atmosphere in 37 °C for 4 hours. Cell death was measured using a Lactate Dehydrogenase release assay. Briefly, plates were spun for 5 min at 250xg and 100 µl of cell supernatant was assayed for Lactate Dehydrogenase release using the Cytotoxicity Detection Kit (Roche) according to the manufacturer's guidelines. To minimise contaminating cell carry-over the supernatant was filtered through a 96-well 0.2 micron AcroPrep™ plate (Pall). LDH release was quantified by reading absorbance at 492 nm and percentage cytotoxicity was calculated using the following formula: 100x [sample – mean (background)] / mean (max target – min target); EC_{50} values were calculated using non-linear regression analysis by GraphPad Prism® software.

Complement-dependent cytotoxicity (CDC) assay

[0095] Viable tumour target cells were incubated in media (RPMI1640 w/o phenol red, Gibco®, plus 5% FCS), with human complement serum (Sigma-Aldrich® #S1764) and various concentrations of antibody (chimeric SC104, humanised SC104 or human IgG_1 isotype, Sigma-Aldrich®, # I5154) in triplicate in 96-well flat well plates (Corning®) in a 150 µl assay consisting of the following final concentrations: target cells, $13.3x10^4$ cells/ml; complement, 15%; antibody range 10 to 0.01 ug/ml. For controls, target cells only were incubated in the absence (target background) or presence (target & complement background) of complement, and complement (complement background) was incubated in media only. Plates were centrifuged for 2 min at 160xg and incubated in humified CO_2 atmosphere in 37 °C for 2-3 hours. Cell death was measured using the CellTiter 96® kit

(Promega®) according to the manufacturer's guidelines including an additional incubation period of 3-4 hours. Death of target cells was quantified by reading absorbance at 492 nm and percentage cytotoxicity was calculated using the following formula: 100x [sample – mean (target & complement background)] / [mean (complement background) – mean (target & complement background)]; EC_{50} values were calculated using non-linear regression analysis by GraphPad Prism® software.

Novel humanised SC104 antibody variants with lower predicted immunogenicity have potent cytotoxicity against human colon tumour cells

[0096] Cytotoxicity against colon tumour cells of humanised SC104 variants with lower predicted immunogenicity was tested in antibody-dependent cell-mediated cytotoxicity and complement-dependent-cytotoxicity assays. The humanised SC104 antibody variants and the chimeric SC104 antibody, but not the human isotype control, had potent antibody-dependent cell-mediated cytotoxicity activity against C170 tumour cells using peripheral blood mononuclear cells of a normal human donor (Figure 13). Similar results were obtained using peripheral blood mononuclear cells from other human donors (data not shown). Figure 14 shows that the same panel of humanised SC104 antibody variants and the chimeric SC104 antibody, but not the human isotype control, had potent complement-dependent-cytotoxicity activity against Colo205 tumour cells using human complement. In addition, all other humanised SC104 antibody variants with lower predicted immunogenicity had potent antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxic activity against Colo205 tumour cells (data not shown). In line with the differential direct cell killing data, the 1U6A-derived SC104 antibody variants showed higher antibody-dependent cell-mediated cytotoxicity and complement-dependent-cytotoxicity activity compared with 1QLR-derived SC104 antibody variants. This hierarchy in immune-mediated cytotoxic activity was unexpected given the similar binding activity of the different SC104 antibody variants (Table 6).

EXAMPLE 6

Immunohistochemistry protocol

[0097] A multi-tumour human tissue microarray containing samples of each tumour type from 10-12 different donors was screened for binding to biotinylated humanised SC104 antibody variants using immunohistochemistry. Biotinylated human IgG_1 isotype was used for negative control staining. The tissue microarray sections were subjected to a series of different antigen retrieval strategies, including modification of temperature, pressure, and pH. In addition, a dilution series of the primary antibody was performed to obtain the best

possible signal to noise ratio. Immunoreactivity by the antibody was graded by visual inspection in a four step scale based on staining intensity and percentage of positive cells.

Humanised SC104 antibody variant binds to various human cancer types

[0098] A number of different cancer types from different human patients were analysed for binding with a humanised SC104 antibody possessing lower predicted immunogenicity (SEQ ID NO:7/SEQ ID NO:50). The humanised SC104 antibody, but not the human isotype control, bound to human colon cancer tissues demonstrating the specificity of the binding conditions used (data not shown). Table 7 summarises that positive membrane staining was found in colon cancer (75%). Surprisingly, positive staining was found in pancreatic cancer (70%) and to a lower extent in ovarian cancers (25%) and lung cancers (16.7%). In contrast, no staining was observed with kidney cancers in the limited sample size analysed. These results indicate that the humanised SC104 antibody with low predicted immunogenicity is useful for tumour diagnosis in indications of colorectal, pancreatic, ovarian and lung malignancies. In addition, one can envisage that the humanised SC104 antibody with low predicted immunogenicity is useful for the therapeutic treatment of colorectal, pancreatic, ovarian and lung cancer in humans. Such anti-tumour efficacy in vivo could be evaluated in mouse tumour xenograft models.

EXAMPLE 7

Effector function enhancement of SC104 antibodies

[0099] Effector function of antibodies can be enhanced by increasing antibody-dependent cell-mediated cytotoxicity or complement-dependent-cytotoxicity or by the combination of antibody-dependent cell-mediated cytotoxicity and complement-dependent-cytotoxicity.

ADCC enhancement

[0100] SC104 antibody variants and chimeric SC104 were engineered for enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) activity using standard modifications to the Fc region of the antibody. Standard methods include for example protein-engineering or glyco-engineering of the Fc region of the antibody.

[0101] As an example of protein-engineering, mutations in the constant heavy chain sequences were generated as described by Lazar et al., 2006 (Lazar, Dang et al. 2006). Specifically, S122D, S181A, I215E mutations were introduced into the Fc sequence SEQ ID NO:52, to form SEQ ID NO:53. A number of humanised antibodies along with the chimeric antibody possessing the enhanced Fc-region SEQ ID NO:52 were tested in ADCC assays following their expression in CHO cells and purification by protein A

chromatography as described in Example 2. ADCC function was measured as described in Example 5.

[0102] As an example of glyco-engineering, CHO cells producing humanised SC104 antibody variants or chimeric SC104 antibody were incubated for 8 to 10 days with kifunensin (0.25µg/ml) according to Zhou et al. (Zhou, Shankara et al. 2008). Subsequently antibody was purified as described in Example 2, and ADCC function was measured as described in Example 5.

[0103] Another example of glyco-engineering used the Potelligent® method as described in Shinkawa T. et al., 2003 (J Biol Chem 278: 3466-73). The variable light and heavy chain regions of the humanised antibody variants were expressed as an IgG1 on a standard constant region backbone. The sequence of the constant heavy chain was GenBank P01857.1 and the sequence of the constant light chain was NCBI accession number P01834. ADCC function was measured as described in Example 5.

Effector enhanced SC104 antibody variants and chimeric SC104 antibody have increased antibody-dependent cell-mediated cytotoxicity

[0104] In a series of experiments protein-engineering or glyco-engineering was applied to increase antibody-dependent cell-mediated cytotoxicity of chimeric SC104 antibody and SC104 antibody variants with low predicted immunogenicity. Compared with the unmodified antibodies, the Fc-engineered (SEQ ID NO:53) or kifunensin-treated SC104 humanised antibody variants and chimeric SC104 antibody showed increased antibody-dependent cell-mediated cytotoxicity against Colo205 tumour cells. Figure 15 shows an example of markedly increased killing of human colon cancer cell WiDr (ATCC accession number CCL-218) by kifunensin-treated humanised SC104 antibody with lower predicted immunogenicity (SEQ ID NO:7/SEQ ID NO:50). Figure 16 shows an example for markedly increased killing of human colon cancer cell Colo201 (ATCC accession number CCL-224) by Potelligent® -treated humanised SC104 antibody with lower predicted immunogenicity (SEQ ID NO:7/SEQ ID NO:94, the sequence of the constant heavy chain and the sequence of the constant light chain were GenBank P01857.1 and NCBI accession number P01834, respectively).

EXAMPLE 8

Prophylactic mouse xenograft HT29 tumour model

[0105] Female BALB/c nude mice were inoculated subcutaneously with $2x10^6$ human colon cancer HT29 cells (ATCC accession number HTB-38). On the same day as tumour

cell inoculation (day 0), mice were randomised based on body weight into two treatment groups (n=10 per group). Each group was treated intraperitoneally with either the vehicle control (PBS, 10 ml/kg) or humanised SC104 antibody (10 mg/kg). The vehicle control and humanised SC104 antibody were administered twice weekly for four weeks. Tumour volume was calculated three times weekly using the following formula: Volume (mm^3) = length x $diameter^2$ x π /6.

[0106] During the course of the study some mice had to be culled due to excessive body weight loss resulting in reduced mouse numbers for vehicle control (n=6) and antibody (n=9) treatment groups. Upon termination of the study (day 27), tumours were excised post-mortem from all mice, cleaned of skin and weighed.

Therapeutic mouse xenograft Colo201 tumour model

[0107] Female athymic nude mice were inoculated subcutaneously with $5x10^6$ human colon cancer Colo201 (ATCC accession number CCL-224). After the tumour volume reached ~110 mm^3 (day 0), mice were randomised into two treatment groups (n=10 per group). Each group was treated intraperitoneally with either the vehicle control (PBS, 10 ml/kg) or humanised Potelligent®-engineered (as described in Example 7) SC104 antibody (10 mg/kg). The vehicle control and humanised Potelligent®-engineered SC104 antibody were administered twice weekly over 38 days. Tumour volume was calculated two times weekly using the following formula: Volume (mm^3) =1/2 (a^2 x b) where 'a' is the smallest diameter and 'b' is the largest diameter.

Potent anti-tumour efficacy of humanised SC104 antibody with lower predicted immunogenicity in vivo

[0108] Treatment of tumour-bearing mice with humanised SC104 antibody with lower predicted immunogenicity (SEQ ID NO:7/SEQ ID NO:50) resulted in significant reduced tumour volume and tumour weight compared with vehicle control treatment in a prophylactic HT29 tumour model (Figure 17). Moreover, in a therapeutic Colo201 treatment model, humanised Potelligent®-engineered SC104 antibody with lower predicted immunogenicity (SEQ ID NO:7/SEQ ID NO:94) showed significant anti-tumour activity (Figure 18).

[0109] One can envisage that humanised SC104 antibody variants with low predicted immunogenicity are useful for the treatment of colorectal cancers in humans either as mono-therapy or in combination with other therapeutic anti-tumour agents, for example chemotherapy, small molecules or biologics.

Table 1. CDR sequences of SC104.

Variable region	CDR number	CDR Sequence
V_H	1	SGYSWH (Kabat) GYSITSGYSWH (AbM)
V_H	2	HIHFSGRPTYNPSLSS
V_H	3	KGKGSDDGLNY
V_L	1	SASSSLSYIH
V_L	2	DTSNLAS
V_L	3	FQGSEYPLT

Table 2. Few initial SC104 humanised antibody variants retained binding activity to SC104 antigen positive human colon cancer cell line C170

SC104 antibody form	V_L (SEQ ID NO) / V_H (SEQ ID NO)	C170
humanised	SEQ ID NO:18/SEQ ID NO:10	<1
humanised	SEQ ID NO:18/SEQ ID NO:17	<1
humanised	SEQ ID NO:7/SEQ ID NO:9	<1
humanised	SEQ ID NO:16/SEQ ID NO:9	<1
humanised	SEQ ID NO: 8/SEQ ID NO:10	<1
humanised	SEQ ID NO:8/SEQ ID NO:17	11.0
humanised	SEQ ID NO:7/SEQ ID NO:15	23.4
humanised	SEQ ID NO:16/SEQ ID NO:15	29.4
humanised	SEQ ID NO:18/SEQ ID NO:12	64.5
humanised	SEQ ID NO:16/SEQ ID NO:11	73.9
humanised	SEQ ID NO:8/SEQ ID NO:12	74.6
humanised	SEQ ID NO:7/SEQ ID NO:11	81.9
humanised	SEQ ID NO:16/SEQ ID NO:13	91.0
humanised	SEQ ID NO:7/SEQ ID NO:13	96.1
chimeric	SEQ ID NO:4/SEQ ID NO:2	100

expressed as percentage binding relative to chimeric SC104 binding; when tested similar data were obtained in other experiments

Table 3. Sequence comparison of framework region immediately prior to Kabat-defined CDR-H1

Source of amino acid sequence	Five amino acids prior to Kabat-defined CDR-H1
Murine donor Sequence	GYSIT
1U6A acceptor sequence	GGSIS
1QLR acceptor sequence	GGSFS

Table 4: Residues I, L, and V at position 29 in combination with residue Y at position 27 in 1U6A framework 1 of variable heavy chain confer highest binding activity of humanised SC104 antibody variants with lower predicted immunogenicity to SC104 antigen positive human colon cancer cell line C170

SC104 antibody form	Residue substitution at position 29	V_L (SEQ ID NO) / V_H (SEQ ID NO)	C170
chimeric	I	SEQ ID NO:4/ SEQ ID NO:2	100.0
humanised	I	SEQ ID NO:7/SEQ ID NO:50	96.5
humanised	L	SEQ ID NO:7/SEQ ID NO:62	79.4
humanised	V	SEQ ID NO:7/SEQ ID NO:70	71.4
humanised	T	SEQ ID NO:7/SEQ ID NO:69	20.3
humanised	M	SEQ ID NO:7/SEQ ID NO:63	15.1
humanised	N	SEQ ID NO:7/SEQ ID NO:64	8.4
humanised	Y	SEQ ID NO:7/SEQ ID NO:72	3.3
humanised	H	SEQ ID NO:7/SEQ ID NO:60	2.5
humanised	R	SEQ ID NO:7/SEQ ID NO:67	1.7
humanised	Q	SEQ ID NO:7/SEQ ID NO:66	1.4
humanised	P	SEQ ID NO:7/SEQ ID NO:65	1.4
humanised	W	SEQ ID NO:7/SEQ ID NO:71	1.4
humanised	A	SEQ ID NO:7/SEQ ID NO:54	<1
humanised	S	SEQ ID NO:7/SEQ ID NO:68	<1
humanised	F	SEQ ID NO:7/SEQ ID NO:58	<1
humanised	C	SEQ ID NO:7/SEQ ID NO:55	<1
humanised	G	SEQ ID NO:7/SEQ ID NO:59	<1
humanised	D	SEQ ID NO:7/SEQ ID NO:56	<1
humanised	E	SEQ ID NO:7/SEQ ID NO:57	<1
humanised	K	SEQ ID NO:7/SEQ ID NO:61	<1

Expressed as mean percentage activity relative to chimeric SC104 calculated from 2 experiments

Table 5. Positive charged residues R and K at position 71 in 1U6A framework 3 of variable heavy chain confer highest binding activity of humanised SC104 antibody variants with lower predicted immunogenicity to SC104 antigen positive human colon cancer cell line C170

SC104 antibody form	Residue substitution at position 71	V_L (SEQ ID NO) / V_H (SEQ ID NO)	C170
chimeric	R	SEQ ID NO:4/ SEQ ID NO:2	100.0
humanised	R	SEQ ID NO:7/SEQ ID NO:50	95.7
humanised	K	SEQ ID NO:7/SEQ ID NO:81	115.7
humanised	H	SEQ ID NO:7/SEQ ID NO:79	83.1
humanised	M	SEQ ID NO:7/SEQ ID NO:83	82.9
humanised	F	SEQ ID NO:7/SEQ ID NO:77	79.6
humanised	A	SEQ ID NO:7/SEQ ID NO:73	70.8
humanised	P	SEQ ID NO:7/SEQ ID NO:85	58.4
humanised	Y	SEQ ID NO:7/SEQ ID NO:91	60.7
humanised	S	SEQ ID NO:7/SEQ ID NO:87	52.1
humanised	Q	SEQ ID NO:7/SEQ ID NO:86	60.1
humanised	C	SEQ ID NO:7/SEQ ID NO:74	47.3
humanised	D	SEQ ID NO:7/SEQ ID NO:75	48.2
humanised	G	SEQ ID NO:7/SEQ ID NO:78	54.1
humanised	T	SEQ ID NO:7/SEQ ID NO:88	43.5
humanised	I	SEQ ID NO:7/SEQ ID NO:80	23.5
humanised	L	SEQ ID NO:7/SEQ ID NO:82	31.9
humanised	N	SEQ ID NO:7/SEQ ID NO:84	25.6
humanised	V	SEQ ID NO:7/SEQ ID NO:89	36.4
humanised	W	SEQ ID NO:7/SEQ ID NO:90	25.3
humanised	E	SEQ ID NO:7/SEQ ID NO:76	22.6

expressed as mean percentage activity relative to chimeric SC104 calculated from 2-3 experiments

Table 6. Humanised SC104 antibody variants with low predicted immunogenicity demonstrate unexpected functional differences in direct killing activity, antibody-dependent cell-mediated-cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activities despite essentially identical binding activities against SC104 antigen positive human colon cancer cells

		Binding		Direct killing	ADCC		CDC
SC104 antibody form	V_L (SEQ ID NO) /V_H (SEQ ID NO)	C170	Colo205	C170	C170	Colo205	Colo205
chimeric	SEQ ID NO:4/ SEQ ID NO:2	100	100	100	100	100	100
humanised	SEQ ID NO:7/SEQ ID NO:25	101.4	104.8	98.9	154.2	120.3	54.1
humanised	SEQ ID NO:7/SEQ ID NO:50	106.8	112.0	89.4	162.5	203.5	136.0
humanised	SEQ ID NO:8/SEQ ID NO:26	99.0	95.8	48.7	32.3	84.4	9.9
humanised	SEQ ID NO:8/SEQ ID NO:38	103.5	104.3	50.3	47.3	nd	20.1

expressed as percentage activity relative to chimeric SC104; nd, not determined; when tested similar data were obtained in other experiments

Table 7. Humanised SC104 antibody variant with low predicted immunogenicity binds to various human tumour types

		Percentage of membranous immunostaining			
Tumour type	Number analysed	Negative	weak	moderate	strong
Colon	12	25	41.7	16.7	16.7
Pancreas	10	30	10	30	30
Ovary	12	75	16.7	8.3	0
Lung	12	83.3	16.7	0	0
Kidney	12	100	0	0	0

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US 6,180,370
US 7,083,784
US 7,217,797
US 7,326,681
US 7,388,081
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SUMMARY OF SEQUENCE LISTING

SEQ ID NO:1: Amino acid sequence of murine SC104 heavy chain variable region.

SEQ ID NO:2: Amino acid sequence of chimeric SC104 heavy chain with mouse variable region and human IgG1 backbone (human IgG1 heavy chain CH1, hinge, CH2 & CH3 domains).

SEQ ID NO:3: Amino acid sequence of murine SC104 light chain variable region.

SEQ ID NO:4: Amino acid sequence of chimeric SC104 light chain with mouse variable region and human light chain constant region.

SEQ ID NO:5: Heavy chain signal sequence.

SEQ ID NO:6: Light chain signal sequence.

SEQ ID NO:7: 1U6A-based SC104 light chain polypeptide sequence incorporating substitution A15P.

SEQ ID NO:8: 1QLR-based SC104 light chain.

SEQ ID NO:9: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence.

SEQ ID NO:10: Kabat-grafted 1QLR-based SC104 heavy chain polypeptide sequence.

SEQ ID NO:11: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence.

SEQ ID NO:12: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1QLR-based SC104 heavy chain polypeptide sequence.

SEQ ID NO:13: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating six substitutions.

SEQ ID NO:14: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating eight substitutions.

SEQ ID NO:15: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating six substitutions.

SEQ ID NO:16: 1U6A-based SC104 light chain polypeptide sequence incorporating three subsitutions.

SEQ ID NO:17: Kabat-grafted 1QLR-based SC104 heavy chain polypeptide sequence incorporating four substitutions.

SEQ ID NO:18: 1QLR-based SC104 light chain polypeptide sequence incorporating two substitutions.

SEQ ID NO:19: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions Q1E and Q46E.

SEQ ID NO:20: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions Q1E and I48M.

SEQ ID NO:21: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions Q1E and V67I.

SEQ ID NO:22: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions Q1E and T68S.

SEQ ID NO:23: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions Q1E and V71R.

SEQ ID NO:24: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions Q1E and E72D.

SEQ ID NO:25: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitution V71R.

SEQ ID NO:26: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitution V71R.

SEQ ID NO:27: Kabat-grafted 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitution V71R.

SEQ ID NO:28: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitution V71R.

SEQ ID NO:29: Kabat-grafted 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y and V71R.

SEQ ID NO:30: Amino acid sequence of chimeric SC104 heavy chain with mouse variable region and human IgG1 backbone (human IgG1 heavy chain CH1, hinge, CH2 & CH3 domains) incorporating CDR-H2 substitution F53W.

SEQ ID NO:31: Amino acid sequence of chimeric SC104 heavy chain with mouse variable region and human IgG1 backbone (human IgG1 heavy chain CH1, hinge, CH2 & CH3 domains) incorporating CDR-H2 substitution F53Y.

SEQ ID NO:32: Amino acid sequence of chimeric SC104 heavy chain with mouse variable region and human IgG1 backbone (human IgG1 heavy chain CH1, hinge, CH2 & CH3 domains) incorporating CDR-H2 substitution F53P.

SEQ ID NO:33: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitutions S64K and V71R.

SEQ ID NO:34: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitutions S64L and V71R.

SEQ ID NO:35: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitutions S64H and V71R.

SEQ ID NO:36: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitutions S64F and V71R.

SEQ ID NO:37: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitutions S64R and V71R.

SEQ ID NO:38: Kabat-grafted 1QLR-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, F29I, T30S, S64K and V71R.

SEQ ID NO:39: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S64K and V71R.

SEQ ID NO:40: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S64L and V71R.

SEQ ID NO:41: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S64H and V71R.

SEQ ID NO:42: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S64F and V71R.

SEQ ID NO:43: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S64R and V71R.

SEQ ID NO:44: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S40P and V71R.

SEQ ID NO:45: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S40P, S64K and V71R.

SEQ ID NO:46: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S40P, S64L and V71R.

SEQ ID NO:47: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S40P, S64H and V71R.

SEQ ID NO:48: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S40P, S64F and V71R.

SEQ ID NO:49: AbM-grafted CDR-H1, Kabat-defined CDR-H2 and CDR-H3 of 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions S40P, S64R and V71R.

SEQ ID NO:50: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71R.

SEQ ID NO:51: Amino acid sequence of chimeric SC104 heavy chain with mouse variable region and human IgG1 backbone (human IgG1 heavy chain CH1, hinge, CH2 & CH3 domains) incorporating substitution N60D.

SEQ ID NO:52: Heavy chain constant region incorporating hinge, CH1, CH2 and CH3 domains.

SEQ ID NO:53: Heavy chain constant region incorporating hinge, CH1, CH2 and CH3 domains, incorporating amino acid substitutions S122D, S181A, I215E.

SEQ ID NO:54: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29A, T30S, S40P, S64K and V71R.

SEQ ID NO:55: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29C, T30S, S40P, S64K and V71R.

SEQ ID NO:56: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29D, T30S, S40P, S64K and V71R.

SEQ ID NO:57: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29E, T30S, S40P, S64K and V71R.

SEQ ID NO:58: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29F, T30S, S40P, S64K and V71R.

SEQ ID NO:59: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29G, T30S, S40P, S64K and V71R.

SEQ ID NO:60: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29H, T30S, S40P, S64K and V71R.

SEQ ID NO:61: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29K, T30S, S40P, S64K and V71R.

SEQ ID NO:62: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29L, T30S, S40P, S64K and V71R.

SEQ ID NO:63: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29M, T30S, S40P, S64K and V71R.

SEQ ID NO:64: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29N, T30S, S40P, S64K and V71R.

SEQ ID NO:65: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29P, T30S, S40P, S64K and V71R.

SEQ ID NO:66: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29Q, T30S, S40P, S64K and V71R.

SEQ ID NO:67: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29R, T30S, S40P, S64K and V71R.

SEQ ID NO:68: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29S, T30S, S40P, S64K and V71R.

SEQ ID NO:69: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29T, T30S, S40P, S64K and V71R.

SEQ ID NO:70: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29V, T30S, S40P, S64K and V71R.

SEQ ID NO:71: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29W, T30S, S40P, S64K and V71R.

SEQ ID NO:72: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, I29Y, T30S, S40P, S64K and V71R.

SEQ ID NO:73: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71A.

SEQ ID NO:74: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71C.

SEQ ID NO:75: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71D.

SEQ ID NO:76: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71E.

SEQ ID NO:77: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71F.

SEQ ID NO:78: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71G.

SEQ ID NO:79: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71H.

SEQ ID NO:80: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71I.

SEQ ID NO:81: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71K.

SEQ ID NO:82: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71L.

SEQ ID NO:83: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71M.

SEQ ID NO:84: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71N.

SEQ ID NO:85: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71P.

SEQ ID NO:86: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71Q.

SEQ ID NO:87: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71S.

SEQ ID NO:88: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71T.

SEQ ID NO:89: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71V.

SEQ ID NO:90: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71W.

SEQ ID NO:91: Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71Y.

SEQ ID NO:92: Heavy chain constant region incorporating hinge, CH1, CH2 and CH3 domains.

SEQ ID NO:93 Light chain constant domain

SEQ ID NO:94 Kabat-grafted 1U6A-based SC104 heavy chain polypeptide sequence incorporating substitutions G27Y, T30S, S40P, S64K and V71R

CLAIMS

1. A V_H antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

(i) the sequence of FR1 is X_1VQLQESGPGLVKPSETLSLTCTVX_2GYSX_3X_4 (SEQ ID: 95) wherein;
X_1 is Q or E
X_2 is S or T
X_3 is I, L or V
X_4 is S or T
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto;

(ii) the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto;

(iii) the sequence of FR2 is WIRQX_5PGKGLX_6WX_7G (SEQ ID NO: 97) wherein
X_5 is S or P
X_6 is Q or E
X_7 is M or I
or a sequence at least 90% similar, preferably at least 90% identical, thereto;

(iv) the sequence of CDR2 is HIHX_8SGRPTYX_9PSLX_{10}S (SEQ ID NO: 98) wherein
X_8 is F, Y or W
X_9 is N or D
X_{10} is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto;

(v) the sequence of FR3 is R$X_{11}X_{12}$IS$X_{13}X_{14}$TAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 99) wherein
X_{11} is V or I
X_{12} is T or S
X_{13} is R or K

X_{14} is E or D
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto with the proviso that the residue at position 6 must be R or K;

(vi) the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto;

(vii) the sequence of FR4 is WGQGTLVTVSS (SEQ ID NO: 101) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

2. A V_H antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence

QVQLQESGPGLVKPSETLSLTCTVSGYSX$_3$SWIRQPPGKGLQWIGRVTIS X$_{13}$ETAKNQFSLKLTSMTAADTAVYYCARWGQGTLVTVSS (SEQ ID NO: 102);
wherein X_3 is I, L or V and X_{13} is R or K;
and the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto;
the sequence of CDR2 is HIHX$_8$SGRPTYX$_9$PSLX$_{10}$S (SEQ ID NO: 98) wherein
X_8 is F, Y or W
X_9 is N or D
X_{10} is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto and;
the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

3. A V_H antibody binding domain as claimed in claim 1 or claim 2 in which the sequence of FR1 is selected from the group consisting of
QVQLQESGPGLVKPSETLSLTCTVSGYSIS, (SEQ ID NO: 103)
QVQLQESGPGLVKPSETLSLTCTVSGYSIT, (SEQ ID NO: 104)
QVQLQESGPGLVKPSETLSLTCTVSGYSLS, (SEQ ID NO: 105)
QVQLQESGPGLVKPSETLSLTCTVSGYSLT, (SEQ ID NO: 106)
QVQLQESGPGLVKPSETLSLTCTVSGYSVS, (SEQ ID NO: 107)
QVQLQESGPGLVKPSETLSLTCTVSGYSVT, (SEQ ID NO: 108)
QVQLQESGPGLVKPSETLSLTCTVTGYSIS, (SEQ ID NO: 109)
QVQLQESGPGLVKPSETLSLTCTVTGYSIT, (SEQ ID NO: 110)

QVQLQESGPGLVKPSETLSLTCTVTGYSLS, (SEQ ID NO: 111)
QVQLQESGPGLVKPSETLSLTCTVTGYSLT, (SEQ ID NO: 112)
QVQLQESGPGLVKPSETLSLTCTVTGYSVS, (SEQ ID NO: 113)
QVQLQESGPGLVKPSETLSLTCTVTGYSVT, (SEQ ID NO: 114)
EVQLQESGPGLVKPSETLSLTCTVSGYSIS, (SEQ ID NO: 115)
EVQLQESGPGLVKPSETLSLTCTVSGYSIT, (SEQ ID NO: 116)
EVQLQESGPGLVKPSETLSLTCTVSGYSLS, (SEQ ID NO: 117)
EVQLQESGPGLVKPSETLSLTCTVSGYSLT, (SEQ ID NO: 118)
EVQLQESGPGLVKPSETLSLTCTVSGYSVS, (SEQ ID NO: 119)
EVQLQESGPGLVKPSETLSLTCTVSGYSVT, (SEQ ID NO: 120)
EVQLQESGPGLVKPSETLSLTCTVTGYSIS, (SEQ ID NO: 121)
EVQLQESGPGLVKPSETLSLTCTVTGYSIT, (SEQ ID NO: 122)
EVQLQESGPGLVKPSETLSLTCTVTGYSLS, (SEQ ID NO: 123)
EVQLQESGPGLVKPSETLSLTCTVTGYSLT, (SEQ ID NO: 124)
EVQLQESGPGLVKPSETLSLTCTVSTGYSVS, (SEQ ID NO: 125) and
EVQLQESGPGLVKPSETLSLTCTVTGYSVT (SEQ ID NO: 126).

4. A V_H antibody binding domain as claimed in any one of claims 1 to 3 in which the sequence of CDR1 is SGYSWH (SEQ ID NO: 96).

5. A V_H antibody binding domain as claimed in any one of claims 1 to 4 in which the sequence of FR2 is selected from the group consisting of WIRQSPGKGLQWIG (SEQ ID NO: 127), WIRQSPGKGLQWMG (SEQ ID NO: 128), WIRQSPGKGLEWIG (SEQ ID NO: 129), WIRQSPGKGLEWMG (SEQ ID NO: 130), WIRQPPGKGLQWIG (SEQ ID NO: 131), WIRQPPGKGLQWMG (SEQ ID NO: 132), WIRQPPGKGLEWIG (SEQ ID NO: 133), and WIRQPPGKGLEWMG (SEQ ID NO: 134).

6. A V_H antibody binding domain as claimed in any one of claims 1 to 5 in which the sequence of CDR2 is selected from the group consisting of HIHFSGRPTYNPSLSS (SEQ ID NO: 135), HIHFSGRPTYNPSLKS (SEQ ID NO: 136), HIHFSGRPTYNPSLLS (SEQ ID NO: 137), HIHFSGRPTYNPSLHS (SEQ ID NO: 138), HIHFSGRPTYNPSLFS (SEQ ID NO: 139), HIHFSGRPTYNPSLRS (SEQ ID NO: 140), HIHWSGRPTYNPSLSS (SEQ ID NO: 141), HIHWSGRPTYNPSLKS (SEQ ID NO: 142), HIHWSGRPTYNPSLLS (SEQ ID NO: 143), HIHWSGRPTYNPSLHS (SEQ ID NO: 144), HIHWSGRPTYNPSLFS (SEQ ID NO: 145), HIHWSGRPTYNPSLRS (SEQ ID NO: 146), HIHYSGRPTYNPSLSS (SEQ ID NO: 147), HIHYSGRPTYNPSLKS (SEQ ID NO: 148), HIHYSGRPTYNPSLLS (SEQ ID NO: 149), HIHYSGRPTYNPSLHS

(SEQ ID NO: 150), HIHYSGRPTYNPSLFS (SEQ ID NO: 151), HIHYSGRPTYNPSLRS (SEQ ID NO: 152), HIHFSGRPTYDPSLSS (SEQ ID NO: 153), HIHFSGRPTYDPSLKS (SEQ ID NO: 154), HIHFSGRPTYDPSLLS (SEQ ID NO: 155), HIHFSGRPTYDPSLHS (SEQ ID NO: 156), HIHFSGRPTYDPSLFS (SEQ ID NO: 157), HIHFSGRPTYDPSLRS (SEQ ID NO: 158), HIHWSGRPTYDPSLSS (SEQ ID NO: 159), HIHWSGRPTYDPSLKS (SEQ ID NO: 160), HIHWSGRPTYDPSLLS (SEQ ID NO: 161), HIHWSGRPTYDPSLHS (SEQ ID NO: 162), HIHWSGRPTYDPSLFS (SEQ ID NO: 163), HIHWSGRPTYDPSLRS (SEQ ID NO: 164), HIHYSGRPTYDPSLSS (SEQ ID NO: 165), HIHYSGRPTYDPSLKS (SEQ ID NO: 166), HIHYSGRPTYDPSLLS (SEQ ID NO: 167), HIHYSGRPTYDPSLHS (SEQ ID NO: 168), HIHYSGRPTYDPSLFS (SEQ ID NO: 169), and HIHYSGRPTYDPSLRS (SEQ ID NO: 170).

7. A V_H antibody binding domain as claimed in any one of claims 1 to 6 in which the sequence of FR3 is selected from the group consisting of RVTISRETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 171), RVTISRDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 172), RVTISKETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 173), RVTISKDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 174), RVSISRETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 175), RVSISRDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 176), RVSISKETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 177), RVSISKDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 178), RITISRETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 179), RITISRDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 180), RITISKETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 181), RITISKDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 182), RISISRETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 183), RISISRDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 184), RISISKETAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 185), and RISISKDTAKNQFSLKLTSMTAADTAVYYCAR (SEQ ID NO: 186).

8. A V_H antibody binding domain as claimed in any one of claims 1 to 7 in which the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100).

9. A V_H antibody binding domain as claimed in any one of claims 1 to 8 in which the sequence of FR4 is WGQGTLVTVSS (SEQ ID NO: 101).

10. A V_H antibody binding domain as claimed in any one of claims 1 to 9 further comprising a constant domain.

11. A V_H antibody binding domain as claimed in claim 10 in which the constant domain has the sequence:-

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 52); or

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 92); or

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPEEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 53).

12. A V_L antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

(i) the sequence of FR1 is EX_{13}VLTQSPGTLSLSX_{14}GERATLSC (SEQ ID NO: 187) wherein,
X_{13} is I or N,
X_{14} is A or P,

or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto;

(ii) the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence or a sequence at least 90% similar, preferably at least 90% identical, thereto;

(iii) the sequence of FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189) or a sequence at least 90% similar, preferably at least 90% identical, thereto

(iv) the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto;

(v) the sequence of FR3 is GIPDRFSGSGSGX$_{15}$DFTLTISRVEPEDFAVYYC (SEQ ID NO: 191) wherein,
X$_{15}$ is T or N
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto;

(vi) the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical, thereto;

(vii) the sequence of FR4 is FGQGTKLEIKR (SEQ ID NO: 193) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

13. A V_L antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence EIVLTQSPGTLSLSPGERATLSCWYQQKPGQAPRLLIYGIPDRFSGSGSGTDF TLTISRVEPEDFAVYYCFGQGTKLEIKR (SEQ ID NO: 194);
and the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto;
the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto and;
the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical, thereto.

14. A V_L antibody binding domain as claimed in claim 12 or claim 13 in which the sequence of FR1 is selected from the group EIVLTQSPGTLSLSPGERATLSC (SEQ ID NO: 195), EIVLTQSPGTLSLSAGERATLSC (SEQ ID NO: 196),

ENVLTQSPGTLSLSPGERATLSC (SEQ ID NO: 197), and ENVLTQSPGTLSLSAGERATLSC (SEQ ID NO: 198).

15. A V_L antibody binding domain as claimed in any one of claims 12 to 14 in which the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188).

16. A V_L antibody binding domain as claimed in any one of claims 12 to 15 in which the sequence of FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189).

17. A V_L antibody binding domain as claimed in any one of claims 12 to 16 in which the sequence of the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190).

18. A V_L antibody binding domain as claimed in any one of claims 12 to 17 in which the sequence of the sequence of FR3 is GIPDRFSGSGSGTDFTLTISRVEPEDFAVYYC (SEQ ID NO: 199) or GIPDRFSGSGSGNDFTLTISRVEPEDFAVYYC (SEQ ID NO: 200).

19. A V_L antibody binding domain as claimed in any one of claims 12 to 18 in which the sequence of the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192).

20. A V_L antibody binding domain as claimed in any one of claims 12 to 19 in which the sequence of the sequence of FR4 is FGQGTKLEIKR (SEQ ID NO: 193).

21. A V_L antibody binding domain as claimed in any one of claims 12 to 20 further comprising a constant domain.

22. A V_L antibody binding domain as claimed in claim 21 in which the constant domain has the sequence TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 93).

23. An antibody comprising a V_H binding domain as claimed in any one of claims 1 to 9 and a V_L binding domain as claimed in any one of claims 12 to 20.

24. An antibody as claimed in claim 23 in which the V_H binding domain and/or the V_L binding domain further comprise a constant domain.

25. An antibody as claimed in claim 24 in which the constant domain of the V_H binding domain has the following sequence:-

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 52); or

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 92); or

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPEEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 53).

26. An antibody as claimed in claim 24 or claim 25 in which the constant domain of the V_L binding domain has the sequence
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC (SEQ ID NO: 93).

27. A V_H antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

(i) the sequence of FR1 is
EVQLQQWGAGLLKPSETLSLTCAVYGYS$X_{18}X_{19}$ (SEQ ID NO: 201)
wherein;
X_{18} is I, L or V
X_{19} is S or T

or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto;

(ii) the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto;

(iii) the sequence of FR2 is WIRQPPGKGLEWX_{20}G (SEQ ID NO: 202) wherein
X_{20} is M or I
or a sequence at least 90% similar, preferably identical, thereto;

(iv) the sequence of CDR2 is HIHX_{21}SGRPTYX_{22}PSLX_{23}S (SEQ ID NO: 98) wherein
X_{21} is F, Y or W
X_{22} is N or D
X_{23} is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto;

(v) the sequence of FR3 is
R$X_{24}X_{25}$ISX_{26}DTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 203) wherein
X_{24} is V or I
X_{25} is S or T
X_{26} is R or K
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto with the proviso that the residue at position 6 must be R or K;

(vi) the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto;

(vii) the sequence of FR4 is WGQGTLVTVSS (SEQ ID NO: 101) or a sequence at least 90% similar, preferably at least 90% identical, thereto;

28. A V_H antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95%, identical to the sequence
EVQLQQWGAGLLKPSETLSLTCAVYGYSX_{18}SWIRQPPGKGLEWIGRVTIS X_{26}DTSKNQFSLKLSSVTAADTAVYYCARWGQGTLVTVSS

(SEQ ID NO: 204);
wherein X_{18} is I, L or V and X_{26} is R or K
and the sequence of CDR1 is SGYSWH (SEQ ID NO: 96) or a sequence at least 80% similar, preferably at least 80% identical, thereto;
the sequence of CDR2 is HIHX$_{21}$SGRPTYX$_{22}$PSLX$_{23}$S (SEQ ID NO: 98) wherein
X_{21} is F, Y or W
X_{22} is N or D
X_{23} is K, L, H, F, R or S
or a sequence at least 90% similar, preferably at least 90% identical, thereto and;
the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

29. A V_H antibody binding domain as claimed in claim 27 or claim 28 in which the sequence of FR1 is selected from the group consisting of EVQLQQWGAGLLKPSETLSLTCAVYGYSIS (SEQ ID NO: 205), EVQLQQWGAGLLKPSETLSLTCAVYGYSIT (SEQ ID NO: 206), EVQLQQWGAGLLKPSETLSLTCAVYGYSLS (SEQ ID NO: 207), EVQLQQWGAGLLKPSETLSLTCAVYGYSLT (SEQ ID NO: 208), EVQLQQWGAGLLKPSETLSLTCAVYGYSVS (SEQ ID NO: 209), and EVQLQQWGAGLLKPSETLSLTCAVYGYSVT (SEQ ID NO: 210).

30. A V_H antibody binding domain as claimed in any one of claims 27 to 29 in which the sequence of CDR1 is SGYSWH (SEQ ID NO: 96).

31. A V_H antibody binding domain as claimed in any one of claims 27 to 30 in which the sequence of FR2 is WIRQPPGKGLEWIG (SEQ ID NO: 133) or WIRQPPGKGLEWMG (SEQ ID NO: 134).

32. A V_H antibody binding domain as claimed in any one of claims 27 to 31 in which the sequence of CDR2 is selected from the group consisting of HIHFSGRPTYNPSLSS (SEQ ID NO: 135), HIHFSGRPTYNPSLKS (SEQ ID NO: 136), HIHFSGRPTYNPSLLS (SEQ ID NO: 137), HIHFSGRPTYNPSLHS (SEQ ID NO: 138), HIHFSGRPTYNPSLFS (SEQ ID NO: 139), HIHFSGRPTYNPSLRS (SEQ ID NO: 140), HIHWSGRPTYNPSLSS (SEQ ID NO: 141), HIHWSGRPTYNPSLKS (SEQ ID NO: 142), HIHWSGRPTYNPSLLS (SEQ ID NO: 143), HIHWSGRPTYNPSLHS (SEQ ID NO: 144), HIHWSGRPTYNPSLFS (SEQ ID NO: 145), HIHWSGRPTYNPSLRS (SEQ ID NO: 146), HIHYSGRPTYNPSLSS (SEQ ID NO: 147), HIHYSGRPTYNPSLKS (SEQ ID NO: 148), HIHYSGRPTYNPSLLS (SEQ ID NO: 149),

HIHYSGRPTYNPSLHS (SEQ ID NO: 150), HIHYSGRPTYNPSLFS (SEQ ID NO: 151), HIHYSGRPTYNPSLRS (SEQ ID NO: 152), HIHFSGRPTYDPSLSS (SEQ ID NO: 153), HIHFSGRPTYDPSLKS (SEQ ID NO: 154), HIHFSGRPTYDPSLLS (SEQ ID NO: 155), HIHFSGRPTYDPSLHS (SEQ ID NO: 156), HIHFSGRPTYDPSLFS (SEQ ID NO: 157), HIHFSGRPTYDPSLRS (SEQ ID NO: 158), HIHWSGRPTYDPSLSS (SEQ ID NO: 159), HIHWSGRPTYDPSLKS (SEQ ID NO: 160), HIHWSGRPTYDPSLLS (SEQ ID NO: 161), HIHWSGRPTYDPSLHS (SEQ ID NO: 162), HIHWSGRPTYDPSLFS (SEQ ID NO: 163), HIHWSGRPTYDPSLRS (SEQ ID NO: 164), HIHYSGRPTYDPSLSS (SEQ ID NO: 165), HIHYSGRPTYDPSLKS (SEQ ID NO: 166), HIHYSGRPTYDPSLLS (SEQ ID NO: 167), HIHYSGRPTYDPSLHS (SEQ ID NO: 168), HIHYSGRPTYDPSLFS (SEQ ID NO: 169), and HIHYSGRPTYDPSLRS (SEQ ID NO: 170).

33. A V_H antibody binding domain as claimed in any one of claims 27 to 32 in which the sequence of FR3 is selected from the group consisting of RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 218), RVTISKDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 219), RVSISRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 220), RVSISKDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 221), RITISRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 222), RITISKDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 223), RISISRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 224), RISISKDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO: 225),.

34. A V_H antibody binding domain as claimed in any one of claims 27 to 33 in which the sequence of CDR3 is KGKGSDDGLNY (SEQ ID NO: 100).

35. A V_H antibody binding domain as claimed in any one of claims 27 to 34 in which the sequence of FR4 is WGQGTLVTVSS (SEQ ID NO: 101).

36. A V_H antibody binding domain as claimed in any one of claims 27 to 35 further comprising a constant domain.

37. A V_H antibody binding domain as claimed in claim 36 in which the constant domain has the sequence:-

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE

VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 52); or

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 92); or

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPEEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 53).

38. A V_L antibody binding domain, the binding domain comprising, in sequence, a first framework region (FR1), a first CDR (CDR1), a second framework region (FR2), a second CDR (CDR2), a third framework region (FR3), a third CDR (CDR3) and a fourth framework region (FR4), wherein:

(i) the sequence of FR1 is EX23VLTQSPATLSLSPGERATLSC (SEQ ID NO: 211) wherein,
X23 is I or N,
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto;

(ii) the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto;

(iii) the sequence of FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189) or a sequence at least 90% similar, preferably at least 90% identical, thereto;

(iv) the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190) or a sequence at least 80% similar, preferably at least 80% identical, thereto;

(v) the sequence of FR3 is GIPDRFSGSGSGX24DFTLTISRLEPEDFAVYYC (SEQ ID NO: 212) wherein,
X24 is T or N
or a sequence at least 90% or at least 95% similar, preferably at least 90% or at least 95% identical, thereto;

(vi) the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical, thereto;

(vii) the sequence of FR4 is FGGGTKVEIKR (SEQ ID NO: 193) or a sequence at least 90% similar, preferably at least 90% identical, thereto.

39. A V_L antibody binding domain, the binding domain comprising a framework sequence and a first, second and third CDR positioned within the framework sequence in which the sequence of the framework regions is at least 90%, preferably at least 95% identical to the sequence EIVLTQSPATLSLSPGERATLSCWYQQKPGQAPRLLIYGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCFGGGTKVEIKR (SEQ ID NO: 213);
and the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188) or a sequence at least 90% similar, preferably at least 90% identical, thereto;
the sequence of CDR2 is DTSNLAS (SEQ ID NO: 140) or a sequence at least 80% similar, preferably at least 80% identical, thereto and;
the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192) or a sequence at least 80% similar, preferably at least 80% identical, thereto.

40. A V_L antibody binding domain as claimed in claim 38 or claim 39 in which the sequence of FR1 is selected from the group EIVLTQSPATLSLSPGERATLSC (SEQ ID NO: 214) or ENVLTQSPATLSLSPGERATLSC (SEQ ID NO: 215).

41. A V_L antibody binding domain as claimed in any one of claims 38 to 40 in which the sequence of CDR1 is SASSSLSYIH (SEQ ID NO: 188).

42. A V_L antibody binding domain as claimed in any one of claims 38 to 41 in which the sequence of FR2 is WYQQKPGQAPRLLIY (SEQ ID NO: 189).

43. A V_L antibody binding domain as claimed in any one of claims 38 to 42 in which the sequence of the sequence of CDR2 is DTSNLAS (SEQ ID NO: 190).

44. A V_L antibody binding domain as claimed in any one of claims 38 to 43 in which the sequence of the sequence of FR3 is

GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC (SEQ ID NO: 216) or GIPDRFSGSGSGNDFTLTISRLEPEDFAVYYC (SEQ ID NO: 217).

45. A V_L antibody binding domain as claimed in any one of claims 38 to 44 in which the sequence of the sequence of CDR3 is FQGSEYPLT (SEQ ID NO: 192).

46. A V_L antibody binding domain as claimed in any one of claims 38 to 45 in which the sequence of the sequence of FR4 is FGGGTKVEIKR (SEQ ID NO: 193).

47. A V_L antibody binding domain as claimed in any one of claims 38 to 46 further comprising a constant domain.

48. A V_L antibody binding domain as claimed in claim 47 in which the constant domain has the sequence TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 93).

49. An antibody comprising a V_H binding domain as claimed in any one of claims 27 to 35 and a V_L binding domain as claimed in any one of claims 38 to 46.

50. An antibody as claimed in claim 49 in which the V_H binding domain and/or the V_L binding domain further comprise a constant domain.

51. An antibody as claimed in claim 50 in which the constant domain of the V_H binding domain has the sequence:-

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 52); or

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF

YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 92); or

ASTKNPDVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPEEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 53).

52. An antibody as claimed in claim 50 or claim 51 in which the constant domain of the V_L binding domain has the sequence TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 93).

53. An antibody comprising two light chains and two heavy chains in which the sequence of the light chains is as shown in SEQ ID NO: 7 and the sequence of the heavy chains is as shown in SEQ ID NO: 50.

54. An antibody comprising two light chains and two heavy chains in which the sequence of the light chains is as shown in SEQ ID NO: 7 and the sequence of the heavy chains is as shown in SEQ ID NO: 94.

55. An antibody as claimed in any one of claims 23 to 26 or 49 to 54 in which the antibody has a reduced level of fucose.

56. A method of treating cancer in a subject wherein the cancer is selected from the group consisting of colorectal, ovarian, pancreatic, prostatic and lung, the method comprising administering to the subject a therapeutically effective quantity of a binding domain or antibody according to any one of claims 1 to 55.

57. A method of detecting the presence of cancer cells in a sample, the method comprising contacting the cell sample with a binding domain or antibody according to any one of claims 1 to 55 and detecting binding of the binding domain or antibody to the cells.