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WO 2019/147152 A1 **US 20190276541 A1**

(58) Field of Search:
Other: **WPI, EPODOC, Patent Fulltext, BIOSIS, MEDLINE, CAS ONLINE**

(54) Title of the Invention: **Humanised antibodies or functional fragments thereof against tumour antigens**
Abstract Title: **An antibody specific for the tumour antigen sialy-Tn (STn)**

(57) An antibody or fragment thereof, wherein the antibody comprises a heavy chain variable region (VH), comprising complementarity determining regions (CDR) selected from SEQ ID NOs 1 to 24 or 49 to 88. The antibody also comprises a light chain variable region (LH), comprising CDR regions selected from SEQ ID NOs 25 to 48 or 89 to 128. The antibody can comprise a humanised heavy and light chain framework region. CDR-3 of the light chain can be mutated. CDR-2 of the heavy chain can be mutated. The antibody can bind the siayl-Tn (STn) antigen and a group of glycans terminated by alpha 2,6-linked sialic acids. The glycans terminated by alpha 2,6-linked sialic acids can comprise STn, 2,6-sialyl T, di-sialyl T, or 2, 6-sialolactosamine. The antibody can be in the form of a single chain variable fragment (ScFv), monoclonal antibody, chimeric antibody, humanized antibody, bispecific antibody, an antibody drug conjugate, or CAR T-cell. A further aspect is a method of detecting a tumour in a subject using the antibody. Further aspects are expression vectors and host cells for producing the antibody.

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Fig 1

SEQ ID NO	Variant (VH or VL sequence)	SEQ ID NO	Variant (VH or VL sequence)
1	mAb_VH-v1	26	mAb_VL-v2
2	mAb_VH-v2	27	mAb_VL-v3
3	mAb_VH-v3	28	mAb_VL-v4
4	mAb_VH-v4	29	mAb_VL-v5
5	mAb_VH-v5	30	mAb_VL-v6
6	mAb_VH-v6	31	mAb_VL-v7
7	mAb_VH-v7	32	mAb_VL-v8
8	mAb_VH-v8	33	mAb_VL-v9
9	mAb_VH-v9	34	mAb_VL-v10
10	mAb_VH-v10	35	mAb_VL-v11
11	mAb_VH-v11	36	mAb_VL-v12
12	mAb_VH-v12	37	mAb_VL-v13
13	mAb_VH-v13	38	mAb_VL-v14
14	mAb_VH-v14	39	mAb_VL-v15
15	mAb_VH-v15	40	mAb_VL-v16
16	mAb_VH-v16	41	mAb_VL-v17
17	mAb_VH-v17	42	mAb_VL-v18
18	mAb_VH-v18	43	mAb_VL-v19
19	mAb_VH-v19	44	mAb_VL-v20
20	mAb_VH-v20	45	mAb_VL-v21
21	mAb_VH-v21	46	mAb_VL-v22
22	mAb_VH-v22	47	mAb_VL-v23
23	mAb_VH-v23	48	mAb_VL-v24
24	mAb_VH-v24	49	mAb_VH-v25
25	mAb_VL-v1	50	mAb_VH-v26

51	mAb_VH-v27	76	mAb_VH-v52
52	mAb_VH-v28	77	mAb_VH-v53
53	mAb_VH-v29	78	mAb_VH-v54
54	mAb_VH-v30	79	mAb_VH-v55
55	mAb_VH-v31	80	mAb_VH-v56
56	mAb_VH-v32	81	mAb_VH-v57
57	mAb_VH-v33	82	mAb_VH-v58
58	mAb_VH-v34	83	mAb_VH-v59
59	mAb_VH-v35	84	mAb_VH-v60
60	mAb_VH-v36	85	mAb_VH-v61
61	mAb_VH-v37	86	mAb_VH-v62
62	mAb_VH-v38	87	mAb_VH-v63
63	mAb_VH-v39	88	mAb_VH-v64
64	mAb_VH-v40	89	mAb_VL-v25
65	mAb_VH-v41	90	mAb_VL-v26
66	mAb_VH-v42	91	mAb_VL-v27
67	mAb_VH-v43	92	mAb_VL-v28
68	mAb_VH-v44	93	mAb_VL-v29
69	mAb_VH-v45	94	mAb_VL-v30
70	mAb_VH-v46	95	mAb_VL-v31
71	mAb_VH-v47	96	mAb_VL-v32
72	mAb_VH-v48	97	mAb_VL-v33
73	mAb_VH-v49	98	mAb_VL-v34
74	mAb_VH-v50	99	mAb_VL-v35
75	mAb_VH-v51	100	mAb_VL-v36

101	mAb_VL-v37
102	mAb_VL-v38
103	mAb_VL-v39
104	mAb_VL-v40
105	mAb_VL-v41
106	mAb_VL-v42
107	mAb_VL-v43
108	mAb_VL-v44
109	mAb_VL-v45
110	mAb_VL-v46
111	mAb_VL-v47
112	mAb_VL-v48
113	mAb_VL-v49
114	mAb_VL-v50
115	mAb_VL-v51
116	mAb_VL-v52
117	mAb_VL-v53
118	mAb_VL-v54
119	mAb_VL-v55
120	mAb_VL-v56
121	mAb_VL-v57
122	mAb_VL-v58
123	mAb_VL-v59
124	mAb_VL-v60
125	mAb_VL-v61
126	mAb_VL-v62
127	mAb_VL-v63
128	mAb_VL-v64
129	mAb_VH-v65
130	mAb_VH-v66
131	mAb_VH-v67
132	mAb_VH-v68
133	mAb_VH-v69
134	mAb_VH-v70
135	mAb_VH-v71
135	mAb_VH-v72
137	mAb_VH-v73
138	mAb_VH-v74
139	mAb_VH-v75
140	mAb_VH-v76
141	mAb_VH-v77
142	mAb_VH-v78
143	mAb_VH-v79
144	mAb_VH-v80
145	mAb_VH-v81
146	mAb_VH-v82
147	mAb_VH-v83
148	mAb_VH-v84
149	mAb_VH-v85
150	mAb_VL-v65
151	mAb_VL-v66
152	mAb_VL-v67
153	mAb_VL-v68
154	mAb_VL-v69
155	mAb_VL-v70
156	mAb_VL-v71

157 mAb_VL-v72
158 mAb_VL-v73
159 mAb_VL-v74
160 mAb_VL-v75
161 mAb_VL-v76
162 mAb_VL-v77
163 mAb_VL-v78
164 mAb_VL-v79
165 mAb_VL-v80
166 mAb_VL-v81
167 mAb_VL-v82
168 mAb_VL-v83
169 mAb_VL-v84
170 mAb_VL-v85
171 mAb_VL-v86
172 mAb_VL-v87
173 mAb_VL-v88

Fig. 2

Figure showing amino acid sequences of the variable chains (VH and VL) of certain humanised variants

Clone	**Amino acids of VH**	**Amino acids of VL**
Parental L2A5	EVQLQESGPGLVKPSQSLSLTCSVIGYSITSGYYWNWIRQ FPGNKLEWMGSINYDGSNIYNPSLKDRISITRDTSKNQFFL KLNSVTTEDTATYYCARGGDYWGQGTSVTVSS	DIVLTQTPAIMSASPGEKVTLTCSASSSVSYMHWFQQK SGTSPKRWIYDTSKLASGVPARFSGSGSGTSYSLTISS MEAEDAAAYYCQQWSSDPPMLTFGAGTKLELK
CBS-V1	QVQLQESGPGLVKPSQTLSLTCTVIGYSITSGYYWNWIRQ PPGKGLEWIGSINYDGSNIYNPSLKSRVTISRDTSKNQFSL KLSSVTAADTAVYYCARGGDYWGQGTTVTVSS	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQK PGQAPRRWIYDTSKRATGVPARFSGSGSGTDYTLTISS LEPEDFAAYYCQQWSSDPPMLTFGGGTKLEIK
CBS-V3	QVQLQESGPGLVKPSQTLSLTCSVIGYSITSGYYWNWIRQ PPGKGLEWIGSINYDGSNIYNPSLKDRVTISRDTSKNQFSL KLSSVTAADTAVYYCARGGDYWGQGTTVTVSS	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQK PGQAPRRWIYDTSKRATGVPARFSGSGSGTDYTLTISS LEPEDFAAYYCQQWSSDPPMLTFGGGTKLEIK
CBS-V2	QVQLQESGPGLVKPSQTLSLTCSVIGYSITSGYYWNWIRQ PPGKGLEWIGSINYDGSNIYNPSLKDRVTISRDTSKNQFSL KLSSVTAADTAVYYCARGGDYWGQGTTVTVSS	DIVLTQSPAILSLSPGERATLSCSASSSVSYMHWFQQKS GQAPKRWIYDTSKLASGVPARFSGSGSGTDYTLTISSLE AEDFAAYYCQQWSSDPPMLTFGGGTKLEIK

Fig. 3a

Humanized L2A5 variants

Amino acid sequence alignment comparing the heavy chain (VH) of the parental antibody (L2A5) to the humanized variants described in the patent application.

Amino acid sequence alignment:

```
Parental      EVQLQESGPGLVKPSQSLSLTCSVIGYSITSGYYWNWIRQFPGNKLEWMGSINYDGSNIY   60
CBS-V1_VH     QVQLQESGPGLVKPSQTLSLTCTVIGYSITSGYYWNWIRQPPGKGLEWIGSINYDGSNIY   60
CBS-V3_VH     QVQLQESGPGLVKPSQTLSLTCSVIGYSITSGYYWNWIRQPPGKGLEWIGSINYDGSNIY   60
CBS-V2_VH     QVQLQESGPGLVKPSQTLSLTCSVIGYSITSGYYWNWIRQPPGKGLEWIGSINYDGSNIY   60
              :****************:*****:****************** **: ***:***********

Parental      NPSLKDRISITRDTSKNQFFLKLNSVTTEDTATYYCARGGDYWGQGTSVTVSS     113
CBS-V1_VH     NPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCARGGDYWGQGTTVTVSS     113
CBS-V3_VH     NPSLKDRVTISRDTSKNQFSLKLSSVTAADTAVYYCARGGDYWGQGTTVTVSS     113
CBS-V2_VH     NPSLKDRVTISRDTSKNQFSLKLSSVTAADTAVYYCARGGDYWGQGTTVTVSS     113
              *****.*:::*******  ***.***:  ***.***************:*****
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Fig. 3b

Humanized L2A5 variants

Amino acid sequence alignment comparing the light chain (VL) of the parental antibody (L2A5) to the humanized variants described in the patent application.

Amino acid sequence alignment:

```
Parental      DIVLTQTPAIMSASPGEKVTLTCSASSSVSYMHWFQQKSGTSPKRWIYDTSKLASGVPAR   60
CBS-V1_VL     EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRRWIYDTSKRATGVPAR   60
CBS-V3_VL     EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRRWIYDTSKRATGVPAR   60
CBS-V2_VL     DIVLTQSPAILSLSPGERATLSCSASSSVSYMHWFQQKSGQAPKRWIYDTSKLASGVPAR   60
              :*****:** :* ****:.**:***************** * :*:******** *:*****

Parental      FSGSGSGTSYSLTISSMEAEDAAAYYCQQWSSDPPMLTFGAGTKLELK  108
CBS-V1_VL     FSGSGSGTDYTLTISSLEPEDFAAYYCQQWSSDPPMLTFGGGTKLEIK  108
CBS-V3_VL     FSGSGSGTDYTLTISSLEPEDFAAYYCQQWSSDPPMLTFGGGTKLEIK  108
CBS-V2_VL     FSGSGSGTDYTLTISSLEAEDFAAYYCQQWSSDPPMLTFGGGTKLEIK  108
              ********.*:*****:* ** ******************.*****:*
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Legend for Figs 3a and 3b:

* (Asterix) positions with a single, fully conserved residue.

: (colon) positions with conservation between amino acid groups of similar properties.

. (period) positions with conservation between amino acid groups of weakly similar properties.

Fig. 4

Humanised variants – amino acid sequence of V_H heavy chain. The H-CDR1, H-CDR2 and H-CDR3 sequences are highlighted in bold.

HUMANIZED VARIANTS - VH CHAIN (CDRs in bold)				
Name in patent	Seq ID	H-CDR1	H-CDR2	H-CDR3
mAb_VH-v1	SEQ ID No 1	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v2	SEQ ID No 2	QVQLQESGPGLVKPSQTLSLTCSVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKD**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v3	SEQ ID No 3	QVQLQESGPGLVKPSQTLSLTCSVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKD**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v4	SEQ ID No 4	EVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWMG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v5	SEQ ID No 5	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v6	SEQ ID No 6	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v7	SEQ ID No 7	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v8	SEQ ID No 8	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v9	SEQ ID No 9	EVQLKESGPALVKPTQTLTLTCTVIGYSIT**SGYYWN**WIRQPPGKALEWMG**SINYDGSNIYNPSLKT**RLTISRDTSKNQFVLTMTNMDPVDTATYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v10	SEQ ID No 10	EVQLKESGPALVKPTQTLTLTCTVIGYSIT**SGYYWN**WIRQPPGKALEWMG**SINYDGSNIYNPSLKT**RLTISRDTSKNQFVLTMTNMDPVDTATYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v11	SEQ ID No 11	EVQLKESGPALVKPTQTLTLTCTVIGYSIT**SGYYWN**WIRQPPGKALEWMG**SINYDGSNIYNPSLKT**RLTISRDTSKNQFVLTMTNMDPVDTATYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v12	SEQ ID No 12	EVQLKESGPALVKPTQTLTLTCTVIGYSIT**SGYYWN**WIRQPPGKALEWMG**SINYDGSNIYNPSLKT**RLTISRDTSKNQFVLTMTNMDPVDTATYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v13	SEQ ID No 13	QVTLKESGPALVKPTQTLTLTCTVIGYSIT**SGYYWN**WIRQPPGKALEWIG**SINYDGSNIYNPSLKT**RLTISRDTSKNQFVLTMTNMDPVDTATYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v14	SEQ ID No 14	QVTLKESGPALVKPTQTLTLTCTVIGYSIT**SGYYWN**WIRQPPGKALEWIG**SINYDGSNIYNPSLKT**RLTISRDTSKNQFVLTMTNMDPVDTATYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v15	SEQ ID No 15	QVTLKESGPALVKPTQTLTLTCTVIGYSIT**SGYYWN**WIRQPPGKALEWIG**SINYDGSNIYNPSLKT**RLTISRDTSKNQFVLTMTNMDPVDTATYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v16	SEQ ID No 16	QVTLKESGPALVKPTQTLTLTCTVIGYSIT**SGYYWN**WIRQPPGKALEWIG**SINYDGSNIYNPSLKT**RLTISRDTSKNQFVLTMTNMDPVDTATYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v17	SEQ ID No 17	EVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWMG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v18	SEQ ID No 18	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v19	SEQ ID No 19	QVQLQESGPGLVKPSQTLSLTCSVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKD**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v20	SEQ ID No 20	EVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWMG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v21	SEQ ID No 21	EVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWMG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v22	SEQ ID No 22	EVQLQESGPGLVKPSQTLSLTCSVIGYSIT**SGYYWN**WIRQPPGKGLEWMG**SINYDGSNIYNPSLKD**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTSVTVSS		
mAb_VH-v23	SEQ ID No 23	EVQLQESGPGLVKPSQTLSLTCSVIGYSIT**SGYYWN**WIRQPPGKGLEWMG**SINYDGSNIYNPSLKD**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTSVTVSS		
mAb_VH-v24	SEQ ID No 24	EVQLQESGPGLVKPSQTLSLTCSVIGYSIT**SGYYWN**WIRQPPGKGLEWMG**SINYDGSNIYNPSLKD**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTSVTVSS		

Fig. 5

Humanised variants – amino acid sequence of V_L light chain. The L-CDR1, L-CDR2 and L-CDR3 sequences are highlighted in bold.

HUMANIZED VARIANTS - VL CHAIN (CDRs in bold)				
Name in patent	Seq ID	H-CDR1	H-CDR2	H-CDR3
mAb_VL-v1	SEQ ID No 25	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v2	SEQ ID No 26	DIVLTQSPAILSLSPGERATLSC**SASSSVSYMH**WFQQKSGQAPKRWIY**DTSKLAS**GVPARFSGSGSGTDYTLTISSLEAEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v3	SEQ ID No 27	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v4	SEQ ID No 28	DIQMTQSPSSLSASVGDRVTITC**RASSSVSYMH**WFQQKPGKAPKRLIY**DTSKLAS**GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v5	SEQ ID No 29	DIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKLAS**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v6	SEQ ID No 30	EIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKRAT**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v7	SEQ ID No 31	DIQLTQSPSSLSASVGDRVTITC**RASSSVSYMH**WFQQKPGKAPKRLIY**DTSKLAS**GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v8	SEQ ID No 32	DIQMTQSPSSLSASVGDRVTITC**RASSSVSYMH**WFQQKPGKAPKRLIY**DTSKLAS**GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v9	SEQ ID No 33	DIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKLAS**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v10	SEQ ID No 34	EIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKRAT**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v11	SEQ ID No 35	DIQLTQSPSSLSASVGDRVTITC**RASSSVSYMH**WFQQKPGKAPKRLIY**DTSKLAS**GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v12	SEQ ID No 36	DIQMTQSPSSLSASVGDRVTITC**RASSSVSYMH**WFQQKPGKAPKRLIY**DTSKLAS**GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v13	SEQ ID No 37	DIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKLAS**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v14	SEQ ID No 38	EIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKRAT**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v15	SEQ ID No 39	DIQLTQSPSSLSASVGDRVTITC**RASSSVSYMH**WFQQKPGKAPKRLIY**DTSKLAS**GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v16	SEQ ID No 40	DIQMTQSPSSLSASVGDRVTITC**RASSSVSYMH**WFQQKPGKAPKRLIY**DTSKLAS**GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v17	SEQ ID No 41	DIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKLAS**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v18	SEQ ID No 42	DIVLTQSPAILSLSPGERATLSC**SASSSVSYMH**WFQQKSGQAPKRWIY**DTSKLAS**GVPARFSGSGSGTDYTLTISSLEAEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v19	SEQ ID No 43	EIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKRAT**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v20	SEQ ID No 44	DIQLTQSPSSLSASVGDRVTITC**RASSSVSYMH**WFQQKPGKAPKRLIY**DTSKLAS**GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v21	SEQ ID No 45	EIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKRAT**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v22	SEQ ID No 46	EIVLTQSPATLSLSPGERATLSC**RASSSVSYMH**WFQQKPGQAPRRLIY**DTSKRAT**GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v23	SEQ ID No 47	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK		
mAb_VL-v24	SEQ ID No 48	DIVLTQSPAILSLSPGERATLSC**SASSSVSYMH**WFQQKSGQAPKRWIY**DTSKLAS**GVPARFSGSGSGTDYTLTISSLEAEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK		

Fig. 6a

Affinity-matured variants – amino acid sequence of V_H heavy chain. The H-CDR1, H-CDR2 and H-CDR3 sequences are highlighted in bold.

AFFINITY-MATURED VARIANTS - VH CHAIN				
Name in patent	Seq ID	H-CDR1	H-CDR2	H-CDR3
mAb_VH-v25	SEQ ID No 49	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v26	SEQ ID No 50	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGTNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v27	SEQ ID No 51	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v28	SEQ ID No 52	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**AGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGHP**WGQGTTVTVSS		
mAb_VH-v29	SEQ ID No 53	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYNWN**WIRQPPGKGLEWIG**SINHDGSNFYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGAY**WGQGTTVTVSS		
mAb_VH-v30	SEQ ID No 54	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGRD**WGQGTTVTVSS		
mAb_VH-v31	SEQ ID No 55	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v32	SEQ ID No 56	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINFDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v33	SEQ ID No 57	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**TGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGEP**WGQGTTVTVSS		
mAb_VH-v34	SEQ ID No 58	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**QGYYWN**WIRQPPGKGLEWIG**SINYDGLNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGRD**WGQGTTVTVSS		
mAb_VH-v35	SEQ ID No 59	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYNWN**WIRQPPGKGLEWIG**SINYDGSNFYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGNL**WGQGTTVTVSS		
mAb_VH-v36	SEQ ID No 60	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**AGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v37	SEQ ID No 61	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**QGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v38	SEQ ID No 62	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGTNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v39	SEQ ID No 63	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**QGYYWN**WIRQPPGKGLEWIG**SINYNGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v40	SEQ ID No 64	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQTPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v41	SEQ ID No 65	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**TGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v42	SEQ ID No 66	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**QGYYWN**WIRQPPGKGLEWIG**SINYDGANIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v43	SEQ ID No 67	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQSPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v44	SEQ ID No 68	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SSHYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v45	SEQ ID No 69	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v46	SEQ ID No 70	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYNWN**WIRQPPGKGLEWIG**SINPDGSNFYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v47	SEQ ID No 71	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v48	SEQ ID No 72	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v49	SEQ ID No 73	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYNGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS		
mAb_VH-v50	SEQ ID No 74	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYSWN**WIRQPPGKGLEWIG**SINHDGSNFYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGNS**WGQGTTVTVSS		

mAb_VH-v51	SEQ ID No 75	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS
mAb_VH-v52	SEQ ID No 76	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**KGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS
mAb_VH-v53	SEQ ID No 77	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYSWN**WIRQPPGKGLEWIG**SINSDGSNFYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS
mAb_VH-v54	SEQ ID No 78	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS
mAb_VH-v55	SEQ ID No 79	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGNS**WGQGTTVTVSS
mAb_VH-v56	SEQ ID No 80	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGTNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGRN**WGQGTTVTVSS
mAb_VH-v57	SEQ ID No 81	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS
mAb_VH-v58	SEQ ID No 82	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SISYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS
mAb_VH-v59	SEQ ID No 83	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQTPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS
mAb_VH-v60	SEQ ID No 84	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS
mAb_VH-v61	SEQ ID No 85	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**QGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDP**WGQGTTVTVSS
mAb_VH-v62	SEQ ID No 86	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGTNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS
mAb_VH-v63	SEQ ID No 87	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**NGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAM**GGAY**WGQGTTVTVSS
mAb_VH-v64	SEQ ID No 88	QVQLQESGPGLVKPSQTLSLTCTVIGQSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGHP**WGQGTTVTVSS

Fig. 6b

Affinity matured variants – amino acid sequence of V_L light chain. The L-CDR1, L-CDR2 and L-CDR3 sequences are highlighted in bold.

AFFINITY-MATURED VARIANTS - VL CHAIN (CDRs in bold)					
Name in patent	Seq ID		H-CDR1	H-CDR2	H-CDR3
mAb_VL-v25	SEQ ID No 89	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK
mAb_VL-v26	SEQ ID No 90	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK
mAb_VL-v27	SEQ ID No 91	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWTADPPMLT**	FGGGTKLEIK
mAb_VL-v28	SEQ ID No 92	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK
mAb_VL-v29	SEQ ID No 93	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK
mAb_VL-v30	SEQ ID No 94	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK
mAb_VL-v31	SEQ ID No 95	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK
mAb_VL-v32	SEQ ID No 96	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK
mAb_VL-v33	SEQ ID No 97	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK
mAb_VL-v34	SEQ ID No 98	EIVLTQSPATLSLSPGERATLSC	**SASSSVSYMH**	WFQQKPGQAPRRWIY	**DTSKRAT**	GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC	**QQWSSDPPMLT**	FGGGTKLEIK

mAb_VL-v35	SEQ ID No 99	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v36	SEQ ID No 100	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v37	SEQ ID No 101	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v38	SEQ ID No 102	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v39	SEQ ID No 103	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v40	SEQ ID No 104	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSIRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWTAHPPMLT**FGGGTKLEIK
mAb_VL-v41	SEQ ID No 105	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v42	SEQ ID No 106	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v43	SEQ ID No 107	EIVLTQSPATLSLSPGERATLSC**SASSTVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWTDDPPMLT**FGGGTKLEIK
mAb_VL-v44	SEQ ID No 108	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v45	SEQ ID No 109	EIVLTQSPATLSLSPGERATLSC**SASSSVSHMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWTVDPPMLT**FGGGTKLEIK
mAb_VL-v46	SEQ ID No 110	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v47	SEQ ID No 111	EIVLTQSPATLSLSPGERATLSC**SASSSVAYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWTADPPMLT**FGGGTKLEIK
mAb_VL-v48	SEQ ID No 112	EIVLTQSPATLSLSPGERATLSC**SASSTVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWTADPPMLT**FGGGTKLEIK
mAb_VL-v49	SEQ ID No 113	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v50	SEQ ID No 114	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v51	SEQ ID No 115	EIVLTQSPATLSLSPGERATLSC**SASQSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWTADPPMLT**FGGGTKLEIK
mAb_VL-v52	SEQ ID No 116	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v53	SEQ ID No 117	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v54	SEQ ID No 118	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWDADRRMLT**FGGGTKLEIK
mAb_VL-v55	SEQ ID No 119	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v56	SEQ ID No 120	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v57	SEQ ID No 121	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTQLEIK
mAb_VL-v58	SEQ ID No 122	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v59	SEQ ID No 123	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWTEDPPMLT**FGGGTKLEIK
mAb_VL-v60	SEQ ID No 124	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**LGGGTKLEIK
mAb_VL-v61	SEQ ID No 125	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v62	SEQ ID No 126	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v63	SEQ ID No 127	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK
mAb_VL-v64	SEQ ID No 128	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDPPMLT**FGGGTKLEIK

Fig. 7

Affinity matured clones: variant, sequence identity number, and amino acid sequence of variable heavy and light chains.

Clone		AA of VH		AA of VL
mAbv1	**SEQ ID NO 1**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITSG YYWNWIRQPPGKGLEWIGSINYDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDYWGQGTTVTVSS	**SEQ ID NO 25**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v25	**SEQ ID NO 49**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITSG YYWNWIRQPPGKGLEWIGSINYDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDPWGQGTTVTVSS	**SEQ ID NO 89**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v26	**SEQ ID NO 50**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITSG YYWNWIRQPPGKGLEWIGSINYDGTNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDPWGQGTTVTVSS	**SEQ ID NO 90**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v27	**SEQ ID NO 51**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITSG YYWNWIRQPPGKGLEWIGSINYDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDYWGQGTTVTVSS	**SEQ ID NO 91**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWT ADPPMLTFGGGTKLEIK
mAb v28	**SEQ ID NO 52**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITA GYYWNWIRQPPGKGLEWIGSINYDGSNIYN PSLKSRVTISRDTSKNQFSLKLSSVTAADTAV YYCARGGHPWGQGTTVTVSS	**SEQ ID NO 92**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v29	**SEQ ID NO 53**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITSG YNWNWIRQPPGKGLEWIGSINHDGSNFYN PSLKSRVTISRDTSKNQFSLKLSSVTAADTAV YYCARGGAYWGQGTTVTVSS	**SEQ ID NO 93**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v30	**SEQ ID NO 54**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITSG YYWNWIRQPPGKGLEWIGSINYDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGRDWGQGTTVTVSS	**SEQ ID NO 94**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK

mAb v31	**SEQ ID NO 55**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITSG YYWNWIRQPPGKGLEWIGSINYDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDPWGQGTTVTVSS	**SEQ ID NO 95**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v32	**SEQ ID NO 56**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITSG YYWNWIRQPPGKGLEWIGSINFDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDPWGQGTTVTVSS	**SEQ ID NO 96**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
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mAb v34	**SEQ ID NO 58**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITQG YYWNWIRQPPGKGLEWIGSINYDGLNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGRDWGQGTTVTVSS	**SEQ ID NO 98**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v35	**SEQ ID NO 59**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITSG YNWNWIRQPPGKGLEWIGSINYDGSNFYN PSLKSRVTISRDTSKNQFSLKLSSVTAADTAV YYCARGGNLWGQGTTVTVSS	**SEQ ID NO 99**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v36	**SEQ ID NO 60**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITA GYYWNWIRQPPGKGLEWIGSINYDGSNIYN PSLKSRVTISRDTSKNQFSLKLSSVTAADTAV YYCARGGDPWGQGTTVTVSS	**SEQ ID NO 100**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
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mAb v38	**SEQ ID NO 62**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITSG YYWNWIRQPPGKGLEWIGSINYDGTNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDPWGQGTTVTVSS	**SEQ ID NO 102**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
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mAb v40	**SEQ ID NO 64**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITSG YYWNWIRQTPGKGLEWIGSINYDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDYWGQGTTVTVSS	**SEQ ID NO 104**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSIRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWT AHPPMLTFGGGTKLEIK
mAb v41	**SEQ ID NO 65**	QVQLQESGPGLVKPSQTLSLTCTVIGQSITTG YYWNWIRQPPGKGLEWIGSINYDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDPWGQGTTVTVSS	**SEQ ID NO 105**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
mAb v42	**SEQ ID NO 66**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITQG YYWNWIRQPPGKGLEWIGSINYDGANIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDYWGQGTTVTVSS	**SEQ ID NO 106**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
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mAb v46	**SEQ ID NO 70**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITSG YNWNWIRQPPGKGLEWIGSINPDGSNFYN PSLKSRVTISRDTSKNQFSLKLSSVTAADTAV YYCARGGDYWGQGTTVTVSS	**SEQ ID NO 110**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
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mAb v61	**SEQ ID NO 85**	QVQLQESGPGLVKPSQTLSLTCTVIGYSITQG YYWNWIRQPPGKGLEWIGSINYDGSNIYNP SLKSRVTISRDTSKNQFSLKLSSVTAADTAVY YCARGGDPWGQGTTVTVSS	**SEQ ID NO 125**	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRR WIYDTSKRATGVPARFSGSGSGTDYTLTISSLEPEDFAAYYCQQWS SDPPMLTFGGGTKLEIK
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Fig 8

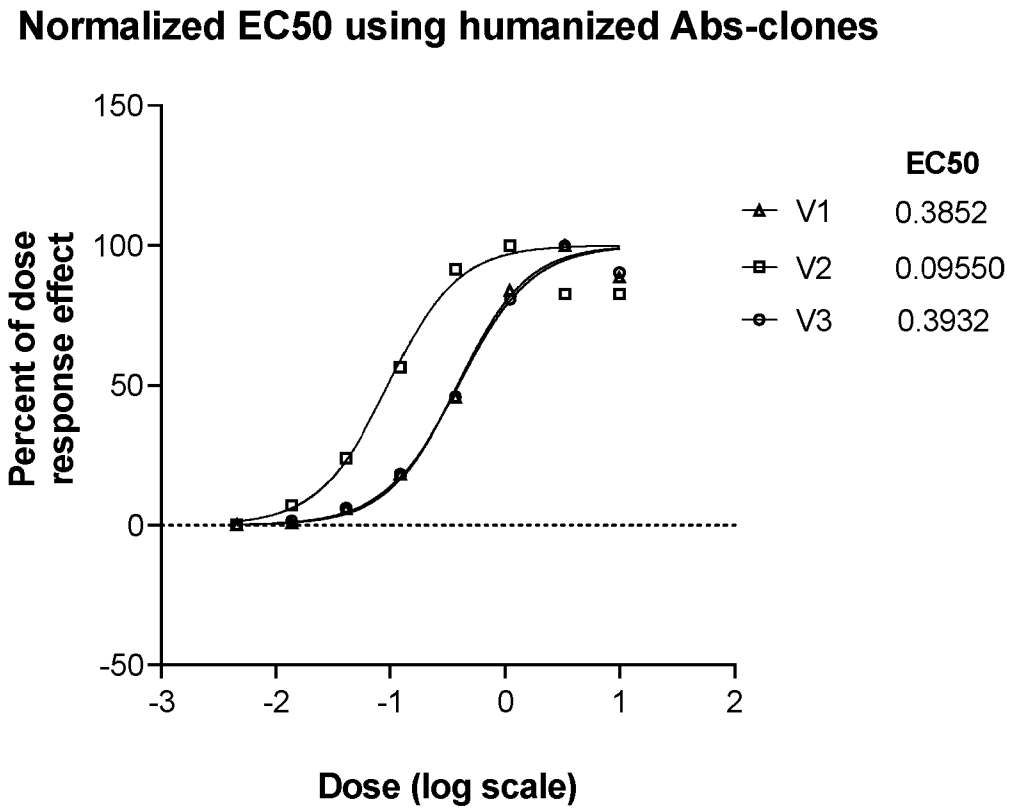


Figure 8. shows EC50 graph of V1-3 humanized clones screened against BSM mucin by ELISA. EC50 was plotted as percentage of dose response effect (y-axis) versus the antibody dose in log scale (x-axis). Clone V2 has a lower EC50 compared to the V1 and V3 clones.

Fig 9

Binding to STn+ cell lines

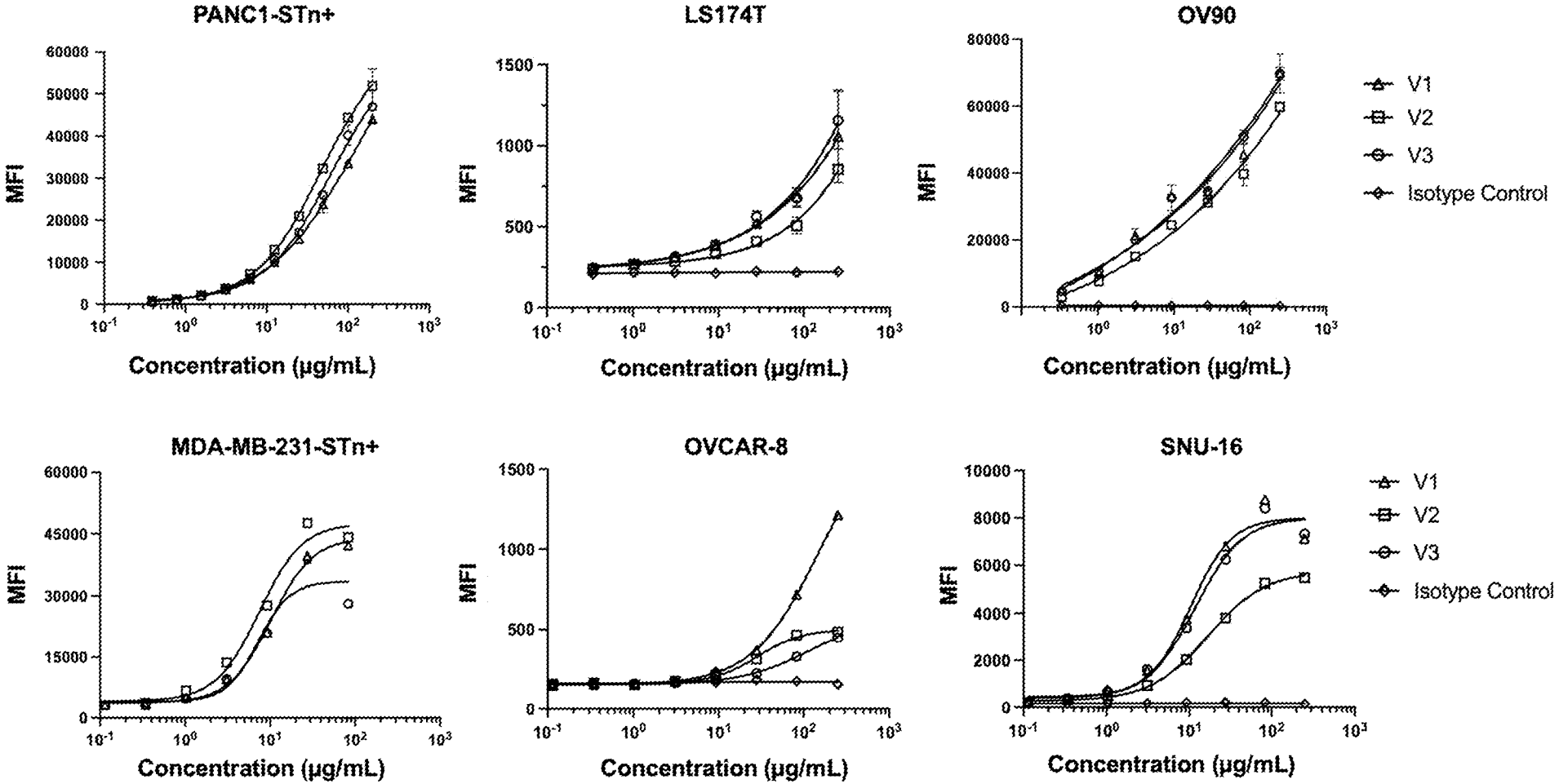


Figure 9. shows binding of humanized antibody clones using six cancer cell lines, expressing different level of STn+. The three variants shown (V1, V2 and V3) maintained high binding to different cell lines expressing STn.

Fig 10

NMR-analysis

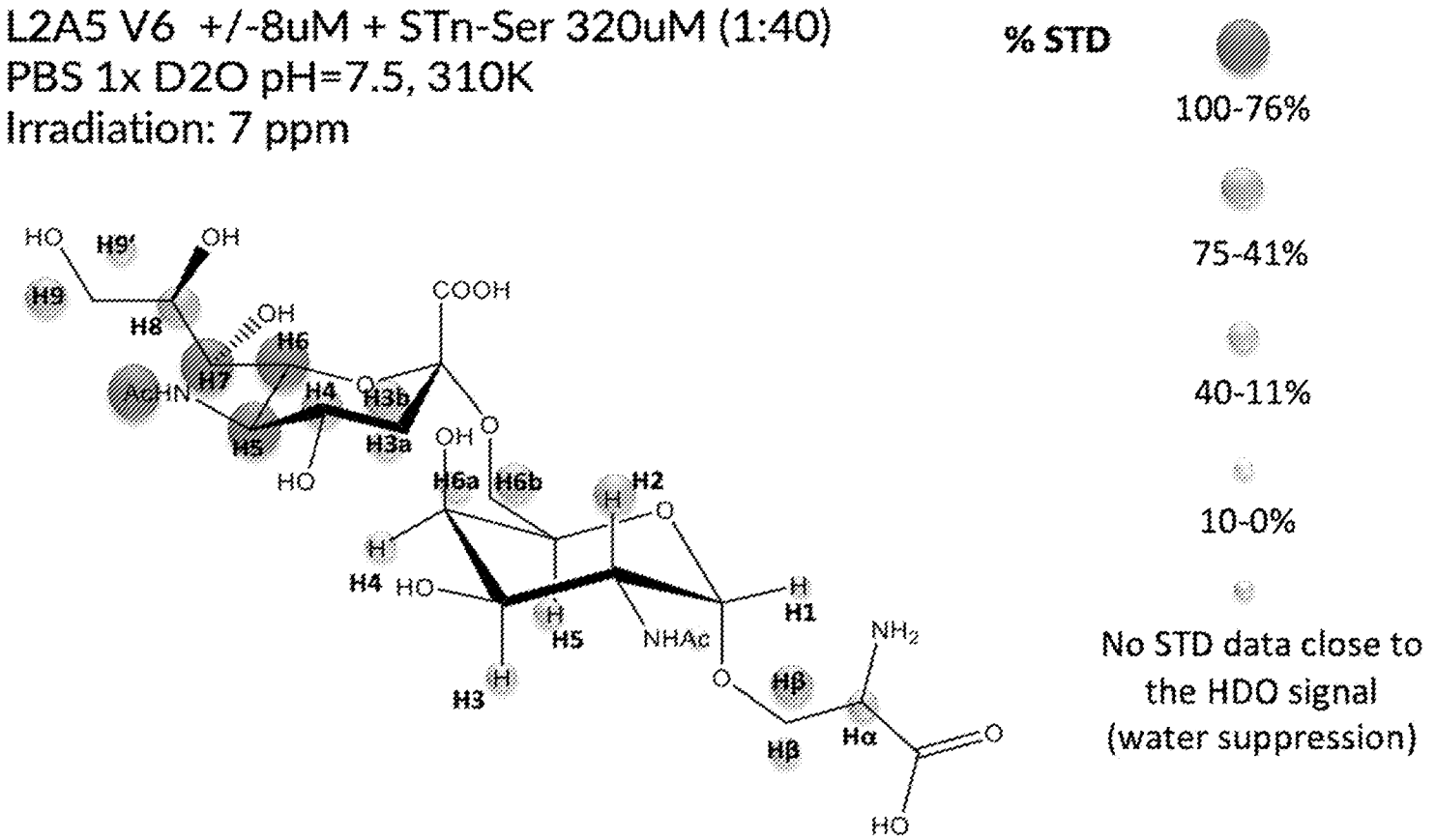


Figure 10. NMR studies can identify the points of contact/interaction between the antibody and glycan molecules. In this experiment, using humanized variant 6 (V6), it was observed that the antibody binds mostly to the sialic acid portion of STn (orange circles), but also interacts with the GalNac portion (lower right side of the scheme)

Fig.11

Surface Plasmon Resonance (SPR)

Ligand	Analyte	Chi² (RU²)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
BSA-STn-serine	CBS-PC	NA	NA	NA	NA	NA
BSA-STn-serine	CBS-V1	2.55E-01	1.74E+05	5.53E-03	3.17E-08	17.8
BSA-STn-serine	CBS-V3	3.30E-01	1.71E+05	5.68E-03	3.32E-08	21.3
BSA-STn-serine	CBS-V2	1.35E-01	1.32E+05	5.19E-03	3.94E-08	13.8

Figure 11. shows SPR can be used to measure the affinity and kinetic parameters of different anti-STn antibodies. BSA-STn was immobilized to the chip and the antibodies were injected over the surface as association phase. CellmAbs CBS humanized clones (V1-V3) showed a high affinity towards BSA-STn, with KD ranging from 29nM to 39nM. No interaction was detected between the positive control clone and BSA-STn.

Fig.12

Glycan-array

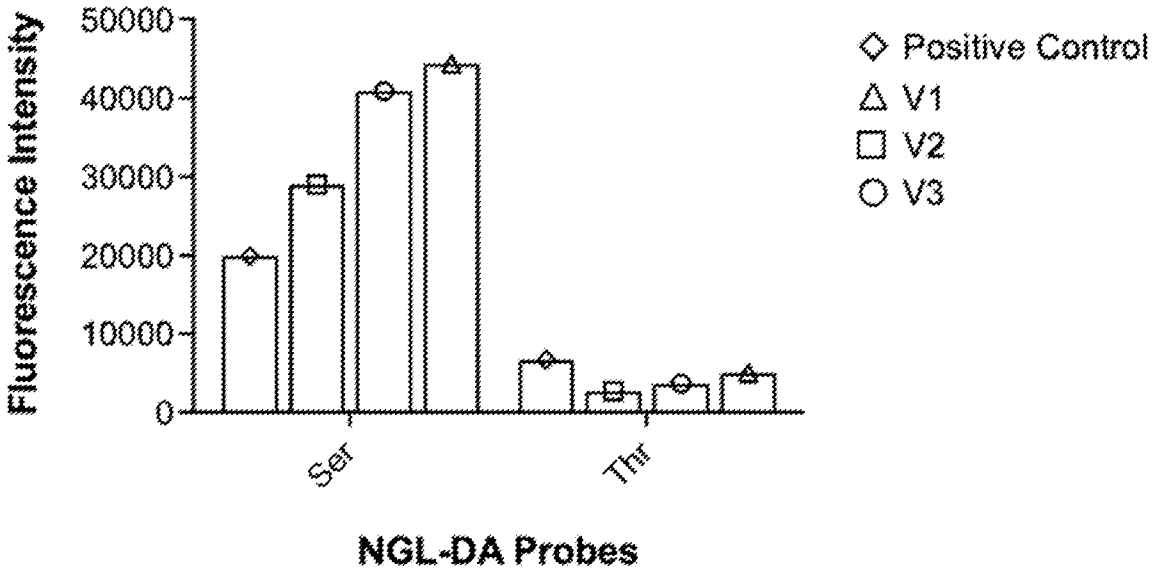
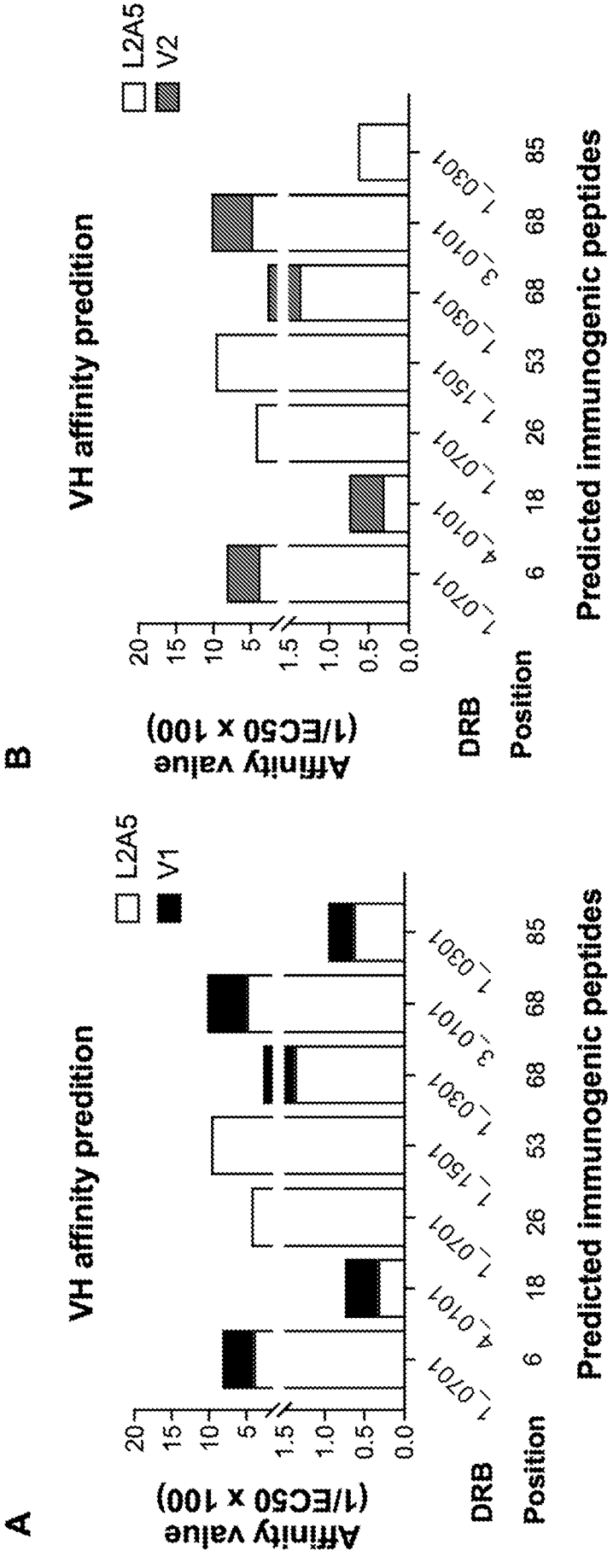


Figure 12. shows the humanized antibody clones binding to different immobilized glycan probes. This assay can detect the binding specificity and the binding intensity of antibodies to a panel of α2-6 sialylated glycans. Humanized mAbs maintained specificity to STn-Ser and STn-Thr probes, confirming that the humanization process did not alter the mAb properties. V1-V3 have increased binding to STn-Ser when compared to "positive control".

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Fig.13

Immunogenicity analysis



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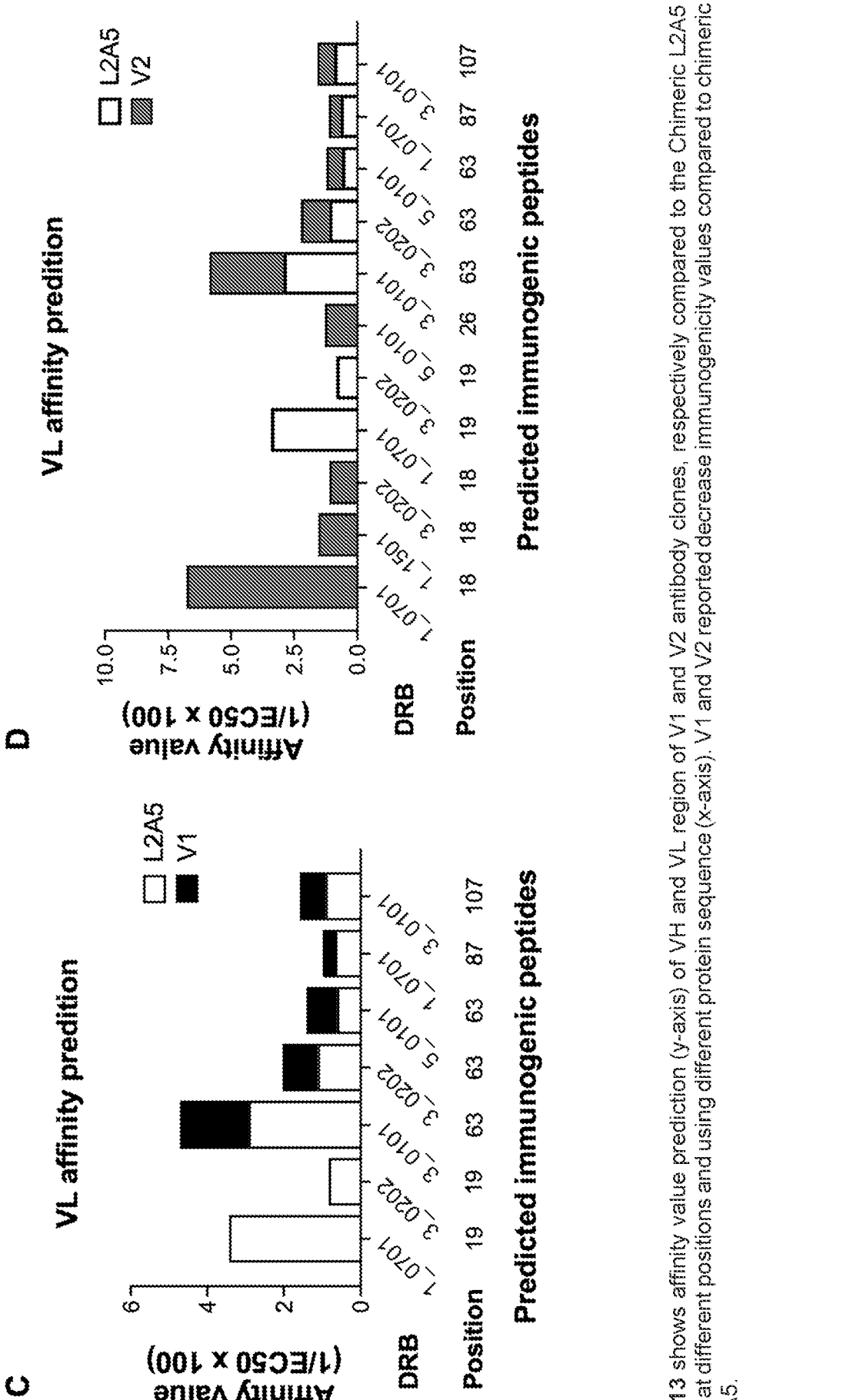


Fig13 shows affinity value prediction (y-axis) of VH and VL region of V1 and V2 antibody clones, respectively compared to the Chimeric L2A5 Ab, at different positions and using different protein sequence (x-axis). V1 and V2 reported decrease immunogenicity values compared to chimeric L2A5.

Figure 14

Internalization assay

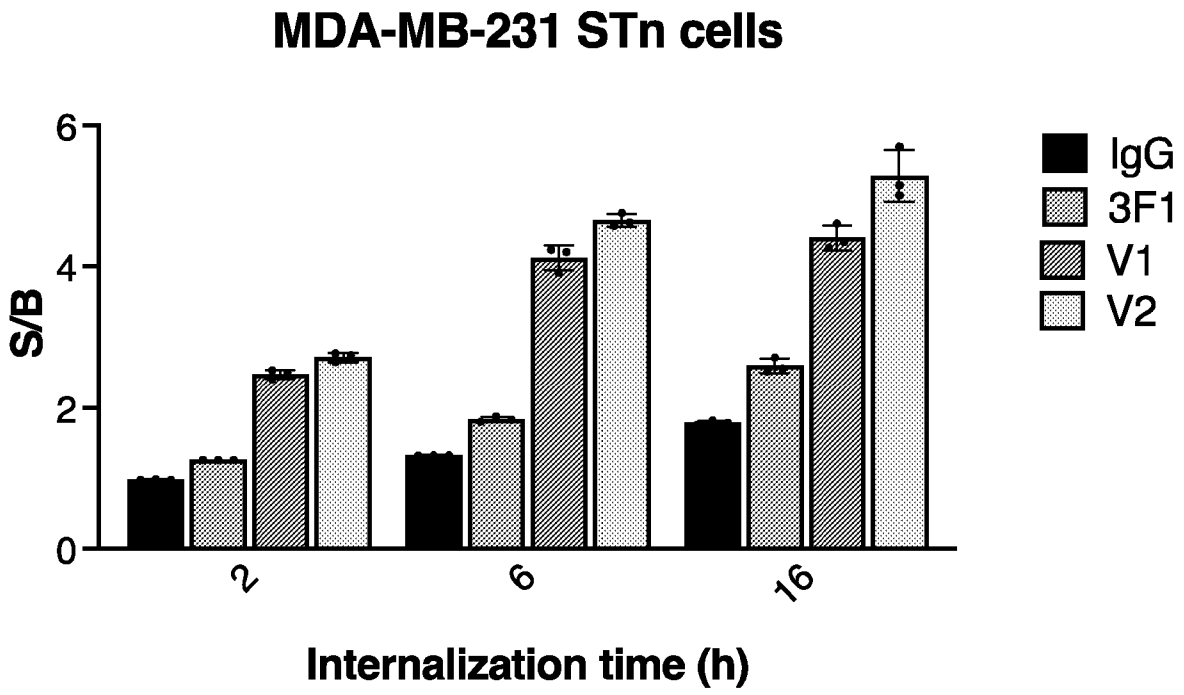


Figure 14. Antibody internalization-assay analysis in breast cancer cell line, MDA-MB231 overexpressing STn transgene, using humanized Abs variants (V1-2) compared to control IgG and 3F1, commercially available Abs. Antibody internalization is reported as signal-to-background ratio MFI (y-axis), normalized to untreated cells at 2- 6 and 16 hours (x-axis)

Fig. 15

Tissue Microarrays- TMAs

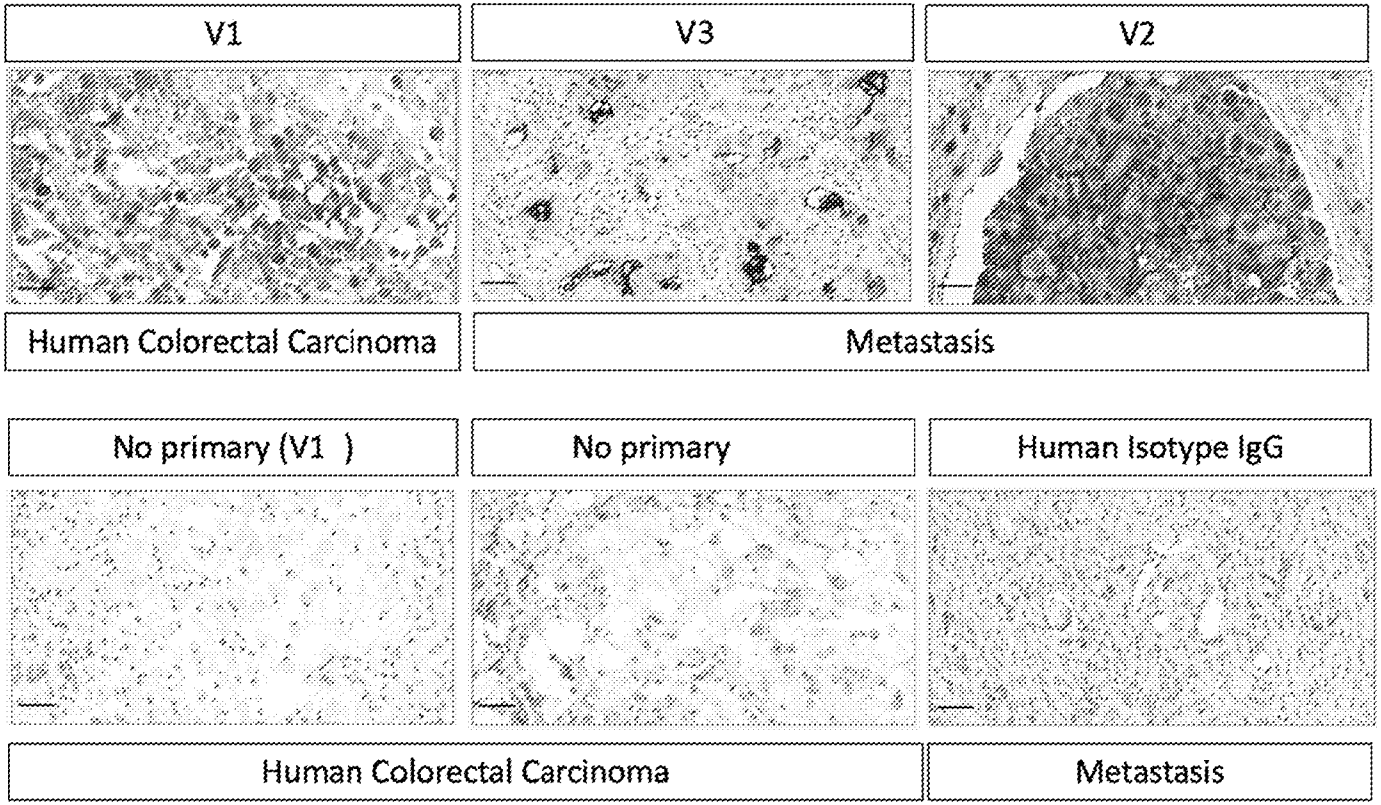


Figure 15. Representative IHC staining of STn using humanized V1-V3 antibody clones in colon carcinoma and different metastatic tissues. The scale bar represents 50um.

Fig. 16

Affinity-matured clones: EC50 ELISA – BSM

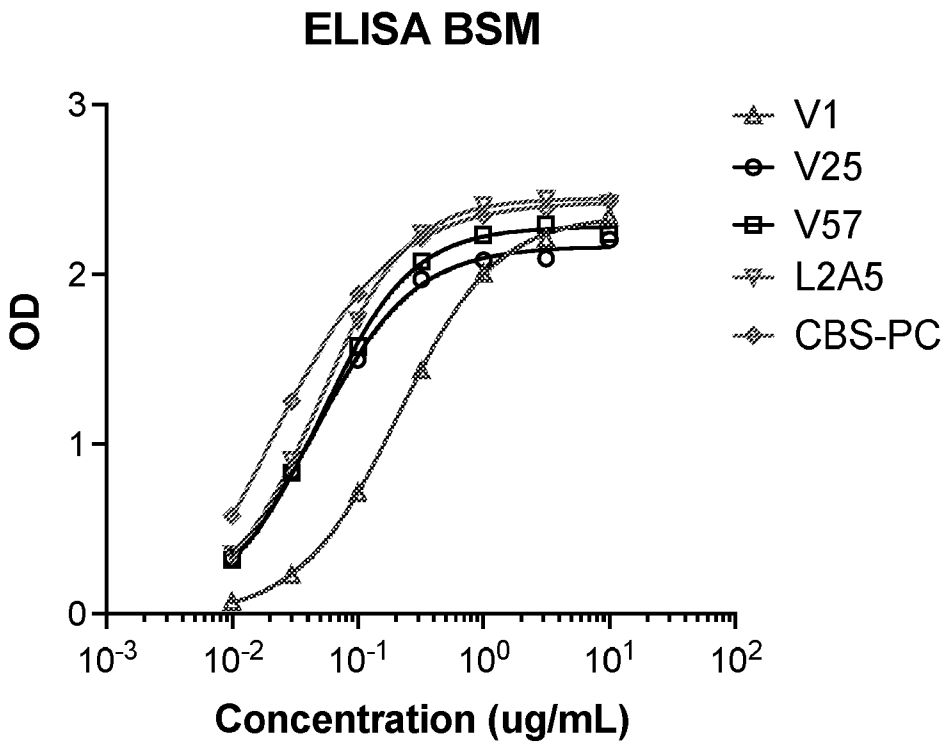


Figure 16. shows antibody binding to BSM mucin detected by ELISA. Binding to BSM is shown as Absorbance (OD) on the y-axis at different antibody concentrations (μ(g/mL) on the x-axis. Clones tested included new variants with point mutations in the CDR (selected after the affinity maturation process).

Fig.17

EC50 cell lines - MDA-MB-231-STn, OV90, SNU16 and COLO205

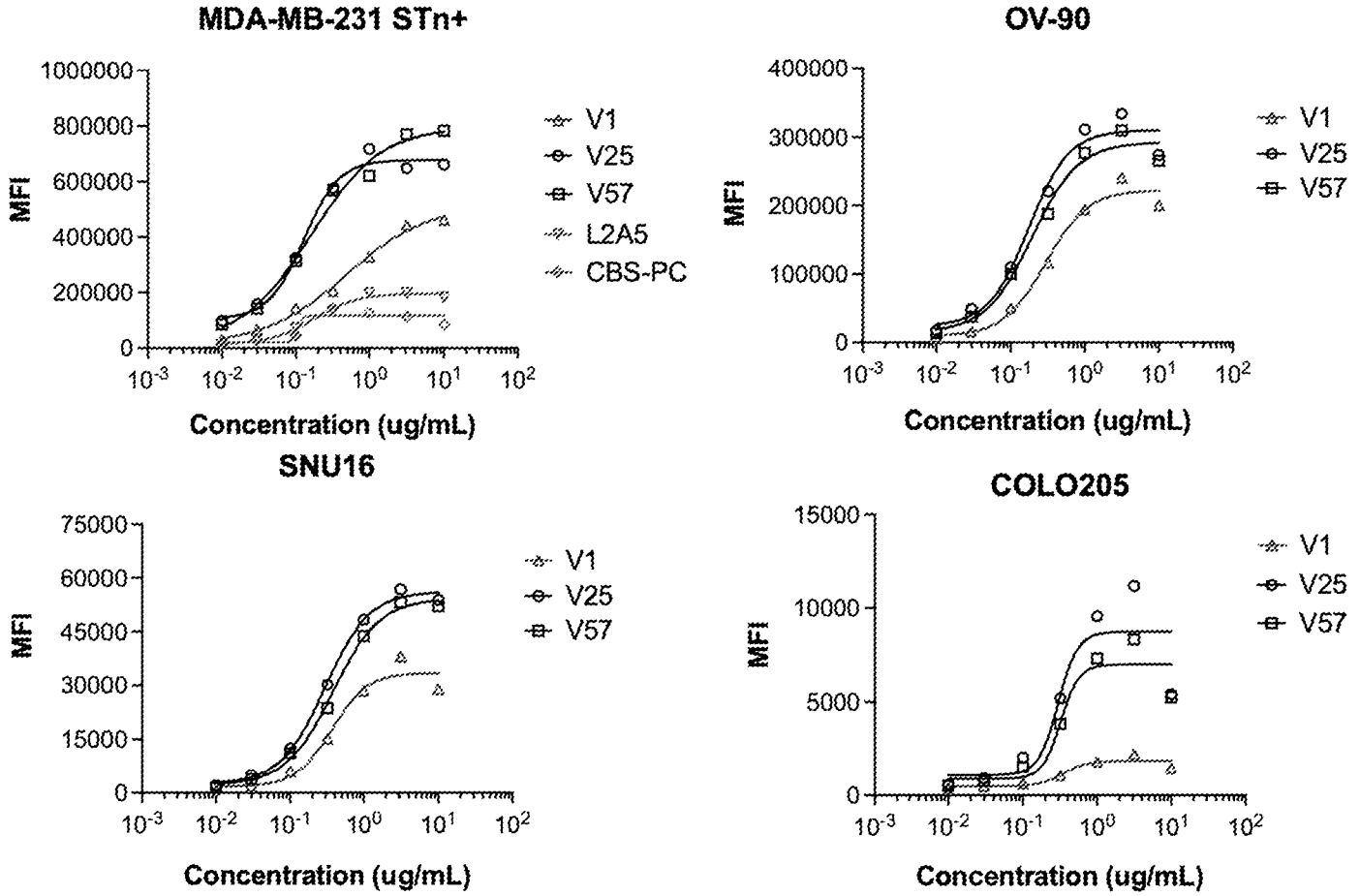


Figure 17. shows binding profile of the certain affinity-matured variants in cell lines with different STn expression levels. Each antibody was titrated using a 7-point concentration (μg/mL) curve and the binding intensity was measured by flow cytometry using a secondary antibody conjugated to a fluorophore.

Fig. 18

Antibody	FACS			TOP MFI
	EC50 [ng/ml]	95% CI EC50 [ng/ml]	R square	
V25	99,23	66,58 to 145,6	0,9798	700000
V46	107,5	67,32 to 168	0,9711	560000
V57	141,6	107,9 to 185,5	0,9915	800000
L2A5-Chimeric	151,4	109,3 to 207,5	0,9864	200000
V1	309,7	218,6 to 434,8	0,9868	350000

Figure 18. shows based on the binding results obtained in MDA-MB-231 STn cells, the EC50 for each antibody was calculated. This table summarizes the EC50 obtained for each clone as well as the maximum MFI detected.

25 09 23

Fig. 19

Glycan- array - affinity-matured clones

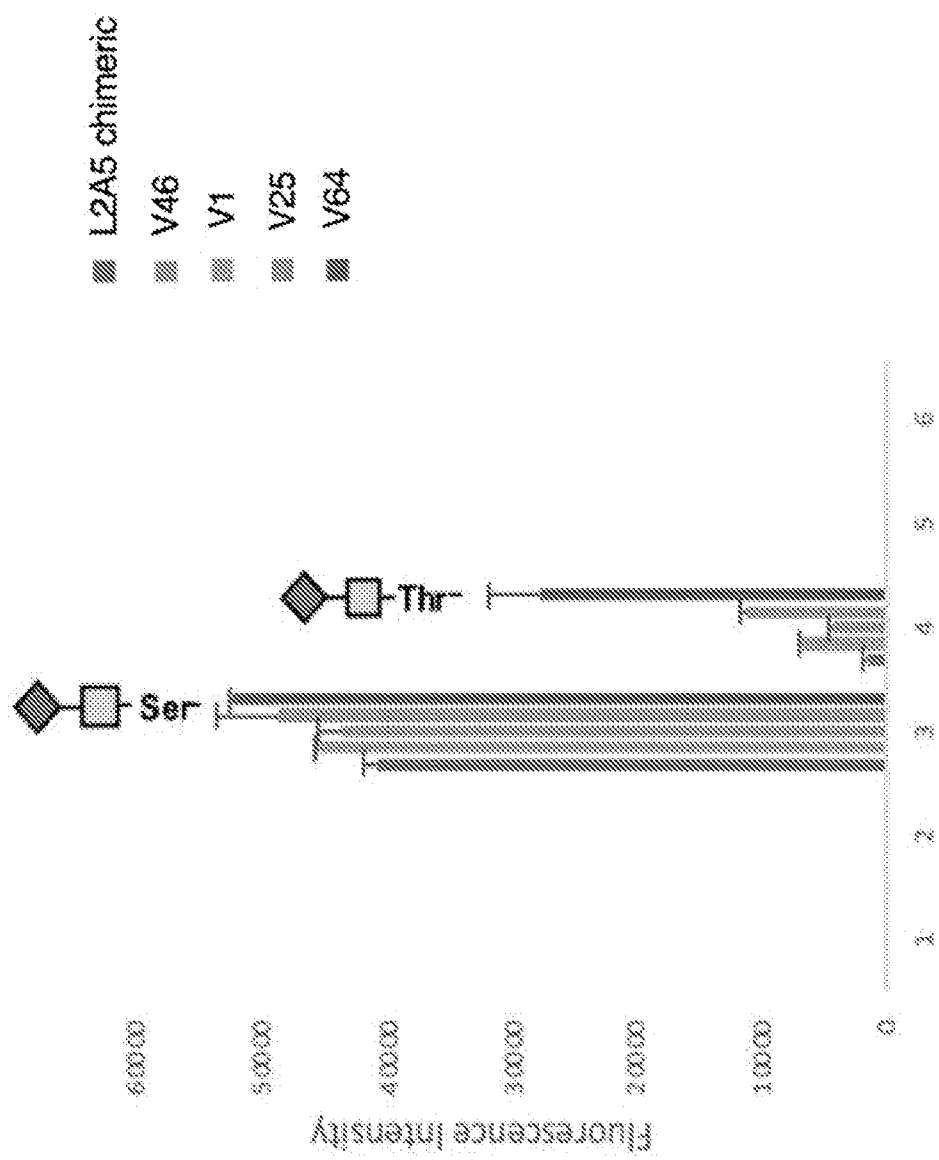


Figure 19. shows binding reported as fluorescent intensity (y-axis) of affinity-matured clones using a glycan array with different sialylated probes (x-axis). Affinity-matured clones retained specificity to STn-serine (probe3) and STn-threonine (probe4).

Fig. 20a

Humanised V1 variants – amino acid sequence of V_H heavy chain. The H-CDR1, H-CDR2 and H-CDR3 sequences are highlighted in bold.

HUMANIZED V1 VARIANTS - VH CHAIN (CDRs in bold)				
Name in patent	Seq ID	H-CDR1	H-CDR2	H-CDR3
mAb_VH-v65	SEQ ID No 129	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDESNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v66	SEQ ID No 130	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDKSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v67	SEQ ID No 131	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDASNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v68	SEQ ID No 132	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYEGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v69	SEQ ID No 133	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYQGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v70	SEQ ID No 134	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYRGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v71	SEQ ID No 135	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYAASNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v72	SEQ ID No 136	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYEESNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v73	SEQ ID No 137	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYFFSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v74	SEQ ID No 138	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYGGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v75	SEQ ID No 139	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYHHSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v76	SEQ ID No 140	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYKKSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v77	SEQ ID No 141	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYLLSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v78	SEQ ID No 142	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDNSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v79	SEQ ID No 143	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYPGSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v80	SEQ ID No 144	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYQQSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v81	SEQ ID No 145	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYRRSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v82	SEQ ID No 146	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYDTSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v83	SEQ ID No 147	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYVVSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v84	SEQ ID No 148	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYWWSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		
mAb_VH-v85	SEQ ID No 149	QVQLQESGPGLVKPSQTLSLTCTVIGYSIT**SGYYWN**WIRQPPGKGLEWIG**SINYYYSNIYNPSLKS**RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR**GGDY**WGQGTTVTVSS		

Fig. 20b

Humanised V1 variants – amino acid sequence of V_L light chain. The L-CDR1, L-CDR2 and L-CDR3 sequences are highlighted in bold.

HUMANIZED V1 VARIANTS - VL CHAIN (CDRs in bold)				
Name in patent	Seq ID	H-CDR1	H-CDR2	H-CDR3
mAb_VL-v65	SEQ ID No 150	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDAPMLT**FGGGTKLEIK		
mAb_VL-v66	SEQ ID No 151	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDKPMLT**FGGGTKLEIK		
mAb_VL-v67	SEQ ID No 152	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSDNPMLT**FGGGTKLEIK		
mAb_VL-v68	SEQ ID No 153	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSEPPMLT**FGGGTKLEIK		
mAb_VL-v69	SEQ ID No 154	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSKPPMLT**FGGGTKLEIK		
mAb_VL-v70	SEQ ID No 155	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSNPPMLT**FGGGTKLEIK		
mAb_VL-v71	SEQ ID No 156	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSQPPMLT**FGGGTKLEIK		
mAb_VL-v72	SEQ ID No 157	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSRPPMLT**FGGGTKLEIK		
mAb_VL-v73	SEQ ID No 158	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSAAPMLT**FGGGTKLEIK		
mAb_VL-v74	SEQ ID No 159	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSEEPMLT**FGGGTKLEIK		
mAb_VL-v75	SEQ ID No 160	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSFFPMLT**FGGGTKLEIK		
mAb_VL-v76	SEQ ID No 161	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSGPPMLT**FGGGTKLEIK		
mAb_VL-v77	SEQ ID No 162	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSHHPMLT**FGGGTKLEIK		
mAb_VL-v78	SEQ ID No 163	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSIIPMLT**FGGGTKLEIK		
mAb_VL-v79	SEQ ID No 164	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSKKPMLT**FGGGTKLEIK		
mAb_VL-v80	SEQ ID No 165	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSLLPMLT**FGGGTKLEIK		
mAb_VL-v81	SEQ ID No 166	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSNNPMLT**FGGGTKLEIK		
mAb_VL-v82	SEQ ID No 167	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSQQPMLT**FGGGTKLEIK		
mAb_VL-v83	SEQ ID No 168	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSRRPMLT**FGGGTKLEIK		
mAb_VL-v84	SEQ ID No 169	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSSPPMLT**FGGGTKLEIK		
mAb_VL-v85	SEQ ID No 170	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSTTPMLT**FGGGTKLEIK		
mAb_VL-v86	SEQ ID No 171	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSVVPMLT**FGGGTKLEIK		
mAb_VL-v87	SEQ ID No 172	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSWWPMLT**FGGGTKLEIK		
mAb_VL-v88	SEQ ID No 173	EIVLTQSPATLSLSPGERATLSC**SASSSVSYMH**WFQQKPGQAPRRWIY**DTSKRAT**GVPARFSGSGSGTDYTLTISSLEPEDFAAYYC**QQWSSYYPMLT**FGGGTKLEIK		

HUMANISED ANTIBODIES OR FUNCTIONAL FRAGMENTS THEREOF AGAINST TUMOUR ANTIGENS

Technical Field

The present invention relates to humanised antibodies or functional antibody fragments thereof, or probes thereof, directed against a group of antigens identified in cancer.

State of the art

The sialyl Tn (STn) is a short O-glycan antigen, a disaccharide consisting of sialic acid linked to N-acetylgalactosamine, i.e. Neu5Acα-2, 6GalNAc, O-linked to Serine or Threonine amino acid residues, in alpha configuration through the GalNAc residue, in a polypeptide chain. This truncated glycan has been detected with different frequencies in different types of carcinomas (Julian, Videira, & Delannoy 2012), while absent in normal healthy tissues. In addition, STn is targeted for metastatic, drug resistant and highly malignant tumours. In this type of tumours, their characteristics are the following:

1) Association of STn expression with early and metastatic cancer cells (Okasaki et al., 2012).

2) Correlation of increased STn expression in patients with poor prognosis, reduced overall survival, and lack of response to chemotherapy (Choi et al., 2000)

3) Evasion of immune-cells surveillance (Carrascal et al., 2014)

Alpha-2, 6 sialic acids are typically truncated cancer biomarkers. Short alpha 2,6 sialylated O-glycans are overexpressed in several types of cancer. They are generally involved in cancer progression and metastasis. In addition, truncate glycans have been reported to contribute to immune evasion through their recognition by a number of immune receptors, such as the sialic acid binding proteins (Siglecs) (Crocke, Paulson, & Varkl, 2007; Micoll et al., 2003).

The present invention provides further antibodies, functional antibody fragments thereof, or probes thereof, that bind specifically to these cancer biomarkers. Besides the specific identification of tumour cells, these antibodies also have the potential to block the recognition of

such ligands by host cell receptors involved in the mechanism underlying tumour progression, including immune tolerance.

A number of antibodies have been approved to treat cancer patients (see, for example: https: //www.cancer.org), and further details of these, and further background to the present invention can be found in WO2019/147152.

WO2019/147152 describes nucleotide sequences encoding a monoclonal antibody (mAb) against the STn and a group of glycans terminated by alpha-2, 6-linked sialic acids. These antigens are short-chain glycans that are overexpressed in cancer but not expressed by normal cells.

Summary of the invention

In a broad aspect, according to the present invention, there is provided an antibody or fragment thereof, or probe thereof, wherein the antibody or fragment or probe comprises:

(a) a heavy chain variable region (VH) wherein the VH comprises complementarity determining regions (CDRs) selected from the group consisting of:

(i) H-CDR1, H-CDR2 and H-CDR3 as shown in any one SEQ ID NOs. 1 to 24 or 49 to 88 respectively

and / or,

(b) a light chain variable region (VL) wherein the VL comprises complementarity determining regions (CDRs) selected from the group consisting of:

(i) L-CDR1, L-CDR2, and L-CDR3 as shown in any one of SEQ ID NOs. 25 to 48 or 89 to 128 respectively.

The fragment may be a functional antibody fragment of the antibody disclosed – that is, retain the ability to bind antigen.

In one aspect, the antibody or fragment thereof may comprise a heavy chain variable region (VH) and a light chain variable region (VL). Constant regions may also be provided, as will be understood.

Thus, in one aspect, the present invention essentially provides engineered antibodies with amino acid changes in the chimeric L2A5 framework regions described in WO2019147152 in order to humanize it. The primary scope of humanization is to engineer antibodies from non-human species, whose protein sequences have been modified to increase their similarity to antibodies normally produced in humans. The present inventors have discovered and produced humanized L2A5 variants, as disclosed in the present application, while retaining binding and specificity to STn whilst showing decreased immunogenicity.

In a further aspect, the invention also describes additional engineered antibodies based on a humanized variant, termed V1 in this application. Through a process of affinity maturation, different point mutations were introduced in the CDRs which enabled the present inventors to generate variants with higher binding affinity to STn. As disclosed in this patent application, several antibody variants have shown increased binding to STn+ cell lines and BSM mucin, with no relevant binding to other glycans.

The present application describes the generation of humanized antibodies, recognizing STn with moderate to high affinity and high specificity – characteristics which generally cannot be predicted in advance. Humanized clones also potentially have decreased immunogenicity, which decreases the chance of human anti-drug antibodies (HAMA) response, increases the therapeutic window and improves the antibody PK profile.

Furthermore, the present application describes the development of high affinity humanized anti-STn antibodies through a process of affinity maturation. For certain therapeutic applications, it is desirable to have antibodies with high affinity to the target antigen, thus improving the antitumor response.

The present inventors have now found a way to improve the antibody affinity and binding to the target, Sialyl Tn (STn), by the generation of new antibodies. These are related to the clone described in patent WO2019147152A1. In particular, the present inventors have provided a number of different humanised antibody clones, including the humanized clone referred to herein with the acronym of V1. Using in particular this humanized V1 clone, the present inventors have also now provided a series of new and different antibodies resulting from an affinity maturation process. These new clones or variants possess differences in the amino

acid sequences and an increase in affinity and binding to the target Sialyl Tn (STn), thus substantially improving over the known antibodies.

The present application thus describes high affinity humanized anti-STn antibodies obtained through a process of affinity maturation. For certain applications, it is highly desirable to have antibodies with high affinity to the target antigen, improving the antitumor response. For instance, this may allow for:

- antibody-drug conjugates or radioimmunoconjugates with increased tumour uptake and antitumor function.

- fine-tuning of bispecific T cell engagers and CAR receptors to induce the desired T cell activation level.

- improved blocking of STn *in vivo*, leading to antitumor responses by restoring the function of immune cells.

Brief description of Figures

Figure 1 shows the sequence ID numbers assigned to the variants disclosed herein, which are reflected in the accompanying sequence listing.

Figure 2 shows the amino acid sequences of the variable chains (VH and VL) of certain humanised variants disclosed herein.

Figure 3a shows the amino acid sequence alignment comparing the heavy chain (VH) of the parental antibody (L2A5) to certain humanized variants described herein.

Figure 3b shows the amino acid sequence alignment comparing the light chain (VL) of the parental antibody (L2A5) to certain humanized variants described herein.

Figure 4 shows the amino acid sequence of the V_H heavy chain of humanised variants disclosed herein.

Figure 5 shows the amino acid sequence of the V_L light chain of humanised antibody variants disclosed herein.

Figure 6a shows the amino acid sequence of the V_H heavy chain of affinity-matured variants disclosed herein.

Figure 6b shows the amino acid sequence of the V_L light chain of affinity-matured variants disclosed herein.

Figure 7 shows the amino acid sequences of the variable chains (VH and VL) of the affinity-matured variants disclosed herein, together with the clone variant name and their corresponding sequence identity no., as reflected in the accompanying sequence listing.

Figure 8 shows normalized EC50 using humanized Abs clones V1, V2 and V3.

Figure 9 shows binding of humanized clones to different STn+ cancer cell lines.

Figure 10 shows NMR analysis of the points of contact/interaction between the antibody and glycan molecules.

Figure 11 shows how Surface Plasmon Resonance (SPR) can be used to measure the affinity and kinetic parameters of different anti-STn antibodies.

Figure 12 shows results of glycan array analysis, using different immobilized glycan probes, to evaluate the binding specificity and the binding intensity of antibodies to a panel of α2-6 sialylated glycans.

Figure 13 A to D show the results from an *in silico* immunogenicity analysis.

Figure 14 shows results from antibody internalization-assay analysis using humanized antibody variants (V3, V1) using MDA-MB231 breast cancer cell line, overexpressing STn.

Figure 15 shows representative IHC staining results using humanized V1, V2 and V3 antibody-clones in colon carcinoma and different metastatic tissues.

Figure 16 shows EC50 results for certain affinity-matured antibody variants binding property to Bovine Submaxillary Mucin (BSM) using ELISA assay and EC50 analysis

Figure 17 shows the binding profile for certain affinity-matured variants using different cancer cell lines, presenting different STn expression levels.

Figure 18 shows the EC50 for certain affinity-matured variants based on binding results obtained in MDA-MB-231 STn breast cancer cell lines.

Figure 19 shows binding results to different types of glycans, using different sialylated probes for certain affinity-matured variants.

Figure 20a shows the sequence ID No and amino acid sequence of the V_H heavy chain of humanised V1 variants disclosed herein.

Figure 20b shows the sequence ID No and amino acid sequence of the V_L light chain of humanised V1 variants disclosed herein.

It will be understood that monoclonal antibodies (mAbs) refers to an antibody that is produced by a single B cell clone. MAbs can be also produced by an hybridoma, which is a hybrid between a B cell and myeloma cell, or cell lines that express recombinant DNA coding for the immunoglobulin heavy and light chain, and therefore will produce a single and specific antibody.

The antibodies may be expressed to the extracellular milieu and then purified from there.

The specificity of an antibody is its ability to react with one antigen or a group of antigens that share a certain epitope. An epitope, also known as antigenic determinant, is the part of an antigen that is recognised by the antibody.

An antibody belongs to the immunoglobulin class of proteins and it is typically an assembling of two identical heavy chains (around 50-70 kDa) and too identical light chains (around 25 kDa). In the amino-terminal of each heavy or light chain there is a sequence: of 100-130 amino acids that code for the variable region. In the carboxyl- terminal of each heavy or light chain there is a sequence that codes the constant region. Typically, each antibody binds the same antigen, i.e. is bivalent.

The antigen-binding fragment (Fab) is the antibody fragment that binds to antigens. Each Fab is composed of one constant and one variable domain from each heavy and light chain of the antibody. The Fragment crystallisable (Fc) region is composed of 2 or 3 domains of the carboxy-terminal of the two heavy chains. While the Fab ensures binding to the antigen, the Fc region ensures that each antibody generates an effector immune response. The Fc region binds to various cell receptors, such as Fc receptors, and other molecules, such as complement proteins, mediating different physiological effects including opsonization to facilitate phagocytosis by phagocytes, cell lysis by natural killer cells, and degranulation of mast cells, basophils and eosinophils.

The term "variable domain'" or "variable region" is the amino-terminal part of the light or heavy chains of an antibody that interacts with the antigen. It typically has a length of about 120 to 130 amino acids in the heavy chain and typically about 100 to 110 amino acids in the light chain. The sequences of each of the variable regions are substantially varied, particularly in the

complementary determining regions (CDRs) responsible for the interaction with the specific antigen. The CDRs are flanked by less varied framework regions (FR). There are typically three CDRs in each of the light and heavy chains. Thus, for example, CDRs L1, L2, and L3 are within the light chain, and CDRs H1, H2 and H3 are within the heavy chain.

The expression "functional antibody fragment or probe" suitably refers to a part of the antibody that includes the variable region of the heavy and the light chain of the antibody, or includes either the variable region of the heavy or the variable region of the light chain of the antibody. For example, a functional antibody fragment or probe retains most or all the binding activity of the initial antibody from which the fragment or probe is derived. Such functional antibody fragments or probes can for example include the single chain Fv (scFv), diabody, triabody, tetra-body and mini-body.

It will be appreciated that the term fragment as used herein in particular relates to fragments of antibodies specifically as described and these form an important aspect of the present disclosure. In this way, a monoclonal or recombinant antibody as provided by the present disclosure may for example be provided as any of the following fragments: (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains; (iv) the dAb fragment which consists of a VH domain; (v) the isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments; and (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site.

Alternatively, as will be understood, an antibody according to the present disclosure may comprise a whole IgG antibody, whereby the antibody includes variable and constant regions.

The term "nucleotide sequences" refers to a sequence of nucleotides of any length, either deoxy ribonucleotides or ribonucleotides or their analogues thereof.

As will be understood, nucleotide sequences can be transcribed to produce mRNA, which is then translated into a polypeptide and/or a fragment thereof.

Further aspects of the invention will now be described. In one preferred aspect, an antibody or functional antibody fragment or probe thereof is provided, wherein the antibody or fragment or probe comprises one of the following pairs of heavy chain CDRs and light chain CDRs:

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 1 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 25;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 2 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 26;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 3 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 27;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 4 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 28;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 5 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 29;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 6 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 30;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 7 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 31;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 8 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 32;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 9 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 33;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 10 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 34;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 11 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 35;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 12 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 36;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 13 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 37;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 14 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 38;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 15 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 39;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 16 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 40;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 17 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 41;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 18 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 42;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 19 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 43;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 20 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 44;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 21 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 45;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 22 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 46;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 23 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 47; or

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 24 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 48.

Sequence identity information identifying the humanised and affinity-matured variants provided by the present invention, including the full VH and VL sequences and the CDR regions for each variant is given in Figures 1 to 6, and in the sequence listing accompanying this application.

The present invention provides humanised and affinity-matured antibody variants which show excellent binding affinity and specificity to the antigen STn, whilst also showing decreased immunogenicity. Humanised variants are disclosed herein by way of SEQ ID Nos 1-24 (variable VH region) and SEQ ID Nos 25-48 (variable VL region), and affinity-matured antibody variants are disclosed herein by way of SEQ ID Nos 49-88 (variable VH region) and SEQ ID Nos 89-128 (variable VL region),

In one preferred aspect, an antibody or functional antibody fragment or probe thereof is provided, wherein the antibody or fragment or probe comprises one of the following pairs of heavy chain CDRs and light chain CDRs:

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 17 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 41;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 20 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 44;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 21 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 45.

In a further aspect, an antibody or functional fragment or probe thereof, is provided, wherein the antibody or fragment or probe comprises one of the following pairs of light chain CDRs and heavy chain CDRs:

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 49 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 89;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 50 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 90;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 51 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 91;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 52 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 92;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 53 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 93;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 54 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 94;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 55 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 95;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 56 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 96;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 57 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 97;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 58 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 98;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 59 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 99;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 60 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 100;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 61 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 101;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 62 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 102;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 63 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 103;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 64 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 104;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 65 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 105;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 66 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 106;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 67 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 107;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 68 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 108;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 69 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 109;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 70 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 110;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 71 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 111;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 72 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 112;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 73 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 113;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 74 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 114;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 75 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 115;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 76 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 116;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 77 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 117;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 78 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 118;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 79 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 119;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 80 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 120;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 81 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 121;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 82 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 122;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 83 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 123;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 84 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 124;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 85 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 125;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 86 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 126;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 87 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 127; or

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 88 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 128.

In a preferred aspect, an antibody or fragment thereof or probe is provided, wherein the antibody or fragment or probe comprises one of the following pairs of heavy chain CDRs and light chain CDRs:

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 49 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 89;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 70 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 110;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 81 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 121; or

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 88 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 128.

In a further aspect of the invention, an antibody or functional antibody fragment or probe thereof is provided, wherein the antibody or fragment or probe comprises in addition to the CDR regions described herein:

(a) a heavy chain variable region (VH) wherein the VH comprises a humanised heavy chain framework region

and / or,

(b) a light chain variable region (VL) wherein the VL comprises a humanised light chain framework region.

The humanised framework regions may, for example, be as further described below. It will be understood that the variable framework regions refer to the sequences surrounding the CDR regions. Thus different combinations of the CDR regions and variable framework regions described herein may be made if desired.

Thus, in one aspect of the present invention, an antibody or fragment thereof is provided, wherein the antibody or fragment comprises:

(a) a heavy chain variable region (VH) wherein the VH comprises a humanised heavy chain framework region as shown in the heavy chain variable region (VH) sequences selected from the group consisting of:

(i) any one SEQ ID NOs. 1 to 24 or 49 to 88 respectively

and / or,

(b) a light chain variable region (VL) wherein the VL comprises a humanised light chain framework region as shown in the light chain variable region (VL) sequences selected from the group consisting of:

(ii) any one of SEQ ID NOs. 25 to 48 or 89 to 128 respectively.

One of the above described humanised heavy chain framework regions may be paired with any one of the humanised light chain framework regions, as desired.

Certain pairings of the framework regions are preferred. In one aspect of the invention, there is provided an antibody or fragment thereof wherein the antibody or fragment comprises one of the following pairs of light chain and heavy chain framework regions, wherein the heavy chain framework region is as shown in the heavy chain variable region (VH) sequences shown below, and wherein the light chain framework region is as shown in the light chain variable region (VL) sequences shown below:

heavy chain framework region of SEQ ID NO. 1 paired with light chain framework region from SEQ ID NO. 25;

heavy chain framework region of SEQ ID NO. 2 paired with light chain framework region from SEQ ID NO. 26;

heavy chain framework region of SEQ ID NO. 3 paired with light chain framework region from SEQ ID NO. 27;

heavy chain framework region of SEQ ID NO. 4 paired with light chain framework region from SEQ ID NO. 28;

heavy chain framework region of SEQ ID NO. 5 paired with light chain framework region from SEQ ID NO. 29;

heavy chain framework region of SEQ ID NO. 6 paired with light chain framework region from SEQ ID NO. 30;

heavy chain framework region of SEQ ID NO. 7 paired with light chain framework region from SEQ ID NO. 31;

heavy chain framework region of SEQ ID NO. 8 paired with light chain framework region from SEQ ID NO. 32;

heavy chain framework region of SEQ ID NO. 9 paired with light chain framework region from SEQ ID NO. 33;

heavy chain framework region of SEQ ID NO. 10 paired with light chain framework region from SEQ ID NO. 34;

heavy chain framework region of SEQ ID NO. 11 paired with light chain framework region from SEQ ID NO. 35;

heavy chain framework region of SEQ ID NO. 12 paired with light chain framework region from SEQ ID NO. 36;

heavy chain framework region of SEQ ID NO. 13 paired with light chain framework region from SEQ ID NO. 37;

heavy chain framework region of SEQ ID NO. 14 paired with light chain framework region from SEQ ID NO. 38;

heavy chain framework region of SEQ ID NO. 15 paired with light chain framework region from SEQ ID NO. 39;

heavy chain framework region of SEQ ID NO. 16 paired with light chain framework region from SEQ ID NO. 40;

heavy chain framework region of SEQ ID NO. 17 paired with light chain framework region from SEQ ID NO. 41;

heavy chain framework region of SEQ ID NO. 18 paired with light chain framework region from SEQ ID NO. 42;

heavy chain framework region of SEQ ID NO. 19 paired with light chain framework region from SEQ ID NO. 43;

heavy chain framework region of SEQ ID NO. 20 paired with light chain framework region from SEQ ID NO. 44;

heavy chain framework region of SEQ ID NO. 21 paired with light chain framework region from SEQ ID NO. 45;

heavy chain framework region of SEQ ID NO. 22 paired with light chain framework region from SEQ ID NO. 46;

heavy chain framework region of SEQ ID NO. 23 paired with light chain framework region from SEQ ID NO. 47; or

heavy chain framework region of SEQ ID NO. 24 paired with light chain framework region from SEQ ID NO. 48.

In a further aspect of the invention, there is provided an antibody or fragment thereof or probe thereof wherein the antibody or fragment or probe comprises one of the following pairs of light chain and heavy chain framework regions, wherein the heavy chain framework region is as shown in the heavy chain variable region (VH) sequences shown below, and wherein the light chain framework region is as shown in the light chain variable region (VL) sequences shown below:

heavy chain framework region of SEQ ID NO. 49 paired with light chain framework region from SEQ ID NO. 89;

heavy chain framework region of SEQ ID NO. 50 paired with light chain framework region from SEQ ID NO. 90;

heavy chain framework region of SEQ ID NO. 51 paired with light chain framework region from SEQ ID NO. 91;

heavy chain framework region of SEQ ID NO. 52 paired with light chain framework region from SEQ ID NO. 92;

heavy chain framework region of SEQ ID NO. 53 paired with light chain framework region from SEQ ID NO. 93;

heavy chain framework region of SEQ ID NO. 54 paired with light chain framework region from SEQ ID NO. 94;

heavy chain framework region of SEQ ID NO. 55 paired with light chain framework region from SEQ ID NO. 95;

heavy chain framework region of SEQ ID NO. 56 paired with light chain framework region from SEQ ID NO. 96;

heavy chain framework region of SEQ ID NO. 57 paired with light chain framework region from SEQ ID NO. 97;

heavy chain framework region of SEQ ID NO. 58 paired with light chain framework region from SEQ ID NO. 98;

heavy chain framework region of SEQ ID NO. 59 paired with light chain framework region from SEQ ID NO. 99;

heavy chain framework region of SEQ ID NO. 60 paired with light chain framework region from SEQ ID NO. 100;

heavy chain framework region of SEQ ID NO. 61 paired with light chain framework region from SEQ ID NO. 101;

heavy chain framework region of SEQ ID NO. 62 paired with light chain framework region from SEQ ID NO. 102;

heavy chain framework region of SEQ ID NO. 63 paired with light chain framework region from SEQ ID NO. 103;

heavy chain framework region of SEQ ID NO. 64 paired with light chain framework region from SEQ ID NO. 104;

heavy chain framework region of SEQ ID NO. 65 paired with light chain framework region from SEQ ID NO. 105;

heavy chain framework region of SEQ ID NO. 66 paired with light chain framework region from SEQ ID NO. 106;

heavy chain framework region of SEQ ID NO. 67 paired with light chain framework region from SEQ ID NO. 107;

heavy chain framework region of SEQ ID NO. 68 paired with light chain framework region from SEQ ID NO. 108;

heavy chain framework region of SEQ ID NO. 69 paired with light chain framework region from SEQ ID NO. 109;

heavy chain framework region of SEQ ID NO. 70 paired with light chain framework region from SEQ ID NO. 110;

heavy chain framework region of SEQ ID NO. 71 paired with light chain framework region from SEQ ID NO. 111;

heavy chain framework region of SEQ ID NO. 72 paired with light chain framework region from SEQ ID NO. 112;

heavy chain framework region of SEQ ID NO. 73 paired with light chain framework region from SEQ ID NO. 113;

heavy chain framework region of SEQ ID NO. 74 paired with light chain framework region from SEQ ID NO. 114;

heavy chain framework region of SEQ ID NO. 75 paired with light chain framework region from SEQ ID NO. 115;

heavy chain framework region of SEQ ID NO. 76 paired with light chain framework region from SEQ ID NO. 116;

heavy chain framework region of SEQ ID NO. 77 paired with light chain framework region from SEQ ID NO. 117;

heavy chain framework region of SEQ ID NO. 78 paired with light chain framework region from SEQ ID NO. 118;

heavy chain framework region of SEQ ID NO. 79 paired with light chain framework region from SEQ ID NO. 119;

heavy chain framework region of SEQ ID NO. 80 paired with light chain framework region from SEQ ID NO. 120;

heavy chain framework region of SEQ ID NO. 81 paired with light chain framework region from SEQ ID NO. 121;

heavy chain framework region of SEQ ID NO. 82 paired with light chain framework region from SEQ ID NO. 122;

heavy chain framework region of SEQ ID NO. 83 paired with light chain framework region from SEQ ID NO. 123;

heavy chain framework region of SEQ ID NO. 84 paired with light chain framework region from SEQ ID NO. 124;

heavy chain framework region of SEQ ID NO. 85 paired with light chain framework region from SEQ ID NO. 125;

heavy chain framework region of SEQ ID NO. 86 paired with light chain framework region from SEQ ID NO. 126;

heavy chain framework region of SEQ ID NO. 87 paired with light chain framework region from SEQ ID NO. 127; or

heavy chain framework region of SEQ ID NO. 88 paired with light chain framework region from SEQ ID NO. 128.

It is the case that the framework regions allow a certain degree of variability in the exact sequence, whilst still allowing for maintenance of function, including binding affinity and specificity. Accordingly, the invention also provides an antibody or fragment or probe thereof as described, wherein the heavy chain framework region and /or the light chain framework region may have 80% or more, preferably 85% or more, sequence identity to the specific sequences recited herein. For example, this may be by way of substitution, addition, or deletion of amino acid residues, with substitution often being preferred. Substitutions may, for example, be conservative amino acid substitutions.

In a preferred aspect of the invention, the antibody or fragment or probe thereof as described herein is such that the heavy chain framework region and /or the light chain framework region have 90% or more, preferably 95% or more, sequence identity to the specific sequences recited herein. For example, this may be by way of substitution, addition, or deletion of amino acid

residues, with substitution often being preferred. Substitutions may, for example, be conservative amino acid substitutions.

In a further aspect of the present invention, there is provided an antibody or fragment thereof or probe thereof wherein the antibody or fragment or probe comprises:

(a) a heavy chain variable region (VH) selected from the group consisting of:

(i) any one SEQ ID NOs. 1 to 24 or 49 to 88 respectively

and / or,

(a) a light chain variable region (VL) selected from the group consisting of:

(ii) any one of SEQ ID NOs. 25 to 48 or 89 to 128 respectively.

Any one of the VH regions may be paired with any one of the VL regions, although certain pairings are preferred.

Thus, in a further aspect, the present invention provides an antibody or fragment thereof or probe thereof as described herein, wherein the antibody or fragment or probe comprises one of the following pairs of heavy chain variable regions (VH) and light chain variable regions (VL):

SEQ ID NO. 1 paired with SEQ ID NO. 25;

SEQ ID NO. 2 paired with SEQ ID NO. 26;

SEQ ID NO. 3 paired with SEQ ID NO. 27;

SEQ ID NO. 4 paired with SEQ ID NO. 28;

SEQ ID NO. 5 paired with SEQ ID NO. 29;

SEQ ID NO. 6 paired with SEQ ID NO. 30;

SEQ ID NO. 7 paired with SEQ ID NO. 31;

SEQ ID NO. 8 paired with SEQ ID NO. 32;

SEQ ID NO. 9 paired with SEQ ID NO. 33;

SEQ ID NO. 10 paired with SEQ ID NO. 34;

SEQ ID NO. 11 paired with SEQ ID NO. 35;

SEQ ID NO. 12 paired with SEQ ID NO. 36;

SEQ ID NO. 13 paired with SEQ ID NO. 37;

SEQ ID NO. 14 paired with SEQ ID NO. 38;

SEQ ID NO. 15 paired with SEQ ID NO. 39;

SEQ ID NO. 16 paired with SEQ ID NO. 40;

SEQ ID NO. 17 paired with SEQ ID NO. 41;

SEQ ID NO. 18 paired with SEQ ID NO. 42;

SEQ ID NO. 19 paired with SEQ ID NO. 43;

SEQ ID NO. 20 paired with SEQ ID NO. 44;

SEQ ID NO. 21 paired with SEQ ID NO. 45;

SEQ ID NO. 22 paired with SEQ ID NO. 46;

SEQ ID NO. 23 paired with SEQ ID NO. 47; or

SEQ ID NO. 24 paired with SEQ ID NO. 48.

In a further aspect of the invention, an antibody or fragment thereof or probe thereof is provided wherein the antibody or fragment or probe comprises one of the following pairs of heavy chain variable regions (VH) and light chain variable regions (VL):

SEQ ID NO. 49 paired with SEQ ID NO. 89;

SEQ ID NO. 50 paired with SEQ ID NO. 90;

SEQ ID NO. 51 paired with SEQ ID NO. 91;

SEQ ID NO. 52 paired with SEQ ID NO. 92;

SEQ ID NO. 53 paired with SEQ ID NO. 93;

SEQ ID NO. 54 paired with SEQ ID NO. 94;

SEQ ID NO. 55 paired with SEQ ID NO. 95;

SEQ ID NO. 56 paired with SEQ ID NO. 96;

SEQ ID NO. 57 paired with SEQ ID NO. 97;

SEQ ID NO. 58 paired with SEQ ID NO. 98;

SEQ ID NO. 59 paired with SEQ ID NO. 99;

SEQ ID NO. 60 paired with SEQ ID NO. 100;

SEQ ID NO. 61 paired with SEQ ID NO. 101;

SEQ ID NO. 62 paired with SEQ ID NO. 102;

SEQ ID NO. 63 paired with SEQ ID NO. 103;

SEQ ID NO. 64 paired with SEQ ID NO. 104;

SEQ ID NO. 65 paired with SEQ ID NO. 105;

SEQ ID NO. 66 paired with SEQ ID NO. 106;

SEQ ID NO. 67 paired with SEQ ID NO. 107;

SEQ ID NO. 68 paired with SEQ ID NO. 108;

SEQ ID NO. 69 paired with SEQ ID NO. 109;

SEQ ID NO. 70 paired with SEQ ID NO. 110;

SEQ ID NO. 71 paired with SEQ ID NO. 111;

SEQ ID NO. 72 paired with SEQ ID NO. 112;

SEQ ID NO. 73 paired with SEQ ID NO. 113;

SEQ ID NO. 74 paired with SEQ ID NO. 114;

SEQ ID NO. 75 paired with SEQ ID NO. 115;

SEQ ID NO. 76 paired with SEQ ID NO. 116;

SEQ ID NO. 77 paired with SEQ ID NO. 117;

SEQ ID NO. 78 paired with SEQ ID NO. 118;

SEQ ID NO. 79 paired with SEQ ID NO. 119;

SEQ ID NO. 80 paired with SEQ ID NO. 120;

SEQ ID NO. 81 paired with SEQ ID NO. 121;

SEQ ID NO. 82 paired with SEQ ID NO. 122;

SEQ ID NO. 83 paired with SEQ ID NO. 123;

SEQ ID NO. 84 paired with SEQ ID NO. 124;

SEQ ID NO. 85 paired with SEQ ID NO. 125;

SEQ ID NO. 86 paired with SEQ ID NO. 126

SEQ ID NO. 87 paired with SEQ ID NO. 127; or

SEQ ID NO. 88 paired with SEQ ID NO. 128.

In the same way as described above, the invention also provides an antibody or fragment thereof or probe thereof as described, wherein the heavy chain variable region (VH) and/or the light chain variable region (VL) may have 80% or more, preferably 85% or more, sequence identity to the specific VH and VL sequences recited herein. For example, this may be by way of substitution, addition, or deletion of amino acid residues, with substitution often being preferred. Substitutions may, for example, be conservative amino acid substitutions.

In a preferred aspect of the invention, the antibody or fragment or probe thereof as described herein is such that the heavy chain variable region (VH) and /or the light chain variable region have 90% or more, preferably 95% or more, sequence identity to the specific sequences recited herein. For example, this may be by way of substitution, addition, or deletion of amino acid residues, with substitution often being preferred. Substitutions may, for example, be conservative amino acid substitutions.

In terms of functionality, the invention provides an antibody or fragment thereof, or a probe thereof, as described herein, that binds to STn and a group of glycans terminated by alpha 2,6-linked sialic acids. The glycans terminated by alpha 2,6-linked sialic acids may comprise for example STn, 2,6-sialyl T, di-sialyl T, or 2,6-sialolactosamine.

An overall group of glycans recognized by the antibody or fragment thereof, or a probe thereof, as described herein includes:

1. Sialyl Tn:

NeuAcα--6GalNAcα / β1-

2. 2,6-sialyl T :

Gal β1-3GalNAcα1 –- NeuAcα2-6

3. di sialyl T:

NeuAcα2-3Galβ1-3GalNAcα1 --- NeuAcα2-6

4. 2,6-sialo-N-acetyllactosamine:

NeuAcα2-6Galβ1-4Glcβ1 -

The sialyl Tn, aka, STn, sialosyl Tn, sialylated Tn, Neu5Ac-α2, 6GalNAcα-O-Ser/Thr, or also referred to as CD175s by the "cluster of differentiation' nomenclature, is the simplest sialylated mucin-type O-glycan. The STn is a truncated O-glycan containing a sialic acid (Neu5Ac) α-2,6 linked (via carbon 6} to N-acetyl-galactosamine (GalNAc) alpha-O-linked to a serine/threonine (Ser/Thr) (Neu5Ac-α2, 6GalNAcα-O-Ser/Thr). The sialylation prevents the formation of various core structures otherwise found in mucin –type O-glycans.

STn is expressed by more than 80% of human carcinomas and is associated with poor prognosis and decreased overall survival in different cancer patients. The biosynthesis of the STn antigen has been linked to the expression of the sialyltransferase ST6GalNAc1, and to mutations or loss of heterozygosity of the *COSMC* gene.

Antibodies that bind to STn with such specificity are particularly interesting because of their high tumour specificity and low or absent reactivity to normal cells, in contrast to many current antibody therapies.

An antibody or fragment thereof or probe thereof as described, is able to specifically bind to STn or a group of alpha-2,6 sialylated glycans, as described herein.

The antibodies or fragments thereof described herein, may be subject to glycan changes at glycosylation sites.

In one aspect of the invention, an antibody or fragment thereof or probe thereof as described herein, may be provided in any suitable form. For example, the antibody or fragment or probe may be provided as a ScFv, monoclonal antibody, chimeric antibody, humanized antibody, bispecific antibody, antibody drug conjugate (ADC) or CAR-T-cell, or other format, as will be understood by the skilled person.

Thus, for example, an antibody or fragment or probe thereof of the present invention, may be provided as a single-chain fragment variable antibody (scFv). This refers to a functional antibody fragment containing only the VL and VH regions, which are joined by a linker, forming a monovalent antigen binding site. Diabodies, tribodies and tetrabodies are antibodies including dimers, trimers or tetramers of scFv, i. e. containing two, three and four polypeptide chains respectively, and forming two, three and four antigen binding sites respectively, which can be the same or different.

The antibody, functional antibody fragment or probes thereof, in the present invention, may have one or more binding sites. If containing more than one binding site, these sites can be identical to one another or can be different. In the case of two different binding sites, the antibody, functional antibody fragment or probe thereof, is named a "bispecific" antibody.

In a further aspect, the present invention thus also provides an antibody drug conjugate (ADC) comprising an antibody or fragment or probe thereof, a linker and a cytotoxic drug, as herein described.

The invention also provides a pharmaceutical composition comprising an antibody or functional antibody fragment or probe thereof, or an antibody drug conjugate (ADC) and a pharmaceutically acceptable carrier, as described herein.

In another aspect, there is provided a method of detecting a tumour biomarker in a patient sample using an antibody or functional antibody fragment or probe thereof, or an antibody drug conjugate (ADC), or a pharmaceutical composition as described herein. The methodology involves the staining of biological samples obtained from a subject with the nucleotide sequences encoding an antibody or functional antibody fragment or probe thereof, or an antibody drug conjugate (ADC), as described herein, under suitable conditions for specific binding to the said antibody. The presence or absence of binding of the said antibody is indicative of tumour cells expressing

cell surface STn, 2,6-sialyl T, di-sialyl T, or 2,6-sialolactosamine. For example, the biological samples analysed, may include isolated cells, or tissue, or tumour derived proteins.

The invention also provides an antibody or functional antibody fragment or probe thereof, or an antibody drug conjugate (ADC), or a pharmaceutical composition for medical use, all of them as herein described. In particular, the antibody as a pharmaceutical composition for medical use aims at being used for treating cancer patients. It is envisaged that various types of tumours may be treated with the variants disclosed herein.

Thus, in some aspects, the antibody, functional antibody fragment or probe thereof of the invention, may be conjugated or fused to one or more diagnostic or therapeutic agents, or any other desired molecules. The resulting conjugated antibody, functional antibody fragment or probe thereof, can be useful to monitor or diagnose the onset development, progression and/or severity of a disease associated with the expression of STn or alpha -2, 6 sialylated glycans.

An antibody or functional fragment or probe thereof of the invention may also be used to detect the expression of STn or alpha-2,6 sialylated glycans in any biological sample using classical immunohistological methods (IHC or immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA), flow cytometry, and immunoblotting.

The antibody, functional antibody fragment or probe thereof of the invention may be included alone, conjugated, or in combination with a pharmaceutical composition, provided in an effective concentration, to wield a therapeutically useful effect, with minimal side effects.

In a further aspect, the invention includes an isolated polynucleotide comprising a nucleic acid sequence, wherein the nucleic acid sequence encodes an antibody or functional antibody fragment or probe thereof, as described herein, in particular the variable heavy chain region of the antibody, the variable light chain region domain of the antibody, or functional antibody fragments or probes thereof.

In a further aspect, the present invention provides an expression vector comprising a polynucleotide encoding an antibody or fragment or probe as described herein. As will be understood, a suitable host cell comprising such an expression vector may be provided.

In accordance with the invention, a method of producing an antibody or functional antibody fragment or probe thereof, as described herein may comprise using such a suitable host cell.

Sequence Liabilities

As part of the present invention, we have also looked at identifying sequence liabilities (i.e. post-translational modification - PTM - sites) in the CDRs of the humanized V1 and affinity-matured clones described herein. We have identified at least one PTM site in the heavy chain and another other in the light chain. To remove the liabilities, it is proposed to introduce a single amino acid alteration in each PTM, therefore changing the CDR.

The highest risk positions have been assessed to be:

In the VL:
•CDR3 position 93/94 "DP" Aspartate Fragmentation site
In the VH:
•CDR2 position 53/54 "NS" Deamidation site.

This risk appears to be specific to the affinity-matured variant mAb-v53.

•CDR2 position 55/56 "DG" Aspartate Isomerisation site.
Accordingly, in one aspect of the invention, any one of the L-CDR3 sequences or VL variable light chain sequences disclosed herein (either humanised or affinity-matured) may be further mutated to replace "D" (aspartic acid / aspartate) at position 93 with any one of the following amino acid residues:

A, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y.
In a further aspect, any one of the L-CDR3 sequences or VL variable light chain sequences disclosed herein (either humanised or affinity-matured) may be further mutated to replace "P" (proline) at position 94 with any one of the following amino acid residues:

A, E, F, H, I, K, L, N, Q, R, T, V, W, or Y.

In one of the changes indicated for position 93 may be paired with any one of the changes indicated for position 94.

In one preferred aspect, any one of the L-CDR3 sequences or VL variable light chain sequences disclosed herein (either humanised or affinity-matured) may be further mutated to replace the "DP" at positions 93 and 94 with any one of the following pairs of amino acid residues:

DA, DK, DN, EP, KP, NP, QP, RP, AA, EE, FF, GP, HH, II, KK, LL, NN, QQ, RR, SP, TT, VV, WW, or YY.

Thus, the VL CDR3 sequence in any of the sequences disclosed herein may be modified in the above way. Mutations DA, DK, DN, EP, KP, NP, QP, or RP may be preferred.
Certain preferred VL sequences incorporating the above sequence liability modifications are shown in Figure 23b, which discloses the variable light chains of humanised variants (v65 to v88) based on V1. These are shown as SEQUENCE ID Nos 150 to 173.
Accordingly to a further aspect of the invention, any one of the H-CDR2 sequences or VH variable heavy chain sequences disclosed herein (either humanised or affinity-matured) may be further mutated to replace "D" (aspartic acid / aspartate) at position 55 with any one of the following amino acid residues:

A, E, F, G, H, I, K, L, P, Q, R, V, W or Y.

In a further aspect, any one of the H-CDR2 sequences or VH variable heavy chain sequences disclosed herein (either humanised or affinity-matured) may be further mutated to replace "G" (glycine) at position 56 with any one of the following amino acid residues:

A, E, F, H, I, K, L, N, Q, R, T, V, W or Y.

In one of the changes indicated for position 55 may be paired with any one of the changes indicated for position 56.
In one preferred aspect, any one of the H-CDR2 sequences or VH variable heavy chain sequences disclosed herein (either humanised or affinity-matured) may be further mutated to replace the "DG" at positions 55 and 56 with any one of the following pairs of amino acid residues:

DE, DK, DA, EG, QG, RG, AA, EE, FF, GG, HH, KK, LL, DN, PQ, QQ, RR, DT, VV, WW, or YY.

Thus, the VH CDR2 sequence in any of the sequences disclosed herein may be modified in the above way. Mutations DE, DK, DA, EG, QG, or RG may be preferred.
Certain preferred VH sequences incorporating the above sequence liability modifications are shown in Figure 23a, which discloses the variable heavy chains of humanised variants (v65 to v85) based on V1. These are shown as SEQUENCE ID Nos 129 to 149.

In a preferred aspect, any one of the L-CDR3 sequences or VL variable light chain sequences disclosed herein (either humanised or affinity-matured) and containing a mutation to replace the "DP" at positions 93 and 94 with any one of the following pairs of amino acid residues:

DA, DK, DN, EP, KP, NP, QP, RP, AA, EE, FF, GP, HH, II, KK, LL, NN, QQ, RR, SP, TT, VV, WW, or YY
may be paired with any one of the H-CDR2 sequences or VH variable heavy chain sequences disclosed herein (either humanised or affinity-matured) and containing a mutation to replace the "DG" at positions 55 and 56 with any one of the following pairs of amino acid residues:

DE, DK, DA, EG, QG, RG, AA, EE, FF, GG, HH, KK, LL, DN, PQ, QQ, RR, DT, VV, WW, or YY.

In a further aspect of the invention, in the affinity-matured variant mAb-v53, it may be advantageous to further mutate this variant to replace "NS" (asparagine / serine) at position 53 and 54 of CDR2 in the variable heavy chain with alternative amino acids. For example, the N may be replaced with one of A, E, F, G, H, I, K, L, P, Q, R, V, W or Y, whilst keeping S at position 54. Alternatively, the S may be replaced with A, E, F, G, H, I, K, L, P, Q, R, V, W or Y whilst keeping N at position 54. Or both could N and S could be changed, such that a combination of the above variations may be used.

Detailed description

Materials and methods

In general, where applicable, a method for the production of the antibodies of the invention can include fusion between two cells producing an hybridoma, introducing a nucleotide sequence of the invention into a host cell, culturing the host cell under suitable conditions and for a sufficient

time for the production of the encoded heavy and/or light chain of the antibody or functional fragment or probe of the invention, following purification of the heavy and/ light chain of an antibody or functional fragment or probe thereof.

Recombinant expression of an antibody or functional antibody fragment or probe thereof of the invention, that binds to STn or a group of alpha-2,6 sialylated antigens, can include the construction of an expression vector containing a nucleotide sequence that encodes the heavy and/or light chain of an antibody or functional antibody fragment or probe thereof of the invention.

The vector can be produced by recombinant DNA technology. Such vectors can also include other coding nucleotide sequences, originating a chimeric antibody sequence. For instance, they may include the nucleotide sequence encoding the constant region of the antibody molecule (see WO 86/05807 and WO 89701036) enabling the expression of a chimera protein, containing the amino acid sequence of the antibody, functional antibody fragment or probe thereof, of the present invention followed by the entire heavy, or light chain, or both the entire heavy and light chains of the antibody

The expression vector can be transferred to a host cell by Transfection/Transduction techniques and the resulting cells produce the antibody or functional antibody fragment thereof of the invention. Thus, the invention includes host cells containing nucleotide sequences encoding the antibody or functional antibody fragment or probe thereof of the invention.

The host cell can be chosen to modify the characteristics of the product derived from the inserted nucleotide sequences.

In one aspect, these host cells can add glycosylation or phosphorylation sites, or other modifications to the coded proteins. For example, the host cells can provide the correct processing and cell trafficking/secretion of the proteins.

Finally, in order to increase the similarity to antibodies normally produced in humans and seek to decrease immunogenicity effects, the present inventors have provided new and improved useful antibody variants, including the humanization of the parental L2A5 antibody, and throughout antibody affinity maturation of a selected humanized variant, named with the acronym of V1, leading to the generation of antibody variants with increased binding and affinity to the target STn.

Antibody clones / variants obtained were characterised in terms of binding to different cancer cell lines, specificity to the target, and immunogenicity as further described below.

In general terms, the methods employed by the inventors, and the specific processes described, in terms of their technical details, will be well understood by those skilled in this field.

Humanization

Variable domain analysis and CDR identification

For the purpose of identifying complementarity determining regions (CDRs) and analysing the closest matching germline sequences the IMGT Domain Gap Align tool was used: http://www.imgt.org/3Dstructure-DB/cgi/DomainGapAlign.cgi

Molecular modelling

Molecular models were built for VH and VL domains based on homology to previously published antibody crystal structures using in-house software. PDB files allow for viewing in any molecular visualization software. Images were generated using PyMol.

Sequence liability analysis

Antibody sequences were analysed for specific liabilities based on published protein motifs. Analysis was performed using an in-house system built in Microsoft Excel. The software used the following motifs where X represents any amino acid apart from Proline:

Risk	Liability	Motif
High	Glycosylation	NXS or NXT
	Free Cysteine	C
	Deamidation	NG, NS or QG
	Isomerization	DG, DP or DS
Medium	Deamidation	NH
	Hydrolysis	NP
	Cleavage	TS, KK, RK or KR
	Transglutaminase	LLQG
	Integrin binding	RGD or KGD
	Plastic binding	FHENSP or WXXW

Gene synthesis and cloning

Variable heavy and variable light domains were designed with appropriate restriction sites at the 5' and 3' ends to enable cloning into Absolute Antibody cloning and expression vectors. Variable domain sequences were codon optimized for expression in human cells. Following gene synthesis the variable domains were cloned into Absolute Antibody vectors of the appropriate species and type. The correct sequence was verified by Sanger sequencing with raw data analysed using DNASTAR Lasergene software. Once confirmed plasmid DNA preparations of the appropriate size were performed to generate a sufficient quantity of high quality DNA for transfection.

Expression and purification

HEK 293 (human embryonic kidney 293) mammalian cells were expanded to the optimum stage for transient transfection. Cells were transiently transfected with heavy and light chain expression vectors and cultured for additional 6 days. Cultures were harvested by centrifugation at 4000 rpm, and filtered through a 0.22 M filter. A first step of purification was performed by Protein A affinity chromatography with elution using citrate pH3.0 buffer, followed by neutralization with 0.5M Tris, pH 9.0. The obtained eluted protein was then buffer exchanged into PBS, using a desalting column. Antibody concentration was determined by UV spectroscopy and the antibodies concentrated as necessary.

Antibody analytics

Antibody purity was determined by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) and HPLC (high performance liquid chromatography). SEC-HPLC was performed on an Agilent 1100 series instrument using an appropriate size exclusion column (SEC). Antibody expression titre was determined by Protein A HPLC.

Humanized Antibody characterization

Different assays were performed to demonstrate whether the humanized variants maintained biophysical properties (specificity, affinity, and internalization) similar to the parental clone. These assays included:

- evaluation of binding to BSM mucin (STn carrier) by ELISA
- evaluation of binding to different STn+ cell lines (flow cytometry)
- NMR studies to understand the antibody interaction with STn-serine glycan
- *in silico* Immunogenicity analysis
- Glycan array to determine antibody specificity
- Affinity measurement by SPR
- TMAs to evaluate mAb binding to patient-derived cancer tissue

Affinity Maturation

Library generation

A bioinformatic analysis of the parental antibody was performed to generate a site directed CDR-mutation library. After homology modelling of the antibody Fv regions, and CDR grafting onto the template, CDR residues possibly involved in antigen binding were identified. For the heavy chain, 16 positions and for the light chain, 14 positions were identified. By analysing a NGS database, commonly used amino acids for the specific germline were identified. Based on this, degenerated codons were designed, introducing mutations at the identified position possibly involved in antigen binding. Amino acids with unfavourable characteristics were generally avoided. The introduction of mutations can be described by a gaussian distribution with an average of four mutations for each antibody chain. Primers were designed based on the degenerated codons and used for introduction of mutations into the antibody sequence. The mutated antibody genes were cloned into Yumab´s scFv phage display vector and three libraries were generated and packaged into antibody-phage particles. A library with a total functional diversity greater than $5x10^8$ cfu was generated. Antibody clones with a functional open reading frame were determined by DNA sequence analysis. Packaging and purification of antibody-phage particles resulted in at least $3x10^{11}$ cfu/ml for each library.

Affinity maturation by *in-vitro* selection

The generated antibody-phage library was used for the affinity maturation by in-vitro selection. The same overall excess of antibody-phage particles to functional size was used for each individual library. The specific amount was pooled into one library for in-vitro selection.

For the first panning round, a biotinylated BSM was used. The antibody phage output of panning round one, generated on the biotinylated protein, was used for a second round on decreasing numbers of STn+ cells to increase the stringency and drive the output towards antibodies with increased affinity. In both panning rounds a negative selection against several negative antigens was performed. Four different strategies were used for affinity maturation by in-vitro selection. By increasing the stringency from strategy one to four, a decreasing amount of eluted antibody-phage particles is expected.

Antibody screening

Eluted antibody-phage particles after panning round two were used for infection of E. Coli. 384 clones from each strategy were selected randomly for antibody screening. In total, 1536 antibody clones were used for production of monoclonal scFv antibodies in the bacterial system. The produced antibody clones were tested for binding activity on the positive and negative cell line. The provided control antibody (IgG) as well as the parental scFv antibody were used as positive control. The parental scFv antibody was identified with a signal to noise ratio of 20, therefore clones with a signal to noise ratio greater than 20 were identified as hits.

Antibody sequencing

210 clones were identified as hits and selected for DNA sequence analysis. Sequence analysis revealed 42 uniquely mutated antibodies. These antibodies showed between one and six mutations in the CDR. Several hotspot mutations were identified indicating preferable mutations at different positions.

In addition, all 42 uniquely mutated antibodies were selected and soluble scFv were produced. The production was used for an ELISA screening on two positive antigens (biotinylated BSM and non-biotinylated BSM), as well as two negative antigens (streptavidin and BSA). The signal to noise ratio between positive and negative antigens was calculated for analysis. Most antibodies showed potent binding to both positive antigens and no binding to the negative antigens. Based on the generated results antibodies were selected for conversion into the final format and production in mammalian cell culture.

Conversion to final format (human IgG1):

Based on the obtained results 20 antibodies were selected for conversion into human IgG1. The antibodies were cloned into Yumab´s mammalian expression vector and produced in mammalian cell culture. The antibodies were purified using protein A affinity chromatography and buffer exchanged to phosphate buffered saline. A quality control was performed by UV/VIS spectrometry and reducing SDS-PAGE. 18 antibodies were successfully produced and showed high purity and integrity. In parallel the parental antibody was cloned in the same format and produced simultaneously.

Affinity ranking

For validation of antibody binding, a titration on the provided positive (MDA-MB-231 STn) and negative cell lines (MDA-MB-231 WT), and also to Bovine Submaxillary Mucin (BSM), was performed. Additional titration experiments were performed with cell lines that naturally express STn (COLO205, SNU16, OV90). All antibodies showed potent and specific binding to the target cells. An EC50 value was calculated. The best antibodies showed an EC50 value around 0,6 nM, whereas the parental antibody was calculated with an EC50 value of 2 nM. The specificity of the affinity-matured antibody clones was evaluated by glycan arrays.

Results

Humanised variants

EC50 ELISA - BSM

In order to evaluate the antibody binding to the target antigen STn, we employed an *in vitro* assay using a mucin that naturally expresses STn: Bovine Submaxillary Mucin (BSM). The humanized variants (V1, V2 and V3) were incubated using BSM-coated wells and binding was evaluated using ELISA assay. Absorbance was detected at 450 nm, as shown in Fig. 8a and a normalized EC50 was then calculated as shown in Fig 8b.

Fig. 8 shows normalized EC50 using humanized clones V1, V2 and V3. An ELISA-assay was performed using Bovine Submaxillary Mucin (BSM). 96 well plates were coated using BSM [3ug/mL], dissolved in 1X PBS.

Fig 8: Absorbance was detected using a microplate reader at 450 nm. Absorbance values were normalized against the background and plotted as a % of the dose response effect versus the antibody dose (log scale) as shown in the graph. The EC50 (in ug/mL) was calculated for each condition.

Based on these results, the humanized clone V2 presented with a lower EC50, compared to clone V1 and V3, thus suggesting better binding activity at lower concentration. Together these results indicate that those humanized variants have good binding properties to the target antigen STn.

Binding to STn+ cell lines

Figure 9 shows the results of binding of humanized clones to STn+ cell lines. The results show that the three variants shown (V1, V2 and V3) maintained high binding to different cell lines expressing STn.

NMR

Figure 10 illustrates how NMR studies are able to identify the points of contact/interaction between the antibody and glycan molecules. In this experiment, using humanized variant 6 (V6), it was observed that the antibody binds mostly to the sialic acid portion of STn (orange circles), but also interacts with the GalNac portion (lower right side of the scheme).

SPR

Figure 11 illustrates how Surface Plasmon Resonance (SPR) can be used to measure the affinity and kinetic parameters of different anti-STn antibodies. BSA-STn was immobilized to the chip and the antibodies were injected over the surface as association phase. The humanized clones tested (V1, V2 and V3) showed a high affinity towards BSA-STn, with KD ranging from 29nM to 39nM. No interaction was detected between a competitor clone and BSA-STn.

Glycan arrays

Figure 12 illustrates that by using a chip with different immobilized glycan probes, this assay can evaluate the binding specificity and the binding intensity of antibodies to a panel of α2-6 sialylated glycans. Humanized mAbs maintained specificity to STn-ser and STn-thr probes, confirming that the humanization process did not alter the mAb properties. V1 and V3 were shown to have increased binding when compared to “positive control”.

***In silico* Immunogenicity analysis**

Depending on the HLA context and other factors, therapeutic antibodies may be immunogenic, inducing an immune response that may lead to premature elimination or neutralization of the molecule (human anti-mouse monoclonal antibody response, or HAMA). In order to analyze the humanized CBS variants and predict their overall immunogenicity compared to the chimeric CBS version (as previously published in WO2019147152A1), in silico tools were used to analyze the antibody sequence. As shown in Fig 13. A-D, the results from this analysis help predict the overall immunogenicity, and we have found it can be used as a guide to further sequence optimization if necessary.

Figure 13 (A-D) illustrates VH and VL affinity prediction in V1 and V2 compared to the Chimeric Ab. Using the NetMHCIIPan 4.0 tool parameters were set with position in protein sequence, %Rank EL (percentile rank of eluted ligand prediction score), BA (Binding Affinity), SB (Strong Binder), WB (Weak Binder). Antibody sequences were uploaded in FASTA format. Affinity values were calculated based on the Binding Affinity (BA) provided by the tool (1/EC50 x 100).

Results obtained from the analysis of the Variable Heavy chain (VH) indicated that both CBS-V1 and CBS-V2 had a decrease in the number of immunogenic peptides compared to the chimeric CBS (chimeric L2A5) antibody (Fig A-B). Finally, the prediction results of the Variable Light chain (VL) indicated that CBS-V1 had a decrease in the number of immunogenic peptides compared to the chimeric CBS (chimeric L2A5) antibody while CBS-V2 showed an increase in the number of immunogenic peptides compared to chimeric CBS (Fig C-D).

Using these *in silico* predictions, with an overall decrease of immunogenic peptides in the humanized clones V1 and V2 seen compared to the Chimeric L2A5 Ab, these results provide evidence that these variants are less immunogenic and therefore more suitable for further development, compared to chimeric CBS.

Internalization assay

The objective of this study was to evaluate the internalization kinetics of CBS anti-STn antibodies in Breast Cancer cell lines overexpressing STn (MDA-MB231-STn). To evaluate this feature we assessed an internalization assay using humanized Abs clones (V1 and V2) together with a negative (IgG) and positive (3F1) antibodies controls as shown in Figure 14.

Figure 14 illustrates results from an antibody internalization-assay analysis using humanized Abs variants (V1, V2) in the Breast Cancer Cell line, MDA-MB231 overexpressing STn. $1x10^5$ target cells (MDA-MB-231 STn+) per well were plated in 96-well plates. Antibodies (10 ug/mL) were incubated for 2h, 6h or 16h with the target cells (triplicates for each condition) and the pHrodo fluorescence was measured by flow cytometry, Human IgG1 [2,59 mg/mL] (Abcam, Lot. GR3218380-20) was used as a negative control. 3F1 [2,57 mg/mL], a commercial anti-STn antibody, was used as a positive control and humanized variants V1 and V2 [1 mg/mL] were tested in the assay. In this assay, the antibodies were labelled with a Zenon pHrodo fluorophore (Invitrogen, Cat.Z25611) that is activated only in low pH conditions found in the early endosome. Antibody internalization was indirectly evaluated by measuring pHrodo fluorescence by flow cytometry. A signal-to-background ratio (S/B) was calculated by dividing the MFI data from each antibody to the background (target cells only) MFI. The S/B data was plotted as mean +/- SD for each time point.

In conclusion, CBS-V1 and CBS-V2 showed higher internalization profiles in MDA-MB-231 STn+ cells when compared to 3F1 mAb. The internalization was time-dependent and showed near-peak signal at 6h, a moderate to fast internalization kinetics

TMAs (tissue microarray)

To quantitatively assess the Ab binding capacity of the newly generated humanized Ab-clones, we performed immunohistochemistry (IHC) using anti-human STn V1, V2 and V3 Abs on TMA slides. In this analysis the following Abs were tested using different TMA panels as Cancer-tumours (40 types, 95 cases) and Cancer metastasis (48 cases). Representative IHC images using V1, V2 and V3, together with the controls (no primary and isotype) are shown in Figure 15.

Figure 15 shows representative IHC staining using humanized V1, V2 and V3 antibody clones in colon carcinoma and different metastatic tissues. Immunohistochemistry (IHC) was performed on formalin-fixed paraffin-embedded (FFPE) human colon carcinoma tissues using a Leica Bond automated immunostainer and 3 anti-STn antibodies V1, V2 and V3 (1:5000) plus negative (no primary antibody control) and Human IgG isotype control with citrate buffer, pH 6.0. Staining was visualized in DAB (brown) and haematoxylin was used as nuclear counterstain (blue). Whole slide images were generated using a Panoramic SCAN (3D Histech). The scale bar in the figures represents 50um.

The results obtained using IHC together with the humanized Abs (V1, V2 and V3), strong affinity to the target antigen STn was denoted in both colorectal cancer and metastatic tissues compared to controls (no primary and human isotype). Interestingly, all the three human variants of anti-STn showed a very similar pattern of binding.

Conclusions - Humanization

Findings and conclusions which may be drawn from the results obtained include the following:

- Humanized clones derived from parental L2A5 have been found to be successfully generated
- Variants V1, V2 and V3 have been shown to maintain high binding and specificity to STn
- V1 and V2 are predicted to have a lower immunogenicity compared to the parental clone
- We have used V1 as a starting point for an affinity maturation process, and further results from this are described below

Affinity-matured clones

Results

EC50 ELISA – BSM

This was performed for affinity-matured clones as described above.

Figure 19 shows EC50 ELISA results for certain clones having point mutations in the CDR regions (selected after the affinity maturation process) – these were screened against BSM mucin by ELISA. The results show that the affinity-matured variants have a lower EC50 compared to the parental V1 clone.

EC50 cell lines - COLO205, SNU16, OV90, MDA-MB-231-STn

This was performed for affinity-matured clones as described above.

Figure 20 shows results of the binding profile of the new affinity-matured antibody variants in cell lines with different STn expression levels. Each antibody was titrated using a 7-point

concentration curve and the binding intensity was measured by flow cytometry using a secondary antibody conjugated to a fluorophore.

Based on the binding results obtained in MDA-MB-231 STn cells, the EC50 for each antibody was calculated, and these are shown in Figure 21. The table summarizes the EC50 obtained for each clone as well as the maximum MFI detected.

Glycan array - affinity-matured clones

This was performed for affinity-matured clones as described above.

Affinity-matured clones were evaluated in a glycan array with different sialylated probes, and the results are shown in Figure 22. It was found that the affinity-matured clones retained specificity to STn-serine and STn-threonine (probes 3 and 4), while showing only residual binding to other probes.

Conclusions - Affinity maturation

Findings and conclusions which may be drawn from the results obtained include the following:

- The introduction of point mutations in specific regions of V1 CDRs generated clones with higher binding affinity
- Despite higher binding affinity, affinity-matured clones are still specific to STn. This is a surprising result.

CLAIMS

1. An antibody or fragment thereof, wherein the antibody comprises:

(a) a heavy chain variable region (VH) wherein the VH comprises complementarity determining regions (CDRs) selected from the group consisting of:

(i) H-CDR1, H-CDR2 and H-CDR3 as shown in any one SEQ ID NOs. 1 to 24 or 49 to 88 respectively

and / or,

(b) a light chain variable region (VL) wherein the VL comprises complementarity determining regions (CDRs) selected from the group consisting of:

(i) L-CDR1, L-CDR2, and L-CDR3 as shown in any one of SEQ ID NOs. 25 to 48 or 89 to 128 respectively.

2. An antibody or fragment thereof according to claim 1, wherein the antibody comprises a heavy chain variable region (VH) and light chain variable region (VL).

3. An antibody or fragment thereof according to claim 1 or 2, wherein the antibody comprises one of the following pairs of heavy chain CDRs and light chain CDRs:

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 1 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 25;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 2 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 26;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 3 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 27;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 4 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 28;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 5 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 29;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 6 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 30;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 7 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 31;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 8 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 32;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 9 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 33;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 10 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 34;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 11 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 35;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 12 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 36;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 13 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 37;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 14 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 38;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 15 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 39;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 16 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 40;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 17 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 41;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 18 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 42;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 19 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 43;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 20 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 44;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 21 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 45;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 22 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 46;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 23 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 47; or

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 24 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 48.

4. An antibody or fragment thereof according to claim 3, wherein the antibody comprises one of the following pairs of heavy chain CDRs and light chain CDRs:

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 1 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 25;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 3 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 27;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 2 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 26.

5. An antibody or fragment thereof according to claim 1 or 2, wherein the antibody comprises one of the following pairs of light chain CDRs and heavy chain CDRs:

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 49 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 89;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 50 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 90;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 51 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 91;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 52 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 92;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 53 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 93;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 54 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 94;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 55 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 95;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 56 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 96;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 57 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 97;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 58 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 98;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 59 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 99;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 60 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 100;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 61 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 101;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 62 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 102;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 63 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 103;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 64 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 104;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 65 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 105;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 66 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 106;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 67 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 107;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 68 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 108;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 69 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 109;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 70 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 110;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 71 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 111;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 72 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 112;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 73 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 113;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 74 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 114;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 75 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 115;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 76 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 116;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 77 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 117;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 78 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 118;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 79 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 119;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 80 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 120;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 81 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 121;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 82 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 122;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 83 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 123;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 84 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 124;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 85 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 125;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 86 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 126;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 87 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 127; or

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 88 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 128.

6. An antibody or fragment thereof according to claim 5, wherein the antibody comprises one of the following pairs of heavy chain CDRs and light chain CDRs:

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 49 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 89;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 70 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 110;

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 81 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 121; or

H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NO. 88 paired with L-CDR1, L-CDR2, and L-CDR3 as shown in SEQ ID NO. 128.

7. An antibody or fragment thereof according to any preceding claim, wherein the antibody comprises:

(a) a heavy chain variable region (VH) wherein the VH comprises a humanised heavy chain framework region

and / or,

(b) a light chain variable region (VL) wherein the VL comprises a humanised light chain framework region.

8. An antibody or fragment thereof according to any preceding claim, wherein the antibody comprises:

(a) a heavy chain variable region (VH) wherein the VH comprises a humanised heavy chain framework region as shown in the heavy chain variable region (VH) sequences selected from the group consisting of:

(i) any one SEQ ID NOs. 1 to 24 or 49 to 88 respectively

and / or,

(b) a light chain variable region (VL) wherein the VL comprises a humanised light chain framework region as shown in the light chain variable region (VL) sequences selected from the group consisting of:

(ii) any one of SEQ ID NOs. 25 to 48 or 89 to 128 respectively.

9. An antibody or fragment thereof according to claim 7, wherein the antibody comprises one of the following pairs of light chain and heavy chain framework regions, wherein the heavy chain framework region is as shown in the heavy chain variable region (VH) sequences shown below, and wherein the light chain framework region is as shown in the light chain variable region (VL) sequences shown below:

heavy chain framework region of SEQ ID NO. 1 paired with light chain framework region from SEQ ID NO. 25;

heavy chain framework region of SEQ ID NO. 2 paired with light chain framework region from SEQ ID NO. 26;

heavy chain framework region of SEQ ID NO. 3 paired with light chain framework region from SEQ ID NO. 27;

heavy chain framework region of SEQ ID NO. 4 paired with light chain framework region from SEQ ID NO. 28;

heavy chain framework region of SEQ ID NO. 5 paired with light chain framework region from SEQ ID NO. 29;

heavy chain framework region of SEQ ID NO. 6 paired with light chain framework region from SEQ ID NO. 30;

heavy chain framework region of SEQ ID NO. 7 paired with light chain framework region from SEQ ID NO. 31;

heavy chain framework region of SEQ ID NO. 8 paired with light chain framework region from SEQ ID NO. 32;

heavy chain framework region of SEQ ID NO. 9 paired with light chain framework region from SEQ ID NO. 33;

heavy chain framework region of SEQ ID NO. 10 paired with light chain framework region from SEQ ID NO. 34;

heavy chain framework region of SEQ ID NO. 11 paired with light chain framework region from SEQ ID NO. 35;

heavy chain framework region of SEQ ID NO. 12 paired with light chain framework region from SEQ ID NO. 36;

heavy chain framework region of SEQ ID NO. 13 paired with light chain framework region from SEQ ID NO. 37;

heavy chain framework region of SEQ ID NO. 14 paired with light chain framework region from SEQ ID NO. 38;

heavy chain framework region of SEQ ID NO. 15 paired with light chain framework region from SEQ ID NO. 39;

heavy chain framework region of SEQ ID NO. 16 paired with light chain framework region from SEQ ID NO. 40;

heavy chain framework region of SEQ ID NO. 17 paired with light chain framework region from SEQ ID NO. 41;

heavy chain framework region of SEQ ID NO. 18 paired with light chain framework region from SEQ ID NO. 42;

heavy chain framework region of SEQ ID NO. 19 paired with light chain framework region from SEQ ID NO. 43;

heavy chain framework region of SEQ ID NO. 20 paired with light chain framework region from SEQ ID NO. 44;

heavy chain framework region of SEQ ID NO. 21 paired with light chain framework region from SEQ ID NO. 45;

heavy chain framework region of SEQ ID NO. 22 paired with light chain framework region from SEQ ID NO. 46;

heavy chain framework region of SEQ ID NO. 23 paired with light chain framework region from SEQ ID NO. 47; or

heavy chain framework region of SEQ ID NO. 24 paired with light chain framework region from SEQ ID NO. 48.

10. An antibody or fragment thereof according to claim 7, wherein the antibody comprises one of the following pairs of light chain and heavy chain framework regions, wherein the heavy chain framework region is as shown in the heavy chain variable region (VH) sequences shown below, and wherein the light chain framework region is as shown in the light chain variable region (VL) sequences shown below:

heavy chain framework region of SEQ ID NO. 49 paired with light chain framework region from SEQ ID NO. 89;

heavy chain framework region of SEQ ID NO. 50 paired with light chain framework region from SEQ ID NO. 90;

heavy chain framework region of SEQ ID NO. 51 paired with light chain framework region from SEQ ID NO. 91;

heavy chain framework region of SEQ ID NO. 52 paired with light chain framework region from SEQ ID NO. 92;

heavy chain framework region of SEQ ID NO. 53 paired with light chain framework region from SEQ ID NO. 93;

heavy chain framework region of SEQ ID NO. 54 paired with light chain framework region from SEQ ID NO. 94;

heavy chain framework region of SEQ ID NO. 55 paired with light chain framework region from SEQ ID NO. 95;

heavy chain framework region of SEQ ID NO. 56 paired with light chain framework region from SEQ ID NO. 96;

heavy chain framework region of SEQ ID NO. 57 paired with light chain framework region from SEQ ID NO. 97;

heavy chain framework region of SEQ ID NO. 58 paired with light chain framework region from SEQ ID NO. 98;

heavy chain framework region of SEQ ID NO. 59 paired with light chain framework region from SEQ ID NO. 99;

heavy chain framework region of SEQ ID NO. 60 paired with light chain framework region from SEQ ID NO. 100;

heavy chain framework region of SEQ ID NO. 61 paired with light chain framework region from SEQ ID NO. 101;

heavy chain framework region of SEQ ID NO. 62 paired with light chain framework region from SEQ ID NO. 102;

heavy chain framework region of SEQ ID NO. 63 paired with light chain framework region from SEQ ID NO. 103;

heavy chain framework region of SEQ ID NO. 64 paired with light chain framework region from SEQ ID NO. 104;

heavy chain framework region of SEQ ID NO. 65 paired with light chain framework region from SEQ ID NO. 105;

heavy chain framework region of SEQ ID NO. 66 paired with light chain framework region from SEQ ID NO. 106;

heavy chain framework region of SEQ ID NO. 67 paired with light chain framework region from SEQ ID NO. 107;

heavy chain framework region of SEQ ID NO. 68 paired with light chain framework region from SEQ ID NO. 108;

heavy chain framework region of SEQ ID NO. 69 paired with light chain framework region from SEQ ID NO. 109;

heavy chain framework region of SEQ ID NO. 70 paired with light chain framework region from SEQ ID NO. 110;

heavy chain framework region of SEQ ID NO. 71 paired with light chain framework region from SEQ ID NO. 111;

heavy chain framework region of SEQ ID NO. 72 paired with light chain framework region from SEQ ID NO. 112;

heavy chain framework region of SEQ ID NO. 73 paired with light chain framework region from SEQ ID NO. 113;

heavy chain framework region of SEQ ID NO. 74 paired with light chain framework region from SEQ ID NO. 114;

heavy chain framework region of SEQ ID NO. 75 paired with light chain framework region from SEQ ID NO. 115;

heavy chain framework region of SEQ ID NO. 76 paired with light chain framework region from SEQ ID NO. 116;

heavy chain framework region of SEQ ID NO. 77 paired with light chain framework region from SEQ ID NO. 117;

heavy chain framework region of SEQ ID NO. 78 paired with light chain framework region from SEQ ID NO. 118;

heavy chain framework region of SEQ ID NO. 79 paired with light chain framework region from SEQ ID NO. 119;

heavy chain framework region of SEQ ID NO. 80 paired with light chain framework region from SEQ ID NO. 120;

heavy chain framework region of SEQ ID NO. 81 paired with light chain framework region from SEQ ID NO. 121;

heavy chain framework region of SEQ ID NO. 82 paired with light chain framework region from SEQ ID NO. 122;

heavy chain framework region of SEQ ID NO. 83 paired with light chain framework region from SEQ ID NO. 123;

heavy chain framework region of SEQ ID NO. 84 paired with light chain framework region from SEQ ID NO. 124;

heavy chain framework region of SEQ ID NO. 85 paired with light chain framework region from SEQ ID NO. 125;

heavy chain framework region of SEQ ID NO. 86 paired with light chain framework region from SEQ ID NO. 126;

heavy chain framework region of SEQ ID NO. 87 paired with light chain framework region from SEQ ID NO. 127; or

heavy chain framework region of SEQ ID NO. 88 paired with light chain framework region from SEQ ID NO. 128.

11. An antibody or fragment thereof according to any one of claims 7 to 10, wherein the heavy chain framework region and /or the light chain framework region have 80% sequence identity to the sequences recited.

12. An antibody or fragment thereof according to any one of claims 7 to 11, wherein the heavy chain framework region and /or the light chain framework region have 90% sequence identity to the sequences recited.

13. An antibody or fragment thereof according to any preceding claim, wherein the antibody comprises:

(a) a heavy chain variable region (VH) selected from the group consisting of:

(i) any one SEQ ID NOs. 1 to 24 or 49 to 88 respectively

and / or,

(a) a light chain variable region (VL) selected from the group consisting of:

(ii) any one of SEQ ID NOs. 25 to 48 or 89 to 128 respectively.

14. An antibody or fragment thereof according to claim 13, wherein the antibody comprises one of the following pairs of heavy chain variable regions (VH) and light chain variable regions (VL):

SEQ ID NO. 1 paired with SEQ ID NO. 25;

SEQ ID NO. 2 paired with SEQ ID NO. 26;

SEQ ID NO. 3 paired with SEQ ID NO. 27;

SEQ ID NO. 4 paired with SEQ ID NO. 28;

SEQ ID NO. 5 paired with SEQ ID NO. 29;

SEQ ID NO. 6 paired with SEQ ID NO. 30;

SEQ ID NO. 7 paired with SEQ ID NO. 31;

SEQ ID NO. 8 paired with SEQ ID NO. 32;

SEQ ID NO. 9 paired with SEQ ID NO. 33;

SEQ ID NO. 10 paired with SEQ ID NO. 34;

SEQ ID NO. 11 paired with SEQ ID NO. 35;

SEQ ID NO. 12 paired with SEQ ID NO. 36;

SEQ ID NO. 13 paired with SEQ ID NO. 37;

SEQ ID NO. 14 paired with SEQ ID NO. 38;

SEQ ID NO. 15 paired with SEQ ID NO. 39;

SEQ ID NO. 16 paired with SEQ ID NO. 40;

SEQ ID NO. 17 paired with SEQ ID NO. 41;

SEQ ID NO. 18 paired with SEQ ID NO. 42;

SEQ ID NO. 19 paired with SEQ ID NO. 43;

SEQ ID NO. 20 paired with SEQ ID NO. 44;

SEQ ID NO. 21 paired with SEQ ID NO. 45;

SEQ ID NO. 22 paired with SEQ ID NO. 46;

SEQ ID NO. 23 paired with SEQ ID NO. 47; or

SEQ ID NO. 24 paired with SEQ ID NO. 48.

15. An antibody or fragment thereof according to claim 13, wherein the antibody comprises one of the following pairs of heavy chain variable regions (VH) and light chain variable regions (VL):

SEQ ID NO. 49 paired with SEQ ID NO. 89;

SEQ ID NO. 50 paired with SEQ ID NO. 90;

SEQ ID NO. 51 paired with SEQ ID NO. 91;

SEQ ID NO. 52 paired with SEQ ID NO. 92;

SEQ ID NO. 53 paired with SEQ ID NO. 93;

SEQ ID NO. 54 paired with SEQ ID NO. 94;

SEQ ID NO. 55 paired with SEQ ID NO. 95;

SEQ ID NO. 56 paired with SEQ ID NO. 96;

SEQ ID NO. 57 paired with SEQ ID NO. 97;

SEQ ID NO. 58 paired with SEQ ID NO. 98;

SEQ ID NO. 59 paired with SEQ ID NO. 99;

SEQ ID NO. 60 paired with SEQ ID NO. 100;

SEQ ID NO. 61 paired with SEQ ID NO. 101;

SEQ ID NO. 62 paired with SEQ ID NO. 102;

SEQ ID NO. 63 paired with SEQ ID NO. 103;

SEQ ID NO. 64 paired with SEQ ID NO. 104;

SEQ ID NO. 65 paired with SEQ ID NO. 105;

SEQ ID NO. 66 paired with SEQ ID NO. 106;

SEQ ID NO. 67 paired with SEQ ID NO. 107;

SEQ ID NO. 68 paired with SEQ ID NO. 108;

SEQ ID NO. 69 paired with SEQ ID NO. 109;

SEQ ID NO. 70 paired with SEQ ID NO. 110;

SEQ ID NO. 71 paired with SEQ ID NO. 111;

SEQ ID NO. 72 paired with SEQ ID NO. 112;

SEQ ID NO. 73 paired with SEQ ID NO. 113;

SEQ ID NO. 74 paired with SEQ ID NO. 114;

SEQ ID NO. 75 paired with SEQ ID NO. 115;

SEQ ID NO. 76 paired with SEQ ID NO. 116;

SEQ ID NO. 77 paired with SEQ ID NO. 117;

SEQ ID NO. 78 paired with SEQ ID NO. 118;

SEQ ID NO. 79 paired with SEQ ID NO. 119;

SEQ ID NO. 80 paired with SEQ ID NO. 120;

SEQ ID NO. 81 paired with SEQ ID NO. 121;

SEQ ID NO. 82 paired with SEQ ID NO. 122;

SEQ ID NO. 83 paired with SEQ ID NO. 123;

SEQ ID NO. 84 paired with SEQ ID NO. 124;

SEQ ID NO. 85 paired with SEQ ID NO. 125;

SEQ ID NO. 86 paired with SEQ ID NO. 126

SEQ ID NO. 87 paired with SEQ ID NO. 127; or

SEQ ID NO. 88 paired with SEQ ID NO. 128.

16. An antibody or fragment thereof according to any preceding claim wherein L-CDR3 is further mutated to replace D (aspartic acid / aspartate) at position 93 with any one of the following amino acid residues:
A, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y.

17. An antibody or fragment thereof according to any preceding claim wherein L-CDR3 is further mutated to replace P (proline) at position 94 with any one of the following amino acid residues:
A, E, F, H, I, K, L, N, Q, R, T, V, W, or Y.

18. An antibody or fragment thereof according to claim 16 wherein any one of the mutations indicated for position 93 is paired with any one of the mutations indicated in claim 17 for position 94.

19. An antibody or fragment thereof according to claim 16, 17 or 18, wherein L-CDR3 is further mutated to replace the DP at positions 93 and 94 with any one of the following pairs of amino acid residues:
DA, DK, DN, EP, KP, NP, QP, RP, AA, EE, FF, GP, HH, II, KK, LL, NN, QQ, RR, SP, TT, VV, WW, or YY.

20. An antibody or fragment thereof according to any one of claims 16 to 19 wherein the light chain variable sequence VL is as shown in any one of SEQUENCE ID NOs 150 to 173.

21. An antibody or fragment thereof according to any preceding claim wherein H-CDR2 is further mutated to replace D (aspartic acid / aspartate) at position 55 with any one of the following amino acid residues:
A, E, F, G, H, I, K, L, P, Q, R, V, W or Y.

22. An antibody or fragment thereof according to any preceding claim wherein H-CDR2 is further mutated to replace G (glycine) at position 56 with any one of the following amino acid residues:
A, E, F, H, I, K, L, N, Q, R, T, V, W or Y.

23. An antibody or fragment thereof according to claim 21 wherein any one of the mutations indicated for position 55 is paired with any one of the mutations indicated in claim 22 for position 56.

24. An antibody or fragment thereof according to claim 21, 22 or 23 wherein H-CDR2 is further mutated to replace the DG at positions 55 and 56 with any one of the following pairs of amino acid residues:
DE, DK, DA, EG, QG, RG, AA, EE, FF, GG, HH, KK, LL, DN, PQ, QQ, RR, DT, VV, WW, or YY.

25. An antibody or fragment thereof according to any one of claims 21 to 24 wherein the heavy chain variable sequence VH is as shown in any one of SEQUENCE ID NOs 129 to 149.

26. An antibody or fragment thereof according to any one of claims 16 to 25 wherein L-CDR3 is further mutated to replace the "DP" at positions 93 and 94 with any one of the following pairs of amino acid residues:
DA, DK, DN, EP, KP, NP, QP, RP, AA, EE, FF, GP, HH, II, KK, LL, NN, QQ, RR, SP, TT, VV, WW, or YY;
and wherein H-CDR2 is further mutated to replace the DG at positions 55 and 56 with any one of the following pairs of amino acid residues:
DE, DK, DA, EG, QG, RG, AA, EE, FF, GG, HH, KK, LL, DN, PQ, QQ, RR, DT, VV, WW, or YY.

27. An antibody or fragment thereof according to any one of claims 16 to 26 wherein L-CDR3 is further mutated to replace the "DP" at positions 93 and 94 with any one of the following pairs of amino acid residues:

DA, DK, DN, EP, KP, NP, QP, or RP;
and wherein H-CDR2 is further mutated to replace the DG at positions 55 and 56 with any one of the following pairs of amino acid residues:
DE, DK, DA, EG, QG, or RG.

28. An antibody or fragment thereof according to any one of claims 13 to 27, wherein the heavy chain variable region (VH) and /or the light chain variable region (VL) have 80% sequence identity to the sequences recited.
29. An antibody or fragment thereof according to claim 28, wherein the heavy chain variable region (VH) and /or the light chain variable region (VL) have 90% sequence identity to the sequences recited.
30. An antibody or fragment thereof according to any preceding claim that binds STn and a group of glycans terminated by alpha 2,6-linked sialic acids.
31. An antibody or fragment thereof according to claim 30 wherein the glycans terminated by alpha 2,6-linked sialic acids comprise STn, 2,6-sialyl T, di-sialyl T, or 2,6-sialolactosamine.
32. An antibody or fragment thereof according to any preceding claim that is subject to glycan changes at glycosylation sites.
33. An antibody or fragment thereof according to any preceding claim that is in the form of a ScFv (single-chain variable fragment), monoclonal antibody, chimeric antibody, humanized antibody, bispecific antibody, an antibody drug conjugate (ADC), or CAR-T-cell.
34. An antibody or fragment thereof according to any preceding claim that is a functional antibody fragment thereof, or a probe thereof, that binds STn and a group of glycans terminated by alpha 2,6-linked sialic acids.
35. An antibody drug conjugate (ADC) comprising an antibody according to any preceding claim, a linker and a cytotoxic drug.
36. A pharmaceutical composition comprising an antibody or fragment thereof according to any one of claims 1 to 34, or an antibody drug conjugate (ADC) according to claim 35, and a pharmaceutically acceptable carrier.
37. A method of detecting a tumour in a subject using an antibody or fragment thereof according to any one of claims 1 to 34, or an antibody drug conjugate (ADC) according to claim 35, or a pharmaceutical composition according to claim 36, the method comprising: staining a biological sample obtained from a subject with the nucleotide sequences encoding an antibody according to any one of claims 1 to 34, or an antibody drug conjugate (ADC) according to claim 35, under conditions suitable for specific binding of the said antibody, wherein the presence or absence of

binding of the said sequences is indicative of the presence or absence of tumour cells expressing cell surface STn, 2,6-sialyl T, di-sialyl T, or 2,6-sialolactosamine, optionally wherein the biological sample comprises isolated calls, or tissue, or tumour derived proteins.

38. An antibody or fragment thereof according to any one of claims 1 to 34, or an antibody drug conjugate (ADC) according to claim 35, or a pharmaceutical composition according to claim 36, for use in medicine.

39. An antibody or fragment thereof according to any one of claims 1 to 34, or an antibody drug conjugate (ADC) according to claim 35, or a pharmaceutical composition according to claim 36, for use in treating cancer.

40. An expression vector comprising a polynucleotide encoding an antibody according to any one of claims 1 to 34.

41. A host cell comprising an expression vector according to claim 40.

42. A method of producing an antibody or fragment thereof according to any one of claims 1 to 34 which method comprises using a host cell according to claim 41.

Intellectual Property Office

Application No:	GB2209332.2	**Examiner:**	Dr Chris Perry
Claims searched:	1-4, 7-9, 11-14 and 16-42 (all in part)	**Date of search:**	23 December 2022

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1-4, 7-9, 11-14 and 16-42	WO 2019/147152 A1 (UNIV NOVA DE LISBOA) See claims 1, 3 and 5
X	1-4, 7-9, 11-14 and 16-42	US 2019/276541 A1 (SIAMAB THERAPEUTICS INC) See claims 1, 5-9, 25 and 29-30

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC, Patent Fulltext, BIOSIS, MEDLINE, CAS ONLINE

International Classification:

Subclass	Subgroup	Valid From
C07K	0016/30	01/01/2006
A61K	0039/395	01/01/2006
A61P	0035/00	01/01/2006
G01N	0033/574	01/01/2006